METHOD FOR COATING A SURFACE

A method of applying a coating of polymeric material to a surface of a substrate comprises the steps of contacting the surface to be coated with a suitable quantity of a solution of one or more polymerisable species and causing the polymerisable species to undergo polymerisation, characterised in that, during polymerisation, the solution and polymeric material formed therefrom are constrained by a mould placed against the surface of the substrate and cooperating therewith to define a chamber. The substrate may be a biosensor transducer.
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TECHNICAL FIELD

The present invention relates to a method for coating surfaces with a defined layer of a polymeric material. In particular, although not exclusively, it relates to the use of such a method in the field of analytical chemistry and biosensors.

BACKGROUND

In the fields of surface analysis and biosensors, it is frequently desirable to produce a coating of a polymeric material, e.g. a polymer gel, on a substrate in order to assess properties of the polymeric material or of substances capable of binding to the polymeric material. The polymer coating may also be used as a means by which further molecular species may be immobilised in order to assess their interaction with subsequently introduced analytes. Typical analytical techniques in which such coatings may be used include biosensors, such as those based on surface plasmon resonance (SPR), ellipsometry, optical waveguides, resonant mirrors and quartz crystal microbalances (QCMs) (see Biomolecular Sensors, Gizeli and Lowe. Published by Taylor and Francis, London; 2002), and other surface interrogation methods such as the scanning probe microscopies.

In the fabrication of molecular imprinted polymers (MIP), a monomer mixture (or a mixture of oligomers or polymers capable of undergoing further polymerisation and/or cross-linking) is polymerised with a template molecule present in solution. The monomer molecules can be said to self-assemble around the template molecule to form a three dimensional matrix which holds cavities that are specific for the template molecule that was present during the polymerisation. An imprint of the template molecule is created in the polymer matrix. By washing the polymer matrix, the template molecules are removed from the matrix and only the cavities remain. Cavities of this kind have been shown to have properties in terms of selectivity and binding
characteristics that are similar to those of biological affinity molecules such as antibodies. These ‘synthetic antibodies’ have a number of properties that are advantageous compared to biological antibodies. Biological antibodies take months to develop in experimental animals, are considered unstable and fragile and are difficult to store with retained functionality. In contrast, synthetic antibodies can be almost instantly created, are chemically stable and can be stored for long periods without any loss in functionality.

While many conventional techniques exist for attaching proteins and antibodies to surfaces, the coating of a polymer layer with affinity properties on a chemical sensor (e.g. biosensor) transducer surface is a more difficult task. The polymer matrix has to be sufficiently thick to receive its functionality and have sufficient binding capacity, but thin enough to be in the sensing reach of the transducer device or at least thin enough for mass transfer processes not to limit the response time of the sensor. In addition, the thickness of the coating should be reproducible in order for the biosensor to have reproducible detection properties.

Percival and co-workers (Molecular imprinted polymer coated QCM for the detection of nandrolone. Analyst 127, 1024-1026; 2002) have manufactured a MIP coated sensor for detection of nandrolone. The molecular imprinted polymer was separately polymerised in organic solvents and then crushed into small particles. The particles were then put in suspension in a solution of PVC in tetrahydrofuran. This suspension was then applied to the sensor surface by means of spin-coating. The spin-coating procedure involves fixation of the sensor surface to a disc that can be rotated at a given number of revolutions per minute. By rotating the disc at high speed, a liquid placed on the surface will spread to form a thin layer, the thickness of which is governed mainly by the rotational speed and the viscosity of the liquid. Consequently, it is possible by this method to coat sensor surfaces with a polymer layer of relatively reproducible thickness.

For other types of MIPs the above approach is, however, less useful. MIPs for small molecules are often mixtures of different functional monomers that are polymerised
together with a template in organic solvents. The polymer that is created is highly cross-linked and often mechanically and chemically robust and can therefore sustain the rough treatment described above. MIPs for larger molecules such as proteins, nucleic acids and polysaccharides and for particles such as viruses and bacteria are fundamentally different in their function and their fabrication, as described in European patent application EP 0797096 A2. Such MIPs have been formed by polymerisation of one, or maybe two, monomers, a cross-linker and the template molecule. The polymerisation takes place in aqueous solution in order to preserve the three dimensional structure of the biomolecule/bioparticle during the polymerisation. The created polymer is significantly less cross-linked than the MIPs for small molecules both because the binding sites of the larger molecules or particles are required to be significantly bigger and also to allow diffusion of larger molecules through the polymer matrix. The polymer has the appearance of a hydrogel, which means that it consists mainly of water. If the water is removed, the three dimensional structure of the gel is changed and the gel shrinks to a fraction of its original volume. Due to the less robust properties of the MIP for proteins and particles, the crushing and spin-coating method described above is not applicable.

