ILLUSTRATED POCKET DICTIONARY OF CHROMATOGRAPHY

PAUL C. SADEK

ILLUSTRATED POCKET DICTIONARY OF CHROMATOGRAPHY

ILLUSTRATED POCKET DICTIONARY OF CHROMATOGRAPHY

Paul C. Sadek, PhD

WILEY-INTERSCIENCE

A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data:
Sadek, Paul Charles.
Illustrated pocket dictionary of chromatography / Paul C. Sadek.
p. cm.
Includes bibliographical references and index.
ISBN 0-471-20021-2 (pbk.)
1. Chromatography–Dictionaries. I. Title.

QD79.C4S33 2005
543′.8′03—dc22

2004045641

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

CONTENTS

PREFACE

Instrument-based chromatography is now, for all practical purposes, a mature and well-established field. The days of the necessity for intensive training on the theories underpinning the technology simply to provide competent use have long since passed. Now the requirement is for the analyst to be competent and proficient in the use of the technology, to produce reliable and defensible results on a routine basis with little or no formal guidance. I have found that this is case in my laboratory, and I believe the basic scenario is the same in laboratories throughout the world.

I have attempted to address these needs in two other texts—through method review and summary in *The HPLC Solvent Guide*, 2nd edition, and through description of the use and maintenance of LCs in *Troubleshooting HPLC Systems: A Bench Manual*.

This text aims at a slightly different need that lies beyond the basic use of chromatographic instrumentation. The commonplace use of chromatographic techniques in analyzing regulated products places an additional burden on the analyst: the proficient use of the basic data-handling terms is also expected. Therefore, the definitions of some fundamental statistical terms and concepts are included.

It should be noted that there are many excellent formal training courses that can assist with these issues, but often, because of the broad nature of the field, they can only cover topics in a limited fashion that may or may not meet the exact needs of the trainee. Outstanding texts also exist that deal with deriving and defining the concepts and equations governing separations, but many analysts to do want to read through pages of theory of derivation to get to the desired equation, only to find that a real-life example is not included.

This text is intended as a companion for those who have limited experience in chromatography but now are required to work in that field. It provides a basis from which the analyst can readily apply a concept without fully understanding the derivation from the math or the fundamental concepts.

The thrust of this text is to provide succinct entries, both definitions and graphic/pictorial aids, from which the analyst can get immediately useful results. Analysts who require the theory behind or the details surrounding a concept are referred to the large set of excellent reference texts listed at the end of the entries.

A list of commonly used acronyms is also presented; they seem to proliferate more rapidly than rabbits, and so, once again, those ana-

lysts who are not long-time familiars of the technology may feel somewhat cast adrift without a ready reference.

In summary, then, this is not a theory or application text in the traditional sense of the word but is truly a guide and support for those analysts who now find themselves required to immediately utilize the technology in their laboratory.

My hope is that this text is small enough to be used readily in the laboratory, a working manual if you will, but large enough to cover the needs of a wide range of analysts.

Paul C. Sadek
Grand Rapids, MI

A

absorbance The mathematical representation for the loss of incident radiant energy intensity (e.g., ultraviolet, visible, infrared) as it passes through the sample is the absorbance, A:

$$A = \log(P_o/P),$$

where P_o is the power of the incident beam and P is the attenuated beam that leaves the sample and impinges the detector. Because absorbance is the logarithm of the ratio of radiant energies, it is unitless. Absorbance is also related to the solution concentration of an analyte through Beer's law: $A = \varepsilon bC$, where ε is the absorptivity of the analyte, b is the cell path, and C is the concentration of the analyte.

absorption (1) The partitioning process for an analyte between the mobile phase and a liquid or liquidlike stationary phase. (2) The process by which incident radiation on a sample is attenuated by interaction with the sample.

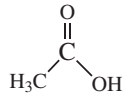
absorptivity A proportionality constant used in the relationship between absorbance and analyte concentration (i.e., Beer's law). Absorptivity is a function of analyte identity, wavelength, solvent composition, and temperature. The units of measure for absorptivity are determined by the units of measure used to express the analyte concentration: When concentration is expressed as moles/liter (molarity) and the path length in centimeters then the molar absorptivity, ε, has units of L/cm·mole.

accuracy The accuracy of a result is defined as how closely the experimentally derived result comes to the "true" or "accepted" value. In the method development process the accuracy of a method is often determined by spiking a matrix blank [a sample containing every component except the analyte(s) of interest] to the desired level and calculating the percent recovery, 100% being expected.

acetic acid (glacial) Molecular weight: 60.1; boiling point: 117.9°C; refractive index (20°C): 1.3716; density (20°C): 1.049 g/mL;

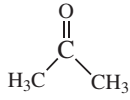
Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

viscosity (15°C): 1.31 cP; polarity index (P′): 6.2; Hildebrand solubility parameter (δ): 12.4; pK_a = 4.7. Miscible with water. Acetic acid is a weak acid that is frequently used as a mobile-phase modifier directly or as a buffer (acetic acid/acetate) in LC, TLC, and CE techniques. Pungent odor.



Acetic acid

acetone Molecular weight: 58.1; boiling point: 56°C; refractive index (20°C): 1.3587; density (20°C): 0.79 g/mL; viscosity (20°C): 0.36 cP; UV cutoff: 330 nm; eluotropic strength (ε^o)—on alumina: 0.56, on silica: 0.53; polarity index (P′): 5.1; Hildebrand solubility parameter (δ): 9.6. Miscible with water. Extremely volatile and flammable. Acetone is known as a "universal" solvent in that it is miscible with an extremely wide range of commonly used polar and nonpolar solvents. [Note that this fact does not imply anything about the solubility of salts and buffers in acetone.] Because of this, acetone may be used as the intermediate solvent when changing from HPLC reversed-phase systems (i.e., aqueous/organic solvents) to normal-phase systems (i.e., nonpolar solvents) and vice versa. Acetone is also used as a solvent in TLC and a sample solvent in GC.



Acetone

acetonitrile Molecular weight: 41.1; boiling point: 82°C; refractive index (20°C): 1.3441; density (20°C): 0.78 g/mL; viscosity (15°C): 0.38 cP; UV cutoff: 190 nm; eluotropic strength (ε^o)—on alumina: 0.65, on silica: 0.52; polarity index (P′): 5.8; Hildebrand solubility parameter (δ): 11.7. Miscible with water. Volatile and flammable. Acetonitrile is one of the most commonly used solvents in reversed-phase HPLC because of its low viscosity and very low UV cutoff. It should be noted that phosphate buffers have a limited solubility in aqueous/acetonitrile solvents. This solubility decreases as the phosphate ion increases in charge (i.e., goes from $H_2PO_4^-$ to PO_4^{3-}). Acetonitrile is not miscible with alkanes. Acetonitrile has found limited use in TLC and GC.

CH_3CN

Acetonitrile

achirality Condition in which a molecule and its mirror image are superimposable (i.e., the molecule does not contain a chiral center). Compare with diastereomer.

activity (1) The activity of an analyte is determined from the concentration of the analyte, [A], and its activity coefficient, γ_A,

$$\text{Activity of A} = \gamma_A[A]$$

(2) Used to describe the extent and strength of surface interactions caused by residual hydroxyl groups (i.e., silanol and aluminol) in adsorption support materials.

activity coefficient, γ_A Number experimentally generated to quantitatively correct an analyte's concentration for the nonideality of the system (i.e., it is not at infinite dilution). In an ideal solution an activity coefficient is 1.0. (*See* activity.) As a case in point, the activity coefficient for a solute in a liquid solution is dependent on the charge density, shape, and size of the solute and the composition of the ionic species in the solvent (e.g., ionic strength). As the concentration and charge density of the ions increase, the activity coefficient decreases.

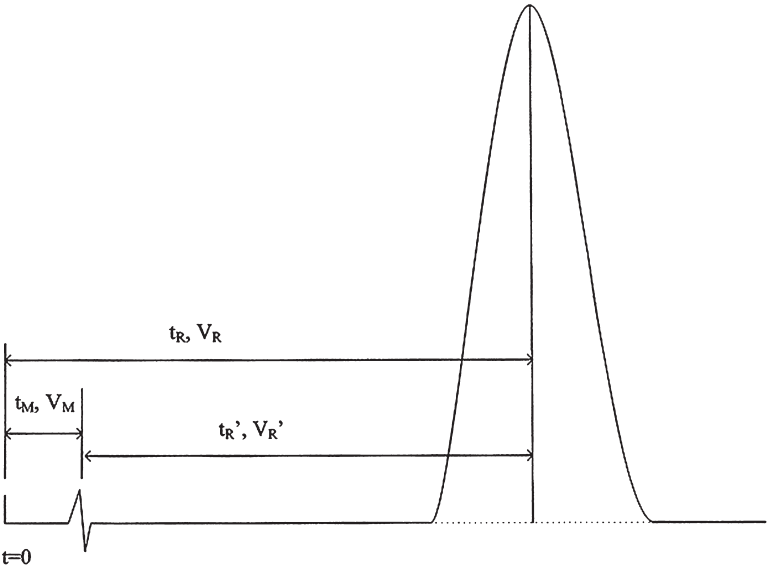
adapter Component used to couple solid-phase extraction columns or reservoirs.

additive *See* mobile-phase modifier.

adjusted retention time, t_R' The adjusted retention time is calculated from the chromatographic retention time, t_R, and the elution time of a nonretained compound, t_M:

$$t_R' = t_R - t_M$$

The adjusted retention time is used to compensate for system-related retention time variability and allows for a rapid comparison of results from instrument to instrument and lab to lab.



The adjusted retention is the time or volume, t_R' or V_R', calculated from the time of injection, $t = 0$, to the time or volume peak maximum of the analyte, t_R or V_R, less the time or volume it takes for an unretained peak to elute, t_M or V_M.

adjusted retention volume, V_R' The adjusted retention volume is calculated from the retention volume, V_R, and the system void volume as determined by a nonretained compound, V_M:

$$V_R' = V_R - V_M$$

For GC work this should not be confused with the corrected retention volume, V_R°, which takes into account the compressibility of a gas.

adsorbent In particular, an adsorbent is a support material used for adsorption chromatography separations (e.g., graphitized carbon, silica, alumina, zirconia). However, adsorbent can sometimes be used in a broader sense to indicate the support material in general.

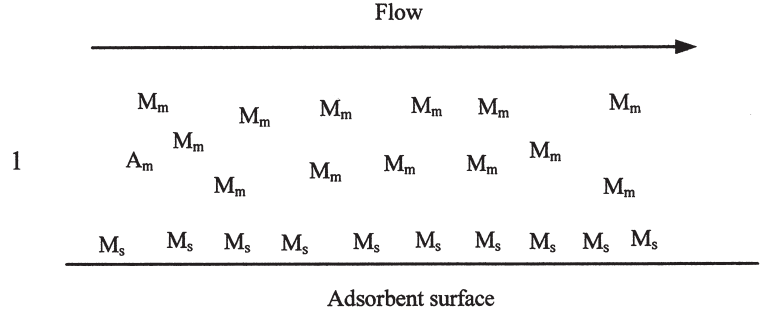
adsorption The process of a molecule transferring from the mobile phase and adhering to the surface of the stationary phase. The adsorption process can be a facile thermodynamic equilibrium, kinet-

ically slow, or irreversible. It is controlled by one or more interactions: hydrogen bonding and van der Waals interactions. The condition of rapid, reversible thermodynamic equilibrium is the desired one in most separations.

adsorption chromatography Adsorption chromatography, also called liquid-solid chromatography (LSC), uses unmodified solid adsorbents such as silica, alumina, and carbon to generate a separation. The retention mechanism for adsorption chromatography is through the reversible equilibrium displacement of mobile-phase components, M, with the analyte, A, on the surface of the adsorbent, s, into the mobile phase, m:

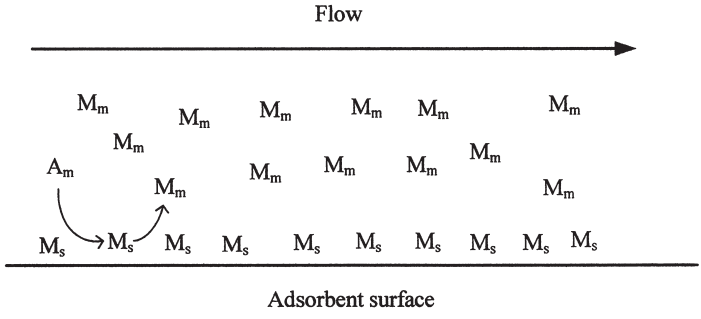
$$A_m + M_s \leftrightarrow A_s + M_m$$

As shown in step 1, the mobile-phase molecules have reached equilibrium with the stationary phase. In step 2, an analyte molecule displaces a surface-sorbed mobile-phase molecule and is retained, as shown in step 3. Elution occurs when the mobile phase displaces the analyte from the surface. This adsorption-desorption action happens numerous times through the elution process. The chemical interactions governing the adsorbent interactions are van der Waals and hydrogen bond.

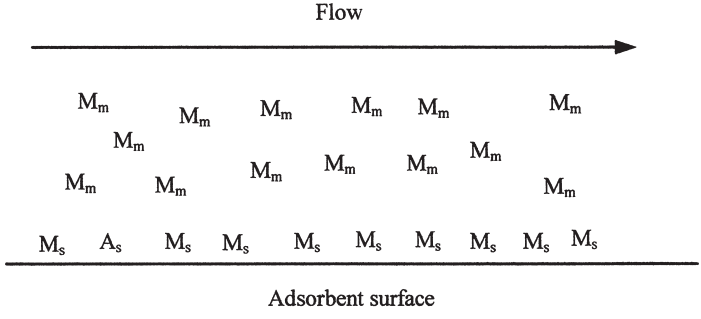


Adsorption chromatography: Step 1 is where the analyte in the mobile phase, A_m, reaches the packed column that is in dynamic equilibrium with flowing mobile phase, M_m and adsorbed M_s. Step 2 is where the analyte displaces ($A_m \rightarrow A_s$) a surface-adsorbed mobile phase ($M_s \rightarrow M_m$). Step 3 shows the analyte being surface adsorbed, A_s. Ultimately, it will be displaced by the mobile phase, and a series of adsorption-desorption steps will occur down the length of the column. The relative amount of time A spends on the surface versus in the mobile phase determines its elution time.

2



3



adsorption isotherm A plot constructed from the equilibrium concentration of an analyte in the mobile phase per mass of sorbent. The shape of the isotherm and the relative concentration of the analyte with respect to the sorbent are factors that determine the elution profile. *See* Langmuir and Freundlich isotherms.

adsorption site A specific area on a stationary phase where the analyte (or any other component of the mobile phase) can interact with the stationary phase. For example, on alumina supports, the adsorption sites are basic, neutral, and/or acidic electron-acceptor aluminol groups. On an octadecyl bonded-phase silica stationary phase the adsorption sites are the residual silanol groups (technically the octadecyl groups are considered to be involved in a partition process). Note that the type and concentration of these sites in a given

column determine, in conjunction with other operational parameters (mobile phase composition, temperature, etc), the degree to which an analyte is retained on the stationary phase.

aerogel A packing material that is produced from a gel that contains a dispersing liquid. The dispersing liquid is removed, leaving a porous gel. The silica-based form of these gels is commonly used for size-exclusion work.

affinity chromatography An LC technique that uses various biologically active substances (e.g., enzymes, substrates) to generate separations, for example, the use of antibodies bonded to the support that interact specifically with a corresponding hormone (drug or peptide, etc). The difficulty with affinity chromatography is in finding conditions where the substrate-enzyme interactions are reversible on a chromatographic timescale. In addition, for bonded enzymes loss of activity on bonding to the surface and maintaining conditions (both use and storage) for long-term separation reproducibility are a challenge.

affinity ligand A material bonded to a support to generate a separation based on affinity interactions. Affinity ligands can be for general substance classes such as lectins (e.g., concanavalin A) for sugars and polysaccharides, dyes (e.g., Cibacron blue) for specific enzyme classes, or biospecific ligands such as an antibody for an antigen.

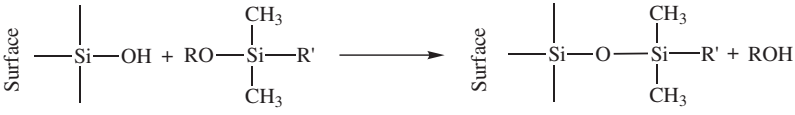
agarose A polymeric polysaccharide used in the separation of biomolecules in both slab electrophoresis and gel-filtration separations.

air peak In GC a nonretained, nonexcluded component (typically air) is used to define the minimum time needed for to travel from the injector to the detector. (This is similar to the void volume in LC systems.)

alkanes A class of compounds that are saturated hydrocarbons. The general formula of a linear alkane is C_nH_{2n+2}. Alkanes may also have branched or cyclic structures. Examples of alkanes frequently used in chromatographic separations are pentane, hexane, and heptane (linear alkanes where n = 5, 6, and 7, respectively), iso-octane (a branched alkane also called 2,2,4-trimethylpentane), and cyclopentane and cyclohexane (cyclic alkanes having rings of 5 and 6 carbons, respectively).

alkenes A class of compounds that are saturated hydrocarbons. The general formula of a linear alkane is C_nH_{2n}. Alkenes may also have branched or cyclic structures. Alkenes do not have wide use as solvents in chromatographic separations but find use as "preservatives" for chlorinated alkane solvents (such as methylene chloride). For this use, amylene (2-methyl-2-butene) and cyclohexene are examples.

alkoxysilanes A group of derivatization reagents used in the preparation of bonded phases. Alkoxysilanes are used in place of chlorosilanes when the bonded-phase functional group itself is reactive toward the chlorosilane.



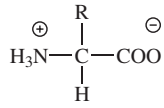
where R is $-CH_3$ or $-CH_2CH_3$ and R' is the bonded phase moiety

Alkoxysilane

alumina A material used as a support in HPLC and TLC. It has the general formula $(Al_2O_3)_x$. Alumina is characterized as basic, neutral, or acidic. Unmodified alumina has an isoelectric point of ~7.5. At pH values above 8 basic alumina can be used as a cation exchange material, whereas below pH 7 it can be used as an anion exchange material. In addition, the activity of an alumina support is defined by the Brockmann activity scale (a measure of the water present on the alumina).

aluminol group An Al-OH terminating group on an alumina surface.

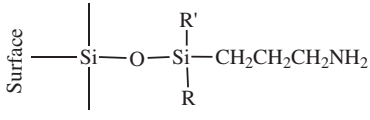
amino acids A class of compounds that are difunctional in nature, having both a basic amine functional group and a carboxylic acid functional group. They are the basic chemical building blocks for peptides and proteins and have the following general chemical structure: where R is a substituent ranging from a simple hydrogen R = H, which describes glycine to, for example, R = $NH_2CH_2CH_2CH_2CH_2$ which describes lysine.



Amino acid (general)

amino acid analysis Amino acid analyses are analytical methods used to characterize peptides and proteins with respect to their amino acid composition. Both LC and GC are used for amino acid analysis. In both cases, the peptide or protein must be hydrolyzed into the basic amino acid constituents. These amino acids are then further treated to enhance detectability (e.g., with *o*-phthalaldehyde for fluorescent HPLC analysis, phenylisothiocyanate for UV HPLC analysis, or boron trifluoride to render carboxylic acids volatile for FID GC analysis).

aminopropyl-bonded phase An aminopropyl-bonded phase has a terminal amine functional group covalently bonded to the surface of the support material.



Aminopropyl bonded phase

For discussion of the R groups, *see* octadecyl bonded phase.

amperometric detector Used in LC work. It consists of an electrode that is part of the detector cell poised at a constant potential versus a reference electrode (e.g., Ag/AgCl). As an electroactive sample moves through the cell, it is either reduced or oxidized (depending on the applied potential and its reduction potential) and a resulting current is generated as the signal, generally read in nano- or microamperes. Note that an auxiliary electrode is present to prevent current flow through the reference electrode. The maximum signal occurs when the rate at which the sample reaches the electrode is diffusion limited. The type and concentration of the supporting electrolyte in the mobile phase are important, as are achieving selectivity

and optimizing sensitivity through the proper choice of the applied potential. Pulsed amperometry is a modified amperometric technique that rapidly increases the applied potential for a very short period of time (milliseconds). The sample signal is obtained over a portion of the pulse in order to reduce system noise.

ampholyte A compound that contains one (or more) acidic functional group *and* one (or more) basic functional group. Amino acids are examples of ampholytes having a carboxylic acid group (—COOH) and a basic amine functional group (—NH_2). Note that molecules that have multiple acid/base functional groups are referred to as polyampholytes. Examples include polypeptides and proteins.

amphoteric support Typically a resin that has both positively and negatively charged functional groups permanently on the surface.

analysis time The time required for any separation for all components of a sample that is injected ($t = 0$) to migrate through the system and detector. Note that in a gradient system additional time is required for the system to return to the original conditions (e.g., the temperature for GC and mobile-phase composition for LC) and completely reequilibrate to the initial conditions.

analyte The component of interest contained in the sample. It is the component that is recovered and analyzed subsequent to all sample preparation steps.

analytical HPLC column Has an inner diameter of 3.0–5.0 mm and ranges from 5 to 30 cm in length. Injection volumes from 1 to 50 μL are typical, with sample loads in the microgram range. Note that as the inner diameter increases in general the resolution, analysis time, operation ease and upkeep, and maximum sample load increase whereas sensitivity decreases.

anion A molecule or atom that bears a negative charge is an anion or anionic. Examples of anions are acetate (CH_3COO^-), phosphate ($H_2PO_4^-$, HPO_4^{-2}, or PO_4^{-3}), hydroxide (OH^-), and chloride (Cl^-).

anion exchange An anion exchange material has a permanent positive charge on the surface and is used to separate anionic compounds (negatively charged species). The type of positive charge (e.g., a primary, secondary, or tertiary amine) and its concentration on the surface (given as meq/g), the anionic analyte to be eluted, and the type and concentration of anionic mobile-phase component that is used to displace the analyte determine the overall elution time and profile. Anion exchange resins are classified as strong or weak depending on their relative affinity to anions.

anode In techniques in which a voltage is applied (i.e., electrolytic, not galvanic), an anode is a positive electrode toward which anions migrate.

area percent A determination of the level of analyte, A_s, compared with the total area of all the sample-related peaks in the chromatogram, $\Sigma(A_s)_i$,

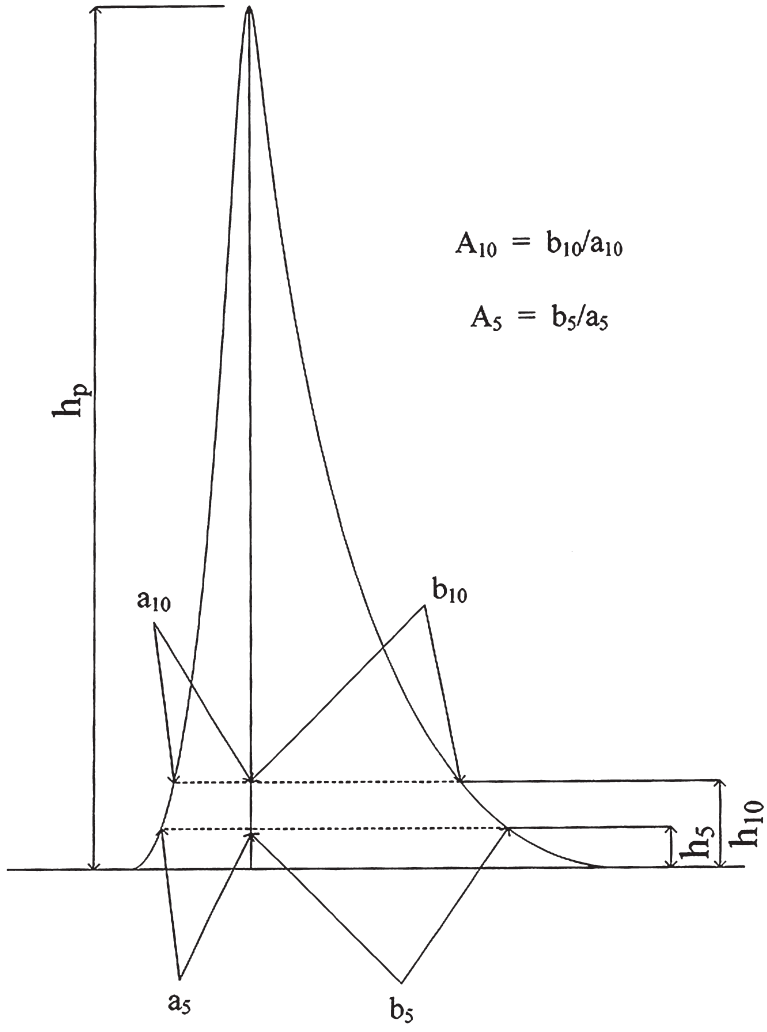
$$\text{area \% of } A_s = [A_s/\Sigma(A_s)_i] \times 100$$

Note that for this method to be effective, all related components in the sample must elute and be detected and all analyte responses must be the same (response/mass).

asymmetric Refers to a carbon atom that has four unique substituents. This asymmetric carbon generates a chiral center.

asymmetry Refers to any elution profile varying from a symmetric Gaussian distribution. It is mathematically calculated as an asymmetry factor and is determined from a defined height on the peak (*see* asymmetry factor, A_x).

asymmetry factor, A_x Calculated as a ratio of the distance between the perpendicular dropped from the peak maximum that elutes after the perpendicular to that which elutes before the perpendicular.



To calculate the asymmetry factor the following must be done: (1) Measure the height of the peak at peak maximum, h_p. (2) Drop a line from the peak maximum that is perpendicular to the baseline. (3) Mark the percent height at which the asymmetry will be determined. (In this case both 10% and 5% are marked.) (4) Draw a line from the front to the back of the elution profile at the chosen peak height. This line is parallel to the baseline. (5) Measure the distances from the peak front and tail to the perpendicular and calculate the asymmetry as: A = b/a.

Mathematically,

$$A_x = b/a$$

and x is the % peak height at which the asymmetry was calculated. Peaks that are perfectly symmetric have A = 1.0, whereas tailed peaks have A > 1.0 and fronted peaks have A < 1.0.

A term The component of the van Deemter and Knox equations that describes the impact that eddy dispersion has on the band broadening process.

atmosphere A unit of measure for pressure in a chromatographic system equaling 14.7 lb/in^2 (psi) or 760 torr.

atmospheric pressure ionization (API) Describes an inlet ionizer to a mass spectrometer that produces ions at atmospheric pressure. This basic inlet is coupled with electrospray or chemical ionization to produce detectable ion fragments.

autosampler An automated injection system that is programmed to inject a preprogrammed volume of sample (or set of samples) one or more times. Some autosamplers can dilute and mix samples before injection. The autosampler takes the place of manual injection and is advantageous with regard to both precision and efficiency.

average Term used interchangeably with the term mean. The average is the best mathematical estimate of the value for related results. *See* mean.

axial diffusion *See* longitudinal diffusion.

azeotrope A mixture of liquids that, under constant conditions, boils at a constant composition (i.e., the liquid and the vapor above it have the same composition). The azeotropic condition is particularly useful for the distillation of mixtures to produce constant-ratio blends.

B

back diffusion Occurs in gas chromatography when the injector septum no longer seals after an injection. This allows air and air-borne contaminants into the system and leads to ghost peaks.

back flush A technique used to effectively clean an LC column from materials that have deposited on the head of the column. This involves reversing the direction of solvent flow in the column and using a very strong solvent to elute the adsorbed material from the head of the column. Effective back flushing requires the use of a strong mobile phase. Note that this may involve a number of steps, especially if a mobile phase with salt-based buffers is used. In any case, never have the back flush effluent go through the detector; pump the effluent directly to waste. Also, for columns that have received long-term use back flushing may be detrimental because the reversed flow may cause a weakened packing bed structure to collapse.

backpressure The measure of the resistance to flow through a chromatographic system. Barring any constriction at the point of entry of the mobile phase into the system, the backpressure is a measure of the pressure of the system at the top of the column. In general, decreased particle size and increased mobile-phase viscosity increase the system backpressure. Common units of pressure are atmospheres, bars, pounds per square inch (psi), or pascals (Pa).

band Term used to describe the analyte envelope as it moves through the stationary phase.

band broadening During the course of the elution process all the factors that contribute to widening the analyte envelope are termed band broadening. Band broadening is a function of phase-transfer processes, extra column volumes, and diffusion phenomena. The larger the band broadening effects, the less efficient the system.

bandwidth (1) Bandwidth, t_w, describes the width of the analyte envelope, along the length of the column or plate, as it elutes from the column; bandwidth is analogous to peak width for the eluted sample that has passed through the detector. (2) Defines the range of

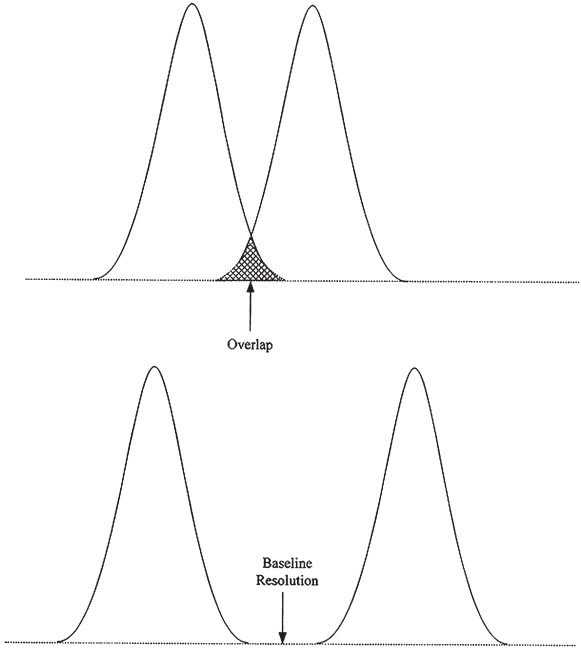
Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

wavelengths that pass through a monochrometer in a UV-visible detector. Linearity is better as bandwidth decreases, but the trade-off is that the radiation that reaches the sample decreases with decreasing bandwidth and so the detection limit generally increases.

bar A unit of measure for pressure equal to ~14.7 psi, 1 atm, or ~0.1 MPa.

baseline The operating baseline is generated by the eluent only through the system. For isocratic or isothermal runs the baseline should be constant with no upward or downward drifting. For a gradient (composition or thermal), a shift in baseline is often expected but should result in a smooth change in the baseline and should be reproducible from run to run.

baseline resolution Occurs when in the case of two adjacent peaks the least-retained peak completely elutes before the later-eluting peak begins to elute.



In the top separation the peaks are only partially resolved. The bottom separation shows baseline resolution, i.e., the elution profile for the first-eluting peak reaches the system baseline before the second peak starting to elute.

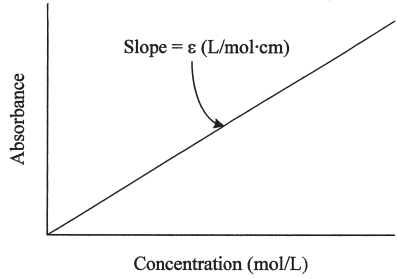
Resolution is mathematically described by a resolution factor and/or a separation factor.

beam-deflection refractive index (RI) detector Uses a split cell: One section of the cell contains the static reference solution (typically the mobile phase) and the other contains the flowing mobile-phase stream. The reference solution is subtracted from the signal of the flowing mobile, and the result is recorded as the detector output. The beam-deflection RI is useful over the entire refractive index range (unlike the Fresnel RI).

Beer's law The mathematical relationship between absorbance, A, and sample concentration, C:

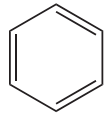
$$A = \varepsilon bC$$

Here ε represents the molar absorptivity (concentration in mol/L and cell length in cm). For quantitative analytical use, a plot of A vs. C is generated and the resulting sample concentration can be read directly from the graph.



Beer's law: The plot of absorbance vs. concentration in this case is linear, with the slope being the molar absorptivity. Note that the value of ε varies with solvent composition and wavelength of operation.

benzene Molecular weight: 78.1; boiling point: 80.1°C; refractive index (20°C): 1.5011; density (20°C): 0.87 g/mL; viscosity (20°C): 0.65 cP; UV cutoff: 280 nm; solubility in water (20°C): 0.18%; water solubility in benzene (25°C): 0.06%. Benzene is an excellent solvent for many organic compounds but is infrequently used today because of its high health hazard. Flammable and volatile.



Benzene

BET test method Named for the developers (Brunauer, Emmett, and Teller), a technique that uses nitrogen adsorption isotherms to determine the surface area of porous materials. Pore volume and pore size distribution can also be derived from these test results.

bias The effect resulting from the presence of a systematic error in the analysis. A bias consistently shifts the obtained result to a greater or lower value than expected. A bias is particularly difficult to diagnose because repeated analysis does not expose bias (unlike random error).

binary mobile phase Functionally defined as a solution that is comprised of two major constituents (e.g., 50/50 v/v methanol/water). Although solvents contain low–level mobile-phase modifiers such as a buffer, the buffer by definition exists as an equilibrium between two forms (e.g., acid and its conjugate base) and so does not technically meet the definition of binary. Therefore, it is less confusing to refer to the major constituents only.

biocompatible Refers to any component (i.e., mobile phase, packing, tubing, etc) that comes into contact with a biomolecule and does not cause irreversible adsorption or denaturation.

bleed (1) The process of release of volatile/soluble components of a GC or HPLC stationary phase. (2) Associated with the decomposition or release of volatile materials from a septum in GC. The most critical aspects of bleed occur in GC and LC/MS applications, where the bonded-phase or septum breakdown leads to the appearance of these moieties in the detector. This is not the same as baseline drift, which is due to the overall change in detector response generated by changes in the elution conditions.

bonded phase The part of a stationary phase that is chemically bound to the support material. A common example is the reaction of chlorodimethyloctadecylsilane with silica to produce an

"octadecyl" bonded-phase material. The stationary phase is often identified by its prevalent functional group, hence "octyl" rather than "dimethyloctyl."

BPC (bonded-phase chromatography) A liquid chromatographic technique based on the use of support materials that are chemically modified to produce stationary-phases of varying polarity and functionality. Bonded phases run the range from reversed phase to normal phase to chiral to affinity, etc.

breakthrough Typically used in flow injection analysis and solid-phase extraction, breakthrough represents the time (or volume) when a defined percentage (e.g., 1%) of the solute elutes from the column.

breakthrough volume For a defined system and set of operating conditions, the volume of eluent needed to elute a defined percentage of solute (e.g., 1%). This parameter is particularly important for flow injection analysis (determines the time between injections) and solid-phase extraction (determines the fraction to discard or collect). It is a function of system volume, flow rate, and the retention of the analyte.

Brockmann activity scale Used in the characterization of alumina support materials and based on the amount of water adsorbed to the support material.

Brockmann Activity	%Added Water	%Water Total
I	0	~2
II	3	~4.5
III	6	~7.5
IV	10	~11.5
V	15	~16.5

brush phase *See* monomeric bonded phase.

B term The component of the van Deemter and Knox equations that describes the impact that longitudinal diffusion has on the band broadening process.

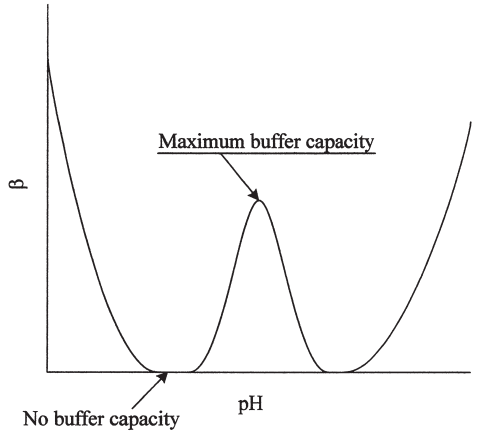
buffer Used to minimize changes in a critical solution parameter when the solution is subjected to change. A typical buffer application

is holding the pH of a solution constant. In this case the buffer is comprised of the acid (e.g., acetic acid) and conjugate base (e.g., sodium acetate). Conversely, a base and its conjugate acid accomplish the same thing. The most effective buffer is obtained when the desired pH is the same as the pK_a of the acid (i.e., [HA] = [A^-], where HA is the acid and A^- is the conjugate base).

buffer capacity (or buffer index), β A quantitative means of calculating the change of a solution pH on the addition of acid or base to the solution. Mathematically the buffer index is written as:

$$\beta = dC_b/d\text{pH (for base addition), or}$$
$$\beta = -dC_a/d\text{pH (for acid addition)}$$

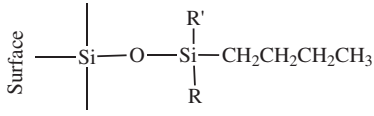
where C_b and C_a are the concentrations of base and acid, respectively.



Buffer capacity: The plot is a representation of the ability of a solution to resist a change in pH when either acid or base is added. Where β has a maximum (i.e., at the pK value for the acid-base conjugate pair) is the pH at which addition of acid or base results in the smallest change in solution pH.

Note that the maximum buffer capacity occurs at the point where pH = pK (equal concentrations of the conjugate acid/base pair) and will increase with overall buffer concentration.

butyl bonded phase, C_4 Has a C_4 functional group covalently bonded to the support material:



Butyl bonded phase

For further discussion of the R groups *see* octadecyl bonded phase.

Common applications for this bonded phase are separations of peptides and proteins because these less hydrophobic bonded phases lead to less denaturing/structural changes in the protein (in part because weaker mobile phases will cause elution). This type of support is typically wide pore (300Å).

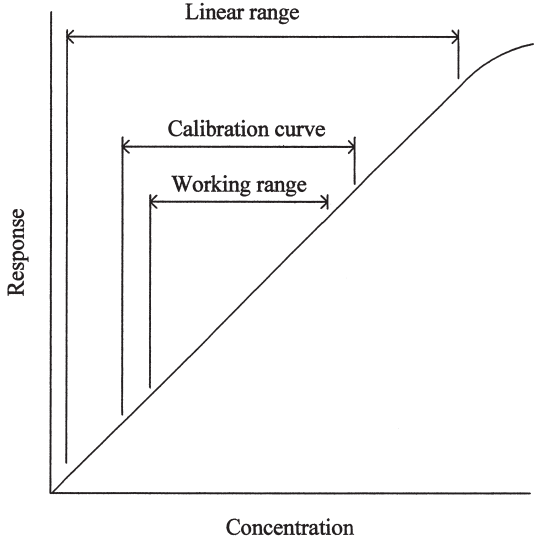
***n*-butyl chloride** Molecular weight: 92.6; boiling point: 78.4°C; refractive index (20°C): 1.4021; density (20°C): 0.89 g/mL; viscosity (20°C): 0.45 cP; UV cutoff: 220 nm; eluotropic strength (ε^o) on alumina: 0.26; polarity index (P′): 1.0; Hildebrand solubility parameter (δ): 8.4; solubility in water (20°C): 0.1%; *n*-butyl chloride solubility in water (20°C): 0.08%. Flammable. *n*-Butyl chloride has limited use in normal-phase LC and TLC separations.

$CH_3CH_2CH_2CH_2Cl$

n-Butyl Chloride

C

calibration curve The resulting plot of the detector response against the analyte concentration. Ideally, the curves are linear over the range used for quantitation, but well-defined and reproducible nonlinear curves are acceptable in cases in which establishing linearity is not possible (e.g., for very low-concentration work) *and* appropriate nonlinear curve-fitting is available.



The calibration curve is established to verify that the linearity across a critical concentration range is achieved. The calibration extremes should extend beyond the lower and upper concentration limits of the working range. Sample concentrations are targeted to fall within the working range to give the most precise and accurate results.

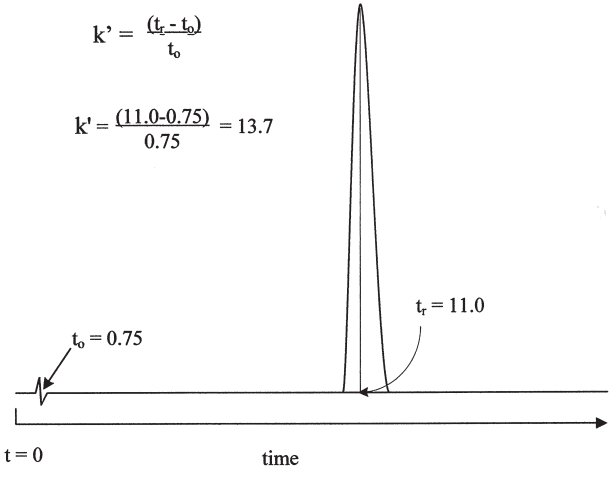
capacity The concentration of sample that is analyzed without overloading the column or the detector.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

capacity factor, k′ The capacity factor is a way of expressing the retention of a compound (as a multiple of the void volume of the system):

$$k' = (t_r - t_o)/t_o$$

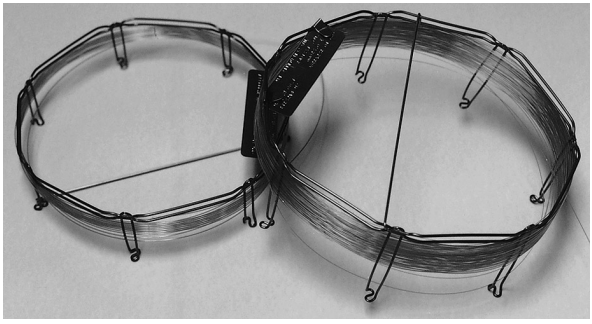
where t_r is the retention time of the retained analyte and t_o is the time required for an unretained nonexcluded peak (i.e., a void volume marker) to elute. *See* retention factor.



Calculation of the capacity factor for a peak depends on determining the void time for the system, t_o (= 0.75), and the retention time for the analyte, t_r (= 11.0) from the chromatogram. The capacity factor is one parameter used to verify peak identity.

capillary column (1) A capillary column that is used in GC is an open fused silica tube that is not filled with a packing material but may be lined with a thin film of liquid phase along the inside wall. The outside of the fused silica is commonly coated with a thin layer of polyimide for protection. The tube inner diameter ranges from 100 to 530 µm, and the liquid-phase film thickness typically ranges from 0.2 to 5 µm. Cross-linking of the liquid support leads to longer-term stability and lower bleed of the column. Column lengths span 10–100 m, with those in the 15 to 30 m range being most common. For a capillary column, the designation of "open tubular" (OT) refers to the class

of columns that have uncoated fused silica supports. When the support is coated, the class of columns is called wall-coated OT (WCOT). Porous open OT (PLOT) columns have a porous support deposited on top of the fused silica material. Finally, support-coated OT (SCOT) columns pack very small support particles in the capillary tube and then the entire packing material is coated with liquid phase. They are rarely used today. These columns are manufactured from fused silica that is clad with aluminum, polyimide, or stainless steel. (2) Various types of capillary columns are used in the field of capillary electrophoretic techniques. These are similar, if not identical, to the type used in GC. (3) Capillary columns for LC work ranges from 0.02 to 0.004 in. in inner diameter and are made from stainless steel.



Left: A 10-m GC capillary column. Right: A 60-m GC capillary column. Note the thinness of the column (lower right column shows end of column) and the cage that the columns are wound around for support. The cage is also used to keep the column from touching the walls of the oven. The metal tag affixed to the left-hand side of the 60-m column is the identity tag for the column (including phase, ID, length, serial number, etc).

capillary electrochromatography (CEC) A technique that uses a packed capillary column and electroosmotic flow to generate a separation.

capillary zone electrophoresis (CZE) A capillary electrophoresis technique that generates a separation based on a potential developed across an open tube fused-silica capillary column.

capillary gel electrophoresis (CGE) A technique that mimics classic slab gel electrophoresis but places the gel within a capillary column.

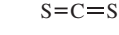
capillary flow The main transfer mechanism for the mobile phase in a thin-layer chromatography plate. This action occurs because of the free energy gain, ΔE, experienced by a solvent entering a capillary:

$$\Delta E = -2\gamma V_m / r$$

where γ is the surface tension of the mobile phase, V_m is the molar volume of the mobile phase, and r is the radius of the capillary.

capillary isoelectric focusing (CIEF) A technique in which a capillary is filled with an established pH gradient solution and the sample is moved, in the presence of solution ampholytes and an applied electric field, to the pH at which its charge is effectively neutralized.

carbon disulfide Molecular weight: 76.1; boiling point: 46.3°C; refractive index (20°C): 1.6280; density (20°C): 1.26 g/mL; viscosity (20°C): 0.37 cP; solubility in water (20°C): 0.22%; water solubility in carbon disulfide (25°C): 0.01%; polarity index (P′): 0.3. Very volatile, highly toxic. Stench. Carbon disulfide is a solvent used in GC for sample desorption from extremely nonpolar surfaces such as charcoal.



Carbon disulfide

carbon load The amount of bonded phase chemically attached to a support material, often determined through the elemental analysis of the carbon level. Carbon load is expressed as the carbon weight per support weight (w/w or w%). The larger the carbon load, the more bonded phase is on the surface. Note that the elemental determination cannot determine between bonded phase (e.g., C18) and end-capping; both are carbon-based. In general, for alkyl bonded phases, the higher the carbon load the more hydrophobic the support material.

carbon tetrachloride Molecular weight: 153.8; boiling point: 76.7°C; refractive index (20°C): 1.4601; density (20°C): 1.59 g/mL; viscosity (20°C): 0.97 cP; UV cutoff: 263 nm; eluotropic strength (ε^o): on alumina—0.18, on silica—0.11; polarity index (P′): 1.7; Hildebrand

solubility parameter (δ): 8.6; solubility in water (20°C): 0.08%; water solubility in carbon tetrachloride (20°C): 0.008%. Volatile, carbon tetrachloride is a solvent used in GC, TLC, and normal-phase HPLC.

CCl_4

Carbon tetrachloride

carrier The support to which an affinity ligand is bonded.

carrier ampholyte Used in capillary isoelectric focusing to produce a pH gradient across the column, thereby forcing the analyte to its point of isoelectric pH.

carrier electrolyte Typically a buffered solution that is used to control the pH and ionic strength of a solution. In capillary electrophoresis carrier electrolyte solution concentrations are generally between 10 and 100 mM. The upper limit results from the generation of too much conductivity, whereas the lower limit defines the injection amount of the sample.

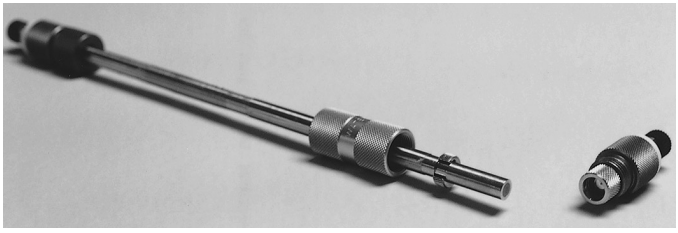
carrier gas The mobile phase used in GC work. An ideal carrier gas is inert to the analyte, column, and detector, is available in a high-purity form, and is safe and economical. The choice of carrier gas has direct implications for the overall efficiency of the GC system. Hydrogen and helium produce the highest efficiency, but for reasons of safety (hydrogen is combustible) and cost (helium is relatively expensive) nitrogen is frequently used, with concomitant loss of efficiency. For capillary columns optimal flow rates range from 10 to 40 cm/s, whereas for packed bed columns flows range from 20 to 60 mL/min.

carryover Refers to the presence of a peak from a previous sample appearing in the current chromatogram. A strong indication of carryover is that the peak reappears in subsequent injections but at lower and lower intensities (as the contamination is swept out of the system). An obvious source of carryover is the incomplete injection of the sample. Less obvious is when an elution time for a run is not long enough to elute all components in the previous sample and they appear in the subsequent injection. Finally, carryover can also be the direct result of components in the chromatographic system that are

not clean. This is particularly crucial when the injection solution changes from a weak solvent to a strong one. The strong solvent often solvates compounds deposited in the injector loop.

cartridge (1) This term has become hopelessly confused and can apply to the syringe-type barrel column (as per EPA documents) or the luer-top/luer-exit configuration. *See* solid phase extraction. (2) The housing used to contain columns with removable endfittings.

cartridge column Consists of the housing, packing, and frits. In essence, it is a column without endfittings. To use the cartridge, it is placed within a cartridge holder that fits snugly around the cartridge to prevent leakage under pressure.



