STUDIES ON GEL FILTRATION

SORPTION PROPERTIES OF THE BED MATERIAL SEPHADEX

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Gel filtration, as described recently[1], is a method that makes possible the separation of substances with different molecular dimensions, and it has been applied with success in our biochemical laboratory. We have used the column technique for desalting protein solutions and for the separation of proteins and other colloids from low-molecular-weight substances[6]. The method has also been used for group separation of protein hydrolysates and biological extracts.

We have observed that, under certain conditions, some substances are adsorbed to the bed material, whereas others may exhibit a negative sorption, at least for some part of the applied quantity. We thought it would be of interest to make a closer investigation of the sorption properties of the bed material, especially the sorption of low-molecular-weight substances.

This paper describes gel filtration experiments with buffering substances, amino acids, purine and pyrimidine derivatives, vitamins, alkaloids and some simple aromatic substances.

PRINCIPLES OF GEL FILTRATION

A theory for gel filtration and a more detailed description of the method and the bed material will be presented by FLODIN[2].

In a packed column one can distinguish two kinds of aqueous phases, one within the gel grains and one surrounding the grains. Let us denote the sum of the internal aqueous volumes of the grains as the inner volume of the column, V_i, the volume of the surrounding aqueous phase as the outer or void volume, V_o, and the partition coefficient for a substance between these two phases as K_D. When a substance is filtered through the column, the elution volume, V_e, is

$$V_e = V_o + K_D V_i$$

A substance submitted to gel filtration is preferentially characterized by its K_D value, which is calculated from the expression above as

$$K_D = \frac{V_e - V_o}{V_i}$$

A complete exclusion from the inner phase is generally obtained for large molecules such as proteins, which are hindered from entering the interior of the grains by

the polysaccharide network. A low-molecular-weight solute such as glycine can diffuse freely into and through the grains and has a K_D value of about 1.

In reality, there are some exceptions to these rules. The calculation of K_D by the above equation is only approximately true. In fact, part of the inner volume, V_i, is water of hydration which is firmly bound to the polysaccharide framework in the gel grains and is inaccessible to the solute molecules. For an accurate determination of K_D, the inner volume should be corrected for the water of hydration.

A large number of substances, inlcuding many of those discussed in this paper, interact with the bed material. Thus, some of them are slightly adsorbed and their elution is delayed, which results in an increased K_D value; others show a negative sorption, and have a lower K_D than expected.

MATERIALS

The filtration experiments were made with columns packed with the cross-linked polysaccharide Sephadex G-25 (manufactured by Pharmacia, Uppsala, Sweden). The characteristics of the bed material were: water regain = 2.9 g water/g dry substance; wet density = 1.099. In the majority of experiments, the grain size in the dry state was 50–100 mesh, and in some experiments it was 200–400 mesh.

Though the bed material should be completely non-ionized, it was found to contain a small amount of ionized groups. These are probably carboxyl groups, and they amount to about 10 μequiv./g dry Sephadex.

The test substances used in the filtration experiments were of analytical grade or a comparable purity.

METHODS

The dry Sephadex was allowed to swell in 0.05 M sodium chloride for half an hour and was then freed from fine-grained material by repeated sedimentations and decantations. The gel grains were poured into a glass tube and packed in the same manner as is described for cellulose columns in zone electrophoresis[3]. A circular filter paper was put on the top of the packed column to protect the surface. Most of the experiments were made on the same column with the dimensions of 3.5 × 35 cm. After equilibration of the column with distilled water or with an electrolyte solution, the substances to be tested were put on the column in a volume of 5.0 ml. Elution was then started with the same aqueous solution as was used for equilibration with a hydrostatic pressure of 60 cm. This gave a flow rate of 2 ml per minute. When an effluent volume somewhat smaller than the void volume of the column had been taken off, the rest of the effluent was collected in fractions of 4.8 ml in an automatic fraction collector. All experiments were carried out at room temperature.

The fractions were analysed by a suitable method: acids and bases by titration with dilute sodium hydroxide and hydrochloric acid, respectively; salts by titration after passage of an anion or cation exchanger; amino acids, except tryptophan and tyrosine, with ninhydrin reagent according to MOORE AND STEIN[4]; tryptophan, tyrosine

and other aromatic substances by measurement of their ultraviolet absorption in a Unicam SP 500 spectrophotometer.

The substances to be tested were separately filtered through the column and the yield and the K_D value determined.

The data necessary for the calculation of K_D are determined in the following way. The elution volume is determined by measuring the effluent volume from the addition of the test solution to the point where the concentration gradient of the eluted substance is maximum. V_o is experimentally determined as the elution volume for haemoglobin, with phosphate buffer (ionic strength $\mu = 0.05$) pH 7.0 as eluant. V_i is calculated from the water regain (W_R) and the dry weight of bed material (a).

$$V_i = a \cdot W_R$$

No correction for the water of hydration is made. A K_D value of 0.8 therefore indicates a non-restricted diffusion in the gel column.

