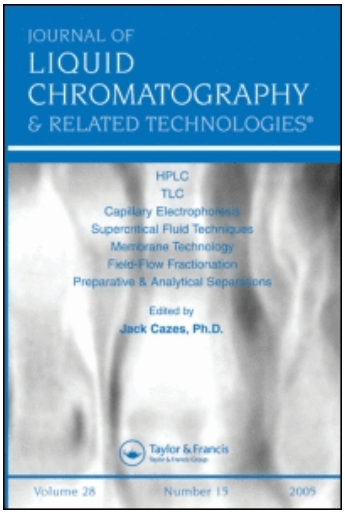

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Nowotnik, D. P. and Riley, A. L. Ml(1992) 'The Determination of Charge of Anionic Tc-99m Radiopharmaceuticals', Journal of Liquid Chromatography & Related Technologies, 15: 12, 2165 — 2174
To link to this Article: DOI: 10.1080/10826079208016332
URL: http://dx.doi.org/10.1080/10826079208016332

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(12), 2165-2174 (1992)

THE DETERMINATION OF CHARGE OF ANIONIC Tc-99m RADIOPHARMACEUTICALS

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ABSTRACT

The absolute charges on several anionic Tc-99m complexes were determined by an HPLC method using a SAX column and sodium sulphate as eluent. Retention times were measured over a range of $[SO_4^{2-}]$, and a non-linear regression technique was employed to determine complex charge. The system was validated using pertechnetate (-1) and the ligand TDG (-2). The charge of Tc-99m DTPA was shown to be -2. The major Tc-99m EHIDA complex has a charge of -1, while the main transient complex of this ligand has a -2 charge. Both of the complexes of Tc-99m TDG have a charge of -2. The method provided near integer values in all cases.

INTRODUCTION

The development of new technetium-99m radiopharmaceuticals for diagnostic imaging has, in recent years, generally followed the systematic approach used for pharmaceuticals; i.e. there has been some attempt relate the biodistribution of new technetium complexes with physical properties (1). As the overall charge of a technetium complex is an important determinant of its biodistribution, knowledge of the charge is

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essential to the development of meaningful structure-distribution relationships (SDRs). However, because of the extremely low chemical concentration of most Tc-99m preparations ($<10^{-6}$M), the charge of a Tc-99m complex is usually inferred from knowledge of the structure of its Tc-99 counterpart (2). This method is not always reliable, as evidence that complexes are the same at both concentration levels generally relies solely upon a comparison of HPLC data (2). Ideally, the charge on Tc-99m complexes should be determined directly from measurements of these complexes.

Owunwanne et al (3) described a method for the determination of charge of anionic Tc-99m complexes by equilibration with anion-exchange resin. Use of conventional liquid chromatography with an anion-exchange stationary phase proved to be a modification of this method which provided greater convenience, particularly in the safe-handling for radioactive materials (4). A further improvement was achieved by Wilson and Pinkerton through the use of HPLC (5). The method described in this paper is an adaptation of the latter HPLC method.

EXPERIMENTAL

Theory

Owanwanne et al (3) developed a method for the determination of charge of a radiopharmaceutical which involved the equilibrium of that radiopharmaceutical between a known weight of an ion exchange resin and an electrolyte containing a competing anion/cation. This method is basically the same as the competitive binding assays used in certain immunoassays. The equilibrium constant (K) in this process can be defined as:

$$K = \frac{(M_r^m)^a \times (A_s^a)^m}{(M_s^m)^a \times (A_r^a)^m} \qquad \text{---------- (1)}$$

Where: M is the activity of a radiopharmaceutical of charge m

A is the activity of a counter ion of charge a

and subscripts r and s denote resin and solution phase, respectively.

This relationship can also be applied to an ion-exchange chromatography system. For an HPLC system, the measured parameter for any substrate will be its retention time (R_t). The factors which contribute to the retention time of a charged substrate using an ion-exchange column and isocratic elution can be broken down as follows:

$$R_t = R_i + R_c + R_o \qquad \text{---------- (2)}$$

where R_i is the retention time attributable to ion-exchange mechanisms, R_c is the retention of the compound due to other mechanisms, and R_o is the retention time of a non-retained compound (void-volume marker). R_i will be governed by the ion-exchange equilibrium between resin-bound substrate and substrate in solution, i.e.

$$R_i = K_i \times \frac{M_r^m}{M_s^m} \qquad \text{---------- (3)}$$

Where K_i is a constant for the column.

