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Spectroscopic study of the reaction mechanism of buspirone interaction with iodine and tetracyanoethylene reagents and its applications

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The reactions between the drug buspirone (busp) in its base form and iodine amphoteric reagent (n-donor and/or σ-acceptor) and with tetracyanoethylene as a π-acceptor reagent (TCNE) have been studied spectrophotometrically at different reactant concentrations, time intervals, temperatures, and with different solvents and wavelengths, with the aim of selecting the conditions that give the most suitable molar extinction coefficients. This study aims chiefly to throw light on the nature of these reactions and to select the most proper conditions for spectrophotometric application of these reagents to determine this biologically active drug used in treating different diseases.

The reaction mechanism involves the formation of busp-I_2 outer and inner sphere complexes. The separated busp-I_2 solid product obtained was investigated using elemental analyses, FT-IR, thermal analyses (TA) and electron ionization mass spectrometry (EI-MS) and was found to be biologically active. The reaction mechanism of busp-TCNE involves the formation of a charge transfer (CT) complex. The analytical parameters of the proposed spectrophotometric procedures were calculated. These procedures were applied in the analysis of busp in its formulations as a drug used to treat psychiatric illnesses. The values of the Sandell sensitivity, standard deviation (SD), relative standard deviation (RSD) and recovery percentage show the high sensitivity of these procedures. This study also presents a promising new busp-I_2 drug derivative that can be used more efficiently for the same purposes as its parent. It gives a clear idea about the possible metabolites and metabolic pathways of busp and its derivative that may occur *in vivo*.

Keywords: spectrophotometric study; buspirone; iodine and tetracyanoethylene reagents; inner- and outer- sphere complexes; CT-complexes; applications

Introduction

The drug Buspirone (busp) is usually a hydrochloride[1] with the general formula $C_{21}H_{31}N_5O_2$.HCl. It has a molecular weight of 421.96 and its IUPAC nomenclature is 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4,5]decane-7,9-dione hydrochloride.[4,5] Its structural formula is given in Figure 1.

Jacob *et al*.[2] made spectrophotometric estimations of busp-HCl in pharmaceutical formulations. Spectrophotometric methods were applied to the analysis of busp tablets; recovery was 98% and excipients did not interfere.

Capillary-zone electrophoresis techniques have been used[3] for the analysis of busp and other non-benzodiazepine agents. The detection limits of each compound and relative standard deviation (RSD) were found to be 0.36 ng and 1.0–1.3% respectively.

Voltametric determination of busp was carried out by Chen *et al*.[4] The calibration graph was linear for 30 nM to 5 μM busp with a detection limit of 5 nM; the RSD (n = 15) at 0.5 μM was 1.5%. Recoveries of busp (0.08–4 μg ml^{-1}) were 90% to 112%.

Differential pulse polarography techniques[5] were used to analyse busp in drug formulations. The calibration graph covered the range 50 to 500 μM and the detection limit was 84.4 μg $L.^{-1}$ Average recovery of 10 mg busp was 100.5 ± 1.5% (n = 10).

Several papers[6–11] have been published concerning the biological activity of busp and its analogues and/or its derivatives. The intrinsic activity and comparative molecular dynamics of busp analogues at the 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors were tested.[6] Clinical studies indicated that 5-HT_{1A} partial agonists have anxiolytic properties. It was found that busp raises blood pressure through activation of the sympathetic nervous system and by direct activation of A_1-drenergic receptors after severe haemorrhage.[7] Effects of chronic treatment with busp on mice under social stress have been studied.[8] The comparison between the *in vivo* potency and intrinsic activity of busp and its metabolites in rats by pharmacokinetic-dynamic modelling had been performed.[9,10] The effects of single, oral doses of diazepam, busp and placebo on auditory event-related potentials have been assessed in healthy volunteers.[11] Some chromatographic techniques have been used effectively in the analysis of busp and its metabolites. Stanly[12] used a combined HPLC-mass spectrometry technique to study the equine metabolism of busp. Kems *et al*.[13] studied busp metabolite structure profile using a LC-MS spectrophotometric protocol. Visy *et al*.[14] performed a comparative pharmacokinetic pilot study of two busp preparations

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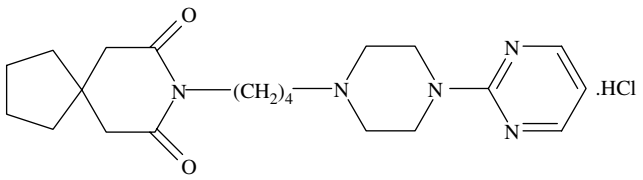

$C_{21}H_{31}N_5O_2HCl$ M.Wt. = 429.96 m.p. = 185-186°C

Figure 1. Structural formula for busp.

by gas chromatography mass spectrometry in dogs. Plasma (1.5 ml) was incubated for 30 min to dissolve protein precipitate and was mixed with 0.5 ml physiological NaCl solution. The mixture was subjected to solid-phase extraction (SPE) using a Baker bond column loaded with 100 mg octadecylsilica gel and eluted with acetonitrile/1% triethylamine.

Lee *et al*. recently applied LC-MS techniques to identify the structure of drug metabolites and related compounds.[15] They illustrated the usefulness of the rapid structure identification of drug metabolites for accelerating drug development by identfying and analysing busp metabolites.[AQ5] Qiao *et al*.[16] made a successful trial to determine busp in human plasma using GC-MS. Heparinized whole blood (5 ml) was centrifuged and 2 ml of the plasma was shaken with a 0.2 ml 0.5M-$Na_2B_4O_7$ buffer of pH 8.5 and 5ml CH_2Cl_2 for 1 min and then centrifuged for 10 min.

