International Journal of Pharmaceutics 396 (2010) 149–155

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Nanosized particles of orlistat with enhanced *in vitro* dissolution rate and lipase inhibition

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ARTICLE INFO

Article history:
Received 4 February 2010
Received in revised form 29 May 2010
Accepted 1 June 2010
Available online 9 June 2010

Keywords:
Orlistat
Obesity
Nanosuspension
Dissolution
In vitro lipase inhibition
Melt emulsification
High pressure homogenization

ABSTRACT

Orlistat is locally acting inhibitor of gastrointestinal lipases which has been developed for the treatment of obesity. The present study was designed with the intent to formulate orlistat in a different way compared to the current practice and investigate its inhibition of gastrointestinal lipases. Orlistat is considered as a technologically problematic and unmanageable substance because of waxy nature, low melting point and low chemical stability. The manuscript presents the critical issues regarding engineering of its nanosuspension with controlled particle size by melt emulsification and high pressure homogenization. In order to formulate dry product, lactose was dissolved in nanosuspension as filler and spray drying has been performed for obtaining the final powder product. Laser diffraction, scanning electron microscopy and atomic force microscopy have been used for orlistat nanosuspension characterization, dissolution studies and lipase inhibition studies were performed to characterize the *in vitro* efficacy of formulated orlistat. The advantage of selected technological procedures is nanosized orlistat with elevated *in vitro* dissolution rate in comparison to raw drug, physical mixture and marketed product. Furthermore, nanosuspension demonstrated significantly higher *in vitro* lipase inhibition in comparison to references. To conclude, the results show new technological solution and remarkable increase of pharmacological effect which could potentially lead to decreasing the dose and consequently dose dependent side effects.

1. Introduction

Obesity is a disease involving body fat storage and body weight gain. The recent health crisis has spurred research in weight control, including studies in diet, exercise, surgery and pharmaceutical preparations. Because obesity is caused by a build-up of fat in the body due to, for example, the over-consumption of high fat food, modern therapeutic approaches are mostly focused on blocking or stimulating various biomolecules and enzymes involved in fat metabolism. Some strategies have encompassed serotonin and noradrenalin re-uptake inhibitors, β3-adrenoreceptor agonists, leptin agonists, melanocortin-3 agonists, cannabinoid (CB1) receptor blockers, and lipase inhibitors (Ballinger and Peikin, 2002; Heck et al., 2000).

Recently, much attention has been given to the development of new medicinal products that could be used in chronic treatment accompanied by a reduced-calorie diet and physical activity. As excess fat consumption is widely thought to be one of the primary causes of obesity, ways of specifically inhibiting triglyceride digestion have been introduced as an approach to reducing fat absorption. Orlistat (Fig. 1), also known as tetrahydrolipstatin (THL), belongs to a class of pharmacological agents, the lipase inhibitors (EMEA, 2005). It exerts its pharmacological activity in the lumen of the stomach and small intestine by binding to the active site of gastric and pancreatic lipases, with consequent inhibition of the systemic absorption of dietary fat. The undigested triglycerides are not absorbed, resulting in caloric deficit and a positive effect in weight control (Hadvary et al., 1988; Henness and Perry, 2006; Kothamasu et al., 2009; Lowe, 1994; Wang and Du, 2009). The recommended dose of orlistat is one 120 mg capsule three times daily, during or 1 h after each main meal containing fats (EMEA, 2005). Recently, an over-the-counter product containing 60 mg orlistat per capsule has been launched and recommended for patients with mild obesity (DeLancey, 2009; EMEA, 2009). The drug acts specifically on digestive lipases *in vivo*, although it can inhibit other human lipases such as lipoprotein and hormone-sensitive lipase *in vitro* (Carrière et al., 2001; Wang and Du, 2009).

Orlistat is a white to off-white crystalline powder with lipophilic properties and very low aqueous solubility within the physiological range of pH (Effat et al., 2007; Kothamasu et al., 2009; Mohammadi et al., 2006). Due to its low water solubility and dissolution rate, only a low level of drug dissolves. Undissolved drug exhibits no, or very limited, therapeutic activity, regardless of whether a systemic or a localized action is desired. Therefore, higher doses have to be

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doi:10.1016/j.ijpharm.2010.06.003

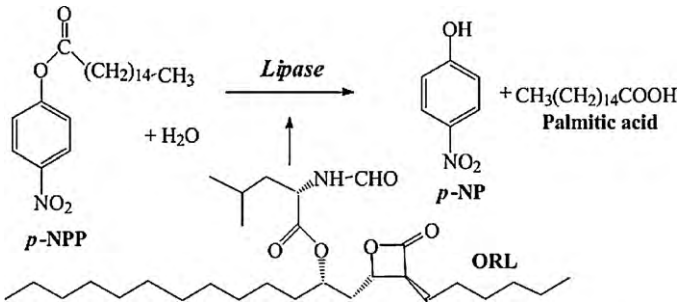

Fig. 1. Hydrolysis of *p*-nitrophenylpalmitate (*p*-NPP) to *p*-nitrophenol (*p*-NP) by pancreatic lipase and inhibition by orlistat (ORL).

administered, which can result in an increased potential for serious side effects (Maeder et al., 2004).

Orlistat is considered in the state of the art as a technologically problematic and unmanageable substance because of its waxy nature, low melting point, at 43 °C, and low chemical stability. Additionally, the crystalline structure of orlistat, which is important for chemical stability, is disadvantageous in terms of bioavailability (Moon and Lee, 2007; Shah and Zeller, 1998).

According to recent patent reports, pharmaceutical compositions containing orlistat have been formulated using various technological processes, such as extrusion and spheronization, micronization and other relatively time-consuming and demanding procedures (Shah, 1998). In contrast, formulations with orlistat can also be produced by relatively simple and rapid methods such as blending and mixing with additives (Barbier et al., 2002). It has been included in film delivery systems (Myers et al., 2009) and soluble fibre tablets (Daggy and Mandel, 2003). The problematic nature of orlistat thus indicates the need for developing a novel drug delivery system that will be able to satisfy most of the formulation and pharmacodynamic requirements. For that reason, we have selected nanosuspension as formulations potentially able to enhance solubility of orlistat and its lipase inhibition.

