A New Formulation of ^{99m}Tc-Macroagreggated Albumin (MAA) Freeze-dried Kit for Lungs Scintigraphy

Alaa K. Jabbar, Adeeb Abass Al-shami*,*
*Husham M. Abdulkarim***
** College of Pharmacy, Almustansiriya University, Iraq*
*** Ministry of Science &Technology, Chemical Research Center, Iraq*

الخلاصة

حضرت عدة طبية جاهزة من تكتلات مجهرية للالبومين (MAA) المعلمة بالتكنيشيوم−99م بتركيب جديد وبطريقة سهلة وسريعة. حضر عالق المايكرواكريكت الالبومين من تجفيف 0.4 ml من مصل الالبومين الانساني 200mg/ml بواسطة السلاين. أضيف حامض الهيدروكلوريك لتحلل الالبومين يتبع هذه الخطوه أضافة القصدير على شكل القصديريت.

(3.5ml) من بولي اثيلين كلايكول (PEG) (1mg/ml) أضيف أيضا كعامل مضاد للتكتلات المجهرية للالبومين. عدلت الدالة الحامضية الى(5.2) بواسطة هيدروكسيد الصوديوم بعدها سخن العالق مع الرج المستمر لغرض تجميع جزيئات الالبومين.

وزعت المادة المحضرة على قناني كل منها تحوي على (1Ml). أضيف (1Ml) من ($Na^{99m}TcO_4$) وحصلنا على نقاوة راديوكيمياوية أكثر من (95%) بعد فترة خمسة عشرة دقيقة من بدأ التفاعل وكانت مستقرة لاكثر من ستة ساعات في حالتها السائلة .

أوضحت نتائج التوزيع البايولوجي على الفئران بان اكثر من(93%) من الجرعة المزروقة تركزت في الرئتين وبينت نتائج التوزيع الحجمي للجزيئات ان (80%) منها تتراوح (8−10 مايكروميتر) وان العدة الجافة والمعقمة والخالية من البايروجين (Sn-MAA) مستقرة لاكثر من ستة اشهر.

Abstract

A new formulation of macro aggregated albumin (MAA) to be labeled with ^{99m}Tc was prepared by a simple and fast method. The suspension of macroagreggated albumin was prepared by diluting 0.4ml human serum albumin (200mg/ml) with normal saline (sterilized and pyrogen free) Concentrated hydrochloric acid was added to denature the protein and this step was followed by the addition of tin in the form of stannite . 3.5ml of polyethylene glycol (PEG) (1mg/ml) was also added to act as antiagglomerating agent. The pH was adjusted to 5.2 with NaOH and the suspension was heated with continuous stirring to get aggregation.

The vials contents were allowed to react with ^{99m}Tc and high radiochemical purity was obtained (greater than 95%) after 15min. ^{99m}Tc-MAA was stable for at least six hours.

The organ distribution data in mice showed that more than 93.0% of the injected dose has accumulated in the lungs with a negligible amount of radioactivity to be detected in the non target organs.

The size distribution data showed that 80.0% of the particles occur in the range of 10—80μm. The freeze-dried preparation of Sn-MAA kits (sterile and pyrogen free) were stable for at least 170 day.

Introduction

Mcaffe and coworkers have used human serum albumin to be labeled with ^{99m}Tc for the scanning of placenta [1]. Then Stern and Mcaffe have performed lungs scintigraphy using ^{99m}Tc-macroaggregated albumin [2]. Later Kavula and Browne were prepared ^{99m}Tc-macroagreggated albumin for the lungs perfusion studies [3]. Pickhardt have proved that the ^{99m}Tc-MAA can be used as significance diagnostic tool of contra lateral lungs perfusion [4, 5].

Gavin performed a study for the preparation of a new Lungs scanning agent with specificity and selectivity by using ^{99m}Tc-Macro aggregated albumin [6].Then this agent was applied for the diagnosis of pulmonary thrombobyltic disease and defect in the contralateral lungs (in case of the unilateral absence of pulmonary perfusion of lungs scintigraphy) [7,8,9].

