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European Journal of Pharmaceutics and Biopharmaceutics 44 (1997) 39–52

European Journal of Pharmaceutics and Biopharmaceutics

An in vitro-in vivo investigation of oral bioadhesive controlled release furosemide formulations[1]

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Received 4 February 1997; accepted 14 May 1997

Abstract

The in vitro controlled release and bioadhesive properties of furosemide formulations were evaluated with standard dissolution tests and with a specially developed model using rabbit intestine. The results showed that the controlled release properties were not affected by the application of the bioadhesive polymer but that the bioadhesive properties were substantially different. In order to assess the gastrointestinal transit time in vivo, a γ-scintigraphy study was performed in six volunteers testing the same controlled release formulation with and without bioadhesive polymer. Plasma levels of furosemide, evaluation of urinary flux and measures of urinary excretion of furosemide in the six volunteers allowed correlations to be made between gastrointestinal transit and furosemide absorption. © 1997 Elsevier Science B.V.

Keywords: Bioadhesion; γ-Scintigraphy; Neutron activation; Controlled release formulation; Gastrointestinal transit; Furosemide; Samarium-153

1. Introduction

A problem frequently encountered with controlled release dosage forms is the inability to increase residence time of the dosage form in the stomach and proximal portion of the small intestine. In the fasted condition, gastric residence of a dosage form is typically short where it is unusual to observe residence times of a single unit dose greater than 1 h. It is also common for dosage forms to transit rapidly through the small intestine in the fed or fasted condition. Rapid GI transit phenomena like this may consequently diminish the extent of absorption of many drugs. Since many drug compounds are absorbed exclusively in the small intestine or in a limited segment of the intestine, it would therefore be beneficial to develop sustained release dosage forms which remain in the stomach for an extended period of time.

Several approaches have been tried to prolong gastric residence, one of which is the use of oral bioadhesive formulations [1]. Generally these formulations are made using mixtures of bioadhesive polymers and the drug or by coating tablets and other dosage forms with the bioadhesive polymers when dissolved in organic solvents [2–4]. These approaches of using adhesives to prolong gastrointestinal residence have ended with equivocal results. The working hypothesis of our research strategy assumed that previous adhesive formulations possibly failed due to the formation of

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[1] In part presented at the 2nd Rencontre–Pharmapeptides, held on October 3–4, 1996 at the Centre Interuniversitaire de Recherche et d'Enseignement, Archamps, France.

PII S0939-6411(97)00100-8

agglomerates when using the first formulation approach. When using the second formulation approach, it is conceivable that the dimensions of the coated dosage form are simply too large, thus resulting in a relatively small amount of the bioadhesive polymer being applied to the dosage form's outer surface relative to its total volume.

Additionally, in many examples found in the literature, the bioadhesive polymer is the same as the polymer controlling the release of the active ingredient [5]. We considered the possibility of having the two functions—bioadhesion and controlled release—separated in the formulation in order to be able to adjust them according to the biological and therapeutic needs of the active ingredient.

To achieve this, we prepared microgranules (125–400 μm) with controlled release properties containing the active ingredient. These microgranules were coated with the bioadhesive polymer by dry direct compression forming slugs which were subsequently crushed. The coated microgranules were then mixed with a disintegrant and a hydrophobic lubricant and dosed in standard hard gelatin capsules. The hydrophobic lubricant prevented the clogging of the bioadhesive microparticles before their hydration and spreading in the gastrointestinal tract [6].

Of several preparations which were developed, one formulation containing furosemide as model drug was tested both in vitro and in vivo. This anthranilic acid derivative is a potent diuretic with a reported oral bioavailability of 64%. The terminal elimination half-life in normal fasted males is approximately 30–120 min and the usual dosage for patients suffering from edema or hypertension is 40 mg given orally as an immediate release formulation twice daily. Site absorption studies in the gastrointestinal tract using a remote control delivery device have shown that furosemide is poorly absorbed when delivered in the colon or the terminal small intestine with a reported 3% colonic absorption relative to a 40 mg oral dose administered in a hard gelatin capsule [7].

The in vitro controlled release and bioadhesive properties of the formulations were evaluated with standard dissolution tests and with a specially developed model using rabbit intestine. A γ-scintigraphy study which radiolabeled the delivery system and correlated position in the gastrointestinal (GI) tract with drug absorption offered useful information for optimizing this delivery system. γ-Scintigraphy has been used extensively as a non-invasive technique for in vivo evaluation of oral dosage forms [8–10]. It has been used to assess the performance of enteric-coated dosage forms, localization of drug-release for targeted delivery systems, to characterize variation in gastrointestinal transit of pharmaceutical dosage forms and also to understand the mechanism of food effect as it relates to gastrointestinal transit [11–14].

The neutron activation radiolabeling technique used in this study involved the incorporation of a stable, non-radioactive isotope (samarium-152 oxide) in the test formulations [15–17]. The stable isotope was subsequently converted to specific levels of the radioactive isotope by neutron bombardment in a suitable nuclear reactor and was demonstrated not to alter the dosage form nor change the drug chemically.

The objectives of the present study were:

1. To prepare controlled release bioadhesive formulations of furosemide having the bioadhesive and the controlled release functions separated.
2. To assess their in vitro performance using a specially developed method for measuring bioadhesion.
3. To assess and compare gastrointestinal transit of two different sustained release oral formulations of furosemide, one of which has proposed bioadhesive character.
4. To draw a correlation between gastrointestinal transit and furosemide absorption, and to evaluate if differences in GI transit may be exploited to develop a controlled release delivery system of furosemide.

The results of this study will be interpreted with emphasis on optimizing an oral sustained release formulation of furosemide and other drugs which show similar absorption characteristics.

2. Experimental

2.1. Material

Furosemide was purchased from Secifarma (Milan, Italy), samarium oxide from Sigma (St. Louis, MO), hydrogenated castor oil, sold as Cutina® HR, from Henkel (Dusseldorf, Germany), saturated polyglycolized glycerides, sold as Gelucire, from Gattefossé (Saint-Priest, France), carbomer, sold as Carbopol® 934 PH, from BF Goodrich (Cleveland OH), Hydroxypropylmethylcellulose (HPMC), sold as Methocel® E4M, from Dow Chemical (Midland, OH), leucine from A. Jinomoto Co. (Kawasaky, Japan), crosspovidone, sold as PVP CL, from BASF (Parsippany, NJ), magnesium stearate from Mallinckrodt (Hennef, Germany). Capsules were purchased from Capsugel (Greenwood, S.C.). All buffers and reagents were of analytical grade and were used without any further purification.

2.2. Methods

2.2.1. Preparation of controlled release, bioadhesive furosemide capsules

The capsules were prepared in three steps: (1) preparation of controlled release microparticles; (2) preparation of bioadhesive controlled release microparticles; and (3) preparation of final capsule formulation.

