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DESIGN AND CHARACTERIZATION OF METFORMIN NANOSUSPENSION BY NANOPRECIPITATION METHOD

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ABSTRACT

Present study was to prepare Metformin nanosuspension and characterization of nanosuspension and the main aim behind to formulate was to increase the permeability of the drug as it belongs to BCS class III. Metformin was selected and was found to increase the *in vitro* diffusion and partition coefficient of the drug. It was prepared by Nanoprecipitation method with high speed homogenizer by using different ratio of stabilizer: polymer. The formulation with the high stabilizer: polymer ratio shows the reduced particle size, more diffusion rate, high % production yield and % entrapment efficiency. The formulations were analysed for FTIR, DSC, PDI, Zeta potential, XRD, SEM and stability studies. The average particle size of optimized batch of nanosuspension was found to be 399nm.

KEYWORDS: Nanosuspension, Metformin, Nanoprecipitation, High speed homogenizer, Partition coefficient.

INTRODUCTION

Pharmaceutical scientists always face challenges for producing a formulation because many of the new chemical complexes that are been discovered are mostly poorly water-soluble drugs[1] thus low saturation solubility and permeability leads to poor bioavailability; this problem is more severe for the drugs belonging to BCS class III. For most of the drugs that are existing in the market are Synthetic molecule that are of weakly acidic or basic in nature demonstrate narrow absorption window which when are given orally.[2] The poor absorption

of the molecule in the gastrointestinal tract is due to the various physiological pH of the GI tract thus a poor permeation leads to poor bioavailability of the drug. If the drug is having poor drug solubility and permeability it will not get absorbed through gastrointestinal tract and will not produce desired pharmacological action in the body.[3]

To get rid of such problems numerous conventional approaches are adopted like use of co-solvents, saltformation, micronization, nano-precipitation, dispersion systems etc. but they have limitation in increasing the solubility and permeability of the drug thus some novel approaches like microemulsion, liposomes, dispersion of solids and inclusion complexes with β-cyclodextrin have been done this shows a stable and effective formulation development. There are many difficulties in such types of techniques and also lack of worldwide applicability to all drugs.[4] Nanotechnology has a vast type of application in the pharmaceutical industries and has gain many attentions in various fields. It is also used in oral, parenteral, transdermal, transmucosal, ocular and pulmonary drug delivery routes over decades. The Nano engineering has been developed and has various pharmaceutical applications.[5]

Nanosuspension has various advantages like increased rate of absorption, increased oral bioavailability, reduction in required dose, reduction in fed/fasted variability, rapid onset of action, suitability for hydrophilic drugs, dose reduction, high drug loading capacity are possible.[5] Nanoprecipitation is simple, robust and easy in scaling up. In this technique combination of two stabilizers were used and one coating polymer was used. Two stabilizers are required for proper stabilization of the system. Long chain water insoluble polymer was used for coating the nanoparticle. Metformin is a well-known drug used as first line treatment in type II diabetes. It is a biguanide used as Anti-Diabetic, *Antihyperglycemic* agent belongs to BCS class III. The chemical name of metformin is 1,1-Dimethyl biguanide. Metformin inhibits the respiratory chain complex 1, which supresses the ATP levels and increases the AMP. Increase in the AMP levels activates AMPK Adenosine Monophosphate-Activated Protein Kinase which inhibit glucose production.[6,7]

MATERIAL

Metformin was purchased from Sigma Aldrich Mumbai, Maharashtra. Tween 80 and Polyvinyl Pyrrolidone was purchased from Research Lab fine chem, Mumbai, Maharashtra, Polyvinyl alcohol was purchased from Evonik, Mumbai, Maharashtra and Ethanol was

purchased from Pallav Chemicals and solvent Pvt Ltd, Mumbai, Maharashtra. All the excipients and solvents used were of analytical grade.

METHOD

Nanosuspensions of metformin were produced using nanoprecipitation method with combination of high speed homogenization. It involves 3 steps.

In step I the two different stabilizers were used. Polyvinyl alcohol was dissolved in distilled water (10ml) by heating at 70ºC for 15min and Tween 80 was dissolved in distilled water (10ml). Thus, by taking both the stabilizer to 1:1 ratio; stabilizer solution was made to stabilize particles.[8] In step II the drug was dissolved in ethanol and later injected into the stabilizer solution drop wise with the help of syringe; which was kept under high speed ultra turrax for homogenization at 5000rpm for 45min.[9] In step III Polyvinyl Pyrrolidone 80mg was dissolved in distilled water(10ml), and this solution was added to homogenized drug-stabilizer mixture to arrest the growth of precipitating particles in the solution at different concentration.[8]

Optimization of Formulation

Polymer**,** stabilizer ratio was optimised by using 2^3 factorial design and was investigated to prepare the nanosuspension formulation.

