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GEL CHROMATOGRAPHY COLUMN SCANNING FOR THE ANALYSIS OF ^{99m}Tc-LABELLED COMPOUNDS

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SUMMARY

The gel chromatography column scanning (GCS) method has been studied, special attention being paid to its suitability for the analysis of ^{99m}Tc-labelled compounds and radio-pharmaceuticals.

The sample to be analyzed was applied at the top of a column filled with Sephadex G gel. Elution was carried out with 0.9% sodium chloride solution with such a volume that all the radioactivity was retained in the column. The column was then sealed and scanned with a 1 mm slit-collimated NaI(Tl) crystal.

The GCS method is discussed and different factors influencing the scanning profile are considered.

The relationship between the position of the activity peak in the scanning profile and the molecular weight of the sample is given for Sephadex G-25 Medium gel.

INTRODUCTION

Gel filtration with Sephadex[1] has been used extensively for studying the chemical state of ^{99m}Tc in labelled compounds and radio-pharmaceuticals[2–9]. In a gel chromatography column filled with Sephadex, molecules larger than the largest pores of swollen Sephadex pass through the bed in the phase outside the gel particles and are thus eluted first. Smaller molecules penetrate the gel particles to a varying extent, depending on their size and shape. Molecules are therefore fractionated on a Sephadex bed in order of decreasing molecular size.

In gel chromatography column scanning (GCS)[10,11], only a small volume of the eluting agent is used, so that none of the radioactive zones is eluted. The distribution of the γ-emitting radionuclides in the column is studied by scanning with a slit-collimated NaI(Tl) detector instead of studying the elution curve by fraction collection.

The GCS method is much less time consuming and is technically less difficult to use than conventional gel chromatography with fraction collection. It also yields more detailed information about the form and distribution of molecular size of ^{99m}Tc-labelled compounds than thin-layer chromatography and can be performed in a closed system at the pH value preferred, and in inert atmosphere if desired. The GCS

method has been shown to be very useful for the identification and quality control of ^{99m}Tc-labelled radio-pharmaceuticals, both during the development of new preparations and in routine use[10,11]. The present work demonstrates the relation to conventional gel chromatography and the fundamental characteristics and parameters of the GCS method when applied to pertechnetate ($^{99m}TcO_4^-$) and ^{99m}Tc-labelled compounds.

EXPERIMENTAL

Chemicals

Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from a technetium-99m generator (Amersham, Great Britain, Code MCC.3). The generator was an alumina column on which molybdenum-99 was absorbed as molybdate ion. Technetium-99m (half-life 6 h) was formed by the radioactive decay of molybdenum-99 (half-life 67 h). When radioactive equilibrium was established (22 h), the pertechnetate ions were eluted with 0.9% (*i.e.*, 0.154 *M*) sodium chloride solution.

The radioactive concentration of technetium-99m in the solutions was of the order of 1 mCi/ml. The chemical concentration of technetium-99m in these solutions was therefore of the order of 10^{-8}–10^{-9} *M*.

Preparation of columns

The Sephadex G gels used are listed in Table I. Each type fractionates within a particular range of molecular weight, determined by the degree of swelling of the gel. The gel powder was permitted to swell in excess of distilled water for 2–6 h standing in a boiling water-bath[1]. After cooling to room temperature, the gel slurry

TABLE I

PHYSICAL PROPERTIES OF DIFFERENT TYPES OF SEPHADEX G GELS

Sephadex	*Dry particle diameter (μm)*	*Bed volume (ml per g dry Sephadex)*	*Fractionation range, dextrans (molecular weight)*	*Comments*
G-10	40–120	2 – 3	<700	
G-15	40–120	2.5– 3.5	<1500	
G-25 Coarse	100–300	4 – 6	100– 5000	Ultra high flow
Medium	50–150	4 – 6	100– 5000	High flow
Fine	20– 80	4 – 6	100– 5000	Laboratory application
Superfine	10– 40	4 – 6	100– 5000	High resolution
G-50 Medium	50–150	9 –11	500– 10000	
G-75	40–120	12 –15	1000– 50000	
G-100	40–120	15 –20	1000–100000	

thus obtained was carefully poured into a glass tube leading down a glass rod. A piece of glass-wool was placed both in the top and in the bottom of the gel bed. The internal diameter of the tube was 15 mm and the length of the gel bed was 22–24 cm.

