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Spectroscopic Studies of Naproxen and Indomethacin Reactions with Copper (II) Reagent and their Micro Determination through Ion - Pair Formation and their Biological Activities

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Abstract: A simple and accurate spectrophotometric method for the determination of naproxen and indomethacin drugs in pure and pharmaceutical dosage forms has been developed. The proposed method is based on the ion-pair formation reaction between copper (II) and the carboxylic group of the drugs. The reactions were studied spectrophotometrically at optimum conditions of, reactant concentration, temperature, time, λ_{max} and pH. Applying of molar ratio method (MRM) on the naproxen and indomethacin copper (II), indicated (1:1) stoichiometric ratio. Validity of Beer's Law was carried out at 260 and 270 nm, for naproxen and indomethacin ion - pairs, respectively. Results of analysis were statistically validated. The linearity ranges were found to be 2.173 to 66.78 and 3.54 to 93.03 µg ml^{-1} of recovery values 99.750 to 100.304 and 99.290 to 100.283 % for naproxen and indomethacin drugs respectively. The low values of standard deviation (SD = 0.003 to 0.038 and 0.006 to 0.031) and relative standard deviation (RSD = 0.0615 to 0.1045 and 0.0158 to 0.0687 %) refer to the accuracy and precision of the proposed method for spectrophotometric micro-determination of these drugs in pure and in their pharmaceutical dosages forms in comparison with official methods. The robustness and procedure validation were assayed within and in-between days. The solid ion - pairs of these drugs with copper (II) have been separated and identified by elemental analyses and different spectroscopic tools in order to prove the proposed reaction scheme. They were found to be biologically active toward some kinds of *Tribolium confusum* common insect species in flour mills and treated areas and their adults. These solid compounds were found to be more biologically active than their parent drugs.

Keywords: Naproxen, Indomethacin, Copper (II) ion - pairs, Spectroscopy, Biological Activities.

1 Introduction

The therapeutic importance of naproxen and indomethacin drugs was behind the development of many analytical methods for their determination in pure and in pharmaceutical formulation samples. These methods include spectrophotometry [1–5], electrochemical methods [6], HPLC [7], and electrophoresis [8]. As compared to the electrochemical, electrophoretic, and chromatographic

Methods, despite of their higher common availability of the instrumentation, the simplicity of procedures, economy, speed, precision and accuracy of the technique still make spectrophotometric methods attractive [9].

Non – steroidal anti –inflammatory drugs (NSAIDs) are drugs with analgesic, antipyretic and anti – inflammatory effects. Naproxen (NAP) and indomethacin (IN) is most famous member of this group of drugs [10]. Naproxen (NAP) is chemically 2-Naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-Methoxy-α-methyl-2- naphthalene acetic acid [11]. Its structural formula is $C_{14}H_{14}O_3$ of mol. Mass 230.26 g mol^{-1}, and its chemical structural is shown in Fig. 1.

Several analytical methods have been published for the determination of NAPS in pharmaceutical preparations and biological fluids. These methods included first derivative non-linear variable-angle synchronous fluorescence

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spectroscopy (22), CE with electro spray mass spectrometry (23), HPLC [12], and capillary isotachophoresis [13] flow-injection analysis (FIA) and FIA by using complex

pharmaceuticals and biological fluids have been reported. They include chromatography [17], spectrophotometry [18], electrochemical [19] and other methods.

