pH-Dependent Dissolving Nano- and Microparticles for Improved Peroral Delivery of a Highly Lipophilic Compound in Dogs

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ABSTRACT RR01, a new highly lipophilic drug showing extremely low water solubility and poor oral bioavailability, has been incorporated into pH-dependent dissolving particles made of a poly(methacrylic acid-*co*-ethylacrylate) copolymer. The physicochemical properties of the particles were determined using laser-light-scattering techniques, scanning electron microscopy, high-performance liquid chromatography, and x-ray powder diffraction. Suspension of the free drug in a solution of hydroxypropylcellulose (reference formulation) and aqueous dispersions of pH-sensitive RR01-loaded nanoparticles or microparticles were administered orally to Beagle dogs according to a 2-block Latin square design (n = 6). Plasma samples were obtained over the course of 48 hours and analyzed by gas chromatography/mass spectrometry. The administration of the reference formulation resulted in a particularly high interindividual variability of pharmacokinetic parameters, with low exposure to compound RR01 ($AUC_{0\text{-}48h}$ of 6.5 µg.h/mL and coefficient of variation (CV) of 116%) and much higher T_{max}, as compared to both pH-sensitive formulations. With respect to exposure and interindividual variability, nanoparticles were superior to microparticles ($AUC_{0\text{-}48h}$ of 27.1 µg.h/mL versus 17.7 µg.h/mL with CV of 19% and 40%, respectively), indicating that the particle size may play an important role in the absorption of compound RR01. The performance of pH-sensitive particles is attributed to their ability to release the drug selectively in the upper part of the intestine in a molecular or amorphous form. In conclusion, pH-dependent dissolving particles have a great potential as oral delivery systems for drugs with low water solubility and acceptable permeation properties.

KeyWords: Nanoparticles, Microparticles, Oral Administration, Poor Water Solubility, pH-Sensitive Polymer

INTRODUCTION

The amount of drug absorbed from the gastrointestinal tract (GIT) into systemic circulation is a result of complex processes and is mainly determined by the chemical structure of the molecule (1). In recent years, research in pharmaceutical chemistry has focused on optimization of in vitro activity of the chemical leads, with less attention given to their physicochemical properties. Particularly, drug design approaches based on combined chemistry and quantitative structure-activity relationships have led to potent new chemicals that tend to be more lipophilic and less water soluble (2,3). Since aqueous solubility is one of the crucial factors influencing drug absorption from the GIT, many newly discovered compounds exhibit low oral bioavailability (despite satisfying permeation capacity) (2,3). Because the oral route is still the dominant and preferred method of administering drugs, the development of novel oral delivery systems allowing increased dissolution rates for highly lipophilic drugs is generating growing interest. Different strategies have been described to achieve this objective. Among these, lipid-based formulations have received much academic and commercial interest based on the assumption that formation of a presolubilized phase and subsequent GIT metabolism of the lipids may facilitate dissolution and absorption of the drug (4). Another strategy has relied on the obvious advantage of increasing the surface area available for dissolution of the drug, as illustrated by drug micronization (5) or microemulsification of lipid vehicles (4). A third strategy is based on the formulation of poorly water soluble drugs in solid dispersions (solid solution or dispersion of the drug in excess of its solubility in a water-soluble matrix) in which the drug is present

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as a polymorph, solvate, or amorphous form that may favor its dissolution (4-7). However, so far, constraints of solubility, potential interaction of the drug with excipients, and physical stability limitations have restricted the use of such formulations (4-7).

Recently, we reported on the performance of pH-sensitive particles made of a poly(methacrylic acid-*co*-ethylacrylate) copolymer (Eudragit L100-55, Röhm GmbH, Darmstadt, Germany) as oral delivery systems for poorly water soluble HIV-1 protease inhibitors (8-11). The described pH-sensitive particles are matrix-type dispersed systems made of a pH-dependent dissolving polymer. These formulations have been shown to dramatically improve the oral bioavailability of HIV-1 protease inhibitors in mice and dogs (8-11). Such performance has been related to the selective release of the drug close to its absorption site in a highly dispersed way and in a molecular or amorphous form (8-11).

