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Original article

INVESTIGATION OF CARBAMAZEPINE-Fe(III) AND 10,11 DIHYDROXCYCARBAMAZEPINE-Fe(III) INTERACTIONS WITH SPECTROPHOTOMETRIC METHOD

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Abstract

Carbamazepine (CBZ) is an antiepileptic drug, with a structure similar to imipramine. In this study, chemical interaction of carbamazepine and 10,11 dihydroxycarbamazepine (CBZ-diol) which is one of the most important metabolites of carbamazepine with Fe(III) were investigated in in-vitro media in order to examine anemia owing to iron deficiency. The data are obtained via investigation of the compounds that form complexes with iron.

As a result of spectrophotometric investigation of the chemical interaction between CBZ and Fe (III) was found to occur in % 10 dioxane - water solvent medium, at the interval of pH= 2-3, at room temperature, in 5 min reaction time, when equal concentrations of CBZ and Fe (III) were used. The interaction reaction of 10,11CBZ-diol with Fe (III) was found to occur optimally in aqueous solution at room temperature, in 5 min reaction time, at the interval of pH=2-3 and concentration of CBZ two times higher than Fe (III) concentration.

It is investigated that whether the interaction reaction of Fe(III)- CBZ and Fe(III)-CBZ-diol to make complexes according to Irwing-Rossoti method by potentiometry.The interaction between CBZ- Fe(III) and CBZ-diol- Fe(III) is a weak interaction reaction rather than being a reaction that forms complexes and this supports the results of spectrophotometric analysis.

Key words: *Carbamazepine, 10,11 Dihydroxycarbamazepine, Fe(III), Chemical interaction, Spectrophotometry.*

Karbamazapin -Fe(III) ve 10,11 Dihidroksikarbamazapine-Fe(III) Etkileşimlerinin Spektrofotımetrik Yöntemle İncelenmesi

Karbamazepin (CBZ) yapısı bakımından imipramin'e benzeyen antiepileptik olarak kullanılan bir ilaçtır. Bu çalışmada CBZ–Fe(III) ve karbamazapinin önemli metabolitlerinden biri olan 10,11 dihidrosikarbamazapin (CBZ-diol) – Fe(III) arasındaki kimyasal etkileşim in-vitro ortamda incelenerek, CBZ ve metabolitinin demir ile kompleks oluşturan maddelere benzer biçimde demir eksikliğine bağlı anemiye neden olup olamayacağı yönünde kimyasal veriler elde edilmeye çalışıldı.

CBZ – Fe (III) arasındaki kimyasal etkileşimin en iyi % 10 dioksan-su çözücü ortamında, pH 2-3 aralığında, oda sıcaklığında, 5 dakika reaksiyon süresinde ve eşit konsantrasyonda CBZ ve Fe (III) ile gerçekleştiği, 10,11 CBZ-diol – Fe(III) arasındaki kimyasal etkileşimin ise en iyi sulu çözeltide, oda sıcaklığında, 5 dakika reaksiyon süresinde, pH 2-3 aralığında ve demirin iki katı CBZ konsantrasyonunda gerçekleştiği bulundu. Ayrıca, etkileşim reaksiyonunun bir kompleksleşme reksiyonu olup olmadığı Irwing-Rossoti metoduna göre potansiyometrik yöntemle incelendi ve CBZ–Fe (III) ve CBZ-diol–Fe(III) arasındaki etkileşimlerin kompleksleşme reaksiyonu değil zayıf bir etkileşim reaksiyonu olabileceği yönünde spektrofotometrik sonuçları destekleyen sonuçlar elde edildi.

Anahtar kelimeler: *Karbamazapin, 10,11 Dihidrokarbamazapin, Fe(III), Kimyasal etkileşim, Spektrofotometri.*

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INTRODUCTION

Carbamazepine (CBZ) structure is similar to imipramine. It's chemical name is 5H-dibenz (b,f) azepine-5-carboxamide CBZ, (Figure 1) is a highly lipophilic, neutral tricyclic compound. It is widely used for the treatment of epileptic seizures, trigeminal neuralgia and psychiatric disorders (1-3). CBZ is metabolized to over 30 metabolites both in rat and humans (4). In humans it is mainly oxidized to major metabolite carbamazepine-10,11- epoxide (CBZ - E; figure 1) The epoxide is mainly hydrolyzed to the 10,11- trans-dihydrodiol metabolite (10,11-dihydro-carbamazepine, (CBZ-diol), (10,11-D; figure 1)) prior to excretion in urine (5). Primary minor metabolites are 9-hydroxymethyl-10-carbamoyl acridan (9-AC; figure 1), 3-hydroxy-carbamazepine (3-OH-CBZ) and 2-hydroxy-carbamazepine (2-OH-CBZ; figure 1) (6).

