Journal of Electroanalytical Chemistry 628 (2009) 43–47

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Insights in the antioxidant activity of diarylamines from the 2,3-dimethylbenzo[*b*]thiophene through the redox profile

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ARTICLE INFO

Article history:
Received 29 October 2008
Received in revised form 5 January 2009
Accepted 8 January 2009
Available online 21 January 2009

Keywords:
Benzo[*b*]thiophene
Diarylamines
Antioxidant properties
Electrochemical assays

ABSTRACT

Cyclic voltammetry was used to evaluate the antioxidant activity of 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes previously synthesized by some of us, comparing their oxidation potentials with those of the well-known synthetic standards (BHA, BHT). Compounds with electron-donating groups on the arylamine moiety have lower $E_{p/2}$ than compounds with electron-withdrawing groups or electron-deficient rings (pyridines). The position of the methoxy group on the arylamine moiety also changes the oxidation potential: lower $E_{p/2}$ for the diarylamines with methoxy groups in the *para* position. Comparing the first peak potential with the ones of BHA and BHT, the diarylamine compounds show lower oxidation potential, and therefore higher reducing power. A reasonable inverse correlation was also observed between the $E_{p/2}$ values and the pEC$_{50}$ values obtained in antioxidant activity chemical assays. It can be generalized that compounds with lower $E_{p/2}$ values have better antioxidant activity (DPPH assay) and higher reducing power.

1. Introduction

During normal metabolism, free radicals are continuously generated in small amounts. Although many of these free radicals are important to normal physiological function, when in excess, they may damage proteins, enzymes and DNA, increasing the risk of diseases such as cancer, Alzheimer's, Parkinson's, angiocardiopathy, arthritis, asthma, diabetes, and degenerative eye disease [1]. To balance the physiological generation of free radicals, cells are equipped with several defence systems, including oxidative enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, or antioxidant compounds such as α-tocopherol, ascorbic acid, carotenoids, and glutathione [2–4].

Antioxidants are widely used in cosmetic, pharmaceutical and food-processing industries to prevent products from aging and decaying, while maintaining the antioxidant health benefit [5]. Among the most widely used synthetic antioxidant compounds are the butylated hydroxyanisole (BHA) and the butylated hydroxytoluene (BHT) that act by blocking free radicals, converting them into stable products via redox reactions [6]. The continuous discovery of new synthetic products with antioxidant activity is of primary importance as they may substitute with advantage the currently used antioxidant compounds.

The reducing properties of diarylamines make them very important as antioxidants, especially as radical scavengers [7,8]. In fact most representative examples of antioxidants are hindered phenols and diphenylamine derivatives [9]. Particularly, the antioxidant properties of 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes were previously reported by us, using several standard techniques including reducing power, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity, inhibition of erythrocyte hemolysis and inhibition of lipid peroxidation using the β-carotene linoleate system. In this series structure–activity relationships (SARs) were established relating the antioxidant activity with the number and position of different substituents on the phenyl ring, with the presence of a pyridine ring and with the positions of its nitrogen atom relative to the N–H bond [10].

Progressively, electrochemical techniques have been tested and developed as an alternative tool, for the evaluation of antioxidant activity, expressed in terms of "antioxidant power", due to their quickness, simplicity and low cost. [5,11–14]. Powerful electrochemical techniques such as cyclic voltammetry can provide a deeper insight into the redox-processes of oxidative stressors and antioxidants. Antioxidant compounds can act as reducing agents and, in solutions, they tend to be easily oxidized at inert electrodes. Based on this fact, some of the previous cited authors established an interesting relationship between electrochemical behaviour of

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doi:10.1016/j.jelechem.2009.01.004

the antioxidant compounds and their resultant “antioxidant power”, where “low oxidation potential” corresponds to “high antioxidant power”. Additionally, the evaluation of the redox properties of the synthesized compounds also allows the establishment of SARs, concerning the influence of different groups on the phenyl ring [15].

In the present work, cyclic voltammetry was used to evaluate the antioxidant activity of 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes, comparing their oxidation potentials with well-known synthetic (BHA, BHT) standards. These results allow the establishment of correlations of the electrochemical behaviour of the compounds and the previously reported antioxidant activity EC_{50} values [10]. In the present study it was also possible to establish some structure–activity relationships according to the type, the number and position of substituents on phenyl ring or to the presence of a pyridine ring.

