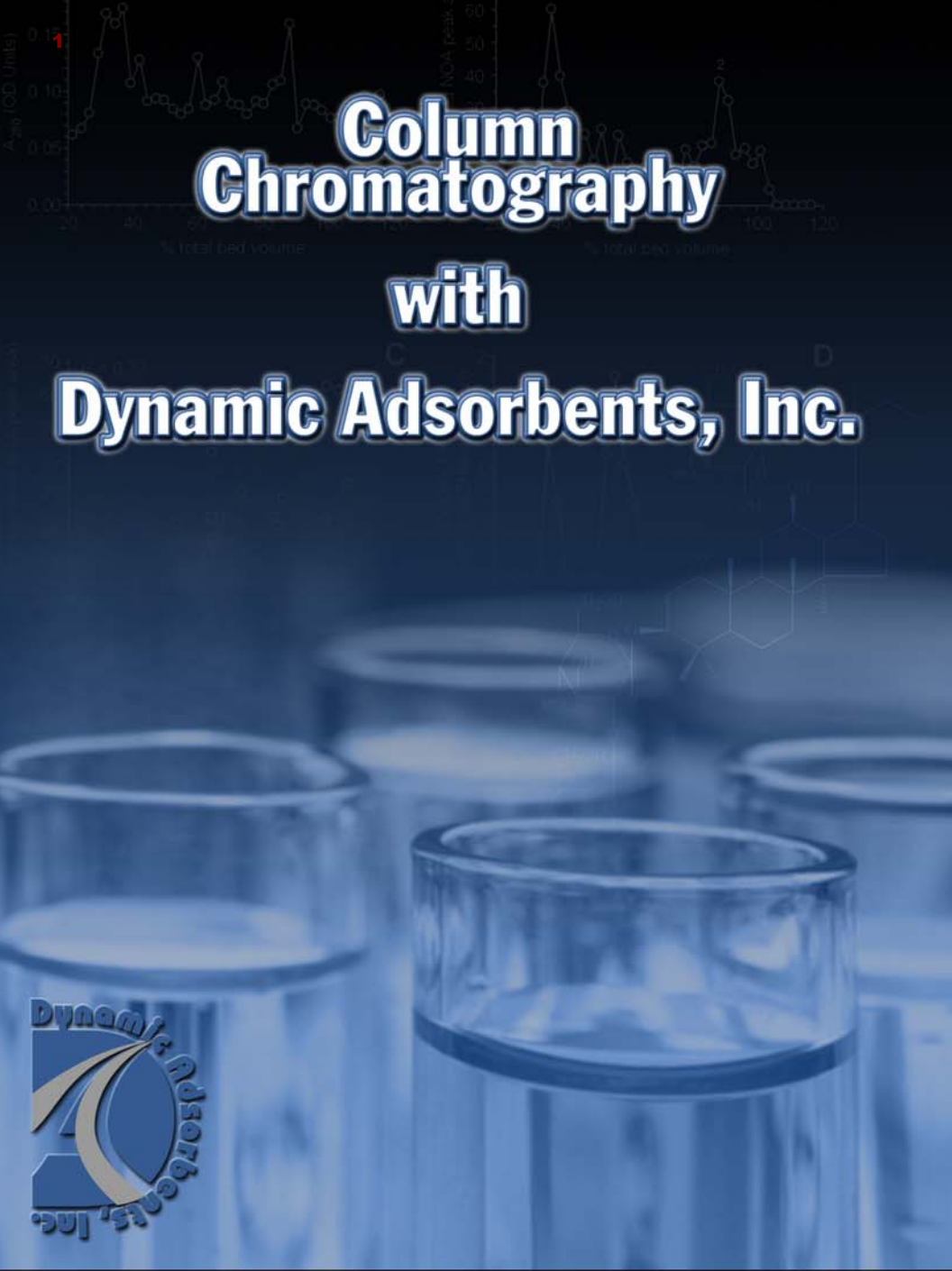
Column Chromatography with Dynamic Adsorbents, Inc.



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The authors recommend the use of DAI Alumina and Silica adsorbents which are manufactured to the specifications detailed in the following pages.

Column Chromatography using DAI Adsorbents

This short primer describing column chromatography is dedicated to users of our fine adsorbents. The objective is not to encompass textbook precision or to introduce the latest developments in the field. This primer presents basic knowledge of the art of chromatography. Using techniques in this primer many common problems may be eliminated. This guideline opens the pathway for the task of chromatography: **The reproducible separation of complex mixtures in a rapid and economical manner.**

Chromatography, a Physical Chemical Separation Technique

Chromatography constitutes a physical chemical technique for separating mixtures of compounds. This separation occurs within a **system** comprising a stationary phase and a **mobile phase.**

The separation is achieved using **reversible equilibrium mechanisms**.

A **resolvable mixture** consists of compounds differing in their **affinities** relative to the **interface** between stationary and mobile phases. These affinities may be measured as (reaction) enthalpies.
Separation can be achieved even if these affinities differ slightly, because each single molecule interacts multiple times with all participants of these **dynamic equilibria.** This dynamic interaction creates the enrichment for similar molecules at well defined **locations.** The location within a given system is specific for each species of molecule.

Each array of such a collection of identical molecules is shaped under the influence of **time** and **flow** into **zones** of compounds. While these zones will initially overlap they move apart during their migration through the system resulting in pure monosubstances.

This primer will address and illustrate the overall concept of chromatographic separation. The tools of chromatography feature elements of art, science and lots of trial and error.

Column Chromatography, a Specific Chromatographic Setup

Column Chromatography is a specific type of Chromatographic method. Separation occurs within a sorbent bed shaped into a cylindrical tube column. The **sorbent bed** comprises the **stationary phase** and is more simply called **sorbent.** The mobile liquid phase is usually called **eluent,** even if with some non-elution techniques (TLC, dry column) an actual elution is omitted.

Sorption environment is a term describing the interaction of all of the physical and chemical forces which occur at the accessible interface formed by the stationary phase in contact with the mobile phase. This is a dynamic, rather than a static interface.

The sorption environment includes the interaction with the constituents of the sample. Some equilibrium mechanisms useful for chromatographic techniques include:

- Adsorption
- Partition
- Ion Exchange
- Gel Permeation.

The **mixture to be** separated **i.e.** the **sample** is brought into a state allowing each component to move freely about and to change freely between stationary and mobile phases: the sample mixture must be in either a liquid or gaseous state.

The Retention

As the mobile phase flows along the physical surface of the stationary phase the irregularly mixed volumes of solute interact with the solid or immobilized liquid surface. This interaction is accomplished by each individual type of solute (sample) attaching to and residing (at/or dissolved) by the stationary phase for a certain period of time. **During this period of physical interaction the front of a given solvent volume continues its migration, leaving interacting solute behind: the molecule is retained for a shorter or longer period of time at the given stationary phase.** Each individual type of molecule exhibits a chromatographic retention pattern which is specific and unique for that molecule. As equilibria between the stationary and mobile phases occur, these equilibria generate a dynamic behavior for the solute molecules. Each molecule has a chance to migrate in the direction of the flow during its stay in the mobile phase. The fraction of time of attachment to this solid or solidified surface is specific for each species of molecules due to the existence of a **multi-step** process. By this mechanism discrete zones of identical molecules eventually develop. These zones possess borders as well as **points of gravity** (peak maxima). As time elapses during their migration within the column, these discrete zones drift apart from each other until only identical molecules are found within the borders of each zone: separation is achieved when a volume increment of pure solvent separates each zone.

The Elution

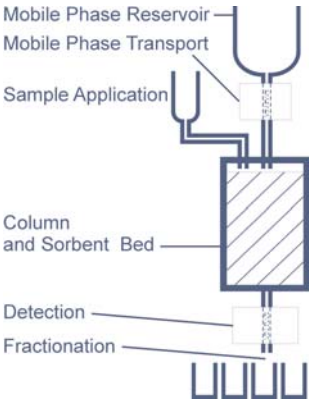
After **a** certain time period, the mobile phase carries individual zones out of the column. This sequence of exit from the system and time of residence within the system is specific for each type of molecule within the given separation environment.

The **eluate** leaving the system is divided into many unique **fractions.** These fractions may then be further analyzed depending upon specific needs.

The Chromatographic Apparatus

Chromatography can be simplified into the following components: (See attached illustration)

- mobile phase reservoir
- sample application and injection system
- column tubing
- detector (plus electronics plus recorder)
- fractionation (receptacle for eluate)



Physical forces allow the mobile phase to flow past the surface of the solid or solidified phase. This flow pattern is accomplished with the aid of tubes of appropriate shape, dimension and construction.

A simple chromatograph is illustrated by a glass tube fitted with a bulb at its upper end while its lower end is closed with a permeable wad of cotton. The cylindrical section (glass tube) is filled with sorbent. The sample to be separated out is applied at the upper end using a pipette. The bulb acts as a reservoir for the mobile phase. The physical force of gravity forces the eluent down the sorbent bed. The migration of the constituents can be detected and the sample separated or fractionated with the aid of a number of glass beakers.

The principles outlined in this sample design illustration equally apply to the most sophisticated and up-to-date technologies which may include sophisticated mobile phases, highly sensitive detectors, and precise and computerized sample injection devices. No matter how sophisticated the chromatographic system the limiting factor in function is the quality of the sorbent utilized in the separation process**. This is because the sorbent alone accomplishes the separation**. For this reason the use of highly standardized sorbents are recommended to achieve the most reliable and reproducible separation results.

The Chromatographic Process

Column Chromatography (CC) is a chromatographic solution utilizing an interaction between mobile and stationary phases to purify desired substances. Rationale design for achieving chromatographic separation maximizes the means for combining a large surface interface of sorbent with the eluent under study. Methods to achieve maximum surface interaction and obtain the best separation include:

1. Using a very long collection tube of very small diameter.
2. Placing a series of small tubes in parallel. A collection of small parallel tubes is formed by the various interstitial volumes of a porous sorbent packing.

Both approaches are in common use.
The separation process within the sorbent bed (the “column”) is influenced by the following parameters:

a. length of column
b. flow rate of the mobile phase
c. specificity of the sorbent particle
d. diameter of the sorbent
e. activity of the sorbent temperature
f. polarity of the solvent
g. amount of sample

The Stationary Phase

The **stationary phase** consists of an **active solid** which is simply the carrier of a separation. The active phase is either **impregnated** to the carrier or **chemically bonded** to it. Active stationary phases are adsorbents, ion exchangers and molecular sieves. Active liquid phases are long-chain hydrocarbons, long-chain alcohols, or water. They are soaked up by a porous substance (carrier) like silica or diatomaceous earth (guhr). The reaction of the Si-OH group of silica gel with long-chain carbon compounds (C_8, C_{18}) having Si-Cl or Si-OR moieties is a prime example of a **chemical bonding.**

The chromatographic properties of a uniform, non-modified solid are governed within narrow limits by the energy pattern of its accessible surface. Therefore, solidified material (i.e. impregnated surfaces on solid supports) allows a wide range of selectivity when very specific separating mediums are used. An equally wide range of modified surfaces are accessible by using compounds which react with solid surfaces such as modified silica.

According to the physical states of the phases used, the following types of chromatographic systems can be utilized:

		1. Mobile Phase	
		liquid	gaseous
2. active suface or stat. Phase	solid	liquid/solid chromatography LSC	gaseous/solid chromatography GSC
	liquid	liquid/liquid LLC	gaseous/liquid GLC

In this primer only systems using at least one liquid phase will be discussed. (In some instances, however, it will be necessary to refer to the gaseous phase for clarity's sake). Some of the terms frequently used are categorized according to the above schematic.

Liquid Chromatography:	liquid/solid or liquid/liquid
Adsorption Chromatography:	liquid/solid
Ion Exchange Chromatography:	liquid/solid
Gel Permeation Chromatography:	liquid/solid
Affinity Chromatography:	liquid/solid
Partition Chromatography:	liquid/liquid
Reverse Phase Chromatography:	liquid/liquid or liquid/solid

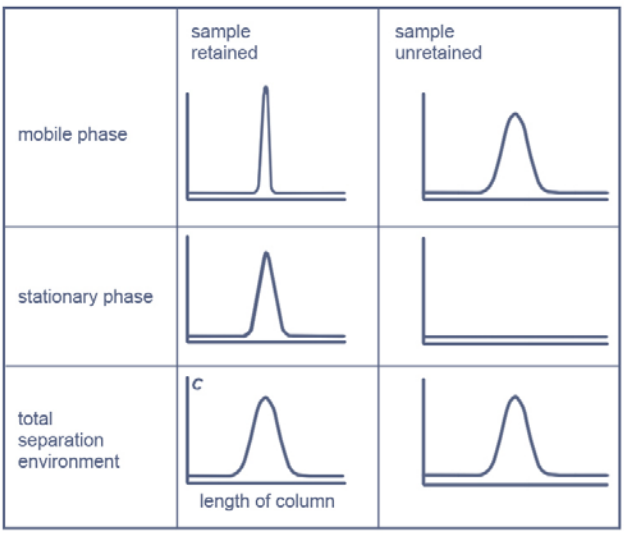
The first approach is useful for gas-liquid chromatographic setups because of the reduced diffusion times and relatively low pressures required for forcing a gas through a long and narrow coated capillary. The process is also useful for gas-solid chromatographic systems where the inner walls of a capillary are coated with a monomolecular layer of an appropriate solid (WCOT).
The second approach uses porous packing. A sorbent bed of this type may be used in both column chromatography and thin-layer chromatography systems.
Column chromatographic systems in general use cylindrical, uniform porous sorbent beds (columns) efficient when their length measures approximately 10 diameters. A chromatogra-phic thin layer is a column of extreme dimensions: a cylinder rolled open on a rigid support, forming a very thin column as long as it is wide.

Substances with and without Retention

For example, a solution containing two types of molecules are contained together in a mixture and need to be separated from each other. In this model, the solution may contain molecules having unique properties which either allow the molecules to interact with the stationary phase or go directly through the stationary phase with no interaction.

If various molecules contained within an initial chromatographic zone are offered to the interfaces of the sorbent bed, two fundament types of molecules can be distinguished:

a) Retained molecules = compound with retention; and
b) Non-retained molecules = compounds without retention = inert compounds = compounds which do not interact with the stationary phase = unretained compound which passes right through the sorbent bed, without any interaction with the sorbent.

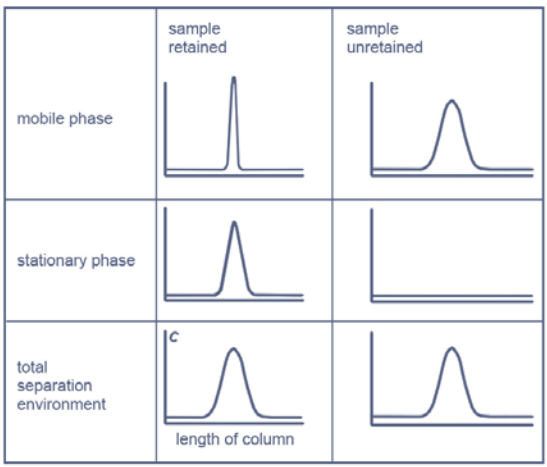


The moment a substance (sample) is applied to the top of a chromatographic bed at T = O a zone or plug of uniform coverage and rectangular shape is generated. If this substance is one of the retained types, then the coverage of the active surface of each sorbent particle within this volume will be uniform. The concentration of this compound within the neighboring mobile phase in direct contact with the solid and located within the appropriate interstitial volume will also be uniform under the aforesaid conditions. Both the coverage of the solid phase and the concentration within the adjoining liquid phase correspond to each other and are governed by the principle of equilibrium.

In the case of an unretained sample, all of its molecules remain within the liquid of the **interstitial volume** on the upper section of the chromatographic bed. Concentration changes are experienced after the liquid comes into contact with the sorbent; i.e. none of the molecules are attracted by the surface forces of the solid.

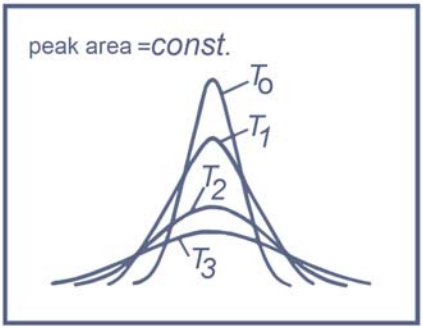
The Peak

The expanse of **a** mixed volume consisting of a sample partitioned solid and liquid phase (non-partitioned in the case of an inert sample) is called a **peak**. In actuality, there is no differentiation regarding the actual location of the sample substance, whether located in the mobile and/or liquid phases. The location of the substance relative to the length of the column and its quantity per unit sorption environment is considered here (concentration vs. column length).



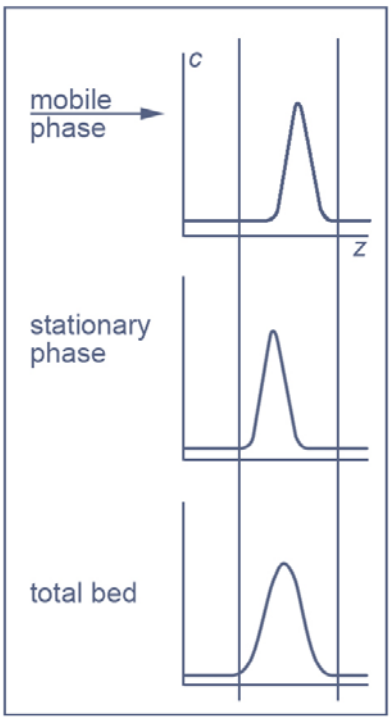
The diagram describes the chromatographic state of a non-eluted sample within the chromatographic bed (not to be mistaken for the concentration profile of an eluted peak). For an eluted peak the concentration of the sample within the eluent is plotted vs. the elution time or the eluted volume (c/t or c/R diagrams), and no longer includes considerations relative to the solid phase.

Using the vantage point of the c/z-plot, the initial peak will be oblong and sharp edged, and should be as narrow as possible for optimum column performance. During its migration from the top of the column towards the bottom, the peak will lose its sharp edges and will finally be transformed into a bell shaped curve. The initial oblong box of bulk sample experiences different interactions over time. To a large extent, diffusion and flow irregularities will cause physical and chemical interaction changes. In the ideal case, a Gaussian curve will be generated. Its base broadens due to physical factors such as time dependence, geometrical shape of the sorbent bed, specific properties of sorbent, temperature, flow and a number of other factors which sometimes cannot be completely specified or pinpointed.



As a rule, (exemptions exist) the width at the base will continuously expand during the separation process. One of the prime tasks in chromatography is to apply the sample at a minimum width, i. e. with maximum concentration, while keeping the peak from broadening during the separation. As the initial peak becomes smaller, resolution is enhanced.

