Analytical Study for the Charge-Transfer Complexes of Pregabalin

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Abstract: Studies were carried out, for the first time, to investigate the charge-transfer reactions of Pregabalin (PRE) as *n*-electron donor with various π -acceptors: 7,7,8,8-tetracyanoquinodimethane (TCNQ), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (chloranilic acid, *p*CA), tetracyanoethylene (TCNE) and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil). Different colored charge-transfer complexes and radical anions were obtained. Different variables affecting the reactions were studied and optimized. The formations of the colored complexes were utilized in the development of simple, rapid and accurate spectrophotometric methods for the analysis of PRE in pure form as well as in its pharmaceutical preparation. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9995-0.9999) were found between the absorbance and the concentrations of PRE in the range of 8-400 $\mu\text{g mL}^{-1}$. The limits of assays detection ranged from 0.60 to 8.11 $\mu\text{g mL}^{-1}$. No interference could be observed from the additives commonly present in the capsules. The methods were successfully applied to the analysis of capsules that contain PRE, with good accuracy and precision; the recovery percentages ranged from 100.19 $\pm$ 0.83 to 100.50 $\pm$ 0.53. The results were compared favorably with the reported method.

Keywords: Pregabalin, Charge-transfer complexes, Spectrophotometry; Pharmaceutical analysis.

Introduction

Pregabalin (PRE); 3-(aminomethyl)-5-methylhexanoic acid, is structurally related to the inhibitory neurotransmitter aminobutyric acid (GABA). They constitute an important group of compounds that are used in the treatment of epilepsy and neuropathic pain¹. Recently, it has been approved for treatment of generalized anxiety disorders in Europe².

Pregabalin is relatively new and therefore, is not yet official in any pharmacopoeia and few analytical methods were reported for its determination. This include³⁻⁷ High Performance Liquid Chromatography (HPLC). Therefore, the aim of the present study was directed to the development of simple, rapid and sensitive spectrophotometric methods for the selective analysis PRE in its pharmaceutical preparation.

The molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely colored charge-transfer complexes, which absorb radiation in the visible region⁸. A variety of electron donating compounds have been reported to yield charge-transfer complexes leads to their utility in the development of simple and convenient spectrophotometric methods⁹⁻¹³. The charge-transfer reaction has not been reported yet for PRE, therefore the aim of the present study was directed to investigate this reaction.

Experimental

Apparatus

A Shimadzu 1601 PC double beam spectrophotometer equipped with 1.0 cm quartz cells with a fixed slit width (2 nm) was used, coupled an IBM-PC computer running by spectrophotometric Shimadzu UVPC with software. -SpectronicTM GenesysTM, UV/VIS Spectrophotometer (Milton Roy Co., USA).

Chemicals and reagents

Pregabalin was a gift from Parke-Davis Warner-Lambert, Germany and was used as working standard. 7,7,8,8-tetracyanoquinodimethane (TCNQ; Aldrich Chem. Co., Milwaukee, USA) was 1 mg mL⁻¹ in acetonitrile and the solution was stable for at least 1 week at 4°C. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; Merck, Schuchardt, Munich, Germany) was 2 mg mL⁻¹ in methanol and it was prepared afresh daily. 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone (chloranilic acid, pCA) from BDH Chemicals (Poole, UK) was 4 mg mL⁻¹ in acetonitrile and the solution was prepared afresh daily. Tetracyanoethylene (TCNE; Nacalai Tesque, Kyoto, Japan) was 2 mg mL⁻¹ in acetonitrile, and it was prepared fresh daily and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil; Sigma Chemical Co., St. Louis, USA) were 5 mg mL⁻¹ in acetonitrile and the solution was prepared afresh daily. All solvents and other chemicals used through this study were of analytical grade.

Pharmaceutical preparation

The commercial capsules used in the present investigation was Lyrica[®] capsules (Godecke AG/Germany under license of Park-Davis), labeled to contain 100 mg Pregabalin per capsule. B.N. 0568077

Preparation of standard and capsule sample solutions

Preparation of stock standard solution

Into a 50 mL calibrated flask, 8-400 mg of PRE was accurately weighed and dissolved in 2 mL methanol, completed to volume with the same solvent (for DDQ) and with acetonitrile (for the

other acceptors). These stock solutions were diluted with the respective solvents to obtain suitable concentrations that lie in the linear range of each particular assay method.

Preparation of capsules sample solution

The contents of twenty capsules were weighed and finely powdered. A quantity of the powder equivalent to 8-400 mg of PRE were transferred into a 50 mL calibrated flask, dissolved in 2 mL methanol, swirled and sonicated for 5 min, completed to volume with the corresponding solvent (as in stock solutions), shaken well for 15 min and filtered. The first portion of the filtrate was rejected and a measured volume of the filtrate was diluted quantitatively with a suitable solvent to yield suitable concentrations lie in the linear range of each particular assay method.

