PREPARATION OF [5-^{125}I]IODOACETAMIDOETHYL AMINONAPHTHALENE-1-SULFONIC ACID IN LIPOSOMES

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SUMMARY

The synthesis of [5-^{125}I]iodoacetamidoethyl aminonaphthalene-1-sulfonic acid ([^{125}I]IAEDANS) was accomplished beginning from sodium [^{125}I]iodide. The product was entrapped within positively charged liposomes after ideal preparatory conditions were determined. The stability of the preparation after exposure to elevated temperatures and light was measured as well as the release rate of [^{125}I]IAEDANS from the liposomes. Liposome-entrapped [^{125}I]IAEDANS is a potential radiopharmaceutical for imaging of infarcted myocardial tissue.

Key Words: liposomes, 5-(iodoacetamidoethyl) aminonaphthalene-1-sulfonic acid, myocardial, Iodine-125

INTRODUCTION

Liposomes bearing a net positive charge on their surface have been reported to accumulate in infarcted myocardial tissue against a flow gradient (1). This finding lead to the suggestion that therapeutic agents could be delivered to infarcted cells by liposomes following coronary artery occlusion. The transport of an impermeable dye, 5-(iodoacetamidoethyl) aminonaphthalene-1-sulfonic acid (IAEDANS) into muscle fibers with positively charged liposomes was accomplished, and it was demonstrated that the dye, after uptake by the fibers, was bound to intracellular actin (2). Furthermore, these liposomes were capable of delivering IAEDANS to infarcted cells in the guinea pig myocardium after experimental coronary ligation (3). Based on this information, it seemed reasonable to employ liposomes for the delivery of radiolabelled IAEDANS as a radiopharmaceutical in the detection of infarcted myocardium. This report describes the preparation of liposome-

0362-4803/83/111265-09$1.00

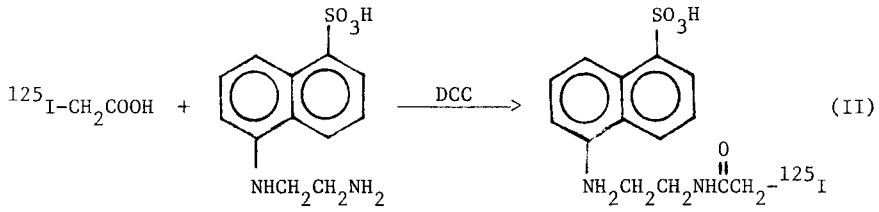
Received August 1, 1983

entrapped [^{125}I]IAEDANS and the stability of this radiopharmaceutical under preparatory conditions. The synthesis of [^{125}I]IAEDANS, which was accomplished by modifying the general procedure of Hudson and Weber (4), was developed so that the synthetic procedure could accommodate the use of sodium [^{123}I]iodide, an isotope of iodine with a shorter half-life than ^{125}I, but possessing superior imaging characteristics for external detection.

RESULTS AND DISCUSSION

The overall scheme for the synthesis of [^{125}I]IAEDANS is illustrated in figure 1. [^{125}I]Iodoacetic acid was prepared by the exchange between iodoacetic acid and sodium [^{125}I]iodide (reaction I). The exchange occurred rapidly at 35° (see figure 2) with up to 99% of the radioiodine being incorporated in the iodoacetic acid within 20 minutes. At 0°, the exchange was only 60% in the same time period. The exchange between sodium [^{125}I]iodide and unlabelled IAEDANS directly

$$Na^{125}I + ICH_2COOH \xrightarrow{CH_3CN} {}^{125}I\text{-}CH_2COOH \qquad (I)$$



(II)

Figure 1. Synthesis of [^{125}I]IAEDANS

was also examined. However, less than 18% of the ^{125}I was found in the IAEDANS after one hour. In addition, these conditions lead to the degradation of the IAEDANS molecule. Therefore, since the radiolabelled IAEDANS could be easily prepared in one hour (according to figure 1), the direct exchange between ^{125}I and IAEDANS was abandoned.

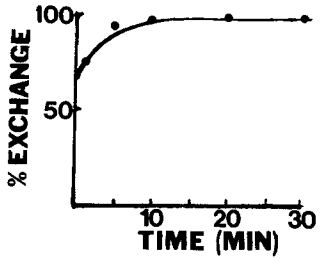


Figure 2. Effect of reaction time on exchange between sodium [^{125}I]iodide and iodoacetic acid.

The condensation of [^{125}I]iodoacetic acid with N-(aminoethyl)-5-naphthylamine-1-sulfonic acid (EDANS) (Reaction II) was catalyzed by dicyclohexylcarbodiimide (DCC) without the esterification step previously described (4). A variety of molar ratios for this reaction were examined, the results of which appear in Table 1. Unfortunately, whenever (^{125}I]iodoacetic acid was the limiting reagent, the products proved extremely difficult to purify. Thus, the reactions outlined in Table 1 led to relatively low specific activity products. We were, however, able to produce enough radioactive material for liposomal encapsulation and subsequent animal studies.

