Understanding the interactions of oleic acid with basic drugs in solid lipids on different biopharmaceutical levels.

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ABSTRACT

There has recently been increasing interest in understanding the impact of intestinal supersaturation on the absorption of poorly water-soluble drugs. Focus has been mostly on the effect of excipients on maintaining drug supersaturation. The aim of the this study was to explore the effects of drug-excipient interactions of an anhydrous formulation, when dispersed in simple buffer media and, in particular, focusing on precipitation kinetics. A solid lipid-based formulation comprising of PEG-32 stearate and an oleic acid (OA) (8:2 w/w) was developed using loratadine (pK_a = 5.3) and carvedilol (pK_a = 7.8) as the model drugs. UV/FTIR spectroscopy and viscometry were used to characterize the drug-OA molecular interactions in solution and the solid formulations were studied using x-ray diffraction, thermal analysis and van't Hoff solubility-temperature plots. Precipitation kinetics of the drug formulations was monitored in real-time in a phosphate buffer (pH = 6.5) using focused beam reflectance measurements. The addition of OA in the formulations resulted in a substantial increase in drug solubility. Although the drug-OA interactions appeared to be partially lost upon dispersion of the formulation, the extent of precipitation markedly decreased compared to the formulations without OA. A precipitation number (P_{nc}) was introduced as a ratio of a relevant residence time of drug in the gastrointestinal (GI) tract to the induction time (the onset of crystalline precipitation). Without OA, P_{nc} was already taking critical values (>1), while the anhydrous formulation was still below saturation for both drugs. The addition of OA resulted in amorphous rather than crystalline precipitates, which could be advantageous for drug re-dissolution and absorption. This study provides an improved understanding of OA and basic drug interactions on different levels of *in vitro* performance for more rational oral formulation development.

KEY WORDS: Drug-excipient interactions, basic drugs, oleic acid, supersaturation, precipitation kinetics, solid lipid-based systems

INTRODUCTION

Poorly water-soluble drugs (PWSD) present a challenge in drug development because of a typically reduced systemic exposure upon oral

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administration. A key approach to enhancing bioavailability of PWSD is to employ lipid-based formulations (LBFs) that may consist of oils, surfactants, and co-solvents (1). The latter excipients are often added to solubilize hydrophobic drugs, while primarily lipophilic compounds may be incorporated in oils alone. The high solvent capacity of an anhydrous formulation generally decreases upon formulation dispersion and digestion in the gastrointestinal tract (GI tract), thereby resulting in drug supersaturation. This supersaturated state is thermodynamically unstable and its extent constitutes the driving force for drug precipitation. In the last decade, there has been a growing interest in 1) the development of different types of supersaturating drug delivery systems (SDDS) (2, 3) that stabilize supersaturation, and also in 2) understanding the mechanisms by which LBFs generate supersaturation (4, 5, 6). Maintenance of drug supersaturation upon aqueous formulation dispersion has been demonstrated in several studies with the inclusion of polymers (7, 8), surfactants (9), and cyclodextrins (10) in drug formulations. If a sufficiently long and stable period of drug supersaturation can be achieved, intestinal drug absorption and bioavailability can be increased (11).

There are currently not many studies on drug supersaturation that are mechanistically oriented and thus there is a need to better differentiate the effects of the excipients on drug solubility (upon dispersion) from the influence on the kinetics of drug precipitation. There is a need for a better holistic understanding of drug-excipient interactions beginning with an anhydrous formulation all the way to the fate of the drug formulation, *in vitro* and *in vivo*. Such a systematic study of drug-excipient interactions has, as yet, not been reported for solid lipid-based formulations. This study was based on this approach examining excipient interactions all the way through to *in vitro* as outlined in Figure 1.

This study employs a solid lipid-based excipient that has recently been introduced for oral use. Polyethylene glycol 32 stearate (PEG-32 S) is a waxy lipid-based excipient, with a melting point at 48°C and Hydrophilic-Lipophilic Balance (HLB) of 16. It belongs to the group of polyoxyl stearates, including PEG-32 mono- and diesters of mainly stearic (octadecanoic), and/or palmitic (hexadecanoic) acid, and free PEG-32 (12). Since PEG-32 esters are hydrophilic surfactants and free PEG-32 is a hydrophilic solvent, this excipient alone would correspond to a type IV formulation in the Lipid Formulation Classification System (13).

The present study focused on the mechanistic understanding of oleic acid (OA) and basic drug interactions in anhydrous solid lipid-based formulations, and upon aqueous dispersion of these systems. In particular, drug-excipient interactions were studied with respect to drug precipitation kinetics. Since it has been demonstrated earlier that various hydrodynamics may have an impact on precipitation kinetics (14), a comparison between two different mixing techniques was carried out (magnetic stirring and standard USP 3 apparatus). A solid lipid-based formulation, comprising PEG-32 stearate and OA in an optimal ratio was developed as a model system. loratadine (pK_a = 5.3, log P = 3.9, intrinsic solubility = 3.31 μg/ml) (15, 16, 17) and carvedilol (pK_a = 7.8, log P = 4.1, intrinsic solubility = 0.512 μg/ml) (15, 16, 18) were selected as basic model drugs and were incorporated at different saturation levels in LBFs with, and without, OA. The formulations were dispersed in a phosphate buffer (0.1 M, pH = 6.5) and the precipitation kinetics was studied using a laser scanning technique, namely Focused Beam Reflectance Measurement (FBRM). Gao *et. al.* (19) were the first to use this technique to analyze drug precipitation. Compared with commonly used light scattering techniques (e.g. nephelometry), FBRM allows *in situ* monitoring of both the dimension and the number of precipitated particles. Finally, the concept of a biopharmaceutically relevant parameter, i.e. a

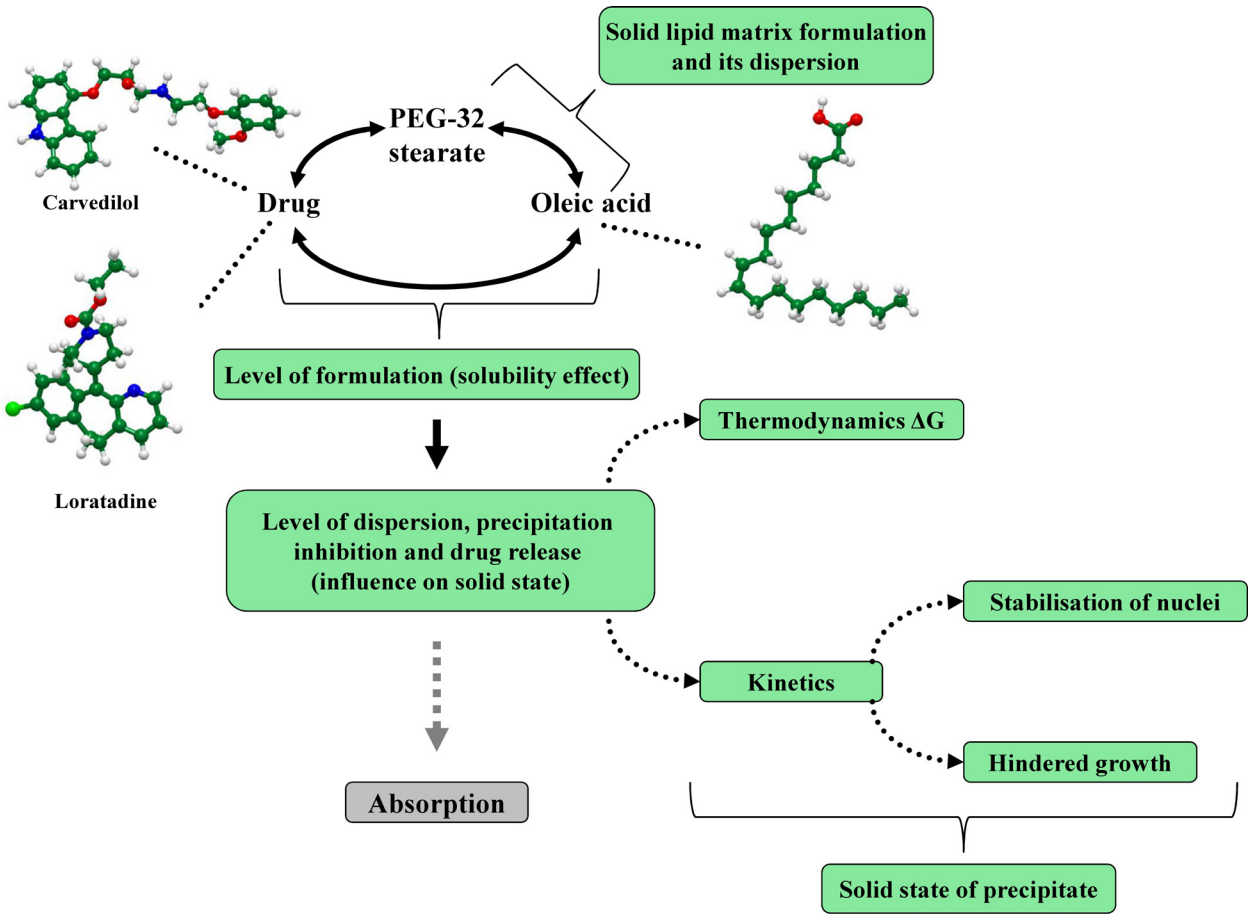

Figure 1 Scheme highlighting the different biopharmaceutical levels of the formulation *in vitro* performance.