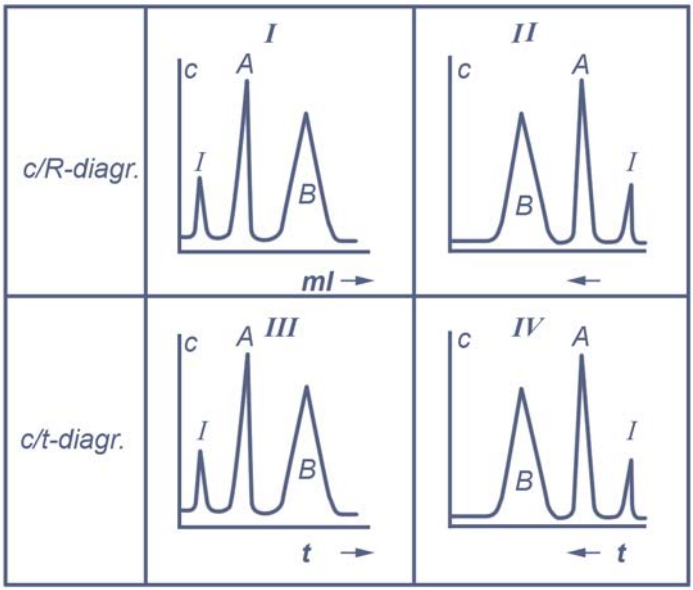
There is only one peak profile for the substance within the mobile phase and one peak profile for the fraction dynamically adhering to the solid phase; considering the sorbent, mobile phase and type of equilibrium used (e. g. adsorption). The peak of the dissolved sample within the mobile phase migrates ahead of the substance attached to the stationary phase. The points of gravity of the two partial peaks under investigation will never come to coincide during the mass transfer through the bed unless the flow comes to a stop. **Therefore, true equilibrium values measured by a static experiment will never exist. The dynamics of the process will never allow true equilibrium while the mobile phase is flowing.**



This phenomenon accounts for peak broadening due to mobile phase flow. It may be reduced but it can never be completely eliminated.
Other inherent properties of the stationary phase have considerable influence on peak broadening. A reduction of these adverse characteristics may be achieved by using standardized sorbents.

The Chromatogram

Peaks resulting from column separations are normally plotted graphically as elution profiles immediately upon leaving the column. Concentrations of compounds dissolved by the eluent are plotted vs. the volume leaving the column or vs. time.

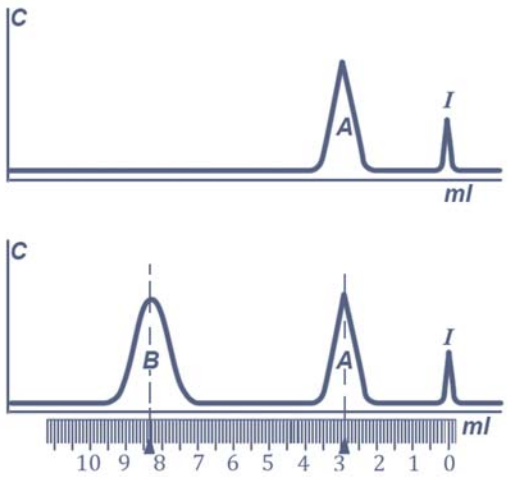


Graphic plots represent the time or volume axis and run on the paper from right to left or vice versa. If the paper leaves the recorder from left to right, plots are written from right to left, such as shown in graphs II and IV above.

Measuring Retention and Resolution

The values of an inert peak (=unretained peak) indicate the dead volume of a column used If volumes of tubing are disregarded, together with time and solvent flow. This peak indicates the location of an imaginary solvent front, depicting the travel of all retained compounds after their application to the column top. More simply, it indicates the “dead time” or the time elapsed while the imaginary solvent front reappears at the base of the chromatographic column.

In the case of one unretained (I) and one retained (A) substance **(R = 29** ml retention volume) the upper chromatogram will be received at the bottom of the column. If an additional substance B with a different retention volume **(R = 83** ml) is present, the lower chromatogram will result. If all 3 compounds leave the column one after another, they are considered separated. If no cross-over contamination is present, the compounds are considered **completely separated**.



In order to facilitate the interpretation of major chromatographic formulae, understanding the following symbols is useful:

t = time in seconds elapsed between two given moments, e. g. application of any solute and the appearance of its peak maximum = retention time

t_0 = time in seconds elapsed between two given moments, e. g. application of an unretained solute and the appearance of its peak maximum: dead time

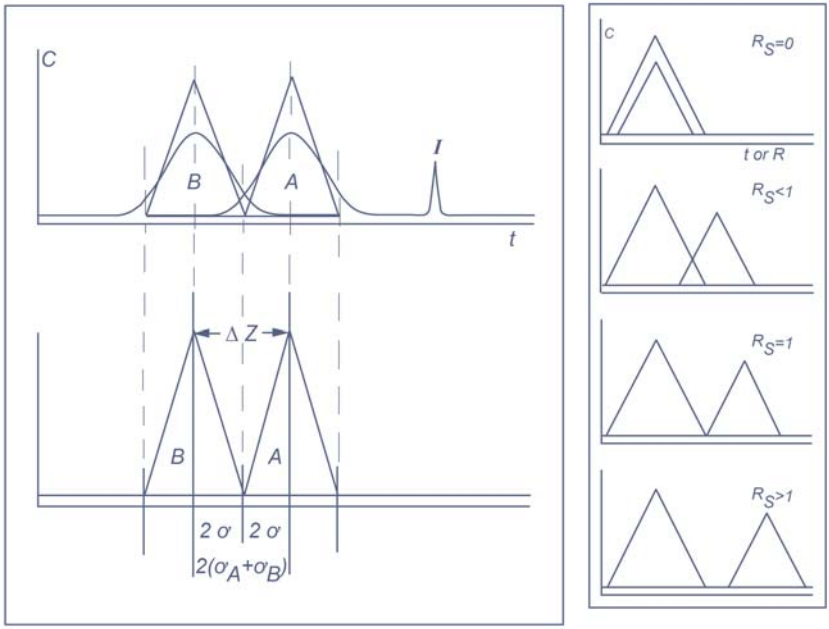
t_ = $t-t_0$ = adjusted retention time = actual time of residence within the chromatographic system.

R = elution volume, measured in ml eluent e. g. from the moment of application of a retained solute until the appearance of the maximum of a retained peak = retention volume

R_0 = elution volume measured in ml eluent, e.g. from the start of application of an unretained solute until the appearance of the maximum point of an **unretained** peak = dead volume.
R R-R_0 = v • t_ = adjusted retention volume
v = flow rate of the eluent, measured in ml/min
v = flow velocity of eluent, measured in mm/sec
z = column length
Az = difference between two peak maxima measured either in time or in volume units
Rs = resolution of two peaks, no dimension.

The degree of separation - the resolution R_s - is a well defined ratio. A chromatogram is used to calculate its value (c/t or c/R).

The peaks used for the determination of R_s are simplified by forming triangles, whereby the tangents to the points of inflexion and their interaction with the baseline are used. The distance between these two points of interaction is called **zone width** or **bandwidth** (e. g. expressed in time units) and measure close to 4 standard deviations of the Gaussian error curve. Each portion of the base line located left of the perpendicular line through the peak of substance A and right of the line through the peak of substance B equals 2 ($2ó_a$ and $2ó_b$).

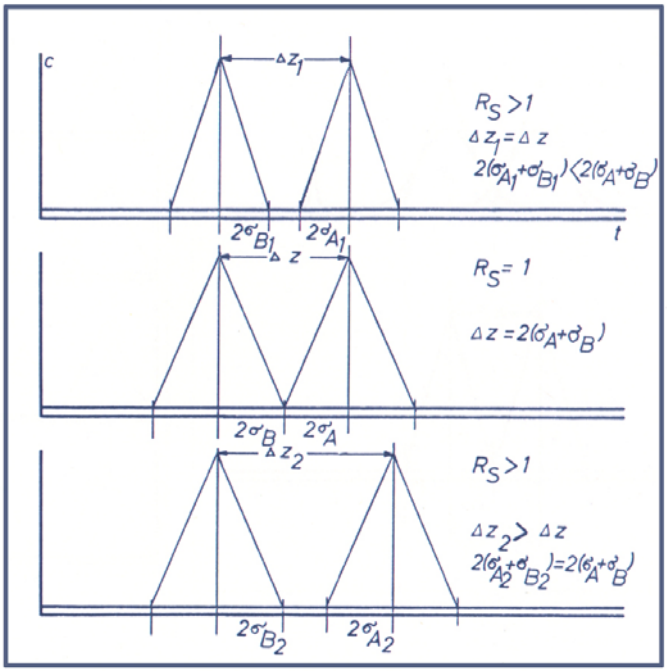


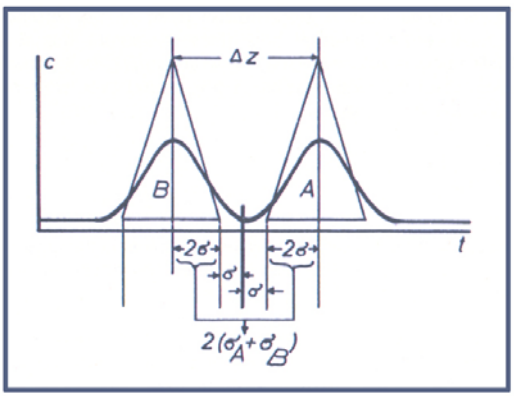
The peaks of each individual triangle marks the retention (time or volume) for the pertinent compound when measured from a standardized point on the chromatogram (e. g. the unretained peak), marking the adjusted retention (time or volume). It is of secondary interest whether to use adjusted or non-adjusted values when computing resolution R_g. The distance of the peak maxima Ä z is placed over two times the sum of the sigmas of the two compounds in question:

$$R_S = \frac{\Delta z}{2(\sigma_A + \sigma_B)}$$

In the case of the triangles of two compounds joining each other at the edges of their base lines, the resolution equals 1 and both compounds are considered completely separated; the maxima is located apart at a distance of 4 (“4 - Ó separation”).

When R_s equals zero, the peaks of the two compounds are placed one over the other where the peak maxima shows an identical position relative to its location over the abscissa.


c
Δz_1
$R_S > 1$
$\Delta z_1 = \Delta z$
$2(\sigma'_{A1}+\sigma'_{B1}) < 2(\sigma'_A+\sigma'_B)$
$2\sigma'_{B1}$
$2\sigma'_{A1}$
t
Δz
$R_S = 1$
$\Delta z = 2(\sigma'_A+\sigma'_B)$
$2\sigma'_B$
$2\sigma'_A$
Δz_2
$R_S > 1$
$\Delta z_2 > \Delta z$
$2(\sigma'_{A2}+\sigma'_{B2}) = 2(\sigma'_A+\sigma'_B)$
$2\sigma'_{B2}$
$2\sigma'_{A2}$


c
Δz
B
A
t
$2\sigma'$
$2\sigma'$
σ'
σ'
$2(\sigma'_A+\sigma'_B)$

When discussing actual curves and considering their (asymptotical – see glossary) approach to the base line, it is evident that some overlapping does exist even with a 4 - ó separation at R_s = 1, and it is easy to understand that there are two factors present, improving the resolution:

a) a larger distance Ä z between the peak maxima, while the bases of the triangles remain
   constant and
b) a reduction of the bases (squeezing of the peaks) while Ä z remains constant.

Not before R_s = 1.5 (“6 - ó separation”) a separation of two substances can be considered really complete. Such a state may be called a “baseline separation”, i. e. the concentration of any two samples in the (even minute) increment of the mobile phase between two peaks does then equal zero. If the fractions are cut exactly where the concentration curves join the baseline, each individual fraction will consist of a solution of a pure compound dissolved in the (pure) mobile phase. Fractions between those two peaks consists only of pure mobile phase.

The separation process within the sorbent bed (the “column”) is influenced by the following parameters:

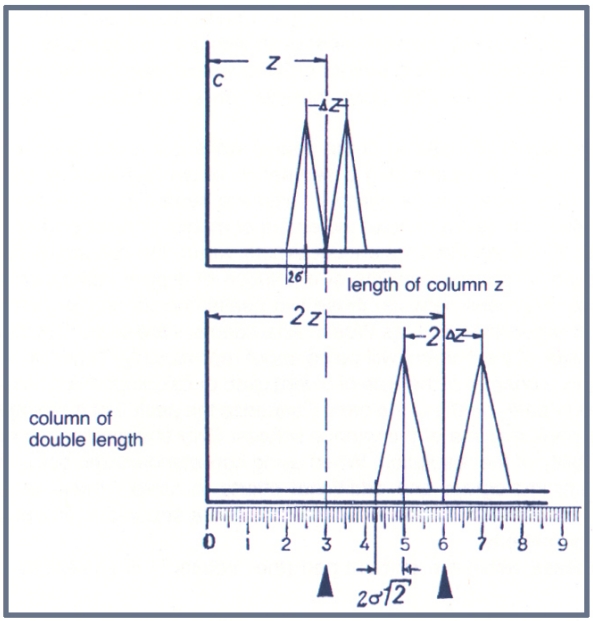
a. length of column
b. flow rate of mobile phase
c. specificity of the sorbent particle
d. diameter of the sorbent
e. activity of the sorbent temperature
f. polarity of the solvent
g. amount of sample

Length of Column

The paramount goal in separation sciences is to have as pure and complete a separation as possible. Physical limitations preclude achieving pure separation. However, knowledge of the variables is essential to assure the best separation.

During the migration through the sorbent bed the difference of the peak maxima (Ä z) grows linear to the length of the path traveled. The peak width at base will change only “2 times when Ä z has doubled.

For this reason, one can conclude that a longer column improves resolution. As the back pressure rises with the length of the column it takes longer for the sample to elute. If the flow is increased to higher values more non-equilibrium results and consequently the peak broadens. If the flow is kept constant, longer time of residence within the bed results, meaning more time is available for diffusion. This will also lend to peak broadening. Therefore, the use of longer and longer columns is not the ultimate solution to achieve a pure separation. A column should never be longer

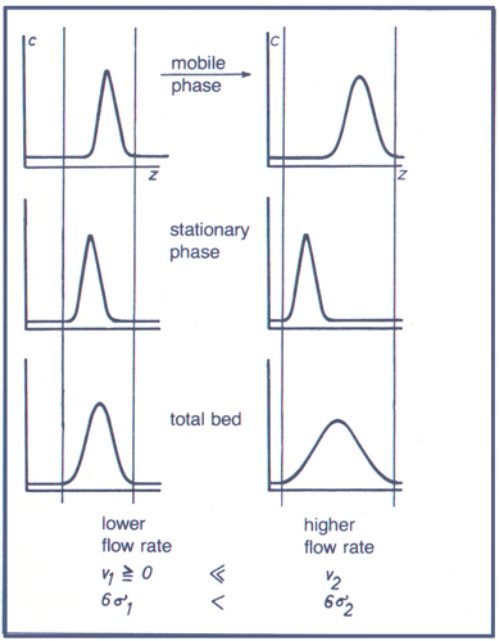


than necessary to achieve the desired separation. Excess column length leads to excess back pressure and an excess time of residence.

Flow Rate of the Mobile Phase

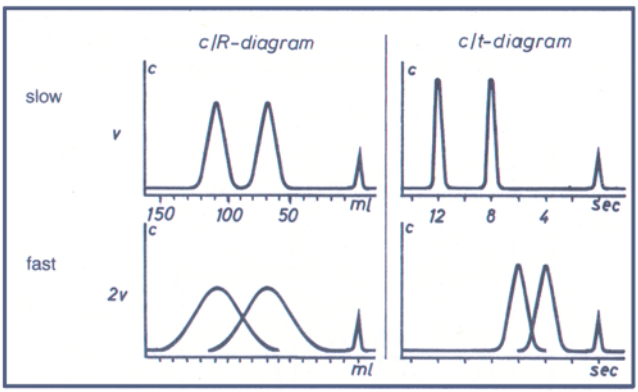
Solute leaves the sorbent **bed** within **a** shorter **time** if the solvent percolates the column at higher flow rates. However, the actual **volume** of mobile phase used until the peak maximum becomes eluted will not differ for higher or lower flow rates. **Sometimes plots of concentrations over elution time are used to disguise (unintentionally) the following fact: c/t-plots do not correctly depict the real situation within the bed of the sorbent. A plot of the concentration over the volume of eluent renders a much better picture of what occurs when solutes migrate through and flow out of the column. When looking at the dynamics of the chromatographic process appreciate that solute in the mobile phase advances faster than solute (loosely) affixed to the surface of the solid or solidified stationary phase.**

The higher the flow rate of the mobile phase, the farther from ideal stationary equilibrium the actual partition of the solute will be between the mobile and stationary phases. The figure below illustrates the situations within the bed (the actual conditions are exaggerated for clarity).



A prime goal for the chromatographer is to optimize the flow rate to a point allowing the solute to reside within the column for a reasonable time period. Higher flow rates result in peak broadening due to a reduction of true equilibrium. However, a shorter time of residence within the sorbent bed helps keep the peak width at the base smaller, since less time is left for the solute to disperse by diffusion forces. Long (strongly) retained peaks will profit most relative to their resolution if high flow rates are used. Little retained solutes benefit from lower flow rates as their resolutions are improved by improved equilibrium conditions and their time of residence within the bed being short. **This is important in order to minimize peak broadening by diffusion**. Equilibrium of the solutes between the two phases should be established achieving just enough “exchange” cycles at the interface in order to achieve the required degree of resolution.

When evaluating a practical separation system, one notices that after the flow rate is doubled separation is completed within half the time previously required, elution volumes remain constant and peak width broadens at the base. If $R_s = 1$ at the lower flow rate, then the resolution changes to $R_s < 1$ at the higher flow rate. In order to maintain good resolution for weakly retained solutes, the flow rate can be increased but only as long as these compounds continue to show sufficient separation. Strongly retained compounds continue to benefit from further increased flow rates as their peaks become narrower due to reduced diffusion time. There exists a maximum resolution for each pair of solutes of comparable polarity at an optimum flow rate. rate. It is not desirable to keep the flow rate lower than this value because peak broadening due to longitudinal diffusion will result from excessive residence



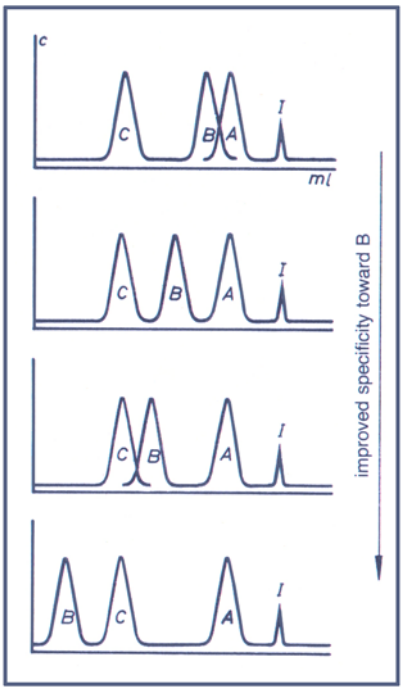
time within the sorbent bed. It is just as undesirable to “shoot” the sample through the bed because non-equilibrium of the solutes between stationary and mobile phases will also cause peak broadening.
Maximum flow rates are limited by the performance of the hardware (pressure pumps, tubing, sealing material, compressibility of the sorbent bed, etc.) and solvent systems (viscosity, compressibility of solvent, temperature of solvent). Higher flow rates can only be produced by higher pressure systems. For practical purposes, when achieving separations (not of theoretical data as the target) it is recommended to use moderate migration rates of the solvent front through the sorbent bed (v = 5 mm/ sec).

The Specificity of the Sorbent

The term “specificity” is frequently used when comparing the separation ability of different chromatographic systems. This is in part a subjective term. One of the reasons for this subjectivity is the fact that the user has little influence on the behavior of the active surface of a sorbent. This behavior is, mostly “built in”, i. e. a result of the production history of the solid.
As there are always a number of parameters at work to achieve separation factors influencing the behavior of the column may often remain hidden. Therefore, the specificity of a certain compound may be attributed erroneously to the sorbent, while other (unknown) parameters may be responsible. There are three basic properties by which the sorbent can be altered to enhance specificity:

a) Transformation of the active sorbent surface in question by derivatization or impregnation.
b) Use of a sorbent of entirely different chemical composition.
c) Physical modification of the active sites at the active surface of a sorbent. This is modified in such a way that only one or a small number of substances will be retained differently while all of the previously used separation parameters prevail.

