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The Use of Charge-Transfer Complexation in The Spectrophotometric Determination of *Amlodipine Besylate*

Ayşegül (Yardımcı) Gölcü [a], Cem Yücesoy [b] and Selahattin Serin [a,c]*

a: KSU, Faculty of Science and Arts, 46100, Kahramanmaraş, Turkey.
b: A.U, Faculty of Pharmazie, 06100, Ankara, Turkey.
c: Ç.Ü, Faculty of Science and Arts, 01330, Adana, Turkey.

Abstract

A simple and sensitive analytical method has been developed for the spectrophotometric assay of amlodipine besylate (ADB) in pure forms and tablets have been described. The method is based on the formation of a charge-transfer complex between the drug and tetrachloquinone (TCQ). This complex exhibit intense absorption bands in the electronic spectrum. The molecular ratio of the reactant in the complex was established and the experimental conditions leading to maximum charge-transfer band was also studied. The reaction proceeds quantitatively at pH 9 and 55°C for 10 min, the absorbance was measured at 346 nm. The method was applied to commercially available tablets and the results were statistically compared with those obtained by UV spectrophotometric method, using Newman-Keuls tests. In our method, Beer's Law limits to 5-25 μg/ml.
The optimum experimental parameters for colour production with reagent were studied and incorporated into procedure.

Keywords: Amlodipine Besylate, Chloranil, Charge-Transfer Complex, Spectrophotometry, Pharmaceutical formulations

1. Introduction

Amlodipine besylate (ADB) is a calcium channel blocking agent with vasodilatory activity similar to that of nifedipine[1] and it was recently introduced for the treatment of angina pectoris and hypertension [2,4]. It is chemically known as 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylicacid,3-ethyl,5-methylesterbesylate [5].

It has been determined in human plasma by HPLC [6] and GC [7,8,9]. Only two spectrophotometric methods have been reported so far for the determination of ADB [10,11]. But the charge-transfer complexes with amlodipine had not been occurred in these methods which reported.

Charge-transfer complex forming reactions used in the determination of electro-donating basic compounds through interaction with δ-acceptors [12-18] or Π-acceptors [19-21], offered sensitive, accurate and easy to carry out analytical methods.

This paper reports a simple and sensitive spectrophotometric method for the ADB in tablets. The method involves the formation of a charge-transfer complex (CTC) between the drug and chloranil (TCQ).

2. Materials and Methods

2.1 Apparatus

Double beam spectrophotometer (Schimadzu 160 A) with 1-cm quartz cuvettes was used.

Tacussel TS 70 N-1 was used for the pH measurements.

2.2 Chemicals

Amlodipine Besylate was kindly provided by Pfizer, İstanbul, Turkey and was used without prior purification. TCQ and other chemicals were purchased from Merck. All chemicals used were of analytical reagent grade quality.

2.2.1 Reagent Solution

TCQ, saturated solution in ethanol. Freshly prepared.

2.2.2 Buffer solution

Sodium tetraborate buffer solution; 0,05 M, ***pH 9.***

2.2.3 Stock Solution of Drug

Prepared by weighing accurately 25 mg of the drug into a 100-ml volumetric flask, dissolving in absolute ethanol:water (1:10) mixture and diluting to volume with distilled water.

2.3 Assay procedure for dosage forms

2.3.1 Tablets

Twenty tablets of ADB were weighed and pulverized to a fine powder. An aliquot equivalent to about 25 mg of ADB is transferred in 50 ml flasks. A suspension with 10 mL of ethanol and 10,0 ml water is shaken for 2 min and filtered to a 100 ml volumetric flask. The first flask is rinsed with small portions of water which are transferred through the same filter paper to the volumetric flask to obtain a solution of 100 ml.

2.4 Recommended Procedure For Pure Forms

Calibration Curves

0,2-1,0 ml standard solution has been transferred to test tubes. 1,0 ml buffer solution and 1,0 ml of chloranil solution are added to each tube. The tubes were maintained at 55°C in a water bath for 10 min. The absorbance of the resulting solution was measured at 346 nm against a reagent blank.

3.Results and Discussion

Chloranil (tetrachloroquinone=TCQ) occured charge-transfer complex with compounds containing primary and secondery aliphatic amines [22,23].

In this work, it has been studied that the TCQ with amlodipin at pH= 7,8,9,10 and 11 reacts or not. Coloured complex formation has been observed only at pH=9 and 10 with the studied effective substance concentration. At the both pH condition reasonable relative standart deviation results were obtained. Since maximum absorbtion values were obtained at pH=9, the experiments were decided to be carried out at that pH (Table 1).

