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Preparation of Solid Dispersion of Everolimus in Gelucire 50/13 using Melt Granulation Technique for Enhanced Drug Release

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Solid dispersion (SD) system of everolimus (EVR) with Gelucire 50/13 (Stearoyl polyoxyl-32 glycerides) was prepared using melt granulation technique with the aim of improving the physicochemical properties and dissolution rate. The solid state characterization using scanning electron microscopy and X-ray powder diffraction, indicated that the drug was homogeneously distributed in the surfactant carrier in a stable amorphous form. The dissolution rate of EVR from the optimized SD composed of the drug, Gelucire 50/13 and microcrystalline cellulose in a weight ratio of 1:5:10, was markedly rapid and higher than that from the drug powder and the market product (Afinitor®, Novartis Pharmaceuticals) in all dissolution mediums tested from pH 3.0 to pH 6.8. The results of this study suggest that formulation of SD with Gelucire 50/13 using melt granulation procedure may be a simple and promising approach for improving the dissolution rate and oral absorption of the anti-cancer agent without the need for using an organic solvent.

Key Words : Everolimus, Solid dispersion, Gelucire 50/13, Dissolution, Melt granulation

Introduction

Everolimus (EVR, Figure 1), the 40-*O*-(2-hydroxyethyl) derivatives of the natural product sirolimus, is administered as a once-daily, oral therapy for the treatment of patients with advanced renal cell carcinoma (RCC) after they fail to respond to sunitinib or sorafenib treatment.[1] EVR is an inhibitor of the mammalian target of rapamycin (mTOR), a component of an intracellular signaling pathway that regulates cellular metabolism, growth, proliferation, and angiogenesis.[2] Recently, the drug was also approved by the US Food and Drug Administration for the treatment of advanced hormone receptor-positive, HER2-negative breast cancer, advanced neuroendocrine tumors of pancreatic origin, renal angiomyolipoma with tuberous sclerosis complex, and subependymal giant cell astrocytoma.[1] In spite of these attractive pharmacological effects, the oral absorption of EVR is challenging owing to its poor solubility in the gastrointestinal tract, unfavorable breakage of the drug in the gastric fluid, and intestinal efflux by P-glycoprotein transporter.[3,4] The solubility of EVR in aqueous medium is below 0.1 mg/mL at 25 °C.

To improve the dissolution rate and oral absorption of the mTOR inhibitor, the pharmaceutical company (Novartis Pharmaceuticals, Basel, Switzerland) developed immediate release tablets (Brand name, Afinitor®) using solid dispersion (SD) with a hydrophilic polymer, hydroxypropyl methyl cellulose (HPMC).[4] In the solvent method, both mTOR inhibitor and HPMC are dissolved in the organic solvents and then spray dried and then pulverized to form the granules. The molecular dispersion of the active substance in a polymeric carrier achieves optimal particle size reduction and surface area enhancement, which result in improved dissolution rates.[5,6] However, a complex process involving the steps of mixing and dissolving a sirolimus derivative and a polymer in an organic solvent, evaporating the solvent, pulverizing dry residues to obtain particulate matter needs to be performed using the conventional solvent method. This process also introduces the residual solvent, which may bring up environmental issues.

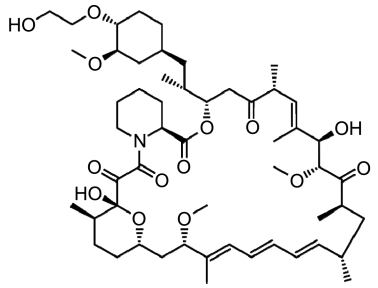

Figure 1. Chemical structure of EVR.

Gelucire® (GLC) is a family of vehicles derived from the mixtures of mono-, di- and triglycerides with polyethylene glycol (PEG) esters of fatty acids. GLC has a wide variety of applications in pharmaceutical formulations as the preparation of SDs, fast release and sustained release formulations with a low melting point (33-65 °C), low toxicity, and wide drug compatibility.[7-10] In particular, GLC-based SD system can be easily prepared by melt granulation technique, a process in which fine agglomeration is obtained through the melting and/or softening of the surfactant carrier at relatively low temperatures, drug dissolution in the molten carrier, followed by drying.[11] The procedure offers some advantages

over the conventional solvent method, since the steps of addition and evaporation of an organic solvent can be omitted. Moreover, from an environmental perspective, it is also a good alternative that does not need the use of organic solvents.

