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Rapidly Dissolving Repaglinide Powders Produced by the Ultra-Rapid Freezing Process

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ABSTRACT

The objective of the study was to produce rapidly dissolving formulations of the poorly water-soluble drug repaglinide using an innovative new technology, ultra-rapid freezing (URF), and to investigate the influence of excipient type on repaglinide stability. Repaglinide compositions containing different types and levels of excipients and different drug potencies (50%-86%) were produced by the URF technology. Repaglinide/excipient solutions were frozen on a cryogenic substrate, collected, and lyophilized to form a dry powder. Surfactants, including sodium dodecyl sulfate, and alkalizing agents such as diethanolamine (DEA) and tromethamine (TRIS) were incorporated into the compositions. Forced degradation of repaglinide was conducted under stressed conditions (eg, elevated temperature, exposure to peroxide) to determine the stability of the drug in such environments. The solubility of repaglinide increased as a function of increasing pH; therefore, incorporation of an alkalizing agent into the URF formulations increased the drug's solubility. Drug instability resulted when the drug was exposed to pH values above 9.0. URF formulations containing alkalizing agents showed no degradation or spontaneous recrystallization in the formulation, indicating that increased stability was afforded by processing. URF processing created nanostructured drug/excipient particles with higher dissolution rates than were achieved for unprocessed drug. Alkalizing agents such as TRIS and DEA, present at levels of 25% to 33% wt/wt in the formulations, did not cause degradation of the drug when processed using URF. URF processing, therefore, yielded fast-dissolving formulations that were physically and chemically stable, resistant to alkali degradation or spontaneous recrystallization in the formulation.

KEYWORDS: Ultra-rapid freezing, dissolution enhancement, stability enhancement, repaglinide, alkalizing agents.

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INTRODUCTION

The biopharmaceutical classification system (BCS) is used to group pharmaceutical actives depending upon the solubility and lipophilicity (permeability) characteristics of the drug. BCS class II compounds are poorly soluble but highly permeable, and they exhibit bioavailability that is limited by dissolution rate.[1] The dissolution rate of BCS class II drug substances may be accelerated by improvement of the wetting characteristics of the bulk powder.[2] Reduction of primary particle size is also critical for increasing the dissolution rate of poorly water-soluble drugs.

Cryogenic processing techniques have been developed to enhance the dissolution rate by creating nanostructured amorphous particles with high degrees of porosity.[3-10] Cryogenic processes allow for a reduction in the primary particle size of drug particles without the intense frictional or mechanical forces involved in ball-milling or other processes relying on frictional comminution or trituration with a mortar and pestle, which can cause degradation of the drug through thermal stress.[11] Previous studies have shown that the cryogenic spray-freezing into liquid (SFL) process produces amorphous solid solutions of drug and excipients.[4] The formation of metastable amorphous solid solutions yields higher energy states for the drug and thus a greater thermodynamic driving force for dissolution.

Hu et al and Vaughn et al have studied the cryogenic SFL technique extensively.[3,4,9] Based on these studies, SFL particles have been shown to have a large specific surface area, producing powders with rapid dissolution. Additionally, the SFL process produces powders that are consistent with a solid solution.[9] SFL powders are formulated with small amounts of surfactant to achieve high drug loadings (50%-86% drug/total solids) while maintaining high dissolution rates. SFL powders require smaller amounts of surfactant to achieve high dissolution rates.[4] These high-drug-loaded SFL powders contain amorphous nanostructured aggregates with high surface area and excellent wettability.

Another cryogenic process, the spray-freeze-drying (SFD) method, typically involves the atomization of a drug-containing solution in gaseous nitrogen above a pool of liquid nitrogen. The fine droplets of drug/solvent are frozen, then lyophilized to remove the solvent. The rapid freezing rates in the cryogenic liquid substrate do not allow for molecular arrangement

into crystalline domains, so SFD processing produces amorphous drug nanoparticle aggregates with improved dissolution rates.[12] The scalability of this type of technology, however, has limited its widespread industrial use.[13,14]

Ultra-rapid freezing (URF) technology involves the use of a solid cryogenic substrate with a thermal conductivity between 10 and 20 W/m degrees K. A solution of the drug is applied to the solid surface of the substrate, where instantaneous freezing takes place. Brownian motion of the particles in solution is slowed significantly, so reactive species have little time to react before being frozen into the solid state. Removal of the frozen particles and lyophilization of the solvent produces stable amorphous drug particles. URF technology has been shown to produce uniform, amorphous, drug particle/excipient aggregates.[15] Additionally, the process is continuous, allowing for improved scale-up applications. A reservoir of boiling cryogenic liquid is not required, allowing for lower operating costs and more convenient operation.

