The optimum pH for the derivative spectrophotometric determination of co-trimoxazole in binary mixtures.

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ABSTRACT

Background: Although the experimental assessment of co-trimoxazole by use of derivative spectrophotmetry underscores the usefulness of this method due to its relative simplicity with which it can be carried out over the official United States Pharmacopoeia (USP) high pressure liquid chromatography (hplc) methods for this drug, suitable optimum conditions ought to be refined for its universal acceptability.

Objective: The objective of the present work was to obtain the optimum pH level for the UV assessment of co-trimoxazole.

Methods: The aqueous solutions of the individual drugs and their binary mixtures were buffered with Sodium Acetate-Acetic Acid buffer in the pH ranges 2-7 and scanned on zero order and on first-order derivative at the wave length between 200- 300nm

Results: At the same drug solution concentrations, spectral shifts occurred with change in pH, especially between the wavelengths 200 and 240nm, only seeming to converge from approximate wavelength 260nm onwards. Absorbance fluctuations were also observed at the same drug concentrations in the pH range 2 to 3.5 and 5 to 7 when the solutions were scanned, even at the wavelength where the spectra seem to converge. However there were no absorbance differences between pH 4 and 5.

Conclusion: The UV spectrophotometric method is dependent on the optimum pH and this has been found to range from 4 to 5.

Key words: hypsochromic shift, auxochromes, sulphamethoxazole, trimethoprim, spectroscopy, pH, UV, pyridines

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INTRODUCTION

The single most used antibacterial agent in Uganda is co-trimoxazole. This drug is a combination of sulphamethoxazole and trimethoprim in the ratio of 5:1. In spite of the strong regulatory control instituted in the country in 1993, it is frequently obtained and used without health workers' advice for various symptoms including simple cough, headache, common colds.[1] Clinically the drug is indicated for infectious diseases, especially those caused by the bacterial family of *Enterebacteriacae*[2, 3]. The combination has been found to have special efficacy in chronic and recurrent infections that include bacterial prostatitis[4], bacterial respiratory infections as in acute exacerbating chronic bronchitis when the infecting organism is *H.influenzae* and *Strep pneumoniae*[5, 6]; acute maxillary sinusitis and otitis media in children[7, 8]; gastro-intestinal infections manifested as shigellosis[9], and genital infections like chancroid. Its high dose has found value in *Pneumocytis carini* and thus ameliorating some symptoms of HIV / AIDS conditions[10].

The easy availability of this drug and its popularity in the community has raised concerns about its general quality in the market, making its quality assessment a mandatory requirement by the National Drug Authority (NDA). In this regard, the development of an assay method that is simultaneously cheap and time saving is a matter of constant necessity. We have recently used a first derivative spectrophotometric method to analyze this drug and showed that it is possible to use a relatively cheap and uncomplicated instrument like a UV spectrophotometer for its analysis. The United States Pharmacopoeia (USP) offers a couple of methods for separately assaying of the different components of the drug; for example it recommends a gravimetric method for trimethoprim and sulphamethoxazole analysis. As for the combination, the method recommended is the use of high-pressure liquid chromatography (hplc) [11].

The cardinal principle employed in spectrophotometric analysis is the absorption of light by specific chemical species and the measurement of emitting colors under the selective UV spectra between 200-380 nm wavelengths. Both sulphamethoxazole and trimethoprim are pyridine derivatives with amino groups that act as power-

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ful auxochromes. When attached directly to a chromophoric benzene system, the amino group is fully saturated, and in this form it is capable of modifying absorption spectra and hence give unreliable readings. In alkaline solution, the absorbing system may be intact, but in acidic solution the amino - H_2N group is replaced by - $+NH_3$, which is considerably less efficient as an auxochrome[12]. Thus in acidic solutions, a hypo chromic effect occurs resulting into a hypsochromic shift.

While derivative spectrophotometry looks promising as a cheap and fast alternative, optimum conditions must still be refined for the method to be universally applicable. The objective of the present work was to assess the optimum pH at which routine first derivative spectrophotometry of co-trimoxazole could be carried out.

