A PROCEDURE FOR GEL FILTRATION OF VISCOUS SOLUTIONS

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INTRODUCTION

Gel filtration with special reference to desalting operations was studied by FLODIN[1]. He investigated some factors influencing the separation of high and low molecular weight compounds, *e.g.* the desalting of proteins. In this work he found that such separations were unfavourably affected by the viscosity of the sample, and for acceptable separations its viscosity should be less than about 5 in relation to the viscosity of the eluant.

The only earlier way of desalting viscous solutions by means of Sephadex® was therefore a batch procedure, which, owing to its low efficiency, must be repeated several times for acceptable results. It was found, however, that if a gel bed of Sephadex G-25 beads was used devoid of liquid in the void space even very viscous solutions could be desalted efficiently.

Such a procedure was best performed with the aid of centrifugal force, and therefore this method was studied mainly, but some alternative methods were also tried, and these are described shortly under the heading "Alternative Techniques".

The centrifugal method has been briefly described in an earlier publication[2] and in a manual for gel filtration with Sephadex[3].

Recently it has been reported that the centrifugal procedure described in this manual has been used successfully for fractionation of concentrated skim milk, whey, and similar biological systems[4].

THE CENTRIFUGAL GEL FILTRATION METHOD

Materials and methods

An MSE basket centrifuge* model 3000 was used. It had a perforated basket with 3000 c.c. cake capacity. The inside of the basket was lined with a 1/32 in. thick sheet of Vyon, porous polythene manufactured by Porous Plastic Ltd., Dagenham Dock, Essex, England.

The inside surface of the Vyon lining was 810 sq cm. The basket radius was 11.3 cm. This value was used for calculation of the centrifugal field.

As bed material for gel filtration the dextran gel Sephadex® G-25 was used in the following particle size grades:

* Measuring & Scientific Equipment, Spenser Street, London, S.W. 1, England.

	Particle size[a] (μ)
Superfine	20– 40
Fine	20– 80
Coarse	100–300

[a] Particles finer than 20 μ passed through the Vyon filter.

Test solutions with a great variety of viscosities from 1.02 to 1770 cP at 20° were prepared with the following solutes dissolved in water.

(1) *Substances of high molecular weight*

Bovine albumin from Poviet Producten N.V., Amsterdam.

Dextran T 250 (a dextran with $\bar{M}_w$ about 250,000).

Dextran sulphate 500 (produced from dextran with $\bar{M}_w$ about 500,000).

DEAE-dextran (produced from dextran with $\bar{M}_w$ about 2,000,000). Native dextran.

All dextran products except the native dextran were commercial products from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

(2) *Substances of low molecular weight*

Sodium chloride, calcium chloride and glucose were *pro analysi* or of a comparable grade of purity.

It is evident that a number of factors govern the centrifugal gel filtration method. Therefore an investigation of the more important ones was performed, and it soon became clear that a desalting experiment should comprise the following steps:

(1) Formation of a gel bed in the centrifugal basket by adding Sephadex beads swollen in water, starting and speeding up the centrifuge.

(2) Spinning off the water in the void space of the gel bed.

(3) Application of the sample and allowing its solutes to equilibrate in the gel filter.

(4) Spinning off the desalted sample.

(5) Washing the gel bed salt free with water.

In the following sections (A–E) some investigations are presented indicating how to perform these steps in order to obtain the best results. The essential features of these steps are summed up as an instruction for the use of the centrifugal method.

A. Optimal bed volume

It was found that the efficiency of a separation was dependent on the amount of Sephadex in the centrifuge, *i.e.* the thickness of the gel bed. This is illustrated by experiments reported in Table I. The experiments were performed with an almost constant sample volume per g of Sephadex G-25 Fine, but with different bed sizes. As shown below (Section C) the optimal sample volume to bed volume ratio was used. The composition of the samples was 20 % Dextran T 250 + 5.3 % NaCl (w/v) and the experiments were performed under otherwise constant conditions.

TABLE I

INFLUENCE OF THE THICKNESS OF THE GEL BED ON THE DESALTING EFFICIENCY

Expt. No.	*Sephadex G-25 Fine (g)*	*Volume of sample per g of Sephadex*	*% NaCl in dry matter of centrifugate*	*Recovery of dextran (%)*	*Separation efficiency (%)*
56	125	0.80	1.7	81	95.0
57	250	0.80	1.1	83	96.6
31	600	0.83	0.023	89	99.92

Full use of the 3000 c.c. cake capacity of the centrifuge is therefore recommended giving a cake thickness of 3–4 cm.

Fig. 1 is a schematic picture of the Sephadex filter packed in the centrifuge basket.

The best way to prepare the gel filter is by pouring the stirred up Sephadex slurry quickly into the basket of the centrifuge and rapidly increasing its speed up to a rather high value.

