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Neutron activation-based gamma scintigraphy in pharmacoscintigraphic evaluation of an Egalet® constant-release drug delivery system

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Abstract

This paper is a report from a pharmacoscintigraphic study with an Egalet® constant-release system containing caffeine and natural abundance samarium oxide. First the formulation was tested in vitro to clarify integrity during irradiation in the nuclear reactor. Then six healthy male volunteers were enrolled into the in vivo study. The in vitro release of caffeine obeyed all the time linear zero-order kinetics. The in vivo release of radioactive Sm_2O_3 consisted of three consequent linear phases with different slopes. The release rate was fastest while the product was in the small intestine and slowest when the product was in the descending colon. In terms of the bioavailability of caffeine, the most important factor seemed to be the residence time in the ascending and transverse colon. A long residence time in these sections led to high AUC values for caffeine.

Keywords: Gamma scintigraphy; Neutron activation; Samarium; Imaging; Controlled release; In vitro–in vivo correlation

1. Introduction

It has become increasingly evident that in vitro studies are inadequate in relation to the development of modified-release drug formulations. In vivo behaviour also needs to be investigated at an early stage. One of the most appropriate means of studying the fates of formulations in the gastrointestinal (GI) tract is gamma scintigraphy (Wilson and Washington, 2000; Newman et al., 2003). In most studies radioactive technetium (^{99m}Tc) or indium (^{111}In) has been used as a marker. These radioisotopes have, however, some disadvantages, especially if modified-release oral formulations are being evaluated. Firstly, both are radioactive, which limits the use of normal manufacturing procedures and equipment. Secondly, the half-life of ^{99m}Tc is short (6 h) in relation to the time needed to study many prolonged-release applications.

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For complex dosage forms a method involving use of a stable isotope during preparation of the product is preferable. Samarium oxide (Sm_2O_3) can be satisfactorily employed in this connection (Parr et al., 1985). Following its incorporation in a formulation during manufacture it can be activated in a neutron flux to $^{153}Sm_2O_3$ with a half-life of 46 h. The method requires access to an appropriate nuclear reactor. Gamma cameras used for diagnostic purposes are suitable for studying drug formulations, and are available in most large hospitals.

The aim of this work was to subject an oral constant-release Egalet® drug delivery system to pharmacoscintigraphic investigation. The in vitro drug release rate from the Egalet® system studied should be constant (zero-order kinetics) (Bar-Shalom et al., 2003). However, no evidence is available about the in vivo behaviour of the system and how it correlates with the pharmacokinetic profile of the drug substance included in the system. In this study caffeine was used for pharmacokinetic evaluation and the fate in the GI tract was investigated by neutron activation-based gamma scintigraphy.

2. Materials and methods

2.1. Study product

The target formulation in this study was Egalet® constant-release system (Egalet a/s, Denmark). The Egalet® system is an injection-moulded drug delivery system. It consists of an impermeable shell enclosing a plug of active drug. The shell is a cylindrical tube open at both ends and is made of cetostearyl alcohol and ethylcellulose. The matrix of the plug comprises a mixture of polyethylene glycol monostearates and polyethylene oxide (Bar-Shalom et al., 2003). The drug-release mechanism is expected to be erosion of the matrix rather than diffusion from the matrix. The formulation contained 8 mg of natural-abundance Sm_2O_3 (Aldrich, USA) of which 26.7% is $^{152}Sm_2O_3$, and 50 mg of caffeine (Ph.Eur.).

2.2. In vitro studies

The Egalet® formulation was subjected to dissolution testing using the paddle method of USP 24. A rotation rate of 100 min^{-1} and a 0.1 M HCl medium (900 ml) were used for the first 2 h. The rotation rate was then reduced to 50 min^{-11} and the medium changed to a phosphate buffer of pH 6.8 (900 ml). Caffeine concentrations in samples were measured spectrophotometrically at 284 nm. Both irradiated and non-irradiated dosage forms were tested.

