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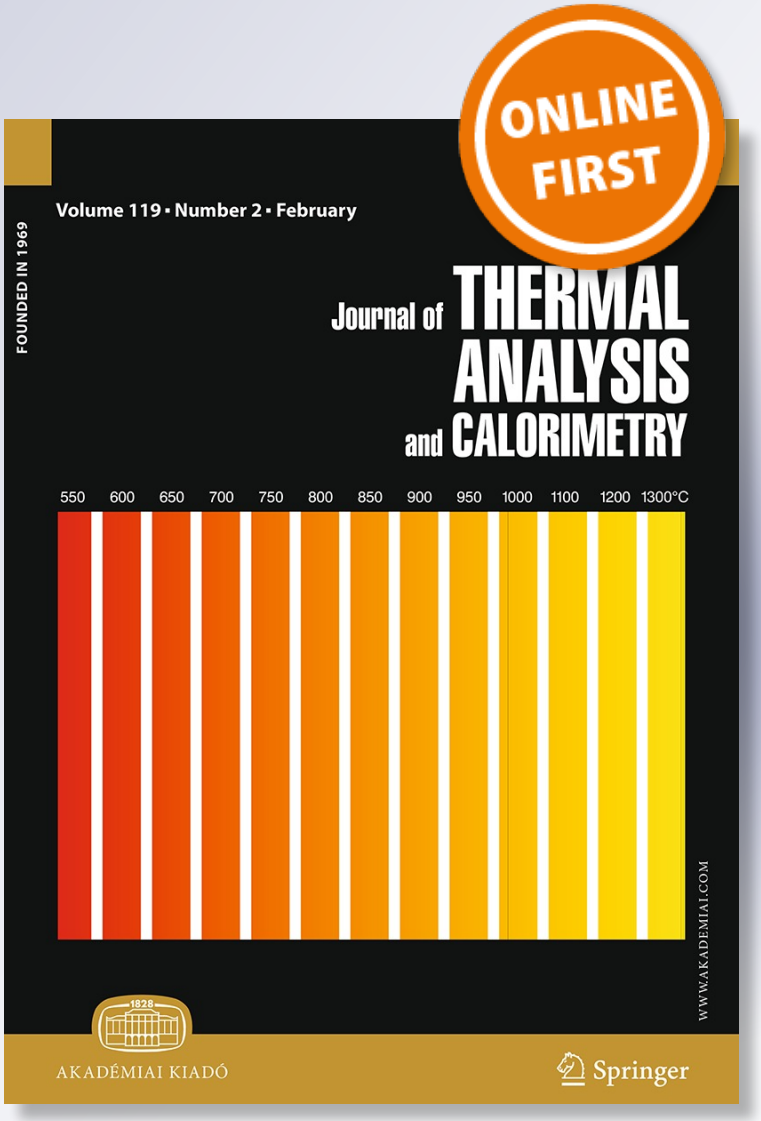

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Preparation and characterization of biologically active novel copper ion-pairs of nonsteroidal anti-inflammatory drugs (NSAIDs)

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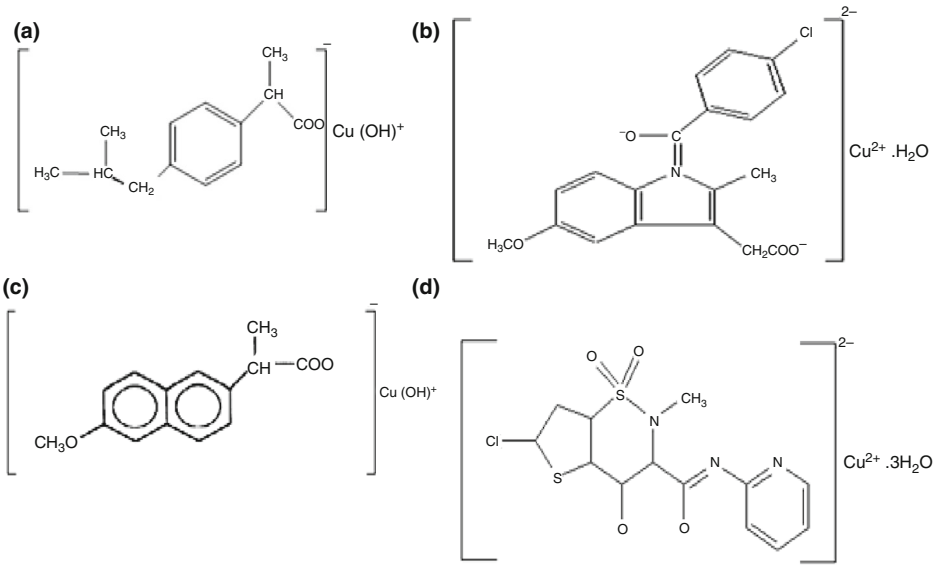
Abstract The present work is concerned with preparation, separation and structure elucidation of solid ion-pairs of nonsteroidal anti-inflammatory drugs in reaction to copper(II) ion. The prepared solid ion-pairs were investigated by different analytical techniques, such as FT-IR, mass and thermogravimetric and differential thermal analyses, in addition to elemental analysis. The general formulae of the prepared ion-pairs were determined. Moreover, the formulae of prepared ion-pairs were proposed and structurally identified. The biological activities of the separated solid ion-pairs toward some kinds of insect species in flour mills in comparison with their drugs were studied, and they were found to be biologically active more than their parent drugs.

Graphical Abstract The present work involved the preparation, separation and structures elucidation of Cu(II) ion-pairs of nonsteroidal anti-inflammatory drugs (NSAIDs). Their proposed structures (a–d) are given.



M. A. Zayed (✉)
Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt
e-mail: mazayed429@yahoo.com

M. I. Nassar
Entomology Department, Faculty of Science, Cairo University, Giza 12613, Egypt

A. M. EL-Gizouli
Chemistry Department, Faculty of Science, Omdurman Islamic University, Khartoum, Sudan

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Keywords Anti-inflammatory drugs (NSAIDs) · Copper–NSAIDs ion-pairs · Thermal · Spectroscopic · Biological activities

Introduction

Inflammation is a common clinical condition [1–4], and rheumatoid arthritis (RA) is a chronic debilitating autoimmune disorder that affects people [5]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been commonly used to reduce pain and inflammation in different arthritic and postoperative conditions [6, 7]. NSAIDs can be classified into several groups. Depending on their chemical structures, NSAIDs are broadly divided into six major classes [8]. In this research, NSAIDs included ibuprofen (IBU), naproxen (NAP), lornoxicam (LOR), and indomethacin (IND). Ibuprofen chemical IUPAC name is (*RS*)-2-(4-(2-methylpropyl) phenyl) propanoic acid. Naproxen (NAP) is chemically 2-naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-methoxy-α-methyl-2-naphthalene acetic acid [8, 9]. Indomethacin has an IUPAC name, 2-{1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl} acetic acid [10–12], and its thermal behavior in different solvents had been discussed [13]. Lornoxicam has an IUPAC name (3*E*)-6-chloro-3-[hydroxyl(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4*H*-thieno[2,3-*e*][1,2] thiazin-4-one 1,1-dioxide [14–16]. The anti-inflammatory activity of NSAIDs is based on the inhibition of prostaglandin synthesis. Prostaglandins are substantially involved in bringing about and maintaining inflammatory processes by increasing vascular permeability and amplifying the effects of other inflammatory mediators such as kinins, serotonin and histamine [17]. Nonsteroidal anti-inflammatory drugs work by inhibiting the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin H_2 (PGH_2). PGH_2, in turn, is converted by other enzymes to several other prostaglandins (which are mediators of pain, inflammation and fever) and to thromboxane A_2 (which stimulates platelet aggregation, leading to the formation of blood clots). The analgesic, antipyretic and anti-inflammatory activity of NSAIDs appears to be achieved mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on platelet aggregation and the gastrointestinal tract [18–20]. However, the role of the individual COX isoforms in the analgesic, anti-inflammatory and gastric damage effects of NSAIDs is uncertain, and different compounds cause different degrees of analgesia and gastric damage [21]. The ratios of selectivity and differences of traditional NSAIDs have previously been evaluated *ex vivo*, although *in vivo* studies have yet to clearly demonstrate the clinical significance of these differences [22]. Little bit literature survey dealt with copper-NSAIDs compounds was given elsewhere [23–26]. Also literature about copper drugs' ion-pairs is scanty. Copper and zinc ion-pairs [27] were extracted from salicylate solution into toluene and determined spectrophotometrically in the organic phase using 1-(2-pyridylazo)-2-naphthol indicator. The determination of copper and zinc was also carried out titrimetrically or by atomic absorption spectrometry after stripping the metal ions from the organic phase. The structures of identical and nonlinear Cu(II) pairs in Y-type zeolites have been studied by EPR spectroscopy. The identical pairs are formed by exchange-coupled Cu(II) ions which have the same symmetry axis and magnetic parameters [28].

