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SORPTION AND CHROMATOGRAPHY OF MACROMOLECULAR SUBSTANCES

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I. INTRODUCTION

The considerable successes recently achieved by sorption and chromatographic methods for the separation, isolation, and purification of substances of low molecular weight have given rise to a series of papers on the fractionation of macromolecular compounds using these methods, and also on the application of chromatographic methods to the analysis of polymer mixtures. Advances in sorption and chromatographic methods as applied to polymers have been due on the one hand to the development of a theory and a laboratory technique of chromatography whereby mixtures of very similar substances can be separated, and on the other to the synthesis of specific polymeric sorbents, mainly ion-exchange resins, the structure of which can be controlled during their preparation. The preparation of cross-linked polymeric sorbents, possessing great intramolecular porosity and capable of absorbing large quantities of macromolecular ions, has been especially important in this connection.

Nevertheless, the development of sorption and chromatographic methods for separating and analysing polymers has encountered a number of difficulties which were of no significance in work with substances of low molecular weight. The first important obstacle was the low rate at which sorption equilibrium is established. Although, in the absorption of substances of low molecular weight, decreasing the grain size of the sorbent and creating conditions under which the boundaries of the chromatographic zones were sharpened enabled the spreading of the bands in the sorption column to be successfully overcome, with macromolecular substances it proved considerably more difficult to sharpen the boundaries of the chromatographic bands. A second circumstance impeding the development of sorption methods in this field is the complicated dependence of the sorption capacity on the molecular weight of the absorbed substances. The capacity increases with increasing molecular weight in a series of homologous polymers of comparatively low molecular weight, and decreases with polymers of relatively high molecular weight[1-5]: on the one hand the usual increase in sorption capacity in conformity with Traube's rule, and on the other a decrease due to the fact that large molecules cannot penetrate the pores of the sorbent. This latter phenomenon, of course, can prove useful in a number of cases for the separation of polymers according to their molecular weights, on the molecular sieve principle.

The dependence of the sorption capacity of polymers on the composition of the solvent in contact with the sorbent has been studied with several materials[4,6,7]. Thus more polystyrene is sorbed by activated charcoal from the comparatively "poor" solvent ethyl methyl ketone than from the "better" toluene[7]. Addition of the very "poor" solvent methanol to ethyl methyl ketone improves the sorption of polystyrene[4]. This phenomenon can be compared with the analogous influence of solubility on the sorption capacity of a substance observed earlier by Tiselius[8].

The rate of sorption of polymers is greatly dependent on their molecular weight. A series of investigations by Yurzhenko *et al.*[9,10] has shown that polyisopropylene, polystyrene, and butadiene–styrene rubber of low molecular weight are sorbed on carbon black much more rapidly than are samples of the same polymers having high molecular weights. The sorption of an unfractionated polymer is accompanied by an increase in the intrinsic viscosity of the solution surrounding the sorbent, indicating selective absorption of the low-molecular-weight fraction[9,10]. The low rate of sorption on carbon black of the high-molecular-weight fraction of butadiene–styrene rubber produces the very interesting phenomenon that, after a certain time,

the total amount sorbed remains unchanged while the viscosity of the solution decreases [11]. This has been explained by the replacement on the sorbent of low-molecular-weight species by those of high molecular weight. The composition of the solvent affects not only the amount sorbed at equilibrium but also the kinetics of sorption. The time required for saturation of the sorbent with the polymer increases on passing from "poor" to "good" solvents [9].

II. ELUTION CHROMATOGRAPHY

The most widespread variant of the chromatographic method is elution chromatography, which consists in sorbing a mixture of substances in the upper part of a column and eluting them with a definite solvent or solution. In such a process each component moves along the column by being repeatedly sorbed and desorbed. The rate at which the substance moves depends on its adsorbability, and is inversely proportional to the adsorption coefficient [12]. As a result of this displacement, the components of the mixture are separated and leave the column in the form of pure fractions of the individual substances or mixed fractions, still incompletely resolved. If the process is carried out with dilute solutions, the concentration in each of the bands falls in direct proportion to the square root of the time, and the band width (the length of column occupied by each band) increases according to the same law. The degree of blurring of the bands depends on the rate of establishment of the sorption equilibrium. With the low rates of sorption observed for polymers a considerable blurring is to be expected.

