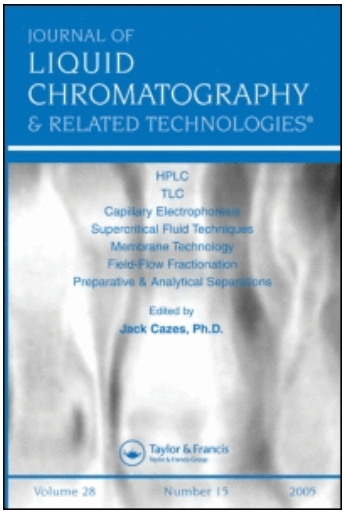


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# Gel Chromatography Column Scanning (GCS) Method of Choice for Quality Control of $^{99m}$Tc-Plasmin Preparations

Lennart Darte[a]; R. Bertil[a]; R. Persson[a]
[a] Radiation Physics Department, Lund, Sweden

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# GEL CHROMATOGRAPHY COLUMN SCANNING (GCS) METHOD OF CHOICE FOR QUALITY CONTROL OF $^{99m}$Tc-PLASMIN PREPARATIONS

Lennart Darte and R. Bertil R. Persson
Radiation Physics Department
Lasarettet, S-221 85 Lund, Sweden



## ABSTRACT

Gel chromatography column scanning (GCS) is useful for testing compounds labelled with gamma-emitting radionuclides. The elution volume used in this technique is so small that no components of the sample are eluted from the column. In the radioactivity distribution of the sealed column various species are recorded in characteristic zones of the column. Plasmin labelled with $^{99m}$Tc is used for scintigraphic detection of deep vein thrombosis. The quality of the $^{99m}$Tc-plasmin preparation has been tested by various methods. The GCS method employing small columns offers a very fast testing procedure and adequate resolution for quality control in routine radiopharmaceutical work.

## INTRODUCTION

Gel filtration has been used extensively for studying the chemical state of labelled compounds and radiopharmaceuticals. Molecules are fractionated on a gel bed in order of decreasing molecular weight and size if no interaction takes place. Some species, however, are firmly bound to the gel and are not eluated from the gel column.

Gel chromatography column scanning, GCS, is useful for samples of gamma-emitting radionuclides. Only a small volume of the developing agent is used so that none of the radioactivity zones are eluted. The distribution of the radioactivity in the column is measured with a scanner or a scintillation camera. The GCS method is much less time consuming and is technically less difficult to use than conventional gel chromatography with fraction collection. The GCS method involves less experimental dis-

turbances, better molecular resolution and better counting statistics than the more commonly used methods of paper chromatography and thin layer chromatography.

The type of gel to be used depends on what molecular weights the labelled compounds have. The gel most widely used and the one suitable for most radiopharmaceuticals is Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden). For separation of high molecular weight compounds and colloids, Sepharose gel is useful.

The GCS method has been related to conventional gel filtration in previous papers (1,2). The method has been used extensively for studying labelling in various $^{99m}$Tc-radiopharmaceuticals. These include the kidney agents Tc-ascorbic acid (1,3-5), Tc-DTPA (3,4,6), Tc-EDTA (7,8) and Tc-unithiol(10); the lung agent Tc-labelled macroaggregates (9 ); the blood agents Tc-HSA (11), Tc-streptokinase (11-13) and Tc-plasmin (11,14-17); the liver agents Tc-Sn-sulphur colloid (2) and Tc-Sb-sulphur colloid (2); the heart and skeletal agents Tc-pyrophosphate (11,18), Tc labelled ethane hydroxy diphosphonate (11) and Tc labelled methylene diphosphonate (11). Fig 1 shows positions of the radioactive zones in the scanning profile of some $^{99m}$Tc-radiopharmaceuticals analysed by columns of Sephadex G-25 Fine gel eluated with 10 ml 0.9 % NaCl-solution.

$^{99m}$Tc-plasmin preparations have been used in a few series of patient investigations of deep vein thrombosis with very promising results (14,19). In these investigations the GCS method was used to analyze the radiochemical purity of each preparation prior to administration. The research work for the development of the $^{99m}$Tc-plasmin preparation (15,16) was performed using columns of 1.5 cm inner diameter and 30 cm in length filled with Sephadex G-25 Fine gel. The testing procedure with such a column takes about half an hour which is often too long for routine clinical work. For more than one year we have used two smaller columns in testing many types of radiopharmaceuticals. In this paper some quality control methods for $^{99m}$Tc-plasmin are compared in order to derive the procedure best suited for routine clinical work.

