Full Paper

Rapid Differential Pulse Voltammetric Determination of Galantamine on the Simple Glassy Carbon Electrode; Alternative for the Standard Methods

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Abstract-   The electrochemical behavior of Galantamine (Gal) in aqueous media has been examined by cyclic and differential pulse voltammetry. The voltammograms were recorded with μA utolab FRA2 Potentiostat-Galvanostat. A three-electrode system was used with a glassy carbon as working electrode, a platinum rod as the counter electrode and an Ag/AgCl as the reference electrode. All tests were carried out under room temperature and N_2 atmosphere. The pH strongly affects the peak potential of Gal. The best analytical response was obtained at pH 2.0. The cathodic peak currents were proportional to Gal concentrations in the range of 0.1–10 mM under the optimized experimental conditions. The detection limit was 0.02 mM. The effect of potential scan rate on the peak potential and peak current of Gal was investigated. The correlation of the peak currents against $v^{1/2}$ (v is the scan rate) is linear, which is very similar to a diffusion-controlled process. Proposed method applied to analysis of commercial tablets, successfully.

1. INTRODUCTION

Galantamine (Gal) (Fig. 1) is an alkaloid that occurs naturally in various plant species of Galanthus and Narcissus [1]. Its IUPAC name is "4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro [3a,3,2-ef] benzazepin-6-ol" and several publications have reported the syntheses of (-)-galantamine and of racemic galantamine [2,3,4].
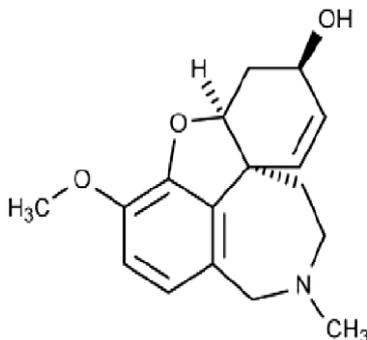
Fig. 1. Structure of 4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H benzofuro [3a,3,2-ef] benzazepin-6-ol (Gal)

Galantamine hydrobromide is an acetylcholinesterase (AChE) inhibitor that is used to moderate and delay the manifestation of Alzheimer's disease (AD) symptoms. Galantamine, commercially available as Razadyne, galantamine hydrobromide, is the most recently approved AChE inhibitor in Europe by the European registration bureau and in the USA by the FDA for the symptomatic treatment of AD [5].

So far, several analytical methods for the determination of pharmaceutical mixtures including voltammetry [6], HPLC [7-10], spectrofluorimetry [11], spectrophotometry [12], capillary electrophoresis [13,14], Flow-injection analysis [15-17], gas chromatography/mass spectrometry [18], thin-layer chromatography/fluorescence detection [19], ion exchange chromatography [20], amperometric determination [21], Far-IR absorption and reflection spectra [22], ion chromatography [23], cyclic voltammetry [24] and liquid chromatography with fluorescence detection [25] had been reported.

Standard analytical methods for galanthamine and other acetylcholinesterase inhibitors that combine, HPLC, on-line coupled UV, MS, and biochemical detection has been published [26].

Electrochemical analytic technique is an attractive method due to simplicity, rapidity, low expense, high sensitivity and possibility of miniaturization. Construction of electrochemical sensors continues to be an area of great interest and a relatively large amount of electrochemical research has been devoted to the development and application of these sensors for the analysis of biologically active compounds [25,27-29].

We assess in this work the benefit of the electroanalytical technique for galantamine determination in order to introducing the new alternative standard method, via analysis of the pharmaceutical samples of the Gal. Recently, the extent of reports regarding Gal determination has significantly increased, and therefore, an efficient alternative method may be of interest.

2. EXPERIMENTAL

2.1. Chemicals and reagents

All reagents were of analytical grade (Fluka or Merck). All solutions were prepared with double-distilled water. Stock solutions of Gal were freshly prepared as required at the desired pH (1.0-13.0) and purged with pure nitrogen gas (99.999%) for 5 min before the voltammetric measurements. During the experiments, nitrogen gas was passed over the surface of test solutions in order to avoid entrance of oxygen into the solution.

