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Statistical optimization applied to the spectrophotometric study of a tolmetin–copper(II) complex*

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Abstract: Tolmetin sodium has been investigated and determined from dosage forms as its Cu(II) complex and method optimized by statistical optimization. The assay was developed using two mathematical statistical models: factorial design and response-surface mapping. The decision to apply experimental design techniques to the development of the method was made after a series of screening experiments revealed that the complex formation and extraction are maximized as a function of supporting electrolyte concentration, concentration of Cu(II) acetate and pH of the reaction mixture. One set of two-level three variable factorial experiments was carried out in order to evaluate the main effect, as well as the interaction among factors. The final step was to optimize the values of variables using response surface design. The best set of conditions was selected for further investigation.

Keywords: *Experimental design; tolmetin sodium; copper(II) acetate; complexometry; spectrophotometry.*

Introduction

Tolmetin [(1-methyl-5-*p*-toluoylpyrrol-2-yl)-acetic acid] is a non-steroidal anti-inflammatory agent with analgesic and antipyretic activities. As the sodium salt dihydrate it is formulated in tablet and capsule forms, both of which are used for the treatment of rheumatoid arthritis and osteoarthritis.

Tolmetin has been determined by a variety of analytical techniques, the most commonly used being colorimetry [1], UV spectrophotometry [2] and gas and liquid chromatography [3–7]. Other methods include thin-layer chromatography [8, 9], fluorimetry [10] and polarography [11]. As an instrumental tool, spectrophotometry offers significant economic advantages over gas and liquid chromatography. This paper is aimed at developing a new, simple, extractive spectrophotometric method for the determination of tolmetin sodium in dosage forms.

There are no data on the use of metal ions as analytical reagents for tolmetin sodium determinations. The aim of this work was to establish: (a) a new extractive spectrophotometric method with the Cu(II) ion as an analytical reagent without prior degradation or derivatization of tolmetin sodium; (b) to investigate the tolmetin–Cu(II) complex and to determine the optimum set of operating conditions for the parameters affecting the analysis by response-surface mapping; and (c) to develop a simple and easily reproducible procedure for routine analysis of tolmetin sodium from dosage forms.

Experimental

Reagents

Tolmetin sodium bulk drug and Tolectin® tablets (200 mg) were obtained by courtesy of McNeil Inc. (New York, USA). Copper(II) acetate, potassium chloride, acetic acid and sodium hydroxide were obtained from Merck A.G. (Darmstadt, Germany). Doubly-distilled water was used.

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Solutions

A freshly prepared (4.0 mg ml^{-1}; 1.27×10^{-2} M) aqueous solution of tolmetin sodium was used as the standard solution for analytical purposes. The calibration curve was prepared with eight solutions with concentrations from 0.5 to 0.7 mg ml^{-1} (1.586×10^{-3} to 2.22×10^{-2} M). A sample solution containing 4.0 mg ml^{-1} of tolmetin sodium was prepared by extracting tolmetin sodium from tablets.

Copper(II) acetate solution (50.0 mg ml^{-1}; 2.5×10^{-1} M) was prepared by dissolving 5.0 g of Cu(II) acetate in 1 ml of water with the addition of 1 ml of 1 M acetic acid and diluting the solution to 100 ml with water. The ionic strength of the final solution was kept constant at 0.2 M by addition of 2 M potassium chloride solution. Acetate buffer solutions covering the pH range 4.0–6.5 were made by mixing appropriate volumes of 1 M sodium hydroxide and 1 M acetic acid solutions.

Apparatus

The solution absorption was recorded on a Specord M 40 Carl Zeiss Jena Spectrophotometer, provided with matched 10-mm quartz cells. Measurements of pH were carried on a Radiometer 22 pH-meter. The pH values were determined with a saturated calomel-glass electrode.

