DESIGN, DEVELOPMENT AND EVALUATION OF NANOSUSPENSIONS FOR ENHANCEMENT OF ORAL BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

PH.D. SYNOPSIS

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The Synopsis ~~and thesis~~ with details as above, may be accepted by the University for evaluation by external examiners.

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(Signature of Supervisor with date)

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Index

1. Title of the thesis

DESIGN, DEVELOPMENT AND EVALUATION OF NANOSUSPENSIONS FOR ENHANCEMENT OF ORAL BIOAVAILABILITY OF POORLY SOLUBLE DRUGS.

2. Abstract

Drug which belongs to BCS Class-II, has poor oral bioavailability due to its limited aqueous solubility. Antihypertensive agents (Candesartan Cilexetil and Telmisartan) as well as atypical antipsychotic agent (Ziprasidone Hydrochloride Monohydrate) with poor water solubility were selected as drug candidates for the research work. In this study, an attempt was made to develop stable nanosuspensions to enhance oral bioavailability of selected drugs. Analytical methods were developed for selected drugs for the estimation of drug in formulations and plasma too. Received gratis samples of selected drugs and stabilizers were subjected for identification and compatibility study by FTIR and DSC. Based on solubility from different solvents and their combinations, methanol was identified as solvent and water as an anti-solvent. Nanosuspensions were prepared using precipitation-ultrasonication method using suitable stabilizers and lyophilized using mannitol as a cryoprotectant according to physicochemical properties of drugs. Various formulation parameters like amount of drug, amount of stabilizers, solvent to anti-solvent ratio as well as process parameters like effect of stirring time, stirring speed, sonication time etc. were screened by Plackett-Burman design to identify key factors producing maximum effect on quality of nanosuspension. Maximum impact producing two factors were considered for further study to optimize the formulation by 3^2factorial design. The optimized formulations of selected drugs were evaluated by various parameters like particle size and size distribution, polydispersity index, zeta potential, solubility study, *in-vitro* dissolution study, gas chromatography for presence of residual solvent and scanning electron microscopy. Optimized formulations were subjected to accelerated stability study according to ICH guidelines. *In-vivo* bioavailability study was also carried out to compare optimized nanosuspensions with available marketed preparations.

3. Brief description on the state of the art of the research topic

In pharmaceutical field, formulation of poorly water-soluble drug has always been a challenging problem and it is a major issue for the development of new dosage form. Around

10% of the present drugs, 40% of the research drugs and 60% of drugs coming directly from synthesis have low solubility 1–10 μg/ml [1-3]. If drug solubility cannot be improved [4], the drug cannot be absorbed through GI tract upon oral administration and cannot exert its pharmacological action on the target tissue. It is due to the phospholipidic nature of cell membranes, thus certain degree of lipophilicity is required for those drug compounds, while in terms of permeability high lipophilicity is beneficial. In most of the cases it translates into poor aqueous solubility [5]. This creates delivery problems such as low oral bioavailability and erratic absorption. Drug solubility can be enhanced using traditional approaches such as co-solvents, salt formation, complexation, or delivery through carriers like liposome, solid-dispersions or micronization [6]. However, in many cases they cannot solve the bioavailability problem. For example, micronization of poorly soluble drugs has been applied for many years to improve dissolution velocity of poorly soluble drugs, but reducing the drug to micron size does not increase the saturation solubility of the drug, and at such a low saturation solubility, as generally observed in the BCS class II drugs, the increment in the dissolution characteristics does not help to a great extent, nanonization has been employed for treating the BCS class II drugs.

When the drug being reduced to nanosized level, there is an increase in the saturation solubility assisted by improvement in dissolution characteristics, which could be attributed to the effective increase in the particle surface area, according to Ostwald–Freundlich equation and Noyes-Whitney equation. Ostwald–Freundlich equation expresses how particle size influences on saturation solubility (*C*s), a compound-specific constant relying only on temperature in a given solvent. Accordingly, *C*s of the drug increases substantially with a decrease of particle size [2,7]. Nanosuspensions have emerged as a promising strategy for an efficient delivery of hydrophobic drugs because of their versatile features such as very small particle size [8].

It is generally considered that compounds with very low aqueous solubility shows dissolution rate-limited absorption. Improvement of aqueous solubility in such case is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the drug, thus, dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug [9,10].

Candesartan cilexetil, one of the selected antihypertensive drugs, is an ester prodrug that is hydrolyzed during absorption from the gastrointestinal tract to the active form candesartan.

The absolute bioavailability for candesartan is about 40% when candesartan cilexetil is given as a solution and about 14% when given as tablets. Peak plasma concentrations of candesartan occur about 3 to 4 hours after oral doses as tablets. Candesartan is more than 99% bound to plasma proteins. It is excreted in urine and bile mainly as unchanged drug and a small amount of inactive metabolites. The terminal elimination half-life is about 9 hours. Candesartan is not removed by haemodialysis [11]. Candesartan Cilexetil is categorized under Angiotensin II Receptor Antagonist, which is white to off-white crystalline powder. It is practically insoluble in water, sparingly soluble in methanol [12].

Telmisartan is categorized under antihypertensive agent - angiotensin II receptor antagonists. It is white or slightly yellowish, crystalline powder, practically insoluble in water, sparingly soluble in strong acid (except insoluble in HCl), soluble in strong base, slightly soluble in methyl alcohol, sparingly soluble in dichloromethane, having melting range 261-263°C. It is considered as BCS Class II drug having low solubility and high permeability. The absolute oral bioavailability is dose dependent about 42% after a 40-mg dose. Telmisartan is rapidly absorbed from gastrointestinal tract with peak plasma concentration 350ng/ml being reached 0.5 to 1hour after oral dose. It is metabolized by conjugation to form pharmacologically inactive acyl glucuronide; the glucuronide of the parent compound is the only metabolite that has been identified in human plasma and urine and also excreted entirely in the feces via bile, as unchanged drug. 99% of drug is bound to plasma proteins. Terminal elimination is reported to about 24 hours [13-16].

Ziprasidone Hydrochloride is categorized under an atypical antipsychotic agent. It is white or slightly pink powder, practically insoluble in water, slightly soluble in methanol and methylene chloride, having melting point 300°C. It is considered as BCS Class II drug having low solubility and high permeability. The absolute bioavailability of 20 mg dose under fed conditions is reported approximately 60%. Ziprasidone Hydrochloride is well absorbed from the gastrointestinal tract with peak plasma concentrations being reached 6 to 8 hours after oral dose. Ziprasidone Hydrochloride is metabolized by aldehyde oxidase and by the cytochrome P450 iso-enzyme CYP3A4. It is excreted mainly as metabolites in the faeces (about 66%) and urine (about 20%); less than 5% of a dose appears as unchanged drug. 99% of drug is bound to plasma proteins. Mean terminal elimination half-life is reported to about 7 hours and volume of distribution is 1.5 L/kg. Peak plasma concentration of Ziprasidone Hydrochloride is about 89ng/ml reaching 2 to 3 hours after oral dose [17-22].

3.1 Research work done related to Nanosuspension by precipitation method

Ref. No.	Drug Name	Author	Reference	Conclusion
23	Nateglinide	Papdiwal A. et. al.	Formulation and Characterization of Nateglinide Nanosuspension by Precipitation Method. International Journal of Pharmaceutical Sciences and Nanotechnology, 2014; 7(4): 2685-2691.	Solubility and dissolution rate of Nateglinide was improved by the preparation of nanosuspension using nanoprecipitation technique.
24	Meloxicam	Raval A. J. et.al.	Preparation and Characterization of Nanoparticles for Solubility and Dissolution Rate Enhancement of Meloxicam. International Research Journal of Pharmaceuticals, 2011; 1(2): 42-49.	Dissolution was improved by preparing stable nanoparticles by combining anti-solvent precipitation and high pressure homogenization approaches in presence of stabilizers and converting into dry powders by spray-drying.

3.2 Research Paper related to Nanosuspension for bioavailability enhancement

Ref. No.	Drug Name	Author	Reference	Conclusion
25	Nitrendipine	Xia D. et. al.	Preparation of stable nitrendipine nanosuspensions using the precipitation–ultrasonication method for enhancement of dissolution and oral bioavailability. European Journal of Pharmaceutical Sciences, 2010; 40(4):325–334.	Nanosuspensions by the precipitation–ultrasonication method demonstrated that C_{max} and $AUC_{0\rightarrow12}$ values of nanosuspension in rats were approximately 6.1-fold and 5.0-fold greater than that of commercial tablets.
26	Carvedilol	Liu D. et. al.	Fabrication of Carvedilol Nanosuspensions through the Anti-Solvent Precipitation–Ultrasonication Method for the Improvement of Dissolution Rate and Oral Bioavailability. AAPS Pharm SciTech, 2012; 13(1), 295-304.	The *in-vivo* test demonstrated that C_{max} and $AUC_{0\rightarrow36}$ values of nanosuspensions were approximately 3.3-fold and 2.9-fold greater than that of the commercial tablets,

3.3 Patents for the nanosuspension and selected drugs

Ref. No.	Patent No.	Title of the patent
27	US005858410A	Pharmaceutical nanosuspensions for medicament administration as systems with increased saturation solubility and rate of solution
28	US 20110124702	Nanosuspension of a poorly soluble drug via micro fluidization process
29	EP1912898	Method for concentrating nanosuspensions
30	US 20120058151	Nano-particulate compositions poorly soluble compounds.
31	US20120135053A1	Nano-particulate Telmisartan compositions and process for the preparation thereof.
32	US 20080193542A1	Injectable depot formulations and methods for providing sustained release of nanoparticle compositions.

