

Research Article

Physico-chemical characterisation of ^{99m}Tc -tin fluoride colloid agent used for labelling white cells

Chris Tsopelas*

RAH Radiopharmacy, Nuclear Medicine Department, Royal Adelaide Hospital, RAH Radiopharmacy, North Terrace, Adelaide, South Australia

Summary

^{99m}Tc -tin fluoride colloid is an agent used to label leucocytes, for the imaging and diagnosis of inflammatory conditions including Crohn's disease. Despite previous investigations, this radiolabelling agent is still poorly characterised. The aim of this work was to examine the process of formation and stability of ^{99m}Tc -tin fluoride colloid using mass spectrometry, membrane filtration and atomic absorption spectrophotometric techniques. Tin-oxide bonds in tin clusters were identified in the stannous fluoride reagent vial by mass spectrometry. From radioactive particle size distribution experiments, the facile disruption of radiocolloid particles with excess oxygen gas contrasted to the partial hydrolysis of Sn(II) during the formation process. Under the standard conditions, 10% of particles were determined as 1–3 μm , and this population coordinated 96% of the ^{99m}Tc added. Colloid particle formation and the reduction of ^{99m}Tc -pertechnetate is discussed. Sodium fluoride may optimise 1–3 μm radioactive particle size, by regulating particle growth. ^{99m}Tc -tin fluoride colloid is affected by positive or negative charge, as either Al, Mo ions or solid membranes, resulting in either coagulation and/or deflocculation of the particles. Copyright © 2006 John Wiley & Sons, Ltd.

Received 23 January 2006; Revised 5 March 2006; Accepted 6 March 2006

Key Words: ^{99m}Tc -tin colloid; particle size; leucocytes; mechanism

Introduction

For almost 20 years ^{99m}Tc -tin fluoride colloid has been used in Australia, and more recently in Europe, to successfully radiolabel patient leucocytes, for the clinical diagnosis of infection and inflammation, particularly inflammatory

*Correspondence to: Chris Tsopelas, Nuclear Medicine Department, Royal Adelaide Hospital, RAH Radiopharmacy, North Terrace, Adelaide, South Australia. E-mail: ctsopela@mail.rah.sa.gov.au

bowel disease. The preparation of this radiocolloid usually involves combining sodium fluoride and stannous fluoride solutions, and then adding a portion of that liquid to diluted ^{99m}Tc -pertechnetate. The radiolabelling reaction is complete in approximately 1 h, when >95% of the initial radioactivity is consumed to form ^{99m}Tc -tin fluoride colloid. In the second phase of the procedure, the radiotracer is incubated with a sample of patient whole blood *ex vivo*, and the neutrophils bind radioactivity either by a phagocytic mechanism¹, or by specific-cell surface adherence.² When radioactive blood is administered back into the same patient, ^{99m}Tc -neutrophils follow their normal course in the circulation and participate in the acute phase response at an inflammatory site.

The product is commercially available as pharmaceutical cold kits, in which the stannous fluoride component can be presented as lyophilised or non-lyophilised forms.^{3–6} The radiocolloid is easily and efficiently prepared from either formulation at room temperature. ^{99m}Tc -tin fluoride colloid was shown to have a particle size of $2.6 \pm 0.5 \mu\text{m}$ by autoradiography of cell suspensions,⁷ or a mean value of $\sim 2.1 \mu\text{m}$ by membrane filtration.³ The particle size has been reported to decrease ($\sim 1 \mu\text{m}$) from shear deflocculation within the syringe needle during its preparation,⁵ or increase ($> 3 \mu\text{m}$) to form macroaggregates either in a static environment⁸ or with prolonged vigorous shaking.⁹ Even though ^{99m}Tc -tin fluoride colloid has been extensively employed in nuclear medicine to radiolabel leucocytes, the chemistry of this radiopharmaceutical agent is not clearly understood. The aim of this study was to investigate the formation and stability of ^{99m}Tc -tin fluoride colloid particles.

