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Radiolabelling of polymer microspheres for scintigraphic investigations by neutron activation. 3. Changes in the physical properties of Eudragit RS-sulphapyridine microspheres from incorporating samarium oxide

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Summary

Eudragit RS microspheres containing sulphapyridine and samarium oxide (Sm_2O_3) have been prepared using a solvent evaporation process. Microspheres containing 1.1% w/w Sm_2O_3 were smaller, had a higher density, and released encapsulated drug significantly more slowly than samples containing no Sm_2O_3. Electron microscopy revealed that in the presence of Sm_2O_3, the size and number of pores in the microsphere surface was markedly reduced, suggesting that it may be exerting an effect at the organic:aqueous interface during microsphere formation.

Introduction

In the first two papers in this series, we reported on the incorporation of samarium oxide (Sm_2O_3) into Eudragit RS-sulphasalazine microspheres, and the effects of irradiation on the microsphere physical properties (Watts et al., 1991, 1993). Sulphasalazine was initially chosen as a potential absorption marker for a biopharmaceutical evaluation of these microspheres in the human colon. However, due to the relatively high dosage requirements of this drug, subsequent studies have focused on the microencapsulation of sulphapyridine, the absorbed colonic metabolite of sulphasalazine. In the colonic metabolism of sulphasalazine by the resident bacterial flora, 5-aminosalicylic acid is also generated. This compound remains largely unabsorbed and exerts a local antiinflammatory action in the colon (Klotz, 1985).

During preliminary investigations of the preparation of Eudragit RS-sulphapyridine microspheres, it became apparent that the presence of Sm_2O_3, even in low concentrations, could have marked effects on their physical properties. This was in contrast to the work with sulphasalazine-containing microspheres, where similar amounts

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of Sm_2O_3 had no apparent effects on microsphere physical properties (Watts et al., 1991). This paper describes the investigation of this phenomenon in more detail and reports on a comparison between the physical properties of Eudragit RS-sulphapyridine microspheres containing Sm_2O_3 and Eudragit RS-sulphapyridine microspheres containing no Sm_2O_3.

Materials and Methods

Materials

Eudragit RS100 (Dumas U.K., Tunbridge Wells, U.K.), sulphapyridine (gift from Rhône-Poulenc, Dagenham, U.K.), natural abundance samarium oxide (Sigma, Poole, U.K.), polysorbate (Tween) 20 (Sigma), sodium hydroxide pellets (BDH, Poole, U.K.), potassium dihydrogen orthophosphate (BDH), dichloromethane (GPR grade) (Rhône-Poulenc), methanol (Analar grade) (Rhône-Poulenc), Specpure™ samarium oxide (Johnson Matthey, Royston, U.K.) and Spectroflux™ 121A (Johnson Matthey) were obtained from the indicated sources.

Methods

Microsphere preparation

1.5 g of sulphapyridine and 0 or 67.5 mg of Sm_2O_3 were added to a solution of 3 g of Eudragit RS in 30 ml of dichloromethane. Sulphapyridine and Sm_2O_3 were insoluble in dichloromethane and formed a suspension. Dispersion of the Sm_2O_3 powder was aided by sonication of the mixture for 10 min. The mixture was then emulsified into 150 ml of 0.05% w/v aqueous polysorbate 20 solution in a glass beaker using an overhead paddle stirrer at 250 rpm. Stirring was continued until all of the dichloromethane had evaporated. Unemulsified drug-polymer agglomerates floating on the liquid surface were decanted from the beaker, and the microspheres collected by filtration, washed with 150 ml of distilled water, and freeze-dried overnight. Three replicate batches without Sm_2O_3 and three batches containing Sm_2O_3 were produced.

Determining microsphere size and density

The weight of dry microspheres in each batch was recorded and the fraction in three size ranges, < 250, 250–500 and > 500 μm, determined by sieving.

To measure the microsphere tap density, a sample from each batch (250–500 μn sieve fraction) was poured into a 10 ml glass measuring cylinder. The cylinder was lightly tapped 20 times and the volume of microspheres measured. The tap density was calculated by dividing the microsphere mass by its volume.

