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Research paper

Gamma scintigraphic evaluation of the fate of microcrystalline chitosan granules in human stomach

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Abstract

In several reports of in vitro studies it has been suggested that the mucoadhesive chitosans could be of value in preparing gastro-retentive formulations. The aim of this study was to obtain direct in vivo evidence of whether microcrystalline chitosan (MCCh) formulations acted as gastro-retentive systems in humans. Neutron-activation-based gamma scintigraphy was used to study gastric residence times of MCCh granules in healthy male volunteers. Possible effects of neutron irradiation on the properties of the MCCh granules were studied in advance, in vitro. In vivo gamma scintigraphic evaluations were carried out with the subjects in a fasted state, using granules containing 95% (F1) or 40% (F2) of MCCh of molecular weight 150 kDa. Reference formulation (F3) was lactose granules. The reference granules passed rapidly from the stomach (mean $t_{50\%}$ 0.5 ± 0.3 h ($n = 5$)). MCCh in granules prolonged gastric residence times of the formulations in only a few cases (in one volunteer in the F1 group ($n = 4$) and in two volunteers in the F2 group ($n = 5$)). Maximum individual $t_{50\%}$ values were 2.1 h (F1) and 2.3 h (F2). It was concluded that the in vivo mucoadhesion of MCCh formulations is erratic, and that the formulations studied are not reliable gastro-retentive drug delivery systems.

Keywords: Microcrystalline chitosan; Mucoadhesion; Gastro-retentive; Gamma scintigraphy; Neutron activation; Samarium

1. Introduction

There have been many attempts recently to develop chitosan formulations for gastro-retentive drug delivery [1–3]. Results of a number of in vitro studies suggest that chitosan could be of value as an excipient in such formulations. Chitosan is a cationic polymer that forms gels in acidic environments, like that in the stomach. The gel formed is mucoadhesive. Chitosan formulations have been shown to adhere to various mucosal preparations, e.g. of porcine and rat intestine [4,5], porcine stomach [6] and porcine oesophagus [6,7]. Although in vitro results have been encouraging, there is very little information on the mucoadhesion of chitosan formulations in the gastrointestinal tract.

During the last few years, our group has studied microcrystalline chitosan (MCCh) in slow-release formulations. Results of in vitro studies by us have shown that MCCh offers advantages over conventional chitosan because gel formation with MCCh is more efficient, and its retardant effects on drug release are more marked [8]. Development of slow-release granules for gastro-retentive drug delivery has been our specific aim. The idea was based on the ability of MCCh base to protonate at acidic pH values, such as those in the stomach. The resulting positively charged chitosan might then interact electrostatically with the negatively charged mucus gel layer and adhere to mucosa [4]. We have carried out bioavailability studies in healthy volunteers to determine whether use of MCCh granules could lead to gastro-retentive formulations [7,9]. To obtain indirect evidence relating

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to possible adherence of the granules to gastric mucosa we used furosemide as a model drug in formulations. Furosemide is site-specifically absorbed, in upper parts of the gastrointestinal tract only [10]. Accordingly, if $AUC_{0-\infty}$ values for furosemide from a slow-release chitosan formulation were similar to or higher than those from an immediate-release formulation, the chitosan formulation concerned would be gastro-retentive. If, on the other hand, there were no adhesion of chitosan to the gastric mucosa, amounts of furosemide absorbed would be markedly reduced, because furosemide would be released beyond the 'absorption window'. In our studies, $AUC_{0-\infty}$ values (mean ± SD) for furosemide (40 mg) from an immediate-release tablet and from granules containing 40% of MCCh (M_w 150 kDa) were 3800 ± 941 μg l^{-1} h and 3450 ± 1180 μg l^{-1} h, respectively [9]. The slight difference in mean AUC values was not statistically significant. The high amount of furosemide absorbed from the MCCh granules suggests that the furosemide could have been released in the stomach. However, variation in $AUC_{0-\infty}$ values between individuals was fairly high (coefficient of variation 34%), suggesting that gastro-retention might not have occurred to similar extents in every volunteer. When release of furosemide from granules was prolonged by increasing the amount or molecular weight of MCCh, mean $AUC_{0-\infty}$ values decreased by 29% for granules containing 95% of MCCh of M_w 150 kDa (to 2700 ± 1070 μg l^{-1} h) and by 50% for granules containing 95% of MCCh of M_w 240 kDa (to 1890 ± 544 μg l^{-1} h) [7]. These findings do not suggest substantial mucoadhesion in the stomach.

In the study reported here, the aim was to obtain direct evidence of what happens to MCCh formulations in the stomach. Gamma scintigraphy was used to study the fates of MCCh granules in the human gastrointestinal tract. A neutron-activation method was used to label the formulations. A stable isotope (^{152}Sm) was incorporated in granules during manufacture, and activated in a thermal neutron flux to a gamma-emitting nuclide (^{153}Sm) prior to administration. The study took place in two stages. Possible effects of the neutron activation process on MCCh were first investigated in vitro, by means of gel-formation and dissolution tests on MCCh granules before and after irradiation. Such investigations were required because irradiation can alter the characteristics of formulations containing polymeric excipients [11,12]. MCCh grades of M_w 150 and 240 kDa were examined. Only MCCh grades the properties of which did not change markedly during irradiation could be used in the in vivo tests. In the second stage of the study, gamma scintigraphic investigations were carried out in vivo. Because we had found in previous studies [7,9] that the amount of MCCh in granules affected the bioavailability of the model drug, the same percentages (40 or 95%) of MCCh were used in the gamma-scintigraphic study reported here.