Table 1. Molar Ratios in the Preparation of [^{125}I]IAEDANS

Exp	$^{125}ICH_2COOH$ (mmol)	DCC (mmol)	EDANS (mmol)	Yield (%) Radiochem.	Chem.	Sp. Act. (μCi/mg)
1	0.027	0.054	0.025	30.7	33.2	85
2	0.050	0.075	0.025	28.1	56.2	46
3	0.050	0.100	0.025	29.0	58.0	46
4	0.060	0.090	0.050	36.8	44.2	38
5	0.100	0.150	0.050	37.3	74.6	23
6	2.00	2.20	1.00	40.8	81.5	--

The effect of temperature and light on the stability of [^{125}I]IAEDANS was measured using thin layer chromatographic analysis. The data expressed in Table 2 indicate that [^{125}I]IAEDANS solutions are relatively stable when stored at 0° but degrade much more rapidly at elevated temperatures.

Table 2. Effect of Temperature on the Percent Decomposition of [^{125}I]IAEDANS

	TIME								
Temp	1 hr	2 hr	6 hr	15 hr	1 d	2 d	3 d	5 d	7 d
0°	2.0	-	2.1	3.2	3.5	4.0	6.0	6.4	9.0
25°	3.5	-	9.1	15.3	24.8	40.0	44.7	56.4	67.0
37°	8.7	16.3	27.9	50.6	67.2	87.3	92.2	-	-

Figure 3 demonstrates that [^{125}I]IAEDANS, known to be a light-sensitive compound, degrades quite markedly in a matter of hours when exposed to light. The effect of the presence of a primary amine on [^{125}I]IAEDANS stability was also studied to determine if the stearylamine present in the liposomal membrane would act to degrade the entrapped radiopharmaceutical. Because stearylamine is not soluble in the buffer systems employed, isopropylamine was used instead to determine if it had any effect on [^{125}I]IAEDANS stability. In both the temperature-dependent and light-dependent stability studies, the effect of isopropylamine on [^{125}I]IAEDANS stability was negligible.

Once the desired product was prepared and tested for decomposition under various conditions, the procedures leading to optimum liposomal entrapment were studied. Of the many procedures available for the production of liposomes, the solvent vaporization method of Deamer and Bangham was chosen to encapsulate [^{125}I]IAEDANS in which a lipoidal solution was slowly injected into a warm aqueous solution containing the radiolabelled product (5). The solvent in which the lipids were dissolved evaporated when injected through the aqueous solution resulting in the spontaneous formation of unilamellar liposomes.

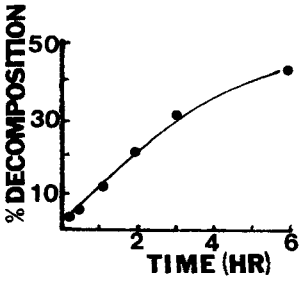


Figure 3. Effect of light exposure on decomposition of [^{125}I]IAEDANS

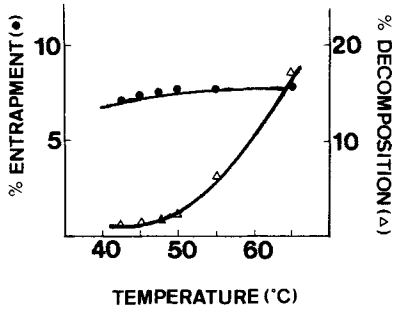


Figure 4. Effect of temperature on the entrapment efficiency and decomposition of liposomal [^{125}I]IAEDANS

For these experiments, n-pentane with a boiling point of 36.1° was used to dissolve the lipids and the percent entrapmen of [^{125}I]IAEDANS in the aqueous phase was determined as a function of temperature. The results appear in figure 4 along with concomitant data on [^{125}I]IAEDANS decomposition during liposome production. These results demonstrate that while the percent entrapment of [^{125}I]IAEDANS does not increase when the temperature of the aqueous phase exceeded 50°C, the decomposition of [^{125}I]IAEDANS rose markedly. Thus, an aqueous phase temperature of 50°C appeared to be optimal for liposome production.