The biological and medical activity of these NSAIDs could be improved and changed by their reaction with copper(II) which actually have pronounced biological activity. Therefore, in this research preparation and structure investigation of NSAIDs–copper novel products are the main targets. Consequently in the present study, NSAIDs–copper ion-pairs are under investigation by elemental, mass spectrometry (MS) and thermal analysis (TA) measurements (TG/DTG and DTA). The biological activities of IBU, NAP, IND and LOR drugs and their solid copper(II) ion-pairs will be tested according to the protocol described by Delobel et al. [29].

Experimental

Materials and reagents

All chemicals used were of the analytical reagent grade (AR) and of highest purity degree available. They included ibuprofen (IBU, mole mass = 206.28 g mol^{-1}), naproxen (NAP, mole mass = 230.26 g mol^{-1}), lornoxicam (LOR, mole mass = 371.82 g mol^{-1}) and indomethacin (IND, mole mass = 357.8 g mol^{-1}); an authentic sample were kindly supplied by Arab Drug Company, Cairo (Egypt), and their melting points were found 75–77, 153–155, 239–240 and 155–160 °C, respectively, to confirm their purity [30]. Copper(II) sulfate pentahydrate, anhydrous sodium carbonate and sodium hydroxide (AR, Prolabo), ammonia solution (4 % v/v), hydrochloric acid, phosphoric acid, acetic acid, boric acid (BDH), disodium salt of ethylenediaminetetraacetic acid–EDTA–(AR) and Murexide indicator were used. Double-distilled water from all glass equipment was usually used in all preparations.

Procedures

Preparation of solid NSAIDs–copper ion-pairs

The drugs ibuprofen (IUB), naproxen (NP), indomethacin (IND) and lornoxicam (LOR) solutions were prepared by dissolving the accurately weighed amount of the pure drug in the appropriate volume of 5×10^{-2} M sodium carbonate, with gentle warming. The solid ion-pairs of drugs with copper(II) were prepared by addition of a solution of appropriate mass of metal salt of 0.125 g (0.5 mmol) copper sulfate pentahydrate in 50 mL water to a 50 mL solution of 0.103 g (0.499 mmol) IBU, 0.1151 g (0.5 mmol) NAP, 0.1789 g (0.5 mmol) IND and 0.186 g (0.5 mmol) LOR, respectively. The resulted solid ion-pairs appeared as colored precipitates. The precipitates were left for 10 min until completely settled. The obtained solid ion-pairs were separated, filtered and crystallized from ethanol. The yield of each solid ion-pair was calculated. The physical properties of these ion-pairs were studied [color, melting point (m.p.) and solubility].

Elemental analyses, spectroscopic, thermal studies of copper ion-pairs

On determination of the metal content of the prepared ion-pairs, accurately weighed portion (0.0501, 0.0252, 0.0054 and 0.0247 g, respectively) of the prepared ion-pair was placed in Kjeldahl flask. A mixture of concentrated nitric and hydrochloric acids (aqua regia, 1:3) was added to a powdered ion-pair with gradual heating. After evaporation of each mixture near dryness and complete digestion, the remained solutions had faint blue color. Each solution was then diluted to a 10 mL with bidistilled water, and the copper content was determined by titration of 1 mL of each solution against 0.01 M standard EDTA solution, using Murexide indicator by recommended procedure [30, 31]. The mole masses of the given ion-pairs were calculated from its copper content titrimetrically determined, using the Eq. (1):

$$W = M \times V \times \text{M.wt.}/1000 \quad (1)$$

where W = mass of solid digested ion-pairs, M = the obtained molarity of Cu(II), V = 10 mL of ion-pair solution, M.wt. = mole mass of digested ion-pairs.

Elemental microanalysis of the separated solid ion-pairs of copper with the drugs, for C, H, N, Cl and S, was performed in the Microanalytical Centre, Cairo University. The performed analyses were repeated twice to check their accuracy. Infrared spectra were recorded on a Perkin-Elmer FT-IR-type 1650 spectrophotometer in wave number region 4,000–400 cm^{-1} as KBr disks. The thermal analyses (TG, DTG and DTA) were carried out in dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of 10 °C min^{-1}, in platinum crucible, using Shimadzu TGA/DTA-50H thermal analyzers. The mass spectra were recorded by the EI technique at 70 eV using MS–5988 GC–MS Hewlett-Packard instrument in the Microanalytical Center, Cairo University. Melting point apparatus (Gallen Kamp, Germany) was used to measure the melting points of solid drugs and their ion-pairs.

Biological activity of drugs and their ion-pairs

Adults of *Tribolium confusum* were laboratory-reared [32] on wheat flour at 27.5 ± 1.5 °C and 70 % ±5 % (R.H.) according to the method of Frederic et al. [33] with some modifications. *T. confusum* adult was topically treated with 10 μ of each compound according to the protocol described by Delobel et al. [29] as follows: Thirty insects divided into three replicates (10 adult/replicate) were topically, and mortality was then monitored after 24 h. Thirty adults of control experiment were used in three replicates without treatment. The adult mortality was estimated according to Abbot [34]. Estimation of LD_{50} values was made using Finney analysis [35].

Results and discussion

Structures study of NSAIDs–copper ion-pairs

During the application of the proposed method using copper(II) reagent for spectrophotometric micro-determination of the selected drugs [36–38], the formation of solid ion-pairs is detected, particularly in high concentrations of drug solutions. These solid ion-pairs were prepared, separated and their structures were elucidated by elemental analyses (EA), infrared spectroscopy (FT-IR), thermal analyses (TG/DTG and DTA) and mass spectrometry (MS).

Elemental analyses

Elemental analyses of the prepared solid ion-pairs are performed and recorded in Table 1. From these results, the general formulae are determined and mol masses are calculated. From these data, it is found that IBU-Cu(II) has the general formula ($CuC_{13}H_{17}O_2{\cdot}OH$) of mole mass = 286.84 g mol^{-1}. NAP-Cu(II) has the general formula ($CuC_{14}H_{13}O_3{\cdot}OH$) of mole mass = 309.81 g mol^{-1}. LOR-Cu(II) has the general formula ($CuC_{13}H_{10}ClN_3O_4S_2{\cdot}3H_2O$) of mole mass = 489.37 g mol^{-1}, and IND-Cu(II) has the general formula ($CuC_{19}H_{15}ClNO_4{\cdot}H_2O$) of mole mass = 438.05 g mol^{-1}. The mole masses of these ion-pairs were actually calculated using Eq. 1 from the

Table 1 Analytical and physical data of copper(II) drug ion-pairs

Ion-pair	Color yield/%	m.p./°C	Elemental analysis Found/calcd %					
			C	H	N	S	Cl	Cu(II)
IBU-Cu(II)/$CuC_{13}H_{17}O_2{\cdot}OH$, mole mass = 286.84 g mol^{-1}	Blue/92.39	230–235	54.77/54.39	6.4/6.62	–	–	–	21.76/22.16
NAP-Cu(II)/$CuC_{14}H_{13}O_3{\cdot}OH$, mole mass = 309.81 g mol^{-1}	Dark green/85.86	235–240	56.79/54.23	4.15/4.52	–	–	–	19.45/20.51
LOR-Cu(II)/$CuC_{13}H_{10}Cl\ N_3O_4S_2{\cdot}3H_2O$, mole mass = 489.37 g mol^{-1}	Yellowish green/88.08	220–225	31.67/31.88	2.68/3.27	7.22/8.58	7.05/7.08	8.69/7.25	12.87/12.99
IND-Cu(II)/$CuC_{19}H_{15}ClNO_4{\cdot}H_2O$, mole mass = 438.05 g mol^{-1}	Faint green/88.13	190–195	51.59/52.05	3.26/3.88	2.97/3.19	–	8.21/8.1	14.27/14.51

M = Cu(II), IBU = ibuprofen drug/$C_{13}H_{17}O_2$, mole mass = 206.29 g mol^{-1}, NAP = naproxen drug/$C_{14}H_{14}O_3$, mole mass = 230.26 g mol^{-1}, LOR = lomoxicam drug /$C_{13}H_{10}ClN_3O_4S_2$, mole mass = 371.82 g mol^{-1} and IN = indomethacin drug/$C_{19}H_{16}ClNO_4$, mole mass = 357.8 g mol^{-1}

determined copper ion content in their moiety obtained after digestion and EDTA titration [30].