2.2. Apparatus

The voltammograms were recorded with µA utolab FRA2 Potentiostat-Galvanostat. A three-electrode system was used with glassy carbon electrode (GC ROD 2 mm/76 mm) as working, a platinum rod as the counter and an Ag/AgCl as the reference electrode (Autolab comp.). All tests were carried out under room temperature. A digital pH meter (Jenway 370) was applied for pH adjustment.

3. RESULTS AND DISCUSSION

3.1. Voltammetric studies on Gal

In this work, glassy carbon was tested as working electrode. As shown in Fig. 2, Gal generates appropriate response on this electrode. The observed wave at 0.712 V (*vs*. Ag-AgCl reference electrode) maybe ascribed to the following process Fig. 3.

3.2. Effect of solution pH

The effect of pH of the Gal solution (2.0 mM) on the electrode potential was investigated. The electrochemical behavior of the Gal was studied in a large pH range between 1.0 and 13.0. The pH value was adjusted gradually using NaOH or HCl.
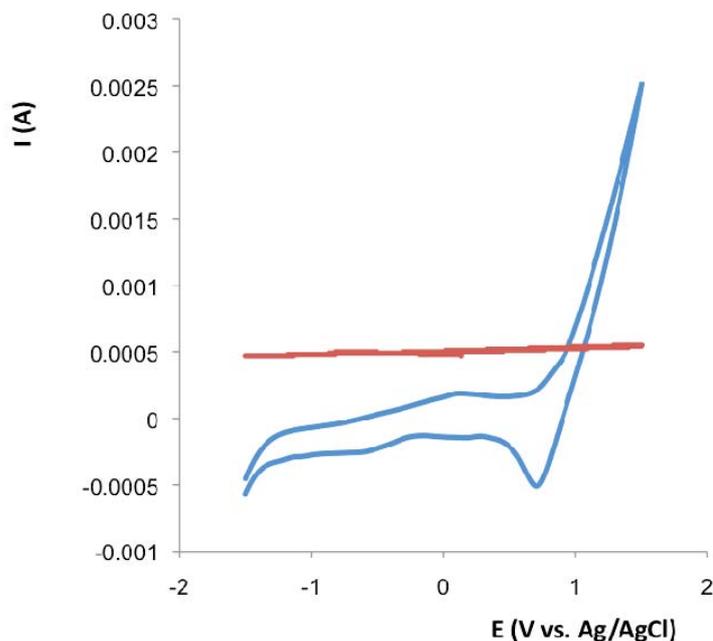
Fig. 2. Cyclic voltammograms of (a) Blank and (b) 5.0 mM Gal on the glassy carbon electrode at pH 2.0 and scan rate 200 mV s^{-1}
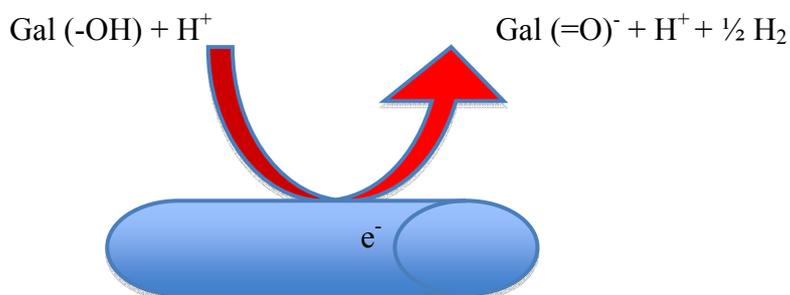
**Fig. 3.** Proposed mechanism for electro-reduction of Gal at the glassy carbon surface