RESULTS

The applicability of the gel filtration method has been shown by FLODIN[2], and experiments with proteins, peptides, and amino acids are described by PORATH[5,7]. The aim of this investigation was to study the behaviour of low-molecular-weight substances, giving special attention to those cases where interaction with the bed material occurs.

To make the following description more clear, the tested substances are divided into five groups. All experiments, however, are made on the same column, and the experimental results are quite comparable.

1. *Buffering substances*

The gel filtration method is a rapid and effective way of desalting solutions containing high-molecular-weight solutes. It can also be used for changing buffering ions in a protein solution[5].

The first experiments in this series were, therefore, made with those substances which can be used as buffers. With the intention of investigating the possibilities of using the method for desalting, most of the experiments were made with distilled water as an eluant (Table I).

All the tested substances except γ-collidine were eluted quantitatively with water. In some cases, a slight adsorption to the bed material occurs. This is most pronounced for sodium and potassium hydroxide and for sodium tetraborate, which are known to form complexes with carbohydrates. The strong adsorption of collidine cannot yet be explained, but collidine can, however, be quantitatively eluted from the column by an electrolyte solution.

2. *Amino acids*

Besides glycine, which is included in Table I, fourteen additional amino acids have been tested (Table II).

TABLE I

BUFFERING SUBSTANCES FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH

Substance	*Quantity (mg)*	*Eluant*	K_D	*Yield (%)*
Sodium hydroxide	40	Distilled water	2.2	97
Potassium hydroxide	56	Distilled water	2.3	92
Ammonium hydroxide	34	Distilled water	0.8	93
Sodium bicarbonate	42	Distilled water	0.8	99
Sodium carbonate	29	Distilled water	0.8	103
Sodium phosphate	142	Distilled water	0.7	95
Sodium tetraborate	101	Distilled water	1.7	101
Sodium acetate	41	Distilled water	0.7	
Sodium citrate	129	Distilled water	0.5	99
Hydrochloric acid	18	Distilled water	0.8	94
Formic acid	23	Distilled water	0.9	88
Acetic acid	30	Distilled water	0.9	98
Citric acid	118	Distilled water	0.9	96
Diethylbarbituric acid	1.3	Distilled water	1.2	98
Glycine	0.8	Distilled water	0.9	91
Triethylamine	100	Distilled water	1.0	
Pyridine	1.5	Distilled water	1.2	97
γ-Collidine	1.5	Distilled water	> 5	
γ-Collidine	1.5	0.05 *M* sodium chloride	0.9	100

TABLE II

AMINO ACIDS FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH

Substance	*Quantity (mg)*	*Eluant*	K_D	*Yield (%)*
Alanine	1.0	Distilled water	0.8	80
Serine	1.4	Distilled water	0.8	92
Leucine	2.4	Distilled water	0.8	94
Methionine	2.2	Distilled water	0.8	90
Proline	3.0	Distilled water	0.8	86
Hydroxyproline	4.8	Distilled water	0.8	97
Phenylalanine	2.5	Distilled water	1.0	91
Tyrosine	7.5	Distilled water	1.1	98
Tryptophan	2.1	Distilled water	1.9	91
Aspartic acid	2.4	Distilled water	0.2	83
Aspartic acid	1.6	0.05 *M* sodium chloride	0.8	95
Glutamic acid	2.3	Distilled water	0.2	91
Glutamic acid	2.3	0.05 *M* sodium chloride	0.8	98
Lysine hydrochloride	2.5	Distilled water	> 3.3	
Lysine hydrochloride	2.5	0.05 *M* sodium chloride	1.0	100
Arginine hydrochloride	3.0	Distilled water	> 13	
Arginine hydrochloride	3.0	0.05 *M* sodium chloride	1.0	97
Histidine hydrochloride	3.0	Distilled water	> 3.6	
Histidine hydrochloride	3.0	0.05 *M* sodium chloride	0.9	107

With distilled water as eluant, most of the amino acids are eluted with a K_D about 0.8, which indicates a free diffusion in the column. K_D does not reach the value of 1.0, because of the water of hydration. The aromatic amino acids show some adsorption to the bed material, and the basic amino acids are strongly adsorbed.

The acid amino acids, on the contrary, are partially excluded from the gel grains. Just as in the case of collidine, these anomalies disappear when the eluant contains an electrolyte.

3. *Nucleotides, nucleosides, purines, and pyridines*

Because of their great biological significance, some purine- and pyrimidine derivatives were tested on the gel column (Table III).

