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Rapid screening of multiple antibiotic residues in milk using disposable amperometric magnetosensors

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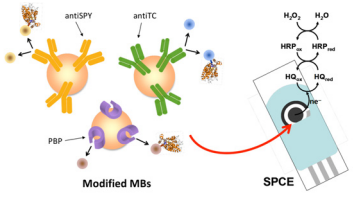
HIGHLIGHTS

- First disposable amperometric magnetobiosensor for screening of multiple antibiotics.
- Discrimination between free and milk samples containing antibiotics at their MRLs.
- Simple and short sample pretreatment and fast detection within 5 min.
- Useful and affordable alternative to classical assays for antibiotic screening in milk.

GRAPHICAL ABSTRACT

Rapid screening of multiple antibiotic residues in milk using disposable screen-printed carbon electrodes (SPCEs), a mixture of 3-target specific modified magnetic beads (MBs) and direct competitive assays using horseradish peroxidase (HRP)-labeled tracers.



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ABSTRACT

Disposable amperometric magnetosensors, involving a mixture of modified-magnetic beads (MBs), for the multiplex screening of cephalosporins (CPHs), sulfonamides (SAs) and tetracyclines (TCs) antibiotic residues in milk are reported for the first time in this work. The multiplexed detection relies on the use of a mixture of target specific modified magnetic beads (MBs) and application of direct competitive assays using horseradish peroxidase (HRP)-labeled tracers. The amperometric responses measured at −0.20 V vs. the Ag pseudo-reference electrode of screen-printed carbon electrodes (SPCE) upon the addition of H_2O_2 in the presence of hydroquinone (HQ) as redox mediator, were used to monitor the extent of the different affinity reactions. The developed methodology, involving a simple and short pretreatment, allowed discrimination between no contaminated UHT and raw milk samples and samples containing antibiotic residues at the maximum residue limits (MRLs). The usefulness of the multiplexed magnetosensor was demonstrated by analyzing spiked milk samples in only 5 min. The results demonstrated that a clear discrimination of milk samples contaminated with antibiotics at their MRL level or their mixtures, allowing the identification of milk not complying with current legislation. These features make the developed methodology a promising alternative in the development of user-friendly devices for on-site analysis to ensure quality control for dairy products.

1. Introduction

Antibiotics are commonly used for the prevention and treatment of several infectious diseases produced by bacteria or as feed additives to promote growth in farm animals [1]. Tetracyclines,

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sulfonamides, and β-lactams are among the most common groups of antibiotics employed to prevent and treat animal diseases in veterinary medicine where they may be used individually or in combination [2,3]. Antibiotic residues enter the milk supply when withdrawal periods are not strictly complimented or when a cow retains antibiotic residues in its system for an extraordinary length of time [4,5]. The presence of antibiotic residues in food holds the risk of undesirable health effects for the consumers, because of the risk of chemical poisoning, allergic reactions, change in consumer perception of the product and the development of mechanisms of bacterial resistance, causing a serious threat to human and animal health [2,6,7]. Moreover, milk and dairy products industries are among the sectors most affected by the presence of antibiotic residues because of important economic losses derived by the inhibitory effect of these biocides in the fermentation processes implied in the production of cultured milk products (e.g., cheese and yoghurt) [8–11].

In order to avoid these serious consequences, food products need to be tested for the presence of residues before consumption and, therefore, MRLs have been established to ensure human food safety; the European Union has stated the MRLs of different antibiotic residues in milk. For β-lactam antibiotics, different MRLs have been fixed according to their potency, being 60 and 100 $\mu g\,kg^{-1}$ the MRLs established for cefapirin (CEF) and ceftiofur (CTF), respectively. For TCs a 100 $\mu g\,kg^{-1}$ MRL has been stated individually; while for SAs a 100 $\mu g\,kg^{-1}$ limit was fixed for their total amount found in sample [12]. Therefore, dairy industry should screen incoming milk for the presence of antibiotics to prevent contaminated milk from entering the food chain [13].

Rapid, accurate and sensitive methods are required to detect antibiotic residues in routine assays. Although numerous screening methods have been proposed as tests for antibiotics determination, many of them have not even been applied to real samples. Common screening methods to detect antibiotic residues in food samples are microbiological tests. These tests, based on the inhibition of microbial growth, are commercially available and also used for routine detection of antibiotics in milk as they are easy to perform, but they are time consuming (requiring several hours) and lack of sensitivity for diverse groups of antibiotics [14]. Enzyme-linked immunosorbent assay (ELISA) methods are the most used immunoanalytical strategies for residue determination [15]. Principal disadvantages of these methods are their frequently high background signals, the large number of required incubation and washing steps and the difficulty to be automated.

