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# Radiolabelling of polymer microspheres for scintigraphic investigations by neutron activation. 1. Incorporation of samarium oxide and its effects on the properties of Eudragit RS: sulphasalazine microspheres.

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## Summary

Eudragit RS: sulphasalazine microspheres containing 0–0.8% w/w samarium oxide ($Sm_2O_3$) have been produced using a conventional emulsification-solvent evaporation technique. Direct quantification of incorporated $Sm_2O_3$ was achieved using X-ray fluorescence spectroscopy (XRF). The efficiency of $Sm_2O_3$ incorporation increased with concentration but microsphere yield, appearance, drug content and drug release rate were unaffected by $Sm_2O_3$ content over the concentration range investigated. Incorporation of $Sm_2O_3$ by solvent evaporation would appear to offer a convenient method for producing microspheres to be radiolabelled by neutron activation for use in scintigraphic studies.

## Introduction

Gamma scintigraphy has been widely used to investigate the distribution of drug delivery systems in the body, and in particular the gastrointestinal transit of orally administered dosage forms (Wilson and Washington, 1988).

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Conventionally, dosage forms for scintigraphic investigation are radiolabelled by incorporating small amounts of radioisotopes such as technetium-99m ($^{99m}Tc$), indium-111 ($^{111}In$) or indium-113m ($^{113m}In$) but there are a number of potential drawbacks in using this technique (Parr et al., 1985). For example, the short-lived nature of some of these radioisotopes (e.g. $t_{1/2}$ $^{99m}Tc = 6$ h) means that the time for production of the dosage form may be limited, and although this can be overcome by incorporating larger quantities of isotope, this has the disadvantage of expos-

ing manufacturing personnel to higher levels of radioactivity. Secondly, since the incorporation of isotope into the dosage form requires designated radioisotope facilities, production of radiolabelled dosage forms in a conventional pharmaceutical production environment is impractical. A third problem is the possible contamination of specialist manufacturing equipment with the radioisotope, potentially putting it out of use until the isotope has decayed. Finally, if manufacturing problems are encountered or if the final dose form does not meet the required specifications, the investigation for which the dosage forms were produced would need to be postponed until a new batch could be made.

Neutron activation techniques, originally developed at the University of Kentucky (Parr et al., 1985), overcome many of these problems (Parr and Jay, 1987; Parr et al., 1990; Hardy et al., 1991). Poorly water-soluble, non-radioactive compounds of elements such as samarium (Sm), erbium (Er), or barium (Ba) are incorporated into the dosage form during manufacture. Prior to administration, the dosage form is exposed to a neutron beam, which converts the stable isotope into a gamma-emitting radioisotope. In this process $^{152}Sm$ is converted to $^{153}Sm$ ($t_{1/2} = 46.7$ h), $^{170}Er$ is converted to $^{171}Er$ ($t_{1/2} = 7.5$ h) and $^{138}Ba$ is converted to $^{139}Ba$ ($t_{1/2} = 1.4$ h) (CRC Handbook of Chemistry & Physics). Dosage forms can thus be produced using conventional manufacturing facilities and shown to meet the required specifications before irradiation.

In this paper we investigate the incorporation of samarium oxide into sustained release polymer microspheres produced by an emulsification/solvent evaporation procedure, a common technique for microsphere manufacture (Watts et al., 1990). These microspheres are excellent candidates for radiolabelling using neutron activation. The successful application of gamma scintigraphy to follow the position of a dosage form clearly depends on the majority of the radioactivity remaining associated with the dosage form. To achieve this by conventional means would involve binding the radioisotope to an ion-exchange resin carrier. The resin would then need to be thoroughly washed to remove unadsorbed radioisotope and dried before incorporation into the microspheres during manufacture. Since the microsphere manufacturing alone takes at least 24 h, the whole radiolabelling process would be lengthy.

In the study described, microspheres were prepared from the water-insoluble acrylic polymer Eudragit RS® (Rohm Pharma GmbH). Sulphasalazine was also incorporated since the studies are part of a larger programme investigating the controlled delivery of drugs to the colon.

The amount of target nuclide incorporated within the preparation is a critical determinant of the activity generated during neutron bombardment. The team at the University of Kentucky have estimated the levels of incorporated target material indirectly by comparison with the activity generated after neutron activation of a known quantity of isotope (Parr et al., 1990). In the present study we have used X-ray fluorescence spectroscopy (XRF) to provide a direct measurement of microsphere samarium content.