Methods

General

Sodium ^{99m}Tc -pertechnetate was obtained from the daily milking of a $^{99}\text{Mo}/^{99m}\text{Tc}$ -generator (Gentech, Australian Radioisotopes, NSW, Australia). Sodium ^{18}F -fluoride was obtained from a manufacturer (Austin Health, VIC, Australia). LWC Kit [A + B] (RAH Radiopharmacy, SA, Australia) contained stannous fluoride as a frozen formulation (vial B), and was used in all experiments. The aluminium (III) ion level ($\mu\text{g}/\text{mL}$) in the generator eluent was determined using a colourimetric test kit (Microquant A1 Aluminium Test, Merck, Darmstadt, Germany). The pH of radiocolloid dispersions was determined using a pH meter (TPS, 901-pH) with a glass electrode (Sentek, p13/40 mm) within 30 min of preparation. The electrode was pre-calibrated with pH standards (4.00, 6.88) and used when the instrument slope exceeded 93%. The following filters were used for membrane filtration: $5 \mu\text{m}$ polycarbonate (PC, Nucleopore, CA, USA), $5 \mu\text{m}$ hydrophilic acrylic copolymer (Versapor[®], Gelman, MA, USA), $3 \mu\text{m}$ PC (Nucleopore), $1 \mu\text{m}$

teflon (PTFE, Gelman), 0.2 μm PC (Nucleopore), 0.2 μm polysulfone (PS, Whatman; CP; Singapore), 0.2 μm cellulose acetate (CE, Advantec MFS; CA; USA), 0.2 μm PTFE (Advantec MFS), and 0.2 μm hydrophilic polyvinylidene fluoride with cellulose ester layer (Durapore[®], Millipore; MA; USA). Colloid size is described by particle diameter (μm). Results from experiments performed $n \geq 3$ are reported as mean \pm standard error.

Mass spectrometry

Mass spectra of tin fluoride samples were obtained by the electrospray technique using a mass spectrometer (Quattro micro, Waters/Micromass, Manchester, UK). Stannous fluoride was thawed under the nitrogen atmosphere of vial B, and undiluted samples were subsequently analysed at 0 and 2 h.

Particle size distribution (PSD)

Atomic absorption (AA) spectrophotometry. Cold colloid was prepared in the same way as ^{99m}Tc -labelling is described below, with the exception that saline replaced ^{99m}Tc -pertechnetate. The tin fluoride colloid samples (2 ml) were filtered through 5, 1 and 0.2 μm PC filters (pre-rinsed with saline, 2 ml) into volumetric flasks (10 ml), and rinsed with saline (1–2 ml). Each filtrate was then diluted to volume with HCl (1 M; 6–7 ml). The samples were analysed for % Sn content by AA spectrophotometry (nitrous oxide-acetylene flame) with reference to an unfiltered, non-radioactive tin fluoride colloid standard.

Radiolabelling and quality control

^{99m}Tc -tin fluoride colloid was prepared using LWC Kit [A + B]. Vial A contained sodium fluoride (6.25 mg) in water for injection (WFI; 5 ml) stored at 2–8°C, and vial B contained SnF_2 (0.64 mg) in WFI (1 ml) stored in a freezer. The standard procedure involved adding vial A (4 ml) to thawed vial B, and after mixing for ~ 10 s, the colourless dispersion was then filtered (0.2 μm , CE) into a sterile vial. This filtrate (0.5 ml) was added to a syringe (5 ml) containing ^{99m}Tc -pertechnetate (40 MBq) in saline (0.9%; 2.5 ml) with an air space (~ 1 ml), and then the contents of the syringe were mixed by rotation (~ 40 rpm) at room temperature for 50–60 min using a rotation apparatus (RSM6 suspension mixer, Ratek Instruments, VIC, Australia). Radiocolloid was used within 30 min of preparation. Radiolabelling efficiency (RE) of the radiopharmaceutical was determined by ascending instant thin layer paper chromatography with normal saline as the eluent. ^{99m}Tc -colloid remained at the origin ($R_f = 0.0$), ^{99m}Tc -pertechnetate migrated with the solvent front ($R_f = 1.0$). Radioactive paper sections (1 cm) were counted in a

γ -counter (Cobra II Auto-Gamma, Canberra Packard, Victoria, Australia) and % RE was calculated as $100\% - \% {}^{99m}\text{Tc}$ -pertechnetate activity.