4. Definition of the Problem

To design, develop and evaluate nanosuspensions for enhancement of oral bioavailability of poorly soluble drugs.

5. Objective and Scope of work

- To perform preformulation study of selected drugs.
- To perform scanning and calibration curve preparation of selected drugs.
- To prepare nanosuspension using precipitation – ultrasonication method technique from selected drugs.
- To identify key factors affecting formulation of nanosuspension by Plackett and Burman Screening Design of experiments
- To optimize other formulation and processing parameters (preliminary studies) by trial and error method.
- To optimize nanosuspension formulation by 3^2 factorial design.
- To characterize developed nanosuspensions by various physicochemical parameters as well as analytical techniques.
- To study *in-vitro* drug release profile of optimized formulation and compare with marketed preparation.
- To perform accelerated stability studies according to ICH guidelines.
- To perform *in-vivo* bioavailability study of optimized formulation and compare with marketed preparation.

6. Original contribution by the thesis

The entire work in this synopsis, is the original work, with research papers publication/presentation as well as GUJCOST funded Minor Research Project – 2015 as the back bone. The details of the associated project and papers are as follows:

6.1 Research work funded by GUJCOST (Annexure-1)

- GUJCOST sanctioned Minor Research Project entitled – 'Development and evaluation of Ziprasidone Hydrochloride loaded Nanosuspension for bioavailability enhancement' with grant worth Rs. 4.75 Lacs (Letter No. GUJCOST/MRP/2014-15 Dated 30/3/2015)

6.2 Paper Presented (Annexure-2)

- Poster entitled, '**Application of Plackett- Burman Screening Design for Optimizing Formulating and Processing Parameters of Ziprasidone Hydrochloride Nanosuspension**' at SERB sponsored two days National Seminar on Bioavailability Enhancement: An Industry Desire and Regulatory Constrains organized by Department of Pharmaceutical Sciences, Saurashtra University, Rajkot on 30th and 31st July, 2016.

6.3 Paper Published (Annexure-3,4,5)

- Review article published in Asian Journal of Pharmacy and Technology (ISSN: 2231–5705) entitled, '**Nanosuspension: An Emerging Trend for Bioavailability Enhancement of Poorly Soluble Drugs**' Year: 2012, Vol. 2: Issue 4, Pages 158-169. **(Annexure-3)**
- Research article is accepted for publication in International Journal of Pharmaceutical Research (ISSN 0975-2366) entitled, '**Screening of Formulating and Processing Parameters on Candesartan Cilexetil Nanosuspension Prepared by Nanoprecipitation-Ultrasonication Technique**' and will be published in October - December, 8[4], 2016 issue.[IJPR/PPT/062016/0012] **(Annexure-4)**
- Research article is accepted for publication in Journal of Pharmaceutical Science and Bioscientific Research (ISSN 2277-3681) entitled, '**Screening of formulating and processing parameters for Ziprasidone Hydrochloride nanosuspension prepared by nanoprecipitation-ultrasonication technique**' with manuscript no. **16RE-6011. (Annexure-5)**

7. Methodology of Research

7.1 Scanning and Calibration curve preparation in methanol and dissolution media of drug

Scanning of selected drugs were performed in methanol as well as its dissolution media to find out absorbance maxima (λ_{max}). Calibration curves were prepared in methanol for measurement of drug content as well as in respective dissolution media for estimation of Cumulative Percent Release of drugs.

7.2 Selection of solvent and anti-solvent

The solubility of selected drugs were studied in different solvents and their combinations. Selection of good and poor solvent was done based upon solubility of drug [33].

7.3 Preparation of nanosuspension by precipitation-ultrasonication method

Nanosuspension was prepared by the precipitation–ultrasonication method. Drug was dissolved in methanol by sonication for 5 mins at room temperature. Different stabilizers were dissolved in water to obtain a series of anti-solvents. Both solutions were passed through a 0.45μm filter. The anti-solvent was cooled to 3°C in an ice-water bath. Then, drug solution was quickly introduced by means of a syringe positioned with the needle directly into stabilizer solution into 40 ml of the pre-cooled anti-solvent at different stirring speed under overhead stirrer to allow the volatile solvent to evaporate at room temperature for 4-5 hours. After precipitation of anti-solvent, sample was immediately transferred to a test tube and was treated with an ultrasonic probe at different time lengths (in mins). The probe with a tip diameter of 6 mm was immersed in the liquid, resulting in the wave traveling downwards and reflecting upwards. Batch size for preparation of nanosuspension was taken 40 ml [25].

Lyophilization of nanosuspension of optimized batch

Nanosuspension was converted into the dry powder by using Lyophilizer. In lyophilization process sample was kept into the chamber and temperature maintained at -80°C and high pressure for 8-10 hrs. After the 6-8 hrs nanosuspension was converted into the dry powder and removed from the chamber and placed in airtight container for further work.

7.4 Selection of stabilizer

Different stabilizers like Polyvinyl Alcohol, PVP K-30, Sodium Lauryl Sulphate, Poloxamer 188 and Poloxamer 407 were screened by preparing nanosuspensions (Table1) and measuring their saturation solubility, mean particle size, poly dispersity index (PDI) and zeta potential [34].

7.5 Drug-Excipient Compatibility Study

The potential physical and chemical interactions between drugs and excipients can affect the chemical, physical, therapeutical properties and stability of the dosage form. FTIR and DSC study were performed for checking of drug-excipient compatibility.

7.6 Plackett-Burman Design [35]

The Plackett-Burman design is suitably used to screen a large number of factors believed to be affecting important product characteristics or attributes, and is generally used during the initial phase of the study. By review of literature five factors were selected to affect the quality of nanosuspension. To identify which factor has its prominent effect on quality, stability as well as efficacy of the nanosuspension, this design was used. A total of 8 experiments were generated for screening of five independent factors namely amount of drug in mg (X_1), amount of stabilizer in mg (X_2), solvent: anti-solvent volume ratio (X_3), stirring speed in rpm (X_4) and sonication time in min (X_5). Saturation solubility in µg/ml (Y_1) and mean particle size in nm (Y_2) were selected as dependent factors.

Net effect of individual factor was calculated from the value of evaluated parameters from following equations,

$$\textbf{Effect of } X_1 = [(Y_1+Y_4+Y_6+Y_7)-(Y_2+Y_3+Y_5+Y_8)]/8$$
$$\textbf{Effect of } X_2 = [(Y_1+Y_2+Y_5+Y_7)-(Y_3+Y_4+Y_6+Y_8)]/8$$
$$\textbf{Effect of } X_3 = [(Y_1+Y_2+Y_3+Y_6)-(Y_4+Y_5+Y_7+Y_8)]/8$$
$$\textbf{Effect of } X_4 = [(Y_2+Y_3+Y_4+Y_7)-(Y_1+Y_5+Y_6+Y_8)]/8$$
$$\textbf{Effect of } X_5 = [(Y_1+Y_3+Y_4+Y_5)-(Y_2+Y_6+Y_7+Y_8)]/8$$

After getting net effect of individual parameters two key parameters were identified which had maximum effect on product characteristics. These two parameters can be selected for

product optimization by factorial design and other three parameters can be optimized by trial and error method.

7.7 Optimization of other Preliminary Parameters

Preliminary parameters were optimized by varying one parameter at a time, while keeping others constant, so that effect of varied parameters could be evaluated. Each batch was repeated thrice (n=3) for the confirmation of repeatability. The parameters were optimized to achieve minimum particle size and maximum saturation solubility. Optimized parameters were solvent to anti-solvent volume ratio (1:4, 1:6, 1:8), stabilizer concentration (30mg, 40mg, 50mg), stirring speed (800RPM, 1000RPM, 1200RPM), sonication time (10min, 20min, 30min)etc.

7.8 Factorial design for optimization of key parameters

A 3^2 factorial design was applied for optimization of key parameters like amount of drug in mg and solvent to anti-solvent volume ratio for Candesartan Cilexetil amount of drug in mg and solvent to anti-solvent volume ratio [36, 37], while for Telmisartan and Ziprasidone Hydrochloride amount of drug in mg and stirring speed [38]. Both particle size and saturation solubility, important features of nanosuspension considered to play a significant role in the formulation performance, were taken as dependent parameters in this study. Multiple regression analysis, contour plots and 3D response surface plots were used to study the main and interaction effects of the variables on the particle size and saturation solubility [additionally CPR at 15min in Ziprasidone Hydrochloride Nanosuspension]. The numbers of experiments required in factorial design studies were dependent on the number of independent variables selected and the number of levels at which they are studied. The response was measured for each trial and then either simple linear equation (1), or interactive equation (2) or quadratic (3) model was fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant terms.

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2} \quad \text{----------(1)}$$

$$\mathbf{Y= b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2} \quad \text{----------(2)}$$

$$\mathbf{Y= b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2} \quad \text{----------(3)}$$

Where, Y is the dependent variable, while b_0 is the intercept, bi (b_1 and b_2), bij (b_{12}) represents the regression coefficient for the second order polynomial equation and Xi

represents the levels of independent formulation variables. Mathematical modeling was carried out by using equation 3 to obtain a second order polynomial equation [39]. The values of dependent variable obtained at various levels of two independent variables (X_1 and X_2) were subjected to multiple regressions to yield a second order polynomial equation. The main effects of X_1 and X_2 represent the average result of changing one variable at a time from its low to high value. The interaction (X_1X_2) shows how the particle size and saturation solubility changed when two variables were simultaneously changed. The larger the magnitude of the **t** value and the smaller the **p** value, the more significant is the corresponding coefficient.