Table No. 1: Optimization of NS formulation.

Formulation No.	Drug (mg)	Tween 80 (mg)	PVA (mg)	PVP (ml)	Ethanol (ml)
NS1	200	160	80	5	15
NS2	200	320	80	5	15
NS3	200	160	160	5	15
NS4	200	320	160	5	15
NS5	200	160	80	10	15
NS6	200	320	80	10	15
NS7	200	160	160	10	15
NS8	200	320	160	10	15

(*NS: Nanosuspension)

From the study it was observed that yield of formulation changes according to Polymer-Stabilizer ratio. Based on this, 2^3 factorial design was accepted to optimize the final batch, The Drug and Ethanol were kept constant and Tween80, PVA, PVP were the independent variable. In present study, percent entrapment efficiency, practical yield and drug release

were dependent variables. The nanosuspension formulations were prepared by using high speed agitator, ultra turrax apparatus.

Characterization of Nanosuspension

Nanosuspension formulations were characterized for λ_{max} value to check the presence of metformin in the nanosuspension, FTIR, DSC, Particle size, Polydispersity index (PDI), zeta potential, SEM, % entrapment efficiency, practical yield.

Determination of λ_{max} of Metformin Nanosuspension Formulations

λ_{max} value of nanosuspension formulation was determined to confirm the presence of metformin in formulation. 2 ml of nanosuspension was added in 10ml Ethanol (1mg/ml). The aliquot (1ml) of this solution was withdrawn and volume was made up to 10 ml. Appropriate dilutions were made with ethanol to give concentration of 10 µg/ml. It was scanned in the range from 200-800nm using UV-visible spectrophotometer (V-530) Jasco, Japan and the spectrum was recorded using spectra manager software to determine λ_{max}.[10]

a) Fourier Transforms Infrared Analysis (FT-IR)

The FT-IR of nanosuspension was obtained on Jasco V-530 FT/IR-4100 spectrometer (Japan) over range of 500-4000 cm^{-1} in dry KBr pellet. Dry KBr (50mg) was finely grounded in mortar and nanosuspension (1-2ml) were subsequently added and softly mixed in order to avoid of the crystals and compressed into disks using hydraulic press. FTIR was used for determination of functional group in the sample of nanosuspension.[9]

b) Differential Scanning Calorimetry (DSC)

The DSC analysis was carried out to confirm compatibility and thermal behaviour of the formulation. The thermal analysis of metformin nanosuspension using a DSC method of sample was by performed using PerkinElmer 4000. Accurately measured sample of nanosuspension were taken up to 1-2ml and sealed in separate aluminium pans before heating at a scanning rate of 10°C/min over the temperature range of 30–300°C. Sample analysis was performed under Nitrogen Purging, flow rate is of 20ml/min.[9]

c) Determination of Production Yield

The production yield of nanosuspension can be determined by using this formula.

$$Production\,Yield = \frac{Practical\ \ Mass}{Theoretical\ \ Mass} \times 100$$

d) Determination of Entrapment Efficiency

Aliquots of nanosuspension solution were subjected to centrifugation at 3000 rpm for 10 minutes. The clear supernatant was siphoned off carefully to separate the untrapped metformin and the absorbance was recorded at λ_{max} 237nm using UV-vis spectrophotometer (V-530) Jasco. The sediment was diluted with the solvent and absorbance was taken at 237nm. Amount of metformin in supernatant and sediment gave a total amount metformin in 1 ml dispersion. The percent entrapment was calculated by using following formula.[11]

$$\%Entrapment = \frac{Amount\ of\ drug\ in\ sediment}{Amount\ of\ drug\ added} \times 100$$

e) Partition Coefficient Determination

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium.

$P_{o/w} = (C_{oil}/C_{water})$ equilibrium

Partition coefficient is a measure of drugs lipophilicity and an indication of its ability to cross bio membrane. The partition coefficient of metformin was determined in n-octanol: water system. 110 ml of saturated solution of metformin and nanosuspension was added in n-octanol and use as stock solution. Then 4 reagent bottles are used labelled as A, B, C, D. In these bottles different concentration was added as shown in **table no 2.** Stopper was placed on each bottle and shaken for 30 min. after shaking the mixture were transfer to separating funnel and allow to stand for 15 mins to separate the layer. Organic and aqueous layer were separated out in dry beakers, later on organic layer of 10 ml was titrated against 0.1N NaoH using phenolphthalein as indicator and aqueous layer of 10 ml was titrated against 0.01N NaoH using phenolphthalein as indicator.[13]

Table No. 2: Different concentration used in partition coefficient.

