CHARGE TRANSFER COMPLEX FORMATION IN SPECTROPHOTOMETRIC AND CONDUCTOMETRIC DETERMINATION OF SOME SULFONAMIDES

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نقترح هنا طريقة للتحليل الطيفي لتقدير السلفونواميدات بشكلها النقي في المستحضرات الصيدلانية تعتمد على تكوين مركب معقد انتقالي الشحنة بين الفينوسافرانين كمتلقي للشحنة وبين السلفونواميدات كمانح لها في محلول الخلات الراصد (أس هيدروجيني 3). وقد تمت دراسة العوامل التي تؤثر على تكوين المركب المعقد انتقالي الشحنة، وتم الحصول على منحنيات معايرة خطية في مدى التركيز 1.07 – 16.70 مكغ/مل عند 25°مئوية وكان أدنى حد للكشف في مدى 0.428- 0.573 مكغ/مل. كما تم دراسة نفس المركبات المعقدة في الوسط المائي وذلك لإجراءات قياسات التوصيل. ويمكن استعمال هذه الطريقة بنجاح للمستحضرات الصيدلانية. ولقد تبين أن معاملات التوصيل المولاري تزداد بزيادة الحرارة (25 – 35°مئوية) في كل الأنظمة التي تمت دراستها ، وأن تكون معقدات السلفونواميدات مع الفينوسافرانين تتم بنسبة 1:1 في كلا الطريقتين.

A spectrophotometric method is proposed for determining sulfonamides in pure form and in pharmaceutical preparations, based on the charge-transfer (CT) complex formation between phenosafranine as an acceptor and sulfonamides as donors in acetate buffer (pH 3). The factors affecting the formation of the CT- complex were studied, linear calibration graphs were obtained in the concentration range 1.07 – 16.70 µg/ml at 25ºC. The minimum detection limit is in the rang 0.428-0.573 µg/ml. The same complexes have been studied in aqueous media for conductometric measurements. The method could be successfully adopted for pharmaceutical preparations. The molar conductivity coefficients were found to increase with increasing temperature (25-35^{0}C) in all the systems studied. The stoichiometry of the sulfonamides - phenosafranine complexes was found to be 1:1 in both techniquecs.

Keyword: Charge transfer, spectrophotometry, conductometry, sulfonamides, phenosafranine

Introduction

The charge transfer (CT) complexes are formed between electron donors, having sufficiently low ionization potential, and acceptors, having sufficiently high electron affinity. The transfer of an electron from a donor to an acceptor is readily possible in the charge transfer process (1). Several similar methods for the analysis of drugs have been developed using different basic dyes for the spectrophotometric determination of some acidic drugs such as, niclosamide, ketoralac trometamine and diclofenac sodium in pharmaceutical preparations (2-5). Krishna *et al.* (6) and Al-Attas (7) studied internal indicators in nitritometric assay of sulfonamides, procaine and benzocaine. Direct current polarography and cyclic voltammetry were used to study the reduction behaviour of tiaprofenic acid with safranine-T (8).

Phenosafranine (PSF) has been previously reported as an analytical reagent in many fields of analysis (9-13). Spectrophotometric and conducto-metric methods were described for the determination of some sulfonamides in pure form and in pharmaceutical preparations in aqueous medium by safranine-O (14) and safranine-T (15). The same methods were also used for the determination of nicotinamide and its derivatives by safranine-O (16).

The present work describes the development of simple, and rapid spctrophotometric and conductometric methods for the quantitative determination of some sulfonamides namely: sulfa-diazine (SDZ), sulfamethazine (SMT), suflametho-xazole (SMX), sulfisoxazole (SFZ), sulfaguanidine (SG), sulfapyridine (SPD) and sulfamerazine (SMR) in pure form and SMX in pharmaceutical

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Table 1. Collective data for the Spectrophotometric method for the determination of Sulfanomides with PSF.

