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*Short Communication*

# Preparation and characterization of Cu(II)–lignocaine complex. Indirect estimation of lignocaine in pharmaceutical preparations by atomic absorption spectroscopy (AAS)

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## Introduction

Lignocaine hydrochloride is a local anaesthetic and is widely used for injections and for local application to mucous membranes [1, 2]. A plethora of names for lignocaine has been used in recent literature [3], for example, lidocaine or 2-(diethylamino)N-(2,6-dimethylphenyl)-acetamide. The interest in the lignocaine complex drew the attention of several workers investigating solutions [4, 5] yet it seems that the complexes in the solid state drew little attention in respect of their structure and geometry.

The success of the atomic absorption spectrometric (AAS) technique has led to the development of indirect methods of analysis and to its application to permit the determination of organic species by AAS [6].

The aim of the present work was to prepare the solid Cu–lignocaine complex, and to characterize it by elemental analysis, TGA, and conductance measurements. The bonding between Cu(II) and lignocaine was confirmed by IR and $^1$H-NMR spectra. Electronic and EPR spectral studies were conducted to explain the geometry of lignocaine molecules around the Cu(II) ion. Also, an indirect method is described for the determination of lignocaine in pharmaceutical preparations by atomic absorption spectroscopy.

## Experimental

All chemicals used in the present investigation were pure BDH products. Copper sulphate solution was standardized by the recommended method [7]. A stock solution of lignocaine was prepared by dissolving a definite weight of lignocaine in the appropriate volume of water.

*Preparation of $[Cu(lignocaine)_2] \cdot 2H_2O$ complex*

A solution of the Cu(II) sulphate (0.001 mole) in distilled water was added dropwise to a solution of lignocaine (0.0022 mole) in the minimum amount of pure ethanol and the reaction mixture was stirred for 24 h. The green crystals obtained were filtered off, washed several times with distilled water to remove any traces of metal ions, then with ethanol (3 × 25 ml) to remove any traces of lignocaine, and finally washed with diethyl ether (2 × 25 ml) and dried *in vacuo*.

The apparatus and working procedures for investigating the solid complex were as described previously [8, 9]. A Beckman DU 8B spectrophotometer was used for spectral measurements. pH values were measured using a Hanna instrument type HI 8417. The atomic absorption measurements were made with a Beckman Model 1301 atomic absorption

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accessory, a DB-grating spectrophotometer equipped with a Beckman potentiometric recorder and a Beckman laminar flow burner. The copper hollow cathode lamp was supplied by Beckman.

*Atomic absorption determination of lignocaine*

To the lignocaine solution, 1.5% $CuSO_4$ solution (1 ml) and 15% $Na_2CO_3$ solution (1 ml) were added; the solution was mixed thoroughly, diluted to a certain volume, and centrifuged. The concentration of copper in the supernatant was measured by atomic absorption spectrophotometry, under the following conditions. Wavelength = 324.7 nm; lamp current = 10 mA; slit width = 0.2 mm; air pressure = 20 lb $in.^{-2}$; acetylene pressure = 3 lb $in.^{-2}$; burner height = 2.5 in.; and flow rate 3.5 ml $min^{-1}$. The concentration of lignocaine was calculated from a calibration curve.

*Determination of lignocaine in an ointment*

A sample containing lignocaine (10 mg) was shaken with $CHCl_3$ (20 ml) and 0.1 M HCl (10 ml) for 1 min and then centrifuged. The organic layer was extracted with 0.1 M HCl (3 × 10 ml); the combined extracts were diluted to 50 ml with 0.1 M HCl, and a 5-ml aliquot was analysed as described above. The results obtained by the proposed method were in good agreement with those obtained by the official method.

## Results and Discussion

On the basis of the analytical data obtained, the Cu(II)–lignocaine complex can be formulated as $[Cu(lignocaine)_2] \cdot 2H_2O$. Calculated (found): % C, 59.34 (59.00); % H, 8.12 (7.80); % N, 9.89 (9.60); % Cu, 11.22 (10.80). This indicates that lignocaine behaves as a mono-anionic bidentate ligand towards the Cu(II) ion. This idea is supported by the low conductance value of Cu(II)–lignocaine complex in DMF (13.00 $ohm^{-1}$ $cm^2$ $mole^{-1}$), indicating that the complex is a nonelectrolyte [10]. Thus, Cu(II) forms with lignocaine a 1:2 (Cu:L) non-ionic complex. The existence of lattice water is supported from the results of thermogravimetric analysis (TGA), where the upper part of the thermogram shows the removal of water molecules from the Cu(II)–complex at 60–75°C. The loss in weight amounts to 6.5% (calculated 6.36%) which corresponds to two water molecules.