2.3. Neutron activation

The Sm_2O_3 was activated to $^{153}Sm_2O_3$ using the FiR 1 250 kW TRIGA nuclear reactor (General Atomics, USA) at the VTT Technical Research Centre of Finland and a neutron flux of $1.1 \times 10^{12} n\,cm^{-2}\,s^{-1}$. The temperature in the irradiation space of the reactor did not exceed 40 °C. Seven items were irradiated at a time. The irradiation time was 4 min, to allow the target radioactivity of 1 MBq to be reached 48 h after irradiation.

2.4. Safety requirements

Gamma spectra and radioactivities were measured to assess the safety of the formulations for use in studies on human. The requirements were in accordance with STUK (Finnish Radiation and Nuclear Safety Authority) guidelines. The as-low-as-reasonably-achievable (ALARA) principle was observed, and exposure to radiation was minimized in all situations. Gamma spectra were measured 24 h after irradiation for one example, using a high-purity germanium semiconductor (model 7229P, Canberra, Belgium) to confirm radioactive purity. The safety requirements were that any net gamma peak area not originating from ^{153}Sm should not exceed 0.3% of the ^{153}Sm main peak at 103 keV, and that the total for net peak areas not originating from ^{153}Sm should not exceed 1% of the ^{153}Sm main peak.

The radioactivity of ^{153}Sm was measured 48 h after irradiation, i.e. immediately before drug administration to volunteers. A Vinten Isocal II radioisotope calibrator (Vinten Instruments Ltd, UK) was used. Six capsules from each batch were studied. The safety requirement was that ^{153}Sm activity should not exceed 1.4 MBq. A radioactivity of 1.4 MBq corresponds with an effective absorbed dose of 1 mSv for each study subject.

2.5. Gamma scintigraphic investigations

Six healthy male volunteers (20–40 years of age) were enrolled in the study. Their weights varied from 70 to 87 kg and their body mass indices (BMI) from 21 to 25 kg m^{-2}. Before the studies, each volunteer was examined physically and subjected to routine haematological testing (haemoglobin, haematocrit, erythrocyte count, total white cell count, red cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet count, sedimentation rate, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase) and urine analysis (pH, protein, glucose). Each volunteer was informed about the possible risks and adverse effects of taking the study formulations. Written informed consent to participate in the study was obtained. The study was carried out in accordance with International Conference on Harmonization (ICH) Good Clinical Practice Guidelines and the Declaration of Helsinki (World Medical Assembly, 1964) and subsequent amendments. The study protocol had been approved by the Finnish National Agency for Medicines and the Ethics Committee of Helsinki University Hospital (HUS). The studies were carried out at Diacor private hospital, which has a radiation safety licence issued by STUK.

Scintigraphic studies were started 48 h after neutron activation. This delay allowed decay of unwanted radioisotopes (primarily ^{24}Na). The lower tip of the sternum and the iliac crests of each study subject were marked with a felt-tip pen, and markers containing ^{57}Co were attached to these locations with adhesive tape. The radioactivities of the markers at the sternum and iliac crest were 0.03 and 0.96 MBq, respectively.

Each volunteer had fasted for at least 12 h, and had been asked to abstain from foods and fluids containing xanthine or caffeine for 48 h prior to drug administration. Xanthine and caffeine ingestion was also forbidden throughout the imaging period. At approximately 8 a.m. subjects in the sitting position were given one Egalet® system with 180 ml of water. Following drug administration, ten anterior and posterior images each of one minute's duration were recorded at intervals of 0.5 (0–5 h), one (5–12 h) and three h (12–24 h), for 24 h. Imaging was undertaken with subjects in the supine position. Between imaging times the subjects could move freely. Scintigrams were recorded at 103 keV (window width ±10%) using a Multispect 2 dual-head gamma camera (Siemens AG, Germany). Collimators were of the LEHR (low energy high resolution) type. The first meal was allowed 4 h after ingestion of the formulation.

Stored scintigrams were used to determine the radioactivities of the products as functions of time. Regions of interest (ROI) relating to counts originating from non-disintegrated drug formulations were drawn manually on anterior and posterior gamma images for each time point. Geometric means of counts relating to the two ROIs were calculated.