Fig. 4 illustrates the dependence of the peak potential and peak current on the pH of the solution. As can be seen, the highest sensitivity was obtained at pH 2.0 (Fig. 4A). The cathodic peak potential corresponding to the Gal reduction shows a linear variation with pH with a slope of –48.5 mV/pH (R^2=0.9901) as shown in Fig. 4B. This suggests that the total number of electrons and the protons taking part in the charge transfer is the same. When the pH value was lower or upper than 2.0, the cathodic peak current of Gal would decrease and peak potentials shift toward positive and negative direction, respectively. Therefore, the results indicate that a solution at pH 2.0 could be chosen for further studies.
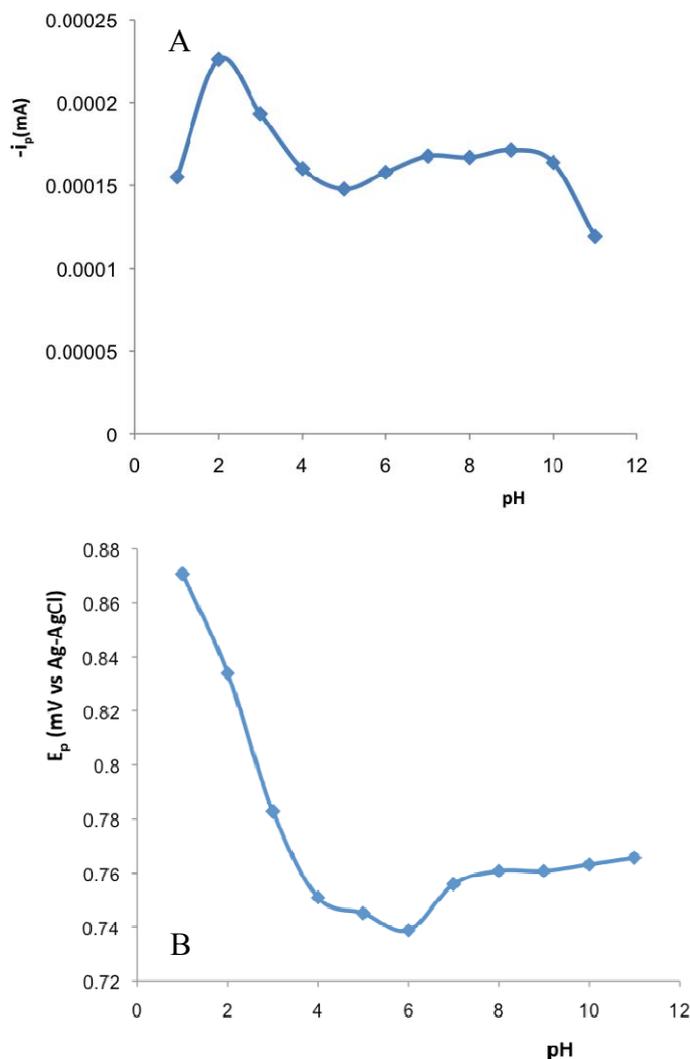
Fig. 4. Influence of pH on the (A) peak current and (B) peak potential of Gal in solution containing 2.0 mM Gal at scan rate 200 mV s^{-1}