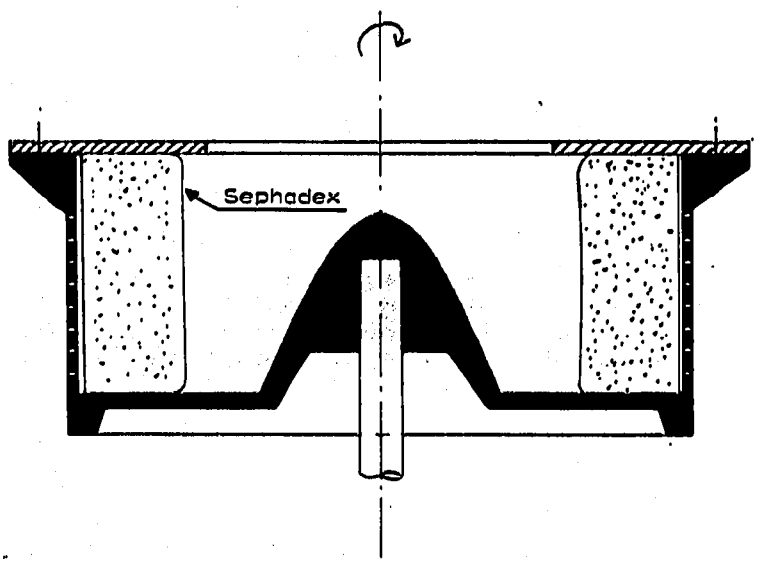

Fig. 1. Diagram of a gel filter packed in a basket centrifuge.

B. Estimation of the centrifugal field required for spinning off the water in the void space

It seemed logical to try to determine the optimal value of this centrifugal field (abbreviated to fv_o) by a stepwise increase of the centrifugal field with determination of the amount of water removed from the bed for each step. However, as shown in Fig. 2 there was a steady removal of water with increasing field, indicating that some internal water was also squeezed out of the Sephadex beads at high centrifugal fields.

Nevertheless, it should be possible to determine the value of fv_o from such a spinning off curve as fv_o should correspond to a condition where the total amount of solvent in the Sephadex slurry poured into the centrifuge except the inner volume of the gel bed (V_{aq}—V_i) had been spun out.

The dotted lines in Fig. 2 illustrate such a determination indicating a fv_o value of 800 $\times$ g. Yet this estimation of fv_o was very uncertain depending on experimental errors and the very slight slope of the curve at the intersectional point. By this method, therefore, only an estimation of the order of magnitude of fv_o could be done.

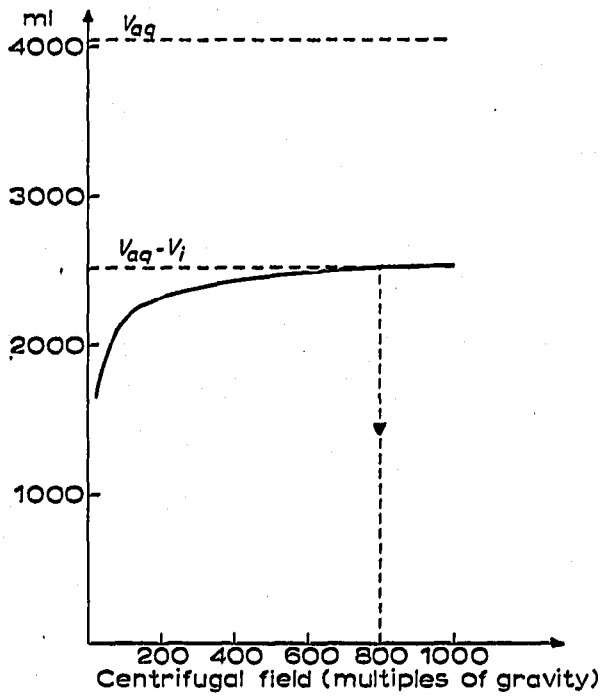

Fig. 2. Removal of water (external and some internal) from a slurry of 582 g (= a) dry Sephadex G-25 Fine beads in 4050 ml of water (= V_{aq}) in a basket centrifuge by a stepwise increase of the centrifugal field. Approximate estimation of the centrifugal field required for spinning off the water in the void space. V_t was calculated from the formula $V_t = a \cdot W_r$ to 1500 ml, where W_r = 2.57 (water regain value[3]).

Another way of estimating fv_o should be to perform experiments at various centrifugal fields with samples containing a completely exluded high molecular weight substance and a salt. If water were to remain in the void space it would dilute the incoming sample, and a too high centrifugal field would probably shrink the beads resulting in absorption of water from the sample. With dextran and sodium chloride as solutes a series of such experiments were performed, the results of which are compiled in Table II.

When the interstitial water was spun off at 950 $\times$ g the dextran content of the centrifugate was practically constant indicating that this centrifugal field was sufficient for spinning off the water in the void space of the gel bed.

A centrifugal field of 1000 $\times$ g in round figures is therefore recommended for this purpose. In reality, however, the conditions were more complicated than hitherto described. For example the colloid osmotic effect of the dextran obviously caused a dilution, since the dextran content of the centrifugate was considerably lower than that of the sample.

