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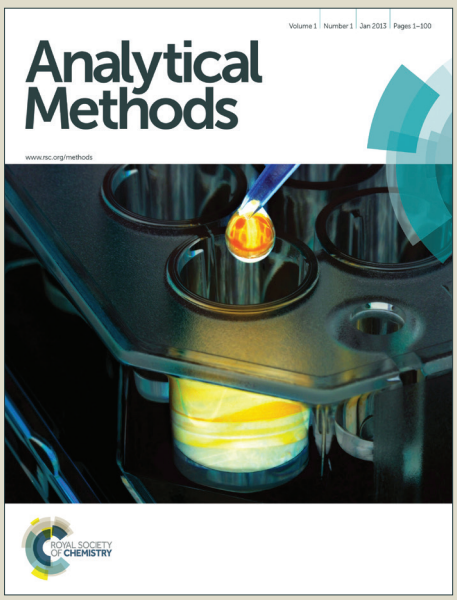

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Screening and authentication of herbal formulations based on microextraction-assisted voltammetry of microparticles

Mariele Martini[a], Leandro Machado de Carvalho[a], Adela Blasco-Blasco[b], Antonio Doménech-Carbó*[b]

[a] Universidade Federal de Santa Maria – UFSM, Campus universitário, Caixa Postal 5051, CEP 97105-970, Santa Maria – RS, Brazil.

[b] Departament de Química Analítica, Universitat de València, Dr. Moliner, 50, 46100 Burjassot (València) Spain

* Corresponding author. e-mail: antonio.domenech@uv.es

Abstract

A simple solid state electrochemical methodology for screening and authentication of herbal formulations is described. The proposed method is based on the record of the voltammetric response, in contact with aqueous buffers, of microparticulate films of antioxidant compounds resulting from micro extraction of dried herbal samples with ethanol or acetone. The obtained voltammetric responses led us to differentiate between diverse active components upon application of bivariate and multivariate chemometric techniques. Resolution of herbal preparations containing of two or more components is possible when well-separated voltammetric signals are recorded. In favorable cases, such characteristic voltammetric signatures can be used for relative quantification of components and synthetic adulterants.

Keywords: Herbal formulations; Vegetal species; Screening; Voltammetry of microparticles.

1. Introduction

In the last decades, the use of herbal formulations has experienced a considerable growth in most countries,[1] thus motivating the appearance of adulterations. The illegal practice of adding non-declared synthetic chemicals such as anorexics, antidepressants, etc. as adulterants in such formulations has received, as recently reviewed,[2] considerable attention.[3-9] Another important problem is the assessment of the vegetal composition of the herbal-based products; i.e., the identification and/or characterization of the plant species effectively constituting the herbal preparation. This is a crucial problem because correct plant identification is the foundation of the safe use of plant based natural health products.[10] Different quality assessment strategies,[11,12] involving chemical profiling[13,14] and characterization of bioactive components or families of components[15,16] and/or marker compounds[17] have been used for this purpose. These strategies are mainly based on different chromatographic techniques coupled to mass spectroscopy detection. Such techniques permit, via application of chemometric methodologies, the development of chemotaxonomic approaches for identifying vegetal species.[18-20]

In this context, electrochemical techniques have been used to detect individual components in herbal formulations, including naturally occurring compounds,[21-24] and synthetic adulterants.[25-28] These techniques involve conventional solution-phase electrochemistry, thus requiring sample pretreatments addressed to the dissolution of the components of the sample into a suitable electrolyte. Complementing these approaches, we have reported a series of solid-state voltammetric methods addressed to the detection[29] and quantitation[30] of chemical adulterants in herbal formulations and screening tomato[31] and tea[32] varieties. Such methods are based on the voltammetry of microparticles (VMP), a solid-state electrochemical technique developed by Scholz et al.[33] which provides analytical information on the composition and structure of sparingly soluble materials.[34,35] This technique has been applied to a variety of organic compounds,[33-36] including the estimation of antioxidant properties of vegetables,[37,38] radical scavenging activity.[39] The current report presents a microextraction-assisted VMP method aimed to the screening, authentication and quality assessment of herbal formulations. The proposed methodology, to some extent based on a holistic-based philosophy,[40,41] involves simple procedures and no sample pretreatment, exploting the electrochemical activity of polyphenolic compounds existing in leaf extracts in organic

solvents. Among the wide variety of components forming the vegetal matter, some bioactive phenolic components, such as anthocyanins, procyanidins, etc. possess electroactive polyphenol motifs, displaying electrochemical responses based on proton-assisted oxidation of catechol units to quinones similar to that of flavonoids.[33-36,42-45] The reported method consists of an *in situ* microextraction-assisted voltammetry of microparticles (VMP) assay. This method differs from the previously reported VMP-based methods[29,30] by the use of a extraction step prompting the detection of the signals from the vegetal components of the sample, in contrast with the above 'dry' methods where the signals of the adulterant chemicals were detected. The relevant point to emphasize is that the proposed methodology can provide authentication criteria from the chemometric analysis of the complete voltammetric pattern of the vegetal components *without the need for identifying and quantifying the individual compounds* existing in the sample, as usually required.[11-20] The detection and quantification of synthetic adulterants from voltammetric data will be also treated. Additional potential benefits from this technique are the possibility of analyzing minimal amounts of sample (micrograms to nanograms, if necessary), the no need of sample treatment and the possibility of field analysis using portable electrochemical equipment.