Other methods for coating a surface exist, such as the one used by Lan Cao and co-workers (Enantioselective sensor based on microgravimetric quartz crystal microbalance with molecular imprinted polymer film. Analyst 126, 184-188; 2001). They apply a small droplet, 5 μL, of the polymerisation mixture to the sensor surface and the droplet is allowed to polymerise in a nitrogen environment for 12 hrs under UV light irradiation. This in situ method for coating a sensor surface is more suited for the large molecule MIPs. The method, however, is limited in terms of how thin the polymer layer can become and it suffers from problems in spreading a small amount of liquid over the sensor surface and achieving a uniform layer. A related method is described by Kugimiya and Takeuchi (Electroanalysis 11, 1158-1160; 1999), wherein the polymerisation mixture is sandwiched between the sensor transducer surface and a glass slide. Such a method does not, however, allow for reproducible control of the thickness of the polymer coating.
An object of the present invention is to provide for a satisfactorily reproducible method for coating sensor, and other substrate, surfaces with polymer layers and which allows for improvements in the polymer layers in terms of consistency of thickness and shape.

SUMMARY OF THE INVENTION

Accordingly, one aspect of the invention provides a method of applying a coating of polymeric material to a surface of a substrate, the method comprising the steps of contacting the surface to be coated with a suitable quantity of a solution of one or more polymerisable species and causing the polymerisable species to undergo polymerisation, characterised in that, during polymerisation, the solution and polymeric material formed therefrom are constrained by a mould placed against the surface of the substrate and co-operating therewith to define a chamber.

The method of the present invention allows for the preparation of a coating of polymeric material on a substrate surface and which is controllable in terms of its shape, thickness, uniformity of thickness and homogeneity. The method is highly versatile, allowing for polymerisations to be conducted with a wide range of polymerisable species, and is relatively straightforward to conduct under controlled, e.g. inert, atmospheric conditions. The term 'polymerisable species', as used herein, includes monomers, oligomers and polymers, provided that the species is capable of undergoing further polymerisation and/or cross-linking.

Preferably, the mould defines a recess having a depth of approximately 0.1 μm to 100μm, more preferably approximately 1μm to 50μm.

The substrate is preferably a biosensor transducer, such as a SPR chip or a QCM. The method has particular advantages when used for the coating of biosensor transducers. The layer or coating of polymer is of defined thickness and can be made very thin, essentially independently of the viscosity of the solution or the resulting polymeric material. The method does not employ high g-forces, unlike spin coating, and thus is considerably milder on denaturable components of the solution such as
biomolecules. The determination of the thickness of the layer of polymeric material is more precise than with spin coating or sandwich coating. This is very important since the thickness of the layer determines the sensibility, response time and general performance of the sensor employing the transducer. Uniformity of the thickness of the coating, eminently possible by means of the method, ensures reproducibility in analysis, both between transducers produced on separate occasions and between separate areas on the same transducer.

In preferred embodiments, the quantity of solution which is contacted with the surface to be coated is in excess of the volume of the chamber.

By employing an excess of the solution of polymerisable species, one can be more sure that the chamber is completely filled. Excess solution can simply be drawn away from the mould and substrate surface by using an absorbent and/or adsorbent medium such as tissue paper. The absorbent and/or adsorbent medium is preferably placed before the mould and substrate surface are brought against each other. Preferably, the mould is constructed in such a way as to allow escape of excess liquid from the chamber, e.g. by having one or more discontinuities in the region of the mould intended to be placed against the substrate surface and through which liquid may be forced or drawn. The solution is preferably an aqueous solution.

The mould may be formed from silica. In such cases, the surface of the mould, or at least a part of that surface which defines the chamber, is preferably modified to increase its hydrophobicity. The modification may be by means of hydrophobic silanes. The silanes may be selected from trifluoropropyltrimethoxysilane and trimethylchlorosilane. The hydrophobic modification of the mould reduces adhesion between the mould and hydrophilic polymeric materials. Alternative materials for the mould itself include polyurethanes, polycarbonates, polytetrafluoroethane and silicones.

It is preferred that the polymeric material is attached to the surface of the substrate by covalent bonding, electrostatic forces and/or physisorption. The covalent bonding of the polymeric material to the surface may be obtainable by the attachment of a
bifunctional linker, capable of reacting with one or more of the polymerisable species, to the surface prior to polymerisation. The bifunctional linker may be selected from thiol-containing compounds and silane-containing compounds.

