Chromatography: A Perspective

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IN 1906 the Russian botanist, Tswett, published in the proceedings of the German Botanical Society (*30*) a description of a new technique for separating the components of complex mixtures. This technique, which was based on the partition of solutes between a stationary solid adsorbent and a moving liquid phase, was dealt with more comprehensively in a later work in Russian (*31*). It consisted simply of pouring a small quantity of the solution of pigments, such as green leaf pigments, on the top of a vertical column of adsorbent and following this with pure solvent, whereupon a series of colored bands formed down the length of the column in a sequence determined by mass relationships and adsorption coefficients. Tswett named his technique the "chromatographic method."

Unfortunately the chromatographic method got off to a bad start. In the first place, Tswett's book was published in Russian, and his original paper was published in a botanical journal, as were several later ones. Hence much of his work did not receive wide notice by chemists. In the second place, many of his publications at that time were somewhat polemic in nature. In reading the abstracts of his publications one cannot help but feel in Tswett a vigorous individualism, which probably grated on the sensibilities of the chemical hierarchy. Tswett had a clear conception of the tremendous scope of his discovery and such implicit faith in the procedure that against all opposition he insisted doggedly upon the presence of two components in chlorophyll and in the "phaeophytin" of that period. But for a lowly botanist to assault thus the whole chemical profession was unthinkable! Although a few isolated remarks appeared in favor of Tswett's work, the chromatographic method fell largely into disrepute, and, according to Zechmeister (*33*), Tswett spent the latter part of his life in misery and poverty.

Chromatography was not revived until 1931, under the stimulus of rather widespread researches on the carotenoids. In the intervening period only infrequent references to the technique can be found. Palmer, in his monograph on the carotenoids (*16*) published in 1922, speaks very highly of the technique and refers to several of Tswett's publications. The importance of the carotenoids as precursors of vitamin A was not realized, however, until 1928, when Euler and co-workers (*8*) pointed out that pure carotene, lycopin, and certain other carotenoids showed the same characteristic reaction with antimony trichloride in chloroform as did vitamin A. They further demonstrated the pronounced activity of carotenoids as growth factors and were able to show a definite relationship (*9*) between the antimony trichloride reaction, carotene content, and vitamin A potency. This new incentive led several investigators to undertake intensive studies in this field, and in 1931 Kuhn and Lederer (*13*) described the separation of carotenoids from several natural sources by "adsorption analysis" on fibrous alumina. This was followed two years later by publications by Karrer and Morf (*11*) and Karrer and Walker (*12*) describing the separation of α- and β-carotenes on calcium oxide or hydroxide. Then in 1934 two articles by Strain (*22, 23*) appeared, setting forth the excellent properties of magnesium oxide as an adsorbent for the separation of α- and β-carotenes. Strain concluded that there was no support for the view that α- and β-carotenes are artifacts arising from the action of the adsorbent. These observations appear to have broken the back of any remaining opposition to the method initiated by Tswett, and numerous publications continued to flow from the pens of Karrer and co-workers, Strain, and others.

From the early 1930's to the present day, chromatography has continued to develop in a multiplicity of directions, aided by numerous ingenious devices and refinements in technique, so that there is no longer a single branch of chemistry that has not profitably employed this separational procedure. In the words of Paul Karrer in his lecture at the International Congress of Pure and Applied Chemistry (London, 1947), quoted recently by Tiselius (*29*):

> No other discovery has exerted such a great influence and widened the field of investigation of the organic chemist as much as Tswett's chromatographic analysis. Research in the field of vitamins and hormones, carotenoids, and numerous other natural components could never have progressed so rapidly and achieved such great results if it had not been for this new method, which has also disclosed the enormous variety of closely related compounds in nature.

It is beyond the scope of this paper to treat individually of each important development or useful variation in the method. An excellent review of the more important developments is given in a recent publication by Strain (*25*). A general perspective of the scope of modern chromatography, as well as some insight into possible future developments, may be gained, however, by considering the process from the standpoint of competing equilibria and by making a classification on this basis. Such a classification assumes complete reversibility in the interactions taking place—a condition occasionally not fulfilled and, for certain applications, not always necessary. But in the general case the stationary adsorbent and the mobile fluid (which may be either gaseous or liquid) compete with each other for the solutes originally present in the fluid phase. Such a classification is given in Table 1.