2. Materials and methods

2.1. Diarylamines

The 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes **2** and **3** (Fig. 1) were previously synthesized by some of us by palladium-catalyzed C–N Buchwald–Hartwig coupling of the also prepared 7-bromo-2,3-dimethylbenzo[*b*]thiophene or 7-amino-2,3-dimethylbenzo[*b*]thiophene **1** (Fig. 1) with methoxylated anilines and 3-aminopyridine or substituted (3-methoxy or 4-cyano) bromobenzenes and 2-bromopyridine, respectively [10].

2.2. Standards and reagents

Standards BHA (2-*tert*-butyl-4-methoxyphenol) and BHT (2,6-di-*tert*-butyl-4-metylphenol) were purchase from Sigma (St. Louis, MO, USA). Tetrabutylammonium perchlorate (TBAP), electrochemical grade, was purchase from Fluka and kept at 30 °C before use. Acetonitrile (CH_3CN), Fisher p.a. grade, was doubled distilled over CaH_2 (Fluka, p.a. grade).

2.3. Instrumentation

Cyclic voltammetry (CV) measurements were performed on an Autolab PGSTAT 302 potentiostat/galvanostat using a closed standard three electrode cell. A platinum disk (BAS, $\phi = 0.314\ cm^2$) was used as the working electrode and a Pt foil as the counter electrode. All potentials are referred to an Ag/AgCl 3 M KCl reference electrode (Methrom).

2.4. Procedure

The diarylamines and standards were studied in CH_3CN solutions containing 1 mM of the compound and 0.1 M TBAP as the supporting electrolyte. Prior to use, the working electrode was polished in an aqueous suspension of 0.3 μm alumina (Beuhler) on a Master-Tex (Beuhler) polishing pad, than rinsed with water. Subsequently, for a chemical treatment, the electrode was placed on ultrasounds bath in HCl 6 M during 1 min, and then in methanol. This cleaning procedure was applied always before any electrochemical measurements. After filling the electrochemical cell, the solution was de-aerated with argon. The voltammetric profiles were then acquired in different intervals in the range between −0.5 and 2 V using scan rates from 0.01 to 1 V s^{-1}. The first and subsequent cycles were recorded in order to access any adsorption phenomena common in organic compounds [16].

3. Results and discussion

The compounds used in the present study are shown in Fig. 1.

The cyclic voltammograms for the different 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes show a typical electrochemical behaviour, with three major irreversible oxidation processes. Occasionally, additional electrochemical waves, observed as shoulders, were found, predominantly at low scan rates. This electrochemical profile resembles the voltammogram of the amino precursor **1**, Fig. 2, leading us to associate the observed oxidation processes with the 7-amino-2,3-dimethylbenzo[*b*]thiophene backbone. Nevertheless, the different substitutions with electron-donating or withdrawing groups on the phenyl ring and the presence of a pyridine ring promote significant changes in these anodic processes as it will be observed and discussed.

The anodic current density for the three mentioned electrochemical processes, increases linearly with the square root of the scan rate, suggesting diffusion controlled responses (Fig. 3). This, together with the absence of the cathodic peak in the reverse scan indicates that the oxidation processes are followed by a chemical reaction, corresponding to an EC mechanism [17].

With continuous cycles the voltammograms reveal some decrease in current density, much more significant for compounds **2c** and **3a**. In fact, after 8–10 cycles at 0.1 V s^{-1} the electroactivity of compound **3a** is almost vanished (Fig. 4). This fact can be explained by the inactivation of the electrode surface probably due to an irreversible adsorption/deposition of the previous oxidized species. In order to re-activate the electrode, it must be removed and submitted to a cleaning procedure described in the experimental section.

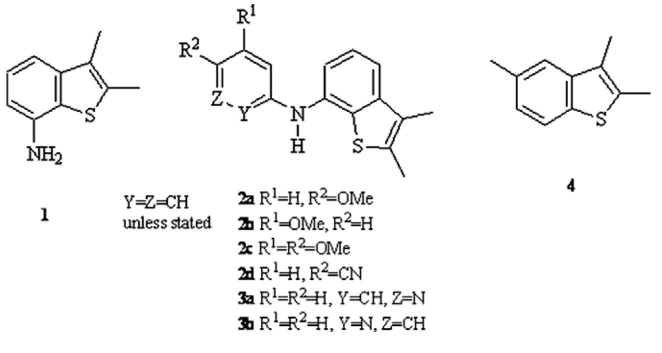

Fig. 1. Structures of the precursor 7-amino-2,3-dimethylbenzo[*b*]thiophene (**1**), of the 7-aryl and 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes **2a–d** and **3a–b**, respectively, and of the 2,3,5-trimethylbenzo[*b*]thiophene (**4**).

