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In vitro and in vivo evaluation of floating riboflavin pellets developed using the melt pelletization process

J. Hamdani, J. Goole, A.J. Moës, K. Amighi *

Laboratoire de Pharmacie Galénique et Biopharmacie, Université Libre de Bruxelles, Campus Plaine, CP 207, Boulevard du Triomphe, 1050 Brussels, Belgium

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Abstract

Floating pellets were prepared using the melt pelletization process in a Mi-Pro® high shear mixer (Pro-C-epT, Belgium). Formulations were based on a mixture of Compritol® and Precirol® as meltable binders and on the use of sodium bicarbonate and tartaric acid as gas-generating agents. Good floating abilities were obtained by using the gas-generating agents in both the inner matrix and the outer coating layer of the pellets. In vitro evaluation of floating capability was performed both by using the resultant weight apparatus and by counting floating pellets at the surface of beakers containing 0.1N HCl solution, in vivo evaluation of floating pellets capabilities was also performed. Riboflavin-containing floating pellets (FRF) were administered orally to nine healthy volunteers versus non-floating pellets (NFRF). Volunteers were divided in two groups, fasted group ($n=4$) 729 kcal and fed group ($n=5$) 1634 kcal as the total calorie intake on the testing day. An increase of urinary excretion of riboflavin was observed when the volunteers were dosed with the floating pellets, especially after feeding. As riboflavin has a narrow window of absorption in the upper part of small intestine, this phenomenon could be attributable to the gastric retention of floating pellets.

Keywords: Melt pelletization; Controlled-release; Floating pellets; Riboflavin; Urinary excretion

1. Introduction

Oral floating dosage forms are developed in order to be retained in the stomach and the upper part of small intestine, assuring a slow delivery of drug above the absorption site. Gastro-retentive devices may be useful for the delivery of many different kind of drugs, especially for optimum delivery of drugs that act locally in the stomach, e.g. misoprostol (Oth et al., 1992), and for stomach-specific antibiotic drug delivery used in the treatment of *Helicobacter pylori* (Burton et al., 1995; Patel and Amiji, 1996; Whitehead et al., 2000).

In this regard, the present work aims to assess the intragastric behaviour in humans of a new multiple unit system produced by the melt pelletization process (Hamdani et al., in press). Riboflavin (RF) was therefore chosen as the drug tracer because its absorption occurs mainly in the proximal small intestine. Moreover, it undergoes very little metabolism and its pharmacokinetics can be investigated by analysis of the urinary excretion following oral administration in humans (Sato et al., 2003, 2004). In this evaluation, riboflavin was used in its sodium salt form: sodium riboflavin 5′-phosphate (RF5′PNa). This Vitamin B_2 derivative is more water-soluble than riboflavin itself but is subject to the same absorption and transport mechanisms. Furthermore, it is excreted in the urine as RF and also has absorption sites mainly limited to the upper region of the small intestine. This allows indirect demonstration of an increase in the residence time of the dosage form above the absorption area: any increase in the gastric transit time of the dosage form increases the quantities of absorbed and eliminated riboflavin (Ingani et al., 1987b).

A previous investigation that included formulation, dissolution and buoyancy tests had shown good in vitro floating capabilities for comparable placebo, tetracycline and theophylline formulations (Hamdani et al., in press). But in order to evaluate the real floating capabilities of floating RF5′PNa pellets on the gastric content and their usefulness in achieving an extended gastric transit, these pellets were compared with non-floating RF5′PNa pellets (control). The in vivo behaviour of RF5′PNa

* Corresponding author. Tel.: +32 2 650 52 52; fax: +32 2 650 52 69.
E-mail address: kamighi@ulb.ac.be (K. Amighi).

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dosage forms was evaluated by means of RF urinary excretion measurements among fasted and fed healthy volunteers.

2. Materials and methods

2.1. Materials

Lactose 450 mesh (DMV International, Netherlands) was used as a diluent. Methocel® K100 (Colorcon, USA) was used as a gel-forming excipient. Sodium bicarbonate and tartaric acid (Federa, Belgium) were used as gas-generating agents. Sodium riboflavin 5′-phosphate (Certa, Belgium) was used as a model drug. Glyceryl palmito-stearate (Precirol® ATO 5) and glyceryl behenate (Compritol® 888) were supplied by Gattefosse (France) and used as lipophilic binders. The binders occur as fine white free-flowing powders.

