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Communication

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Spacer layer engineering for ultrasensitive Hg(II) detection on surface plasmon-coupled emission platform

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Abstract: In this work, we demonstrate for the first time the ultrasensitive detection of Hg^{2+} ions with femtomolar sensitivity in water samples with the use of the surface plasmon-coupled emission (SPCE) platform. The use of portable network diagnostic tools for water security and integrated water shed management is a topic of recent research interest. In this context, the current study explores Hg^{2+} monitoring using a rhodamine-6G (Rh6G) derivative bearing a monothiospirolactone mounted onto a SPCE substrate. Thus far, the limit of detection for mercury ions by the conventional fluorescence technique has been 0.15 nᴍ. However, we have achieved 1 fᴍ Hg^{2+} detection using silver nanoparticle-based spacer layer engineering on an SPCE sensor chip. Using this technology, a field device can be fabricated for rapid, ultrasensitive, multi-analyte detection (of contaminants) in water samples.

Keywords: fluorescence enhancements; mercury sensing; silver nanoparticles; spacer engineering; surface plasmon-coupled emission.

1 Introduction

Access to potable drinking water, essential for human sustenance, is a foremost concern in the 21st century. This situation has risen as a large extent of water resources is affected by geogenic and anthropogenic sources. Heavy metal ions in this context are particularly long-term hazardous toxicants on account of their high toxicity, retention, and accumulation in the human body. Their influence on the environment and nature is disparaging [1–3]. Among the transition and heavy metal ions, mercury contamination is of research interest due to its widespread contamination and its origin form: mining [4], waste incineration, fossil fuel combustion [5], Portland cement manufacturing, volcanic eruptions [6], and oceanic emissions [7], to name a few. Mercury is employed in several applications that include batteries, fluorescent light bulbs, compact fluorescents, thermometers, thermostats, amalgam in dental fillings, thimerosal in vaccines, and automotive switches. Mercury is known for its severe genotoxic, immunotoxic, and neurotoxic effects [8]. Organic mercury, especially, is highly soluble in water and passes easily through the biological membranes, causing damage to the central nervous and endocrine systems, and is found to be more toxic than the inorganic mercury salts [9]. This necessitates rapid and sensitive detection of mercury ions with a specific focus on data collection networks for an integrated water management approach.

There are different well-established protocols for mercury estimation: inductively coupled plasma spectroscopy, atomic absorption spectroscopy, molecular absorption spectroscopy, and electro-analytical techniques, to name a few [10]. However, these techniques involve significant sample preparation time scales, need cumbersome equipment, require trained personnel, and are financially heavy and not suitable for a resource-limited setting. In this regard, fluorescence spectroscopy offers an alternative and rapid method for metal ion screening. A variety of fluorescent chemosensors have been developed based on rhodamine derivatives [11–15], fluorescein derivatives [16–18], NO_2S_2-donor macrocycles [19], naphthalimide [20], oligonucleotides [21], genetically engineered cells [22], nanomaterials [23], foldamers [24], and DNAzymes

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[25]. Among the abovementioned chemosensors, rhodamine-based fluorescence detection has been preferred due to the well-understood photochemical processes, low cost, long-wavelength (>500 nm) absorption or emission, high molar absorption coefficient, and quantum yield of these derivatives. These optical sensors have an interesting metal ion response, a turn "on/off" behavior. In the absence of the metal ions, the molecule prefers the spirolactam ring closed, with little or no absorption in the visible region. However, in the presence of the metal ions, chelation occurs, resulting in ring opening and simultaneous creation of a highly absorbing, fluorescent complex.