Compound	λ max-(nm)	Range, μg ml^{-1}	ε x 10^4	a x10^{-4}	s x 10^{-3}	Regression equation[a] Slope (b)	intercept (a)	r	SD, %
Sulfadiazine (SDZ)	273	1.25 – 15.02	4.53	1.81	5.53	0.021	0.138	0.9958	0.49
Sulfamethazine (SMT)	272	1.39 – 16.70	5.02	1.80	5.55	0.020	0.038	0.9997	0.45
Sulfamethoxazole (SMX)	272	1.27 – 15.20	4.90	1.93	5.17	0.021	0.029	0.9946	0.45
Sulfisoxazole (SFZ)	272	1.34 – 14.70	4.85	1.81	5.51	0.021	0.010	0.9998	0.47
Sulfaguanidine (SG)	270.5	1.07 – 12.85	4.28	1.99	5.01	0.022	0.003	0.9998	0.46
Sulfapyridine (SPD)	271.5	1.25 – 12.47	4.96	1.98	5.03	0.021	0.069	0.9903	0.47
Sulfamerazine (SMR)	272	1.43 – 14.32	5.06	1.77	5.66	0.020	0.060	0.9997	0.47

[a] A = a + bc where c is the concentration in μg ml^{-1} .
ε = Molar absorptivity, 1 mol^{-1} cm^{-1}.
a = Specific absorptivity, ml g^{-1} cm^{-1}.
s = Sandell sensitivity, μg ml^{-2} / 0.001A.
r = Correlation coefficient.
SD, % = Standard deviation.

Pharmaceutical preparations by phenosafranine dye in aqueous medium.

Experimental

Chemicals and Solutions:

The compounds (SDZ), (SMX), (SMT), (SFZ), (SG), (SPD) and (SMR) with a purity of 99% were obtained from Aldrich. PSF (Microscopic materials) was obtained from BDH, England.

Pharmaceutical formulations were obtained from the local market, Bactrim tablets (400 mg SMX/ tablet) and Bactrim suspension (200 mg SMX/ 5ml), F. Hoffmann-La Roche Ltd., Basel-Switzerland, Septrin tablets (400 mg SMX/tablet), Wellcome Foundation Ltd., London-England, and Septazole suspension (200 mg SMX/ 5ml), Alexandria Pharmaceutical Company, Alexandria-Egypt.

Stock solutions of salfanoides (1x10^{-3} M) were prepared by dissolving the calculated amounts in ethanol-deionized water (5:95, v/v). The solution of PSF (1x10^{-2} M) was prepared in deionized water. Acetate buffer solutions (pH 3-5) were prepared using 0.2 M solutions of acetic acid and sodium acetate(17). The stock standard solution of salfonamides remain stable for two-three weeks at 4^{0}C in the dark, but PSF remains stable for more than four weeks if kept in the dark.

Equipments:

UV/VIS Spectrophotometer UV-1601 with personal spectroscopy software version 3.7, connected to Shimadzu TCC-240A controller, was used for the spectrophotometric measurements. Hanna Research HI 8820N conductivity meter connected to an ultrathermostat, temperature was controlled to ± 1.0°C, used for conductance measurements. Jenway pH-Meter model 3015 was used with accuracy ± 0.02 pH unit.

Procedures

1.Spectral Measurements

Calibration graph

The spectra of CT-complexes were scanned over the range of 200-650 nm in solutions containing 0.3 ml of 10^{-3} M of salfanomides, 0.3 ml of 10^{-3}M of PSF and 4 ml of acetate buffer of pH 3, diluted with deionized water to 10 ml against a blank of buffer solution of the same used buffer. Plot the absorbance versus the concentration of the CT-complex and prepare a calibration graph.

2. Conductometric Titrations

Calibration graph

An aliquot 10 ml of 10^{-3} M of the sulfa drug solution was transfered to the conductivity cell and titrated with 10^{-2} M PSF- solution. The conductance was recorded after thorough stirring following each addition (2min. intervals) and plotted *versus* the volume of the titrant.

Pharmaceutical Preparations

A. Tablets:

Twenty tablets were weighed to determine the average weight of a tablet and was finely powdered. Into a 100 - ml measuring flask the amount was transfered and dissolved in ethanol-water mixture (50:50, v/v) by shaking for 10 min, then mixed well and filtreted if necessary. An accurately measured volume of the filtrate was transfered to a 50 ml-measuring flask and completed to the mark with deionized water. Solutions of lower concentrations were prepared by appropriate dilution with deionized

water. Then it was proceeded as detailed under ″spectral measurements or conductometric titrations″, starting from: "The spectra of CT-complexes ……". The drug concentration was obtaned from the computed regression equation (table 1).

B. Suspension:

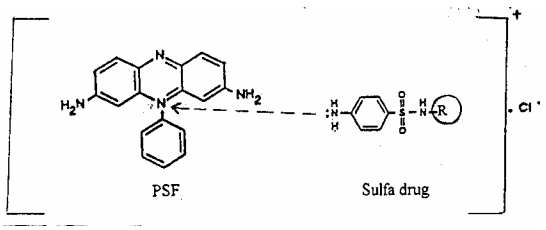
An aliquot [5 ml of the suspension (200 mg SMX /5ml)] was diluted with 10 ml of ethanol by shaking 10 min., then mixed well and completed to 50 ml in a measuring flask using deionized water. Solutions of lower concentrations were prepared by appropriate dilution with deionized water, then proceeded as detailed under ″spectral measurements or conductometric titrations″, starting from "The spectra of CT-complexes ……". The mass of the drug was computed from the calculated regression equation (table 1).