## MATERIALS AND METHODS

### GCS method

The method requires only minor special equipment and is very simple to perform (column specifications in table 1):

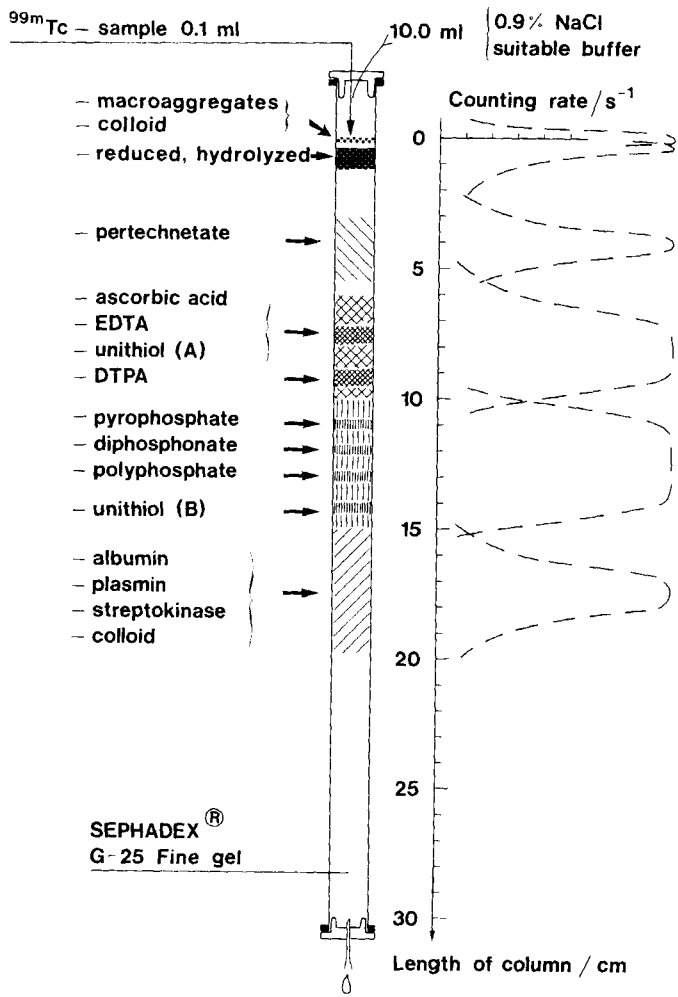


FIGURE 1

Principle of gel chromatography column scanning (GCS). The figure shows positions of some $^{99m}$Tc-radiopharmaceuticals on a Sephadex G-25 Fine column after eluting with 10 ml 0.9 % NaCl solution.

1. Preparing the gel slurry. Add Sephadex G-25 Fine into a large volume of distilled water with stirring and allow to swell overnight or boil for 2-3 hours.

2. Packing the column. Use a tube of inert transparent material of 1-2 cm internal diameter and 10-30 cm height. Insert a stopper with

TABLE 1

Comparison of time lengths for testing procedures

| GCS | Type of gel | Dimensions Length x Diameter (cm) | Sample volume (ml) | Elution volume of 0.9 % NaCl (ml) | Elution time (min) | Recording time: Scanner (min) | Scintillation camera (min) |
|---|---|---|---|---|---|---|---|
| | Sephadex G-25 Fine | ca 30x1.5 | 0.10 | 10.0 | 15 | 15 | <2 |
| | Sephadex G-25 Medium | 12.5x0.9 | 0.05 | 1.8 | <2 | 10 | <2 |
| | Sephadex G-25 Medium | 5.5x1.5 | 0.05 | 1.8 | <2 | 5 | <2 |
| TLC | Adsorbent | Dimensions Length x Width (cm) | Sample volume (µl) | Developing solvent | Developing time (min) | Recording time: Scanner (min) | Well type scint. counter (min) |
| | Silica gel plate | 20x5 | 5 | MEK | ca 45 | 10 | |
| | Silica gel strip | 8x1 | 2 | MEK | <2 | | <4 |
| | Silica gel plate | 20x5 | 5 | 0.9 % NaCl pH 2 | ca 40 | 10 | |
| | Silica gel strip | 8x1 | 2 | 0.9 % NaCl pH 2 | <2 | | <4 |



a needle and a wet glass wool plug (or polyethylene filter) into the bottom. Pour the gel slurry into the column, allow to settle, and fill again until a solid gel column of the desired height is obtained. Mount a second wet glass wool plug at the top of the gel bed and maintain it perfectly horizontal. The column is then carefully washed with a solution of 0.9 % NaCl. A prepared column can be used many times.

3. A testing procedure.

a) Initial state resulting in column saturation is obtained with 0.9 % NaCl/HCl solution having the same pH as the sample to be analyzed.

b) The sample ($\lesssim$ 0.1 ml) is applied to the top of the column and allowed to soak into the top glass wool until the solution is no longer visible. The elution is performed immediately afterwards with the same eluent as used for achieving the initial state. The elution volume is chosen to exclude radioactive components

from being eluted from the column and to ensure that the interesting radioactivity distribution spans the entire column length.

c) When elution is complete, the radioactivity distribution in the column is studied either by scanning with a 1-mm slit-collimated NaI(Tl) detector (e.g. Fig 2) or by measuring the whole column with a scintillation camera (e.g. Fig 3). The fraction of each labelled component of the sample is calculated as the net number of counts recorded in the zone in question normalised to the total number of counts recorded over the entire distribution.

