Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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High throughput molecularly imprinted polymers based electrochemical nanosensors for point-of-care diagnostics of COVID-19

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ABSTRACT

The importance of early diagnosis of infectious disease has been revealed well by the COVID-19 pandemic. The current methods for testing SARS-CoV-2 mainly utilize biorecognition elements. The process of production of these biorecognition elements is not only tedious, time-consuming but also costly. The molecularly imprinted polymers recently have gained considerable attention as they are stable and also offer high selectivity and specificity than conventional labels. The present review discussed the MIPs-based electrochemical nano-sensors diagnostic of SARS-CoV-2.

1. Introduction

The importance of cost-effective, portable, rapid and specific diagnostic devices for detection of infectious agents has well proven in the current COVID-19 pandemic. The Severe Acute Respiratory Syndrome Virus (SARS-CoV-2) hailed from the beta coronavirus family comprises of 4 structural and 16 non-structural proteins in addition to positive-strand RNA genome [1,2]. The Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the main diagnostic modality for COVID-19 [3]. The delay in testing results, need for sophisticated instruments and skilled personnel using conventional techniques such as RT-PCR along with hurdles in demand and supply of diagnostics for COVID-19 created havoc in the current pandemic. The Point-of-Care Testing (POCT) devices have recently been shown great importance in the timely detection of infectious agents in patients [4,5]. The fabrication of these POCT devices utilizes mainly the biorecognition elements (BREs) which are costly to produce and limits sensor shelf life [6]. To overcome these limitations, recent advancement in material science with respect to polymer technology has led to the development of highly selective Molecularly Imprinted Polymers (MIPs). MIPs structurally mimics the antibody and are hence also termed plastic antibodies [7,8]. The mixture of functional monomer, cross-linkers and initiators along with suitable template in appropriate solvent led to the synthesis of MIPs. After synthesis, removal of the template from MIP leaves behind the template-specific binding sites which enable selective binding of the analyte [9]. The integration of Molecularly Imprinting Technology (MIT) with electrochemical technique is considered as a valuable approach for effective sensing purposes [10]. The MIP-based electrochemical nanosensors are based on the principle of measuring the charge transfer passed by a redox probe *via* thin MIP film. Electropolymerization allows controlled and rapid deposition of polymer films with tunable thickness [8]. MIP-based electrochemical detection of COVID-19 is illustrated in Fig. 1

The present review discussed the currently available MIPs-based electrochemical nanosensors for the detection of SARS-CoV-2. Besides, a brief insight on synthesis and advantages of MIPs over conventional labels has also been discussed. This review would enable researchers about the novel strategies for the design and fabrication of next-generation diagnostic devices.

2. MIPs advantages over conventional labels

Many (BRESs) like DNA, RNA, aptamers, enzymes are used to detect the analyte by binding specifically to the target analyte. There are certain drawbacks associated with the usage of these BRESs, which include instability for a long time, high cost, storage at a specific temperature, limited production at a time etc. This raises the demand for the artificial recognition element which can mimic the BRESs and can

