

## Preparation and evaluation of floating risedronate sodium Gelucire<sup>®</sup> 39/01 matrices

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Incorporation of bisphosphonates in the lipid reduces gastric irritation. Only gastric retention with sustained release allows the drug to reach the duodenum and jejunum and improves the availability of bisphosphonates. Risedronate sodium and Gelucire<sup>®</sup> 39/01 floating matrices were prepared using melt solidification. The sustained release floating matrices were evaluated for *in vitro* and *in vivo* floating ability and *in vitro* drug release. Ageing of the matrices was studied by differential scanning calorimetry, hot stage polarizing microscopy, scanning electron microscopy and *in vitro* drug release. Ageing causes changes in the crystal structure of Gelucire<sup>®</sup>, which is responsible for an increase in drug release.

*Keywords:* risedronate sodium, Gelucire<sup>®</sup> 39/01, hot stage polarizing microscopy, ageing, scanning electron microscopy

Osteoporosis and Paget's disease of bone are major problems in women and geriatric patients where antiresorptive agents are normally recommended. Despite their benefits, bisphosphonates suffer from very poor oral bioavailability (1–2%) (1–3). The low bioavailability is likely due to the extensive ionization and highly hydrophilic nature of bisphosphonates, which prevent the transcellular transport across intestinal epithelium and favor the paracellular route (4–5). Higher localized concentration of bisphosphonates has resulted in severe gastro-intestinal side effects such as dysphagia, esophagitis and gastric ulceration (6).

To improve the bioavailability of bisphosphonates, structural modification of the bisphosphonate molecule, use of absorption enhancers and design of drug delivery systems have been attempted (7–11). Surfactants such as sodium lauryl sulphate and medium chain fatty acid esters were found to increase bisphosphonate absorption (13, 14). Comparative evaluation of different enhancers was carried out by Raiman *et al.* (11). It was observed that enhancers acting on tight junctions are most effective compared to other categories. Bisphosphonates are best absorbed from the upper GI tract (duodenum and jejunum) (4, 6). Gastroretentive hydrogel with sustained release was designed by

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Flashner-Barak *et al.* (12) for delivery of bisphosphonates to enhance bioavailability. The system was composed of a gel forming agent with superdisintegrant and tannic acid. Lipids are considered as an alternative to polymer in the design of sustained drug delivery systems due to their advantages such as the low melt viscosity (thus avoiding the need of organic solvents for solubilization), absence of toxic impurities such as residual monomer catalysts and initiators, potential biocompatibility and biodegradability and prevention of gastric irritation by forming a coat around the gastric irritant drug (13–14). Recently, Kumar *et al.* (15) demonstrated the use of amphiphilic lipid glyceryl mono oleate (GMO) for the design of the floating matrix system. Lipids have a special advantage as carriers for drugs having irritant properties (16–17).

Gelucire® is a family of vehicles derived from the mixtures of mono-, di- and triglycerides with polyethylene glycol (PEG) esters of fatty acids. These are available with a range of properties depending on their hydrophilic-lipophilic balance (HLB) and melting point range (33–65 °C) (18–19). They have a wide variety of applications in pharmaceutical formulations. These are used in the preparation of fast release and sustained release formulations. Gelucire® containing only PEG ester (Gelucire® 55/18) is generally used in the preparation of fast release formulations, which contain only glycerides or mixture of glycerides. PEG esters (Gelucire® 54/02, 50/13, 43/01, 39/01) are used in the preparation of sustained release formulations (20–21).

Major objectives of the present study are to prepare and evaluate sustained release floating bisphosphonate-Gelucire® 39/01 matrices with respect to risedronate sodium, [1-hydroxy-2-(3-yrindinyl) ethylidene] bis-phosphonic acid] monosodium salt, as a model bisphosphonate. The prepared matrices were evaluated for *in vitro* and *in vivo* floating ability and *in vitro* drug release. *In vivo* floating ability of the formulation was studied in healthy human volunteers by  $\gamma$ -scintigraphy. Effect of ageing on Gelucire® 39/01 was evaluated by hot stage microscopy (HSM), scanning electron microscopy (SEM) and differential scanning calorimetry (DSC).

