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(54) Title: MOLECULARLY IMPRINTED SCINTILLATION POLYMERS

(57) Abstract: A molecularly imprinted polymer having specific binding sites, which polymer compriese at least one component for energy transfer located in proximity to said binding sites. There is also described a method of preparing and the use of such a molcularly impritned polymer.

MOLECULARLY IMPRINTED SCINTILLATION POLYMERS

Field of the invention

The present invention relates to a molecularly imprinted polymer having specific binding sites, a method of preparing a molecularly imprinted polymer having specific binding sites and use of a molecularly imprinted polymer according to the invention.

Background of the invention

Specific binding materials are of critical importance in the development of sensors and assays. Biological antibodies, receptors and single strand DNA segments display very high binding affinity and specificity towards the corresponding antigens, agonists/antagonists and complementary DNA sequences. These biological binders have been widely used in sensitive detection of target analytes, both for clinical purposes and in drug developments. Since biomacromolecules are not stable, and in many cases are difficult to produce, synthetic materials that mimic the binding characteristics of these biomolecules have been intensely studied to provide useful affinity materials for applications in separation and analysis.

Among the most practicable approaches is molecular imprinting of synthetic polymers. By molecular imprinting, co-polymerisation of functional monomers and cross-linking monomers is carried out in the presence of a molecular template, which results in a rigid polymer matris embedding the template. Removal of the template reveals binding sites specific to the template or its close analogues. Molecularly imprinted polymers are much more stable than biological receptors, and much easier to produce. They have great potential to replace or supplement biological receptors in all affinity-related applications.

In molecular imprinting, the assembly of the template-functional monomer complex prior to and during the

polymerisation reaction, as well as the re-binding of the template by the obtained polymer is driven by various molecular interactions between the template and the functional monomers.

Wulff and Poll has described a method of using reversible covalent binding for molecular imprinting of an optically active compound, as well as the use of the polymer for separation of an optically active antipode from a racemate mixture (Wulff, G.; Poll, H.-G. Makromol. Chem. 1987, 188, 741-748). US patent 5310648 describes the use of metal chelating functional monomers for preparation of an imprinted polymer matris. More favourably, non-covalent interactions have been used in the PCT applications WO 93/09075 and WO 98/07671 for preparation of a chiral solid-phase chromatography material containing molecular imprints of an optically pure enantiomer to be separated. In addition to separation, special interests have been focused on using imprinted polymers for the development of sensors and assays, in which synthetic polymers are used to replace biological macromolecules such as antibodies and receptors. In this way the lifetime of the sensors and the affinity reagents for assays are greatly increased, whereas production costs are reduced.

PCT application WO 94/11403 describes a method for producing molecularly imprinted polymers as artificial antibodies, and methods for using these artificial antibodies in therapeutic and diagnostic applications. Analogous to the heterogeneous immunoassays, the amount of bound, radioisotope-labeled analyte is quantified after separation of the unbound fraction by centrifugation or filtration. The separation steps are tedious and difficult to automate, which prevents high throughput in handling large amount of samples. It is desirable that no separation step is used when detecting a target analyte with a molecularly imprinted polymer.

The prerequisite of separation when using imprinted polymers in assays is due to the fact that binding of the analyte does not induce any directly detectable physico-chemical changes in the polymeric materials. Imprinted polymers are only used as affinity adsorbents to specifically isolate the target analyte, which is then quantified using routine analytical techniques. However, when imprinted polymers are put in physical contact with appropriate transducers, binding of a target analyte causes physicochemical responses (change in mass, resistance, capacitance, refractive index etc.) that are translated into various sensor signals. US patent 5910286 describes a chemical sensor comprising an acoustic wave transducer and a layer of molecularly imprinted polymer. Binding of the target molecule to the polymer layer affects the propagation of the acoustic wave in a medium, whereby a sensor response is obtained.

Physical attachment of imprinted polymers to transducers is easy to carry out. However, this often leads to sensors showing rather poor selectivity, since non-specific binding also leads to generation of sensor signal. In a more sophisticated manner, imprinted polymers generate sensor signals only when a target is specifically bound to the polymers. This is obtainable by using a specialised functional monomer that either releases an indicator, or displays an altered optical characteristic upon binding of the target molecule. US patent 6063637 describes sensors for use in detecting sugars. The sensor is composed of a metal complex that binds to the target molecule and releases a proton. Measurement of the released proton provides an indirect indication of the target molecule concentration. PCT application WO 96/41173 describes use of fluorescent functional monomers for signal generation. Binding of the target analyte changes fluorescent characteristic of the imprinted polymer, which is detected with a fluorescent spectrophotometer. However, these specialised functional monomers are

scarcely applicable to a broad range of different target analytes, to which however many "universal" functional monomers have demonstrated to provide satisfactory binding specificity.