The purpose of the current study is to formulate and optimize an SD system of EVR in the surfactant carrier (GLC 44/14 or 50/13) using melt granulation method. Physicochemical properties of SDs were characterized with an emphasis on surface morphology, crystallinity and chemical stability. Moreover, dissolution profiles of the mTOR inhibitor from the SD systems were investigated under various conditions in comparison with those of an intact drug alone, and market product (Afinitor®).

Experimental

Materials. EVR was purchased from Biocon Ltd. (India, purity over 99.4%). GLC 44/14 (Lauroyl polyoxyl-32 glycerides, melting point 44 °C, hydrophilic-lipophilic balance 14) and GLC 50/13 (Stearoyl polyoxyl-32 glycerides, melting point 50 °C, hydrophilic-lipophilic balance 13) was kindly provided by Gattefosse (Cedex, France). Microcrystalline cellulose (Avicel PH 102) was obtained from FMC Corporation (Philadelphia, USA). All organic solvents were high-pressure liquid chromatography (HPLC) grade and all other chemicals were reagent grade.

Preparation of EVR-loaded SD Formulations. SDs in various weight ratios of drug to the carrier were prepared by melt granulation method.[11] EVR (50 mg) was added to the molten base comprising either GLC 50/13 or 44/14 as listed in Table 1. The blend was heated 10 °C above the melting point of each carrier for 5 min with continuous magnetic stirring. The mass was crushed, ground gently with a mortar and pestle and passed through a 500 μm sieve. For the preparation of F7, microcrystalline cellulose was added to the drug-containing molten solution, followed by continuous blending for 10 min.

Preparation of EVR and GLC Physical Mixtures. GLCs are a waxy pellet, and hence these were crushed to fine particles firstly to prepare the physical mixture (PM). PMs of EVR with either GLC 44/14 or 50/13, in a 1:3 weight ratio of drug to the carrier, were prepared by blending them by triturating for 10 min followed by sieving (500 μm).

Drug Content and Uniformity. A sample containing 10 mg of the drug was dissolved in 10 mL of acetonitrile (0.25 mg/mL) and sonicated for 10 min. Then the samples were centrifuged at 12,000 rpm for 10 min, and the supernatants were analyzed by the HPLC analysis. The quantitative determination of EVR was accomplished by HPLC using the acetonitrile-phosphate buffer ($NaH_2PO_4 \cdot H_2O$ 0.11 w/w%) (40:60) as a mobile phase at a flow rate of 1.2 mL/min. The HPLC system consisted of a UV detector (L-2400), a pump (L-2130), a data station (LaChrom Elite, Hitachi, Japan), and a 15 cm C_{18} column (Shiseido, Tokyo, Japan). The column eluent was monitored at 275 nm, and the peak of the mTOR inhibitor was separated with a retention time of 7.5 min.

Table 1. Compositions (mg) of EVR-loaded SD formulations

	F1	F2	F3	F4	F5	F6	F7
EVR	10	10	10	10	10	10	10
GLC 44/14	30	-	-	-	-	-	-
GLC 50/13	-	30	10	50	100	200	50
MCC[a]	-	-	-	-	-	-	100

[a]MCC indicates microcrystalline cellulose (Avicel 102).

Scanning Electron Microscopy (SEM). The samples were coated with a thin gold layer by an automatic magnetron sputter coater system (Jeol MSC201, USA). Then, SEM photographs were taken by a scanning electron microscope (Joel JSM 6510 SEM, USA) operated at an acceleration voltage of 15 kV.

X-ray Powder Diffraction (XRD). XRD observation of the samples was performed at room temperature with an X-ray diffractometer (Ultima IV, Rigaku Corp., Japan). Monochromatic Cu Kα-radiation ($\lambda = 1.5418$ Å) was obtained with a Ni-filtration and a system of diverging and receiving slides of 0.5° and 0.1 mm, respectively. The diffraction pattern was measured with a voltage of 40 kV and a current of 30 mA over a 2θ range of 3–40° using a step size of 0.02°.

***In vitro* Drug Release Test.** *In vitro* dissolution test was performed using USP-24 Type 2 dissolution test apparatus (DST-600A, Fine Scientific Instruments, Korea). Drug powder, SDs and the market product with 10 mg of EVR were placed in the dissolution vessel containing 900 mL of dissolution medium (pH 3.0, pH 4.0, pH 6.8 and water) maintained at 37.0 ± 0.5 °C and stirred at 50 rpm. Aliquots (4 mL) were collected periodically and replaced with fresh and pre-warmed dissolution medium. The samples were centrifuged at 12,000 rpm for 10 min, and the supernatants were diluted with acetonitrile for HPLC analysis.

