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Urea co-inclusion compounds of 13 *cis*-retinoic acid for simultaneous improvement of dissolution profile, photostability and safe handling characteristics

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Abstract

13-*cis* Retinoic acid (*cis*-RA), a synthetic retinoid used in the treatment of severe acne, is known to exhibit extremely low aqueous solubility and high photosensitivity. In this study, urea, a well-known adductor for linear compounds, was successfully employed for the adduction of *cis*-RA — a substituted cyclic organic compound. Formation of urea inclusion compounds was confirmed by FTIR, DSC and XRD. A modified Zimmerschied calorimetric method was employed for the estimation of the minimum amount of rapidly adductible endocyte (RAE) required for adduction of *cis*-RA in urea. Urea–*cis*-RA–RAE inclusion compounds containing varying proportions of guests were prepared and their thermal behaviour studied by DSC. The inclusion compounds were found to have an improved dissolution profile as demonstrated by an overall increase in the dissolution efficiency. An accelerated photostability study, conducted as per Q1B ICH guidelines, revealed that co-inclusion of *cis*-RA in urea delayed photo-degradation of the drug when compared with that of the pure drug. The results suggest the possibility of exploiting co-inclusion of the drug in a urea host lattice for improved solubility, stability and reduced handling problems for *cis*-RA.

Introduction

13-*cis* Retinoic acid or isotretinoin (*cis*-RA; Figure 1A) is a synthetic retinoid, which has found clinical application in the systemic treatment of severe calcitrant cystic acne vulgaris and in cases of persistent and recurrent moderate acne (Peck et al 1979; Farrell et al 1980; Jones 1989). Its action is believed to be mediated by the inhibition of sebum production and the reduction of sebaceous gland size. It also induces changes in skin lipids, follicular keratinization and the inflammation associated with acne (Marsden et al 1984; Shalita 1984). In addition, *cis*-RA has been reported to reverse the process of chemical carcinogenesis by eliminating preneoplastic lesions and has been found useful in the prevention of epithelial cancers (Lippman et al 1993). *cis*-RA is known to have adverse effects typical of chronic hypervitaminosis A and teratogenic effects (Chen 1985), therefore its use has been restricted to non-pregnant females and patients unresponsive to the conventional acne therapies.

cis-RA is a lipophilic drug (log P = 6.6 (Nankervis 1993)) and is sparingly soluble in water. It is poorly absorbed after oral administration and should be taken with food. In general, retinoids are unstable compounds, being sensitive to oxygen, heat and light (Tan et al 1992; Lucero et al 1994; Brisaert et al 1995). Their stability is, therefore, of pharmaceutical interest. Theoretically, upon photo-exposure, each of the double bonds in the conjugated polyene chain portion of the retinoid moiety can undergo isomerization to give both mono-*cis* and multiple-*cis* isomers but due to steric hindrances, some of these isomers may not be thermodynamically stable at room temperature and tend to isomerize to more stable forms (Motto et al 1989; Bempong et al 1995). Hence, the photostationary state consists of a mixture of all the possible geometric combinations, including the original form. Some of the effects of retinoic acid depend on the geometry of the polyene chain. For example, *cis*-RA and all-*trans* retinoic acid display differential effects on the mRNA levels of retinoic acid receptors (Haq et al 1991). Also, there are differences in the receptor binding of the various isomers (Sundquist et al 1993). Therefore, the development of novel formulations characterized by improved dissolution profile and

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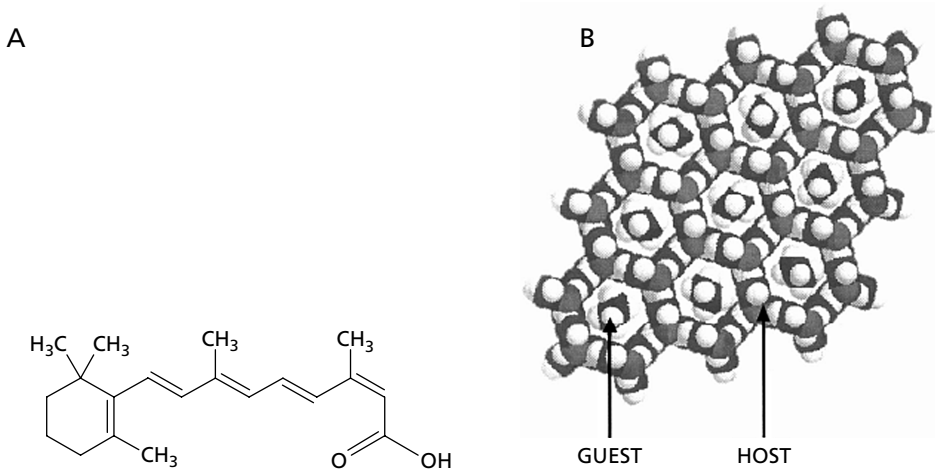

Figure 1 Molecular structure of 13-*cis* retinoic acid (A) and the hexadecane–urea inclusion compounds (B) showing nine tunnels with van der Waals radii, viewed along tunnel axis (reproduced from Harris (1997) by permission of the Royal Society of Chemistry.

reduced sensitivity towards retinoic acid photoisomerization seems to be important.

