FORMULATION DEVELOPMENT OF ORLISTAT NANOCRYSTALS: *IN VITRO* CHARACTERIZATION AND *IN VIVO* STUDIES

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ABSTRACT

Poor solubility of orlistat limits its luminal concentration and hence needs to be administered in higher doses, leading to drug related side effects. The aim of the present research was to investigate nanocrystallization approach to increase the solubility of orlistat using melt extrusion and high-pressure homogenization (HPH) methods. The effect of factors like type and amount of polymer, homogenization pressure and time, and number of cycles on orlistat solubility was investigated. A ~10-fold increase in the solubility of orlistat was attained using OPo11N with a subsequent increase in the dissolution rate of the drug. Poloxamer 188-orlistat nanocrystals (OPo11N) as compared to pure orlistat led to a decrease in $T_{90\%}$(20 mins for OPo11N and 51 mins for marketed sample). *In vivo* studies in female Sprague Dawley (SD) rats showed that post one month of oral administration the total cholesterol and low-density lipoproteins of female SD rats remained unchanged compared to the control group. The triglycerides content and high-density lipoproteins levels were significantly increased with increase in the treatment time i.e. 12 weeks compared to the group treated with pure orlistat drug. In conclusion, the NC approach could serve as an effective formulation strategy for solubility enhancement of orlistat.

Keywords: Nanocrystals, orlistat, high-pressure homogenization, lipid profile, solubility enhancement

INTRODUCTION

Oral delivery of drug remains the most convenient route of administration due to ease of administration, high patient compliance, cost effectiveness and flexibility in dosage form design[1]. Low solubility and dissolution form a rate-determining step in bioavailability of BCS class II and IV drug[2].

Orlistat, a gastric-lipase inhibitor, has limited aqueous solubility due to its lipophilic side chain. Orlistat's administration is thus recommended to be supplemented with a fatty meal to aid its absorption and be selectively available for lipases in the gastric region. This availability of orlistat is limited by its aqueous solubility. To circumvent this issue, commercial orlistat capsules use sodium dodecyl sulfate (SDS), which leads to its availability to inhibit gastric lipases. However, use of SDS in formulation leads to inflammation of stomach mucosa. Additionally, the low aqueous solubility of orlistat limits its efficacy, leading to an increase in the dose administered, which in turn is responsible for drug and excipient associated side effects. Thus, it is recommended to increase the solubility of orlistat, which will avoid the intake of fatty diet for its absorption and use of toxic excipients like detergents[3].

Approaches like salt formation, solid dispersion, use of surfactant, prodrugs, incorporation in cyclodextrin complex and micronization have been investigated to improve the drug solubility. Micronization, a process of reducing the particle size of the drug to increase the effective surface area, remains a primary approach towards increasing the drug solubility. However, micronization does not always yield a significant increase and thus nanonization serves as an alternative approach.

Nanosuspensions, a class of colloidal dispersions of pure drug particles in an outer liquid phase, are formulated either alone or with a combination of pharmaceutical

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excipients[4]. For instance, nanocrystals are carrier free drug cargoes with particle size ranging from 80-100 nm that are formulated using 'bottom up' and 'top down' method[5,6]. Bottom-up technique involves precipitation and nanocrystallization of drug based on the molecular form. Techniques like precipitation, supercritical-fluid emulsification, microemulsification, and gas anti-solvent (GAS) technique serves as the bottom-up process in nanocrystallization. Besides, the 'top down' technique involves grinding of drug particles into nanoparticles through mechanical force, like media milling technique and high-pressure homogenization (HPH).

Nanoparticle precipitation by the anti-solvent, a direct and simple method in the preparation of drug nanocrystals, is usually difficult to control in terms of the particle size and thus, addition of surfactant becomes mandatory. Sodium lauryl sulfate, cyclodextrins and polyethylene glycols are widely used stabilizers in the formulation of nanosuspensions[3]. The current paper thus aims at increasing the solubility of orlistat using pharmaceutically approved stabilizers and without the need of a fatty diet and toxic excipients like detergents. Additionally, minimal information is reported on *in vivo* effect of orlistat on lipid profiles. The authors assumed that nanocrystallization of orlistat, a BCS class II drug, may show some improved or altered lipid profiles. Hence, the authors proposed to study the influence of orlistat nanocrystals on plasma lipid profiles.

MATERIALS AND METHODS

Materials

Orlistat was provided as a generous gift sample by Macleods Pharmaceuticals Ltd., Mumbai. Poloxamer 188 (P188) and polyethylene glycol 6000 (PEG 6000) were gifted by BASF India Ltd, Navi Mumbai and Mohini Organics Pvt. Ltd., Mumbai, respectively. Acetonitrile and *o*-phosphoric acid were purchased from SD Fine Chemicals and were used without further purification unless indicated. All other reagents were of analytical grade and used without further purification.

