

## EXTRACTION-SPECTROPHOTOMETRIC DETERMINATION OF BENZOCAINE BY DICYCLOHEXYL-18-CROWN-6 AND CALMAGITE

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### Abstract

A simple and sensitive extraction-spectrophotometric method for rapid and accurate determination of benzocaine in pure and dosage forms was developed. Benzocaine was effectively extracted as a 1:1 ion pair complex with dicyclohexyl-18-crown-6 and calmagite in acidic media into chloroform, followed by spectrophotometric determination at 486 nm. Molar absorptivity of the ternary complex at this wavelength was  $5.25 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ . Beer's law was obeyed over the range 1.65-132  $\mu\text{g/ml}$  of benzocaine and the minimum detectable amount of the drug was 0.6  $\mu\text{g/ml}$ . The method was successfully applied to the determination of benzocaine in a cream sample.

**Keywords:** Benzocaine; DC18C6; Calmagite; Extraction; Spectrophotometry

### Introduction

Benzocaine is a local anesthetic of ester type which is now in a wide clinical use [1]. It has been proposed that the drug penetrates cell membranes in its uncharged form and binds to putative intracellular receptors [2]. Various spectrophotometric [3-7] and chromatographic [8,9] methods for determination of benzocaine have been reported. The official methods for determination of the drug in its dosage form include nitritometric and UV spectrophotometric procedures [10-12]. The former is tedious time consuming and the latter lacks selectivity. We have previously reported the use of different crown ether-indicator systems in extraction-spectrophotometric determination of  $\text{K}^+$  [13],  $\text{NH}_4^+$  [14],  $\text{Cu}^{2+}$  [15],  $\text{Hg}^{2+}$

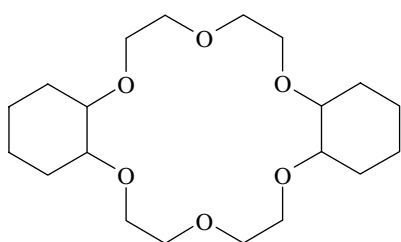
[16] and  $\text{Pb}^{2+}$  [17] ions. In this paper we report a simple, rapid and sensitive method for the determination of benzocaine in pure and dosage forms. The method is based on the quantitative extraction of dicyclohexyl-18-crown-6 benzocaine-calmagite ternary complex from acidic-media into chloroform, followed by spectrophotometric measurement at 486 nm. To the best of our knowledge, this is the first use of macrocyclic ligands in spectrophotometric analysis of local anesthetics.

### Experimental Section

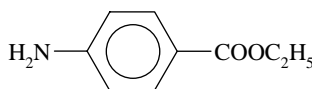
#### Chemicals

Reagent grade dicyclohexyl-18-crown-6 (DC18C6)

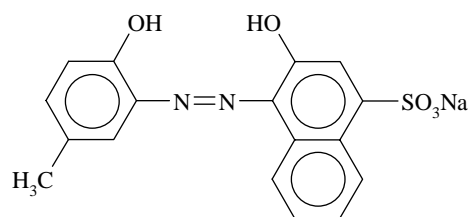
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DC18C6



Benzocaine



Calmagite

and calmagite were obtained from Merck chemical company and used without any further purification except for vacuum drying over  $P_2O_5$ . Reagent grade benzocaine and creams containing 5.0% of the drug were obtained from Tehran-Daru Pharmaceutical Company (Tehran, Iran) and used as received. Extra pure chloroform and ethanol and analytical grade sulfuric acid (all from Merck) were used as received. A working standard solution of 0.01 M benzocaine was prepared by dissolving 8.25 mg of pure drug in 5 ml of 1:1 (v/v) water-ethanol mixture. Doubly distilled water was used throughout.

#### Apparatus

A Shimadzu 265 UV-Vis spectrophotometer was used for obtaining the electronic and for the absorbance measurements at fixed wavelengths.

#### Procedure

An aliquot of the sample solution containing 1.65–132  $\mu\text{g}$  of benzocaine was placed in a calibrated 10-ml volumetric flask. To this was added 1 ml of  $2.0 \times 10^{-3}$  M calmagite and 0.5 ml of 0.5 M sulfuric acid. Then diluted to the mark with water. The solution was transferred into a 50-ml separatory funnel, 5 ml of chloroform containing  $2.0 \times 10^{-4}$  M DC18C6 was added and the mixture was shaken vigorously for 5 min. The phases were allowed to separate, the organic layer was collected and its absorbance was measured at 486 nm against a reagent blank.