Another way to circumvent the separation requirement is to utilise the principle of proximity energy transfer, more specifically proximity scintillation. PCT application WO 91/08489 describes a support body for use in scintillation proximity radioimmunoassay, the support body being constructed of a scintillation material having biological binders coupled to its surface such as antigens, antibodies, etc, which are capable of selective binding of a target analyte. Several patents and patent-applications (US 4271139; US 4568649; EP 1007971 A1) have been related to embodiments of the technique using various scintillation materials and assay formats. The selective binding materials in these patents and patent applications are all derived from biological macromolecules such as antibodies, receptors, enzymes, etc. One major disadvantage is, however, that the biological binding materials are unstable and not easy to produce. It is highly desirable that more stable binding materials are used to replace the biological macromolecules in scintillation proximity assays. Although molecularly imprinted polymers have been used in many analytical applications, their use in proximity scintillation assay has been difficult to realize.

Summary of the invention

The present invention relates in a first aspect to a molecularly imprinted polymer having specific binding sites, which polymer comprises at least one component for energy transfer located in proximity to said binding sites.

In one embodiment the component for proximity energy transfer is chemically incorporated into the polymer. In another embodiment the component for proximity energy transfer is bound to the surface of the polymer.

In a further embodiment, said component is a scintillator. In yet another embodiment, said scintillator comprises a reactive group. In still another embodiment, said reactive group comprises at least one C=C bond.

In one embodiment the scintillator is 2,5-diphenyloxazole or a derivative thereof.

In another embodiment, said reactive group comprises at least one of the groups -COOH, -CHO, -OH and $-NH_2$.

In a further embodiment the polymer is an organic polymer comprising as a main component at least one polymer chosen from the group comprising polyacrylate, polystyrene, polyurethane, polyanaline and polyamide. In yet another embodiment the polymer is an inorganic polymer obtained from alkoxides of silicon, aluminum or titanium.

In one embodiment the polymer has a configuration chosen from the group comprising monolith, irregular particles, thin films, membranes, microspheres and beads. In another embodiment the polymer has been polymerised in situ within wells of a microtitre plate or on a microchip.

In still another embodiment the polymer comprises two scintillators.

In yet another embodiment the polymer comprises an aromatic substance, which assists in exciting the component for proximity energy transfer.

In a further embodiment the distance between the component for proximity energy transfer and the binding sites of the polymer is within the range 0-50 μm.

In another aspect the invention relates to a method of preparing a molecularly imprinted polymer having specific binding sites, comprising

incorporation into and/or conjugation with the polymer of a component for proximity energy transfer.

In one embodiment, said method comprises the steps:

copolymerisation of functional monomers in the prescence of at least one template molecule using conven-

tional polymerisation techniques, which monomers comprise at least one component for proximity energy transfer; and

removal of the template molecule.

In another embodiment, said method comprises the steps:

copolymerisation of functional monomers and at least one reactive monomer carrying an optionally protected, reactive group in the prescence of at least one template molecule using conventional tecniques;

chemical conjugation of the reactive group of the polymer and a reactive group of the component for proximity energy transfer; and

removal, before or after said conjugation, of the template molecule.

In yet another embodiment the component used for proximity energy transfer is a scintillator. In still another embodiment the scintillator comprises at least one C=C bond. In a further embodiment the scintillator is 2,5-diphenyloxazole or a derivative thereof.

In one embodiment the reactive group of the scintillator is chosen from the group comprising -COOH, -CHO, -OH and $-NH_2$.

In another embodiment the reactive group of the monomer is chosen from the group comprising -COOH, -CHO, -OH and $-NH_2$.

In still another embodiment the polymerisation is performed so that a polymer with a configuration chosen from the group comprising monolith, irregular particles, thin films, membranes, microspheres and beads is obtained. In yet another embodiment the polymerisation is performed in situ within the wells of a microtitre plate or on a microchip.

In one embodiment two different scintillators are in use. In another embodiment an aromatic substance, which assists in exciting the component for proximity energy transfer, is incorporated into the polymer. In still another embodiment the incorporation of the second scin-

tillator and/or the aromatic substance is obtained by physical absorption or by chemical linkage.

In still another aspect the invention relates to use of a molecularly imprinted polymer according to the invention or a polymer prepared according to a method of the invention in a proximity scintillation assay.

In yet another aspect the invention relates to use of a molecularly imprinted polymer according to the invention or a polymer prepared according to a method of the invention for screening of combinatorial libraries.

In a further aspect the invention relates to use of a molecularly imprinted polymer according to the invention or a polymer prepared according to a method of the invention for use in sensors.

In a last aspect the invention relates to use of a molecularly imprinted polymer according to the invention or a polymer prepared according to a method of the invention for in situ monitoring of radioactive metabolites or enzymatic reactions.

In one embodiment of the use of a molecularly imprinted polymer according to the invention or a polymer prepared according to a method of the invention an imaging system is used for quantifying the fluorescence signal. In another embodiment the imaging system is a Charge Coupled Device (a CCD camera).