Stability Test. Stability studies were conducted by placing powdered samples in stoppered glass vials and storing in stability chambers maintained at 40 °C and 75%RH for accelerated stability and 25 °C and 60%RH for long-term stability, respectively. Samples were removed after 1 month and tested for changes in the drug content (%).

Results and Discussion

Preparation and Characterization of SDs. The SDs of EVR in GLCs were prepared by melt granulation technique to enhance the dissolution rate and to minimize the manufacturing and/or environmental issues associated with the solvent method. The melt granulation method did not require the use of organic solvents for the preparation of dispersion system, whereas both the drug and the carrier needed to be dissolved in a sufficient amount of solvent in case of the solvent method. In particular, when HPMC is used as a carrier for SD, a mixture of dichloromethane and ethanol is usually added to dissolve the drug and the carrier as HPMC has a low solubility in ethanol.[12,13] However, dichloromethane is classified as a Class II solvent,[14] whose usage should be avoided whenever possible.[15] The drug content in SD

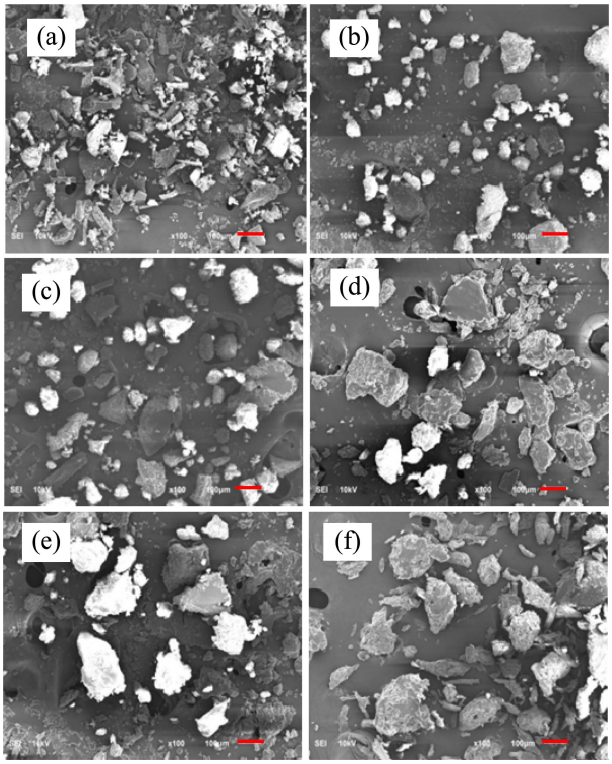

Figure 2. SEM micrographs for (a) raw material of EVR, (b) GLC 50/13, (c) physical mixture of drug and GLC 50/13 at the ratio of 1:3, SDs of (d) F2 and (e) F7, and (f) intact microcrystalline cellulose. Scale bars indicate 100 μm.

formulations was almost equal (97.2 to 100.1%) with low values of standard deviation, indicating that the drug was uniformly distributed in the hydrophilic carrier with the melt granulation process without any drug degradation and/or precipitation (Data not shown).

The solid state of EVR-GLC dispersion was characterized by scanning electron microscopy (SEM). Figure 2 shows SEM pictures of the raw material of the drug, pulverized GLC 50/13, and its corresponding PM and SD (F2). The drug crystals seemed to be irregular fragment in shape and their size ranged from 5-100 μm (Figure 2(a)). Typical appearance of drug powder and GLC 50/13 (Figure 2(b)) were observed in the photomicrographs of the PM (Figure 2(c)). On the other hand, in case of SD, it was difficult to determine the presence of drug crystals (Figure 2(d)), indicating that the drug crystals appeared to be incorporated into molten mass of the carrier at the molecular level. The appearance of SDs prepared using GLC 44/14 (F1) and GLC 50/13 in different ratios (F3, F4, F5, and F6) was almost same to that of F2 (Data not shown). The SEM images of the F7 formula showed that rough surfaces of fibrous microcrystalline cellulose (Figure 2(f)) were appeared to be covered with SD of EVR, and microcrystalline cellulose was white in color (Figure 2(e)).