Infrared analysis of solid ion-pairs

Infrared spectrum of solid IBU and its solid ion-pair The FT-IR spectra of ibuprofen drug solid ion-pair with copper(II) were achieved in the wave number ranged from 4,000 to 400 cm^{-1}, as shown in Fig. 1a. The significant frequencies are interpreted using two references [39, 40]. The FT-IR of IBU refers to the bands of ν $OH_{stretch}$ (at 3,017 cm^{-1}), ν OH_{bend} (at 2,543 cm^{-1}), ν C–O_{bend} (at 421 cm^{-1}) of ν COO (at 937 cm^{-1}) and of ν benzene ring (at 1,070–1,181 cm^{-1}). These bands are shifted to higher values of wave numbers in the corresponding IBU-Cu ion-pair[ν $OH_{stretch}$ (at 3,422 cm^{-1}), ν OH_{bend} (at 2,870 cm^{-1}), ν C–O_{bend} (at 549 cm^{-1}) of ν COO (at 1,513 cm^{-1})], except that of benzene ring which is shifted to lower values of wave numbers (ν benzene ring at 1,070–1,118 cm^{-1}). These data mean that carboxylic group is strongly shared in the formation of IBU-Cu ion-pair via electrostatic attraction, which leads to lowering of the electron density over the benzene ring as a result of electron withdrawing. There is another peak appeared at 588 cm^{-1}, which may be attributed to the ν Cu–O bond. These data confirm the proposed structure of IBU-Cu in Fig. 2a.

Infrared analysis of NAP solid ion-pair Figure 1b shows the FT-IR spectra of naproxen solid ion-pair with copper(II). The well-defined characteristic peaks of naproxen drug at 3,191, 2,579, 478 and 964 cm^{-1} are assigned to the ν $(OH)_{str}$, ν $(OH)_{bend}$, ν (C–O) bend and ν COO, respectively. The ν values of benzene ring (1,081–1,114 cm^{-1}) are shifted to lower values of wave number (1,022–1,111 cm^{-1}), respectively, as result of ionic bonding in solid NAP-Cu(II) ion-pair. These frequencies are 3,734 cm^{-1} ν $(OH)_{str}$, 2,971 cm^{-1} ν $(OH)_{bend}$, 567 cm^{-1} ν (C–O) bend and 1,265 cm^{-1} ν COO and are assigned to the same characteristic groups. The formation of new bond at 471 cm^{-1} is attributed to Cu–O bond. All these experimental evidences confirm the participation of carboxylic group COO^- of naproxen drug in the formation of solid NAP-Cu(II) ion-pair via electrostatic attraction as given by the proposed structure in Fig. 2b.

Infrared analysis of IND solid ion-pair Figure 1c shows the FT-IR spectra of indomethacin drug (IND) solid ion-pair with copper(II). The well-defined characteristic peaks of IND drug at 3,020, 1,227, 914, 1,597 and 1,699 are assigned to the ν $(OH)_{str}$, ν $(OH)_{bend}$, ν $(C–O)_{bend}$, ν (COO), ν and ν (C=O, amide), respectively. These peaks of IND drug are shifted to another value of wave number in case of solid IND-Cu(II) ion-pair as a result of electrostatic

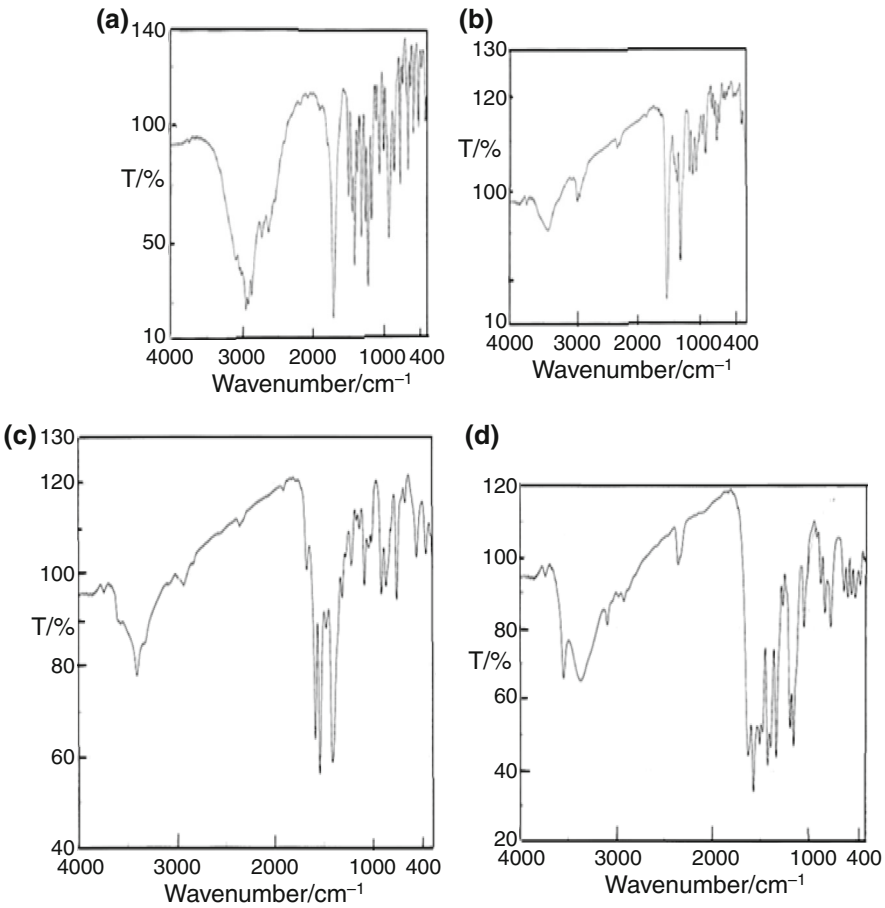


Fig. 1 FT-IR spectra of **a** solid IBU-Cu(II) ion-pair, **b** solid NAP-Cu(II) ion-pair, **c** solid IND-Cu(II) ion-pair, **d** solid LOR-Cu(II) ion-pair

attraction except those of aromatic rings. These frequencies are 3,743, 1,285, 1,049, 1,543 and 1,591 cm^{-1}, respectively, for the same characteristic peaks. This means that the electronic density around hetero-five-membered nitrogen rings is withdrawn away due to sharing of carboxylic (COO^{-}) group in the formation of solid IND-Cu(II) ion-pair as given by the proposed structure in Fig. 2c. The appearance of new band at wave number 564 cm^{-1} may be attributed to the formation of the Cu–O bond.

Infrared analysis of LOR solid ion-pair Figure 1d shows the FT-IR spectra of LOR solid ion-pair with copper(II). In case of LOR, FT-IR shows –*v* OH (str.) at 3,600, *v* C–O (bend.) at 1,200, –*v* C=O (amide) at 1,680, *v* CONH at 1,700 cm^{-1}, respectively. The pyridyl group of LOR drug shows *v* values at 1,581, 1,030, 991 and 604 cm^{-1} due to its different modes of vibrations. In case of FT-IR of LOR-Cu, these bands are shifted to wave numbers values as *v* OH (at 3,650 cm^{-1}), *v* NH amide (at 3,400 cm^{-1}), *v* C–O (amide) (at 1,155 cm^{-1}), ν C=O (amide) (at 1,628 cm^{-1}) and *v* CONH (at 1,670 cm^{-1}). The modes of vibrations of pyridyl group are shifted to lower values of wave number range 963–786 cm^{-1}. This shift confirms the sharing of both amide group in enol form and OH of heterocyclic ring via electrostatic attraction in the formation of LOR-Cu as given by the proposed structure in Fig. 2d. This means that the electron cloud around pyridyl group was withdrawn away due to sharing of amide group of LOR in solid ion-pair formation via electrostatic attraction.

