INTRODUCTION

Potentiometric sensors constitute major category of electrochemical sensor and are commonly represented by ion-selective electrodes (ISEs), with typical commercial form as pH sensors or glass electrode apart from large variety of commercially available ISEs that may be helpful in direct determination of ion concentration in liquid samples of different nature. Potentiometric measurements with ISEs are performed versus some stable and well-defined reference electrode contacting the sample solution through a liquid junction. In ISEs, the signal is generated by charge separation at the interface between ion-selective membrane and the solution due to selective partitioning of ionic species between these two phases. In classical ISEs, the arrangement is symmetrical or unblocked configuration which means that the membrane separates two solutions, the test solution and the inner solution with constant concentration of ionic species. The electrical contact to an ISE is provided by a reference electrode (usually Ag/AgCl) in contact with the internal solution that contains
chloride ions at constant concentration. The measured ISE potential is the sum of the two reference electrode potentials, the membrane potential constituted by boundary potentials at each membrane/solution interface and a possible diffusion potential which may be caused by an ion concentration gradient within the ion-selective membrane phase. Such configuration of potentiometric sensors is configured as double-barrel configuration, and the ISE interface remained unblocked due to use of two internal filling solutions that severely restrict the practical usability especially to use only in upright position with further restriction concerning miniaturization of potentiometric sensors of particular concern. Such limitations have been searched for further improvement to further miniaturize sensors and improve their versatility and ruggedness for application outside the laboratory environment, and have led to the development of solid-contact sensors such as coated-wire electrodes (CWEs) and ion-selective field-effect transistors (ISFETs). In the 1970s, the CWE was introduced in which an ion-selective membrane was directly contacted with a metal wire (James, Carmack, & Freiser, 1972); thus, ISE interface remained blocked constituting the innovation on solid-state ISEs. ISEs with solid inner contact are considered as asymmetrical. Taking into consideration that potentials generated at each membrane interface are included into the overall sensor signal response, it is clear that to obtain a solid-contact ISE with a stable electrode potential, it is required to have a fast and thermodynamically reversible ion to electron transduction system that may fix the potential at the solid-contact/membrane interface (Bobacka, Ivaska, & Lewenstam, 2008). In case of all-solid-state electrodes based on mixed heavy metal/silver halides and/or chalcogenides, this ion to electron transduction is performed by an upper contact layer where silver salts and silver metal are mixed together (Vlasov & Bychkov, 1984). In case of polymer membrane electrodes, the intermediate transduction layer may be formed by electropolymerized conducting polymers, such as polypyrroles and polypiophenones, which are electroactive materials with mixed electronic and ionic conductivity as reported in our earlier disclosure (Pandey, Prakash, Srivastava, & Seth, 1998a, 1998b). Doped electropolymerized polymer membrane as solid contact between ion-sensing membrane and metal contact is susceptible to potential drift during subsequent measurement and required further improvement in membrane technology acting as bridge between metal and sensing membrane and is disclosed in these applications. The use of non-specific ion carrier-doped siloxane-polyindole nanodispersion undergoing self-assembly into thin film is disclosed as suitable membrane contact between metal contact and ion-sensing membrane (Pandey & Pandey, 2017). The electrical contact to an ISE is provided by a reference electrode (usually Ag/AgCl) in contact with the internal solution that contains chloride ions at constant concentration, thus again limiting usability of solid-state working ISE in real application and again requiring a solid contact in the form of suitable membrane and avoiding the need of internal solution that contains chloride ions at constant concentration. The present disclosure provides innovation on developing novel thin films, one acting as solid contact between metal and PVC master membrane to develop solid-state working ISE and other film acting as protecting layer over reference electrode to maintain constant potential difference across reference electrode to yield all-solid-state potentiometric sensors and disclose in current disclosure with further advancement over earlier report (Pandey et al., 1998a, 1998b).

