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RESEARCH PAPER

# Studies of Floating Dosage Forms of Furosemide: In Vitro and In Vivo Evaluations of Bilayer Tablet Formulations

Nurten Özdemir,[1,*] Sefika Ordu,[1] and Yalçin Özkan[2]

[1]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ankara, 06100, Tandogan-Ankara, Turkey*
[2]*Gülhane Military Medical Academy, Department of Pharmaceutical Technology, 06018, Etlik-Ankara, Turkey*

**ABSTRACT**

*For the purpose of enhancement the bioavailability of furosemide (FR), a floating dosage form with controlled release of FR was designed in this study. Because of the lower solubility of active material in the gastric medium, it was first enhanced by preparing an inclusion complex of FR with beta-cyclodextrin (β-CD) in a 1:1 proportion using the kneading method. Following the design of dosage form, bilayer floating tablets were prepared. After dissolution rate studies were performed using the continuous flow-through cell method, the formulation that provided delivery of active material near the target profile was given to six healthy male volunteer subjects, and in vivo tests were performed. It was determined by radiographs that floating tablets prepared by adding $BaSO_4$ stayed in the stomach for 6 hr. Further, values of the area under the plasma concentration-time curve (AUC) obtained with the floating dosage form were about 1.8 times those of the conventional FR tablet in blood analyses; maximum and minimum plasma concentrations were also found to be between the desired limits. In urine analyses, the peak diuretic effect seen in classical preparations was decreased and prolonged in floating dosage forms. Also, a considerably significant correlation was detected between in vivo results and in vitro data of the dissolution rate, and it was concluded that the modified continuous flow-through cell method is usable for in vitro dissolution rate tests of floating dosage forms.*
***Key Words:*** *Bioavailability; Controlled release; Floating dosage forms; Furosemide; Solid dispersion.*

* To whom correspondence should be addressed. E-mail: nozdemir@pharmacy.ankara.edu.tr

## INTRODUCTION

It is known that the short stay of active material released from controlled-release oral preparations in the region that they are absorbed, specifically in the gastrointestinal (GI) tract, leads to bioavailability problems. Thus, to prolong the passage time of the preparations through the GI tract, it has been suggested that (*a*) addition of certain fatty acids such as triethanolamine and cetyl palmitate to the formulations used since it is known that they may reduce the gastric emptying rate; (*b*) preparation of bioadhesive systems by adding polymers to the formulations, which may attach to the surface of GI epithelium; and (*c*) development of floating dosage forms that may remain on the contents of the stomach because of they have a lower density than that of the stomach (1).

Floating dosage forms (hydrodynamic balanced systems, HBS) are oral dosage forms of tablets, capsules, or microbeads and contain hydrocolloids that allow floating by swelling. Effervescent granules or floating chambers may also be added to the formulations to provide floating (2–4).

For the following instances, it has been suggested that an active material should be formulated in the form of an HBS to enhance its bioavailability: (*a*) having dissolution and/or stability problems in the fluids of the small intestine, (*b*) being effective locally in the stomach, and (c) being absorbed only in the stomach and/or upper part of the small intestine (2).

It has been reported that furosemide (FR), the active material used in the present study, has a bioavailability problem, and it initially shows an adverse temporary peak diuretic effect (5,6). To eliminate such an effect, various efforts have been made for the preparation of FR in prolonged-release forms. However, it has been reported that the bioavailability of such preparations has been decreased to 40–60% compared to conventional tablet forms (5).

Although it has not been shown for humans, it has been pointed out in all animal studies of bioavailability that there may be regions in the stomach and/or upper the part of the small intestine in which FR is specifically absorbed; it has been thought that the short stay of controlled release preparations in this specific region of absorption leads to bioavailability problems (7). Accordingly, this study was designed to enhance the bioavailability of FR by prolonging its duration in the stomach via the floating dosage forms with controlled release.

