AJPS, 2010, Vol. 7, No.1

A new preparation of colloidal ^{99m}Tc-Tin phosphate stabilized with CMC as lyophilized kit for bone Marrow Scintigraphy

Alaa, K. Jabbar*, Husham, M. Abdul-Kareem**

College of pharmacy, Al-mustansirya University, Baghdad, Iraq
****Chemical research center, Ministry of science & technology, Iraq***

الخلاصة

حضر مستحضر غروي من فوسفات القصدير المعلم بنظير التكنيشبوم−99م بطريقة جديدة وسهلة و أعتمدت الطريقة على تسخين فوسفات الصوديوم ثنائي الهيدروجين في وسط متعادل مع كاربوكسي مثيل سليلور كعامل أستقرارية ثم برد الى درجة حرارة الغرفة واضيف اليه كلوريد القصديروز, أوضحت نتائج التحليل الكيمياوي بان النقاوة الراديوكيمياوية أكثر من 99 % مع توزيع حبيبي حجمي مناسب.

أشارت نتائج التوزيع البايولوجي أن أكتر من 30% من الجرعة المعطاة تتركز في نخاع العظم في حين تتركز كمية قليلة جدا في الاعضاء الاخرى مما تدل على ان المستحضر مناسب جدا في أستخدامه لتشخيص وتصويرنخاع العظام.

Abstract

The new preparation of colloidal Tin phosphate labeled with technisum-99m for bone marrow studies. The method was applied with heating sodium bi hydrogen phosphate with carboxy methyl cellulose (CMC) as stabilizer in neutral medium then cooed at room temperature, followed by adding stannous chloride as stannite. The chemical analysis indicates that the radiochemical purity is greater than 99% with suitable particle size distribution.

The results of biodistribution indicate that the preparative agent was accumulated with higher concentration in bone marrow reach to 30% of total injected dose and negligible percent in non target organs. The results meaning that the new preparative agent is a good agent for using as bone marrow scintigraphy compared with others.

Introduction

Bone marrow scintigraphy is useful parameter for the evaluation of various hematological disorders as well as the detection of early bone metastases of malignant tumor. Most of radio−colloidal which have been employed for

bone marrow imaging are not ideal agents. Colloidal Gold-198 is previously used for "REC"studies and Iron-52.

Also used for imaging hematopoietic tissues but are not preferable due to the high radiation exposure. However, these isotopes have a number of disadvantages. They have restricted availability because they are a cyclotron produced isotopes .Indium-111 can be obtained in a high yield with a half-life of 2.81 days which is a short period for the purpose of distribution to allow bone marrow scintigraphy [1,2] . Moreover, it is not widely used because it could be bound to, or transferred [3], and accumulated in placenta with slow blood clearance [4].

Among the known ^{99m}Tc-colloidal radiopharmaceuticals like ^{99m}Tc-sulphur colloid was applied for liver and spleen scanning with low bone marrow uptake [5]. ^{99m}Tc-phytate was not suitable agent for bone marrow imaging [6]. ^{99m}Tc-antimony sulfide colloidal agent was proposed as an excellent scanning agent for bone marrow but it was high retention in the blood which was led to limit for clinical acceptance, moreover, the method of preparation involved many hard steps [7]. McAfee and coworkers were prepared ^{99m}Tc-minimicroaggregated albumin in the formulation to give the highest bone marrow accumulation about six times than of ^{99m}Tc-sulphur colloid agent [8]. Hyman and coworkers were improved the bone marrow uptake of ^{99m}Tc-minimicraggregated albumin in the baboon by a factor of three in comparison with ^{99m}Tc-sulphur colloid and by a factor of two for both ^{99m}Tc-antimony colloid and ^{99m}Tc-microaggregated albumin. ^{99m}Tc-Sn-phosphatepoly vinylpyrollidone stabilized colloid was the agent of choice for bone marrow scanning but it was critically in heating during preparation and multi-steps that was not convenient agent for clinical use [9, 10].

The two important factors which have deterred the wider application of radiocolliods as clinical agents for measuring phagocytic and digestive functions of the reticuloedorthelial system, firstly, optimal particle size distribution and secondly, reproducibility of the method for preparing the suspensions. A particle size of less than "50nm" is useful for the same purposes and also for the liver, Spleen, and bone marrow scanning agents [11].The specific preparation of ^{99m}Tc-Tinphospate agent was prepared by Stern and coworkers but in long procedure[12].