Radiolabelling with sodium ${}^{18}\text{F}$ -fluoride. The procedure involved adding sodium ${}^{18}\text{F}$ -fluoride (25 MBq) diluted in WFI (1 ml) to vial B (1 ml) and after ~ 10 s of mixing, the dispersion was filtered ($0.2\ \mu\text{m}$, CE) into a vial that was rotated (~ 40 rpm) for 50 min at RT. RE of the product was determined by ITLC with normal saline as the eluent. Stannous ${}^{18}\text{F}$ -fluoride colloid remained at the origin ($R_f = 0.0$), and sodium ${}^{18}\text{F}$ -fluoride was identified to migrate with the solvent front ($R_f = 1.0$) in reference experiments. Radioactive paper sections were counted in the γ -counter over 300–800 keV for 15 s/section, without decay correction. % RE was calculated as $100\% - \% \text{ sodium } {}^{18}\text{F}\text{-fluoride activity}$.

Radioactive PSD (RPSD). Radioactive products were analysed by membrane filtration using $5\ \mu\text{m}$ PC, $3\ \mu\text{m}$ PC, $1\ \mu\text{m}$ PTFE, and $0.2\ \mu\text{m}$ PS, CE or PTFE filters. All filters were pre-rinsed with saline (2 ml). Typically, a radiocolloid sample (0.2 ml) was filtered, then the filter was rinsed with saline (2 ml). The filter and filtrates were each counted to determine the % activity above and below the filter size, and the values were used to calculate % RPSD. % RPSD was also determined for ${}^{99m}\text{Tc}$ -colloid prepared: (i) without the $0.2\ \mu\text{m}$ filtration step; (ii) in an oxygen-free atmosphere, in a sterile nitrogen-filled vial (10 ml); (iii) with excess oxygen gas bubbled into the dispersion during 50 min.

Influence of sodium fluoride. The level of sodium fluoride during the ${}^{99m}\text{Tc}$ -labelling procedure was varied to: 0.0 mg (WFI; 1 ml), 1.0 mg in WFI (1 ml), or 5.0 mg in WFI (1 ml).

Influence of filter membranes. As above, ${}^{99m}\text{Tc}$ -colloid samples (0.2 ml) were each filtered with $5\ \mu\text{m}$ Versapor[®] and $0.22\ \mu\text{m}$ Durapore[®] membranes and then rinsed. The filter and filtrates were each counted to determine the % RPSD.

Influence of cations. ${}^{99m}\text{Tc}$ -tin fluoride colloid (3 ml) prepared under standard conditions contained 22 mg of NaCl. The following salts were added to the radiotracer in separate experiments: (i) normal saline (9 mg/ml; 2 ml; pH 6.3); (ii) calcium chloride dihydrate in saline (1 mg/ml; 0.8 ml; pH 6.5); (iii) potassium chloride in saline ($47\ \mu\text{g}/\text{ml}$; 0.8 ml); (iv) sodium sulphate in saline ($47\ \mu\text{g}/\text{ml}$; 0.8 ml); (v) aluminium potassium sulphate dodecahydrate in saline ($47\ \mu\text{g}/\text{ml}$; 0.2 ml or 0.4 ml or 0.8 ml; pH 6.1) or (vi) disodium molybdate dihydrate in saline ($46\ \mu\text{g}/\text{ml}$; 0.8 ml; pH 6.2) at room temperature (22°C) for 5 min. Each dispersion was filtered ($3\ \mu\text{m}$ PC; $1\ \mu\text{m}$ PTFE) and the extent of

particle deflocculation was determined as the percentage of total counts in the filtrate.

