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Two spectrophotometric methods for estimation of clarithromycin in pharmaceutical formulations and human plasma based on charge transfer complexes

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ABSTRACT

Two novel, simple, and sensitive spectrophotometric methods were applied successfully to the determination of clarithromycin (CLM) in pure, pharmaceutical dosage form, and humane plasma with good accuracy and precision. The methods were based on chrage transfer complexes (CTCs) with both iodine (I_2) as σ-acceptor and tetracyanoethylene (TCNE) as π-acceptor. The complexes were stable at least for two days after its formations. The orange and yellow CTC species have an absorption maxima at 363 and 420nm for I_2 and TCNE, respectively, with a molar absorptivity between 2985 to 3497 and 6877 to 9872 l mol^{-1} cm^{-1}. The stoichiometries of the CTCs were defined by Job's, molar ratio and striaght line methods and were found to form 1:1 stable stoichiometric complexes. Also, the formation constants and thermodynamic parameters(ΔH^o, ΔS^o, and ΔG^o) of the resulting CTCs were determined. The optimum reaction conditions and other analytical parameters were also investigated. The developed methods were found to be linear over concentration ranges of 35-135 and 15-95μg ml^{-1} with limit of detection 0.12 and 0.18μg ml^{-1} for iodine and TCNE methods, respectively. The methods were shown to be applicable to the determination of CLM in human plasma.

KEYWORDS

Charge transfer complexes;
Clarithromycin;
Iodine;
TCNE;
UV-vis;
Drug analysis.

INTRODUCTION

Clarithromycin (CLM), 6-o-methylerythromycin, is a semi-synthetic macrolide antibiotic with good antimicrobial activity against a wide range of gram-positive and gram-negative organisms(Figure 1). It is widely used for the treatment of Mycoplasmas, Haemophilus influenzae, Chlamydia species and Rickettsia[1,2]. Various analytical methods have been developed to determine clarithromycin in formulations and biological samples, such as spectrophotometric[3,4], chromatographic[5-7], and electrochemical[8,9] methods. The chromatographic methods reported recently have lower detection limit, but suffer from the long analysis time, expensive instrumentation and complex sample preparation which is inappropriate in clinical studies with large numbers of samples. Methods of electrochemical and amperometric detection also suffer from time-consuming problems in both sample preparation steps and the chromatography.

The study of charge-transfer (CT) interactions between various electron donors and σ- and π-acceptors

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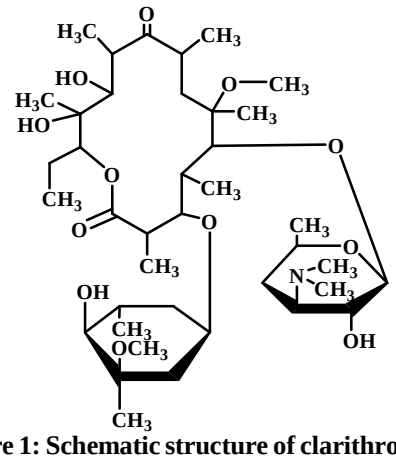

Figure 1: Schematic structure of clarithromycin

had attracted considerable interest and growing importance. This is due to the important role of charge-transfer complexes(CTCs) played in biological systems[10-12] and for quantitative estimations of drugs[13-16]. The CTC as an analytical method has been the focus of interest of many recent studies for its high sensitivity, good analytical selectivity, easy and less time consuming comparing with the above methods. Also, CTCs of a drug molecule usually absorb in the visible range and thus lead to easy detection and estimation of the drug in complex matrixes.

Iodine (I_2) and tetracyanoethylene (TCNE) are strong σ- and π- acceptors drugs, respectively. They well known for its electron-accepting properties which may be deduced from molecular orbital consideration and have been used as models acceptors to investigate the electron-donating properties of organic molecules[17,18]. In human biology, iodine is required for the biosynthesis of the thyroid hormones, triiodothyronine and thyroxin, which regulate metabolic rate. During the past few decades the CT complexation of iodine and TCNE with a wide variety of drugs molecule have been the subject of extensive research[19-23].