Precipitation number (P_{nc}), by the ratio of a relevant residence time of drug in the GI tract to the induction time (the onset time of crystalline precipitation) is introduced.

MATERIALS AND METHODS

Materials

Loratadine and carvedilol were purchased from AK Scientific Inc. (Union City, CA, USA) and PEG-32 stearate was a gift from Gattefossé SAS (Lyon, France). Oleic acid vegetable oil (extra pure) was supplied by Merck (Darmstadt, Germany). Sodium phosphate monobasic anhydrous and sodium hydroxide (pellets) were obtained from Sigma-Aldrich (Steinheim, Germany), hydrochloric acid (0.5 M) from J. T. Baker (Deventer, Holland), and potassium phosphate monobasic from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile of high-pressure liquid chromatography (HPLC) grade was purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). The air-filled thermoplastic capsules (VegaGels®), used for drug release testing, were produced at the Aenova facility at Swiss Caps AG (Kirchberg, Switzerland).

Methods

Preparation of solid systems

Mixtures of PEG-32 S with OA at different weight ratios (10:0, 9:1, 8:2, 7:3, 6:4) were prepared by heating to 20°C above their melting points, mixing and cooling to room temperature (RT). Other ratios of PEG-32 S and OA did not solidify and remained liquid or

semisolid at RT, and were therefore not considered further in this study.

Characterization of solid systems

X-ray Diffraction (XRD)

XRD of PEG-32 S and of PEG-32 S/OA mixtures were analyzed at RT using a diffractometer Phaser D2 (Bruker, Karlsruhe, Germany) with a LYNXEYE detector and EVA software. The source of radiation was Co Kα at 30 kV, 10 mA, the measurement range was 5-50° 2θ using a step size of 0.02° and a step time of 0.5 seconds. The sample was automatically rotated on a holder at 15 RPM. An XRD and polarizing light microscopy (Olympus BX61, Tokyo, Japan) was additionally used to determine the solid-state properties of the precipitated drugs upon dispersion of the formulation in a phosphate buffer.

Differential Scanning Calorimetry (DSC)

The thermal characteristics of PEG-32 S and of the solid mixtures were determined using a differential scanning calorimetry (DSC 8500, Perkin Elmer, Santa Clara, CA, USA). The samples (at an average weight of 8 mg) were accurately weighed in hermetically sealed aluminum pans (50 µl), held at an initial temperature (20°C) for 5 minutes, then heated at a rate of 10°C min^{-1} from 20°C to 80°C under nitrogen gas purging (20 ml/min). A DSC was also used to examine the drug-loaded formulations (saturation level of PEG-32 S or of PEG-32 S/OA 8:2 w/w, S = 1.5, i.e. 150% w/w of drug equilibrium solubility at 37°C) to detect any crystalline drug.

Particle size measurements following aqueous dispersion of solid systems

The PEG-32 S and PEG-32 S/OA mixtures were dispersed in 0.025 M HCl (pH = 1.6), or in a phosphate buffer (0.1 M, pH = 6.5) at 37°C to measure particle size. Dynamic laser light scattering was used with a Zetasizer Nano ZS (Malvern, Worcestershire, United Kingdom). Each sample was accurately weighed in a dust-free glass vial and 20 g of the dispersion medium (heated to 37°C) was added (1:200 w/w). Prior to the measurements, the dispersions were mixed for 10 minutes at 100 RPM in a water bath (37°C) and passed through a coarse filter (0.45 µm) to ensure the absence of dust particles. The mean particle size (Z-average diameter) of each dispersed sample was calculated from the volume size distribution. For these measurements aqueous viscosity provided a good approximation of the diluted dispersions. All experiments were repeated in triplicate using fresh samples. This method was also used to examine the particle size of the dispersed drug-loaded PEG-32 S/OA (8:2 w/w) formulations (at saturation level of PEG-32 S/OA 8:2 w/w, S = 0.8).

Spectroscopic and rheological characterization of drug-excipient molecular interactions

UV spectroscopy

The UV spectra of OA and of the drug-OA mixtures were scanned using a SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA, USA). Both loratadine and carvedilol were mixed in dark glass vials with OA at 80% w/w of their S_{eq} (equilibrium solubility in OA at 37°C), purged with nitrogen, and left for one hour at 37°C while magnetically stirring (300 RPM). An aliquot (300 µl) of each sample was added to a 96-well microplate and UV spectra were recorded over a wavelength range of 200-700 nm.

Mechanical chip-based rheology

The viscosity profiles of the drug-OA mixtures were analyzed using a new mechanical chip-based (MEMS) capillary rheometer (mVROC™ RheoSence, San Ramon, CA, USA). It measures the viscosity from the pressure drop of a sample as it flows through a rectangular slit. Each drug was dissolved in OA in dark glass vials at concentrations of 0%, 5%, 10%, 15%,

20%, and 25% (w/w). The mixtures were purged with nitrogen and left for one hour at 37°C using magnetic stirrers at 300 RPM. A glass syringe (Hamilton 81260 SYR 500 µl) was loaded with the sample and placed inside a thermal jacket (25±0.5°C). When the temperature was stable, the sample was pumped to flow (at a shear rate of 500 s^{-1}) through the channel of the chip. The pressure drop was detected by a sensor (cell m-VROC A-10) and the viscosity was calculated using the m-VROC Control Software™. All experiments were carried out in triplicate.

FTIR

The infrared spectra of the drugs, OA, and drug-OA mixtures (prepared as described previously) were recorded using a Diamond ATR (attenuated total reflection) accessory (MKII Golden Gate™, Specac Inc, Woodstock, GA, USA) using a Bio-Rad Excalibur FTS 3000 MX spectrophotometer (Bio-Rad, Cambridge, MA, USA). The analysis depth of the surface was approximately 1 µm and all spectra were calculated from 32 scans (each containing 2038 points), in the wavelength number range of 645-4000 cm^{-1}. Data were acquired with the Resolutions Pro software v. 5.2.0 (Agilent Technologies, Santa Clara, CA, USA).

Testing of the anhydrous drug-loaded solid systems

Van't Hoff solubility study

The PEG-32 S/OA mixture (8:2 w/w), which upon dispersion, resulted in the smallest particle size with lowest polydispersity index (PDI) was selected for the solubility study. Drug solubility was determined at different temperatures (55°C, 60°C, 65°C, and 70°C), and the value at 37°C was extrapolated using van't Hoff plots. An excess amount of loratadine or carvedilol was added to dark glass vial containing approximately 5 g of melted vehicle. The vials were tightly closed with rubber cap under nitrogen purging (2 minutes). The mixtures were agitated using magnetic stirrers at 300 RPM in water baths at controlled temperatures for 72 hours. Drug solubility was measured after 24, 48, and 72 hours to ensure that equilibrium was reached. Samples were withdrawn, filtered through 0.45 µm PVDF syringe filters (Titan3, SMI-LabHut LTD, Gloucester, UK), and diluted with a phosphate buffer (20 mM, pH = 2.5 in weight ratios) for HPLC analysis. All solubility values were determined in triplicate.

The solubility of loratadine in OA was measured directly at 37°C, and these samples were prepared as for the drug solubility studies in the vehicles. In contrast, it was not feasible to determine the solubility of carvedilol in OA at 37°C using magnetic stirring in glass vials, due to the high viscosity of the mixture. Therefore, an excess amount of carvedilol was added to Eppendorf® tubes (1.5 ml) containing OA, and the mixtures were shaken using a Thermomixer comfort (Eppendorf AG, Hamburg, Germany) at 1400 RPM and 37°C for 72 hours. Every 24 hours, the samples were centrifuged (n = 3) at 16 100 x g (37°C, 30 minutes) using a Centrifuge 5415 R (Eppendorf AG, Hamburg, Germany). Finally, the concentration of the compound in the supernatant was determined by HPLC analysis following dilution.

The maximum supersaturation ratios, SR^M, of the dispersed formulations in phosphate buffer (pH = 6.5) were calculated according to $SR^M = C/C^*$, where C is the maximum concentration of solubilized drug and C^* is the solubility in the dispersion of formulation in phosphate buffer (1:100 w/w), respectively (20).

Drug loading of solid systems

The drug-loaded systems were prepared as follows. The PEG-32 S and PEG-32 S/OA mixture (8:2 w/w) were heated to 20°C above their melting point, and added to the previously weighed drug in dust-free dark glass vials. Following a heating phase (1 hour at 80°C agitated using a magnetic stirrer), the samples were cooled to RT (21). Drug loads are

expressed as saturation levels (*S*) of PEG-32 S or of PEG-32 S/OA (8:2 w/w), referring to the equilibrium solubility of loratadine or carvedilol (in the formulation at 37°C), respectively.

Zeta potential measurements of dispersed drug-loaded solid systems

For measurements of zeta potential a Zetasizer Nano ZS (Malvern, Worcestershire, United Kingdom) was used. The PEG-32 S/OA (8:2 w/w), drug-free and drug-loaded (at 80% w/w of S_{eq}), were dispersed (1:200 w/w) in 0.025 M HCl (pH = 1.6) or in a phosphate buffer (0.1 M, pH = 6.5) at 37°C for 10 minutes at 100 RPM. Each dispersed sample was passed through a coarse filter (0.45 μm), poured into a transparent cuvette with electrodes at each end, and inserted in a slit through which a laser was beamed. An electric field was applied to the cell and the Zetasizer measured the electrophoretic mobility of particles. The zeta potential was calculated and all measurements were carried out in triplicate at 37°C.

