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ORIGINAL ARTICLE

Enhanced *ex vivo* intestinal absorption of olmesartan medoxomil nanosuspension: Preparation by combinative technology



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KEYWORDS

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Abstract The purpose of this study was to develop nanosuspension based on combinative technology to enhance the intestinal absorption of Olmesartan medoxomil (OLM), a potent antihypertensive agent with limited oral bioavailability. Two combinative approaches were employed and then characterized. *In vitro* intestinal absorption of OLM nanosuspension and plain OLM was studied using non-everted rat intestinal sac model. Optimal OLM nanosuspension was prepared by a combination of ball milling and probe sonication using stabilizer, Poloxamer 407. The formula exhibited particle size of 469.9 nm and zeta potential of -19.1 mV, which was subjected to *ex vivo* studies. The flux and apparent permeability coefficient in intestine from OLM nanosuspension was higher than the plain drug, thereby suggesting better drug delivery.

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Abbreviations: OLM, olmesartan medoxomil; P407, Poloxamer 407; HPH, high pressure homogenization; PDI, polydispersity index

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1. Introduction

Drug solubility is a crucial factor limiting the therapeutic advantage of many potent drugs because of low oral bioavailability. The conventional approach to enhance oral bioavailability of drugs with very low aqueous solubility includes use of co-solvents, salt formation, pH adjustment, emulsions and micellar dispersions, micronization and complexation with cyclodextrins (Lawrence and Rees, 2000; Nakano, 2000; Stella and Rajewski, 1997). These approaches are useful, but possess some limitations such as the use of large amount of excipients and sophisticated equipment. An alternative method is nanonization of drug and stabilization using stabilizers, termed as nanosuspension (particle size in nanometer range). Nanosuspensions are reported to increase saturation solubility due to reduced particle

size and increased surface area, contributing to enhanced dissolution and eventually increased bioavailability (Kesiosoglou et al., 2007; Kocbek et al., 2006; Liversidge and Cundy, 1995). Broadly, there are two methods for preparation of nanosuspension, bottom-up (precipitation) and top-down (Rabinow, 2004; Merisko-Liversidge et al., 2003). The top down technologies are based on particle fragmentation to submicron units and include ball milling and high-pressure homogenization (Keck and Muller, 2006; Jacob et al., 2000). Top-down method is widely accepted to reduce particle size of drug and proved to be successful; however, combinative technologies are recently used to produce even smaller particles or reduce the processing time to prepare nanosuspension. Combinations of ball milling, lyophilization or precipitation with high pressure homogenization (HPH) are the reported methods under combinative technologies (Salazar et al., 2012a, 2013b).

Olmesartan medoxomil (OLM) is a selective AT1-subtype angiotensin-II receptor antagonist used for the treatment of hypertension (Warne and Jarvis, 2002). The aqueous solubility of OLM is $< 7.75 \mu\text{g/ml}$ and oral bioavailability of the tablet is only 26% in healthy humans (Prajapati et al., 2013). The unabsorbed drug leads to gastrointestinal side effects such as abdominal pain, dyspepsia, gastroenteritis and nausea. The nanosuspension of OLM was reported to enhance its bioavailability (Thakkar et al., 2011). In the present study, an effort was made to prepare nanosuspension of OLM by a combination of top down methods and evaluate the effectiveness of combinative methods in enhancing the intestinal absorption of OLM.

2. Methods

2.1. Preparation of nanosuspension

The nanosuspensions of OLM were prepared by combinative technologies. Two methods were employed in the present study viz. ball milling followed by probe sonication and high speed homogenization followed by probe sonication. Various concentrations of stabilizers such as PVA and Poloxamer 407 (P407) were used to stabilize the nanosuspensions (Table 1). Briefly, 30 mg of OLM was dispersed in 15 ml of stabilizer solution and homogenized (Polytron PT 1300D, Singapore) or ball milled (PM100, Retsch, Germany) followed by probe

Table 1 Preparation of nanosuspensions of OLM.