The aim of this study is to prepare ^{99m}Tc-Tinphosphate precolloid as a good bone marrow scanning agent with accurate, simple and short procedure of preparation.

Materials and Methods

The preparation of T in (II)-Phosphate pre-colloidal which give rise to the best marrow uptake was carried by using chemicals of commercial sources without further purification.

Preparation of colloidal kit:

The colloidal was prepared by heating a mixture of an aqueous solutions of sodium bi hydrogen phosphate dihydrate"$NaH_2PO_4.2H_2O$ "10mg/ml" and Carboxy methyl cellulose low viscosity sodium salt "CMC" "4 mg/ml" in neutral medium for a few minutes the cooled to room temperature , followed adding 15 mg/ml of stannous chloride ($SnCl_2 . 2H_2O$ in 0.2N HCl). The pH was adjusted to 3.0 with phosphate buffer; nearly clear color solution was obtained. The solution was filtered through 0.1 µm membrane filter. 2 ml of aliquots were dispensed in vial that contains of each 0.166 mg sodium bi hydrogen phosphate, 0.300 mg stannous chloride dehydrates and 4.66 mg carboxy methyl cellulose "CMC".

Radiochemical analysis:

Paper chromatography:

The paper chromatography was used for determination of radio-colloidal Tin-phosphate labeled with technisum-99m. Radiocolloidal samples were spotted at the lower egad of a 2x20 cm Whatman No.1and developed in 85% methanol, after chromatographic strips were scanned by a thin layer scanner (LB 2723 Berthoid Dunschcht-scanner-II). The strips were cut into lower and upper halves and the counted in a well type Scintillation gamma counter (Berthoid MAG 312 West-Germany).

Gel Chromatography Column Scanning Technique (GCS):

Sepharose® (AB pharmacia, Sweden) was used for determination of different fractions of free and bound technisum-99m. A sample volume of 0.1-0.3 ml was applied on the top of the column (1.5 in diameter).The column was filled Sepharose-cl 6B up to a height of 33 cm and eluted with 10ml of 0.9% NaCl solution. The column was sealed and scanned in the horizontal position with a 1mm slit collimated NaI (Tl) crystal detector. The scanning profile was obtained on a recorder and as a digital printout. Reduced hydrolyzed form of ^{99m}Tc-Colloidal and/ or large particles was found within the distance of 0.0---3.0 cm at the top of the gel, 99mTc-pertechnitate in the zone of 4.0----6.0 cm below and ^{99m}Tc-Sn-phosphate colloid in 7.0---19.0 cm below the top of the gel. This profile gave information on the size distribution of the labeled colloidal compound and the presence of other Technisum-99m labeled species in the preparation.

Organ Distribution:

The organ distribution studies were performed in white Newzealand rabbits (weight range1-2 kg). The 250 µci of radiocolloid dosage were injected

via an ear vein in volumes of 0.2 ml. the rabbits were sacrificed 15 and 60 min. post injection. Urine collection was performed after the removal of the bladder, and samples of blood were taken from the dissected animals, other organs of interest like Liver, Lungs, Spleen, Stomach, and Kidneys were taken out. The femur was removed and its bone marrow sample was collected. Samples of marrow, muscle and bone were weighted and counted with other organs of interest in a well scintillation counter. The organ distribution data obtained were normalized to the total administered activity and the physical decay of technetium-99m was taken in consideration .Blood volume and total bone marrow weight were approximately estimated as 5% and 1.6% of whole body weight respectively, whereas 43% for muscle and 10% for bone.

Results

The term of radiochemical purity was developed to show the percentage of technisum-99m bound with Sn-phosphate and the reduced hydrolyzed form of ^{99m}Tc-colloid (large particles), these fractions are remained at the origin when the paper chromatography was used. The results of analysis were shown in (fig-1). The pH dependent was studied in different intervals of pH (3.0, 3.5, 4.0, 5.0, 5.6, 6.0, 7.0), it was clear that the highest radiochemical purity of radio-colloid is greater than 99% was obtained at pH between 3.0 to 3.5 which is determined by paper chromatography. The radiochemical purity of radio-colloid obtained 95.17% to 98.54% when the pH values were raised from 4.0 to 7.7. These results indicated that the effect of pH value in the range of study was insignificant (Table-1).

