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Preparation and Evaluation of Floating Risedronate Sodium–Gelucire® 43/01 Formulations

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ABSTRACT Single and multi-unit floating matrices of risedronate sodium were prepared using Gelucire® 43/01 by melt solidification and melt granulation technique, respectively. The controlled release floating matrices were evaluated for in vitro and in vivo floating ability and in vitro drug release. Effect of aging on Gelucire® 43/01 was evaluated by hot stage microscopy (HSM), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), in vitro floating ability, and in vitro drug release. Multi-unit system obtained has shown initial burst release, which was suppressed in single unit system. Both single- as well as multi-unit systems showed increase in rate of drug release on aging due to changes in the properties of the Gelucire® 43/01. Multi-unit matrices obtained by melt granulation were relatively easier for scale up and advantageous if the initial burst release does not cause any significant clinical adversity.

KEYWORDS Risedronate sodium, Single-unit, Multi-unit, Drug release, Gelucire, Hot stage microscopy, Floating

INTRODUCTION

Antiresorptive agents are normally recommended for osteoporosis and Paget's disease of bone in women and geriatric patients (Delmas & Meunier, 1997; Fleisch, 1997; Johansen et al., 1996). Bisphosphonates constitute a major class of oral antiresorptive drugs that are considered to be stable analogs of pyrophosphate (P–O–P). The basic P–C–P structure of bisphosphonates allows many possible variations by changing the two lateral chains on the carbon atom. Bisphosphonates have shown very low bioavailability (1–2%). The low bioavailability is likely to be due to the high hydrophilicity and extensive ionization of bisphosphonates, which prevents transcellular transport across intestinal epithelium and favors the paracellular route (Gert et al., 1995; Lin, 1996; Twiss et al., 1994). Higher localized concentration of bisphosphonates has resulted in severe gastro-intestinal side effects such as

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dysphagia, esophagitis, and gastric ulceration (DeGroen et al., 1999; Lufkin et al., 1994).

To improve the bioavailability of bisphosphonates, structural modification of bisphosphonate molecule, use of absorption enhancers, and design of drug delivery systems have been attempted previously. The lipophilic prodrugs of clodronic and etidronic acid were systhesized by Niemi et al. (1999, 2000). Complexation with calcium ions was considered responsible for the low bioavailability of bisphosphonates, therefore ethylene diamine tetra acetic acid (EDTA) was used for the enhanced bioavailability of bisphosphonates (Green et al., 1997). Surfactants such as sodium lauryl sulphate and medium chain fatty acid esters were found to increase bisphosphonate absorption (Boulenc et al., 1995; Lindmark et al., 1998). Comparative evaluation of different enhancers was carried out by Raiman et al. (2003). It was observed that enhancers acting on the tight junctions were most effective over other categories. Gastroretentive hydrogel with sustained release was designed by Flashner-Barak et al., for the delivery of bisphosphonates to enhance bioavailability (Flashner-Barak et al., 2002). The system composed of a gel forming agent with superdisintegrant and tannic acid.

Lipids are considered as alternative to polymer in the design of controlled drug delivery systems due to their advantages like 1) low melt viscosity, there by obviating the need of organic solvents for solubilization, 2) the absence of toxic impurities such as residual monomers catalyst and initiators, 3) the potential biocompatibility and biodegradability and prevention of gastric irritation by forming a coat around the gastric irritatic drug (Bowtle, 2000; Porter & Charman, 2001). Recently, Kumar et al. (2004) have demonstrated the use of amphiphilic lipid glyceryl mono oleate (GMO) for design of floating matrix system (Ahlgren et al., 2000; Kumar et al., 2004; Pouton, 1985). These matrices, due to high viscosity cubic phase, served as a controlled release system. Lipids have a special advantage as a carrier for drugs having gastric irritant properties (Ahlgren et al., 2000; Pouton, 1985).

Gelucire® is a family of vehicles derived from mixtures of mono-, di-, and triglycerides with polyethylene glycol (PEG) esters of fatty acids. Gelucires® are available with a range of properties depending on their hydrophilic–lipophilic balance (HLB) 1–18 and melting point (33°–70°C) range (Aïnaoui & Vergnaud, 1998; Aïnaoui et al., 1997; Sheu et al., 2001; Sutananta et al., 1994). They have wide variety of applications in pharmaceutical formulations. The Gelucire® containing only PEG ester (Gelucire® 55/18) are generally used in preparation of fast release formulations and those containing only glycerides or mixtures of glycerides and PEG esters (Gelucire® 54/02, 50/13, 43/01, 39/01) are used in preparation of sustained release formulations (Barker et al., 2003; Dennis et al., 1990). Gelucire® 43/01 is a class of highly hydrophobic lipid with HLB 1 and melting point 43°C. Gelucire® 43/01 comprises of mixture of saturated triglycerides of different fatty acids C_8 (3%), C_{10} (2%), C_{12} (29%), C_{14} (2%), C_{16} (17%), and C_{18} (36%) melting at 43°C. Extreme hydrophobicity of Gelucire® 43/01 is attributed to absence of PEG esters, which in turn provides release-retarding ability. Sutananta et al. reported sustained release single unit matrices using Gelucire® 43/01, where only 1.7% theophylline was released over a period of 20 hours (Sutananta et al., 1995). Therefore, attempts are required to increase drug release, for which two approaches are attempted in the present work. The first approach is to increase surface area and decrease matrix resistance by designing a multi-unit system; and the second approach is incorporation of release enhancers in the single-unit system. Single- and multi-unit systems have their own advantages and limitations.