Shown on the right-hand side of the column is a disassembled cartridge column endfitting. Note that the frit is internal to the column tube. This means that the frit cannot be replaced in a cartridge column as it can in a conventional endfitting column. Because there is no ferrule, another mechanism for setting the endfitting to the column is used: a C ring that fits into a grove on the column tubing. The cartridge endfitting is finger tightened (note knurled body on the two endfitting pieces.) An assembled endfitting is shown on the left-hand side of this column.

cathode In techniques where voltage is applied (i.e., electrolytic, not galvanic), a cathode is a negative electrode toward which cations migrate.

cation A molecule or atom that bears a positive charge.

cation exchange A cation-exchange material has a permanent negative charge on the surface and is used to separate cationic compounds (positively charged species). The type of negative charge (e.g., carboxylate, sulfonate) and its concentration on the surface, expressed in milliequivalents/gram, the substance to be eluted, and

the type/concentration of the cationic mobile-phase additive used to displace the substance determine the overall retention time and elution profile. Cation-exchange resins are classified as strong (e.g., sulfonate) or weak (e.g., carboxylate).

certificate of analysis (COA) A document that accompanies a material that represents the test results for a specific lot or batch of material with respect to the actual specifications developed for that material. A COA is a guarantee that the delivered material is consistent with past lots of material and will possess the same attributes/properties in the future. A COA is also sometimes referred to as a certificate of compliance.

chain length A parameter used to define the number of carbons in the bonded phase, for example, octyl (eight carbons), aminopropyl (three carbons terminating an amine group), and butyl (four carbons).

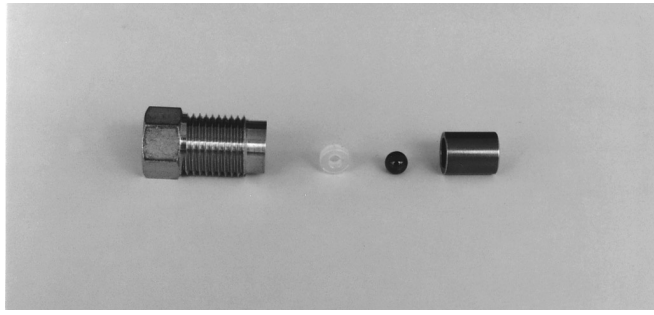
channeling Denotes the presence of an empty volume (or void) present within the body of a packed column that ultimately leads to band broadening. In extreme cases peak splitting may also occur. Channeling is often the result of poor packing, physical shock to the column, and extended use. The result of channeling is a decrease in column efficiency, especially as increased tailing.

char The solid residue left from a completed pyrolysis process.

check sample *See* control sample.

check valve A component in LC systems that "directs" solvent flow from the reservoir, through the column, to the detector by preventing backflow during pump reciprocation. Check valves are paired and typically affixed to the pump head. The inlet check valve is positioned between the solvent reservoir and the pump head. It is open and permits flow of solvent from the reservoir to the pump head when the piston reciprocates (i.e., draws out from the pump head). The inlet check valve then closes as the piston pushes into the pump head, thereby preventing solvent from flowing back into the reservoir. The outlet check valve is positioned between the pump head and the injector. It is open and permits flow of solvent from the pump head to the injector when the piston pushes into the pump. The outlet check valve then closes as the piston reciprocates, thereby preventing solvent from being pulled back from the column.

Three different manufacturers' check valves. Note that they all have a different sealing mechanism on the bottoms. Left, a hard plastic disk; center, a flexible plastic O ring; right, a metal disk. Also note that the threading is different, thereby preventing use in more than one type of pump.



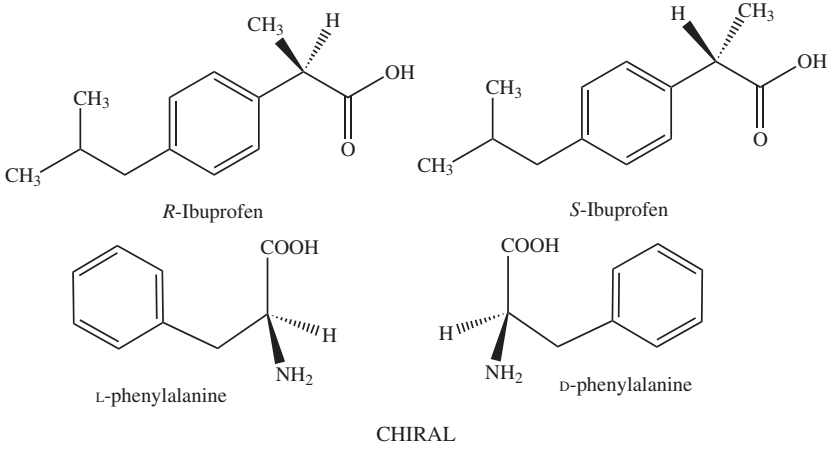
An exploded view of a check valve. From left to right: housing, upper seat, ball, retaining disk. Not seen is the second seat recessed into the disk.

chemical ionization (CI) In chemical ionization the source produces accelerated electrons that pass through the volatilized sample. A reagent gas is also bled into the source along with the sample. The reagent gas is present at a high concentration compared with the sample such that it is preferentially ionized. The resulting reagent gas ions then chemically react with the sample to produce ions that are then analyzed by their mass-to-charge ratio. A typical reagent gas is methane, which reacts with a sample molecule, S, as follows:

$$CH_4 + e^- \rightarrow CH_4^+ + S \rightarrow CH_4 + SH^+$$

chemisorption An adsorption process that results in an irreversible chemical interaction of the analyte with the sorbent surface.

chiral A chiral molecule is one that is spatially arranged such that the molecule and its mirror image are not superimposable. Chirality is often conceptualized as right- and left-handedness. It is also explicitly designated as part of some chemicals' nomenclature (e.g., D- and L-phenylalanine, R- and S-ibuprofen). Regardless of the designation, the indication that there is a difference in the molecules is readily determined by the way in which a pure enantiomer polarizes light. In this case the D indicates right-handed polarization (dextrorotatory) and L indicates left-handed polarization (levorotatory).



chiral recognition The basis for the separation of enantiomeric molecules. It describes the differential interaction of a chiral bonded phase with one of the pair of enantiomers and is the basis of the chromatographic separation. The stronger interaction leads to longer retention.

chiral stationary phase (CSP) An immobilized functional group that can generate different elution times between enantiomeric forms of a compound because of spatial orientation effects. There are five commonly recognized categories of CSPs: (1) steric brush phases (e.g., Pirkle-type columns), (2) Host-guest cavity phases (e.g., cyclodextrins and crown ethers), (3) Helical polymers (e.g.,

celluloses), (4) Chiral affinity proteins (e.g., bovine serum albumin), and (5) Ligand exchangers (e.g., amino acids fully complexed with transition metal ions such as copper).

chlorinated solvents Chlorinated solvents have found extensive use as liquid-liquid extraction solvents (e.g., carbon tetrachloride, chloroform, methylene chloride), as sample solvents in GC (methylene chloride), and as mobile-phase components in LC and TLC (chloroform, methylene chloride, butyl chloride) and SEC (chlorobenzene and 1,2-dichlorobenzene, 1,2,4-trichlorobenzene). It should be noted that many chloroalkane solvents are unstable (e.g., methylene chloride, chloroform) and decompose into free radical compounds and HCl. Both the free radical compounds and HCl can be reactive toward analytes and cause degradation of columns. To counteract this, many manufacturers add "preservatives" such as amylene and cyclohexene that are present to react with the decomposition products. Aromatic chlorinated solvents (e.g., chlorobenzene) are stable and are often used in high-temperature SEC analyses. The biggest drawback to their use is their incompatibility with UV detectors, because the cutoff wavelength is typically 290 nm or above.

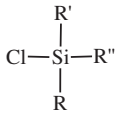
1-chlorobutane *See* *n*-butyl chloride.

chloroform Molecular weight: 119.4; boiling point: 61°C; refractive index (20°C): 1.4458; density (20°C): 1.49 g/mL; viscosity (20°C): 0.57 cP; UV cutoff: 245 nm; eluotropic strength (ε^o): on alumina—0.40, on silica—0.26; polarity index (P′): 4.1; Hildebrand solubility parameter (δ): 9.2; solubility in water (20°C): 0.8%; water solubility in chloroform (20°C): 0.056%. Very volatile. Chloroform is frequently used in liquid-liquid extractions, is routinely used as a sample solvent in GC and TLC applications, and receives limited use in HPLC and SEC applications. Because of its susceptibility for degradation, chloroform is sold with amylene and ethanol (up to 2%) added as preservative.

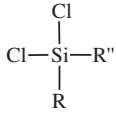
$CHCl_3$

Chloroform

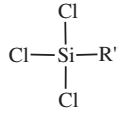
chlorosilanes A class of compounds having the general structure:



Monochlorosilane



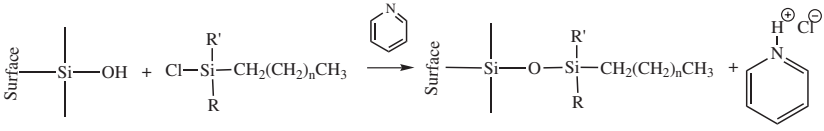
Dichlorosilane



Trichlorosilane

chlorosilane

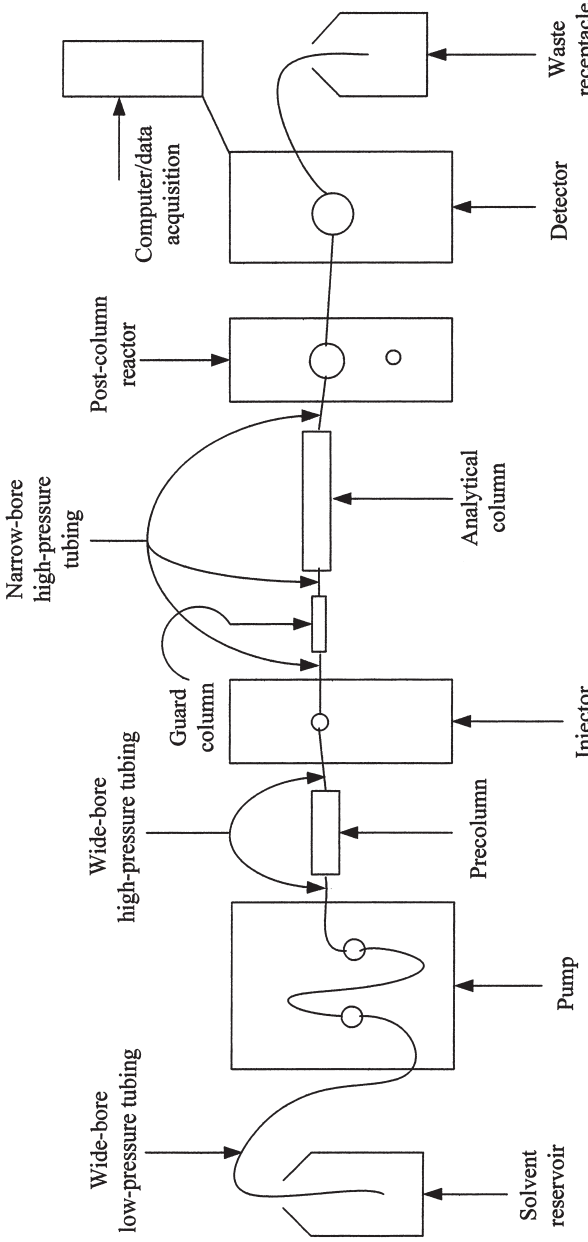
Chlorosilanes are used in the chemical modification (derivatization) of silica support materials. In these cases R and R′ are chloride and/or a short-chain alkane (e.g., methyl, isopropyl, etc) and R″ is the functional group of interest such as octadecyl, octyl, methylphenyl, etc. Monochlorosilanes are used to produce monolayer bonded phases, whereas di- and trichlorosilanes are frequently used to product polymeric layer bonded phases. The general reaction of a chlorosilane with the silica surface is (the example is for a general dimethylalkyl bonded phase).



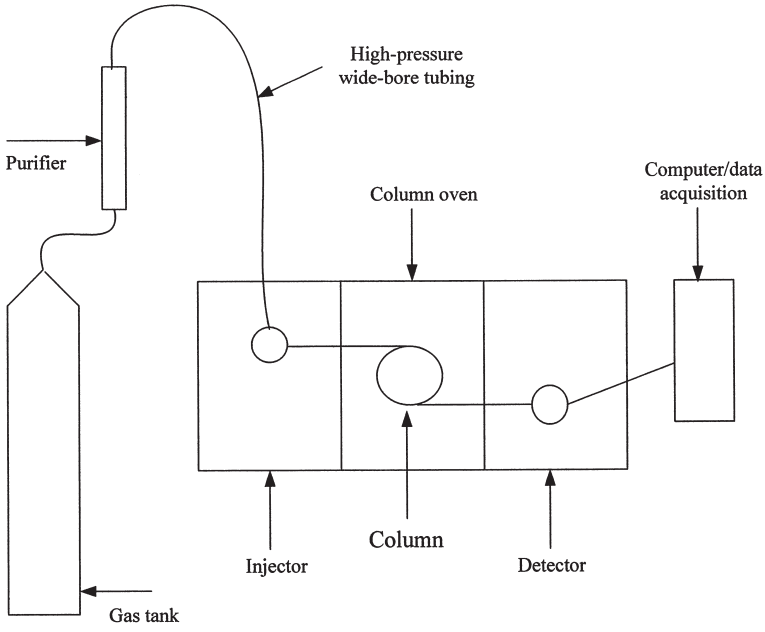
Chlorosilane reaction

chromatogram The detector response vs. time profile that is generated during a separation.

chromatograph (1) An instrument that is designed to generate a reproducible separation. A basic chromatograph is comprised of a mobile-phase control system, an injector, a column, and a detector. (2) As a verb, to conduct a process that enables separation of species by elution through a chromatographic stationary phase.



HPLC chromatograph. This schematic representation shows all required components needed for operation of the system as well as some additional nonessential ones, namely, the precolumn, the guard column, and the postcolumn reactor. These are included only to indicate the relative positional relationship to other components in the system.



GC chromatograph. This schematic representation shows all required components needed for operation of the system. Very specialized components can be added before the injector such as purge and trap or headspace analysis units. These are used to meet special sample needs.

chromatography A series of techniques that are used to separate multiple components in a samples based on relative affinities of these components between the mobile phase and the stationary phase.

chromophore A functional group that is present in a compound that absorbs radiation in the operating range of a UV-visible detector (typically 190–800 nm).

circular thin-layer chromatography *See* radial thin-layer chromatography.

coating The process by which a liquid stationary phase is adsorbed to the support material. The coating process is typically associated with packed-bed GC columns. The coating is expressed as a weight percent (compared with the weight of the support material). To create

a coated support, the appropriate liquid stationary phase is dissolved in a volatile solvent and then slurried with the support material. The solvent is then slowly evaporated from the slurry, thereby depositing the stationary phase on the solid support material.

coating efficiency The calculation for coating efficiency is used to estimate how closely the performance of a GC column approaches its theoretical limit:

$$\% \text{ coating efficiency} = (H_{\text{min}}/H_{\text{actual}}) \times 100$$

where H_{min} and H_{actual} are the height equivalent to a theoretical plate and the actual theoretical plate minimum, respectively.

coefficient of determination, r^2 The coefficient of determination is a mathematical determination of how strongly a dependent variable is correlated to the independent variable. The value r^2 is calculated from the square of the correlation coefficient, r. Perfectly correlated data have an r^2 (or r) value of 1.0 or −1.0. For many methods in which detector response and concentration are expected to have a linear correlation, minimum acceptable r^2 values are typically >0.95.

coelution A condition in which two or more peaks are not baseline resolved from one another. The resolution factor is a mathematical representation of how well peaks are resolved.

cold trapping The process of cold trapping, that is, holding the column temperature at least 100°C below the boiling point of the lowest boiling component of a sample, is used to condense and concentrate the sample into a very narrow band on the GC column. Elution is then accomplished by raising the column temperature.

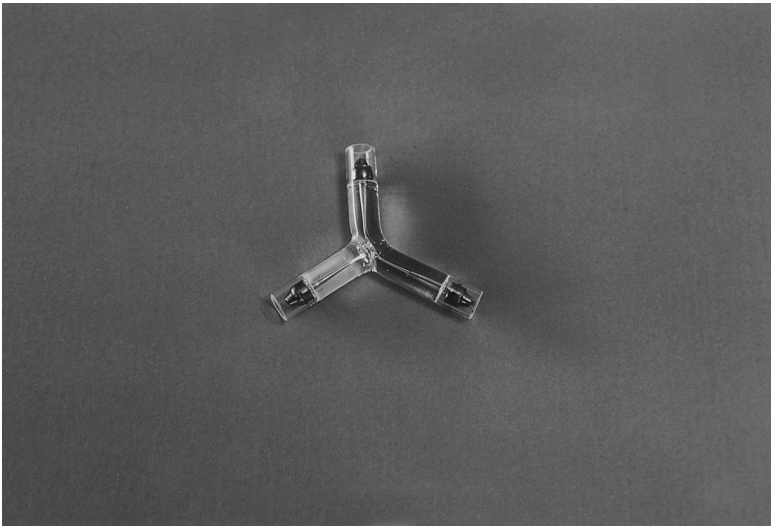
column bank A term used in size exclusion chromatographic separations when more than one column is connected in series in order to generate sufficient molecular weight discrimination.

column efficiency A measure of the overall performance of a column with respect to peak shape, retentivity, resolution, and speed. In a very general way, a column is considered more efficient if it generates more theoretical plates, a more symmetric peak and can resolve many analytes in a short period of time.

column length, *L* The distance from the column inlet to its outlet that contains stationary phase.

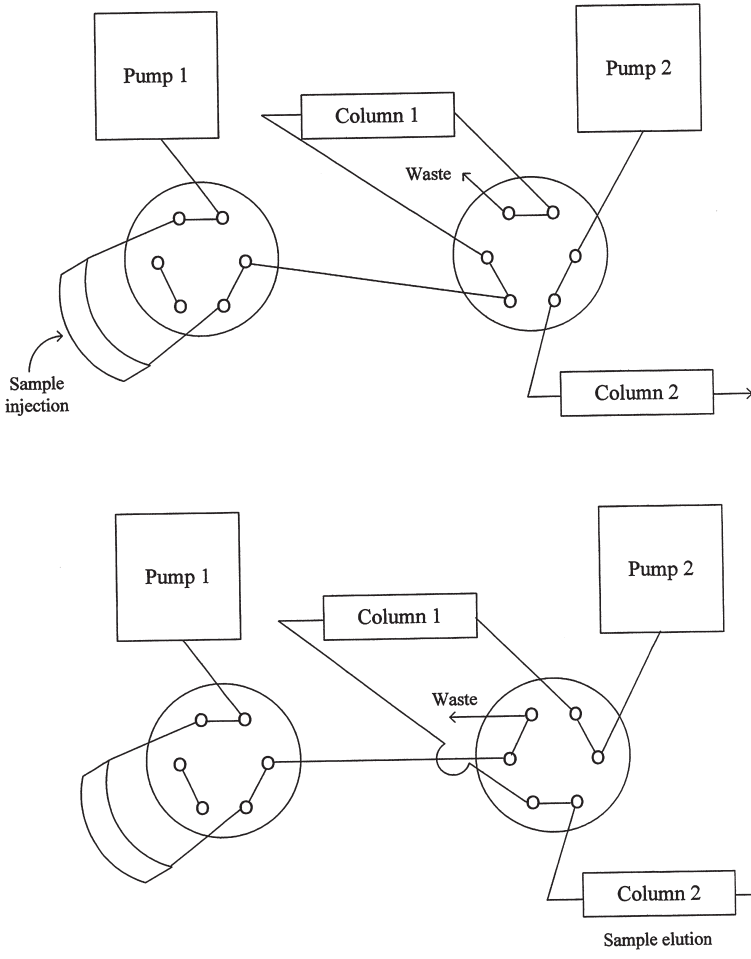
column oven In most conventional GC systems the column is housed in a sealed area, the oven, that is heated to produce the temperature gradient. Note that part of the heating and cooling process in a GC separation involves the heat transfer from the heating element in the oven to the gas around the column and ultimately to the column itself. Therefore, extremely rapid temperature increase rates are not possible (e.g., a typical temperature gradient falls into the 1–20°C/min range).

column splitter Used in gas chromatography to take the effluent from one column and split it to two detectors. This enables the analyst to achieve dual confirmation or increase the range of analytes determined.



The column splitter is used on the end of a GC column in order to direct the effluent to two different detectors. This is used for dual confirmation analyses on a single injection.

column switching Used to (1) prevent unwanted or column-damaging components in the sample from reaching the separation column or (2) generate trace enrichment of a component by concentrating the analyte band at the head of a column. The switching system consists of two or more columns and one or more valve placed in series. Flow is directed by switching the valves and redirecting the flow stream.



Column switching is an elaborate form of sample preparation in which a sample is loaded onto column 1 with a mobile phase generated by pump 1. Once the unwanted contaminants, etc., are flushed through column 1 to waste, the flow path is switched so that pump 2 now elutes sample on column 1 in the opposite direction and onto column 2, where further separation occurs. Often columns 1 and 2 and mobile phases from pumps 1 and 2 are very different in order to trap the analytes of interest on column 1 and then separate them on column 2 with a very different mobile phase.

column volume, V_c The volume contained within an unpacked column tube:

$$V_c = \pi r^2 L$$

where r is the column radius and L is the column length.

component (1) A unique and identifiable constituent in a defined phase. (2) A unique and well-defined part of an instrument.

compressibility A measure of how much a liquid or gas changes volume as a result of changing pressure. Liquids typically have a compressibility of 0.005–0.015%/bar, and adjustments for this change are not necessary. Gases, on the other hand, are strongly affected by pressure changes, and a compressibility factor is used to correct for the change.

compressibility factor, j The flow of a gas through a GC system is not constant because the gas stream volume is considerably compressed at the inlet of the column, where pressure is the greatest compared with atmospheric pressure at the exit of the column. This change is compensated for by the use of a compressibility factor:

$$j = 3\left[(p_i/p_o)^2 - 1\right] \Big/ 2\left[(p_i/p_o)^3 - 1\right]$$

where p_i is the inlet pressure and p_o is the outlet pressure. Therefore, the average flow rate, F, through a column is :

$$F = j \times F$$

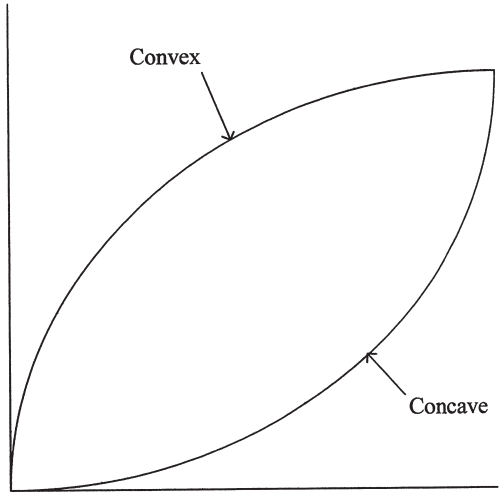
where F is the measured flow rate.

concave Describes a nonlinear gradient having a positive slope that tends toward infinity:

A concave gradient is, for the %B or strong solvent, mathematically described as:

$$\%B = (t/T)^n \times 100$$

where t is the time since the start of the concave gradient, T is the total time for the gradient, and n defines the steepness of the gradient.



concentration A unit of measure used to describe the level of analyte in a sample. Common concentration units are molarity (M): mol/L; percent (%): g/100 g sample; parts per million (ppm): μg/g sample; normality (N): equivalents/L.

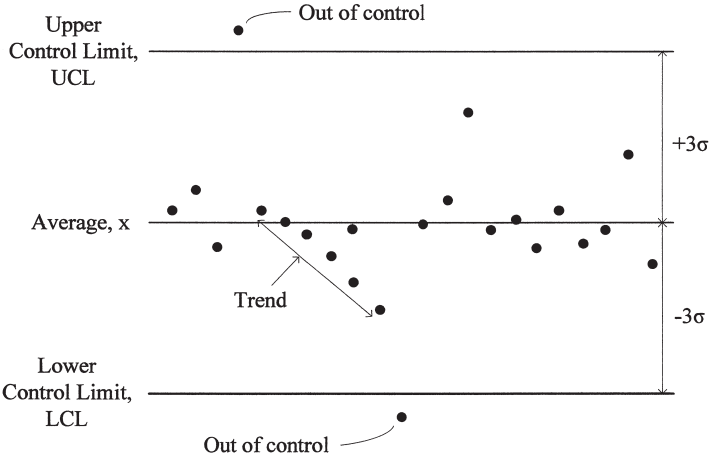
concentration detector A nondestructive detector whose signal is not affected by the rate at which the sample is passed through it. For example, a thermal conductivity detector or a UV detector will register the same response over time if the flow is stopped with the sample in the detector cell (not necessarily true for a mass detector).

conditioning The term used in GC work to describe the process that removes adsorbed species (e.g., solvents) from liquid-solid columns before their use. In HPLC conditioning refers to the process of equilibrating the column with the mobile phase (e.g., ion-pair reagents). In GC conditioning involves running the column through a series of temperature programs that run the entire temperature range that will be routinely used for analyses. In HPLC numerous column volumes of mobile phase are passed through the column to condition it. In SPE conditioning is the step that is used to wet the packing material with solvent.

conductivity detector Detector is used in LC work to monitor the mobility of ions in solution under an applied electrical field. The

sample therefore must bear a charge. No reduction or oxidation occurs. The output is in nano- or microsiemens. Most separations that utilize a conductivity detector are done by ion exchange, and so the concentration and charge of the buffer affect detector noise. Higher charge or concentration leads to higher noise. The use of a suppressor column neutralizes some of the charge and lowers system noise.

control chart Used to monitor the performance of a system versus critical parameters. It has a target value, an upper control limit (UCL), and a lower control limit (LCL). Some charts also highlight upper and lower warning limits. When a system is operating under control, the monitored parameter will fall between the UCL and LCL. A control chart enables the operator to readily assess whether there is a trend in the data, an offset, or a bias, any of which can start a corrective action before the system surpasses either limit (and is out of control).



A control chart is a visual means for identifying when a system or process is in or out of control. To establish this chart, numerous experiments need to be run in order to generate an average value and the deviation for the process. Once that is accomplished, ongoing plots of results are done and out-of-control points are readily identified. In addition, trends become readily apparent, a trend being where three or more consecutive data points change in the same direction. Trending is the result of ongoing change in the system, such as column breakdown, lamp intensity changes, etc., and indicates that preventive maintenance should be conducted before an out-of-control result is generated. For chromatographic systems control charts may be generated for resolution, efficiency, response factors, etc.

controlled pore glass A silica-based support that is used in the synthesis of DNA and as an LC support of ion-exchange, size-exclusion, and affinity separations. Controlled pore glass offers high and reproducible surface areas, high mechanical strength, and narrow and reproducible pore size distribution.

control limit An established value for a parameter used to indicate when the sample value exceeds or falls below this value. Control limits are effective when they are used to monitor critical parameters of a product over long periods of time. These limits mark the acceptable operational limits of a process or characteristics of a product and are part of a control chart. In most cases control limits are set for maximum acceptable values (upper control limit, UCL) and minimum acceptable values (lower control limit, LCL). If any parameter falls outside the control limits, the process is stopped. Some control limits for HPLC and GC could be efficiency (number of theoretical plates, LCL only), resolution (R_s value, LCL only), or peak asymmetry (tailing and fronting, UCL and LCL).

control sample A sample that has already been analyzed and is subsequently stored so that the analyte concentration does not change over time. It is run along with current samples as a way to independently verify that the entire method, from sample preparation to analysis, is working consistently over time. Also called a check sample.

convex Describes a nonlinear gradient having a positive slope that ultimately tends toward zero (*see* figure under concave). A concave gradient is, for the %B or strong solvent, mathematically described as:

$$\%\mathrm{B} = 1 - (1 - t/T)^n \times 100$$

where t is the time since the start of the concave gradient, T is the total time for the gradient, and n defines the steepness of the gradient. *See* concave.

corrected retention volume, V_R^o Term most frequently used in GC; the result of the fact that flow is not constant throughout the system (*see* compressibility factor). V_R^o is calculated as:

$$V_R^o = j \times V_R$$

where V_R is the retention volume obtained directly from the chromatogram.

correlation coefficient, *r* A mathematically determined value of how closely a dependent variable is linearly related to the independent variable. Perfectly correlated data have an r value of 1.0 (direct relationship) or −1.0 (inverse relationship). For many methods in which detector response and concentration are expected to have a linear correlation, minimum acceptable r values are typically >0.97. To make a reasonable determination of the correlation between the variables, it is typical for at least five equally spaced standard concentrations that span the anticipated linear range to be analyzed and the responses plotted against concentration.

coulometric detector Used on LC; similar to an amperometric detector except that the redox reaction is driven to 100% completion. The importance here is that under these conditions, the total number of coulombs transferred (i.e., the integrated peak area) is directly related to the moles of analyte present in the sample through Faraday's law:

$$\text{moles} = Q/nF$$

where Q is the charge transferred, n is the number of moles of electrons used, and F is Faraday's constant.

countercurrent chromatography (CCC) A technique that uses two immiscible liquid phases, one stationary and one moving through it, to separate, concentrate, or purify analytes.

counter ion (1) Used to compete with the sample ion for charged surface sites on an ion-exchange column. Retention times and peak shapes are strongly affected by the identity and concentration of the counter ion. (2) Any charged species that, when associated with its opposite charge, produces a neutral complex. For example either a sodium (Na^+) or a potassium (K^+) ion is a counter ion to the monobasic phosphate ion ($H_2PO_4^-$).

coverage The level of stationary phase that is deposited or bonded to a support material. In LC coverage is expressed as micromoles/gram or % carbon (w/w), whereas in GC it is weight percent or film thickness.

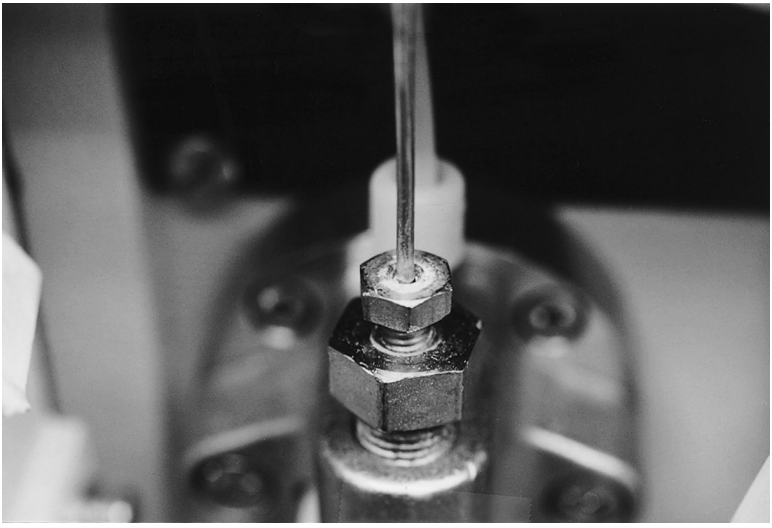
Craig countercurrent distribution The Craig countercurrent distribution apparatus was used to perform numerous distinct sequen-

tial liquid-liquid extractions. The probability that a solute will appear in the nth tube after r transfer events is:

$$P_{n,r} = r!\, p^n q^{(r-n)} \big/ (n!(r-n)!)$$

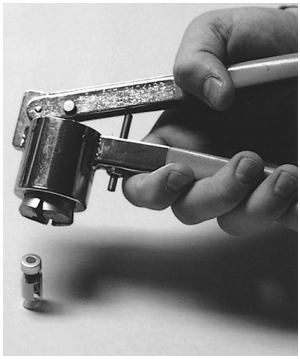
where p is the probability that the analyte will be transferred and q that it will not be transferred.

creep The by-product of liquid leak in an LC union when the mobile phase or sample contains dissolved salts. The sign of creep is a white deposit along the union.



The white salt deposit along the outlet check valve union is creep. This can indicate that salts have deposited in other internal areas of the system as well.

crimper/crimp top A crimp top is a nonthreaded cap used as a bottle closure. The associated vial has a thick lip that the crimper forms the cap around to form a seal.



The crimping tool is used to seal the cap to the vial.

critical micelle concentration (CMC) The concentration at which surfactant (surface active agents that have a hydrophilic head and hydrophobic tail) molecules change their state from predominantly independently dispersed to mostly aggregated. The aggregated surfactant molecules are called micelles. Formation of micelles in solution often produces dramatic changes in the physical and chemical properties of the solution such as surface tension and conductivity.

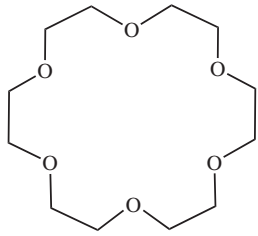
critical point The temperature and pressure at which a substance becomes a critical fluid, that is, the point where a gas can no longer be converted into its corresponding liquid occurs at the substance's critical point.

critical pressure, p_c The lowest pressure that would cause a substance to liquefy when held at its critical temperature. *See* SCF (supercritical fluid).

critical temperature, T_c The maximum temperature at which a substance in its gas form can be converted to its corresponding liquid by an increase in pressure. *See* SCF (supercritical fluid).

cross-linking When polymeric support material is prepared from monomers, the monomers are chemically bonded together through a process called cross-linking. A commonly used cross-linking reagent is divinylbenzene used in the production of polystyrene-divinylbenzene copolymers that are base resins for many ion-exchange support materials.

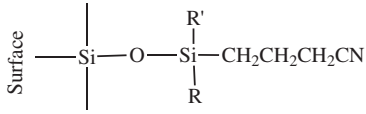
crown ethers A set of heterocyclic polyethers that form pockets of a unique diameter allowing the ether to interact preferentially with ions having a positive charge and diameter close to that of the ring. Crown ethers are grafted to support surfaces and are used as ion-exchange materials or as host-guest cavity chiral phases. These cyclic ethers typically range from 4 to 6 ether units, and the intervening carbon bridge is often ethyl or propyl. These hydrocarbon bridges can be derivatized to include phenyl or acetyl functional groups.



18-crown-6

C term The component of the van Deemter and Knox equations that describes the impact that mass transfer between and in phases has on the band broadening process.

cyanopropyl bonded phase A cyanopropyl bonded phase has this functionality chemically bonded to the support silica:



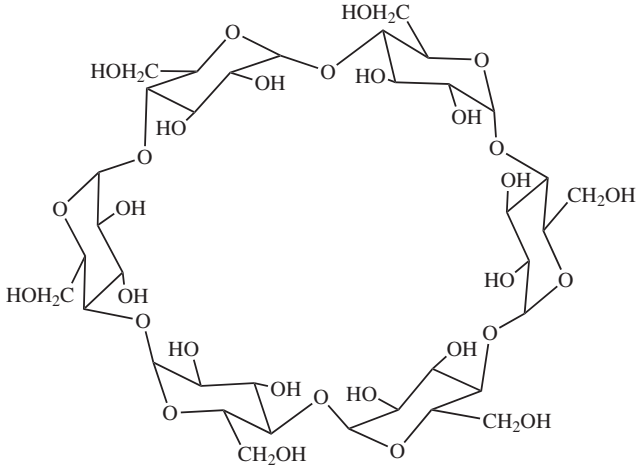
Cyanopropyl bonded phase

For further discussion of the R groups, *see* octadecyl bonded phase.

It should be noted that because of the low polarity of this phase it has been effectively used as both a normal-phase support and a reversed-phase support.

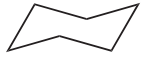
cycle time A term used to define the time interval that detectors need to acquire a full scan of data. For example, a scanning UV detector may acquire spectral data from 200 to 400 nm or a mass spectrometer may scan from mass 40 to 400.

cyclodextrin A cyclic α(1 → 4)-linked D-glucopyranose having six, seven, or eight units. The resulting forms are called α-, β-, and γ-cyclodextrin, respectively. Cyclodextrins have been effectively used as mobile-phase modifiers and as host-guest cavity chiral bonded phases.



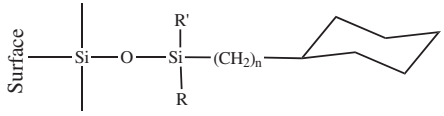
α-Cyclodextrin

cyclohexane Molecular weight: 84.2; boiling point: 81°C; refractive index (20°C): 1.4262; density (20°C): 0.78 g/mL; viscosity (15°C): 1.0 cP; UV cutoff: 200 nm; eluotropic strength (ε^{o}): on alumina—0.04, on silica—0.04; polarity index (P′): 0.0; Hildebrand solubility parameter (δ): 8.2; solubility in water (25°C): 0.006%; water solubility in cyclohexane (20°C): 0.01%. The most common use for cyclohexane is in GC and TLC, where small volumes of solvent are used. In HPLC, the cost of cyclohexane compared to hexane is significant and so cyclohexane is less frequently used. In rare instances there is an enhanced specificity/selectivity in the LC separation.



Cyclohexane

cyclohexyl bonded phase A cyclohexyl bonded phase has this functionality chemically bonded to the support silica:

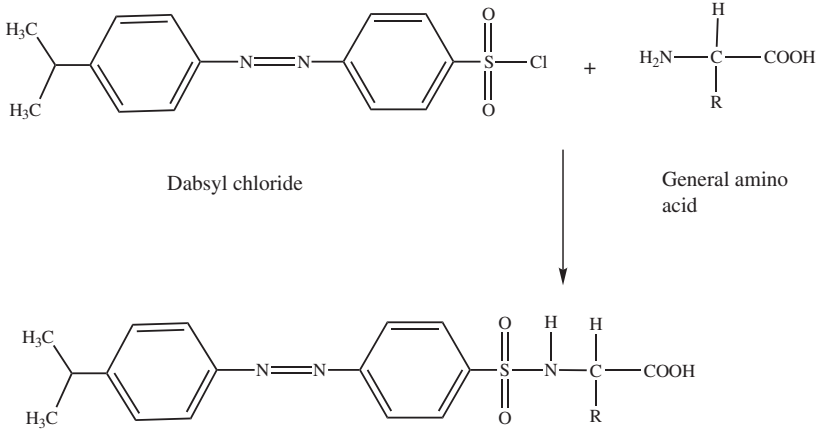


Cyclohexyl bonded phase, (n=0-3)

For further discussion of the R groups, *see* octadecyl bonded phase.

D

dabsyl chloride (DABS-Cl) [4-(dimethylamino)azobenzene-4′-sulfonyl chloride] A derivatizing reagent used for amino acids and primary alcohols to produce a product that absorbs in the UV range.



Dalton's law States that for a fixed volume the pressure generated by any mixture of gases is the sum of the pressures contributed by the individual gases alone:

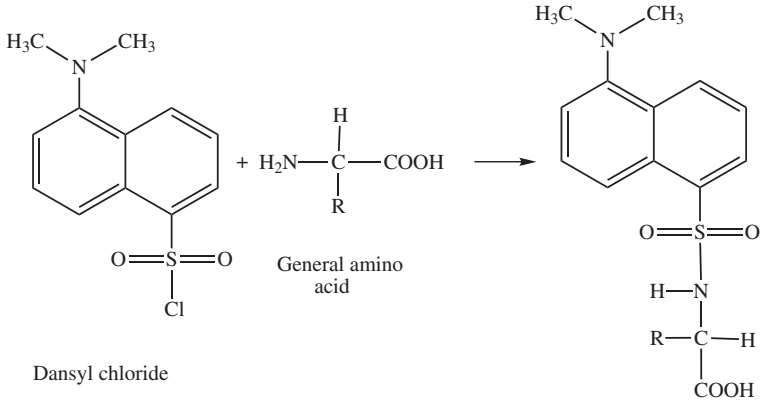
$$P_{tot} = \sum P_i$$

where P_{tot} is the total pressure and P_i is the pressure due to the ith component gas in the mixture. This can also be expressed in mole fraction, x_i, through the use of the ideal gas law ($PV = nRT$):

$$P_{tot} = (\sum x_i)RT/V$$

dansyl chloride (5-*N*,*N*-dimethylamino-1-naphthalenesulfonyl chloride) A derivatizing reagent used for primary amines to produce a product with UV absorbance or fluorescence. For fluorescence, $\lambda_{ex} \approx 350\,nm$ and $\lambda_{em} \approx 455\,nm$.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2



Dansyl chloride

Darcy's law Describes the relationship between mobile phase velocity, v, and the pressure gradient in a packed column, dP/dx:

$$v = (B_o dP)/(\varepsilon \eta dx)$$

where B_o is the specific permeability coefficient for the packed column, ε is the interparticle porosity, and η is the mobile-phase viscosity.

deactivation (1) The process of removing unwanted strong interaction sites in RPLC. (2) The process of removing active sites in adsorption chromatography (underivatized silica) through the adsorption of water. In bonded phase work, the presence of underivatized inhomogeneous silanol groups is minimized through treatment of the base silica with acid to extract out metals. In this case it has positive effects on the chromatography, especially with respect to the peak shape of strong hydrogen-bonding molecules (e.g., primary amines). In adsorption chromatography, the acid/base and hydrogen bonding interactions provided by the silanols are key to many separations, and so eliminating this interaction is not desired. However, because of the inhomogeneity of the silanols it is often advantageous to deactivate the most active sites through the addition of a low and constant level of water in the mobile phase. This produces efficient and reproducible chromatographic elution profiles. Note that too much water decreases retention times and resolution.

dead volume, V_M The volume in the system that is in the mobile-phase flow path and is not swept by the passage of the mobile phase through the system. *Also see* hold-up volume, V_M.

degassing Before use in HPLC, solvents undergo degassing to remove dissolved gases. Dissolved gases can lead to the following problems: Bubbles get trapped throughout the HPLC system, most notably (1) in the check valves (lead to inconsistent flow) and (2) in the detector, causing erratic baseline pulsing or unwanted signals (e.g., O_2 absorbs at $\lambda < 210$ nm and reacts in an electrochemical detector). Degassing can be accomplished through one of many techniques: sparging (with He or Ar), sonication, refluxing and applying a vacuum. Whereas refluxing is the most effective technique, it is also incompatible with mixed solvents and is impractical (from a time and safety point of view). Sonication with vacuum seems to be the most effective, coupled with a helium blanket blown across the top of the reservoir.

denaturation The loss of the three-dimensional structure of a protein. This process is often irreversible. In chromatographic separations, denaturation can be caused by such things as high or low pH, ionic strength, organic solvent level, and the type of stationary phase. This is a difficult problem because many organic solvents cause denaturation when present at the 15–25% v/v level and much higher organic levels are needed to cause elution. The use of a butyl bonded phase seems to be most compatible with preservation of the protein structure.

densitometer Frequently used for quantitative work in thin-layer chromatography. The source light (often UV) is transmitted through the back of the plate and up through the adsorbed packing. The plate is scanned by moving it past the source from the point of sample application to the solvent front. The change in absorbance is read as a function of the position on the plate.

density, *d* The density is the weight per unit volume for a given substance or sample. Typical units are grams/milliliter.

derivatization The process of chemically modifying either (1) a packing material surface to change its polarity or (2) an analyte through the addition of a specific functional group and increase detection specificity or sensitivity.

derivatizing reagent Used to react with a compound or material in order to change the chemical and/or physical properties of that compound or material. Examples include the derivatization of silica to create bonded phase supports or of compounds to incorporate chromophores or fluorophores or render them volatile.

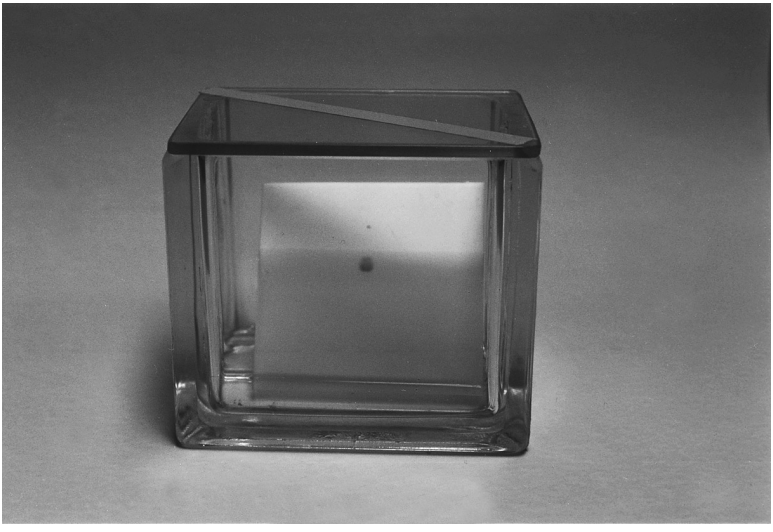
desalting The process by which simple salts are removed from high-molecular-weight compounds.

detection The process by which an analyte is monitored as it passes through the detector. Detection can be obtained by choosing chemical and physical properties such as absorptivity, refraction, and molecular weight.

detector The detector is the component of an instrument that generates the signal from the passage of the analyte through it.

determinate errors Errors in a measurement that are not due to random error and hence cannot be identified through statistical analysis. Bias and offset errors are examples of determinate error.

developing chamber An airtight container that is used to hold the developing solvent and plate in thin-layer chromatography. Note that consistent separation requires that the chamber be saturated with the developing solvent and that the solvent level stay beneath the point of application (i.e., the sample spot) of the sample on the TLC plate.

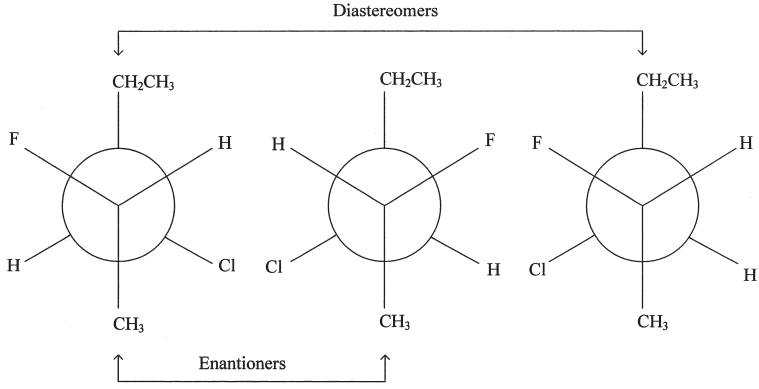


The picture shows a thin-layer plate partially developed. The solvent level is the dark line running near the bottom of the plate. The solvent from is the gray line about 75% of the way up the plate. The sample spot has moved well up the plate and has resolved into two spots, a smaller leading and a larger trailing.

deviation Also called the residual; the measure of how much an individual result differs from the mean, x (or expected result). For the following set of results, 6.1, 6.4, 6.2, 6.2, 6.1, the mean (or average) is 6.2. Therefore, the deviation for each of the individual results is –0.1, + 0.2, 0.0, 0.0, –0.1, respectively.