The solution, prior to polymerisation, commonly comprises one or more species of polymerisable monomers, a cross-linking agent, a catalyst and/or a polymerisation initiator. The monomer may be selected from acrylamides, agarose, methacrylate, substituted acrylamides, substituted methacrylates, substituted or unsubstituted ethacrylates, substituted or unsubstituted acrylates and 3-aminophenylboronic acid. The cross-linking agent may be selected from N,N'-methylenebisacrylamide, piperazinediacrylamide, N,N'-bisacrylylcystamine, N,N'-diallyltartardiamide and glutaraldehyde. The initiator may be ammonium persulphate. The catalyst may be N,N,N',N'-methylenthlenediamine.

The polymerisable species may, alternatively, be suitable for polymerisation to form cross-linked polypeptylene glycols, such as polyethylene glycol, cross-linked polyethylene imine, cross-linked carbohydrates or cross-linked polypeptides, such as albumin, fibrinogen or streptavidin. A preferred cross-linking agent in such cases is glutaraldehyde.

One or more of the polymerisable species may contain a functional group suitable for subsequent coupling of a biomolecule to the polymeric material. The functional group may be selected from carboxyl groups, amino groups, hydroxyl groups, vinyl groups, aldehyde groups and thiol groups.

Polymeric coatings containing functional groups are particularly useful for the further immobilisation of biomolecules for subsequent analytical studies. Techniques for immobilisation of biomolecules are well known in the biosensor field. Hydrophilic polymeric materials in general are especially advantageous since they minimise the non-specific adsorption of proteins to the substrate surface.

 Preferably, the polymeric material takes the form of a gel. In certain embodiments, the polymeric material is substantially saturated with water so as to form a hydrogel.
The use of a water-saturated polymer, or hydrogel, has the advantage that the total available surface of the sensor can be greatly enhanced. This increases the sensitivity of a biosensor employing a transducer coated with such a polymeric material.

In preferred embodiments of the method, the surface of the substrate is brought into contact with the solution by dispensing the solution onto the surface, the mould being subsequently placed against the surface of the substrate. The advantage of this manner of carrying out the method is improved control. An alternative manner is to disperse the solution into the mould so as to fill the mould and then to place the substrate surface against the mould, thereby defining the chamber.

The polymerisation may be carried out under an inert atmosphere. This has the advantage of avoiding oxygen which may inhibit certain polymerisation reactions. The inert atmosphere may be a nitrogen or argon atmosphere.

The solution and/or the polymeric material formed therefrom may be maintained in a water-saturated atmosphere. Such conditions preserve the water content of the polymeric material and solution. This is important for conservation of the three-dimensional conformation of the polymeric material, particularly when a hydrogel is intended to be prepared.

The mould may comprise a porous or perforated material. Such a mould allows the controlled diffusion of an additional reactant to the polymerisation chamber. An inhibitor compound may be added to such a mould and may diffuse therefrom to inhibit polymerisation of the solution in the region adjacent the mould. This allows further control of the thickness of the coating of polymeric material and can be used to prevent polymerisation immediately adjacent the mould, thereby reducing mould-polymeric material adhesion.

In certain embodiments, the solution includes one or more species of template molecules or particles for the formation of a molecular imprinted polymeric material. Preferably, in such embodiments, the polymeric material is subjected to a washing step
following polymerisation in order to remove the template molecules or particles. In certain embodiments, one of the template molecules is a polypeptide. Alternatively or additionally, one of the template molecules or particles may be a polynucleotide, carbohydrate, virus or microorganism.

In another aspect, the invention provides a method for coating a surface of a biosensor transducer with a polymeric material, characterised in that a mould is used to shape the polymeric material on the surface.

Also provided by the present invention, in a related aspect, is a method of applying a coating of polymeric material to a surface of a substrate, the method comprising the steps of contacting the surface to be coated with a suitable quantity of a liquid or semi-solid dispersion of the polymeric material and allowing the polymeric material to adhere to the surface, characterised in that, whilst the polymeric material is undergoing adhesion to the surface, a mould is placed against the surface of the substrate and cooperates therewith to define a chamber by means of which the polymeric material is shaped.