Results

General

The concentration of aluminium (III) ions in the generator eluent (10 ml) was measured at five occasions during the study and found to be $0.30 \pm 0.06 \mu\text{g/ml}$, a value below the manufacturer's specification ($5 \mu\text{g/ml Al}^{3+}$) and the British Pharmacopoeial limit of $2 \mu\text{g/ml Al}^{3+}$.¹⁰ The pH of ^{99m}Tc-tin fluoride colloid was found to be 6.1 ± 0.1 ($n = 9$).

Mass spectrometry

Molecular cations that contained tin were assigned to the following fragments. *M/e*: $\text{Sn}_4\text{F}_5(\text{OH})_2$ (609), $\text{Sn}_4\text{F}(\text{OH})_5$ (584), $\text{Sn}_3\text{F}_3(\text{OH})_2$ (451), $\text{Sn}_3(\text{OH})_4$ (428), $\text{Sn}_3\text{F}(\text{OH})_2$ (413), Sn_2F_3 (297), $\text{Sn}_2(\text{OH})_2$ (274), Sn_2F (259), SnF (139), SnOH (137), SnH (121). The sequence of molecular ions of $t = 0$ and 2 h samples were identical. The peak intensity ratios of $\text{Sn}_4\text{F}(\text{OH})_5$ to $\text{Sn}_4\text{F}_5(\text{OH})_2$, $\text{Sn}_3(\text{OH})_4$ to $\text{Sn}_3\text{F}_3(\text{OH})_2$, $\text{Sn}_2(\text{OH})_2$ to Sn_2F_3 , were higher in the $t = 2$ h sample (at room temperature) spectrum by an average of $68 \pm 7\%$, than in the $t = 0$ h sample spectrum.

PSD

Preliminary experiments with size excluded filtrates (0.2, 1 and 5 μm PC filters) using ultraviolet spectrophotometry found this analysis technique to be unsuitable for assaying stannous fluoride. Atomic absorption spectrophotometry gave % tin content for each of the following size-excluded filtrates: 5 μm : $100.1 \pm 1.6\%$ ($n = 15$); 3 μm : $100.6 \pm 1.5\%$ ($n = 15$); 1 μm : $89.8 \pm 1.6\%$ ($n = 22$); 0.2 μm : $100.8 \pm 2.0\%$ ($n = 23$); and 0.02 μm : $51.1 \pm 1.5\%$ ($n = 7$). These values were used to calculate the PSD of tin fluoride colloid (Table 1). The trimodal population comprised of 10% particles in the 1–3 μm range, 45% of particles were 20–200 nm, and 47% of tin was < 20 nm as soluble and/or particulate material.

Table 1. PSD of tin fluoride colloid

Size range (μm)	AA
> 5	Not detected
3–5	Not detected
1–3	10.3%
0.2–1	Not detected
0.02–0.2	44.5%
<0.02	46.6%

Radiolabelling efficiency (RE)

The RE of ^{99m}Tc -tin fluoride colloid was $98.8 \pm 0.4\%$ ($n = 11$). After mixing concentrated Na^{18}F with stannous fluoride (vial B) for 50 min, $99.9 \pm 0.0\%$ ($n = 3$) radioactivity was detected at the solvent front by ITLC, indicating colloidal material at the strip origin was absent of radioactive (and non-radioactive) fluoride.

% RPSD – influence of sodium fluoride

Results of the RPSD of ^{99m}Tc -tin fluoride colloid when prepared under the standard (oxygen-containing) or oxygen-free (nitrogen) atmosphere, are shown in columns 4 and 5 of Table 2, respectively. Under the standard conditions where ~ 1 ml of air ($\sim 21\%$ as oxygen) was present in the syringe, more than 96% of the ^{99m}Tc -activity was found to be associated with the 1–3 μm size particles, plus minor populations of 3–5 μm or 0.2–1 μm . The oxygen-free atmosphere gave 95% 1–3 μm particles. A lower sodium fluoride level resulted in a decreased population of the 1–3 μm ^{99m}Tc -particles, as well as an increase in larger and smaller particle size ranges (cf. 5 mg versus 1 mg versus 0 mg NaF). In the absence of a 0.2 μm filtration step, 12% of radioactive particles detected were $> 3 \mu\text{m}$, indicating that larger particles were present in the cold kit shortly after it was thawed. Excess oxygen bubbled into the dispersion yielded $> 90\%$ activity as less than 1 μm particles.