Measuring procedure

The sample volume was limited to 0.20 ml in order to obtain good resolution;

this volume is about 0.5% of the bed volume. This sample size was also large enough to avoid any dependence on local variations in the sample solution and is so small that its expenditure is often acceptable for testing radio-pharmaceuticals in routine use. This sample size is also generally large enough to give the scanning profile good statistical significance.

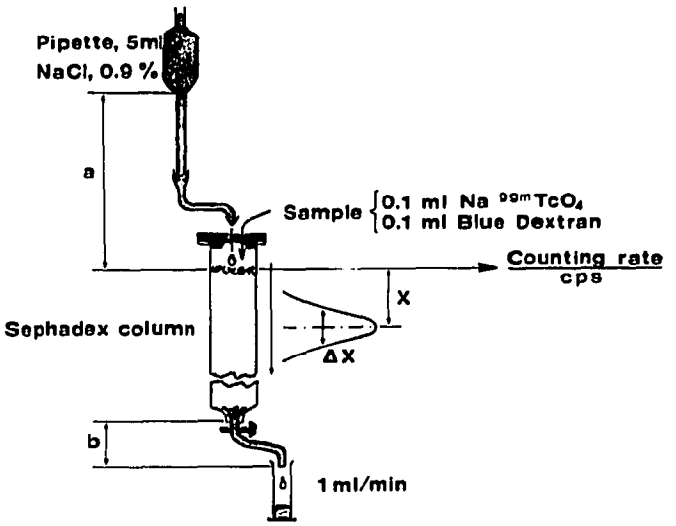

Fig. 1. Principles of the experimental arrangement used for the gel column operation.

The principles of the experimental arrangement are illustrated in Fig. 1. The scanning-profile of free pertechnetate was studied for different elution volumes. The sample, which consisted of 0.10 ml of $Na^{99m}TcO_4$ and 0.10 ml of Blue Dextran 2000, was applied at the top of the column. The elution was then carried out with 0.9% sodium chloride solution. The flow-rate was about 1 ml/min (except for Sephadex G-100), which was obtained by adjusting the height of the pipette (a in Fig. 1) and the outlet level (b in Fig. 1) of the column. For each 5.0 ml of eluent, the column was sealed and scanned in horizontal position with a 1 mm slit-collimated NaI(Tl) crystal (5 mm thick, 50.8 mm diameter) with a beryllium window. The distance between the top of the gel bed and the maximum of the recorded activity peak, X, and the width at the half-maximum of the peak, ΔX, were measured in the scanning profile thus obtained on a recorder. The distance between the top of the gel bed and the centre of the Blue Dextran 2000 zone was also measured, by observing the blue colour. The fraction of ^{99m}Tc activity present in each centimetre of the column was determined in order to permit quantitative comparisons to be made. The number of counts recorded per centimetre was thus recorded on a printer, and after subtraction of background values, this number was divided by the net sum of the number of counts recorded for the entire length of the column.

RESULTS AND DISCUSSION

Migration of $^{99m}TcO_4^-$ through the column

A set of scanning profiles was obtained for each column, describing the trans-

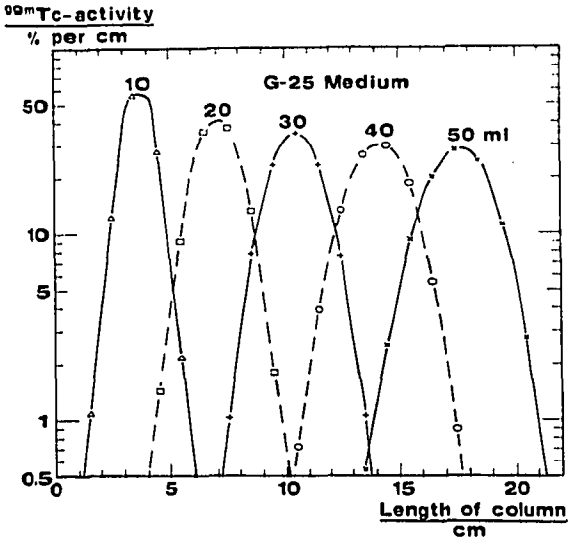

Fig. 2. Scanning profiles of $^{99m}TcO_4^-$ recorded in a column filled with Sephadex G-25 Medium, after elution with different volumes of 0.9% sodium chloride solution.