TABLE 1

Primary mode of action	Class	Type of competitive interaction:		Representative phenomena*
		Mobile phase	Solid phase	
Ionic: (solubility product, instability constant, etc.)	1	Ions	Ions	Ion exchange
	2	Complex	Ions	Ion exchange
	3	Ions	Complex	Inorganic chromatography
	4	Complex	Complex	(Not reported)
	5	Ions	Dispersion	Fractional decomposition methods
	6	Complex	Dispersion	Fractional decomposition methods
	7	Dispersion	Dispersion	Diffusion of colloids, macromolecules
	8	Ions	Association	Electrokinetics
	9	Dispersion	Ions	(Not reported)
	10	Dispersion	Complex	(Not reported)
Nonionic: H-bonding; dipole, and/or induced dipole; geometry of molecules (partition coefficients)	11	Association	Association	Organic (and partition) chromatography
	12	Association	Dispersion	Catalytic polymerization
	13	Association	Ions	Catalytic dissociation
	14	Association	Complex	(Not reported)
	15	Complex	Association	(Not reported)
	16	Dispersion	Association	Salting-out adsorption

* The items in this column are admittedly incomplete and dependent primarily on the author's limited knowledge. The reader may be able to suggest phenomena that could be included in the spaces labeled "not reported."

In this table the following definitions apply:

Ion: A solute particle having a definite electrical charge and characterized by a simple structure and subcolloidal dimensions.

Complex: The product of a reversible interaction between a solute particle of simple structure and a complexing agent to form a new species. The combination usually involves linkages of an auxiliary, or coordinate, character. The complex may itself be a simple structure (charged or uncharged), or it may be a relatively large and complicated aggregation of particles appearing as a colloidal dispersion, as a precipitate, or as an immobile deposit on the solid phase.

Dispersion: The product of a reversible interaction of a solute with another solute, or with the solvent itself, to produce a species having a decreased association with the solvent. This decrease in association becomes evident in the aggregation of particles to form a colloidal dispersion or a visible precipitate. In this discussion the term dispersion is not intended to include those dispersons arising from a process of complexing as described in the preceding paragraph. It does, however, include dispersions of proteins, silicates, soaps, dyes, organic polymers, and other particles which may or may not be ionically charged, depending upon pH, adsorption or occlusion of ions, presence of dissociable groups, etc.

Association: A mutual attraction between a solute particle and another substance (either in the liquid or solid phase, or both), or between two solute particles of the same species, which does not involve the establishment of fixed bonds. This interaction is characterized, rather, as a loosely bound complex of indeterminate composition arising from the geometry of the molecules involved, from dipole and induced dipole interactions, from resonating electrostatic attractions, or from other imperfectly understood factors. The "bond energies" involved, insofar as this term may be applied here, lie in the neighborhood of 2 to 8 kilocalories per mole, as contrasted with the much greater bond energies involved in the types of aggregation discussed previously.

A casual study of these types of interaction will reveal that the selection is to some extent arbitrary. It is difficult or impossible to assign quantitative definitions to these terms. Yet the phenomena they represent are real, and their selection is based on their suggestiveness of these phenomena in accordance with current usage. Consequently, they are employed here as descriptive titles rather than as terms of exact definition. As such, they are paired in Table 1 to form sixteen possible combinations that theoretically may give rise to competing equilibria by means of their relationships to the mobile phase and the solid phase. The title gives the key to the type of relationship existing. Each pair describes a class. Several of the classes represented will be recognized as already popular in chromatographic methods. Others are just being introduced, or have not as yet been suggested. It is the purpose of the following paragraphs to consider briefly the development and prospects of chromatography as related to these classes.

Class 1: Chromatography by ion exchange has been widely studied. Schwab and co-workers have done considerable pioneering in this field (*19, 20*) and have applied inorganic chromatography as an adjunct in microanalysis (*18*). Perhaps one of the most impressive applications of this method is the concentration of the isotopes of potassium, lithium, and nitrogen by Taylor and Urey (*27, 28*) using columns of zeolites. The relative adsorption affinities of ions appear to depend primarily on valence, degree of hydration, and basicity. The separation of zirconium and hafnium on an ion exchange resin has been announced recently (*26*), development being carried out with 6 M hydrochloric acid. The more basic hafnium appears first in the effluent.