The XRD patterns of drug powder, the surfactant carrier, and SD prepared by melt granulation method are shown in Figure 3. No diffraction peak of EVR (Figure 3(a)) was observed, while GLC 50/13 exhibited some crystallinity as

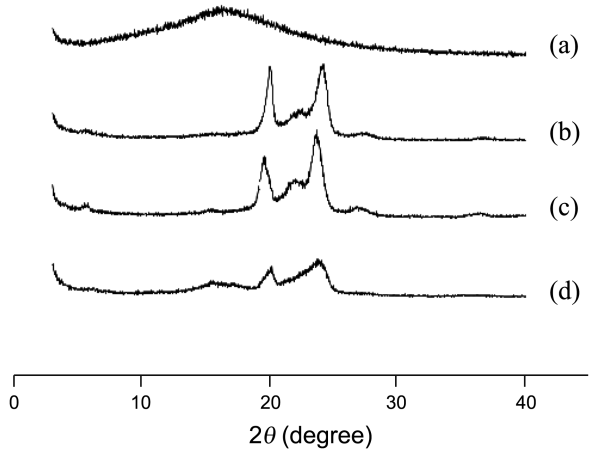

Figure 3. XRD diffractograms for (a) raw drug material, (b) GLC 50/13, and SD of (c) F4 and (d) F7.

indicated by the two characteristic peaks of high intensity at 19.26 and 23.50 at 2θ (Figure 3(b)). And the XRD pattern of SDs including F2 (Figure 3(c)) and F7 (Figure 3(d)) was quite analogous as that of the carrier itself, with no other distinctive peaks. This result suggests that EVR itself exists in an amorphous state, and the crystallinity of the drug remained in an amorphous state in the SD formulas, regardless of the presence of microcrystalline cellulose.

Dissolution Profiles of EVR from SDs. The dissolution profile of EVR from the drug powder, PMs and SDs with 44/14 and −50/13 in a weight ratio of 1:3 in distilled water are presented in Figure 4. In distilled water, PMs of GLC 44/14 and −50/13 showed dissolution profiles similar to those of the intact drug; after 2 h, approximately 30-35% of the drug was released. On the other hand, the release rate of EVR from SDs (F1 and F2) was significantly higher than that from the drug powder and/or PMs with the same ratio of drug to the carrier. In particular, the greater dissolution enhancement of EVR was achieved with the GLC 50/13-based formula (F2) compared to the GLC 44/14-based formula

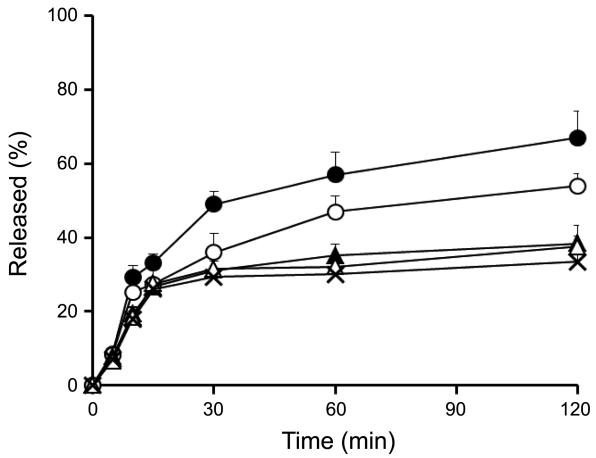

Figure 4. Dissolution profiles of the drug released from SD systems based on GLC 44/14 (F1, ○) or 50/13 (F2, ●), PMs with GLC 44/14 (△) or 50/13 (▲), and drug powder (×) in distilled water. Data represent mean ± SD (n = 3).

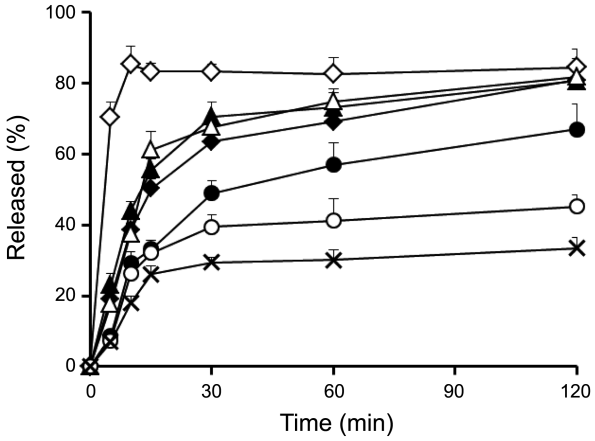

Figure 5. Dissolution profiles of EVR from GLC 50/13-based SD systems in distilled water prepared with different ratios of drug to GLC 50/13; 1:1 (F3, ○); 1:3 (F2, ●); 1:5 (F4, ▲); 1:10 (F5, △); 1:20 (F6, ◆), and prepared with microcrystalline cellulose at the ratio of 1:5:10 (F7, ◇), and drug powder (×). Data represent mean ± SD (n = 3).