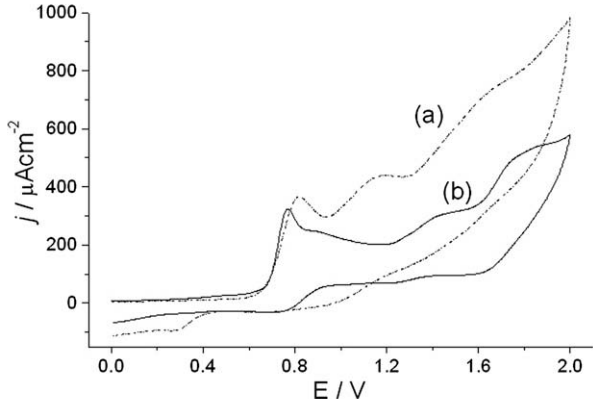

Fig. 2. Cyclic voltammogram of 1 mM arylamines/0.1 M TBAP solutions with a Pt electrode at 0.1 V s^{-1}. (a) Compound **1**; (b) compound **2a**.

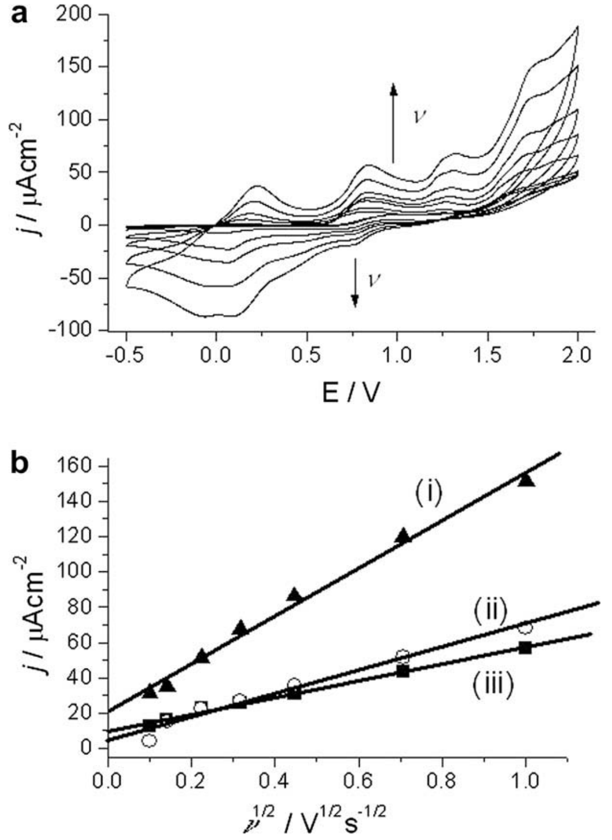

Fig. 3. (a) Cyclic voltammogram of 1 mM of compound **2c**/0.1 M TBAP solutions with a Pt electrode at scan rates in the range of 0.01 to 1 V s^{-1}. (b) Linear plot of the anodic current densities *vs* square root of scan rate: (i) First; (ii) second and (iii) third anodic peaks.

The deposition of chemical species onto the electrode surface is common during the oxidation of many organic species such as thiophene, pyrrole, aniline and other benzene derivatives, leading sometimes to an irreversible deposition of electroactive species such as monolayers or polymers [18–22]. In this study we have also noticed that when cycling the compounds at fast scan rates (above 0.2 V s^{-1}) a new reversible electrochemical process is detected at potentials around 0 V (Figs. 3a and 5), which increases in intensity upon cycling. This behaviour is typical for a deposition of an electroactive species in the electrode surface and is associated with the increase of the "modified electrode" active area. This fact, common for all compounds, is most evident for the methoxy derivatives, **2a** and **2c**, probably due to some structural/electronic effects of these ring substituents. However, if the electrode is rinsed, then placed and scanned in a supporting electrolyte solution, the electroactivity is not observed. We must conclude that the interactions of this new species with the platinum electrode are not strong enough to maintain it at the electrode surface, and it was probably washed up during the solution/electrode transfer.

