Extractive Spectrophotometric Determination of Microgram Amounts of Codeine, Narcotine, Papaverine & Thebaine by Solochrome Green V 150

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A direct spectrophotometric method for the quantitative determination of microgram amounts of codeine, narcotine, papaverine and thebaine is described. The method is based on the formation of ion-pair complexes between these alkaloids and Solochrome Green V 150 and measurement of the absorbance of chloroform extract at 520 nm. The spectral characteristics, the pH values for maximum extraction, composition of the complexes and the effect of various foreign substances have been described.

Trace determination of various opium alkaloids is one of the topical problems in organic analysis because of their toxicological and pharmacological importance. Most commonly employed methods are indirect and time-consuming. A few direct spectrophotometric methods based on the use of acid dyes are available for the determination of codeine and papaverine but these are apparently not applicable to narcotine and thebaine.

A search for acid dyes for the determination of various opium alkaloids revealed that the dye, Solochrome Green V 150, forms extractable ion-pair complexes with codeine, narcotine, papaverine and thebaine, and the colour of the extract can be used for their spectrophotometric determination. Quantitative determination of morphine by this reagent is not possible because of very poor extraction of the complex into organic solvents. Since the extracted ion-pairs obtained with four of the five opium alkaloids absorb at the same wavelength, the alkaloids need to be separated prior to their determination in a mixture.

Absorbance measurements were carried out using a Bausch and Lomb Spectronic-20 spectrophotometer.

Solochrome Green V 150 dye was obtained from the Imperial Chemical Industries Limited, Calcutta. Samples of pure opium alkaloids were procured from Central Forensic Science Laboratory, Hyderabad. Walpole and Clarke and Lub buffers were used. All the other chemicals used were of analytical grade.

Procedure — An aliquot of the alkaloid solution containing 0.025-0.1 mg of alkaloid was transferred to a separating funnel and 5 ml of 10⁻¹⁻⁻⁻ M aqueous dye solution and 10 ml of suitable buffer solution added. The mixture was extracted by shaking with 15 ml of chloroform for 1 min. The chloroform layer was separated and the absorbance measured at 520 nm.

The unique feature of the reagent Solochrome Green V 150 is its universality and convenience for the determination of four of the five major opium alkaloids in spite of the fact that it requires their separation when present together. Most of the commonly associated impurities do not interfere and the sensitivity is better than that obtained in the commonly used methods. Since the dye is not extracted into chloroform the absorbance of the reagent blank is negligible.

Aqueous solutions of Solochrome Green V 150 have a maximum absorbance at 490 nm. Change in pH of the solution does not affect the λ_max of the dye. The dye is not extracted into chloroform at any pH in the range 1-11. The ion-pair complexes of the four alkaloids extracted in chloroform show a maximum at 520 nm and change in pH does not affect this maximum. Of the three halogenated solvents, chloroform, 1,2-dichloroethane and dichloromethane, chloroform has been found to be the best extractant. The pH values for maximum extraction of ion-pair of alkaloids into chloroform are given in Table 1. About one min of shaking is sufficient for complete extraction. Excess of shaking sometimes results in emulsion formation. The colour of the extract is stable for about 24 hr, after which sedimentation starts. The compositions of ion-pair complexes of four alkaloids as found by continuous variation and mole ratio methods, the concentration range in which the Beer’s law is obeyed and Sandell’s sensitivity are listed in Table 1. Five-fold excess of the dye is required for maximum colour development. Concentrated solutions of the dye result in emulsion formation. The precision of the method for the determination of various opium alkaloids was found to be ±2%. The effect of foreign substances has been checked. Citric acid, acetylsalicylic acid, sodium salicylate, sodium benzoate, sodium diethyl barbiturate and saccharine sodium do not interfere even in ten-fold excess. A statistical evaluation

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>pH of maximum Beer’s law extraction (in µg)</th>
<th>Composi-</th>
<th>Sandell’s sensitivity (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range of the complex (Alk: Dye)</td>
<td></td>
<td>(abs. unit)</td>
</tr>
<tr>
<td>Codeine</td>
<td>6-0 50-1000 1:1</td>
<td>2.5/cm²/0.002</td>
<td></td>
</tr>
<tr>
<td>Narcotine</td>
<td>2-0 5-300 1:2</td>
<td>0.5/cm²/0.005</td>
<td></td>
</tr>
<tr>
<td>Papaverine</td>
<td>2-0 0-5-30 1:2</td>
<td>0.05/cm²/0.004</td>
<td></td>
</tr>
<tr>
<td>Thebaine</td>
<td>3-6 5-200 1:2</td>
<td>0.2/cm²/0.005</td>
<td></td>
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</tbody>
</table>
of the results of determination of the alkaloids by the dye (Soluochrome Green V 150) has been given in Table 2.