% RPSD – influence of filter membranes

The membrane composition of the filter used in the filtration test, did affect the RPSD results. The positive charged membrane surfaces (Table 3) gave higher levels of either larger radiocolloid (67% $> 5 \mu\text{m}$) or smaller radiocolloid (25%; $< 0.22 \mu\text{m}$), indicating particle coagulation or deflocculation had occurred with each membrane, respectively. In conjunction with the results from

Table 2. % RPSD of ^{99m}Tc -tin fluoride colloid prepared \pm sodium halide

Size range (μm)	% RPSD					
	0 mg NaF ($n = 3$)	1 mg NaF ($n = 3$)	5 mg NaF ($n = 6$)*	5 mg NaF, $-\text{O}_2$ ** ($n = 3$)	5 mg NaF, $-0.2 \mu\text{m}$ *** ($n = 3$)	5 mg NaF, excess O_2 ($n = 3$)
> 3	$7.8 \pm 0.6\%$	$8.3 \pm 0.6\%$	$3.1 \pm 0.3\%$	$2.4 \pm 0.1\%$	$11.7 \pm 0.5\%$	$4.9 \pm 0.8\%$
1–3	$75.9 \pm 1.5\%$	$87.5 \pm 0.6\%$	$95.6 \pm 0.4\%$	$95.4 \pm 0.1\%$	$86.9 \pm 0.5\%$	$3.9 \pm 0.3\%$
< 1	$16.3 \pm 0.9\%$	$4.2 \pm 0.1\%$	$1.2 \pm 0.4\%$	$2.2 \pm 0.1\%$	$1.4 \pm 0.7\%$	$91.2 \pm 1.0\%$

*1% $> 5 \mu\text{m}$, 2% 3–5 μm , 96% 1–3 μm , 1% 0.2–1 μm , 0% $< 0.2 \mu\text{m}$ (standard conditions).

**1% $> 5 \mu\text{m}$, 1% 3–5 μm , 95% 1–3 μm , 2% 0.2–1 μm , 0% $< 0.2 \mu\text{m}$ (oxygen-free atmosphere).

***9% $> 5 \mu\text{m}$, 3% 3–5 μm , 87% 1–3 μm , 1% 0.2–1 μm , 0% $< 0.2 \mu\text{m}$ (no filtration step).

Table 3. Filter membrane adsorption of ^{99m}Tc -tin fluoride colloid

Pore size (μm)	Filter membrane composition	Membrane surface charge	Filter activity	<i>n</i>
5	PC	Neutral	$0.9 \pm 0.1\%$	6
5	Versapor [®]	Positive	$66.2 \pm 3.0\%$	6
0.2	PTFE	Neutral	$99.0 \pm 0.2\%$	3
0.2	PS	Neutral	$99.4 \pm 0.1\%$	6
0.2	CE	Neutral	$99.9 \pm 0.1\%$	3
0.22	Durapore [®]	Positive	$75.2 \pm 3.4\%$	4

Table 2, ^{99m}Tc -tin fluoride colloid was stable after exposure to the neutral membranes polycarbonate, teflon, cellulose acetate and polysulfone.

% RPSD – Influence of ions

The effect of soluble ions on the radiocolloid stability is shown in Table 4. A surplus of sodium chloride (18 mg) in the standard preparation did not significantly affect the RPSD, where 94–96% of radiocolloid was 1–3 μm in size. Addition of Ca^{2+} resulted in a small increase of $\sim 6\%$ larger particles, but most were unchanged in the 1–3 μm range. The potential ^{99m}Tc -generator impurities Al^{3+} and MoO_4^{2-} did alter the RPSD after a brief exposure time. Lower levels ($< 19 \mu\text{g}$) of aluminium ion had no effect on the RPSD but at 38 μg , there was minor deflocculation and coagulation. The counter ions K^+ and SO_4^{2-} from aluminium potassium sulphate, each had little effect on altering the radiocolloid size, with the anion increasing the $< 1 \mu\text{m}$ particles by 1–2%. Molybdate caused more coagulation compared to Al^{3+} , to the extent of 15% and the dispersion was colourless.