Drug precipitation testing upon dispersion and release from capsules

Drug precipitation upon aqueous dispersion

Drug precipitation upon dispersion in the phosphate buffer (0.1 M, pH = 6.5; 1:100 w/w) was analyzed using a Lasentec FBRM D600L probe (Lasentec, USA). This laser scanning technique uses an in-process probe, which detects the chord length distribution of particles. The general measurement principle of focused beam reflectance measurements (FBRM) was described by Ruf *et al.* (22). The detection is limited to particles with a chord length of 1 μm. For calculation of the induction time, only chord lengths of less than 10 μm were monitored in order to follow the number of evolving particles over time, while avoiding the noise from counts of aggregated particles. In contrast to that, when the effect of OA on precipitation was studied, all chord lengths and their particle size distributions (PSD) were considered. Both PEG-32 S and PEG-32 S/OA (8:2 w/w) were loaded with loratadine or carvedilol at the following saturation levels of PEG-32 S or of PEG-32 S/OA (8:2 w/w): 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.3, 1.5, and 1.7. Due to the ranges of interest, these saturation levels were non-equally distributed. The probe was positioned at an angle of 30° in a thermostated glass vessel (37°C) and the accurately weighed drug formulation was added to the dispersion medium. The dispersion was stirred at 500 RPM and the particle size of precipitated drug was recorded every 2 seconds for 8 hours using the iC FBRM software v. 4.0 (Mettler-Toledo AutoChem, Columbia, MD, USA). All formulations were dispersed in a phosphate buffer (pH=6.5) and measured in triplicate. From each precipitation curve, the onset time of crystalline precipitation (induction time, τ_{ind}) was determined at the crossing point of the tangent of the precipitation curve and the time axis. Subsequently, a Precipitation number (P_{nc}) was defined for each saturation level of PEG-32 S or of PEG-32 S/OA (8:2 w/w) using Equation 1:

$$P_{nc} = t_{res}/\tau_{ind} \qquad \text{Eq. 1}$$

where t_{res} is a relevant drug residence time in the GI tract as approximated by Amidon *et al.* at 180 minutes (23).

Drug release testing

Compendial release testing from soft capsules was determined for a potential effect of the different hydrodynamics or of the shell material on drug precipitation kinetics. Drug formulations (PEG-32 S and PEG-32 S/OA (8:2 w/w)) loaded with loratadine or carvedilol were studied both at a saturation level of 1.5. A reciprocal cylinder BioDis® apparatus (RRT 8, CALEVA Ltd, Dorset, England) was thermostated at 37 ± 0.5°C. Each vessel contained 200 ml of phosphate buffer (0.1 M, pH = 6.5). A mesh size of 420 μm was selected (top and bottom mesh) for the glass cylinders that were dipping at a rate of 20 dpm. Two manually filled soft capsules were placed in

each glass cylinder, due to their lower fill mass (~ 0.6 g) compared to the capsules obtained from a machine-filling process (~ 1.0 g). Prior to each experiment, the air-filled capsules were weighed before and after manual filling with the drug formulations to determine the exact fill mass in each capsule. At predetermined time points, a 1 ml sample was taken using a syringe, immediately filtered through a 0.45 µm PVDF syringe filter (Titan3, SMI-LabHut LTD, Gloucester, UK) and analyzed using HPLC. The removed volume was replaced each time with 1 ml of fresh medium. The total time of analysis was 180 minutes to approximate the relevant residence time of the drug in the GI tract. All experiments were performed in triplicate.

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography analysis employed a LiChrospher 60, RP select B 125-4 (5 µm) column (Merck, Darmstadt, Germany), a degasser (G1379 B), an isocratic pump (G1310A), an autosampler (G1329A), and a variable wavelength detector (G1314B). The mobile phase consisted of acetonitrile and 20 mM phosphate acetate buffer, pH = 2.5 (40:60, v/v). Flow rate was 1 ml/min with the injection volume of 10 µl and the detection wavelengths were 248 nm and 280 nm for loratadine and carvedilol, respectively. The retention times were 6 minutes for loratadine and 4 minutes for carvedilol, respectively.

Data analysis

The temperature-dependent drug solubility in solid systems (PEG-32 S and PEG-32 S/OA (8:2 w/w)) was analyzed at different temperatures with n = 3. The Statgraphics Centurion XVI ed. Professional Program from Statpoint Technologies Inc. (Warrenton, Virginia, USA) was used for the van't Hoff linear regression and prediction of drug solubility at 37°C. A good correlation was assumed if the R^2 value was greater than 0.99.

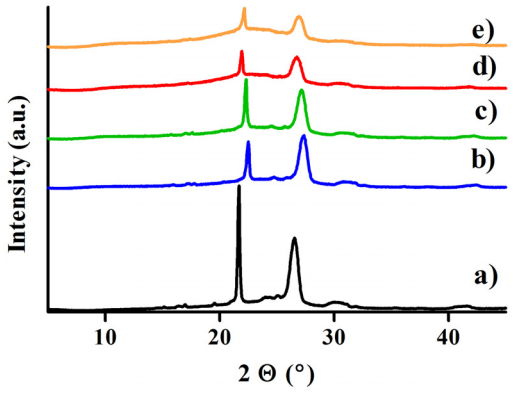

Figure 2 X-ray diffraction patterns of the solid systems containing different concentrations of oleic acid in PEG-32 S/OA mixtures (w/w). Oleic acid concentrations: (a) 0%, (b) 10%, (c) 20%, (d) 30%, and (e) 40% (w/w).

RESULTS

Characterization of solid drug-free systems

In preliminary experiments, OA was added to PEG-32 S at different w/w ratios to determine the physical consistency of the mixtures at RT. Visual observation of the mixtures (stored for two days at RT) indicated that when the concentration of OA was not greater than 40% w/w, the mixtures solidified and no phase separation occurred, whereas higher concentrations of the liquid OA were deemed as less suitable for a solid lipid-based formulation.

X-ray Diffraction (XRD)

Figure 2 shows the x-ray diffractograms of PEG-32 S and PEG-32 S/OA mixtures. The presence of peaks in the mixtures at the same angle (2θ) as those of PEG-32 S confirmed that the crystallinity of PEG-32 S was maintained in the formulations. A gradual decrease in peak heights was observed as the concentration of OA increased. This can be interpreted as lowering the concentration of crystalline lipid phase by increasing the amount of OA. The extent of crystallinity of PEG-32 S in PEG-32 S/OA mixtures could be approximated by the crystallite size(s). The determination of

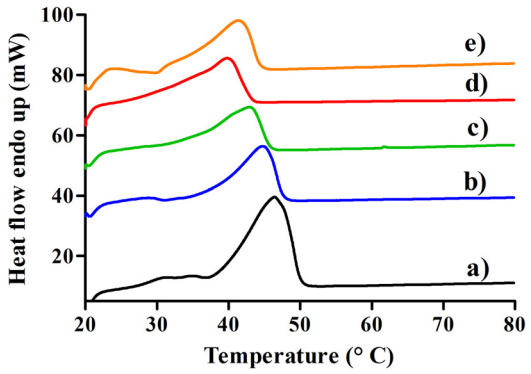

Figure 3 Differential scanning calorimetry (DSC) thermograms of the solid systems containing different concentrations of OA in PEG-32 S/OA mixtures (w/w). Oleic acid concentrations: (a) 0%, (b) 10%, (c) 20%, (d) 30%, and (e) 40% (w/w).

crystallite size(s) from XRD patterns are based on the widths of the diffraction peaks. Due to the complexity of added OA, this task was beyond the scope of the present work. However, observations made by Patel *et al.* suggested that the addition of OA resulted in the decrease of the crystallite size (24).

Differential Scanning Calorimetry (DSC)

The DSC thermogram of PEG-32 S exhibited a characteristic endothermic peak at 47°C (Figure 3 a). A gradual decrease in the onset of melting endotherms was observed, as the concentration of OA in the mixtures with PEG-32 S increased (Figure 3). Similar changes were observed for the melting peak maximum of the mixtures.

