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Radiolabelling of polymer microspheres for scintigraphic investigations by neutron activation. 2. Effects of irradiation on the properties of Eudragit RS-sulphasalazine microspheres

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Summary

Eudragit RS-sulphasalazine microspheres containing up to 0.8% w/w samarium oxide (Sm_2O_3) have been irradiated with neutrons as a potential method for radiolabelling. Following irradiation, no leaching of $^{153}Sm_2O_3$ from the microspheres was detectable during in vitro dissolution testing. Using HPLC, sulphasalazine appeared to be stable to irradiation. Irradiation resulted in little apparent change in the molecular weight of Eudragit RS, although there were marked changes in the macroscopic and microscopic appearance of the microspheres. The physical changes in the microspheres most obviously manifested themselves in a considerable increase in the rate of sulphasalazine release; the time for 50% of the encapsulated drug to be released was at least halved for all but one microsphere sample. The mechanism by which drug release was enhanced was unclear.

Introduction

In a recent publication, we described the successful preparation of Eudragit RS-sulphasalazine microspheres containing samarium oxide (Sm_2O_3) (Watts et al., 1991a). It was considered that the incorporation of Sm_2O_3 followed by neutron irradiation may be a suitable method for microsphere radiolabelling, ultimately allowing us to use gamma scintigraphy to evaluate their biopharmaceutical performance.

In our studies we have utilised natural abundance Sm_2O_3, the composition of which is listed in Table 1. In the neutron irradiation process, neutron absorption leads to mass changes in the Sm isotopes. The isotope of interest for gamma scintigraphic imaging is ^{153}Sm, which is generated from the capture of neutrons by ^{152}Sm. ^{153}Sm is unstable and decays with a half-life of 46.7 h into ^{153}Eu (CRC Handbook of Chemistry and Physics, 1987). Decay is accompanied by the emission of β-particles and γ-rays. The only other noteworthy isotopic conversion arising from the irradiation of

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TABLE 1

Isotopic composition of naturally-occurring samarium oxide

Isotope atomic number	% abundance
144	3.1
147	15.1
148	11.3
149	13.9
150	7.4
152	26.6
154	22.6

Sm_2O_3 is the generation of ^{155}Sm from ^{154}Sm. ^{155}Sm is a high energy β-emitter, but since it has a half-life of just 22 min (CRC Handbook of Chemistry and Physics, 1987) it rapidly decays to insignificant levels and thus has little effect on the radiation dosimetry of the material. The ability to use natural abundance material is one of the attractions of Sm_2O_3 for neutron activation studies. In contrast, for compounds incorporating other atoms suitable for neutron activation, such as erbium, the low yield of activity from the naturally abundant material requires the use of expensive enriched material for dosage form radiolabelling (Digenis and Sandefer, 1991).

For neutron activation to be of practical use as a method for radiolabelling a pharmaceutical dosage form, it is clearly essential that the process has minimal effects on its physical and functional properties. However, a number of studies reported in the literature have demonstrated that both the incorporation of isotope and subsequent neutron irradiation can have effects on the properties of dosage forms.

For example, barium sulphate concentrations above 0.33% w/w and erbium oxide or Sm_2O_3 concentrations above 10% w/w were found to significantly reduce the hardness of lactose tablets. Following irradiation, the hardness of tablets containing isotope increased, while tablets containing no isotope were unaffected. For tablets with and without isotope, a direct correlation was observed between the irradiation time and the time for tablet disintegration (Parr and Jay, 1987).

The performance of enteric-coated erythromycin pellets containing erbium oxide has been assessed before and after neutron irradiation. The amount of erythromycin released in vitro after 30 min was significantly reduced compared to non-irradiated pellets, although there was no difference in release after 60 min. Irradiation also caused a small decrease in the acid resistance of the pellet coating, which may have resulted from an increase in coat porosity (Parr et al., 1990).

Sustained-release matrix tablets containing ibuprofen have been radiolabelled by incorporation of erbium oxide followed by neutron irradiation. The in vitro drug release rate was found to increase slightly following irradiation (Parr et al., 1987).

A recent publication has reported the incorporation of ^{165}Ho-acetylacetonate, a potential radiotherapeutic agent, into poly(lactic acid) microspheres produced by solvent evaporation. As a result of neutron irradiation, to convert ^{165}Ho to ^{166}Ho, the molecular weight of the poly(lactic acid) was markedly reduced (Mumper and Jay, 1992).

