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Design and Characterization of Nanocrystals of Quercetin for Solubility and Dissolution Enhancement

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ABSTRACT

In the pharmaceutical formulation water solubility is most affecting property to the stability and bioavailability of drug. If the drug is insoluble in both aqueous and organic solvents then it is very difficult to deliver the drug in a sufficiently bioavailable form. Although some approaches are available to increase the bioavailability of poorly soluble drugs having drawbacks like not only low drug loading but also the large doses hence, it is a challenge to formulation researcher to overcome such problems. Nanonisation is one of the solution to poorly soluble drugs. It leads the solubility and more biologically available and safer dosage form of poorly soluble and bioavailable drugs. Quercetin is also a water insoluble drug with low bioavailability hence; in the present study by using anti-solvent precipitation method nanocrystals were formulated. Then the prepared nanocrystals are subjected to particle size analysis, FTIR study, X-ray diffraction, DSC, and in-vitro dissolution study. The nanocrystals are possible with less particle size with slightly changes in the crystallinity. It has shown that, drug increases the saturation solubility with enhanced dissolution profile study.

Keywords: **Quercetin, nanocrystals, solubility, anti-solvent, dissolution profile.**

1. INTRODUCTION

Quercetin is a plant flavonol from the flavonoid groups. It has wide range of biological & pharmacological activities such as antitumor [1], antibacterial [2], antioxidant, anti-inflammation [3], obtains protection against osteoporosis, pulmonary & cardiovascular diseases [4]. It is possible to be developed as an active pharmaceutical ingredient (API) for the different formulations, on the other hand its partial use due to poor solubility & low rate of dissolution leading to low bioavailability [5,6].

Most of the drugs demonstrate such low solubility that micronization does not lead to improve the solubility and dissolution rate of the drugs. Due to micronization to nanonization drug nanocrystals gained much more interest to enhance the solubility of drug [7]. By the formulation of nanocrystals particle size reduction is done by nanosizing has been applied to improve the solubility [8,9]. Due to high drug payload capacity, use of minute quantity of excipients, higher chemical stability, lower toxicity, easy scale up and manufacturing nanocrystals gained increased interest to enhance the solubility compare to other nano-particulate system [10]. Drug nanocrystals are 100% drug load capacity with crystalline nanosized particles. They are formulated as a liquid dispersion and contains the raw drug stability with a polymer and surfactant [11,12].

The aim of the present study to decrease the particle size of the drug which is enhances the solubility of the drug with increased solubility. The particle size reduction was done by anti-solvent precipitation method. The solubility study and *in-vitro* dissolution profile of the obtained nanocrystals were compared with the results of pure drug. The polymer used to prepare the nanocrystals is beta-cyclodextrin which is a solubility enhancer. The prepared nanocrystals were evaluated for particle size analysis; solubility study to confirm the increase in solubility of formulation as compared with pure drug, crystalline state was also assayed by using X-ray diffraction study method and dissolution profile study.

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2. MATERIALS AND METHODS

2.1 MATERIALS

Quercetin was purchased from India mart, beta-cyclodextrin was purchased from Aarti chemicals (Solapur) India, DMSO & ethanol are analytical grade.

2.2 PREPARATION OF QUERCETIN NANOCRYSTALS

The nanocrystals were prepared by anti-solvent precipitation method. The preparation process involves two steps [13].

Step 1: Preparation of drug solution in organic solvents: Concentration of drug solution was prepared by preparing solution of drug in organic solvent (based on solubility of drug in particular solvent).

Step 2: Addition of a drug solution in water: Nanocrystals were prepared by adding the required quantity of water containing polymer (beta-cyclodextrin) with continuous stirring. Immediately, particles precipitated from the anti-solvent & milk like suspension was formed which was then filtered & dried. The drying was carried out using tray dryer at 40^0C.

3. CHARACTERIZATION

Nanocrystals obtained by above method screening their solubility. Quercetin nanocrystals showing improved solubility was further studied for drug content, particle size distribution, X-Ray Diffraction study, DSC study & dissolution profile study.

3.1 PARTICLE SIZE DETERMINATION BY USING MICROSCOPE

The average particle size distribution of selected batches of nanocrystals was determined by using SAGLO SOFT-GEL Micro-Imaging Adaptor.

3.2 SCANNINF ELECTRON MICROSCOPY

Scanning electron microscopy (Zeiss EVO 18, Mumbai) was used to analyze particle size and surface morphology. The surface morphology of nanocrystals was examined at pH 7.4. The samples were mounted on aluminum mount and then were critical point dried, sputter-coated with 9nm of gold/palladium and imaged using scanning electron microscope.

