A DICTIONARY OF CHROMATOGRAPHY

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Sources of Diagrams

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Introduction

The enthusiasm with which my previous book, *A Dictionary of Spectroscopy*, was greeted by all but a few reviewers has encouraged me to attempt the same type of presentation for chromatography.

Despite efforts to standardize terms and nomenclature[278,320] there is still much confusion in this realm. Where possible I have sought to use terms which appear to have become generally accepted, and at the same time to indicate how these are inter-related throughout the different forms of chromatography. Because detectors receive a variety of names in the chemical literature I have included as many of these names as possible and cross-referenced them in every case to the name that appears to be most frequently employed.

I would emphasize that this book, like *A Dictionary of Spectroscopy*, is not intended for the person who considers himself, or herself, an expert in the theory and practice of the subject. It has been written to provide a source of reference to the student, laboratory technician or general scientist seeking rapid information on chromatography. As such it not only includes the main equations covering theoretical chromatography, but also lists the equipment available for carrying out chromatography in all of its forms.

One of my objects in writing this book is to save people from the labours of having to wade through several advanced books on the subject to obtain adequate answers to relatively straightforward questions. At the same time I have tried to provide adequate references to both journals and textbooks in order that more detailed studies can be pursued where required. The various abbreviations employed throughout the book, such as h.p.l.c. and t.l.c., are explained in the appropriate place in the alphabetical order of the text.

I would be grateful for any constructive suggestions for inclusion of additional data or for the drawing of my attention to any errors that may have been introduced, in the hope that the necessary improvements can be made in any future edition.

Ronald C. Denney
January 1976

Symbols

The following symbols and letters are employed in the equations throughout the book:

A_i	peak area for solute i
A_R	detector response factor
a	weight of dry gel
a_i	proportionality factor for solute i
C_1	recorder sensitivity
C_2	reciprocal chart speed
C_3	mobile phase flow rate
C_G	coefficient of mass transfer in gas phase
C_S	coefficient of mass transfer in stationary liquid phase
c	solute concentration
D	detector output
D	dielectric constant
D_G	diffusion coefficient in gas mobile phase
D_M	diffusion coefficient in mobile phase
D_S	diffusion coefficient in stationary liquid phase
d_f	film thickness of stationary liquid phase
d_p	average particle size
d_R	retention distance on chromatogram
E	cohesive energy
$\boldsymbol{E}$	electric field strength
E_u	lower limit of detectability
F_o	mobile phase flow rate
H	height equivalent to a theoretical plate
h	reduced plate height
I	detector current
j	column pressure gradient correction factor
K	distribution (or partition) coefficient
K_{av}	proportion of gel medium available to solute
K_d	distribution coefficient (on gels)
k	capacity factor (or capacity ratio or partition ratio)
L	column length
M	migration value
M_r	molecular weight
n	number of plates
n_{eff}	effective number of plates
p	pressure
p_i	column inlet pressure
p_o	column outlet pressure
Q	quantity of sample
Q	molecular ionization constant
Q_E	nett charge on particle
R	gas constant
R_B	retention factor relative to Butter Yellow

R_F	retention factor (or relative front)
R_M	relative movement for related compounds
R_s	resolution
R_{st}	retention factor relative to a standard
R_u	background noise
r	radius of particle
r_o	radius of capillary
S	sensitivity
S	separation factor
S_r	solvent regain
T	temperature
t	time
t_M	retention time for non-sorbed species
t_R	retention time
t_R^0	corrected retention time
t_R'	adjusted retention time
$\bar{u}$	average linear gas velocity in column
u_E	electrophoretic mobility
u_i	inverse speed of chart paper
V	volt
V	volume
V_e	elution volume
V_g	specific retention volume
V_i	inner volume
V^l	molal volume of liquid
V_M	volume of mobile phase in column, dead (interstitial) volume of column. retention volume for air
V_m	volume of gel
V_N	nett retention volume
V_o	void (outer) volume
V_R	retention volume for solute
V_R'	adjusted retention volume
V_R^0	corrected retention volume
V_S	liquid stationary phase volume
V_t	total volume
w	peak width
Δw	width at half peak height
w_i	weight of sample i
w_L	weight of stationary liquid phase in column
w_r	water regain
x	molar fraction of solute
α	relative retention
α_G	retention volume ratio
β	phase ratio
γ	labyrinth factor
δ	solubility parameter
ε^0	solvent strength
ζ	zeta potential
η	viscosity
$[\eta]$	limiting viscosity number
λ	packing constant
μ	statistical mean
μ	micro
ν	reduced velocity
ρ	specific gravity of solvent
σ	standard deviation
σ_d	detector sensitivity
σ_c	sensitivity of chart recorder
Φ	Flory Fox constant