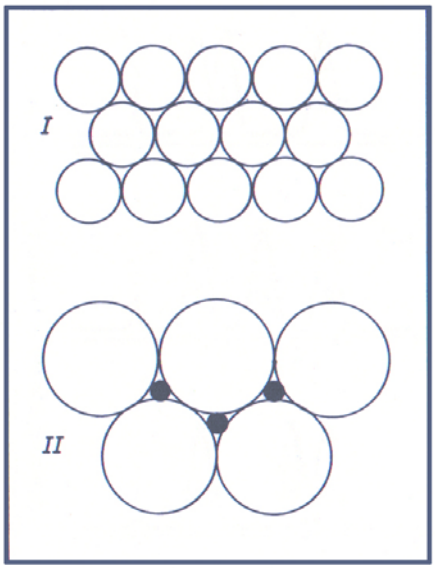
Actually, case a) is only made up of the generation of a completely new interface and has nothing to do with an improvement of the specificity of the sorbent: It represents the synthesis of a different sorbent.
As case b) uses also an entirely different chemical species, only case c) shows the characteristics of a change of specificity which can be attained for instance by heat processing, aging an interface or removing undesirable minor contamination. Specificity per se will never act only positively in the direction of achieving “better resolution”. It should, theoretically, influence only **one** definite substrate. If this sort of specificity helps to shift one peak (B) of predominant interest away from (another) peak(s) (A), it may help to improve this resolution of the (pair of) solutes (A-B) in question. At the same time, however, this specifically retained peak (B) may come closer to some other component (C) and contaminate this fraction of eluate. It would worsen the resolution (B-C).
Only in the rare case when a change of selectivity moves a certain peak into a space between two other peaks (I - A - ß - C) does it exert an overall positive effect on the entire separation. The schematics below depicts what is commonly known as “specificity”. The goal simply is to provide a means to capture or elute each chemical species separately.



Standardized sorbents differ from non-standardized products by maintaining their specificity in combination with a great number of solute molecules from lot to lot and over many years, ensuring high reproducibility.

The Particle Diameter of the Sorbent

Spheres with uniform diameter give the most regularly packed column. (hexagonally densest packing) is unquestioned (case I). A more densely packed sorbent bed can be made by incorporating tiny spheres fitting into the interstices between the larger spheres (case II).

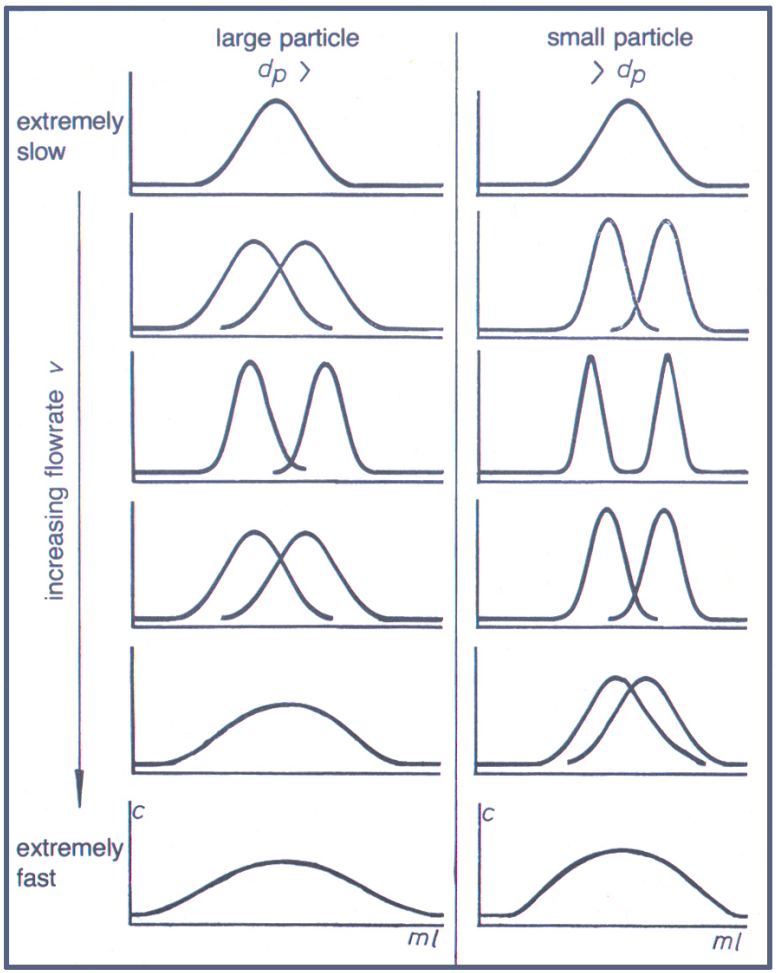


Similar reasoning also applies to irregularly shaped particles, especially when very small particles come close to the shape of a globe. The actual size (particle diameter) and the particle size distribution of an adsorbent play important roles in regulating column packing, pressure, and volume:

1. As the particle size decreases, the density of the column packing increases.
2. The denser the column, the more pressure required to force the volume through the column per unit time.
3. The smaller the particle size, the more volume of the bed is filled with solid; leaving smaller void, interstitial volumes to be penetrated by a flowing thinner film of mobile phase.

The major advantages for using a thin film is the short distance which an individual molecule will travel while randomizing between the liquid and mobile phases. Achieving an equilibrium state of partitioning for molecules between these two phases is completed within a shorter period of time. Additionally, the dynamic exchange of each molecule between the two phases can be repeated more often within unit time. By doing so each molecule has a better chance (more often!) of finding its proper place within the bulk of its own kin; thus increasing resolution. By using smaller particles while maintaining the same effective flow rate, better equilibrium values can be obtained, with a resulting narrower peak.

In the case where good separation is obtained with large particles, switching to finer particles allows higher flow rates and speeds the separation. A shorter time of residence of solutes within the solvent bed additionally increases resolution for the longer retained solutes by reducing longitudinal diffusion. In the example where all separation paramaters except the flow rate are kept constant, a chromatogram of two solutes changes is provided when a fine sorbent is compared with a coarse sorbent:



Accordingly one should choose from the following particle cuts:

1. **Coarse particles:** recommended for columns without pumping devices for the mobile phase (gravity columns).
2. **Fine particles:** used if liquid is pumped through the sorbent bed.

The flow rates of the mobile phase are optimized after the separation system is adjusted relative to the:

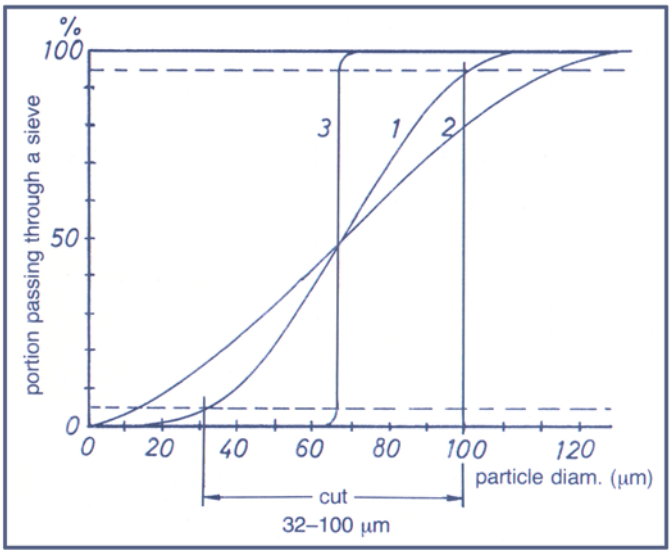
- sorbent
- activity of the sorbent
- particle size of the sorbent
- length and width of the sorbent bed
- viscosity and temperature of the solvent

Pump technology limits the applicability for smallest particles, longest sorbent beds, and highest viscosities of the mobile phase. Chromatographs become very complicated if a pressure higher than 500 bars is used to achieve separation.
Sieve cuts used for various pressure ranges with high performance separations are preferably standardized according to the (metric) German Industrial Standard (DIN = Deutsche Industrie Norm). (The mesh system is less useful, especially when dealing with fine particles.)
It is imperative to use sorbents free from fines to prevent the sorbent bed from clogging. Furthermore, the use of coarse particles should also be avoided as they impede uniform packing of the column.

When analyzing particle cuts, a plot of the particle passing through a (real or hypothetic) grid of a sieve over the particle diameter is of great help. Very often, however, one fails to consider the difference between a plot of weight vs. diameter and a plot of the particle number vs. diameter; making false comparisons. The plot of weight vs. diameter is preferred.

The following cuts have proven advantageous.

Cut (microns)		approxim. pressure range (bars)
200–500	long, technical use columns	up to 10
100–200	analytical and prep. gravity columns	up to 2
63–200	low pressure columns	up to 5
32– 63	analyt. & prep. columns	
18– 32	analyt. & prep. columns with medium pressure	5–10
7– 12	high pressure analyt.	20–100
3– 6	columns	100–500

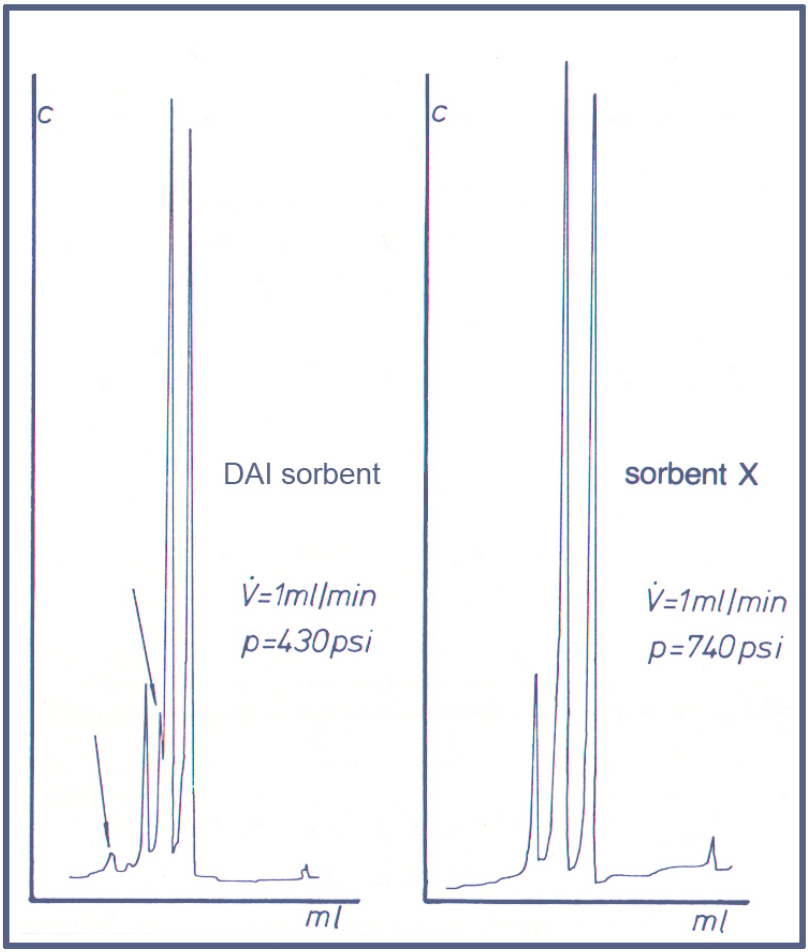


Standardized sorbents (curve 1) differ from non-standardized material (curve 2) in that the inclination of the sigmoidal curve is steeper with the standardized and in having only a low percentage of excess fines. They are also free from coarse particles which could impede homogeneous packing of the sorbent bed.

The back pressure of particle-filled columns can be calculated, assuming the collection of particles to have a “mean diameter.”

dp/dz = 2 const • r\ • v/d£; whereby
p = pressure
z = length of column
v = flow velocity of solvent front
dp = particle diameter
r| = viscosity of solvent.

If the performance of two columns is compared (keeping all parameters of the separation constant) a column with a higher back pressure (composed of smaller particles) should theoretically provide a better resolution due to its smaller “mean particle diameter”.
Excess fines contained in non-standardized cuts clog the column at some sections while excessively coarse particles cause overly loose packing at some other sections of the sorbent bed. Both conditions have negative effects on resolution. Curve 3 shows a so-called “monodisperse” phase, allowing a column to be packed with optimal uniformity and minimal back pressure for a given particle diameter. It has already been mentioned that it is technically difficult to produce such narrow cuts. Well standardized sorbents - such as DAI adsorbents which are manufactured to demanding standards - come relatively close to highly narrow particle size distributions.

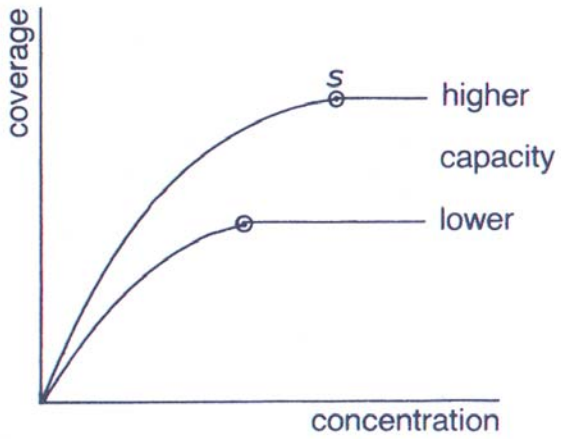


When comparing a column made of Silica DAI with competitors silica X, and keeping all parameters, flow rate, constant (v = 1 ml/min), the DAI column demonstrates lower back pressure and better resolution. As determined by the lower back pressure, Silica DAI should have a larger particle and a poorer resolution, while Silica X should be composed of smaller particles and better resolution. However, DAI has both lower back pressure and better resolution because Silica DAI has a purer ("narrower") particle cut. Its particle distribution comes closer to the ideal monodisperse phase. **DAI adsorbents provide superior performance because of highly standardized particle size distribution.**

Sorbents with Activity and with Constant Activity

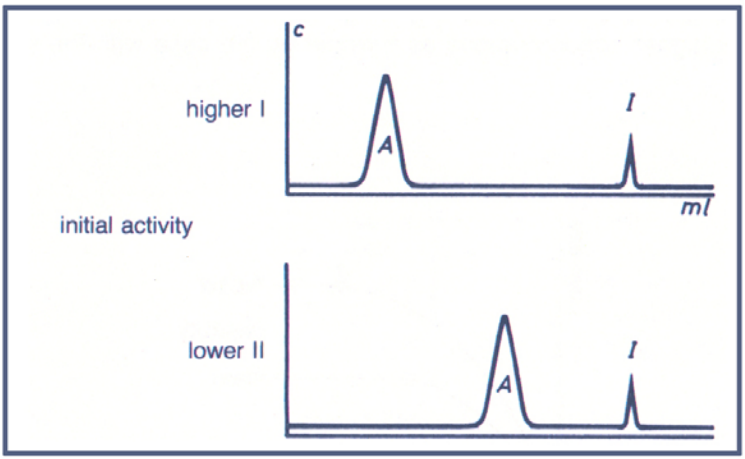
The term “activity” is used very liberally for describing a number of effects encountered in daily chromatographic work.

Sometimes one refers to the “active sites” on the surface of a stationary solid phase, attributing them to a number of phenomena. In other instances, the term activity is used to define the ratio of solute used per unit sorbent, i. e. a measure for the coverage of the sorbent surface. A higher capacity means that a sorbent can take up more solute per unit surface (or weight) over the entire range of concentrations of solute offered to “react” with it. A sorbent’s point of saturation (S) is reached at higher concentrations as with low activity sorbents. The separation of a mixture into its individual components is only possible if the individual components in a combination of stationary and mobile phase possess different adsorption/desorption properties.

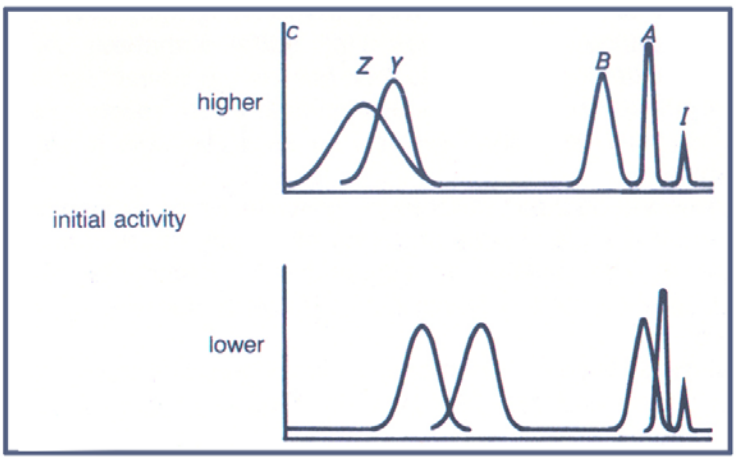


Sorbents having well defined and of highest possible activity – such as Alumina DAI Activity Super I and Silica DAI, Active - are preferentially used for purification purposes. Frontal analyses and bulk applications using agitated reactors are the main types of applications for high activity = high capacity sorbents.
A third meaning for the term “activity” defines the capability of a sorbent to retain certain individual molecules or groups of similar molecules within a chromatographic column. When dealing with adsorption chromatography, high activities (high **initial activities)** are required whenever sorbents are standardized relative to their behavior when in contact with a number of different solvents. Such standardization is essential to produce material which “reacts” identically when used in an identical reaction system. As an example such reproducibility is critical for the purification of pharmaceuticals.

A sorbent operated by elution techniques with higher activity should retain solutes better than less active material. For example when examining two sorbents being exposed to an identical solute, they exhibit the following properties: a sorbent with a high initial activity (case I) shows overall longer retention while the sorbent with a low initial activity (case II) exhibits overall shorter retention. (See chromatograms below).



DAI adsorbents are standardized according to Brockmann and Schodder (see glossary). Due to this standardization, all chromatographic separations are absolutely reproducible over years when employing these products.
Highly active sorbents are used when samples (A, B) with little retention (time or volume) are separated. An extremely high activity will cause excessive retention volumes; even for small quantities of retained samples. Eventually, high activities prohibit separation of strongly retained samples due to peak broadening, poor equilibrium and/or by longitudinal diffusion.



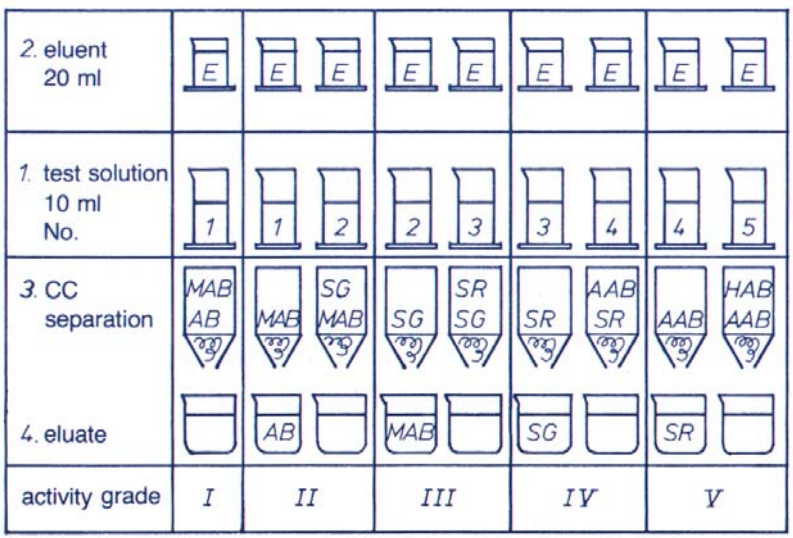
A separation of longer (stronger) retained samples (Y, Z), can be done more effectively after appropriate deactivation of the sorbent or with a sorbent of lower initial activity. One may expect a poor separation throughout the entire activity range (from highly active to almost completely deactivated sorbents) if non-standardized sorbents are used. Sorbents with high initial activity and thorough standardization - DAI adsorbents - are practical throughout the entire range of activities, not only at discrete activity grades or **steps** (such as Brockmann I, II etc.) Standardized sorbents are also practical at well defined intermediary levels along an activity **curve**. These activity curves are accessible by meticulously migrating from one activity grade to the other while continuously adding small increments of deactivator to the sorbent.