To evaluate this drug delivery concept in the present study, the new chemical entity RR01 has been incorporated into pH-sensitive particles in nanometer and micrometer sizes. Suspensions of the particles and a reference formulation consisting of a suspension of the free drug have been administered orally to Beagle dogs. In this study, the advantage of pH-sensitive formulations over other formulations (eg, lipid-based or solid dispersion formulations) with regard to manufacturing and stability features is also highlighted.

MATERIALS AND METHODS

Materials

RR01 (Novartis Pharma AG, Basle, Switzerland) is a lipophilic substance with a logP value of 3.89 (n-octanol/phosphate buffer pH 7.4), almost insoluble in water (0.09 mg/L; pKa 2.78), and with a molecular weight > 600 d. The polymeric material used to prepare the particles was a poly(methacrylic acid-*co*-ethylacrylate) copolymer with a monomer molar ratio of 1:1 (Eudragit L100-55, USP/NF methacrylic acid copolymer Type C, Röhm GmbH, Darmstadt, Germany), soluble in intestinal fluid above pH 5.5, and with a molecular weight of 250 kd (Figure 1). Poly(vinyl alcohol) (PVAL) with a molecular weight of 26 kd (Mowiol 4-88, Hoechst, Frankfurt/Main, Germany) was used as an emulsifier to prepare the nanoparticles.

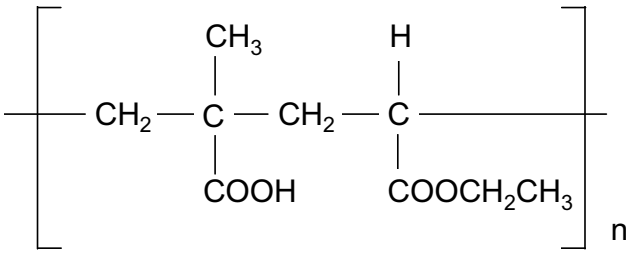

Figure 1. Chemical structure of pH-sensitive Eudragit L100-55.

Hydroxypropylcellulose (Klucel HF, Hercules, Wilmington, DE) was used as a suspending agent in the reference formulation. Benzyl alcohol and methanol (Fluka, Buchs, Switzerland) were of analytical grade.

Nanoparticle preparation, purification, and freeze-drying

RR01-loaded nanoparticles were prepared using the emulsification-diffusion method (12, 13). An aqueous phase (179 g) containing 12% (wt/wt) PVAL as the stabilizing agent was added under mechanical stirring (1600 rpm) to a benzyl alcohol phase (94 g) containing 14.2% (wt/wt) of a solubilized mixture of compound RR01 and Eudragit L100-55 in ratios ranging from 1:19 to 1:1. Because benzyl alcohol is miscible only at a ratio of 1:25 (wt/vol) with water, an oil-in-water emulsion was obtained after complete addition of the aqueous phase. The emulsion was then diluted with 2900 g of pure water to induce total diffusion of benzyl alcohol in the aqueous phase, leading to the formation of spherical nanoparticles (12,13).

The nanoparticulate suspension was purified and concentrated by cross-flow filtration using a Sartorcon Mini device (Sartorius, Göttingen, Germany) mounted with a polyolefin cartridge filter (100 nm pore size). A total volume of 28 L of pure water was used to remove benzyl alcohol and free PVAL (14).

The purified nanoparticulate suspension (330 g) was divided in 2 glass flasks, which were frozen at -60°C in an ethanolic bath under rapid rotation and freeze-dried for 24 hours at 0.05 mbar in a Lyolab BII (Secfroid, Aclens, Switzerland), with a condenser temperature of -60°C ± 4°C. The production yield was calculated as the ratio of the final amount of nanoparticles recovered after

freeze-drying to the sum of initial amounts of polymer and drug.

Microparticle preparation

RR01-loaded microparticles were produced in a laboratory-scale spray dryer (Model 190, Büchi, Flawil, Switzerland). A methanolic solution (300 g) containing 5% (wt/wt) of a mixture of compound RR01 and Eudragit L100-55 in ratios ranging from 1:19 to 1:1 was spray dried (0.5 mm nozzle) using the following parameters: inlet temperature 50°C; outlet temperature 37°C to 42°C; aspirator setting 15; pump setting 4 mL/min; spray-flow 500 NL/h. The production yield was calculated as the ratio of the final amount of microparticles recovered after spray drying to the sum of initial amounts of polymer and drug.

