

## Original Research Article

# Charge-Transfer Reaction of Cediranib with 2,3-Dichloro-3,5-dicyano-1,4-benzoquinone: Spectrophotometric Investigation and Use in Development of Microwell Assay for Cediranib

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### Abstract

**Purpose:** To investigate the charge-transfer (CT) reaction between cediranib (CRB) and 2, 3 - dichloro-5, 6- dicyano -1, 4 - benzoquinone (DDQ) and employment of the reaction as a basis for the development of a novel 96 - microwell spectrophotometric assay for CRB.

**Method:** The reaction of CRB and DDQ was investigated in different solvents of varying dielectric constant and polarity index. The reaction resulted in the formation of a red-colored product. Spectrophotometric investigations confirmed that the reaction proceeded through to the formation of a (CT) complex.

**Results:** The molar absorptivity of the CT complex was linearly correlated with the dielectric constant and polarity index of the solvent; the correlation coefficients were 0.801 and 0.858, respectively. The association constant of the complex was  $0.5 \times 10^3 \text{ L mol}^{-1}$  in 2-propanol. The reaction conditions were optimized for the development of the microwell assay for CRB. The assay limits of detection and quantitation were 6.8 and 20.6  $\mu\text{g/well}$ , respectively. The assay was validated as per the guidelines of the International Conference on Harmonization (ICH) and successfully applied to the analysis of CRB in its bulk and dosage forms with good accuracy and precision.

**Conclusion:** The developed assay has high throughput and consumed minimum volume of organic solvent; thus, it reduces the exposure of the analysts to the toxic effects of organic solvents, and should significantly lower the analysis cost.

**Keywords:** Cediranib, 2,3-dichloro-3,5-dicyano-1,4-benzoquinone, Charge-transfer reaction, Spectrophotometry, Microwell assay, High throughput analysis.

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## INTRODUCTION

Cediranib (CRB, Fig 1) is a novel small-molecule; potent inhibitor of vascular endothelial growth factor (VEGF) receptor tyrosine kinases [1-3]. Extensive literature survey revealed that only one liquid chromatography–mass spectrometric method was available for determination of CRB

in biological fluids [4]. This fact promoted our interest in the development of a new method for quality control (QC) of CRB.

The charge-transfer (CT) reaction between the electron-donating and electron-accepting molecules has considerable importance in different fields of chemistry [5-8]. CT reactions

are generally associated with the formation of intensely colored CT complexes, which usually absorb radiations; consequently, a new light absorption band occurs at much longer wavelength than in the spectra of the CT complex components [9]. The rapid formation of these complexes leads to their widespread utility in the development of spectrophotometric methods for analysis of many pharmaceutical molecules [10] and extensive studies involving CT reactions have been conducted [10 - 12]. The benzoquinone derivatives have  $\pi$ -orbitals of the benzoquinone ring, which have electron-accepting ability. This ability increases with the introduction of electron-withdrawing groups like chloro-, nitro-, cyano, etc., on the benzoquinone ring [13]. These groups have strong electronegativity and large Hammett constants [14]. 2, 3 - dichloro- 5, 6- dicyano -1, 4 - benzoquinone (DDQ, Fig 1) acts as a strong  $\pi$ -electron acceptor in studying the CT reactions of electron donors [15].

The present study was devoted to the investigation of the CT reaction between CRB and DDQ and employment of the reaction as a basis in the development of a novel 96 - microwell high throughput spectrophotometric assay for CRB.

## EXPERIMENTAL

### Chemicals and materials

Cediranib (CRB) standard was obtained from Weihua Pharma Co. Ltd. (Zhejiang, China) and

was used as received; the purity of the investigated compound was > 99 %. DDQ Sigma-Aldrich Corporation, (St. Louis, MO, USA). Solvents and excipients were of analytical grade (Fisher Scientific, California, CA, USA).