Table 1
Composition of furosemide formulations tested

Material	Composition (% w/w)		
	Formulation 1	Formulation 2	Formulation 3
Furosemide	36.55	12.07	14.20
Samarium oxide	2.81	0.93	1.09
Hydrogenated castor oil	27.37	9.01	10.61
Saturated polyglycolized glycerides	18.27	6.04	7.10
Carbomer	—	28.05	33.00
HPMC	—	28.05	33.00
Leucine	—	0.85	1.00
Polyvinylpyrrolidone crosslinked	10.00	10.00	—
Magnesium stearate	5.00	5.00	—

(1) Controlled release microparticles were prepared mixing for 5 min furosemide, samarium oxide and a part of hydrogenated castor oil in a Tonazzi mixer-kneader. After mixing, the blend was kneaded in the same equipment with a melted mixture (80°C) containing polyglycolized glycerides and the remaining hydrogenated castor oil. The kneading was continued for 10 min. The product was then crushed and granulated through an Erweka oscillating granulator. Afterwards, the mixture was manually sieved collecting fractions having a size range of 125–400 μm.

(2) Bioadhesive controlled release microparticles were prepared by dry granulation of bioadhesive polymeric materials with the previously described controlled release granulate of furosemide. Carbomer and Methocel were mixed in a Turbula mixer with the controlled release particles and this blend was pressed in a rotary press using 1 cm^2 flat punches in order to obtain 1.12 cm slugs. The slugs were then crushed and granulated using an Erweka oscillating granulator and the product manually sieved in order to obtain 200–600 μm sized particles. This method allowed production of furosemide controlled release microparticles that were uniformly coated with an amount of bioadhesive polymer greater than that which can be obtained by standard film coating processes using solvents.

(3) The final capsule formulation was prepared by mixing the bioadhesive controlled release microgranulate in a Turbula mixer equipment with crosspovidone and magnesium stearate. A weighed amount of the final blend containing 40 mg of furosemide was manually filled into hard gelatin capsules (size No. 1).

The formulations tested had the compositions reported in Table 1.

2.2.2. In vitro release test

The in vitro dissolution test was performed according to USP XXIII paddle method at 37 ± 1°C and 50 rpm. As dissolution medium, USP XXIII phosphate buffer pH 5.8 was used. Samples were taken at 0, 1, 2, 4, 8, 12, 16, and 24 h and evaluated for furosemide content by UV spectrophotometry at 274 nm.

2.2.3. Bioadhesion test

An in vitro method using isolated intestinal mucosa from New Zealand white rabbits (1.8–2.3 kg) was developed [18]. Animals were euthanized via pentobarbital sodium injection, and after making a midline abdominal incision of the skin, the small intestine was exposed and a segment of desired length (3.5 cm) was excised. The lumen was rinsed with Ringer's Lactate solution.

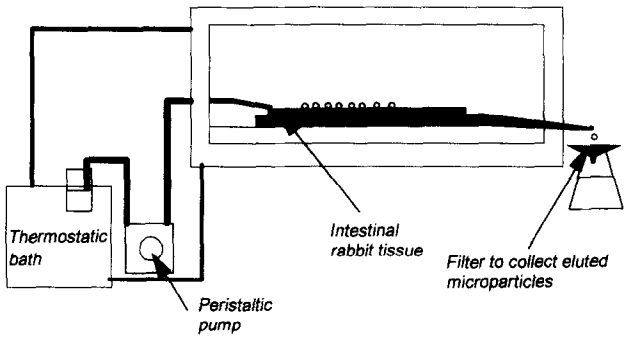


Fig. 1. In vitro model to evaluate bioadhesion.

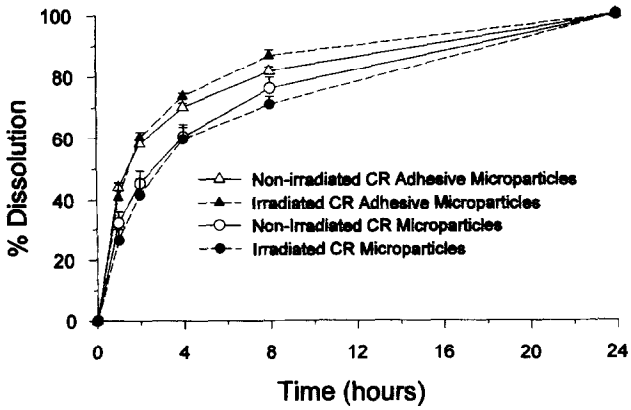


Fig. 2. In vitro dissolution profiles of CR and CR bioadhesive formulations before and after neutron activation.

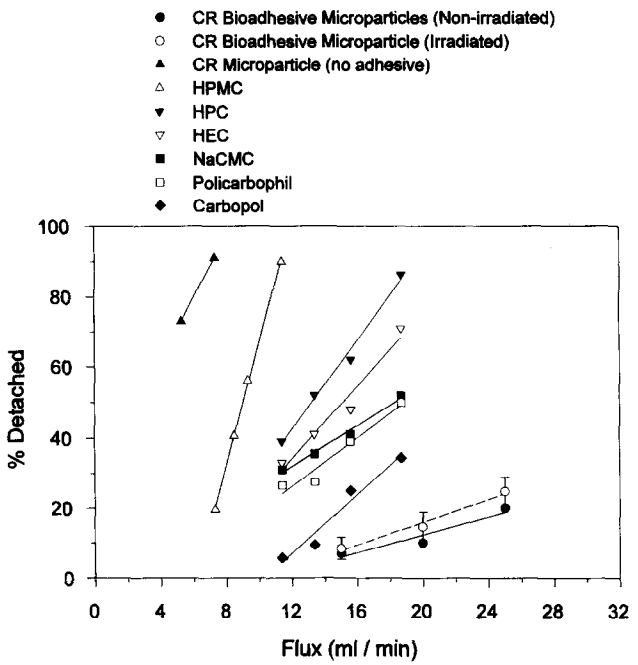


Fig. 3. Polymers and furosemide formulations tested with in vitro bioadhesion test. Furosemide CR bioadhesive was tested before and after neutron activation.

Before testing the in vitro adhesiveness of the formulation, the intestinal tissue was sliced laterally and attached to a polycarbonate support which was then positioned into a cylindrical cell heated at 37°C as shown by schematic representation in Fig. 1. The tissue was washed with a fixed volume of distilled water and an exact mass of bioadhesive microgranules, corresponding to 1–3 mg of furosemide, was placed on it. The microparticles were maintained on the excised intestine for 2 min before elution was started in order to allow the adhesive polymer to hydrate. Following this period of incubation, the microparticles were eluted with distilled water using a peristaltic pump over constant time period (10 min) while the flux of the water (ml/min) was varied in separate tests. Washed off particles were collected and the furosemide levels were determined by UV analyses at 274 nm in order to determine the exact percentage of detached particles.

2.2.4. In vivo study design and formulations tested

The study was an open-label, two-period, two treatment crossover study in six healthy male volunteers (age 23–34 years, and 70.0–84.1 kg). Each subject received the following treatments in a randomized order:

Treatment A: control sustained release granulate, 1 × 40 mg furosemide capsule following a minimum 10 h fast (Formulation 1).

Treatment B: bioadhesive sustained release granulate, 1 × 40 mg furosemide capsule following a minimum 10 h fast (Formulation 2).