The present invention is equally applicable to the coating of a substrate surface with fully or partially pre-prepared polymeric material. The liquid or semi-solid dispersion of the polymeric material preferably comprises a solution or suspension of the material in a solvent which may be aqueous or non-aqueous. Preferably, the dispersion is a gel. In some embodiments, the dispersion of the polymeric material is substantially non-cross-linked, a cross-linking agent and a catalyst and/or an initiator being added once the dispersion has been brought into contact with the substrate surface. The mould can then be placed against the substrate surface such that cross-linking of the polymeric material, e.g. to form a gel, may take place within the chamber. In such cases, the process of adhesion of the polymeric material to the substrate surface may be regarded as taking place during the cross-linking process. The use of the mould with fully or partially pre-prepared polymeric materials provides advantages in terms of control of shape and thickness of the polymeric coating, as discussed above. In addition to the monomers listed above, preferred monomers for use in this aspect of the
The invention includes 4,4'-diisocyanatodiphenylmethane, bisphenol A, and 4,4'-diaminodiphenyl ether. In addition to the crosslinkers listed above, other cross-linking agents which may be used include phloroglucinol, triethanolamine and melamine. This aspect of the invention is also particularly suitable for use with the polyalkylene glycols, polyethylene imine, carbohydrates and polypeptides listed above. These species may readily be cross-linked on the substrate surface, e.g. using glutaraldehyde. The method of this aspect of the invention may also be used for the formation of a molecular imprinted polymeric material, in which case the dispersion includes one or more species of template molecules or particles, as described above.

The invention also provides, in a further aspect, a substrate having a coating of a polymeric material applied to one or more of its surfaces and obtainable by a method as described above.

In yet another aspect, the invention provides a mould for use in a method as described above, the mould comprising a block having formed in one or more surfaces a recess bounded by a ridge, the ridge being capable of contact with the surface of a substrate such that a chamber may be defined by the surfaces of the recess and the surface of the substrate, the depth of the recess, measured from the level of the ridge, being between 0.1 μm and 100 μm and the shape of the recess corresponding with the shape of the desired coating of polymeric material.

The mould is preferably formed from a material susceptible of shaping by milling, photolithography or dry or wet etching. High precision milling is capable of providing resolution in the μm region whereas photolithography and etching allow for a resolution of below 0.1 μm in terms of depth and of the order of a few μm in directions parallel to the surface being treated. Alternatively or additionally, the mould may be generated by first creating a master using one or more of the techniques described above and then making the mould itself by conforming a compliant polymer to the master surface, the polymer and master then being separated. Suitable compliant polymers include silicones, polyurethanes, polycarbonates and polytetrafluoroethane.
The depth of the recess is preferably substantially uniform. This has advantages in terms of manufacturing simplicity and from the point of view of homogeneity of the polymeric material characteristics across a given coating.

The surfaces of the recess preferably have a low surface roughness. The mould manufacturing techniques described above are typically capable of producing silica mould surfaces having a roughness of 100nm or less in terms of overall depth of irregularities. Low surface roughness reduces adherence of the polymeric material to the mould.

The ridge of the mould may have one or more discontinuities by means of which excess solution may escape from the chamber. The advantage of such a design is the ease of removal of excess solution from the mould and substrate surface prior to polymerisation, as mentioned above.

In a related aspect, the invention also provides the use of a mould as described above for shaping a coating of polymeric material on the surface of a substrate.

In addition, the invention provides a kit of parts comprising a biosensor transducer and a mould for placement against the surface of the transducer to cooperate therewith to define a chamber, the chamber being suitable for the containment, in use, of a solution of one or more polymerisable species which can be caused to undergo polymerisation so as to form a coating of polymeric material on the surface of the transducer.

The invention will now be described in more detail by way of example only and with reference to the appended drawings, of which:

Figure 1 shows a perspective view of a mould according to the invention and fabricated by a wet etching procedure;

Figure 2 shows a transverse section of the mould of Figure 1 adjacent a QCM biosensor transducer in a) perspective view and b) side elevation;
Figure 3 shows the results of an analysis of haemoglobin binding to a MIP coating on a QCM biosensor transducer; and

Figure 4 shows the etch pattern for a multitude of different moulds manufactured on the same silica disc using the wet etching procedure.

DESCRIPTION OF PREFERRED EMBODIMENTS

The mould of the present invention can be manufactured by different high precision fabrication methods, for instance milling or dry or wet etching. For the etching methods, an oxidised (1000°C in air and water vapour) silica disc can be patterned with a photo resist polymer film with the shape desired for the mould. The oxide layer of the silica disc can then be selectively removed by etching (hydrofluoric acid) in the pattern expressed by the photo resist polymer. This means that the mould can be given the depth corresponding to the thickness of the oxide layer, which can be varied between 0.1 μm and a few μm.

