



Disposable electrochemical sensor for rapid determination of heavy metals in herbal drugs

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Abstract

Analysis of herbal drugs and extracts need rapid and affordable methods to assure the quality of products. The application of the electrochemical sensors in the field of quality control of herbal drugs, herbal drug preparations and herbal medicinal products appears very promising, advantageous and alternative to conventional methods due to their inherent specificity, simplicity and for the fast response obtained. This paper presents a proposal about the application of disposable electrochemical sensors associated with electroanalytical instrumentation for the detection of heavy metal analysis in herbal drugs. In particular samples of St. John's wort were analysed applying anodic stripping voltammetry. The content of Cd and Pb were evaluated. The ICP spectroscopy was used as reference method.

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1. Introduction

Among different target analytes for quality control of herbal drugs, heavy metals play an important role. Recently, in the European Pharmacopoeia [1], a monograph concerning the analysis of heavy metals in herbal drugs and fatty oils by absorption spectrometry has been reported. As alternative to traditional spectrochemical techniques for the detection of heavy metals in routine analysis of herbal drugs we propose the use of

electroanalytical techniques coupled with disposable screen-printed electrodes. Low cost and rapid multielement measurements are thus possible.

The use of the screen-printing procedure for the development of sensor and biosensor is gaining consideration in the field of analytical chemistry as shown by the increasing number of scientific papers describing modifications and applications of these tools and the launch of commercial products based on this technology [2–5]. Screen-printed electrodes are planar devices, based on different layers of inks printed on a plastic or ceramic substrate.

Screen-printing technology is particularly attractive for the production of disposable sensors. This is especially important in order to overcome

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the phenomenon referred to as ‘electrode fouling’, which is one of the main drawbacks of electrochemical sensors.

Different inks can be used to print electrodes and therefore different properties of the final sensor can be achieved. The inks can be printed on several kinds of supports like glass, ceramic and plastic sheets. Different inks are commercially available and some of them are based on noble metals (Au, Pt, Ag, etc.). However, for these inks a high firing temperature (850–1200 °C) is necessary and the overall process becomes cumbersome. Very interesting materials for printing electrochemical sensors are the polymeric carbon-based inks, because of their very low firing temperature (from room temperature to 120 °C) and because they can be printed on plastic sheets. Carbon can also be mixed with different compounds (mediators, enzymes, and metal particles) and therefore modified sensors can be produced. In the literature some carbon electrodes modified with catalytic particles have been described and they showed an improvement in the detection of hydrogen peroxide with respect to simple carbon electrodes. Using this modification strategy, ruthenized screen-printed electrodes have been produced; these devices have been used in a simple and rapid method for the determination of anticholinesterase pesticides [6–8]. Other electrode modification strategies for pesticides detection are reported in Ref. [9].

In this paper we describe the use of mercury coated graphite screen-printed electrodes as sensors for heavy metal determination.

These applications are based on graphite screen-printed electrodes as working electrode and auxiliary electrode and a silver reference electrode (Fig. 1).

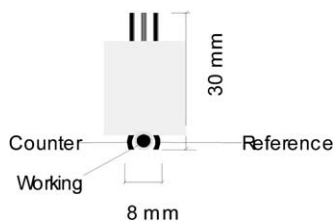


Fig. 1. Scheme of the screen-printed electrodes.

The electrochemical technique used is the square wave (SW) anodic stripping voltammetry. The method proposed consists of three main steps. First, a thin mercury film (TMF) is pre-plated onto the surface of the working electrode. Then, a preconcentration step is performed: the mercury film graphite electrode is held at a negative potential (generally it is 300–500 mV more negative than the reversible potential, calculated from the Nernst equation, for the least easily reduced ion to be determined). The metal ions in the sample solution are thus reduced at such negative potential and concentrated into the mercury film.

Finally the amalgamated metals are measured by applying a positive potential scan and measuring the peak currents produced as the system reaches the oxidation potentials of the metals; the anodic peak current is proportional to the concentration of the metal in the amalgam, and thus in the sample [10].

2. Experimental

2.1. Reagents

Mercury chloride, Suprapur grade hydrochloric, nitric, acetic, perchloric acids were purchased from Merck (Italy). The water used for preparation of solutions was from a Milli-Q System (Millipore, Italy). Heavy metal stock solutions were prepared by diluting lead(II) and cadmium(II) standard solutions AAS grade (Fluka, Italy).

