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Determination of Triamcinolone Acetonide in Pharmaceutical Formulation and Human Serum by Adsorptive Cathodic Stripping Voltammetry

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Electrochemical behavior of triamcinolone acetonide (TAA) has been studied by cyclic voltammetry at a hanging mercury drop electrode in universal buffers of pH 2–11. Due to the interfacial adsorption of triamcinolone acetonide onto the mercury surface, a fully validated square-wave adsorptive cathodic stripping voltammetric procedure was developed for its determination in the bulk form. Under optimized conditions, the stripping voltammetric peak current of triamcinolone acetonide showed a linear dependence on TAA concentration over the range 1×10^{-9}–9×10^{-8} mol L^{-1}. The mean percentage recovery of TAA was 99.84 ± 0.64, the detection limit (LOD) was 3×10^{-10} mol L^{-1}, and the quantitation limit (LOQ) equaled 1×10^{-9} mol L^{-1}. The proposed procedure was successfully applied in the determination of triamcinolone acetonide in Kenacort® tablets and in human serum spiked with TAA without any pretreatment and/or time-consuming extractions prior to the analysis. LOD and LOQ corresponding to the determination of triamcinolone acetonide in spiked human serum were 7.5×10^{-10} mol L^{-1} and 2.5×10^{-9} mol L^{-1}, respectively.

Badano elektrochmiczne zachowanie acetonidu triamcinolonu (TAA) metodą cyklicznej woltamperometrii na elektrodzie rtęciowej w przedziale pH 2–11. Ponieważ stwierdzono adsorpcję TAA na powierzchni rtęci, do oznaczania tego związku zastosowano procedurę wykorzystującą wiszącą elektrodę rtęciową i adsorpcyjną, katodową woltamperometrię strippingową. W warunkach optymalnych otrzymano liniową zależność między wysokością piku woltamperometrycznego i stężeniem TAA w zakresie 1×10^{-9}–9×10^{-8} mol L^{-1}. Średni odzysk wyniósł 99.84 ± 0.64 %, granica wykrywalności 3×10^{-10} mol L^{-1} a granica oznaczalności 1×10^{-9} mol L^{-1}. Opacowaną procedurę zastosowano do oznaczania TAA w tabletkach Kenacort® i w ludzkiej surowicy krwi po dodaniu analitu. Nie było porzeby przeprowadzenia wstępnych działań chemicznych i ekstrakcji. Granicę wykrywalności i granicę oznaczalności w surowicy krwi oznaczono odpowiednio jako $7{,}5 \times 10^{-10}$ mol L^{-1} i $2{,}5 \times 10^{-9}$ mol L^{-1}.

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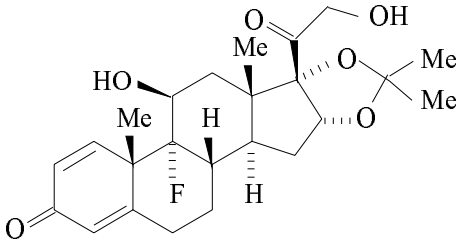
Triamcinolone acetonide (TAA) {(11β, 16α)-9-fluoro-11, 21-dihydroxy-16, 17-[1-methylethylidenebis(oxy)]pregna-1-4-diene-3, 20-dione} is a potent glucocorticoid used for antiinflammatory treatment, *e.g.* in patients with bronchial asthma or rheumatoid arthritis [1].



Structure of TAA molecule

Triamcinolone is not a major metabolite of triamcinolone acetonide in humans [2], however 6β-hydroxytriamcinolone acetonide is a major metabolite found in urine of rats, monkeys, and dogs [3].

Several methods for determination of triamcinolone acetonide, including spectrophotometry [4–7], high-performance liquid chromatography [8–11], and gas chromatography [12, 13] have been described in the literature. Most of them require sample pretreatment and time-consuming solid-phase extraction steps prior to the analysis, as well as expensive reagents and equipment, which are too expensive for routine pharmaceutical analyses and pharmacokinetic studies.