If a deeper mould is required, the silica mould can be further processed by etching in potassium hydroxide at 80°C. The KOH solution etches approximately 1 μm per minute and the depth can thereby be given by the incubation time.

A possible shape of the mould is displayed in Figure 1. Preferably, the mould comprises a recess with a shape that corresponds to the shape of the surface that is to be coated. The depth of the recess is important since it governs the thickness of the polymer film. The microfabrication techniques allow for the depth of the mould to be set to between 0.1 and 100 μm. The desired thickness is dependent on analyte and application and on which type of transducer is utilised. The mould 11 comprises a substantially flat surrounding plate 12, a substantially flat raised annular ridge 13 and a recess 14 bounded by the ridge 13.

If the mould is made out of silica, the surface of the created mould is hydrophilic. The polymer, in the case of typical MIPs for biomolecules or particles, is also hydrophilic. Since it is important that the created polymer layer stays on the sensor surface, the
polymer should not adhere significantly to the mould. Adhesion to the mould can be prevented by modifying the mould surface to become hydrophobic, which can be achieved by treatment of the silica surface with hydrophobic silanes such as trifluoropropyltrimethoxysilane or trimethylchlorosilane.

In biosensor applications, the polymer layer has to be well attached to the transducer surface for the sensing principle to work. There are different strategies to achieve this: covalent bonding to the surface, electrostatic attachment and physisorption (utilising mainly Van der Waals forces).

Physisorption is the simplest to accomplish, but requires some care if a stable and reproducible attachment to the surface is to be ensured. If the polymer layer consists of a hydrophilic polymer such as polyacrylamide, stable attachment to a gold-coated surface can be accomplished simply by cleaning the gold surface before the coating process. The cleaning of the gold surface requires relatively harsh washing conditions to remove the organic material that is normally physisorbed to the surface from the environment (often referred to as environmental carbon). Feasible washing methods include dry oxidising methods such as oxygen plasma cleaning, UV/Ozone cleaning and Corona cleaning. Wet cleaning methods include Piranha solution (1:3 v/v 30% H₂O₂:Concentrated H₂SO₄ for ten minutes) or a solution of 1:1:5 30% H₂O₂: Concentrated NH₃: Deionised water, in which the gold surface is immersed for 5 minutes at a temperature of 75°C. All washing methods require extensive rinsing in water and the coating procedure should be started as soon as possible after the washing to prevent adsorption of a new layer of environmental carbon.

Covalent bonding to the surface can be accomplished by using a bifunctional linker which is attached covalently to the surface and has the ability to copolymerise with the polymerisation mixture. Molecules suitable for linking between a gold-coated sensor surface and a polymeric material formed from vinyl-containing monomers, e.g. a polyacrylamide gel, include n-alkylthiol compounds having polymerisable groups, such as vinyl or epoxy groups, located at the opposite end of the molecule to the thiol group. An example of such a molecule is allylmercaptan. The thiol end group of
allylmercaptan spontaneously forms a strong bond to the gold surface, thereby creating a monolayer on the surface. The vinyl end-group can then take part in the polymerisation, forming a multitude of bonds between the gold surface and the polyacrylamide matrix. Many other linkers are possible and should be chosen with regard to the sensor surface material and to the polymerisation mixture. Certain end groups bind to certain surfaces; thiol groups are suitable for gold, silane end-groups bind to glass surfaces and many other surfaces. Plastics surfaces may require activation, e.g. by means of a plasma (such as an oxygen plasma), in order to achieve satisfactory covalent bonding. The end-group intended to bind to the polymer should be a reactive or polymerisable group that can take part in the polymerisation. The reactivity of the group should be similar to or higher than that of the polymerisation mixture.

Electrostatic attachment of the polymer to the sensor surface can be accomplished by introducing charged groups on the sensor surface and introducing oppositely charged groups in the polymer matrix.

The polymerisation mixture is normally composed of a monomer, a crosslinker, a catalyst and a polymerisation initiator and in some cases a template molecule. Possible monomers are acrylamides, agarose, methacrylate, substituted acrylamides, substituted methacrylates, acrylic acid and 3-aminophenylboronic acid. Possible crosslinkers are N,N’-methylenebisacrylamide, piperazinediacrylamide, N,N’-bisacrylylcystamine, N,N’-diallyltartardiamide and glutaraldehyde. A possible initiator and catalyst system is ammoniumpersulphate and TEMED (N,N,N’,N’-methylethlenediamide).