## Materials and Methods

### *Materials*

Eudragit RS® (Rohm Pharma, Darmstadt, Germany), sulphasalazine (Sigma, Poole, U.K.), natural abundance samarium oxide (26.7% $^{152}Sm$) (Sigma), polysorbate (Tween) 20 (Sigma), methylene chloride (GPR grade) (Rhone-Poulenc, Dagenham, U.K.), acetone (Analar grade) (Rhone-Poulenc), sodium hydroxide (BDH, Poole, U.K.), potassium dihydrogen orthophosphate (BDH), Specpure® samarium oxide (Johnson Matthey, Royston, U.K.), Spectroflux® 121A (Johnson Matthey).

### *Methods*

*Microsphere manufacture.* Microspheres were produced by an emulsification-solvent evaporation procedure. Eudragit RS (4 g) was dissolved in 40 ml of methylene chloride. Sulphasalazine (2 g) and 0, 10, 20, 40 or 60 mg of samarium oxide ($Sm_2O_3$) were added to form a suspension. Dispersion of the $Sm_2O_3$ into the drug-polymer mixture was aided by sonication in an ultrasonic bath for 5–10 min. The drug-polymer-$Sm_2O_3$ mixture

was then dispersed into 200 ml of 0.1% w/v aqueous Tween 20 solution in a glass beaker using an overhead paddle stirrer (250 rpm). Stirring was continued for 4–5 h in a fume cupboard, until all of the solvent had evaporated. The microspheres were collected by filtration, rinsed with 150 ml of distilled water and freeze-dried overnight. A 250–500 $\mu$m sieve fraction of each microsphere formulation was used for subsequent studies.

*Microsphere drug content.* An accurately weighed sample of 4–6 mg of each microsphere formulation was placed into a 100 ml volumetric flask and 5 ml of acetone added to dissolve the microsphere polymer matrix. The sulphasalazine was dissolved and the Eudragit RS precipitated by making to 100 ml with 0.05 N aqueous sodium hydroxide solution. The precipitated polymer was removed by filtration (1 $\mu$m membrane filter) and the UV absorbance at $\lambda_{max} = 458$ nm measured. The drug concentration was calculated with reference to an absorbance: concentration plot of sulphasalazine in 0.05 N sodium hydroxide solution containing 5% v/v acetone.

*Efficiency of samarium oxide incorporation.* The concentration of $Sm_2O_3$ in the completed microspheres was determined by XRF analysis using the method below.

For each microsphere sample, 0.4 g was weighed into a Pt/Au crucible. The crucible was ignited at 500 °C for 12 h followed by further ignition at 1000 °C for 15 min to remove all of the organic matter, i.e., drug and polymer. The crucibles were cooled and 2.60 g of Spectroflux® 121A added prior to fusing the sample at 1000 °C for 1 h. The molten sample was then poured onto a 32 mm brass die maintained at 225 °C and pressed with a brass plunger. Standards containing 0, 0.15, 0.3, 0.55, 0.75 and 1.0% w/w $Sm_2O_3$ were prepared by directly fusing $Sm_2O_3$ with Spectroflux® 121A.

Concentrations of $Sm_2O_3$ were determined using a Philips PW1400 XRF spectrometer fitted with a Rh X-ray tube. The Sm concentration was determined using the Sm $L\alpha_1$ line, a $LiF_{200}$ diffracting crystal and a scintillation counter. X-ray emissions for both peak and background were counted for 200 s. The $Sm_2O_3$ concentration of the samples was determined from the net intensities (peak minus background) by reference to the calibration performed with the six prepared standards. The calibration plot of corrected counts against concentration for the $Sm_2O_3$ standards was linear over the concentration range used ($r = 0.999$).

*Drug release from the microspheres.* Microsphere samples (20–30 mg) were added to 500 ml of 0.05 N pH 7 phosphate buffer in a dissolution apparatus (USP apparatus 2). We estimated the solubility of sulphasalazine in pH 7 phosphate buffer at 37 °C to be in excess of 0.13 mg/ml and thus sink conditions were always maintained. 10 ml samples of buffer were withdrawn at 30, 60, 120, 180, 240, 300 and 360 min, passed through a membrane filter (1 $\mu$m) and the UV absorbance at $\lambda_{max} = 359$ nm measured. Any microspheres retained on the filter were returned to the dissolution vessel with 10 ml of fresh buffer. The drug concentration was determined with reference to a calibration curve of sulphasalazine in pH 7 phosphate buffer. Each dissolution test was performed in duplicate.