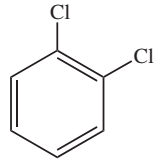
dextrans Anhydroglucose polymers developed for use as packing materials for low-pressure LC separations.

diastereomers Configurational isomers that are nonsuperimposable on one another and are not mirror images of one another.



diatomaceous earth Also known as kieselguhr; a silaceous material formed from the debris of diatoms. It is used as a packing material for packed-bed GC columns, cleanup columns, and TLC plates. The composition of diatomaceous earth varies with its source but commonly consists of ~90% SiO_2 and ~10% of other oxides such as Fe_2O_3, Na_2O, Al_2O_3, and CaO.

1,2-dichlorobenzene Molecular weight: 147.0; boiling point: 180°C; refractive index (20°C): 1.5514; density (20°C): 1.306 g/mL; viscosity (25°C): 1.32 cP; UV cutoff: 295 nm; Polarity index (P′): 3.1; Hildebrand solubility parameter (δ): 10.0; solubility in water (20°C): 0.013%; water solubility in 1,2-dichloromethane (25°C): 0.31%. 1,2-Dichlorobenzene has its major use as a solvent in high-temperature size-exclusion chromatography.



1,2-Dichlorobenzene

dichloromethane (methylene chloride) Molecular weight: 84.9; boiling point: 39.8°C; refractive index (20°C): 1.4241; density (20°C): 1.3391 g/mL; viscosity (25°C): 0.44 cP; UV cutoff: 233 nm; eluotropic strength (ε^o): on alumina—0.42, on silica—0.30; polarity index (P′): 3.1; Hildebrand solubility parameter (δ): 9.7; solubility in water (20°C): 1.6%; water solubility in dichloromethane (20°C): 0.24%. Dichloromethane is extensively used as a sample solvent in GC work. For environmental samples this is typically due to the fact the dichloromethane is used to extract organics from water in a liquid-liquid extraction step. Dichloromethane is also used at low levels in normalphase LC and TLC work. In all cases it is preserved with an alkene such as amylene or cyclohexene because it is prone to free radical degradation.

CH_2Cl_2

Dichloromethane

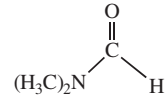
diethylaminoethyl (DEAE) A common substituent used as a weak anion-exchange functional group.

diethyl ether *See* ethyl ether.

diffusion The process by which molecules are driven away from areas of high concentration to lower concentration (via chemical potential differences). *See* Fick's first law of diffusion; Fick's second law of diffusion.

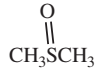
diffusion coefficient, D A quantitative measure of how rapidly a molecule moves through a defined matrix. The value for D is dependent on analyte, temperature, pressure, matrix, etc. The units for D are square centimeters/second. Common gas in a gas diffusion coefficients range from 0.01 to 3 cm^2/s, whereas solute in a liquid diffusion coefficients range from 10^{-7} to 10^{-5} cm^2/s.

dimethyl formamide (DMF) Molecular weight: 73.1; boiling point: 153°C; refractive index (20°C): 1.4305; density (20°C): 0.95 g/mL; viscosity (20°C): 0.92 cP; UV cutoff: 268 nm; polarity index (P′): 6.4; Hildebrand solubility parameter (δ): 11.5. Miscible with water. Combustible. Dimethyl formamide is a rarely used solvent in HPLC and GPC.



Dimethyl formamide

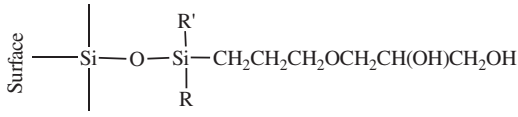
dimethyl sulfoxide (DMSO) Molecular weight: 78.1; boiling point: 189°C; refractive index (20°C): 1.4783; density (25°C): 1.096 g/mL; viscosity (20°C): 2.24 cP; UV cutoff: 268 nm; eluotropic strength (ε^{o}): on alumina—0.62, on silica—0.41; polarity index (P′): 7.2; Hildebrand solubility parameter (δ): 12.8. Miscible with water. DMSO is readily adsorbed through the skin! DMSO is used sparingly as a mobile-phase component in HPLC work because of its high UV cutoff and viscosity but is used more often in sample preparation. This is especially true for peptide and protein work because DMSO is not as strong a denaturing agent as other more commonly used HPLC solvents.



Dimethyl sulfoxide

diode-array detector (DAD) *See* PDA (photodiode-array detector).

diol bonded phase The common structure for a diol-bonded phase is two hydroxyl groups on the last two carbons of a hexylether functional group. Diol phases are rarely used in HPLC and find infrequent use in SPE for sample cleanup.



Diol bonded phase

For further discussions of the R and R′ groups, *see* octadecyl bonded phase.

disk A solid-phase extraction format in which the packing material (typically 3–7 μm in diameter) is intercalated into a PTFE mesh. Sample is drawn through the disk with an apparatus similar to a solvent filtering unit.

displacement The process that best describes the retention mechanism for liquid-solid (adsorption) chromatography.

distillation A method used to separate components of a liquid through differences in their boiling points.

distribution The representation of the frequency with which individual measurements yield different results is the distribution of the data.

distribution coefficient, D (1) The chromatographic distribution coefficient (also called the partition coefficient), K_D, is the ratio of the equilibrium concentration of a species associated with the stationary phase (C_s) to that in the mobile phase (C_m):

$$K_D = C_s/C_m$$

(2) A more general solution distribution coefficient is used to describe the stoichiometric ratios of a compound that partitions between two definable and distinct phases. In liquid-liquid extractions the value of D for a given species X partitioned between phases A and B is written as:

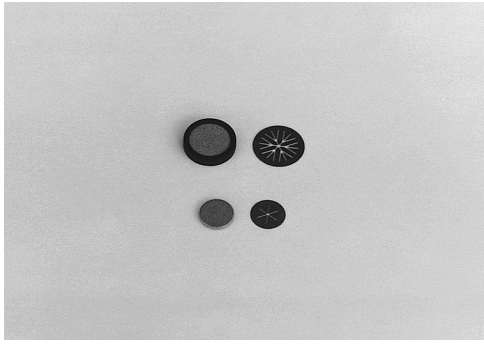
$$D = [X]_B/[X]_A$$

where B is typically the organic phase and A is the aqueous phase. Also note that the distribution coefficient also takes into account species that dissociate or associate. For example, a diprotic acid can

have three forms, H_2A, HA^-, and A^{2-}, when in solution. As a consequence, a value for D can be developed as:

$$D = [H_2A]_B / ([H_2A]_A + [HA^-]_A + [A^{2-}]_A)$$

distributor, distributor plate A thin stainless steel disk that is placed between the inlet column endfitting and the inlet frit. The star pattern in the distributor is to facilitate the spreading of the sample over a wider area of the inlet frit and hence the column.



Two sets of frits-distributors are shown with the distributor on the right. Note the "star-like" pattern that runs from the center to the edge of the distributor. This provides an channel of little of no resistance to flow and allows the sample to spread across the top of the column packing.

double peak Systems that are grossly contaminated or have severely compromised packing beds can generate a split or double peak.

drift The average slope of the detector baseline over the course of an analysis; can be caused by incomplete column equilibration, changes in temperature of mobile-phase composition, system leaks, and contamination of the sample or mobile phase.

drift tube A component of an evaporative light scattering detector that evaporates the mobile-phase from the analyte before it enters the light-scattering cell. The drift tube operating temperature must be optimized for both the mobile-phase composition and the flow rate in order for the detector to generate reproducible results.

dry packing The dry packing technique is used to pack non-wetted packing material into columns (compare with slurry packing). Dry packing is commonly used for packed-bed GC columns, precolumns in HPLC, and medium- and low-pressure LC columns. To effectively pack these columns, the column is tamped or vibrated in order to completely settle the material.

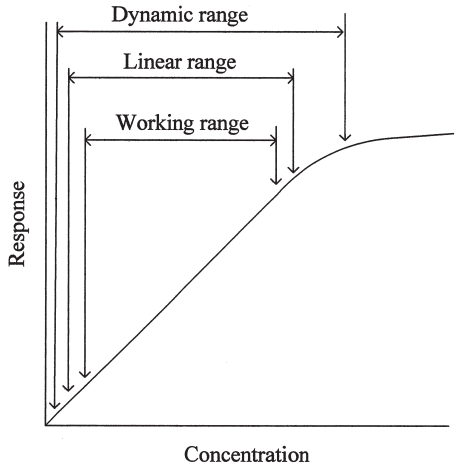
dual-beam detector Dual-beam detectors can be dual beam in space or time. Regardless of how the beam is split, the background signal is compensated for and the analyte signal is continuously corrected for the background noise. This results in lower overall signal noise.

dual-channel detection Used when a detector cannot provide positive compound identification/confirmation by itself. For example, a flame ionization detector produces a retention time for identification but in a complex mixture many compounds could coelute. To create positive identity/confirmation, the effluent from the column can either be split to two detectors or go through a series of nondestructive detectors. The second detector should respond to a different characteristic of the analyte. Some detectors provide strong dual confirmation. For example, GC/MS provides both retention time and mass information simultaneously.

dwell time The time required for a mixed solvent to leave the gradient mixing chamber and arrive at the head of the column.

dwell volume The volume in an HPLC system between the gradient mixing chamber and the head of the column. If the dwell volume varies considerably between systems, then the analyte elution time will be affected. This is because the actual gradient profile (at the same flow rate) reaches the head of the column later in a system with a larger dwell volume. In essence, this has effectively increased the initial "hold" time for the gradient and all elution time will be longer under these conditions.

dynamic range The range of analyte concentration that a detector gives a changing output. This does not mean that the response must be linear. As shown in the diagram, the linear and working ranges of the detector for the analyte are subsets of the dynamic range.



The dynamic range for a detector includes non-linear portions of the response versus concentration plot. The limit to the dynamic range is that there must be a quantitative difference in the response of two different concentrations. On the low-concentration part of the range the system noise is the limiting factor. On the high-concentration part of the range the saturation of the detector, solubility of the analyte, or chromatographic overloading of the system may be the limiting factors. In fluorescence work self-absorption is also a potential factor.

E

Eddy diffusion Also called axial diffusion, a band-broadening effect caused by the flow stream splitting around particles in the packed bed of a column. It is a component of the A term in the van Deemter and Knox equations.

effective plates, N_{eff} The calculation of the number of effective plates generated by an analyte is determined by correcting for the non-retained volume, t_o:

$$N_{eff} = 5.54[(t_r - t_o)/w_{1/2}]^2$$

where $w_{1/2}$ is the peak width at half-height and t_r is the retention time of the analyte. Essentially, N_{eff} is N corrected for the system void volume.

effective plate height, H_{eff} H corrected for the void volume (as are effective plates, N_{eff}) and calculated as:

$$H_{eff} = L/N_{eff}$$

where L is the column length.

efficiency Column (or more precisely system) efficiency is inversely proportional to the dispersion (broadening) of a peak that elutes through it. The mathematical determination of efficiency is accomplished through calculation of the number of theoretical plates (N) or height equivalent to a theoretical plate (HETP).

effluent The entire mobile-phase stream that exits the column.

Ehrlich's reagent [*p*-(dimethylamino)benzaldehyde, DMBA], Ehrlich's reagent is a derivatizing reagent used for the postcolumn derivatization of primary amines and numerous sulfa drugs that contain a primary amine (e.g., sulfadimidine, sulfanilamide, sulfadiazine). Detection is at 450 nm.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

electric field strength, E In capillary electrophoresis techniques a potential is applied across the length of the column. The resulting field strength, E, is described as:

$$E = (E_i - E_d)/L$$

where E_i is the potential at the injector end of the column, E_d is the potential at the detector end of the column, and L is the column length.

electrochemical detector (ECD) These detectors are used in LC and are of three basic types, all based on different aspects of electrochemically generated "current": (1) amperometric, which measures current from the oxidation or reduction of the analyte, (2) conductometric, which measures the change in solution conductivity as the sample passes through a fixed electric field, and (3) potentiometric, which measures the potential developed at an electrode as the sample passes through the detector.

electrokinetic potential *See* zeta potential, ζ.

electron-capture detector (ECD) The electron-capture detector is used in GC. It utilizes a β-radiation source (most commonly ^{63}Ni) to ionize the carrier gas and thereby produce a constant stream of electrons that generate a signal current. This current is measured and is attenuated as analyte molecules pass through, causing a decrease of current reaching the detector. This attenuation is recorded as the output signal. The extent of the decrease in current is dependent on the electron-capture cross section of the analyte and its concentration. The linear range of an ECD is ~10^5 with a minimum sensitivity of around 10^{-9} g. Very effective electron-capture functional groups include halogen atoms (e.g., chloride, bromide), sulfur, phosphorus, and nitro groups.

electron-impact ionization (EI) An EI source causes molecular ionization through the production of electrons that are accelerated across the flow path of volatilized sample. The analyte is charged and accelerated into a mass spectrometer detector:

$$A + e^- \rightarrow A^+ + \text{fragments}$$

An EI source is not particularly effective at producing molecular ions (i.e., the molecular weight of the parent compound or its protonated form). Chemical ionization sources are used for molecular ion formation.

electroosmotic flow (EOF) v_{eo} The flow of mobile phase through a capillary that is the result of an applied potential at the capillary wall (i.e., a zeta potential). EOF is mathematically defined as:

$$v_{eo} = \varepsilon\zeta E/\eta$$

where ε is the dielectric constant, ζ is the zeta potential, E is the applied voltage, and η is the viscosity.

electroosmotic mobility, μ_{eo} The flow velocity for the analyte generated at a set field strength, E:

$$\mu_{eo} = v_{eo}/E$$

electrophoresis A technique that separates molecules based on differing charge densities and molecular size/shape through the application of an applied electric field (potential).

electrophoretic mobility, μ The mobility of an ion, that is, the velocity at which an ion travels across a support material, is determined by its charge/volume ratio, three-dimensional structure, degree of solvation, and viscosity of the carrier solution. Mathematically this is represented as:

$$\mu = v/E = q/6\pi\eta r$$

where v is the ionic velocity, E is the applied potential, q is the net charge of the ion, η is the solution viscosity, and r is the radius of the solvated ion.

electrophoretic velocity, v_{ep} The rate at which an ion migrates through the support system at a fixed potential:

$$v_{ep} = L/t$$

where L is the length of the support (cm) and t is the migration time (s).

electrospray ionization (ES) An electrospray ionization source generates a fine aerosol that is charged by poising a potential across the exit needle. No thermal input, other than that generated by the applied potential, is used to produce ionization. The produced ions are then accelerated into the mass analyzer for charge/mass separation. This technique is very gentle and in general produces parent molecular weight data. Molecular ions often bear multiple charges, and so the mass-to-charge ratio is affected accordingly.

eluate The entire effluent that flows from a column.

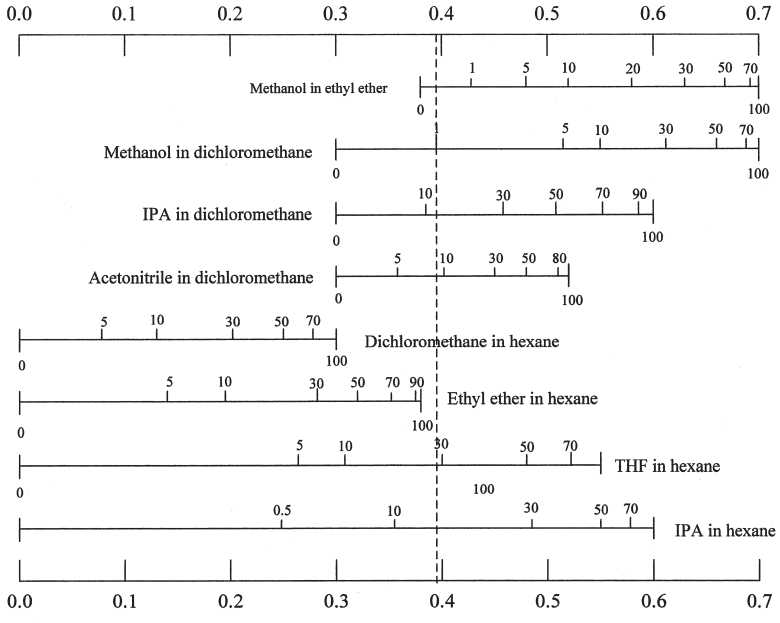
eluent The component in the separation system that moves the sample along the column.

eluite The portion of the sample that is analyzed, that is, the analyte.

elutriation A technique used to fractionate particles based on their density/shape and resulting movement in a flowing stream (liquid or gas).

eluotropic series Lists solvents according to their elution strength (i.e., the shorter an analyte retention time, the higher the eluotropic strength). This scale was developed for liquid-solid supports such as silica and alumina, with pentane the weakest and water the strongest commonly used solvents.

eluotropic strength, ε^o An experimentally derived number that represents the relative elution strength of a solvent on a defined sorbent material. This scale was originally developed for alumina and silica, with the weakest solvent, pentane, having an $\varepsilon^o \equiv 0$. See individual solvent listings for values. Note that nomograms have been developed in order for analysts to readily find solvent mixtures with equivalent, or isoeluotropic, strengths. It must be realized, however, that this equivalency only predicts relative elution strength and cannot be used to predict selectivity effects.



ε^o on silica

A nomogram such as this is used in the rapid determination of mobile-phase compositions that are of approximate eluotropic strength. To use, connect the same value on top and bottom scale (here ε^o ~0.39). Where the line intersects solvent compositions of the same ε^o is where similar mobile-phase strength mixtures exist. In this example only dichloromethane-hexane and ethyl ether-hexane mixtures are inappropriate. Note that eluotropic strength does not indicate anything about selectivity differences.

elute To move solutes through a support material.

elution The process involved in moving an analyte band through or along a support material.

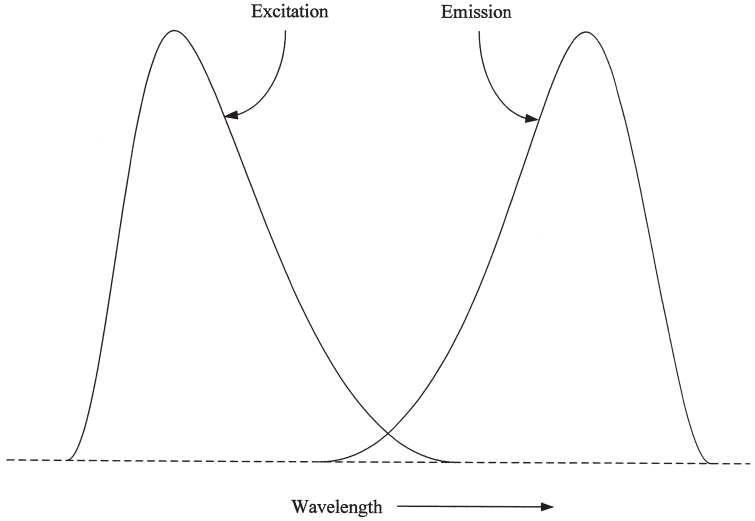
elution profile The resulting detector response vs. time output. The elution profile, when reproducible, is used for peak identification, system performance evaluation, and quantitation. These profiles are typically generated from primary standard compounds, and samples are compared against them.

elution volume, V_R The volume of the mobile phase required to elute an analyte from the column, calculated as:

$$V_R = F \times t_r$$

where F is the flow rate and t_r is the retention time at the peak maximum.

emission wavelength, λ_{em} The wavelength that is designated for monitoring the radiation emitted from a fluorescent molecule. Note that the λ_{em} is always at a longer wavelength than the excitation wavelength, λ_{ex}.

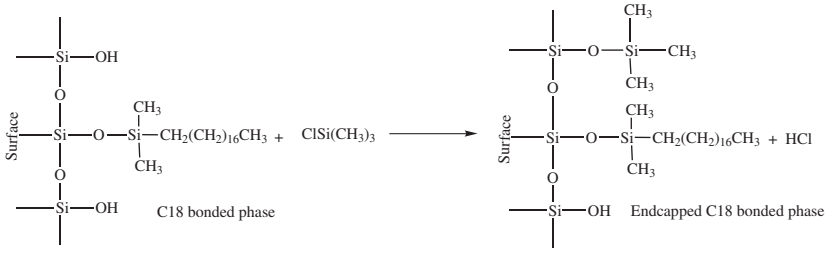


In a very simplistic sense a fluorescent molecule has an excitation manifold that is of higher energy than the emission profile. This is because energy is lost through various mechanisms before the molecule reaches the appropriate excited state from which to fluoresce (*see* fluorescence). Many excitation-emission profiles are mirror images with overlap. As a consequence, a wavelength far enough away from this overlap should be chosen for quantitative work.

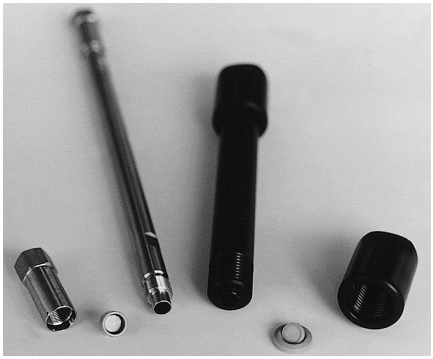
enantiomers Two compounds that differ in "handedness". *See* chiral.

endcapping The process used to reduce the number of residual silanol groups on the surface of a support material. Special reagents

such as chlorotrimethylsilane [$ClSi(CH_3)_3$] or hexamethyldisilazane [$HN(Si(CH_3)_3)_2$] are used. The small size of these reagents as compared with an octyl- or octadecyl-bonded phase allows them to reach and react with residual silanols interspersed between the larger bonded moieties.

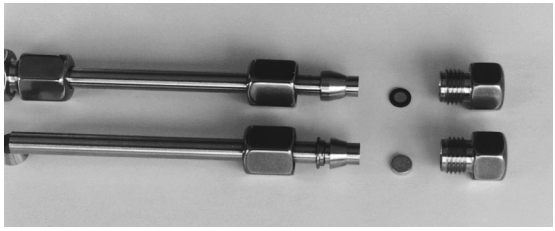


endfitting A part of a column that (1) allows for column connection to the injector and detector and (2) holds frits that prevent packing material from leaving the column when mobile phase flows through the column.



Both columns have endfittings that attach directly to the column tube, i.e., the tube itself is threaded. The column on the left is manufactured from stainless steel and the column on the right from PEEK. Note that the frit for the stainless steel column is actually press-fit into a cap that fits on top of the column, whereas the frit for the PEEK column fits inside a deformable O ring that seals when the column endfitting is tightened to the column tube.

Both columns are manufactured from stainless steel, but the endfitting on the left terminates as a "male" union and that on the right as a "female" union.



Two "female" column endfittings are shown in an exploded view. The top column uses a single-piece ferrule and a plastic-encased frit. The bottom column uses a two-piece ferrule and a plain frit.

error An estimate of the range around a result within which the true value lies. In essence,

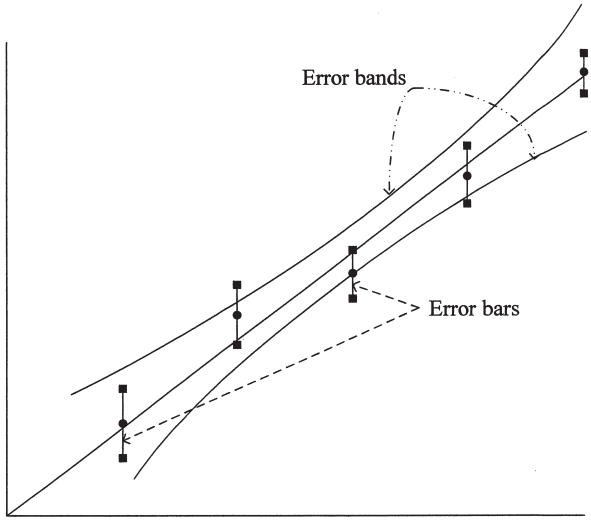
$$x_{\text{true}} = x_{\text{eperimental}} \pm \text{error}$$

error, absolute The difference between the experimentally obtained result, x, and the true result, X:

$$\text{absolute error} = X - x$$

error bar A line through each plotted result that estimates the range within the expected value lies. An error bar should be generated for every parameter that has an error associated with it. However, it is assumed that the independent variable has negligible

error associated with its accepted value. In a very simplistic fashion, the error in the obtained response of the unknown (dependent variable, y) is transferred to the standard curve and the resulting range of x is determined.



There are two representations of estimated error: Error bars provide an estimate of the associated error with an individual datum. The error band represents an estimate of the error associated with data along a best-fit curve.

error propagation When more than one measurement occurs during the course of an analysis, each measurement step will have an error propagated through to the answer. For example, an assay that includes weight determination, dilution, injection volume, peak integration, and concentration determination from a calibration curve will have at least five independent sources of error that will be reflected in the overall uncertainty of the final reported value.

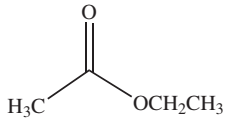
error, relative The absolute error divided by the true value, X:

$$\text{relative error} = (\text{absolute error})/X$$

ethers Ethers have the general functional group R—O—R′ and can be symmetric (e.g., $CH_3CH_2OCH_2CH_3$, diethyl ether or ethyl ether), asymmetric [e.g., CH_3—O—$C(CH_3)_3$, methyl *tert*-butylether], or cyclic

(e.g., THF). Many ethers are unstable and readily form peroxides that, if they become concentrated enough, can pose an explosion hazard. As a consequence, many ethers have preservatives added to them that prevent the buildup of peroxides in the solvent. Nevertheless, the laboratory stock of ethers should be routinely used or replaced as per the documented shelf life instructions of the manufacturer.

ethyl acetate Molecular weight: 88.1; boiling point: 77°C; refractive index (20°C): 1.3724; density (20°C): 0.90 g/mL; viscosity (20°C): 0.45 cP; UV cutoff: 260 nm; eluotropic strength (ε^o): on alumina—0.58, on silica—0.48; polarity index (P′): 4.3; Hildebrand solubility parameter (δ): 9.1; solubility in water (20°C): 8.7%; water solubility in ethyl acetate (20°C): 3.3%. Ethyl acetate is most commonly used in TLC, liquid-liquid extractions, and sample preparation. Because of its moderately high UV cutoff, its use in HPLC is as a low-percentage mobile-phase component.



Ethyl acetate

ethyl ether, diethyl ether Molecular weight: 74.1; boiling point: 35°C; refractive index (20°C): 1.3524; density (20°C): 0.71 g/mL; viscosity (20°C): 0.24 cP; UV cutoff: 215 nm; eluotropic strength (ε^o): on alumina—0.38, on silica—0.43; polarity index (P′): 2.8; solubility in water (20°C): 6.9%; water solubility in ethyl acetate (20°C): 1.3%. Ethyl ether is a very common solvent used in liquid-liquid extraction because of the ease with which it can be evaporated. It is also used to a lesser degree in TLC and HPLC. Ethyl ether is prone to forming peroxides and is often preserved with ethanol. High volatility and flammability make special handling precautions necessary.

$H_3CH_2COCH_2CH_3$

Ethyl ether

evaporative light-scattering detector (ELSD) Useful for analytes that are nonvolatile and have no chromophores or fluorophores. In an ELSD the column effluent is nebulized, vaporized to produce

desolvated particles (or partially solvated droplets), and analyzed through the resultant 90° scattering of a laser source.

exchange capacity A term used for ion exchange supports that is defined as the number of equivalents of ion that the support can adsorb per gram of support. Exchange capacity can be expressed on a dry weight or wet (volume) basis. The latter is done because many ion exchange supports are organic resins that swell dramatically when they are solvated (some by as much as 50%). Typical exchange capacities range from 50 μeq/g to 5 meq/g.

excitation wavelength, λ_{ex} The wavelength that is designated for incident radiation on a fluorescent molecule such that emission will occur. (*See* fluorescence.) The relationship between the excitation and emission wavelengths is shown in the figure under emission wavelength. Note that there is overlap between the two manifolds and that the excitation wavelength is always shorter than the emission wavelength.

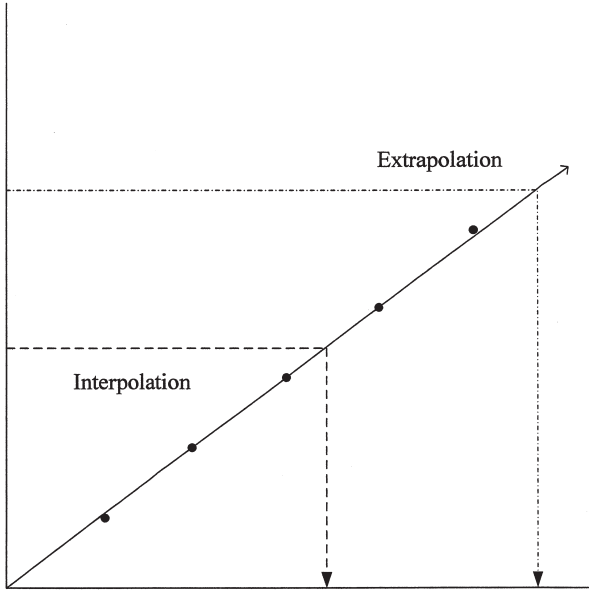
exclusion limit A parameter used in size-exclusion chromatography that represents the lowest molecular weight that is excluded from all pores in the packing material. This means that a column rated as a 10^4 molecular weight limit will elute all molecular weights at or above 10^4 at the same volume, that is, the void volume of the system. *See* molecular weight calibration curve.

exclusion volume, V_e Defined by the volume in a size-exclusion column exclusive of the pore volume, that is, the interstitial column volume only. Molecules that are too large to penetrate any pores in the packing material still have access to the volume external of the pores, the interstitial volume. In the exclusion volume *all* excluded molecules elute at the same time.

external standard Method used when as instrument operation is stable and reproducible over time. To work successfully, the external standard material itself must be a high-purity form of the analyte of interest. This material is used to generate a working curve from which the analyte concentration is determined. For the external standard method, standards are run before and after the sample series and for longer runs between samples. This allows the analyst to assess system performance continuously.

extracolumn volume Any volume in the system from the injector to the detector (inclusive) is extracolumn volume. Extracolumn volume decreases the overall efficiency of a separation because each source adds to the total variance (i.e., width) of a peak. These volumes should be kept to a minimum by using the narrowest appropriate inner diameter connecting tubing, shortest lengths of connecting tubing, smallest detector cell volume, zero-dead volume unions, etc.

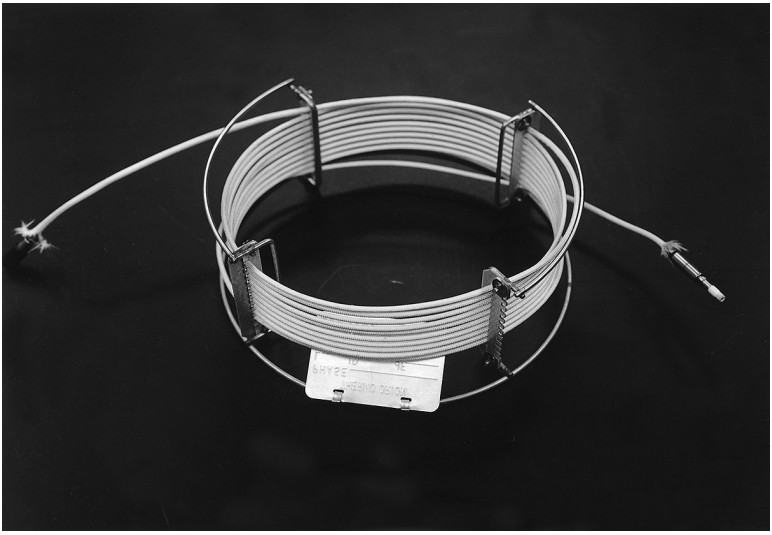
extrapolation The process of extending a curve beyond an established set of data points. For extrapolated results to be of analytical value a number of assumptions are made: (1) The mathematical representation of the data does not change past the data set used to generate it; (2) the chemical nature of the analyte does not change; (3) the detector responds in the same fashion; (4) the sources of error are equal to or less than that found within the bounds of the data set.



Working outside the defined working curve as defined by known standards (extrapolation) is dangerous because there is no guarantee of linearity (or conformance to any other best-fit parameter). Interpolation always gives the most reliable result.

F

fast gas chromatography (FGC) Uses very short capillary columns (≤10 m) that are encased in a stainless steel mesh housing that fits snuggly around the column. The housing is ballistically heated (up to 20°C/s), thereby cutting run times by up to 80%. Temperature re-equilibration is also decreased (there is not a large oven volume from which to dissipate heat).



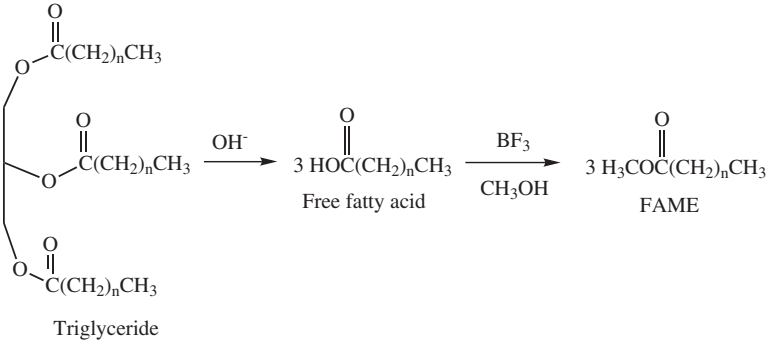
A capillary GC column is housed within the braided metal sheath. Because heat is transferred extremely quickly to the column in this configuration very steep temperature gradients are attainable.

fast liquid chromatography (FLC) Uses short (≤5 cm) and narrow (≤3 mm) columns containing 3 to 5-μm particles to produce separations that range from seconds to a few minutes.

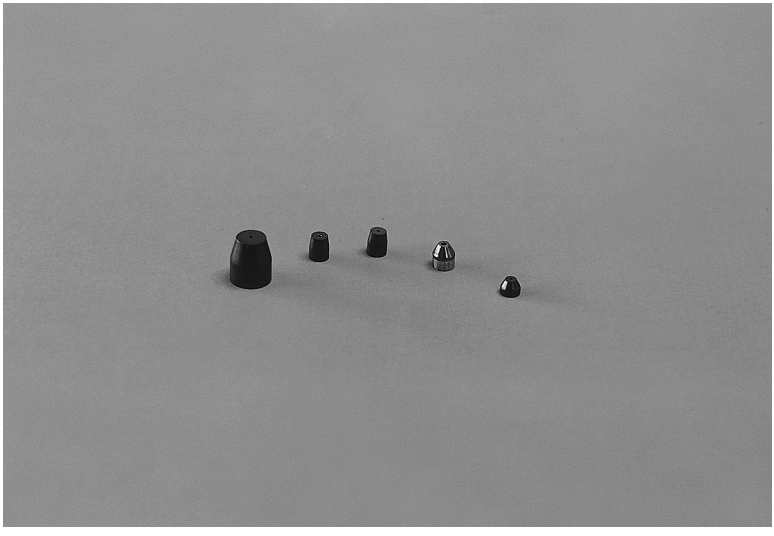
fast protein liquid chromatography (FPLC) A low-pressure technique used to separate proteins.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

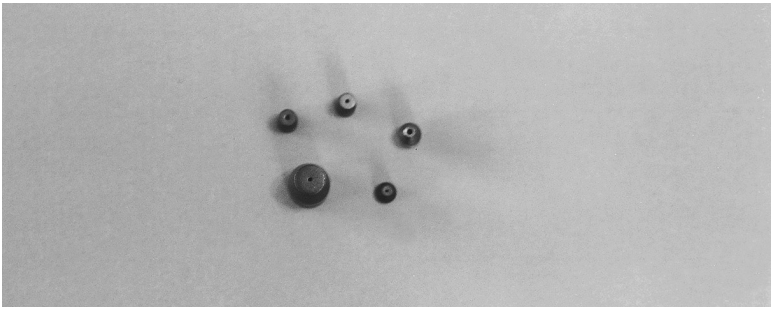
fatty acid methyl ester analysis (FAME) The analysis of carboxylic acids by gas chromatography, especially fatty acids, is an important technique. The analysis requires that the sample, often present as a triglyceride, is hydrolyzed and the resulting free fatty acid esterified to produce a volatile product.



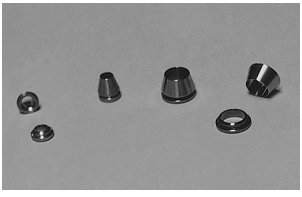
ferrule A component of a separation system that fits over tubing and is deformed inside a compression fitting (typically by tightening a nut into the union) such that the union forms a leak-free flow path.



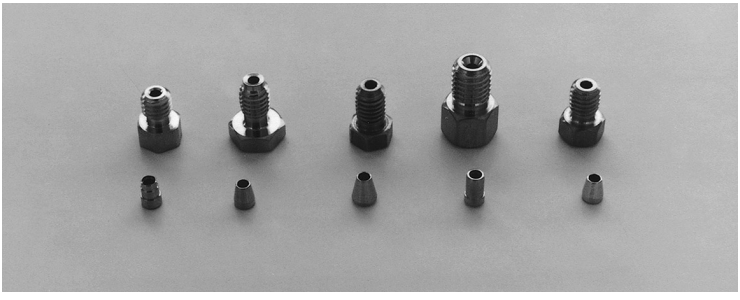
Left to right: three vespel ferrules for different size connectors, one graphite ferrule, and a blended vespel ferrule. Graphite is extremely soft and deforms well to create a leakproof union. It typically cannot be resealed. Vespel is a hard material that often can be resealed.



Same as previous figure but note that the holes are different sizes in order to accommodate different column outer diameters.



A series of two-piece ferrules used for 1/8″ or 1/4″ copper and stainless steel tubing connections in GC.



A series of HPLC ferrules and associated nuts used for 1/16″ tubing connections. Each ferrule-nut combination is for a specific supplier. Left to right: Rheodyne, Waters, Valco, SSI, Parker.

Ferrules are typically one piece in HPLC; unions are made from stainless steel, PEEK, and other solvent-resistant polymers that can tolerate high pressure. Some high-pressure ferrules are two piece. In some cases, the nut and ferrule are made together to form a component that can be tightened by hand. Common materials of construction for GC ferrules are graphite, vespel, and vespel-graphite blended material. For gas unions, copper tubing with brass ferrules and fittings are used.

Fick's first law of diffusion This law defines the variation of mass flow through a surface as a function of the concentration gradient:

$$\partial N/\partial t = -\mathrm{D}(\partial n/\partial l)$$

where N is the number of molecules, t is the time, D is the diffusion coefficient, n is the concentration of molecules, l is the diffusion distance, and $\partial n/\partial l$ is the concentration gradient. Note that $\partial N/\partial t$ is also called the flux or mass flow per unit time. The negative sign indicates that the flux is in the direction of lower concentration.

Fick's second law of diffusion This law describes the longitudinal diffusion that occurs in a chromatographic column:

$$\partial N/\partial t = \mathrm{D}(\partial^2 n/\partial l^2)$$

Rearranged and solved for mass flux, n becomes:

$$n = \left[N/2(\pi \mathrm{D}t)^{1/2}\right]e^{-(l^2/4\mathrm{D}t)}$$

which describes a Gaussian-like curve where N is the number of molecules, t is the time, D is the diffusion coefficient, n is the concentration of molecules, and l is the diffusion distance. This law describes the time rate of change of concentration with respect to the spatial rate of change along the concentration gradient.

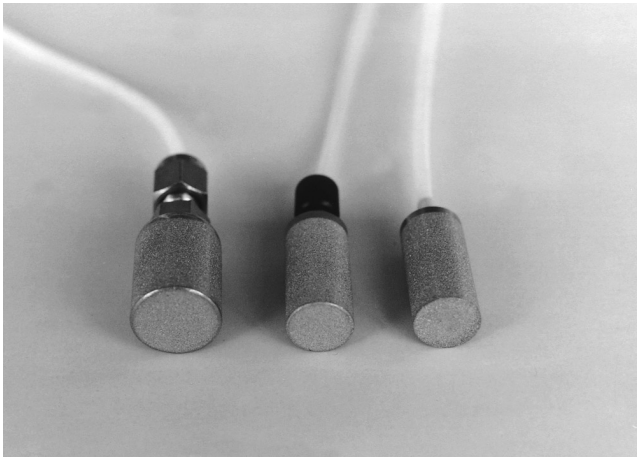
field-flow fractionation (FFF) A technique used for the separation and characterization of high-molecular-weight and particulate species. The separation is generated in an open flow channel across which an applied field is applied. These external fields include gravitational force fields, etc.

film thickness, d_f A measure of how much liquid stationary phase is deposited on the support surface. Film thicknesses typically range from 0.25 to 5 µm. Thin films offer rapid phase transfer, minimize peak broadening, and maximize resolution and peak capacity. Thick films generate less resolution but offer much higher sample loading capacity.

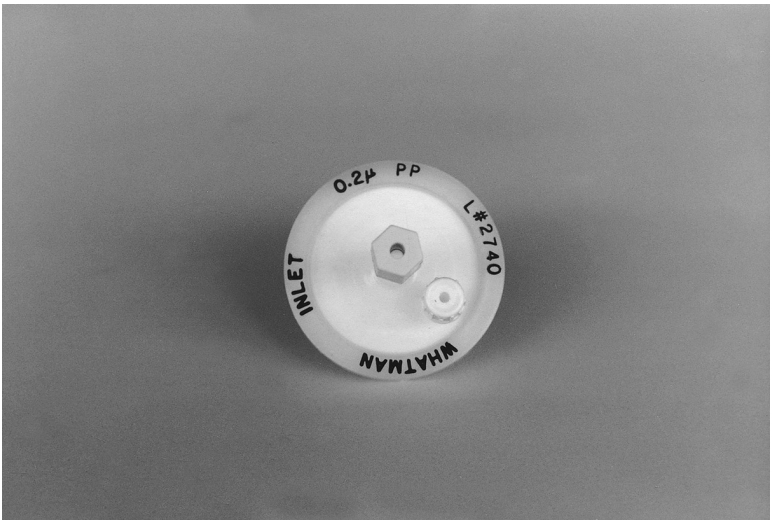
filters (1) Porous materials that are used to remove particulates from solvents, samples, and mobile phases. See individual filter type for further discussion. (2) Used in spectrophotometers to restrict the range of wavelengths transmitted to the sample.

It is important that one realizes that the material of construction for the filter must be compatible with the solvent used. Also, the average pore size in the filter, the porosity (how many pore/unit surface area), and the total available surface area are important filter characteristics. There are two basic types of spectral filters: band pass and cutoff. A band-pass filter transmits a defined upper and lower value of wavelengths, whereas a cut-off filter transmits wavelengths either above or below a set wavelength.

filter, inlet Removes particulate from the mobile phase before it enters the instrument. In HPLC the inlet filter is typically a sintered glass or porous stainless steel component that is attached to the end to the inlet tube. This assembly then rests in the reservoir. Filters can also be placed in a special housing above the reservoir. In this setup the filter is often connected to a vacuum so that the solvent is both filtered and degassed before it reaches the pump. In GC work the filter is typically in line after the gas tank and removes particulates and any aspirated oils, etc., before they reach the GC unit.

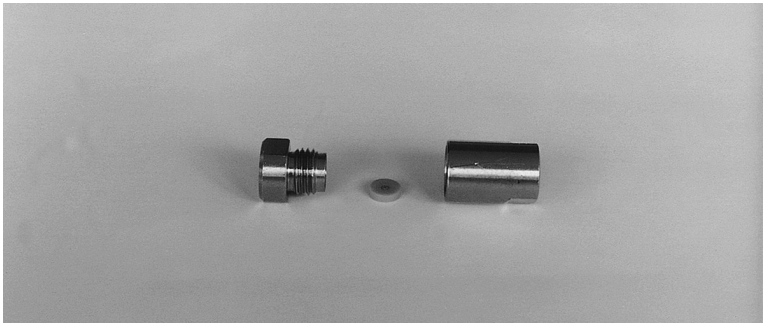


Stainless steel inlet filters. Left to right: female union, male union, nozzle-ended. All tubing is wide-bore PTFE to facilitate flow to the pump head.



This is a combination inlet filter (0.2-μm PTFE) and degassing filter. An aspiration tube is connected through the off-center union, which pulls a vacuum through the filter and degasses the mobile phase as it is pulled into the pump head.

filter, in-line Used in HPLC; placed between the outlet filter and the injector. These filters should also be placed after the pressure transducer so that if and when they clog, the increased pressure can



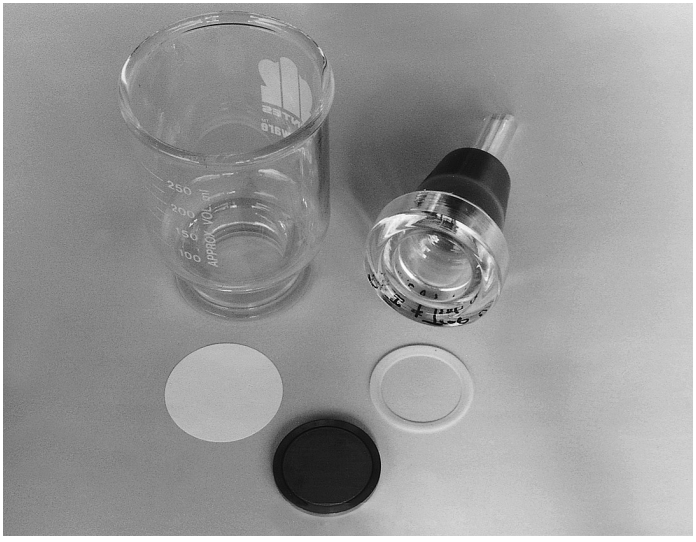
This in-line filter consists of an embedded stainless steel frit held in a low-volume housing. In-line filters are positioned between the pressure transducer and the injector and are there to remove particulates generated by the piston seals and other components in the pump.

be used to pinpoint the problem component. In-line filters are specifically designed to remove particulates generated by the piston seal (as it ages), etc. In-line filters are constructed from stainless steel or titanium and placed in a special high-pressure holder.