Instead of using the c/R plot of an elution chromatogram for the determination of the activity of a sorbent, the use of the Brockmann and Schodder test [Chem. Ber. **74B**, 73 (1941)] is simpler and just as reliable. This test provides reliable information from highest to low activity levels. A standardized volume of individual pairs of dyes, dissolved in a standard solvent mixture (= eluent) to a standardized concentration is applied to a 50 mm long sorbent bed 15 mm in diameter. An initial peak is produced. This peak is then developed using a standard volume of eluent. The various activity grades are determined by measuring the eluted or non-eluted sample peaks according to their position relative to the top of the column. (See glossary).

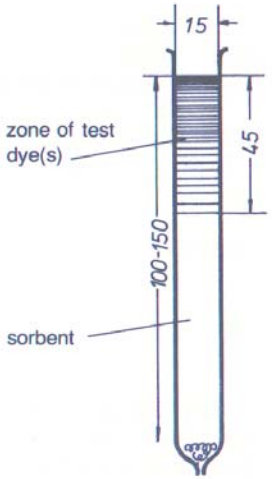
E = Eluent = solvent = 1 vol. of pure benzene, plus 4 vol. of pure petroleum ether.
Test solution: each 20 mg test dye per 50 ml of E (e. g.: 20 mg AB + 20 mg MAB + E ad 50 ml).

Soln. #1: azobene (AB) + p-methoxyazobenzene (MAB)
Soln. #2: MAB + Sudan yellow (SG)
Soln. #3: SG + Sudan red (SR)
Soln. #4: SR + p-aminoazobenzene (AAB)
Soln. #5: AAB + p-hydroxyazobenzene (HAB).

Ten ml of the appropriate solution is carefully applied to the top of the sorbent bed using a pipette. Elution is done with 20 ml of eluent. Due to the location of the various dyes on or eluted from the column, the activity grades can be determined according to the schematics below.



DAI suggests an improved procedure for simplifying the method, providing better precision. According to DAI's method, 10 to 15 cm long glass columns are used, reducing elution from the bed. The distance between the top of the sorbent bed and the visually discernable bottom most section of each peak defines the zones of test. With the aid of this procedure all intermediate activity statuses from the previous rigid Brockmann grades become accessible to a well defined assay. This procedure makes fine tuning of the adsorbent possible while deactivating the sorbent under absolute control by gradually decreasing energy statuses.
If more precise activity statuses are given for such a sorbent the Brockmann grade (as determined by the pair of dyes used) should be mentioned and the distance (in mm) between the foremost border of the foremost migrating dye and the top of the sorbent bed should be added.



In the above example the sorbent shows a Brockmann activity of I/45. The test is performed using #1 solution, giving an activity grade I. The distance of migration is 45 mm, indicating the fine tuned position between activity grade I/O and I/50 for the production of the usual Brockmann grades I-V. One should choose deactivator to reach grade I/30, II/30, III/30 etc.
DAI absorbents are standardized to a high degree with all 3 surface types of alumina (acid basic, neutral) showing identical initial activity. When deactivated with exact amounts of deactivator volumes (e. g. water), DAI adsorbents' exhibit identical lower activity statuses.
Each status, including the old Brockmann grades, are predetermined and reliably reproduced.
The following deactivation instructions are valid for all Act. I DAI Alumina:

Activity	I	II	III	IV	V
% of water **added** (w/w)	0	3	6	10	15

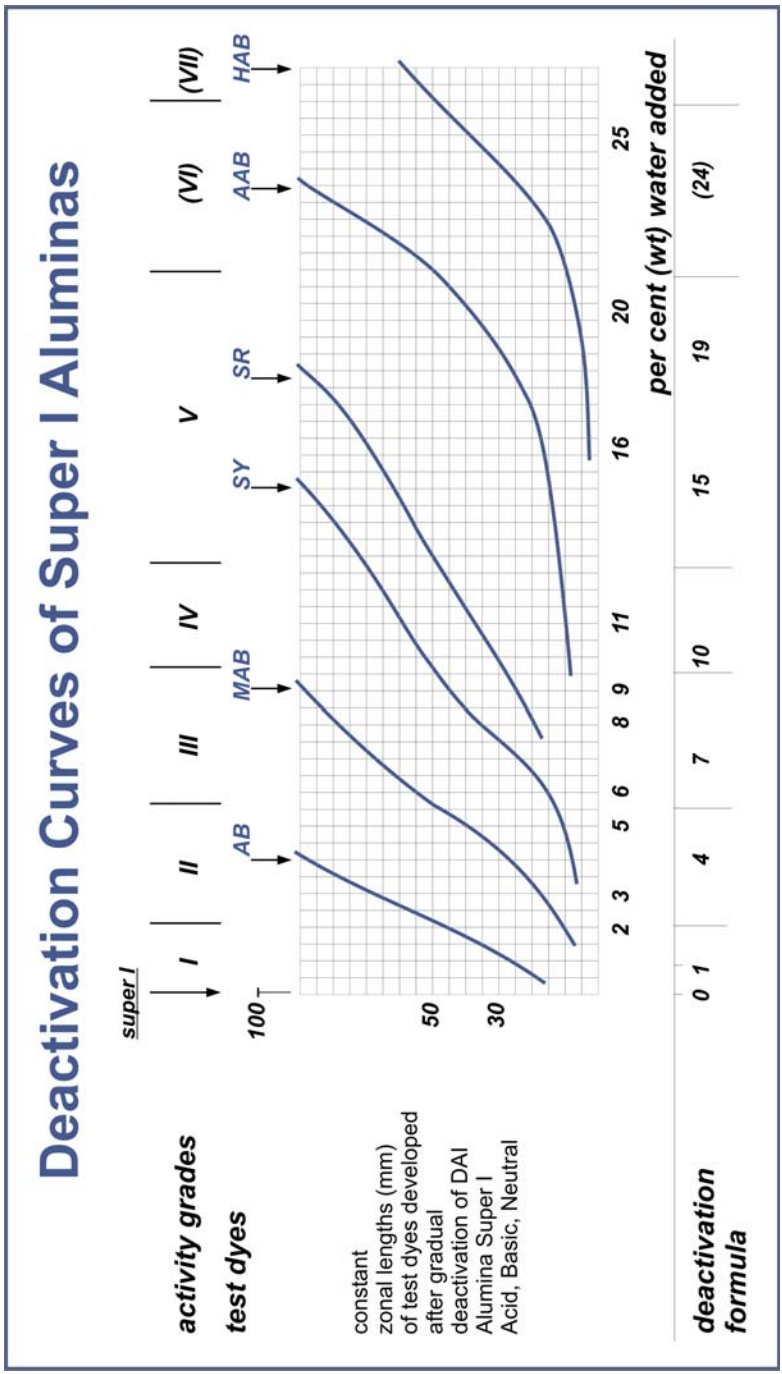
Super I Alumina is absolutely **"deactivation constant"** due to its unique production process. Each of the three surface types (acid, basic, neutral) retain identical initial activities of 1/16 ±2 mm, measured by the #1 solution (AB and MAB). Furthermore, each of the three surface types also show an identical sensitivity towards the addition of a "deactivator" (such as water); **they are deactivation constant.** The addition of a determined amount of water leads reliably to identical lower activity, regardless of the surface type of Super I Alumina used.
The deactivation instruction below is valid for Super **I** Alumina:

Activity	Super I/16	I/30	II/30	III/30	IV/30	V/30
% of water **added** (w/w)	0	1	4	7	10	19

Alumina having constant performance characterizes the above mentioned fine adjustment along the activity scale. Due to a proprietary activation process it is possible to add two more grades to the Brockmann activity scale: grades VI and VII.

The deactivation constant is extremely stable and when stored in air tight containers maintain its original properties for years.

Deactivation of an inorganic, oxide sorbent is very simple:
The deactivator (predominantly water, but also glycol, glycerol, etc.) is weighed or pipetted and added to the sorbent contained in a tight-closing bottle. After the addition of the appropriate amount of deactivator, the bottle is closed and the mixture shaken until all lumps disappear. (When using glycerol or glycol it is advisable to heat the mixture to 100°C for a few hours in a closed vessel). After cooling to room temperature the deactivated absorbent is ready. Longer storage is favorable, as longer equilibration time ensures a more homogeneous deactivation by further isothermal distillation on the surface.
If very small increments of water are added to Super I Alumina, and if each sample is assayed according to Brockmann using a refined method, **deactivation curves** of the following kind are achieved:



Different deactivation guidelines are provided for adding water to Silica, Active

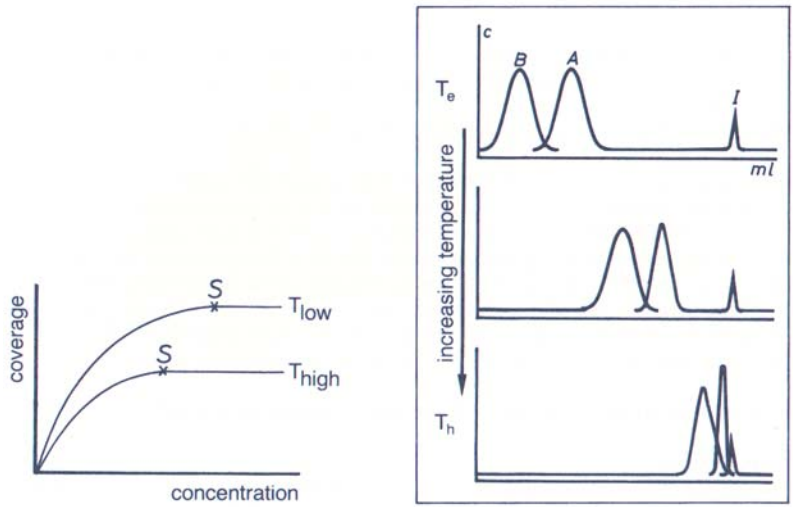
Activity	I	II	III	IV	V
% of water **added** (w/w)	0	10	12	15	20

As the initial activity of activated silica corresponds to requirements of the Brockmann-I-test, the above deactivation procedure provides reproducible deactivation. Since the Brockmann test constitutes a standardized method for gravity flow type sorbents, flow rate and particle size must be considered as variables.

When used with confined particles deactivation will fail as the flow through the sorbent bed approaches zero. Small particle size adsorbents offered by DAI deliver identical separation abilities for large and small particle materials. Thus, all sorbents live up to reproducible and reliable standards. All energy changes at the active sites for both the sample and the solvent are always identical when brought in contact with deactivators. Using DAI adsorbents it is easy to scale up and/or scale down between small pore and large pore columns, as well as columns using large and small particle cuts.

The Temperature

In the case of a static experiment – the establishment of an adsorption isotherm – it can be demonstrated that the coverage of the solid surface F (mg sample/mg sorbent) will be reduced over the entire concentration range with a rise in temperature.



The point of saturation (S) is shifted to lower concentrations at higher temperatures. This temperature gradient exists with both adsorption and reversed phase systems. With rising temperatures the retention volumes for the various solutes decreases. Generally, the resolution and distance between the peak maxima (Äz) decreases, accordingly.

It may be advantageous to utilize higher temperatures in order to elute longer retained samples more quickly or to avoid the change to a more polar mobile phase. If elevated temperatures are used during a chromatographic separation, undesired reactions of all components of the system are more likely to occur than at lower temperatures. As a corollary, the separation of less retained samples and those tending to undergo deterioration should preferably be done at the lowest possible temperature level. The lowest possible temperature is governed by the viscosity and the melting point of the solvent(s) used. High temperatures may reduce the elution volume of strongly retained samples, may cut diffusion time, and improve the resolution. For adsorption systems, the boiling point of the solvent will limit the temperature while an upper limit of 60° C exists when working with reversed phase materials.

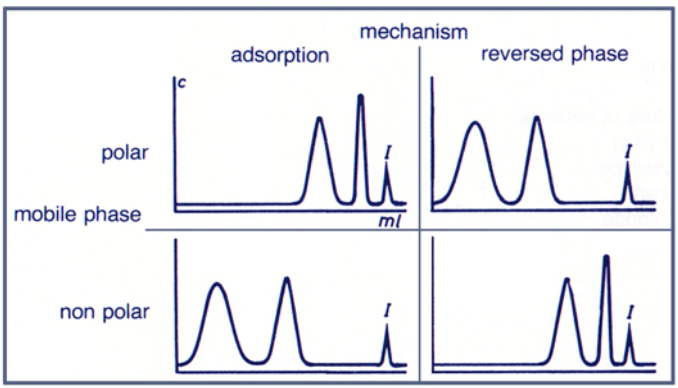
The Polarity of the Solvent

A major component for achieving a separation and fine tuning various interactions within the separation environment is the solvent (and/or the solvent mixture).

For chromatographic purposes, the term “polarity” is simple and meaningful. The term is meant to describe the dielectric constant of a solvent suitable to establish a series of solvents while changing elution forces.

Fluoralkane
n-Pentane
Isooctane
Petroleum ether
n-Hexane
n-Heptane
Cyclohexane
Cyclopentane
Carbon sulfide
Carbon tetrach bride
Amylchloride
i-Propylether
Toluene
Chlorobenzene
Benzene
Chloroform (free of ethanol)
Diethyl ether (dry)
Methylene Chloride
Tetrahydrofuran
Ethylene dichloride
Methylethyl ketone
Dioxan
Ethyl acetate
Pyridine
Acetone
Amylalcohol
Dimethylsulfoxide
Nitromethane
Acetonitrile
i-Propanol
Ethanol
Methanol
Ethylene glycol
Acetic acid
Water

A solvent is considered “non-polar” if it has limited interaction with sorbent, does not provoke solute to move back into the mobile phase, and resides on the sorbent. In contrast, water is a highly polar liquid which interacts very strongly with an active surface (liberation of considerable high heat energy when brought into contact).
Pentane is considered non-polar. Solute dissolved in pentane is completely adsorbed by sorbent. As this series characterizes the elution strength, it may also be called the **elutropic series.** This general series is adjusted specifically to each sorbent. There is no fundamental difference in a solvent property if used for reversed phase chromatography or for partition and adsorption chromatography. For reverse phase and partition, the series is preferentially called **“mixotropic series”.**
For adsorption systems retention decreases (the retention volumes become smaller) the more polar a solvent. With reversed phase systems the opposite case is experienced. The more polar the eluent used, the longer the retention (more solvent is used to wash the solute off the column).

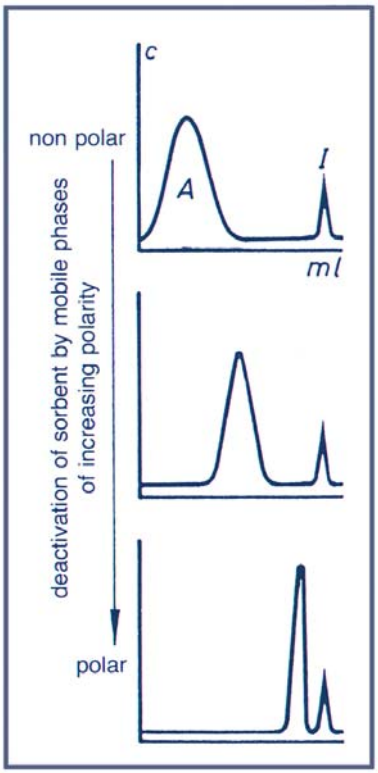


The necessity to **standardize the solvent** system is too often neglected. This type of standardization includes a number of parameters, depending upon the level of purity. Purity may mean an absence of contaminants relative to the optical transparency. It may also mean the absence of colloidal solids, absence of moisture and absolute uniformity of the solvent (mono-substance) or all of these properties together. An absolute mono-substance is the ideal eluent for chromatography. There is considerable experience demonstrating a clean-up of solvents by contact with active sorbents; percolation through beds of active alumina or silica - especially Super I activity Alumina DAI or Silica DAI Active. The use of standardized solvents may remove many unwanted situations in the chromatographic process.

Deactivation and Constant Deactivation Behavior

In the laboratory separations are more complex than a simple system comprising sample, one uniform solvent and an unmodified sorbent. Simple systems of this kind may be used when weakly retained compounds are separated. Here, a non-polar solvent and a highly active sorbent surface are demonstrated. A great number of cases call for a properly adjusted sorbent; a deactivated sorbent. Deactivation can be obtained by adding polar liquid (water, glycerol, glycol) to the sorbent. Be aware that each participating liquid or dissolved molecule offered to the surface of the sorbent will “react” with the sorbent. Besides the molecules of the bulk solvent or mixture, **all contaminants** struggle for their position at some active site. Even small portions of polar contaminant contained in non-polar solvents may alter the properties of the interface. The addition of “modifiers” makes use of this property. Evolvement of heat - the liberation of sorption energy - can easily be measured after the addition of solvent. The rise of temperature is a qualitative measure of the degree of deactivation accomplished. There are a number of physical means to measure the degree of residual activity after the surface is exposed to various species of molecules and the energy differences between the active and the deactivated state of a sorbent.

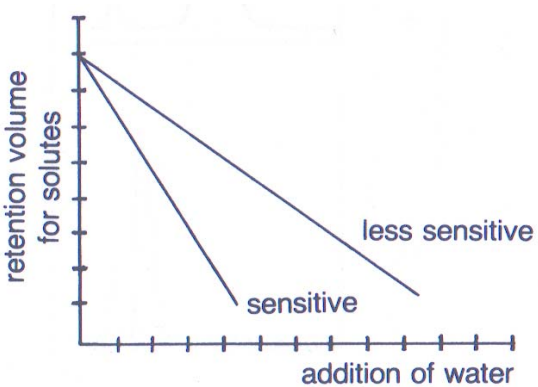
If the retention volume of sample A is determined, a certain retention volume specific for this compound is found. Assuming one uses a solid phase of high activity (not yet modified by any type of previous deactivation) and eluents of different polarities, one may expect the following chromatograms:

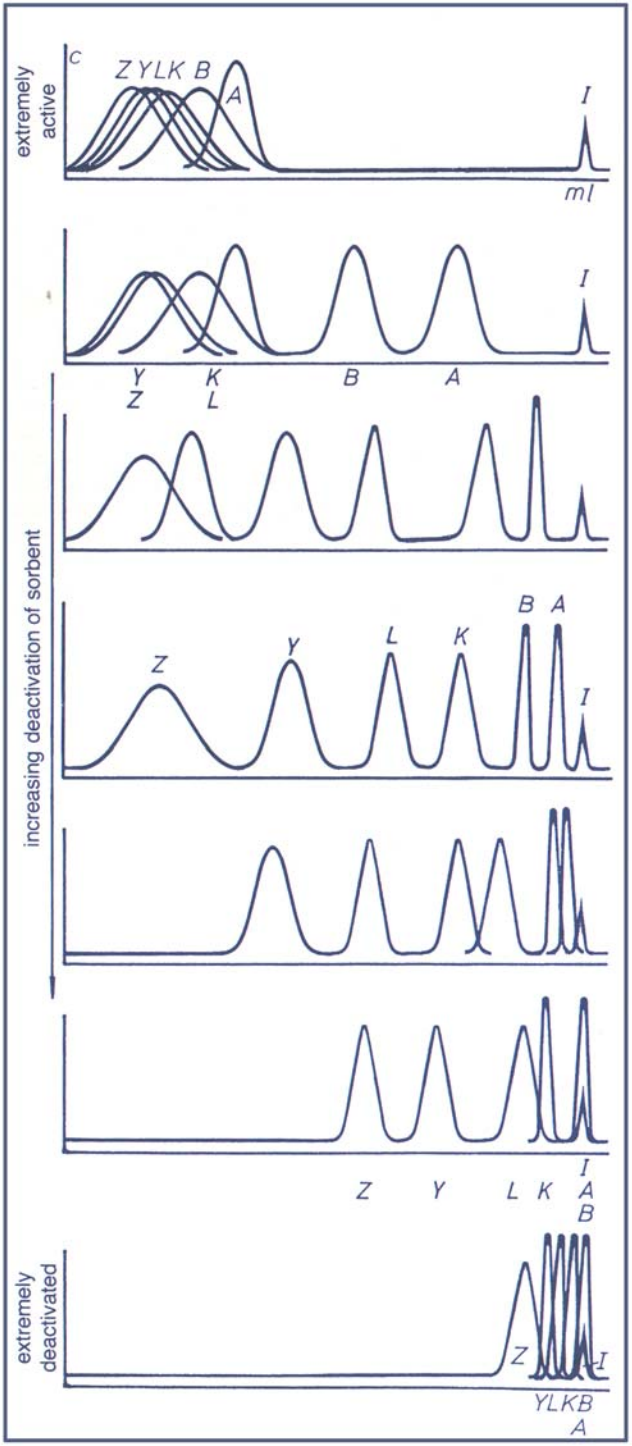


Finding the best suited solvent system may require some trial-and-error. It is recommended to start the search for the best suited eluent for both adsorption and reversed phase (partition) systems with a medium polarity liquid. For adsorption systems start with chloroform or methylene chloride. After an initial test one can decide whether to use a more or less polar solvent for the next trial. The optimum eluent will be found by stepwise limiting the number of possible solvents within the sequence of the elutropic series. If the use of mixtures appears inevitable, care should be taken to use solvents close to each other along the elutropic series. Despite published literature on the behavior of eluents in contact with separating surfaces, a faster or more reliable or more elegant method than the above trial and error technique does not exist. A realization and/ or improvement of the separation of two substances or of a complex mixture may not always be accomplished by the mere variation of the solvent(s) used. A well defined change of the surface structure and surface energy of the solid phase can be of great help. This change can be accomplished by adding specific deactivators to the sorbent powder prior to filling the column (tube). The most effective, simple, and stable deactivators for oxidic sorbents are water, glycol and glycerol. A highly active sorbent's activity can be lowered in small increments by a measured addition of the deactivator, e. g. water.

