



(51) International Patent Classification:

G01N 33/00 (2006.01) B01J 20/281 (2006.01)

B01J 20/26 (2006.01) B32B 3/30 (2006.01)

B01J 20/28 (2006.01)

(21) International Application Number:

PCT/SG2017/050340

(22) International Filing Date:

05 July 2017 (05.07.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10201701587P 28 February 2017 (28.02.2017) SG

(71) Applicant: NATIONAL UNIVERSITY OF SINGAPORE [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(72) Inventors: LI, Fong Yau, Sam; c/o National University of Singapore, Faculty of Science, Department of Chemistry, 21 Lower Kent Ridge Road, Singapore 119077 (SG). LIN, Xuanhao; c/o National University of Singapore, Faculty of

Science, Department of Chemistry, 21 Lower Kent Ridge Road, Singapore 119077 (SG). LI, Haiyan; c/o National University of Singapore, Faculty of Science, Department of Chemistry, 21 Lower Kent Ridge Road, Singapore 119077 (SG).

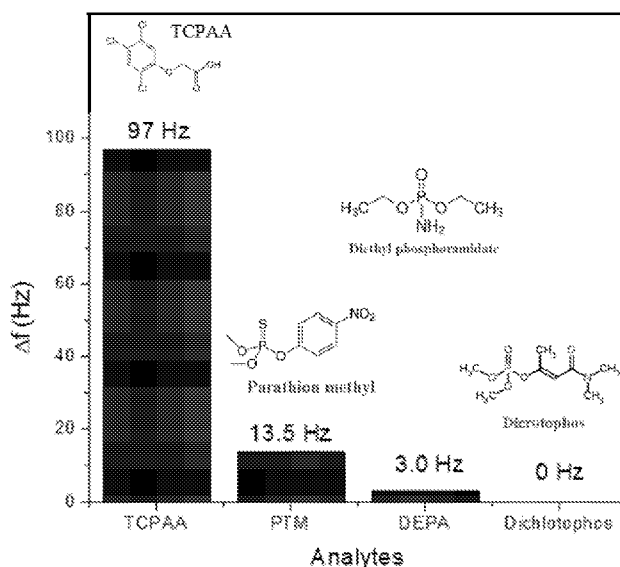
(74) Agent: PATEL, Upasana; Marks & Clerk Singapore LLP, Tanjong Pagar, P O Box 636, Singapore 910816 (SG).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: A METHOD OF MAKING A MOLECULARLY IMPRINTED POLYMER SENSOR

Figure 9



PDA/TCPAA 4/1
Bake 0.5 h
Soak 14 h

All analytes in ~ 10 μM
(PCTAA 13.3 μM, PTM 9.9 μM, DEPA 10.0 μM, Dichlorophos 10.0 μM)

(57) Abstract: There is provided a method of making a molecularly imprinted polymer (MIP) sensor comprising: preparing a MIP solution; providing the MIP solution onto a surface of a sensing substrate to form a MIP film; exposing the MIP film to a gas to initiate gas phase polymerisation of the hydrophilic monomer host comprised in the MIP film; annealing the MIP film; and removing template molecules comprised in the MIP film, wherein removing comprises extracting the template molecules from the MIP film to form cavities in the MIP film. There is also provided a MIP sensor made from the method of the present invention, as well as a method for detecting and/or quantifying a target molecule using the MIP sensor.



TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

A method of making a molecularly imprinted polymer sensor

Technical Field

The present invention relates to a method of making a molecularly imprinted polymer sensor and a method for detecting and/or quantifying a target molecule using the
5 molecularly imprinted polymer sensor.

Background

Currently, molecularly imprinting is through an in-situ but diffusion-dependent inclusion of template molecules into polymers during polymerisation. During the polymerization
10 process, the dissolved monomers become solid phase polymers while the template molecules are in aqueous solution (liquid phase). Template molecules in the liquid phase may have difficulty to diffuse and be entrapped into the solid polymer phase. Accordingly, many template molecules may still remain in the solution while only a few of them are in the matrix of polymer. This limits the available imprinted molecular
15 cavities in the prepared MIP sensor.

There is therefore a need for an improved method of making a molecularly imprinted polymer sensor.

Summary of the invention

The present invention seeks to address these problems, and/or to provide an improved
20 method of making a molecularly imprinted hydrophilic polymer sensor.

In general terms, the invention relates to a method of making a molecularly imprinted hydrophilic polymer sensor which is highly selective to hydrophilic and acidic analytes. The hydrophilic polymer-based molecularly imprinted polymer sensor may include some non-covalent interactions, electrostatic interactions such as ion-ion, ion-dipole
25 and/or dipole-dipole interactions, hydrogen bonding and π - π interactions between the hydrophilic polymer comprised in the sensor and the target molecules. The advantage of the molecularly imprinted hydrophilic polymer sensor made from the method of the present invention is that it has good selectivity, reliability and high sensitivity to the target molecules. Further, the molecularly imprinted hydrophilic polymer sensor made
30 from the method of the present invention also allows for fast detection of the target molecules. The method is also a low-cost and simple preparation method for making the molecularly imprinted hydrophilic polymer sensor.

According to a first aspect, the present invention provides a method of making a molecularly imprinted polymer (MIP) sensor comprising:

- preparing a molecularly imprinted polymer solution comprising a hydrophilic monomer host, template molecules and a first solvent, wherein the template molecules are hydrophilic and/or acidic and the first solvent is water or a non-aqueous solvent;
- providing the molecularly imprinted polymer solution onto a surface of a sensing substrate to form a molecularly imprinted polymer film;
- exposing the MIP film to a gas to initiate gas phase polymerisation of the hydrophilic monomer host;
- annealing the MIP film; and
- removing the template molecules from the MIP film, wherein the removing comprises extracting the template molecules from the MIP film using a second solvent to form cavities in the MIP film, wherein the monomer host when polymerised is insoluble in the second solvent, and wherein the template molecule is soluble in the second solvent.

The hydrophilic monomer host may be any suitable hydrophilic monomer. For example, the monomer host may comprise one or more monomers or a combination of a monomer with other co-monomers. For example, the monomer host may comprise a monomer with a functional group selected from the group comprising, but not limited to, amine, imine, amide, carboxyl, hydroxyl, carbonyl and thiol. According to a particular aspect, the hydrophilic monomer host may comprise dopamine (DA). According to another particular aspect, the hydrophilic monomer host may comprise dopamine (DA) and its co-monomer. For example, the co-monomer may be diethyl phosphoramidate (DEPA).

The template molecule may be any suitable hydrophilic and/or acidic template molecule. For example, the template molecule may be selected from the group consisting of, but not limited to: 2,4,5-trichlorophenoxyacetic acid (TCPAA), 2-(2,4,5-trichlorophenoxy)propionic acid, 2,4-dichlorophenoxyacetic acid, 2-(2,4-dichlorophenoxy)propionic acid, 4-(2,4-dichlorophenoxy)butyric acid, 4-chlorophenoxyacetic acid, Fenoxaprop-P, Fluazifop, Fluazifop-P-butyl, Haloxyfop, 2-methyl-4-chlorophenoxyacetic acid (MCPA), Mecoprop, 4-(4-chloro-2-

5 methylphenoxy)butanoic acid (MCPB), Fluroxypyr, Trichlopyr, 2-naphthoxyacetic acid, bromoxynil, loxynil, clopyralid, dicamba, imazapyr, imazaquin, imazethapyr, quinmerac, picloram, bentozone, fomesafen, imazosulfuron, trifensulfuron-methyl, metsulfuron-methyl, metosulam, cycloxydim, fludioxonil, and combinations thereof. According to a particular aspect, the template molecule may be TCPAA.

10 The first solvent may be water or any suitable non-aqueous solvent for the purposes of the present invention. For example, the first solvent may be, but not limited to, water, methanol, ethanol, 1-propanol, 2-propanol, n-butanol, 2-butanol, 2-methyl-2-propanol, 2-methyl-1-propanol, 1-pentanol, isomers thereof, dimethylformamide (DMF), dimethylacetamide (DMAc), tetrahydrofuran (THF), methyl ethyl ketone (MEK), acetonitrile, tetramethyl urea, dimethyl sulfoxide (DMSO), butanone, trimethyl phosphate, or a combination thereof.

15 The sensing substrate may be any suitable sensing substrate for the purposes of the present invention. For example, the sensing substrate may be one which is capable of indicating changes in at least one of: resistance, capacitance, mass, colour, resonance frequency and surface plasmon resonance shift. In particular, the sensing substrate may indicate changes in mass.

20 According to a particular aspect, the MIP film formed during the providing may comprise a self-assembled film of the hydrophilic monomer host and the template molecules. In particular, the MIP film formed during the providing may be a dry film. The MIP film formed may have a suitable thickness. For example, the thickness of the MIP film formed may be 0.01-10 μm .

25 The exposing may be under any suitable conditions. For example, the exposing may be carried out at a suitable temperature and for a pre-determined period of time. According to a particular aspect, the exposing may be at a temperature $\leq 90^\circ\text{C}$. The gas to which the MIP film is exposed to during the exposing may be any suitable gas for the purposes of the present invention. In particular, the gas may be any suitable gas to initiate polymerisation of the hydrophilic monomer host. Even more in particular, the gas may be ammonia vapour or amine vapour. The ammonia vapour or amine vapour
30 may be from any suitable source.