Particle size measurements following aqueous dispersion of solid systems

To select the optimal ratio of PEG-32 S and OA, aqueous formulation dispersions were considered. A minimal particle size and polydispersity index of prepared dispersions of PEG-32 S/OA mixtures were viewed as desirable from a biopharmaceutical viewpoint. Two aqueous media were selected that were mimicking an acidic stomach environment and an intestinal pH. As shown in Figure 4, PEG-32 S : OA w/w ratio had a substantial influence on both particle size and polydispersity. The dispersed mixture with 20% w/w OA exhibited the smallest particle size, as well as, the lowest polydispersity, in both dispersion media and

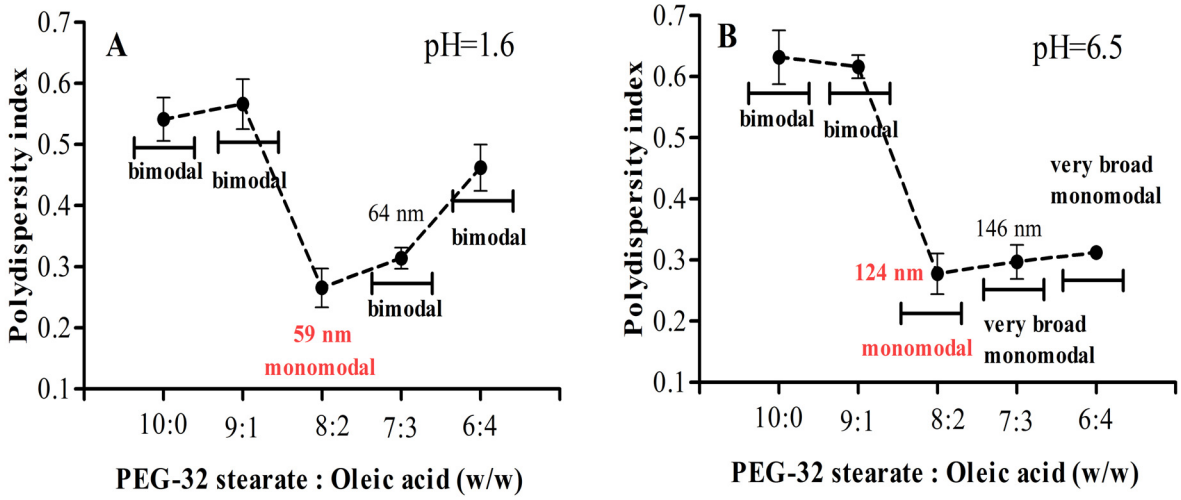

Figure 4 Effect of PEG-32 S : OA ratio on particle size and polydispersity index (PDI) analyzed in (A) 0.025 M HCl (pH = 1.6) and in (B) phosphate buffer (pH = 6.5) (n = 3). The particle size is specified only for systems with a PDI ≤ 0.3.

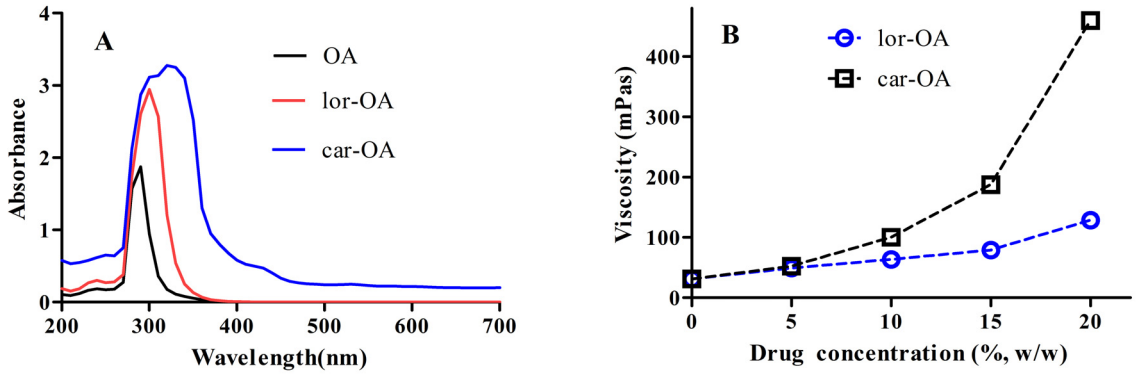

Figure 5 Shift of the maximum in UV spectrum and a broadening of the UV bands (A), and increase in viscosity profiles (B) indicate the presence of loratadine-OA and carvedilol-OA molecular complexes (n = 3, error bars are within markers).

was therefore selected for further evaluations.

Characterization of solid drug-loaded systems
Drug-excipient interaction in oily mixtures

Loratadine and carvedilol typically absorb in organic solvents (e.g. methanol) at 288 nm and at 242 nm, respectively (25, 26). In the present study both drugs were dissolved in OA and a considerable shift in the peak maxima and a broadening of the UV bands, compared to the pure OA was shown (Figure 5A). This indication of a molecular interaction between the excipient and the basic drug was also supported by a rheological study. An increase in viscosity was noted for increasing amounts of drug (Figure 5B). The oily system was probably becoming a partially organized system due to the interaction, which was more pronounced in the case of the carvedilol- compared to loratadine-OA solutions. These findings indicated a molecular complex in oily mixtures.

The IR spectrum of OA showed a characteristic vibrational band at 1700 cm^{-1} (C=O stretching vibrations) and at 3000-2700 cm^{-1} (O-H stretching vibrations) (Figure 6). No changes for the hydroxyl group band were observed in either spectra of drug-OA molecular complexes (lor-OA and car-OA). In contrast, the drug-OA interaction could be assumed from the changes of both drug spectra. In the lor-OA spectrum a shifting and broadening of the characteristic bands at 1200 cm^{-1} and 1700 cm^{-1} was noted, representing the stretching vibrations of C-O and C=O, respectively. Broadening/shifts of bands around 1200 cm^{-1} could also indicate changes in the amine (1250-1020 cm^{-1}) and aromatic amine (1342-1266 cm^{-1}) groups of loratadine. Additionally, changes in the 1700 cm^{-1} region could be attributed to the stretching of the C=N group. This could suggest that the "basic" groups of the drug also interacted with OA, most probably through hydrogen bonding with the carboxyl groups of OAs. For carvedilol, the relevant N-H bonds at 1600 cm^{-1}

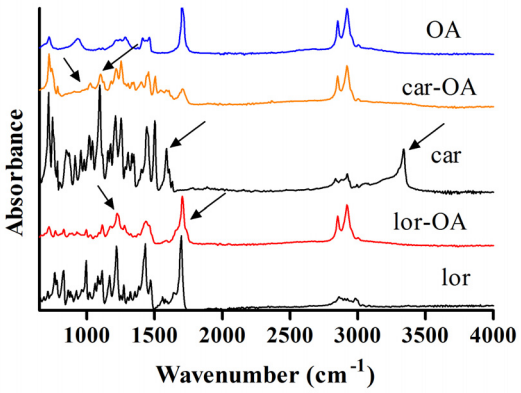

Figure 6 FTIR spectra of loratadine, carvedilol, OA and of their binary drug-OA mixtures (bands of interest are indicated by arrows).

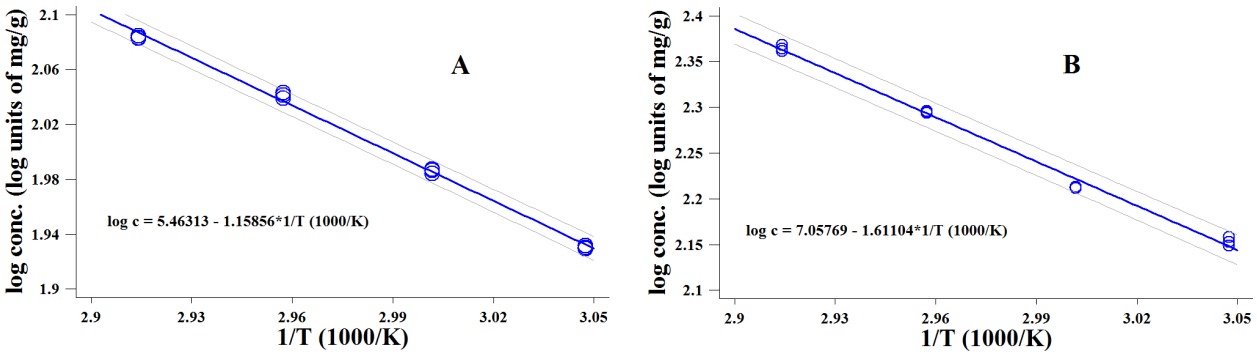

Figure 7 Plot of van' t Hoff model of loratadine solubility in (A) PEG-32 S (linear regression, R^2= 0.9969; *P*<0.05) and in (B) PEG-32 S/OA (8:2 w/w) (linear regression, R^2= 0.9944; *P*<0.05). Outer lines show 95% prediction limits.

and at below 3500 cm^{-1} practically disappeared in the car-OA spectrum. Moreover, the car-OA spectrum displayed the changes in the region between 1200 cm^{-1} and 1000 cm^{-1} that correspond to C-O stretching in ethers and primary alcohols. Both changes strongly indicate the interaction between ether and amino groups in the carvedilol and carboxyl group of OA.

Solubility study

PEG-32 S and PEG-32 S/OA (8:2 w/w) were solid at RT, as well as, at 37°C. To determine drug solubility in these solid systems, it was measured at elevated temperatures and extrapolated to lower temperature using the van't Hoff model (27) (Figures 7 and 8). The extrapolated solubility at a temperature of 37°C was of interest regarding the biopharmaceutical fate of the formulation.

Table 1 shows the extrapolated drug solubility values in PEG-32 S and PEG-32 S/OA (8:2 w/w), as well as the drug solubility in OA determined at 37°C. OA demonstrated remarkable drug solubility for both loratadine and carvedilol.

