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# Solubility enhancement of olmesartan medoximil by spray drying technique

**Kirti K. Kamble* and Kakasaheb R. Mahadik**

*Department of Quality Assurance Techniques, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandawane, Pune, Maharashtra, India*

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**ABSTRACT**

*Olmesartan medoxomil (OM) is an angiotensin II receptor blocker. It is practically insoluble in water and has an oral bioavailability of 26% with a terminal half life of approximately 13 hours. In the present study, a spray drying technique has been used to prepare solid dispersion (SD) of OM with Polyvinyl pyrrolidone (PVP K) 30 and Gelucire® 50/13 with silicon dioxide (Aerosil®200) as carrier to improve drug solubility. The SDs were characterized in comparison with pure drug and corresponding Physical mixture (PM) in the same ratio by using drug content, saturation solubility, scanning electron microscopy (SEM), diffuse reflectance infrared transform spectroscopy (DRIFTS), X-ray powder diffraction (XRPD) and in vitro drug release. SDs were further subjected to aging at room temperature ($30^0$C / 60% RH) for three months and characterized for in vitro drug release and presence of crystallanity using XRPD.Absence of pure OM peaks in XRPD suggests transformation of crystalline OM into an amorphous form. DRIFT spectra revealed presence of hydrogen bonding interactions in solid dispersion. Significant improvement in dissolution of SDOMGA compared to SDOMP, crystalline OM, spray dried OM, and physical mixtures of drug with carrier. Therefore solid dispersion by spray drying with multifunctional excipient Gelucire® 50/13 can stabilize OM in amorphous form, improve solubility and prevent hydrolysis providing better alternative to conventional stabilizers like PVPK30.*

**Keywords:** PVPK30, Gelucire® 50/13, Silicon dioxide, dissolution

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## INTRODUCTION

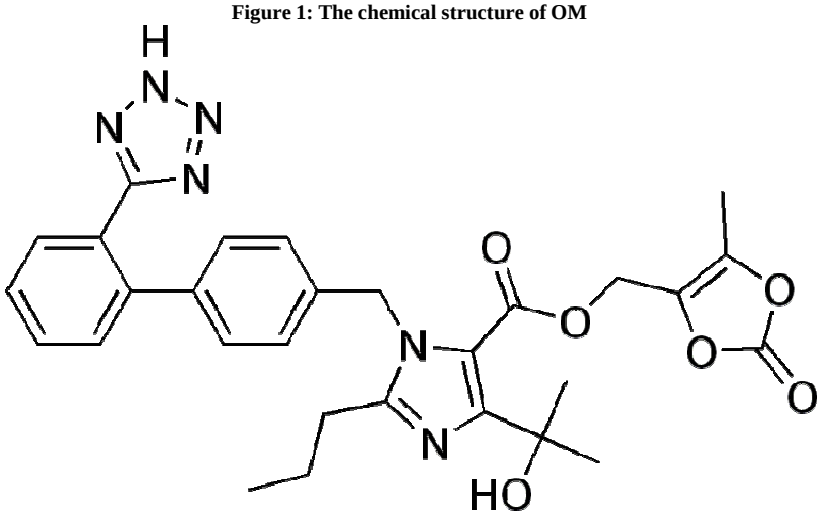
Solubility of many newly discovered drugs is a severe hurdle in formulation and development, due to poor solubility in aqueous and organic media, which lead to poor or varying bioavailability after oral administration. According to BCS system, class II and class IV drugs shows poor solubility and dissolution is rate limiting factor for their absorption [1]. Solid dispersion is most widely used technique to improve dissolution rate and bioavailability of poorly water soluble drugs [2, 3]. Chiou and Riegelman defined solid dispersion as the dispersion of one or more active ingredient in an inert carrier matrix at solid state prepared by the melting (fusion), solvent or melting-solvent method [4]. Various strategies investigated to enhance solubility in solid dispersions like fusion (melting), solvent evaporation, lyophilization (freeze drying), melt agglomeration process, extruding method, spray drying technology, use of surfactant, electro static spinning method and super critical fluid technology [5].

Hydrophilic carriers which are most commonly used in the preparation of solid dispersion are polyvinylpyrrolidone [6], polyethylene glycols [7], polyvinylalcohol [8], colloidal silicon dioxide [9] and lipids such as Gelucire® ( polyglycolized glycerides)[10,11].

Gelucire® is a family of vehicles derived from the mixtures of mono-, di- and triglycerides with polyethylene glycol (PEG) esters of fatty acids. These are available with a range of properties depending on their HLB (1-18) and melting point range (33–65 °C) [12, 13]. Gelucires improves dissolution of drug by improving wettability, reduction in particle size and reduction of crystallinity of drug [14, 15]. Polyglycolized glycerides are low melting point excipients produces sticky and tacky mass when processed by conventional spray drying technique. To overcome this problem they are used with high melting lipid carriers like compritol, sterotex K NF [16] or adsorbent like silicon dioxide which has surface silanol groups; may be able to form hydrogen bonds with drug molecules during formation of SDs and enhances dissolution of drugs [17, 18].