Class 2: An important refinement of simple ion exchange chromatography is described in considerable detail by Spedding, Boyd, Tompkins, and others (*21*). These investigators employed buffered citrate solutions in the chromatographic development of rare earth mixtures on synthetic ion exchange resins. The competition between resin and complexing agent for the individual elements gives rise to a separation depending mainly on relative basicities. It was found possible to separate in a comparatively small number of operations various mixtures of rare earth elements into spectroscopically pure individuals. To obtain a similar result by older methods requires as much as a thousand fractional recrystallizations. The chromatography of fission product mixtures has led to the first positive chemical identification of isotopes of element 61 (*14*).

Class 3: By reversing the system described as Class 2 and selecting a complexing agent for the solid phase it is again possible to achieve separation of ions. Erlenmeyer and Dahn (*7*) have pioneered in this method, employing 8-hydroxyquinoline as the "adsorbent." The position of several metals are recognizable on the column by the colored complexes they form. The pH of the liquid phase is an important factor, since the stabilities of the complexes are directly related to it. The use of this method does not seem to be widespread, possibly because of the limited number of complexing agents possessing favorable characteristics and also because even 8-hydroxyquinoline itself tends to be displaced from the solid phase during the process of development. It would seem that these difficulties could be overcome by "fixing" the complexing agent irreversibly on another solid. Silica, for example, might be precipitated in the presence of 8-hydroxyquinoline to form an "oxinated" silica with the desired characteristics. Much developmental work is obviously possible.

Class 4: Finally, competing equilibria may be established between a complex in the mobile phase and a complex in the solid phase. A mixture of citrate complexes, for example, might be chromatographed on a column of 8-hydroxyquinoline. Here again, the separation will depend on the basicity of the complexed ion, and the sequence of zones should be predictable on the basis of ionic crystal radii. While this method increases the separational possibilities included under the first three classes, no work seems to have been done on it.

Class 5: A solution of cations by chromatographic development may be induced to precipitate in colloidal form through a gradual increase in pH. The colloidal material, being less mobile than the original ions, is retained in the interstices of the adsorbent as a "zone." This mechanism has been pointed out recently by Meinhard and Hall (*15*), and it is felt that some of the previously reported inorganic separations regarded as proceeding by simple ion exchange in reality occur by this process. The method is limited at present primarily to analytical applications on a micro or semimicro scale. Its success will depend on refinements in technique which lead to reliable quantitative estimations.

Class 6: The system described as Class 5 may be modified by first complexing the original ions. The mechanism will then involve decomposition of the complex prior to the precipitation of a zone. Separations will be based on the relative stabilities of complexes and the mobility of the complexing agent. This system should not be confused with Class 15, in which the complex is adsorbed as such on the column. Where the chromatography of simple ions does not lead to a useful separation, a complexing agent may be employed to modify both the sequence and the sharpness of definition of the zones. Little work appears to have been done on this type of separation (*10*) and the formation of a less mobile species by hydrolysis seems to have been largely overlooked.

Class 7: Chromatographic methods so far do not seem to have found much application in the separation of proteins and other macromolecules or molecular aggregates. In this classification the extent of aggregation of the colloid in the liquid phase is often different from, and usually less than, its aggregation when in contact with the solid adsorbent. Unfortunately, proteins are often irreversibly adsorbed and may suffer denaturation at the solid-liquid interface. Adsorption studies on enzymes initiated by Willstätter (*32*), and his school, have resulted in certain successful separations on alumina and silica gel, but such adsorbents cannot be applied generally. An obvious suggestion here, and one that apparently has not been reported, is the chromatography of protein dispersions using as the immobile phase a solid protein structure. Such a structure would seem to be capable of a maximum degree of specificity combined with a maximum degree of "gentleness" toward the adsorbate.

A somewhat different application of this method has been employed recently in the purification of zirconyl salts (*1*). This procedure depends on the formation of the hydrous oxide of zirconium when zirconyl nitrate is dissolved in water at moderate concentrations. The hydrous oxide exists in colloidal form and is nonionic. By passing the solution through an ion exchange column all ions existing as impurities are retained, while the zirconyl colloid appears quantitatively in the effluent. A similar process has been indicated (*4*) for the commercial preparation of silica dispersions. A solution of sodium silicate is developed on an acid-treated ion exchange resin, which retains the sodium ion and releases a salt-free colloidal silicic acid.