3.3. Influence of scan rate

The effect of potential scan rate between 25 and 400 mV s^{-1} (Fig. 5) on the cathodic peak current of Gal was also evaluated. A linear relationship was found between I_{pc} and the square root of the scan rate ($v^{1/2}$) in the part (50-250 mV s^{-1}) of the examined range ($R^2=0.9887$, slope=0.0205). By increasing scan rate to 400 mV s^{-1} leads to decreasing the peak currents for Gal. This is evidence that the kinetic of electron transfer on the electrode surface is not sufficiently fast as consequence of the nature of the matrix whose internal resistance is considerable. For scan rates higher than 400 mV s^{-1}, the voltammetric profiles become distorted, which can be associated with a slow electron transfer rate. From these results, a scan rate of 200 mV s^{-1} was chosen for further studies since it results in voltammograms with better peak definition.
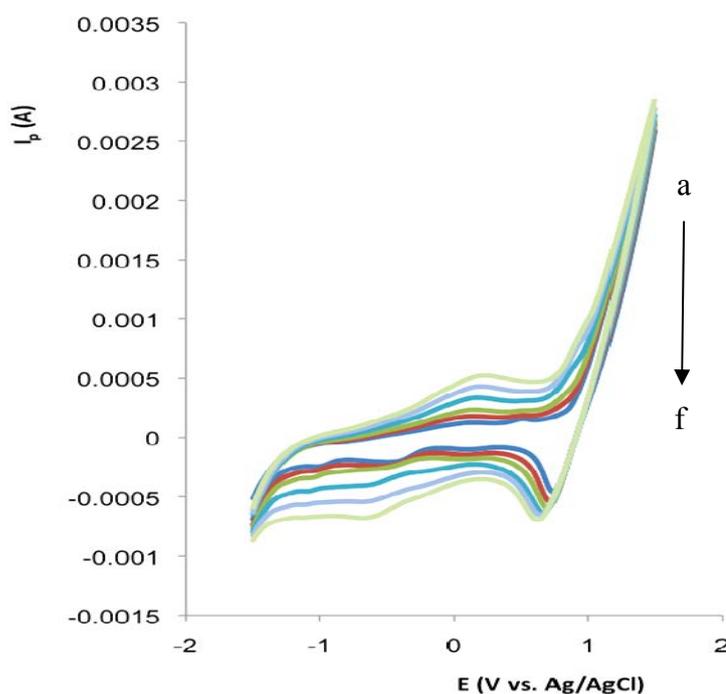
Fig. 5. Variation of peak current with potential scan rates; (a) 25 (b) 50 (c) 100 (d) 200 (e) 300 and (f) 400 mV s^{-1} in pH 2.0 and 2.0 mM Gal

3.4. Effect of supporting electrolyte

Table 1 summarizes the effect of the different supporting electrolytes on the peak current and potential of Gal at optimized condition. Accumulative data show that any noticeable changes in wave parameters including peak current and potential. Therefore, KCl was used in further tests.

Table 1. Effect of the different supporting electrolytes on the peak current and potential of Gal

Supporting electrolyte	I_p (mA)	E_p (mV)
KCl	-0.219	810
KNO_3	-0.192	783
$NaNO_3$	-0.215	773

3.5. Calibration curve and repeatability

After optimizing the operating conditions for the working electrode, cyclic voltammetric measurements were carried out in solutions containing different Gal

concentrations in the range of 0.01–35 mM (Fig. 6). The electrode has a linear dynamic range 0.1–10 mM, with R^2=0.9959 and a detection limit of 0.02 mM (three times the standard deviation of the blank/slope). The repeatability of the voltammetric measurements was evaluated making five successive cyclic voltammograms of the working electrode at optimized conditions. A Gal concentration level of 3.0 mM was selected to examine the repeatability of the voltammetric sensor. For this study, relative standard deviation was 2.2%, indicating that the electrode presents good stability and repeatability, within a confidence level of 95%.
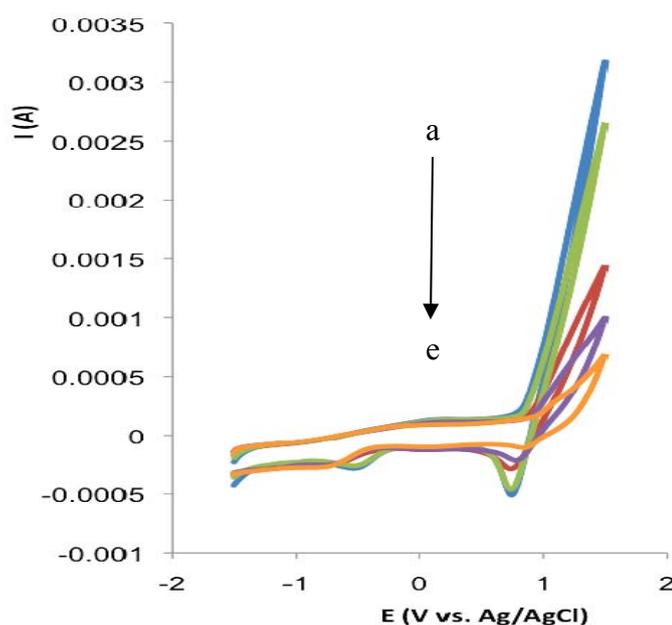
Fig. 6. Variation of peak current with concentration of Gal at pH 2.0 and scan rate of 200 mVs^{-1}; (a) 35.0 (b) 10.0 (c) 1.00 (d) 0.100 and (e) 0.0100 mM Gal

