Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information

Electrochemical Sensing of Estradiol Benzoate Using Hydroxyapatite with Three-dimensional Channels Frameworks

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Experimental details

Materials and reagents

A cation-exchange membrane, Nafion N-117, was purchased from DuPont (USA). Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and methanol were all purchased from Sinopharm. 17β-estradiol-3-benzoate (E2B, 98%) was purchased from TCI. The 1×PBS buffer solution (Hyclone™) was used as supporting electrolyte. All the chemicals used in the present work were of analytical grade and used without any further purification. Millipore (Milli-Q) ultra-pure water was used throughout the work.

Preparation of HAp

0.5 M $Ca(NO_3)_2$ and 0.3 M $NH_4H_2PO_4$ (the pH was adjusted to be 11 beforehand) solutions were injected into two cylindrical polypropylene containers respectively, Figure S1, and separated by the tightly fixed cation-exchange membrane. The crystallisation process was carried out at 50˚C without stirring. After holding for 1 to 7 days, the membrane was recovered after gently rinsed with deionized water for a few times, and then dried in air at 40 ˚C for 12 hr. The phase structure was identified by a powder X-Ray Diffraction meter (XRD; D/ruax 2550PC).

Fabrication of the HAp-CPE

The HAp modified carbon paste electrode (HAp-CPE) was prepared by mixing the HAp and graphite powder (CP) to an optimum HAp/CP ratio of 20% by weight (w/w) using paraffin oil as a binder. A portion of the resulting paste was packed firmly into commercial CPE shell with a PTFE cylindrical tube (geometric area = 0.1256 cm^2) by utilising a copper wire to make electrical contact. The electrode was washed with ultra-pure water and ethanol and then dried at room temperature.

Electrochemical determination

A conventional three-electrode system, consisting of the HAp-CPE working electrode, 1 cm×1 cm platinum gauze counter and saturated calomel electrode (SCE) reference electrode in a 20 mL of glass container, were utilised for the electrochemical investigations. The mixture of methanol and phosphate buffer solution (PBS) at a certain ratio was used as electrolyte. The cyclic voltammetry (CV) behaviour of HAp-CPE was recorded by using an Electrochemical Working Station (CHI-660e, China) under ambient conditions.

Electrochemical analysis

For a totally irreversible electrode process, the relationship between the potential (E_r) and scan rate (v) is expressed as follows:

$$E_r = E^0 + (RT/\alpha nF)\ ln\ (RTk^0/\alpha nF) + (RT/\alpha nF)\ ln\ v$$

where α is electron transfer coefficient, n is electron transfer number, R is the universal gas constant, F is the Faraday constant and T is temperature of the solution. According to the slope of E_r *vs.* ln v plots, αn values for both the methanol:PBS ratios were calculated to be 0.24 and 0.33, respectively. Generally, α is assumed to be 0.5 in totally irreversible electrode process. Therefore, the electron transfer numbers (n) in electrochemical reduction of E2B under the mentioned two environments are 0.48 and 0.66, respectively. This is vaguely close to the estimated electrochemical transfer process being one, based upon the structure of E2B, where the electrochemical reduction of E2B undergoes a one proton and one electron process likely at the –C=O site forming –C-OH. The disparity in the deuced number of electrodes is likely due to the system being a mixture of both adsorbed, diffusional and thin-layer electrochemical processes in operation indicating that the use of this equation/analysis is not strictly valid.

Table S1. Interplanar spacing (*d*) values for (211), (112) and (002) planes (corresponding to the strongest three diffraction peaks) in each sample. The lattice constants for them were obtained by cell refinement*

Days	PDF#	d_{211} /nm	d_{112} /nm	d_{002} /nm	a /nm	c /nm
3	73-0293	0.2818	0.2780	0.3439	0.9432	0.6881
5	86-0740	0.2792	0.2771	0.3416	0.9352	0.6882
7	09-0432	0.2816	0.2794	0.3449	0.9418	0.6884

* These data were collected by Jade 6.5 software.

Figure S1. The bespoke designed device for HAp fabrication in this work (a) and schematic crystal growth principle (b)

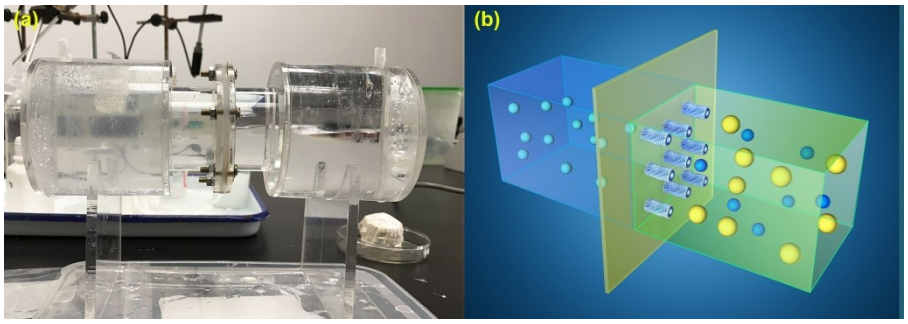

Figure S2. Cyclic voltammetric curves of 7 day-HAp used in the fabrication of HAp-CPE in the mixture of methanol: PBS= 10:90 (v/v) without E2B (blank) and the same mixture containing 1 μg/mL E2B.