Because of our interest in the trend of CTCs and studied their spectral properties[23-25], we investigated the interaction of iodine and TCNE with CLM in dichloromethane (CH_2Cl_2) solutions at different temperatures. The study extended to include the determination of CLM in pharmaceutical preparations and human plasma through CT complexation.

EXPERIMENTAL

Chemicals, reagents and instruments

CLM and white crystalline TCNE (from Sigma-Aldrich, Taufkirchen, Germany) were of the highest purity available and used without any further purification. Resublimed iodine of analytical grade was supplied from Merck (Darmstadt, Germany) and was used as received. All solvents and reagents were of analytical grade. Claritt® tablets (250mg CLM) were prepared by Tabuk Pharmaceutical Manufacturing Co. (Tabuk, Saudi Arabia).

A Shimadzu 1601 PC spectrophotometer with quartz cells of 1cm optical path length was used for recording absorption spectra. All UV-vis spectra were recorded within the wavelength range 200-700nm using the same solvent in the examined solution as a blank. Samples were measured five times at the same temperature. For thermodynamic studies, the apparatus was equipped with a temperature controlled cell holder. Both sample and blank compartment were kept at constant temperature by a Shimadzu TCC-240A thermostat which allowed the temperature to be maintained constant to $\pm 0.1^0$C.

Standard solutions

CLM: A stock solution of CLM at a concentration of 1×10^{-2}M was prepared in volumetric flask by dissolving CLM powder accurately weighed in CH_2Cl_2 and diluting up to 10 ml with the same solvent.

Iodine: Fresh solution of iodine 1×10^{-2}M was prepared by dissolving precisely weighted in the appreciate volumetric flask of CH_2Cl_2.

TCNE: A standard solution of TCNE 1×10^{-2}M was prepared by dissolving 12.8 mg of pure TCNE in a 10ml volumetric flask using CH_2Cl_2.

Sample preparation

Twenty Claritt® tablets were weighed and powdered. Quantities equivalent to 20mg CLM were dissolved in 80ml of CH_2Cl_2 with stirring for 30min. Solutions were filtered in a 100ml volumetric flask and filled to the mark. Working sample solutions were prepared by dilutions in the appropriate way.

Human plasma samples

Human plasma samples collected in EDTA sample tubes (from healthy drug-free volunteers) were spiked

with CLM, vortexed and centrifuged at 1800rpm for 10min to separate the plasma component. A 250µl of plasma samples were transferred to a clean sample tube and 20µl of 0.25 M NaOH was then added. The solutions were vortexed briefly then 1.5ml of ethyl ether were added and vortexed for 5min and centrifuged for another 5min at 4000rpm. The organic layers were transferred into other clean tubes and dried under a stream of N_2 at room temperature. The residues were reconstituted with 250µl CH_2Cl_2 and the CT reactions were carried out.

RESULTS AND DISCUSSIONS

Absorption spectra

The electronic absorption spectra of mixtures of iodine in dichloromethane with different concentration of CLM are shown in figure 2. While none of the reactants show any considerable absorbance in the 300-450nm range, addition of the CLM to iodine results in two absorption bands in this region, presumably due to the formation of CLM-iodine complex. The spectra of the mixture (Figure 2) show bands characteristic of free iodine and complexed iodine. The latter has two absorption bands, the hypsochromically shifted iodine band in the region of 295nm and a CT band at 360nm. The limiting value of this shift is at about 295-300nm, which is the characteristic absorption of the I_3^- ions in solution[26]. Therefore, it can be concluded that the polarization of the iodine molecule in the complex increases to the extent where a clear-cut separation of the band occurs with the resulting formation of triiodide ion. As can be seen from the figure 2, the intensity of 295 and 360nm bands increased markedly with the concentration of CLM. The observed of the CT band and the subsequent formation of the I_3^- ion in solution are most probably due to a transformation of the initially formed outer complex into an inner electron donor accepter (EDA) complex followed by a fast reaction of the resulting inner complex with iodine to form a triiodide ion [27,28]. By analogy with other n-σ* complexes[29,30] we conclude that the following reaction occur between the CLM and iodine in solution as shown in SCHEME 1.