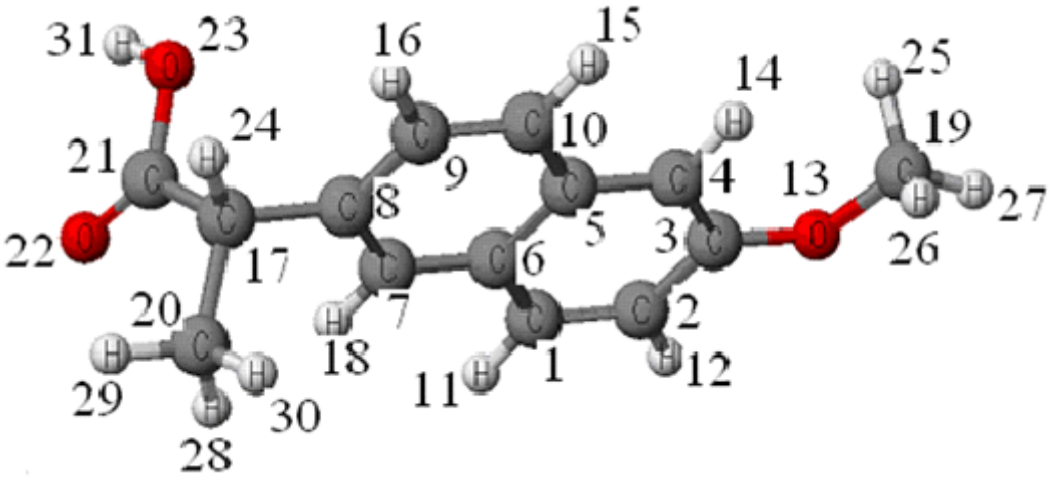


Fig. 1: Normal stereo structure of NAP and numbering system.

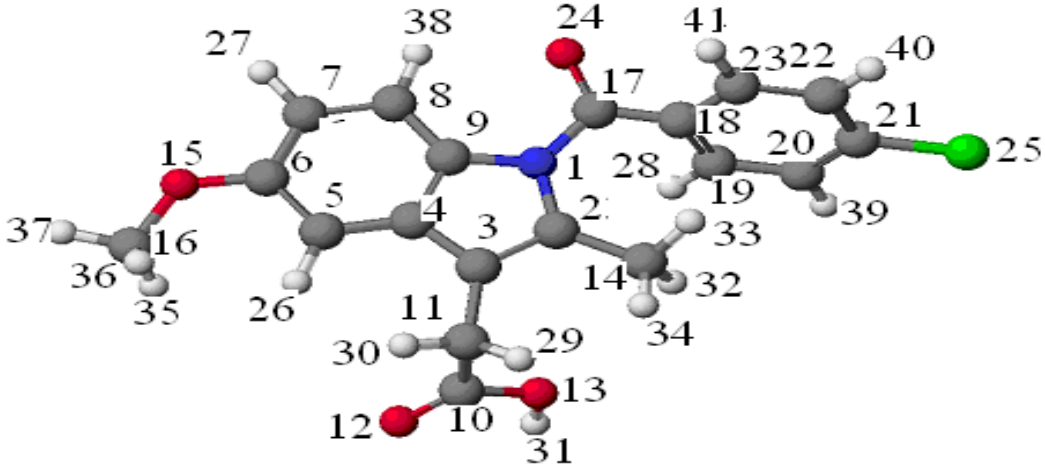


Fig. 2: Normal stereo -structure of IN and numbering system

formation of NAPS [14].

Indomethacin has an IUPAC name, 2-{1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl} acetic acid [15] and structural formula given by Fig. 2.

It also has general formula $C_{19}H_{16}ClNO_4$ and mol. Mass = 357.787 g mol^{-1} [16].

Several methods for the determination of indomethacin in

2 Experimental

2.1 Materials

All reagents of analytical grade were used without further

purification throughout this research. Authentic samples of naproxen and indomethacin drugs were supplied by Arab Drug Co. (Egypt) were used. Tablets of naproxen (Nap. Tablets) and indomethacin (Indocid Tablets) specified as 50 mg /tablet and 25 mg / tablet respectively were produced by Sedico (Egypt). Ammonia solution (4 % v/v), phosphoric acid, acetic acid, boric acid were supplied by BDH respectively. Also, anhydrous sodium carbonate and sodium hydroxide (AR) were supplied by BDH.

2.2 Solutions

$5X10^{-2}$ M solution of anhydrous sodium carbonate was prepared by dissolving accurate weight in distilled water by using 250 mL volumetric flask. 10^{-3} M stock solution of copper sulphate reagent was prepared by dissolving accurate weight in double distilled water by using 100 mL volumetric flask. Universal buffer solutions were prepared as recommended by Briton and Robinson [20].

2.2.1 Standard Stock Solutions

10^{-3}M (230.4 µg. mL^{-1}, 358 µg. mL^{-1}) of standard stock solutions of pure naproxen and indomethacin drugs were prepared separately in two 250 mL volumetric flasks by dissolving 0.0576 and 0.0895 g respectively in sufficient amount of 0.05 M Na_2CO_3 solution and the volume of each solution was completed to the mark with double distilled water.

2. 2. 2 Sample Solutions

For pharmaceutical preparation, ten tablets specified as (250 mg /tablet) and (25 mg /tablet), for NAP and IN, respectively, were weighed and powdered separately. Equivalent amount of powder to one tablet of each separate drug was weighed and dissolved in sufficient amount of 0.05 M Na_2CO_3 solution. The solution of each drug was transferred into separate 50 mL volumetric flask and the volume completed to the mark with double distilled water.