The annealing may be under any suitable conditions. For example, the annealing may comprise annealing the MIP film for a pre-determined period of time at a pre-determined temperature. According to a particular aspect, the pre-determined period of time may be ≥ 5 minutes. According to a particular aspect, the pre-determined
5 temperature may be $\geq 35^{\circ}\text{C}$. In particular, the pre-determined temperature may be 110°C and the pre-determined period of time may be 72 hours.

The removing may be under any suitable conditions for the purposes of the present invention. The second solvent used in the removing may be any suitable solvent. For example, the second solvent may comprise an alcohol, a liquid organic acid, a
10 surfactant, DI water or the like. In particular, the second solvent may comprise a solvent selected from the group consisting of, but not limited to: methanol, dilute acetic acid, sodium dodecyl sulphate, DI water, and a combination thereof.

According to a particular aspect, the method may further comprise:

- drying the MIP film following the providing;
- 15 - rinsing the MIP sensor following the removing; and/or
- drying the sensor following the rinsing.

The drying following the providing and the drying following the rinsing may be performed under any suitable conditions. For example, the drying may comprise air-drying or nitrogen-drying. The drying may be at a suitable temperature, such as $\leq 60^{\circ}\text{C}$.
20 In particular, the drying may be at room temperature.

The rinsing following the removing may be by using any suitable solvent. According to a particular aspect, the rinsing may be by using deionised water.

According to a second aspect, the present invention provides a molecularly imprinted polymer (MIP) sensor made according to the first aspect. In particular, the MIP sensor
25 may be suitable a molecularly imprinted hydrophilic polymer sensor suitable for detecting and/or quantifying the presence of hydrophilic and/or acidic target molecules.

According to a third aspect of the present invention, there is provided a method for detecting and/or quantifying a target molecule using the molecularly imprinted polymer sensor. The method comprises:

- exposing the molecularly imprinted polymer sensor to a sample of fluid containing or thought to contain the target molecule, thereby allowing the target molecule, if present, to be received within cavities of the sensor; and
 - detecting the presence of and/or quantifying the amount of the target molecule bound to the cavities of the sensor using electrochemical, acoustical, spectroscopic, optical or indirect chromatographic techniques,
- wherein the target molecule is a hydrophilic and/or acidic molecule.

Brief Description of the Drawings

In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary embodiments, the description being with reference to the accompanying illustrative drawings. In the drawings:

Figure 1A shows the non-covalent interactions between dopamine and TCPAA molecules and Figure 1B shows the non-covalent interactions between dopamine cations and TCPAA anions;

Figure 2 shows the general principle of dopamine polymerisation;

Figure 3 shows the response of non-imprinted PDA polymer (NIP) sensor to 3.40ppm TCPAA. F3 represents the 3rd overtone frequency of the NIP sensor and D3 represents the 3rd overtone dissipation of the NIP sensor. F3 was used for the ΔF calculation;

Figure 4 shows the response of PDA MIP sensor with 25% imprinted TCPAA molecular cavities when contacted with 3.40ppm TCPAA for 70 minutes. F3 represents the 3rd overtone frequency of the MIP sensor (PDA/TCPAA=4/1) which was used for the ΔF calculation;

Figure 5 shows the low response (line F3) of PDA MIP sensor when the annealing was before the exposing but not after the exposing;

Figures 6 shows the low response (line F3) of PDA MIP sensor when surface ammonia after ammonia exposure process was washed away by DI water;

Figure 7 shows the low response (line F3) of PDA MIP sensor when the sensor was made from simultaneous polymerization and template inclusion in aqueous solution;

5 Figure 8 shows the low response (line F3) of PDA MIP sensor when DEPA was used as template and target molecules;

Figure 9 shows the selectivity of the PDA MIP sensor to different analytes;

Figure 10A shows interactions between PDA chains and TCPAA target molecules and Figure 10B shows interactions between PDA and TCPAA analyte anions;

10 Figure 11 shows the sensitivity of the PDA MIP sensor; and

Figure 12 shows the response of PDA/PDEPA co-polymer based MIP sensor to 3.0 ppm 4-CPA at (a) Day 1 and (b) Day 7.

Detailed Description

As explained above, there is a need for an improved method of making a molecularly
15 imprinted hydrophilic polymer sensor.

The present invention provides a method of making a hydrophilic polymer-based sensor. In particular, the sensor made from the method of the present invention is a molecularly imprinted hydrophilic polymer sensor which shows good response in detecting hydrophilic analytes. Further, the sensor made also exhibits a fast response
20 time in detecting target analytes, a low limit of detection, which is comparable to limits of detection using other sensors which may be more difficult to make or which require a longer time to detect the analytes. It is also more cost-effective to make the sensor of the present invention, as well as using a scalable and a reproducible method since the method is based on molecular imprinting.

25 In particular, the method of the present invention provides a pre-assembly approach to allow template molecules and monomers to interact with each other for a sufficient period of time and lock such interactions in a dry film before polymerization to maximize the inclusion of the template molecules within the film and align the template

molecules well with the monomers so that the imprinted molecular cavities are more precise. The method also provides for a gas phase post-polymerization of the dry film of the template molecules and monomers so that the molecular pre-assembly is disturbed during the polymerization process. In this way, the maximum cavities may be achieved from the template molecules, thereby forming a better sensor.

Molecular imprinting is a technique to produce molecule specific receptors analogous to those receptor binding sites in biochemical systems. A molecularly imprinted polymer (MIP) is a polymer that is formed in the presence of a template or target analyte molecule producing a complementary cavity that is left behind in the MIP when the template is removed. The MIP demonstrates affinity for the original template molecule over other related and analogous molecules.

According to a first aspect, the present invention provides a method of making a molecularly imprinted polymer (MIP) sensor comprising:

- preparing a molecularly imprinted polymer solution comprising a hydrophilic monomer host, template molecules and a first solvent, wherein the template molecules are hydrophilic and/or acidic and the first solvent is water or a non-aqueous solvent;
- providing the MIP solution onto a surface of a sensing substrate to form a MIP film;
- exposing the MIP film to a gas to initiate gas phase polymerisation of the hydrophilic monomer host;
- annealing the MIP film; and
- removing the template molecules from the MIP film, wherein the removing comprises extracting the template molecules from the MIP film using a second solvent to form cavities in the MIP film, wherein the monomer host when polymerised is insoluble in the second solvent, and wherein the template molecule is soluble in the second solvent.

For the purposes of the present invention, hydrophilic monomer host may comprise a hydrophilic monomer unit which is capable of polymerising into a hydrophilic polymer. The hydrophilic monomer host may comprise co-monomers. According to a particular

aspect, the hydrophilic monomer may be a combination of a monomer unit and a co-monomer unit.

The hydrophilic monomer host may be any suitable hydrophilic monomer. For example, the hydrophilic monomer host may comprise one or more monomers, a co-monomer, or a combination of a monomer with other co-monomers. For example, the monomer host may comprise a monomer with a functional group selected from the group comprising, but not limited to, amine, imine, amide, carboxyl, hydroxyl, carbonyl and thiol.

According to a particular aspect, the hydrophilic monomer host comprises dopamine (DA). According to another particular aspect, the hydrophilic monomer host comprises dopamine (DA) and its co-monomer. For example, the co-monomer may be diethyl phosphoramidate (DEPA).

The template molecule may be any suitable hydrophilic and/or acidic template molecule. For example, the template molecule may be selected from the group consisting of, but not limited to: 2,4,5-trichlorophenoxyacetic acid (TCPAA), 2-(2,4,5-trichlorophenoxy)propionic acid (Fenoprop), 2,4-dichlorophenoxyacetic acid, 2-(2,4-dichlorophenoxy)propionic acid (Dichloroprop), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), 4-chlorophenoxyacetic acid, Fenoxaprop-P, Fluazifop, Fluazifop-P-butyl, Haloxypyr, 2-methyl-4-chlorophenoxyacetic acid (MCPA), Mecoprop, 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB), Fluroxypyr, Trichlopyr, 2-naphthoxyacetic acid, bromoxynil, loxynil, clopyralid, dicamba, imazapyr, imazaquin, imazethapyr, quinmerac, picloram, bentazone, fomesafen, imazosulfuron, trifluralin-methyl, metsulfuron-methyl, metosulam, cycloxydim, fludioxonil, and combinations thereof. According to a particular aspect, the template molecule may be TCPAA.

The template molecule may also comprise homologous molecules, homologs, of the target molecule which the sensor is going to be used for detecting and/or quantifying. Homologs of the target molecules may include molecules that are similar to the target molecule in various attributes such as, but not limited to, size, electrostatic potentials, electronegativity, charge density, chemical bonding potential, and molecules that have similar shapes to the target molecule. Homologs may include isomers and stereoisomers of the target molecule.

