REVIEW ARTICLE

CHROMATOGRAPHY AND ITS APPLICATIONS IN PHARMACY

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THE extensive use of chromatography in so many unrelated fields of chemistry during the past two decades attests to its present importance as one of the most valuable of laboratory techniques. A number of comprehensive works and reviews[1,2,3,4,5,6,7,8,9,10] dealing with its history, methods and applications is now available, and it is intended here to survey only some recent developments in technique and a small selection of their applications to pharmaceutical problems.

PRINCIPLES

Chromatography is essentially a process for the fractionation of mixtures by continuous partition between two phases, one of which is moving past the other. In principle, there appears to be no restriction on the nature of the mobile and stationary phases nor on the types of equilibria involved in the partition. In practice, the most important applications have involved a liquid mobile phase and a liquid or solid stationary phase.

A solution of the mixture to be fractionated is placed on top of a column of adsorbent and slowly passed through; according to the affinity of the components of the mixture for the adsorbent, they are held in zones at the top of the column. Passage of more solvent through the column, by fractional elution and re-adsorption, develops the chromatogram by separating the zones of adsorbed material throughout the column, the more strongly adsorbed substances being nearer the top of the column. If the separate zones are readily located by their colour, in ultra-violet light or by means of a reagent streaked along the extruded column, they are separated by division of the column and eluted with a suitable solvent. As an alternative technique elution development is employed; further solvent is passed through the column in order to elute the components successively as shown by suitable chemical or physical tests. Where these methods are inapplicable, the eluate is collected in arbitrary fractions and each fraction is examined separately. In partition chromatography,[6,7,8,9,10] the column consists of an inert carrier on which is distributed a solvent immiscible with the mobile solvent. Similar principles are exploited in paper chromatography,[6,7,8,9,10] where selective adsorption on paper or partition between the mobile phase and the water present in paper, which has been equilibrated with the saturated vapours of both phases, operate to effect the fractionation.

Theoretical treatments[6,7,11] of adsorption and partition chromatography have proved of little value in experimental work, since no theory has so

far considered all the factors known to be important. The most satisfactory treatment appears to be that of Martin and Synge[11] in which distillation theory is applied to the operation of a column. Although experimental verification of theories of chromatography is available under standard conditions, the optimum conditions for a chromatographic separation are usually worked out empirically.

A useful characteristic of a given set of conditions in a chromatographic system is obtained from the ratio:

$$\frac{\text{distance moved by solute}}{\text{distance moved by solvent}} = R_F \text{ or } R$$

The R_F value is of more particular use in partition chromatography, especially on paper, since it is then related to the partition coefficient[6] which remains roughly constant over a relatively wide range of solute concentrations, whereas in adsorption chromatography continuous variation of R with concentration is usual. R_F values are, however, occasionally subject to variations due to extraneous factors and it would appear preferable to make observations of values relative to a suitable standard substance. The sequence of substances on an adsorption column is another useful descriptive and analytical character since, although the sequence may be altered by changes in the solvent and adsorbent,[9] under standardised conditions it is reproducible.

Methods

The general methods of experiment are described in the standard works.[1,2,3,4] Although there may be no clear fundamental distinction between adsorption and partition chromatography, it still remains convenient to consider them separately.

Adsorption Chromatography

Adsorbents. Amongst the following commonly used adsorbents of diminishing order of activity: alumina, magnesium oxide, calcium sulphate, calcium oxide, talc, calcium phosphate, calcium carbonate and lactose, together with those whose relative activity has not been thoroughly examined, namely silica, kieselguhr, paper, starch, clays and charcoal, there are enormous variations in adsorptive capacities and specificities which may vary further with the solvents used. A number of methods for the standardisation,[12] activation and deactivation of alumina and other adsorbents[13] are now available. Very active alumina is made by heating pure aluminium hydroxide at 380° to 400° C. for 3 hours; for the removal of alkali, commercial alumina is boiled with successive quantities of water until soluble alkali is removed, then washed with methanol and reactivated at 160° to 200° C./10 mm. Hg. pressure.[7] In choosing an adsorbent,[4] the load of mixture to be fractionated should be low so that extensive development is possible; in practice, it is frequently possible to fractionate 1 g. of a mixture on about 30 g. of adsorbent, but the column load influences the degree of fractionation. Several cases of chemical change, such as the aminolysis and oxidation of amino-acids,[14]

occurring on adsorption columns have been reported and in some instances this phenomenon has been exploited for preparative purposes.[15]

Solvents. Special purification of solvents is not usually necessary. Fractionation of mixtures of similar substances is often enhanced by elution development with a series of solvents of increasing polarity which have powers of elution in the following ascending order: light petroleum, carbon tetrachloride, *cyclo*hexane, ether, acetone, benzene, esters, chloroform, alcohols, water (at various *p*H values), pyridine and liquid organic acids. Excellent examples of this technique, known as the *durchlaufmethode*, are provided by recent work on *Strophanthus* glycosides.[16] Hydroxylic solvents may cause difficulty because of their solvent action on the adsorbent; thus alumina is appreciably soluble in methanol and in water.