Numerous citations report solvent/drug/excipient compositions being frozen in liquid or gaseous nitrogen or other cryogenic fluids. All of these approaches face the same challenge in transferring the heat necessary to cool and freeze the solution forming the drug particle domains. The heat transfer is forced to pass through a gas film at the surface of the particle. This imparts a rate-limiting step in the heat transfer and defines the maximum freeze rate.[16]

URF technology overcomes the limitation of transferring heat through a gas film by eliminating the gas interface element and using direct contact with the cryogenic substrate. Drug solutions that come into direct contact with a boiling liquid cryogenic substrate transfer heat through a gas bubble film until the temperature of the particle comes into thermal equilibrium with the liquid at its boiling point.[17] Note that conduction is improved by using a cryogenic material with a high thermal conductivity, density, and mass relative to the solution being frozen so as to maintain the surface temperature and heat transfer rate while the solution is being frozen. With URF technology, the thickness of the freezing solution may be controlled to fix its minimum freezing rate since the freezing rate drives the particle formation and determines the freezing solution's characteristics and, hence, drug particle formation.

Repaglinide, a BCS class II compound for treatment of type II diabetes,[18-21] was chosen as a model drug to study dissolution enhancement of drug formulations formed using URF technology. Preformulation studies indicate that a pH-dependent dissolution profile for repaglinide exists, with the drug having a greater aqueous solubility at higher pH. Since this drug is needed to regulate postprandial glucose levels, the drug should ideally dissolve rapidly in the stomach (where the pH of the contents can be low). Additionally, the drug undergoes hydrolysis at high pH, owing to the drug's instability in basic solutions. URF technology can produce stable amorphous drug formulations with high dissolution rates, so repaglinide would be good candidate for the application of URF. The objective of this study was to use URF technology to prepare repaglinide as a stable formulation with a stable amorphous character. Additionally, we hoped that the incorporation of alkalizing agents as well as nonpolymeric surfactants would allow for the production of a rapidly dissolving yet chemically stable repaglinide powder formulation.

Materials and Methods

Materials

Micronized repaglinide was purchased from Kingchem (Lot 040701; Allendale, NJ). Repaglinide US Pharmacopeia (USP) reference standard (Rockville, MD), 200 mg (Lot FOB265, Cat 1600813), was a gift from the Dow Chemical Co (Midland, MI). Sodium dodecyl sulfate (SDS) was supplied by Pierce Chemicals (Rockford, IL). Tromethamine (TRIS) buffer, *t*-butanol, sodium phosphate monobasic, potassium phosphate dibasic, citric acid monohydrate, and diethanolamine (DEA) were purchased from Spectrum Chemicals (Gardena, CA). Hypromellose (Methocel E5) was purchased from Dow Chemical Co (Midland, MI). High-performance liquid chromatography (HPLC)–grade acetonitrile and methanol were obtained from EM Science (Gibbstown, NJ). Acros Organics (Geel, Belgium) supplied 1,3-dioxolane.

Preformulation Studies: pH Solubility Study

The solubility of repaglinide in different buffered media at various time points and 37°C was investigated in this study, based on methods described elsewhere by Higuchi and Connors.[22] Buffer species were prepared, ranging in pH from 1 to 9. For acidic media, 0.1N HCl (pH 1.1) was used, as well as 0.1M sodium phosphate (pH 3), and the USP dissolution medium containing citric acid and sodium phosphate (pH 4.5). For neutral pH values, a 0.1M sodium phosphate buffer (pH 7) was used, while for basic conditions, a 0.1M TRIS buffer was used (pH 9). In addition to the 5 types of buffer solutions prepared above, solutions of each buffer containing 0.1% and 0.2% SDS were prepared. The procedure for determining the pH solubility of the repaglinide was as follows: 10 mg of repaglinide was weighed into each of 36 vials. A 10-mL aliquot of each type of buffer (containing 0%, 0.1%, or 0.2% SDS) was added to each of the drug-containing vials and allowed to equilibrate at 37°C with shaking (Environ Orbital Shaker, Lab-Line Instruments, Melrose Park, IL) for 12, 24, and 48 hours. A 1-mL aliquot was taken from each sample and filtered through a 0.2-μm Whatman PTFE syringe filter (Florham Park, NJ) at 12, 24,

Table 1. Results of the Repaglinide Forced Degradation Study*

Forced Degradation Condition	Reaction Time (hr)	Total % Repaglinide Recovered	% Peak 1 Area (RRT = 0.27)	% Peak 2 Area (RRT = 0.42)	% Peak 3 Area (RRT = 0.69)	% Peak 4 Area (RRT = 0.78)	% Peak 5 Area (RRT = 1.24)	Total % Peak Area Impurity
Acid (1N HCl, 25°C)	1	92.1	5.1	1.1	ND	ND	ND	6.2
Base (1N NaOH, 25°C)	0.25	86.3	3.5	8.2	1.7	ND	ND	13.4
Base (0.1M TRIS, 25°C)	1	88.7	1.4	5.6	2.8	1.2	ND	11.0
Oxidation (6% hydrogen peroxide)	1	91.9	ND	4	4.7	0.8	2.1	11.6
Thermal (1 mg% in methanol, 60°C)	6	81.1	6.7	5.2	2.9	3.1	0.7	18.6
HCl/SDS insoluble mass	—	89.3	2.7	3.3	1.4	ND	ND	7.4
US Pharmacopeia repaglinide reference standard	—	99.82	ND	ND	0.07	0.04	ND	0.11

*RRT indicates relative retention time; ND, not detected; TRIS, tromethamine; SDS, sodium dodecyl sulfate.

and 48 hours. Each sample was analyzed using HPLC for the repaglinide concentration using the method reported by Gandhimathi et al and Krishna Reddy, et al.[23,24] The equilibrium solubility of repaglinide was determined in each type of buffer.

