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European Journal of Pharmaceutics and Biopharmaceutics 43 (1997) 159–164

European Journal of Pharmaceutics and Biopharmaceutics

The influence of thermal neutron irradiation on the in vitro characteristics of ASA oral dosage forms Validation of neutron activation

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Received 15 April 1996; accepted 24 June 1996

Abstract

This paper deals with the effects of the neutron irradiation procedure on the in vitro performance of various commercially available products on the Scandinavian market: paracetamol crystals coated with ethyl cellulose, acetylsalicylic acid (ASA) crystals coated with ethyl cellulose and compressed into tablets, and ASA crystals coated with ethyl cellulose (raw product). The primary objective of the work was to observe the impact of irradiation on complex formulations, manufactured under industrial scale conditions. All formulations, without any incorporation of lanthanide isotope, were irradiated in the JEEP II reactor (Institute of Energy Technology, Kjeller, Norway) and subjected to several in vitro tests. The irradiation was performed in a neutron flux of 1.1×10^{13} n/cm per s for 2, 4, 7 or 15 min. Irradiation of the microencapsulated paracetamol crystals up to 4 min caused no significant damage as compared with the reference. An increase in the exposure time, however, tends to decrease the dissolution rate. In spite of that, the changes were too small to conclude nonequivocally that radiation damage had occurred. Distinct differences of disintegration time and dissolution rate after irradiation were found in the formulation of ASA crystals microencapsulated with ethyl cellulose compressed in rapidly disintegrating tablets. Irradiation up to 7 min prolonged the disintegration time, but unexpectedly accelerated the dissolution. A 15 min exposure caused severe radiation effects, inhibiting drug release almost totally. Tablet hardness increased considerably after irradiation for 15 min, as compared with tablets not irradiated. Based on the present findings and subsequent work, it was concluded that irradiation damage was probably due to degradation of the ethyl cellulose and changes in the crystalline structure of the ASA. Unlike the tablets, drug release of the corresponding granules was accelerated after exposure to the neutron beam for 15 min. This behaviour was attributed to the different density of the materials. Irradiation of the granules for 15 min appeared to cause similar damage as irradiating tablets for 2 min, since the extent of interaction between exposed material and neutron particles depends directly on the density. © 1997 Elsevier Science B.V.

Keywords: Paracetamol crystals; Acetylsalicylic acid crystals; Ethyl cellulose

1. Introduction

The physiological behaviour of a pharmaceutical dosage form is usually predicted by assessment with in vitro pharmacopoeial methods, as, e.g. disintegration and dissolution tests. However, the influence of the preparation itself on the gastrointestinal passage is more complex, so that conclusions based on such simplified methods may be misleading. The technique of γ-scintigraphy has over the last decade, made significant contributions to the understanding of the fate of various dosage forms in the gastrointestinal tract. The performance and behaviour of, e.g. oral dosage forms have been studied in vivo using external scintigraphic imaging techniques.

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PII S0939-6411(96)00008-2

Such procedures require dosage forms radiolabelled with a γ-emitting radionuclide possessing a short half-life to minimise the radiation exposure to the subjects involved in the tests. The dosage forms are usually radiolabelled by incorporating a radionuclide such as ^{99m}Tc or ^{111}In during the preparation.

In many cases, it is not convenient to use a radionuclide marker with a short half-life, particularly for dosage forms requiring a relatively long time for their preparation, such as enteric coated tablets. The implementation of alternative methods has been critically reviewed [1]. Parr et al. [2,3] first described the application of neutron activation for radiolabelling intact dosage forms to overcome the limitation of the aforementioned labelling methods. Neutron activation has proved successful for monitoring the fate of both oral and rectal dosage forms in vivo. During the manufacturing process, a stable nuclide is added to the formulation. Prior to administration, the dosage form is exposed to thermal neutrons producing a radionuclide emitting gamma rays in situ.

