Flow Injection Amperometric Determination of Ascorbic Acid Using a Photoelectrochemical Reaction after Immobilization of Methylene Blue on Muscovite

Yusuf DİLGİN, Zekerya DURSUN, Gürel NİŞLİ
Ege University, Faculty of Science, Department of Chemistry,
35100 Bornova, İzmir-TURKEY

The photoelectrochemical determination of ascorbic acid (AA) was studied based on the photochemical reduction of methylene blue (MB) in 0.1 M phosphate buffer (pH 7.0). MB was used as a redox mediator for the modification of a carbon paste electrode (CPE) due to its facile reducible-oxidizible behaviour. Muscovite, which has a layered structure, was found to be a good and stable supporting material for the immobilization of MB by an ion exchange reaction. The dye is strongly retained and not easily leached from the matrix. MB was reduced to nearly quasi-reversible at the modified carbon paste electrode (MCPE). The oxidation peak potential of leuco-methylene blue (LMB) shifted from –100 mV to 50 mV in the presence of AA. For photoelectrochemical amperometric studies, the operational potential was kept constant at + 50 mV according to the oxidation of LMB, which was produced from the chemical reaction between AA and MB on the modified electrode surface. A laboratory-built flow cell system was constructed for the direct irradiation of the electrode surface with a 500-W halogen lamp. The optimum conditions for the flow injection (FI) amperometric determination of AA were 1.5 mL/min flow rate, 50 μL sample loop and 50 cm transmission tubing length and at a frequency of 60 samples per hour. AA could be determined in the concentration range 1.0 x 10^{-6}-1.0 x 10^{-4} M by using a photoelectrochemical FI method. The detection limit of this method was 1.0 x 10^{-8} M. The relative standard deviation of five replicate injections of 6.0 x 10^{-5} M AA was 2.0 % in photoelectrochemical FI. The results obtained by the proposed procedure are in good agreement with those established using the triiodide procedure for the AA determination of pharmaceutical products.

Key Words: Photoelectrochemical analysis, flow injection analysis, ascorbic acid, muscovite, methylene blue.

Introduction

Ascorbic acid (AA), a water soluble vitamin, is an important micronutrient and plays many physiological roles. AA and its oxidized form, dehydroascorbic acid (DHAA), are biologically active where AA is involved extensively as an antioxidant. Many methods have been reported for the determination of AA such as

titration with oxidants[1], the indirect spectrophotometric method[2], enzymatic analysis[3] and electrochemical analysis[4,5].

Chemically modified electrodes (CMEs) show significant potential for applications in the electroanalytical chemistry of some redox processes. Various methods are available for the modification of electrodes by electrocatalytically active organic or inorganic compounds[6-8]. In recent years, phenoxazines and phenothiazines have been widely used as a redox mediator by immobilization onto layered solids[9-11].The most commonly used layered host materials are metal phosphates[12,13], clays[14], silicates[15], titanates[16] and mica[17]. Some dye compounds such as, methylene blue (MB) and Prussian blue have been shown to be effective electron acceptors for AA at glassy carbon electrode (GCE)[18,19], or carbon paste electrode (CPE)[20]. The electrochemical oxidation of AA at GCE or CPE is irreversible and has a large overpotential, thus decreasing its use in analytical processes. MB has a reversible electrode reaction by a two-electron transfer. This property of MB has led to its use as a redox mediator for the determination of AA by decreasing the overpotentials of the analyte both in MB aqueous solution[18] and by immobilization onto a host-layered compound[20]. Several flow injection (FI) methods with electrochemical detection have also been widely used for AA determination using oxidants such as copper phthalocyanine[5], Prussian blue[19] in modified electrodes and periodate[5], and MB[18] in a carrier stream. A Prussian blue film modified on GCE was prepared electrochemically and was used for amperometric FI determination of AA, but the mediator potential was found to be quite high (+270 mV) compared to the applied potential of AA. Similar studies on the amperometric FI detection of AA were investigated[19,21-24]. However, the photoamperometric FI detection of AA has not been studied using a modified electrode. Only one study based on the photochemical reduction of MB using a carrier stream has been applied to the amperometric FI determination of AA at unmodified GCE. This method seems to be simple, but the consumption of MB was high due to the continuous transporting in the carrier stream. The sensitivity of the method was also poor (1.0×10^{-4}M).

Recently, MB has been immobilized into α-zirconium phosphate (ZrP) by an intercalation reaction[20]. The amount of MB immobilization into ZrP was influenced in the presence of n-butylamine (nba). The material was used as a modifier for a CPE and then it was applied to the photoelectrochemical oxidation of AA. However, the sensitivity of the modified electrode was found to be poor. Mica is another popular model substrate for surface studies due to its availability in sheet form and its cation exchange properties. Muscovite mica (ideal formula $K_2Al_4(Al_2Si_6O_{20})(OH)_4$, M = 796.6) is a chemically inert aluminosilicate mineral; mucovite OH groups are buried 0.4 nm below the surface[25]. Due to the smooth, well-defined surface of muscovite sheets, they are widely used as substrates for surface force measurements[26]. Although MB is adsorbed on mica via ion exchange[27], the MB^+/K^+exchange was quite slow[28] and MB-adsorbed muscovite has not been used as an electrode material. The main purposes of this study were to (1) optimize MB immobilization onto muscovite and then apply for the preparation of modified carbon paste electrode (MCPE), (2) construct a new flow-cell for the photoelectrocatalytic FI investigation of AA at MCPE and (3) apply of the above procedures to the determination of AA in pharmaceutical products.

