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Preparation and characterization of Metaxalone nanoparticles prepared by High Pressure Homogenization.

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ABSTRACT

The aim of present work was to enhance the dissolution of poorly soluble drug metaxalone by particle size reduction. Metaxalone nanoparticles are obtained by high pressure homogenization followed by drying, which are characterized for mean particle size (MPS), polydispersity-index(PDI), zeta-potential(ZP), X-ray diffraction(XRD), Differential scanning calorimetry(DSC), Fourier-transform infra-red(FTIR), scanning electron microscopy (SEM), flow properties, saturation solubility and in-vitro release. The MPS of nanoparticles was observed to be less than 200 nm. The negative ZP indicates the stable nanoparticles obtained by sufficient adsorption of the stabilizers onto drug surface. The XRD and DSC show the retention of drug crystallinity. Out of three drying methods, SD and SG have obtained stable nanoparticles with improved flow properties. Nanoparticles increased the drug solubility by approximately 4 folds with Hydroxy propyl methyl cellulose and sodium lauryl sulfate as surface stabilizers. In-vitro release studies showed a remarkable increase in rate of drug release from 3 % (pure drug) to 34-36 % (nanoparticles) after 15 minutes and at the end of dissolution study almost 95 % of drug dissolved when compared to only 30 % of pure drug. The combining methods of HPH followed by SD/SG was observed to be promising method to produce stable nanoparticles of metaxalone with remarkable increase dissolution rate.. Results from this study suggest that these metaxalone nanoparticles may be a potential candidate for oral administration with quick onset of action for relief of acute painful musculoskeletal conditions.

Keywords: Metaxalone, Nanoparticles, homogenization, spray drying, spray granulation, lyophilization.

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INTRODUCTION

Oral route of administration is the most common and preferred route for drug delivery. But due to limited drug absorption due to poor solubility of drugs results in poor oral bioavailability. Thus the efficacy of the drug depends upon the bioavailability which in turn depends on the solubility of the drug molecules. Thus the solubility of the drug is one of the important features of the molecule to achieve the drug efficacy.

BCS class II drugs are having low solubility and high permeability These BCS class II drugs are not a good candidate for oral delivery if not aimed at increasing the solubility and the rate of dissolution. There are different techniques for improving the drug solubility such as micronization, nanonization, use of co-solvents, surfactants, complexation, solid dispersions etc[1]. For BCS class II drugs the solubility or rate of dissolution can be increased by increasing the particle surface area or use of solubilizing agents such as co-solvents or surfactants.

For some of the poor soluble drugs, it has been observed that reduction of the particle size to micron level is not sufficient to increase the solubility or rate of dissolution and thereby the oral bioavailability. In such cases reduction of particle size to nanoscale level i.e. < 1 µm or 1000 nm, have shown increase in the oral bioavailability. Below 1,000 nm, the saturation solubility becomes a function of the particle size, leading to an increased saturation solubility of nanocrystals, which in turn increases the concentration gradient between gut lumen and blood, and consequently the absorption by passive diffusion[2]

Now-a-days nanoparticle technology is wide spread and of high interest in pharmaceutical development[3]. Nanonization is a process wherein the drug particle size is reduced at the nanoscale level so that it can result in improved drug solubility and pharmacokinetics, and simultaneously may reduce the systemic side-effects. There are different methods [4,5,6,7] used for preparing nanoparticles or nanonization of drug such as Bead or Pearl milling[8], Homogenization[9,10], Emulsification-solvent evaporation technique, Spray drying, nanoprecipitation etc. Two main disadvantages with the bead milling process include the erosion of the beads during the milling process resulting in the impurity formation and adherence of the product to the inner surface of the milling chamber. Whereas the high pressure homogenization method is a simple technique. The principle in the homogenization involves the process wherein the drug suspension is forced through the tiny homogenization gap with a pressure up to 4000 bar. The high pressure homogenizers combine both pressure and mechanical forces to achieve a uniform and consistent particle size. Usually homogenization method is influenced by applied pressure, number of

homogenization cycles and temperature. Important advantage of the high pressure homogenization is high quality, productivity nanosuspensions with little batch to batch variation, with very low microparticle content in the product are obtained[11]. Further when compared to pearl milling, the contamination due to erosion from the wall of the homogenizer is at very low level.

Metaxalone has a chemical name 5-[(3,5-dimethyphenoxy)methyl]-2-oxazolidinone. Metaxalone is a white to almost white, odorless crystalline powder freely soluble in chloroform, soluble in methanol and in 96% ethanol, but practically insoluble in ether or water. Metaxalone is a skeletal muscle relaxant and is indicated as an adjunct to rest, physical therapy, and other measures for the relief of discomforts associated with acute, painful musculoskeletal conditions.

Metaxalone is present in a commercial product having the brand SKELAXIN®, as tablets containing 400 mg or 800 mg of the drug. The usual dosing for adults and children over 12 years of age is one tablet, taken three to four times daily.

It has been reported that when poorly soluble, hydrophobic drugs such as metaxalone are orally administered, the rate of dissolution is slow resulting in poor absorption and tremendous food effect. However if such drugs are to be administered in oral dosage forms for clinical indications such as pain, rapid onset of therapeutic activity is desirable. In such case, the slow rate of dissolution and absorption may put limitations on their therapeutic utility. Painful, musculoskeletal conditions require prompt relief. Therefore, compositions that exhibit quick onset of action are desirable. Metaxalone is an insoluble drug and further has relatively high dosing requirements. Thus, there is need in the art for novel compositions of the metaxalone which are having quick onset of action. Improvement in solubility and thereby the bioavailability of metaxalone may also lead to reduction in the administered doses to achieve a desired therapeutic activity.

MATERIALS AND METHOD

Materials

Metaxalone was obtained from Dr Reddys Laboratories limited. The excipients such as Poly vinyl pyrrolidone [PVP] supplied by BASF, sodium lauryl sulfate [SLS] supplied by JRS, Mannitol from Rouquette Pharma, Lactose supplied by Meggle pharma, Hydroxy propyl cellulose [HPC] supplied by; Hydroxy propyl methyl cellulose [HPMC] supplied by; Polyvinyl alcohol [PVA] supplied by, Polyethylene glycol [PEG] supplied by; Poloxamer supplied by BASF are taken from Dr Reddys Laboratories limited. Lyophilizer, Spray drier, Fluid bed Granulator, High pressure homogenizer are the equipment used for manufacturing nanosuspension and drying. All other solvents and

reagents used were of HPLC or analytical grade. Different instruments used for characterization are from Dr Reddys Laboratories Limited.

METHODS:

Preparation of nanosuspension

The important challenge in preparing the nanosuspensions is physical stability of the nanosuspension. It is well established theory that by reducing the particle size, the surface area is increased which is used to enhance the rate of dissolution rate. However this increase in the surface energy can cause the nanometer sized drug particles to spontaneously aggregate and form into a more thermodynamically stable state[12]. Thus to prepare physically stable nanoparticles various stabilizers are used which stabilize the nanoparticles by steric stabilization and/or ionic/electrostatic stabilization. It is very important that the stabilizers is not interacting chemically with the drug and act merely adsorbing onto the surface of the drug particles. Another important parameter to be considered in obtaining the physically stable nanoparticles is Ostwald ripening resulting from crystallization of the active at uncontrolled rate leading into growth of the particle size[13] Ostwald ripening may be reduced by controlling choice of surface stabilizers, particle size, particle size distribution, solids content. Hence it is very important to select suitable surface stabilizers.

In the present study, high pressure homogenization process was used to prepare the nanoparticles. Different surface stabilizers such as PVP, SLS, HPC, HPMC, PVA, PEG, poloxamer have been evaluated in stabilizing the metaxalone nanoparticles. Different composition of the nanoparticles has been prepared as disclosed in the Table 1 by using different combination of the surface stabilizers. For preparing the suspension of each composition the surface stabilizers are dissolved in purified water with continuous stirring. Then slowly the drug metaxalone is dispersed in the stabilizer solution with continuous stirring. Then the drug suspension is subjected to high shear homogenization for about 30 minutes to prevent lump formation. Then the drug suspension is subjected to high pressure homogenization for about 120 minutes at 1000 bar pressure. While preparing the drug suspension with PVA [MX-04], as PVA was not getting dissolved in purified water, it was heated at 60 °C water bath for about 15-20 minutes, then PVA was completely dissolved. After cooling to room temperature, the drug was dispersed in the polymer solution.

Table 1: Compositions of metaxalone drug suspension.

	MX-01 % (w/v)	**MX-02**	**MX-03**	**MX-04**	**MX-05**	**MX-06**
Metaxalone	5	5	5	5	5	5
Sodium lauryl sulfate [SLS]	0.5	0.5	0.5	0.5	0.5	0.5
Hydroxy propyl methyl cellulose [HPMC]	5	-	-	-	-	-
Poly vinyl pyrrolidone [PVP]	-	5	-	-	-	-
Hydroxy Propyl Cellulose [HPC]	-	-	5	-	-	-
Poly Vinyl Alcohol [PVA]	-	-	-	5	-	-
Poly Ethylene Glycol [PEG]	-	-	-	-	5	-
Poloxamer	-	-	-	-	-	5
P.Water	qs	qs	qs	qs	qs	qs

And while preparing the drug suspension with Poloxamer, lot of foam was obtained when subjected to stirring followed by high shear homogenization. Hence this drug suspension was subjected to high pressure homogenization after the foam is completely subside.

Production of dried nanoparticles

In general, the solid dosage forms are preferred due to their convenience and physical stability. Drug nanosuspensions are the liquid states wherein the drug in nanoscale is in suspended state. The drug particles in this state are highly unstable, hence it has to be converted into dried form. The liquid nanosuspensions can be converted into solid powder by freeze drying or lyophilization; spray drying, pelletization, spray granulation[14]. Usually before drying the nanosuspensions, redispersants are added to the suspension in-order to prevent the aggregation of particles during solidification and also to achieve adequate redispersion in water[15]. Thus overall it is important that the drug nanoparticles preserve its properties of dissolution and/or solubility characteristics upon redispersions. In the present work, three different techniques have been used to convert the nanosuspensions into dry powder form such as lyophilization, spray drying and spray granulation in order find suitable drying technique, because sometimes due to the changes occurring in the drying process results in the formation of irreversible aggregates which are unable to redisperse into nanoparticles upon dissolution thus the advantage of nanoformulations are lost[16]. Extensive research has been carried out to know the effect of the process variables on the redispersibility of the dried nanoparticles[17,18,19,20]. The major challenge with the nanosuspension is preservation of physical and the chemical stability of the nanoparticles in the aqueous medium even after converting into the dried nanoparticles. It has been observed that the nanoparticles are prone for physical instability (crystal growth and formation of aggregates) and chemical instability (drug degradation). It has been reported in the literature that on storage of nanosuspension at room

temperature, Ostwald ripening occurred. As the nanosuspensions were afflicted by Ostwald ripening and settling, drying of nanosuspension is adopted as a strategy to circumvent these stability issues.

The nanosuspension prepared with the steric stabilizers PVP, HPC, HPMC and PEG with the combination of electrostatic or ionic stabilizer SLS has been subjected to the three different drying processes and compared.

Spray granulation

Spray granulation is a process wherein the drug nanosuspension is sprayed onto the micronized substrate[21]. Granulation technique is known to improve the flow properties of the powder. It can be carried out by top spray or bottom spray granulation. Spray granulation of the nanosuspension was carried out in GPCG-1.1; FR463; PtamGlatt fluid bed coater. The fluid bed dryer was pre-heated prior to the start of the spraying process. Through this method of drying the nanosuspension MX-01 with two different redispersants (substrate) mannitol and lactose have been evaluated Spray granulated (bottom spray) was carried out with the Inlet temperature: 70°C, Product temperature: 45 °C, exhaust temperature 40 °C, atomization pressure 1.1 bar, spray pump speed ranging from 3-5 rpm, blower drive speed 40, air flow 18 CFM, spray rate 2-4 g/minute; The nanoparticles obtained after spry granulation with mannitol are termed as MX-07A and with lactose MX-07B.