The enclosed figure depicts the influence of the deactivator on the separation and shows general types of chromatograms received while a complex mixture of strongly (Z, Y), mild (K, L) and weakly (A, B) retained solutes are separated using an identical solvent system which are incrementally deactivated.

In order to perform an incremental deactivation pattern, start with sorbents of closely standardized initial activity. An initial activity can only be achieved by using standardized adsorbents. Only adsorbents exhibiting an extremely well standardized initial activity - DAI adsorbents - may successfully, reproducibly and reliably be deactivated in the aforementioned manner. Not only does standardized initial activity govern the performance of an adsorbent, but it is greatly influenced by the deactivation behavior. For this reason the sensitivity of an adsorbent toward various deactivators must be very closely standardized. A constant deactivation behavior is equally important for other liquid phases brought into contact with the oxidic surface. **A surface owning a constant "reactivity" towards water molecules will also show a similar constant reactivity of its surface with the molecules of other (liquid) compounds.**



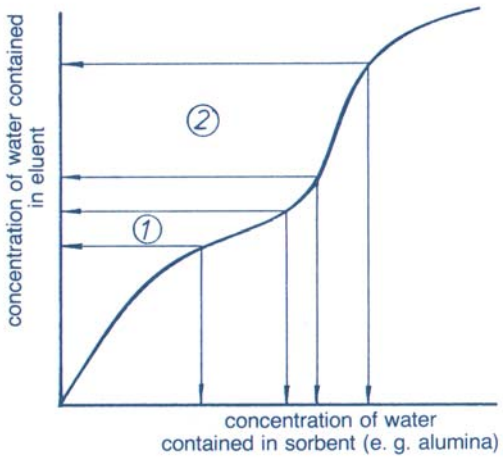


Despite identical initial activity, virtual standardization and the use of an identical mobile phase, chromatogram output may not always be the same. Only if both the initial activity and the deactivation behavior of an adsorbent are standardized to the highest degree will identical, reproducible and reliable separations be achieved.
Sorbents showing a constant deactivation behavior (when deactivated with water) will lend a constant result to any separation technique and elution system. This type of constant behavior within a vast region of "separation environments" is one of the unique features of DAI adsorbents. Curves shown in the illustration "Deactivation Curves of Super I Aluminas" indicate the sensitivity of Super I activity alumina towards various standard dye mixtures on the Brockmann scale. These sensitivities (slopes) are maintained constant due to a unique proprietary production process.
Many separations are achieved using a constant (=isocratic) elution system throughout the entire elution process. These relatively simple systems should be restricted to no more than 2 different types of solvents. Sometimes it is difficult to find these systems because it appears simpler to just add a third component. The search for simple systems is most rewarding because of their inherent stability and reproducibility. The use of solvent gradients is limited to exceptions; in real life settings gradients require more complicated technology, longer setting times and larger liquid volumes are required to regenerate and restitute a column to its starting conditions. Additionally, be aware that it is not difficult to generate the gradient in the feed line to the column. The difficulty lies in the control of the actual separation conditions within the sorbent bed while the gradient migrates through the column (demixing, formation of polarity gradients, tailing of the composition, etc.)
Attend to each component of the solvent system selecting aspecific sites on the surface of the adsorbent where adsorption energy is best liberated. By this mechanism, solvent mixtures generate a number of specific interfaces. Each component gives its specific contribut-ion to the final deactivation status of the sorbent and never overlooks the fact that each contaminant of the mobile phase mixture acts the same way.
There are two fundamentally different ways of packing a column:

1. Dry-packing
2. Wet-packing

The column may be considered equilibrated under two conditions: if the mobile phase is used for packing and if a large amount of liquid, relative to the solid phase is used. As discussed with the isocratic solvent systems, all solvents used to wet-pack a column or wet a dry packed column struggle for their site and seat on the surface of the solid. All columns packed without using the mobile phase of the pertinent separation process must be "conditioned" before use. A sufficiently large volume of eluent flows through the column prior to sample application to establish the desired equilibrium.
Establishment of equilibrium may improve separation, may provoke separation or may, in rare cases, be detrimental to a separation. In some cases, a separation is only received when sample and eluent are applied to a **dry bed.** In other cases, resolution is best during the **first few** runs and eventually fades. In order to introduce more reliability from the the solvent system, especially when doing scale-up work or when working on pilot plant and plant scale, it is necessary to control equilibria. Reproducible conditions are relatively simple to achieve. A technique for the establishment of static sorption isotherms is illustrated.

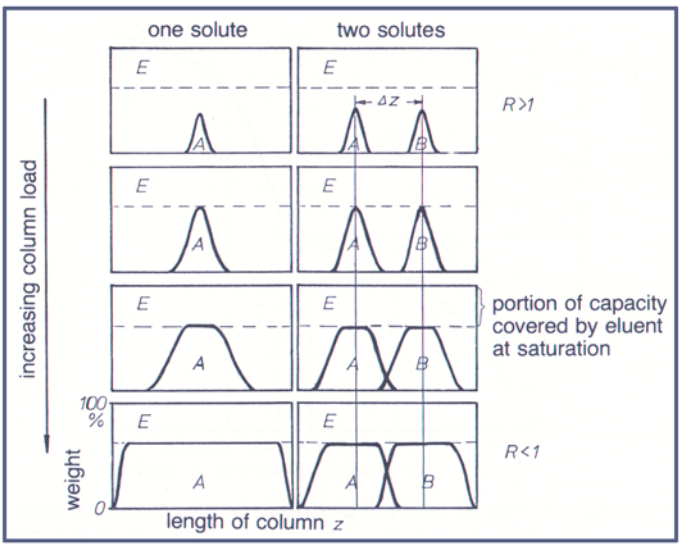
Shake a number of sorbent samples plus solvent mixture (S, + S_2...) plus deactivator (D) within a certain concentration range of deactivator and determine appropriate equilibrium concentra-tions of D in the supernatant and calculate the concentration of D on the solid. A plot of the concentration of D within the liquid phase over D on the solid surface yields valuable information.



Both the sorbent and the liquid phase are adjusted at any point on the curve relative to constituent D. The bulk sorbent is loaded with the equilibrium proportion of D prior to its use and the pertinent percentage of D is mixed with the bulk mobile phase while in the tank. If the column is filled with a pre-equilibrated sorbent and if the appropriate equilibrium solvent is used, then it is no longer necessary to “condition” columns before the first separation. This process eliminates the possibility of generating unwanted and uncontrolled gradients within the sorbent bed during the run. Sorbent as well as solvent are now fine-tuned for the separation. An additional advantage lies in the interpretation of the steepness of the slope(s) of the curve and of the plateau(s). Steepness and plateaus indicate where the solvent and adsorbent are most sensitive to a change in the concentration of D. This knowledge allows for further fine tuning for a successful separation to occur. The most stable sections of this isotherm for the sorbent and mobile phase are then selected in order to choose the most stable separation environment. If certain plateaus are used, they give rise to additional stability and to improved reproducibility of the system. It is advisable to adjust the separation environment to such areas of additional stability. Adsorbents - such as Alumina Super I - are standardized to a high initial activity and to a stable deactivation behavior; adding simplicity to many laboratory and plant scale separations. These separation characteristics can easily be controlled and they respond favorably to fine-tuning the mobile phase. Should insufficient or non-standardized sorbents be used, unreliability, poor reproducibility or adverse conditions may be expected.

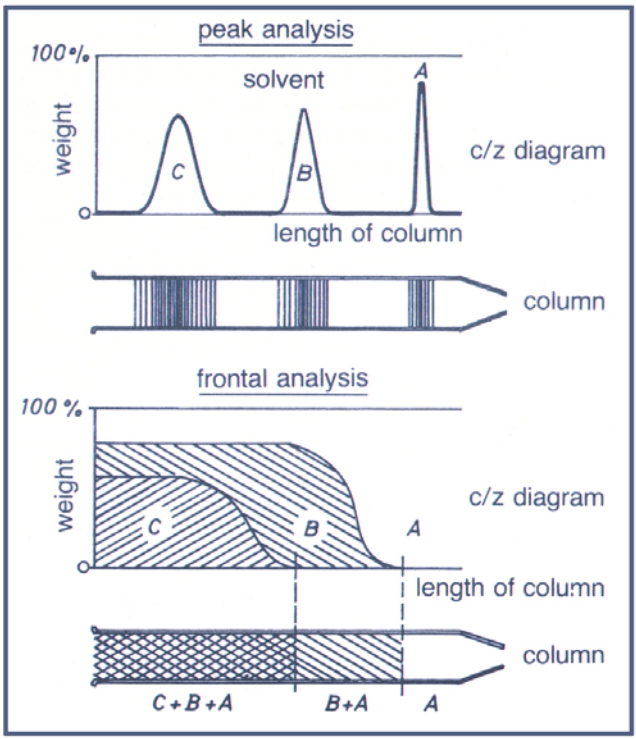
The Capacity

Peak technique or frontal analysis?
When discussing issues related to the capacity of a sorbent bed first clarify whether a peak technique is used for achieving separation or whether plugs of samples are to be conveyed through the sorbent bed using the frontal analysis technique.
When using the peak technique always use a mobile phase as a vehicle for the sample. This is not necessarily the case with frontal analysis. Here, one component or the sample components may act as their own mobile phase.
The figure below shows the schematics of action of a chromatographic bed:



A sorbent bed's entire capacity is used up as soon as the mass of one peak requires the total volume of the sorbent bed. This case is impractical since separating two components cannot be achieved if one peak requires the entire surface present. However, it is easily derived from the fact that the base of the peak within a column broadens not only by dynamic and time dependent effects, but also immediately after coverage of a certain volume of the sorbent bed reaches the point of saturation. (It reaches the ceiling of the uptake of a certain unit volume of the bed). If more sample is deposited, more sorbent is needed, eventually requiring the complete sorbent bed. Given a system where all separation parameters are kept constant except sample load and given two solutes, these solutes show a difference of location in their peak maxima. If the load of the sample is increased the bases of the two peaks broaden. While the original resolution R > 1, the resolution would drop to R < 1 and finally approaches R = 0. It is useful to determine the capacity limit of a column in such a way that this limit is reached as soon as the sample load is so high that the separation of two solutes is no longer "sufficient". The term **"overload"** is often used in this context. An overload is expected to occur as soon as "the resolution is impeded". These intangible definitions give rise to the question of who and what specifies the degree of decrease in resolution and when will the value of **R** no longer be permissible.

It is possible to determine and compare the quality and capacity of different lots of "identical" adsorbents using identical parameters (such as composition of eluent, temperature etc.) and setting a certain value for the resolution. For this purpose, two (or more) solutes are separated and the dependence of the resolution upon the load is measured. **Maximum load measured at a given limit for permissible degree of resolution is an important measure for quality and economy of preparative columns (sorbent beds). Prep columns must be operated close to the maximum capacity for economic reasons.** Many times research must be done to reveal the flaw(s) of the chromatographic process, leading the chromatographer to mistrust their own separation procedures. Only standardized adsorbents provide the required degree of dependability and reliability necessary for high-quality chromatographic results.
When using the frontal analysis technique, only one pure substance can be eluted from the system. This pure substance migrates through the sorbent bed the fastest and demonstrates the largest difference of polarity relative to all the other constituents present in the original mixture. It is necessary to define "capacity" for this technique in order to be able to calculate sorbent requirements and yields. The capacity of a column will be expressed in "ml eluate", counting from the first drop of effluent A from the column until the



first appearance of “contamination” caused by substance B, eluted from the next column. Therefore, capacity is dependent upon detectability of the second component; B. Columns used for frontal analysis are, as a rule, dry-packed. For this reason, high temperatures are experienced after the sample is loaded onto the top of the sorbent bed. There exists a perpetual generation of heat (adsorption) at the front while the following bulk sample wets the column. Since the capacity of a sorbent is considerably lower at elevated temperatures, extra care must be taken to apply the sample in a slow manner in order to let most of the heat diffuse. In some cases, the column must be jacketed and cooled until, the first drop leaves the bottom of the sorbent bed. Immediately after the front reaches the bottom and the bed has cooled, the column may be operated at a higher flow rate. Whenever the column is run too fast at excessively high temperatures, the column can no longer be regenerated. Once capacity is lost, it is gone forever. This is because the entire surface area of the bulk adsorbent is (partially or completely) loaded with molecules which would have been retained at the column top if the column had been operated in the appropriate manner.

Frontal analysis is predominantly used for solvent purification. Superactive Alumina DAI, Act. I Alumina DAI and Silica DAI Active are best suited for frontal analysis. To achieve high economy, the capacity of the contaminant should be as large as possible. When using oxidic adsorbents such as Superactive Alumina DAI, Act. I Alumina DAI and Silica DAI Active, a purification will work at its optimum if:

- the compound to be purified is as non-polar as possible; and
- the contamination is as polar as possible. (See glossary)

Whenever this situation is present, the residual contamination left in the purified compound will be lowest and the contaminants will slowly migrate through the column and show a sharp front. Alumina Super I is the first choice for the purification of a large number of liquids due to its high separation capacity. Alumina Super I demonstrates twice the capacity of Alumina Act. I for comparable impurities.

Highly Standardized Aluminum Oxides for Chromatography[7]

Aluminum Oxides for Chromatography with Elevated Pressure[4]
Alumina N 3—6[2]
Alumina N 7—12
Alumina N 10—18
Alumina N 18—32
Alumina N 32—63

Active Aluminum Oxides for Chromatography with Elevated Pressure[4]
Alumina A 18—32, active[3] Alumina
N B 18—32, active Alumina N 18—
32, active Alumina N 32—63,
active

Aluminum Oxides for Preparative and Classic Column Chromatography[5]
Alumina **A —** Super **1**
Type **W 200**
Alumina **B —** Super **1**
Type **W 200**
Alumina **N —** Super **1**
Type **W 200**
Alumina **A, act. 1**
Alumina **B,** act. **1**
Alumina **N,** act. **1**
Alumina, act. II—III **ace.** to Brockmann
Alumina **R**
(For Radioisotope Techniques)
Alumina DCC[6]
(For Dry-Column Chromatography)

Accessories (for Dry-Column Chromatography)
Nylon-Film Tubing, **40** mm **0**

A = anionotropic = acid B = basic
N = neutral

[2] Affixed numbers indicate particle cut, measured in microns.
[3] If small particle cuts were reactivated to correspond to Activity I on the Brockmann scale, "active" is indicated.
[4] for product specifications see Leaflet
[5] for product specifications see Leaflet
[6] for product specifications see Leaflet
[7] for packing sizes etc. ref to p. 64 and p. 65

Highly Standardized Silica Gels for Chromatography[7]

Silica Gels for Chromatography with Elevated Pressure[4]
Silica 3—6[2]
Silica 7—12
Silica 10—18
Silica 18—32
Silica 32—63

Active Silica Gels for Chromatography with Elevated Pressure[4]
Silica 18—32, active[3]
Silica 32—63, active

Silica Gels for Preparative and Classic Column Chromatography[5]
Silica 0—63
Silica 32—100
Silica 63—100
Silica 63—200
Silica 100—200
Silica 200—500

Active Silica Gels for Preparative and Classic Column Chromatography[5]
Silica 32—100, active
Silica 63—100, active
Silica 63—200, active
Silica 100—200, active (formerly “for adsorption”)
Silica 200—500, active
Silica DCC[6] (for Dry-Column Chromatography)

Accessories (for Dry-Column Chromatography) Nylon Film Tubing, 40 mm 0

1 “Silica DAI” is the new synonym for “Silica Gel DAI”
2 Affixed numbers indicate particle cut, measured in microns.
3 If particles are activated to correspond to Activity I on the Brockmann scale, “active” is indicated.
4 for product specifications see Leaflet AL 20
5 for product specifications see Leaflet AL 16
6 for product specifications see Leaflet AL 15
7 for packing sizes ref to p. 64 and 66

Notes Regarding Applications

Alumina R

This absorbent is specifically developed for medical and nuclear diagnostics. Among other uses it is recommended for the production of various generators where one isotope must be retained while the other is eluted.

Artifacts

One of the main targets in chromatography (besides the separation itself) is the effort to avoiding changes in the molecular structure of either the eluent or the constituents of the sample. Therefore, great care should be taken to evaluate possible reaction mechanisms within the chromatographic environment prior to the actual separation. Very frequently these reactions are initiated by acid or basic sites of the sorbent. These reactions are enhanced when noticeable amounts of moisture are present. Besides the ionic activity of an adsorbent, a rearrangement of the structure of an adsorbed molecule can also be affected by excessively high activity of a sorbent. In view of these facts, a deactivation of alumina and silica sorbents to the lowest possible level (without jeopardizing the separation) is always advisable. See deactivation, p. 27, and: G. Hesse. Molecular rearrangements within a chromatographic column, Analyt. Chem. 277, 5 (1965).

Pharmacopeias

The European Pharmacopeia and other pharmacopeias list procedures for column chromatographic determinations. Most of them relate to alkaloid containing drugs. The property of basic or weakly basic alumina to split the salt of an alkaloid into its free base and the acid anion in alcoholic aqueous medium is used. The alkaloid base is eluted from the column and quantitated according to standard procedures. Contaminants remain adhered to the sorbent at the top of the column.

Liquid Chromatography

Adsorbents listed are used for liquid chromatography, making efficient use of improved resolution with small sorbent particles. Due to decreased permeability of the column involved, higher to highest pressure is required to force the eluent through the sorbent bed. **DAI adsorbents excel in liquid chromatography due to narrow particle cut, low back pressure and combined with excellent resolution and reproducibility.** Alumina and Active Silica are used for the purification of solvents in the practice of chromatography and spectroscopy. Superactive Alumina encompasses higher capacities and provides higher yields when impurities and peroxides are removed, saving both time and money.