Procedure

To 2 ml of tolmetin sodium solution placed in an Erlenmeyer flask fitted with a ground glass stopper, 2 ml of Cu(II) acetate solution, 1 ml 2 M potassium chloride and 4 ml of acetate buffer solution are added. Finally, 5 ml of chloroform was added and the Erlenmeyer flask stoppered. After shaking the reaction mixture for 10 min the green-coloured chloroformic layer was separated in a separatory funnel and the absorbance measured at 696 nm, against a reagent blank. This procedure was employed for measuring the absorption spectrum and for determination of tolmetin sodium from bulk drug and tablets.

Results and Discussion

The characteristics of the complex

Tolmetin sodium reacts with Cu(II) acetate in aqueous solution at pH 4.6–6.8 to form a green, chloroform-extractable complex. The absorption spectrum of the extracted complex was recorded in the spectrum region from 500–900 nm. Maximum absorbance of the complex occurred at 696 nm (Fig. 1). Under the same experimental conditions the chloroformic extract of a mixture of Cu(II) acetate, potassium chloride and acetate buffer solution did not asbsorb in this spectral region.

The amount of complex produced and the reaction rate were considerably influenced by the pH of reaction mixture. The complex was only produced at pH values above 4.6. The general trend observed was a gradual increase in absorbance to pH 5.7, followed by a steady decrease in absorbance after pH 6.0 (Fig. 2), probably because of the concurrent reaction of Cu(II) hydrolysis; therefore, the optimum conditions for complex formation were found in the pH 5.7–6.0 range.

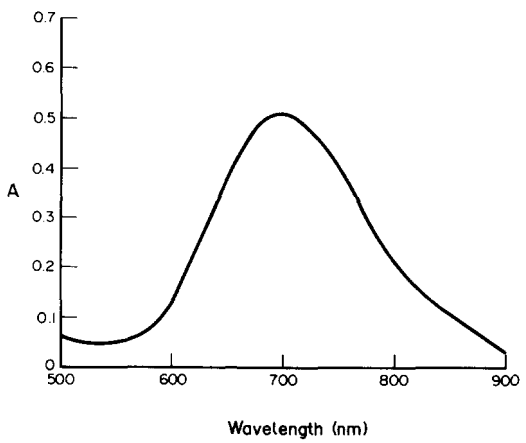

Figure 1
Absorption spectra of tolmetin–Cu(II) complex in chloroform. [Tolmetin] = 1.27×10^{-2} M; [Cu(II)] = 2.5×10^{-1} M; pH 5.8; μ = 0.2 M.

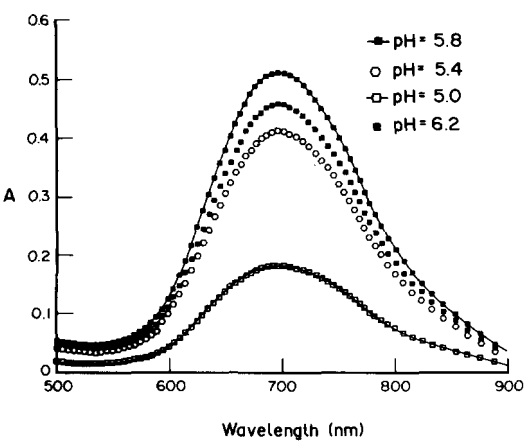

Figure 2
The effect of pH on complex formation and extraction. [Tolmetin] = 1.27×10^{-2} M; [Cu(II)] = 2.5×10^{-1} M; μ = 0.2 M.

As the shape of the absorption curves and positions of the absorption maximum did not vary with pH, it was concluded that only one type of complex is formed in this pH range.

The effect of Cu(II) concentration showed that the absorbance increased for molar ratios up to 20:1 of Cu(II):tolmetin sodium. The use of a higher reagent concentration did not produce an increase in absorbance. The complex was formed immediately and quantitatively extracted after 5 min. Its absorbance remained unchanged with longer period of extraction.