Spray Drying:

Spray drying process is a process wherein the dry powder is obtained from the liquid suspension by rapidly drying in presence of hot air. Spray drying process was used to convert the liquid nanosuspension into dry powder form[22]. Basically the principle works like this: liquid or substance will be sprinkled into a hot cylinder by a sprayer. During the downfall the liquid will evaporate. The products that fall down is solid powder which is separated from the air by a cyclone collector. In spray dryers the material that has to be dried will suspend in the air that is to say that the liquid changes in to a misty fog (atomized) which has a large surface area. The atomized liquid will be exposed to a steam hot air in a drying room. The liquid evaporates fast and the solids recover as powder that exists of fine, hollow spherical parts. To nanosuspension mannitol was added with stirring until uniform suspension was obtained. The resultant suspension was spray dried using Buchi mini spray dryer B-290 with inlet temperature 140 °C, outlet temperature of -74 °C, aspirator settling -70 mbar inlet nitrogen pressure 5 kg/cm2and liquid suspension feed rate 6 ml/minute.

Spray drying was carried out for different metaxalone compositions such as MX-01, MX-02, MX-03, MX-04 and termed as MX-010, MX-09, MX-08, MX-011 respectively after spray drying.

Freeze drying

The principle of freeze drying method is that small amounts of a product will be frozen in and thereafter it will be placed under vacuum. Through vacuum the frozen liquid sublimates. The ice immediately changes in to vapour, without to defrost first, also called sublimate. During process the outside part will be the first part that dewaters. After that the water is removed closer and closer till the core of the product. Hereby the structure of the product stays intact. Due to the vacuum the ice will evaporate immediately without turning in to water again. The quality of freeze dried products is usually of high quality, mainly because the temperature stays low during the whole process. To the nanosuspension from the batches MX-01, MX-02, MX-03, MX-05, mannitol redispersant added and subjected to lyophilization technique and termed as MX-012, MX-013, MX-014, MX-015 after lyophilization. The lyophilization process is carried out by the process wherein the nanosuspension was taken in round bottom flask after sonicating for 5 minutes. Then in a tray dry ice (liquid carbon dioxide) was taken and to this acetone was added. In this the round bottom flask [RBF] was rotated, then the sample freezes immediately to the walls of the round bottom flask. Then these RBF is attached to the lyophilizer maintained at -500 mTorr and condenser temperature between -80 to -85 °C and left overnight for drying the sample.

CHARACTERIZATION OF THE NANOSUSPENSION AND DRIED NANOPARTICLES

The nanosuspension obtained was characterized for mean particle size, zeta potential and Polydispersity index. The dried nanoparticles are characterized for mean particles size, zeta potential, poly dispersity index, X-ray powder diffraction, differential scanning calorimeter, Fourier transform infra-red spectroscopy, Scanning electron microscopy, flow properties, saturation solubility, dissolution profile.

Particle size

The mean particle size (d50) of the nanosuspension before drying was estimated in triplicate by using Zeta sizer- Nano ZS, Malvern Instruments UK) at room temperature. A refractive index of 1.65 was used for particle size analysis. The samples were adequately diluted with deionized water and placed in electrophoretic cell and measurement was carried out with help of software.

The particle size of drug in dried nanoparticles was analyzed by adding dried nanoparticles in water so that the surface stabilizers, re-dispersants are dissolved in water leaving drug in dispersed state, followed by dilution with water to obtain suitable concentrations for measurement in the same manner as carried out for the nanosuspension.

Zeta Potential

The surface charges of the nanoparticles is studied by zeta potential. Value of the surface charge determine the stability of the nanosuspension. A prerequisite to achieve an enhancement of oral bioavailability with drug nanocrystals is that crystals are finely dispersed in the gut and do not aggregate. In case they start aggregation, the bioavailability decreases with increasing aggregate formation. This is attributed to the fact that they lose special properties of nanoparticles such as their adhesive property to the mucosal wall. Therefore it is necessary to prepare nanosuspensions with a physical stability as high as possible. Surface charge properties of the nanosuspensions are studied through zeta potential. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions[23,24] and a minimum of ±20 mV for steric stabilization[25]. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential[26].

Zeta potential of the nano suspension has been analyzed in Malvern zeta sizer after diluting nanosuspension with water to obtain suitable concentration for measurement. Further the zeta potential of the dried nanoparticles is obtained by adding water to the dried nanoparticles and diluted with water to obtain suitable concentration for measurement. The diluted sample was added in specialized zeta cell and the same procedure as that of particle size was carried out.

X-ray Powder diffraction (XRD)

X-ray powder diffraction measurements were carried out on samples using a diffractometer (X'Pert MPD Model, Phillips, Holland). The results were recorded over a range of 0–40° (2θ) using the Cu-target X-ray tube and Xe-filled detector. The operating conditions were: voltage 40 kV; current 30 mA; scanning speed 1/min. Metaxalone and its dried nanoparticles obtained by lyophilization, spray granulation and spray drying has been characterized by XRD to study their morphological changes.

Differential Scanning Calorimetry (DSC)

DSC scans of the Metaxalone drug and dried nanoparticles obtained by lyophilization, spray granulation, spray drying, have been studied using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 20°C/min under dry air flow (100 ml/min) between 25° C and 220° at 10°C/minute.

Fourier Transform Infra-Red (FTIR) Spectroscopy

Due to the complex interaction of atoms with in the molecule, IR absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristic IR absorption at specific narrow frequency range. Multiple functional groups may

absorb at one particular frequency range but a functional group often gives rise to several characteristic absorptions. Stretching & bending vibrations are varied. Thus, the spectral interpretations should not be confined to one or two bands only actually the whole spectrum should be examined .The pure drug metaxalone and dried nanoparticles obtained by lyophilization, spray granulation, spray drying have been studied on the sample prepared in Potassium Bromide (KBr) disks, using Shimadzu Fourier Transform Infra-Red spectrometer. The powder blends for IR spectra was prepared by blending sample with KBr in 1:100 ratio and were scanned over wave number range of 400 to 4000 cm-1.

Scanning Electron Microscopy [SEM]:

The morphology of the raw metaxalone, nanosuspension and nanosized metaxalone was examined with the scanning electron microscopy, operated at the low vacuum with a LFD detector and 60 Pa pressure. First, double sided carbon tape was stuck onto the clean aluminium stub. Then the freshly prepared nanosuspension or dried nanoparticles was applied on the carbon tape and excess was tapped off using nitrogen gas. 5nm thick gold coating was applied onto the sample. Metaxalone, the dried nanoparticles obtained by the different drying methods such as lyophilization, spray granulation and spray drying has been characterized by SEM analysis for studying the morphology of the dried nanoparticles.

FLOW PROPERTIES OF THE DRIED NANOPARTICLES.

To determine the flow properties of the dried nanoparticles, four parameters such as angle of repose, bulk density, tapped density, Carr's Index and Hausner ratio have been determined.. The angle of repose was determined by allowing dried nanoparticles to flow through the funnel which is fixed to the stand at a height of approximately 2 cm from the surface on a plane surface. The flow was continued till the pile formed touches the stem tip of the funnel. Height of the pile was determined and bottom surface of the pile which is roughly circular shape is drawn and the radius of the pile was measured. Angle of repose was then calculated by using the following equation (I).

$$\tan \theta = h / r \qquad \text{(I)}$$

Where, θ = angle of repose, h = height of the pile, r = average radius of the powder cone

If the value of the angle of repose is less than 25 then it shows excellent flow and if the value is between 25-30 it shows good flow and if the value is more than 30 it indicates that the dried nanoparticles have poor flow properties.

The bulk density of dried nanoparticles was determined by placing approximately 25 g of the dried nanoparticles into graduated measuring cylinder and the volume occupied by the powder method was recorded. The tapped density of the dried nanoparticles was determined by measuring the

volume occupied by the dried nanoparticles after tapping continuously until there is no further change in the volume.

The Carr's Index (CI) value gives an indication of powder flow; It is determined by the following equation (II). If the Carr's Index value is less than 25% indicates a good flow whereas, a value greater than 25% indicates a poor flow.

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density -Bulk density X 100}}{\text{Tapped density}} \quad \text{(II)}$$

Hausner ratio was calculated from tapped **a**nd bulk density using the following equation (III). A Hausner ratio value less than 1.20 is indicative of good flow whereas, a value > 1.5 indicates poor flow.

$$\text{Hausner Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad \text{(III)}$$

Saturation Solubility

Saturation solubility is a compound-specific constant only depending on the temperature and the properties of the dissolution medium. However, below a size of approximately 1–2µm, the saturation solubility is also a function of the particle size. Saturation solubility of plain drug (Metaxalone and dried nanoparticles obtained by lyophilization, spray granulation, spray drying were carried out in 0.1 N HCl, 4.5 phosphate buffer, 6.8 phosphate buffer and purified water. Excess amount of the drug or dried nanoparticles have been added to 100 ml of media maintained at 37 °C and shaken on rotary shake flask for a period of 24 hours. The samples were taken into centrifuge tube and centrifuged for about 10 minutes at 4000 rpm. The supernatant was collected and filtered through 0.22 µm nylon membrane filter, diluted with diluents (methanol and acetonitrile in 1:1 ratio) and analyzed using HPLC (waters Alliance HPLC system USA) method. Diluent is prepared by mixing buffer: acetonitrile in 65:35 ratio with sonication followed by centrifugation at 4000 rpm for about 10 minutes. Buffer is prepared by dissolving potassium dihydrogen phosphate in water followed by adding triethyl amine and adjust pH to 2.5±0.05 by phosphoric acid. The solution was filtered through 0.45 µm Durapore PVDF membrane filter and was analyzed by HPLC. The mobile phase is same as diluent. Chromatographic separation was accomplished using an150x4.6 mm, X terra RP-8, C-8, 5 µmcolumn. The mobile phase was

pumped at a flow rate of 1.0 ml/minute during analysis and maintained at a column temperature of 25 °C and detection wavelength of 230 nm.

In-Vitro Dissolution Study:

The dissolution profiles of plain drug metaxalone and dried nanoparticles was determined in a USP apparatus II in 900ml phosphate buffer pH 6.8. The dissolution media was maintained at 37±0.5°C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 400 mg. Dried nanoparticles equivalent to 400 mg was taken for analysis. At specified time intervals (15, 30, 45, 60, 90 minutes) 5ml of dissolution media were withdrawn and replaced with an equal volume of the fresh medium to maintained at 37° C to maintain a constant total volume. Samples were filtered through a 0.22µm nylon membrane filter (Millipore, Bedford, MA) and assayed for drug content in the same manner as carried out for saturation solubility.

RESULTS AND DISCUSSION

Particle Size and Polydispersity index [PDI]

High pressure homogenization was used to reduce the particle size with different surface stabilizers at 1000 bar pressure for about 120 minutes. The nanosuspension was characterized for particle size, poly dispersity index and zeta potential. The mean particle size (MPS)i.e D50 of the input metaxalone drug used to prepare the nanosuspension was 19.4 µ. Particle size and poly dispersity index has been shown in Table 2. It has been observed that the MPS of the drug in suspension at initial stages (before subjecting to HPH] for different batches reduced when compared to input metaxalone. This probably because the particle size is reduced to submicron level due to high shear homogenization process for about 30 minutes. After starting the HPH process, for every 15 minutes the sample was collected for characterization. From the results it has been observed that as the homogenization time period was increasing up to 120 minutes the particle size was reduced. After 120 minutes of homogenization process, the suspension was continued for homogenization for about 30 minutes, but there was no significant change in the particle size. Out of different combination of surface stabilizers, it has been observed that the combination of steric polymer HPMC and electrostatic polymer SLS has produced the least mean particle size of 94nm followed by HPC, PVP and PEG with mean particle size of 164 nm; 178 nm; 187 nm respectively. The MPS of PVA and Poloxamer was high after 120 minutes of homogenization and no further reduction has been observed on further homogenization.