Class 8: When a solid does not contain individual ionic centers of attraction its activity toward the passage of a solution of ions is manifested in the electrokinetic effect. Here the ions are associated with the solid in a postulated electric double layer. A recent description of the process is given by Bikerman (*2*). The effect becomes prominent only at very low concentrations and, in the case of uni-univalent electrolytes, is proportional to the concentration$^{-1/2}$. It is difficult to see how such a system could be made the basis of a useful chromatographic method, especially in view of the high potency of ion exchange media. It is interesting, however, to consider this effect in relation to the migration of ions in soils and other geological formations.

Class 9: The chromatography of a colloidal dispersion which combines ionically with the adsorbent has been suggested for proteins using an ion exchange resin (*29*). The reaction of the colloid with ion exchange media might be predictable on the basis of its interaction with other ionic species. It is impossible, however, to assess the potential value of such a method in the absence of experimental data. An interesting possibility is present in the case of insoluble inorganic salts. If an aqueous dispersion of barium sulfate, for example, is chromatographed on an acid-treated cation exchange resin, the barium ion should be adsorbed while sulfuric acid appears in the effluent. Development of the column may be continued using a different acid, the net result being a barium salt whose original anion has been replaced. The chromatographic separation thus accomplished is one of cations from anions. A separation of a mixture of cations obviously can take place at the same time. Such an application should find use in certain analytical schemes where sulfate, or other anions, are objectionable and difficult to eliminate.

Class 10: If for the ion exchange resin of Class 9 a complexing agent is substituted, still another chromatographic system is obtained. If it is desired to chromatograph on 8-hydroxyquinoline a mixture of metals obtained as difficultly soluble salts, one may add them as a dispersion directly to the column rather than resorting to additional operations designed to convert them to a more soluble form. Using a buffer solution as the developing solvent, the original anions may be carried into the effluent and the metal zones themselves may be separated. Such a method, of course, has only a limited usefulness.

Class 11: Chromatography owes its present popularity primarily to its tremendous success in the field of organic chemistry. In this classification the usurping effects of ionic charges are present to a negligible degree only, and separations become dependent on the associations of the solutes with solvent and adsorbent. Since differences in degree of association may arise from minute structural alterations, separations are based on structure alone. The separation of *cis*- and *trans*-isomers, and of optically active isomers, eloquently demonstrates the potency of this method. One of the foremost publications on chromatography, especially with respect to organic separations, is the book by Zechmeister and Cholnoky: *Die chromatographische Adsorptionsmethode* (*34*). In spite of the extensive studies made in this realm, chromatography still remains largely an art rather than a science (*24*).

Partition chromatography also may be placed in this category. Here the "solid" phase is actually a stationary immiscible liquid phase, and the rate of migration of the solute is a function of its partition coefficient in the two liquids. The filter paper chromatography of protein hydrolyzates initiated by Consden, Gordon, and Martin (*3*) is an example of this method that has attained widespread use. It should be pointed out, however, that the partition chromatography mechanism is valid in a more general sense. It is generally agreed from independent experiments that retention of liquid at a solid-liquid interface may extend to a depth of as much as a hundred molecules or more, provided the solid is "wetted" by the liquid. In viscous flow such a layer may be regarded as a "stationary" phase. Further, the structure of the stationary liquid is distinctly modified by the proximity of the adsorbent, thus completing the resemblance to an immiscible liquid phase. It will be seen, therefore, that the difference between ordinary chromatography and partition chromatography depends primarily on the selection of media and not on the mechanism involved.

Class 12: The catalytic effect of an adsorbent on a given solute molecule occasionally results in polymerization to a species which forms colloidal aggregates. This material may be adsorbed irreversibly or it may wander down the column at a sufficiently modified rate that a separation may be effected from another solute not similarly acted upon. The use of such a method is distinctly limited, especially since the polymerization process is in most cases irreversible.

Class 13: An adsorbent may also cause catalytic dissociation of a solute molecule with the formation of an ionic species in the adsorbate. Here again the process may be irreversible. By development with a sufficiently polar solvent, however, recombination of ions may occur, resulting in an equilibrium process. Obviously, in certain cases such a recombination may give rise to a more complex mixture than the original material. The process has been made the basis of various separations, however, in certain types of partition chromatography where a solvolytic ionization takes place in the aqueous phase. Elsden (*5*), for example, has separated mixtures of fatty acids in chloroform on a silica gel column, using bromcresol green as a column indicator.

