

Chapter-12
Influence of Inclusion in
Urea on Sensitivity
towards Moisture

12.1 Introduction

Knowledge of the water content of pharmaceutical solids (proteins, drugs, and excipients) is essential to obtain a solid dosage form with optimal chemical, physical, microbial and shelf-life properties. Water content influences the chemical stability, microbial stability, flow properties, compaction, hardness, and dissolution rate of dosage forms of pharmaceuticals, proteins, biopharmaceuticals, nutraceuticals and phytochemicals. (Kontny and Zografí, 1995). Moisture transfer from gelatin capsule shells to the hygroscopic fill contents or to low RH environment may result in brittle capsule products (Chang *et al*, 1998). Sorption of moisture from the manufacturing environment by hygroscopic granules can lead to a sticking problem during compression. The effect of moisture and humidity on the degradation kinetics of various drug substances has also been reported. Moisture plays two primary roles in catalyzing chemical degradation processes. First, water participates in the drug degradation process itself as reactant, leading to hydrolysis, hydration, isomerization, or other bimolecular chemical reactions. In these cases, the degradation rate is directly affected by the concentration of water hydronium ions, or hydroxide ions. Second, water adsorbs onto the drug surface and forming a moisture sorbed layer in which drug is dissolved and degraded. Water adsorption may also change the physical state of drugs, thereby affecting their chemical reactivity. Thus, water affects drug degradation indirectly by providing a favorable environment for degradation (Yoshioka and Stella, 2000).

Hygroscopicity

Hygroscopicity is the ability of a material to interact with moisture from the surrounding atmosphere. In general materials unaffected by water vapors are termed non-hygroscopic while those in dynamic equilibrium with water in the atmosphere are hygroscopic. Callahan *et al.* (1982) have classified the degree of hygroscopicity into four classes:

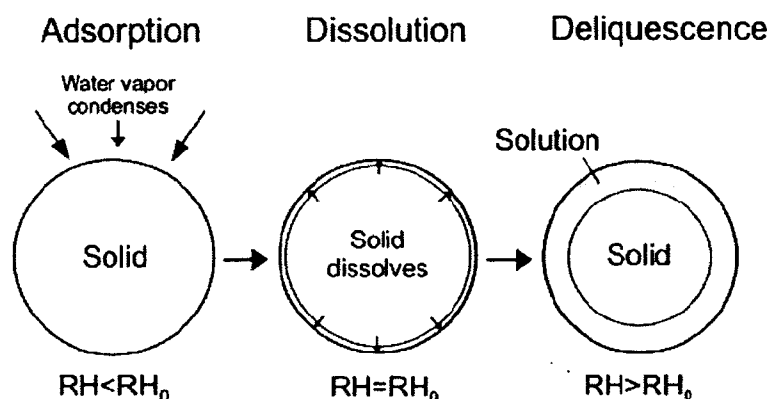
1. *Non-hygroscopic solids*: no increase in water content at < 90% RHs, and increase after storage for one week above 90% RH is less than 20%.
2. *Slightly hygroscopic solids*: no moisture increase at < 80% RHs and increase after one week storage less than 40% at > 80% RH.
3. *Moderately hygroscopic solids*: moisture increase $\leq 5\%$ after storage at below 60% RH, and after storage for one week at > 80% RH is less than 50% moisture.

4. *Very hygroscopic solids*: moisture content increase may occur at 40-50% RH

and increase may exceed 30% after storage for one week > 90% RH.

Normally adsorbed water does not affect the solid to any huge extent before it condenses at the particle surface (Van Campen *et al*, 1983). At high humidity solid water-soluble particles at the surfaces begin to dissolve in adsorbed water vapors and form a saturated solution. This will eventually lead to the deliquescence of the solid. Because a solution is being created, the moisture content rises significantly (Kontny and Zografi, 1995). The RH at which deliquescence first occurs is characteristic of the individual solid and its storage temperature, and it is commonly termed the critical relative humidity (RH_0), which is the RH over the saturated solution of the substance (Kontny *et al*, 1987). The phenomenon of deliquescence is important in pharmaceutical systems because the exposure of solids to humidities above RH_0 results in the formation of a liquid phase where chemical reactions may be accelerated or physical changes catalyzed. Deliquescence and capillary condensation are capable of dissolving water-soluble components (Ahlneck and Zografi, 1990). Condensed water, which is produced during deliquescence, continues to dissolve the solid as long as a sufficiently high RH is maintained: actual dissolution of water-soluble crystalline substances does not occur below RH_0 . Typically poor water-soluble compounds have critical relative humidity values around 90% at 25 °C and when solubility increases, RH_0 decreases (Kontny and Zografi, 1995). The steps in the uptake of water vapors by water-soluble solids are presented in Fig. 12.1.