Results and discussion

1. The Spectrophotometric Method

Scanning of the λ_{max}

Violet charge transfer complexes developed upon mixing equimolar solutions of PSF and each of the sulfanomides in aqueous media. Figure 1 (curves a-d) represents the spectra of the formed complexes having absorption maxima (λ_{max}) in the UV-region of 270-273 nm with an appreciable increase in absorbance of both PSF or the sulfa drugs. This band is attributed to charge-transfer (CT) complex formed, which may be represented by the formula:



pH and Sequence of Addition of Reagents

In a trial to elucidate the optimum media for the quantitative determination of sulfhonamides using aqueous solution and acetate buffer of different pH values ($_p$H 3-5). It was found that acetate buffer of pH 3 (14) is the most suitable for the measurements. The optimum amuont of buffer was found to be 4 ml as it gave marginally the highest and stable absorbance.

Stoichiometry

The stoichiometry of the complexes formed between PSF and sulfa drugs was investigated at pH 3 by the molar ratio and continuous variation methods (18). Both revealed a composition of the complexes occurs as 1:1-ratio (PSF:drug). The complexes are labile where the broad shape of the curve was observed to indicate that the inflection at this ratio may be attributed to the weak binding forces in the C-T complexes due to the bulky size of the two molecules (14,15,19).

Standing Time and Temperature

The complexes were formed instantaneously and the absorbance remained stable for~2 hours in day light and 24 hours in the dark. The complexes were stable at 20-40°C, but 25°C, was chosen for all measurements. The studies proved that absorbance of all complexes is not affected by the presence of the following cations and anions:
Ca^{2+}, Zn^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Eu^{3+}, Mn^{2+}, Cr^{3+}, Hg^{2+} , ClO_3^-, Br^-, $(CH_2COO)_2^-$, BO_2^-, $C_4H_4O_6^{2-}$, NO_3^-, SO_3^{2-}, $B_4O_7^{2-}$, I^-, HCO_3^-, CH_3COO^-, $H_2PO_4^-$, CN^-, $C_2O_4^{2-}$, $C_6H_5O_7^{3-}$, $S_2O_4^{2-}$, Cl^-, OH^-, $BO_2H_2O_2^-$, SO_4^{2-}, $S_2O_5^{2-}$, SO_3^{2-}, $S_2O_3^{2-}$, NO_2^-. On the contrast the ions (Fe^{2+}, $(C_6H_4COH)COO^-$, $Cr_2O_7^{2-}$, $C_7H_5O_2^-$, $COO.C_6H_4COO^-$) caused interference with the absorbance (increase) of all the complexes studied.Under the above mentioned conditions absorbance against concentration data (linear calibration graphs) are shown in Table 1. The method was found to be precise, accurate, rapid and simple for the determination of sulfonamides in the pure form but it was not successful for the pharmaceutical preparations (Bactrim, and Septrin tablets, septazole and Bactrim suspension) which may be due to the presence of trimethoprime (TMP).

2. The Conductometric Method

The molar conductivity coefficient, $\sigma_{\square}$, has been calculated using Gutmann equation (20).

$$\sigma_M = \frac{1}{\alpha_M} \cdot \frac{\sigma p - \sigma_0}{\sigma_0} \qquad (1)$$

Where α is the dissociation constant of the complex, M is the molar concentration of the titrant at the conductivity peak where σ = σp and σ_0 is the linearly interpolated conductivity background read off as a base line joining the conductivities of pure donor and acceptor solutions.

The increase in σ_p values with increase of temperature is consistent with the findings of Hassib and Issa (1), that the rate of electron transfer in complexes is enhanced by increase in temperature, giving rise to greater number of ions in solution. The decrease in conductance with rise of temperature indicates a fewer number of ions carrying current at the stoichiometry of the complex. Such behavior may be explained on the basis that with rise of temperature, intermolecular charge transfer within the molecular complex formed is stronger than intermolecular charge transfer leading to the shift towards the formation of undissociated complex. This is the case of charge transfer complexes formed between Schiffs bases bearing electron-releasing groups and strong acceptors (1).