The absorption spectra of solutions containing CLM and TCNE together exhibit new absorption at longer

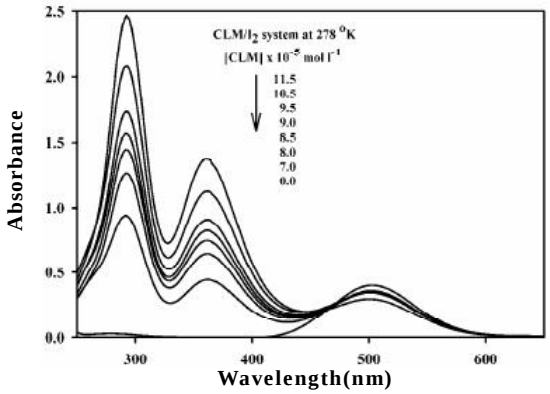

Figure 2: Electronic absorption spectra of CTC for CLM with 5×10^{-4}mol l^{-1} iodine in CH_2Cl_2

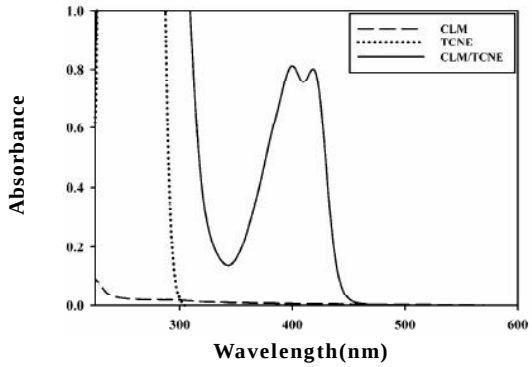

Figure 3: UV-vis spectra of 5×10^{-4}mol l^{-1} of TCNE, 1×10^{-4} mol l^{-1} of CLM tablet, and CTC of CLM/TCNE in CH_2Cl_2 at 5^0C

$$D + I_2 \rightleftharpoons DI_2 \quad \text{Fast (I)}$$
$$\text{I (outer complex)}$$
$$DI_2 \rightleftharpoons (D-I)^+ I^- \quad \text{Slow (II)}$$
$$\text{II (inner complex)}$$
$$D(-I)^+I^- + I_2 \rightleftharpoons (D-I)^+ + I_3^- \quad \text{Fast (III)}$$
$$\text{III} \qquad \text{IV}$$

SCHEME 1: The mechanistic pathway for CTC of CLM/I_2 system

wavelength than either the drug (λ_{max}=227nm) or the acceptor (λ_{max}=275nm) alone. The new and broad absorption indicates that the formation of electron donor acceptor (EDA) complex. The CLM is relatively electron rich and TCNE is relatively electron poor compound. When a solution containing both an electron rich and electron poor compound, they tend to associate with one another in a loose interaction known as EDA complex. The CTC of CLM with TCNE in dichloro methane gave yellow color at 420 and 401 nm as shown

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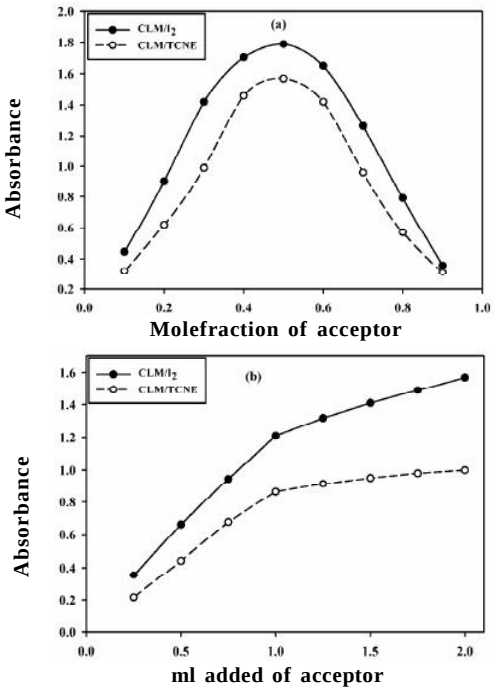

