In Situ Synthesis of Plasmonic Core MIPs on U Bend Optical Fibers for LSPR Sensing of Small Molecule Contaminants in Food and Environment: An Illustration With Bisphenol A

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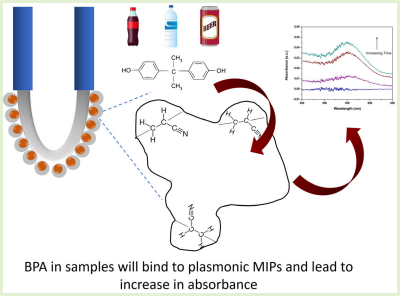
***Abstract*—Detection and remediation of emerging water contaminants such as active pharmaceutical ingredients, endocrine disrupting compounds (EDCs), and polyaromatic hydrocarbons is a global challenge. The current study reports a label-free and biorecognition element-free detection strategy for the detection of such organic compounds in aqueous specimens with Bisphenol A (BPA) as a model analyte. A novel in situ molecular imprinting technique on gold nanoparticles (AuNPs), immobilized on U-bend optical fibers is demonstrated for localized surface plasmon sensing. The molecularly imprinted polymer film was directly formed on allyl mercaptan-modified gold nanoparticles. With an optimized ratio of 1:12:20 of the template: monomer: crosslinker, the sensor could detect BPA in the linear range from 30 ng/mL to 10 μg/mL (R^2 = 0.95) in deionized water and from 50 ng/mL to 50 μg/mL (R^2 = 0.96) in bottled and canned soft, alcoholic and aerated beverages, fruit juices and ice tea with negligible cross sensitivity from possible interferents. This technology is thus of utility to analyze BPA, and similar organic molecules in wastewater, food, and beverages and indicate the fitness of food and water for human consumption.**



***Index Terms*—Clean water, good health, localized SPR (LSPR), optical fiber, plasmonic molecular imprinted polymer (MIP), sensor design.**

I. Introduction

ENDOCRINE disrupting compounds (EDCs) are a class of organic compounds that over the years have emerged as persistent contaminants in the environment [1]. These compounds such as 17β estradiol, estrone, Bisphenol A (BPA), nonylphenol, parabens, phthalates, and others interfere with the normal functioning of the endocrine system and produce adverse reproductive, neurological, cardiovascular, and developmental effects in humans and animals [2], [3]. Of particular importance in this list is BPA which is known to mimic the hormone estrogen. BPA is widely used in manufacturing polycarbonate plastics, as coatings on canned and bottled beverages, baby feeding bottles, epoxy resins, and others which are extensively used worldwide on a daily basis. Consumption of food and beverages packaged in BPA-laden containers, combined with improper disposal measures of the used containers have led to their emerging persistence in the environment, water, and food in concentrations of a few ng/L to a few hundred of μg/L [4]. Low- and middle-income countries with high population densities have seen a high persistence of these in surface and drinking water. Conventional water treatment plants are neither able to detect nor remove BPA from aqueous media [2]. There is, thus, a pertinent need to develop portable and affordable devices for quantitative estimation of BPA to decide on fitness for human use. Worldwide accepted gold standard methods of detection of EDCs involve a modality of high performance chromatography,

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usually coupled with advanced mass spectroscopic detectors. These analyses require state-of-the-art sophisticated laboratory facilities, trained personnel, extensive libraries, and reagents and are time intensive. Since the concentrations of these compounds are in the range from pg/mL to a few hundred μg/mL in environmental specimens, an additional sample preconcentration step using solid phase extraction is often necessary. In a study by Tsalbouris et al. [5], molecularly imprinted polymers-based solid phase extraction was used in tandem with high-performance liquid chromatography-diode array detector (HPLC-DAD) for the detection of BPA in alcoholic and nonalcoholic beverages. However, quantitative estimation of such compounds requires calibration of the system with suitable isotopes which makes the overall detection expensive and limits the deployability for extensive and point-of-use screening. Thus, such analysis is seldom routinely performed online on production lines for these compounds, while humans continue to be on constant chronic exposure to these molecules. Point-of-use sensors are the next best solutions for rapid screening of compounds such as BPA. Most of these technologies use a biorecognition element such as whole cells [6], polynucleotides [7], or enzymes [8], most of which have very specific physiochemical stability criteria for which they are tested. These coupled with complex transduction techniques [9] are often reported to potentially compete with the chromatography-coupled mass spectroscopic techniques [10] in terms of sensitivity, accuracy, and detection limits. However, the inherent disadvantages of sample preparation and sensor storage limit the endpoint of use.

