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RESEARCH ARTICLE

A NOVEL ELECTROCHEMICAL BIOIMPRINTED SENSOR OF BUTYL PARABEN ON A MODIFIED CARBON PASTE ELECTRODE WITH SAFRANINE-O CAPPED TO SILVER NANOPARTICLES

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ABSTRACT

A novel molecularly bioimprinted polymer (bioMIP) sensor was constructed through an efficient one step electropolymerization technique, using the natural dye safranine-O (Sa) capped with silver nanoparticles (AgNPs). The dsDNA was electrochemically entrapped into poly- (safranine-O) capped with silver nanoparticles (poly-(Sa@AgNPs)) film (molecularly bioimprinted polymer), deposited potensiodynamically on the surface of carbon paste electrode (CPE), using cyclic voltammetry (CV). The morphology and performance of bioimprinted polymer were characterized by electron scanning microscopy (SEM. The analytical performance of the bioimprinted sensor was also studied. The modified electrode presented very good reproducibility, satisfactory stability, as well as high sensitivity and selectivity compared to the conventional DNA and electrochemical sensors. Furthermore, the square wave voltammetric peak current was linear to butyl paraben mass concentration in the range 0.362 to 100 μg L^{-1}, with a detection limit of 0.109 μg L^{-1}. The bioimprinted sensor was successfully applied to the determination of butyl paraben in real cosmetic samples with satisfactory recovery ranging from 97.3 to 100.2 %, demonstrating its feasibility for practical application.

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INTRODUCTION

Molecularly imprinted polymers (MIPs) is a convenient methodology for the preparation of synthetic polymers. They employ a target molecule as the template, facilitating selective recognition sites to recognize the target molecule in preference to others with similar structures (Turiel and Martín-Esteban, 2010). The preparation procedures mainly involve positioning functional monomers around the template molecules, forming a complex with the template, followed by polymerization and the template removal (Haupt and Mosbach, 2000). The ease of preparation, the low cost, the ability to 'tailor' recognition material for target analytes, and the stability under harsh conditions make MIPs attractive as recognition elements in electrochemical sensing (Rezaei *et al*., 2013; Yeh and Ho, 2005). MIPs are synthesized in situ on the electrode surface by electropolymerization techniques (Rezaei *et al*., 2014; Da Silva *et al*., 2014). Bulk polymerization methods suffer from some intrinsic limitations, such as the poor site accessibility, time-consuming, high diffusion barrier, and complicated preparation process (Luo *et al*., 2013). The electrochemical deposition methods, on the other hand, provide a simple, repeatable, and green method for the formation of thin and uniform polymer film on the electrode's surface (Peng *et al*., 2000; Kan *et al*., 2012). MIPs, though, suffer from limitations such as long response time, low density of imprinted sites, slow diffusion of the analytes across the MIP film, heterogeneous distribution of imprinted sites, and low sensitivity (Skrdla *et al*., 2006). An excellent design for their improvement is the formation of a thin MIP layer on the surface of nanomaterials with a high surface to volume ratio (Zhang *et al*., 2010). In order to increase the electroactive surface area and reduce overpotential, metal nanoparticles have been used as supporting materials in the preparation of MIP sensors (Wang *et al*., 2011). Metal nanoparticles with unique properties such as good biocompatibility, high conductivity, and high surface-to-volume ratio are also attractive materials for sensors and biosensors applications (Lai *et al*., 2013). Thus, silver nanoparticles (AgNPs) incorporated into various matrices have intensively been investigated in order to expand their utility in nanomaterials, biomedical (Shrivastava *et al*., 2009) and sensor applications (Sharma *et al*., 2009). Generally, the synthesis of AgNPs by the reduction of $AgNO_3$ in solution involves two steps such as nucleation and growth (Lee and El-Sayed, 2006). The rate of nucleation and growth of AgNPs decides the dimension of the final products. The dimension of the fine sized AgNPs can be altered by different control parameters such as concentration of reactants, reductant, pH and