In our previous paper in this series, the incorporation of Sm_2O_3 over the concentration range investigated (0–0.8% w/w) was found to have no obvious effects on microsphere drug content, physical appearance or drug release rate (Watts et al., 1991a). In this second paper we report on the changes in physical and drug release properties resulting from neutron irradiation of the Eudragit RS-sulphasalazine microspheres.

Materials and Methods

Materials

Eudragit RS100 (Dumas U.K., Tunbridge Wells, U.K.), sulphasalazine (Sigma, Poole, U.K.), natural abundance samarium oxide (Sigma), polysorbate (Tween) 20 (Sigma), potassium dihydrogen orthophosphate (BDH, Poole, U.K.), sodium hydroxide (BDH), dichloromethane (GPR grade) (Rhône-Poulenc, Dagenham, U.K.) and methanol (HPLC grade) (Rhône-Poulenc) were obtained from the indicated sources.

TABLE 2

Composition of the Eudragit RS:sulphasalazine:samarium oxide microsphere formulations prepared for neutron irradiation

Formulation	Samarium oxide content (% w/w)	Sulphasalazine content (% w/w)	Eudragit RS content (% w/w)
1	0	31.8	68.2
2	0.08	31.1	68.8
3	0.24	31.8	68.0
4	0.61	31.5	67.9
5	0.80	31.9	67.3

Methods

Microsphere preparation and characterisation

Using an emulsification-solvent evaporation process, five batches of Eudragit RS microspheres were prepared containing 31–32% w/w sulphasalazine and 0–0.8% w/w Sm_2O_3 (see Table 2). Full details of the microsphere preparation technique and the assay for sulphasalazine and Sm_2O_3 content are provided elsewhere (Watts et al., 1991a).

Microsphere irradiation

Microspheres were irradiated at the Universities Research Reactor, Risley, Cheshire, U.K. A 500 mg sample of each batch (250–500 μm in diameter) was placed into a polypropylene tube and irradiated at a neutron flux of 10^{12} neutrons cm^{-2} s^{-1} for a period of 90 min. The radioactivity generated was measured 21 h after irradiation using an isotope calibrator set to detect ^{153}Sm (Type 238, D.A. Pitman, Surrey, U.K.). The delay between irradiation and measurement of activity allowed time for the decay of any short lived isotopes present in the sample, in particular ^{155}Sm.

Leaching of radioactivity

The extent to which the radiolabel dissociated from the microspheres during in vitro dissolution was assessed by the following method. 45 mg of irradiated microspheres (0.8% w/w Sm_2O_3 content) were measured for ^{153}Sm activity and placed into 500 ml of pH 7 phosphate buffer in a single vessel USP type 2 dissolution apparatus (37°C, agitation at 100 rpm). The dissolution fluid contained 0.02% w/v polysorbate 20 to aid microsphere wetting. At 1-h intervals over an 8 h period, the paddle in the dissolution vessel was stopped. The microspheres were allowed to sink to the bottom of the vessel and a 1 ml sample of buffer was drawn into a syringe. It was ascertained beforehand that the sedimentation of Sm_2O_3 was very much slower than the microspheres due to its small particle size (measurements using laser diffraction indicated that the majority of the Sm_2O_3 particles were below 10 μm in diameter). Thus, this technique avoided sampling of microspheres, but allowed sampling of any dissociated Sm_2O_3. The 1 ml sample was transferred to a vial and the radioactive counts recorded for a period of 10 s (Mini-Assay Type 6-20, Mini-Instruments, Essex, U.K.). At each time interval, the counts were also recorded from a reference buffer.

Drug stability

The stability of sulphasalazine to neutron irradiation was assessed by HPLC analysis of drug extracted from the microspheres before and after irradiation. To extract the drug, an accurately weighed sample of microspheres was placed in a 100 ml volumetric flask and 10 ml of methanol added to dissolve the polymer matrix. The polymer was precipitated and the drug dissolved by making to volume with 0.001 N sodium hydroxide solution, Prior to analysis, the samples were passed through a 0.1 μm membrane filter to remove precipitated polymer. 20 μl samples were injected manually onto the C_{18} reverse-phase HPLC column (Hichrom, Reading, U.K.). A mobile phase of 47.5% v/v methanol in pH 6 phosphate buffer was used at a flow rate of 1.3 ml/min (LC pump, Kontron, Switzerland). Peaks were detected spectrophotometrically at 254 nm (Kontron Uvikon 720 LC) and processed by an integrator (SP4270, Spectra Physics, San Jose, CA). Each microsphere batch was assayed twice.