Experimental

Apparatus

Voltammetric measurements were carried out using a Metrohm 694 stand and 693 processor. A Tacussel PRT 500 LC potentiostat in conjunction with a GRSO Tacussel recorder was used for photoelectrochemical FI amperometric studies. The three-electrode system consisted of a home-made modified CPE (i.d. 3 mm), platinum wire electrode and saturated calomel electrode (SCE). The photoelectrochemical FI determination of AA was carried out using a MCPE embodied in a new laboratory-built flow cell. The pH was adjusted by means of a Metrohm E 510 pH-meter with a combined glass electrode. FTIR (Perkin Elmer 1600 series FTIR) was used for the IR spectra of samples as KBr discs. A Shimadzu UV 160A spectrophotometer was used for spectrophotometric measurements. An eight-channel microtube MP-3 (Eyela, Tokyo Rikakikai) peristaltic pump supplied with polyethylene tubing (0.75 mm i.d.) was used for the propulsion of the carrier stream. The sample was introduced into the system by means of a Rheodyne 7125 sample injection valve with 50 μL loop volumes. A 500-W halogen lamp was used for electrode surface irradiation. The structure of the muscovites was photographed with a LEICA DM LSP Polarizan microscope.

Reagents, solutions and sample preparation

All reagents (MB, HNO_3, C_2H_5OH, NaOH, NH_3 n-butyl amine (nba), $Na_2HPO_4.2H_2O$, $NaH_2PO_4.12H_2O$, KIO_3, KI, $Na_2S_2O_3$, H_2SO_4, starch) were of analytical grade. A stock solution of AA (1.0×10^{-2} M) was prepared daily in deoxygenated ultrapure water (Elga 18.2 MΩ cm, UK) or in phosphate buffer solution of pH 7.0 and stored in a dark and cool place. Other concentrations were obtained by diluting the stock solution with ultrapure water. Buffer solutions were prepared from sodium dihydrogenphosphate (0.1 M), and disodiumhydrogenphosphate (0.1 M). Natural muscovite was purchased from Dokuz Eylül University Department of Geological Engineering. The solid pharmaceutical products Redoxon (Roche) and Sandoz (Novartis) were dissolved in phosphate buffer (pH 7.0) solution by grinding and diluting the resulting solution to adjust the concentration to that required by the experimental conditions adopted. The triiodide procedure was used as a standard method for determining AA[29]. The I_3^- reagent was prepared from 50 mL 10^{-2} M KIO_3, 2.0 g KI and 10 mL 0.5 M H_2SO_4 and 10 ml of a 10-fold diluted sample solution was added to the I_3^- reagent, and then this solution was titrated with 0.07 M $Na_2S_2O_3$ using starch indicator.

Preparation of muscovite and its pretreatment

Natural muscovite was separated as a thin sheet and washed first with concentrated HNO_3, then with pure water to remove the acidity and finally with ethanol. The material was kept at 150°C in an oven for 24 h. The dried muscovite was ground in an agate mortar then sieved to obtain various particle sizes for pretreatment. A 0.2 g 100 mesh muscovite was mixed with 2 ml of phosphate buffer and 0.2 mmole MB by stirring for 24 h. In the pretreatment step, the above procedure was repeated by adding various amounts (0.2, 0.0, 1.0, 2.0, 4.0, and 8.0 mmole) of nba to investigate its effect. In an alternative procedure, the pretreatment of muscovite by nba was also carried out before the adsorption of MB. The materials were then washed many times with ultrapure water to remove excess MB and dried at 110°C. The final material was used for the preparation of the MCPE.

Adsorption capacity of muscovite for MB

To get the adsorption rate, suspensions obtained from 50 mg muscovite in 10 mL 2.0 x 10^{-4} M MB were stirred for the desired time interval (3, 5, 24, 54 and 96 h) for each suspension. Each suspension was centrifuged at 2000 rpm for 30 min using a Janetzki centrifuge T5. Each centrifugate was diluted and measured at 664 nm using spectrophotometry.

In order to measure the adsorption capacity of muscovite with MB, various amounts of muscovite in the range 5-200 mg were added to vials each containing 2 x 10^{-4} M MB. Then these mixtures were stirred for 6 h and centrifuged. Each centrifugate was diluted and measured at 664 nm spectrophotometrically. This procedure was repeated for muscovite which had been pretreated with nba. For the pretreatment of muscovite, 2.5 mL nba and 1.5 g muscovite was mixed in a vial and the suspension stirred. Then the solid was dried at room temperature.

Preparation of working electrodes

Unmodified carbon paste (CP) was prepared from 3.25 g of spectroscopic grade graphite powder by mixing with 1.75 g mineral oil in an agate mortar. The modified carbon paste was prepared by the thorough mixing of spectroscopic-grade graphite powder and muscovite or MB-adsorbed muscovite with mineral oil in various composition ratios. The unmodified CP was rigorously packed into a glass tube (3 mm i.d. and 5 cm length). The modified CP was packed as a thin layer (at least 2 mm) onto the bottom of the glass tube containing the CP. Electrical contact to the paste was established via a thin Pt wire (1 mm i.d). The electrode surface was polished and smoothed using weighing paper until a shiny surface was obtained, and then washed with ultrapure water. For each set of experiments, extruding a small plug of the paste by pushing with a copper rod generated a fresh electrode surface.

Construction of photoamperometric flow-cell

A new photoamperometric flow-cell was constructed from a Teflon block with a transparent window for direct electrode surface irradiation. The three-electrode system was embodied into the flow-cell (Figure 1).
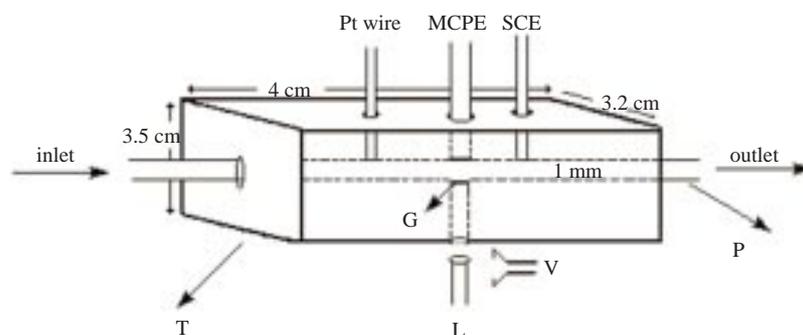
Figure 1. Design for laboratory-built flow-cell used for photoamperometric FI studies. T: teflon block, L: light source V: ventilation, P: transmission tube, G: glass window.