Table 2: Mean particle size (MPS) and Polydispersity index (PDI) of the nanosuspension.

	Initial		**15 min**		**30 min**		**45 min**		**60 min**		**120 min**	
	MPS	PDI	MPS	PDI	MPS	PDI	MPS	PDI	MPS	PDI	MPS	PDI
MX-01	2245	0.9	1147	0.8	475	0.5	303	0.3	127	0.2	94	0.2
MX-02	2415	1	1434	0.6	788	0.7	399	0.69	196	0.4	178	0.4
MX-03	2687	0.94	1678	0.8	845	0.4	674	0.47	220	0.3	164	0.3
MX-04	2501	0.96	1944	0.9	450	0.9	489	0.45	345	0.6	333	0.5
MX-05	2342	1	1700	1	974	1	754	0.5	400	0.5	187	0.6
MX-06	2845	1	1821	0.9	970	0.9	668	0.6	441	0.5	437	0.3

The poly dispersity index gives fair understanding about the particle size distribution in the nanosuspension. PDI value of less than 0.5 shows narrow particle size distribution[27]. PDI also followed the same trend as the particle size. The PDI also reduced as the homogenization time was increasing. MX-01 has shown very narrow particle size distribution with PDI value of 0.2.

To convert the nanosuspension into solid dosage form, first the liquid nanosuspension has to be converted into powder form, hence nanosuspension was subjected for drying. Since the batches MX-01, MX-02, MX-03, MX-05 has shown the mean particle size less than 200 nm, these batches were subjected for drying. It is very essential that the nanoparticles retain their size and properties even after drying also, hence it is very important to evaluate which is the suitable drying method. In this study all these four batches have been subjected for drying by three methods, ie lyophilization, spray granulation and spray drying method. The dried nanoparticles also has been characterized for particle size, PDI and the ZP. Table 3 shows the mean particle size and PDI of the dried nanoparticles at initial and after storing at room temperature(RT) for about 1 month.

Table 3: Mean particle size and PDI of the dried nanoparticles of metaxalone.

	lyophilization				**Spray granulation**				**Spray drying**			
	Initial		RT (1M)		Initial		RT (1M)		Initial		RT (1M)	
	MPS	PDI	MPS	PDI	MPS	PDI	MPS	PDI	MPS	PDI	MPS	PDI
MX-01	97	0.24	165	0.38	101	0.32	111	0.35	93	0.22	99	0.28
MX-02	149	0.30	274	0.67	198	0.35	235	0.4	161	0.45	189	0.41
MX-03	157	0.26	194	0.57	165	0.36	183	0.37	155	0.34	166	0.35
MX-05	171	0.54	263	0.4	195	0.4	203	0.42	180	0.44	180	0.49

It has been observed that all the three process of drying has successfully produced the nanoparticles when dispersed in water. Dried nanoparticles produced by all the three drying process shown the MPS of less than 200 nm at initial stages (i.e. soon after drying). Out of all the batches the batch MX-01 has produced the least particle size by the three processes. And the spray drying process has shown the least particle size for all the compositions and on storage also there was no significant increase in the particle size. From the PDI data it has been observed that the nanoparticles dried by lyophilization shown broad particle size distribution on storage and for the

nanoparticles dried by spray granulation and spray drying shown the narrow particle size distribution. Out of all the batches MX-01 has shown narrow particle size distribution when dried by spray drying process.

Zeta potential [ZP]

The major challenge faced during the process of preparing the nanosuspension by different process is their stability because with the reduction in the particles size there is increase in surface area which usually lead to aggregation causing physical instability. Suitable selection of surface stabilizers would address this problem[28]. Zeta potential of the nanosuspension for all the dried nanoparticles have been generated by using Zetasizer and shown in table 4.

Table 4: Zeta potential of the nanosuspension and dried nanoparticles at initial and on stability

	Nanosuspension		**Lyophilized**		**Spray granulated**		**Spray dried**	
	Initial	RT (1M)	Initial	RT (1M)	Initial	RT (1M)	Initial	RT (1M)
MX-01	38	35	25	22	30	31	38	37
MX-02	31	22	28	19	20	24	31	24
MX-03	28	21	26	24	34	28	34	31
MX-04	25	14	-	-	-	-	-	-
MX-05	31	18	29	16	24	27	34	37
MX-06	24	11	-	-	-	-	-	-

The ZP of the drug suspension before homogenization was observed to be 27. After homogenization for about 120 minutes, it has been observed that zeta potential of the nanosuspension is increased when the steric stabilizer is HPMC, PVP, HPC and PEG. However on storage the zeta potential of the nanosuspension was reduced showing instability. Hence these nanosuspensions should be converted into powder form because in presence of water these nanoparticles try to flocculate and form larger particles. It has been observed that the zeta potential of the dried nanoparticles is reduced when compared to the nanosuspension at initial stages. This could be because of mannitol added to the nanosuspension before drying, which mask the surface of the nanoparticles. When compared to the lyophilization both spray granulation and spray drying have shown good stability, but spray dried particles have shown highest stability with high zeta potential.

From the above data, it has been observed that the combination of HPMC and SLS have formed a stable nanoparticles of metaxalone with the least particle size and highest zeta potential. With respect of different processes, both spray drying and spray granulation have obtained stable nanoparticles, but the spray dried nanoparticles have obtained highest zetapotential.

Solid state characterization

Since the drug particles have been subjected to high pressure, turbulence and shear forces during homogenization process. And further the nanosuspension is subjected to drying process the drug particles are exposed to different conditions such as high temperature and pressure. Hence it is important to study the impact of these pressure, temperature and forces on the nature of the drug particles after size reduction and drying. The dried nanoparticles have been subjected to different solid state characterization studies as described below

X-Ray Diffraction [XRD]

X-ray diffraction is used to study the phase change as a function of stress and temperature and to determine the crystallinity of the drug before and after homogenization followed by drying. Metaxalone and the dried nanoparticles prepared by different process of drying and the bulking agents/redispersants used has been characterized for XRD. Figure 1 shows the comparative XRD pattern of metaxalone, mannitol, lactose and spray granulated nanoparticles (MX-7A with mannitol and MX-7B with lactose). Metaxalone is crystalline material showing sharp intense peak at that the 2theta value of4.41; 13.34 and 17.86. Out of these three peaks, the peak at 4.41 2-theta value is the more intense peak. The redispersants used before drying also shown crystallinity**.** The XRD diffraction pattern of MX-07A and MX-07B showed the characteristic peaks at 4.572; 13.749; 17.361 and 4.521, 13.439, 17.121 respectively. Since the major intense peak of metaxalone (2-theta of 4.41) in both the compositions is differing by 0.162 and 0.111 2-theta value which is within the acceptable range as per United states pharmacopeia (USP) [i.e 2-theta value within 0.2 difference], it has been concluded that both the spray granulated compositions retained its crystallinity after size reduction and drying process also even though the other two characteristics peaks are slightly shifted.

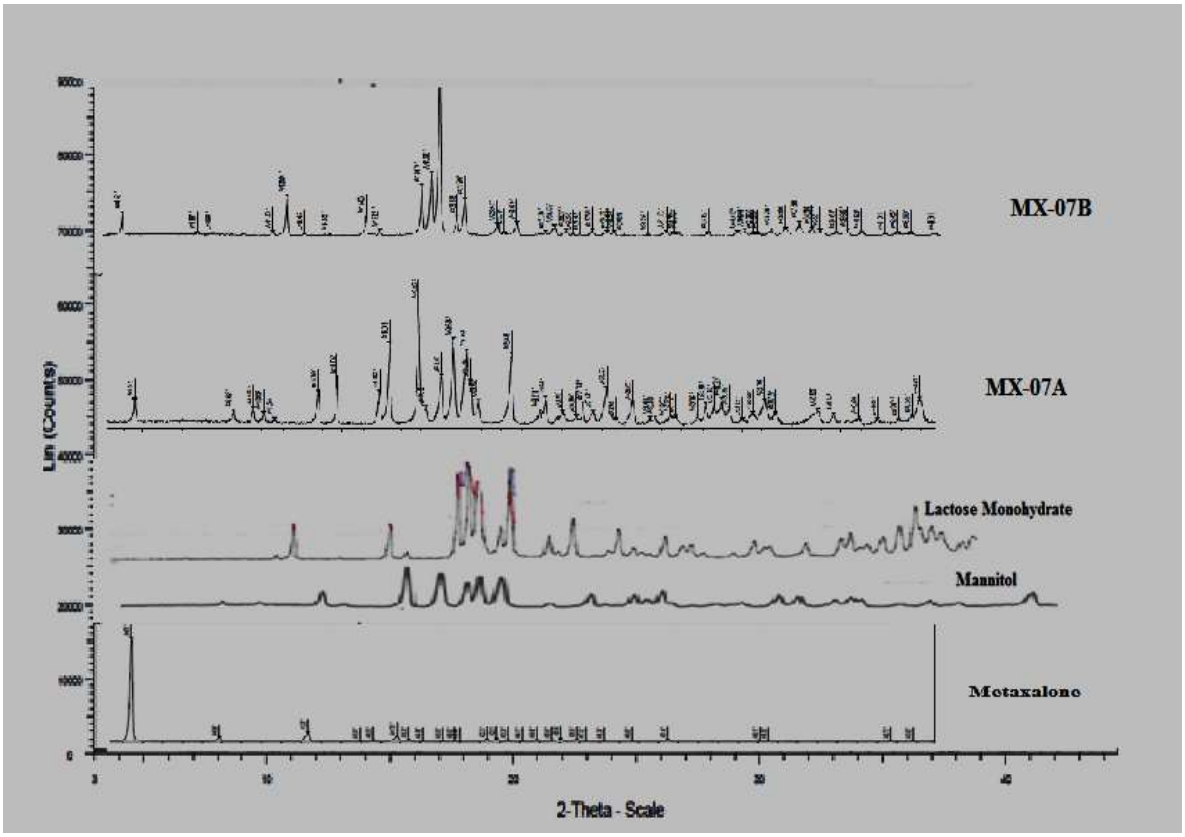

Figure 1: XRPD patterns of spray granulated samples: Metaxalone, MX-07A, MX-07B, Mannitol and Lactose.

The XRD pattern of different spray dried samples (MX-08, MX-09, MX-010, MX-011) for different compositions of metaxalone with the combination of surface stabilizers are represented in Figure 2. The spray dried nanoparticles with HPC shows the characteristics peaks of metaxalone at 4.42, 13.38, 17.882; with PVP at 4.354; 13.28, 17.809; with HPMC at 4.459, 13.383, 17.899; with PEG at 4.40, 13.359, 17.93. Since all the main characteristic peaks of metaxalone are retained in the spray dried nanoparticles, it has been concluded that metaxalone has retained the crystallinity after spray drying process.