Goldthwaite *et al*.[17] successfully used liquid chromatography-chemical reaction interface mass spectrometry as an alternative to radioisotopes for quantitative drug metabolism studies. A 100 µL portion of the supernatant from the incubation of 15N/14C busp with liver slices was injected onto a Zorbax 5 µm Rx-C8 column (15 cm ×4.6 mm i.d.) with gradient elution (0.5 ml/min) with 0.05 M ammonium acetate in methanol and 0.05 M ammonium acetate.

Due to the importance of busp as a drug used in the treatment of several diseases, the aim of the present study was to examine the mechanism of its reactions as an electron n-donor with iodine (amphoteric) and tetracyanoethylene (TCNE) as sensitive acceptor reagents. There is little literature about this subject. The study also aimed to use these reactions in the spectrophotometric microdetermination of this drug in some of its pharmaceutical preparations.

Experimental

Instruments

The spectrophotometric measurements were recorded using a Shimadzu 1700 pharma double-beam recording spectrophotometer with 1 cm quartz cells. The IR spectra were recorded in the Micro-analytical Centre of Cairo University as KBr disks using a Perkin Elmer FT-IR Spectrophotometer M 1430 with a wavelength range of 400–4000 cm^{-1}.

The ^{1}HNMR spectra were measured using a Gemini 2000 Switzerland instrument and the samples were dissolved in dimethyl sulfoxide (DMSO) and measured in Central Lab of the Chemistry Department, Faculty of Science, Cairo University. EI-MS spectra were obtained using a Shimadzu GC-MS- Qp 1000 EX quadruple mass spectrometer (70 eV) within a scan range of m/z = 50–550.

Thermal analyses (TA) were made using a conventional thermal analyser (Shimadzu DSC system and 30 series TG-50). The mass losses of a 5 mg sample and heat response of the change of the sample were measured from room temperature up to 600 °C. The heating rate, in an inert argon atmosphere, was 10 °C min^{-1}. These instruments were calibrated using indium metal as a thermal stable material. The reproducibility of the instrument reading was determined by repeating each experiment more than twice.

Procedures

To different aliquots containing (0.1–1.5 ml) busp-base (0.4 mg ml^{-1}), 1 ml of 4×10^{-3} M I_2 solution was added. On the other hand, 0.3 ml of 5×10^{-3} M TCNE solution was added to 0.04–0.56 ml of working busp-base solution (2 mg ml^{-1}). On studying the effect of solvent on the spectra of reaction mixtures, the volume of each mixture was completed in 10 ml measuring flask by different solvents. The stoichiometry of these reactions was also studied using the molar ratio (MRM)[18,19] and Jobs continuous variation (CVM) methods.[20]

The absorption spectra of the resulting complexes in all cases were scanned in the 250–800 nm wavelength range against blank solutions, prepared in the same manner, without drug. The best wavelength (λ_{max}), which gave the highest ε value, was selected.

Validity of Beer's law

The validity of Beer's law of drugs reactions with I_2 and TCNE reagents was checked in which 1 ml of 4×10^{-3} M I_2 or 0.3 ml of 5×10^{-3} M TCNE was added to regularly varied concentration of busp-base (2–28 µg ml^{-1}), or (6–36 µg ml^{-1}) respectively in 10 ml suitable solvent in a measuring flask. The absorbance values were plotted against the concentration ranges of the drug to give straight lines passing through the origin. These straight lines and/or calibration lines can be used for the microdetermination of drugs in their pharmaceutical products.

The absorbance values of the coloured complexes formed were also measured under different conditions, especially λ_{max}, time, and temperature, against a blank of each complex mixture prepared in a similar way without drug.

Daily measurements

In daily measurements, the applicability of the proposed spectrophotometric procedures and the reproducibly of the results in case of busp-base-I_2 or busp-base–TCNE procedure was tested, and 7 replicates at six or seven different concentrations of busp-base of 4–24 or 10–32 µg ml^{-1} were carried out in the two procedures respectively. The absorbance values of the samples were measured under the optimum conditions daily for four days and the results obtained were recorded to make statistical calculations of SD, RSD, and percentage recovery of the results.

Spectrophotometric microdetermination of drugs in their pharmaceutical preparations

Twenty tablets containing drug formulations were accurately weighed and the average weight of the tablets was calculated. The tablets were crushed to a fine powder. A portion of the powder equivalent to 100 mg of drug was dissolved in 100 ml of suitable solvent. The resulting solutions were filtered through a filter paper of Whatman no. 1 and washed with a suitable solvent. The filtrate was collected and diluted to the final volume of 100 ml with the same solvent. The microdetermination of each drug

was carried out using the same procedure with the previously prepared calibration curves.

Busp-base of 4–20 or 6–36 μg ml^{-1} was extracted from busp tablets using suitable solvent and transferred into 10 ml measuring flask. To the extracts obtained, 1 ml of 4×10^{-3} M I_2 or 0.3 ml of 5×10^{-3} M TCNE was added. The volume of each mixture was completed to the mark with the suitable solvent and the solution was left to stand for 10 min at room temperature. The absorbance of the mixture was measured at 294 nm in the I_2-procedure or at 415 nm in the TCNE procedure. The unknown drug concentration was calculated from the corresponding previously prepared calibration curve for each procedure. The tolerance limit of excipients (e.g. glucose, fructose) had been tested using ten fold concentrations of these fillers.