The influence of the substituents in the arylamine moiety can be observed through the potential shift in the anodic peaks for the first oxidation processes. For this peak the presence of the methoxy group in the *para* or *para* and *meta* position, compounds **2a** and **2c**, decrease the oxidation potential if compared with the amino precursor **1**. On the contrary, the presence of a cyano group in **2d** leads to a significant increase in the oxidation potential (Fig. 6a).

The potential drift can be explained in terms of resonance: the methoxy group stabilizes the oxidized species through the electron-donating effect; the cyano group (electron-withdrawing) promotes a positive shift in the peak potential due to the destabilization of the oxidized form.

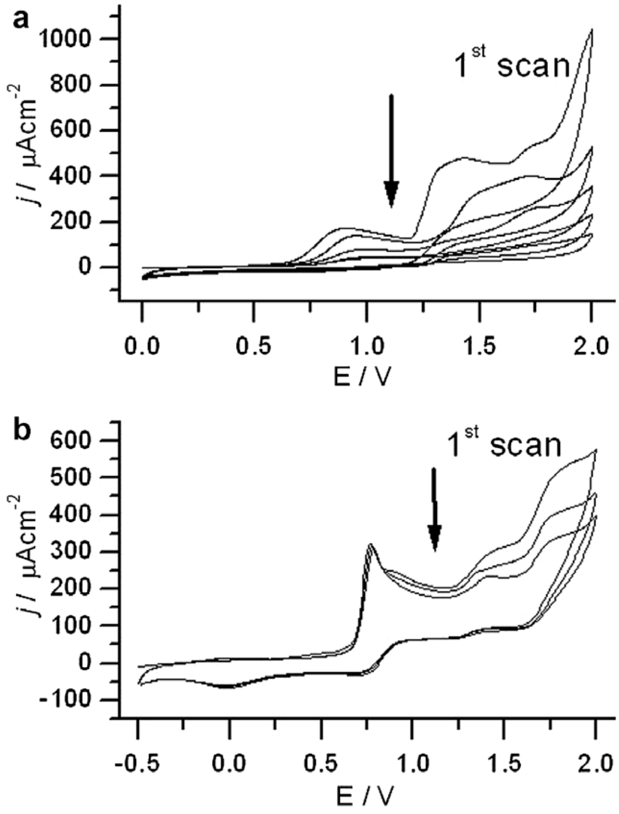

Fig. 4. Cyclic voltammograms of 1 mM arylamines/0.1 M TBAP solutions with a Pt electrode at 0.1 V s^{-1}. (a) Compound **2a** – 3 scans; (b) compound **3a** – 5 scans.

For the di(hetero)arylamino compounds **3a** and **3b**, the position of linkage of the pyridine ring with the benzothiophene moiety does not seem to influence significantly the first redox process (Fig. 6b). In fact, the voltammograms for these compounds are similar to the one observed for compound **2b** (Fig. 6a), with the methoxy in the *meta* position, with an $E_{p/2}$ between those of compounds **2a** and **2d**. Since this first oxidation process is present in the amino precursor **1** and is influenced by the aryl moiety with *para* substituents due to electron delocalization, it should correspond to the removal of an electron from the amine group. The following two oxidation processes should then be associated with the oxidation

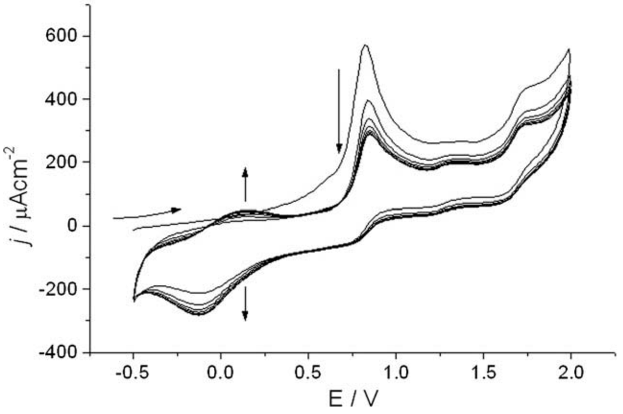

Fig. 5. Cyclic voltammogram of 1 mM compound **2a**/0.1 M TBAP solutions with a Pt electrode at 0.5 V s^{-1}.