Substituting equation 3 into 1 gives:

$$(R_i)^a \times \frac{(A_s^a)^m}{(A_r^a)^m} = \text{constant} \qquad \text{---------- (4)}$$

As the concentration of substrate is very much less than counter ion, the resin-bound concentration of the counter ion is essentially constant over a wide range of solution concentrations. Therefore:

$$(R_i)^a \times (A_s^a)^m = \text{constant} \qquad \text{---------- (5)}$$

Taking logs and rearranging gives:

$$\log (R_i) = \text{constant} - \frac{m}{a} \log (A_s^a) \qquad \text{---------- (6)}$$

Hence, the charge could be determined if it were possible to plot log (R_i) against log (A_s^a) by measuring R_i over a range of counter ion concentrations. However, the observed retention time has to be corrected for R_c and R_o. In determinations involving only a change in A_s^a, R_c and R_o should remain constant. Therefore, the relationship between log (R_t) and log (A_s^a) will be curvilinear, but it will convert to a linear relationship by subtracting a value from all observed R_ts which equals R_c and R_o. This is the basis for our determination of m. Linear regression of log (R_t - x) vs log (A_s^a) with candidate values of x were performed to determine a value of x which provides a maximum value of R, the correlation coefficient. These calculations were performed using a programmed Excel spreadsheet, which determined R and m values with small increments (+0.001) in the value of x until R reaches its maximum value (a similar technique was recently described for the determination of column void volume from retention data on a homologous series of alkylbenzenes (6)). At this point, $x = R_c + R_o$, and the slope of the line equals $\frac{m}{a}$.

An alternative method for the estimation of charge can be derived from equation 6, by taking anti-logs, and rearranging, viz:

$$(R_t = (R_c + R_o) + \frac{\text{constant}}{(A_s^a)^{m/a}} \qquad \text{---------- (7)}$$

Thus, from equation 7, a plot of R_t vs $\frac{1}{(A_S^a)^{m/a}}$ will be linear, with the intersection at the y-axis being equal to $R_C + R_O$. Again, it is not possible to derive a linear plot from equation 7 without knowing the value for one of the variables; in this case, m. A similar non-linear technique was employed to solve equation 7 as was used for equation 6. For equation 7, the value for m is incremented (+0.001) in plots of R_t vs $\frac{1}{(A_S^a)^{m/a}}$ until the maximum value for the linear correlation coefficient R is obtained.

Materials

The ligand thiodiglycolic acid (TDG) was purchased from Aldrich Chemical Co., and twice recrystallised from water prior to use. ^{99m}Tc (as pertechnetate) was obtained from a commercial $^{99}Mo/^{99m}Tc$ generator (Amersham International plc). ^{99m}Tc complexes, ^{99m}Tc-DTPA and ^{99m}Tc-EHIDA were prepared from lyophilized kits (Amersham International) according to the manufacturer's instructions. The ^{99m}Tc complexes of TDG were made as described previously (7).

Chromatographic Procedure

The HPLC system used in this study comprised of an Altex 410 pump, a Rheodyne injector, an HPLC Technology Ltd. SAX column (250 x 4.6 mm), and standard UV and radiometric detection systems. Aqueous sodium sulphate was employed as eluent. The column was prepared by eluting sequentially with methanol, water, then with 50 mM aqueous sodium sulphate for 8 hours at 1 mL/min to ensure equilibration with sulphate.

For a determination of R_t, the column was equilibrated with the desired concentration of aqueous sodium sulphate for 30 minutes at 1 mL/min prior to

conducting the determination. Retention times of test substrates were determined at a flow of 1 mL/min, in duplicate. Tc-99m complexes were detected with a standard radiometric detector. The ligand thiodiglycolic acid was detected by UV at 240 nm.

RESULTS AND DISCUSSION

The HPLC method described previously by Wilson and Pinkerton (5) for the determination of the charge of technetium anions was also based on the relationship between retention time and counter ion strength given as equation 6, above. In that study, R_i was determined by subtraction of the column dead-time, R_O, (determined using $^{22}NaCl$ or MeOH) from the observed retention time. Therefore, that method made the assumption that the sole mechanism of retention of the test substances was ion-exchange. This clearly could lead to errors in the determination of charge if other retention mechanisms were present. Therefore, we opted to use a non-linear method for the determination of charge which includes R_O and a term R_C, which accounts for the contribution to overall retention of the solute which does not vary with counter ion strength; i.e. all retention mechanisms other than ion-exchange.

Wilson and Pinkerton's HPLC method (5) employed an AE-Pellionex-SAX precolumn coupled to 140mm column packed with Aminex A-29, with aqueous sodium acetate as eluent. In conjunction with the HPLC Technology Ltd. SAX column, acetate proved to be inadequate as a counter ion, leading to extremely long retention times (unreported data). Sulphate was selected for this study for its greater ionic strength (8) and poor complexing ability. This system provided retention times <30 minutes for all compounds studied, as shown in the following table:

The determination of charge of the standard compounds, pertechnetate (-1) and the free ligand thiodiglycolic acid (-2) gave values which were in good agreement with the known charges of these compounds (Table 2). Russell et al (4) determined the charge

Table 1. Observed retention times of standards and test complexes

Sulphate Concentration mM	Observed retention times (R_t) in minutes						
	TcO_4^-	Tc-DTPA	Tc-EHIDA		TDG	Tc-TDG	
			#1	#2		#1	#2
100	4.32	9.53		5.70	5.6	4.59	5.19
50		13.33		6.16	7.0	5.28	6.34
35		16.62			8.0	5.80	7.16
25		20.25	5.51	7.51	9.75	6.59	8.38
15	6.21	31.60	5.72	9.55	13.4	8.52	11.71
10	7.52		6.05	11.47	17.3	10.89	15.74
7.5			6.61	13.47	21.75	13.17	19.69
5.0	9.37	80.68	6.85	18.21			
3.5							
2.0	13.26						
1.0	18.59						
0.5	24.97						

Table 2. Results from the calculation of charge using equations 6 and 7.