Figure 4: (a) Continuous variation plot for CLM/I_2 and CLM/TCNE systems at 363 and 420nm, respectively. (b) Photometric titration curve for the CLM/I_2 and CLM/TCNE reactions in CH_2Cl_2 at 363 and 420 nm, respectively

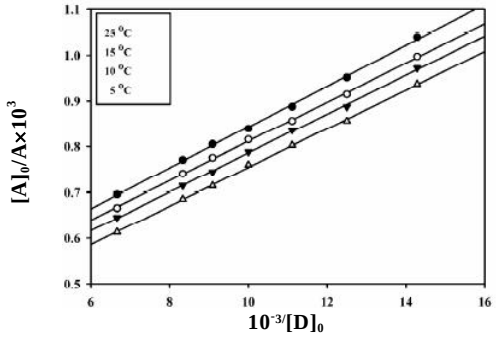

Figure 5 : Benesi-Hildebrand plots for CTC of CLM/I_2 system at different temperatures

in figure 3. The new, low energy absorptions observed in solutions containing both a donor and an acceptor have been described by Mulliken[31] as CT transitions involving the excitation of an electron on the donor to an empty orbital on the acceptor gaving n-π* transition.

The stoichiometry of the complexes were carefully determined using different methods. Molar ratio and Job's method of continuous variation[32,33] indicated a 1:1 complexation ratio shown in figure 4. Application of qualitative straight line method[34] gave also straight

TABLE 1 : Maximum absorption wavelength λ_{CT}, equilibrium constant K_{CT}, and molar bsorption coefficient ε_{CT} values for CLM complexes with I_2 and TCNE in CH_2Cl_2 at different temperatures

Acceptor T/ ^{0}K	λ_{CT} (nm)	K_{CT} (l mol^{-1}) ×10^3	ε_{CT} (l mol^{-1} cm^{-1})	K_{CT} (l mol^{-1}) × 10^3	ε_{CT} (l mol^{-1} cm^{-1})	r
		Bensi-Hildebrand equation		Scott equation		
I_2	363					
298		8.67±0.87	2985.89	8.79±0.79	2973.96	0.9989
288		9.89±0.65	3072.52	9.84±0.59	3095.42	0.9993
283		10.51±0.94	3244.77	10.43±1.05	3234.87	0.9992
278		11.10±1.12	3497.30	11.21±0.98	3513.92	0.9992
TCNE	420					
298		2.86±1.21	6877.25	2.95±0.97	6885.94	0.9987
288		3.43±1.08	7411.44	3.39±1.31	7414.27	0.9986
283		3.91±0.77	8359.24	3.88±0.68	8278.25	0.9993
278		4.26± 0.56	9872.98	4.25±0.71	9986.23	0.9990

line with slope ranging from 0.97 to 0.99. In the case of CLM/TCNE system, the relative intensities of the two bands do not vary with temperature in the range of 25-5^{0}C. Thus, the bands are not associated with the presence of two 1:1 isomeric structures[23]. Moreover, using multiwavelength linear regression of concentration-dependent absorption data[35], no evidence is found of significant concentrations of 2:1(D_2A) complexes. Therefore, the band multiplicity may arised from a wide range energetically accessible conformations of the CLM/TCNE complex involving the overlap of the lowest unoccupied molecular orbital (LUMO) of the acceptor with the either the highest occupied molecular orbital (HOMO)or HOMO-1 of CLM. The 1:1 complexation ratio mention that the drug have only one strong basic or electron donating center.

A linear regression was also obtained according to the Benesi-Hildebrand and Scott relations[36,37] for the estimation of the formation constant (K_{CT}) and molar extinction coefficient (ε_{CT}) (Figure 5). The K_{CT} and ε_{CT} values for the formed CTCs of the drug with I_2 and TCNE in dichloromethane at 5,10,15, and 25^{0}C were listed in TABLE 1. The relations depend on the experimental conditions that one of the two component species should be present in large excess. Either donor or acceptor concentration should be larger than the other. The complexes show high values of both the K_{CT} and ε_{CT}. The high values of K_{CT} in generally, reflects high stabilities of the formed CTCs as a result of the expected high donation of such CLM which contain a