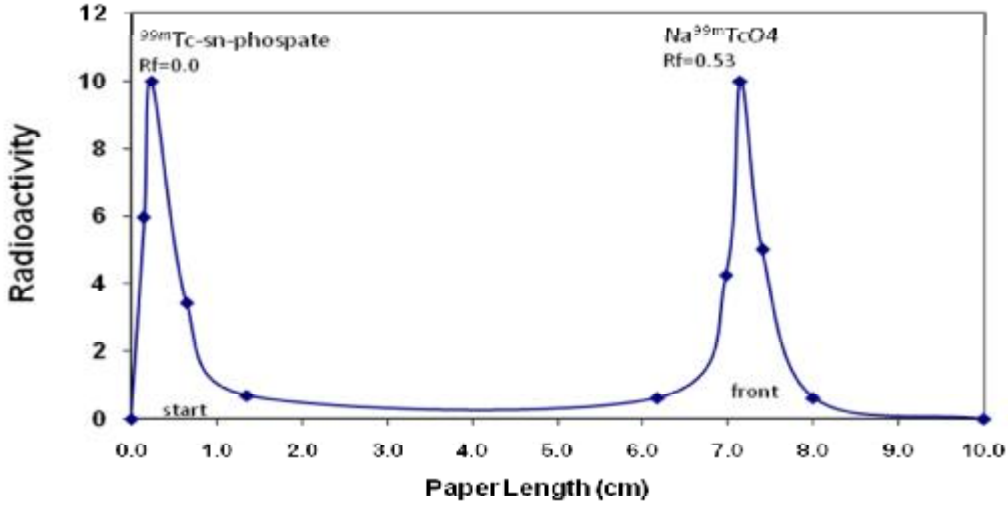

Fig-1: Radiochromatogram of 99mTc-phosphate -CMC colloid & 99mTc-pertechnitate on paper whattman No.1 developed with 85% methanol

pH Value	Radiochemical purity	
	^{99m}Tc-Sn-phosphate	Free $Na^{99m}TcO_4$
3.0-3.5	99.64	0.36
4.0	98.54	1.46
5.0	98.48	1.58
5.6	95.17	4.83
6.0	98.28	1.72
7.0	97.70	2.3

Table-1: Effect of pH value on the radiochemical purity of ^{99m}Tc-Sn-phosphate-CMC by paper chromatography.

Many parameters were influenced the rate of blood clearance and distribution of colloids including the numbers and the size of particles, presence of stabilizers, surface active agents, competing colloids , the chemical nature of the colloid surface , distribution of charge surface and electrophoresis mobility[20, 21,22]. The organ distribution of ^{99m}Tc-phoshpate-CMC stabilized colloid in rabbits at 60min. after injection is shown in (fig-3), expressed as percent dose in the whole organ. The highest accumulation was found in the liver and bone marrow, with much lower uptake in bone, spleen, blood, muscle, kidneys and lung. Table-2 shows the (percent dose per gram of marrow: percent dose per gram of tissue). The biodistribution of ^{99m}Tc-phosphate-CMC in rabbits was studied as a function of time after $Na^{99m}TcO_4$ addition. It is evident that the organ distribution was performed on the radio colloidal formation after 15 min. of labeling is not the optimal organ distribution and not obtaining the suitable particle size and stable colloidal preparation due to the reaction of $Na^{99m}TcO_4$ with per colloidal preparation may not be complete. The residual blood activity of ^{99m}Tc-phosphate-CMC at 60 min. following administration was about 1.24% which is appreciably lower than^{99m}Tc-Colloid rang from 1.8% to 14.4% [7]. The bone marrow uptake of ^{99m}Tc-Sn-phosphate-CMC stabilized colloid was found to be (30%) which is higher than ^{99m}Tc-Sn-phosphate-PVP stabilized colloid (26.7%) [10], it high enough for scanning. This value was greater than that of other ^{99m}Tc-Colloid.