2.6. Pharmacokinetic evaluation

Just before drug administration a cannula was inserted into the forearm vein of each subject and a blank blood sample was taken. After each imaging period a 10 ml blood sample was taken for measurement of caffeine levels. Blood levels of the drug were determined by means of gas chromatography, using a method developed at the National Public Health Institute, Laboratory of Substance Abuse, which is a clinical chemistry laboratory accredited by the Finnish authorities. The analytical method was validated according to Shah et al. (2000).

3. Results and discussion

The in vitro dissolution curve for caffeine from the Egalet® constant-release formulation is shown in Fig. 1. Drug release took place over 10 h and closely followed zero-order kinetics, as the manufacturer had indicated it should. Irradiation changed the in vitro dissolution profile slightly. During the first 2 h the release rate was greater than from formulations that had not been irradiated, but subsequently the curves were largely parallel. It is well known that irradiation can accelerate drug release, especially from formulations that contain polymeric excipients (Watts et al., 1993; Waaler et al., 1997; Säkkinen et al., 2004). However, we decided that the change in dissolution profile was so minimal that conclusions could still be drawn concerning the fate of the product studied, as determined by means of gamma images.

Fig. 2 shows the fate of the Egalet® system in volunteer 04. The gastric residence time was 1 h. Four

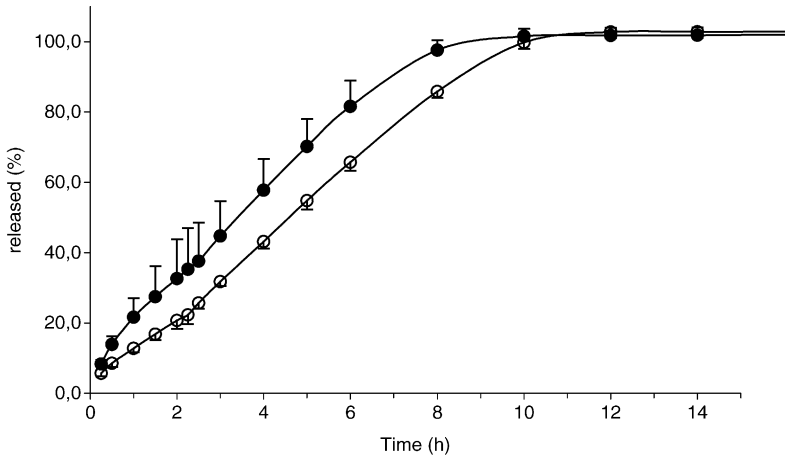

Fig. 1. Dissolution of caffeine from non-irradiated Egalet® systems (○) and Egalet® systems irradiated (●) for 4 min ($n = 6$). Bars indicate standard deviations. Effects of irradiation are most marked during the first hours of dissolution. The dissolution medium was 0.1 M HCl for the first 2 h, then phosphate buffer pH 6.8 for the next 22 h. Rotation rates were 100 and 50 min^{-11}, respectively.