A standard lunch meal was provided at 5 h post dose (cheeseburger, potato chips, decaffeinated soft drink) and a standard dinner meal was eaten at 11 h post dose. To maintain hydration from the diuretic effects of furosemide, subjects were required to drink orange juice (8 oz) at 2, 4, 6, and 10 h post dose.

2.2.5. γ-Scintigraphy and radiolabeling

Formulations 1 and 2 described in Table 1 were used for the in vivo study and in vitro assessment. The third formulation (Formulation 3) was used only to test the effects of neutron activation on the in vitro bioadhesion characteristics of the preparations. In each unit dose containing 40 mg furosemide, samarium oxide was incorporated at the time of manufacture in each drug formulation so that each dose contained 3.0 mg of non-radioactive Sm_2O_3. Doses were radiolabeled via neutron activation (thermal flux $= 8 \times 10^{13}$ neutrons cm^{-2}/s, 7 s) to produce radioactive samarium -153 ($t_{1/2} = 46.7$ h, $\gamma = 103$ keV).

Formulations 1 and 2 were repackaged as single unit doses in separate polyethylene envelopes which were then heat-sealed. Individual polyethylene envelopes were identified by a lot number specific to the formulation being irradiated, thus, the possibility of mixing samples was avoided. Formulation 3 was comprised of the controlled release granules containing the bioadhesive excipient but without the disintegrant and lubricant; this formulation was repackaged in polyethylene packets and neutron activated ($t = 7$ s) and was used for in vitro bioadhesion studies.

Treatment A was the control CR formulation 1 containing no bioadhesive excipient. The control formulation was manually weighed and filled into hard gelatin capsules (113 mg per capsule) and unit doses were sealed into individual polyethylene packets. Capsules, 30, were neutron activated ($t = 7$ s) and used for in vitro dissolution tests.

Treatment B was the bioadhesive CR formulation 2. The bioadhesive formulation was manually weighed and filled into hard gelatin capsules (301 mg per capsule) and unit doses were sealed into individual polyethylene packets. Capsules, 30, were neutron activated ($t = 7$ s) for in vitro evaluation.

For each of the two periods of the in vivo study, 12 capsules for treatment A and treatment B were neutron activated for 7 s. A single capsule was administered to each subject approximately 48 h post neutron activation when each capsule contained approximately 17–20 μCi of ^{153}Sm.

2.2.6. Dose administration, sample collection and in vivo γ- scintigraphy

Volunteers reported to the study unit the morning of dose administration after at least a 10 h fast. Prior to ingesting the radiolabeled capsule, two external markers

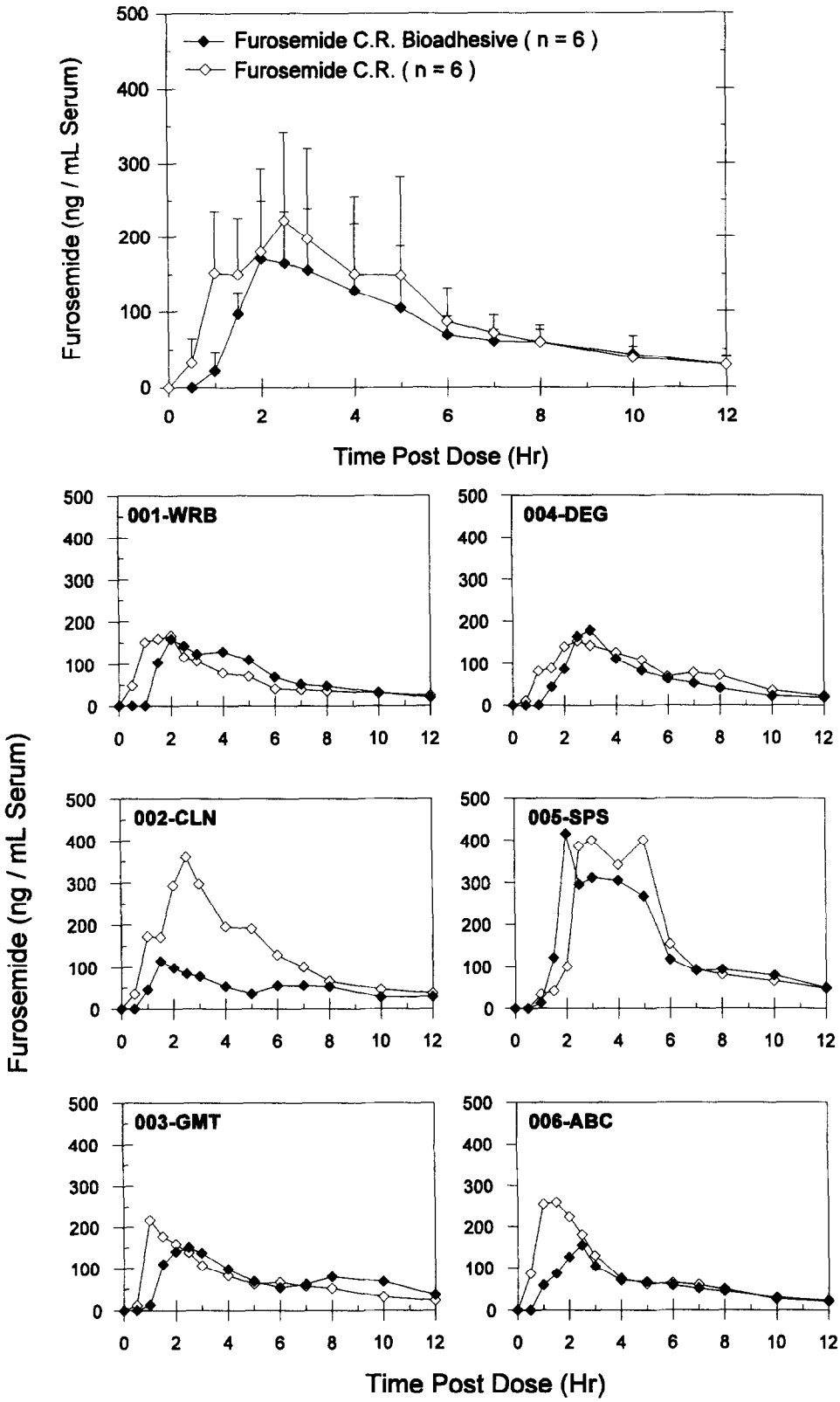


Fig. 4. Mean ($n = 6$) and individual serum concentrations following oral administration of 40 mg furosemide from a CR formulation with and without bioadhesives.

(2–4 μCi ^{153}Sm) were placed on each subject to facilitate consistent patient positioning in front of the γ camera. The first marker was placed on the patient's right side of chest, 7–10 cm below the nipple, and a second marker was placed on the left anterior superior ileac spine (approximately the hip bone). The scintigra-

Table 2
Mean basic pharmacokinetic values ($n = 6$) following oral dose of 40 mg furosemide in fasted subjects from C.R. and C.R. bioadhesive formulations

		AUC 0–12 h (ng × h)/ml	C_{max} (ng/ml)	T_{max} (h)	Onset of absorption (h)
Treatment A (controlled release formulation)	Mean	1170.24	259.73	2.40	0.60
	S.D.	448.80	101.82	1.40	0.20
Treatment B (bioadhesive formulation)	Mean	911.43	193.15	2.20	1.10
	S.D.	438.33	110.35	0.40	0.30

phy analysis software (ScinWin™, PC Solutions, Louisville, KY) uses these external markers to automatically align and register the image to the same relative position, thus, variations in patient positioning or actual patient movement is removed. Drug doses were taken with 240 ml of water, and subjects were immediately positioned supine beneath the scintillation camera (Siemens Basicam, low energy parallel hole collimator, 103 keV/15% window). Subjects were imaged for 8 min at 30 min intervals until it was confirmed that radioactivity had entered the colon. Thereafter, imaging was hourly through 12 h. Data were acquired in 1 min increments and stored on computer generating a time-activity study (Cardiac Medical Systems Software, Springfield, WI).