## EXPERIMENTAL

### Materials

Risedronate sodium was obtained as a gift sample from Fleming Laboratories Ltd. (India). Gelucire® 39/01 (semi-synthetic glycerides, waxy solid, m.p. = 39 °C, HLB = 01), Compritol® 888 ATO (atomized glyceryl behenate, white powder, m.p. = 70 °C, HLB = 02), Precirol® ATO 5 (atomized glyceryl dipalmitostearate, white powder, m.p. = 54 °C, HLB = 02) were generous gifts from Gattefosse s.a. (France). Caprol PGE 860 (nonionic, nonalkoxylated oleic acid esters of decaglycerol) was supplied by Abitec Corporation (USA). Licap capsules (hard gelatin capsule specially designed for lipid formulations, size 0) were obtained as a gift sample from Capsugel (India).

### Preparation of matrices

The matrix systems were prepared by melting Gelucire® and Caprol PGE 860 at 10 °C above the melting point of Gelucire®. Risedronate sodium (30 mg) was added to the molten mass under stirring. The amount of Caprol PGE 860 varied from 40 mg to 80 mg

per formulation. The homogeneous molten mixture was filled into Licap capsules using a preheated glass Pasteur pipette and allowed to solidify at 4 °C. The capsules were equilibrated to room temperature for 6 h before evaluation. The compositions of risedronate sodium-Gelucire® 39/01 single-unit matrices with Caprol PGE 860 are shown in Table I.

Table I. Composition of risedronate sodium Gelucire® matrices

Formulation	Risedronate sodium	Gelucire® 39/01	Caprol PGE-860
F00	–	400 mg	40 mg
F01	30 mg	400 mg	40 mg
F02	30 mg	400 mg	50 mg
F03	30 mg	400 mg	60 mg
F04	30 mg	400 mg	70 mg
F05	30 mg	400 mg	80 mg

### *In vitro floating ability*

The floating ability of the matrices was determined by using the USP 24 (22) type II (Electrolab TDT-06P, India) dissolution method at 50 and 100 rpm maintained at  $37 \pm 0.3$  °C in 900 mL of 0.1 mol L<sup>-1</sup> HCl. The matrices were placed in the medium and the floating times were measured by visual observation (23).

### *γ-Scintigraphy*

*In vivo* floating ability was studied by  $\gamma$ -scintigraphy in healthy male human volunteers, 25–30 years of age and 55–65 kg body mass. They were non-alcoholic, non-smokers and were not taking any other medication. <sup>99m</sup>Tc  $3.7 \times 10^6$  Bq was uniformly mixed with the molten Gelucire® containing the drug and Caprol PGE 860 and the mass was poured into Licap capsules and frozen at 4 °C. Each volunteer ingested the capsule (Batch F02) orally along with water after taking a light breakfast in the morning. The capsules were visualized using a gamma camera (GE Millennium MPR Gamma Camera, Israel). Images were taken with volunteers in supine position immediately after administration of the formulation (0 h) and at intervals of 1, 2, 3, 4, 5 and 6 h. The human ethical committee approved the protocol of the study.

### *Effect of ageing*

The formulations were stored at room temperature for 30 days and the effect of ageing on Gelucire® 39/01 was studied using HSM, SEM, DSC and *in vitro* drug release.

### *Hot stage microscopy (HSM)*

Hot stage microscopy was conducted using a Mettler Toledo FP82HT hot stage (Switzerland) assembled on a Leica DMLP polarizing microscope equipped with a Leica

MPS-30 camera (Germany). The different types of samples were observed under the microscope using a scanning speed of 2 °C min<sup>-1</sup>. Changes in the sample morphology (melting – crystallization) were noted as a function of temperature. Three types of samples were prepared for the physical evaluation of matrices:

- (i) freshly solidified samples: placebo Gelucire® matrices were prepared and stored for 12 h at room temperature in order to avoid effects due to the thermal history;
- (ii) aged samples: samples were stored up to 30 days at room temperature (25 °C) in order to detect any physical ageing effect associated with glyceride bases;
- (iii) untreated samples: samples without any special treatment (as received from supplier).

### *Scanning electron microscopy (SEM)*

The surface of fresh and aged matrices was coated with a thin gold-palladium layer using a sputter coater unit (VG-Microtech, UK) and the surface topography was performed with a Cambridge Stereoscan S120 scanning electron microscope (Cambridge instrument, UK).