Nevertheless, there are limitations in the conventional fluorescence technique, including low collection efficiency (<1%) arising due to its isotropic emission, interference by scattered light, photobleaching of the fluorophore, and a congested fluorescence emission spectrum on account of spectral broadening. In this context, surface plasmon-coupled emission (SPCE), as a technique that alters the behavior of excited fluorophores in proximity to nanometer-thick metal films, has gained importance. SPCE is a near-field phenomenon that results when a few-nanometer-thick layer containing radiating dipoles is positioned above a thin silver film (50 nm) that couples >50% of the fluorescence (1% in conventional fluorescence platform) into the surface plasmons. Due to the outcoupling conditions of the surface plasmons (electronic oscillations on metal thin films), the light passing through a transparent prism is polarized directional emission with significant background suppression [26, 27]. To augment the coupling of fluorescence, we have introduced an additional spacer layer sandwiched between the fluorophore and the silver thin film. Our spacer layer engineering has presented significant enhancements (in excess of thousand fold) in SPCE, with the use of hybrid spacers that include low-dimensional carbons, biomaterials, semiconductor nanoparticles, noble metal nanoparticles, and their composites [28–31]. These novel SPCE substrates have found convergent application in monitoring of $LpPLA_2$ (coronary heart disease marker at picomolar concentration), organic dye systems (at femtomolar concentration), and dopamine and DNA (at attomolar concentration) [28–34]. In the present study, we extend the capability of this platform for ultrasensitive monitoring of mercury ions in water.

2 Materials and methods

Rhodamine-6G (Rh6G) and polyvinyl alcohol (PVA) were purchased from Sigma Aldrich, St. Louis, MO, USA. $HgCl_2$, HPLC-grade acetonitrile, and tetrahydrofuran (THF) were procured from Merck Specialties Private Limited, Mumbai, Maharashtra, India. Thiourea, phosphorus oxychloride, and 1,2-dichloroethane were sourced from SD Fine Chemicals, Mumbai, Maharashtra, India. Silver nanoparticles (AgNPs, 20 nm) were acquired from BBI Solutions, Redding, CA, USA. Pyrex slides coated with a 50-nm-thick silver thin film and a 5-nm-thick silica top layer were purchased from EMF Corp., Ithaca, NY, USA. Millipore water (18 MΩ cm^{-1}) was used to prepare all the solutions for the current study.

2.1 Synthesis of the Rh6G derivative (Rh6G D)

A two-step preparation of the rhodamine derivative was carried out, as reported earlier [14]. In the first step, Rh6G (3 g, 6.3 mmol) was added to a solution of NaOH (4.8 g, 0.12 mol) in a mixture of ethanol (30 ml) and water (60 ml). The solution was refluxed for 12 h and then cooled to room temperature, followed by the evaporation of the ethanol in vacuum. The pH of the solution was adjusted to 6–7 using 2 M HCl. The precipitate obtained was filtered, washed several times with water, and dried to obtain A.

In the second step, to the stirred solution of A (2 g, 4.8 mmol) in 1,2-dichloroethane, 3 ml phosphorus oxychloride was added dropwise. The reaction mixture was refluxed for 4 h, followed by cooling and evaporation in vacuum. The crude acid chloride was then dissolved in 6 ml THF and added dropwise to a solution containing thiourea (1.52 g, 20 mmol), 12 ml triethyl amine in a mixture of 50 ml THF and 10 ml water at room temperature. The solvent was removed under reduced pressure after stirring the solution overnight. Water (50 ml) was added, and the precipitate formed was filtered, washed with water several times, and dried to obtain Rh6G D. The reaction scheme is presented in the Figure 1A and B illustrates the SPCE instrumental setup showing the following: A – 532 nm laser for excitation, B – aperture for adjustment, C – silver slide for mounting the sample, D – hemi-cylindrical prism, E – filter, and F – detector.