Blood samples were collected from a venous catheter into a vacuum tube (7 ml, no anticoagulant) at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10 and 12 h post dose. Blood samples were centrifuged (3000 rpm, 10 min) within 30 min after being drawn, and serum was separated and immediately frozen and stored at −20°C until the samples were analyzed by HPLC.

Pooled urine samples were collected at the following time intervals: 0–2 h; 2–4 h; 4–8 h and 8–12 h. The total volume of urine output during each time interval was recorded and a 30 ml aliquot was stored at −20°C until analyzed by HPLC.

2.2.7. Scintigraphic analysis

The sequential computer generated images were reviewed for each subject and regions of interest (ROI) were drawn to represent the stomach, jejunum, ileum and cecum/colon (ScinWin™ Scintigraphy Analysis Software, PC Solutions, Louisville, KY). All counts were background and decay corrected. Dynamic gastrointestinal transit plots were generated which depicted the relative percent of radioactivity in each region of interest versus time. The serum concentration of furosemide was then overlaid on these GI transit curves.

2.2.8. Furosemide analyses in plasma and urine

Urine and plasma samples were analyzed with a validated method by HPLC [19]. Detectors were used in series to detect furosemide by fluorescence and the internal standard (phenobarbital) by ultraviolet absorption.

3. Results and discussion

3.1. In vitro dissolution and bioadhesion results

The dissolution profiles of formulations 1 and 2, before and after irradiation, are shown in Fig. 2.

It can be noticed that the in vitro dissolution of furosemide from the bioadhesive formulation was slightly faster than the drug release from formulation which had no bioadhesive coating. This small increase in the rate of drug release was attributed to the hydrophilic properties of hydroxypropylmethyl cellulose and carbomer which may have acted as wetting agents. Also note that there was no statistical difference in the dissolution profiles before and after irradiation. The observed release profiles are typical of controlled release matrix system where release is faster in the first hours with a subsequent slower rate of release at later time points. Content uniformity of each formulation was also unaffected by the neutron activation procedure.

The in vitro bioadhesion of several formulations using different polymers is represented in Fig. 3.

Bioadhesion was evaluated by increasing the water flow across the isolated rabbit intestine. The percentage of detached particles was plotted versus the water flux (ml/min) in order to obtain the adhesion curves for several different formulations containing different proposed bioadhesive polymers (Fig. 3). Controlled release particles of furosemide without bioadhesive coating were tested as a reference. Regression analysis of the individual curves in Fig. 3 made it possible to calculate the water flow needed to detach 50% of particles from the mucosa surface over a fixed time interval. Polycarbophil, carbomer, and sodium carboxymethylcellulose (NaCMC) exhibited the best in vitro adhesive characteristics. Hydroxypropylmethylcellulose (HPMC) alone had poor adhesive properties, but when used in combination with carbomer, the overall adhesion was increased. Also from the curves shown in Fig. 3,

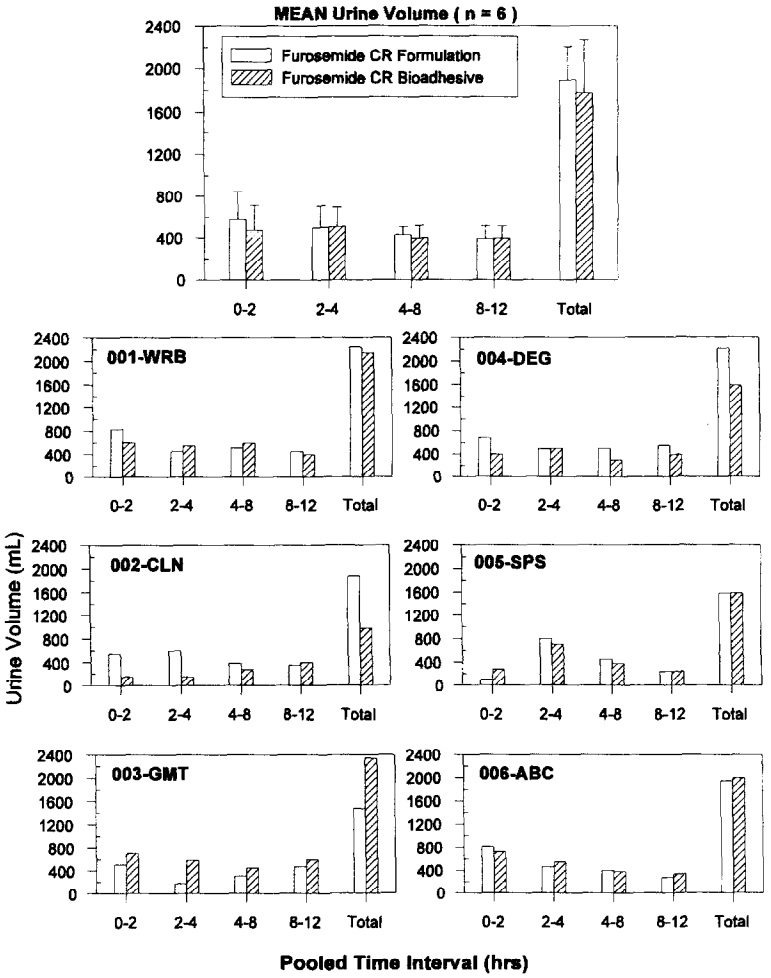


Fig. 5. Mean ($n = 6$) and individual urine output (ml) following oral dose of 40 mg furosemide from a controlled release formulation with and without bioadhesives.

hydroxypropyl (HPC) and hydroxyethylcellulose (HEC) demonstrated moderate adhesiveness.

For the in vivo study with furosemide, carbomer in combination with hydroxypropylmethylcellulose was chosen. Fig. 3 also depicts the in vitro adhesion data using formulation 3 (same bioadhesive polymers as formulation 2) before and after irradiation. The results showed that there was no difference in adhesion, indicating that neutron bombardment did not change this property.

3.2. Samarium-153 production

Non-enriched samarium oxide was added to each formulation so that individual unit doses had the equivalent of 3.0 mg samarium oxide. A 7 s irradiation (neutron flux = 8×10^{13} neutrons cm^{-2}/s) was calculated to produce 25.4 μCi samarium-153 at 48 h post activation. The experimentally determined samarium-153 production was approximately 75% of the calculated level (19.02 $\pm$ 1.03 μCi for formulation 1 and 18.91 $\pm$ 1.15 μCi for formulation 2; $n = 30$ for each batch at 48 h after neutron activation).