Heterocyclic substances, except for some of the nucleotides, are also adsorbed to the bed material. In water, the nucleotides are almost completely excluded from the gel grains, but this behaviour is normalized when the elution is done with an electrolyte solution.

TABLE III

PURINE AND PYRIMIDINE DERIVATIVES FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH

Substance	*Quantity (mg)*	*Eluant*					
		Distilled water		*0.05 M sodium chloride*		*Phosphate, $\mu = 0.05$, pH 7*	
		K_D	*Yield (%)*	K_D	*Yield (%)*	K_D	*Yield (%)*
Ribonucleic acid	3.1	0.0	90				
Diphosphopyridine nucleotide	2.8	0.0	100	0.8	81		
Adenosine triphosphate	2.0	0.0	74	0.6	94		
Adenylic acid	1.4	0.1	85	1.2	99		
Guanylic acid	1.7	0.4	82	1.3	99	0.9	82
Cytidylic acid	1.9	0.1	82	0.8	96	0.7	
Uridylic acid	1.3	0.1	98	0.8	100	0.7	93
Adenosine	1.1	1.7	100	1.8			
Guanosine	1.3	1.6	93			1.8	82
Cytidine	1.3	1.2	93			1.2	
Uridine	1.2	1.0	94			1.0	
Inosine	1.2	1.2	96	1.3	101		
Adenine	0.6	2.2	89	2.4	99		
Cytosine	0.8	1.6	93			1.4	
Uracil	0.8	1.1	84			1.2	
Hypoxanthine	1.0	1.6	103	1.6	101		
Xanthine	1.3	1.8	88				
Dimethylxanthine	1.2	1.3	100				
Trimethylxanthine	1.2	1.1	96				

4. *Vitamins and alkaloids*

Table IV shows the results of experiments made with some vitamins and alkaloids.

As was the case for collidine and for the basic amino acids, the basic substances in this series of experiments are strongly adsorbed to the bed material in distilled water. When the elution is carried out with a solution containing an electrolyte, however, even those substances that are most strongly adsorbed in distilled water are recovered quantitatively, showing only a faint reversible adsorption.

TABLE IV

VITAMINS AND ALKALOIDS FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH

Substance	*Quantity (mg)*	*Eluant*	K_D	*Yield (%)*
Ascorbic acid	1.4	Distilled water	0.9	103
Riboflavin	0.6	Distilled water	1.6	93
Nicotinamide	2.0	Distilled water	1.4	95
Thiamine hydrochloride	2.3	Distilled water	> 2.9	
Thiamine hydrochloride	2.3	0.05 *M* sodium chloride	1.0	91
Pyridoxine hydrochloride	1.6	Distilled water	> 3.3	
Pyridoxine hydrochloride	1.6	0.05 *M* sodium chloride	1.0	104
Codeine hydrochloride	10.1	Distilled water	> 3.5	
Codeine hydrochloride	12.0	0.05 *M* sodium chloride	1.2	99
Morphine hydrochloride	14.0	Distilled water	> 3.3	
Morphine hydrochloride	12.1	0.05 *M* sodium chloride	1.3	101
Quinine sulphate	11.9	Distilled water	> 2.9	
Quinine sulphate	12.0	0.05 *M* sodium chloride	1.6	102

5. *Some simple aromatic compounds*

From the preceding experiments, one can conclude that substances having an aromatic or heterocyclic structure as well as those of basic nature will cause some interaction with the bed material, resulting in delayed elution. The experiments with aromatic substances were extended with some additional examples, which are summarized in Table V.

TABLE V

AROMATIC SUBSTANCES FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH

Substance	*Quantity (mg)*	*Eluant*	K_D	*Yield (%)*
Benzoic acid	5.0	Distilled water	0.5	
Salicylic acid	1.3	Distilled water	0.3	103
Anthranilic acid	2.6	Distilled water	0.6	
Sulphanilic acid	0.6	Distilled water	0.3	103
Picric acid	0.8	Distilled water	0.4	100
Cinnamic acid	0.3	Distilled water	0.3	109
Phthalic acid	6.0	Distilled water	1.1	98
Salicylic acid	2.0	0.05 *M* sodium chloride	1.6	95
Sulphanilic acid	0.7	0.05 *M* sodium chloride	1.1	103
Picric acid	0.6	0.05 *M* sodium chloride	2.5	94
Picric acid	0.6	Phosphate pH 7	2.7	97
Phenol	3.2	Distilled water	0.7	101
Phenol	4.0	0.05 *M* sodium chloride	1.7	97
Aniline	4.0	Distilled water	1.5	98
Benzyl alcohol	52.5	Distilled water	1.3	95
Salicyl alcohol	3.9	Distilled water	1.4	103

These experiments indicate that all aromatic compounds are adsorbed to some extent to the bed material. The early elution of acids with water is once more established. This negative sorption is avoided when the elution is made with a salt solution.

