*E. histolytica* antigen in serum or in faeces (coproantigen) is a positive indication of infection. ELISA and RIA for antigen detection are expensive, time-consuming, and require sophisticated apparatus and imported reagents. CIEP, although slightly less sensitive than ELISA and RIA, can be performed with easily available materials even by paramedical personnel. Ganguly *et al*[1] have reported 26.8% positive cases among amoebic liver abscess patients and no positivity in non-amoebic controls. Our results are different: a much higher positivity (93.9%) was recorded in cases of amoebic liver abscess. The earlier workers have also not detected serum antigen in the other categories of amoebiasis patients, viz. amoebic hepatitis, amoebic cyst passers and amoebic colitis, whereas we have reported 87.5%, 0% and 84.6% positivity respectively for these categories.

Financial help to RKA from ICMR, New Delhi, is thankfully acknowledged.

1 January 1988; Revised 29 July 1988

1. Ganguly, N. K. *et al., Ind. J. Med. Res.*, 1980, **71**, 213.
2. Diamond, L. S., *J. Parasitol.*, 1968, **54**, 1047.
3. Singh, B. N., Das, S. R. and Dutta, G. P., *Curr. Sci.*, 1973, **42**, 227.
4. Singh, B. N., Dutta, G. P. and Das, S. R., *Curr. Sci.*, 1974, **43**, 71.
5. Das, S. R., Kidwai, S. A. and Gupta, A. K., *J. Biosci.*, 1979, **1**, 255.
6. Auernheimer, A. H. and Atchely, F. O., *Am. J. Clin. Pathol.*, 1962, **38**, 548.
7. Krupp, I. M., *Am. J. Trop. Med. Hyg.*, 1974, **23**, 27.

## SIMPLE COLORIMETRIC ESTIMATION OF FUROSEMIDE IN DOSAGE FORMS—PART I

PRADEEP MISHRA, D. KATROLIA and R. K. AGRAWAL
*Department of Pharmaceutical Sciences, Dr Hari Singh Gour Vishwavidyalaya, Sagar 470 003, India.*

FUROSEMIDE (4-chloro-*N*-furfuryl-5-sulphamoylanthranilic acid) is a well-known diuretic. It is estimated officially by titrimetry and UV spectrophotometry[1]. Other methods include colorimetry[2–6], potentiometry[7], iodometry[8], gas chromatography[9] and HPLC[10]. None of the reported methods involves complexation. As anthranilic acid is supposed to undergo complexation with metals, it was thought worthwhile to exploit this property for the estimation of Furosemide in marketed formulations. The present communication deals with the application of the formation of a green-coloured complex of copper with Furosemide to estimate the latter in marketed formulations. The complex was found to have absorbance maximum at 742 and 730 nm in methanol and ethyl acetate respectively.

*Chemicals and reagents*

All the chemicals used were of AR and GR grade, of BDH or Sarabhai, unless otherwise specified. Furosemide, received as gift sample from M/s Hoechst India Ltd, Bombay, was used without further purification.

A 1% (w/v) cupric acetate solution was prepared in methanol:water mixture (3:2) and the pH was adjusted to between 3 and 4 with dilute acetic acid for the estimation in methanol. For the estimation in ethyl acetate, an aqueous solution of cupric acetate (1% w/v, pH adjusted to 3–4 with acetic acid) was used. All spectral measurements were made on a Shimadzu UV-150 double-beam spectrophotometer.

*Estimation in methanol*

The standard solution of Furosemide (10 mg/ml) was prepared in methanol. Aliquots (10–50 mg Furosemide) were transferred to 10 ml volumetric flasks, 2 ml of cupric acetate solution were added, and the volume made up with methanol. The absorbances were measured at 742 nm against the reagent blank. The reagent blank was prepared without adding the drug solution. The calibration curve was plotted in the range 1–5 mg/ml of the drug.

*Estimation in ethyl acetate*

The standard solution of Furosemide (10 mg/ml) was prepared in ethyl acetate. As above, aliquots (10–50 mg Furosemide) were transferred to 10 ml volumetric flasks and volume made up with ethyl acetate. The contents were transferred to a separating funnel containing 5 ml of cupric acetate prepared for the purpose, and the funnel was shaken. The layers were allowed to separate and the ethyl acetate layer was collected. Absorbances were measured at 730 nm and the calibration curve was plotted as in the earlier case.

To find out whether the developed green colour is sufficiently stable to allow absorbance measurements, absorbance studies were carried out every 15 min for an hour on five samples in both solvents. The results showed that the developed colour was stable for more than an hour.