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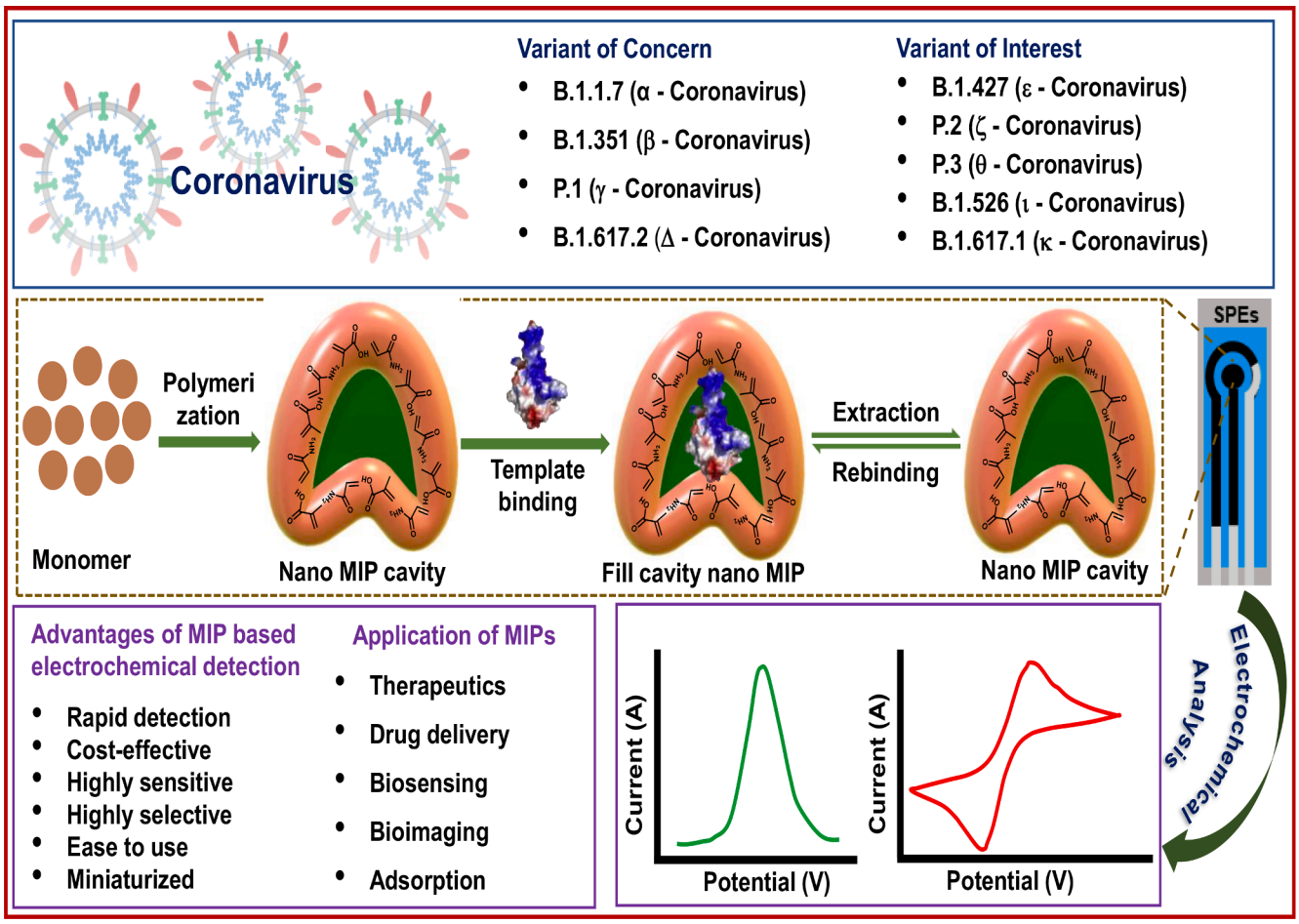

Fig. 1. Schematic representation of MIP-based detection of SARS-CoV-2.

selectively detect the analyte. [11,12]. The main properties of MIPs and the various templates used for MIPs along with the published literature are depicted in Fig. 2.

3. MIP-based electrochemical nanosensors for detection of SARS-CoV-2

Recently, developed MIPs for SARS-CoV-2 recognition and binding have shown potential for wide applications, including fabrication of MIPs-based sensors. The MIP-based antibody represents high selectivity towards the receptor binding domain (RBD) of SARS-CoV-2 even in the presence of the RBD of similar coronavirus [12]. Therefore, it can be potentially exploit for fabrication of MIP-based sensors.

The SARS-CoV-2 specific nanoMIP was prepared as an alternative for natural antibodies, which can detect the RBD sensitively and selectively. The synthesis is represented in Fig. 3 **(I),** dot blot showed the selectivity of nanoMIP towards real virus detection. Fig. 3 **(II) (A)**, showing the positive control of SARS-CoV-2 spike protein, **(B)** and **(C)** displayed viral particles of SARS-CoV-2, **(D)** showing negative control of viral culture media only and **(E)** displays the reference control of conjugated polymer nanoparticles. Dot blot further demonstrated that nanoMIP can selectively detect SARS-CoV- 2. The nanoMIP offers LOD as low as 5 fg/mL and is also found to be stable at high temperatures. Using these nanoMIPs, the next generation electrode can be synthesized and can also utilize for Internet-of-Things and POCT diagnostics [13].

For instance, a more sensitive, portable electrochemical sensor was developed to detect the SARS-CoV-2 nucleoprotein (ncovNP). The sensor was fabricated onto the gold-based thin-film electrode (Au-TFE). The Au-TFE was further modified by 4-aminothiophenol (4-ATP), the cleavable linker layer was prepared using 3,3′ -dithiobis [sulfosuccinimidyl propionate] (DTSSP). The ncovNP immobilization was done by drop-casting ncovNP on the cleavable linker modified electrode. Further, the m-phenylenediamine was electrochemically deposited on the electrode. The imprints on the surface of the electropolymerized electrode were done using 2-mercaptoethanol (2-ME) which enables the release of ncovNP by leaving the imprints behind.