Sm_2O_3 content

The amount of Sm_2O_3 entrapped within the microspheres was determined using X-ray fluorescence spectroscopy, in a manner described previously (Watts et al., 1991).

Sulphapyridine content

An accurately weighed quantity of microspheres estimated to contain 3–5 mg of sulphapyridine was placed into a 100 ml volumetric flask. 5 ml of methanol was added to dissolve the Eudragit RS, and the flask made to volume with 0.05 N sodium hydroxide solution. 25 ml of this solution was further diluted to 100 ml with 0.05 N sodium hydroxide solution, filtered (0.45 μm membrane filter) and the UV absorbance measured at 247 nm. Drug concentrations were calculated with reference to a concentration-absorbance curve of sulphapyridine in 0.05 N sodium hydroxide.

Drug release rate

Drug release was determined using a USP type 2 dissolution apparatus. Microsphere samples were placed into dissolution vessels containing 500 ml of pH 7 phosphate buffer at 37°C (0.02% w/v Tween 20 was added to aid microsphere wetting), and agitated at 100 rpm. 10-ml samples were withdrawn from the dissolution vessels at regular intervals, passed through a 1 μm membrane filter and the UV absorbance at 247 nm measured. Any microspheres retained on the filter were returned to the dissolution vessel with 10 ml of fresh buffer. The drug concentration was calculated with reference to a concentration-ab-

sorbance plot of sulphapyridine in pH 7 phosphate buffer.

Electron microscopy

Electron micrographs of the microsphere surface and interior structure were obtained using low temperature (cryogenic) scanning electron microscopy (Philips 505 SEM/Hexland CT1000A cryotransfer station).

Results and Discussion

Microsphere characterisation

Table 1 lists the physical characteristics of the microsphere batches. There was little difference in yield between the two sets of samples. However, the tap density of the microspheres containing Sm_2O_3 was 30% higher than for the Sm_2O_3-free samples. Physical differences were also apparent from handling the samples. The Sm_2O_3-free samples were brittle and easily powdered between the fingers, whereas the samples containing Sm_2O_3 were considerably harder in consistency.

The presence of Sm_2O_3 may have slightly influenced the microsphere sulphapyridine content. The drug content of all three batches containing Sm_2O_3 ($33.6 \pm 0.8\%$) was marginally higher than for those without ($31.3 \pm 0.3\%$).

There were also clear differences in the microsphere size distributions (Fig. 1). In contrast to the Sm_2O_3-free samples, in microspheres containing Sm_2O_3, the proportion greater than 500 μm in diameter was reduced, and the proportion smaller than 250 μm was increased.

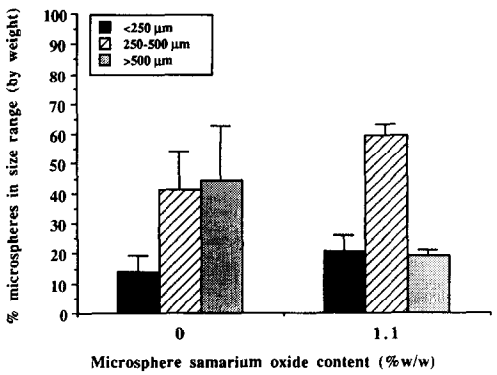

Fig. 1. Effect of samarium oxide incorporation on the size distribution of Eudragit RS-sulphapyridine microspheres (mean of three batches).

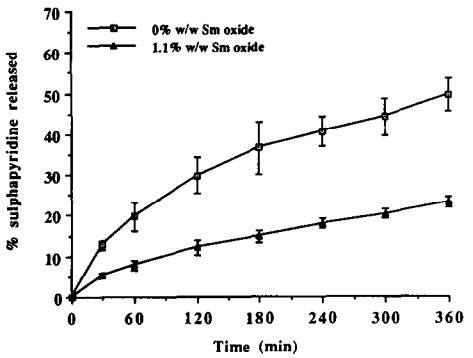

Fig. 2. Effect of samarium oxide incorporation on the rate of sulphapyridine release from Eudragit RS-sulphapyridine microspheres (mean of three batches).