Voltammetric and FI amperometric procedure

A solution of 25 mL phosphate buffer (pH 7.0) was placed in a thermostatic voltammetric cell. The solution was purged with nitrogen for at least 10 min before starting the measurements. During all cyclic (CV) and differential pulse (DP) voltammetric measurements, the flow of gas was passed over the solution. The i-E curves were recorded in the potential range of –1.0 to + 1.0 V vs. SCE using unmodified or modified CPEs. The effect of AA concentration was studied by adding its solution into the buffer and then the i-E curves were recorded with or without irradiation onto the MCPE surface. In the photoelectrochemical FI experiments, phosphate buffer solution as a carrier stream, 50 μL sample loop, 1.5 mL/min flow rate and 50 cm transmission tubing length were used. A schematic diagram of the FI configuration is shown in Figure 2. A 500-W halogen lamp was used for the irradiation of the MCPE surface. Various concentrations of AA or dissolved and diluted pharmaceutical samples were injected to the carrier stream either directly or under irradiation of the electrode surface. The peak currents were measured in phosphate buffer solution at + 50 mV constant potential (vs. SCE).
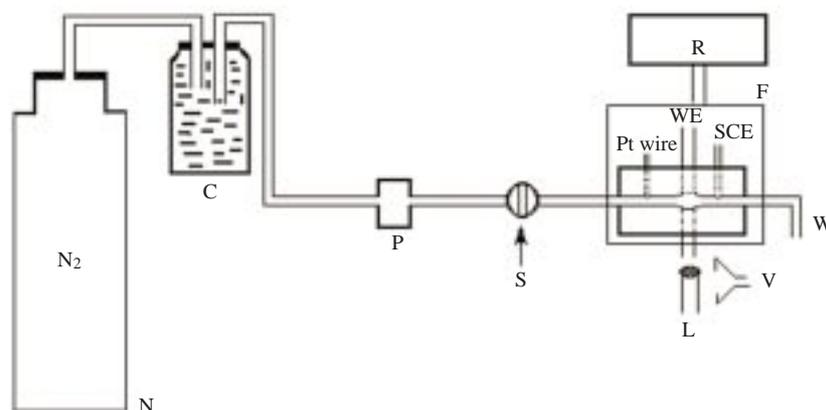
Figure 2. Schematic diagram of the flow injection amperometric (FIA) manifold: N: nitrogen gas, C: carrier stream, P: peristaltic pump, S: sampling valve, F: flow-cell (detector), L: light source, V: ventilation, W: waste, R: recorder, WE: working electrode.

Results and Discussion

Characterization of MB adsorbed muscovite

Surface characterization of MB adsorbed on muscovite was made using FTIR, a polarizable microscope and voltammetric techniques. MB-adsorbed muscovites were first characterized by infrared spectroscopy and compared to the corresponding unmodified solid materials. The results are shown in Figure 3. There is no peak at muscovite and nba treated muscovite, and even nba has CH_3 and NH_2 groups. An nba-treated and MB-adsorbed muscovite sample was characterized by CH_3 and aromatic C-H vibrations of MB at 2928 and 1600 cm^{-1} respectively. The peak intensities of MB were increased depending on nba ratio due to the increase in the adsorption of MB. A 40-fold blown-up photograph from the Polarizan microscope showed that MB adsorption on muscovite was carried out (Figure 4), and nba plays an important role in the adsorption of MB on muscovite as an increasing factor. Although nba seems to be a nonionic molecule, it has a dipole character that can be easily adsorbed on muscovite then it can exchange with MB cation. Muscovite mica

is a 2:1 layered aluminosilicate and each 2:1 layer consists of two tetrahedral silica sheets sandwiching one octahedral alumina sheet. Since on average one silicon atom out of four in tetrahedral sheets is replaced by aluminium, the layers are negatively charged, and can hold both interlayer cation in the structure[25] and the electropositive side of nba. Another possibility is attachment through the proton acceptor property of muscovite due to the OH^- groups in the structure because alkyl amine groups tend to be protonated with the Si-OH group in the solid phase. The nba could be attached to the layer of muscovite during the pretreatment process and doffer suitable sites the for the binding of MB onto the muscovite. Various organic cationic compounds can absorb onto the muscovite surface and possibly also within its interlayer like in clay and ZrP. Generally, intercalation was mentioned in many works done with ZrP, clay and especially in the case of synthetic mica[30−32], which has a sheet structure similar to that of muscovite. The preliminary XRD powder results indicated that there was no major change in the interlayer distance of the muscovite sheets, although there was a minor change in the crystal structure.