port of $^{99m}TcO_4^-$ through the column. Some of the normalized profiles for a Sephadex G-25 Medium column are shown in Fig. 2, *i.e.*, the percentage of activity per centimetre of all of the activity in the column after elution with different volumes of 0.9% sodium chloride solution. The distance between the top of the gel bed and the maximum of the recorded activity peak, X, increases linearly with the elution volume, V, as shown in Fig. 3. Fig. 3 also shows the relationship between the distance between the top of the gel bed and the centre of the Blue Dextran 2000 zone for various elution volumes, V. There is no significant difference in the latter relationship between different types of gel in the region studied.

Only very low flow-rates could be obtained for Sephadex G-100, and it was also difficult to use the same column for repeated trials. For this reason, only a few measurements were performed with Sephadex G-100, but the results agree reasonably well with the Sephadex G-75 line in Fig. 3.

The precision in the evaluation of the distances from the top of the gel bed after elution are, on average, ± 0.1 cm for the activity peak and ± 0.5 cm for the centre of the zone of Blue Dextran 2000. There are also other uncertainties due to differences in the packing of the columns and to differences in the application of the sample, which are more difficult to estimate.

The distribution coefficients, K_{av} and K_d, for pertechnetate can be calculated in order to enable a comparison to be made between the parameters recorded in gel chromatography column scanning and those obtained in conventional gel chromatography[12]. K_{av} is related to the distribution of the $^{99m}TcO_4^-$ ions between the mobile phase and the total gel phase by the equation

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} \qquad (1)$$

where

V_e = Elution volume of a component, *i.e.*, the volume of eluent measured from the application of the sample to the elution of the component in maximum concentration.

V_0 = Void volume, *i.e.*, the volume between the gel particles. It is determined as the elution volume of Blue Dextran 2000.

V_t = Total bed volume, composed of the gel matrix volume, V_m, the internal volume inside the particles of gel, V_i, and the void volume, V_0, *i.e.*, $V_t = V_m + V_i + V_0$.

For substances that are neither completely excluded, such as Blue Dextran 2000, nor able to diffuse freely, only a fraction of the inner volume is available for diffusion. This can also be described by the equation

$$V_e = V_0 + K_d \cdot V_i \tag{2}$$

The K_d value of a substance can thus be calculated from the equation

$$K_d = \frac{V_e - V_0}{V_i} \tag{3}$$

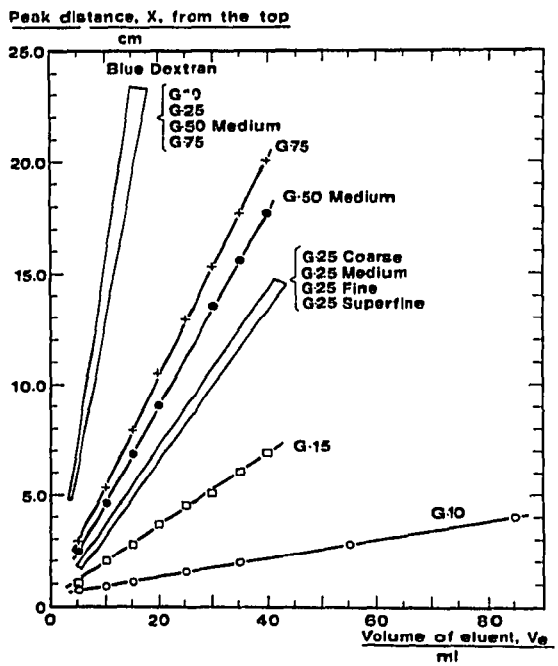

Fig. 3. Distance between the top of the gel bed and the maximum of the recorded activity peak for $^{99m}TcO_4^-$, X, as a function of the elution volume, V, for different types of Sephadex gel. The diagram also shows the distance between the top of the gel bed and the centre of the Blue Dextran 2000 zone as a function of the elution volume.