OM is a prodrug ester. In vivo it is completely and rapidly de-esterified to active metabolite olmesartan via an enzyme, arylesterase [20]. OM belongs to BCS class II. It is practically insoluble in water and has an oral bioavailability of 26% with a terminal half life of approximately 13 hours [21]. In the present study, a spray drying technique has been used to prepare solid dispersion of OM with PVPK30 and Gelucire® 50/13 with silicon dioxide (Aerosil® 200) as carrier to improve drug solubility and bioavailability. Few attempts have been made to improve solubility of OM by complexation with Cyclodextrin [29], with poly ethylene glycol 4000 (PEG 4000), HPMC K4, HPMC K100, Poloxamer-407 and crospovidone [30], controlled release floating tablet [31] and crystal-co-agglomeration technique [32]. But slow process of complexation, high molecular weight of cyclodextrins and crystalline nature of drug may limit their practical utility.

**Figure 1: The chemical structure of OM**




In the present study, a spray drying technique has been used to prepare solid dispersion of OM with PVPK30 and Gelucire® 50/13 with silicon dioxide dioxide (Aerosil® 200) as carrier to improve drug solubility and bioavailability. The SDs were characterized in comparison with pure drug and corresponding PM in the same ratio by using drug content, saturation solubility, scanning electron microscopy (SEM), diffuse reflectance infrared transform spectroscopy (DRIFTS), X-ray powder diffraction (PXPD),and in vitro drug release. SDs were further subjected to aging at room temperature $30^0$C / 60% RH for three months and characterized for in vitro drug release and presence of crystallanity using XRPD. To avoid frequent repetition of long phrases, we abbreviate the compositions of prepared material as follows: Pure olmesartan medoxomil as OM, Solid dispersion as SD, Physical mixture as PM, spray dried pure drug as AOM, solid dispersion of olmesartan medoxomil with Gelucire® 50/13 and silicon dioxide (adsorbent) as SDOMGA and with PVPK30 as SDOMP. Corresponding physical mixture as PMOMGA and PMOMP.

## MATERIALS AND METHODS

### Materials

OM was obtained as a gift sample from Glenmark Pharmaceuticals Ltd. (Mumbai, India), Gelucire®50/13 ( stearoyl Macrogoglycerides ) EP, solid pastilles, nominal mp = 47–50 $^0$C, HLB = 13 were generous gift from Gattefossĕ (St. Priest, Cedex, France), Silicon dioxide (Aerosil® 200) (Degussa, Dusseldorf, Germany) was supplied by Get-Rid

Pharmaceuticals Ltd. (Pune, India). PVPK30 (BASF, Ludwigshafen, Germany). All other chemicals and solvents were of analytical grade and used as received.

**Preparation of SDs and PMs**

OM either alone or in combination with PVP K30 (1:1) and Gelucire® 50/13(1:1) was dissolved in sufficient amount of dichloromethane to obtain 10% (w/v) solutions. To the clear solution of OM and Gelucire® 50/13, silicon dioxide (Aerosil®200) (one parts by weight of OM) was slowly added to obtain uniform suspensions. Spray drying of these suspensions were carried out using laboratory scale spray dryer (LU-222 Advanced model, Labultima, Mumbai, India) under following set of conditions: inlet temperature, 48–50$^0$C; outlet temperature, 38–40$^0$C; feed rate, 4–6 ml/min; atomization air pressure 2 kg/cm$^2$ and aspiration −150mmWC.

PMs in the same ratios were also prepared by physically mixing drug and excipients thoroughly for 10 min in a mortar until a homogeneous mixture was obtained. All the samples were passed through fine mesh (150 μm) and stored in desiccated environment until further study.

**Drug Content**

The percentage drug content in SDs were estimated by dissolving quantities equivalent to 10 mg of powder in 10 ml methanol, vortex for 10 min and filtered through 0.45μm membrane filter, appropriately diluted with distilled water and the UV absorbance were recorded at 252 nm by using UV-visible spectrophotometer (Jasco V-530, Japan). The percentage drug content was recorded by slope method.

**Saturation Solubility**

The saturation solubility of pure drug OM, AOM, SDs and PMs were determined by equilibrating excess powder in distilled water and Phosphate buffer (pH 6.8). The suspensions were stirred for 48 hours on a rotary incubator shaker (C24KC refrigerated incubator shaker, New Brunswick Scientific Co., New Jersey, USA) at the room temperature. The solutions were then centrifuged (Eppendorf-5810 R, Japan) at 4000 rpm for 10 min; supernatant was filtered through 0.45μm membrane filter, appropriately diluted and analyzed for OM spectrophotometrically at 252 nm.

**Scanning electron microscopy (SEM)**

Samples were mounted on a double faced adhesive tape and sputtered with thin gold palladium layer by sputter coater unit (JOEL, JFC, Tokyo, Japan) and surface topography were analyzed with a scanning electron microscope (JEOL-JSM, 6360, Tokyo, Japan)

**X-ray powder diffraction (XRPD)**

PXRD patterns of pure drug OM, AOM, SDs and PMs were recorded on X-ray diffractometer (D8 Advance, Brucker, USA) with Cu line as source of radiation. The samples were analyzed in 2θ angle range of 5 to 50$^0$. The range and the chart speed were 2×10$^3$ CPS and 10 mm/degree 2θ, respectively.

**Diffuse reflectance infrared fourier transform spectroscopy (DRIFTS)**

The DRIFTS spectra's were obtained, after appropriate background subtraction; using an FTIR spectrometer (FT/IR-4100, Jasco, Japan). The sample was mixed with dry potassium bromide and was scanned from 4000–600 cm$^{-1}$, Jasco spectra manager ver.2 was used for data acquisition and analysis.