S. no.	Code	Stabilizer	Concentration (%)	Time of probe sonication (min)
1	BM1	Poloxamer 407	0.5	15
2	BM2	Poloxamer 407	0.5	10
3	BM3	Poloxamer 407	0.25	15
4	BM4	Poloxamer 407	0.25	10
5	BM5	Poloxamer 407	0.125	15
6	BM6	Poloxamer 407	0.125	10
7	BM7	Poloxamer 407	0.1	15
8	BM8	Poloxamer 407	0.1	10
9	BM9	PVA	0.5	30
10	BM10	PVA	0.25	30
11	BM11	PVA	0.25	15
12	HSH1	Poloxamer 407	0.125	15
13	HSH2	PVA	0.25	15

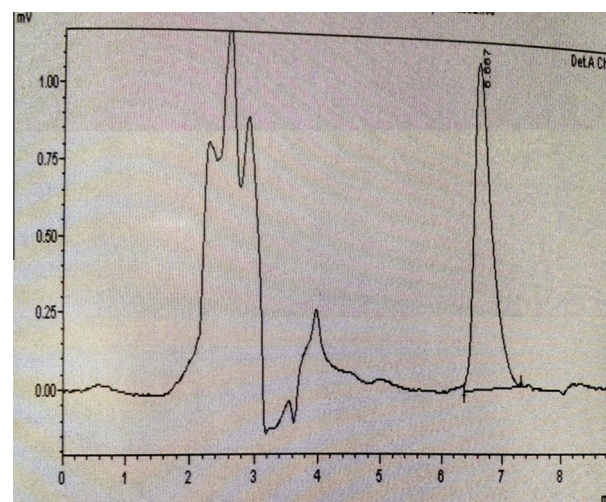


Figure 1 HPLC chromatogram of olmesartan medoxomil.

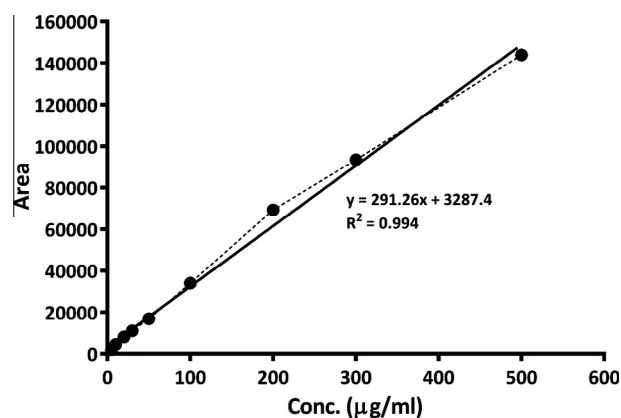


Figure 2 Calibration curve of OLM using HPLC-UV method.

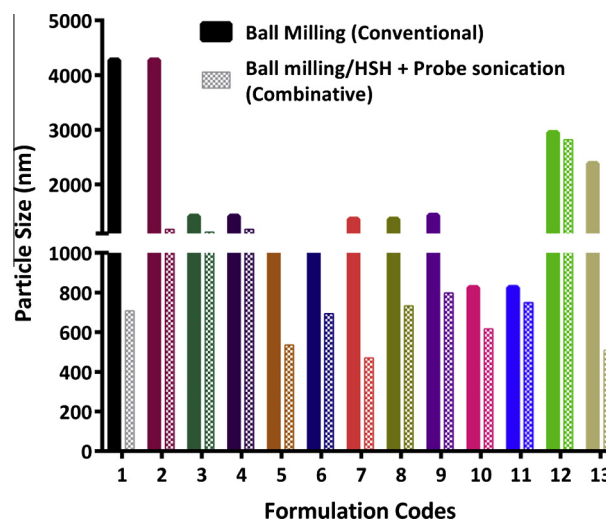


Figure 3 Particle size of nanosuspensions after conventional and combinative technologies.

Table 2 Characterization of nanosuspensions of OLM.

S. no.	Code	Ball milling		Ball milling + probe sonication		
		PS (nm)	PDI	PS (nm)	PDI	ZP (mV)
1	BM1	4275	0.443	707.8	0.495	-23.6
2	BM2			1177	0.638	-23.6
3	BM3	1418	0.770	1127	1.0	-3.73
4	BM4			1178	0.275	-3.56
5	BM5	1032	0.789	534.9	0.570	-19.3
6	BM6			693.4	0.544	-19.1
7	BM7	1360	0.660	469.9	0.439	-19.1
8	BM8			732.5	0.478	-18.6
9	BM9	1432	0.782	797.2	0.837	-3.09
10	BM10	824.7	0.692	616.5	0.561	-4.99
11	BM11			749.1	0.861	-4.3
		HSH	HSH + probe sonication			
12	HSH1	2947	0.503	2816	0.640	-4.96
13	HSH2	2380	0.511	509.4	0.450	-21.3