In a review of the method, Meseguer, Buri and Gurny list a number of nuclides which have been subject to systematic investigations [4].

The choice of nuclide to be used is based on several considerations, including: the half-life of the radioactive nuclide formed by the neutron flux; the γ and β emission energy of the nuclides in question; and the time needed for the transport of the dosage form from the irradiation facilities to the site of investigation.

To achieve a desired level of activity, the amount of isotope as well as the irradiation time may exceed a critical level, affecting the physical properties of the preparation. The γ and β yield of the radioactive nuclides formed may be unimportant compared with the impact of the irradiation process itself.

The aim of the present study was to examine the influence of neutron irradiation on a number of in vitro characteristics of complex dosage forms, manufactured under industrial scale conditions.

2. Experimental—material and methods

2.1. Materials

Two controlled release preparations authorised in Norway (Globentyl®, Nycomed N-Oslo) and Sweden (Paracetamol ER®, Astra, S-Södertälje), respectively, were chosen from market products. Both formulations consist of microencapsulated drug granules. Globentyl® tablets are formed of compressed acetylsalicylic acid (ASA) crystals coated with ethyl cellulose. Paracetamol ER sachets contain paracetamol crystals coated with an ethyl and hydroxypropylmethyl cellulose membrane as drug-release modifying polymer.

In addition, investigations were performed using the raw-product of Globentyl® consisting of microencapsulated ASA granules.

2.2. Irradiation procedures

All samples were irradiated at the JEEP II reactor (Institute of Energy Technology, N-Kjeller). Before irradiation the content of one Paracetamol ER® sachet or a sample of 2.5 g raw product, respectively, was transferred into a plastic ampoule. Samples of 20 Globentyl® tablets or four plastic ampoules were placed into a polypropylene tube (rabbit) and conveyed by a rabbit system to the reactor core and irradiated with thermal neutrons for the selected time, i.e. 2, 4, 7 or 15 min, respectively, at 1.1×10^{13} neutrons/cm per s. Internal evaluations have shown the day to day variation in the flux to be in the order of 1–2%. After irradiation, the tablets/granules were stored behind a lead-shield until the radioactivity decreased below 400 000 Bq.

2.3. Tablet hardness of Globentyl® tablets

Tablet hardness was determined using a 'Schleuniger Bruchfestigkeitstester' (Schleuniger Productronic AG, CH-Solothun) which calculates hardness by measuring the force needed to crack the tablet in half.

2.4. Disintegration of Globentyl® tablets

Tablet disintegration time was determined by placing one tablet in each chamber of the USP disintegration apparatus [5] which was then placed into 900 ml 0.1 N hydrochloric acid. The time needed for all granules to pass through the mesh was measured.

2.5. Colour changes after irradiation

The appearance of a number of irradiated tablets (usually 10), was compared with that of the same number of non-irradiated tablets.

2.6. Salicylic acid content in Globentyl® tablets

A defined quantity of a crushed tablet was transferred to a 25 ml volumetric flask and diluted with methanol. The flask was shaken for about 2 min, the content filtered through a 0.22 μm filter and analyzed by high pressure liquid chromatography (Shimadzu, JP-Kuyoto). Determination of the salicylic acid content was performed by injecting 30 μl of each sample onto a Bondapack C 18 column 300 × 3.9 mm I.D., 10 μm average particle size (Waters, USA-Milford, MA), and monitoring the absorbance at 267 nm. A mobile phase consisting of 460 ml methanol, 540 ml distilled water and 10 ml concentrated acetic acid was used at a flow

rate of 1 ml/min. Under these chromatographic conditions, salicylic acid eluted at around 7 min.