Several studies have been undertaken for BPA detection in canned beverages and food products. Taguchi et al. [11] reported one of the first studies using molecular imprinted polymer (MIP)-coupled surface plasmon resonance (SPR) for the detection of BPA. The study reported a detection limit of 1 mM but diverse analyte samples and cross-sensitivity analysis were not reported. However, such transduction schemes are immune to electrostatic and electromagnetic interferences and are thus likely to offer robust alternatives for point-of-use sensing applications [12]. Wang et al. [13] reported a MIP on a magnetic covalent organic framework coupled with quantum dots for fluorescence quenching-based detection of BPA. The authors have reported an experimental limit of detection (LoD) of 25 μg/L but the complexity of materials and transduction may limit the point-of-use deployment of such a sensing scheme. Some similar studies utilizing MIPs for optical detection of BPA are summarized in Table I. Although optical approaches are robust, the LoDs achieved are on the higher side, and hence, these technologies may be tailored for BPA detection in stored agro products or gray water.

Through this study, we report an in situ method of molecular imprinting on gold nanoparticles immobilized optical fibers for localized SPR (LSPR) based sensing of small molecules. The surface of gold nanoparticles was first modified with allyl mercaptan to achieve $=CH_2$ bonds. The fiber was then dipped in a prepolymerization solution consisting of the template, monomer, and crosslinker in a certain ratio. Polymerization was thermally initiated with benzoyl peroxide (BPO) as the polymerization initiator and a uniform imprinted polymer layer was formed around the gold nanoparticles. This imprinted film acts as the recognition layer for the detection of BPA. Through this study, we report on a generic scheme for the design of U-bend optic fiber sensors with a simple modification of the gold nanoparticles for the detection of EDCs circumventing the use of a biorecognition element. With the developed sensor, it was possible to detect BPA in bottled water, bottled beverages, and tea. The linear range of detection was 30 ng/ml to 10 μg/ml in water ($R^2 = 0.95$) and 50 ng/ml to 50 μg/ml in bottled aerated beverage ($R^2 = 0.96$). Although the concept is illustrated with BPA as a model EDC, it may be easily extended for specific detection of all aforesaid EDCs in a multiplexed sensor array format in tandem with the handheld device reported earlier [14]. This technology has immense potential in the screening of EDCs in bottled and canned food and beverages.

TABLE I
MOLECULAR IMPRINTED POLYMER BASED SENSORS IN LITERATURE

Sensing method	MIP composition	Real samples tested	Detection range	LoD	Ref
Fluorescence quenching	Carbon-dot embedded mesoporous silica-based MIP	Canned croaker, sardine, orange juice, hawthorn,	25 ng/mL to 2 µg/mL	16 ng/mL	[15]
Fiber-optic evanescent wave sensor	methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA)	Mineral water in plastic bottles	3 ng/mL to 5 µg/mL	1.7 ng/mL	[16]
Surface enhanced Raman spectroscopy	MAA, EGDMA	Mineral water in plastic bottles	100 ng/mL to 1 µg/mL	70 ng/mL	[17]
Fluorescence quenching	MAA, EGDMA based MIP coated on Cr2O3 nanoparticles	Baby bottle, river water, well water	10 ng/mL to 1 µg/mL	1.5 ng/mL	[18]
Localized surface plasmon resonance	Acrylonitrile, EGDMA based MIP directly synthesized on immobilized gold nanoparticles	Soft aerated beverage, alcoholic beverage, fruit juice, bottled tea and mineral water	30 ng/mL to 10 µg/mL	30 ng/mL	This study

