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Study of Solubility Enhancement of Quercetin by Inclusion Complexation with Betacyclodextrin

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Abstract:-

The main purpose of the present study was to formulate the inclusion complexes of Quercetin with β-CD by solvent evaporation method and to check whether the solvent evaporation methods enhances the solubility of the drug as compared to another methods present. Quercetin was being procured from the PE Chem and was being mixed with βCD which was obtained from Loba chemicals, in this method the methanol was use as solvent from Merck Specialties Private Ltd. Various methods of inclusion complexes are present out of which three methods were used, solvent evaporation, physical mixing and kneading method.Various ratios of Quercetin-β-CD were being used. Quercetin-β-CD complexes were characterized by phase solubility study, UV spectroscopy, solubility study, XRD, DSC, *In-vitro* dissolution study and, FTIR analyses. Dissolution studies were performed for all inclusion complexes and the results were compared with those obtained for pure drug and their physical mixtures. The phase solubility analysis shows that there is a formation of 1:1molar inclusion complex of the drug with β -cyclodextrin. The stability constant of Quercetin: β-CD (1:1 and 321 M^{-1}).F.T.I.R. and DSC results shows interaction occur in the drug and polymer. The inclusion complex prepared by the solvent evaporation method exhibits an overall best result.

Key words: Quercetin, Cyclodextrin, Inclusion complex, Solvent Evaporation, Kneading, Physical mixing, Complexation.

INTRODUCTION:-

Quercetin is one of the important bioflavonoids present in more than twenty plant material which is known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities[1,2]. In spite of this wide spectrum of pharmacological properties, the use of Quercetin in the pharmaceutical field is limited due to its poor aqueous solubility [3,4]. These properties of QUR result in poor bioavailability, poor permeability, instability, and extensive first-pass metabolism before reaching the systemic circulation [5]. Therefore, it is highly necessary to develop new dosage forms of QUR with increased solubility and improved bioavailability. The drug delivery systems, such as inclusion complexes to increase its solubility and improve its bioavailability. Cyclodextrin inclusion complexation forms host-guest complex by weak intermolecular interaction lead to enhancing in solubility and bioavailability of poorly soluble drugs [6].

One of the main purpose to include cyclodextrin results in enhancement of solubility and dissolution rate off the lipophilic drugs in aqueous media, results in improved bioavailability[7,8]. This technique has shown the solubility on various drugs such as celecoxib and rofecoxib [9,10]

Over the years a variety of techniques like pH adjustment, co-solvent addition, surfactant addition, etc. have been studied and widely used to improve the solubility and dissolution of water insoluble drugs. The formation of inclusion complex with nontoxic agents is also a type of manipulation used to improve the dissolution properties of drugs [11].

Drugs with solubility less than 0.1 mg/ml usually shows dissolution limited bioavailability [12]. Also, if the drug is too water soluble, it poses a problem with the partitioning from the aqueous environment to the lipophilic biological membranes thereby making the bioavailability permeation limited [13].

Many other techniques such as complexation by kneading,[14,15] freeze-drying,[16] co-precipitation, [17] solvent evaporation, use of pro-drugs, formation of water-soluble complexes and soluble salt formation have been used for improving dissolution, solubility and thus the bioavailability of the drug. The solvent evaporation method provides advantage to use a lipophilic drug with hydrophilic polymer. Solvent evaporation method shows good encapsulation efficiency of hydrophilic polymers [18]. Their is an important role in the formulation development of the cyclodextrin and poorly soluble drug, due to their effect on solubility, dissolution rate, chemical stability and absorption of drug[6,19]. The objective of the present study is to find the better possibility for improving the solubility and dissolution rate of Quercetin by complexation with β-cyclodextrin and also to compare the different complexation methods.

Cyclodextrins have been used extensively as such complexation agents. Cyclodextrins are cyclic oligomers of dextrose or its derivatives. Drug solubility increase by forming an inclusion complex with the non-polar region of the drug molecule (guest) being introduced into the cavity of the cyclodextrin molecule (host) [13]. Furthermore, cyclodextrin is being used as a taste masking agent and reduce bad smelling of drug[20-24].

