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Monodisperse radioactive polystyrene latex microspheres have been prepared by attachment of chromium-51 to latex spheres. Labeling was by emulsion polymerization of chromium acetylacetonate dissolved in styrene monomer using commercially available microspheres. This was accomplished with two successive polymerization steps; radiation excitation and radical polymerization initiated with potassium persulfate. After attachment of the label the particle suspension was purified by repeated centrifugation wash cycles to remove labile radioactivity. Results indicate a radioactive binding yield of greater than 80% to the particles. The stability of the label was tested in *in vivo* and *in vitro* leaching studies. In these tests, the activity leaching rate was estimated to be less than 0.2% per day.

Labeling of monodisperse polystyrene microspheres with tightly bound ^{51}Cr

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introduction

Inert radioactive monodisperse particles in a size range suitable for efficient deposition in the respiratory tract have been developed for use in a test of respiratory tract clearance. These tracer microspheres are deposited in the lungs of rats by inhalation and the clearance patterns from the respiratory tracts of control animals and animals exposed to air pollutants are compared. Other applications for such radiolabeled monodisperse particles include calibration of instruments, studies of particle behavior, including deposition and resuspension phenomena and as tracers of air-flow patterns.

Since it was necessary to have reproducible sizes from several labeling batches, commercially available microspheres from Dow Chemical (Midland, Michigan) of 1.099 micron nominal diameter were chosen as the initial material. These spheres are often used for the calibration of aerosol instruments and are well characterized in the literature.⁽¹⁾

As for the selection of the radioisotope, it had to form a chelate complex which is stable at a neutral pH, be relatively non-toxic, decay by gamma emission and have a half-life of at least several days. For these reasons, radioactive chromium-51 was selected. The chromium (III)

state of this element is readily converted to chromium acetylacetonate. Its physical half-life is 27.8 days and it decays by electron capture, emitting only gamma radiation of 0.32 Mev energy. A practical goal was to develop high yield procedures which would allow the radiochemist to work with small quantities of radioactive material.

methods

A flow diagram of the general procedure used to label the polystyrene latex particles is shown in Figure 1. An approximate time interval is listed for each procedural step.

An initial objective of the reaction chemistry was the development of experimental procedures which would convert the Cr (III) state, with high reproducible yields, into a chromium acetylacetonate complex. Figure 2(a) shows the assumed chemical reaction. The basic procedure, obtained from others, as modified to increase labeling yields by elevation of the temperature of the solution during the formation of the complex.⁽²⁾

Carrier-free radioisotope (30 mCi) as CrCl_3 in 0.1 normal hydrochloric acid was injected into a

Figure 1 — Flow diagram for labeling of polystyrene latex particles with ^{51}Cr .

25 x 200 millimeter test tube and diluted to 8-10 milliliters with deionized water. A small magnetic stirring bar was used for light agitation. A pH electrode fitted with a capillary injection tube was inserted into the test tube and the pH of the solution was adjusted to 5.0 with 0.2 normal sodium hydroxide by injection through the capillary tube. Two-tenths milliliter of acetylacetone (2,4 pentanedione) was added and agitated by shaking for 1-2 minutes until thorough homogeneity was attained. The solution was further neutralized until a steady pH of 6.5 - 7.0 was obtained. The pH electrode was withdrawn and rinsed lightly into the reaction solution with deionized water. The solution was placed in a constant temperature bath at 65-70°C without agitation for one hour.

After cooling for 1.5 hours, the acetylacetonate-chromium complex was extracted with 10 milliliters of benzene which had been thoroughly shaken with the reaction products. A modified 25 milliliter pipette was used to facilitate this separation. The extraction was repeated with 5 milliliters of benzene and the two extracts passed through a fluoromembrane filter (0.45 micrometer pore size) and evaporated to dryness at 65°C under a clean filtered air stream.

Figure 2 — Assumed chemical reactions during the labeling procedure: (a) represents the formation of the chromium-acetylacetonate complex; (b) represents the radiation ionization and emulsion polymerization phases.

Figure 3 — Purification of labeled latex particles by sequential centrifugation.

During the extraction with benzene, a small amount of suspended white colloid formed in the aqueous phase.

The benzene extract was clear to slightly yellow after filtering and evaporation of the benzene extract left a yellow-red residue. A small amount of radioactivity could become airborne with the solvent vapor during drying, so adequate ventilation during the benzene evaporation was necessary. Yield measurements were made by radioactive counting of the aqueous solution and the extracted benzene solution under identical counting geometry and comparing the measured activities. Reproducible chelation yields averaging 90% uptake of the chromium have been obtained.