The optimum adsorption time of MB to muscovite was found to be 6 h (Figure 5). While the adsorption capacity of untreated muscovite was 18 μmol/g muscovite, pretreatment by nba afforded an increased adsorption capacity of 30 μmol/g muscovite (Figure 6). The adsorption isotherms obtained from these data were fitted to Langmuir equations and showed that the MB distribution on the muscovite was homogeneous.
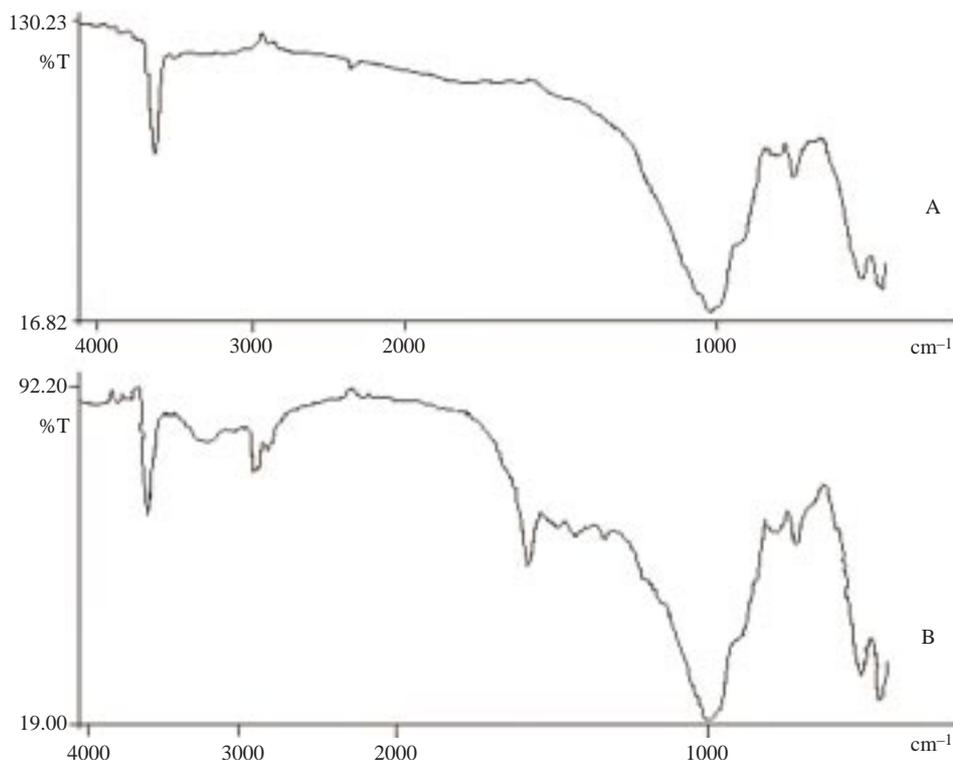
Figure 3. IR spectra of muscovite treated with A) 8 mmole nba, B) 8.0 mmole nba and 0.2 mmole methylene blue (MB).
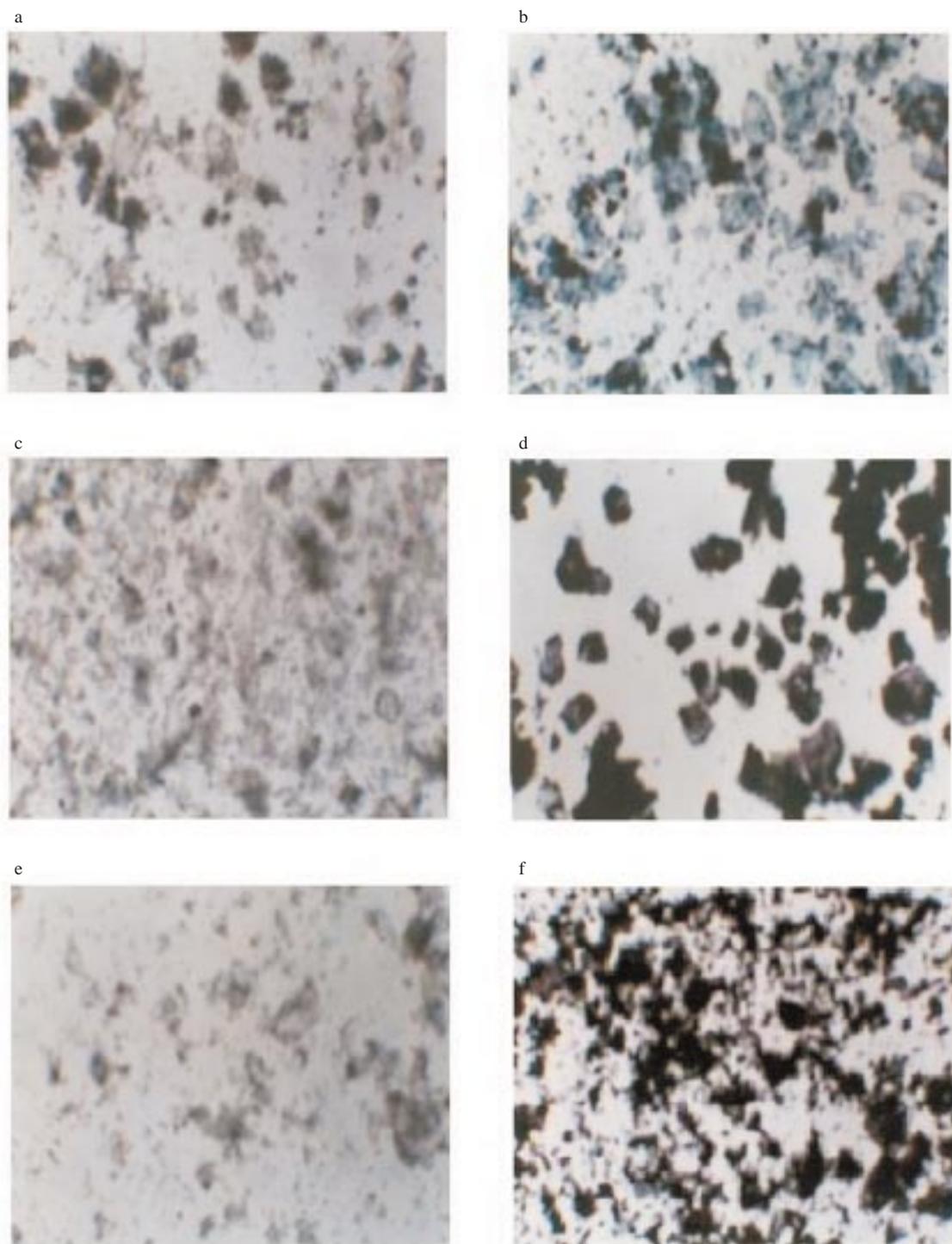
Figure 4. The photographs obtained from a Polarizan microscope a) natural muscovite, b) muscovite in the presence of 0.2 mmole MB, c) muscovite in the presence of 4 mmole nba, d) muscovite in the presence of 4 mmole nba, and 0.2 mmole MB, e) muscovite in the presence of 8 mmole nba, and f) muscovite in the presence of 8 mmole nba and 0.2 mmole MB.