Preparation of nanosuspension

Drug and polymers (P188, PEG 6000 and combination of P188 and PEG 6000) were added to water (drug: polymer::1:1) and solubilized by increasing the temperature to around 55 °C (maintained for 30 minutes) with continuous magnetic stirring at 500 rpm for 30 minutes on a magnetic stirrer (Remi 5MLH,Remi Electronic Ltd).The solution was homogenized using high-pressure homogenizer (HPH), (APV Rennie Lab homogenizer, Denmark) to obtain a nanosuspension. The homogenization pressure, cycles and time (mins) are mentioned in Table III. The resulting nanosuspension with 10 % mannitol as a cryoprotectant was kept at -80 °C overnight, frozen and transferred to the lyophilizer (Lab India freeze dryer, Lyoquest, India) operating under low pressure of 100mTorr at a temperature of -30 °C to obtain a lyophilized powder. The lyophilized nanocrystals of orlistat-P188 (OPo11N), orlistat-PEG6000 (OPe11N) and orlistat-P188-PEG6000 (OPoPe11N) were stored in a desiccator until evaluation.

Characterization of nanosuspension

Powder X-ray diffraction (XRD) (X'PertPro, Malvern Panalytical Ltd.) was carried out using PAN analytical technique to determine the crystallinity of orlistat and the as-prepared nanocrystals. The nanocrystals were evaluated for physiochemical properties like visual appearance and re-dispersibility[7]. Particle size of the nanosuspension was determined by photon correlation spectroscopy using Zetasizer (Malvern Instrument Model Nano ZS 90). The particle morphology was analyzed a using scanning electron microscopy (SEM) by loading a few drops of nanosuspension on a carbon coated grid followed by air drying and SEM analysis[7,8]. Additionally, drug content and percent yield were determined for nanosuspensions using the method described by Yong *et al* [8]. Briefly, for percent drug content, the nanocrystals equivalent to 5 mg orlistat were dissolved in methanol and quantified using HPLC and calculated using the formula:

$$\% \text{ Drug content} = \frac{\text{Amount of drug in nanocrystals}}{\text{Therotical amount of drug in nanocrystals}} \text{ x } 100$$

Contact angle measurement

The contact angle is measured by a liquid drop method to determine the wettability of a liquid on solid surface by the Young's equation. Briefly, on a glass slide, using Ramé-hart contact angle telescope-goniometer and contact angle of water as the control, the hydrophillicity of orlistat, its physical mixture (PM) with polymers, and lyophilized nanocrystals with cryoprotectants was measured[9]. As a rule, the lesser the contact angle with the glass side, the more is the wettability, subsequently higher the solubility.

Saturation solubility determination

Saturation solubility of the orlistat nanocrystals with selected polymers in ratio 1:1 w/w was carried out at pH 6 with 3 % SLS and 0.5 % NaCl. Briefly, in a 25 mL conical flask, 10 mL of dissolution media was taken and excess amount of nanocrystals were added. The flasks were thermostatically controlled in an orbital flask

shaker (Electrolab, India) at 25 °C for 24 h. The resultant dispersions were filtered and quantified using HPLC. Saturation solubility of orlistat nanocrystals was evaluated in triplicate and compared to pure orlistat.

In vitro dissolution studies

Nanocrystals equivalent to 120 mg of pure orlistat in a size 0 gelatin capsule were taken for dissolution studies. The dissolution was carried out in triplicate as per the USP protocol in USP type II paddle type dissolution apparatus. The dissolution media was 900 mL of pH 6 buffer with 3 % SLS and 0.5 % NaCl maintained at 37 ± 0.5 °C and stirred at 75 rpm. Aliquots (3 mL) were withdrawn at predetermined time intervals and filtered followed by HPLC analysis. An equal volume of fresh dissolution medium was replaced to maintain the sink conditions. The release was presented as % drug release vs time (mins). *In vitro* dissolution of pure orlistat as well as marketed product of orlistat were used as controls (Reshape Capsules, Meyer Organics Pvt. Ltd).

Stability study

Orlistat nanocrystals (OPo11N, OPe11N, and OPoPe11N were subjected to stability studies for three months[10] with conditions specified in ICH guidelines. The orlistat nanocrystals were packed in a 5 mL amber colored glass vials sealed and crimped with an aluminum closure. After three months, the nanocrystals were evaluated for their physiochemical properties, % drug content and *in vitro* release profile.