## EXPERIMENTAL

### Materials

Furosemide was supplied by Deva A.S. (Istanbul, Turkey); in addition, the following were used in the study: β-cyclodextrin (Sigma, St. Louis, MO); hydroxypropylmethylcellulose 4000 (HPMC 4000) mPa·S (Fluka, Deisenhofen, Germany); hydroxypropylmethylcellulose 100 (HPMC 100) mPa·S Fluka; carboxymethyl cellulose (CMC) (Merck, Darmstadt, Germany); polyethylene glycol 400 (PEG400) (Merck); and Di-Pac (Amstar, New York). All other materials and solvents were analytical grade.

### Formulation

The target values for the dosage form to achieve were calculated from the pharmacokinetic parameters of the drug and were as follows (8–10): sustaining dose 44 mg, zero-order release rate 6.98 mg/hr, and dosage interval 8 hr. Because the solubility of active material at pH 1.2 was very low, it was enhanced first by preparing solid dispersions of FR with β-CD in a 1:1 proportion using the kneading method (11).

The polymers HPMC 4000, HPMC 100, and CMC were employed in formulations for the control of delivery of active material and for the matrix formation. Floating was provided by adding an effervescent mixture of sodium bicarbonate with citric acid.

Using the direct pressing method, tablets were compressed in a hand press with a 12-mm diameter punch. To begin, the mixture that provided floating was placed on the die cavity, and a preparatory pressing was made. Thereafter, the second component that contained the active material and polymers was added, and the final tablets were pressed with a compression force of 16 MPa. It was determined that the hardness of the tablets that were pressed at this pressure was 11 strong-cobb.

### Floating Capability

Tablets were placed in a 400-ml flask at pH 1.2, and both the time needed to go upward and float on the surface of the fluid and the floating durations were determined (12).

### In Vitro Dissolution Studies

The continuous flow-through cell method was used in the dissolution rate studies to provide the sink condition

in the dissolution medium. As the dissolution medium, artificial gastric fluid of pH 1.2 without enzymes with surface tension reduced to 45–50 $mNm^{-1}$ by adding polysorbate 20 with a concentration of 0.02% was employed to simulate in vivo conditions (13).

Dissolution rates were studied using a powder form of FR/β-CD complex (195 mg) equivalent to 40 mg of FR in flow rates of 3, 6, 9, and 16 ml/min to establish the flow rate that would be used. The flow rates to provide the sink condition were determined as 9 and 16 ml/min, and it was decided that a flow rate of 9 ml/min would be used in the entire study to facilitate the work.

Formulations prepared were subjected to dissolution rate tests for 8 hr, and the amounts of active material were measured spectrophotometrically at a wavelength of 274 nm.

The dissolution rate data obtained were evaluated relating to compatibility to the kinetics of zero order, first order, RRSBW distribution, and $Q \rightarrow \sqrt{t}$, and the results were used in an attempt to explain the mechanism of the release of active material from preparations (14,15).

### In Vivo Studies

The in vivo tests discussed below were made on six healthy male volunteer subjects whose ages were between 25 and 32 years, who weighed between 60 and 71 kg, and who were 1.60 to 1.70 m tall.

#### Determination of Staying Duration of Floating Tablets in the Stomach

For X-ray detection, 10 mg of $BaSO_4$ was added to the part of the final formulation that provides controlled release (the amount of $BaSO_4$ that allows visibility by X-ray, but does not preclude the floating of tablets was experimentally calculated). Labeled tablets were given to subjects with 200 ml of water after a light, 308 kcal breakfast. Following ingestion, gastric radiography was undertaken at 0.5, 1, 2, 3, 4, and 6 hr, and the duration the tablets stayed in the stomach was determined.

#### Blood Analysis of the Amount of Active Material

Every 15 days, the prepared floating dosage form and commercial FR tablet (Furomid®) as a control were given to the subjects on an empty stomach with 200 ml of water. Each subject took 100 ml of water hourly. Following a light breakfast after 2 hr and then a light midday meal around noon, blood samples (5 ml each) were taken at hours 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24, and the sera was removed by centrifugation.