Discussion

This study was designed to further characterise ^{99m}Tc -tin fluoride colloid, primarily on the basis of size. The results are taken together and discussed in terms of the synthesis of radiocolloid particles as well as their stability to the ionic environment.

Radiocolloid formation

Under the standard conditions to prepare ^{99m}Tc -tin fluoride colloid, the product of high radiolabelling efficiency (99%) was obtained consistently. Radioactive particle size analyses by membrane filtration measured more than 95% of radiocolloid particles as 1–3 μm . When the 0.2 μm filtration step was omitted from the preparation, a higher proportion of larger ($> 3 \mu\text{m}$) ^{99m}Tc particles was detected, in agreement with an earlier report.¹¹ These results indicate this filtration step is important for initially removing the larger

Table 4. % RPSD of ^{99m}Tc -tin fluoride colloid after the addition of ions

Size range (μm)	RPSD after \pm ions (mass of salt above 22 mg Na^+)*									
	Na^+ , 0 mg ($n = 3$)	Na^+ , 18 mg ($n = 3$)	K^+ , 38 μg ($n = 3$)	Ca^{2+} , 1 mg ($n = 3$)	Al^{3+} , 9 μg ($n = 3$)	Al^{3+} , 19 μg ($n = 3$)	Al^{3+} , 38 μg ($n = 3$)	MoO_4^{2-} , 37 μg ($n = 3$)	SO_4^{2-} , 38 μg ($n = 3$)	
> 3	3.1 \pm 0.3%	5.9 \pm 0.1%	4.5 \pm 0.8%	8.9 \pm 1.3%	3.1 \pm 0.5%	3.0 \pm 0.4%	13.0 \pm 0.8%	14.9 \pm 0.8%	3.6 \pm 0.2%	
1–3	95.6 \pm 0.4%	94.0 \pm 0.1%	94.9 \pm 0.9%	90.5 \pm 1.2%	96.5 \pm 0.4%	96.7 \pm 0.4%	77.4 \pm 0.8%	80.6 \pm 0.6%	94.2 \pm 0.6%	
< 1	1.2 \pm 0.4%	0.2 \pm 0.1%	0.6 \pm 0.1%	0.5 \pm 0.1%	0.4 \pm 0.1%	0.3 \pm 0.1%	9.6 \pm 0.5%	4.5 \pm 0.3%	2.3 \pm 0.4%	

* standard preparation contains 22 mg NaCl.

particles, and leaving behind <200 nm particles available for reaction with ^{99m}Tc -pertechnetate.

In the synthesis of colloidal particles, two major events occur in sequence that include primary particle formation and growth.¹² If ^{99m}Tc -pertechnetate is also present in solution, there is the simultaneous reduction and coordination of ^{99m}Tc to the particle matrix. The primary colloidal particles are formed when concentrated areas of stannous fluoride molecules in solution, or clusters, ultimately precipitate (nucleation). They behave as templates for further growth, in a process that involves the accumulation of remaining soluble molecules at the sol surface. Primary particles of ^{99m}Tc -tin colloid were reported as having a ~ 0.4 μm diameter,⁵ or a mean particle size of 234 ± 22 nm at reconstitution that increases to 2.3 ± 0.8 μm with agitation 4 h later.¹³ For ^{99m}Tc -tin fluoride colloid, the primary colloidal particles exist in the filtrate after the 0.2 μm filtration step, and prior to ^{99m}Tc -pertechnetate reconstitution. This is supported with evidence from the PSD data derived from non-radioactive membrane filtration experiments, and mass spectrometry that identified higher order Sn ions. Most ($\sim 90\%$) of the particle population was assayed to be <200 nm in diameter, and the remainder was in a tight size range of 1 – 3 μm . The low percentage (10%) of these particles accounted for $>95\%$ of radioactivity when ^{99m}Tc -pertechnetate was used in the procedure.