In vivo studies

Female Sprague Dawley[11] within a weight range of 180-190 g were procured from Bharat Serum and Vaccines Ltd, Thane, India and were kept under standard conditions of temperature and relative humidity with 12 h light/dark cycle. The animals were fed on standard commercial diet and water *ad libitum*. The Institutional Animal Ethical Committee approved the experimental protocols as per CPCSEA guidelines through the research project no. CPCSEA/IAEC/BNCP/P-15/2015.

For induction of obesity, the animals were fed with a high fat diet (HFD) for 8 weeks with a free access to HFD and water. The composition of HFD (g kg^{-1}) is given in Table I[12]. Food intake and water intake was measured daily and body weight was measured on weekly basis. The animals were screened for induction of obesity by analyzing parameters for obesity such as body weight and biochemical parameters (triglycerides, cholesterol, HDL, LDL)[13-15].

Table I: Composition of high fat diet (HFD)

Sr. No.	Ingredient	Quantity (g kg^{-1})
1	Normal pellet diet	365 g
2	Lard	310 g
3	Casein	250 g
4	Cholesterol	10 g
5	DL-methionine	3 g
6	Yeast powder	1 g
7	NaCl + Vitamins A, D3, E & K mixture	1 g and 2-3 drops day^{-1}

The animals were grouped randomly into four groups with ten rats in each as given in Table II. One group of normal animals served as control and was fed with a normal pellet diet. Another group of thirty rats was fed with high fat diet to induce obesity. The rats were dosed orally once daily for a period of 4 weeks (from 8th week to 12th week). Food consumption and body weight were recorded every week for each animal. Minimal information is reported on *in vivo* effect of orlistat on lipid profiles. The authors assumed that nanocrystals of orlistat, may show some improved or altered lipid profiles. At pre-determined time intervals (day 0, 8th week and 12th week), blood sample was withdrawn through retro-orbital plexus of the rats and serum cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides were measured using standard commercial kits (Transasia Bio-Medicals Ltd., Germany)[16].

Table II: Group distribution of SD rats

Group	Type of treatment	No. of animals
1	Normal (lean) control	10
2	Obese control	10
	Obese rats administered orally 2 mg kg^{-1} plain orlistat daily	10
	Obese rats dosed with OPo11N nanocrystals (equivalent to 2 mg kg^{-1} orlistat) daily	10

Statistical analysis

The data shown here represents the mean±SD value for n- number of experiments. Number of experiments in case of formulation optimization equals to three, whereas in case of *in vivo* studies, n represents the number of animals equal to 10 (in each group). Statistical analysis was carried out using one-way

ANOVA, followed by Tukey's test for comparison against the desired groups. The graphs were prepared using GraphPad Prism 7®.

RESULTS AND DISCUSSIONS

Preparation of nanocrystals

We also evaluated β-cyclodextrin complexation (weight-by-weight basis), and hot-melt extrusion approach for orlistat solubility. A ~3-fold and 6.5-fold increase in orlistat solubility was observed with 1:1 and 1:3 (drug:β-cyclodextrin) ratio, respectively; and a ~3-fold and ~6-folds increase in case of 1:1 and 1:3 (drug:polymer ratio) ratio, respectively (data unpublished). On the other hand, nanocrystals prepared by combination of melt method and HPH method enhanced the solubility of orlistat by ~15-fold. The nanocrystals were prepared using P188 and PEG 6000 individually or in combination at varied concentrations (0.5 %, 1 % and 2 %) (Table III).

Nanocrystals with lower polymer concentration and pressure below 10000 psi were unstable because high molecular weight polymers like PEG 6000 require high pressure for efficient size reduction and coating[17]. Thus, at 1 % polymeric concentration (P188, PEG600, P188: PEG6000) at 10000 psi and 13 cycles, the nanocrystals were found to be stable (batch A4, A10, and A16). The nanocrystals prepared using 0.5 % polymer concentrations were unstable due to agglomeration due to cohesive forces between the orlistat molecules. Interestingly, increasing the polymer concentration to 2 % yielded stable crystals; however, a high polymer concentration led to increase in the particle size due to increase in the overall deposition over the individual nanocrystals. Nanocrystals with 1 % polymer concentration were stable with <300 nm particle size in case of nanocrystals with each polymer (determined by dynamic light scattering). Thus, nanocrystals at 1 % polymeric concentration, batch A4 (OPo11N), batch A10 (OPe11N) and batch A16 (OPoPe11N) were optimized