In another embodiment of the use of a molecularly imprinted polymer according to the invention or a polymer prepared according to a method of the invention, arrays of photomultiplier tubes are used for scintillation counting.

Brief description of the drawings

Figure 1 schematically shows the use of a molecularly imprinted scintillation polymer according to the present invention for detection of a target analyte.

Figure 2 shows examples of functional monomers and cross-linking monomers used in preferred embodiments of the present invention.

Figure 3 shows examples of scintillators used in preferred embodiments of the present invention.

Figure 4 shows binding of tritium-labeled (S)-propranolol to increasing amounts of a molecularly imprinted scintillation polymer and a control polymer in an aromatic solvent.

Figure 5 shows binding of tritium-labeled (S)-propranolol to increasing amount of a molecularly imprinted scintillation polymer and a control polymer in an aqueous solvent.

Figure 6 shows a displacement assay of (S)-propranolol with a molecularly imprinted scintillation polymer in an aromatic solvent. ΔCPM is the decrease in proximity scintillation counts (counts per minute) caused by the unlabeled analyte.

Figure 7 shows a displacement assay of (S)-propranolol with a molecularly imprinted scintillation polymer in an aqueous solvent. CPM and CPM_0 are the proximity scintillation counts (counts per minute) in the presence and absence of the unlabeled analyte respectively.

Detailed description of the invention

The present invention relates to molecularly imprinted scintillation polymers carrying a "universal reporter" group. More particularly, the reporter is a scintillator that is chemically bound to the imprinted polymer in proximity to the specific binding sites. Signal generation is based on the principle of proximity scintillation. When a radioisotope-labeled analyte binds to the polymer, the analyte is brought into close enough proximity to a scintillator for the β- or γ-radiation from the radioactive decay to stimulate the scintillator to emit fluorescence. In contrast, the unbound labeled analyte is too far away from the scintillator and unable to induce any fluorescence. In the present invention the scintillator used does virtually not affect the binding of the target analyte by the functional monomer, but it rather senses the binding event by which the radioactive

analyte is brought close. The scintillator can, because of this, be combined with various functional monomers, and is therefore applicable to a large variety of target analytes. The scintillator is compatible with both the covalent and the non-covalent imprinting strategies.

The imprinted scintillation polymers are useful in two aspects: (1) in a direct detection or quantification of a target analyte that co-exists with other molecules in a radioisotope-labeled sample; (2) in a competitive assay of a target analyte wherein a radioisotope-labeled analyte or its analogue is used as a tracer, whose binding is inhibited by the unlabeled target analyte in a sample.

The molecularly imprinted scintillation polymers according to the invention comprise tailor-made recognition sites obtained by molecular imprinting and at least one scintillator for generation of the binding signal. The scintillator can be chemically incorporated into or bound to the surface of the polymer matris and is in close proximity (typically within 50 μm) to the recognition sites. Figure 1 schematically shows the principle of an application of the molecularly imprinted scintillation polymers for detection of a template molecule, or a molecule closely related to the template.

The recognition sites, or specific binding sites of the polymer are obtained by polymerising functional monomers and, optionally, cross-linking monomers, in the presence of a template molecule, whereby non-covalent or covalent interactions are formed between said functional monomers and said template molecule, and removing said template from the molecularly imprinted polymer. The recognition sites are utilised to specifically bind a target analyte of interest, where said target is the same template molecule, or is a molecule closely related to the template. Examples of functional monomers and cross-linking monomers are listed in figure 2.

In the present invention, the term scintillator can be defined as a phosphorescent or fluorescent molecule that generates a flash of light when excited by an ionising particle such as a β-particle or a photon. In this case the reactive group of the scintillator contains a C=C bond, so that the scintillator can be co-polymerised into the matrix of an imprinted polymer during the imprinting reaction. Preferably the scintillator is a derivative of 2,5-diphenyloxazole. By scintillator is also meant a scintillant molecule that has a reactive group, whereby the reactive group is used for chemical immobilisation of the scintillant molecule on the polymer matris. In this later case the reactive group is e.g. $-NH_2$, -OH, -COOH, or -CHO, which can be used for chemical immobilisation of the scintillator on a previously synthesised molecularly imprinted polymer. Said chemical immobilisation reactions can be carried out prior to, or after removal of the template molecules from the polymers. Figure 3 shows examples of scintillator.

In the present invention the term polymer covers both organic and inorganic polymers. Examples of organic polymers are those based on polyacrylate, polystyrene, polyanaline and polyurethane. Said polymers may be cross-linked to various extents. The polymers are obtained by conventional polymerisation reactions, for example free radical polymerisation or condensation polymerisation. An example of an inorganic polymer is a silica gel obtained by hydrolysis of precursor monomers e.g. alkoxysilanes that are commonly used for preparation of silica particles.

