AN INVESTIGATION OF THE KINETICS OF FORMAMIDINE SULFINIC ACID (FSA) DEGRADATION IN THE PREPARATION OF TECHNETIUM-99m RADIOPHARMACEUTICALS

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SUMMARY

Technetium-99m glucoheptonate complex was prepared using formamidine sulfinic acid (FSA) as a reducing agent. The fraction of technetium which was complexed to glucoheptonate remained at less than 20 percent for the first 10 minutes. After 20 minutes, the complexed fraction was greater than 95 percent and remained at this level after 24 hours. The lag phase was attributed to the kinetics of the base catalyzed degradation of FSA to urea and bisulfite. The overall rate of FSA degradation was determined and found to be dependent on the concentration of base and the initial concentration of FSA. The rate of production of one of the degradation products, urea, was also measured and found to be dependent on the concentration of base, but independent of the initial FSA concentration. A reaction scheme for the degradation of FSA was proposed and the reaction rate constants for the products of this reaction were determined.

Key Words: Technetium-99m, Formamidine Sulfinic Acid, Reducing Agent

INTRODUCTION

The majority of diagnostic procedures in nuclear medicine utilize the radionuclide technetium-99m which has favourable imaging characteristics. In order to obtain the desired tissue and organ localization it is necessary to complex technetium-99m to carrier molecules. The most stable form of technetium has a valence of +7. To achieve complexation by ligands, reduction of technetium to a valence of +3, +4 or +5 is necessary.

The most commonly used reducing agent for the preparation of technetium-99m radiopharmaceuticals is the stannous ion, usually utilized as the chloride salt. However, stannous chloride is useful only in acidic or neutral media. Recently, other reducing agents, including formamidine sulfinic acid (FSA), have been suggested as alternatives to stannous chloride.(1)

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A major advanatage of FSA is that it is a strong reducing agent in alkaline medium. The use of FSA allows the binding of technetium to ligands which form stable complexes at basic pH (5).

Provokov, et. al.(2) have measured the reduction of FSA in normal sodium hydroxide. The reduction potential was dependent on hydroxide concentration and temperature, reaching a maximum value at 95° C. FSA, in an aqueous solution at 25° C., or lower, underwent a molecular rearrangement. Upon addition of sodium hydroxide, the FSA decomposed to give sodium bisulfite and urea.

Rasinskaya, et. al.(3) reported that the concentration of FSA in neutral solutions at 50° C. the concentration potential was 300 mV in the 50° - 90° C. range. The addition of sodium hydroxide increased the reduction potential, but reduced the stability of FSA. The optimum FSA to sodium hydroxide ratio was found to be 1:5 or 1:6 at an initial FSA concentration of 2.0 - 4.0 grams per litre.

McGill and Tindstrom (4) reported the use of FSA in the reduction of cadmium. They confirmed the presence of dithionite and urea as degradation products of FSA in alkaline medium and proposed a reaction sequence for the degradation of FSA (Figure 1).

FSA has been utilized in our laboratory for the preparation of technetium-99m glucoheptonate (5). This preparation was found to have high radiochemical purity and stability at levels of radioactivity up to 37 GBq and concentration of 1.9 GBq per ml.

In order to utilize FSA for the preparation of other radiopharmaceuticals it is necessary to further examine the chemical properties of this reducing agent.