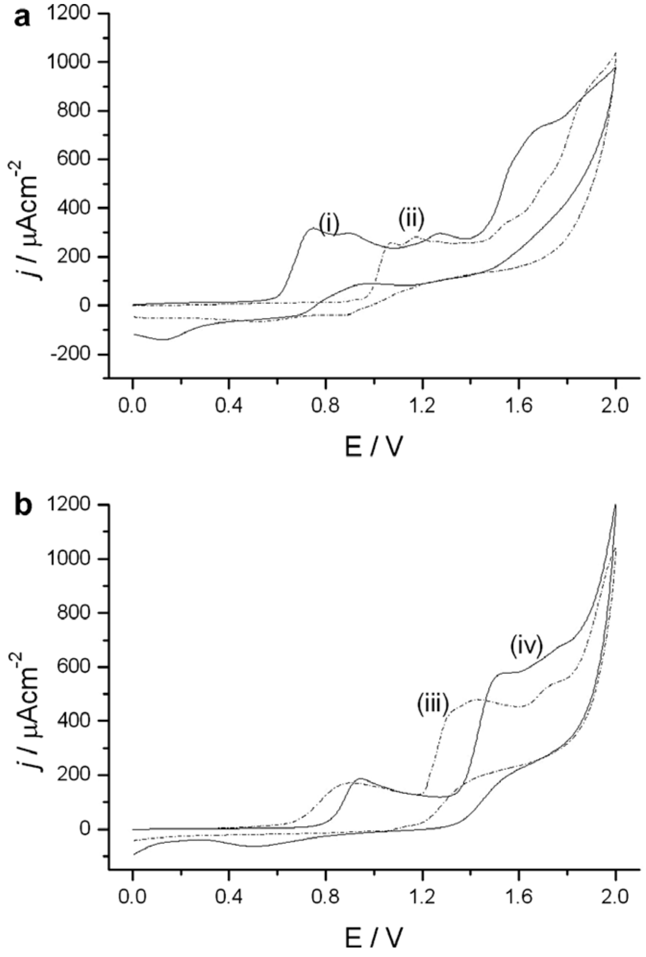

Fig. 6. Cyclic voltammograms of 1 mM arylamines/0.1 M TBAP solutions with a Pt electrode at 0.1 V s^{-1}. (i) Compound **2b**; (ii) compound **2d**; (iii) compound **3a** and (iv) compound **3b**.

of the benzothiophene structure. In order to check this latter suggestion we performed the cyclic voltammetry for compound **4**, a methylated benzothiophene without the amino group. The voltammogram for this compound presents only two irreversible oxidation peaks at high potential, 1.49 and 1.84 V, corroborating the previous attribution of the three oxidation peaks present in compounds **1**, **2** and **3**.

In order to assert the potentiality of cyclic voltammetry as a technique for the antioxidant properties evaluation, the voltammetric results for the first anodic process, expressed in terms of the oxidation potential ($E_{p/2}$), were plotted against the antioxidant activity (DPPH assay) and the reducing power results obtained for these compounds and published elsewhere [10] (Fig. 7; Table 1). Additionally the redox properties of the 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes (in terms of the potential of the first anodic wave) were compared with those of the synthetic antioxidant standards, BHA and BHT. The di(heteroaryl)amines in study revealed lower oxidation potential, and therefore higher reducing power (Table 1).

A reasonable inverse correlation (Fig. 7) was observed between the $E_{p/2}$ and the pEC_{50} values for both methods (DPPH scavenging activity and reducing power). In general it can be observed that compounds with lower $E_{p/2}$ values have better antioxidant activity and higher reducing power, as it was observed for other types of antioxidant compounds like flavanoids [23,24]. Although lower values of $E_{p/2}$ were found to correlate with increased antioxidant

