

Influence of the radioactive concentration of the *in vitro* stability of Tc-99m(Sn)-pyrophosphate

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The *in vitro* stability of $^{99m}\text{Tc}(\text{Sn})$ -pyrophosphate solution was examined in dependence on the preparation conditions of the samples, the radioactive concentration of ^{99m}Tc in the kit and the time elapsed after labeling. The samples without any protection are highly unstable. The formation of ^{99m}Tc -pertechnetate does not depend on the radioactive concentration. Nitrogen purging provides protection in the case of low radioactive concentrations (37 MBq $^{99m}\text{Tc}/\text{ml}$) but exhibits no effect at higher concentrations. The best stabilization was achieved by using ascorbic acid. A concentration of 60 $\mu\text{g}/\text{ml}$ keeps the content of ^{99m}Tc -pertechnetate below 1% during six hours after labeling, even in solutions of high radioactive concentrations (740–814 MBq/ml). Gentisic acid is less effective. Concentrations about ten times higher than required using ascorbic acid are needed to keep the content of ^{99m}Tc -pertechnetate below 5% during six hours.

Key words: pyrophosphate, technetium-99m, ascorbic acid, gentisic acid, chemical stabilization.

The first step in the production of compounds labeled with ^{99m}Tc is its reduction, usually by stannous ions. Lower, less stable, positively charged states of technetium readily form complexes with different chelating agents. For the routine clinical practice, ligand, reductant and some additives (if needed) are prepared and delivered in the freeze-dried form under vacuum or in an inert atmosphere. The end user should only add ^{99m}Tc activity.

It is known that the radiochemical purity of tin-reduced ^{99m}Tc -labelled radiopharmaceuticals is time dependant. Therefore, their *in vitro* stability is a very important parameter. It should be sufficiently high to ensure their confident and economical use during a reasonable long time after preparation. This time span, denoted also as the shelf life, is usually up to six hours in most nuclear medical departments. During this time the radiochemical purity should preferably be $\geq 95\%$.

The decomposition of ^{99m}Tc -radiopharmaceuticals can be induced by the presence of some agents present in the ^{99m}Tc eluate¹ and/or in the inactive components of the kit. Enhanced investigations were devoted to the eximination of the

effects of the products of the radiolysis of water whereby different free radicals and molecular products, like hydrogen peroxide, are formed.

In a previous paper,² several methods aimed at maintaining a high radiochemical purity of $^{99m}\text{Tc}(\text{Sn})$ -pyrophosphate (PyP) solutions were tested. Oxygen was partially excluded by nitrogen purging and the effect of some chemical stabilizers was also examined. These experiments were performed at low radioactive concentrations of ^{99m}Tc so that the decomposition of the complex was triggered by its exposure to air ("oxygen" conditions) or by the addition of hydrogen peroxide.

However, in routine practice, much higher technetium activities for labeling are needed. In this paper the applicability of these protection methods was examined over a broad region of radioactive concentrations of ^{99m}Tc . The effects of nitrogen purging or the addition of either ascorbic or gentisic acid into the PyP solution containing up to 814 MBq ^{99m}Tc (22 mCi) per ml of the kit were investigated. The aim was to show if these methods would still be effective enough and if the concentrations of the chemical stabilizers which were found sufficient at low radioactive concentrations would still be applicable when the labeling was performed under these experimental conditions.

EXPERIMENTAL

Tetrasodium pyrophosphate anhydrous (PyP, Fluka), L(+)-ascorbic acid (Merck), stannous chloride dihydrate (Merck) and 2,5-dihydroxybenzoic (gentisic) acid (Fluka) were commercial p.a. grade chemicals. For the experiments, fresh solutions of PyP dissolved in $0.154 \text{ mol dm}^{-3}$ NaCl (self-made) and SnCl_2 dissolved in conc. HCl and diluted in bidistilled water were used. The other reagents were also prepared fresh. The ratio ligand/reductant was kept constant so that their concentrations in the kit were $4 \times 10^{-2} \text{ mol dm}^{-3}$ PyP and $9 \times 10^{-4} \text{ mol dm}^{-3}$ SnCl_2 .