Electrolytes are important for maintaining cell signalling, kidney function, homeostasis, and body fluid balance. Electrolyte levels can fluctuate due to exercise, diet, disease, poisoning, and organ failure, making their monitoring invaluable for healthcare assessment. Solid-state ion-selective electrodes (ISE) that incorporate H+ ionophores and solvent polymeric membranes have been shown to be efficacious for metal cation determination (Bakker, Bühlmann, & Pretsch, 1997). In contrast to liquid-based ISEs, solid-state ISEs require less maintenance and are compatible with microfabrication and array construction methods. Bühlmann and Stein introduced the use of 3D macroporous carbon electrodes, which were prepared from colloidal sphere templating (3DOM), as a solid contact for PVC-doped valinomycin-sensing membrane-based K+ detection (Lai, Fierke, Stein, & Bühlman, 2007). The crucial point in designing a solid-state ISE is innovation in solid contact that subsequently controls the referencing parameter for reliable potentiometric sensing. Accordingly, we have designed a solid contact derived from hydrophobic-doped polyindole/polycarbazole membrane (Pandey et al., 1998a, 1998b) for assembling valinomycin-impregnated PVC matrix membrane suitable to detect potassium ion in fluid. The subsequent improvement was made extending the ionophore to develop urea biosensor (Pandey et al., 1998b). The innovation in the device is in developing solid contact, which was achieved through electropolymerization of indole/carbazole monomer thus restricting the site for solid-state contact development and was limited to only specified electrode surface. Further, the membrane was macroporous causing the leaching out of the dopant/ion carrier from the membrane matrix that subsequently restricted the practical implementation and subsequent commercialization of as-made ISE. Accordingly, there is a need of a soluble polymeric nanodispersion having potentiality for (a) assembling over any substrate for yielding thin mesoporous/nanoporous membrane under ambient conditions; (b) compatibility of polymeric nanodispersion with suitable ion carrier that subsequently allows encapsulation of the same within assembled thin membrane and (c) hydrophobicity of resulting membrane from polymeric nanodispersion for subsequent casting PVC matrix membrane. Fortunately, we have recently developed a nanodispersion in volatile organic solvent as siloxane-polyindole-gold nanoparticle (Pandey & Pandey, 2017) that undergoes self-assembly as superhydrophobic surface meeting all criteria for yielding the required membrane for designing working ISE of practical significance and is reported in this article.

Potentiometric sensing involves the participation of a working electrode referred as ion-selective electrode (ISE) together with a reference electrode, and the potentiometric signal with respect to reference electrode is monitored to realize the selective sensing of targeted ions. One of the requirements for a reference electrode is of an internal filling solution and also a double junction to exclude the contribution of ions present in internal filling solution containing anions especially
chloride ion when Ag/AgCl referencing is used. Accordingly, designing of a solid-state potentiometric sensor for a cation again requires a suitable reference electrode made without internal filling solution to exclude the need of two junctions during referencing to yield potentiometric signal proportional to the concentration of desired ions for which sensor is designed. To meet this requirement, a nanostructure thin membrane derived from functional alkoxysilanes impregnated with p-toluenesulfonyl chloride assembled over silver/silver chloride reference electrode has been disclosed. In addition, the sol–gel film may further be modified with chloro-functional alkoxysilane to yield all-solid-state reference electrode required for potentiometric operation and is reported in this invention.