In this paper we have described a sensitive stripping voltammetric procedure for determination of TAA and for testing content uniformity in its dosage form. Stripping voltammetry is widely applied in drug analysis; it usually involves a simple preconcentration step, and also most of the excipients do not interfere in the determination. Up to now, no stripping voltammetric procedure for determination of triamcinolone acetonide has been described. We have utilized a simple and sensitive square-wave adsorptive cathodic stripping voltammetry to determine TAA in a bulk form, in pharmaceutical formulation, and in spiked human serum without the necessity of sample pretreatment and/or time-consuming extraction steps prior to the analysis.

EXPERIMENTAL

Solutions and reagents

A 1×10^{-3} mol L^{-1} stock standard solution of triamcinolone acetonide (Sigma, St. Louis, MO, USA) in ethanol (Merck) was prepared and stored at 4°C. Working solutions of TAA (concentration range:

10^{-6}–10^{-4} mol L^{-1}) were prepared daily by appropriate dilution of the stock solution with ethanol directly before use. A Mettler balance (Toledo–AB104, Switzerland) was used for weighing solid materials.

A series of universal buffer solutions of pH 2–11 was prepared by mixing equal volumes of 0.04 mol L^{-1} orthophosphoric, acetic, and boric acids. pH of the obtained buffers was adjusted with appropriate volumes of 0.2 mol L^{-1} NaOH solution. All chemicals were of analytical-reagent grade and were used without further purification. A Crison pH-meter (Crison, Barcelona, Spain) served for pH measurements. Deionized water was obtained from a Purite-Still Plus deionizer connected to an AquaMatic double distillation water system (Hamilton Laboratory Glass LTD, Kent, UK).

Apparatus

Computer-controlled Electrochemical Analyzers, Models 263A and 394–PAR, (Princeton Applied Research, Oak Ridge, TN, USA) controlled *via* 270/250 PAR software were used for voltammetric measurements. A three-electrode configuration (303A–PAR) was incorporated into a micro-electrochemical cell and comprised a hanging mercury drop electrode (HMDE) as a working electrode (surface area = 0.026 cm^2), an Ag/AgCl/KCls reference electrode, and a platinum wire auxiliary electrode. A magnetic stirrer (305–PAR) and a stirring bar were used to provide convective transport during the accumulation step.

Solutions of Kenacort® tablets

Ten tablets of Kenacort® (Bristol–Myers Squibb Company, New York–Cairo), with a declared content of 4 mg of TAA per tablet, were weighed and average tablet mass was determined. Then, they were grounded to a homogeneous fine powder in a mortar. An amount of the powder equivalent to the mass of one tablet was accurately transferred to 70 mL of ethanol (Merck) in a 100 mL-in-volume calibrated flask. The mixture was then sonicated for about 10 min and then the volume was made up to the mark with ethanol. Afterwards, the solution was filtered through a 0.45 µm Milli-pore filter (Gelman, Germany). Desired concentrations of TAA were obtained by accurate dilution of the obtained solution with ethanol. The solution was directly analyzed according to the general analytical procedure.

Solutions of spiked human serum

Serum sample from healthy volunteer was stored frozen until assay. To each of 10 centrifugation tubes containing TAA at a certain concentration, 0.1 mL of human serum sample and 1.0 mL of ethanol were added, and the contents were mixed well. Ethanol was added to denature and precipitate the protein. The solutions were centrifuged (Eppendorf centrifuge 5417C, Hamburg, Germany) for 3 min at 14000 rpm to separate the precipitated proteins. Clear supernatant was filtered through a 0.45 µm Milli-pore filter to obtain protein-free human serum spiked with various concentrations of TAA (10^{-6}–10^{-4} mol L^{-1}). Spiked human serum sample was transferred carefully into a 10 mL-in-volume calibrated flask and the volume was made up to the mark with the universal buffer (pH 2.5) as a supporting electrolyte. Finally, the sample was introduced into the micro-electrochemical cell. The solution was then analyzed according to the general analytical procedure without pretreatment and/or extraction.