The amount of active material was determined by high-performance liquid chromatography (HPLC) using μBondapak $C_{18}$ (Waters, Milford, MA) column at a flow rate of 1.0 ml/min. Methanol and 0.01 M sodium acetate (35:65) were used as the mobile phase and sodium cephalotin as the internal standard (16,17). The chromatograms of both the standard and the samples were analyzed by a computer program, and the concentrations of active material were estimated.

#### Pharmacokinetic Analysis

Parameters including AUC, $C_{max}$, and time to reach maximum plasma concentration $T_{max}$ were estimated using a computer. Statistical analyses were performed using a Student $t$ test with $p < .05$ as the minimal level of significance.

#### Analysis of Urine Data

After the standard tablets of FR and the developed floating dosage form were given to subjects as well as in the fasting state of as control group, the diuretic effect was determined by measuring the volumes of urine that were collected at hours 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24.

## RESULTS AND DISCUSSION

### Formulation

In the present study, a dose structure was designated using the pharmacokinetic data of FR; accordingly, the amount of active material in the dosage form was estimated as 44 mg, the dosage interval as 8 hr, and the rate constant of release of active material as 6.98 mg/hr.

Because the solubility of active material at the gastric medium of pH 1.2 at 37°C is as low as 30.27 mg/ml, it was enhanced to 91.88 mg/ml by preparing an inclusion complex of FR with β-CD (11). Thus, controlled release limited by solubility was precluded, and delivery of active material from the preparation was controlled by the formulations.

All formulations were prepared as two-layer tablets. The first layer provided floating and contained the mixture of sodium bicarbonate and citric acid to form air bubbles and HPMC 4000 as a matrix material to retain the air bubbles. The second layer (release layer) provided controlled release of active material and contained active material and HPMC 100 as hydrophilic matrix material.

***Table 1***

*Formulations Used in the Study*

| Ingredient (mg) | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ | $F_9$ | $F_{10}$ | $F_{11}$ | $F_{12}$ | $F_{13}$ | $F_{14}$ | $F_{15}$ | $F_{16}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FR/β-CD (1:1) | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 |
| HPMC 100 | 100 | 100 | 100 | 80 | 70 | 50 | 50 | 50 | 50 | 30 | 30 | 20 | 30 | 20 | 30 | 20 |
| Lactose | | | | | | | | | | | | | 20 | 20 | | |
| PEG 6000 | | 20 | 30 | | | 30 | | 50 | | | 30 | | | | | |
| PEG 10000 | | | | | | | | | 50 | | | | | | | |
| Di-Pac | | | | | | | | | | | | | | | 20 | 20 |

Since it is known that the compression force applied to tablets affects the onset time of floating (18), tablets were pressed first with different compression forces, with their effect on the onset time examined. It was found that the less the compression force was, the shorter the time to onset of floating. For example, when the tablet coded $F_5$ was pressed with a compression force of 16 MPa, it began to float in 20 min, whereas at a force of 32 MPa, the time was prolonged to 45 min. It was thought that the tablets with lower strength drew water to the structure more easily, precipitating the effervescent effect to begin. Therefore, a compression force of 16 MPa as the lowest force to provide a tablet with an adequate strength was used in the pressing of all tablets.

The ideal amounts of both effervescent mixture and polymer for the floating layer were estimated by determining the onset time of floating. In an attempt to shorten the onset time by increasing the concentration of effervescent mixture, it was observed that tablets were dispersed; on the other hand, lower concentrations caused this duration to prolong. The best proportion for floating was found to be 250 mg for HPMC 4000, 34 mg for sodium bicarbonate, and 28 mg for citric acid. After the determination of this arrangement of effervescent powders, formulations given in Table 1 were prepared to determine the proportion of polymer needed to provide delivery of active material near the target profile from the release layer that contained active material. Delivery of active material in tablets prepared using HPMC 4000 was very low; besides, it was observed that the formulations with CMC were dispersed after a certain time. When HPMC 100 was employed to increase the delivery by lowering the viscosity of the polymer used, it was observed that the delivery of active material significantly increased (Figs. 1a and 1b). Figure 2 shows the linear relation between the amount of HPMC 100 and zero-order release rate constant of active material.