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temperature. Therefore, a number of nanoparticles could be obtained by changing the parameters like different stabilizing agents and reaction conditions. The AgNPs have also been synthesized by various chemical reduction methods using ascorbic acid, citrate and $NaBH_4$ (Prabakaran and Pandian, 2015; Tan *et al*., 2015). But, these methods require huge quantity of chemicals and controlling the particle size is difficult. Lately, these drawbacks have been removed by using sunlight as the source of motivator. Sunlight acts as an external motivator to control the size of AgNPs, which would provide sufficient energy to get the particle of required size. The use of sunlight has nontoxic nature, is nonpolluting and simple in chemical processes (Rastogi and Arunachalam, 2011; Luo, 2007). For the synthesis of nanoparticles, the usage of an organic dye as a stabilizing agent gives improved properties rather than other stabilizing agents such as polymers (Prabakaran *et al*., 2013), leaf (Krishnaraj *et al*., 2010), fruit extracts (Singh *et al*., 2013), glucose (Pulit and Banach, 2013) and carbon materials (Prabakaran *et al*., 2013). Moreover, the dye is a better stabilizing agent than the above mentioned agents because, it has special ionic, polar, non-bonding functional groups (-azo, -sulphite, -hydroxyl and -nitro) and is highly a conjugated π–bonded system (Liu *et al*., 2012; Wu *et al*., 2011; Zhai and Efrima, 1996). However, only few reports of using sunlight as source mediator and natural dyes as stabilizers in the synthesis of AgNPs.

Furthermore, water-soluble dyes, such as methylene blue (Ensafi, 2003), methylene green (Wang and Dong, 2000), carmine (Liu *et al*., 2006) and other dye derivatives have been widely used as mediators to catalyze the reduction or oxidation of organic molecules. Safranine (Sa) is a water-soluble red-colored phenazine type dye used widely in paper and pharmaceutical industries. However, safranine polymer has rarely been used as a modifier of electrode surfaces or a mediator to facilitate the electrochemical reaction of compounds (Liu, 2010). As well, DNA is an interesting biomolecule, which has recently attracted much attention because of its ability to bind small ligand molecules with high affinity and specificity. Small molecules interact with DNA by covalent (chemical modification of various DNAconstituents) and reversible non-covalent (electrostatic, groove binding, and intercalation). Intercalation occurs when a planararomatic ring system is inserted between DNA base pairs and unwinds the DNA helical twist to accommodate the intercalators.

Parabens are esters of p-hydroxybenzoic acid and are used extensively as antimicrobial preservatives in food industry, cosmetic products as well as in pharmaceutical preparations and are considered as endocrine disruptors. Parabens, which are present in these products, could be released continuously in the watery environment from the domestic sewages. Consequently, increased concern exists for their long-term consequences in humans (Harvey and Everett, 2004). Various analytical techniques have been used in the determination of parabens, such as the high performance liquid chromatography (Thomassin *et al*., 1997), gas chromatography (Altria and Bestford, 1996), capillary electrophoresis (Thomassin *et al*., 1997), and solid phase extraction (Tuncagil *et al*., 2010). Even though these techniques have high precision with low limits of detection, they are costly, time-consuming and relatively complex, since they require preconcentration of the analyte. On the other hand, electrochemical sensors, used in the detection of parabens (36-38), are fast, cheap, selective, sensitive, and simple in their use and they do not require preconcentration steps. The aim of the present study was the development of novel, sensitive, selective, environmentally friendly and also cost effective bioimprinted sensors. Thus, dsDNA had been entrapped within poly-(Sa) capped with AgNPs (Sa@AgNPs) network to improve the selectivity and sensitivity of the measured signals. The combination of DNA with MIP sensors (bioimprinted sensor) allowed the preparation of a novel selective tool for the determination of interested analyte in complex mixtures.