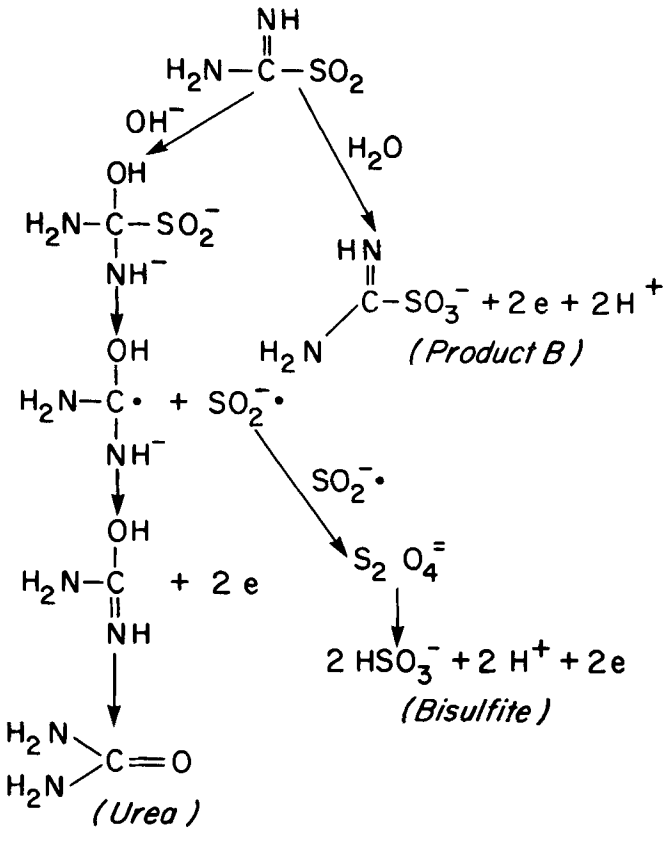


Figure 1
A proposed reaction scheme for the degradation of FSA (modified from McGill and Lindstrom (4).

METHOD

Technetium-99m glucoheptonate was prepared according to the following procedure.

To 1.5 ml calcium glucoheptonate (200 mg/ml) add 18.5 ml of a solution of technetium-99m sodium pertechnetate in 0.9% sodium chloride followed by 0.5 ml of formamidine sulfinic acid (10 mg/ml). The pH is adjusted to 12 using 0.01 N sodium hydroxide. The final volume is approximately 20 ml.

The fraction of technetium-99m which was complexed to glucoheptonate was determined using thin layer chromatography (Gelman S. G. Type) developed in n-butanol.

Ultraviolet Spectrophotometry (Unicam 5000) was used to monitor the disappearance of FSA. The ultraviolet spectra was recorded at ambient temperature using 1.00 cm matched quartz cells with water as the reference material. A neutral FSA solution exhibits an absorbance peak at 270 nm. At this wavelength FSA conforms to Beer's law for concentrations of 0.001 to 0.5 mg/l. If FSA is dissolved in alkaline media a second absorbance peak appears at 316 nm which is the wavelength at which dithionite absorbs.

Urea concentrations were determined using a modification of the method of Berthlot (6). This procedure utilizes the enzyme urease and is specific for urea.

RESULTS AND DISCUSSION

The binding of technetium-99m glucoheptonate in the presence of FSA was monitored by thin layer chromatography (Figure 2). There was a lag phase in the first 10 minutes during which the proportion of total activity bound to glucoheptonate remained at less than 20 percent with the remainder being present as pertechnetate. After 10 minutes the binding increased rapidly and was greater than 95 percent by 20 minutes.

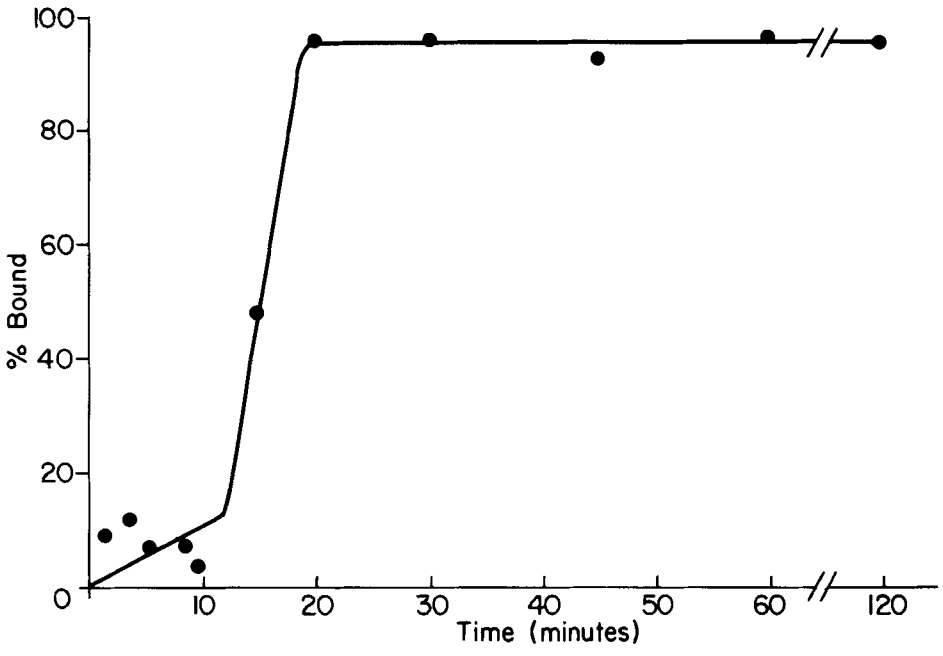
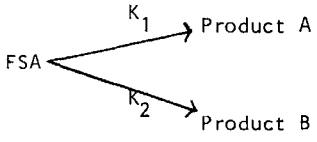