Bottle	Distilled water (ml)	Stock solution (ml)	n-Octanol
A	50	40	10
B	50	30	20
C	50	20	30
D	50	10	40

f) Particle Size Analysis

The particle size of nanosuspension was measured using Malvern Zetasizer. Before running the sample, the nanosuspension were dispersed in distilled water to confirm that light scattering signal (as indicated by particles count per second). The analysis was carried out at room temperature, keeping the angle of detection at 90°. The average particle size was measured.[12]

g) Zeta Potential

The zeta potential of optimized batch was measured at least three times using Malvern Zetasizer at 25 ± 0.5°C. The average values were employed.[14]

h) X-Ray Diffraction Analysis

X-ray diffraction analysis of optimized batch was studied by using Bruker AXD8 diffractometer. The diffractogram can be used to confirm the crystalline or amorphous nature of sample. The study was confirmed by powder X-ray diffractometer at continuous scan range of 2θ = 5 - 60; the operating voltage and current were 40 (kV) and 30 (mA) respectively.[15]

i) Scanning Electron Microscopy

In order to examine the particle surface morphology and shape, scanning electron microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using a Carlzeiss Supra-5 Scanning Electron Microscope (Germany) operated at 20 kV. The smallest size nanosuspension was used for determining surface morphology.[16]

j) Stability Studies

The aim of stability studies is to predict the shelf life of the product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. Optimized formulation of nanosuspension was selected for stability studies. According to ICH guidelines nanosuspension formulation was stored in glass bottles at 25°C/60% RH and 40°C/75% RH for 3 months. These samples were analysed and checked for changes in physical appearance and % entrapment efficiency at 0, 1, 2 and 3 months.[17]