Repaglinide Degradation Study

The accelerated degradation of repaglinide was studied upon exposure to a series of harsh environments. Samples of bulk repaglinide were subjected to acidic, basic, thermal, and oxidative stress conditions, as recommended by the International Conference on Harmonization.[25] Acid degradation was accomplished by weighing out ~1 mg on a microbalance scale to the 1/1000 mg (Mettler Toledo M3/36, Hightstown, NJ, calibrated by Aldingen Co, validated through September 2007) repaglinide into a 100-mL volumetric flask, dissolving it in 5 mL of methanol, then exposing it to 10 mL of 1N HCl for 1 hour at 25°C. The reaction was then quenched with 10 mL of 1N NaOH and diluted to volume with water. Base degradation involved weighing out ~1 mg repaglinide, on the microbalance mentioned above, into a 100-mL volumetric flask, dissolving it in 5 mL of methanol, then exposing the drug solution to 10 mL of 1N NaOH for 15 minutes at 25°C. The reaction was quenched with 10 mL of 1N HCl, then diluted to volume with water. Additionally, forced base degradation was performed using 0.1M TRIS base, similar to the NaOH degradation above. About 1 mg of repaglinide, weighed on the microbalance mentioned above, was added to a 100-mL volumetric flask and dissolved in 5 mL of methanol. The drug solution was exposed to 10 mL of the basic 0.1M TRIS (pH 9.0) solution for 1 hour at 25°C. The reaction was quenched with 0.1N HCl after completion of the reaction. Oxidative degradation occurred by weighing out 1 mg of repaglinide on the microbalance mentioned above, dissolving it in 5 mL of methanol, then adding 1 mL of 30% hydrogen peroxide (H_2O_2) to the solution (total hydrogen peroxide concentration prior to dilution was 5% peroxide), allowing it to be exposed for 1 hour at 25°C. Finally this solution was diluted to a 100-mL volume with water (to a final H_2O_2 of 0.3% H_2O_2). Thermal degradation was completed with repaglinide in methanolic solution (1 mg %wt/vol) at 60°C for 6 hours. A 1-mL aliquot was taken from each sample, filtered through a 0.2-μm Whatman PTFE syringe filter (Florham Park, NJ), and injected into the chromatograph. The chromatographic purity of the resulting stressed solutions was analyzed by HPLC (chromatographic procedure detailed below under "Dissolution Performed Under Sink Conditions") to determine the extent of degradation and to identify the primary degradation products or impurities left over after drug synthesis.[23,24] The results of the forced repaglinide degradation study are shown in Table 1, with the USP standard chromatographic purity results given for comparison.

Preparation of URF Formulations

Repaglinide formulations were processed using URF technology. Repaglinide, which is poorly water soluble, was dissolved in 1,3-dioxolane (12.5% wt/vol), an organic solvent that is water-miscible. Water-soluble excipients in the formulations (SDS, TRIS) were dissolved in either water or a water/*t*-butanol (30:70 vol/wt) cosolvent system to produce a 0.01% to 0.02% wt/vol solids loading in the aqueous phase. *T*-butanol, with a melting point of 24°C to 27°C, was employed to raise the freezing point of the final drug solution. The 1,3-dioxolane and water/*t*-butanol solutions were mixed to produce a final solids content of ~1% wt/vol,

Table 2. Summary of URF Formulations*

Formulation	Components	Ratio
URF-A	REP:SDS	1:1
URF-B	REP:SDS:DEA	1:0.5:0.5
URF-C	REP:SDS:DEA	1:0.17:0.17
URF-D	REP:SDS:TRIS	1:0.5:0.5
URF-E	REP:SDS:TRIS	1:0.17:0.17
Co-ground physical mixture A	REP:SDS	1:1
Co-ground physical mixture D	REP:SDS:TRIS	1:0.5:0.5

*URF indicates ultra-rapid freezing; REP, repaglinide; SDS, sodium dodecyl sulfate; DEA, diethanolamine; TRIS, tromethamine.

and the resulting organic/aqueous cosolvent system was frozen on the cryogenic substrate maintained at –45°C. The frozen droplets were then removed from the substrate and lyophilized using a Virtis Advantage Tray Lyophilizer (Virtis Co, Gardiner, NY), removing all of the frozen solvent to produce a powder. The formulations were packaged in hermetically sealed glass containers under dry nitrogen. Table 2 shows the compositions investigated in this study, including the drug-to-excipient ratios.

Preparation of Co-Ground Physical Mixtures

Co-ground physical mixtures containing repaglinide (control) with varying proportions of excipients, corresponding to 2 of the URF compositions listed in Table 2, were mixed by geometric dilution in a mortar and pestle.

