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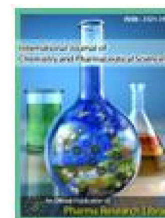

Research Article Open Access

Preparation and Evaluation of Urea Co-Inclusion Complexes of COQ10 for the Simultaneous Enhancement of Dissolution Profile and Its Stability

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A B S T R A C T

Coenzyme Q10 is known as ubiquinone, ubiquinol, and ubidecarenone, an endogenous essential molecule present in every cell in the body. It is a fat-soluble, vitamin-like benzoquinone compound and has an essential role in the production of cellular energy in the form of ATP. However it has poor aqueous solubility and also suffers from photo instability. In the present study, urea, a well-known adductor for linear compounds was successfully utilized for inclusion of Coenzyme Q10 through a modified technique using oleic acid as rapidly adducible endocyte. Formation of Coenzyme Q10 urea co-inclusion compounds was confirmed by FTIR, DSC and XRD. Optimization of formulation in the preparation of Coenzyme Q10 urea co-inclusion compounds was achieved by a 2^2 level factorial design. The co-inclusion compounds were found to exhibit good content uniformity. Through the formation of co-inclusion compounds of urea; it results in achieving steep improvement in the dissolution profile of Coenzyme Q10 and marked increase in its photo stability.

Keywords: Adduction, Content uniformity, Dissolution, Coenzyme Q10, Factorial design Urea inclusion compounds.

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1. Introduction

Coenzyme Q10 is a fat-soluble, vitamin-like nutrient present in every organ of the body. It is a series of compounds which were first described and named by Crane and coworkers [1, 2]. It is also known as ubiquinone because its chemical structure is that of a quinone and it is ubiquitously presence in nature. The chemical structure of coenzyme Q10, $C_9H_{10}O_4$, 2, 3- dimethoxy-5-methyl-1, 4-benzoquinone, was first reported by Folker's group [3]. It is essential for cell respiration and electron transfer, helping to control the production of energy in the heart cells. It also acts as a powerful antioxidant and membrane stabilizer that prevents cellular damage resulting from normal metabolic processes. Although CoQ10 is available naturally in our diet, e.g. in beef, eggs, fish and organ meats, however its use as a dietary supplement has increased dramatically in the last decade. Due to its relatively large size and having a long side chain comprised of 10 isoprene units which contributes to its high molecular weight (863.34 g/mol) as well as its hydrophobic nature, it is poorly and slowly absorbed from gastrointestinal tract [5]. It is widely accepted that the bioavailability of CoQ10 may be improved with an increase of its water solubility and a number of researchers have attempted to enhance its absorption by creating a more water-miscible formulations. Many different approaches have been used to improve the in vitro dissolution of CoQ10. Some approaches include preparation of nanoparticles into which CoQ10 has been incorporated [6], solubilization in a blend of sorbitan monooleate, polysorbate 80, medium chain triglycerides, propylene glycol, alpha tocopherol and poly vinyl pyrrolidone [7], preparation of redispersible dry emulsion [8], solid dispersion of CoQ10 with Eudragit [9], fine oil-in-water emulsion by development of self-emulsifying drug delivery system [10] and self-nanoemulsifying drug delivery system [11].

An inclusion compound is a unique form of chemical complex in which one molecule is enclosed within another molecule or structure of molecules. [12] Inclusion compounds were first observed by Mylius in 1886 as unusual complexations occurring between hydroquinone and various volatile compounds [13]. Although urea inclusion compounds has been known for a long time, but attracted considerable attention of researchers over the past few years because of their interesting and dynamic physicochemical properties [14-15]. Guest molecules containing benzene or cyclohexane rings do not form inclusion compounds with urea, presumably because these structural components are too wide to fit comfortably inside the tunnel [16]. If however benzene carries a long chain substituent, then an inclusion compound may be formed because the long chain of this compound is readily adducted and apparently the unit cell can easily withstand the distortion caused by an occasional benzene group[17]. Similarly a non-adductible endocyte like 3-methyl heptane form an adduct with urea only when a rapidly adductible endocyte serves as a pathfinder [18]. The endocytes having a sufficiently long n-alkane chain and hence easily adductible within urea channels are termed as rapidly adductible endocytes (RAE) while substituted or cyclic endocytes which are known to be non-adductible in urea due to their large molecular size and shorter alkyl chains are named normally non-adductible endocytes. The host substructure consists of a hexagonal framework of hydrogen bonded urea with open, essentially infinite, parallel, nonintersecting tunnels (diameter 5.5-5.6 $\mathring{A}$) which completely enclose guest molecules [19]. A large number of long straight chain compounds such as fatty acids, alkanes, alkenes, alcohols, amino acids, monoesters, and diesters can be exploited as rapidly adductible endocyte. But, dimerization of fatty acids in urea inclusion compounds leads to improved stability profile of the fatty acid-urea inclusion compounds adduct as compared to those of n-aliphatic compounds. In addition of this oleic acid has also been used as a penetration enhancer in transdermal formulations and to improve the bioavailability of poorly water-soluble drugs in tablet formulations. In the present study, the possibility of co-inclusion of a small proportion of CoQ10 in the presence of a suitable RAE i.e. oleic acid was investigated for the improvement of its dissolution and photo stability profile.