TABLE II

DESALTING EXPERIMENTS IN WHICH VOID WATER AND SAMPLES WERE SPUN OUT AT VARIOUS CENTRIFUGAL FIELDS

Expt. No.	*Sample*				*Centrifugate*						*Desalting efficiency (%)*
	Vol. (ml)	*g per 100 ml of*		*% NaCl in dry matter*	*Centrifugal field* (multiples of gravity)*	*Vol. (ml)*	*Dextran T 250*		*NaCl*		
		Dextran T 250	*NaCl*				*g per 100 ml*	*yield (%)*	*mg per 100 ml*	*% in dry matter*	
46	500	19.9	5.2	20.7	250	100	7.9		0.7	0.009	
						100	9.7		0.7	0.007	
						100	12.2		0.7	0.006	
						100	14.3		0.7	0.005	
						145	15.6		0.7	0.004	
					Total	545	12.2	67	0.7	0.006	99.98
44	500	19.9	5.2	20.7	550	100	14.8		0.7	0.005	
						100	15.2		0.7	0.005	
						100	17.4		0.9	0.005	
						100	17.6		0.9	0.005	
						82	18.3		1.0	0.005	
					Total	482	16.6	80	0.9	0.005	99.98
31	500	20.3	5.3	20.7	950	100	16.5		2.6	0.016	
						100	16.8		3.6	0.022	
						100	17.0		4.0	0.023	
						100	17.2		4.4	0.025	
						132	17.0		4.9	0.029	
					Total	532	16.9	89	4.0	0.023	99.92
47	500	19.9	5.2	20.7	1500	100	21.0		0.8	0.004	
						100	20.3		1.4	0.007	
						100	19.1		2.0	0.011	
						100	17.5		3.5	0.020	
						108	16.2		3.8	0.023	
					Total	508	18.5	94	2.3	0.012	99.95

* In each experiment the same centrifugal field was used for spinning out both void water and the dextran solution.

C. Application of sample

Optimal centrifugal field for sample application and equilibration. It was found that for a good separation the sample must remain in the gel bed for a considerable time and it should, therefore, be applied at a low speed of the centrifuge.

The best desaltings were obtained with such conditions, and 50–60 × *g* seemed most convenient for both the application and the equilibration of a sample. A centrifugal field of about 60 × *g* is therefore recommended for sample application and equilibration.

Sample volume. In order to determine the maximum sample volume which could be retained by the gel bed at a centrifugal field of about 60 × *g* the experiment illustrated by Fig. 3 was performed.

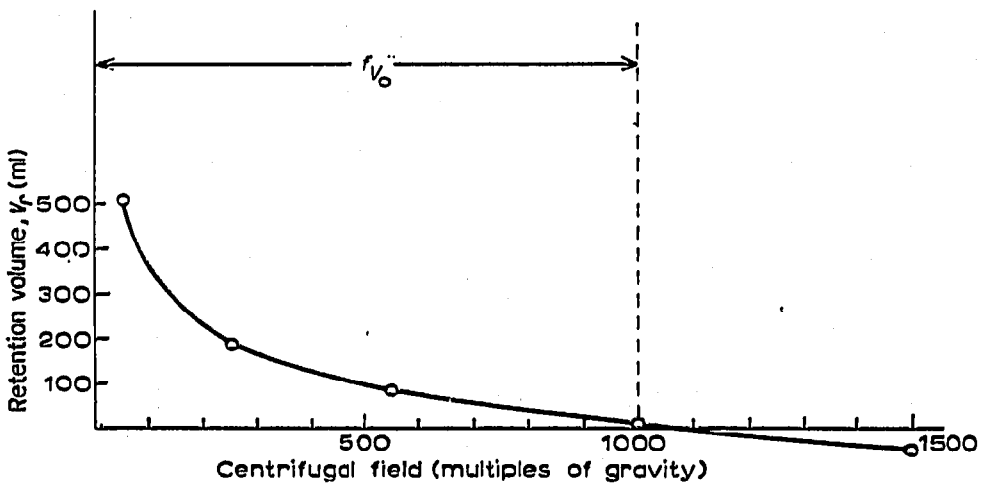

Fig. 3. Retention volume, of water, at increasing centrifugal field for a 3 l gel filter of Sephadex G-25 beads.

In this experiment the gel (Sephadex G-25 Fine) was first packed and the void water was spun off at 1,000 × *g*. The speed of the centrifuge was then reduced to 60 × *g* and 1,000 ml of water were added. The centrifugal field was kept constant until no more water came out of the gel filter. Half the added amount of water could be spun off at this field. The gel filter thus retained 500 ml of added water at 60 × *g*.

By increasing the centrifugal fields stepwise to 1,000 × *g* the remaining amount of added water was gradually spun off. The correlation between the retention volume of the gel bed and the centrifugal field is shown in Fig. 3.

A suitable sample volume should therefore be 500 ml. These findings were also confirmed for a sample with a viscosity of about 100 cP as illustrated by the following experiment.

