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Labelling Study of Sodium Citrate With Tc^{99m} and The Bological Behaviour Of The Labelled Complex as Kidney Functioal Agent

Khalida, S. M.*

** Department of Chemistry, College of Science, University of Wassit*

Abstract:

The objective of this study is to optimize the parameters for the development of a procedure for preparing Sn-sodium citrate label with Tc^{99m}, of providing a stable complex after mixing with Tc^{99m}-pertechnetate and to find the potential of technetium- 99m (Tc^{99m}) citrate complex as a new kidney functional agent. The labeling conditions of Tc^{99m} citrate complex using stannous chloride dehydrate as a reducing agent for pertechnetate have been described. The gel chromatography scanning (GCS) method reveals that the labeling efficiency of Tc^{99m}-citrate complex is promoted by raising the pH of the preparation to (pH=4) using 1N NaOH. The optimal amounts of the reactants in the preparation to obtain labeled and stable complex with high kidney uptake were found to be (1mg) sodium citrate and (100 µg) $SnCl_2.2H2O$. The results show that high labeling yield (≥95%) for the labeled complex. The data of bio distribution experiments in the laboratory animals (mice), clear high radioactivity accumulation of labeled complex in kidney at 5 minutes post injection. The results of bio distribution of labeled complex have shown a good biological behavior with low radioactivity accumulation in the non-target organs (blood, liver and other organs).

الخلاصة :

أن الهدف من هذه الدراسة هو التوصل الى طريقة لتعليم مادة سترات الصوديوم بالتكنيشيوم 99م والحصول على معقد مستقر ومعرفة كفائته لاستخدامه في تشخيص وظيفة الكليتين. تم دراسة ظروف تعليم مادة سترات الصوديوم بنظير التكنيشيوم 99م باستخدام كلوريد القصديروز كعامل مختزل للبرتكنتات. ان كفاءة المعقد المعلم الناتج تم تقديرها بطريقة كروموتوغرافيا الهلام وكروموتوغرافيا الورقة وقد دلت النتائج على ان اعلى ناتج تعليم واستقرارية للمعقد اضافة الى اعلى نسبة تجمع للمعقد المشع في الكليتين قد تم التوصل اليها عندما استخدم 1 ملغرام من سترات الصوديوم و100 مايكروغرام من كلوريد القصديروز وعند الاس الهيدروجيني 4. أظهرت النتائج الى ان اعلى ناتج تعليم للمعقد يساوي او اكثر من 95% أما التجارب التي اجريت على الحيوانات المختبرية (الفئران) قد اوضحت الى ان اعلى تجمع للمعقد المعلم في الكليتين يكون بعد 5 دقائق من زرق الحيوان المختبري.

لقد أظهرت نتائج التوزيع البايولوجي بان المعقد المعلم يمتلك سلوك بايولوجي جيد مع قلة تجمعه في الاعضاء غيرالمستهدفة كالدم والكبد واعضاء اخرى.

Introduction:

Radionuclides have been used to study renal function clinically since the introduction of the radioisotope renogram by Tapeline and Kimball [1]. This use is mainly directed at the excretory functions of the kidney that involve glomerular filtration and tubular secretion. Glomerular filtration is a process that be quantified by the measurement of the rate of renal clearance of a particular substance in the blood. The ideal indicator must meet the following criteria: i) free filtration through the glomerular capillary membranes; ii) no secretion or absorption by the renal tubules; iii) not metabolize by the kidney; iv) no binding to plasma proteins; v) nontoxic and inert; and vi) measurable with high accuracy. Radiochemical purity is an additional requirement when radio labeled agents are used [2].Tc99m is the most commonly used medical isotope. It is estimated that every year 30 million patients undergo Tc99m procedures around the world [3]. Additional benefits of the medical isotope Tc99m are the low

dose required for medical imaging and its short half-life of only six hours, which ensures that it does not remain in the body very long. Moreover, the historically low cost of Tc-99m has made it attractive [4]. Tc99m is obtained from the decay of its parent isotope molybdenum-99 (Mo99). It was discovered in 1937 [5]. Different Tc99m compounds can be used to verify the function of different organs. Tc99m glucoheptonate (Tc99m GH) was developed by Agha N. H., Al-Hilli, H.A.Karim [6] and 2, 3-dimercapto succinic acid labeled with Tc99m was developed by Lin, et.al of the medi-physics crop [7]. Tc99m diethylentriamine pentad acetic acid (Tc99m DTPA) was developed by Havser W., Atkins, and H.L. [8]. Tc99m sodium citrate as a renal imaging agent was introduced in 1973 [9].The new preparation is not toxic; in addition it can be rapidly and simply prepared by a single step method. The biological behavior (organ distribution and toxicity of Tc99m Sn-Citrate) have been studied in mice.This study investigates the labeling of sodium citrate with Tc99m using SnCl2.2H2O as a reducing agent. This preparation appears to be specifically useful as renal function agent.

Materials and Methods:

Sodium citrate was obtained from (BDH chemicals Ltd. Poole-England), stannous chloride dehydrates was obtained from (Riedel De Haen AG- Germany), and what man 3 mm paper was obtained from (What man Ltd. Kent-England)

Preparation of Sn- Citrate complex $[Sn_3(Citrate)_2]$:

Tc^{99m} citrate was prepared by mixing 1.0 ml. (1mg/ml) of sodium citrate solution with different amounts of $SnCl_2.2H_2O$ (50, 100, and 200) at pH (3.2, 4.0, and 5.0) into 10 ml. vial. To the content of the vial, 5-10 mCi (2-4) ml. of Tc^{99m} elute (Radiochemical center, Amersham) was added and after 30 min. of equilibration a sample was analyzed.