### Preparation of standard CRB solutions

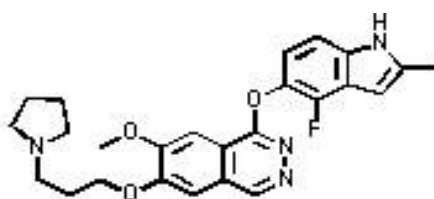
Into a 5 ml calibrated flask, 10 mg ( $2.22 \times 10^{-5}$  mole) of CRB was accurately weighed, dissolved in 2 ml methanol, and completed to volume with the same solvent. This stock solution ( $4.44 \times 10^{-3}$  mol L<sup>-1</sup>) was diluted with the appropriate solvent to obtain CRB concentrations suitable for the corresponding study.

### Determination of association constant

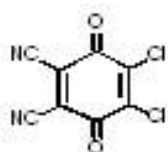
Series of CRB solutions ( $0.89 \times 10^{-4}$  -  $5.5 \times 10^{-4}$  mol L<sup>-1</sup>) were mixed with DDQ solution of a fixed concentration ( $4.4 \times 10^{-3}$  mol L<sup>-1</sup>); both CRB and DDQ solutions were prepared in 2 - propanol as this solvent gave the highest readings and was not harmful to the microwell-plate transparency. These mixed solutions were allowed to reach equilibrium (~5 min) at room temperature ( $25 \pm 2$  °C). The absorbance of the solutions was measured at 460 nm against reagent blanks treated similarly. The measurements were conducted on UV-1601 PC double-beam ultra-violet-visible spectrophotometer (Shimadzu, Kyoto, Japan).

### Preparation of CRB-containing dosage form sample solution

An accurately weighed amount (45 mg) of CRB was mixed with the excipients: microcrystal-line



**Cediranib (CRB):** 4-[(4-fluoro-2-methyl-1H-indol-5-yl)oxy]-6-methoxy-7-[3-(pyrrolidin-1-yl)propoxy]quinazoline



**DDQ:** 2,3-Dichloro-3,5-dicyano-1,4-benzoquinone

**Figure 1:** The chemical structure and chemical names of cediranib (CRB) and 2, 3 - dichloro- 5, 6- dicyano -1, 4 - benzoquinone (DDQ)

cellulose (50 mg), colloidal silicon dioxide (10 mg), anhydrous dibasic calcium phosphate (5 mg), sodium starch glycolate (5 mg), and magnesium stearate (5 mg). The laboratory-prepared sample was dissolved in 5 ml of methanol, swirled and sonicated for 5 min, and filtered. The first portion of the filtrate was rejected, and a measured volume of the filtrate was diluted with methanol to yield CRB concentrations in the range of 100 - 1000 µg/ml. These samples were analyzed by the proposed assay applying the microwell assay procedure.

### Microwell assay procedure

Accurately measured aliquots (100 µL) of the standard or sample solution containing varying amounts of CRB (100 – 1000 µg/mL) were transferred into wells of 96 - microwell assay plates (Corning/Costar Inc., Cambridge, USA). Solutions were dispensed by adjustable 8 – channel - pipette (Sigma Chemical Co., St. Louis, MO, USA). One hundred microliters of DDQ solution (0.1 %, w/v) was added, and the reaction was allowed to proceed at room temperature (25 ± 2 °C) for 5 min. The absorbances of the resulting solutions were measured at 460 nm by the microwell-plate reader (ELx 808, Bio-Tek Instruments Inc. Winooski, USA) empowered by Gen5 software (provided with the instrument). Blank wells were treated similarly except 100 µL of methanol was used instead of the sample, and the absorbances of the blank wells were subtracted from those of the sample wells.