Sample: 1,350 ml of a solution containing 19.8 g of Dextran T 250 and 5.1 g of sodium chloride per 100 ml corresponding to 20.4 % sodium chloride in the dry matter.

Centrifugate: Ten centrifugate fractions were collected. The curve in Fig. 4 indicates the average salt content for integrated fractions, while the dotted curve represents the average salt content of the first six fractions plotted in the same way. From the efficiency of the desalting it follows that, during the equilibration stage, the gel filter could only hold a portion of the sample, corresponding to about 500 ml centrifugate volume, or the same as the retention volume for water according to

Fig. 3. For a filter bed of 3000 ml a sample size of about 500 ml is, therefore, recommended for a good desalting. In many cases, however, a larger sample volume may give a sufficient desalting.

Sample feed rate. Sample feed rates from 10 to 500 ml/min have been tested. The best results were obtained with a fast feed rate. The reason for this seems to be that the sample is evenly spread over the whole inner surface of the gel bed at a fast feed rate. The highest feed rate mentioned above, *i.e.* 500 ml/min, is recommended: It is also time-saving.

Equilibration time at optimal centrifugal field for gel filtration. The time for equilibration of the sample in the gel bed greatly influenced the separation. This is illustrated by three experiments in Table III. In each of them a 500 ml sample was fed to the centrifuge. The sample contained 19.8 g of Dextran T 250 and 5.4 g of sodium chloride per 100 ml, the latter representing 21.4 % of the total dry matter of the sample. The viscosity of the sample was about 100 cP/20°.

With an equilibration time longer than 8 min the content of sodium chloride of the centrifugate was not appreciably lowered. In most cases an equilibration time of about 15 min was used, which was sufficient even for the most viscous sample tested.

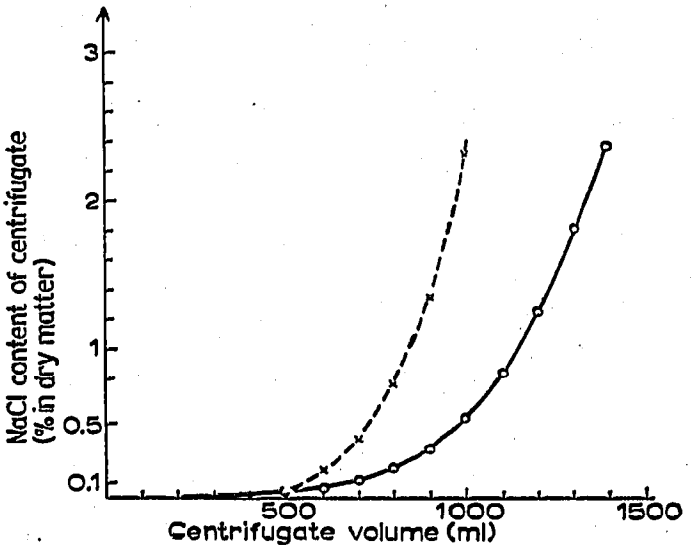

Fig. 4. Desalting of a 1350 ml sample with a viscosity about 100 cP/20° on a 3 l gel filter of Sephadex G-25 Fine beads. The sample contained 19.8 g of Dextran T 250 and 5.1 g of sodium chloride per 100 ml (20.4 % of sodium chloride in the dry matter). Continuous curve is salt content for integrated centrifugate fractions. Dotted curve is salt content of the first six centrifugate fractions.

TABLE III

THE RELATION BETWEEN THE EQUILIBRATION TIME AND THE DESALTING EFFICIENCY

Expt. No.	*Sample feed time (min)*	*Equilibration time after feed at 60 × g (min)*	*Centrifugate, sodium chloride in % of total dry matter*	*Recovery of dextran (%)*
26	3/4	0	11.4	89
27	1	4	2.2	89
28	1	8	0.033	90

To obtain a sufficient retention of samples with different viscosities and also to avoid excessive resistance to flow through the gel bed it was necessary to select different grades of Sephadex G-25 for different viscosity ranges. As a result of performed experiments the following preliminary viscosity ranges seemed recommendable for the coarse, fine and superfine grades of Sephadex G-25:

Viscosity of the sample (cP at 20°)	*Grade of Sephadex G-25*
1–50	Superfine
5–200	Fine
> 200	Coarse

D. Spinning off the desalted sample

To avoid retention of liquid in the void space the centrifugate should be spun out from the gel filter in a centrifugal field at least amounting to fv_o, *i.e.* at about 1,000 × *g*. According to a great number of experiments, see Table IV, this centrifugal field was also sufficient for spinning off the centrifugate with a good recovery of its high molecular weight solutes.

The course of the spinning off curve for dextran, the recovery of dextran and the remaining salt content have been determined for different samples and different grades of Sephadex G-25.