Chromatographic methods require skilled personnel, have a low throughput, are quite expensive, and demand long and tedious sample pretreatment procedures [14,16], thus being restricted to confirmatory purposes. Other available methodologies include photoluminescence detection, spectrophotometry, capillary electrophoresis and even titrimetry [17,18]. These are time consuming and not useful for field analysis.

Despite several surface plasmon resonance (SPR) methods having been proposed as useful analytical microarrays for the simultaneous detection of antibiotic residues in milk [7,14,16], none of them has reached the real market as a rapid, on-field test for milk analysis, probably because of the high cost of the detection systems, they are not fast enough, or because of the complicated procedures involved in the detection.

The combination of advances in electrochemistry with the potential of immunochemical methods allows the implementation of electrochemical immunosensors that exhibit important advantages related with the analytical characteristics mentioned above for screening methodologies and, therefore, emerging as attractive alternatives for the determination of antibiotics. In fact, our group has recently developed several electrochemical affinity sensors for the determination of antibiotics in milk [19–22]. However, although there is a strong need for reliable methods suitable for the simultaneous analysis of multi-class antibiotic residues for routine monitoring purposes, most of the electrochemical immunosensors and the reported analytical procedures are still focused on the detection of a single antibiotic family. To the best of our knowledge, there is no report of an electrochemical immunosensor able to detect residues of three different antibiotic families in milk simultaneously.

This paper describes for the first time the development of an amperometric affinity-magnetosensor to perform a rapid screening of CPHs, SAs and TCs antibiotic residues in raw cow's milk. Multiplex screening was achieved using disposable electrodes, a mixture of three specifically targeted bead sets and competitive binding between sample antibiotic and HRP-labeled specific tracers. The electrochemical detection, carried out after the magnetic capture of the modified MBs on a SPCE, used HQ as electron transfer mediator and H_2O_2 as the enzyme substrate. The magnetosensor performance, including the characteristics regarding the individual determination of different antibiotics and antibiotics mixtures are evaluated. The developed magnetosensor was successfully applied to the analysis of low concentration level-spiked UHT and raw milk samples after a simple pretreatment step consisting of sample heating at 80 °C during 1 min for thermal inactivation of the endogenous lactoperoxidase (LPO). The obtained results demonstrated that the developed disposable magnetosensor is useful for the screening of milk samples, as a rapid method to ensure that milk containing drug residues is not accepted at dairy plants and processed into finished dairy products.

2. Materials and methods

2.1. Apparatus and electrodes

Amperometric measurements were performed with a CHI812B potentiostat (CH Instruments) controlled by software CHI812B. All measurements were carried out at room temperature.

The transducers employed were screen-printed carbon electrodes (SPCEs) (DRP-110, DropSens) consisting of a 4-mm diameter carbon working electrode, a carbon counter electrode and a Ag pseudo-reference electrode.

A Bunsen AGT-9 Vortex was used for the homogenization of the spiked samples. A magnetic separator Dynal MPC-S (product no. 120.20) was purchased from Dynal Biotech ASA (Norway) and a constant temperature incubator shaker from Ivymen-Comecta was also used. A homemade heating plate was used for the thermal treatment of raw milk.

2.2. Reagents and solutions

All the reagents used were of the highest available grade. Tween®20, hydroquinone (HQ), hydrogen peroxide (30%, w/v), sulfamerazine (SMR), sulfadiazine (SDZ), sulfachloropyridazine (SCP), oxytetracycline hydrochloride (OTC), tetracycline hydrochloride (TC) and ceftiofur (CTF) were purchased from Sigma-Aldrich. Chlortetracycline hydrochloride (CTC), sulfapyridine (SPY) and cefapirin sodium (CEF) were from Fluka. Sodium di-hydrogen phosphate, di-sodium hydrogen phosphate, sodium hydrogen carbonate, potassium chloride, sodium chloride and dimethyl sulfoxide (DMSO) were purchased from Scharlau. Sodium carbonate anhydrous was acquired from Panreac. Stock solutions (2.0 mg mL^{-1}) of different antibiotics were prepared in DMSO (SPY, SMR, SDZ, SCP and CTF), in PBST (TC and CEF) or in carbonate buffer (CTC and OTC). Antibiotic working solutions were prepared daily upon dilution of the stock solutions in milk matrix.