When water-soluble additives such as PEG 6000, PEG 10000, lactose, or Di-Pac were added into the formulations, release of active material increased as a result of

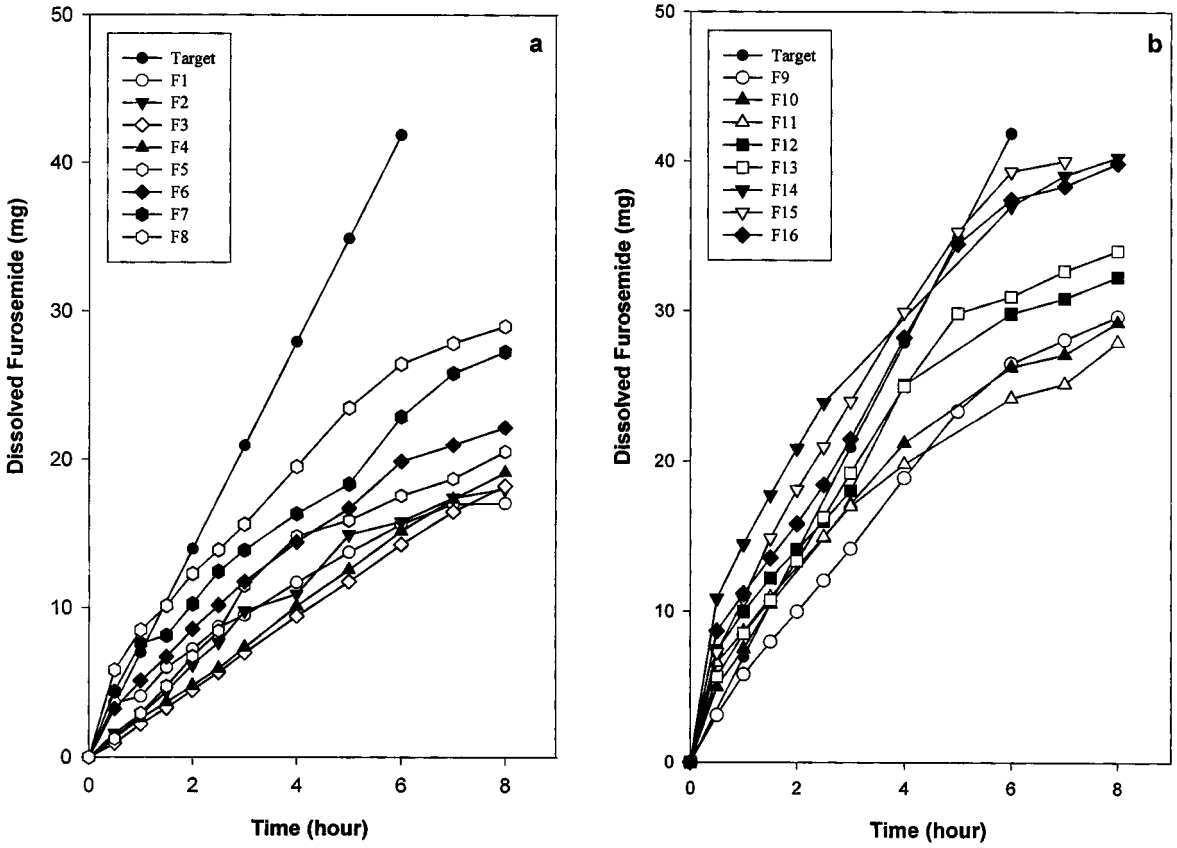


**Figure 1.** Dissolution profiles of the formulations.

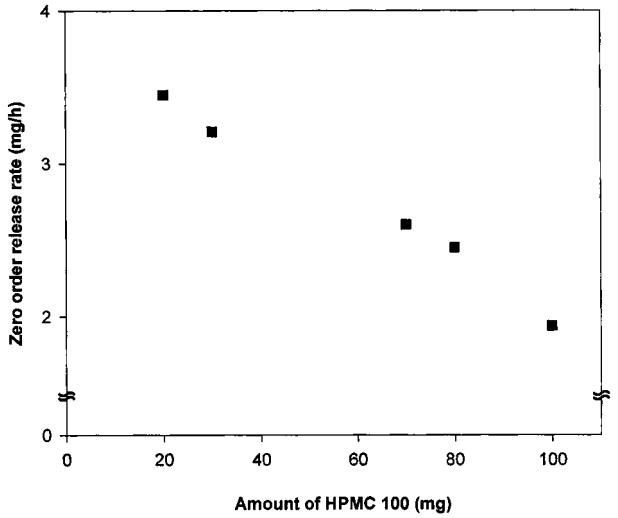


**Figure 2.** The relationship between the amount of HPMC 100 and the zero-order release rate constant.

more rapid swelling of polymer, which has a hydrophilic nature.

A delivery nearest to the target profile was obtained with formulations $F_{15}$ and $F_{16}$, both prepared using Di-Pac. The compatibility of the formulations to the kinetics of zero order, first order, RRSBW, and $Q \rightarrow \sqrt{t}$ was evaluated (Table 2). The rate constant of zero-order release with formulation $F_{15}$ was found to be 6.21 mg/hr. The $F_{15}$ formulation was accepted as the final formulation owing both to shortness of the onset time of floating (15 min) and to nearness of the rate constant of release to the intended one (6.98 mg/hr).

Accordingly, the final formulation for in vivo studies was as follows: for the floating layer, 250 mg of HPMC 4000, 30 mg of sodium bicarbonate, and 28 mg of citric acid; for the release layer, 195 mg of FR/β-CD (1:1), 30 mg of HPMC 100, and 20 mg of Di-Pac.

## In Vivo Studies

Following ingestion of the final formulation prepared by the addition of $BaSO_4$ to the release layer, the durations the tablets stayed in the stomach were examined by radiograms, and it was estimated that tablets stayed in the stomach for 6 hr (Fig. 3).

The bioavailability data obtained are shown in Table 3; the plasma concentration-time curve of both the floating dosage form and the conventional FR are shown in Fig. 4. As seen on Fig. 4, there is a significant difference between the two tablets. The floating dosage form was

***Table 2***

*The Kinetic Assesment of Release Data*