**Dissolution study**

The dissolution studies were performed using USP type II dissolution test apparatus (Electrolab TDT-08L, Mumbai, India). The samples equivalent to 40 mg of OM were placed in the dissolution vessel containing 900 ml phosphate buffer (pH 6.8) maintained at 37±0.5$^0$C and stirred at 50 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through 0.45 μ membrane filter, a concentration of OM was determined spectrophotometrically at 252 nm. Analysis of data was done using PCP-Disso software (V3, Poona College of Pharmacy, Pune, India.).

**Effect of ageing**

The SDs were stored at room temperature 30$^0$C/60% RH for 3 months and the effect of ageing on the SDs were studied by measuring in vitro drug release and presence of crystallinity using XRPD studies.

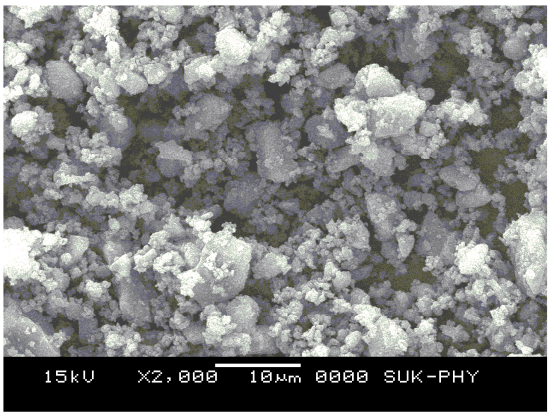
## RESULTS AND DISCUSSION

OM alone and in various ratios (1:0.5, 1:1 and 1:2) with PVP K30 or Gelucire® 50/13 & Aerosil 200 as adsorbent was spray dried. Depending on powder characteristics, drug content and saturation solubility, dissolution profile; optimum ratio of 1:1 parts by weight of OM and PVPK30 and 1:1:1 parts by weight of OM, or Gelucire® 50/13 & Aerosil® 200 was finalized.

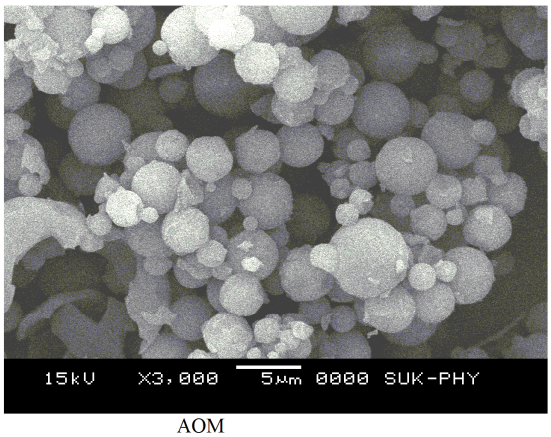
Production yield of AOM and SDOMP (1:1) was 50% and about 90% respectively. Drug content of AOM and SDOMP was 98 ± 1 % (w/w) and 97.5 ±1 % (w/w).

Regarding to SD of OM with Gelucire® 50/13, at lowest proportion of Gelucire® (1:0.5) and at lowest possible temperature ($30^0$C), the product could not obtained due to sticking of product to the walls of drying chamber. This indicates limitation of spray drying technique. Spray freeze drying is one of the techniques to disperse drugs in lipid excipients but it lacks commercial feasibility [22]. The outlet temperature should not exceed the melting temperature of Gelucire so dichloromethane was the only solvent of choice because of its low boiling point and OM and Gelucire® 50/13 were easily soluble in it.

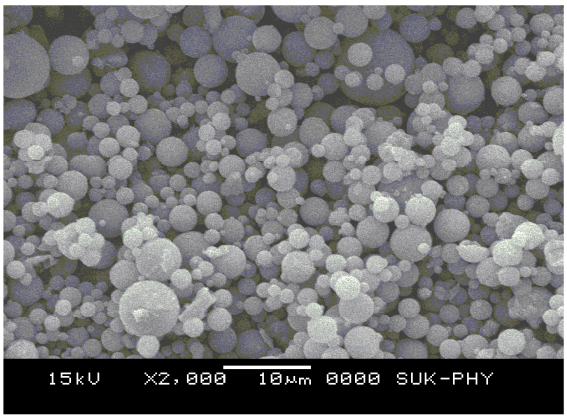
**Figure 2 : SEM of OM, AOM, SDOMP and SDOMGA**



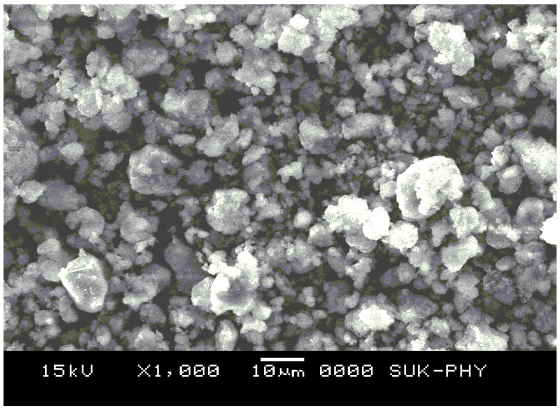

OM




AOM




SDOMP




SDOMGA

SD of OM, Gelucire® 50/13 and silicon dioxide in the ratio 1:1:1 is free flowing powder with 80-85% yield and 98.5 ± 1% (w/w) drug content. The saturation solubility for pure drug OM was 0.18±0.01 mg/ml in phosphate buffer (pH 6.8). All the rest test samples showed increased solubility over crystalline OM. The difference in saturation solubility of PM and SD is probably attributed to reduction in particle size during processing; and presence of amorphous form in SDs as compared with PMs in which drug was present in crystalline form.