Wearable sensors are a new paradigm in point-of-care (POC) diagnostic, allowing for minimally invasive monitoring of physiological functions and elimination of biological fluid transfer between patient and device; these devices are capable of providing real-time analysis of a patient’s condition. Novel epidermal electronic devices consisting of printed flexible circuits that can be stretched and bent to mimic skin elasticity can perform electrophysiological measurements such as measuring temperature and hydration as well as monitoring electrical signals from brain and muscle activity (Kim et al., 2011). Temporary tattoos integrated with screen-printed electrodes directly attached to the skin were recently reported to measure lactate through sweat (Jia et al., 2013). Microneedle-enabled analysis systems are capable of minimally invasive interrogation due to their ability to puncture the skin’s stratum corneum and access interstitial fluid while not interacting with deeper layers of the skin, which contains tissues that are associated with pain, blood flow, or sensation (El-Laboudi, Oliver, Cass, & Johnston, 2013). For example, glass microneedle arrays were used to create pores in the skin in order to extract interstitial fluid by means of a vacuum bell jar for glucose detection with commercially available glucose strips (Wang, Cornwell, & Prausnitz, 2005). A strong correlation was shown between intravenous glucose concentrations and those within dermal tissue. In another study, the surfaces of solid gold microneedles were functionalized with antibodies, which were used to collect non-structural protein-1 (an early marker for dengue virus infection) in mice. When inserted in the skin, the functionalized microneedles bound the protein and remained attached; once the needles were removed from the animal, further ex vivo analysis was performed (Muller, Corrie, Coffey, Young, & Kendall, 2012). Several examples involving microneedles and electrochemical detection have also been recently reported, including packing of hollow microneedles with enzyme-filled carbon pastes to amperometrically detect glucose or glutamate (Windmiller et al., 2011) and use of a multiplexed microneedle device to simultaneously measure glucose, lactate, and pH (Miller et al., 2012). Recently, we have disclosed invention on microneedle arrays for biosensor and drug delivery system (Wang et al., 2017) in which each microneedle includes a protruded needle structure including an exterior wall forming a hollow interior and an opening at terminal end of the protruded needle structure that exposes the hollow interior and a probe inside the exterior wall to interact with one or more chemical or biological substances that come in contact with probe via the opening to produce a probe signal, an array of wires that are coupled to probe of array of hollowed needle, respectively, each wire being electrically conducting to transmit the probe sensing signal produced by a respective probe and the process on biosensing through amperometric sensing comprising of working electrode, counter electrode, and reference electrode connected through screen-printed electrode coupled to electrochemical processing unit. In this case, either drug delivery could be directly achieved or the analyte may be sensed through polarizing the working electrode and monitoring the oxidation/reduction of analyte at the surface of working electrode. The present research is intended to develop potentiometric sensor for sensing potassium ion in biological fluid analogous to that reported earlier (Pandey et al., 1998a, 1998b) with further advancement in designing microneedle-based sensor assembly where hollow microneedles are assembled/fixed over flexible/rigid printed electrode covered with suitable polymer coating having ablation for casting sensing membrane over printed electrode and other polymer ablation for converting the printed electrode into reference electrode that may be explored as wearable sensors. The present disclosure provides the development of flexible printed electrodes in pair where one of them may be converted into working solid-state ISE and other into solid-state Ag/AgCl reference electrode through the use of two solid membrane contacts derived from tetraphenylborate-containing siloxane-polyindole-gold nanoparticle nanodispersion for working ISE and p-toluenesulfonyl chloride-impregnated organically modified silicate membrane for reference electrode. The use of functional alkoxysilanes as solid contact may further be manipulated to control the property of working ISE and also reference electrode. For example, 3-chloropropyltriethoxysilane may further be used to modify the sol–gel film fixed over Ag/AgCl reference electrode to further improve the referencing potential during potentiometric ion sensing. In addition, the printed electrode may be configured into microneedle assembly as disclosed in US Patent 9743870B2, 2017, most suitable for transdermal ion sensing.

2 | EXPERIMENTAL

2.1 | Chemical and instruments

For all experiments, analytical grade chemicals and doubly distilled water were used. The membrane matrix high-molecular-weight polyvinylchloride (PVC; n = 1,100); the anionic additive potassium tetrakis (4-chlorophenyl)borate (KTPcB); the plasticizers 2-nitrophenyl-octyl ether (o-NPOE), triis ethylhexylphosphate (TEHP), bis(2-ethylhexyl) adipate (DOA), dioctylphthalate (DOP) and bis (2 ethylhexyl) sebacate (DOS); valinomycin; and the solvent THF were obtained from Sigma-Aldrich Co. Keithley Multimeter (model 2000) connected to a PC was used for conventional potentiometric measurements.
2.2 | Formation of all-solid-state potentiometric sensors