*IR and $^1$H-NMR spectra*

The bonding of lignocaine to Cu(II) ion was investigated by comparing the IR and $^1$H-NMR spectra of $[Cu(lignocaine)_2].2H_2O$ complex with those of the free ligand.

*IR Spectra.* The IR spectra of the complex exhibit a broad band at 3420–3460 $cm^{-1}$ that could be attributed to $\nu_{OH}$ of lattice water associated with complex formation. The IR spectra of the dehydrated complex show that the $\nu_{OH}$ enolic, $\nu_{CO} \cdot \delta_{OH}$ and $\nu_{C-OH}$ bands within the range 2970, 1670, 1420 and 1120 $cm^{-1}$, respectively, disappear on complex formation [11]. The $\nu_{NH}$ band situated at 3290 $cm^{-1}$ in the spectra of free lignocaine still lies at the same position in the IR spectra of the complex indicating that the NH group is not participating in the complex formation. The new bands appearing in the spectrum of the Cu–lignocaine complex at 490 and 380 $cm^{-1}$ are assigned to $\nu_{M-O}$ and $\nu_{M-N}$, respectively. Thus, the bonding between Cu(II) ion, and lignocaine ligand can take place through proton displacement from the enolic OH group while the $N:(C_2H_5)_2$ group would contribute by co-ordination to form the complex [11].

*$^1$H-NMR.* The $^1$H-NMR spectrum of the complex in DMSO ($d_6$) using TMS as internal standard reveals the following.

(1) The $\delta_{OH}$ enolic signal which appears in the spectra of free lignocaine at 6.20 ppm is not observed in the spectrum of the complex. This is supported by the disappearance of the $\delta$-$CH_2$ signal at 4.22 ppm in the spectrum of Cu(II) chelate, and the appearance of the CH signal at 4.48 ppm, whereas the NH signal located at 10.22 ppm in the spectra of free ligand still lies at the same position in the spectra of Cu(II) complex.

(2) The new signal observed in the spectrum of Cu(II) chelate at 4.91 ppm could be assigned to water molecules associated with complex formation.

(3) The $\delta$Ar-$CH_3$, $\delta$CH or $\delta CH_2$-$CH_3$, and $\delta CH_2$-$CH_3$ signals which are observed in the spectrum of lignocaine at 2.12, 7.02, 7.04, 3.06 and 1.42 ppm display obvious shifts (by 0.05–0.1 ppm) down field in the spectrum of the Cu(II) complex. Thus the conclusions obtained from $^1$H-NMR spectra are in line with the

observation obtained from IR spectra that the bonding between the Cu(II) ion and the lignocaine molecule can take place through a covalent bond with the enolate oxygen, and a co-ordination bond with the $N(C_2H_5)$ group.

*Electronic and EPR spectra*

*Effect of pH.* The absorption spectra of Cu(II)–lignocaine complex was studied at different pH values in 50% DMF solution, (Fig. 1). At pH 3–6.5 the spectra exhibit a broad band situated at 740 nm the solution has a green colour. On increasing the pH of the medium >7.5 the broad band is shifted to a shorter wavelength at 570 nm, the colour of the solution is changed into blue, and the absorbance increases gradually until it attains a maximum at pH 10.0 then decreases again. The increased tendency for complex formation and the shift of the band with increasing pH is probably due to the shift in the equilibrium in favour of the formation of different types of complexes. Thus in acidic and neutral media a green complex is observed whereas at pH >7.5 a blue complex become the predominant species. Many attempts to obtain the blue complex in the solid form failed.

Job's method of continuous variation [12] and the molar ratio method [13] were used to determine the composition of the copper–lignocaine complex. The results obtained indicated the formation of two types of complexes, 1:2 (M:L) complex in an acidic medium and 1:1 (M:L) in an alkaline medium (pH 10).

The electronic spectra of the Cu(II) complex in DMF solution exhibit three bands with $\lambda_{max}$ at 400,570 and 740 nm with $\epsilon_{max}$ $1.2 \times 10^4$, $0.8 \times 10^4$ and $0.09 \times 10^4$ $cm^{-1}$ $mole^{-1}$, respectively. The first band can be assigned as a CT band of the ligand whereas the last two bands could be assigned to the $^2B_{1g} \rightarrow {}^2B_{29}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions of the Cu(II) ions, respectively.