**Table 1 : Saturation solubility's of different formulations of OM**

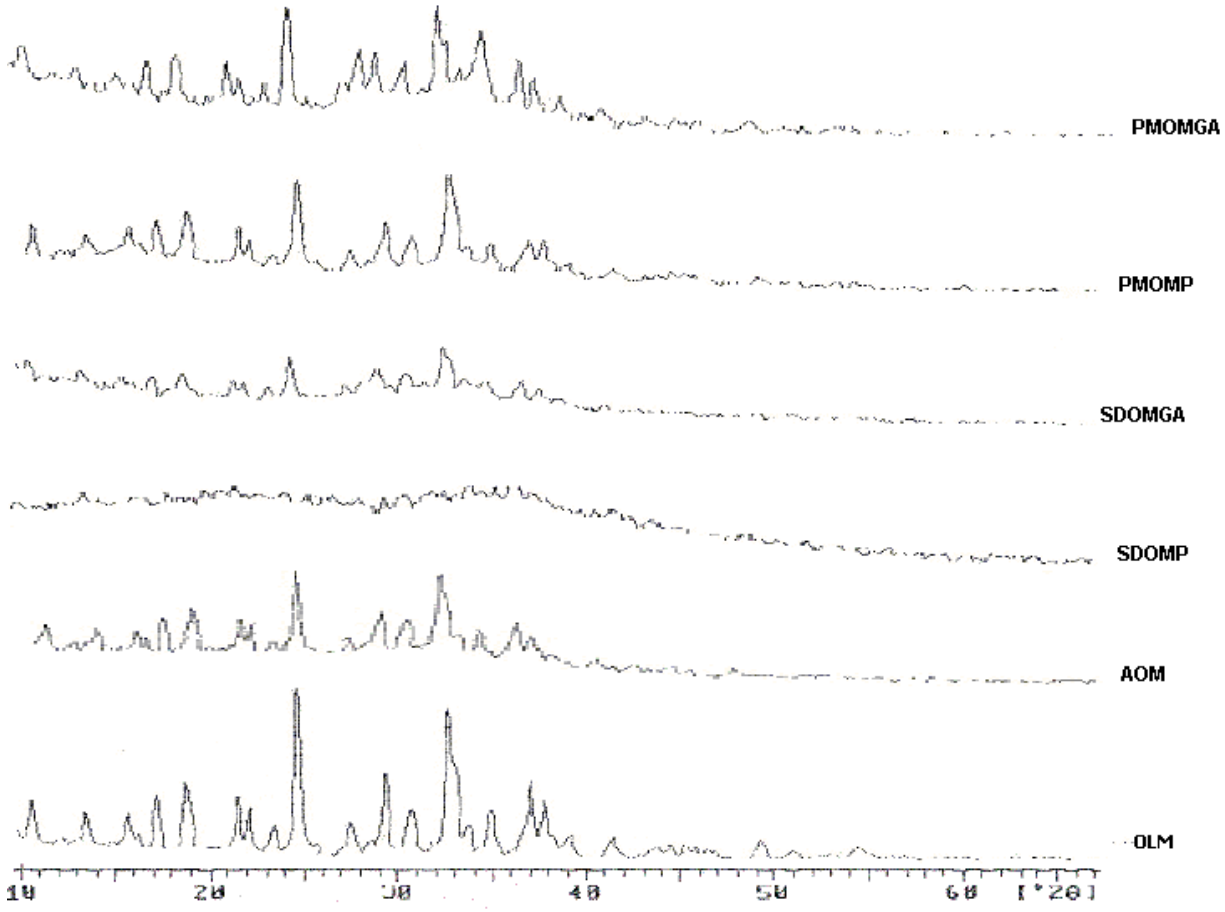
| Type of formulation | Saturation solubility in distilled water (mg/ml) ± SD | Saturation solubility in phosphate buffer pH 6.8 (mg/ml) ± SD |
|---|---|---|
| Pure drug | 0.10±0.03 | 0.18±0.01 |
| AOM | 0.37±0.06 | 0.48±0.09 |
| PMOMP | 0.14±0.05 | 0.29±0.08 |
| PMOMGA | 0.25±0.06 | 0.36±0.07 |
| SDOMP | 0.82±0.13 | 1.05±0.24 |
| SDOMGA | 0.94±0.20 | 1.26±0.12 |

*Mean ± S.D., n=3*

**SEM**

SEM of Pure OM exhibited some large crystals with few microparticles, which might have been generated due to micronization or any other process of size reduction at the time of manufacturing. AOM and SDOMP showed smooth and spherical particles of varying size with smooth surfaces. SDOMGA particles looked like an irregular shaped matrices which suggest that particle shape and surface topography is changed during formation of effective solid dispersion system. These findings demonstrate that reduction in particle size, increased surface area and close contact between drug and carrier may be responsible for the enhanced solubility of drug in SDs.