Nevertheless, in a number of cases, beginning with the work of Mark and Saito [13], it has proved possible to effect the chromatographic separation of polymers according to their molecular weights. Polystyrene of low molecular weight ($M = 10^4$) could be eluted with toluene from a column of activated charcoal mixed with celite [7]. Tetralin, however, elutes both the polystyrene fraction of low molecular weight, which leaves the column at the start of the process, and that of high molecular weight ($M = 10^6$). Fractionation was most successfully accomplished by the successive application of a series of eluent mixtures of toluene and tetralin containing these solvents in the proportions 3 : 1, 1 : 1, and 1 : 3 (Fig. 1). The increase in molecular weight observed on passing up the column, both here and in other investigations [14-16], contradicts the fall in sorption capacity with increase in molecular weight [3,4] predicted by the theory of chromatography [12], according to which components which are sorbed to a smaller extent are the first to move along the column. These contradictory results can be ascribed to different laws for the sorption of polymers, to the fact that in different experiments sorption capacity is determined at different stages in the establishment of sorption equilibrium [16], or even, finally, to the effect of additional factors in the chromatographic process, such as precipitation of the polymer in the column [7].

Different types of polymer sorbed on a column may be completely separated by elution with a solvent. This has been achieved in the case of poly(vinyl chloride) and poly(vinyl acetate) on an activated charcoal column [17]. When isobutyl ketone is employed as eluent, poly(vinyl chloride) is the first to emerge from the column, and only after a considerable time does the acetate appear.

In order to separate rubbers by another variant of the chromatographic method, paper chromatography, the filter

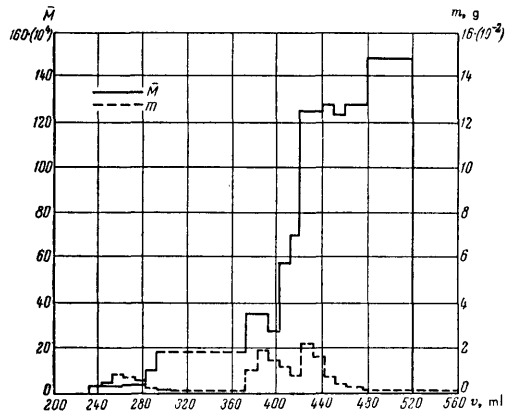


Fig. 1. Chromatographic fractionation of polystyrene on an activated charcoal column. Eluent solution consists of mixtures of toluene and tetralin (3 : 1, 1 : 1, and 1 : 3). $\bar{M}$ = mean molecular weight of polymer in the fraction; m = quantity (in grammes) of polymer in the fraction; v = volume (in ml) of effluent.

paper must be specially treated with a 5% solution of methyltrichlorosiloxane in benzene [18]. The hydrophobic paper obtained in this way retained benzene in its pores instead of the usual solvent – water – employed in paper chromatography. Cyclohexanone was used as the mobile solvent. Guayule and synthetic butadiene–styrene rubbers were separated by this means, the latter rubber being displaced at the greater rate. This method has been used also to investigate the changes in properties undergone by rubbers on milling. Paper-chromatographic fractionation of poly(vinyl chloride) and poly(vinyl acetate) has been accomplished in the usual way without hydrophobic treatment [17], using isobutyl ketone as the mobile phase. While the poly(vinyl chloride) moves with the solvent front in this system, the poly(vinyl acetate) spot remains stationary. The sorption of polymers on paper may be the controlling factor in this process.

The fractionation of stereoisomers of low molecular weight by chromatographic methods is based on the use of optically active sorbents. On an analogous principle, Natta *et al.* [19] used insoluble isotactic polypropylene sorbents of high molecular weight in order to separate soluble polypropylene into fractions of different degrees of stereoregularity. It was established in these experiments that molecules possessing great stereospecificity and forming highly crystalline polymers are linked firmly to such a sorbent. Components of a low degree of crystallinity are comparatively easily eluted with isopropyl ether. Samples of crystallinity varying from 2.5% to 30% were obtained in these experiments from a stereoblock polypropylene of 22% crystallinity. On such a sorbent, fractionation takes place predominantly according to the degree of stereoregularity, and to a lesser extent according to molecular weight. The chromatographic analysis is most conveniently carried out on a column containing silica gel on which the polypropylene of high molecular weight has been deposited.

III. FRONTAL CHROMATOGRAPHY

In view of the difficulties involved in the desorption of sorbed polymers, Claesson developed a frontal chromatographic method for determining the molecular-weight distribution of polymers[2]. The frontal method which he worked out together with Tiselius[20,21], is a process of continuous filtration of a multicomponent solution through a sorbent column. As a result of the different adsorbabilities of the components, each of them appears at the outlet of the column at a quite definite time, after a certain volume of solution has passed through the column. This latter quantity is an important characteristic defining the dynamic sorption properties of a particular component, and when it relates to a column containing 1 g of sorbent it is termed the retention volume. For dilute solutions this quantity is constant for a given component and a given sorbent.