Table 1 Comparison of carvedilol and loratadine solubility in PEG-32 S, OA, and PEG-32 S/OA (8:2 w/w) at 37°C

	SOLUBILITY (mg/g)	
	Carvedilol	**Loratadine**
PEG-32 S *	117.7 ± 4.8	53.4 ± 1.7
OA	372.8 ± 11.3	253.2 ± 0.5
PEG-32 S/OA (8:2 w/w)*	153.9 ± 6.5	73.1 ± 4.3

* extrapolated by linear van't Hoff regression from higher temperatures

Following the solubility study, drug-loaded formulations at different saturation levels of

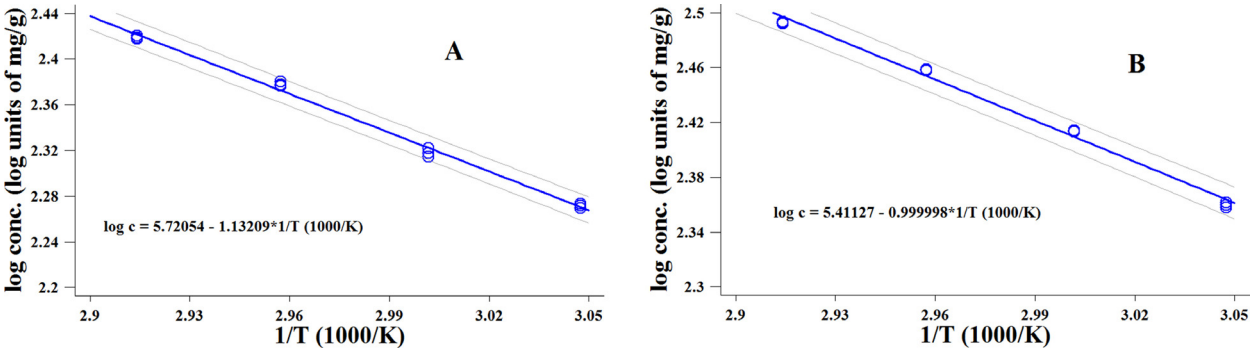

Figure 8 Plot of van' t Hoff model of carvedilol solubility in (A) PEG-32 S (linear regression, R^2 = 0.9944, *P*<0.05) and in (B) PEG-32 S/OA (8:2 w/w) (linear regression, R^2 = 0.9927, *P*<0.05). Outer lines show 95% prediction limits.

PEG-32 S or of PEG-32 S/OA (8:2 w/w) were prepared. The formulations loaded with drugs at supersaturated level (S = 1.5) were analyzed using DSC for the presence of crystalline drugs. The characteristic drug melting peaks in all tested formulations were not observed (Figure 9), which confirmed the absence of crystalline drug.

Drug-loaded PEG-32 S/OA formulations (S = 0.8) were analyzed for particle size following aqueous dispersion. In contrast to the dispersed drug-free formulation, an increased polydispersity index (PDI = 0.4 for pH = 1.6, and PDI = 0.32 for pH = 6.5) and similar Z-average were observed for all examined dispersion. This indicated that the presence of drug probably disturbed the optimized structure of the drug-free system.

Zeta potential measurements of dispersed drug-loaded solid systems

The zeta potential of the dispersed systems was studied to explore the drug-excipient interactions on this biopharmaceutical level (Table 2). Loratadine is a weak base (pK_a = 5.3) and was expected to have a neutral form in a phosphate buffer (pH = 6.5). The zeta potential of the dispersed lor-PEG-32 S/OA in a phosphate buffer was negative. The result was similar to the negative zeta potential of the drug-free formulation under the same conditions. In contrast, the dispersed

Table 2 Zeta potential of drug-free and drug-loaded PEG-32 S/OA (8:2 w/w), dispersed (1:200 w/w) in phosphate buffer (0.1 M, pH = 6.5) and 0.025 M HCl (pH = 1.6) at 37°C

	Zeta potential (mV)		
	PEG-32 S/OA (8:2 w/w)	PEG-32 S/OA (8:2 w/w) loaded with loratadine (S 0.8)	PEG-32 S/OA (8:2 w/w) loaded with carvedilol (S = 0.8)
Phosphate buffer (pH = 6.5)	-7.9 ± 1.0	-6.6 ± 1.5	-0.1 ± 0.1
0.025 M HCl (pH = 1.6)	-0.8 ± 0.5	3.9 ± 0.5	11.2 ± 0.4

carvedilol-loaded formulation (car-PEG-32 S/OA; carvedilol pK_a = 7.8, OA pK_a = 9.85 (28)) resulted in a neutral zeta potential in the phosphate buffer. A positive zeta potential was determined in 0.025 M HCl for both drug formulations, which was in line with the expected protonation and charge of the basic drugs at such a low pH. For the drug-free system dispersed in the acidic medium (0.025 M HCl) zeta potential was almost neutral.

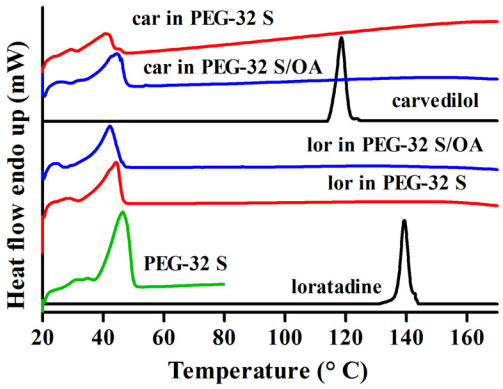

Figure 9 Differential scanning calorimetry (DSC) thermograms of the supersaturated solid lipid-based formulations (S = 1.5) show the absence of the crystalline drug 2 hours upon loading.

Drug precipitation testing upon dispersion and release from capsules

Drug precipitation upon aqueous dispersion

For the preliminary testing, an acidic medium (pH=1.6) was used for the dispersion and analysis of drug precipitation of both drug-loaded formulations (PEG-32 S and PEG-32 S/OA (8:2 w/w)). During the first 30 minutes upon dispersion, no drug precipitation was observed for either drug-loaded formulation. The higher pH of a simulated intestine (phosphate buffer, pH=6.5) was therefore selected, which is more challenging regarding drug supersaturation and reflects the relevant site of drug absorption. As a control, drug-free PEG-32 S and PEG-32 S/OA (8:2 w/w) were also dispersed, but either no FBRM signal or a very low signal (up to 50 counts) was detected. The drug-containing dispersion samples were analyzed to determine the induction times that marked the onset of drug precipitation. Figure

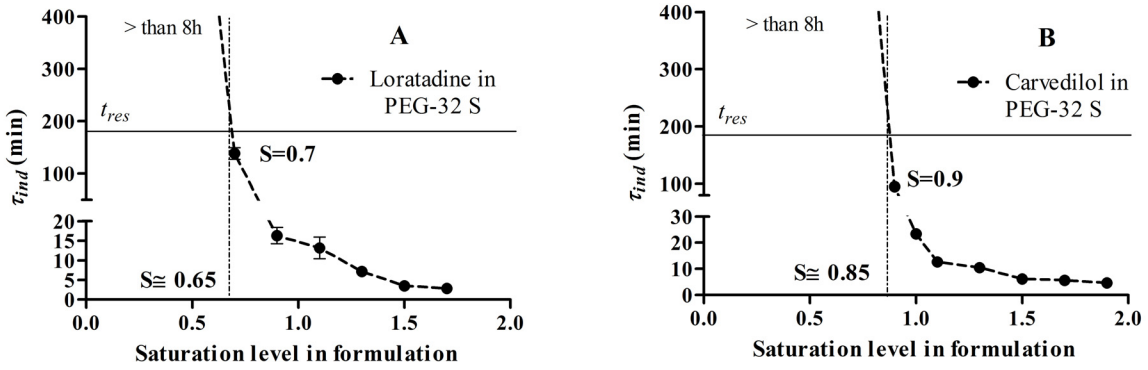

Figure 10 The effect of different saturation levels in the anhydrous formulation of (A) loratadine and (B) carvedilol on the induction times upon dispersion (pH = 6.5). The horizontal line presents a relevant residence time t_{res} (180 min) of the drug in the gastrointestinal tract. The vertical line determines the critical saturation level in the formulation for which drug precipitation starts within t_{res}.

10 shows the results for the dispersions of the formulations without OA. A decrease in the induction time was observed for both drugs with increasing drug saturation. For loratadine loaded in PEG-32 S, the lowest saturation level of PEG-32 S in the anhydrous formulation to exhibit precipitation upon dispersion was 0.7 (considering a measurement limit of 8 hours). The corresponding results for carvedilol showed precipitation detected within the observation time only at a (formulation) drug saturation of 0.9 and beyond. Considering the physiologically relevant residence time of 180 minutes, it was possible to extrapolate a critical formulation saturation level of PEG-32 S at which drug precipitation is expected. These critical values were about 0.65 for the loratadine formulation and 0.85 for the PEG stearate mixture with carvedilol.

Subsequently, dispersions of PEG-32 S/OA (8:2 w/w) were analyzed to explore the effects of OA on drug precipitation. Formulations of both drugs did not show any precipitation in the range of unsaturated formulations. The marked effect of OA as a potential precipitation inhibitor was also of interest to explore in supersaturated systems. A reference drug saturation of 1.5 was selected and FBRM results of the dispersions can be inferred from Figure 11. The particle size distribution (PSD) profiles from the supersaturated systems indicated that the incorporation of 20% w/w of OA lowered the extent of drug precipitation but could not prevent it entirely. This effect was more pronounced for the carvedilol formulations, as shown in Figure 11B. The chord length counts were greatly reduced in the formulation containing OA and the maximum particle size may have slightly shifted to ~10 μm (at t = 3 hours). For loratadine, particle size distributions appeared to be very similar for both tested formulations (Figure 11A). The addition of OA also demonstrated a reduction of the extent of drug precipitation in this system.

