ABSTRACT
Diacerein is a poorly water-soluble drug with relatively low bioavailability. The effect of β-cyclodextrin on the aqueous solubility and dissolution rate of diacerein was investigated. Phase solubility profile indicated that the solubility of diacerein was significantly increased in presence of β-cyclodextrin and it was classified as A L-type, indicating the 1:1 stoichiometric inclusion complexes. The complexes formed were quite stable. The solid complexes prepared by physical mixture, co-evaporation/solid dispersion, kneading method and precipitation method was characterized using differential scanning calorimetry, powder x-ray diffractometry and FTIR. In vitro study showed that the solubility and dissolution rate of diacerein was significantly improved by complexation with β-cyclodextrin.

Key words: Diacerein, β-cyclodextrin, Formulation, Phase solubility profile, Characterization.

INTRODUCTION
Diacerein is an anthroquinone derivative 9, 10-dihydro-4, 5 bis (acetyl)-9, 10-dioxo-2-anthracene carboxylic acid, mainly used in osteoarthritis. From the anthronoid, Diacerein and its metabolites Rhine brake both the production of zylokine (IL-1, IL-6, IL-16 TNFa) which at the point of the inflammation cascade and proteolytic enzyme. Additionally diacerein stimulates the synthesis of cartilage components such as proteoglycane, glykosaminoglycane and hyaluronsaure1-2. It is not official in any pharmacopoeia. The solubility of a poorly soluble drug can be altered in many ways, such as modification of drug crystal forms, addition of co-solvents, addition of surfactant, and complexation with cyclodextrin etc. Among these possibilities the cyclodextrin approach is of particular interest. Diacerein is very sparingly soluble in water (0.01 mg/ml). The poor solubility and wettability of diacerein give rise to difficulties in pharmaceutical formulation meant for oral or parental use, which may lead to variation in bioavailability. To overcome these difficulties, increase in the aqueous solubility of diacerein is an important goal hence in this investigation inclusion complexation of diacerein was tried with β-cyclodextrin with the aim to improve its pharmaceutical properties (i.e. aqueous solubility and dissolution properties).

Cyclodextrin are cyclic (α-1, 4)-linked oligosaccharides of α-D-glucopyranose, containing a relatively hydrophobic central cavity and hydrophilic outer surface. Owing to lack of free rotation about the bonds connecting the glucopyranose units, the cyclodextrin are not perfectly cylindrical molecules but the toroidal or cone shaped. Based on this architecture, the primary hydroxyl groups are located on the narrow side of the cone shape, while the secondary hydroxyl groups are located on the wider edge. During the past two decades, cyclodextrin and their derivatives have been
of considerable interest in the pharmaceutical field because of their potential to form complexes with a variety of drug molecules. Cyclodextrin are used to increase the solubility of water insoluble drug through inclusion complexes formulation. The hydrophobic cavity of cyclodextrin is capable of trapping a variety of molecules within to produce inclusion complexes. Many advantages of drugs complex with cyclodextrin have been reported in scientific literature which includes-increased solubility, enhanced bioavailability, improved stability, masking of bad test or odor, reduced volatility, transformation of liquid or gas into solid form reduced side effect, and the possibility of a drug release system, etc. The solid state characteristics of the drug after preparation and during storage will depend on the processing variables as well as the characteristics of the system. When the objective of the formulation is to attain faster dissolution rates, presence of amorphous of the drug and improved dissolution rate would therefore be a result of presence of amorphous high energy forms of the drug as well as the ability of the cyclodextrin to form a soluble complexes with the drug, the performance of the product over the shelf life would depend on the ability of the cyclodextrin to prevent crystallization of the amorphous drug to its stable crystalline forms. However the use of cyclodextrin in the solid oral dosage forms is limited to low dose drugs with large stability constant due to mass limitations of oral dosage units.

In this study, an attempt was made to improve the solubility and dissolution rate of diacerein by complexing with β-Cyclodextrin, thereby increasing its bioavailability and therapeutics efficiency. The complex of diacerein with β-cyclodextrin was prepared by using Physical mixture, co-evaporation/solid dispersion, kneading method and precipitation method at 1:1 stoichiometric ratio. The characterization of drug with β-Cyclodextrin using differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD) and FTIR, in vitro aqueous solubility and dissolution rate profile of complexes were performed.