2. Experimental

2.1. Reagents and solutions

Aqueous acetic acid/sodium acetate buffer solutions in concentration 0.25 M and pH 4.75 and 0.10 M potassium phosphate buffer at pH 7.0 were used as supporting electrolytes for electrochemical measurements using nanopure water and reagents from Panreac (Barcelona, Spain). Electrochemical experiments were performed at 298 ±1 K in a CH cell using either a laboratory CH I660 potentiostat (Cambria Scientific, Llwynhendy, Llanelli UK) or Ivium CompactStat portable equipment (Ivium Technol. B.V., Eindhoven, The Netherlands). A BAS MF2012 glassy carbon working electrode (GCE) (geometrical area 0.071 cm^2), a platinum wire auxiliary electrode and an Ag/AgCl (3M NaCl) reference electrode were used in a typical three-electrode arrangement. Voltammetric measurements were performed with a freshly-prepared sample-modified

GCE. In order to test the applicability of portable equipments, no degasification of the electrolyte was carried out.

For electrode conditioning, ca. 500 mg of leaf fragments of the herbal preparation were powdered with an agate mortar and pestle during 2 min adding 0.5 mL of ethanol, acetone or chloroform (HPLC grade, Carlo Erba). 50 μL of the resulting suspension were dropped onto the GCE surface. After solvent evaporation in air, the electrode was inserted into the electrochemical cell and electrochemical runs were performed. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used as detection modes. SECM experiments were performed with CH 920c equipment using a microdisk platinum electrode tip (CH 49, diameter 20 μm) and a Pt substrate electrode (geometrical area 0.018 cm^2). A 2.0 mM solution of $K_4Fe(CN)_6$ in 0.25 M acetic acid/sodium acetate aqueous buffer at pH 4.75 was used as a redox probe. The bipotentiostat mode was used to apply potentials to the tip (E_T) and the electrode substrate (E_S). The rate of scanning of the tip over the substrate was 20 μm/s for all experiments, the distance between tip and substrate being of the order of the tip electrode radius.

2.2. Samples

The herbal formulations (n = 25) analyzed in this work were acquired from pharmacies in different regions of Brazil and were received by mail. The samples were stored at room temperature and used as received for analysis. We documented the labeled components for each formulation and then electrochemically analyzed. Prior to voltammograms of microparticulate films prepared from leaf extracts, the powdered sample was analyzed as already described,[2,9,29] in order to discard the presence of chemical adulterants. Samples of raw herbs of the different species from different areas of Brazil were collected and authenticated. Such herbal samples were dried at 60 ºC and ground to a fine powder. The botanic components of the samples are summarized in Table 1. These include plants from a variety of taxonomic groups, including two types of algae. A stereoscopic light microscope Leica GZ6 (X10-X50) was used for the optical examination of the samples and, in several cases, the separation of individual herbal components.

3. Results and discussion

3.1. General voltammetric pattern

Figure 1 shows the cyclic voltammograms of microparticulate films on glassy carbon electrode prepared from ethanolic extracts of 'Pholia magra' (*Cordia ecalyculata*) and *Centella asiatica* immersed into air-saturated 0.10 M aqueous potassium phosphate buffer at pH 7.00. Upon scanning the potential from 0.0 V vs. Ag/AgCl in the positive direction, anodic peaks, labeled as A_{pph}, are recorded at potentials between +0.2 and +1.2 V. These signals correspond to apparently irreversible electron transfer processes as denoted by the absence of coupled cathodic signals in the subsequent negative-going potential scan where only the ubiquitous signal at ca. –0.40 V corresponding to the reduction of dissolved oxygen, C_{ox}, was recorded. This voltammetry, as judged from abundant experimental data on flavonoids, flavones and other polyphenolic compounds,[21,24,31,32,34-36,42-47] can be unambiguously attributed to the proton-assisted oxidation of compounds of this type.

It should be emphasized that, in contrast with previously reported methodologies for VMP adulterant analysis in herbal preparations,[29,30] in which the signals of the adulterant were detected, the methodology proposed here involves the record of the electrochemical response of the components of the vegetal matter.

The voltammetric response of herbal formulations consisted mainly of anodic signals in the potential range between 0.0 and +1.2 V, attributable to the oxidation of solid polyphenolic compounds deposited onto the electrode surface. In order to test the solid state nature of such electrochemical processes, SECM experiments were performed on microparticulate deposits of the ethanolic extract of samples immersed into $K_4Fe(CN)_6$ solution in acetate buffer. In these experiments, it was applied to the tip electrode a potential (E_T) sufficiently positive to promote the oxidation of $Fe(CN)_6^{4-}$ under diffusion-controlled conditions (+0.30 V). As illustrated in Fig. 2 for the case of sample **24**, when no potential was applied to the substrate electrode (Fig. 2a), the particles of the insulating compounds deposited on the base Pt electrode appear as negative feedback features (in blue) forming more or less irregular aggregates on the electrode surface. Upon applying a potential to the substrate (E_S) sufficiently positive to promote

the oxidation of polyphenolic compounds (Fig. 2b), the periphery of the grains becomes modified, as was expected for solid-state redox processes involving ion-permeable materials. These redox processes involve the coupled transfer of electrons and ions through the three-phase particle/base electrode/electrolyte boundary.[48-51] In the case of of insoluble organic compounds in contact with aqueous electrolytes, proton-assisted solid state processes occur.[52,53]

Square wave voltammetry provides well-resolved peaks for films prepared from both ethanolic and acetone leaf extracts yielding voltammetric patterns able to characterize the different vegetal species. This can be seen in Figure 3 where voltammograms of microparticulate films prepared from ethanolic extracts of samples **24** (*Caralluma adscendens*), **40** (*Phaseolus vulgaris*) and **21** (*Cordia ecalyculata*) are shown. In all cases, well-defined voltammetric responses were obtained consisting of intense anodic peaks at +0.23 and +0.61 V for *Caralluma adscendens*, a broad peak at +0.40 V for *Phaseolus vulgaris* and peaks at +0.30 and +0.75 V for *Cordia ecalyculata*. As expected, the voltammetric profiles of acetone and chloroform extracts differ from those recorded from ethanolic extracts, as can be seen on comparing the series of voltammograms for samples nominally containing *Caralluma adscendens* in Figs. 4a-c and 4d-f.