The formation of ^{99m}Tc -pertechnetate in the labeled PyP solution was followed in the samples prepared and treated under the following experimental conditions:

- a) kit prepared directly from the reactant solutions and the experiment were performed without any protection ("oxygen" conditions);
- b) kit prepared from solutions of PyP and SnCl_2 previously purged with nitrogen ("nitrogen" conditions); during the experiments the samples were kept in an air atmosphere;
- c) to the kit solution prepared under "oxygen" conditions, small volumes ($\leq 1 \text{ ml}$) of an aqueous solution of either ascorbic or gentisic acid of known concentrations were added; during the experiments the samples were also kept in an air atmosphere.

Labeling was performed by the addition of ^{99m}Tc -eluate obtained by elution of the high activity $^{99}\text{Mo}/^{99m}\text{Tc}$ generators (Vinča Institute of Nuclear Sciences, Laboratory for Radioisotopes).

The elutions were performed in 24-hours intervals using self-made saline solution. The labelings were performed after not longer than 20 min after the elution of the generators.

The experiments were carried out in the following ranges of initial radioactive concentrations of ^{99m}Tc : 18.5–37 MBq (0.5–1 mCi), 370–444 MBq (10–12 mCi), 555–630 MBq (15–17 mCi) and 740–814 MBq (20–22 mCi) per ml of the kit sample.

The final pH of the samples was 7–7.5.

In definite time intervals after addition of ^{99m}Tc activity (15 min, 3 and 6 h) the content of ^{99m}Tc -pertechnetate was determined using ascending paper chromatography (Whatman No.1) with

acetone as the mobile phase. In this system ^{99m}Tc(Sn)-PyP and ^{99m}Tc-hydrolyzate remain at the start ($R_f=0$), while ^{99m}Tc-pertechetate migrates with the solvent front ($R_f=1$). After developing, the paper strips were cut into 1-cm pieces and measured in a gamma scintillation counter (Gamma 3 33, ICN).

RESULTS AND DISCUSSION

It was supposed that among the products of water radiolysis, hydrogen peroxide is the species mainly responsible for the deterioration of the ^{99m}Tc(Sn)-PyP. It could cause the oxidation of the stannous ions, thus preventing the reduction of the heptavalent technetium, and/or the re-oxidation of the already reduced ^{99m}Tc. The extent of its formation depends on the decay time, the radioactive concentration of ^{99m}Tc and the pH of the kit solution.³ It was found that at pH 6 and an initial radioactive concentration of about 7.4 GBq/ml, approximately 0.5 µg H₂O₂/ml is formed when the eluate was allowed to stand for 1.5 h.⁴ Thus, it could be expected that a significant quantity of hydrogen peroxide is generated, particularly when eluates of high radioactive concentrations are allowed to stand for many hours before use.

The yield of hydrogen peroxide also depends on the amount of oxygen dissolved in the water. Hence, the simplest method to protect the labeled preparation would be to suppress the peroxide formation by excluding oxygen. This can be done by purging the reactant solutions with nitrogen ("nitrogen" conditions). It is also important to ensure that no air is introduced into the kit vial.

Table I presents the data obtained with ^{99m}Tc(Sn)-PyP prepared under "nitrogen" conditions for the various ranges of radioactive concentrations of ^{99m}Tc in the kit solution. In practice it is difficult to prevent the introduction of air into the vial. So, in our experiments, this protection method was performed under the most unsuitable conditions. It was restricted to the preparation of the kit solution, *i.e.*, nitrogen purging of the reactant solutions only. After labeling the samples were kept in an air atmosphere.

TABLE I. Formation of ^{99m}Tc-pertechetate in ^{99m}Tc(Sn)-PyP in dependence on the experimental conditions and the time elapsed after labeling. Concentrations: 4×10^{-2} mol dm⁻³ PyP and 9×10^{-4} mol dm⁻³ SnCl₂. (Oxygen: samples prepared directly from the reactant solutions; Nitrogen: reactant solutions previously purged with nitrogen). Initial ^{99m}Tc radioactive concentration ranges: 18.5–37 and 370–444 MBq/ml of the kit

Time after labeling, h	Initial radioactive concentration, MBq ^{99m} Tc/ml of the kit	Content of ^{99m} Tc-pertechetate, %	
		"Oxygen" samples	"Nitrogen" samples
0.25	18.5–37	2.8±1.2	1.0±0.2
	370–444	4.4±1.4	3.8±1.12
3	18.5–37	10.1±0.8	2.4±0.9
	370–444	9.2±1.2	8.4±1.2
6	18.5–37	14.7±1.2	5.7±2.2
	370–444	14.2±2.5	–

For comparison, the content of hydrogen peroxide in the samples prepared without any protection ("oxygen" conditions) is shown. These samples were prepared simply by dissolving and dispensing of the corresponding chemicals.