II. MATERIALS AND METHODS

A. Materials

Acrylonitrile, methacrylic acid (MAA), azobisisobutyronitrile (AIBN), and p-nitrophenol (P-NTP) were procured from Sisco Research Laboratories. Ethylene glycol dimethacrylate (EGDMA), (3-aminopropyl), triethoxysilane (APTES), BPA, benzoyl peroxide BPO, allyl mercaptan, gold (III) chloride, ampicillin (AMP), ascorbic acid (AA), chlorpyrifos (CPS), ciprofloxacin (CIP), trisodium citrate hydrate, and β-estradiol (E2) were purchased from Sigma Aldrich (Merck).

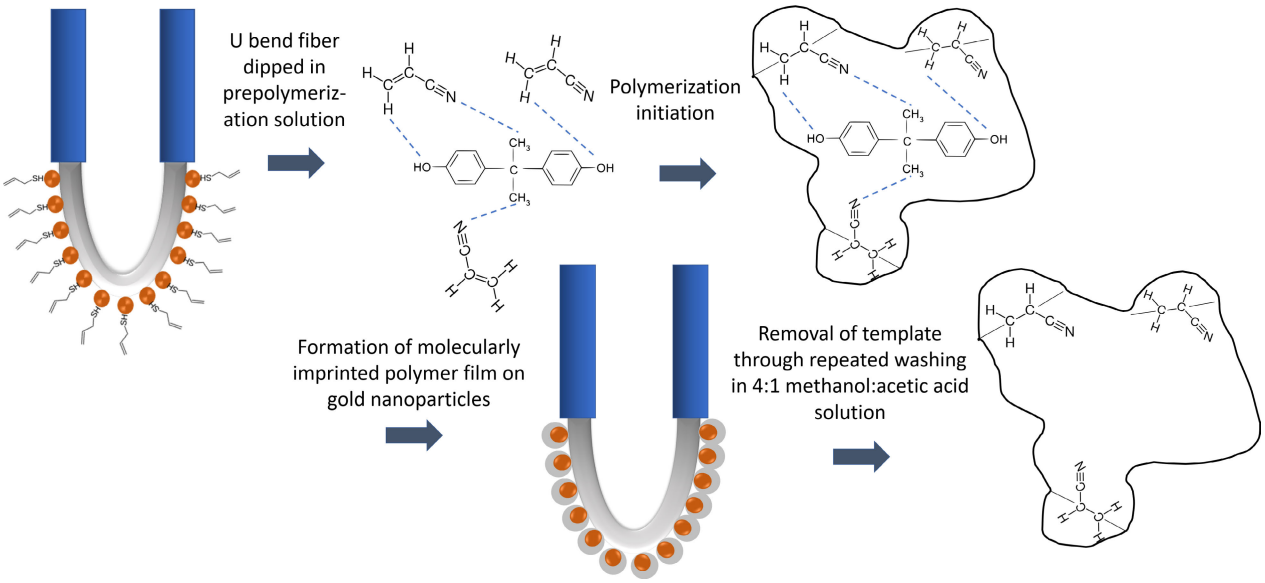

Fig. 1. Schematic of the method of formation of MIP layer on U-bend optical fibers.

All aqueous solutions were made in deionized (DI) water from a MilliQ filtration plant.

B. Preparation of U Bend Optical Fibers

High –OH, silica core, multimode optical fibers of 200-micrometer core diameter were procured from Thorlabs[1], USA, and cut into 40 cm strips. The central region of the optical fibers (2 cm) was dejacketed, decladded and U bent using a butane flame. The bend diameter was maintained at 1.5 mm for optimal sensitivity. The fibers were cleaned by successively incubating in 1 M sodium hydroxide solution for 30 min, 1:1 (v/v) of methanol and concentrated hydrochloric acid for 60 min followed by activation of –OH groups using sulphochromic acid. The fibers were incubated in 1% APTES solution (made in 1:1 (v/v) glacial acetic acid and ethanol) for 10 min and baked at 110 °C for 45 min to generate amine groups on the fiber surface. Citrate-stabilized spherical gold nanoparticles of approximate dimension 20 nm were synthesized by the Turkevich method. Briefly, 5 μl of gold (III) chloride solution was added to DI water under constant stirring and heated till boiling, followed by the addition of 2 mg/ml trisodium citrate dehydrate (reducing and capping agent). Heating was continued under constant stirring till a bright red solution of gold nanoparticles was obtained. Gold nanoparticles were immobilized on the silanized U bend fibers to an OD of 2 at 530 nm (peak plasmonic wavelength of the gold nanoparticles).