2. Materials and methods

2.1. Materials

Microcrystalline chitosans (MCCh) (Novasso, Finland) of mean molecular weight (M_w) 150 and 240 kDa were studied. The approximate extent of deacetylation was 75% for both grades. The MCCh had been manufactured from conventional chitosan in accordance with specifications [13], using a continuous method [14]. The MCCh available is a base. It dissolves only at acidic pH levels, unlike chitosan salts, which are readily soluble even at higher pH values. Other excipients were lactose (Pharmatose DCL 21, DMV International, Netherlands) and polyvinylpyrrolidone (PVP K25, Fluka Chemie, Switzerland). Natural-abundance samarium oxide (Sm_2O_3) (purity 99.9%) (Aldrich, USA) was used for radiolabelling. It contains 27% of the ^{152}Sm isotope, which can be converted by nuclear activation to the gamma-radiation-emitting ^{153}Sm isotope, by neutron capture. The half-life of ^{153}Sm is 46.3 h. Furosemide (Ph.Eur.) was used as a model drug.

2.2. Formulations for in vitro studies

The compositions of the granules used in the in vitro studies are shown in Table 1. Furosemide was used as model drug in the MCCh granules to allow possible effects of neutron irradiation on the chitosan polymer to be evaluated via dissolution testing. Ingredients (for batches of 50 g) were mixed carefully in a mortar. Resulting powder masses were moistened with granulating fluid (a 2.5% aqueous solution of acetic acid (q.s.)), to obtain MCCh matrix granules. Masses were granulated through a 2.0-mm sieve, and the granules obtained dried on trays for 48 h at room temperature. The size fraction 1.18–1.68 mm was separated by sieving and used in the study. The granules were dispensed into capsules for the irradiation process. Size-0 gelatine capsules (Ph.Eur.) were filled by volume. The mass of the content of each capsule was 260 mg. Four batches of capsules were prepared. The first batch contained granules G1 in capsules, and the second granules G3. Batches 3 and 4 were manufactured in such a way that each capsule contained 4 mg of Sm_2O_3. Capsules of batch 3 contained a mixture of G1 and G2 granules, in the ratio 180 to 80 mg,

Table 1
Compositions of granules (G) used in the in vitro studies

	M_w 150 kDa		M_w 240 kDa	
Formulation	G1	G2	G3	G4
MCCh	95%	95%	95%	95%
Furosemide	5%	–	5%	–
Sm_2O_3	–	5%	–	5%

MCCh, microcrystalline chitosan; M_w, molecular weight.

respectively. Capsules of batch 4 were prepared similarly but with G3 and G4 granules.

2.3. Formulations for in vivo gamma scintigraphic studies

The compositions of the granules used in the in vivo studies in human subjects are shown in Table 2. Two MCCh formulations (F1 and F2) and a reference lactose formulation (F3) were studied. Powder masses (in batches of 50 g) containing MCCh were granulated using a 2.5% aqueous solution of acetic acid, as described in Section 2.2. The granulating fluid for the lactose formulation (F3) was purified water. Granules were dispensed into size-0 gelatine capsules (Ph.Eur.) by volume. The masses of the contents of individual capsules were 260 mg (F1), 290 mg (F2) and 370 mg (F3). Each capsule contained 4 mg of Sm_2O_3.

2.4. Neutron activation

Capsules containing granules were irradiated using a 250-kW TRIGA Mark II nuclear research reactor (General Atomics, USA) at the VTT Technical Research Centre of Finland. The neutron flux was 1.2×10^{12} cm^{-2} s^{-1}. Seven to eight capsules were irradiated at a time. For the granules used in the in vitro studies (Table 1) irradiation times were 4 and 8 min. Formulations containing Sm_2O_3 were subjected separately to the irradiation process. For the granules used in the in vivo studies in human subjects (Table 2) the irradiation time was 5.5 min. This allowed the target radioactivity of the formulations (not more than 1.4 MBq) to be reached 48 h after irradiation.

2.5. In vitro studies

2.5.1. Gel formation studies

Effects of irradiation on gel formation by chitosan in vitro were studied using granules containing MCCh of different M_w (Table 1). These gel-formation investigations were quality control studies. If gels were formed differently by MCCh after neutron activation, this could indicate that the irradiation had altered polymer structure. Tests on irradiated granules were carried out after the radioactivity of the samples had declined to background levels. Minimum decay time was 10 days. Gel formation in three samples was studied in parallel at pH 1.2 by means of light microscopy (Leica DMLB 020-519.511, Leica Mikroskopie und Systeme, Germany). The granules were attached to a Petri dish with double-sided adhesive tape, and 15 ml of hydrochloric acid buffer of pH 1.2 (USP 24) were added. Granule areas were measured at intervals using imaging software (Leica Qwin Imaging Systems, Germany) and converted to granule equivalent diameters (d_{ekv}). Hydration and gel-formation of MCCh was expressed in terms of increase in granule relative diameter (REL d_{ekv}) as a function of time. Statistical analyses were carried out using Student's *t*-test relating to independent groups, for REL d_{ekv} values after 30 and 60 min of hydration.

Table 2
Granule formulations used in the in vivo gamma scintigraphic studies

	Formulation		
	F1	F2	F3
MCCh M_w 150 kDa	95%	40%	–
Lactose	3.4%	58.6%	96.4%
Sm_2O_3	1.6%	1.4%	1.1%
PVP	–	–	2.5%

The granules were dispensed into size-0 gelatine capsules, each containing 4 mg of samarium oxide.

2.5.2. Drug release studies

Effects of irradiation on drug release from chitosan granules in vitro were studied using granules containing MCCh of different M_w (Table 1). These drug release studies were also quality control studies. If drug release from a formulation took place differently after neutron activation, this could also indicate that the irradiation had altered polymer structure. Drug release was studied by means of dissolution tests, using the basket method (USP 24) (Distek Premiere 5100 Apparatus, Distek, USA). The speed of rotation was 100 min^{-1}. Tests on irradiated granules were carried out after the radioactivity of the samples had declined to background levels. Minimum decay time was 10 days. Granules were placed in baskets in such a way that the theoretical amount of drug in each basket was 10 mg. Granules in baskets were placed in hydrochloric acid buffer pH 1.2 (USP 24) (7.5 ml) for 1 h, after which samples were transferred to dissolution vessels containing phosphate buffer, pH 5.8 (USP 24) (1000 ml, 37 ± 0.5 °C). Amounts of drug released by four samples in parallel were determined spectrophotometrically. The dissolution apparatus was connected to a flow-through spectrophotometer (Ultrospec 4000, Pharmacia Biotech, UK) via a peristaltic pump (Icalis PCP490, Icalis Data Systems, UK). Absorbances at 274 nm were recorded automatically, using dissolution software (Icalis Data Systems, UK), and converted to percentages of drug released as a function of time. Statistical analyses were carried out using Student's *t*-test relating to independent groups, for $t_{50\%}$ values for drug release.