As the measure of the efficiency of the applied stabilization procedure, an arbitrary upper limit of 5% of ^{99m}Tc -pertechnetate was taken. This means that a given procedure is considered satisfactory if not more than 5% of ^{99m}Tc -pertechnetate is formed in the kit solution during six hours.

From the results shown in Table I it can be concluded that the samples prepared under the "oxygen" conditions are highly unstable. The formation of ^{99m}Tc -pertechnetate does not depend on the radioactive concentration. Similar values were obtained for all the examined ranges of radioactive concentrations.

Nitrogen purging of the reactant solutions is efficient only at low ^{99m}Tc radioactive concentrations (up to 37 MBq/ml). Under these conditions the $^{99m}\text{Tc}(\text{Sn})\text{-PyP}$ is stable for up to six hours after labeling. However, at higher radioactive concentrations of ^{99m}Tc this protection method is no longer efficient.

The second stabilization procedure was the addition of either ascorbic or gentisic acid into the PyP solution which was then labeled with ^{99m}Tc . The samples were prepared under "oxygen" conditions. The contents of these chemical stabilizers given in Table II and III represent the necessary concentration minimum of the given stabilizer to achieve the desired protecting effect.

TABLE II. Effect of ascorbic acid on the formation of ^{99m}Tc -pertechnetate in $^{99m}\text{Tc}(\text{Sn})\text{-PyP}$ prepared under "oxygen" conditions, in dependence on the initial ^{99m}Tc radioactive concentration and the time elapsed after labeling. Concentrations: $4 \times 10^{-2} \text{ mol dm}^{-3}$ PyP and $9 \times 10^{-4} \text{ mol dm}^{-3}$ SnCl_2

Initial radioactive concentration, MBq $^{99m}\text{Tc}/\text{ml}$ of the kit	Content of ascorbic acid, $\mu\text{g}/\text{ml}$ of the kit	Time after labeling, h		
		0.25	3	6
18.5–37	5	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.1
370–444	10	0.1 ± 0.02	0.3 ± 0.1	0.4 ± 0.1
555–630	30	0.7 ± 0.2	0.4 ± 0.2	1.0 ± 0.2
740–814	60	0.1 ± 0.05	0.4 ± 0.1	0.4 ± 0.1

In Table II the influence of ascorbic acid on the stability of $^{99m}\text{Tc}(\text{Sn})\text{PyP}$ is given. It can be seen that this chemical is a very efficient stabilizer already at low concentrations. Even in the highest range of the radioactive concentration of ^{99m}Tc (740–814 MBq/ml) only 60 μg of ascorbic acid per ml of the kit is sufficient to keep the content of ^{99m}Tc -pertechnetate below 1% during six hours.

The effect of gentisic acid is presented in Table III. The result show that, in comparison with ascorbic acid, the efficiency of gentisic acid is lower. Already at the lowest radioactive concentration range the content of pertechnetate in the presence of 50 μg of gentisic acid was 1–2%. In the highest examined range

(750–814 MBq ^{99m}Tc/ml) 500 µg/ml was needed to keep the content of ^{99m}Tc-pertechnetate below 5% during six hours.

TABLE III. Effect of gentisic acid on the formation of ^{99m}Tc-pertechnetate in ^{99m}Tc(Sn)-PyP prepared under "oxygen" conditions, in dependence on the initial ^{99m}Tc radioactive concentration and the time elapsed after labeling. Concentrations: 4×10^{-2} mol dm⁻³ PyP and 9×10^{-4} mol dm⁻³ SnCl₂

Initial radioactive concentration, MBq ^{99m} Tc/ml of the kit	Content of gentisic acid, µg/ml of the kit	Time after labelling, h		
		0.25	3	6
18.5–37	50	1.2±0.2	0.8±0.2	1.8±0.2
370–444	80	1.0±0.2	2.7±0.4	6.7±2.1
370–444	120	1.0±0.2	2.7±0.5	2.9±0.5
740–814	300	—	3.6±0.6	5.6±0.5
740–814	500	0.6±0.2	1.7±0.3	2.9±0.5

CONCLUSIONS

The results show that the unprotected ^{99m}Tc(Sn)-PyP kit solution (prepared under "oxygen" conditions) is unstable. Already in the first hour after labeling a considerable amount of ^{99m}Tc-pertechnetate is found. Its formation seems to be practically independent of the radioactive concentration.