C. Preparation of Molecularly Imprinted U Bend Optical Fibers

Gold nanoparticle-coated optical fibers were incubated in 10 mM allyl mercaptan (in ethanol) for 12 h to form $=CH_2$ group on the gold nanoparticles as per the protocol described in Matsui et al. [19]. After modification of the optical fibers with allyl mercaptan, the probes were dipped in a pre-polymerization solution consisting of the template, monomer, and cross-linker, mixed in a certain ratio.

TABLE II
TEMPLATE: MONOMER: CROSS-LINKER RATIOS FOR POLYMERIZATION

Method	Ratio	Monomer type	Template (mM)	Monomer (mM)	Cross-linker (mM)	Imprinting Factor
M1	1:3:5	Acrylonitrile	50	150	250	3
M2	1:4:20	Acrylonitrile	15	60	300	8.2
M3	1:4:20	Methacrylic acid	15	60	300	8
M4	1:12:20	Acrylonitrile	6.25	75	125	No MIF formation
M5	1:12:20	Acrylonitrile	12.5	150	250	9.8
M6	1:12:20	Methacrylic acid	12.5	150	250	9.4

It is important to maintain a certain ratio of template: monomer: cross-linker to achieve maximum sensitivity and selectivity. Different ratios of the polymerization ingredients (Table II) were mixed to form the imprinted layer on the gold nanoparticles. To prepare the pre-polymerization solution, first, the monomer acrylonitrile and template BPA were added to ethanol (in ratios indicated in Table II) and sonicated for 40 min.

This was followed by the addition of crosslinker EGDMA and polymerization initiator BPO to the above solution and sonication for 40 min. The optical fibers were dipped in the pre-polymerization solution and then heated at 75 °C to thermally initiate the process of polymerization. The polymerization process took around an hour to form a suspension of white polymer in the bulk. Because of the presence of $=CH_2$ group on the gold nanoparticles coated on the optical fiber, a thin layer of molecularly imprinted polymer was formed on the nanoparticles as well. The template was then removed from the imprinted polymer layer on the optical fiber via repeated washing in 4:1 methanol: acetic acid solution. The optical fibers were then dried overnight at

[1]Registered trademark.