Repeatability was tested upon performing five replicate measurements (including the entire extraction/electrode modification process) for each specimen. As illustrated in Fig. 4a, showing three replicate experiments on sample **24** using the same base electrode, the maximal dispersion in peak potentials was of ±5 mV in all cases. The robustness was tested using three different glassy carbon electrodes and also comparing the voltammograms recorded at the laboratory equipment with those performed with the portable equipment. Comparison of data for samples **24**, **40** and **21** in Figs. 3a-c (laboratory equipment) and Figs. 3d-f (portable equipment), respectively, reveals essentially identical voltammetric profiles.

It is pertinent to note that, as is characteristic of the VMP methodology, the peak currents vary from one experiment to another because the amount of electroactive compounds transferred to the electrode surface cannot be controlled; however, the proportion between the different extracted components is the same so that the ratio of currents at each pair of potentials remains constant. This permits to define species-

characteristic features and prompts the use of such current ratios for quantification (*vide infra*).

Examination of the voltammograms in Figs. 4a,d (sample **24**) with those in Figs. 4b,e (samples **19**, **25**, **34**) and Fig. 4c (sample **32**) suggests that, although the voltammograms of all samples nominally containing *Caralluma adscendens* were similar, some significant differences between them exist. Examination of such voltammograms permits grouping it in three types: the voltammograms of samples **24**, **27** and **84** consist of two sharp peaks at +0.23 and +0.61 V, whereas in the voltammograms of samples **19**, **25**, **34**, **69**, **71** and **89** the first peak is weakened and resolved into two components. Finally, this last feature is retained in the voltammograms of samples **20**, **32** and **53** while the peak at +0.61 V is lowered. These features can be associated to the presence of three different varieties of *Caralluma adscendens*: *adscendens*, *fimbriata* and *attenuate*. The possibility of a rapid identification of such varieties, all three being, as recently studied by Pullahiah et al.,[54] of pharmacological value, illustrates the potential interest of VMP for screening purposes.

3.2. Characterization of varieties and mixtures

As far as the amount of electroactive compounds extracted from the herbal formulations and transferred onto the electrode surface cannot be exactly controlled, the ratios between the currents measured at selected potentials (peak currents in particular) rather than the values of those currents have to be used for pattern recognition. On first examination, bivariate and multivariate analysis can be used to discriminate different species. Combining the voltammetric data recorded for films of leaf extracts in EtOH and acetone, one can increase the set of available data to be used for testing the composition of different herbal formulations using pattern recognition strategies. Ideally, if each voltammogram provides a series of current values, $I(E)$, at k potentials, using the extracts for j solvents, a matrix of dimensions $k \times j$ can be constructed for each species or variety and used for chemometric purposes.

A first example of this kind of analysis is shown in Fig. 5, where the $I(270)/I(330)$, $I(330)/I(720)$ and $I(1000)/I(720)$ ratios are combined in a three-dimensional diagram

were the samples corresponding to *Caralluma adscendens* var. *adscendens*, *Caralluma adscendens* var. *fimbriata* and *Caralluma adscendens* var. *attenuata*, fall in clearly separated regions. The resulting diagram offers comparable discriminating ability to that obtained with Principal Component Analysis (PCA) applied to chromatographic data for *Cyathula officinalis*[55] and combined chromatography and infrared spectroscopy for discriminating varieties of *Radix Paeoniae*.[56]

Following pattern recognition criteria reported in literature,[57] normalized voltammograms were constructed as representations of the ratio between $I(E)$, and the maximum peak current, I_{max}, vs. the applied potential E. Combining the data for voltammograms recorded from ethanolic and acetone leaf extracts, plots of $(I(E)/I_{max})_{Acet}$ vs. $(I(E)/I_{max})_{EtOH}$ provide graphs which should be characteristic of the involved vegetal species. As can be seen in Fig. 6a,b, the representations of $(I(E)/I_{max})_{Acet}$ vs. $(I(E)/I_{max})_{EtOH}$ for two replicate experiments on films of leaf extracts of *Caralluma adscendens* var. *adscendens* show a common pattern. This pattern, however, differs significantly from those obtained for herbal formulations **53** (Fig. 6c) and **89** (Fig. 6d), constituted by the other varieties of *Caralluma adscendens*, thus confirming the possibility of an electrochemical discrimination between them.

The identification of individual components in mixtures is favored by the fact that, using the VMP methodology, the responses of microparticulate deposits of different compounds behave independently.[33-35] In several cases, species-characteristic characteristic signals are recorded. For instance, ethanolic extracts of *Garcinia gummi-gutti* produces a sharp signal at ca. +0.90 V, whereas abacateiro and *Rhamnus purshiana* yields peaks at +0.36 and +0.55 V, respectively (see Supplementary materials). The appearance of such peaks permits the identification of the individual components of an herbal mixture in favorable cases. In several others, however, the presence of strongly overlapped signals conditions seriously the resolution of multicomponent systems.

An example of favorable mixture resolution is illustrated in Fig. 7, where the voltammograms of microparticulate films prepared from acetone extracts of samples **40** (Fig. 7a, *Phaseolus vulgaris*), **33** (Fig. 7b, *Garcinia gummi-gutti*) and 21 (Fig. 7c, *Cordia ecalyculata*), are compared with that of sample **37**. This last is composed of a

mixture of the above herbal components, as denoted by the presence in the voltammogram in Fig. 7d of signals at +0.15 and +0.28 V (*Cordia ecalyculata*), +0.57 (*Phaseolus vulgaris*) and +0.75 V (*Garcinia gummi-gutti*).

