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Extractive Spectrophotometric Methods for Determination of Ambroxol Hydrochloride using Bromophenol blue (BPB) and Bromocresol Green (BCG)

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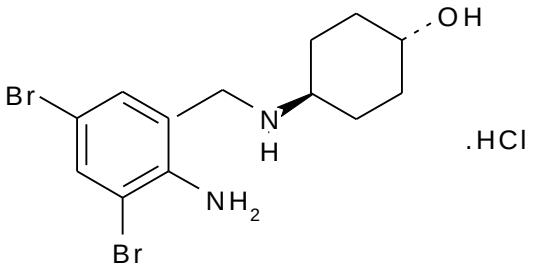
Summary: Two simple, sensitive, rapid and accurate extractive spectrophotometric methods were developed for the determination of ambroxol hydrochloride. The proposed methods are based on ion pair formation of the drug with bromophenol blue (BPB) and bromocresol green (BCG) and subsequent extraction into methylene chloride. The BPB extracted ion-pair complex has a maximum absorption at 412 nm with a molar absorptivity of $1.28x10^4$ l. mol^{-1}. cm^{-1}. Beer's law optimum range is 0.4-19 μg/cm^3 and Ringbom linear range amounts to 6.2-16.6 μg/cm^3 and the extracted BCG ion-pair complex has a maximum absorption at 414 nm with a molar absorptivity of $1.6x10^4$ l. mol^{-1}. cm^{-1}. Beer's law optimum range is 0.4-37 μg/cm^3 and Ringbom linear range of 4.2-24.8 μg/cm^3 was confirmed. The detection limits are 0.019 and 0.021 μg/cm^3 with BPB and BCG, respectively. The specific absorptivity, and Sandell sensitivity are also given. The methods have been applied to the determination of the drug in commercial tablets and capsules.

Introduction

Ambroxol hydrochloride, trans-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol hydrochloride [15942-05-9] is a mucolytic drug that is used for the treatment of lung diseases(1).



Ambroxol hydrochloride

Several different methods have been used for the determination of ambroxol hydrochloride including high performance liquid chromatography (HPLC)[2-7], gas chromatography (GC)[8,9], liquid chromatographic method coupled with mass spectrophotometry[10], capillary electrophoresis[11], capillary isotachophoresis[12], ion-selective plastic membrane electrodes[13], UV spectrophotometry[14,15] and automatic flow injection extraction spectrophotometry based on the formation of ion-pair with bromothymol blue[16]. The purpose of this work is to investigate systematically the formation and extraction behaviour of ion-pairs of ambroxol with bromophenol blue (BPB) and bromocresol green (BCG) in order to develop useful spectrophotometric methods for determination of the drug.

Experimental

Apparatus

The spectral measurements were carried out using a PYE UNICAM SP1750 UV-visible recording spectrophotometer using matched quartz cells of 1 cm path length. Measurements of pH were carried out with a digital Schott Gerate pH meter model, CG 820 equipped with a combined glass calomel electrode.

Reagents and materials

All chemicals used were of analytical grade. Twice distilled water was used throughout all experiments. Pure grade ambroxol hydrochloride was supplied by chemical industries development Co., (CID), Giza, Egypt. The pharmaceutical preparation, ambroxol tablets 30 mg/tablet was provided by Glaxo Welcome Pharm. Co., Cairo, Egypt, and Muco S.R. capsules (75 mg/capsule) was provided by Rameda Pharm. Co., 6^{th} of October city, Egypt. The dyestuffs were used as 0.001 M solution of bromophenol blue (BPB) (molecular weight, 669.96) and bromocresol green (BCG) (molecular weight, 698.04) prepared by dissolving the accurately weighed amounts in the least amount of ethanol and then completed to the final volume with bidistilled water.

As a test solution 10^{-3} M ambroxol hydrochloride was prepared in twice distilled water. The buffer series used was the the modified Britton and Robinson universal buffers[17].