TABLE 2 : Thermodynamic parameters of CTCs of CLM with I_2 and TCNE in CH_2Cl_2

CTC	$-\Delta H^o$/KJ mol^{-1} $\times 10^3$	$-\Delta S^o$/J mol^{-1} K^{-1}	$-\Delta G^o$ (298 K)/KJ mol^{-1}	r
CLM/ I_2	8.53±0.29	46.78±1.02	22.46±0.57	0.996
CLM/ TCNE	13.97±0.32	19.23±0.86	19.71±0.36	0.995

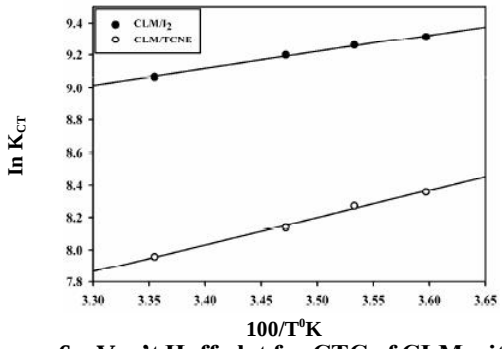

Figure 6 : Van't Hoff plot for CTC of CLM with I_2 and TCNE at 5, 10, 15, and 25°C in CH_2Cl_2

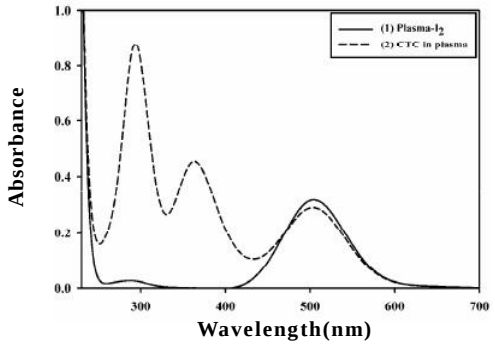

Figure 7 : Electronic absorption spectra for CTC of CLM/ I_2 in humane plasma. (1) Extracted plasma spiked with 5×10^{-4}mol l^{-1} I_2; (2) CTC of extracted plasma spiked with CLM/I_2 system; [CLM]=5×10^{-5}mol l^{-1}

high number of oxygen atom and methylene groups also the presence one nitrogen atom. The observed decrease in formation constant values with rise in temperature indicates the exothermic nature of the interaction between the studied acceptors and CLM.

Thermodynamic parameters(ΔH^o and ΔS^o) of the CTCs have been determined from the temperature dependence of stability constants by Van't Hoff equation[38] as shown in figure 6. The parameters thus obtained were presented in TABLE 2 and these values showed that the complexation is thermodynamically favoured. The enthalpy change of the complexation also reveals that the CTC formation between the CLM and the used acceptors is of exothermic in nature. The ΔG^o values of the complexes were calculated from Gibbs free energy of formation according to the equation 1:

$$\Delta G^o = -RT \ln KCT \quad (1)$$

The obtained results reveal that the CTC formation process is exothermic and spontaneous.

Optimization of experimental conditions

The effect of reaction variables was carefully studied. Three variables were found to affect the intensity of the resulting colors; reagent concentration, solvents, and development time and temperature.

Effect of reagents concentrations

The effect of CT reagent concentration was examined over a range of 1×10^{-4}-1×10^{-3}mol l^{-1}. A constant and maximal absorbance was obtained at reagent concentration 5×10^{-4}mol l^{-1} for I_2 and TCNE.

Effect of solvents.

The solvents studied were CH_2Cl_2, $CHCl_3$, and CCl_4 for iodometric determination method. Methanol, *iso*propanol, acetone, and acetonitrile were additionally tested for the second determination method of CLM by TCNE. The studies show that dichloromethane is the best solvent for the highest absorption intensity.

Effect of development time and temperature

Sample solutions containing CLM and the blank were treated identically with the reagent at temperature ranging from 5 to 25^{0}C. The results obtained indicated that CLM reagent complex was formed at 5^{0}C and the maximum absorbance was attained immediately after mixing the reagents. Longer heating times decreased color intensity which is probably due to partial decomposition of the colored product. The absorbance of the complex remained constant for two days, after which it began to slowly fade.