2.3 Procedures

Spectrophotometric study of naproxen (NAP) and indomethacin (IN) drugs with copper sulphate reagent is involved for selection of proper conditions, such as λ_{max}, pH, temperature, molar ratio method (MRM) and abeyance to Beer's Law. The selected proper parameter is that gives the highest molar absorptivity (ε, L mol^{-1} cm^{-1}).

2.3.1 Preparation of Working Standard Solution and Construction of Calibration Curves

Working solutions with different concentrations were prepared by pipetting 0.1 – 5 mL of pure standard naproxen drug solution into separate 10 mL volumetric flasks, 5 ml of 10^{-3} M copper reagent was added and each volume completed to the mark with double distilled water. Also, solution with different concentrations were prepared by pipetting 0.1 – 1.9 mL of pure standard indomethacin drug solution into separate 10 mL volumetric flasks, 2 mL of 10^{-3} M copper reagent was added and each volume completed to the mark with distilled water. Spectrophotometric measurements were recorded at 260 and 270 nm respectively. The calibration curves were constructed and molar ratio method (MRM) [21] was applied.
Solutions of 3.5, 12.66, 17.27 and 7.2, 39.4, 64.4 µg ml^{-1} of naproxen and indomethacin drug solutions respectively were used for micro – determination of pharmaceutical formulations with the same manner of standard drugs. Spectrophotometric measurements were studied within (intra) and in between (inter) days in order to investigate the validation and robustness of the applied methods.

2.4 Preparation of Solid Ion-Pairs

The solid ion-pairs of naproxen and indomethacin drugs with copper (II) were prepared by addition of a solution of appropriate weight of metal salt of 0.125 g (0.5 mmol) copper sulphate penta hydrate in 50 ml water to a 50 ml solution of 0.1151 g (0.5 mmol) NAP and 0.1789 g (0.5 mmol) IN , respectively. Appropriate weight of each drug dissolved in 2 ml of 0.05 M Na_2CO_3 and bidistelled water. The resulted solid products appeared as colored precipitates. The precipitates leaved for 10 mins until completely settled. The obtained solids were separated, filtered and washed with suitable solvent using a Hearch funnel of suitable pores. Moreover, the obtained compounds were dried in vacuum desiccators. The yield of each solid complex was calculated. The physical properties of these compounds were studied (color, mp, solubility, etc.). Elemental analyses (C, H, and N) and FT-IR were made at the Micro - analytical Center of Cairo University. The performed analyses aimed to elucidate structures of the prepared ion-pairs.

2.4.1 Elemental Analyses and Spectroscopic Studies

a- Determination of the metal content of the prepared complexes.

Accurately weighed portion (0.0501, 0.0247g respectively) of the prepared complexes was placed in two separate Kjeldahl flasks. A mixture of concentrated nitric and hydrochloric acids (aquaregia, 1:3) added to powdered complex 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 bidistelled water and the copper content was determined by titration of 1 mL of each solution against 0.01M standard EDTA solution, using Murexide indicator by recommended procedure [21]. The molecular weights of the given compounds were calculated from its copper content titrimetrically determined, after acid digestion, using the equation (1):

W = M X V X M. Wt. / 1000 (1) Where W = weight of solid digested complex, M = the obtained molarity of Cu (II), V = 10 ml of complex solution, M.Wt. = molecular weight of digested complex.

b- The other elements like C, H, N, X, and S were analyzed and determined in Micro Analytical Center of Cairo University.

2.4.2 Biological Activity of Drugs and Their Copper (II) Ion Pairs

a-Insects and commodity

Adult of *Tribolium confusum* were laboratory reared on wheat flour at 27.5 ±1.5ºC and 70% ± 5% (R.H.) according to the method of Frederic *et al.* [22] with some modifications.

b- Bioassay and statistical analysis:

T. confusum adult was topically treated with 10 µ of each compound according to the protocol described by Delobel *et al.* [23] as follows: Thirty insects divided on three replicates (10 adult/replicate) were topically and mortality was then monitored after 24 hr. Thirty adults of control experiment were used in three replicates without treatment. The adult mortality was estimated according to Abbot [24]. Estimation of LD50 values was made using Finney analysis [25].