**Figure 3 : XRPD patterns of OM, AOM, SDOMP, SDOMGA, PMOMP and PMOMGA**




**XRPD**

XRPD of pure OM showed numerous distinctive peaks in the region of 10 to $40^0$ (2θ) at $18.950^0$, $21.820^0$, $24.950^0$, $29.605^0$ $31.075^0$ and $33.195^{0,}$ indicating that pure OM is highly crystalline in nature. PMs with PVP or Gelucire® 50/13 showed characteristics diffraction peaks of OM, but there was significant decrease in intensity of some major

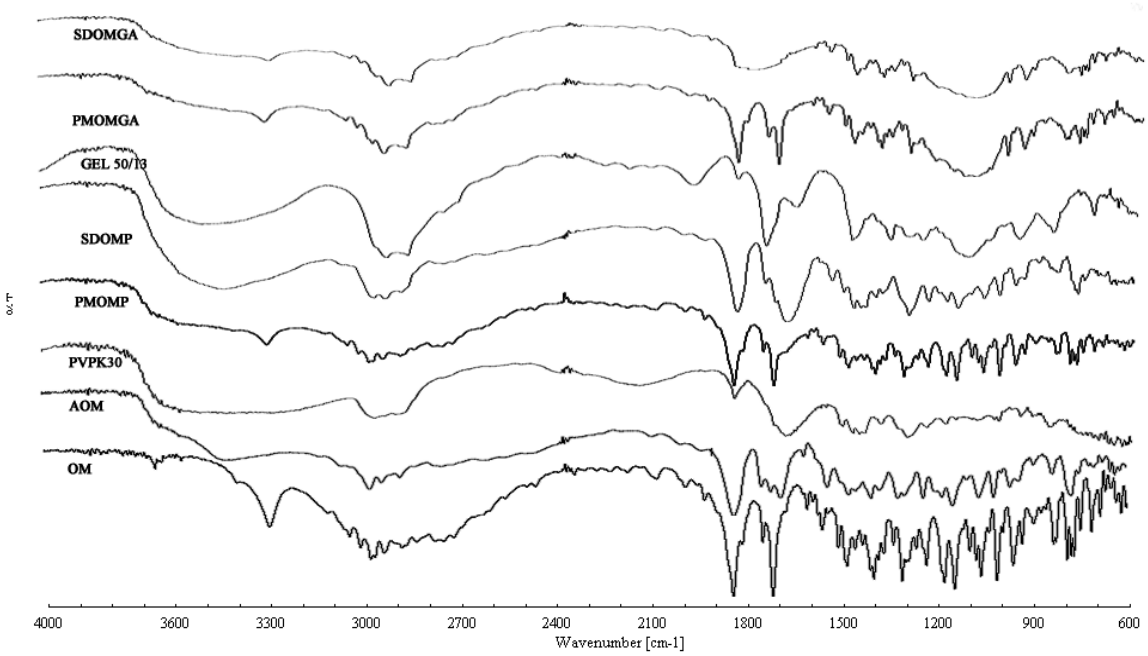
OM crystalline peaks suggesting that there was partial loss of crystallinity, may be due to physical presence of amorphous excepients and grinding with carrier and adsorbent.

On the other hand the XRPD patterns of AOM and SD are prepared by spray drying were completely different from those of pure OM and PMs. It showed hollow, broad and diffuse maxima, caused by relatively random arrangement of the constituent molecules, which produced poorly coherent scatters. This confirmed the transformation of crystalline OM into an amorphous OM.

**DRIFT**

To study the possible interaction of OM with the carriers, DRIFT spectroscopy was carried out. The spectrum of OM was characterized by peaks at 3650 cm–1 (OH stretching), 3290cm–1 (NH stretching), 2927 cm–1 (aliphatic CH stretching), 1830 cm–1 (ester stretching), 1703 cm–1 (acetate stretching) and 1527-1446 cm–1(C=C stretching). In case of spray dried OM (AOM) , a slight shift in –NH stretching vibrations (3450 cm-1) and broadening of C=O stretching vibrations was observed, suggesting possibility of intermolecular hydrogen bonding between adjunct OM molecules.

**Figure 4 : DRIFT spectra of OM, AOM, SDOMP, SDOMGA, PMOMP and PMOMGA**




The FT-IR spectrum of PVP showed a characteristic absorption band at 1660 cm–1 (C=O) and a very broad absorption band at 3435 cm–1 due to the presence of water. The absorption bands corresponding to crystalline OM were observed for the physical mixtures with PVP, suggesting that there is no interaction between pure OM and PVP. In contrast, absorption bands corresponding to the C=O stretching bands at 1650 cm–1 were not observed, and the symmetrical and asymmetrical N–H stretching vibrations of OM at 3290 cm–1 were broadened in the SDCAP spectra. [23] PVP has two functional groups, i.e. =N– and C=O, that can potentially form hydrogen bonds with the drug. However, stearic hindrance precludes the involvement of nitrogen atoms in intermolecular interactions, thus making the carbonyl group more favorable for hydrogen bonding. These results suggest that the N–H functional groups of OM act as a hydrogen donor while the C=O of PVP is a hydrogen acceptor, forming hydrogen bonds in SD and resulting in the formation of stable amorphous form of OM. Ideally, the DRIFT spectra of PMs should be equivalent to addition spectrum of excipients and the crystalline drug. However, the overall spectrum of and PMOMGA appeared to be influenced by the incorporation of silicon dioxide. The presence of a broad prominent peak at 1107 cm–1 (strong Si–O linkage) is characteristic of silicon dioxide.[17] As stated earlier, the hydrogen bonding potential of silanol groups in the local environment of silica is well documented, so there is always a possibility that during preparation of PMs the –NH group of OM can form very weak hydrogen bonds with the