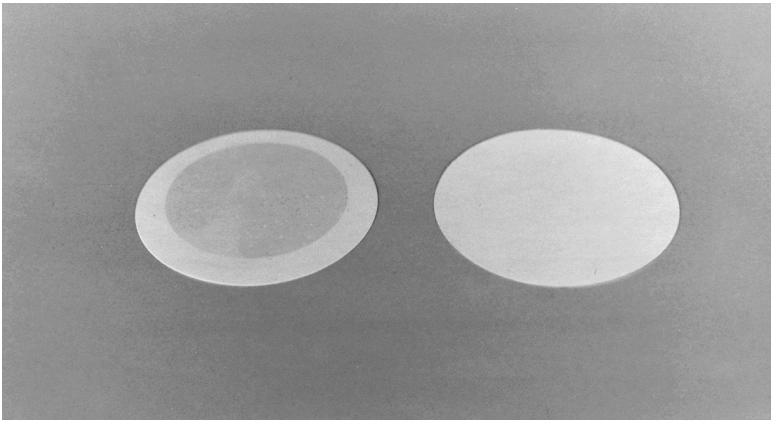
filter photometers Unlike a fixed wavelength detector, a filter photometer uses a deuterium lamp and controls the operating wavelength through the use of band-pass filters.

filter, solvent Used to remove particulates from the solvent before its being placed in a solvent reservoir. Although serving a function similar to that of the inlet filter, the solvent filter removes a large fraction of particulates so that maintenance of the inlet filters is greatly reduced. Solvent filters should be used is two circumstances: (1) salts or materials that contain particulate matter are added to the solvent, or (2) the solvent purity (with respect to particulates) is in question. With many high-purity solvents today, filtering the sample in the laboratory often adds more particulates than it removes. If there is any question, then contact the manufacturer.

A fully assembled solvent filter unit. The solvent is placed in the top reservoir and pulled, by vacuum, through a filter. The filtered solvent is collected in the side-armed flask.

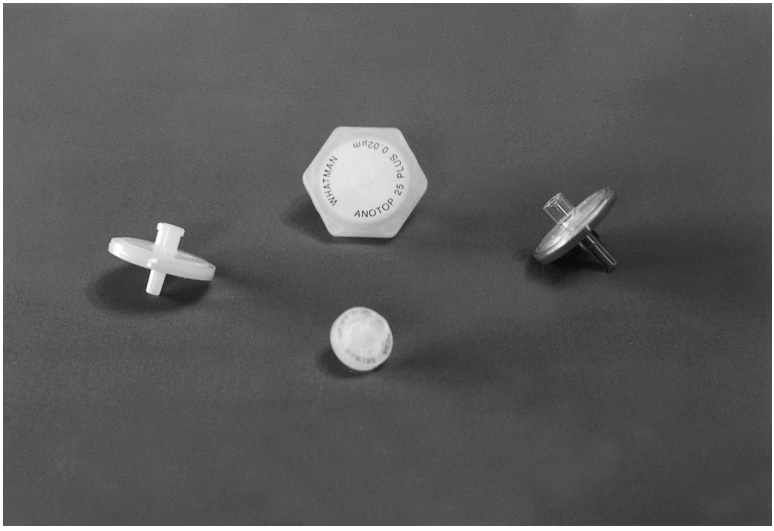


The solvent reservoir and filter assembly are comprised of the (from 10 o'clock clockwise) reservoir, the holder base, a sealing O ring, a mesh filter support, and the filter. Not pictured is the clamp that holds the assembly together.



A used filter (left) and an unused filter (right). Note the significant level of residue collected on the used filter.

filter, syringe Attaches to the end of a syringe and is used to remove particulates from samples. Filter syringes are one-time use filters. They come in a wide range of materials (e.g., cellulose, glass, nitrocellulose, PTFE) to make them compatible with a wide range of solvents and solutions. They come in a wide range of diameters and pore sizes. Correct selection of composition, diameter, and pore size is important for overall performance and reproducibility.



Syringe filters of different diameters and pore sizes. The most common filter pore diameters are 0.45 and 0.2 µm. Note that the top filter is 0.02 µm. Construction of the housing is typically polypropylene or polyethylene, and all these are connected to a syringe via a Luer port.

fixed-wavelength detector Utilizes a lamp that emits a fixed wavelength lamp such as cadmium (λ = 229 nm), zinc (λ = 214 nm), mercury (λ = 313, 340, 365, 405, 426, 546 nm), and deuterium (λ = 214, 229, 254, 280, 313, 340, 365 nm). Filters are used to prevent unwanted wavelengths through the system.

flame-ionization detector (FID) A destructive mass flow detector used in GC analyses. The sample is burned in the detector, and the current generated by the sample combustion generates the signal. For hydrocarbon compounds, the FID signal is approximately proportional to the number of carbons in the sample. The linear range

is 10^6, and the detection limits are in the parts per billion range. Because of the very low detection limits of this detector the input gases to the detector must be hydrocarbon free to keep the background noise at an acceptable level. The FID has a linear range of approximately 10^7.

flashback In GC systems using a vaporization inlet, flashback is the condition in which the sleeve volume is overloaded because of the large volume of expansion that occurs as a liquid sample is converted to a gas. This expansion causes the vapors to move back to the top of the inlet and recondense, leading to low and irreproducible results.

Florisil A patented magnesium silicate (Mg_2SiO_3) used predominantly in sample cleanup.

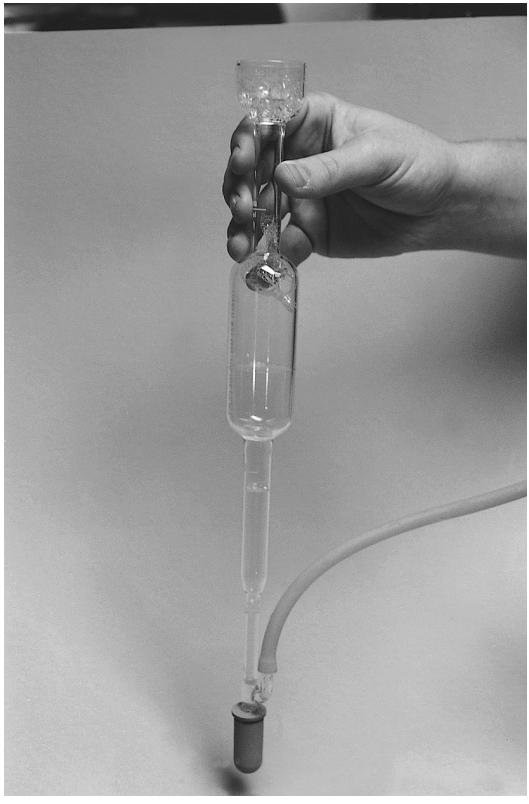
flow restrictor Most commonly used in SCF work and placed after a column to maintain constant system pressure during a separation.

flow controller Used in GC systems to produce a constant gas flow in the system even though the resistance to flow may vary with time (e.g., as temperature changes during a gradient).

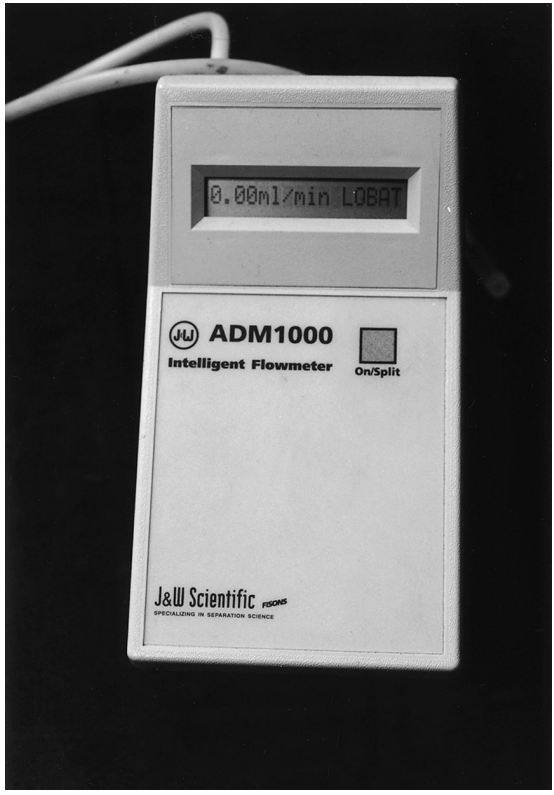
flow injection analysis (FIA) The basic FIA technique is based on the injection of a sample into a nonsegmented continuous carrier stream that brings the analyte to the detector. Selectivity is either based on the purity of the sample (no other component in the system that the detector responds to) or a selective reaction (e.g., enzymatic or derivatization) of the analyte of interest. In more complex FIA systems there can be segmentation between samples. This is often accomplished through the injection of an air bubble between sample injections. Carry over and sample adsorption to the tubing can cause irreproducibility. Quantitation can be done either by peak height or peak area. Standards must be run along with samples.

flow meter Used to determine the volume (mL/min) flow rate of gases through the GC system. Two common flow meters are (1) soap bubble and (2) electrical sensor. The soap bubble flow meter is a manual operation in which a soap bubble moves up a calibrated volume tube and the time to move a given volume is recorded and from that the rate is calculated. The electrical sensor provides an automatic digital readout of the flow rate. Note that the flow rate is

determined at the detector exit (detector off) and at the split exit port (if split injections are made). Accurate measurement and reproduction of the flow rate generate reproducible chromatographic elution profiles.



A bubble meter is a visual means for determining flow rate in GC. The bottom tube is connected to a by-pass port on the GC to adjust the gas flow/composition correctly. Note the bubble approximately halfway up the tube. Determining the time it takes for the bubble to move between volume markings on the tube allows for the calculation of flow rates.



This is an electrical flow meter (with low batteries!). The tube is connected to a by-pass port on the GC, and the flow rate is read directly from the face.

flow path Describes the exact route a molecule follows as it elutes through a column. The existence of multiple flow paths is a contributing factor to peak broadening (eddy diffusion).

flow rate, F A measure of the speed at which the mobile phase is traveling through the system. In HPLC work flow rates typically range from 0.5 to 2 mL/min, in SEC from 0.5 to 5 mL/min, in capillary GC from 0.1 to 1 mL/min, and for packed-bed from GC 75 to 90 mL/min.

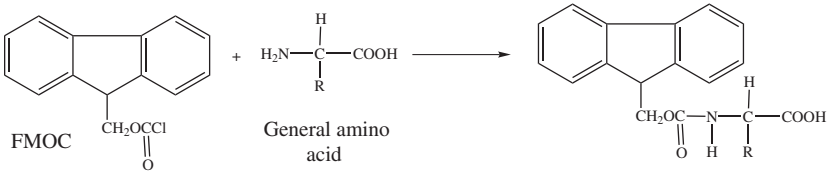
flow resistance parameter, Φ The flow resistance parameter is dimensionless and is calculated from the specific permeability, B_o, and the particle diameter, d_p:

$$\Phi = d_p^{\,2}/B_o$$

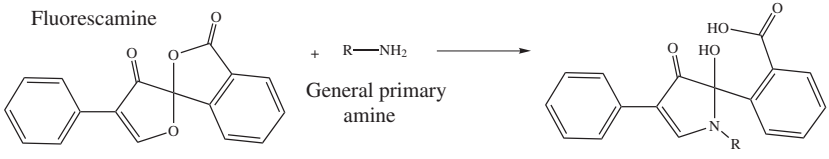
The parameter provides a means to compare column performance in terms of overall resistance to mobile-phase flow.

flow sensitivity Detectors that have baseline responses to mobile-phase flow rate are termed flow sensitive. This is true for mass detectors such as refractive index.

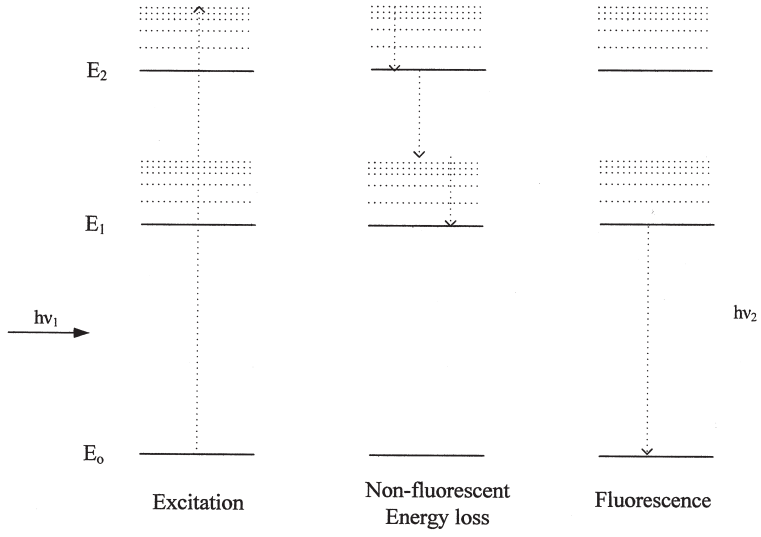
9-fluorenylmethyl chloroformate (FMOC) A derivatization reagent used to produce a fluorescent product from its reaction with primary amines; commonly used in amino acid analyses. $\lambda_{ex} \sim 260$ nm, $\lambda_{em} \sim 305$ nm.



fluorescamine A derivatization reagent used to produce a fluorescent product from its reaction with primary amines. $\lambda_{ex} \sim 390$ nm, $\lambda_{em} \sim 475$ nm.



fluorescence The process by which energy that is absorbed by atoms/molecules is converted into emitted light. The process is threefold: excitation, relaxation to a "stable" excited state, emission. Note that the emission energy is higher (i.e., occurs at lower wavelength) than the emission energy. Also note that the efficiency of fluorescence emission event is always less than 100% (radiationless energy losses are often significant competing pathways for energy loss).

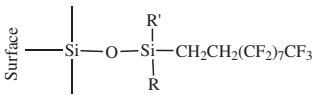


Fluorescence is a two-step process: excitation through energy absorption as a photon followed by emission from a "stable" excited state. Between the excitation and emission could be a series of radiation (IR) and radiationless (heat) loss mechanisms. Therefore, no fluorescence event is 100%. Note that the fluorescence wavelength is always longer (less energy) than the excitation wavelength (energy is lost).

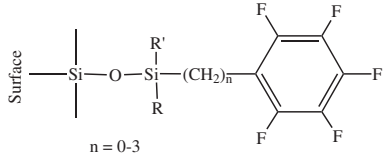
fluorescence detector Set up to monitor fluorescence emission from an excited-state molecule. The detector consists of an excitation source, a flow cell, and a detector element. The detector element is placed at a 90° angle to the source beam. Slits prevent stray light from entering the detector or striking the detector element. Fluorescence is one of the most sensitive detection techniques because the baseline output is theoretically zero. This, coupled with its sensitivity (only a limited number of molecules fluoresce), makes it a powerful analytical technique.

fluoroalkyl-bonded phases A class of bonded phases that have perfluorinated ends to the bonded phase such as heptadecylfluorodecyl. These bonded phases are very hydrophobic but have unique selectivity for polarizable compounds.

Fluorophenyl bonded phases



Heptadecafluorodecyl bonded phase

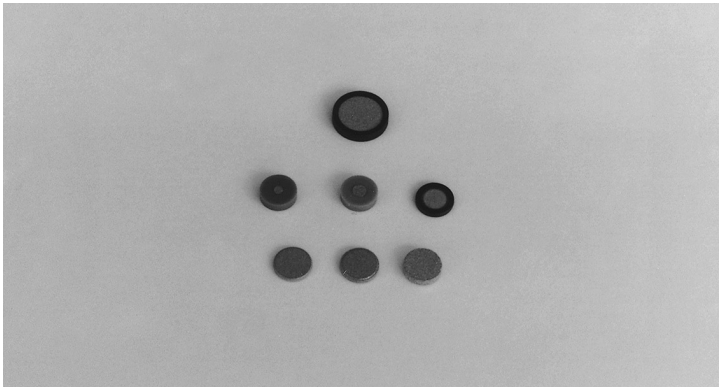


Perfluorophenyl bonded phase

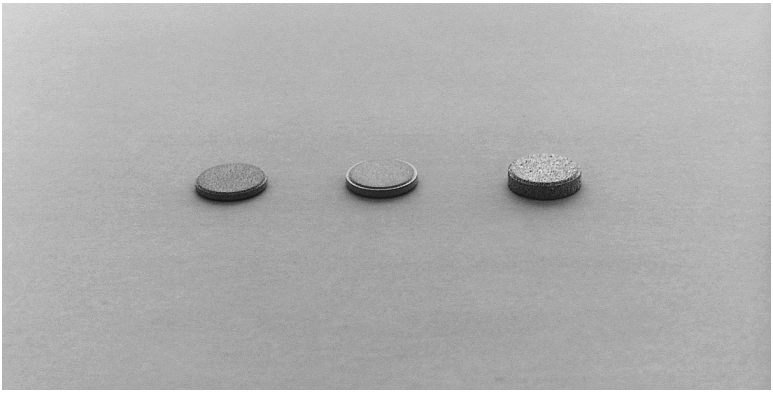
fluorophore A functional group that will fluoresce when the proper wavelength radiation is used to excite it.

Fresnel refractive index detector The Fresnel RI detector uses a prism gasket that creates two separate cells and a plate. The incident beam is split and sent through the two cells, one of which contains the static reference solution (often the mobile phase). The other goes through the flowing mobile-phase cell. The signals are subtracted from one another, and the result is recorded as the detector output. This type of RI is the most sensitive, but separate prisms must be used in order to cover the entire RI range (i.e., changed).

frit A porous component often constructed from stainless steel or titanium that is placed at the top and bottom of packed columns and holds the packing bed in place. Frits are also used in in-line filters.



Frits are porous membranes typically constructed from stainless steel or titanium. They are available in a wide range of diameters, thicknesses, and porosities. Column frits are used to keep the packing bed in place. Frits are used in in-line filters to trap particulates.



Three frits of varying thickness.

fronting The condition in which the elution profile of a peak is skewed before the peak maximum. *See* asymmetry.

Freundlich isotherm Describes the distribution of an analyte between the stationary phase, C_s, and the mobile phase, C_m, as:

$$C_s = KC_m^N,$$

where K is the Freundlich distribution coefficient and N is the Freundlich exponent. When N = 1, a linear isotherm results.

G

gas-liquid chromatography (GLC) This technique utilizes a gas as the mobile phase and a solid support material that is coated with a liquid stationary phase. The stationary phase must have a high boiling point (to prevent it from volatilizing off the support and out of the column). Liquid stationary phases include such materials as polysiloxanes and polyethyleneglycols. These are derivatized with various functional groups such as methyl, phenyl, and cyano, to produce phases of varying polarity.

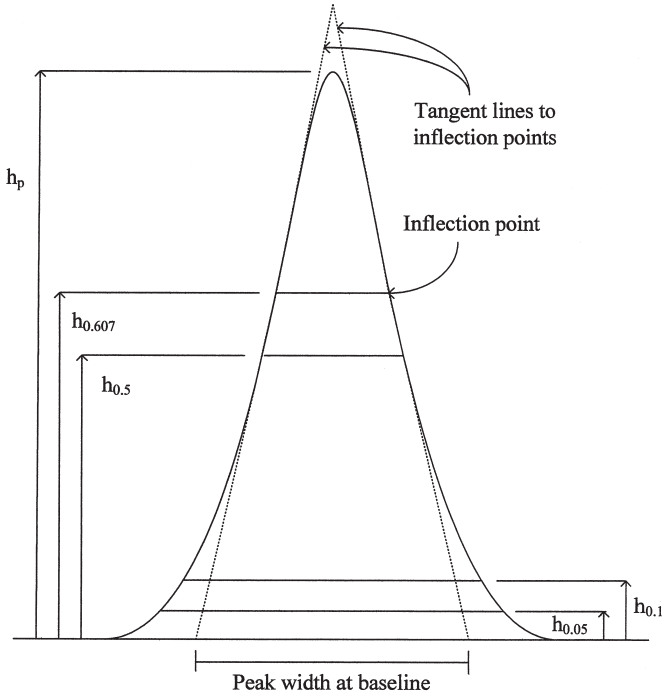
gas-solid chromatography (GSC) This technique utilizes a gas mobile phase and an unmodified adsorbent support such as silica or alumina for generating separation.

Gaussian curve The Gaussian curve is generated from the Gauss function:

$$F_{x,\sigma} = [1/\sigma\sqrt{2\pi}]e^{-(x-x_i)^2/2\sigma^2}$$

where x is the center of the distribution (mean) and σ is the width (deviation) of the distribution. This equation is used to describe chromatographic theory from which analytical parameters such as retention time, area, and symmetry are calculated.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2



The shape and some fundamental characteristics of a Gaussian curve.

gel The material that provides a physical support and overall structure to the stationary phase is often composed of a gel. It is through this packed gel bed that samples travel. Gels such as polyacrylamide, agarose, dextrans, and cellulose are used in electrophoretic work, whereas silica gels are used in HPLC, TLC, packed-bed GC, etc.

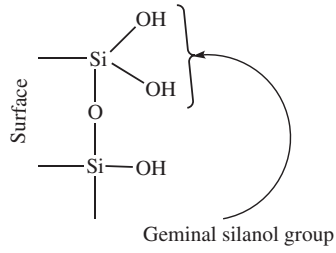
gel-filtration chromatography (GFC) A subset of size-exclusion chromatography techniques using aqueous mobile phases to separate analytes according to their molecular weight.

gelling The point in the silica polymerization process where the individual particles become large enough and concentrated enough to fuse together is where gelling begins. Gelling is also the process by which gel electrophoretic supports establish a stable porous structure. For silicas the gelling process is controlled by temperature, pH

[and the reagent(s) used to control pH], subsequent chemical treatment, and washing.

gel-permeation chromatography (GPC) A subset of size-exclusion chromatography techniques using nonaqueous mobile phases to separate analytes according to their molecular weight.

geminal Literally means "couple" or "twin" and is used specifically to describe the condition on a silica surface where one silicon atom terminates with two hydroxyl groups.



ghost peak An unexpected chromatographic peak that is irreproducible in elution time, shape, and intensity and cannot be directly linked to any component in the sample that is injected. Ghost peaks can be generated by a number of situations: (1) solvent/sample solution mismatches (i.e., the solvent and sample solution do not match in composition), (2) mobile-phase impurities (most commonly seen in gradient work), (3) carryover and in-line mixing effects (e.g., dirty tubing, injector, column), and (4) septum bleed and aging.

glass wool Used as an insert into GC injector sleeves to prevent nonvolatile components from entering the column. This also traps particulates generated by the septum or other components. Glass wool is often silanized in order to remove active sites.

Golay equation Describes the dispersion (peak broadening) as:

$$H = 2D_m/v + (1 + 6k' + 11k'^2)r^2/24(1 + k')D_m + 2k_i d_f^2 v/3(1 + k_i)^2 D_s$$

where D_m is the diffusion coefficient of the analyte in the mobile phase, v is the velocity of the mobile phase, k' is the capacity factor for the analyte, r is the radius of the column, d_f is the stationary phase

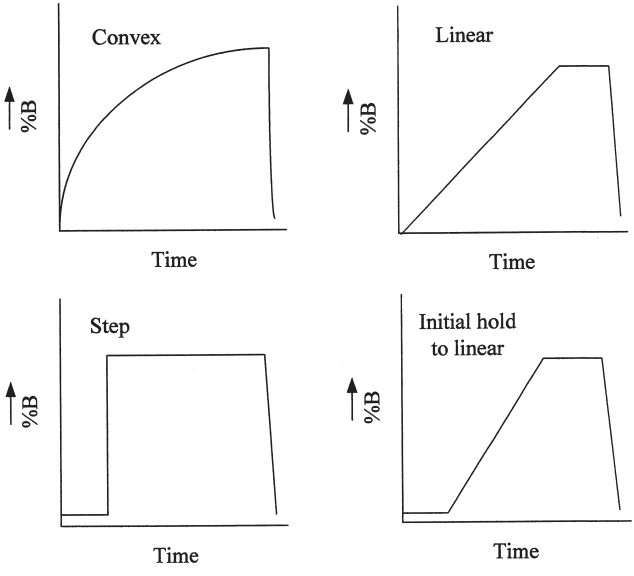
thickness, and D_s is the diffusion coefficient for the analyte in the stationary phase. More simply:

$$H = B/v + (C_s + C_m)v$$

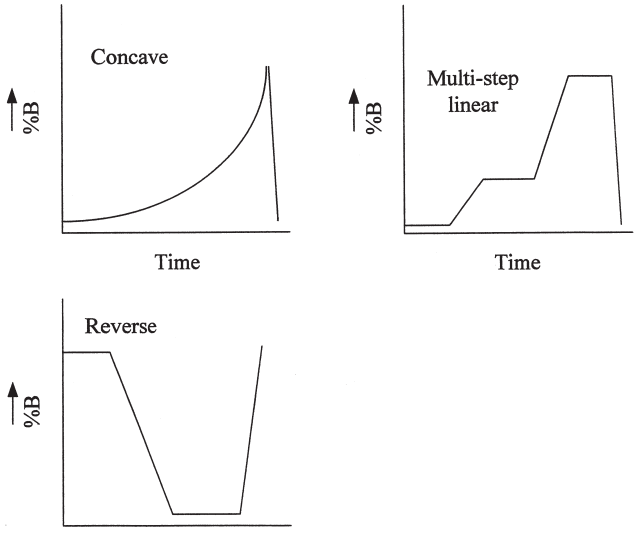
See van Deemter equation for more details as to the meaning of the A and B constants.

gradient A gradient denotes that there is a parameter in the separation that is changed with time in order to affect the elution. In HPLC a gradient refers to a change in the mobile-phase composition with time, whereas in GC a gradient refers to the change in temperature with time. In either case gradient profiles can be linear, step, nonlinear, convex, concave, etc.

gradient profile The way in which a parameter is varied with time can be described through its gradient profile. Some common HPLC solvent gradient profiles are shown below:



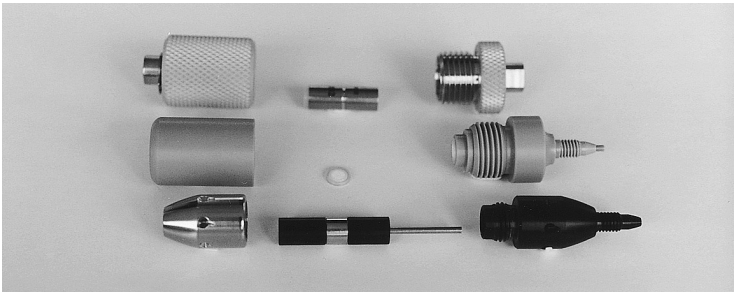
Continued



In HPLC the solvent gradients can take on a wide variety of shapes from linear, to multistep linear, to step, to curved. A gradient profile is chosen to maximize overall system efficiency: shortest run time with acceptable peak shape and resolution.

graphitized carbon A packing material that is produced from high-purity carbon and used in adsorption separations. This support provides unique separation capability for isomers.

guard column Placed between the injector and the "working" column, that is, the column used to produce the separation and also



Three different configurations of guard columns are shown. The left and right components are parts of the housing. The center component contains the packing material. Note that the packing material should be identical to the packing material used in the column the guard column protects.

typically expensive, to protect the working column from sample excipients and contamination. Guard columns are typically short (1–5 cm) and contain packing material that is identical to that in the working column. Guard columns are much less expensive than the working column and are, therefore, sacrificial columns.

H

head pressure, Δp The measure of the resistance to mobile-phase flow at the head of the column. This term includes all sources of resistance to flow after the column as well (e.g., connector tubing, detector, etc). Compare with backpressure. Head pressure is measured by a pressure transducer that is placed in the mobile phase path just before the injector. It is a function of flow rate, mobile-phase temperature, mobile-phase composition, column length and diameter, and packing material characteristics.

headspace analysis All headspace techniques involve the extraction, collection, and analysis of volatile components in a liquid sample. Analysis is often coupled to a GC. Common types of headspace analyses include static and purge and trap.

heart cutting A purification technique used in semipreparative and preparative LC that takes a slice of the peak near the peak maximum in order to obtain the highest purity fraction from the peak.

Henderson–Hasselbach equation This equation is derived from acid-base dissociation equilibrium constants, K_a:

$$K_a = [A^-][H^+]/[HA]$$

where $[A^-]$, $[H^+]$, and [HA] are the solution concentrations of the conjugate base, hydronium ion, and undissociated acid, respectively. Rearrangement yields the final form of the Henderson–Hasselbach equation:

$$pH = pK_a + \log([A^-]/[HA])$$

where pH and pK_a are the –log of $[H^+]$ and K_a, respectively.

***n*-heptane** Molecular weight: 100.2; boiling point: 98.4°C; refractive index (20°C): 1.3876; density (20°C): 0.68 g/mL; viscosity (20°C): 0.41 cP; UV cutoff: 200 nm; eluotropic strength (ε^o) on alumina—0.01,

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

on silica—0.00; polarity index (P′): 0.1; Hildebrand solubility parameter (δ): 7.4; solubility in water (20°C): 0.003%; water solubility in heptane (20°C): 0.01%. Volatile and flammable.

$CH_3CH_2CH_2CH_2CH_2CH_2CH_3$

Heptane

Heptane is used sparingly in GC as a sample solvent and in LC as a normal-phase mobile-phase component because of its high cost relative to hexane. Although alkanes are considered very stable, they do degrade over time when in contact with oxygen, heat, and light.

***n*-hexane** Molecular weight: 86.2; boiling point: 68.7°C; refractive index (20°C): 1.379; density (20°C): 0.66 g/mL; viscosity (20°C): 0.31 cP; UV cutoff: 195 nm; eluotropic strength (ε^o) on alumina—0.01, on silica—0.00; polarity index (P′): 0.1; Hildebrand solubility parameter (δ): 7.3; solubility in water (20°C): 0.014%; water solubility in hexane (20°C): 0.01%. Volatile and flammable. Hexane is a solvent commonly used as a sample solvent in GC and a mobile-phase component in LC and TLC. It should be kept in mind that the commonly purchased high-purity hexane is typically a mixture of approximately 85% *n*-hexanes and 99.9% hexanes; in essence a C_6 hydrocarbon isomer blend. Although alkanes are considered very stable, they do degrade over time when in contact with oxygen, heat, and light.

$CH_3CH_2CH_2CH_2CH_2CH_3$

Hexane

high-performance liquid chromatography (HPLC) A liquid chromatography technique that uses columns packed with particles that are 3–10 μm in diameter. To generate constant flow through such a packed bed requires that the mobile phase be forced through by a pump, often at pressures exceeding 500 psi. Smaller particle diameters lead to higher efficiency and high-pressure mobile-phase delivery to shorter analysis times.

high-performance thin-layer chromatography (HPTLC) A technique based on the same principles as thin-layer chromatography except using smaller particles (5–15 μm) and an adsorbent thickness of only ~150 μm. In general, the reduced thickness of the adsorbent generates a faster separation and the smaller particle diameter higher efficiency.

high-pressure gradient pump Utilizes multiple high-pressure pumps to deliver different solvents into a gradient mixing chamber that is positioned after the pumps. The gradient itself is therefore generated under high pressure. The advantage is that there is low dwell volume for the system. The disadvantage is the fact that the heat of mixing occurs immediately before the mobile-phase interaction with the sample and the cost associated with multiple high-pressure delivery pumps.

Hildebrand solubility parameter, δ Derived from the cohesive energy density of a solvent, C:

$$C = (\Delta H - RT)/V_m$$

where ΔH is the heat of vaporization for the solvent, R is the gas constant, T is the temperature, and V_m is the molar volume of the solvent. The solubility parameter, δ, is:

$$\delta = \sqrt{C}$$

The units for δ are $\sqrt{cal/}\sqrt{cm}$. This parameter was derived from the fact that solubility should be a function of similar cohesive energies and so it is assumed that solvents with similar δ values would be miscible in each other. See individual solvents for their δ value.

hold-up volume, V_M Represents the entire volume of the system that is accessible to a small molecule. The system hold-up volume, determined through the use of an unretained/unexcluded compound, includes the volume in the injector, connecting tubing, column, detector, etc. The column hold-up volume includes the interstitial and pore volumes, V_i and V_p, respectively:

$$V_M = V_i + V_p$$

homolog and homologous series A homolog is a molecule that is related to others through a fixed and constant change. For example, *n*-butane, *n*-pentane, *n*-hexane, and *n*-heptane form a homologous series based on a methylene group (—CH_2—) increment. Kovat's retention index is based on the use of homologous series for a wide range of stationary phases. HPLC is also used to stand for high-pressure liquid chromatography.

hydrodynamic volume A critical parameter for size-exclusion chromatography because it is defined as the total volume swept out by a molecule when it is completely rotated around its central axis. Therefore, the only case when the true molecular volume matches the hydrodynamic volume is in the case of a perfect sphere.

hydrogel A direct product of post-polymerization sineresis. This type of gel is further hardened by heating to form a xerogel.

hydrolytic stability Any bonded phase that comes in contact with aqueous solutions is susceptible to having bonded phase groups cleaved from the surface because of chemical reaction with water. The degree to which the phase is susceptible to hydrolysis is a measure of the hydrolytic stability.

hydrophilic In general, refers to samples and stationary phases that are compatible with high-percentage aqueous solutions. Hydrophilic literally means "water-loving."

hydrophobic In general, refers to samples and stationary phases that are incompatible with high-percentage aqueous solutions, that is, are compatible with nonpolar solutions. Hydrophilic literally means "water-fearing."

hydrophobic effect In highly aqueous solvents, hydrogen bonding between water molecules is a strong and energetically favored state. When nonpolar molecules are added to these systems they are "expelled" from the solvent so that the hydrogen bonds can be reformed and the most energetically favored state can be realized.

hydrophobic interaction chromatography (HIC) An LC technique that uses low-polarity support materials and varying ionic-strength mobile phases to generate separation of macromolecules (e.g., proteins) that are incompatible with organic solvents. Elution is generated by decreasing the salt concentration. To increase the hydrolytic stability a number of routes have been taken: Add bulky groups to the derivatizing silane [i.e., Cl-Si$(C(CH_3)_3)_2$R vs. Cl-Si$(CH_3)_3)_2$R]; endcap the support material; form multiple bonding sites to the surface, produce a polymeric bonded phase.

hydrostatic injection The process used in capillary electrophoresis that utilizes differential pressure, such as gravity, to make an injection. In this case the sample vial is positioned above the

column and the head of the column is inserted under the sample meniscus. Sample is drawn into the column for a set period of time (not by predetermined volume) in order to apply the correct volume of sample to the column.

hydroxyapatite A calcium hydroxyphosphate mineral used as a highly specialized stationary phase for the separation of nucleic acids and proteins.

hyphenated techniques Analyses that combine two or more instrumental techniques to produce one analytical system. Examples include GC-FID (gas chromatography-flame ionization detection) and LC-MS-MS (liquid chromatography-mass spectrometry-mass spectrometry detection).

I

ideal gas law For a perfect, or ideal gas, the relationship between pressure, P, volume, V, amount of component, n, and temperature, T, is:

$$PV = nRT$$

where R is defined as the gas constant. This relationship has particular importance in GC, where elution is accomplished through a temperature gradient over a typical range from 50 to 250°C.

immiscible Two liquid components that form separate layers on mixing at one or more proportions are immiscible. Immiscibility should not be confused with solubility. For example, even though ethyl ether and water are immiscible, they are soluble in one another: Ethyl ether is soluble to 6.9% in water (at 20°C) and water is soluble in ethyl ether to 1.3% (at 20°C).

immobilization The process of bonding an affinity ligand to a support material. Because the analyte/support interaction in affinity chromatography is based on specific three-dimensional interaction the immobilization process is critical in that it cannot alter the interaction site.

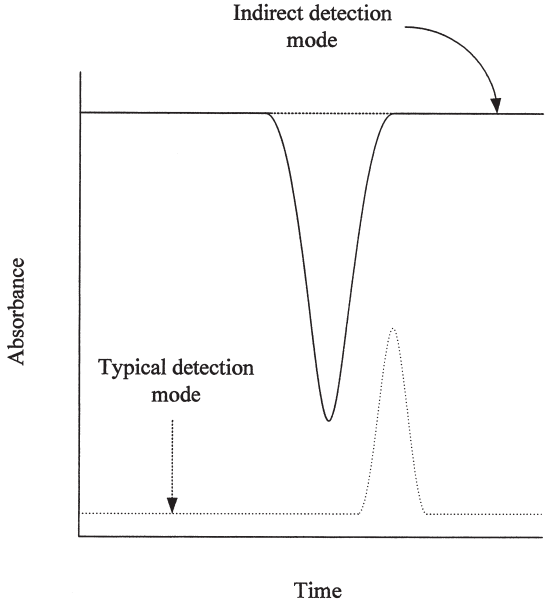
imprinted phase A stationary phase that has been created by using a molecule as a template from which the phase retains its shape "cavity." Phases prepared in this fashion will show a high degree of specificity, or at least exhibit high selectivity. The largest drawback to imprinted phases is their slow mass transfer characteristics that lead to heavy band broadening.

inclusion volume, V_i Represents the interstitial volume and all pore volume available in a size-exclusion column. The inclusion volume represents the entire volume accessible to analytes that have an effective molecular diameter less than the pore diameter in the support material.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

indeterminate error Random error that can be identified by statistical means and then minimized or eliminated.

indirect detection For analytes that do not generate a response from the detector, a reagent that does generate a response is placed in the mobile phase. Now when the analyte passes through the detector the response is attenuated and a "negative" peak is recorded. For example, an analyte that has no UV-chromophore may be detected through the addition of a mobile-phase modifier that does absorb in the UV. The resulting chromatogram will generate a negative peak from a high baseline signal:



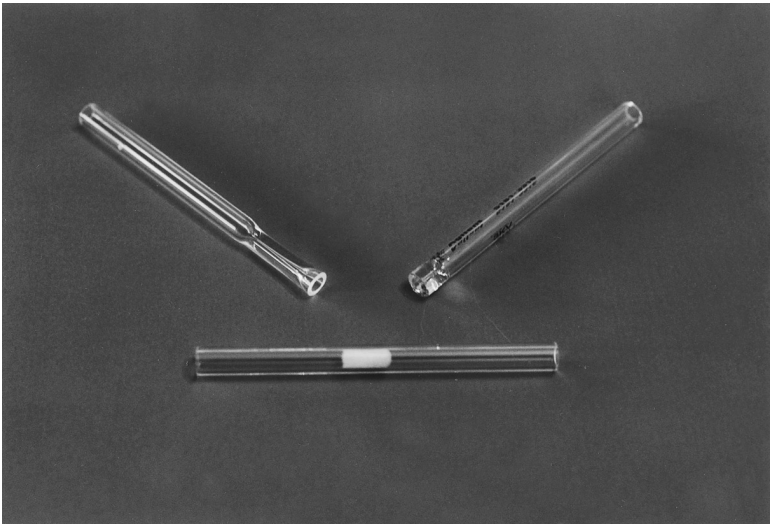
Indirect detection makes use of a mobile phase that is modified to generate a large and constant signal, in this case absorbance. The analyte does not generate a response, and so when it enters the detector the signal actually decreases. Data systems that can integrate negative peaks are needed to support this mode of detection. Note that the bottom curve represents a typical absorbance response where the mobile phase has little or no absorbance and the analyte generates a signal.

infinite diameter effect Occurs when a sample plug is injected directly into the column packing of a column that has dimensions (i.e.,

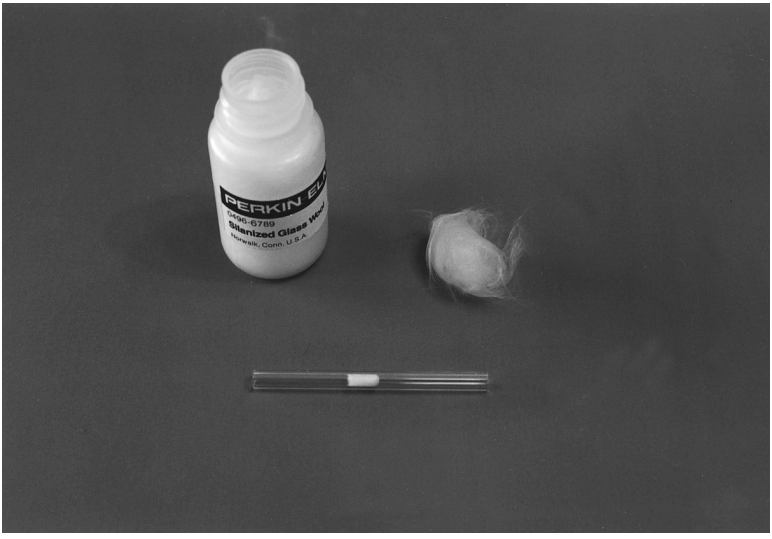
length and inner diameter) such that the plug dimensions resulting from normal band broadening events never spread the plug enough for it to reach the column wall. This condition gives higher overall column efficiency compared with typical injection plugs that are spread across the column.

injection The event that transfers the sample from a static position (e.g., contained in a sample vial or injector loop) outside the mobile phase. For HPLC and GC the instant an injection occurs is defined as time zero: $t = 0$.

injector liner A component of the GC injector housing that is frequently made from glass. Its purpose is to prevent sample contact with the hot stainless steel surface of the injector. Injector liners are often partially filled with passivated glass wool. The glass wool acts as a filter for debris from the septum and other nonvolatile materials that may get into the injector. Injector liners have different internal configurations that are designed to more effectively handle samples of varying thermal expansion, etc.



Three examples of GC injector liners are presented. They vary from a long horn end (top left) to a constricted outlet (top right) to a straight bore (lower middle) that is wadded with glass wool.



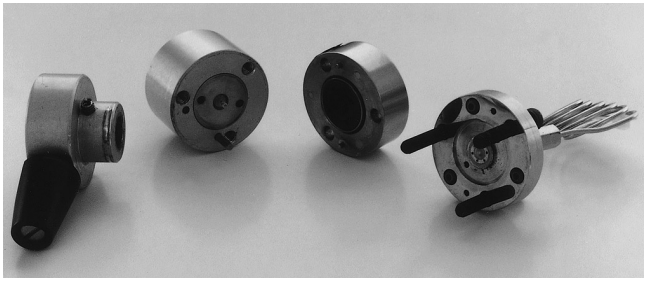
This straight-bore GC liner is wadded with glass wool. The glass wool is often silanized to prevent analyte adsorption. The glass wool is present to trap particulates from septa breakdown and oils/contaminants from connectors, gas tanks, etc.

injector port A component of a manual injector that guides the syringe needle into the injector and makes a tight seal.

This part of an HPLC injector valve guides the syringe needle up to the rotor seal. The injector port has a pliable plastic tip that forms a seal around the syringe and to the rotor seal.

injector valve The component in a liquid chromatograph that stores a sample until injection, in the loop, and changes the position of the loop from static to in the flow path on injection.

This is an example of a manual HPLC injector valve. It consists of a port, an injection lever, a body, a rotor seal, a stator, and a loop.



This is an exploded view of a manual HPLC injector. Left to right: injection lever, body, rotor seal, stator, and loop. The injector port (see injector port figure) abutting the rotor seal creates a leak proof seal. Both parts need periodic replacement.

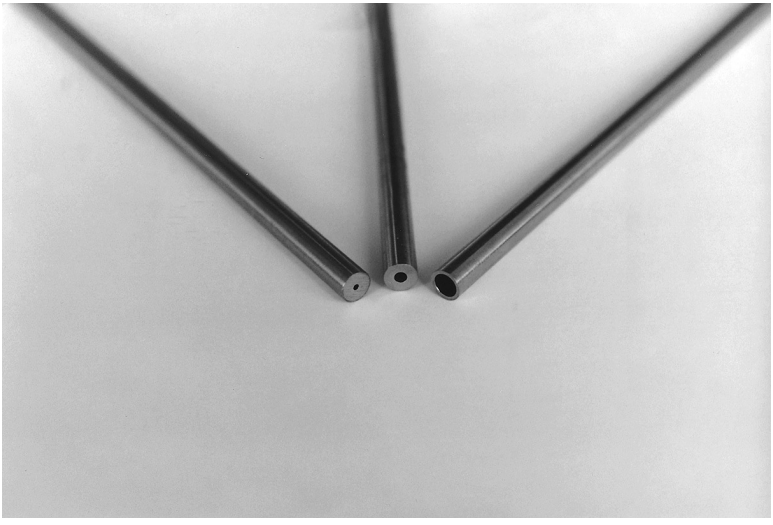
inlet The top (head) of a column or the reservoir-side check valve in a dual-check value pump system.

in-line filter *See* filter, in-line.

inner diameter (ID) The actual cross-sectional distance across the open interior of a tube through which mobile phase flows. This is an important parameter in at least three components: connecting tubing, column tubing, injector tubing. The volume-length relationship is shown in the table below.