MATERIAL AND METHODS
Chemicals
Diacerein was obtained as a gift sample from Macleods Pharmaceutical Pvt. Ltd. Mumbai India and β-Cyclodextrin was from Universal Medicament Pvt. Ltd. Nagpur. All other ingredient and solvent used were of analytical grade.

Preparation of solid inclusion complexes
The following inclusion complexes of diacerein and β-cyclodextrin were prepared at 1:1 molar ratio.

Physical mixture
The physical mixture (PM) of diacerein and β-cyclodextrin in 1:1 molar ratio was prepared by mixing individually components that had previously been sieved through sieve no 60.

Solid dispersion/co-evaporated dispersion method
Inclusion complex of diacerein and β-cyclodextrin in 1:1 molar ratio was prepared by dissolving the drug in methanol and β-cyclodextrin (CD) was dissolved in water separately. The CD solution then added to the drug solution and stirred to attain equilibriums. The resulting solution was evaporated to dryness.
Kneading method
Inclusion complex of diacerein and β-cyclodextrin in 1:1 molar ratio was prepared by the CD, not by dissolving but kneaded like a pest with small amount of water to which the drug component has been added. Drug component can be dissolved in a small amount of methanol in which CD has been suspended. Several hours of girding of paste in mortar result in evaporation of solvent and formation of powder like complex.

Precipitation method
Inclusion complex of diacerein and β-cyclodextrin in 1:1 molar ratio was prepared by drug and CD, which dispersed in water and the solution was heated to obtain concentrated, viscous and translucent liquid. The solution was left to give a precipitation of inclusion complex. Precipitate obtained was separated and dried to get solid inclusion complex.

Phase solubility studies
The phase-solubility technique permits the evaluation of the affinity between β-Cyclodextrin and diacerein in water. Phase solubility studies were performed according to the method reported by Higuchi and Connors. Diacerein, in amount that exceeded its solubility, was taken into vial in which 25 ml of distilled water (pH 6.8) containing various concentration of β-Cyclodextrin (1-5 mmol) was added. These flasks were sealed and shaken at 25°C for 48 hours. This amount of time is considered sufficient to reach equilibrium. Subsequently, the aliquots were withdrawn, using a syringe and samples were filtered immediately through a whatman filter paper and approximately diluted. A portion of the sample was analyzed by UV spectrophotometer (UV-1700, Shimadzu, Japan) at 258 nm against blank prepared in the same concentration of β-cyclodextrin in water. The solubility experiments were performed in triplicate.

The apparent stability constant (Kc) according to the hypothesis of 1:1 stoichiometric ratio of complex was calculated from the phase-solubility diagrams using the following equation:

\[ K_{11} = \frac{\text{slope}}{S_0 (1 - \text{slope})} \]

The slope was obtained from the initial straight line portion of the plot of Diacerein concentration against β-Cyclodextrin, and S0 is the equilibrium solubility of Diacerein in water.

Powder X-ray diffractometry
The powder X-RD patterns of drug, β-Cyclodextrin, and complexes were recorded by using automated Philips Holland PW 1710 scanner with filter Cu radiation over the interval 5-60°/2θ. The operation data were as follows: voltage 35 kV, current 20 mA, filter Cu and scanning speed 1°/min.

Differential scanning calorimetry
The samples were analyzed by DSC using a Mettler Toledo SR system. The samples (5 mg each) were placed into pierced aluminum container. The studies were performed under static air atmosphere in the temperature range of 20°C to 400°C at a heating rate of 10°C/min. The peak temperatures were determined after calibration with standard.

Fourier transform infrared (FTIR) spectroscopy
Fourier transform IR spectra were recorded on FT/IR-4100 type A. The spectra were recorded for Diacerein, β-Cyclodextrin physical mixture, Solid dispersion, kneaded and precipitation system. Samples were prepared in KBr disc (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm⁻¹, resolution was 4 cm⁻¹.
**In Vitro dissolution studies**

The dissolution patterns of the complexes were compared with those of pure drug. The dissolution studies were performed according to the USP XXIII, using rotating basket method. The sample, corresponding to 100mg of Diacerein, were placed into hard gelatin capsules. The dissolution medium was 900ml of Phosphate buffer (pH 7.2). The stirring speed of the paddle was 100 rpm, and the temperature was maintained at 37°C ± 0.5°C. The samples (1 ml) were withdrawn at various time intervals, which was filtered through whatman filter paper and analyzed by UV spectrophotometer at 258 nm.