2.2. Methods

2.2.1. Pellet manufacture

Floating (FRF) and non-floating (NFRF) pellets were prepared in a vertical small-laboratory-scale high-shear mixer (Mi-Pro®, Pro-C-epT, Belgium). The production conditions have been discussed in previous works (Hamdani et al., 2002, in press). Formulations and manufacturing conditions are shown in Table 1. The temperature of the heating jacket was kept constant throughout the whole process (45 °C). The product temperature, the torque, the impeller speed (IS) and chopper speed (CS) were monitored during pellet manufacture. In order to avoid excessive particle-size increase and/or agglomeration during the pelletization step, the product temperature was carefully controlled and ensured by flowing cooling air (2–3 m^3/h) through the bowl lid. Finally, a very short (2 min) "coating step" completed the process. For this step, the machine was stopped in order to add the coating mix (Precirol® and gas-generating agents) to the pellets, then the process was restarted with the heating jacket temperature, IS, CS and the cooling air flow kept identical to the experimental conditions used during the massing/pelletization step. The duration of the whole pellet manufacturing process was around 40 min.

Table 1
Formulation and manufacturing conditions of riboflavin floating and non-floating pellets

Formulation (%, w/w)		Manufacturing conditions
FRF		
Matrix (250 g)		*Granulation*
Sodium riboflavin 5′-phosphate	12	IS: 1800 rpm
Precirol®	15	CS: 130 rpm
Compritol®	53	Heating jacket: 45 °C
$NaHCO_3$	8	*Pelletization*
Tartaric acid	7	IS: 800 rpm
Methocel K100	5	CS: 4000 rpm
Coating (70 g)		MT: 18 min
Precirol®	71	Heating jacket: 45 °C
$NaHCO_3$	18	*Coating*
Tartaric acid	11	IS: 800 rpm
		CS: 4000 rpm
		CT: 5 min
		Heating jacket: 45 °C
NFRF		
Matrix (250 g)		*Granulation*
Sodium riboflavin 5′-phosphate	12	IS: 1800 rpm
Precirol®	15	CS: 130 rpm
Compritol®	15	Heating jacket: 45 °C
Lactose450 Me ad	100	*Pelletization*
		IS: 800 rpm
		CS: 4000 rpm
		MT: 25 min
		Heating jacket: 45 °C

2.2.2. In vitro evaluation of floating capabilities

Only pellets with size fractions in the range of 1250–2000 μm (sieve analysis) were considered for the in vitro floating evaluation studies.

2.2.2.1. Counting method. The method has been described by Ichikawa et al. (1991). Briefly, a precise number, between 100 and 150, of pellets was immersed in 70.0 ml of 0.1N HCl containing 0.05% (w/v) Polysorbate 20 in a 100 ml beaker maintained at 37 °C. Then, the beaker was kept shaking horizontally at a speed of 100 cycles/min for 23 h. The liquid surface of the beaker was then checked for floating pellets submerged beneath other pellets. When such undesirable phenomena were observed, the beaker was gently shaken in order to gain monolayer of floating pellets on the liquid surface. The number of floating pellets was then estimated by photographing the liquid surface in the beaker and counting the number of floating pellets on the picture. Experiments were performed in triplicate and the percentage of floating pellets was calculated by Eq. (1):

$$\text{Floating pellets (\%)} = \left(\frac{\text{number of floating pellets at the measured time}}{\text{initial number of the pellets}}\right) \times 100. \quad (1)$$

2.2.2.2. Resultant weight method. The resultant weight (RW) of the pellets was measured at known time intervals using the apparatus and method of Timmermans and Moës (1990a,b). The medium was 0.1N HCl containing 0.05% (w/v) of Polysorbate 20. Experiments were performed in triplicate at 37 °C. The pellets were placed in a basket which acted as a sample holder and which was attached to the resultant-weight apparatus (Fig. 1). As described by Timmermans and Moës (1990a,b), the resultant-weight apparatus enables in vitro monitoring of the total force F acting vertically on an immersed object. This force F determines the RW of the object in immersed conditions and can be used to quantify its floating or non-floating capabilities. As described by Eq. (2), the magnitude and the direction of force F, and hence of the RW, correspond to the vectorial sum of the buoyancy (F_{buoy}) and gravity (F_{grav}) forces acting on the object.