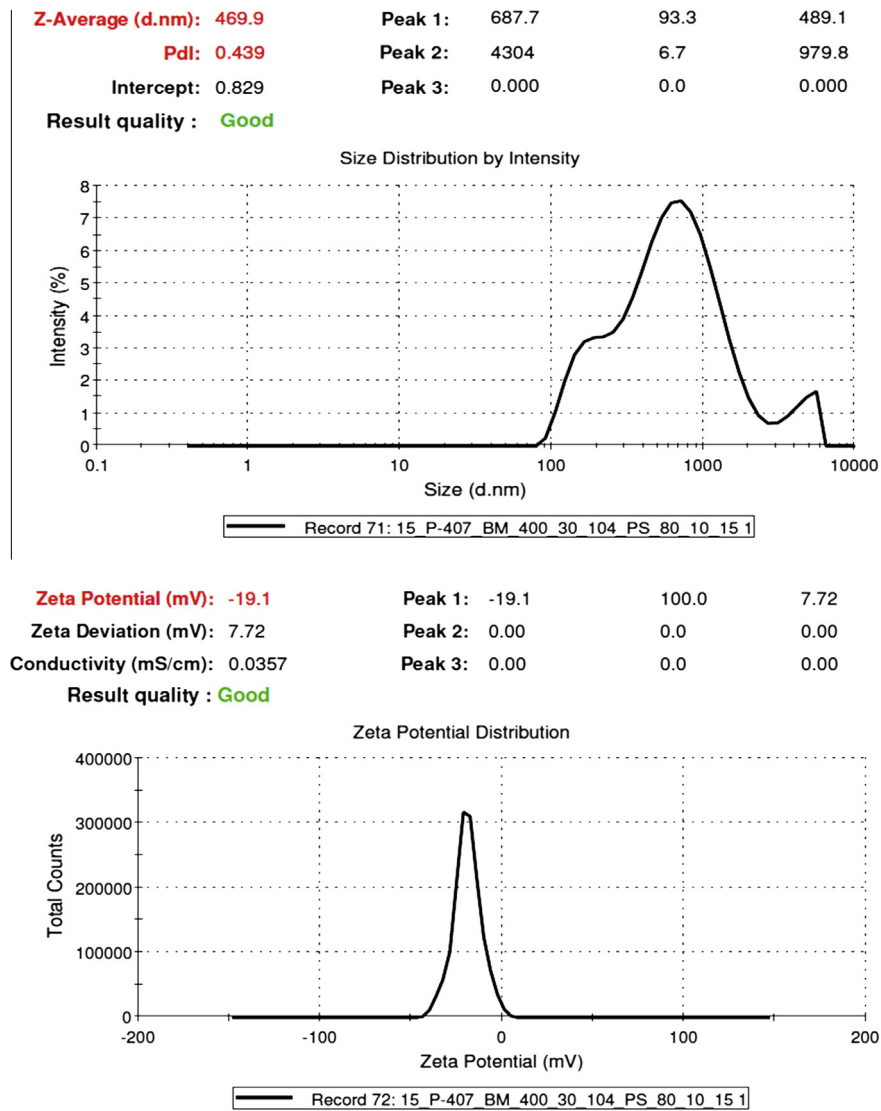


Figure 4 Particle size and zeta potential of the selected nanosuspension, BM7 using Malvern zetasizer.

Table 3 Drug content of OLM nanosuspension by external standard method.

S. no.	Group	Area	Drug amount (%)
1	Pure drug	34108	100
2	Nanosuspension	21509	63.06

sonication. The dispersion was homogenized at 17,000 rpm for 30 min, whereas, ball milling was done at 400 rpm for 30 min using 5 mm SS balls. The effect of variation in time of sonication (10, 15 or 30 min) on the particle size was evaluated.

2.2. Particle size, polydispersity index (PDI) and zeta potential

The particle size (PS), PDI and zeta potential (ZP) of the prepared nanosuspensions were assessed using Malvern zetasizer. The nanosuspension of OLM was selected based on particle size and zeta potential. The selected nanosuspension was lyophilized using mannitol as a cryoprotectant to carry out FT-IR, DSC and drug content.