In general, the discrimination of the components of a binary mixture can be made when both are in relatively high concentrations in the sample. The proposed methodology can be applied to samples formed by more than two components in favorable cases where all the individual constituents display well-defined voltammetric signals at clearly separated potentials.

Absolute quantification can be obtained using standard addition-dilution methods previously described.[30] In general, however, only the relative amount of the components of the sample is required for quantitation of herbal preparations. In the cases of two components A and B producing well-separated voltammetric peaks, the quantification can be obtained from the ratio between the respective peak currents or peak areas. This procedure requires, however, previous calibration, because the specific current response (in terms of peak current/concentration or peak area/concentration ratios, I_{pJ}/c_J and A_{pJ}/c_J, J = A,B) of each component is different, so that:

$$\frac{c_A}{c_B} = h\left(\frac{I_{pA}}{I_{pB}}\right) = H\left(\frac{A_{pA}}{A_{pB}}\right) \qquad (1)$$

Here, h and H represent constants depending on the components A and B and the electrochemical parameters (potential scan rate, square wave frequency, etc.). An equivalent quantification can be applied for the case of synthetic adulterants added to the herbal formulation, described in the following section.

3.3. Detection of synthetic adulterants

In order to test the possibility of detecting synthetic adulterants in herbal preparations, the voltammetric response of samples spiked with different typical adulterants[2] was recorded. When clearly separated voltammetric peaks appear for the adulterant and the genuine herbal components, peak current or peak area values for the individual signals

can be used for quantification.[34,35] In several cases, however, the signals for the adulterant and the herbal components are strongly overlapped so that in the mixtures of the components a unique voltammetric wave was recorded. This is the case of sibutramine, a frequent adulterant[2,58] in formulations of *Caralluma adscendens*. As illustrated in Figure 8, the electrochemical responses of sibutramine, sample **19** and their mixtures were similar, the voltammetric signal being positively shifted on increasing the proportion of sibutramine.

To quantify the sibutramine/sample ratio, two easily measurable parameters were used, both previously described in VMP literature:[34] the peak potential and the ratio between the currents at two selected potentials. Figure 9a illustrates the variation of the peak potential, E_{pd}, (measured after semi-derivative convolution of the voltammograms), with the percentage of sibutramine in the drug-sample **24** mixtures whereas Fig. 9b depicts the variation of the ratio between the currents at 700 and 800 mV, $I(700)/I(800)$ on the sibutramine/(sample **24**) mass ratio. The above representations can be used as calibration graphs for determining the concentration of the synthetic adulterant in a sample of unknown composition. Interestingly, both representations approach to linearity at low concentrations of sibutramine. This situation facilitates the determination of the adulterant in real cases, where low concentrations of the same can be expected.

The variation of E_{pd} on the concentration (in % w/w) of sibutramine, c, can be fitted to the empirical equation E_{pd} (mV) = (650±1) + (2.28±0.05)c – (0.0092±0.0008)c^2 (N = 5; r = 0.9992). In the case of the current ratio, one can assume that the intensity of the measured signals at the potentials I and II, I_I, I_{II}, corresponds to the sum of the independent contributions of the adulterant (A) and the sample components (B) and that such contributions are proportional to their respective concentrations in the sample so that $I_J = g_{JA}c_A + c_{JB}c_B$ (J = I, II). Then, introducing the respective proportionality coefficients between currents and concentrations, the current ratio, I_{II}/I_I, ratio can be expressed as a function of the ratio of the concentrations of A and B, c_A/c_B, as:

$$\frac{I_{\mathrm{I}}}{I_{\mathrm{II}}} = \frac{\left(\frac{g_{\mathrm{IA}}}{g_{\mathrm{IIB}}}\right)\left(\frac{c_{\mathrm{A}}}{c_{\mathrm{B}}}\right) + \left(\frac{g_{\mathrm{IB}}}{g_{\mathrm{IIB}}}\right)}{1 + \left(\frac{g_{\mathrm{IIA}}}{g_{\mathrm{IIB}}}\right)\left(\frac{c_{\mathrm{A}}}{c_{\mathrm{B}}}\right)} \qquad (2)$$

Assuming that all g_{JK} (J = I, II; K = A, B) coefficients have similar values, the above equation tends at low $c_{\mathrm{A}}/c_{\mathrm{B}}$ values to a linear variation between the current ratio and the concentration ratio given by:

$$\frac{I_{\mathrm{I}}}{I_{\mathrm{II}}} = \left(\frac{g_{\mathrm{IA}}}{g_{\mathrm{IIB}}}\right)\left(\frac{c_{\mathrm{A}}}{c_{\mathrm{B}}}\right) + \left(\frac{g_{\mathrm{IB}}}{g_{\mathrm{IIB}}}\right) \qquad (3)$$

From data in Fig. 8b, one can obtain $g_{\mathrm{IA}}/g_{\mathrm{IIB}}$ = 0.0444±0.0001 and $g_{\mathrm{IB}}/g_{\mathrm{IIB}}$ = 0.700±0.006. At high values of $c_{\mathrm{A}}/c_{\mathrm{B}}$, Eq. (2) tends to $I_{\mathrm{II}}/I_{\mathrm{I}} = g_{\mathrm{AI}}/g_{\mathrm{AII}}$, so that, from the above set of data, we obtain $g_{\mathrm{AI}}/g_{\mathrm{AII}}$ = 1.90±0.02.