The reference formulation at a drug saturation level of PEG-32 S or of PEG-32 S/OA (8:2 w/w) of 1.5 was further explored for time evolution of particle counts. Counting primarily short chord lengths (< 10 μm) was focused on the early particle growth phase with minimal noise from potential aggregates. Interestingly, a spontaneous nucleation was observed for both drugs loaded in PEG-32 S/OA (8:2 w/w). Figure 12A shows the precipitation kinetics of the dispersed loratadine formulation (S = 1.5). A high initial count rate was detected that slowed down to a much lower plateau

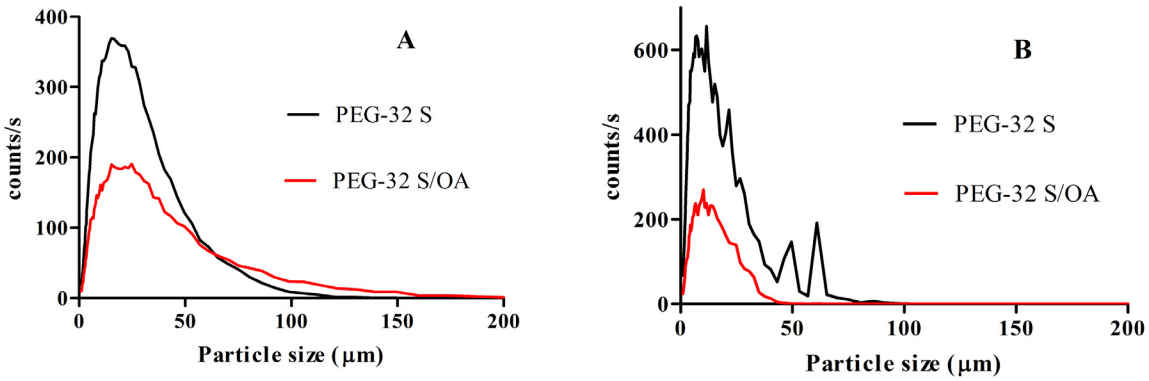

Figure 11 Mean particle size distribution (PSD) profiles observed at t = 3h upon dispersion of solid lipid-based formulations loaded with (A) loratadine and (B) carvedilol at S = 1.5.

compared to the formulation without OA. Also carvedilol in PEG-32 S/OA (8:2 w/w) (S = 1.5) demonstrated only a high initial rate that was followed by almost constant count rates after about 20 minutes. The count rate plateau from this OA-containing formulation was again substantially lower than observed with carvedilol-loaded PEG-32 S. Interestingly, these mixtures of PEG stearate and drugs did not precipitate spontaneously, but appeared to have short induction times. Therefore OA influenced the extent, as well as, kinetics of drug precipitation.

For oral drug delivery, it is also important to analyze the solid-state properties of the precipitated drugs. Figure 13 shows the X-ray diffraction patterns of the precipitates and of crystalline drugs as used in the solid formulations (S = 1.5, at t=3 hours). These X-ray patterns demonstrated that both drugs maintained their crystallinity following the precipitation of the dispersed drug-loaded PEG-32 S. These precipitates were also investigated using polarizing light microscopy and no different crystal morphology was observed. Birefringent needles and cubic shapes

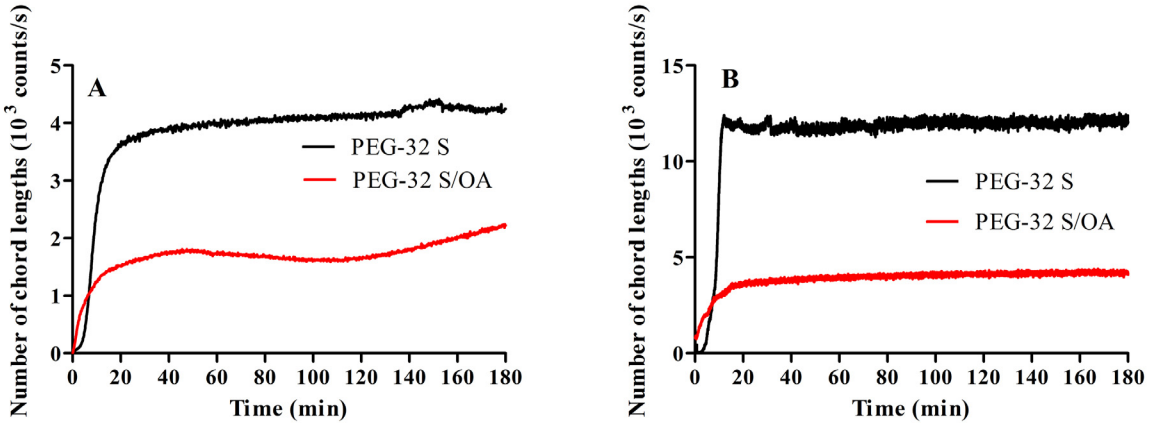

Figure 12 Effect of the drug-OA interaction on the type of precipitation kinetics of (A) loratadine and (B) carvedilol, both loaded at a saturation level of 1.5 in PEG-32 S and PEG-32 S/OA (8:2 w/w), respectively.

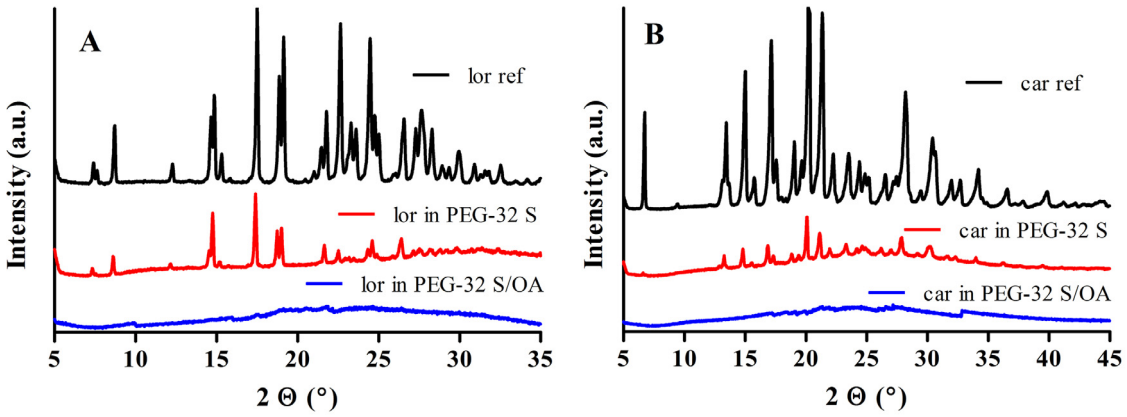

Figure 13 X-ray diffraction patterns of (A) loratadine powder as reference (black line), loratadine precipitated from PEG-32 S (red line), and from PEG-32 S/OA (blue line), and (B) carvedilol powder as reference (black line) and carvedilol precipitated from PEG-32 S (red line), and from PEG-32 S/OA (blue line). All solid formulations were supersaturated (S = 1.5) and the precipitated drugs were analyzed following 3 hours of dispersion in phosphate buffer (pH = 6.5).

for loratadine and carvedilol precipitates, respectively, were observed. This situation was in contrast to adding OA, since the drug precipitates from the dispersed PEG-32 S/OA (8:2 w/w) systems were amorphous as shown by the X-ray patterns in Figure 13. However, for the loratadine precipitate some isolated crystals were also observed under the polarized microscope, while no crystalline drug was found in the carvedilol precipitate.

The amorphous precipitates demonstrated that OA not only changed the type of precipitation kinetics, but also influenced the solid state of both drugs upon dispersion. Tables 3 and 4 provide an overview of the drug saturation levels of PEG-32 S or PEG-32 S/OA (8:2 w/w) studied for their precipitation kinetics. For the calculation of maximum supersaturation ratios (SR^M) (20), determination of the equilibrium drug solubilities in the dispersion medium was needed.

The solubility of loratadine was 0.25 ± 0.01 mg/ml and 0.52 ± 0.02 mg/ml for the dispersion medium containing PEG-32 S and PEG-32 S/OA (8:2 w/w), respectively. For carvedilol, the corresponding solubilities were 0.91 ± 0.03 mg/ml and 1.68 ± 0.06 mg/ml, respectively. Additionally, for each studied saturation level of PEG-32 S or of PEG-32 S/OA (8:2 w/w), the Precipitation number (P_{nc}) was determined using Equation 1.

This number assessed the biopharmaceutical relevance of a supersaturation following dispersion of a formulation. However, it is important to note that the focus was only on the crystalline precipitates because amorphous precipitates were deemed less problematic for drug absorption (21). Amorphous drugs typically have a high solvent pressure and easily result in a supersaturated state, which is a biopharmaceutical advantage over crystalline material (29, 30).

For both drugs loaded in PEG-32 S, precipitation upon dispersion started already with anhydrous formulations below saturation. A critical saturation level of PEG-32 S was 0.7 for loratadine and 0.9 for carvedilol. Observed drug precipitation within a relevant physiological time span was reflected by precipitation numbers increasing beyond unity as seen in Tables 3 and 4.