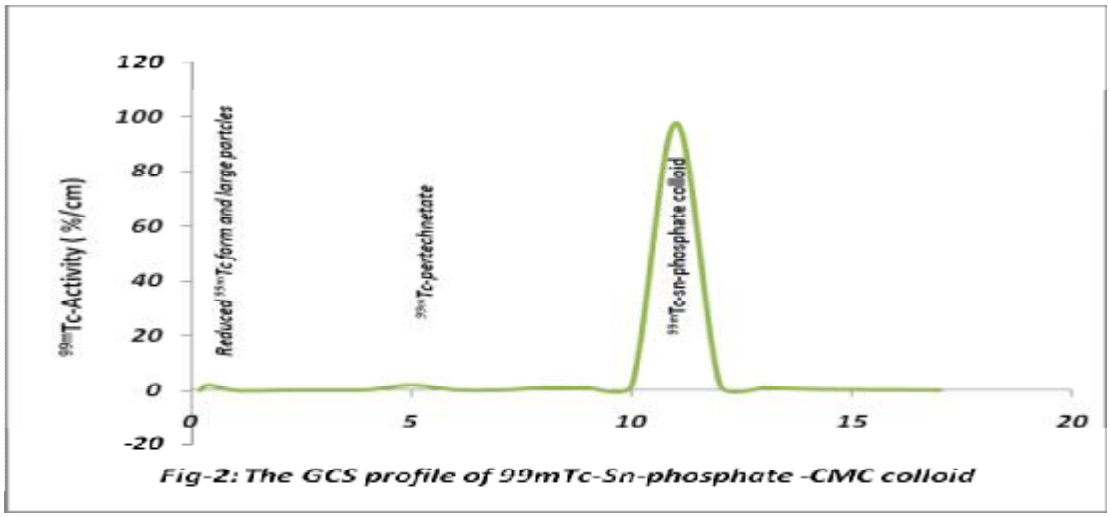

Fig-2: The GCS profile of 99mTc-Sn-phosphate -CMC colloid

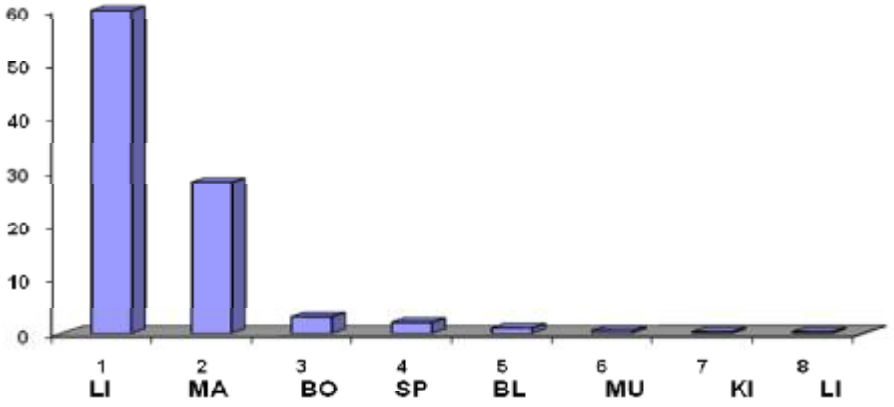

Fig.3: Organ distribution of 99mTc-Sn-phosphate-CMC colloid in rabbite at one hour post injection.(Liver"Li",Marrow"MA",Bone"BO", Spleen"SP",Blood"BL",Muscle "MU",Kidney"KI",Lungs"LU".

Discussion

The radiochemical purity of the radio colloid in the pH range of 3.0 to 3.5 was conformed by the (GCS technique) using Sepharose C1-6B.

The formation rate of the radio-colloidal fractions depending on the time reaction after addition of $Na^{99m}TcO_4$ .The fraction of activity was remained at the top of the column due to the presence of the large particles. These large particles undergo decomposition to produce smaller radio-colloidal particles as previously reported [16,17] and it is not related to the presence of reduced hydrolyzed form of $Na^{99m}TcO_4$ (Fig-2).

However, the rate of formation of radio-colloid after 60 min. was yielded sufficient and optimal particle size with high radiochemical yield to obtain good bone marrow uptake. The relatively slow formation rate of the ^{99m}Tc-phosphate–CMC stabilized colloid is reckoned to the formation of $Na^{99m}Tc$ (v) O_4 [18, 19] to be bound to the pre-colloidal preparation.

The ratio of Marrow: liver of our preparation (when expressed as persent dose per organ) was 0.43 with only 1.62% appeared in the urine; this fact reveals that accumulation of this colloidal preparation in marrow is high. These results are supported by the data obtained when using one gram of muscle (97.67, 45.5, and 1115.3) respectively.

Our conclusion of the Sepharose C1-6B is the recommended gel for assessment of the radiochemical purity of various radio-colloidal preparations. Carboxy methyl cellulose is an excellent stabilizing agent for stabilized the colloidal preparation of Sn-phosphate. The preparation is an excellent bone marrow agent compared with other commercial agents [23, 26].