2. Materials and Methods

Materials

Coenzyme Q10 was procured from Samex Overseas, Surat, Gujarat, India; the following materials were of analytical grade, urea crystals, extra pure (E. Merck, Mumbai, India), oleic acid (Rankem, New Delhi, India), methanol A.R. grade (Rankem) were used. All other reagents used in experimentation were of analytical grade.

Methods

Excipients Compatibility Studies

The powdered mixture of urea and CoQ10 in the ratio of 1:1 was dissolved in methanol, dried in air and kept in desiccators for 24 hours before conducting the FTIR (IR 200 ThermoNicolet, Madison, USA) study employing KBr disc technique. The spectrum was recorded over a range of 4000–400 cm^{-1}. The graphs obtained through FTIR spectral analysis of CoQ10 and urea and their physical mixture (Fig. 1) reveal that the characteristic peaks of CoQ10 which are evident in spectra of pure drug are also present in the spectra of CoQ10-urea physical mixture. This confirms that urea and CoQ10 were present in the form of physical mixture and no significant chemical interaction has taken place between them. Shifting of some of the characteristic peaks however confirms weak ven-der wall attraction or weak-dipole-dipole electrostatic attraction/repulsion between the specific functional groups of these compounds. [20]

Preparation of Urea Inclusion Compounds of CoQ10

1 gm of CoQ10 was dissolved in 30 ml methanol containing 3 gm urea by vigorous shaking. Subsequently 0.5 gm oleic acid was added as RAE to the above solution; this led to immediate precipitation of crystals. The solution was allowed to stand at room temperature for 2–3 hour. Crystals

were separated from the mother liquor by vacuum filtration, dried and packed in suitable containers [21].

Determination of Entrapment Efficiency of CoQ10-Urea Inclusion Compounds

1 mg of CoQ10 was dissolved in 1 ml ethanol and this solution was further diluted up to 100 ml so as to obtain 10 µg/ml and subsequently 0.5 ml, 1.5 ml, 2 ml, 3 ml, and 4 ml to obtain the concentration in range of 5 µg/ml, 15 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml respectively.. The spectrum of solution with concentration of 10µg/ml was recorded against ethanol as blank. After scanning the solutions between the wavelength 200-400 nm the $_{max}$ came out to be at 274 nm.

The resulting absorbances obtained were plotted against the corresponding concentrations to obtain the standard curve. Raw complex were washed with the n-hexane so as to remove uncomplexed drug. For the estimation of above mentioned parameters the quantity of the CoQ10-Urea inclusion compounds, equivalent to 50 mg of CoQ10 were transferred into a 100-ml volumetric flask using ethanol. The contents of the flask were shaken for about 10 min and made up to volume, mixed and filtered so as to obtain a 10µg/ml solution and then analyzed with UV spectrophotometer at a wavelength of 274 nm. [22]

Content uniformity analysis

Determine the content of CoQ10 in each of 10 equal amount sample taken at random using the method given in the monograph or by any other suitable analytical method. (IP 2007) [28].The preparation complies with the test if each individual content is 85 to 115 per cent of the average content.

Characterization of Urea Inclusion Compounds

FTIR Analysis

The FTIR spectra of the samples was recorded using FTIR spectrophotometer (IR 200 ThermoNicolet, Madison, USA) employing KBr disc technique and all samples were scanned over a range of 400–4000 cm^{-1}.