Elution at various pH values

In order to investigate the possibility of using acid and alkaline solutions as eluants, some experiments were made with elution at extreme pH values. It would also be of interest to see how a shift in pH might influence the sorption effects. Collidine, sulphanilic acid and tryptophan were chosen as representative substances.

TABLE VI

COLLIDINE, SULPHANILIC ACID, AND TRYPTOPHAN FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH AT DIFFERENT pH VALUES

Eluant	*pH*	*Collidine 2.0 mg*		*Sulphanilic acid 0.5 mg*		*Tryptophan 2.0 mg*	
		K_D	*Yield (%)*	K_D	*Yield (%)*	K_D	*Yield (%)*
0.01 *M* hydrochloric acid	2.0	0.8	101	0.9	93	2.2	100
1.0 *M* acetic acid	2.4	0.8	99	0.9	100	2.0	91
0.02 *M* phosphate	7.0	1.0	101	1.1	100	2.1	100
0.05 *M* triethyl-ammonium carbonate	9.0	1.5	98	0.9	100	1.7	96
0.01 *M* ammonium hydroxide	10.6	1.5	86	0.4	99	0.5	96
0.01 *M* sodium hydroxide	12.0	1.5	93	0.5	99	0.7	99

TABLE VII

SOME AROMATIC AND HETEROCYCLIC SUBSTANCES FILTERED THROUGH A COLUMN OF SEPHADEX G-25, 50–100 MESH, WITH 0.01 *M* AMMONIUM HYDROXIDE pH 10.6 AS ELUANT

Substance	*Quantity (mg)*	*Ammonium hydroxide*		*Sodium chloride*
		K_D	*Yield (%)*	K_D
Adenylic acid	1.4	0.1	96	1.2
Guanylic acid	1.3	0.1	97	1.3
Cytidylic acid	1.6	0.1	96	0.8
Uridylic acid	1.5	0.1	95	0.8
Picric acid	0.5	0.4	98	2.5
Salicylic acid	2.2	0.5	101	1.6
Adenosine	1.0	1.8	100	1.8
Cytosine	0.7	1.3	99	
Inosine	1.2	0.3	100	1.3
Adenine	0.5	1.2	98	2.4
Phenol	3.7	1.0	100	1.7
Benzyl alcohol	50.0	1.3	96	
Riboflavin	0.8	0.8	88	
Nicotinamide	2.1	1.3	100	
Thiamine hydrochloride	1.5	0.3	95	1.0
Pyridoxine hydrochloride	1.6	0.7	98	1.0

It is evident that one gets a quantitative recovery over the whole pH range, and that the sorption properties of the bed material varies with pH.

It is interesting to find that the sorption properties are so drastically changed at high pH values. The negative sorption of acid substances is more accented and the

adsorption of aromatic substances seems to be decreased. The experiments with alkaline solution (0.01 M ammonium hydroxide) as eluant were therefore extended to include other aromatic and heterocyclic substances.

For comparison, K_D values from experiments in 0.05 M sodium chloride are included in Table VII.

The exclusion of acid substances on elution with distilled water or alkaline solution is valid only when small quantities are filtered through the column. For large quantities, the main portion of the acid is eluted in a normal way, *i.e.* with the same K_D as when an electrolyte solution is used as eluant. However, in these cases, too, the elution starts too early, and the zone is spread out in a large effluent volume. This is illustrated in Fig. 3a, where 50 mg of picric acid is filtered through the column with water. In the corresponding experiment in Table V, the picric acid had $K_D = 0.4$, and 100 % of the acid was eluted within an effluent volume equal to the volume $V_o + V_i$. In this case, the K_D value is 1.6, and only 6.5 % of the applied acid is eluted within the same volume. Analogous experiments have been made with sulphanilic acid and salicylic acid with distilled water as well as diluted ammonium hydroxide as eluant, and similar results were obtained.

A similar quantitative limitation has also been found for those basic substances which are strongly adsorbed to the bed material on elution with distilled water. Experiments have been made with frontal analysis of collidine and alkaloids, but the capacity of the column for these substances has not been determined.

Filtration experiments with mixtures of low-molecular-weight substances

In all the previous experiments, the tested substances are separately filtered through the column. We shall now discuss some experiments made with mixtures of these test substances. The first example relates to the experiments reported in Table VI.