Due to a higher permeability of paracellular route in the upper G.I. tract, it is advisable to formulate bisphosphonates as gastroretentive form. Mucosal irritation has further needs of the formulation development task. Floating systems with lipidic coating have been formulated to overcome these problems. The lipid coat of hydrophobic triglyceride Gelucire® 43/01 will retard drug release as well reduce gastrointestinal irritation. So the major objectives of the present study were to prepare and evaluate sustained release single- and multi-unit floating bisphosphonate-Gelucire® 43/01 formulations considering risedronate sodium, [1-hydroxy-2-(3-pyridinyl)ethylidene]bis[phosphonic acid] monosodium salt, as model bisphosphonates (Gert et al., 1995). The prepared formulations were evaluated for in vitro, in vivo floating ability and in vitro drug release. In vivo floating ability of the formulations was studied in healthy human volunteers by γ-scintigraphy. Effect of aging on Gelucire® 43/01 were evaluated by hot

stage microscopy (HSM), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), in vitro floating ability, and in vitro drug release.

MATERIALS

Risedronate sodium (white crystalline powder, m.p.=238–240°C, freely soluble in water, maximum dose 30 mg once daily) was obtained as a gift sample from Fleming Laboratories Ltd., India. Gelucire® 43/01 (waxy solid, m.p.=43°C, HLB=01), Compritol® 888 ATO (white powder, m.p.=70°C, HLB=02), Precirol® (white powder, m.p.=54°C, HLB=02) were generous gifts from Gattefosse, Cedex, France. Caprol PGE-860 (oleic acid esters on decaglycerol) was supplied by Abitec Corporation, Jenesville, WI, USA. Licaps® capsules (size 0, hard gelatin capsule specially designed for lipid formulations) were obtained as a gift sample from Capsugel, Mumbai, India.

METHODS

Preparation of Matrices

Multi-unit Matrix Systems

Risedronate sodium and Gelucire® 43/01 granules were prepared by melt granulation technique in the ratio of 1:1 to 1:3 parts by weight. Gelucire® 43/01 was melted in a jacketed vessel at 60°C. Risedronate sodium was added to the molten Gelucire® 43/01 mass under stirring with the propeller blade at 100 rpm using constant speed stirrer (Eurostar, Power Control-visc P4, IKA Labortecnik, Germany) and allowed to solidify at 4°C. The solidified mass was passed through 1000 μm sieve to get uniform-sized granules. The granules were filled into Licaps® capsules.

Single-unit Matrix Systems

The single unit matrix systems were prepared by melting Gelucire® 43/01 in a glass beaker at 60°C. Risedronate sodium (30 mg) and Caprol PGE 860 were added to the molten mass under stirring with the propeller blade at 100 rpm using constant speed stirrer to obtain a homogenous mass. The amount of Caprol PGE 860 varied from 40 mg to 80 mg per formulation. The homogeneous molten mixture was filled into Licaps® capsules using a preheated glass pasteur pipette and allowed to solidify at 4°C. The capsules were equilibrated to room temperature (25°C) for 6 h before evaluation. The compositions of risedronate sodium–Gelucire® 43/01 single-unit matrices with Caprol PGE 860 are shown in Table 1.

Floating Characteristics

In Vitro Floating Ability

In vitro floating ability of the formulations (in triplicate) were determined by using the USP 24 type II dissolution test apparatus (TDT-06P Electrolab, Mumbai, India) at 50 and 100 rpm maintained at 37±0.2°C in 900 mL of 0.1 N HCl (pH 1.2). The Licaps® capsules of single- and multi-unit formulations were placed in the medium and the floating times were measured by visual observation (Iannuccelli et al., 1998).