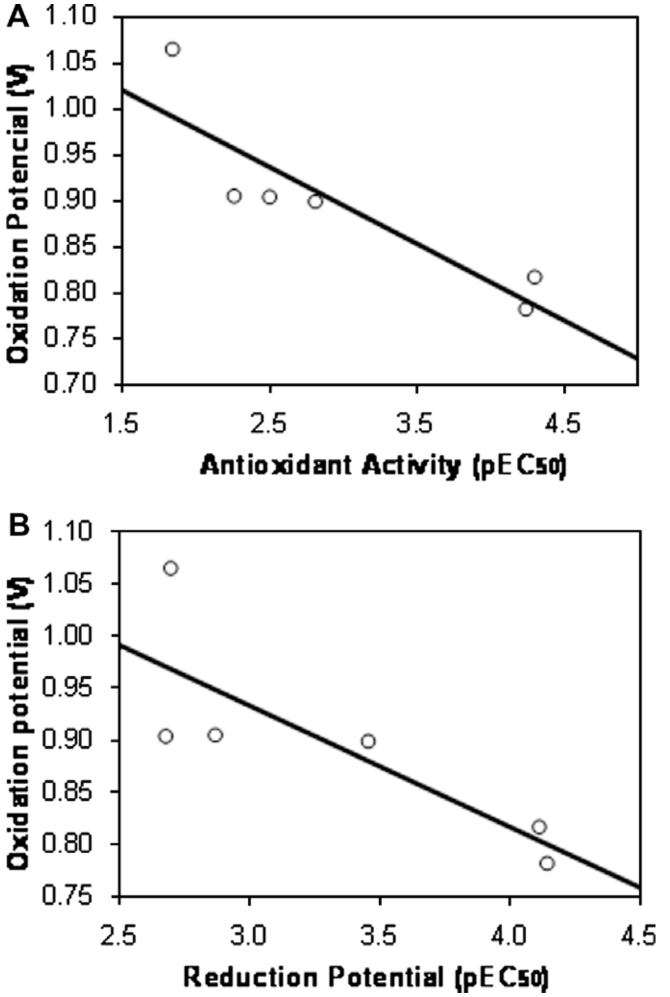

Fig. 7. Correlation between the electrochemical data (oxidation potential) and: (A) antioxidant activity (B) reducing potential. EC_{50} values are given in M for antioxidant activity and for reducing potential. Inverse correlations were obtained between the oxidation potential and (A) antioxidant activity ($r_2 = 0.785$) and (B) reducing potential ($r_2 = 0.655$).

activity and reducing power, the correlation coefficients observed were relatively poor. This may indicate that the oxidation potentials of the 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes studied are not the only factor responsible for their antioxidant activity. In a recent QSAR (quantitative structure-activity relationship) study the antioxidant activity of di(hetero)arylamines derivatives of benzo[*b*]thiophenes was also correlated to

Table 1
Electrochemical results from cyclic voltammetry of the diarylamines solutions (potential of the first anodic wave), and pEC_{50} values obtained for DPPH method and Reducing power (RP).

Compound	$E_{p/2}$ (V)	pEC_{50} (DPPH)	pEC_{50} (RP)
1	0.82	–	–
2a	0.77	4.30	1.66
2b	0.91	2.81	1.00
2c	0.74	4.24	1.64
2d	1.07	1.84	0.25
3a	0.90	2.26	0.46
3b	0.94	2.50	0.27
BHA	1.024	–	–
BHT	1.433	–	–

the presence and position of electronegative and polarizable atoms at the inner sphere of the compounds [25].

4. Conclusions

The redox profile of the diarylamines studied showed three typical irreversible anodic processes. The first was associated to the arylamine moiety, corresponding to the lowest oxidation potential, and the latter to the oxidation of the benzo[*b*]thiophene structure. These observations pointed to the importance of the arylamine presence for the antioxidant properties since it introduces an oxidation process at lower potentials. It can be concluded that the presence of an electron-donating group (OMe) in the *para* position relative to the NH (**2a**) decreases the $E_{p/2}$ values while a methoxy group in the *meta* position (**2b**) increases this potential. When two methoxy groups, one in the *para* and other in the *meta* position, are present (**2c**) the $E_{p/2}$ compares with the one for compound **2a**. The presence of an electron-withdrawing group ($C{\equiv}N$) in the *para* position (**2d**) increases significantly the $E_{p/2}$ value. The same effect is observed for the pyridine (electron-deficient ring) derivatives (**3a** and **3b**) but in a lower extent.

Acknowledgements

The authors are grateful to Foundation for Science and Technology (Portugal) for financial support, to the research Centres, to the research Project POCI/QUI/59407/2004 and to the PhD grants attributed to R.M.V.A (SFRH/BD/27430/2006) and R.C.C. (SFRH/BD/29274/2006).

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