TLC methods

Conventional methods of thin layer chromatography for radiopharmaceutical work were used. Determination of the fraction of free $^{99m}$Tc-pertechnetate was carried out using silica gel developed in me-



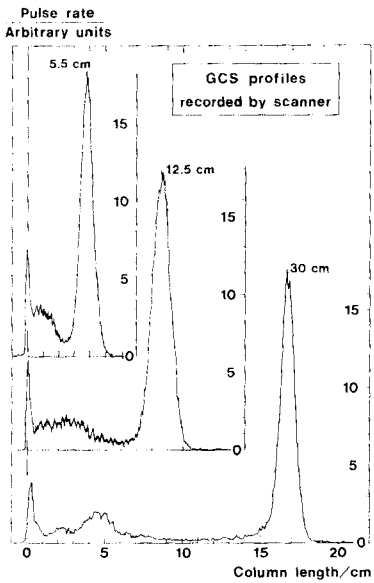


FIGURE 2

GCS profiles from various types of columns after testing a $^{99m}$Tc-plasmin preparation using 0.9 % NaCl eluent of pH value 2. The profiles have been recorded by a scanner.

I. Sephadex G-25 Medium, 5.5x1.5 cm, 1.8 ml.

II. Sephadex G-25 Medium, 12.5x0.9 cm, 1.8 ml.

III. Sephadex G-25 Fine, 30x1.5 cm, 10 ml.

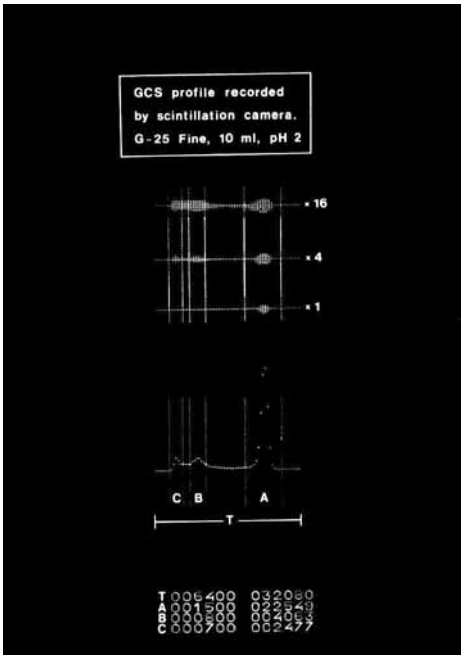


FIGURE 3

GCS profile observed from a Sephadex G-25 Fine 30x1.5 cm column for a $^{99m}$Tc-plasmin preparation using 10 ml 0.9 % NaCl eluent of pH value 2. The brightness, profile and digital displays shown were recorded by a scintillation camera.



thyl ethyl ketone, partly with 20 x 5 cm plate and partly with 8 x 1 cm strip. Determination of the unbound, reduced $^{99m}$Tc fraction was carried out with the same type of plate and strip but developed in 0.9 % NaCl at the pH value 2.1. After development the plates were scanned with the slit-collimated detector, and the strips were cut into two pieces and counted in a well type scintillation detector.

Labelling method

The method of labelling plasmin with $^{99m}$Tc was first studied in detail in our Department (15,16). NOVO Industry A/S, Denmark afterwards prepared kits according to our specifications. The preparation of $^{99m}$Tc-plasmin from these kits is performed as follows:

1. Adjust the pH value by the addition of 0.2-0.3 ml 0.1 M HCl.
2. Add $^{99m}$Tc-pertechnetate (5-50 mCi) to a final volume of 3.5 ml.

The preparation which has pH value 2 is ready for use in patient studies after 45 minutes and is stable for more than 30 hours. Approximately 0.1-1 ml preparation(0.5 mCi)is generally used per patient.

## RESULTS AND DISCUSSION

The labelling yield and the radiochemical purity of the $^{99m}$Tc-plasmin preparation were analysed by various methods. The time length of the testing procedure is indicated in Table 1. The developing time for a TLC plate is approximately three times the elution time of a 30 cm GCS column. The recording times are approximately the same. The small TLC strips and the small GCS columns offer very rapid procedures for quality control.