The quantity of lipid material used in the liposome production procedure also had an effect on the percent entrapment of [^{125}I]IAEDANS. Each ampule of lipid solution contained 10 μmole of dipalmitoylphosphatidyl choline (DPC), 0,5 μmole of palmitoyl lysophosphatidyl choline (PLC), and 1.0 μmole of stearylamine (SA) dissolved in a mixture of 0.1 ml methanol and 5 ml n-pentane. The effect of the number of ampules used on the entrapement of [^{125}I]IAEDANS is illustrated in figure 5. Note that as the number of ampules increases, so does

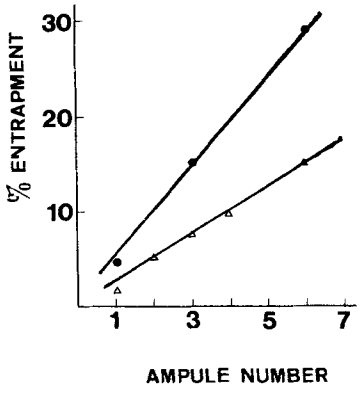


Figure 5. Effect of lipid quantity (number of ampules used) on entrapment efficiency of liposomal [^{125}I]IAEDANS. The aqueous phase consisted of [^{125}I]IAEDANS 13.0 mg (●) or 1.3 mg (△) in 2 ml phosphate buffer. Each ampule consisted of a mixture of DPC (10 μmole), PLC (0.5 μmole), and SA (1.0 μmole) redissolved in 0.1 ml methanol and 5 ml n-pentane.

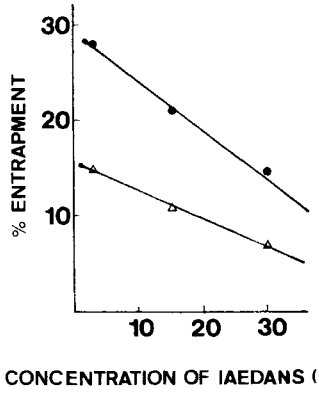


Figure 6. Effect of [^{125}I]IAEDANS concentration on entrapment efficiency using 3 ampules (Δ) or 6 ampules (●) of lipids, (see figure 5 for composition of ampule).

the percent liposomal entrapment of [^{125}I]IAEDANS, regardless of 13.0 mg or 1.3 mg of the radiolabelled product is used (although entrapment is more efficient using the former amount). However, the percent entrapment decreased as the concentration of [^{125}I]IAEDANS in the aqueous phase was increased (figure 6). This held true for the experiments in which either 3 or 6 ampules of lipid solution were used.

After the [^{125}I]IAEDANS was synthesized and entrapped in liposomes, the release of the radiopharmaceutical from the liposomes at 35° (in the dark) was studied. A freshly prepared sample of the liposomal suspension was diluted and divided into several fractions, each being incubated in phosphate buffer (pH 7.4) for various periods of time. After incubation, the samples were applied to a Sephadex G-50 column and the percent [^{125}I]IAEDANS released from the liposomes was calculated by dividing the amount of activity due to free (not trapped) [^{125}I]IAEDANS by the total activity applied to the column. The results of this study appear in figure 7. Note that at t = 0 immediately after the liposomes were diluted and divided, approximately 8-10% release was observed. After storing these liposomes at 0° for 4 days, still only 8-10% release was observed. Apparently, this small amount of release of [^{125}I]IAEDANS from the liposomes is due to dilution since undiluted liposome samples demonstrate no release upon rechromatograph with Sephadex. In addition, undiluted samples stored at 0° for 2 days showed less than 1% release of the radiolabelled compound.

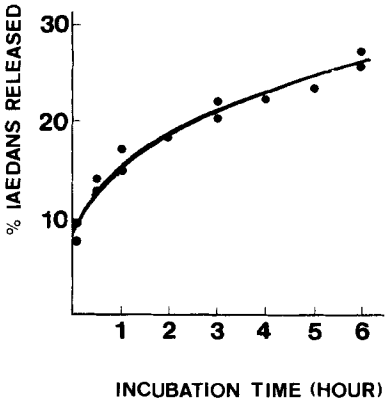


Figure 7. Effect of incubation time on the release of [^{125}I]IAEDANS from liposomes at 35°.

Positively charged liposomes containing [^{125}I]IAEDANS are currently being evaluated in our laboratories as a myocardial scanning agent for the detection and evaluation of infarcted myocardium in an animal model. The procedures described here can be applied to the synthesis of IAEDANS with an I-23 label for future imaging studies.

METHODS

Reagents. Sodium [^{125}I]iodide (high conc.) in 0.1 N NaOH was purchased from New England Nuclear. Iodoacetic acid (Aldrich)was purified by recrystallization from ethyl ether and petroleum ether. Acetonitrile (MCB, spectrophotometric grade), 1,5-Edans (Aldrich), DL-α-dipalmitoyl phosphatidylcholine (Sigma), L-α-palmitoyl lysophosphatidylcholine (Sigma), stearylamine (Sigma), dicyclohexylcarbodiimide (Aldrich), Sephadex G-50 particle size 20-80 μ (Sigma) and all other chemicals were reagent grade. Chloroform, n-pentane and methanol were distilled before use.