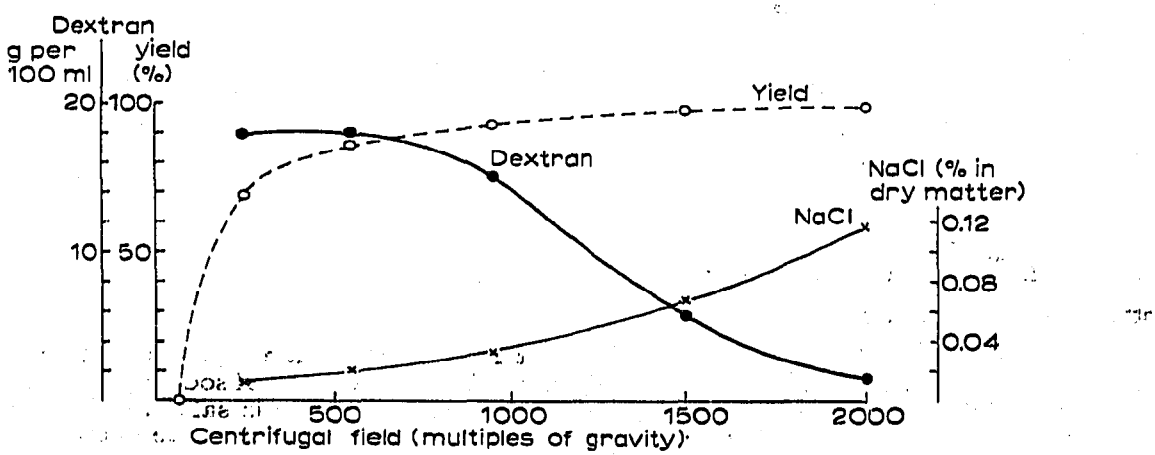

Fig. 5. Spinning-off curves for Dextran T 250 and sodium chloride by a stepwise increase of the centrifugal field. A centrifugal field of 950 × *g* was used for spinning off external water from a 3 l gel bed of Sephadex G-25 Fine beads.

Typical curves illustrating such data are shown by Fig. 5. For all grades of Sephadex G-25 the character of these curves was the same. They have the following characteristics in common:

(a) The dextran content declines from about 500 × *g* indicating that the beads begin to shrink, probably because of compression, at this centrifugal field.

(b) The salt content of the centrifugate steadily increases with increasing centrifugal field.

According to Fig. 5 the recovery of dextran from the centrifugate can be further improved by increasing the centrifugal field beyond 1,000 × *g* but at the expense of a slightly higher salt content and increased dilution.

On the other hand, if too low a centrifugal field is used for spinning off interstitial water before the sample is applied, the shape of the dextran curve is altered considerably because the sample is diluted by remaining interstitial water. Fig. 6 illustrates such conditions when interstitial water was spun off at 550 × *g*.

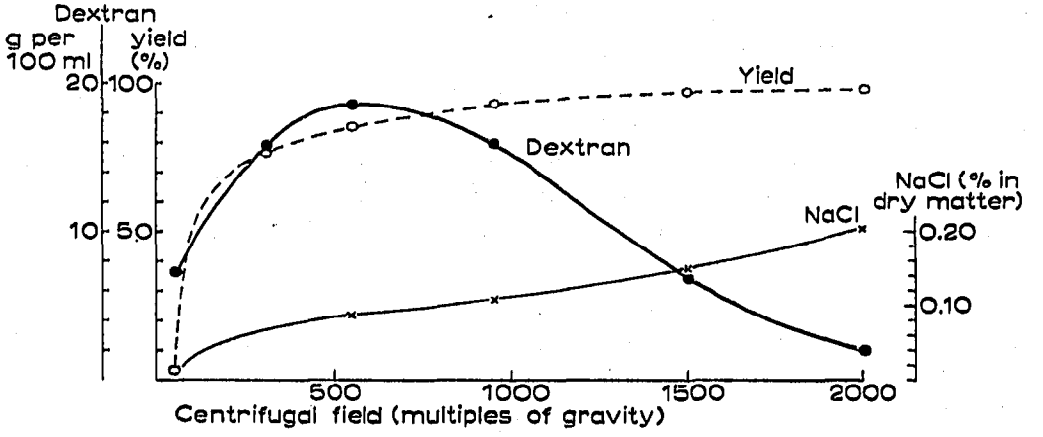

Fig. 6. Spinning-off curves for Dextran T 250 and sodium chloride by a stepwise increase of the centrifugal field. A centrifugal field of 550 × *g* was used for spinning off the external water from a 3 l gel bed of Sephadex G-25 Fine beads.

E. Washing the gel bed

Solutes which have penetrated the beads are best washed out in a low centrifugal field or at standstill in the following manner.

The speed of the centrifuge is lowered to about 60 × *g* and solutes which have penetrated the gel beads are rinsed out of the gel with distilled water at a rate not below 1 l/min for the fine grade of Sephadex G-25 and requiring about 6 l of distilled water or 2 × V_t for a virtually complete elution.

For the coarse grade of Sephadex G-25, however, the rinsing is best performed with the centrifuge at rest. The gel cake, which then flows down to the bottom of the basket, is rinsed either with running water or by repeated washing.