The first solvent may be any suitable solvent for the purposes of the present invention. According to a particular aspect, the first solvent may be water or a non-aqueous solvent. In particular, the first solvent may be an alcohol. Examples of the first solvent include, but is not limited to, water, methanol, ethanol, 1-propanol, 2-propanol, n-butanol, 2-butanol, 2-methyl-2-propanol, 2-methyl-1-propanol, 1-pentanol, isomers thereof, dimethylformamide (DMF), dimethylacetamide (DMAc), tetrahydrofuran (THF), methyl ethyl ketone (MEK), acetonitrile, tetramethyl urea, dimethyl sulfoxide (DMSO), butanone, trimethyl phosphate, or a combination thereof. Even more in particular, the first solvent is methanol.

The preparing may comprise mixing the hydrophilic polymer host, the template molecules and the first solvent. According to a particular aspect, various orders of addition and mixing of the hydrophilic polymer host, template molecule and first solvent may be used. The amount of hydrophilic polymer host, template molecule and first solvent added to the MIP solution may depend on the hydrophilic polymer host, the template molecule and the first solvent being used. In particular, the amounts of hydrophilic polymer host, template molecule and first solvent added to the MIP solution is also selected such that the thickness of the MIP film formed from the MIP solution is $\leq 10 \mu\text{m}$.

The providing may comprise using any suitable method to place a suitable amount of the prepared MIP solution onto a surface of a sensing substrate. In particular, the providing may comprise immobilising a suitable amount of MIP solution onto a surface of the sensing substrate. The sensing substrate may be any suitable sensing substrate for the purposes of the present invention. For example, the sensing substrate may be one which is capable of indicating changes in at least one of: resistance, capacitance, mass, colour, resonance frequency and surface plasmon resonance shift. In particular, the sensing substrate may indicate changes in mass.

Accordingly, the sensing substrate may be any suitable sensing substrate for the purposes of the present invention. In particular, the sensing substrate may be selected such that the sensing substrate is suitable for being able to indicate the change in a measureable property of the polymer host during the detection of target molecules. For example, the sensing substrate may be a quartz crystal substrate, a metal-based substrate, an oxide-based conductive glass substrate, and the like.

According to a particular aspect, the sensing substrate may be a quartz crystal substrate. The quartz crystal substrate may be coated, for example with silver, gold, or the like. The quartz crystal may be suitable for quartz crystal microbalance. Alternatively, the sensing substrate may be a metal-based substrate. The metal-based substrate may be suitable for surface plasmonic resonance (SPR). Another example of a suitable sensing substrate may be indium tin oxide (ITO) or fluoride-doped tin oxide (FTO) conductive glass substrate suitable for electrochemical sensing systems.

The providing may comprise coating the MIP solution onto a surface of the sensing substrate. The coating may be by any suitable coating method. For example, the coating may be by electrospinning, dip coating, laser deposition, spin casting, dipping, direct dropping or a combination thereof. Even more in particular, the coating comprises quantity controlled dropping of the MIP solution onto a surface of a sensing substrate.

According to a particular aspect, the surface of the sensing substrate onto which the MIP solution is provided may be cleaned prior to the providing. The cleaning of the surface may be by any suitable method using any suitable solvent. For example, the solvent may be methanol.

Following the providing, the MIP film formed on the surface of the sensing substrate is allowed to dry. The drying may be under any suitable conditions. According to a particular aspect, the MIP film formed during the providing may comprise a self-assembled film of the hydrophilic monomer host and the template molecules. Therefore, the drying may be under suitable conditions to enable the hydrophilic monomer host and template molecules to self-assemble. The drying may be at a suitable temperature. For example, the drying may be at a temperature of $\leq 60^{\circ}\text{C}$. In particular, the drying may be at $15-60^{\circ}\text{C}$, $20-55^{\circ}\text{C}$, $25-50^{\circ}\text{C}$, $30-45^{\circ}\text{C}$, $35-40^{\circ}\text{C}$. Even more in particular, the drying may be at $20-25^{\circ}\text{C}$. According to a particular embodiment, the drying may be by air drying or nitrogen drying.

The hydrophilic monomer host and template molecules may self-assemble due to the interactions between them. For example, the interactions may be as shown in Figures 1A and 1B. In particular, TCPAA molecules may have hydrogen bonding (bond energy $5-30\text{ kJ/mol}$) and $\pi-\pi$ interactions ($0-50\text{ kJ/mol}$) with dopamine as shown in Figure 1A.

TCPAA molecules may also dissociate to TCPAA anions and protons which may be captured by dopamine molecules to become dopamine cations. TCPAA anions may have hydrogen bonding, π - π interactions and electrostatic interactions (5-350 kJ/mol; ion-ion, ion-dipole, dipole-dipole) with dopamine cations as shown in Figure 1B. It is because of these non-covalent interactions that dopamine molecules/ions and TCPAA molecules/ions are able to self-assemble to form a MIP film with well-organised molecules/ions.

The MIP film formed during the providing may be a dry film. The MIP film formed may have a suitable thickness. For example, the thickness of the MIP film formed may be 0.01-10 μm . In particular, the thickness of the MIP film formed may be 0.1-9.5 μm , 0.5-9.0 μm , 1.0-8.5 μm , 1.5-8.0 μm , 2.0-7.5 μm , 2.5-7.0 μm , 3.0-6.5 μm , 3.5-6.0 μm , 4.0-5.5 μm , 4.5-5.0 μm . Even more in particular, the thickness of the MIP film formed may be 0.4 μm .

Once the MIP film is formed, the MIP film may then be exposed to a gas to initiate gas phase polymerisation of the hydrophilic monomer host. The exposing may be under any suitable conditions. For example, the exposing may be carried out at a suitable temperature and for a pre-determined period of time. The pre-determined period of time may be ≥ 5 minutes (min). For example, the pre-determined period of time may be 5 – 1440 min, 10-1140 min, 15-960 min, 20-840 min, 30-720 min, 45-600 min, 60-480 min, 75-360 min, 90-120 min, 100-110 min. In particular, the pre-determined period of time may be 120 min.

According to a particular aspect, the exposing may be at $\leq 90^\circ\text{C}$. For example, the exposing may be at $20-90^\circ\text{C}$, $25-85^\circ\text{C}$, $30-80^\circ\text{C}$, $35-75^\circ\text{C}$, $40-70^\circ\text{C}$, $45-65^\circ\text{C}$, $50-60^\circ\text{C}$. In particular, the exposing may be at room temperature.

The gas to which the MIP film is exposed to during the exposing may be any suitable gas for the purposes of the present invention. In particular, the gas may be any suitable gas to initiate polymerisation of the hydrophilic monomer host. For example, the gas may be ammonia vapour, ammonia gas, monoethanolamine, diethanolamine, triethanolamine, or any other suitable amine vapour. The gas may be with or without the addition of water vapour. Even more in particular, the gas may be ammonia vapour. The ammonia vapour may be from any suitable source. For example, the ammonia

vapour may be from aqua ammonia liquid or ammonia gas with or without moisture. In particular, the ammonia vapour is from aqua ammonia.

The exposing may comprise sealing the MIP film and the ammonia vapour for the pre-determined period of time at a pre-determined temperature. The sealing may be by any
5 suitable method. For example, a paraffin film or any other suitable material may be used for the sealing. After the pre-determined time has passed, the seal may be removed. The MIP film may show some brown spots, indicating that polymerisation of the hydrophilic monomer host may have initiated. The ammonia vapour is then removed.

10 After the ammonia vapour has been removed, the MIP film is then annealed. The annealing may be under any suitable conditions. For example, the annealing may comprise annealing the MIP film for a pre-determined period of time at a pre-determined temperature. In particular, the annealing enables further polymerisation of the MIP film. The annealing also enhances the adhesion of the MIP film to the surface
15 of the sensing substrate. Without the annealing, the MIP film may be flushed away during the removing step.

The complete polymerization of the hydrophilic monomer host is critical for the good stability of the MIP sensor. This is because any residue of the hydrophilic monomer host may cause further polymerisation after the template molecules are removed and
20 may therefore cause a change in the imprinted molecular cavities.

According to a particular aspect, the pre-determined period of time for the annealing may be ≥ 5 minutes. For example, the pre-determined period of time may be 0.5-120 hours, 1-100 hours, 5-90 hours, 10-80 hours, 15-75 hours, 20-70 hours, 25-60 hours, 30-55 hours, 35-50 hours, 40-45 hours. In particular, the pre-determined period of time
25 may be 72 hours.

According to a particular aspect, the pre-determined temperature for the annealing may be $\geq 35^{\circ}\text{C}$. For example, the pre-determined temperature may be $35-150^{\circ}\text{C}$, $50-130^{\circ}\text{C}$, $60-120^{\circ}\text{C}$, $70-115^{\circ}\text{C}$, $80-110^{\circ}\text{C}$, $90-100^{\circ}\text{C}$. In particular, the pre-determined temperature may be 110°C .

According to a particular aspect, the annealing may be carried out at 110°C for up to 72 hours.

Figure 2 shows a general polymerisation scheme of dopamine. In particular, dopamine molecules are first oxidised to dopaminequinone, which further forms 5,6-dihydroxyindole through cyclization and rearrangement. 5,6-dihydroxyindole is then further polymerised to PDA.