### 3.6. Analysis of real samples using proposed method

In order to evaluation of glassy carbon electrode performance in quantitative analysis of Gal, Differential Pulse Voltammetry (DPV) was applied to mentioned commercial drugs and hand making samples. Fig. 7 shows the excellent differentiation ability of proposed voltammetric method in quantitative analysis of 8, 20 mg tablets and 5, 14 mg hand making formulations.

The proposed method used for assay of Gal in commercial tablets produced by Sobhandarou pharmaceutical company (Table 2). It is evident from the table that the proposed method is very sensitive and has good accuracy with narrow toleration. From economic point of view, the proposed method is simple, rapid and all the analytical reagents are inexpensive and are available in any analytical laboratory. So, it is a good alternative to the accepted standard methods.

T **Table 2.** Determination of Gal in pharmaceutical formulations using the proposed method

Sample[a]	Stated content (mg)	Gal (mg)/ Found	Recovery (% of nominal value)
Gal, 8 mg	8.0	8.3	103.75
Gal, 20 mg	20.0	20.6	103.00

[a] Sobhandarou pharmaceutical company, Rasht, Iran
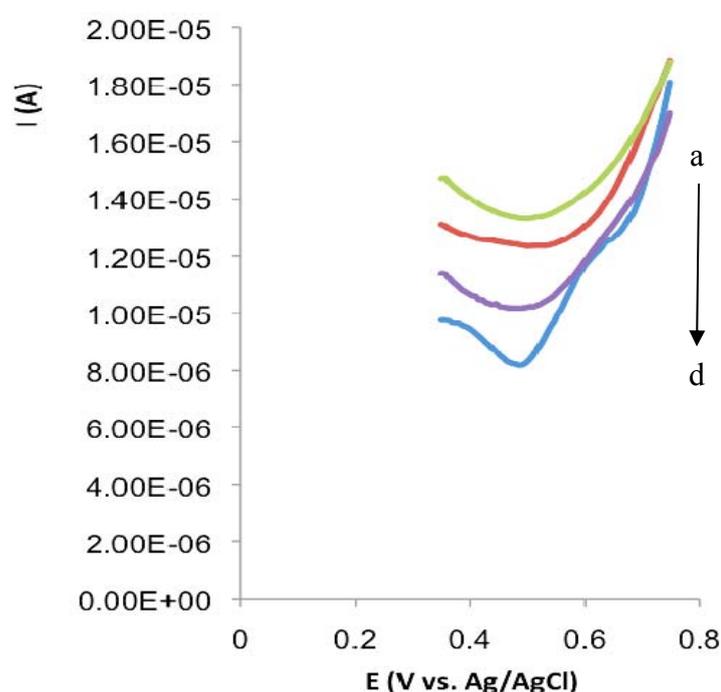
Fig. 7. Differential pulse voltammograms of commercial tablets; (b) 8 (d) 20 and hand making samples; (a) 5 (c) 14 mg Gal in pH 2.0 at glassy carbon electrode

4. CONCLUSION

The proposed method describes a rapid method for the Gal assay without tedious steps that are necessary in chromatographic standard methods. Hence, it can be recommended for the routine quality control of the studied drug either in bulk or in pharmaceutical formulations. The proposed method was based on the reduction of Gal by appropriate potential at the glassy carbon electrode. The principal advantage of the proposed method is its simplicity and rapidity. The proposed method offers advantages in that only a small amount of drug or dosage formulations are required for analysis.

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REFERENCES

[1] S. Lopez, J. Bastida, F. Viladomat, and C. Codina, Phytochem. Anal. 13 (2002) 311.