X-ray Powder Diffraction

A Philips 1710 x-ray diffractometer (XRD) with a copper target and nickel filter (Philips Electronic Inst, Mahwah, NJ) was used to obtain XRD results for the samples. Powders were mounted on aluminum stages with glass bottoms and smoothed to a level surface. The XRD pattern of each sample was measured from 10 to 50 degrees 2-theta using a step increment of 0.1 2-theta degrees and a dwell time of 1 second at each step.

Conventional Differential Scanning Calorimetry

Samples weighing 8 to 15 mg were added to an aluminum pan, which was then crimped and sealed. These samples were evaluated on a TA Instruments model 2820 differential scanning calorimeter (DSC; New Castle, DE). Samples were heated at a rate of 10°C/min from 25°C to 250°C using a conventional DSC ramping method. Samples were purged with nitrogen gas at 150 mL/min. Melting points and phase transitions were measured at the peak minimum of each DSC thermogram. The DSC was calibrated with an indium standard. The DSC was used to determine the melting point and purity of repaglinide bulk and standards (data not shown).

Scanning Electron Microscopy

A Hitachi S-4500 field emission scanning electron microscope (Hitachi, Ltd, Tarrytown, NY) was used to obtain scanning electron micrographs (SEMs) of the powder samples. Samples were gold-palladium sputter-coated for 30 seconds prior to viewing under an acceleration voltage of 5 kV.

Dissolution Performed Under Sink Conditions

Dissolution testing of the samples was performed according to the USP 27 Apparatus II (Vankel Model VK6010 Dissolution Tester with a Vanderkamp Model VK650A heater/circulator, Varian, Inc, Palo Alto, CA). Dissolution parameters were set up according to the USP monograph for repaglinide tablets, using 50 mM citric acid/100 mM sodium phosphate dibasic buffer with a pH of 4.5. Powder samples corresponding to a repaglinide content of 2 to 5 mg were introduced into 900 mL of dissolution medium. A sample volume of 5 mL was collected at 3, 7, 11, 15, 20, 25, and 30 minutes (n = 6) using a VK8000 autosampler (Varian, Inc, Cary, NC). There was no medium replacement. Paddle speed and bath temperature were maintained at 50 rpm and 37°C, respectively. The collected aliquots were filtered, then analyzed, with HPLC at a wavelength of 240 nm using a Shimadzu LC-10 liquid chromatograph (Shimadzu Corp, Columbia, MD) equipped with a Waters Symmetry C18 5-μm 4.6 × 150 mm reverse-phase column (Waters Corp, Milford, MA). The 10 mM phosphate mobile phase buffer consisted of 10 mM potassium phosphate monobasic dissolved in deionized water with the pH adjusted to 3.5 with o-phosphoric acid. The mobile phase was composed of the 10 mM phosphate buffer and acetonitrile in a 50/50 proportion. The repaglinide peak eluted with an average retention time of 6 minutes at a flow rate of 1 mL/min.[23] Sink conditions were maintained throughout the dissolution studies at <10% of solubility as determined in the pH solubility study.

Dissolution Performed Under Supersaturation Conditions

Supersaturated dissolution was performed using a small-volume dissolution apparatus equipped with a paddle stirring mechanism (Vankel VK6010 Dissolution Tester, Varian Inc, Palo Alto, CA).[26] Amounts of drug compositions were weighed out corresponding to ~25 times the aqueous solubility of repaglinide (~53 mg repaglinide in each sample). The dissolution medium was the same as described above (eg, 50 mM citric acid/100 mM sodium phosphate dibasic

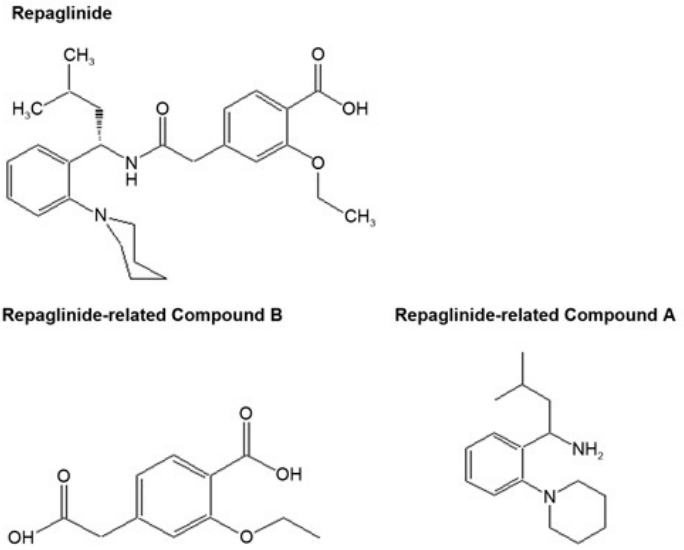

Figure 1. Structure of repaglinide, repaglinide-related compound B, and repaglinide-related compound A.

pH 4.5), using 100 mL of medium for this procedure. Paddle speed and bath temperature were maintained at 50 rpm and 37°C. A 1-mL aliquot of sample was taken manually at 2, 5, 10, 20, 30, 60, 120, and 1440 minutes, with no medium replacement. This aliquot was filtered through a 0.2-μm Whatman nylon filter, and a 0.5-mL aliquot of this solution was immediately diluted in 1 mL of acetonitrile and analyzed for repaglinide concentration using the HPLC procedure described above.