Drug release rate

The microsphere drug release rate was determined before and after irradiation using a USP

type 2 dissolution apparatus. Samples were released into 500 ml of pH 7 phosphate buffer containing 0.02% w/v Tween 20 (37°C), and agitated at 100 rpm. 10-ml samples were withdrawn from the dissolution flasks at regular intervals, passed through a 1 μm membrane filter and the UV absorbance at 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 calculated with reference to a concentration:absorbance plot of sulphasalazine in phosphate buffer.

Polymer molecular weight analysis

The effect of irradiation on the stability of Eudragit RS was determined by molecular weight analysis of polymer extracted from pre- and post-irradiated microspheres. The microspheres were placed in a test tube and dissolved in methanol. The tube was then centrifuged to separate sulphasalazine from polymer solution. The clear polymer solution was pipetted into a glass evaporating dish and the methanol allowed to evaporate. The precipitated polymer was then redissolved in chloroform at an approximate concentration of 2 mg/ml in preparation for molecular weight analysis.

The polymer molecular weight was determined by a sedimentation equilibrium ultracentrifugation technique using a Beckman Model E analytical ultracentrifuge (Morgan et al., 1990).

Low temperature scanning electron microscopy

Surface and sectioned electron micrographs were obtained of samples of non-irradiated and irradiated microspheres from the batch containing no Sm_2O_3. Low temperature (cryogenic) scanning electron microscopy (Philips SEM 505/Hexland CT1000A cryotransfer station) was used to minimise damage to the samples from electron beam irradiation.

Results and Discussion

Microsphere characterisation

There were obvious physical changes in the irradiated microspheres apparent on removal from the reactor. All of the samples had lightly caked into a single mass. Although this mass could be largely broken-up by gentle shaking of the sample containers, the microspheres were passed through a 500 μm sieve to remove any aggregates prior to further evaluation. The microspheres had also undergone a minor darkening in colour and appeared to have slightly shrunk. These changes may have been a result of the heat of the reactor, since the samples would have been exposed to a temperature of about 50–60°C for the 90 min period of irradiation. This temperature will have exceeded the glass transition temperature of Eudragit RS (42°C) (Jones et al., 1991).

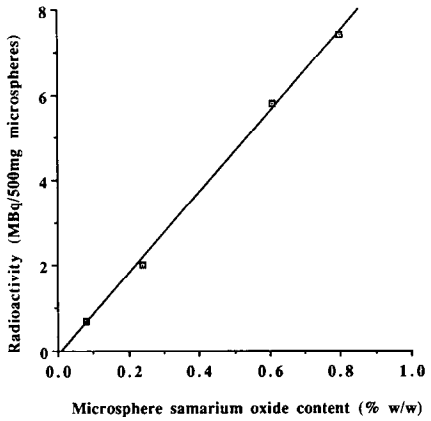


Fig. 1. ^{153}Sm activity generated by neutron irradiation of samarium oxide-containing Eudragit RS-sulphasalazine microsphere samples (radioactivity measured 21 h after irradiation).

The ^{153}Sm activity generated in each of the four Sm_2O_3-containing samples is presented in Fig. 1. There was an excellent correlation ($r = 0.999$) between assayed Sm_2O_3 content and radioactivity, indirectly confirming that the precision of X-ray fluorescence assay used for determining Sm_2O_3 content (Watts et al., 1991a) was high. To produce satisfactory scintigraphic images in a human subject, it would probably be necessary to administer approx. 2 MBq of ^{153}Sm, which in this study corresponded to a Sm_2O_3 content of approx. 0.2% w/w in 500 mg of microspheres. However, this amount of activity could equally be generated by longer irradiation of microspheres having a lower Sm_2O_3 content or shorter irradia-