Recently, attempts have been made to improve dissolution behaviour and photostability of *cis*-RA using cyclodextrin inclusion compounds (Lin et al 2000; Yap et al 2005), as inclusion in liposome matrix (Thoma & Joachan 1992; Ioele et al 2005) and as niosomes (Manconi et al 2003). Photostability studies have been performed on different formulations like lotions (Brisaert & Plaizier-Vercammen 2000), pharmaceuticals (Teraoka et al 2001) and cosmetics (Wang 2000). In this work, an attempt has been made to improve the photostability and dissolution profile of solid *cis*-RA by co-inclusion of the drug in a urea host lattice.

cis-RA is regarded as a hazardous drug and requires safe handling by personnel involved in the preparation, processing, administration and disposal of the drug as per Occupational Safety and Health Administration (OSHA) guidelines. It is strongly recommended that the personnel should wear gloves and adequate necessary clothing while handling the drug. However, adduction can cause altered physical properties of any guest molecule, resulting in reduced handling problems of a compound when included (Weber 1986) and also toxic and hazardous substances becoming safer (Cross et al 1973). Hence, urea inclusion compound formation is proposed to impart safe handling characteristics to *cis*-RA in addition to improvement in the dissolution profile and photostability.

Among the various solid inclusion compounds that have been studied in recent years, the urea inclusion compounds have received particular attention in view of the wide range of fundamental physicochemical phenomena that they exhibit, including incommensurate structural properties, order–disorder phase transitions, molecular motion and properties relating to one-dimensional confinement (Takemoto & Sonoda 1984; Hollingsworth & Harris 1996). In the conventional urea inclusion compounds, the host structure comprises a hydrogen-bonded arrangement of urea molecules that contain hexagonal, non-intersecting, linear, parallel tunnels (Figure 1B) (Harris 1997). The diameter of the urea tunnel varies between about 5.5 and 5.8 Å as a function of the position along the tunnel and this structure is stable only when the tunnels are filled with a dense packing of guest molecules. Linear molecules are included along the tunnel in an extended planar zigzag conformation (Smith 1952; Frank 1975).

The conventional urea channel structure is formed only with guest molecules possessing a sufficiently long alkane chain and only a limited degree of substitution of this chain is allowed (Redlich et al 1950). Compounds having long chains with very little branching may form characteristic adducts in urea. The bulkier molecules, like benzene, are not accommodated by the tunnel and these compounds form no adducts. However, 1-phenyloctadecane forms an adduct with urea. The long chain of this compound is readily adducted and apparently the unit cell can easily withstand the distortions caused by an occasional benzene group (Schlenk 1949; Findlay 1962). 3-Methyl heptane, a normally non-adductible endocyte (NNAE), forms an adduct with urea only when a more slender hydrocarbon (e.g. n-C_6H_{14}, a rapidly adductible endocyte) serves as a so-called pathfinder (Schlenk 1949). The endocytes possessing a sufficiently long n-alkane chain, and hence easily adductible within urea channels, are named herein rapidly adductible endocytes (RAEs) while sufficiently substituted or cyclic endocytes, which are known to be non-adductible in urea, are named normally non-adductible endocytes (NNAE) (Thakral & Madan 2008).

Recently, co-inclusion of amiloride hydrochloride, enalapril maleate and glipizide, along with RAE, in urea has achieved steep improvement of dissolution profiles of these drugs (Thakral & Madan 2007a, b, 2008). In this study, an attempt has been made to simultaneously improve dissolution profile, photostability and safe handling characteristics of *cis*-RA (NNAE drug) through co-inclusion along with a suitable RAE in urea (Thakral & Madan 2006). A number of long straight-chain compounds, such as fatty acids, alkanes, alkenes, alcohols, amino acids, monoesters and diesters, can be employed as the RAE. However, dimerization of fatty acids in urea inclusion compounds leads to improved stability of the fatty acid–urea inclusion compounds adduct as

compared with those of n-aliphatic compounds (McAdie 1963). Moreover, oleic acid is reported to increase the bioavailability of hydrophobic drugs (Stella et al 1978). Hence, oleic acid was selected as the RAE for the purpose of co-inclusion of *cis*-RA in urea.

Materials and Methods

Materials

cis-RA (isotretinoin) was supplied by ABL Biotechnologies Ltd (Chennai); all other chemicals were of HPLC or analytical grade.

Preparation of urea inclusion compounds of *cis*-RA with RAE

cis-RA (0.5 g) was dissolved in 30 mL methanol containing 5 g urea by slight heating. Subsequently, 0.6 g oleic acid was incorporated into the solution. Immediate precipitation of crystals of urea co-inclusion compound was observed. The solution was allowed to stand at room temperature for 2–3 h. Crystals were separated from the mother liquor by vacuum filtration, dried and packed in light resistant containers (Madan & Grover 1993; Bajaj & Madan 1994; Madan 1994). The entire experiment was conducted in subdued light.

Characterization of urea inclusion compounds

The FTIR spectra of the samples were recorded on a FTIR spectrophotometer (IR 200 ThermoNicolet, Madison, USA) using the KBr disc technique and all samples were scanned over a range of 400–4000 cm^{-1}. The resolution of the IR spectra was 4 cm^{-1}. Thermal analysis of the crystals was conducted using a DSC Q10 V 9.0 (275) (Waters Ltd, Vienna, Austria) TA system equipped with a computerized data station. DSC analysis was carried out at a heating rate of 10°C min^{-1} from 40°C to 200°C in an atmosphere of nitrogen gas by passing at a flow rate of 60 mL min^{-1}. DSC was calibrated using indium metal with a melting endotherm at 156.89°C. All samples (~3 mg) were heated in a crimped aluminium pan sealed by application of minimum possible pressure and an empty aluminium pan was utilized as the reference. X-ray diffractograms of the crystals were obtained using an X-ray diffractometer (Philips, X'Pert Pro, PW 3050/PW 3071 (Lelyweg, The Netherlands), using nickel filtered Cu-Kα1 radiations (λ= 1.540598 Å), voltage 40 kV, current 30 mA. Diffractograms were run at a scanning rate of 2° min^{-1} over a 2θ range of 10–80°.