Following the annealing, the MIP film may be allowed to cool to room temperature. Once the MIP film has cooled, the template molecules are removed from the MIP film. The removing may be under suitable conditions for the purposes of the present invention. When the template molecule is removed, it may leave behind a MIP film with cavities complementary in shape and functionality to the template molecule, which can rebind, in the cavities, a target identical to the original template molecule. For example, the removing may be by extraction with a second solvent. The imprinted molecular cavities may be on the surface of the MIP film and inside of the MIP film. In particular, the second solvent is selected such that the polymer host is insoluble in the second solvent and the template molecules are soluble in the second solvent.

According to a particular aspect, the extracting may comprise soaking the sensing substrate with the MIP film on the surface of the sensing substrate in the second solvent for a pre-determined period of time. The second solvent may be any suitable solvent. For example, the second solvent may comprise an alcohol, a liquid organic acid, a surfactant, DI water or the like. In particular, the second solvent may comprise a solvent selected from the group consisting of, but not limited to: methanol, dilute aqueous acetic acid, sodium dodecyl sulphate, DI water, and a combination thereof. For example, the dilute aqueous acetic acid may be 1% acetic acid. Alternatively, the template molecule may be evaporated from the MIP film if the second solvent has a lower boiling point than the template molecule.

Once the template molecules have been removed from the MIP film, the MIP sensor may be rinsed and dried. The rinsing may be by using any suitable solvent. For example, the rinsing may be by using deionised water.

The drying following the rinsing may be performed under any suitable conditions. For example, the drying may comprise air-drying or nitrogen-drying. The drying may be at a

suitable temperature, such as $\leq 60^{\circ}\text{C}$. In particular, the drying may be at room temperature.

According to a second aspect, the present invention provides a molecularly imprinted polymer (MIP) sensor made according to the first aspect. In particular, the MIP sensor
5 may be suitable a molecularly imprinted hydrophilic polymer sensor suitable for detecting and/or quantifying the presence of hydrophilic and/or acidic target molecules.

The MIP sensor may be a device that simultaneously monitors one or more target molecules. According to a particular aspect, the MIP sensor may be read visually. According to another particular aspect, the MIP sensor may be coupled to electronics
10 that read the MIP film on the sensing substrate and report wirelessly to a central facility. Alternatively, the MIP sensor may be incorporated into a portable and/or handheld device for measurement and processing onsite. The polymer host and the synthesis of the MIP film for each target molecule may be determined by the physical and/or chemical characteristics of the target molecules. For example, the MIP sensor
15 may comprise one or more MIP films, wherein each MIP film within the MIP sensor, such as a test strip, may be specific to a single target molecule.

According to a third aspect of the present invention, there is provided a method for detecting and/or quantifying a target molecule using the molecularly imprinted polymer sensor. The method comprises:

- 20
- exposing the molecularly imprinted polymer sensor to a sample of fluid containing or thought to contain the target molecule, thereby allowing the target molecule, if present, to be received within cavities of the sensor; and
 - detecting the presence of and/or quantifying the amount of the target molecule bound to the cavities of the sensor using electrochemical,
25 acoustical, spectroscopic, optical or indirect chromatographic techniques,
- wherein the target molecule is a hydrophilic and/or acidic molecule.

The interaction between a polymer host and a target molecule in a MIP may involve associations between the polymer host and the target molecule. The binding interaction
30 may exploit other various forces in conjunction with shape recognition, but the interaction between polymer host and the target molecule can include any interactions between the target molecule and the polymer host. According to a particular aspect,

the target molecule may be as described above. In particular, the target molecule may be TCPAA.

According to a particular aspect, the detecting using electrochemical, acoustical, spectroscopic or optical techniques may comprise measuring a change of a measurable property of the MIP film, wherein a change comes about when the target molecule is detected in the MIP film. The change of the measureable property may be a change in capacitance, resistance, colour, mass, resonance frequency, surface plasmon resonance shift or the like.

The present invention will be exemplified by the following non-limiting examples.

Example 1

Preparation of PDA MIP sensor

The MIP method was used to prepare a PDA-based sensor and 2,4,5-trichlorophenoxy acetic acid (TCPAA) was used as template molecules. 20 µL of a prepared solution comprising 1 mL of methanol, 0.01 g of PDA, and 0.0025 g of TCPAA, was coated onto a gold-coated quartz crystal chip (Au surface diameter 9.5 mm; bare chip base frequency 4.98 MHz). The resulted film had a thickness of 0.4 µm, which was suitable for use on the QSense Quarts Crystal Microbalance (QCM) platform.

For QCM, the mass change of the MIP sensor with and without the adsorption of target molecules follows the QCM mass-frequency effect, Sauerbrey equation (Eq. 1):

$$\Delta F = -2.26 \times 10^6 \times F^2 \times \frac{\Delta m}{A} = -\frac{2f_0^2 \Delta m}{A\sqrt{\mu\rho}} \quad (\text{Eq. 1}),$$

where ΔF is the measured frequency change (Hz), F & f_0 – fundamental resonance frequency (MHz), Δm – mass adsorbed, A – area coated by MIP film, μ - shear modulus of quartz, ρ - density of quartz.

The general procedure used for the preparation of the PDA MIP sensor was as follows:

- 1) clean Au-coated quartz chip with methanol flush;

- 2) prepare methanol solution of dopamine and TCPAA;
- 3) place 20-200 μ L of solution onto chip Au surface;
- 4) air dry the sample at room temperature;
- 5) expose the chip to ammonia vapour;
- 5 6) remove the ammonia vapour;
- 7) anneal the chip in an oven;
- 8) wash/soak the chip with methanol; and
- 9) dry the chip to obtain the PDA MIP sensor.

When the above method was repeated without the addition of PTM, the PVDF film
10 formed was a non-imprinted polymer (NIP) film. The method formed a NIP sensor.

Both the PDA-based MIP sensor and the NIP sensor were then exposed to TCPAA to test how well the sensors detected the TCPAA molecules. As explained above, the frequency change ΔF was measured as a correlation of the amount of TCPAA molecules detected by the sensor.

15 The NIP film of the NIP sensor absorbed only very limited amounts of TCPAA as can be seen in Figure 3. In particular, the frequency change ΔF of the NIP sensor was 8.0 Hz in 70 minutes when contacted with 3.40 ppm (13.31 μ M) PDA.

When the original PDA/TCPAA ratio was 4/1 and the annealing time in a 60°C oven was 19 hours, the prepared MIP sensor had ΔF of 249.0 Hz when contacted with 3.40
20 ppm TCPAA for 70 minutes (see Figure 4). The MIP sensor had a much higher (31.1 times) value of ΔF compared to the NIP sensor, which made the sensor sensitive for the detection of the targeted analyte.

Annealing the MIP film has two functions: (i) to promote polymerization of dopamine; and (ii) enhance adhesion of the film to the substrate. annealing before the exposure to
25 the ammonia vapour only enhanced the adhesion between the MIP film and the sensing substrate surface. If the annealing was done before the exposure to the

ammonia vapour, but not after it, the prepared PDA MIP sensor had a low response of 17.0 Hz to the 3.40 ppm TCPAA solution as seen in Figure 5. The ammonia at the surface after exposure was critical for the further polymerization of dopamine during the annealing. If the surface ammonia after exposure was washed away by deionised water followed by the annealing, the prepared PDA MIP sensor also had a low response of 11.0 Hz to the 3.40 ppm TCPAA solution (see Figure 6).

Simultaneous polymerisation and template inclusion was also tried in aqueous solution, but the PDA sensor chip had a low response of 2.5 Hz to 3.40 ppm TCPAA as shown in Figure 7.

PDA sensor was also tested using another amine-based molecule, diethyl phosphoramidate (DEPA). DEPA was used as template and target molecule. The sample preparation process was kept the same as for TCPAA. PDA/DEPA ratio was 4/1. The resulted PDA sensor had a low response of 8.5 Hz to 1.53 ppm DEPA as seen in Figure 8. When the methanol solution was replaced by aqueous solution during the sensor preparation, the PDA sensor also had a low response to DEPA.

Optimisation of polymerisation conditions

Dopamine polymerisation time during the annealing step was optimised as shown in Table 1. The annealing temperature was set at 60°C.

Baking time (hr)	ΔF (Hz)
0.5	97.0, 46.0, 54.0 (bad stability)
1	30.0 (± 2)
2	37.0 (± 2)
15	38.0 (± 2)
72	249.0 (± 5)

Table 1: Annealing time and 3.40 ppm TCPAA analyte response for the PDA/TCPAA film (4/1; 60°C)

When polymerisation (annealing) time was short as 30 minutes, the PDA sensor had high response but low stability as the performance decayed within a week. The reason for this was mainly the unpolymerised dopamine monomer residue which continued to polymerise after the annealing process and template removal process, causing big changes to the molecularly imprinted cavities. The longer the annealing time, the more

stable the PDA sensor was. With 72 hours annealing, the PDA sensor was stable and had a high response to the TCPAA target molecules.

Optimisation of template removal

For TCPAA template removal, methanol, 1% acetic acid, DI water and some other solutions were effective by flushing or soaking. 1% acetic acid soaking time was optimised as shown in Table 2.