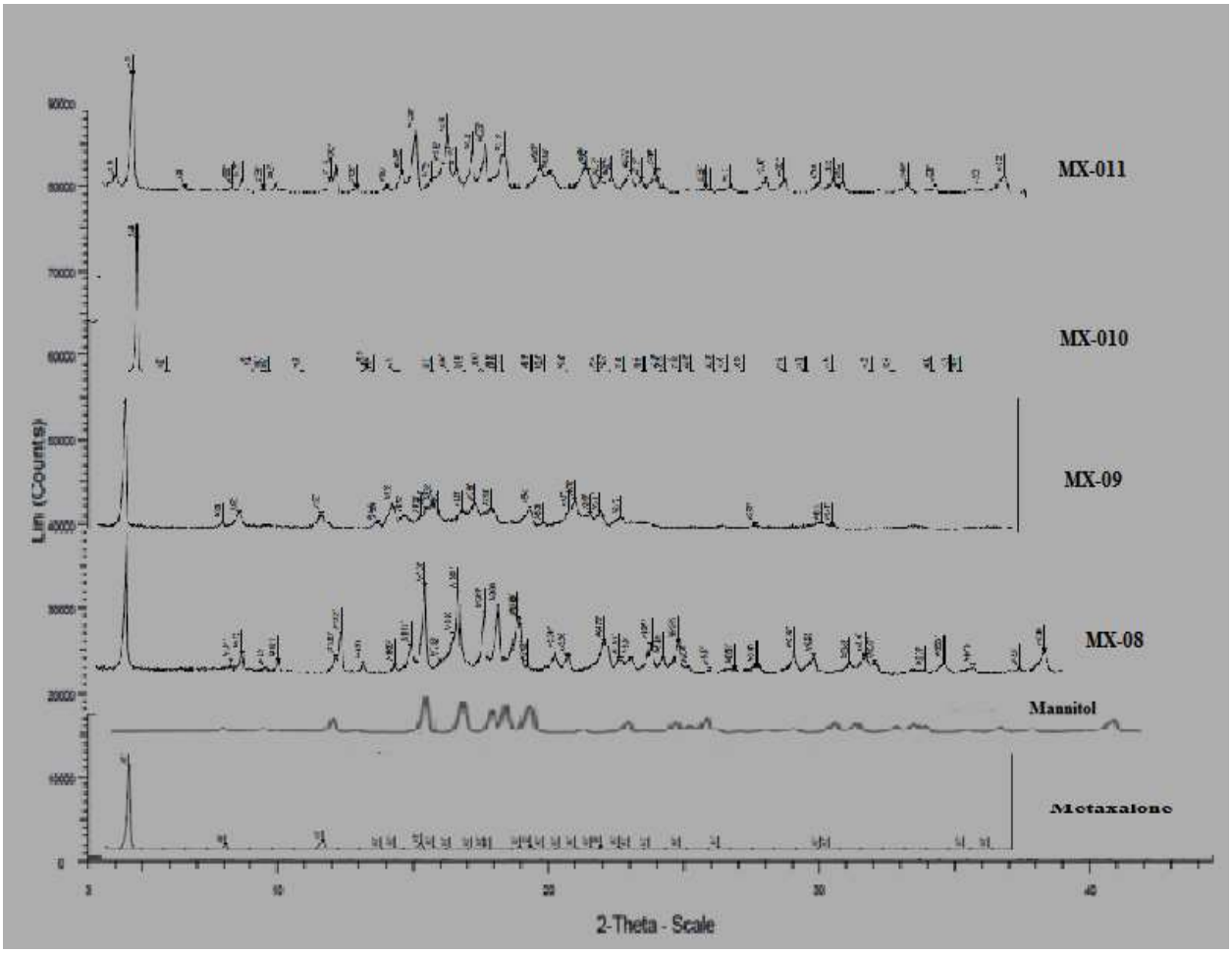

Figure 2 Shows the XRD patterns of spray dried samples in comparison with metaxalone and Mannitol.

Similarly the lyophilized nanoparticles have been evaluated for the change in the crystallinity after lyophilization process by XRD [Figure 3]. All the compositions after lyophilization has retained the characteristic peaks; MX-012 has shown metaxalone characteristic peaks 2-theta value of 4.435, 13.375, 17.881; MX-013 shows at 4.488, 13.435, 17.981; MX-014 shows at 4.452, 13.380, 17.896 and MX-015 shows at 4.451, 13.32, 17.87 respectively. Hence from the XRD analysis it has been concluded that the metaxalone after particle size reduction and drying its nanosuspension by three different processes also retained its crystallinity. The additional peaks are contributed by excipients such as mannitol and lactose.

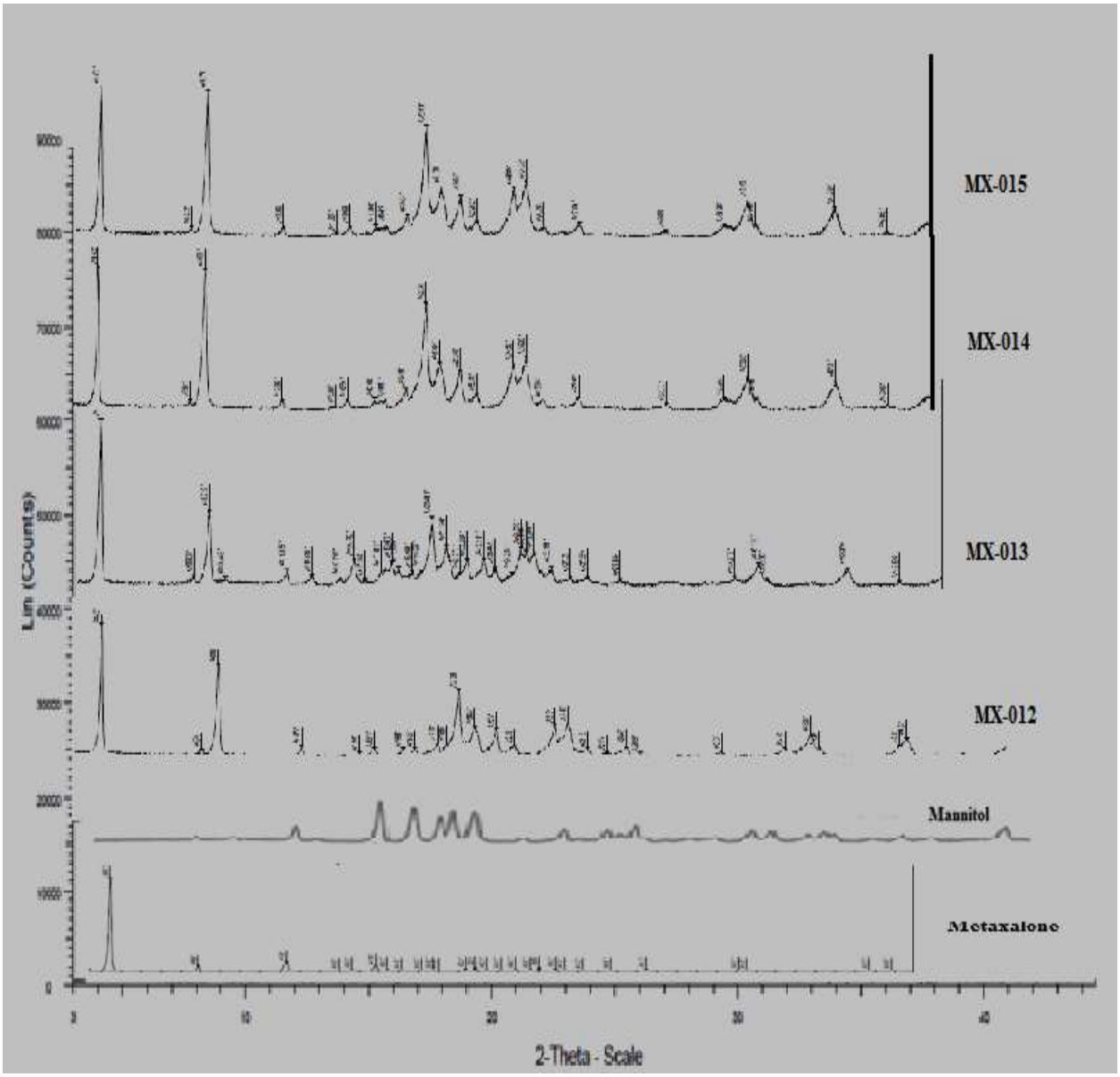

Figure 3: XRPD patterns of lyophilized samples: Metaxalone, MX-012, MX-13, MX-014, MX-015, Mannitol.

Differential Scanning Calorimetry [DSC] Analysis:

In order to observe effect of thermal changes on the metaxalone nanoparticles, dried nanoparticles are subjected to DSC analysis. The DSC plots obtained for metaxalone and nanoparticles prepared by drying three different process ie spray granulation (MX-07A & MX-07B), spray dried (MX-08, MX-09, MX-10, MX-011) and lyophilized (MX-012, MX-013, MX-14, MX-015).

The DSC of metaxalone showed a single sharp endotherm at 122.23 °C. The endotherm peaks of the excipients used are reported in literature for example mannitol show endotherm at 168.4°C[29] PEG between 60-65 °C[30] corresponding to its melting point. Pure povidone shows glass transition temperature at 168°C and broad endotherm at 100 °C shows loss of water because of extreme

hygroscopic nature of PVP[31]. HPMC shows broad endotherm at 72 °C, HPC shows broad small endotherm peak at 83.9 °C[32] Lactose monohydrate shows a sharp endotherm at 140°C due to loss of monohydrate and the melting endotherm around 220 °C. In contrast, after spray drying, lactose became amorphous as evidenced by the exothermic peaks and this was in agreement with the previous report[33.] It has been observed that the characteristic endotherm peak of the metaxalone was retained after drying by three processes. This further confirms the XRD interpretation that irrespective of any of the drying methods used, metaxalone retained its crystallinity.

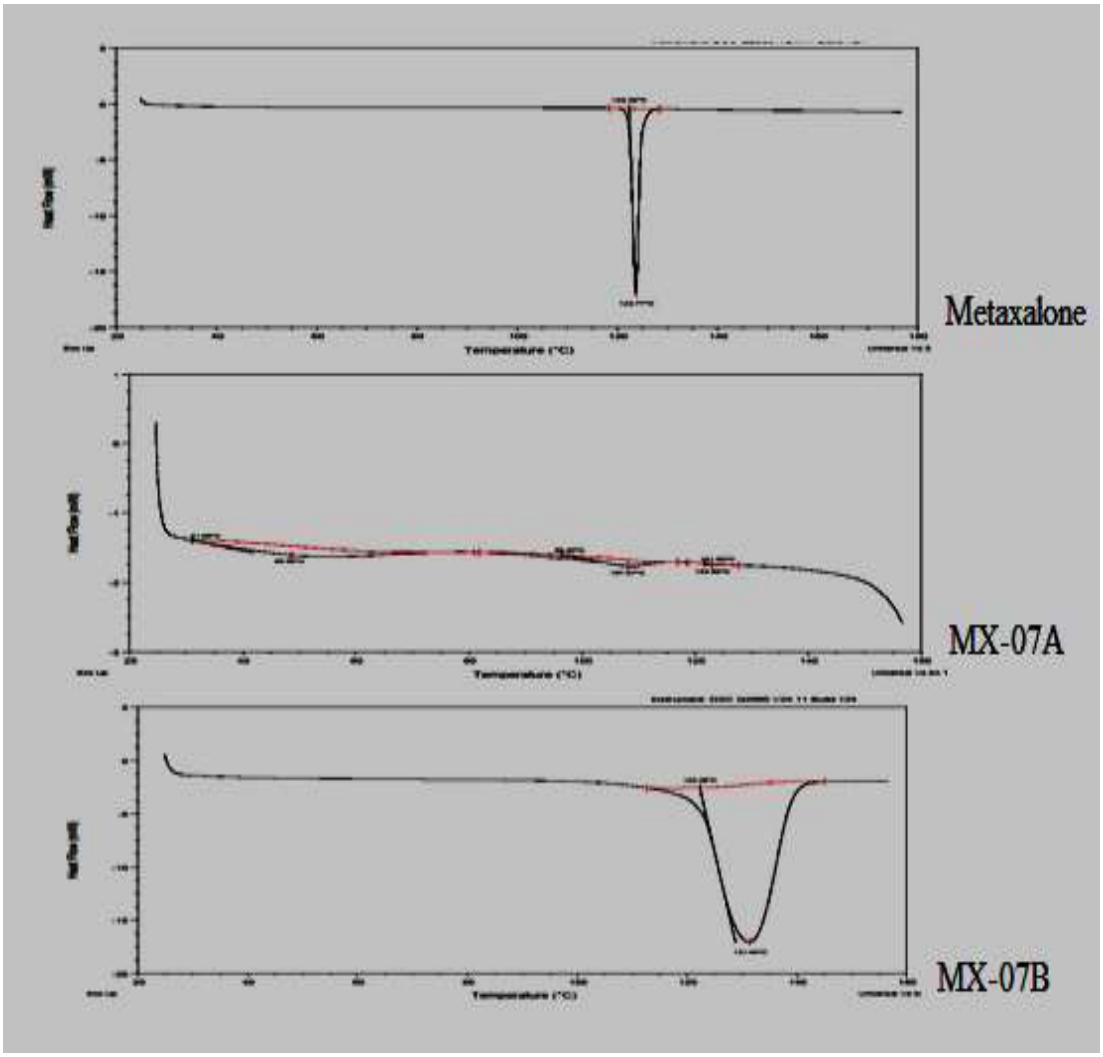

Figure 4: DSC of metaxalone and spray granulated nanoparticles.