Determination of minimum ratio of RAE and NNAE for formation of co-inclusion compounds with urea

The minimum amount of RAE required for adduction of *cis*-RA per unit quantity of urea was determined by modified Zimmerschied calorimetric method based on the measurement of the temperature increase following addition of increments of RAE to a methanolic solution of urea containing excess of the drug (Zimmerschied et al 1950; Madan 1994). A small-mouthed silvered Dewar flask, fitted with a rubber stopper bearing a thermocouple capable of being read to 0.01°C, served as the calorimeter. Hence, 10 g urea, 5 g *cis*-RA and 25 mL methanol were shaken gently in the calorimeter until an equilibrium temperature was attained. Initially increments of 0.1 mL, and subsequently of 0.5 mL of RAE were successively incorporated into the calorimeter. The calorimeter was shaken after each addition to facilitate an early attainment of the equilibrium and the equilibrium temperature was recorded. A plot of temperature rise versus amount of RAE revealed the minimum amount of oleic acid utilized in the formation of co-inclusion compounds of *cis*-RA and oleic acid in urea (Madan 1994).

Preparation of urea inclusion compounds containing varying proportions of *cis*-RA and RAE

A number of urea–*cis*-RA–oleic acid co-inclusion compounds (cRAICs) containing varying proportions of *cis*-RA and oleic acid were prepared by the above method. The relative proportion of *cis*-RA:RAE used for preparation of different inclusion compounds is listed in Table 1. DSC scans of these inclusion compounds were carried out at the temperature range of 40–200°C.

Assay procedure

HPLC chromatograms were run on a Shimadzu prominence fitted with an LC-20 AD pump, connected to a UV-Vis detector, SDA-20A set at 280 nm. A Phenomex Luna C-18 ODS (5 μm) column (250 mm × 4.6 mm) was utilized. Acetonitrile 95% (v/v) and 5% (v/v) of a 1% (w/v) aqueous ammonium acetate solution was delivered at a flow rate of 1.1 mL min^{-1}. The injection volume was 20 μL. A computer was connected to the detector for data acquisition and peak area and retention time calculation. *cis*-RA was found to elute at a retention time of ~5.5 min.

Content determination and content uniformity analysis

Accurately weighed amounts of *cis*-RA–RAE–urea co-inclusion compounds (cRAICs) containing varying proportions of

Table 1 Drug loading and content uniformity of different *cis*-RA–RAE–urea co-inclusion compounds containing varying proportions of drug and RAE

Product	RAE:drug	Drug loading (as percentage of complex)	Percent drug claimed[a]
cRAIC-1	0.4:1	16.2	99.4 ± 0.3
cRAIC-2	0.6:1	15.3	96.3 ± 0.5
cRAIC-3	0.8:1	14.6	98.6 ± 0.6
cRAIC-4	1:1	12.9	96.4 ± 0.5
cRAIC-5	1.4:1	8.4	95.8 ± 0.3
cRAIC-6	1.8:1	5.6	97.6 ± 0.7

[a] Mean + s.d. for ten randomly drawn samples of inclusion compounds.

cis-RA and RAE were dissolved in methanol and suitably diluted to contain a concentration of ~10 μg mL^{-1}. The drug content was determined by HPLC. For the purpose of content uniformity analysis, exactly weighed amounts of all *cis*-RA–urea co-inclusion compounds containing equivalent of 20 mg of the drug were dissolved in methanol and suitably diluted and assayed by HPLC. Ten randomly drawn samples were used for content uniformity study for each inclusion compound.

Dissolution rate studies

Dissolution study was conducted using USP dissolution apparatus II, in 900 mL of water, maintained at 37 ± 0.5°C at a speed of 100 rev min^{-1}. The quantity of *cis*-RA (20 mg) and of inclusion complexes cRAIC-2 and cRAIC-4 containing an amount of drug equivalent to 20 mg was added to dissolution medium. At predetermined time intervals (2, 5, 10, 20, 30, 45 and 60 min) 5 mL of the samples were withdrawn with volume replacement. The samples were filtered through a 0.45-μm membrane filter, appropriately diluted with methanol and analysed for drug content by HPLC. A cumulative correction was made for the removed samples while determining total amount of drug dissolved. All experiments were performed in triplicate.

Statistical methods

The dissolution rates of *cis*-RA, cRAIC-2 and cRAIC-4 were compared by statistical analysis using one-way analysis of variance (all values were normally distributed). If a significant difference was found, comparisons of the means of individual groups were performed using Tukey's Multiple Range Test at a statistical significant level of $P < 0.05$ (Bolton & Bon 2004).

Photostability study of *cis*-RA and its urea co-inclusion compounds

Photodegradation process was performed in Neutronics Photostability study apparatus Model NEC/09RSPS, equipped with a cool white fluorescent lamp and near UV fluorescent lamp, option 2 according to the ICH Guidelines Q1B for photostability testing (ICH 1997). The apparatus was set up with an electronic device for both irradiation and temperature controlling inside the box. Irradiance power was set to overall illumination of 5.2 Klux h^{-1} and near UV energy of 1.3 W 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 *cis*-RA and of urea co-inclusion compound, cRAIC, were spread as a thin layer in glass Petri dishes (ø 6 cm). The Petri dishes were placed in the photostability chamber sufficiently apart to avoid shadowing and irradiated with both visible and UV lamps. The samples were withdrawn from the chamber after every 24 h for up to 6 days. The withdrawn samples were immediately sealed hermetically in a completely light-resistant packaging and stored in a refrigerator.