7.9 Checkpoint analysis

A checkpoint analysis was performed to confirm the utility of established response surface plots and contour plots in the preparation of nanosuspension. Values of independent variables (X_1 and X_2) were selected and corresponding values of dependent variables were calculated by substituting the values in the reduced polynomial equation. Nanosuspensions were prepared experimentally by taking the amounts of the independent variables (X_1 and X_2) on the same checkpoints. Checkpoint cum optimized batch was prepared three times and mean values were determined. Difference of theoretically computed values of particle size as well as saturation solubility and the mean values of experimentally obtained for both responses were compared.

7.10 Evaluation of Nanosuspensions

- Saturation solubility [40]
- Particle size and PDI [41]
- Zeta potential [41]
- Dissolution study [42]
- Drug content
- Scanning electron microscopy (SEM)
- Accelerated stability study as per ICH Guidelines [43]
- Residual solvent by Gas – Chromatography
- *In-vivo* Bioavailability study

8. Results of Candesartan Cilexetil Nanosuspension

Scanning and calibration curve was prepared in methanol in the range of 5-30µg/ml by UV-Visible spectrophotometer showed λ_{max} at 254 nm and regression equation was found to be **Y = 0.0295X + 0.011** with regression co-efficient **0.9994** for UV absorption spectrum of Candesartan Cilexetil. Scanning and calibration curve was prepared in 0.05 M Phosphate Buffer, pH 6.5 in the range of 4-16µg/ml by UV-Visible spectrophotometer showed λ_{max} at 259 nm and regression equation was found to be **Y = 0.0501X - 0.0116** with regression co-efficient **0.9992.** Selection of solvent and anti-solvent showed that drug had highest solubility **(5.31mg/ml) in methanol** and least solubility **(0.00119 mg/ml) in water**, so they were selected as solvent and anti-solvent respectively. Different stabilizers like Polyvinyl Alcohol, PVP K-30, Sodium Lauryl Sulphate, Poloxamer 188 and Poloxamer 407 were screened from which **PVP K-30** was selected by subjecting nanosuspension for measurement of their saturation solubility, mean particle size, poly dispersity index (PDI) and zeta potential. FTIR and DSC study of drug, stabilizer and physical mixture indicated compatibility of ingredients.

Results of Plackett-Burman screening design revealed that **solvent: anti-solvent ratio as well as amount of drug** were found to be promising formulating parameters having prominent effect on quality of Candesartan Cilexetil nanosuspension, so they were selected as independent factors X_1 and X_2 respectively. Mean particle size (Y_1) and saturation solubility (Y_2) were selected as dependent factors for 3^2 factorial design for optimization of the formulation. By using Minitab 17.0 software CFD-8 was found to be optimized batch. Desirability of optimized batch was 1.0. Optimized batch had following formulation and process parameters which is shown in table.

Table 1: Formulation and process parameters for optimized batch

Amount of candesartan cilexetil	20mg
Amount of PVP K-30	50mg
Solvent to anti-solvent volume ratio	1:15
Stirring speed	1200RPM
Stirring Time	4Hrs
Sonication Time	30min
Amount of lyophilizer	70mg (1:1 Ratio, Total Solid : Mannitol)

Results were obtained for evaluation parameters of optimized batch like, the mean particle size and PDI of nanosuspension were 242.7nm and 0.345 respectively while the zeta potential and saturation solubility were −32.98mVand 111.85µg/ml, respectively. Drug content was found to be 101.01%w/w. The in-vitro dissolution of candesartan cilexetil was 97.13%w/w was obtained within 2 min. Residual solvent methanol was observed 171.87ppm which was less than 3000ppm described by ICH guidelines for class-2 solvent. The Surface Topology as measured by Scanning Electron Microscopy of pure drug was found to be long, thin and flat with particles larger (5-32µm) in size. However after formulation, particles became smaller (about 300nm) which were adsorbed on the surface of mannitol used as cryoprotectant may be by hydrophobic interaction. The *in-vitro* dissolution rate of candesartan cilexetil was significantly increased as compared to marketed formulation by reducing the particle size. Stability study, according to ICH guideline (25°C ± 2°C and 65%RH ± 5%RH) revealed that there is no significant physical and chemical change after 6 months evaluated by mean particle size, zeta potential, CPR at 2 min and drug content of the lyophilized formulation The *in-vivo* test demonstrated that the C_{max} and $AUC_{0\rightarrow24}$ values of nanosuspension in rats were greater than that of marketed formulation respectively.

9. Results of Telmisartan Nanosuspension

Scanning and calibration curve was prepared in methanol in the range of 2-20µg/ml by UV-Visible spectrophotometer showed λ_{max} at 296 nm and regression equation was found to be **Y = 0.51X + 0.011** with regression co-efficient **0.999** by UV absorption spectrum of Telmisartan. Scanning and calibration curve was prepared in Phosphate Buffer, pH 7.5 in the range of 2-20µg/ml by UV-Visible spectrophotometer showed λ_{max} at 296 nm and regression equation was found to be **Y = 0.0422X - 0.0038** with regression co-efficient **0.999.** Selection of solvent and anti-solvent showed that drug has the highest solubility **(3.329mg/ml) in methanol** and least solubility **(0.012mg/ml) in water**, so they were selected as solvent and anti-solvent respectively. Different stabilizers like Polyvinyl Alcohol, PVP K-30, Sodium Lauryl Sulphate, Poloxamer 188 and Poloxamer 407 were screened from which **Poloxamer 407** was selected by subjecting nanosuspension for measurement of their saturation solubility, mean particle size, poly dispersity index (PDI) and zeta potential. FTIR and DSC study of drug, stabilizer and physical mixture indicated compatibility of ingredients.

Results of Plackett-Burman screening design revealed that **amount of drug as well as stirring speed** were found to be promising formulating and processing parameters having

prominent effect on quality of Telmisartan nanosuspension, so they were selected as independent factors X_1 and X_2 respectively. Mean particle size (Y_1) and saturation solubility (Y_2) were selected as dependent factors for 32 factorial design for optimization of formulation. By using Minitab 17.0 software TFD-6 was found to be optimized batch. Desirability of optimized batch was 0.9629. Optimized batch had following formulation and process parameters which is shown in table.

Table 2: Formulation and process parameters for optimized batch

Amount of Telmisartan	15mg
Amount of Poloxamer 407	50mg
Solvent to anti-solvent volume ratio	1:8
Stirring speed	1200RPM
Stirring Time	4Hrs
Sonication Time	30min
Amount of lyophilizer	65mg (1:1 Ratio, Total Solid : Mannitol)

Results were obtained for evaluation parameters of optimized batch like, the mean particle size and PDI of nanosuspension were 328.0 nm and 0.477 respectively while the zeta potential and saturation solubility were −30.36mVand 100.16µg/ml, respectively. Drug content was found to be 99.54%w/w. The *in-vitro* dissolution of telmisartan was 98.24%w/w was obtained within 2 min. Residual solvent methanol was observed 192.27ppm which was less than 3000ppm described by ICH guidelines for class-2 solvent. The Surface Topology as measured by Scanning Electron Microscopy of pure drug was found to be long, thin and flat with particles larger (0.5-15µm) in size. However after formulation, particles became smaller (about 300nm) which were adsorbed on the surface of mannitol used as cryoprotectant by hydrophobic interaction. Stability study, according to ICH guideline (25°C ± 2°C and 65%RH ± 5%RH) revealed that there is no significant physical and chemical change after 6 months evaluated by mean particle size, zeta potential, CPR at 2 min and drug content of the lyophilized formulation The *in-vivo* test demonstrated that the C_{max} and $AUC_{0\rightarrow24}$ values of nanosuspension in rats were greater than that of marketed formulation respectively.

10. Results of Ziprasidone Hydrochloride Nanosuspension

Scanning and calibration curve was prepared in methanol in the range of 10-60µg/ml by UV-Visible spectrophotometer showed λ_{max} at 317 nm and regression equation was found to be **Y = 0.0149X + 0.0133** with regression co-efficient **0.999** by UV absorption spectrum of

Ziprasidone Hydrochloride. Scanning and calibration curve was prepared in 0.05M Phosphate Buffer, pH 7.5 in the range of 10-60µg/ml by UV-Visible spectrophotometer showed λ_{max} at 318 nm and regression equation was found to be **Y = 0.0142X - 0.0012** with regression co-efficient **0.9994.** Selection of solvent and anti-solvent showed that drug has highest solubility **(2.443mg/ml) in methanol** and least solubility **(0.022mg/ml) in water**, so they were selected as solvent and anti-solvent respectively. Different stabilizers like Polyvinyl Alcohol, PVP K-30, Sodium Lauryl Sulphate, Poloxamer 188 and Poloxamer 407 were screened from which **Poloxamer 407** was selected by subjecting nanosuspension for measurement of their saturation solubility, mean particle size, poly dispersity index (PDI) and zeta potential. FTIR and DSC study of drug, stabilizer and physical mixture indicated compatibility of ingredients.

Results of Plackett-Burman screening design revealed that **amount of drug** as well as **stirring speed** were found to be promising formulating parameters having prominent effect on quality of Ziprasidone Hydrochloride nanosuspension, so they were selected as independent factors X_1 and X_2 respectively. Mean particle size (Y_1), saturation solubility (Y_2) and CPR at 15min (Y_3) were selected as dependent factors for 3^2 factorial for optimization of formulation. By using Minitab 17.0 software ZFD-6 was found to be optimized batch. Desirability of optimized batch was 0.9035. Optimized batch had following formulation and process parameters which is shown in table.