The potential impact of new technology in production of ^{99m}Tc-MAA kits is not clear. The technique suited for highly radiochemical yield, more stable, lyophilized form and good scintigraphy are more important for the clinical evaluation of kit [10]. Researchers and their coworkers have been continued to improve the preparation and production of techniques that can be yielded a good lungs scanning agent [11, 12].

Some complicated methods to prepare a suitable particle size of aggregated albumin with the range of target uptake [13].

Several improvments had been studied in the procedures for production of Sn-MAA lyophilized kit with suitable particle size distribution had been achieved [14—16].

Our aim was to produce a macro aggregated albumin kit by a simple, fast and reproducible procedure with relatively high radiochemical purity, suitable particle size, long shelf-life and high lungs and low liver uptake.

Experimental

Chemicals:

All chemicals used in this work were of the highest analytical grade obtained from their commercial sources and without any further purification. Deionized-distilled water was used for the preparation. The chemicals were applied:

Human serum albumin (HSA) purchased from Sigma, Chimica "USA". Polyethylene glycol (PEG) "M. Wt: 4000 dalton" was obtained from Fluka, Chime" AG".Sodium pertechnitate ($Na^{99m}TcO4$) from Amersham Co. "U.K". Methanol, Soduim chloride, sodium hydroxide, hydrochloric acid, stannous chloride and silica gel plates were obtained from (BDH) , Chemicals, Ltd, Pool, England) .Light microscope "Columbus", Haemocyto-meter, Scintillation counter(Gamma Zint BF 5300 ,Berthoid,FRD) and Thin layer scanner (Berthoid, Germany).

Preparation of ^{99m}Tc-MAA:

1. 0.4ml of human serum albumin (200 mg / ml) was diluted with 3.6 ml of isotonic sailine. The solution was mixed with 4.0 ml Conc. hydrochloric acid and rapid stirring was applied for 10 min. using a magnetic bar for denaturation of albumin. 2.5ml of this solution was diluted to 47ml with distilled-water.
2. 10 mg of Tin oxide was suspended with 10ml of 5N NaOH and heated to boiling point for complete dissolution of Tin oxide, the stannite was filtered using a millipore filter (0.45).
3. 2.5ml of stannite solution was added to the diluted "HSA" solution drop by drop with continuous stirring. This step was followed by the addition of 3.5ml polyethylene glycol "PEG" (1mg/ml) and the whole mixture was diluted to 98ml with distilled water. The pH was adjusted to 5.0-5.2 using 0.1N NaOH.
4. The later preparation was heated in a water bath with slow and continuous agitation for 10min. at 76-78^{o}C. Two hours later the suspension was decanted and the supernatant was resuspended by the same volume of sodium chloride solution (conc. 4mg / ml) and PEG (conc. 0.035 mg/ml). This step was repeated at least one time and 2ml portions of the suspension were then placed in 17ml vials for lyophilization.
5. 3ml of Na $^{99m}TcO_4$ eluante containing 6mci were added to the vial and after shaking for 15sec., the radiochemical purity was different time intervals (15, 30, 60, 120, 240, 360 min). The highest radiochemical purity was obtained at 15min. after the addition of Na $^{99m}TcO_4$ elunate to the lyophilized kit. The process was carried out under aseptic conditions.

Particle size distribution:

The determination of the particle size of different batches of Sn-MAA preparations was performed using a light microscope and Haemocyto-meter.

Radio analytical method:

Thin-layer chromatography (TLC) technique was used for determination of the pertechnetate fraction and the ^{99m}Tc-MAA fraction together with reduced hydrolyzed form of ^{99m}Tc using silica gel plates developed with 85% methanol or 0.9% sodium chloride.

Paper chromatography was also applied for the determination of radiochemical purity using the same solvents.

Both plates and strips were scanned using (thin-layer) scanner (Berthoid, Germany).

Biological distribution:

Organ distribution of ^{99m}Tc-MAA preparation was performed using (5) white male mice (BALB/C) weighing (28-30gm), for both lyophilized, non lyophilized kits, Aliquots of 0.1ml containing (10-15 µci) was injected intravenously through the tail vein and the mice were sacrificed using diethyl ether at different time intervals (5,10,60,180 min. and 20 hours).

The accumulation in various organs was measured using a well-type scintillation detector (Gama Zint BF.5300, Berthoid, Germany).