### *Differential scanning calorimetry (DSC)*

Thermograms of fresh and aged matrices were obtained using a Mettler-Toledo DSC 821<sup>e</sup> instrument (Mettler-Toledo) equipped with an intracooler. Zinc/indium standards were used to calibrate the DSC temperature and enthalpy scale. The powdered sample of beads was hermetically sealed in an aluminum pan and heated at a constant rate of 5 °C min<sup>-1</sup>, over a temperature range of 25–55 °C. Inert atmosphere was maintained by purging nitrogen at a flow rate of 50 mL min<sup>-1</sup>.

### *In vitro release*

The release studies of fresh and aged formulations (30 days) were performed using USP 24 (22) type II dissolution test apparatus. Matrices were placed in the dissolution vessel containing 0.1 mol L<sup>-1</sup> HCl maintained at 37 ± 0.3 °C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through Whatman filter paper 41, the concentration of risedronate sodium was determined spectrophotometrically at 261.2 nm. Release studies were performed in triplicate. Analysis of data was done the using 'PCP Disso v 3' software, India.

## RESULTS AND DISCUSSION

Bisphosphonates are highly water-soluble hard drugs (not easily metabolized *in vivo*), which are absorbed by paracellular routes and therefore show poor absorption. Due to higher permeability of the paracellular route in the upper GI tract, it is advisable to formulate bisphosphonates in a gastroretentive form. Mucosal irritation of the drug

due to high-localized concentration worsened further the formulation development task. Floating matrices with lipid coating were formulated to overcome these problems. The lipid coat of hydrophobic triglyceride Gelucire® 39/01 will retard drug release as well reduce GI irritation. Gelucire® 39/01 comprises a mixture of hemi-synthetic glycerides of different fatty acids melting at 39 °C. Extreme hydrophobicity of Gelucire® 39/01 is attributed to the absence of PGE esters, which in turn provides release-retarding ability. Lipids having a low hydrophilic lipophilic balance (HLB), for example Precirol® ATO 5 (HLB = 02), Compritol ATO 888 (HLB = 02) and Gelucire® 39/01 (HLB = 01), have been tried for the preparation of floating matrices. Among these lipids Gelucire® 39/01 has shown a good floating property of retarding the drug release. The hydrophobic nature of the excipient is thus responsible for the floating behavior but all excipients with low HLB do not ensure floating. For example, similar matrices prepared using Compritol and Precirol separately did not exhibit the floating property. Therefore, apart from hydrophobicity, density also plays an important role in designing floating matrices using a lipophilic excipient.

The floating ability of the matrices was studied using the USP 24 type II (22) method at 50 and 100 rpm. The study showed that the amount of Gelucire® effects the floating property and that there is no effect of stirring speed on the floating property of the matrices. The amount of Gelucire® used is 400 mg for 30 mg drug, below which the matrix did not float (data not shown). All the batches from Table I floated all the time during the *in vitro* release study. There was no floating lag time for any of the batches.

The  $\gamma$ -scintigraphy study of the matrices in human volunteers showed that the formulation remained in the stomach for about 6 h. Drug release profiles of risedronate sodium from Gelucire® matrices in 0.1 mol L<sup>-1</sup> HCl are shown in Fig. 1. Addition of Caprol PGE-860 (nonionic, nonalkoxylated polyglycerol type surfactant used as emulsifier) in

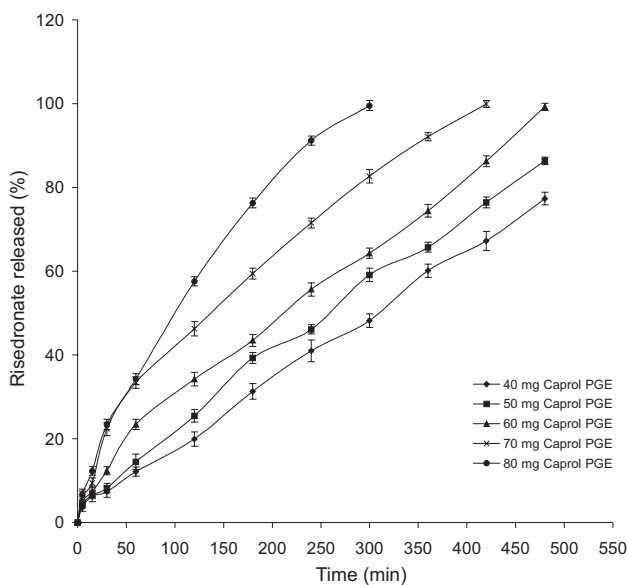


Fig. 1. Release profiles of risedronate sodium from the matrices, showing the effect of increasing the Caprol PGE 860 amount. Each point refers to mean  $\pm$  SD ( $n = 3$ ).

the matrices improved the drug release. As the amount of Caprol PGE-860 increased, the release was found to increase. The surfactant has been previously reported to possess the property to loosen the tight junction and to enhance paracellular penetration (24). Thus, the use of Caprol PGE-860 in the formulations may also enhance the drug availability to the body by enhancing paracellular penetration.