silanol groups of A200. All major peaks of carbonyl stretching vibrations (C=O) were absent and amine peaks (-NH) were broadened in the DRIFT spectra of SDCAGA. This indicated the possibility of hydrogen bonding between OM and the carriers. However, the site of interaction of Gelucire® would be expected to be in the C=O group, affecting the N–H vibration, [24] In fact, the interaction may possibly also occur between the C=O group of OM and the –OH group of Gelucire® [16] This was supported by the significantly decreased intensity of peaks corresponding to the carbonyl stretching vibrations. Although both the carriers show formation of a stable SD with OM by hydrogen bonding, the functional groups involved are different. While in hydrogen bonding of OM with PVP an -NH is involved, with Gelucire® both C=O and -NH functional groups are involved. A200 may also take part in hydrogen bonding with OM. However, no such interaction was observed in SDOMGA. Since A200 is dispersed in DCM it is not freely available for hydrogen bonding interactions. This is in accordance with the findings of Raghavan et al.[25]

**Dissolution study**

OM was characterized by only 29.48±2.03 % drug release within 60 min. AOM showed 40.47±1.86 % release within 60 min. This improvement in dissolution could be attributed to the presence of amorphous form of OM, as confirmed by XRPD studies. Spray drying generated amorphous particles with enhanced thermodynamic escaping tendency leads to faster dissolution rate and higher solubility. PMs showed slight improvement in the drug release and saturation solubility which probably attributed to an improvement of wetting and to local solubilization by the excipients in the diffusion layer [26]. PMOMGA showed slight increase in dissolution over PMOMP, may be due to the wetting characteristics of Gelucire® and solubalization through micelle formation [27]

Both the SDs showed statically improved dissolution ($p<0.05$) compared to OM and AOM. Within first 10 min the SDs exhibited a higher burst of OM from the SDs. This was might be due to many possible mechanisms such as reduction in particle size, increase in surface area of drug, conversion to amorphous form as confirmed by XRPD studies (fig 3), solubilisation effect due to presence of carriers and improved wettability and dispersibility of drug from dispersion [27,28].