A

Absolute detector sensitivity
This is the change necessary in the physical parameter to obtain full-scale deflection of the recorder at maximum detector sensitivity for a defined level of noise (*q.v.*). It is employed particularly in connection with h.p.l.c. detectors. *See also* Relative sample sensitivity; Sensitivity; Signal-to-noise ratio.

Absolute retention volume
See Nett retention volume.

Absorption
Penetration in bulk of one material throughout a second material is termed absorption. It is usually associated with the formation of fairly strong chemical bonds and is rarely reversible at ambient temperatures. Dissolution of gases in liquids frequently involves absorption forces. The main difference between this and adsorption (*q.v.*) is that the former involves fairly uniform penetration of the absorbent matrix, whilst the latter occurs at the surface, and the forces involved in adsorption are very much less than the chemical forces of the absorption process.

Acidic cation exchanger
See Cation exchanger.

Activation of adsorbents
In general the activity of the adsorbents (*q.v.*) employed in t.l.c. and other forms of adsorption chromatography is increased as the water content is reduced. To obtain the greatest degree of activity, kieselguhr (*q.v.*) and silica gel (*q.v.*) plates are usually dried at 100 °C for one hour. They are then stored in a dry chest until required as the activity is lost by adsorption of atmospheric moisture. Alumina (*q.v.*) plates vary greatly in activity depending upon the period of time and temperature at which drying has been carried out. The activity of alumina

columns and plates is established by use of the Brockmann scale (*q.v.*) of dyes.

Adjusted retention time t'_R
See Retention time.

Adjusted retention volume V'_R
This is the retention volume (*q.v.*) for the substance less the retention volume for a non-sorbed species:

$$V'_R = V_R - V_M$$

In g.c. the non-sorbed species is air, and the value for the adjusted retention volume is therefore the retention volume corrected for the dead volume (*q.v.*) in the sample port, detector and connections.

Adsorbents
Any material which will adsorb one substance in preference to another from solution can be employed as an adsorbent in adsorption chromatography. As a result a very wide range of materials has been used. Those most commonly employed for g.s.c. include activated carbon, silica gel (*q.v.*) and microporous polymers. For t.l.c. the most common are alumina (*q.v.*), cellulose, kieselguhr (*q.v.*) and silica gel. In some cases the adsorbents for t.l.c. contain a small amount of added gypsum which acts as a binder (for example in *Silica gel G*). To assist detection of zones they may also contain a fluorescing indicator.

Adsorption
This is a process which occurs at the surface of a liquid or solid as a result of the attractive forces between the adsorbent and the solute. These forces may be physical, such as van der Waals forces (*q.v.*), or weakly chemical, as in the case of hydrogen bonding (*q.v.*). Physical adsorption is associated with low heats of adsorption whilst chemical adsorption usually involves higher energy changes. As a result chemical adsorption is stronger than physical adsorption.

Adsorption probably occurs at all surfaces and interfaces to some extent, but for use in separative processes it is common to employ

porous substances possessing large effective surface area to mass ratios.

Adsorption processes such as those in g.s.c. are normally carried out at higher temperatures than are the corresponding separations by partition on g.l.c. The higher temperatures are necessary in order to obtain separations in a reasonable period of time from the g.s.c. column.

Adsorption chromatography

This form of chromatography employs an adsorbent such as silica gel (*q.v.*) or alumina (*q.v.*) as a solid stationary phase, and either a liquid mobile phase (in t.l.c. and adsorption column chromatography) or a gas mobile phase (in g.s.c.). Separation is dependent upon the different extents to which solutes are adsorbed by the solid: the less strongly adsorbed materials travel faster than the strongly adsorbed materials.