## Results and Discussion

$Sm_2O_3$ was successfully encapsulated into the microspheres. The efficiency of incorporation is recorded in Table 1 and, in general, increased with $Sm_2O_3$ concentration. XRF proved to be an excellent method for the assay of microsphere $Sm_2O_3$ content, with a level of 0.08% w/w being measurable. The encapsulation efficiency of

TABLE 1

*Efficiency of incorporation of samarium oxide into sulphasalazine-Eudragit RS microspheres as measured by XRF*

| Microsphere samarium oxide content (% w/w) | | Incorporation efficiency (%) |
|---|---|---|
| Theoretical | Assayed | |
| 0.17 | 0.08 | 47 |
| 0.33 | 0.24 | 73 |
| 0.67 | 0.61 | 91 |
| 1.00 | 0.80 | 80 |

$Sm_2O_3$ is not primarily a function of its solubility since it is only appreciably soluble at acidic pH. (The aqueous solubility of $Sm_2O_3$ is only 0.054 mg/100 ml (Parr et al., 1990)). The difference between theoretical and assayed samarium content may reflect difficulty in the dispersion of the $Sm_2O_3$ powder into the drug-polymer solution prior to microsphere production. Despite sonication, agglomerates of $Sm_2O_3$ could sometimes still be seen in the drug-polymer solution prior to mixing into the surfactant solution.

Incorporation of $Sm_2O_3$ into the microspheres did not appear to significantly affect their formation or composition. The proportion of drug and polymer successfully formed into microspheres was in all cases in excess of 80% w/w (Table 2) and typically at least 30% by weight of any given microsphere batch fell between 250 and 500 $\mu$m in size. Drug levels in the microspheres were similar at all samarium concentrations at between 31 and 32% w/w (Table 2). This high efficiency of drug encapsulation (90–94%) reflected the low aqueous solubility of sulphasalazine with only small amounts of drug dissolving out of the drug-polymer phase into the surrounding surfactant solution during microsphere production. Similar values for drug content of Eudragit RS: sulphasalazine microspheres have been recorded previously (Watts et al., 1991). All of the microsphere samples had a similar physical appearance.

TABLE 2

*Effect of samarium oxide content on microsphere yield and sulphasalazine content*

| Microsphere samarium oxide content (% w/w) | Microsphere yield (g) | Sulphasalazine content (% w/w) | Sulphasalizine incorporation efficiency (%) |
|---|---|---|---|
| 0 | 5.1 | 31.8 | 95.5 |
| 0.08 | 4.9 | 31.1 | 93.4 |
| 0.24 | 5.4 | 31.8 | 95.5 |
| 0.61 | 5.0 | 31.5 | 94.6 |
| 0.80 | 5.4 | 31.9 | 95.8 |

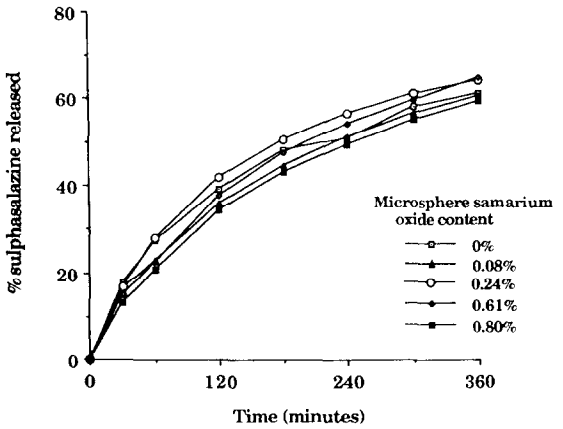


Fig. 1. Rate of release of sulphasalazine from Eudragit RS microspheres containing different concentrations of samarium oxide.

Fig. 1 shows the release profiles of sulphasalazine from the five microsphere batches. The rate of drug release was similar for all of the samples and thus appeared to be unaffected by the incorporation of $Sm_2O_3$. The sustained release properties of the formulations are clearly demonstrated and after 6 h approx. 60% of the encapsulated drug had been released.

## Conclusions

In this paper we have described a convenient and straightforward technique for incorporating a target nuclide (samarium oxide) into microspheres produced by a solvent evaporation technique. XRF provided a direct method for assaying the level of incorporated label.

Clearly it is desirable that the incorporation of a radiolabel should not adversely affect the physical properties of the dosage form, in particular the drug release profile. In this respect, the presence of $Sm_2O_3$ in the Eudragit RS microspheres did not affect the rate of sulphasalazine release over the concentration range investigated. These microspheres are intended to be used for in vivo gamma scintigraphic experiments, although the solubility of $Sm_2O_3$ in acidic media (e.g. gastric fluid) would effectively restrict their use to intestinal investigations.

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