On the day of HPLC analysis, an accurately weighed amount of the sample was washed into a volumetric flask with mobile phase and suitably diluted to contain a concentration of ~20 μg mL^{-1}. The content of *cis*-RA in each sample dilution was analysed by the HPLC method. The complete HPLC analysis was conducted under subdued light. The entire experiment was conducted in duplicate.

Results and Discussion

In this work, the possibility of simultaneous improvement of the dissolution profile, photostability and safe handling characteristics of *cis*-RA through co-inclusion with RAE in urea has been explored. *cis*-RA, a normally non-adductible endocyte, was incorporated into urea adduct in the presence of a RAE (oleic acid). The minimum proportion of RAE required to form the adduct was estimated, and the physico-chemical properties of the resulting adducts were investigated.

Characterization of urea inclusion compounds

Incorporation of a small amount of oleic acid into a methanolic solution of urea and *cis*-RA led to an immediate precipitation of fine needle-shaped, uniformly coloured crystals (cRAICs (cis-retinoic acid inclusion compounds)).

Figure 2 demonstrates the IR spectra for pure *cis*-RA and crystals from solution of urea–*cis*-RA and RAE (cRAIC). c*is*-RA is characterized by bands around 1700–1500 and 1300–1100 cm^{-1} that correspond to C = O and C – O stretching vibrations (Yap et al 2005). The IR spectra of cRAIC crystals exhibit peaks at frequencies characteristic of the hexagonal form of urea (3410 cm^{-1} and 3226 cm^{-1} (out-of-phase and in-phase NH vibrations); occurrence of 4 bands at 1675–1590 cm^{-1} (due to CO stretching and NH_2 bending vibrations); slight raising of the skeletal out-of-phase bending frequency at 791 cm^{-1}; and symmetric C–N frequency increased from 1000 cm^{-1} to 1014 cm^{-1}), which indicate presence of hexagonal channel structure of urea (Fischer & McDowell 1960; Durie & Harrisson 1962). The IR spectra of cRAIC crystals also demonstrated certain bands, some of which can be attributed to the presence of guest species (e.g. a strong absorption band at 2925 cm^{-1}) attributed to asymmetric CH_2 stretch (both oleic acid and *cis*-RA), at 1463 cm^{-1} (in plane O-H band). However, the host lattice in an inclusion compound is known to give a vibrational spectrum that inevitably obscures some of the guest molecule bands (Davies 1984). Thermograms for pure *cis*-RA and cRAIC crystals are presented in Figure 3. Pure *cis*-RA exhibits a single melting endotherm at 173.8°C. However, the thermogram of cRAIC showed that these crystals melt incongruently in two steps—the first step is attributed to the collapse of the hexagonal form of the urea inclusion compound to yield the guest moiety and tetragonal solid urea, while the second step is the melting of tetragonal urea (McAdie 1963). Figure 4 shows the powder X-ray diffraction patterns of pure *cis*-RA, pure tetragonal urea and urea co-inclusion compound of *cis*-RA with RAE (cRAIC). Pure *cis*-RA is crystalline, as demonstrated by the sharp and intense diffraction peaks. The diffractogram of urea crystals also depicts peaks characteristic of the tetragonal form of urea, mainly characterized by the peak at 22° 2θ (interplanar spacing at 4.04 Å) (Radell & Connolly 1960). However, the diffractogram of crystals of cRAIC shows peaks