In recent years, nanoparticle engineering has been seen as a promising approach for the enhancement of drug solubility (Rabinow, 2004; Műller and Keck, 2004; Keck and Müller, 2006; Kocbek et al., 2010). An outstanding feature of nanosuspensions is the increase in saturation solubility, and consequently an increase in dissolution rate of the compound, caused by small sized particles (Kelvin effect) with greater specific surface area. In general, saturation solubility is a compound-specific constant, and is temperature dependent. However, due to increased dissolution pressure, as described by Kelvin's equation, the saturation solubility increases below a particle size of approximately 1000 nm (Kocbek et al., 2006; Műller and Keck, 2004). Moreover, similarly to other nanoparticles, nanosuspensions show increased adhesiveness to tissue (Ponchel et al., 1997).

Nanosuspensions in aqueous or non-aqueous vehicles can be produced by bottom-up (e.g. precipitation) or top-down (e.g. wet milling) processes. High pressure homogenizers such as the piston gap homogenizer have proved to be a highly successful in nanosuspension formation (Keck and Müller, 2006; Ambrus et al., 2009; Dolenc et al., 2009). Furthermore, the melt emulsification method, traditionally used to prepare solid lipid nanoparticles, has been adapted for production of nanosuspensions by Kocbek et al. (2006). The particular advantage of this method is the avoidance of organic solvents during production. It is less time-consuming and leaves no unprocessed active compound, which can occur in the case of wet milling. Additionally, the method is useful for drugs with melting points below 100 °C (Kocbek et al., 2006).

Most publications on orlistat have been focused primarily on clinical studies, and only a few deals with technological aspects and *in vitro* efficacy. Its crystal morphology has been reported, but very few data exist regarding solubility and dissolution rate, and their impact on lipase inhibition. Our main goal has been to design an oral orlistat nanoformulation with greater efficacy and potency than the raw substance and the already registered and marketed product. In order to achieve a faster and more potent effect, the main emphasis must be on establishing increased solubility and dissolution rate.

2. Materials and methods

2.1. Materials

Orlistat was obtained from Ranbaxy (Sinnar, India), sodium dodecyl sulphate (SDS) and acetonitrile from Merck KgaA (Darmstadt, Germany), Tween® 80 from Fluka (Buchs SG, Switzerland) and polyvinylpyrrolidone K-30 from BASF (Germany). Tris(hydroxymethyl) aminomethane for pH 8.4 buffer, 4-nitrophenylpalmitate (*p*-NPP) as lipase substrate, anhydrous sodium acetate ($C_2H_3NaO_2$), lipase powder from porcine pancreas (L3126, Type II, 358 units/mg protein using olive oil or 50 units/mg protein using triacetin), sodium deoxycholate and other bile salts used in reaction mixtures, and sodium taurodeoxycholate were obtained from Sigma–Aldrich (Steinheim, Germany). Potassium sodium tartrate tetrahydrate, for pH 4.0 buffer, and calcium chloride were from Merck (Frankfurt, Germany). All other chemicals were of analytical grade and used as received. Water was purified by reverse osmosis.

2.2. Reagents for lipase inhibition

Solution I – Solution I has been prepared by mixing 41 mM Tris buffer, pH 8.4 with 1.8 mM sodium deoxycholate and 7.2 mM sodium taurodeoxycholate, just before experiments.
Solution II – Solution II consisted of 0.1 mM $CaCl_2$ in 1.6 mM tartrate buffer, pH 4.0, and was prepared just before experiment.
Solution for enzymatic reaction: Mixture of Solution I and Solution II in volume ratio 8.5:1.5.
Substrate solution: 4-Nitrophenylpalmitate (*p*-NPP) was dissolved in acetonitrile to give a stock solution with a concentration of 10 mM. Eventually, stock solution was diluted with Solution for enzymatic reaction to give a final concentration of 0.25 mM and stored at −20 °C.
Lipase suspension: Lipase powder from porcine pancreas was suspended in a Tris buffer pH 8.4 in a concentration of 5 mg/ml. The suspension was centrifuged and the supernatant was used for enzymatic reaction.
Buffer solution pH 4.5: Buffer solution pH 4.5 was prepared according to the prescription from Ph. Eur. 6th Ed. (*2.9.3 Dissolution test for solid dosage forms*). 2.28 g of Tris was mixed with 1.77 g of anhydrous sodium acetate. Prepared mixture was dissolved in 1 l of hydrochloric acid solution pH 1.5.

2.3. Methods

2.3.1. Preparation of nanosized orlistat

Orlistat nanosuspension was prepared by the melt emulsification method. Raw orlistat (2.5 g) was dispersed in 100 ml aqueous solutions of various stabilizers (Tween® 80, PVP K-30, poloxamer 188 and SDS) in different concentrations. The dispersion was stirred during heating to approximately 50 °C, above the orlistat melting point ($T_m \approx 43$ °C), for 2–3 min. The dispersion, in the form of a hot emulsion, was transferred to a heated high pressure homogenizer (HPH; APV-2000, Invensys, Denmark) and homogenized at 300/30 bars (first/second valve) and 50 °C for 10 cycles. The homog-

enized emulsion was cooled slowly to room temperature to solidify and form nanosized particles.

2.3.2. Spray drying

Lactose (7.5 g) was dissolved in cold nanodispersion, prepared with 2.5 g orlistat and 0.5 g SDS, one day after its preparation. The dispersion was then spray dried (Büchi B290, Flawil, Switzerland). The inlet air temperature was set to 70 °C, the aspirator to 100% and pump to 10%. The outlet air temperature was 36 °C. The spray dried product was filled into capsules size 0 containing 60 mg of orlistat, equivalent to the product on the market.