2.5.3. Gamma spectra and radioactivity

Gamma spectra and radioactivity were measured to determine the safety of the formulations used in the in vivo studies in human subjects (Table 2). Safety requirements were set in accordance with the guidelines established by STUK (Radiation and Nuclear Safety Authority of Finland). The ALARA- (as-low-as-reasonably-achievable-) principle was observed, and exposure to radiation was minimized in every situation.

The gamma spectrum was measured 24 h after irradiation using an HPGe (high-purity germanium)

semiconductor detector (model 7229P, Canberra, Belgium) at the VTT Technical Research Centre of Finland. One capsule from each batch irradiated was studied. The safety requirements were that any net peak area not originating from ^{153}Sm should not be greater than 0.3% of the ^{153}Sm main peak area at 103 keV, and that the total for the net peak areas not originating from ^{153}Sm should not be greater than 1% of the ^{153}Sm main peak area at 103 keV.

Activity of ^{153}Sm was measured 48 h after irradiation, immediately before administration of formulations to volunteers. A CRC-35R Radioisotope Calibrator (Capintec, USA) was used for measurement. Six capsules from each batch irradiated were studied. The safety requirement was that ^{153}Sm activity should not exceed 1.4 MBq. Radioactivity of 1.4 MBq would have resulted in an effective absorbed dose for each study subject of 1 mSv (STUK).

2.6. In vivo gamma scintigraphy

2.6.1. Subjects

Three groups of five healthy male volunteers participated in gamma scintigraphic studies. The ages of the volunteers ranged from 20 to 39 years. Their weights varied from 62 to 97 kg and their body mass indices (BMI) from 19 to 27 kg m^{-2}. Subjects were non-smokers. Before the studies, each volunteer was examined physically, and subjected to routine haematological testing (Hb, HCR, B-Eryt, B-Leuc, ESR, S-Alat, S-Asat, S-AFOS, S-GT) and urine analysis (U-pH, U-Prot, U-Gluc). Each volunteer was informed about possible risks and adverse effects of taking the study formulations. Written informed consent to participation in the studies had been obtained. The investigations were 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 in the HUS Nuclear Medicine Division, which has a radiation safety licence issued by STUK. The study protocol was drawn up in accordance with the guidelines established by STUK, and the ALARA principle was observed. During the study a labelled formulation was administered only once to each study subject (single dose study), so that the effective absorbed dose for the subject could not exceed 1 mSv.

2.6.2. Procedure

Gamma scintigraphic studies were carried out 48 h after neutron activation. This time period 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 the locations with adhesive tape. The activities of the markers at the sternum and iliac crests were 0.03 and 0.96 MBq, respectively. A capsule containing ^{153}Sm-labelled granules (F1, F2 or F3 (Table 1)) was administered to each volunteer in a sitting position, with 180 ml of water, at 08:00 or 12:00 h. Each volunteer had fasted overnight for at least 12 h, and had been asked to abstain from alcohol, and from xanthine- and caffeine-containing foods and fluids for 48 h prior to administration of the study formulation. The volunteers were not allowed to eat or drink during the imaging period. The study protocol was designed to eliminate as many variables as possible that could affect mucoadhesion in the stomach and/or gastric emptying of the formulations, e.g. to eliminate effects of food and beverages, and other factors (such as medication, disease, age).

After administration of the formulation, anterior and posterior images, each of 1-min duration, were recorded continuously for the first 30 min, after which six images, each of 1-min duration, were recorded every 15 min for the next 3–4 h. The ^{57}Co marker at the tip of sternum remained in place throughout the scintigraphic study. The higher-activity markers at the iliac crests were kept in place only for the first minute of each imaging period and then removed, because scattered radiation from them could have negatively affected image quality. Gamma pulses were detected at 70 and 103 keV (window width ± 10%) by means of a dual-head gamma camera (ADAC Forte, ADAC Laboratories, USA) equipped with low-energy general-purpose (LEGP) collimators. During imaging each subject lay supine beneath the gamma camera. At other times they could move freely.

2.6.3. Data analysis

Scintigrams were used to determine formulation activities in regions of interest (ROI). ROIs (relating to the stomach) were drawn manually on gamma images for each time point (of a fixed size for paired anterior and posterior images), and counts relating to ROIs were calculated using Hermes software (version 3.7, Nuclear Diagnostics, Sweden). Geometric means of counts in paired anterior and posterior images were calculated. Gastric emptying of the formulations was expressed in terms of remaining relative counts (REL_{counts}) in each ROI as a function of time. Lag times before the onset of gastric emptying were obtained directly from individual gastric emptying curves. REL_{counts} between 0.90 and 0.10 were used to determine the gastric-emptying rate constant (k) by means of linear regression analysis. Times at which half of the granules had left the stomach ($t_{50\%}$) were calculated and used in evaluating gastric-residence times. Statistical analyses were carried out using non-parametric Kruskal–Wallis analysis of variance.

3. Results and discussion

3.1. Effects of neutron activation in vitro

The effects of the neutron activation process on the properties of chitosan grades depended on M_w (Figs. 1 and 2).