For quantification purposes, the adulterant/sample ratio can be estimated as:

$$\frac{c_{\mathrm{A}}}{c_{\mathrm{B}}} = \frac{\left(\frac{g_{\mathrm{IIB}}}{g_{\mathrm{IA}}}\right)\left(\frac{I_{\mathrm{I}}}{I_{\mathrm{II}}}\right) - \left(\frac{g_{\mathrm{IB}}}{g_{\mathrm{IA}}}\right)}{1 - \left(\frac{g_{\mathrm{IIA}}}{g_{\mathrm{IA}}}\right)\left(\frac{I_{\mathrm{I}}}{I_{\mathrm{II}}}\right)} \qquad (4)$$

while the mass fraction of adulterant, f_{A} (= $c_{\mathrm{A}}/(c_{\mathrm{A}}+c_{\mathrm{B}})$) will be:

$$f_A = \frac{\left(\frac{g_{\mathrm{IIB}}}{g_{\mathrm{IA}}}\right)\left(\frac{I_{\mathrm{I}}}{I_{\mathrm{II}}}\right) - \left(\frac{g_{\mathrm{IB}}}{g_{\mathrm{IA}}}\right)}{1 - \left(\frac{g_{\mathrm{IB}}}{g_{\mathrm{IA}}}\right) + \left[\left(\frac{g_{\mathrm{IIB}}}{g_{\mathrm{IA}}}\right) - \left(\frac{g_{\mathrm{IIA}}}{g_{\mathrm{IA}}}\right)\right]\left(\frac{I_{\mathrm{I}}}{I_{\mathrm{II}}}\right)} \qquad (5)$$

Accordingly, f_{A} (or $c_{\mathrm{A}}/c_{\mathrm{B}}$) can be calculated for a sample of unknown composition from experimental $I_{\mathrm{II}}/I_{\mathrm{I}}$ data providing that the values of the $g_{\mathrm{IB}}/g_{\mathrm{IIB}}$, $g_{\mathrm{AI}}/g_{\mathrm{AII}}$, and $g_{\mathrm{AI}}/g_{\mathrm{AII}}$ ratios are determined from calibration experiments such as illustrated in Fig. 9b. From which, the detection limit for sibutramine detection was evaluated of 2% wt of the drug.

Conclusions

Microparticulate films of extracts of herbal components in herbal preparations display, in contact with aqueous electrolytes, characteristic voltammetric responses. Such responses, attributable to the oxidation of the extracted electroactive polyphenolic compounds can be used for screening and authentication of herbal formulations. Varieties (var. *adscendens*, *fimbriata* and *attenuata*) of *Caralluma adscendens* were discriminated voltammetrically both in the laboratory and in field using portable equipment. The proposed method, which does not require laborious sample pretreatment, permits a fast and sensitive way to characterize the components of herbal formulations. In favorable cases, the analysis of voltammetric curves permits the detection of the presence of two or even more than two-component herbal mixtures and the addition of synthetic adulterants. This requires that the individual components are present in relatively high proportions and that the corresponding voltammetric signals become clearly separated. The relative quantification of different pairs of components is in general possible for binary mixtures even in the case of strongly overlapped voltammetric signals, thus illustrating the capabilities of electrochemical analysis in the pharmacological domain.

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Table 1. Botanic species in herbal formulations in this study.

Family	Species	Common name
Asteraceae	*Cynara carduculus* L.	Alcachofra
Apocyanaceae	*Caralluma adscendens* Wall.	Edible, karallamu, caralluma
Fabaceae	*Phaseolus vulgaris* L. *Senna alexandrina* Mill. (*Cassia angustifolia* Vahl.)	Judía, Alubia, Fesol, Faba, fabe Sen, Casia, Cassia Officinalis
Rhamnaceae	*Rhamnus purshiana* DC.	Cáscara sagrada, cascara buckthorn, bearberry
Clusiacea	*Garcinia gummi-gutta* L (Roxib.)	Tamarindo, brindleberry, Malabar tamarind
Lauraceae	*Persea Americana* Mill.	Abacateiro, Aguacate
Boraginaceae	*Cordia ecalyculata* Vell.	Bugre, colita, gomita, café do mato, Pholia Magra®, Pocha magra
Apiaceae	*Centella asiatica* L.	Centella Asiática, Gotu Kola, Antanan, Pegaga, Brahmi
Rutaceae	*Citrus aurantium* L.	Naranjo amargo
Theaceae	*Camellia sinensis* Kuntze	Te verde, green tea
Fucaceae	*Fucus vesiculosus* L.	Fucus, sargazo vejicoso
Phormidiaceae	*Arthrospira maxima* Setch.	Espirulina

Figures

Figure 1. Cyclic voltammograms of microparticulate films on glassy carbon electrode prepared from ethanolic extracts of: a) 'Pholia magra' (= *Cordia ecalyculata*, Lamiales, Boraginaceae); b) *Centella asiatica* (Apiales, Apiaceae) immersed into air-saturated 0.10 M aqueous potassium phosphate buffer at pH 7.00. Potential scan rate 50 mV/s.

Figure 2. SECM map colors of a microparticulate deposit of an ethanolic extract from sample **24** on Pt substrate immersed into 2.0 mM $K_4Fe(CN)_6$ plus 0.25 M HAc/NaAc, pH 4.75. a) E_T = +0.30 V; E_S = 0.00 V.

Figure 3. Square wave voltammograms of microparticulate films on glassy carbon electrode prepared from ethanolic extracts of samples: a,b) **24** (*Caralluma adscendens*); c,d) **40** (*Phaseolus vulgaris*) and e,f) **21** (*Cordia ecalyculata*) obtained from laboratory (a,c,e) and portable (b,d,f) equipments. Potential scan initiated at −0.25 V in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

Figure 4. Square wave voltammograms of microparticulate films on glassy carbon electrode prepared from a-c) ethanolic and d-f) acetone extracts of samples nominally containing *Caralluma adscendens* immersed into air-saturated 0.10 M aqueous potassium phosphate buffer at pH 7.00. Samples a,d) **24** (three replicate voltammograms), b,e) **25** (accompanied by samples **19** and **34** in b)), c,f) **32**. Potential scan initiated at −0.25 V in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz. Three replicate experiments are superimposed in a).