Table 3: Formulation and process parameters for optimized batch

Amount of Ziprasidone Hydrochloride (Equivalent to 15mg of Ziprasidone Base)	16.95mg
Amount of Poloxamer 407	50mg
Solvent to anti-solvent volume ratio	1:8
Stirring speed	1200RPM
Stirring Time	4Hrs
Sonication Time	30min
Amount of lyophilizer (1:1 Ratio, Total Solid : Mannitol)	66.95 mg

Results were obtained for evaluation parameters of optimized batch like, the mean particle size and PDI of nanosuspension, were 218.0 nm and 0.456 respectively while the zeta potential and saturation solubility were −32.1mV and 76.25µg/ml respectively. Drug content

was found to be 100.46%w/w. The *in-vitro* dissolution of Ziprasidone Hydrochloride was obtained 96.61%w/w within 15 min. Residual solvent methanol was observed 73.53ppm in optimized batch which was less than 3000ppm described by ICH guidelines for class-2 solvent. The Surface Topology as measured by Scanning Electron Microscopy of pure drug was found to be long, thin and flat with particles larger (2-27µm) in size. However after formulation, particles became smaller (about 200nm) which were adsorbed on the surface of mannitol used as cryoprotectant may be by hydrophobic interaction. Stability study, according to ICH guideline (25°C ± 2°C and 65%RH ± 5%RH) revealed that there is no significant physical and chemical change after 6 months evaluated by mean particle size, zeta potential, CPR at 15 min and drug content of the lyophilized formulation. The *in-vivo* test demonstrated that the C_{max} and $AUC_{0\rightarrow12}$ values of nanosuspension in rats were greater than that of marketed formulation respectively.

11. Achievements with respect to objectives

- Selected drugs were BCS Class II drugs so problem was there with poor saturation solubility which was increased with nanosuspension formulations
- Similarly BCS Class II drugs shows dissolution as rate limiting step but here nanosuspensions revealed maximum drug release obtained within 2 – 20 mins and indicating better performance compared to marketed formulations.
- The *in-vivo* bioavailability study proved that the C_{max} and $AUC_{0\rightarrow24}$ values of nanosuspension in rats were greater than that of commercial formulations.
- The optimized lyophilized product was physically and chemically stable upto 6 months when tested according to ICH guidelines.

12. Conclusion

Nanosuspension was prepared successfully from selected drugs by combination of precipitation and ultrasonication technique and lyophilized using mannitol as a cryoprotectant according to physicochemical properties of drugs. Analytical methods were developed for selected drugs for the estimation of drug in formulations and dissolution media. Solvents and anti-solvents were selected based upon solubility of drug in respective solvents and their combination. Stabilizers were selected by preparing nanosuspension and evaluated with suitable parameters. Compatibility study were carried out using FTIR and DSC studies and showed drug- excipient compatibility. Various formulation parameters like effect of

stabilizers, solvent to anti-solvent ratio as well as processing parameters like effect of stirring time, stirring speed, sonication time etc. were screened by Plackett-Burman design to identify key factors producing maximum effect on quality of nanosuspension. Maximum impact producing two factors were considered for further study to optimize the formulation by 3^2 factorial design. The optimized formulations were evaluated by various parameters like mean particle size, polydispersity index, zeta potential, saturation solubility study, *in-vitro* dissolution study, gas chromatography for presence of residual solvent and scanning electron microscopy. Optimized formulations were subjected to accelerated stability study according to ICH guidelines and found to be physically and chemically stable for 6 months. *In-vivo* bioavailability study of optimized nanosuspensions was also carried out and results revealed improved C_{max} and AUC compared with marketed preparations.

13. Copies of papers published and a list of all publications arising from the thesis

13.1 Published / Accepted Papers----------As per point 6.2 and 6.3----------

13.2 Papers arising from thesis

Sr. No.	Probable Title	Probable Journal
1	Application of Plackett- Burman Screening Design for Optimizing Formulating and Processing Parameters of Ziprasidone Hydrochloride Nanosuspension	International Journal of Pharmaceutical Sciences and Nanotechnology
2	Formulation and Evaluation of Candesartan Cilexetil Loaded Nanosuspension for Bioavailability Enhancement.	Indo American Journal of Pharmaceutical Research
3	Design, development and evaluation of nanosuspension for enhancement of oral bioavailability of telmisartan.	AAPS SciTech
4	Development and evaluation of nanosuspension formulation for oral bioavailability enhancement of Ziprasidone Hydrochloride.	Drug Development and Industrial Pharmacy

14. Patents (if any) ---------- NA----------

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No: GUJCOST/ MRP/ 2014-15/

30th March, 2015

Ms. Jalpa S. Paun
Department of Pharmaceutics
B.K.ModyGovt.Pharmacy College,
Polytechnic Campus,
Near AjiDam,Rajkot

Sub: Award of GUJCOST Minor Research Project Grant for the proposal on "Development and evaluation of Ziprasidone Hydrochloride loaded Nanosuspension for bioavailability enhancement" By Ms. Jalpa S. Paun

Dear Madam,

Warm Greetings from Gujarat Council on Science and Technology (GUJCOST), Gandhinagar.

With reference to your project proposal on Development and evaluation of Ziprasidone Hydrochloride loaded Nanosuspension for bioavailability enhancement and subsequent presentation before the Expert Committee, GUJCOST is pleased to inform you that your proposal has been sanctioned for an amount of Rs. 4,75,000/- for two years' time period.

As per the GUJCOST guidelines and for the disbursement of the grant, a Memorandum of Understanding (M.O.U) has to be signed between the Principal/ Head of the Institute, Principal Investigator of the project and GUJCOST on Rs. 100/- stamp paper with Notary registration. A draft MOU has been enclosed herewith for your reference.

We request you to please send us the signed copy of the MOU at the earliest so that necessary financial assistance may be released.

Tanking you and with best regards.

Yours sincerely,

(Narottam Sahoo)

Encl.: As above

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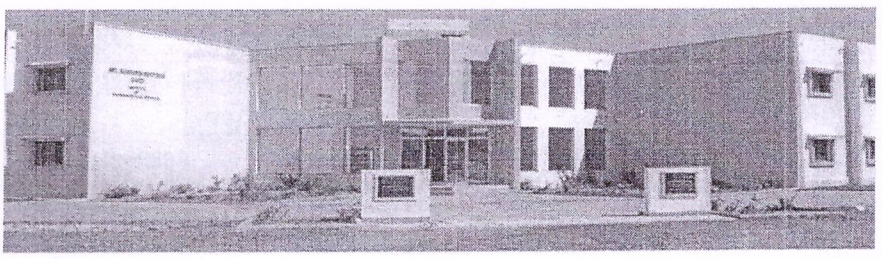

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PP-22

APPLICATION OF PLACKETT–BURMAN SCREENING DESIGN FOR OPTIMIZING FORMULATING AND PROCESSING PARAMETERS OF ZIPRASIDONE HYDROCHLORIDE NANOSUSPENSION

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ABSTRACT

Low oral bioavailability of poorly water-soluble drugs poses a great challenge during drug development. Poor water solubility and low dissolution rate are issues for the majority of upcoming and existing biologically active compounds. Ziprasidone Hydrochloride (ZH) is BCS class-II drug having low solubility and high permeability. The aim of the present investigation was to identify critical formulating and processing parameters which influences on quality of the nanosuspension. Nanosuspension formulation of a poorly soluble drug was developed using nanoprecipitation-ultrasonication technique. A total of 8 experiments were generated for screening 5 independent factors namely the amount of Ziprasidone Hydrochloride (mg) (X_1), amount of stabilizer (mg) (X_2), solvent to anti-solvent volume ratio(X_3), stirring speed (rpm) (X_4) and sonication time (min) (X_5). Mean particle size (nm) (Y_1), saturation solubility (µg/ml) (Y_2) and CPR at 15 min (%) (Y_3) were selected as dependent factors.The obtained results showed that nanosuspension prepared with the Poloxamer 407 has improved saturation solubility as compare to all other stabilizers. Result also revealed that concentration of drug and stirring speed were found to be promising formulating and processing parameters having prominent effect on quality of Ziprasidone Hydrochloride nanosuspension.

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REVIEW ARTICLE

Nanosuspension: An Emerging Trend for Bioavailability Enhancement of Poorly Soluble Drugs

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ABSTRACT:
Drug effectiveness is influenced by a crucial factor like solubility of drug, independence of the route of administration. Most of the newly discovered drugs coming out from High-throughput screening are failing due to their poor water solubility which is major problem for dosage form design. Now a day, nanoscale systems for drug delivery have gained much interest as a way to improve the solubility problems. Nanosuspension technology is a unique and economical approach to overcome poor bioavailability that is related with the delivery of hydrophobic drugs, including those that are poorly soluble in aqueous media. Design and development of nanosuspension of such drugs is an attractive alternative to solve this problem. Preparation of nanosuspension is simple and applicable to all poorly soluble drugs. A nanosuspension not only solves the problem of solubility and bioavailability but also alters pharmacokinetic profile of the drug which may also improve safety and efficacy. This review article takes account of introduction, advantages, properties, formulation consideration, preparation, characterization and application of the nanosuspensions.
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KEYWORDS: Nanosuspensions, Poorly soluble drugs, Drug Delivery, Bioavailability, Solubility enhancement.