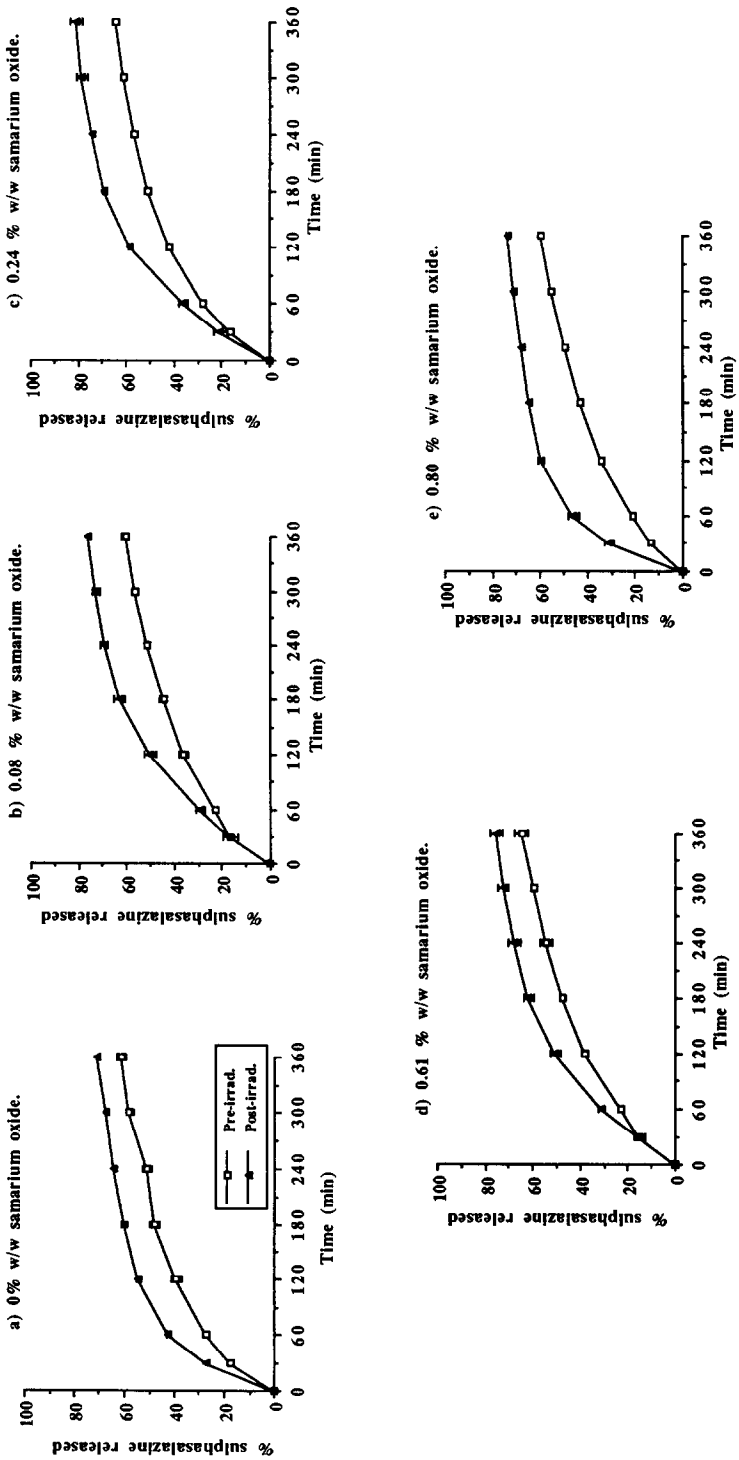


Fig. 2. (a–e) Release rate of sulphasalazine from Eudragit RS-sulphasalazine microspheres containing 0–0.80% w/w samarium oxide before and after neutron irradiation.

TABLE 3

Effect of neutron irradiation on the sulphasalazine content of Eudragit RS:sulphasalazine:samarium oxide microsphere formulations, measured using HPLC

Microsphere samarium oxide content (% w/w)	Microsphere sulphasalazine content (% w/w)	
	Before irradiation	After irradiation
0	31.1 ± 0.2	31.0 ± 0.1
0.08	31.2 ± 0.3	31.3 ± 0.1
0.24	31.8 ± 0.3	31.3 ± 0.6
0.61	31.0 ± 0.4	30.3 ± 0.2
0.80	30.3 ± 0.7	29.8 ± 0.2

tion of microspheres with a higher Sm_2O_3 content.

Leaching of radioactivity

Throughout the 8 h period of the dissolution experiment the radioactive counts in the dissolution buffer remained at the same level as the reference buffer. Loss of counts due to radioactive decay would have been less than 12% over this period due to the long half-life of ^{153}Sm. At the end of the 8 h dissolution period, spectrophotometric assay of the dissolution buffer revealed that 75% of the encapsulated sulphasalazine had been released. Therefore, although a large proportion of the drug had dissolved from the microsphere matrix, the radiolabel remained in place.

Drug stability

The results of the microsphere drug stability assay are presented in Table 3.

For the pre-irradiated samples, there was generally good agreement with the drug content values obtained from the spectrophotometric assay (Table 2). Following irradiation, the assayed sulphasalazine contents were essentially unchanged (Table 3). No additional peaks were detected and there were no changes in the elution time of the sulphasalazine peak.

Sulphasalazine therefore appeared to be stable to neutron irradiation under the chosen conditions. This is in agreement with other neutron irradiation studies, where ibuprofen (Parr et al., 1987), erythromycin (Parr et al., 1990), 5-aminosalicylic acid (Digenis and Sandefer, 1991) and naproxen (Hardy et al., 1991) have all been shown not to undergo significant radiolysis.