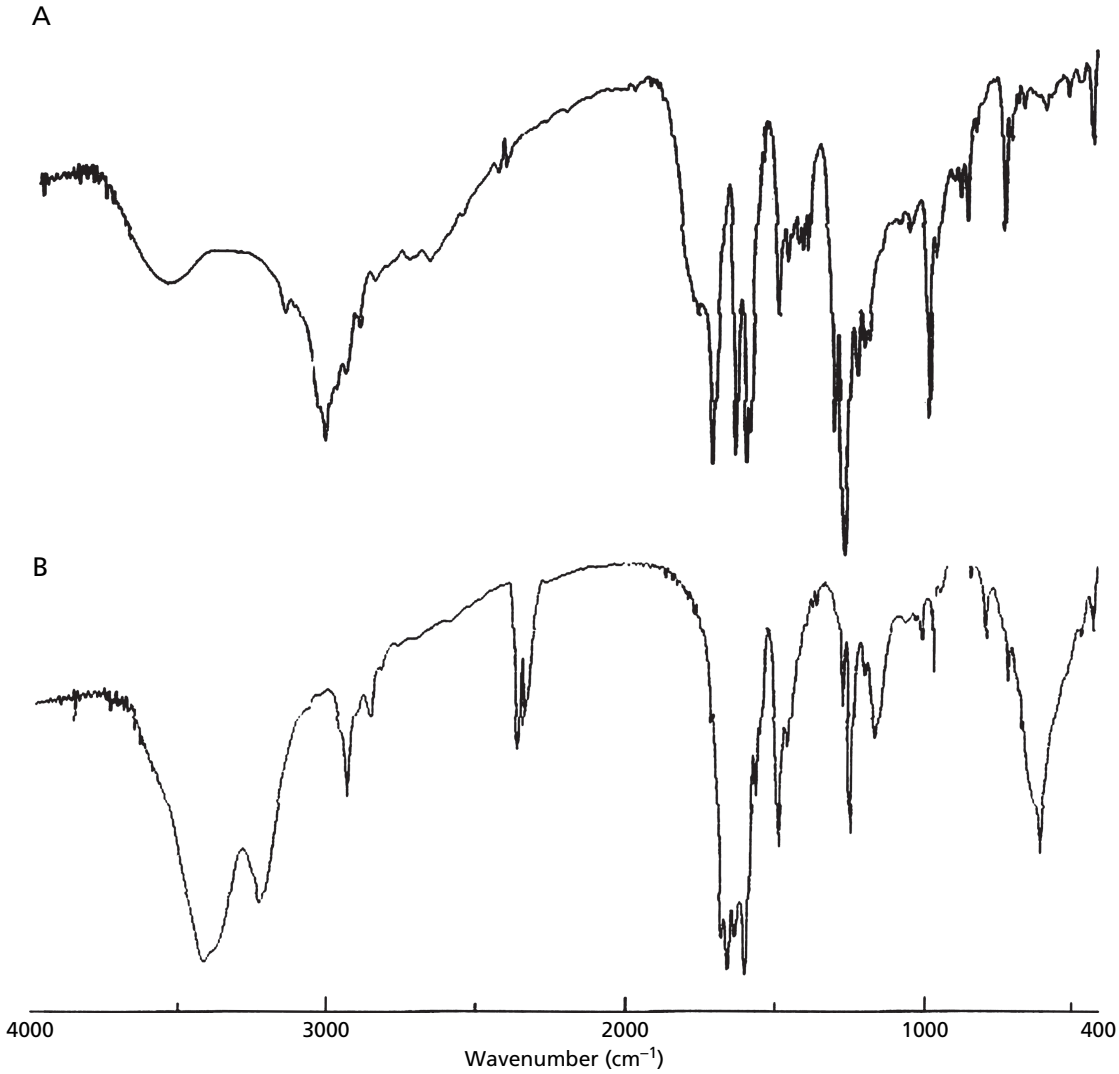

Figure 2 IR spectra for 13 *cis*-RA (A) and *cis*-RA–RAE–urea co-inclusion compounds (cRAIC).

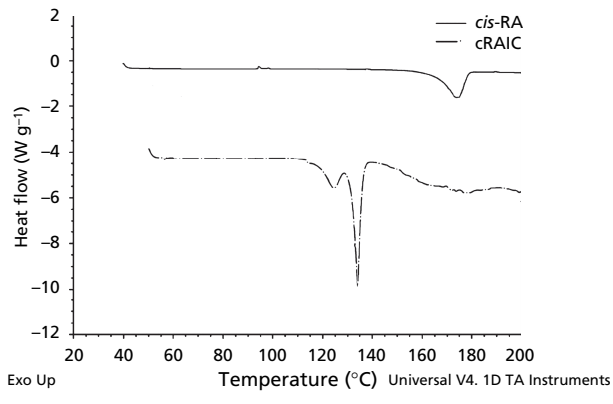

Figure 3 DSC thermograms of *cis*-RA and *cis*-RA–RAE–urea co-inclusion compounds (cRAIC).

characteristic of the hexagonal form of urea (i.e. peaks at 21.4°, 26.4°, 24.4° and 77.8° 2θ (interplanar spacings at 4.13, 3.38, 3.63 and 7.24 Å) (Brodman & Radell 1967). The absence of major peaks of *cis*-RA further indicates that the guest molecules are 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 the bulky guests (Stuart 1956).

Minimum ratio of RAE and NNAE for inclusion of NNAE in urea

The temperature rise on addition of successive small increments of oleic acid to a methanolic solution of urea and *cis*-RA was plotted (Figure 5). The curve typically demonstrated events in the following sequence: an initial temperature rise, followed by intermediate final temperature, subsequent temperature rise and then achievement of a final temperature. The minimum amount of RAE required for adduction of