1% acetic acid soaking time (h)	ΔF (Hz)
2	40, 43
15	46 (± 2)
24	46 (± 2)

Table 2: Optimising 1% of acetic acid soaking time for the PDA sensor (PDA/TCPAA film (4/1; annealing time 0.5 hours at 60°C)

It can be seen that for 1% acetic acid, soaking for 2 hours was sufficient. The soaking time may be longer or shorter than 2 hours and it did not affect the PDA sensor performance.

Optimisation of PDA/TCPAA ratio

Different PDA and TCPAA template molecule ratios were also optimised as shown in Table 3.

PDA/TCPAA ratio	ΔF (Hz)
8:1	66.0 (± 3)
4:1	249.0 (± 5)
2:1	135.0 (± 3)
1:1	134.0 (± 3)

Table 3: Optimising PDA/TCPAA ratios for PDA sensor performance (annealing time 19 hours)

It can be seen that 4/1 of PDA/TCPAA was found to perform the best. Other ratios like 2:1 and 1:1 also performed quite well. PDA/TCPAA less than 1:1 could also be possible. The more template molecules, the more possible cavities will be created. However, the imprinted cavities may collapse over the time due to insufficient PDA polymer.

Sensor selectivity

PDA sensor selectivity was also studied. It was highly selective to TCPAA, while had low response to other pesticide molecules such as parathion methyl, diethyl phosphoramidate and dicotophos (see Figure 9). The good selectivity of PDA to TCPAA was due to the imprinted TCPAA molecular cavities and non-covalent interactions between PDA and TCPAA target molecules. The interactions between PDA polymer chain and TCPAA molecules include hydrogen bonding, π - π interaction, and van der Waals force (Figure 10A). Van der Waals force is not shown in the Figure 9. In aqueous solution, TCPAA could also dissociate into TCPAA anions and protons which bind to nitrogen atoms of PDA chains forming positively charged ammonium cations. TCPAA anions and ammonium cations in PDA also have strong electrostatic force (Figure 10B).

Sensor sensitivity

The PDA sensor preparation conditions for the sensitivity test were PDA/TCPAA 4/1, annealing at 60°C for 19 hours, soaking time 2 hours for removal of TCPAA template molecules using in 1% of acetic acid and aging 7 days of the ready-to-use PDA sensor. The calibration curve had a R^2 of 0.99998 and slope 16.4920 (Figure 11). Limit of detection (LOD) of 15.1 ppb and limit of quantitation (LOQ) of 45.7 ppb were achieved from the prepared PDA sensor.

Example 2

Characterisation of PDA copolymer MIP sensor

The co-polymers of dopamine (DA) and diethyl phosphoramidate (DEPA) were synthesized using the method as described in Example 1 with the exception being the monomer host added in the MIP solution. The weight ratio of DA to DEPA may be 20:1 to 1:20, preferably 5:1 to 1:5. The co-polymerization also be done by pH adjustment, addition of oxidizing agents, or exposure to ammonia or oxidizing gases.

The fabricated PDA/PDEPA co-polymer MIP was evaluated with 3.0 ppm of 4-CPA as the target molecule using QCM, which showed 20 Hz and 18 Hz of response in 15 minutes at Day 1 and Day 7, respectively (Figure 12). This demonstrated the feasibility of PDA co-polymer MIP sensor.

The present examples show that the molecularly imprinted polydopamine-based or polydopamine copolymer based sensors have good selectivity, stability, reliability and high sensitivity. In particular, the preparation method of the MIP sensors is unique in
5 that it comprises pre-self-assembly of monomer and template molecules, gas phase initiation of polymerization, and post-self-assembly polymerization. Advantages of the method of the present invention include the making of MIP sensors with good selectivity, stability, reliability, high sensitivity, unique PDA sensor film, unique sensing of hydrophilic and acid analyte molecules, fast analysis, easy preparation of sensors,
10 low sensor cost and analysis cost.

Whilst the foregoing description has described exemplary embodiments, it will be understood by those skilled in the technology concerned that many variations may be made without departing from the present invention.

Claims

1. A method of making a molecularly imprinted polymer (MIP) sensor comprising:
 - preparing a molecularly imprinted polymer solution comprising a hydrophilic monomer host, template molecules and a first solvent, wherein the template molecules are hydrophilic and/or acidic and the first solvent is water or a non-aqueous solvent;
 - providing the molecularly imprinted polymer solution onto a surface of a sensing substrate to form a molecularly imprinted polymer film;
 - exposing the MIP film to a gas to initiate gas phase polymerisation of the hydrophilic monomer host;
 - annealing the MIP film; and
 - removing the template molecules from the MIP film, wherein the removing comprises extracting the template molecules from the MIP film using a second solvent to form cavities in the MIP film, wherein the monomer host when polymerised is insoluble in the second solvent, and wherein the template molecule is soluble in the second solvent.
2. The method according to claim 1, wherein the MIP film formed during the providing comprises a self-assembled film of the monomer host and the template molecules.
3. The method according to claim 1 or 2, wherein MIP film formed during the providing is a dry film.
4. The method according to any preceding claim, wherein the gas to which the MIP film is exposed during exposing is ammonia vapour or amine vapour.
5. The method according to any preceding claim, wherein the exposing is carried out at $\leq 90^{\circ}\text{C}$.
6. The method according to any preceding claim, wherein the annealing comprises annealing the MIP film for a pre-determined period of time at a pre-determined temperature.

7. The method according to claim 6, wherein the pre-determined period of time is ≥ 5 minutes.

8. The method according to claim 6 or 7, wherein the pre-determined temperature is $\geq 35^{\circ}\text{C}$.

9. The method according to any of claims 6 to 8, wherein the pre-determined temperature is 110°C and the pre-determined time is 72 hours.

10. The method according to any preceding claim, wherein the thickness of the MIP film is $0.01\text{-}10\text{ }\mu\text{m}$.

11. The method according to any preceding claim, wherein the hydrophilic monomer host comprises a monomer with a functional group selected from the group comprising amine, imine, amide, carboxyl, hydroxyl, carbonyl and thiol.

12. The method according to claim 11, wherein the hydrophilic monomer host comprises dopamine (DA) or a co-polymer of DA and diethyl phosphoramidate (DEPA).

13. The method according to any preceding claim, wherein the template molecules comprise: 2,4,5-trichlorophenoxyacetic acid (TCPAA), 2-(2,4,5-trichlorophenoxy)propionic acid, 2,4-dichlorophenoxyacetic acid, 2-(2,4-dichlorophenoxy)propionic acid, 4-(2,4-dichlorophenoxy)butyric acid, 4-chlorophenoxyacetic acid, Fenoxaprop-P, Fluazifop, Fluazifop-P-butyl, Haloxyfop, 2-methyl-4-chlorophenoxyacetic acid (MCPA), Mecoprop, 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB), Fluroxypyr, Trichlopyr, 2-naphthoxyacetic acid, bromoxynil, loxynil, clopyralid, dicamba, imazapyr, imazaquin, imazethapyr, quinmerac, picloram, bentazone, fomesafen, imazosulfuron, trifluralin-methyl, metsulfuron-methyl, metosulam, cycloxydim, fludioxonil, and combinations thereof.

14. The method according to any preceding claim, wherein the template molecule is 2,4,5-trichlorophenoxyacetic acid.

15. The method according to any preceding claim, wherein the first solvent comprises water, methanol, ethanol, 1-propanol, 2-propanol, n-butanol, 2-butanol, 2-methyl-2-propanol, 2-methyl-1-propanol, 1-pentanol, isomers thereof,

dimethylformamide (DMF), dimethylacetamide (DMAc), tetrahydrofuran (THF), methyl ethyl ketone (MEK), acetonitrile, tetramethyl urea, dimethyl sulfoxide (DMSO), butanone, trimethyl phosphate, or a combination thereof.

5 16. The method according to any preceding claim, wherein the second solvent comprises a solvent selected from the group consisting of: alcohols, liquid organic acids, surfactants, DI water, and a combination thereof.

10 17. The method according to any preceding claim, further comprising drying the MIP film following the providing.

18. The method according to any preceding claim, further comprising rinsing the MIP sensor following the removing.

15 19. The method according to claim 18, further comprising drying the sensor following the rinsing.