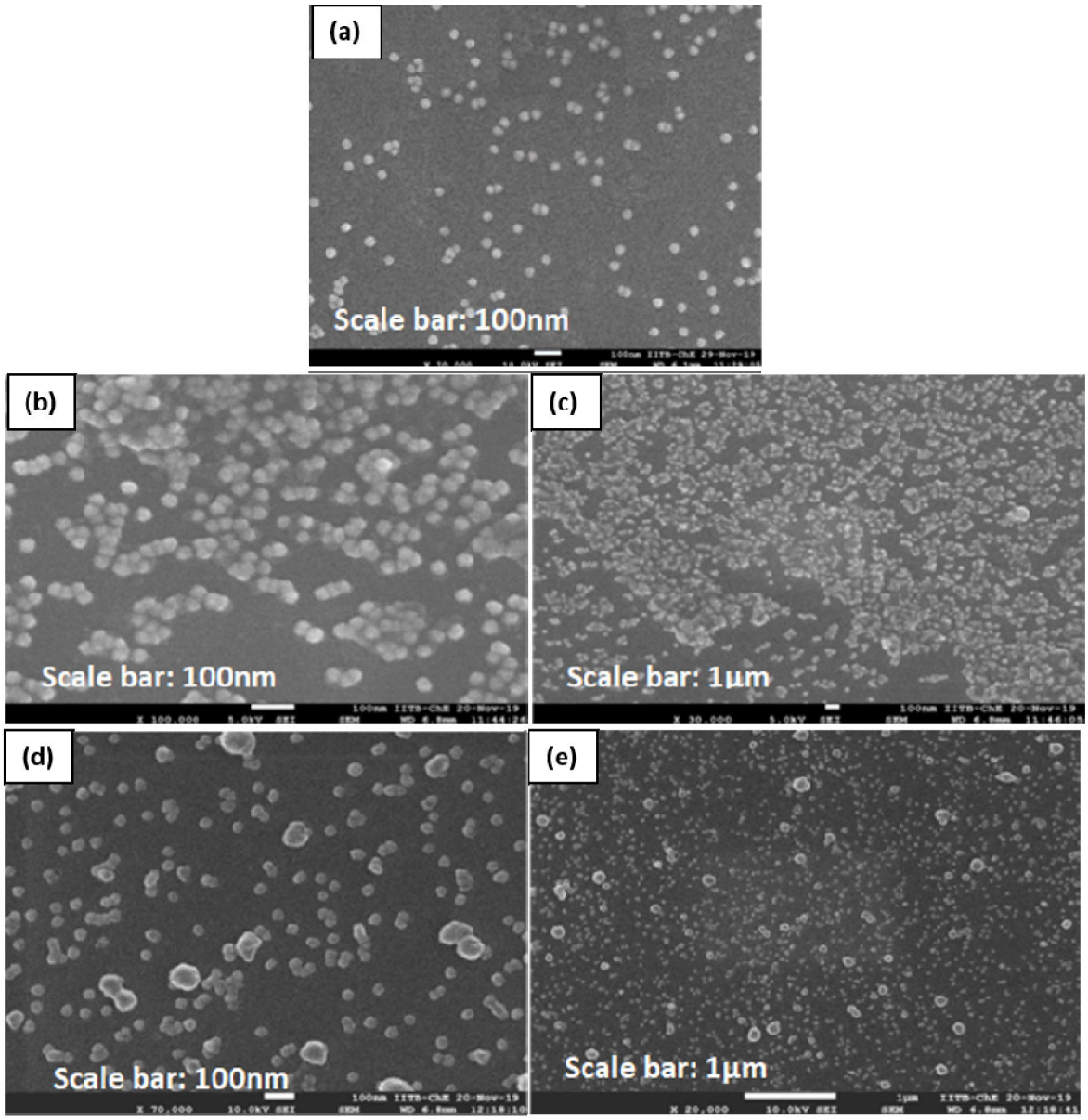

Fig. 2. (a) FEG-SEM image of gold nanoparticle coated U-bent optical fiber. (b) and (c) FEG-SEM image of a polymer layer on AuNP-coated U-bent optical fiber as per method M5. (d) and (e) FEG-SEM image of a polymer layer on gold nanoparticle-coated U-bent optical fiber as per method M4.

room temperature and used. Non imprinted polymer (NIP) coated U-bent optical fibers were also prepared in a similar way, without the template, and the results are demonstrated in the supplementary section, Fig. 1.

The MIP-modified probes were then tested for their response to BPA (in deionized water) in the optical setup as described in Section II-B. Optical fibers modified as per method M4 did not show any response to the analyte. However, the sensors prepared as per the other methods did show a response toward 10 μg/ml of BPA. An increase in absorbance with a maxima at 550 nm was observed.

To compare the performance of the six sensors (M1 to M6), an imprinting factor (IF) was defined as follows:

Imprinting factor = Response of MIP-coated sensor to 10 μg/ml of BPA/response of NIP-coated sensor to 10 μg/ml of BPA

BPA should ideally not bind to NIP coated sensor and produce no increase in absorbance. Hence, the higher the IF, the better the sensor. The IFs were calculated and the values are depicted in Table II. An increase in IF was observed as the concentration of the monomer was increased with respect to the template. The highest IF was observed for the ratio 1:12:20 for both acrylonitrile and MAA. Thus, method M5 was selected as the optimum protocol for the development of sensors for the detection of BPA.