Table III: Various batches tried for preparation of nanocrystals

No.	Drug: Stabilizer ratio (%)	Stabilizer	Time (min)	Cycle	Pressure (psi)	Results
A1	1:1	P188 (OPo11N)	5	13	5000	Unstable
A2	1:1		5	13	7000	
A3	1:0.5		5	13	10000	
A4	1:1		5	13	10000	Stable
A5	1:2		5	13	10000	Stable but increase in particle size
A6	1:1		2.5	7	10000	Unstable
A7	1:1	PEG 6000 (OPe11N)	5	13	5000	
A8	1:1		5	13	7000	
A9	1:0.5		5	13	10000	
A10	1:1		5	13	10000	Stable
A11	1:2		5	13	10000	Stable but increase in particle size
A12	1:1		2.5	7	10000	Unstable
A13	1:1	P188+ PEG6000 (OPoPe11N)	5	13	5000	Unstable
A14	1:1		5	13	7000	
A15	1:0.5		5	13	10000	
A16	1:1		5	13	10000	Stable
A17	1:2		5	13	10000	Stable but increase in particle size
A18	1:1		2.5	7	10000	Unstable

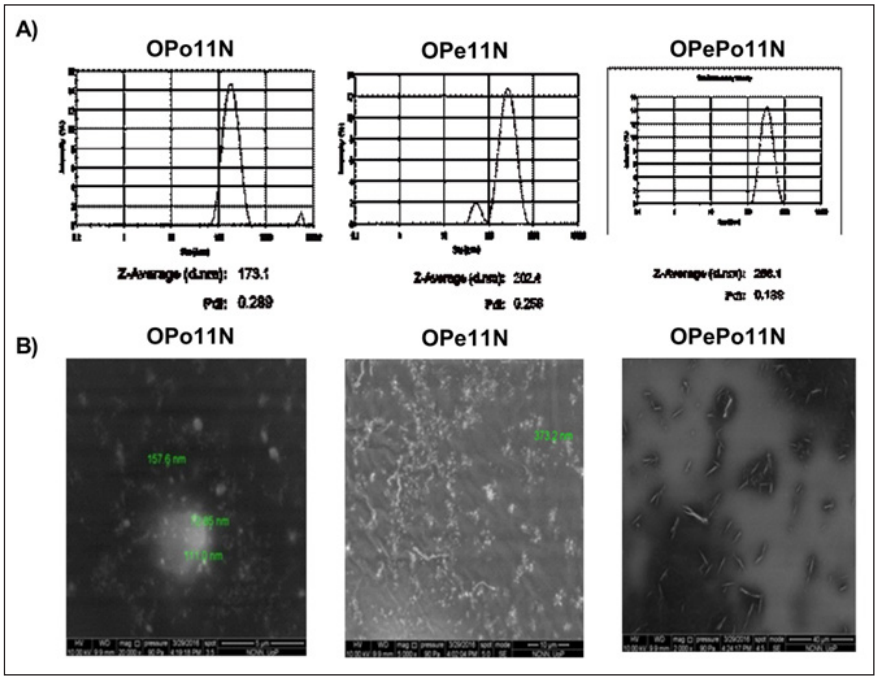

Fig. 1: A) Particle size of nanocrystals and B) SEM images of nanocrystals

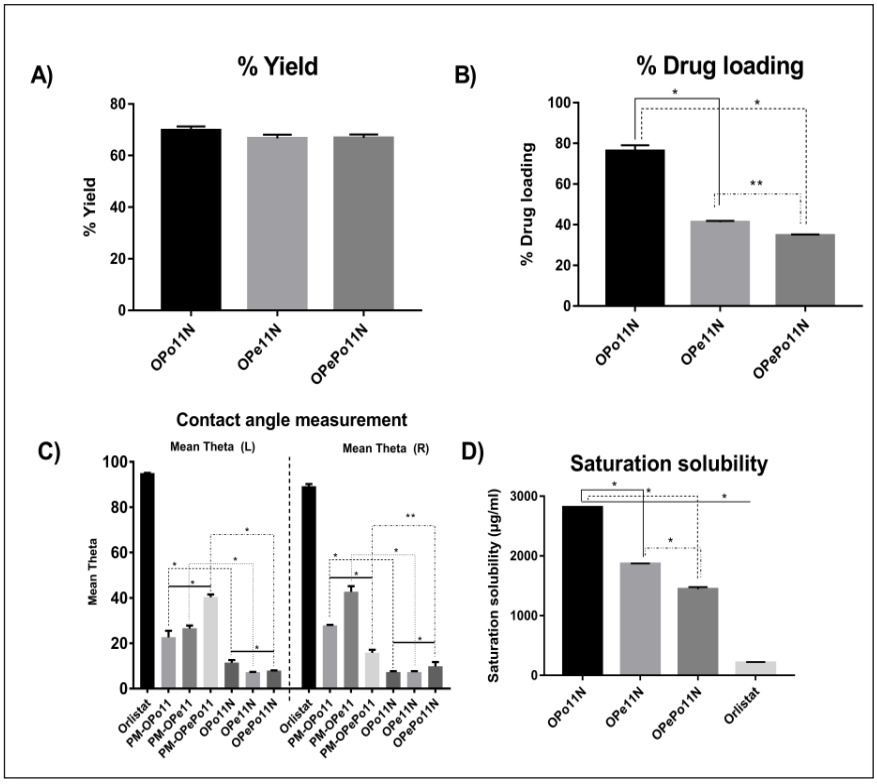

Fig. 2: A) % yield of nanocrystals, B) % drug loading of orlistat in nanocrystals, C) Contact angle measurement of nanocrystals in comparison to orlistat (PM stands for physical mixture of orlistat and polymer) and D) saturation solubility of orlistat-NCs: saturation solubility of nanocrystals of orlistat with Poloxamer188, PEG600 and Combination of Poloxamer188 and PEG 6000 showed 5-13 times increase in solubility. *p<0.0001, **p<0.05

for further evaluation based on stability of nanosuspension and its particle size.

Characterization of nanocrystals

Particle size analysis using Malvern zeta sizer (Fig. 1A) revealed OPo11N, OPe11N and OPoPe11N were in the size range of 173 nm to 288 nm with PDI below 0.3. Nanocrystals with P188 (OPo11N) showed smallest particle size (173.1 nm) compared to OPe11N (202.4 nm) composed of PEG 6000 and OPoPe11N (288.1 nm) consisting of combination of P188 and PEG 6000. A trend of increase in the particle size is due to an increase in the