3.3. Furosemide serum concentration profiles

Fig. 4 shows mean and individual furosemide serum profiles for all six subjects and treatments. Initial review of the individual curves indicated that the onset of absorption for the bioadhesive formulation 2 tended to be later than the control formulation 1. Furthermore, the absorption rate of the bioadhesive formulation 2 also appeared to be slower in five of the six subjects (with exception of subject 005-SPS). Several of the individual curves also indicated a plateau region where the rate of furosemide input was approximately equal to furosemide elimination.

The pharmacokinetic parameters derived from the furosemide curves in Fig. 4 are represented in Table 2. Furosemide AUC, C_{max} and T_{max} were not statistically different between treatments, however, the onset of absorption was shown to be statistically later ($P = 0.013$. paired t-test) for the bioadhesive formulation where the onset of absorption was 0.6 $\pm$ 0.2 and 1.2 $\pm$ 0.3 h for the control and bioadhesive formulation, respectively. Mean furosemide AUC's were 1170.2 $\pm$ 448.5 and 911 $\pm$ 438.3

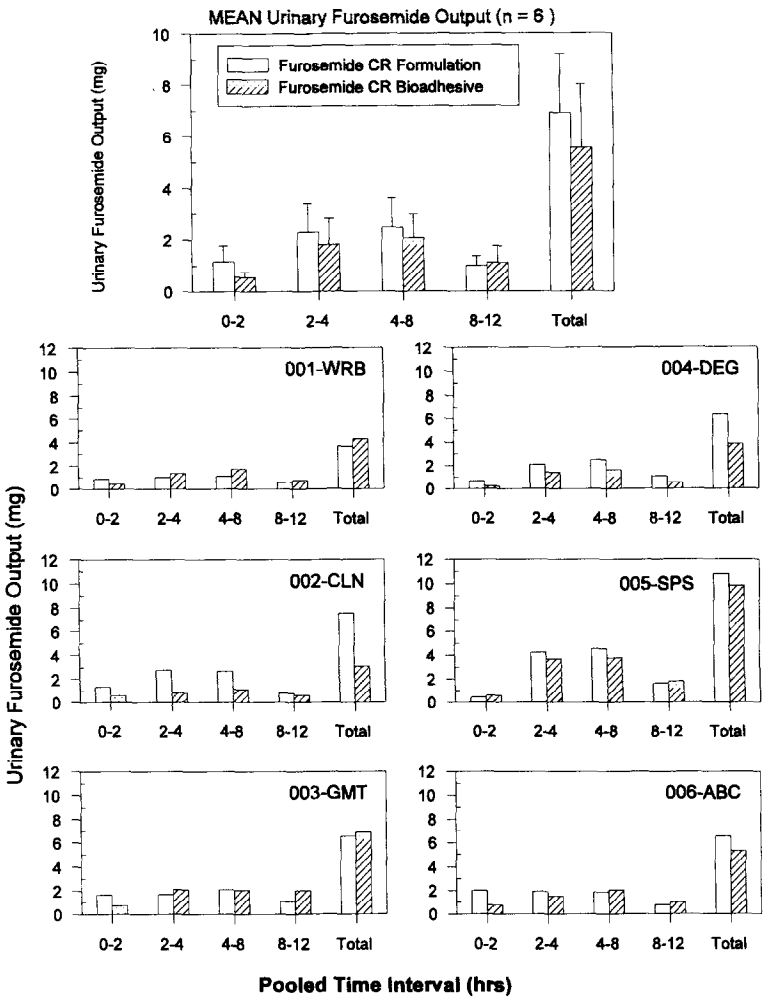


Fig. 6. Mean ($n = 6$) and individual urinary furosemide excretion (mg) following oral administration of furosemide (40 mg) in a controlled release formulation with and without bioadhesives.

ng × h/ml for treatments A and B, respectively, and although not statistically different (P-value = 0. 160, paired t-test), three of six subjects (subjects 2, 4 and 6) had a reduced AUC of 20% or greater following oral administration of the bioadhesive formulation. Possible reasons for such a reduction will be addressed with discussion of gastrointestinal transit data.

3.4. Urine production and fraction of furosemide excreted unchanged

Since the site of action of furosemide is the luminal surface of the ascending limb of the loop of Henle, the fraction of dose excreted unchanged in the urine represents the fraction which is potentially available for pharmacological action. Intravenous doses of furosemide to healthy subjects result in 50–80% of the dose excreted unchanged, whereas after oral dosing, the dose excreted unchanged is 20–55% [20]. Urine production at defined time intervals is shown by a bar graph in Fig. 5.

The greatest diuresis was observed with either the 0–2 h sample or the 2–4 h sample. These data are otherwise unremarkable and no statistical differences between treatments were noted. Furosemide urine excretion is shown graphically in Fig. 6.

The amount of furosemide excreted unchanged was statistically different (P-value = 0.030, paired t-test) in the 0–2 h pooled sample where the amount of furosemide found in the urine from the controlled release formulation 1 (no bioadhesive) was 1.159 ± 0.611 mg, while the bioadhesive formulation 2, had approximately half that amount (0.560 ± 0.193 mg). The lower amount of furosemide in the 0–2 h sample following oral administration of the bioadhesive formulation was observed in five of six subjects (Fig. 6, exception of subject 005-SPS).

This observation was also consistent with the delay in furosemide absorption reported in Fig. 4. The percentage of unchanged furosemide excreted in the urine 0–12 h for the entire collection interval was $17.2 \pm 5.7\%$ and $13.8 \pm 6.2\%$ for the control and bioadhesive

formulation, respectively. This was less than the literature reports of 20–55% of unchanged drug following oral administration of furosemide, but it was still sufficient to elicit the diuretic response observed in the 0–2 h and 2–4 h urine collection.

There was no statistical difference between treatments in the total amount of furosemide excreted for the entire pooling interval (0–12 h). However, the same three subjects who showed at least a 20% decrease in furosemide serum AUC's also had at least a 20% reduction of furosemide excreted into the urine following administration of the bioadhesive formulation. A good correlation ($r^2 = 0.842$) between the furosemide $AUC_{0\ 12\ h}$ and total furosemide urinary excretion (0–12 h) was demonstrated graphically by Fig. 7. Possible reasons for intra-subject changes in furosemide absorption will be discussed with reference to changes in intra-subject gastrointestinal transit times.

3.5. Gastrointestinal transit and relationship to furosemide absorption

Discussion of gastrointestinal transit data will attempt to address the following questions:

1. Do the gastrointestinal transit times of the control release granulate (treatment A, formulation 1) differ from the GI transit times observed with the bioadhesive formulation (treatment B, formulation 2)? Do the two formulations move through the GI tract in a different manner?
2. When the bioadhesive formulation was administered, can the later onset of furosemide absorption and lower urinary furosemide excretion at the 0–2 h pooling interval be explained by differences in GI transit?
3. Can changes in intra-subject gastrointestinal transit explain the intra-subject decrease in furosemide serum AUC and urinary excretion observed in three subjects?