γ-Scintigraphy

In vivo floating ability of single-(Batch F03) and multi-unit formulations (1:3) were studied by γ-scintigraphy in six healthy human male volunteers of age 25–30 years and 55–65 kg body weight with no clinical significant illness in the last two months. They were non-alcoholic, non-smokers, and were not taking any other medication for at least one week. ^{99m}Tc 0.1 milli curie was uniformly mixed with the molten formulations and solidified at 4°C. The formulations were filled into the Licaps®. The volunteers were asked to swallow these Licaps® capsules with 180 mL of water in the morning. The formulations were

TABLE 1 The Compositions of Risedronate Sodium–Gelucire® 43/01 Matrices

Sr. no.	Risedronate sodium	Gelucire® 43/01	Caprol PGE
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

visualized using a gamma camera (GE Millennium MPR Gamma Camera, Israel) immediately after administration of formulation, and at intervals of 1, 2, 3, 4, 5, and 6 h. The human ethical committee approved the protocol of the study.

Release Studies

The release studies were performed using USP 24 type II dissolution test apparatus under sink condition. The formulations (in triplicate) were placed in the dissolution vessel containing 900 mL 0.1 N HCl maintained at 37±0.2°C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through whatman filter paper 41, concentration of risedronate sodium was determined spectrophotometrically (V530 Jasco, Japan) at 261.2 nm. Analysis of data was done using PCP Disso V 3.0 software (Pune, India).

Aging of Gelucire® 43/01

Effect of aging was studied by HSM, SEM, DSC, in vitro floating ability, and in vitro drug release.

Hot Stage Microscopy

HSM was conducted using Mettler Toledo FP82HT hot stage (Switzerland) assembled on Leica DMLP microscope equipped with Leica MPS-30 camera (Germany). Different samples were observed under the microscope under scanning speed of 2°C/min. Changes in the morphology were noted as a function of temperature. Three types of samples were prepared for the physical evaluation of Gelucire® 43/01:

- *Freshly solidified samples:* Placebo Gelucire® 43/01 matrices (without drug and Caprol PGE 860) were prepared and stored for 6 h at room temperature in order to avoid effects due to the previous thermal history.
- *Aged samples:* Placebo matrices were stored up to 30 days at room temperature in order to detect any physical aging effect associated with glyceride base.
- *Untreated samples:* Gelucire® 43/01 without any special treatment (as received from the supplier).

Scanning Electron Microscopy

The surface of the fresh and aged formulations were coated with a thin gold-palladium layer by sputter coater unit (VG-Microtech, UK), and the surface topography was analyzed with a Cambridge Stereoscan S120 scanning electron microscope (SEM, Cambridge, UK).

Differential Scanning Calorimetry

Thermograms of untreated sample, placebo, fresh and aged Gelucire® 43/01 matrices were obtained using a Mettler-Toledo DSC 821^e instrument equipped with an intracooler. Indium standard was used to calibrate the DSC temperature and enthalpy scale. A piece of matrices was hermetically sealed into a pierced aluminum crucible and heated at a constant rate of 5°C/min, over a temperature range of 25–65°C. Inert

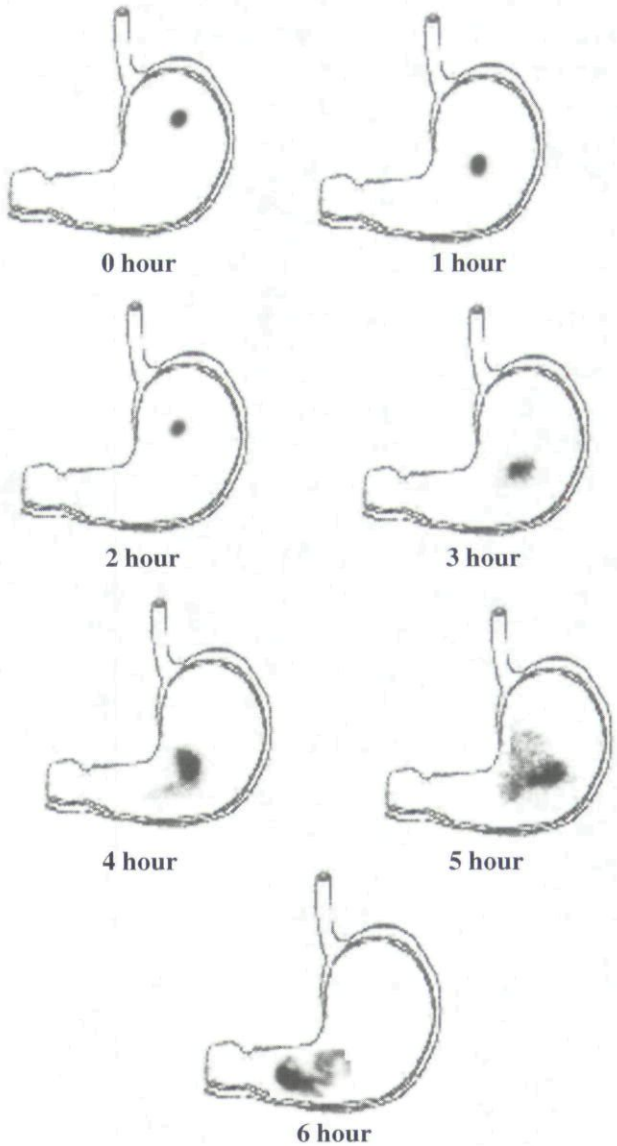

FIGURE 1 Gamma Scintigraphs of Single-Unit Matrices of Risedronate Sodium–Gelucire® 43/01 (Batch F03) Showing Floating Ability in Human Stomach.

atmosphere was maintained by purging nitrogen at the flow rate of 50 mL/min.