2.5 Instruments

Spectrophotometer, spectronic model 601-Milton Roy (USA), UV – Vis with matched quartz cell of 1 cm optical length was used for spectrophotometric measurements in the wavelength range of 200 – 800 nm. Automatic micropipette (10 – 100 µL) model Accu pipet USA, was used to measure the very small volumes, Glass micro pipettes were used to measure the large volumes, Sensitive analytical balance [0.0001g, SCALTEC (Germany)], pH/ mV-meter Model 701 A/digital ion analyzer and Magnetic stirrer theromostated hot plate (VELP-Europe). Infrared spectra were recorded on a Perkin-Elmer FT-IR type 1650 spectrophotometer in the region 4000 - 400 cm^{-1} as KBr discs.

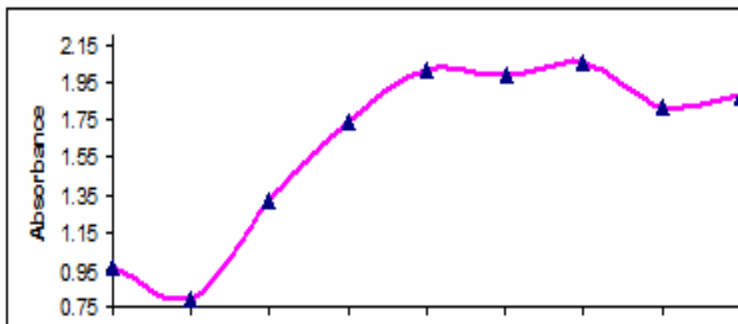
3 Results and Discussion

3.1 Selection of Suitable Wavelength

The spectra of naproxen (NAP), copper (II) reagent and that of NAP – Cu (II) product show that NAP λ_{max} = 230 nm, copper (II) reagent has λ_{max} = 810 nm and NAP – Cu (II) has a λ_{max} = 260 nm. Therefore, the selected wavelength to do further study for the reaction of NAP and Cu (II) product (260 nm) occurs faraway from that of both drug and reagent. The spectra of indomethacin (IN) and that of IN – Cu (II) product show that IN λ_{max} = 225 nm and IN – Cu (II) has a λ_{max} = 270 nm. Therefore, the selected wavelength to do further study for the reaction of IN and Cu (II) product (270 nm) occurs faraway from that of both drug and reagent.

3.2 Effect of pH on Spectra of Drugs and Reaction Products

From the data in Figures (3 and 4), it is clear that the suitable pH is 6 - 7 and 6.0 for studying NAP - Cu (II) and IN - Cu (II) ion-pairs respectively.



3.3 Effect of Temperature on the Drug Reaction Products

The effect of temperature on the drug reaction products indicates that the suitable temperatures for the NAP – Cu (II) and IN Cu (II) products are 50 and 45 ^{o}C respectively.

3.4 Stoichiometry of Reagents and Drugs

The stoichiometric ratio of each drug with the given reagent was determined by the molar ratio method [26]; the results referred to the formation of 1:1 [R]: [drug] ion - pairs/or metal complexes.

The analytical parameters for spectrophotometric micro – determination of pure NAP and IN drugs are shown obtained results in Table 1.

These data show that the recovery values were in the ranges of 99.75 – 100.304 %, 99.290 – 100.283 % for NAP and IN drugs, respectively. The Sandell sensitivity (S) was found to be $3.2X10^{-4}$ and $9.12X10^{-7}$ μg.cm^{-2}, respectively. The LDL and HDL were found to be 2.173 – 66.78 and 3.54 – 93.03 μg.ml^{-1} for NAP and IN drugs, respectively. The SD values were in the ranges of 0.003 – 0.038, 0.006 – 0.031, respectively. In addition to, the RSD values were in the ranges of 0.0615 – 0.1045 %, 0.0158 – 0.0687 %, respectively. These values refer to the accuracy and precession of the suggested methods in micro - determination of both drugs in pure states.

3.5 Validity of Beer,s, Law

Under the optimum conditions describe above Beer's law is valid in the concentration ranges of 2.30 – 66.78 and 3.6 – 93.03 μg ml^{-1} for NAP and IN respectively. Above these limits, negative deviations were observed. The correlation

coefficient values were found to be 0.9986 and 0.9992, for NAP and IN drug products respectively. Therefore, these standard methods can be used for micro - determination of these drugs within and in between days in order to assay validity and robustness of the suggested procedures.