Results and Discussion

pH Solubility Study

Figure 1 shows the chemical structure of repaglinide along with some possible hydrolysis products predicted by Krishna Reddy et al.[24] Figure 2 shows the results of the pH solubility study in terms of solubility versus pH in the presence of SDS. Results from the pH solubility study showed much higher solubility of the drug at pH values greater than 7. Repaglinide exhibits 2 pK_a values of 4.19 and 5.78,[27] and being a weakly acidic compound, the drug is ionized at higher pH values, owing to its higher aqueous solubility at higher pH values. During the pH solubility study, it was observed that an insoluble mass was formed in the vials containing 0.1N HCl and 0.1M sodium phosphate pH 3 with 0.1% and 0.2% SDS. This instability resulted from the SDS's hydrolyzing to lauryl alcohol and sodium bisulfite in the acidic solution.[28] The un-ionized repaglinide could bind to or dissolve into the oily and immiscible phase that was present in the acidic aqueous solutions (lauryl alcohol), causing the formation of the water-insoluble mass in the vial. This phenomenon was observed in only the acidic solutions containing SDS and repaglinide. This insoluble mass was isolated and dissolved in the HPLC mobile phase and analyzed using the HPLC method described above for repaglinide and impurity content. These results are presented in Table 1.

All of the samples from the pH solubility study were analyzed by HPLC. Repaglinide was observed to be relatively stable under acidic conditions (1N HCl, 37°C, 1 hour). In samples exposed to higher pH (0.1M TRIS buffer, pH 9), impurity peaks were observed after 1 hour. Table 1 shows degradation of repaglinide in TRIS buffer after 1 hour. From these data, it can be concluded that repaglinide, although more soluble at higher pH, is also less stable at higher pH.

Forced Degradation Study

Results of the forced degradation study showed chemical instability under all stressed conditions, most notably under the base degradation condition (pH 12, 1N NaOH). Alkaline instability was expected because of the degradation of the drug that was observed in the pH solubility study. After 15 minutes of reaction time in 1N NaOH, repaglinide degraded more than 13%. It is hypothesized that some of the degradation observed in basic conditions results from the base-catalyzed hydrolysis of the amide bond in the drug molecule.[24] Oxidative conditions created by adding hydrogen peroxide to the drug solution, and thermal stresses caused by heating the solution, also yielded degradation peaks in the chromatograms. Repaglinide appears to be most resistant to degradation when exposed to acidic conditions. Few degradation peaks were observed in the HPLC chromatograms using 1N HCl at 25°C for 1 hour. Table 1 shows the results of the forced degradation study, including the amount of repaglinide remaining after forced degradation and retention times for degradants/impurities observed under accelerated conditions.

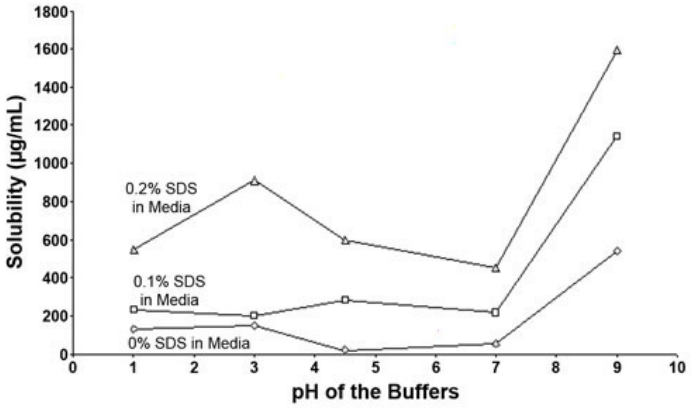

Figure 2. Solubility of repaglinide (μg/mL) in different pH buffers with differing percentages of SDS added to the buffer. SDS indicates sodium dodecyl sulfate.

Table 3. Potency and Impurity Analysis of REP and REP URF Formulations*

Sample	Total REP Recovered (%)	% Peak Area 1 (RRT = 0.58)	% Peak Area 2 (RRT = 0.71)	% Peak Area 3 (RRT = 0.78)	% Peak Area 4 (RRT = 1.29)	% Peak Area 5 (RRT = 2.32)	% Peak Area 6 (RRT = 3.45)	Total % Peak Area Impurity
URF-B	99.65	0.07	0.07	0.05	0.05	0.05	0.06	0.35
URF-C	99.62	0.05	0.06	0.08	0.07	0.06	0.06	0.38
URF-D	99.69	0.05	0.06	0.04	ND	0.05	0.04	0.24
URF-E	99.72	0.05	0.06	0.05	0.04	0.05	0.06	0.31
Bulk REP	99.54	0.09	0.07	0.07	0.07	0.08	0.08	0.46
USP REP reference standard	99.82	ND	0.07	0.04	ND	ND	0.05	0.16

*REP indicates repaglinide; URF, ultra-rapid freezing; RRT, relative retention time; ND, not detected; USP, US Pharmacopeia.