$$F = F_{buoy} - F_{grav} = d_f g V - d_s g V = (d_f - d_s) g V = (d_f - M/V) g V \quad (2)$$

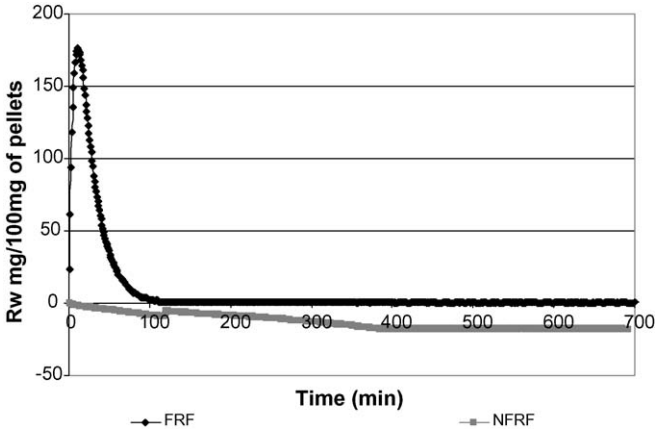

Fig. 1. Resultant weight values for floating (FRF) and non-floating (NFRF) riboflavin pellets.

F is the total vertical force (resultant weight of the object), g the acceleration of gravity, d_f the fluid density, M the object mass and V is the object volume.

It was assumed that pellets have good floating capabilities if the resultant weight values are still greater than zero after 800 min and if the percentage of floating pellets is still up to 60% or more after 8 h.

2.2.3. In vitro dissolution test

The drug release determinations were carried out at 37 °C using USP 24 no. 2 dissolution testing apparatus (paddle), at a rotational speed of 60 rpm. The media used was 900 ml phosphate buffer (0.05 M) at pH values of 1.5, 3.0 and 6.5, containing 0.05% (w/v) of Polysorbate 20. The drug release from pellets was determined at 445 nm using an Agilent 8453 UV–vis Dissolution Testing System (Agilent, USA). The percentages of the drug released were measured at fixed time intervals and averaged ($n = 5$). The samples were protected from light to avoid RF alteration. Only pellets with size fractions in the range of 1250–2000 μm were considered for the dissolution studies.

As the riboflavin pellet formulations (FRF and NFRF) were designed to be administered in vivo to healthy volunteers, it was important to ensure the similarity of the in vitro dissolution profiles of those formulations before the in vivo study. The comparison of the profiles was based on the measure of similarity factor f_2 (Shah et al., 1998). The compared dissolution profiles were obtained under the same test conditions and the dissolution time points for both profiles were the same. As indicated by Shah et al. (1998) for controlled release products, 1, 3, 5 and 8 h were fixed as dissolution time points for comparison of the profiles.

2.2.4. Measurement of riboflavin concentration in urine

The level of riboflavin in urine was measured by using a spectrofluorimetric method recommended by Burch et al. (1948) and adapted by Ingani (1987a). The fluorescence of the solutions was measured at emission and excitation wavelengths of, respectively, 552 and 445 nm, using a Perkin-Elmer LS 50 B (Bucks, Germany) fluorescence spectrophotometer. Briefly, 4 ml of urine were introduced in a centrifugation tube and 0.4 ml of pH 3.5 acetic buffer (3.25 M) was added. The oxidation was started by the addition of 1.0 ml of a $KMnO_4$ solution (4%, w/v). After 1 min, the excess of $KMnO_4$ was reduced by 0.1 ml of a H_2O_2 solution (30%). The residue was then extracted by addition of 4 g of ammonium sulphate and 3.0 ml of benzylic alcohol saturated with water. The tubes were shacked for 3 min and centrifuged at 3000 tours/min for 5 min. One milliliter of the supernatant was taken and diluted in 7.0 ml of a solution containing 45% (v/v) of ethanol in a solution of acetic acid and sodium acetate at 0.1N each. The sample concentration was calculated considering the slope and the intercept of a riboflavin calibration curve (0.5, 1.0, 2.0, 3.0, 5.0 and 10.0 μg/ml).