The position of the $\lambda_{max}$ and the shape of the band indicate square planar geometry around the Cu(II) ion [14].

The x-band EPR spectrum at 1000 K of $[Cu(lignocaine)_2] \cdot 2H_2O$ complex exhibits an intense broad signal with no obvious hyperfine splitting (Fig. 2). The $g_{eff} = 2.0613$ together with the shape of the EPR spectra are relevant to square planar geometry around the Cu(II) ion [14]. Based on the knowledge obtained from elemental analysis, conductance TGA, IR spectra $^1$H-NMR electronic, and EPR spectra, the bonding between lignocaine, and Cu(II) ions can be formulated as shown in Scheme 1.

*Indirect determination of lignocaine by atomic absorption spectroscopy*

Although several colorimetric methods had been developed for the determination of lignocaine through its copper complex, no atomic absorption spectrometric procedure was previously carried out.

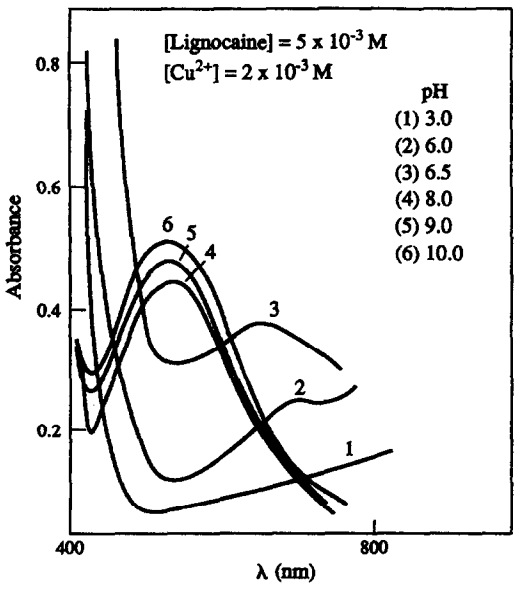



**Figure 1**
Effect of pH on copper–lignocaine complex.

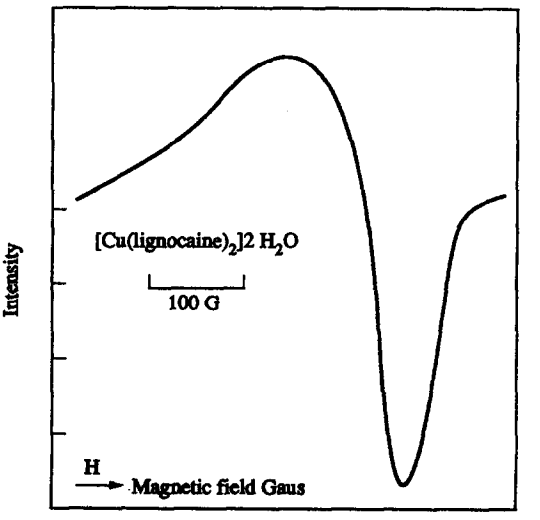
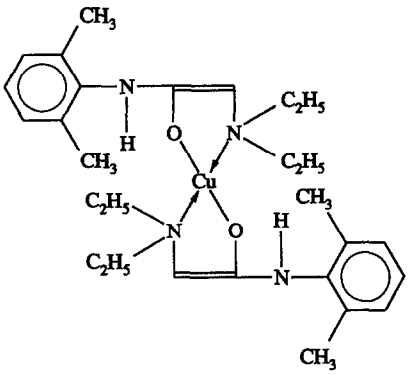



**Figure 2**
*x*-band EPR spectra of $[Cu(Lignocaine)_2] \cdot 2H_2O$ complex at 300 K.



**Scheme 1**

Lignocaine was allowed to react with Cu(II) ions, present in a solution of pH 10 in $Na_2CO_3$ medium where the respective Cu(II) complex is formed. This complex was directly aspirated, atomized in the instrument, and the absorbance was due to that reacted with lignocaine, while the excess of copper carbonate was precipitated.

Thus, the lignocaine content can be easily calculated with the aid of pre-drawn calibration curves of the copper–lignocaine complex. To calculate the lignocaine content the following equation was used:

$$Y_{lig} = \frac{X \cdot V\ W_{lig}}{W_{Cu}} = 4.545 \cdot X.V,$$

where $Y_{lig}$ = concentration of lignocaine, $X$ = concentration of Cu(II)–ion, $V$ = total volume, $W_{lig}$ = molecular weight of lignocaine and $W_{cu}$ = atomic weight of Cu(II) ion.