MATERIALS AND METHODS:-

Materials

Quercetin (98.5%) was obtained from PC chem India. βCD was being procured form Loba Chemicals. Methanol was being purchased form Merck Specialties Private Limited. All of the above chemicals were of analytical grade.

Methods

Preparation of inclusion complexes:-

β-cyclodextrin was being used to prepare the Quercetin and β-cyclodextrin inclusion complexes in the ratio of 1:1,1:2,1:4,1:8, using physical mixing, kneading and solvent evaporation method.

Physical Mixing Method:-

In physical mixing, Quercetin and β-cyclodextrin were weighed and mixed evenly in a motor with light trituration. Trituration was being carried out for an hour until a homogenous mixture is being obtained. The mixture then was being passed through the sieve of 65 mesh size and kept in a closed container [25, 26].

Kneading method:-

In kneading method [14], β-cyclodextrin and Quercetin were being weighed as required for the various ratios and then β-cyclodextrin was being converted into a paste by adding small portions of water and then the Quercetin was being added in the paste and continuously kneaded for three h. An appropriate quantity of water was being added to maintain the consistency. Then paste was being dried in a hot air oven at 30-40ºC for 24 h. The dried powder was then powdered into a fine powder and stored in a closed container [25,26].

Solvent evaporation method:-

In the solvent evaporation method Quercetin was dissolved in 25 ml of methanol while β-cyclodextrin was dissolved in 50 ml of distilled water. The two solutions were mixed and stirred for an hour. Methanol was evaporated off by heating at 40ºC under constant stirring in an orbital incubator shaker. Water was then removed by keeping in a hot air oven at 40ºC for 24 h. The mixtures were then grounded using mortar pestle and then sieved through the sieve of mesh size 65, then the mixture was kept in a closed container [25].

Phase solubility study:-

The phase solubility study was carried by the Higuchi and Connors method[27,28]. An excess amount of QUR(1g) was added to 50ml distilled water in which various concentration of β-CD(1,2,4,8 mM)was added. The flask was then shaken continuously at 30˚C for 72 h. Samples of 3 ml were withdrawn and filtered through Whatman filter paper and then diluted up to 10 ml with distilled water and assayed at 372nm(UV 1800, Shimadzu Corporation, Japan).

By using phase solubility diagram, stability constant (Kst) was calculated using equation:

Kst=slope⁄S0 (1-slope).

The slope was being obtained from the plot of Quercetin concentration against β-CD concentration.

Where, Kst is stability constant,

S0 is the intercept.

Solubility studies:-

Solubility studies were performed by adding an excess amount of mixture (1g) in 50ml distilled water. The flask was agitated at 120 rounds per min in orbital incubator shaker and the temperature was being maintained at 30ºC for 72 h. Samples of 3ml were withdrawn and filtered through Whatman filter paper and diluted up to 10 ml and were being assayed at 372 nm by UV spectrophotometer (UV 1800, Shimadzu Corporation, Japan).

$$\text{Solubility} = \frac{\text{Absorbance-Intercept}}{\text{Slope}}$$

In vitro dissolution studies:-

In vitro dissolution studies were performed under sink condition for both QUR and mixture using USP type II dissolution apparatus (Electro Lab TDT-08L, Mumbai, India). Dissolution rate study of optimized mixture was conducted in 900ml distilled water at 50 rpm and temperature conditions were 37ºC±0.5ºC. The amount of powder equivalent to 20 mg of drug weighed and added to the dissolution medium and 3ml aliquots were withdrawn at 10, 20, 30, 40, 50, 60 min time intervals and filtered through Whatman filter paper. The initial volume was maintained by adding 3 ml of fresh dissolution medium. The sample was withdrawn and drug concentration in the samples was being assayed at 372 nm using a double beam UV visible spectrophotometer (1800, Shimadzu, Japan).