All-solid-state reference electrode can be made on any conductive solid surface. We used screen-printed electrode (SPE) in two-electrode configuration as disclosed in Indian Patent application 201811035930. The typical process for the fabrication of SPE consists of five stencils of specially designed geometry as shown in Figure 1, made up of cloth made from polyester or stainless steel cloths of mesh size 160/360. The screen printer used for printing conducting ink using these screen are indigenous screen printer. The conducting ink was printed using these stencils fixed in screen printer using squeeze and flood bar at appropriate pressure with complete alignment printing exposure superimposing on each other. The first printer is made with metal ink silver mainly over electrical connection followed by graphite ink leading to track 1 and track 2 as shown in Figure 1. Track 2 is screen-printed with Ag/AgCl ink. The next printing is carried out by masking inks made from dielectric ink with specially designed stencil to leaving hole A and hole B as shown in Figure 1. Track 1 having Ag/AgCl with hole B is used for making all-solid-state reference electrode, and track 2 with hole A is used for casting all-solid-state working electrode. The hole B on track 1 with Ag/AgCl is further modified with a sol–gel suspension 3. The suspension 3 is made by mixing five different components: (a) methanolic solution of 3-aminopropyltrimethoxysilane, (b) methanolic solution of 2-(3,4-epoxycyclohexyl) ethyltrimethoxysilane, (c) PVA solution in DD water, (d) methanolic solution of p-toluenesulfonfyl chloride and (e) 0.1 N HCl. These components are homogenized by ultrasonication, and 20-50 µl of the homogeneous suspension is allowed through drop-casting over Ag/AgCl layer followed

FIGURE 1 Schematic diagram showing the fabrication of screen-printed electrode
by drying for appropriate time to yield thin film of organically modified silicate. A ethanol solution of 3-chloropropyltriethoxysilane was drop-cast over organically modified silicate layer followed by controlled drying that finally yields the fabrication of all-solid-state reference electrode. Hole A on track 2 is initially modified with a thin film made from suspension 1. The suspension is made in three steps: (a) 3-APTMS solution in acetone using either chloroform or acetone as solvent was stirred continuously for 6-12 hr at room temperature. The colourless solution turns to yellow indicating the polymer synthesis. To this medium, tetrachloroauric acid was added and left for 4-5 hr that allows the formation of siloxane–gold nanoparticles; (b) indole monomer was dissolved in acetone (25 mM) and mixed with 25 mM solution of HAuCl₄ in acetone. The mixture is then stirred continuously for over 2-6 hr to yield self-assembled siloxane–polyindole–gold nanoparticles. (c) the equal fraction of siloxane–gold nanoparticles made as described in (a) or (b) was mixed with polyindole–gold nanoparticles as described in section (c) and stirred for few minutes. The suspension is then left undisturbed for over 2-6 hr. The appearance of brown-red colour indicated the formation of polyindole-AuNP suspension. 50 µl of 1 mg/ml solution of tetraphenylborate was added in 200 µl of siloxane–polyindole–gold nanoparticle suspension and homogenized through sonication leading to the formation of suspension 1. 20-50 µl of this suspension was drop-cast over the conductive track of hole A and allowed to dry for 2-3 hr resulting in the formation of membrane 1 acting as solid-state contact between conductive metal layer and PVC master membrane. Suspension 2 forming PVC master membrane was made as followed; the solution of PVC casting membrane was made in dried THF of the composition: PVC fine powder (56 mg); valinomycin (1 mg); 1-(2-nitrophenoxy)octane (0.12 ml); potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (0.75 mg); and THF (2 ml). After complete dissolution of the membrane material, 50 µl of the solution was added to hole A over membrane-1. The solvent (THF) was allowed to evaporate slowly over a 12-20-hr period at room temperature under controlled drying condition. This ISE has been tested for the potassium ion in the range of the typical concentrations, that is 1 × 10⁻⁶ to 5 × 10⁻¹ M, and was found to be very stable for several sets of measurements. The printed electrode made from hole A and hole B is now ready for potentiometric sensing of potassium ion in the given samples. This ion sensor may further be convertible into wearable potentiometric device by fixing microneedle assembly designed and developed at North Carolina State University through 3D printing technology. Microneedle includes a protruded needle structure including an exterior wall forming a hollow interior and an opening at terminal end of the protruded needle structure that exposes the hollow interior and a probe inside the exterior wall to interact with one or more chemical or biological substances that come in contact with probe via the opening to produce a probe signal.