The proposed structures of drugs' ion-pairs

Depending upon the FT-IR data previously discussed together with conductivity measurements data, the proposed structural formulae of the prepared copper(II) ion-pairs are given in Fig. 2a–d. In order to confirm these structures, thermal analyses (TA) and mass spectra of these compounds were performed. Full description of TA is given in Figs. 3 and 4, and schemes 1, 2, 3, and 4.

Thermal analyses of solid ion-pairs Thermogravimetric (TG/DTG) analysis of solid IBU-Cu(II) ion-pair is employed to provide quantitative information on mass losses due to thermal decomposition as a function of time and temperature. The thermal analyses data of solid IBU-Cu(II) ion-pair are shown in Figs. 3a and 4a. From TG/DTG curve as

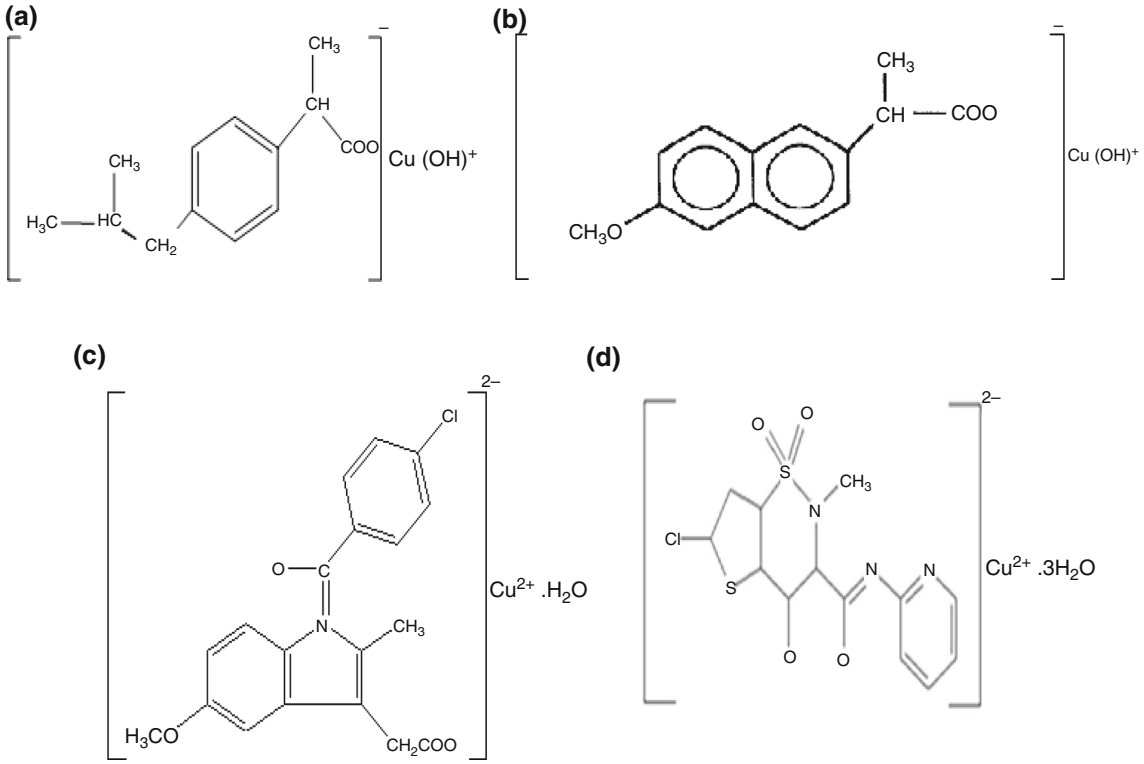


Fig. 2 Proposed structure of **a** solid IBU-Cu(II) ion-pair, **b** solid NAP-Cu(II) ion-pair, **c** solid IND-Cu(II) ion-pair, **d** solid LOR-Cu(II) ion-pair

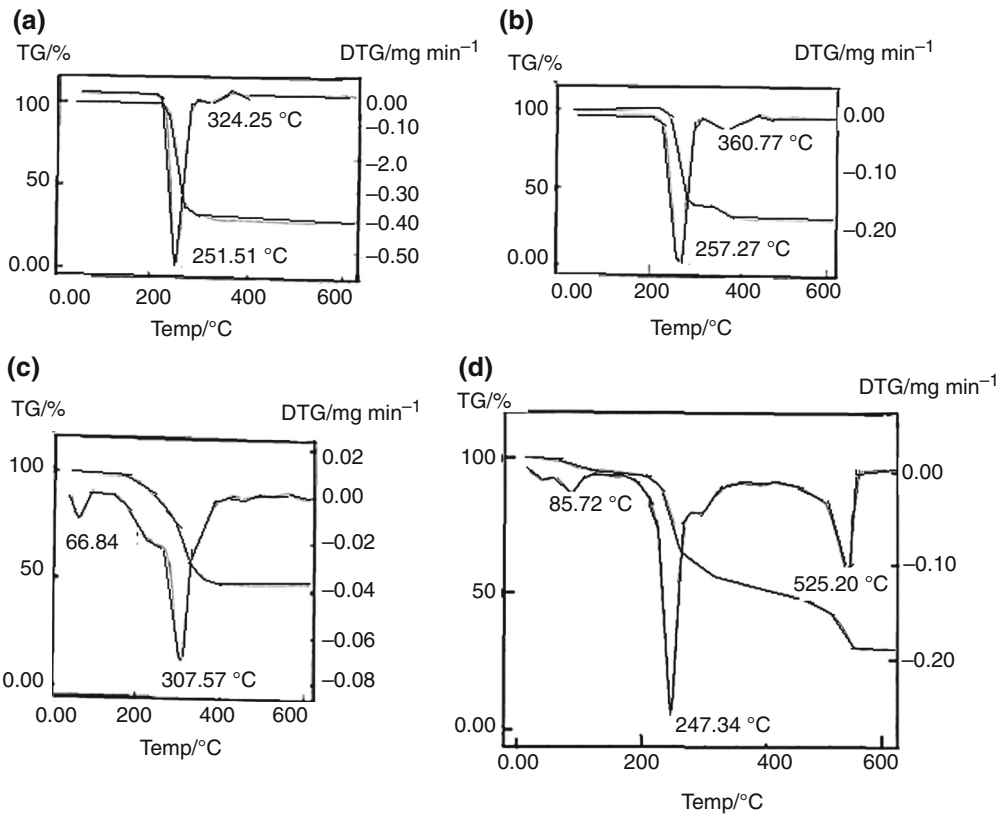


Fig. 3 TG and DTG of **a** solid IBU-Cu(II) ion-pair, **b** solid NAP-Cu(II) ion-pair, **c** solid IND-Cu(II) ion-pair, **d** solid LOR-Cu(II) ion-pair