$[MM^+]_0 = 2.10^{-4}$ M $A_0 = 40$ μmol/g muscovite
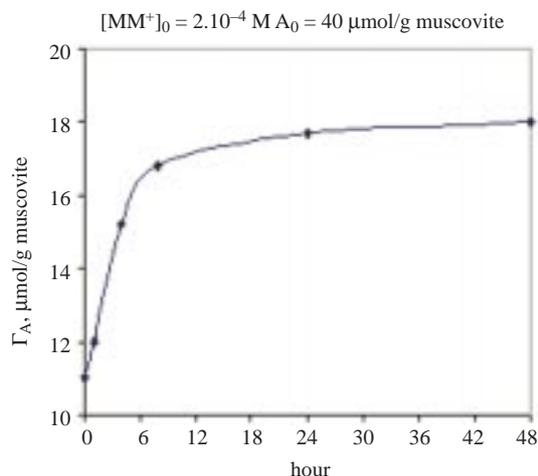
Figure 5. Adsorption rate of MB^+ (2.10^{-4} M 10 mL) on muscovite (50 mg), Γ_A is the equibliurium MB^+ adsorption.

A. $[MM^+]_0 = 1.10^{-4}$ M

B. $[MM^+]_0 = 1.10^{-4}$ M
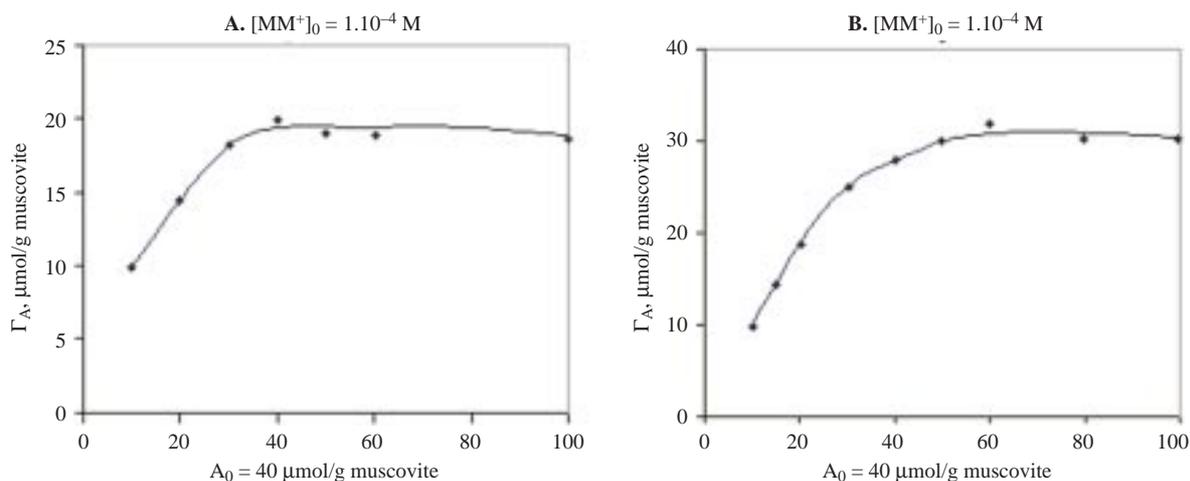
Figure 6. Dependence of the equibliurium MB^+ adsorption (Γ_A) on the initial amount of MB^+ (A_o) for A) muscovite B) pretreatment muscovite with nba.

A stable and constant differential pulse peak current was obtained with the MCPE, which was kept in the buffer solution during consecutive scanning for one interval (Figure 7). The voltammetric signals showed that MB was stable and not rediffused from the electrode surface to the buffer solution. In contrast, adsorbed MB on an unmodified CPE was found to be unstable and the back diffusion of MB from the CPE surface was observed to be rapid (Figure 8)

Cyclic voltammograms recorded with the MCPE also displayed a nearly quasi-reversible electrode process in pH 7.0 phosphate buffer solution, which is similar to the case of MB in buffer solution at a bare CPE. In contrast, no significant current was obtained by using muscovite modified CPE alone (Figure 9). It was also shown that there is no electroactive group on the muscovite surface. MB was reduced at the MCPE at –280 mV giving LMB which was oxidized at –100 mV. By comparison, AA is oxidized irreversibly at 360 mV at the unmodified CPE. AA reaction with MB onto the MCPE surface gave LMB and its oxidation potential was shifted from –100 mV to +50 mV and the peak height was also increased by increasing AA concentration. As can be observed, the detection peak potential for AA oxidation at MB