Drug release rate

Fig. 2a–e compares the drug release profiles of the five microsphere samples before and after irradiation. In all cases, the drug release rate was markedly enhanced following neutron irradiation, and with one exception (Fig. 2d), the time for 50% of the sulphasalazine to be released was at least halved.

Since some of the physical changes observed in the irradiated microspheres may have been heat-induced, the effect of heat alone on the microsphere drug release rate was established. 200 mg from a sample of Eudragit RS microspheres containing 30.7% w/w sulphasalazine was weighed into a pierced polypropylene tube and placed in an oven at 75°C for a period of 90 min. At the end of this period, the microspheres had lightly aggregated and darkened in colour. These effects were similar to those noted in the irradiated samples. There was a 1.3% loss in weight as a result of heating. Measured by light microscopy, the mean microsphere size had been reduced from 408 ± 61 to 349 ± 64 μm (n = 50). However, compared to an untreated sample, sulphasalazine was released more slowly from the heat-treated microspheres (see Fig. 3). The reduction in drug release rate for the heat-treated sample appeared to be primarily a result of impaired microsphere wetting during the early stages of dissolution.

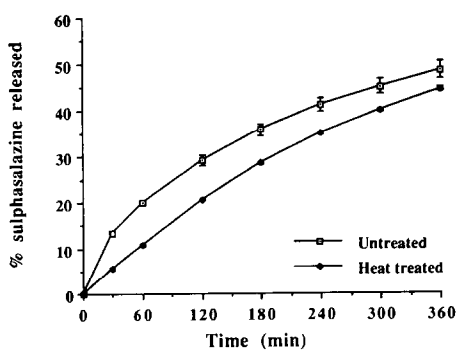


Fig. 3. Effect of heat treatment on the release rate of sulphasalazine from a sample of Eudragit RS-sulphasalazine microspheres.

