Voltammetric Determination of Salicylic Acid in milk samples using Copper wire electrodes in Alkaline Medium

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RESUMO

O uso de analgésicos é bastante comum na nossa sociedade, porém, quando esse medicamento é utilizado no tratamento pós-parto de vacas, o ácido salicílico (AS) resultante da metabolização do fármaco, é detectado no leite bovino, caracterizando um agente contaminante, dado que a sua presença no leite é proibida. Nesse contexto o desenvolvimento de novos métodos analíticos para quantificar o AS é essencial. Esse estudo teve como objetivo quantificar o AS em diferentes amostras de leite utilizando Voltametria de Pulso Diferencial, com eletrodo de cobre em meio alcalino. Foram testadas seis amostras de leite (Cru, Integral, Semidesnatado, Desnatado, Fortificado com multivitaminas, Integral sem lactose), tratadas com soluções de $ZnSO_4$ 15 % (m:v) e de NaOH 2,5 mol L^{-1}, utilizando banho ultrassônico e centrifugação. O eletrodo de trabalho foi um fio de Cu (Ø = 1,3 mm), o auxiliar um fio de Pt e Ag/AgCl/KClsat como referência.

Palavras-chave: Voltametria de Pulso Diferencial, Ácido Salicílico, Leite.

Introdução

The use of analgesics containing acetylsalicylic acid (ASA), popularly known as aspirin, is quite common. However, when administered for postpartum treatment of cows, ASA is metabolized into salicylic acid (SA), which is absorbed by the animal and excreted through the milk. In addition, SA is sometimes used illegally as a preservative in milk, which is not permitted by current regulations. The illegal presence of SA to this matrix compromises both food quality and consumer health (1). In this context, the development of sensitive, simple, and environmentally friendly analytical methods becomes necessary. Voltammetric techniques offer several advantages, including high sensitivity, operational simplicity, and low instrumentation cost (2). The aim of this study was to quantify SA in six different milk samples using Differential Pulse Voltammetry (DPV) and a miniaturized copper electrode while employing low volumes of reagents and non-organic solvents during pretreatment.

Experimental

The electrodes used were a Cu wire (Φ = 1.3 mm) as working electrode, a Pt wire as auxiliary electrode and $Ag/AgCl/KCl_{sat}$ as reference electrode. The supporting electrolyte was a 0.1 mol L^{-1} NaOH solution.

Sample treatment

The treatment of the milk sample consisted of a 500 µL aliquot of refrigerated milk transferred to a 1.5 mL polypropylene microcentrifuge tube, to which 100 µL of a 15% (m:v) $ZnSO_4$ aqueous solution and 400 µL of a 2.5 mol L^{-1} NaOH solution were added. The mixture was left in an ultrasonic bath for 10 min and subsequently centrifuged at 12,000 rpm for 5 min. Then, a 500 µL aliquot of the clear liquid resulting from centrifugation is collected using a micropipette and transferred to an electrochemical containing 4.5 mL of ultrapure water, where the reading is performed. For the recovery tests, 5 µL of $ZnSO_4$ are replaced by 5 µL of AS standard solution. Six milk samples were tested: Raw, Whole, Semi-skimmed, Skimmed, Fortified with multivitamins and Whole lactose-free.

Resultados e Discussão

The sample pretreatment used utilizes two dilutions steps, an initial 1:1 dilution of the original sample and a 1:10 dilution. In the second step the extract is mixed with ultrapure water in the electrochemical cell, resulting in a NaOH concentration of 0.1 mol L^{-1}, the optimized supporting electrolyte concentration. These sequential dilutions increase the method's effective Limit of Detection (LOD) and Limit of Quantitation (LOD) to 60 µmol L^{-1} and 200 µmol L^{-1}, respectively.

Differential pulse voltammograms were recorded over a range of AS concentrations in the final extracts from the six analyzed milk samples, then calibration curves were constructed and the corresponding sensitivities obtained for each extract are shown in Figure 1.

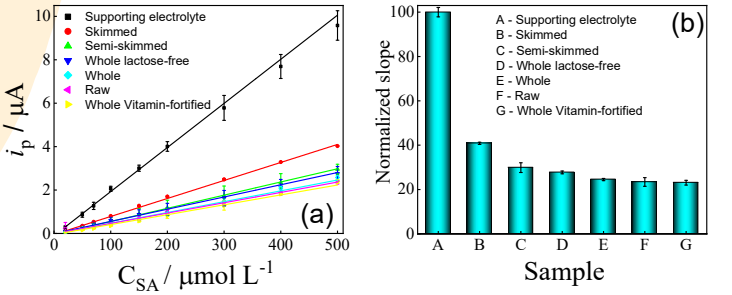

Figure 1. (a) Calibration curves obtained by DPV in the final milk extracts. (b) Normalized sensitivity obtained for each milk extract. Sensitivities were normalized relative to the sensitivity obtained in supporting electrolyte (defined as 100%).

As shown in Figure 1, the sensitivity achieved in the supporting electrolyte was significantly higher than that obtained with the milk extracts, indicating that the pretreatment did not fully eliminate matrix effects. The highest sensitivity was observed in the skimmed milk extract, suggesting a significant influence of lipid content on AS sensitivity. In general, it was observed that the sensitivity of the extracts decreased by more than 50% compared to the supporting electrolyte. Consequently, the standard additions calibration method was employed for AS determination in spiked milk samples, to compensate for matrix effects.

To evaluate the accuracy of the proposed method, addition-recovery experiments were performed using the six analyzed milk samples. Each sample was spiked with 138 mg L^{-1} of AS, corresponding to 50 µmol L^{-1} in the electrochemical cell. Table 1 shows AS concentrations and recovery percentages for each analyzed sample. With recovery percentages ranging from 91 to 107 % with RSD values between 2.1 and 5.3 %, meeting the AOAC performance requirements for standard methods at a spiked concentration of 100 mg L^{-1}, where expected recoveries fall with the range of 90 to 107 %, and the expected RSD is ≤ 5.3 % (3). Statistical analysis using the t-test at a 95% confidence level did not exceed the critical values, indicating no evidence of systematic errors in the method. These results demonstrate the accuracy of the proposed voltammetric method for AS determination in milk samples.

Table 1. Recovery percentages and determined SA concentrations in spiked milk samples.

Milk sample	Added (mg L^{-1})[a]	Found (mg L^{-1})[b]	Recovery (%)	t^c
Skimmed	138	146 ± 8	106 ± 6	1.7
Semi-skimmed	138	141 ± 3	102 ± 2	1.7
Whole lactose-free	138	139 ± 4	101 ± 3	2.9
Whole	138	148 ± 5	107 ± 3	3.4
Raw	138	124 ± 5	91 ± 3	4.0
Whole vitamin-fortified	138	141 ± 7	102 ± 5	0.7

[a]Added to the sample, resulting in a concentration of 50 µmol L^{-1} (6.9 mg L^{-1}) in the electrochemical cell. Typical concentrations of SA used as a milk adulterant range from 400 to 500 mg L^{-1} (4). [b]Average value ± standard deviation (n = 3). [c]$t_{critical}$ = 4.30 for 2 degrees of freedom and 95% confidence level (5).

Conclusões

The developed sample pretreatment offers some advantages, including low sample and reagent consumption, elimination of organic solvents, and short processing time. The pretreatment used represents a promising tool for AS quantification. This study provides a sensitive, selective, and eco-friendly electrochemical method for AS determination in milk, with broader potential applications in milk analysis.

Agradecimentos

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