Particle size analysis

The particles were redispersed in pure water by vigorous vortex stirring. The mean diameter and polydispersity index (expressed using a 0-to-9 scale) of the nanoparticle size distribution was determined by photon correlation spectroscopy using a Coulter Nanosizer (Coulter Electronics Ltd, Harpenden, UK). The particle size distribution of the microparticles was determined by laser light diffraction using a Mastersizer (Malvern Instrument, Malvern, UK). Particle sizes are expressed as the weighed mean of the volume distribution D [4,3]. Each value resulted from a triplicate determination.

Particle morphological examination

Morphology of the particles was examined by scanning electron microscopy (SEM). The particles were redispersed in pure water by vigorous vortex stirring, followed by short sonication (< 30 seconds) in the case of microparticles. A drop of the dispersion was spread over a SEM stub, dried in a desiccator, and coated with a thin layer of gold in a cathodic evaporator. Morphological evaluation of the particles was conducted using a JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan).

Evaluation of drug content and entrapment efficiency

The drug loading of the particles was determined by high-performance liquid chromatography analysis. The system used was an LC Module I plus system (Waters, Milford, MA) equipped with a Nucleosil 100-5 C18 column (5 μm particle size, 250 mm long, and 4 mm inner diameter; Macherey-Nagel Gmbh & Co., Düren, Germany). The mobile phase consisted of 72:28 (vol/vol) methanol:0.001M phosphate buffer (pH 8.0). In a typical experiment, 25 mg of dry particles were dissolved in 20 mL of mobile phase. The resulting solution was filtered through a 0.45 μm filter (Durapore, HVLP, Millipore, Switzerland), and 10 μL were injected into the column. The flow rate was 1 mL/min with a run time of 20 minutes. Ultraviolet detection was performed at 224 nm. The entrapment efficiency was calculated as the ratio of the experimental drug loading (wt/wt) determined as described below to the initial percentage (wt/wt) of compound RR01 in the formulation. Each determination was repeated twice.

X-ray analyses

X-ray analyses were performed on the polymer, compound RR01, their physical mixture, and the RR01-loaded particles. Diffraction powder patterns were measured with a Guinier camera FR 552 (Enraf Nonius, Delft, The Netherlands) using a Co Kα1 radiation and digitized with an LS-18 line scanner.

In vivo study on peroral absorption

Six healthy male Beagle dogs (4 to 7 years old) weighing 10 kg to 12 kg were used for the study. Shortly before being administered, nanoparticles and microparticles were suspended in pure water. The reference formulation was prepared by suspending compound RR01 in a 0.5% aqueous Klucel HF solution. Each animal received randomly a single dose (1.2 g/kg) of reference formulation (equivalent of 3.5 mg/kg of drug), aqueous dispersion of pH-sensitive nanoparticles (4.3 mg/kg of drug), or aqueous dispersion of pH-sensitive microparticles (4.1 mg/kg of drug) according to a 2-block Latin square design (n = 6). Administration was performed by means of a gastric tube that was immediately rinsed with 20 mL of pure water. A wash-out period of at least 6 days was imposed between 2 dosings in the same animal. The dogs were fasted for about 18 hours prior and 6 hours after administration, but had free access to water throughout the experiment. This experiment was approved by the Veterinary Committee of the Canton of Basle, Switzerland.

Blood samples (approximately 2.7 mL each) were collected from the cephalic vein before (t = 0 hours) and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 hours postadministration, into heparinized syringes. Plasma was obtained by centrifugation of the blood samples, and was frozen and stored below -18°C until analysis. The drug was determined in plasma by a gas chromatographic/mass spectrometric method (Novartis Pharma AG procedure). The limit of quantitation of the method was 6.3 ng/mL plasma. The area under the plasma concentration-time curve from 0 to 48 hours ($AUC_{0\text{-}48h}$) was determined by the trapezoidal calculation method. The maximal plasma concentration (C_{max}) and the time of maximal plasma concentration (T_{max}) were directly determined from the graphs. Individual dose-normalized $AUC_{0\text{-}48h}$ were calculated as the ratio of $AUC_{0\text{-}48h}$ to the corresponding drug dose.