Neutral Alumina

Neutral Alumina DAI is devoid of ionic particles which could change the pH-value of an aqueous slurry. **They are uniform, non-exchanging particles, while other products simply provide mixtures of anion plus cation exchanging alumina. This feature is important because possible catalytic interactions with ion sensitive substrates are minimized, thus keeping rearrangements of unknown samples at a minimum. These products are manufac-tured using unique DAI processes.** See G. Hesse, I. Daniel and G. Wohlleben, Angew. Chem. 64, 103 (1952).

Technical Adsorbents

Adsorbents in commodity quantities are available in 50 kg and 100 kg containers. Alumina of all three surface modifications as well as Silica in various particle cuts are available. Special particle size cuts and/or adsorbents providing specific properties can be custom tailored according to customer specifications.

Dry Column Chromatography

This technique facilitates an up scaling of TLC analytical separations to a preparative size operation. Columns are dry packed using specifically developed sorbent and thin nylon tubing. Nylon tubing is available in flat rolls. Creases can be removed from these tubes by either blowing hot air through them with a hair dryer or rinsing them with acetone and hot water. Dry column chromatography is covered by applicational examples Nos. 4-11, of this primer.

Applicational Examples

Examples No. 1-4 are intended for use as introductory experiments for column chromatography work. Examples No. 5-25 constitute excerpts from literature where DAI adsorbents (active Alumina and Silica) were recommended. For more detail refer to the original cited literature. Although the products are only referred as “alumina” or “silica” it is easy to find the appropriate DAI product by studying the latest DAI product line.

1. The separation of crystal violet from uranin as an example for the demonstration of the difference between basic and acid alumina and the influence of the eluent.

25 mg of crystal violet and sodium salt of fluorescein (oHnOsNa uranin) are dissolved in 50 ml of 98 % aqueous ethanol. An aqueous solution of the same concentration is also prepared. Two columns of 10mm diameter and 200 mm length are prepared using basic and acid alumina DAI respectively. Each 6 ml of the solution in alcohol and water, respectively, is applied to different columns. Separation is achieved as indicated on the graph below. These examples demonstrate how properties of the various surfaces (basic and acid) and solvents influence the development and separation of identical solutes.

solvent	Al_2O_3	development
ethanol	basic	Crystal violet eluted by 20 ml of ethanol. Fluorescein remains adsorbed as a short, orange zone. Elution of fluorescein by 20 ml of water.
	acid	Crystal violet eluted by 20 ml of ethanol. Fluorescein remains adsorbed at a broad yellow zone. Water gives additional broadening of zone but does not effect elution.
water	basic	50 ml of water elute fluorescein. Crystal violet is retained on the column as a narrow zone.
	acid	Crystal violet is eluted by 20 ml of water. Fluorescein is smeared along the entire column, but only small portions of it are eluted by water.

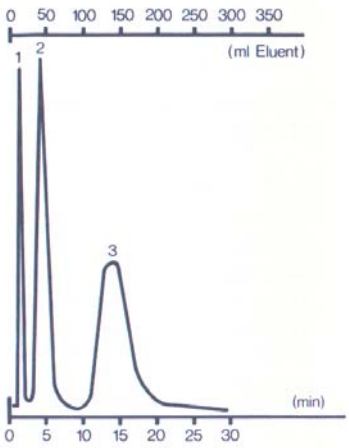
2. Separation of cis- and trans-azobenzene on silica gel plates[3,30]

Two spots of equal size of commercial azobenzene (dissolved in benzene) are applied to a TLC plate coated with activated Silica Gel DAI TLC. After drying, one half of the plate is covered with black paper and then exposed for 1 -2 hours to sunlight or, to ultra-violet light (quartz lamp). The plate is then placed in a chamber with cyclohexane:benzene (3:1) causing the solution to climb 10 cm up the plate. Two spots will now appear - one near the starting point (cis-azobenzene) and another with a Rp value of 0.7 (trans-azobenzene). The cis-azobenzene spot is appreciably visible on the illuminated side, as compared to the shielded side.

3. Liquid chromatographic separation of benzene, amidopyrin and antipyrin

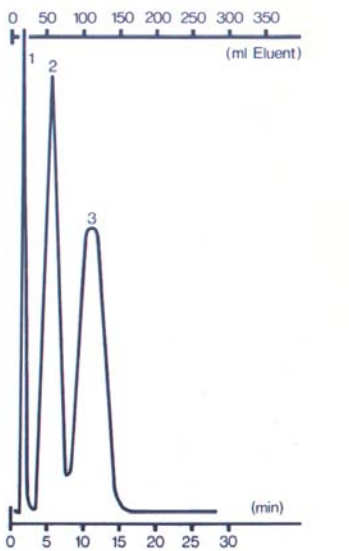
The following separations were achieved in a liquid chromatographic system using neutral alumina and silica of 32-63 microns as an adsorbent.

Substances Separated	Sample (mg)	Retention Volume (ml)
1. benzene	0.1	15
2. amidopyrine	0.1	40
3. antipyrine	0.1	135



Operating conditions: Sample dissolved in:
iso-octane/methylene chloride
Adsorbent used:
Alumina. Act. Super I, Neutral,
32-63 microns
Stationary Phase: 10% water
Eluent:
iso-octane: methylene chloride = 6:4
Sample Volume: 0.2 ml Flow Rate: 10 ml/min
Operating Pressure:
27 psi Column Size: 9 mm x 300 mm
Temperature:
20° C Detection: UV detector 251 nm

Substances Separated	Sample (mg)	Retention Volume (ml)
1. benzene	0.1	15
2. amidopyrine	0.1	35
3. antipyrine	0.1	105



Operating conditions: Sample dissolved in:
iso-octane/methylene chloride
Adsorbent used: Silica 32-63 Stationary Phase:
25% triethanolamine
Eluent:
iso-octane: methylene chloride = 2:8
Sample Volume: 0.2 ml Flow Rate: 10 ml/min
Operating Pressure:
28 psi Column Size: 9 mm x 300 mm
Temperature: 20° C Detection: UV detector 251 nm

4. "Dry-Column Chromatography": This is a preparative chromatographic **technique** with resolution similar to thin-layer Chromatography[17' 18>19]

Love uses activity alumina grade II-III, with a fluorescent indicator for short-wave UV light as his medium for dry-column systems.

According to his papers, "Dry-Column" technique separations are comparable to those performed by thin-layer chromatography, and are carried out rapidly.

Alumina DAI and Silica DAI for Dry-Column Chromatography allow for direct transfer of TLC-plate or microscope slide conditions to column scale purification. They can be used in glass columns or thin Nylon film tubing (transparent to short-wave UV light) of 10-50 mm in diameter.

The empty column is filled with adsorbent and the mixture ready for separation is deposited on the top of the column. The solvent for developing the chromatogram moves down the dry column by **capillary action**. The separation is finished after the solvent reaches the bottom of the column (usually after 15-30 minutes). The Nylon tube (thin film) can be cut to remove and isolate the separated fractions.

5. The separation of ferrocene, acetyl ferrocene and diacetyl ferrocene. A dry-column experiment.

The dry column technique exhibits many advantages over classic column chromatography. Dry column chromatography is simple, requires little time for set up and uses small amounts of solvents. Originally, DCC was used to separate mixtures received from the acylation reaction of ferrocene. This example is very attractive as the components differ from each other by color and because they can be completely resolved from each other. The separation can easily be visually monitored as the solvent migrates through the column. The separation accomplished through DCC is very simple when the best suited solvent mixture is determined on a small scale using TLC.

Experimental Details

The crude mixture is obtained by reacting ferrocene with acetic anhydride and with phosphoric acid. Acetyl ferrocene is the main product with small quantities of diacetyl ferrocene and non-reacted ferrocene present. The chromatographic separation may further be improved by slightly heating the initial reaction mixture. For the same reason it is recommended to avoid an excess of sodium hydrogen carbonate during the neutralization step. Cotton or glass wool is placed at the bottom of a chromatographic glass tube, approx. 20 in. long and ¾ in. wide. 35 g of DAI Alumina for DCC is filled into the glass tube using a funnel. The column is tapped so that the bed settles tightly.

The top of the bed must be absolutely level. The reaction mixture is dissolved with approx. 5 ml. of acetone and mixed with 2 g. of DCC adsorbent. Acetone is evaporated until the adsorbate powder flows freely. This dry powder is filled gently onto the top of the sorbent bed, leveled and topped by another 5 mm layer of pure DCC adsorbent. Development is started by slow (drop by drop) addition of methylene chloride until a solvent overhead of 5 cm develops. This liquid overhead is maintained as long as the solvent front migrates to the bottom of the column. As soon as the front reaches a distance of 15 mm from the bottom, solvent delivery is stopped and the overhead is removed using a pipette. Immediately after the front reaches the bottom, the column is removed from the clamp, and placed level so that the R_f values can be determined right away: Ferrocene (yellow zone), acetyl ferrocene (red/orange zone), and (if present) diacetyl ferrocene (brownish zone). The different zones are cut out of the column, using knife and spatula and transferred into glass beakers. The adsorbates are eluted 3 times, each time using 20-30 ml. of ether. Average yield of acetyl ferrocene is 30% of the theoretical yield.

6. Thin-Layer Chromatography - Dry-Column **Chromatography**[15]

It is not always possible to receive pure substances from reaction products by recrystallization or distillation. Dry-Column Chromatography (DCC) is a reliable method separating products and for the purification of organic substances. Unlike thin-layer Chromatography (TLC; 10-100 143.), grams of substance can be separated by means of this technique.

To define the best conditions for the separation of a substance mixture first separate by TLC. Use TLC-plates DAI Silica Gel F 254/366 (20 x 20cm.; thickness of layer 0.25 mm), or glass plates coated with Aluminum Oxide DAI neutral TLC (dried at approx. 130 °C. for 0.5 hours; with Fluorescent Indicator Green for wave length 254 nm.; thickness of layer 0.25 mm). The layer is divided into 10 vertical tracks by scratching off the carrier material (lines 1 mm wide). According to the usual TLC-method, the substance mixture is dissolved in a solvent and a drop of the mixture is applied to the starting points of the individual tracks. At the left, right, and upper edge of the plate the adsorbent is scratched off to form a 1 cm wide margin. The plate having been prepared is then fixed within a horizontal TLC-chamber (according to Geiss and Schlitt), the adsorptive layer facing the vaporization-trough. The latter is subdivided into 10 small troughs, corresponding exactly to the 10 vertical sections of the plate. The individual troughs contain hexane, carbon tetrachloride, benzene, chloroform, diethyl ether, ethyl acetate, acetone, n-propanol, ethanol, and methanol [(eluotropic series) see glossary]. Each of the vertical sections of the plate is now vaporized for one hour with the respective solvent. Following vaporization, the adsorptive layer is separated from the troughs with a slide in-between each division. Next, the plate is brought into contact with a solvent, e. g. cyclohexane, or - when separating highly polar substances - methanol. Separation is complete once the solvent front nearly reaches the opposite edge of the plate. After drying the plate at room temperature, it is inspected under UV-light, at 254 nm. and 366 nm. successively. Visible spots are marked with conditions for the best separation recorded.

Since the quality of the separation is often improved by employing the above-mentioned TLC-chamber as opposed to conventional separation chambers (using the same combination of solvents), the separation of the substance mixture should be repeated in the conventional chamber on the basis of the same conditions, using either TLC-plates or TLC-sheets DAI

Silica Gel F 254/366, or the above-mentioned neutral aluminum oxide layer. The solvent placed in the separating chamber consists of a mixture of cyclohexane or methanol and a suitable vaporization agent, e. g. ethyl acetate, using a 1:1 ratio. If satisfactory separation is achieved, then the conditions are adopted for the separation of larger quantities by means of DCC.

The separating column consists of a nylon tube 1 m long and closed at its lower end by means of a stapler. A quantity of Aluminum Oxide or Silica Gel DAI for Dry-Column Chromatography, equivalent to the 300-fold weight of the substance to be separated is filled into the tube and compacted by repeatedly tapping the bottom of the column.

The concentrated solution of the substance mixture is mixed with a tenfold weight of the previously used adsorbent and the solvent is distilled using a rotary evaporator. The obtained dry mixture is deposited as an even layer on top of the column and covered with another 2 cm of adsorbent. The column is sealed with a small pad of glass wool and the bottom is pierced with a needle. The appropriate solvent mixture is applied using a dropping funnel, forming a constant liquid head of about 2 cm. When the solvent front has reached the bottom of the column the separation is complete. The column is inspected under UV-light successively at 254 nm. and 366 nm. The zones detected are marked and cut with a razor blade. The substances are obtained by extraction of the zones with a suitable solvent and concentrated by means of a rotary evaporator.

Nylon tubes of different radii are used, depending on volume. The radius required is determined by the following data:

Most favorable length of column: 90 cm.
Ratio substance-adsorbent: 1:300 (to 1:500)
1000 ml. Silica Gel DCC = 500 g.
1000 ml. Aluminum Oxide DCC = 900 g.
Nylon film tubing available*: 30, 50, 80, 100, 120 mm flat 0; thickness of wall 0.04 mm.

A quantity of 300 g or 600 ml of adsorbent is required for the separation of 1 g of substance mixture on Silica Gel DCC. The tube should have a diameter of 50 mm.

7. Pre-separation of essential oils and similar complex substance mixtures for GC analysis by means of modified dry-column Chromatography[14]
It is frequently impossible to achieve complete separation of all components of complex mixtures, such as essential oils. A pre-adsorptive separation by means of column chromatography offers a solution to this problem. This method allows the exclusion of substance groups by taking advantage of the different polarities of individual fractions. **Chromatography is the procedure of choice for purification of all plant alkaloids.**

This useful procedure ensures the avoidance of frequently occurring artifacts and allows a preliminary separation into 5 fractions of different polarity. Figure 1 shows the slightly polar fractions (1 and 2) obtained by elution with pentane or benzene, using a silica gel column for dry-column chromatography. The polar fractions of the mixture are obtained by cutting the bed into 3 parts, and subsequent elution with ether:methanol = 8:2 (v/v). This results in a separation into 5 fractions of different polarity. By using standardized silica gel for dry-column chromatography with adjusted water content, additional deactivation of the sorbent is not necessary.

Equipment consists of a chromatographic tube of 250 mm in length. The lower end of the tube is closed by a ground glass-adapter with a fused sinter glass filter, and a one-way stopcock with a fused vacuum outlet piece. Ground round bottom flasks are used as receivers.

The adsorption tube is packed with Silica Gel DAI for Dry-Column Chromatography, compacted by vibration to a height of 10 cm. Two ml. of a 10%-solution of essential oil in n-pentane or n-hexane is applied to the top of the column and covered by a 1 cm-layer of the silica gel. This is followed by elution with 150 ml. of n-pentane which must pass completely through the column (= fraction 1). Then the receiver is changed and development is carried out by means of 65 ml of benzene (= fraction 2). The column, run dry is divided - starting from the bottom - in a 4:3:3 ratio; the individual silica gel-zones are scratched out with a spatula. Each zone is immediately suspended in a 10 ml solution consisting of ether:methanol (8:2). Fractions 3, 4, and 5 are similarly obtained.

Fractions 3-5 are processed separately. After transferring the suspension into the chromatographic apparatus (described above) allow the liquid to drain off, wash with 30 ml. of ether:methanol mixture (8:2), extract twice with 40 ml of water to remove the methanol and dry the ether phase by means of Na_2SO_4. Concentrate the fractions to 2 ml under reduced pressure and N_2-atmosphere.

Fraction 1 contains the apolar compounds (hydrocarbons). Fraction 2 comprises the slightly polar compounds, such as esters of monocarboxylic acids of medium length chain. Compounds found in fraction 3 are, for instance, acetates of aliphatic alcohols of medium length chain. Fraction 4 contains aliphatic aldehydes and ketones and fraction 5 contains the most polar compounds (alcohols, etc.).

An example of the separation of a test-mixture essential rue oil by means of gas chromatography is offered.

8. Analysis of the essential oil of Ocimum gratissimum L.[6]

O. gratissimum is a labiate which widely grows throughout Western Africa and used by homeopathic physicians for medicinal benefits. There are eugonol, citral, and thymol containing varieties. The thymol-containing group represents a “living pharmacy”. Decoctions of oil leaves are used for the treatment of jaundice. Drops prepared from singed leaves are used for the treatment of colds and for tropical malaria. A thorough analysis of the plant’s contents demonstrates therapeutic benefits based primarily on mono- and sesquiterpenes. [Hydrocarbons (terpenes) with 10 carbon units which may be linear or contain rings (C_{10}, H_{16}) and 15 carbon atoms, respectively].

Essential oil is obtained through water-steam-distillation. After reduction of the high thymol-content of the oil by sodium hydroxide solution, separation into hydrocarbons and oxygenized compounds is performed on Aluminum Oxide DAI for Dry-Column Chromatography according to the method by Loev and Goodman.

The pre-separation by means of dry-column Chromatography is necessary for complex mixtures such as essential oils. The effectiveness of this chromatographic procedure is comparable to thin-layer chromatography, making the isolation of a pure substance (thymol methyl ether) possible.

The fractions are separated by gas Chromatography into individual components and identified by means of IR-, UV-, and partly NMR-spectrums.

In previous experiments oil was separated using hexane and acetone on silica gel 0.2-0.5 mm in a glass column. By using dry-column chromatography, considerable time and material is saved. To this effect, 280 g of Aluminum Oxide DAI for Dry-Column Chromatography is packed by vibration in a nylon-film tubing of 100 cm length and a diameter of 32 mm (= 500 mm flat 0; product no. 09612). The bottom of the tube is closed, filled with glass wool, and perforated. One ml of oil and 200 ml of methylene chloride is chromatographed in the nylon tube. Detection under UV 254 nm. is performed and the tube is cut into 3 fractions, according to identified zones.

Desorption is done for each fraction with 100 ml of ether. Hexane is more effective for the isolation of thymol methyl ether.

9. Studies on acetylene compounds[29]

Following the reaction of 2-butynyl-phenyl sulfone with cyclohexanone all extracts are united, washed, dried and the solvent evaporated. The residue is separated using Silica DAI for Dry Column Chromatography contained in nylon tubing, 5 cm in diameter and 100 cm long. A free flowing adsorbate of the residue with DCC silica is placed on top of the sorbent bed. The chromatogram is developed using benzene:petroleum ether = 5:1 (v/v) for the mobile phase. After the solvent front has reached the bottom of the column, the nylon column is cut into 6 equal sections, using a razor blade. The second and third section from the bottom will be separately eluted with the mobile phase. The solvent is evacuated and the residue recrystalized from alcohol. The lower section yields colorless prisms of a m. p. near 86-87° C, while the upper section yields colorless prisms of a m. p. near 75-76° C. For further analytical details see literature reference.