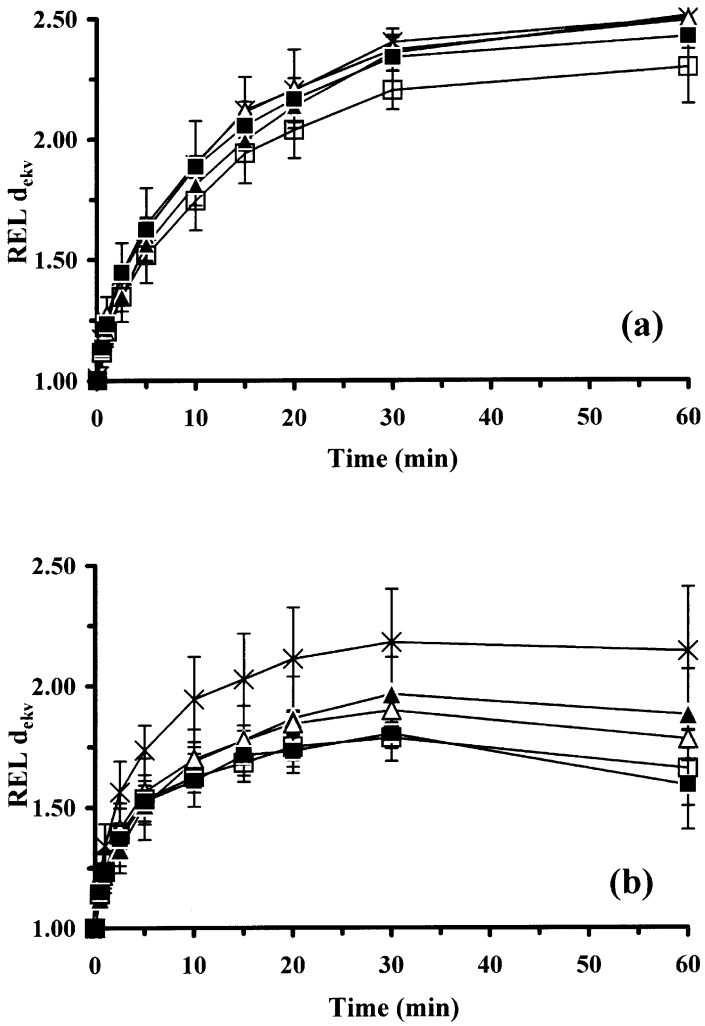

Fig. 1. Effects of neutron activation process on diameters of swollen granules (REL d_{ekv}) containing 95% of microcrystalline chitosan (MCCh) of molecular weight (a) 150 kDa and (b) 240 kDa at pH 1.2 (mean ± SD; $n = 3$). Irradiation time: ×, non-irradiated; ▲, 4 min; ■, 8 min; △, 4 min with Sm_2O_3; □, 8 min with Sm_2O_3.

Fig. 1 shows the effects of irradiation on gel formation by MCCh in granules ($n = 3$). With MCCh of M_w 150 kDa, hydration and gel formation was similar before and after irradiation. In contrast, granules containing MCCh of M_w 240 kDa increased less in diameter (REL d_{ekv}) after irradiation, indicating that neutron activation had reduced hydration and gel formation. REL d_{ekv} values after 8 min of irradiation were statistically significantly different ($P < 0.05$) from those for the reference (non-irradiated) granules. Fig. 2 shows the effects of neutron activation on furosemide release from MCCh granules ($n = 4$). Drug release was not markedly affected when MCCh of M_w 150 kDa was used. In contrast, irradiation increased drug release from granules containing MCCh of M_w 240 kDa. The effects of irradiation on gel formation by chitosan are evidently reflected in drug release. $t_{50\%}$ values for drug release after 4 or 8 min of irradiation ($t_{50\%}$ 23.5 ± 3.3 and 15.8 ± 3.5 min, respectively) were statistically significantly different ($P < 0.05$) from those for the reference granules ($t_{50\%}$ 30.0 ± 2.5 min) with MCCh of M_w 240 kDa.

Fig. 2b shows that the effect of neutron irradiation on MCCh of M_w 240 kDa depend on the irradiation dose. The effect of irradiation on drug release was most marked after 8 min of irradiation, i.e. after the highest neutron irradiation dose. $t_{50\%}$ values after 8 min of irradiation (15.8 ± 3.5 min) were statistically significantly different ($P < 0.05$) from those for the granules that had been irradiated for 4 min (23.5 ± 3.3 min). Drug release was fastest when the formulations contained Sm_2O_3. $t_{50\%}$ values decreased ($P < 0.01$) to 9.0 ± 0.4 and 7.4 ± 0.4 min when Sm_2O_3 had been simultaneously subjected to neutron activation for 4 or 8 min, respectively. In such cases the granules had also been exposed to gamma radiation from neutron capture.

The findings described above show that the effects of neutron activation on the properties of polymeric excipients such as chitosan need to be examined before gamma scintigraphic studies in vivo. The findings that gels are formed less readily from MCCh following irradiation and that drug release is enhanced suggest that irradiation may result in degradation of chitosan polymer. Our findings