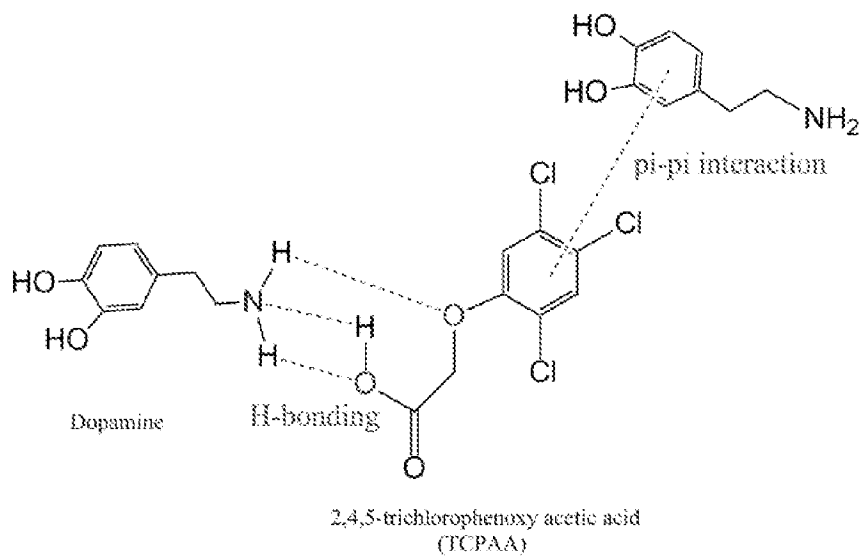
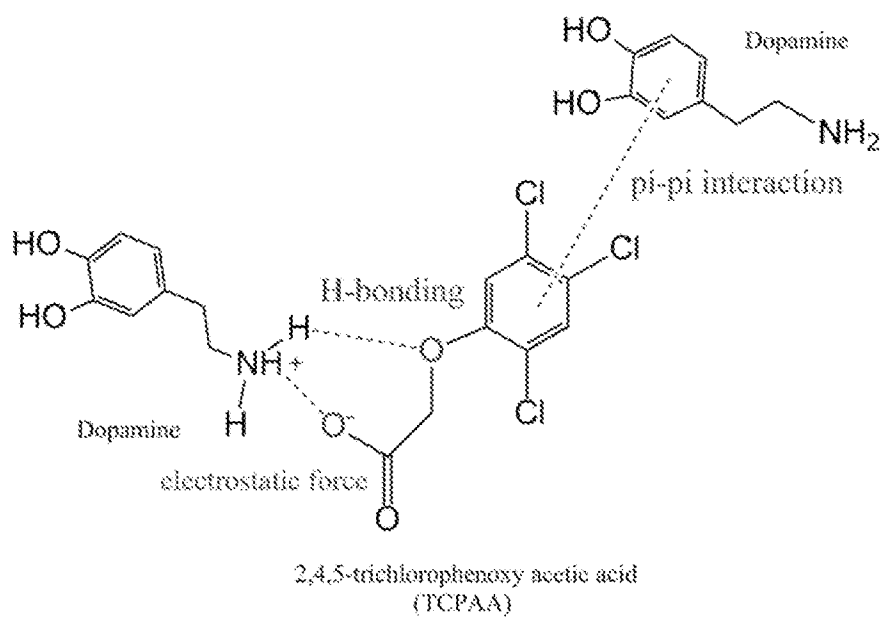
20. A molecularly imprinted polymer (MIP) sensor made from the method of any of the preceding claims.

20 21. A method for detecting and/or quantifying a target molecule using the molecularly imprinted polymer sensor according to claim 20, the method comprising:

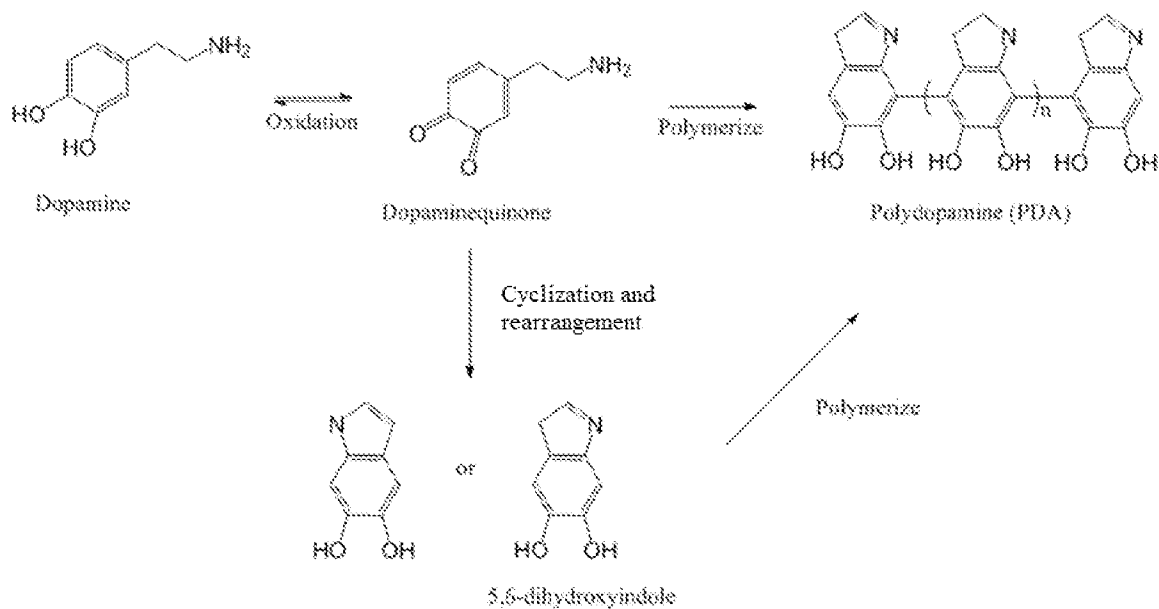
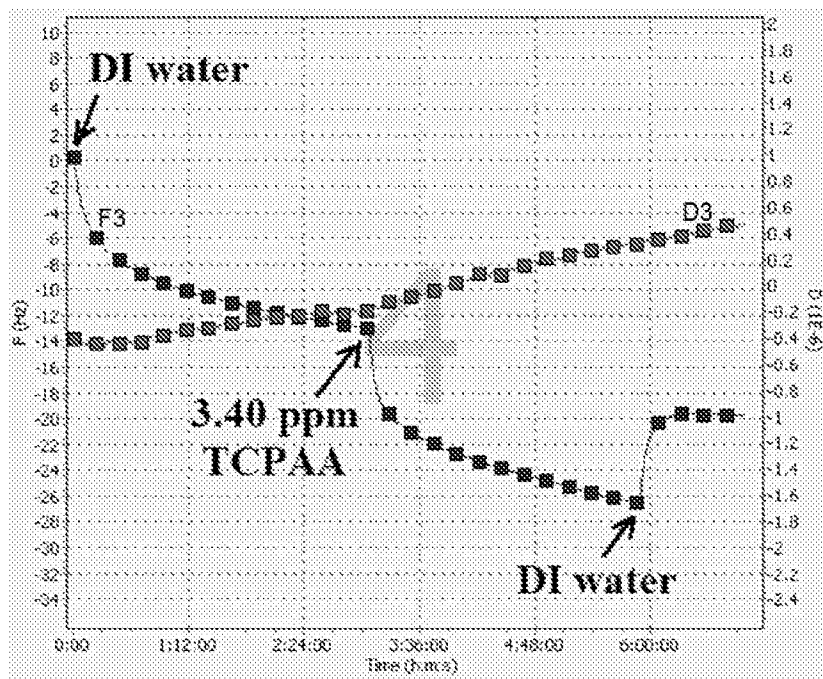
- exposing the molecularly imprinted polymer sensor to a sample of fluid containing or thought to contain the target molecule, thereby allowing the target molecule, if present, to be received within cavities of the sensor formed by the template molecules; and
- detecting the presence of and/or quantifying the amount of the target molecule bound to the cavities of the sensor using electrochemical, acoustical, spectroscopic, optical or indirect chromatographic techniques,

30 wherein the target molecule is a hydrophilic and/or acidic molecule.