molecular weight of the polymers corresponding to its size, with P188 having the least. Fig. 1B illustrates the SEM images of the orlistat nanocrystals with Poloxamer 188, confirming the amorphous nature of OPo11N. PEG6000 gave a mixture of amorphous and crystalline, while the combination PEG 6000 and P188, gave complete crystalline structures. Hence, OPo11N possesses comparatively better solubility than the OPe11N and OPoPe11N, which is discussed later. Percent yield and percent drug content of nanocrystals shown was highest in case of OPo11N as compared to OPe11N and OPoPe11N as given in Fig. 2A and 2B, respectively.

Contact angle measurement and saturation solubility

Contact angle measurement was carried out on a plain glass surface using Ramé-hart contact angle telescope-goniometer to check wettability that gives a correlation with the solubility. The contact angle of water was found to be 56±0.2° (positive control). Hydrophilicity of orlistat, physical mixtures of orlistat:polymer (1:1) and nanocrystals was determined by preparing pellets of orlistat, orlistat: polymer physical mixture and nanocrystals. The low water solubility of the orlistat decreases the wetting of the glass

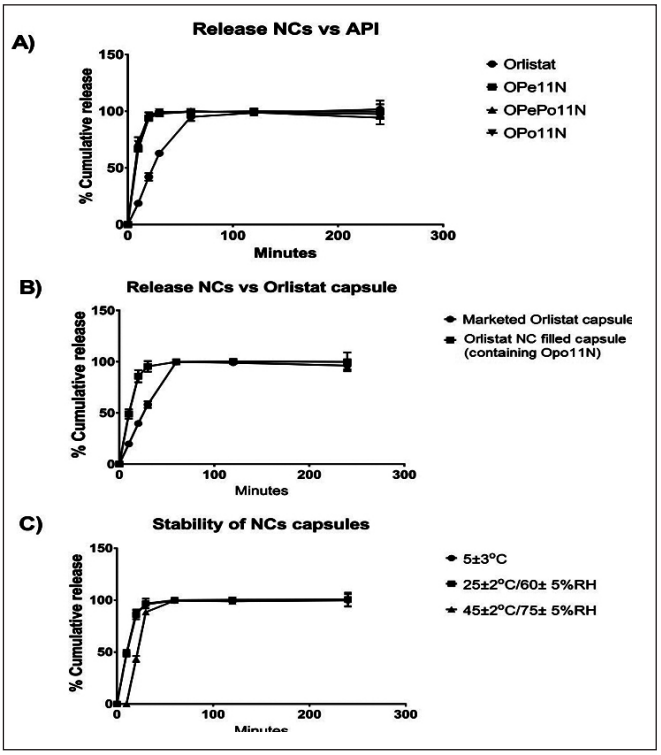

Fig. 3: A) *In vitro* release profile of nanocrystals: *In vitro* release profile of orlistat nanocrystals showed complete release within 20 minutes for all the three nanocrystals. Hence, the release profile of orlistat nanocrystals proves the faster dissolution was contributed to conversion of orlistat into nanometer size range, B) Drug release profile of orlistat NCs in capsules v.s. marketed orlistat capsule (~95 % of drug release is achieved in NCs in 30 mins as compared to orlistat capsule that is achieved in ~60mins), C) *In vitro* release profile of orlistat NCs post 3 months of stability studies as per ICH guidelines