Cmpd	From equation 6				From equation 7		
	charge	Rc +Ro	R	intercept	charge	Rc +Ro	R
TDG	-1.93	4.16	0.9998	2.09	-1.89	4.04	0.9998
Tc-TDG #1	-2.08	3.97	0.9999	1.88	-2.04	3.91	0.9999
Tc-TDG #2	-2.14	4.23	0.9998	2.13	-2.12	4.17	0.9999
TCO4-	-1.13	3.21	0.9992	1.18	-1.07	2.89	0.9995
Tc-DTPA	-2.00	5.79	0.9998	2.58	-1.96	5.35	0.9999
Tc-EHIDA #1	-2.25	5.21	0.9877	1.05	-1.00	4.29	0.9791
Tc-EHIDA #2	-2.21	5.19	0.9989	1.90	-2.01	4.97	0.9997

of renal function radiopharmaceutical, Tc-99m DTPA (9), to be -2 by an LC method. As shown in table 2, the HPLC method confirms that result.

The HIDAs ("Hepatobiliary IminoDiAcetic acids") are a class of ligands (Fig.1.) originally devised by Loberg et al (10) which were found, when complexed to Tc-99m, to possess rapid liver uptake and clearance. This property indicated that these compounds could be useful for hepatic function imaging. A large number of HIDAs were produced, with the aims of minimizing renal clearance, interference from bilirubin, and hepatic

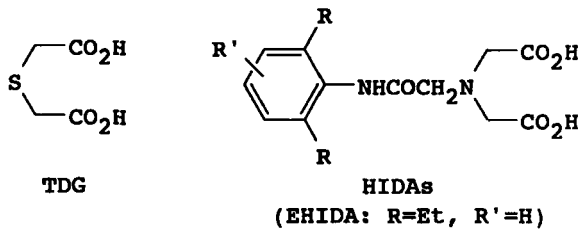

Figure 1. The structures of TDG and HIDA ligands.

transit times (11,12). All HIDAs form a Tc(III) complex, with 2 ligands per metal, leading to an overall charge of -1 (13).

From table 2, it can be seen that determination of the charge of Tc-EHIDA using equations 6 and 7 gave quite different values, with the value of charge from equation 7 being in excellent agreement with that previously reported. This was the only example where use of the two equations gave different values of charge. Presumably the conversion to logarithms of the observed retention times of Tc-EHIDA #1, which showed little variation with counter ion strength, resulted in poor precision in using equation 6 for charge determination.

A transient technetium EHIDA complex is also formed (14) immediately after complex formation, which appears to convert rapidly to the lipophilic anionic (-1) hepatobiliary agent. As shown in table 2, the transient species (#2) possesses a charge of -2. While the structure of this complex has not been identified, the determined charge of -2 for this complex provides some additional evidence of its structure, but still leaves open more than one possibility, e.g. $Tc(III)(EHIDA)_1(OH)_3$, $Tc(III)(EHIDA)_2(OH)$.

Thiodiglycolic acid (TDG) forms two hydrophilic anionic technetium complexes (15,16) which were studied in laboratory animals (7) and in man (17) as renal function agents. The primary complex (#1) formed at room temperature and displayed renal clearance at the glomerular filtration rate (GFR), while the secondary complex (#2, formed in high yield by heating the 'kit' used to form complex #1) cleared at a faster rate.

This indicated that the #2 complex might be clinically useful as a technetium-replacement for Hippuran, a radioiodinated radiopharmaceutical used to assess effective renal plasma flow (ERPF).

Complexes with renal clearance rates greater than GFR generally do so by tubular secretion, a process which requires recognition of certain molecular features (18). The structures of the technetium TDG complexes have not been determined. This study has shown that both complexes possess a charge of -2, which leaves open the possibility that the two technetium TDG complexes are stereoisomers, and only the #2 complex is recognised by the receptors for tubular secretion.

Use of HPLC for the determination of the charge on anionic technetium complexes provides a rapid and reliable method. As mixtures of Tc-99m complexes (from a single ligand) are frequently encountered, this chromatographic method does not require prior separation of mixtures and/or purification of individual complexes. With the test compounds examined in this study, the values of absolute charge obtained were generally close to integer. The results in Table 2 demonstrate that (as R_0 is constant for the system), the contribution of other retention mechanisms (R_c) cannot be ignored when using ion-exchange HPLC for the determination of charge.

ACKNOWLEDGEMENT

The authors wish to thank J. Minshall for technical assistance with this study.

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