2.3.3. Physico-chemical characterization of orlistat nanosuspension

2.3.3.1. Determination of solubility. Orlistat (200 mg) was added to 50 ml of purified water containing SDS at 1, 2, and 3% (w/v), and stirred on a magnetic stirrer at 25 °C. After 1, 2 and 4 days of stirring, samples were filtered through a 0.2 μm CE filter (Sartorius AG, Goettingen, Germany) and the amount of dissolved orlistat determined as described below.

2.3.3.2. Particle size analysis. Particle size distribution was determined using a laser diffractometer, Mastersizer X, equipped with a small sample dispersion unit (Malvern Instruments, Worcestershire, UK). Samples were dispersed in orlistat-saturated water. Particle size distribution typically includes D (v, 0.1), D (v, 0.5) and D (v, 0.9), which represent the volume percentage of particles below a determined size (μm). This technique requires the refractive index (RI) of the substance, which was determined using Saveyn's method (Saveyn et al., 2002). Solutions of orlistat were prepared in ethanol over a range of concentrations from 1 to 25% (w/w). The RI of the solutions was measured using a refractometer (Jena J78, Carl Zeiss, Germany) and extrapolated to 100% concentration to yield the solid RI (20).

2.3.3.3. Scanning electron microscopy (SEM). A scanning electron microscope (SEM, Supra 35 VP-24-13, Carl Zeiss, Germany), with a secondary detector was operated at an acceleration voltage of 0.6 kV. SEM samples were prepared by the droplet evaporation technique. A droplet of nanosuspension was deposited on double-sided carbon tape (diameter 12 mm, Oxon, Oxford Instruments, UK), dried at room temperature. A SEM sample of raw orlistat was prepared by direct deposition of powder on double-sided carbon tape.

2.3.3.4. Atomic force microscopy (AFM). Droplets of orlistat nanosuspension were deposited on freshly cleaved mica and dried 15 min in a covered Petri dish at room temperature in order to avoid dust contamination. Nanoparticles were imaged by tapping-mode AFM, using a silicon cantilever in force modulation mode (PPP-FMR-10, Nanosensors, f_0 = 75 kHz, c = 2.8 N/m) on an Agilent AFM 5500 microscope (Agilent, Santa Clara, USA). Topography, amplitude and phase data were collected simultaneously for all samples. Image analysis was carried out using the Pico-image software package.

2.3.3.5. Determination of orlistat concentration by HPLC. Orlistat was quantified using a HPLC system Agilent 1100 series (Hewlet Packard, Waldbron, Germany) equipped with a C18 column (150 mm × 4.6 mm, 5 μm Symmetry, Thermo, USA) at 25 °C. The mobile phase consisted of acetonitrile:water (950:50, v/v) with 0.1% (v/v) phosphoric acid (85% R) at a constant flow rate of 1.5 ml/min. The eluent was monitored at 205 nm. The response was linear between 10–200 mg/l. All samples were analyzed in triplicate.

2.3.4. In vitro studies

2.3.4.1. Dissolution. Capsules with spray dried product containing nanosized orlistat were dissolved using a paddle method on a validated dissolution system VanKel (VK 6000, New Jersey, USA) at a rotational speed of 75 rpm and 37 ± 0.5 °C. Capsules, fixed on sinkers, were transferred to 900 ml of different dissolution media containing various concentrations of SDS (1, 2 and 3%, w/v). 5 ml samples were withdrawn at predetermined intervals and filtered through 0.2 μm CE filters (Sartorius AG, Goettingen, Germany). The first 4 ml of filtrate was rejected before collecting the sample in the vial. The concentration of sample was determined as above. Raw orlistat (ORL), physical mixture of raw orlistat with lactose and SDS (PM) in the same ratio as used for preparation of spray dried product with nanosized orlistat, and marketed product Alli® 60 mg (GlaxoSmithKline, UK) were used as references.

2.3.4.2. Lipase inhibition.

2.3.4.2.1. Sample preparation for lipase inhibition assay. Samples were prepared for lipase assay using the USP dissolution apparatus II. The type of dissolution medium included acetate buffer pH 4.5, according to the Ph Eur 6th Ed. Raw orlistat (ORL), physical mixture of orlistat with excipients (PM) and Alli® capsules as reference and capsules with nanosized orlistat (NS), each form fixed on sinkers, were placed in vessels containing 900 ml of the dissolution medium. The vessels were stirred at 75 rpm. The temperature of the dissolution media was kept at 37 °C throughout the dissolution study. Aliquots (10 ml) were taken at 5, 15, 30, 45, 60 and 90 min, and centrifuged at 17,000 rpm for 5 min (Sigma 3K30 centrifuge, Germany); supernatants were used for lipase inhibition analysis. All samples were analyzed in triplicate.

2.3.4.2.2. In vitro assay for determination of lipase inhibition. The method according to Lee et al. was optimized for assaying lipase activity. The basic principle involved hydrolysis of the chromogenic ester (*p*-NPP) and quantification of the yellow coloured *p*-nitrophenol product (Lee et al., 1993).

Enzyme assays were performed in a 96-well microplate by adding 78 μl of substrate solution, 20 μl of lipase suspension (c = 5 mg/ml) and 2 μl of orlistat dissolution sample in pH 4.5. After 20 min pre-incubation of inhibitor with lipase in the presence of bile salts, 100 μl of substrate *p*-NPP was added and absorbance measured immediately at 405 nm, then at 60 s intervals for 60 min at 37 °C, using a Tecan Safire® microplate reader (Tecan® Trading AG, Switzerland). Positive (80 μl of solution for enzymatic reaction, 20 μl of lipase suspension and 100 μl of substrate) and negative (100 μl of solution for enzymatic reaction and 100 μl of substrate) controls were analyzed at the same time. Activity and inhibition of lipase with orlistat were obtained from % of activity of lipase = $\text{slope}_{\text{avg}}$ (sample)/$\text{slope}_{\text{avg}}$ (positive control) × 100, and % of inhibition = 100 − % of activity of lipase.