The dried chromium complex was dissolved in 0.2 milliliter of freshly distilled styrene monomer in a 25 x 200 millimeter test tube. A dark red solution was formed at this point. Two milliliters of 1.099 micrometer nominal diameter polystyrene microspheres in a water suspension (10% weight/volume) were pipetted into the test tube and an additional 3 milliliters of deionized water injected into the solution. The mixture was agitated gently with a wrist shaker for 5 hours at room temperature.

Five milliliters of deionized water were added and the monomer-metal complex and microspheres were exposed to an 11,000 Curie cesium-137 gamma-ray source. This was to induce hydrogen stripping/ionization reactions that form carbon radicals on the latex spheres. A dose of about 13 million rads, achieved in 19 hours, was empirically found to be near optimum for inducing attachment of label to the spheres. Longer exposure, and hence greater dose, strongly discolored the spheres and shorter exposure times reduced the binding yield. To further enhance binding to the microspheres, approximately 10 milligrams of potassium persulfate radical initiator was added and the suspension shaken in a constant temperature bath at 65-70°C for 4 to 6 hours. Figure 2 (b) represents the assumed chemical reaction. During the gamma-ray ionization procedure, the particles tended to turn off-white or slightly yellow in color. Following the solution polymerization step, a light build up of coagulated particles was evident on the vial walls and some white clusters were floating in the suspension. After emulsion polymerization, the suspension was diluted to 80 milliliters with deionized water and passed through a coarse filter. This procedure filtered the clusters from the suspension. Dilution to this volume brought the PSL weight to volume ratio to 0.25%; this was the concentration that was used for storage of labeled particles. The mixture was then ready for washing or purification, further dilution and aerosolization. A "transfer" yield was measured at this point to determine the fraction of radioactivity not lost to the walls of the beaker or to the filter. The average transfer yield was greater than 90%.

The washing or purification stage of this experiment consisted of centrifuging to separate monodisperse particles from radioisotope in solution or in suspension. Centrifugation was performed without compacting all of the solids in the bottom of the centrifuge tube. Effective centrifugation time, speed and number of wash cycles was determined by trial and error. A 20 milliliter sample at 0.25% weight/volume concentration was spun at 65-400 g's for 20 minutes. The centrifuge tube had a diameter of 2.9 cm and a height of 11.5 cm; the bottom 1.7 cm of the tube was tapered. This procedure

settled the majority of the particles to the tube bottom, leaving a light particle cloud in the bottom 5 milliliters of the liquid. The upper 15 milliliters of the supernatant was removed, the particle fraction re-diluted, resuspended by brief ultrasonic agitation and centrifugation repeated. The supernatant fractions contained the nonbound activity plus some latex particles. By counting a standard sample of supernatant and plotting its activity versus wash cycle, the removal of non-bound radioactive material was followed (Figure 3). About 4 wash cycles are sufficient to reduce supernatant activity to near background but six were routinely used. After the last resuspension with fresh water, three drops of 5% sodium lauryl sulfate surfactant were added to help stabilize the suspension.

Figure 4 — Electron micrograph of polystyrene latex microspheres labeled with ^{51}Cr . The count median diameter of the larger particles is 1.4 microns. The smaller particles seen in the background contain less than 2% of the total particle radioactivity; the origin of these particles is most likely from dried residues of the reaction chemistry and dissolved materials in the nebulized suspension.

results and discussion

The particles were aerosolized and evaluated for size distribution using electron microscopy and cascade impactors, and for stability of the radiolabel in rats using intraperitoneal injection, intratracheal instillation and aerosol inhalation as well as *in vitro* leaching studies.

The particles were nebulized from the suspension, dried, charge equilibrated and passed into a chamber. The nebulizer used was a Lovelace-type (Aries, Inc., Davis, California). Proper dilution (0.01% weight/volume) of the microspheres before aerosolization was necessary to prevent formation of a large number of airborne multiplets. Samples for electron microscopy were obtained using a point-to-plane electrostatic precipitator (Aries, Inc., Davis, California), operated at a sampling rate of 100 cc/min.⁽³⁾ The labeled aerosol (Figure 4) was relatively monodisperse with a count median diameter of 1.4 microns. The increase in size from 1.099 microns occurs during the repolymerization steps in the labeling process. Small particles, less than 0.3 microns in diameter, containing less than 2% of the total particle radioactivity (measurement of back-up filter on cascade impactor) are also seen in the background of the photograph. They were most likely aerosolized dried surfactant and chromium residue from the reaction chemistry that resulted from drying water droplets that did not contain PSL particles.