Figure 5. Three dimensional diagram constructed from the values of $I(270)/I(330)$, $I(330)/I(720)$ and $I(1000)/I(720)$ obtained from the square wave voltammograms of ethanolic extracts of blanks and samples of *Caralluma adscendens* var. *adscendens*, *fimbriata* and *attenuata* in phosphate buffer in conditions such as in Fig. 3.

Figure 6. Plots of $(I(E)/I_{max})_{Acet}$ vs. $(I(E)/I_{max})_{EtOH}$ for: a,b) two replicate experiments on films of leaf extracts sample **24** (*Caralluma adscendens* var. *adscendens*) and samples c)

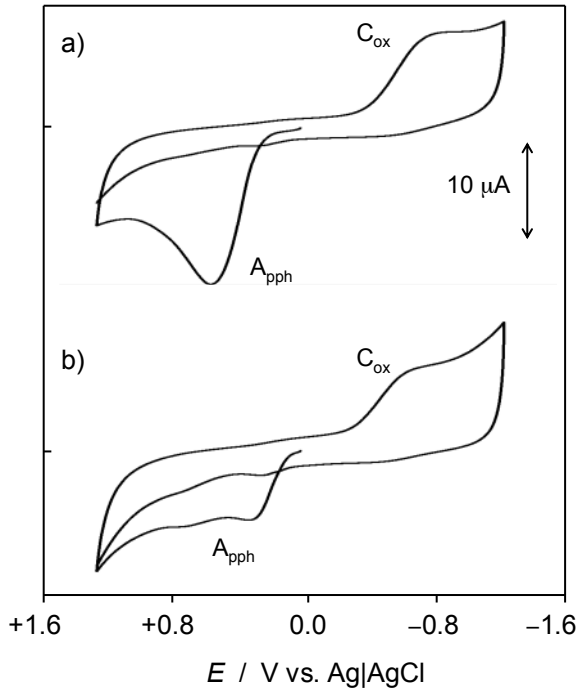
53 and d) **89** immersed into air-saturated 0.10 M aqueous potassium phosphate buffer at pH 7.00. From square wave voltammograms such as in Fig. 4.

Figure 7. Square wave voltammograms of microparticulate films on glassy carbon electrode prepared from acetone extracts of samples: a) **40**; b) **33**; c) **21**; d) **37** in contact with air-saturated 0.10 M aqueous potassium phosphate buffer at pH 7.00. Potential scan initiated at −0.25 V in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz. C: *Cordia ecalyculata*; P: *Phaseolus vulgaris*; G: *Garcinia gommi-gutti*.

Figure 8. Positive-going scan square wave voltammograms of microparticulate films on glassy carbon electrode prepared from ethanolic extracts of: a) sibutramine, sample **24** spiked with b) 16.5% w/w and c) 32.5% w/w sibutramine, and d) sample **24**. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

Figure 9. Variation of a) the peak potential measured after semi-derivative convolution of the voltammograms, E_{pd}, on the percentage of sibutramine and b) the ratio between the currents at 700 and 800 mV, $I(700)/I(800)$, on the sibutramine/sample mass ratio in drug-sample **24** mixtures. From voltammograms in Fig. 8. The dotted line represents the linear tendency at low sibutramine concentrations.

Figure 1.



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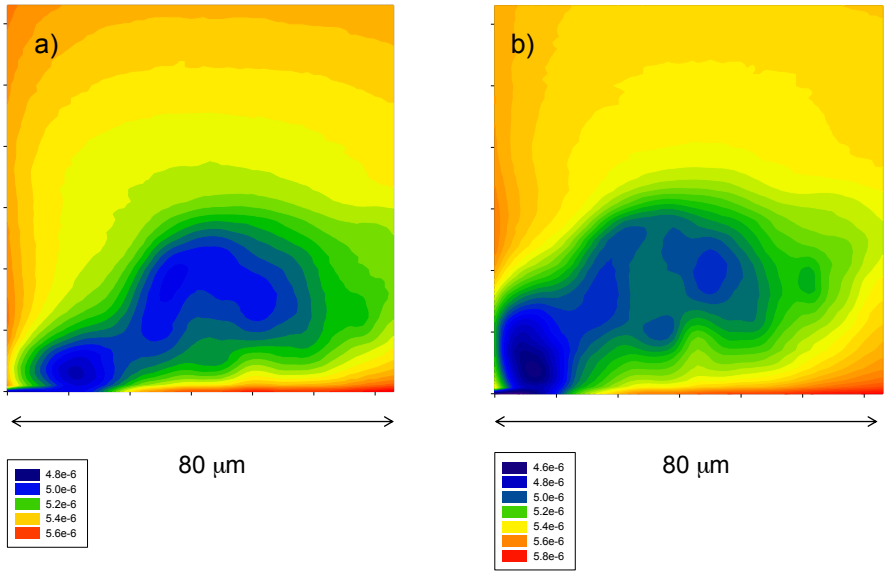