2.7. Dissolution

2.7.1. Globentyl® tablets

Drug release from Globentyl® tablets was determined both in water (pH 6.9) and 0.1 N hydrochloric acid on a total of 12 tablets per irradiation group. Dissolution tests were performed in a dissolution apparatus (USP apparatus II) [5]. The apparatus used was a Sotax AT6 (Sotax AG, Switzerland) with a constant temperature water bath at 37 ± 0.5°C. The Sotax AT6 was connected on-line to a spectrophotometer (Ultrospec II, LKB, S-Uppsala), interfaced with a PC (Commodore PC 20-II, Software TDS, LBK, S-Uppsala). One tablet, containing 500 mg ASA, was released into the dissolution media, and agitated at 100 rpm. Samples were withdrawn (flow 2 ml/min) , filtered (GDG/F Whatman International, UK-Maidstone) and measured (267 nm) at programmed time intervals. The drug concentrations were calculated using a calibration curve for ASA in the corresponding dissolution media.

2.8. Raw-product (globentyl)

A portion of 520 ± 0.4 mg of raw product containing approximately 500 mg of ASA was mixed with 900 ml of 0.1 N Hydrochloric acid. To facilitate the wetting of the granules, 0.01% of Polysorbat 80 was added to the dissolution media. Dissolution tests were performed on a total of 12 aliquot samples using the same conditions as described above.

2.9. Paracetamol ER®

The content of one sachet was added to 1000 ml of water (pH 6.9). Samples were measured (249 nm) using the system described above and calculated using a calibration curve of paracetamol in water (pH 6.9).

2.10. Statistical treatment of the results

The Weibull equation [6] was fitted to the release profiles. Due to the release profiles and the sampling schedule, the F_u (amount released after infinite time), was fixed to 100% for the release from the Globentyl® tablets.

The calculated parameters and measured values from the hardness and disintegration tests were compared using a multivariate analysis of variance, canonical discriminant analysis in the SAS-system (SAS Institute US, Cary, NC).

Table 1
Hardness of irradiated globentyl tablets (in kp, $n = 10$)

Irradiation-group	R-0 min	R-2 min	R-4 min	R-7 min
M	6.9	9.7*	7.0	8.6*
SD	0.4	1.6	0.6	1.1
CV (%)	5.2	16.7	9.1	12.5

* Value significantly different from non-irradiated tablets ($P = 0.05$).

3. Results

3.1. Globentyl® tablets

3.1.1. Hardness

The results of the tablet hardness tests are shown in Table 1. There were significant differences between non-irradiated and some of the irradiated tablets. The unchanged hardness of the tablets irradiated for 4 min compared with the significant increase for tablets irradiated 2 and 7 min, is inexplicable. Irradiating globentyl tablets for 15 min produced unbreakable tablets. This irradiation treatment was therefore, excluded from the further statistical treatment of the data obtained.

3.1.2. Disintegration

Irradiation significantly prolongs the disintegration of the globentyl tablets, as can be seen in Table 2. As in the hardness test, the tablets irradiated for 15 min had to be excluded from the evaluation. For these tablets the disintegration test was discontinued after 24 h without any sign of disintegration. The main difference between disintegration of irradiated and non-irradiated tablets was that whereas the non-irradiated produced a fast disintegration into light snowflake-like particles which easily passed the mesh, irradiated tablets produced larger yellowish parts which were retained on the mesh for some time.

3.1.3. Colour changes

Although the tablets were distinctly brown following 15 min irradiation, no clear changes in appearance could be detected for tablets irradiated 2–7 min.

3.1.4. Salicylic acid content

There were no differences in the salicylic acid content of the non-irradiated and irradiated tablets.

Table 2
Disintegration of irradiated globentyl tablets (in min, $n = 6$)

Irradiation-group	R-0 min	R-2 min	R-4 min	R-7 min
M	0.3	3.3*	3.8*	3.1*
SD	0.0	1.4	1.4	1.3
CV (%)	0.0	42.6	36.7	4.1

* Value significantly different from non-irradiated tablets ($P = 0.05$).