Organ	% administered dose/organ after labeling	
	15 minute	60 minute
Blood	2.96	1.24
Liver	44.45	60.58
Lungs	0.17	0.39
Spleen	0.37	1.95
Stomach	0.24	0.05
Kidneys	2.22	0.74
Urine	13.97	1.62
Muscle	3.97	0.65
Bone	12.82	2.86
Marrow	18.98	29.92
Marrow/Liver	0.426	0.495
1g marrow/1ml blood	20.07	97.67
1g marrow/1g bone	9.28	45.45
1g marrow/ 1g muscle	134.43	1115.28

Table-2: Organ distribution of ^{99m}Tc-phosohate_CMC colloid "60" min. Post injection in rabbits as a function of labeling time.

References

1 - Engstedt, L.; Franzen, S. and Lonsson, L. et al. (1958). Acta Radio. (49) 66.
2 - Merrick, M.V.; Grodon-Smith, E.C.; Lavender, J.P. and Szur, L. (1975). J.Nucl. Med. (16) (66-68).
3 - Goodwith, D.A.; Goode, R.; Brown, L. et al. (1971). Radiology, (100)175-179.

4 - Graber, S.E.; Hurley, P.J. and Heyssel, R.M. et al. (1970). Prec. Sec.Exp. Biol. Med. (133) 1093-1096.
5 - Bekerman, C. and Gottchalk, A. (1973). J. Nucl. Med. (12) 237.
6 - Robert, G.; Hamilton, G. and Philip, O. et al. (1977). J. Nucl. Med. (18) (563-565).
7 - Nagai, K.; Ito, Y. and Otsuka, N. et al. (1981). Int. J. Nucl. Med. Boil. (8) (85-89).
8 - Mcafee, J.G. and Subramanian, G.J. (1982). J. Nucl. Med. (23) (21-28).
9 - Heyman, S.; Davis, A.M. and Shuikin, P.M. et al. (1979). Proc. 2nd. Int. Symp. Radiopharmaceuticals, New York, Soc. Nucl. Med. (593-601).
10 - Faiz-Ur-Rehman and Shama-Uz-Zaman et al. (1986). Appl. Radiat. Isot. (37): (249-255).
11 - Alvarez, J.; Subramanian, G. and Rhodes, B. A. et al. (1975). Radiopharmaceuticals, New York, Soc. Nucl. Med. (102-107).
12 - Stern, H.S.; Mcafee, J.G. and Subramanian, G. (1966). J. Nucl. Med. (7): 665.
13 - Al-hilli, H.M.; Agha, N.H. and Al-jumili, I.F. et al. (1986). J.Radioanal. Nucl. Chem. Letters, (104) (5) (273-286).
14 - Person, R.B.; Stand; S.E. and Knoos, T. (1978). J. Radioanal.Chem, (43): (275-286).
15 - Subramanian, G. and Mcafee, J.G. (1972). J. Nucl. Med.13 (947-950).
16 - Illum, l. and Davis, S.S. (1987). Life sci., (40): 1553-1560.
17 - Sankovic, D.; Maksiv, T. and Djokk, D. et al. (2008). J. Nucl. Med., 232 (3) (2008): (601-604).
18 - Jones, R.J. (1992). Radiology, 183(2) (381-322).
19 - Creab, D. (1990). Can. Assoc. Radiol. 4(1): 51.
20 - Atkins, H.L.; Hauser, W. and Richards, P. (1970). J. Reticuloendothl. Soc., (8): 176-184.
21 - Doson, E.L. and Jones, H.B. (1953). Acta Med. Scand (suppl 273): (144): (1-71).
22 - MaCfee, J.G. and Subramanian G. (1969). In Clinical scintillation imaging "2nd ed L. M. Freeman and P. M. Johnson Eds, New York, Grune and Stration (64-70).
23 - Daza, F.L. and Toylar, A.I. Semin. (1986). Nucl. Med. (15) (3): (239-259).
24 - Jankovic, L.J. and Djokk Divna, D.J. (2005). Nucl. Med. Comm. (26) (415-419).
25 - Aharoni Dvora; Krausz Yodphuto and Elstein Debran et al. (2002). J. Nucl. Med., (27) (7): (503-509).
26 - Hodgson, N.; Zabel, P. and Mattar, A.G. et al. (2002). Annals Surgical oncology, (8) (2): (133-137).