Preparation of busp-I_2 solid product

Drops of iodine solution were added to the drug solution in 10 ml of ethanol (96%) until a greenish-yellow solid product appeared as a precipitate. The precipitate was left for 10 min until it had completely settled. Iodine solution was added continuously until precipitation was complete and filtered through small circular Whatman no. 1 filter paper in a Hirsch funnel. The crude product was recrystallized from dichloromethane and petroleum ether and dried in a vacuum desiccators. The yield of the final product was 86%.

Results and Discussion

Absorption spectra, effect of solvent, effect of time, effect of temperature, sequence of addition of reactants and the effect of interfering materials

Different solvents were tested as reaction media for interaction between busp- basic drug and I_2 as a σ-acceptor in the tri- iodide form and the π- acceptor TCNA (Table 1). 1,2-Dichloroethane and acetonitrile were selected as suitable solvents for busp-I_2 and busp-TCNA products respectively which refer to their polarities. The absorption spectrum of the reaction product between busp-basic drug and triiodide ion is given in Figure 2. It shows that both reactants have maximum wavelengths (520 nm for I_2 and 250 nm for basic drug) far from that of the complex formed (λ_{max} = 294 and 360 nm). Therefore λ_{max} = 294 is selected for studying all reaction conditions between the basic drug and I_2, because it has the highest ε value of 3.8×10^4 L.mol^{-1}.cm^{-1}.

The absorption spectra of busp-base with TCNE reagent as an acceptor to form CT- complex are shown in Figure 3 and Table 1. These spectra show that busp-base-TCNE CT-complex has two main peaks at 396 nm ($\varepsilon = 1.137 \times 10^4$ L. $mole^{-1}$ cm^{-1}) and at 415 nm ($\varepsilon = 1.147 \times 10^4$ L. $mole^{-1}$. cm^{-1}) in acetonitrile solvent. The drug and the reagent have no absorption at these wavelength values. It is therefore appropriate to select either 415 nm or 396 nm as the maximum wavelength to do further spectrophotometric studies for the CT complex of busp-base-TCNE in acetonitrile that was formed. The sequence of addition of reactants shows the results:

1. Drug + reagent + solvent (acetonitrile) $\varepsilon = 4.10 \times 10^3$ L.mol^{-1}.cm^{-1} at 415 nm
2. Drug + solvent + reagent $\varepsilon = 3.100 \times 10^3$ L.mol^{-1}.cm^{-1}.
3. Reagent + drug + solvent $\varepsilon = 3.990 \times 10^3$ L.mol^{-1}.cm^{-1}.
4. Reagent + solvent + drug $\varepsilon = 1.283 \times 10^3$ L.mol^{-1}.cm^{-1}.

Table 1. Peak position ($\lambda_{max.}$) and molar absorptivity of busp-I_2 and busp-TCNE complexes in polar and non-polar solvents

Solvent	$\lambda_{max.}$ (nm)		ε (L.mol^{-1}. cm^{-1})	
	I_2	TCNE	I_2	TCNE
Dichloroethane	418	360	20.85×10^3	45.50×10^2
		399		45.83×10^2
	294	300	38.412×10^3	56.83×10^2
Dichloromethane	360	418	10.737×10^3	32.58×10^2
		399		32.83×10^2
	293	300	68.91×10^2	22.625×10^3
Acetonitrile	360	415	27.500×10^3	11.37×10^3
	355	396	11.47×10^3	26.250×10^3
Chloroform	291	290	11.32×10^2	97.62×10^2
Methanol	358	—a	15.57×10^3	
		—b		
		—b	29.62×10^3	
		288		

[a] Interference from reagent
No reaction occurred [b]

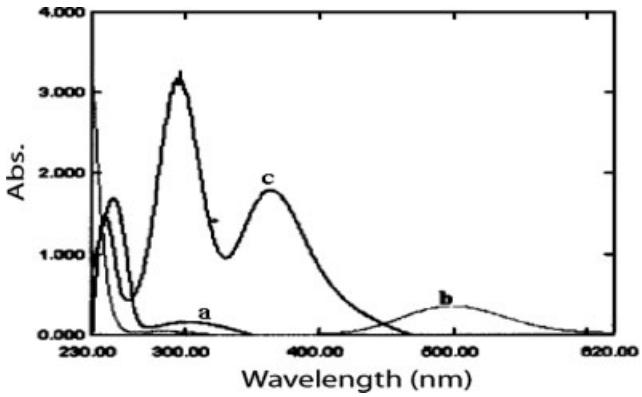

Figure 2. Absorption spectra of: (a) 8×10^{-5} M busp; (b) 4×10^{-4} M iodine; (c) 8×10^{-5} M busp.-iodine complex in 1,2-dichloroethane.

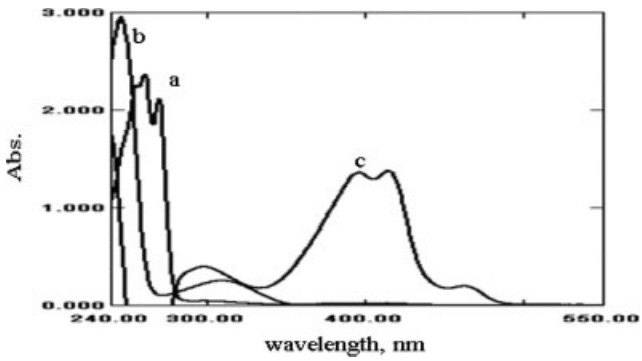

Figure 3. The spectra of: (a) busp-TCNE CT-complex; (b) busp drug; (d) TCNE reagent.