A seven-stage cascade impactor (Aries Inc., Davis, California) was used to obtain aerodynamic diameter and geometric standard deviation of the aerosol size distribution.⁽⁴⁾ The average aerodynamic diameter was 1.5 microns with a geometric standard deviation of 1.2.

The radioactive label stability, or the ability of the particles to retain their radioactive tag, was tested in rats using several methods. A summary of the leaching studies is shown in Table I. The

TABLE I
Results of Leaching Tests

Test	% Leached/Day
Intraperitoneal injection (excreta)	< 0.2
Intraperitoneal injection (organs other than abdominal wall)	< 0.1
Intratracheal instillation (organs other than respiratory and GI tract)	< 0.1
Aerosol inhalation (organs other than respiratory and GI tract)	< 0.1
IN VITRO	< 0.1

first test used involved intraperitoneal injection of an aqueous suspension of labeled particles. Intraperitoneal injection of polystyrene latex microspheres has been used by others to test for leaching of radioactivity.^(2,5)

The particles themselves are expected to be retained in the abdomen while any chromium acetylacetonate complex is assumed to be eliminated from the abdomen. To further test for destruction of the complex, which should liberate free chromium within the organism, the injected animal was sacrificed after 7 days and various organs counted for chromium activity. Feces and urine were also collected over the 7 day period post injection and measured for radioactivity. The excreted activity was compared to a standard sample of the material, as injected into the animal, diluted to the same volume and geometry of the excreta. Results show that less than 1% of the retained activity was found in the organs counted after 7 days and the total amount excreted was less than 2%. The majority of activity was contained in the "abdominal wall", which contained the muscle, skin, fur and inner membranes of the wall of the abdomen.

Another test of label stability involved instillation of a small quantity of the particles into the lung via the trachea to determine leaching characteristics under lung-tissue conditions. The animal was sacrificed after 24 hours and several organs counted for activity. Ideally, only the lungs and gastro-intestinal tract should contain any activity. Less than 0.1% of the activity was observed in the combined activity of the other organs. Aerosol inhalation tests were used to confirm the tracheal instillation data. After sacrifice at 24 hours postinhalation exposure, no significant activity above background was found in any organ except the lungs and gastrointestinal tract. All of these tests are indicative that the activity was strongly bound and did not separate from the particles when subjected to the chemical environments within the living animal.

The leaching rate of the ⁵¹Cr from the polystyrene latex microspheres was also observed in *in vitro* experiments. A fiberglass filter on which an aerosolized sample of the particles had been deposited was placed between two membrane filters (pore size of 0.65

micrometer) and sandwiched between two polypropylene rings. The total exposed area of the filters was 26 cm². The sample was placed in a beaker which contained 300 milliliters of Ringer's solution plus bovine serum albumin at a 0.4% weight/volume concentration. The temperature of the solvent was maintained at 37°C and the pH of the solution was adjusted to 7.2 with small amounts of hydrochloric acid and sodium hydroxide at the beginning of the experiment. The beaker was covered with a waxed plastic seal to prevent large pH changes during the experiment. At pre-determined times aliquots of the solvent were taken and analyzed for the amount of radioactivity which had gone into solution. The leaching rate of the ⁵¹Cr from the polystyrene particles under these conditions was determined to be less than 0.1% per day.

conclusions

The procedure for labeling the polystyrene latex microspheres with ⁵¹Cr is straight-forward provided the necessary equipment is available. An important step in the procedure is gamma-ray irradiation of the suspension. Elimination of this step will significantly decrease the overall labeling yield and possibly the label stability. Also, deviation from the particle-monomer-surfactant ratios used in the procedure is likely to cause a decrease in the labeling yield and alter the stability of the particle suspension.

If the procedure is followed as given, the chromium-51 will be tightly bound to the polystyrene latex microspheres. The results from the *in vivo* and *in vitro* leaching tests show that radioisotope does not readily separate from the particles when subjected to the chemical environments in rats for up to seven days.

The labeled particles have been used in tests of lung clearance in nearly 1000 rats over a two year period. Reproducibility of the properties of the labeled particles has been excellent from batch to batch. Thus far about 20 batches of particles have been labeled.

Stored suspensions of labeled particles, kept refrigerated, appear to be useful for as long as the radioactivity persists; i.e. several months. After long periods of storage, the particle suspension should be washed via centrifugation.

as previously described, in order to remove nonbound radioactivity.

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