Supplementary Information

Detection of Progesterone in Aqueous Samples by Molecularly Imprinted Photonic Polymers

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MIPs Sensors Fabrication

The elution of progesterone was conducted via two steps, first; by soaking the film and replacing the solution every 30 min, for 3 h with different volume ratios of acetic acid to ethanol 1:9 (1 mL AA + 9 mL ethanol), 1: 5.67 (1.5 mL AA + 8.5 mL ethanol), and 1:2.33 (3 mL AA + 7 mL ethanol). The effect of each elution solution was evaluated by measuring the peak wavelength shift in their UV-Vis reflectance spectra, in accordance with the properties of the photonic molecular imprinted films, second; was conducted with respect to the washing time, as a shorter time for target removal is more desirable for the entire process. The elution was carried out by washing the film with 1:9 (v: v) acetic acid: ethanol solutions, for 3 hours and 4 hours, both with replacement of clean solutions every 30 min The shift in peak wavelength and the reflectance was measured also after every wash during the experiments, so the removal is completed when no further shift change is noticed in the film.

Fabrication optimization study

Effect of the time in Hydrofluoric Acid bath

The creation of substantial macropores in molecularly imprinted films is highly favorable for mass transportation and fast accessibility to recognition sites. This 3D highly ordered films can be formed by the removal of silica particle and making interconnected pores in an inverse opal structure.

The silica particles were eliminated by immersion in 5% HF bath. The effect of etching time was investigated by soaking the films in the acid solution for 24 hrs, 36 hours, and 48 hrs, followed by exhaustive rinsing with deionized water. The resulting porous films were characterized by their reflectance spectra in the UV-visible range, identifying the peak wavelength and determining the shift of the Bragg's diffraction peak after each etching time

Effect of the elution solvent composition (Acetic Acid: Ethanol ratio)

The analyte is removed to create specific binding cavities, compatible in shape, size, and functionality group in the imprinted film. The elution of progesterone was conducted with a solution of acetic acid in ethanol, by soaking the film and replacing the solution every 30 min, for 3 h. Different volume ratios of acetic acid to ethanol were investigated: 1:9 (1 mL AA + 9 mL ethanol), 1: 5.67 (1.5 mL AA + 8.5 mL ethanol), and 1:2.33 (3 mL AA + 7 mL ethanol). The effect of each elution solution was evaluated by measuring the peak wavelength shift in their UV-Vis reflectance spectra, in accordance with the properties of the photonic molecular imprinted films. After the treatment,

visual inspection was conducted for each film, in order to check whether the film was damaged, or detached from the slide. Thus, the faulty films were discarded and not used in future tests.

Effect of the washing time

The optimization of the target removal process from the MIPs was also conducted with respect to the washing time, as a shorter time for target removal is more desirable for the entire process. The elution was carried out by washing the film with 1:9 (v: v) acetic acid: ethanol solutions, for 3 hours and 4 hours, both with replacement of clean solutions every 30 min. The shift in peak wavelength was measured after every 30 min during the experiments. A visual inspection to the film quality was conducted to observe if the film was either damaged or detached; the films showing obvious signs of damage were discarded.

Standard Solutions Preparation

The stock solution of progesterone was prepared by dissolving 10 mg in 100 ml methanol and diluted with de-ionized water to the concentration of 100 ppb. The standard working solutions were prepared by diluting (100 ppb) with ultrapure water with concentration from (1-20) ppb.