Determination of plazma levels of the CBZ metabolites is required in pharmacokinetic and metabolic studies, and the routine analysis of CBZ-E along with CBZ may provide optimal therapeutic monitoring of CBZ treatment. The most widely used methods of analysis for CBZ is liquid chromatography with UV (7-13) or liquid chromatography-elektrospray mass spectrometry (14-16). Determination of two main metabolites of CBZ (CBZ- E and CBZ-diol) (17,18) and determination of carbamazepine in pharmaceutical preparation using high performance liquid chromatography and derivative spectrophotometry (19).

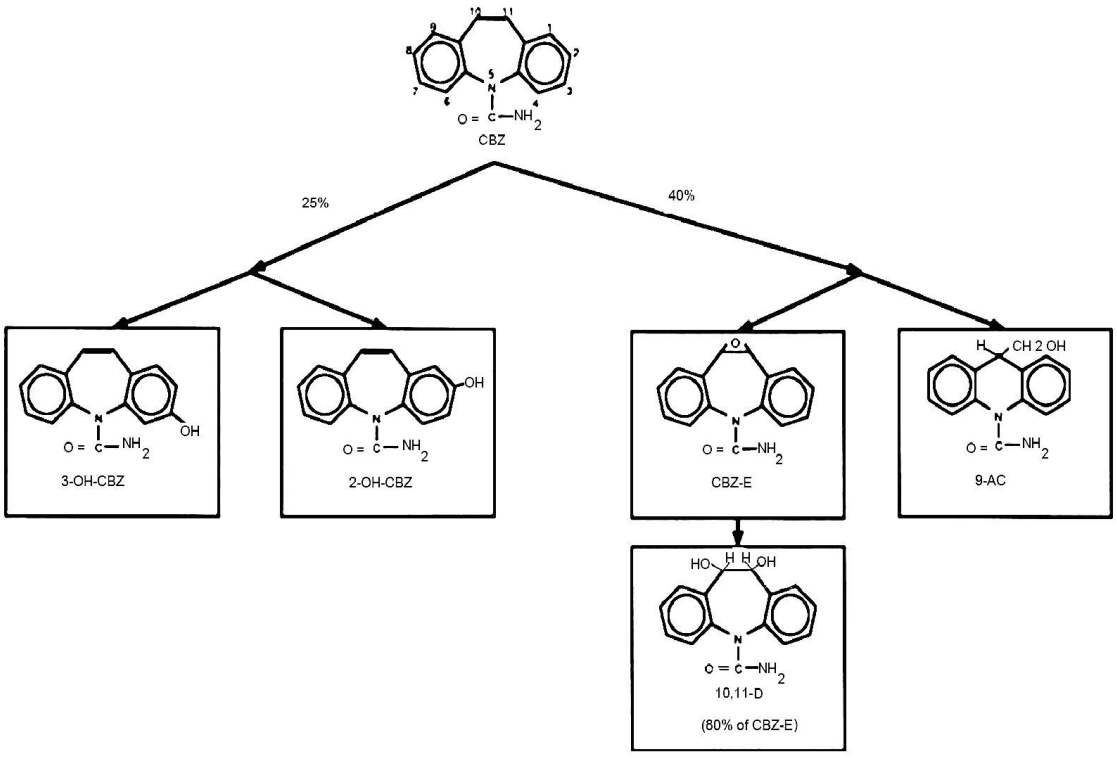

Figure 1. Principal metabolic pathways of CBZ in humans.

EXPERIMENTAL

Material

Carbamazepine USP standard was supplied from Yeni İlaç-Turkey and 10,11-dihydro-carbamazepine (CBZ-diol) USP standard was supplied from Novartis. All reagents were of analytical grade. $FeNO_3.H_2O$ (Merck), $NaCH_3COO$ (Merck), CH_3COOH (Merck), Tris (Merck), HCl 37 % (Merck), NaOH (Merck), pH = 4.01 and pH = 7.00 WTW STP 10 technical buffer solutions, GLF-2004 distilled water apparatus, WTW Sentix electrode, WTW Inolab terminal 3 pH metre, Shimadzu UV-1601 Spectrophotometer, Shimadzu Ax200 balance, Radiometer TIM800 titration manager, ABU 901 autoburette and HI 1131B combination pH electrode were used. Computer calculations were performed on the pH-metric data. All the solutions were prepared by using distilled water.

