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Radiochemical purity and particles number determinations of modified ^{99m}Tc-macroaggregated albumin

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Abstract: A new procedure for the aggregation of human albumin and ^{99m}Tc-labelling of the prepared macroaggregated albumin are presented. Simple methods for quantifying of all the radiochemical impurities existing in ^{99m}Tc-MAA were tested. Thus, 85 % methanol was used as the mobile phase in paper and ITL chromatography with Whatman N^{o} 1 and ITLC-SA strips. A system of two solvents (acetone and 1 M NaCl or 0.9 % NaCl) was used for 3 MM paper, ITLC-SA and ITLC-SG strips and silica gel plates as the stationary phase. Low-voltage paper electrophoresis with Whatman 3 MM paper sheets soaked in barbiturate buffer and the gel chromatography column method (Sephadex G-25) were also applied. Filtration through syringe filters, proposed by European and Yugoslav Pharmacopoeia, was performed for comparison. The application of the mentioned tests lead to consistent results for the labelling efficiency (> 98.5 %) and percent radiochemical impurities of ^{99m}Tc-MAA. Determination of the particles number in a counter chamber and their size distribution under a light microscope with a calibrated ocular scale gave the result of 300000 –350000 particles per 1 mg of HA. This confirmed that the human albumin macroaggregates prepared by our new procedure is remarkably improved and convenient for routine diagnostic purposes.

Keywords: human albumin macroaggregates, ^{99m}Tc-labelling, quality control.

INTRODUCTION

Many radiopharmaceuticals are currently used for various nuclear medicine tests. Since they are administered to humans, they should satisfy the same important criteria as conventional drugs, namely they should be sterile and pyrogen free and they should undergo all quality control measurements required of conventional drugs.[1–3] Some of radiopharmaceuticals meet most of the requirements for their use, while others warrant further development or replacement.

Human albumin macroaggregates (MAA) labelled with ^{99m}Tc is a radiopharmaceutical widely used for the diagnosis of lung diseases and radionuclide venography.[4] The addition of ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) obtained from ^{99}Mo-^{99m}Tc radionuclide generator enables the simple preparation of ^{99m}Tc-MAA.

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Human albumin macroaggregates were prepared in kit form for the first time around 1970.[5,6] Now this radiopharmaceutical usually contains a lyophilized preparation of aggregated human albumin, stannous chloride and other substances as preservatives or stabilizers, depending on the manufacturer. The particle size ranges from 10 μm to 100 μm, which must be checked prior to the administration to humans. The widespread use of ^{99m}Tc-MAA in everyday diagnostic practice in nuclear medicine necessitated the development of simple, rapid and economic methods of its quality control.[7–11]

In the Laboratory for Radioisotopes, The Institute of Nuclear Sciences "Vinča", MAA has been prepared since 1978.[12] The procedure for preparation was further developed and changed but the results of MAA quality control suggested some non-uniformity in their quality. This paper presents a new procedure obtained as the result of investigations to optimize the aggregation conditions (pH, concentrations of human albumin and tin (II), temperature of aggregation and stirring rate). Now this radiopharmaceutical has the most favourable composition of a lyophilized preparation with glycine as a new stabilizer. The standard procedure for the determination of the number of lymphocytes was used for the determination of particles number of ^{99m}Tc-labelled MAA.[13] Besides the quality control methods proposed in USP (XXIII),[14] European pharmacopoeia (1997)[15] and Pharmacopoeia Yugoslavica (2000),[16] we assayed using more detailed radiochromatographic methods for the determination of labelling efficiency and radiochemical purity of ^{99m}Tc-MAA. For this purpose, the current radiochromatographic and electrophoresis methods for the detection of all impurities in ^{99m}Tc-labelled radiopharmaceuticals were evaluated. In this way, rapid and simple methods for the determination of the labelling efficiency and radiochemical purity were established.