2.2. Printing of the electrodes

Electrodes were printed with a model 245-screen-printer from DEK (Weymouth, UK) using inks obtained from Acheson Italiana (Milan, Italy). A graphite based ink (Electrodag 423), a silver ink (Electrodag 477 SS RFU), a silver/silver chloride ink (AV 459), and an insulating ink (Electrodag 6018 SS) were used.

The inks were deposited, on a polyester flexible substrate, in a film of controlled pattern and thickness to obtain overlapping layers. At first the silver tracks were printed, then the graphite pad was positioned over part of the silver track;

and finally the insulating layer was deposited with openings that allow electrical contact with the circuit at one end and the analyte solution at the other end.

After each printing step the inks were heated at 110 °C for 10 min for polymerization.

2.3. Electrochemical analysis

All the experiments were carried out using an Autolab PGSTAT 10 System (Ecochemie, Utrecht, Netherlands). SW voltammetry conditions were: conditioning potential -0.3 for 60 s, deposition potential -1.1 V for 120 s, equilibration time 30 s, SW amplitude 28 mV, step potential 3 mV, frequency 15 Hz. HCl 0.1 M was used as supporting electrolyte.

2.4. Mercury thin film deposition onto the screen-printed electrode

The screen-printed electrode was used as a substrate for a TMF, the mercury film was preplated from a stirred mercury(II) chloride solution, 80 mg/l, in hydrochloric acid 0.1 M, by holding the electrode at -1.1 V for 2 min. The potential was then switched to -0.2 V for a cleaning period of 2 min.

2.5. Sample preparation and analysis

In this work, samples of St. John's wort were analysed and the content of Cd, Pb were evaluated. The ICP spectroscopy was used as reference method.

To mineralise the herbal samples (St. John's wort leaves and flowers), a nitric-perchloric digestion was used. The digestion was performed in a closed vessel. At 0.1 g of sample were added 3.2 ml of nitric acid and 0.8 ml of perchloric acid; everything was heated up at 200 °C until a white residual is formed. The process was concluded adding 5 ml of supporting electrolyte solution (0.1 M of HCl).

Metal content values by ICP were obtained from a private certified laboratory, where an ICP Spectrometer Optima 3000 SC (Perkin–Elmer) was used, after a microwave digestion of the

samples. The precision of the ICP determination was approximately 10%.

3. Results and discussion

3.1. Electrode characterization

The round shaped working electrode was examined using scanning electron microscopy (SEM) (Fig. 2). The thickness of the carbon layer is 20 μm , while the silver layer is 5–8 μm . The surface analysis shows a very irregular profile. This is confirmed by the electrochemical evaluation of the actual surface area of the electrode that is 1.7 times larger than the geometric area.

Some impurities are often present in commercial graphite inks: this is a major drawback of the use of commercial inks, especially if the impurity leads to electrochemical interferences. The presence of electroactive groups was revealed by cyclic voltammetry. The scan was performed in phosphate buffer (pH 7.4) + KCl 0.1 M, with a scan rate of 10 mV/s (Fig. 3). However, these interferences are negligible, using the optimised experimental condition for heavy metal analysis, described below.

The use of a Ag/AgCl ink rather than the bare Ag ink for the reference electrode surface is based on the assumption that, even if at positive potentials the silver may form silver chloride when chloride is present in the solution, this may lead to a possible instability of the reference electrode and hence of the electrochemical potential held at the working electrode. The difference of potential of the silver/silver chloride ink and the silver ink with respect to a 'real' silver/silver chloride reference electrode, saturated KCl as internal solution, was electrochemically tested. The potentials of both of them were stable for more than 30 min. Therefore, the silver ink was used as material for the reference electrode fabrication speeding up the preparation procedure.

