Green Synthesis, Structural Characterization and Biological Activity of Diclofenac-Urea Co-Crystal

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ABSTRACT

Co-crystals have become an area of research interest for pharmaceutical drug improvements due to their characteristic nature in modifying the physico-chemical properties of active pharmaceutical ingredients (APIs) with the aid of a co-former. Co-crystals improve the solubility, dissolution rate, melting point, and stability of the API without changing the chemical identity nor diminishing the biological activity or the therapeutic effect of the API. This work reports the green synthetic approach to synthesize Diclofenac-Urea (DUREA) co-crystal using a benign solvent and stirring at room temperature, all in an environmentally friendly manner. The synthesis of DUREA was accomplished by reaction of a 1:1 molar ratio of diclofenac potassium and urea and the product gave a white crystalline compound with an excellent yield (91%). The product (DUREA) was structurally characterized using melting point, UV/VIS, and FT-IR spectrophotometry. The antimicrobial activity of the co-crystal was tested against Salmonella typhi and Proteus mirabilis.

Keywords: Co-crystal, Diclofenac, Urea, Characterization, Antimicrobial, FTIR.

1. Introduction

Recent findings and ongoing pharmaceutical research have proved the need to improve the efficacy and physico-chemical properties of pharmaceutical compounds. Due to this fact, pharmaceutical co-crystals have been an area of research focus for many scientists attributable to the need to improve the raw materials incorporated in drug manufacturing for better physico-chemical conditions. Active pharmaceutical ingredients (APIs) are biologically active and mostly found in the state of low solubility, stability and dissolution rate which may result to poor bioavailability and this is a major concern in the development of co-crystals.

Co-crystals are formed by two or more molecular or ionic compounds combining at a known stoichiometric ratio, having a different crystallographic form than the precursor compounds [1,2], and appear as a unique crystal structure different from the individual molecules that make up its structure. In solid states, they are stable under ambient temperature and pressure conditions [3].

Co-crystals can improve the bioavailability [4] and physico-chemical properties of API effectively without changing the chemical properties and biological functions of the API [5]. They result as molecular complexes that contain two or more components by the formation of π-π interaction, hydrogen bonding, Van der Waals forces, and other non-covalent bonds [6].

Different methods have been utilized in the synthesis of co-crystals such as cooling crystallization, reaction crystallization [7], slow evaporation, slurry crystallization [8], mechanochemical methods [9], grinding, as well as evaporation [10]. Currently, co-crystals are been applied in different science-related fields such as pharmacology, agriculture, food, and cosmetic industries. Studies are increasingly showing the important part that co-crystals play in today’s modern pharmaceutical industries towards improving the physico-chemical properties of APIs and
enhancing their efficacy. Thus, lots of articles have been reviewed, published, and cited with the sole aim of designing, characterizing and developing novel co-crystals with desired properties.

1.1. API and Co-former

Diclofenac potassium is a non-steroidal anti-inflammatory drug (NSAID), used as antipyretic, anti-inflammatory, and analgesic agents [11], indicated in the relief of all grades of pain and inflammations associated with a wide range of conditions, such as acute muscle pain, dysmenorrhea, arthritic conditions, pyrexia, gout, and migraines, [12],[13]. Contrary to the action of many traditional NSAIDs, diclofenac inhibits cyclooxygenase (COX)-2 enzyme with greater potency than it does COX-1 [14]. However, diclofenac is poorly water soluble as an active pharmaceutical ingredient. Urea, employed as a co-former in the synthesis of DUREA is a molecule that serves an important role in nitrogen containing compounds. It is a colourless, odourless solid, highly soluble in water and organic solvents, and practically non-toxic [15].

In medicine, urea has been studied as a diuretic, and was first used by W. Friedrich in 1892 [16]; it was also used to treat hyponatremia and was found safe [17]. Whilst, one of the challenging factors in co-crystal development is the selection of a co-former, urea has been selected as a pharmaceutically acceptable compound capable of improving the solubility of diclofenac.

Though few researchers have reported the co-crystals of NSAIDs with urea, this research displays the green synthesis of diclofenac-urea co-crystal, and its antimicrobial evaluation and characterization by spectroscopic techniques to identify the structure of the newly formed compound using UV/Vis and FT-IR spectrophotometry.

![Chemical structure of diclofenac and urea](image)

**Scheme 1.** Chemical structure of (a) diclofenac and (b) urea

2. Materials and Methods

2.1. Materials

Diclofenac potassium (99%) was a gift from Sidom Pharmaceuticals Nigeria. Urea was received as a gift from the Department of Chemistry, Federal University Otuoke, Nigeria, and analytical grade ethanol (98%) from Merck Germany. All other reagents and chemicals used were of analytical reagent grade and were used as such without any further purification. Double distilled water was used where required.

**Synthesis of Durea**

Diclofenac Potassium (0.296 g, 1 mmol) and urea (0.060 g, 1 mmol) were dissolved in 10 ml ethanol in a round bottom flask and stirred using a magnetic stirrer for 4 hours. There was no colour change and no precipitate formed.
The solution was filtered into a 250 ml beaker, covered and the filtrate was kept for 4 days to evaporate slowly and for crystal formation. The filtrate formed a white crystalline compound. Yield: 91%.