5. Solvent + drug + reagent $\varepsilon = 1.200 \times 10^3$ L.mol^{-1}.cm^{-1}.
6. Solvent + reagent + drug $\varepsilon = 1.525 \times 10^3$ L.mol^{-1}.cm^{-1}.

From these values it is clear that the best sequences for the addition of reactants are the first and then the third.

The effect of time (T in minutes) on the formation of the busp-I_2 and busp-TCNE products was studied carefully at temperatures of 25 °C to 30 °C and at $\lambda_{max} = 294$ nm and at 415 nm respectively for the reaction products. These results show that the maximum absorbance attained at 25 °C ($\varepsilon = 0.25 \times 10^4$ L. mole $^{-1}$.cm^{-1}) after 20 mins, and the colour of the reaction product busp-I_2 (Figure 2c) (yellowish-green) remained unchanged for at least one day. Time also has a pronounced effect at the beginning of the reaction between busp-base and TCNE reagent up to 10–15 mins at which the absorbance of the formed CT- complex attains the highest ε value of approximately 1.455×10^4 L.mole^{-1} cm^{-1}.

The effect of temperature on the spectra of reaction between busp- I_2 in 1, 2-dichloroethane (at 10 min) and busp-TCNE in acetonitrile (at 20 min) was studied at temperature range 10 to 70 °C. The data obtained show that the absorbance increased with the increase of temperature and the temperature attained maximum effect at 20–30 °C. In order to apply the proposed procedures to determine busp in pharmaceutical preparations using I_2 as σ- acceptor and TCNE as π- acceptor it is important to study the effect of fillers present in pharmaceutical forms. These fillers are glucose, lactose and starch. The tolerance limit was taken as the amount of interfering material that caused an absorbance error not exceeding ±3% and an error in percentage recovery of no more than ±2%. The results obtained show no interference effect on using 10-fold amount of fillers.

Stoichiometry of the reaction of busp drug with I_2 and TCNE reagents and the reactions mechanisms

The stoichiometric ratio of busp to I_2 or TCNA reagent [R] is obtained by applying both the molar ratio method (MRM)[18,19] and Jobs continuous variation method (CVM).[20] The data obtained refer to the stoichiometric ratio [R]/[Drug] = 1:1.

Spectrophotometric determination of busp-basic drug using iodine and TCNE reagents

Validity of Beer's law

The variation of the absorbance values of the reaction with the change in the pure drug concentration in the range 2–40 μg ml^{-1} shows that Beer's law is valid over the concentration range 4–24 μg ml^{-1} of busp drug with the iodine method and in the range 6–36 μg ml^{-1} with the TCNE method (Table 2). To select the more accurate linear concentration range; Ringbom sensitivity was applied. The absorbance percentage is plotted against – log [drug] to obtain the correct concentration limits, which were found to be 4–10 μg ml^{-1} and 6–14 μg ml^{-1} of busp using I_2 and TCNE reagents respectively. The mean recovery values obtained ranged from 98.31% to 100.12% (Table 2). These values indicate the successful use of the proposed spectrophotometric methods for the microdetermination of the pure drug form. The low values of the calculated SD (0.053 to 0.182) and the RSD (0.29% to 1.69%) for n = 7 indicate the high accuracy and precision of the proposed procedure. This is also supported by the calculated Sandell sensitivity values (S = 0.00258 to 0.02525 μg cm^{-2}), which indicated the high sensitivity of the procedures used in the spectrophotometric microdetermination of busp by I_2 and TCNE reagents.

Table 2. Analytical parameters for microdetermination of busp by I_2 and TCNE methods

	I_2	TCNE
λ_{max} (nm)	294	415
Beers law limit,μg.ml^{-1}	4–24	6–36
Ringbom optimum concentration range, μg.ml^{-1}	4–10	6–14
Molar absorptivity, l mol^{-1} cm	38.412×10^3	11.47×10^3
Sandell Sensitivity, μ g cm^{-2}	0.00258	0.0025 25
Regression equation:		
(a)- Slope(specific absorptivity)	0.1026	0.041
(b)- Intercept	0.0333	−0.0652
(c)- Correlation Coefficient	0.9991	0.9939
Standard deviation*	0.053–0.157	0.082–0.182
Recovery%	98.67–100.3	98.31–101.12
Calculated F-value (4.28)**	1.19–3.08	1.068–3.54
Calculated t-value (2.447)**	1.05–1.95	−2.13–+0.591

* Seven replicates
** Theoretical values at 95% confidence limit

Table 3 shows the results of the daily RSD for different concentrations of the drug obtained from experiments carried out over a period of four days. The reproducibility of the results was obtained using seven replication experiments with seven different concentrations of basic busp drug (4–24 or 12–28 μ g ml^{-1}). These results indicate that the proposed methods are highly reproducible and consequently I_2 and TCNE can be successfully applied as specific reagents for microdetermination of the pure basic busp drug.

Application of the proposed spectrophotometric procedures

The proposed spectrophotometric procedure was used to determine busp via its reaction with I_2 and TCNE reagents under optimum conditions in buspar tablets coming from the Saudi Arabian local markets. The results are shown in Table 4. These data show that the concentration of busp determined by the proposed procedures is very near to that obtained by applying the standard procedure.[21] In order to check the correlation between the suggested and standard official procedures,[21] it is required to conduct f-tests for all results (Table 4). The calculated SD = 0.05 to 0.157, RSD = 0.28 to 1.64%, and recovery percentage = 99.23 to 100.2% for 4–20 μg ml^{-1} busp indicated the reliability, accuracy and precision of the suggested procedure and its success and robustness in the analysis of the drug in its pharmaceutical forms.