RESULT

a) FTIR

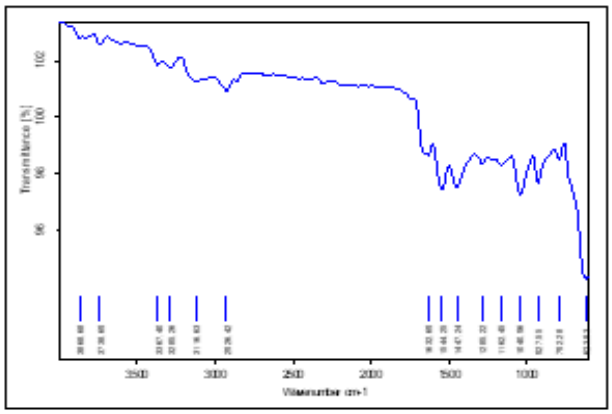

Fig no 1: Metformin

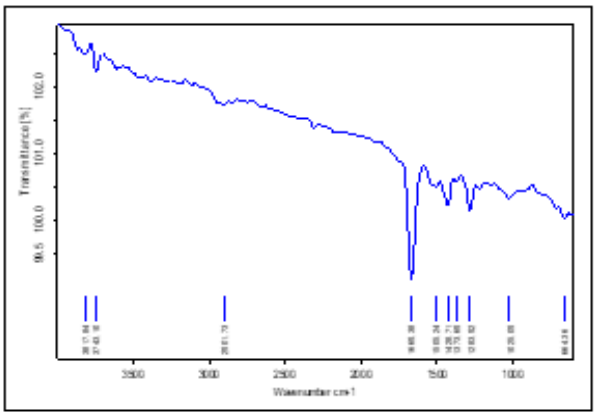

Fig no 2: PVA

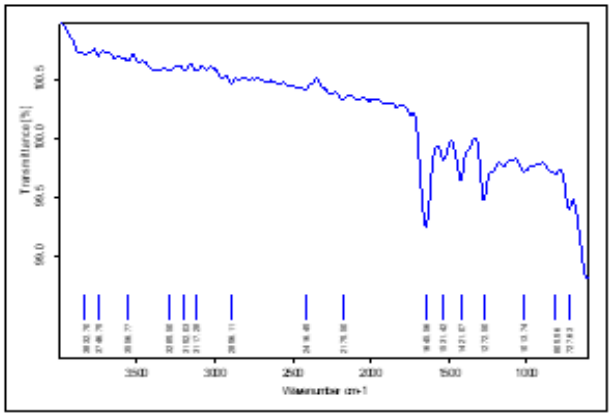

Fig no 3: PVP

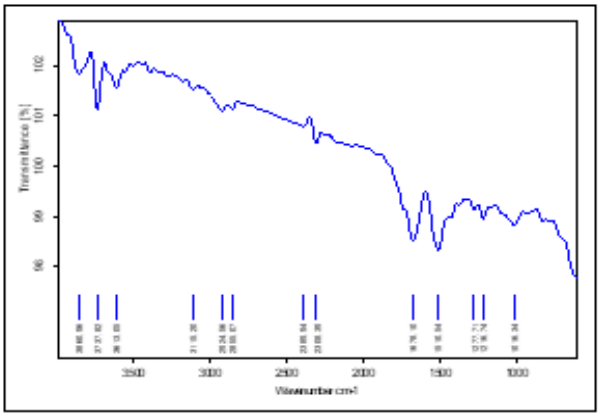

Fig no 4: Physical Mixture

For evaluating the compatibility between drug and excipients FTIR spectra were recorded. FTIR spectra of Metformin, PVA, PVP and physical mixture were observed; the spectra reflects important structural elucidation like functional groups present in the compound. The details of spectra of Metformin, PVA, PVP and physical mixture was as follows **(fig no 1-4 and table no 3).** From the obtained result we can conclude that there was no chemical interaction between drug molecules and excipients and the drug was stable in the polymeric mixture.

Table No. 3: FT-IR interpretation of Spectra.

Sr. No.	Functional group	Frequency (cm-1)
Metformin	N-H Stretch	3116.63
	C-H Stretch	2926.42
	C=N Stretch	1632.69
	C-N Vibration	1162.45
	CH3 Bending	1447.24

PVA	C-H Stretching	2901.73
	C-C Multiple bond stretching	1669.38
	C-O Stretching	1283.92
	O-H Tertiary	1373.69
	-CH_2- Bending	1428.17
PVP	C-H Stretching	2896.11
	C-C Non-conjugated	1645.56
	C=O Stretching	1645.56
	C-N Vibration aromatic primary	1272.50
	-CH_2- Bending	1421.07
Physical mixture	N-H Stretch	3115.20
	C-H Stretch	2924.56
	C=N Stretch	1678.10
	C-N Vibration	1216.74
	C-O Stretch	1277.71
	C=O Stretch	1678.10