adsorbed onto muscovite (MCPE) was also shifted 310 mV to more negative potentials than unmodified CPE. In addition, there is an increase in anodic peak current, corresponding to the oxidation of LMB to MB in pH 7.0 phosphate buffer solution using DP voltammetry in Figure 7. The catalytic activity of the redox couple at +50 mV obviously mediates the oxidation of AA and reduces the overpotential observed on CPE electrodes. The reaction rate was increased by the irradiation of the MCPE surface, which gave the highest photocurrent in the presence of AA (Figure 9). The reaction mechanism at the electrode surface is shown below.
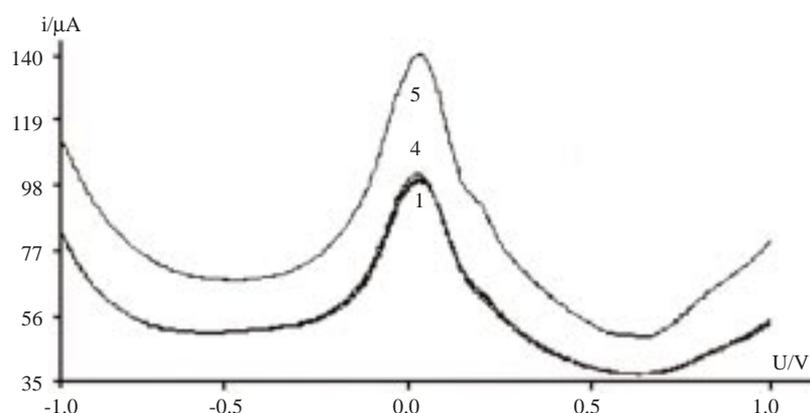
Figure 7. DP voltammograms recorded at MCPE in phosphate buffer; time dependence (1-4) at 1-min intervals and in the presence of 1.0×10^{-4} M AA with irradiation (5).
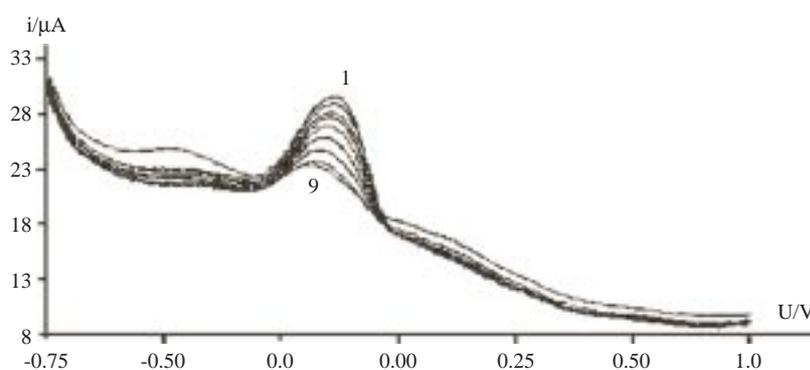
Figure 8. DP voltammogram obtained after 24 h immersion of the unmodified carbon paste electrode into 1.0×10^{-2} M MB solution containing 0.1 M phosphate buffer solution (pH 7.00) (1) time dependence, during consecutive scanning at 1-minute intervals (2-9).
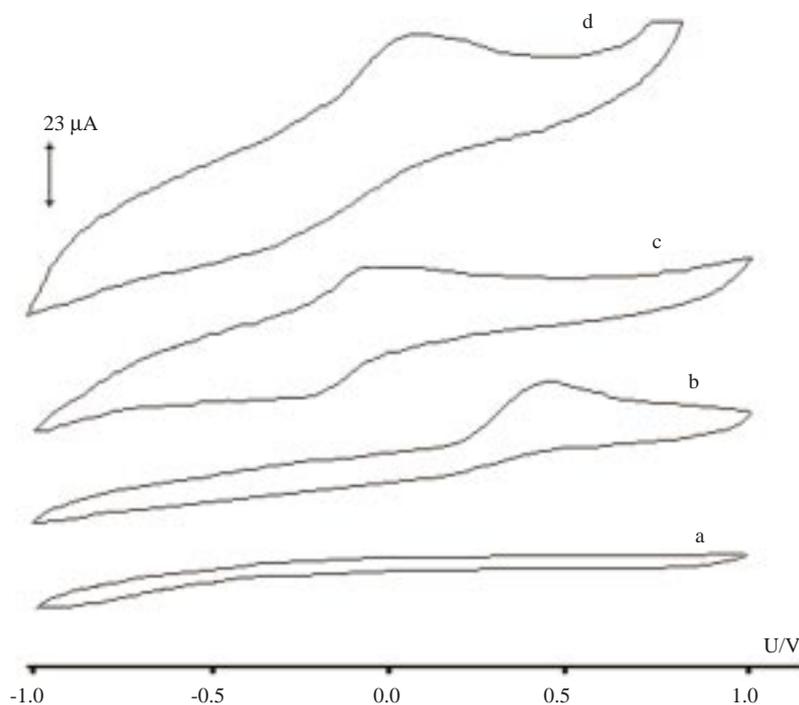
Figure 9. Cycling voltammograms in 0.1 M phosphate buffer (pH 7.0) using a) unmodified carbon paste electrode (CPE), b) in the presence of 1.0×10^{-3} M AA, c) MB adsorbed muscovite modified CPE (MCPE), and d) MCPE in the presence of 1.0×10^{-3} M AA and c and d were recorded by electrode surface irradiation. The scan rate was 60 mV s^{-1}.

$$MB \xrightarrow{h\upsilon} MB^* \qquad\qquad \text{Photon excitation}$$

$$MB^* + AA \longrightarrow L - MB + DHAA \quad \text{Chemical reaction}$$

$$L - MB \underset{}{\overset{-2e^-}{\rightleftharpoons}} MB \qquad\qquad \text{Electrode reaction}$$
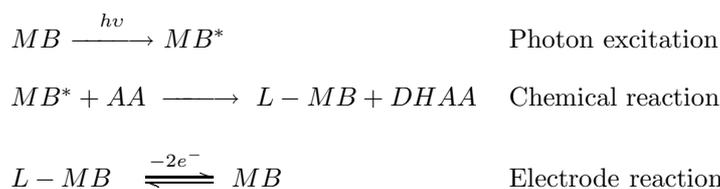
Photoelectrochemical Flow Injection Amperometric Detection of AA

In the photoelectrocatalytic amperometric study, preliminary experiments showed higher peak currents than those of the amperometric flow study. The newly constructed flow-cell gave good light permeability for the irradiation of the MCPE surface. In the FI amperometric electrocatalytic detection of AA, the optimum oxidation potential of LMB was found to be +50 mV, which is far from the AA oxidation potential (360 mV). The flow rate, sample volume and transmission tube length were optimized at 1.5 mL min^{-1}, 50 μL and 50 cm respectively. For the photoamperometric measurements, the effect of irradiation time was investigated. The segmented irradiation of the MCPE surface for about 45 s was preferred as it minimized the heating effect of the lamp on the flow system. The peak current at +50 mV was selected with respect to the oxidation of LMB via surface-mediated charge transfer instead of AA. The surface irradiation causes the overall reaction rate of the system to be mainly controlled by the photoelectrocatalytic reaction, not by diffusion. The design of the flow-cell responded well enough when the electrode surface was exposed to the light source at a distance of 20 cm from the electrode surface during the injection and dispersion of AA.