The results of quality control can be seen in Table 2 and Figures 2-3. In testing a radiopharmaceutical with TLC two TLC systems are generally necessary to determine $^{99m}$Tc-pertechnetate and unbound, reduced $^{99m}$Tc fractions. For $^{99m}$Tc-plasmin preparations the saline sys-



TABLE 2

Comparison of quality control results for a $^{99m}$Tc-plasmin preparation

| Method of quality control | Fractions recorded in various zones $^{99m}$Tc-plasmin | $^{99m}$Tc-red.-hydr. | $^{99m}TcO_4^-$ |
|---|---|---|---|
| GCS, 30 cm column<br>scanner<br>scintillation camera | 14-21 cm zone<br>66 %<br>70 % | 0-2 cm zone<br>7 %<br>8 % | 2-6 cm zone<br>14 %<br>13 % |
| GCS, 12.5 cm column | 6.5-11 cm zone<br>72 % | 0-1 cm zone<br>7 % | 1-4 cm zone<br>15 % |
| GCS, 5.5 cm column | 2.5-5.5 cm zone<br>72 % | 0-0.5 cm zone<br>$\lesssim$11 % | 0.5-2.0 cm zone<br>$\lesssim$15 % |
| TLC, SG + MEK<br><br>20x5 cm plate<br>8x1 cm strip | $R_F \simeq 0$ | $R_F \simeq 0$ | $R_F \simeq 1.0$<br><br>13 %<br>17 % |
| TLC, SG + 0.9% NaCl<br><br>20x5 cm plate<br>8x1 cm strip | $R_F \simeq 0$<br><br>95 %<br>93 % | $R_F \simeq 0$ | $R_F \simeq 1.0$ |

tem, which has been used successfully for many other radiopharmaceuticals, can not be used to measure unbound, reduced $^{99m}$Tc, probably due to absorption of $^{99m}$Tc-plasmin at the application point. On the other hand, the methyl ethyl ketone system is useful in determination of the $^{99m}$Tc-pertechnetate fraction. Earlier tests have shown poor reproducibility for the small strips in routine testing of $^{99m}$Tc-plasmin preparations. In the GCS technique only one testing system is enough. $^{99m}$Tc-reduced-hydrolyzed, $^{99m}$Tc-pertechnetate and other $^{99m}$Tc-labelled impurity in addition to $^{99m}$Tc-plasmin are separated into various zones on the column (Figure 4). Since the $^{99m}$Tc-plasmin preparation is rather unstable in 0.9% NaCl of neutral pH value (15) the elution must be performed with pH value 2 to avoid serious errors (Table 3, Figure 4).



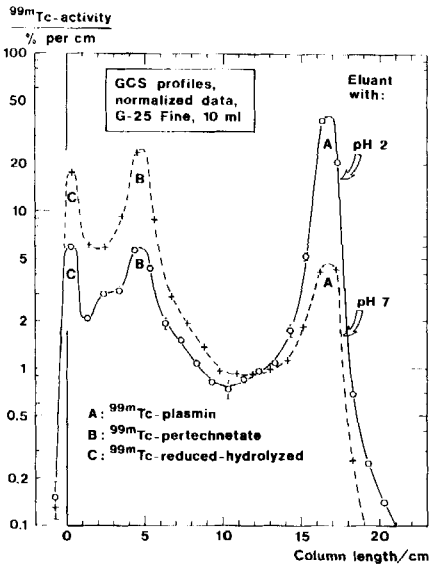


FIGURE 4

GCS profiles of Sephadex G-25 Fine 30x1.5 cm columns for a $^{99m}$Tc-plasmin preparation using 10 ml 0.9 % NaCl eluent with pH values 2 and 7. The distributions are shown as the percentages of the total numbers of counts/cm of the columns.

TABLE 3

The importance of correct pH value for the eluant during the testing procedure

| GCS column | Fraction recorded in the $^{99m}$Tc-plasmin zone at pH value 2 | pH value 7 |
|---|---|---|
| 30 cm (Figure 4) | 66 % | 12 % |
| 12.5 cm | 72 % | 27 % |
| 5.5 cm | 72 % | 19 % |

Resolutions observed with various GCS columns are compared in Figure 2. In the 5.5 cm column $^{99m}$Tc-reduced-hydrolyzed and $^{99m}$Tc-pertechnetate components cannot always be resolved. It is believed that the resolution of the 12.5 cm column is adequate for quality control in routine radiopharmaceutical work. Because of the availability of scintillation cameras in nuclear medicine departments their use for recording columns offers a very rapid testing procedure with nearly the same resolution as obtained with a slit-collimated scanning detector (Table 2, Figure 3). The whole testing procedure with a 12.5 cm column including the attainment of the initial state thus takes less than 15 minutes.

The results from the present experiment agree with observations from more than 100 tests of $^{99m}$Tc-plasmin preparations using small columns run in parallel with some other quality control method. The small columns have proved to be very reliable with good reproducibility of the results. They can also be used for quality control of most other kinds of radiopharmaceuticals labelled with $^{99m}$Tc or other gamma-emitting radionuclides.



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