2.2.5. In vivo study design

Nine healthy male subjects (age range 23–45 years; weight range 70–96 kg) participated after giving informed consent. The study, approved by the local Ethical Committee (Erasme Hospital, Brussels, Belgium), involved a double blind randomized design in which the study of the floating pellets and the comparison with the control were carried out on the same subjects. The study was conducted by administrating to each subject both floating and control systems on two separate sessions. The FRF pellets and the NFRF pellets (amount of RF5′PNa 20 mg) were filled in opaque hard gelatine capsules. The volunteers were separated into two groups: a fasted group and a fed group (Table 2). In order to avoid any variation of the gastric residence time of the dosage forms depending on the subject postures, the volunteers were not authorized to be lying down during the session day. There was at least 6 days washout between each administration.

After an overnight fast of 12 h, each of the healthy volunteers followed the standard meals protocol described in Table 2. The urine samples were obtained pre-dose and at scheduled times following administration of 1, 2, 3, 4, 6, 8, 14 and 23 h. Subsequently, cumulative amount of excreted riboflavin in urine was measured. The time 0 was considered as the time at which the subjects dosed. The pre-dose sample was necessary in order to evaluate the amount of Vitamin B2 in each subject that was provided from his normal diet.

3. Results and discussion

3.1. In vitro floating evaluation and drug releasing properties

Before experiments on healthy volunteers, it seems to be evident that the real floatability of riboflavin pellets should be assessed in vitro. In this regard, the FRF pellets were compared to NFRF for in vitro floating capabilities by the resultant weight method and the counting procedure. As shown in Fig. 1, FRF pellets presented positive resultant weight values thanks to their floating capabilities which result from the presence of a mixture of sodium bicarbonate and tartaric acid in both the inner and the outer surface of pellets (Table 1). Furthermore, as described in a previous work (Hamdani et al., in press), the formulation should contain Methocel K100 in the inner matrix pellets as a swellable excipient that entraps CO_2 bubbles. On the other hand, Fig. 1 shows negative resultant weight values for non-floating

Table 2
Feeding regimens used throughout the study

Timing	Fasted condition ($n=4$)	Fed condition ($n=5$)	Excretion of urine
7.45 a.m.			Pre-dose sample
8.00 a.m., breakfast	–	200 ml orange juice, 3 slices toast, 10 g margarine, 20 g jam, 50 g light cheese, 150 ml tea + 6 g sugar	
8.30 a.m.	Administration of a capsule with 200 ml water	Administration of a capsule with 200 ml water	t_0 (capsule administration)
9.30 a.m.			$t_{1\,h}$
10.20 a.m.	200 ml water	85 g fruit pie, 150 ml tea ± 6 g sugar	
10.30 a.m.			$t_{2\,h}$
11.30 a.m.	200 ml water	200 ml water	$t_{3\,h}$
12.30 p.m.			$t_{4\,h}$
12.45 p.m., lunch	200 ml vegetables soap, 150 g chicken, 50 g tomatoes sauce, 100 g potatoes, 175 ml water, 150 ml tea + 6 g sugar	200 ml vegetables soap, 150 g chicken, 50 g tomatoes sauce, 100 g potatoes, 175 ml water, 150 ml tea + 6 g sugar	
2.30 p.m.			$t_{6\,h}$
3.15 p.m., snack	75 g apple pie, 150 ml tea + 6 g sugar	75 g apple pie, 150 ml tea + 6 g sugar	
4.30 p.m.			$t_{8\,h}$
Total energy (kcal)	729	1634	
Night			
10.30 p.m.	Free	$t_{14\,h}$	
7.30 a.m.		$t_{23\,h}$	

pellets (NFRF) synonymous with sinking pellets. Those results were corroborated by the counting results (Fig. 2): the percentage of FRF pellets that were still floating after 8 h comprised between 70 and 80%, whereas the percentage of control pellets (NFRF pellets) that were floating after 30 min fell to 0%, from the results of a counting test (results not shown). The reason for that a percentage of the FRF pellets was sinking after 8 h can be explained by the relatively low floating forces developed by our formulations and by the progressive lost of CO_2 from the pellets.