Differential Scanning Calorimetry analysis

Thermal analysis of the crystals was performed using a DSC Q10 V 9.0 (275), Waters Ltd., Vienna, Austria. TA system with a differential scanning calorimeter equipped with a computerized data station. 2 mg sample were heated in crimped closed aluminum pan at scanning rate of 108°C /min from 40 to 160°C in an atmosphere of nitrogen gas by passing at a flow rate of 60 ml/min. An empty aluminum pan was used as the reference pan. DSC was calibrated using Indium metal with a melting endotherm at 156.898 $^{\circ}$C.

Powder X-Ray Diffraction Study

X-ray diffractograms of the complex were recorded using X-ray Diffractometer (Philips, X'Pert Pro, PW 3050/PW 3071, Lelyweg, The Netherlands). Experimental settings were: Nickel filtered Cu Ka radiations (=1.540598 A°), voltage 40 kV, current 30 mA and scanning rate 2°/min over a 2 range of 10–80°.

Optimization of Inclusion Compounds

Optimization refers to the art and science of allocating available resources to the best possible effect [23]. In order to design the best formulation of inclusion complexes that would give maximum entrapment and dissolution rate in the entire pH range a 2^2 factorial design was used to optimize the amount of oleic acid and urea against a fixed amount of CoQ10 in the inclusion compounds. A factor is an assigned variable such as concentration, temperature, lubricating agent, drug treatment, or diet. The levels of a factor are the values or designations assigned to the factor. Amount of urea and oleic acid was taken each at two levels i.e., maximum (3 mg for urea and 0.5 mg for oleic acid) and minimum (2 mg for urea and 0.25 mg for oleic acid) for 1 mg of drug, to obtain four formulations [24].Amount of drug incorporated in each formulation was 50 mg.

Dissolution Studies

In an ideal situation, an oral dosage form should be tested in-vitro throughout the entire physiological pH 1to 7.8 of the entire GIT in order to simulate the in-vivo conditions [11]. Many studies have reported that maximal concentration of CoQ10 is achieved 6 h after a single dose of formulations [25, 26]. The in-vitro dissolution studies were carried out in USP Type II dissolution apparatus (Lab India DS 8000). 50 mg of CoQ10 or equivalent amount of inclusion complexes were taken in 900 ml of 0.1 N HCl and phosphate buffer pH 7.4 & 6.8 as dissolution medium and subjected to dissolution studies. The paddle was rotated at 50 rpm for 6 hours during which 10 ml samples were withdrawn at predetermined time interval and the medium was replenished with fresh medium. These samples were filtered through whatmann filter paper (0.45µ) and analyzed for drug released.

Stability Studies

Photodegradation process was performed in Photostability study apparatus (Thermolab ES2000 UV), equipped with a cool white fluorescent lamp and near UV fluorescent lamp, option 2 according to the Q1B ICH Guidelines for photostability testing (ICH 1997) (http://www.ikev.org). Irradiance power was set to overall illumination of 5.2 Klux h^{-1} and near UV energy of 1.3W h m^{-2}.Temperature and relative humidity inside the chamber were maintained at 25°C and 60%, respectively, throughout the study. A weighed quantity of finely powdered CoQ10 and its urea co-inclusion compounds were spread as a thin layer in glass Petri dishes (diameter 6 cm). The Petri dishes were placed in the photostability chamber sufficiently apart to avoid shadowing and irradiated with visible lamps. The samples were withdrawn from the chamber after every 24 hour for up to 6 days. The withdrawn samples were immediately analyzed in subdue light environment.

3. Results and Discussion

Characterization of urea inclusion compounds

Preparation of inclusion compounds of CoQ10 in urea in the presence of oleic acid as a suitable RAE was attempted. Addition of small amount of oleic acid as the RAE to a methanolic solution of urea and CoQ10 led to immediate precipitation of fine needle-shaped, yellowish orange crystals. The formation of inclusion compound was confirmed as follows:

FTIR Analysis

FTIR studies were carried out for urea and CoQ10 alone as well as co-inclusion complexes and their physical mixture (Fig 1). In the absence of guest molecule, urea crystallizes in a tetragonal arrangement showing characteristics [29]. IR spectra of inclusion complex exhibited vibrations that are characteristic of the hexagonal channel structure of urea [30, 31] that is out-of-phase NH stretching vibrations at 3412 cm^{-1} and in-phase NH stretching vibrations at 3218 cm^{-1}. Urea inclusion complex also depicted some peaks that can be attributed to the presence of guest moieties, that is oleic acid (2925 cm^{-1} (asymmetric CH_2 stretch), 2853cm^{-1} (symmetric CH_2 stretch). Similar observations were made regarding the occurrence of four bands between 1675 and 1590 cm^{-1} (due to CO stretching and NH_2 bending vibrations), slight raising of the skeletal out-of-phase bending frequency at 789 cm^{-1}, and symmetric C-N frequency increased from 1000 cm^{-1} to 1026cm^{-1}, which are all characteristic of the hexagonal channel structure of urea. These findings indicate transformation of uncomplexed tetragonal urea to hexagonal form containing guest moieties. The above findings are based on inclusion complex prepared using oleic acid as RAE. The FTIR spectra of complex prepared without the use of RAE shows peaks which are characteristics of tetragonal urea at 3428 cm^{-1} 3331 cm^{-1} and 3254 cm^{-1} and it also contains the peaks of CoQ10 at 1670 cm^{-1}, 1587 cm^{-1}, 1263 cm^{-1},1148cm^{-1} and 1079 cm^{-1} [30, 31]. So it can be concluded that as CoQ10 contains 10 unit long isoprene chain which cannot be adducted in urea without the help of suitable pathfinder i.e. RAE.