The rate of movement of the chromatographic zone boundary for a substance in a column can be easily calculated from the mass continuity equation for the substance[12]

$$\frac{\partial c}{\partial x} + \alpha \frac{\partial c}{\partial v} + \frac{\partial m}{\partial v} = 0, \qquad (1)$$

where c is the concentration of the substance at a given cross-section of the column, x the distance from the top of the column, v the volume of solution which has run through the column, α the volume of the pores in a column of height 1 cm and cross-section 1 cm^2, and m the amount of substance absorbed by the sorbent at the given cross-section of the column calculated for the quantity of sorbent occupying unit height of the column.

From Eqn. (1) the rate of displacement along the column of a point corresponding to a constant concentration of the substance is defined by the equation

$$\left(\frac{\partial x}{\partial v}\right)_c = \frac{1}{\alpha + \frac{\partial m}{\partial c}}, \qquad (2)$$

provided that the substance has a definite sorption isotherm

$$m = f(c). \qquad (3)$$

The theory of chromatography shows that, in the case of a sorption isotherm concave to the concentration axis (Langmuir and Freundlich types), a sharp leading edge is formed when a solution of the substance is filtered through a column. In other words, the instant the substance appears at the outlet from the column, its concentration increases abruptly to that in the initial solution. It can be shown from Eqn. (1) that the rate of displacement of the sharpened leading edge is given by the equation

$$\left.\frac{\partial x}{\partial v}\right|_{fr} = \frac{1}{\alpha + m/c}. \qquad (4)$$

All these relationships, which hold for a one-component system, become more complicated with solutions which contain several substances. In the presence of polymer homologues in the solution, the laws of motion of the components along the column are defined by a set of equations of type (1)

$$\frac{\partial f_\omega}{\partial x} + \alpha \frac{\partial f_\omega}{\partial v} + \frac{\partial m_\omega}{\partial v} = 0, \qquad (5)$$

for all polymer homologues having molecular weights lying between M and $M \pm dM$, where $f_\omega(M)dM$ is the concentration of each polymer homologue. The Langmuir sorption isotherm equation for each such component is of the form

$$m_\omega(M) = \frac{k(M) f_\omega(M)}{1 + \int_0^\infty l(M)\, f_\omega(M)\, dM}, \qquad (6)$$

where k and l are constants depending on the properties of the polymer and sorbent molecules.

It is difficult to find a general solution for the movement of a system of polymer homologues along the column. With dilute solutions, however, the equation of the sorption isotherm is simplified and becomes linear

$$m_\omega(M) = k(M) f_\omega(M). \qquad (7)$$

In this case, since the amount of each polymer homologue sorbed depends only on its concentration, it is possible to solve each material balance Eqn. (5) separately, as has been demonstrated in a number of cases[12]. For linear adsorption isotherms the front edge and the point of constant concentration then move with the same velocity

$$\left.\frac{\partial x}{\partial v}\right|_{fr} = \left(\frac{\partial x}{\partial v}\right)_c = \frac{1}{\alpha + m_\omega(M)/f_\omega(M)}. \qquad (8)$$

Thus all polymer homologues move along the column at a rate depending only on the properties of the given polymer fraction and those of the sorbent, independently of their concentration and of the total concentration of polymer in the solution. Under these conditions the concentration of each component increases in the column from zero to a limiting value equal to the concentration of these substances in the initial solution.

Thus when a volume v of solution is run through the column, all components for which the retention volume v_0 is less than v appear in the filtrate; at any given instant the mixture arriving at the outlet from the column contains all the components eluted from the column at a concentration equal to their concentration in the original solution. This means that an extremely simple relation exists between the molecular-weight distribution of the polymer and the elution curve for frontal analysis.

With polymers containing no fractions of low molecular weight, the adsorbability should fall with increase in molecular weight[2,3]. The theory of fractionation can also be applied to substances showing an inverse relation between sorption capacity and molecular weight. In conformity with Eqn. (8) the velocity of the front should thus be greater the higher the molecular weight, and therefore an inverse relation should exist between the retention volume v_0 of the polymer homologue and its molecular weight

$$M = \gamma(v_0). \qquad (9)$$

The equation to the elution curve for the frontal process can be written

$$\frac{C}{C_0} = \psi(v_0), \qquad (10)$$

where C and C_0 are the concentrations of the polymer at the outlet from the column and in the initial solution respectively.