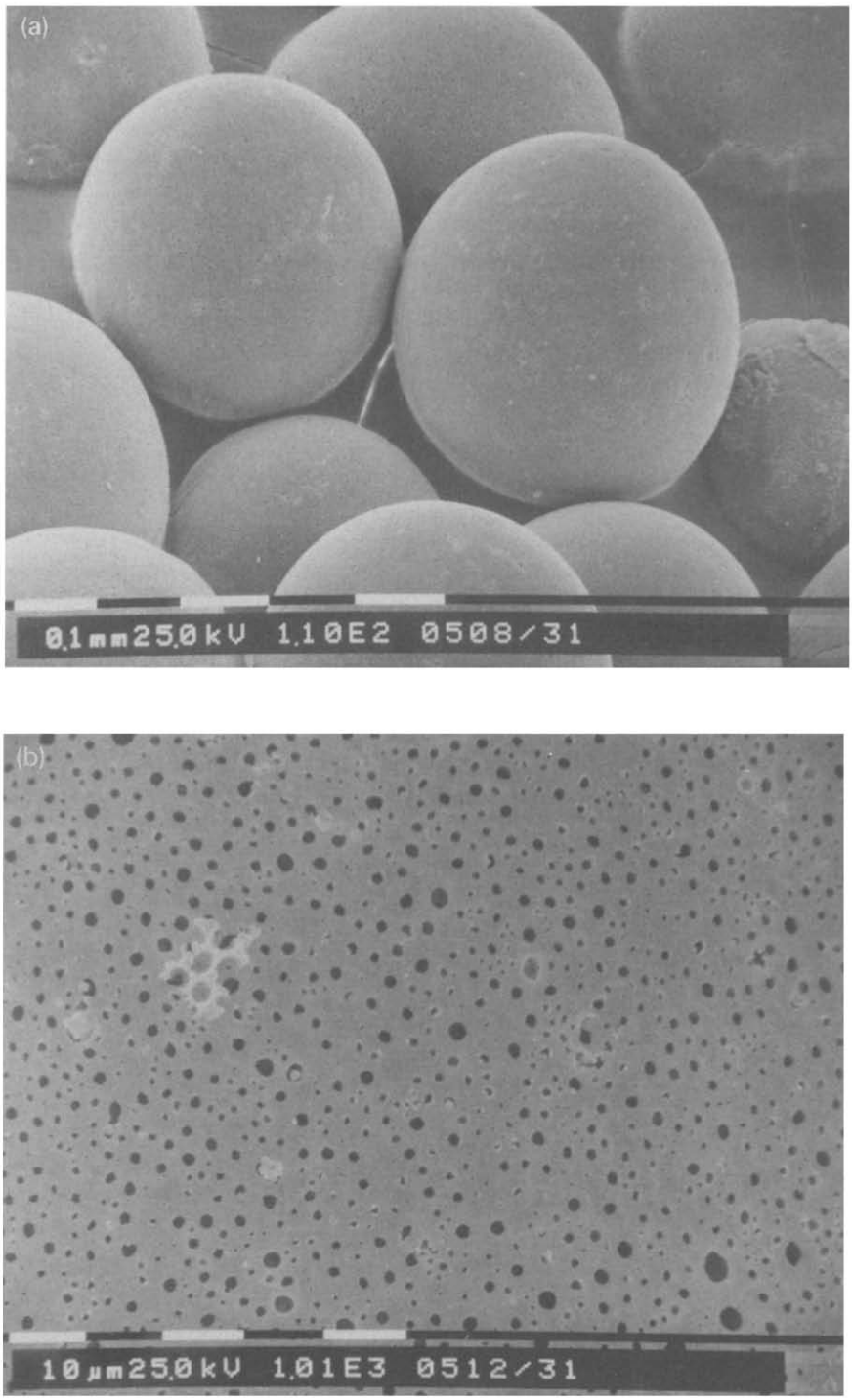
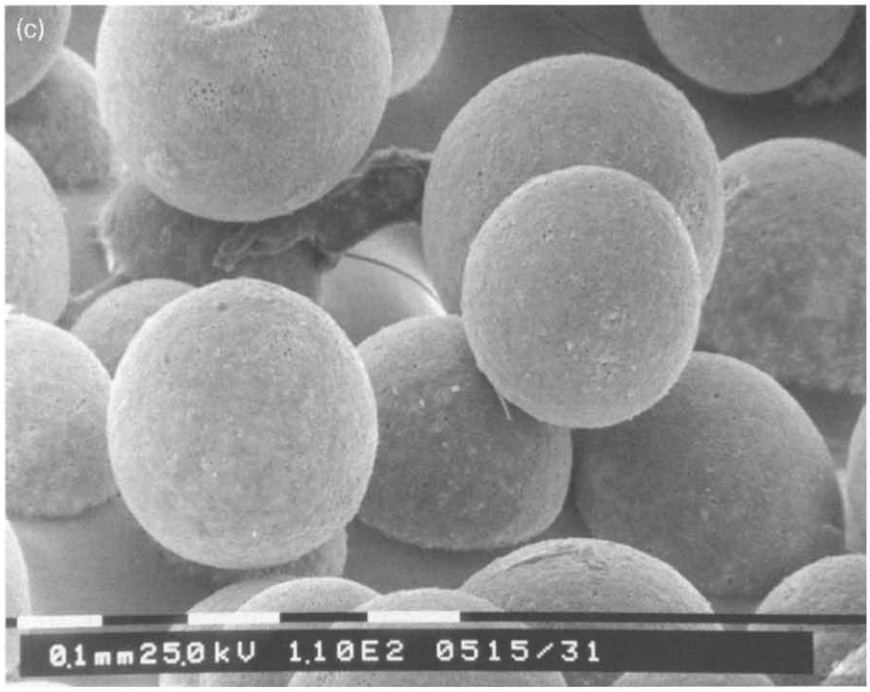


Fig. 4. Electron micrographs of the surface of Eudragit RS-sulphasalazine microspheres. (a) Non-irradiated microspheres (magnification ×110); (b) non-irradiated microsphere (magnification ×1000); (c) irradiated microspheres (magnification ×110); (d) non-irradiated microsphere (magnification ×1000).