The peak signal could be detected even for a 1.0 x 10^{-8} M AA injection. The peak height was increased by increasing thesing the AA concentration to 1.0 x 10^{-7} M (Figure 10). In injections of more concentrated AA, increases in the peak height were not as high as expected, but the responses did show linearity upon increasing AA concentration. The linear concentration range in the photoelectrochemical FI and FI amperometric (without electrode surface irradiation) was 1.0 x 10^{-6}-8.0 x 10^{-5} M (Figure 11) and 5.0 x 10^{-6}-1.0 x 10^{-4} M (Figure 12) respectively.
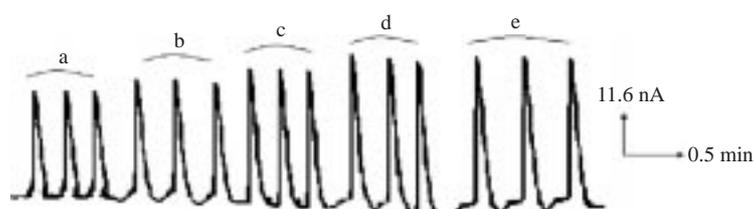
Figure 10. Photoamperometric response of AA a) 1.0, b) 3.0, c) 5.0, d) 8.0 and e) 10.0 x 10^{-8} M with carrier stream of 0.1 M phosphate buffer (pH 7.00), 50 μL sample injection volume, 1.5 mL min^{-1} flow rate and +50 mV constant potential vs SCE. A 500 W halogen lamp was used.
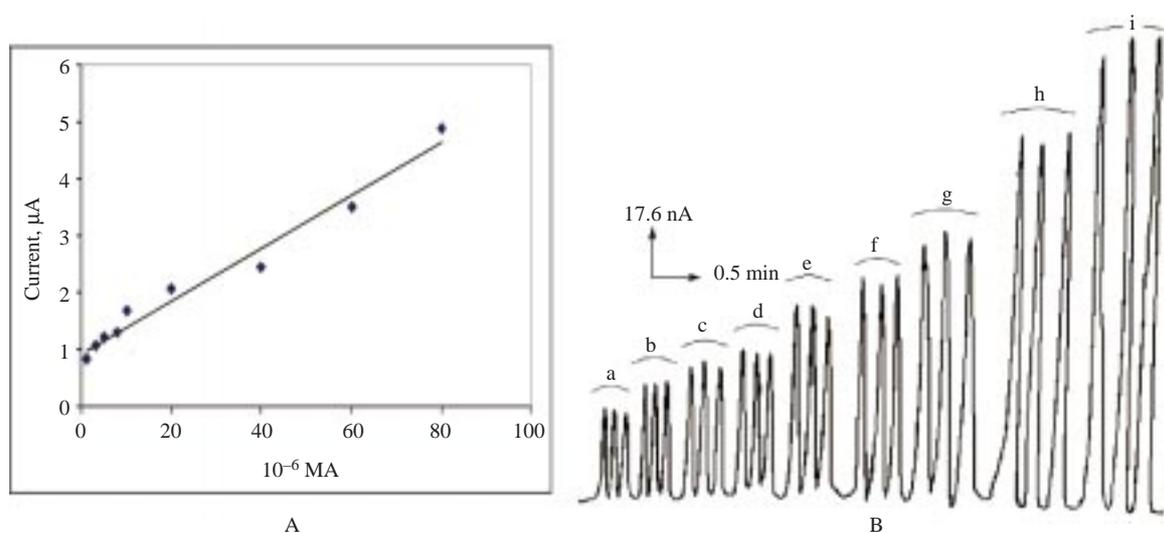
Figure 11. Dependence of peak height on AA concentrations in the diagram (A) and fiagram (B): a) 0.1, b) 0.3, c) 0.5, d) 0.8, e) 1.0, f) 3.0, g) 5.0, h) 8.0 and i) 10.0 x 10^{-5} M with carrier stream of 0.1 M phosphate buffer (pH 7.00), 50 μL sample injection volume, 1.5 mL min^{-1} flow rate and 50 mV constant potential vs SCE using photoelectrochemical FI amperometric system with 500 W halogen lamp.
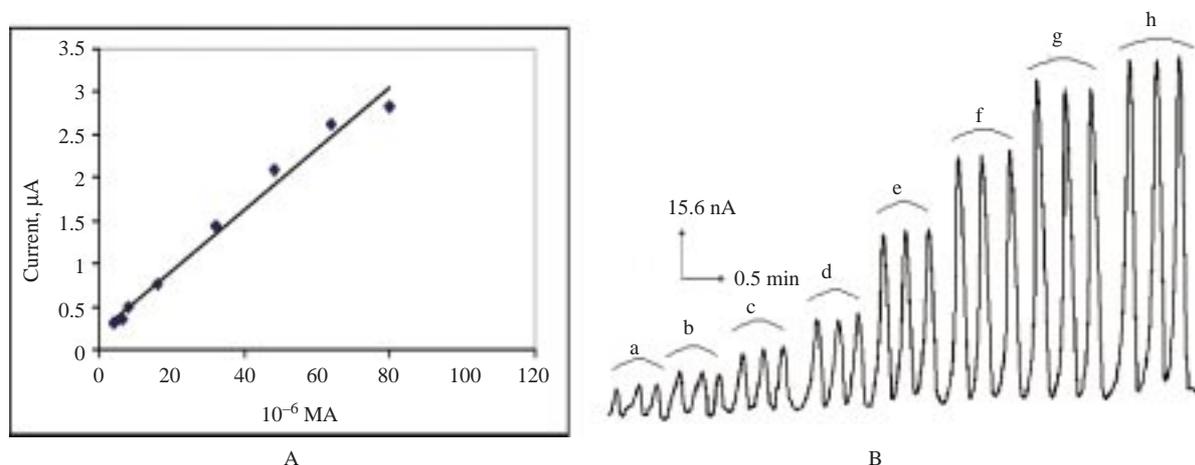
Figure 12. Dependence of peak height on AA concentrations in the diagram (A) and fiagram (B): a) 0.5, b) 0.8, c) 1.0, d) 2.0, e) 4.0, f) 6.0, g) 8.0, and h) 10.0 $\times 10^{-5}$M with a carrier stream of 0.1 M phosphate buffer (pH 7.00), 50 μL sample injection volume 1.5 mL min^{-1} flow rate and 50 mV constant potential vs. SCE using only an FIA system, without a halogen lamp.