MATERIALS AND METHODS

Apparatus

The experimental process of this work was carried out in our laboratory as previously described[13]. Camspec M350 UV-Visible double-beam spectrophotometer with matched 1-cm quartz cuvettes and a fixed slit width (2nm) connected to an IBM-PC Computer loaded with Camspec WindowsTM was used for all the absorbance measurements and treatment of data. A ***Metrohm 692-pH/***ion meter was used for the pH measurements.

Reagents and preparation of their concentrations

Ethanol (90%) and Acetic acid (99.8%), NaOH, Sodium acetate, all of analytical grade were purchased from commercial sources. Ethanol (10%) was prepared by adding sufficient distilled water to the mark, to 11ml of 90% Ethanol in a 1L volumetric flask. Acetic Acid (37%) was prepared by adding 37ml of acetic acid to a 100ml volumetric flask and diluting it to the mark with distilled water.

Sulphamethoxazole powder (Glaxo laboratories Limited, India, 99.46%) and Trimethoprim (Jiangdu Xinghai chemicals Co. Ltd, China, 100.0%) were used as supplied. Co-trimoxazole tablets (Farmaceutici Ecobi S.A.S Ronco Serivia (GE) - Italy, Lot No. 901E) were assayed. Filter paper (Whatman No. 1) was used to filter the solutions.

Preparation of the buffer solution

Double distilled water, freshly prepared, was used for all the appropriate dilutions. About 7(6.9)g of sodium Acetate was mixed with 3.0 ml of Acetic Acid (37%) and the mixture was diluted to 1L with water. Twenty ml volumes of the resulting solution were adjusted upwards or downwards with 0.1 NaOH or Acetic Acid to produce buffer solutions in the pH range 2-7.

Preparation of the stock solution

Sulphamethoxazole and trimethoprim standard stock solutions ($100\mu gm1^{-1}$) were prepared in aqueous ethanolic solution (10%) and kept below 5°C in a refrigerator for not more than 10 days.

Standard working solutions

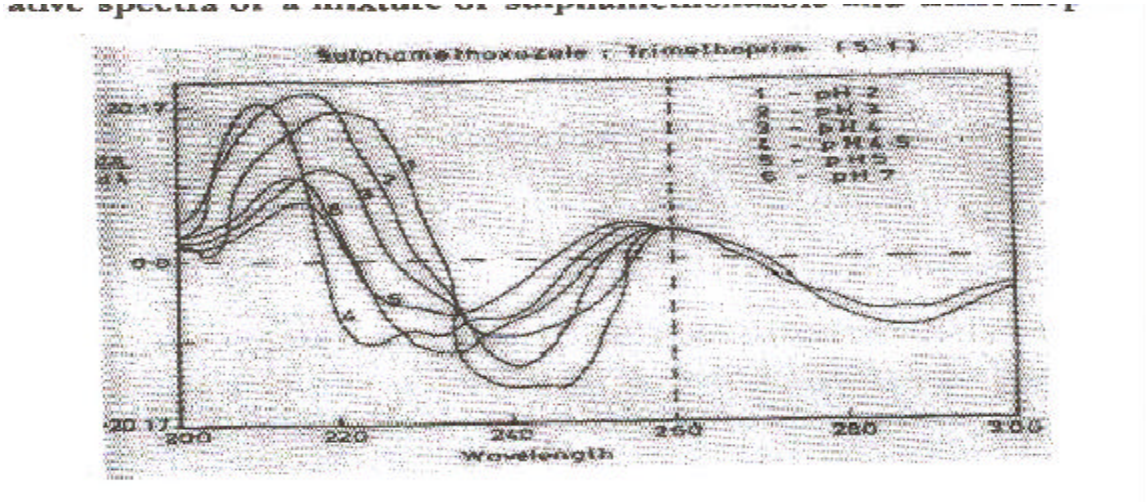
Three concentration levels 5, 10 and $20\mu gm1^{-1}$ of the two drugs were prepared from the stock solutions . For the $10ìgml^{-1}$ working solutions 5 ml volumes of each stock solution were measured into seven 50 ml flasks. Then 5.5 ml ethanol (90%) was added in each flask, followed by 10 ml of the buffer solutions (pH range 2-7) to each of the flasks. Distilled water was then added to the 50ml mark to yield the final concentration of $10\mu gm1^{-1}$. As similar procedure using appropriate amounts was used to obtain the final concentrations of 5 and $20\mu gm1^{-1}$. In order to make a 5:1 ratio of mixtures, 25ml of sulphamethoxazole and 5ml of trimethoprim were mixed. Suitable spectroscopic settings were made and scanning of the drug solutions was done over the range 200nm - 300nm, against aqueous ethanol (10%) at the various pH ranges. The instrument was set at a scan speed of 135nm/min, scanning data interval 0.2nm; and span of 50s. The absorbance and D_1-curves of the solutions (n=5) were obtained and sulphamethoxazole and trimethoprim determined.