Procedure for calibration curve

Into a series of 50 ml separating funnels, 5ml buffer solutions of pH 3.00 or pH 3.65 and 5 ml or 2 ml 10^{-3} M of BPB or BCG, were introduced. Appropriate volumes of 0.001 M drug solution (0.05 ml to 0.70 ml in case of BPB and 0.1 ml to 0.8 ml in case of BCG) was added to each funnel and mixed well, the funnels were shaken vigorously with 9 ml methylene chloride for 1 min., then allowed to stand for separation of the two phases. The organic phase was collected, dried over anhydrous sodium sulphate then filtered through a Whatman No. 42 filter paper and completed to 10 ml with methylene chloride. The absorbance was measured at the corresponding λ max after 15 min., in case of BPB and 1 hour in case of BCG against blank solution containing the same ingredients except the drug. Standard calibration curves were thus constructed.

Procedure for drug formulations

Twenty tablets or capsules were accurately weighed and powdered in a mortar, an amount equivalent to one tablet or capsule was dissolved in about 50

ml bidistilled water, then filtration through a Whatman No. 42 filter paper was performed. The filtrate was transferred into 250 ml measuring flask and completed to the mark with bidistilled water. The assay was completed using the procedure described above.

Results and Discussion

Ambroxol hydrochloride forms ion-pairs with bromophenol blue (BPB) and bromocresol green (BCG) and these ion-pairs are quantitatively extracted into methylene chloride. The polarity of the solvent affects both extraction efficiency and absorbance intensity. Several water immiscible organic solvents including chloroform, carbon tetrachloride, toluene, benzene, ethylene chloride and methylene chloride were tried. The most convenient solvent found to produce the highest absorbance extraction power and stability of colour was methylene chloride. The absorption spectra of the ion-pairs extracted into methylene chloride is shown in Fig. 1. The ion-pairs with BPB and BCG absorb maximally at 412 and 414 nm, respectively, the reagent blanks under similar conditions showed no absorption.

The effect of pH on the formation of the ion-pairs of ambroxol hydrochloride with BPB and BCG was studied using Britton and Robinson niversal buffers. The results indicate that the absorbance value of methylene chloride extract of the ion-pairs with BPB and BCG were constant within the pH ranges 2.5-3.5 and 3.5-3.8 respectively, Fig. 2. Thus all measurements were carried out at pH 3.00 and pH 3.65 with BPB and BCG, respectively.

The intensity of the colour formed reaches a maximum after 15 min. in case of BPB and 1 hour in case of BCG and remains stable for more than 10 hours in both cases. The effect of dyestuff concentrations was studied by adding different amounts of dyestuff (1-5 ml 10^{-3} M) to constant amount of the drug. It was found that maximum absorbance obtained with 3 ml 10^{-3} M

in case of BPB and 2 ml in case of BCG. Shaking times ranging from 0.5 to 5 min. did not cause any change in the absorbance, indicating that the equilibrium between the two phases can be attained rapidly with high reproduciblity.