RESULTS

The methodological experiments described led to the following standard procedure for centrifugal gel filtration:

(1) For a centrifuge with a 3 l cake capacity 600 g (dry weight) of a suitable grade of Sephadex G-25 beads are swollen in 4 l of water for 1 h. The basket is loaded with the stirred up slurry and the speed of the basket is rapidly increased.

(2) External water is spun off from the gel bed at 1,000 × *g*.

(3) A 500 ml sample is added in 1 min at 60 × *g* and is allowed to equilibrate in the gel bed for 15 min.

(4) The "desalted" sample is spun off at 1,000 × *g*.

(5) For the fine and super fine grades of Sephadex G-25 the gel bed is washed

salt free at 60 × *g* with 1–2 l of water/min. For the coarse grade the washing is made with the basket at rest.

Experiments according to the centrifugal method

In Table IV a series of experiments have been tabulated. These have been arranged to demonstrate separations for samples with viscosities from low to high values successively requiring the three different grades of Sephadex G-25 from superfine for a dilute solution of albumin, fine for more viscous solutions, to coarse necessary for a very viscous solution of native dextran.

For the superfine and fine grades, however, the results were unsatisfactory, if the viscosities of the samples were too high (expt. Nos. 70 and 20). The reason for this seems to be an overloading of the gel bed resulting in some kind of "break-through", as appreciable amounts of liquid were spun off at 60 × *g*; for samples of lower viscosities no centrifugate was spun off at this equilibration stage. For these grades the viscosities of the samples should at least be less than about 80 and 450 cP respectively.

The results of Table IV indicate that with an appropriate grade of Sephadex G-25 very good separations were obtained. In most cases the centrifugate volumes differed very little from the sample volumes. The centrifugates could therefore be desalted once more with small dilutions (compare expt. Nos. 14 and 15).

Summing up the results of Table IV the conclusions are that samples within the whole viscosity range up to about 2,000 cP could be "desalted" efficiently with the centrifugal procedure for gel filtration.

Most desaltings described in this paper and performed according to the centrifugal method were done with samples containing about 20 g of Dextran T 250 per 100 ml having a viscosity of about 100 cP at 20°.

As a comparison a similar desalting experiment was done utilizing the column technique, Fig. 7a. The viscosity of the sample was 115 cP/20° and as shown by the figure, the desalting failed completely. Fig. 7b shows the normal behaviour for the desalting of a low viscosity sample on the same column.

ALTERNATIVE TECHNIQUES

As mentioned in the introduction the gel filtration procedure with a gel bed devoid of interstitial water can be carried out by other means than by the centrifugal force method. Up to now the following methods have been tried:

(1) Vacuum filtration on a Büchner-funnel or a sintered glass filter.

(2) Mechanical compression of the gel bed in a thick-walled syringe with filter bottom.

(3) Blocking the void space of a gel filter column by a liquid immiscible with the solvent used for swelling the beads*.

The centrifugal method is, however, superior to all these alternative methods, possibly with the exception of the second, using mechanical compression of the gel bed which could be a good alternative for very small samples.

The use of vacuum filtration may also be a recommendable method for samples

* The method proposed by Mr. BJÖRN SÖDERQVIST is gratefully acknowledged.

TABLE IV

EXPERIMENTS ACCORDING TO THE CENTRIFUGAL METHOD

Abbreviations: S = sample; C = centrifugate; Na-Dx-S = Dextran Sulphate 500; DEAE-Dx = DEAE-Dextran.

Expt. No.	*Sephadex G-25 grade*	*Sample*		*Content of (A) (g/100 ml) in*		*Content of (B) in % of dry matter in*		*Volume in ml of*		*Viscosity of sample*		*Remaining (B) in C (%)*	*Recovery of (A) in C (%)*
		Large molecules (A) excluded by the beads	*Small molecules (B) penetrating the beads*	*S*	*C*	*S*	*C*	*S*	*C*	*temp. (°C)*	*cP*		
62	Superfine	Albumin	Sodium chloride	0.91	0.86	69.3	0.95	500	530	20	1.02	0.4	100
70	Superfine	Dextran T 250	Sodium chloride	18.7	16.7	21.1	4.5	500	475	20	85	14.8	85
12	Fine	Dextran T 250	Sodium chloride	4.92	4.84	20.8	0.60	500	476	20	5.5	2.2	93
14	Fine	Dextran T 250	Sodium chloride	10.1	9.57	20.4	0.30	500	488	20	18	1.1	92
15	Fine	Dextran T 250	Sodium chloride	9.57	9.32	0.3	0.04	460	446	20	16	0.1	87
51	Fine	Na-Dx-S	Sodium chloride	9.36	6.39	10.7	0.05	500	593	20	20	0.33	81
42	Fine	DEAE-Dx	Calcium chloride	9.19	5.97	8.5	0.00	500	605	20	47	0	79
31	Fine	Dextran T 250	Sodium chloride	20.2	16.9	20.7	0.02	500	532	20	110	0.08	89
55	Fine	Dextran T 250	Glucose	20.0	17.2	5.0	traces	500	520	20	110	traces	90
33	Fine	Dextran T 250	Sodium chloride	24.6	18.7	21.0	0.01	500	565	20	220	0.04	86
20	Fine	Dextran T 250	Sodium chloride	29.2	22.5	21.3	10.8	500	563	20	460	39	87
11	Coarse	Dextran T 250	Sodium chloride	20.0	17.7	20.7	0.53	500	528	20	110	1.9	93
67	Coarse	Native Dextran	Sodium chloride	24.7	17.8	20.0	0.02	495	600	25	1770	0.06	87