Apparatus and experimental methods. The simple device described by Martin[6] is extremely efficient for the preparation of a uniformly packed column. A slurry of adsorbent and solvent of a creamy consistency is poured into the chromatogram tube and thoroughly homogenised by a few rapid strokes of a perforated disc mounted by its centre on a long, thin metal rod. The disc should fit the tube closely and the diameter of the perforations should be not more than about 1/16 in. The disc is brought to within about 1 inch of the bottom of the tube and then moved slowly downwards; this causes the solid to pack beneath it. Rapid homogenising strokes are followed by slow packing strokes until the whole column is packed. With such columns, it is often necessary to accelerate the rate of flow of developing solvent by regulated, positive pressure or suction. Even formation of the zones is facilitated by a low rate of flow, uniform pressure and the use of a long column; small temperature variations appear to have little effect. Unevenness in the development of the zones in large columns is often unavoidable, but is greatly reduced in a multiple column of 2 or 3 units with interposed mixing cells.[17,18] Extrusion of the developed column may be difficult, especially when the adsorbent has not been packed as a slurry; for this reason, the use of slightly tapering tubes has been recommended. Amongst the modifications of the usual apparatus for special purposes,[19,20] a column of filter-paper discs or strips,[21] applied hitherto mainly in the resolution of mixtures of enzymes,[22] may have extensive applications. A film of adsorbent fixed to a microscope slide by means of starch has been employed in microchromatography.[23]

Location of zones. For the detection of zones of adsorbed, colourless substances on a column, fluorescence in ultra-violet light, the formation of dark bands on a fluorescent adsorbent[24,25] or streak reagents[1,26] may be appropriate. In the location of fluorescent substances, quartz apparatus is not normally required, but quartz tubes are necessary for the observation of fluorescence-quenching on columns of fluorescent adsorbent. Such an adsorbent, suitable for the detection of substances adsorbing between 230 and 390 mμ, is prepared by mixing silicic acid with half its weight of kieselguhr ("Celite 535") and adding 2·5 per cent. each of fluorescent zinc silicate and zinc sulphide. After development, the zones are observed in ultra-violet light in a darkened room.

Streak reagents are applied with a brush as a longitudinal streak on the extruded column; sensitive reagents for aliphatic and aromatic amines, alcohols, phenols, ketones, acids, nitro compounds, nitramines, unsaturated hydrocarbons and vitamin A have recently been carefully evaluated.[26] The progress of a chromatographic fractionation may frequently be followed by a continuous recording of changes in the physical properties of the issuing eluate; for this purpose changes in conductivity,[27] refractive index[28] and ultra-violet absorbancy ratios[29] have been exploited.

When these methods for the detection of colourless substances are inapplicable, it is usual to employ elution development. Several efficient devices for the automatic collection of the eluate fractions are available.[27,30]

Partition Chromatography

The original partition column, intended for the separation of acetylated amino-acids, consisted of a column of silica gel on which the stationary phase, water, was supported; the mobile phase was a water-immiscible solvent saturated with water (chloroform with 1 per cent. of *n*-butanol). The individual partition coefficients of the components of the mixture are usually the determining factors in this type of fractionation.[7] Modifications of the stationary and mobile phases and of the support for the stationary phase have led to the application of this technique to a wide range of mixtures.

Types of partition column. It appears that to avoid displacement from the support by the mobile phase, the stationary phase must be the more polar. As a consequence water, buffers[31,32] or aqueous solutions of acids and bases[33,34,35] have been most frequently utilised as the stationary phase and a water-immiscible solvent as the mobile phase. The use of a stationary phase other than water or an aqueous solution, together with an appropriate mobile phase, has been successful in some instances[7]; thus *n*-butanol supported on cellulose acetate, nitromethane supported on silica with *n*-hexane or methanol as mobile phase and other systems[36,37] have been employed. Recently reversed-phase partition chromatographic systems, in which the less polar solvent of a given pair forms the stationary phase, have been described; these have involved the use of kieselguhr treated with dichlorodimethylsilane[38] and of powdered glass.[39] Silica,[40] kieselguhr, starch, powdered cellulose, pulped filter-paper, charcoal and powdered glass are efficient supports for the stationary phase of partition columns. Of these, selected grades of kieselguhr ("Hyflo Super Cel" and "Celite 535") are probably the most generally useful.