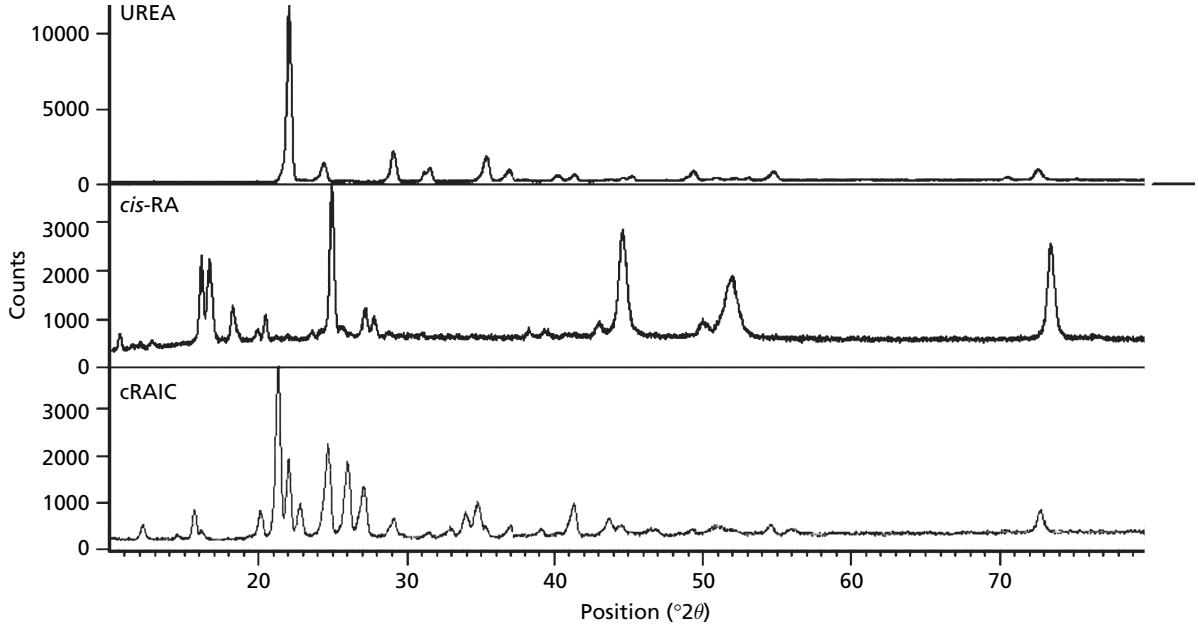

Figure 4 X-ray diffraction patterns of tetragonal urea, *cis* RA and *cis*-RA–urea co-inclusion compounds (cRAIC).

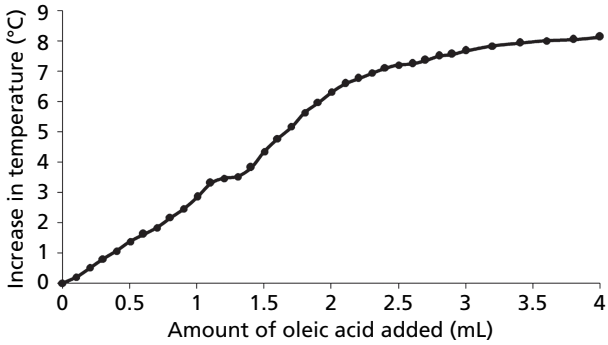

Figure 5 Plot showing increase in temperature following addition of successive increments of RAE to methanolic solution of urea and *cis*-RA.

cis-RA in urea was calculated from the point of intersection of the lines of extrapolation of the initial rate of temperature rise and intermediate final temperature. The second stage of temperature rise is due to displacement of NNAE with RAE as demonstrated by the fact that the overall temperature rise is similar to that of RAE alone (Zimmerschied et al 1950). The minimum ratio of RAE-to-drug for adduction of *cis*-RA in urea was determined to be 0.392:1.

On the basis of calculations of the minimum ratio of RAE:*cis*-RA, urea inclusion compounds containing varying proportion of RAE and drug (Table 1) were prepared and utilized for further investigations.

Characterization of inclusion compounds containing varying proportions of cis-RA and RAE

DSC thermograms of different cRAICs containing varying proportions of *cis*-RA and RAE are shown in Figure 6. The disappearance of the melting endotherm at ~174°C indicates the absence of the crystalline form of *cis*-RA and inclusion of the drug into the host lattice. In all cases a low-temperature endotherm was observed, which corresponds to the complex decomposition and to the release of guest molecules and solid tetragonal form of urea. The influence of the relative proportion of RAE on the heat of decomposition was studied statistically and the data revealed the following regression equation:

$$\triangle H = 18.637R + 16.171 \ (r^2 = 0.9827) \quad (1)$$

where $\triangle H$ is the heat of decomposition of co-inclusion compound and R is the relative proportion of RAE per unit weight of *cis*-RA.

Thus, an increase in proportion of RAE in the adduct leads to corresponding enhancement in the heat of decomposition of the resulting inclusion compounds and makes it more stable. *cis*-RA, being a cyclic substituted moiety, is presumably too wide to fit inside the hexagonal tunnels formed by the urea host. However, in the presence of RAE, *cis*-RA is co-included along with RAE in urea. This inclusion of bulky guests would, in turn, lead to local distortion of the urea channel structure in the vicinity of the cyclic ring, the extent of steric strain on the host corresponding to the proportion of NNAE incorporated in the inclusion compound.

Content determination and content uniformity analysis

The maximum amount of *cis*-RA that can be included in urea in the presence of the RAE was calculated on the basis of the mathematical equation proposed by (Redlich et al 1950) and was found to be ~22%. However, *cis*-RA, being a cyclic and

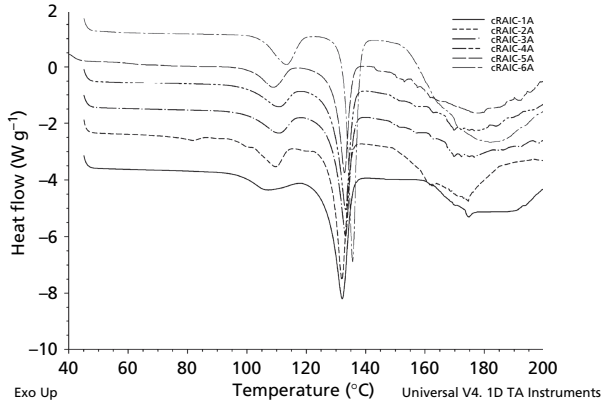

Figure 6 DSC thermograms of *cis*-RA–RAE–urea co-inclusion compounds containing varying proportions of *cis*-RA and RAE.

substituted molecule, was expected to show divergence from this particular value. Experimental determinations led to the finding that the content of *cis*-RA in urea co-inclusion compounds with different ratios of RAE:*cis*-RA ranged from 5.6% to 16.2%. Table 1 shows that the content of *cis*-RA in urea co-inclusion compounds with different ratios of drug:RAE ranged from 95.5 to 99.8% of the claimed amount of drug. Therefore co-inclusion of drug in a urea lattice appears applicable to development of a formulation with high content uniformity.