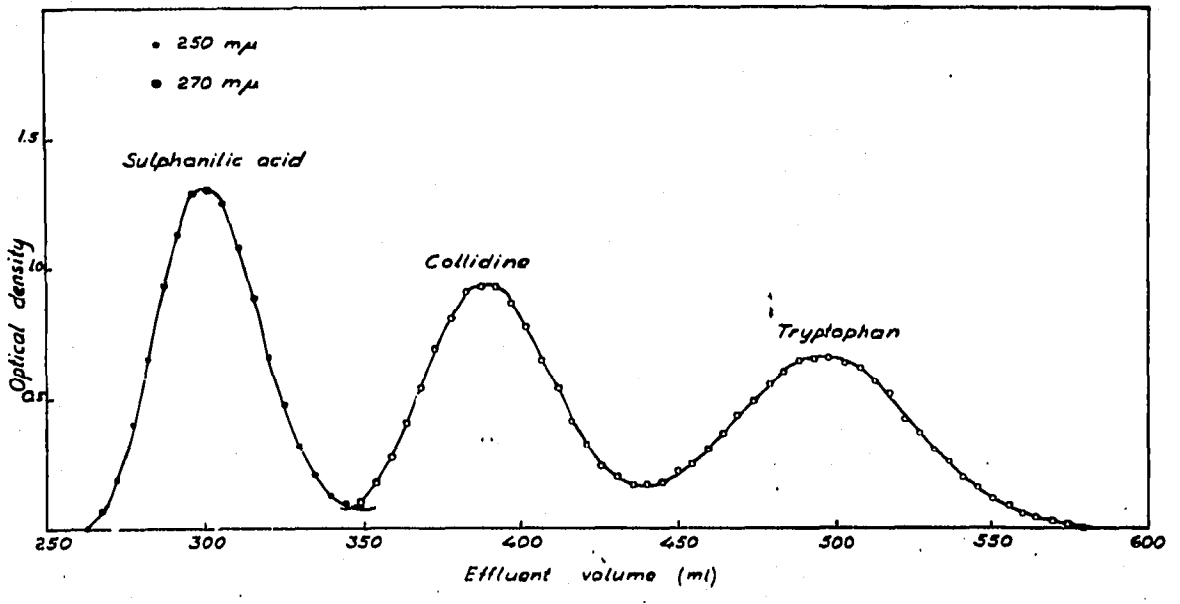

Fig. 1. 0.6 mg sulphanilic acid, 2.0 mg collidine, and 2.0 mg tryptophan filtered through a column (3.5 × 35 cm) of Sephadex G-25, 50–100 mesh. Eluant: 0.05 M triethylammonium carbonate pH 8.0.

At pH 8–9 it should be possible to separate the three substances: sulphanilic acid, collidine, and tryptophan. Fig. 1 shows the elution diagram when the eluant is 0.05 M triethylammonium carbonate at pH 8.0. The K_D values 0.8, 1.4, and 2.0 correspond well to those obtained for the three substances in separate experiments.