surface, whereas an increase in wetting is observed in case of nanocrystals (Fig. 2C). A 10 to 13-fold decrease in the hydrophobicity of orlistat nanocrystals was observed leading to an increase in aqueous solubility. The mean theta of physical mixture of P188 (PM-OPo11), PEG 6000 (PM-OPe11) and combination (PM-OPoPe11) was found to be significantly lower ($p<0.0001$) than that of orlistat. In addition, a further significant improvement in wetting was also observed in between the nanocrystals (Fig. 2C) at $p<0.0001$, which is due to the amorphous nature of the nanocrystals.

Saturation solubility of orlistat and nanocrystals evaluated in phosphate buffer 7.4 (with or without 3 % SLS and 0.5 % NaCl) revealed a significant increase in the solubility compared to orlistat. An approximate 15-fold, 10-fold, and 7.5-fold increase in solubility was observed at $p<0.0001$ compared to orlistat (Fig. 2D), attributed to the formation of amorphous complexes, as confirmed by SEM (Fig. 1B) and XRD (Fig. 4). Additionally, a ~2-fold and 1.5-fold increase in solubility was observed in OPe11N than OPo11N and OPoPe11N, respectively.

In vitro release of nanocrystals

A 90 % drug release was achieved in 20 minutes in case of OPo11N, OPe11N, and OPoPe11N; however, in case of pure orlistat ~95% of orlistat was released at ~51 minutes. The higher % drug release in case of nanocrystals is attributed to the formation of the amorphous nanocrystals of orlistat (Fig. 3A). *In vitro* release of orlistat nanocrystals was improved as compared to the marketed sample. A comparative drug release profile of orlistat capsules and orlistat nanocrystals in capsules is given in Fig. 3B. A comparative XRD of nanocrystals with pure orlistat is given in Fig. 4.

Pure orlistat showed its crystalline nature, indicated by the distinctive peaks at angles 5.8, 18.5, 19.5 and 22.3±0.2degrees. Nanocrystals of orlistat indicate a

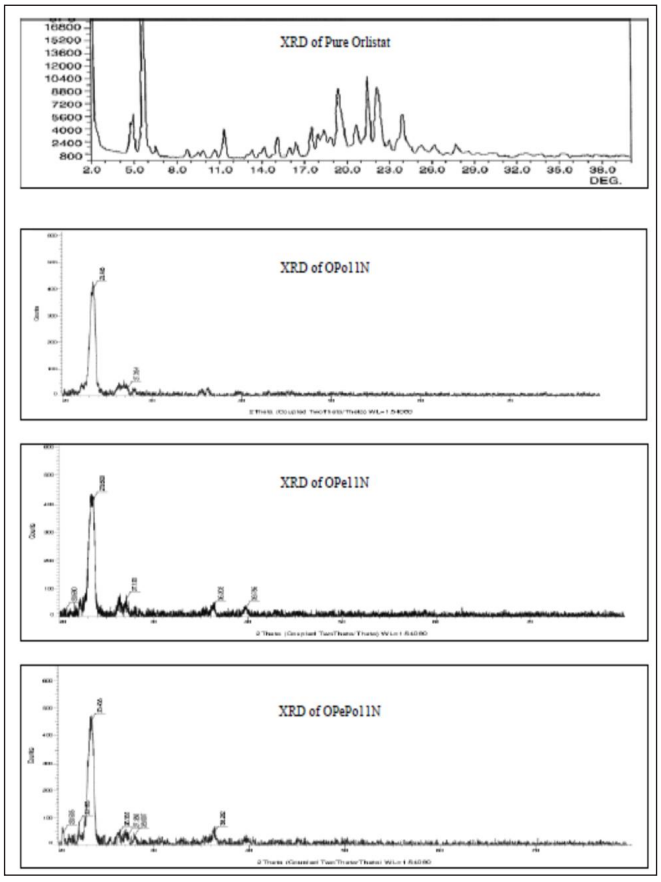

Fig 4: XRD Analysis of pure orlistat, OPo11N, OPe11N and OPoPe11N, respectively

Table IV: Results of appearance and % drug content of nanocrystals after 3 month stability studies