The introduction of adsorption chromatography is usually credited to Michel Tswett[291,292], although his work on the separation of plant pigments was published at about the same time as Day's report[67] on the separation of coloured oils by percolation through earth. Real credit for the first useful studies on adsorption columns should, however, go to Reed[248] whose work, published in 1893, included separations both of iron(III) chloride and copper(II) sulphate, and of alkaloids on columns of powdered kaolin.

Adsorption detector

See Thermal adsorption detector.

Adsorption isotherm

In adsorption chromatography the distribution coefficient (*q.v.*) is dependent upon the concentration of the substance in solution. At a constant temperature the distribution of the solute between the solid adsorbent stationary phase and the liquid mobile phase can be represented in a graphical form as the adsorption isotherm, being a pictorial presentation of the concentration in the mobile phase (per unit volume) in equilibrium with the corresponding concentration in the stationary phase (per unit weight). The shape of the adsorption isotherm is a guide to the performance of the solute and adsorbent

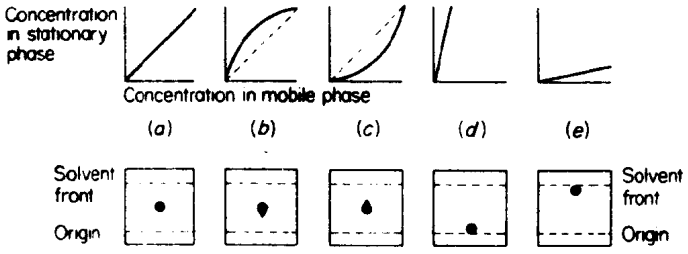

Figure A1. Relationship between adsorption isotherm and sample movement on thin layer chromatography

under t.l.c. conditions, as shown in Figure A1. The ideal linear isotherm, representing constant distribution between the two phases at all concentrations, is rarely achieved over a large concentration range as all substances in solution affect each other. The convex isotherm (Figure A1*b*) gives rise to tailing bands or spots, whilst the concave isotherm (Figure A1*c*) produces leading bands or spots. It is tailing of this type which frequently makes resolution in adsorption chromatography so difficult. Figure A1*d* and A1*e* indicates how the angle of the line is related to the R_F value (*q.v.*) for the solute.

With zeolites (*q.v.*) the adsorption isotherm is a measure of the amount of material adsorbed related to the applied pressure.

Aerogels

If removal of the dispersing agent from a gel system can be achieved without shrinkage of the gel structure the rigid form obtained is called an aerogel. In practice this name is now applied to any rigid structure that can be employed for gel permeation chromatography (*q.v.*) even if it is not strictly a gel. The term not only includes silica gel beads but also the modern glass aerogels[128] prepared by heat treating borosilicate glass to produce channels with regular dimensions of 75–2000 Å (7.5–200 nm) in the glass powder. These rigid glass and silica gel aerogels are of particular importance for gel permeation work carried out by h.p.l.c. as they retain their structures even under pressure, whilst xerogels (*q.v.*) will collapse.

Affinity chromatography

This is a procedure first described by Campbell *et al.*[43] in 1951 and used to obtain a highly selective purification of bio-molecules by employing a medium consisting of a gel matrix combined with an enzyme or nucleic acid[63]. The biospecific adsorbent is prepared by coupling a specific ligand (such as an enzyme, antigen or hormone), for the macromolecule of interest, to a water-insoluble carrier by means of a free carboxyl, amino or phenolic group[120]. By making the column selective or specific in this way only those molecules able to bind with the immobilized ligand are retarded and held on the column[308,315], to be later released in a purified state as required.

Agar-agar

This polysaccharide is obtained by extracting red seaweed; it is soluble in hot water and on cooling the solution forms a firm gel. Although agar itself has been employed for chromatographic separations[235], it is more commonly separated into its two main components—Agarose (*q.v.*), the neutral component, and agaropectin, which contains carboxylic and sulphonic acid groups[7].

Agarose

The neutral component of agar-agar (*q.v.*) is known as Agarose and is obtained after acidic components have been precipitated by acetyl pyridinium chloride[144] or by fractional precipitation with polyethylene glycol[254].