2.2 Fabrication of the SPCE substrate

An aqueous 40 mM stock solution of $HgCl_2$ was prepared initially. Aqueous solutions of various concentrations ranging from 400 nM to 4 fM were prepared by serial dilution. A 2% PVA aqueous solution was prepared by mild heating, and a 4 mM solution of Rh6G D was made using acetonitrile. Rh6G D (1 mM) in 1% PVA was equilibrated with different concentrations of mercury ions and

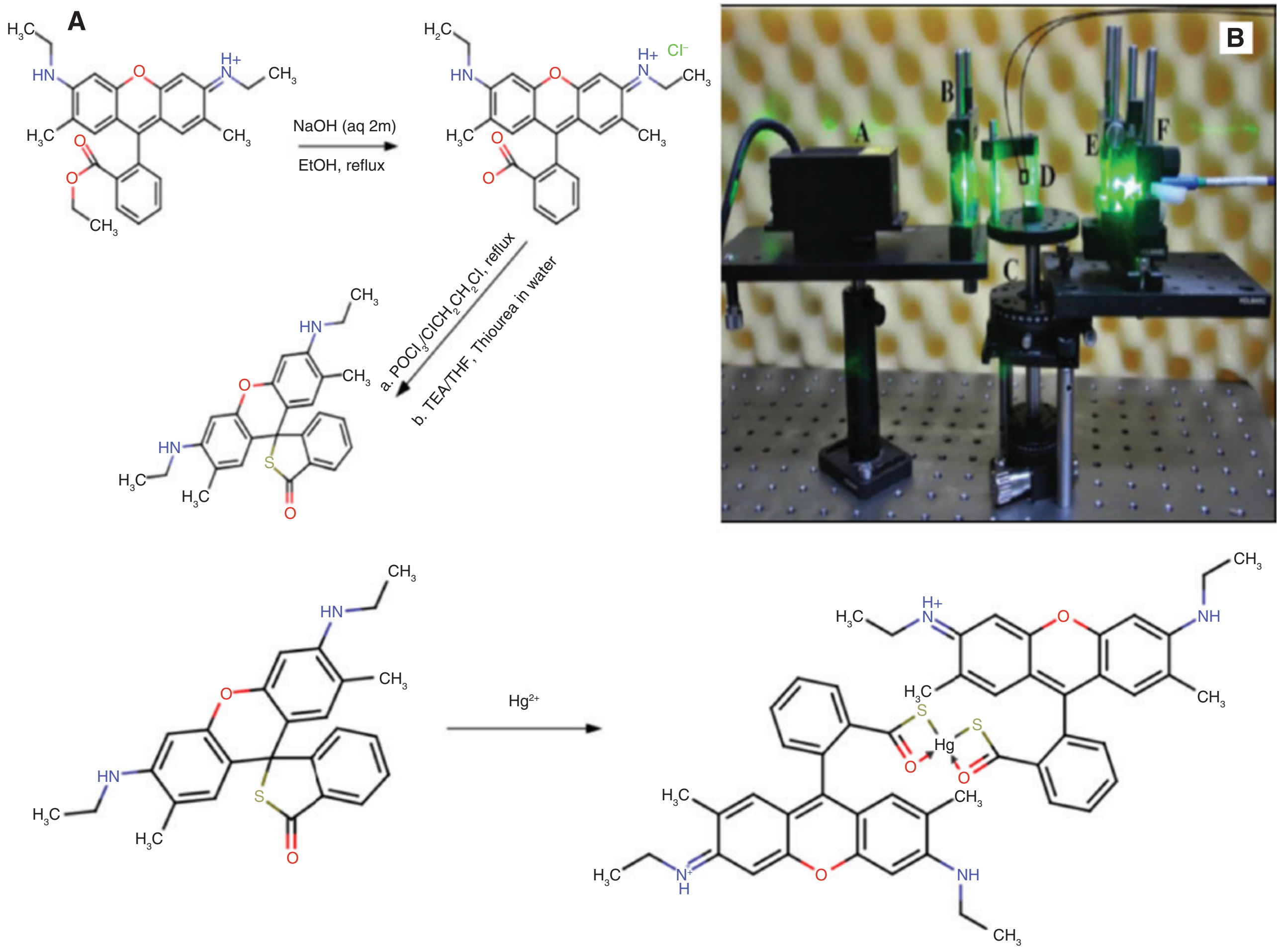

Figure 1: (A) Reaction scheme for the preparation of Rh6G D; (B) SPCE instrumental setup (A – 532 nm laser, B – aperture, C – silver slide, D – hemi-cylindrical prism, E – filter, and F – detector); and (C) Rh6G D-Hg^{2+} complex formation.

