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Short Communication

Acemetacin and Indomethacin Detection using Modified Carbon Microelectrodes

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Abstract- In the present work, mercury thin film modified carbon microelectrodes were tested towards detection of the pharmacological compounds acemetacin and indomethacin. The compounds were characterized electrochemically by adsorptive stripping differential pulse and square wave voltammetry (AdSDPV and AdSSWV). Best results were obtained via AdSDPV for both compounds (Acemetacin: LOD=1.05×10^{-7} M, LOQ=3.51×10^{-7} M; Indomethacin: LOD=1.34×10^{-7} M, LOQ=4.47×10^{-7} M). These results are comparable to earlier reports using classical hanging drop mercury electrodes, but with environmental advantages due to the limited mercury amount that is used and the easy way to recover it. Detection and quantification limits are lower than the obtained with currently used analytical techniques.

Keywords- Acemetacin, Indomethacin, Electroanalytical techniques, Microelectrodes, Thin mercury film

1. INTRODUCTION

The widespread use of pharmacological compounds and personal care products, in particular in western societies, led to a new concern regarding its disposal into the

environment. Efficient pharmaceutics monitoring through electroanalytical techniques is, as a consequence, currently being extensively pursued aiming low cost, simple and fast, but still reliable, analyte detection [1]. Acemetacin and indomethacin are two non-steroidal anti-inflammatory drugs with very similar structure and properties (see Fig. 1). These are used in the treatment of inflammatory and degenerative diseases of articulations, possessing anti-pyretic and analgesic properties [2-4]. Acemetacin and indomethacin detection have been performed by classic analytical tools such as liquid chromatography, spectrophotometry, fluorometry or UV spectroscopy, although some examples using electroanalytical methods have been also reported, such as the use of hanging mercury drop electrode (HMDE) [2-3,5–10]. HMDE, however, has been banned internationally because of the high mercury quantity that is needed. In contrast, to coat a microelectrode, only a minimal quantity of mercury is necessary, which is electrochemically recovered by chronoamperometric dissolution, with no environmental contamination.

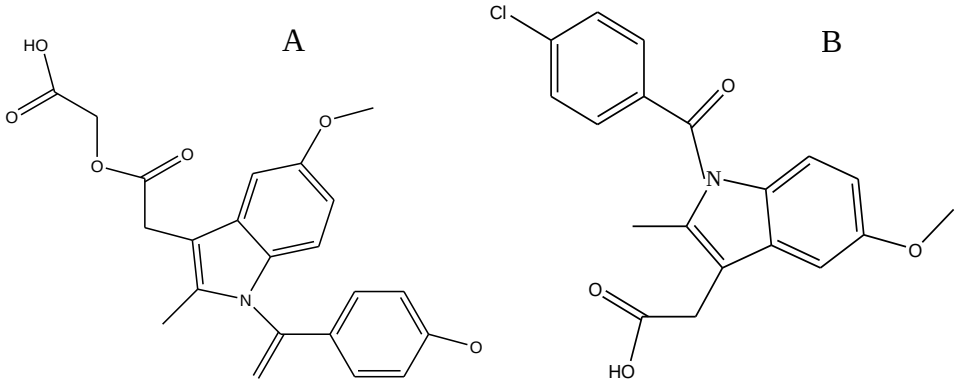

Fig. 1. Structures of **(A)** Acemetacin and **(B)** Indomethacin (Chem3D Pro, CambridgeSoft©)

In the present work, thin mercury film modified carbon microelectrodes were tested towards the detection of the individual drugs and their mixture by adsorptive stripping differential pulse voltammetry (AdSDPV) and adsorptive stripping square wave voltammetry (AdSSWV). The sensitivity of both AdSDPV and AdSSWV was determined through the determination of the detection and quantification limits (LOD and LOQ, respectively) using the statistical method described by Miller and Miller [11-12].

2. EXPERIMENTAL

Supporting electrolyte was 0.04 M Britton-Robinson buffer (boric, phosphoric and acetic acid), pH 7.6. Acemetacin and indomethacin solutions were prepared from dilutions of the respective stock solutions of 1 mM. These were prepared by dissolution of the appropriate

drug mass in a 50/50 (V/V) solution of ethanol and Britton-Robinson buffer, assessing the remaining volume with Britton-Robinson buffer. Mix solutions were prepared with addition of volumes of the drugs' solutions so that the final concentration of both drugs in the mix solution was equal. All reagents were p.a. grade. The electrochemical assays were performed using an AUTOLAB PGSTAT128N in a one-compartment cell in a three electrode configuration. Working electrode was carbon microelectrode with 10µm diameter, counter and reference electrodes were a platinum wire and a saturated calomel electrode (SCE), respectively. Prior to the assays, the microelectrode was polished using 1.0 and 0.3 µm alumina and submitted to an ultra-sound bath for 60 s. Counter and reference electrodes were rinsed with milli-Q water. Solutions were purged with an argon flux for 15 min. Modified carbon microelectrode with a thin mercury film was used, deposited potentiostatically at 0V for 60s from a 6 mM $HgCl_2$/ 1 mM KNO_3/ 0.5% HNO_3 solution. Indomethacin and acemetacin concentrations ranged from 0.005 to 0.5 mM. SWV and DPV were performed with the correspondent parameters: SWV, v=20.4 mV/s, f=8Hz, ΔE=0.02505 V and DPV, v=25.5 mV/s, ΔE=0.02505 V using for both t_{dep}=90 s and E_{dep}=-0.6 V.