b) DSC

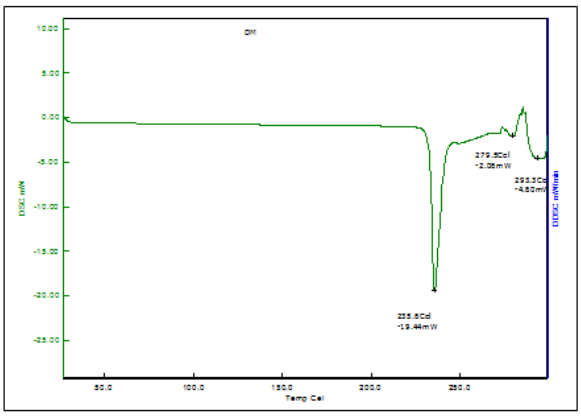

Fig no 5: DSC curve of Metformin

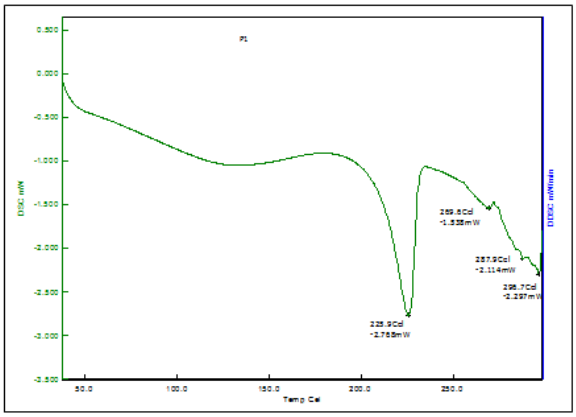

Fig no: 6 DSC curve of PVA

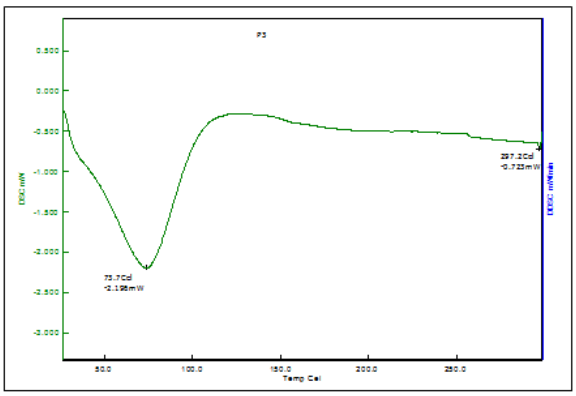

Fig no 7: DSC curve of PVP

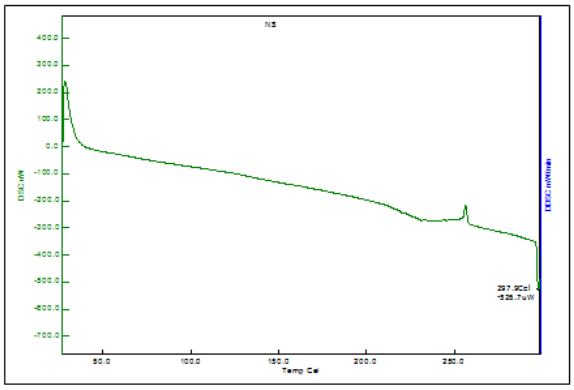

Fig no 8: DSC curve of NS8

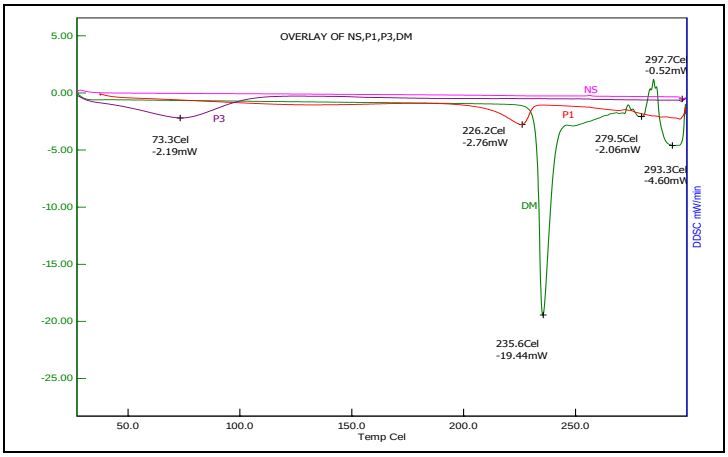

Fig. No. 9: Overlay of DSC.

The thermal analysis of metformin nanosuspension using a DSC method of sample was by performed using PerkinElmer 4000. The scanning rate of 10°C/min over the temperature range of 30–300°C. The DSC technique gives an idea about the physical features of the sample as crystalline or amorphous nature and explains a possible interaction between drug and other compounds in nanosuspension. According to the thermograms, Metformin shows sharp endothermic peak at 235.6°C, PVP shows broad endothermic peak at 73.3ºC which shows loss of water and PVA shows slight crystallinity with endothermic peak at 225.5°C. The thermogram of Nanosuspension shows Single endothermic peak at 297.7ºC. Thus, the change in the thermogram seen only a single peak is observed. The sharp peak is shifted to short peak at high melting point. This change indicates dehydration of the pure drug and change in the particle size[16] as observed in **fig no 5-9.**

Determination of Production Yield and % Entrapment Efficiency

Table No. 4: Production Yield and % Entrapment Efficiency.

Sr. No	Formulation	Production yield (%)	% Entrapment efficiency
1	NS1	61.66	53.03
2	NS2	56.33	66.66
3	NS3	68	33.78
4	NS4	64.3	46.6
5	NS5	52	24.51
6	NS6	65.42	35
7	NS7	56.57	68.3
8	NS8	75.14	71.8

Partition Coefficient Determination

Partition coefficient is used to measure the drug lipophilicity and an indication of its ability to cross bio membrane. Partition coefficient of metformin and nanosuspension was found to be -

1.206 and 1.1501. Thus, partition coefficient increases due to Nanosuspension as shown in **table no: 5.**

Table No. 5: Partition coefficient of Metformin and Nanosuspension.