Fig. 12.1 Water vapor adsorption and deliquescence of water soluble solid particle (modified from Van Campen *et al*, 1983)



Water associated with a substance is classified as either free or bound. Free water (sometimes called mobile or unbound) is loosely adsorbed on the surface of the substance and has the properties of bulk water. Bound water is directly or tightly associated with a material and is not readily available for chemical interaction with other species. Additionally, some water is less tightly bound, with properties reflecting a much higher level of structure than bulk water but less than that of tightly bound water. Thus, the amount of free water rather than the amount of total water is critical to the chemical and physical stability of a drug substance that is moisture sensitive (Cartensen, 1988).

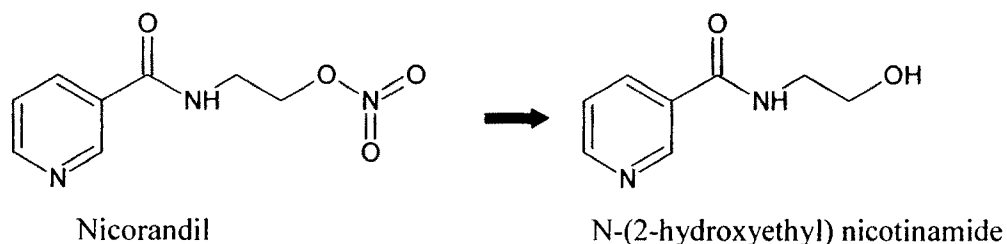
12.2 Hygroscopic nature of urea

Solid urea is stable at room temperature and atmospheric pressure (Kirk-Othner, 1982). It can be stored safely with no loss in quality under normal circumstances. However, urea has a critical relative humidity of 72.5% at 30 °C. Thus at relative humidity of the surrounding atmosphere above the particular value, urea is hygroscopic and begins to absorb moisture from the atmosphere (Admas and Merz, 1929).

12.3 Nicorandil: a moisture sensitive drug

In the solid crystalline state, nicorandil is stable under conditions of extreme dryness, but when it is exposed, although for short periods of time and room temperature, even at low humidity, a considerable humidity ensues. The hydrolysis of nicorandil is catalyzed by three factors, each influencing the others, namely, the increased percentage of moisture in the product in powder state, the temperature and the storage period (Veronesi *et al*, 1996).

The progressive degradation of nicorandil entails the hydrolysis of inorganic ester contained in the molecule, with consequent liberation of nitric acid and N-(2-hydroxyethyl) nicotinamide, a compound that is not pharmacologically active at all considerable dosages, as evidenced by the substantial decrease in the content of active ingredient and therefore of the pharmacological activity (Veronesi *et al*, 1996).



Influence on Moisture Sensitivity

The degradation of nicorandil in the presence of water is so rapid that in aqueous solution at 5%, the product loses, at 60 °C and at pH 7 in only 12 hours almost 20% of its titre. To the contrary, the powder in the dry state and under the same conditions of temperature, does not exhibit, during the same period, appreciable such semi-quantitative variations.

The same disadvantages and drawbacks reported for the raw material nicorandil are evidenced, albeit with more reduced intensity, during the preparation of conventional oral dosage forms but the aforesaid phenomena of degradation attains alarming levels during the subsequent storage periods.

The use of completely moisture proof material for packaging of tablets proves not only relatively expensive, but also insufficient to prevent the absorption of moisture, and therefore to avoid the known degradation mechanism.

12.4 Determination of Sensitivity Towards Moisture

Nicorandil was shown to be successfully co-included in the narrow hexagonal channels of urea in the presence of a suitable RAE (oleic acid, in the present instance). In the present investigation, influence of co-inclusion of nicorandil in urea channels on its moisture sensitivity was studied. In addition to the above, the hygroscopicity of tetragonal and hexagonal form of urea was also investigated.