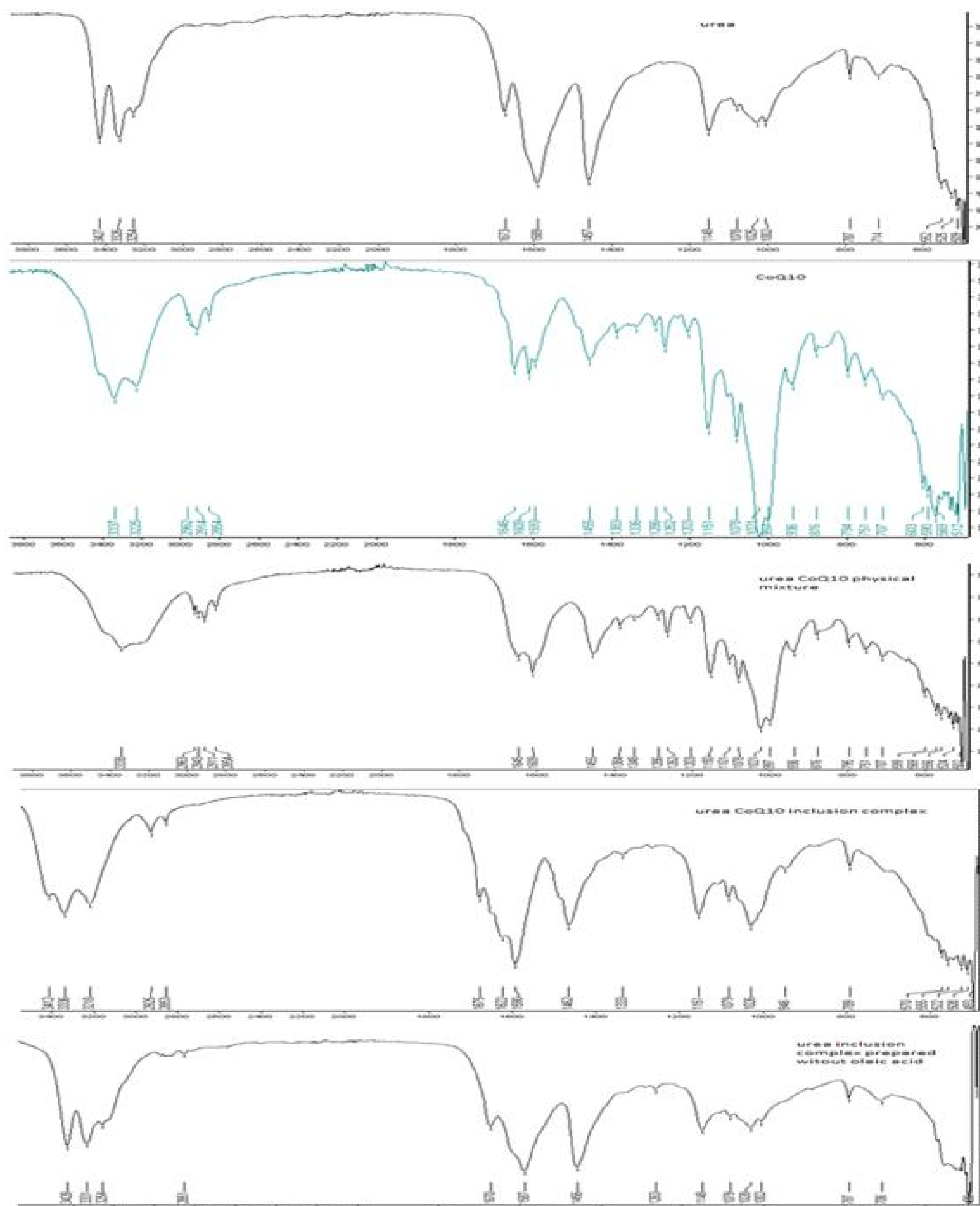


Figure 1: FTIR Spectra of urea, coenzyme Q10, physical mixture and inclusion complex with RAE and without RAE

X-Ray Diffraction Studies of CoQ10- Urea Co-Inclusion Complex:

Diffractional peaks relevant to crystalline CoQ10 were not detectable in inclusion compound product, indicating that the guest molecules were trapped and isolated from one another in the honeycomb network of urea and do not contribute to the crystal structure except for slight distortions of the hexagonal channels caused by bulky guests (Fig 2). The diffractogram of hexagonal urea was characteristically distinguishable from that of the pure tetragonal form of urea, indicating a change in the crystalline form of urea. Prominent peaks at 4.07319 Å, 3.54527 Å, 3.88894 Å, 3.20628 Å, 7.09791Å and 3.77039 Å in the decreasing order of their intensities [29] reveal the presence of hexagonal urea forming adducts. Absence of peaks characteristic of tetragonal form of urea further indicates the transformation of tetragonal form of urea to hexagonal channels containing endocytes. Diffractional peaks relevant to tetragonal urea at 3.96104 Å, 3.60246 Å, 3.5912 Å, 2.80926 Å and 2.52039 Å (Keller 1948) were found in diffractogram of inclusion complex which is formulated without RAE. So the above findings implicitly established the utility of oleic acid for inclusion of CoQ10 in urea beyond any reasonable doubt (Table 1).

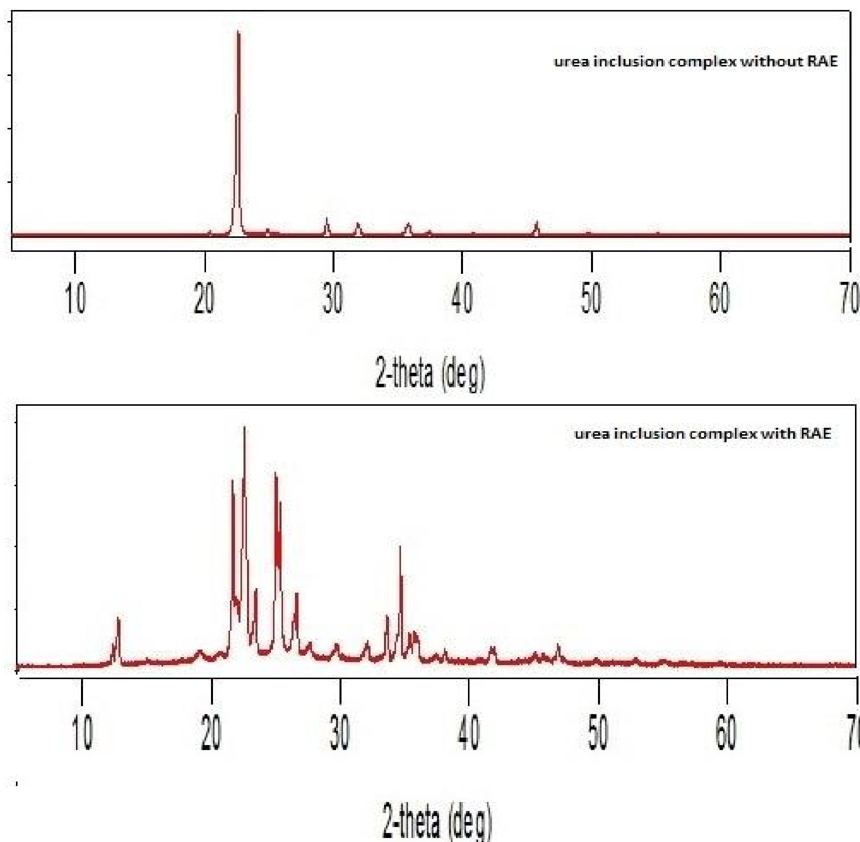


Figure 2: X-Ray Diffractogram of CoQ10- Urea Co-Inclusion Complex without RAE and with RAE