Figure 2
Percentage of technetium-99m bound to glucoheptonate vs time.

The lag phase seen in the complexing of technetium to glucoheptonate may be due to the present of trace amounts of contaminants which are stronger oxidizing agents than technetium and thus are preferentially reduced by the FSA. Another possible explanation is that the reducing species is not FSA itself, but one or more of its degradation products. If this is the case, the technetium will not be reduced until sufficient quantities of this product are produced. In order to investigate this possibility it is necessary to examine the kinetics of the degradation of FSA.

Assuming the following mechanism for the base catalyzed degradation FSA (7):



the rate equation is:

$$(1) \qquad \frac{d[FSA]}{dt} = k_1[FSA] + k_2[FSA] = K[FSA]$$

where $k_1 + k_2 = K$

by integration:

$$(2) \qquad \frac{\ln [FSA]_o}{[FSA]} = Kt$$

$$(3) \qquad [FSA] = [FSA]_o \, e^{-Kt}$$

The rate of formation of product A can be expressed as:

$$(4) \qquad \frac{d[A]}{dt} = k_1 [FSA] = k_1 [FSA]_o e^{-Kt}$$

and the integration:

$$(5) \qquad [A] = [A]_o + \frac{k_1}{K} [FSA]_o \quad (1-e^{-Kt})$$

since $A_o = 0$

$$(6) \qquad [A] = \frac{k_1}{K} [FSA]_o \quad (1-e^{-Kt})$$

and likewise for product B:

$$(7) \qquad [B] = \frac{k_2}{K} [FSA]_o \quad (1-e^{-Kt})$$

From equation (2) a plot of ln FSA_o / FSA vs time will result in a straight line (with a slope which is equal to K.) From equation (6) a plot of urea vs $(1-e^{-Kt})$ will yield a straight line with a slope equal to $\frac{k_1}{K} FSA_o$. Using these two plots, it is possible to calculate K, the rate constant for the disappearance of FSA and k_1, the rate constant for the production of product A.

A 0.05 mg/ml solution of FSA was prepared in 0.01 sodium hydroxide. The change in FSA concentration was monitored using ultraviolet spectrophotometry. A plot of ln FSA_o / FSA vs time resulted in a straight line (Figure 111). The slope of this line, which is equal to K, the overall rate constant, was determined using the Method of Least Squares and was found to be 0.0438 ± 0.002. The correlation coefficient for this line was 0.984.

Six solutions of 0.05 mg/ml of FSA in 0.01 N sodium hydroxide were incubated at room temperature. At 0, 5, 10, 20, 30, and 60 minutes the pH of the solutions