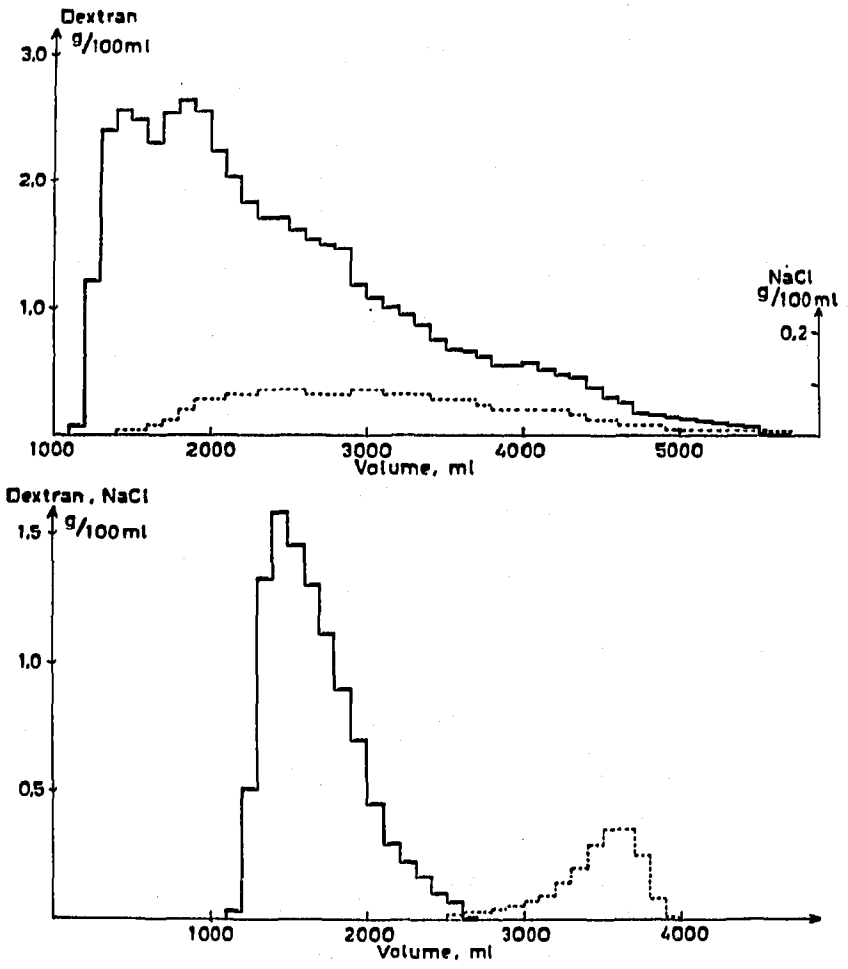

Fig. 7. Desalting on a 7.4 × 90 cm column of Sephadex G-25 Coarse of 200 ml samples containing 1.0 g/100 ml of sodium chloride. (a) 24.3 g/100 ml of Dextran fraction 789 A, viscosity 115 cP/20° (upper diagram); (b) 5.3 g/100 ml of Dextran fraction 789 A, viscosity 4.7 cP/20° (lower diagram)

with moderate viscosities as this method only requires simple, generally available laboratory equipment.

The drawback of this procedure is that it is rather laborious. Besides, it is almost impossible to eliminate the water in the void space completely, resulting in a more dilute filtrate and a lower recovery of excluded solutes than in the centrifugal method. The separation is, however, quite as good in both cases. The procedures are illustrated by the following examples.

Method 1

A bed of water-swollen Sephadex G-25 Fine beads was packed on a glass filter with 75.4 sq. cm filter surface and as much as possible of the void liquid was sucked out of the gel bed by vacuum. Suction inevitably produced cracks in the bed which had to be repacked several times. The final filter cake, which was not affected by suction, was rather dense. The bed height was 5 cm and the volume of the filter cake about 380 ml.

As much as possible of external water had now been sucked out and the bed was ready for sample application. Without suction 126 ml of a solution containing

19.8 g of Dextran T 250 and 4.7 g of sodium chloride was added on the top of the bed. The sample, which had a viscosity of 100 cP/20°, slowly entered the bed (50 min were required). The sample was then forced out of the bed by vacuum.

The recovery of dextran was 75 % and the salt content was reduced from 19.3 to 0.03 % in the dry matter.