3.3 CRYSTALLINE STATE EVALUATION

Crystalline state evaluation of nanocrystal formulation was done by using Powder X-Ray Diffraction (PXRD) [14].

POWDER X-RAY DIFFRACTION (PXRD)

PXRD diffractograms of quercetin nanocrystals & pure drug were recorded using analytical XRD Bruker D8. It is equipped with Kα radiation (40 kv, 20 ma). The scanning of the quercetin nanocrystals and pure quercetin was done from 5^0 to 50^0 2Θ respectively.

3.4 FTIR STUDIES OF QUERCETIN NANOCRYSTALS [15]

FT-IR spectra of pure drug (Quercetin) and nanocrystals of drug were carried out to investigate physicochemical compatibility of drug with polymer. Structural elucidation was done using Thermofischer Scientific Nicolet IS-10.FTIR analysis of pure quercetin & quercetin nanocrystals were performed & spectrum was obtained using FT-IR.

3.5 DIFFERENTIAL SCANNING COLORIMETRY

DSC thermo gram of pure drug and drug loaded nanocrystals was performed to determine the thermal properties of the drug and that of formulation. The thermo gram was covered by heating from 32.00^0 C to 400.00^0 C at 12^0 C/min. the vessel is constantly purged with nitrogen at a rate of the 200 ml/min by using SDT Q600v20.9.

3.6 SOLUBILITY DETERMINATION OF NANOCRYSTALS [16]

10 mg of nanocrystals were dissolved in the 10 ml of distilled water. Then the sample was homogenized for 1 min by sonication and centrifuged at 21,000 X G force. Four ml supernatant was taken and analyzed by spectrophotometer VU-visible. The absorbance was measured at 255.8 nm.

3.7 STABILITY STUDY

Stability study of the prepared nanocrystals (optimized formulation) were carried out by storing at 4^0 C in refrigerator, room temperature at 30^0 C±2^0C /65%±5% RH and at 40^0 C±2^0C /65%±5% RH in humidity control oven for thirty days. Parameters residual percent drug content were carried out. The

results of quercetin content after 30 days the stability testing at different storage condition.

3.8 IN-VITRO RELEASE STUDY [17]

The release rate of quercetin nanocrystals was determined using USP Dissolution testing apparatus II (Basket type). The dissolution test was performed using 900 ml of phosphate buffer pH 7.4 at 37±0.5^0 & 100 rpm speed. Aliquot (sample) (5ml) of the solution was withdrawn from the dissolution apparatus every 15 min interval for first 1 h, & every 30 min intervals for next 2hrs. Aliquot was replaced with phosphate buffer pH 7.8. The sample were filtered & suitably diluted. Absorbance value of these solutions was measured at 257nm using UV spectrophotometer. The percentage drug was calculated.

4. RESULTS & DISCUSSION

4.1 PARTICLE SIZE DISTRIBUTION

The particle size distribution of formulated quercetin nanocrystals was carried out using SAGLO SOFT-GEL Micro-Imaging Adaptor. It was found that the prepared nanocrystals were found in irregular shape and polydispersed type of distribution as shown in figure 1.

Fig.1 Particle size analysis of Quercetin Nanocrystals

4.2 SCANNING ELECTRON MICROSCOPY STUDY

Surface morphology of nanocrystals was investigated by scanning electron microscopy. The representative SEM photographs of the nanocrystals are shown in figure 2. It was observed by SEM analysis that the nanocrystals were appearing irregular shape and the particle size of the quercein nanocrystals is 500nm.

Fig. 2 SEM image of Quercetin Nanocrystals

4.3 PXRD STUDY OF QUERCETIN NANOCRYSTALS

The X-ray diffraction pattern of the original quercetin displayed the presences distinct peaks are 2Θ, 10.73^0, 12.33^0, 15.87^0, 24.41^0, 26.50^0, and 27.40^0 which suggested that high crystalline form of the drug. At the slightly different peak intensities lyophilized quercetin shoes same results. In the X-ray powder diffractograms nanocrystallinity is known to cause such effects. Hence, the results showed by X-ray diffraction pattern confirmed that quercetin nanocrystals were found in the crystalline state. X-ray pattern of the Quercetin and Quercetin Nanocrystals are shown in figure 3 & 4.