Apparatus and experimental methods. The apparatus and experimental techniques employed with adsorption columns are used with partition columns. After thorough trituration of the stationary phase and supporting solid by stirring with a rod in a beaker, the column is packed by Martin's method. In addition to the methods for the detection of the zones applicable to adsorption columns, suitable indicators may be incorporated in the stationary phase, particularly when mixtures of acids or bases are being examined. In elution development of a partition

column, the load of mixture undergoing fractionation is relatively low and variation in the partition coefficients with concentration may cause widening of the bands. Automatic measurement of changes in the physical properties of the eluate has frequently been employed to obtain a continuous record of the course of fractionation.[27] Adsorption on the supporting material of a partition column usually leads to a reduction in the operating efficiency of the column and may cause difficulty.[41] In some cases it may however play an important part in effecting fractionation.

Paper Chromatography

Partition chromatography on paper[40] has developed rapidly and there is now an extensive literature devoted to the subject.[3,10] In this process, a small spot of solution containing 1 μg. to several mg. of the mixture is placed near the top of a strip of filter-paper, the end of the paper near the spot is inserted in a trough containing a solvent saturated with water, and the whole is suspended in a suitable chamber whose atmosphere is saturated with the vapours of water and of the solvent. When the solvent has flowed a suitable distance down the paper, the position of the solvent front is marked and the paper is dried. The components of the fractionated mixture are then treated with an appropriate reagent, usually applied by spraying, and finally characterised by comparison of their R_F values with the simultaneously determined values for authentic materials. Caution must be exercised in the characterisation of compounds solely on the basis of their R_F values.

In developing a two-dimensional paper chromatogram, the spot of solution is placed near one corner of a square sheet of filter-paper and one edge is inserted in the solvent trough. After the solvent has flowed nearly to the opposite edge of the sheet, the paper is removed, dried and developed at 90° to the direction of flow of the first solvent with a second solvent in the trough. By using two solvents, the degree of fractionation is greatly enhanced. The relative importance of partition and adsorption in effecting fractionation in paper chromatography is doubtful in many cases[8,42] and ion-exchange may have some significance in this connection.[43]

Apparatus and experimental methods. Of the various grades of filter-paper, Whatman No. 1 to 5 appear to be most satisfactory[44]; for larger scale work, the use of thicker paper, made from Whatman No. 1 and blotting paper, has been described.[45] The scope of paper chromatography may be extended by impregnation of filter-paper with various substances to modify the properties of the stationary phase; amongst the substances applied in this way are phosphate buffer,[46] alumina,[47,48] silica,[49] rubber latex[50] and resin.[51] The solvents employed are usually partially miscible with water and of relatively low volatility, such as *n*-butanol, collidine, lutidine, piperidine, furfuryl alcohol, liquefied phenol, although all types of solvent have been used and good results have been obtained with water-miscible solvents.[42,52]

Many ingenious modifications of the original experimental techniques have been evolved.[53] The most important general modification involves

development by capillary ascent,[54] a device which has the merit of greater simplicity in operation; simple forms of apparatus for this purpose,[55] especially for exploratory experiments,[56] have been described. Amongst the modifications in detail of the original procedure are convenient forms of solvent troughs,[57] chambers[58] and methods for the simultaneous development of a number of paper strips[54,59] and sheets.[60] A rapid method employing discs of filter-paper is available[61] and for substances which require prolonged development a pad of cellulose may be attached to the end of the paper strip.[62] Since after fractionation on paper the separated materials can usually be eluted without loss,[63] quantitative work on paper depends largely on the availability of analytical methods sufficiently sensitive for the examination of the eluted materials.