Method 2

A thick-walled syringe was made of perspex as ordinary glass syringes did not stand the pressure applied during the procedure. In the bottom of the syringe a Vyon-filter was put in as a support for the gel. An ordinary piston with 21 mm diameter fitted into the syringe, and the compression required was obtained with a spindle press.

With the syringe in a vertical position water-swollen Sephadex G-25 Fine beads were packed to a bed height of 6.7 cm, gel volume 23 c.c. 10 ml of water was pressed out of the gel with the piston, which then was removed allowing the gel bed to expand. 4.07 ml of a sample with a viscosity of about 100 cP was added on top of the bed. The sample contained 19.9 g of Dextran T 250 and 5.2 g of sodium chloride per 100 ml, the latter amounting to 20.7 % of the dry matter of the sample. The piston was inserted again after 17 min and the sample was pressed out from the void space. Three fractions were collected and analyzed (Table V).

TABLE V

A DESALTING EXPERIMENT WITH MECHANICAL COMPRESSION OF THE GEL BED

Fraction No.	*Filtrate*			
	ml	*Dextran: T 250 (g/100 ml)*	*g*	*NaCl (mg)*
1	2.1	8.48	0.178	0
2	2.2	16.05	0.353	0
3	1.7	11.30	0.192	0.33
Total	6.0		0.723	0.33

Dextran recovery: 89 %.
Sodium chloride: 0.05 % in the recovered dextran.

The third fraction evidently contained some salt solution which had been squeezed out of the gel.

Method 3

This method has hitherto only been applied to samples of low viscosities. The best results have been obtained with *n*-butyl alcohol as the void space blocking solvent.

An experiment was performed in the following way: Sephadex G-25 Fine beads were allowed to swell in water and packed in a glass column (2.5 cm I.D.) giving a bed height of 18.5 cm. The water above the gel surface was drained and *n*-butyl alcohol saturated with water was added carefully to the bed. The alcohol was allowed to percolate

through the column carrying with it droplets of water from the void space until the void volume had been replaced by the alcohol.

A 19 ml sample containing 0.53 g of Dextran T 250 and 0.13 g of sodium chloride per 100 ml of water was then layered on the gel bed, which was slowly drained. After 4 h 300 ml of *n*-butyl alcohol and 19 ml of aqueous solution had been collected. This solution was now completely salt-free and 75 % of the dextran was recovered.

DISCUSSION

For solutions of high viscosities the new procedure for gel filtration with Sephadex G-25 described in this article has proved to be far superior to the column procedure.

The reason for this insensitivity to the viscosity of the sample must be that a gel bed free from water in the void space is used. Various techniques can be applied to accomplish the procedure but, as already discussed under the heading "Alternative techniques", the centrifugal technique is the best one.

Certainly the alternative techniques can be improved considerably, and pneumatically operated; for instance, the technique using mechanical compression of the gel bed could be a very attractive method for small samples.

On a laboratory scale the centrifugal technique is very convenient: no special equipment is necessary, other than a basket centrifuge. Not only is the operation simple, but there is no need to watch for "desalted" fractions.

Another advantage is that the centrifugal technique seems suitable for scaling up for industrial use and in this respect it is also of importance that the operation of the centrifuge can be automated[5].

For estimation of the capacity of the centrifugal procedure the total process time or cycle time is of great interest.

With the centrifuge used the cycle time was 2–4 h depending on the viscosity of the sample. However, no efforts were made to reduce these cycle times to obtain optimal capacities. It seems possible to reduce the cycle time appreciably, at least, in some cases. Thus MORR *et al.*[4] reported a cycle time of only 30 min, which would mean a capacity of at least 1 l of sample per h for a 3 l basket centrifuge. With commercially available centrifuges, capacities 100 times that value could be obtained.

SYMBOLS

fV_o = Centrifugal field for elimination of the liquid from the void space, multiples of gravity.
V_{aq} = Total water volume for an experiment.
V_i = Total inner volume of the Sephadex G-25 beads forming the gel filter.
V_o = Void volume of gel filter.
V_r = Retention volume of gel filter in a centrifugal field.
V_t = Volume of gel bed.

SUMMARY

A procedure for gel filtration of viscous solutions with Sephadex® G-25 beads has been developed with the following characteristic features.

A bed of such beads is packed and the liquid in the void space is eliminated. The sample is fed into the empty void space and a separation of solutes penetrating the beads from excluded solutes takes place. After equilibration the solution in the void space is removed and the beads are washed.

The procedure is best carried out in a basket centrifuge, but other types of apparatus can also be used, for instance a vacuum filtration set or a syringe for mechanical compression of the bed.

Contrary to conventional gel filtration in columns the centrifugal procedure is useful for gel filtration of viscous solutions with viscosities up to at least 2000 cP.

The centrifugal procedure is well suited for desalting of high molecular weight polymers on a preparative scale because concentrated solutions can be handled with little dilution of the polymer. The procedure seems suitable also for industrial application, and the operation can be automated.

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