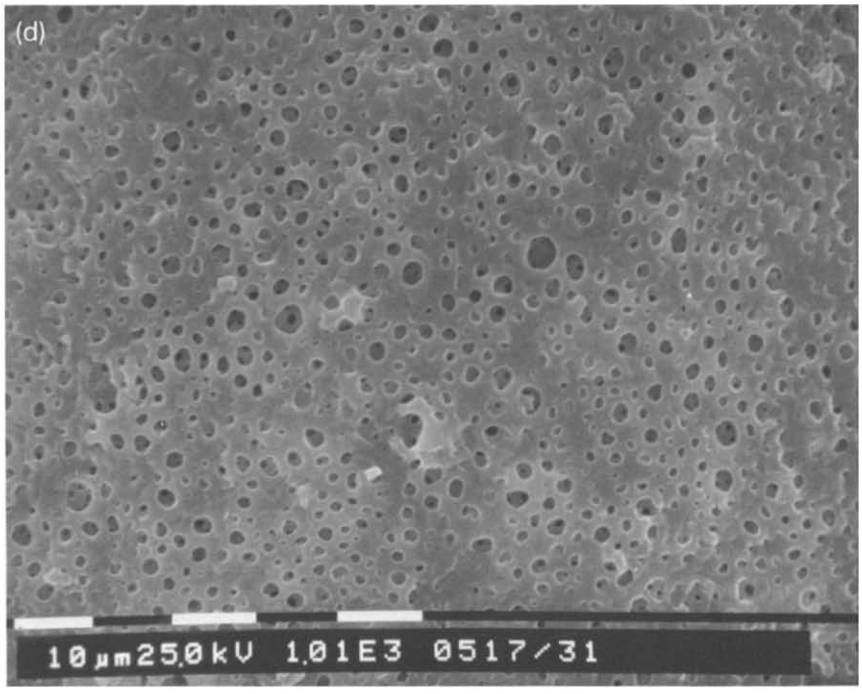


Fig. 4 (continued).

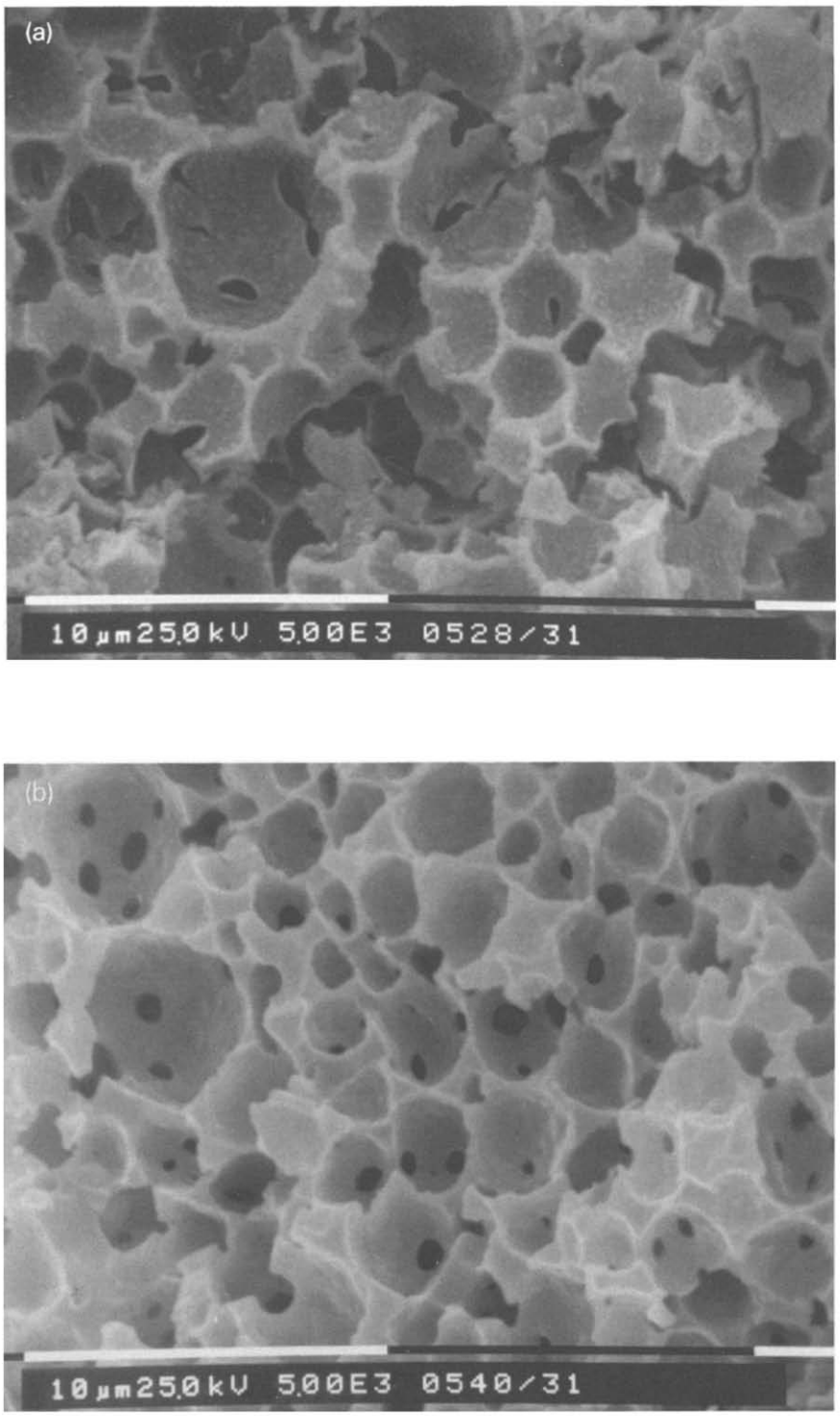


Fig. 5. Electron micrographs of the internal structure of Eudragit RS-sulphasalazine microspheres. (a) Non-irradiated microsphere (magnification ×5000); (b) irradiated microsphere (magnification ×5000).