Location of zones. For the detection of colourless substances on a paper chromatogram, a sensitive test revealing a number of different substances is most valuable. Ninhydrin is most commonly employed for amino-acids on paper strips and sheets[64]; iodine in ethanolic solution or as vapour may be used for the location of amines, amino-acids, amino-alcohols, guanidines, purines, pyrimidines and alkaloids[65]; carbohydrates can be detected by ammoniacal silver nitrate,[7,66] by an acidified ethanolic solution of β-naphthylamine containing a trace of ferric sulphate[67] and by solutions of phenols in acidified *n*-butanol[68]; fluorescence in ultra-violet light or the quenching of fluorescence[69] have been exploited for purines and pyrimidines,[70] pterins,[71] amino-acids and peptides[72] and porphyrins.[6] By heating the paper to incipient charring many substances can be located.[61] Certain substances, such as antibiotics and growth factors, can be detected by their biological effect when the paper is incubated in contact with agar seeded with a selected organism.[73,74] Compounds containing radioiodine,[75,77] radiocarbon,[78] radiosulphur[79] and radiophosphorus[80] are detected by means of a Geiger counter or by development of an autoradiograph formed when the paper is placed in contact with X-ray film. Light transmittancy,[81] refractive indices of a liquid in contact with the paper[82] and X-ray and electron diffraction patterns of the substances after leaching[83] should be capable of wide application. In paper chromatography salts may separate into ions and cause difficulty in the detection of other substances.

Method of Tiselius

In the special techniques elaborated by Tiselius,[84] a solution of the mixture is forced upwards through a column of adsorbent and the course of fractionation is followed by a continuous record of the refractive index of the liquid leaving the column. When the process is carried out as a *frontal analysis*,[85] as with fatty acids, no developing solvent is used and only the first fraction is likely to be pure; for this reason, *elution analysis*, employing a developing solvent, or *displacement analysis*,[7,86,87] employing a substance of greater affinity for the adsorbent, are to be preferred for preparative purposes. Displacement development has been applied in the fractionation of sugars, amino-acids and peptides on charcoal columns.[86]

Structural Differences and Chromatographic Separations of Organic Compounds

Adsorption chromatography is usually the most selective technique for the fractionation of mixtures of weakly polar molecules; partition chromatography is applicable to substances which can be distributed between immiscible solvents. No general relationship has been deduced between molecular architecture and the ease of separation of organic compounds,[7,9] because of the complexity of the attractive forces in chromatographic systems, of variations in the state of molecules in different chromatographic systems and of the frequently unexpected effects of polar groups.[88] The information summarised in Table I[9] indicates that the degree of separation depends on the functional groups and is least when molecules differ in features which produce least difference in the respective attractions between the solute and the two phases.

TABLE I

Difference in structure	Ease of separation
Isomerism of acyclic compounds	poor to fair
Isomerism of acyclic and cyclic compounds	poor to fair
Position of centres of unsaturation	poor to fair
Isomerism of conjugated and unconjugated compounds	fair to good
Number of centres of unsaturation	good
Geometrical isomerism	fair to good
Optical isomerism[89]	poor
Molecular dimensions	poor to fair
Number of polar groups	good
Polarity of functional groups	good

Applications

A comparison of chromatography with other important general methods for the separation of mixtures such as distillation, sublimation, fractional crystallisation, partition and chemical separations shows that it possesses two extremely valuable features; it is applicable to small quantities of material and the conditions of operation normally cause no change in the components of the mixture being separated. Its applications to pharmacy are now so extensive that no more than a general indication of its value in pharmaceutical analysis and as a preparative and analytical method in a number of groups of substances can be given here.

Pharmaceutical Analysis

When the necessary experimental procedures have been studied in detail, chromatographic methods are of particular value in dealing with three types of analytical problems; (1) tests for homogeneity of substances liable to contamination with chemically similar substances; (2) the identification of pharmaceutical substances and preparations; (3) the determination of the individual components of complex mixtures or of substances in dilute solution. Rigorous standardisation of all the possible variables is of the greatest importance because of the frequently unpredictable effects of minor variations in chromatographic processes.

Tests for homogeneity are of particular value in the standardisation of substances obtained from natural sources, such as alkaloids, glycosides

steroids and lipoids. The presence of ergotaminine in ergotamine may be demonstrated by the appearance of two fluorescent bands on an alumina column using chloroform as developing solvent[90]; contamination of ergometrine with ergometrinine is readily shown by their separation on a paper strip.[91] Similar examples are afforded by the chromatographic detection of photolytic decomposition products of riboflavine[92] and of the homogeneity of cardiac glycosides.[93,94]