Coating procedure:
The polymerisation mixture is prepared, including all components except the initiator, and is de-airated to remove all oxygen from the mixture (since oxygen is an inhibitor for many polymerisation reactions). The initiator is added to the de-airated solution and is allowed to mix with the solution. A droplet is applied to a sensor area and is spread over the whole surface. The hydrophobically coated mould is placed on the droplet,
thereby defining the thickness of the polymer layer and forcing excess liquid out to the periphery of the mould.

The mould is kept in place until the polymerisation is completed. When the polymerisation is completed the mould is removed and the polymer layer is ready for use or, in some cases, ready for further processing steps.

In some applications, a porous media such as a Kleenex cloth is placed around the mould to extract excess liquid from the polymerisation zone. In order to fixate the mould in its position on top of the sensor surface, a glass weight may be glued to the silica mould. The glass weight helps with placing the mould in the correct position and ensures that the mould comes into firm contact with the sensor surface. This ensures that the polymer layer is formed to the correct thickness.

The interaction between mould and sensor surface is illustrated diagrammatically in Figure 2. The mould 11 is brought into contact by means of the annular ridge 13 with the surface of the sensor transducer, generally indicated 21. The sensor transducer shown is a QCM, comprising a quartz crystal disc 22 bearing gold electrodes 23a,b. The mould 11 is lowered onto the sensor transducer (the transducer having previously had polymerisation mixture applied to the electrode 23a) such that the ridge 13 contacts the quartz disc 22 adjacent the perimeter of the electrode 23a. A chamber is thus formed by the inner wall 13a of the ridge 13, the recess 14, the electrode 23a and a narrow annular portion of the quartz disc 22. Polymeric material is formed within this chamber, thus resulting in the coating of the entire electrode 23a with a polymeric layer of uniform thickness.

Since many polymerisations are inhibited by oxygen, in some applications it may be necessary to perform the coating procedure under an inert atmosphere in order to achieve the correct polymerisation conditions. The inert atmosphere may be nitrogen or argon.
Some polymer coatings, such as the protein MIPs described above, are best kept saturated with water since drying may induce structural changes in the polymer matrix. These structural changes may damage the intended properties of the polymer. The polymerisation mixture and the created polymer may be kept in wet conditions by saturating the surrounding atmosphere with water. The evaporation of water will thereby be reduced, preserving the water content of the polymer or polymerisation mixture.

In some applications, it may be advantageous to make the mould in a porous or perforated material in order to allow for controlled diffusion of a reactant to the polymerisation zone. This reactant may be an inhibitor, which is transferred to the polymerisation mixture in a controlled manner in order to stop the polymerisation in the zone close to the mould, thereby reducing the adhesion of the polymer to the mould. The inhibitor may also be added in order to reduce the film thickness. The controlled diffusion of inhibitor from the mould reduces the thickness of the polymer by inhibiting the polymerisation in the zone closest to the mould.

EXAMPLE - Polyacrylamide molecular imprinted polymer for detection of Haemoglobin

An Attana 100 standard gold coated 10MHz quartz crystal was immersed in a solution of 1:1:5 30% H₂O₂: Concentrated NH₃: Deionised water at 75°C for 5 minutes, in order to remove all organic residues physisorbed to the crystal’s gold surface. Subsequently, the crystal was rinsed extensively in deionised water and was dried in a stream of nitrogen before it was immediately processed further, as described below.

A polymerisation mixture consisting of 112mg acrylamide monomer and 6mg N, N' Methylenebisacrylamide crosslinker was prepared in 1760 μL of phosphate buffer (pH 7.4). TEMED (N,N,N',N'-methylenebridiamide) initiator solution of 5% v/v was added to the polymerisation mixture in an amount of 40 μL. Finally, 160 μL of Haemoglobin solution of 50mg/ml was added to the mixture, following which the solution was thoroughly mixed and de-airated.
A silica mould was prepared with wet etching in potassium hydroxide to receive a depth of 10 μm. The mould had an annular flat ridge (13 of Figure 1) having a width of 0.5mm and extending 10 μm from a flat silica plate (10x10mm², 12 of Figure 1). The inner diameter of the ridge was 5 mm, compared to the 4 mm of the sensor surface of the quartz crystal. A 15 mm glass rod of 6 mm diameter was attached to the non-etched rear of the mould to serve as a handle and to give the mould sufficient weight for the coating process. The mould was washed extensively in ethanol and was then dried in nitrogen. To make the mould more hydrophobic, the mould was incubated in trimethylchlorosilane for 1 hour and was then allowed to dry.