From the above considerations it follows that $\psi(v_0)$ is the weight fraction of substances of molecular weight exceeding

M (a single-valued function of v_0). Consequently there exists a function

$$F(M) = 1 - \psi(v_0) = 1 - \frac{C}{C_0}, \qquad (11)$$

which represents the weight fraction of all polymer homologues of molecular weight equal to or less than M.

In order to study the molecular-weight distribution, it is thus necessary to determine the form of the frontal elution curve $\psi(v_0)$ and to plot the graph of $f_\omega = \partial F(M)/\partial v$ against v_0. Since the above arguments hold only for dilute solutions, the elution curves obtained experimentally must be extrapolated to zero concentration. On Claesson's suggestion this is effected by extrapolating along straight lines intersecting the elution curves in characteristic points. The intercepts on each such straight line are then extrapolated to zero concentration. On this basis Claesson obtained a series of curves representing the molecular-weight distributions in polymers. Three

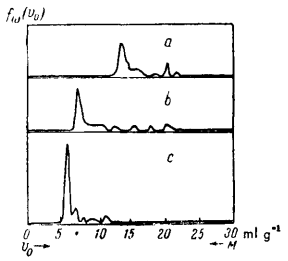


Fig. 2. Determination of molecular weight distribution in poly(methyl methacrylate) by frontal sorption analysis:
a) polymer prepared with benzoyl peroxide; *b*) and *c*) commercial specimens; v_0 = volume of effluent (retention volume); M = molecular weight; $f_\omega(v_0)$ = molecular weight distribution function.

curves of this type for different samples of poly(methyl methacrylate) are shown in Fig. 2. The dependence of f_ω on v_0 along these curves is quite definite, whereas the relation between v_0 and M is extremely rough, since the scale factor of the horizontal axis in terms of molecular weight must be determined in independent experiments on fractionated samples (dependence of v_0 on M). Nevertheless, the curves obtained, even without a knowledge of the v_0–M relation, give valuable information of the presence of maxima on the molecular-weight distribution curve (owing to the inverse character of the above relation), and are also useful for the comparison of polymers of the same type. A more important defect of the proposed method is evidently the requirement of equilibrium, common to all relations deriving from the theory of chromatography. The low rate of sorption of substances of high molecular weight can greatly distort the pattern of sorption and lead to an erroneous analysis of the data. A low rate of filtration of the solution through the column is absolutely essential in such experiments.

IV. NEW CHROMATOGRAPHIC METHODS

New procedures have recently been worked out to improve the power of chromatographic methods to separate polymer mixtures. One of them, the moving capacity gradient or gradient elution method, was suggested by Harteck and others [22-24] and the theory was worked out by Zhukhovitskii *et al.* [25]. This process consists in eluting the substances from the sorption column with a solution of continuously changing composition. The gradual replacement of the solvent or solution is accomplished in such a way that the sorption capacity for each substance continuously falls with time at each cross-section of the column. It is no longer sufficient to use only one material balance Eqn. (1) to represent this process, but a set of equations must be considered

$$-\frac{\partial c}{\partial x} = \alpha \frac{\partial c}{\partial v} + \frac{\partial m}{\partial v}, \qquad m = \varphi(c, \rho), \qquad (12)$$

where ρ is a parameter defining the sorption capacity for a given concentration of the substances but with a variable solution composition.

The solution to this set of equations indicates that the zone for each component should contract to a very narrow band. Thus, the gradient elution method can lead to greatly improved chromatographic separations.

Using this principle, Williams was able to separate two fractions of nylon of molecular weight 150 000 and 350 000 on a celite column [26]. Gradient elution was carried out with a solution of formic acid of continuously increasing concentration. The fraction of low molecular weight was eluted first (hydrolysis of the polymer took place during chromatography). Williams' work on the fractionation of polysiloxanes on activated charcoal, based on the same principle, was even more successful [27]. In these experiments the polymethylsiloxane was separated into a series of fractions, the first of which to leave the column contained components of low molecular weight. A continuous increase in molecular weight of the siloxane during chromatographic separation occurred when a set of solvents of increasing solvent power was used (methyl alcohol–ethyl alcohol–ethyl ether).