The identification of pharmaceutical substances and preparations depends upon the determination of R_F values or on observations of the sequence of the distribution of the components of a complex mixture on a column. In one of the methods for the identification of a sulphonamide, the sodium salt, after development on paper with aqueous methanol, is located by spraying with copper sulphate solution and characterised by the colour of the stain and its R_F value.[95] The recognition of the individual alkaloids of ergot has hitherto depended upon careful measurements of a series of physical constants, particularly optical rotation in different solvents. A simple solution to this problem is provided by paper chromatography of the amino-acids liberated on hydrolysis of the alkaloids.[91] The characterisation of galenical preparations[96,97,98,99] and crude drugs[100] by their behaviour on columns is a reliable and simple procedure when the conditions have been carefully standardised; some of the observed variations, as for example with extracts of male fern,[101] may be due to the preparations themselves. The scope of such methods is found empirically; thus whereas certain varieties of jalap can be distinguished,[102] attempts to identify aloe from different sources in this way were unsuccessful.[103]

In quantitative analysis, chromatography is employed in the isolation of the desired material in a form suitable for its determination by a standard chemical, physical or biological method. *Datura stramonium* has been assayed by adsorption on an alumina column of the alkaloids in an ethereal extract of the alkalinised drug, followed by elution with ethanol, evaporation to remove volatile bases and titration of the remaining total alkaloids.[104] This method yields results in agreement with those obtained by the usual procedure and is simpler in operation. The individual alkaloids of solanaceous drugs can be isolated quantitatively from partition chromatogram columns.[32,105] In the control of purity of salts of physiologically active bases, it is desirable to determine the base. For this purpose, solvent extraction can be conveniently replaced by chromatography on appropriately standardised alumina.[106]

Anthraquinone-containing drugs, for which analytical procedures have been hitherto unsatisfactory, can be standardised by isolation of the hydroxyanthraquinones on a magnesium oxide–kieselguhr column; these are then eluted and determined spectrophotometrically.[103] Further examples of quantitative processes are mentioned below and it appears probable that these methods are capable of extension to such problems as the determination of thyroxine in thyroid and the assay of injections and lamellæ.

Naturally occurring pigments. Chromatography has been extensively

used in the isolation, characterisation and determination of the 60 or so carotenoids at present known and continues as the method of choice in the examination of new materials for carotenoids.[107] Examination of urinary and fæcal porphyrins[108] and bile pigments[109] by chromatographic methods are now diagnostic aids.[110]

Steroids. The *durchlaufmethode* has been most frequently employed in the fractionation of colourless steroids on adsorption columns.[111,112,113] An example of the use of coloured derivatives[114] is afforded by the separation of the 2:4-dinitrophenylhydrazones of androsterone, testosterone, œstrone and progesterone on alumina, which is developed first with benzene, then with acetone and light petroleum and finally with chloroform. Separations on paper[115,116] have been effected by conversion of steroids to water-soluble derivatives, for example by reaction with Girard's reagent.[117] In the assay of progesterone in oily solution, the ketosteroid is isolated on a paper strip, located by the *m*-dinitrobenzene–potassium hydroxide reaction, extracted and determined spectrophotometrically.[118]

Amino-acids, peptides and proteins. The applications of chromatography to amino-acids, peptides and proteins has formed the subject of several reviews.[6,7,8,9,10,119] Recent developments in work on this group of compounds has included extension of the qualitative and quantitative methods for amino-acids[85,120,121,122,123]; the examination of peptides,[124] proteins[125] and enzymes,[126,127] insulin,[128,129] liver fractions,[7,130,131] of urinary excretion of amino-acids[132]; nitrogen metabolism of the thyroid,[75,79] of micro-organisms[133] and of enzyme systems[134] and studies of adrenocorticotrophins.[135]

Carbohydrates. The value of chromatographic methods in carbohydrate chemistry[3,4,5,6,7,8,9,10,136] provides an excellent illustration of their capabilities in the separation of stereoisomers. By means of adsorption[137,138] and partition[139] columns and paper strips[140,141] numerous separations of monosaccharides, polysaccharides and their derivatives have been effected. In the quantitative determination of sugars[142,143,144] and their derivatives[145] on paper, the mixture is developed with butanol on a broad strip and the positions of the zones are located by spraying a narrow strip cut from the side of the broad strip. By matching the sprayed strip with the remainder, the unsprayed zones can be marked and cut out; each sugar is then extracted with water and determined by an oxidation titration.