References

Extraction of Hg(II) with Caproic Acid

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The extraction of Hg(II) into chloroform containing hexanoic acid has been studied. The effect of various variables like metal and acid concentrations and pH of the aqueous phase on extraction has been investigated. These studies indicate that the extracting species is monomeric and involves four hexanoic acid molecules per Hg(II) ion; two of the acid molecules being utilized for solvation. The corresponding extraction equilibrium can be written as Hg^{2+}+2(HR)_{2}O \leftrightarrow (HR)_{2}2HR+2H^{+}.

In recent years considerable interest has grown in the extraction of metals by aliphatic monocarboxylic acids \(^{12-14}\). Schweitzer et al. \(^{13-14}\) have studied the extraction of some metals in caproic acid systems and observed that the metals are extracted as solvated metal carboxylates either in monomeric or polymeric form. A survey of literature revealed that practically no work has been done on the identification of extracting Hg(II) carboxylate species only the extraction behaviour with some carboxylic acids is studied. Fletcher and Wilson \(^{6}\) and Alexperove et al. \(^{7}\) reported the extraction of Hg(II) with naphthenic acid and assigned it a position in a selectivity series of metals. Plaksir and Anisimova \(^{8}\) investigated the extraction of Hg(II) from nitric acid solution by C\(_{4}\)-C\(_{4}\) fatty acids.

In the present communication the extraction behaviour of Hg(II) hexanoate in chloroform is reported. Effect of various variables such as acid and metal ion concentrations and pH of the aqueous phase on extraction has been investigated. Using these data the extracting mercury(II) species is identified and the equilibria involved are discussed.

Mercuric nitrate (GR) was dissolved in doubly distilled water, and to this a few drops of conc. HNO\(_{3}\) were added to prevent hydrolysis. The solution was standardized with sodium chloride using a phenolphthalein indicator. Caproic acid was standardized by usual acid-alkalimetric titration. All other reagents were also of AR grade and used as such. Hg radioisotope supplied by Bhabha Atomic Research Centre, Bombay (India), was used as a tracer.

In each set equal volumes (10 ml) of the aqueous solution \(1 \times 10^{-4}M\) Hg(II) or any other concentration) and organic phases \((0-25M\) or any other concentration of caproic acid) were shaken at room temperature, \((20\pm 2^\circ)\). The ionic strength of the aqueous phase was maintained at \(0-1M\) by adding NaNO\(_{3}\) solution. After equilibration the two phases were separated and the pH of the aqueous phase determined by a Cambridge bench pH-meter. Suitable aliquots of the two phases were counted using a NaI(Tl) well-type scintillation counter and the distribution ratio calculated by the usual method.

The data on the extraction of Hg(II) by caproic acid dissolved in chloroform have been analysed following the method of Tanaka et al. \(^{8}\).

The total concentration of mercury in the organic phase is given by Eq. (1)

\[
\log C_{Hg,o} = j\log C_{Hg,w} - \log \alpha(Hg) - 2 \log [H^+] + \log \sum_{x=0}^{3} K_{xH} [HR]_{x}^{2} \left[1 + \frac{x}{2}\right]_{0}^{1/2} + \log j \quad ... (1)
\]

where \(C_{Hg,o}\) and \(C_{Hg,w}\) denote total mercury concentrations in organic and aqueous phase respectively, \(HR\), caproic acid and \(\alpha(Hg)\), the side reaction coefficient taking into account the hydrolysis as well as complexation of Hg\(^{2+}\) in the aqueous phase.

In the above equation, the concentration of caproic acid \(\alpha(HR)\) being kept constant, a plot of \(\log C_{Hg,o} vs (\log C_{Hg,w} - \log \alpha(Hg) - 2 \log [H^+] )\) should be linear with a slope of \(j\) from which the degree of polymerization is determined.

When only one species \((HR)_{x} HR)\) is predominant in the organic phase, Eq. (1) is simplified to

\[
\log C_{Hg,o} = j\log C_{Hg,w} - \log \alpha(Hg) - 2 \log [H^+] = j + \log K_{H} + j(1 + x/2) \log [HR]_{o} \quad ... (2)
\]

According to this expression the plot of experimentally determinable values \(\log C_{Hg,o} = j\log C_{Hg,w} - \log \alpha(Hg) - 2 \log [H^+]\) against \(\log [HR]_{o}\) provides