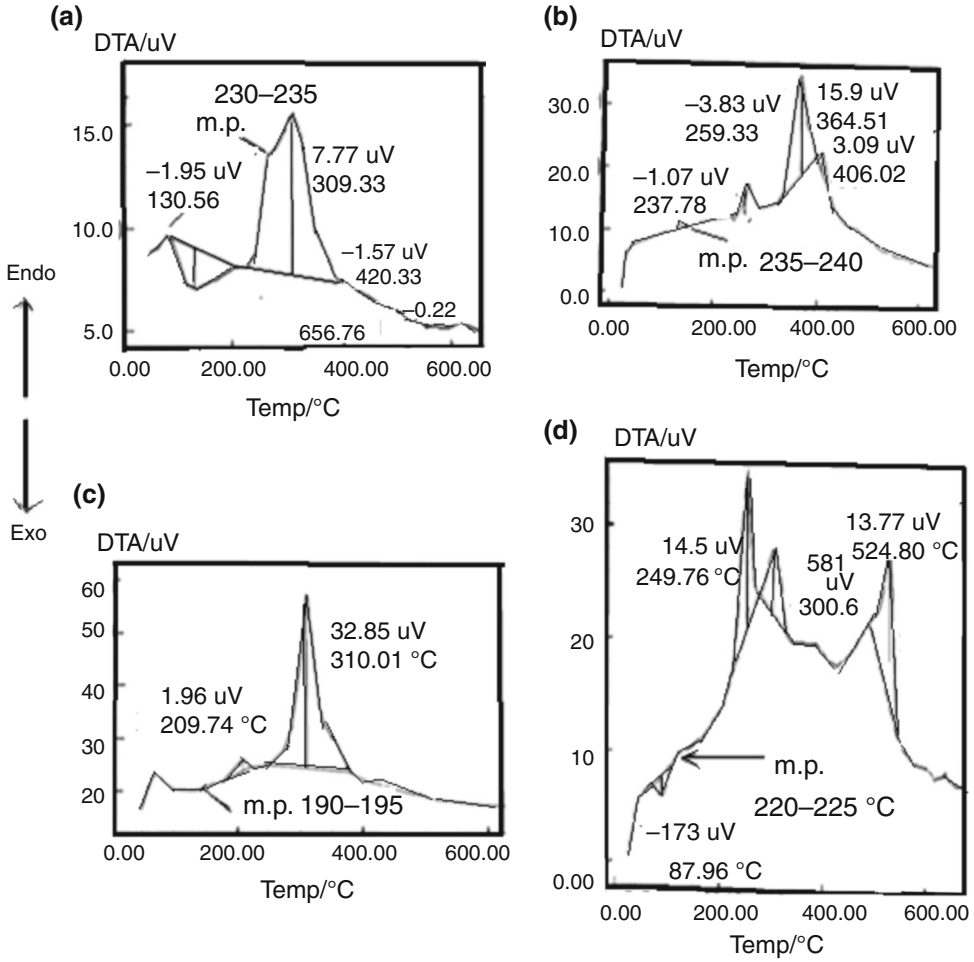
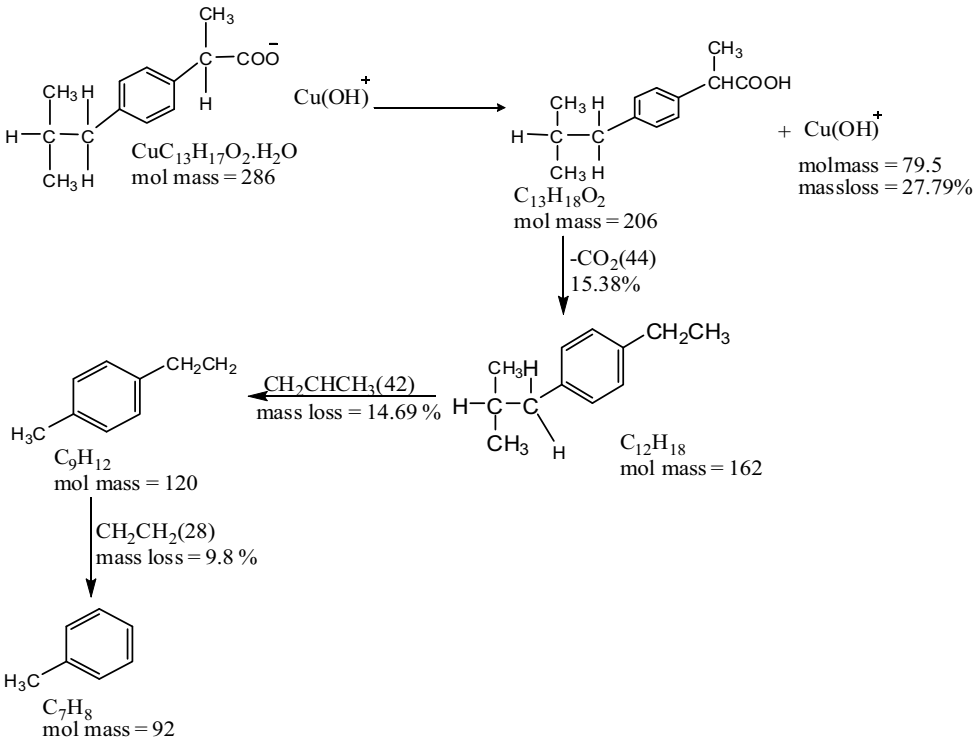


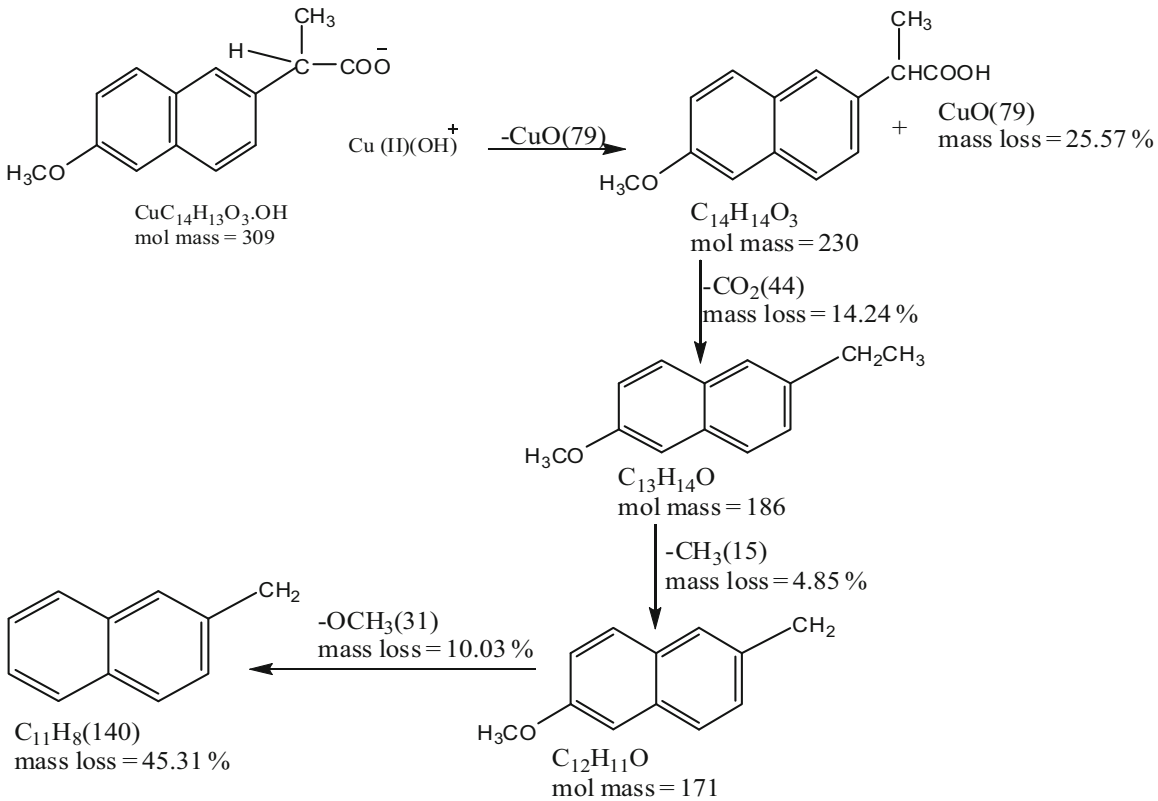
Fig. 4 DTA of **a** solid IBU-Cu(II) ion-pair, **b** solid NAP-Cu(II) ion-pair, **c** solid IND-Cu(II) ion-pair, **d** solid LOR-Cu(II) ion-pair

shown in Fig. 3a, it is clear that the main mass loss occurs within the temperature range 200–260 °C, T_{peak} DTG = 251.51 °C (mass loss = 66.500 %). The second mass loss occurs within the temperature range 300–350 °C and centered at 324.45 °C (mass loss = 3.722 %). The total mass loss is 70.22 %. The remainder part may be due to the stable residue of CuO (28 %). From DTA curve (Fig. 4a), it is clear that thermal decomposition of IBU-Cu(II) ion-pair occurs in three regions. The first exothermic peak within temperature range 70–210 °C, at 130.56 °C, may correspond to the loss of COOH group, as a result of its chemical rearrangement into CO_2 molecule, of calculated mass loss = 15.38 %. The second endothermic peak within temperature range 220–410 °C, at 309.33 °C, may correspond to the loss of CH_2CHCH_3 molecule of calculated mass loss = 14.69 %. The third small endothermic peak within temperature range 420–450 °C, T_{peak} DTA = 420.33 °C, may correspond to the loss of CH_2CH_2 molecule of calculated mass loss = 9.8 %. The proposed thermal decomposition of IBU-Cu(II) is shown in Scheme 1. Small endothermic peak appeared at 230 °C may be attributed to m.p. of this ion-pair as assigned in Fig. 3a.

