Use of stannous methylene diphosphonate for labelling red blood cells with ^{99m}Tc

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Summary. A technique is described for in vitro labelling of red blood cells with ^{99m}Tc using stannous methylene diphosphonate as the reducing agent. The labelling yield has been found to be > 95% after three washes with normal saline.

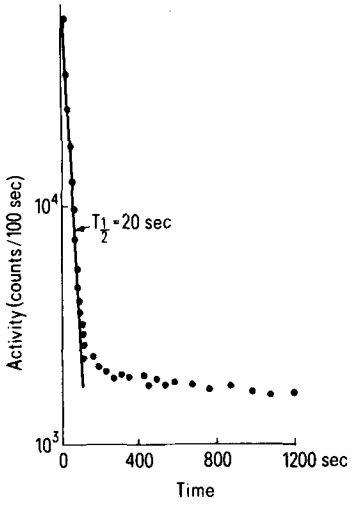
A number of methods have been described in the literature for labelling of red blood cells (RBC) with technetium-99m (^{99m}Tc) using stannous chloride as the reducing agent[3–5]. More recently reduction of ^{99m}Tc-pertechnetate has been achieved using stannous pyrophosphate[6, 7]. This work is a preliminary report on a newer agent, namely, stannous methylene diphosphonate (Sn-MDP) which has been prepared in kit form[8] for use as the reducing agent in the first stage of the labelling process.

Materials and methods. The contents of the Sn-MDP kit (containing 5 mg MDP and 0.3 mg SnF_2) were reconstituted in 5 ml of nitrogen-purged saline. Aliquots of this solution comprising of 1, 6, 30, 100 and 300 µg of tin were each dispensed into separate tubes containing 2 ml of whole blood with heparin added as anticoagulant. The mixture was incubated for 5 min at room temperature with gentle shaking by hand, after which it was centrifuged and the buffy coat and plasma layer removed. The required amount of ^{99m}Tc-pertechnetate activity (in 1 ml volume) was added and the cells again incubated for 5 min at room temperature. After centrifuging the clear supernatant was separated from the labelled RBC layer, and the amount of ^{99m}Tc activity in each fraction determined.

Labelling yield of ^{99m}Tc-RBC shown as a function of tin content for washed and unwashed cells

Amount of Tin (µg)	No washing	3 washes with saline
300	24.4 ± 3.2	98.1 ± 0.9
100	23.0 ± 1.9	97.9 ± 1.1
30	38.3 ± 3.4	97.7 ± 1.6
6	39.9 ± 1.8	97.7 ± 2.0
1	45.9 ± 2.1	97.6 ± 1.0

Each value is the mean of 5 observations ± 1 SD.



Disappearance of ^{99m}Tc-RBC from the canine tibia.

The procedure described above was repeated but this time the RBC's were washed and centrifuged 3 times with nitrogen purged saline before adding the ^{99m}Tc activity, and the cells incubated at room temperature as before.

Results and discussion. The data in the table shows the labelling efficiency attained with this method for both washed and unwashed cells, and also for variable amounts of tin present. It can be seen that the labelling yield increases as the quantity of tin decreases, but that this yield is unaffected by the tin content after washing 3 times with normal saline. The saline was purged with nitrogen in order to provide an oxygen-free atmosphere and hence prevent any re-oxidation of the reduced ^{99m}Tc. The method can be described as a 2-stage-process: pretinning of the red cell membrane followed by rapid reduction and binding of the ^{99m}Tc within the cell, possibly to the haemaglobin molecule. Washing with saline serves to remove any traces of unbound tin trapped between the cells, as any reduction of ^{99m}Tc outside the cells will simply lead to a decrease in the labelling yield.

^{99m}Tc-labelled RBC's have been used for a number of investigations including red cell survival studies, RBC mass determination, spleen scanning and placental localization. As part of another study these cells have been labelled and used as a vascular marker to aid compartmental analysis following washout of a radiotracer from a given organ.

The accompanying figure illustrates a semilogarithmic plot of activity shown as a function of time following i.a. injection into the canine tibia. A NaI(Tl) crystal scintillation probe was placed over a region of the leg and the washout recorded after injection of ^{99m}Tc-labelled RBC. The activity cleared very rapidly from the field of view of the detector with a half-time of 20 sec, falling to less than 5% of the peak value within 2 min after injection. Beyond this time the curve showed a plateau effect due to rapid equilibration within its volume of distribution. This equilibrium was maintained for the remaining period of observation (20 min) indicating that tagging with ^{99m}Tc resulted in a stable red cell complex, in vivo, with little, if any, elution of activity out of the cells during this period.

In conclusion a highly reproducible Sn-MDP kit has been prepared for use not only as a bone scanning agent, but that the reducing properties of this kit has been put to further use in the process of red cell labelling with ^{99m}Tc.

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