Method
Spectrophotometric

In order to find out the optimum solvent system for the interaction of CBZ-Fe(III); $6.00x10^{-4}$ M CBZ stock solution that was prepared using 1,4-dioxane (diox) and ethanol (EtOH), was diluted to 10 mL using solvent mixtures in various ratios in the range of % 10-100. So that the final concentrations of each CBZ solution was $6.00x10^{-5}$ M. The optimum ratio of the solvent system was determined through measuring the absorbance of each diluted solution, against its reference. The spectra pertaining to the interaction of $6.00x10^{-5}$ M CBZ and $6.00x10^{-5}$ M Fe(III) in mixture for 5, 15, 30 and 60 minutes, were obtained by using $6.00x10^{-5}$ M CBZ, $6.00x10^{-5}$ M Fe(III) and solvent mixture as reference solutions, in order. The spectra of the mixtures that were prepared as explained above, were obtained at 20, 30, 37, 45 and 60°C against their reference solutions. The optimum pH was found by working with the solutions prepared as in the previously determined optimum conditions, in the pH range of 2.00-8.00. In order to find out the optimum reagent (carbamazepine) ratio for the interaction of CBZ-Fe(III), a series of solutions where CBZ/Fe(III) molar ratio was changed between 1-10 were prepared, while Fe(III) concentration was kept constant at $6.00x10^{-5}$ M, using the previously determined optimum conditions. Then the spectra of each mixture was obtained against its reference solution.

The absorbances of $6.00x10^{-5}$ M CBZ-diol and $3.00x10^{-4}$ M Fe(III) in mixture were obtained after 5, 15, 30 and 60 minutes by using $6.00x10^{-4}$ M CBZ-diol, $6.00x10^{-4}$ M Fe(III) and solvent mixture as reference solutions, in order. The spectra of the mixtures prepared as explained above, were obtained 5 min after heating the solution to 20, 30, 37, 45 and 60°C against their reference solutions. The optimum pH was found by working with the solutions prepared as in the previously determined optimum conditions, in the pH range of 2.00-8.00. In order to find out the optimum reagent ratio (CBZ-diol) for the interaction of CBZ-diol-Fe(III), a series of solutions where CBZ-diol /Fe(III) molar ratio was changed between 1-10, while Fe(III) concentration was kept constant at $6.00x10^{-4}$ M were prepared using the previously determined optimum conditions. Then the spectra of each mixture was obtained against its reference solution.

Potentiometric

It was investigated that whether the interaction reaction of Fe(III)- CBZ and Fe(III)-CBZ-diol to make complexes according to Irwing-Rossoti method (20-22) by potentiometry.In order

to determine the protonation constant the solution including $HClO_4$ and ligand (carbamazepine) + $HClO_4$ were titrated potentiometrically using 0.1 N NaOH. Average n_A values were calculated from the titration curves.

For the calculation, the following equation is given below:

$$\mathbf{n_A} = y + [(V_1 - V_2)(N + C)] / [(V_0 + V_1)C_L]$$

Where:

V_0 = initial volume (mL)	: 25.00
N = Molarity of the base (NaOH)	: 0.1000 N
C = $HClO_4$ concentration,	: 0.0030 M
C_L = Ligand (carbamazepine) concentration	: 0.0107 M
Y = Number of protons given for carbamazepine	: 0

V_1, V_2 = NaOH volumes of V_1 and V_2 were read from the titration curves which contain $HClO_4$ and ligand + $HClO_4$. Although the titration curves that show the interaction between Fe(III)-CBZ and Fe(III)-CBZ-diol were different than the curves of CBZ and CBZ-diol (Figure 2,3) the difference between them was very small. Therefore protonation and formation constants could not been calculated.

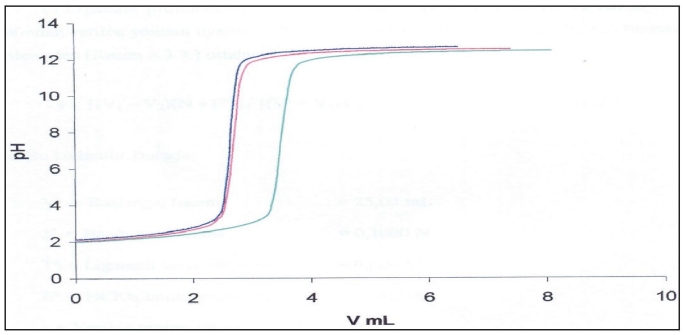

Figure 2. Potentiometric titration curves of $1.00.10^{-2}$ M $HClO_4$ solution(__), $3.00.10^{-3}$ M CBZ solution(__) and $3.00.10^{-3}$ M CBZ -$1.00.10^{-3}$ M Fe (III) mixture(__).