| | Zero Order | | First Order | | RRSBW | | | $Q - \sqrt{t}$ | |
|---|---|---|---|---|---|---|---|---|---|
| Formulation | $kr^0$ | $r^2$ | $kr$ | $r^2$ | $T$ (min) | β | $r^2$ | $k$ | $r^2$ |
| $F_1$ | 1.94 | .988 | $5.92\times10^{-2}$ | .983 | 1260.04 | 0.71 | .977 | $1.61\times10^{-2}$ | .987 |
| $F_2$ | 2.31 | .990 | $6.97\times10^{-2}$ | .979 | 807.91 | 1.00 | .992 | $1.58\times10^{-2}$ | .986 |
| $F_3$ | 2.33 | .998 | $6.89\times10^{-2}$ | .998 | 817.11 | 1.15 | .999 | $1.24\times10^{-2}$ | .980 |
| $F_4$ | 2.45 | .998 | $7.37\times10^{-2}$ | .996 | 837.83 | 1.07 | .996 | $1.38\times10^{-2}$ | .974 |
| $F_5$ | 2.61 | .945 | $8.15\times10^{-2}$ | .970 | 624.44 | 1.13 | .983 | $2.01\times10^{-2}$ | .983 |
| $F_6$ | 2.60 | .980 | $8.65\times10^{-2}$ | .994 | 723.90 | 0.83 | .997 | $2.47\times10^{-2}$ | .994 |
| $F_7$ | 2.67 | .974 | $8.37\times10^{-2}$ | .986 | 711.46 | 0.74 | .993 | $2.34\times10^{-2}$ | .987 |
| $F_8$ | 3.22 | .978 | $13\times10^{-2}$ | .992 | 457.36 | 0.77 | .983 | $45\times10^{-2}$ | .989 |
| $F_9$ | 3.71 | .978 | $15\times10^{-2}$ | .993 | 422.76 | 1.02 | .995 | $4.22\times10^{-2}$ | .988 |
| $F_{10}$ | 3.21 | .958 | $13\times10^{-2}$ | .991 | 425.64 | 0.82 | .997 | $4.68\times10^{-2}$ | .994 |
| $F_{11}$ | 2.76 | .969 | $11\times10^{-2}$ | .993 | 518.75 | 0.87 | .992 | $4.30\times10^{-2}$ | .996 |
| $F_{12}$ | 3.45 | .961 | $16\times10^{-2}$ | .984 | 349.52 | 0.77 | .971 | $5.90\times10^{-2}$ | .980 |
| $F_{13}$ | 4.02 | .935 | $19\times10^{-2}$ | .985 | 308.10 | 0.93 | .983 | $6.41\times10^{-2}$ | .981 |
| $F_{14}$ | 3.97 | .935 | $30\times10^{-2}$ | .997 | 175.76 | 0.83 | .981 | $10\times10^{-2}$ | .986 |
| $F_{15}$ | 6.21 | .995 | $32\times10^{-2}$ | .976 | 216.33 | 0.94 | .979 | $8.99\times10^{-2}$ | .991 |
| $F_{16}$ | 4.57 | .962 | $31\times10^{-2}$ | .980 | 219.82 | 0.94 | .943 | $8.84\times10^{-2}$ | .973 |