EXPERIMENTAL

Preparation of human albumin macroaggregates

Aggregation of human albumin (20 % HA, National Blood Transfusion Institute, Belgrade) was performed in a double walled glass vessel, at a constant temperature (343 ± 1) K, maintained by circulating thermostated water (thermostat model VEB MLW Prufgerate-Werk, Medingen, Germany). The mixture of HA in acetate buffer (pH 5) was stirred at 350 rpm for 30 min. Subsequently, $SnCl_2 \times 2H_2O$ (Merck, p. a.) in 0.1 M HCl was added and the stirring was continued for a further 15 min. After cooling, the supernatant was decanted and the human albumin macroaggregates were rinsed three times with acetate buffer. The aggregates were separated by centrifugation (5 min at 2000 g). The prepared suspension was diluted with acetate buffer and glycine (Merck, p.a.) was added. The suspension after partitioning through 18-gauge needles was lyophilised. The complete operation was carried out in a laminar airflow hood and under aseptic conditions. The used equipment had been previously sterilized, and all necessary solutions were sterilized by membrane filtration using 0.20 μm cellulose acetate dispensable syringe filters (Millipore Corporation, USA). The obtained MAA in kit form was a sterile, pyrogen-free preparation with 8.8 mg macroaggregates per vial (7 mg HA before aggregation), 0.275 mg $SnCl_2 \times 2H_2O$ and 2.5 mg glycine per vial. The ^{99m}Tc-labelling of MAA was carried out by adding $^{99m}TcO_4^-$ from a ^{99}Mo-^{99m}Tc radionuclide generator (Laboratory for Radioisotopes, INS "Vinča"), to the vial and shaking vigorously for a few minutes.

Radiochemical purity

For the measurements of the labelling efficiency of ^{99m}Tc-MAA, instant thin-layer chromatography (ITLC) combined with paper chromatography, paper low-voltage electrophoresis and gel filtration were ap-

plied. In these methods small aliquots of the radiopharmaceutical preparation (≈37 kBq) were spotted on a paper strip (Whatman), ITLC-SA or ITLC-SG strips (Gelman Instrument Company, Ann Arbor, Michigan, USA) made of glass fiber impregnated with polysilicic acid or silica gel, respectively, as well as TLC-plates covered with silica gel. Paper chromatography was performed using Whatman N^{o} 1 (2 × 16 cm) with 85 % (w/v) methanol (Merck, p.a.) and Whatman 3 MM (2 × 16 cm) with two solvents: acetone (Merck, p.a.) and 1 M NaCl, (Merck, p.a.). The ITLC-SA or ITLC-SG strips (2 × 16 cm), as well as the TLC-plates (2 × 16 cm) were developed first in acetone till 15 cm and after air drying in 0.9 % (w/v) NaCl or 1 M NaCl till 7 cm. One centimeter cuts were counted in a gamma-scintillation counter and the labelling efficiency of ^{99m}Tc-MAA was determined.

Low-voltage paper electrophoresis (220 V, 2 h) was performed applying a radioactive sample (≈ 37 kBq) on Whatman 3 MM paper sheets soaked in barbiturate buffer, pH 8.6. After electrophoresis, the distribution of the activity along the paper strips was determined by measuring 1-cm dried pieces in a gamma-scintillation counter. The percentage of radioactivity of each fraction was determined from its ratio to the total radioactivity of the strip.

Gel filtration was performed by passing a 0.1-ml aliquot (≈ 37 kBq) of ^{99m}Tc-MAA through a Sephadex G-25 column (Pharmacy fine, Upsala, Sweden) with 0.9 % (w/v) NaCl as the eluting solvent. Since it was not possible to scan the column, it was eluted with 35 ml of 0.9 % (w/v) NaCl. One-millilitre aliquots were collected in vials and the radioactivity of each aliquot was measured in a gamma counter.

Pharmacopoeia Yugoslavica 2000 (Ph. Yug. V) proposes the determination of non-filterable radioactivity using dispensable syringe filters. In our examination, 0.3-ml aliquots (≈ 37 kBq) of ^{99m}Tc-MAA were passed through cellulose acetate dispensable syringe filters with 0.20 μm and 0.45 μm pore size polyethersulfone membrane filters (Whatman), followed by washing with 20 ml 0.9 % (w/v) NaCl.