Dissolution rate studies

Figure 7 exhibits the dissolution profiles for pure *cis*-RA and its urea co-inclusion compounds released in water. The dissolution profiles were evaluated by the dissolution efficiency (DE) parameter at 5 min and 60 min. DE is a model-independent parameter and is employed to compare

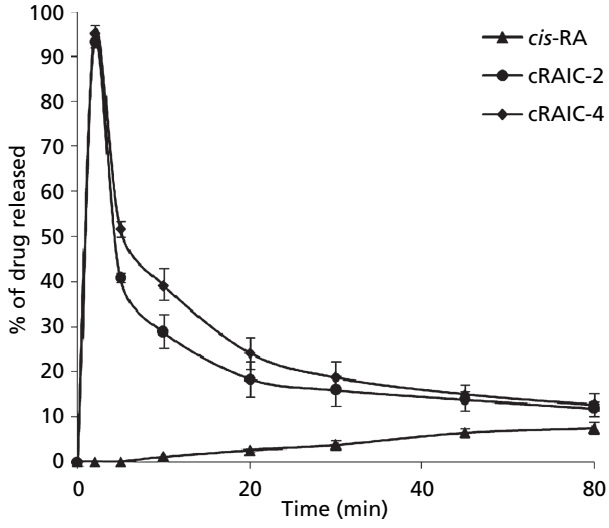

Figure 7 Dissolution profile of pure *cis*-RA and its co-inclusion compounds with urea containing varying proportions of *cis*-RA and RAE. Each point is the average of three measurements; error bars indicate ± s.d.

the dissolution profiles of two different formulations (Khan 1975). It is calculated according to the formula:

$$DE_T = \int_0^T y_t.dt / y_{100}.T \quad (2)$$

Where DE_T is DE at time T, y_t is percent of drug dissolved at any time t, y_{100} denotes 100% dissolution and the integral represents the area under dissolution curve between time zero and T. The extent of pure *cis*-RA released was found to be quite low ($DE_5 = 0.00$ and DE_{60} ~0.03), which may be attributed to the hydrophobicity of the drug. Co-inclusion of the drug in urea provided a faster release of the drug content as exhibited by a DE_5 value of ~0.64 for the two inclusion compounds containing varying proportions of drug and RAE. There was a marked improvement in the initial dissolution profile as manifested by the immediate release of drug content into the dissolution medium. Co-inclusion of drug in urea resulted in an almost instantaneous release of the drug, as exhibited by release of ~94% of content within 2 min. However, this immediate drug release in the dissolution medium was followed by a subsequent fall in the amount of drug content in the solution and DE_{60} was found to be as low as ~0.22, though better than the same DE_{60} for pure drug. When urea co-inclusion compound system is exposed to an aqueous medium, the urea hexagonal host lattice dissolves rapidly and leads to an immediate release of the included guest (i.e. drug and oleic acid) in the instant case at the molecular level. In the instant case, a supersaturation ratio of ~7.5 is developed upon addition of the contents to the dissolution medium. However, such a high supersaturation can not be sustained (Miller 1984) and it will lead to the spontaneous/primary nucleation for obvious reasons. However, as cis-RA is known to possess limited water solubility, the initially released drug molecules subsequently tend to crystallize out of the dissolution medium in excess of the drug's solubility, owing to non-sink conditions prevailing in the dissolution media. Since *cis*-RA is a lipophilic moiety and is known to permeate rapidly through biological barriers, therefore a concentration built up at the actual site of dissolution may not be actually achieved in-vivo. Thus, complete dissolution followed by rapid permeation of the drug may be expected in-vivo.

Statistical analysis

One-way analysis of variance followed by Tukey's Multiple Range Test revealed that the mean of the dissolution profile of *cis*-RA was significantly different from the means for cRAIC-2 and cRAIC-4 ($P < 0.05$). However, the mean of dissolution profiles of cRAIC-2 and of cRAIC-4 were not found to be statistically different at a significance level of $P < 0.05$, which suggested that the difference in composition of adduct had no effect of the dissolution profile of the resulting inclusion compounds.

Photodegradation profile of *cis*-RA in solid state

Figure 8 shows the content of *cis*-RA left undecomposed in the drug sample and in its urea co-inclusion compound (cRAIC) when exposed simultaneously to both the cool white fluorescent and UV lamp as per Q1B photostability study

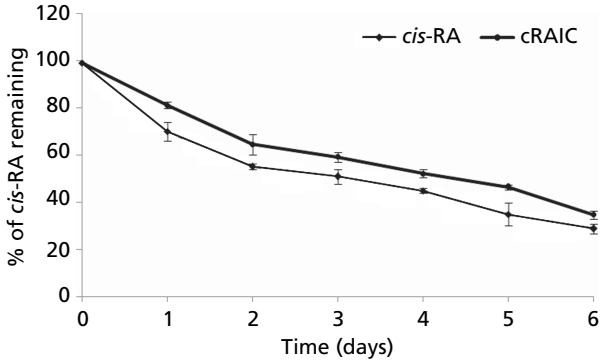

Figure 8 Figure showing amount of *cis*-RA left undecomposed after photo-exposure in samples of pure drug and of its urea co-inclusion compounds.

guidelines. The bright crystals of *cis*-RA were found to have been converted into a sticky lumpy mass on photo-exposure. On the other hand, cRAIC samples were found to retain their free-flowing characteristics, even after 144 h of the combined photo-exposure to both UV and visible light. The figure clearly indicates that the drug co-inclusion with RAE in urea reduces the photodegradation process if compared with that of the pure solid drug. While the sample of pure drug was found to retain only ~70% and ~34% of residual drug content after irradiance with UV and visible light exposure for 24 and 120 h, respectively, the same amount of photo-exposure resulted in a residual drug concentration of ~81% and ~46%, respectively, for the co-inclusion complex of *cis*-RA.