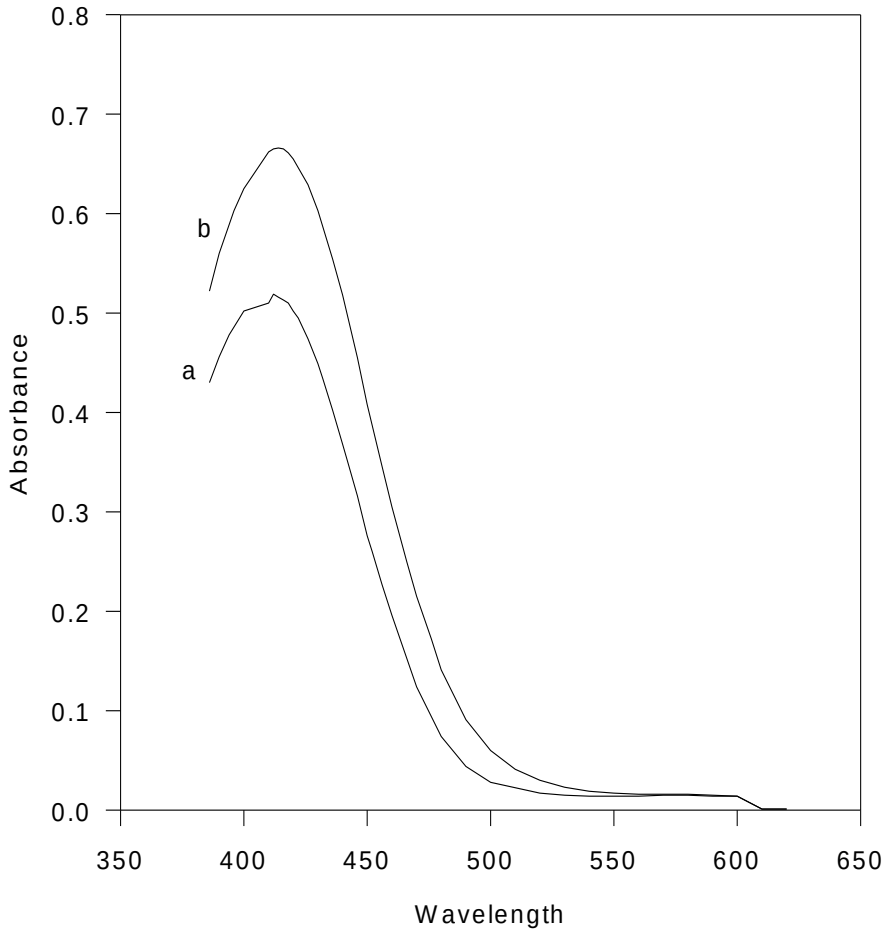


Fig.1 Absorption spectra of ambroxol hydrochloride complex extracted into methylene chloride using (a) BPB and (b) BCG
ambroxol hydrochloride = 4×10^{-5} M, BPB = 3×10^{-4} M
BCG = 2×10^{-4} M

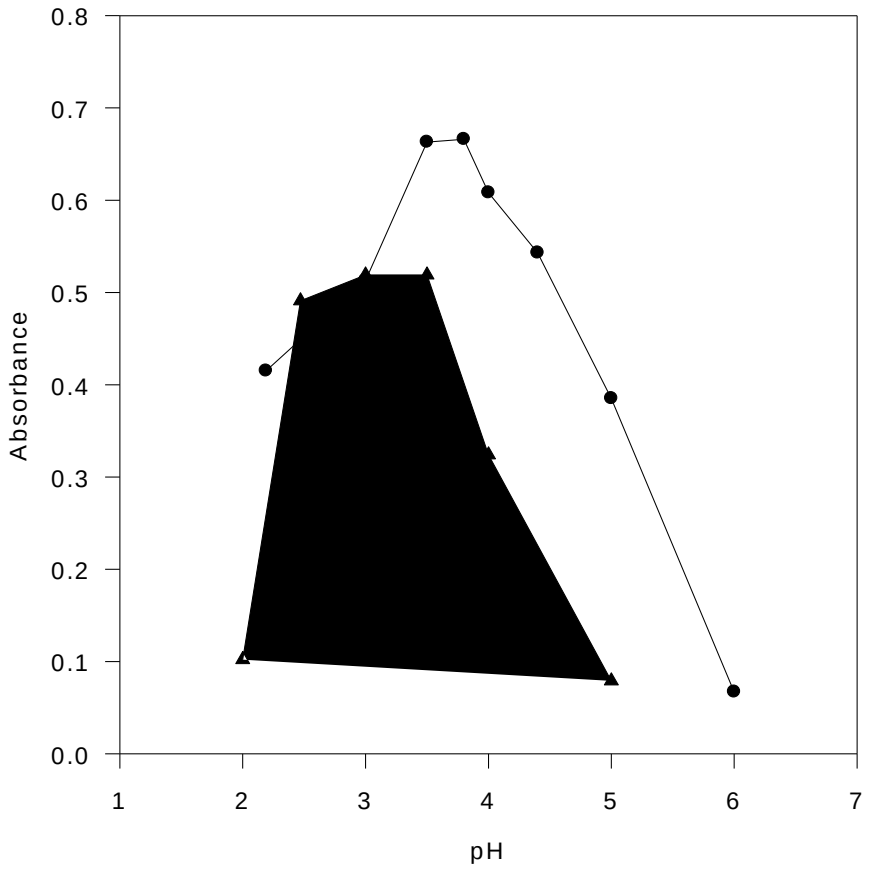
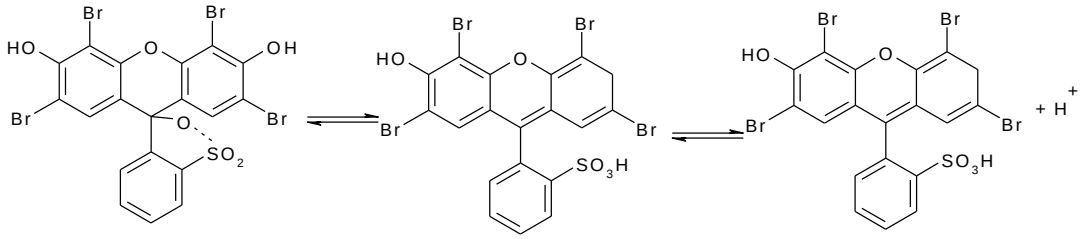