In Vitro Release

In vitro release of aged formulations (30 days) was checked to detect any aging effect on the drug release retarding property of Gelucire® 43/01.

RESULTS AND DISCUSSIONS

Yield and drug content of the granules were found to be in the range of 95–99% and 98–102% w/w respectively. The in vitro floating ability of both systems showed that the amount of Gelucire® affected

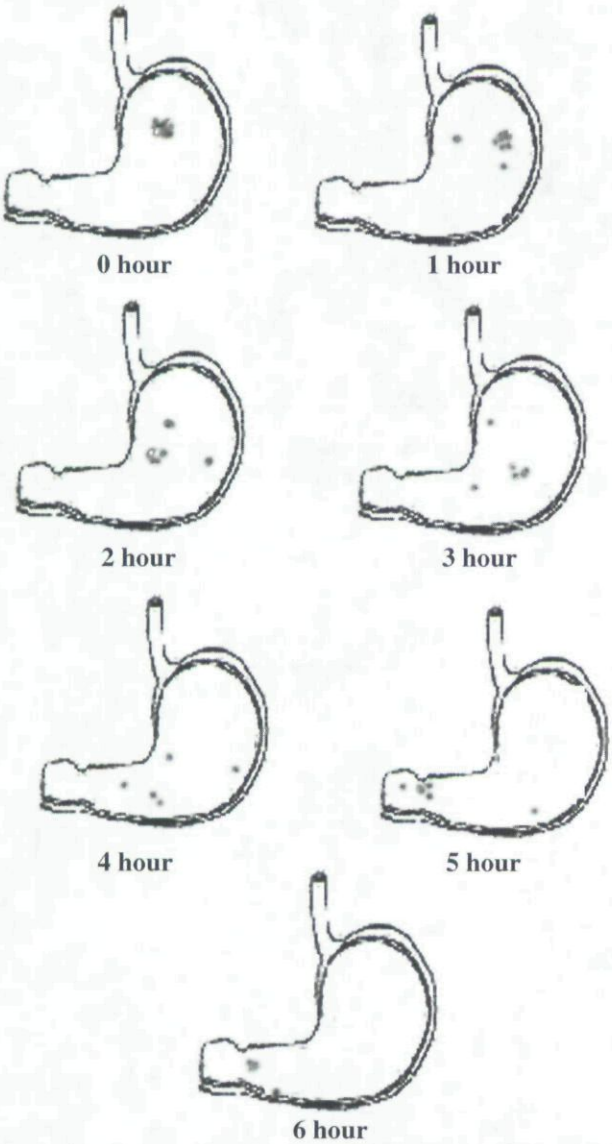

FIGURE 2 Gamma Scintigraphs of Multi-Unit Formulation of Risedronate Sodium–Gelucire® 43/01 (1:3) Showing Floating Ability in Human Stomach.

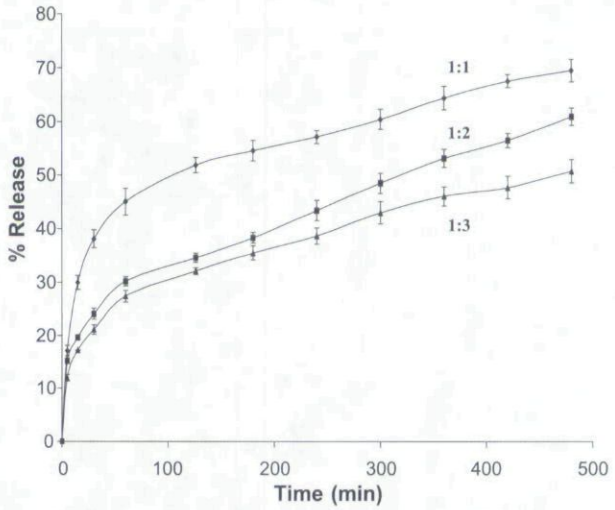

FIGURE 3 Release Profiles of Risedronate Sodium from the Multi-Unit System Showing the Effect of Increasing Gelucire 43/01 Ratios. Each Point Refers to Mean±SD (n=3).