Drug content:-

The drug content of the QUR in mixture was determined using powder equivalent to 20 mg and was dissolved in distilled water up to 100 ml. the solution was then filtered using Whatman filter paper no 42 and measured at 372 nm using a double beam UV visible spectrophotometer (1800, Shimadzu, Japan)**.**

$$\%\ \text{Drug content} = \frac{\text{Measured drug amount in complex}}{\text{Amount of complex taken}} \times 100$$

Fourier Transform Infrared Spectroscopy:-

The FTIR spectra were being carried for drug and solid complexes with FTIR spectrophotometer. The samples were prepared by using KBr This mixture was compressed in a form of a pellet by applying 10 tons of pressure hydraulic press. The pellets were scanned over a wave number range of 4000 to 400cm^{-1}.

Powder X-Ray Diffractometry:-

X-ray diffraction was carried out using Philips Analytical X-Ray BV (PW 1710)at room temperature in which cobalt was used as anode material and graphite monochromatic was operated at a voltage of 40Kv. The samples were analyzed in 2θ angle range; process parameters were set as: scan size of 0.025º (2θ), scan step time of 1.25 s and time of acquisition of 1 h.

Differential Scanning Calorimeter:-

The DSC thermogram pattern was recorded on the thermal analyzer. The mixture sample was heated in closed aluminum crimped cells at a heating rate of 10ºC/in the 30-300ºC temperature range under a nitrogen atmosphere at a flow of 50ml/min.

RESULTS AND DISCUSSION:-

Phase Solubility Studies:-

To determine the stoichiometric proportion of QUR with β-cyclodextrin, Phase solubility study was carried out. The phase solubility diagram of QUR is being shown in Figure 1. The phase solubility diagram obtained shows that there is an increase in the solubility of Quercetin with the increase in the β-cyclodextrin concentration. According to the Higuchi and Connors theory[27, 28] this shows an

(AL) type diagram; this may attribute the formation of soluble 1:1 QUR-BCD inclusion complexes. Stability constant obtained for QUR was found to be 321.24 M^{-1}. It was reported that stability constant (Ks) value between 50-5000 M^{-1} is considered suitable for the improvement of stability and solubility of the poorly soluble drugs [25].

Solubility studies:-

The solubility values of QUR are being shown in the Table 1, irrespective of the preparation used. There were three different methods used in the solubilization of QUR, they were by kneading, physical mixing, solvent evaporation, in which solvent evaporation method produces the highest solubility of QUR with β-cyclodextrin, followed by kneading and physical mixing.

In vitro Dissolution:-

Dissolution characteristics of QUR and β-cyclodextrin complexes are given in Table 2. More than 90% of the drug was released within 60 min. the values of the solvent evaporation were much higher as compared to that of the physical, kneading method. Figure 2 describes the *in vitro* dissolution of the complexes

Drug content:-

The percent drug content in each of the inclusion complex is being determined. The drug content by kneading, physical mixing and solvent evaporation is being shown in the Table 3. From this, it was found that the drug content of a 1:1 solvent evaporation method was found to be the better batch as compared to other batches. Hence this batch was being considered the finalized optimized batch.

FTIR:-

Infrared spectra of QUR, as well as those of its mixture with CDs, are presented in Figure 3(A). Infrared spectra of QUR as well as of the other mixture it was found that a absorption of characteristics carbonyl band was seen at 1663 cm^{-1} assigned to aromatic ketonic carbonyl stretching. The characteristic peaks of Quercetin were observed at 3666.38, 1006, 2975 and 1663 cm^{-1} which corresponded to the functional group present in the structure of the drug, were 3666.38 is of O-H stretching, 1006 is of C-O stretching, 2975 is of $–CH_3$ streching. The FTIR of drug/β-CD was being compared with mixtures and drugs. It was found that the characteristic aromatic carbonyl stretching band of solvent evaporation showed at 1694.78 cm^{-1} in the case of kneading method the shift was being at 1692.36 $cm^{-1}$and physical mixture shows the characteristic band at 1693.92cm^{-1}. Along with the reduced intensity of the same band. To confirm the existence of the complex as a new compound, there were changes in the characteristic band as compared to that of pure drug [16].