Solid Gelucire® exists in three crystalline forms (melted gelucire on cooling forms unstable form II which is converted into metastable form I after 3–6 h at RT, which is finally converted into stable form I' after several days at RT). The effects of ageing on the placebo Gelucire® 39/01 matrices (Batch F00) were studied using HSM, DSC, SEM. The SEM microphotographs of fresh (form I) and aged matrices (form I') are shown in Fig. 2. After ageing, the sample showed significant changes on the surface, which may be due to changes in the crystal structure of Gelucire®. DSC thermograms of fresh (6 h after preparation) placebo Gelucire® matrices and after 30 days are shown in Fig. 3. Thermograms of fresh (form I) and aged (form I') samples have shown significant differences. The DSC thermograms of freshly prepared matrices showed two melting endotherms at 37 and 38 °C while in the case of aged matrices a single endotherm at 40 °C was observed. This shift in melting temperature may be correlated to changes in the crystal structure of Gelucire®. These results are in correlation with the observations of hot stage microscopy. HSM of untreated, fresh and aged placebo matrices are shown in Fig. 4. The HSM microphotograph shows the presence of some unmelted portion even at 47 °C in the aged sample. The energy required for melting increases with ageing, which may be attributed to changes in the crystal structure. This may be attributed to crystallization of glycerides during ageing. This slow crystallization of glycerides is responsible for changes in the crystal structure, showing surface roughness.

*In vitro* release from aged formulations (30 days) was observed to detect any ageing effect on the drug release retarding property of Gelucire®.

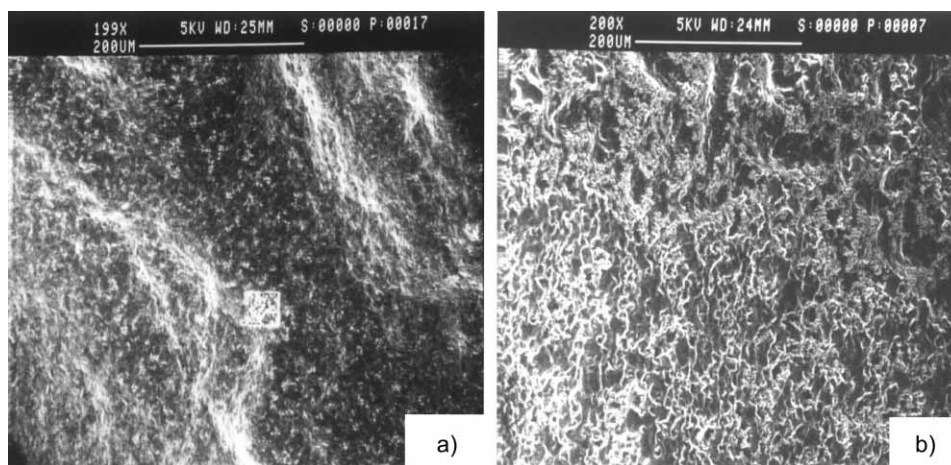


Fig. 2. Scanning electron microscopy images of: a) surface of fresh matrices (199 X), b) surface of aged matrices (200 X).

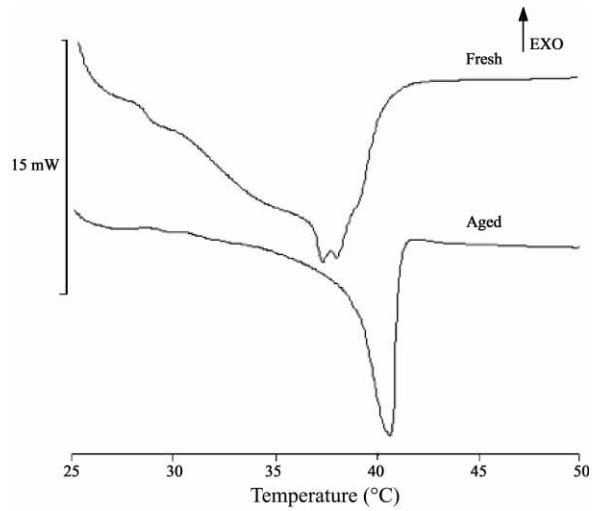


Fig. 3. DSC thermograms of placebo Gelucire® matrices at room temperature: a) fresh (day 0), b) aged (30 days).