Drug release testing

Initial release tests (USP 3 apparatus) used anhydrous formulations with PEG-32 S or

Table 3 Loratadine doses, corresponding saturation levels of the anhydrous formulations, calculated maximum supersaturation ratios (SR^M), and Precipitation numbers (P_{nc}) used for the drug precipitation analyses

MODEL FORMULATION	DOSE (mg/g)	SATURATION LEVEL OF ANHYDROUS FORMULATION (*S*)	MAXIMUM SUPERSATURATION RATIO (SR^M) UPON DISPERSION (1:100 w/w)	PRECIPITATION NUMBER (P_{nc}) at pH=6.5
Loratadine in PEG-32 S	32	0.6	1.3	< 1 (< 0.38)
	37.4	0.7	1.5	>1 (1.31 ± 0.10)
	48.1	0.9	1.9	> 1 (11.15 ± 1.51)
	58.8	1.1	2.4	> 1 (14.08 ± 2.95)
	69.4	1.3	2.8	> 1 (25.63 ± 4.41)
	80	1.5	3.2	> 1 (52.14 ± 7.53)
	90.8	1.7	3.6	> 1 (64.00 ± 6.93)
Loratadine in PEG-32 S/OA	58.5	0.8	1.1	< 1
	65.8	0.9	1.3	< 1
	80.4	1.1	1.6	*
	95	1.3	1.8	*
	109.6	1.5	2.1	*
	124.3	1.7	2.4	*

* Different type of kinetics and an amorphous precipitate

Table 4 Carvedilol doses, corresponding saturation levels of the anhydrous formulations, calculated maximum supersaturation ratios (SR^M), and Precipitation numbers (P_{nc}) used for the drug precipitation analyses

MODEL FORMULATION	DOSE (mg/g)	SATURATION LEVEL OF ANHYDROUS FORMULATION (*S*)	MAXIMUM SUPERSATURATION RATIO (SR^M) UPON DISPERSION (1:100 w/w)	PRECIPITATION NUMBER (P_{nc}) at pH=6.5
Carvedilol in PEG-32 S	94.2	0.8	1	<1 (< 0.38)
	105.9	0.9	1.2	> 1 (1.97 ± 0.16)
	117.7	1.0	1.3	> 1 (7.74 ± 0.50)
	129.5	1.1	1.4	> 1 (14.36± 1.81)
	153	1.3	1.7	> 1 (17.17± 0.82)
	176.5	1.5	1.9	> 1 (29.37 ± 2.91)
	200.1	1.7	2.2	> 1 (32.14 ± 4.18)
	223.6	1.9	2.5	> 1 (39.24 ± 6.17)
Carvedilol in PEG-32 S/OA	153.9	1.0	0.9	< 1
	169.3	1.1	1.0	*
	200.1	1.3	1.2	*
	230.9	1.5	1.4	*
	261.6	1.7	1.6	*
	292.4	1.9	1.7	*

* Different type of kinetics and an amorphous precipitate

PEG-32 S/OA (8:2 w/w) that were below drug saturation. These experiments did not reveal any drug precipitation for either drug (data not shown). It must be noted that the given aqueous formulation dilution (1:160 w/w), specific hydrodynamics, as well as, the capsule made a difference to the dispersion experiments using FBRM. Therefore the main interest became to compare the formulations at a supersaturated reference level of 1.5. Figure 14 shows the drug release profiles of the formulations for the model drugs (loratadine and carvedilol).

Both drugs precipitated during the drug release testing and their kinetic profiles showed clear differences. A fast dispersion was observed for the different formulations once the thermoplastic capsules opened. Therefore, the systems had in common that the maximum

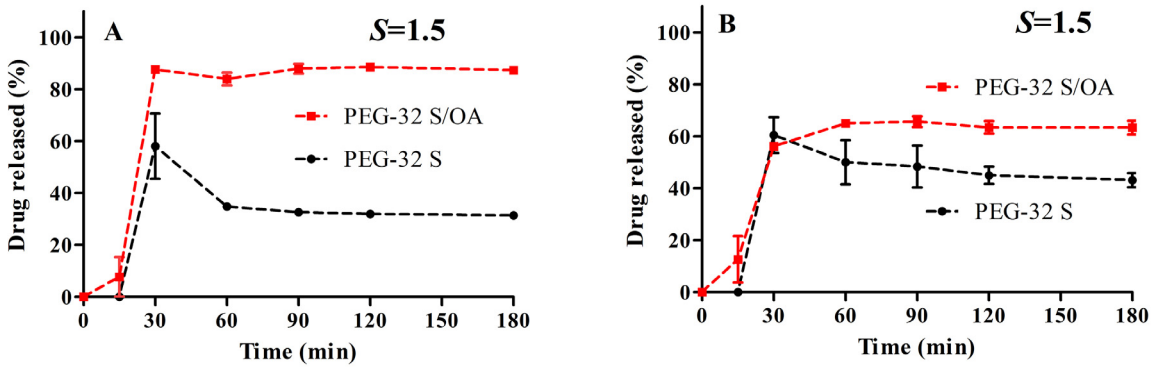

Figure 14 Drug release profiles of (A) loratadine, and (B) carvedilol, formulated in PEG-32 S and PEG-32 S/OA (8:2 w/w) and encapsulated in VegaGels®, analyzed in USP 3 apparatus in phosphate buffer (pH = 6.5), 20 dpm, at 37 ± 0.5° C (n = 3).

drug concentration was reached within 30 minutes (Figure 14). The OA-containing system reached a maximum of released drug at 90% for loratadine and for carvedilol it leveled off at 70%. This plateau level of the drug concentration remained constant during the 3 hours of analysis. In contrast to that, the maximum of released drug for PEG-32 S formulations was reached at around 60%, followed by fast drug precipitation and marked lowering of concentrations for both drugs. Moreover, the resulting maximum drug supersaturation ratios (SR^M) during release testing were calculated. For loratadine SR^M values were 3.2 and 2.1 for the PEG-32 S formulation and OA-containing formulation, respectively. For carvedilol, the corresponding values were 2.0 and 1.4, respectively.

DISCUSSION

Mechanistic understanding of drug-excipient interactions on different biopharmaceutical levels could be a way to improve the development of oral dosage forms. However, until now there has been a lack of systematic studies of drug-excipient interactions in solid lipid-based systems. As detailed in Figure 1, the focus of this study was on the OA interactions with basic drugs in an anhydrous formulation, upon aqueous dispersion, and particularly with respect to precipitation kinetics, as well as, the evolving solid state of the precipitate.

Level of the anhydrous formulation

First, a solid system containing PEG-32 S and OA in optimal ratio (8:2 w/w) was developed which demonstrated optimal dispersion with respect to particle size and polydispersity. The reduction of particle size could indicate forming of mixed micelles that may be beneficial for robust drug absorption. The smallest particle sizes were targeted, as it proved to be relevant for increased bioavailability of some PWSDs (31). However, such size effects may not always be relevant for drug bioavailability (32).

Second, the drug-OA interactions were characterized in oily mixtures. It is well understood that intermolecular forces are often strong enough to control physical properties, such as, viscosity (33). This was also confirmed in the present study, e.g., the pronounced intermolecular forces between OA and the basic drugs resulted in increasing viscosity with increasing amounts of both drugs (Figure 5B). The molecular interactions between OA and the model bases were also supported by the results of the spectroscopic studies. The shift and the broadening of the UV band of drug-OA mixtures clearly indicated interactions

between the two components. The nature of the drug-OA interactions was further characterized using FTIR spectroscopy. As shown in Figure 6, there were no changes in the band of the hydroxyl group of OA (3000-2700 cm^{-1}). This could be attributed to the higher OA content in the mixtures (~ 1:5 w/w drug : OA ratio). Tertiary amines (such as those in loratadine) show no characteristic bands, but should they undergo protonation, they would become secondary amines, and the matching bands would appear. In the present study, this did not occcur. A spectrum shifting and broadening of the characteristic bands at 1200 cm^{-1} and 1700 cm^{-1} were observed in the lor-OA, showing stretching vibrations of C-O and C=O, respectively. This could indicate hydrogen bonds between OA and both carboxyl groups of loratadine. Similar observations were reported by Nacsa *et al.* in a study of loratadine interacting with a cyclodextrin (34). However, broadening/shifts of IR bands in the same region (around 1200 cm^{-1} and 1700 cm^{-1}) could also indicate changes in the amine and the stretching of the (aromatic) C=N group. Therefore, it could not be excluded that the "basic" groups of loratadine were also involved in the interactions with OA, especially since there was excess OA in the lor-OA mixture. For carvedilol, the relevant N-H bonds at 1600 cm^{-1} and below 3500 cm^{-1} disappeared in the car-OA spectrum. Moreover, changes in the region between 1200-1000 cm^{-1} were observed, corresponding to C-O stretching in ethers and primary alcohols. In a classical sense, this could be interpreted as deprotonation of N-H groups and change of environment around C-O groups. Thus, spectroscopic results indicate several possible intra- and inter-molecular interactions of drug and OA.

OA demonstrated a substantial influence on the solubility of both loratadine and carvedilol (Table 1). These results are in line with the observations reported by Patel *et al.* for lumefantrine-OA formulations (35). In summary, the findings here indicate that the strong drug-OA interactions can be viewed as molecular complex formations on the level of the anhydrous formulation.