**RESULTS**

**Phase solubility study**

The phase-solubility diagram for the complex formation between diacerein and \(\beta\)-cyclodextrin presented in fig 1. This plot showed that the aqueous solubility of drug increase linearly as a function of \(\beta\)-Cyclodextrin concentration. The linear host-guest correlation with slope of less than 1 suggested the formation of a 1:1 complex with respect to \(\beta\)-cyclodextrin concentration. The apparent stability constant, \(K_c\), obtained from the slope of linear phase solubility diagram was found to be 299.5 mol\(^{-1}\)\(\cdot\)7,8,16.

![Phase solubility diagram](image)

**Powder X-ray diffraction**

The diffraction pattern of the complex was supposed to be clearly rather than the superimposition of each of the components in the true inclusion complexes. Crystallinity was determined by comparing some respective peak heights in the diffraction patterns of the binary system with those of a reference. The powder X-RD patterns for the diacerein, \(\beta\)-cyclodextrin and complexes are presented in fig 2. As a consequence of the coincidence of diffraction peaks between diacerein and \(\beta\)-cyclodextrin, characteristic peaks of diacerein those situated at 0° and 40° (2\(\theta\)), were used for conformation studies. The complexes showed all characteristics peaks corresponding to the drugs, but with lower intensity concentration and possible reduction in crystallinity due to complexes. The diffraction patterns of the physical mixture, solid dispersion, kneaded and precipitation system showed simply the sum of each component, indicating the presence of diacerein in the crystalline state. The relationship used for the calculation of crystallinity was relative degree of crystallinity (RDC) = \(I_{sam} / I_{ref}\) where \(I_{sam}\) = the peak heights of the sample investigated and \(I_{ref}\) = the peak heights at the angle for the reference with the highest intensity\(^6,7,17\). Pure drug peak at 10.44° 2\(\theta\) was used for containing the RDC of physical mixture, solid dispersion, kneaded and precipitation system. The RDC values of corresponding binary system were 0.998, 0.811, 0.601 and 0.374 respectively.
Diacerein

(A)

β-Cyclodextrin

(B)
Precipitation method

Kneading method
Fig. 2: XRD Spectra of diacerein - β-cyclodextrin system. XRD spectra of diacerein (A), β-cyclodextrin (B), precipitation method (C), kneading method (D), solid dispersion/co-evaporated method (E) and physical mixture (F). Differential scanning colorimetry:
The thermal behavior of physical mixture, solid dispersion, kneaded and precipitation system was similar to untreated samples, indicating that the physical mixture, solid dispersion, kneaded and precipitation system processes was not substantially affect their solid state properties. The DSC thermograms for the diacerein, β-cyclodextrin and complexes are presented in fig 3. Diacerein exhibits a characteristic endothermic fusion peak at 249.7°C, hence no polymorphs of diacerein were found. Further β-cyclodextrin showed a brand effect at 315.6°C. The DSC curve of drug showed a small and shallow endothermic segment in the melting of the drug indicating reduction in drug crystallinity due to complexation.6-7.
Fig 3: DSC thermogram of diacerein - β-cyclodextrin system.
DSC thermogram of β-cyclodextrin and kneading method (A), DSC thermogram of solid dispersion/co-evaporated method and diacerein (B) and DSC thermogram of precipitation method and physical mixture.
Fourier transform infrared (FTIR) spectroscopy. Since FTIR is highly sensitive method of analysis all spectra of complexes showed the other changes from parent spectra, i.e. diacerein, β-cyclodextrin and complexes are presented in fig 4. No significance change in the IR spectra of physical mixture, solid dispersion, kneaded and precipitation system diacerein- β-cyclodextrin complexes were observed except for the broading of peaks. The broading of peak was probably due to the restriction of bending and stretching vibration of the Diacerein due to the β-cyclodextrin cavity. The FTIR spectra of complexes showed significant shift of carbonyl absorption band at 1769 cm$^{-1}$ assigned to carboxyl carbonyl stretching$^{18}$. 
Fig. 4: FT-IR spectra of diacerein-β-cyclodextrin system. FT-IR spectra of diacerein and β-cyclodextrin (A), FT-IR spectra of precipitation method and solid dispersion/co-evaporated method (B) and FT-IR spectra of kneading method and physical method (C).
Drug content
UV spectrophotometry was used to determine the drug content of the binary system of the β-cyclodextrin: drug molar ratio (1:1), in all system sample of the binary system were dissolved in Phosphate buffer (pH 7.2) which was diluted to obtain ~ 10 µg/ml. The β-cyclodextrin drug ratio would therefore remain 1:1 in the final solution to calculate the drug content. The Drug content in all the system was found to be 49.86 % w/w to 52.5% w/w (99.77 % to 105.01%)7.