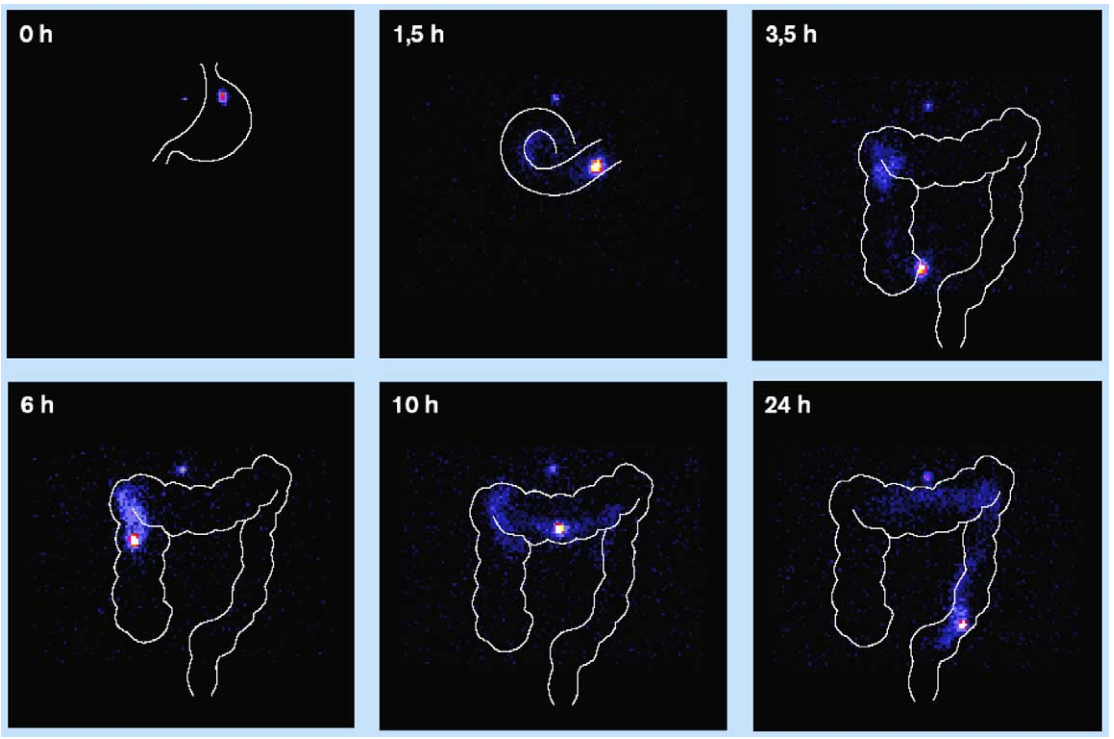

Fig. 2. Scintigrams relating to the Egalet® system in subject 04 at 0, 1.5, 3.5, 6, 10 and 24 h after administration. The dosage form is visible in the stomach (top left), at the end of the duodenum (top centre), at the ileo-caecal junction (top right), in the ascending colon (bottom left), in the transverse colon (bottom middle) and in the descending colon (bottom right), with varying amounts of released samarium oxide in front of and behind the dosage form.

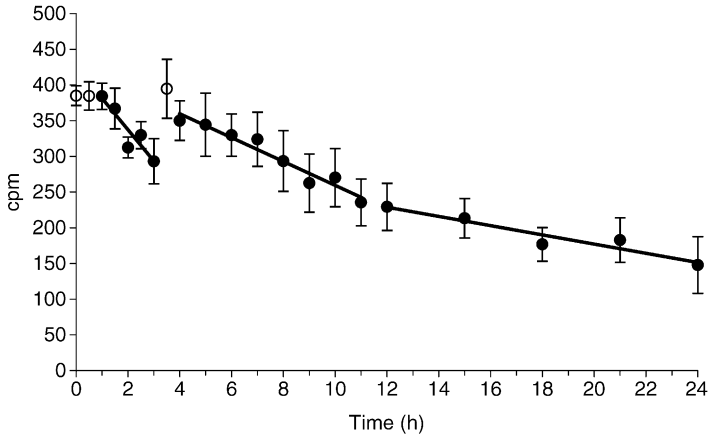

Fig. 3. Mean numbers of gamma counts per minute (cpm) in the six Egalet® subjects. Bars indicate standard errors of mean. Linear fit lines are shown for the three phases evident (small intestine, ascending and transverse colon, descending colon).

hours after administration the formulation had already reached the ileo-caecal junction, i.e. the transit time through the small intestine had been 3 h. As seen in Fig. 3 it was evident that release of radioactive Sm_2O_3 during the first hour in the stomach was minimal, but that after it had begun it continued throughout the 24 h imaging period. It is evident that the Egalet® formulation remained for some time at the ileo-caecal junction but that radioactive Sm_2O_3 released passed into the colon. Six hours after administration the Egalet® system had caught up with released Sm_2O_3 at the hepatic flexure. Subsequently, radioactivity was detected both in front of and behind the Egalet® system in the colon. Twenty-four hours after administration the Egalet® dosage form had arrived in the lower part of the descending colon, but radioactivity was still evident in the transverse colon. The overall transit time of the intact Egalet® dosage form was less than that of the fine-particulate Sm_2O_3. This finding is in accordance with that in the study by Hardy et al. (1985), in which the transit time for a single-unit tablet was shorter than that for a concomitantly administered multiple-unit pellet formulation.