Result and discussion

The term of radiochemical purity is applied to show the percentage of ^{99m}Tc-MAA and reduced hydrolyzed form of ^{99m}Tc. Both fractions remain at the origin when thin-layer and paper chromatography has been used [12]. The migration distance of three components ^{99m}Tc-MAA, Reduced hydrolyzed form of ^{99m}Tc and pertechnitate ($Na^{99m}TcO_4$) were shown in (fig.1). The migration distance of two components ^{99m}Tc-MAA and reduced hydrolyzed form stay at 2cm far from the origin while the pertechnitate ($^{99m}TcO4^{--}$) at 10cm from the origin of strip.

Several experiments were performed using the lyophilized kits to determine the particle size of Sn-MAA. The particles distribution of Sn-MAA kit is shown in (fig.2). The particle size between (10-30 µm) were maximum percentage , this range of particles is more suitable for targeting the agent to get the maximum distribution of the kit dose to lungs with small amount of dose to reach liver.

The formation rate of ^{99m}Tc-MAA was followed of labeling for six hours due to short half-life of ^{99m}Technisum. Table-1 shows that the rate of labeling of Sn-MAA kit with $Na^{99m}TcO_4$ within 15min. produced labeling yield of 99.60% within four hours which is indicated that the labeled compound stable more than six hours.

Many parameters were also investigated like method of denaturation of protein, quantity of human serum albumin, Tin content as stannite, rate and time of stirring and their temperature through the aggregation process.

The results of the organ distribution experiments for lyophilized form at different time intervals after injection to mice are shown in table-2, when the maximum accumulation of ^{99m}Tc-MAA was detected in lungs as target organs within 10min. post injection. The results indicated that the maximum fraction of the dose (97.46 %) reached to the lungs within 10min. and stayed in organ for about three hours, which give an advantage for using the kit in different patients

about the labeling time. The labeling yield and lungs uptake were very high during the time of study.Table-3 shows the result of the stability of lyophilized Sn-MAA kit which was followed for 170 days, where the lungs uptake was 95.56%.

Toxicity test was performed on the same kind of the mice using different doses of the final product as represented in table-4. The results indicate that the safety factor (lethal dose) of the three elements were high in four groups of mice, which reached to 583.2 in group four.

The resultants kit is characterized by:

1. The labeling of Sn-MAA is carried out in the single step process.
2. Simple and fast method for the preparation of Sn-MAA.
3. Reproducible with the optimal particle size distribution.
4. The shelf-life of Sn-MAA kits is convenient.
5. The radiochemical yield is highly consistent.
6. The lungs uptake is very high with low liver uptake.

The preparation of Sn-MAA kit for labeling with Na $^{99m}TcO_4$ involves the following advantages:

1. The kit is contained a low quantity of Tin (44µg/preparation).
2. The PEG was used for the first time as anti-agglomerating agent for macro-aggregated particles with particle size (10-100 µm).