General analytical procedure

The known volume of the standard stock solution of TAA was pipetted into a calibrated flask and the volume was made up to 10 mL with the universal buffer of pH 2.5. Concentration of ethanol in the final solution was 1%. This solution was placed in the electrochemical cell and deoxygenated with pure nitrogen

for about 10 min in the first cycle and for 30 s in each consecutive cycle. During measurements nitrogen was allowed to flow over the solution. Accumulation of TAA onto the HMDE surface was performed at –0.5 V *vs* Ag/AgCl/KCls for 130 s under continuous stirring of the solution at 400 rpm on a magnetic stirrer. Then, the solution was left quiescent for 5 s to equilibrate and afterwards voltammograms were recorded by scanning the potential towards negative direction applying square waveform. Calibration plot was constructed under optimum conditions.

RESULTS AND DISCUSSION

Cyclic voltammetry

Electrochemical behavior of TAA at HMDE was studied by cyclic voltammetry at the scan rate of 100 mV s^{-1} in the universal buffer of various pH (2–11) containing 20% (v/v) of ethanol. The results indicated reduction of >C=O group at C_3 of the A ring in the analyte's molecule. The reaction proceeded either *via* consumption of two electrons in two one-electron steps at pH ≤ 9, or in a single two-electron step at pH > 9. Another >C=O group located at C_{20} in the side chain was reduced at more negative potentials *via* consumption of two electrons at pH 5–9. The >C=O group at C_3 of the A ring of TAA molecule was easier to reduce than that at C_{20} of the side chain due to the presence of steric hindrance at C_{20} [14]; therefore the former group was reduced at less negative potentials.

Adsorption of TAA onto HMDE electrode was recognized in cyclic voltammograms (Fig. 1, curve c) recorded in 1×10^{-6} mol L^{-1} solution of the analyte in the universal buffer of pH 2.5 after prior adsorptive accumulation of the drug at –0.5 V for 30 s (Fig. 1, curve b). After accumulation, well-defined single irreversible cathodic peak was obtained, which corresponded to the reduction of carbonyl group at C_3 (curve b). Significant enhancement of the peak current in the first scan (curve b) compared to that in the subsequent scan obtained at the same mercury drop (curve c), or to that recorded after prior accumulation under open circuit conditions (curve a), has confirmed adsorption of TAA onto mercury electrode. Adsorption of TAA was also identified by measuring the peak current (i_p) in the universal buffer (pH 2.5) at various scan rates n (50–500 mV s^{-1}) after prior accumulation of TAA onto HMDE at –0.5 V for 30 s. The log i_p *vs* log *v* plot was linear and the corresponding regression equation is: $\log i_p = 0.85 \log v - 1.85$ ($r = 0.998$). The slope of 0.85 may be considered as close to the expected theoretical value of 1.0 for an ideal case of surface-adsorbed species [15] with some contributions from diffusion.