Small intestine residence values were not statistically different between the control (treatment A) and bioadhesive (treatment B) formulations. The mean time for 50% of the radioactive marker to transit through the small intestine ($SITT_{50}$) was 210 ± 51 and 208 ± 89 min for the control and bioadhesive formulations, respectively. Gastric emptying was also not statistically different. However, there was a tendency for most subjects to have a later initial gastric emptying with the bioadhesive formulation as compared with the control formulation. The first observed gastric emptying times for the control and bioadhesive formulations were 17 ± 5 and 41 ± 17 min, respectively (no statistical significance, P-value = 0.063). Regardless of the lack of statistical significance, the delay in the onset of furosemide absorption and reduced furosemide urinary excretion observed from the bioadhesive formulation at the 0–2 h pooling interval was presumably caused by a concomitant delay in initial gastric emptying of the bioadhesive formulation.

To underscore the lag time observed for initial stomach emptying, intra-subject comparisons of the gastric emptying curves are shown in Fig. 8.

The individual gastric emptying profiles in Fig. 8 demonstrated the tendency for the bioadhesive formulation to delay initial gastric emptying in all of the subjects with exception of one individual (002-CLN). The lag time in gastric emptying of the bioadhesive formulation was also carried over through the remainder of the GI tract where a slight delay was observed in the entrance and exit of radioactivity in other segments of the GI tract. This observation is supported with reference to the mean gastrointestinal transit curves for the stomach, jejunum, ileum and colon which are depicted in Fig. 9.

Collectively, these data suggest that the cause for the later onset of furosemide absorption and reduced urinary excretion at the 0–2 h pooled sample from the bioadhesive formulation was caused by a simultaneous delay in initial gastric emptying. With the limited data presently available, it is difficult to ascribe an exact cause for the observed lag time in gastric emptying. It is possible that the formulation performed in vivo as a true bioadhesive, however, such in vivo adhesive properties were evidently not strong enough to overcome the powerful gastric contractions during phase III of the migrating motor complex. This statement is supported by the rapid rate of gastric emptying observed in subjects 1, 3 and 5 once gastric emptying was initiated (Fig. 8). Another plausible reason for delayed gastric emptying may originate from a slower rate of in vivo hydration of the bioadhesive formulation which in turn resulted in later dispersal towards the antrum where stomach emptying is initiated. This latter explanation is consistent with observations we have made utilizing similar excipients where the formulation is 30% or more of the rate controlling polymer.

Either explanation is supported by the scintigraphic images from a representative subject No. 1 shown in Fig. 10 and Fig. 11 for the control and bioadhesive formulations, respectively.

Comparison of the images in these two figures shows that the controlled release formulation containing no adhesives (Fig. 10) was in the jejunum at the 30 min imaging sequence which coincided with the onset of furosemide absorption. Conversely, the bioadhesive formulation (Fig. 11) was situated high in the gastric fundus at the 30 min interval with spreading observed throughout the fundus by 1.0 h. Significant gastric emptying of the bioadhesive formulation was not ob-

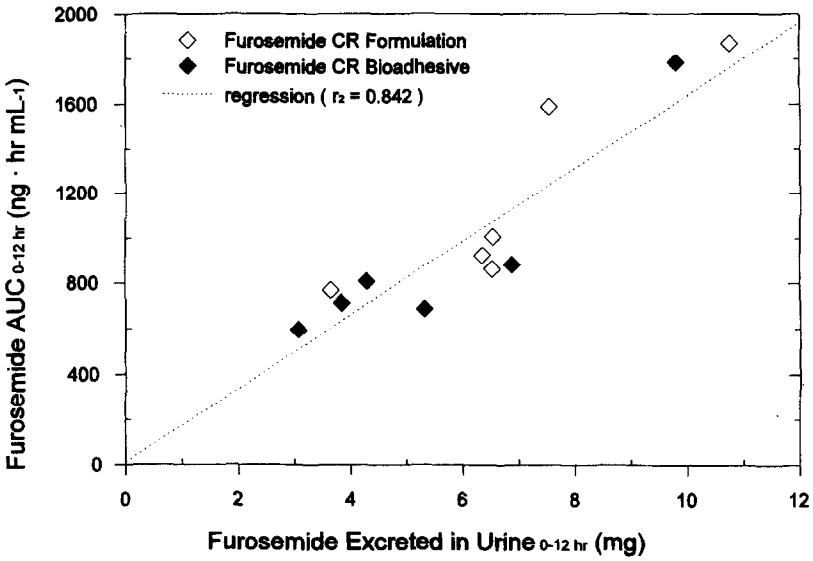


Fig. 7. Correlation between individual furosemide serum AUC0-12 h and excretion of unchanged drug into the urine following oral administration (40 mg) of a CR formulation and a CR bioadhesive formulation.

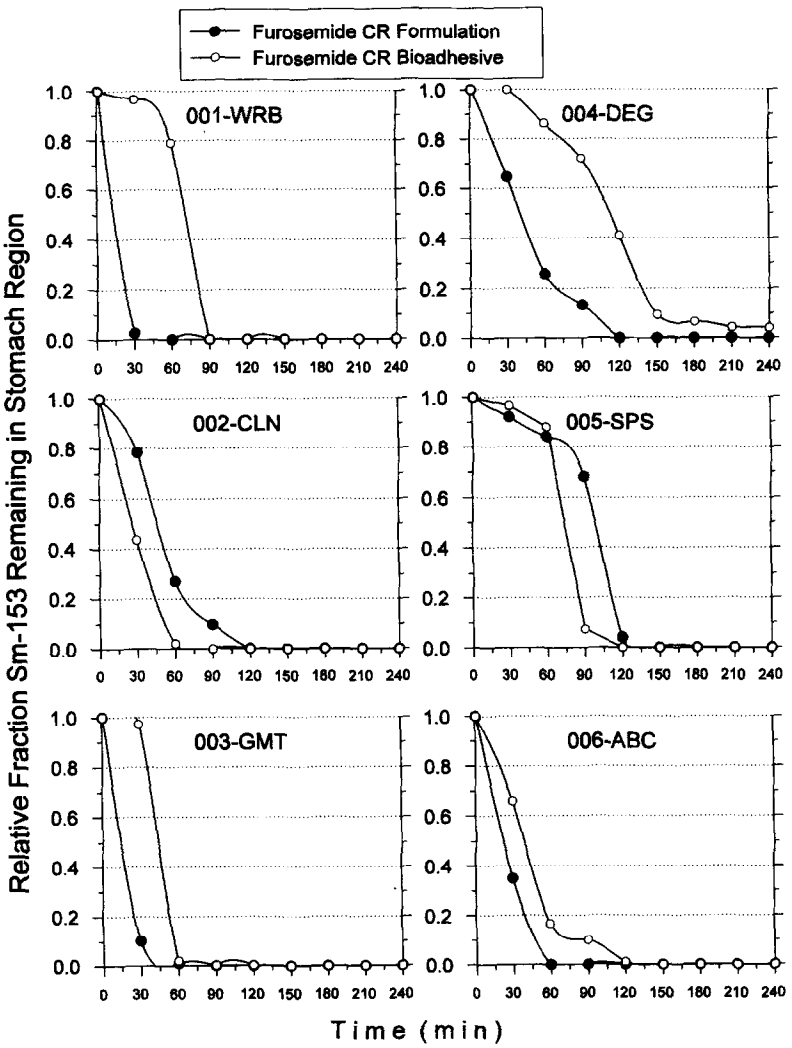


Fig. 8. Intra-subject gastric emptying comparisons of samarium-153 labeled controlled release formulations of furosemide (40 mg) with and without bioadhesives.