In Vitro dissolution studies
Since the presence of β-cyclodextrin showed that there was decreased in extinction coefficient of the drug, since the β-cyclodextrin is highly water soluble it was expected to instantly dissolved in the medium under the condition of dissolution test. The release rate profile was drawn as the cumulative percent release on y-axis and time on x-axis which showed in fig 5. It showed that 40% of the drug was released in 20 min, and up to 75% after 60 min. Physical mixture shows release up to 25% of the drug in 20 min and up to 54% after 60 min whereas pure drug exhibited the release of 15% after 30 min and not more than 42% after 60 min. These quantities contrast with the markedly two fold increase in the release of dispersion, kneaded and precipitation system6-7.

DISCUSSION
From the result it is observed that the solubility of diacerein in the presence of β-cyclodextrin can be classified as the A2 type. This indicated that the complexes at 1:1 ratio are adequately stable. In case of complexes, a diffraction pattern was similar to that of pure drug β-cyclodextrin, but with a reduction in peak intensities. This confirms partial complexes formation i.e. some part of diacerein entrapped in β-cyclodextrin cavity. The DSC thermogram for the complexes

![Dissolution Rate Profile](image-url)

Fig. 5 : Dissolution rate profile of diacerein - β-cyclodextrin system.
showed the persistence of the endothermic peak of diacerein for the physical mixture, solid dispersion, kneaded and precipitation system product. The thermal behavior of physical mixture, solid dispersion, kneaded and precipitation system is similar to the untreated samples, indicating that the physical mixture, solid dispersion, kneaded and precipitation system processes did not affect their solid state properties. For physical mixture, solid dispersion, kneaded and precipitation system complexes, there is reduction in peaks intensity, this can be explained on the basis of major interaction between the drug and β-cyclodextrin. Furthermore, the characteristic endothermic effect of β-cyclodextrin and diacerein is slightly shifted to lower temperature for the physical mixture, solid dispersion, kneaded and precipitation system complexes, indicating that diacerein got complex with β-Cyclodextrin.

The enhancement in dissolution profile has been attributed due to the formation of inclusion complexes in the solid state and reduction in the crystallinity of the product, as conformed by powder X-RD study. The dissolution rate increase for the physical mixture, solid dispersion, kneaded and precipitation mixtures is due to the wetting effect of the β-cyclodextrin, this effect is more evident for the kneaded product, where the mixing process between the two components is more intensive. The effect of complexation with β-cyclodextrin on the solubility of diacerein can be explained in terms of the reduction in the crystallinity of the drug caused by the solid dispersion, kneaded and precipitation process and the inclusion into the hydrophobic cavity of the β-cyclodextrin. The FTIR spectra of physical mixture, solid dispersion, kneaded and precipitation system significant shift of carbonyl absorption band at 1769 cm⁻¹ assigned to carboxyl carbonyl stretching. This may be indicative that the drug monomeric dispersion, as a consequence of the interaction with cyclodextrins through hydrogen bonding, which could result in its inclusion into the hydrophobic cavity of the β-cyclodextrin.

ACKNOWLEDGMENT
The authors are grateful to Dr. Abhay Dharamsi, Principal and Staff KMCH college of Pharmacy Coimbatore, for providing facilities to carry out the research work. They are also thankful to Macleods Pharmaceutical Pvt. Ltd. Mumbai and Universal Medicament Pvt. Ltd. Nagpur, Department of Pharmaceutical Sciences University Campus, Nagpur for providing the gift sample and help in performing characterization studies.

REFERENCES
1. Dr. Alferd kelment, Tara news. “Osteoarthrose and Structure modifying agent”.
6. Derle D, Boddu SHS, Mager M. Studies on the Preparation, Characterization and Solubility of β-Cyclodextrin-Starnidazole