Figure 4 shows the comparison of two different compositions of the metaxalone nanoparticles, one with mannitol (MX-07A) and other with lactose (MX-07B) dried by spray granulation technique. From their DSC plots it has been observed that both the compositions retain the metaxalone endotherm peak. In MX-07A small endotherm peak corresponding to drug is shown at 122.9 °C and broad endotherm at 160 °C corresponding to mannitol and small endotherm at 107°C may be

due to loss of water in SLS. MX- 07B the drug peak is broader and observed at higher temperature of 131.44 °C, this is probably because of the interaction of drug with lactose and merge of drug peak with the dehydration peak of lactose monohydrate.

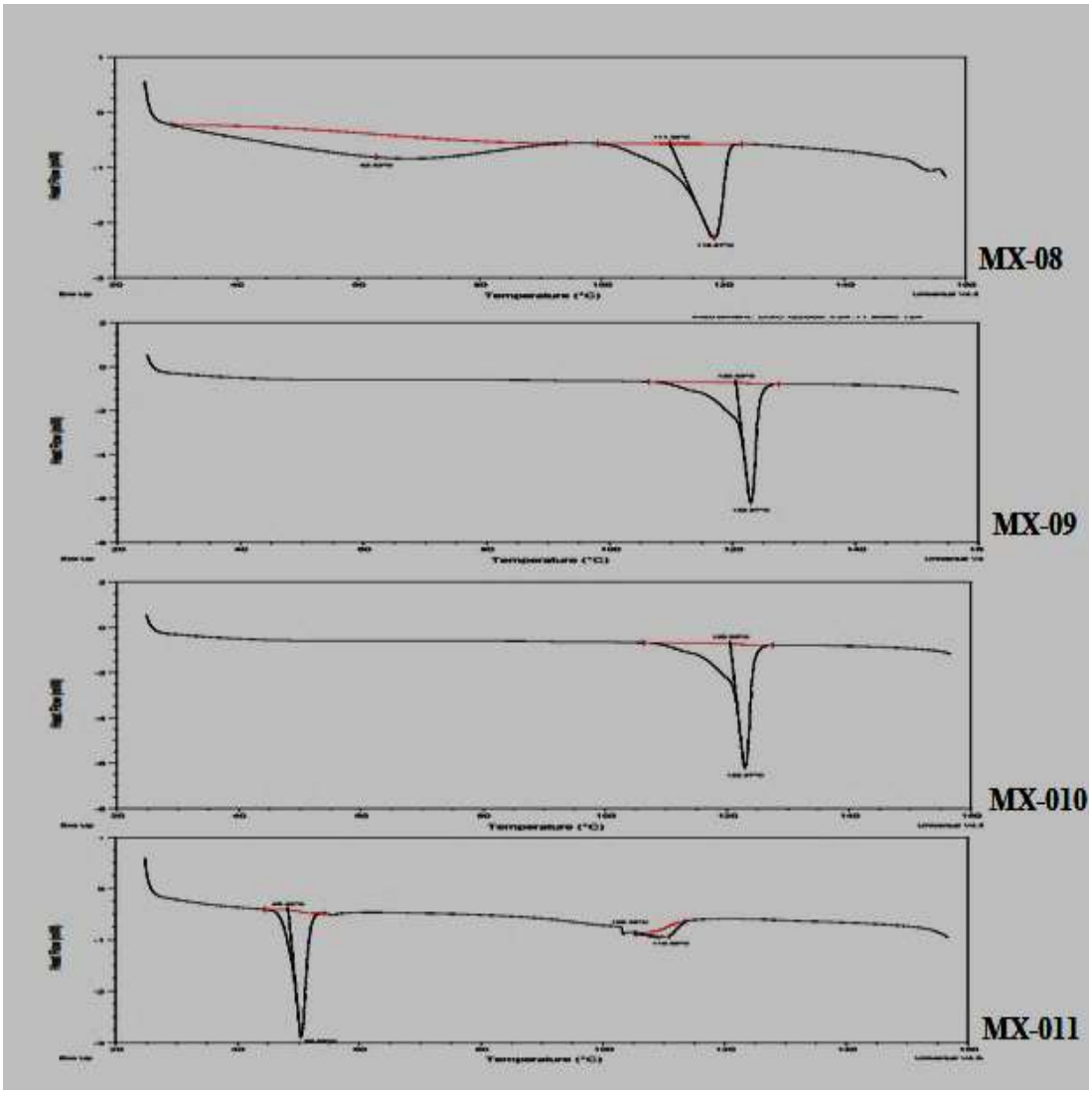

Figure 5: DSC of metaxalone and spray dried nanoparticles.

From the DSC of compositions of the spray dried samples (Figure 5) and the lyophilized samples (Figure 6) four different primary stabilizers such as PVP, HPMC, HPC and PEG are compared. From the spray dried samples it has been observed that the metaxalone related endotherm peak is retained in almost all the formulation, but it is slightly shifted when the stabilizer is PVP, HPC and PEG, this probably due to the interaction of these stabilizers with the drug metaxalone. However when the stabilizer is HPMC, the drug peak is shown at 122°C showing its compatibility with the drug.

From the DSC plot of the lyophilized samples, it has been observed that all the compositions have shown the drug peak. The composition with HPMC surface stabilizer has shown endotherm at 121.89 which is close to the metaxalone reference peak, but the other compositions have shown slight shift in the endotherm peaks this is probably due to chemical interaction of drug with excipients used. From the above results it has been observed that among the surface stabilizers evaluated, HPMC appears to the suitable redispersant for preparing the metaxalone nanoparticles.

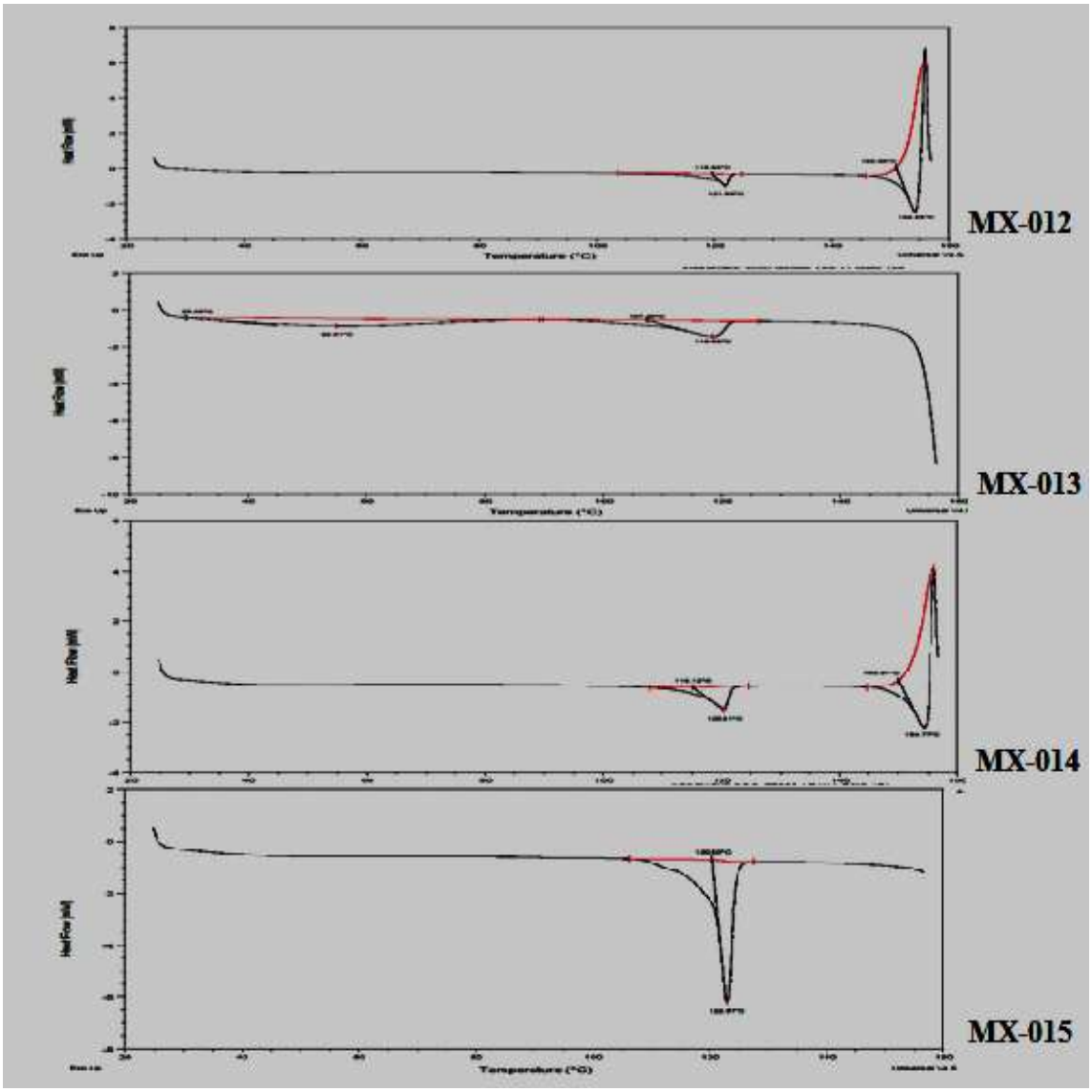

Figure 6: DSC of metaxalone and lyophilized nanoparticles.

FTIR spectroscopy:

Infrared (IR) provide structural information on a molecular level. The spectra are based on unique molecular vibrations that occur within a compound; hence the different fundamental molecular vibrations of differing polymorphic forms will give each its own unique fingerprint spectrum from

which it may be identified. Any change in the structure of the drug metaxalone due to interaction between metaxalone and excipients has been studied.

Metaxalone shows the characteristic IR peaks 3283 cm-1 due to N-H stretch in the secondary amine, 1736 cm-1 due to C=O stretch due to carbonyl group, peaks between 2880-2950 cm-1 due to C-H stretch in alkanes; between 1450-1480 cm-1 due to C-H bend alkanes and characteristic peaks between 1000-650 cm-1 due to C-H bend in alkenes and between 1320-1000 due to C-O stretch in ether. These characteristic peaks are shown in Figure 7 in comparison with different dried nanoparticles. .From the comparative IR spectrums, it has been identified that metaxalone shows a sharp peak at 3283 cm-1. While the same peak in the formulation MX-07A is slightly broader and in MX-07B is broadened, this probably may be because of the hydrogen bond interaction between the amine and the carbonyl group.