To the best of our knowledge, the use of a bioimprinted sensor to determine butyl paraben based on its interactions with dsDNA has not yet been reported. The bioimprinted sensor was evaluated under optimum condition to confirm its electrochemical analytical properties such as linearity, conductivity, selectivity, stability and reproducibility. Moreover, it was applied for the analysis of the butyl paraben in real cosmetic samples.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade unless stated otherwise and used as received. Acetic acid and potassium ferrocyanide, used were purchased from Merck (Darm-stadt, Germany). Ethanol, butyl paraben (pharmaceutical secondary standard), methyl paraben (analytical standard), ethyl paraben (certified reference material), bisphenol A (certified reference material), mineral oil (for IR spectroscopy), double stranded calf thymus ds-DNA (highly polymerized) and graphite poeder (<20 μm, synthetic) were obtained from Sigma-Aldrich (St Luis, USA). Safranine-O was purcased from Panreac. Silver nitrate was obtained (99.8%) was obtained from Ducela Biochemie (The Netherlands). The dsDNA was dissolved in phosphate buffer (pH 7.0) containining 20 $x10^{-3}$ mol L^{-1} NaCl and the stock solution (0.5 g L^{-1}) kept frozen. Purity of DNA was checked by monitoring the ratio of the absorbance at 260–280 nm. The solution gave a ratio of A_{260} / A_{280} > 1.8, indicating that dsDNA was sufficiently free from protein (Cao *et al*., 1999). All aqueous solutions were prepared with double-distilled water. Stock solutions of 0.5 g L^{-1} of bytyl paraben were prepared after weighing a certain amount of the compound and dilution in ethanol.

Instrumentation

Electrochemical measurements were performed with a Potentiostat–Galvanost at μ AutoLab (Echo Chemie, The Netherlands) controlled by GPES 4.9.0005 Beta software. A conventional three-electrode cell consisted of a platinum wire counter electrode, a 3 mol L^{-1} KCl saturated Ag/AgCl reference electrode, a carbon paste electrode of 3 mm inner and 9 mm outer diameter of the PTFE sleeve, as well as the proposed bioimprinted sensor as the working electrode were used. The pH of all solutions was measured using a Consort C830 pH meter (Consort bvba, Belgium). The carbon paste electrode was prepared by thoroughly mixing by hand adequate amounts of graphite powder and paraffin oil of 75/25 mass ratio. A portion of the resulting mixture was packed into the bottom of the PTFE sleeve. The surface was polished to a smooth finish manually on a piece of weighing paper before use. Electrical contact was established via stainless steel screws. The electrochemical cells were cleaned with diluted nitric acid and rinsed with double-distilled water. Ultrapure

nitrogen was used to de-aerate the solutions by purging the dissolved oxygen for 15 min prior to each experiment. All experiments were performed at abient temperature.

Synthesis of EBT Stabilized AgNPs

Silver nanoparticles were synthesized by adopting a green approach using safranine-O as a stabilizing agent as well as reducing agent. 0.024 g (0.14 x10^{-3} mol L-1) of $AgNO_3$ and 0.160 g (0.46 x10^{-3} mol L^{-1}) of safranine were dispersed in 25 mL of 0.025 g (0.625 x10^{-3} mol L^{-1}) NaOH (pH 10.0) in a 100 mL beaker with stirring, for about 5 min and then exposed to visible light for 30 min in static condition. The solution colour changes from wine red to brownish yellow colour indicating the formation of silver nanoparticles. The resultant reaction mixture was then kept at normal room condition and washed with water and ethanol and dried in vacuum desiccator.

Preparation of bioimrinted sensor

The interaction between the ds-DNA and butyl paraben in solution phase was carried in phosphate buffer solution (0.5 mol L^{-1} and pH 7.0) containing 20 x10^{-3} mol L^{-1} NaCl, 0.5 x10^{-3} g L^{-1} ds-DNA and 1.0 x10^{-3} g L^{-1} butyl paraben. The mixture was stirred for 300 s. Next, safranine capped with silver nanoparticles was added to the mixture and sonicated for 180 s. Cyclic voltammetric electrodeposition from −0.80 V to +1.30 V for 15 cycles with a scan rate of 0.30 V s^{-1} was performed in imprinted solution for the fabrication of bioimprinted sensor. As a control electrode, the non-bioimprinted polymer (NBIP) electrode was prepared under the same experimental procedure without adding parabens. Then, the electrode was dried at room temperature for 30 min. After that, the modified electrode was immersed in ethanol:acetic acid (8:2 v/v) solution for 10 min to remove the template from polymer.