For optimal signal generation, a secondary scintillator can be incorporated into the imprinted polymer, or admixed with the imprinted scintillation polymer used in an assay. The secondary scintillator is a phosphorescent or fluorescent molecule that is excited by the primary scintillator, and emits a flash of light at a longer wavelength. Typical secondary scintillators are those

commonly used in liquid scintillation counting, or derivatives of them containing a reactive group. Chemical incorporation of said secondary scintillator can be carried out in the same step as that in which the primary scintillator is incorporated, or achieved in separate post-imprinting steps.

The present invention also provides a further method for introducing an aromatic substance into molecularly imprinted scintillation polymers. Said base component assists to transfer the radioisotope decay into a short wavelength radiation, which is able to excite the primary scintillator for generation of a fluorescence signal. Said aromatic substance may be chemically linked to the imprinted polymer, typically by co-polymerisation of aromatic monomers or cross-linking monomers during the imprinting reaction, or be physically absorbed into the polymer matris. The aromatic substance is typically an aromatic solvent, when it is physically absorbed into the polymer matris. This is especially useful when the measurements are carried out in a non-aromatic solvent. Said aromatic solvent is confined within the polymer matris when the latter is transferred into the assay solvent, e.g. an aqueous or a highly polar organic solvent. When the measurement is carried out in an aromatic solvent, for example in toluene, the solvent itself may serve as the base component.

Thus, the present invention relates to molecularly imprinted scintillation polymers comprising:

(a) Specific binding sites generated by a molecular imprinting reaction. The molecular interactions driving the assembly of functional monomer – template complex during the imprinting, and recognition of the target analyte by the imprinted polymer may be covalent, non-covalent, or a combination of both.

(b) At least one scintillator covalently fixed in close proximity (within 50 μm) to the specific binding sites of the imprinted polymer. Chemical fixation of the

scintillator is obtained in two ways: (1) by introducing a scintillator into an imprinting mixture, so that the scintillator is co-polymerised with the other monomers during the imprinting reaction; (2) by chemical immobilisation of a reactive scintillator to a previously synthesised molecularly imprinted polymer.

During the co-polymerisation of a scintillator with other imprinting components, the scintillator does not interfere with the functional monomer-template interaction, and is randomly incorporated into the growing polymer chain. The imprinting reaction may be a free radical reaction, an ionic reaction, an oxidation-reduction, an electrochemical reaction, or other polymerisation reactions including hydrolysis polymerisation of inorganic precursor monomers. The imprinting polymerisation may be initiated by heat, UV radiation, γ-radiation, electrochemical potential, acid hydrolysis, or by other chemical means. After the polymerisation, the template is removed and the obtained polymer is worked up following standard procedures.

To improve signal detection, said scintillator is optionally a combination of a primary and a secondary scintillator. Alternatively, the secondary scintillator is incorporated into said polymer, which has a chemically bound primary scintillator, by using the physical absorption method described above. Optionally an aromatic monomer, more specifically styrene or divinylbenzene, is used in the imprinting reaction to provide a chemically linked base component for better scintillation response in non-aromatic solvents. The aromatic monomer may be the same as the functional monomer or the cross-linker. Alternatively, the optional base component may be co-impregnated with the secondary scintillator into the imprinted polymer by using the physical absorption method.

For the chemical immobilisation of a scintillator on an imprinted polymer, an imprinted polymer is initially synthesised. The imprinted polymer may carry additional

reactive groups that can be used for coupling of a scintillator. Alternatively a small fraction of binding groups in the imprinted polymer is used for coupling of the scintillator. The coupling reaction may be carried out prior to, or after removal of the template molecule from the imprinted polymer. Optionally, a secondary scintillator and an aromatic substance are also immobilised in the same way.

The molecularly imprinted scintillation polymers of the present invention are synthesised in various configurations including monoliths, irregular particles, microspheres, membranes, films, and monolayers. The imprinted polymers can also be synthesised in situ in microtitre plate wells. The imprinting reactions are typically similar to those of established ones, except that either the imprinted polymer is further treated by chemical or physical means to incorporate scintillators, or the scintillator is incorporated into the polymer chains during the imprinting reaction.

The molecularly imprinted scintillation polymers of the present invention can be synthesised in the form of microparticles or microspheres. Preferably, the microparticles and microspheres then have an average diameter of 0.01-10 μm. The imprinted polymers are synthesised using a precipitation polymerisation method described in PCT application WO 00/041723.

The imprinted scintillation polymers of the present invention can also be synthesised in situ in microtitre plate wells or on microchips. The polymers may be in the form of continuous films or separate spots. More specifically the thickness of the polymer layer is controlled during preparation to be less than 50 μm.