2.3. HPLC method

The analytical method for ACV was developed and validated as per the ICH Q2 (R1) guideline. The RP-HPLC–UV method was employed to estimate the drug content in the nanosuspension and intestinal absorption. The method was validated by injecting 20 µl of the standard drug samples at the flow rate of 0.5 ml per minute to the C₁₈ ODS column. The mobile phase consisted of a mix of acetonitrile:phosphate buffer, pH 6.2 (41:59 v/v). The peak of the drug was measured using a UV-detector at 250 nm. The run time was 9 min and the retention time was 6.6 min. The chromatogram is shown in Fig. 1.

2.4. Drug content

The freeze dried nanosuspension was analyzed by dispersing a weighed amount in methanol followed by sonication and filtering through 0.22 µm filter. The amount of drug was determined by HPLC. The drug content was determined by two methods, external standard and calibration curve method (Fig. 2). The equation for calibration curve is presented beneath.

$$y = 291.26x + 3287.4 \quad (1)$$

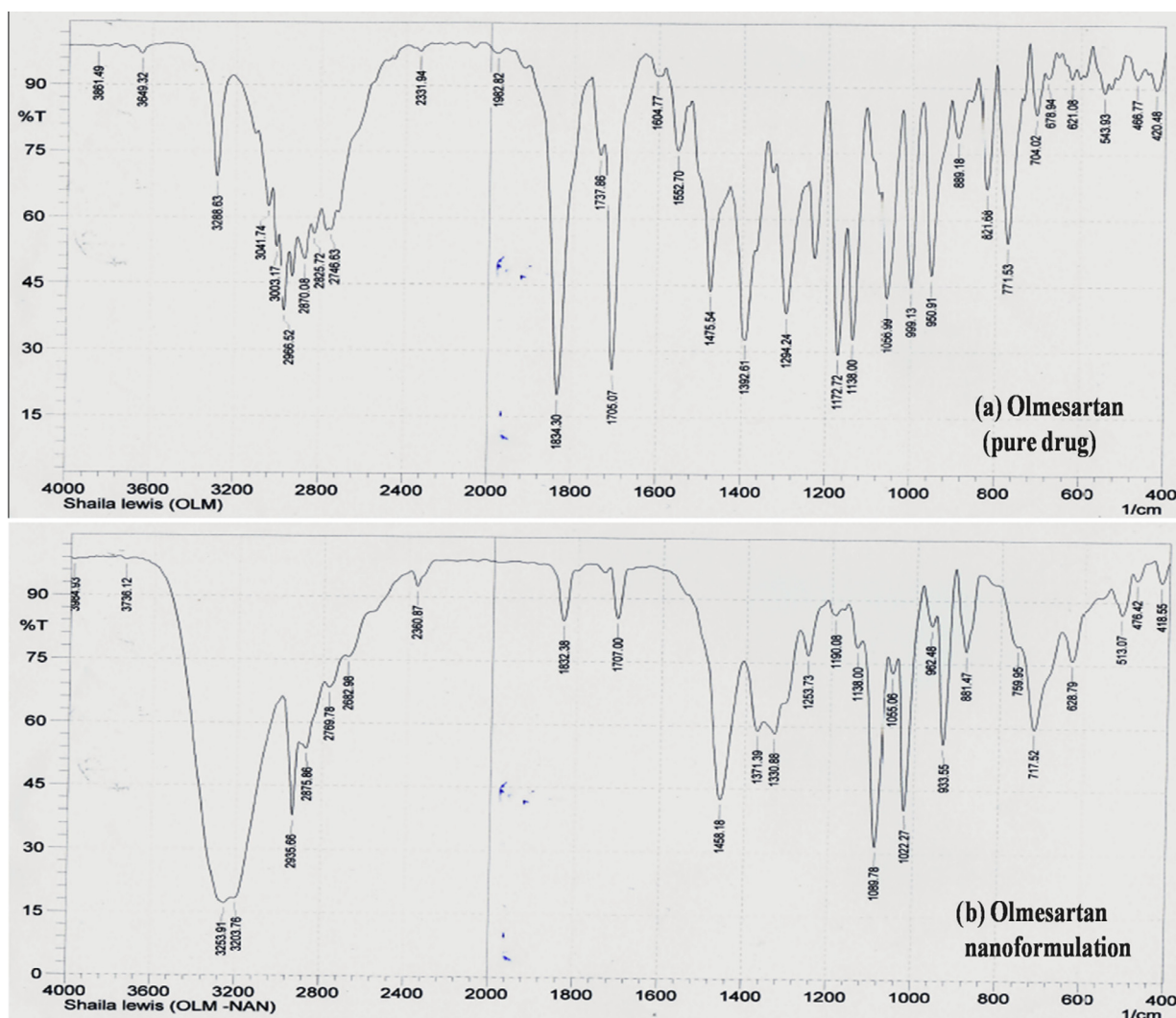


Figure 5 FT-IR spectra of OLM and its freeze dried nanoformulation.