Drug release rate

Fig. 2 shows the drug release profiles of the two sets of microsphere samples. The incorporation of Sm_2O_3 appeared to dramatically suppress the rate of drug release. For the samples containing no Sm_2O_3 54% of the encapsulated drug had been released after 6 h, whereas in the same time period, just 28% of encapsulated drug had been released from the samples containing Sm_2O_3. The suppressed drug release rate of the latter samples may in part have been related to their higher density.

Electron microscopy

Surface electron micrographs of samples from the Sm_2O_3-free and Sm_2O_3-containing microspheres are shown in Figs 3a,b and 4a,b, respectively. The Sm_2O_3-free sample contained many large surface holes (Fig. 3a) which were absent in the sample containing Sm_2O_3 (Fig. 4a). Higher magnification views revealed the size and number of pores to be greater in the Sm_2O_3-free samples (Fig. 3b vs Fig. 4b).

In contrast to the surface features, the internal structure of the two sets of microspheres did not differ greatly. Both sets consisted of a spongy drug-polymer matrix containing a number of large

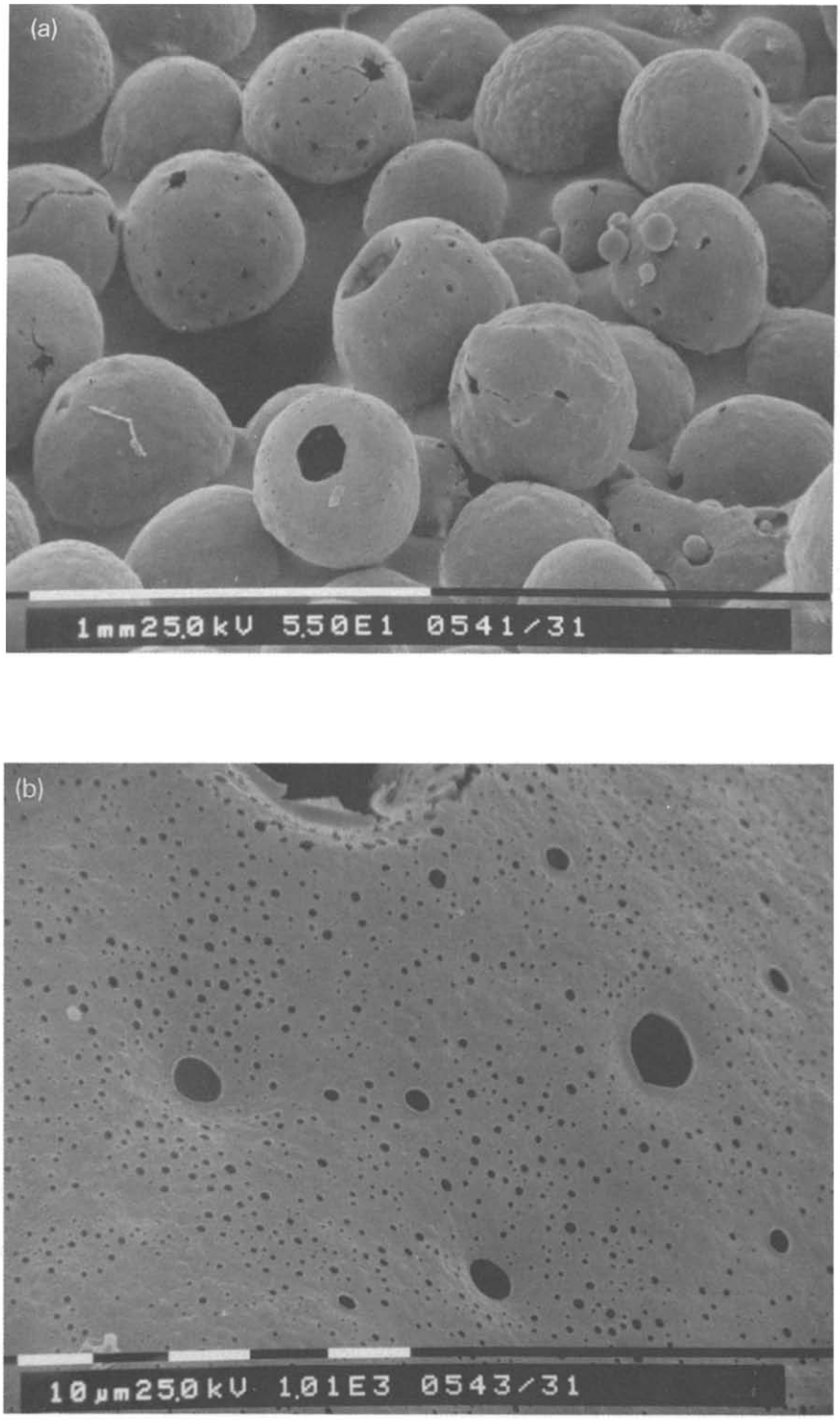

Fig. 3. Electron micrographs of Eudragit RS-sulphapyridine microspheres containing no samarium oxide. (a) View of microsphere population (magnification ×55); (b) view of microsphere surface (magnification ×1000).