Solid State Characterization of Bulk Drug Substance

The melting point of repaglinide was 129.97°C, as measured by DSC (data not shown). This was similar to the reported melting point of 130°C to 131°C.[27] HPLC analysis of the bulk drug substance confirmed the potency at >99%, with few degradation peaks seen in the chromatograms (Table 3), when compared with the USP repaglinide reference standard. Degradation peaks accounted for less than 0.1% of the total repaglinide peak area individually and less than 0.5% of the total repaglinide peak area in total, conforming to USP specifications. SEMs showed that repaglinide existed in a long needle-shaped crystal habit, with a wide particle size distribution, the average particle size being 20 to 23 μm (Figure 3). X-ray diffraction of the bulk powder yielded characteristic crystalline peaks at 12.5, 15, 25.25, 27.85, and 35.85 2-theta.

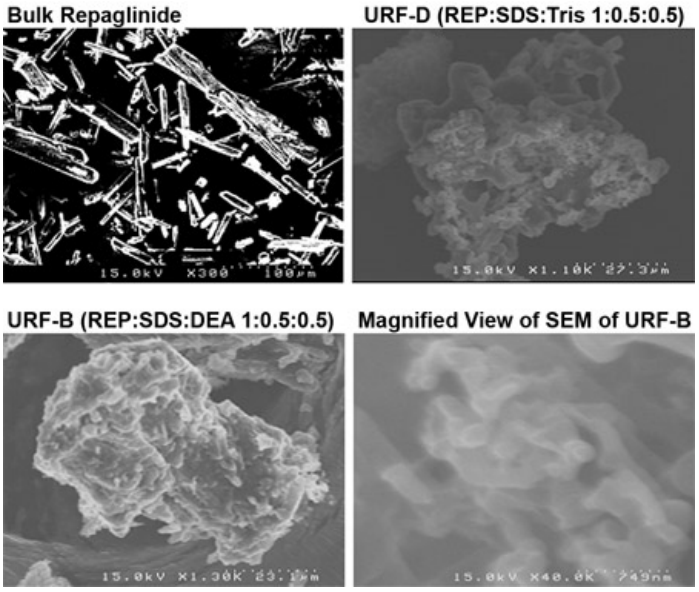

Figure 3. SEMs of REP and REP-URF formulations. URF indicates ultra-rapid freezing; REP, repaglinide; SDS, sodium dodecyl sulfate; TRIS, tromethamine; DEA, diethanolamine; SEMs, scanning electron micrographs.

Dissolution Results (Sink Conditions)

Dissolution rates of URF formulations, bulk drug substance, and repaglinide tablets were evaluated at sink conditions according to the USP monograph for repaglinide tablets. SEMs of URF formulations revealed a porous network of nanostructured aggregates with significantly reduced particle sizes over those of the bulk drug (Figure 3). The physical particle morphology, therefore, is responsible for the URF powders' having a higher dissolution rate than the bulk repaglinide drug substance did. Results of the sink dissolution testing are shown in Figure 4. Upon addition of bulk repaglinide to the dissolution medium, the powder did not wet but remained floating at the air-liquid interface. Incorporation of a wetting agent, SDS,[29-31] into the URF formulation (URF-A) resulted in rapid wetting of the powder upon addition to the dissolution medium, with the formulation rapidly sinking into the medium and wetting upon initiation of the dissolution test. The level of SDS studied during dissolution was ~2.5 mg SDS in 900 mL of dissolution medium, which is below the critical micelle concentration of SDS. Based on

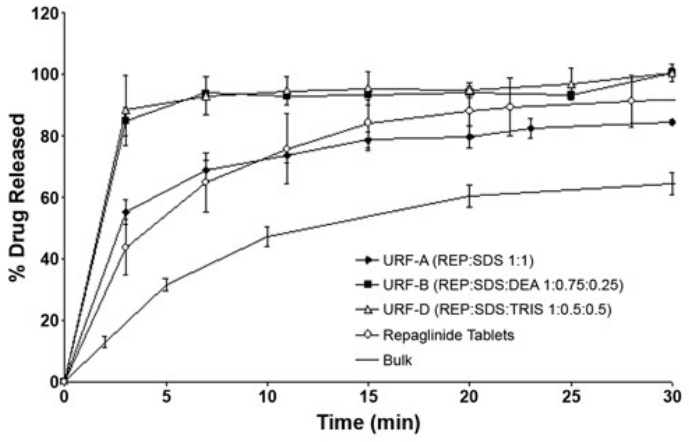

Figure 4. Dissolution performed under sink conditions: effect of adding an alkalizing agent to the URF formulation. URF indicates ultra-rapid freezing; REP, repaglinide; SDS, sodium dodecyl sulfate; DEA, diethanolamine; TRIS, tromethamine.