The Fig. 3 **(III)** represents the calibration plot of the developed sensor at the concentration range of 2–111 fM in lysis buffer (LB). The inset figure shown in Fig. 3 **(III)** depicts the DPV curve used as base to construct the calibration plot. The LOD & LOQ were found to be 15 and 50 fM respectively. As shown in Fig. 3 **(IV)**, the developed sensor showed many folds higher response against the ncovNP than the other proteins present. The calibration of the developed sensor was done with spiked samples of known concentration. The calibration plot is presented in Fig. 3 **(V)**, showing the linear concentration of ncovNP in the range of 0.22 to 333 fM. With the average data of 4 patients, the LOD and LOQ was found to be 27 fM and 90 fM respectively. The data demonstrates that the sample with value more than 0.22 fM will be considered as Covid-19 positive. Furthermore, the cross-selectivity and selectivity of the sensor for ncovNP against S1(a subunit of spike

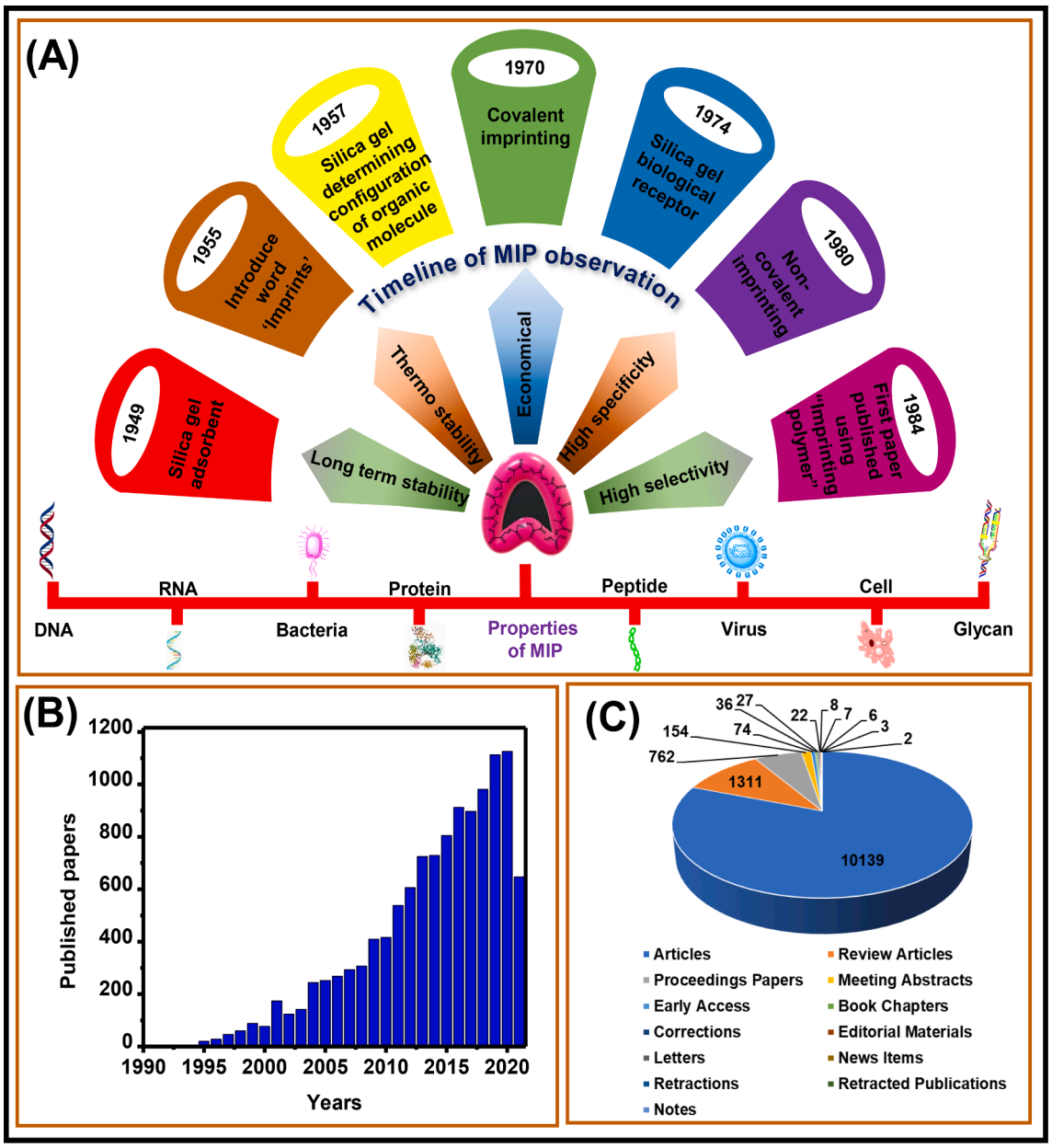

Fig. 2. Representation of (A) various templates, properties along with the early history of MIPs. (B) and (C) number of year-wise published literature and its types [Data was obtained from "Web of Science" with "Molecularly Imprinted Polymer" entered as "Subject" in the search box (Last access date: 10.08.2021)]

protein) were shown in Fig. 3 **(VI)**. The experimental data indicated that the developed sensor could be used for the analysis of the real sample where all other interfering proteins will be present [14]. Some recent work on MIPs based electrochemical nano-sensors for the detection of various viruses is described in Table 1.

4. Current challenges and future perspectives

Despite all the other advantages mentioned above, MIPs have some limitations which include the re-agglomeration of nanomaterials, the effect of external factors on its synthesis [20]. Certain limitations can be improved using computational designing and smart pre-analysis [21]. Although, MIT is a very well-known technology, still commercialization is a major concern. Further, integration of MIP-based electrochemical sensors with Internet-of-Thing could pave a way to tackle the shortage of diagnostic devices in the current situation of high demand. In the future, we look forward to the commercialized MIP-based devices not only for viral detection but in many other fields.

5. Concluding remarks

The huge demand of affordable, highly specific and sensitive POC devices for early detection of infection can be suffices using MIP-based electrochemical sensors in the current COVID-19 pandemic. This review discusses about the advantage of MIPs over conventional labels. The unique properties of MIPs for SARS-CoV-2 makes them undoubtable