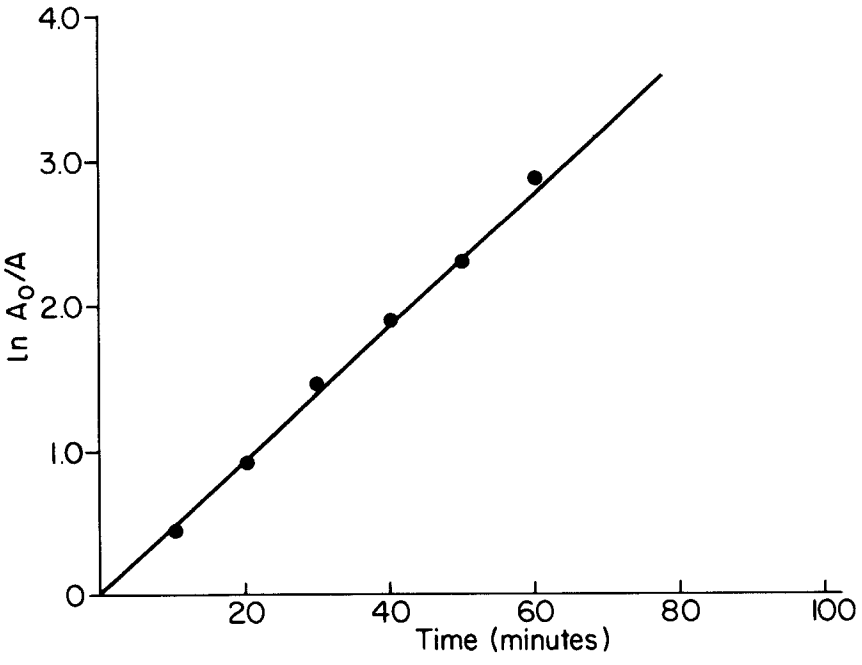


Figure 3
The natural log of the initial absorbance divided by the absorbance at time t vs time (0.5 mg per ml FSA in 0.01 N osdium hydroxide).

were adjusted to pH 5-6 in order to "stop" the production of urea. A plot of urea concentration vs $1-e^{-Kt}$ was obtained and found to be linear. (Figure 1V). The slope of this line, which is equal to $\frac{k_1}{K} FSA_o$, was determined to be 1.2×10^{-3} min $^{-1}$ by the Method of Least Squares. The value k_1 was calculated to be 0.023 min $^{-1}$. The correlation coefficient for this plot was 0.977.

The reaction rate constant k_2 was calculated using the equation $K = k_1 + k_2$ and found to be 0.021 min $^{-1}$. The rate of production of dithionite was not determined since a plot of the change of absorbance at 316 nm vs $1-e^{-Kt}$ does not yield a straight line due to the simultaneous oxidation of dithionite to bisulfite.

The overall reaction rate constant (K) was determined at various concentrations of sodium hydroxide (Table 1). It was found that a hydroxide concentration of less than 0.01 N the relationship of $\ln FSA_o / FSA$ vs time was no longer linear. At these concentrations, the sodium hydroxide is not in great excess and the molar ratio of FSA to sodium hydroxide is greater than 1:10. Under these conditions sodium hydroxide is no longer acting as a catalyst and the reaction rate equations described previously no longer apply.

As the concentration of base was increased from 0.01 N to 0.02 N, the overall reaction rate constant decreased suggesting that the increased amount of sodium hydroxide was inhibiting the reaction.

The rate of production of urea (k_1) was also found to be dependent on the sodium hydroxide concentration. The rate constant, (k_2) which was obtained indirectly using K and k_1, was found to be independent of the concentration of base added.

K, k_1 and k_2 were determined at different initial FSA concentrations. The sodium hydroxide concentration was 0.06 N in all cases. The results in Table 11 show that K, the overall reaction rate, is dependent on the initial FSA concen-

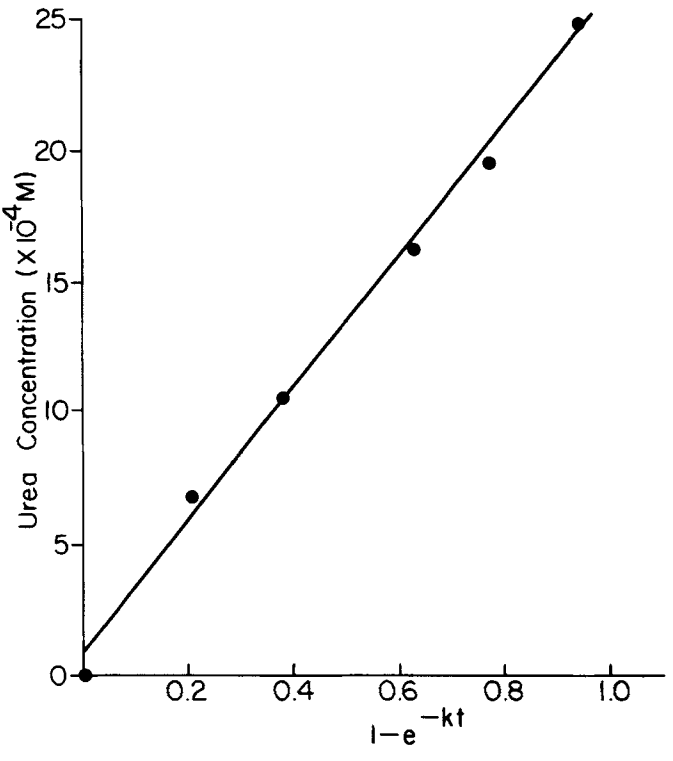


Figure 4
Urea concentration vs $1-e^{-Kt}$ (0.05 mg per ml 0.01 N sodium hydroxide).