[2] B. M. Trost, and W. Tang, Angew. Chem. Int. Ed. Engl. 41 (2002) 2795.

[3] C. Guillou, Angew. Chem. Int. Ed. Engl. 40 (2001) 4745.

[4] M. Node, Angew. Chem. Int. Ed. Engl. 40 (2001) 3060.

[5] J. Marco-Contelles, M. do Carmo Carreiras, C. Rodeŕıguez, M. Villarroya, and A. G. Garćıa, Chem. Rev. 106 (2006) 116.

[6] F. Mattivi, U. Vrhovsek, and G. Versini, J. Chromatogr. A 855 (1999) 227.

[7] M. M. Yust, J. Pedroche, J. G. Calle, J. Vioque, F. Millan, and M. Alaiz, Food Chem. 85 (2004) 317.

[8] J. Yang, S. Wang, J. Liu, and A. Raghani, J. Chromatogr. A 1156 (2007) 174.

[9] V. P. Hanko, and J. S. Rohrer, Anal. Biochem. 308 (2002) 204.

[10] S. Oshite, M. Furukawa, and S. Igarashi, Analyst 126 (2001) 703.

[11] K. K. Verma, and A. Jain, Talanta 35 (1988) 35.

[12] G. Chen, J. S. Cheng, and J. N. Ye, J. Fresenius' Anal. Chem. 370 (2001) 930.

[13] J. Elek, D. Mangelings, T. Ivanyi, I. Lazar, and Y.V. Heyden, J. Pharm. Biomed. Anal. 38 (2005) 601.

[14] N. T. Abdel-Ghani, A. F. Shoukry, and S. H. Hussein, J. Pharm. Biomed. Anal. 30 (2002) 601.

[15] S. Hanaoka, J. M. Lin, and M. Yamada, Anal. Chim. Acta 409 (2000) 65.

[16] M. I. Evgen'ev, S. Yu. Garmonov, L. Sh. Shakirova, and E. V. Degterev, Pharm. Chem. J. 36 (2002) 559.

[17] J. P. Zanetta, A. Pons, C. Richet, G. Huet, P. Timmerman, Y. Leroy, A. Bohin, J. P. Bohin, P. A. Trinel, D. Poulain, and J. Hofsteenge, Anal. Biochem. 329 (2004) 199.

[18] N. Kato, T. Kojima, S. Yoshiyagawa, H. Ohta, A. Toriba, H. Nishimura, and K. Hayakawa, J. Chromatogr. A 1145 (2007) 229.

[19] G. Ravindran, and W. L. Bryden, Food Chem. 89 (2005) 309.

[20] G. P. Jin, and X. Q. Lin, Electrochem. Commun. 6 (2004) 454.

[21] G. Dovbeshkoa, and L. Berezhinsky, J. Mol. Struct. 450 (1998) 121.

[22] R. L. G. Norris, P. J. Ravenscroft, and S. M. Pond, J. Chromatogr. B 661 (1994) 346.

[23] J. Saurina, S. H. Cassou, E. Fabregas, and S. Alegret, Analyst 124 (1999) 733.

[24] C. D. Andrade, J. A. Rufian-Henares, S. Jimenez-Perez, and F. J. Morales, Food Chem. 98 (2006) 580.

[25] A. Walcarius, J. Solid State Electrochem. 10 (2006) 469.

[26] K. Ingkaninan, C. M. de Best, R. van der Heijden, A. J. P. Hofte, B. Karabatak, H. Irth, U. R. Tjaden, J. van der Greef, and R. Verpoorte, J. Chromatogr. A 872 (2000) 61.

[27] L. Moreno, A. Merkoçi, S. Alegret, S. Hernández-Cassou, and J. Saurina, Anal. Chim. Acta, 507 (2004) 247.

[28] G. Heriem, S. Monney, V. Blondeau-Patissier, B. Fahys, and T. Gharbi, Electrochim. Acta 54 (2009) 6797.

[29] K. Majumder, and S. Bhattacharya, Polyhedron 18 (1999) 3669.