then spin coated onto the SPCE substrate. Spin coating was carried out on the silver slide at 3000 rpm for 60 s to obtain a 30-nm polymer overcoat. These substrates were attached to a BK7 hemi-cylindrical prism using an index matching fluid and mounted onto a rotating stage. The slides using AgNPs were also fabricated in the same manner, with a layer of AgNPs in PVA spin coated prior to spin coating the fluorophore.

3 Results and discussion

On the addition of Rh6G D to a solution containing Hg^{2+} ions, chelation of Hg^{2+} with the sulfur and oxygen atoms results in ring opening of the monothiospirolactone (in the derivative). The formation of the complex is due to the inherent affinity of Hg^{2+} toward sulfur [14]. The Rh6G D forms a 2:1 stoichiometric complex with the mercury ions,

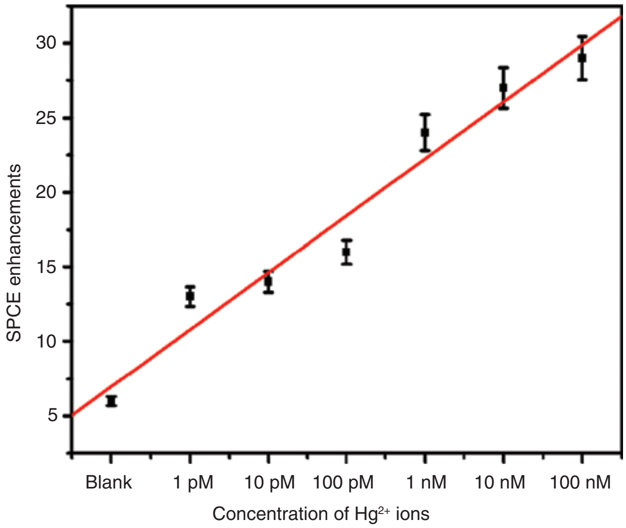

Figure 2: Concentration plot indicating SPCE enhancements for different Hg^{2+} concentrations.