12.4.1 Water sorption study

The water sorption properties of pure urea, complexed urea (inclusion compound of urea with oleic acid only) and co-inclusion compound of urea with nicorandil in the presence of oleic acid (NRDIC) were determined gravimetrically before and after storage at 30 °C under conditions of various relative humidities in a desiccator. The various RH conditions were achieved in desiccators using saturated salt solutions. Samples in triplicate in open glass vials were allowed to equilibrate in the desiccator and samples were weighed at room temperature after regular interval storage. The water sorption behavior was evaluated from the average weight increase and was expressed as water content (% per starting weight). The results are expressed in **Table 12.1**.

12.4.2 Influence on moisture sensitivity of nicorandil

Nicorandil drug and its co-inclusion compound with urea in the presence of oleic acid were exposed to 60 % RH at 30 °C for a period of one week. At regular intervals samples were withdrawn and sealed hermetically. Nicorandil aqueous solutions have

Table 12.1 Moisture uptake by samples of urea, co-inclusion compounds of urea with nicorandil (NRDIC) and urea-oleic acid inclusion compounds (UOA) when exposed to varying relative humidity at 30 °C.

| Time in days | Relative humidity | | | | | | | | | | | | | | | | | |
|--------------------|-------------------|-------|-----|------|-------|------|------|-------|------|------|-------|------|------|-------|------|------|-------|------|
| | 60 | | | 74 | | | 78 | | | 80 | | | 83 | | | 92 | | |
| | Urea | NRDIC | UOA | Urea | NRDIC | UOA | Urea | NRDIC | UOA | Urea | NRDIC | UOA | Urea | NRDIC | UOA | Urea | NRDIC | UOA |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 43.2 | 21.5 | 13.8 | 49.1 | 25.3 | 18.4 | 70.4 | 27.4 | 21.6 | 78.1 | 26.9 | 23.5 | 83.8 | 34.7 | 31.1 |
| 2 | 0 | 0 | 0 | 43.2 | 25.2 | 17.9 | 52.3 | 31.4 | 22.5 | 74.2 | 34.3 | 24.3 | 81.5 | 35.1 | 26.7 | 90.4 | 39.8 | 34.2 |
| 3 | 0 | 0 | 0 | 43.2 | 26.1 | 19.7 | 52.3 | 31.4 | 24.1 | 74.2 | 34.3 | 25.7 | 81.5 | 35.1 | 27.8 | 90.4 | 41.3 | 35.4 |
| 4 | 0 | 0 | 0 | 43.2 | 26.1 | 19.7 | 52.3 | 31.4 | 24.1 | 74.2 | 34.3 | 25.7 | 81.5 | 35.1 | 27.8 | 90.4 | 41.3 | 35.4 |
| 5 | 0 | 0 | 0 | 43.2 | 26.1 | 19.7 | 52.3 | 31.4 | 24.1 | 74.2 | 34.3 | 25.7 | 81.5 | 35.1 | 27.8 | 90.4 | 41.3 | 35.4 |
| 6 | 0 | 0 | 0 | 43.2 | 26.1 | 19.7 | 52.3 | 31.4 | 24.1 | 74.2 | 34.3 | 25.7 | 81.5 | 35.1 | 27.8 | 90.4 | 41.3 | 35.4 |
| 7 | 0 | 0 | 0 | 43.2 | 26.1 | 19.7 | 52.3 | 31.4 | 24.1 | 74.2 | 34.3 | 25.7 | 81.5 | 35.1 | 27.8 | 90.4 | 41.3 | 35.4 |

been reported to be stable when content determination is done by UV spectrophotometric analysis. Hence, the content determination for the stability studies was performed by HPLC method. In the present study, following HPLC method (Bachart and Fung, 1993) was followed:

HPLC chromatograms were run on a Shimadzu prominence fitted with LC-20 AD pump, connected to a UV- Vis. detector, SDA-20A set at 280 nm. A Phenomex Luna C-18 ODS (5 μ m) column (250 mm X 4.6 mm) was utilized. The mobile phase consisted of filtered 45 % methanol-water, which was delivered at a flow rate of 1.0 ml/min. The injection volume was 20 μ l. Pure water (purified by milli-Q system) was used as solvent. A computer was connected to the detector for data acquisition and peak area and retention time calculation. In the chromatogram resulting from this HPLC system, nicorandil was found to elute at the retention time of \sim 4.658 min. **Fig. 12.2** shows scan of typical chromatogram for nicorandil sample. The calibration curve exhibiting relationship between peak area and concentration of sample is presented in **Figure 12.3**.