Table 1
The pH effect to formation of CTC

	A B S O R B A N C E				
Sample No	pH=7	pH=8	pH=9	pH=10	pH=11
1	*	*	0,521	0,468	*
2	*	*	0,520	0,465	*
3	*	*	0,524	0,475	*
4	*	*	0,518	0,480	*
5	*	*	0,527	0,469	*
x	-	-	0,522	0,471	-
SD	-	-	0,004	0,006	-
%SD	-	-	0,761	1,273	-

* No colour

Coloured complex formation occurs at room temperature therefore the equilibrium time of the reaction was investigated. In order to determine the equilibrium time, the colour insenties after 10-20-30-40 minutes were compared to each other (Table 2).

Table 2
The effect of heat and heating time to colour formation

Sample No	Heat (°C) X Time(min)	Absorbance (Recovery %)
1	20 X 10	0.475 (92.4)
2	20 X 20	0.499 (97.1)
3	20 X 30	0.506 (98.4)
4	20 X 40	0.482 (93.8)
5	55 X 5	0.472 (91.8)
6	55 X 10	0.514 (100)
7	55 X 15	0.493 (95.9)
8	65 X 5	0.478 (92.9)

To determine the effect of heat on the reaction rate, the the colour intensities of series at 55°C 5-10-15 min and at 65°C 5 min, were also compared (Table 2).The maximum absorbance were obtained by heating for 10 min at 55°C (A= 0,514). The experiment at room temperature showed very close absorbance to the maximum values (A= 0,506). The followed experiments gave the results in very short time therefore the optimum conditions were determined by heating 10 min at 55°C. The determined conditions were applied to the serial which forms coloured complex in 30 min at room temperature.

In order to determine the buffer amount to keep pH constant, buffer solution of 0.5, 1.0, 1.5, 2.0, 2.5 ml were added respectively and the results were compared. It has been obsorved that the series with 0.5 and 2.5 ml of buffer solution do not show colour reaction (Table 3).

Table 3
The effect of added buffer solution (pH=9)
to colour formation

	ABSORBANCE Buffer (ml)				
Sample No	0,5	1,0	1,5	2,0	2,5
1	*	0,510	0,527	0,503	*
2	*	0,525	0,519	0,510	*
3	*	0,499	0,491	0,495	*
4	*	0,517	0,511	0,505	*
5	*	0,521	0,499	0,494	*
x	-	0,514	0,509	0,501	-
SD	-	0,010	0,015	0,007	-
%SD	-	1,954	2,953	1,40	-

*No colour

High absorbance values were obtained by addition of 1.0 and 1.5ml of buffer solution. It has been decided to the addition of 1.0 ml buffer solution in the experiment due to the lower relative standart deviation of 1.0 ml buffer added serial.

In order to determine the reagent amount required for optimum colour formation, saturated chloranil solution of 0.5, 1.0, 1.5, 2.0, 2.5 ml were added and the results were compared (Table 4).

Table 4
The effect of reagent amount to colour formation

	A B S O R B A N C E Chloranil (ml)				
Sample No	0,5	1,0	1,5	2,0	2,5
1	*	0,521	0,504	0,520	*
2	*	0,514	0,497	0,511	*
3	*	0,517	0,510	0,528	*
4	*	0,527	0,493	0,524	*
5	*	0,526	0,499	0,515	*
x	-	0,521	0,501	0,520	-
SD	-	0,006	0,007	0,007	-
%SD	-	1,152	1,403	1,353	-

*No colour

The series with 0.5 and 2.5 ml of reagent showed no colour reaction. It has been thought that addition of 0.5 ml of reagent gives unsufficient reagent concentration to form complex. It could not be explained not ot form complex by adding 2.5 ml of reagent. The relative standart deviations of the series added 1.0, 1.5 and 2.0 ml of reagent found very close to each other. Comparison of the absorbance values of the series showed that addition of 1.0 ml of reagent was enough to form complex.

Having determined the optimum conditions, the series obtained at room temperature for 30 min and at 55°C for 10 min were also investigated in forms of colour stability (Table 5).