TABLE II

RELATIONS BETWEEN X AND V, AND ΔX AND $\sqrt{X}$, FOR PERTECHNETATE WITH VARIOUS TYPES OF GEL *(g)*

Sephadex	*Peak distance from top* ($X^{Tc} = a_g^{Tc} \cdot V + b_g^{Tc}$)		*Width at half-maximum* ($\Delta X = k_g^{Tc} \cdot \sqrt{X} + l_g^{Tc}$)	
	a_g^{Tc} *(cm/ml)*	b_g^{Tc} *(cm)*	k_g^{Tc} *($\sqrt{cm}$)*	l_g^{Tc} *(cm)*
G-10	0.041 ± 0.004	0.5 ± 0.1	0.39 ± 0.09	0.1 ± 0.1
G-15	0.16 ± 0.01	0.4 ± 0.2	0.43 ± 0.08	0.2 ± 0.1
G-25, Coarse	0.35 ± 0.02	0.0 ± 0.2	1.2 ± 0.1	−0.4 ± 0.3
G-25, Medium*	0.35 ± 0.02	0.0 ± 0.2	0.94 ± 0.09	−0.4 ± 0.3
G-25, Medium*	0.43 ± 0.01	0.2 ± 0.2	0.56 ± 0.07	0.3 ± 0.2
G-25, Fine	0.35 ± 0.02	0.0 ± 0.2	0.57 ± 0.08	−0.2 ± 0.1
G-25, Superfine	0.35 ± 0.02	0.0 ± 0.2	0.31 ± 0.03	0.0 ± 0.1
G-50, Medium	0.44 ± 0.01	0.3 ± 0.2	0.63 ± 0.08	0.2 ± 0.2
G-75	0.49 ± 0.01	0.5 ± 0.2	0.52 ± 0.08	0.7 ± 0.2

* Different batches of gel.

The transport of the sample through a column can, according to Fig. 3, be described by a linear relationship for each type of gel (g) and each compound (i) (Table II):

$$X^i = a_g^i \cdot V + b_g^i, \tag{4a}$$

where X^i is the peak distance from the top of the gel bed and V is the corresponding volume of eluent. Consider two occasions giving two points on this line:

For the column-length used, X_0, the elution volume for Blue Dextran 2000 can also be determined from Fig. 3. This is the void volume, V_0, of the column. The $^{99m}TcO_4^-$ peak is at X^{Tc} in this column for this elution volume. Thus

$$X^{Tc} = a_g^{Tc} \cdot V_0 + b_g^{Tc} \tag{4b}$$

When the $^{99m}TcO_4^-$ peak is just at X_0, the elution volume is V_e. Thus

$$X_0 = a_g^{Tc} \cdot V_e + b_g^{Tc} \tag{5}$$

Eqns. 1, 4 and 5 give

$$K_{av} = \frac{\left(\frac{X_0 - b_g^{Tc}}{X^{Tc} - b_g^{Tc}}\right) - 1}{\left(\frac{V_t}{V_0}\right) - 1} \tag{6}$$

The total bed-volume, V_t, is calculated from the dimensions of the column, *i.e.*, $V_t = X_0 \cdot \pi \cdot (1.5/2)^2$, which is about 40 ml.

The corresponding expression for the calculation of K_d is obtained from eqns. 3–5:

$$K_d = \frac{\left(\frac{X_0 - b_g^{Tc}}{X^{Tc} - b_g^{Tc}}\right) - 1}{\left(\frac{V_t}{V_0}\right)} \quad (7)$$

The V_t/V_0 values (Table III) were calculated both from statements of the manufacturer[12] and by experimental estimations[13].

The values of K_{av} and K_d for $^{99m}TcO_4^-$ in different types of gel are calculated from eqns. 6 and 7, and are given in Table III.

The difference between the distribution coefficients K_d and K_{av} becomes smaller the more heavily swollen is the type of gel that is being considered. High values of the distribution coefficients imply interaction of the sample with the gel phase[12,14].

TABLE III

DISTRIBUTION COEFFICIENTS, K_d AND K_{av}, FOR PERTECHNETATE IN DIFFERENT GELS

The HETP and the maximum number of peaks that can be separated (n) on the columns are also given.