## RESULTS

### Absorption spectral characteristics

The absorption spectrum of CRB solution (5.5 × 10<sup>-4</sup> mol L<sup>-1</sup>, in methanol) was recorded. It was obvious from the spectrum that CRB exhibited a maximum absorption peak (λ<sub>max</sub>) at 320 nm. CRB solution (5.5 × 10<sup>-4</sup> M) was mixed with DDQ solution (4.4 × 10<sup>-3</sup> mol L<sup>-1</sup>), and the interaction of CRB with DDQ was allowed to proceed at room temperature (25 ± 2 °C), and the absorption spectrum of the reaction mixture was recorded against DDQ reagent blank solution. A red-colored product was obtained showing absorption maximum at 460 nm, and the reaction went to completion very rapidly.

### Effect of solvent on the interaction of CRB with DDQ

The interaction of CRB with DDQ was allowed to proceed in different solvents of varying dielectric

constants and polarity indices and the absorption spectra were recorded. The molar absorptivity (ε) was determined in each solvent; the obtained values are given in Table 1.

### Association constant for CRB-DDQ CT complex

The association constant was evaluated at room temperature (25 ± 2 °C) and at the λ<sub>max</sub> of CRB-DDQ complex (in 2-propanol as this solvent gave the highest reading and did not affect the transparency of the microwell of the assay plate, as acetrontrile did) using the Benesi-Hildebrand equation (Equation 1) [19]:

$$A_0/A^{AD} = 1/\epsilon^{AD} + 1/K_c^{AD} \cdot \epsilon^{AD} \times 1/D_0 \dots\dots\dots (1)$$

where [A<sub>0</sub>] is the molar concentration of the acceptor (DDQ); [D<sub>0</sub>] is the molar concentration of the donor (CRB); A<sup>AD</sup> is the absorbance of the complex formed at the λ<sub>max</sub>; ε<sup>AD</sup> is the molar absorptivity of the complex; K<sub>c</sub><sup>AD</sup> is the association constant of the complex (L mol<sup>-1</sup>). Upon plotting the values [A<sub>0</sub>]/A<sup>AD</sup> against 1/[D<sub>0</sub>], a straight line was obtained, from which the association constant, correlation coefficient, and the molar absorptivity (ε) of the CRB-DDQ complex were calculated. The intercept of the fitting line corresponded to 1/ε<sup>AD</sup> and the association constant was calculated from the slope value of the line and the derived value of ε<sup>AD</sup>. The molar absorptivity and association constant of the complex (in 2-propanol) were found to be 1 × 10<sup>3</sup> L mol<sup>-1</sup> cm<sup>-1</sup> and 0.5 × 10<sup>3</sup> L mol<sup>-1</sup>, respectively. The standard free energy change (ΔG<sup>o</sup>) of the CT complex was calculated and found to be 3.7 × 10<sup>3</sup> kcal mol<sup>-1</sup>.

### Optimization of assay conditions

The optimization of experimental conditions for carrying out the reaction in the 96-microwell assay plate was investigated by altering each reaction variable in turn while keeping the others constant. The reaction was carried out in methanol and all the measurements were carried out by the plate reader at 460 nm. The studied conditions were: concentration of DDQ reagent, reaction time, and temperature. The studied range of these conditions and the optimum value that was selected for the assay development are given in Table 2.

It is worth mentioning that methanol was considered as optimum solvent for carrying out the reaction in the microwell assay plates although acetonitrile gave the highest ε value

**Table 1:** Effect of solvents on the position and intensity of absorption of the reaction mixtures of CRB with DDQ

Name	Solvent		$\epsilon (\times 10^3)^c$
	DEC <sup>a</sup>	PI <sup>b</sup>	
Acetonitrile	37.5	5.8	3.28
Methanol	32.7	5.1	2.87
Ethanol	24.6	5.2	2.75
Acetone	20.7	5.1	2.34
2-Propanol	19.9	3.9	2.24
Dichloromethane	10.5	3.5	2.03
Dichloroethane	9.1	3.1	0.51
Toluene	2.4	2.4	0.22
Chloroform	2.2	4.8	0.10

<sup>a</sup> DEC = dielectric constant; values were obtained from reference 18; <sup>b</sup> PI = Polarity index; values were obtained from reference 19; <sup>c</sup>  $\epsilon$  = molar absorptivity; values are expressed as  $L \text{ mol}^{-1} \text{ cm}^{-1}$

(Table 1). This was because of the negative effect of acetonitrile on the transparency of the assay plate material.