3. Results and discussion

Since orlistat expresses its effect locally in the stomach and upper jejunum, it could be important to increase the concentration of dissolved drug there. According to this, higher lipase inhibition in the gastro intestinal tract (GIT) could be achieved by using nanosized orlistat formulation. The main benefit of nanosuspension formulations is their increase in drug solubility due to smaller particles and higher dissolution rate as a consequence of higher specific surface area. Furthermore, orlistat nanoformulation could provide better adhesiveness on tissue and prolonged effect on lipase inhibition in comparison to conventional raw compound. Reduction of dose and dose dependent side effects could be another and essential benefit of the designed formulation.

Table 1
Particle size in particular volume fraction of raw orlistat, orlistat nanosuspension, and redispersed spray dried formulation with nanosized orlistat.

	Particle size (μm)		
	D (v, 0.1)	D (v, 0.5)	D (v, 0.9)
Raw orlistat	38.51	87.32	168.04
Orlistat nanosuspension	0.10	0.26	0.75
Redispersed spray dried formulation with nanosized orlistat	0.11	0.32	1.26

3.1. Preparation and physico-chemical characterization

The selection of an appropriate stabilizer is crucial in the development of nanosuspensions and is specific for each drug candidate. In pre-formulation experiments, Tween® 80, PVP K-30, poloxamer 188 and SDS were tested as stabilizers in concentrations from 0.1 to 2% (w/w). According to particle size, SDS proved to be the most appropriate candidate. It has been utilized as a wetting agent in marketed orlistat products, further confirming it as the recommended option. Dispersion with 0.5% (w/w) SDS and 2.5% (w/w) orlistat resulted in the preferred particle size and were used for further experiments. Greater numbers of droplets can be formed by increasing the amount of drug with higher specific area and consequently higher interfacial energy. Further increase in drug content led to formation of an unstable emulsion with larger drug droplets. Lower SDS concentrations also resulted in less stable formulations with larger particles.

These preliminary experiments showed that the combination of high pressure homogenisation with the melt emulsification method and with SDS as stabilizer are appropriate for preparing nanosuspensions with controlled particle size distribution. The size of orlistat particles was determined 24 h after preparation by laser diffraction (LD), using an experimentally determined value of refractive index of 1.45 (data not shown). The particle size of raw orlistat ranged from 38.51 to 168.04 μm, and of nanoformulated orlistat from 100 to 750 nm (Table 1).

The conversion of nanosuspension into a dry state is a very important step, in terms of long-term stability. Due to low chemical stability of orlistat in water nanosuspensions were prepared fast and dried immediately. Usually, two methods are used, spray drying and lyophilisation. The former is generally preferred in the pharmaceutical industry, since it is less time and energy consuming. Spray drying of orlistat nanosuspension has to be carefully controlled as the temperature of the dried product should not reach or exceed the melting temperature of orlistat. Low inlet temperature and low feed rate, combined with high air flow, resulted in a low product temperature (36 °C), conditions adequate for drying the formulation. The spray dried product was collected and stored in a humidity resistant container. Values of the size distributions D (v, 0.1), D (v, 0.5) and D (v, 0.9) of redispersed spray dried product with nanosized orlistat were increased only slightly (Table 1), confirming the suitability of the method. The yield of nanosuspension preparation and spray drying was approximately 77% and the content of orlistat 23.1 ± 0.3%, which is practically the same as theoretical (23.8%). HPLC analyses of our dried samples have shown no additional peaks which would indicate the degradation of orlistat. Additionally particle size analyses of redispersed spray dried product have confirmed that the physical stability expressed as particle size did not change significantly during spray drying.

The size and shape of raw and nanosized orlistat particles were confirmed by SEM and AFM microscopy. SEM imaging can be very demanding because of the low melting point of orlistat. Due to the vacuum and exposure to electrons in SEM, images have to be taken as quickly as possible, otherwise melting can occur. The morphology of samples can differ significantly (Fig. 2). Particles of raw orlistat exhibit large aggregates of needle shaped crystals (Fig. 2a) which probably behave like large crystals since the size, obtained by light diffraction, was very large (Table 1). Fig. 2b shows orlistat nanoparticles produced by melt emulsification followed by spray drying with lactose. Spherical or sphero-cylindrical particles are seen, with a large population of particles with size in the range of 100 nm.

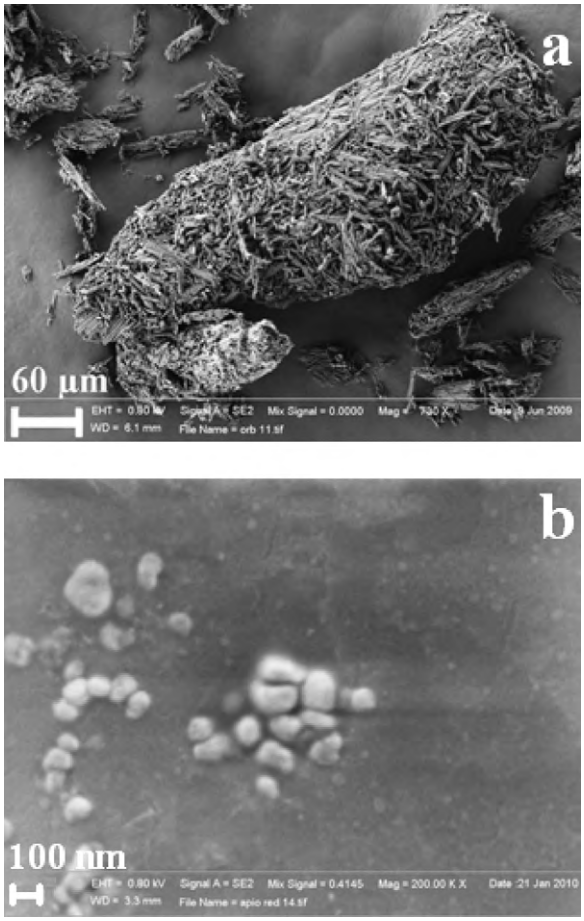

Fig. 2. SEM images of raw (a) and nanosized orlistat (b).