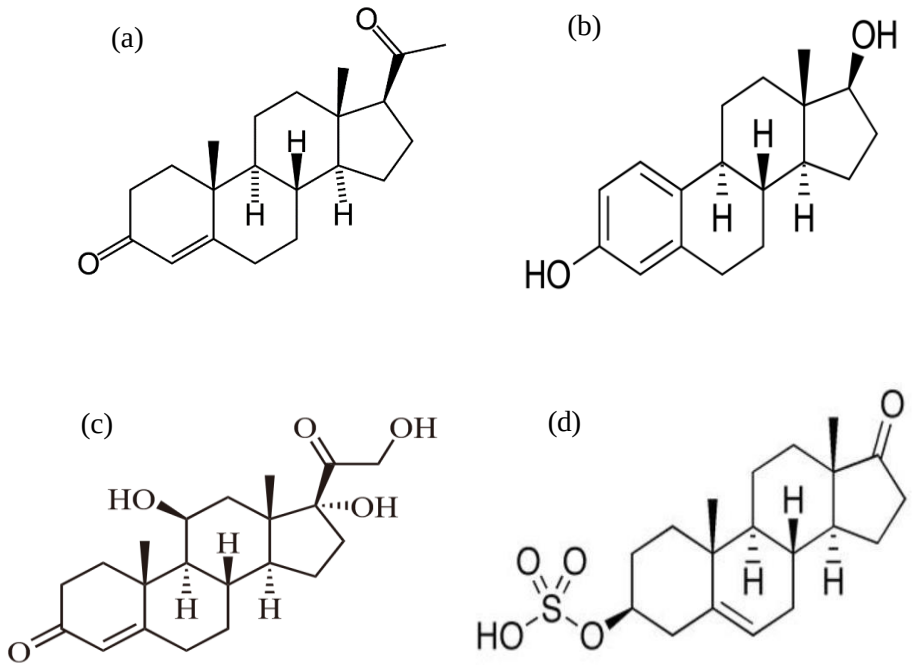

Figure S.1. Chemical structure of (a) Progesterone, (b) 17-β Estradiol, (c) Hydrocortisone, (d) Dehydroepiandrosterone-Sulfate (DHEA-S)

Optical response

PG attachment into the photonic MIP binding sites modified the Bragg diffraction spectra of the films due to swelling and refractive index changes, producing the optical signal. The Bragg equation relates the diffraction peak (λ_{max}) for the polymer with its morphology (Eq.S.1):

$$\lambda_{max} = 1.633 \left(\frac{d}{m}\right)\left(\frac{D}{Do}\right)\left(n_a^2 - \sin\theta^2\right)^{0.5} \qquad \text{(S.1)}$$

where d is the SiO_2 particle dimater, m is the Bragg diffraction order, (D/D_0) is the degree of swelling of the polymer (D in the equilibrium state and D_0 in the reference state) , n_a is the average refractive index of the hydrogel, and θ is the beam angle of incidence. The reflectance spectrum of the films was collected over a range of λ between 200–800

nm, using a double-beam UV–visible spectrophotometer (Cary 60, Varian) with a Harrick Scientific's Specular Reflection Accessory (ERA-30G) for measurement of reflectance at a fixed angle of 30 degrees.

Analytical methods

A PG stock solution was prepared by dissolving 10 mg in 100 ml methanol (100 ppm) followed by dilution with de-ionized water to a concentration of 100 ppb. The standard working solutions were prepared by diluting the 100 ppb stock solution with ultrapure water to concentration from 1-20 ppb.

All experiments were carried out at room temperature, 25± 0.5 ^{0} C. The concentration of progesterone stock solution was determined by a Waters Alliance 2695 High Performance Liquid Chromatography (HPLC) system coupled with Waters Acquity TQ triple quadrupole mass spectrometer (MS/MS). The analytes were separated by a Phenomenex (Torrance, CA) Kinetex C18 (100mm x 4.6 mm; 2.6 µm particle size) reverse-phase column. The mobile phase consisted of 10 mM ammonium acetate and 0.1% formic acid in water (A) and 100% acetonitrile (B). The gradient conditions were 0 – 0.5 min, 2% B; 0.5-7 min, 2- 80% B; 7.0 -9.0 min, 80-98% B; 9.0 – 10.0 min, 2% B; 10.0 – 15.0 min, 2% B at a flow rate of 0.5 mL/min. The ion source in the MS/MS system was electrospray ionization (EI) operated in the positive ion mode with capillary voltage of 1.5 kV. The ionization sources were programmed at 150°C and the desolvation temperature was programmed at 450°C. The MS/MS system was in the multi-reaction monitoring (MRM) mode with the optimized collision energy. The ionization energy, MRM transition ions (precursor and productions), capillary and cone voltage, desolvation gas flow, and collision energy were optimized by the Waters IntelliStart™ optimization software package. The retention time, calibration equations, and limits of the detection for the analyses of PG are summarized in **Table S.1**.

