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

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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 (Kesisoglou et al., 2007; Kocevak 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 μg/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 sonication for 15 min with an interval of 10 min (Table 1). The results were compared with the conventional method i.e., ball milling followed by probe sonication for 15 min.

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                            |
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     | 1127        | 1.0       | −3.73   |
| 3      | BM3  | 1418         | 0.770     | 1178        | 0.275     | −3.56   |
| 4      | BM4  | 1032         | 0.789     | 534.9       | 0.570     | −19.3   |
| 5      | BM5  | 1360         | 0.660     | 693.4       | 0.544     | −19.1   |
| 6      | BM6  | 1432         | 0.782     | 797.2       | 0.837     | −3.09   |
| 7      | BM7  | 824.7        | 0.692     | 616.5       | 0.561     | −4.99   |
| 8      | BM8  | 824.7        | 0.692     | 749.1       | 0.861     | −4.3    |
| 9      | BM9  | HSH          | HSH + probe sonication |
| 10     | BM10 | 2947         | 0.503     | 2816        | 0.640     | −4.96   |
| 11     | BM11 | 2380         | 0.511     | 509.4       | 0.450     | −21.3   |

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

\[
y = 291.26x + 3287.4
\]

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           |

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

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

| Groups                              | Pure drug | Nanoformulation |
|-------------------------------------|-----------|-----------------|
|                                     | 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          |

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).

\[
P_{app} = \frac{dQ}{dt} + \frac{1}{A + C_0}
\]  

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](https://via.placeholder.com/150)  

DSC thermograms of OLM and its freeze dried nanoformulation.

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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 Kreb’s-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 × 10⁻⁸ cm/s and 0.18 × 10⁻⁸ cm/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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