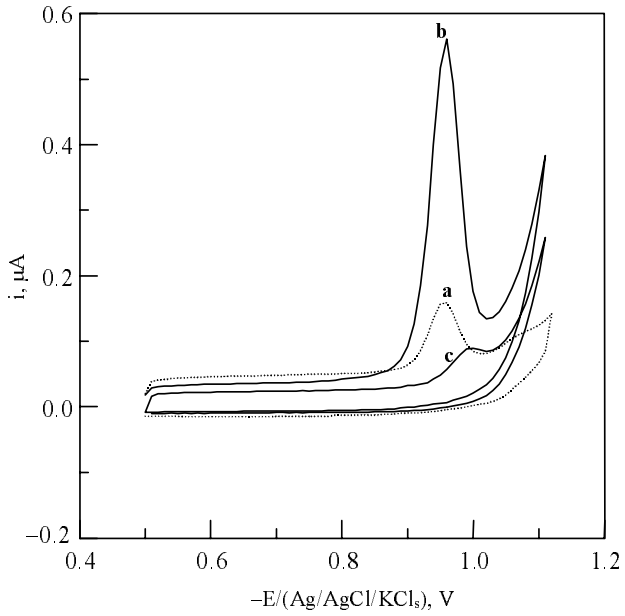


Figure 1. Cyclic voltammograms of 1×10^{-6} mol L^{-1} TAA in the universal buffer of pH 2.5 at scan rate 100 mV s^{-1} recorded after adsorptive accumulation of TAA at the open circuit (a), at $E_{acc.} = -0.5$ V for 30 s (b), (c) – repetitive cycle at the same mercury drop

Surface coverage of the electrode, Γ_0 (defined as the amount of reactant adsorbed onto the mercury electrode surface, mol cm^{-2}) was calculated using the expression: $\Gamma_0 = Q / nFA$, where Q is the amount of charge consumed in the surface process estimated from integration of the voltammetric peak area (1×10^{-6} mol L^{-1} TAA at pH 2.5) corrected for the residual current [16], n is the total number of electrons consumed during reduction (n = 2), and A is the surface area of mercury electrode (0.026 cm^2). By dividing the number of coulombs transferred (742.2 nC) by the conversion factor of nFA, surface coverage of 1.48×10^{-10} mol cm^{-2} was obtained. This means that each adsorbed TAA molecule occupies 1.12 nm^2.

Square-wave stripping voltammetery

Square-wave adsorptive cathodic stripping (SWAdCS) voltammetric response of 1×10^{-6} mol L^{-1} TAA in the universal buffer of pH 2–11 was recorded after prior accumulation of the analyte onto HMDE at –0.5 V for 30 s. SW voltammograms showed similar behavior to that observed in cyclic voltammograms over the entire pH range. At pH = 2.5 the peak was sharper and better developed; hence all the subsequent measurements were carried out in the universal buffer of pH 2.5.

The influence of instrumental conditions (frequency f, scan increment ΔE_s and pulse-amplitude a) on the magnitude of peak current of 1×10^{-6} mol L^{-1} TAA in the universal buffer (pH 2.5) after adsorptive accumulation onto HMDE at –0.5 V for 30 s was examined. At ΔE_s = 10 mV and a = 25 mV peak current increased linearly over the frequency range 10–80 Hz. At f = 80 Hz and a = 25 mV peak current increased linearly with the scan increment (ΔE_s) up to 10 mV. Even higher peak currents were obtained by increasing pulse amplitude (a) up to 25 mV.

The effect of accumulation potential ($E_{acc.}$) on SWAdCS voltammetric peak of 1×10^{-6} mol L^{-1} TAA at pH 2.5 was examined over the range from –0.2 to –0.7 V after prior adsorptive accumulation of the analyte onto HMDE for 30 s. The peak was the best developed and the highest over the potential range from –0.4 to –0.6 V (Fig. 2). At lower and higher potentials the peak current decreased. This decrease might be attributed to desorption of the drug at either higher or lower potentials with respect to the zero charge potential, which in turn corresponds to the strongest adsorption of uncharged organic molecules. Finally, accumulation potential of –0.5 V (*vs* Ag/AgCl /KCls) was chosen for further studies.