the floating property of single unit matrices and that there was no effect of stirring speed on the floating property of the single- and multi-unit matrices. The amount of Gelucire® used was 400 mg for 30 mg drug for single-unit matrices, below which the matrix did not float (data not shown). All the batches floated all the time during the in vitro release study. There was no buoyancy lag time for any of the batches. The hydrophobic nature of the Gelucire® 43/01 was responsible for floating behavior, but all low HLB excipients did not ensure floating, as similar formulations prepared using Compritol® and Precirol® separately did not show floating property. Therefore, apart from hydrophobicity, density of Gelucire® 43/01 also played an important role in design of floating system using lipophilic excipient. The water

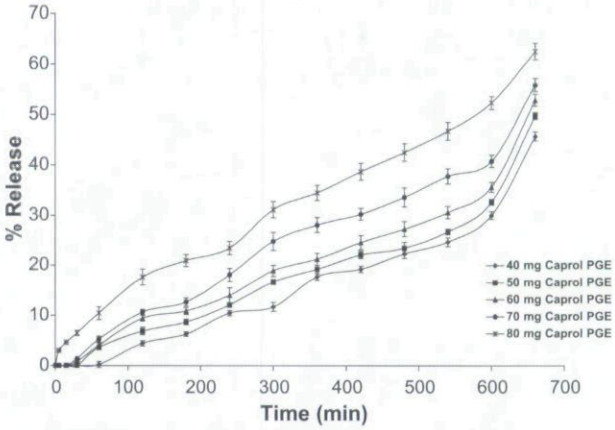

FIGURE 4 Release Profiles of Risedronate Sodium from the Single-Unit Matrices Showing the Increasing Effect of Caprol PGE 860 Amount. Each Point Refers to Mean±SD (n=3).

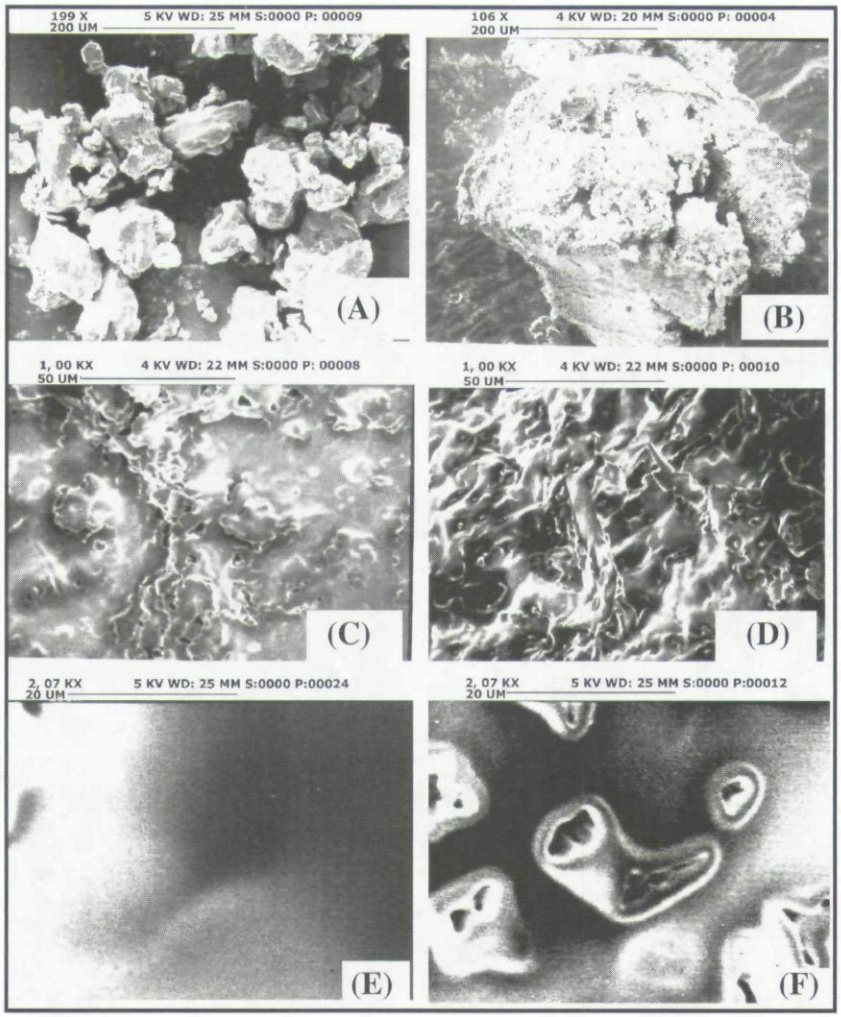

FIGURE 5 Scanning Electron Microscopy Images of: (A) Risodronate Sodium, Original Magnification ×199; (B) Melt Granule, ×106; (C) Surface of Fresh Multi-Unit System, ×1000; (D) Surface of Aged Multi-Unit System, ×1000 X; (E) Surface of Fresh Single-Unit Matrices, ×2007; (F) Surface of Aged Single-Unit Matrices, ×2007.