3.6 Intra and Inter- day Measurements

Table 2 shows the obtained values of within day determination for different concentrations of the pure drugs drugs, respectively. The SD values were in the ranges of 0.003 – 0.038 and 0.006 – 0.031, respectively. Also, the RSD values were in the ranges of 0.0615 – 0.1045 and 0.0158 – 0.0687%, respectively. These values refer to the high accuracy, reliability and precision of the applied procedures.

The in-between day results during application of the proposed procedures are shown in Table 3.

Table 1: The analytical parameters for spectrophotometric determination of pure **NAP** and **IN** drugs by the proposed method.

Parameters	drugs	
	NAP	**IN**
Reagent	Cu(II)	Cu(II)
Temperature(ºC)	50	45
λ_{max} (nm)	260	270
pH	6 – 7	6
Beer's law (µg.ml^{-1})	2.30 – 66.78	3.6 – 93.03
LDL (µg.ml^{-1})	2.173	3.54
HDL (µg.ml^{-1})	66.78	93.03
R^2	0.9986	0.9992
Regression equation (Y)	Y = 0.5319x + 0.1051	y = 1.0137x + 0.2205
Molar absorptivity (l mol^{-1}cm^{-1})	6.175 X103	1.096X10^4
SD	0.003 – 0.038	0.006 – 0.031
RSD %	0.0615 – 0.1045	0.0158 – 0.0687
Sandell sensitivity(µg.cm^{-2})	3.2X10^{-6}	9.12X10^{-7}
Recovery %	99.750 – 100.304	99.290 – 100.283

The data of in- between day measurements of pure drugs by proposed method for five replicates of each drug concentration refer to the recovery values were in the ranges of 99.88 – 100.217 and 99.28 – 100.27 % for NAP and IN drugs, respectively. The SD values were in the ranges of 0.002 – 0.018 and 0.006 – 0.028, respectively. Also, the RSD values were in the ranges of 0.0194 – 0.0463 and 0.0156 – 0.0625 %, respectively. These data indicate the accuracy, validity and robustness of the suggested procedures.

Tables (4 and 5) show the results obtained for the by proposed method for five replicates of each drug concentration.

These data show that the recovery values were in the ranges of 99.75 – 100.310 and 99.290 – 100.283 % for NAP and IN

and Indocid – 25 mg/tablet) by the proposed and official methods [27].

The recovery values of NAP obtained by proposed method were ranged between 98.49 – 100.481 %, but for NAP obtained by reported official method was 96.33%. Also, SD of NAP obtained by proposed method was ranged between 0.0067 – 0.0751, but SD for NAP obtained by reported official method was 1.09 [27]. On the other hand, the obtained results by proposed method for IN determination, as in Table 5, it is found that the recovery values were

Table 2: Within day spectrophotometric micro-determination of pur

drug	[wt] taken (µg.ml-1)	[wt] found (µg.ml-1) ± SD	Recovery (%)
NAP	2.30	2.303 ± 0.003	100.000 – 100.261
	6.9	6.908 ± 0.013	99.930 – 100.304
	11.51	11.513 ± 0.017	99.880 – 100.174
	16.12	16.118 ± 0.038	99.750 – 100.223
	20.72	20.723 ± 0.033	99.860 – 100.174

between 99.29 – 100.226 % and SD values were between 0.011 – 0.040. But the recovery values obtained by reported official method [28] were 99.92 and 100.20%. This study revealed that the proposed method for determination of NAP and IN drugs are simple, selective and sensitive with reasonable precision and accuracy more or less on the same level like official ones. Moreover, the proposed method can be used as alternative method for the routine determination of NAP and IN drugs in pure and in their pharmaceutical dose forms.

3.7 Structure Investigation of Solid Compounds

The separated solid copper – drug ion - pairs were analyzed by elemental analyses and found to have general formulae of NAP - Cu (II) ($CuC_{14}H_{13}O_3$. OH) and that of IN - Cu(II) ($CuC_{19}H_{15}ClNO_4.H_2O$). The data obtained are given in Table (6).

The structural formulae of the solid ion - pairs had been studied by comparison of FT-IR of the drugs and of these products. The FT-IR of NAP refers to the bands of νOH ,

Table 4: Spectrophotometric micro – determination of **NAP** drug mg/tablet) by proposed and official methods.