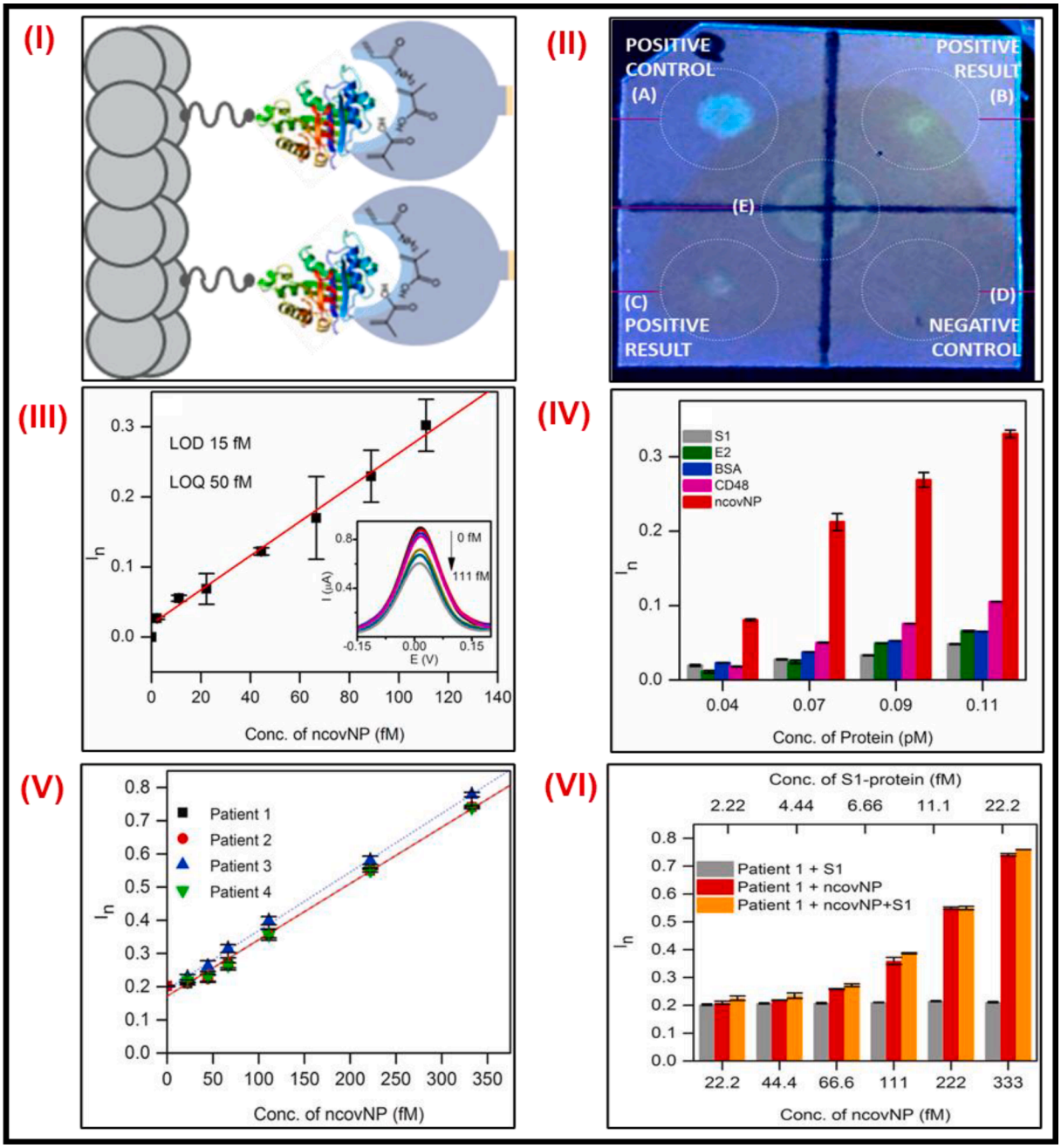

Fig. 3. **(I)** Nano MIP imprinting demonstrating the polymerisation step of the production process. **(II)** Dot blot demonstrating live virus detection. **(III)** Calibration plot of ncovNP sensor obtained at the low concentration range of ncovNP (2–111 fM) in LB. The inset shows typical DPV curves used to construct the calibration plot. **(IV)** Selectivity test of ncovNP sensor showing its responses against the different proteins (S1, E2 HCV, BSA, CD48 and ncovNP) **(V)** The calibration plots of ncovNP sensors obtained against COVID-19 negative samples in UTM, 20-fold diluted with LB and spiked with 22.2, 44.4, 66.6, 111, 222, 333 fM of ncovNP. **(VI)** Cross-selectivity test of ncovNP sensor against S1, and mixture of ncovNP and S1 protein in COVID-19 negative sample in UTM 20-fold diluted with LB. Reprinted with permission [13,14].

Table 1
MIPs based electrochemical nano-sensors for the detection of SARS-CoV-2 and other viruses.

Analyte (virus)	Sensing Platform	Monomer	Template	Polymerization Techniques	Electrochemical Techniques	Sample	Linearity	LOD	References
SARS-CoV-2	Au-TEF/ ncovNP-MIP	Phenylenediamine	ncovNP	Electropolymerization	DPV	Nasopharyngeal swab	2.22–111 fM	15 fM	[14]