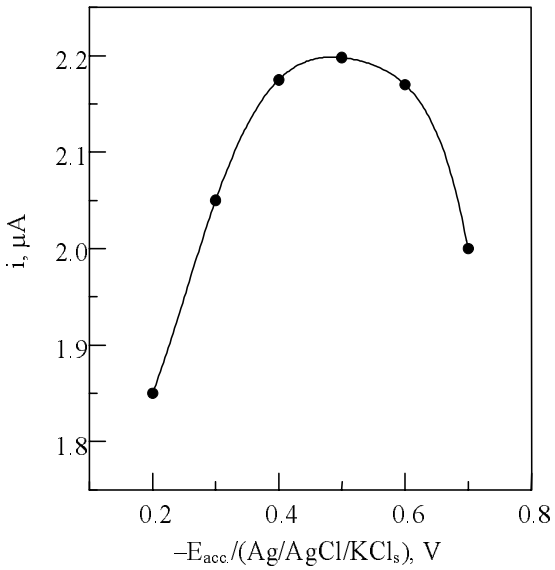


Figure 2. The effect of accumulation potential (E_{acc}) on SWAdCS voltammetric peak current of 1×10^{-6} mol L^{-1} TAA in the universal buffer of pH 2.5; $t_{acc.}$ = 30 s, f = 80 Hz , E_s = 10 mV, and a = 25 mV

The effect of accumulation time ($t_{acc.}$) at –0.5V on the peak current was investigated for various bulk concentrations of TAA (1×10^{-6}, 5×10^{-7}, 1×10^{-7} and 1×10^{-8} mol L^{-1}) in the universal buffer of pH 2.5. As shown in Figure 3, for 1×10^{-6}, 5×10^{-7} and

1×10^{-7} mol L^{-1} TAA solutions the magnitude of the peak current depended linearly on the accumulation time up to 50, 70, and 130 s, respectively. For 1×10^{-8} mol L^{-1} TAA solution, the peak current magnitude increased linearly with the accumulation time in the whole applied range (curve d). Apparently, lower concentration of the analyte requires longer accumulation time. On the one hand, the choice of accumulation time was dictated by the sensitivity required. On the other hand, the SW signal increased with the increase of mercury electrode area (0.01–0.026 cm^2). In this study, HMDE of an area of 0.026 cm^2 was used. The influence of the waiting time was also considered and waiting time of 5 s was applied.

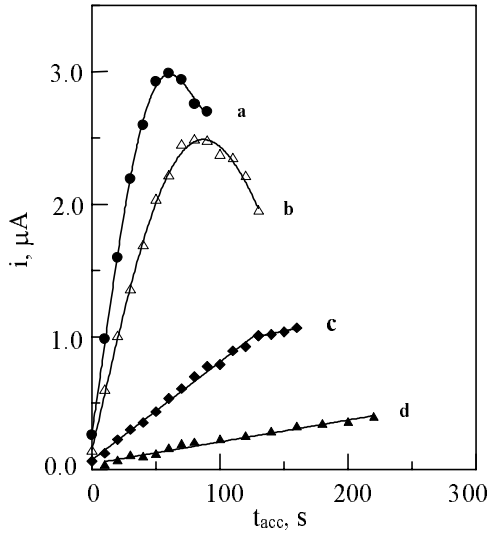


Figure 3. The effect of accumulation time (t_{acc}) on SWAdCS voltammetric peak current of: (a) 1×10^{-6} mol L^{-1}, (b) 5×10^{-7} mol L^{-1}, (c) 1×10^{-7} mol L^{-1} and (d) 1×10^{-8} mol L^{-1} TAA in the universal buffer of pH 2.5; $E_{acc.} = -0.5$ V; f = 80 Hz , ΔE_s = 10 mV and a = 25 mV

Accordingly, the optimum operational parameters of the proposed SWAdCS voltammetric procedure were as follows: $E_{acc.} = -0.5$ V, $t_{acc.} \leq 130$ s, f = 80 Hz, ΔEs = 10 mV, a = 25 mV, and a universal buffer of pH 2.5 as the supporting electrolyte.

Validation of the procedure

Validation of the proposed SWAdCS voltammetric procedure for the assay of bulk TAA was examined *via* evaluation of linearity, limit of detection (LOD), repeatability, reproducibility, precision, selectivity, robustness, and intermediate precision [17]. Linear calibration plots were constructed over various concentration ranges and

referred to different accumulation times (Tab. 1). Regression equations corresponding to the calibration plots exhibited good linearity (Tab. 1), thus confirmed validity of the proposed procedure for determination of TAA.