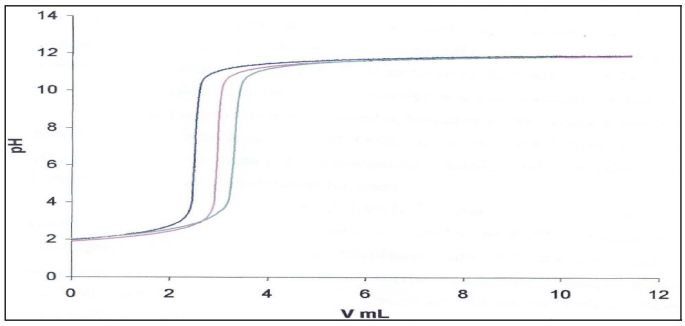

Figure 3. Potentiometric titration curves of $1.00.10^{-2}$ M $HClO_4$ solution(__), $6.00.10^{-3}$ M CBZ-diol solution(__) and $6.00.10^{-3}$ M CBZ -$1.00.10^{-3}$ M Fe (III) mixture (__).

RESULTS AND DISCUSSION

As a result of the spectrophotometric investigation of the chemical interaction pertaining to CBZ-Fe(III), the maximum interaction was observed at $6.00x10^{-5}$ M Fe(III) and $6.00x10^{-5}$ M CBZ concentrations, at room temperature, pH=2,50 in %10 dioxane solution, and after 5 minutes reaction time.The peak that shows the interaction was demolished when carbamazapin concentration is two fold higher than Fe(III) concentration and eventually disappeared when carbazapamine concentration was increased to higher levels the same concentration (figure 4). The maximum interaction reaction of 10,11 dihydroxycarbamazepine with Fe (III) was found to occur in aqueous solution at room temperature, after 5 min reaction time, at the interval of pH=2-3 and the suitable CBZ-diol concentration was found twice higher than Fe(III) concentration for Fe(III) - CBZ-diol interaction (figure 5).

When the spectrum of $6.00x10^{-4}$ M CBZ-diol and $3.00x10^{-4}$ M Fe(III) mixture, which is obtained against $6.00x10^{-4}$ M CBZ-diol reference solution, was compared with the spectrum of $3.00x10^{-4}$ M Fe(III), it was observed that the peak at 197 nm was shifted to 244 nm (Figure 6). Also when the spectrum of the mixture was obtained against Fe(III) reference solution, it was observed that the CBZ-diol peak at 224 nm was shifted to 248 nm (Figure 7). These significant wavelength shifts suggest that there is an interaction between Fe(III) and CBZ-diol.

It was investigated that whether the interaction reaction of Fe(III)- CBZ and Fe(III)-CBZ-diol to make complexes according to Irwing-Rossoti method by potentiometry. Although the titration curves that show the interaction between Fe(III)-CBZ and Fe(III)-CBZ-diol were different than the curves of CBZ and CBZ-diol (Figure 2,3) the difference between them was very small so it could not been calculated. However even the presence of that small difference indicates that there is a weak interaction and this supports the results of spectrophotometric analysis.

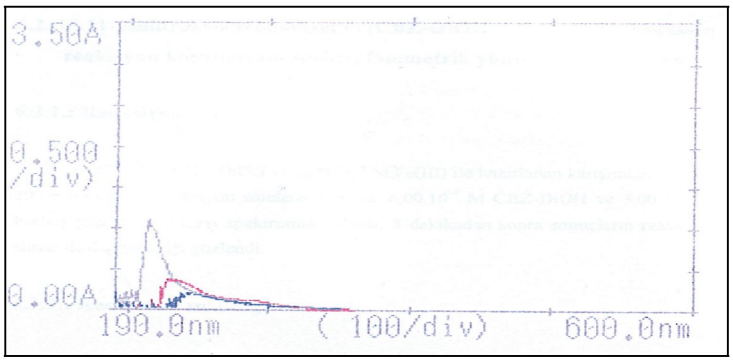

Figure 4. The spectrum of $6.00.10^{-5}$ M CBZ-$6.00.10^{-5}$ M Fe (III) mixture against $6.00.10^{-5}$ M CBZ solution (__), the spectrum of $1.20x10^{-5}$ M CBZ-$6.00.10^{-5}$ M Fe (III) mixture against $1.20.10^{-5}$ M CBZ solution (__) and the spectrum of $1.80.10^{-5}$ M CBZ-$6.00.10^{-5}$ M Fe (III) mixture against $1.80.10^{-5}$ M CBZ solution.