1/7

Figure 1A**Figure 1B**

2/7

Figure 2**Figure 3**

3/7

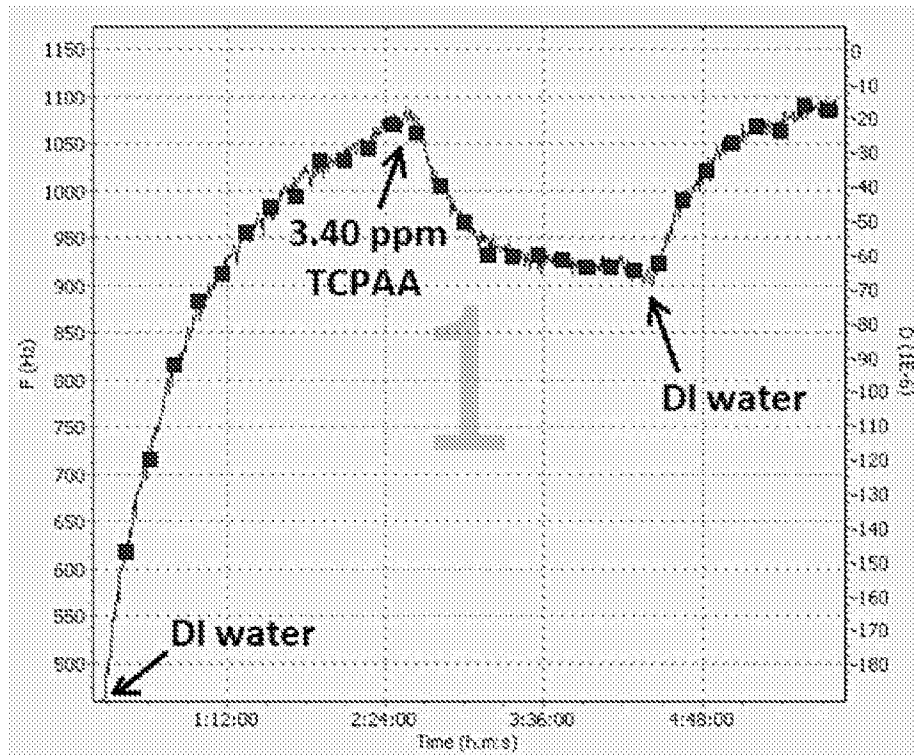
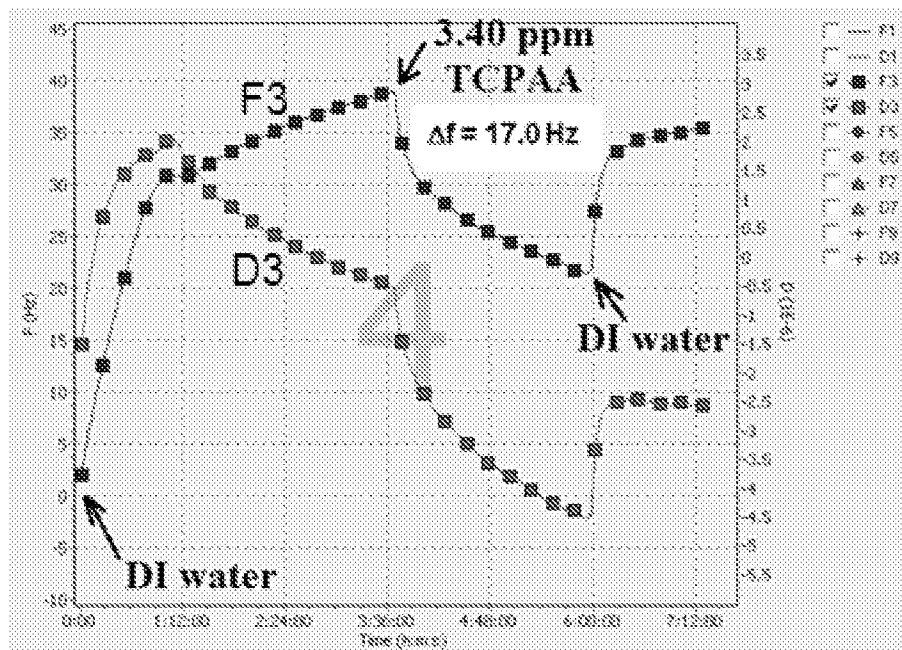
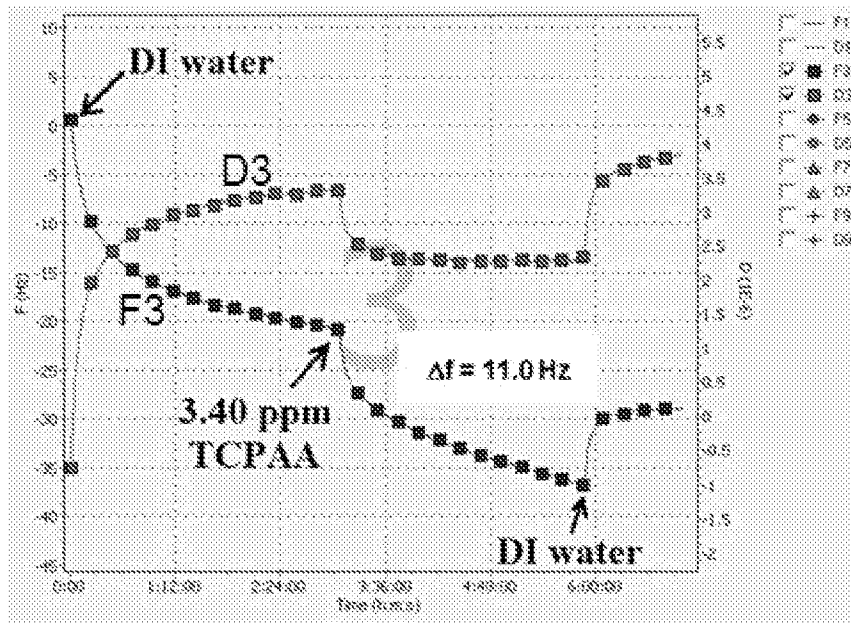
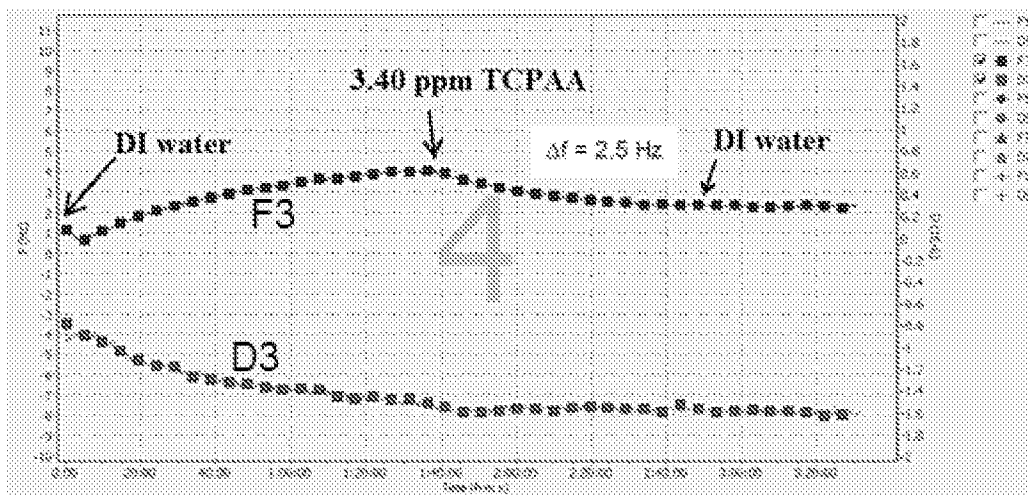
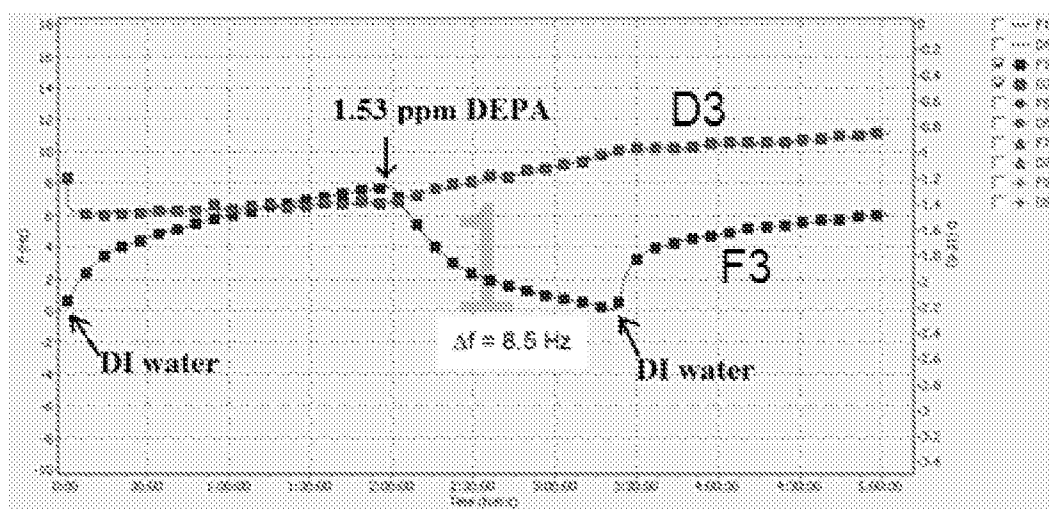
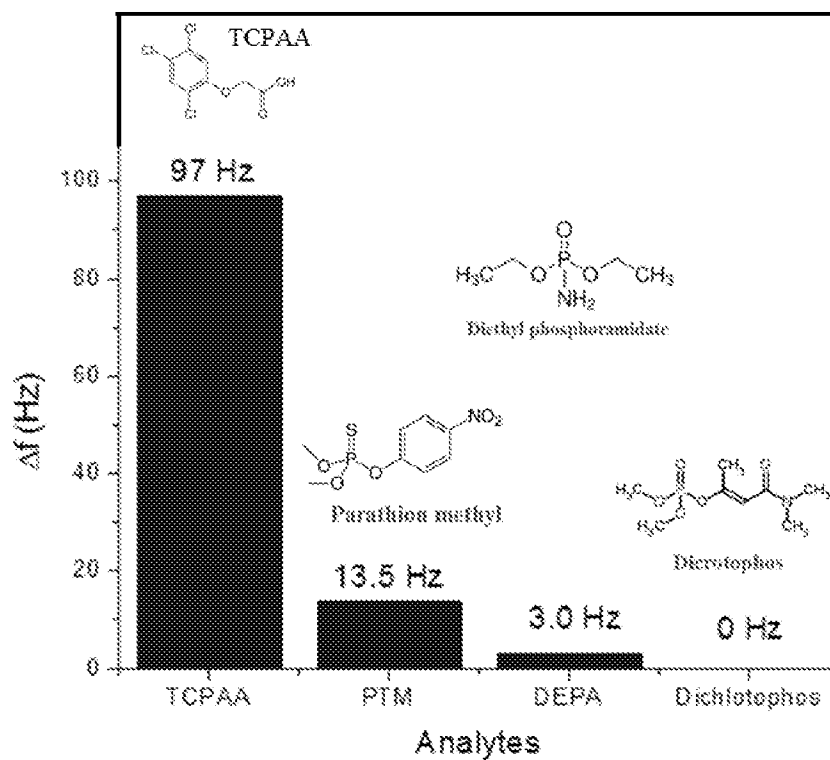
Figure 4**Figure 5**