Fig. 3 shows mean detected gamma counts per minute in the small intestine and colon for all six volunteers. No marked decrease in counts was evident during the first hour after administration. One hour was the mean gastric residence time for the six subjects. When Egalet® dosage forms were in the small intestine, from one to three hours after administration, numbers of counts in the ROI declined linearly. The mean transit time through the small intestine of intact Egalet® dosage forms was about 2 h. This is less than the 3 ± 1 h for single-dosage form preparations recorded in the literature (Davis et al., 1986; Coupe et al., 1991). However, it has been reported that shorter transit times are not unusual, particularly in individuals who regularly engage in high-intensity sport (Wilson, 2003). Four of the six volunteers fell into this category.

Four hours after administration, the Egalet® systems had reached the colon. Subsequently, again, a linear decline in counts was observed until 12 h after administration. During this time the units were in the ascending or transverse colon. From the mean time-point of 15–24 h numbers of counts also decreased, approximately linearly, but at lower rates. Fig. 1 shows that in vitro release of the model drug (caffeine) took place in accordance with linear kinetics throughout. In vivo release of the marker ($^{153}Sm_2O_3$) was also linear, but clearly triphasic. The rate was greatest as long as the product remained in the small intestine. It declined markedly after passage into the colon, and was lowest in the descending colon. Linear fitting to mean values was carried out in three phases, using the method of least squares. Factors for the fit-lines corresponding to the three phases were found to be 43.9 cpm h^{-1} (small intestine, $R^2 = 0.85$), 16.5 cpm h^{-1} (ascending and transverse colon, $R^2 = 0.96$) and 6.4 cpm h^{-1} (descending colon, $R^2 = 0.92$).

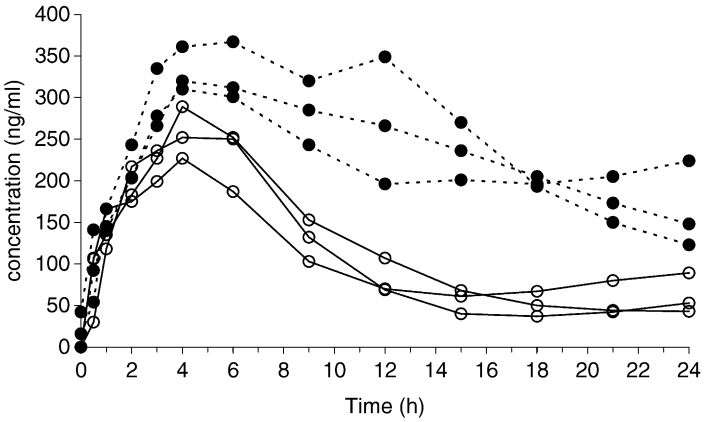

Fig. 4. Individual time–concentration curves for caffeine plasma levels in the six Egalet® subjects. The curves are divided into two groups, reflecting differences in the absorption of caffeine with time.

Fig. 4 shows individual time–concentration curves for caffeine for all the six study subjects. Two groups, each containing three subjects, can be distinguished. In both groups absorption of caffeine started very quickly. All subjects had measurable concentrations in the blood as early as 15 min after drug administration. In this respect the results differ slightly from those obtained by means of gamma images, where it was approximately 1 h before it became evident that $^{153}Sm_2O_3$ was being released from the Egalet® dosage forms. This is understandable because caffeine is freely soluble in water but Sm_2O_3 is insoluble. In both groups maximum drug concentrations (C_{max}) were achieved about 4 h after drug administration. Subsequently, however, the curves for the two groups started to separate. In one group of three study subjects drug levels declined fairly rapidly. In the other group of three subjects caffeine concentrations remained at markedly higher levels for up to 24 h.