In one embodiment of the invention the obtained polymer is used for detection of a target analyte present in a radioisotope-labelled sample, or in a displacement assay of a target analyte using a radioisotope-labelled probe. In many cases the radioactive probe may be the

labelled analyte or its analogue. For these purposes the target analyte, or an analogue of the analyte is used as the template for synthesising the molecularly imprinted scintillant polymer, so that binding sites specific to the target analyte are generated in the imprinted polymer. Binding of the radioactive analyte or probe induces fluorescence emission, which can be quantified with standard scintillation counter, or analysed with appropriate imaging systems.

In another embodiment of the invention the obtained polymer in microtitre plate format is used for analysing large amount of samples. The imprinted scintillation polymers are deposited in microtitre plate wells, to which the samples to be analysed are added. Direct detection of the radioactive analyte, or quantification of displacement of the radioactive probe by the target analyte, can be carried out with arrays of photomultiplier tubes or with a Charge Coupled Device (a CCD camera).

In a further embodiment of the invention the imprinted scintillation polymers are used for screening combinatorial libraries, where the polymers having specific binding sites for an agonist, an antagonist, or an enzyme inhibitor, are used to replace biological receptors for the affinity-based screening experiments. The radioisotope-labelled agonist, antagonist, or inhibitor is used as the probe to search for potential leads that bind to the imprinted polymers. High throughput screening is obtainable using arrays of photomultiplier tubes or imaging systems such as a CCD camera.

In yet another embodiment the scintillation polymer is used for in situ monitoring of radioactive metabolites or enzymatic reactions, where the imprinted scintillation polymer, specific to an intermediate or a product, is used as a chemosensor for monitoring the specific compounds.

Experimental part

Synthesis of scintillation monomers

Scintillation monomers are synthesised from 2,5-diphenyloxazole derivatives carrying a reactive group. The scintillation monomers are co-polymerised into the polymer matris during an imprinting reaction.

Example 1

Synthesis of 4-hydroxymethyl-2,5-diphenyloxazole acrylate (scintillator 6, see Figure 3)

4-hydroxymethyl-2,5-diphenyloxazole (scintillator 2, see Figure 3) is synthesised according to a literature method (Hamerton, et al., Chem. Mater. 2000, 12, 568).

4-hydroxymethyl-2,5-diphenyloxazole (1.9 g, 7.57 mmol) and triethylamine (0.917 g, 9.084 mmol) are dissolved in dichloromethane (30 ml). The solution is cooled in an ice water bath while acryloyl chloride (0.821 g, 9.084 mmol) is slowly added during stirring. The solution is stirred at 0°C for 2 h, after which it is washed with 1 M HCl (30 ml). The organic layer is separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product is purified on a silica column using ethyl acetate to yield colourless crystals (0.784 g, 34%). ^1H NMR ($CDCl_3$): δ (ppm) 8.10-8.20 (m, 2H, aromatic), 7.70-7.77 (m, 2H, aromatic), 7.36-7.56 (m, 6H, aromatic), 6.50 (dd, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.4 Hz, *trans*-$CH=CH_2$), 6.21 (dd, 1H, J_{trans} = 17.3 Hz, J_{cis} = 10.4 Hz, -$CH=CH_2$), 5.88 (dd, 1H, J_{cis} = 10.4 Hz, J_{gem} = 1.4 Hz, *cis*-$CH=CH_2$), 5.39 (s, 2H, -CH_2O-).

Synthesis of molecularly imprinted microparticles carrying a co-polymerised scintillator

Non-covalent interactions are utilised for preparing a molecularly imprinted scintillation polymer. A scintillator is incorporated by co-polymerisation together with the functional monomer and the cross-linking monomer. A precipitation polymerisation method is used to generate discrete polymer microparticles.

Example 2

Synthesis of a molecularly imprinted scintillation polymer (MISP 1) specific for (S)-propranolol

(S)-propranolol (0.784 mmol), methacrylic acid (1.567 mmol), trimethylolproprane trimethacrylate (1.567 mmol), 4-hydroxymethyl-2,5-diphenyloxazole acrylate (0.784 mmol) and α,α´-azoisobutyronitrile (0.152 mmol) are dissolved in anhydrous toluene (45 ml). The solution is saturated with dry nitrogen gas, and sealed under a nitrogen atmosphere. The solution is transferred into a water bath pre-set at 60°C to initiate the free radical polymerisation. The reaction continues at 60°C for 16 h. Following the polymerisation, the polymer microparticles are dissected by brief ultrasonic treatment and collected by centrifugation. (S)-propranolol is removed by repeated solvent extraction of the polymer in methanol containing 10% acetic acid. The polymer microparticles are finally washed with acetone and dried in vacuum. A non-imprinted scintillation polymer (NISP 1) is synthesised under identical condition except omission of the template, (S)-propranolol.

The obtained polymer microparticles have an average diameter of 0.6-1 μm, which is determined by scanning electron microscopy. Surface areas of the microparticles are approximately 7 m^2g^{-1}, which is determined by nitrogen absorption measurement. Elemental analysis confirms that the imprinted polymer (MISP 1) and non-imprinted polymer (NISP 1) contain approximately equal amounts of scintillator (Table 1).