The ionic strength also played a significant role by its influence on the shape and intensity of the recorded peaks. The effect of ionic strength on complex formation was followed in the range 0.1–0.4 M. At an ionic strength of 0.2 M, optimally shaped spectra were recorded.

Optimum conditions for complex formation

To achieve maximum sensitivity, optimum conditions should be utilized. In order to investigate the effect of each factor and their interaction, a three variable, two-level, full-factorial design was chosen [12]. For reaction of the Cu(II) ion with tolmetin, the dependent variable, was the absorbance of the extracted complex. The independent variables were pH, ionic strength and concentration of Cu(II) acetate. Different levels of these variables were selected in order to maximize the information that could be obtained from experimental data. The design matrix (Table 1) shows the eight treatment combinations of a low (−) and high (+) level of factors.

Table 1
Factorial design

Trial	Factor level* A	B	C	Absorbance
1	−	−	−	0.1335
2	+	−	−	0.4253
3	−	+	−	0.2016
4	+	+	−	0.4671
5	−	−	+	0.1634
6	+	−	+	0.4550
7	−	+	+	0.2209
8	+	+	+	0.5098

*Low (−) and high (+) levels of the following factors:

Factor		Value	
		Low (−)	High (+)
A	[pH of reaction mixture]	4.6	5.8
B	[ionic strength]	0.1 M	0.2 M
C	[Cu(II) concentration]	0.16 M	0.20 M.

From the estimate of factors effect (Table 2), the tolmetin–Cu(II) complex was found to be less sensitive to variation in the levels of Cu(II) concentration and ionic strength, than in the variation of pH. The effect of pH was therefore the main effect.

Table 2
Estimates of factor effects

ave	=	0.32208
A	=	0.28445
B	=	0.05555
AB	=	−0.00725
C	=	0.03040
AC	=	0.00580
BC	=	0.00060
ABC	=	0.00590

In order to increase the sensitivity of the method, a response surface diagram was developed to identify the optimum experimental conditions. A response-surface can simultaneously represent two independent and one dependent variable when a mathematical relationship between variables is known or can be assumed [12]. Since a 25-fold excess of Cu(II) concentration led to a quantitative conversion of tolmetin into the complex and use of a higher reagent concentration did not produce an increase in absorbance, the Cu(II) concentration was held constant at 0.25 M. Two remaining independent variables together with the absorbance as the dependent variable was used for response-surface mapping. Ten solutions were examined (pH 5.2, 5.8, 6.4 and μ = 0.1, 0.2, 0.3 M, with a replicate at the mid-point pH 5.8, μ = 0.2 M) and according to the experimental data model fitting methods gave the equation for the relationship between pH and μ

$$A = -13.557 + 0.1227\mu + 4.8000\text{pH} - 1.6920\mu^2 - 0.4111\text{pH}^2 + 0.0808\mu\text{pH}. \quad (1)$$

This assumed response-surface and its simpler form as a contour of constant response in two-dimensional factor space are given in Fig. 3. The negative estimates of μ and pH coefficients cause the estimate response-surface to fold downward quadratically in both factors, μ and pH, although less rapidly in μ than in pH.

Maximum response is obtained with pH = 5.8 and μ = 0.19 M. Thus pH 5.8 and μ = 0.19 M were used as the working conditions.