Some diagrams for purine and pyrimidine derivatives eluted with phosphate

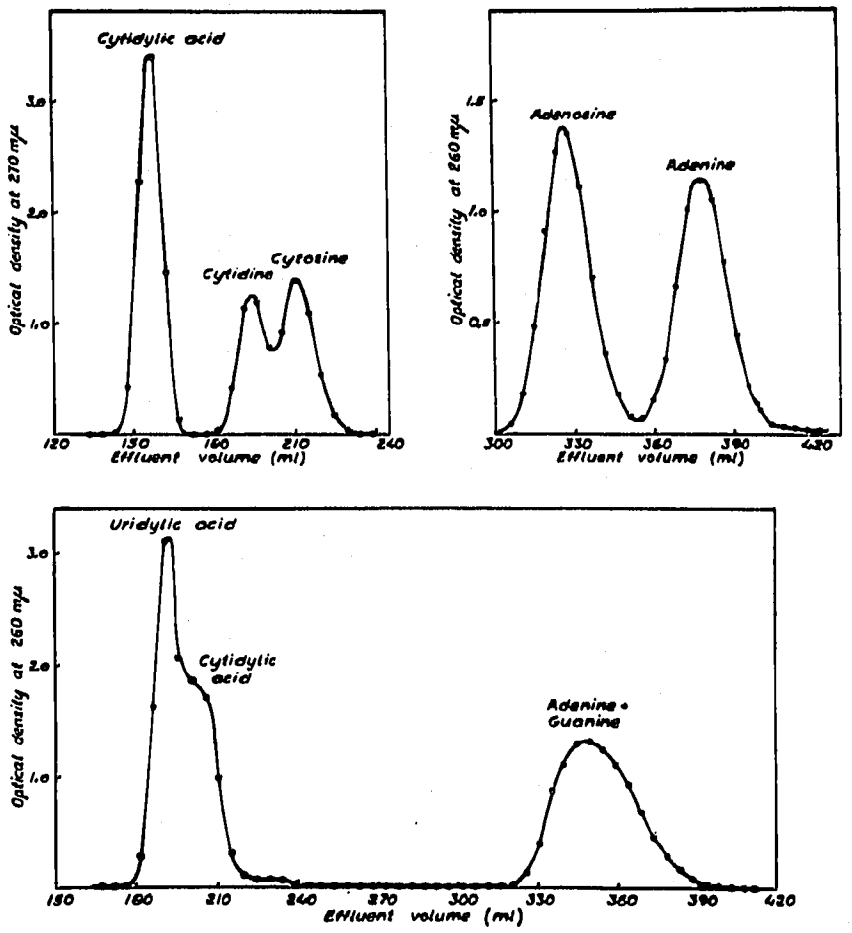

Fig. 2. Purine and pyrimidine derivatives filtered through a column (3.0 × 30 cm) of Sephadex G-25, 200–400 mesh. Eluant: Sodium phosphate, pH 7.0 (μ = 0.05).

buffer (μ = 0.05) are given in Fig. 2. The third diagram in this figure shows the separation of a mixture corresponding to an acid hydrolysate of ribonucleic acid. In agreement with the results listed in Table III, separation is only possible between the nucleotides and the bases.

It has been mentioned earlier that the exclusion of acids from the gel grains when elution is carried out with distilled water is valid only when very small amounts are filtered through the column. This is exemplified for picric acid in Fig. 3a. If the test solution contains 25 mg sodium chloride as well as 50 mg picric acid, the diagram b in Fig. 3 is obtained. The peaks are hydrochloric acid and sodium picrate, respectively. This time the zone is not spread out as in the preceding experiment, but the picrate will attain a partition equilibrium in the inner and outer water phase when passing through the column. A similar result is also obtained with collidine when the

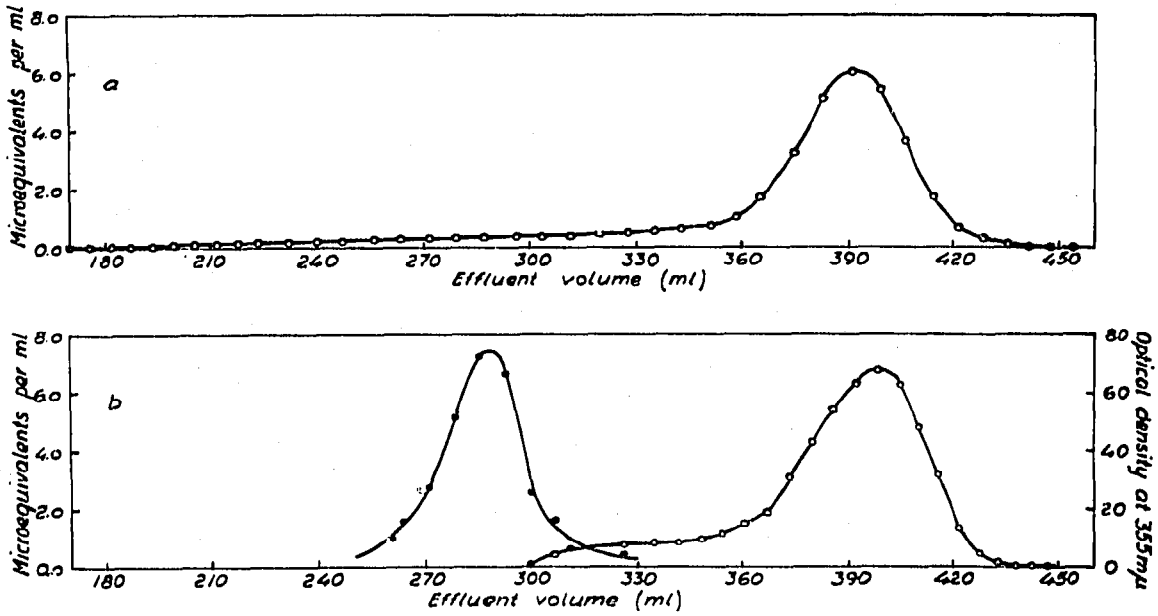

Fig. 3. (a) 50 mg picric acid (b) 50 mg picric acid and 25 mg sodium chloride filtered through a column (3.5 × 35 cm) of Sephadex G-25, 50–100 mesh. Eluant: Distilled water. o picrate; • chloride ions.