III. Results and Discussions

A. Characterization

Gold nanoparticle-coated and molecularly imprinted optical fibers were analyzed with field emission gun scanning electron microscopy FEG-SEM (JEOL JSM 7600F) to confirm the formation of the imprinted film. Fig. 2(a) is an image of an AuNP-coated U-bent optical fiber sensor. Fig. 2(b)–(e) depicts the formation of imprinted film as per methods M5 and M4, respectively. As evident from the images, patches of polymer

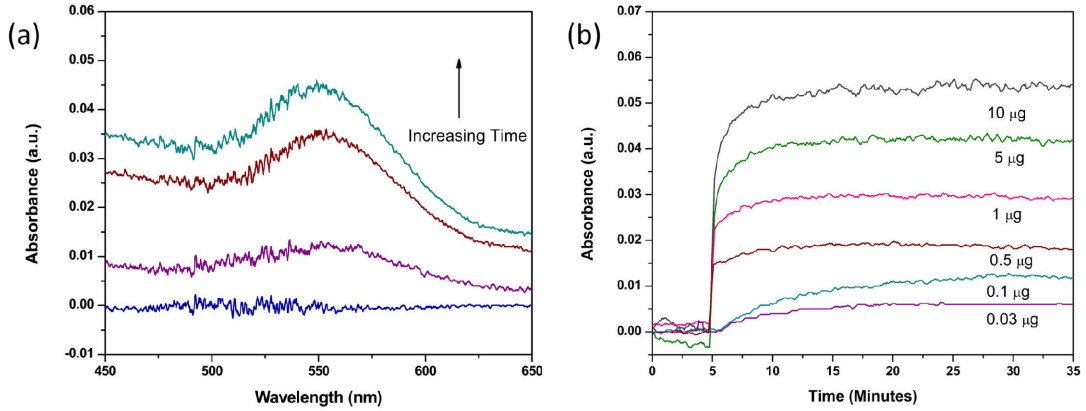

Fig. 3. (a) Change in absorbance spectrum of MIP modified probe when subjected to 5 μg/ml BPA. (b) Time-varying response of the sensor to different concentrations of BPA.

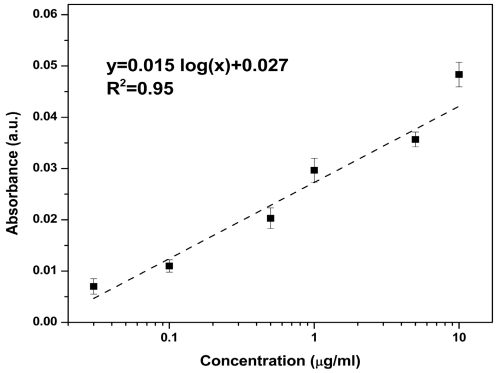

Fig. 4. Calibration curve for BPA spiked in deionized water. All readings were taken in triplicates and the error bar indicates standard error.

were formed in the case of M4 whereas a uniform layer of polymer was formed by M5.

B. Detection of BPA in Deionised Water

The MIP-modified optical fibers as per method M5 were tested for detection of the analyte BPA. The absorbance spectrum with a maxima at 550 nm is depicted in Fig. 3(a). The response of the sensor probes for different concentrations of BPA from 30 ng/ml to 10 μg/ml, spiked in deionized water, was recorded [Fig. 3(b)] and a linear calibration curve with R^2 = 0.95 was obtained (Fig. 4).

C. Selectivity of the Sensor

Sensor selectivity studies were performed with a few other small molecules likely to be present in real matrices, such as wastewater, fruit juices, or soft beverages. For this purpose, 10 μg/ml of 17β estradiol (E2), a hormone; P-NTP, a structurally similar molecule to BPA; AMP and CIP, two of the most commonly used antibiotics; AA, a small molecule present in fruit juices and soft drinks; and CPS, a pesticide, were chosen and the sensor response was recorded in triplicates. The results as depicted in Fig. 5(a), exhibit excellent selectivity of the sensor toward BPA.