In order that equilibrium and kinetic factors in the sorption of polymers should act in the same direction, Mould and Synge [28] proposed the use of an electrokinetic ultrafiltration method, the idea of which was to move the polymer mixture through a porous block, under the action of electro-osmosis for example. Under these conditions both equilibrium and kinetic factors favour the displacement of the low-molecular-weight fractions. This method has been used to effect a molecular-weight separation of polysaccharides at a collodion membrane.

Considerable progress in the fractionation of polymers has been achieved by the use of a new chromatographic method – precipitation chromatography – previously developed for substances of low molecular weight [29]. Application of this method to the separation of polymer homologues is based on the well-known fractional precipitation of polymers by the addition of "poor" and "good" solvents to the solution. We owe to Williams and several other investigators [26,30-33] the successful realisation of the fractionation of polymers by precipitation and dissolution as a chromatographic column process. The process involves allowing the polymer solution to flow from the upper, hotter layer of a column of the finely ground material to the lower,

cooler layer. If the solution is saturated, in equilibrium with the solid at the top of the column, and the temperature coefficient of solubility is positive, a precipitate is formed at the bottom. Such a displacement of substance along the column is analogous to the ordinary sorption chromatographic process, but with the only difference that repeated sorption and desorption are replaced by precipitation and dissolution. If there is no change in composition of the solvent during the process, and the temperature falls linearly along the column, the material balance equation for each cross-section of the column can be written

$$-\frac{dc}{dx}=\left(\frac{\partial m}{\partial v}\right)_x \qquad K\frac{dt}{dt}=\left(\frac{\partial m}{\partial v}\right)_x, \tag{13}$$

where t is the temperature, K is a constant defining the temperature gradient along the column, m is the amount of the substance in the precipitate, and the remaining symbols have the same significance as in Eqn. (1). From Eqn. (13) we obtain

$$\left(\frac{\partial x}{\partial v}\right)_m=-K\frac{dc/dt}{(\partial m/\partial t)_v}. \tag{14}$$

Thus the rate of movement of a zone is determined by the temperature coefficient of the solubility and the distribution of the substance along the column.

In actual fractionations which have already been carried out by precipitation chromatography, the effect of the temperature gradient is supplemented by gradient elution based on the introduction of a solution of variable composition into the column, beginning with a "poor" solvent and gradually adding a "good" solvent. A chromatographic apparatus operating on such a principle is shown in Fig. 3. The column is filled with a suspension of glass powder in the "poor" solvent. The main part of the column A is placed in an aluminium jacket B, the upper part of which is heated by means of the heater C, while the lower part is cooled with water (cooling coil D). Thus the process takes place with a constant temperature gradient in the column. The mixed eluent of variable composition enters the upper part of the column from the mixer E, initially filled with the "poor" solvent. The "good" solvent, added to the mixer, continuously changes the composition of the solvent entering the column.

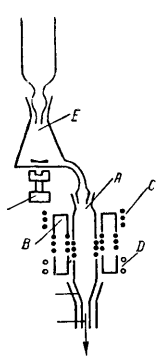


Fig. 3. Apparatus for fractionation of polymers by precipitation chromatography.

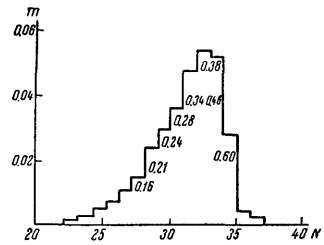


Fig. 4. Fractionation of polystyrene by precipitation chromatography. N = No. of fraction; m = amount of substance in fraction. The numerals on the graph indicate the specific viscosity of the fractions (in 0.5% solution in toluene).

The result of the fractionation of a sample of polystyrene of molecular weight 108 000 by the method described here is shown in Fig. 4. The solvents used were ethyl alcohol and ethyl methyl ketone. The values shown along the curve for the viscosities of the polymer fractions indicate that the molecular weight of the polystyrene continuously increases as the solution leaves the column.

Precipitation chromatography enables very narrow fractions of polymer homologues to be further resolved[33]. Clearly the method has great possibilities.