Sample	Proposed method		Official method (27)	
	[Drug] µg mL^{-1}		[Drug] µg mL^{-1}	
	Taken	Found± SD	Taken	Found
Napriu	3.5	3.4539 ± 0.0067	*2.00	*2.89
	8.10	8.0591 ± 0.0139		

(at 3191- 2968 cm^{-1}), $\nu OH_{bend.}$ (at 2579-2487 cm^{-1}) and $\nu C\text{-}O_{bend.}$ (at 599 - 478 cm^{-1}), νCOO (at 964 - 744 cm^{-1}) and ν benzene ring (at 1458 - 1024 cm^{-1}). These bands are shifted

to higher values of wave numbers in the corresponding NAP - Cu ion – pair [$\nu OH_{stretch}$ (at 3734 - 3680 cm^{-1}), νOH_{bend} (at 3426 - 2971cm^{-1}), $\nu C\text{-}O_{bend}$ (at 962 - 567 cm^{-1}), νCOO (at 887 - 751cm^{-1})] except that of benzene ring is shifted to lower values of wave numbers [νbenzene ring (at 1265 – 922 cm^{-1})]. These data means that carboxylic group (COO^{-}) strongly shared in formation of NAP - Cu ion – pair, which leads to lowering of the electron density over the benzene ring. Appearance of new peak at 62-588 cm^{-1}, may be attributed to the νCu-O bond formation. These data confirm the proposed structure of NAP – Cu (II) in Fig (5).

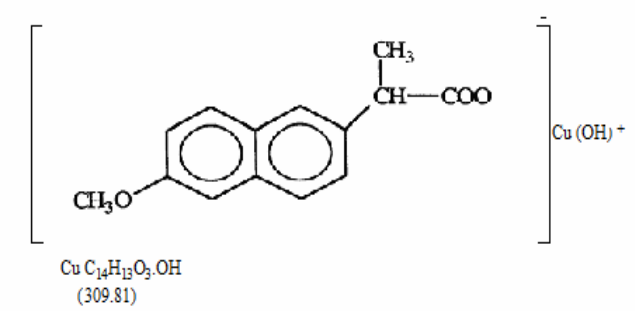


Fig. 5: Proposed structure of NAP – Cu (II) ion pair in solution.

The IN drug FT-IR shows ν OHstr. (at 3020 – 3744 cm^{-1}), ν $C\text{-}O_{bend}$ (at 1023 – 1227 cm^{-1}), ν COO (at 914 – 747 cm^{-1}), ν benzene ring(at 1597 – 1071 cm^{-1}) and ν $C{=}O_{amide}$ (at 1699-1597 cm^{-1}). In case of IN – Cu (II) ion – pair, these bands are shifted to the higher values of wave numbers ν OHstr. (at 2930 - 3743 cm^{-1}), ν C-Obend. (at 1174 - 1285 cm^{-1}), ν COO (at 1049-1144 cm^{-1}), ν benzene ring(at 1543 - 1224 cm^{-1}) and ν $C{=}O_{amide}$ (at 1591 - 1543 cm^{-1}). The appearance of new peak at 564-467 cm^{-1} may be attributed to the νCu-O bond formation. These data confirm the proposed structure of IN – Cu (II) in Fig (6).

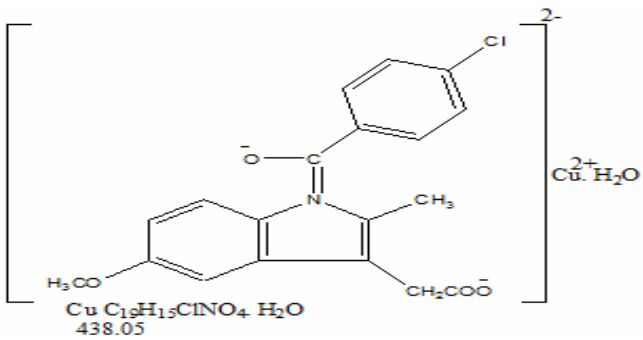


Fig. 6: Proposed structure of solid IN-Cu (II) ion pair in solution.

3.8 Biological Activities of Drugs in Comparison with Their Copper Compounds

The biological activities of naproxen, indomethacin drugs and their solid copper ion - pairs were determined according to the protocol described by Delobel et al. [23]. *Tribolium confusum* is the most common and destructive insect species in flour mills and treated areas. Adult of *Tribolium 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. [22] with some modifications. T. *confusum* adult was topically treated with 10µg of each compound (NAP, IN, NAP – Cu(II) and IN – Cu(II)) according to Delobel et al. [23] protocol as follows: Thirty insects divided on three replicates without treatment. The adult mortality was estimated according to Abbot [24]. Estimation of LD50 values was made using probit analysis by Finney [25]. The obtained results are shown in Table (7).