Particle counting and size measurements

The number of particles was determined in a counter chamber under a Zeiss Standard GFL microscope. A 0.1 ml aliquot of human albumin macroaggregates coloured with 0.1 % Rose Bengal solution was introduced into the counter chamber, the particles were allowed to settle for 1 min and then observed under the microscope. Their size distribution was checked under a microscope with a calibrated ocular scale.

RESULTS AND DISCUSSCION

Radiochemical purity

The radiochemical purity of a radiopharmaceutical is the fraction of the total radioactivity in the desired form. Several radiochemical impurities can exist in ^{99m}Tc-MAA preparations, such as a colloid reduced and hydrolysed fraction (^{99m}Tc-Sn colloid), free pertechnetate ($^{99m}TcO_4^-$) and a soluble ^{99m}Tc-albumin (^{99m}Tc-HA).[10] Each of these components are characterized by R_f value, which is defined as the ratio of the distance the component had traveled to the distance the solvent front had advanced from the original point of application of the investigated compound. As no single ideal method exists to determine all the mentioned radiochemical impurities during the same experiment, different stationary and mobile phases were combined. The radiochromatographic results are presented in Tables I and II. The results are expressed as mean values of at least six tests with the standard deviations also given. The yields (%) represent the amounts of the mentioned radiochemical impurities and hence the labelling yields of ^{99m}Tc-MAA. The obtained chromatographic results confirmed that most of the ^{99m}Tc activity remained at the application spot on the paper, ITLC strips or silica gel plates. Technetium-99m pertechnetate is the only radiochemical impurity determined by the paper and ITL chromatographic methods in 85 % methanol.

TABLE I. Radioanalytical paper and ITL chromatography of ^{99m}Tc-MAA with 85 % methanol

Solid phase	Yield (%) $R_f = 0.0$	Yield (%) $R_f = 0.36 – 0.45$	Yield (%) $R_f = 0.73 – 0.85$
Whatman N^o 1	80.1 ± 2.2	19.9 ± 2.2	–
ITLC-SG	91.7 ± 2.4	–	8.3 ± 2.4

TABLE II. Radioanalytical paper and ITL chromatography of ^{99m}Tc-MAA with acetone and 1 M NaCl (*0.9 % NaCl)

Solid phase	Yield (%) $R_f = 0.0$	Yield (%) $R_f = 0.4 – 0.5$	Yield (%) $R_f = 0.9 – 1.0$
Whatman 3 MM	98.4 ± 0.4	1.5 ± 0.3	0.1 ± 0.1
ITLC-SA*	98.8 ± 0.2	0.8 ± 0.1	0.4 ± 0.1
ITLC-SG	98.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
ITLC-silica gel	99.7 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

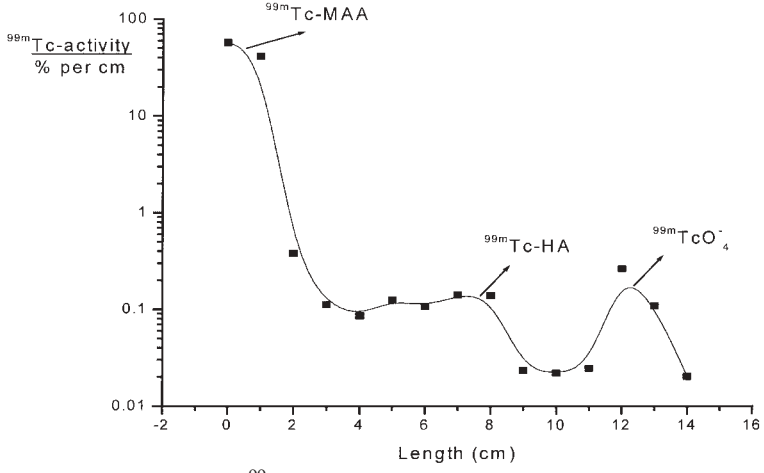

Fig. 1. Distribution of radioactivity of ^{99m}Tc-MAA along the electropherogram (barbiturate buffer pH = 8.6, 220 V, 2 h)