3.2. Anodic stripping for metal ions determination

The mercury coated screen-printed electrodes were used for heavy metal determination coupled with voltammetric stripping analysis. Mercury was

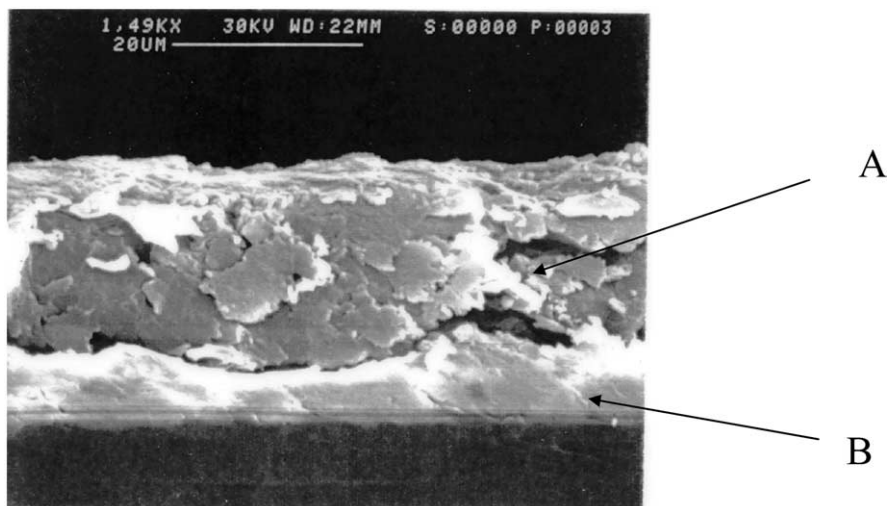


Fig. 2. Cross-section of the SEM of the screen-printed electrode graphite layer. (A) Carbon layer, (B) silver layer.

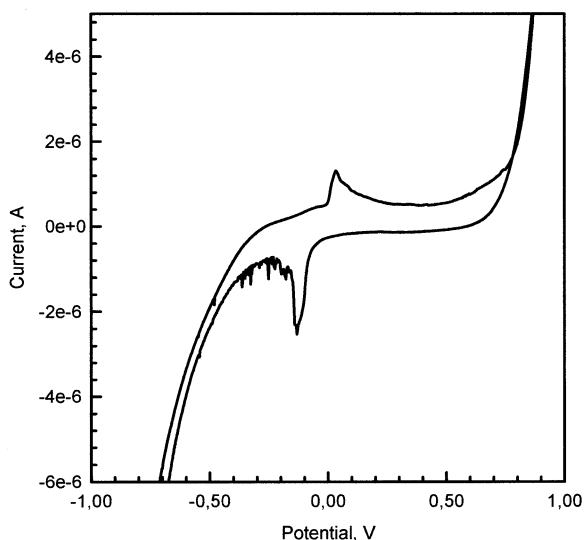


Fig. 3. Electrochemical characterisation of screen-printed working electrode: cyclic voltammogram in phosphate buffer+KCl 0.1 M.

plated on the carbon electrode surface of the screen-printed electrode, by application of a plating potential in a solution of HgCl_2 . The thickness of the mercury film (L) may be calculated using the equation:

$$L = 2.4 \times 10^{-5} it / r^2$$

where i is the mercuric ion plating current, t is the plating time, and r is the geometrical radius of the electrode surface ($r = 1.5 \text{ mm}$). The film thickness increases linearly with the plating time (Fig. 4). A mercury plating time of 2 min was chosen for further experiments.

The metal ion peak current depends on various parameters of the deposition and stripping steps, as well as on the characteristics of the metal ion to