Powder XRD

Powder X-ray diffractometry is a useful method for the detection of cyclodextrin complexation in powder state[29]. Figure 4 shows the X-Ray diffraction patterns of QUR and corresponding complexes with cyclodextrin. Crystallinity is determined by comparing representative heights in the diffraction patterns. The powder XRD pattern of QUR drug is shown in the Figure 4(A) sharp peak present is the evidence of the highly crystalline nature of the drug the peaks were observed at 15º,17º and 26.94º of 2θ values. In the case of the complexes, it refers to a new solid phase with low crystallinity, which indicates inclusion complex formation. Amorphization was instead induced by a solvent evaporation method, where this shows a large diffraction peak in which it is no possible to distinguish the characteristic peaks of the flavonoids. These results confirm that QUR/β-CD (SE) no longer present as a crystalline material and their solid complexes exist as an amorphous state.

DSC:-

To confirm the formation of the solid complex, thermal behavior of QUR/β-CD solid complexes was studied using DSC. are shown in Figure 5 shows, DSC thermograms of QUR solid complexes. The DSC thermograms of QUR Figure 5(A). shows an endothermic peak at 320.8ºC corresponding to its melting point. β-CD alone showed a broad endothermic peak representing a loss of water molecule, a dehydration process. The thermograms of physical mixture and complexes are different from the pure drug, which gives clear evidence that there is a formation of the complexes. The disappearance of endothermic peaks with these systems gives clear evidence that there is the formation of the complexes of QUR and β-CD. The endothermic peak shifted from 121.25ºC to 112.55ºC of solvent evaporation method in Figure 5(E),while also peak changed in kneading method Figure 5(D) and physical mixing Figure 5(C). This shows that there is a formation of inclusion complex in all the cases.

Table 1: Solubility data of QUR and QUR/βCD

CODE	QC:BCD	SOLUBILITY
β-CD0:(QUR)	QUR: No βCD(1:0)	0.003±0.02
β-CD 1:1(PM)	QUR:βCD(1:1)	0.33±0.1
β-CD 1:4(PM)	QUR:βCD(1:4)	0.15±0.15
β-CD 1:8(PM)	QUR:βCD(1:8)	0.06±0.1
β-CD 1:1(KM)	QUR:βCD(1:1)	0.33±0.11
β-CD 1:2(KM)	QUR:βCD(1:2)	0.15 ±0.005
β-CD 1:4(KM)	QUR:βCD(1:4)	0.09 ±0.02
β-CD 1:8(KM)	QUR:βCD(1:8)	0.05 ±0.01
β-CD 1:1(SE)	QUR:βCD(1:1)	0.401±0.001
β-CD 1:2(SE)	QUR:βCD(1:2)	0.27±0.015

Table 2: The *In-vitro* Dissolution of QUR/ βCD of various methods.						
TIME (HR)	**%CR(1:1SE)**	**%CR(1:2SE)**	**%CR(1:1PM)**	**%CR(1:2PM)**	**%CR(1:1KM)**	**%CR(1:2KM)**
0	0	0	0	0	0	0
1	54	39.91304348	9.391304348	16.43478261	21.13043478	30.52173913
2	66.03913043	51.87391304	21.1826087	25.9173913	35.33478261	49.47391304
3	75.49565217	56.63478261	35.33478261	40.05652174	49.5	58.96956522
4	82.59130435	68.4	42.45652174	51.87391304	54.27391304	68.41304348
5	87.32608696	82.55217391	58.93043478	61.33043478	58.99565217	80.20434783
6	94.39565217	92.02173913	75.45652174	68.42608696	68.41304348	92.00869565