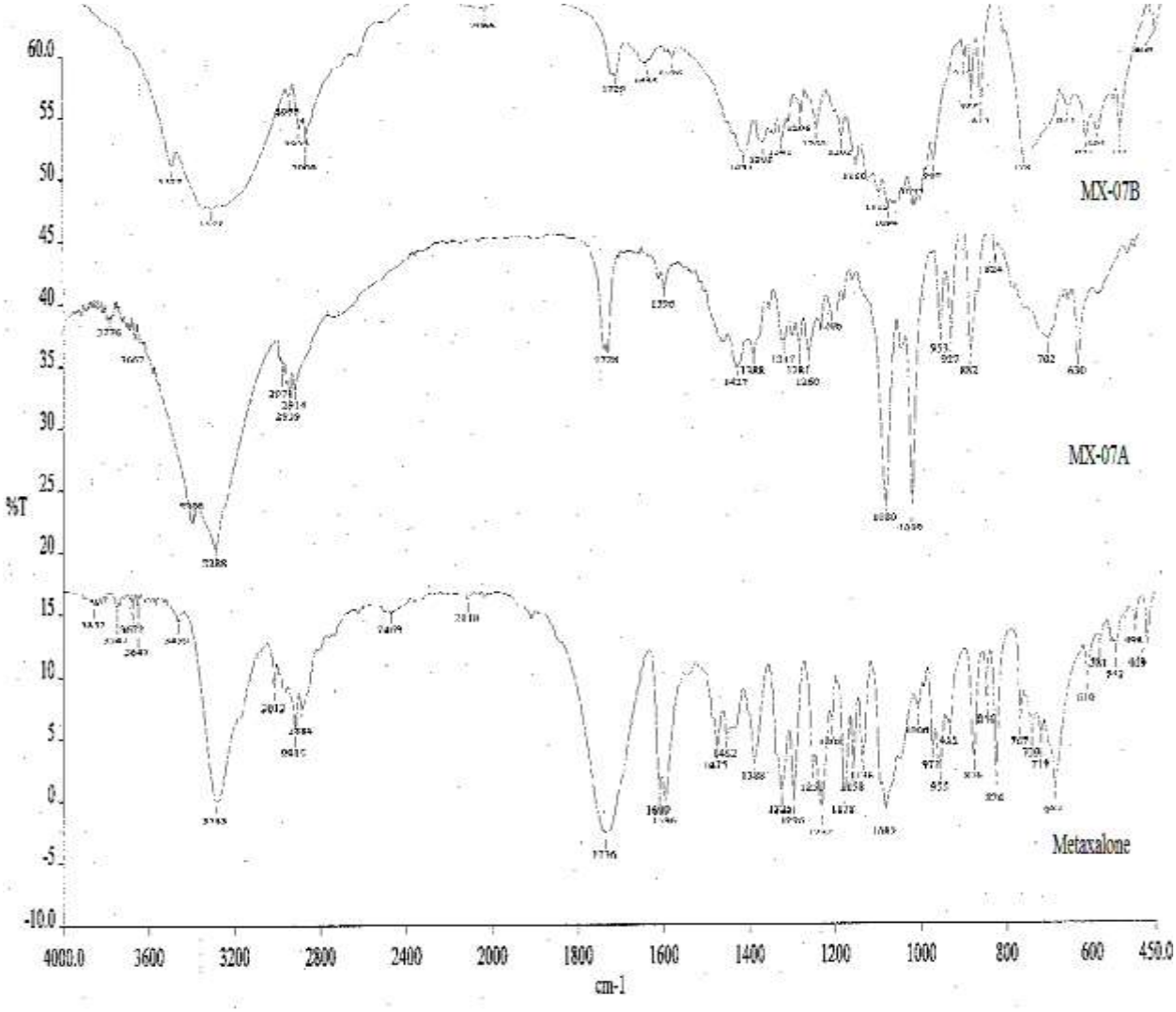

Figure 7: Comparative IR spectra of metaxalone with compositions dried by spray granulation MX-07A and MX-07B.

The IR spectra of the metaxalone compositions dried by spray drying and lyophilization process has been illustrated in Figure 8 and Figure 9 respectively. It has been clearly observed that all the four compositions are overlaying over each other and when compared with the active, it has been observed that peak at N-H stretch is broadened when compared to the pure API, probably arising due to inter or intra molecular hydrogen bonding **.**

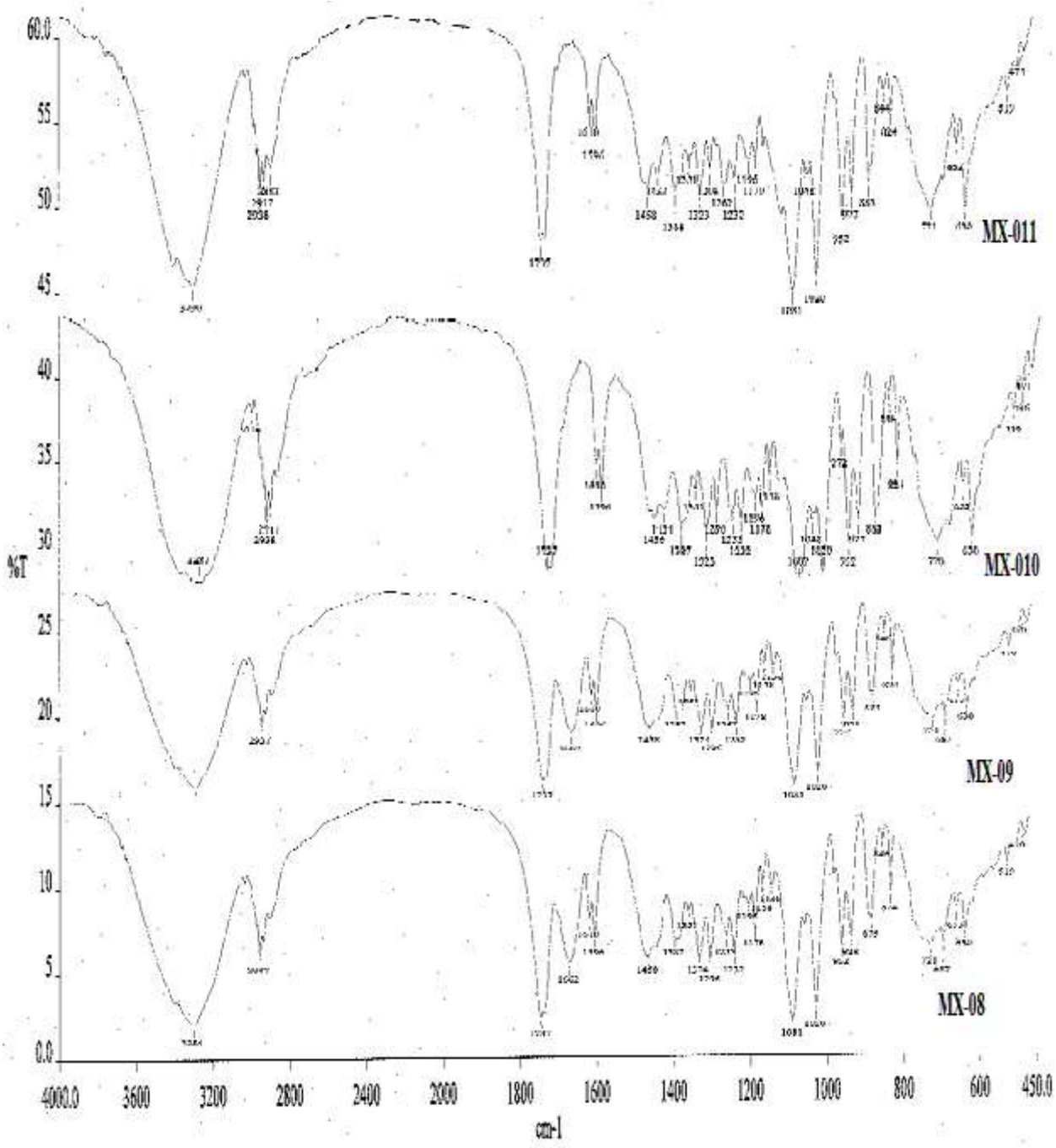

Figure 8: Comparative IR spectra of metaxalone with compositions dried by spray drying MX-08, MX-09, MX-010 and MX-011.

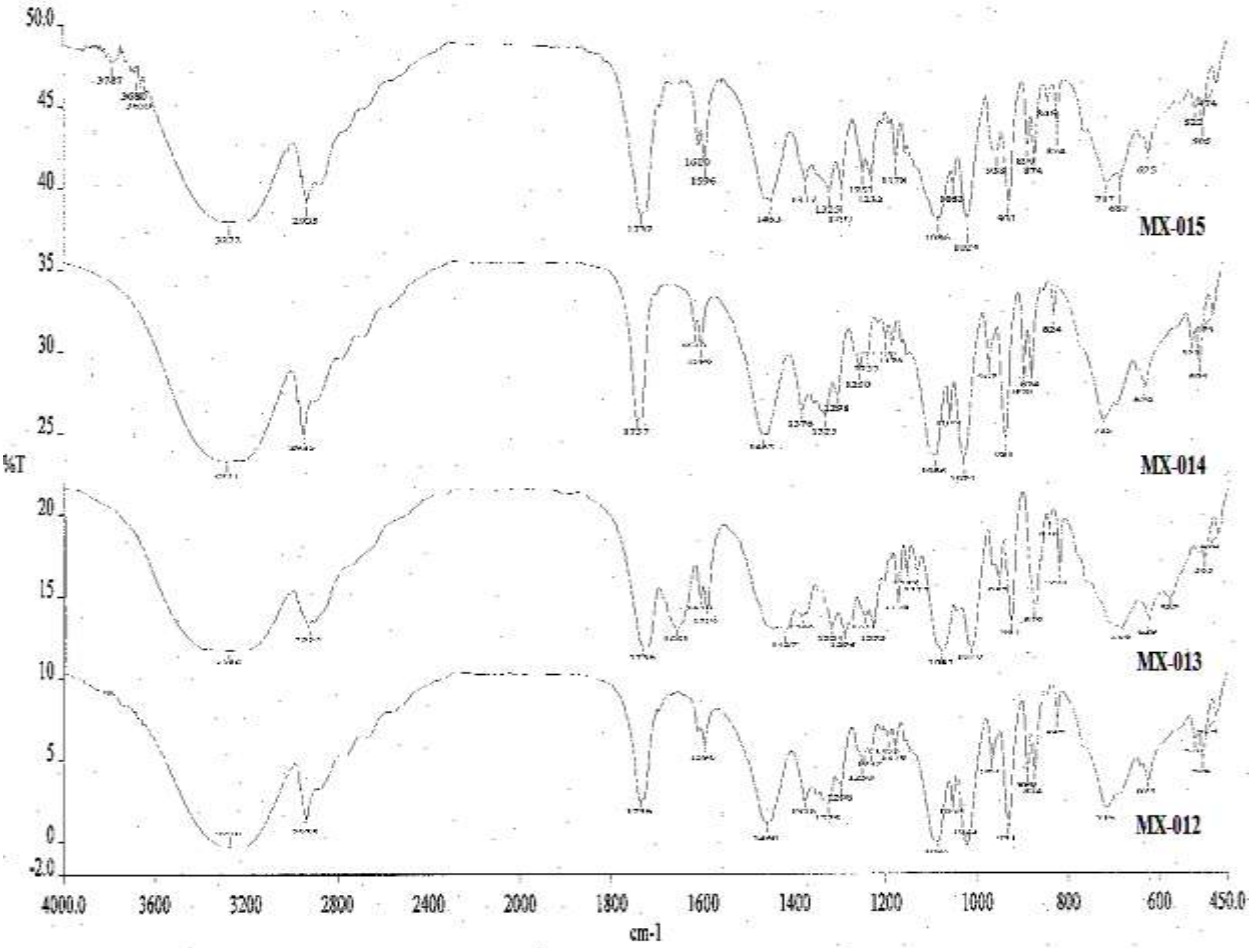

Figure 9: Comparative IR spectra of metaxalone with compositions dried by lyophilization MX-12, MX-13, MX-014 and MX-015.

From all the above infra red spectra, it has been observed that there is no appreciable change in the positions of the characteristic bands of the drug either in dried nanoparticles when dried by any of the three processes. Since there is no change in the nature and position of the bands in the dried nanoparticles except broadening of the some of the peaks, it can be concluded that the drug maintains its identity without going any chemical interaction with the excipients used.

Scanning electron microscopy

During the process of preparing the nanoparticles, the drug particle size is reduced with impact of different pressure or forces which may also result in change of the shape, surface etc. Electron microscopy is one of the powerful methods for determining attributes of the nanoparticles. It also helps in validating the reliability of particle size characterization techniques such as laser diffraction or dynamic light scattering. SEM produces the three dimensional pictures of the nanoparticles which will enable us to study about the morphology or surface of the nanoparticles.