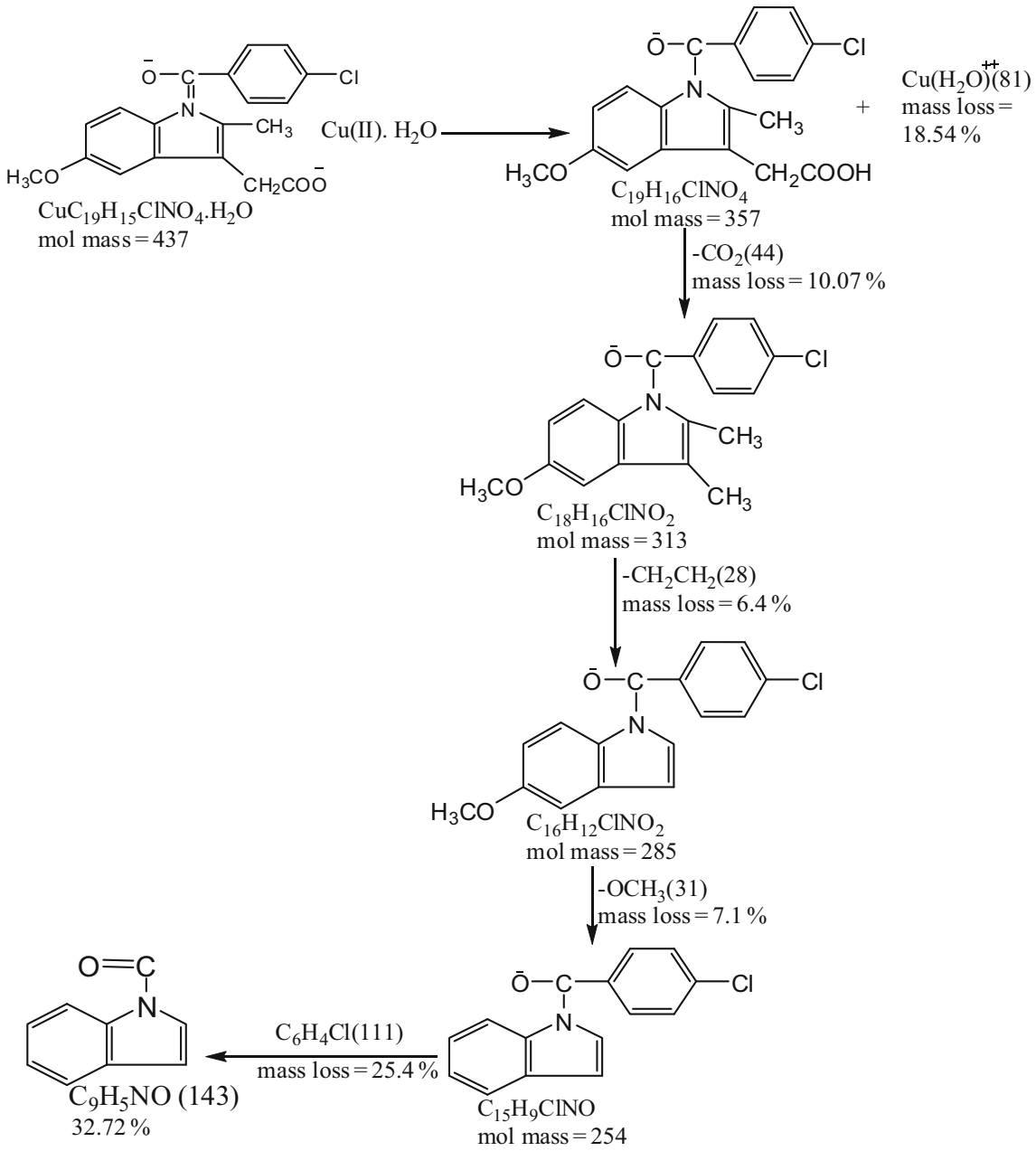
Thermogravimetric (TG/DTG) analysis of solid NAP-Cu(II) ion-pair is employed to provide quantitative information on mass losses due to thermal decomposition as a function of time and temperature. The thermal analyses data of solid NAP-Cu(II) ion-pair are shown in Figs. 3b and 4b. From TG/DTG curve as shown in Fig. 3b, it is clear that the thermal decomposition of NAP-copper(II) ion-pair occurs within two steps. The first peak occurs within the temperature range 200–290 °C and at T_{peak} DTG = 259.29 °C, with practical mass loss of 61.06 %. The second peak occurs within the temperature range 300–400 °C and T_{peak} DTG = 360.77 °C, with mass loss of 9.427 %. The total mass loss = 70.487 %. The remainder part may be attributed to CuO residue. From DTA curve (Fig. 4b), it is clear that the small exothermic peak is due to the loss of COOH group, which chemically rearranged into CO_2 molecule, of mass loss = 14.24 %. Thermal decomposition of NAP-Cu(II) ion-pair occurs in

Scheme 1 Proposed thermal decomposition of IBU-Cu(II) solid ion-pair





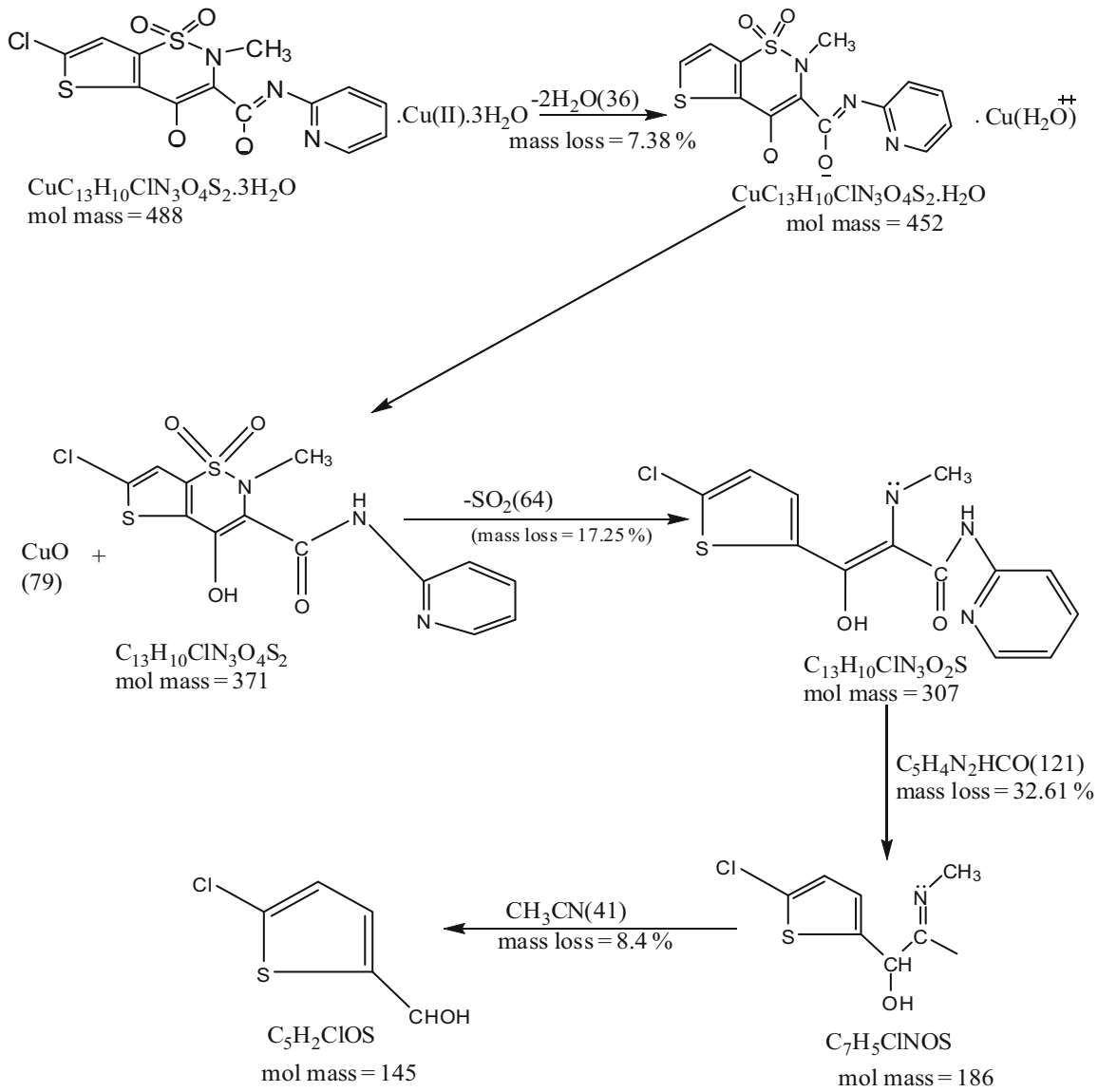
Scheme 2 Proposed thermal decomposition of solid NAP-Cu(II) ion-pair