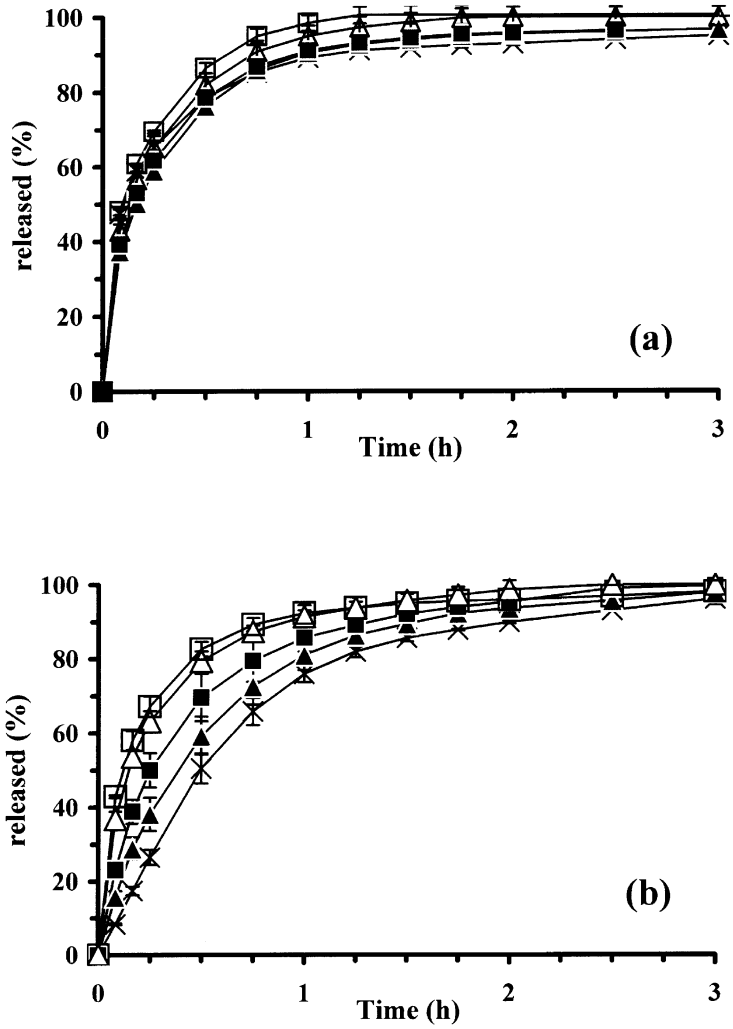

Fig. 2. Effects of neutron activation process on release of furosemide from granules containing 95% of microcrystalline chitosan (MCCh) of molecular weight (a) 150 kDa and (b) 240 kDa at pH 5.8 after 1 h of pre-treatment at pH 1.2 (mean ± SD; $n = 4$); Irradiation time: ×, non-irradiated; ▲, 4 min; ■, 8 min; △, 4 min with Sm_2O_3; □, 8 min with Sm_2O_3.

accord well with those in previous studies (e.g. of ethyl celluloses by Waaler et al. [15], of HPMCs and pectins by Ahrabi et al. [11] and of MCCh by us), indicating that irradiation could cause chain scission of polymers with glycoside linkages. Our results with MCCh-based formulations show that high-M_w MCCh was particularly sensitive to irradiation, and that the effects of irradiation became more marked as the irradiation dose increased. However, irradiation did not affect the properties of MCCh of M_w 150 kDa. MCCh of this grade was therefore chosen in the formulations to be tested in vivo (Section 3.3).

3.2. In vitro safety tests

Gamma spectra and radioactivity were studied using the formulations intended for the in vivo human studies. Gamma spectra for all formulations (F1, F2 and F3) after 24 h of decay time related mainly to gamma radiation originating from ^{153}Sm. A small peak of radioactive sodium (^{24}Na) was found in some samples. Net peak areas originating from ^{24}Na were less than or equal to 0.11% (F1), 0.10% (F2) and 0.08% (F3) ($n = 1$) of the ^{153}Sm peak area. Because the half-life of the isotope is only 15 h, activity originating from ^{24}Na was insignificant after 48 h, when the formulations were administered to volunteers. All other activity detected originated from natural background radiation. Activity of ^{153}Sm after 48 h of decay time was below 1.4 MBq for F1, F2 and F3. Mean radioactivities were equal to or below 0.91 MBq (F1), 0.99 MBq (F2) and 1.08 MBq (F3) ($n = 6$). Safety requirements were met with every formulation.

3.3. In vivo gamma scintigraphy

Results relating to the fates of formulations F1, F2 and F3 in the human stomach are shown in Figs. 3–5 and Tables 3 and 4. The MCCh formulations F1 and F2 were evaluated to determine if they acted as gastro-retentive systems.