RESULTS AND DISCUSSION

Nanoparticle and microparticle preparation

The emulsification-diffusion and spray-drying methods allowed the preparation of nanoparticles and microparticles, respectively, with optimal drug entrapment efficiency (~100%), even using a drug:polymer ratio as high as 1:1 (Table 1), and with excellent batch-to-batch reproducibility. This outcome is of great interest and puts these systems at an advantage over, for example, lipid-based formulations, because poorly water-soluble drug substances are often also limited with respect to their solubility in suitable lipophilic solvents (4).

Table 1: Characteristics and Production Yield of pH-sensitive Nanoparticles and Microparticles Produced by Emulsification-Diffusion and Spray-Drying

Formulation	Mean Size ± SD (nm)	Drug Loading % ± SD (wt/wt)	Production Yield %
Drug:polymer ratio 1:19			
Nanoparticles	292 ± 22 (3)*	4.97 ± 0.04	90
Microparticles	10 900 ± 200	5.14 ± 0.03	68
Drug:polymer ratio 1:1			
Nanoparticles	297 ± 6 (2)*	49.83 ± 0.06	98
Microparticles	9900 ± 500	50.12 ± 0.10	69

*** Polydispersity index (0-9 scale)**

The production yields ranged around 95% for nanoparticles and around 70% for microparticles. The latter value was particularly satisfying knowing the generally low production yields encountered with laboratory-scale spray dryers (15). In the case of the emulsification-diffusion method, the production yield remained high in spite of the relatively high solid content (14.2%) of the organic phase, which proved to be an advantage over other preparation methods (16). Freeze-dried nanoparticles were easy to redisperse (no aggregation was encountered), most probably because of the presence of residual PVAL at their surface (12,13). The microparticles exhibited rather poor wettability because no surfactant was used during preparation. For morphological and size examination, this problem was circumvented by vigorous vortex stirring followed by short sonication (< 30 seconds) in small volumes of water.

Nanoparticle and microparticle size and morphology

Under scanning electron microscope examination, nanoparticles exhibited spherical morphology and smooth surfaces as well as a monodispersed size distribution (data not shown), confirming results obtained by photon correlation spectroscopy (Table 1). Neither the size nor the morphology were influenced by the drug loading. The microparticle size distribution and mean size are presented in Figure 2 and Table 1.

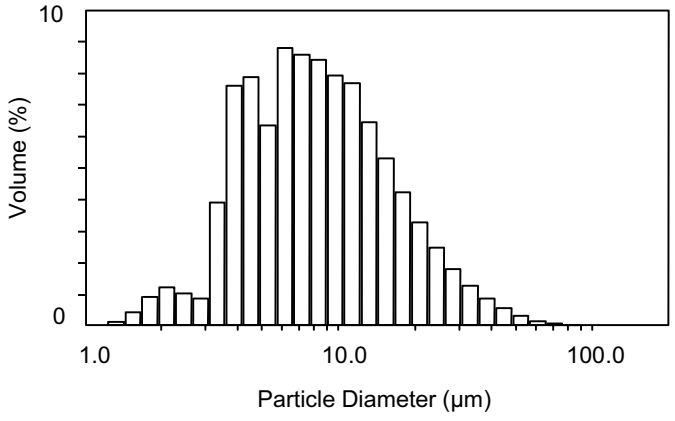

Figure 2. Size distribution of Eudragit L100-55 microparticles as assessed by laser light diffraction (drug loading 50%).