The results of the paper electrophoresis are presented at Fig. 1. Low-voltage electrophoresis in barbiturate buffer is a convenient method for the determination of the labelling efficiency and radiochemical purity of ^{99m}Tc-MAA. Human albumin macroaggregates labelled with technetium-99m remained at the origin; non-aggregated ^{99m}Tc-HA migrated (6 – 7 cm), as well as free TcO_4^- (12 – 13 cm) in the anodic direction. The percentage of radioactivity of each fraction was determined from their ratio to the total radioactivity of the strip. The investigations of 27 different batches of human albumin macroaggregates labelled with technetium-99m showed that ^{99m}Tc-MAA was a radiopharmaceutical of high radiochemical purity (99.7 ± 0.4 %). The results of radiochemical purity studies with gel filtration confirmed that the maximum of ^{99m}Tc activity was retained at the top of the column. The percentages of filtrated radioactive impurities were determined by equation:

$$\% \text{ radiochemical impurities} = \frac{\text{filtrate radioactivity(cpm)}}{\text{(gross syringe, cpm)} - \text{(residual syringe, cpm)}} \times 100$$

where cpm is counts per minute. These experiments showed that 98.5 ± 0.9 % of the total radioactivity results from ^{99m}Tc-labelled MAA and 1.5 ± 0.2 % is related to a colloid reduced and hydrolysed fraction (≈ 0.6 % ^{99m}Tc-Sn colloid, free pertechnetate (≈ 0.6 % $^{99m}TcO_4^-$) and soluble ^{99m}Tc-albumin (≈ 0.3 % ^{99m}Tc-HA)).

The results of the investigation of non-filterable radioactivity are presented in Table III as mean values of six experiments for each syringe filter together with standard deviations. As it can be seen the results of both series of experiments are very similar and are in good agreement with the values proposed by Ph. Yug. V (> 90 %).

TABLE III. Radiochemical purity determination of ^{99m}Tc-MAA by filtration

The type of syringe filter	Membrane bound ^{99m}Tc-MAA (%)	Radiochemical impurities in filtrate (%)
0.20 μm cellulose acetate membrane filter	99.3 ± 0.4	0.7 ± 0.4
0.45 μm polyethersulfone membrane filter	99.4 ± 0.1	0.6 ± 0.1

The literature data, as well as our previous experience dealing with human albumin macroaggregates labeled with ^{99m}Tc, show that a great percentage of the labelled particles adhered to the plastic syringes. Therefore, the used aliquots of the preparation were measured prior and after being spotted on strips, columns or syringe filters. These results showed that 14.8 ± 3.0 % of radioactivity adhered to the used syringe (1-ml tip syringe, Zdravlje, Leskovac, Yugoslavia).

The number of particles and their size distribution were determined in human albumin macroaggregates in 16 squares of the counter chamber. The average number of particles per preparation unit (5 ml, 7 mg HA) was estimated to 2160000 – 2450000 that is 300000 – 350000 particles per 1 mg of HA. More than 80 % of the particles were in the size range from 40 – 60 μm, less than 1 % were greater than 100 μm and no particles greater than 150 μm were founded.

CONCLUSION

The results obtained by different techniques show that the used chromatographic, electrophoresis and gel filtration methods are appropriate for routine quality control of ^{99m}Tc-MAA. They allow the determination of all three radiochemical impurities in this radiopharmaceutical. Only the results of paper chromatography and ITLC-SG in 85 % methanol were not in accordance with the results obtained by the other methods. The stationary phase (paper and ITL chromatography on SA or SG strips and silica gel plates) did not affect the reproducibility of the separation of the investigated radiochemical impurities by the two solvents. These results are in good accordance with electrophoresis, gel chromatography as well as syringe filterability results with values 98.5 % – 99.7 % of total radioactivity assignable to ^{99m}Tc-MAA. Moreover, ITL-chromatography with acetone and

0.9 % NaCl or 1 M NaCl is a simple and quick method appropriate for routine quality control of ^{99m}Tc-MAA. This confirmed that human albumin macroaggregates prepared by the new procedure and labelled with ^{99m}Tc are a radiopharmaceutical of high labelling efficiency and radiochemical purity, with a satisfactory number of particles of the required size. In this way the MAA prepared in the Laboratory for Radioisotopes are remarkably improved. Their correct qualities enable good results in routine application in diagnostic or nuclear medicine to be attained.