Analysis method:

Radiochemical purity analysis was performed in order to identify quantitatively the various chemical species of Tc^{99m} that may be present in the final preparation. The analysis of Tc^{99m} citrate was performed as followed:

A. Gel chromatography column scanning technique (GCS) using sephadex G25F.
B. Paper chromatography (P.C.) using 3 mm papers as stationary phase with acetone as mobile phase.

Gel chromatography column scanning technique:

Gel filtration using sephadex (AB Pharmacia fine chemicals) was used for detection Tc^{99m}-fractions in the preparation. A column with 13mm was filled with swollen sephadex G25 fine. The sample to be analyzed was applied on the top of the gel in suitable volume (0.1-0.2ml) then developed with 15 ml saline (0.9% NaCl solution). The column was sealed and scanned with a slit (1mm) collimated NaT (II) crystal.

Paper chromatography:

The method employed whattman 3mm paper as the stationary phase with acetone as mobile phase. This method was used to separate the free pertechnetate from the Tc^{99m} complex. Tc^{99m} sodium citrate remains at the application point with R_f value of (0.0), while free pertechnetate migrates with solvent fronts with an R_f (1.0).

Animal studies:

Organ distribution was evaluated in 18 Swiss albino mice. 50µCi samples of Tc^{99m} sodium citrate were given intravenously via the tail vein of the mice. The organs of interest were collected after killing the animals at different time intervals post injection and counted using a well-type scintillation counter (Automatic Gamma sample changer LB. MAG 312 Berthold-Italy).

Results:

Samples of 0.1-0.2 ml. were analyzed with the GCS method at different

time intervals (15, 30, and 180 minutes) and the results obtained from the profiles are summarized in table (1). The data show that the formation rate of Tc99m citrate is very fast, regardless of the amount of SnCl2.2H2O, followed by subsequent degradation of the complex with the time. The labeling yield of Tc99m citrate complex as a function of pH = 4.0 was studied using 1N sodium hydroxide solution for pH adjustment. The results of variation in the pH which are summarized in table (2) show that the optimal pH for obtaining a high yield of Tc99m citrate complex was found to be 4.0.

The data presented in table (3) have shown that a high labeling yield could be obtained when; 1mg of Na-citrate is used in the preparation. The formation rate and the stability data of Tc99m-complex have shown in table (4).

Bio kinetic Investigation:

Progress in nuclear medicine has resulted from both, a new imaging technology and the availability of high specificity radiopharmaceutical therefor, it is worthy to consider the target organ-to-background ratio in the evaluation of a new radiopharmaceuticals. Basically the in vivo studies in the intact animal are an absolute necessity prior to the commencement of human investigation. Therefore, to start the in vivo studies of Tc99m citrate preparation, the organ distribution as a function of sacrifice time has been performed in mice. The data summarized in table (5) have shown the Organ distribution data of Tc99m citrate in mice.

$SnCl_2.2H_2O$ µg	Tc^{99m} citrate com. After 15 min.	Tc^{99m} citrate com. After 30 min.	Tc^{99m} citrate com. After 180 min.
50	**98.7**	**97.5**	**96.5**
100	99.7	99.5	99.0
200	99.0	98.0	98.0

Table-1: Effect of $SnCl_2.2H_2O$ weight on labeling yield of Tc^{99m} citrate complex

pH	%Tc^{99m} citrate complex
3.2	99.5
4.0	100
5.0	98.0

Table-2: Effect of pH on labeling yield of Tc^{99m} citrate complex

Na-citrate mg	%Tc^{99m} citrate complex
1	100
2	99.7

Table-3: Effect of Na-citrate weight on labeling yield of Tc^{99m} citrate complex

Time (min.)	%Tc^{99m} complex
15	99.7
30	99.5
180	99.0

Table-4: Formation rate and stability of Tc^{99m} citrate complex

Organs	3 min.	5 min.	30 min.
Blood	3.2	9.6	0.7
Liver	1.9	4.0	1.2
Lungs	0.07	0.03	0.2
Spleen	0.005	0.02	0.1
Stomach	0.007	0.05	0.1
Intestine	2.23	6.0	2.23
Kidneys	5.7	12.0	1.2
Kidneys/Liver	3.0	3.0	3.0

Table-5: Organ distribution data of Tc^{99m} citrate in mice.

Discussion:

The main parameters regarding the formation of Tc^{99m} citrate complex of high labeling efficiency and stability in the preparation were studied in detail. Various amounts of $SnCl_2.2H_2O$ and a constant amount of Na-citrate were mixed with Tc^{99m} eluate to obtain a high labeling yield of Tc^{99m} citrate complex with a considerable stability.

It is evident from the results that rate of formation of the labeled complex is relatively fast and it is remained stable up to four hours without appreciable degradation. The degree and the rate of degradation of the complex in the preparation were found to be dependent on the concentration of $SnCl_2.2H_2O$.

The results show that the activity accumulation in the kidneys reach a maximum of 5 min. post injection and at the same time, the blood and liver activity showed the minimal.

This indication a good renal specificity of Tc^{99m} citrate complex, since the kidney/ blood and kidney/liver activity ratios approaching factors 1 and 3 by 5 min. after injection.

From these results the optimum values were chosen to formulate preparation to be used as a rental agent.

We conclude from this study the possibility of labeling of sodium citrate with Tc^{99m} and obtained a stable complex that can be prepared a freeze dried kit from this complex used as kidney functional agent.

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