(F1), in which the release rate of EVR was approximately 67% after 2 h. The drug release from F1 was only about 54% after 2 h. Therefore, we used the GLC 50/13 as a primary surfactant carrier to formulate SDs for further studies.

Figure 5 shows the dissolution profiles of EVR in distilled water from SD systems at different ratios of drug to GLC 50/13. GLC 50/13 is commonly used as a SD carrier to improve the dissolution rate of various water-insoluble drugs. In the SD systems with GLC 50/13, as the proportion of the surfactant carrier was increased, drug dissolution rates were increased and they reached a plateau at a ratio of 1:5 (F4), achieving more than 80% drug release after 2 h (Figure 5). No significant differences in the release of EVR were found between F4–F6. According to the Nernst–Brunner and Levich modification of the Noyes Whitney dissolution model equation, the dissolution rate of a drug is proportional to its effective surface area,[16,17] and the decrease in the diffusion layer thickness by reduction in the particle size, would further result in accelerated dissolution.[18] The enhanced dissolution rate of F4 can be attributed to the increase in effective surface area and saturation solubility, and the decrease in the diffusion layer thickness by particle size reduction at the molecular level.

Microcrystalline cellulose was used as an excipient in the GLC 50/13-based SD product (F4) to impart good flow properties to the powder to overcome the sticky and tacky nature of GLCs, facilitating drug dissolution in the aqueous medium. In a preliminary study, granules prepared with the drug, GLC 50/13, and microcrystalline cellulose at a ratio of 1:5:2.5 or 1:5:5 had a sticky consistency, and hence they could not produce a free-flowable powder (Data not shown). Instead, the SD prepared with 100 mg of microcrystalline cellulose (F7), at the ratio of 1:5:10, provided uniform and free-flowing granules. In the release test, F7 was rapidly disintegrated and dispersed within 5 min in distilled water, providing more than 85% of EVR release within 10 min (Figure 5). On the other hand, only 44% of drug was released from F4 within 10 min, although over 80% drug release was achieved after 2 h. According to the above drug release results, the optimized formula for the drug, GLC 50/13 and microcrystalline cellulose at a ratio of 1:5:10 (F7) was finally established to develop immediate release oral dosage form of EVR.

Dissolution studies were further performed for the drug powder, F7 formula, and commercial product (Afinitor®) using various dissolution mediums (distilled water, pH 3, pH 4, and pH 6.8) and the results are shown in Figure 6. The release of EVR from the raw material was pH-independent and showed an incomplete dissolution behavior. In pH 3.0, 4.0, 6.8 and distilled water, the amounts of EVR released were only 29-34% after 60 min. The amounts of EVR released from the F7 dispersion were also pH-independent, but noticeably higher than those released from the raw material, achieving more than 80% of release of EVR within 10 min in all mediums. Also, about 70% of the drug incorporated was released in all mediums after 5 min, which was higher than that released from the commercial tablet, which exhibited 36-45% of drug release within 5 min.

Chemical Stability of EVR-loaded SD. The stability of SD prepared by melt granulation process was compared to that of the drug powder itself. The drug was extremely susceptible to oxidative degradation,[19] and the amount of EVR was decreased to 93.5% at 25 °C, 60%RH conditions and to 72.4% at 40 °C, 75%RH conditions after 1 month storage (Table 2). On the other hand, the degradation rate of the mTOR inhibitor was considerably slower than that of drug itself, although a 7.4% decrease in the residual drug content was found in accelerated storage conditions. These results indicated that the amorphous drug was further stabilized in the SD system as a consequence of drug-GLC interactions and/or by incorporation into the carrier. However, a further study investigating the stabilization mechanism of the excipients is needed.