HIV-p24	GCE/ MIPs/ MWCNTs	Acrylamide	HIV-p24	Surface polymerization	DPV	Serum	1.0×10^{-4} to 2 ng cm^{-3}	0.083 pg cm^{-3}	[15]
Zika	SPE/GO-Au/SIP	Acrylamide Methacrylic acid Methyl methacrylate N-vinylpyrrolidone	Zika Viral RNA	Self-polymerization	CV	Serum	10^{-3} to 10^{2} PFU mL^{-1}	2×10^{-3}PFU mL^{-1}	[16]
HIV-1	GCE/ MIP/ CNF-Bi	Pyrrole	NBD-556@gp120	Electropolymerization	DPV	Plasma	0.002 to 0.05 ng mL^{-1}	0.0003 ng mL^{-1}	[17]
Dengue	SPCE/PS/ MIP	Dopamine	Dengue NS1 protein	Self-polymerization	EIS	Serum	1 to 200 ng mL^{-1}	0.3 ng mL^{-1}	[18]
Dengue	SPCE/ MIP	4-Aminophenol	Dengue NS1 protein	Electropolymerization	EIS	Serum	50 to 200 μgL^{-1}	29.3 µg L^{-1}	[19]

*Glassy Carbon Electrode- GCE, Multi Walled Carbon Nanotubes- MWCNTs, Screen Printed Electrode- SPE, Graphene Oxide- GO, Suface Imprinted Polymer- SIP, Carbon Nanofragment and Bismuth Oxides-CNF-Bi, Polysulfone -PS, SPCE- Screen-Printed Carbon Electrodes

useful material for fabrication of next generation POCT sensors for detection of SARS-CoV-2.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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