The chemical composition of ^{99m}Tc -tin fluoride colloid is currently unknown. Stannous fluoride is a weak acceptor molecule towards neutral donor molecules,¹⁴ or in the presence of air at room temperature, yields tin oxyfluoride.¹⁴ Many other hydrolysis products have been reported¹⁵ including $\text{SnO}\cdot\text{SnF}_2\cdot 3\text{H}_2\text{O}$, $\text{Sn}_2\text{OF}_2\cdot x\text{H}_2\text{O}$ ($x = 0, 3, 4, 6$) and $3\text{SnO}\cdot 2\text{SnF}_2\cdot 6\text{H}_2\text{O}$. The mass spectrometry data identified Sn–O bonds in the stannous fluoride formulation, before the addition of sodium fluoride or ^{99m}Tc -pertechnetate. Further hydrolysis occurred at room temperature over time, based on the detection of more molecular ion $\text{Sn}_4\text{F}(\text{OH})_5$ than $\text{Sn}_4\text{F}_5(\text{OH})_2$ in the 2 h sample. However, excessive oxidation of Sn(II) by oxygen gas resulted in dissociation of radiocolloid into smaller particles ($>90\%$ <1 μm , Table 2). The hydrolysis reaction rather than oxidation, appears to be important in ^{99m}Tc -tin fluoride colloid formation and growth, yielding Sn–O–Sn or $\text{Sn}^{\text{II}}\text{–OH}$ bonds, although not exclusively throughout the particle structure. Obviously, there must be sufficient Sn(II) at the particle surface to reduce ^{99m}Tc -pertechnetate during radiolabelling. Other colloidal tin compounds include stannic oxide (SnO_2) and stannic acids (H_2SnO_3 , $\text{H}_{10}\text{Sn}_5\text{O}_{15}$).¹⁵ Perhaps an earlier report of ‘inconsistent and unsatisfactory’ ^{99m}Tc -products¹⁶ obtained from single kit formulations, could be explained by the presence of excessive oxidised tin.

During the preparation of ^{99m}Tc -tin fluoride colloid, the mixing/agitation step was achieved by rotation of the radioactive syringe containing a mobile

air bubble. When the procedure was repeated in the absence of oxygen, the RPSD of the product was essentially identical to that radiocolloid prepared under standard conditions. This means the oxygen in the air space did not significantly contribute to colloid oxidation, and supports hydrolysis by water in the aqueous dispersion.

The ionic species SnF_3^- , SnF^+ and Sn_2F_5^- are known to exist in a solution of stannous fluoride dissolved in a molar excess of fluoride ions,¹⁷ and they would be expected to form after a combination of the LWC Kit reagents. However there was no reaction between the contents of vial B and $^{18}\text{F}^-$, indicating fluoride ions do not comprise the tin fluoride colloid matrix, and that sodium fluoride in vial A during the standard procedure provides sodium cations for the particle surface. Ionised atoms at the surface must be negatively charged to bind Na^+ , and these are likely to be oxygen from $\text{Sn}-\text{O}^-$ bonds. The electrostatic surface charge is the electrical double layer, and this inherent property governs the stability and binding characteristics of a colloid. Tin fluoride colloid particularly, binds by surface adhesion to glass¹³ or to different cell types in whole blood⁷ such that the particle structure is conserved.