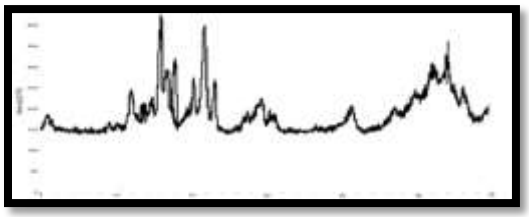

Fig. 3 X-ray powder diffraction spectrum of Quercetin

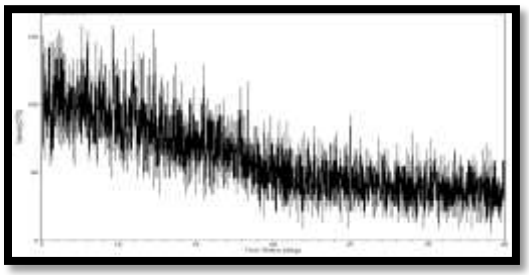

Fig. 4 X-ray powder diffraction of Quercetin Nanocrystals

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4.4 FTIR STUDY OF QUERCETIN NANOCRYSALS

To investigate physicochemical compatibility between the drug and polymer FTIR study was carried out. Any interaction between the drug and polymer can be detected by IR spectroscopy. The IR spectrum of quercetin nanocrystals were compared with the IR spectrum pure quercetin. The results were showed that there the polymer was compatible with the pure drug. The similarities between FTIR spectra of coarse quercetin and nanosized quercetin show no chemical changes between quercetin and excipients. The IR spectrum of quecetin and quercetin nanocrystals is shown in figure 5 & 6. This was compared with functional group frequencies as shown in the table 1.

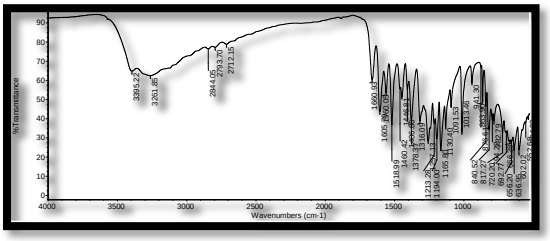

Fig. 5 FTIR study of pure Quercetin

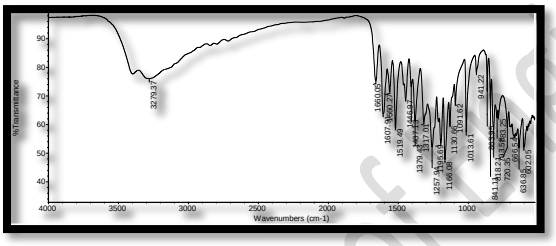

Fig. 6 FTIR study of Quercetin Nanocrystals

Table I: FT-IR spectra interpretation

Functional groups	Abs. frequency region	Quercetin	Quercetin Nanocrystals
O-H	3200-2700	3395.22	3279.37
C=C	1600-1660	1518.99	1519.49
C=O	1770-1650	1660.93	1607.91
C-H	1440-1320	1406.86	1379.43
C-N	1230-1020	1013.46	1166.08
C-Cl	600-800	817.27	720.35

4.5 DIFFERENCIAL SCANNING CALORIMETER

The DSC report in fig. 7 shows the quercetin used in this formulation had a sharp melting endothermic peak at 316^0 C followed by degradation supporting the melting point of quercetin. However, no melting point peak was exhibited in the curve of formulation i.e. quercetin nanocrystals. These results suggested that the drug was dispersed throughout the polymer.

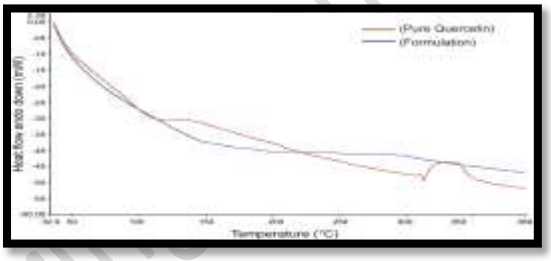

Fig. 7 DSC curve of Quercetin and Quercetin Nanocrystal

4.6 SOLUBILITY STUDY OF QUERCETIN NANOCRYSTAL

In this study, solubility of quercetin was found to be 2.5±0.03µg/ml & the solubility of the formulated quercetin nanocrystals was found to be 12.08±0.09µg/ml (Figure 8). This study indicated that formulation of quercetin nanocrystals by anti-solvent precipitation method enhanced the saturation solubility of quercetin approximately 5 times (12.09±0.09µg/ml) than pure quercetin (2.5±0.03µg/ml).