TABLE 1

Elemental analysis of scintillation polymers

Polymer	C (%)	H (%)	N (%)
MISP 1	63.2	7.7	0.79
NISP 1	63.1	7.5	0.80

Example 3

Synthesis of a molecularly imprinted scintillation polymer (MISP 2) specific for (S)-propranolol

(S)-propranolol (0.602 mmol), methacrylic acid (0.784 mmol), trimethylolproprane trimethacrylate (0.784 mmol), 4-hydroxymethyl-2,5-diphenyloxazole acrylate (0.157 mmol) and α,α´-azoisobutyronitrile (0.073 mmol) are dissolved in anhydrous toluene (40 ml). The solution is saturated with dry nitrogen gas, and sealed under a nitrogen atmosphere. The solution is transferred into a water bath pre-set at 60°C to initiate the free radical polymerisation. The reaction continues at 60°C for 16 h. Following the polymerisation, the polymer microparticles are dissected by brief ultrasonic treatment, and collected by centrifugation. (S)-propranolol is removed by repeated solvent extraction of the polymer in methanol containing 10% acetic acid. The polymer microparticles are finally washed with acetone and dried in vacuum. A non-imprinted scintillation polymer (NISP 2) is synthesised under identical condition except omission of the template, (S)-propranolol.

The obtained polymer microparticles have an average diameter of 0.6-1 μm, which is determined by scanning electron microscopy. The surface areas of the microparticels are approximately 7 m^2g^{-1}, which is determined by nitrogen absorption measurement. Elemental analysis confirms that the imprinted polymer (MISP 2) and non-imprinted polymer (NISP 2) contain approximately equal amount of scintillator (Table 2).

TABLE 2

Elemental analysis of scintillation polymers

Polymer	C (%)	H (%)	N (%)
MISP 2	62.4	7.6	1.3
NISP 2	62.8	7.6	1.1

Synthesis of molecularly imprinted microspheres comprising a co-polymerised scintillation monomer and an aromatic substance

Non-covalent interactions are utilised for preparing a molecularly imprinted scintillation polymer. A scintillation monomer and an aromatic substance are incorporated by co-polymerisation with the functional monomer and/or the cross-linking monomer. A precipitation polymerisation method is used to generate discrete polymer microspheres.

Example 4

Synthesis of a molecularly imprinted scintillationpolymer (MISP 3) specific for (S)-propranolol, having divinylbenzene as the aromatic substance

(S)-propranolol (0.529 mmol), methacrylic acid (1.05 mmol), divinylbenzene (4.20 mmol), 4-hydroxymethyl-2,5-diphenyloxazole acrylate (0.262 mmol) and α,α´-azoisobutyronitrile (0.097 mmol) are dissolved in anhydrous acetonitrile (40 ml). The solution is saturated with dry nitrogen gas, and sealed under a nitrogen atmosphere. The solution is transferred into a water bath pre-set at 60°C to initiate the free radical polymerisation. The reaction continues at 60°C for 16 h. After the polymerisation, the polymer microspheres are collected by centrifugation. (S)-propranolol is removed by repeated solvent extraction of the polymer in methanol containing 10% acetic acid. The polymer microspheres are finally washed with acetone and dried in vacuum. A non-imprinted polymer (NISP 3) is synthesised under identical condition except omission of the template, (S)-propranolol.

The obtained polymers are in the form of microspheres having an average diameter of 0.6-2.8 μm, which is determined by scanning electron microscopy.

Example 5

Synthesis of a molecularly imprinted scintillation polymer (MISP 4) specific for (S)-propranolol, having styrene as the aromatic substance

(S)-propranolol (0.602 mmol), methacrylic acid (0.392 mmol), styrene (0.392 mmol), trimethylolproprane trimethacrylate (0.784 mmol), 4-hydroxymethyl-2,5-diphenyloxazole acrylate (0.157 mmol) and α,α´-azoisobutyronitrile (0.073 mmol) are dissolved in anhydrous acetonitrile (40 ml). The solution is saturated with dry nitrogen gas, and sealed under a nitrogen atmosphere. The solution is transferred into a water bath pre-set at 60°C to initiate the free radical polymerisation. The reaction continues at 60°C for 16 hours. After the polymerisation, the polymer microspheres are collected by centrifugation. (S)-propranolol is removed by repeated solvent extraction of the polymer in methanol containing 10% acetic acid. The polymer microspheres are finally washed with acetone and dried in vacuum. A non-imprinted scintillation polymer (NISP 4) is synthesised under identical condition except omission of the template, (S)-propranolol.

Immobilisation of the scintillator on molecularly imprinted microspheres

Molecularly imprinted scintillation polymers are prepared by immobilisation of a reactive scintillator on previously synthesised molecularly imprinted polymers.