Results

Figure 1 shows the effect of change in pH in the solvent used for ultraviolet measurements of sulphamethoxazole and trimethoprim of the same concentrations ($10ìgml^{-1}$) The spectral changes and absorbance values are quite striking, especially between wavelengths of 200 -258nm. From the wavelength 259 onwards on the UV range no further changes are observed.

In the figure it is observed that the absorption value for sulphamethoxazole (ëmax = 265nm) and trimethoprim (ëmax = 271nm) remain fairly unchanged between pH 4 and pH 5.

Fig. 1: A first-derivative spectra of a mixture of sulphamethoxazole and trimethoprim (5:1)



DISCUSSION

Both the identification and quantification of compounds by absorption spectrophotometry is dependent on the absorbance and spectral shapes of the chemical species being measured. In the present work it was observed that in both sulphamethoxazole and trimethoprim, spectral shape changes occurred with changes of pH. Figure 1 shows the striking changes of the same concentration, especially between wavelengths of 200 -258nm. From the wavelength 259 onwards little further changes are discernable. These changes were seen with all the concentration ranges 5-20ìgml^{-1} of the drug solutions.

Ar'ev[14] and Makevich, et al[15] observed spectral shifts as a result of electrochemical changes of the solutions in different laboratory situations. One of these changes is solubility itself, leading to the number of density fluctuations in the water, which creates conditions favorable for the acceptance of the hydrophobic additive. These workers noted that the solvent exerts an intense influence on the quality and shape of the spectrum in uv-spectroscopic measurements. Kalnin'sh[16] examined the spectral shifts of the charge transfer bonds for the complexes of phenol with the electron acceptor chloranil due to hydrogen bond formation between the phenol and solvent. It was shown that the electronic excitation of the complex results in much lower H-bond energies

In figure 1 it is observed that the absorption value for sulphamethoxazole (ëmax = 265nm) and trimethoprim (ëmax = 271nm) change with both low and high pH. However it can be noted that the absorbance remains fairly unchanged between pH 4 and pH 5. Measuring the infra-red (IR) absorption spectra of cation exchange in different ionic forms, Uglyanskaya, et al[17] showed that the spectra of the acidic forms do not show continuous absorption of energy. More fundamentally, Uglyanskaya and co-workers[17] showed that in aqueous solutions, the pH exerts an intense effect on ionisable chromophores due to the differing extent of conjugation in the ionized and the non-ionized chromophore.

In addition to the above, the amino groups in both sulphamethoxazole and trimethoprim are able to act as effective auxochromes. These amino groups when fully saturated are capable of modifying absorption spectra and hence give unreliable readings, especially in the acidic environment. In acidic settings H_2N is replaced by $+NH_3$ that is considerably less efficient as an auxochrome[12].

In our previous work we showed that the optimum wavelength at which sulphamethoxazole could be measured in the first derivative spectrophotometry was 237.6nm. The changes observed are notable in both absorbance and derivative values. For trimethoprim, which was measured at 259nm, the changes here seem negligible. However specific assessment, marching the pH and absorbance at this wavelength also showed fluctuations. This has special importance in spectrophotometric measurement since the assay is made in the binary mixture, if one component is affected then the entire assay can be rendered unreliable. Here we have used the individual components of the drug co-trimoxazole to demonstrate the behavior of the individual agents against the changes in pH. In the figure we used the composition ratio 5:1 (sulphamethoxazole:trimethoprim) as in most pharmaceutical preparations. However the use of the individual components would have been sufficient since the characteris-

tics of the individual components are the ones responsible for the behavior in the binary mixture. In conclusion, since this study makes use of absorption values of these compounds at wavelengths other than their ëmax, there is need to optimize and reproduce the pH conditions under which measurements are taken.

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