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Design, optimisation and characterisation of cisapride nanosuspension

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Abstract
For the past few decades, there has been a considerable research carried out in the field of Nanotechnology especially in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Nanotechnology in the form of nanoparticles has great potential in the drug delivery field. A poorly water-soluble drug indicates insufficient bioavailability following oral administration, resulting in fluctuating plasma level. Nanosuspensions of poorly water-soluble drugs are known to increase the oral bioavailability. In the present work, Cisapride Nanosuspension was prepared by Precipitation technique using Tween 80 as stabiliser. Formulation 5 was the best formulation with a % Entrapment Efficiency of 93.42%, % Drug Release of 95.6%, Zeta Potential of -17.3mV, Polydispersity of 0.222 and Particle Size of 438nm. Thus Formulation 5 was selected as the optimised formulation.

Keywords: nanotechnology, nanosuspension, cisapride, precipitation technique, optimisation

1. Introduction
Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} m [1]. It is estimated that more than one third of the compounds being developed by the pharmaceutical industry are poorly water soluble [2]. An important property of a drug substance is solubility especially aqueous system solubility [3]. Many formulation approaches are available to solve the problems of low solubility and low bioavailability of drugs [4]. The conventional approaches include micronisation, use of fatty solutions, use of penetration enhancer or co-solvents, surfactant dispersion method, salt formation, precipitation, etc., but still, these techniques having limited utility in solubility enhancement for poorly soluble drugs [5]. Additional approaches are vesicular system like liposomes, dispersion of solids, emulsion and microemulsion methods and inclusion complexes with cyclodextrins which show beneficial effect as drug delivery system but major problems of these techniques are lack of universal applicability to all drugs [6]. The drug microparticles/micronized drug powder is transferred to drug nanoparticles by techniques like Bottom-Up Technology and Top-Down Technology [7]. The major goals in designing nanoparticle drug delivery system is to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen [8].
Nanosuspensions are colloidal dispersed solid of nanosized drug particles stabilized by surfactants [9]. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1 μm in size. Size reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility [10].
Cisapride is a gastroprokinetic agent, a drug that increases motility in the upper gastrointestinal tract [11]. It acts directly as a serotonin 5-HT_4 receptor agonist and indirectly as a parasympathomimetic. Cisapride has been used for the treatment of gastroesophageal reflux disease (GERD). It also increases gastric emptying in people with diabetic gastroparesis [12].

2. Materials and Methods
Cisapride, Polyvinylpyrrolidone (PVP K30), Acetone, Tween 80 (Himedia, Hyderabad), Lutrol-Poloxomer (Torrent Pharmaceutical Ltd). All other materials and reagents used were of analytical grade of purity.

2.1 Preparation of Cisapride nanosuspension:
Nanosuspensions were prepared by the nanoprecipitation technique. Cisapride was dissolved in acetone at room temperature. This was poured into water containing different combinations of Tween 80, PVP K30 and Lutrol maintained at room temperature and subsequently stirred on magnetic stirrer to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 hour followed by sonication for 1 hour. Thus, the nanosuspension was formed and preserved for further use.

Table 1: Formulation Table for the Preparation of Cisapride Nanosuspension

Ingredients	F1	F2	F3	F4	F5	F6
Cisapride	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
PVP	50 mg	50 mg	50 mg	50 mg	75 mg	75 mg
Lutrol (Poloxamer)	-	1 mg	1 mg	2 mg	2 mg	2 mg
Tween 80	1 mg	-	1 mg	1 mg	1 mg	2 mg
Acetone	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Water QS	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