Table 3: Drug content of QUR		
CODE	**QC:BCD**	**% DRUG CONTENT**
PM(1:1)	QUR:βCD(1:1)	66%
PM(1:2)	QUR:βCD(1:2)	60.2%
KM(1:1)	QUR:βCD(1:1)	67.2%
KM(1:2)	QUR:βCD(1:2)	45.4%
SE(1:1)	QUR:βCD(1:1)	**82.5%**
SE(1:2)	QUR:βCD(1:2)	79.9%

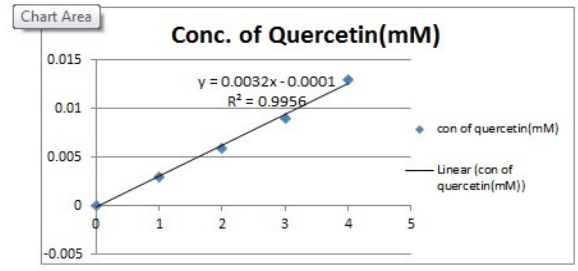

Figure 1: Phase Solubility diagram of QUR in aqueous solution of βCD

Figure 1. Phase Solubility diagram of quercetin in aqueous solution of βCD.

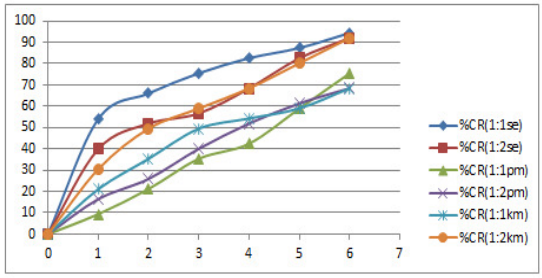

Fig 2. The Invitro Dissolution of Quercetin/ βCD of various methods

Figure 2. The Invitro Dissolution of quercetin βCD of various methods

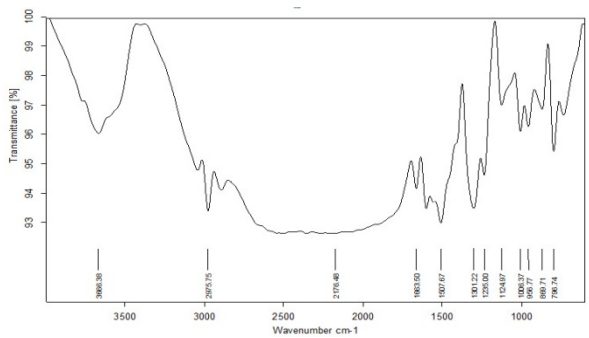

Figure 3(A) IR of Quercetin

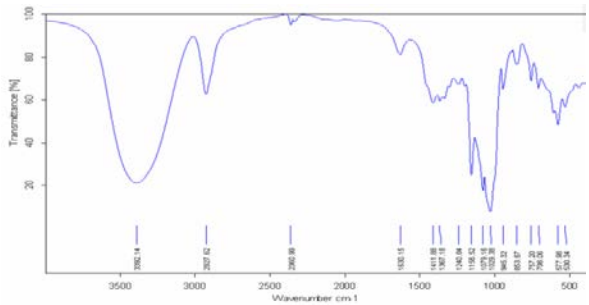

Figure 3(B) IR of BCD

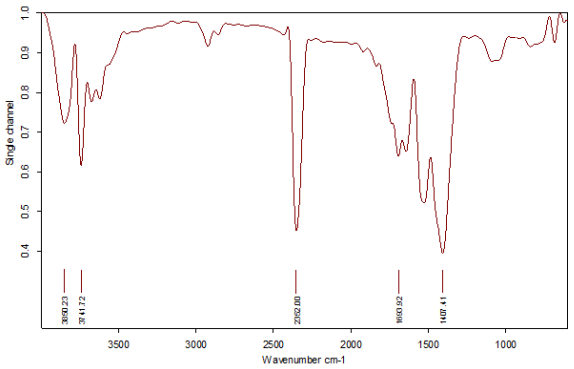

Figure 3(C) IR of physical mixing

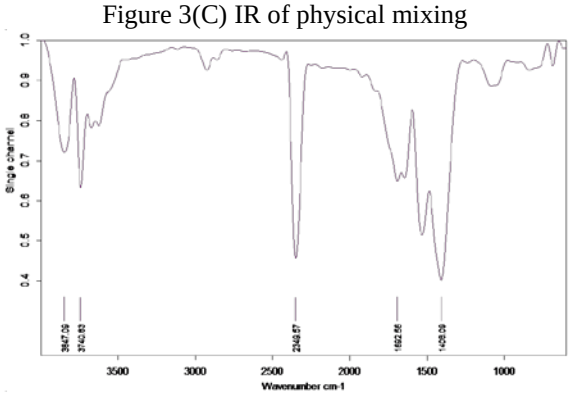