Figure 12.2 Representative HPLC chromatogram of nicorandil.

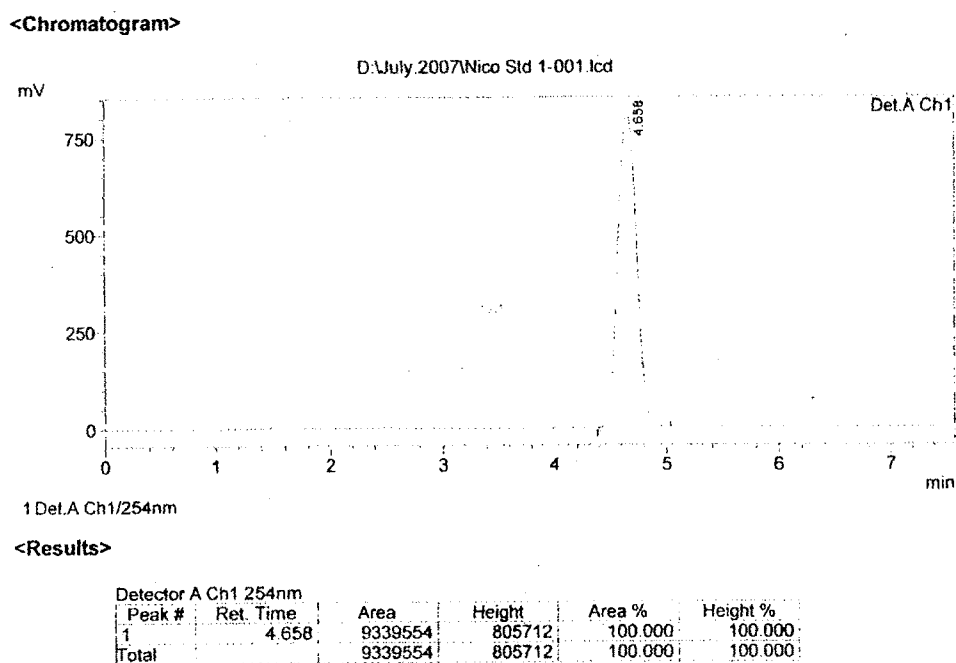
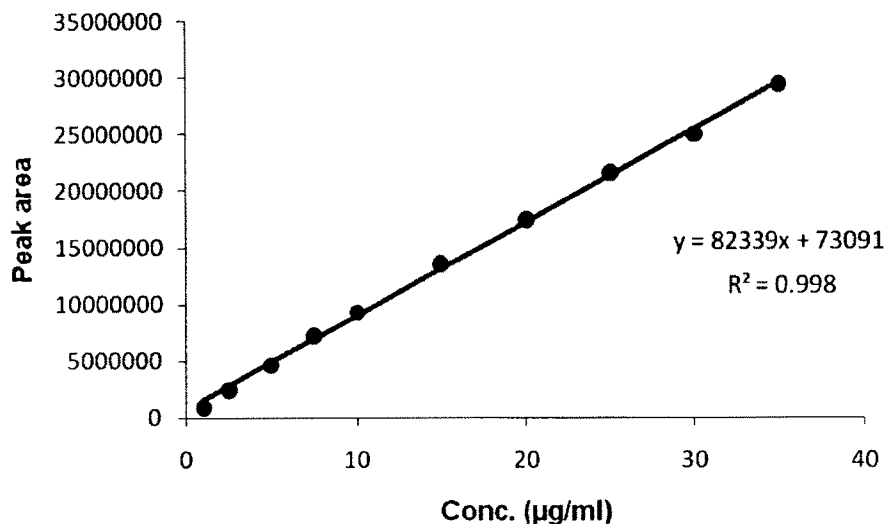


Figure 12.3 Calibraton curve for estimation of nicorandil by HPLC method.

On the day of HPLC analysis, accurately weighed amount of drug sample and of NRDIC were dissolved in water and diluted appropriately to contain a concentration of ~ 10 $\mu\text{g/ml}$. The content of nicorandil in each sample dilution was analyzed by HPLC method. The complete experiment was conducted in duplicate.