TABLE 1 *

Rate Constants for the Degradation of FSA

Initial FSA Concentration ($M \times 10^{-4}$)	K ($min^{-1} \pm SD$)	k_1 ($min^{-1} \pm SD$)	k_2 ($min^{-1} \pm SD$)
9.26	0.046 ± 0.001	0.030 ± 0.004	0.016 ± 0.004
18.52	0.040 ± 0.001	0.032 ± 0.004	0.008 ± 0.004
27.80	0.039 ± 0.003	0.031 ± 0.003	0.008 ± 0.004
37.00	0.036 ± 0.003	0.032 ± 0.005	0.004 ± 0.005

* initial NaOH concentration was 0.06 M in all cases.

TABLE 2 *

Rate Constants for the Degradation of FSA

Initial Sodium Hydroxide Concentration (M)	K (min^{-1} ± SD)	k_1 (min^{-1} ± SD)	k_2 (min^{-1} ± SD)
0.01	0.044 ± 0.004	0.023 ± 0.004	0.021 ± 0.004
0.02	0.055 ± 0.001	---------	---------
0.06	0.046 ± 0.001	0.025 ± 0.002	0.021 ± 0.002
0.10	0.037 ± 0.001	0.016 ± 0.001	0.021 ± 0.002

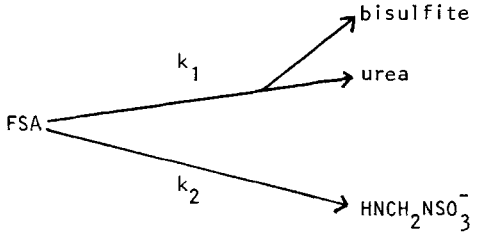
* initial FSA concentration was 4.6×10^{-4} M in all cases.

tration. The rate constant, k_1, determined by monitoring the production of urea, was found to be independent of initial FSA concentration and k_2 was found to be dependent on the initial FSA concentration.

The rate of production of urea is k_1. The reaction pathway which results in the production of urea is dependent on the hydroxide concentration and is not dependent on the initial FSA concentration. If the proposed reaction scheme given in Figure 1 is correct, bisulfite is a co-product of this reaction pathway. The production of bisulfite would also be expected to be dependent on hydroxide concentration and independent of the initial FSA concentration.

The rate constant k_2 was found to be independent of hydroxide concentration and dependent on the initial concentration of FSA. The oxidative rearrangement of FSA in aqueous solutions should be independent of pH but dependent on the initial FSA concentration. Product B then corresponds to the product $HNCH_2NSO_3^-$ in the proposed reaction scheme (Figure 1).

The degradation of FSA, therefore, can be written as:



Our experience with Tc-99m glucoheptonate (FSA) has shown that the reduction of technetium and the formation of the Tc-99m glucoheptonate complex, using FSA as a reducing agent, is not instantaneous in contrast to the formation of Tc-99m glucoheptonate using stannous chloride as a reducing agent. Using stannous chloride, the complex is formed virtually instantaneously in neutral or slightly acidic media. Our investigation of the kinetics of the base

catalized degradation of FSA has shown that the half time of the reaction is in the order of 15 minutes. In order to initiate the reaction an initial pH of 9.5 or greater is required. To effectively utilize FSA as a reducing agent for the preparation of other technetium radiopharmaceuticals, it is necessary to take the kinetics and chemical requirements of the formation of the reducing species into consideration. If this is done, FSA is potentially useful as a reducing agent for other technetium radiopharmaceuticals, especially those utilizing ligands which form stable complexes in alkaline media.

As has been noted in Baldas and Pajer (8), complexation of reduced technetium-99m by FSA or its degradation products is possible. Under the conditions utilized in this work, the complexation of technetium-99m by FSA or its degradation products is low even in the presence of excess quantities of FSA. However, the possibilities of radiochemical impurities occuring, especially if the primary ligand is weak, should always be considered.

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