Reaction mechanism between I_2 and busp drug and the identified reaction product

The absorption spectra of the busp-I_2 product in dichloroethane (Figure 2c) display two maximum wavelengths at 294 (ε = 3.84 X10^4) and 360 nm (ε = 2.085 X10^4), which may be attributed to the formation of two absorbing species[22] of inner and outer sphere complexes[23] leading to the formation of an ion pair as

Table 3. Between-day precision of the microdetermination of busp by I_2 and TCNE method at selected optimum conditions

I_2 method					TCNE method				
Recovery%	Found µg.ml^{-1}	Taken µg.ml^{-1}	R.S.D.(%)	S.D.*	R.S.D.(%)	S.D.*	Recovery%	Found µg.ml^{-1}	Taken µg.ml^{-1}
98.75	3.95	4.0	1.37	0.054	0.94	0.111	98.58	11.83	12
100.25	8.02	8.0	1.13	0.091	0.92	0.146	99.44	15.91	16
99.42	11.93	12	0.89	0.106	1.18	0.213	100.22	18.04	18
100.44	16.07	16	0.85	0.136	0.91	0.203	100.95	22.21	22
97.60	19.52	20	0.77	0.151	0.83	0.200	100.75	24.18	24
98.67	23.68	24	1.10	0.260	1.12	0.311	98.68	27.63	28

* Seven replicates

Table 4. Microdetermination of bus-base in pharmaceutical preparations (buspar) using the proposed (I_2, and TCNE) methods and official method[21]

I_2 method							TCNE method						
Taken µg.ml^{-1}	Found µg.ml^{-1} Proposed$_1$	official$_2$ methods	S.D$_1$.*	S.D$_2$*	F-test**	t-test**	Taken µg.ml^{-1}	Found µg.ml^{-1} Proposed$_1$	official$_2$ methods	S.D$_2$*.	S.D$_1$.*	t-test**	F-test**
6.0	5.92	5.96	0.053	0.066	1.57	10	10	10.06	10.03	0.106	0.082	+0.591	1.672
8.0	8.01	7.94	0.086	0.079	1.193	14	14	13.92	14.06	0.082	0.154	−2.13	3.54
10	10.03	9.97	0.067	0.082	1.49	16	16	16.18	16.01	0.178	0.151	+1.924	1.390
14	13.99	14.06	0.088	0.154	3.08	20	20	19.89	19.96	0.129	0.078	−1.224	2.721
18	18.05	18.16	0.157	0.095	2.73	24	24	23.98	24.17	0.182	0.174	−1.995	1.096
20	19.89	19.96	0.054	0.078	2.10	32	32	31.46	31.62	0.236	0.244	−1.247	1.068

* Seven replicates
** Theoretical values at 95% confidence limit, F = 4.28, t = 2.447

given by the following equations:

$$D + I_2 \rightarrow D - I_2 \text{ (outer sphere complex)}$$
$$D - I_2 \rightarrow [D - I]^+ I^- \text{ (Inner sphere complex)}$$
$$[D - I]^+ I^- + I_2 \rightarrow [D - I]^+ I_3^- \text{ (ion-pair)}$$

The formation of I_3^- ions is attributed to the transformation of an outer sphere complex to an inner sphere one, liberating iodide ions as a result of reactions between iodine σ-acceptor and busp drug (D) as a donor. The reaction product at high concentration of reactants was separated in a solid form using a procedure recommended by Zayed *et al.*[24]

Identification of the structure of the busp-I_2 product

This study aimed chiefly to identify the separated product of the reaction between busp-HCl drug and I_2 as tri-iodide form. The results are used to confirm the possible reaction mechanism of these compounds. This study involves elemental microanalyses (C, H, N, mol wt.), thermal analyses (TGA, DTG, and DSC), FT-IR, ^{1}HNMR, and Electron ionization mass spectrometry (EI-MS).

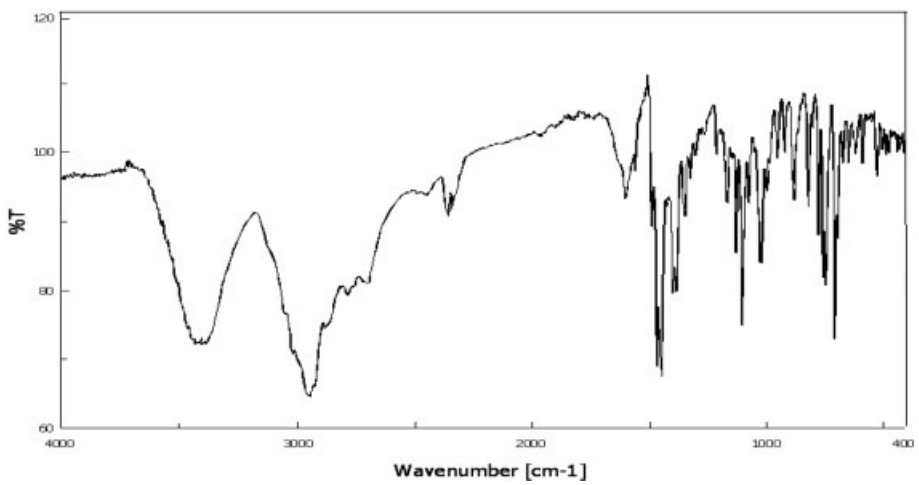

Figure 4. The FT- IR spectra of busp I_2 product.