AFM is capable of scanning the surfaces in controlled environmental conditions and is complementary to SEM imaging. Dispersing nanoparticles on a substrate (mica) is essential, for several reasons. Factors such as exposure time and dilution of the particle dispersion can be taken into consideration. Other parameters, such as the interfacial and electrostatic energy associated with the nanoparticles, tend to cause them to clamp together or to keep far apart. Also, hydrophilic and hydrophobic interactions between particle, substrate and solution can cause agglomeration and coalescence (Kirat et al., 2005).

Diluted water dispersions of nanosized orlistat were used for sample preparation, which was conducted in the same way as for SEM imaging. Water was chosen as the appropriate solvent, given the poor solubility characteristic of orlistat. A diluted nanosuspension resulted in characterization of individual nanoparticles, 15 min after air drying on muscovite mica as a substrate (Fig. 3). The drawback of the method is that some particles could still be dissolved in the dispersive medium due to the high dilution and could later crystallize during drying. Step height measurement clearly confirms the irregular particle shape with particle size 160.6 ± 44 nm

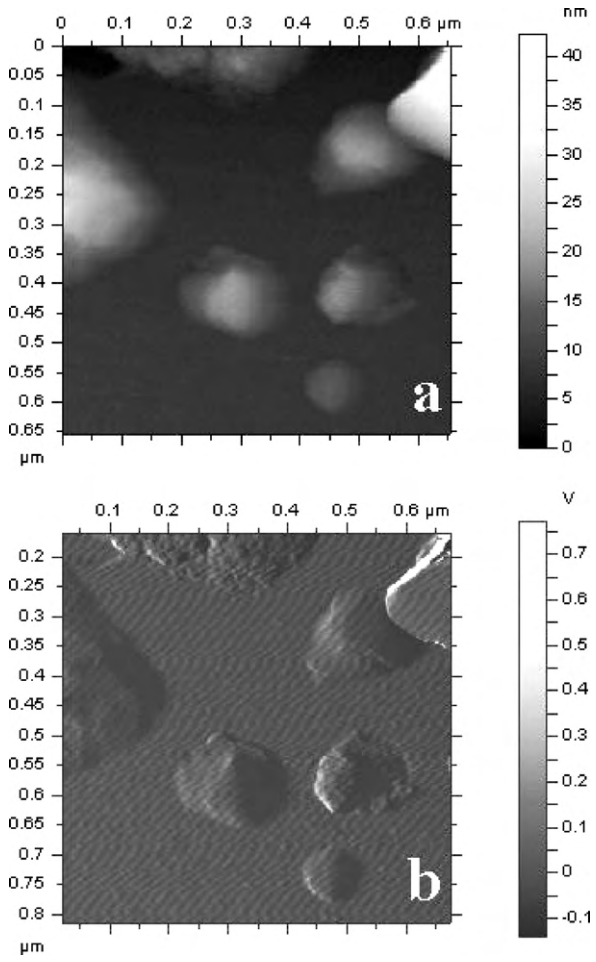

Fig. 3. AFM images of nanosized orlistat: topography 2D (a) and amplitude image 2D (b): orlistat nanoparticles imaged by tapping mode in air.

(Fig. 4), in good agreement with the results obtained by SEM.

3.2. Solubility and dissolution studies

Knowledge of drug substance solubility in different media is important in developing an appropriate dissolution method, which is a very challenging issue in the case of drugs with low water solubility. Dissolution studies are usually performed under "sink conditions" which normally occur in a volume of dissolution medium that is at least 3–10 times the saturation volume (Ph. Eur. 6th Ed.). In particular situations, media with non-sink conditions might also be used, if they are shown to be more discriminating or provide reliable data which otherwise can only be obtained by the addition of surfactants (Müllertz, 2007). According to the solubility results (Table 2), 900 ml of medium with SDS 1% or more would ensure sink conditions for dosage forms with 60 mg of orlistat. The solubility of orlistat increased with higher SDS concentration (Table 2). Solubility in water without any SDS was below the limit of detection of the HPLC method (2 mg/l).

Table 2
Orlistat solubility in the media with various concentration of sodium dodecylsulphate.

SDS (%)	0% NaCl *c* (mg/l)	0.5% NaCl *c* (mg/l)
1	0.95 ± 0.06	1.25 ± 0.07
2	1.80 ± 0.01	2.15 ± 0.04
3	2.30 ± 0.03	2.90 ± 0.05

An additional object of interest was the impact of NaCl on the solubility of orlistat. Electrolytes are often added to the dissolution medium to mimic the ionic strength in the GIT. The addition of inorganic salts increases surface activity and strengthens the adsorption of surfactants at the boundary between phases. Inorganic electrolytes influence all the colloidal properties of surfactants, increasing their micelle forming ability, solubilizing action, and wetting. The introduction of electrolytes also decreases the critical micelle concentration of ionogenic surfactants (Kolesnikova and Glukhareva, 2009). The addition of NaCl to the SDS medium increased the solubility of orlistat, in agreement with reported data (Table 2).

It is important to establish an *in vitro* dissolution test that measures the product performance, especially for poorly water soluble drugs. Dissolution testing is mainly utilized to develop and evaluate new formulations by determining the rate of drug release from dosage forms, estimating the stability of such formulations, monitoring product consistency and assessing formulation changes.