Sr. No.	Sample	Solvent System	Partition Coefficient	Log P
1	Metformin	n-Octanol/ Distilled water	0.0622	-1.206
2	Nanosuspension		12.74	1.1501

Drug Release Profile

Metformin drug release from nanosuspension was taken in a Franz diffusion cell apparatus the drug release from nanosuspension was determined using a donor compartment containing the known quantity (2ml) of the nanosuspension containing equivalent to 10 mg of drug compared with the plain drug which was then placed on to a Semi- permeable layer of denatured egg shell for permeation of the formulation. Receiver compartment was filled with 27 ml of Phosphate buffer 7.4 pH at 37 ± 1°C for 8 hrs. The contents of the cell were agitated on a magnetic stirrer. Samples were withdrawn periodically and replaced with an equal volume of fresh Phosphate Buffer 7.4 pH. Samples were diluted suitably & metformin content was determined by UV method at 237nm.[18] Drug release profile of nanosuspension and metformin was carried out, and the cumulative amount of drug release was found to be 55.69% and 42.03% after a period of 2hr for nanosuspension NS8 batch and metformin respectively as shown in **table no 6** and **fig no 10.**

Table No. 6: Drug release of Metformin and Nanosuspension.

Time (min)	%CDR	
	Metformin	Nanosuspension
0	0	0
15	14.31	28.24
30	22.84	32.53
45	25.3	34.2
60	29.33	38.36
75	31.59	41.04
90	35.12	46.54
105	38.02	52.13
120	42.03	55.69

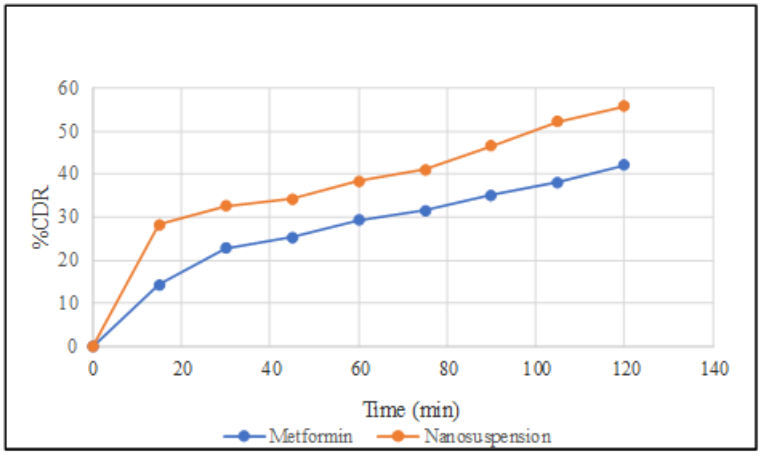

Fig. No. 10: Graph showing comparison between %CDR in Metformin and Nanosuspension NS8.

Particle Size, Polydispersity Index Zeta-Potential

The particle size was determined by using Zetasizer ver.7.12 (Malvern Instruments Ltd). The average particle size of nanosuspension particle should be in the range of 200-600 nm. The average particle size of optimized NS8 metformin nanosuspension batch was found to be 399 nm. A PDI of 0.5 shows Narrow particle size distribution.[19] The polydispersity index was found to be 0.556. As shown in **fig no 11.**

The zeta potential confirms the stability of the formulation. It indicates the repulsive force between the adjacent particles. Thus, smaller the particles or molecule high zeta potential is needed for its stability to resist the particle aggregation. The result of zeta potential showed that surface potential of the nanosuspension particle was found to be -40.7mV as shown in **fig no 12.** The particle having negative charge on their surface provide stable formulation.[20]