On the other hand, as the in vivo evaluation of floating capability is based on the determination of the amount of riboflavin in urine, the in vitro dissolution behaviour of FRF and NFRF pellets must be similar. Dissolutions tests were evaluated in phosphate buffers (0.05 M) mediums at pH values of 1.5, 3.0 and 6.5, as shown in Fig. 3. After an initial burst effect, a sustained release of RF5′PNa occurs. As the process of drug release from lipidic Gelucire matrices with a very low HLB values (close to 1, such as Compritol® and Precirol®) attempts to be controlled by diffusion (Aïnaoui et al., 1997; Aïnaoui and Vergnaud, 1998), drug release from such pellets depends on the drug solubility. As an example, the water solubility of RF5′PNa at pH 3.8 is 43 mg/ml (Merck, 1996). This high water solubility of the drug was also greatly responsible for the large burst effect observed on the dissolution profiles. Moreover, slightly higher burst effects and faster RF releases were obtained from both the floating and the non-floating pellets in the phosphate buffer medium simulating the upper intestine (pH 6.5) in comparison to those simulating the stomach (pH 1.5 and 3.5). On the other hand, the presence of the effervescent mix in the floating pellets (FRF) formulations made them more hydrophilic than non-floating pellets (NFRF). Consequently, the dissolution profile of FRF pellets was higher than NFRF pellets. However, the similarity factor (f_2) (Shah et al., 1998) for the dissolution curves (FRF and NFRF pellets) obtained at pH 1.5, was superior to 50 and was, respectively, of 46 and 38 for dissolution tests conducted at pH 3.0 and 6.5.

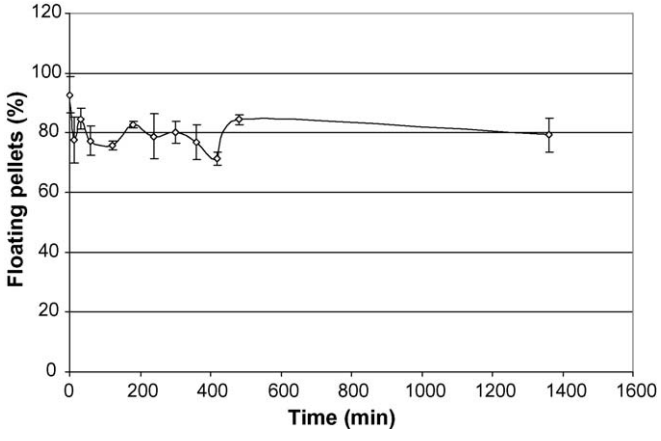

Fig. 2. Percentages of floating riboflavin pellets at the surface of beakers.

The similarity between the dissolution curves of floating and non-floating pellets could be considered as acceptable and in vivo evaluation could be performed.

3.2. In vivo floating evaluation

The aim of this study was to compare floating capabilities of FRF pellets with NFRF ones. The hypothesis is that, as long as floating pellets remain in the stomach or in the upper part of the small intestine, the riboflavin absorption can be promoted. Con-