Class 14: The employment of a solid phase which forms a complex with an organic solute does not appear to have been reported. Picric acid is suggested here as a complexing agent which might be used as the "adsorbent." Since it is soluble in organic solvents, it must first be rendered immobile either by the introduction of hydrophilic groups into the molecule or by irreversible adsorption on a suitable solid. Such a modified adsorbent may then be used in the chromatography of aromatic hydrocarbons, ethers, etc., the separation depending upon the relative stabilities of the corresponding picrates. Inasmuch as such picrates are often highly colored, the progress of a zone down the column may be readily traced. Other similar complexing agents include *sym*-trinitrobenzene, picrolonic acid, styphnic acid. Mercury salts might be used in the chromatography of certain unsaturated compounds; and such materials as calcium chloride and aluminum chloride, could be used in the separation of aliphatic ethers. For the separation of halogen derivatives, the use of ion exchange resins containing amine and imine groups is possible. All of these systems require essentially anhydrous conditions in order to avoid complications of an ionic character.

Class 15: The solubility of various metal complexes, such as the dithizonates, in organic solvents has led to a number of extraction procedures of analytical value. Little has been done, however, with the chromatography of such solutions. The separation of heavy metal dithizonates on alumina has been reported (*6*) as partially successful, but the use of other complexes in this manner has not been described. It is felt that further investigation of this method should be rewarded by useful separations which are especially applicable in trace-metal analysis.

In this same category partition chromatography is also possible. Such a system would involve the percolation of an organic solution of complexes through a column of silica gel, or other suitable water-retentive adsorbent. It might be suggested that this method be included in Class 4 except that here we are concerned primarily with the association of complexes rather than the competition between two different complexing agents. These systems may be reversed by employing an organic solvent in the solid phase and using an aqueous solution of complexes for the mobile phase.

The chromatography of aqueous solutions of metal complexes on hydrophilic adsorbents which have been pretreated with the complexing agent has been described (*35*). Where these complexes are ionic in character (e.g., the metal ammine complexes) this system becomes one of simple ion exchange and properly belongs in Class 1; where they are uncharged (e.g., soluble chelate complexes) the migration of the complex is governed primarily by its association with the adsorbent and may be included here. The developing solvent in these cases also contains the complexing agent, and the separation of zones results from a competition between the aqueous phase and the adsorbent for the complexed material. That is, dissociation of the complex itself is not a material factor in the process, as it is in Classes 2, 3, and 4. These fields of application are untouched.

Class 16: The chromatography of colloidal dispersions is beset with a number of difficulties, including the possibility of the presence of an almost limitless number of molecular species (e.g., polymers) and of widely varying degrees of aggregation. A phenomenon has been suggested recently by Tiselius (*29*), however, which promises to assist materially in the chromatography of proteins, namely, "salting-out" adsorption. Proteins of low adsorption affinity may be strongly bound to the solid phase in the presence of a salting-out agent such as ammonium sulfate. The exact nature of the association of the protein with the adsorbent is not known, but where the strength of association varies with different proteins the basis of a chromatographic separation is established. The elution of separate proteins may be carried out simply by varying the salt concentration in the developing solvent. The success of the method requires, of course, complete reversibility among the species present.

Some progress in the chromatography of particulates ranging from virus to bacterial size has been made, and its application has been demonstrated recently (*17*) in the separation of certain subcellular, enzymatically active granules. This separation was accomplished on columns of Celite, using aqueous solutions of sodium chloride as developing solvents. The mechanism here, also, is apparently one of salting-out adsorption, inasmuch as the original adsorption was accomplished under the influence of a much higher salt concentration than was used in the development of the column. Obviously, such a procedure is of value in amplifying, or supplanting, centrifugal methods of separation.

It will be apparent to investigators familiar with chromatographic methods that the scheme advanced above is somewhat oversimplified. It often happens, for example, that two or more of the types of interaction given here occur simultaneously in varying degrees on a single chromatogram. Conversely, because the exact nature and properties of certain solute particles are not well understood, it becomes difficult to place their chromatographic behavior in a specific class. It is also apparent that many of the significant advances in instrumentation have been omitted. For a description of these, the reader is referred to the several excellent reviews and monographs on the subject.

This tabulation is designed primarily to fulfill two purposes. It is intended, first, to point out those directions in which the greatest advances in chromatography have taken place and to reveal some of the reasons for these advances. Second, it is intended to suggest other applications which have not been tried, or which have been only briefly examined, with the hope of indicating some probable areas of future research.

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