Table 1. Regression parameters of calibration plots corresponding to SWAdCS voltammetric determination of bulk TAA in the universal buffer of pH 2.5, $E_{acc.} = -0.5$ V, f = 80 Hz, $\Delta E_s = 10$ mV and a = 25 mV, at 25°C

t_{accc}, s	Linearity range, mol L^{-1}	Regression equation (i_p (μA) = bC (μmol L^{-1}) + a)	r	LOD, mol L^{-1}
30	$5 \times 10^{-9} - 2 \times 10^{-6}$	$i_p = 2.60\ C + 0.080$	0.998	1.50×10^{-9}
60	$3 \times 10^{-9} - 9 \times 10^{-7}$	$i_p = 4.96\ C + 0.114$	0.996	9.01×10^{-10}
130	$1 \times 10^{-9} - 9 \times 10^{-8}$	$i_p = 8.80\ C + 0.136$	0.999	3.00×10^{-10}

LOD was calculated from the calibration plots obtained after previous accumulation of TAA onto HMDE for different times. For the calculation of LOD, one used the expression: 3 SD/b [17], where SD is the standard deviation of the blank (or the intercept of the calibration line), and b is the slope of the calibration plot. The results given in Table 1 confirm reliability of the proposed SWAdCS voltammetric procedure for determination of bulk TAA.

Repeatability, reproducibility, precision, and accuracy [17] of the analysis performed applying the proposed procedure were estimated from four replicate determinations of 5×10^{-8} and 7×10^{-8} mol L^{-1} bulk TAA ($t_{acc.} = 130$ s and $E_{acc.} = -0.5$ V) within one day (intra-day assay) and within three days over a period of one week (inter-day assay). Satisfactory mean percentage recoveries (R, %) and relative standard deviations (RSD) for intra- and inter-day precision and accuracy were achieved (Tab. 2).

Table 2. Results of intra- and inter-day assays of bulk TAA applying SWAdCS voltammetric procedure in the universal buffer of pH 2.5; $t_{acc.} = 130$ s (n = 4)

Added, mol L^{-1}	Day	Intra-day precision			Inter-day precision		
		R%	Precision RSD %	Accuracy RE. %	R%	Precision RSD %	Accuracy RE. %
5×10^{-8}	1	99.62	0.386	–0.38	99.69	0.55	–0.31
	2	99.84	0.640	–0.16			
	3	99.60	0.570	–0.40			
7×10^{-8}	1	99.72	0.173	–0.29	99.58	0.70	–0.42
	2	100.1	1.500	0.10			
	3	98.91	0.640	–1.085			

Good agreement between added and determined amounts of TAA indicated satisfactory reproducibility, precision, and accuracy of the proposed procedure for determination of bulk TAA.

To estimate selectivity of the described method, TAA was determined in 5×10^{-8} mol L^{-1} TAA solution and in a standard solution of Kenacort® tablet containing 5×10^{-8} mol L^{-1} TAA [17]. Determinations were preceded with adsorptive accumulation of the analyte onto HMDE at –0.5 V for 130 s. No significant differences in the recoveries and relative standard deviations were observed in the absence (99.84 ± 0.64%) and presence (99.78 ± 0.51%) of excipients. Thus, the proposed SWAdCS voltammetric procedure can be considered selective.