Table 5
Colour Stability of CTC

	A B S O R B A N C E		
Heat x Time	First Measure	30.min (± variation%)	60.min (± variation)
20° x 30'	0,506	0,505 (-% 0,2)	0,516 (+%1,9)
55° x 10'	0,514	0,512 (%-0,4)	0,514 (-%0,0)

Table 6

Absorption Values of Standart ADB solutions for TCQ method

ADB (μg/ml)	0,0*	5,0	7,5	12,5	7,5	2,5	25,0
Absorbance (20° x30')	0,288	0,187	0,279	0,474	0,667	0,849	0,956
Absorbance (55° x10')	0,250	0,201	0,308	0,514	0,701	0,863	0,974

*: Blank

The experiment carried out at room temperature showed no colour change in first 30 min but in second 30 min 1.9% increase has been observed in the colour intensity. The colour of the serial obtained by heating at 55°C has been constant for 60 min. Consequently under the both conditions colour has been within the acceptable error limits for 60 min. Depending on this results, it has been decided that the chloranil method can be applied on larger sample series.

Following the stability tests, calibration curves have been plotted by using 5-25 μg/ml amlodipine added standard solution at room temperature and 55°C, respectively (Table 6). The equations obtained by least square method are as follows:

For 20°C, 30 min $y=3.8.10^{-2}x - 5.8x10^{-3}$ (r=0.9999)

For 55°C, 10 min $y=3.8.10^{-2}x + 2.3x10^{-3}$ (r= 0.9992)

These show that the method has given correct results for both conditions with 5-25 μg/ml amlodipine. It has been decided that plotting calibration curves for each experiment was necessary due to the colour reactions were effected by condition of media.

Molar absorbtivitiy of colour has been calculated as follow (Table 7).

For 20°C 30 min ε= 21418

For 55°C 10 min ε= 22661

Table 7
Molar absorptivity (ε) values of CTC.

	20^{o} X 30'	55^{o} X 10'
ε	21209.54	22797.42
ε	21096.12	23288.91
ε	21504.43	23319.15
ε	21614.61	22716.41
ε	21398.57	21751.44
ε	21685.90	22094.22
ε	21418.20	22662.23
SD	230.36	632.08
%SD	1.08	2.80

The method has been applied to the samples containing 10 mg amlodipine and the results (Table 8) were compared using Newman-Keuls test [24] as statisties by spectrophotometric method supplied by Pfizer company (Table 9).

Table 8
Analysis of amlodipine (Norvasc) Tablets

	F O U N D (mg)		
ADB in Tablets (mg)	Chloranil I	Chloranil II	UV Method
10	9,14	9,84	9,80
10	10,14	9,95	9,81
10	9,34	9,82	9,77
10	9,23	9,99	9,85
10	9,18	9,99	9,72
10	9,42	9,78	9,89
x	9,41	9,90	9,81
SD	0,37	0,09	0,06
%SD	3,93	0,91	0,60

*I: (20°C x 30')
II: (55°C x 10')

Table 9

Comparative studies for amlodipine dosage forms

	TCQ-I	UV	TCQ - II
TCQ-I	-	0.40**	0.49**
UV	-	-	$8.83.10^{-2}$

** Meaningful difference for p = 0.01.

There has been no significant difference at pH= 0.05 level between the results of serial forming coloured complex by heating at 55°C and the results of serial using UV method. Depending on this, it has been decided that this method can be applied safely on determination of amlodipine by forming coloured charge-transfer complex at 55°C. The serial studied at room temperature showed significant difference at p=0.01 level with both those at 55°C and those used UV method. In this case it has been decided that the colour forming at room temperature will not use to determination of amlodipine.

Chemistry of Coloured Species:

In this method, the formation of charge-transfer complex is based on the basic nature of the drug (ADB), which under specified experimental conditions forms a charge-transfer complex with the tetrachloroquinone (TCQ).The stoichiometric balance between ADB and TCQ was investigated using Job's Continous Varitation method [25]. The results obtained showed that 1:2 complexes were formed between ADB and TCQ. The structural representation of the complex is shown in Scheme 1. Fig.1 shows the UV-VIS absorbtion spectra of ADB–TCQ charge-transfer complexes.