Scheme 3 Proposed thermal decomposition of Indo-Cu(II) ion-pair

three consecutive thermal steps. The first peak within temperature range 250–270 °C, as very sharp exothermic peak at 259.33 °C, corresponds to the loss of CH_2CH_2 radical which may be chemically rearranged into CH_3 radical of mass loss = 4.85 %. The second peak within temperature range 320–390 °C, as sharp endothermic peak at 364.51 °C, may correspond to the loss of OCH_3 radical of mass loss = 10.03 %. The third peak at the temperature range 390–420 °C, as small endothermic peak at T_{peak} DTA = 406.02 °C, may correspond to the loss of $C_{11}H_8$ molecule of mass loss = 45.31 %. This concluded that the proposed thermal decomposition of NAP-Cu(II) ion-pair occurs in four consecutive thermal steps as shown in Scheme 2. Very faint endothermic peak appeared at 235 °C may be related to m.p. of this ion-pair (Fig. 3b).

Thermogravimetric (TG/DTG) analysis of solid IND-Cu(II) ion-pair is employed to provide quantitative information on mass losses due to thermal decomposition as a function of time and temperature. The thermal analyses data of solid IN-Cu(II) ion-pair are shown in Figs. 3c and 4c. It is clear from TG/DTG (Fig. 3c) that this ion-pair decomposed within temperature range 50–100 °C. The first mass loss of this compound occurred at T_{peak} DTG = 66.84 °C (mass loss = 1.366 %), and the main



Scheme 4 Proposed thermal decomposition of solid LOR-Cu(II) ion-pair

second mass loss occurs within temperature range 250–400 °C (mass loss = 50.273 %), T_{peak} DTG = 307.52 °C. The total mass loss is 51.639 %. From DTA curve (Fig. 4c), it is clear that thermal decomposition of solid IND-Cu(II) ion-pair occurs in three endothermic steps. The first peak may refer to rupture of $Cu(H_2O)^{++}$ hydrated cation from the entity of this compound at 66.64 °C, and at the end of the process, it may be changed into CuO at high temperature. The second step occurs within the temperature range 170–230 °C, T_{peak} DTG = 209.74 °C, which may correspond to the loss of CO_2 gas molecule coming from decomposition of COO^- cation, together with CH_2CH_2 and OCH_3 radicals of mass loss = 23.57 %. The third main peak appears within temperature range 250–380 °C, at T_{peak} DTG = 310.01 °C, which may correspond to the loss of C_6H_4Cl, of mass loss = 25.4 %. Therefore, the total mass loss = 48.97 % (practical loss = 51.639 %), and the proposed thermal decomposition of IND-Cu(II) may be represented by Scheme 3. Very faint endothermic peak appeared at 190 °C may be related to m.p. of this ion-pair (Fig. 3c).

The thermal analyses data of solid LOR-Cu(II) ion-pair are shown in Figs. 3d and 4d. The TG/DTG curve of solid LOR-Cu(II) ion-pair as given in Fig. 3d shows decomposition steps. These peaks occur in the temperature range 50–100 °C, at T_{peak} DTG = 85.72, 170–270, and 247.34 °C, respectively. The total mass losses are 66.502 %. The remainder part may be due to formation of stable copper oxide. The inspection of the DTA curve (Fig. 4d) of solid LOR-Cu(II) ion-pair refers to small exothermic peak in the

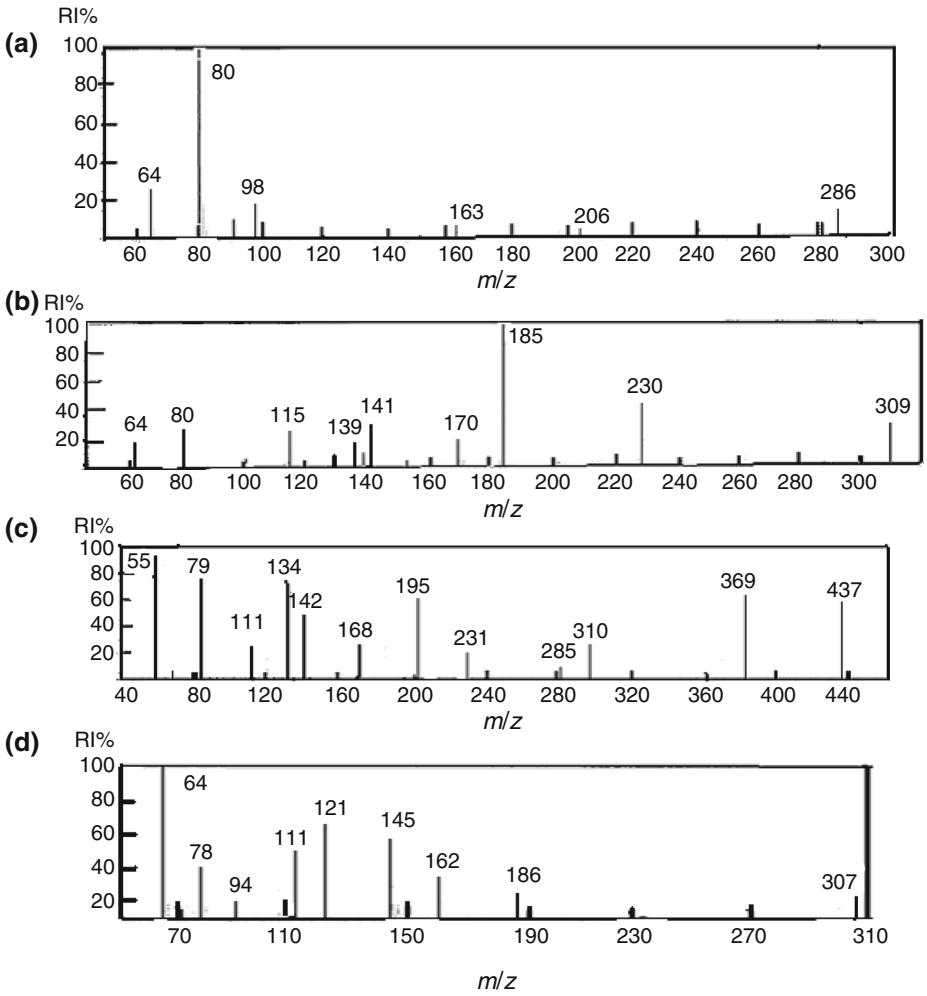


Fig. 5 Mass spectra of **a** solid IBU-Cu(II) ion-pair, **b** solid NAP-Cu(II) ion-pair, **c** solid IND-Cu(II) ion-pair, **d** solid LOR-Cu(II) ion-pair

temperature range 70–120 °C of T_{peak} DTA = 87.96 °C. This may be attributed to the thermal rupture of the hydrated $Cu(H_2O)_3^{++}$ from the moiety of the LOR-Cu(II) ion-pair followed by the loss of $2H_2O$ molecules (calcd. mass loss = 7.38 %). At the end of thermal process at high temperature, the remainder $Cu(H_2O)^{++}$ radical cation changed into stable CuO. The second peak occurs at the temperature range 200–255 °C, as a sharp endothermic peak at 249.7 °C, which may correspond to the loss of SO_2 gas molecule (calcd. mass loss = 17.25 %) as a result of SO_2 group rupture. The third peak occurs within the temperature range 260–320 °C, as small endothermic peak at T_{peak} DTA = 300.64 °C, which may correspond to the loss of $C_5H_5N_2CO$ molecule (calcd. mass loss of 32.61 %). These results concluded that the thermal decomposition of LOR-Cu(II) ion-pair occurs in consecutive thermal steps as shown in Scheme 4. Very faint endothermic peak appeared at 220 °C may be related to m.p. of this ion-pair (Fig. 3c).