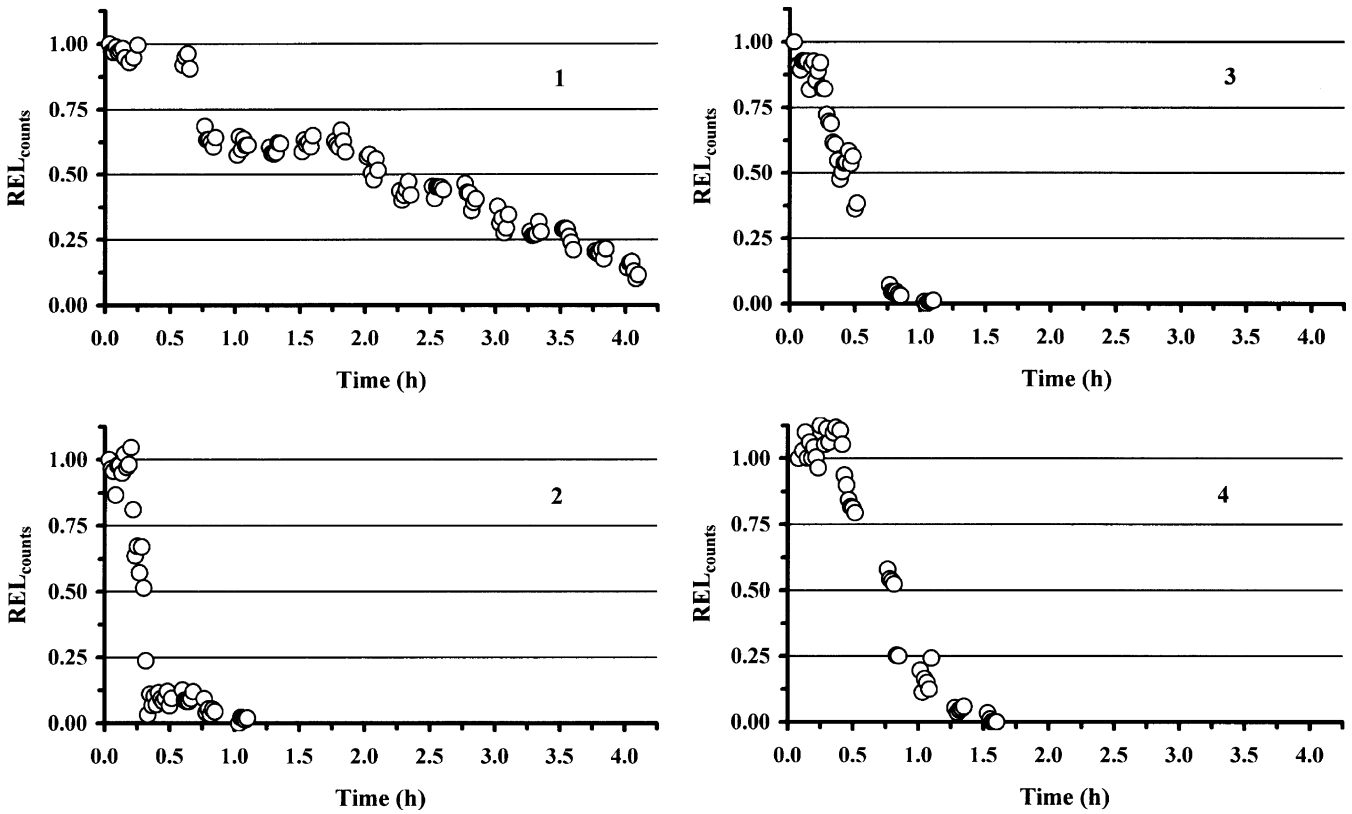

Fig. 3. Individual curves for gastric emptying of microcrystalline chitosan (MCCh) granules (F1; MCCh 150 kDa 95%, see Table 2 for details) dispensed in gelatine capsules.

The reference lactose formulation F3 was expected not to exhibit gastro-retentive characteristics. Individual curves for gastric emptying are shown for 14 volunteers. The number of volunteers in the study was initially 15 but results relating to one were excluded because the gelatine capsule and its contents (F1) adhered to the volunteer's oesophagus for nearly 2 h, and the results relating to gastric emptying of the formulation in this volunteer were clearly different from those with other subjects in the study. The results are published as a separate case report [16].

Table 3
Lag time (h) before the onset of gastric emptying of granules dispensed in gelatine capsules

Subject	F1 MCCh 150 kDa 95%	F2 MCCh 150 kDa 40%	F3 Lactose
1	0.77	0.78	0.19
2	0.22	0.26	0.40
3	0.25	0.20	0.42
4	0.43	0.16	0.12
5	–	0.24	0.77
Mean	0.42	0.33	0.38
± SD	0.25	0.26	0.25
Median	0.34	0.24	0.40

For details of formulations F1, F2 and F3 see Table 2.

Fig. 5 shows individual curves relating to gastric emptying of lactose granules (F3). From the steeply declining curves it is evident that gastric emptying of this formulation was fairly rapid for all five volunteers, as expected. Lag times before the onset of gastric emptying were, in general, fairly short (on average about 20 min) (Table 3). Rate parameters relating to gastric emptying are shown in Table 4. The rate constant (k) for gastric emptying varied from 4 h^{-1} to about 1 h^{-1}, which means that it took from 15 min to about 1 h for the formulation to be cleared from the stomach. Gastric emptying took place in most cases in small and frequent boluses. This is well illustrated in Fig. 5, in which there are several observation points at the steeply declining portions of the gastric emptying curves (e.g. those for subject no. 3).

Figs. 3 and 4 show individual curves relating to gastric emptying of MCCh formulations (F1 and F2) in nine volunteers. Lag times before the onset of gastric emptying varied about from 15 to 45 min (Table 3). Thereafter, gastric emptying of granules began to take place in small boluses. In contrast to the findings with the reference granules, gastric emptying of MCCh granules was prolonged on three occasions out of nine (Figs. 3 and 4), in subject 1, who received formulation F1, and in subjects 1 and 5, who received formulation F2. Rate constants (k) in these subjects (F1, subject 1, 0.16 h^{-1}; F2, subject 1, 0.45 h^{-1}; F2, subject