*Determination of Furosemide in dosage forms*

*Tablets*

The average weight of 10 tablets was determined and the tablets powdered in a glass mortar to a fine powder. Accurately weighed powder equivalent to 200 mg of Furosemide was taken and extracted with 3 × 10 ml quantities of methanol. The methanol extracts were collected after filtration and volume made up in a 50 ml volumetric flask. An aliquot of this solution containing 10–30 mg of the drug was taken in a 10 ml volumetric flask and the procedure described above for obtaining a calibration curve was followed for estimation in methanol. For the estimation in ethyl acetate, the solvent used for extraction was ethyl acetate. An aliquot containing 10–30 mg of the sample was taken in a 10 ml volumetric flask and the procedure above was repeated.

*Injections*

To 10 ml of injection solution, 1 ml of dilute hydrochloric acid was added. The drug was extracted with 3 × 5 ml portions of ethyl acetate. For estimation in ethyl acetate, the extracts were transferred to a 25 ml volumetric flask and volume made up with ethyl acetate. An aliquot containing 10–50 mg of drug was taken and the procedure for tablets was followed.

For the estimation in methanol, the combined extracts were evaporated to dryness on a water bath. The residue was quantitatively transferred to a 25 ml volumetric flask and volume made up with methanol. An aliquot containing 10–50 mg of drug was taken and the estimation procedure was repeated.

The estimation of the drug in the marketed dosage forms was also carried out by the official method[1], which involves measuring absorbance of Furosemide in alkali medium at 271 nm. The results are given in table 1.

As the estimation of Furosemide is based on the green colour of the complex, the estimation of copper in the complex was undertaken with a view to establish metal : ligand ratio.

*Synthesis and estimation of copper complex*

Cupric acetate solution (0.0025 M) was prepared in distilled water and the pH was adjusted to 3–4 with acetic acid. The solution was then added to a solution of Furosemide (0.005 M) in methanol with constant stirring. The green-coloured complex separated out, and was filtered and washed several times with distilled water acidified with acetic acid to free it from cupric acetate. The precipitate was then dried at 110°C.

The copper content of the complex was determined by the established procedure[11] after extracting out the total metal in minimum quantity of nitric acid. The metal to ligand ratio was found to be 1 : 2. On this basis, the structure shown in figure 1 is proposed for the complex.

The proposed method is very simple and involves the use of a simple colorimeter; the official method involves the more sophisticated spectrophotometer. The good recovery of the drug (98–100%) and the sensitivity (1–5 mg/ml) make this method a more practical alternative.

**Table 1** *Estimation of Furosemide in dosage forms*

| | Claimed amount (mg) | Found by* proposed method (mg) | Recovery (%) | Found by* reported method (mg) | Recovery (%) |
|---|---|---|---|---|---|
| In methanol | | | | | |
| Tablets A | 40 | 39.60 | 99.00 | 39.80 | 99.50 |
| B | 40 | 38.90 | 97.25 | 39.20 | 98.00 |
| Injection | 10 mg/ml | 9.90 | 99.00 | 9.90 | 99 00 |
| In ethyl acetate | | | | | |
| Tablets A | 40 | 39.10 | 97.75 | 39.30 | 98.25 |
| B | 40 | 39.00 | 97.50 | 39.40 | 98.50 |
| Injection | 10 mg/ml | 9.75 | 97.50 | 9 90 | 99.00 |

*Average of results of three experiments.

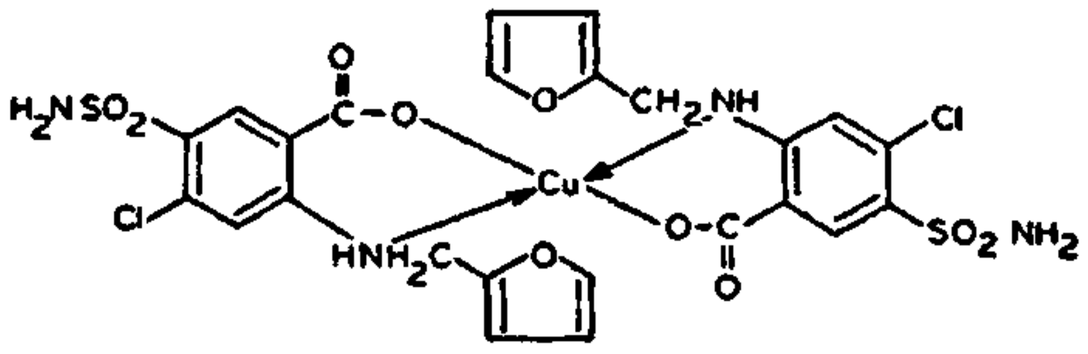

**Figure 1.** Proposed structure of Furosemide–copper complex.

The library facilities provided by the Director, Defence Research Development Establishment, Gwalior, are gratefully acknowledged. Thanks are also due to M/s Hoechst India Ltd, Bombay, for the gift of Furosemide.