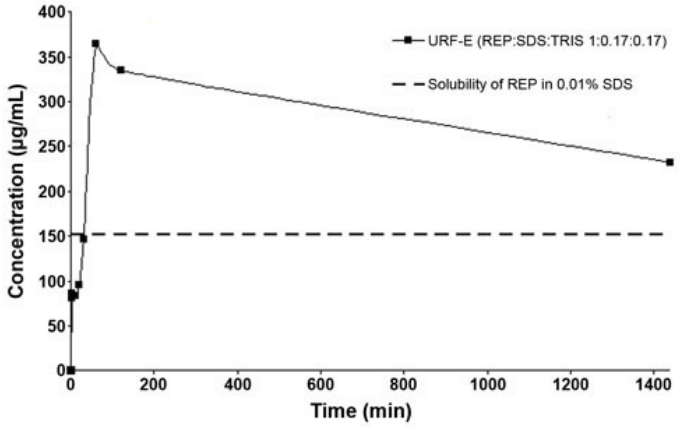

Figure 5. Dissolution of URF-E performed under supersaturated conditions, pH 4.5 citrate/sodium phosphate buffer, 50 rpm, 37°C, 100 mL, paddle method. URF indicates ultra-rapid freezing; REP, repaglinide; SDS, sodium dodecyl sulfate; TRIS, tromethamine.

results from the solubility study, a higher-pH medium would result in a greater driving force for dissolution because of an increase in the equilibrium solubility. In the case of pH 9, for example, the solubility would be increased by a factor of ~25 times the equilibrium solubility at pH 4.5. DEA and TRIS were employed as alkalizing agents in formulations URF-B and URF-E, respectively. When these alkalizing agents are added to the URF formulations, the dissolving drug particles should experience an increase in the surrounding microenvironmental pH, allowing for faster dissolution. Results shown in Figure 4 confirm a significant increase in dissolution rate compared with that of URF-A with SDS alone (no alkalizing agent).

Within the first 5 minutes, 90% and 88% of the repaglinide in URF-B and URF-E, respectively, was dissolved in the dissolution media, compared with ~60% and 55% for URF-A and repaglinide tablets, respectively, further indicating the advantage of an increase in local environmental pH. The bulk drug showed only ~30% drug release after 5 minutes, because of the poor wettability of the powder. Complete (100%) dissolution of URF-B and URF-E was observed after 30 minutes, while repaglinide tablets and URF-A showed only 90% and 85% release after 30 minutes, respectively. All formulations tested, including repaglinide tablets, showed an increase in dissolution over bulk repaglinide, which showed only ~63% release after 30 minutes.

Dissolution Results (Supersaturated Conditions)

The goal of the dissolution study at supersaturated conditions was to determine (1) the metastable solubility of amorphous URF repaglinide in 4.5 dissolution buffer and (2) the length of time repaglinide remained at a supersaturated concentration before recrystallization of the drug occurred.[32]

Figure 5 shows the results of the dissolution conducted under supersaturated conditions of formulation URF-E, which contained the alkalizing agent TRIS. URF formulations showed increased solubility of the amorphous drug in the dissolution medium, followed by slow reprecipitation over 24 hours. The equilibrium solubility of crystalline repaglinide in the dissolution medium (accounting for 0.01% SDS added to the medium from the formulation itself) was determined to be 150 µg/mL. URF-E quickly produced a supersaturated solution, achieving a maximum concentration of ~2.5 times the equilibrium solubility. The dissolution medium remained supersaturated over a 24-hour period, during which the concentration fell to only ~1.5 times equilibrium solubility. Since supersaturation is the main driving force for nucleation and growth, the elevated concentration produced during the first 60 minutes of dissolution led to reprecipitation of particles from solution. As can be concluded from these results, the amorphous form of repaglinide with an alkalizing agent and SDS as a surfactant present in the formulation was important in achieving and maintaining supersaturated levels of repaglinide in the dissolution medium.

Figure 6 shows the results of the supersaturated dissolution study of formulation URF-A, containing drug and surfactant (repaglinide:SDS, 1:1 ratio). Compared with the similar physical mixture, URF-A exhibited a much faster dissolution rate. Crystalline drug in the physical mixture approached the measured equilibrium solubility of the drug in dissolution medium (accounting for 0.03% SDS added to the medium from the formulation itself). The amorphous URF-A formulation, however, reached a maximum concentration of ~600 µg/mL within 20 minutes, corresponding to a supersaturation level of 2.4 times equilibrium solubility. Upon reaching the maximum supersaturation, the drug quickly