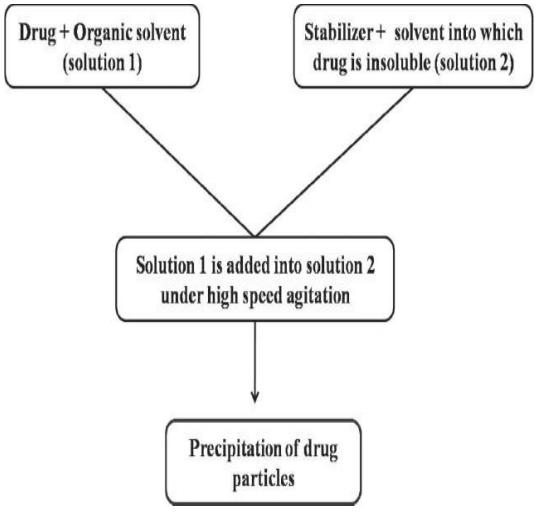

Fig 1: Flow Chart for Preparation of Nanosuspension Evaluation of Nanosuspension

2.2 Evaluation of Nanosuspension

- **Drug Content**

About 1 ml of nanosuspension preparation was taken and diluted appropriately with 0.1N HCl and the drug content of the samples were estimated by UV-Visible spectrophotometer at 276nm.

- **Redispersibility**

Redispersibility of nanosuspension stored in vials were determined by tilting the vial bottle up and down with hand till the sediment was uniformly dispersed in aqueous phase and the number of times tilted was noted and rated as fast, medium and slow. The following grading was given:

a) 1-2 times-Very fast
b) 2-5 times-Fast
c) 5-10 times-Medium
d) More than 10 times-Slow

- **Saturation Solubility Studies**

It was carried out by adding excess amount of the sample to distilled water (2 ml) and the samples were subjected to shaking in screw-capped vials for 24 hrs. The samples were further taken in test tubes and centrifuged at 1000 rpm for 10 mins after that samples were filtered through 0.22 µm membrane filter and the filtrate was diluted appropriately with distilled water and the drug content was estimated in UV-Visible spectrophotometer at 276nm.

- **Evaluation of Mean Particle Size and Particle Size Distribution**

The mean size of Cisapride nanosuspensions were measured by photon correlation spectroscopy (PCS) using a Zetasizer nano-ZS. The mean particle size and the span of particle size distribution (polydispersity index PI) are two important characteristic parameters because they affect the saturation solubility, dissolution rate, physical stability, even in-vivo behavior of nanosuspensions.

- **Evaluation of Surface Charge (Zeta Potential)**

Zeta potential gives certain information about the surface charge properties and further the long-term physical stability of the nanosuspensions. The particle size distribution and zeta potential of nanoparticles were analyzed by photon correlation spectroscopy (PCS) using a Zetasizer nano-ZS. The samples were placed into disposable plastic cuvette, and all measurements were carried out at 25° C.

- **Scanning Electron Microscopy**

The shape and surface morphology of the nanoparticles were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

- **Evaluation of % Entrapment Efficiency**

The Nanosuspension with known amount of drug incorporated was centrifuged at done at speed of 15000 rpm for 30 minutes. The supernatant solution was separated and the free drug present in the supernatant was analysed by UV-Visible spectrophotometer at 318 nm using a calibration curve. The amount of drug not entrapped in the supernatant was calculated. The amount of drug entrapped and percentage entrapment was determined from drug not entrapped. Standard deviation was determined for 3 trials. The entrapment efficiency was calculated as follows:

$$\text{Entrapment efficiency} = \frac{\text{Total Drug content} - \text{Free dissolved Drug}}{\text{Drug amount used}} \times 100$$

- **Evaluation of % Drug Release Study**

The dissolution test was performed using a USP XXIV rotating paddle apparatus with a Pharmatest PTW SIII (Pharma Test, Hamburg, Germany) at 37 °C and a rotating speed of 100 rpm in 500 ml of Phosphate buffer solution pH 6.88 and methanol, within the concentration range of 2-16 µg/ml and 2-18 µg/ml respectively. Certain amounts of drugs were dispersed in the dissolution medium. An aliquot of 5 ml was withdrawn at different time intervals with a sterile syringe filter (pore size 0.22 µm). The withdrawn volume was replenished immediately with the same volume of the pre warm (37°C) dissolution medium in order to keep the total

volume constant. Percent of cisapride dissolved at various time intervals was withdrawn and assayed by UV spectroscopy at λmax=276nm and calculated from the regression equation generated from the suitably constructed calibration curve.