Chemically it is a high molecular weight polysaccharide[7] formed from β-D-galactopyranose and 3,6-anhydro-α-L-galactopyranose units, with a composition corresponding to $[C_{12}H_{14}O_5(OH)_4]_n$. The gel is used for separating substances with high molecular weights in the range 10^3–10^8 and for studies on viruses, phages and bacteria. Only aqueous solutions can be employed as organic solvents destroy the gel structure[75].

Alkali metal flame ionization detector

See Thermionic detector.

Alumina

Chemically represented as $Al_2O_3.xH_2O$, alumina is one of the most common materials employed as a stationary phase (*q.v.*) in adsorption chromatography. It has a large surface area and a high porosity providing a retentive, basic surface suitable for separating neutral and basic compounds. It acts as an adsorbent by forming hydrogen bonds (*q.v.*) between its own hydroxyl groups and functional groups on the solute. It is frequently used with no other additives, but may also contain 5% gypsum as a binder (Alumina G), and/or fluorescent indicators for viewing spots under ultraviolet light. The activity of alumina, which is determined by the Brockmann scale (*q.v.*) decreases with its water content. It is usually available in five grades, the most active being grade I.

Alumina G

See Alumina.

Ambient operation

This term is used most commonly in connection with h.p.l.c., referring to operation of the chromatograph at room temperature without the application of temperature programming (*q.v.*) or elevated temperature. Flow programming (*q.v.*) and gradient elution (*q.v.*) are usually carried out under ambient conditions. *See also* Sub-ambient operation.

Amino acid analyser

Amino acids are differentially retarded, separated and eluted in a regular sequence according to their basic, neutral and acidic characteristics by a very specialized application of ion exchange chromatography (*q.v.*). The process, first described by Spackman *et al.*[273] in 1958, may employ as many as five ion exchange columns and either a series of buffer solutions or a gradient buffer mixture[237]. The detection of individual amino acids in the eluate is carried out photometrically by the measurement of absorbances at 440 nm and 570 nm from colours produced between the amino acids and ninhydrin reagent at 100 °C. Amino acid analysers are manufactured commercially and compact systems have been designed[129,130].

Ampholytes

These are substances which carry both positive and negative charges

(they are amphoteric). Typical ampholytes are amino acids and proteins which possess both $—COO^-H^+$ and $—NH_3^+OH^-$ groups and for which the structure at any time is *p*H dependent in the following manner:

$$H_2N—\underset{\displaystyle R}{\underset{|}{CH}}—COO^- \rightleftharpoons H_3N^+—\underset{\displaystyle R}{\underset{|}{CH}}—COO^- \rightleftharpoons H_3N^+—\underset{\displaystyle R}{\underset{|}{CH}}—COOH$$

at alkaline *p*H — at isoelectric point — at acid *p*H

Separation of such substances by electrophoresis (*q.v.*) is very *p*H dependent.

Amphoteric ion exchange resins

Special bipolar ion exchange resins carrying both positive and negative ionic active groups are known as amphoteric ion exchange resins. Both types of active group are able to participate in ion exchange processes, frequently carried out simultaneously on the same solution. Studies have shown[214] that the single groups react independently and stoichiometrically.

They consist of a linear cross-linked mixture of polymers comprising a cation exchanger entwined within an anion exchanger. This structure is achieved by polymerizing the cation exchanger monomer within the structure of the previously formed anion exchanger producing the 'snake cage' structure required for ion retardation (*q.v.*). Such resins are employed to remove electrolytic impurities from liquids[134] by the preferential retardation of the electrolyte due to the formation of ion pairs with the resin.

Anion exchangers

Any ion exchanger (*q.v.*) solely capable of exchanging anions is known as an anion exchanger. The synthetic ion exchange resins used for this purpose are insoluble polymeric bases which produce insoluble salts as a result of the ion exchange operation. There are two main categories of anion exchanger—the strong base exchangers and the weak base exchangers. A typical strong anion exchanger is prepared from the polymerization of styrene and divinylbenzene, followed by chloromethylation and treatment with an appropriate secondary or tertiary amine.