Conductometry could be successfully and simply used for the quantitative determination of organic compounds by using reactions involving change in ionic concentration of analytical reagents, change in ionic volume or formation of CT- complex (19) Therefore sulfonamides were titrated against PSF in aqueous media. The reagent concentration was nearly 10 times that of the drug solution to minimize dilution effects during the titration. In addition, a dilution correction factor based on considering the conductivity as a linear function of dilution was also made using the equation:

$$X_{Corr} = X_{obs\,[(V_1+V_2)/V_1]} \qquad (2)$$

Where X_{Corr} and X_{obs} are the corrected and observed conductance, respectively, V_1 is the initial volume and V_2 is the volume of added reagent (21). $1x10^{-2}$M solution of PSF was used to achieve a constant and highly stable conductance reading after 2 minutes intervals and to minimize the dilution effect on the conductivity throughout the titration. The CT-complex formed on adding the reagent is soluble in the media.

The conductometric method, using safranine O in aqueuos media, was described for the determination of most of the investigated sulfa drugs (14). In this work, PSF is used for conductometric determination of the mentioned sulfonamides, where straight lines were obtained, intersecting at molar ratio of 1:1 (PSF:drug). Increases in conductance before and after end-point are attributed to PSF added and to the increase in chloride ion concentrations. Other parameters such as viscosity, dielectric constant, solvation effect and ion- pair formation might also affect the shape of curve.

Analysis of drugs in pharmaceutical preparations

The spectrophotometric method based on the charge-transfer complex between sulfa drugs and PSF proved to be sensitive, accurate and precise for the pure compounds. But it was not successfully adoptable for determining sulfonamides in pharmaceutical preparations. On the other hand, the conductometric titration has the advantage of being simple, accurate and reproducible for pure and dosage forms without interference from excipients. Beer's law limits, molar absorptivities, regression equation, standard deviations and correlation coefficients obtained by linear squares treatment of the results are given in Table 1. The performance of the present method was compared with the reference method (6). Mean values obtianed in Student's t-and F-tests showed the absence of any systematic error in the method Table 3. Table 2 and Table 3 show the results of conductometric titration of sulfa drugs in pure form and in pharmaceutical preparations. The data prove that this method can be used successfully for the analysis of such compounds.

Table 2 . Conductometric determination of sulfonamides in pure form with PSF.

compound	mg		% Recovery	±SD *
	Taken	Found		
Sulfadiazine	27.53	27.48	99.82	0.05
		27.53	100.00	
		27.58	100.81	
Sulfamethazine	30.62	30.58	99.86	0.04
		30.58	99.89	
		30.65	100.10	0
Sulfamethoxazole	27.86	27.85	99.96	0.08
		27.98	100.43	
		27.82	99.86	5
Sulfisoxazole	29.40	29.44	100.14	0.04
		29.46	100.20	
		29.37	99.90	7
Sulfaguanidine	23.56	23.54	99.92	0.04
		23.57	100.40	
		23.49	99.70	0
Sulfapyridine	27.42	27.42	100.00	0.07
		27.54	100.44	
		27.42	100.00	6
Sulfamerazine	31.49	31.47	99.94	0.02
		31.51	100.06	
		31.52	100.10	6

* No. of determinations = 3

Table 3 . Determination of SMX in pharmaceutical preparations with PSF by conductometric method.

Drugs	mg			± SD**
	Taken	Found	Recovery %	
Bactrim tablets	40	40.10	100.25	
		40.40	101.00	0.002
		40.20	100.50	
	4	4.020	100.50	
		4.050	101.25	0.021
		4.010	100.25	
Septrin tablets	40	40.10	100.25	
		40.30	100.75	0.002
		40.40	101.00	
	4	3.950	98.75	
		4.010	100.25	0.03
		4.990	99.75	
Reference method[6] Student t[a] 2.122 (2.776)[b] *F*[a]1.44(19)[b]	200	198.00	99.00	
		198.10	99.05	0.10
		198.20	99.10	
Septazole suspension	20	20.10	100.570	
		20.02	100.556	0.021
		19.98	99.900	
	2	2.020	101.0	
		1.990	99.50	0.015
		2.010	100.5	
Bactrim suspension	20	19.95	99.75	
		19.99	99.95	0.021
		19.98	99.90	
	2	1.990	99.50	
		2.010	100.5	0.015
		2.020	101.0	

**No. of determinations = 3 .
[a] Comparison with Krishna method (6).
[b] Values in parenthesis are the theoretical t-and *F*-values at p = 0.05 .

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