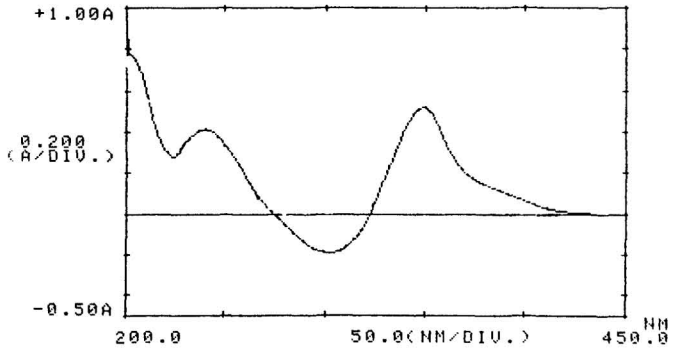
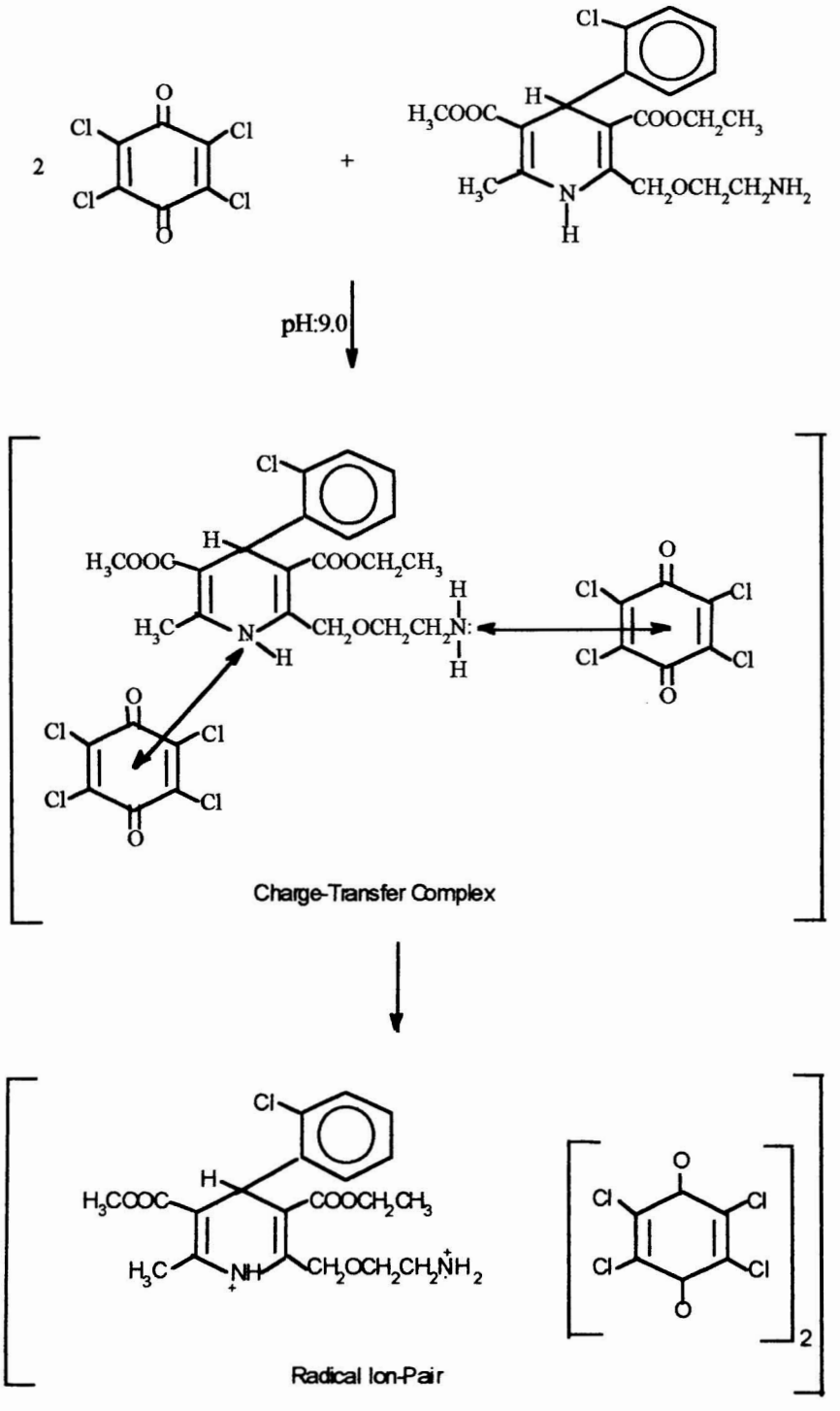


Fig.1. The UV-Visible absorption spectra of ADB-TCQ charge-transfer complex.



Scheme I

Conclusions

Amlodipine possesses a primary amine group on the side-chain attached to the dihyropyridine ring. Colorimetric assay method based on the reaction of the amine group with chloranil was developed. Chloranil gives with amlodipine a colored charge-transfer complexe, if heated 55°C for 10 min. The calibration equation for 5-25 μg/ml amlodipine besylate is y=0,038x – 0,006 (r=0,9992). Since the colored complexe is stable for 60 min, the method can be applied to large series of samples.

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