Pure drug metaxalone, nanosuspension and its dried nanoparticles prepared by three different drying processes were been analyzed for surface appearance, particle size and shape by scanning electron microscopy. As shown in Figure 10, pure metaxalone showed rectangular shaped like crystalline particles with particle size ranging from 10 to 100 microns. After the particle size reduction the drug particles changed into small irregular particles showing the reduction of particle size.

Figure 10, 11 and 12 shows the SEM pictures of spray granulated (with mannitol and lactose), spray dried and lyophilized nanoparticles respectively. When we compare the SEM images of 7A and 7B in Figure 10, it has been observed that the particles using mannitol are almost close to spherical in shape with rough surface whereas the 7B using lactose is showing the irregular shaped particles as clusters or loose aggregates of the particles. Hence mannitol shows better properties in term of forming the spherical particles, hence subsequent batches have been prepared by using mannitol as redispersant.

The SEM images of nanoparticles obtained by spray drying are shown in Figure 11. The nanoparticles obtained with HPC(MX-08), HPMC(MX-010)as primary stabilizer have shown perfect spherical globule shaped nanoparticles with smooth surface but the nanoparticle prepared by using the PVP (MX-09) and PEG (MX-011) as stabilizers have shown large aggregates of smaller particle size owing to its instability. The nanoparticles prepared by lyophilization particles using HPMC (MX-012) and PVP (MX-013) have been characterized for SEM as shown in figure 12. These images shows loose aggregate of needle shaped particles.

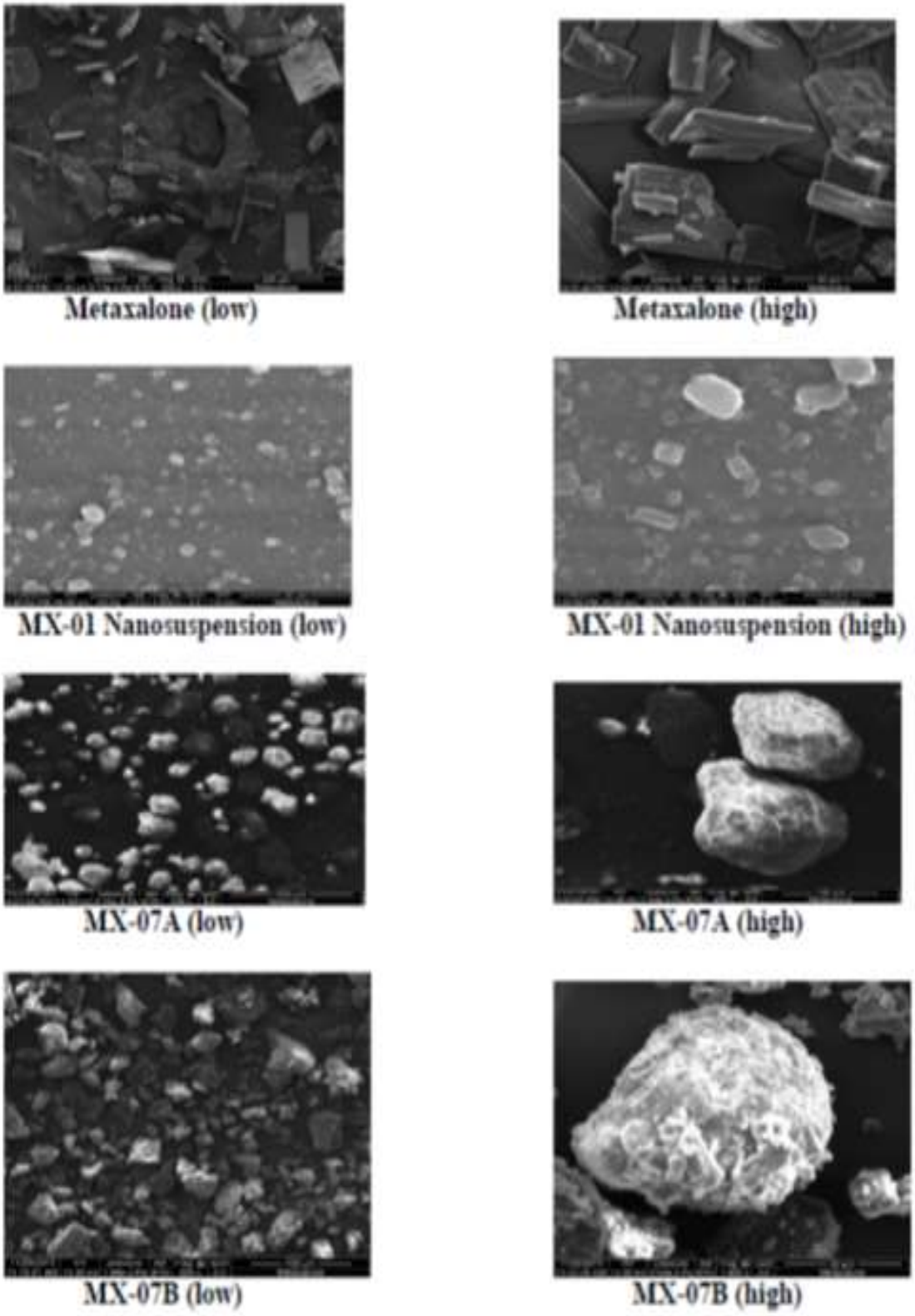

Figure 10: SEM pictures of the metaxalone, nanosuspension (MX-01) and the nanoparticles dried by spray granulation with mannitol (MX-07A) and lactose (MX-07B) as redispersant at low and high magnification.

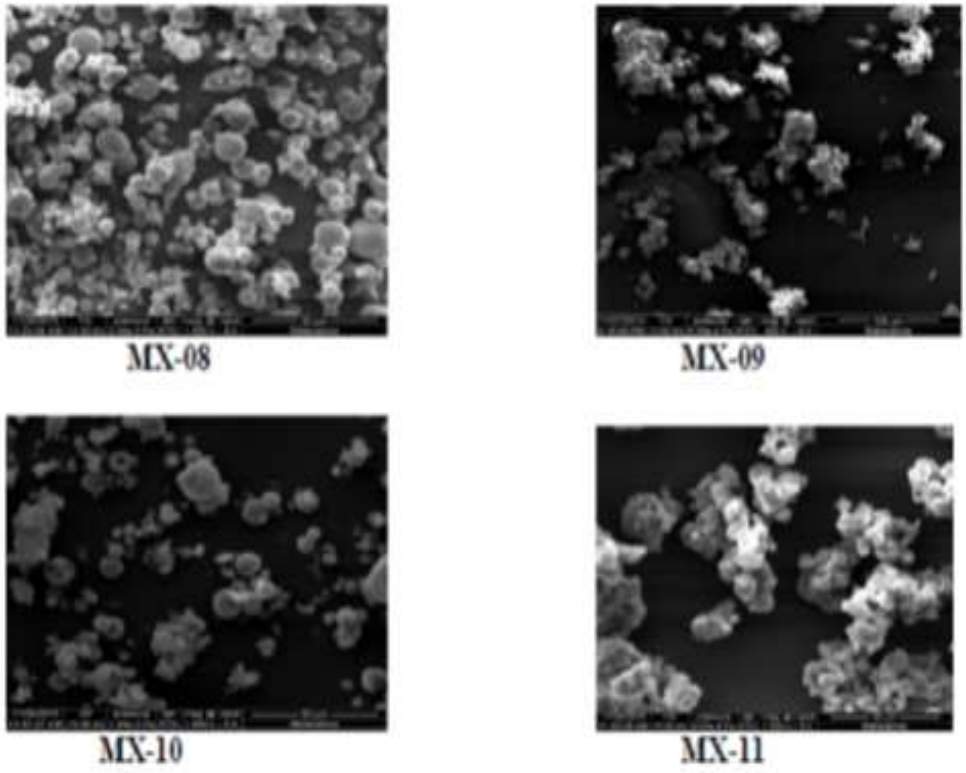

Figure 11: SEM pictures of the nanoparticles dried by spray drying process

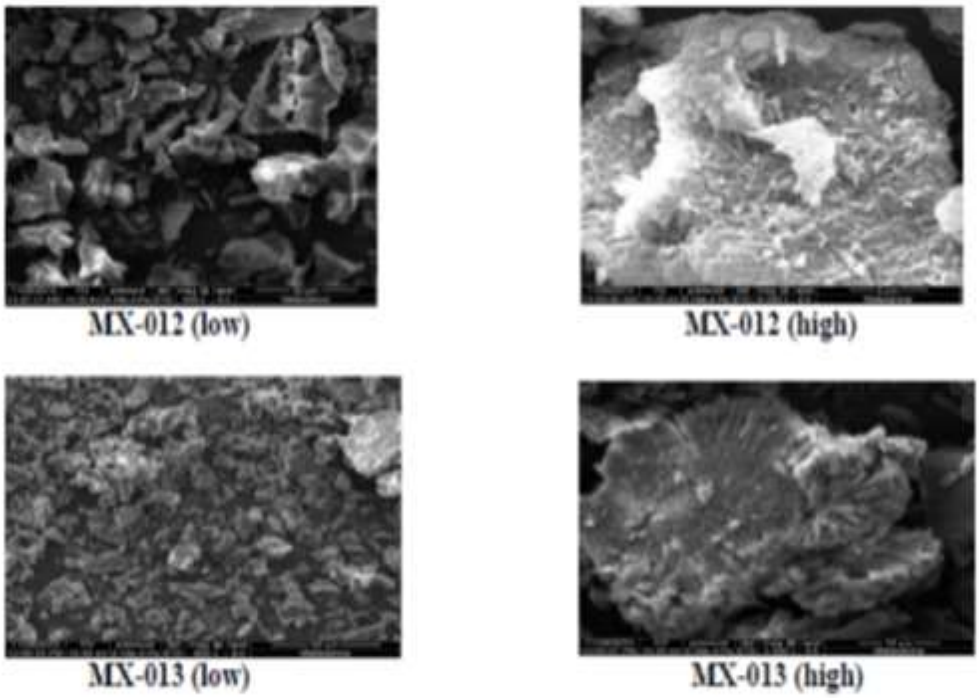

Figure 12: SEM pictures of the nanoparticles dried by lyophilization using HPMC (MX-012) and PVP (MX-013) using mannitol as redispersant at low and high magnification.

When we compare the different techniques for drying the nanoparticles for the composition using HPMC and SLS as surface stabilizer using mannitol as redispersant ie MX-07A, MX-010 and MX-012 it has been observed that the spray drying and spray drying was successful in producing the smooth shaped spherical particles. Lyophilization was not successful in obtaining desired particle shape and surface. Apart from HPMC, HPC also showed the smooth surface when spray dried. The spray dried nanoparticles have shown deposition of redispersant on the nanoparticles and spray granulated nanoparticles have shown the layering of the metaxalone nanoparticles onto the surface of redispersant used as the substrate, thus creating the hydrophilic microenvironment favorable to increase the solubility of the poorly soluble drugs.

Flow properties of dried nanoparticles

Flow properties are usually lessened as their size decreases. Flow properties of dried nanoparticles prepared by three processes have been determined. Figure 13 shows the data generated for dried nanoparticles such as angle of repose, Carr's Index and Hausner ratio compared with the drug.