Table S.1 Precursor and productions selected for progesterone analysis by HPLC-MS/MS (LODs = limits of detection)

Compound	RT (min)	Molecular ions (m/z)	Product ions (m/z)	Polarity	Linear Equation	Correlation coefficients (R square)	Collision Energy	Cone Voltage (V)	LOD (µg/L)
Progesterone	11.124	315.01	96.85	ES+	y = 7470.8x	0.9976	Tune	30	1.23

Analysis of manufacturing parameters

The interparticle capillary forces between the colloids depend on the evaporation rate of the solvent in suspension. Furthermore, a balance must be achieved between the solvent evaporation and the colloid influx rate to

the meniscus region on the substrate. The variation of ambient temperature during colloidal crystal formation between 50°C and 70 °C showed a fluctuating effect on the colloidal crystals arrays arrangement, determined by the peak wavelength (Figure S.2 a). The results didn't demonstrate a clear trend; however, a large variability in peak wavelength was observed for the tests at 60 and 65°C, which incidentally corresponded to days when the relative humidity was higher. Due to the hygroscopic nature of ethanol, water molecules would be absorbed in the solvent and the suspended particles, resulting in lower the repulsive interaction forces and a more disorganized approach of colloids to the glass substrate in the form of small aggregates instead of single particles to form the deposits.

The degree of removal of the silica NPs after polymerization was assessed (Figure S.2 b): for a time in HF of 36 hrs, the SiO_2 NP removed from the polymer films led to a shrinkage in the imprinted cavities and cause a volume change in the hydrogel film porosity and resulted in a significant change of the Bragg diffraction peak. The 36 hr time was superior to 24hr as it demonstrated higher shift values, evidencing better removal of silica particles without producing damage to the polymer film that was observed after 48 hrs immersion in the HF bath. In the PG removal process, three different washing ratios of acetic acid: ethanol (v:v) were considered: 1:9 (1 mL AA + 9 mL ethanol), 1: 5.67 (1.5 mL AA + 8.5 mL ethanol), and 1:2.33 (3 mL AA + 7 mL ethanol) volume. The results of the reflectance spectra of the films with washing ratio (1:9) (Figure S.2 c) showed a maximum shift toward shorter wavelength of visible spectra region to an average λ_{max} value of 395±15 nm, which reflects the removal of the analyte from the binding sites and the shrinking of the hydrogel film. The (1.5:8.5) washing ratio produced a poor quality with wide peaks and not clearly defined maximum, as well as some damaged films. With increasing ratio of acetic acid: ethanol up to 3:7, the films appeared to be totally damaged. For this reason, the ratio of 1:9 of ethanol/acetic acid solution and elution time of 3hrs were considered in all fabrication processes. Elution time of 3 and 4 hours were compared, with replacement of the solution every 30 min. Previous work in the laboratory showed that 3 hours were needed to produce significant elution of a chemically similar target molecule. The results showed that 3 hrs washing time had high efficiency of target molecules elution from the MIPs matrix. Longer times proved to be excessive exposure to the acetic acid and ethanol solution, as the 4 hrs washing time caused damage to the film polymeric structure and led to the shift back to higher wavelengths (Figure S.2 d).