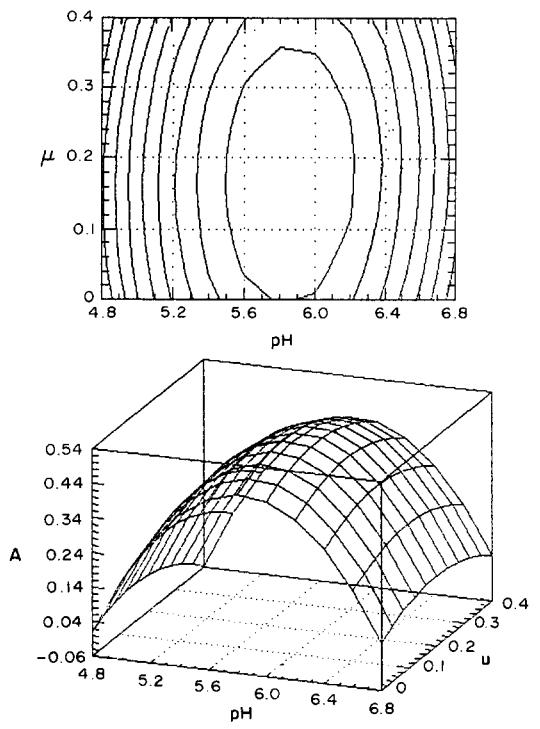

Figure 3
The absorption response of tolmetin–Cu(II) complex in chloroform, with respect to the pH and ionic strength or reaction mixture. [Tolmetin] = 1.26×10^{-2} M.

Since the factors chosen did have a significant effect on response, the variance in the data set accounted by the factors as they appear in the model was larger than the variance of the residuals (Table 3). If the factors had no effect upon the response these variances would be expected to have the same value. It was confirmed by the Fisher variance ratio for significance of the regression i.e. significance of the factor effect. $F = 7.979$ ($F_{crit} = 6.26$) was signifiant at the 95% level of confidence.

The test for the lack of fit compared the variance due to the lack of fit with the variance due to purely experimental uncertainty. $F_{lof} = 29.496$ ($F_{crit} = 215.7$), which was not significant.

The conclusion was that there was not a significant amount of the variation in the measured responses and measured responses that could be explained by the model. As expected, the residuals were very small. The coefficient of multiple determination (R^2) was 0.9794 indicating that the factors explained the data very well. If the degrees of freedom are taken into account the adjusted R^2 is 0.9536.

Stoichiometry of the complex

The stoichiometric ratio of tolmetin to Cu(II) in the complex was determined by the application of Job's method of continuous variations. The concentration of aqueous tolmetin sodium and Cu(II) acetate solutions was 10^{-2} M. Nine solutions containing tolmetin sodium and Cu(II) acetate in various molar ratios with the total volume held at 5 ml. The extraction was performed with 5 ml of chloroform and the absorbance measured at 696 nm. The curve obtained (Fig. 4) had a maximum at a molar ratio $x_{max} = 0.33$, which indicates the formation of a 1:2 complex. The composition of the complex was confirmed by the molar ratio method. The curve obtained had an intercept at tolmetin:Cu(II) = 2:1.

The determination of the composition and relative stability constant of the complex was carried out at pH 5.8 and $\mu = 0.2$.

Relative stability of the complex

The relative stability constant of the complex was determined by the Job's method of non-equimolar solutions [13]. The total volume in each experiment was 12 ml, with five-fold and 10-fold excesses of Cu(II) acetate (p).

The value of relative stability constant, log

Table 3
Analysis of variance for variables and for the full regression

Source of variation	Sum of squares	d.f.	Mean square	*F*-ratio
μ	0.00249696	1	0.00249696	6.21
pH	0.00729833	1	0.00729833	18.15
μ^2	0.00574435	1	0.00574435	14.28
pH^2	0.06080253	1	0.06080253	151.19
$\mu \times$ pH	0.00002772	1	0.00002772	0.07
Model	0.077978	5	0.0152740	37.98
Error	0.00160866	4	0.00040217	29.49
Lack of fit	0.00159068	3	0.0053023	
Purely experimental uncertainty	0.00001798	1	0.00001798	
Total (corr)	0.0779785	9		