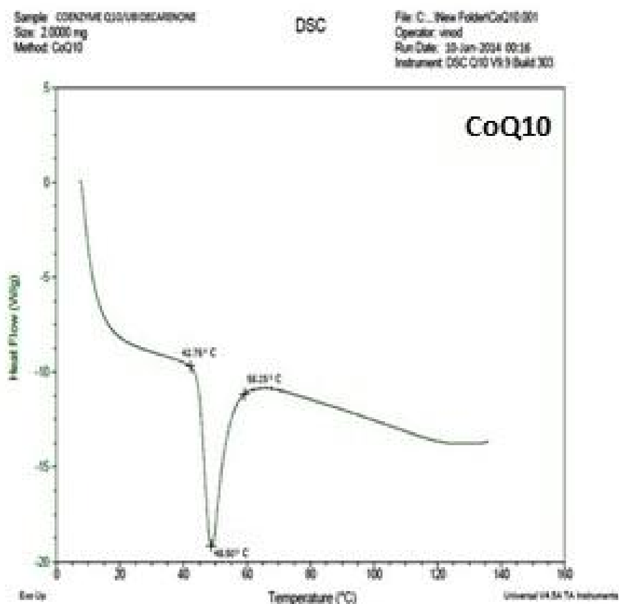
Table 1: Peak List of X-Ray Diffractograph of CoQ10-Urea Co-Inclusion Compounds with RAE

Urea inclusion complex with RAE		**Urea inclusion complex without RAE**	
d(ang.)	Rel. int. I	d (ang.)	Rel. int. I
6.8434	24.74	3.96104	60.82
4.07645	68.12	3.92664	100
4.07319	27.31	3.60246	4.56
3.91731	100	3.5912	3.44
3.88894	47.26	3.03524	11.49
3.77039	32.06	2.80926	6.21

3.54527	78.91	2.80118	3.67
3.49748	58.56	2.52039	4.22
3.35789	22.13	2.50547	8.79
2.57783	24.61	1.98241	8.94

Differential Scanning Calorimetry (DSC) Studies

The thermal behaviour of urea inclusion complex was studied to confirm the formation of inclusion complexes. The thermogram (Fig 3) of the drug shows a characteristic endotherm corresponding to the melting point of the crystalline drug (i.e. 48°C). CoQ10 urea inclusion complex thermogram shows that the urea crystals melt incongruently in four steps. The first step involves the collapse of the hexagonal form of the urea inclusion compound to yield the guest moiety and tetragonal solid urea, while the later steps involve melting of tetragonal urea. The first step (121.98°C) involves the collapse of the hexagonal form of urea inclusion compound to yield the guest moiety and tetragonal solid urea while the later steps (125.16°C, 130.86°C 135.06°C) is attributed to melting of tetragonal urea [32,33]. The thermogram of the inclusion compound does not show a peak of CoQ10, implying inclusion of the drug into the urea lattice [34].