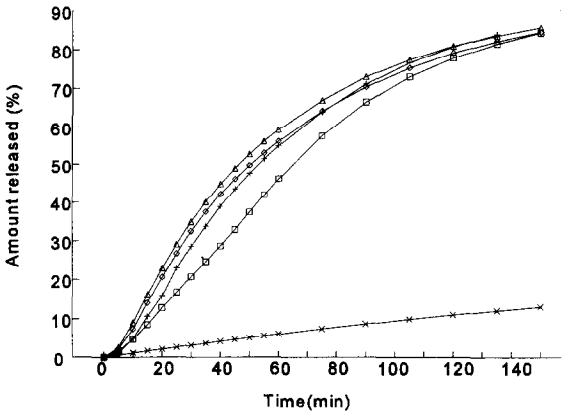

Fig. 1. Release profile from globentyl tablets in water (mean value of raw data, $n = 12$): □, R-0 min; +, R-2 min; ◇, R-4 min; △, R-7 min; and X, R-15 min.

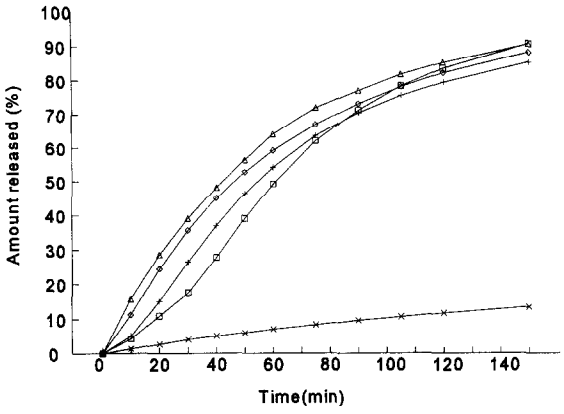

Fig. 2. Release profile from globentyl tablets in HCl (mean value of raw data, $n = 12$): □, R-0 min; +, R-2 min; ◇, R-4 min; △, R-7 min; and X, R-15 min.

3.1.5. Dissolution in water

As is depicted in Fig. 1, the release of ASA from the irradiated tablets differed significantly from the non-irradiated tablets. The most marked difference was the prolonged release from tablets irradiated for 15 min. In comparison, tablets irradiated 2–4 min differ markedly from controls, since irradiation produced a faster release. The Weibull model was fitted to the release data. The estimated parameters are shown in Table 3. Due to the slow release from the tablets irradiated for 15 min, estimation of the Weibull parameters for these tablets was not possible, and consequently left out of the subsequent statistical evaluation. Multivariate analysis of the parameters showed that there was a significant reduction for both Td and β for the irradiated tablets in comparison with the non-irradiated ones.

3.1.6. Dissolution in HCL

As can be seen from Fig. 2, and Table 4, the alterations in the release profile as a consequence of irradiation, are the same as in water. And also in this case, the statistical evaluation showed a significant reduction in Td and β following irradiation from 2 to 7 min.

Table 3
Mean Weibull-parameters for release in water ($n = 12$)

Irradiation-group		T_d (min)	β	T_0 (min)
R-0 min	M	83.5	1.2	4.2
	SD	13.3	0.3	4.2
	CV (%)	15.9	26.6	101.9
R-2 min	M	65.5	0.9	8.0
	SD	18.0	0.2	3.7
	CV (%)	27.5	18.8	46.1
R-4 min	M	68.3	0.8	7.8
	SD	7.5	0.1	2.9
	CV (%)	11.0	8.7	37.3
R-7 min	M	62.0	0.8	12.1
	SD	9.5	0.1	4.1
	CV (%)	15.3	11.6	33.7

3.2. Globentyl® raw product

As can be seen from Fig. 3 and Table 5, the effects of irradiation on the raw product had an opposite effect as compared with the compacted tablets. Irradiating the powder for 4 min prolonged the release, whereas a 15 min irradiation increased the rate of release. In contrast, an almost insoluble system was produced by irradiating the tablet.