1 July 1988; Revised 7 September 1988

1. *The United States Pharmacopoeia*, 21st Revision, 1985; *The national formulary*, 16th edn, 1985, United States Pharmacopoeial Convention, Inc., Twinbrook Parkway, Rockville, USA, p. 452.
2. Elsayed, M. A. and Nwakamma, C. O., *Pharmazie*, 1979, **34**, 251; *Chem. Abstr.*, 1979, **91**, 199008r.
3. Casassas, E. and Fabregas, J. L., *Anal. Chim. Acta*, 1979, **106**, 151; *Chem. Abstr.*, 1979, **90**, 210205e.
4. Abdine, H., Korany, M. A., Wahbi, A. M. and El Yazbi, F., *J. Drug Res.*, 1980, **12**, 37; *Chem. Abstr.*, 1981, **95**, 863772.
5. Moussa, B. A., Kousy, E. L. and Nagacaa, M., *Egypt. J. Pharm. Sci.*, 1983, **23**, 21; *Chem. Abstr.*, 1986, **104**, 116172e.
6. Matsuda, R., Takeda, Y. and Shouji, *Bunseki Kagaku*, 1986, **35**, 151; *Chem. Abstr.*, 1986, **105**, 12215y.
7. Malecki, F. and Staroscik, R., *Anal. Chim. Acta*, 1982, **139**, 353; *Chem. Abstr.*, 1982, **97**, 78998d.
8. Shukla, I. C., Ahmad, S., Singh, D. and Shrivastava, M. K., *Indian J. Pharm. Sci.*, 1983, **45**, 249.
9. Hackett, L. P. and Dusci, L. J., *Clin. Toxicol.*, 1977, **11**, 353.
10. Carr, K., Rane, A. and Froelich, J. C., *Anal. Abstr.*, 1978, **85**, 6D71.
11. Vogel, A. I., *A text book of quantitative inorganic analysis*, 4th edn, ELBS & Longman, New York, 1978, p. 155.

## NATURE OF THE APICAL CAP IN *TRENTEPOHLIA*

G. N. HARIHARAN and
K. V. KRISHNAMURTHY
*Department of Botany, Bharathidasan University, Tiruchirapalli 620 024, India.*

THE green alga *Trentepohlia* (order *Chaetophorales*) is not only a free-living member growing on tree barks and rocks, but also enters into symbiotic association with fungi to form lichens such as *Coenogonium*[1]. The alga is characterized by branched filamentous thallus and the terminal cell of each branch has special and prominent apical caps.

The apical cap was described as pectic in nature as early as 1911 by West and Hood[2]. This has been taken for granted by all subsequent investigators and the standard textbooks[1,3] of even recent date have described the cap as pectic, citing West and Hood[2].

In a recent study of the lichen *Coenogonium* where the phycobiont is *Trentepohlia*, the present authors found that the caps were very prominent and scored negative in standard histochemical reactions that are specific for pectin, such as toluidine blue O at pH 4.5[4] and alkaline hydroxylamine hydrochloride[4]. The cap was also negative to ruthenium red[5]. It was, however, positively stained by zinc-chlor-iodide[4] which stains cellulose blue. This reaction and the absence of staining with toluidine blue O were also noticed in the characteristic stratified walls of ordinary vegetative cells of this alga. In other words, the apical cap of this alga is of the same chemical nature as the stratified wall of vegetative cells, i.e. cellulosic.

The authors thank the Department of Environment and Forests, New Delhi, for financial assistance.

1 July 1988; Revised 19 September 1988

1. Prescott, G. W., *The algae: A review*, Reprinted edn, Bishen Singh Mahendra Pal Singh, Dehradun, India, 1984.
2. West, G. S. and Hood, D. E., *New Phytol.*, 1911, 10, 241.
3. Chapman, V. J. and Chapman, D. J., *The algae*, English Language Book Society and Macmillan, London, 1975.
4. Krishnamurthy, K. V., *Methods in plant histochemistry*, S. Viswanathan Printers and Publishers Pvt. Ltd, Madras, 1988.
5. Johansen, D. A., *Plant microtechnique*, McGraw-Hill, New York, 1940.