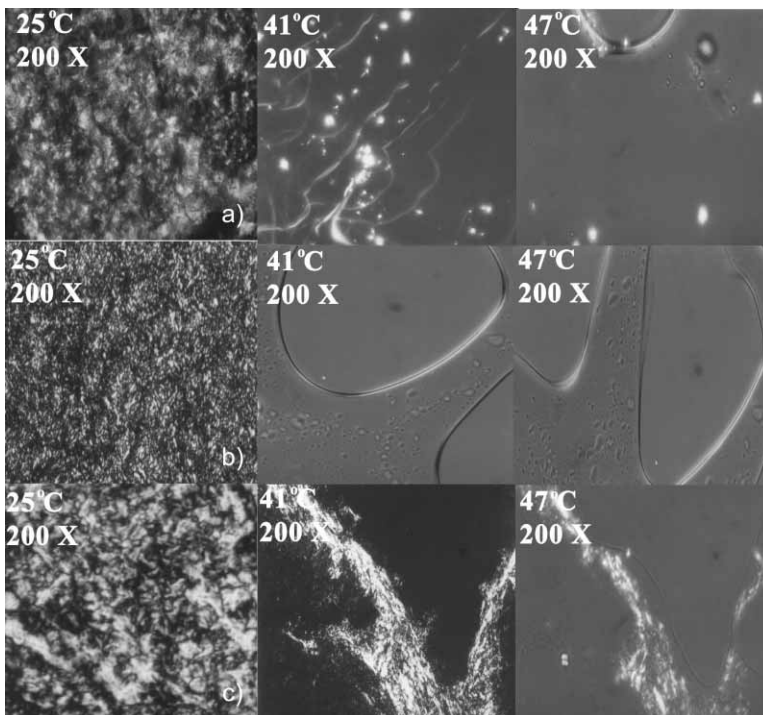


Fig. 4. HSM microphotographs of Gelucire® 39/01: a) untreated samples, b) fresh placebo matrices (1 day), c) aged placebo matrices (30 days).

In the present study, it was also observed that the release-retarding activity of Gelucire® is reduced during ageing. It may be attributed to the conversion of form I to form I'. The SEM microphotographs of form I' showed more surface roughness compared to form I (Fig. 2). Surface roughness may lead to faster drug release, which does not have any impact on the floating ability. The aged formulation showed a similar floating ability as freshly prepared formulations (data not shown).

## CONCLUSIONS

Owing to its extreme hydrophobicity and low density, Gelucire® 39/01 may be considered an appropriate carrier for designing sustained release floating drug delivery systems of risedronate sodium. SEM, HSM, DSC showed that ageing of Gelucire® 39/01 is responsible for an increase in drug release. Further attempts are required to stabilize Gelucire® 39/01 during ageing.

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## S A Ž E T A K

### Priprava i evaluacija plutajućih matriksa rizedronat natrija s Gelucire® 39/01

BHASKAR CHAUHAN, SHIAM SHIMPLI, KAKASAHEB R. MAHADIK i ANANT PARADKAR

Uklapanje bisfosfonata u lipide smanjuje iritaciju želuca. Samo zadržavanje u želucu s uspošenim oslobađanjem omogućava da ljekovita tvar dospije u duodenum i jejunum i povećava bioraspoloživost bisfosfonata. Rizedronat natrij i Gelucire® 39/01 plutajući

matriksi pripravljeni su metodom taljenja i očvršćivanja. Proučavana je sposobnost plutanja pripremljenih matriksa *in vitro* i *in vivo*, te oslobađanje ljekovite tvari. Starenje matriksa proučavano je diferencijalnom pretražnom kalorimetrijom, polarizirajućom mikroskopijom s vrućom pločom i pretražnom elektronskom mikroskopijom. Starenje uzrokuje promjene u kristalnoj strukturi Gelucire® zbog kojih se povećava oslobađanje ljekovite tvari.

*Ključne riječi:* rizedronat natrij, Gelucire® 39/01, polarizirajuća mikroskopija s vrućom pločom, starenje, pretražna elektronska mikroskopija

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