Nanocrystals	Appearance	Initial % drug content	After 3 months		
			25± 2°C/ 60± 5 % RH	5 °± 3 °C	40± 2 °C/75±5 % RH
OPo11N	White powder	77.70 ± 1.2	74.91 ± 0.9	75.11± 0.4	78.03±1.9
OPe11N	White powder	40.74 ±0.008	42.02±3.1	40.92±1.2	38.33±1.09
OPoPe11N	White powder	34.2±1.7	36±0.08	31.43±2.8	38.10±0.48

significant reduction in the crystallinity due to the loss of crystalline nature of during the process of nanocrystal formulation. A complete or partial amorphous state was achieved with the orlistat nanocrystals (OPo11N, OPe11N and OPoPe11N), as indicated by their diffraction patterns with the intensities lower than the pure drug, which might have contributed for improved solubility and dissolution rate.

Stability studies

Based on the results, the nanocrystals approach exhibited a promising enhancement in the solubility and thereby the *in vitro* drug release. The orlistat nanocrystals (OPo11N, OPe11N and OPoPe11N) subjected to stability studies as per the ICH guidelines for a three-month period were evaluated for changes in physiochemical properties and *in vitro* drug release.

Appearance, % drug content and *in vitro* drug release of nanocrystals

Orlistat nanocrystals on stability exhibited no apparent change in the physiochemical properties of the nanocrystals. The nanocrystal powder retained its white color and flow properties (data not shown). In addition, the drug content (Table IV) did not vary significantly as compared to the initial value.

The *in vitro* drug release profiles of nanocrystals also did not differ significantly at the end of three months (Fig. 3C) demonstrating the stability of the nanocrystals to retain drug at the accelerated conditions.

FORMULATION OF SOLID DOSAGE FORM CONTAINING NANOCRYSTALS

The OPo11N nanocrystals were filled in size 0 hard gelatin capsules (generous gift from ACG Capsules Pvt. Ltd.) with orlistat equivalent to 120 mg. The amorphous nature of the OPo11N renders poor flow characteristics and thus, addition of 1 % microcrystalline cellulose, 0.5 % talc and magnesium stearate as diluent, glidant and lubricant, respectively, improved the flow properties of NCs and aided capsule filling.

In vitro release of orlistat nanocrystals filled capsules

In vitro release profile of OPo11N in capsule dosage form showed $T_{90\%}$ within 24.58±1.05 mins as shown in Fig. 3B. The release rate of orlistat capsule was slightly delayed compared to plain OPo11N due to the presence of glidant and diluents in the capsule dosage form.

Stability studies of solid dosage form

OPo11N (equivalent to 120 mg orlistat) filled capsules were kept for stability studies at temperature and humidity conditions as per ICH guidelines and were further evaluated for *in vitro* drug release after 3 months. *In vitro* release profiles of nanocrystals in hard gelatin capsules after 3 months stability studies were found to be satisfactory and stable at 25± 2 °C/ 60± 5 % RH, 5 °±3 °C (Fig. 3C). However, capsules stored at 40±2 °C/ 75±5 % RH showed delay in disintegration of capsule. Thus, storage condition of 40±2 °C/ 75±5 % RH was found to be unsatisfactory for orlistat-nanocrystals capsules. Interestingly, this delayed behavior was not observed in case of OPo11N. Thus, probably, the diluent, lubricant or glidant is responsible for the interaction leading to the delayed release.

IN VIVO STUDIES

Female Sprague Dawley rats were used for the *in vivo* studies of orlistat. After 8th week, high fat diet induced rats were orally dosed with plain orlistat and orlistat nanocrystals for a period of 4 weeks as discussed above. Blood samples withdrawn at 0 day, 8th week and 12th week were evaluated for lipid profile using commercial Erba kits for biochemical parameters like serum triglyceride, cholesterol, HDL-cholesterol and LDL- cholesterol.

High fat diet induced obese rats (Group II, III and IV) recorded a significant increase in the body weight (data not shown) and biochemical parameters (serum triglyceride, cholesterol, HDL and LDL) compared to control group (Group I). At the end of 8th week with attainment of a remarkable increase in weight and lipid profile (week 8) due to the high fat diet, the animals were