The obvious explanation of why there were two pharmacokinetic groups is illustrated in Fig. 5. A typical study subject from each of the two groups was selected. In subject 04 caffeine blood levels declined fairly quickly. In subject 05 caffeine concentrations remained high for longer. From gamma images, the total residence time in the ascending colon and

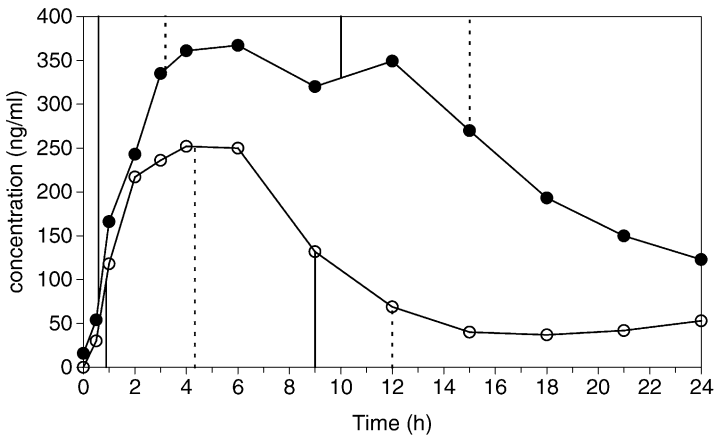

Fig. 5. Caffeine plasma levels in two Egalet® subjects (● = 04 and ○ = 05), one from each group. Vertical lines mark transitions from stomach to small intestine, to ascending colon, to transverse colon and to descending colon for each subject.

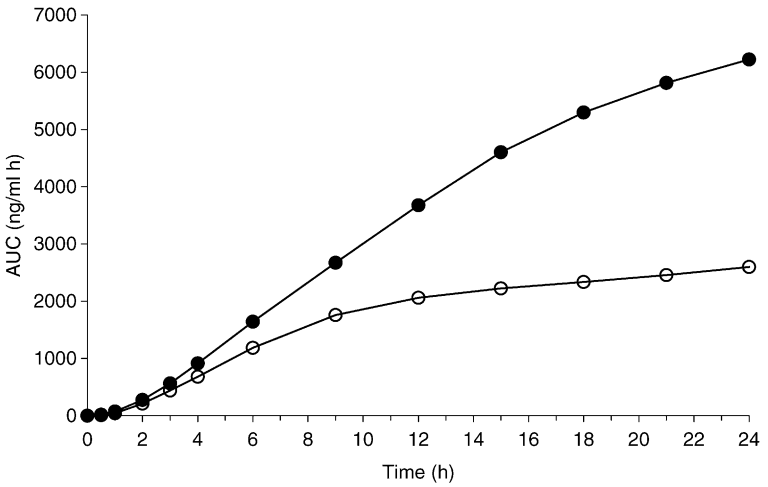

Fig. 6. Cumulative area-under-curve values for two Egalet® subjects (● = 04 and ○ = 05) as functions of time.

transverse colon was about 7.5 h in subject 04 but about 12 h in subject 05. Slow transit through the proximal and central parts of the colon would seem to result in more prolonged drug absorption. The descending colon, perhaps because its content is more viscous than the contents of the ascending colon and transverse colon, would seem to be a less favourable site for drug release and subsequent absorption. Fig. 6 shows cumulative area-under-curve (AUC) values for the two subjects as functions of time. The curve describing subject 05 is close to linear for up to 18 h after administration, i.e. not only in vitro drug dissolution but also in vivo drug absorption in this volunteer followed almost zero-order kinetics, as intended. In subject 04 a linear increase in AUC values lasted only for about 9 h. The more rapid transit of the product to the descending colon in subject 04 than in subject 05 may explain the difference between the two volunteers. More simply, the in vitro–in vivo correlation was very good in subject 05 but not in subject 04. Under in vivo conditions the rate of transit of the delivery system through the colon is more important than the rate of drug release. As far as the bioavailability of caffeine is concerned, the most important factor seemed to be the residence time in the ascending and transverse colon. Long residence times in these sections led to high AUC values for caffeine.

4. Conclusions

All formulations administered left the stomach under fasting conditions within 1 h, and the mean time of transit through the small intestine was about 2 h, i.e. shorter than the times commonly cited in the literature. This may have been because of the nature of the study subjects. Most regularly engaged in high-intensity sport. Transit times from the ileo-caecal junction to the splenic flexure ranged from 8 to 12 h. The variation in this parameter was greater than variations in previously mentioned parameters.