### Validation and application of the assay

Under the above mentioned optimum reaction conditions, the calibration curve for the analysis of CRB by the proposed assay was constructed. The regression equation for the results was derived using the least-squares method. Beer's law plot (10-concentration levels,  $n = 5$ ) was linear in the range of 10 – 100  $\mu\text{g/well}$ . The calibration equation was:  $A = 0.0386 + 0.0089 C$  ( $r = 0.9995$ ), where A, C and r are the absorbance, concentration of CRB ( $\text{mg/well}$ ) and correlation coefficient, respectively (Table 3). The limits of detection (LOD) and quantitation (LOQ) were determined [25] using the formula:  $\text{LOD or LOQ} = \kappa \text{SDa/b}$ , where  $\kappa = 3.3$  for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The LOD and LOQ values were 6.8 and 20.6  $\mu\text{g/well}$ , respectively. The intra- and inter assay precisions were assessed at three CRB concentrations (20, 50 and 80  $\mu\text{g/well}$ ). The relative standard deviation (RSD) values did not exceed 2 %, indicating good precision of the assay. This good level of precision was attributed to the accuracy of the volumes that were concomitantly dispensed in the microwells by multi-channel pipettes, and completeness of the reaction in a small volume (200  $\mu\text{L}$ ). The high-precision property of the assay made it suitable for its routine application in QC laboratories for determination of CRB samples (bulk and dosage forms). In addition, the accuracy, robustness and ruggedness of the assay were evaluated and all the results were satisfactory as per the ICH guidelines [25]. A summary for the quantitative and validation parameters of the assay is given in Table 3. The assay was applied to the analysis of CRB-containing laboratory-made dosage forms. The

mean percentage recovery, relative to the nominated amounts, obtained by the proposed assay was  $98.23 \pm 2.58$  %. This good recovery values indicated the reliability of the proposed assay for routine application for the accurate determination of CRB in pharmaceutical QC laboratories.

**Table 3:** Quantitative parameters for the analysis of CRB by the microwell spectrophotometric assay based on the CT reaction of CRB with DDQ

Parameter	Value
Linear range ( $\mu\text{g/well}$ )	10 – 100
Intercept	0.0386
Standard deviation of intercept	0.0184
Slope	0.0089
Standard deviation of slope	$3 \times 10^{-4}$
Correlation coefficient	0.9995
LOD ( $\mu\text{g/well}$ )	6.8
LOQ ( $\mu\text{g/well}$ )	20.6
Precision (RSD, %)	< 2
Accuracy (%)	> 98

**Table 2:** Optimization of experimental conditions for the microwell spectrophotometric assay for CRB based on its formation of colored CT complex with DDQ

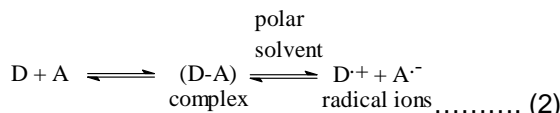
Condition	Studied range	Optimum
DDQ conc. (% w/v)	0.02 – 0.2	0.1
Solvent	Different <sup>a</sup>	Methanol
Reaction time (min)	2 – 40	5
Temperature ( $^{\circ}\text{C}$ )	25 – 60	25
$\lambda_{\text{max}}$ (nm)	400 – 650	460

<sup>a</sup> Solvents used as in Table 1

## DISCUSSION

The results showed us that the absorption intensity of the new absorption maximum (460 nm) increased with CRB concentrations in the interaction solution. This continuous increase in the absorbance of the new absorption band with

(the reaction time and) CRB concentrations was indicative of the formation of CRB - DDQ product. The new absorption band was attributed to the complete electron transfer from CRB (D) to DDQ (electron acceptor; A) accompanied by the formation of colored radical acceptor anion (DDQ<sup>-</sup>) [16], which was formed by the dissociation of an original donor-acceptor (D - A) complex (equation 2).