INTRODUCTION:
Bioavailability is defined as the rate and extent to which the active ingredient is absorbed from a drug product and becomes available at the site of action.[1] From a pharmacokinetic perspective, bioavailability data for a given formulation provide an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the bioavailability data for a solution, suspension or intravenous dosage form. In addition, bioavailability studies provide other useful pharmacokinetic information related to distribution, elimination, effects of nutrients on absorption of the drug, dose proportionality and linearity in pharmacokinetics of the active and inactive moieties. Bioavailability data can also provide information indirectly about the properties of a drug substance before entry into the systemic circulation, such as permeability and the influence of pre-systemic enzymes and/or transporters.

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Bioavailability of a drug is largely determined by the properties of the dosage form, rather than by the drug's physicochemical properties, which determine absorption potential. Differences in bioavailability among formulations of a given drug can have clinical significance; thus, knowing whether drug formulations are equivalent is essential.

Poorly water soluble drugs are increasingly becoming a problem in terms of obtaining satisfactory dissolution within the gastrointestinal tract that is necessary for good oral bioavailability. It is not only existing drugs that cause problems but it is the challenge to ensure that new drugs are not only active pharmacologically but have enough solubility to ensure fast enough dissolution at the site of administration, often the gastrointestinal tract.[2]

FACTORS AFFECTING BIOAVAILABILITY:
Low bioavailability is most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs. Solid drugs need to dissolve before they are exposed to be absorbed. If the drug does not dissolve readily or cannot penetrate the epithelial membrane (eg, if it is highly ionized

and polar), time at the absorption site may be insufficient. In such cases, bioavailability tends to be highly variable as well as low[3]. Age, sex, physical activity, genetic phenotype, stress, disorders (eg, achlorhydria, malabsorption syndromes), or previous GI surgery (eg, bariatric surgery) can also affect drug bioavailability.

IMPROVEMENT OF BIOAVAILABILITY:

Improvement of bioavailability of poorly water soluble drug remains one of the most challenging aspects of drug development. By many estimates up to 40% of new chemical entities discovered by the pharmaceutical industry today are poorly water soluble compounds.[4]

Together with the permeability, the solubility behavior of a drug is a key determinant of its bioavailability. There have always been certain drugs for which solubility has presented a challenge to the development of a suitable formulation for oral administration. Examples are griseofulvin, digoxin, phenytoin, sulphathiazole etc. With the recent arrival of high throughput screening of potential therapeutic agents, the number of poorly soluble drug candidates has risen sharply and the formulation of poorly soluble compounds for delivery now presents one of the most frequent and greatest challenges to formulation scientists in the pharmaceutical industry.

Consideration of the modified Noyes-Whitney equation provides some hints as to how the dissolution rate of even very poorly soluble compounds might be improved to minimize the limitations to oral availability.[5] The main possibilities for improving dissolution according to this analysis are:

- To increase the surface area available for dissolution by decreasing the particle size of the solid compound,
- By optimizing the wetting characteristics of the compound surface,
- To decrease the diffusion layer thickness,
- To ensure sink conditions for dissolution and,
- To improve the apparent solubility of the drug under physiologically relevant conditions.[6]

A fundamental step in the solubilization of drug compound is the selection of an appropriate salt form, or for liquid drugs, adjustment of pH of the solution. Traditional approaches to drug solubilization include either chemical or mechanical modification of the drug molecule, or physically altering the macromolecular characteristics of aggregated drug particles.

Improvement of bioavailability can be obtained by following measures:

- Addition of solubilizing excipients
- Inclusion complexes
- Polymorphism
- Lipid-based emulsion systems
- Salt form
- Solid dispersions
- Particle size reduction etc.

NEED OF NANOSUSPENSION FOR BIOAVAILABILITY ENHANCEMENT:

Nevertheless, pharmacokinetic studies of BCS class – II drugs showed that they have a low oral bioavailability, which may be due to poor water solubility of drug. There are many classical pharmaceutical ways to improve drug dissolution rate such as dissolution in aqueous mixtures with an organic solvent,[7] formation of ß-cyclodextrin complexes,[8] solid dispersions[9] and drug salt form.[10]

During last 20 years a new technology, reducing drug particle size, has been developed to increase drug dissolution rate. According to Noyes–Whitney equation, drugs with smaller particle size have enlarged surface areas which lead to increase dissolution velocity. Higher the dissolution rate together with the resulting higher concentration gradient between gastrointestinal lumen and systemic circulation could further increase oral bioavailability of drugs.[11] A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for oral, topical, parenteral or pulmonary administration. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm.[12] In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosized particles can increase solution velocity and saturation solubility because of the vapor pressure effect. In addition; the diffusional distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. Increase in surface area as well as concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. Dissolution experiments can be performed to quantify the increase in the saturation solubility of a drug when formulated into a nanosuspension.[13]

The stability of the particles obtained in the nanosuspension is attributed to their uniform particle size which is created by various manufacturing processes. The absence of particles with large differences in their size in nanosuspensions prevents the existence of different saturation solubilities and concentration gradients;

consequently preventing the Oswald ripening effect. Ostwald ripening is responsible for crystal growth and subsequently formation of microparticles. It is caused by a difference in dissolution pressure/saturation solubility between small and large particles. Molecules diffuse from the higher concentration area around small particles which have higher saturation solubility to an area around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles.

ADVANTAGES OF NANOSUSPENSIONS:

The major advantages of nanosuspension technology are:[14]

- Provides ease of manufacture and scale-up for large scale production,
- Long-term physical stability due to the presence of stabilizers,
- Oral administration of nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability,
- Rapid dissolution and tissue targeting can be achieved by IV route of administration,
- Reduction in tissue irritation in case of subcutaneous/intramuscular administration,
- Higher bioavailability in case of ocular administration and inhalation delivery,
- Drugs with high log P value can be formulated as nanosuspensions to increase the bioavailability of such drugs,
- Improvement in biological performance due to high dissolution rate and saturation solubility of the drug,
- Nanosuspensions can be incorporated in tablets, pellets, hydrogels and suppositories are suitable for various routes of administration,
- The flexibility offered in the modification of surface properties and particle size, and ease of post-production processing of nanosuspensions enables them to be incorporated in various dosage forms for various routes of administration, thus proving their versatility.

INTERESTING SPECIAL FEATURES OF NANOSUSPENSIONS: [15]

- Increase in saturation solubility and consequently an increase in the dissolution rate of the drug.
- Increase in adhesive nature, thus resulting in enhanced bioavailability.
- Increasing the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility.
- Absence of ostwald ripening, producing physical long term stability as an aqueous suspension.
- Possibility of surface-modification of nanosuspensions for site specific delivery.

CRITERIA FOR SELECTION OF DRUG FOR NANOSUSPENSIONS:

Nanosuspension can be prepared for the API that is having either of the following characteristics:[16]

- ✓ Water insoluble but which are soluble in oil (high log P) **OR** API are insoluble in both water and oils
- ✓ Drugs with reduced tendency of the crystal to dissolve, regardless of the solvent
- ✓ API with very large dose

METHODS OF PREPARATION FOR NANOSUSPENSIONS:

Milling techniques (Nanocrystals or Nanosystems)

Media milling:

Media milling is a technique used to prepare nanosuspensions.[11,12, 17-19] Nanocrystal is a patent protected technology developed by Liversidge et al. In this technique, the drug nanoparticles are obtained by subjecting the drug to media milling. High energy and shear forces generated as a result of impaction of the milling media with the drug provide the necessary energy input to disintegrate the microparticulate drug into nanosized particles. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then the milling media or pearls are rotated at a very high shear rate. The major concern with this method is the residues of milling media remaining in the finished product could be problematic for administration.[17]

FORMULATION OF NANOSUSPENSION[17]

Table 1: Formulation Consideration for nanosuspension

Excipients	Function	Examples
Stabilizers	Wet the drug particles thoroughly, prevent Ostwald's ripening and agglomeration of nanosuspensions, providing steric or ionic barrier	Lecithins, Poloxamers, Polysorbate, Cellulosics, Povidones
Co-surfactants	Influence phase behavior when micro emulsions are used to formulate nanosuspensions	Bile salts, Dipotassium Glycerrhizinate, Transcutol, Glycofurol, Ethanol, Isopropanol,
Organic solvent	Pharmaceutically acceptable less hazardous solvent for preparation of formulation.	Methanol, Ethanol, Chloroform, Isopropanol, Ethyl acetate, Ethyl formate, Butyl lactate, Triacetin, Propylene carbonate, Benzyl alcohol.
Other additives	According to the requirement of the route of administration or the properties of the drug moiety	Buffers, Salts, Polyols, Osmogens, Cryoprotectant etc.

Nanosuspensions are produced by using high-shear media mills or pearl mills. The mill consists of a milling chamber, milling shaft and a recirculation chamber. An aqueous suspension of the drug is then fed into the mill containing small grinding balls/pearls. As these balls rotate at a very high shear rate under controlled temperature, they fly through the grinding jar interior and impact against the sample on the opposite grinding jar wall. The combined forces of friction and impact produce a high degree of particle size reduction. The milling media or balls are made of ceramic-sintered aluminium oxide or zirconium oxide or highly cross-linked polystyrene resin with high abrasion resistance. Planetary ball mill is one example of the equipment that can be used to achieve a grind size below 0.1 μm.