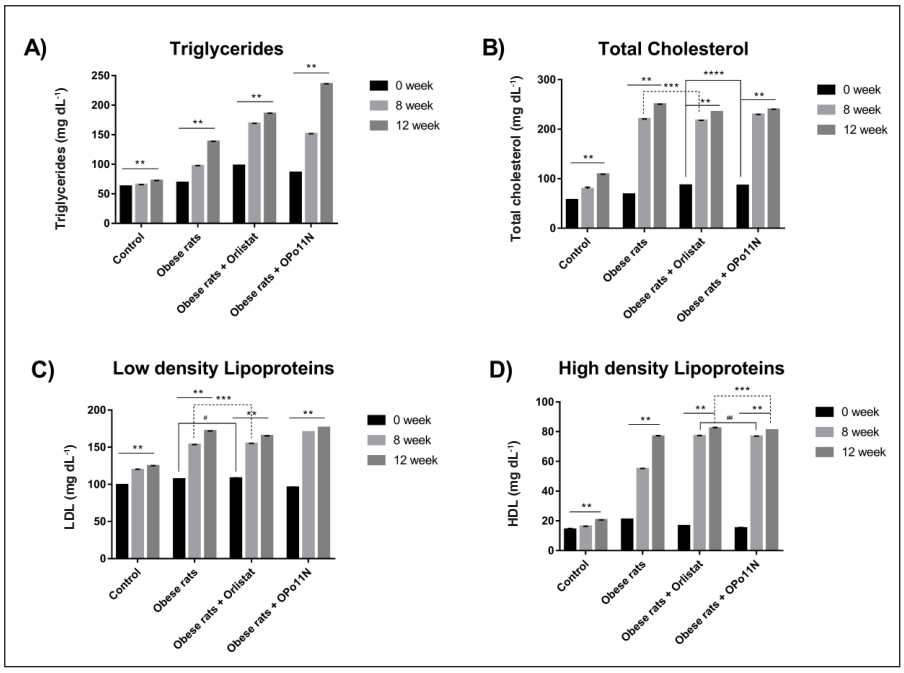

Fig. 5: Animal data of control group and high fat diet induced obesed rats
****p<0.0001, ***p<0.05, ****p=0.9665, #p=0.1549, ##p=0.6508**

dosed once daily for one month. Orlistat (2 mg kg^{-1}) and orlistat-NCs (OPo11N) dose equivalent to 2 mg kg^{-1} were given to the animals orally. Following one month of treatment (8^{th} to 12^{th} week), the serum profile of the animals was measured (Fig. 5). There was a significant increase in the obesity markers with time ($p<0.0001$). The serum levels for triglycerides, HDL, LDL and cholesterol increased significantly with time ($p<0.05$), showing that one month of treatment was not sufficient in controlling the obesity markers and implying the necessity of prolonged dosing. The gastrointestinal lipase inhibitory activity of orlistat is possible when administered during or after fatty meal to enhance solubilization of orlistat in gastric lumen. Orlistat further decreases the dietary triglyceride absorption and partially inhibits triglyceride breakdown and absorption of free fatty acids and monoglycerides. Reports suggest that food intake exhibits minimal effect on HDL-cholesterol, however, inconsistent effects are seen. Due to minimal information available upon *in vivo* behavior of orlistat related formulations, the authors proposed to evaluate the *in vivo* behavior of orlistat nanocrystals. However, no major observation could be concluded from the *in vivo* studies. The reason could be probably due to the need for a long-term orlistat administration to influence the lipid profiles. In such short-term studies, the authors thus suggest that monitoring of unaltered fatty acid excreted in fecal matter would be desirable. Thus, a further extension of this could include quantification of the lipid parameters in the excreta of the animals to establish a positive correlation with the promising enhanced *in vitro* solubility. Nevertheless, an improved solubility and dissolution profile of OPo11N vs orlistat reveals a ~10-fold increase in solubility, thereby potentially emphasizing the solubility enhancement characteristic of nanocrystallization as a solubility augmenting approach.

CONCLUSION

The nanocrystals effectively increased the solubility of orlistat, as evident by the saturation solubility and *in vitro* drug release against the marketed sample. P188 was found be an efficient polymer of choice based on percent drug content, yield and *in vitro* drug release. A probable mechanism involved could be solubilization effect of the carrier and conversion of drug from crystalline to amorphous form. Results suggested that nanocrystals prepared by modified homogenization technique enhanced the solubility and dissolution rate of orlistat in water. However, *in vivo* studies did not yield significant results in lowering of plasma lipid levels in comparison to marketed sample because of the short period of nanocrystals administration. Further studies designed to analyze the reduction of fatty acids excretion in the fecal matter of the animal's will help in extrapolating with the enhanced *in vitro* drug release and enhanced solubility of orlistat and develop a better correlation. This study opens up a new avenue to further this nanocrystal approach, especially in case of orlistat as it increases its solubility ~3-fold than β-CD complexation and hot-melt extrusion approach and ~10-fold as compared to pure orlistat. Thus, we propose nanocrystallization as a platform technology for enhancing the solubility of BCS class II drugs.

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