General analytical procedure

One milliliter of the standards or sample solutions of PRE ($80\text{--}4000\ \mu\text{g mL}^{-1}$) was transferred into 10 mL calibrated flasks. One milliliter of the acceptor solution was added, and the reaction was allowed to proceed at room temperature ($25\pm 5^\circ\text{C}$) for 10 min (in case of chloranil and TCNE), 15 min (in case of TCNQ). The reactions in case of DDQ, and *p*CA were achieved instantaneously. The solutions were diluted to volume with methanol (for DDQ), and with acetonitrile (for the other acceptors). The absorbance of the resulting solutions were measured at the wavelengths of maximum absorption (842, 456, 535, 412 and 521 nm for TCNQ, DDQ, *p*CA, TCNE, and chloranil, respectively) against reagent blanks treated similarly.

Results and Discussion

Spectral characteristics of the reaction

The interaction of PRE with polyhaloquinone and polycyanoquinone π -acceptors in non-polar solvents such as dichloroethane was found to produce colored charge-transfer complexes. In polar solvents such as methanol or acetonitrile, complete electron transfer from PRE, as an electron donor, to the acceptor moiety (A) takes place with the formation of intensely colored radical ions, according to the following scheme:

The interaction of PRE with π -acceptors at room temperature gave colored chromogens showing different absorption maxima at 842, 456, 535, 412, and 521 nm for TCNQ, DDQ, *p*CA, TCNE and chloranil, respectively (Figures 1, 2). The predominant chromogen with TCNQ in acetonitrile is the bluish-green colored radical anion, which exhibits strong absorption maxima at 842, 825, 762 and 742 nm. These bands may be attributed to the formation of the anion $\text{TCNQ}^{\bullet-}$, which was probably formed by the dissociation of an original donor-acceptor (D-A) complex with PRE.

Chloranilic acid (*p*CA) exists in three ionic forms, the neutral yellow-orange H_2A at very low pH, the violet HA^- which is stable at pH 3 and a colorless A^{2-} , which is stable at high pH; these transformations are illustrated in the following scheme:

Since the interaction of PRE with *p*CA in acetonitrile gave a violet product, it might be concluded that HA^- was the form of *p*CA involved in the reaction described herein. With TCNE, the characteristic shaped absorption band of TCNE radical anion with reported maximum in acetonitrile at 412 nm was not found. Instead, a duplet at 394 nm and 414 nm was formed which corresponds to the 1,1,2,3,3-pentacyanopropeneide (PCNP) anion, which is more preferable than TCNE anion, in quantitative analysis¹⁴.

The relative sensitivity of the five acceptors employed in the present analytical work may be attributed to their difference in electron affinities, as well as the conditions employed in the reaction (reagent concentration, reaction time and solvent), (Table 1).

Figure 1. Absorption spectra of the reaction products of PRE with each of TCNE (1), chloranil (2) and TCNQ (3). Concentrations of PRE were 250, 400 and 24 $\mu\text{g mL}^{-1}$ in case of TCNE, chloranil and TCNQ, respectively. Solutions were in acetonitrile.

Figure 2. Absorption spectra of the reaction products of PRE with each of DDQ (1) and *p*CA (2). Concentrations of PRE were 70 and 120 $\mu\text{g mL}^{-1}$ in case of DDQ and *p*CA, respectively. Solutions were in case of DDQ methanol and in acetonitrile in case of *p*CA.

Table 1. Optimum conditions for the charge-transfer interaction of pregabalin with different acceptors

Acceptor	Acceptor concentration, mg mL ⁻¹	Solvent	Time, min	λ_{max} , nm
TCNQ	1.0	Acetonitrile	15	842
DDQ	2.0	Methanol	At once	456
<i>p</i> CA	4.0	Acetonitrile	At once	535
TCNE	2.0	Acetonitrile	10	412
Chloranil	5.0	Acetonitrile	10	521

Optimization of reaction conditions

Effect of reagent concentration

The results of variations in the reagents concentrations indicated that 1 mL of the concentrations indicated in Table 1 were the optimum concentrations. The higher concentrations of the reagents may be useful for rapidly reaching equilibrium, thus minimizing the time required to attain maximum absorbance at the corresponding wavelengths of maximum absorbance.

Effect of solvent

In order to select the most appropriate solvent, the reactions were carried out in different solvents. Small shifts in the position of the maximum absorption peak were observed, and the absorption intensities were also influenced. Methanol gave maximum sensitivity in case of DDQ. Acetonitrile was considered as an ideal solvent for the other π -acceptors. This is because it offered maximum sensitivity, which was attributed to the high dielectric constant of acetonitrile that promotes maximum yield of radical anions, in addition to its high solvating power for acceptors¹⁵.