Dry co-grinding:

Nanosuspensions prepared by high pressure homogenization and media milling using pearl-ball mill are wet–grinding processes. Recently, nanosuspensions can be obtained by dry milling techniques. Successful work in preparing stable nanosuspensions using dry-grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media has been reported.[20-22]

Itoh et al reported the colloidal particles formation of many poorly water soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinyl pyrrolidone (PVP) and sodium dodecyl sulfate (SDS). Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxyl propyl methylcellulose (HPMC) and cyclo-dextrin derivatives have been used.[23-25] Physico-chemical properties and dissolution of poorly water soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug.[26,27] Dry co-grinding can be carried out easily and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable amorphous solid can be obtained.

Advantages:

- Media milling is applicable to the drugs that are poorly soluble in both aqueous and organic media.
- Very dilute as well as highly concentrated nanosuspensions can be prepared by handling 1mg/ml to 400mg/ml drug quantity.
- Nanosize distribution of final nanosized products.

Disadvantages:

- Nanosuspensions contaminated with materials eroded from balls may be problematic when it is used for long therapy. (Wet milling technique)
- The media milling technique is time consuming.
- Some fractions of particles are in the micrometer range.
- Scale up is not easy due to mill size and weight.

High Pressure Homogenization:

Homogenization in Aqueous media (Dissocubes)

Homogenization involves the forcing of the suspension under pressure through a valve having a narrow aperture. Dissocube technology was developed by Muller et al. in which, the suspension of the drug is made to pass through a small orifice that results in a reduction of the static pressure below the boiling pressure of water, which leads to boiling of water and formation of gas bubbles. When the suspension leaves the gap and normal air pressure is reached again, the bubbles shrink and the surrounding part containing the drug particles rushes to the center and in the process colloids, causing a reduction in the particle size. Most of the cases require multiple passes or cycles through the homogenizer, which depends on the hardness of drug, the desired mean particle size and the required homogeneity.

Scholer et al. prepared atovaquone nanosuspensions using this technique.[28] To produce a nanosuspension with a higher concentration of solids, it is preferred to start homogenization with very fine drug particles, which can be accomplished by pre-milling.

Homogenization in Non Aqueous Media (Nanopure):

Nanopure is the technology in which suspension is homogenized in water-free media or water mixtures.[29] In the Dissocubes technology the cavitation is the determining factor of the process. But, in contrast to water, oils and oily fatty acids have very low vapor pressure and a high boiling point. Hence, the drop of static pressure will not be sufficient enough to initiate cavitation.

Patents covering disintegration of polymeric material by high- pressure homogenization mention that higher temperatures of about 80°C promoted disintegration, which cannot be used for thermo labile compounds. In nanopure technology, the drug suspensions in the non- aqueous media were homogenized at 0°C or even below the freezing point and hence are called "deep-freeze" homogenization. The results obtained were comparable to Dissocubes and hence can be used effectively for thermo labile substances at milder conditions.

Advantages:

- Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.
- Ease of scale-up and little batch-to-batch variation.[30]
- Narrow size distribution of the nanoparticulate drug present in the final product [31]
- Allows aseptic production of nanosuspensions for parenteral administration.
- Flexibility in handling the drug quantity, ranging from 1 to 400mg mL^{-1}, thus enabling formulation of very dilute as well as highly concentrated nanosuspensions.

Disadvantages:
- Prerequisite of micronized drug particles.
- Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.

Precipitation Method:
Using a precipitation technique, the drug is dissolved in an organic solvent and this solution is mixed with a miscible anti-solvent. In water-solvent mixture the solubility is low and the drug precipitates. Mixing processes vary considerably. Precipitation has also been coupled with high shear processing. The nanoedge process (is a registered trademark of Baxter International Inc. and its subsidiaries) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy.[32]

Nanoedge:
The basic principles of Nanoedge are the same as that of precipitation and homogenization. A combination of these techniques results in smaller particle size and better stability in a shorter time. The major drawback of the precipitation technique, such as crystal growth and long-term stability, can be resolved using the Nanoedge technology. Rapid addition of a drug solution to an anti-solvent leads to sudden super-saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super-saturation when the solubility of the amorphous state is exceeded. The success of drug nanosuspensions prepared by precipitation techniques has been reported.[32-35] In this technique, the precipitated suspension is further homogenized, leading to reduction in particle size and avoiding crystal growth. Precipitation is performed in water using water-miscible solvents such as methanol, ethanol and isopropanol. It is desirable to remove those solvents completely, although they can be tolerated to a certain extent in the formulation. For an effective production of nanosuspensions using the Nanoedge technology, an evaporation step can be included to provide a solvent-free modified starting material followed by high-pressure homogenization.

Nanojet technology:
This technique, called opposite stream or nanojet technology, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure. The high shear force produced during the process results in particle size reduction. Equipment using this principle includes the M110L and M110S microfluidizers (Microfluidics).

The major disadvantage of this technique is the high number of passes through the microfluidizer and the product obtained contains a relatively larger fraction of microparticles.

Emulsions as templates:
Apart from the use of emulsions as a drug delivery vehicle, they can also be used as templates to produce nanosuspensions. The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent. Such solvents can be used as the dispersed phase of the emulsion. There are two ways of fabricating drug nanosuspensions by the emulsification method. In the first method, an organic solvent or mixture of solvents loaded with the drug is dispersed in the aqueous phase containing suitable surfactants to form an emulsion. The organic phase is then evaporated under reduced pressure so that the drug particles precipitate instantaneously to form a nanosuspension stabilized by surfactants. Since one particle is formed in each emulsion droplet, it is possible to control the particle size of the nanosuspension by controlling the size of the emulsion droplet. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading in the emulsion. Originally, organic solvents such as methylene chloride and chloroform were used.[36]

However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Relatively safer solvents such as ethyl acetate and ethyl formate can still be considered for use.[37,38]

The emulsion is formed by the conventional method and the drug nanosuspension is obtained by just diluting the emulsion. Dilution of the emulsion with water causes complete diffusion of the internal phase into the external phase, leading to instantaneous formation of a nanosuspension. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of di-ultrafiltration in order to make it suitable for administration. However, if all the ingredients that are used for the production of the nanosuspension are present in a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspension.

Advantages:
- Use of specialized equipment is not necessary.
- Particle size can easily be controlled by controlling the size of the emulsion droplet.
- Ease of scale-up if formulation is optimized properly.

Disadvantages:
- Drugs that are poorly soluble in both aqueous and organic media cannot be formulated by this technique.
- Safety concerns because of the use of hazardous solvents in the process.
- Need for di-ultrafiltration for purification of the drug nanosuspension, which may render the process costly.

- High amount of surfactant / stabilizer is required as compared to the production techniques described earlier.

The production of drug nanosuspensions from emulsion templates has been successfully applied to the poorly water-soluble and poorly bioavailable anti-cancer drug mitotane, where a significant improvement in the dissolution rate of the drug (five-fold increase) as compared to the commercial product was observed.[39]

Microemulsions as templates:
Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant and co surfactant.[40]

Their advantages, such as high drug solublization, long shelf life and ease of manufacture, make them an ideal drug delivery vehicle. Recently, the use of microemulsions as templates for the production of solid lipid nanoparticles[41] and polymeric nanoparticles[42] has been described. Taking advantage of the micro emulsion structure, one can use microemulsions even for the production of nanosuspensions.[43] The drug can be either loaded in the internal phase or preformed microemulsions can be saturated with the drug by intimate mixing. The suitable dilution of the microemulsion yields the drug nanosuspension by the mechanism described earlier. The influence of the amount and ratio of surfactant to co surfactant on the uptake of internal phase and on the globule size of the microemulsion should be investigated and optimized in order to achieve the desired drug loading. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of di-ultrafiltration in order to make it suitable for administration. However, if all the ingredients that are used for the production of the nanosuspension are present in a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspension. The advantages and disadvantages are the same as for emulsion templates. The only added advantage is the need for less energy input for the production of nanosuspensions by virtue of microemulsions.

Supercritical fluid method:
Supercritical fluid technology can be used to produce nanoparticles from drug solutions. The various methods attempted are rapid expansion of supercritical solution process (RESS), supercritical anti-solvent process and precipitation with compressed anti-solvent process (PCA). The RESS involves expansion of the drug solution in supercritical fluid through a nozzle, which leads to loss of solvent power of the supercritical fluid resulting in precipitation of the drug as fine particles. In the PCA method, the drug solution is atomized into a chamber containing compressed CO_2. As the solvent is removed, the solution gets supersaturated and thus precipitates as fine crystals. The supercritical anti- solvent process uses a supercritical fluid in which a drug is poorly soluble and a solvent for the drug that is also miscible with the supercritical fluid. The drug solution is injected into the supercritical fluid and the solvent gets extracted by the supercritical fluid and the drug solution gets supersaturated. The drug is then precipitated as fine crystals. The disadvantages of the above methods are use of hazardous solvents and use of high proportions of surfactants and stabilizers as compared with other techniques, particle nucleation overgrowth due to transient high supersaturation, which may also result in the development of an amorphous form or another undesired polymorph.[44]

POST-PRODUCTION PROCESSING:
Post-production processing of nanosuspensions becomes essential when the drug candidate is highly susceptible to hydrolytic cleavage or chemical degradation. Processing may also be required when the best possible stabilizer is not able to stabilize the nanosuspension for a longer period of time or there are acceptability restrictions with respect to the desired route. Considering these aspects, techniques such as lyophillization or spray drying may be employed to produce a dry powder of nano-sized drug particles. Rational selection has to be made in these unit operations considering the drug properties and economic aspects.[17]

CHARACTERIZATION OF NANOSUSPENSION:
Mean particle size and particle size distribution
The mean particle size and particle size distribution are important characterization parameters as they influence the saturation solubility, dissolution velocity, physical stability as well as biological performance of nanosuspensions. It has been indicated by Muller and Peters (1998) that saturation solubility and dissolution velocity show considerable variation with the changing particle size of the drug.[13] Photon correlation spectroscopy (PCS) can be used for rapid and accurate determination of the mean particle diameter of nanosuspensions. Moreover, PCS can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1–0.25 indicates a fairly narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution. No logarithmic normal distribution can definitely be attributed to such a high PI value. Although PCS is a versatile technique, because of its low measuring range (3nm to 3µm) it becomes difficult to determine the possibility of contamination of the nanosuspension by micro particulate drugs (having particle size greater than 3µm). Hence, in addition to PCS analysis, laser diffractometry (LD) analysis of nanosuspensions should be carried out in order to detect as well as quantify the drug

microparticles that might have been generated during the production process.