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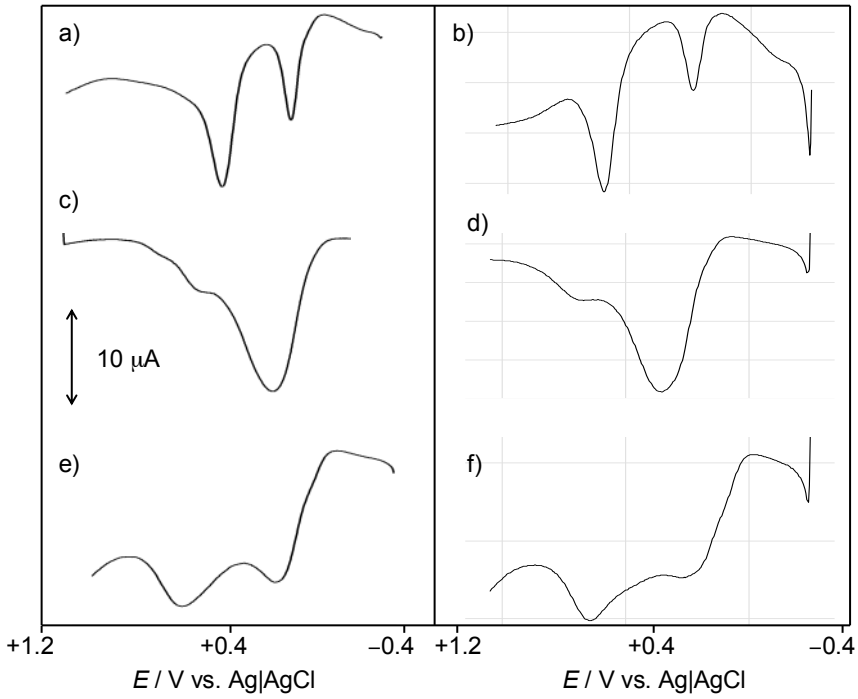

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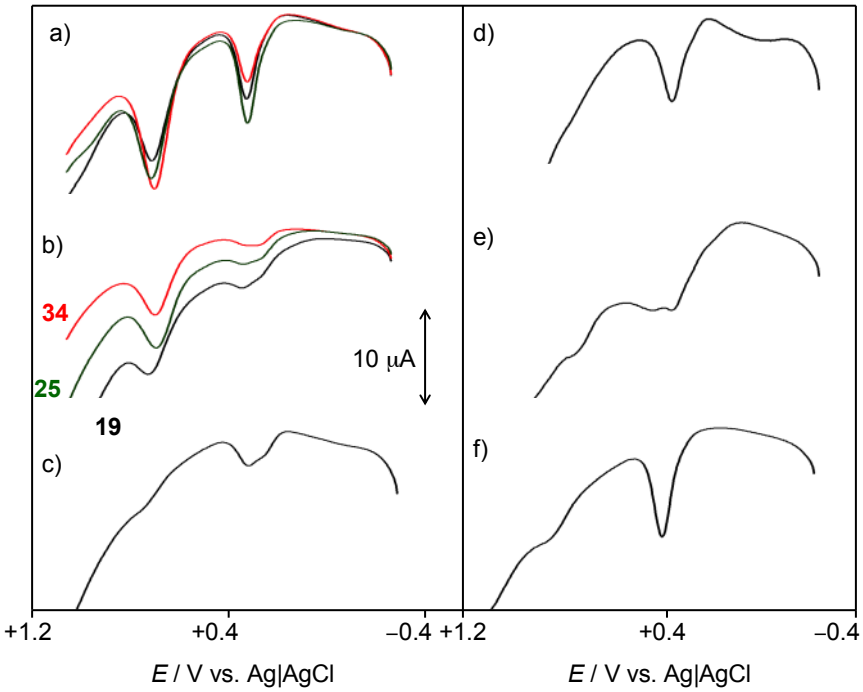

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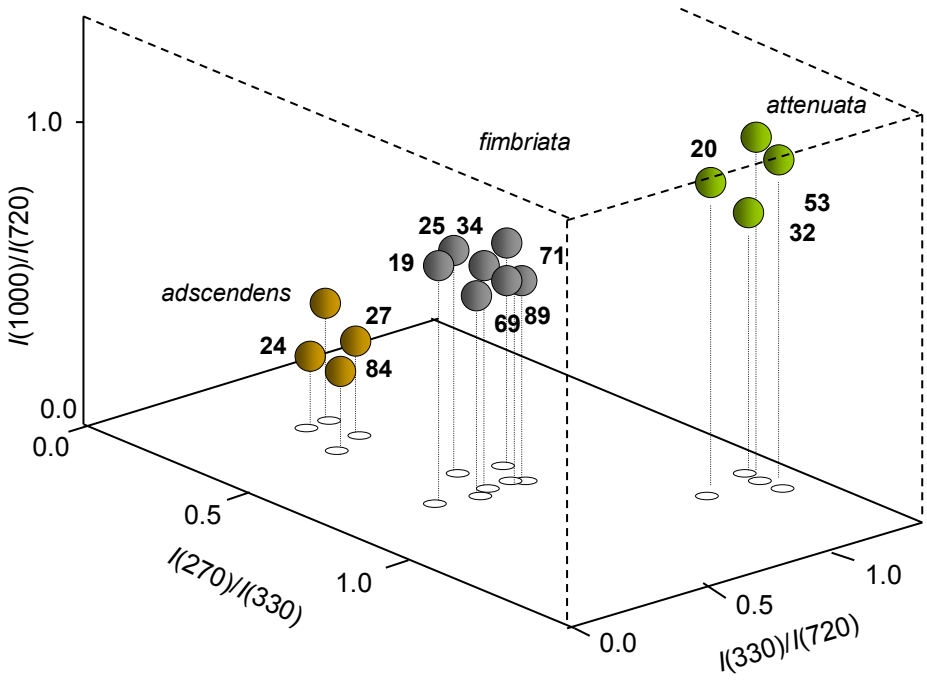