3. RESULTS AND DISCUSSION

The mercury thin film is applied via chronoamperometry, using a constant potential of 0V for 60s from a dilute solution of Hg (II) salt. Since a microelectrode is used, a very small amount of mercury is applied, approximately 2 ng for each film. After the measurement, the mercury is completely removed potentiostatically through the application of +1.7 V for 90 s, back again to the mercury salt solution. After application of the thin mercury film, AdSDPV and AdSSWV were performed with the modified microelectrode. Results attained with both techniques were quite similar. The reduction peak of acemetacin is observed at slightly higher potential values than the reduction peak of indomethacin, as can be observed in Fig. 2. Before the study of acemetacin and indomethacin concentration variation, two experimental parameters were optimized: the deposition time on the working electrode surface and the deposition potential. After several variations of these parameters for both techniques (DPV and SWV) with the modified microelectrode, it was found that the best experimental condition was a deposition time of 90 s at -0.6 V.

The current intensity of the reduction peaks increase with the analyte concentration in solution, as expected. Looking at the mean current intensity values of acemetacin and indomethacin, it is possible to observe some loss of linearity of the plotted data, in particular for indomethacin. The behaviour is linear up to 0.5 mM for acemetacin and 0.35 mM for indomethacin (see Fig. 3).

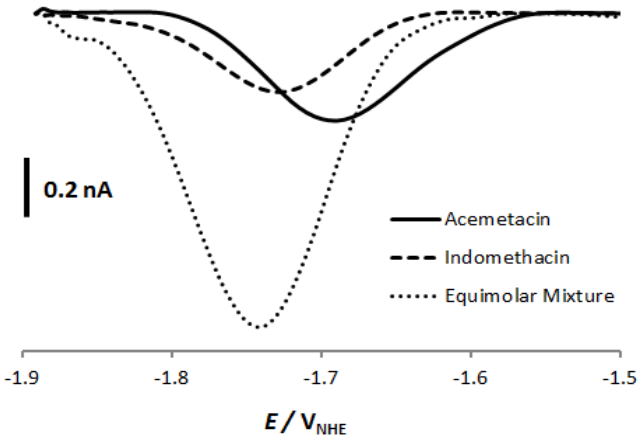

Fig. 2. DPV assays of 0.35 mM acemetacin, indomethacin and its equimolar mixture using the modified mercury thin film carbon microelectrode

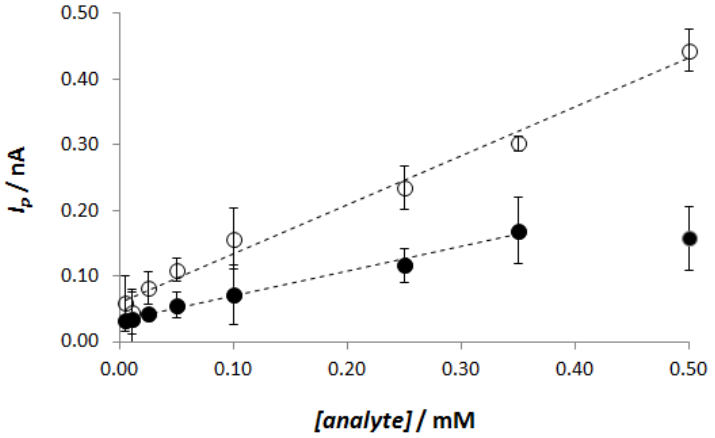

Fig. 3. Current intensity values as function of the analyte concentration, for acemetacin (○) and indomethacin (●) attained by AdSDPV

Best results were obtained via AdSDPV for both compounds (Acemetacin: LOD=1.05×10^{-7} M, LOQ=3.51×10^{-7} M; Indomethacin: LOD=1.34×10^{-7} M, LOQ=4.47×10^{-7} M). Calculated LOD and LOQ values are comparable with indomethacin detection with HMDE [2] and more sensitive than LODs and LOQs determined with spectrophotometric and liquid chromatographic techniques reported in the literature for both compounds [3,5]. AdSDPV and AdSSWV assays of solutions containing both drugs rendered only one reduction peak, correspondent to the reduction of both acemetacin and indomethacin (see Fig. 2). Since the reduction potentials of both drugs are very close, it is not possible to separate these drugs by these two electroanalytical techniques using the given conditions, as reported earlier in the literature for the HMDE [4]. Another interesting feature found is that the sum of the peak intensity values of the individual drug signals is larger than the signal of the equimolar mixture (see Fig. 4).