D. Calibration for Detection of BPA in Aerated Soft Beverages

BPA is extensively used as a plasticizer for the production of polycarbonate plastics and resins. By coming into contact with BPA-containing materials, such as plastic bottles or resin lining in cans, BPA is introduced into tinned or bottled liquids. The sensor was tested for the detection of BPA in aerated soft drinks. For calibration of the sensor, soft drinks packaged in BPA-free bottles were used. A sample of the drink was first kept in the open for 30 min to bubble out the air. Different concentrations of BPA were then spiked and detection was carried out in triplicates for each concentration. The absorbance was found to increase due to the binding of BPA on plasmonic MIP on the optical fiber, with a peak at 550 nm. A linear calibration curve (in the logarithmic scale) was obtained in the range of 50 ng/mL to 50 μg/mL with an R^2 value of 0.96 [Fig. 5(b)].

E. Recovery Studies

Commercially produced bottled and canned apple juice, bottled and canned beer, bottled iced tea, and bottled water were commercially procured and used to evaluate the performance of the sensor in real analyte specimens. Three different sensors prepared as per the method M5, as illustrated earlier, were used to test each concentration of each specimen, and the relative standard deviations (RSDs) were noted. For fruit juice, beer, and tea, the calibration curve was obtained for aerated beverages, and for bottled water, the calibration curve obtained for deionized water was used for the recovery studies. In all cases, the recovery was within 15% while the RSD was also found to be less than 10%, as summarized in Table III. An overestimation was seen in the case of beer, possibly because of its 15% alcohol content. The results thus hold promise for the point-of-use deployability of our developed sensor for diverse alcoholic and nonalcoholic, canned and bottled beverages.

F. Discussion

A simple, effective method of synthesis of plasmonic core MIPs directly on optical fibers has been demonstrated in this study. Since the imprinted layer is directly formed on immobilized AuNPs, additional steps in biosensor design involving bioconjugation linker chemistries were reduced. Selection of the ratios of the template: monomer: cross-linker is an important optimization that is required to form a uniform

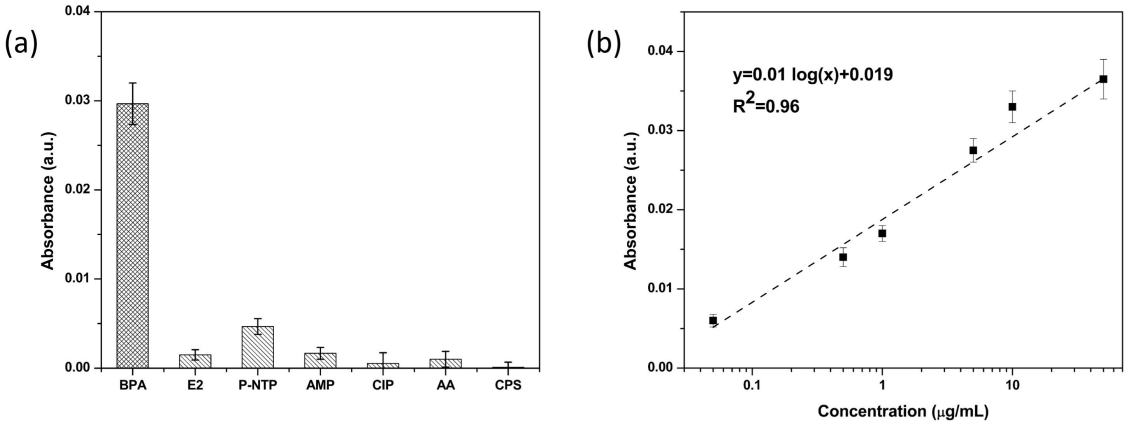

Fig. 5. (a) Selectivity analysis of the developed sensor to 17β estradiol (E2), P-NTP, AMP and CIP, AA, and CPS. 1 μg/mL of BPA was used while 10 μg/mL of all other reagents were used (b) calibration curve for BPA spiked in aerated soft beverage. All readings were taken in triplicates and the error bar indicates standard error.