V. POLYELECTROLYTES

Ion-exchange methods for the sorption of polymers have two main advantages over sorption methods based on van der Waals interaction between sorbent and sorbate: the existence of a large number of synthetic ion-exchange resins, possessing a great variety of properties, and also the possibility of specifying beforehand the sorption capacity of polyelectrolytes. The latter parameter depends on the formation of an electrovalent bond between specific groups in the polymers and in the sorbents. The synthesis of ion-exchange resins possessing considerable intramolecular porosity has enabled sorbents to be produced which absorb large quantities of polyelectrolytes. Such materials have been synthesised using very small quantities of cross-linking agent, for example divinylbenzene, the substance most widely used for combining linear polymeric molecules into three-dimensional structures. By varying the degree of cross-linking of the ion-exchanger, sorbents can be produced which are able to absorb small ions but not large ions. On this principle – the "molecular" or "ion-exchanger" sieve principle – various ionised substances can be separated according to their molecular weights. The simplest task here is to isolate polyelectrolytes from simple ions. For example, Amberlite IR-4B is an excellent sorbent for galacturonic acid, but it does not sorb polygalacturonic acid[34]. Polymannuronic (alginic) acid and its monomer behave similarly. The products of the enzymatic hydrolysis of polygalacturonic acid are absorbed by Amberlite IR-4B to an increasing extent as hydrolysis proceeds. The behaviour of phosphoric and polymetaphosphoric acids on this sorbent follows the same pattern. The definite effects observed

with monomer–polymer systems become much less clearly defined with polymer homologues which are linear polyelectrolytes (polysulphostyrene[35]), although these too have been fractionated on a set of columns.

A new principle for the separation of ionised from unionised substances, that of ionic exclusion[36], based on the greater ability of the latter to penetrate the grains of a swollen ion exchanger, has been employed to separate weak and strong polyelectrolytes[37]. Lignosulphonic and weak polyacids in sulphite liquors have been separated by this method. Application of the molecular sieve principle as well has enabled this system to be fractionated also into polyelectrolytes of low and of high molecular weight.

VI. SORPTION OF PROTEINS BY ION-EXCHANGE RESINS

Biologically active polymers – proteins and nucleic acids – occupy a special place among soluble polyelectrolytes. Sorption methods have long been successfully applied to the purification and separation of these classes of compounds, especially proteins and enzymes. The numerous investigations in this field have been reported in great detail in a number of papers and books, especially in the reviews by Zittle[38] and by Moore and Stein[39]. The first of these papers discusses the equilibrium and the kinetic aspects of the sorption of proteins on both mineral and organic sorbents. The second gives a detailed account of work in the field of column chromatography. Finally, the application of chromatographic methods to the separation and analysis of proteins and nucleic acids has been reviewed by Sober and Peterson[40]. To avoid duplication, and since the results obtained during the past two years do not involve new principles, readers are referred to the above reviews. However, no mention is made in these papers of recent work on the mechanism and the laws governing the sorption of proteins by ion-exchange resins. An account of this work is given below.

The sorption of proteins by ion-exchange resins is governed by two main factors: the size of the absorbed ions and the occurrence of intermolecular interactions of opposite sign (*e.g.* Coulombic attraction and repulsion, resulting from the simultaneous presence of both positive and negative charges in proteins).

Application of the molecular-sieve principle enables proteins of high molecular weight to be separated from those of low molecular weight. Insulin, a protein of molecular weight 12 000, is sorbed in large amounts on sulphonated ion-exchange resins having a swelling factor of about 4.[41] Under the same conditions blood-serum proteins are absorbed in very small amounts. The synthesis of more cross-linked (less swollen) sulphonated resins provides an opportunity for the separation of polypeptides and proteins by this method, as well as of oligopeptides of different molecular weights[42].

Fractionation of proteins on the molecular-sieve principle using the hydrogen form of sulphonated resins should be carried out at 2°–5° to avoid catalytic hydrolysis of the peptide link. Denaturation phenomena are usually absent: desorbed insulin, for example, retains its biological activity. The preparation of sulphonated resins having swelling factors of 6–12 has led to the production of sorbents which now absorb blood-serum albumin and globulin in appreciable quantities (up to 10–20 mg g^{-1}).

However, the establishment of sorption equilibrium on resins of normal grain size is very slow, in this case taking several days or even weeks. A substantial increase in the capacity of sulphonated cation-exchange resins for sorbing proteins, and a marked reduction in the time required for the establishment of equilibrium, are achieved by using sorbents of very small particle size[43] (less than 0.05 mm). Under these conditions a considerable number of proteins are absorbed in amounts exceeding 100 mg g^{-1}. The adsorbabilities of different proteins and also the effect of the medium on sorption characteristics cannot be described in terms of the usual ion-exchange mechanism. However, a recently developed theory of the sorption of dipolar ions (amino-acids[44]) has provided a unified explanation of many unexpected experimental facts observed in the sorption of proteins.