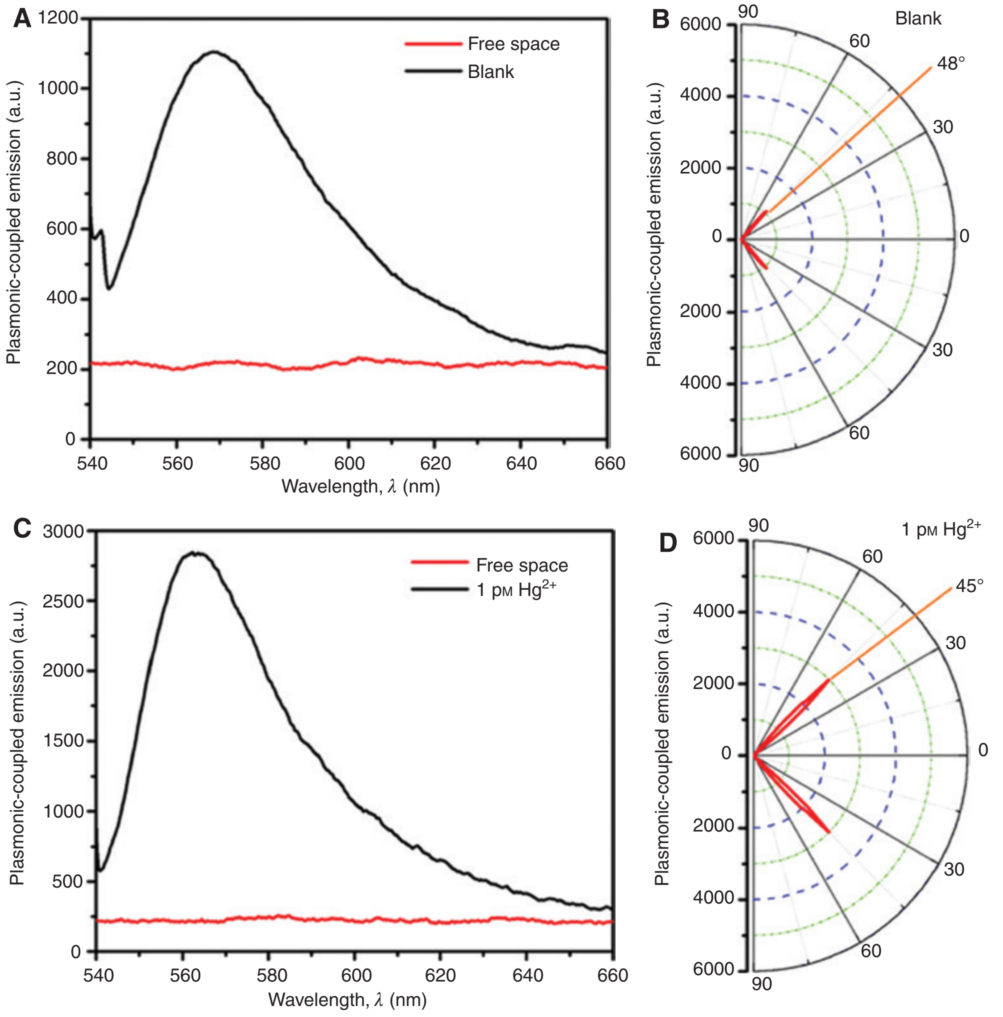

Figure 3: (A) SPCE and FS intensity of Rh6G D in the absence of Hg^{2+}; (C) SPCE and FS intensity of Rh6G D in the presence of Hg^{2+}; and SPCE radial plots for (B) blank and (D) 1 pм Hg^{2+}.

as depicted in Figure 1C. The Rh6G D in the presence of the Hg^{2+} ions consequently behaves as a fluorescent turn-on switch.

In a recent report, a limit of detection (LOD) of 0.15 nм Hg^{2+} ions was achieved using Rh6G D in conventional fluorescence spectroscopy [35]. However, in this study,

Table 1: SPCE enhancements and angularity obtained for different Hg^{2+}concentrations using Rh6G D (with and without an AgNP spacer layer).

Concentration of $Hg^{2}+$	Without AgNP spacer layer		With AgNP spacer layer	
	SPCE enhancements	Angle (°)	SPCE enhancements	Angle (°)
100 nм	35	45	82	55
10 nм	28	46	67	55
1 nм	26	44	64	56
100 pм	17	45	60	55
10 pм	15	46	58	55
1 pм	13	45	56	56
100 fм	–	–	45	55
10 fм	–	–	34	54
1 fм	–	–	20	55
Blank	6	48	10	50

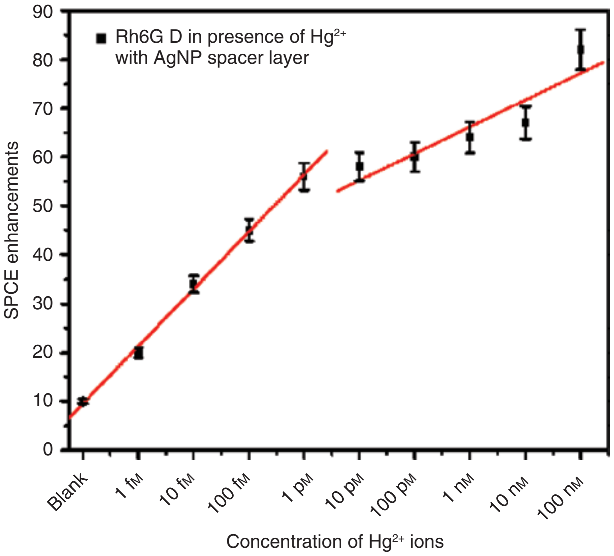

Figure 4: Concentration plot indicating AgNP spacer layer-dependent SPCE enhancements for different Hg^{2+} concentrations.