Table 7: Effect of NAP and IN drugs and solid copper-drug ion - pairs on the Tribolium confusum insects.

Drug solid ion - pairs		
Conc. %	NAP	IN
10	15	17
30	30	30
50	58	56
LD_{50}	48	46
Control	00	00
Solid copper - drug ion - pairs		
Conc. %	NAP – Cu(II)	IN – Cu(II)
20	7	5
30	20	10
50	38	18
Control	00	00

he most biologically effective on *T. confusum* which caused 17, 30, and 56% mortalities after adult treatments with the concentration of 10, 30 and 50 of IN comparison to no effect

on the control. On the other hand, NAP drug showed a similar mortality (15, 30, and 58%) on *T. confusum* with the same concentrations. The order of toxicity (LD_{50}) values was found 48 and 46 % of NAP and IN drugs, respectively. On the other hand, the prepared solid copper-drug ion - pairs, showed that solid IN – Cu(II) ion – pair was most biologically active than solid NAP – Cu(II) ion - pair. It is also concluded that the presence of cupric ions in moiety of these solid ion - pairs enhanced biological activities of these drugs [29,30]. This may be attributed to copper essential biological activity. The enhanced biological activity of both LOR drug and it copper complex may be attributed to the extra bioactivity of sulpher atoms in entity of this drug and its product.

4 Conclusions

The proposed method could be applied efficiently for spectrophotometric micro –determination of NAP and IN drugs under investigation in pure and in dosage forms. Also, the proposed method requires less time for analysis, provide better RSD, LDL, HDL and high recovery percentages. Moreover, the proposed method is simple, low cost and could be easily used in routine analysis of these drugs; needs no expensive reagents. The solid copper – drug ion - pairs have been separated and identified by elemental analyses and FT-IR spectroscopic technique and they were found to be biologically active toward some kinds of *Tribolium confusum* common insect species in flour mills and treated areas and their adults. The solid copper ion - pairs were found to be more biologically active than parent drugs. The biological activities of solid copper ion – pairs may be attributed to the presence of copper (II) ion in the moiety of these compounds; which is biologically active cation.

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References

[1] C. S. P. Sastry, P. Y. Naidu, and S. S. N. Murty, Spectrophotometric methods for the determination of omeprazole in bulk form and pharmaceutical formulations. Talanta., **44 (7)**, 1211–1217(1997).

[2] D. Castro, M. A. Moreno, S. Torrado, and J. L. Lastres. Comparison of derivative spectrophotometric and liquid chromatograpic methods for the determination of omeprazole in aqueous solutions during stability studies. J. Pharm. Biomed. Anal.,**21(2)**, 291–298(1999).

[3] K. Karljikovic-Rajic, D. Novovic, V. Marinkovic, and D. Agbaba. First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. J. Pharm. Biomed. Anal., **32(4-5)**, 1019–1027(2003).

[4] A. M. Wahbi, O. Abdel-Razak, A. A. Gazy, H. Mahgoub, and M. S. Moneeb. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. J. Pharm. Biomed. Anal., **30 (4)**, 1133–1142(2002).

[5] H. K. Li, W. B. Zhou, and Y. Li. Spectrophotometric determination of omeprazole based on the charge transfer reaction between omeprazole and chloranilic acid. Guangpu Shiyanshi., **21**, 646–649(2004).

[6] A. M. Qaisi, M. F. Tutunji, and L. F. Tutunji. Acid decomposition of omeprazole in the absence of thiol: a differential pulse polarographic study at the Static Mercury Drop Electrode (SMDE). J. Pharm. Sci., **95(2)**, 384–391(2006).

[7] United States Pharmacopeia 24, United States Pharmacopeial Convention, The Board of Trustess. (2000).

[8] T. Pérez-Ruiz, C. Martínez-Lozano, A. Sanz, E. Bravo, and R. Galera, Determination of omeprazole, hydroxyomeprazole and omeprazole sulfone using automated solid phase extraction and micellar electrokinetic capillary chromatography. J. Pharm. Biomed. Anal., **42(1)**, 100–106(2006).

[9] F.S. Rojas, and C.B. Ojeda, Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008: A review, Analytica Chimica Acta., **635(1)**, 22-44(2009).

[10] C. Reichel, R. Brugger, H. Bang, G. Geisslinger, Brune, Molecular cloning and expression of a 2 – arylpropionyl – coenzyme A epimerase: a key enzyme in the inversion metabolism of Ibuprofen. Mol. Pharmacol., **51(4)**, 576 – 82(1997).