Dissolution of capsules with spray dried orlistat nanoformulation (NS), raw orlistat (ORL), physical mixture (PM) and marketed 60 mg product (Alli®) was studied in media with SDS concentrations (1–3%) that ensured sink conditions. NS released approximately 50% of the orlistat in 15 min in 1% SDS and more than 90% in 2% and 3% SDS media (Fig. 5). In the same time and under the some conditions, Alli® released only approximately 5, 10

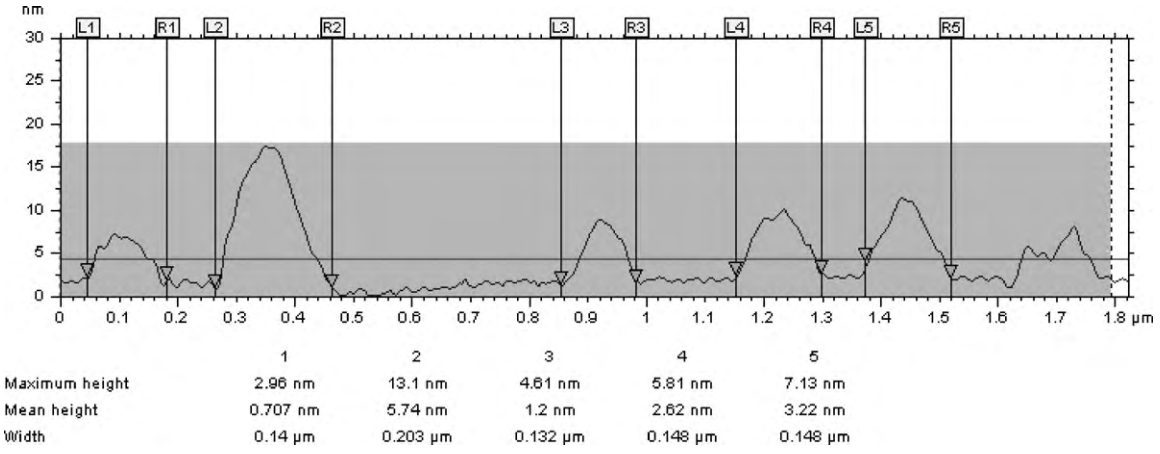

Fig. 4. Step height measurement of an orlistat nanosuspension deposited and dried on freshly cleaved mica. The average particle size obtained by AFM analysis is 160.6 ± 44.7 nm.

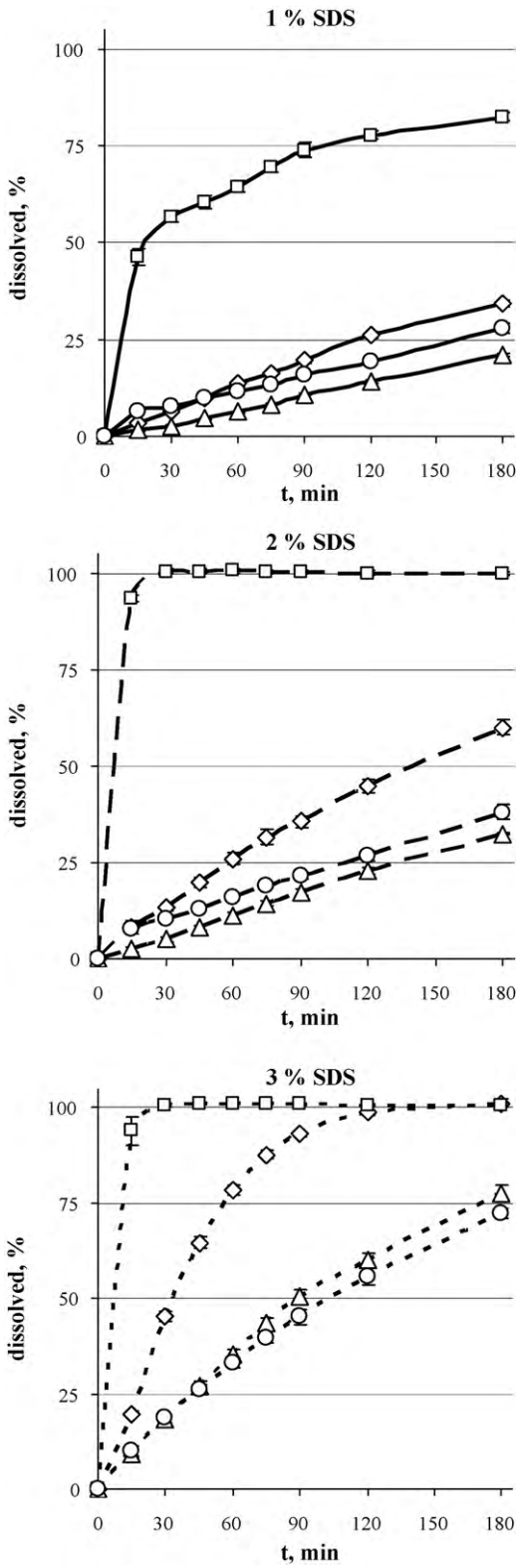

Fig. 5. The dissolution profiles of spray dried formulations with nanosized orlistat (NS, -□-), raw orlistat (ORL, -△-), physical mixture (PM, -◘-) and marketed product (Alli®, -◇-). The results were obtained with the paddle dissolution test in 1% (—), 2% (– –) and 3% SDS (- - -). Error bars of three repeats are less than ±2%.

and 20% of orlistat. Complete dissolution of NS was observed after 3 h in 1% SDS medium and after only 30 min in 2% and 3% SDS. In the case of Alli®, the dissolution profile in 3% SDS showed complete release of orlistat after 2 h. As expected, dissolution of raw orlistat and in physical mixture was similar but slower than that of Alli® in all dissolution media. The dissolution profiles of physical mixture (orlistat/SDS/lactose 2.5/0.5/7.5) confirmed that the increase in the dissolution rate of NS was mainly accounted by the decrease in particle size.

There are no pharmacopoeial monographs or dissolution tests for orlistat. However, FDA suggests that an appropriate test for capsules with 120 mg of orlistat would be the USP II test using 900 ml of 3% SDS in 0.5% sodium chloride solution, pH 6.0 and 75 rpm. The dissolution profiles of NS, Alli® and ORL in 1, 2 and 3% SDS with 0.5% NaCl were also determined (data not shown). In all the media, nanosized orlistat was completely dissolved in 15 min. The dissolution rate was accordingly significantly higher than with other formulations used for reference.