To the polymerisation mixture 40 μL of ammonium persulphate solution (10% by weight in phosphate buffer) was added. The solution was carefully mixed and a 10 μL droplet was applied to the freshly cleaned and dried crystal. The mould was immediately placed on top of the droplet, whereby a 10 μm layer was formed on the sensor surface and excess liquid was forced to the periphery of the crystal. The polymerisation was allowed to finish and the mould was gently removed after 20 minutes. The coated crystal was then rinsed in water.

To remove the haemoglobin template molecule the crystal was washed in sodium dodecylsulphate (SDS) for 24 hours under stirring. The SDS was removed by subsequent washing in deionised water for 24 hours. The crystal was then dried and was inserted in an Attana 100 quartz crystal microbalance analysis system. The analysis system was started with phosphate buffer (pH 7.4) as running buffer and the surface was exposed to subsequent samples containing bovine serum albumin and haemoglobin at 1 mg/ml. With the exception of a small initial saturation of the sensor with BSA, the sensor shows a high specificity towards the haemoglobin samples as shown in Figure 3.

The significant binding of the haemoglobin to the polymer-coated QCM is indicated by the large negative shift in the resonance frequency of the crystal on each application of this protein.

Figure 4 shows how readily a variety of different mould designs can be produced by etching from a single silica disc. In particular, the mould designs need not have circular
recesses, as described above, but may have rectangular 41 or square 42 recesses. The choice of shape of recess will depend largely upon the analytical application. A mould design in which the ridge contains discontinuities to allow escape of excess polymer dispersion or polymerisation mixture is shown at 43.
1. A method of applying a coating of polymeric material to a surface of a substrate, the method comprising the steps of contacting the surface to be coated with a suitable quantity of a solution of one or more polymerisable species and causing the polymerisable species to undergo polymerisation, characterised in that, during polymerisation, the solution and polymeric material formed therefrom are constrained by a mould placed against the surface of the substrate and cooperating therewith to define a chamber.

2. A method according to claim 1 wherein the mould defines a recess having a depth of 0.1 μm to 100 μm.

3. A method according to claim 2 wherein the depth of the recess is 1 μm to 50 μm.

4. A method according to any of claims 1 to 3 wherein the substrate is a biosensor transducer.

5. A method according to any of claims 1 to 4 wherein the quantity of solution which is contacted with the surface to be coated is in excess of the volume of the chamber.

6. A method according to any preceding claim wherein the mould is formed from silica.

7. A method according to claim 6 wherein the surface of the mould, or at least a part of the surface which defines the chamber, is modified to increase its hydrophobicity.

8. A method according to claim 7 wherein the modification is with hydrophobic silanes.
9. A method according to claim 8 wherein the silane is selected from trifluoropropyltrimethoxysiliane and trimethylchlorosilane.

10. A method according to any preceding claim wherein the polymeric material is attached to the surface of the substrate by covalent bonding, electrostatic forces and/or physisorption.

11. A method according to claim 10 wherein the covalent bonding of the polymeric material to the surface is obtained by the attachment of a bifunctional linker, capable of reacting with one or more of the polymerisable species, to the surface prior to polymerisation.

12. A method according to claim 11 wherein the bifunctional linker is selected from thiol-containing compounds and silane-containing compounds.

13. A method according to any preceding claim wherein the solution, prior to polymerisation, comprises one or more species of polymerisable monomers, a cross-linking agent and a catalyst and/or a polymerisation initiator.

14. A method according to claim 13 wherein the monomer is selected from acrylamides, agarose, methacrylate, substituted acrylamides, substituted methacrylates substituted or unsubstituted ethacrylates, substituted or unsubstituted acrylates and 3-aminophenylboronic acid.

15. A method according to claim 13 or claim 14 wherein the cross-linking agent is selected from \textit{N,N'-methylenebisacrylamide}, piperazinediacylamide, \textit{N,N'-bisacrylylcystamine}, \textit{N,N'-diallyltartardiamide} and glutaraldehyde.

16. A method according to any of claims 11 to 15 wherein the initiator is ammonium persulphate.

17. A method according to any of claims 11 to 16 wherein the catalyst is \textit{N,N,N',N'-methylenelethylenediamide}. 
18. A method according to any of claims 1 to 13 wherein the polymerisable species are suitable for polymerisation to form cross-linked polyalkylene glycols, cross-linked polypeptides, cross-linked polyethylene imine or cross-linked carbohydrates.

19. A method according to any preceding claim wherein one or more of the polymerisable species contains a functional group suitable for subsequent coupling of a biomolecule to the polymeric material.