Electroanalytical measurements

For electrochemical measurement, butyl paraben was firstly accumulated on the sensor surface for 5 s. The electrochemical performance of the bioimprinted sensor was characterized by cyclic voltammetry (CV) and square wave voltammetry (SWV). The CV measurements were performed between −0.8 and +1.3 V at a scan rate of 0.10 V s^{-1} in phosphate buffer pH 7.0. The SWVs were obtained scanning the electrode potential between-0.5 V and +1.3 V with a fequency of 10 Hz, a step potential of 0.045 V and a modulation amplitude of 0.10005 V in a phosphate buffer pH 7.0. The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction using a peak width of 0.1. Repeated measurements were carried out following renewal of the CPE surface by cutting and polishing the electrode.

RESULTS AND DISCUSSION

First, the template (butyl paraben) interacted with dsDNA prior to polymerization. Fig. 1 displays the square wave voltammograms of butyl paraben prior and after the interaction with dsDNA. Thus, butyl paraben had one oxidation peak at +0.730 V (curve b), while dsDNA at +1.093 V (curve c). With the addition of increasing amount of dsDNA into butyl paraben solution, the oxidation peak current of butyl paraben was decreased (curves d and e). A successful imprinting requires the presence of interactions between template and functional groups in the polymer matrix. Imprinting studies pointed to the importance of tailoring additional functional groups, in order to improve the selectivity of imprinting polymers. The role of these functional groups is to assist in the creation of the specific binding cavities complementary to the imprint species, similar to the activesite in an enzyme. In this study, improved imprinting properties are obtained as a result of cooperative interactions between the dsDNA, the functional monomer and the template. It is known that planar aromatic ring systems act as intercalators which stack between dsDNA base pairs leading to significant π–π interaction.

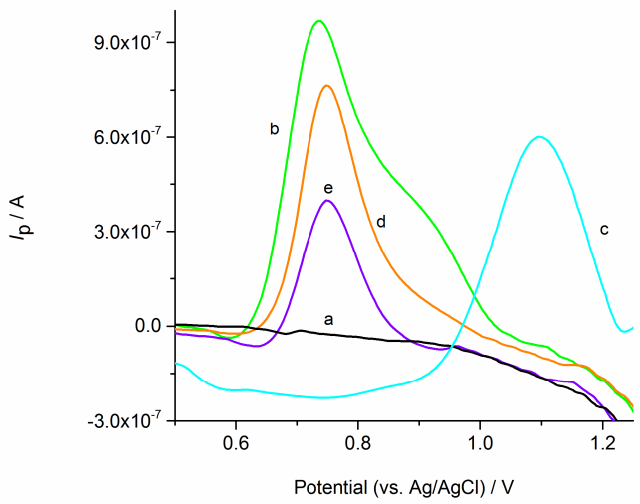

Fig. 1. Square wave voltammograms of (a) bare CPE, (b) 1.0 μg L^{-1} butyl paraben before the interaction with dsDNA on CPE, (c) 2.5 μg L^{-1} dsDNA before the interaction with butyl paraben on CPE, (d) 1.0 μg L^{-1} butyl paraben after the interaction with 2.5 μg L^{-1} dsDNA on CPE and (e) 1.0 μg L^{-1} butyl paraben after the interaction with 5.0 μg L^{-1} dsDNA on CPE. Conditions: as mentioned in the material and methods section