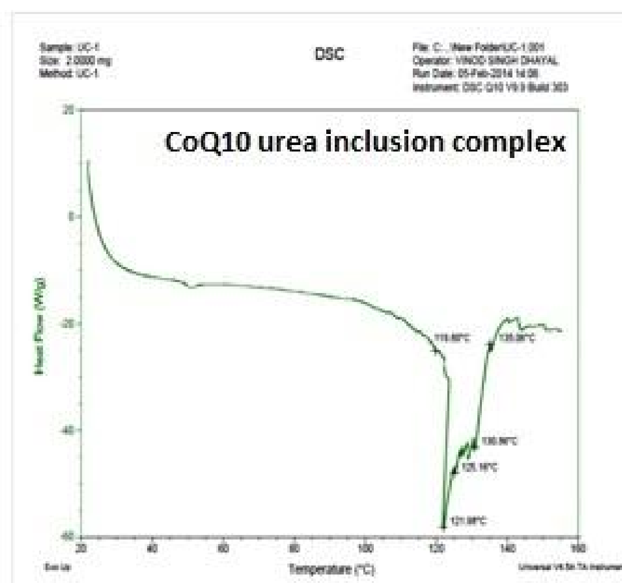


Figure 3: DSC thermo gram of CoQ10 and its inclusion complex

Dissolution Rate Studies

When co-inclusion compounds of urea come in contact with an aqueous dissolution medium, the urea lattice dissolves almost immediately and thus results in instantaneous release of the included drug at a molecular level. Also, inclusion complex of a drug along with RAE in urea leads to weakening/distortion of urea host lattice, manifested as subsequent increase in the dissolution rate. Pure CoQ10 and its urea co-inclusion complexes were subjected for dissolution in phosphate buffer pH 6.8 and phosphate buffer pH 7.4 and 0.1 N HCl. Different preparations containing varying proportions of urea and oleic acid against a fixed proportion of CoQ10 according to 2^2 factorial design were prepared and dissolution studies were carried out in phosphate buffer pH 6.8 and phosphate buffer pH 7.4 and 0.1 N HCl along with the pure drug. On the basis of the %drug released plotted against time it was revealed that although the presence of both urea and oleic acid is significant for enhancing the dissolution rate of CoQ10, the presence of urea was more significant than that of oleic acid. Comparative drug released from pure and its inclusion complex is given in the table. The dissolution rate of inclusion complexes increased up to 65.35143%, 52.44000%,50.115% from 11.73048%, 9.22500% and 7.507% for the pure drug in phosphate buffer pH 6.8 phosphate buffer pH 7.4 and 0.1 N HCl after 6 hours respectively (Table 2).

Table 2: % Cumulative Release of pure CoQ10 and its Urea Co-Inclusion Complex in phosphate buffer pH 6.8 and phosphate buffer pH 7.4 and 0.1 N HCl

Time (hrs)	% Cumulative Release Of pure CoQ10			% Cumulative Release of optimized Urea Co-Inclusion Compounds		
	0.1N HCl	Phosphate buffer pH 7.4	Phosphate buffer pH 6.8	0.1N HCl	Phosphate buffer pH 7.4	Phosphate buffer pH 6.8
0.5	1.26	5.67000	5.742857	10.71	17.73000	20.22857
1	3.164	6.27300	7.606667	21.269	24.85700	29.36762
2	4.279	7.69200	9.061905	32.934	33.05100	35.52
3	5.676	8.40600	9.932381	38.516	39.08300	47.90857
4	6.81800	8.67700	10.64000	45.14600	47.60800	60.00190
5	6.982	8.95000	11.18286	48.425	50.10300	62.45238
6	7.507	9.22500	11.73048	50.115	52.44000	65.35143

Content uniformity analysis

The contents of optimized complexes were found to vary from 96.1 to 99.2% of the claimed amount of drug. Therefore co-inclusion compounds of drug in urea lattice exhibit good content uniformity and hence can be exploited for the development of a better quality pharmaceutical formulation.

Photo Stability Studies

CoQ10 is a light-sensitive compound and urea inclusion complexes are well known hosts with the ability to significantly improve photo-stability of the photo labile compound. Therefore, effect of UV irradiation on CoQ10 degradation was investigated as described. CoQ10 is yellow to orange crystalline powder, and upon exposure to light, CoQ10 gradually decomposes, and the colour changes to dark yellow. Protection from UV light-induced decomposition by urea inclusion complexes has been demonstrated for many substances. There was a marked decrease in the total amount of decomposed drug in the urea co-inclusion complexes sample when compared to the corresponding samples of its pure CoQ10. Pure drug was found to retain only 46.33333% of residual drug content after irradiance with visible light exposure for 7 days, the same amount of photo-exposure resulted in a residual drug concentration of 69.16667% for the co-inclusion complexes of CoQ10.

4. Conclusion

In the present study, CoQ10 was selected as suitable normally non-adductible endocytic drugs candidate known for poor solubility in the body fluid and sensitivity towards photo exposure. Urea inclusion compounds of CoQ10 have been prepared, to meet these objectives. The dissolution rate of inclusion complexes increased significantly. This would not only ensure better bioavailability of the drug, but also a faster onset of action. There was a marked decrease in the total amount of decomposed drug in the urea co-inclusion complexes sample when compared to the corresponding samples of its pure CoQ10. So the need of hour is to get benefit from exploiting the unique capability of urea inclusion compounds.

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