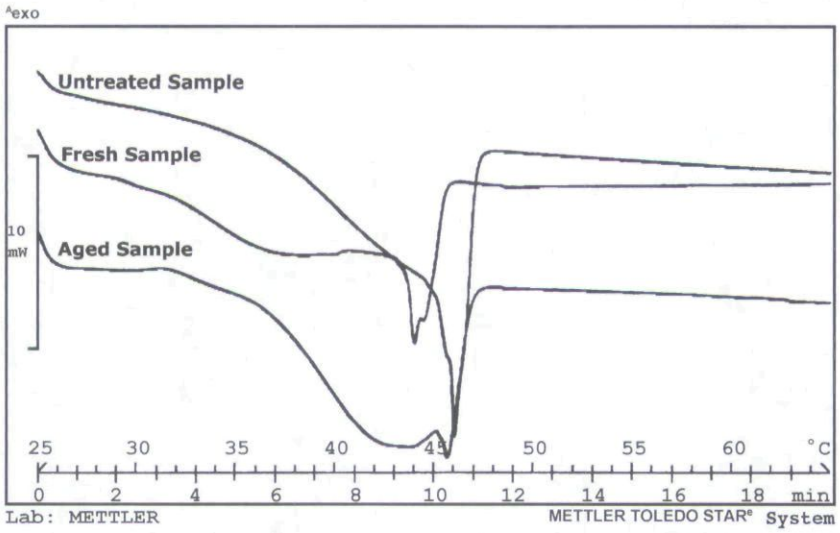

FIGURE 6 DSC Thermograms of Placebo Gelucire® 43/01 Matrices: (A) Untreated Sample; (B) Fresh Sample (0 Day); (C) Aged Sample (30 Days; R.T.).

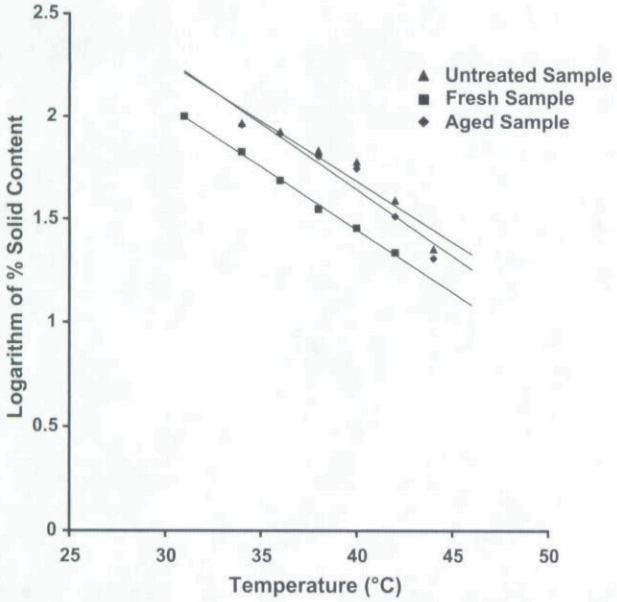

FIGURE 7 Semi-log Solid Fat Content as a Function of Temperature of Gelucire® 43/01. (A) Untreated Samples; (B) Fresh Sample; (C) Aged Sample.

uptake mechanism may not play a role in Gelucire® 43/01 because of its extreme hydrophobic nature. γ-Scintigraphy study of the single- (Fig. 1) and multi-unit (Fig. 2) floating systems showed that the formulation remains in the stomach for about 6 h. Considering the results of Fig. 1, and physiological emptying of the stomach, the single unit system stayed there much longer than 6 h. It might be due to higher lipid content and bigger size of single unit system. Figure 3 shows the release profiles of risedronate sodium from Gelucire® 43/01 floating granules. As the amount of Gelucire® 43/01 was increased in the multi-unit system the release rate was found to decrease and, also from the dissolution profiles, it has been clearly shown that burst release was observed initially. This may cause gastric irritations.

To prevent burst release, single unit matrices were designed containing a release enhancer Caprol PGE 860. Caprol PGE 860 is nonionic, nonalkoxylated polyglycerol type surfactant (HLB 11) used as emulsifier. The surfactants have been reported to possess the property to loosen the tight junction and enhance paracellular penetration (Lindmark et al., 1998). The amount of release enhancer in matrices plays an important role to obtain desired drug release. Addition of Caprol PGE 860 in the matrices improved the drug release. Figure 4 shows the release profiles of risedronate sodium in 0.1 N HCl from Gelucire® 43/01 floating single-unit matrices containing different amounts of Caprol PGE 860. As the amount of the emulsifier was increased, the release was found to increase. There was almost no release of risedronate sodium (<1%) over a period of 8 h from plain Gelucire® 43/01 matrices.