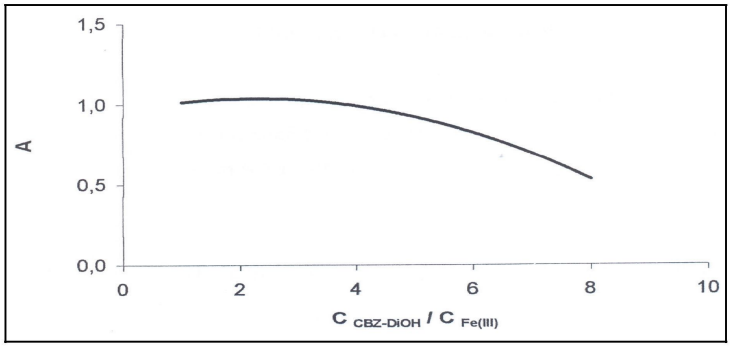

Figure 5. The optimum reagent ratio (CBZ-diol) for the interaction of CBZ-diol and Fe(III).

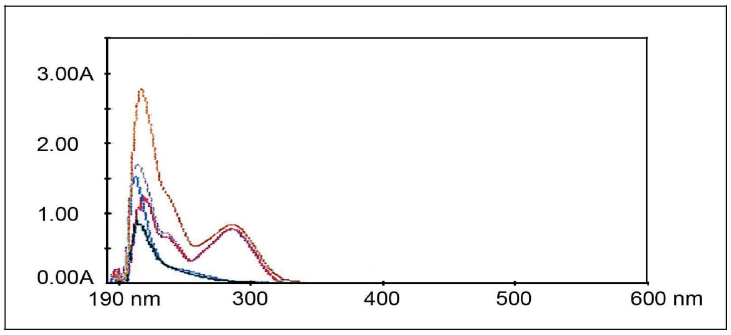

Figure 6. (__) The spectrum of $6.00.10^{-5}$ M CBZ solution against 10% dioxane solvent, (__) the spectrum of $6.00.10^{-5}$ M CBZ- $6.00.10^{-5}$ M Fe(III) mixture against $6.00.10^{-5}$ M Fe(III) reference solution, (__) the spectrum of $6.00.10^{-5}$ M Fe(III) solution against 10% dioxane solvent, (__) the spectrum of $6.00.10^{-5}$ M CBZ - $6.00.10^{-5}$ M Fe (III) mixture against $6.00.10^{-5}$ M CBZ reference solution (__) the spectrum of $6.00.10^{-5}$ M CBZ- $6.00.10^{-5}$ M Fe(III) mixture against 10% dioxane solvent.

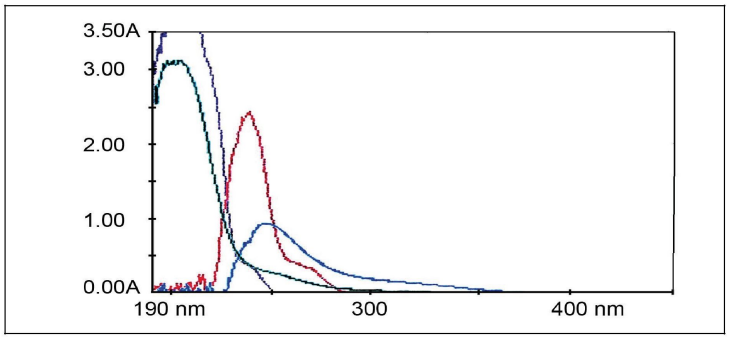

Figure 7. (__) The spectrum of $6.00.10^{-4}$ M CBZ-diol solution against water, (__) the spectrum of $6.00.10^{-4}$ M CBZ-diol - $3.00.10^{-4}$ M Fe(III) mixture against $3.00.10^{-4}$ M Fe(III) reference solution, (__) the spectrum of $3.00.10^{-4}$ M Fe(III) solution against water, (__) the spectrum of $6.00.10^{-5}$M CBZ-diol- $3.00.10^{-4}$ M Fe(III) mixture against $6.00.10^{-4}$ M CBZ-diol reference solution.

CONCLUSION

As a result of this study, an interaction was found between CBZ, CBZ-diol and Fe(III), but the results suggest that, the interactions between CBZ- Fe(III) and Fe(III)- CBZ-diol is a weak interaction reaction rather than being a reaction that forms complexes. Therefore this weak interaction is not enough to cause an anemia related to Fe(III) defficiency.

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