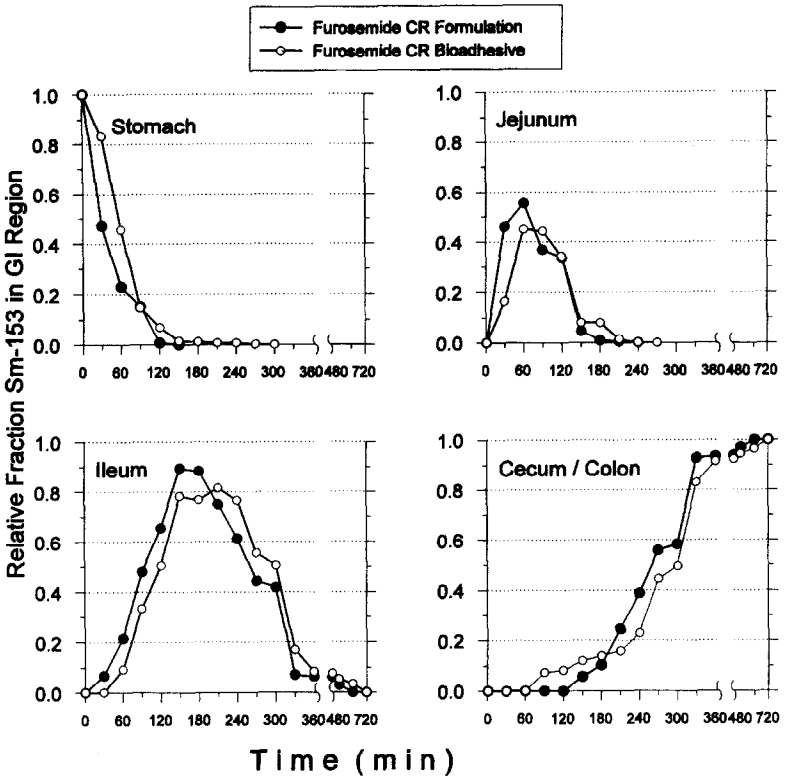


Fig. 9. Mean gastrointestinal transit curves ($n = 6$) of samarium-153 labeled controlled release formulations of furosemide (40 mg) either with or without bioadhesives.

served until 1.5 h post dose which coincided with the first detectable serum furosemide levels. Thus, the images in Fig. 10 and Fig. 11 help to pictorially demonstrate the different manner in which gastric emptying was initiated, and further demonstrates the delayed gastric emptying observed with the bioadhesive formulation. Subject 005-SPS was the only individual who did not show a delay in the onset of absorption between the control and bioadhesive formulation (Fig. 4). The reason for the lack of delay was derived from the relatively late gastric emptying of the control formulation in subject 005-SPS as compared with the bioadhesive formulation (Fig. 8).

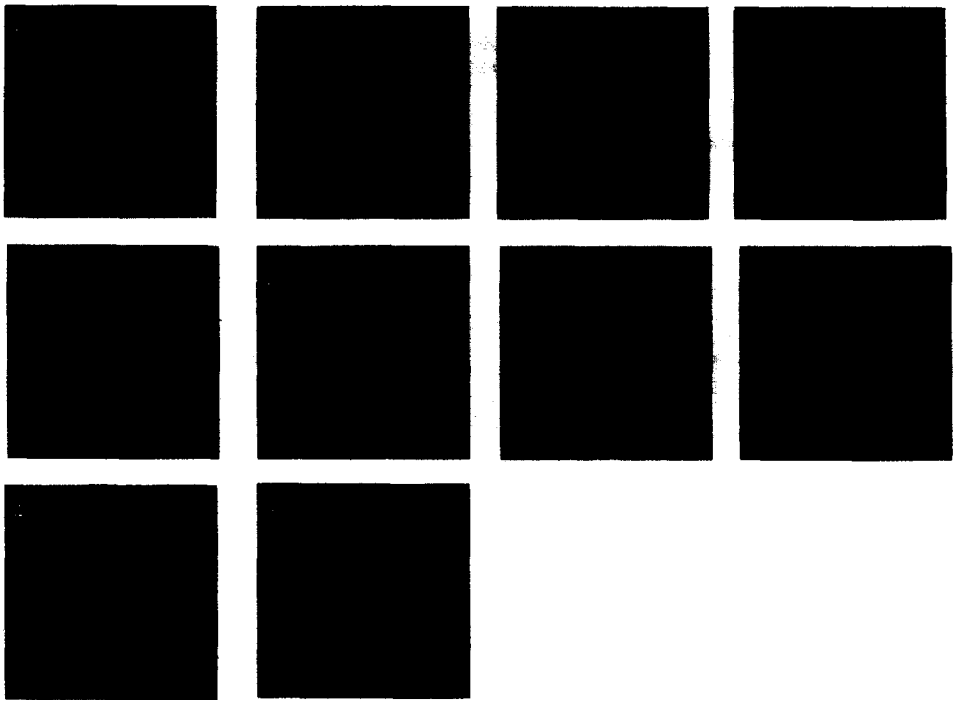
3.6. Possible explanations for intra-subject variability of furosemide absorption

The absorption and bioavailability of furosemide is known to be erratic which has been credited to originate from a multitude of factors including underlying diseases, differences in tablet batches, calculations based either on urine or plasma data, and the presence or absence of food [20]. Some literature references have described that furosemide is most efficiently absorbed from the stomach. This particular study was done using the rat model where rapid absorption was noted from the stomach at pH 3 [20]. Progression down the GI tract from the stomach to the duodenum to the jejunum showed a slower absorption as the pH rose from 3 to 5 [20]. It was stated that this pH dependence appeared to exert greater influence on the absorption of furosemide than the increased surface area of the small intestines.

From past experiences of conducting scintigraphic studies which correlate drug absorption with GI transit, it is highly unusual to observe systemic drug levels where the absorption process has been implicated primarily in the stomach. This does not exclude the fact that gastric residence may positively or negatively affect the extent of absorption of some drugs [21], but it has been our experience that the onset of drug absorption typically correlates with initial gastric emptying of the radioactive marker and entry of the drug into the small intestine. The data from the present study also support such past experiences. Furosemide was not detected in the serum until the radioactive marker had entered the small intestine, furthermore, the elimination phase of furosemide frequently corresponded with the arrival of the radioactive marker in the cecum and colon region where furosemide absorption is known to be poor [7].