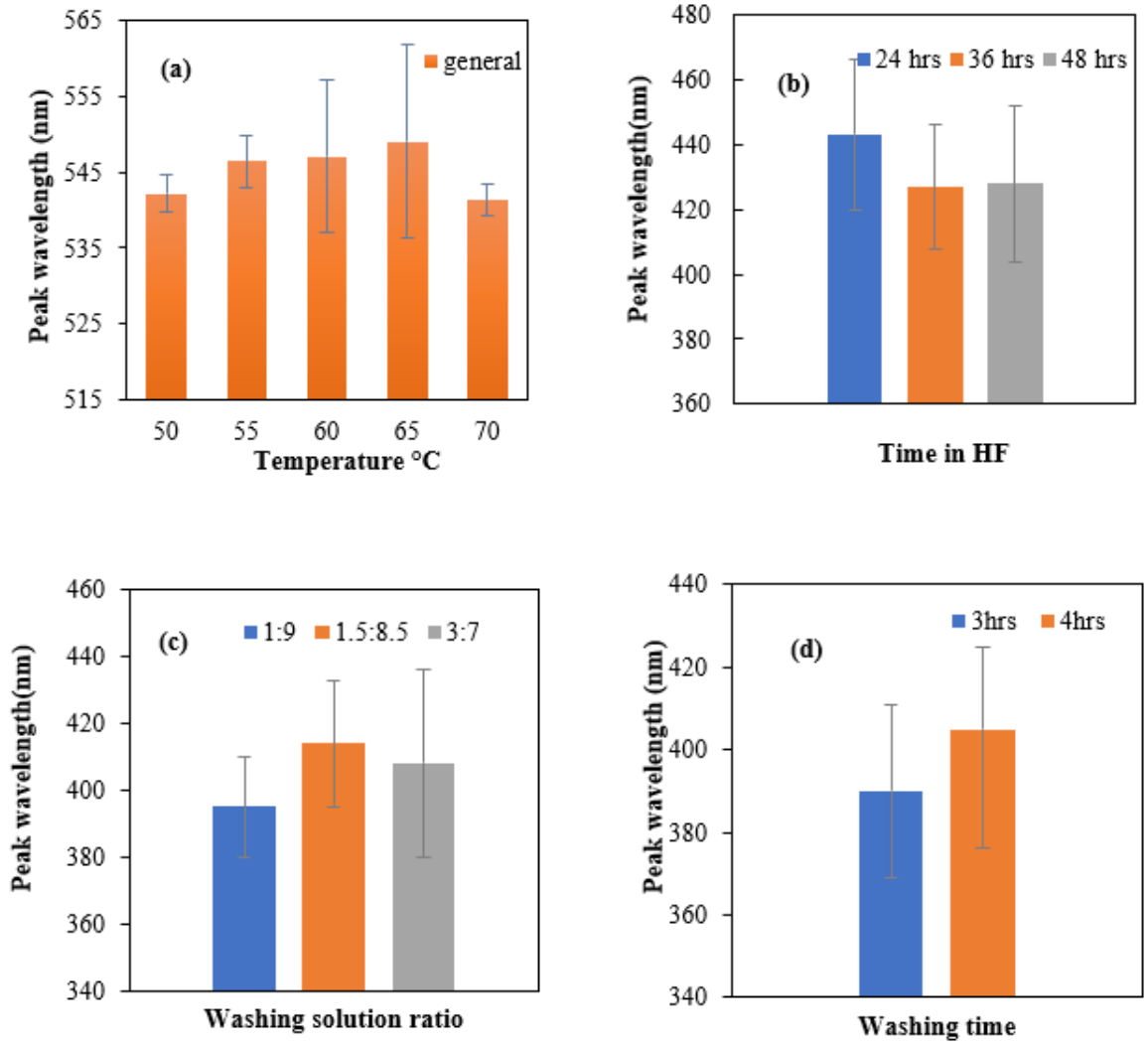

Figure S.2. Average peak wavelength at different manufacturing parameter levels: (a) temperature of the deposition chamber;(b) time in 5% HF bath; (c) solvent composition (acetic acid: ethanol ratio); (d) washing time.