It was previously reported that Na^+ increases the aggregation rate of tin fluoride colloid prepared after 0.2 μm filtration.⁵ In these experiments surplus sodium chloride or potassium chloride had no effect on the RPSD (Table 4, Na^+ 0–18 mg and K^+ 38 μg), unlike sodium fluoride (0–5 mg) that decreased the <1 μm particle population from 16 to 1%, respectively, due to aggregation into 1–3 μm particles. Interestingly, the >3 μm particles decreased by 8–3% over the same mass range of NaF, respectively, implying that a minor effect of fluoride ions in the particle forming environment could also be regulating particle growth. Furthermore, it is unclear whether the growth phase is defined as an association of many 200 nm template particles to produce one larger 1–3 μm particle, or that each template particle enlarges to a diameter of 1–3 μm . From the literature, colloid deflocculation or aggregation from physical manipulation indicates certain chemical bonds in the colloid are weak,⁵ and implies that particle growth might involve the association of many template particles. Despite the ambiguity of the tin fluoride growth mechanism, one event must occur after exposure to $^{99\text{m}}\text{Tc}$ -pertechnetate: Sn(II) in the particle matrix reduces $^{99\text{m}}\text{Tc}$ to a favourable oxidation state for coordination with charged atoms such as oxide on the surface.

Radiocolloid stability

Membrane filtration was employed to examine the size distributions of $^{99\text{m}}\text{Tc}$ -tin fluoride colloid. This rapid and reliable technique has the advantages of measuring the size of bulk particles in their traditional environment, and it avoids the use of an energy beam (cf: microscopy) for the analysis. The method

measures size as volume diameter and assumes particles are approximately spherical, but is limited if the sample contains irregularly shaped particles including laminates or fibrils.¹² Membrane filtration was previously used to examine the hydrophobic colloids ^{99m}Tc -antimony trisulphide and ^{99m}Tc -rhenium sulphide.¹⁸ Both of these agents were poorly adsorbed to membranes of varying compositions, including those which were charged. In contrast, ^{99m}Tc -tin fluoride colloid was found to be incompatible with the positively charged membranes (Table 3), where a substantial portion of the total particles became coagulated or deflocculated. This radiocolloid was unaffected by the neutral filter membranes cellulose acetate, teflon, polysulfone and polycarbonate. When metal ions were added to the radioactive particles, the higher oxidation state Al^{3+} and MoO_4^{2-} species altered size to yield a mixture of smaller and larger particles, the trivalent ion causing more disruption to the population of 1–3 μm particles when 38 μg was present. Molybdates are known to be reduced by Sn(II) in dilute acid to afford blue coloured Mo_2O_5 ;¹⁹ however, this adduct was not formed under the conditions used here, and rather, the molybdate anion influenced the aggregation to 15% of > 3 μm ^{99m}Tc -tin fluoride colloid particles.

These results show that ^{99m}Tc -tin fluoride colloid is sensitive to certain ions in its environment. Its preparation occurs in dilute sodium chloride (0.9%) at pH 6.1, in an ionic solution where the zeta potential (surface charge) is at a minimum and where particle diameter is slightly altered.⁵ Thus the presence of Na^+ and Cl^- ions, but not H^+ ions, are favourable to the electrical double layer which is integral to colloid stability.

Conclusions

Tin-oxo bonds were identified in the stannous fluoride cold kit formulation by mass spectrometry, as fragment ions that contained up to four tin atoms. These bonds were a result of hydrolysis in the aqueous medium, rather than from oxygen gas in the air bubble during the mixing step. The extent of stannous hydrolysis could not be determined, but it is likely to be limited because stronger oxidation conditions resulted in almost complete disruption of radiocolloid into smaller particles. During the preparation, the filtration step removed coarse particles to provide primary particles for further growth in the presence of ^{99m}Tc . A small portion (10%) of the total particle population was 1–3 μm , that bound 96% of radiometal when ^{99m}Tc -pertechnetate was added. Sodium fluoride was necessary to optimise the 1–3 μm radioactive particle size, possibly because fluoride ion regulates particle growth. ^{99m}Tc -tin fluoride colloid was sensitive to charge in the environment, when exposed to solid filter membranes or soluble ions (Ca^{2+} , Al^{3+} , MoO_4^{2-}), where either aggregation and/or deflocculation ensued.

Acknowledgements

Daniel Jardine at the Flinders Advanced Analytical Laboratory (FAAL), Flinders University of South Australia, for running the mass spectra of the stannous fluoride samples is gratefully acknowledged.

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