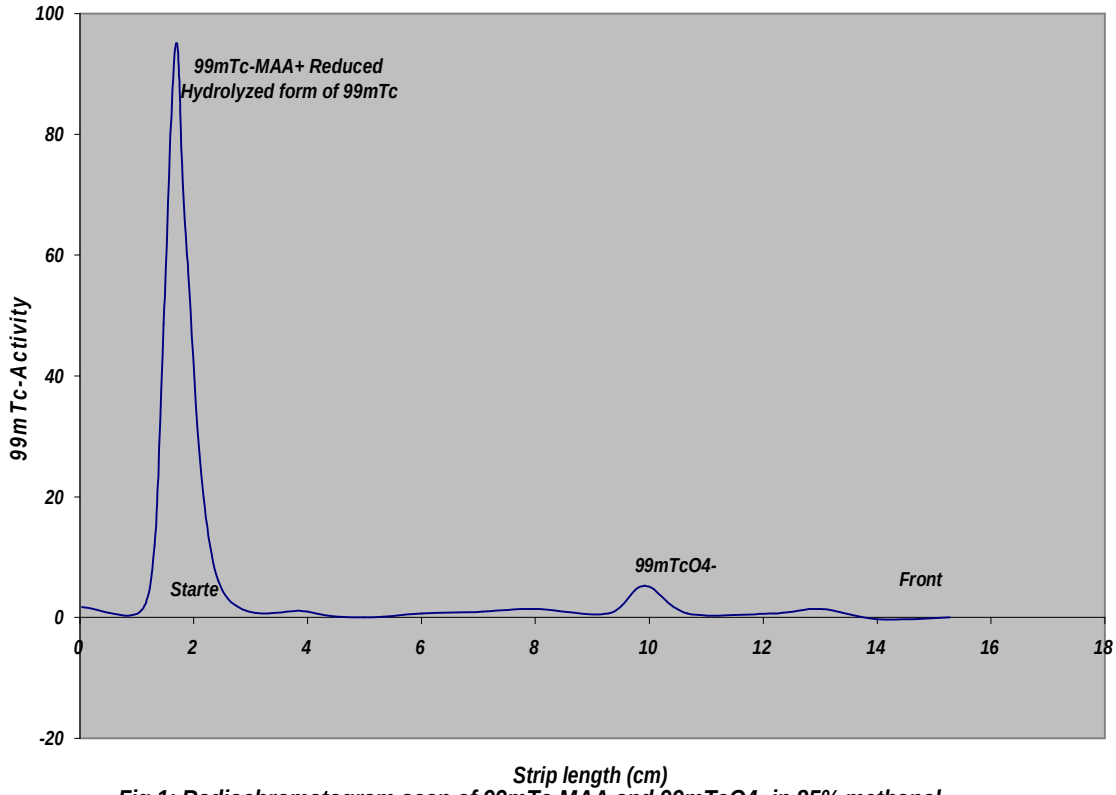

Fig.1: Radiochromatogram scan of 99mTc-MAA and 99mTcO4- in 85% methanol.

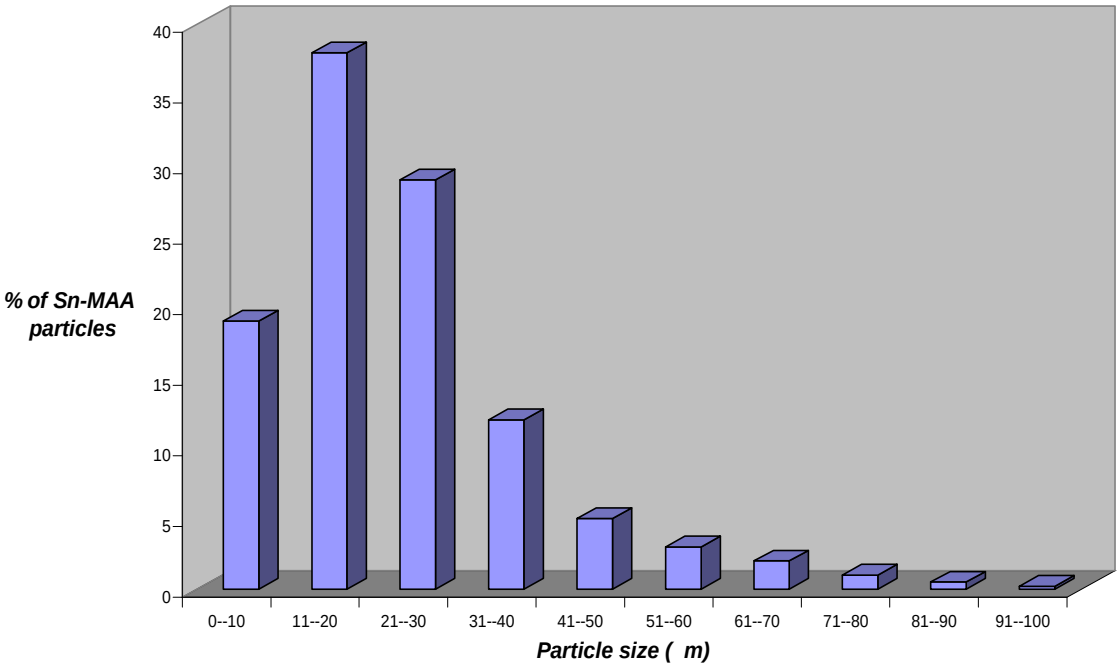

Fig.2: Size distribution of Sn-MAA particles

Labeling time(min.)	(%) ^{99m}Tc-MAA	(%) Na $^{99m}TcO_4$
15	96.79	3.21
30	97.32	2.68
60	98.92	1.08
120	98.83	1.17
240	99.60	0.4
360	99.09	0.91

Table.1: The formation rate of ^{99m}Tc-MAA determined by paper chromatography developed in saline.