20. A method according to claim 19 wherein the functional group is selected from carboxyl groups, amino groups, hydroxy groups, vinyl groups, aldehyde groups and thiol groups.

21. A method according to any preceding claim wherein the polymeric material is substantially saturated with water so as to form a hydrogel.

22. A method according to any preceding claim wherein the surface of the substrate is brought into contact with the solution by dispensing the solution onto the surface, the mould being subsequently placed against the surface of the substrate.

23. A method according to any preceding claim wherein the polymerisation is carried out under an inert atmosphere.

24. A method according to any of claims 1 to 23 wherein the solution and/or the polymeric material formed therefrom are maintained in a water-saturated atmosphere.

25. A method according to any preceding claim wherein the mould comprises a porous or perforated material.
26. A method according to claim 25 wherein an inhibitor compound is added to the mould and diffuses therefrom to inhibit polymerisation of the solution in the region adjacent the mould.

27. A method according to any preceding claim wherein the solution includes one or more species of template molecules or particles for the formation of a molecular imprinted polymeric material.

28. A method according to claim 27 wherein one of the template molecules is a polypeptide.

29. A method according to claim 27 or claim 28 wherein one of the template molecules or particles is a polynucleotide, carbohydrate, virus or microorganism.

30. A method according to any of claims 27 to 29 wherein the polymeric material is subjected to a washing step following polymerisation in order to remove the template molecules or particles.

31. A method for coating a surface of a biosensor transducer with a polymeric material, characterised in that a mould is used to shape the polymeric material on the surface.

32. A method of applying a coating of polymeric material to a surface of a substrate, the method comprising the steps of contacting the surface to be coated with a suitable quantity of a liquid or semi-solid dispersion of the polymeric material and allowing the polymeric material to adhere to the surface, characterised in that, whilst the polymeric material is undergoing adhesion to the surface, a mould is placed against the surface of the substrate and cooperates therewith to define a chamber by means of which the polymeric material is shaped.
33. A method according to claim 32 wherein the dispersion of the polymeric material is substantially non-cross-linked, a cross-linking agent and a catalyst and/or a polymerisation initiator being added once the dispersion has been brought into contact with the substrate surface.

34. A substrate having a coating of a polymeric material applied to one or more of its surfaces and obtainable by a method according to any of claims 1 to 32.

35. A mould for use in a method according to any of claims 1 to 32, the mould comprising a block having formed in one or more surfaces a recess bounded by a ridge, the ridge being capable of contact with the surface of a substrate such that a chamber may be defined by the surfaces of the recess and the surface of the substrate, the depth of the recess, measured from the level of the ridge, being between 0.1 \( \mu \text{m} \) and 100 \( \mu \text{m} \) and the shape of the recess corresponding with the shape of the desired coating of polymeric material.

36. A mould according to claim 34 wherein the block is formed from a material susceptible of shaping by milling, photolithography or dry or wet etching.

37. A mould according to claim 34 or claim 35 wherein the material of the mould is silica.

38. A mould according to any of claims 34 to claim 36 wherein the depth of the recess is substantially uniform.

39. A mould according to any of claims 34 to 37 wherein the surfaces of the recess have a low surface roughness.

40. A mould according to any of claims 34 to 38 in which the ridge has one or more discontinuities by means of which excess solution may escape from the chamber.
41. A mould according to any of claims 34 to 39 in which the material of the mould is porous or perforated.

42. A mould according to claim 40 containing a polymerisation-inhibiting compound capable, in use, of diffusion from the mould into the solution adjacent the surfaces of the recess.

43. A mould according to any of claims 34 to 41 wherein one or more of the surfaces of the recess of the mould is modified to increase its hydrophobicity.

44. A mould according to any of claims 34 to 42 wherein the depth of the recess is 1 μm to 50 μm.

45. Use of a mould according to any of claims 34 to 43 for shaping a coating of polymeric material on the surface of a substrate.

46. A kit of parts comprising a biosensor transducer and a mould for placement against the surface of the transducer to cooperate therewith to define a chamber, the chamber being suitable for the containment, in use, of a solution of one or more polymerisable species which can be caused to undergo polymerisation so as to form a coating of polymeric material on the surface of the transducer.
Figure 1
Figure 3

Haemoglobin Selective Polymer Coating on QCM

![Graph showing frequency vs time with BSA and bovine haemoglobin injections at specific time points.](image-url)
A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 B05D1/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N B05D

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search

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Date of mailing of the international search report

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