4. Conclusions

The results from this study which correlated the gastrointestinal transit of a water insoluble radioactive marker (samarium-153 oxide) with absorption of



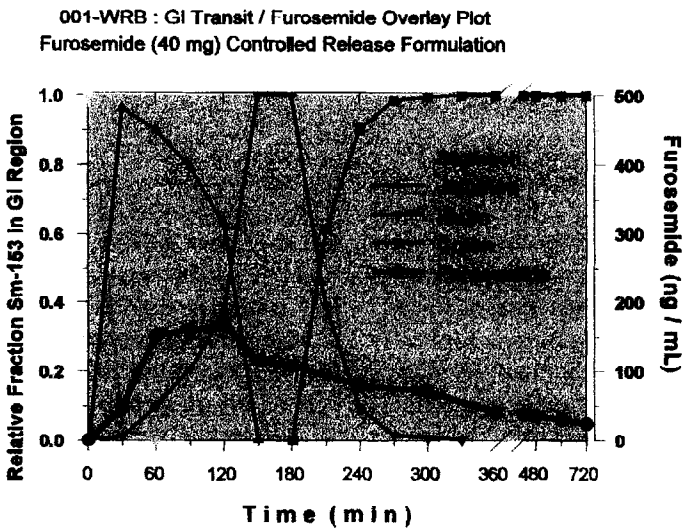


Fig. 10. Scintigraphic images through five hours post dose and GI transit/furosemide overlay plot for subject 001-WRB following oral dose of furosemide (40 mg) administered as a controlled release formulation in a fasted condition.

furosemide from a controlled release (CR) formulation and the same CR formulation with the addition of a bioadhesive polymer coating indicated that drug release from each formulation was controlled. This was demonstrated by the lack of a high C_{max} which is characteristic of immediate release formulations. Sustained furosemide levels were also achieved as confirmed by individual furosemide profiles which were predominantly flat through 5–8 h post dosing. Increased urine output from the furosemide formulations was most evident in the 0–2 h and 2–4 h collection interval. Diuresis was less from 4–12 h post dose. There was no statistical difference in gastrointestinal transit values between the control and bioadhesive formulations. However, the initial gastric emptying times of the bioadhesive formulation tended to be later as compared with the control formulation. This difference may have been statistically significant with a larger subject population. The delayed onset of gastric emptying of the bioadhesive formulation resulted in a later onset of furosemide absorption and also a decreased amount of furosemide excreted in the urine for the first pooling

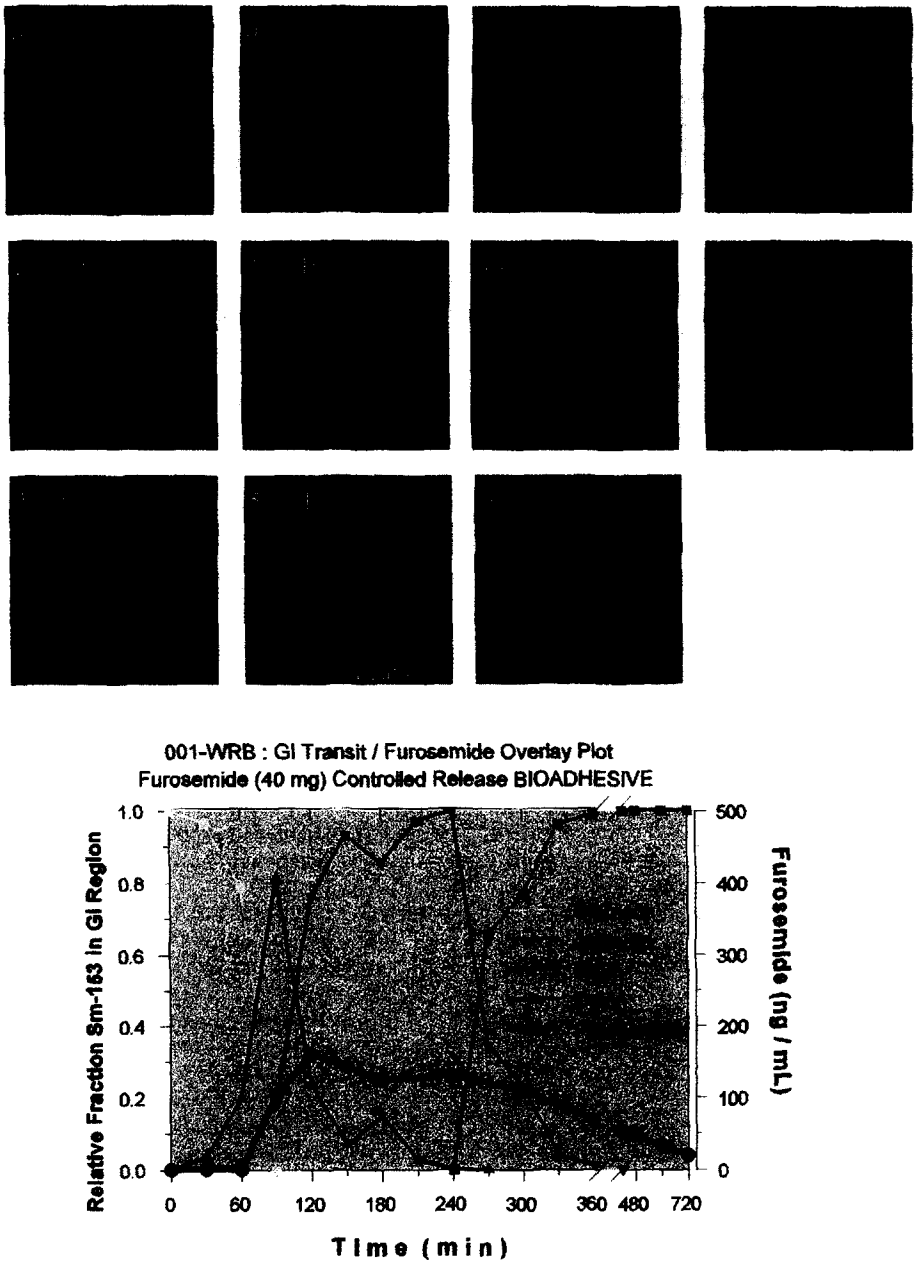


Fig. 11. Scintigraphic images through 5 h post dose and GI transit/furosemide overlay plot for subject 001-WRB following oral dose of furosemide (40 mg) administered as a controlled release Bioadhesive formulation in a fasted condition.

interval (0–2 h). Other than the manner in which gastric emptying was initiated, the two formulations showed no consistent differences in transit through the small intestine. In some instances the formulations were observed to spread widely throughout in the small intestine, while in others the material moved as a well-defined mass. Due to the limited sample size, it was not possible to determine whether the manner in which the material moved through the small intestine was formulation specific.

The amount of furosemide recovered in the urine in this study (7.7–26.9% of the dose) indicated that furosemide absorption was incomplete as compared with the fraction of the dose excreted from an immediate release formulation (20–50% of the dose).

Gastrointestinal transit/furosemide overlay plots demonstrated that the onset of drug absorption coincided with arrival of the radioactive marker in the small intestine. Drug levels remained relatively constant in many subjects until the radioactive marker moved into the cecum and colon where furosemide absorption is poor. Arrival of the material to the colon typically corresponded with the elimination phase of furosemide. Intra-subject changes in furosemide absorption between

treatments was possibly associated with intra-subject variation in gastric emptying and small intestine transit. Since it was shown that the bioadhesive formulation did not significantly change gastric emptying, sustained drug absorption from these systems will have the greatest probability of success for drugs which also show a degree of absorption from the colon.

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