Level of dispersion, precipitation inhibition and drug release

To better understand the drug-OA interactions following aqueous dispersion, the zeta potential of dispersed drug-free and drug-loaded formulations in acidic environment (pH = 1.6) and at an intestinal pH of 6.5 were studied (Table 2). At a low pH, both drug bases were expected to be protonated, resulting in a positive zeta potential of their formulation dispersions and indicating that both drugs were associated with surface of micelles. In the same acidic medium, the dispersion of the drug-free formulation exhibited an almost neutral zeta potential probably because fatty acids on the surface would be predominantly in their neutral form. In the phosphate buffer, the zeta potential of the dispersed drug-free formulation was slightly negative, which is a common observation for dispersed glyceride oils or other systems with low dielectric constant. The zeta potential of the dispersed loratadine-containing formulation was also slightly negative, reflecting the neutral form of the weak base (pK_a = 5.3). For the dispersed carvedilol-loaded formulation (carvedilol pK_a= 7.8), the net charge was almost neutral. Here, the negative charge observed with the drug-free formulation was probably suppressed by the presence of protonated carvedilol at the surface of the micelles. Thus, a fraction of protonated drug was evidently interacting with the colloidal surfaces, while some other drug may have also partitioned as charged molecules into the bulk solution.

Subsequently, it was important to evaluate the effect of OA on drug precipitation. As evident from Tables 3 and 4, the dispersions of both model drugs loaded either in PEG-32 S or OA-containing systems resulted mostly in supersaturated solutions. While the dispersed PEG-32 S precipitated already as unsaturated formulations (S<1 in the anhydrous formulation), OA-containing systems precipitated only at (formulation) drug

saturation of 1.1 and beyond. The addition of OA increased the drug solubility equilibrium in the dispersion medium. As a consequence, the degree of supersaturation upon dispersion of OA-containing systems was decreased, which in turn reduced the driving force for drug precipitation. This mechanism of precipitation inhibition is well known for using surface active excipients.

Interesting findings were also inferred from a comparison of the maximum supersaturation ratios (SR^M) between the different formulations for a given drug. As mentioned earlier, the addition of OA almost doubled the solubilizing capacity of the dispersion medium (see section 3.4.1.). As a consequence, although the drug loading was much higher for the OA-containing formulations (at the same *S*), the corresponding SR^M values upon dispersion were lower compared to SR^M in systems without OA. Moreover, both systems started to precipitate at corresponding SR^M values (Tables 3 and 4). Here the report by Rodriguez-Hornedo *et al.* who studied critical supersaturation in spontaneous drug precipitations becomes interesting (36). Such critical supersaturation for spontaneous nucleation was compared between systems containing different solvents. When the drug solubility was in a two or threefold regimen (as in this study), the critical supersaturation was similar. However, when the drug solubility was five-fold or higher, clearly reduced values of critical supersaturation were observed.

Recently, Anby *et al.* showed (practically spontaneous) danazol precipitation upon dispersion of various formulations, when SR^M values were greater than 2 (37). The authors differentiated this situation from lipolysis-induced drug precipitation, where another critical value of SR^M was proposed. Our results of supersaturation upon dispersion demonstrated that, for values less than 2, the Precipitation number became critical (i.e., > 1). Certainly, a different time span was considered here as opposed to nearly spontaneous precipitation. Interesting was the finding of a different critical SR^M value for the two bases. This implies that critical SR^M values for precipitation might be useful to compare formulations, but may be rather drug specific. In addition, the systems reported here contained considerably higher drug amounts (especially in all supersaturated formulations), which could have been relevant for precipitation kinetics.

As shown in Figures 11 and 12, the OA influenced the extent of precipitation and its kinetics. The can be compared to the findings of Gao *et al.* who reported that the amount of surfactant (Tween® 80) in the formulation dictated the initial degree of drug (AMG 517) supersaturation, and therefore, its precipitation kinetics (19). In the present study, there was substantially less precipitation when the OA was added to the formulation. The precipitation kinetics of these OA-containing formulations was, however, characterized by a high initial rate. The portion of drug that precipitated almost spontaneously may have been a primarily solubilized base in the bulk. Some drug-OA interactions may also have occurred in the bulk but it was mainly on the colloidal level, where molecular interactions were assumed. This direct drug-OA interaction on the level of micelles was probably one mechanism of nucleation inhibition. OA could have further reduced the interfacial energy of forming drug nucleates and it may have interfered with the particle growth process. The latter effect may, however, be more pronounced for polymeric precipitation inhibitors as reported elsewhere (7, 38).

Besides the extent of precipitation and its kinetics, the solid state of the evolving material can be of crucial biopharmaceutical importance. If the drug precipitates in a crystalline form in the intestinal environment, it is often problematic, since re-dissolution of drug crystals is typically rather slow. In the present study, the precipitates of both drugs in PEG-32 S were crystalline (Figure 12).

The presence of OA affected the solid state of precipitates, resulting in an amorphous material. Another recent study by Stillhart *et al.* also found an amorphous precipitate of carvedilol in a digested formulation medium, whereas the pure formulation dispersion (no lipolysis) produced crystalline material (39). It could have been because of the generation of fatty acids during lipolysis that an interaction with carvedilol enabled an amorphous precipitate. In this study the presence of a fatty acid in the anhydrous formulation could be a beneficial formulation strategy to target amorphous drug precipitates. Thomas *et al.* recently showed the advantage of such amorphous precipitates from lipid-based formulations using halofantrine as a model drug in *in vivo* studies (21).

A biopharmaceutically relevant dimensionless parameter, the Precipitation number (P_{nc}) is introduced here. It is the ratio of the drug residence time in the GI tract (t_{res}) to the induction time of crystalline precipitation (τ_{ind}). The induction time is a parameter characteristic to chemical engineering and the comparison with a physiologically relevant time was meaningful. The precipitation kinetics were studied using FBRM in a closed system without a drug absorption sink. This is a typical aspect of a simplified *in vitro* test and may result in an overestimation of the calculated P_{nc}. However, although both loratadine and carvedilol are fast permeating drugs, the focus was more on the effects of OA on the drug precipitation kinetics rather than attempting to make absolute predictions for *in vivo* drug absorption. All dispersed drug-loaded PEG-32 S formulations resulted in supersaturated solutions (SR^M>1). However, not all precipitated within a relevant intestinal transit time, which was reflected by different P_{nc} values. Therefore, the P_{nc} provides a simple tool for quick screening of different formulations and/or drug loadings in formulations with respect to a relevant physiological time frame. It is important to compare crystalline precipitates, whereas the amorphous precipitates obtained from the OA-containing formulations were not directly comparable using P_{nc}.

The importance of applied hydrodynamics in the precipitation evaluation should be mentioned. As shown above, already unsaturated drug-loaded systems (anhydrous formulations without OA) were precipitating upon aqueous dispersion, when rigorous mixing of 500 RPM during FBRM testing was used. For the drug release testing using USP 3 apparatus, 20 dpm, the precipitation occurred only for supersaturated systems (S = 1.5). The release rate of loratadine appeared to be strongly influenced by the presence of OA and marked precipitation was noted in the absence of the excipient. This excipient effect was also observed for carvedilol, but it was less pronounced. Such drug comparison on the same supersaturation level is meaningful to standardize the driving force of nucleation, but it is accompanied by different drug loads. Moreover, drug solubilities were different, that is, carvedilol exhibited much higher solubility compared to loratadine. This certainly affected drug release under non-sink conditions, so the extent of the excipient effect for different drugs should be interpreted carefully.

Tables 3 and 4 allow comparing formulations at similar doses at the resulting maximal supersaturation. OA always had a clear effect which would be expected in a release experiment comparing formulations at a constant dose level. For absolute values, some care is needed when different *in vitro* tests are compared. Recently, Carlert *et al.* explored two different hydrodynamics for *in vitro* precipitation studies of a basic BCS class II drug (AZD0865) (40). They observed that precipitation rates were remarkably slower in the shaking model (85 cycles/min, amplitude 2 cm) compared to the stirring model (USP 2 mini-vessel set up, paddle speed at 150 RPM). The various shears were likely to be the main difference also in the FBRM experiments presented here, compared to the release analysis using the USP 3 apparatus. The effects of the capsule shell may have been less critical given the rather fast immediate drug release profiles.

CONCLUSION

The aim of the current study was to better understand the effects of basic drug-OA interactions in a solid lipid-based system on different biopharmaceutical levels, i.e., an anhydrous formulation and subsequent aqueous dispersion. Of particular interest was the influence on the drug precipitation kinetics and on the solid-state properties of obtained precipitates following dispersion.

On the level of the anhydrous formulation, it was likely that the drug-OA molecular complexes were formed, which led to a substantial increase of solubilized drugs in the formulations. Once the formulations were dispersed, a clear complex formation was no longer apparent and most likely the interactions were indeed partially lost. However, some interactions were still present upon dispersion on the level of evolving micelles, and probably also within the bulk solution. OA clearly influenced supersaturation and the extent of drug precipitation, as well as, its kinetics. Most importantly, OA acted as a precipitation modifier, since it induced an amorphous precipitate that was otherwise crystalline without this excipient. Such an *in situ* forming amorphous system could be considered a novel formulation strategy to deliver poorly soluble basic drugs.

It was further concluded that relevant drug-excipient interactions should be studied separately for the different levels of biopharmaceutical testing. This appears to be critical for an improved biopharmaceutical understanding and also with respect to a more rational selection of systems in pharmaceutical formulation development. For such development purpose, the introduction of a Precipitation number could be useful for screening lipid-based candidates in formulations. Future studies should address other drug-excipient systems for different biopharmaceutical levels that may also include an absorption step. An improved understanding of drug-excipient interactions would also advance the field of biopharmaceutical drug absorption modeling.

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