Mass spectra of solid ion-pairs Full description of mass spectra of these copper ion-pairs is given in Fig. 5. The mass spectrum of IBU-Cu(II) ion-pair is recorded using GC–MS technique as illustrated in Fig. 5a. This spectrum is helpful in the interpretation of the formation of some fragment ions in the present mass spectrum. The signal that appears at m/z = 286 (RI = 16.83 %) refers to the appearance of the molecular ion $[Cu\ C_{13}H_{17}O_2{\cdot}H_2O]^+$. The signal that appears at m/z = 206 (RI = 2.47 %) refers to the appearance of the fragment ion $[C_{13}H_{18}O_2]^+$. The base peak in the spectrum that appears at m/z = 80 (RI = 100 %) is mainly due to the formation of $[C_6H_7]^+$ ion. Other important ion observed in the mass spectra at m/z = 163 (RI = 3.76 %) refers to the formation of $[C_{12}H_{18}]^+$ ion. Fragmentation of solid IBU-Cu(II) ion-pair by thermal and mass technique is similar where the rupture takes place at the weakest bond positions.

The mass spectrum of NAP-Cu(II) ion-pair is recorded using GC–MS technique as illustrated in Fig. 5b. This spectrum is helpful in the interpretation of the formation of some fragment ions in the present mass spectrum. The signal that appears at m/z = 309 (RI = 32 %) refers to the appearance of the molecular ion $[Cu\ C_{14}H_{13}O_3{\cdot}OH]^+$. The

moderate intensity reflects the stability of the molecular ion of NAP-Cu(II) following EI. The base peak in the spectrum that appears at m/z = 185 (RI = 100 %) is mainly due to the formation of $[C_{13}H_{14}O]^+$ ion as secondary process. Other important ions are observed in the mass spectra at m/z = 170 (RI = 7.7 %), at m/z = 141 (RI = 29.22 %) and at m/z = 80 (RI = 35 %), respectively. These fragment ions may be due to the formation of $[C_{12}H_{11}O]^+$, $[C_{11}H_8]^+$ and [Cu OH]$^+$, respectively.

The mass spectrum of IND-Cu(II) ion-pair is recorded using GC–MS technique as illustrated in Fig. 5c. The signal that appears at m/z = 437 (R = 63.43 %) may be due to the molecular ion [Cu $C_{19}H_{15}ClNO_4{\cdot}H_2O]^+$. The moderate intensity reflects the stability of the molecular ion of IN-Cu(II) following EI. The hydrated copper ion may be firstly separated from the moiety of this main molecular ion. The main molecular ion looses carboxylic group as CO_2 gas, leading to the formation of fragment ion $[C_{17}H_{10}ClNO_3]^+$ observed in the mass spectra at m/z = 310 (RI = 20.0 %). The base peak in the spectrum that appears at m/z = 55 (RI = 93.6) may be due to the formation of HCN and CO gases as a result of the formation of $[HC{\equiv}N]^+$ and $[CO]^+$ fragment ions. Other important fragment ions are appeared at m/z = 285 (RI = 11.88 %) and at m/z = 111 (RI = 33.87 %), respectively. These fragment ions may be due to the formation of $[C_{16}H_{12}ClNO_2]^+$ and $[C_6H_4Cl]^+$, respectively. Fragmentation of solid IND-Cu(II) ion-pair by thermal and mass technique is similar where the rupture takes place at the weakest bond positions.

The mass spectrum of LOR-Cu(II) ion-pair is recorded using GC–MS technique as illustrated in Fig. 5d. By careful inspection of EI–MS of LOR-Cu(II) ion-pair, main molecular ion of this compound is non-detectable, which may be due to its instability, as it starts to decompose in thermal analyses at 87.96 °C. Therefore, all detected fragment ions are coming from the LOR molecule itself after separation of copper-hydrated cation, $Cu(H_2O)_3^{++}$, that appear at m/z = 121 (RI = 62 %). The base peak appears at m/z = 64 (RI = 100 %) is attributed mainly to the loss of fragment ion SO_2^+ as sulfur dioxide gas from the entity of LOR molecule leading to the formation of $C_{13}H_{10}ClN_3O_2S^+$ unstable fragment ion (m/z = 307, RI = 19.2 %). This unstable fragment ion gives the ion C_7H_5ClNOS (m/z = 186, RI = 20 %). The signal that appears at m/z = 145 (RI = 56.48 %) refers to the appearance of the fragment ion $[C_5H_2ClOS]^+$. Other important ions are observed in the mass spectra at m/z = 121 (RI = 66.59 %) and at m/z = 78 (RI = 38 %), respectively. These fragment ions may be due to the formation of $[C_5H_5N_2O]^+$ and $[C_5H_4N]^+$, respectively.

Fragmentation of solid LOR-Cu(II) ion-pair by thermal and mass technique seems to be very similar where the bond rupture takes place at the weakest bond positions started with SO_2 group followed by the rupture of the other side chains. It is clear from the comparison between TA and MS of both LOR drug and its LOR-Cu(II) ion-pair that metabolites of the drug is highly affected by the presence of Cu(II) which make the drug molecule unstable and easily decomposed.

Biological Activity of solid drugs and their ion-pairs

The biological activities of ibuprofen, naproxen, indomethacin and lornoxicam drugs and their solid copper ion-pairs were determined (Table 2) according to the protocol described by Delobel et al. [29]. *T. confusum* is the most common and destructive insect species in flour mills and treated areas. Adults of *T. confusum* were laboratory-reared on wheat flour at 27 ± 1.5 °C and 70 % ±5 % (R.H.) according to the method of Frederic et al. [33] with some modifications. *T. confusum* adult was topically treated with 10 µg of each compound (IBU, NAP, IND, LOR and their Cu(II) ion-pairs) according to Delobel et al. [29] protocol as follows: Thirty insects were divided into three replicates without treatment. The adult mortality was estimated (Table 2) according to Abbot [30]. Estimation of LD_{50} values was made using probit analysis by Finney [35]. From the obtained results in Table 2, it found that LOR drug is the most biologically effective on *T. confusum* which caused 21, 40 and 73 % mortalities after adult treatments with the concentration of 10, 30 and 50 of LOR compared to no effect on the control. On the other hand, IBU drug showed least mortality (8, 20 and 41 %) on *T. confusum* with the same concentrations. The order of toxicity (LD_{50}) values is found to be 55, 48, 46 and 35 % of IBU, NAP, IN and LOR drugs, respectively. On the other

Table 2 Effect of ibuprofen, naproxen, indomethacin and lornoxicam drugs and its Cu(II) ion-pairs on the *Tribolium confusum* insects

Conc %	IBU	NAP	IN	LOR
Drug compounds				
10 s	8	15	17	21
30	20	30	30	40
50	41	58	56	73
LD_{50}	55	48	46	35
Control	00	00	00	00
Conc %	**IBU-Cu(II)**	**NAP-Cu(II)**	**Indo-Cu(II)**	**LOR-Cu(II)**
Cu(II) ion-pairs				
10	21	7	5	3
30	50	20	10	20
50	86	38	18	41
Control	00	00	00	00

IBU ibuprofen, *NAP* naproxen, *LOR* lornoxicam, *Indo* indomethacin

hand, the solid prepared Cu(II)-drug ion-pairs showed that LOR-Cu(II) ion-pair is most biologically active than other ion-pairs. It is also concluded that the presence of cupric ions in moiety of these solid ion-pairs enhanced biological activities of these drugs. This may be attributed to copper essential biological activity. The enhanced biological activity of both LOR drug and its copper ion-pair may be attributed to the extra bioactivity of sulfur atoms in the entity of this drug and its product.

Conclusions

This research successfully concerned with the preparation, separation and structures elucidation of solid ion-pairs of nonsteroidal anti-inflammatory drugs (NSAIDs) in reaction to copper(II) ion. Comparison between MS and TA of solid copper ion-pairs with those of their drugs provides further information about TA and MS fragmentation pathways. From the application of both experimental techniques on investigation of solid ion-pairs, it is concluded that the copper ion found in entities of these compounds reasonably directs their TA and MS fragmentation pathways. This is also of great effect on their metabolites in in vivo systems. The biological activities of drugs and their copper(II) solid ion-pairs toward some kinds of insect species in flour mills were studied. It was found that ion-pairs of the drugs with copper(II) were found to be more biologically active than drugs themselves. This may be attributed to the biological activity of copper(II) in their entities. These ion-pairs are freely soluble in aqueous media than parent drugs, from which spray for killing different kinds of flying or creeping insects can easily be prepared.

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