10. Assay of aflatoxins[24]

When assaying for the presence of aflatoxins one must first separate out substances that may interfere with fluorescence. Fatty matter and plant dyes also interfere with fluorescence. Very laborious pre-cleaning procedures may be used. Dry-column Chromatography (DCC), offers an approach which includes solutes having Rp-values similar to those of aflatoxins. DDC excludes other contaminants that originate from food and feed material. Aflatoxins are extracted from the source sample using 70% acetone. The majority of contaminants are precipitated by lead acetate. The raw aflatoxins extracted are then pre-cleaned using a silica column and chloroform:methanol = 97:3 (v/v). Interfering substances still present in the eluate can almost completely be separated from the aflatoxins by DCC. **Any extract, independent of the method by which it is obtained, can be treated by DCC.**
A nylon tube 50 mm in diameter of appropriate length to hold 70 g of Silica DAI for DCC and some liquid overhead is packed after cotton is inserted to its base. Small holes are punched at the location of the cotton allowing for air flow while the solvent migrates down the column. Nylon tubing is used because of inertness for chromatographic eluents. Before usage, the column is tapped or vibrated allowing the sorbent to tightly settle. Development is performed using either chloroform:acetone: N-hexane = 85:15:20 (v/v) and stopped as soon as the front has almost reached the bottom. The column is viewed using UV light at 360 nm and fluorescent zones are marked. They can be clearly differentiated from zones retained at the top of the column. All homologs of aflatoxin are located at one common zone. This zone is cut out of the bulk column using a sharp knife. This section is then transferred into a glass column previously filled with 2 cm of dehydrated sodium sulphate. The column is then eluted with 150 ml of chloroform: methanol = 145:5 (v/v). Next, the solvent is evaporated and redissolved using 5 ml of chloroform. The solution is subjected to TLC and quantitation is performed by either visual comparison with standards or by fluorometry or fluorodensitometry.

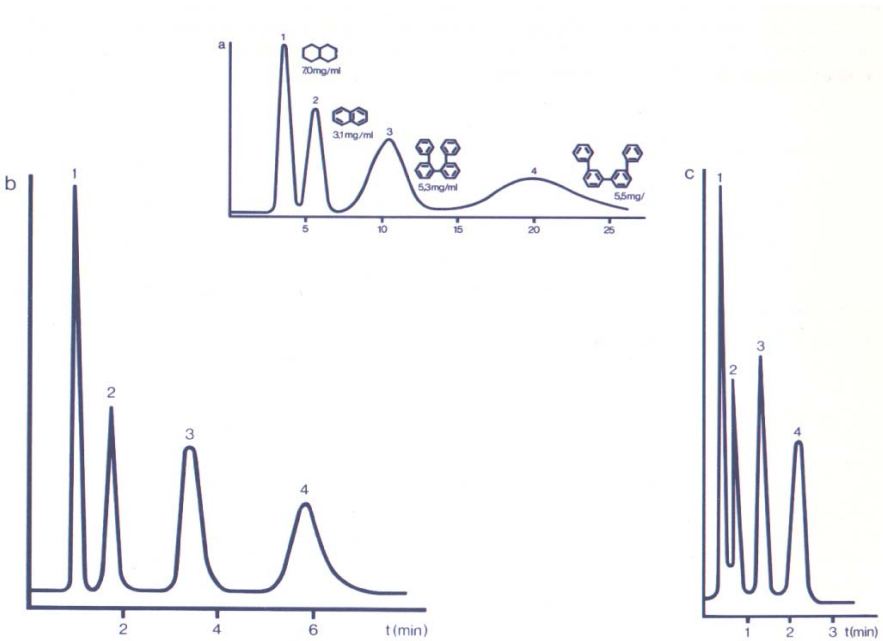
11. The determination of griseofulvin using potassium dichromate dissolved in sulfuric acid[1]

Griseofulvin is assayed by dissolving in concentrated sulfuric acid and the subsequent addition of potassium dichromate. This procedure yields a dark red color reaction. Omission of potassium dichromate causes the formation of an intensively yellow colored solution. To determine the structure of the reaction products the precipitate of a preparative test reaction is filtered, washed and dissolved in chloroform. The solution is then washed and the active ingredient partitioned into a solution of sodium carbonate by shaking. Subsequently, the liquid phase is acidified and the sample precipitated. The precipitate is extracted by chloroform and its constituents separated using a DCC column filled with Silica DCC (1.5 x 50 cm). Prior to filling the column DCC Silica is further deactivated by the addition of 10% (w/w) water. The mobile phase consists of a combination of chloroform:acetone:formic acid= 98:1:1 (v/v).

12. An exact and rapid method for the determination of polycyclic hydrocarbons

High speed analytic liquid chromatographs require fine-grained adsorbents with narrow mesh cuts. This is shown by the diagrams a, b, and c. Diagram a) refers to Aluminum Oxide DAI neutral, Act. I, b), and c) refer to Alumina DAI N 18. The experimental details are shown in the table below. In case b) and c) - though using shorter columns and in only a fifth or a tenth of the time - an excellent separation of the polycyclic hydrocarbons is achieved.

	a	b	c
Column	$A1_2O_3$ neutral, Act I (70-150 µ) 6% H_2O added (W/W)	Alumina N 18 (18-300 µ) 6% H_2O added (W/W)	Alumina N 18 (18-300 µ) 6% H_2O added (W/W)
Instrument	Waters Assoc. Liquid Chromatograph, Model ALC-201, x 32 Attenuation	Waters Assoc. Liquid Chromatograph, Model ALC-201, x 8 Attenuation	Waters Assoc. Liquid Chromatograph, Model ALC-201, x 8 Attenuation
Dimensions of the column	100 cm x 2.3 mm inner diameter	50 cm x 2.3 mm inner diameter	20 cm x 2.3 mm inner diameter
Solvent	Hexane	Hexane	Hexane
Flow rate	0.82 ml/min	1.5 ml/min	1.5 ml/min
Injection volume	20 µl	20 µl	10 µl



13. Chromatographic determination of sodium **and potassium** hydroxide **contents of electroplating baths13**

NaOH and KOH concentrations can be determined after appropriate conversion to the respective chloride salts. Separation is performed using solutions containing approximately 0.5% of each solute applied with 34 bar pressure. A column measuring 2.5 x 100 cm is filled with basic alumina of 50-80 micron size. NaCl and KCl can be separated within 20-30 minutes if the samples are within a 0.04 to 1-molar concentration range.

14. Reactions of toluene sulphonates on an alumina surface23

This work studied the chemistry of sulphonate esters on the surface of Activity I Alumina. Results cover the influence of active Alumina on primary and secondary acyclic or cyclic toluene-p-sulphonate esters. The esters are dissolved in carbon tetrachloride and added to samples of acid, basic and neutral aluminium oxide. The mixtures are stirred and digest for 24 hours at 25°C. Aliquots are sampled at standardized time intervals and analyzed. Isolation is achieved by repeated extraction of the oxide using carbon tetrachloride, eluting all cracked compounds. Subsequent extraction using ether separates alcohol compounds. A trans -1,2-cleavage is the preferred pathway. With exo-norbornyl toluene-p-sulfonate a 1,3-cleavage and hydrolysis occurs, retaining the original configuration.

15. A sulfur analog of the croconate dianion27

4,5-Dimethylmercapto-4-cyclopentone-1,3-dion, dissolved in dry dioxane is refluxed together with selenium dioxide. Selenium is filtered off and the solvent evaporated to yield dark violet crystals. These crystals are dissolved in methylene chloride and resolved on a 1 cm x 30 cm column consisting of 30 g of Silica DAI 100-200 microns. A violet zone of 1,1'-bi (3,3' 4,4'-tetramethylmercapto-2,2', 5,5'-tetraoxo) -cyclopentenylidene elutes first and constitutes 22 % of the yield, followed by a red zone of 4,5-dimethyl-mercapto-4-cyclopentene-1, 2, 3-trion, and yields 46% of the starting material.

16. Metal complexes of tetrapyrrol ligands4- 5

Metal chelates are produced by reducing methylation of zinc octaethylporphinate. Each is separated using **a** column of neutral Alumina DAI, activity grade III. A recrystalization of these orange, yellow-red and red compounds is accomplished without any difficulty. Metal acetylacetonates are also reacted with octaethyl porphins. This complexing is accomplished using triple and quaternary charged ions. Acetelylacetonates are soluble in organic solvents. All complexes are separated using Alumina Neutral, grades III and IV.

17. C-17-oxidation of clavin alkaloids having primary alcohol hydroxy moieties[21]

Dihydro-lysergol-1 is used as a model. Oxidation in the presence of cyclohexanone leads to 6-methyl -8-(2-oxo-cyclohexylidene methyl)-ergoline-1 and dihydro-lysergic acid-1. Alkaloids are oxidized yielding the pertinent carbonic acids. The product of the aldol condensation is isolated by precipitation and extracted by acid aqueous solution with further extraction of the aqueous phase by benzene. This latter step removes the aldol auto-condensation products of cyclohexanone. The remaining aqueous solution is neutralized by adding diluted ammonia and basic constituents are extracted using methylene chloride. The methylene chloride extract is then washed with water and evaporated leaving a concentrated solution. Run this solution through a column consisting of 30 g Silica DAI 100-200 with methylene chloride used as the eluent. The resultant solution is evaporated and the desired compound is recrystalized with the addition of benzene.

18. The separation of cyclic nucleoside monophosphates from other nucleotides using alumina columns

This method uses Alumina sorbent neutralized by tris-HCI buffer. None of the 3', 5'-cyclic nucleotides, nucleosides, purines, pyrimidines are retained by the sorbent, while all other nucleotides remain adsorbed. Thus, 3', 5'-nucleotide monophosphates can be separated from other nucleotides. A glass column measuring 9 mm x 15 cm is filled with 1 g of Alumina N DAI. This column is moisturized with 0.05 m drops of tris-HCI-buffer. Next, the column is flushed with 15 ml of buffer. The effluent of the column is kept at a pH of 7.6. Since the 3', 5'-cyclic nucleotides are the only P-containing substances eluted, radiolabeled ^{32}P- labeled nucleoside monophosphate constitutes a precursor useful for the determination of cyclase enzymes. This method is valid for determining the activities of adenylcyclase and guanylcyclase.

19. A reliable method for the determination of estriol in pregnant urine[9]

This method is based upon acid hydrolysis, extraction, methylation, and chromatographic separation. The determination can be completed within one working day. In order to obtain total control over possible losses during the entire procedure, tritium labeled estriol-16-glucoside-uronate is added to the non hydrolysed urine. The mixture **is** methylated and extracted by benzene. Next, the extract is washed twice by distilled water and subjected to column chromatography using neutral Alumina. A fraction containing estriol-3-methyl ether is eluted using benzene:ethanol **= 94:6** (v/v), evaporated and the residue dissolved in methanol. A colorimetric quantitation is done using the Kober reaction.

20. The human gonadotropin cycle[10]

Urinary estrogen and pregnandiol concentrations was measured daily from adult females during their menstrual cycle. Urine is hydrolyzed in an acid medium and extracted by toluene. This extract is washed by an aqueous solution of NaCl in NaOH and distilled water. The solvent is evaporated and the residue dissolved by benzene. This solution is subjected to column chromatography using neutral Alumina DAI, with the fraction containing pregnandiol being evaporated. Cholesterol acetate is added and following an acetylation in dry pyridine, the mixture is quantitated using gas chromatography.

21. Chromatographic separation of phospholipids using alumina and solvents containing ammonium salts[21]

Although a number of methods exist to determine phospholipids the results remain unsatisfactory. The authors used standardized DAI neutral Alumina adjusted to activity grade IV. They report for the first time the admixture of ammonium salts in solvent achieved a separation of most acid phospholipids.
A mixture of phospholipids is extracted from sheep liver and purified by the addition of chloroform and an aqueous solution of calcium chloride. All phospholipids are transformed into calcium salt. An average of 1 mg of phospholipid is extracted per gram of raw liver. A column (diameter: length = 1:5) is slurry-packed with alumina with a solvent mixture consisting of chloroform:methanol:water = 66:33:3.2 (v/v). The liquid sample (0.5 mg per gram adsorbent) is dissolved by the same solvent mixture and charged on the column. Approximately 4 void volumes of the column elutes all simple lipids and phosphatidyl choline, while 5 volumes of chloroform:methanol:wa-ter=25:25:4 (v/v) elutes sphingomyeline and lysophosphatidyl choline and 9 volumes of chloroform:methanol:water=10:10:3 (v/v) elutes ethanolamine phospholipids. Other solvent systems are used for the elution of acid phospholipids. As the most mild elution conditions are used, salt is still found in the solvent. The column is eluted by 10 volumes of a mixture of chloroform:ethanol:70mM-NH_4NO_3 = 18:25:1 (v/v) of 5.1 pH-value, to allow phosphatidyl inositol and cardiolipin to leave the column. Finally, phosphatidyl serine is eluted by 4 volumes of chloroform:ethanol:70mM-ammonium acetate = 18:25:1 (v/v) at a pH=7.8. For larger samples, columns of 600 g and 1000 g are used (diameter: length = 1:2-3). Identical sample loads and identical solvent systems are used. The separation is completed in 10 hours.

22. Adsorption elution chromatography of alkylbenzenes using alumina columns[22]

This paper describes the separation of mono alkylbenzenes using neutral Alumina DAI. Adsorbent is deactivated by adding 0.5% (wt.) water. The glass column has an inner diameter of 4 mm, a length of 100 cm and is filled with 15 g of deactivated adsorbent, 0.5 – 1 mg of sample, causing the dissolved eluent to charge at the top of the column. The eluent consists of a mixture of N-pentane and cyclohexane, and detected spectrophotometrically.

The scope of this paper inquired into the structural properties of alkyl benzenes such as chain length, with the possibility for predicting retention volumes in a given chromatographic environment studied. Good separation is achieved between molecules owning normal C-chains and isomers at the a-position relative to the aromatic ring, with theoretical calculations being demonstrated as accurate.

23. An assay of polycyclic aromatics contained in vaseline[2]

Vaseline used for either cosmetic or pharmaceutical applications must pass stringent purity requirements. Mineral origin impurities contained in Vaseline with carcinogenic potential must be assayed and removed. Sharp absorption bands of aromatics in the UV region close to 270 nm indicate the presence of these impurities. Absence of these bands indicates a level of lower than 10^{-6} g aromatics per gram of sample. If the portion of carcinogen present within the bulk of aromatics is assumed to be 1%, then a lack of bands means a carcinogen level lower than 10^{-8} g/g. The sensitivity of this test can be improved by separating aromatics from all other paraffins. This procedure is discussed by various authors. As aromatics show considerable fluorescence in the ultraviolet region they are easily detected. The challenge is a test method for substances showing fluorescence at 366 nm. 3.4-Benzopyrene is the test substance and quinine sulfate is used as the standard. Samples of original mineral oils and samples containing 3,4-benzo-pyrene are prepared in 1% cyclohexane. Silica DAI 100-200 is used for the separation of those polycyclic aromatics showing fluorescence. Products purified by the above percolation show a decrease or no fluorescence when eluted from the column. All of the 3.4-benzyopyrene added is retained by the adsorbent.

24. Sampling and analytical procedure for polycyclic aromatics contained in exhausts[11]

Many polycyclic aromatic hydrocarbons are formed by incomplete combustion of organic compounds besides CO_2, CO and H_2O. As some of them appear to be carcinogenic, an assay for each individual component is important. The carcinogenic activity of automobile condensate exhaust is attributed to the organic compounds owning 5 and 6 ring structures. A prerequisite for a well functioning assay is developing a method to quantitatively dephlegmate and collect aromatics with boiling points in the temperature range from 340° C (phenanthrene) to 580° C (coronene, di-benzopyrene). A multi-step method for collecting 3-to7-ring aromatics is given. A first enrichment is accomplished after the deposits are collected using cooled filters at low temperature. Matter thusly collected is dissolved in acetone. Water is added to the solution and extraction is achieved using cyclohexane. The cyclohexane solution is extracted using a mixture of dimethyl formamide: water = 9:1 (v/v).

Next, the DMF-phase is diluted by a sufficient amount of water to replenish its original volume. This diluted phase is extracted by cyclohexane. Cyclohexane is washed with water, dried and the solution volume is reduced to 1 ml by evaporation. This remaining 1 ml is charged to the top of a 10 x 220 mm column consisting of Silica DAI 100-200 Active, previously deactivated by the addition of 15 percent water (w/w). Cyclohexane is used as an eluent. This step is followed by another pre-separation using a molecular sieve. The resulting fraction containing the high molecular weight aromatics is separated using a column filled with Alumina N DAI Super I previously deactivated by the addition of 5.4 per cent water (w/w). A 10 x 75 mm column is used. Detection is done by spectrophotometry. Test solutions are used to determine the exact retention volumes of the 12 aromatics which are the major constituents.

25. The chromatographic separation of (carcinogenic) polycyclic **aromatics**[25]

A great number of procedures for the collection and separation of polycyclic aromatics using column, paper, thin layer and gas chromatography are discussed in this review.

Frequently, dusty or sooty matter must be extracted. Most organics are extracted by benzene. This solvent however excludes fluorimetric quantitation. Cyclohexane exhibits selectivity for the polycyclic aromatics. Spectrograde isooctane, cyclohexane, and N-pentane are well suited when the absorption or fluorescence of the compounds is required for quantitation. Chloroform, methylene chloride, dichloroethane and nitromethane should be omitted. No guidelines exist for the minimum time required for each individual extraction process. Twenty three methods are listed, employing column chromatography for separation and for quantitative determination of compounds. Column dimensions vary from 8 x 30 mm to 12.5 x 600 mm. Active Alumina is preferred while some authors use Active Silica. Among basic, neutral and acid alumina, most authors prefer basic and neutral preparations. Seventeen TLC-methods referenced here use nearly identical solvent systems as column procedures. Basic and neutral alumina layers are preferred, although silica, silica G and silica GF are used. Most layers are activated prior to separation. Comparing paper and thin layer chromatography, the authors point out that TLC is unexcelled because of short development time, sharpness of separation, sensitivity and the possibility of using more aggressive spray reagents. A number of gas chromatographic separations are also discussed in this review.