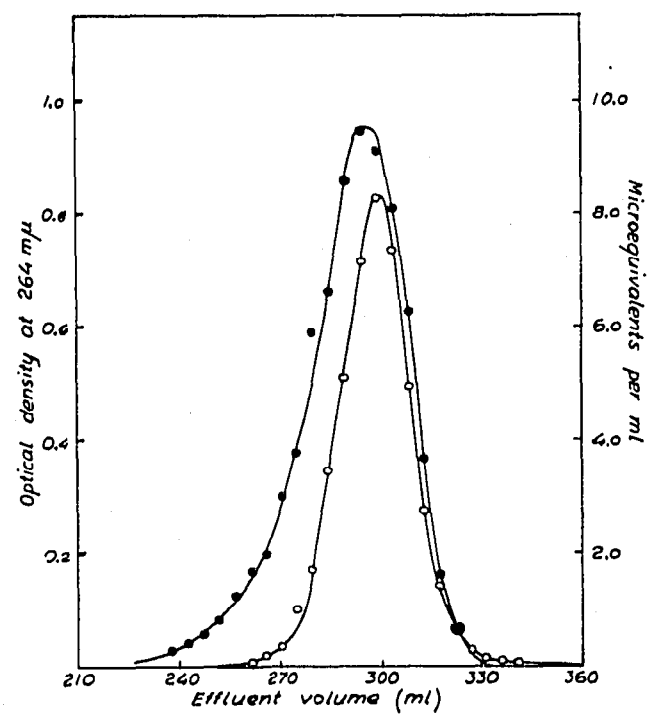

Fig. 4. 3 mg collidine and 25 mg sodium chloride filtered through a column (3.5 × 35 cm) of Sephadex G-25, 50–100 mesh. Eluant: Distilled water. o collidine; • chloride ions.

test solution contains an electrolyte. Fig. 4 shows an experiment where 3 mg collidine and 25 mg sodium chloride are washed through the column with water. Usually the collidine is strongly adsorbed to the bed material, but, in the presence of an electrolyte

in the test solution, collidine is eluted without any appreciable adsorption, just as if the elution had been carried out with an electrolyte solution.

A further example of the normalization of the elution conditions is given in Fig. 5. A mixture of 20 mg glycine, 20 mg methionine, 20 mg tyrosine, 20 mg tryptophan, 20 mg aspartic acid, 20 mg lysine hydrochloride, and 20 mg arginine hydrochloride

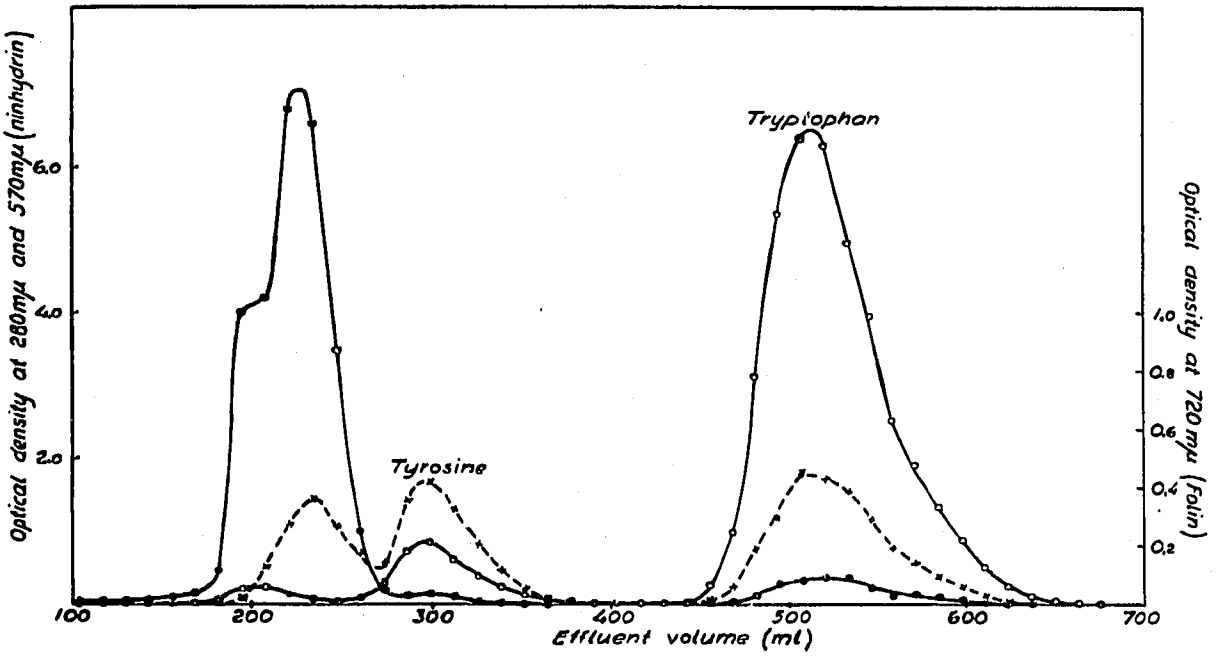

Fig. 5. 20 mg glycine, 20 mg methionine, 20 mg tyrosine, 20 mg tryptophan, 20 mg aspartic acid, 20 mg lysine hydrochloride, and 20 mg arginine hydrochloride in 15.0 ml of water filtered through a column (3.5 × 40 cm) of Sephadex G-25, 50–100 mesh. Eluant: Distilled water. o Optical density at 280 mμ; • 570 mμ (ninhydrin reagent); × 720 mμ (Folin-Ciocalteu reagent).