**Figure 5 : Dissolution profiles of OM, PMOMP, PMOMGA, AOM, SDOMP and SDOMGA**

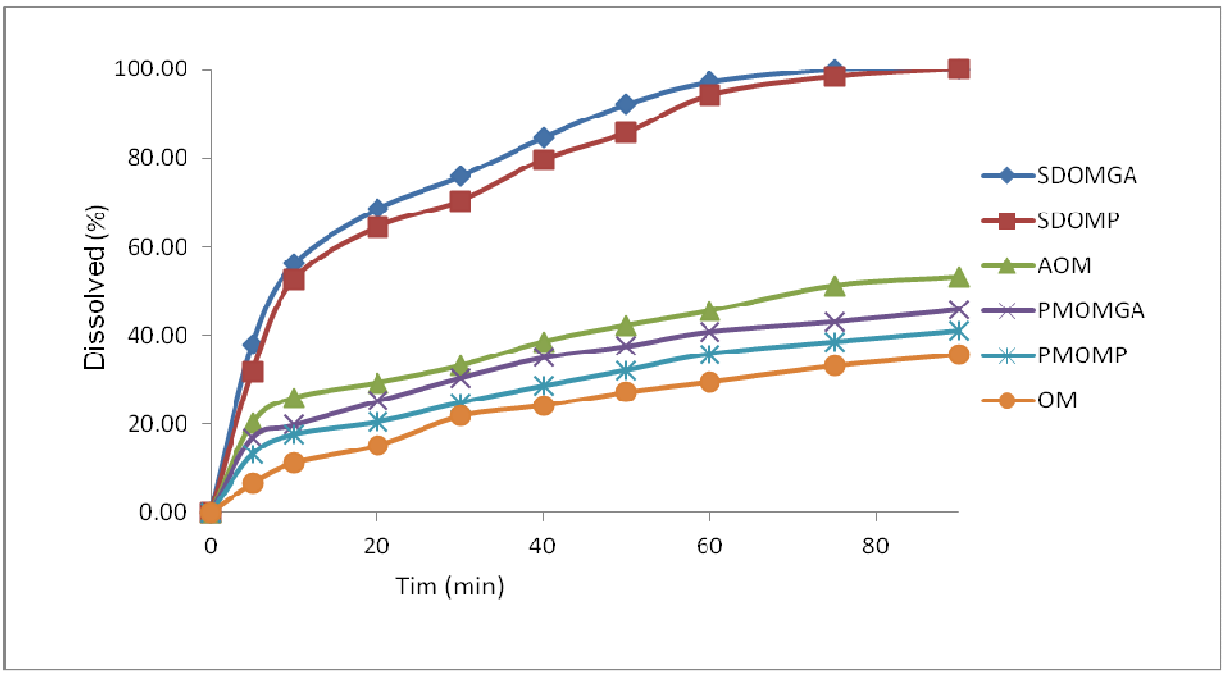


**Figure 6: Dissolution profiles of OM, PMOMP, PMOMGA, AOM, SDOMP and SDOMGA after 3 months**

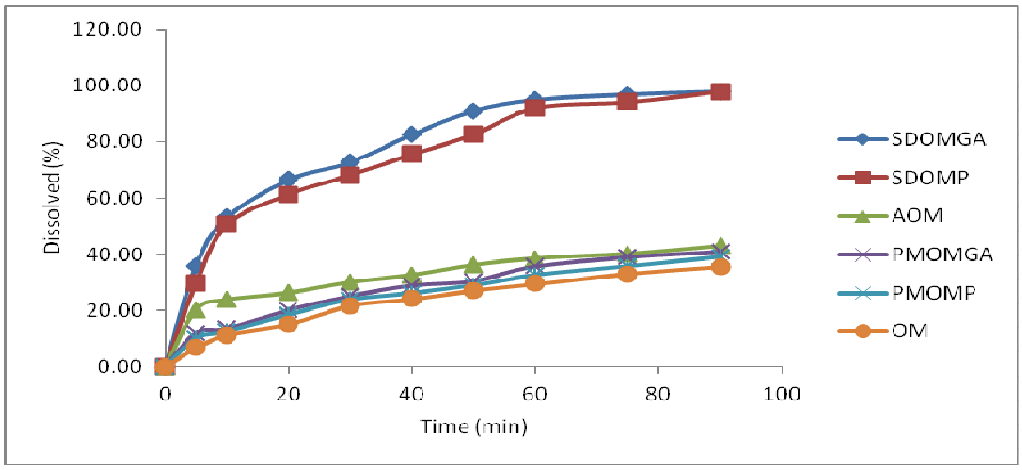
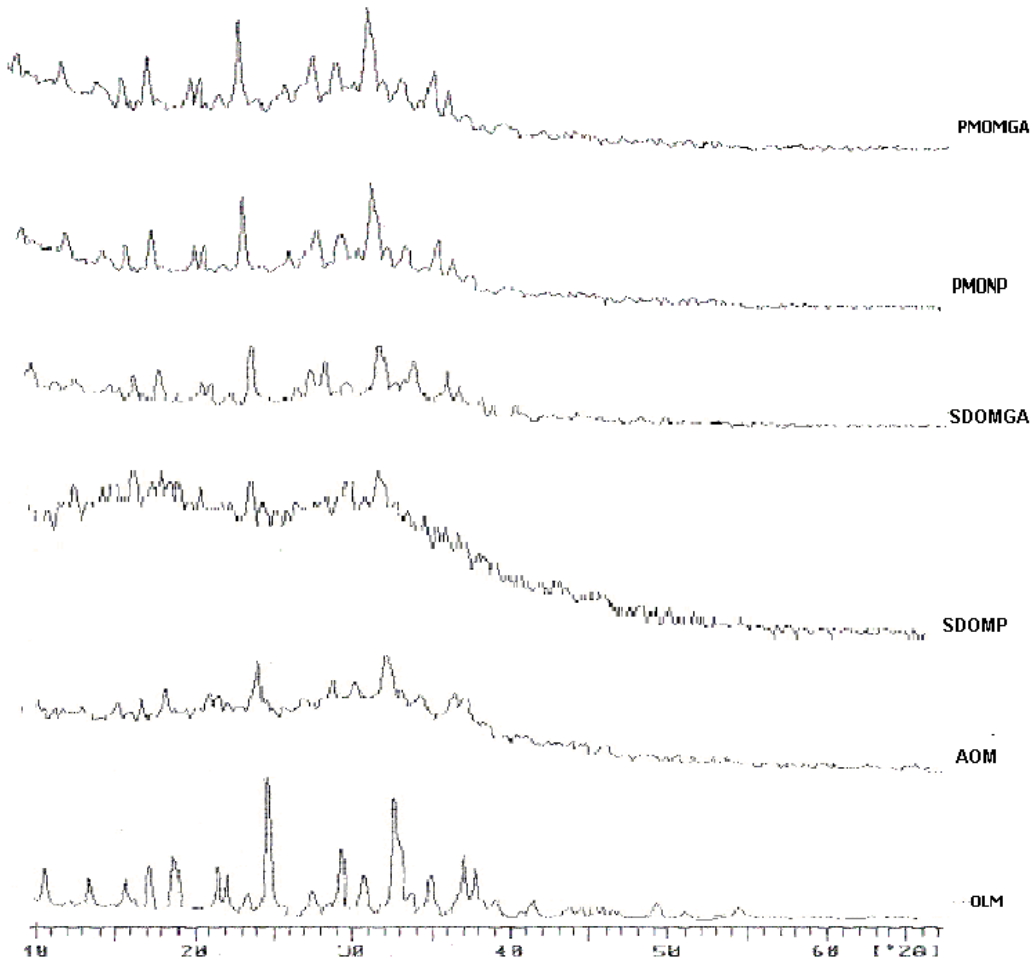



**Figure 7 : XRPD patterns of OM, AOM, SDOMP, SDOMGA, PMOMP and PMOMGA after 3 month**




**Stability studies**

It is well known that amorphous drugs formulated in the form of SDs tend to recrystallize on storage. For the present study, ambient temperature and relative humidity ($30^0$C / 60% RH) were selected. During aging study there was

gradual decrease in dissolution rate of AOM , over the period of 3 months as compared to freshly prepared samples. XRPD patterns of AOM showed characteristic diffraction peaks of crystalline drug confirmed destabilization of amorphous state of OM. The consistent existence of amorphous nature of OM in SDs was evidenced from no significant decrease in dissolution rate as compared to freshly prepared samples over the period of 3 months. This improved stability in case of SDOMP could be due to antiplasticizing property of PVP and hydrogen bonding of drug with PVP. In case of SDOMGA due hydrogen bonding between OM & Gelucire® 50/13 and adsorption on the surface of amorphous silicon dioxide with potential hydrogen bonding, exhibiting additional mechanism of stabilization.