Fig. 2. Effect of pH on the absorbance of the ambroxol ion pair with (a) BPB and (b) BCG
[ambroxol] = 4×10^{-5}M [BPB] = 3×10^{-4}M
[BCG] = 2×10^{-4}M

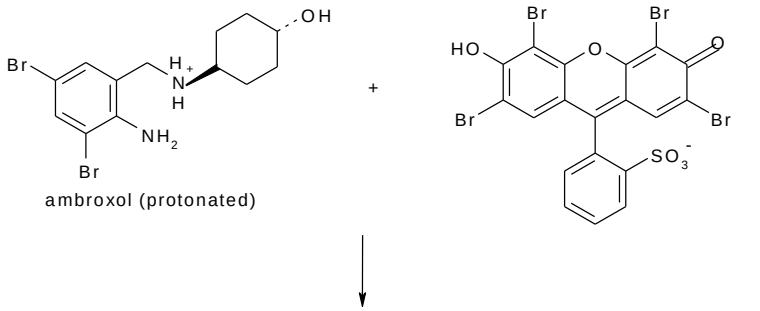
d

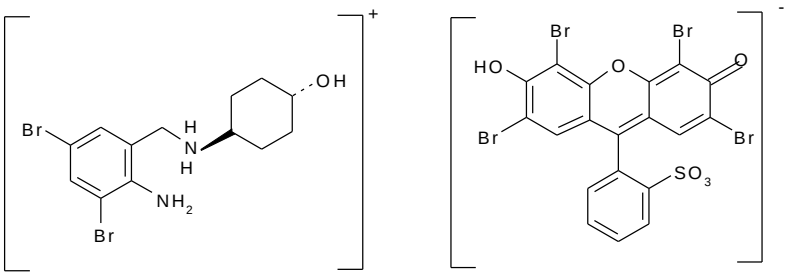
The colour of BPB and BCG is due to the opening of lactoid ring and subsequent formation of quinoid group. It is supposed that the two tautomers are present in equilibrium but due to the strong acidic nature of the sulphonic acid group, the quinoid form must be predominant[18]. Finally, the protonated ambroxol hydrochloride forms ion-pairs with the dyestuffs which are quantitatively extracted into methylene chloride. The possible reaction mechanisms are given in scheme 1.



bromophenol blue
(lactoid ring)

(quinoid ring)





ambroxol-bromophenol blue complex

Scheme (1)

Stoichiometry of the ion-pair

The composition of the ion-pair formed between ambroxol and BPB or BCG has been established applying the continuous variation method[19], it indicates the composition of the ion-pair to be 1:1 in both cases. The stability constants were also calculated[20] using the data of the continuous variation method, log β_n was found to be 5.89 and 6.43 in case of BPB and BCG, respectively. The values of log βn indicate that the complexes are fairly stable.