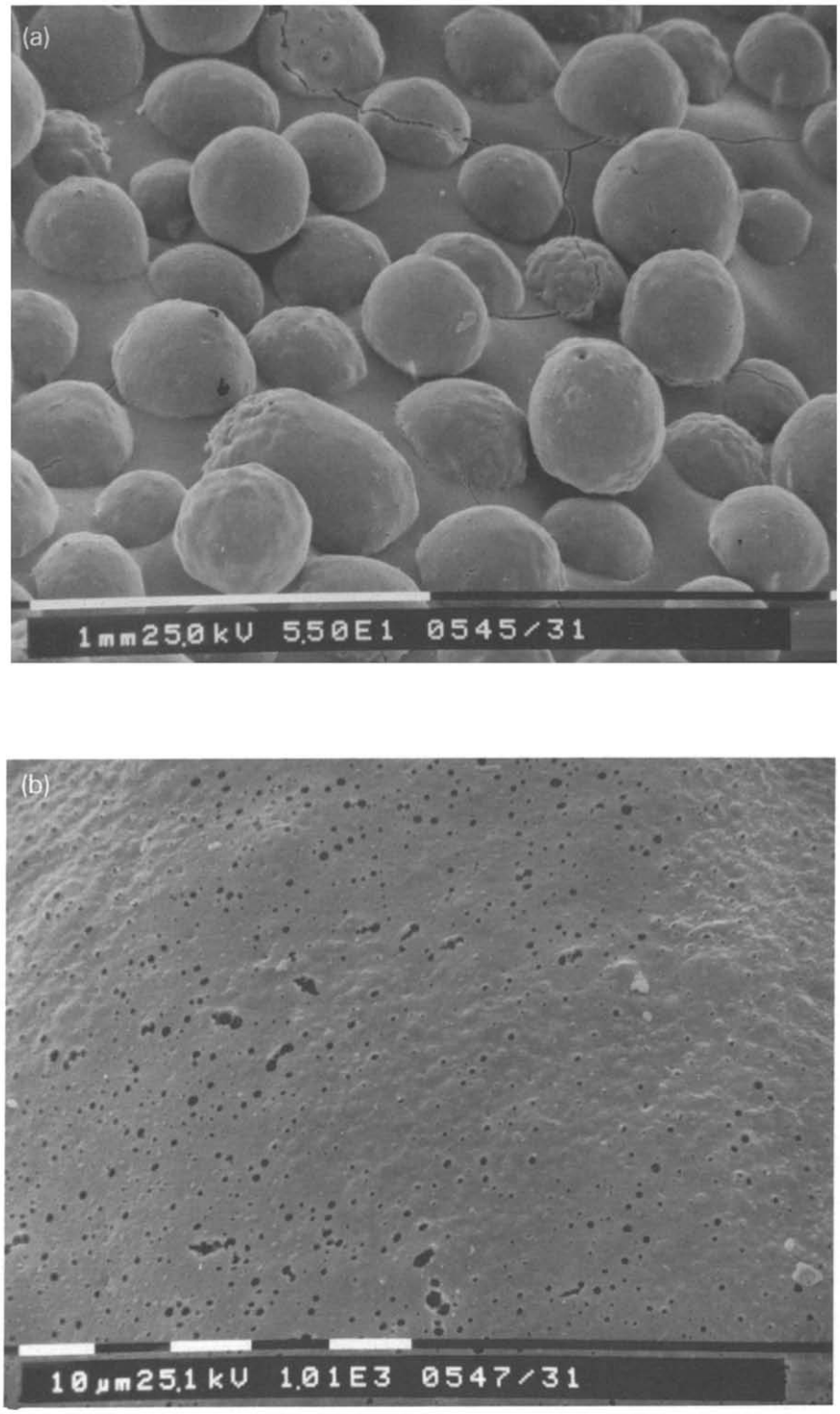

Fig. 4. Electron micrographs of Eudragit RS-sulphapyridine microspheres containing 1.15% w/w samarium oxide. (a) View of microsphere population (magnification ×55); (b) view of microsphere surface (magnification ×1000).