Robustness [17] of analytical results indicates their resistivity to some changes of experimental conditions. In order to study robustness in our method, the influence of small variations of pH (2.5–3), accumulation potential (–0.45 – –0.55 V), and accumulation time (120–130 s) on recovery and standard deviation was examined by determination of 5×10^{-8} mol L^{-1} bulk TAA. The obtained mean percentage recoveries (99.84 ± 0.64–98.2 ± 0.61) were not significantly affected by variations of experimental conditions. Consequently, the proposed SWAdCS voltammetric procedure was regarded reliable for determination of bulk TAA and can be considered robust. Intermediate precision of the proposed procedure was examined by determining 5×10^{-8} mol L^{-1} TAA under the same operational conditions at different times by two different analysts and using two PAR potentiostats, Models 263 A, Lab. (1) and 394, Lab. (2). Mean percentage recoveries obtained in Lab. 1 (99.84 ± 0.64) and Lab. 2 (97.94 ± 0.74), as well as day-to-day recovery (99.69 ± 0.55–98.22 ± 0.60) were found reproducible, as there was no significant difference between recovery and relative standard deviation values.

Assay of Kenacort® tablets

The proposed SWAdCS voltammetric procedure was successfully applied to the assay of TAA in Kenacort® tablets without the necessity of sample pretreatment and/or time-consuming extraction. The results were obtained using the calibration plot. They confirmed applicability of the proposed procedure to the determination of TAA in Kenacort® tablets (Tab. 3). Validity of the proposed procedure was further assessed applying standard addition method to pre-analyzed tablet solutions spiked with three different standard TAA solutions. The results were compared to those obtained applying spectrophotometric method [7]. The calculated F-value (Tab. 3) did not exceed the theoretical one. Thus, there was no significant difference between the proposed and literature methods with respect to reproducibility. Also, no significant difference was noticed between these two methods with respect to accuracy and precision (Tab. 3).

Table 3. Assay of Kenacort® tablets by SWAdCS voltammetric procedure (t_{acc} = 130 s) and literature spectrophotometric method [7]

Declared, mg/tablet	4.0
Recovery by the proposed method (R % ± RSD) (Calibration plot method)	99.78 ± 0.51
Recovery by the proposed method (R % ± RSD) (Standard addition method)	100.04 ± 0.40
Recovery by the literature method (R % ± RSD) (Standard addition method)	99.97 ± 0.17
F-value	5.54
t-test	1.05

Theoretical values of F-test and t-test at 95% confidence level (for $n_1 = n_2 = 4$) are 6.6 and 2.45, respectively.

Assay of spiked serum samples

Human serum sample was spiked with TAA, which was subsequently quantitatively determined by SWAdCS voltammetric procedure ($t_{acc.}$ = 30–130 s and $E_{acc.}$ = –0.5 V) without sample pretreatment and/or time-consuming extraction prior to the analysis. Representative voltammograms corresponding to various concentrations of TAA in human serum after 130 s adsorptive accumulation are shown in Figure 4. Regression parameters of the corresponding calibration plots for TAA determined in human serum samples after different accumulation times are given in Table 4. Mean percentage recovery and relative standard deviation was 102.35 ± 2.4 for six replicate measurements.

Table 4. Regression parameters of the calibration plots of TAA in spiked human serum obtained by the proposed SWAdCS voltammetric procedure (n = 6)

t_{acc}, s	Linearity range, mol L^{-1}	Regression equation (i_p (μA) = bC (μmol L^{-1}) + a)	r	LOD, mol L^{-1}
30	$7 \times 10^{-9} – 8 \times 10^{-7}$	i_p = 2.20 C + 0.075	0.998	2.1×10^{-9}
60	$4 \times 10^{-9} – 6 \times 10^{-7}$	i_p = 4.03 C + 0.164	0.996	1.2×10^{-9}
130	$2.5 \times 10^{-9} – 8 \times 10^{-8}$	i_p = 7.48 C + 0.228	0.998	7.5×10^{-10}

CONCLUSION

A square-wave stripping voltammetric procedure for the determination of triamcinolone acetonide has been described. The reduction of the analyte at a HMD electrode was preceded by the adsorptive accumulation of the analyte. The described procedure was fully validated according to the analytical parameters established by the United States Pharmacopoeia. The procedure was successfully applied to the determination of TAA in pharmaceutical formulations and in human serum. The described SWAdCS voltammetric procedure is fast, simple, selective, highly sensitive, and inexpensive.

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