Inner diameter (in.)	Radius (cm)	Volume of 10 cm section (μL)
0.004	0.00508	0.81
0.007	0.00889	2.48
0.01	0.0127	5.07
0.02	0.0254	20.3
0.04	0.0508	81.1

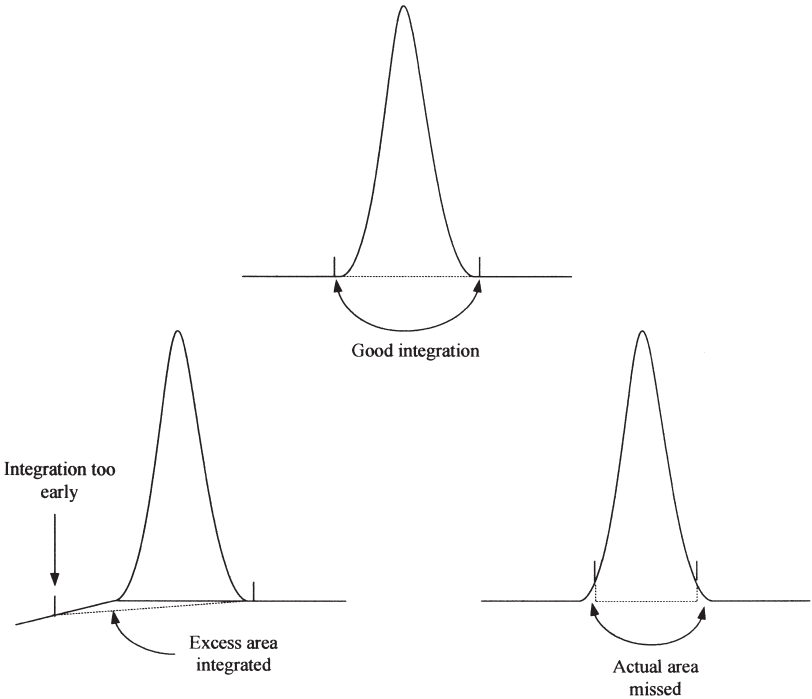
Note that the use of about 4 in. of 0.01-in. ID tubing leads to an equivalent added extra-column volume as a sample injection of 5 μL! Keep the lengths of all connecting tubing after the injector and before the detector as short as possible. In HPLC connector tubing, the choice of ID is dictated by use. For tubing before the pump, it is usually a large ID on the order of 0.02 in. The tubing is meant to provide unrestricted flow from the pump. PTFE is frequently used because of its chemical resistivity, flexibility, and ease of use. From the pump to the injector, large-ID stainless steel is used. Once again, the tubing is used to provide an unrestricted flow from the pump to the injector. However, in this case the tubing must be able to withstand high pressures (>4000 psi) and so stainless steel is often used. For the connec-



Left to right: 1 mm-, 2 mm- and 4.6-mm inner diameter HPLC column tubing. All are constructed from stainless steel and are ¼ in. in outer diameter.

tions from the injector to the column and on to the detector, small-ID stainless steel is used, often in the range of 0.005 in. This minimizes extra-column volume and the peak broadening associated with it. For injectors, the volume contained is crucial and so the appropriate ID must be used in order for the proper volume to be contained for injection (see table).

integration The process of determining the area under a peak. Integration is composed of two distinct events: Integration is started and then terminated. These events are often linked to the change detector signal (i.e., the slope). This becomes very difficult when



The top profile shows a good integration where the entire peak is included and the integration points (i.e., the vertical lines) are near the start and end of the peak. In the bottom left example, there is a rising baseline to the peak. The integration triggers too early, giving an area that is larger than the true peak area. In the bottom right example, the triggers occur too late and not all of the peak is integrated, leading to an underestimated peak area.

peaks have very shallow rises or declines (such as extreme fronting or tailing). Because the integrated area of a peak is often used in analyte quantitation, reproducible integration is critical to generating acceptable results.

internal standard An internal standard method is used when the reproducibility of the analytical instrument is not as precise as needed. An internal standard is a compound that is similar in chemical/physical properties to the analyte of interest, does not coelute with any other component in the sample, is stable and obtainable in a high-purity form, and has an acceptable linear range. An internal standard is added to a sample before analysis and is then quantitated against its standard curve to determine its overall recovery. If the recovery is not 100% of that expected, then it is assumed that the recovery of the analyte is the same and the result is corrected accordingly.

internal surface reversed phase (ISRP) An internal surface reversed-phase support is a porous packing material that is derivatized on the surfaces external to the pores with a nonadsorptive hydrophilic bonded phase and derivatized on the pore interior with a hydrophobic bonded phase. This type of column is also called a Pinkerton column. It is used in the analysis of complex samples containing protein and smaller analytes (such as drug compounds). The protein is excluded from the pores and does not adsorb to the packing surface, whereas the smaller compounds enter the pores and are retained in the reversed-phase mode.

interpolation The process used to determine an unknown result from its response/output as compared against a series of standards whose responses/outputs bracket the result. *See* extrapolation.

interstitial porosity, ε_o Represents the volume contained within the column but outside the packing material, V_e, with respect to the total column volume, V_c:

$$\varepsilon_o = V_e/V_c$$

interstitial velocity, u_e The velocity of the mobile phase moving outside the packing material particles represents the elution volume of an analyte that is totally excluded from the pore volume:

$$u_e = F/A\varepsilon_o$$

where F is the flow rate, A is the cross-sectional area of the column, and ε_o is the interstitial porosity.

interstitial volume, V_e The volume in a column excluding the volume of the packing material (and its pore volume).

intraparticle porosity, ε_i The fraction of volume of the packing material itself, V_{pack}, that is pore volume, V_{pore}:

$$\varepsilon_i = V_{pore} / V_{pack}$$

intraparticle volume, V_i Also called pore volume; the volume of the pores contained within the packing material. This value is experimentally determined through the use of mercury porosimetry or the BET nitrogen adsorption method.

intrinsic viscosity, $[\eta]$ Used in the generation of a universal calibration curve (i.e., a viscometric detector monitors this) for polymers:

$$[\eta]M = f(V_e)$$

where M is the molecular weight and $f(V_e)$ denotes a function of the elution volume. The intrinsic viscosity is the limiting value of the reduced viscosity at infinite dilution of the analyte. Alternatively:

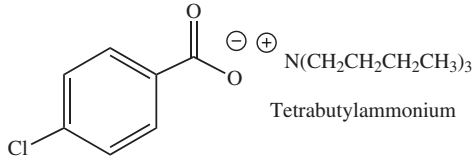
$$[\eta] = \lim_{c \to 0} \eta_{sp} / c,$$

where $\eta_{sp} = (\eta - \eta_o)/\eta_o$ and η is the viscosity of the solution and η_o is the viscosity of the solvent.

ion-exchange chromatography (IEC) An LC technique that generates a separation through ionic interaction. The support material has a permanent positive or negative change and retains analytes of the opposite charge. Elution is accomplished through adding competing ions (same charge as analyte) to the mobile phase.

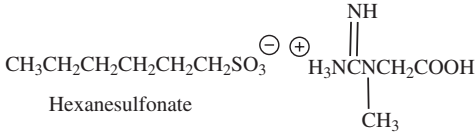
ion-exchange supports Materials that have permanent charged functional groups on the surface. Anion-exchange supports (those supports used to separate anions) have positively charged groups, whereas cation-exchange supports have negatively charged groups.

ion-pair reagent Used in LC separations, often reversed phase, to give charged analytes a longer retention time through the formation of an ion-pair complex with a mobile-phase modifier. For example, deprotonated carboxylic acids can form ion pairs with quaternary amines:



4-Chlorobenzoate Ion pair amine

and protonated amines can form ion pairs with sulfonic acids: The result is an overall uncharged ion pair that is now more retained than the ion itself.



Hexanesulfonate Ion pair acid

Creatine

ion suppression Used for peak shape enhancement of weakly ionizable analytes such as primary and secondary amines and carboxylic acids. The pH of the mobile phase is adjusted to below the pK_a of the analyte to achieve suppression.

ion-trap mass analyzer The ion trap is a monopole mass analyzing unit that utilizes radio frequencies (rf) to create a stable volume within the ring electrode. Selected mass-to-charge (m/z) ratios are moved from the analyzer to the detector by changing the rf in a controlled manner.

irregular packing material The term "irregular" refers to the shape of the base support material used in the column. As the term suggests, the particles do not have a uniform shape (as contrasted to spherical support materials). These particles are often manufactured

by milling larger particles down to smaller sizes. Sieving and other sizing techniques are then used to produce a product with a desired average particle size. Irregular particles are infrequently used in HPLC work, having been replaced by spherical supports. Irregular particles are commonly found in semipreparative and preparative columns, solid-phase extraction columns and cartridges, and TLC plates because of the lower cost of production compared with spherical materials.

irreversible adsorption Occurs when the interactions of the analyte with any component of the column, usually the packing material, are so strong that the mobile phase cannot cause it to elute.

isobaric Refers to the condition in which the mobile phase is held at a constant pressure.

isoconfertic Refers to the condition in which the mobile phase is held at a constant density.

isocratic elution An HPLC system generating a separation under conditions in which composition of the mobile phase does not change with time. Contrast with gradient.

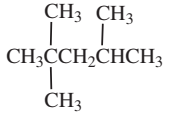
isoelectric point, pI The pH at which the effective net charge for an ampholyte (or polyampholyte) is zero. For a compound that has one base and one acid functional group, for example, an amino acid, the isoelectric point can be calculated directly from the pK_a values of the carboxylic acid and the protonated amine:

$$pI = \left[pK_{a,\text{-COOH}} + pK_{a,\text{-}NH_3^+} \right] / 2$$

It should be noted that at the pI for a species the solubility is typically at its lowest.

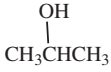
isoeluotropic Two solvents that generate the same average elution time for a range of analytes are termed isoeluotropic. These mobile phases would also be said to have the same solvent strength. Note that this determination must be done on identical column packing with all operating parameters identical (except for the solvent). Also note that although the *average* retention for the test set of analytes is the same, the retention of a particular group of analytes (say phenolics) may be greater in one system over another.

iso-octane, 2,2,4-trimethylpentane Molecular weight: 114.2; boiling point: 99.2°C; refractive index (20°C): 1.3914; density (20°C): 0.69 g/mL; viscosity (20°C): 0.50 cP; UV cutoff: 215 nm; eluotropic strength (ε^o): on alumina—0.01, on silica—0.01; polarity index (P′): 0.4; Hildebrand solubility parameter (δ): 6.9; solubility in water (25°C): 0.0002% water; water solubility in iso-octane (20°C): 0.006%. Volatile and flammable. Iso-octane is used as a solvent in GC analyses and as solvent in HPLC and TLC.



Iso-octane

isopropyl alcohol, 2-propanol (IPA) Molecular weight: 60.1; boiling point: 82.3°C; refractive index (20°C): 1.3772; density (20°C): 0.79 g/mL; viscosity (20°C): 2.4 cP; UV cutoff: 205 nm; eluotropic strength (ε^o): on alumina—0.82, on silica—0.60; polarity index (P′): 4.3; Hildebrand solubility parameter (δ): 11.5. Miscible with water. Volatile and flammable. IPA is known as a "universal" solvent in that it is miscible with an extremely wide range of commonly used polar and nonpolar solvents. [Note that this fact does not imply anything about the solubility of salts and buffers in IPA.] Because of this quality, IPA is used as the intermediate solvent when changing from HPLC reversed-phase systems (i.e., aqueous/organic solvents) to normal-phase systems (i.e., nonpolar solvents) and vise versa. IPA is also used as a mobile-phase component in both reversed- and normal-phase LC separation. However, the high viscosity of IPA-water mixtures in the 20–60% range lead to unacceptably high operating backpressure.



Isopropyl alcohol

isopycnic Refers to the condition in which the mobile phase is held at constant density (typical parameter monitored in SCF work).

isotachophoresis The separation technique that is based on constant-speed electrophoresis. In isotachophoresis, unlike CZE, the buffer is heterogeneous across the support and is composed of a leading electrolyte (one that moves faster than the analyte) and a terminating electrolyte (one that moves more slowly than the analyte) .

isotherm The relationship between the concentration of a substance S in the mobile phase, C_{mp}, and its concentration in the stationary phase, C_{sp}, is defined by the substance's isotherm under those operating conditions. Under ideal conditions the distribution of S between phases can be represented as:

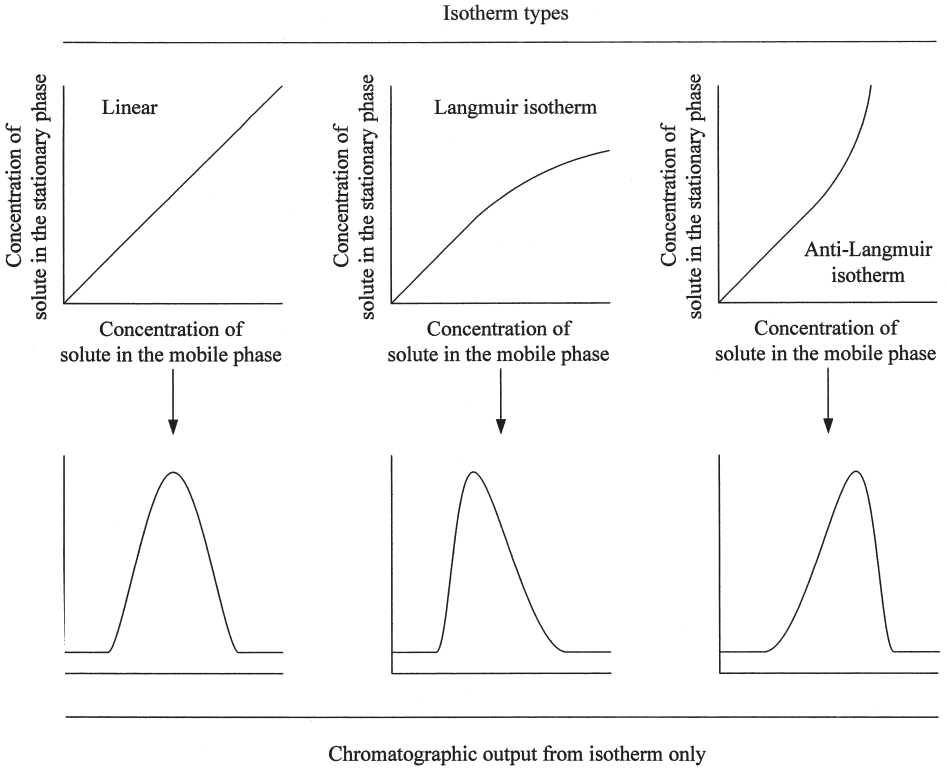
$$S_{mp} \leftrightarrow S_{sp}$$

The equilibrium distribution between phases is mathematically represented as:

$$K_{eq} = [S_{sp}]/[S_{mp}] = C_{sp}/C_{mp}$$

in this case a linear relationship and, hence, a linear isotherm. Nonlinear isotherm relationships can also be described mathematically. Some more common representations are Langmuir and Freundlich isotherms. *See* figure next page.

isothermal The operating condition in GC where the column temperature does not change over the course of the separation is termed an isothermal separation.



Examples of linear, Langmuir and anti-Langmuir isotherms and their effect on peak shape (no other interactions present).

J

Johnson noise Generated in electrical circuitry because of the presence of resistors and is proportional to the square root of the resistance present: Noise (in V) $\propto \sqrt{R}$.

joule heating Conduction of electrical current through an electrolytic solution generates heat due to the forced movement of ions in opposing directions and results in Joule heating (also called Ohmic heating). From a practical point of view this heating leads to alterations in the solution viscosity and irreproducible profiles. In the extreme, the solvent may actually boil, causing trapped air bubbles, etc. Therefore, to avoid this the heat generated must be dissipated from the system throughout the entire separation process.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

K

katharometer detector *See* TCD (thermal conductivity detector).

kieselguhr *See* diatomaceous earth.

Knox equation Describes the mathematical relationship between the reduced plate height, h, and the reduced velocity, v, in a chromatographic system:

$$h = Av^{1/3} + B/v + Cv$$

where A, B, and C represent different physical influences on peak tailing. The practical implication of this equation, and others like it, e.g., the van Deemter equation, is that they predict a flow rate at which the smallest h value is obtained (correlates to the largest N value and greatest efficiency). Unfortunately, this v value is often so slow that it is impractical for routine separations. The compromise therefore is to increase throughput (increase v) at the expense of lost efficiency.

Kovat's retention index, I Used to compare therelative retention of compounds regardless of column and laboratory through the use of the following equation:

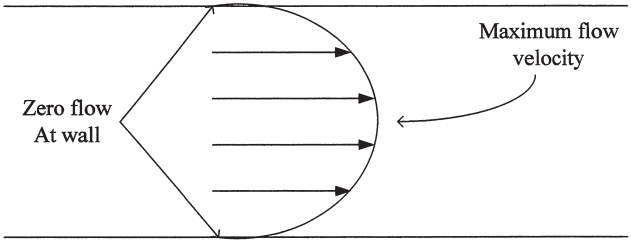
$$I = 100\,(\log V_{N,x} - \log V_{N,n})/(\log V_{N,n+1} - \log V_{N,n}) + 100n$$

where $V_{N,x}$ is the retention volume of the analyte, $V_{N,n+1}$ and $V_{N,n}$ are the retention volume for two adjacent homologous n-alkanes (e.g., n-hexane and n-heptane) that bracket the retention volume of the analyte, and n is the number of carbons in the alkane.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

L

laminar flow A condition found where a fluid in a confined area (e.g., tubing) is moving at a velocity such that the flow profile forms a smooth and well-defined series of streamlines that are invariant with time.



Laminar flow is a smooth flow profile with the linear velocity at the walls being zero and the maximum flow velocity at the center.

lamp Term often used interchangeably with the term "source"; indicates the component in a detector that is responsible for generating a constant energy flux through the sample. For single fixed-wavelength lamps the output is determined by the choice of element. For example, detection of $\lambda = 229$ requires a cadmium lamp.

langmuir isotherm An isotherm describes the equilibrium distribution of a solute molecule between the stationary phase, C_s, and the mobile phase, C_m, or $K = C_s/C_m$. The surface concentration in a Langmuir isotherm is mathematically expressed as:

$$C_s = [K \times Q \times C_m]/[1 + (K \times C_m)]$$

See isotherm.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

leading electrolyte The electrolyte that contains the highest-mobility analyte ion in an isotachophoretic separation (*see also* terminal electrolyte).

leak test Done to make sure that all unions and seals are properly set. In GC gas leaks are often discovered by using a drop of a soap-water or IPA-water mixture at the union and looking for air bubbles. If bubbles appear, then the union is not properly set. In LC a piece of tissue paper is placed in contact with the union. If there is a leak, then solvent will be wicked up into the tissue, indicating that the union is incomplete.

least squares For sets of data that are expected to be linearly related, the technique used to determine the line that best fits the data is called the least-squares fit of the line (also called linear regression of the line). The least-squares technique minimizes the sum of the squares of the dependent variable value from that of the determined line. The resulting relationship is expressed as $y = mx + b$, where y is the dependent variable, m is the slope of the line, x is the independent variable and b is the y-intercept value.

ligand A molecule that forms strong chemical interactions with another atom or molecule. The ligand can be bound to a support material as in affinity chromatography or free in solution. In affinity chromatographic separations there are two classes of ligand: high-specificity ligands that strongly bind to one molecule or one class of closely related molecules, or general ligands that bind to entire classes of compounds (e.g., lectins are ligands for sugar separations). In a more general sense a ligand is an organic molecule (such as ethylenediaminetetraacetic acid, EDTA) that binds to metal ions:

$$\mathrm{EDTA}^{4-} + \mathrm{Ni}^{2+} \leftrightarrow \mathrm{NiEDTA}^{2-}$$

light-scattering detector cell The light-scattering cell generates a response based on the amount of laser light scattered 90° from the beam path by particles flowing through it. The detector responds as $A = am^x$, where a is the response factor for each individual analyte, m is the solute mass, and x is the slope of the response.

linear flow velocity, *u* The velocity at which a component moves through a column is called its linear velocity and is mathematically expressed as:

$$u = L/t$$

where L is the column length and t is the residence time of the component in the column.

linear regression The process through which the slope and intercept of a linearly correlated set of data are determined. One of the techniques used is least squares.

linear velocity, *u* The rate at which the mobile phase moves through a length of column. The linear flow rate is calculated from:

$$u = \mathrm{F}/\varepsilon\mathrm{A}$$

where F is the flow rate, ε is the column porosity, and A is the cross-sectional area of the column tubing.

liquid chromatography (LC) A technique that utilizes a liquid mobile phase to generate separations.

liquid-phase loading This term applies to the liquid stationary phase that is present on a GC support that is used in a GLC separation. It is expressed as a weight percent:

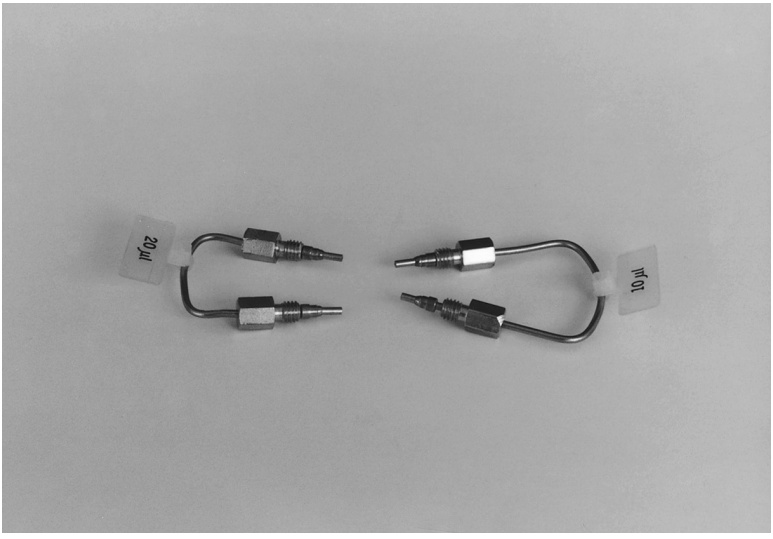
$$\%\ \text{liquid loading} = [(\text{wt of liquid phase})/(\text{wt of liquid phase} + \text{wt of support material})] \times 100$$

liquid-solid chromatography (LSC) *See* adsorption chromatography.

loading (1) The amount of stationary phase adsorbed or bonded to a support material. This is often expressed as a weight percent. (2) The amount of sample injected onto a column is referred to as the sample loading.

longitudinal diffusion A band-broadening effect that is the result of the chemical potential gradient caused by the concentration differences within the eluting sample band and the adjacent mobile phase. It is part of the B term in the van Deemter and Knox equations.

loop A part of an injector that contains the sample before injection. In some injectors the sample needle acts as the loop as well. Injector loops can be internal or external to the body of the injector. Large volumes (≥2 μL) are typically external. Loops are constructed from stainless steel and PEEK.



This component of the HPLC injector valve contains the sample that is presented by the syringe. These are 20-µL and 10-µL stainless steel loops. Loops are also constructed from PEEK.

low-angle laser light scattering detector (LALLS) Measures the light scattered from a high-intensity laser source by macromolecules in the effluent. Through a plot of $CK/R\phi$ versus concentration an intercept of 1/MW is obtained, where C is the solute concentration, K is the function of an optical parameter of the macromolecule, and $R\phi$ is the Rayleigh scattering at low angle ϕ.

low-pressure gradient pump Forms a gradient through the use of solenoids connected to each reservoir and a mixing block that is positioned before the pump. In this case only one high-pressure pump is needed and therefore is advantageous over a high-pressure gradient pump from a cost consideration. The major disadvantage is that the dwell volume is considerable in these types of systems.

M

macroporous Refers to polymerized resins (e.g., polystyrene-divinylbenzene) that are formed in a solvent where the monomers are soluble but the polymer is not. The result is a high-mechanical-stability spherical porous material that has included solvent. Removal of the entrapped solvent results in the finished product.

macroreticular resin A resin that is cross-linked such that a highly porous particle with large average diameter pores is produced. (*See* microreticular.) These materials are used for the separation of larger analytes and as ion-exchange supports.

make-up gas Used to help increase the flow rate of the sample eluting from the column through the detector. This is particularly crucial for split injection samples.

Mark–Houwink equation Defines the relationship between the molecular weight of a polymer, MW, and the intrinsic viscosity of a dilute solution of the polymer, [η]:

$$[\eta] = K \times (\mathrm{MW})^{a}$$

where K and a are constants for a given polymer type and solution composition.

mass flow detectors A destructive detector whose signal is in part derived from the rate at which a sample moves through the detector cell. As an example, a flame-ionization detector burns a sample to generate a signal so that if the flow through the detector were stopped the signal would rapidly decrease to zero. (This is not the case with a concentration detector.)

mass spectrum A plot of the ion abundance versus the mass-to-charge ratio (m/z) that is generated in a mass spectrograph.

mass transfer The process of a compound moving between phases (or through a given phase). Resistance to mass transfer and

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

transfer in a stagnant phase are contributors to zone spreading (increased peak width) and are captured as the C term in the van Deemter and Knox equations.

matrix blank A sample that contains all the components of a sample, in concentrations that are expected in the sample, except for the compounds that are to be analyzed. A matrix blank is used to determine whether or not there are any matrix components that will ultimately interfere with the analysis (e.g., coelute).

McReynolds phase constants Developed to establish a systematic ordering of GC stationary phases with respect to specific solute interactions. This can then be used to predict the change in the retention index, ΔI, for the tested phase with respect to squalene:

$$\Delta I = aX' + bY' + cZ' + dU' + eS'$$

where a, b, c, d, and e are assigned constant values associated with a specific test probe molecule (e.g., benzene, nitropropane, etc) and X′, Y′, Z′, U′, and S′ refer to a molecule's aromaticity, proton donor/acceptor characteristics, proton acceptor characteristics, dipole interactions, and strong proton acceptor characteristics, respectively. Tables of the values for each of these constants were generated for a wide range of nonpolar to very polar stationary phases.

mean, *x* The mean value for any set of related data is the best estimate of the value of the results and is mathematically determined by:

$$x = \sum x_i / n$$

where Σ represents the summation of all data points 1–n and x_i is the value of the ith datum in the set of n data points. For example, for the set of data 6.1, 6.4, 6.2, 6.2, and 6.1, x is 6.2 [i.e., (6.1 + 6.4 + 6.2 + 6.2 + 6.1)/5].

median The middle value in a set of rank-ordered data. For example, for the set of data 6.1, 6.4, 6.2, 6.2, and 6.1, the rank order is 6.1, 6.1, 6.2, 6.2, 6.4 and the median is 6.2.

mesh and mesh sizes A mesh is used to sieve particles having a wide size distribution. The mesh size is determined by the number of meshes in the sieve and is characterized with respect to the largest

diameter of a particle that can pass through it. Therefore, the number of meshes in the sieve is inversely proportional to the particle size it will allow to pass through. Two basic mesh scales are in use: US Standard and Tyler. Some examples are given below:

US Standard	Eq. Particle Diameter (μm)
50	300
80	180
140	106
270	75
400	38

methanol, methyl alcohol Molecular weight: 32.0; boiling point: 64.7°C; refractive index (20°C): 1.3284; density (20°C): 0.79 g/mL; viscosity (20°C): 0.55 cP; UV cutoff: 205 nm; eluotropic strength (ε^o): on alumina—0.95, on silica—0.73; polarity index (P′): 5.1; Hildebrand solubility parameter (δ): 13.7. Miscible with water. Volatile and flammable. Methanol is a very commonly-used solvent in reversed phase LC and purge & trap GC.

CH_3OH

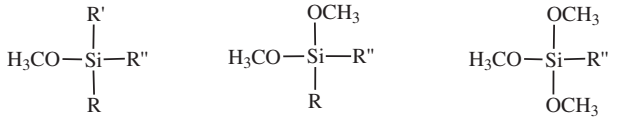
Methanol

method development The formalized process by which a set of separation criteria are determined such that with a defined set of parameters the same separation profile is achieved. The process of method development can be qualitative or quantitative. An effective method development protocol sets out well-defined success criteria, for example, minimum resolution between peaks, peak asymmetry and response factor ranges, minimum column efficiency (N), total analysis time, linearity, linear range, and reproducibility. The finished separation is optimized to meet all these criteria.

method validation The process that follows method development and sets strict limits on the reproducibility, robustness, and ruggedness of the method. This validation process often includes multiple sample preparations within one lab, matrix blank analysis (to ensure that there is no coeluting peak in the matrix that will interfere with the analyte of interest), multiple laboratory, analyst, and instru-

ment performance evaluation, limits of detection, specificity, selectivity, accuracy and precision, repeatability, ruggedness, and robustness.

methoxysilanes A class of compounds that are used to chemically modify silica support material and have the basic structure:



Monomethoxysilane Dimethoxysilane Trimethoxysilane

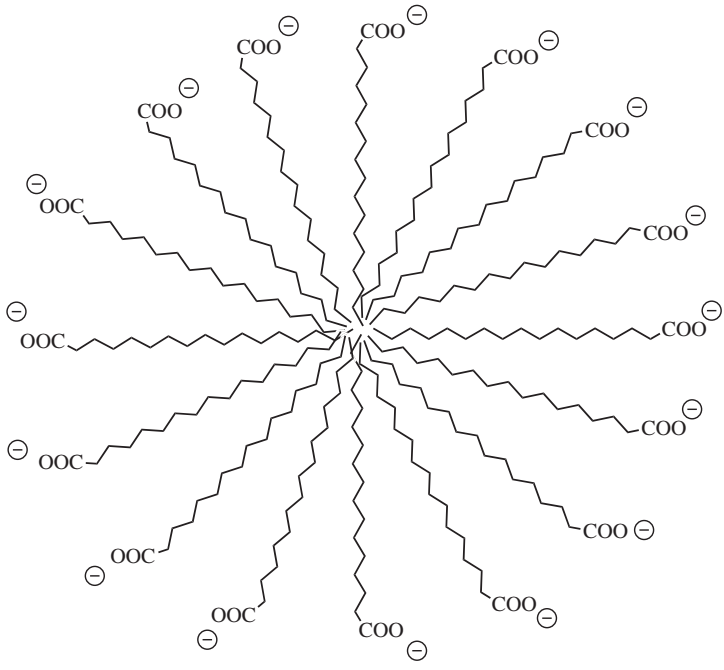
Methoxysilanes

where R and R′ can be methoxy groups or, more typically, methyl (or other alkyl) groups. R″ defines the type of bonded phase that is present; for example, when R″ = $-CH_2CH_2CH_2CN$ the phase is a cyano phase. Methoxysilanes are used when the terminal functional group, in this case cyano, is reactive with a chlorosilane functional group.

methyl alcohol *See* methanol.

micellar electrochromatography (MECC or MEKC) A capillary electrophoresis technique that is used to separate neutral analytes through the application of a wall potential (i.e., using electroosmotic flow). A surfactant is added to the liquid phase in a concentration large enough to produce micelles. The movement of the micelles coupled with the transfer of analyte into and out from the micelle generates the separation.

micelle An agglomeration of compounds that have distinct and separate hydrophobic and polar (hydrophilic) functionalities. Agglomeration occurs above a certain critical concentration in solution. For example, surfactants such as fatty acids in soaps have a hydrophobic portion (the long-chain alkyl group) and a polar group (the carboxylic acid) that enable the formation of a micelle when the aqueous solution concentration is high enough.



Micelle

microbore Columns that have inner diameters (ID) <2 mm are considered microbore columns. The packing material used in these columns is usually ≤3 μm in diameter. Flow rates are typically ≤0.2 mL/min (because of the very high backpressure generated in the system). These systems are especially suited for LC/MS work, limited sample situations, or separations requiring high peak capacities. From an operation point of view, as the inner diameter and column length decrease, the sensitivity of the method increases (less dilution of the sample) but the difficulty of normal maintenance increases (easy to damage or contaminate).

microporous Refers to polymerized resins (e.g., polystyrene-divinylbenzene) that form particles of a size and shape that are dependent on the rate of polymerization (controlled by concentration, addition rate, temperature, etc). The result is a porous, pressure-sensitive, irregularly shaped particle.

microreticular A microreticular resin is produced through a cross-linking such that small pores result. *See* macroreticular.

migration time, t_m The time required for an analyte to travel from the injector to the detector in a capillary electrophoresis column under an applied voltage, V:

$$t_m \approx 1/V$$

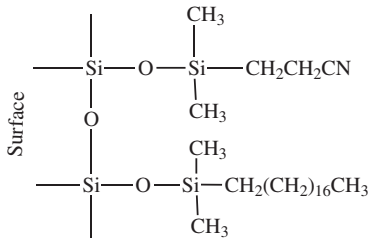
minimum detectability Synonymous with limit of detection, minimum detectability defines the lowest analyte signal that can be distinguished from system noise.

miscible Two liquid components that can be mixed together in all proportions at a given temperature without forming two separate layers are termed miscible.

miscibility number, M Developed to offer a quick way of determining whether or not a given pair of solvents is miscible. Each solvent is assigned an experimentally determined miscibility number ranging from 2 to 29; 2 is very polar and 29 is nonpolar. Any pair of solvents that have M numbers differing by <15 are miscible at room temperature. With a difference of >17, they are immiscible. A value of 16 means that miscibility is strongly temperature dependent around room temperature.

mixed-bed Refers to a column (or support) that is prepared containing two or more distinct types of stationary phases. An example would be a blend of an ion-exchange packing with a reversed-phase packing. The resulting mixed-bed column would provide a mixed-mode separation based on ion-exchange and hydrophobic retention mechanisms (*see* mixed mode).

mixed mode Refers to any separation that is the result of more than one retention mechanism. This can be intentional or unintentional. As an example, a column can be packed with an ion-exchange packing mixed with a reversed-phase packing (intentional mixed mode). This type of bonded phase is more difficult to make reproducibly but offers a wide range of potential selectivities not available on single-moiety bonded phases. Conversely, a reversed-phase support can have residual silanol groups that can lead to mixed-mode separation: hydrophobic and silanophilic (often unintentional).



Mixed-mode

mobile phase In HPLC, TLC, and CZE the mobile phase is a liquid that moves through/past a stationary phase and causes separation. In GC separations, the mobile phase is a gas (often called the carrier gas).

mobile-phase modifier *See* modifier.

mobility *See* electrophoretic mobility, μ.

modifier A component added to the mobile phase, typically at a low level, to alter its chemical and/or physical properties and enhance the efficiency of the separation.

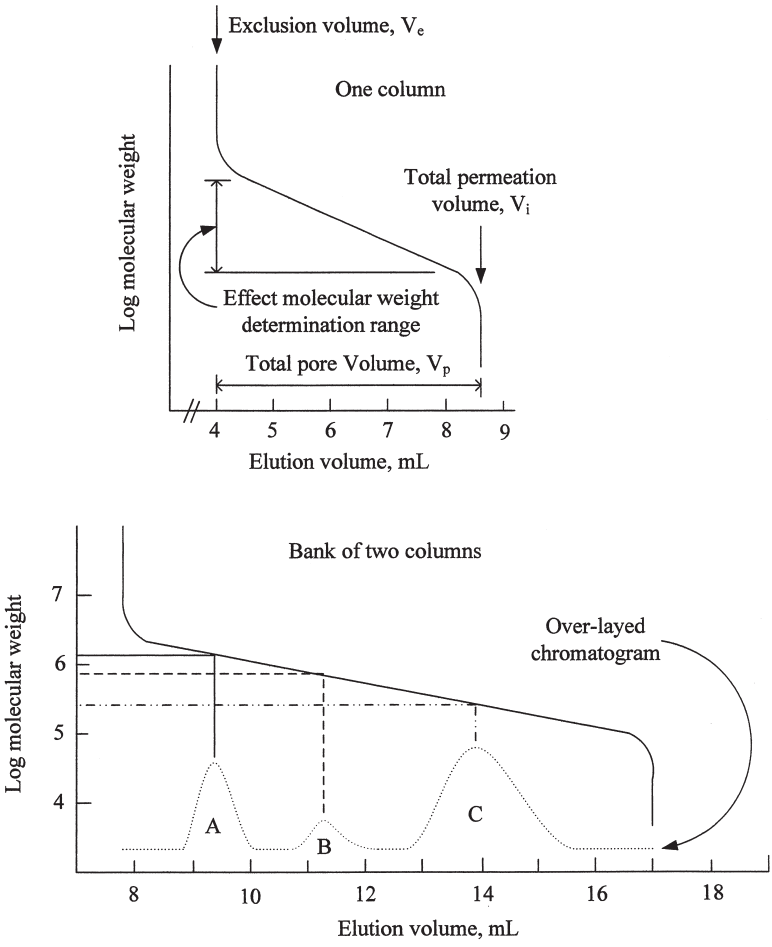
molar absorptivity, ε A parameter that appears in Beer's law ($A = \varepsilon bC$) and is a function of the degree of interaction that a specific analyte has with the incident radiation at a known wavelength in a defined solvent conditions (composition, pH, temperature, etc).

The units for ε are $L/[mol \cdot cm]$ (when b, the cell path length, is in cm, and the concentration, C, is molarity). Another term used for molar absorptivity is the molar extinction coefficient.

molarity, M A unit of measure of concentration; units are moles/liter.

molecular imprinting The process of creating stationary phases that have exact analyte negative images grafted onto them.

molecular weight calibration curve The plot of molecular weight versus retention volume is typically used to characterize the molecular weight distribution for polymeric sample. A representative plot is shown below. The calibration curve should be made with a series of standards that are comprised of the same material as that



Top: A representation of a calbration curve generated by one column. The exclusion volume, V_e, represents the volume in the system that is accessible to all analytes of a molecular weight too high to access any pore volume. No molecular weight determination can be made here. The effective molecular weight range, V_e to V_i, represents the total accessible pore volume (here 8.5 – 4 = 4.5 mL). The total permeation volume is the total volume accessible to any molecule under a certain molecular weight. Bottom: A representation of a bank of two columns that were used to generate the the top curve, in series. The total volume is double that for a single volume, but the effective molecular weight is the same. This generates more discrimination between molecular weights. An elution profile is superimposed on the calibration curve, the mean molecular weights being A $10^{6.1} = 1.2 \times 10^6$; B $10^{5.9} = 7.9 \times 10^5$; C $10^{5.4} = 2.5 \times 10^5$. Note that, unlike typical reversed-phase HPLC, the large molecules elute first because no partition or adsorption occurs, only separation based on size.

being tested. The range of molecular weights that are analyzed is determined by the total exclusion volume (in the above case compounds that have a molecular weight of 10^4 or above are too large to penetrate any pores in the packing material) to the total inclusion (or permeation) volume (at a molecular weight of 10^2 or less, all pores are accessible to the sample). It should be noted that not only the size but also the shape of the sample molecule is critical to determining the access to pores of various diameters (not just the molecular weight).

molecular weight distribution For compounds that do not have one defined molecular weight (e.g., benzene has a molecular weight of 77 g/mol), such as polymers, the molecular weight distribution defines the molecular weight range for the sample and the amount of material present in the sample for each molecular weight segment. Some of the ways polymer distributions are described are by the average molecular weight, z-average molecular weight, z+1-average molecular weight, etc.

moment Statistical moments are used to mathematically define the elution profile (or distribution) and are in essence the concentration (C) time (t) function of the profile.

Zeroth moment, peak area, A; $A = \int C dt$
First moment, retention time, t_r; $t_r = [1/A] \int Ct dt$
Second moment, variance or peak width, σ^2; $\sigma^2 = [1/A] \int Ct^2 dt - t_r^2$

monochrometer A component in a spectrophotometer that is used to produce a narrow range of radiation (e.g., a narrow spectral wavelength band) that passes through the sample. For conventional spectrophotometric detectors, the monochrometer is either a diffraction grating or a prism, is responsible for separating the source radiation into spatially separated wavelengths, and is placed before the sample cell. The exact bandwidth is determined by the placing and width of the inlet. In a photodiode array detector there is no true monochrometer because the separation of wavelengths occurs after the radiation passes through the sample. The bandwidth is determined by the angle subtended by an individual diode with respect to its position and distance from the prism.

monodisperse Defines a sample that has a very narrow distribution of a property, such as molecular weight distribution for a polymer or particle diameter for a support material.

monolithic support Monolithic supports are based on the production of a unitary porous structure formed in situ and thereby exactly conforming to the column housing.

monomeric bonded phase A monomeric bonded phase has individual moieties chemically attached to one binding site on the support surface. These supports are commonly prepared from monochloro- or monoalkylsilanes and are also referred to as brush phases. These supports are typically very reproducible from batch to batch and have good mass transfer characteristics. *See* polymeric bonded phase.

multidimensional chromatography Refers to either (1) two or more columns used in series to produce a separation (e.g., LC and GC) or (2) a separation that is run in two directions (e.g., TLC).

N

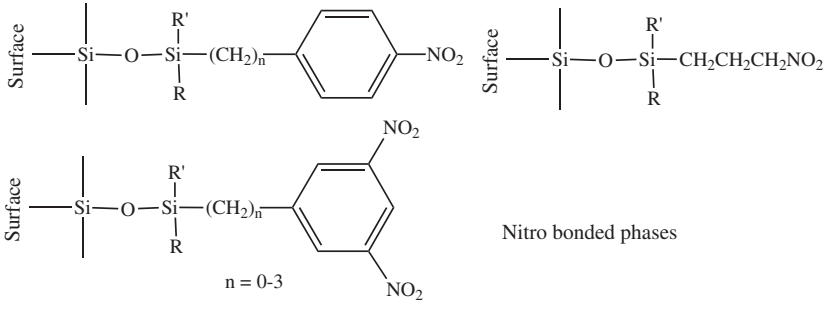
narrowbore Columns that have an inner diameter of approximately 2–4 mm are classified as narrowbore columns. The packing material used in narrowbore columns typically ranges from 2 to 5 μm in diameter and the lengths range from 3 to 10 cm. Flow rates used range from 0.3 to 1.5 mL/min.

nebulizer Used to turn a liquid flowing stream into a uniform dispersion of small droplets (i.e., aerosol) that ultimately enter the detector. Nebulizers are found at the interface of a column effluent and the detector inlet. Two types of detectors that utilize nebulizers are mass spectrometers and evaporative light scattering detectors.

net retention volume, V_N Calculated from the adjusted retention volume, V_R' and the compressibility factor, j:

$$V_N = j \times V_R'$$

nitro-bonded phase Nitro bonded phases range from alkyl- to phenyl based. The phenyl based may have one or more substituted nitro group. The advantage of this type of column is that the π-electron acceptor characteristic of the NO_2 group gives unique selectivity to highly conjugated and aromatic compounds.



Nitro bonded phases

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

nitrogen-phosphorus detector (NPD) Utilizes the ionization caused by heating a cesium- or rubidium-salted bead such that it preferentially reacts with nitrogen and phosphorus atoms. Detection limits are about 10^{-12} g and a linear range of 10^3 or 10^4 can be obtained.

noise Any signal output from the detector that is not a direct response to the analyte. There are many different sources of noise, some that are controllable or minimizable and some that are not. The most important aspect to noise is that it is the ultimate limiting factor in establishing the precision of a measurement. From a more practical viewpoint noise is the determining factor in establishing system detection limits. Noise can be experimentally determined in a number of ways.

noise, long-term Noise is noise that cycles over long times, for example, the duration of an analysis. This is not to be mistaken for drift because long-term noise is not necessarily confined to moving in one direction as drift typically is.

noise, short-term Noise that cycles or pulses over short time intervals. Short-term noise is due to electrical detector noise (e.g., Johnson flicker and shot noise) and is the ultimate limit for establishing detection limits because it overlies all other signals.

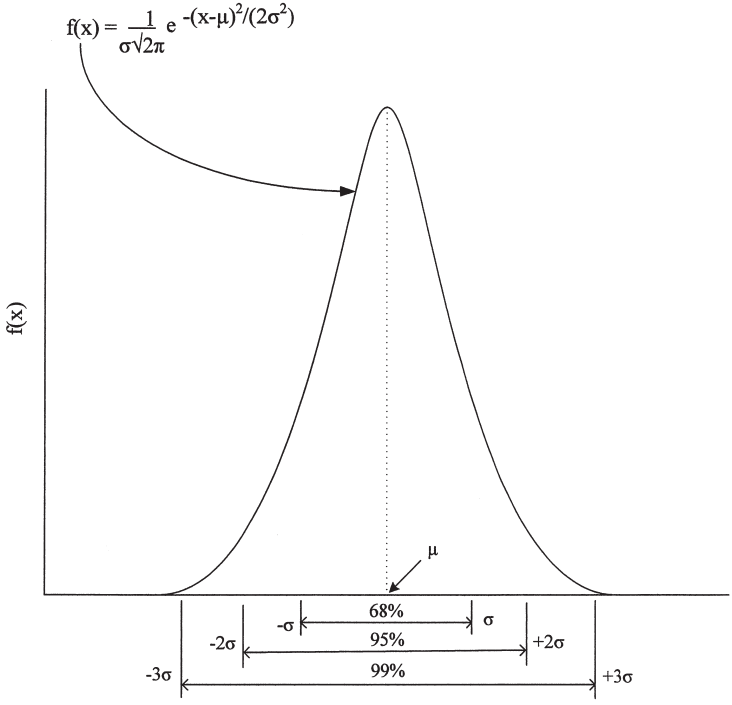
nonaqueous reversed-phase chromatography (NARP) Reversed-phase separations are typically conducted with a water/organic mobile phase. However, for analytes that are extremely hydrophobic or incompatible with water, a mobile phase that contains no water is used and the separation is termed "nonaqueous reversed phase."

nonporous supports Contain no porous character at all but may be chemically modified like other bonded phase supports. These supports have extremely low surface areas as compared with analogous porous material. Typical nonporous supports are silica- or resin based.

normal distribution A perfectly symmetric distribution of data that is mathematically described by the Gauss function:

$$f_{x,\sigma} = [1/\sigma\sqrt{2\pi}]e^{-(x-xi)2/2\sigma 2}$$

where x is the center of the distribution (mean) and σ is the width deviation of the distribution.



A normal (or Gaussian) curve is defined by the exponential function given in the picture. The profile also lists the mean value (μ), the deviation from the mean (σ), and the area under the curve for the range (+/–σ).

normality, N A unit of concentration that is defined as the gram equivalent weight of active substance per liter of solution. For example, a hydrochloric acid (HCl gram molecular weight is 36.5 g/mol) titration of sodium hydroxide has 1 equivalent H^+ ion per 1 equivalent of OH^- (the reaction stoichiometry). Therefore, a 0.1 N HCl solution contains 3.65 g/L of HCl. Alternatively, sulfuric acid (H_2SO_4 gram molecular weight of 98.1 g/mol) has 2 equivalent H^+ and so 0.1 N H_2SO_4 contains 4.91 g/L of H_2SO_4 for the same reaction with NaOH.

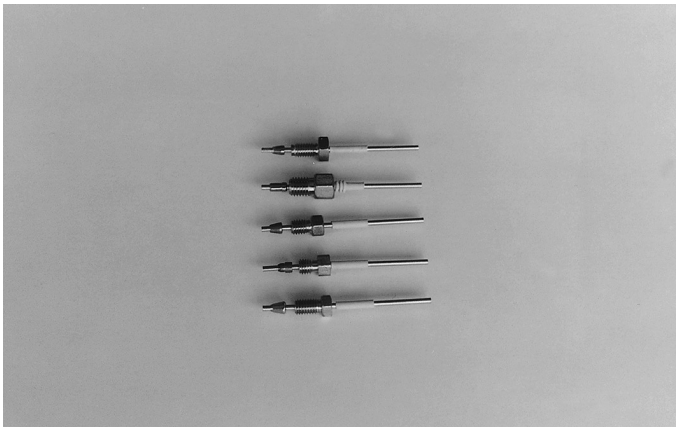
normal phase The term came about after the development of bonded phases that had a nonpolar character (e.g., octadecyl). A simple way to remember this is that the original underivatized support materials (e.g., silica, alumina) for LC had polar surfaces and nonpolar (or polar-modified nonpolar mobile phase). These then were the usual or normal-phase combinations used in a separation. Contrast that to the nonpolar bonded phase (octadecyl) and the polar hydroorganic mobile phases used to generate an elution and the term for these became reversed phase. The present-day meaning has blurred somewhat with the advent of nonaqueous reversed-phase separations, highly polar bonded phases, and other more specialized separations, but in general the terminology and the distinction still exist for many mainstream separations.

number-average molecular weight, M_n Determined from the elution profile generated in an SEC separation and mathematically determined by:

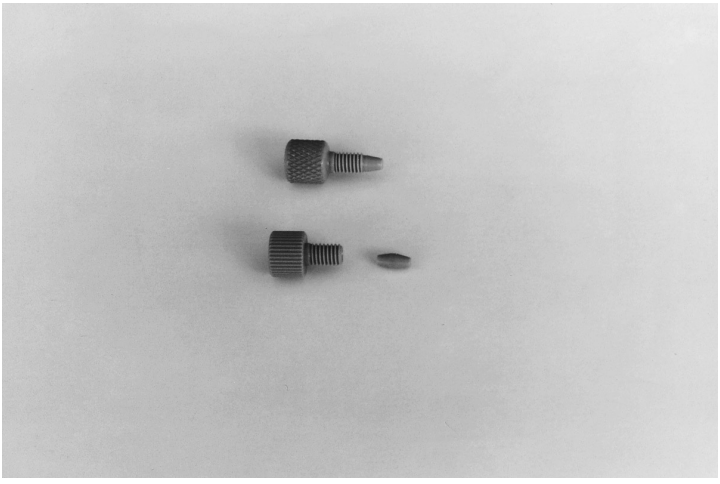
$$\begin{aligned} M_n &= (n_1M_1 + n_2M_2 + \ldots + n_iM_i)/(n_1 + n_2 + \ldots + n_i) \\ &= \sum(n_iM_i)/\sum n_i \end{aligned}$$

where n_i represents the number of molecules having molecular weight M_i. Summation occurs over all possible M_i molecular weights in the sample.

nut A component, typically threaded, that along with the compression fitting and deformable ferrule, creates a leak-tight union around tubing (e.g., connecting, column, etc).