Sephadex	V_t/V_0 *used in the calculations*[12,13]	K_d	K_{av}	*HETP (mm)*	n
G-10	1.15 ± 0.12	29 ± 7	21 ± 3	0.3 ± 0.1	6 ± 1
G-15	1.36 ± 0.14	6 ± 1	5.0 ± 0.7	0.4 ± 0.2	5.6 ± 0.9
G-25, Coarse	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	2.2 ± 0.6	3.1 ± 0.3
G-25, Medium*	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	1.3 ± 0.3	3.6 ± 0.3
G-25, Medium*	1.19 ± 0.12	1.9 ± 0.6	1.5 ± 0.1	0.7 ± 0.2	4.6 ± 0.5
G-25, Fine	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	0.5 ± 0.1	5.3 ± 0.7
G-25, Superfine	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	0.18 ± 0.04	8.2 ± 0.9
G-50, Medium	1.25 ± 0.13	1.8 ± 0.5	1.46 ± 0.08	0.8 ± 0.2	4.3 ± 0.5
G-75	1.40 ± 0.14	1.3 ± 0.4	1.20 ± 0.06	0.8 ± 0.2	4.3 ± 0.5

* Different batches of gel.

Resolution

The factors that determine the possibility of resolving adjacent peaks in the scanning profile of the column are the distance between the peaks and the sharpness of the peaks. The way in which the sharpness of a $^{99m}TcO_4^-$ peak depends on the traversed column length for different types of gel can be seen in Figs. 4 and 5. The width at half-maximum of the recorded activity peak, ΔX, is used to measure the sharpness of the peak. The measuring-points for Sephadex G-100 lie in the vicinity of the Sephadex G-75 line in Fig. 4. The precision in the evaluation of ΔX is, on average, ±0.05 cm. Even columns with the same type of gel show small differences in the slopes and the positions of the lines. The ΔX value thus seems to depend on

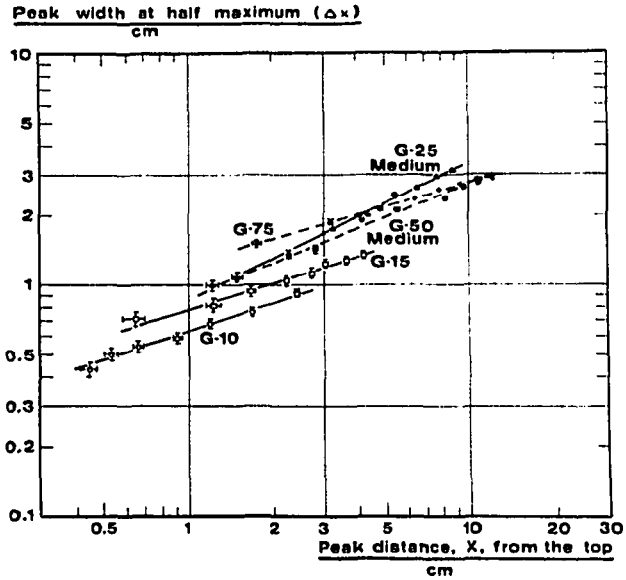

Fig. 4. Width at half-maximum of the recorded $^{99m}TcO_4^-$ activity peak, ΔX, for different types of Sephadex gel as a function of the traversed column length, X.