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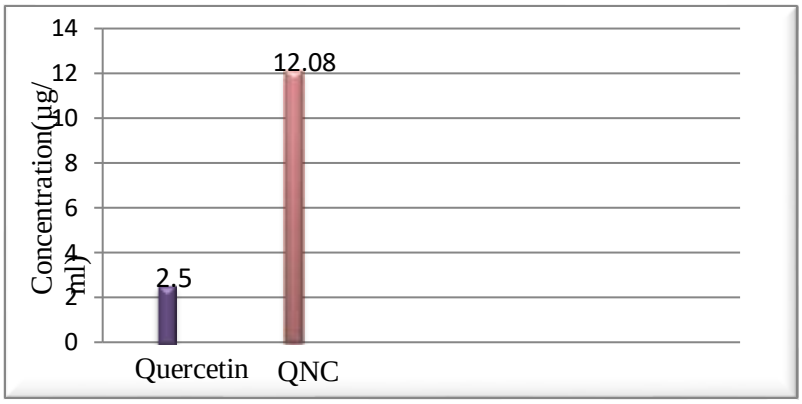

Fig. 8 Solubility studies of Quercetin Nanocrystals (QNC)

4.8 *IN-VITRO* RELEASE STUDY OF QUERCEIN NANOCRYSTALSI

The dissolution profile of the pure quercetin & formulated quercetin nanocrystals were studied. The results showed that quercetin nanocrystals with beta-cyclodextrin exhibited dissolution profiles about 93.57 % within 180 min as compared to pure quercetin (dissolution profile 27.58 %) within the same time (Figure 9). In this study the dissolution of the pure quercetin showed a very poor dissolution rate. The greatest increase in the dissolution rate is exhibited by quercetin nanocrystals. Hence, compared to the pure quercetin quercein nanocrystals shows increased dissolution rate.

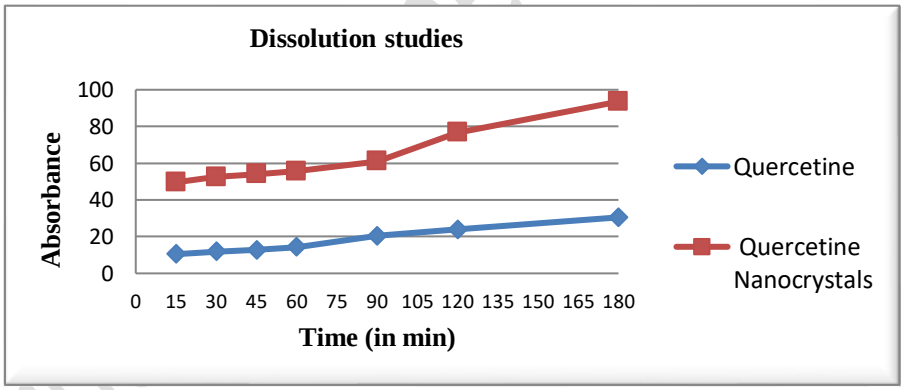

Fig. 9 *In-vitro* dissolution study of Quercetin Nanocrystals

4.9 STABILITY STUDY

From stability studies it was confirmed that nanocrystals of quercetin nanocrystals remained more stable at 4^0 C. The maximum instability of nanocrystals was observed at 40 ± 2^0 C (Table 2).

Table 2 Stability study of Quercetin Nanocrystals

% quercetin content at 4^0C	% quercetin content at 30^0 C±2^0C/65%±5% RH	% quercetin content at 40^0 C±2^0C/65%±5% RH
72.65	72.56	66.78

5. CONCLUSION

In the present study, the nanocrystals technique has been specifically used to increase the solubility &

dissolution rate of the poor water soluble quercetin. Anti-solvent precipitation method was used to prepare Quercetin Nanocrystals in order to improve the quercetin solubility & dissolution property. Particle size distribution (SEM) of formulated nanocrystals showed range in 500nm using anti-solvent precipitation method. Therefore, this study indicated that anti-solvent precipitation method is cost effective & simple precipitation method. FTIR study showed that is no interaction materials used in the preparation of quercetin nanocrystals. XRPD and DSC data revealed that there is a crystalline nature in prepared quercetin nanocrystals as compared to pure quercetin. Solubility study indicated that quercein nanocrystals enhanced the solubility of quercetin produced by precipitation method. *In-vitro* dissolution study showed that greatest increase in the dissolution rate by quercetin nanocrystals.

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