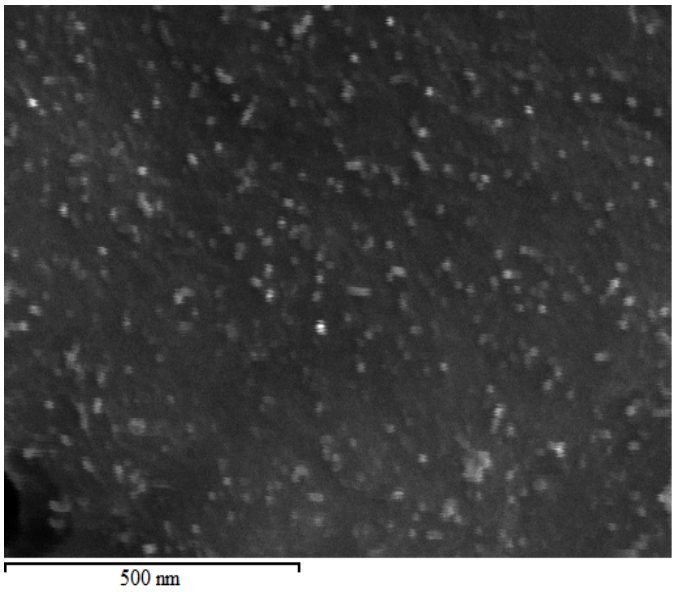

Fig. 2. SEM image of the bioimprinted sensor

As it was shown in Fig 1 the decrease of butyl's paraben oxidation peak current proved the existence of interaction between bytyl paraben and dsDNA. Furthermore, the peak potential of butyl paraben shifted to more positive values with increasing the mass concentration of dsDNA, indicating that

these molecules interact through intercalation (Palecek and Bartosik, 2012; Carter *et al*., 1981). Biomolecules, including dsDNA, can be entrapped into the polymer network during electropolymerization. The electrochemical entrapment of dsDNA is simple, rapid, reagentless, and it is a one-step technique that can be performed in aqueous solutions under mild conditions (Prabhakar *et al*., 2007). One of the main role of the Sa is to form a three-dimensional structure, remainin unchanged after removal of the template. Moreover, the use of Sa has been proposed in order to wrap around the template during polymerization and introduce additional functional groups that can interact with the template. Consequently, the phenyl and NH groups in poly-(Sa) could possibly interact with butyl paraben via π–π interactions and hydrogen bonding. In adittion, AgNPs has been used to improve the low density of imprinted sites into MIPs, the slow diffusion of the analytes across the MIP film, and the low sensitivity. The surface morphology the bioimprinted sensor was evaluated, using SEM (Fig. 2). As shown in Fig. 2, globular shaped Ag nanoparticles were uniformly distributed in the bioimprinted layer.

SWV oxidation peak current was measured as a function of butyl paraben mass concentration at least three times under the selected operational parameters (Fig. 3). The oxidation peak curren of butyl paraben increases with butyl paraben's mass concentration as can be seen in Fig. 4. This evolution can be understood in terms of variation in the bioimprinted polymer's electroactivity in the presence of increasing amounts of target. The calibration plot of butyl's paraben mass concentration versus peak current was found to be linear over the range of 0.362 to 100.0 μg L^{-1} with the regression equation of I_p (μA) = 0.188 (±0.001)γ (μg L^{-1}) + 0.007 (±0.007), r^2= 0.9998. For concentrations higher than 100.0 μg L^{-1}, the peak current remained constant, which could be explained by saturation of sensor response, due to the limited number of preformed functionalized cavities. The detection limit (LOD) was estimated as 3 *s*/*m* and found to be 0.109 μg L^{-1} and the quantification limit (LOQ) was calculated as 10 *s*/*m* and was found to be 0.362 μg L^{-1} (*s* representing the standard deviation of the peak current (n = 3) and *m* is the slope of the calibration curve).

Table 1. Comparison of different detection techniques of butyl paraben

Electrode	Linear range	LOD	LOQ	Ref
In_2O_3 nanobricks glassy carbon electrode (GCE)	0.14 μmol L^{-1} to 2.4 μmol L^{-1}	0.08 μmol L^{-1}	0.26 μmol L^{-1}	(36)
Multi-wall nafion (NAF) modified GCE	10 μmol L^{-1} to 100 μmol L^{-1}	1.1 μmol L^{-1}	10 μmol L^{-1}	(37)
MIP GCE	5 μmol L^{-1} to 80 μmol L^{-1}	0.2 μmol L^{-1}	5 μmol L^{-}	(38)
Bioimprinted sensor	0.362 μg L^{-1} to 100 μg L^{-1} or 0.0017 μmol L^{-1} to 0.5148 μmol L^{-1}	0.109 μg L^{-1} or 0.0006 μmol L^{-1}	0.362 μg L^{-1} or 0.0017 μmol L^{-1}	This study