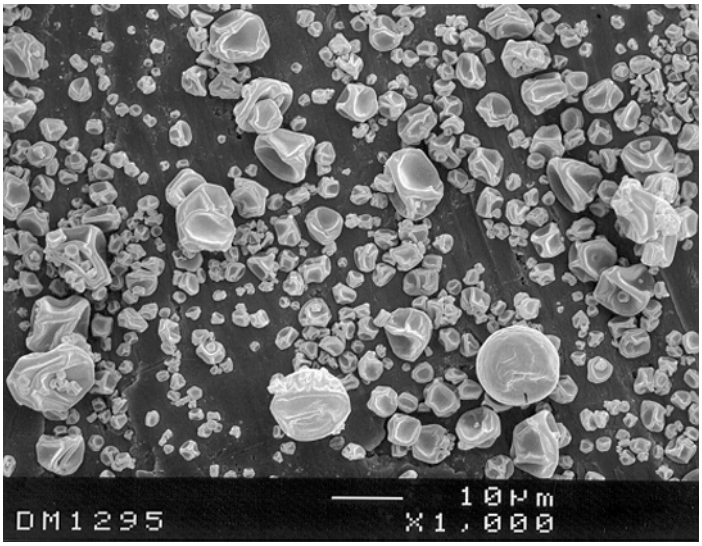

Figure 3. Scanning electron micrograph of Eudragit L100-55 microparticles (drug loading 50%).

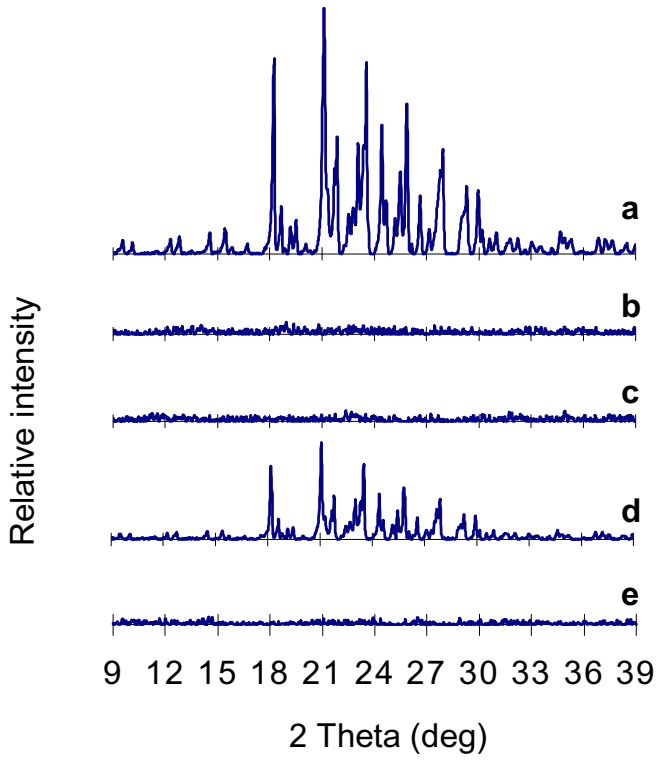

Figure 4. X-ray diffraction pattern of (a) compound RR01, (b) Eudragit L100-55 nanoparticles (drug loading 50%), (c) Eudragit L100-55 microparticles (drug loading 50%), (d) physical mixture of compound RR01 and Eudragit L100-55 (50% of compound RR01), and (e) Eudragit L100-55.

For both drug-polymer ratios (1:1 and 1:19), the spray-dried microparticles exhibited shriveled surfaces (Figure 3), apparently derived from originally spherical particles distorted by loss of internal volume as a result of solvent evaporation (15,17).

Physical state and stability of the drug within the particles

X-ray analyses were performed to establish the physical state of both the polymer and drug in the particle formulations (8,10). Nanoparticles and microparticles loaded with 50% of compound RR01 were analyzed in the same ratio as a physical mixture of compound RR01 and polymer immediately after production (Figure 4) and after 12 months of storage at 4°C (data not shown because of identical nature). In both cases, the original crystal structure of compound RR01 was not found in the nanoparticles or in the microparticles, in spite of the relatively high drug loading of the particles. In contrast, the diffraction pattern of the physical mixture could be clearly explained as a superimposition of the patterns of the pure components.

The absence of crystallinity in the nanoparticles and microparticles indicated that compound RR01 was amorphous or molecularly dispersed within the polymeric matrix. The absence of recrystallization even after 12 months of storage indicated the physical stability of the drug within the polymeric matrices. This feature is particularly advantageous considering the problems of physical instability frequently reported with other types of formulations. For example, in conventional solid dispersion-based formulations, the manufacturing process, the drug concentration and the storage duration can greatly alter the physical state of the drug in the formulation, with unreliable effects on the drug dissolution rate (7). In the case of pH-sensitive particles, we appear to have overcome such a limitation.