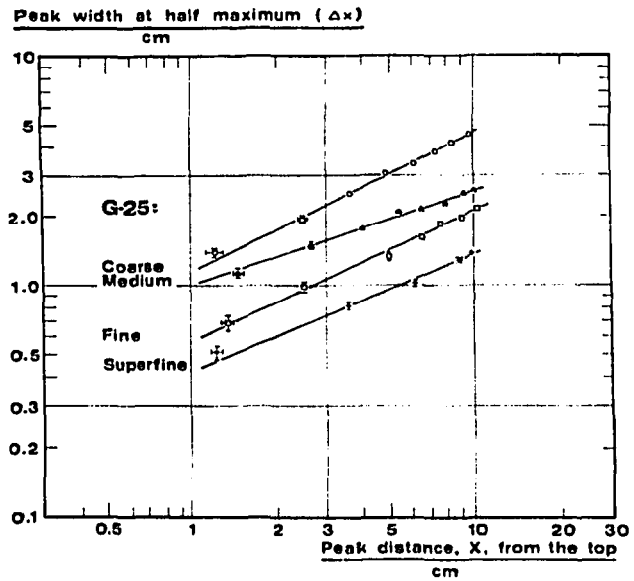

Fig. 5. Width at half-maximum of the recorded $^{99m}TcO_4^-$ activity peak, ΔX, for different grades of particle size of Sephadex G-25 gel as a function of the traversed column length, X.

small differences in the packing of the column and small variations in the application of the sample.

The broadening of peaks in gel chromatography is measured in terms of the height equivalent to a theoretical plate (HETP)[12,13,15]. If the elution-curve has the

shape of a Gaussian error curve, the HETP can be represented by the following equation[15]:

$$\text{HETP} = \frac{l_{\text{gel}}}{\text{N}} = \frac{l_{\text{gel}}}{\left(\frac{V_e}{\sigma_{V_e}}\right)^2} \tag{8}$$

where l_{gel} ($= X_0$) is the length of the column, N is the number of theoretical plates of the column and σ_{V_e} is the standard deviation of the peak in the elution volume curve. Thus the width at half-maximum, ΔV_e, of the peak in the elution curve is given by

$$\Delta V_e = 2\sigma_{V_e} \sqrt{2 \ln 2} \tag{9}$$

The HETP can be expressed in terms of the GCS distances. For the column length, X_0, eqn. 5 gives

$$\Delta X_0 = a_g^{\text{Tc}} \cdot \Delta V_e \tag{10}$$

From eqns. 5, 8, 9 and 10 the HETP can be calculated as

$$\text{HETP} = \frac{1}{8 \ln 2} \cdot \frac{X_0}{\left(\frac{X_0 - b_g^{\text{Tc}}}{\Delta X_0}\right)^2} \tag{11}$$

The average value of the slopes of the lines is 0.5, in both Fig. 4 and Fig. 5, *i.e.* the peak width at half-maximum, increases approximately proportional to the square root of the traversed length of the column. This relationship (Table II) can also be expressed by the equation

$$\Delta X = k_g^{\text{Tc}} \sqrt{X} + l_g^{\text{Tc}} \tag{12a}$$

and thus for the column length X_0:

$$\Delta X_0 = k_g^{\text{Tc}} \sqrt{X_0} + l_g^{\text{Tc}} \tag{12b}$$

From eqns. 11 and 12

$$\text{HETP} = \frac{X_0}{8 \ln 2} \cdot \left(\frac{k_g^{\text{Tc}} \cdot \sqrt{X_0} + l_g^{\text{Tc}}}{X_0 - b_g^{\text{Tc}}}\right)^2 \tag{13}$$

The peak capacity, n, which is the maximum number of peaks that can be separated on a given column, can also be used as a measure of the resolution of the system[16,17]. According to Giddings[16]

$$n = 1 + 0.2 \sqrt{N} \tag{14}$$

where N is the number of theoretical plates. Equations (8) and (14) give

$$n = 1 + 0.2 \sqrt{\frac{X_0}{\text{HETP}}} \tag{15}$$

The values of HETP and n for $^{99m}TcO_4^-$ in different types of gel are calculated from eqns. 13 and 15, and are given in Table III.

It is possible to compare the resolutions of different gel columns by comparing HETP or n values for a test substance. The resolution is evidently correlated to the size of the gel particles (Tables I and III). The smallest dry particle diameter gives the best resolution for types of gel that have the same degree of swelling. For types of gel with the same dry particle diameter, the least degree of swelling gives the best resolution.