The calibration graph was prepared by the procedure described above. Beer's law was valid over the concentration range 1–10 μg $ml^{-1}$ of lignocaine. The Sandell sensitivity [16] of the reaction as calculated from Beer's law data was 0.091 μg $cm^{-2}$ and the corresponding molar absorptivity was $3.18 \times 10^3$ l $mol^{-1}$ $cm^{-1}$. In order to determine the accuracy and precision of the method, solutions containing three different concentrations of lignocaine HCl were prepared and five absorbance measurements were performed on each reaction product obtained. The results are given in Table 1. The overall relative standard deviation of these 15 determinations was 1.52%.

Lignocaine ointment contains excipients which may interfere with general AAS such as panthenol, dimethicone and antihistaminic drugs. No interference was observed due to the presence of pyridoxine HCl, naphazoline, and dimethicone. In contrast, adrenaline and panthenol showed strong interference with the lignocaine determination. The results are shown in Table 2. Accordingly, these compounds should be removed by separation. The extraction of lignocaine HCl in chloroform was found to be effective for elimination of the interference effect. The chloroform extract containing lignocaine HCl only was then assayed.

**Table 1**
Accuracy and precision of the AAS method

| Solution no. | Lignocaine (μg) Added | Found ± SD | RSD % |
|---|---|---|---|
| 1–5 | 5.0 | 5.00 ± 0.09 | 1.80 |
| 6–10 | 10.0 | 10.05 ± 0.17 | 1.69 |
| 11–15 | 30.0 | 29.90 ± 0.32 | 1.07 |
| Mean | | | 1.52 |

**Table 2**
Effects of diverse components on the determination of lignocaine by AAS*

| Additive | Concentration (μg $ml^{-1}$) | Recovery of lignocaine (%) |
|---|---|---|
| Pyridoxine HCl | 1 | 100 |
| | 10 | 100 |
| Naphazoline HCl | 1 | 100 |
| | 10 | 100 |
| Dimethicone | 1 | 100 |
| | 10 | 100 |
| Adrenaline | 1 | 105 |
| | 10 | 120 |
| Panthenol | 1 | 104 |
| | 10 | 120 |

*Concentration of lignocaine = 10 μg $ml^{-1}$.

The above method was successfully applied to raw materials and the ointment. The method was compared with the USP method. The results obtained when the two methods were applied to lignocaine itself and the pharmaceutical preparations are shown in Table 3. These results indicate that the proposed method is in good agreement with the USP method. The proposed AAS method is simpler and less time consuming than that of the USP [17].

A comparison of the proposed method with the method described by Nerin *et al.* [18], which involves the solvent extraction of the ternary complex of lignocaine and the in-

**Table 3**
Determination of lignocaine HCl alone and in pharmaceutical preparations by AAS and USP method

| Sample | | Labelled amount (mg) | | Percentage of labelled content found* | |
|---|---|---|---|---|---|
| | | Taken | found | AAS | USP |
| Lignocaine HCl | 1 | 2 | 2.002 | 100.1 ± 1.54 | 99.8 |
| (pure) | 2 | 5 | 4.987 | 99.74 ± 1.22 | 99.8 |
| | 3 | 10 | 9.909 | 99.09 ± 1.41 | 99.2 |
| Lignocaine ointment | 4 | 2 | 2.020 | 101.1 ± 1.66 | 100.1 |
| (2%) | 5 | 5 | 4.950 | 99.0 ± 1.52 | 99.04 |
| (Nile Co., Egypt) | 6 | 10 | 9.980 | 99.9 ± 1.36 | 98.85 |
| Lignopanthen | 7 | 2 | 1.990 | 99.5 ± 1.63 | 99.0 |
| (2%) | 8 | 5 | 4.950 | 99.0 ± 1.24 | 98.93 |
| (Nile Co., Egypt) | 9 | 10 | 9.902 | 99.02 ± 1.12 | 99.1 |
| Contaderm | 10 | 2 | 2.016 | 100.5 ± 1.48 | 99.9 |
| (October pharm.) | 11 | 5 | 4.995 | 99.9 ± 1.28 | 99.81 |
| | 12 | 10 | 9.950 | 99.0 ± 1.99 | 99.7 |

*Mean of five measurements.

organic complex ($CO(SCN)_4^{2-}$), indicate that the proposed method is more rapid and is simple, sensitive and accurate.

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