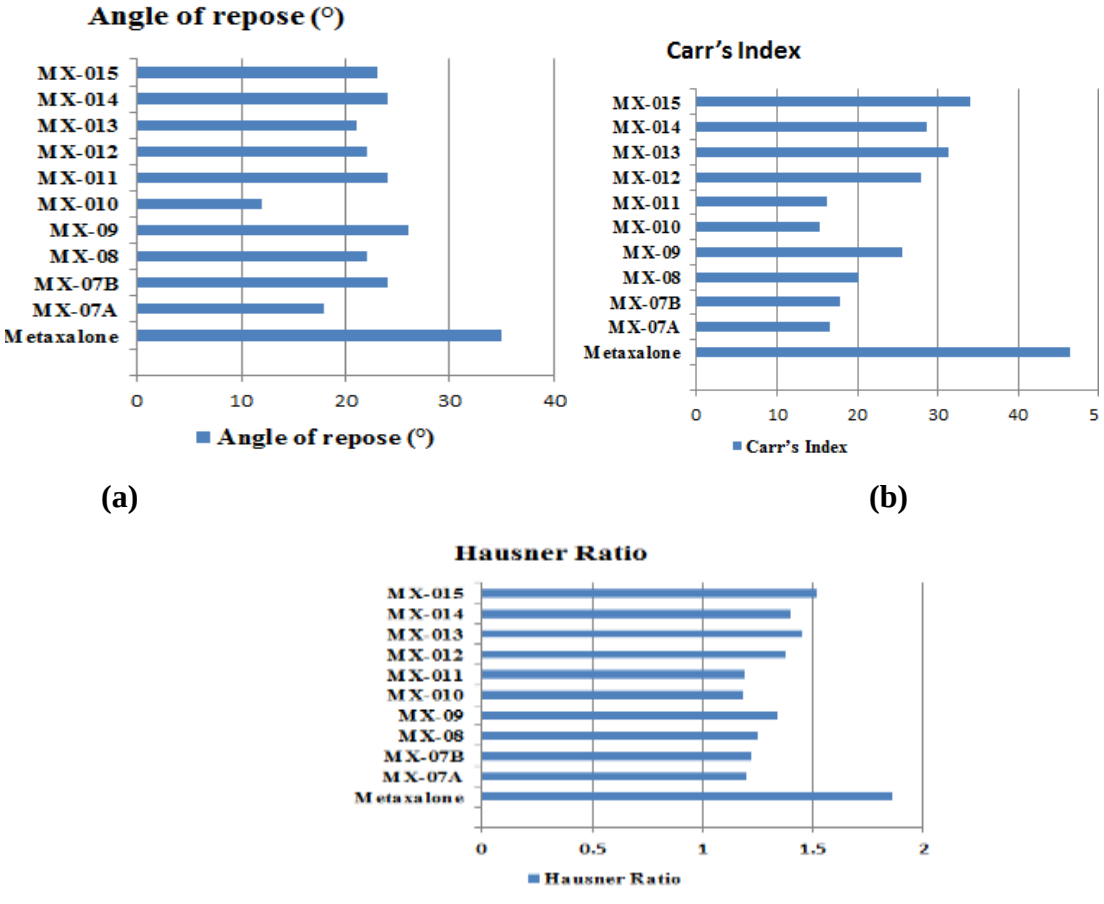

Figure 13: Flow properties of dried nanoparticles obtained by different drying process a) Angle of repose b) Carr's compressibility Index c) Hausner ratio.

From figure 13, it has been observed that pure drug metaxalone as such exhibited very poor flow properties with angle of repose of 32 °; Carr's index of 46 and hausner ratio of 1.8. After drying by all the three processes, the flow properties of dried metaxalone nanoparticles have been significantly improved however when compared to pure drug. Among the spray granulated nanoparticles, the nanoparticles obtained by lactose has shown less flow properties when compared to that of mannitol, this could be because of the monohydrate of lactose resulting in the formation of aggregates and contributing to poor flow.

In the spray dried nanoparticles, the improved flow properties has been observed for MX-010 with angle of repose, Carr's index, hausner ratio of 12, 15 and 1.18 respectively. This is because of good adsorption of the surface stabilizers over the metaxalone nanoparticles during the spray drying process resulting in the spherical particles. All the lyophilized samples have exhibited very poor flow properties.

Saturation Solubility:

The aqueous solubility is important because most of the gastrointestinal tract is aqueous environment. Maintenance of the supersaturation condition in areas of the gastrointestinal tract where drug absorption occurs can provide increased bioavailability, for an improved therapeutic effect. The saturation solubility of the metaxalone nanoparticles prepared y using HPMC, PVP, HPC and PEG and dried by lyophilization, spray granulation and spray drying was evaluated in 0.1 N HCl, acetate buffer pH 4.5, phosphate buffer pH 6.8, purified water at physiological temperature (37 °C) and compared with the metaxalone drug. The saturation solubility of drug nanoparticles was significantly higher than metaxalone unmilled microparticles at all pH conditions. Solubility of pure drug in 0.1NHCl (0.191 mg/ml), 4.5 phosphate buffer (0.192 mgml); 6.8 phosphate buffer 0.183 mg/ml) and in water (0.184 mg/ml). This data indicates that there is slight difference in the solubility of metaxalone in acidic media and water, but the difference is not significant to tell that the drug exhibits pH dependent solubility. Hence it is concluded that the drug exhibits pH independent solubility. Further from the solubility data of the dried nanoparticles it has been observed that, saturation solubility of metaxalone in nanoparticles has been significantly improved by particle size reduction followed by drying (spray drying and spray granulation) when compared to the pure drug metaxalone. In all the medias the composition with HPMC and SLS (MX-07A and MX-010) as surface stabilizers has shown significant improvement in solubility. The aqueous solubility of MX-07A and MX-010 was 0.72 (3.9 folds) and 0.59 mg/ml (3.2 folds) respectively. Subsequently the compositions prepared by HPC and SLS also shown good improvement in the

solubility. The increase in the solubility is attributed to the lowest particle size followed by creating the hydrophilic microenvironment by drying the nanosuspension.

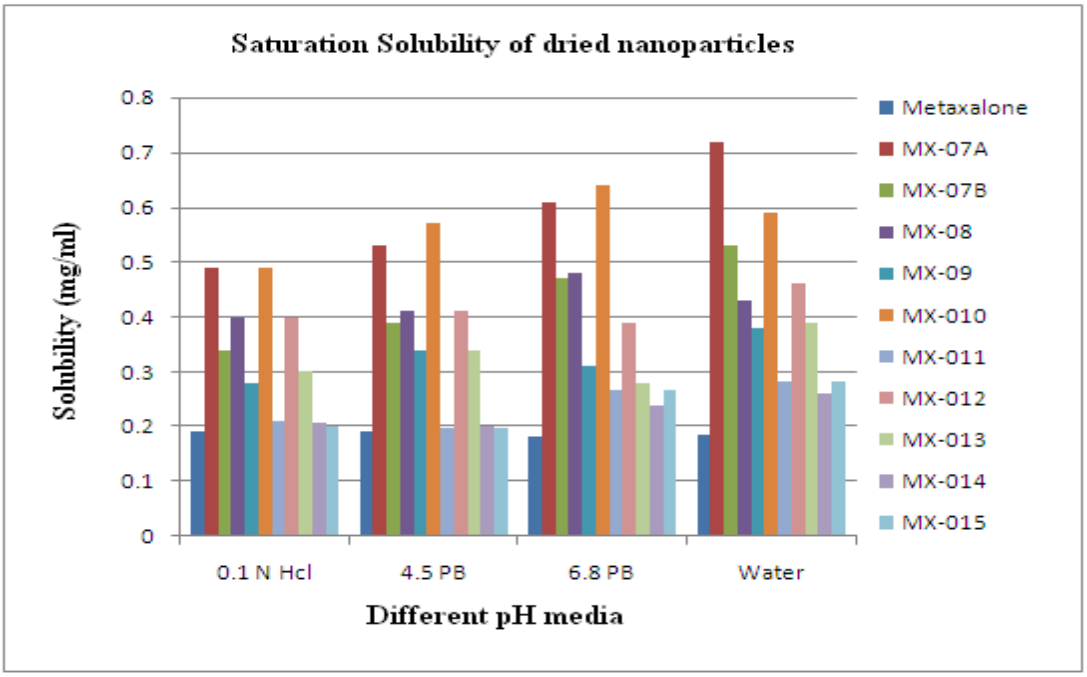

Figure 14 shows the saturation solubility of the dried nanoparticles

In-vitro dissolution

The profiles shown in Figure 15 illustrated the dissolution rates of drug metaxalone and the dried nanoparticles obtained by three different drying processes. It has been observed that the nanosized drug particles shown increase in the rate and extent of dissolution in comparison with the pure drug especially in initial stage of dissolution ie within 15 minutes; It has found that only 29% of drug metaxalone was dissolved whereas nanoparticles by all processes has shown improvement in rate and extent of dissolution with more than 60 % dissolved within 1 hour when dried spray drying or spray granulation. The rate of dissolution by spray drying or spray granulation has shown 5 folds increase whereas lyophilization shown 2-3 folds increase in the dissolution rate when compared to pure drug..

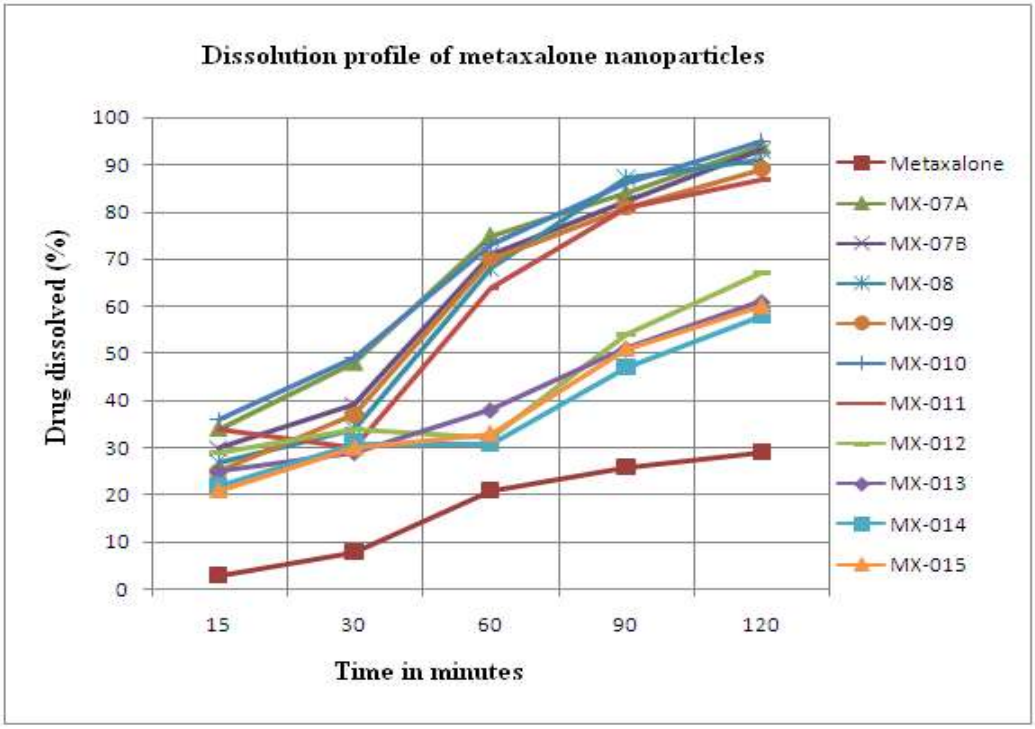

Figure 15 shows the dissolution profile of the dried nanoparticles in comparison with drug

CONCLUSION

The solubility and the dissolution of the BCS class II drug metaxalone was enhanced by preparing the stable nanoparticles by high pressure homogenization and converting into dry powder form. Among different surface stabilizers evaluated the combination of HPMC and SLS has produced stable nanoparticles. Out of three techniques evaluated spray drying and spray granulation were successful in producing stable nanoparticles with significant increase in saturation solubility and dissolution rate. Mannitol has shown better redispersability than lactose. Stability of dried nanoparticles were evaluated by XRD, DSC, FTIR, SEM. XRD and DSC have shown retention of the drug crystalline nature thus contributing to the long term stability. Reduction of the particle size have shown dramatic increase in the rate and extent of drug dissolution due to increase in the surface area and by creating the hydrophilic microenvironment around the drug nanoparticles thus these nanoparticles may be a explored as a potential candidate for oral administration with quick onset of action for relief of acute painful musculoskeletal conditions

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