Error analysis

The results, which are summarized in Tables II and III, show how a good correlation between GCS parameters and the parameters of conventional gel chromatography can be obtained. The largest contribution to the errors (about 95% confidence limits) in K_d and K_{av} are due to the uncertainties of the slopes, partly for the Blue Dextran 2000 line, and partly for the $^{99m}TcO_4^-$ lines in Fig. 3. In addition, the applied uncertainties in the V_t/V_0 ratios used give rise to about a 10–15% error in K_d. The largest contributions to the errors in HETP and n are due to the uncertainties in the linear relationship between ΔX and $\sqrt{X}$ (Table II). The accuracy of the determined parameters can consequently be increased by using larger column lengths, and by using a more exact start-and-stop procedure for the elution of the column. The greatest improvement in the determination of K_d and K_{av} will, however, probably be obtained by a higher precision in measuring the void volume, V_0. Variations in the results when different columns with the same type of gel but from different batches of gel were used, were studied only for Sephadex G-25 Medium. This type of variation is not included in the errors given in Tables II and III.

CONCLUSION

This investigation has shown that it is possible to correlate the characteristics and elution parameters which affect conventional gel chromatography to the GCS method. A column diameter of approximately 15 mm was found to be optimal for the purposes of GCS. A diameter of less than 10 mm gave poor resolution, and a diameter of more than 20 mm necessitated the use of extremely large amounts of gel. Measurements with different column lengths showed that the best length for a Sephadex G-25 bed is 300 mm for an elution volume of 15 ml.

The column must be packed carefully, and the application of the sample must be reproducible in order to avoid affecting the resolution or the traversed length of the activity. The pH of the eluent can influence the scanning profile owing to variations in the absorption and the interaction properties of the column. The correlation between, on the one hand, the increased amount of eluent and, on the other hand, broadening of peaks and increased distance between peaks, was demonstrated in this investigation with $^{99m}TcO_4^-$ samples. Variation in the flow-rate of the eluent affects

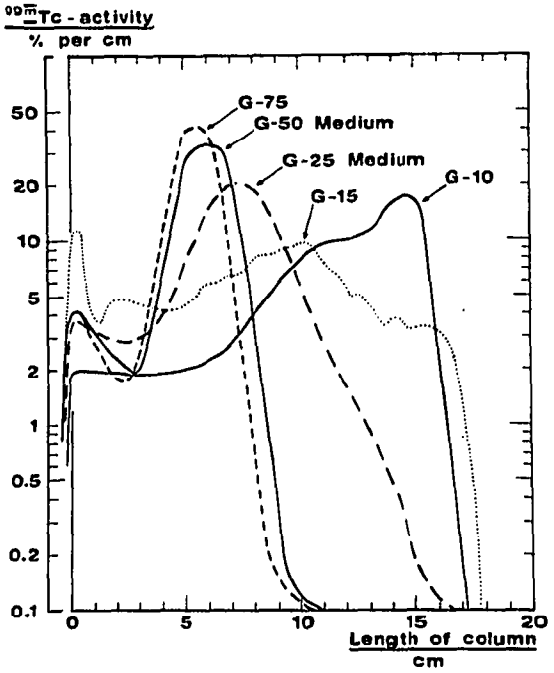

Fig. 6. GCS profiles for ^{99m}Tc–iron ascorbate complex with different types of gel and an elution volume of 10 ml of 0.9% sodium chloride solution.

resolution (especially for large molecules) and possibly even has a small effect upon the traversed length of the activity. A low flow-rate gives the best resolution. The purpose of the measurements defines the range of variation of the flow-rate which can be accepted. One thus uses a high flow-rate (up to 5 ml/min) for routine purposes.

The type and the particle size of the gel define the scanning profile and the possible resolution. The molecular-weight range is important when choosing the type of gel to be used. When using the method for a ^{99m}Tc-labelled complex, the molecular weight is often under 5000. The scanning profiles for a ^{99m}Tc–iron ascorbate complex (molecular weight about 300–400) prepared according to Persson and Strand[10] are shown in Fig. 6. The performance of the scanning profile is similar for Sephadex G-25, G-50 and G-75, but differs from that of Sephadex G-15 and G-10. Sephadex G-25 Medium is to be preferred because of its easy use, and it shows no ageing effect. Sephadex G-10 and G-25 Medium have therefore been the most common types of gel used in the GCS method[18].