Sacrificing Time (min.)	Percentage dose / Organ				
	Lungs	Liver	Kidneys	Spleen	Caracas
5	95.50	2.50	0.30	0.10	1.60
10	97.50	0.60	0.30	0.20	1.40
60	87.20	3.30	1.60	0.10	8.80
180	86.30	3.70	1.60	0.10	8.30
1200	72.10	8.30	2.90	1.40	15.30

* Mean of five mice.

Table.2: Organ distribution of ^{99m}Tc-MAA as a function of time in mice*, radiochemical purity 99.00%.

Time after production (Day)	Labeling yield(%)	Percentage dose / organ			
		Lungs	Liver	Kidneys	Caracas
1	97.00	94.00	1.50	0.50	4.00
12	99.7	97.50	0.50	0.20	1.80
34	99.60	96.50	1.00	0.30	2.20
80	97.50	95.00	2.00	0.50	2.50
170	97.00	95.50	2.50	0.30	1.7

* Mean of five mice.

Table.3: The determination of the self-life of Sn-MAA lyophilized kit by measuring the radiochemical yield and organs biodistribution of ^{99m}Tc-MAA in mice*(time reaction 15min.)

Group number	Kit content (mg) / injected dose			Safety Factor
	Tin	HSA	PEG**	
1	2.3331	23.333	4.082	58.3
2	6.999	69.999	12.248	174.9
3	11.665	116.655	20.414	291.6
4	23.331	233.31	41.412	583.2

* Ten mice for each group.
** Polyethylene glycol (M. Wt. 4000 Dalton)

Table.4: The safety factor of Sn-MAA kit in mice*.

References

1. Mcafee, J. G.; Stern, H. S.; Fueger, G. F.; Baggish, M. S.; Hoizman, G. B. and Zolle, I. (1964). J. Nucl. Med., (5) 936.
2. Stern, H. S.; Zolle, I. and Mcafee, J. G. (1965). Int. J. ppl. Radiat. isotopes, (16) 283.
3. Kavula, M. P. and Brwone, Jr. A. IAEA, SM-171/42.
4. Pickhardt, P. J. and Fischer, K. K. (1998). Differential diagnosis, AJR, (517) 145-150.
5. Pickhardt, P. J. (1997). Clin. Nucl. Med., (22) 484-485.
6. Gavin, D. Neill. (1971). Int.J. Appl.Radiat. isotopes, (22) 42-44.
7. Shin, W. J. and Pulmano, C. (1996). Clin. Nucl. Med., (21) 465-468).
8. Wei-Jen Shih and Jain-Kui Han (1999). Rad. Med., 17(4) 327-328.
9. Makoto Hosono, Kikuo Machida et al, (2002). Annals of Nuclear Medcine,16(8) 577—581.
10. United States Patent, Mailnckrodt Chemical Works, St. Louis, 3,863, 004, 45 Jan. 28, 1975.
11. Ilyas, M.; Haider, KH.; Saeeda, H.; Javed, M.; Shams, Z. and Sameera, C. (1998). Medical Journalof Islamic ACADEMY OF Science, 11(4), 131—138.

12. Divnad Joklc, Drina Jankovic and Tatjana Maksin,(2002),J. Serb. Chem. Soc.,67(8-9)573—579.
13. Max, S. Lin and Savl, H. Winchell (1972). J. Nucl. Med.,13 (12) 28.
14. Philip, J.; Robbins, D. L.; Fortman, Jennine and Lewis, T. (1973). Int. J. Appl. Radiat. Isotopes, (24) 481.
15. Paoll, DE. T.; Nicoling, J. O. and Radicella, R. (1966). Int. J. Appl. Radiat. Isotopes, (17) 551.
16. Al-janabi, M. A. A.; Yousif, Z. M. and Kadim, A.H. M. (1983). Int. J. Appl. Radiat. Isotopes, 34 (10)1473.