Figure 3(D) IR of kneading

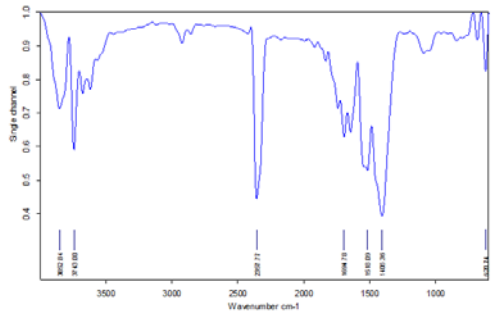

Figure 3(E) IR of solvent evaporation

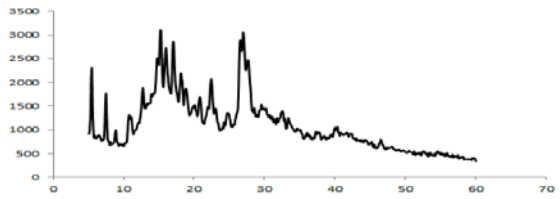

Figure 4(A) XRD of QUR

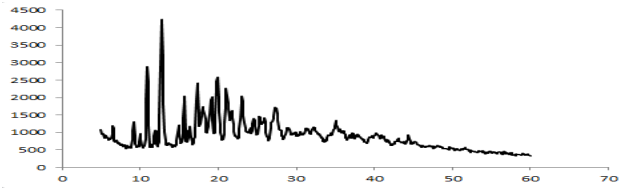

Figure 4(B) XRD of BCD

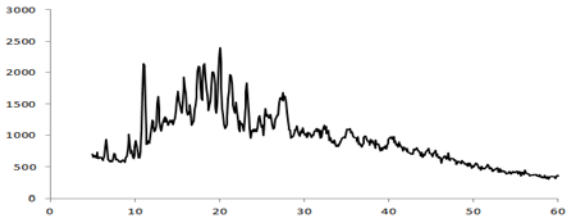

Figure 4(C) XRD of PM

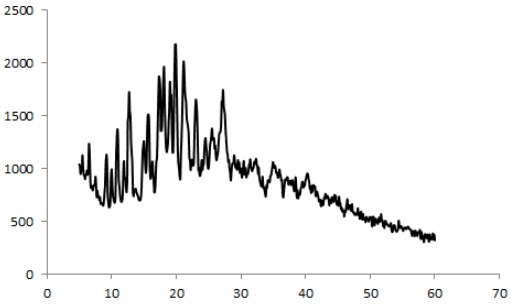

Figure 4(D) XRD of KM

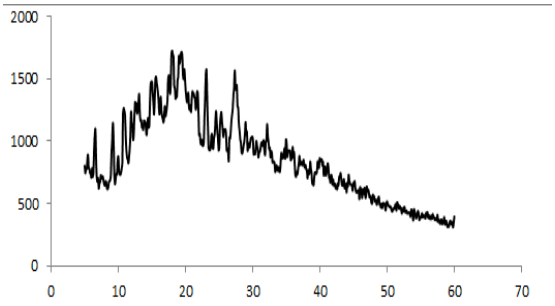

Figure 4(E) XRD of SM

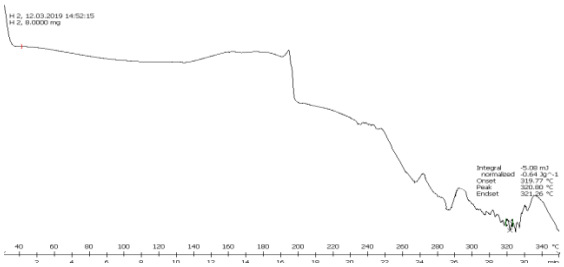

Figure 5(A) DSC of QUR

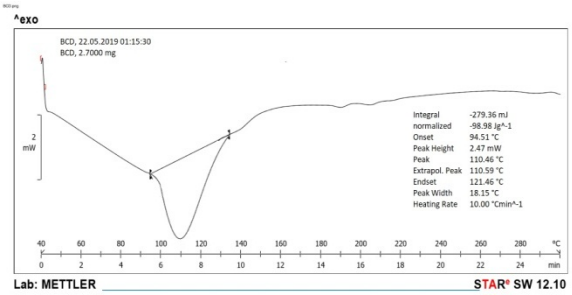

Figure 5(B) DSC of βCD

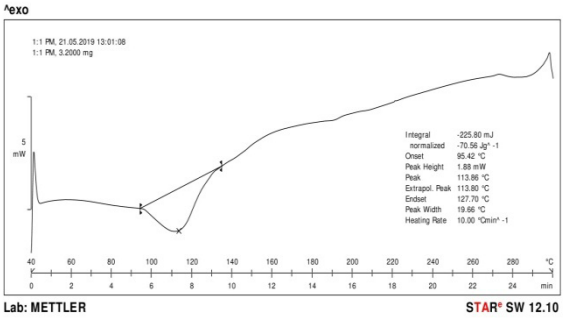

Figure 5(C) DSC of PM

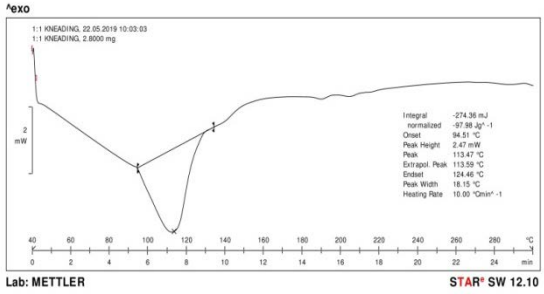

Figure 5(D) DSC of KM

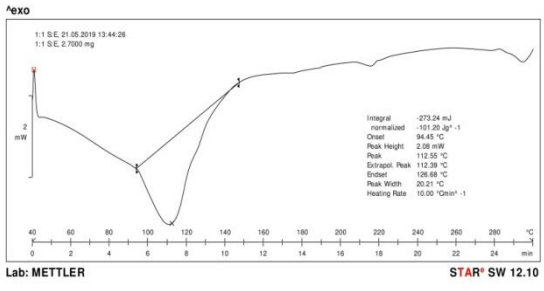

Figure 5(E) DSC of SM

CONCLUSION:-

The phase solubility diagram that is Figure 1 shows the complex formation between QUR and β-CD. The solubility of QUR increased linearly with a slope as a function of β-CD concentration. The phase solubility diagram Figure 1 can be classified as type (A) according to Higuchi and Connors[27,28]. It is assumed that the increase in solubility observed was due to the formation of a 1:1 M inclusion complex. The stability constant (Kc) of QUR and β-CD complex (1:1) was calculated as 321.24 M^{-1}.

The three preparation methods, physical trituration, kneading and solvent evaporation, could increase the solubility and dissolution rate of QUR via the formation of the inclusion complex with β-CD. The most effective method in terms of QUR solubilization also with drug content was solvent evaporation method. Crystalline form of QUR was converted to amorphous form through inclusion complexation.

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