Table 4 Characteristic peaks of OLM nanoformulation and pure drug in FT-IR spectra.

Groups	Pure drug		Nanoformulation (cm^{-1})
	Reported (cm^{-1})	Observed (cm^{-1})	
Broad intermolecular hydrogen bond, O—H stretch	3288	3288	3253
Aliphatic C—H stretch	2972	2966	2935
C=O of carboxylic group	1706	1705	1707
C—N stretch	1474	1475	1458
In plane O—H bend	1389	1392	1371
C—O—C stretch	1028	1056	1055

2.5. Fourier transform-infrared spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC)

The FT-IR spectra and DSC thermograms of the freeze-dried OLM nanoformulation and pure drug were obtained to evaluate the drug-excipient interaction and crystallinity of the drug in the nano-system.

2.6. *Ex vivo* intestinal absorption – non-everted sac method (Bothiraja et al., 2012; Shishu and Maheshwari, 2010)

Ex vivo studies were performed on fasted male Wistar rats weighing 250–300 g.

The study was approved by the Institutional Animal Ethics Committee (No.–IAEC/KMC/48/2014), Manipal University. The animals were sacrificed and intestine (duodenum) was isolated and washed with Kreb's-Ringer solution. The non-everted tissue of 6 cm length was tied at one end and filled with 1.1 ml drug/formulation solution containing 10 mg drug and was tied at the other end, making the sac. The sac was immersed in the Kreb's-Ringer solution contained in the beaker. The aliquots of 1 ml solution were removed and replaced with Kreb's-Ringer solution at 30, 45, 60, 90 and 120 min time interval. The drug concentration in the aliquots was analyzed using HPLC. The outcomes were analyzed statistically applying student's *t*-test at $p < 0.05$. The permeability coefficients were determined using Eq. (2).

$$P_{\text{app}} = dQ/dt + 1/(A + C_0) \quad (2)$$

The slope of the linear portion of the plot was considered permeation flux (dQ/dt).

3. Results and discussion

3.1. Characterization of prepared nanosuspensions of OLM

The particle size was compared after ball milling/HSH and ball milling/HSH along with probe sonication. It was found that a combination of ball milling and probe sonication resulted in smaller particles than ball milling alone (Fig. 3). HSH along with probe sonication was also successful in case of P407 but not with PVA. This indicates that ball milling or HSH (pre