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Quick Reference to all DAI Adsorbents:

Adsorbents for Column Chromatography	Packing g	particle size range in µm: 3–6	7–12	10–18	18–32	0–63	32–63	32–100	63–100	63–200	100–200	200–500
Silica	10	●	●	●	●							
	100	●	●	●	●							
	500					●	●	●	●	●	●	●
	1 000						●			●	●	
	25000					●	●		●	●		●
Silica, active	10				●							
	100				●							
	500						●	●	●	●	●	●
	25 000										●	
Silica, spec. types	500									DCC		
	3000									DCC		
	25000									DCC T		T
Alumina	10	N	N	N	N							
	100	N	N	N	N							
	500						N					
Alumina, active	10				N							
	100				A B N							
	500						N					
Alumina, Act. 1	500									A B N		
	1000									A B N		
	5000									A B N		
	50000									A B N		
Alumina — Super 1	500									A B N		
	1000									A B N		
	5000									A B N		
	50000									A B N		
Alumina, spec. types	500									DCC Br R		
	5000									DCC Br		
	50000									DCC Br R T		

DCC = for Dry-Column Chromatography
A = acid alumina
B = basic alumina
N = neutral alumina
Br = Act. II–III according to Brockmann
R = for radioisotope techniques
T = for plant purposes (different types)

Please ask for special information on TLC materials and precoated plates and aluminum backed sheets

Adsorbents for Column Chromatography:

Highly Standardized Aluminum Oxides	Activity	pH-Value about	Particle Range		Water Soluble Matter %	Bulk Weigth About g/ml
			µm	mesh		
Alumina N 3 — 7		7.5	3 — 6		<0.1	0.9
Alumina N 7 — 12		7.5	7 — 12		<0.1	0.9
Alumina N 10 — 18		7.5	10 — 18		<0.1	0.9
Alumina N 18 — 32		7.5	18 — 32		<0.1	0.9
Alumina N 32 — 63		7.5	32 — 63		<0.1	0.9
Alumina A 18 — 32, active	active	4.5	18 — 32		<0.1	0.9
Alumina B 18 — 32, active	active	10	18 — 32		<0.1	0.9
Alumina N 18 — 32, active	active	7.5	18 — 32		<0.1	0.9
Alumina N 32 — 63, active	active	7.5	32 — 63		<0.1	0.9
Alumina A — Super 1	Super 1	4.5	50 — 200	70 — 290	<0.1	0.9
Alumina B — Super 1	Super 1	10	50 — 200	70 — 290	<0.1	0.9
Alumina N — Super 1	Super 1	7.5	50 — 200	70 — 290	<0.1	0.9
Alumina A, Akt. 1	1	4.5	50 — 200	70 — 290	<0.1	0.9
Alumina B, Akt. 1	1	10	50 — 200	70 — 290	<0.1	0.9
Alumina N, Akt. 1	1	7.5	50 — 200	70 — 290	<0.1	0.9
Alumina Akt II — III acc. Brockman	II — III	10	50 — 200	70 — 290	<0.2	0.9
Alumina R 3 — 7		4.3	50 — 200	70 — 290		0.9
Alumina DCC 3 — 7	III/20 mm	neutral	63 — 200	70 — 230		0.9
Dynamic Adsorbents, Inc.		**Tailor-made Alumina for Plant Scale Use**				

Adsorbents for Column Chromatography:

Highly Standardized Silica Gels	Activity	pH-Value about	Particle Range		Water Soluble Matter %	Bulk Weigth About g/ml
			µm	mesh		
Silica		6.5 — 7	3 — 6		<0.2	0.4
Silica		6.5 — 7	7 — 12		<0.2	0.4
Silica		6.5 — 7	10 — 18		<0.2	0.4
Silica		6.5 — 7	18 — 32		<0.2	0.4
Silica		6.5 — 7	32 — 63		<0.2	0.4
Silica	active	6.5 — 7	18 — 32		<0.2	0.4
Silica	active	6.5 — 7	32 — 63		<0.2	0.4
Silica		6.5 — 7	<63	<230	<0.2	0.3
Silica		6.5 — 7	32 — 100		<0.2	0.5
Silica		6.5 — 7	63 — 100	150 — 230	<0.2	0.5
Silica		6.5 — 7	63 — 200	70 — 230	<0.2	0.5
Silica		6.5 — 7	100 — 200	70 — 150	<0.2	0.4
Silica		6.5 — 7	200 — 500	30 — 70	<0.2	0.5
Silica	active	6.5 — 7	32 — 100		<0.2	0.5
Silica	active	6.5 — 7	63 — 100	150 — 230	<0.2	0.5
Silica	active	6.5 — 7	63 — 200	70 — 230	<0.2	0.5
Silica	active	6.5 — 7	100 — 200	70 — 150	<0.2	0.4
Silica	active	6.5 — 7	200 — 500	30 — 70	<0.2	0.5
Silica DCC	III/30 mm	6.5 — 7	63 — 200	70 — 230		0.5
Dynamic Adsorbents, Inc.			**Tailor-made Silica Gels for Plant Scale Use**			

Adsorbents for Column Chromatography

Catalog No.	Particle Size	Qty.
Silica Active, 60A (Continued)		
2749	18-32u	10g
2805	18-32u	l00g
2750	32-63u	500g
2766	32-100u	500g
2767	63-100u	500g
2769	63-200u	500g
02751-05	100-200u	500g
02751-1	100-200u	1kg
02751-2	100-200u	2.5kg
02751-5	100-200u	5kg
02751-25	100-200u	25kg
2770	200-500u	500g
Silica Gel MPLC, 60A		
04668-05	0-63u	500g
04668-1	0-63u	1kg
04668-2	0-63u	2.5kg
04668-5	0-63u	5kg
04668-25	0-63u	25kg
2745	18-32u	10g
2757	18-32u	l00g
02830-05	18-32u	500g
02830-1	18-32u	1kg
02830-2	18-32u	2.5kg
02830-5	18-32u	5kg
02830-25	18-32u	25kg
02759-05	32-l00u	500g
02759-1	32-l00u	1kg
02759-2	32-l00u	2.5kg
02759-5	32-l00u	5kg
02759-25	32-l00u	25kg
Silica Gel Classic Column, 60A		
04660-05	63-l00u	500g
04660-1	63-l00u	1kg
04660-2	63-l00u	2.5kg
04660-5	63-l00u	5kg
04660-25	63-l00u	25kg
04667-05	63-200u	500g
04667-1	63-200u	1kg
04667-2	63-200u	2.5kg
04667-5	63-200u	5kg
04667-25	63-200u	25kg
02761-05	100-200u	500g
02761-1	100-200u	1kg
02761-2	100-200u	2.5kg
02761-5	100-200u	5kg
02761-25	100-200u	25kg
02809-05	200-500u	500g
02809-1	200-500u	1kg
02809-2	200-500u	2.5kg
02809-5	200-500u	5kg
02809-25	200-500u	25kg

Adsorbents for Column Chromatography

Catalog No.	Particle Size	Qty.
Silica Gel		
Silica for TLC 5-15u, 60A		
04671-05		500g
04671-1		1kg
04671-2		2.5kg
04671-5		5kg
04671-25		25kg
04674-05	with Gypsum	500g
04674-1	with Gypsum	1kg
04674-2	with Gypsum	2.5kg
04674-5	with Gypsum	5kg
04674-25	with Gypsum	25kg
04677-05	with F-254	500g
04677-1	with F-254	1kg
04677-2	with F-254	2.5kg
04677-5	with F-254	5kg
04677-25	with F-254	25kg
04680-05	with Gypsum and F-254	500g
04680-1	with Gypsum and F-254	1kg
04680-2	with Gypsum and F-254	2.5kg
04680-5	with Gypsum and F-254	5kg
04680-25	with Gypsum and F-254	25kg
Silica for Prep TLC, 60A		
04682-1	with Gypsum and F-254	1kg
04682-5	with Gypsum and F-254	1kg
Silica for HPLC and Flash Grade		
2790	5u	10g
2791	5u	l00g
2793	l0u	l0g
2794	l0u	l00g
2796	15u	l0g
2797	15u	l00g
Silica Flash, 60A		
02826-05	32-63u	500g
02826-1	32-63u	1kg
02826-2	32-63u	2.5kg
02826-5	32-63u	5kg
02826-25	32-63u	25kg
Silica Wide Pore (150 A)*		
03227-05	63-200 micron*	500g
03227-1	63-200 micron	1kg
03227-2	63-200 micron	2.5kg
03227-5	63-200 micron	5kg
03227-25	63-200 micron	25kg
Silica Wide Pore (200 A)*		
03227-05	63-200 micron*	500g
03327-1	63-200 micron	1kg
03327-2	63-200 micron	2.5kg
03327-5	63-200 micron	5kg
03327-25	63-200 micron	25kg
Silica Wide Pore (500 A)*		
03427-05	100-250 micron*	500g
Silica Wide Pore (1000 A)	inquire for availability and price	
Silica Active, 60A		
2749	18-32u	10g
2805	18-32u	l00g
2750	32-63u	500g
2766	32-100u	500g

Adsorbents for Column Chromatography

Catalog No.	Particle Size	Qty.
Alumina Wide Pore (Continued)		
591974	25u	l00g
591975	32-63u	1kg
591976	63-100u	1kg
591977	100-200u	1kg
591978	50-200u	1kg
Alumina Dry Column Chromatography		
04514-05	F-254	500g
04514-5	F-254	5kg
04514-50	F-254	50kg
Silica Dry Column Chromatography		
04530-05	F-254	500g
04530-1	F-254	1kg
04530-3	F-254	3kg
04530-5	F-254	5kg
04530-25	F-254	25kg
04630-25	w/o F-254	25kg
Drysphere Desiccant		
01001-05	w/o Indicator	500g
01001-10	w/o Indicator	10kg
01001-25	w/o Indicator	25kg
01001-50	w/o Indicator	50kg
01005-05	20% Indicator	500g
01005-10	20% Indicator	10kg
01005-25	20% Indicator	25kg
01005-50	20% Indicator	50kg
01006-05	100%Indicator	500g
01006-10	100%Indicator	10kg
01006-25	100%Indicator	25kg
01006-50	100%Indicator	50kg
01010-1	w/o Indicator	1kg
Nylon Tubing		
9652	1" Flat Outside Diameter x 20 Meters	
9653	1 1/2" Flat Outside Diameter x 20 Meters	
9654	2" Flat Outside Diameter x 20 Meters	
9655	2 1/2" Flat Outside Diameter x 20 Meters	
9656	3" Flat Outside Diameter x 20 Meters	
9662	6" Flat Outside Diameter x 20 Meters	

Adsorbents for Column Chromatography

Catalog Number	Particle size	Qty.
Alumina Acid for TLC		
04347-1	5-15u	1kg
04347-50	5-15u	50kg
Alumina with Gypsum		
04413-1	5-15u	1kg
04413-50	5-15u	50kg
Alumina for PCB Removal	**(Alumina C)**	
02103-1	50-200u	1kg
02103-50	50-200u	50kg
Alumina for Pyrogen Removal		
02120-1	50-200u	1kg
02120-50	50-200u	50kg
Alumina for Bio-Mass Clean-Up		
02300-1	50-150u	1kg
02300-5	50-150u	5kg
02300-25	50-150u	25kg
02300-50	50-150u	50kg
Alumina for Process Clean-Up	**(Scavenger)**	
04100-1	150-600u	1kg
04100-5	150-600u	5kg
04100-25	150-600u	25kg
04102-1	600-1200u	1kg
04102-5	600-1200u	5kg
04102-25	600-1200u	25kg
04104-1	1200-2400u	1kg
04104-5	1200-2400u	5kg
04104-25	1200-2400u	25kg
Alumina for Decolorization		
05005-1	30-200u	1kg
05005-5	30-200u	5kg
05005-25	30-200u	25kg
05005-50	30-200u	50kg
Alumina for Dioxin Analysis		
05788-05	50-200u	500g
05788-1	50-200u	1kg
05788-5	50-200u	5kg
05788-25	50-200u	25kg
Alumina for Arsenic Removal		
995500-98		25kg
Alumina for Radioactive		
06031-05	50-150u	500g
06031-50	50-150u	50kg
Specialty Sorbents		
9602	Polyamide For CC	250g
9603	Polyamide For TLC	250g
9604	Polyamide Prep Scale	250g
9605	Polyamide Large Scale	
9804	Florisil PR, 60-100 Mesh	500g
Alumina Wide Pore		
591371	5u	10g
591372	l0u	10g
591373	15u	10g
591374	25u	l00g
591375	32-63u	1kg
591376	63-100u	1kg
591377	100-200u	1kg
591378	50-200u	1kg
591971	5u	10g
591972	l0u	10g
591973	15u	10g

Adsorbents for Column Chromatography

Alumina	2007 Catalog	
Catalog Number	**Particle size**	**Qty.**
Alumina Basic Act I		
02078-05	50-200u	500g
02078-1	50-200u	1kg
02078-5	50-200u	5kg
02078-50	50-200u	50kg
Alumina Neutral Act I		
02135-05	50-200u	500g
02135-1	50-200u	1kg
02135-5	50-200u	5kg
02135-50	50-200u	50kg
Alumina Acid Act I		
02159-05	50-200u	500g
02159-1	50-200u	1kg
02159-5	50-200u	5kg
02159-50	50-200u	50kg
Alumina Basic Super I		
04577-05	50-200u	500g
04577-1	50-200u	1kg
04577-5	50-200u	5kg
04577-50	50-200u	50kg
Alumina Neutral Super I		
04589-05	50-200u	500g
04589-1	50-200u	1kg
04589-5	50-200u	5kg
04589-50	50-200u	50kg
Alumina Acid		
04601-05	50-200u	500g
04601-1	50-200u	1kg
04601-5	50-200u	5kg
04601-50	50-200u	50kg
Alumina Act II-III		
04694-05	50-200u	500g
04694-5	50-200u	5kg
04694-50	50-200u	50kg
Alumina Neutral		
2142	5u	10g
2148	10u	10g
2151	15u	10g
02156-1 [Flash Grade]	15-40u	1kg
02156-25 [Flash Grade]	15-40u	25kg
02157-05 [Flash Grade]	25u	500g
02061-05 [Flash Grade]	32-63u	500g
02061-1 [Flash Grade]	32-63u	1kg
02061-5 [Flash Grade]	32-63u	5kg
02061-25 [Flash Grade]	32-63u	25kg
Active Alumina Neutral	**for HPLC Flash Grade**	
2059	18-32u	500g
02062-05	32-63u	500g
Active Alumina Acid	**for HPLC Flash Grade**	
2063	18-32u	l00g
Active Alumina Basic	**for HPLC Flash Grade**	
2065	15-40u	500g
02262-1	32-63u	1kg
02262-25	32-63u	25kg
Alumina Basic for TLC		
04341-1	5-15u	1kg
04341-50	5-15u	50kg
Alumina Neutral for TLC		
04344-1	5-15u	1kg
04344-50	5-15u	50kg

Glossary

Absorption: a physical or chemical phenomenon or a process in which atoms, molecules, or ions enter some bulk phase - gas, liquid or solid material. This is a different process from adsorption, since the molecules are taken up by the volume, not by surface. A more general term is sorption which covers adsorption, absorption, and ion exchange.

Adsorbent: A material (such as alumina or silica gel) having the ability to extract certain substances from gases, liquids, or solids by causing the substances to adhere to its internal surface without changing the adsorbent physically or chemically.

Adsorption: The action of a material in extracting a substance from the atmosphere (or a mixture of gases and liquids) and gathering it on the surface in a condensed layer; the process is not accompanied by physical or chemical change.

Angstrom (Å): A unit of length equal to one hundred-millionth (10^{-8}) of a centimeter, used especially to specify radiation wavelengths. (In chromatography, an angstrom measures the adsorbent pore diameter).

Asymptotical: a line, usually a straight line, which is continually approached by a curve that never meets the line.

Bandwidth (zone width or peak width): represent retention dimensions (time or volume) parallel to the baseline. If the baseline is not parallel to the axis representing time or volume, then the peak-widths are to be drawn parallel to this axis. Three peak-width values are commonly used in chromatography. Peak width at base is the segment of the peak base intercepted by the tangents drawn to the inflection points on either side of the peak. Peak width at half height is the length of the line parallel to the peak base at 50% of the peak height that terminates at the intersection with the two limbs of the peak. Peak width at inflection points is the length of the line drawn between the inflection points parallel to the peak base.

Brockmann Activity Scale: Measures the percentage of water added to the adsorbent based upon weight/weight relationships between water and the adsorbent. See charts below.

Standard Activity	I	II	III	IV	V
% of water added	0	3	6	10	15

Super Activity	I/16	I/30	II/30	III/30	IV/30	V/30
% of water added	0	1	4	7	10	19

Silica Activity	I	II	III	IV	V
% of water added	0	10	12	15	20

Capillary Action (capillarity): the interaction between contacting surfaces of a liquid and a solid that distorts the liquid surface from a planar shape.

Chemical Bond: mechanism whereby atoms combine to form molecules. There is a chemical bond between two atoms or groups of atoms when the forces acting between them are strong enough to lead to the formation of an aggregate with sufficient stability to be regarded as an independent species.

Chromatogram: A graphical or other presentation of detector response, concentration of analyte in the effluent or other quantity used as a measure of effluent concentration versus effluent volume or time.

Chromatography: Chromatography is a separations method that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the components in a mixture. A column holds the stationary phase (alumina, silica gel) and the mobile phase (liquid mixture) carries the sample through it. Sample components that partition strongly into the stationary phase spend a greater amount of time in the column and are separated from components that remain in the mobile phase and pass through the column faster. As the components exit from the column they can be quantified by a detector and/or collected for further analysis.

Column Chromatography (CC): A solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase). The theory of column chromatography is analogous to that of thin-layer chromatography. The most common adsorbents - silica gel and alumina - are the same ones used in TLC. The sample is dissolved in a small quantity of solvent (the eluent) and applied to the top of the column. The eluent, instead of rising by capillary action up a thin layer, flows down through the column filled with the adsorbent. Just as in TLC, there is an equilibrium established between the solute adsorbed on the silica gel or alumina and the eluting solvent flowing down through the column.

Desiccant: A substance (adsorbent) used to withdraw moisture from other materials. Although the removal of large quantities of water is done by evaporation, aided by moving air currents and by elevated temperature, the last traces of moisture are often held very tightly and do not evaporate readily. Furthermore, evaporation ceases when the moisture content of the material is reduced to that of the drying-air current. For final drying, a desiccant is used. It may react with water chemically or retain water through capillarity of adsorption. The drying agent is placed directly into the gas or liquid to be dried; solid materials are placed in a desiccator, a closed vessel in which moisture diffuses to the desiccant through the dry desiccator atmosphere. A desiccant loses potency as it takes on water; often it can be renewed by heating. Desiccants which form hydrates can be selected to maintain certain levels of low humidity in a closed vessel. Among the more important types of solid desiccants are silica gel, activated alumina, anhydrous calcium sulfate, magnesium perchlorate, oxides (of barium and calcium), and activated carbon.

Desorption: the ability for a chemical to move with the mobile phase. The more a chemical desorbs, the less likely it will adsorb, thus instead of sticking to the stationary phase, the chemical moves along the solvent front.

Dry Column Chromatography (DCC): A modern chromatographic technique allowing easy and rapid transfer of the operating parameters of analytical thin layer chromatography (TLC) to preparative column chromatography (CC). The dry-column technique bridges the gap between preparative column chromatography and analytical thin-layer chromatography.

Dynamic Equilibrium: a condition in which several processes act simultaneously to maintain a system in an overall state that does not change over time.

Eluent: the liquid or gas entering a chromatographic bed (e.g. a column) used to effect a separation by “elution”.

Eulotropic Series: a relative ranking of liquid chromatography solvents ranging from non-polar to very polar properties in order of their eluting power.

Flash Chromatography: A rapid form of preparative column chromatography –Prep LC based upon “an air pressure driven hybrid of medium and short column chromatography optimized for rapid separation.” This approach was pioneered by W.C. Still at Columbia University, and described in J. Org Chem. 43, 2923 (1978). Separation was based upon the relatively inexpensive apparatus used.

Dynamic Adsorbents, Inc. Family of Flash Cartridges

Alumina
Silica
Bonded Phases
Custom Phases

Industry's cleanest, lowest metal content adsorbents, Standardized production, Reproducible lot to lot.

DAI's Alumina Superior in Selectivity and Versatility




Application	Description of Application	Recommended Alumina
ALKALOIDS	Isolation from ergot-, opium-, rauwolfia-, and other alkaloids	Basic, medium activity, Specialty
ANTIBIOTICS	Isolation, purification	Neutral
ESSENTIAL OILS	Removal of terpenes	Basic, Neutral
PLANT EXTRACTION	Isolation of active substances	Basic, Neutral, Acid
DEHYDRATION OF ORGANIC SOLVENTS	Use in HPLC solvents, removal of H2O	Basic. highly active Drysphere™
ENZYMES	Purification	Neutral
GLYCOSIDES	Isolation of digitalis-, strophantus-, glycosides, etc.	Neutral
REMOVAL OF LEAD	Cations from water	See Specialty Types
HORMONES	Isolation and purification of synthetic products, of ketosteroids from neutral materials. etc.	Neutral
PURIFICATION OF ORGANIC SOLVENTS	for analytical and technical purposes	Basic, highly active
OILS	Clarification of fatty oils, separation of fatty acids	Basic
PCB'S	Remove from solvents, Transformer oils	Alumina "C "
REMOVAL OF PEROXIDES	from organic solvents	Basic, highly active
REMOVAL OF PYROGENS	from injectable solutions and infusions	Alumina P
TAXOLS AND NEUTRACEUTICALS	Various medicinal extracts from plants, etc.	Alumina Biomass & Decolorization

VERSUS

Superior Adsorption

Alumina	
30 Parameters for Selectivity	
A.	3 pH ranges: acid, basic, and neutral
B.	2 surfaces areas for standard Chromatography…Alumina: 150 m^2/gm and 200 m^2/gm
C.	5 Brockman activity ranges: I, II, III, IV, V

Silica Gel	
Parameters for Selectivity	
A.	pH ranges 6.5 to 7.5
B.	2 Brockman activity ranges: I and II