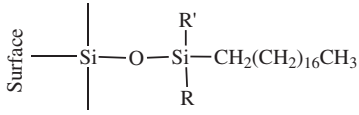
The photograph shows various nut-ferrule assemblies that have been set into the same-make union. Note the difference in the length and pitch of the nut threads (this makes nuts from one manufacturer incompatible with others). Also note the distance that the tubing extends from the ferrule is different. Obviously, a nut-ferrule couple fourth from the top will not seal well in a union design for a nut-ferrule set shown second from the top (different tubing set distances, different ferrule lengths, and different threading of the nut).



There are numerous nut-ferrule combinations. Pictured here are one-piece and two-piece assemblies.

O

octadecyl-bonded phase, ODS, C18 An octadecyl bonded phase has a C18 functional group covalently bonded to the surface of the support material.

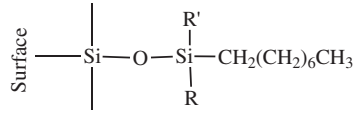


Octadecyl bonded phase

The side groups R and R′ are commonly methyl groups but can also be more bulky alkanes (e.g., isopropyl). The identification of these groups is often omitted in the description of the bonded phase, hence, octadecyl-bonded phase and not dimethyloctadecyl-bonded phase. In other instances, the R groups can be reactive functional groups such as —OH. These groups are used as points from which to make polymerized supports or are unmodified to add polarity to the surface.

octanol-water partition coefficient, $P_{o/w}$ Used to estimate the hydrophobicity of compounds. The importance of this parameter is that it is an accepted regulatory estimate of the tendency of an organic compound to bioaccumulate in living cells. Reversed-phase chromatography has been used as a rapid alternative method for determining $P_{o/w}$.

octyl-bonded phase, C8 An octyl bonded phase has a C8 functional group covalently bonded to the surface of the support material.



Octyl bonded phase

For discussion of the R groups, *see* octadecyl-bonded phase.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

Offord's equation Relates the electrophoretic mobility of molecules, μ_{em}, to their net charge, Z, and their molar mass (g/mol):

$$\mu_{em} = Z/[M^{3/2}]$$

on-column detection With on-column detection, the detector is positioned such that the analyte is detected while it is within the packed bed of the column. This typically requires the construction of a special "window" along the column wall or insertion of a probe. The advantage is that a considerable amount of band broadening can be eliminated in this fashion.

optimization The process of changing operating parameters in s system in order to fulfill all pre-established separation requirements (e.g., run time, resolution, peak shape). Typical requirements for a separation include one or more of the following: run time, resolution, peak shape, sensitivity, selectivity, specificity, linearity and linear range, precision and accuracy, ruggedness, robustness, etc.

overload An overload situation occurs when the concentration of the analyte (of sample) exceeds the capacity of the column or the linear range of the detector. Column overload refers to the condition in which the concentration of the analyte exceeds the capacity of the column precluding the rapid and reversible transfer of the analyte from the mobile phase to the stationary phase. Peak shape is adversely affected and often results in pronounced fronting. In a detector, overload occurs when there is no further change in response (detector output) for an increase in analyte concentration.

over-pressured layer chromatography (OPLC) A thin-layer technique in which the sorbent layer is firmly pressed against a cover membrane and solvent is forced through the sorbent layer. This technique is like HPC that the solvent is "pumped" through the sorbent, but it is still planar chromatography. This technique has the advantage over HPLC that viscous solvents can be used and over TLC that the development of the chromatogram is faster.

oxidation The process by which a compound (or atom) loses an electron.

P

packing In LC columns, packed-bed GC columns, and many sample preparation components (e.g., SPE), the process of placing the packing material into the column. Dry packing is commonly used in packed-bed GC columns and sample preparation components, whereas slurry packing is used for HPLC columns.

packing material The material contained within LC, packed-bed GC, and sample preparation components. Packing material is also called support material, packing, and stationary phase. Packing materials vary in particle size and shape, chemical identity, base material, pore size, and pore volume.

paired-ion chromatography (PIC) An LC technique used to separate charged or chargeable analytes on a reversed-phase support by creating an associated molecule through the addition of oppositely charged ions to the mobile phase. The resulting complex is overall charge neutral and has a much longer retention than the charged species. Quaternary amines are frequently used for anionic analytes and sulfonic acids for cationic analytes. *See* ion-pair reagent.

paired-ion reagents *See* ion-pair reagent.

particle diameter, d_p Often the average diameter obtained from the distribution of the particle sizes in a given production batch of material. Although the average diameter of the packing material is important, the range of sizes is also critical to the overall efficiency of a column manufactured from this material and cannot be determined from the particle diameter information alone.

partition chromatography An LC technique that utilizes a liquid as a stationary phase, typically adsorbed to the support surface. This is difficult to do because the stationary phase bleeds off the column over time, resulting in an ever-changing amount of stationary phase present in the column. Current theory also places bonded phases in

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

this category. It should be noted that the retention mechanism for partition chromatography is different from adsorption chromatography in that adsorption chromatography is exclusively surface mediated and partition chromatography models an analyte partially or completely entering the stationary phase. For partition-based separations the C term in the van Deemter equation (mass transfer component) is very important.

partition coefficient *See* distribution coefficient, D.

parts per billion, ppb A unit of concentration expressed as ng analyte/g sample or g analyte/10^9 g sample, etc.

parts per million, ppm A unit of concentration expressed as μg analyte/g sample or g analyte/10^6 g sample, etc.

pascal, Pa A unit of measure, in newtons/square meter, used for backpressure displays in some chromatographic systems. 1 MPa is approximately 10 atm or 150 psi.

path length, b The distance that the source radiation travels through the sample. Path length is an explicit variable in Beer's law: $A = \varepsilon bC$. The direct impact is that absorbance is directly proportional to path length.

peak area The total area under the peak elution profile, above the baseline, and between the integration start and stop markers. *See* integration.

peak broadening The result of all dispersion processes present in the chromatographic system that increase the width of a peak as it moves through the system. The extent of peak broadening is represented as the sum of the variances (σ^2) of all independent contributors:

$$\sigma^2_{tot} = \sigma^2_{inj} + \sigma^2_{col} + \sigma^2_{det} + \sigma^2_{ct}$$

where σ^2_{tot} is the total system variance, σ^2_{inj} is due to the injector, σ^2_{col} is due to the column, σ^2_{det} is due to the detector, and σ^2_{ct} is due to connecting tubing and unions.

peak capacity, n The maximum number of peaks that can be adequately resolved (defined by analyst) within a defined time frame is the peak capacity of the system. When the resolution is taken as unity (R = 1) the estimation for peak capacity becomes:

$$n \approx 1 + 0.25[N^{1/2} \times \ln(1 + k'_i)]$$

where N is the number of plates for the ith peak and k'_i is the capacity factor for the ith peak. Conceptually the peak capacity can be seen to increase with increasing system efficiency (N, theoretical plates) because higher N leads to comparable narrower peak widths.

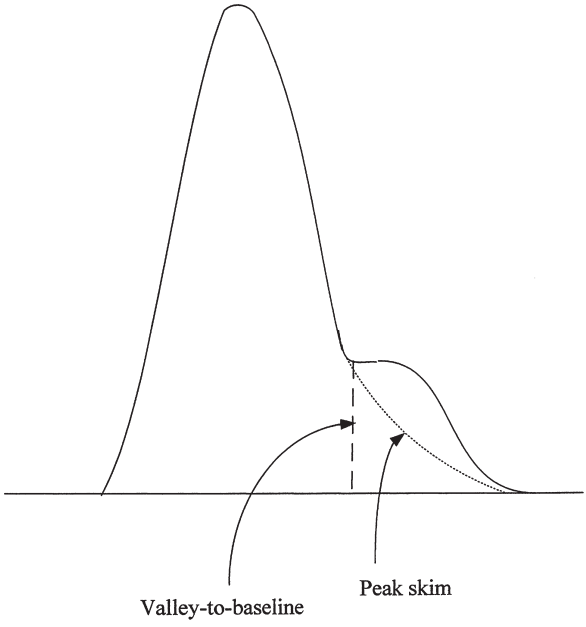
peak height, h_p The distance, in arbitrary response units (R), of a peak from its maximum response to the baseline. For a perfectly nonsloping baseline the line connecting the peak maximum and the baseline forms a 90° angle to the baseline. For sloping baselines, the connecting line is drawn between the peak maximum and an extended line from the nonsloping part of the baseline. *See* Gaussian curve.

peak maximum Occurs when the detector response attains its greatest response for a unique analyte. The retention time is generated from the peak maximum. A peak "maximum" may also be the most negative response for an analyte if the detector response can be positive or negative as in the case of a refractive index detector. *See* peak height, h_p.

peak purity A mathematical comparison of elution spectra between a known and a sample. When normalized spectra and overlap perfectly, then the sample and standard are concluded to be the same. If not, then multiple points along the spectra are compared and a purity level determined therefrom.

peak shape The elution profile generated by the detector once the analyte has passed through the chromatographic system. Statistical moments and other parameters (asymmetry, width, etc.) are also used to quantitatively describe the peak shape.

peak skimming A technique used for deconvolving peaks that are incompletely resolved.



When there is overlap between peaks a number of techniques are used to estimate the separate areas of the peaks. Peak skimming uses an exponential curve from the largest peak through the smaller peak. A valley-to-baseline method drops a line that is perpendicular to the baseline from the valley between the two peaks.

peak variance, σ^2 A direct statistical measure of peak width (i.e., second moment) for Gaussian peaks.

peak width, *w* A measure of how long it takes for the analyte profile to pass through the detector. *See* Gaussian curve. The peak width is an important parameter because it is used to determine column efficiency (theoretical plates, N), resolution (R_s), etc. For nonsymmetrical peaks, the peak width is determined by direct measurement.

PEEK Polyetheretherketone. PEEK is a polymer that has become frequently used in systems where stainless steel causes problems (e.g., metal ion analysis and some protein/peptide analyses). Connecting tubing, injector loops, and even column tubing are now fabricated from PEEK.

pellicular Defines a particle that has a solid nonporous core and a thin porous coating atop the core. Pellicular materials are ideal for use in precolumns or in separations that do not require high overall efficiencies.

pentane Molecular weight: 72.2; boiling point: 36.1°C; refractive index (20°C): 1.3575; density (20°C): 0.63 g/mL; viscosity (20°C): 0.23 cP; UV cutoff: 190 nm; eluotropic strength (ε^o): on alumina—0.0, on silica—0.0; polarity index (P′): 0.0; solubility in water (20°C): 0.04%; water solubility in pentane (20°C): 0.009%, Extremely volatile and flammable. Pentane is rarely used in HPLC or TLC because of its very high volatility. It is more commonly used as a sample solvent of GC.

$$CH_3CH_2CH_2CH_2CH_3$$

Pentane

perfluorotributylamine (PFTBA) A compound commonly used in the calibration and tuning of a quadrapole MS detector coupled to a GC.

$$(CF_3CF_2CF_2CF_2)_3N$$

PFTBA

perfusion chromatography An LC technique that is used for the separation of macromolecules. It uses support material that has very large pores (≥300 Å) so that the macromolecules can access them.

permeation The ability of an analyte to enter/exit pores in the packing material; implies that there is no interaction of the analyte with the packing material surface. Permeation is the primary "retention" mechanism that is sought in size exclusion separations.

permeability, B_o A measure of the resistance a column generates to the flow of mobile phase through it and is mathematically described as:

$$B_o = \left[d_p{}^2 \varepsilon^3\right] / 180[1-\varepsilon]^2$$

where ε is the porosity and d_p is the particle diameter.

petroleum ethers Alkyl hydrocarbons produced by taking defined boiling range fractions from crude oil distillates. It should be noted here that "ether" does not refer to the chemical class of compounds, R—O—R′, but rather to the high volatility of many of the petroleum ether fractions. These fractions are defined by a boiling range, a typical range used in GC work being 30–60°C. In this range the fraction consists of mainly pentanes (boiling point ~35°C) and hexanes (boiling point ~70°C). Fractions are also prepared over narrower ranges and continue up to about 200°C.

pH The pH scale is a logarithmic measure of the hydronium ion, H^+, concentration in a solution:

$$pH = -\log[H^+]$$

In practice a common error made in the preparation of organic/aqueous buffer solutions is that the pH is "measured" after mixing the solvents rather than obtained in the aqueous phase alone (before mixing). Keep in mind that glass pH electrodes are designed to respond to hydronium ion concentration in water; the hydration layer in the electrode is adversely and irreproducibly altered by the presence of the organic modifier. Best practice is to prepare the buffer in the aqueous phase (at a concentration high enough to compensate for the dilution with the organic solvent) and then mix with the organic solvent.

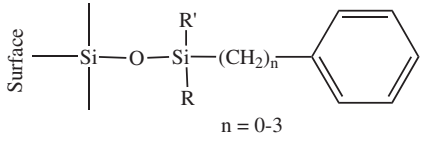
phase A part of a system that is physically distinct and bounded, that is, forms a definable interface with one or more other parts (phases) of the system. Chromatographic systems utilize phases to effect separation, being liquid-liquid (e.g., countercurrent chromatography and liquid-liquid extraction), liquid-solid (e.g., HPLC and TLC), and liquid-gas (e.g., GC and distillations).

phase ratio, β The volume of the mobile phase, V_M, with respect to the volume of the stationary phase, V_S:

$$\beta = V_M/V_S$$

For capillary GC open-tubular columns, $\beta = (r - 2d_f)/2d_f$, where r is the column radius and d_f is the thickness of the film (i.e., the stationary phase).

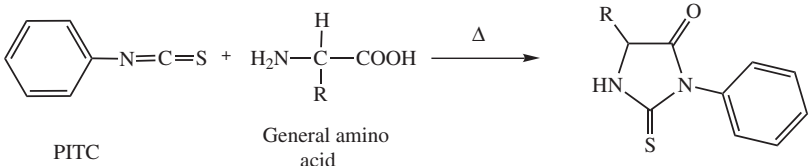
phenyl-bonded phase A phenyl-bonded phase has a phenyl functional group covalently bonded to the surface of the support material.



Phenyl bonded phase

For discussion of the R groups, *see* octadecyl-bonded phase.

phenylisothiocyanate (PITC) Best known as a derivatizing reagent for amino acids:



PITC

General amino acid

PITC reaction

but can be used for compounds with 1° amine functional groups. Subsequent analysis is typically carried out in the UV region (e.g., 254 nm).

phenylthiohydantoin (PTH) The chemical class of compounds that result from the reaction of phenylisothiocyanate (PITC) with 1° amines. *See* PITC (phenylisothiocyanate).

photoionization detector (PID) Uses a high-energy photon source to ionize the analyte, A. The reaction is:

$$A + h\nu \rightarrow A^+ + e^-$$

where $h\nu$ is the photon and e^- is the released electron. This reaction produces a current that is related to the analyte concentration. The detector offers selectivity in that only analytes containing easily photoionized functional groups such as aromatic hydrocarbons and some organosulfur compounds produce a response. Only a small fraction of the analyte molecules are ionized, and so for quantitative analyses the detection must be done under conditions that generate reproducible results.

phosphoric acid Molecular weight: 98.0. The following are for 85% solution in water: density (20°C): 1.88 g/mL; miscible with water.

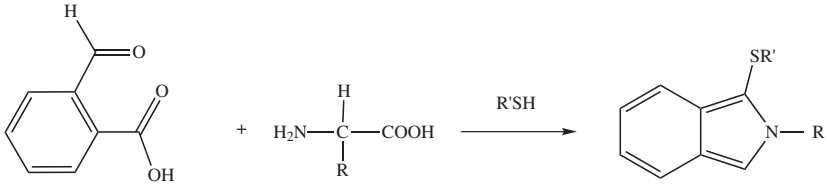
H_3PO_4

Phosphoric acid

The three pK_a values are: pK_1: 2.1, pK_2: 7.2, pK_3: 12.3. Phosphoric acid and its alkali salts (e.g., sodium and potassium) are frequently used in LC separations as solution buffers. Note, however, that the solubility of phosphate salts in acetonitrile is limited.

photodiode-array detector (PDA) A PDA detector uses a deuterium lamp for the UV spectrum and a tungsten lamp for the visible spectrum. The PDA detector differs from the single-wavelength detector in that all the source light passes through the sample. Discrete wavelengths are then generated by a dispersive element (e.g., prism) that is placed between the sample cell and a series of detector diodes. Each diode subtends a fixed wavelength range depending on its distance from the element, the angular position from the element, and the dispersive power of the element.

***o*-phthalaldehyde (OPA)** A derivatizing reagent used to react with 1° amines: OPA is commonly used for the derivatization and analysis of amino acids and other primary amine compounds. Fluorescence detection is done with excitation at 240 nm and emission at 455 nm.

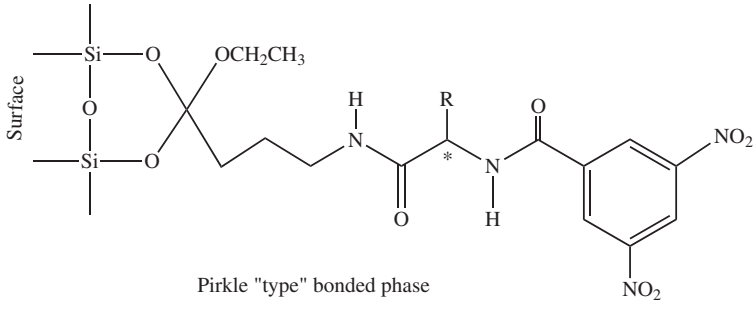


OPA

General amino acid

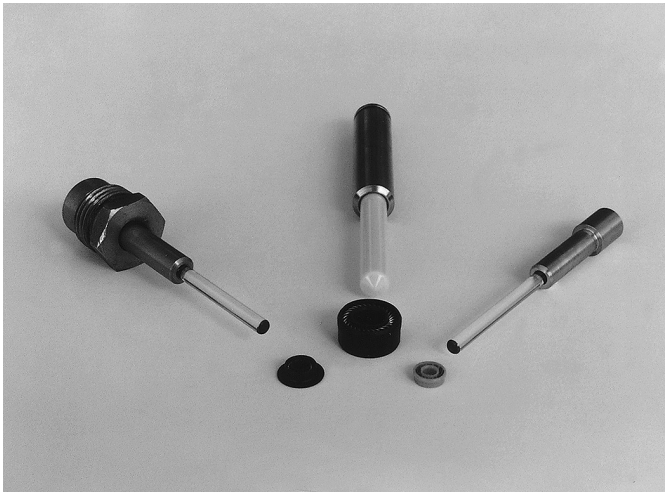
pinkerton column *See* ISRP (internal surface reversed phase).

Pirkle column Named for its inventor, the original Pirkle column was a monomeric bonded phase that had the following structure: This bonded phase and many others having similar construction are chiral in nature (*denotes the chiral center) and are used in the separation of enantiomers.



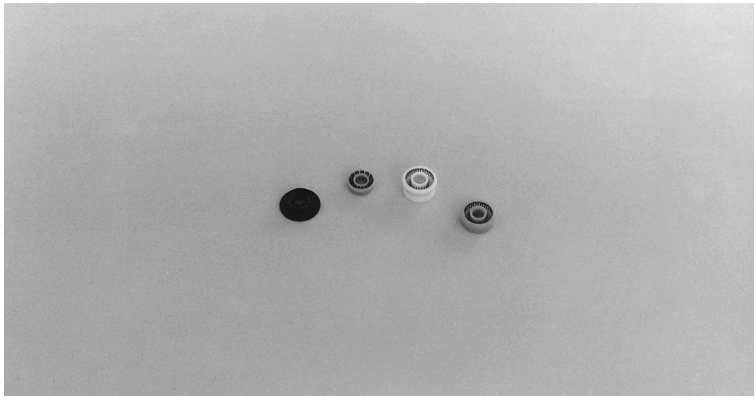
Pirkle "type" bonded phase

piston A finely machined component of an HPLC pump that moves into and out from the piston head. Its movement forces mobile phase flow through the system. Many pistons used in analytical LCs are constructed from sapphire. For semipreparative and preparative units stainless steel and ceramic pistons are more common.



Three different piston-piston seal combinations are shown above. The left and right sets are for an analytical HPLC system; the middle is from a column-packing pump. Note that the larger diameter of the center pump will allow for faster flow rates (displaces more volume per piston stroke).

piston seal A component that forms a high-pressure leakproof seal between the pump head and the piston. The piston seal is often manufactured from chemical-resistant and compressible polymers. These have an imbedded coil that applies pressure to the internal (piston) and external surface (pump head).

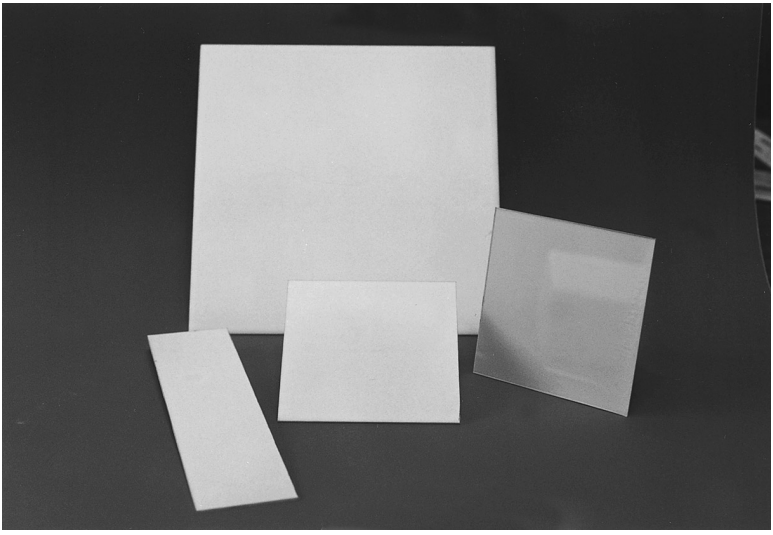


Four different configurations of piston seals are shown here. They represent different manufacturer systems. Note that the materials of construction vary as well.

placebo *See* matrix blank.

planar chromatography A separation that is developed on a support material that is essentially two dimensional, for example, thin-layer chromatography. Also see TLC

plate (1) The component of a thin-layer chromatography product that provides support for the sorbent material. The support material is typically made from glass, plastic, or, less commonly, aluminum. The types of phases used in TLC are the same as those used in HPLC, but the particle size for TLC is typically larger. (2) *See* theoretical plate, N.

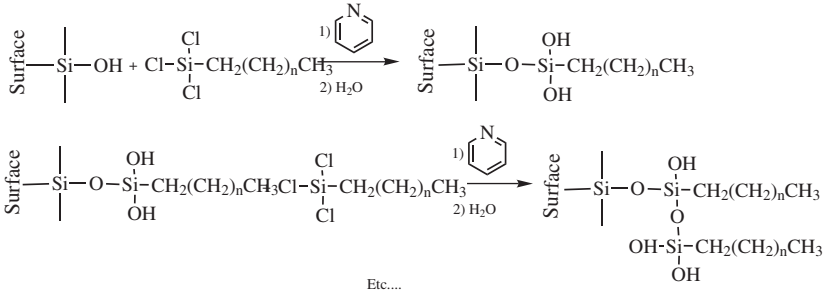


Three different sized thin-layer plates are shown: lower left 5 × 20 cm, center 10 × 10 cm, and center top 20 × 20 cm. The sorbent is silica in each case. The rightmost plate is reversed to show the glass support.

plate theory Originally developed by Martin and Singh to provide a mathematical description of a chromatographic elution curve. Many others have modified and added to this basic theory through the years.

polyacrylamide Produced from the copolymerization of acrylamide and *N*,*N*′-methlenebisacrylamide. The resulting copolymer is a porous material that is used as an aqueous size-exclusion chromatographic support.

polymeric bonded phase A polymeric bonded phase has multiple attachments through a single bonding site on the surface. This is accomplished by using multifunctional silanes (e.g., trichloro- or trialkylsilanes). The polymer is built out from the surface as follows: The advantage of the polymeric phase over monomeric phases is that the polymeric phase offers a higher level of hydrophobicity and a lower number of accessible silanol groups. The disadvantages are the greater mass transfer effect on peak broadening (C term in van Deemter equation) and the overall poorer reproducibility between batches.



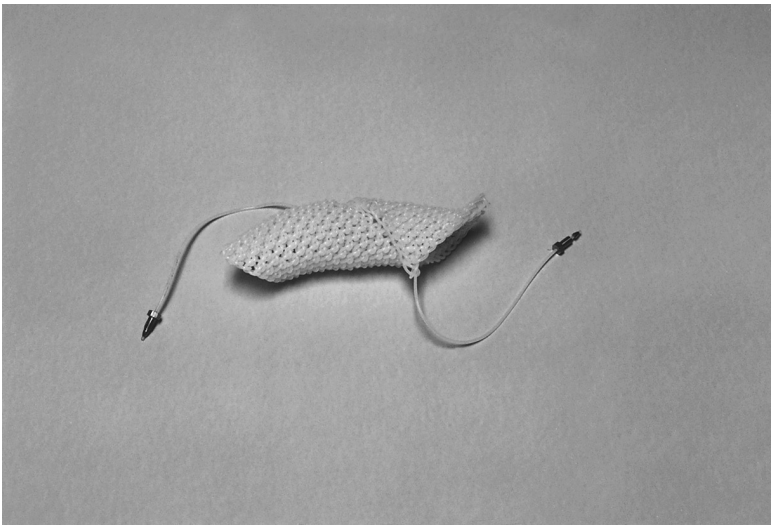
Polymeric bonded phase

polydispersity, M_w/M_n The polydispersity for a sample is a measure of how tight the polymeric distribution is around the avearage molecular weight M_n. A polymer is monodisperse when each molecule has the identical molecular weight M_w, or $M_w = M_n$ and $M_w/M_n = 1.0$.

polystyrene-divinylbenzene (PS-DVB) A versatile support that is used for gel permeation and reversed-phase separations and as an ion-exchange material when ionic functional groups are bonded to its surface.

pore volume, V_i The pore volume of a packing material is the volume internal to the particles and is expressed in milliliters/gram of material. This value is frequently determined by mercury porosimetry or BET adsorption isotherm. Not surprisingly, pore volume is directly proportional to pore size.

post-column reactor (PCR) Used to change the properties of an analyte such that specificity is obtained and/or sensitivity and detection limits are improved. PCRs are placed in the flow path after the column, and the reagent is pumped into the system through a tee. The flow rate must be matched to the system flow rate and the reaction time must be long enough to make sure that reaction is complete. In many cases a reactor coil (heated or illuminated) must be added to ensure that the reaction takes place. This leads to substantial broadening of the analyte peak.



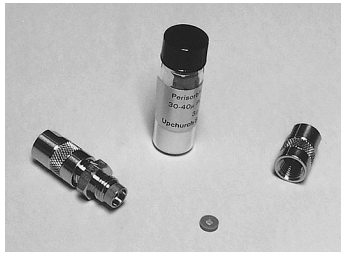
Reactor coils such as this are used to (1) give a long enough time to allow for a chemical reaction to go to completion before reaching the detector and/or (2) be placed in a heating bath or irradiation chamber to initiate or increase the reaction rate.

pounds per square inch, psi A unit of measure for backpressure in a chromatographic system, where 14.5 psi = 1 atm = 760 torr.

pre-adsorbent A thick 2 to 4 cm band of material running along the bottom of a thin-layer chromatography plate. It is made from an inactive material. The pre-adsorbent therefore allows for higher sample loading with no chromatography occurring before the active sorbent phase. An added benefit is that as the sample moves from the pre-adsorbent layer to the active sorbent the sample band is sharpened, resulting in a more efficient separation.

precision A measure of how reproducibly a method generates the same result for replicate determinations. The overall precision of a method consists of the precision of the sample preparation step, the analysis step, and the data processing step.

precolumn Generally placed between the outlet check valve of the pump and the pressure transducer. It has the same bonded phase identity as the analytical column. It is used to protect the analytical column



An HPLC precolumn is used as a sacrificial column when harsh/reactive mobile phases are used. The packing material should match the identity of the analytical column but can be made from much less expensive large-particle material (30–40 μm). The column is dry packed and flushed with mobile phase to remove air before attaching to the system.

from particularly aggressive solvents (e.g, high and low pH, etc). The premise of the precolumn is that it acts as a "sacrificial" column. The aggressive solvent dissolves the material in the precolumn, thereby becoming saturated with the bonded phase, hence the reason for the bonded phase needing to match the analytical column phase. It should be noted that the particle size is often much larger for the precolumn because system efficiency is unaffected (it is placed before the injector). The 40- to 60-μm particles found in many solid-phase extraction columns are acceptable. Precolumns are typically dry-packed and then equilibrated with mobile phase off-line.

preparative (HPLC) Columns that have an ID >2 cm and lengths >25 cm are used to process large samples masses and are termed preparative columns. For preparative columns the packing is usually ≥40 μm, flow rates are >10 mL/min, and injection volumes are >0.1 mL.

preparative thin-layer chromatography (TLC) To increase the sample load capacity over a normal TLC plate, a preparative TLC plate has a a thicker sorbent layer (500–2000 μm vs. ~250 μm). Larger plates, height and width, may also be used. In preparative TLC the bands are identified and the sorbent containing the band is scraped from the plate. The analyte is then desorbed from the sorbent by placing the sorbent is a very strong solvent. The solvent is removed or exchanged for a long-term storage solvent.

pressure Pressure in chromatographic systems is reflective of the resistance that the system (in particular the column) has to forced mobile-phase flow through it. An operational diagnostic for the proper

functioning of a chromatographic system is often indicated through the pressure generated at the head of the column. This is typically referred to as head pressure (GC) or backpressure (LC). The pressure is determined through the use of a pressure transducer situated after the pump and before the injector. Common units of measure for pressure are atmosphere (atm), bar, pounds per square inch (psi), or Pascals (Pa).

1-propanol *See* *n*-propyl alcohol.

2-propanol *See* isopropyl alcohol.

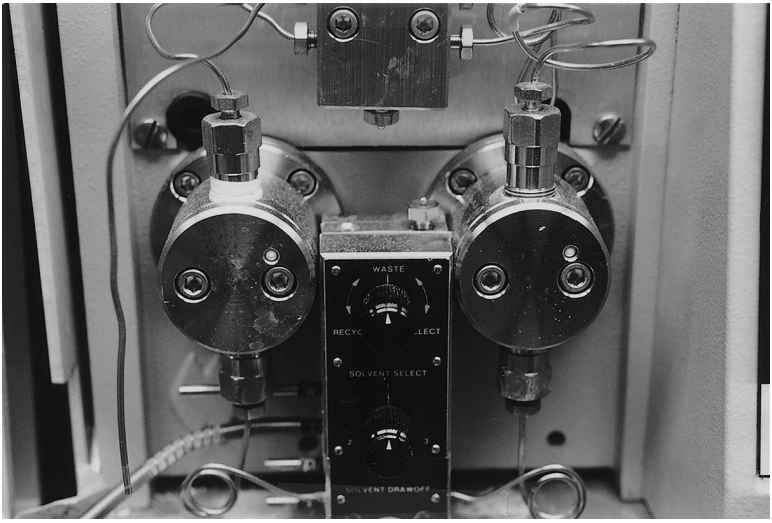
***n*-propyl alcohol** Molecular weight: 60.1; boiling point: 97.2°C; refractive index (20°C): 1.3856; density (20°C): 0.80 g/mL; viscosity (20°C): 2.3 cP; UV cutoff: 210 nm; eluotropic strength (ε^o): on alumina—0.82; polarity index (P′): 4.3; Hildebrand solubility parameter (δ): 12.0; solubility in water (20°C): 0.04%; water solubility in *n*-propyl alcohol (20°C): 0.009%. Extremely volatile and flammable.

$CH_3CH_2CH_2OH$

n-Propyl alcohol

polytetrafluoroethylene (PTFE) A polymer of $(CF_2CF_2)_n$ that has high chemical resistivity, low adhesiveness, elasticity, and a wide useful temperature range. These characteristics make this material ideal for routine low-pressure work in HPLC. The patented trademark is Teflon®. Note that PTFE is permeable to oxygen and that this could be a problem when using an electrochemical detector (depending on the operating applied potential) or in very low UV work (<210 nm).

pump A component of an LC system that generates the mobile-phase profile and maintains (i.e., isocratic) or reproducibly alters (i.e., gradient) it throughout the course of a separation. Through its design a pump ensures that solvent flow direction and rate are maintained as invariant as possible. Pumps vary in delivery rate ranges from 0.01 mL/min for microbore work to 5–10 mL/min for GPC/SEC work to >10 mL/min for prep work. Pumps can be single piston (isocratic work) or dual piston (or single piston and compensating piston for gradient work).



Shown is an HPLC dual piston pump. Note the two pump heads that house separate pistons. The purpose of the dual piston system is to (1) allow for gradient generation and (2) produce a low-pulse flow.

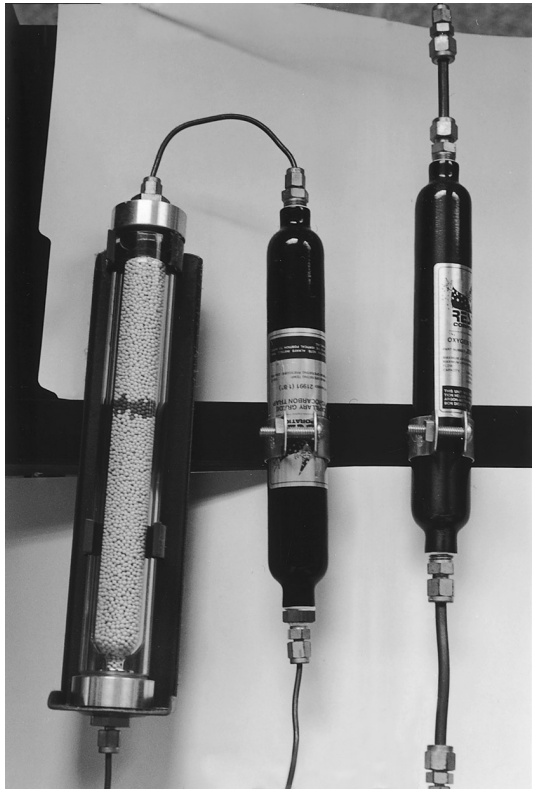
pump head A finely machined block (often 316 stainless steel) that consists of a threaded port for setting inlet and outlet check valves, the interior chamber that "stages" mobile phase, a bored hole that allows for passage of the piston into and out from the piston head, and a recessed area for the piston seal. *See* pump.

purge and trap, P & T A dynamic gas-liquid extraction technique that is used before analysis by GC. A sample is extracted by continuously passing a constant flow gas through it. The volatile components are purged from the sample and then are trapped on a strong adsorbent material. Adsorption is often enhanced by cooling the trapping adsorbent. Once the sample is purged, then the trap is rapidly heated and the effluent is directed onto the GC column where separation takes place. The P & T method allows for extremely low level detection (low parts per billion to high part per trillion) and is frequently the method of choice for environmental volatile analysis.

purifier Tank or cylinder gases can often be the source of a wide range of impurities: oils, carbon dioxide, oxygen, water. To remove

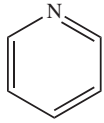
these impurities gas purifier (or scrubber) columns are placed in line after the gas source and before entering the instrument.

Note that the purifiers should be mounted vertically so that as the column material settles no voids are created.



These three purifiers are placed after the gas supply tank to remove oxygen, hydrocarbons, and water (right to left). Inlet to this manifold from the gas tank is at the right.

pyridine Molecular weight: 79.1; boiling point: 115.3°C; refractive index (20°C): 1.5102; density (20°C): 0.98 g/mL; viscosity (20°C): 0.95 cP; UV cutoff: 330 nm; eluotropic strength (ε^o): on alumina—0.71; polarity index (P′): 5.3; Hildebrand solubility parameter (δ): 10.7. Miscible with water. Flammable liquid with a disagreeable odor.



Pyridine

pyrogram A program that is the resulting chromatogram generated by a pyrolysate (a compound undergoing pyrolysis).

pyrolysis The controlled thermal decomposition process that is used for the characterization of nonvolatile polymers and other high-molecular-weight materials.

pyrolysis gas chromatography A GC technique that analyzes the breakdown compounds generated by a pyrolysis event.

pyrolyzate The product of the pyrolysis process.

Q

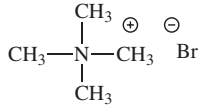
quadrupole mass analyzer A quadrupole is constructed from four hyperbolic rods set at precisely determined right angles to one another. A DC voltage (+ or −) is applied to alternating rods and rapidly alternated. A radio frequency (rf) is applied along the rods as well. By adjusting the DC voltage and the rf signal, various mass-to-charge ratios (m/z) are passed through the quadrupole and strike the detector.

quantum efficiency, φ_f A measure of the fraction of excited molecules that will fluoresce:

$$\varphi_f = k_f/(k_f + k_i)$$

where k_f is the rate constant for the fluorescence process and k_i represents the rate constant for all competing radiative pathways.

quaternary alkyl amine A quaternary amine has the following general structure:



Tetramethylammonium bromid
quaternary amine

The methyl form (R = R′ = R″ = methyl) is grafted to a support and is a common form for strong anion exchange sites. Hydroxide salts of free compounds, for example, tetrabutylammonium hydroxide, are used as ion-pair reagents.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

R

racemate Equal parts of the (+) and (–) forms of enantiomers result in a racemic mixture. These mixtures do not polarize light as a pure enantiomer would.

radial compression The technique of radial compression is used on columns that are fabricated with flexible walls. Application of the radial pressure minimizes wall effects during the separation and thereby decreases peak broadening and increases overall operational efficiency.

radial thin-layer chromatography A TLC technique that spots a sample in the center of a circular plate. Developing solvent is applied to the spot through a wick. The sample is carried out radially from the center spot as the solvent moves away from the wick.

radioactivity detector Used in HPLC to monitor radioisotopically labeled analytes (e.g., ^{3}H, ^{131}I, ^{13}C). The decay mechanism of the atom determines the type of detector that is used. For example, β-decay atoms can be detected directly through β-radiation or indirectly through scintillation derived from the disintegration event. The cells for this type of detector are typically quite large (or fractions need to be collected), and so the overall efficiency of the system is low.

Raman scatter The scatter/emission of exciting wavelengths at fixed energy differences. This scatter produces background signal for fluorescence analyses.

random errors Those uncertainties that are part of a result because of sampling and testing variability.

range The upper and lower limits for analyte concentration for which a detector gives an acceptable response. The range is determined by the operational requirements set by the analyst for the method; it is always equal to or greater than the working range.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

Raoult's law Relates the partial gas pressure at a given temperature for component A in a liquid, p_A, as a function of its mole fraction in the liquid, x_A, and its pressure above its pure liquid form at that temperature, P_A:

$$p_A = x_A P_A$$

This is based on ideal gas behavior.

Rayleigh scattering In Rayleigh scattering, the incident and emitted photons are the same. This creates a nonzero background for fluorescence detectors.

reciprocation The process of the piston moving into and out from the piston head. Many HPLCs operate with a spring-loaded piston in contact with an eccentric cam. As the cam rotates, the piston is forced into the piston head at a fixed rate. The flow rate is then a function of the volume displacement and the rate of reciprocations/minute.

recovery The recovery of an analyte is calculated as the amount of material reaching the detector as compared with the theoretically expected amount and is usually expressed as a percentage:

$$\text{recovery} = \{[\text{amount detected}]/[\text{amount expected}]\} \times 100$$

High recovery is a requirement for most method validations.

recycle The process of returning solvent from the effluent path to the reservoir. Solvent consumption is a significant ongoing cost of LC analyses. To conserve solvent, the detector output is used as a trigger to start/stop the detector effluent into the reservoir. The triggers are at the beginning and ending of a peak. For recycling to be effective, the system must:

(1) be isocratic (i.e., the effluent composition matches the reservoir composition) and
(2) not introduce contamination or gas into the mobile phase.

reduced plate height, *h* Calculated from the height equivalent to a theoretical plate, H, and the particle diameter, d_p as follows:

$$h = H/d_p$$

This parameter is used to compare the relative column efficiencies for columns having packing materials with different particle size.

reduced pressure, P_r In supercritical fluids the ratio of the system pressure, P, to the fluid's critical pressure, P_c:

$$P_r = P/P_c$$

reduced temperature, T_r In supercritical fluids the ratio of the system temperature, T, to the fluid's critical temperature, T_c, is the reduced temperature:

$$T_r = T/T_c$$

reduced velocity, ν Calculated from the linear velocity, u, the diffusion coefficient of the analyte, D, and the packing material particle diameter, d_p:

$$\nu = ud_p/D$$

reduced linear velocity, v The rate at which an analyte moves one particle diameter along a column due to flow with respect to the rate due to diffusion. Mathematically reduced linear velocity is expressed as:

$$v = u \cdot d_p/D_m$$

where u is the linear flow velocity (distance/time), d_p is the particle diameter (distance), and D_m is the diffusion coefficient (distance^2/time) of the analyte in the mobile phase. Note that ν is unitless and is the primary variable in the Knox equation, which is used to determine the reduced plate height for a system (as a function of changing ν).

reduction The process of a compound (or atom) gaining electrons.

re-equilibration For any analysis that changes elution conditions during the course of a separation, the system must return to the fully equilibrated initial conditions. This process is called re-equilibration. The time needed to reach initial conditions is called the re-equilibration time and can be quite lengthy. For example, it may take 10–15 min for a GC column temperature to return to the starting temperature, and for LC it may require similar lengths of time to reach the initial mobile phase composition and reestablish column equilibrium with the mobile phase.

reflux A distillation process whereby volatilized portions of the sample are returned to the sample in order to recycle and produce higher-purity distillate.

refractive index, n The refractive index of a material is calculated as the ratio of the speed of light in a vacuum, c, to that in the substance, v: n = c/v. The refractive index is a function of wavelength, temperature, and pressure and therefore is often denoted as $n_D^{20°C}$ (assumed standard pressure), where D represents the sodium D-line and a temperature of 20°C.

refractive index detector Monitors the change in the refractive index between a reference solution (or solvent) and the sample. The output is therefore in refractive index units (often 1×10^{-6} to 1×10^{-4} full scale). There are two fundamental types of refractive index detectors: Fresnel and beam deflector. In both cases the chromatograms can generate both positive and negative peaks (see figure). Both require significant warm-up times to equilibrate. In both cases the system temperature needs to be carefully controlled because small temperature variation leads to significant baseline drift. In order for this to occur it is best to work at least 5° above ambient temperature (to allow the temperature control unit to operate effectively). For all practical purposes gradient elution is incompatible with refractive index detection; there is no effective way of rapidly compensating for the reference solution composition change.

refractive index effect In UV-visible detectors, the change of the refractive index of the effluent passing through the column can generate a "peak" that is not due to an absorbing species but is due to a refractive index effect. This effect is most commonly observed near the void volume and when a sample solvent varies considerably in composition from the mobile phase into which it is injected. Remember that the changes in the refractive index of a solution cause the incident beam to change refraction and thereby alter the intensity of radiation reaching the detector, hence a "peak."

regeneration (1) Column regeneration typically has a specific meaning of restoring column performance back to or near its original efficiency. For example, ion-exchange columns are regenerated by flushing with an appropriate counterion and normal- and reversed-phase LC columns are regenerated by back flushing with appropriate strong solvents. (2) Column regeneration is also sometimes the term

used to signify the reequilibration step needed after a gradient to return the system back to its initial operating conditions.

repeatability Measured by the precision generated through the replicate analysis of one sample by one analyst on one instrument.

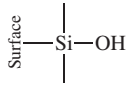
reproducibility The measure of precision that is generated by replicate analysis of one sample between analysts, instruments, and laboratories.

reservoir A container that is used to contain and protect the solvents that are drawn into the pump. Reservoirs are designed to prevent UV light from striking and degrading the solvent, retard the readsorption of gas in the solvent, and prevent particulates from getting into the solvent. Reservoirs must be chemically compatible with the solvent and should be cleaned before every use.

Three reservoirs are shown. The silver disks are sparging lines (streaming argon or helium into the solvent to produce a continuously degassed solvent). The white cylinders are the inlet filters, in this case made from glass.

reservoir, sample The component of a solid-phase extraction column that is above the top frit and acts as the sample "holder" before vacuum or pressure being applied. Larger volumes may be attained through the attachment of empty reservoirs (say 10–30 mL) to the top of the column through an adapter.

residual silanol groups Silanol groups that are left on the silica support surface after the bonding (both primary bonded phase and endcapping, if done). Free silanol groups have the structure:



Residual silanol group

For many silica support materials the normal concentration of silanol groups on the surface is ~8–9 μmol/m^2. Surface silanols can be free, vicinal, and geminal (see vicinal and geminal entries). The presence of residual silanol groups is responsible in part for the extreme tailing that can be generated when amine-containing compounds are separated. Mobile-phase modifiers such as trifluoroacetic acid and triethylamine are added to the mobile phase to help prevent interaction of the analyte with the residual silanol groups and thereby reduce/eliminate tailing.

resin An organic polymeric support material used in various separations (e.g., IEC, GPC). Resins can be either macro- or microreticular, which is a function of their manufacture and determines many of the resins' physical characteristics. Resins are made from the polymerization of various materials such as polystyrene with divinylbenzene, methacrylate, latexes, dextrans. These materials, either as is or further modified through the addition of functional groups, are then used as basic support materials in ion-exchange, size-exclusion, and other types of separations.

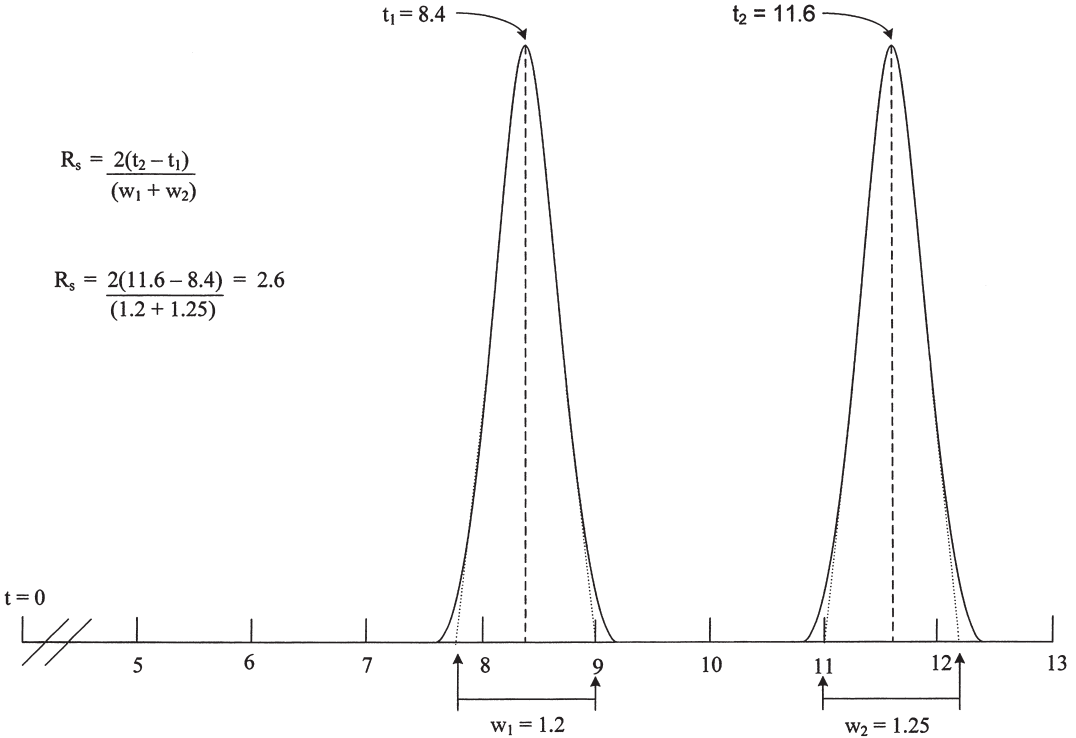
resolution, R_s or R A quantitative measure of the distance between two adjacent peaks. It can be expressed in terms of retention time difference as a multiple of summed peak widths:

$$R_s = 2(t_2 - t_1)/(w_1 + w_2)$$

where t_2,w_2 and t_1,w_1 are the retention times, peak, widths for the second (latest) eluting peak and first eluting peak, respectively. Conversely, resolution can be expressed in terms of column parameters:

$$R_s = (\sqrt{N}/4)(\alpha - 1)(k'/[k' + 1])$$

where N is the theoretical plates (column efficiency), α is the separation factor (system selectivity), and k′ is the capacity factor (column retentivity).