2.3 | Potentiometric measurement

The screen-printed electrode is dipped in 0.05 M Tris-HCl buffer pH 7.2 for potentiometric measurement. The potentiometric sensor made in two-electrode configuration of screen-printed electrodes is dipped in an electrochemical cell as disclosed in Indian Patent (Pandey, 2018). The output from two-electrode configuration of potentiometric sensor was recorded with Keithley Multimeter 2000 connected to a PC. Varying concentration of KCl was added, and new steady-state potential after reaching from each addition is recorded and plotted as shown in Figure 2a. A plot of potential versus log[C] of potassium ion was used to construct a calibration plot for sensing potassium ion (Figure 2b).

3 | RESULTS AND DISCUSSION

3.1 | Evaluation of all-solid-state potentiometric sensors

All-solid-state potentiometric sensor is made from screen-printing microfabrication technology. Screen-printed electrodes as shown
in Figure 1 are printed in two-electrode configuration consisting of two conducting tracks: (a) graphite-conducting electrode and (b) Ag/AgCl electrode. Graphite-conducting track is converted into working electrode made from ionophore-impregnated PVC matric membrane, whereas the Ag/AgCl electrode is converted into solid-state reference electrode avoiding the need of an internal filling solution which is one of important additions of present contribution. In order to eliminate the need of internal reference solution, it is necessary to achieve constant dipolar potential across the reference electrode. To meet this requirement, a membrane containing p-toluenesulfonyl chloride was cast on Ag/AgCl electrode from silicate precursors as suspension 3 made from five components: (a) methanolic solution of 3-aminopropyltrimethoxysilane, (b) methanolic solution of 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane, (c) PVA solution in DD water, (d) methanolic solution of p-toluenesulfonyl chloride and (e) 0.1 N HCl. The silicate precursors allow to form of p-toluenesulfonyl chloride-impregnated thin membrane capable of maintaining constant dipolar potential. In order to further improve the constant recovery of dipolar potential, an ethanolic solution of 3-chloropropyltriethoxysilane was drop-cast over organically modified silicate layer followed by controlled drying that finally yields the fabrication of all-solid-state reference electrode.

Graphite-conducting electrode of Figure 1 is converted into working electrode as ISE based on blocked interface meeting the geometry of coated-wire electrode (James, Carmack, & Freiser, 1972). Such ISE again requires the existence of constant dipolar potential across the membrane/solid-state contact. In order to meet this requirement, an ion-exchange membrane made from suspension-1 is cast on graphite-conducting electrode acting as bridge between ion-sensing membrane and solid-state graphite-conducting electrode. Suspension 1 is made from a siloxane polymer–polyindole–gold nanoparticle sol in acetone. Non-specific ion exchanger like sodium tetrathylborate is added into suspension 1 and drop-cast to yield a non-specific ion exchange membrane that may act as bridge between graphite electrode and ion-sensing membrane impregnated with valinomycin to yield constant dipole across the ion-sensing membrane. The PVC membrane casting solution is made by dissolving PVC powder in THF followed by adding plasticizer, non-specific ion exchanger, potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, and specific ion carrier, valinomycin, and drop-cast over membrane made from siloxane polymer composite. After careful drying and incubating on aqueous solution containing potassium ion, all-solid-state potassium ion sensor is obtained for potentiometric potassium ion sensing and the sensitivity of the same may be calculated from Nernst equation:

\[
\text{EMF} = \text{E}_0 + \text{E}_J + S \log a_i
\]

where

\[
\begin{align*}
\text{EMF} &= \text{electromotive force generated by the difference of membrane potential} \\
\text{E}_0 &= \text{standard potential (mV)} \\
\text{E}_J &= \text{liquid-junction potential (mV)} \\
S &= \text{Nernstian slope (59.16}/z_i\text{ mV/decade change in concentration for H}_2\text{O at 25°C)} \\
z_i &= \text{charge number of ion } i \\
a_i &= \text{activity of ion } i \text{ in the sample solution (mol/L).}
\end{align*}
\]

3.2 | Response of potassium ion sensor

Potentiometric response as a function of potassium ion concentrations is obtained as shown in Figure 2. The response time of the sensor is very fast to the order of <10 s. A linear relation between EMF and Log [K+] is recorded. The response display linear relation between 0.5 and 10 mM potassium ion as shown in Figure 2b. The finding shown in Figure 2 reveals excellent performance of all-solid-state potassium ion sensor for practical application. The reproducibility of potentiometric sensor has been recorded. The sensor responded >95% initial response for subsequent 20 measurements justifying excellent practical usability of as-made hydrophobic ion-exchange film that maintains
constant dipolar potential across membrane–solution interface and solid-state reference electrode junction in the absence of internal filling solutions in each case.

3.3 | Clinical trials

Blood samples from 25 patients collected at pathological laboratories for routine analysis were used for sensing potassium ions. After potassium content in serum samples was determined by both the potassium sensors presented in this paper and the routine hospital electrolyte analyser, the results were analysed and compared as shown in Figure 3a. Excellent correlation coefficient justifies that testing results by the potassium sensors and the routine hospital electrolyte analyser are in agreement.

3.4 | Stability

The long-term stability of the potentiometric sensor was evaluated by continuously measuring freshly prepared potassium ion solutions with different concentrations (1, 2, 5 and 9 mM) for 50 days. The potassium ISEs were stored in a dry state at room temperature. The sensitivity (slope of the calibration curve) was calculated according to three assay results every day. The long-term stability of the sensors is illustrated in Figure 3b. The variation of the slope of calibration plots is <2.8 mV/decade over 50 days, showing fairly good stability for a typical set of ISEs.

3.5 | Application in transdermal ion sensing

The screen-printed electrodes based on all-solid-state sensor can be transformed into a microneedle-assembled probe having great potentiality for transdermal sensing. Biocompatible material of class 2a commonly used for hearing aid implants previously demonstrated the compatibility of this material with two-photon lithography as well as evaluated growth of human epidermal keratinocytes and human epidermal fibroblasts on this material. Integration of the microneedle with a solid-state potassium ion sensor was achieved by writing a hollow microneedle onto a removable substrate which could fit within a predefined recess on the solid-state sensor shown in Figure 4. A LCD-based potentiometric device has been developed dedicated to potentiometric operation in body fluid making the system portable for biomedical applications. A detailed investigation of transdermal sensing is underway.

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4 | CONCLUSION

The article describes the fabrication of all-solid-state potentiometric sensor avoiding the need of an internal filling solutions requires to achieve constant dipolar potential during potentiometric operation. This is the first demonstration of all-solid-state ion sensor that exhibited a detection range from $10^{-5}$ to $10^{-2}$ M with a near Nernstian slope of 57.5 mV per decade and rapid stabilization (~10 s) most suitable for analysing potassium ion in body fluid. All-solid-state ISE can be further assembled with microneedle converting the device into a transdermal devices with dedicated electronics. The finding demonstrated innovative design at first stage. The future improvement innovating programmed potentiometric recording along with further improvement in microneedle-assembled sensing of serum electrolyte is underway that will lead the use of current technology for routine clinical application.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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