Effect of reaction time

The optimum reaction time was determined by monitoring the color development at room temperature (25 ± 2 °C). Complete color development was attained instantaneously with DDQ and *p*CA, or after 10-15 min with other acceptors (Table 1). The developed colors remained stable at room temperature for at least a further 30 min.

Development and validation of the analytical methods

Calibration curves, linearity and sensitivity

Under the specified optimum reaction conditions, the calibration curves for PRE with the different analytical reagents employed in the present work were constructed. The regression equations for the results were derived using the least-squares method. In all cases, Beer's law plots were linear with very small intercepts and good correlation coefficients in the general concentration range of 8-400 $\mu\text{g mL}^{-1}$ (Table 2). The limits of detection were 0.60-8.11 $\mu\text{g mL}^{-1}$.

Table 2. Comparative parameters for the charge-transfer reaction of pregabalin with various acceptors

Parameter	<i>p</i> CA	TCNQ	Chloranil	TCNE	DDQ
Linearity range, $\mu\text{g mL}^{-1}$	40-200	8-24	50-400	50-300	20-70
Slope (b)	0.0061	0.0257	0.0027	0.0052	0.0159
S.D. of slope (S_b)	5.8×10^{-5}	0.0003	3.0×10^{-5}	0.00007	0.00025
Intercept (a)	0.0511	0.0706	0.0649	-0.0654	-0.0202
S.D. of intercept (S_a)	0.0077	0.0052	0.0073	0.0136	0.01205
Correlation coefficient (r)	0.9999	0.9998	0.9995	0.9996	0.9995
Coefficient of determination (r^2)	0.9997	0.9996	0.9997	0.9993	0.9990
Molar absorptivity, $\text{Lmol}^{-1} \text{cm}^{-1}$	1.01×10^3	7.25×10^3	7.82×10^3	7.71×10^3	2.51×10^3
Detection limit, $\mu\text{g mL}^{-1}$	0.60	2.27	8.11	7.90	3.88
Quantitation limit, $\mu\text{g mL}^{-1}$	2.00	7.58	27.03	26.33	12.93
Lower 95% confidence limit	98.72	98.85	98.27	98.26	98.53
Upper 95% confidence limit	101.17	101.02	100.68	100.59	101.80

Precision

The precisions of the assays (within-assay and between-assay) were determined for PRE concentrations cited in Table 3. The within-assay precision was assessed by analyzing six replicates of each sample as batch in a single assay run, and the between-assays precision was assessed by analyzing the same sample, as triplicate, in two separate assay runs. The assays, gave satisfactory results; the relative standard deviations (R.S.D.) were less than 2% (Table 3). This level of precision of the proposed methods was adequate for the quality control analysis of PRE.

Table 3. Precision of the proposed methods for analysis of pregabalin (PRE)

Method	PRE, $\mu\text{g mL}^{-1}$	Within-assay, n=6		Between-assay, n=6	
		Mean $\pm$ S.D	R.S.D	Mean $\pm$ S.D	R.S.D
TCNQ	16.0	16.09 $\pm$ 0.24	1.50	15.43 $\pm$ 0.17	1.06
DDQ	50.0	51.74 $\pm$ 0.68	1.36	48.76 $\pm$ 0.65	1.30
<i>p</i> CA	100.0	98.48 $\pm$ 0.70	0.70	100.09 $\pm$ 0.88	0.88
TCNE	200.0	198.47 $\pm$ 0.82	0.41	200.79 $\pm$ 0.80	0.40
Chloranil	120.0	120.69 $\pm$ 0.84	0.70	118.87 $\pm$ 0.79	0.66

Specificity and interference

The proposed spectrophotometric methods have the advantages that the measurements in all of these methods are performed in the visible region, away from the UV-absorbing interfering substances that might be co-extracted from PRE-containing dosage forms. Potential interference by the excipients in the dosage forms was also studied. Samples were prepared by mixing known amount (50 mg) of PRE with various amounts of the common excipients such as starch, glucose, lactose, and magnesium stearate. The results (Table 4) revealed that no interference was observed from any of these excipients with the proposed methods. The absence of interfering from these excipients was attributed to the extraction with organic solvent prior to the analysis.