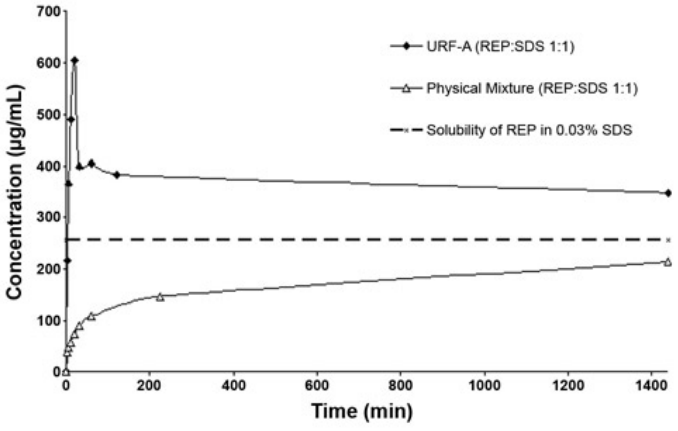

Figure 6. Dissolution of URF-A and REP/SDS physical mixture under supersaturated conditions: pH 4.5 citrate/sodium phosphate buffer, 50 rpm, 37°C, 100 mL. URF indicates ultra-rapid freezing; REP, repaglinide; SDS, sodium dodecyl sulfate.

reprecipitated out of solution, which reduced the supersaturation to ~1.5 times equilibrium solubility in less than 2 hours. Similar to formulation URF-E, in URF-A the SDS present in the drug powder was able to reduce the interfacial tension between the dissolution medium and the drug formulation, which allowed the amorphous drug to dissolve quickly and the solution to remain supersaturated at 1.4 times the equilibrium solubility even after 24 hours.

The ability of these formulations to supersaturate the dissolution media highlights the possibility for increased bioavailability from an amorphous drug form. The metastable solubility of an amorphous drug form may be as high as 100 times greater than that of its crystalline form.[33,34] If the concentration of drug in solution is significantly increased, the higher chemical potential will lead to increased flux across an exposed membrane,[35] resulting in blood levels that are much higher for an amorphous drug form than for an identical crystalline form. URF formulations presented in this work were highly amorphous and supersaturated buffered media up to 24 hours, showing physicochemical stability. These properties should lead to high concentration levels during gastrointestinal tract transit and thus significant improvement in bioavailability.

X-ray Diffraction

URF formulations were evaluated for their physicochemical stability. Samples of each formulation were tested after preparation, using x-ray diffraction (Figure 7). Formulations of amorphous solid solutions have inherent instability when certain wetting agents are used to enhance dissolution. The use of poloxamer polymers for their wetting ability has produced formulations with fast dissolution rates,[36,37] but these formulations tend to spontaneously recrystallize in the presence of humidity. The formulations presented in this study do not contain polymers but do show an amorphous character. XRD patterns of URF formulations show none of the characteristic crystallinity peaks observed in the bulk drug substance. The amorphous character of the dispersed repaglinide in the formulations is retained, and spontaneous recrystallization of the drug in the solid state is inhibited by using the URF process.

Chromatographic Purity and Potency

URF formulations were also evaluated for chromatographic purity. Repaglinide shows instability at pH values greater than 9, so base-catalyzed degradation was evaluated after URF formulations were produced. Impurities in each formulation were calculated by comparing the peak areas of each impurity peak against the peak areas of the repaglinide peak in the HPLC chromatograms. Results from chromatographic purity tests for selected formulations are shown in

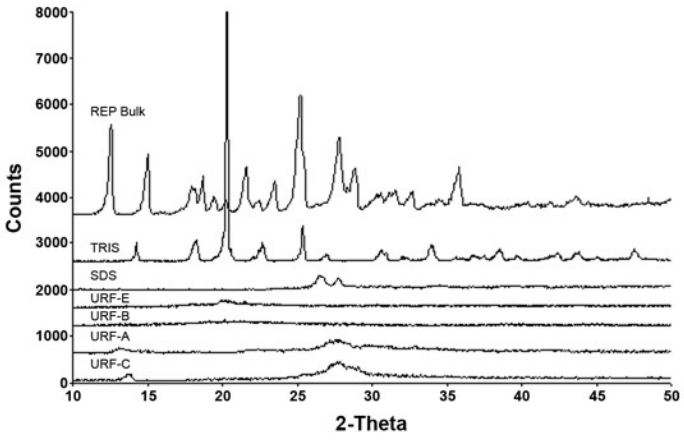

Figure 7. X-ray diffractograms of REP, excipients, and REP-URF formulations. REP indicates repaglinide; TRIS, tromethamine; SDS, sodium dodecyl sulfate; URF, ultra-rapid freezing.

Table 3. The formulations and the repaglinide standard had comparable percent impurities, probably residuals from drug synthesis. Therefore, further degradation of the repaglinide was not observed in the URF formulations, even though they contained an alkalizing agent. Rapid freezing rates allowed for the formation of solid solutions of the drug, reducing Brownian motion of the drug/excipient particles, making the formulations resistant to the degradation experienced by the drug in liquid solutions.[7]

CONCLUSIONS

Preformulation results showed instability of repaglinide to alkali conditions, but URF formulations containing alkalizing agents showed no increased amounts of degradation products. Additionally, these formulations showed a faster rate of dissolution and higher supersaturated dissolution concentration than did the equivalent physical mixtures, because of their amorphous character indicated by XRD results. The rapid freezing of the drug/cosolvent mixture produces a stable formulation, in addition to being a scalable and continuous process. Using URF technology, we were able to process repaglinide into stable formulations with high dissolution rates that should show greater bioavailability than the crystalline bulk drug.

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