Amino-acids and peptides of low molecular weight display a number of sorption properties denied to ions bearing one or more charges of the same sign. Thus, the amounts of dipolar ions (amino-acids) absorbed by the hydrogen form of sulphonated resins are approximately hundredfold greater than the amounts absorbed by the same resins in its salt form. Since the constant for the exchange of hydrogen ions with sodium ions on sulphonated resins is close to unity, this sorption phenomenon observed with amino-acids cannot be regarded as due to ion exchange. This, together with many other observations, has led to the following mechanism for the sorption of dipolar ions (amino--acids

$$R_1SO_3^- H^+ + H_3N^+ R_2COO^- = R_1 SO_3^- H_3N^+R_2 COOH. \qquad (15)$$

It follows that dipolar ions absorbed on the hydrogen form of sulphonated cation exchangers are converted into cations. When a neutral solution surrounds the sorbent, this can only be explained as a large sorption effect causing a marked change in properties of the sorbed ion. The desorption of amino-acids by solutions of mineral acids is an ordinary ion-exchange process, since in such an experiment the amino-acid occurs as the cation both in the sorbed state and in solution. The sorption of an amino--acid on a sulphonated cation exchanger in the salt (*e.g.* sodium) form cannot take place in accordance with Eqn.(15). Consequently it is not the cation of the amino-acid but the zwitterion (dipolar ion) which should be present in the sorbed state on the salt form of resins. In conformity with this, the sorption capacity of sulphonated resins for zwitterions of low molecular weight is negligible: electrostatic attraction is accompanied by repulsion due to the presence of charges of like sign on sorbent and sorbate.

The quantity of zwitterions sorbed on sulphonated cation-exchange resins in the salt form can be substantially increased by diminishing in some way the effective repulsion caused by the negative charge on the carboxyl group. This can be achieved by decreasing the degree of ionisation of the carboxyl groups, changing from aqueous to aqueous–acetone solutions, by increasing the ionic strength of the solution and creating a screening shell around the ionised group, or finally by increasing the distance between the amino and carboxyl groups, using polypeptides instead of amino-acids[44]. In the last case the sorbed ion can without difficulty orient on the sorbent in such a way that the repulsion effect is diminished. The above considerations enable us to assess the sorption capacities of proteins on the salt and the hydrogen forms of sulphonated resins in terms of the distance between the amino and the carboxyl groups in the sorbed proteins. Zwitterions in which groups

TABLE. Sorption of proteins by the sulphonated cation-exchange resin SBS.

Protein	Sorption of protein, mg g^{-1}; resin in		Protein	Sorption of protein, mg g^{-1}; resin in	
	H form	Na form		H form	Na form
γ-Globulin	171	0	Adrenocorticotropic hormone	117	63
Serum albumin	145	0	Myogen	47	26
Ovalbumin	96	0	Chymotrypsinogen	90	105
Insulin	125	30			

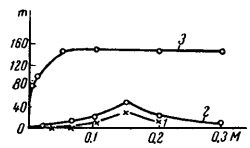


Fig. 5. Sorption of proteins from solutions containing NaCl on SBS sulphonated resins in the sodium form (m = quantity of protein sorbed, mg g^{-1} resin; M = molarity of NaCl):
1) serum albumin; 2) γ globulin; 3) insulin.

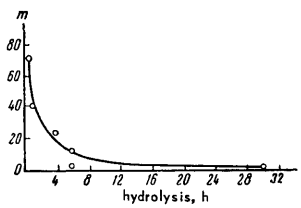


Fig. 6. Dependence of sorption of hydrolysis products of serum globulin on extent of hydrolysis; m = sorption expressed in mg-equiv. of nitrogen in the absorbed hydrolysis products (carboxylic cation-exchanger KMT in hydrogen form).

having unlike charges (NH_3^+, COO^-) are close together should be sorbed mainly on the hydrogen form of resins, and should not be sorbed, or be sorbed to a small extent, on resins in the salt (sodium) form. When the distance between the groups is relatively great (or in the presence of a great excess of positive charges), sorption of the proteins should occur on both salt and hydrogen forms of the resins. The data in the Table show that proteins can belong to either class of substances[43].

Biologically active proteins should belong predominantly to the second class of substances, which are sorbed on the salt forms of resins. Indeed, Coulombic attraction to other protein molecules in a solution of low ionic strength can occur only with proteins of this type. If, however, one of the interacting protein molecules contains oppositely charged groups situated close together, mutual attraction is possible only if the functional groups in the second macromolecule have the correct topography.