CV could be useful to characterize the stepwise fabrication process of the bioimprinted sensor. Fig. 3 shows the CV and SWV results the bioimprinted sensor. As it can be seen in Fig 3, when the electrode was coated with the bioimprinted film, two oxidation peaks at about -0.097 V and +0.498 V of poly-(Sa@AgNPs) was observed (curve a), while the oxidation peak of butyl paraben was inhibited, which indicated that the bioimprinted film covered the entire surface of the electrode and blocked the interfacial electron transfer between poly-(Sa@AgNPs) and butyl paraben. The extraction of template molecules resulted in the formation of recognition cavities, which enhance the diffusion of butyl paraben through the polymer layer and increased the current response (curve b) and the oxidation peak of butyl paraben became obvious at +0.777 V. After the bioimprinted sensor was immersed in butyl paraben solution for 5 s, the peak current of the poly-(Sa@AgNPs) oxidation peaks decreased (curve c), as well as butyl paraben's oxidation peak desapeared, which could be ascribed to the rebinding of butyl paraben into the cavities and limited chances for the electron transfer on the electrode surface. As shown in curve d, coating of the NBIP film on the CPE surface had led to further decreace of the poly-(Sa@AgNPs) oxidation peaks. This can be related to the decrease in electrode conductivity in the presence of dense layer of polymer without recognition cavities. To obtain the best efficiency for imprinted sensor, the effect of several parameters including electropolymerization scan cycles, MIP composition, template mass concentration, pH, incubation and extraction time were investigated. All parameters were evaluatedon the basis of the change of butyl paraben oxidation peak current (I_p).

More discussion regarding the effect of various parameters on the current response has been given in the *Supplemental Information* in the same section. The quantitative analysis of butyl paraben using bioimprinted was performed SWV. The

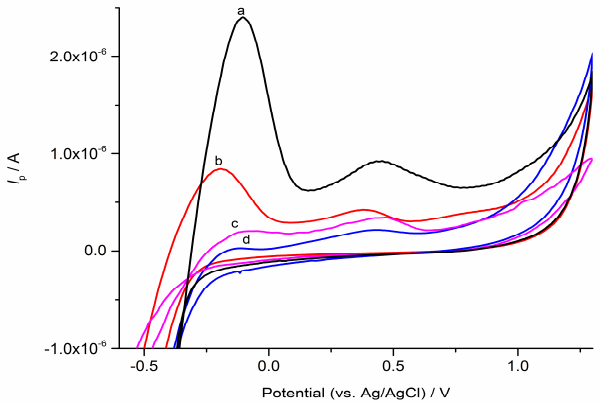

Fig. 3. Cyclic voltammograms of (a) bioimprinted sensor before template removal, (b) bioimprinted sensor after removal of template, (c) bioimprinted sensor after 5 s incubating in 1.0 μg L^{-1} butyl paraben solution and (d) NBIP. Conditions: potential scan range = -0.8 V to +1.3 V, scan rate = 10 mV s^{-1} and step potential = 6 mV

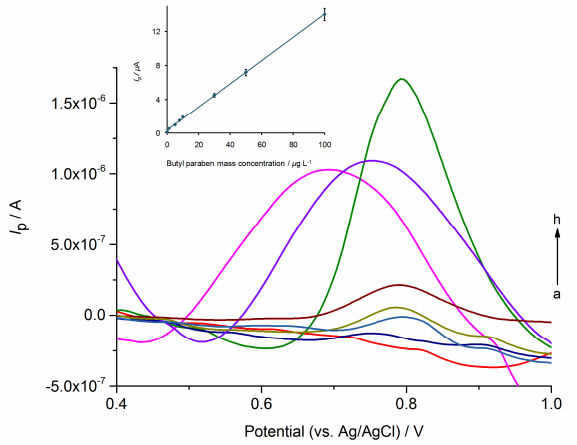

Fig. 4. Square wave voltammograms of bioimprinted sensor after incubation in 0.362 (a), 1.000 (b), 5.000 (c), 8.000 (d), 10.000 (e), 30.00 (f), 50.00 (g) and 100.0 (h) μg L^{-1} of butyl paraben solution. Inset is the calibration curve of bioimprinted sensor in different concentrations of butyl paraben solutions. Conditions: as mentioned in the material and methods section