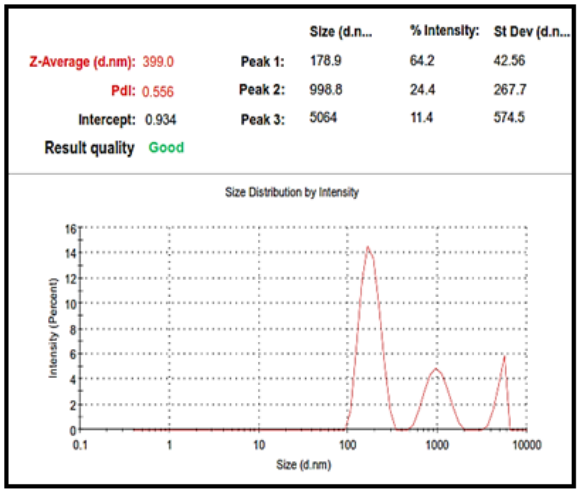

Fig no 11: Graph of Mean Particle size

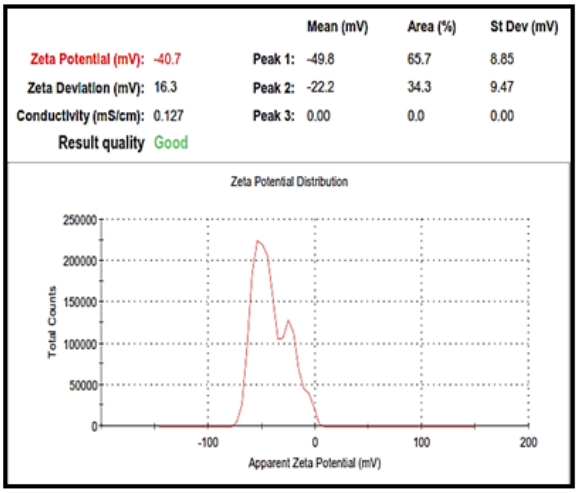

Fig no 12: Graph of Zeta potential

X-ray Diffraction Analysis

XRD patterns of metformin showed sharp peaks at 2θ-scattered angles of 17º, 22º, 23º, 28º and 35º, these peaks indicate the crystalline nature of drug as shown in **fig no 13.** These crystalline speaks were decreased in the nanosuspension, indicates amorphous nature of the drug after entrapment. Intensities of drug peaks were also decreased in the formulation. This reduced.

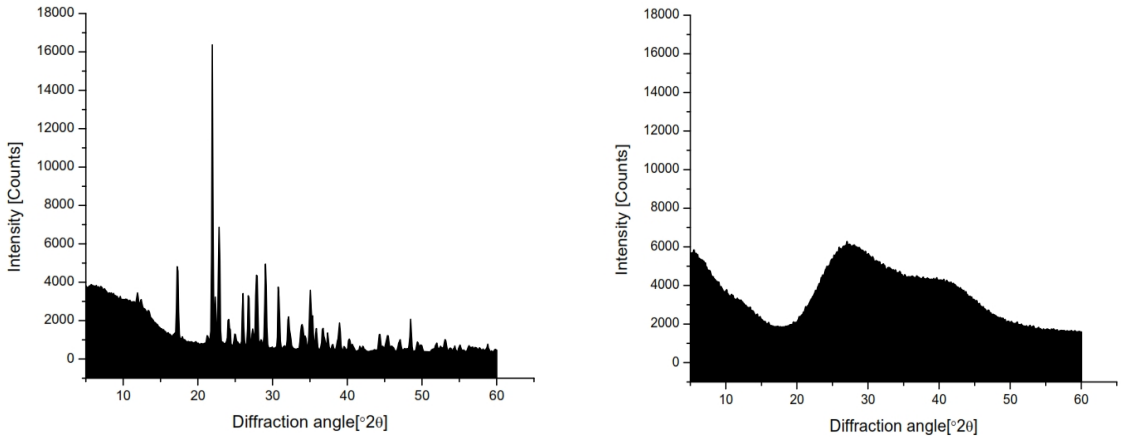

Fig. No. 13: XRD Diffractogram of Metformin Fig no 14: XRD Diffractogram of (NS8).

intensity indicates the decreased crystallinity of drug as shown in **fig no 14.** Thus, the intensity of the peaks was lower than that of metformin probably due to interaction between excipients and metformin, and also seemed to be influenced by the preparation method of nanosuspensions. Similar observations were also reported for other compounds in the literature.[21]

SEM

SEM results of metformin nanosuspension were detected by using Carlzeiss Supra-5, Germany. The results of nanosuspension show the polydisperse particle as confirmed by polydispersity index, the variation in particle size can be attributed to hydrogen-bond-induced aggregation and agglomeration but not to Ostwald ripening. The coat of PVP can be observed in **fig no 15** Thus, PVP is a long chain polymer that helps for coating the drug.[8] **Fig no 17** showed that crystalline state of metformin was still preserved in the nanosuspensions which indicated that metformin was stable.[21]

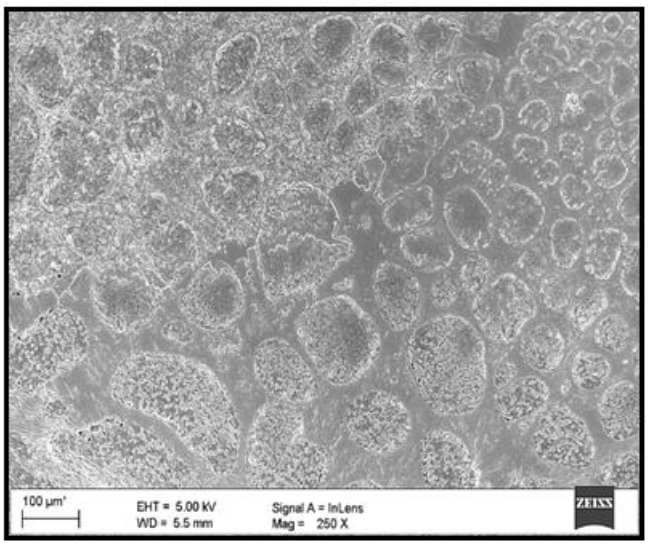

Fig no 15: SEM image of Nanosuspension (A)