TABLE III
RECOVERY STUDIES OF THE SENSOR

Sample		Spiked Concentration (μg/mL)	Measured Concentration (μg/mL)	Recovery (%)	RSD (%)
Fruit Juice	Bottled	1	0.91	91	7.69
	Canned	1	0.89	89	5.62
Beer	Bottled	1	1.10	110	9.11
	Canned	1	1.06	106	9.42
Tea	Bottled	1	0.89	89	7.86
	Bottled	10	8.5	85	9.41
Water	Bottled	1	0.95	95	4.21
	Bottled	10	9.3	93	4.30

MIP film and ensure detection of the analyte with minimum cross-sensitivity. An initial ratio of 1:3:5 yielded a poor IF of 3, which could be because of the lesser number of noncovalent interactions between the template and the monomer. Hence, increasing the template: monomer ratio to 1:4 and further to 1:12 led to a better IF. The concentration of the cross-linker also plays an important role in the formation of a MIP layer MIF. Excess crosslinker may make the polymer too rigid for molecules to fit in whereas too little cross-linker might make the polymer too flexible and thus, compromise specificity.

Hence, an optimized ratio of 1:12:20 was found to have the best imprinting factor. The concentration of the monomer and crosslinker in the pre-polymerization solution was also a deterministic factor in the formation of the MIF. Lesser concentrations of the polymerization precursors (as in M4) led to the formation of patchy polymer coats on the optical fiber, whereas, doubling the concentrations (as in M5) led to the formation of a uniform film. This could be because of the presence of a lesser number of monomer molecules and crosslinker to interact with allyl mercaptan on the AuNPs. Furthermore, acrylonitrile was found to produce a slightly better IF than MAA. A possible explanation for this could be the presence of a larger number of hydrogen bonding sites with the template in acrylonitrile and steric obstruction of the methyl groups in MAA. The sensors are demonstrated for robust use in diverse aqueous specimen matrices. Moreover, they exhibited minimal cross sensitivity toward another phenolic compound, such as P-NTP, and required no sophisticated storage requirements. No change in binding properties of the sensor was observed upto 50 days of storage at room temperature. The LoD in deionized water was found to be 30 ng/mL whereas in beverages, LoD was found to be slightly higher at 50 ng/mL. This is because the overall organic load in beverages is higher than in water which implies that the analyte of interest is deterred by a higher concentration gradient to reach the active sensor substrate. This leads to loss of sensitivity as well as LoD. The findings are similar to our earlier work [20]. There exists further scope for lowering the LoD. Possible avenues include optimization with a mixture of monomers to achieve more functional groups that are complementary to that of the template and further exploration in the combination of the template: monomer: cross-linker ratios.

IV. CONCLUSION

The current study demonstrates a novel in situ method of molecular imprinting on gold nanoparticle-modified U bend optical fibers for LSPR-based detection of BPA. This technology can easily be extended to EDCs in general. Molecular imprinted film was successfully formed on an allyl group modified gold nanoparticle-coated optical fiber sensor. A template: monomer: cross-linker ratio of 1:12:20 yielded the highest imprinting factor and was found to be the optimum ratio for MIP based sensor. The response of the sensor to BPA was found to be log linear over the range of 30 ng/ml to 10 μg/ml in water ($R^2 = 0.95$), with an experimentally established LOD of 30 ng/ml and in the range of 50 ng/ml to 50 μg/ml in aerated bottled soft drink ($R^2 = 0.96$). Thus, the sensor could be utilized for the measurement of BPA in canned and bottled beverages. The developed sensor is of utility in quality control for the measurement of BPA in bottled and canned beverages to decide on its suitability for human consumption.

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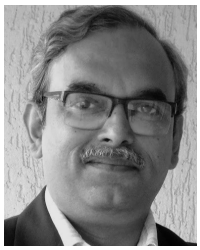

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