Adherence to Beer's law

Beer's law was verified and found to be satisfactorily obeyed for 0.4-19 and 0.4-37 μg/cm^3 of ambroxol hydrochloride in case of BPB and BCG methods, respectively. The linear regression equations for calibration graphs were:A = 0.008 + 0.0309 C, with a correlation coefficient of linear regression of 0.9989 in case of BPB and A = 0.008 + 0.0386 C with a correlation coefficient of linear regression of 0.9994 in case of BCG. (The unit of C is μg/cm^3). The values of correlation coefficient indicate good linearity. The molar absorptivity (ε), specific absorptivity (a) [21] Sandell sensitivity (S) [22], limit of detection[23] and Ringbom linear range were calculated and their values are listed in Table 1. These values indicate that the methods are highly sensitive.

Effect of diverse ions

A systematic study of the effect of foreign species present along with ambroxol hydrochloride on the determination of ambroxol hydrochloride at 16.60 μg/cm^3 level was undertaken. This study was carried out by using the proposed procedures and adding a known amount of foreign species to 16.6 μg/cm^3 ambroxol hydrochloride. The results indicate that up to 50 fold of glucose, fructose, lactose, maltose and glycine do not interfere in case of BCG while in case of BPB up to 40 fold of lactose and maltose and up to 20 folds of glucose, fructose and glycine do not interfere.

Table 1 Optical data conditions and cumulative data for extractive spectrophotometric determination of ambroxcl hydrochloride using BPB and BCG

Parameter	BPB	BCG
λ max (nm)	412	414
pH	3.00	3.65
Beer's law linear range µg/cm^3	0.4-19	0.4-37
Ringbom linear range µg/cm^3	6.2-16.6	4.2 -24.8
Linear regression equation	A = 0.008 + 0.0309 C	A = 0.008 + 0.0386 C
ε	1.28 X 10^4	1.60 x 10^4
a	0.0309	0.0386
S	0.0324	0.0259
s	0.008	0.011
r	0.9989	0.9994
D. L. µg/cm^3	0.190	0.021

ε : Molar absorptivity (L mol^{-1} cm^{-1})
a : Specific absorptivity (ml g^{-1}.cm^{-1})
S : Sandell sensitivity (µg.cm^{-2})
s: Standard deviation
r : Correlation coefficient
D.L. Detection limit (µg/cm^3)

Analysis of pharmaceutical preparations

In order to establish the validity of the proposed methods, pharmaceutical preparations were analysed. The data in Table 2 show that the assay results were in good agreement with values for the normal contents with those using UV spectrophotometric method [14]. Student t- and F- tests (at 95% confidence level) were applied [23], the calculated t- values ranged from 0.204 to 1.254 which is lower than the tabulated value at 95% confidence level and 7-degree of freedom (2.262), the F-values were found to be in the ranges from 1.56 to 2.57 which are lower than the tabulated values at 95% confidence level. This means that there is no significant difference between the proposed methods and the reference method.

Table 2. Determination of ambroxol hydrochloride in pharmaceutical preprations.

Trade name	*Labelled amount mg/tablet or capsule*	*Proposed methods* BPB	BCG	*Ref. method [14]*
Ambroxol tablets	30			
mg found		29.6	29.65	
Mean recovery % ± S D		98.67 ± 1.95	98.84 ± 2.095	99.05 ± 1.307
F- Ratio		2.23	2.57	
t- test*		0.332	0.174	
Muco S.R. capsules	75			
mg found		73.16	73.58	
Mean recovery % ± S D		97.55 ±1.148	98.11± 0.490	97.79±0.502
F- Ratio*(9.12)		5.23	1.05	
t- test**(2.3 65)		0.416	0.964	

Average of five determinations for the proposed methods and four determinations for the reference method
*Tabulated F-values at 95% confidence level.
** Tabulated t-value at 95% confidence level and 7 degree of freedom

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