3.3. Paracetamol ER® granules

Although the formulation of the paracetamol and that of the globentyl granules are almost identical, the release from paracetamol is not affected by irradiation, as can be noted in Fig. 4.

4. Discussion

Several factors determine the time of irradiation needed for a dosage form to achieve a desired level of activity at the time of its administration (e.g. 50 μCi).

Table 4
Mean Weibull-parameters for release in HCL ($n = 12$)

Irradiation-group		T_d (min)	β	T_0 (min)
R-0 min	M	74.6	1.4	4.8
	SD	6.2	0.2	2.9
	CV (%)	8.3	11.9	60.5
R-2 min	M	66.4	0.9	10.8
	SD	9.6	0.1	4.1
	CV (%)	14.5	15.0	37.6
R-4 min	M	63.7	0.9	4.6
	SD	12.4	0.1	1.7
	CV (%)	19.5	10.8	36.5
R-7 min	M	57.4	0.9	2.5
	SD	5.5	0.1	2.4
	CV (%)	9.7	10.5	93.9

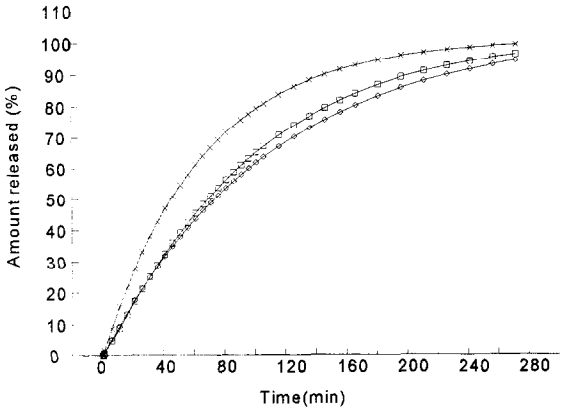

Fig. 3. Release profile from globentyl granules in HCl following Weibull-Transformation ($n = 12$): □, R-0 min; ◇, R-4 min; and X, R-15 min.

At a defined flux, the necessary load (mg) of the dosage form mainly depends on the neutron capture cross-section of the nuclide used. Other factors which have to be taken into account include unfavourable activation of other formulation components and the time required for the transport of the dosage form from the site of irradiation to the imaging facilities. The range of irradiation times used in this study was chosen from published work, taking different neutron flux into consideration and allowing for possible transport. Assuming the incorporation of 2 mg erbium oxide into a tablet (weight 600 mg) irradiation for 2, 4, 7 an 15 min in a neutron flux of 1.1×10^{13} n/cm per s would result in an activity of 50 μCi 1, 8, 14 and 22 h after the irradiation. The radiation exposure a subject would receive after oral administration of a tablet with this activity is comparable to the radiation exposure received from, e.g. X-ray examination of the GI or chest.

This study was performed without the incorporation of any marker. The reason was that we wanted to examine a complex formulation manufactured under industrial scale conditions. Furthermore, previous work [7–9] performed on the effect of incorporation of stable markers, demonstrated that adding small amounts (0.3–1% W/W) of erbium or samarium oxide does not significantly affect the integrity of the dosage form. In these publications, it has also been shown that the effect of the radioactivity of the activated isotope on in vitro behaviour was negligible, compared with the effect of the irradiation procedure.

A possible explanation for the observed effects could be the elevated temperature inside the pile reactor (up to 60°C). The effect of heat was not tested specifically for the preparations studied in this paper. But exposure of similar preparations to 60°C for 15 min did not produce any alterations in the in vitro characteristics [10].