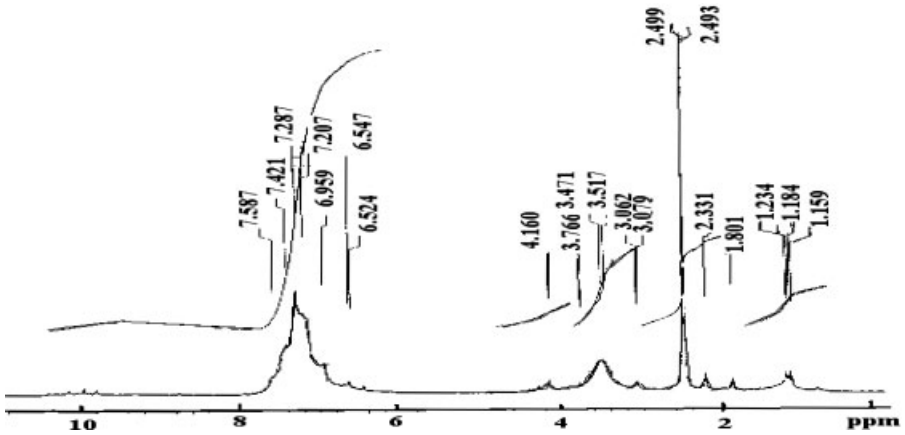

Figure 5. The ^{1}HNMR spectra of busp I_2 product.

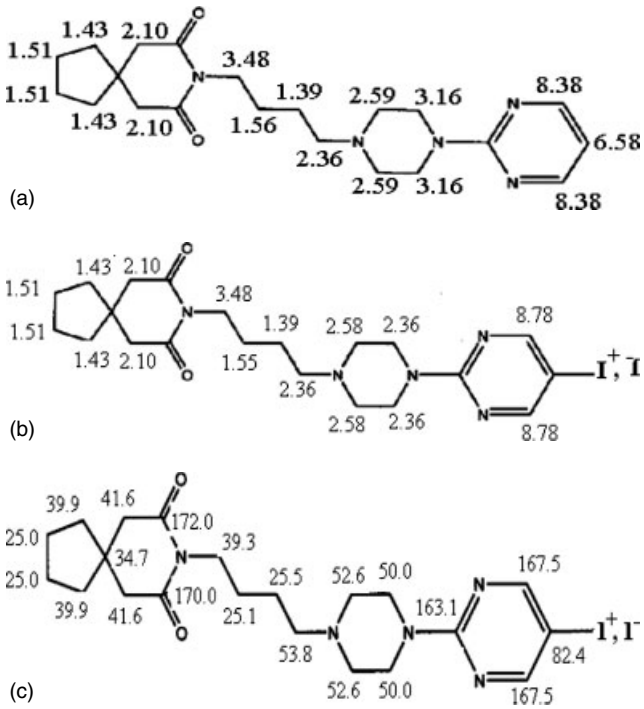

Figure 6. (a)The estimated ^{1}HNMR of busp parent; (b)the estimated ^{1}HNMR of busp-I_2 inner sphere complex; (c) the estimated ^{13}CNMR of busp-I_2 inner sphere complex.

Microanalysis of the reaction products of busp- I_2 and busp-HCl yielded C% = 33.29, 33.07%, H% = 3.89, 4.00%, N% = 8.22, 7.93% and I% = 33.0, 32.99% (calculated: C% = 32.88, H% = 3.88, N% = 9.06%, I% = 33.6%). The proposed general formulae due to this elemental analysis are: $C_{21}H_{30}I_2N_5O_2$ (outer complex) or $C_{21}H_{30}IN_5O_2^+ I^-$ (inner complex) with a molecular mass of 640.

FT-IR spectra of busp-I_2

The FT-IR spectrum of the busp-I_2 product (Figure 4) shows sharp bands in the wave number range 414–505 cm^{-1} which may be attributed to the different modes of C–I bond in the heterocyclic piprazine ring. The other bands at 522–793 cm^{-1} may be assigned to different modes of C–N and/or C═N bonds actually affected by the presence of iodine substituent in the piprazine ring. This explains the shifts of these bands from their positions in the parent busp spectrum. The bands at 1023–1583 cm^{-1} may be attributed to υC–H different modes. The bands at 1649.8–1714 cm^{-1} may be assigned to different modes of C–O and/or C═O bonds in the product skeleton. The bands at 2857–3984 cm^{-1} may be attributed to water and/or amino group different modes of vibrations.

^{1}HNMR spectra of the busp-I_2 product

The ^{1}HNMR of the spectra of the product is shown in Figure 5, and the theoretically predicted protons of both busp (Figure 6a) and busp–I_2 product (Figure 6b) are given. The protons of the aliphatic side chain appear (Figure 5a) at 1.55, 139, 2.36 and 3.48 ppm; protons at 2.59 and 3.16 ppm originate from the piprazine ring; at 6.58 and 8.38 ppm from the 2-pyrimidine ring and at 1.51, 1.43 and 2.10 ppm from protons of the azospirine decadione ring system. The ^{1}HNMR of the product (Figure 6b) is actually affected by the iodine atom in the pyrimidine ring in which the protons appear at 8.6 ppm instead of 8.38 ppm and disappear at 6.56 ppm due to the substitution of its proton by an iodine atom. The estimated ^{13}CNMR spectra (Figure 6c) confirm the proposed structural formula of the busp-I_2 product.