12.5 Result and discussions

12.5.1 Stability of urea in tetragonal and hexagonal form

Table 12.1 exhibits the moisture increase when the samples of urea, urea inclusion compound with oleic acid and urea nicorandil-oleic acid inclusion compounds were exposed to conditions of varying humidity. The observations are presented as scatter diagram for each sample in **Figures 12.4 to 12.6**.

Retrofit analysis of the data in **Table 12.1** and **Figures 12.4 to 12.6** reveals the following:

- Urea is not hygroscopic at normal humidity levels as exhibited by negligible increase in water content in all sample even after exposure for one week at 60 %.
- Urea tends to absorb moisture from atmosphere when exposed to humidity levels of ~ 74 % RH. The increase in moisture content was observed for only 1 day, after which equilibrium seemed to be attained and only marginal increase in moisture contents was noted.

Influence on Moisture Sensitivity

- Increase in relative humidity of atmosphere led to increase in moisture uptake by urea sample.
- In comparison to pure tetragonal urea, inclusion compound of urea with oleic acid (UOA) was found to be much more stable as exhibited by substantial fall in moisture uptake by these samples at all humidity levels.
- Though inclusion complex formation led to increase moisture stability for urea, this does not seem to affect the critical relative humidity of urea as demonstrated by moisture uptake by UOA samples at humidity levels of ~74 %.
- Co-inclusion of nicorandil in urea complex along with oleic acid led to reduction in moisture stability of NRDIC (as compared to that of UOA). However, NRDIC was found to exhibit much reduced moisture uptake as compared to that of pure tetragonal urea.

Thus, the inclusion compounds of urea, in which urea exists in its hexagonal form exhibit quite reduced moisture sorption and improved moisture stability as compared to that of the pure tetragonal form of urea. The fact that urea inclusion compounds are more stable than the tetragonal form of urea has already been supplemented by thermal analysis study. As formation of urea inclusion compound is associated with release of heat of decomposition, the compounds possess appreciably improved stability as compared to that of urea. This characteristic has been further strengthened by demonstration of reduced sensitivity of hexagonal urea towards moisture uptake.

In addition to above, heat of decomposition and hence stability of a urea inclusion compound has been reported to be dependent upon shape and length of guest molecules. Bulky guests with ring substitution are known to distort the hexagonal channel in the vicinity of aromatic ring and hence reduce stability of the resulting compound. This characteristic has been further manifested by increase moisture sensitivity of NRDIC as compared to that to UOA. Thus, increasing relative proportion of RAE in the urea inclusion compound can lead to further increase in stability towards moisture uptake by the resulting inclusion compound.

Figure 12.4 Moisture uptake by urea as a function of time at different relative humidity at 30 °C.

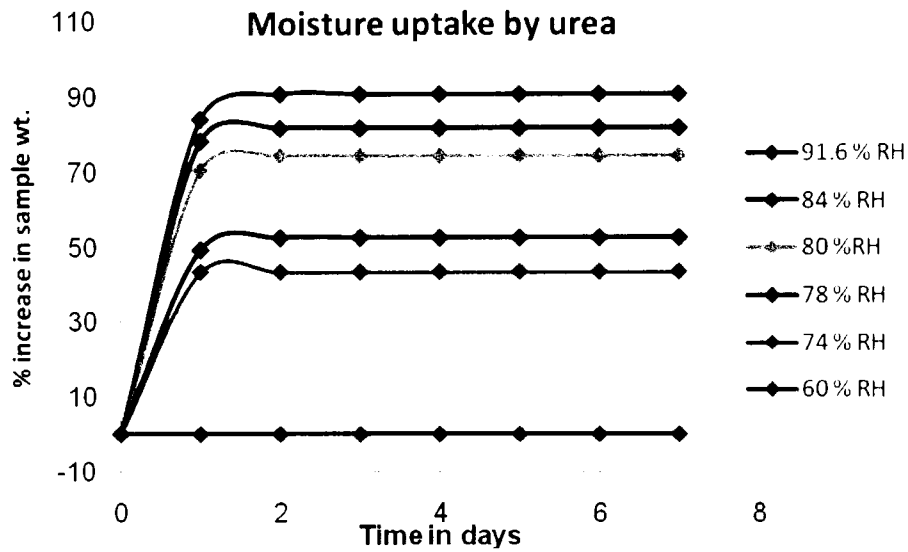


Figure 12.5 Moisture uptake by co-inclusion compound of nicorandil and oleic acid (NRDIC) as a function of time at different relative humidity at 30 °C.