Sink conditions have been established with 1% SDS in water, which is therefore an adequate dissolution medium for 60 mg orlistat capsules. This medium is quite different from those orlistat pharmaceutical formulations used, and is therefore a more discriminating and pertinent tool for dissolution testing than media with higher SDS concentrations.

3.3. Lipase inhibition studies

The most important property of orlistat is its very potent inhibition of lipase (Sternby et al., 2002). IC_{50} is characteristic of the inhibitors; however it depends also on the substrate and lipase, their concentration and temperature. Lipase inhibition studies are often used to mimic the conditions in the GIT and are used to predict the efficacy of lipase inhibitors. The major advantages of the method applied in this study are the following: a more relevant, from the *in vivo* point of view, medium (buffer pH 4.5) used for dispersing the orlistat capsules (Lewis and Liu, 2009.), avoidance of addition of large amount of surfactants such as SDS for improving poor solubility and wettability of orlistat in water, and simulation of the conditions in the GIT (presence of bile salts in solution for lipase assay). Furthermore, the large volume dissolution approach (900 ml) for sample preparation makes the method more suitable as a discriminatory quality control test. Finally, the method could be performed using regular UV–vis spectrophotometry due to increase of the volumes of solutions used in the assay.

The degree of lipase inhibition differs significantly between the nanosized orlistat in capsules and the references (Fig. 6). Nanosized orlistat rapidly achieved saturated concentration and caused 90% reduction of pancreatic lipase activity in 5 min after the gelatinous capsule had been dissolved and redispersed in medium. Disintegration of orlistat pellets from Alli® was slow and incomplete over 90 min, resulting in significantly lower lipase inhibition.

A moderate dose of orlistat (60 mg) has been shown to reduce 90% of gastric and pancreatic lipase activity, if it is adequately mixed with the meal (Sternby et al., 2002). Furthermore, when the same dose was given as a capsule, the reduction in lipolytic activity was markedly less, indicating the inadequate accessibility of the drug in this form. Other clinical studies have confirmed enhanced inhibitory effect of micronized orlistat in comparison to orlistat pellets (Carrière et al., 2001).

The results of the *in vitro* experiments with the orlistat nanoformulation strongly suggest the superiority of such a formulation in *in vivo* conditions (fed-state conditions) because of the rapid dispersal and increased surface area of nanosized orlistat. The reduction of lipase activity from current *in vitro* experiments is significantly greater than equivalent results from *in vivo* clinical tests, in accordance with reported data. This could be explained in terms of

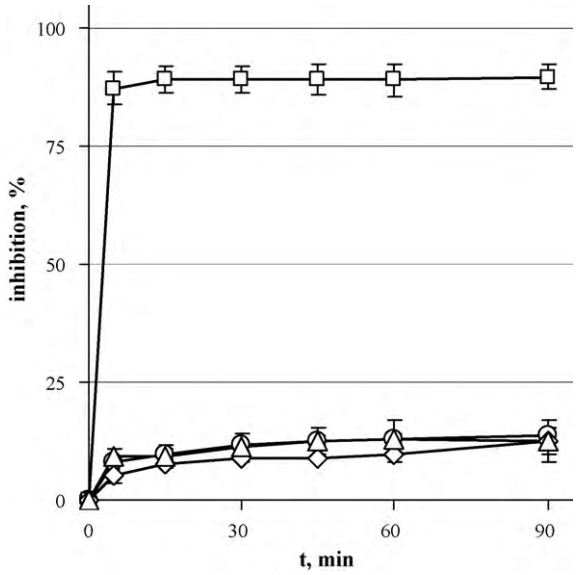

Fig. 6. Inhibition of lipase by nanosized orlistat formulation (NS, -□-), raw orlistat (ORL, -△-), physical mixture (PM, -¤-) and marketed product (Alli®, -◇-). Sample preparation was conducted in buffer pH 4.5 without added SDS.

physiological factors such as different duration of gastric emptying, excretion of bile, different motility of jejunum, and pathological complications which could significantly decrease the percentage of pancreatic lipase inhibition *in vivo* (Carrière et al., 2001; Sternby et al., 2002).

4. Conclusion

The melt emulsification method, with high pressure homogenization, is shown to be a useful approach for producing a nanosuspension of a poorly soluble drug with low melting point. Because of the demanding physical properties of orlistat, the selection of an appropriate stabilizer and production parameters are essential for the nanosuspension preparation. A nanosuspension in the form of a dry powder was obtained by spray drying with lactose. It exhibited a much greater *in vitro* dissolution rate than both the raw drug and the marketed product. Importantly, the nanosuspension demonstrated much greater *in vitro* lipase inhibition than the reference products. All these findings support the possibility of decreasing the dose *in vivo* and consequently the dose dependent side effects.

Acknowledgements

The research was supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia. The authors thank Prof. Odon Planinšek for his support with scanning electron microscopy and Prof. Roger H. Pain for critical reading of the manuscript. The authors wish to express their gratitude to Polpharma for the supply of active substance.

References

Ambrus, R., Kocbek, P., Kristl, J., Sibanc, R., Rajkó, R., Szabó-Révész, P., 2009. Investigation of preparation parameters to improve the dissolution of poorly water-soluble meloxicam. Int. J. Pharm. 381, 153–159.

Ballinger, A., Peikin, S.R., 2002. Orlistat: its current status as an antiobesity drug. Eur. J. Pharmacol. 440, 109–117.

Barbier, P., Hadvary, P., Lengsfeld, H., 2002. Orlistat compositions. US 2002/0035089. Hoffman-La Roche Inc.