The relative standard deviation in the photoelectrochemical FI and FI for a standard solution of 6.0 x 10^{-5} M AA was less than 2.0% RSD (n = 5) and 1.7% RSD (n = 5) respectively. The linear range and sensitivity for the photoelectrocatalytic flow injection analysis were improved about 61% compared with those obtained without irradiation flow injection analysis. Using the method in the optimum conditions, AA was determined in two pharmaceutical product samples (Table). The results obtained with this new procedure are in good agreement with those established using the triiodide procedure.

Table. The amounts of ascorbic acid (AA) in pharmaceutical tablets determined by proposed electrocatalytic and photoelectrocatalytic methods and standard triiodide procedure.

Sample procedure (mg)	Electrocatalytic procedure (mg)	Photoelectrocatalytic procedure (mg)	Triiodide
Redoxan	10.9 ± 0.8	10.56 ± 0.7	9.55 ± 0.7
Sandoz	9.57 ± 1.2	9.75 ± 1.2	10.2 ± 0.5

Conclusion

Several previous studies have demonstrated that AA can be determined using MB[18] or Prussion Blue[19] in solution or on an electrode surface by FI amperometric determination. However, their sensitivities were found to be poor. In the present study, the photoamperometric determination of AA by MB-adsorbed muscovite MCPE was for the first time described and a new photoelectrochemical flow cell was constructed. It was shown that the first flow cell construction was the most suitable and it may be further developed in future studies. The modified electrode displayed a photoelectrocatalytic effect on the oxidation of AA. The sample frequency was 60 samples per hour.

Acknowledgements

References

1. S.M. Sultan, **Talanta 40** 593 (1993).

2. L.E. Leon and J. Catapano, **Anal. Lett. 26(8)**, 1741 (1993).

3. L.T. Jin, G.Z. Zhao and Y.Z. Fang, **Chem. J. Chinese Uni**. **15** (1994).

4. P.C. Pandey, S. Upadhyay, H.C. Pathak and C.M.D. Pandey, **B. Electrochem**. **15: (11)**, 439 (1999).

5. Z. Dursun, "**Preparation of Modified Electrodes and Applied to New Voltammetric Techniques**" Ph.D. Thesis, Ege University, İzmir, Turkey 1999.

6. B. Nalini and S.S. Narayana, **Anal. Chim. Acta 405**, 93 (2000).

7. A.M. Yu and H.Y. Chen, **Anal. Chim. Acta 344**, 181 (1997).

8. J. Wu, J. Suls and W. Sonsen, **Electrochem. Commun. 2**, 90 (2000).

9. J.A. Cooper, R.G. Campton and M. Wu, **Anal. Chem. 70**, 2922 (1998).

10. F.D. Munteanu, L.T. Kubota and L. Gorton, **J. Electroanal. Chem. 509**, 2 (2001).

11. L.T. Kubota and L. Gorton, **Electroanal. 11** 719 (1999).

12. A. Malinuskas, T. Ruzgas and L. Gorton, **J. Electroanal. Chem. 484**, 55 (2000).

13. L.T. Kubota and L. Gorton, **J. Solid State Electr. 3**, 370 (1999).

14. B. Ballarin, R. Seeber, D. Tonelli and C. Zanardi, **Electroanal. 12(6):** 434 (2000).

15. L.T. Kubota, F. Gouvea, A.N. Andrade, B.G. Milagres and D.D.O. Neto, **Electrochim. Acta 41 (19):** 1465 (1996).

16. E.F. Perez, L.T. Kubota, A.A. Tanaka and D.D.O. Neto, **Electrochim. Acta 43**, 1665 (1998).

17. D. Fischer, W.R. Caseri and G. Hahner, **J. Colloid Interf. Sci. 198(2):** 337 (1998).

18. L.E. Leon, **Talanta 43**, 1275 (1996).

19. S.S.L. Castro, V.R. Balbo, P.J.S. Barbeira and N.R. Stradiotto, **Talanta 55** 249 (2000).

20. J.A. Cooper, K.E. Woodhouse, A.M. Chippindale and R.G. Compton, **Electroanal. 11 (17):** 1259 (1999).

21. J.H. Khorasani, M.K. Amini, H. Ghanei and S. Tangestaninejad, **Iran. J. Chem. Chem. Eng. 20(2):** 66 (2001).

22. R.C. Matos, L. Angnes, M.C.U. Araujo and T.C.B. Saldanha, **Analyst 125(11):** 2011 (2000).

23. H.B. Mark, H. Zhang, S.K. Lunsford, O. Ceylan, A.I. Khaskelis, N. Atta, A. Galal, S. Hausner, J.F. Rubinson, G.C. Russell, H. Zimmer and G.P. Kreishman, **Anal. Chim. Acta 385(1-3):** 281 (1999).

24. E.M. Garrido, J.L.F.C. Lima and C.D. Matos, **Farmaco 50 (12):** 881 (1995).

25. M.A. Osman and U.W. Suter, **J. Colloid Interf. Sci. 214**, 400 (1999).

26. J. Penasky, H.M. Schneider, S. Granick and C.R. Kessel, **Langmuir 11**, 953 (1995).

27. R.A. Shelden, W.R. Caseri and U.W. Suter, **J. Colloid Interf. Sci. 157**, 318 (1993).

28. M.A. Osman and U.W. Suter, **J. Colloid Interf. Sci. 224** 112 (2000).

29. F.J. Welcher, **Standard Methods of Chemical Analysis**, Sixth Edition, Volume Two, Part B, London, 1967.

30. M. Danjo, M. Tsuhako, H. Nakayama, T. Eguchi, N. Nakamura, S. Yamaguchi, H. Nariai and I. Motookata, **B. Chem. Soc. Jpn. 70**, 59 (1997).

31. T. Shichi and K. Takagi **J. Phtoch. Photobio. C: Photochemistry Reviews 1**, 113 (2000).

32. Y. Kanzaki, M. Hayashi, C. Minami, Y. Inoue, M. Kogure, Y. Watanabe and T. Tanaka, **Langmuir 13 (14):** 3674 (1997).