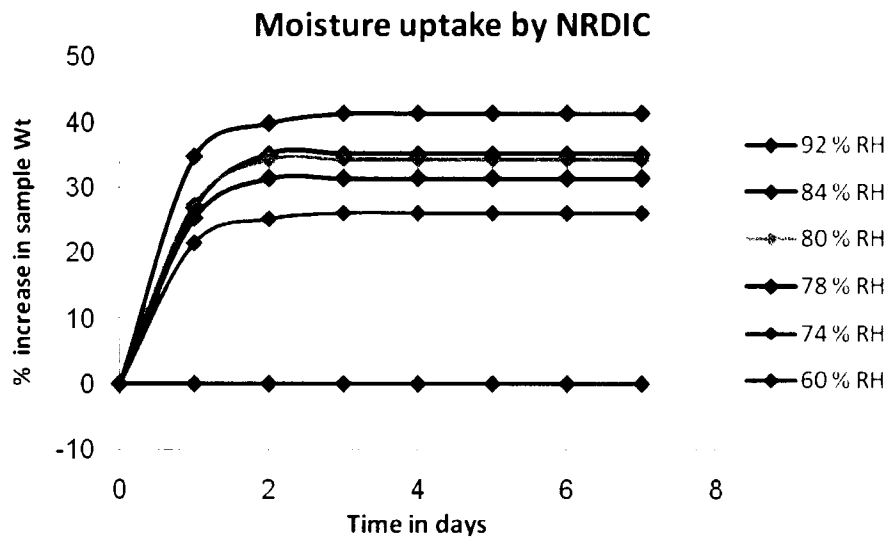
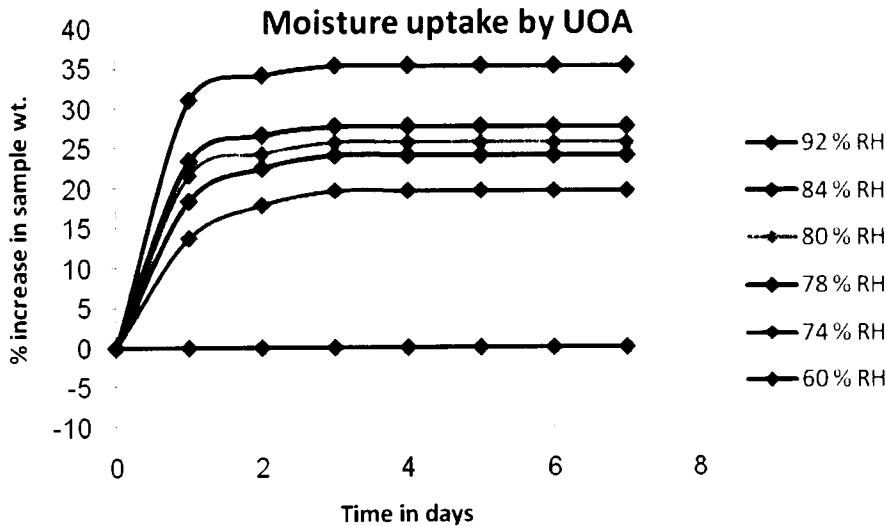


Figure 12.6 Moisture uptake by oleic acid- urea inclusion compound as a function of time at different relative humidity at 30 °C.



12.5.2 Moisture sensitivity study of Nicorandil and its urea inclusion compounds.

Nicorandil drug and its co-inclusion compound with urea in the presence of oleic acid were exposed to 60% RH at 30 °C for one week. The contents of nicorandil present in samples drawn at various time intervals are listed in Table 12.2 and the data is plotted in Figure 12.7.

Figure 12.7 Plot showing percentage of Nicorandil remained intact in samples exposed to 60% RH at 30 °C.