Summarising the findings of the present study, globentyl tablets appear to be especially sensitive to the irradiation process. In spite of the prolonged disintegration time found for irradiated tablets, irradiation for 7 min results in an increase in the dissolution rate. In contrast, irradiation of the tablets for 15 min causes an almost complete blockage of drug release and disintegration. The stickiness of the granules observed both during the disintegration process of the tablets and after irradiation of the raw product may contribute to the altered in vitro behaviour. The observed changes in the in vitro behaviour may be explained by a combination of several radiochemical reactions. Sisman and Bopp [11] noted that a predominant chain scission occurs in the substituted as well as the unsubstituted cellulose chain upon exposure to pile radiation. According to these authors, the 1,4-α-glucosyl unit is one of the structures easily split by ionising radiation. This may explain the dissolution behaviour of globentyl tablets since a degradation of the cellulose chain as described above would reduce the diffusion barrier and thus, produce a faster release of the ASA. Radiophysical processes influencing the drug release may be caused by partial distortion of the crystalline structure of the ASA. Damage of the lattice structure may occur through expulsion of atoms by neutrons, thereby transferring some of their energy to the atoms (Frenkel defects). Accumulation of such defects can finally cause partial melting of the substance [12]. Since the frequency of lattice defects is proportional to the density of the material and since melting processes have a considerable impact on the in vitro behaviour of single unit dosage forms, the different behaviour of the raw product and tablets can be explained. As observed, sticky granules were produced when irradiating the raw product for 15 min, whereas the tablet hardness increased dramatically and the tablet became almost unbreakable. On the other hand, although the raw products of globentyl and paracetamol ER are quite similar in their formulations, irradiation of paracetamol ER up to 15 min did not change their in vitro behaviour at all.

Acknowledgements

Abstracted in part from a thesis submitted by G.S. Lisether to the Christian-Albrechts-Universität, Kiel. This study was in part supported financially by the Scientific Fund of NMD, the Royal Norwegian Council for Scientific and Industrial Research and Nycomed Pharma A/S, Oslo. The authors wish to thank M. Pharm. P.O. Bremer, B. Andreassen and O. Johanssen at the Institute for Energy Technology, Kjeller for irradiation of the samples. We are grateful to Dr C. Graffner for valuable discussions and Dr Bærheim Svendsen proofreading this manuscript and for valuable comments. We acknowledge Dr Clive Wilson for

Table 5
Mean Weibull-parameters for release in HCL ($n = 12$)

Irradiation-group		F_u (%)	T_d (min)	β	T_0 (min)
R-0 min	M	101.4	98.3	1.1	0.0
	SD	0.5	1.3	0.0	0.0
	CV (%)	0.5	1.3	0.9	—
R-4 min	M	103.1	110.2	1.0	0.0
	SD	0.4	1.7	0.0	0.0
	CV (%)	0.4	1.5	1.3	—
R-15 min	M	101.1	65.7	1.0	0.0
	SD	0.4	1.4	0.0	0.0
	CV (%)	0.3	2.2	0.6	—

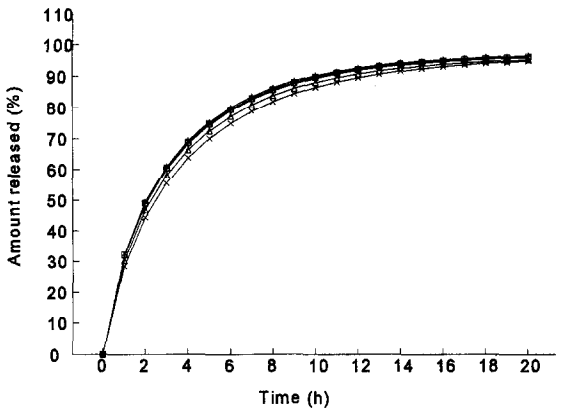

Fig. 4. Release profile from paracetamol granules in HCl following Weibull-Transformation ($n = 12$): □, R-0 min; +, R-2 min; ◇, R-4 min; △, R-7 min; and X, R-15 min.

his comments and pertinent criticism of this work. The technical assistance of Helge Gundersen is gratefully acknowledged.

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