The effects of aging on the Gelucire® 43/01 were studied using HSM, DSC, SEM, in vitro floating ability, and in vitro drug release. The SEM photographs of fresh and aged single-unit matrices of 43/01 are shown in Fig. 5. The surface showed formation of pores in aged matrices, with smooth surface in fresh matrices. The SEM photograph of the granules has shown presence of lipid on the surface. The surface of the fresh granule showed smooth patches of lipid on the surface. After aging, the sample showed significant change in the surface, which might be due to changes in the properties of the Gelucire® 43/01 (Fig. 5).

DSC thermograms of untreated Gelucire® 43/01, placebo fresh Gelucire® 43/01 matrices (6 h after preparation), and placebo aged Gelucire® 43/01 matrices (30 days) samples are shown in Fig. 6. Thermograms of untreated, fresh, and aged samples have shown significant difference. The ΔH_f values have increased upon aging. The untreated sample has shown melting endotherm at 44°C which might be associated with the low melting glycerides present in the sample, whereas the glycerides melted over a wide range in aged sample as compared to fresh one. In the fresh sample, melting onset occurs at temperature

TABLE 2 Effect of Gelucire® 43/01 Aging on Enthalpy and Regression Analysis Data of Semi-log Plot Between Solid Fat Content and Temperature

		Regression analysis			
Sample	ΔH_f (Jg^{-1})	Intercept	Slope	R^2	Significance
Untreated sample	104.28±2.69	4.0365	−0.0589	0.9104	0.002
Fresh (0 days)	124.48±1.26	3.8851	−0.06091	0.9970	0.000
Aged (30 days)	129.85±2.09	3.7284	−0.05219	0.8810	0.002

significantly lower than the aged sample, whereas endset temperature is slightly higher in aged sample. From DSC thermograms it is clear that after aging a material structure becomes more uniform; however, there are still some differences in interaction, which might be reflected as multiple peaks as shown in aged samples at 45.6°C.

As the endotherm area is proportional to the solid content, DSC data has been converted in terms of solid fat content. A semi-log plot of solid fat content Vs temperature (T) is shown in Fig. 7. The data fitted well in following model:

$$\% \text{ Solid content} = Me^{-BT} \quad (1)$$

where M and B are constant and B reflects the rate of decrease in solid content. The regression analysis data is summarized in Table 2. Thus, logarithmic decrease occurred in the solid content in all the samples. The rate of decrease in solid content was higher in fresh sample as compared to the untreated and aged sample. This observation was in accordance with the observation by Sutananta et al. (1994).

In the fresh samples, part of the glycerides melted up to 40°C (71.5%) are significantly higher than the aged sample (44.6%). These results were in correlation with the observations of HSM. HSM of untreated, fresh, and aged placebo matrices are shown in Fig. 8. It has been observed that complete melting of the freshly prepared Gelucire® 43/01 matrices occurred at

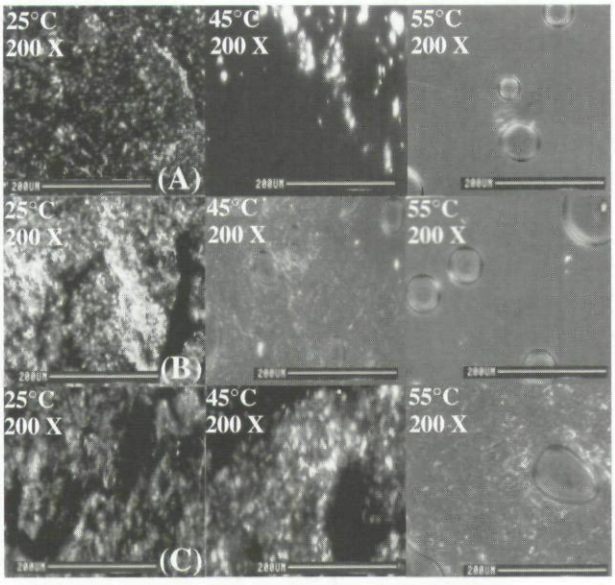

FIGURE 8 HSM Microphotographs of Gelucire® 43/01: (A) Untreated Samples; (B) Freshly Prepared Placebo Gelucire® 43/01 Matrices; (C) Aged Placebo Gelucire® 43/01 Matrices.