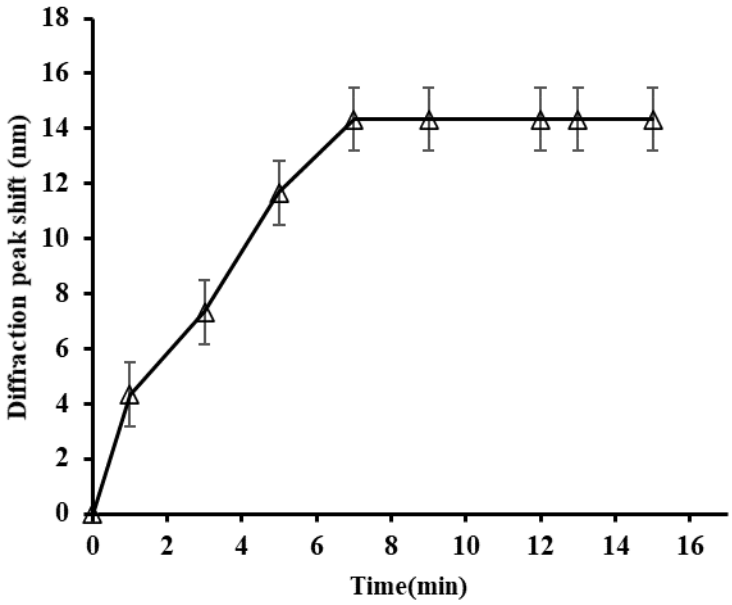

Figure S3. Kinetic response of the progesterone MIP; initial progesterone concentration 20 μg L^{-1} (n=6).

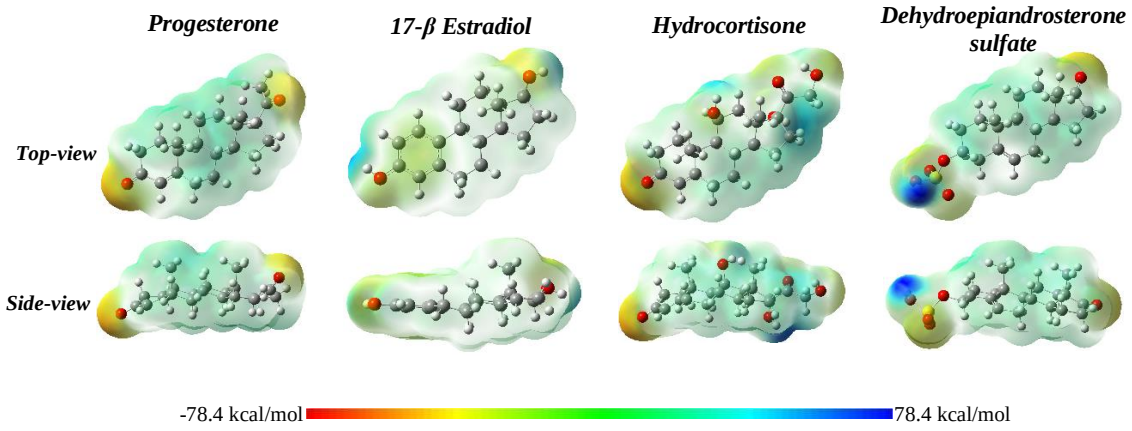

Figure S.4. Top and side views of the molecular electrostatic potential surface of the selected hormones (iso-electron density value of 0.001 au).

Table S.2 Parameters calculated from the molecular electrostatic potential representations mapped against iso-electron density value of 0.001 au.

	Progesterone	17-β Estradiol	Hydrocortisone	Dehydroepiandrosterone sulfate
Volume ($Å^3$)	424.4	358.8	449.4	443.5
Minimal ESP value (kcal/mol)	-54.0	-36.7	-55.7	-45.4
Maximal ESP value (kcal/mol)	27.3	59.41	71.7	79.6
Surface area ($Å^2$)	342.5	302.2	357.6	366.9
Positive surface area ($Å^2$)	258.9	179.6	243.2	263.5
Negative surface area ($Å^2$)	83.6	122.6	114.4	103.4
Nonpolar surface area ($Å^2$) (|ESP| <= 10 kcal/mol)	91.6 (26.7 %)	188.2 (62.3 %)	124.2 (34.7 %)	107.6 (29.3 %)
Polar surface area ($Å^2$) (|ESP| > 10 kcal/mol)	250.9 (73.3 %)	113.9 (37.7 %)	233.4 (65.3 %)	259.3 (70.7 %)
Molecular polarity index (MPI) (eV)	0.709	0.460	0.734	0.692