### Results and Discussion

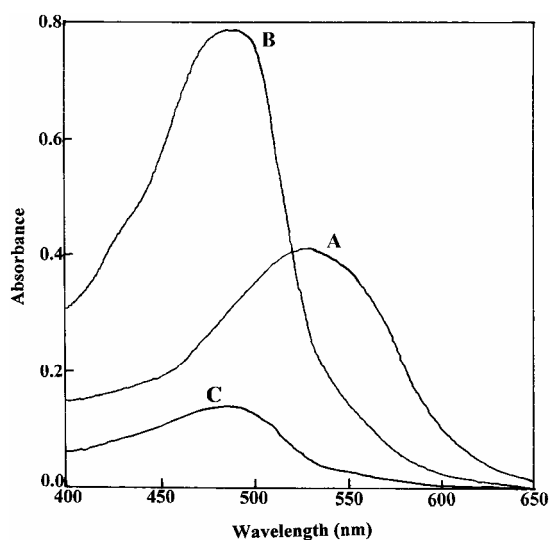
The absorption spectra of calmagite in the absence and presence of benzocaine in acidic media were recorded (Fig. 1A). Both solutions showed similar spectra having a maximum at 530 nm. The same solutions were then extracted with chloroform containing DC18C6 and the corresponding spectra in

organic phase were recorded (Fig. 1B and C). As it is seen, the extracted complex of benzocaine shows a maximum at 486 nm with a molar absorptivity of  $5.25 \times 10^3$  l/mol/cm, whereas the reagent blank shows a negligible absorbance at this wavelength. In this case, calmagite as a large polarizable molecule can act as a suitable counter ion [18] for the efficient extraction of benzocaine<sup>+</sup>-DC18C6 complex as a ternary neutral adduct into the organic phase. The existence of 1:1 stoichiometry for the benzocaine-crown ether complex was confirmed by the continuous variations method (Fig. 2).

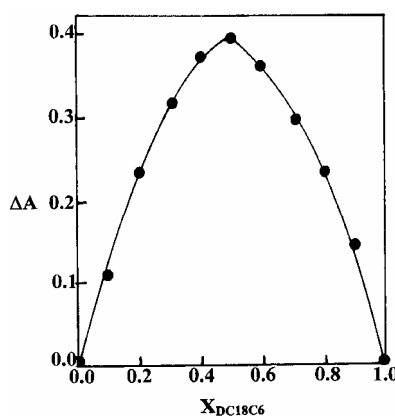
Benzocaine as a neutral molecule can be converted to a  $R-NH_3^+$  cation in acidic medium. The cation thus formed is able to form a relatively stable 1:1 complex with DC18C6 [19,20]. Upon formation of a neutral lipophilic ion pair with calmagite, the resulting complexed cation can be readily extracted into the chloroform phase. Thus, the influence of the nature and amount of acid on the extraction of the drug was investigated. Among different mineral acids tested, sulfuric acid showed the best results. The optimum concentration of sulfuric acid for the quantitative extraction of benzocaine was also investigated and the results are shown in Figure 3. As seen, maximum extraction occurs in the acid concentration range  $1.5 \times 10^{-2}$  –  $3.0 \times 10^{-2}$  M, beyond which the absorbance is decreased. At lower acid concentrations, the fraction of benzocaine in its cationic form is low, while at higher acidity, the amount of calmagite in its univalent anionic form is diminished [21].

The influence of calmagite concentration on the extraction of benzocaine is shown in Figure 4. It was found that the maximum absorbance occurs at a calmagite concentration of  $2.0 \times 10^{-4}$  M. A further excess of calmagite causes a gradual decrease in absorbance as a result of some emulsion formation during the extraction process.

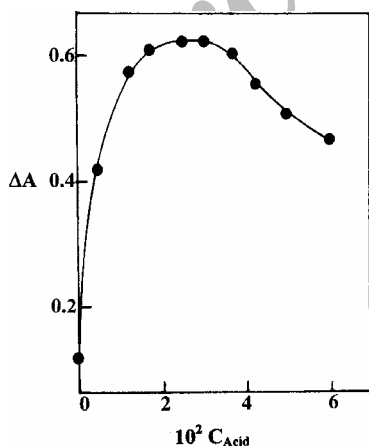
The effect of DC18C6 concentration on the extraction of benzocaine was investigated and the results are shown in Figure 5. It is obvious that the absorbance of ternary complex in the organic phase



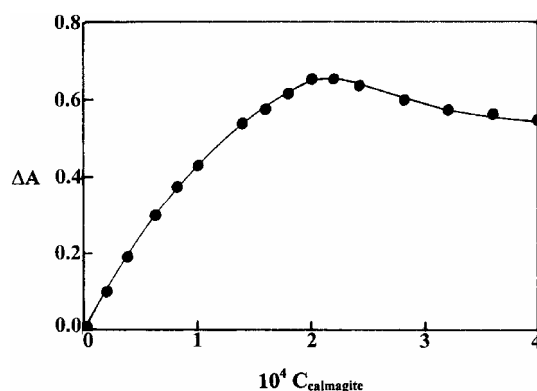
**Figure 1.** Absorption spectra of: 1,  $2.0 \times 10^{-4}$  M calmagite in  $2.5 \times 10^{-2}$  M  $H_2SO_4$  solution; 2, benzocaine-DC18C6-calmagite complex in chloroform; 3, reagent blank in chloroform.