For a particular solid, the appropriate kinetic equation for decomposition is usually obtained by analysing the experimental decomposition data using the existing kinetic forms to see which one gives the best fit (Ng 1971). Hence the experimental data obtained on photostability testing were subjected to various kinetic equations proposed for the characterization of decomposition in the solid state (Glass et al 2004). The kinetic equations, in their differential and integral forms, along with the correlation coefficient obtained for each equation for both the sample (i.e. the drug) and its urea co-inclusion compound with urea are listed in Table 2.

Retrofit analysis of Table 2 revealed that among all the kinetic equations investigated, the best fit for both the drug sample and its urea co-inclusion compound was obtained with the unimolecular decay equation. Furthermore, it has been suggested that sorting out mechanisms by statistical analysis is dangerous (Carstensen 1990); hence from the data obtained, it is difficult to definitely assign a single reaction model for this study.

The photochemistry within an inclusion complex involve features that may be quite distinct from those of the uncomplexed substances since the interior of the cavity constitutes an isolated environment where the included species are usually present as single molecules restricting the photochemistry to intermolecular events (Glass et al 2004). In the case of *cis*-RA, photodecomposition has been attributed to mainly isomerization along double bonds in the side chain and to the oxidation of the unsaturation within the ring, as exhibited by the types of photodecomposition products isolated from a photodegraded drug sample. The containment of guest moiety within urea channels may tend to restrict its isomerization and also to prevent direct availability of incident photons to conjugated double bonds, which provide necessary energy for isomerization. Thus, urea inclusion compound formation tends to reduce the photosensitivity of included guest species.

Inclusion of a bulky guest is known to cause distortions in the vicinity of the cyclic ring in the surrounding hexagonal channels formed of urea molecules. These distortions, in turn, weaken the intermolecular hydrogen bonds and tend to destabilize the host lattice structure. Increasing the proportion of RAE in the co-inclusion compound will reduce the frequency of these distortions and may lead to improved stability of the urea lattice as a whole. Thus, though inclusion of *cis*-RA leads to some improvement in photostability, improved photoprotection can be further expected by incorporation of increased proportion of RAE in the co-inclusion compound. Moreover, use of a suitable RAE, which is more photo-sensitive than the drug itself, can lead to further photo-protection of the included drug

cis-RA is regarded as a hazardous drug and requires safe handing by personnel involved in its preparation, processing, administration and disposal (OSHA). However, in the instant case, *cis*-RA molecules are entrapped and enclosed in the tunnels of the urea host lattice, therefore they might not be available for coming into contact with the skin during the routine handling of urea inclusion compound containing c*is*-RA. As a consequence the personnel engaged in handling of a hazardous drug such as c*is*-RA may not be exposed to the hazardous drug after inclusion in urea. Accordingly, *cis*-RA will cease to be hazardous as long as the urea host lattice remains intact and inclusion of *cis*-RA in urea may ultimately improve its safe handling characteristics for obvious reasons.

Table 2 Various kinetic equations used to describe decomposition in solid state, and the correlation coefficient for photodecomposition of pure drug *cis*-RA and its urea co-inclusion compound

Kinetic equation	Differential form	Integral form	Fit (r^2) for the equation	
			***cis*-RA**	**c**RAIC
Unimolecular decay	$d\alpha/dt = k(1-\alpha)$	$-\ln(1-\alpha) = kt$	0.9785	0.9799
Prout-Tompkins equation	$d\alpha/dt = k\alpha(1-\alpha)$	$\ln[\alpha/(1-\alpha)] = kt + C$	0.9694	0.9424
Modified Prout-Tompkins equation	$d\alpha/dt = k\alpha^{1-1/k}(1-\alpha)^{1+1/k}$	$\ln[\alpha/(1-\alpha)] = k \ln t + C$	0.9588	0.9703

α = fractional decomposition of drug at time t; k = rate constant.

Conclusion

cis-RA, a highly lipophilic and photolabile drug, is known to be hazardous and hence frequently raises formulation problems. In an effort to simultaneously improve the dissolution profile, photostability and safe handling of the drug, co-inclusion compounds of the drug in urea were investigated in this study. Appreciable photo-protection of *cis*-RA was observed when the drug was co-included with RAE in a urea host lattice. Almost instantaneous release of *cis*-RA was observed in-vitro. However, *cis*-RA in excess of its solubility subsequently crystallized out owing to non-sink conditions prevailing in the dissolution media. Since *cis*-RA is a lipophilic moiety and is known to permeate rapidly through biological barriers, a concentration built up at the actual site of dissolution may not be actually achieved in-vivo. Thus complete dissolution followed by the rapid permeation of the drug can naturally be expected in-vivo. Moreover, *cis*-RA, which is regarded as a hazardous drug, may be rendered safe because of its entrapment and enclosure in the tunnels of the urea host lattice following inclusion in urea. As a consequence, personnel engaged in the handling of any hazardous drug, such as *cis*-RA, may not be exposed to the drug after inclusion in urea. Simultaneous improvement of photostability, dissolution profile and safe handling characteristics offer urea co-inclusion compounds as a valuable tool for the development of safe and effective pharmaceutical formulations of *cis*-RA.

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