The results of various measurements with Sephadex G-25 Medium columns (diameter 15 mm, elution volume 15.0 ml) on ^{99m}Tc-labelled compounds and Blue Dextran 2000 are shown in Fig. 7. The correlation of the distance between the top of the gel bed and the maximum of the recorded activity peak, X, and the molecular weight of the compound, M, are given by the equation

$$X \approx (11 \log_{10} M) - 19 \qquad (16)$$

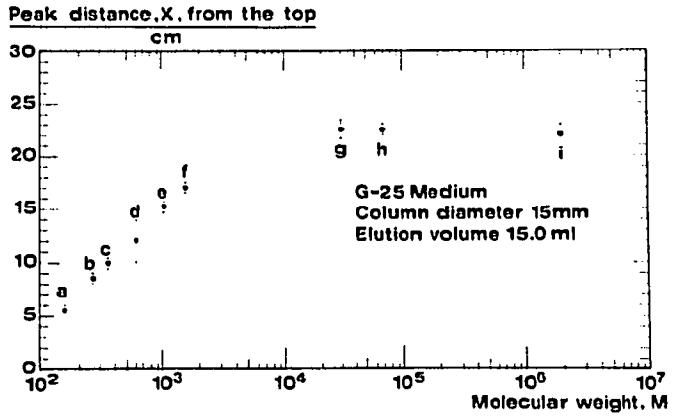

Fig. 7. Relationship of the distance between the top of the gel bed (Sephadex G-25 Medium) and the maximum of the recorded activity peak, X, to the molecular weight of different ^{99m}Tc-labelled compounds, M, and Blue Dextran 2000. Compounds: a = pertechnetate; b = ^{99m}Tc–hydrazine complex[18]; c = ^{99m}Tc-ascorbate[11]; d = ^{99m}Tc–DTPA (diethylene triamine pentaacetate) complex[11]; e, f = ^{99m}Tc–polyphosphate[11]; g = ^{99m}Tc–streptokinase[18]; h = ^{99m}Tc–albumin[11]; i = blue Dextran 2000[1].

This relationship can also be used to estimate the molecular weight of the labelled compound. It can, however, be expected that X is also dependent on the size, the chemical structure, charges, affinity for the gel, etc.

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REFERENCES

1 *Sephadex Gel Filtration in Theory and Practice*, Pharmacia Fine Chemicals, Uppsala, Sweden, 1973.
2 P. Richards and H. L. Atkins, *Jap. Nucl. Med.*, 7 (1968) 165.
3 R. B. R. Persson and K. Lidén, *Int. J. Appl. Radiat. Isot.*, 20 (1969) 241.
4 W. C. Eckelman, G. Meinken and P. Richards, *J. Nucl. Med.*, 12 (1971) 596.
5 W. C. Eckelman and P. Richards, *J. Nucl. Med.*, 13 (1972) 202.
6 W. C. Eckelman, G. Meinken and P. Richards, *J. Nucl. Med.*, 13 (1972) 577.
7 P. E. Valk, C. A. Dilts and J. McRae, *J. Nucl. Med.*, 14 (1973) 235.
8 M. W. Billinghurst, *J. Nucl. Med.*, 14 (1973) 793.
9 W. Hauser, H. L. Atkins and P. Richards, *Radiology*, 101 (1971) 637.
10 R. B. R. Persson and S. E. Strand, in *Radiopharmaceuticals and Labelled Compounds*, I.A.E.A., Vienna, 1973, p. 169.
11 R. B. R. Persson, in G. Subramanian (Editor), *Proceedings of International Symposium on Radiopharmaceuticals*, Society of Nuclear Medicine, New York, 1974.
12 H. Determann, *Gel Chromatography*, Springer-Verlag, Berlin, 1969.
13 H.-J. Zeitler and E. Stadler, *J. Chromatogr.*, 74 (1972) 59.
14 B. Gelotte, *J. Chromatogr.*, 3 (1960) 330.
15 P. Flodin, *J. Chromatogr.*, 5 (1961) 103; P. Flodin (Editor), *Dextran Gels and Their Applications in Gel Filtration*, Pharmacia, Uppsala, Sweden, 1962, pp. 39–41.
16 J. C. Giddings, *Anal. Chem.*, 39 (1967) 1027.
17 N. Yoza, *J. Chromatogr.*, 86 (1973) 325.
18 R. B. R. Persson, to be published.