the LOD of 1 fM Hg^{2+} ions was achieved, highlighting the sensitivity of the SPCE platform in comparison with the conventional fluorescence technique. Two experiments were carried out as part of this study. Firstly, the detection of Hg^{2+} ions was studied using plain Rh6G D encapsulated in the polymer matrix, followed by the use of AgNPs as the spacer layer on the SPCE sensor chip for augmented SPCE enhancements.

In the first set of experiments, Hg^{2+} ions at 1 pM were detected with a 13-fold enhancement of fluorescence over the free space (FS) emission. A 35-fold enhancement was achieved in the presence of 100 nM concentration of Hg^{2+} over its FS emission. The fluorescence of Rh6G D in the absence of Hg^{2+} was about 6-fold higher compared to FS. Figure 2 presents the SPCE enhancements achieved for different concentrations of Hg^{2+} ranging from 100 nM to 1 pM. The fluorescence spectrum of Rh6G D in the absence of Hg^{2+} (blank) along with its FS spectrum have been plotted in Figure 3A, and B shows the SPCE angularity plot with

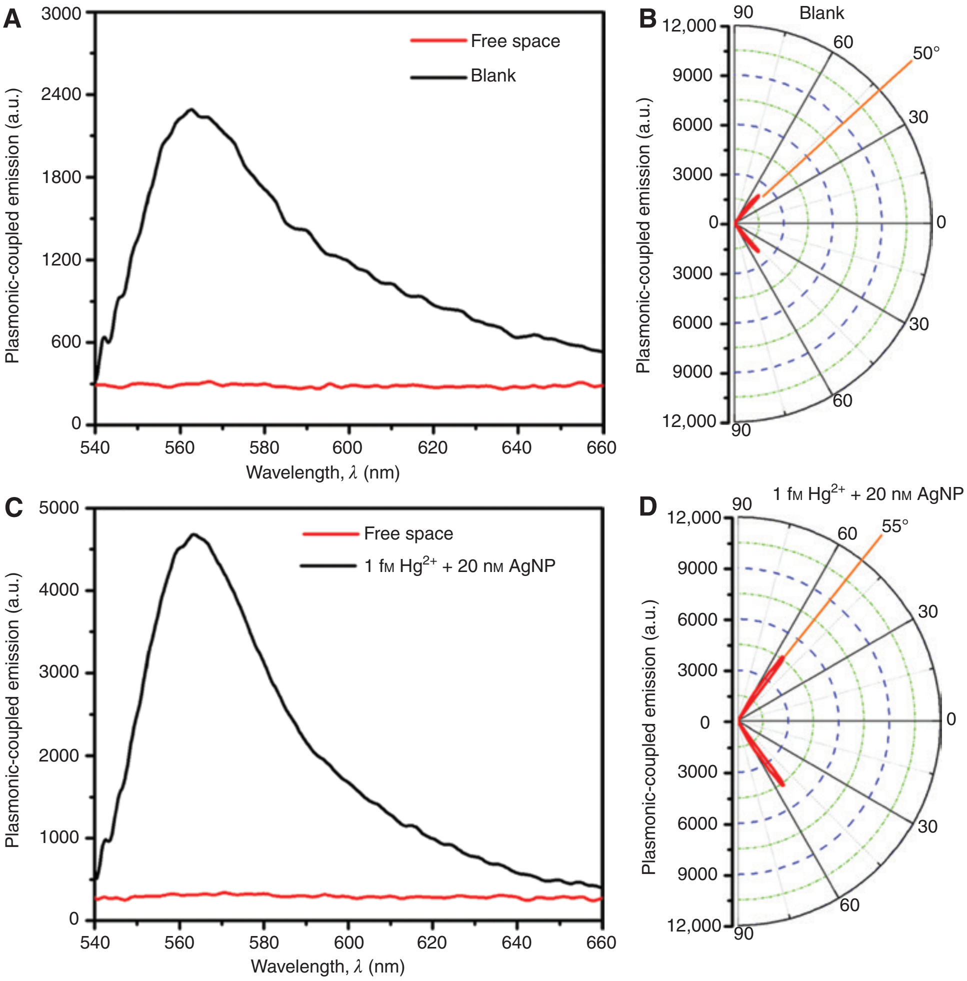

Figure 5: In the presence of the AgNP spacer layer, (A) SPCE and FS intensity of Rh6G D in the absence of Hg^{2+}; (C) SPCE and FS intensity of Rh6G D in the presence of Hg^{2+}; and SPCE radial plots for (B) blank and (D) 1 fM Hg^{2+}.

emission at 48°. Figure 3C shows the fluorescence spectrum of Rh6G D in the presence of Hg^{2+} (1 pм) along with its FS spectrum, and its SPCE angularity (45°) has been plotted in Figure 3D.

In the second set of experiments, an LOD of 1 fм for Hg^{2+} ions was achieved with the use of an AgNP spacer layer. The effect of AgNPs on the coupling efficiency of the radiating dipole with the surface plasmons has been well understood in earlier reports [33, 36]. In the current study, 20 nm AgNPs in 1% PVA were used as the spacer layer. Spacer engineering resulted in amplification of the SPCE enhancements as well as improved sensitivity for Hg^{2+} detection. The enhancements in the SPCE signal for both sets of experiments have been tabulated (Table 1). The SPCE enhancements for different concentrations of Hg^{2+} ranging from 100 nм to 1 fм in the presence of AgNPs have been plotted in Figure 4. The two slopes in this figure have been obtained for Hg^{2+} concentrations in two different ranges: lower (1 fм–1 pм) and higher (10 pм–100 nм). This particular occurrence is not uncommon, as it has already been reported in earlier studies pertaining to mercury determination with the use of glucose biosensors [37]. Figure 5A (blank) and Figure 5C (1 fм Hg^{2+}) present the fluorescence intensity plots obtained in the presence of the AgNP spacer. Figure 5B and D present the radial plots for blank and 1 fм concentration of Hg^{2+}. Further, the emission was significantly p-polarized in the SPCE region, 95% in the absence and 97% in the presence of AgNPs.

However, the localized surface plasmon resonance of the AgNPs (distributed uniformly throughout the sample matrix) helps in the effective coupling of photons from the radiating dipole with the silver thin film. This results in ultra-amplification of the fluorescence emission and significantly alters the LOD of Hg^{2+} ions as seen from the second set of experiments. Currently, studies are under way to analyze multiple analytes (contaminants) in water for comprehensive water quality monitoring, to be achieved with this plasmonic device.

4 Conclusions

Rapid and sensitive detection of mercury ions has a great impact on the management of community health and the environment on account of its ubiquitous presence and high toxicity. However, current fluorescence-based technologies have their limitations in sensitivity on account of poor signal collection efficiency. The use of the SPCE platform and an engineered sensor chip (spacer layer) in this context has significantly aided in the achievement of enhanced sensitivity, resulting in femtomolar detection of Hg^{2+}. The ability of AgNPs to increase the coupling efficiency of the fluorophore was utilized to achieve fluorescence enhancements in excess of 80-fold. We are in the process of prototyping a multi-analyte sensing portable device for rapid, low-cost online water monitoring and, in turn, realize an integrated water management approach.

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Conflict of interest: The authors declare no conflict of interest.

Supplementary data: The following is the supplementary data related to this article: additional information concerning the SPCE measurements and results are available as Supporting Information (SI).

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