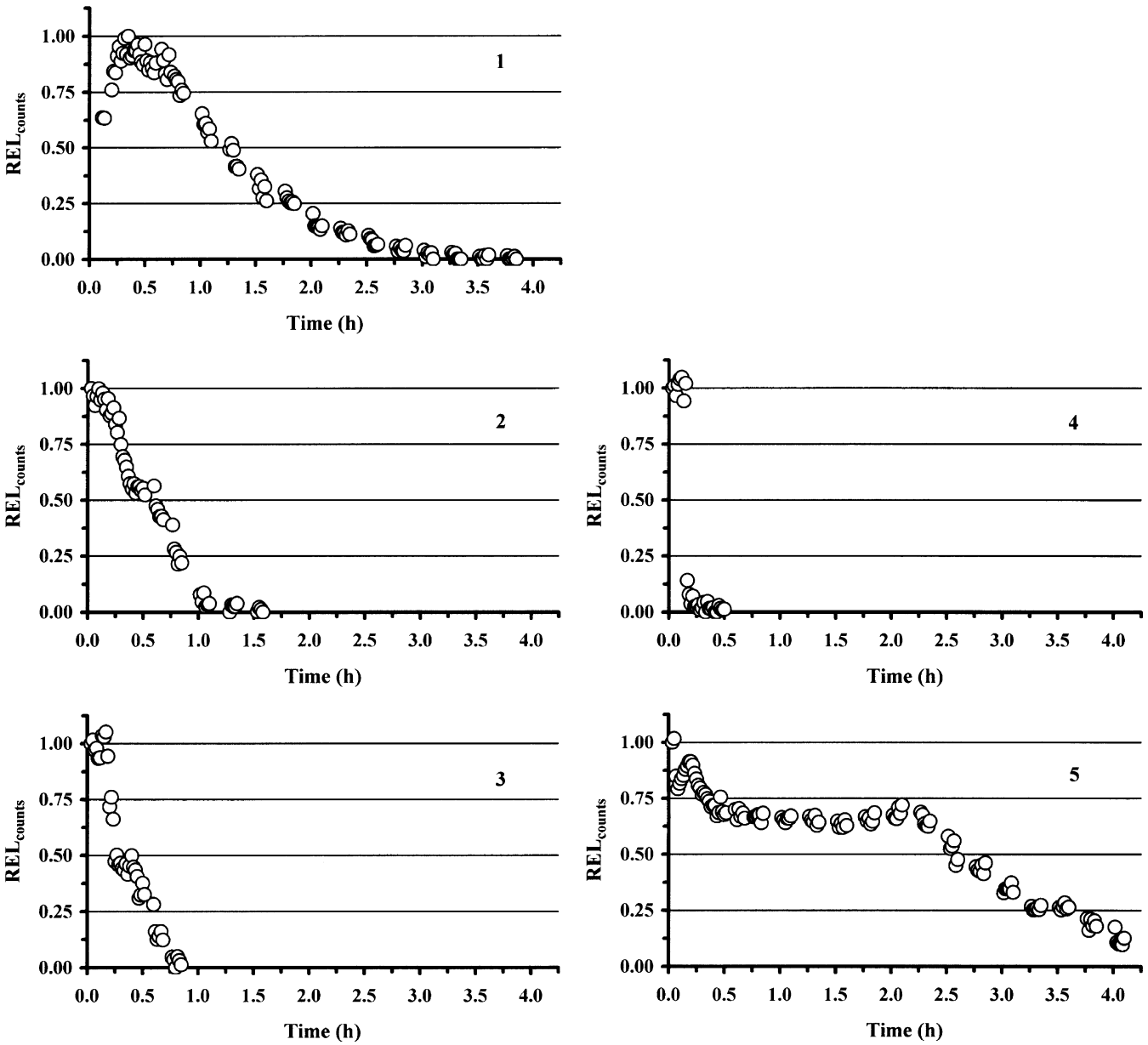

Fig. 4. Individual curves for gastric emptying of microcrystalline chitosan (MCCh) granules (F2; MCCh 150 kDa 40%, see Table 2 for details) dispensed in gelatine capsules.

5, 0.15 h^{-1}) were markedly lower than those for the reference formulation (F3, 0.91–4.04 h^{-1}) (Table 4). From the gamma images it could be concluded that the gastric residence time of MCCh granules was obviously prolonged by adhesion of granules to gastric mucosa, because the granules remained in the same region of the stomach. However, in the remaining six volunteers who had received MCCh granules (F1 or F2) gastric-emptying curves were fairly similar to those for the reference formulation (F3). In one subject the capsule containing MCCh granules (F2) cleared from the stomach very rapidly after administration, before the capsule could disintegrate (Fig. 4, subject 4).

Mean data relating to the formulations are shown in Tables 3 and 4. The parameter most commonly used in evaluations of the gastric residence time of dosage forms is the $t_{50\%}$ value, which includes the lag time before gastric emptying starts. In our study, mean lag times were similar (differences not significant) for formulations F1, F2 and F3 (Table 3). In this case the $t_{50\%}$ values therefore directly reflect changes in rates of gastric emptying of the formulations. Mean $t_{50\%}$ values for the MCCh formulations were 0.89 ± 0.81 h ($n = 4$) (F1) and 0.93 ± 0.87 h ($n = 5$) (F2), and 0.49 ± 0.30 h ($n = 5$) for the reference formulation (Table 4). Variation between results for the different individuals was extremely high, and there are no statistically