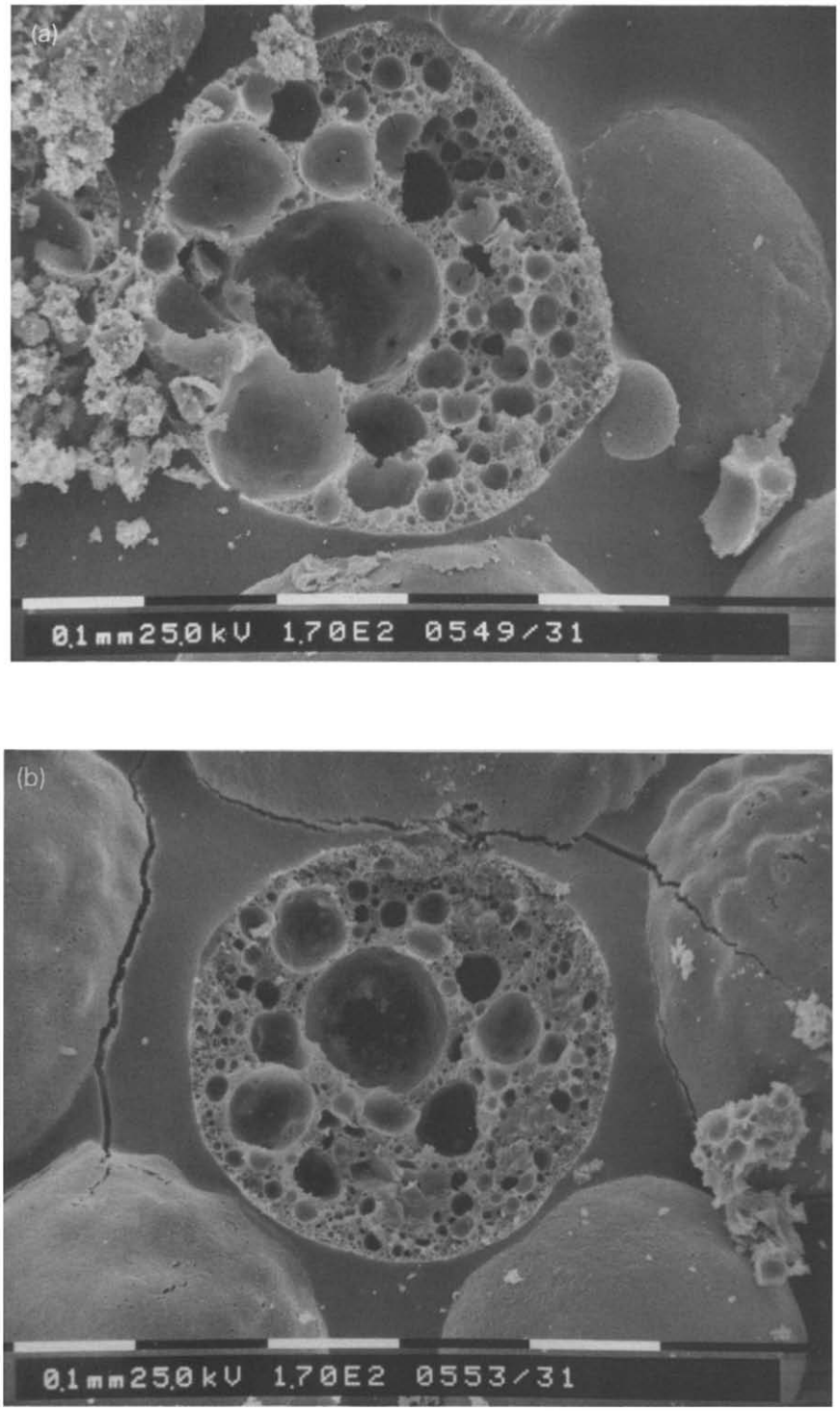

Fig. 5. Electron micrographs of the interior of Eudragit RS-sulphapyridine microspheres. (a) Microsphere containing no samarium oxide (magnification ×170); (b) microsphere containing 1.15% w/w samarium oxide (magnification ×170).

TABLE 1

Physical properties of the Eudragit RS-sulphapyridine microsphere formulations

Samarium oxide content (% w/w)	Sulphapyridine content (% w/w)	Yield (g) (g/ml)	Tap density
0	31.3±0.3	3.7±0.2	0.26±0.01
1.15±0.8	33.6±0.8	3.5±0.3	0.34±0.02

cavities. However, the size and number of cavities appeared to be greater in the Sm_2O_3-free microspheres (Fig. 5a) compared to the Sm_2O_3-containing microspheres (Fig. 5b), which was consistent with the differences in density.

It would therefore appear that the reduced drug release rate from microspheres containing Sm_2O_3 might have been a result of lower surface and internal porosity.

Conclusions

In this paper, we have demonstrated that the presence of only a small amount of Sm_2O_3 in Eudragit RS-sulphapyridine microspheres can have a dramatic effect on their physical properties, most apparent in a markedly reduced drug release rate. It is not entirely clear how Sm_2O_3 is exerting these changes, but its presence in the microspheres resulted in increased density and reduced surface porosity, which might both be expected to reduce the drug release rate. A reduction in surface porosity, combined with a reduction in microsphere size suggests that Sm_2O_3 may be exerting effects at the surface of the forming microspheres. The adsorption of certain finely-divided solids at oil-water interfaces is a well known phenomenon and materials such as hydrated aluminium silicate, aluminium magnesium silicate and carbon black may be used for stabilising emulsions (Martin et al., 1983). Furthermore, two other finely divided solids, magnesium stearate and aluminium tristearate, have been used as stabilising agents in the preparation of Eudragit microspheres by solvent evaporation (Goto et al., 1985, 1986).