Figure 6**Figure 7**

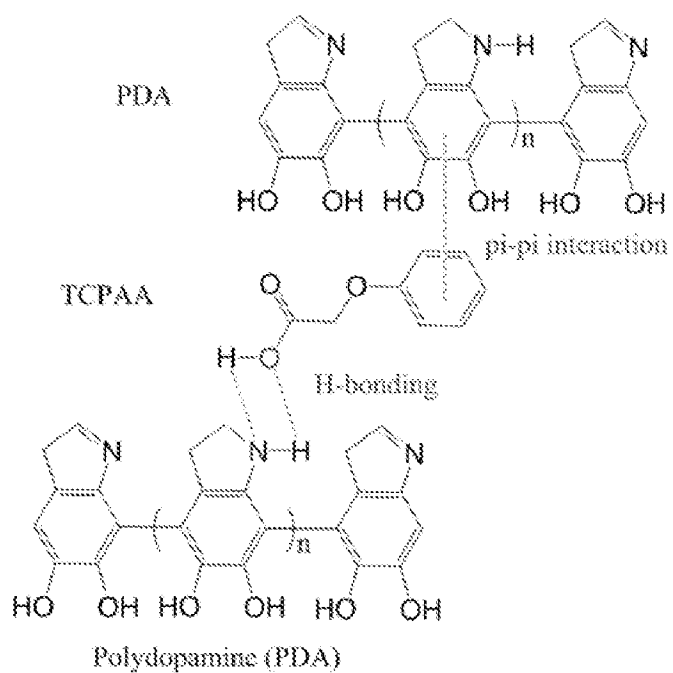
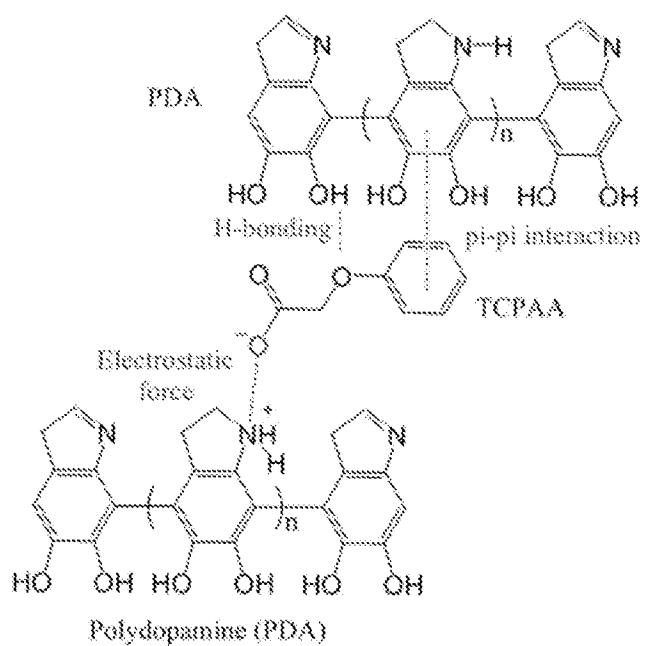
5/7

Figure 8**Figure 9**

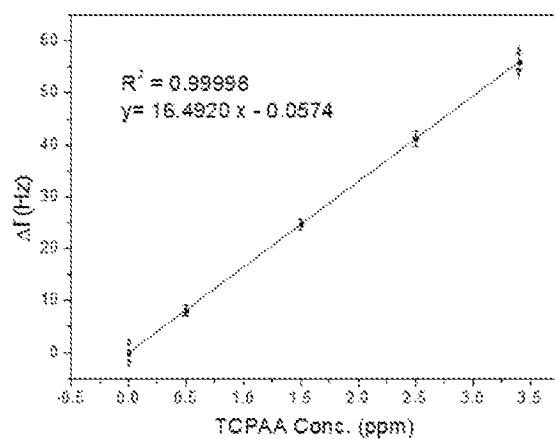
PDA/TCPAA 4/1
Bake 0.5 h
Soak 14 h

All analytes in ~ 10 uM
(PCTAA 13.3 uM, PTM 9.9 uM, DEPA 10.0 uM, Dichlorophos 10.0 uM)

6/7

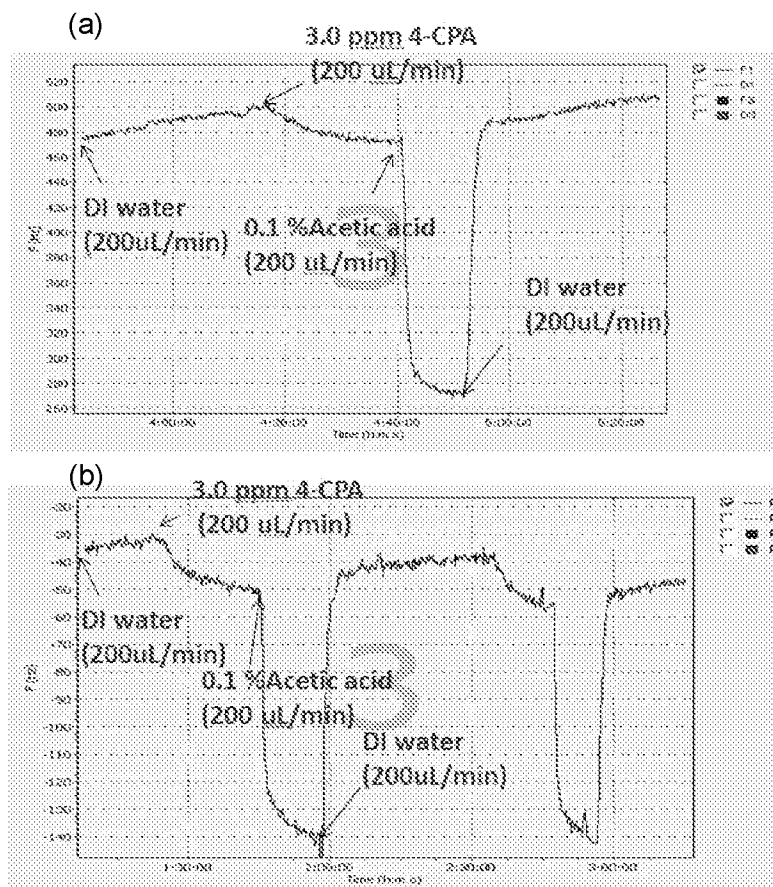
Figure 10A**Figure 10B**

7/7

Figure 11

LOD: 15.1 ppb
 LOQ: 45.7 ppb

PDA/TCPAA 4/1
 Bake 19 h
 Soak 2 h
 Aging 7 days

Figure 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2017/050340

A. CLASSIFICATION OF SUBJECT MATTER

See Supplemental Box

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, CAS registry: registry number search based on working examples, MIP, dopamine, substrate, QCM, hydrophilic, gas phase, and like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHOU, W. ET AL., A quartz crystal microbalance sensor based on mussel-inspired molecularly imprinted polymer. <i>Biosensors and Bioelectronics</i> , 16 July 2010, Vol. 26, No. 2, pages 585-589 [Retrieved on 2017-09-10] <DOI: 10.1016/J.BIOS.2010.07.024> Sections 2.3 and 3.1-3.7	1-21
X	CN 103424381 A (NANCHANG UNIVERSITY) 4 December 2013 Example 1 of the original non-English language document (a machine translation is enclosed only for your reference)	1-21
X	CN 101776635 A (JIANGNAN UNIVERSITY) 14 July 2010 Examples 1-4 of the original non-English language document (a machine translation is enclosed only for your reference)	20-21
X	US 2012/0234699 A1 (HONG, C. ET AL.) 20 September 2012 [0048]-[0049]	20-21

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

*Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
10/09/2017 (day/month/year)

Date of mailing of the international search report
19/09/2017 (day/month/year)

Name and mailing address of the ISA/SG

Intellectual Property Office of Singapore
IPOS
51 Bras Basah Road
#01-01 Manulife Centre
Singapore 189554
Email: pct@ipos.gov.sg

Authorized officer

Cao Ye (Dr)

IPOS Customer Service Tel. No.: (+65) 6339 8616

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2017/050340

Supplemental Box

(Classification of Subject Matter)

Int. Cl.

G01N 33/00 (2006.01)

B01J 20/26 (2006.01)

B01J 20/28 (2006.01)

B01J 20/281 (2006.01)

B32B 3/30 (2006.01)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2017/050340

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 103424381 A	04/12/2013	NONE	--
CN 101776635 A	14/07/2010	WO 2011/095033 A1	11/08/2011
		US 2012/0285833 A1	15/11/2012
US 2012/0234699 A1	20/09/2012	TW 201237406 A1	16/09/2012