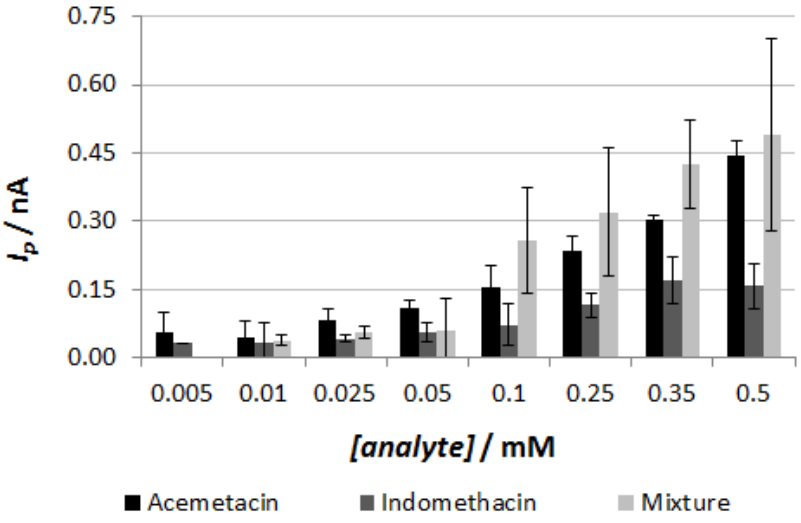

Fig. 4. Mean current intensity values of the reduction peaks of (A) acemetacin, (B) indomethacin and (C) its equimolar mixture, attained by AdSDPV. Note: due to background noise during the measurements, it was not possible to obtain the peak current for 0.005 mM of the equimolar mixture

Taking as an example the concentration value of 0.35 mM both in acemetacin and indomethacin, with "total" concentration of the equimolar mixture being 0.7 mM, the question remains whether the result for the mixture is due to loss of linearity or to an interaction effect between the two drugs present in solution at the same time. Forlower concentrations, the sum of the signals is within the range of linearity. As an example, for concentration 0.1 mM, the sum of the individual drug signals and the equimolar mixture signal are very close (sum=0.23; mixture=0.26) and the difference between the two values lies within the calculated error for this particular concentration (standard deviation=0.1). However, even within the range of linearity, the mixture current signal is always inferior to the sum of the individual drugs, which points to an effect of possible interactions between the two molecules. A comparison between bare and modified carbon microelectrodes was also performed for the same experimental conditions. Although the compounds could also be successfully detected using the bare carbon microelectrode, lower current intensities are achieved. As an example, for 0.1 mM of acemetacin, the current signal of bare carbon microelectrode is less intense (0.148 nA) compared to the mean current intensity value rendered by microelectrodes modified with thin mercury film (0.156 nA), as can be observed in Fig. 5. Hence, it is safe to assume that the application of the thin mercury film enhanced the detection of the drugs, lowering the detection and quantification limits.

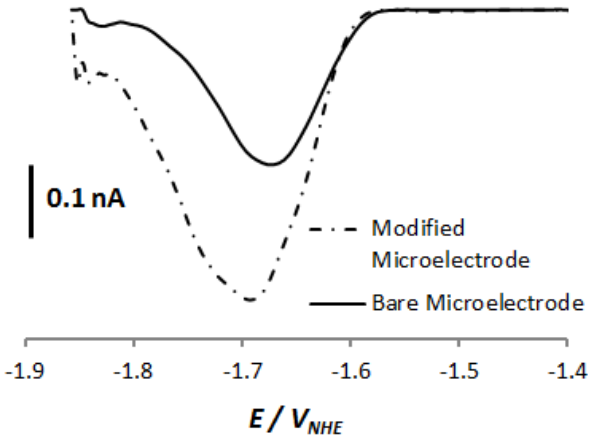

Fig. 5. DPV assays of 0.1 mM acemetacin using bare and mercury thin film modified carbon microelectrode

4. CONCLUSION

Thin mercury film carbon microelectrodes were used coupled with fast electrochemical cathodic stripping techniques to study indomethacin and acemetacin individually and in mix solutions containing both compounds. Best results were obtained via AdSDPV for both compounds (Acemetacin: LOD=1.05×10^{-7} M, LOQ=3.51×10^{-7} M. Indomethacin: LOD=1.34×10^{-7} M, LOQ=4.47×10^{-7} M). Calculated LOD and LOQ values are comparable with indomethacin detection with HMDE and more sensitive than LODs and LOQs determined with classic analytical methods for both compounds. Also, as reported earlier in the literature [4], within the mixture it was not possible to discriminate the individual signals of the two compounds.In agreement with previous reports, within the mixture, and using the tested experimental conditions, it was not possible to discriminate the individual signals of the two compounds. Although the methodology can benefit from further optimization, under the tested experimental conditions, thin mercury films have shown to enhance the compounds' detection, compared to bare carbon microelectrode assays and compared to the currently used techniques for analysis. The present study rendered good preliminary results, capable of evolving into real sample testing, which will be performed as next step.

Acknowledgements

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