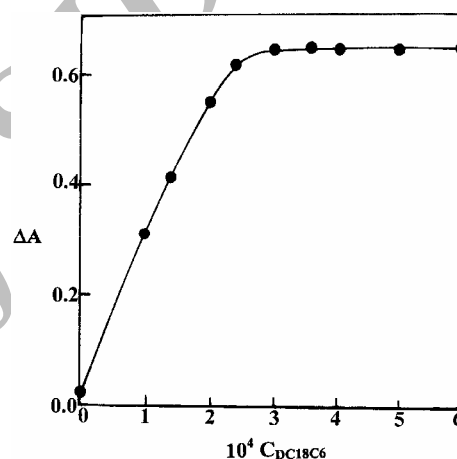
**Figure 2.** Continuous variations plot for benzocaine cation-DC18C6 system.



**Figure 3.** Effect of  $H_2SO_4$  concentration on the extraction of benzocaine. Conditions: benzocaine  $33 \mu\text{g/ml}$ ; calmagite,  $2.0 \times 10^{-4}$  M; DC18C6,  $2.010^{-4}$  M.



**Figure 4.** Effect of calmagite concentration on the extraction of benzocaine. Conditions: benzocaine  $33 \mu\text{g/ml}$ ;  $H_2SO_4$ ,  $2.5 \times 10^{-2}$  M; DC18C6,  $2.0 \times 10^{-4}$  M.



**Figure 5.** Effect of DC18C6 concentration in chloroform on the extraction of benzocaine. Conditions: benzocaine,  $33 \mu\text{g/ml}$ ;  $H_2SO_4$ ,  $2.5 \times 10^{-2}$  M; Calmagite,  $2.0 \times 10^{-4}$  M.

increases with an increase in DC18C6 concentration in chloroform. Maximum extraction occurs at a macrocycle concentration of  $2.4 \times 10^{-4}$  M. A further excess of DC18C6 has no considerable effect on the fraction of complex extracted.

The ionic strength of the aqueous solution, adjusted by NaCl, was found to affect the extraction of benzocaine with DC18C6 and calmagite into chloroform. There is an inverse relation between the extent of extraction and the ionic strength of the aqueous phase. Similar results have been reported in the literature [13-17,22].

The extraction of benzocaine under the conditions recommended in the procedure part is rapid. A shaking time of 5 min was found to be sufficient for the complete extraction of benzocaine. Longer shaking times did not have any considerable effect on the absorbance measured.

The extraction process was performed at optimal experimental conditions with some organic solvents such as chloroform, toluene, diethyl ether, carbon tetrachloride and methyl isobutyl ketone. Among these organic solvents tested, chloroform was found to be the best one because of some advantages such as effective and fast extraction, increased absorptivity of the mixed complex and low cost and availability of the solvent.

The effect of the number of extractions on the extraction efficiency of the system was studied. It was found that a single extraction with 5 ml chloroform can afford the quantitative extraction of benzocaine.

The existence of excess amounts of common excipients such as propylene glycol, glycerin, talc, starch, calcium carbonate and lactose caused no spectral interference during determination and, consequently, does not influence the extraction efficiency of benzocaine from acidic solutions.

A calibration graph for benzocaine was constructed under the optimal experimental conditions discussed. Beer's law was obeyed over the concentration range of 1.6-132 µg/ml of benzocaine at 486 nm. The molar absorptivity of benzocaine-DC18C6-calmagite complex in chloroform was calculated as  $5.25 \times 10^3$  l/mol/cm. The minimum detectable amount of the drug was found to be 0.6 µg/ml. The relative error (95% confidence level) for 33 µg/ml of benzocaine was 2.2% (10 replicates).

In order to evaluate the applicability of the proposed method to real samples, it was applied to the determination of benzocaine in an ointment from Tehran-Daru pharmaceutical company (Tehran, Iran). One gram of the sample was dissolved in 3 ml 0.5 M H<sub>2</sub>SO<sub>4</sub>, completely transferred into a 10-ml volumetric flask and diluted to the mark with water. After proper dilution, the amount of benzocaine in the resulting solution was determined by the proposed method. The amount of the drug in the sample was found to be (5.28±0.12)% which is in satisfactory agreement with the declared amount of 5.0% by the manufacturer and confirmed by the BP standard method [11].

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