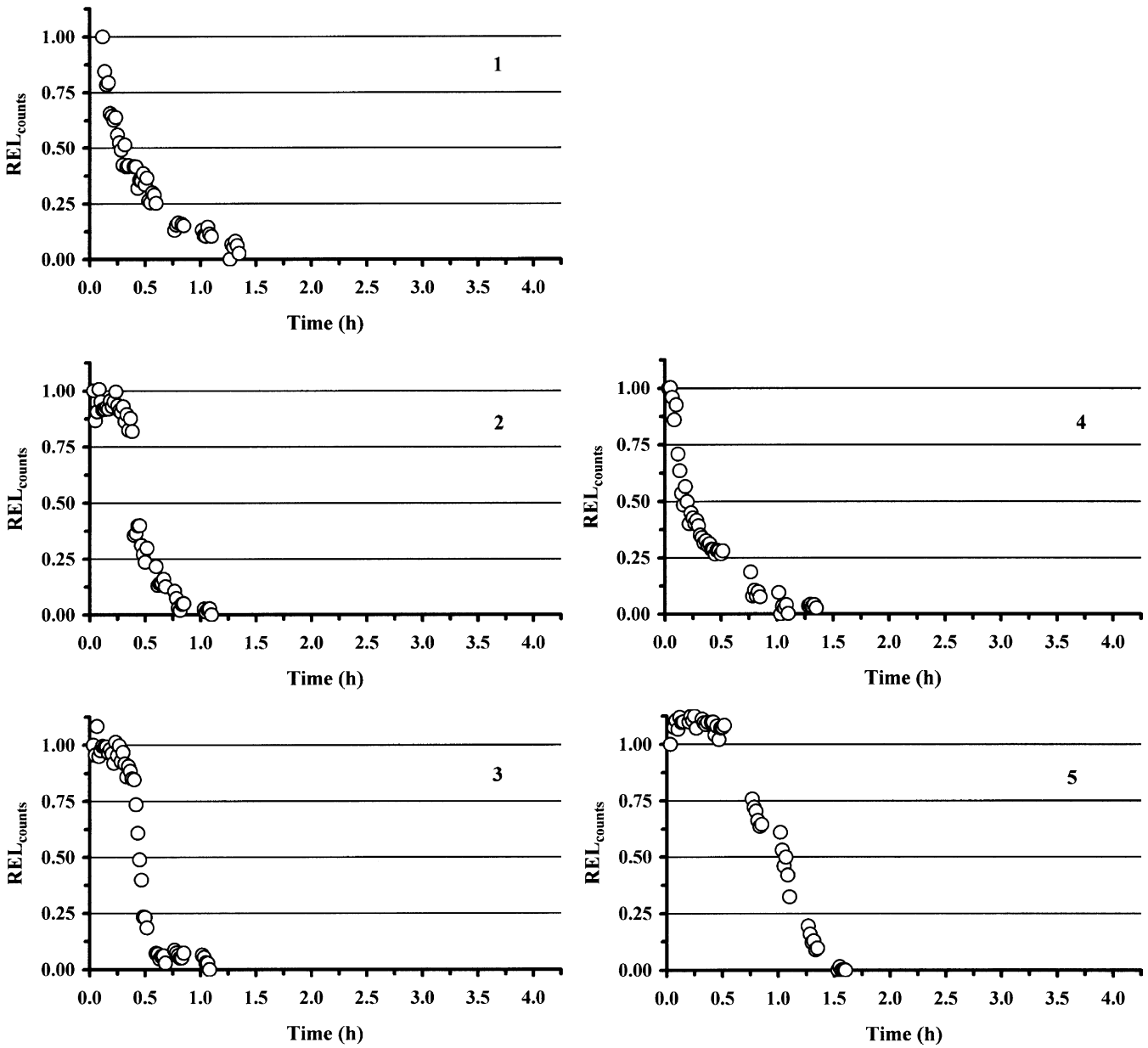

Fig. 5. Individual curves for gastric emptying of lactose granules (F3; see Table 2 for details) dispensed in gelatine capsules.

significant differences between the formulations with respect to gastric residence time or gastric emptying rate. It is obvious that in most cases adhesion of MCCh granules to the gastric mucosa either failed to take place or the MCCh had not adhered strongly enough in vivo. The two MCCh formulations, one containing 95% of MCCh and the other 40%, did not differ statistically significantly from each other: even a high percentage of MCCh in a formulation did not increase the frequency of adherence.

The results of our gamma scintigraphic investigations accord well with results of our previous bioavailability studies with furosemide formulations, suggesting that gastric residence times of MCCh granules may only occasionally be prolonged [7,9]. On the other hand, results of several in vitro studies on chitosan, including our own [7], have suggested that chitosans could be valuable as mucoadhesive excipients. These results show that adhesion of MCCh formulations in the human gastrointestinal tract is very erratic, with adherence to gastric mucosa occurring in only a third of instances. Gastro-retention was rare, even though the subjects lay supine during the first 30 min, when gastric emptying would be expected to be slower than if the subjects had been upright [17]. Granules were administered to subjects in the fasting state, when gastric pH would be expected to be lowest [18]. In such environments chitosan base would be expected to be highly ionized, and adhesion could have occurred between the positively charged chitosan and the negatively charged mucus gel-layer. However,

Table 4
Parameters relating to gastric emptying of granules dispensed in gelatine capsules

Subject	F1 MCCh 150 kDa 95%			F2 MCCh 150 kDa 40%			F3 Lactose		
	$t_{50\%}$	k	r	$t_{50\%}$	k	r	$t_{50\%}$	k	r
1	2.07	0.16	−0.951	1.34	0.45	−0.976	0.34	1.04	−0.941
2	0.29	4.48	−0.945	0.56	0.92	−0.962	0.43	2.65	−0.848
3	0.45	1.41	−0.947	0.34	1.14	−0.921	0.45	4.04	−0.921
4	0.77	1.09	−0.954	0.15	18.24	−0.964	0.23	0.91	−0.861
5	–	–	–	2.27	0.15	−0.915	1.00	1.12	−0.977
Mean	0.89	1.78		0.93	4.18		0.49	1.95	
± SD	0.81	1.87		0.87	7.87		0.30	1.36	
Median	0.61	1.25		0.56	0.92		0.43	1.12	

$t_{50\%}$, time (h) at which 50% of the granules have left the stomach; k (h^{-1}), gastric-emptying rate constant; r, correlation coefficient. For details of formulations F1, F2 and F3 see Table 2.

the physiological conditions in the stomach may be such that gastro-retention of the formulations is hard to achieve. First, exposure of chitosan to soluble mucins in the stomach lumen before it has a chance to interact with the mucus gel-layer could limit the adhesive properties of the formulations [6]. Secondly, the layer of mucus gel on which adhesion could take place is continually eroding, which might hinder gastro-retention of adhesive systems [19]. Many polymers known for their excellent in vitro mucoadhesive properties (e.g. polyacrylic-acid-based polymers) have failed to exhibit gastro-retentive properties in studies in human beings [20,21].

Some formulation-related factors could also have affected the gastro-retentive properties of the granules, namely granule size and administration of the granules in gelatine capsules. In a previous study Remuñan-López et al. [2] evaluated chitosan microspheres as gastro-retentive formulations in mice. They found that microspheres approximately 10 μm in diameter first adhered to the murine gastric mucosa, then were internalized by the mucosa for several hours, thus overcoming limitations imposed by mucous turnover. In other studies small ion-exchange resin particles (between 90 and 125 μm) were also gastro-retentive in the human stomach [22,23]. One means of improving the in vivo performance of MCCh granules might be to make them markedly smaller. The effect of administering granules in gelatine capsules also needs to be studied. It has been suggested that administering formulations containing mucoadhesive polymers in gelatine capsules could make the gastro-retentive properties of the formulations less marked [24]. Gelatine in a capsule shell could interact with a mucoadhesive polymer during dissolution, and thus diminish the adhesive properties of the formulation.

4. Conclusions

The aim in the study reported was to obtain direct evidence of the fates of MCCh formulations in the human stomach, and to determine whether such formulations could be valuable as gastro-retentive drug delivery systems. However, the results showed that adhesion of the MCCh formulations studied in the human gastrointestinal tract was very erratic, with adhesion to the gastric mucosa in only one third of cases. As far as the kinds of formulations studied are concerned, behaviour was not sufficiently reproducible.

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