The sorption of proteins by sulphonated ion-exchange resins in salt form can be substantially intensified by increasing the ionic strength of the solution (Fig. 5). Nevertheless, the amounts of proteins sorbed under these conditions are small. Another way of increasing the sorption of proteins on the sodium sulphonate form of a resin is to use aqueous–acetone solutions[43].

Carboxylic cation exchangers sorb proteins in accordance with somewhat different laws. As zwitterions, many proteins are not sorbed on the salt forms of these exchangers[45], and the majority are sorbed in only small amounts. Nevertheless, proteins can be sorbed in large amounts on special types of carboxylic resins in the hydrogen form, which can swell greatly. The resin KMT, which incorporates a small quantity of cross-linking agent, proved to be such a cation exchanger. However, additional complications arise which are not observed in the sorption of proteins by sulphonated resins. Thus it has been shown that amino-acids are not sorbed by the same KMT resin in the hydrogen form, although it sorbs proteins. As the proteins are hydrolysed (Fig. 6), the sorption of the resulting polypeptides diminishes. This is the opposite rule to that observed in the molecular-sieve phenomenon, for the sorption capacity increases instead of decreasing with growth in the molecular weight of the polypeptides. There are grounds for assuming that this is connected with the formation of hydrogen bonds between the peptide groups of the polypeptides and the functional groups in the sorbent[46].

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THE DISCOVERY OF ELEMENT 103

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In April 1961, as the result of numerous experiments, a team from California University Radiation Laboratory consisting of A. Ghiorso, T. Sikkeland, A. Larsh, and R. Latimer obtained evidence of a new artificial synthetic element of atomic number 103.[1,2] In honour of the founder of their laboratory, Nobel prizewinner E. O. Lawrence, they proposed to name this new element lawrencium (symbol Lw).

Element 103 was obtained by the bombardment of californium with boron ions accelerated to 70 MeV energy by the linear accelerator HILAC. An alpha-activity was observed to result from this irradiation which could only be explained by the disintegration of a new element with atomic number 103. However, this activity is so small that it amounts to no more than several alpha-particles an hour. For this reason it is not yet possible to observe the mendelium disintegration products, which would confirm the atomic number of the element responsible for the new activity. Nonetheless, on the basis of nuclear data this activity can be positively ascribed to the element with atomic number 103. The method used by the team to obtain and identify the radiations is shown schematically in Fig. 1. In principle, the method is based on the approach used in the preparation of element 102. The californium target weighed 3 μg and had the following isotopic composition:

^{249}Cf — 3.3%, ^{250}Cf — 32.8%, ^{251}Cf — 12.3% and ^{252}Cf — 50.8%.

The californium was deposited on a nickel foil 0.0015 mm thick in the form of a circle 2.5 mm in diameter. The success of the experiment was strongly dependent on the purity of the target; the purification consisted of meticulous use of ion-exchange columns and specially pure reagents. The last stage in the purification of the target from undesirable traces of lead and bismuth was accomplished by heating the target *in vacuo* by electron bombardment. Traces of lead and bismuth must be reduced to the minimum, since when these elements are irradiated by heavy ions they give a large yield of elements disintegrating with a half-life of 25 sec and emitting alpha-particles of energy 8.8 MeV. This activity can completely obscure the alpha-activity of element 103.

The current of ^{10}B or ^{11}B ions passing through the very thin target was not allowed to exceed 0.5 μA, to avoid melting the target. The atoms formed in the bombardment were ejected from the target into the surrounding

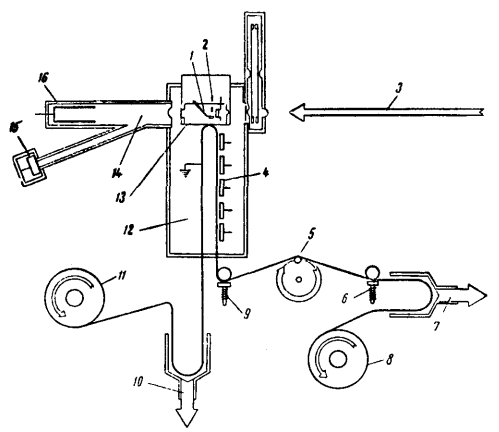


Fig. 1. Diagram of the apparatus for obtaining element 103: 1) target (-400 V); 2) collimator; 3) beam of heavy ions; 4) silicon alpha-particle counters; 5) motor; 6) brake; 7) suction; 8) take-up spool; 9) brake; 10) suction; 11) feed spool; 12) helium; 13) casing (-150 V); 14) vacuum; 15) silicon counter for measuring ion beam energy; 16) Faraday cylinder.