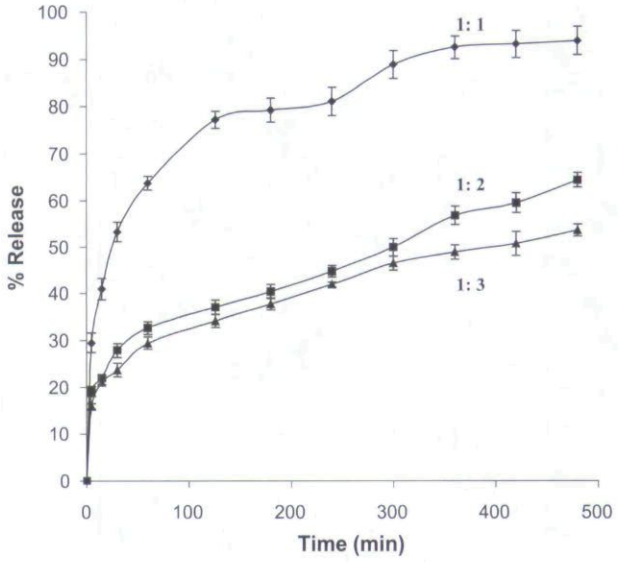

FIGURE 9 Release Profiles of Risedronate Sodium from the Multi-Unit System Showing Effect of Aging (30 Days). Each Point Refers to Mean ± SD (n=3).

45°C. HSM microphotograph showed the presence of some unmelted portion even at 45°C in aged sample. The energy required for melting increased with aging, which might be attributed to phase transformation such as crystallization of glycerides during aging. This slow crystallization of glycerides was responsible for many changes in the properties of the Gelucire® 43/01, showing surface roughness. This shift in melting temperature might be due to changes in the properties of the Gelucire® 43/01.

In vitro release from aged formulations (30 days) was observed to detect any aging effect on the drug release retarding property of Gelucire® (Figs. 9 and

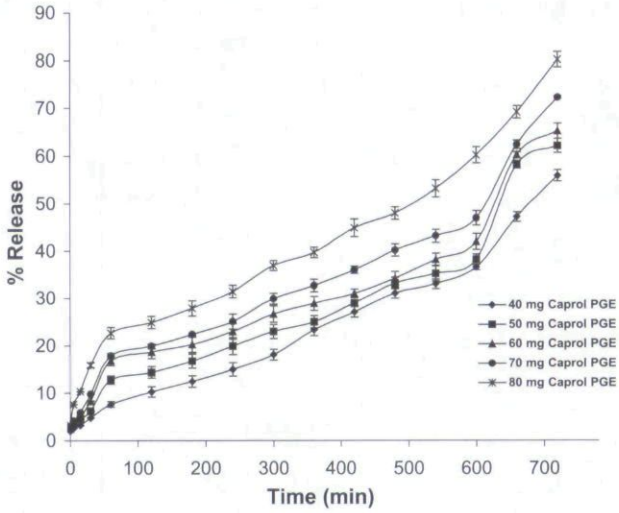

FIGURE 10 Release Profiles of Risedronate Sodium from the Single-Unit Matrices Showing Effect of Aging (30 Days). Each Point Refers to mean ± SD (n=3).

TABLE 3 Effect of Aging on Drug Release from Single- and Multi-unit Matrix Systems

Formulations	One day	30 days
Multi-unit	$T_{50\%}$	$T_{50\%}$
1:1	129.4 min	30.1 min
1:2	298.8 min	248.2 min
1:3	468.5 min	393.3 min
Single-unit	$T_{40\%}$	$T_{40\%}$
F01	640.2 min	625.3 min
F02	630.2 min	605.1 min
F03	610.3 min	585.2 min
F04	582.3 min	470.2 min
F05	470.2 min	363.1 min

10). In the present study, it was observed that due to aging, release-retarding activity of Gelucire® 43/01 was reduced in both single- as well as multi-unit systems. In the multi-unit system, the effect was significantly less for batches containing drug: Gelucire® 43/01 in the ratio of 1:2 and 1:3 as compared to 1:1, whereas in single-unit system, significant increase in the release rate was observed for batches containing the higher amount of surfactant, which might be attributed to faster erosion of the matrix. Similarly, release enhancement was found to be more in aged samples containing higher surfactant concentration, which might be due to phase transformation of Gelucire® 43/01 during aging (Table 3). Thus single- as well as multi-unit systems showed release enhancement during aging. This might be because the lipid material is transforming to the more crystalline form. But this nature does not have any impact on the floating ability. Floating ability of all the aged formulation remains the same as the initial formulation (data not shown).

In comparison with single-unit systems, multi-unit systems offered advantages such as low lipid content and ease of manufacture. The melt granulation technology has been developed to a level where scale up and commercialization is possible. The only draw back of multiunit system was initial burst effect, significance of which should be clinically evaluated in comparison with a single-unit system.

CONCLUSION

Gelucire® 43/01 may be considered as an appropriate carrier for designing of sustained release floating drug delivery systems due to its extreme hydrophobicity and low density. The single- and multi-unit floating systems of risedronate sodium using Gelucire® 43/01 may be considered as controlled release delivery systems with reduced gastro-intestinal irritation and increased bioavailability. Caprol PGE, a high HLB emulsifier, acted as release enhancer for a single-unit system. SEM, HSM, and DSC showed aging of the Gelucire® 43/01 which was responsible for an increase in drug release. Single-unit systems did not show burst release as in case of multi-unit system.

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