$kr^0$ = zero-order release rate constant; $kr$ = first-order release rate constant; $T$ = value stands for the time for 63.2 % release of the drug; β = shape factor; $k$ = rate constant obtained from $Q-\sqrt{t}$ kinetic; $r^2$ = determination coefficient.

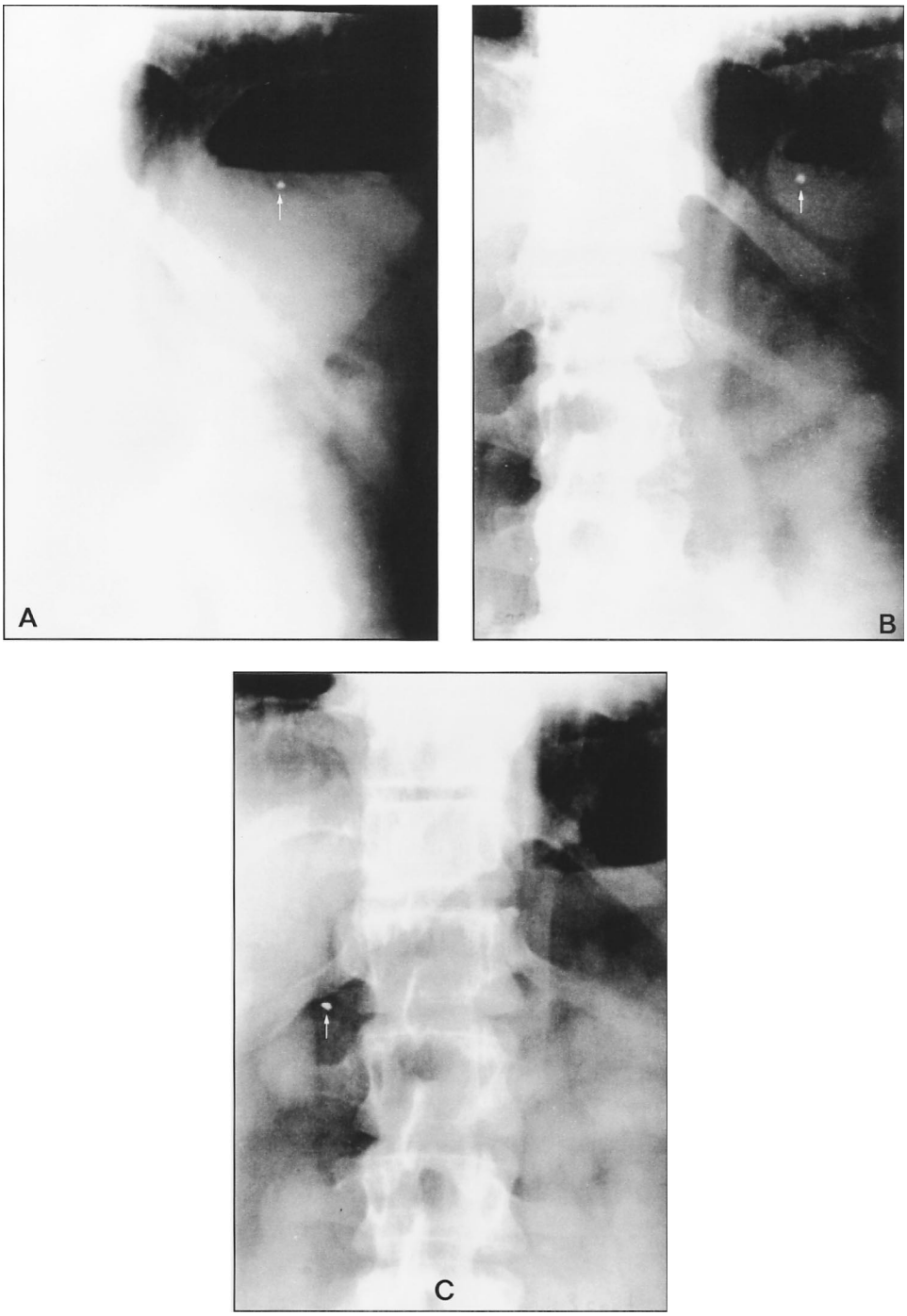


**Figure 3.** X-ray photographs of the floating tablets in the stomach at (A) 1 hr, (B) 3 hr, and (C) 6 hr after administration to the healthy volunteers.

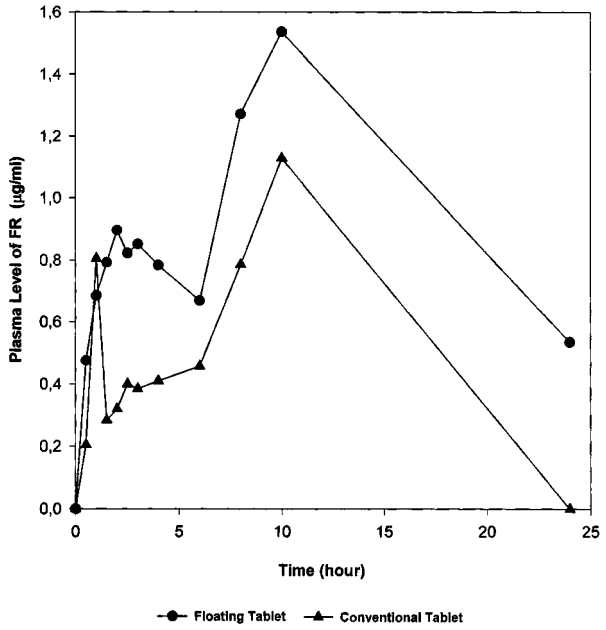