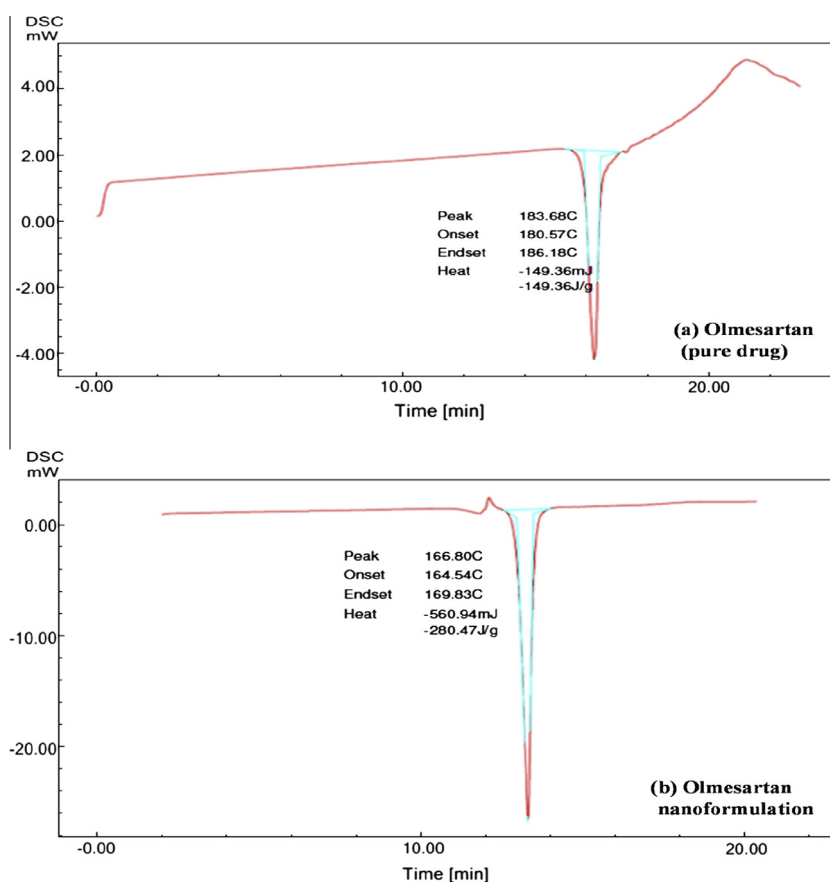


Figure 6 DSC thermograms of OLM and its freeze dried nanoformulation.

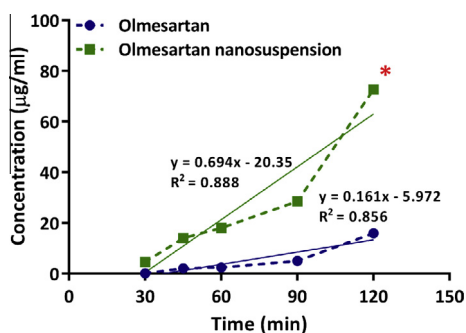


Figure 7 *Ex vivo* intestinal absorption of OLM using non-everted intestinal sac method. *Indicates significant difference in variances of two groups at $p < 0.05$ using student's *t*-test.

milling) produces the crack on the surface of particle which leads to smaller particle size after homogenization at high pressure. Ball milling produced particles of size near to 1μ which further led to smaller particles after probe sonication in accordance with the earlier report (Peterson, 2010). An increase in the concentration of stabilizers increased the particle size. The zeta potential of P407-stabilized nanosuspensions was observed to be in the acceptable range (+20 to -20 mV) as compared to PVA-stabilized nanosuspensions. The nanosuspension (BM7) stabilized with 0.1% P407 and 15 min probe sonication after 30 min ball milling showed particle size of 469.9 nm and acceptable zeta potential of -19.1 mV (Table 2 and Fig. 4). Thus, BM7 was selected for further studies. The particle size increased to 900 nm after lyophilization.

3.2. Drug content

The drug content of selected OLM nanosuspension (BM7) was found to be 63.06% and 62.56% by external standard and calibration curve methods, respectively (see Table 3).

3.3. FT-IR and DSC

There was no considerable change in peaks in the OLM nanoformulation as compared to pure drug, which indicated no interaction between drug and excipients (Fig. 5). The peaks are depicted in Table 4 and complied with earlier reported values (Sasidhar et al., 2013). In DSC thermograms, the endotherm of pure drug was observed at 183 °C, whereas the endotherm decreased to 166 °C in nanoformulation (Fig. 6). This indicated that there was a slight decrease in the crystallinity of OLM nanocrystals.

3.4. Ex vivo intestinal absorption

The absorption was estimated through the duodenum (proximal part) of the intestine, as OLM mostly gets absorbed through the duodenum (Kang et al., 2012). The drug permeated across the intestinal wall and its concentration was measured in the Krebs-Ringer solution. The absorption of drug in nanosuspension was observed to be significantly increased as compared to pure drug (Fig. 7). The permeability coefficients of nanosuspension and pure drug were found to be $0.72 \times 10^{-8} \text{ cm}^2/\text{s}$ and $0.18 \times 10^{-8} \text{ cm}^2/\text{s}$ respectively. The observations suggest that the reduction in particle size leads

to increase in the permeation of the drug particles and eventually improves the absorption of the drug.

4. Conclusion

It is observed in the present study that the combinative technologies reduce the particle size of the drug more efficiently than the conventional approach with lesser processing time. The prepared nanosuspension showed the optimum particle size and zeta potential. In *ex vivo* intestinal absorption, the absorption and permeability of OLM nanosuspension were observed to be increased as compared to pure drug. This shows that there was better reduction in particle size resulting in increased surface area which increased the permeation and eventually increased the absorption. However, pharmacokinetic and pharmacodynamic studies are required to further support the finding.

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