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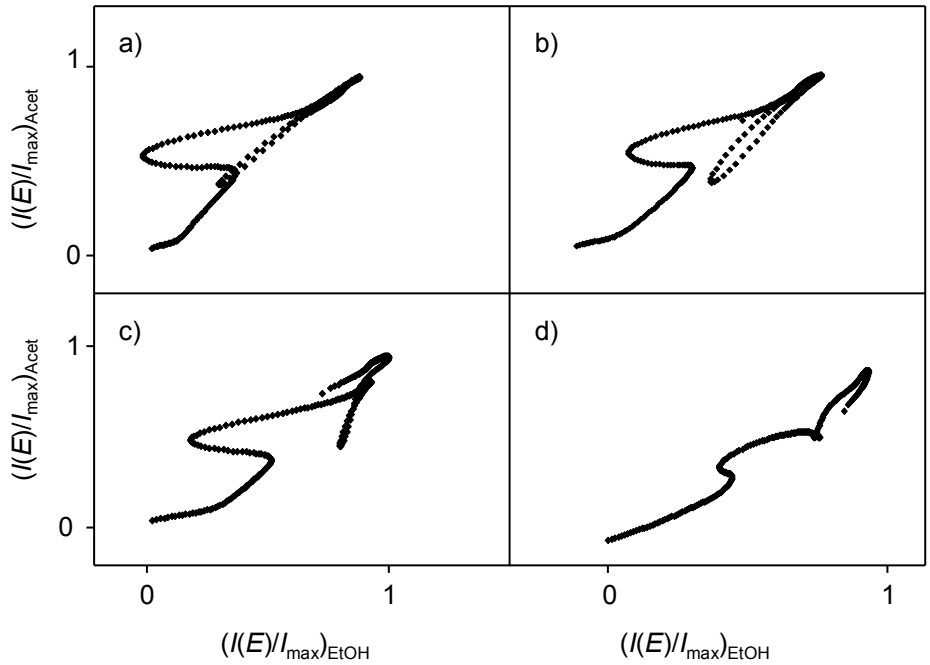

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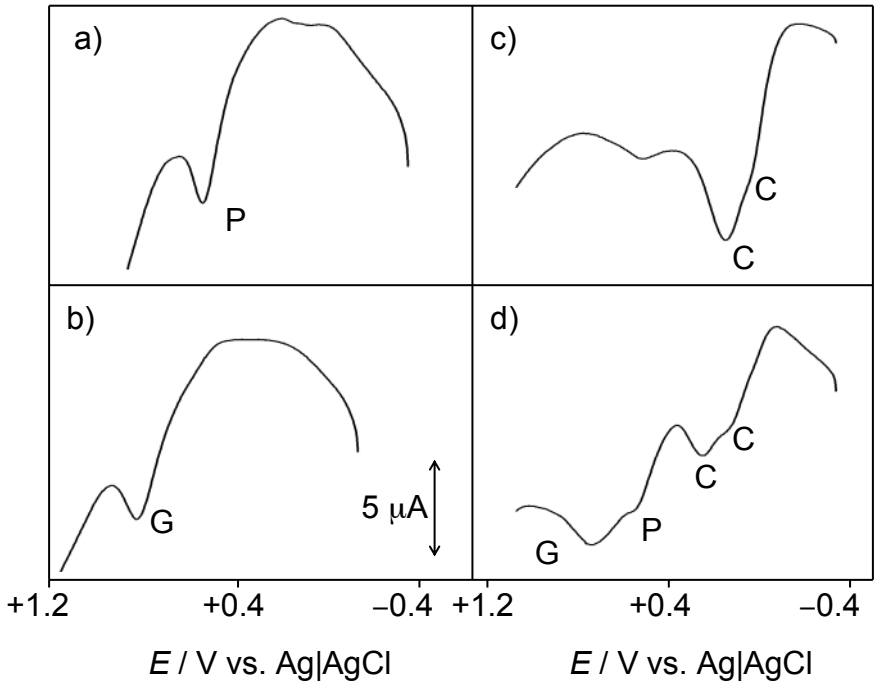

Figure 8.

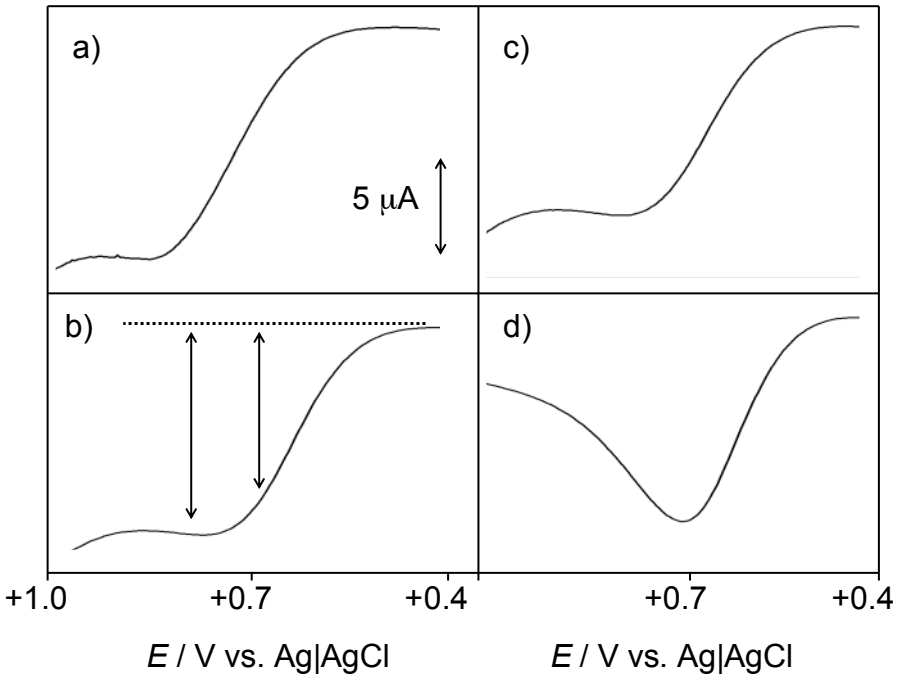

Figure 9.