Conclusion

To improve the dissolution rate and oral absorption of EVR, a novel SD system was prepared by incorporating mTOR inhibitor into GLC 50/13 using the melt granulation technique. The fabrication process was very simple without the need for using an organic solvent. The optimized SD formulation consisted of EVR, GLC 50/13 and microcrystalline cellulose at a weight ratio of 1:5:10. The SD structure

Table 2. Drug content (%)[a] of the optimized SD (F7) and drug powder in ambient conditions of 25 °C, 60% RH and in accelerated conditions of 40 °C, 75% RH at 1 month

	Drug powder	F7
Initial	99.5 ± 1.1	98.9 ± 1.4
25 °C, 60% RH at 1 month	93.5 ± 1.1	97.5 ± 0.3
40 °C, 75% RH at 1 month	72.4 ± 3.6	91.5 ± 0.5

[a]Values represent mean ± SD (n = 3).

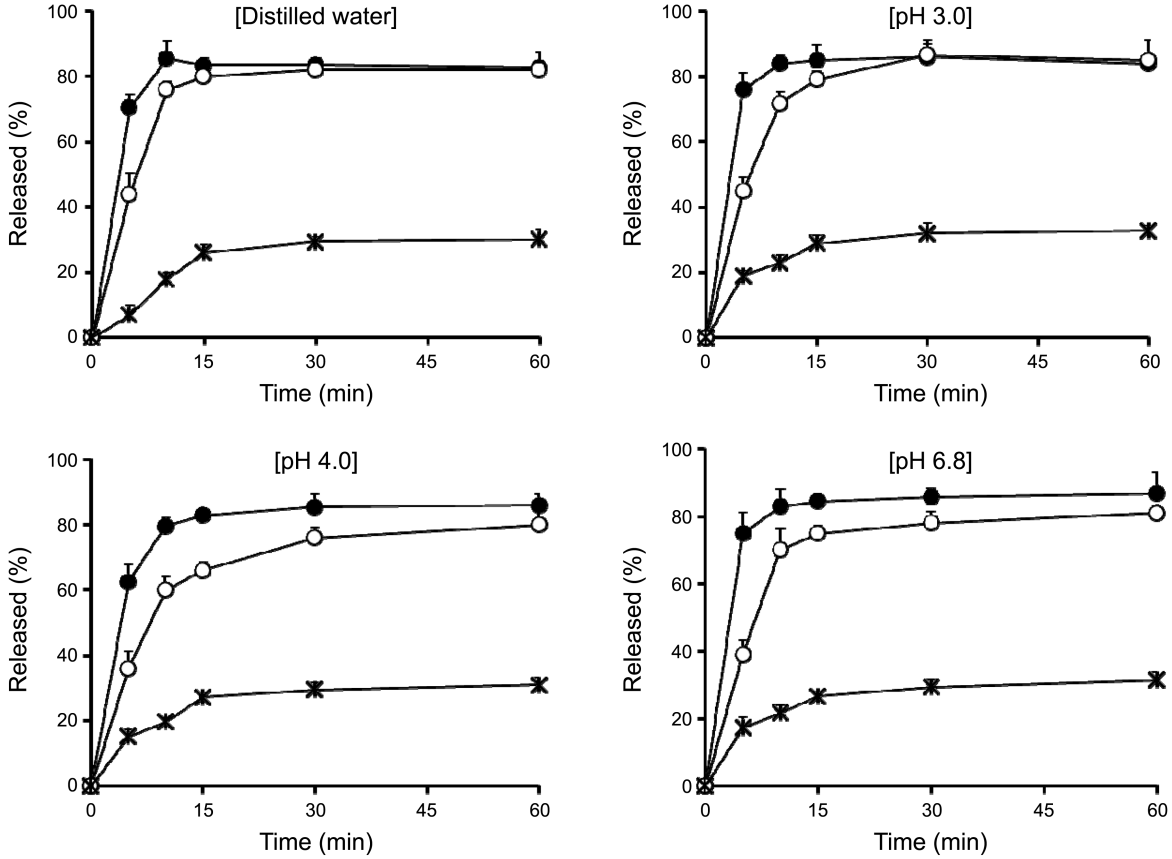

Figure 6. Comparative dissolution profiles of EVR from F7 (●), Market product (○) and drug powder (×) in different pH media. Data are expressed as mean ± SD (n = 3).

showed a remarkably higher dissolution rate in all dissolution mediums compared to that of the intact drug and the market product (Afinitor®), and it demonstrated that more than 80% of initially loaded active ingredient was released within 10 min. On the basis of these results, it can be suggested that the SD formulation using GLC 50/13 can be promising for the development of an immediate release tablet of the mTOR inhibitor.

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