Table 4. Analysis of pregabalin (PRE) in the presence of common excipients by different methods

Ingredient	Recovery, % $\pm$ S.D				
	TCNQ	DDQ	pCA	TCNE	Chloranil
Starch	99.58 $\pm$ 0.48	97.97 $\pm$ 0.69	99.38 $\pm$ 1.01	100.94 $\pm$ 0.28	99.78 $\pm$ 0.30
Glucose	100.48 $\pm$ 1.58	100.39 $\pm$ 1.02	97.45 $\pm$ 0.93	99.67 $\pm$ 1.67	100.53 $\pm$ 1.76
Lactose	99.31 $\pm$ 0.49	99.93 $\pm$ 0.92	99.75 $\pm$ 0.77	99.53 $\pm$ 0.84	99.87 $\pm$ 1.87
Acacia	97.38 $\pm$ 0.30	97.39 $\pm$ 0.38	100.29 $\pm$ 0.53	99.75 $\pm$ 1.85	79.43 $\pm$ 1.92
Mag. Stea.	98.88 $\pm$ 1.28	100.36 $\pm$ 1.17	99.39 $\pm$ 0.67	100.68 $\pm$ 1.57	99.11 $\pm$ 0.11

Ruggedness and robustness

The ruggedness of the proposed methods was assessed by applying the procedures using two different instruments (Showing in Section 2) in two different laboratories at different elapsed time. Results obtained from lab-to-lab and day-to-day variations were found to be reproducible as R.S.D. did not exceed 2%. Robustness of the procedures was assessed by evaluating the influence of small variation of experimental variables: concentrations of acceptor reagent, and reaction time, on the analytical performance of the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results; recovery percentages were 99.0-101.9% $\pm$ 0.92-1.65. This provided an indication for the reliability of the proposed methods during routine work.

Quantification

At fixed experimental conditions, the intensity of absorption at the specified wavelengths was found to be a function of the concentrations of the investigated drug. In all cases studied, Beer's law plots were linear with very small intercepts. For comparison, the HPLC reported method⁶ was applied. Statistical analysis of the results obtained (Table 5), indicated that the proposed procedures were accurate and precise as the reported method.

Table 5. Statistical analysis of the results obtained for assay of authentic pregabalin (PRE) drug using the proposed methods compared with the reported HPLC method⁶

Parameter	Reported	TCNQ	DDQ	pCA	TCNE	Chloranil
X	99.58	99.94	100.16	100.08	99.42	99.48
$\pm$ S.D	0.69	0.88	1.56	1.26	1.11	1.15
n	4	6	6	6	6	6
V	0.48	0.77	2.43	1.59	1.23	1.32
t		0.83	0.71	0.74	0.26	1.06
F		1.60	5.06	3.31	2.56	2.30

Four and Six determinations were used for the reported and the reference methods, respectively. The tabulated values of t and F at 95% confidence limit are t=1.83 and F=4.95.

Application of the method to the analysis of capsules

The obtained satisfactory validation results made the proposed procedures suitable for the routine quality control analysis of PRE. The proposed and reported methods⁶ were applied to the determination of PRE in its capsules. The results obtained by the proposed methods were statistically compared with those obtained by the reported method. The obtained mean values

of the labeled amounts ranged from 100.19 ± 0.83 to 100.50 ± 0.53 (Table 6). In the *t*- and *F*-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated similar precision and accuracy in the analysis of PRE in its capsules. It is evident from these results that all the proposed methods are applicable to the analysis of PRE in its capsules with comparable analytical performance. However, the critical recommendations of some of these methods might be based on the experimental conditions (*e.g.* reaction time), and the ultimate sensitivity that determines the amount of specimen required for analysis. For example, the methods involving DDQ and *p*CA are recommended whenever rapid analysis is required; this because they have very short reaction time. The method involving TCNQ is recommended, as high sensitivity is required on the expense of the analysis time.

Table 6. Determination of pregabalin in Lyrica[®] capsules by the proposed and reported HPLC method⁶

Parameter	Reported	TCNQ	DDQ	<i>p</i> CA	TCNE	Chloranil
X	100.17	100.29	100.19	100.38	100.43	100.50
±S.D	0.76	0.73	0.83	0.64	0.77	0.53
N	4	5	5	5	5	5
V	0.58	0.53	0.69	0.41	0.59	0.28
t		0.24	0.42	0.46	0.51	0.77
F		1.07	1.22	1.38	1.03	2.05

Four and five determinations were used for the reported and the reference methods, respectively. The tabulated values of *t* and *F* at 95% confidence limit are *t*=1.86 and *F*=6.16.

Conclusions

The charge-transfer complexation reaction of pregabalin (PRE) as electron donor and some electron acceptors has been investigated. The obtained complexes were studied by visible spectrophotometry. The obtained colored complexes were utilized in the development of simple, rapid, and accurate spectrophotometric methods for the analysis of PRE in pure forms as well as in capsules. The proposed methods are superior to the previously reported UV-based spectrophotometric methods, as the measurements are performed in the visible region, away from the UV-absorbing interfering excipients that might be co-extracted from PRE containing dosage form. In addition, the proposed methods used a spectrophotometer, which is available in all quality control laboratories, and they involve very simple procedures.

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