Scheme 2. Schematic synthesis of Durea co-crystal and 3D structure

3. Result and Discussion

White crystalline solids of diclofenac-urea co-crystals (DUREA) have been obtained by magnetically stirring an equimolar mixture of diclofenac and urea in ethanol. Table 1 and 2 depict the physico-chemical parameters and solubility of DUREA in different solvents respectively. DUREA exhibits a lower melting point than diclofenac potassium but a higher melting point than urea, the co-former. DUREA shows improved solubility in ethanol, methanol, and water when compared with diclofenac because of the co-former urea. The improved aqueous solubility of DUREA was conferred by the aqueous solubility of urea.

Table 1. Physical characteristics of compounds and co-crystal

| Compound    | Colour | Melting Point °C | Appearance |
|-------------|--------|------------------|------------|
| Diclofenac K| White  | 283-285          | Powder     |
| Urea        | White  | 133-135          | Crystalline|
| DUREA       | White  | 180-192          | Crystalline|

Table 2. Solubility of API, co-former and formed co-crystals in different solvent

| Compound    | Distilled Water | Ethanol | Methanol | Acetone |
|-------------|-----------------|---------|----------|---------|
| Diclofenac K| S1S             | S       | S        | S1S     |
| Urea        | S               | S       | S        | S       |
| DUREA       | S               | S       | S        | S       |

Key: S1S = slightly soluble, S = soluble, I = insoluble, VS = very soluble

Just like the parent API and its co-former, DUREA displays no antimicrobial activity (data not shown). However, the formation of co-crystal of diclofenac-urea led to improved solubility of DUREA in different solvents which in part will help improve its dissolution rate and tabletability. UREA, being a highly water soluble compound and
capable of forming hydrogen bond networks increased the solubility of diclofenac after forming DUREA by hydrogen bonding.

Fig.1 shows the UV spectra of urea, diclofenac potassium and DUREA co-crystal. The UV-Vis spectra show the $\pi-\pi^*$ absorption maxima of both urea and diclofenac in the DUREA co-crystal, providing evidence for the formation of DUREA co-crystals. A new peak at 300 nm is assigned to the C=O···H-N hydrogen bond chromophore in DUREA co-crystals.

![Fig.1. UV spectra of urea, diclofenac potassium and DUREA co-crystal](image)

FTIR spectra of diclofenac potassium and DUREA co-crystal are presented in Fig.2. Table 3 summarizes the assignment of the FTIR diagnostic bands of DUREA. The spectra of DUREA when compared with the API diclofenac potassium display the FTIR bands of diclofenac with new bands arising from urea hydrogen bonding with diclofenac. In agreement with Oxley and coworkers [18], the FTIR band at 2359 cm$^{-1}$ is due to symmetric stretching from hydrogen bonding of diclofenac C=O with urea N-H. This is also consistent with C=O···H-N hydrogen bonding found in many small-molecule studies and proteins as elucidated by Sagle et al. [19].

**Table 3. Diagnostic FTIR bands of the DUREA**

| Wavenumber (cm$^{-1}$) | Assignment                                              |
|------------------------|---------------------------------------------------------|
| 3431                   | N-H stretching of diclofenac                           |
| 3352                   | N-H stretching of urea                                 |
| 2962                   | C-H aromatic stretching of diclofenac                  |
| 2359                   | COO stretch                                             |
| 1668                   | C=O stretching vibration indicative of C=O···H-N hydrogen bonding of diclofenac-urea |
### Table 1: FTIR assignments

| Wavenumber (cm⁻¹) | Assignment                                      |
|-------------------|-------------------------------------------------|
| 1622              | C=O deformation vibration stretching of urea    |
| 1575              | Diclofenac asymmetric COO stretching             |
| 1556              | C-O stretching vibration of urea                 |
| 1199              | C-N stretching vibration of diclofenac          |
| 771, 748          | C-Cl stretching vibration of diclofenac         |
| 1290              | C-N stretching vibration of urea                 |

### Fig. 2. FTIR spectra of diclofenac potassium and DUREA co-crystal

#### 4. Conclusion

Cocrystallization is an opportunity to modify physico-chemical properties of active pharmaceutical ingredients in order to obtain drug substances with superior properties such as melting point, tabletability, solubility, stability, bioavailability, and permeability while preserving the intrinsic activity of the active pharmaceutical ingredient. In this work, a new co-crystal of diclofenac, a water insoluble drug has been successfully synthesized and characterized by UV and FTIR spectroscopy. The new DUREA co-crystal is stable and more soluble in water, ethanol, methanol, and acetone. Though, DUREA display no antimicrobial activity, the increased aqueous solubility of DUREA imparted by urea will help obviate the poor solubility disadvantage of diclofenac potassium.

### Declarations

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#### Competing Interests Statement

The authors declare no competing financial, professional and personal interests.
**Consent for publication**

Authors declare that they consented for the publication of this research work.

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