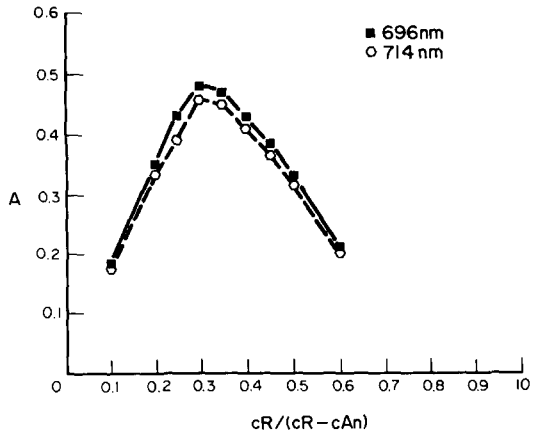

Figure 4
Job's curve of equimolar solutions for the tolmetin–Cu(II) complex in chloroform. [Tolmetin] = [Cu(II)] = 10^{-2} M; pH = 5.8; μ = 0.2 M.

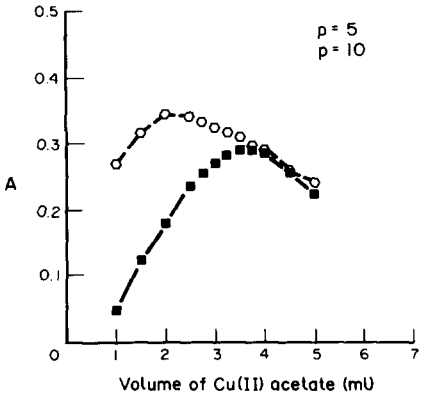

Figure 5
Job's curve of non-equimolar solution at 696 nm. [Tolmetin] = 5 × 10^{-3} M; pH 5.8; μ = 0.2 M.

K′ = 7.385 was confirmed by the Sommer's method [14] using Job's curve of equimolar solutions (Table 4).

Table 4
Conditional stability constant of the tolmetin–Cu(II) complex*

Job's method of non-equimolar solutions*			
[Cu(II)]	p	X_{max}	log K′
2.5 × 10^{-2}	5	0.308	7.54
5.0 × 10^{-2}	10	0.917	7.23
		Mean:	7.385

Sommer's method*				
log K′	log K'_{min}	log K'_{max}	SD	RSD (%)
7.534	7.456	7.601	0.08	1.68

*Conditions: pH 5.8; μ = 0.19; t = 25 ± 0.5°C.

Validation of the method

The method was tested for linearity, precision, sensitivity and reproducibility. Absorbance responses were linearly related to concentration of tolmetin sodium in the range 0.1–1.4 mg ml^{-1} (3.172 × 10^{-4}–4.441 × 10^{-3} M) calculated in the final solution for extraction, with a detection limit of about 41.08 μg ml^{-1} [15]. The regression equation was $y = -0.01746 + 0.6684x$, the correlation coefficient being $r = 0.999$ ($n = 8$), indicating excellent linearity.

The precision of the method was checked at three different concentrations. The RSD (n = 7) varied from 1.60 to 2.98% for concentrations of tolmetin sodium from 2 to 6 mg ml^{-1}.

The applicability of the method for the assay of simple dosage forms was examined by analysing Tolectin® tablets. The recovery was 99.4% (n = 10) relative to the labelled strength of those preparations and the RSD was 2.01%.

The statistical analysis of the results obtained in the determination of tolmetin sodium as a pure compound and from dosage forms are shown in Table 5.

The developed method can be recommended for the routine analysis of tolmetin

Table 5

Sample (n = 10)	Concentration of solutions (mg ml^{-1})	Found (mg ml^{-1})	SD (mg)	RSD (%)	S_x	Recovery (%)
Tolmetin sodium bulk drug	2.0	2.00	0.061	2.98	0.023	100.0
	4.0	4.00	0.064	1.60	0.024	100.0
	6.0	6.00	0.021	1.74	0.008	100.0
Tolectin® tablets (200 mg)	4.0	0.398	0.012	2.01	0.004	99.4

sodium in aqueous solutions and tablets since it is rapid and simple and the results are reproducible.

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