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A Comparative Investigation of Various Sephadex G-Types and Bio-Gel P-10 in the Gel Chromatographic Analysis of ^{99m}Tc-Labeled 1-Hydroxy Ethylidene-1,1 Diphosphonate

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Introduction

THE properties of Sephadex G-25 as a gel chromatographic medium for the analysis of ^{99m}Tc-chelates as well as the influence of the composition of the eluent have been studied by a number of investigators.[1–5] It has been demonstrated that artefacts can be introduced by the chromatographic system. Two sources of error can be distinguished: decomposition of the ^{99m}Tc-complex by competitive interaction of the gel matrix with the technetium ion, and dissociation of the complex by dilution of the ligand solution. In order to avoid the first effect the use of Bio-Gel instead of Sephadex has been proposed.[4] Dilution can be avoided by the use of eluents with the same concentration of reagents (exclusive technetium) as used in the preparation of the complex.[3]

In this paper we present the results of a study of the matrix effect of various Sephadex G-gels in the analysis of ^{99m}Tc-diphosphonate. Results obtained with the alternative gel, Bio-Gel P-10, are also reported.

Experimental

Chemicals

^{99m}Tc-pertechnetate was eluted from a 16–24 days old ^{99m}Tc-generator (New England Nuclear) with physiological saline. A 1.55×10^{-2} M diphosphonate solution was prepared by dissolving 1-hydroxy ethylidene-1,1 disodium diphosphonate (Henkel A.G., West Germany) in water. A solution of 2.65×10^{-3} M Sn Cl_2 (Baker analysed) was prepared daily. All solutions were purged by nitrogen. The investigated Sephadex gels were G-15, G-75 and G-150 with particle diameters 40–120 μm and G-25, medium (Pharmacia Fine Chemicals). Bio-Gel P-10, 200–400 mesh, and Blue Dextran 3000 were obtained from Bio-Rad Laboratories. Other chemicals were commercial reagent grade materials.

Gel chromatographic equipment

The dimensions of the gel bed of the Sephadex and Bio-Gel columns were 1.4 × 18 cm. The flow rate of the eluent was adjusted in such a way that the time interval during which the ^{99m}Tc-diphosphonate is in contact with the gel was always 3 h except in some separate experiments at very low ligand concentrations. In the latter case the contact time was about 1 h.

Procedure

The ^{99m}Tc-diphosphonate was prepared by stannous chloride reduction of $^{99m}TcO_4^-$ in the presence of diphosphonate. One ml of the 1.55×10^{-2} M diphosphonate solution was mixed with 1 ml of the 2.65×10^{-3} M Sn Cl_2 solution. To this mixture 2.5 ml of the $^{99m}TcO_4^-$ eluate was added, and the pH was raised to about 7.3 with a NaOH solution. The reaction mixture was allowed to equilibrate for 15 min.

The ^{99m}Tc-complex was analysed on Sephadex G-15, G-25, G-75, G-150 and Bio-Gel P-10 columns using an eluent with the same concentration of reagents (exclusive technetium) as used in the preparation. The whole experiment, inclusive the preparation, was performed in triplicate.

Results

The results of the experiments on the matrix effect are presented in Table 1.

When saline instead of the complexing material was used in the analysis with a Sephadex G-75 column the percentage of labeling was found to decrease from 92.2 ± 1.8 to 73.4 ± 3.0.

Discussion and Conclusions

The water regain value represents the amount of water taken up by 1 g dry gel during swelling.[6,7] Hence it is a suitable parameter to represent the (inverse) concentration of hydroxyl groups of the Sephadex gels, which are supposed to interact with technetium complexes.[2,3] Table 1 shows a clear correlation between the water regain value (and thus the concentration of hydroxyl groups) and the observed labeling percentage for Sephadex G-25, G-75 and G-150: the labeling percentage increases with decreasing concentration of hydroxyl groups. The labeling percentage obtained with Sephadex G-15 is higher than that obtained with Sephadex G-25. The cause of this phenomenon is probably that the interior surface of the gel beads is inaccessible to the complex. (The ^{99m}Tc-diphosphonate is eluted in the void volume on this gel.)

From these results it might be concluded that if Sephadex gels are to be used for the analysis of ^{99m}Tc-complexes, a Sephadex type with a high water regain value is the best choice. However, the time needed for the analysis is then relatively long owing to the low attainable flow rate with such soft gels.[6] The increased time interval during which the complex is in contact with the gel matrix leads to an enhanced decomposition.[5] To verify this effect the ^{99m}Tc-diphosphonate was also analysed at a higher flow rate (i.e. a contact time of the complex of about 20 min) on Sephadex G-25. The labeling percentage was found to be 96.0 ± 1.8%, i.e. an increase of about 10%

TABLE 1. Percentage of label recovered as a function of the water regain value of the gel

Gel	Water regain value g H_2O/g dry gel	Percentage of label recovered, with its 90% probability interval
Sephadex G-15	1.5	90.3 ± 1.6
Sephadex G-25	2.5	86.3 ± 1.2
Sephadex G-75	7.5	92.5 ± 0.9
Sephadex G-150	15.0	97.7 ± 0.8
Bio-Gel P-10	4.5	99.3 ± 1.1

(see Table 1). Therefore a Sephadex type with a low gel porosity, allowing a high flow rate, is a better choice.

In Table 1 it can also be seen that the gel matrix of Bio-Gel P-10 does not compete for the technetium ion of the complex. This is in agreement with the results of BILLINGHURST and PALSER[4] and JOHANNSEN[5] who observed little or no decomposition of various technetium complexes when Bio-Gel was used as gel medium. Thus the use of Bio-Gel instead of Sephadex must be recommended in order to obtain a minimal matrix effect.

The experiment with the physiological saline as eluent clearly demonstrates that the ^{99m}Tc-diphosphonate decomposes by dilution during gel chromatography on Sephadex. This phenomenon was already demonstrated by STEIGMAN *et al.*[3] for ^{99m}Tc-gluconate. A number of authors[4,5] used saline as eluent in combination with Bio-Gel as gel medium for the analysis of various ^{99m}Tc-chelates. From their results it may be concluded that decomposition of the investigated complexes (among which ^{99m}Tc-diphosphonate[5]) by the saline eluent is of minor importance in the case of Bio-Gel. However, in an attempt to analyse the ^{99m}Tc-diphosphonate prepared at very low diphosphonate and Sn(II) concentrations (5×10^{-5} M and 10^{-5} M, respectively), we found that the complex decomposed completely on Bio-Gel when saline was used as eluent. When the complex was eluted with the same concentration of reagents (exclusive technetium) as used in the preparation, practically all of the technetium activity appeared in the complex fraction. It follows, that only when the ^{99m}Tc-diphosphonate is prepared at higher reagent concentrations, as is usual in medical practice, then saline can be used in conjunction with a Bio-Gel column.[5] In this case the concentration of the excess of complexing agent probably remains large enough during chromatography to prevent decomposition by dissociation.

In conclusion, it is recommended to use always an eluent consisting of the complexing reagents of the same concentration as used in the preparation of the complex.

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