All radioactive samples were assayed with a Packard Model 1208 Auto-Gamma Scintillation Spectrometer or a Victoreen Model 888 dose calibrator.

[^{125}I]Iodoacetic Acid. Iodoacetic acid (2.3 mg, 0.012 μmole was dissolved in 0.05 ml of acetonitrile after which 0.01 ml of 0.1N NaOH containing 1.0 mCi of sodium [^{125}I]iodide was added. The mixture was allowed to run at 35° with stirring for 20 minutes in the dark. The progress of the reaction was monitored by thin layer chromatography(silica gel; chloroform: acetone:ethanol = 2.2:1).

[^{125}I]IAEDANS. These reactions were carried entirely in the dark. The [^{125}I] iodoacetic acid obtained from the above reaction was cooled to 5° after which a solution of dicyclohexylcarbodiimide (DCC, 144.5 mg, 0.67 mmole) in 0.35 ml CH_3CN was added with stirring over a two minute period. The resulting N,N´ - dicyclohexylurea precipitate appeared after a short time. A solution of N - (aminoethyl)-5-naphthylamine-1-sulfonic acid (1,5-Edans, 29.3 mg, 0.11 mmole) in 0.11 ml of CH_3CN and 0.11 ml of 1.0 N NaOH was added to the mixture and in a typical experiment was followed after five minutes by the addition of 0.02 ml of 1.0 N NaOH. The mixture was stirred for another ten minutes after which the urea was filtered off. To the filtrate was added 2.0 ml of CH_3CN and 0.03 ml of 2.0 N HI. The mixture was held at 5° for ten minutes after which the precipitate was filtered and washed with a small volume of CH_3CN. The precipitate was dissolved in a mixture of 0.45 ml of 1/15 M monopotassium phosphate solution and 9.0 ml of CH_3CN. The small amount of undissolved urea was filtered off and to the filtrate was added 0.03 ml of 2.0 N HI. The mixture was held at 5° for ten minutes after which the precipitate was filtered, washed with CH_3CN and dried.

In a reaction using larger amounts of non-radioactive materials, the product was subjected to analysis: $C_{14}H_{15}N_2O_4SI$, Calc: C, 38.72; H, 3.48; N, 6.45; S, 7.38; I, 29.23. Found: C, 38.76; H, 3.49; N. 6.37; S, 7.43; I, 29.38. In addition, the infrared spectrum of the product was identical to authentic material.

Stability of [^{125}I]IAEDANS. One mg of [^{125}I]IAEDANS was dissolved in 0.2 ml of phosphate buffer (pH 7.4) and kept at a specified temperature and time in the dark. Decomposition of the product was monitored by thin layer chromatography as previously described followed by a radiochromatograph of the developed plate. In the same manner, the [^{125}I]IAEDANS solution was monitored for stability upon exposue to light at 25°.

Preparation of liposomes. Cationic unilamellar liposomes composed of dipalmitoyl phosphatidylcholine, palmitoyl lysophosphatidylcholine and stearylamine in a molar ratio of 10 : 0.5 : 1 were prepared according to the method of Deamer and Bangham (5). These reagents were dissolved in $CHCl_3$, sealed in glass ampules under N_2, and stored under refrigeration. Thin layer chromatography was used to check lipid purity before

use. Prior to liposome preparation, the $CHCl_3$, was removed under reduced pressure and the lipids were redissolved in a mixture of n-pentane (5 ml) and methanol (0.1 ml). The lipid solution was injected via a 22 gauge needle through an aqueous [^{125}I]IAEDANS solution preheated to 50° in a Liebig condenser with an internal diameter of 1 cm at a rate of 0.25 ml/min. The resulting liposomal suspension was passed through a 1.0 μm polycarbonate filter (Nuclepore Corp.) to remove large aggregates. The liposome-entrapped [^{125}I]IAEDANS was separated from the free compound by gel filtration chromatography using a Sephadex G-50 column (6 g, 1.3 x 33 cm) which was eluted with phosphate buffer (pH 7.4) with a flow rate of 1.7 ml/min. The effluent was collected as 2 ml fractions and assayed with a gamma scintillation counter. The fractions containing the liposomes were collected and centrifuged at 10,000 X G for 15 minutes to increase the concentration of the liposome suspension for animal experiments.

In vitro release studies. Samples of liposome-entrapped IAEDANS suspended in phosphate buffer (ph 7.4) were incubated at 37° for varying lengths of time. The preparation was then passed through a Sephadex G-50 column and eluted with the buffer solution. The release of [^{125}I]IAEDANS from the liposomes was calculated from counting the effluent fractions.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the financial support of the American Heart Association - Kentucky Affiliate and the Isabell Kircher Fund.

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