Example 6

Synthesis of molecularly imprinted microspheres carrying a reactive moiety for coupling of a scintillator (MIP 1)

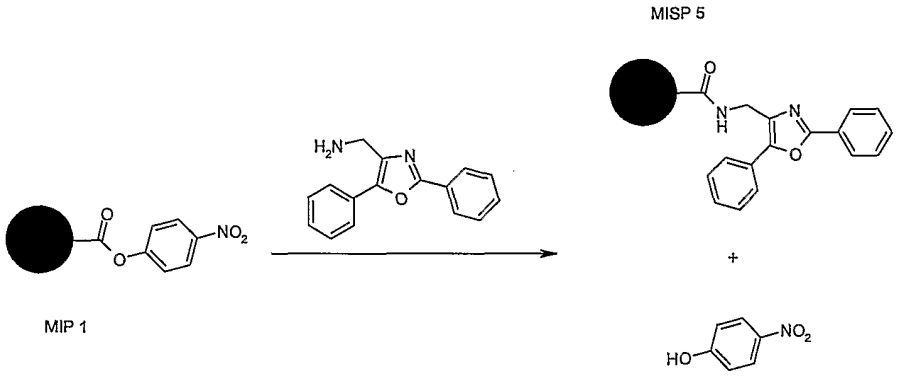
To a borosilicate glass tube are added 17β-estradiol (0.734 mmol), methacrylic acid (1.884 mmol), trimethylolproprane trimethacrylate (1.884 mmol), 4-nitrophenylacrylate (0.942 mmol) and 2-hydroxy-2-methyl-1-phenyl propan-1-one (1.0 mmol) dissolved in anhydrous acetonitrile (40 ml). The solution is saturated with dry nitrogen gas, and sealed under a nitrogen atmosphere. At 20°C, the reaction mixture is exposed to UV irradiation at 350 nm for 4 h. Following the polymerisation, the polymer microspheres are collected by centrifugation. The polymer microspheres are washed with acetonitrile and finally dried in vacuum. A non-imprinted polymer (NIP 1) is synthesised under

identical condition except omission of the template, 17β-estradiol.

Example 7

Preparation of molecularly imprinted scintillation microspheres (MISP 5) and non-imprinted scintillation microspheres (NISP 5) by immobilization of a scintillator on MIP 1 and NIP 1 via the reactive moieties

4-Aminomethyl-2,5-diphenyloxazole (scintillator 3, see Figure 3) is coupled to the molecularly imprinted microspheres (MIP 1) using the following reaction:



The molecularly imprinted microspheres (MIP 1, synthesised in Example 6) (200 mg) are suspended in 12 ml of 1 M solution of a reactive scintillator, 4-aminomethyl-2,5-diphenyloxazole dissolved in acetonitrile. The suspension is gently stirred at 20°C for 24 h. The polymer microspheres are separated by centrifugation and repeatedly washed with methanol containing 10% acetic acid (v/v) to remove the template molecule. The polymer microspheres are subsequently washed with acetone and dried in vacuum. No yellow colour is observed when the treated microspheres are added to 1 M NaOH, which confirms complete immobilisation of the scintillator. The non-imprinted scintillation microspheres (NISP 5) are prepared similarly by treatment of the non-imprinted microspheres (NIP 1).

Synthesis of molecularly imprinted scintillation polymers using sol-gel chemistry

Molecularly imprinted inorganic polymers are synthesised by hydrolysing functional organosilane monomers and tetraethoxysilane in the presence of a template molecule. A scintillation monomer, ICPS-PPO adduct (scintillator 10) is incorporated during the imprinting reaction.

Example 8

Synthesis of surface-imprinted scintillationsilica particles (MISP 6) specific for pinacolyl methylphosphonate using a water-in-oil microemulsion technique

A saturated ammonia solution is obtained by passing ammonia gas into ethanol at 20°C for 6 h. A template molecule, pinacolyl methylphosphonate (0.604 g), a functional organosilane, *N*-(3-triethoxysilylpropyl)-4,5-dihydroimidazole (0.133 g) and the scintillation monomer, ICPS-PPO adduct (0.080 g) are dissolved in ethanol (25 ml), and the solution is added into 37 ml of the saturated ammonia solution under stirring. A nonionic surfactant, polyoxyethylene(5) nonylphenyl ether (NP-5, 0.5 g), cyclohexane (13.5 ml) and water (1.44 ml) are subsequently added. The mixture is stirred at 20°C for 30 min. Tetraethoxysilane (TEOS, 3.36 g) is then added. The reaction mixture is stirred for 24 h at 20°C. The resulting silica particles are separated by centrifugation and then washed sequentially with the following solvents: water/ethanol (2/8, v/v), acetic acid/ethanol/water (3/3/4, v/v/v), water/ethanol (2/8, v/v), and ethanol. The particles are then dried in vacuum. The non-imprinted scintillation silica particles (NISP 6) are synthesised under identical condition except omission of the template, pinacolyl methylphosphonate.