Polymer molecular weight analysis

It was considered that the enhanced drug release from the irradiated microspheres may have been a consequence of radiation-induced polymer damage, resulting in a modification of microsphere structure and an increase in the rate of drug diffusion.

It is well known that polymers are sensitive to irradiation (Swallow, 1973; Spenlehauer et al., 1988; Mumper and Jay, 1992). The potential effects are complex and can result in backbone or side-chain scission and/or cross-linking and, in addition, the degree of damage can be influenced by many other factors such as the oxygen and moisture content of the sample (Swallow, 1973; Hartas et al., 1991).

For Eudragit RS extracted from the microspheres before irradiation, the relative weight average molecular weight (M^w), determined using ultracentrifugation, was 10000. Following irradiation this had increased to 12000. Since the margin of error of these determinations was of the order of $\pm 10\%$, it is conceivable that a small increase in molecular weight may have occurred during irradiation, due to a low degree of polymer cross-linking. However, it should be appreciated that a small change in average molecular weight may hide a greater change in the overall molecular weight distribution. Thus, in theory, a considerable rearrangement in molecular structure resulting from both cross-linking and chain scission could take place, yet the average molecular weight apparently show little change.

Electron microscopy

Low temperature/cryogenic SEM micrographs of both the microsphere surface and internal structure were recorded for one of the microsphere samples before and after irradiation. Fig. 4a–d compares the surface structures of the Sm_2O_3-free batch at $110\times$ and $1000\times$ magnification.

The surface of the non-irradiated microspheres was smooth and contained many pores (Fig. 4a and b). Following irradiation, the smooth surface had been replaced by a softened/melted appearance (Fig. 4c and d). The size and density of pores appeared largely unchanged following irradiation. As with the macroscopic changes noted earlier, the changes in microscopic appearance were probably a result of the heat exposure during irradiation.

The samples were then sectioned to view the internal microsphere structure. In the non-irradiated sample, the microsphere drug-polymer matrix had a porous, honeycomb appearance (Fig. 5a). This type of structure has been noted before in Eudragit RS microspheres produced by solvent evaporation (Watts et al., 1991b). This honeycomb internal structure appeared to be unchanged in the irradiated microspheres (Fig. 5b).

Conclusions

Our studies have demonstrated that Sm_2O_3 incorporation followed by neutron irradiation can be used to radiolabel microspheres produced by the solvent evaporation process. However, although the presence of Sm_2O_3 itself had no apparent effect on the microsphere properties (Watts et al., 1991a), in this paper we have demonstrated that the neutron irradiation process resulted in marked physical changes.

The change of greatest consequence resulting from neutron irradiation was a considerable enhancement in the rate of drug release from the microspheres. Although it was initially considered that this enhancement may have resulted from radiation damage to the polymer stucture, molecular weight analysis was inconclusive. Macroscopic and microscopic changes in the physical appearance of the microspheres were evident, but it is unclear how these might have enhanced the drug release rate. Some of the changes in microsphere appearance may have been the result of heating during the irradiation process, which by itself was found to reduce the microsphere drug release rate.

If neutron activation is to be used as a means for radiolabelling microspheres for scintigraphic evaluation, an increase in drug release rate of the magnitude seen in this study would be unacceptable. However, since for a given neutron flux rate, the amount of radiation damage will depend on the period of irradiation, a reduction in the

irradiation time may reduce the degree to which the drug release rate is enhanced. In the case of sulphasalazine, a pharmacokinetic study might require a dose of at least 500 mg of drug to be administered, which, at a 30% drug loading, would be provided by approx. 1500 mg of microspheres. In this study, more than 21 MBq/1500 mg was generated in the microspheres containing 0.8% w/w Sm_2O_3. Therefore, a dose of 2 MBq of radioactivity/1500 mg of microspheres, sufficient for scintigraphic imaging, could be achieved by a 90% reduction in the irradiation time.

In summary, for neutron activation to be a viable means for radiolabelling Eudragit RS microspheres, the quantity of Sm_2O_3 incorporated and the irradiation time need to be carefully chosen. In this way it may be possible to minimise adverse effects on the microsphere drug release performance and allow the technique to be applied in preparing microspheres for scintigraphic evaluation.

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