Carrière, F., Renou, C., Ransac, S., Lopez, V., De Caro, J., Ferrato, F., De Caro, A., Fleury, A., Sanwald-Ducray, P., Lengsfeld, H., Beglinger, C., Hadvary, P., Verger, R., Laugier, R., 2001. Inhibition of gastrointestinal lipolysis by orlistat during digestion of test meals in healthy volunteers. Am. J. Physiol. Gastrointest. Liver Physiol. 281, 16–28.

Daggy, B.P., Mandel, K.G., 2003. Lipastatin derivative—soluble fiber tablets. US 2003/023077. GlaxoSmithKline.

DeLancey, S., 2009. FDA Issues Early Communication About Ongoing Safety Review of Weight Loss Drug Orlistat. FDA News Release, Available online on: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm180057.htm.

Dolenc, A., Kristl, J., Baumgartner, S., Planinšek, O., 2009. Advantages of celecoxib nanosuspension formulation and transformation into tablets. Int. J. Pharm. 376, 204–212.

Effat, S., Hassan, J., Abbas, K.Z., Bettina, Z., 2007. HPLC analysis of orlistat and its application to drug quality control studies. Chem. Pharm. Bull. 55, 251–254.

EMEA, 2005. Scientific Discussion, Available online on: http://www.ema.europa.eu/humandocs/PDFs/EPAR/Xenical/106698en6.pdf.

EMEA, EMEA, 2009. European Public Assessment Report (EPAR) Alli, EMEA/H/C/854.

Hadvary, P., Lengsfeld, H., Wolfer, H., 1988. Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. Biochem. J. 256, 357–361.

Heck, A.M., Yanovski, J.A., Calis, K.A., 2000. Orlistat, a new lipase inhibitor for the management of obesity. Pharmacotherapy 20, 270–279.

Henness, S., Perry, C.M., 2006. Orlistat: a review of its use in the management of obesity. Drugs 66, 1625–1656.

Keck, C.M., Müller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur. J. Pharm. Biopharm. 62, 3–16.

Kirat, K., Burton, I., Dupres, V., Duprene, Y., 2005. Atomic force microscopy in the characterization of drug nanoparticles. J. Microsc. 218, 199–207.

Kocbek, P., Baumgartner, S., Kristl, J., 2006. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int. J. Pharm. 312, 179–186.

Kocbek, P., Teskač, K., Brožič, P., Lanišnik-Rižner, T., Gobec, S., Kristl, J., 2010. Effect of free and in poly(ε-caprolactone) nanoparticles incorporated new type 1 17β-hydroxysteroid dehydrogenase inhibitors on cancer cells. Curr. Nanosci. 6, 69–76.

Kolesnikova, E.N., Glukhareva, N.A., 2009. The influence of an electrolyte on micelle formation in aqueous solutions of sodium monoalkyl sulfosuccinates. Phys. Chem. Surf. Phenom. 93, 2322–2324.

Kothamasu, S.S., Sah, A., Asapu, U.S., Arumalla, M.R., 2009. Orlistat pharmaceutical formulations. WO 2009/039157. Dr. Reddy's Laboratories Ltd.

Lee, Y.P., Chung, G.H., Rhee, J.S., 1993. Purification and characterization of Pseudomonas fluorescens SIK W1 lipase expressed in Escherichia coli. Biochim. Biophys. Acta. 1169, 156–164.

Lewis, D., Liu, D., 2009. A Novel In Vitro Assay Capable of Monitoring the Inhibitory Effect of Orlistat Dosages, Available online on: http://www.aapsj.org/abstracts/NBC_2009/NBC09-000376.PDF.

Lowe, M.E., 1994. Pancreatic triglyceride lipase and colipase: insights into dietary fat digestion. Gastroenterology 107, 1524–1536.

Maeder, K., Scheibler, L.C., Steffen, H., 2004. Pharmaceutical compositions having depressed melting points. US 6,730,319. Hoffmann-La Roche Inc.

Mohammadi, A., Haririan, I., Rezanour, N., Ghiasi, L., Walker, R.B., 2006. A stability-indicating high performance liquid chromatographic assay for the determination of orlistat in capsules. J. Chromatogr. A 1116, 153–157.

Moon, J.M. Lee, H.-A., 2007. Pharmaceutical formulation with high stability and dissolution and manufacturing process. WO 2007/021073. Boram Pharm. Co., Ltd.

Müller, R.H., Keck, C.M., 2004. Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. J. Biotechnol. 113, 151–170.

Müllertz, A., 2007. Biorelevant dissolution media, solvent systems and their selection in pharmaceutics and biopharmaceutics. AAPS Series, Biotechnology: Pharmaceutical Aspects, pp. 151–177.

Myers, G.L., Hilbert, S.D., Boone, B.J., 2009. Film delivery system for tetrahydrolipstatin. US 2009/0104270. Hoffmann & Baron, LLP.

Ponchel, G., Montisci, M.J., Dembri, A., Durrer, C., Duchêne, D., 1997. Mucoadhesion of colloidal particulate systems in the gastro-intestinal tract. Eur. J. Pharm. Biopharm. 44, 25–31.

Rabinow, B.E., 2004. Nanosuspensions in drug delivery. Nat. Rev. Drug Discov. 3, 785–796.

Saveyn, H., Mermuys, D., Thas, O., van der Meeren, P., 2002. Determination of the refractive index of water-dispersible granules for use in laser diffraction experiments. Part. Syst. Charact. 19, 426–432.

Shah, N., Zeller, M., 1998. Tetrahydrolipstatin containing formulations. WO 98/34607. Hoffman-La Roche AG.

Sternby, B., Hartmann, D., Borgstroom, B., Nilsson, P., 2002. Degree of *in vivo* inhibition of human gastric and pancreatic lipases by orlistat (tetrahydrolipstatin, THL) in the stomach and small intestine. Clin. Nutr. 21, 395–402.

Wang, Z., Du, J., 2009. Therapeutic potential of lipase inhibitor orlistat in Alzheimer's disease. Med. Hypotheses 73, 662–663.