## CONCLUSION

Solid dispersions of poorly water soluble drug Olmesartan medoxomil (OM) were successfully prepared by spray drying technique, using PVP or Gelucire® 50/13 with silicon dioxide as an adsorbent. DRIFT spectroscopy revealed the possibility of hydrogen bonding interactions in solid dispersions which was supported by XRPD observations. In vitro dissolution study showed a significant increase in dissolution rate of solid dispersions as compared to pure OM, spray dried OM and physical mixtures of drug with carrier. During aging study, slight decrease in dissolution was observed with evidence of very slight crystallinity. Thus present study demonstrates high potential of spray drying technique to improve dissolution rate of poorly soluble olmesartan medoxomil by using PVPK30 or Gelucire® 50/13 with silicon dioxide as adsorbent.

**Acknowledgements**
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## REFERENCES

[1] Lobengerg R, Amidon GL. *Eur J Pharm Biopharm*; **2000**, 50, 3-12.
[2] Sekiguchi K, Obi N. *Chem. Pharm. Bull*; **1996**, 9, 866-872.
[3] Ford JL. *Pharm Acta Delvopment*; **1986**, 61, 69-88.
[4] Chiou WL, Riegelman S. *J Pharm Sci*; **1971**, 60, 1281-1303.
[5] Sharma DK, Joshi SB. *Asian journal of pharmaceutics*; **2007**, 1, 9-19.
[6] Ambike AA, Mahadik KR, Paradkar A. *Int J Pharm*; **2004**, 282, 151–162.
[7] Doshi DH, Ravis WR, Betageri GV. *Drug Dev Ind Pharm*; **1967**, 23, 1167-1176.
[8] Suzuki H, Sunada H. *Chem Pharm Bull*; **1998**, 46, 482–487.
[9] Takeuchi H, Nagira S, Yamamoto H, Kawashima Y. *Powder Technology*; **2004**, 141, 87-195.
[10] Abdul- Fatteh AM, Bhargava HN. *Int J Pharm*; **2002**, 235, 17-33.
[11] Finia A, Moyanob JR, Gine´sb JM, Martinezb JIP, Rabasco AM. *Eur J Pharm Biopharm*; **2005**, 60, 99–111.
[12] Aïnaoui A, Vergnaud JM. *Int J Pharm*; **1998**, 169, 155–162.
[13] Sutananta W, Craig DQM, Newton JM. *Int J Pharm*; **1994**, 111, 51–62.
[14] Dordunoo SK, Ford JL, Rubinstein MH. *Drug Dev Ind Pharm*; **1991**, 17, 1685–1713.
[15] Leuner C, Dressman J. *Eur J Pharm Biopharm* **2000**; 50, 47–60.
[16] Chouhan B, Shimpi S, Paradkar A. *AAPS Pharm Sci Tech*; **2005**; 7(1), 1-8.
[17] Chouhan B, Shimpi S, Paradkar A. *Eur J Pharm Sci*; **2005**, 26, 219–230.
[18] Dhumal RS, Biradar SV, Aher S, Paradkar AR. *J Pharm Pharmacol*; **2009**, 61, 743-751.
[19] Norwood D, Branch E III, Smith B, Honeywell M. *Drug Forecast*; **2002**, 27(12),611-618.
[20] Mire DE, Silfani TN, Pugsley MK. J *Cardiovasc Pharmacol*; **2005**, 46(5), 585–93.
[21] VonBergmann K, Laeis P, Puchler K. *J Hypertensive*; **2001**, 19(1), 33-40.
[22] Eldem T, Speiser P, Hincal A. *Pharm Research*; **1991**, 8, 47–54.
[23] Taylor LS., Zografi G. *Pharm Research*; **1997**, 14, 1691–1698.
[24] Passerini Nadia, Beatrice P., Mariarosa M., Dario V., Beatrice A., Cristina C.and Lorenzo R. *J Pharm Sci*; **2002**, 91, 699–707.
[25] Raghavan SR., H. J. Walls and Saad A. Khan. *Langmuir*; **2000**, 16, 7920–7930.
[26] Vippagunta SR, Maul KA, Tallavajhala S, Grant DJW. *Int J Pharm*; **2002**, 236, 111–123.
[27] Leuner G, Dressman *Eur J Pharm Biopharm*; **2000**, 50, 47–60.
[28] Broman E, Khoo C, Taylor LS. *Int J Pharm*; **2001**, 222, 139–151.

[29] R.L.C. Sasidhar, S. Vidyadhara, G.V. Maheswari, B. Deepti and Srinivasa Babu. *Advances in Biological Research*; **2013**, 7 (2), 32-41.
[30] A. Abdul Hasan Sathali and J. Jayalakshmi. *J. Curr. Chem. Pharm. Sc*; **2013**, 3(2), 123-134
[31] Sruthy.P.N, Anoop K.R. *International Journal of Pharmacy and Pharmaceutical Sciences*; **2013**, 5(3), 691-696
[32] Ashwini A. Yadav, Dhanashri S. Yadav, Poonam S. Karekar, Yogesh V. Pore, Pankaj Gajare, , *Pelagia Research Library Der Pharmacia Sinica*; **2012**, 3 (2), 160-169