Oral administration of nanoparticles and microparticles in dogs

The mean plasma concentration profiles of the test drug following the administration of the reference and particle formulations are shown in Figure 5. The corresponding pharmacokinetic parameters are summarized in Table 2. The individual dose-normalized $AUC_{0\text{-}48h}$ values are presented in Figure 6.

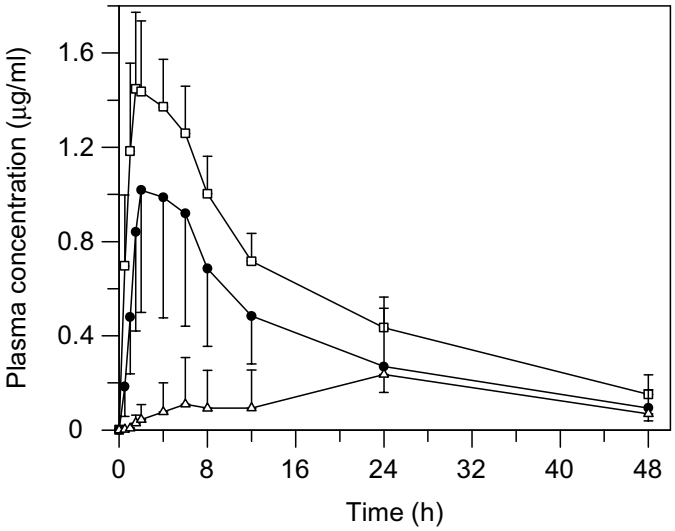

Figure 5. Plasma concentration profiles of compound RR01 after oral administration of the reference formulation (-△-), Eudragit L100-55 nanoparticles (-□-), and microparticles (-●-) (drug loading 5%) to fasted dogs; mean ± SD (n = 6).

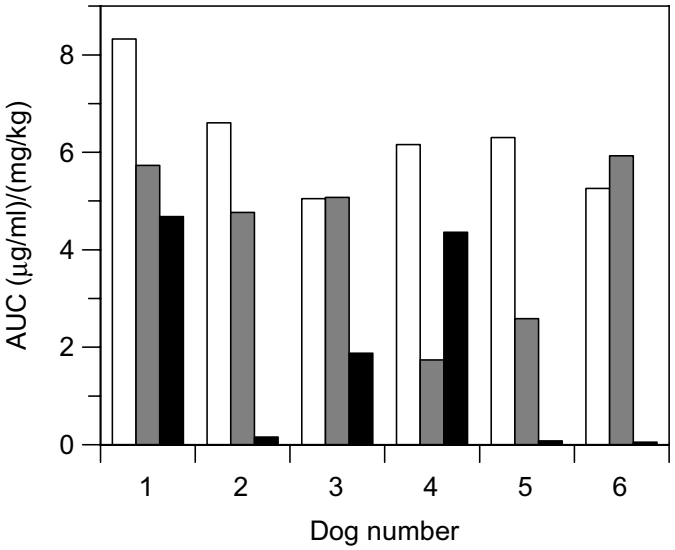

Figure 6. Individual dose-normalized $AUC_{0\text{-}48h}$ values calculated from the plasma concentrations of compound RR01 obtained after oral administration of the reference formulation (■), Eudragit L100-55 nanoparticles (□), and microparticles (▩) (drug loading 5%) to fasted dogs.

The administration of the Klucel suspension (reference formulation) to the dogs resulted in a particularly high interindividual variability in terms of all of the pharmacokinetic parameters calculated (Table 2). The T_{max} values varied considerably between 4 hours and 24 hours. C_{max} and $AUC_{0\text{-}48h}$ values were associated with a coefficient of variation (CV) of 113% and 116%, respectively. With the pH-sensitive particle formulations, the absorption of compound RR01 was faster (T_{max} = 1.5 - 6 hours) and a pronounced peak was observed in all plasma profiles (Figure 5).