[11] Haque T., Takulder MMU., Laila S., Fatema K., Development and validation of RP-HPLC method for simultaneous estimation of Naproxen and Ranitidine Hydrochloride. Pak J. Pharm. Sci., **23(4)**, 379-383(2010).

[12] Mikami, E.T., Goto, Ohno, T., Matsumoto, M., Nishida, M. Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphtylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography. J. Pharm. Biomed. Anal., **23**, 917-525(2000).

[13] Sener, E., Tuncel, M., Aboul-Enein, H.Y. Rapid determination of naproxen sodium in pharmaceutical formulations by flow injection analysis (FIA) using UV-detection, J. Liq. Chromatogr.& Rel. Technol., **26 (3)**, 401- 408(2003).

[14] Van Haselen RA., Fisher PAG. A randomized controlled trials comparing topical piroxicam gel with a homeopathic gel in osteoarthritis of the knee. Rheumatol., **39**, 714-9(2000).

[15] Bhavsar SM., Patel DM., Khandhar AP., Patel CN. Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form. J. Chem. Pharm. Res., **2(2)**, 563-572(2010).

[16] Radovan C., Dascalu D., Vlaicu I., Chiriac V., Ciorba A. Determination of indomethacin at a boron doped diamond electrode. The 12th Symposium on analytical and environmental problems, Szeged (Romania) 26 September (2005).

[17] Stolarczyk, M., krzek, J. and Rzeszutko, W. Application of derivative spectrophotometry to simultaneous determination of indomethacin and 5 – methoxy -2 – methyl – 3 –indolacetic acid in metindol injections. J.AOAC Int. 2004, 87:592-595.

[18] Celia Reguera, M. Cruz Ortiz, M. Julia Arcos. Differencial Pulse Voltammetric Simultaneous Determination of Four Anti-Inflammatory Drugs by Using Soft Modelling, Electroanal. 2002, 14, 1699-1706.

[19] H. T. S. Britton and R. A. Robinson, CXCVIII.—Universal buffer solutions and the dissociation constant of veronal, J. Chem. Soc. 1931, 1456-1462.

[20] D.C. Harris, Quantitative Chemical Analysis, 7th ed.; W.H. Freeman & Co.; New

[21] F. Gressent; I. Rahioui; Y. Rahbe, Characterization of a high – affinity binding site for the pea albumin 1b entomotoxin in the weevil sitophilus oryzae, sitophilus granarius and sitophilus zeamais. Eur. J. Biochem. 2003, 270, 2429 – 2435.

[22] B. Delobel, A. Grenier, J. Gueguen, E. ferrasson, M. Mbailao, Utilisation D, un Plypeptide Derive D, une Albumine PA1b de Legumineuse Comme Insecticide. French Patent. 1998, 9805877.

[23] W. S. Abbott. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 1925, 18, 256-267.

[24] D.J. Finney, Probit analysis. 3rd ed. London, Cambridge University Press (1971). York , 228-237 and 303-318 (2007).

[25] W.C. Vosburgh, G.R.Cooper, Complex ions. I. The identification of complex ions in solution by spectrophotometric measurements. J. Am. Chem. Soc. (1941), 63(2), 437– 442.

[26] Gondalia RP, Dharamsi AP., Spectrophotometric simultaneous estimation of sumatriptan succinate and naproxen sodium in tablet dosage forms. Int. J. Pharm. Biomed Sci. 2010, 1(2), 24-26.

[27] Noreen F , Aman T, Mumtaz A. and Nazir S, spectrophotometric determination of indomethacin using partial least square method, Proc. Pak. Acad. Sci. 2007, 44(3), 173 – 179.

[28] I. Iakovidis, I. Delimaris, S. M. Piperakis, Copper and its complexes in medicine: a biochemical approach, Molecular biology International, Vol. 2011, Review (2011) 1-33.

[29] M.A. Zayed, M. F. Hawash, M. El-Desawy and Ali M.M. El-Gizouli,Investigation of Naproxen Drug using Mass Spectrometry, Thermal Analyses and Semi-Empirical Molecular Orbital Calculation, Arabian Journal of Chemistry (2017) 10, 351–359

[30] M. A. Zayed, Ali M. EL-Gizouli, Spectrophotometric study of Ibuprofen and Lornoxicam drug reactions with copper (II) reagents in solution, J. Pharm. Appl. Chem. (2017) 3 (1), 75-82.