Thermal analyses (TA) of busp-I_2 product

The TGA and the differential thermogravimetry (DTGA) of busp-I_2 product are shown in Figure 7a and DSC is shown in Figure 7b. The TGA and DTG results for the product show four main stages of weight loss. The first one occurs at 120–300 °C of practical weight loss = 37.14% centered at 135.7 °C as given by DTG. It required an energy E = −47.98 J/g and appears as a very sharp endothermic peak at 129.9 °C in DSC.

This weight loss may be attributed to the loss of the aliphatic side chain $(CH_2)_4 + C_2H_2NO + I$ of mole mass = 56 + 56 + 127 = 239 (calculated weight loss = 37.30%) leaving two heterocyclic ring systems. The second weight loss of 19.05% occurs at 300 °C–420 °C centred at 311 °C as given by DTG. This weight loss appears as three endothermic peaks at 212.23 °C, 238.44 °C and 262 °C followed by a sharp exothermic peak centred at 313 °C. This means that this loss followed by chemical changes as result of loss of the second iodine atom of mole mass = 127 (calculated weight loss = 19.8%). The third practical weight loss of 29.7% occurs at 420 °C–540 °C centred at 527 °C, as given by DTG. This weight loss appears as a

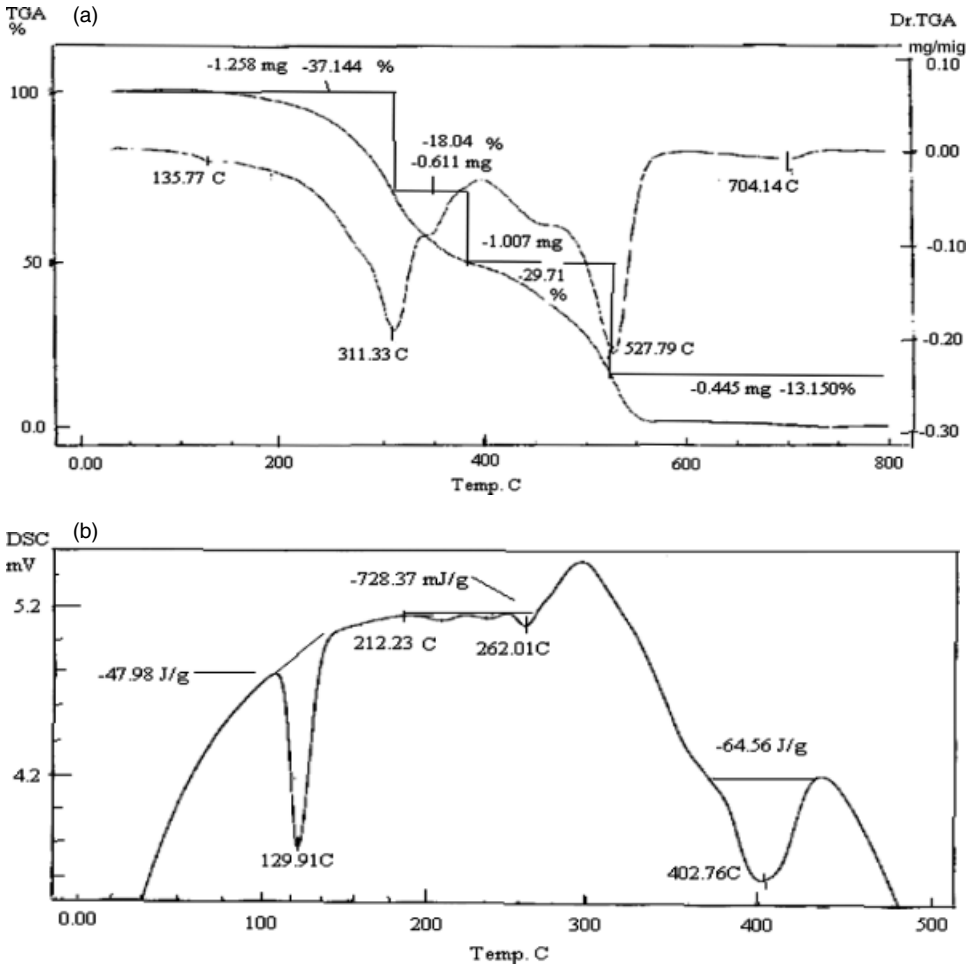

Figure 7. (a) The TGA and Dr.TGA of busp-I_2 product; (b)The DSC of the busp-I_2 product.

sharp endothermic peak in DSC centered at 402.76 °C and required energy = −64.56 J/g. This thermal weight loss may be attributed to the loss of $C_4H_8N_2$+ $C_7H_{10}O$ of mole mass = 194 (calculated weight loss = 30.3%) as a result of decomposition of both hetero ring systems. The fourth practical weight loss, 13.15%, occurs at 540 °C–800 °C and is centred at 704 °C as given by DTG. It appears as a broad exothermic peak due to the vigorous decomposition and complete loss of the remaining part of the pyrimidine ring of the formula $C_4H_2N_2$ of mole mass = 78. The Σ practical weight loss = 98.6% and the Σ calculated weight loss = 99.6%.