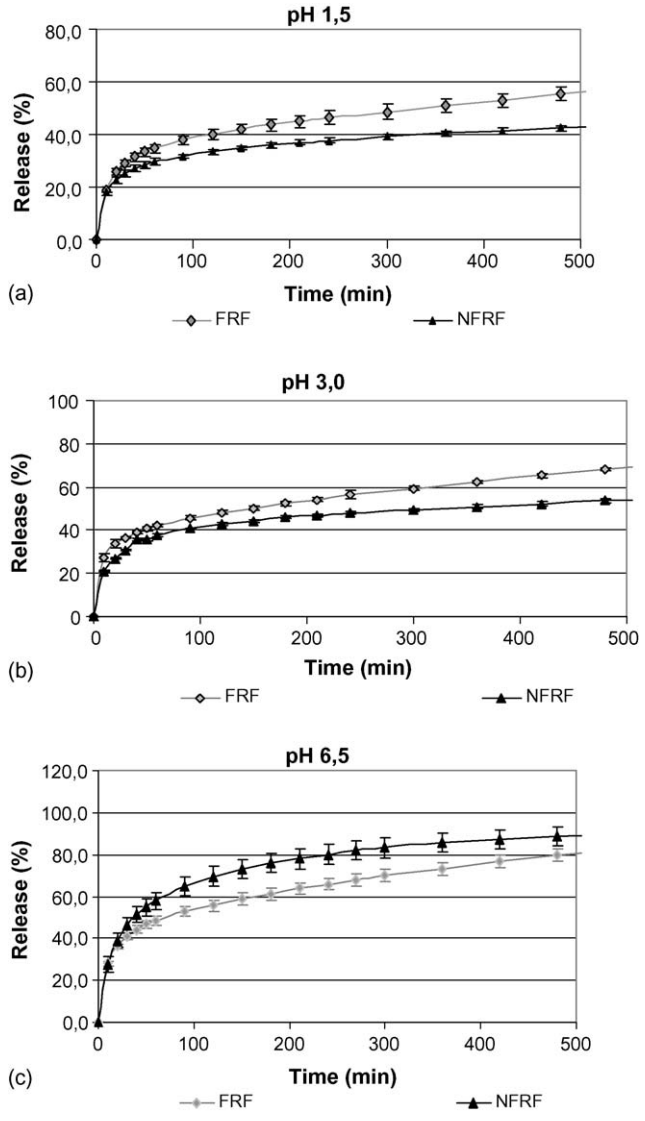

Fig. 3. Drug release of FRF pellets and NFRF pellets in a phosphate buffer 0.05 M at: (a) pH 1.5, (b) pH 3.0 and (c) pH 6.5 (mean ± S.D., $n = 5$).

sequently, the amount of excreted riboflavin should be higher after the administration of FRF pellets than after the administration of NFRF pellets by the volunteers, especially after feeding. Results shown in Table 3 describe the cumulative urinary excretion (mg) after 23 h for each subject, the mean of cumulative RF excretion for each group (feeding and fasting group) is also mentioned. When compared to NFRF results, this cumulative urinary excretion was systematically higher after the administration of FRF pellets, whatever the ingestion protocol might be, fasted or fed. In fact, the statistical comparison (Student's *t*-test) of the cumulative excretion of riboflavin by fasted volunteers did not show a significant difference between floating pellets and non-floating pellets ($p = 0.056$). However, inside the fed group, a significant difference ($p < 0.05$) existed between the cumulative amount of RF excreted after dosing the volunteer with FRF and with NFRF forms. The FRF pellets superiority, in terms of floating abilities, can be illustrated by Fig. 4. Those figures indicate that the urinary excretion of riboflavin in the

Table 3
Cumulative urinary excretion from t_0 to $t_{23\,h}$ for each subject

Subject	FRF cumulative riboflavin excretion (mg)	NFRF cumulative riboflavin excretion (mg)
Fasted condition		
1	24	16
2	17	14
3	24	13
4	14	11
Mean ± S.D.	20 ± 5	14 ± 2
Fed condition		
1	21	13
2	30	14
3	22	19
4	24	21
5	30	16
Mean ± S.D.	26 ± 4	17 ± 3

period under fed and fasted conditions following administration of FRF pellets was generally higher compared with NFRF pellets. Nevertheless, this tendency was even more pronounced in the fed subject group compared with the fasted one. Hence, as shown by Fig. 4, the amounts of riboflavin in the urine were significantly higher ($p < 0.01$) for volunteers dosed by FRF pellets than subjects dosed by NFRF pellets, especially after ingestion of meal (the period between 3 and 6 h post-dosing). This phenomenon could be attributable to the buoyancy properties of FRF pellets in the stomach. This buoyancy, in accordance with literature (Davis et al., 1984, 1987; Moës, 1993) seemed to be enhanced after feeding. Consequently, as shown by Table 3, the cumulative urinary excretion of riboflavin was higher for FRF