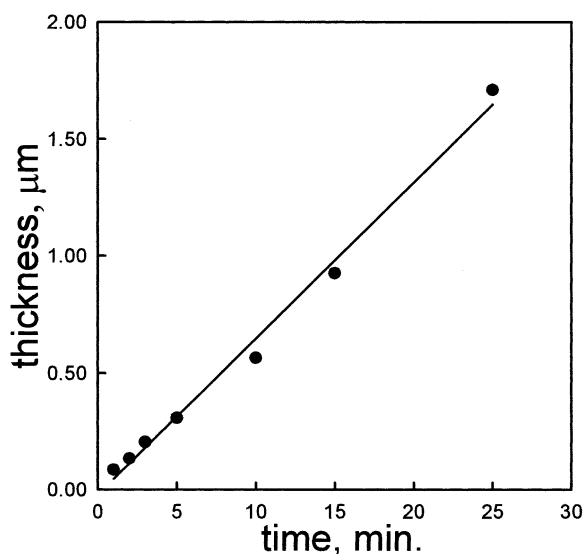
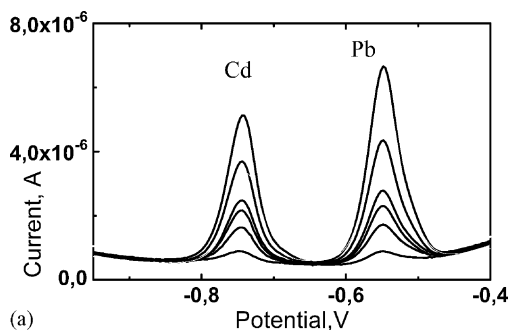
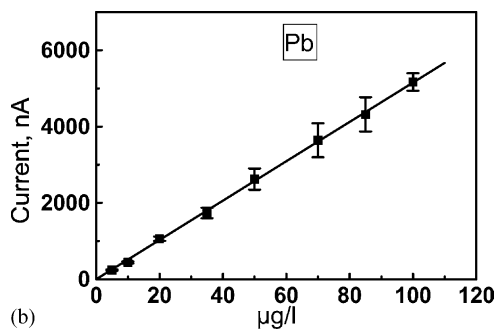


Fig. 4. Influence of the plating time on the mercury film thickness.

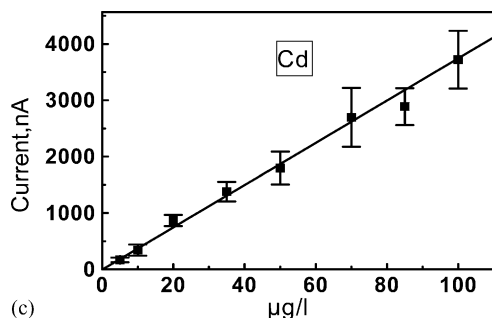
be detected and the electrode geometry. The optimisation of these parameters was reported elsewhere [11]. With the optimised parameters a multielemental analysis can be performed in less than 3 min (Fig. 5). In Fig. 5a is reported the electrochemical profile of a standard solution containing the metals (lead and cadmium) in a range of 5–100 $\mu\text{g/l}$. In Fig. 5b and c are reported the calibration plots for lead and cadmium, respectively. Each point is the mean of the value



(a)



(b)



(c)

Fig. 5. Typical results obtained for a mixture of different heavy metals (lead and cadmium) using the disposable sensor. The voltammograms (5a) and the calibration plots (lead, 5b; cadmium, 5c) are reported.

obtained with three different electrodes. The relative standard deviation at 10 ppb of lead is 10% with three different electrodes. The relative standard deviation of three repetitions using the same strip is 5%.

3.3. Results with real samples

Real samples obtained from St. John's wort were tested, evaluating the metal content (Cd and Pb), after digestion of the sample.

The electrochemical analysis was performed using screen-printed electrodes and the results are reported in Table 1. The results obtained by the electrochemical screen-printed sensors are in agreement with those of the reference method (ICP spectroscopy).

The main advantages of the electrochemical approach are: low analysis costs, fast response time, very low detection limits, simultaneous analysis of different metals. For all of these reasons can be proposed as new analytical tool in traces analysis of heavy metals of herbal drugs.

4. Conclusions

Screen-printed sensors offer the specificity and sensitivity of laboratory-based procedures.

The test can then be performed in field thus avoiding the delays associated with centralised laboratories. Similar advantages are being realised in other sectors such as food and environmental analysis and process control.

These devices is a promising tool for herbal drug analysis due to the possibility to fulfil some demand that the classic methods of analysis do not attain. Original characteristic turns the screen-printing technology a possible methodology to be applied with real samples. Some advantages as high selectivity and specificity, relative low cost of construction and storage, potential for miniaturization, facility of automation and simple and portable equipment construction for a fast analysis and monitoring in platforms of raw material reception, quality control laboratories or some stage during the food processing.

Table 1
Comparison of thin mercury film electrode (TMFE) and ICP results testing herbal samples

Sample	TMFE		ICP	
	Cd (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
1	0.05	0.6±0.1	≤0.1	≤0.1
2	0.2±0.1	0.4±0.1	0.15	0.9
3	0.8±0.1	5.7±0.2	0.35	5.8
4	1.5±0.1	13±2	1.3	19

For stripping analysis herbal samples were treated by wet digestion (see text).

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