The dissociation of the (D - A) complex was promoted by the high ionizing power of the polar solvent and the resulting peak in the absorption spectra of the reaction mixture were similar to the maxima of the radical anion of the acceptor obtained by the iodide reduction method [16].

The interaction in polar solvents with high dielectric constants (e.g. acetonitrile, methanol, ethanol, etc) produced CT complexes with  $\epsilon$  values higher than those produced in low-polar solvents with low dielectric constants (e.g. dichloromethane); however the reaction did not proceed at all in non-polar solvent (e.g. chloroform). The values of  $\epsilon$  correlated with both the dielectric constants [17] and polarity indices [18] of the solvents in which the reaction was performed; correlation coefficients were (r) = 0.801 and 0.858 for dielectric constants and polarity indices, respectively. This was attributed to the complete electron transfer from CRB (electron donor; D) to DDQ (electron acceptor; A) that took place in the polar solvents. Among all the solvents tested, acetonitrile offered the highest  $\epsilon$  value. This was attributed to its high dielectric constant that promoted maximum yield of radical anion of DDQ, in addition to its high solvating power for both DDQ and CRB.

The relatively low value of the association constant was attributed to the common feature for CT complexes with  $\pi$ -acceptors because of the dissociation of the original donor-acceptor complex to the radical anion [9].

The standard free energy change ( $\Delta G^\circ$ ) of the CT complex is related to its association constant and it can be calculated by the following formula (equation 3):

$$\Delta G^\circ = - 2.303 RT \log K_c \dots\dots\dots (3)$$

where  $\Delta G^\circ$  is the standard free energy change of the complex (kcal mol<sup>-1</sup>), R is the gas constant (1.987 calories degree/mole), T is the absolute

temperature in Kelvin ( $^\circ\text{C} + 273$ ) and  $K_c$  is the association constant of the complex (l/mole).

Based on the aforementioned successful formation of a red-colored CT complex between CRB and DDQ, the reaction was used as a basis for the development of CT-based photometric assay for the pharmaceutical quality control (QC) of CRB. The conventional CT-based photometric methods are not automated and consequently their analysis throughput is low, thus their applications in QC laboratories are limited. Moreover, these methods suffer from the consumption of large volumes of organic solvents, which leads to high analysis cost, and more importantly, the incidence of exposure of the analysts to the toxic effects of the organic solvents [19-23]. Therefore, the present study was devoted to employing the CT reaction of CRB in the development of novel photometric method with high analysis throughput that can reduce the consumption of organic solvents in the QC of CRB.

In previous studies, Darwish *et al* [24] successfully adopted a microwell plate reader equipped with photometric detection in the development of high-throughput photometric assays for measuring the active drug contents in pharmaceutical preparations. For these reasons, the present study was designed to employ this methodology for CRB; the reaction was carried out in 96 - microwell plates (200  $\mu\text{L}$  volume).

## CONCLUSION

This is the first report on the description of the CT reaction between CRB and DDQ. The as-say provides a high analytical throughput that can facilitate the processing of a large number of samples in a relatively short time. This property is attributed to the use of multi-channel pipettes for efficient dispensing of the solutions, carrying out the analytical reaction in 96 - well plates (as reaction vessels), and measuring the color signals in the 96 wells at ~ 30 s by the plate reader. In addition, the assay can reduce the consumption of organic solvents, thus reducing the exposure of analysts to the toxic effects of organic solvents, and will lead to lowering of analysis cost when the method employed in pharmaceutical quality control laboratories.

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