EI-MS of busp-I_2 product

The EI-MS spectrum of the solid product of busp.-I_2 is shown in Figure 8 and contains similar features compared to the parent busp drug except for selected ions, e.g., at m/z = 108 (RI = 38.8%), m/z = 127 (RI = 15.2%) and m/z = 128 (RI = 30.3%). It also shows a molecular ion at m/z = 386 (RI = 32.6%) that is assigned to the protonated molecule of busp $[M+H]^+$. The appearance of the peak at m/z = 386 refers to the molecular ion $C_{21}H_{31}N_5O_2^+$ (mole mass 385.46) of the main molecule without the iodine substituted atom that appeared in the skeleton of the product and without the HCl molecule that was originally present in the busp-HCl drug molecule. This is may be attributed to the lower stability of the product and the volatility of iodine atom. It is confirmed by the appearance of the peaks at m/z = 127 of iodine and m/z = 128 of HI. The other difference between EI-MS of busp and its I_2-product is the appearance of the peak at m/z = 56 (RI = 100%) as a base peak in the parent spectra, while it appears at m/z = 56 (RI = 59%) in the product. The base peak in the product appears at m/z = 177 (RI = 100%). The low intensity of the fragment ion of m/z = 56 in the product may reflect the easy rupture of the aliphatic side chain $(CH_2)_4^+$ (mole mass = 56) in the product than in comparison to the parent.

The appearance of the base peak at m/z = 177 (RI = 100%) may be attributed to the formation of the fragment ion $C_6H_4NO_2^-(CH_2)_4^+$ (mole mass 178) as a stable fragment after decomposition of the left-hand side heterocyclic ring in the product. This fragment ion also appeared at m/z = 177 (RI = 94%) in case of busp, which reflects the lower stability of the parent drug molecule. The stability of this fragment ion in the product may be attributed to the presence of the iodine atom, which concentrates the electron cloud on the pyrimidyl piprazine heterocyclic part of the product. The appearance of the fragment ion at m/z = 122 (RI = 31.5%) may be explained by the formation of pyrimidyl ion $C_6H_8N_3^+$ in the same way as in the parent drug (RI = 61.4%). The appearance of the peak at m/z = 222 (RI = 26.4%) in EI-MS of the product and at m/z = 222 (RI = 12%) in the parent drug and at m/z = 222.1 (RI = 46%) obtained by the ESIMS/MS technique is explained by the formation of the fragment ion $C_{13}H_{20}NO_2^+$ as a result of the decomposition of the azospirone

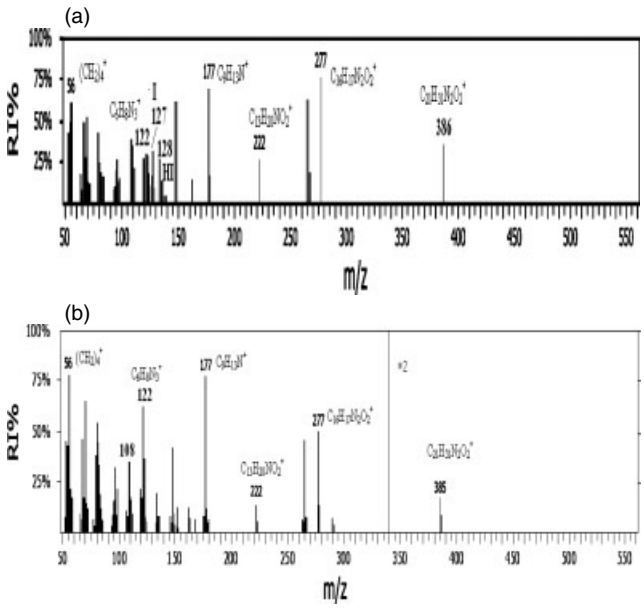

Figure 8. (a) The EI-MS of busp-I_2 inner sphere complex; (b) the EI-MS of busp.

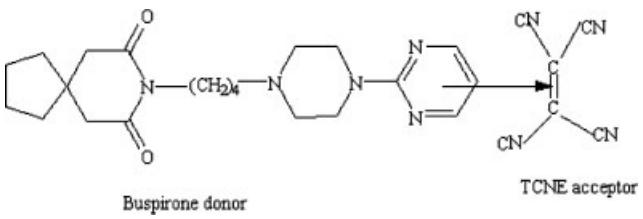

Figure 9. The proposed structure of busp-TCNE- CT-complex.

decane dione heterocyclic ring system. The appearance of the peak at m/z = 277 (RI = 98.3%) in the EI-MS of the product and at m/z = 277 (RI = 49.8%) in the parent is explained by the formation of the fragment ion N-butyl azaspirone decane dione cyclopropane ($C_{16}H_{17}N_2O_2^+$). It is clear from the values of RI that this part is more stable in the product than in the parent as a result of the presence of the substituted iodine atom in the product. The data obtained by thermal analyses (TA) and EI-MS together with ^{13}CNMR data give the proposed structural formula (Figure 5c).[25]

The biological activity of this product was tested by recommended procedure[26] on rats and mices. This study shows the promising efficiency of the busp-I_2 product in remedy of most cured rates (80% of 50 rats and 50 mice) by suitable injection doses (10–30 μg ml^{-1} daily within one month). These results encouraged us to do further more specific studies via cooperation with pharmacist and biologists to shed more light on its biological activity in vivo systems.

In the last few years, different charge transphere complexing agents such as tetracyanoethylene (TCNE) as a π-acceptors have been used for the pharmaceutical assay of donors drugs (D).[27,28] Polar solvents such as acetonitrile were reported[29,30] to promote complete transfer of charge from donor (D) to π-acceptor (R) such as busp basic drug as donor (D) of ratio 1 : 1 (D: R) which has been proved by MRM and CVM.[18–20] The busp-TCNE-CT-complex takes the proposed formula given by Fig 9.

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