Table 2. Recovery studies of butyl paraben by standard solutions in cosmetics using fabricated bioimprinted sensor (n = 3)

Sample	Added (μL)	Detected (μg L^{-1})[a]	Recovery (%)	RSD (%)
Ystheal Contour Yeux Levres®(Avene)	0	0.024	-	-
Ystheal Contour Yeux Levres®(Avene)	25	0.049	97.3	2.8
Ystheal Contour Yeux Levres®(Avene)	50	0.075	98.5	3.1
Ystheal Contour Yeux Levres®(Avene)	75	0.112	105.2	4.6

[a]Average of three replicates.

The analytical characteristics of the bioimprinted sensor for the determination of butyl paraben were compared with previous reported techniques (Table 1). From Table it is obvioius that the proposed bioimprinted sensor had lower detection limit compared to the reported ones. Furthermore, it had suffieciently broad linear range. The validation of the developed bioimprinted sensor was carried out to evaluate the selectivity and specificity in presence of some potential interfering substances having similar structure as butyl paraben. Under selected conditions, the interference test was performed in the presence of 100-fold concentration of various endocrine disruptors' viz., phenol, bisphenol-A, ethyl paraben and methyl paraben. The results obtained show that they do not interfere on the signals of butyl paraben, with the obtained recovery ranging from 101.3 to 103.5 %, indicating that the fabricated sensor is suitable for detection of butyl paraben. The reproducibility of the proposed bioimprinted sensor was investigated by determining the butyl paraben solution (15.00 μg L^{-1}) with five different imprinted sensors which prepared independently under the same conditions. The relative standard deviation (RSD) of 3.2% was obtained, which indicated good sensor-to-sensor reproducibility.The storage stability of the bioimprinted sensor was also investigated by measuring the current change for 15.00 μg L^{-1} butyl paraben at regular intervals (2 day) for a period of three weeks. The sensor retained 98.5 % of its initial response current after storage for three weeks at the refrigarator.

This demonstrated that the bioimprinted sensor has acceptable storage stability. The good stability of the bioimprinted polymer might be attributed to the better integration of dsDNA with Sa@AgNPs matrix during electrochemical entrapment. The real sample analysis for the detection of butyl paraben was done by analysing a commercial product. A 0.1 g of cream was dissolved in 25 mL of ethanol by ultrasonic agitation for 30 min and then the sample solution was centrifuged for 10 min in order to remove the excipients and finally transferred into 100 mL volumetric flask and make up with phosphate buffer of pH 7.0 to obtain 10 mg mL^{-1} solution of cosmetic sample. The amountof butyl paraben present in the cream was determined by standard addition method at the developed bioimprinted sensor. The reliability of the proposed method was verified by calculating the recovery by standard addition method using three different standard samples (1.000, 10.00 and 50.00 μg L^{-1}). The recovery was obtained in the range of 97.3 to 105.2 % with the RSD value less than 5% (Table 2) indicates high accuracy and precision of the developed method for the determination of butyl paraben in cosmetic samples.

Conclusion

The proposed approach demonstrates a valid route for the preparation of a new generation of MIP sensors based on bio-materials (ds-DNA), which has been focused on enhancement of selectivity of imprinted polymers. The bioimprinted film was syn-thesized by electrochemical entrapment of ds-DNA and AgNPs in the poly-Sa network through one-step electropolymerization on the surface of the modified carbon paste electrode. The proposed bioimprinted sensor, which was used to determine butyl paraben was the first to be reported. The high selectivity towards butyl baraben of the proposed bioimprinted sensor compared to the conventional electrochemical sensor was obtained by the cooperative interactions between functional groups in the DNA and poly-Sa. The obtained bioimprinted sensor provided large electrochemically active surface area, excellent reproducibility, good selectivity and sensitivity. Moreover, this new bioimprinted sensor has great potential in the practice sample analysis in complex matrix such as cosmetic samples.

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Appendix A. Supplementary Information

Supplementary information associated with this article can be found, in the online version.

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