Glycosides. Alumina columns have recently been applied in a systematic study of a number of glycosides, such as those of *Strophanthus*,[16,146] *Adenium multiflorum*[147] and *Acokanthera venenata*[148]; two cardiac glycosides have similarly been obtained from *Adenium Hougel*.[149] A digitalis extract, mixed with methanol and chloroform, afforded on an alumina column three zones which by repetition of the process were eventually fractionated into gitoxigenin, digitoxigenin, gitoxin, digitoxin, purpurea glycosides A and B and four new glucosides.[150,151] Similar separations can be effected on paper strips.[152] Rutin can be separated quantitatively from other flavanol-3-glycosides in quantities of 10 to

40 μg. by paper chromatography, using *n*-butanol, acetic acid and water as developing solvent.[153]

Vitamins. Adsorption columns are applied in the estimation of fat-soluble vitamins for their purification prior to spectrophotometric or colorimetric determination.[154,155,156] Precautions must be taken against the decomposition of the vitamins, especially on alumina. Vitamin A in fish-liver oil is converted to the alcohol, fatty acids are removed and the vitamin is purified, with less than 2 per cent. loss, on a dicalcium phosphate column, using ether–light petroleum as developing solvent, and determined spectrophotometrically after elution.[157] Vitamin D is separated from other steroids, vitamin A and carotenoids on a column of magnesium oxide and kieselguhr.[158] A method for the determination of the vitamins B_{12} in fermentation liquors and liver extracts involves resolution of the vitamins on paper strips and a microbiological assay[73,159]; paper chromatography is being used extensively in structural studies of these vitamins.[160] Chromatographic methods continue to yield valuable results in the isolation, characterisation and determination of other vitamins and allied substances.[74,161,162,163,164,165,166]

Antibiotics. Chromatography has been exploited in the isolation of many antibiotics, both on the laboratory and industrial scales.[34,167,168,169] Paper methods have proved especially successful for their characterisation and assay[7,35,46] and in structural studies.[170,171] In one method for the assay of penicillins,[172] filter-paper is soaked in 30 per cent. potassium phosphate buffer of *p*H 6 to 7 and dried in air; a spot of solution of the mixed sodium salts is then developed on the strip with wet ether and the zones are located by placing the strip on agar inoculated with *Bacillus subtilis*; mixtures of pure penicillins are used as reference standards in the quantitative interpretation of the results. By suitable modification, this type of process is made applicable to the examination of penicillin culture filtrates,[173] streptomycin[73] and other antibiotics.[174,175] *Streptomyces* cultures have been investigated[176] using paper impregnated with toluene-*p*-sulphonic acid, followed by a photographic procedure[177] to obtain a permanent record of the results of a biological test. Paper chromatography has resulted in important advances in the elucidation of the structures of the bacterial antibiotics which are peptides.[178]

Alkaloids. As work on less readily accessible plant material extends, adsorption,[179,180,181,182,183,184,185,186] partition[41,187,188,189] and paper[190,191] chromatographic methods are being increasingly applied to the isolation, characterisation and estimation of alkaloids. A method which appears to be capable of wide application is illustrated by the fractionation of the Reineckates of alkaloids of calabash curare on an alumina column.[192] Several alkaloidal assay processes involving the use of adsorption columns have been described.[104,106,193,194] For the isolation of pomegranate alkaloids a partition column consisting of phosphate buffer distributed on kieselguhr is used.[41] Morphine is quantitatively separated from the ψ-morphine and coloured degradation products formed in injections of morphine by elution with ethanol from an alumina column.[188] The scope of these methods in alkaloidal chemistry is indicated by their use

in the separation of cinchona alkaloids,[195] in the identification of curare alkaloids,[196] in the degradation of germitrine on alumina,[197] in the quantitative isolation of morphine, codeine and diamorphine from viscera[198] and in the isolation of new bases from *Colchicum autumnale*.[199]

Anthraquinone derivatives. Cascara[200] and aloe[201] have been assayed by isolation of certain of their anthraquinone derivatives on adsorption columns; similar columns have been used in the examination of *Frangula* extract.[202] For paper chromatograms, an acetone solution of the anthraquinone derivatives is developed with light petroleum saturated with methanol and the spots are located by heating after spraying with methanolic magnesium acetate.[203] A comprehensive method for the determination of emodins in drugs is based on similar principles.[204]

COMMENT

Chromatography has further important applications in several large groups of substances, such as organic acids, lipoids and inorganic compounds, and in numerous separative problems which do not correspond to the simple classification adopted here. Mere consideration of the complexity of the mixtures and analytical problems to which it has been successfully applied does not adequately demonstrate its remarkable usefulness. Its true value becomes more evident from an appraisal of the deductions which have been made from the results of chromatographic experiments; recent developments in knowledge of the structures of polysaccharides, proteins and nucleic acids provide appropriate illustrations.

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