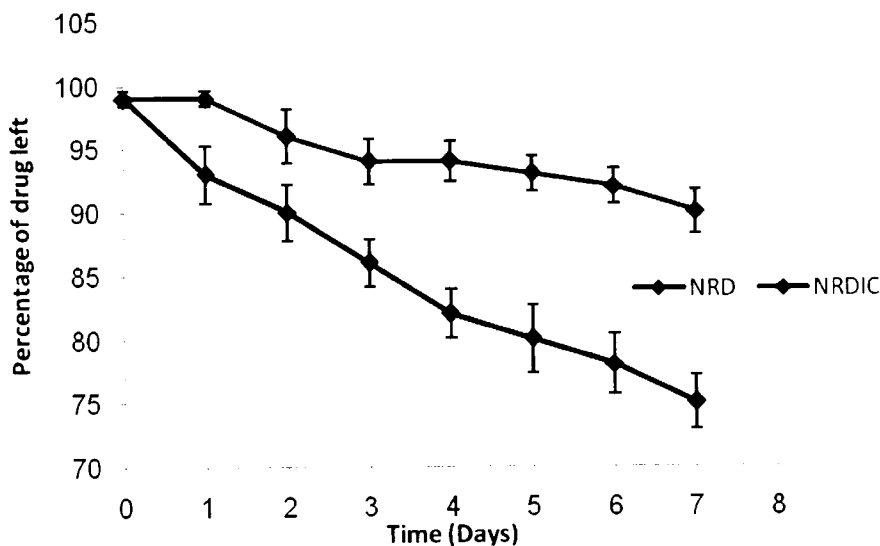


Table 12.2 Percentage of Nicorandil left intact when the drug and its urea co-inclusion compounds were exposed to an atmosphere of 60% RH at 30 °C

| Time in days | Nicorandil | | | NRDIC | | |
|--------------|------------|------|-------|-------|------|-------|
| | 1 | 2 | Mean | 1 | 2 | Mean |
| 0 | 99.6 | 98.4 | 99 | 99.6 | 98.4 | 99 |
| 1 | 91.5 | 95.6 | 93.35 | 98.4 | 99.6 | 99 |
| 2 | 92.6 | 88.2 | 90.4 | 98.4 | 94.2 | 96.3 |
| 3 | 83.8 | 97.6 | 85.7 | 92.3 | 95.8 | 94.05 |
| 4 | 84.1 | 80.3 | 82.2 | 95.2 | 92.1 | 93.65 |
| 5 | 77.6 | 82.9 | 80.25 | 94.2 | 91.5 | 92.85 |
| 6 | 80.3 | 75.6 | 77.95 | 93.1 | 90.3 | 91.7 |
| 7 | 77.5 | 73.2 | 75.35 | 88.6 | 92.1 | 90.35 |

The data clearly reveals when stored in atmosphere of 60 % RH at 30 °C, pure drug demonstrated ~ 75 % of drug remaining intact. Similar exposure of co-inclusion compounds NRDIC led to some reduction in the rate of degradation as manifested by ~ 90 % of drug content left in the sample. Thus, the inclusion of moisture sensitive drug e.g. NRD in urea lattice afforded protection from atmospheric moisture content. The drug being included in the hexagonal channels formed by urea molecules is not exposed to atmosphere and hence manifests reduced degradation. Inclusion of guest moieties containing a cyclic/ring structure has been shown to cause distortions in the vicinity of cyclic moiety. These distortions weaken the host structure and may cause some of the included drug to be exposed to atmosphere. These distortions, however, can be minimized by increasing the relative proportion of RAE in inclusion compound and hence inclusion compounds of nicorandil with still reduced moisture sensitivity can be further developed.

12.6 Conclusion

Urea though quite stable at normal temperature and pressure, is known to be hygroscopic at humidity levels above ~ 72 % RH. Inclusion with a linear long-chain guest moiety has been reported to improve stability of urea. In the present investigation, it was found to that this improved stability is also manifested as substantially reduced moisture uptake when urea in hexagonal form is exposed to an atmosphere with higher humidity levels. Inclusion of NNAE along with RAE was observed to enhance the extent of moisture uptake, though it was still quite less than that of uncomplexed tetragonal urea. Further, urea co-inclusion compound formation was also observed to protect a moisture sensitive NNAE drug (i.e. nicorandil, in the present study) from atmospheric degradation. The guest moieties, contained within hexagonal channels of urea molecules were shielded from exposure to moisture in atmosphere and hence demonstrated improved stability.