The common protection procedure of nitrogen purging was effective only for kit solutions of low radioactive concentration. Taking arbitrarily the formation of 5% of free ^{99m}Tc-pertechnetate during six hours after labeling as a tolerable level, it can be seen that this method is reliable for at least 3 h. However, already in the next higher radioactive concentration range (370–444 MBq ^{99m}Tc/ml), this method of stabilization is not applicable.

The antioxidants ascorbic and gentisic acids can be successfully used for the stabilization of ^{99m}Tc(Sn)-PyP. However, already in the lowest radioactive concentration range ascorbic acid is more efficient. A similar conclusion can also be reached by comparing the results of the kit stability at higher concentrations of ^{99m}Tc radioactivity. By increasing the concentration of ascorbic acid up to 60 µg/ml, the content of ^{99m}Tc-pertechnetate is max. 1% even when the radioactive concentration was 814 MBq/ml.

Gentisic acid was found to be less efficient. In addition, the content of gentisic acid should be about ten times higher than that of ascorbic acid. When the radioactive concentration of ^{99m}Tc was raised up to 740–814 MBq/ml, it was necessary to add 500 µg of gentisic acid per ml of the kit solution to keep the content of ^{99m}Tc-pertechnetate below 5%. At these concentrations of gentisic acid a coloration of the kit solution appeared. It would be necessary to examine whether this content of stabilizer has eventually some side-effects, *e.g.*, a change in the biodistribution.

Generally, it was observed that with increasing radioactive concentration the content of both chemical stabilizers also has to be increased. This increase is much higher than expected taking into account only the supposed radiation-induced production of hydrogen peroxide. This indicates that the assumed reaction mechanism in the kit solution of high radioactive concentration is much more complex.

The difference in the efficiency of ascorbic and gentisic acid is probably due to their chemical structure. Ascorbic acid system is a combination of various components with different chemical properties. Some of them are very efficient radical scavengers. The reactions of gentisic acid, a derivative of salicylic acid, under the given experimental conditions need further investigation.

ИЗВОД

УТИЦАЈ РАДИОАКТИВНЕ КОНЦЕНТРАЦИЈЕ НА *IN VITRO* СТАБИЛНОСТ Tc-^{99m}(Sn)-ПИРОФОСФАТА

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Испитивана је *in vitro* стабилност раствора ^{99m}Tc(Sn)-пирофосфата (PyP) у зависности од услова припреме узорка, радиоактивне концентрације ^{99m}Tc и времена протеклог од обележавања. Узорци без заштите су нестабилни. Садржај ^{99m}Tc-пертехнетата у њима расте са временом практично независно од радиоактивне концентрације. Заштита барботирањем азотом раствора реактанта је ефикасна само код ниских радиоактивних концентрација (37 MBq/ml) док код виших нема више ефекта. Антиоксиданси аскорбинска и 2,5-дихидрокси бензоева киселина утичу на стабилност обележеног препарата. Аскорбинска киселина је ефикаснија. И код највећих испитиваних радиоактивних концентрација (740–814 MBq/ml) потребно је само око 60 µg/ml да садржај ^{99m}Tc пертехнетата не пређе 1% током целог испитиваног периода (6 h). 2,5-Дихидрокси бензоева киселина је мање ефикасна и потребне су знатно веће концентрације да садржај ^{99m}Tc-пертехнетата у препарату не пређе 5% током периода испитивања. Са повећањем радиоактивне концентрације повећава се и концентрација хемијских стабилизатора потребна да би се постигао тражени заштитни ефекат.

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REFERENCES

1. J. Vučina, D. Vuga, N. Vanlić-Razumenić, *J. Radioanal. Nucl. Chem., Letters* **186** (1994) 333
2. J. L. Vučina, *J. Serb. Chem. Soc.* **62** (1997) 137
3. V. J. Molinski, *J. Int. Appl. Radiat. Isotopes* **33** (1982) 811
4. J. L. Vučina, Lj. M. Jaćimović, S. M. Milenković, *Radiol. Jugosl.* **17** (1983) 387.