It is noteworthy that in a previous study investigating the encapsulation of sulphasalazine, no effects on the microsphere drug release rate were observed when incorporating up to 0.8% w/w Sm_2O_3 (Watts et al., 1991). Although the amount of Sm_2O_3 was higher in the sulphapyridine microspheres described here, it is possible that additional factors determine the degree to which Sm_2O_3 affects the microsphere structure and drug release rate, such as the physical properties of the drug being encapsulated, e.g., hydrophobicity or particle size.

Our studies investigating the neutron activation of microspheres have demonstrated that the avoidance of detrimental effects on microsphere performance requires a careful balance between the two factors determining the amount of radioactivity generated, i.e., the quantity of activatable material incorporated (e.g., Sm_2O_3) and the irradiation time. In this paper we have shown that if the amount of Sm_2O_3 incorporated is too high, the microsphere drug release may be suppressed. Conversely, for sulphasalazine-containing microspheres, high neutron exposure resulted in an increased drug release rate (Watts et al., 1993). With the sulphapyridine microspheres, one means of minimising the suppression of drug release rate would be to replace the natural abundance Sm_2O_3 (27.7% ^{152}Sm) with enriched material (95 + % ^{152}Sm). The difference in ^{153}Sm content between enriched and non-enriched material would effectively allow the amount of Sm_2O_3 incorporated to be reduced by 70%, yet to generate a given amount of radioactivity, the neutron exposure would remain unchanged.

The microsphere structural changes induced by the incorporation of Sm_2O_3 may be of more general interest in microencapsulation. The surface-active properties of such materials may make them of use as an additional means of modifying the drug release pattern of microspheres prepared by the solvent evaporation process.

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