Method calibration

A long series of standard solutions of CLM with different concentrations were examined under the optimized conditions. The methods were found to be linear in the ranges of 35-135 and 15-95µg/ml^{-1} with weighed regression described in equations 2 and 3 for iodine and TCNE methods, respectively.

$$A = 12246C - 0.4271, r = 0.9994 \quad (2)$$

$$A = 9373.3C - 0.0638, r = 0.9992 \quad (3)$$

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TABLE 3 : Results obtained by the adopted CTC methods for the analysis of CLM in tablets and plasma

Samples	Concentration	Mean recovery ± RSD(%)[a]	
	μg ml^{-1}	I_2 method	TCNE method
Claritt® tablet	5.00	98.2 ± 1.04	98.4 ± 1.2
	15.00	99.4 ± 1.14	98.5 ± 0.90
	25.00	100.4 ± 0.97	100.2 ± 1.25
Spiked plasma	5.00	98.3±0.91	98.7 ± 1.09
	15.00	98.9±1.32	99.2 ± 0.83
	25.00	99.1±0.22	100.1 ± 0.26

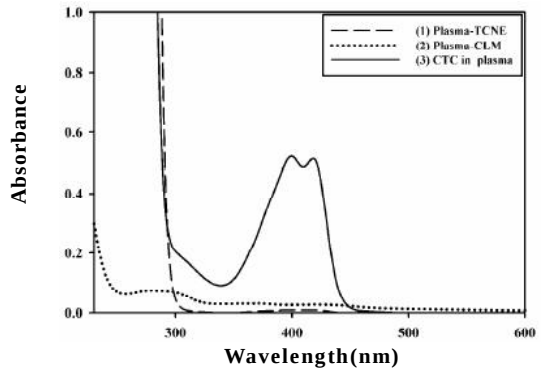

Figure 8: UV-vis spectra for CTC of CLM/TCNE in humane plasma. (1) Extracted plasma spiked with 5×10^{-4}mol l^{-1} TCNE; (2) Extracted plasma spiked with 5×10^{-6}mol l^{-1} CLM; (3) CTC of extracted plasma spiked with CLM/TCNE system

where A is the peak area, *C* is the concentration in μg ml^{-1} and r is the correlation coefficient. The limit of detection (LOD) for CLM was found to be 0.12μg/ml^{-1} for iodine and 0.18μg/ml^{-1} for TCNE method. These levels are adequate for the determination of the tested drug in biological samples[5].

The placebo samples were examined and no peak was recorded indicating the methods are selective in the presence of excipients usually found in tablets formulation. In addition, the methods were applied to the synthetic pharmaceutical samples. The recoveries obtained were in the range of 98.3-101.4% indicating the reliability of the results obtained from the proposed methods. The methods were also applied to the tablets formulation (TABLE 3) and the typical spectra were depicted in figures 2 and 3. To examine the intra-day precision, five runs were conducted in three consequent days for different concentrations of CLM in tablets formulation. The RSD did not exceed 1.30%.

Analysis of spiked human plasma

The high sensitivity attained by the proposed methods allowed the determination of the studied drug in spiked human plasma samples figures 7 and 8. As can be shown, no interferences were recorded between the plasma sample matrix and the drug under study. Also, the accuracy was assessed by investigating the recovery of CLM at three concentration levels (five replicates of each concentration) (TABLE 3). The results showed average percentage recoveries ranged from 98.3-100.1 with RSD <1.32 for both methods, indicating good accuracy and precision.

CONCLUSIONS

New spectrophotometric methods for the assay of CLM in pharmaceuticals and biological samples were adopted and successfully optimized. In these methods, CLM reacts with iodine and/or TCNE in dichloromethane at different temperatures to form the new colored and stable CTCs. The proposed methods enjoy the advantages of high sensitivity, good analytical selectivity, easy and less time consuming comparing with other published methods. Therefore, the presented methods are suitable to be adopted for pharmacokinetic studies and routine estimation of CLM in human plasma.

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