The way for calculating resolution is presented in the figure above. Baseline resolution occurs at ~$R_s = 1.2$ for peak of equal height.

response factor The response factor for any given analyte in a specified system is defined as the peak area (or height)/concentration. In instances where (1) the system is extremely stable and reproducible, (2) the sample concentration is near that of an external standard, and (3) the concentration is well within the linear range of the method, the response factor can effectively be used as a system suitability factor.

response index, α A proposed method for comparing how close a detector response to its ideal response:

$$\log R = \alpha \log C + b$$

where R is the detector response, C is the analyte concentration, and b is a constant. In essence, the response index is the slope of the plot of log R versus log C.

response time, τ A parameter that is uniquely tied to and different for every type of detector. It is typically defined as the time required for the detector to attain a percent of the final response signal level (98%). Too slow a response time leads to peak distortion.

restrictor Used to maintain pressure in a chromatographic system. For example, a restrictor might be placed between the pressure transducer and the injector when low-viscosity solvents (e.g., hexane) are used in LC so that the check valve system seats properly and keeps flow constant and in the direction of the pump to the detector.

retention factor, k The ratio of the amount of solute in the stationary phase, N_{sp}, compared to the amount of solute in the mobile phase, N_{mp}:

$$k = N_{sp}/N_{mp}$$

The retention factor is most commonly used in describing GC equilibria and is a strong function of film thickness (k increases as film thickness increases).

retention time, t_r The time required for a solute to travel from injection to the detection for set instrument conditions. The value for t_r is designated as occurring at the peak maximum.

retention time window A predefined elution time range that is established for a unique analyte and specific operating conditions in order to assign peak identification. Instrument operational stability (e.g., pump, injector, column, detector, integrator) can also readily be assessed in this fashion.

retention volume, V_r The retention time, t_r, multiplied by the flow rate F:

$$V_r = t_r \times F$$

reversed phase Term derived from the fact that original LC separations were conducted on polar supports (e.g., silica) using nonpolar to slightly polar mobile phases (e.g., hexane or 99/1 v/v hexane/methylene chloride); these conditions were "normal." Reversed phases have nonpolar supports (e.g., octadecyl) and polar hydroorganic mobile phases (e.g., 60/40 v/v water/methanol). Reversed-phase supports, in part because of the wide range available, are by far the most commonly used in LC separations. It should be noted that with the advent of polar bonded phases (e.g., diol) in conjunction with hydroorganic mobile phases the clear distinction between normal and reversed phase has blurred.

Reynolds' number, Re A dimensionless parameter that estimates the relative internal and viscous strengths in a flowing liquid:

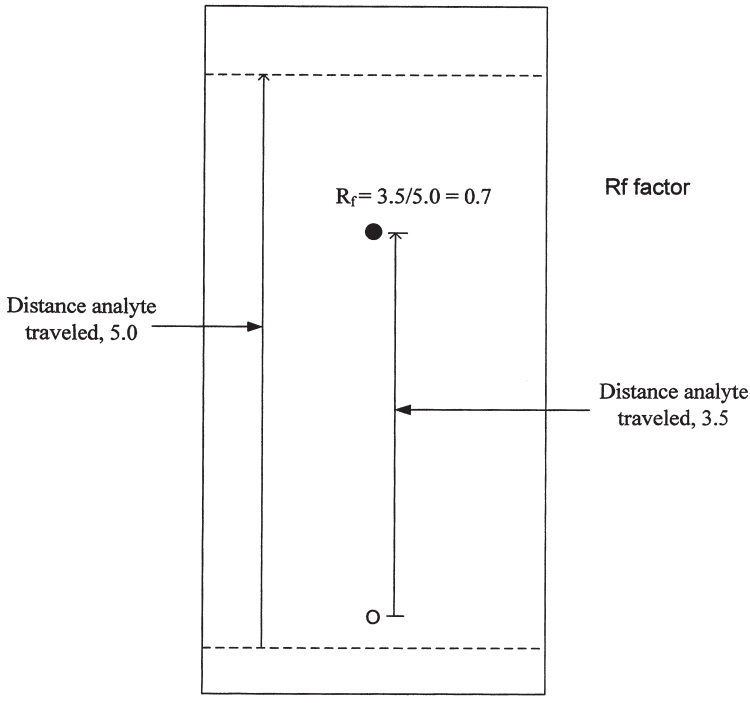
$$Re = \rho v d / \eta$$

where ρ is the fluid density, v is the fluid velocity, d is the tube diameter, and η is the fluid viscosity. The Reynolds' number is used to predict whether flow is laminar or turbulent. A low number signifies laminar flow.

R_f value The thin-layer chromatography parameter that defines the position of the analyte band on the plate:

$$R_f = \frac{\text{(distance of the analyte band from initial spot)}}{\text{(distance to the solvent front from the initial spot)}}$$

Note that it is important that the solvent from never reach the top of the plate. If this happens, then an R_f value cannot be calculated.



The Rf factor is used in thin-layer chromatography to identify a peak based on its migration up the plate. The method for calculating Rf is shown above.

rise time The time required for a detector output to go from 10→90% of the final detector output is termed the detector rise time.

robustness Refers to how effectively the analysis results remain constant when operational variables (e.g., temperature, pH, mobile phase composition [small changes], etc) are purposely changed in a controlled fashion. A robust method produces results that are essentially invariant under the type of variability that occurs from everyday preparations and operation.

Rohrschneider polarity scale, P* Classifies nonpolar and polar stationary phases used in GC by utilizing a logarithmic retention ratio scale based on *n*-butane, b, and butadiene, bd:

$$P^* = a\left[\left(\log\left(V_p^{bd}/V_p^{b}\right)\right) - \left(\log\left(V_u^{bd}/V_u^{b}\right)\right)\right]$$

where a is a constant used to adjust P to 100 for the most polar phase, oxydipropionitrile, u is the nonpolar phase, and p is the polar phase.

rotation planar chromatography (RPC) A TLC technique uses centrifugal force to accelerate the solvent flow from the center of the plate. *See* radial thin-layer chromatography.

ruggedness The ruggedness of a method is a measure of how susceptible a result is to changing one operating parameter in the system. Ruggedness is determined through the purposeful and controlled modification of operating variables over the expected range of variability. For example, if an HPLC separation is optimized with a 60/40/0.1 v/v/v methanol-water-trifluoroacetic acid mobile phase, then the effect of a change on the methanol-to-water ratio of ±1% and the TFA ratio ±0.02% will be determined. If these changes have no effect *on the analytical result* (not on the retention times) in terms of all the acceptability parameters being met, then the method is rugged with respect to these operating variables.

S

salting-in The process of creating a high concentration salt solution such that it increases the solubility of an analyte in that solution. The result may be that the analyte is solublized into the solution or drawn from another phase into the solution.

salting-out The process of creating a high concentration salt solution such that it decreases the solubility of an analyte in that solution. The result may be that the analyte is partitioned into another phase or precipitated out of solution altogether.

sample Can refer to the original material that is processed for testing as well as the actual solution being analyzed.

sample capacity The maximum amount of sample that can be injected into a system without the concentration itself affecting the elution profile. Sample capacity is limited by the amount of sample both that the column can accept (i.e., overloading the equilibrium sites on the surface) or that the detector can respond to without saturation. In the first case the peak shows fronting and in the second flat-topped or shaped-distorted maxima for a peak.

sample, check A sample that has previously been analyzed and its result is valid and stable over time. A check sample is prepared and analyzed along with current samples in order to confirm that analyst, instrument, and method are all working as expected.

sample preparation Any or all of a series of steps taken to generate a solution compatible with the analytical method. Sample preparation techniques include those that clean up, concentrate, derivatize, or any combination of these. In one of its simplest forms sample preparation may just involve sample dilution.

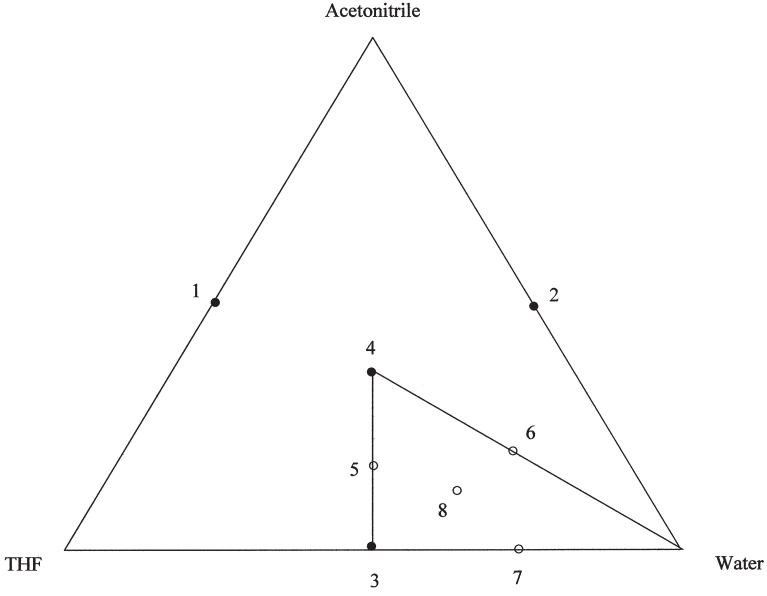
scrubber *See* purifier.

sedimentation The process of separating particles by size and density through their settling velocity.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

selectivity (1) A measure of how effectively a column/mobile phase separates a pair of consecutively eluting peaks. Mathematically the selectivity is often denoted as the separation factor, α, minus one: $\alpha - 1$. The selectivity is an explicit term in the determination of the resolution between peaks, R_s. (2) A detector that responds to a particular class of compounds having a unique chemical or physical property. An example is a UV detector that only responds to molecules having a chromophore that absorbs at a set wavelength.

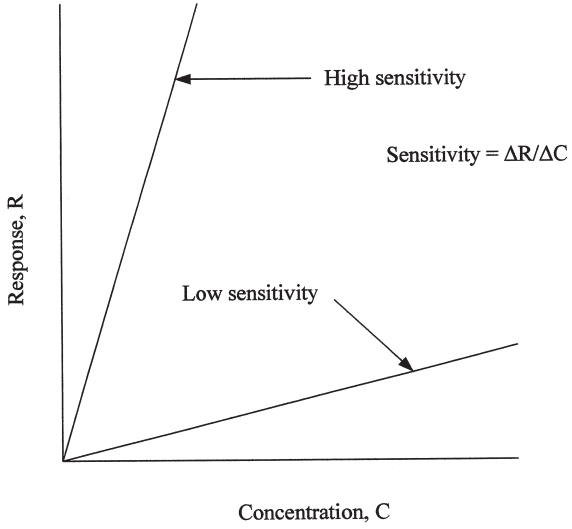
selectivity triangle Developed to provide a structured way of optimizing an adsorption-based separation (i.e., on silica or alumina). The apices were labeled with critical solvent characteristics to reflect the most important/dominant solute/sorbent interactions. Solvent were then arranged within the triangle to assist with the selection process.



A visualization technique for optimizing a separation presented here is a triangle. Solvent compositions are designated for each apex and chromatograms run for each of these points and compositions 1–4 (e.g., 1 is 50/50 v/v acetonitrile/THF, 2 is 50/50 v/v acetonitrile/water, etc). The best chromatographic results then determine the next solvent compositional iteration which in this case is 5–8. Further iterations are carried out until the desired separation is obtained. Obviously, pure solvent does not have to occupy an apex. For example, the lower left apex might just as well have been 50/25/25 v/v/v water/acetonitrile/THF. In that case 1 = 25/62.5/12.5 v/v/v water/acetonitrile/THF.

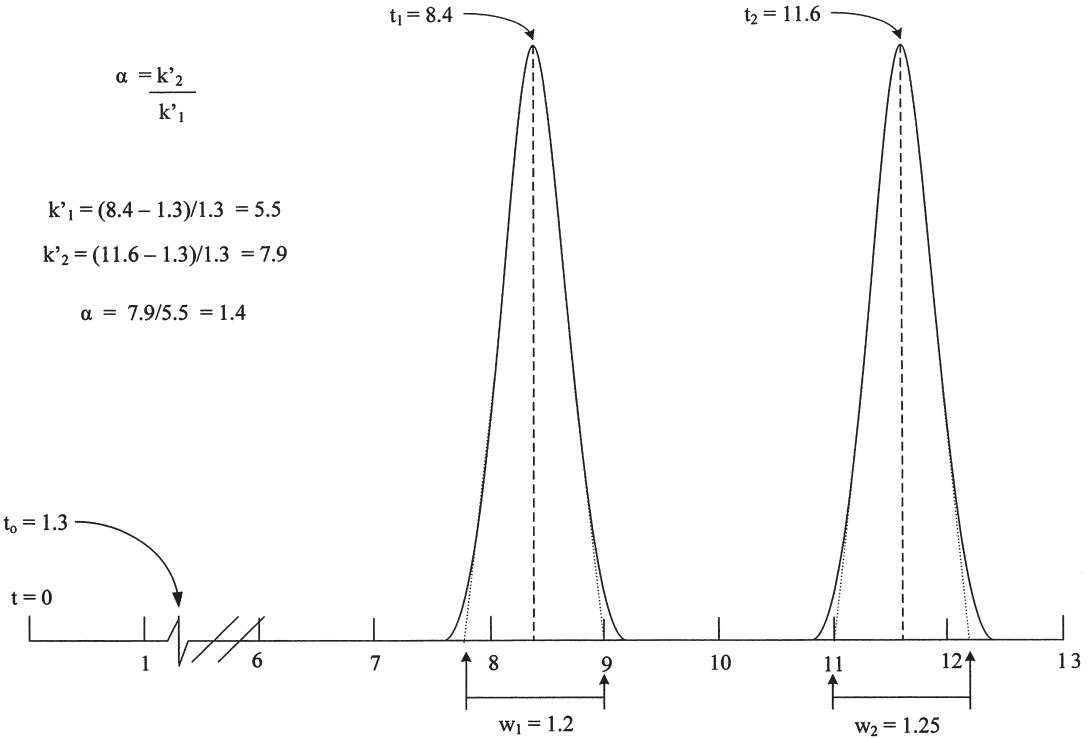
semipreparative HPLC Uses columns that have inner diameters of 6–20 mm and injection masses in the 10 to 1000-mg range. This technique is used to conduct sample fractionation and purification on a laboratory scale.

sensitivity The slope of the detector response, R, versus the analyte concentration, C, i.e., sensitivity = $\Delta R/\Delta C$ for concentration-based detectors and R versus mass flux, m, i.e., sensitivity = $\Delta R/\Delta m$, for mass-sensitive detectors. High sensitivity allows for better differentiation between analyte concentrations but often results in a narrow working range. Conversely, a low-sensitivity method generates a wide working range but a decreased ability to differentiate concentrations.



The slope of the response versus concentration curve is the sensitivity of a technique. High-sensitivity analyses typically have linear responses over narrower concentration ranges. Lower-sensitivity analyses have linear responses over wider concentrations ranges, but differentiation between concentrations becomes more difficult because detector error is often more significant in the determination. The best working area is at the highest sensitivity possible while still maintaining a linear curve that extends past the working and calibration curves.

separation factor, α The ratio of the capacity factors for two adjacent peaks, where k'_2 is the later-eluting peak:

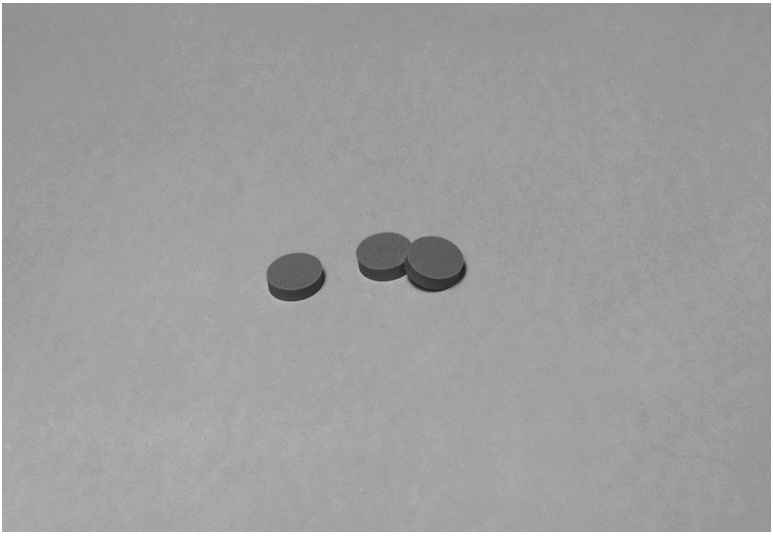


Separation factor: This parameter is another way to describe the resolution between two peaks. The capacity factor for the latest-eluting peak is always in the numerator so that α is always >1. To determine α, the system void time must be determined. The relevant equations are shown above.

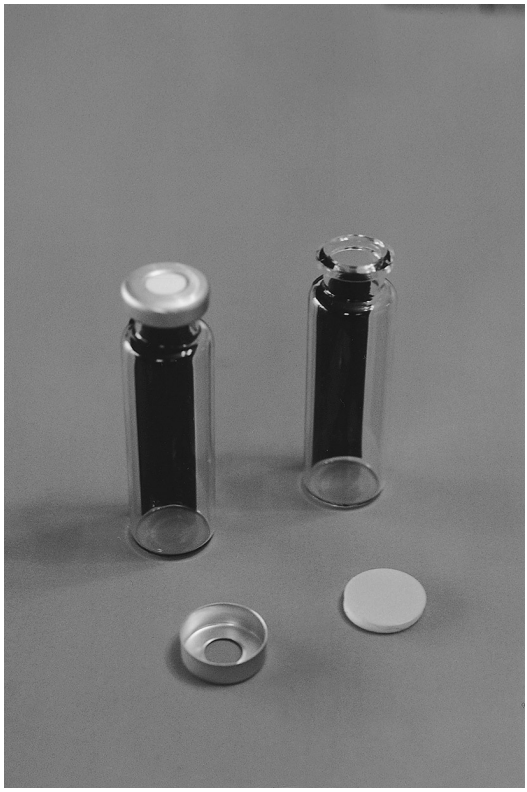
$$\alpha = k'_2/k'_1$$

Per this definition, α is always greater than or equal to 1.

septum A component that is used to allow a syringe to enter a container and withdraw/input material but prevent gas from entering or escaping after the action is complete. Septa are also used in sample vials. For HPLC and GC the septa are commonly PTFE clad to prevent the sample solvent from extracting material from the support material. Septa are also used in headspace vials. Finally, septa are used in GC injector ports and permit the injector syringe to place the sample in the injector port (or on the column) and yet prevent gas from escaping through the septum. Common materials of construction include silicone rubber and PTFE-lined silicone rubber. These septa must be temperature inert because injector temperature are often held at 200°C or higher.



Typical GC injector inlet septa. These are not typically lined with PTFE or any other material. The correct polymer must be stable at the maximum injector port temperature used in order to minimize bleed.



Septa are also used for sealing vials. In this case, the septum is lined with PTFE. The inert liner should be directed so that it is in contact with the sample or sample vapor. In this case the septum is used with a crimp-cap vial.

septum bleed Septa are made from a wide range of materials (e.g., silicon rubber), that may not be fully cross-linked. Therefore, on heating various volatile components from the septum are released into the carrier gas flow and may appear as a peak in the chromatogram. These materials are all rated to different temperature levels.

septum purge A slow flow of carrier gas that passes under the septum and internal to the injector and is used to flush out-gassed components from the septum (*see* septum bleed) away from the column.

shrinking The process by which a dry resin or gel expels solvent and contracts. Particle size and pore diameter are strongly affected by this, and so before use a resin must be fully equilibrated with the operating solvent. *See* swelling.

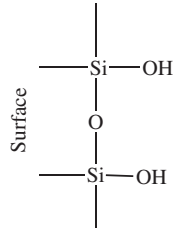
shot noise Generated in an electrical circuit because of the current flowing through the detector collector and proportional to the square root of the current: shot noise = $\sqrt{I}$. This form of noise is typically much less than Johnson noise.

sieve A unit that has a series of precisely placed screens (or meshes) that form apertures through which particles less than or equal to the aperture in size. Two sieve systems are commonly used: US Standard and Tyler. *See* mesh.

sieving A physical method for separating particles based on size.

signal The output of a detector that is the response to an analyte eluting through it is the signal associated with the analyte.

silanol group Terminal hydroxyl groups on the surface of the silica support material. The resulting pH for these groups depends on the purity of the silica and its structure. Normally a 1% in water suspension generates a resulting solution pH of 4–10.



Silanol groups

silanophile A component that generates strong interactions with residual silica surface groups. Basic compounds such as primary and secondary amines are strong silanophiles. Silanophilic interactions are usually seen as peak tailing. Competing molecules such as triethylamine are often added to the mobile phase to diminish or eliminate these interactions.

silica, silica gel A material composed of silicon dioxide, SiO_2. Silica is a typically support material used in HPLC and GC columns. It is stable except at high pH or in hydrofluoric acid (HF) solutions. For HPLC silica is prepared from the controlled polymerization of silicic acid, $Si(OH)_4$. Final HPLC-grade silica is porous, pellicular or nonporous, spherical or irregular in shape, derivatized or underivatized, activated or base deactivated.

silicic acid A commonly used starting material used in the formation of silica with the chemical formula $Si(OH)_4$.

siloxane bonds The siloxane bond is the principle structural bond in silica gel: ≡Si—O—Si≡.

silylation Describes the reaction between a silica derivatizing reagent and the surface silanol group.

sineresis The process that occurs during the postpolymerizarion aging of silica; water and unbound ions are exuded from the silica gel, leaving a smaller more rigid structure called a hydrogel.

single-beam detector In a single-beam absorbance detector the source light passes through the sample directly to the detector device. Changes in source intensity, noise, and drift are uncompensated. *See* dual-beam.

slope The slope (m) of a plot is the change in the dependent variable (Δy) per change in the independent variable (Δx), or $m = \Delta y/\Delta x$. In analytical work plots of detector response (R) versus analyte concentration (C) give rise to a slope of $\Delta R/\Delta C$. This represents the sensitivity of the analysis to the analyte and is also called the response factor for the analyte. The general equation for a linear relationship between x and y is:

$$y = mx + b$$

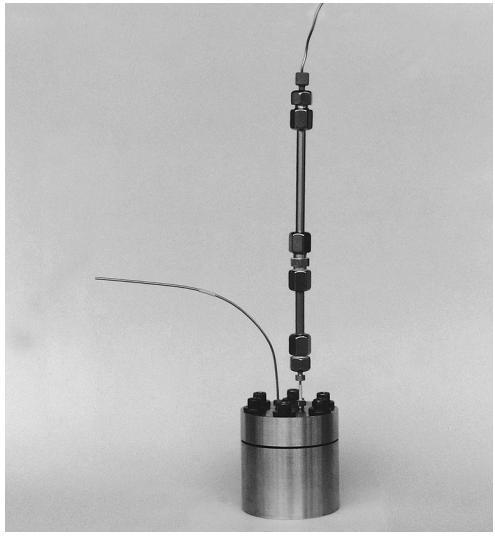
where b is the y-intercept.

size-exclusion chromatography (SEC) An LC technique that generates a separation based solely on analyte size. This technique is commonly used to characterize polymers. The molecular weight is determined through the creating of a MW versus retention time plot. *See* molecular weight calibration curve. SEC techniques include gel

permeation (e.g., polystyrene divinylbenzene support) and gel filtration (e.g., silica support). The important characteristic in any SEC method is the there is no adsorption or partitioning of the analyte onto the support surface; all separation is due to accessing pore volume or not.

skew *See* asymmetry.

slurry packing To reproducibly and effectively place packing material into columns of narrow inner diameter, the packing material is often suspended in a solvent and then pumped under high pressure into the column. This is slurry packing.



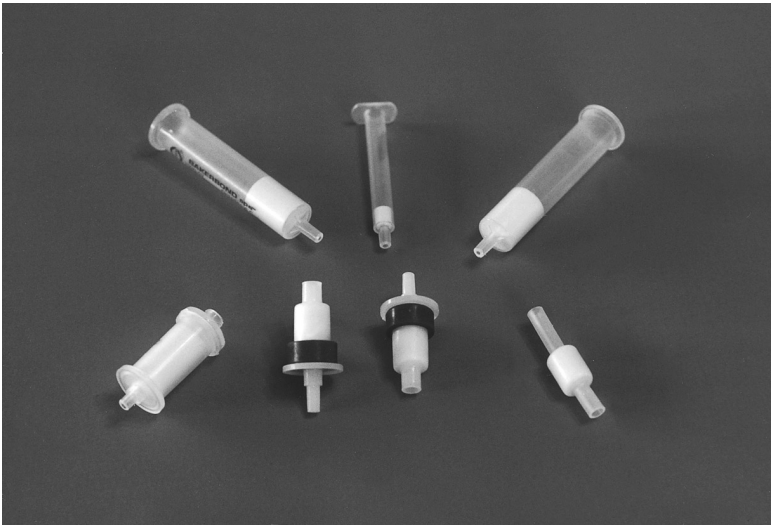
To generate a well-packed and highly efficient HPLC column dry packing is unacceptable. Therefore, packing material is suspended in solution and pumped at high pressure into the column tube. This picture shows one configuration for upward slurry column packing. The slurries packing material is placed in the lower "bomb," which is sealed. The analytical column (top column) is connected above the bomb with a spacer column and is terminated with a fritted endfitting. High pressure is built up with no flow and then released to rapidly pack the column.

Snell's law (of refraction) Snell's law mathematically describes the relationship between the angles of incidence, ϕ_1, and the angle of refraction, ϕ_2, for substances with refractive indices of n_1 and n_2, respectively:

$$n_1 \sin\phi_1 = n_2 \sin\phi_2$$

soap chromatography *See* ion-pair chromatography.

solid-phase extraction (SPE) SPE is a column sample preparation technique that concentrates and separates analyte for other sample components.



Shown above are some common SPE column (top row) and cartridge (bottom row) configurations. The columns are processed by pulling a vacuum. Vacuum manifolds having 6–24 ports have been produced in order to process multiple samples simultaneously. Cartridges are commonly used when a low number of samples are processed. These are fit to the end of a Luer-Lok syringe and processed by applying positive pressure. Finally, the column and cartridge designs have also been designed for use in automated SPE processing units.

solubility limit The maximum amount of substance that can be dissolved under a set of conditions is defined as the solubility limit of the substance.

solubility parameter, δ Mathematically defined as:

$$\delta = (\Delta E_v / V)^{1/2}$$

where ΔE_v is the vaporization energy of the compound and V is its volume. Therefore, the unit of measure for δ is $\sqrt{cal/cm^3}$. The solubility parameter correlates strongly with polarity; the higher the polarity, the higher the δ value. A scale of δ was developed by Hildebrand (the solubility paramter scale).

solubility product, K_{sp} Determined by the equilibrium concentration of ions in a saturated solution. Mathematically the solubility product for a salt nXmY is:

$$K_{sp} = [X^{a+}]^n [Y^{b-}]^m$$

where $[X^{a+}]$ is the cation concentration with charge a^+ appearing n times in the salt and $[Y^{b-}]$ is the anion concentration with charge b^- appearing m times in the salt. For example, the solubility product for $Ca_3PO_4^2$ is $K_{sp} = [Ca^{2+}]^3[PO_4^{3-}]^2$.

soluble A compound that can be dissolved is called soluble. Note that soluble indicates a level that is different from miscible, which means soluble in all proportions. As an example, chloroform is soluble in water to 0.8% at 20°C.

solute A minor component that is dissolved in a solvent.

solvent The major component of a liquid mixture that contains solutes.

solvent polarity index, P′ Experimentally developed by Snyder by comparing the ratio of the capacity factor for a given solute when changing from one solvent to another:

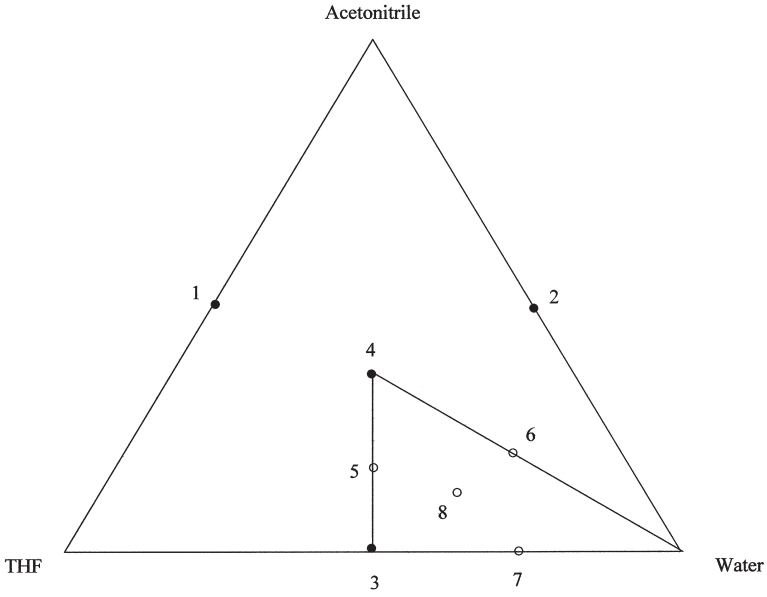
$$k'_2/k'_1 = 10^{(P_1 - P_2)/2}$$

See individual solvent entries for their P' value.

solvent selectivity groups Developed to classify solvents with respect to their primary intermolecular interactions with an analyte. These interactions included pure proton acceptor, pure proton donor, and large dipole moment.

solvent selectivity triangle Originally was developed from the solvent selectivity groups with each apex of the triangle representing one of the critical intermolecular interactions. Now a solvent selec-

tivity triangle is an optimization tool with any three solvent, or solvent blends, occupying the apices. To include more variables the triangle can be expanded into a pyramid, and so on. The basic optimization strategy remains the same.



A visualization technique for optimizing a separation presented here is a triangle. Solvent compositions are designated for each apex and chromatograms run for each of these points and compositions 1–4 (e.g., 1 is 50/50 v/v acetonitrile/THF, 2 is 50/50 v/v acetonitrile/water, etc). The best chromatographic results then determine the next solvent compositional iteration which in this case is 5–8. Further iterations are carried out until the desired separation is obtained. Obviously, pure solvent does not have to occupy an apex. For example, the lower left apex might just as well have been 50/25/25 v/v/v water/acetonitrile/THF. In that case 1 = 25/62.5/12.5 v/v/v water/acetonitrile/THF.

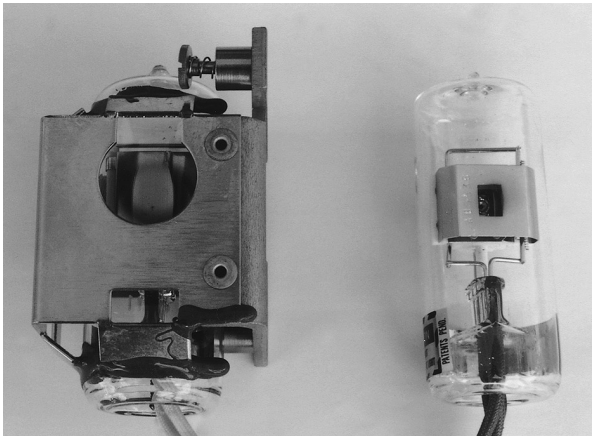
solvent strength, ε In adsorption chromatography the capacity factor for a given analyte is a function of the solvent strength of the mobile phase:

$$\log(k'_1/k'_2) = A_a(\varepsilon_2^o - \varepsilon_1^o)$$

where the subscripts 1 and 2 refer to mobile phases 1 and 2, respectively, and A_a is the surface area of the analyte.

sorbent Synonymous with the packing material: It is what provides adsorption or partition sites for analyte phase transfer.

source A generator of an output that is altered when it is directed through a sampled. For example, a UV source can be a "continuous" source that gives high-intensity output from 190 to 400 nm (such as deuterium) or a high-intensity line emission source such as zinc (214 nm), cadmium (229 and 336 nm), and mercury (254 nm and others).



Two UV sources are shown above. The left source is prealigned in a housing that is locked into the source chamber in the detector. The right source must be aligned once it is placed into the source chamber.

spacer arm Used in affinity and chiral chromatographic supports to move an enzyme-active or 3-D interaction site far enough away from the surface so as to offer the analyte rapid and unhindered access.

specification Defines the target level of a given chemical or physical test along with acceptable upper and/or lower limits for that test for a particular material. For example, if the limit of free salicylic acid in aspirin is 0.3%, then all results must be lower than this limit (upper bounded limit). The specification for aspirin itself is a purity level with a target of 100% and an acceptable range of ±10% (90–110%).

specificity Refers to a detector, column, or system that interacts with only one compound.

specific permeability, B_o Represents the resistance to mobile phase flow through the column and for a packed column is a function of the particle size, d_p, and porosity, ε:

$$B_o = F\eta L/\pi r^2 \Delta p = \varepsilon^3 d_p{}^2/180(1-\varepsilon)^2$$

where F is the flow rate, η is the viscosity, L is the column length, and Δp is the pressure drop. This relationship is derived from the Kozeny–Carman equation. For an open tube:

$$B_o = d_c 2/32$$

where d_c is the column diameter.

spherical, spherical packing The term spherical refers to the shape of packing materials used in HPLC columns (compare to irregular). In the case of spherical packing materials the diameter ranges from 1 to 15 μm.

split/splitless injector Used in GC to flush a fixed percentage (expressed as a split ratio) of the sample to waste. This is done to prevent overloading the column with sample and yet utilize routine syringes.

split ratio The ratio of carrier gas volume that is vented to waste as compared with that which is sent to the column. For example, a typical split ratio ranges from 20:1 to 100:1 (vented:on column). Therefore, the chromatographic result must be multiplied by the split ratio to compensate for the fraction that was vented to waste in order to obtain the correct final result.

spotting The process of applying a sample to any thin layer support. Effective-spotting requires a concentrated sample and a small resulting spot.

stagnant mobile phase Any part of the mobile pahse that is within the support particle and is not freely swept when the mobile phase is flowing through the column. It is a contributing factor to the C term in the van Deemter equation (i.e., peak broadening).

stainless steel An iron-based alloy that, depending on the type and amount of other materials, has varying tensile strength, resistance to corrosion, etc. Most chromatographic components that are fabricated from stainless steel (e.g., tubing, unions, end fittings, frits) are made from 316 stainless steel, which contains 2–3% molybdenum (corrosion inhibition).

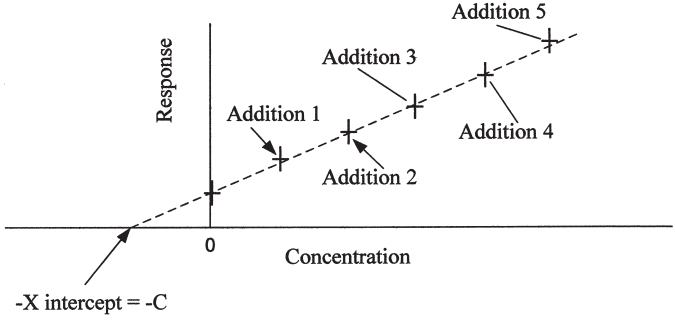
standard deviation, σ A measure of the uncertainty present in a set of related results. To calculate σ use:

$$\sigma = \sqrt{\Sigma(x_i - x)^2 (n-1)}$$

where x_i is the individual ith datum, x is the mean, and n is the total number of data points in the set. As an example, for the set of data 6.1, 6.4, 6.2, 6.2, 6.1, $x = 6.2$ and the deviations from the mean for each point are –0.1, +0.2, 0, 0, and –0.1, respectively. Therefore, $\sigma = \sqrt{[(-0.1)^2 + (0.2)^2 + 0^2 + 0^2 + (-0.1)^2]/(5-1)} = \sqrt{0.06/4} = 0.12$.

standard A material of known identity and purity that is used to establish the establish the concentration of the same compound in a sample. Standards can be primary (i.e., certified by an organization recognized as being capable of producing certified primary standards), secondary (or traceable, meaning that they are assayed against a certified primary standard material), or "off the shelf" such that the manufacturer's certificate of analysis is used as substantiation as to the material's purity. Primary standards give the highest degree of certainty and are frequently the standard employed in the analysis of regulated materials.

standard additions The method of standard additions is a quantitative technique where a series of known amounts of the analyte are added to the sample. These preparations are then analyzed and the following plot generated:



The standard addition technique takes the response from a sample (plotted here where x = 0) and a series of known concentrations are added to the sample (here 5 additions are made). The sample is analyzed after each standard addition, and the result is plotted at the concentration added. The extrapolated line through the x-axis gives the negative concentration in the original sample.

Extrapolation of the data through to the x-axis yields the analytical result.

standard, external Known purity materials that match the identity of the analyte. These standards are analyzed separately from the sample (contrast with internal standard). External standards are effective in systems where the overall stability is extremely high. These standards are used to make up calibration curves that cover the expected range of the analyte in the sample. The sample concentration is then calculated directly from the curve established by the external standards.

standard, internal A compound that is similar to the analyte of interest, is added to the sample before analysis, and is completely resolved from all other peaks in the elution profile. The purpose of an internal standard is to compensate for any variability that may occur during the preparation, separation, and analysis processes. Although not mandatory, an internal standard typically (1) is chemically similar, (2) elutes near the analyte, (3) is commercially available in high purity form, (4) is stable and unreactive under the conditions of preparation and analysis, and (5) is not in the sample itself.

standard operating procedure (SOP) A written document that formally details the manner in which a process, protocol, test, method, or operation is to be performed so that a peer knowledgeable in the field can understand and apply these instructions to gain valid results.

static headspace In this form of headspace analysis, a fixed volume of liquid sample is placed in a septum-sealed vial. This vial is heated to a predetermined temperature and allowed to equilibrate. Once equilibrium is reached a sample is taken by syringe from the gas layer above the liquid in the sample vial and injected into the GC instrument. *See* purge & trap. The static headspace method is reproducible and inexpensive but does not have as low a detection limit as a dynamic headspace method such as Purge & Trap.

stationary phase The retentive component(s) of the packing material. In adsorption chromatography the packing material itself is the stationary phase, whereas in partition chromatography the adsorbed liquid phase or bonded phase is the stationary phase.

steric hindrance Relates to the bulkiness of a molecule, which limits the accessibility of other molecules to a certain space or area. In bonded phase work this is particularly important because the size of a C18 chain ultimately limits the percentage of the surface silanols

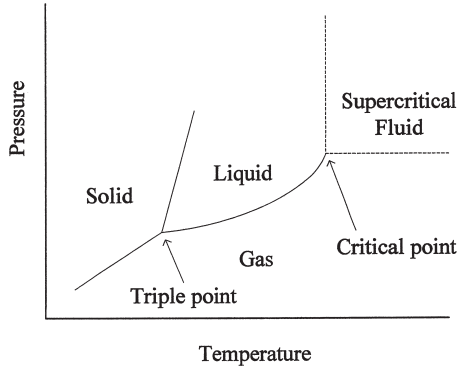
that are reacted. Hence, all other things equal, it is expected that there would be more unreacted silanols on a non-endcapped C18 support than a corresponding butyl support.

Stokes radius Also called the hydrodynamic radius, the Stokes radius is the effective radius of a molecule in the solvent, including any associated solvent molecules.

storage solvent Used to keep the packing material surface wetted and protected from contamination. Manufacturers typically recommend the type of storage solvent that should be used with their columns. For example, an ion-exchange column storage column will most likely have a defined salt type and concentration whereas a C18 bonded phase will use an organic solvent (such as acetonitrile, etc). A very important aspect of the storage solvent is to prevent air from entering the packing bed.

surface coverage The percent of active surface sites (e.g., silanol groups) that are converted to the bonded phase. This is typically derived from the theoretical number of silanol sites/m^2 for the surface (~8 µmol silanol/m^2).

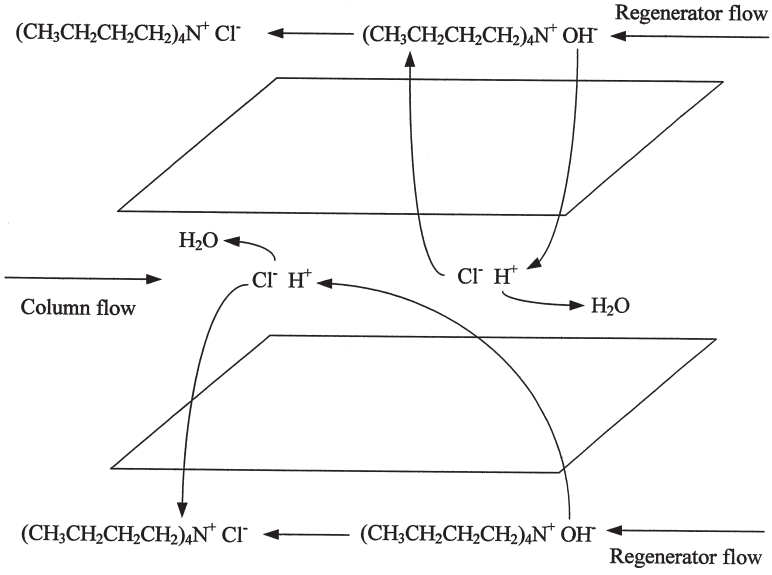
supercritical fluid (SCF) Any compound that is held at a temperature and pressure above its critical point, leaving it to exist as a single phase. A supercritical fluid is intermediate in properties to a liquid and gas.



A supercritical fluid is neither a liquid nor a gas but has intermediate qualities to the two. The critical point designates the temperature and pressure at which the substance exhibits supercritical fluid properties. The triple point is the temperature and pressure at which the solid, liquid, and gas forms of a substance coexist.

supercritical fluid chromatography (SFC) Utilizes a supercritical fluid as the mobile phase and offers a means of effectively analyzing compounds that are thermally labile and incompatible with GC or have limited solubility in liquids used as LC mobile phases.

suppressor A component used in an ion-exchange chromatographic system. It is positioned between the column and the detector and "neutralizes" the effluent before it reaches the detector and thereby dramatically reduces background noise.



A suppressor is used in conductivity detectors to reduce signal background generated by the ions in the mobile phase. In the case of a hydrochloric acid eluent, a tetrabutyl ammonium hydroxide solution is used to exchange in the suppressor column and produce water in the column (thereby reducing the ion concentration and signal generated by the mobile phase) and remove the chloride and the tetrabutyl ammonium chloride. Suppressor column solutions must be replenished periodically.

surface area The surface area of a porous packing material is commonly determined through the calculation of nitrogen isotherms run at −19°C (*see* BET test method). Surface area is typically expressed as m^2/g of packing. High-surface-area packing materials are desirable because this is where the solute equilibrium occurs. Surface areas

range from 10 to 450 m^2/g corresponding to pore sizes of 4000–50 Å, respectively.

surrogate A compound that is not part of the sample matrix and is added to a sample before the preparation step. Its recovery is monitored along with other sample analytes, and its overall recovery is a direct evaluation of the performance of the entire method. In many respects a surrogate is similar to an internal standard in that it cannot be in the sample matrix, should be similar in chemical/physical properties to the analyte(s) of interest, and cannot coelute with any component in the sample. The difference is that a surrogate is not used to "correct" a result as an internal standard is.

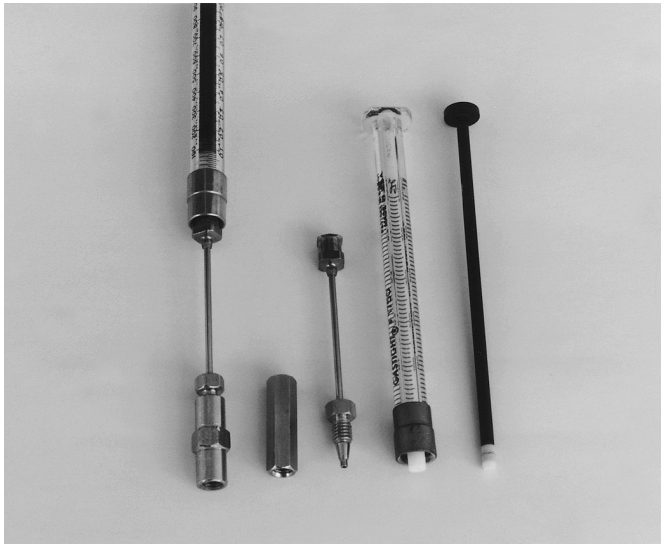
swelling The process by which a dry resin or gel imbibes solvent and expands. Particle size and pore diameter are strongly affected by this, and so before use a resin must be fully equilibrated with the operating solvent. *See* shrinking.

system suitability A term used to describe a series of tests that are conducted over the course of an analysis to support the fact that the system was operating within acceptable parameters during the analysis. Some parameters that could be monitored include: resolution, tailing, and response factor. Standards and check samples (previously analyzed samples having predetermined values) are also part of system suitability testing.

symmetry The measure of how closely the peak elution profile is a mirror image around the peak maximum. For a chromatographic peak the departure of a peak from being perfectly symmetric is represented through the use of an asymmetry factor.