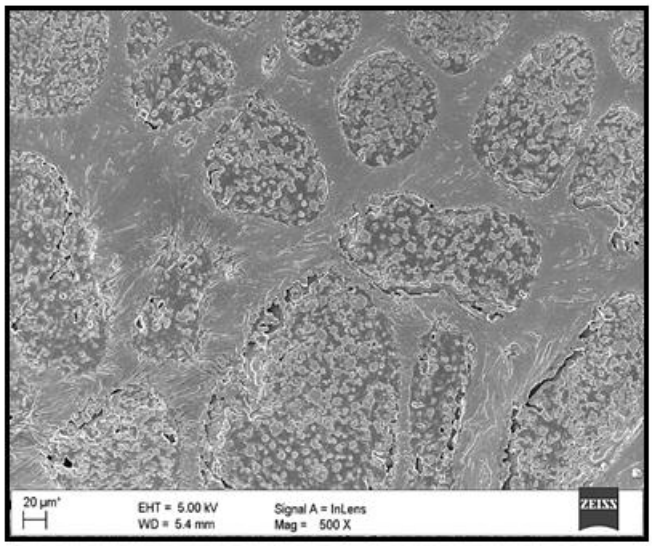

Fig no 16: SEM image of Nanosuspension (B)

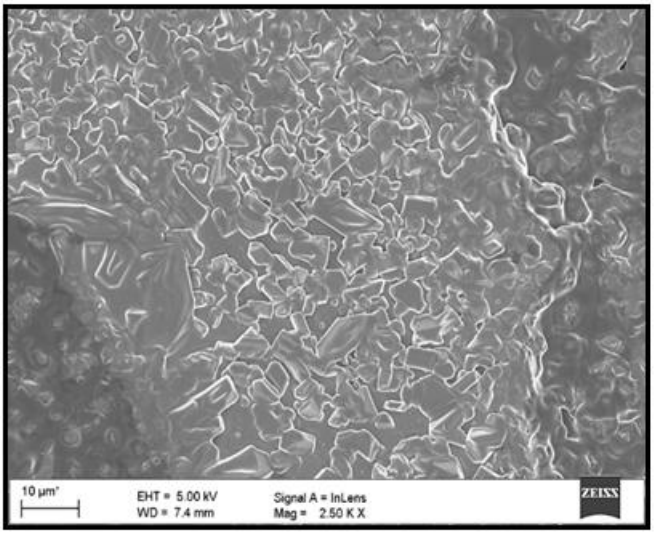

Fig no 17: SEM image of Nanosuspension (C)

Stability Study

The purpose of stability study is to provide evidence on how the quality of drug product varies with time under the influence of a variety of environmental factor such as humidity, temperature, and light. According to ICH guidelines nanosuspension formulation was stored in glass bottles at 25°C/60% RH and 40°C/75% RH for 3 months. These samples were analysed and checked for changes in physical appearance and % entrapment efficiency at 0, 1, 2 and 3 months. As shown in **table no 7.**

Table No. 7: Stability Study of Nanosuspension.

Stability Condition	Formulation	Physical stability				% Entrapment efficiency			
		No. of months				No. of months			
		0	1	2	3	0	1	2	3
25°C/60% RH	Nanosuspension	No change in Appearance				71.8%	69.70%	68.53%	67.25%
40°C/75% RH	Nanosuspension	No change in Appearance				71.8%	69.66%	68.41%	67.18%

CONCLUSION

It is known that metformin impairs lactate clearance of the liver through the inhibition of complex I of the mitochondrial respiratory chain. So, lactic acidosis associated with the use of metformin is predominantly due to the lack of lactate's clearance rather than to an increased production. The low bioavailability and short half-life of metformin make the necessity for the development of nanosuspension of metformin to Increase in permeability which results in increase in bioavailability and minimize frequency, drug accumulation, minimizing side effects and enhancing patient compliance.

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