Various methods are available for particle size measurement.[45] Laser diffractometry yields a volume size distribution and can be used to measure particles ranging from 0.05–80 µm and in certain instruments particle sizes up to 2000µm can be measured. The typical LD characterization includes determination of diameter 50% LD (50) and diameter 99% LD (99) values, which indicate that either 50 or 99% of the particles are below the indicated size. The LD analysis becomes critical for nanosuspensions that are meant for parenteral and pulmonary delivery. Even if the nanosuspension contains a small number of particles greater than 5–6 µm, there could be a possibility of capillary blockade or emboli formation, as the size of the smallest blood capillary is 5–6 µm. It should be noted that the particle size data of a nanosuspension obtained by LD and PCS analysis are not identical as LD data are volume based and the PCS mean diameter is the light intensity weighted size. The PCS mean diameter and the 50 or 99% diameter from the LD analysis are likely to differ, with LD data generally exhibiting higher values. The nanosuspensions can be suitably diluted with deionized water before carrying out PCS or LD analysis.

Crystalline state and particle morphology:
The assessment of the crystalline state and particle morphology together helps in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nano sizing. Additionally, when nanosuspensions are prepared drug particles in an amorphous state are likely to be generated. Hence, it is essential to investigate the extent of amorphous drug nanoparticles generated during the production of nanosuspensions. The changes in the physical state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis[30,31] and can be supplemented by differential scanning Calorimetry.[46] In order to get an actual idea of particle morphology, scanning electron microscopy is preferred.[31]

Particle charge (zeta potential):
The particle charge is of importance in the study of the stability of the suspensions. Usually the zeta potential of more than ±40mV will be considered to be required for the stabilization of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ± 20mV of zeta potential is required.

Surface charges can arise from (i) ionization of the particle surface or (ii) adsorption of ions (such as surfactants) onto the surface. Typically, the surface charge is assessed through measurements of the zeta potential. Zeta potential is the potential at the hydrodynamic shear plane and can be determined from the particle mobility under an applied electric field.[47] The mobility will depend on the effective charge on the surface. Zeta potential is also a function of electrolyte concentration.

Solubility study:
The solubility can also define as the ability of one substance to form a solution with another substance. The substance to be dissolved is called as solute and the dissolving fluid in which the solute dissolve is called as solvent, which together form a solution.

The main advantage associated with the nanosuspensions is improved saturation solubility. This is studied in different physiological solutions at different pH. Kelvin equation and the Ostwald-Freundlich equations can explain increase in saturation solubility. Determination of this parameter is useful to assess *in vivo* performance of the formulation also.[48]

In vitro dissolution study:
Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. It can be considered as a specific type of certain heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid surface.[49]

In vitro dissolution screening should be the first line of biopharmaceutical evaluation of nano-formulations. Since oral nano-formulations are designed to disperse in the stomach contents, dissolution in Simulated Gastric Fluid (SGF) should provide an initial estimate of the dissolution rate enhancement. For insoluble compounds, where dissolution is expected to mainly occur in the intestinal region, further *in vitro* testing in simulated intestinal media will provide additional insight on expected bio-performance. Several reports in the literature report an increased *in vitro* dissolution rate for nanosized APIs. However one should keep in mind that the small particle size for nano-formulations may pose additional needs in terms of analytical sample handling and processing to ensure that no undissolved API is assayed during the dissolution test. Filtering through smaller pore size filters or (ultra)centrifugation to separate un-dissolved API has been implemented in the literature to address this issue.[50]

Stability of Nanosuspensions:
Stability of the suspensions is dependent on the particle size. As the particle size reduces to the nanosize the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening effect and improving the stability of the suspension by providing a steric or ionic barrier. Typical examples of stabilizers used in

nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones etc.[51]

In-vivo biological performance:

The establishment of an *in-vitro/in-vivo* correlation and the monitoring of the *in-vivo* performance of the drug is an essential part of the study, irrespective of the route and the delivery system employed. It is of the utmost importance in the case of intravenously injected nanosuspensions since the nanosuspensions: a promising drug delivery strategy *in-vivo* behaviour of the drug depends on the organ distribution, which in turn depends on its surface properties, such as surface hydrophobicity and interactions with plasma proteins.[52-55] In fact, the qualitative and quantitative composition of the protein absorption pattern observed after the intravenous injection of nanoparticles is recognized as the essential factor for organ distribution.[52-56] Hence, suitable techniques have to be used in order to evaluate the surface properties and protein interactions to get an idea of *in-vivo* behaviour. Techniques such as hydrophobic interaction chromatography can be used to determine surface hydrophobicity,[57] whereas 2-D PAGE[52] can be employed for the quantitative and qualitative measurement of protein adsorption after intravenous injection of drug nanosuspensions in animals.

APPLICATIONS OF NANOSUSPENSIONS IN DRUG DELIVERY:

Parenteral administration:

From the formulation perspective, nanosuspensions meet almost all the requirements of an ideal drug delivery system for the parenteral route. Since the drug particles are directly nanosized, it becomes easy to process almost all drugs for parenteral administration. Hence, nanosuspensions enable significant improvement in the parenterally tolerable dose of the drug, leading to a reduction in the cost of the therapy and also improved therapeutic performance. The maximum tolerable dose of paclitaxel nanosuspension was found to be three times higher than the currently marketed Taxol, which uses Cremophore EL and ethanol to solubilize the drug.[58]

Nanosuspensions can be administered via different parenteral administration routes ranging from intra-articular via intra peritonal to intravenous injection. For administration by the parenteral route, the drug either has to be solubilized or has particle/globule size below 5µm to avoid capillary blockage. In this regard, liposomes are much more tolerable and versatile in terms of parenteral delivery. However, they often suffer from problems such as physical instability, high manufacturing cost and difficulties in scale-up. Nanosuspensions would be able to solve the problems mentioned above. In addition, nanosuspensions have been found to increase the efficacy of parenterally administered drugs.[29]

Oral administration:

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. The efficacy or performance of the orally administered drug generally depends on its solubility and absorption through the gastrointestinal tract. Hence, a drug candidate that exhibits poor aqueous solubility and / or dissolution rate limited absorption is believed to possess slow and/or highly variable oral bioavailability. Danazol is poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a nanosuspension as compared to the commercial danazol macrosuspension Danocrine. Danazol nanosuspension led to an absolute bioavailability of 82.3%, where as the marketed danazol suspension Danocrine was 5.2% bioavailable.[11]

Nanosizing of drugs can lead to a dramatic increase in their oral absorption and subsequent bioavailability. Improved bioavailability can be explained by the adhesiveness of drug nanoparticles to the mucosa, the increased saturation solubility leading to an increased concentration gradient between gastrointestinal tract lumen and blood as well as the increased dissolution velocity of the drug. Aqueous nanosuspensions can be used directly in a liquid dosage form and a dry dosage form such as tablet or hard gelatin capsule with pellets. The aqueous nanosuspension can be used directly in the granulation process or as a wetting agent for preparing the extrusion mass pellets. A similar process has been reported for incorporating solid lipid nanoparticles into pellets. Granulates can also be produced by spray drying of nanosuspensions.[29]

Ophthalmic drug delivery:

Nanosuspensions could prove to be vital for drugs that exhibit poor solubility in lachrymal fluids. Suspensions offer advantages such as prolonged residence time in a cul-de-sac, which is desirable for most ocular diseases for effective treatment and avoidance of high tonicity created by water soluble drugs. Their actual performance depends on the intrinsic solubility of the drug in lachrymal fluids. Thus the intrinsic dissolution rate of the drug in lachrymal fluids controls its release and ocular bioavailability. However, the intrinsic dissolution rate of the drug will vary because of the constant inflow and outflow of lachrymal fluids. One example of a nanosuspension intended for ophthalmic controlled delivery was developed as a polymeric nanosuspension of ibuprofen.[59] This nanosuspension is successfully prepared using Eudragit RS100 by a quasi-emulsion and solvent diffusion method.

Nanosuspensions of glucocorticoid drugs; hydrocortisone, prednisolone and dexamethasone enhance rate, drug absorption and increase the duration of drug action.[60] To achieve sustained release of the drug for a stipulated time period, nanosuspensions can be incorporated in a suitable hydro-gel base or mucoadhesive base or even in ocular inserts. The bio-erodible as well as water soluble/permeable polymers possessing ocular tolerability[61] could be used to sustain the release of the medication. The polymeric nanosuspension of flurbiprofen has been successfully

formulated using acrylate polymers such as Eudragit RS 100 and Eudragit RL 100.[62-64] The polymeric nanosuspensions have been characterized for drug loading, particle size, zeta potential, in-vitro drug release, ocular tolerability and *in-vivo* biological performance. The designed polymeric nanosuspensions revealed superior *in-vivo* performance over the existing marketed formulations and could sustain drug release for 24 h. The scope of this strategy could be extended by using various polymers with ocular tolerability.