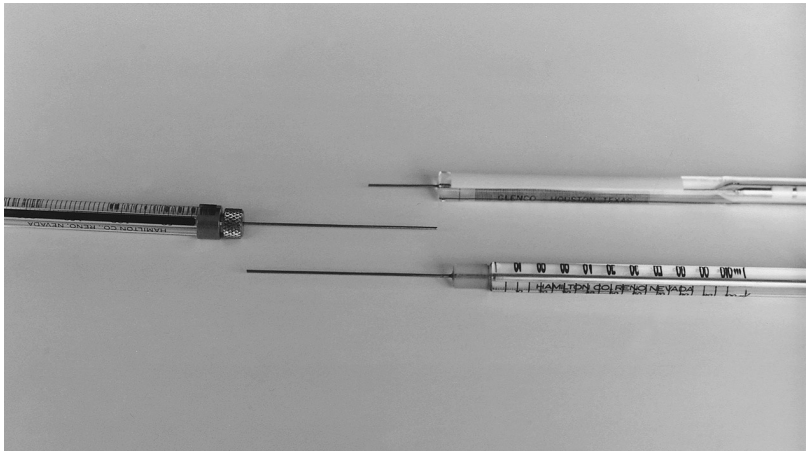
syringe Used in most GC injection systems and in manual HPLC injection systems to introduce the sample into the mobile phase flow stream (GC) or the injector loop (HPLC). A syringe consists of a body (e.g., glass) that is precision bored, a matched plunger (e.g., stainless steel), and a needle (stainless steel). The barrel is marked for volume, and the plunger is used to draw up and expel sample from the barrel. For GC the needle is beveled to make it easier to puncture the septum, whereas in HPLC the needle is often squared so that it forms a seal with the injector port inlet. Gastight syringes often have the plunger terminating with a ribbed PTFE cap to prevent gas from leaking out between the barrel and the plunger. For samples that are ≥1 µL in volume, a plunger-in-barrel is used. For samples that are

A



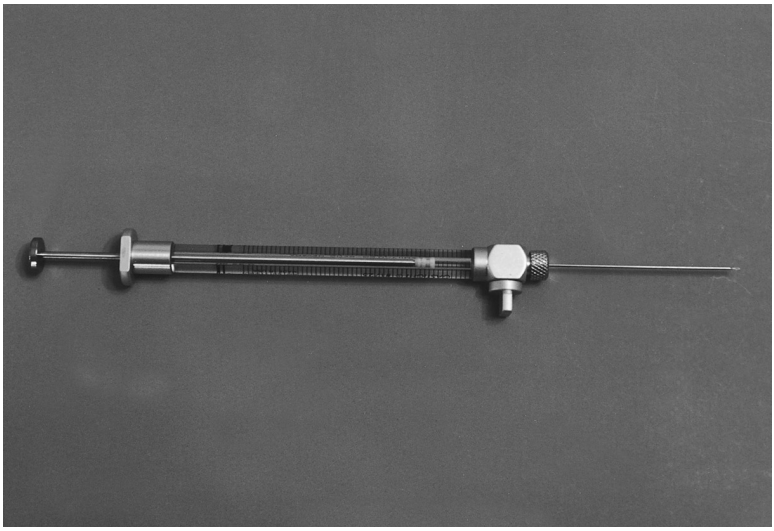
An assembled syringe with a stainless steel tubing adapter is shown at left. The individual components are shown from left to right: ZDV union, Luer-Lok adapter, syringe barrel, PTFE-tipped plunger.

B



Three type of syringes are shown here. All have square-tip needles and are compatible for use in HPLC injectors. The left-hand syringe has a detachable needle. The right-hand syringes have permanent needles of different lengths.

C



This is an example of a gastight syringe. Note the PTFE-tipped plunger, the open-close valve, and the pointed-end needle for puncturing septa.

≤1 μL, a plunger-in-needle is used (i.e., the sample does not enter the barrel).

syringe barrel Contains all components of a solid-phase extraction column: frits and packing material. Typical materials of construction include polypropylene, glass, or PTFE. Also see syringe.

systematic error One that cannot be determined from repetitive analysis. Rather, this type of error consistently over- or underestimates the true value. *See* bias.

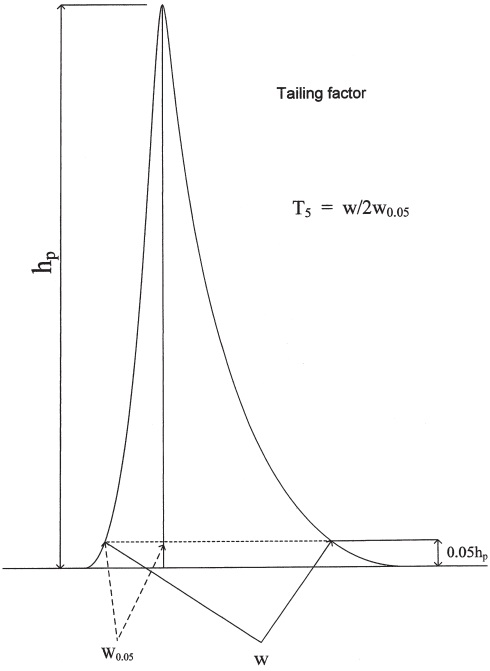
system peak The result of a solvent mismatch between the sample solution and the mobile phase and is often seen as a set of paired peaks. Any time that the solvent used to inject the sample is not identical to the composition of the mobile phase and the mobile phase generates a "non zero" detector response, a system peak may be observed. There are frequently two peaks, one positive-going (due to zone enrichment of a mobile-phase component) and the other negative-going (due to depletion of a mobile-phase component).

T

tailing A condition in a chromatographic elution profile where the peak distribution is skewed after the peak maximum (*see* asymmetry). Mathematically, tailing is quantitated through the use of an asymmetry factor. Tailing is often due to one or more of the following: nonideal equilibria between the mobile and stationary phases (i.e., nonlinear isotherms), slow kinetic desorption from the surface, leaks, voids at the top of the column, column bed imperfections, or pockets of dead volume in the system.

tailing factor, T Related to the asymmetry factor but explicitly defined in the US Pharmacopoeia as:

$$T_{0.05} = w_{0.05}/2w_a$$



The tailing factor was developed by the USP as a parameter to define peak symmetry. The appropriate equation and place on the peak to determine T is shown above.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

tar The liquid residue left from a pyrolytic process.

tee A type of union that is used to combine two flow streams.

A tee is used to bring two separate flow sources together (or connect a pressure-monitoring device). Pictured here is a high-pressure HPLC tee that could be used for connection of a post-column reactor to the column effluent line.

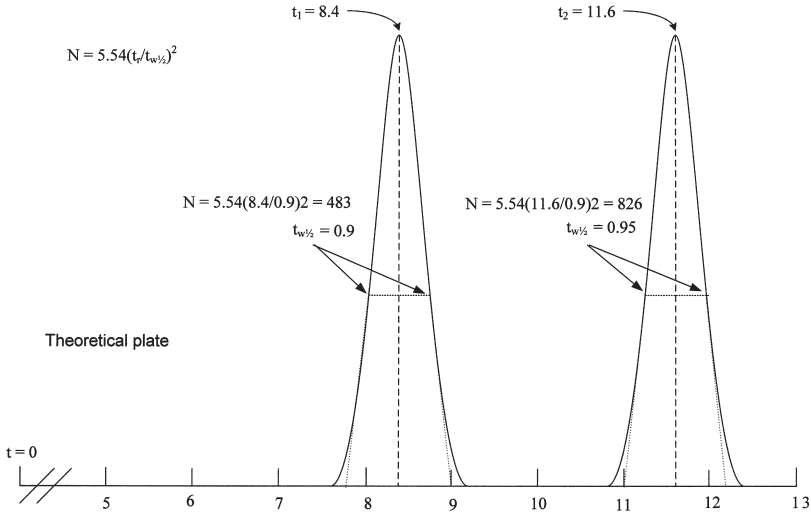
terminal electrolyte The electrolyte that contains the lowest mobility sample ion in an electrophoretic system. Together with the leading electrolyte it brackets the sample migration distance. *See* leading electrolyte.

tetrahydrofuran (THF) Molecular weight: 72.1; boiling point: 66°C; refractive index (20°C): 1.4072; density (20°C): 0.89 g/mL; viscosity (20°C): 0.55 cP; UV cutoff: 212 nm; eluotropic strength (ε^{o}): on alumina—0.45, on silica—0.35; polarity index (P′): 4.2; Hildebrand solubility parameter (δ): 9.1. Miscible with water. THF is a frequently used low-percentage constituent in HPLC work. It is commonly used in GPC. It provides high solubilization, offers unique selectivity, and also has a low viscosity. However, THF rapidly forms peroxides when

exposed to air and light. Preserved THF is incompatible with most UV work.

Tetrahydrofuran

theoretical plate, N A measure of column efficiency. The value is calculated for each peak and is dependent on peak retention and width:



The diagram shows one method of calculating theoretical plates for a peak. Note that this represents the number of plates per column. Hence, if a 25 cm column were used in this example, then this number needs to be multiplied by four to get plates/meter, a common figure used by manufacturers. N can also be calculated from other widths in the peak but different constant values (not the 5.54) would be used.

$$N = C(t_r / w_x)^2$$

where C is a constant derived from which the peak width, t_{wx}, is taken. Here the subscript x refers to the percent peak height. Manufacturers report this figure in plates per column or plates per meter. For example, if a 25 cm column has a peak that generates 4000 theoretical plates, the the corresponding plates per meter would be: (4000 plates/25 cm) × (100 cm/m) = 16,000 plates/m.

thermal conductivity detector (TCD) A nondestructive concentration detector used in GC. The detector measures the difference in the conductivity generated by the sample as compared against the carrier gas along. This difference causes a resistive imbalance on the filaments of a Wheatstone bridge system and a signal is generated from the change in resistance. The TCD is most effective for nonorganic analytes such as NH_3, CO_2, H_2O, etc. The lower detection limits are quite high compared with other GC detectors, in the 10 ppm range. This leads to use with packed-bed columns. These columns can tolerate high sample loadings as compared with capillary columns. The linear range of the TCD is about 10^4.

thermospray ionoization (TS) Uses a volatile charged component of the mobile phase to initiate the ionization of the analyte. A common reagent is ammonium salt:

$$A + NH_2^+ \rightarrow [A \cdot NH_2^+] \rightarrow [AH^+] + NH_3$$

Ionization occurs in an ion source after a aerosolized spray is formed by passing the sample through a capillary inlet. After passing through the source and becoming charged, the ions are then accelerated to the detector. This is a ionization process that is "gentle" and can effectively form molecular ions.

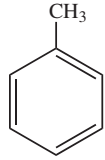
thin-layer chromatography (TLC) A technique that uses a sorbent applied to a planar support, called a plate, for separation. The sample is applied to the sorbent that is contained in a sealed container called a developing tank. The solvent moves up the support through capillary action and causes the analytes to migrate up the sorbent based in the differential affinity between the solvent and the sorbent. Standard TLC uses a sorbent that is prepared from 10- to 60-μm particles that is applied to the plate in a thickness of ~250 μm. The standard plate dimensions are in the 10 cm × 10 cm to 20 cm × 20 cm range.

time constant, τ A measure of the speed of response for a detector (typically the time required to reach 63% of total deflection). Too large a time constant leads to peak distortion but dampens the effect of short-term noise.

titania An adsorption chromatography support that is comprised of repeating TiO_2 units.

titanium A material used in the construction of column frits and components of HPLC systems where metal ion contamination needs to be minimized.

toluene Molecular weight: 92.1; boiling point: 110.6°C; refractive index (20°C): 1.4969; density (20°C): 0.87 g/mL; viscosity (20°C): 0.59 cP; UV cutoff: 284 nm; solubility in water (20°C): 0.074%; water solubility in toluene (20°C): 0.074%. Toluene is primarily used in GPC work. It is nonreactive and does not boil at the lower temperatures at which readily available low-molecular-weight alkanes do. The high cutoff precludes the use of toluene in many HPLC UV methods.



Toluene

tortuosity The diffusion rate of a solute through a porous material is a function of the path it follows, and the number/types of collisions it undergoes is a measure of the tortuosity of the flow path.

tortuosity factor, γ A mathematical value assigned to the tortuosity for an analyte expressed as the effect in the analyte diffusion coefficient in an unimpeded solution, D, versus that in the constricted flow path, D_T:

$$D_T = \gamma D_T$$

The value of γ in packed beds is often 0.6–0.7.

total ion chromatogram (TIC) Collects and displays the intensity of all ions that are detected across the entire defined molecular weight range. TICs give the greatest amount of total information with respect to the ions generated by the sample. However, many of these ions are of no importance and so the data collected are superfluous to the analysis at hand. *See* single ion monitoring.

total permeation volume, V_i The entire system volume (interstitial and pore) accessible to all molecules that can enter all available pores. This is usually expressed in terms of a molecular weight such that for all molecular weights less than this value, the elution volume is the same and equal to V_i. *See* molecular weight calibration curve.

Total porosity, ε_T The ratio of the total mobile phase volume, V_M, to that of the total column volume, V_C:

$$\varepsilon_T = V_M / V_C$$

This value can also be expressed in terms of the intraparticle porosity, ε_i, and the interstitial porosity, ε_e:

$$\varepsilon_T = \varepsilon_e + \varepsilon_i(1 - \varepsilon_e)$$

transmittance The ratio of transmitted radiation intensity, I, to that of the incident radiation intensity, I_o, is defined as the transmittance, T:

$$T = I / I_o$$

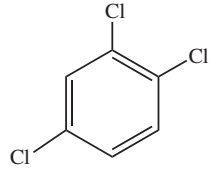
The attenuation of I_o is due to the absorption of the radiation by one or more components of the sample. It should be noted that the relationship between transmittance and absorbance is $A = \log (I_o/I) = \log (1/T)$.

Trennzahl separation number (TZ) Estimates how many paeks (of similar width) will fit between two other peaks of an homologous series:

$$TZ = (t_{r2} - t_{r1}) / (w_{1/2,2} + w_{1/2,1}) - 1$$

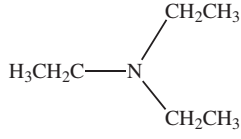
where t_{r2} and t_{r1} are the retention time of the latest- and earliest-eluting series peaks and $w_{1/2,2}$ and $w_{1/2,1}$ are their peak widths at baseline, respectively.

1,2,4-Trichlorobenzene Molecular weight: 181.5; boiling point: 213.5°C; refractive index (20°C): 1.5717; Density (20°C): 1.46 g/mL; UV cutoff: 308 nm; solubility in water (20°C): 0.0025%; water solubility in 1,2,4-trichlorobenzene (20°C): 0.02%. 1,2,4-Trichlorobenzene is almost exclusively used for high-temperature GPC work. It is nonreactive and stable. The high cutoff precludes the use of 1,2,4-trichlorobenzene in many HPLC UV methods.



1,2,4-Trichlorobenzene

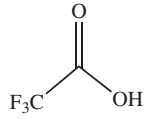
triethylamine (TEA) Molecular weight: 101.2; boiling point: 89.5°C; refractive index (20°C): 1.4010; density (20°C): 0.73 g/mL; viscosity (25°C): 0.36 cP; polarity index (P′): 1.8; solubility in water (20°C): 5.5%; water solubility in triethylamine (20°C): 4.6%. Triethylamine is commonly used in reversed-phase HPLC as a basic mobile phase modifier. TEA interacts with residual silanol groups on the support surface and limits the interaction of a basic analyte with these sites thereby decreasing peak tailing.



Triethylamine

trifluoroacetic acid (TFA) Molecular weight: 114.0; boiling point: 71.8°C; refractive index (20°C): 1.2850; density (20°C): 1.53 g/mL; viscosity (20°C): 0.93 cP; UV cutoff (0.1% solution in water): 210 nm. pK_a: 0.3. Miscible with water. Very volatile with a strong pungent smell. Avoid contact with skin and eyes. Trifluoroacetic acid is a commonly used acidic mobile phase modifier in reversed-phase HPLC. Because it is a very strong acid it is useful for protonating those

compounds that are relatively strong acids that phosphoric and acetic acids cannot effectively protonate. TFA's solubility in a wide range of solvents is also an advantage (compared with phosphates and acetates). It should be noted that TFA is very volatile and will leave solution over extended periods of time. Therefore, solvents containing low levels (e.g., 0.1% or less) should be freshly prepared and sparingly degassed before use.



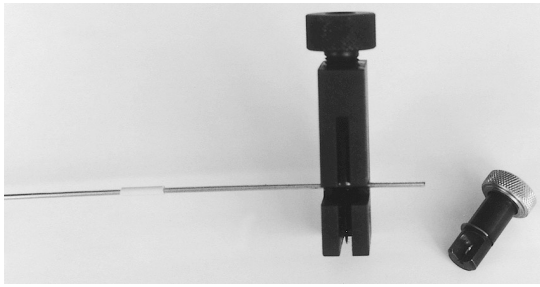
Trifluoroacetic acid

triple point The temperature and pressure at which a substance is present at equilibrium as its solid, liquid, and gas. *See* SCF (supercritical fluid).

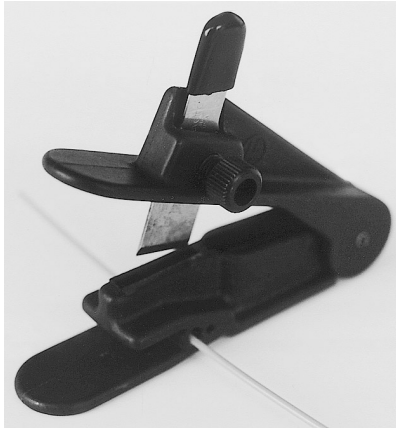
Triple-quadrupole MS/MS Consists of three quadrupoles in series. The first transmits ions (parent ions) in the same fashion as a single-quadrapole instrument. The second MS contains a collision gas that generates additional ions (daughter ions) from those selected by the first quadrapole. These daughter ions are then sent to the third quadrupole for final mass/charge filtering and detection.

tubing Used as a connector, with appropriate unions, for many components in chromatographic systems: gas tank and GC, solvent reservoir and pump head, column and detector, etc. Tubing is also used to contain packing material for HPLC work.

tubing cutter Used to produce square-cut metal and plastic tubing for use as connectors in chromatography instruments.



The is a cutter used for HPLC 1/16″ tubing. A similar but larger model is used for GC tubing (1/8″ and 1/4″). The tubing is pressed between a flat plate and a cutting wheel and rotated. The top knob is used to increase pressure at the cutting wheel works into the tube. The implement to the right is used for opening the tube after cutting . . . it has a sharp tip which remove stainless steel burrs.



This model is used for plastic tubing. The tubing is slipped through a hole under the blade. This hole is the same diameter as the tube so that a square cut results. The blade is then pushed down through the tube to generate the cut.

turbulent flow A condition in which a fluid flowing in a confined space generates a flow profile that is random and fluctuating. In chromatography this leads to unwanted peak broadening. *See* laminar flow.

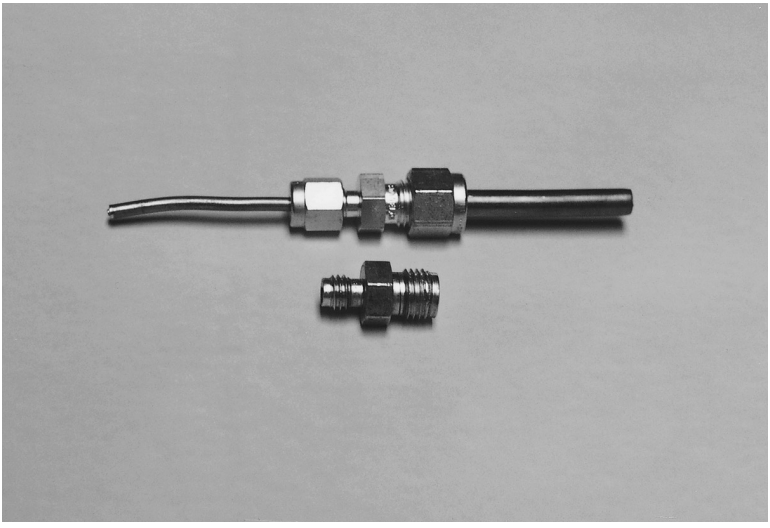
Two-dimensional thin-layer chromatography, 2D-TLC In 2D-TLC the plate is developed in two directions that are 90° from one another with two different solvents.

The technique starts like normal TLC . . . the sample is developed up the plate. However, the spot needs to be near the edge of the plate. Once developed the plate the solvent is allowed to evaporate from the plate and the plate is rotated 90° and developed in a second solvent system.

U

uncertainty The uncertainty in a result represents how much error is associated with the result. The uncertainty is the total propagated error introduced by each measurement or handling step that occurs in the preparation and analysis of a sample. One way to express this uncertainty is through the standard deviation, σ. Therefore, for the result x, the associated uncertainty is $x \pm \sigma$.

union, reducing Establishes a leak-free connection between tubing of different outer diameters.



The union and fittings used to change from a 1/8″ to a 1/4″ copper tube is shown here. The actual reducing union is shown at the bottom.

universal detector Capable of generating a signal for nearly every analyte. The most common universal detector is a refractive index detector. Only in the case where an analyte's refractive index perfectly matches that of the solvent will no signal be generated.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

universal solvent Miscible with a wide range of polar and nonpolar solvents (such as acetone and isopropyl alcohol). Note that this applies only to solvents and not to buffer-modified solvents, mobile-phase modified systems, or solutions containing salts. These solvents are used to convert systems from normal phase to reversed phase use (and vice verse).

UV cutoff The wavelength at which the absorbance of the solvent in a 1-cm cell (versus air) is equal to unity.

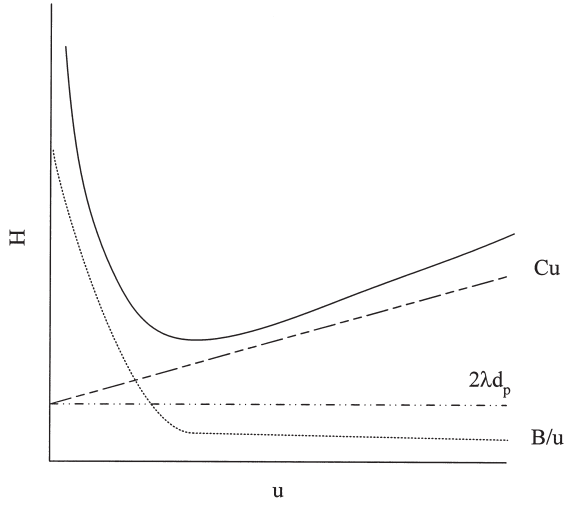
V

vacancy chromatography This LC technique utilizes an indirect detection method for quantitation.

vacuum manifold An apparatus that is used in conjunction with the simultaneous processing of multiple SPE samples.

validation The process of statistically determining that the results obtained by conducting a defined test protocol meet predetermined accuracy, precision, linearity, repeatability, recovery, robustness, and ruggedness values.

van Deemter equation A mathematical equation defining the relationship between the plate height, H, in a column and the linear velocity of the mobile phase through the column, *u*:



This diagram shows the individual contributions (various dashed lines) to the plate height in the van Deemter equation. The total H is shown by the solid curve.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

$$H = \mathrm{A} + \mathrm{B}/u + \mathrm{C}u$$

where A, B and C represent the different physical influences contributing to peak spreading.

van der Waals interactions There are three basic types of van der Waal interactions: dipole-dipole, dipole-induced-dipole, and induced-dipole-induced-dipole. Other than hydrogen-bond interactions, these are the interactions that effect/generate chromatographic separations because they describe solvent/solvent, solvent/solute, solute/solute, solvent/sorbent, and solute/sorbent interactions.

van't hoff curve Generated through the plot of log V′ versus 1/T, where V′ is the corrected retention volume and T is the system temperature in °K. From a thermodynamic derivation of the retention process:

$$\log \mathrm{V}' = -\Delta \mathrm{H}_\mathrm{o}/\mathrm{RT} + \Delta \mathrm{S}_\mathrm{o}/\mathrm{R} - \Delta \mathrm{V}_s,$$

where ΔH_o and ΔS_o are the standard enthalpy and entropy changes associated with the chromatographic equilibrium constant, K.

vaporization The critical phase change from liquid to gas that is utilized for liquid sample introduction into a GC system. Note that this fact is a serious limitation to the applicability of GC for samples because the analyte of interest must be stable when volatized at high temperature.

variable A measurable (or observable) characteristic of an analyte/sample/event. Some common operating variables for GC are temperature, flow rate, split ratio, injection volume, column type/length, etc.

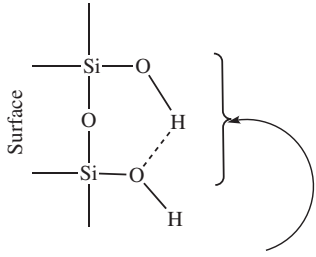
variable-wavelength detector Uses a deuterium lamp for UV work and a tungsten lamp for visible work and a monochrometer (grating or prism) to select the operating wavelength.

variance, σ^2 Mathematically the variance is the square of the deviation, σ, of individual result from the mean divided by the total number of results in the set. For the following set, 6.1, 6.4, 6.2, 6.2, 6.1, the mean is 6.2, the deviations are –0.1, +0.2, 0.0, 0.0, –0.1 and

$$\sigma^2 = \left[(-0.1)^2 + (0.2)^2 + (0.0)^2 + (.0.0)^2 = (-0.1)^2\right]/5 = 0.012$$

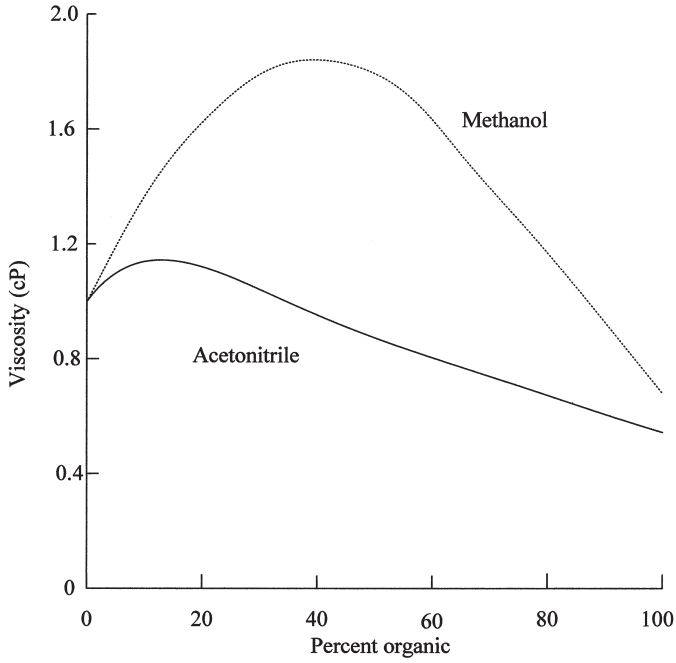
vespel A polyamide polymer that is used in capillary GC column unions mainly as the ferrule. Vespel is not easily deformed and therefore is excellent for high-pressure use. It can also sometimes be reused (unlike graphite ferrules). Modified vespel comes impregnated with 10–40% carbon.

vicinal Literally means "close to" or "nearby" and is used in the description of surface silanol groups. As opposed to isolated noninteractive silanol groups, vicinal silanols groups are spatially close enough to hydrogen bond with one another:



Vicinal silanol group

viscosity, η A measure of the mobile-phase resistance to flow. The system operating pressure is directly proportional to the viscosity of the mobile phase. Viscosities of mixtures are not derived from the mole percent in the mix and the pure viscosity for each component. As seen below, viscosity is a complex function and is dependent on strong interactions between components in the mobile phase (such as hydrogen bonding, etc).



This diagram is to show that the viscosity is not a simple linear function of the composition of two solvents. In the case of alcohols, here methanol, this increase in viscosity is nearly double that pf pure water. This has major implications for the operating backpressure if a water/methanol gradient is used over this composition range. In essence, the backpressure will nearly double before decreasing toward the viscosity of pure methanol.

viscometry detector Measures the intrinsic viscosity of a solution through the use of one or more capillaries across which the pressure drop is measured (through a differential pressure transducer). For a signal capillary the pressure drop, Δp, is defined by Poiseiulle's law:

$$\Delta p = 8\eta F/\pi r^4$$

where η is the viscosity, F is the flow rate and r is the radius of the capillary. The single-capillary design is very sensitive to flow rate fluctuations. The multiple-capillary systems are used to compensate for this issue.

visualization reagent/technique In thin-layer chromatography a visualization reagent is used to make an analyte band appear on a plate. The reagent used can be compound- or class specific (e.g., ninhydrin for amines) or general (sorbent contains a fluorescent label that is quenched by the presence of the analyte).

void An empty volume found at the top of the packing bed of a column. Voids often occur at the top of a column, where packing material is slowly dissolved and carried away by the mobile phase. Empty volume that occurs within the column packing bed is called channeling. Both result in decreased efficiency, often seen as tailing. Severe enough voids/channeling will result in split peaks.

void time, t_o The time required for an unretained unexcluded analyte to travel from the injector to the detector.

void volume, V_M Determined from the void time, t_o, and flow rate, F:

$$V_M = t_o \times F$$

Note that this is not the column volume alone but includes the injector, connecting tubing, and detector volume as well.

W

water Molecular weight: 18.0; boiling point: 100°C; refractive index (20°C): 1.3330; density (20°C): 0.998 g/mL; viscosity (20°C): 1.00 cP; UV cutoff <190 nm. Water is a major constituent in many reversed-phase HPLC analyses. It is a deactivating material in normal phase separations (nonbonded phase). Water is available to meet many requirements (e.g., USP, ACS, pyrogenfree, etc), and so it is imperative that the proper quality be used for the separation at hand.

weight-average molecular weight, M_w The weighted average mathematically represented as:

$$M_w = (w_1M_1 + w_2M_2 + \ldots + w_iM_i)/(w_1 + w_2 + \ldots + w_i)$$
$$= \sum(w_iM_i)/\sum w_i$$

where w_i represents the weight of each molecule, M_i.

wide-bore gas chromatography columns Columns having inner diameters of 0.53 μm are classified as wide bore. Note that HPLC columns typically do not have this classification.

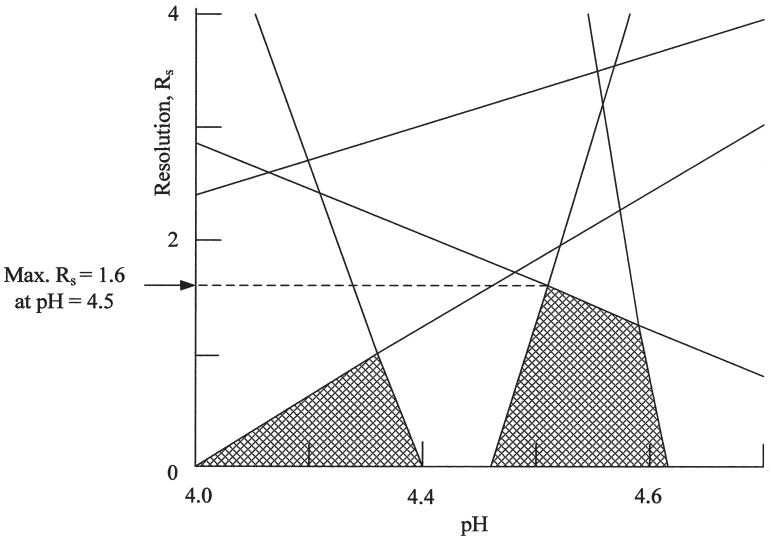
Wilke–Change equation Used to calculate the diffusion coefficient of a solute A in solvent B, D_{AB} (in cp):

$$D_{AB} = \left[7.4 \times 10^{-8}(\varphi_B M_B)^{1/2} T\right]/[\mu_B V_A]^{0.6}$$

where φ_B is an associations factor for B, M_B is the molecular weight of B, T is the temperature (°K), μ_B is the solvent viscosity, and V_A is the molar volume of A at its boiling point.

window diagram A visual optimization technique in the resolution of multiple peaks that are affected by one operating parameter. The operating parameter is changed in a precisely controlled fashion, and the resulting resolution for each pair of analytes in the mixture is plotted against the changed parameter. The point of intersection having the highest mutual resolution is the optimal operating point.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2



A window diagram is a visual optimization technique used to find the operational parameter that gives, in this case, the best resolution. Each line represents the resolution between two peaks in a chromatogram. Since there are six lines, this indicated that there are three analytes being considered and the resolution for AB, BC, and AC are plotted. Maximal resolution is at pH = 4.5 and gives an R_s of 1.6.

X

xerogel The end-product silica gel after polymerization, sineresis, and dehydration is called a xerogel. This is the form used for HPLC packing materials.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

Z

Z-average molecular weight, M_z Represents the second moment of the weight distribution for a sample and is mathematically represented as:

$$M_z = \left(w_1 M_1^{\,2} + w_2 M_2^{\,2} + \ldots + w_i M_i^{\,2}\right) \Big/ \left(w_1 M_1 + w_2 M_2 + \ldots + w_i M_i\right)$$
$$= \sum \left(w_i M_i^{\,2}\right) \Big/ \sum w_i M_i$$

where w_i represents the weight of each molecule, M_i. This method is very sensitive to changes in the high-molecular-weight region of the sample. It is very susceptible to error when noise is present in the system.

Z + 1-average molecular weight, M_{z+1} Represents the third moment of the weight distribution for a sample and is mathematically represented as:

$$M_{z+1} = \left(w_1 M_1^{\,3} + w_2 M_2^{\,3} + \ldots + w_i M_i^{\,3}\right) \Big/ \left(w_1 M_1^{\,2} + w_2 M_2^{\,2} + \ldots + w_i M_i^{\,2}\right)$$
$$= \sum \left(w_i M_i^{\,3}\right) \Big/ \sum w_i M_i^{\,2}$$

where w_i represents the weight of each molecule, M_i. This method is very sensitive to changes in the high-molecular-weight region of the sample. It is very susceptible to error when noise is present in the system.

zeolite A porous alkali-aluminum silicate that is used in specialized separations.

zero dead volume (ZDV) Refers to union and fittings that are machined in such a way as to minimize added extracolumn volume to the system.

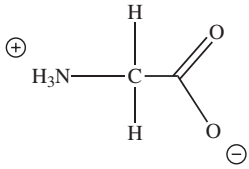
zeta potential, ζ The electrical potential established at the shear plane/double layer interface of a system where an external voltage is applied. In capillary electrophoresis the zeta potential represents

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

the potential difference between fixed charges on the capillary wall. This difference affects the electroosmotic mobility and drives the separation.

zirconia A packing material that has a basic repeating structure of ZnO_2 units. Zirconia is extremely stable to a wide range of pH. It is typically modified through the cross-linking of surface-adsorbed polymers containing reacting terminal groups. These groups then act as grafting sites for bonded phase groups (e.g., octadecyl).

zwitterion Formed from an ampholyte when the proton on the acid moiety is transferred to the base, resulting in a molecule that bears positive and negative separated charges. Therefore the zwitterionic form of glycine is:



Zwitterion

ACRONYMS

AA	atomic absorption
AFID	alkali flame-ionization detector
AOAC	Association of Official Analytical Chemists
ANOVA	analysis of variance
ASE	accelerated solvent extraction
ASTM	American Society for Testing and Materials
ACN	acetonitrile
APCI	atmospheric pressure chemical ionization (mass spectrometry)
API	atmospheric pressure ionization (mass spectrometry)
AU	absorbance unit
AUFS	absorption units full scale
BET	Brunauer, Emmett, Teller method
BOD	biological oxygen demand
BP	bonded phase, British Pharmacopoeia
BSA	*Bis*-trimethylsilylacetamide, bovine serum albumin
BSTFA	*N*,*O*-bis(trimethylsilyl)trifluoroacetamide
CCC	countercurrent chromatography
CD	cyclodextran
CE	capillary electrophoresis
CEC	capillary electroosmotic chromatography, capillary electrochromatography
CGE	capillary gel electrophoresis
cGMP	Current Good Manufacturing Practices
CI	chemical ionization (mass spectrometry)
CIEF	capillary isoelectric focusing
CITP	capillary isotachophoresis
CL	chemiluminescence
CMC	critical micelle concentration, carboxymethylcellulose
COD	chemical oxygen demand
COV	coefficient of variation
CPG	controlled-pore glass
CSP	chiral stationary phase
CZE	capillary zone electrophoresis

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

DAD	diode array detector
DCM	dichloromethane (methylene chloricle)
DEAE	diethylaminoethyl
DL	detection limit (d.l.)
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DNPH	dinitrophenylhydrazine
DQ	design qualification
ECD	electron capture detector; electrochemical detector
EDTA	ethylenediaminetetraacetic acid
EI	electron impact (ionization mass spectrometry)
EKC	electrokinetic chromatography
ELSD	evaporative light scattering detector
EOF	electroendoosmotic flow, electroosmotic flow
EPA	Environmental Protection Agency
EPC	electronic pressure control
ES	electrospray (mass spectrometry)
EtOAc	ethyl acetate
FAB	fast atom bombardment (mass spectrometry)
FAME	fatty acid methyl ester
FCC	Food Chemical Codex
FDA	Food and Drug Administration
FFF	field-flow fractionation
FID	flame ionization detector
FMOC	2-fluorenylmethyl-*N*-chloroformate
FPD	flame photometric detector
FSOT	fused-silica open tubular (column)
FTIR	fourier transform infrared (spectroscopy)
GC	gas chromatography
GFC	gel filtration chromatography
GLC	gas-liquid chromatography
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GPC	gel permeation chromatography
GSC	gas-solid chromatography
HECD	hall electrolytic conductivity detector
HDPE	high-density polyethylene
HETP	height equivalent to a theoretical plate
HFBA	heptafluorobutyric anhydride
HIC	hydrophobic interaction chromatography

HILIC	hydrophilic interaction chromatography
HMDS	hexamethyldisilazine
HPCE	high-performance capillary electrophoresis
HPLC	high-performance liquid chromatography
HPTLC	high-performance thin layer chromatography
IC	ion chromatography
ICH	International Conference on Harmonization
ICP	inductively coupled plasma
ID	inner diameter, identification
IDL	instrument detection limit
IEC	ion-exchange chromatography
IIC	ion-interaction chromatography
IQ	installation qualification
IPA	isopropyl alcohol
IPC	ion-pair chromatography
IR	infrared (detector)
ISO	International Organization for Standardization
KOT	knitted open-tubular (reactor)
LALLS	low-angle laser light scattering (detector)
LAN	local area network
LC	liquid chromatography
LCL	lower control limit
LDPE	low-density polyethylene
LDV	low dead volulme (fitting or union)
LIMS	laboratory information management system
LLC	liquid-liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
LSC	liquid-solid chromatography
MAGIC	monodisperse aerosol-generating interface for chromatography
MALDI	matrix-assisted laser desorption and ionization (mass spectrometry)
MALLS	multiangle laser light scattering (detector)
MDL	minimum detection limit, method detection limit
MDQ	minimum detectable quantity
MECC/MEKC	micellar electrokinetic chromatography
MEK	methylethyl ketone

MPM	mobile phase modifier
MS	mass spectrometry
MSD	mass selective detector
MSTFA	*N*-methyl(trimethylsilyl)-*N*-trifluoroacetamide
M*t*BE	methyl *t*-butyl ether
MTBSTFA	*N*-*tert*-butylsilyl-*N*-methyltrifluoroacetamide
MW	molecular weight
NBD	7-nitrobenzo-2-oxa-1,3-diazole (4-halide)
ND	not detected
NF	national formulary
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance (detector)
NP	normal phase
NPD	nitrogen-phosphorus detector
NPLC	normal-phase liquid chromatography
OD	outer diameter
ODS	octadecyl silica (C_{18} bonded phase support)
OPA	*o*-phthalaldehyde
OPLC	overpressured layer chromatography
OQ	operation qualification
OTC	over the counter
PAD	pulsed amperometric detector
PAGE	polyacrylamide gel electrophoresis
PAH	polyaromatic hydrocarbon
PBB	polybrominated biphenyl
PCB	polychlorinated biphenyl
PDA	photodiode array (detector)
PEEK	polyetheretherketone
PFPA	pentafluoropropionic anhydride
PFTBA	perfluorotributylamine
PGC	pyrolysis gas chromatography
PIC	paired-ion chromatography
PID	photoionization detector
PITC	phenylisothiocyanate
PLOT	porous-layer open tubular (column)
PM	preventive maintenance
PNA	polynuclear aromatic (hydrocarbon)
PQ	performatonce qualification
PSDVB	polystyrene-divinylbenzene
PTFE	polytetrafluoroethylene

PTH	phenylthiohydantoin
PVC	polyvinyl chloride
QSRR	quantitative structure-retention relationship
RCRA	Resource Conservation and Recovery Act
RI	refractive index
RF	response factor
RP	reversed phase
RPC	rotation planar chromatography
RPLC	reversed-phase liquid chromatography
RPTLC	reversed-phase thin-layer chromatography
RRF	relative response factor
RSD	relative standard deviation
SAX	strong anion exchange
SCX	strong cation exchange
SCOT	support-coated open tubular (column)
SD	standard deviation
SDS	sodium dodecyl sulfate
SEC	size-exclusion chromatography
SFC	supercritical fluid chromatography
SIM	selected (or single) ion monitoring (mass spectrometry)
SLS	sodium lauryl sulfate
S/N	signal-to-noise (ratio)
SOP	standard operating procedure
SS	stainless steel
SPE	solid-phase extraction
SPME	solid-phase microextraction
SRM	standard reference material
TBAH	tetrabutylammonium hydroxide
TCD	thermal conductivity detector
TEA	triethylamine
TEMED	*N*,*N*,*N*,*N*-tetramethylethylenediamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetamide
THF	tetrahydrofuran
TIC	total ion chromatogram
TLC	thin-layer chromatography
TMBS	trimethylbromosilane
TMCS	trimethylchlorosilane

TMS	trimethylsilane, trimethylsilica bonded phase
TOF	time of flight
TS	thermospray (mass spectrometry)
TSD	thermionic specific detector (NPD)
UCL	upper control limit
UHMPE	ultrahigh molecular (weight) polyethylene
USP	United States Pharmacopoeia
UV	ultraviolet (detector)
VOC	volatile organic compounds
WAX	weak anion exchange
WCX	weak cation exchange
WCOT	wall-coated open tubular (column)
ZDV	zero dead-volume (fitting or union)

REFERENCES

Capillary electrophoresis

K.D. Altria (ed.), *Capillary Electrophoresis Guidebook: Principles, Operation and Applications*, Humana Press, Totowa, NJ, 1996.

P. Camilleri (ed.), *Capillary Electrophoresis: Theory and Practice*, 2nd ed., CRC Press, Boca Raton, FL, 1997.

F. Foret, L. Krivankova and P. Bocek, *Capillary Zone Electrophoresis*, Wiley & Sons, New York, 1993.

M.G. Khaledi, *High-Performance Capillary Electrophoresis: Theory, Technique and Applications*, Wiley & Sons, 1998.

R. Weinberger, *Practical Capillary Electrophoresis*, Academic Press, San Diego, CA, 2000.

Countercurrent chromatography

W.D. Conway and R.J. Petroski (eds.), *Modern Countercurrent Chromatography*, Volume 93 in ACS Symposium Series, ACS Washington DC, 1995.

N.B. Mandava and Y. Ito, *Countercurrent Chromatography*, Volume 44 in Chromatographic Science Series, Marcel-Dekker, New York, 1988.

J.-M. Menet and D. Thiebaut (eds.) *Countercurrent Chromatography*, Marcel-Dekker, New York, 1999.

Field-flow fractionation

J. Janca, *Field-Flow Fractionation: Analysis of Macromolecules and Particulates*, Volume 39 in Chromatographic Science Series, Marcel-Dekker, New York, 1987.

M.E. Schimpf, K. Caldwell and J.C. Giddings (eds.), *Field-Flow Fractionation Handbook*, Wiley-Interscience, New York, 2000.

Gas chromatography

P.J. Baugh, *Gas Chromatography: A Practical Approach*, IRL Press/Oxford University Press, New York, 1993.

R.L. Grob (ed.), *Modern Practice of Gas Chromatography*, 3rd ed., Wiley-Interscience, New York, 1995.

W. Jennings, E. Mittelfehldt and P. Strempe, *Analytical Gas Chromatography*, 2nd ed., Academic Press, San Diego, CA, 1997.

H.M. McNair and J.M. Miller, *Basic Gas Chromatography*, Wiley & Sons, New York, 1998.

Illustrated Pocket Dictionary of Chromatography, by Paul C. Sadek.
ISBN 0-471-20021-2

High-performance liquid chromatography

B.A. Bildingmeyer, *Practical HPLC Methodology and Applications*, Wiley & Sons, New York, 1992.

P.R. Brown and R.A. Hartwick (eds.), *High Performance Liquid Chromatography*, Wiley & Sons, Volume 98 in Chemical Analysis Series, New York, 1989.

E. Katz, R. Eksteen, P. Schoenmakers and N. Miller (eds.), *Handbook of HPLC*, Volume 78 in Chromatographic Science Series, Marcel-Dekker, New York, 1998.

J.H. Knox (ed.), *High-Performance Liquid Chromatography*, Edinburgh University Press, Edinburgh, UK, 1982.

S. Lindsay, *High Performance Liquid Chromatography*, 2nd ed., part of Analytical Chemistry by Open Learning, Wiley & Sons, New York, 1992.

V.R. Meyer, *Pitfalls and Errors of HPLC in Pictures*, Hüthig GmbH, Hiedelberg, 1997.

V.R. Meyer, *Practical High-Performance Liquid Chromatography*, 2nd ed., Wiley & Sons, New York, 1994.

U.D. Neue, *HPLC Columns: Theory, Technology and Practice*, Wiley-VCH, New York, 1997.

D. Parriott (ed), *A Practical Guide to HPLC Detection*, Academic Press, San Diego, CA, 1993.

P.C. Sadek, *Troubleshooting HPLC Systems: A Bench Manual*, Wiley & Sons, New York, 2000.

R.P.W. Scott, *Silica Gel and Bonded Phases*, Wiley and Sons, New York, 1993.

L.R. Snyder, J.J. Kirkland and J.L. Glajch, *Practical HPLC Method Development*, 2nd ed., Wiley-Interscience, New York, 1997.

Solid-phase extraction and microextraction

J.S. Fritz, *Analytical Solid-Phase Extraction*, Wiley-VCH, New York, 1999.

J. Pawliszyn, *Solid Phase Microextraction: Theory and Practice*, Wiley-VCH, New York, 1997.

N.J.K. Simpson (ed.), *Solid-Phase Extraction: Principles, Techniques and Applications*, Marcel-Dekker, New York, 2000.

S.A. Scheppers Wercinski (ed.), *Solid Phase Microextraction: A Practical Guide*, Marcel-Dekker, New York, 1999.

E.M. Thurman and M.S. Mills, Solid-Phase Extraction: Principles and Practice, Volume 147 in Chemical Analysis Series, Wiley & Sons, New York, 1998.

Thin-layer chromatography

F. Geiss, *Fundamentals of Thin-Layer Chromatography: Planar Chromatography*, Hüthig GmbH, Heidelberg, 1987.

E. Hahn-Dienstrop, *Applied Thin-Layer Chromatography: Best Practices and Avoidance of Mistakes*, Wiley-VCH, New York, 2000.

J. Sherma and B. Fried, *Handbook of Thin-Layer Chromatography*, 2nd ed., Volume 71 in Chromatographic Science Series, Marcel-Dekker, New York, 1996.

J.C. Touchstone, *Practice of Thin-Layer Chromatography*, Wiley-Interscience, 1992.

Miscellaneous

J. Cazes (ed.), *Encyclopedia of Chromatography*, Marcel-Dekker, New York, 2001.

G.W. Ewing, *Analytical Instrumentation Handbook*, 2nd ed, Marcel-Dekker, New York, 1997.

J.C. Giddings, *Unified Separation Science*, Wiley-Interscience, New York, 1991.

B.L. Karger, L.R. Snyder and Cs. Horvath, *An Introduction to Separation Science*, Wiley & Sons, New York, 1973.

C.A. Poole, *The Essence of Chromatography*, Elsevier Health Sciences, Amsterdam, The Netherlands, 2002.

Journals

Chromatographia
Journal of Chromatographic Science
Journal of Chromatography
Journal of High-Resolution Chromatography
Journal of Liquid Chromatography and Related Techniques
Journal of Microcolumn Separations
LC/GC