**Figure 4.** Comparison of the mean plasma levels of furosemide after oral administration of conventional tablet and floating tablet.

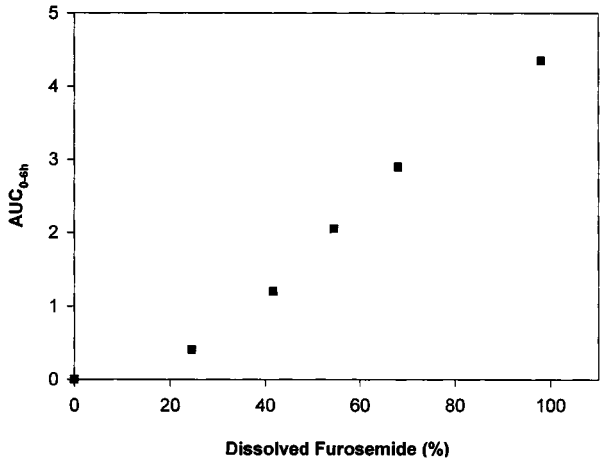


**Figure 6.** The relationship between the AUC and the dissolved furosemide (%).

designated such that $C_{max}$ was 1.66 µg/ml and $C_{min}$ was 0.3 µg/ml; the obtained concentration values in blood with this preparation were found between these intervals. Values of $AUC_{0\text{-}24}$ obtained with the floating dosage form were about 1.8 times more than for the conventional FR tablet.