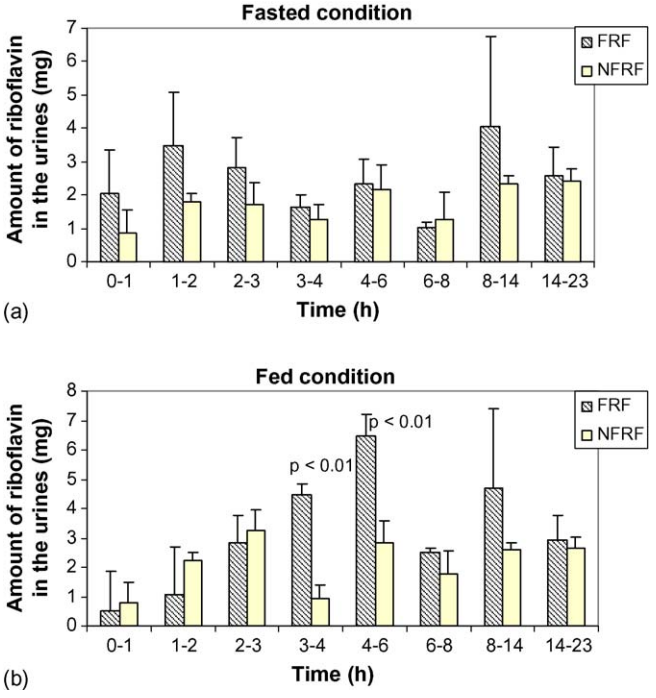

Fig. 4. Amount of riboflavin in urine under: (a) fasted conditions (mean ± S.D., $n = 4$) and (b) fed conditions (mean ± S.D., $n = 5$).

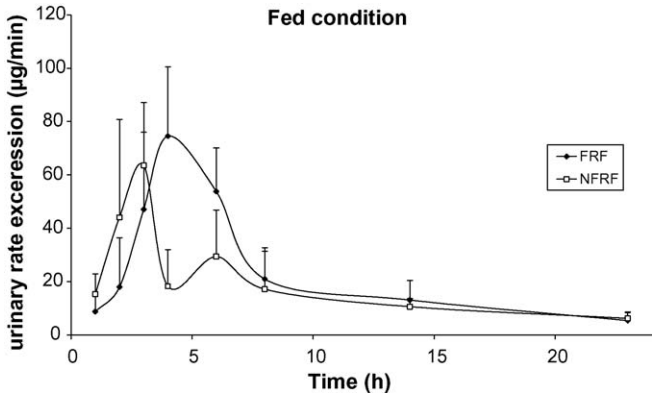

Fig. 5. Comparison of urinary excretion rate values of floating pellets and non-floating ones in the fed group (mean ± S.D., $n = 5$).

pellets (26 ± 4 mg) than for NFRF pellets (17 ± 3 mg). On the other hand, a 1 h delay was observed between the peaks of the urinary excretion rate values of floating pellets in comparison with that for non-floating ones in the fed group (Fig. 5). This phenomenon was not observed in the fasted group (result not shown). This suggests that FRF pellets might be able to float on the stomach sufficiently in the fed condition and that this phenomenon could lengthen the gastric residence time and delay drug arrival at the absorption site. In addition, FRF enabled an increase in the drug absorption rate as the floating pellets in the stomach gradually sank and arrived at the absorption site. Consequently, multiple-unit floating systems seem to be possibly beneficial in enhancing riboflavin absorption. Those results are in accordance with literature. Sato et al. (2003, 2004) had administered orally riboflavin-containing floatable microballoons to healthy volunteers under fed and fasted conditions and subsequently investigated the urinary exertion of riboflavin. They found that the excretion half-time of microballoons was prolonged significantly by feeding. Furthermore, microballoons afforded significantly higher urinary excretion of riboflavin relative to that observed for non-floating dosage forms in both the fasted and the fed conditions.

4. Conclusions

Floating riboflavin pellets (FRF) and non-floating riboflavin ones (NFRF) were administered orally to human healthy volunteers under fed and fasted conditions. Subsequently, an analysis of urinary excretion of riboflavin was performed in order to evaluate, indirectly, the floating capability of pellets. As riboflavin has a narrow absorption window in the upper part of the small intestine, the floating properties of pellets may be responsible of enhanced absorption and elimination of riboflavin. Under fed and fasted conditions the urinary excretion of riboflavin in the period following administration of FRF pellets was generally higher than following administration of NFRF pellets. It may therefore be concluded that FRF pellets float in vivo. However, dosing the subjects after a meal seems to enhance the gastroretentive time of the pellets over the food gastric emptying.

Hence, after demonstrating in a previous work (Hamdani et al., in press), the in vitro floatability of oral multiple unit dosage forms based on a very simple formulation, the present paper assessed the actual in vivo floatability of riboflavin pellets developed by using the very short and ecological process of melt pelletization which was broadly described previously (Hamdani et al., 2002, in press).

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