dissolved in 15.0 ml of water was put on the column and eluted with distilled water. All the aliphatic amino acids were eluted simultaneously with a K_D about 1, which is comparable to elution with electrolyte solution (Table II). The aromatic amino acids, tyrosine and tryptophan, were weakly adsorbed. The total recovery was quantitative.

CONCLUSIONS

The main effect in gel filtration using Sephadex as bed material is the separation of molecules of different size. This has been convincingly shown in other papers[1,2,5–7]. However, the data given in this paper show that a variety of other factors may influence the result of an experiment. These factors are most pronounced for low-molecular-weight substances, but in some cases they may also be relevant for substances of high molecular weight.

For an adequate discussion and a better understanding, it seems reasonable to separate these secondary effects into two groups.

A. Adsorption to the bed material, which is related to the structure of the test substances and is largely independent of the properties of the solvent.

B. Superimposed effects, which depend on the conditions of the column, *i.e.* the ionic strength and pH of the aqueous phases.

A. Among the inorganic ions tested, hydroxyl and borate ions are adsorbed. However, the experimental material is small, and there may be other ions which interact strongly with the gel matrix. For organic compounds, aromatic and heterocyclic substances have a greater tendency to be adsorbed than aliphatic substances. Basic groups in a molecule seem to increase, and acid groups to decrease, the adsorption.

B. These effects appear when a column is equilibrated with distilled water and are manifested in a strong adsorption of some basic substances and an exclusion of some acid substances from the interior of the gel grains. The effects are completely eliminated when the elution is made with an electrolyte solution, or when the test solution contains an electrolyte. However, an exclusion of acid substances is also obtained when elution is made at pH $>$ 10.

There is also another distinction between the two groups *A* and *B*. The effects in *B* are active only for very small quantities of test substance, while the adsorption of aromatic and heterocyclic substances (effect in *A*) is obtained for much larger quantities, giving symmetrical peaks which indicate linear adsorption isotherms. This circumstance and the observations mentioned above make it appear that the effects under *B* are caused by the small amount of ionized carboxylic groups in the bed material.

With these facts in mind, one can predict the result of a gel filtration experiment even with a rather complex mixture. There is always a slight retention of aromatic and heterocyclic substances. Only in very few cases will there be a strong adsorption of some basic compounds and a negative sorption of acid compounds, *i.e.* when the experiment is made in complete absence of other ionized substances. If other ionized substances are present, or if the elution is made with an electrolyte solution, these particular sorption effects disappear. The best way to avoid these superimposed effects is to perform the experiments in a medium containing at least a small amount of electrolyte.

There may, however, be instances when the secondary effects are of great value. Figs. 1, 2 and 5 are examples of a pure adsorption chromatography. A combination of adsorption chromatography and the molecular sieve effect is also of interest[5].

It is assumed that low-molecular-weight substances with K_D values about 0.8 are those which pass the column without sorption and that a correction of the inner volume V_i for the water of hydration would give $K_D = 1$. Also in batch experiments with some of these substances $K_D = 0.8$ is obtained over a wide concentration range[2].

In spite of the secondary effects discussed in this paper, Sephadex seems to be very suitable as column material in gel filtration. None of the tested substances is irreversibly adsorbed to the column, recoveries are almost quantitative in all experiments, and the reproducibility is fairly good. Furthermore, the mechanical and chemical stability of the bed material is quite satisfactory.

The majority of experiments reported in this paper are made on the same column without any detectable change in its properties.

SUMMARY

1. The principle of the gel filtration method is given, and experiments with buffering substances, amino acids, purine and pyrimidine derivatives, vitamins, alkaloids and some simple, aromatic substances are described.

2. The data presented demonstrate a good reproducibility. K_D values and recoveries are given in almost all experiments.

3. The experiments show that the bed material, Sephadex, in addition to the molecular sieve effect, gives a slight adsorption of aromatic and heterocyclic substances.

4. Depending upon the ionic strength and the pH of the gel column, a strong adsorption of some basic substances and a negative sorption of some acidic substances may occur. These effects are easily eliminated by the proper choice of experimental conditions.

5. The column material Sephadex is very useful for gel filtration. It does not seem to give any irreversible adsorption, and more than one hundred experiments have been performed on the same column without any detectable change in its properties.

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