Pulmonary drug delivery:

Nanosuspensions may prove to be an ideal approach for delivering drugs that exhibit poor solubility in pulmonary secretions. Currently such drugs are delivered as suspension aerosols or as dry powders by means of dry powder inhalers. The drugs used in suspension aerosols and dry powder inhalers are often jet milled and have particle sizes of microns.

Because of the microparticulate nature and wide particle size distribution of the drug moiety present in suspension aerosols and dry powder inhalers, some disadvantages are encountered: like limited diffusion and dissolution of the drug at the site of action, rapid clearance of the drug from the lungs, less residence time for the drugs, unwanted deposition of the drug particles in pharynx and mouth. [65,66] The ability of nanosuspensions to offer quick onset of action initially and then controlled release of the active moiety is highly beneficial and is required by most pulmonary diseases. Moreover, as nanosuspensions generally contain a very low fraction of microparticulate drug, they prevent unwanted deposition of particles in the mouth and pharynx, leading to decreased local and systemic side-effects of the drug. Additionally, because of the nanoparticulate nature and uniform size distribution of nanosuspensions, it is very likely that in each aerosol droplet at least one drug nanoparticle is contained, leading to even distribution of the drug in the lungs as compared to the microparticulate form of the drug. In conventional suspension aerosols many droplets are drug free and others are highly loaded with the drug, leading to uneven delivery and distribution of the drug in the lungs. Nanosuspensions could be used in all available types of nebulizer. However, the extent of influence exerted by the nebulizer type as well as the nebulization process on the particle size of nanosuspensions should be ascertained.

Bioavailability enhancement:

Drug with poor solubility or permeability in gastrointestinal tract leads to poor oral bioavailability. Nanosuspension resolves the problem of poor bioavailability by solving the problem of poor solubility, and poor permeability across the membranes. Dissolution rate was increased in diclofenac when formulated in nanosuspension form from 25% to 50% in SGF and H_2O while in case of SIF it was increased from 10% to 35% as compared to coarse suspension.[67] Bioavailability of poorly soluble, a COX-2 inhibitor, celecoxib was improved using a nanosuspension formulation. The crystalline nanosized celecoxib alone or in tablet showed a dramatic increase of dissolution rate and extent compared to micronized tablet. Spironolactone and budesonide are poorly soluble drugs. The higher flux contributes to the higher bioavailability of nanosuspension formulation. The bioavailability of poorly soluble fenofibrate following oral administration was increased compared to the suspensions of micronized fenofibrate.[68] Significant difference ($p< 0.05$) was observed between the fluxes from saturated solution Vs nanosuspension at all concentrations of surfactant. Oral administration of micronized Amphotericin B did not show any significant effect. However administration in nanosuspension form, showed a significant reduction ($P < 0.5\%$) of the liver parasite load by 28.6%, it indicates that the nanosuspension of amphotericin B has high systemic effect and superior oral uptake in nanosuspension form.[69]

The poor oral bioavailability of the drug may be due to poor solubility, poor permeability or poor stability in the gastrointestinal tract (GIT). Nanosuspensions resolve the problem of poor bioavailability by solving the twin problems of poor solubility and poor permeability across the membrane. Bioavailability of poorly soluble oleanolic acid, a hepato-protective agent, was improved using a nanosuspension formulation. The therapeutic effect was significantly enhanced, which indicated higher bioavailability. This was due to the faster dissolution (90% in 20 min) of the lyophilized nanosuspension powder when compared with the dissolution from a coarse powder (15% in 20 min).[29]

Target drug delivery:

Nanosuspensions can also be used for targeted delivery as their surface properties and *in vivo* behavior can easily be altered by changing either the stabilizer or the milieu. Their versatility, ease of scale up and commercial product enable the development of commercial viable nanosuspensions for targeted delivery. The engineering of stealth nanosuspensions by using various surface coatings for active or passive targeting of the desired site is the future of targeted drug delivery systems. Targeting of *Cryptosporidium parvum*, the organism responsible for cryptosporidiosis, was achieved by using surface modified mucoadhesive nanosuspensions of bupravaquone.[70,71] Similarly, conditions such as pulmonary aspergillosis can easily be targeted by using suitable drug candidates, such as amphotericin B, in the form of pulmonary nanosuspensions instead of using stealth liposomes.[72](Review 8)

Nanosuspensions can also be used for targeting as their surface properties and changing of the stabilizer can easily alter the *in vivo* behavior. The drug will be up taken by the mononuclear phagocytic system to allow regional-specific delivery. This can be used for targeting anti-mycobacterial,

fungal or leishmanial drugs to the macrophages if the infectious pathogen is persisting intracellularly.[73]

Topical formulations:

Drug nanoparticles can be incorporated into creams and water-free ointments. The nanocrystalline form leads to an increased saturation solubility of the drug in the topical dosage form, thus enhancing the diffusion of the drug into the skin.[74-78]

Mucoadhesion of the nanoparticles:

Nanosuspension containing drug nanoparticles orally diffuse into the liquid media and rapidly encounter the mucosal surface. The particles are immobilized at the intestinal surface by an adhesion mechanism referred to as "bioadhesion." From this moment on, the concentrated suspension acts as a reservoir of particles and an adsorption process takes place very rapidly. The direct contact of the particles with the intestinal cells through a bioadhesive phase is the first step before particle absorption.[66] The adhesiveness of the nanosuspensions not only helps to improve bioavailability but also improves targeting of the parasites persisting in the GIT.

MARKETED PRODUCTS BASED ON NANOSUSPENSION:

All the products based on nanosuspension have been approved by the FDA from the year 2000 on. All listed products are based on top-down approaches, eight relying on media milling and one on high- pressure homogenization. Although the bottom-up approaches hold tremendous potential with respect to improving bioavailability in obtaining smaller particle sizes (< 100nm) and amorphous drug particles, no commercial application of these systems has yet been realized. A third remarkable point is that all commercial products are intended for oral delivery. This is an illustration of the general preference of the oral route, since it avoids the pain and discomfort associated with injections and is more attractive from a marketing and patient compliance perspective. Finally, the major advantage of nanocrystals for oral delivery is generally regarded as being on the increased specific surface area of the particles. However, EMEND® and Triglide™ are formulated as nanosuspension to reduce fed/fasted variability.[79]

CONCLUSION:

Nanotechnology is an incredible field in the medicine. Since solubility is a crucial factor for drug effectiveness, it is a challenging task to formulate any poorly soluble drug in the industry in conventional dosage forms. Nano-technique is simple; fewer requirements of excipients are there for formulation of dosage form. Attractive features, such as reduction of particles size up to submicron level lead to a significant increase in dissolution velocity as well as saturation solubility. Improved bio-adhesiveness, versatility in surface modification and ease of post-production processing have widened the applications of nanosuspensions for various routes. Nanosuspension technology can be combined with traditional dosage forms: tablets, capsules, pellets and also can be used for parenteral products. Production techniques such as media milling and high-pressure homogenization have been successfully employed for large scale production of nanosuspensions. The advances in production methodologies using emulsions or micro emulsions as templates and precipitation method have provided still simpler approaches for production but with limitations. Further investigation in this regard is still essential. Some of the patented commercially productive technologies have been reviewed and if the patent period ends for such techniques there would be a revolutionary advancement in formulation of poorly water soluble drugs.

Table 2: Current marketed pharmaceutical products based on nanocrystals.[80]

Product	Drug Compound	Company	Manufacturing Technique	Technology
RAPAMUNE®	Sirolimus	Wyeth	MM	Elan Nanocrystals®
EMEND®	Aprepitant	Merck	MM	Elan Nanocrystals®
TriCor®	Fenofibrate	Abbott	MM	Elan Nanocrystals®
MEGACE®ES	Megestrol Acetate	PAR Pharmaceutical	MM	Elan Nanocrystals®
Avinza®	Morphine Sulphate	King Pharmaceutical	MM	Elan Nanocrystals®
Focalin®XR	Dexmethylphenidate Hydrochloride	Novartis	MM	Elan Nanocrystals®
Ritalin®LA	Methylphenidate Hydrochloride	Novartis	MM	Elan Nanocrystals®
Zanaflex Capsules™	Tizanidine Hydrochloride	Acorda	MM	Elan Nanocrystals®
Triglide™	Fenofibrate	First Horizon Pharmaceutical	HPH	Skye Pharma IDD® - P Technology

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This is to certify that research article entitled "Screening of Formulating and Processing Parameters on Candesartan Cilexetil Nanosuspension Prepared by Nanoprecipitation-Ultrasonication Technique." *by* **Jalpa S. Paun,and Hemraj M. Tank**, *have been accepted by journal and published in October - December, 8[4] 2016 issue.*

With Regards

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This is to certify that research manuscript of Ms./Mr. Jalpa S. Paun entitled 'Screening of Formulating and Processing Parameters for Ziprasidone Hydrochloride Nanosuspension Prepared by Nanoprecipitation-Ultrasonication Technique' with manuscript number: **JPSBR16RS6012**, has been received, reviewed and accepted for publication in Journal of Pharmaceutical Science and Bioscientific Research (ISSN 2277–3681), in Volume 6, Issue 6: November 2016 after getting reviewed by two reviewers of journal. The JPSBR Impact Factor is 2.633.

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Thank You,

Dr. N. V. Shah

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