The interindividual variability in terms of C_{max} and $AUC_{0\text{-}48h}$ was much lower than for the reference formulation, as characterized by the CV values (Table 2). Calculation of the bioavailability of the particulate formulations relative to the reference formulation was judged not to be meaningful because of the very high interindividual variability of the reference formulation. However, all the individual dose-normalized $AUC_{0\text{-}48h}$ values calculated for the particle formulations were higher than those calculated for the reference formulation, except in one dog (Figure 6).

As in previous studies (8-11), these results are attributed to the selective release of compound RR01 in a highly dispersed molecular or amorphous form (Figure 4) close to its anticipated absorption site (ie, in the upper part of the intestine where pH values normally range around 5.5). The pH-sensitivity of Eudragit L100-55 particles has been previously demonstrated through in vitro experiments mimicking pH conditions within the GIT (8,11). In the present study, evaluation of the drug release from the particles was problematic, owing to the extremely poor water solubility of compound RR01. Because the release study should be conducted in sink conditions, there were technical difficulties in evaluating in vitro dissolution kinetics of the drug, and the use of surfactants or organic solvents in dissolution testing media was not possible without altering the dissolution pattern of the particles themselves. However, in vitro dissolution of Eudragit L100-55 particles in buffered aqueous media was shown to occur almost instantly at pH 5.5 and above, as expected (8,11).

With respect to drug exposure and interindividual variability, the nanoparticulate formulation was the most efficient (Figures 5 and 6, Table 2). The mean $AUC_{0\text{-}48h}$ achieved with the microparticulate formulation amounted for 68% of that obtained with the nanoparticulate formulation. This result may be attributed to a possible difference of structure between the particles, inherent to the different production methods used (spray drying in one case, emulsification-diffusion in the other case). It may also indicate that the particle size plays an important

Table 2: Pharmacokinetic Parameters of the Test Drug Administered Orally to Fasted Beagle Dogs as Control Suspension and Incorporated into Eudragit L100-55 Particles; Mean (CV, n = 6)

Formulation	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-48h} (μg.h/mL)	Normalized AUC_{0-48h} (μg.h/mL)/(mg/kg)
Control	3.5	4 - 24	0.3 (113)	6.5 (116)	1.9 (116)
Nanoparticles	4.3	1.5 - 6	1.5 (18)	27.1 (19)	6.3 (19)
Microparticles	4.1	1.5 - 6	1.1 (48)	17.7 (40)	4.3 (40)

role in the absorption of compound RR01. However, the higher interindividual variability associated with the microparticles suggested that the degree of dispersion of the particles may also account for these results. As previously mentioned, the wettability of the nanoparticles is much higher than that of the microparticles because of the presence of PVAL residues at the surface of the nanoparticles (12,13). Optimal microparticle redispersion can be achieved by vigorous vortex stirring and short sonication with small amounts of microparticles in small volumes of pure water. However, it appeared that optimal redispersion of the microparticles could not be achieved with the larger amounts and volumes required for oral administration. To circumvent this problem, a microparticulate formulation with optimized redispersibility has been developed and is being evaluated. It is anticipated that this new preparation will provide optimal plasma concentration of compound RR01 with a lower interindividual variability than that obtained in the present study. This would be of great interest considering scaling-up and industrial issues, given the relative ease of preparation of microparticles by spray drying.

CONCLUSION

An ideal oral formulation of a poorly water soluble drug would maximize bioavailability (provided sufficient drug permeability is present), enable dose proportionality, and give reproducible plasma concentration-time profiles (4). The results obtained in this study, combined with previous results (8-11), indicate that this goal may be achieved by administering drugs in pH-dependent dissolving particles. Combining several properties favorable to drug dissolution and intestinal absorption, these preparations successfully induced efficient absorption of drugs with different structures and pharmacological activities (8-11). pH-sensitive particles provide a convenient alternative for administering high doses of drug without the limitations encountered with other formulations (eg, lipid-based or conventional solid dispersion systems, which frequently face manufacturing and stability problems) (4,6,7). In addition, the described particles have the advantage of being constituted of a material commonly used in conventional oral formulation. In this study, the potential of pH-sensitive particles for oral delivery of poorly water soluble compounds was further demonstrated, which may provide new perspectives on a wide spectrum of potent compounds for which pharmaceutical development has been hampered as a consequence of poor aqueous solubility.

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