3. Result and Discussion

- **Physical Appearance**

Physical appearance of the received Cisapride was complies with BP standard. It was white crystalline powder.

- **Melting point**

The melting point of Cisapride was done by capillary method. Meting point found after triplicate study was 108-110˚C complies with pharmacopeias specification.

- **FTIR Spectroscopy**

The drug was identified by FTIR spectrum of sample which shows the characteristic absorption of various functional groups of Cisapride.

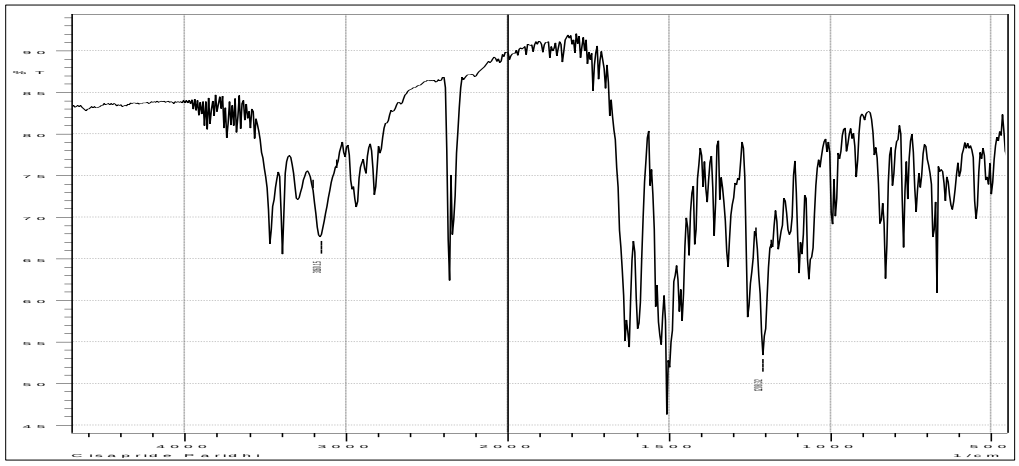

Fig 2: FTIR spectrum of Cisapride

Table 2: Positions of some characteristic absorption of Cisapride

Wave number (cm^{-1})	Characteristic absorption
3250-3300	N-H stretching
2960	CH alkane stretching
1650	CO-NH stretching (C=0)
1500	NH bending
1208	CN aromatic amine

3.1 Light absorption by UV Spectrophotometer

The absorption maxima of 0.002% w/v solution in methanol of Cisapride is found to be 276 nm which complies with the given specification.

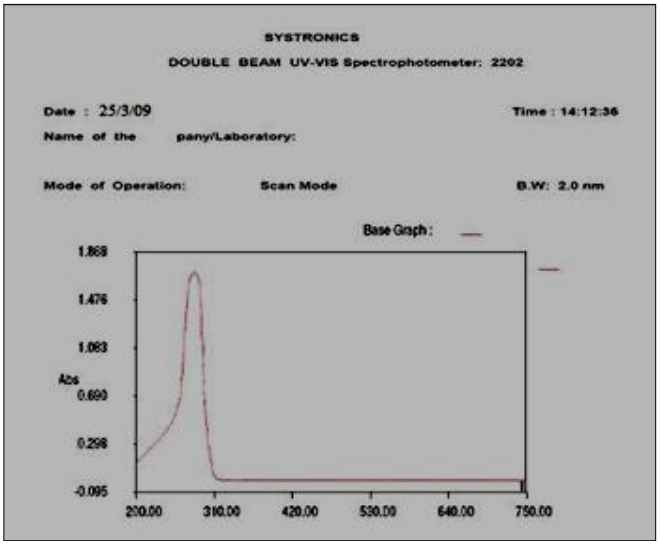

Fig 3: Light absoption by UV Spectrophotometer of Cisapride

3.2 Partition Coefficient

Partition coefficient is a measure of drug lipophilicity and an indication of its ability to cross the biological membrane. The partition coefficient value of Cisapride was found to be 3.84 which complies with the standard