The bioavailability of caffeine in each study subject depended primarily on the time of transit of the dosage form concerned through the colon: long transit times in the ascending colon and transverse colon were reflected in high bioavailabilities and vice versa. There was no time lag in relation to commencement of absorption of caffeine, which is soluble in water, but release in vivo of $^{153}Sm_2O_3$, which is insoluble in water, was not clearly evident until 1 h after drug administration. Release of this water-insoluble marker means that the mechanism governing the behaviour of the Egalet® constant-release system must involve erosion rather than diffusion. Dissolution of caffeine in vitro invariably followed zero-order kinetics very closely. Release of $^{153}Sm_2O_3$ in vivo was triphasic: the rate of release diminished when the Egalet® dosage form moved from the small intestine to the colon, and

again when it moved from the transverse colon to the descending colon.

It is evident that variations in biological factors have much greater effects on the pharmacokinetic profile of a constant-release drug delivery system than any minor change in the characteristics of the dosage form. Where modified-release formulations are concerned, it may be unrealistic to presume that correlations exist between in vitro and in vivo characteristics. The greatly simplified conditions of in vitro tests can never simulate the continual changes that take place in the human GI tract. The natural abundance Sm_2O_3 could be appropriately utilised in the present study. This is an advantage because isotope enriched $^{152}Sm_2O_3$ is much more expensive.

Acknowledgements

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References

Bar-Shalom, D., Slot, L., Lee, W.W., Wilson, C.G., 2003. Development of the Egalet technology. In: Rathbone, M.J., Hadgraft, J., Roberts, M.S. (Eds.), Modified-Release Drug Delivery Technology. Marcel Dekker, New York, pp. 263–271.

Coupe, A.J., Stanley, S.D., Wilding, I.R., 1991. Variation in gastrointestinal transit of pharmaceutical dosage forms in healthy subjects. Pharm. Res. 8, 360–364.

Davis, S.S., Hardy, J.G., Fara, J.W., 1986. Transit of pharmaceutical dosage forms through the small intestine. Gut 27, 886–892.

Hardy, J.G., Wilson, C.G., Wood, E., 1985. Drug delivery to the proximal colon. J. Pharm. Pharmacol. 37, 874–877.

Newman, S.P., Hirst, P.H., Wilding, I.R., 2003. New developments in radionuclide imaging for assessing drug delivery in man. Eur. J. Pharm. Sci. 18, 19–22.

Parr, A., Jay, M., Digenis, G.A., Beihn, R.M., 1985. Radiolabeling of intact tablets by neutron activation for in vivo scintigraphic studies. J. Pharm. Sci. 74, 590–591.

Säkkinen, M., Marvola, J., Korpela, H., Lindevall, K., Lipponen, M., Kekki, T., Ahonen, A., Marvola, M., 2004. Gamma scintigraphic evaluation of the fate of microcrystalline chitosan granules in human stomach. Eur. J. Pharm. Biopharm. 57, 133–143.

Shah, V.P., Midha, K.K., Findlay, J.W., Hill, H.M., Hulse, J.D., McGilveray, I.J., McKay, G., Miller, K.J., Patnaik, R.N., Powell, M.L., Tonelli, A., Viswanathan, C.T., Yacobi, A., 2000. Bioanalytical method validation—a revisit with a decade of progress. Pharm. Res. 17, 1551–1557.

Waaler, T., Sande, S.A., Müller, B.W., Schüller-Lisether, G., 1997. The influence of thermal neutron irradiation on the in vivo characteristics of ASA oral dosage forms: validation of neutron activation. Eur. J. Pharm. Biopharm. 43, 159–164.

Watts, P.J., Wilson, C.G., Davies, M.C., Melia, C.D., 1993. Radiolabelling of polymer microspheres for scintigraphic investigations by neutron activation. 2. Effects of irradiation on the properties of Eudragit RS-sulphasalazine microspheres. Int. J. Pharm. 98, 63–73.

Wilson, C.G., 2003. Colonic drug delivery. In: Rathbone, M.J., Hadgraft, J., Roberts, M.S. (Eds.), Modified-Release Drug Delivery Technology. Marcel Dekker, New York, pp. 217–222.

Wilson, C.G., Washington, N., 2000. Gamma scintigraphy in the analysis of the behavior of controlled release systems. In: Wise, D.L. (Ed.), Handbook of Pharmaceutical Controlled Release Technology. Marcel Dekker, New York, pp. 551–565.