According to the measurements of urine volume (Fig. 5), the peak diuretic effect seen in conventional prepara-

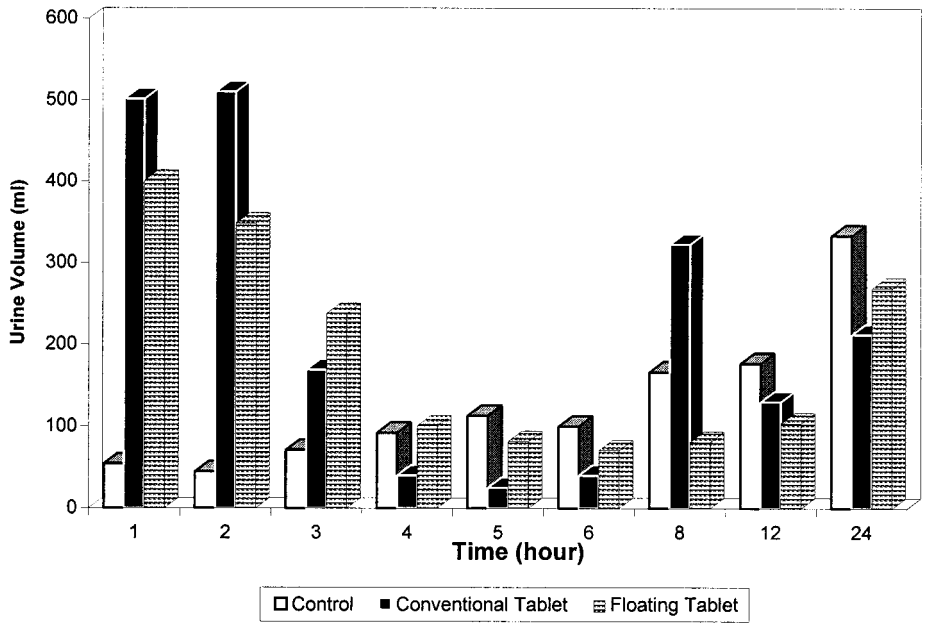


**Figure 5.** Urine volume obtained at different time intervals after administration of conventional and floating tablets.

***Table 3***

*Comparison of the Bioavailability Parameters*

| Formulation | $t_{max}$ (hr) | $C_{max}$ (μg/ml) | AUC (μg · hr/ml) 0–8 hr | 0–10 hr | 0–24 hr |
|---|---|---|---|---|---|
| Conventional tablet | 1.10 | 0.807–1.129 | 3.46 ± 0.052 | 5.38 ± 0.06 | 13.28 ± 0.15 |
| Floating tablet | 2.10 | 0.897–1.537 | 6.30 ± 0.17 | 9.10 ± 0.14 | 23.62 ± 0.10 |

Mean ± SD; $n = 6$.
$t_{max}$ = time to reach maximum serum concentration; $C_{max}$ = maximum serum concentration; AUC = area under the time-concentration curve.

tions disappeared in the floating dosage forms. As a desired effect, however, it has been observed that the diuretic effect was longer and weaker.

When AUC values of the floating dosage form were plotted against the data of in vitro percentages of delivered active material to detect whether there was a correlation between in vivo results of blood analyses and in vitro data for the dissolution rate, a considerably significant correlation was obtained (Fig. 6). This meaningful evidence indicates that the method used for the in vitro dissolution rate may be used in the testing of such preparations.

## CONCLUSION

In this study, floating dosage forms with controlled release were prepared, and both in vivo and in vitro results were studied. The in vivo–in vitro correlation obtained indicates that the method used for the in vitro dissolution rate is an ideal method for the testing of such dosage forms. Showing that the bioavailability of FR has been excessively increased compared to conventional forms and that absorption of FR taken place vastly in stomach and upper part of small intestine, in vivo results are also important.

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