ИЗВОД

ОДРЕЂИВАЊЕ РАДИОХЕМИЈСКЕ ЧИСТОЋЕ И БРОЈА ЧЕСТИЦА КОД МОДИФИКОВАНОГ ^{99m}Tc-МАКРОАГРЕГАТА АЛБУМИНА

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У раду је приказан нови поступак агрегације људског албумина и обележавање добијених макроагрегата албумина технецијумом-99m. У циљу одређивања присутних радиохемијских нечистоћа добијеног ^{99m}Tc-MAA примењују се једноставне радиохемијске методе. Тако се 85 % метанол користи као мобилна фаза при папирној (Whatman N^{o} 1) и хроматографији на танком слоју (ITLC-SA). При употреби 3 MM папира, ITLC-SA, ITLC-SG трака и силика-гел плоча као мобилна фаза користи се систем од два растварача: ацетон и 1 M NaCl или 0.9 % NaCl. У раду су такође коришћене нисконапонска електрофореза са тракама Whatman 3 MM папира поквашеним у барбитуратном пуферу, као и гел-хроматографија на колони (Sephadex G-25). У циљу поређења добијених резултата коришћена је и метода филтрације кроз одговарајуће филтре, како је прописано Европском и Југословенском фармакопејом. Резултати добијени за принос обележавања и садржај свих присутних радиохемијских нечистоћа применом наведених метода су у сагласности са прописаним. Одређивање броја добијених честица и њихове расподеле по величини је показало да се од 1 mg људског албумина коришћеног за агрегацију добија 300000 – 350000 честица. Ови резултати потврђују да је квалитет макроагрегата људског албумина припремљених новим поступком знатно побољшан. Након обележавања технецијумом-99m добијен је радиофармацеутик високе радиохемијске чистоће (> 98,5 %), прописаног броја честица са одговарајућом расподелом по величини, те он у потпуности испуњава све услове за рутинску примену у дијагностичке сврхе.

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REFERENCES

1. G. Subramanian, B. A. Rhodes, J. F. Cooper, V. J. Sodd, *Radiopharmaceuticals*, Society of Nuclear Medicine Inc, New York, 1975, p. 265
2. C. B. Sampson, *Textbook of Radiopharmacy. Theory and Practice*, II Ed., Gordon and Breach Science Publishers, USA, 1994, p. 111
3. G. B. Saha, *Fundamentals of Nuclear Pharmacy*, Springer – Verlag, New York, 1979, p. 117
4. *Ibid*. p 96
5. T. Honda, I. Kazem, N. Croll, L. W. Brady, *J. Nucl. Med.* **11** (1970) 580
6. S. Persano, J. Wagner, *Publication IAEA*, Wien, 1978, p. 517
7. W. C. Eckelman, *J. Nucl. Med.* **17** (1976) 865
8. L. Darte, B. R. Persson, L. Soderbom, *Nucl. Med.* **15** (1976) 80

9. E. K. J. Pawels, R. I. J. Feitsma, *Eur. J. Nucl. Med.* **2** (1977) 97
10. J. R. McLean, *J. Nucl. Med.* **19** (1978) 1045
11. V. Jovanović, T. Maksin, D. Konstantinovska, B. Zmbov, J. Čvorić, *J. Radioanal. Chem.* **59** (1980) 239
12. V. Jovanović, J. Čvorić, O. Jovanović, Č. Teofilovski, *Nucl. Med.* **17** (1978) 66
13. A. Johnstone, R. Thrope, *Innunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1987, p. 88
14. *USP (XXIII)*, USP Convention Inc., Rockville, Md, (1999)
15. *III European Pharmacopoeia* (1997)
16. *Pharmacopoeia Yugoslavica*, (Ph. Yug. V), (2000).