3.3 Calibration plot in phosphate buffer pH 6.8 and Methanol

UV spectra of Cisapride solution in Phosphate buffer pH 7.4 and methanol shows absorbance maxima (λ max) at wavelength 273nm, and 276nm respectively. The standard curves of drug were prepared in Phosphate buffer pH 6.8 and methanol, within the concentration range of 2-16 µg/ml and 2-18 µg/ml respectively. A straight line with Regression coefficient (R^2) 0.980 and 0.981 obtained for Phosphate buffer pH 6.8, methanol respectively which indicates that drug follows Beer's law and signifying that a linear relationship existed between absorbance and concentration of drug.

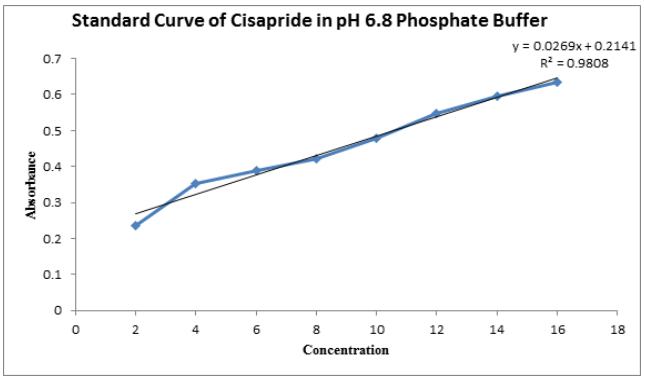

Fig 4: Standard Curve of Cisapride in pH 6.8 Phosphate Buffer

3.4 Particle size and Polydispersity index

The particle size of all prepared nanosuspension were measured by photon correlation spectroscopy and reported. The particles were found to be in the range of 438nm to 832nm. The optimized batch F5 had a Z-average particle size of 438nm with 0.222 Polydispersity index, which indicate the particles are uniform distribution. 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.

3.5 Zeta potential

Zeta potential analysis was performed to get the information about the surface properties of the nanocrystals. The zeta potential of the prepared nanosuspension for optimized batch F5 was found to be -17.3mV. Zeta potential gives certain information about the surface charge properties and further the long-term physical stability of the nanosuspensions. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient.

3.6 % Entrapment Efficiency

The % Entrapment Efficiency of the prepared nanosuspension for optimized batch F5 was found to be 93.42%

3.7 % Drug Release

The % Drug Release of the prepared nanosuspension for optimized batch F5 was found to be 95.6%

Table 3: Evaluation Table for the Preparation of Cisapride Nanosuspension

Parameters	F1	F2	F3	F4	F5	F6
Colour	Off white	Off white	Off white	Off white	Off white	Off white
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Particle Size (nm)	832	621	589	522	438	603
Polydispersity Index	0.524	0.351	0.263	0.258	0.222	0.261
Zeta Potential (mV)	-11.2	-13.5	-15.5	-17.1	-17.3	-14.3
% Entrapment Efficiency	58.3%	67.81%	72.13%	83.3%	93.42%	90.12%
% Drug Release	92.2%	94.3%	93.1%	93.5%	95.6%	94.1%

4. Conclusion

The best nanosuspension study shows that nanosuspension Formulation 5 was the best formulation with a % Entrapment Efficiency of 93.42%, % Drug Release of 95.6%, Zeta Potential of -17.3mV, Polydispersity of 0.222 and Particle Size of 438nm. Thus Formulation 5 is the optimised formulation.

Preparation of nanoparticle drug delivery systems can improve the solubility and bioavailability of poorly water soluble drugs. Formulation of poorly water-soluble drugs has always been a challenging problem for scientists. Nanosuspensions represent a promising alternative to current delivery systems aspiring to improve the biopharmaceuticals performance of drugs with low water solubility. Nanosuspensions will continue to enable novel applications in drug delivery and solve problems associated with the delivery of poorly soluble drug.

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