Titration of tritium-labelled template with increasing amounts of molecularly imprinted scintillation polymers

A fixed amount of tritium-labelled template molecule is incubated with increasing amounts of molecularly imprinted scintillation polymer. Equilibrium is established after binding and the samples are analysed by proximity scintillation counting. The counting results (CPM, counts

per minute) are proportional to the amount of bound labelled template.

Example 9

Evaluation of binding performance of a molecularly imprinted scintillation polymer (MISP 2) by proximity scintillation counting

In a serie of 500 µl polypropylene microcentrifuge tubes, increasing amounts of polymer microparticles (MISP 2 and NISP 2) synthesised in example 3 are suspended in toluene containing 0.5% (v/v) acetic acid. Tritium-labelled (S)-propranolol (2 pmol) is added and the volume topped with the same solvent to 500 µl. The microcentrifuge tubes are incubated at 20°C for 2 h. After incubation, the microcentrifuge tubes are transferred into 6 ml insert counting vials and counted with a model 2119 RACKBETA β-radiation counter (LKB Wallac, Sollentuna, Sweden). Both the imprinted polymer and the non-imprinted polymer are tested. The titration curves for the two polymers are shown in Figure 4. As can be seen, the imprinted polymer binds much more of the tritium-labelled analyte than the control, whereby a much higher scintillation signal (CPM) is obtained.

Example 10

Evaluation of binding performance of a molecularly imprinted scintillationpolymer (MISP 3) by proximity scintillation counting

In a serie of 500 µl polypropylene microcentrifuge tubes, increasing amounts of polymer microparticles (MISP 3 and NISP 3) synthesised in example 4 are suspended in 25 mM citrate buffer (pH 6.0) containing 50% (v/v) acetonitrile. Tritium-labelled (S)-propranolol (2 pmol) is added and the volume topped with the same solvent to 500 µl. The microcentrifuge tubes are incubated at 20°C for 2 h. After incubation, the microcentrifuge tubes are transferred into 6 ml insert counting vials and counted with a model 2119 RACKBETA β-radiation counter (LKB Wallac, Sollentuna, Sweden). Both the imprinted polymer

and the non-imprinted polymer are tested. The titration curves for the two polymers are shown in Figure 5. As can be seen, the imprinted polymer binds much more of the tritium-labeled analyte than the control, whereby a much higher scintillation signal (CPM) is obtained.

Example 11

Displacement assay of (S)-propranolol in an aromatic solvent using a molecularly imprinted scintillation polymer (MISP 2)

To a serie of 500 µl polypropylene microcentrifuge tubes are added 0.2 mg molecularly imprinted scintillation polymer microparticles (MISP 2) synthesised in example 3. (S)-Propranolol and (R)-propranolol are dissolved in toluene containing 0.5% (v/v) acetic acid, diluted with the same solvent to various concentration, and added into the microcentrifuge tubes. To each of these tubes are finally added the tritium-labelled (S)-propranolol (2 pmol), and the volume adjusted to 500 µl with toluene containing 0.5% (v/v) acetic acid. The samples are incubated at 20°C for 2 h, after which they are transferred into 6 ml insert counting vials and counted with a model 2119 RACKBETA β-radiation counter (LKB Wallac, Sollentuna, Sweden) to estimate the bound fraction of the labelled analyte. Figure 6 shows calibration curves for the template molecule, (S)-propranolol, and for its enantiomer, (R)-propranolol. As seen from the figure, the chemical sensing polymer (MISP 2) displays favourable chiral selectivity when used in an aromatic solvent, as the optical antipode of the template yields much less sensor signal (ΔCPM).

Example 12

Displacement assay of (S)-propranolol in an aqueous solvent using a molecularly imprinted scintillation polymer (MISP 3)

To a serie of 500 µl polypropylene microcentrifuge tubes are added 0.2 mg molecularly imprinted scintillation microspheres (MISP 3) synthesised in example 4. (S)-

propranolol and (R)-propranolol are dissolved in 25 mM citrate buffer (pH 6.0) containing 50% (v/v) acetonitrile, diluted with the same solvent to various concentration, and added into the microcentrifuge tubes. To each of these tubes are finally added the tritium-labelled (S)-propranolol (2 pmol), and the volume adjusted to 500 µl with 25 mM citrate buffer (pH 6.0) containing 50% (v/v) acetonitrile. The samples are incubated at 20°C for 2 h, after which they are transferred into 6 ml insert counting vials and counted with a model 2119 RACKBETA β-radiation counter (LKB Wallac, Sollentuna, Sweden) to estimate the bound fraction of the labelled analyte. Figure 7 shows calibration curves for the template molecule, (S)-propranolol, and for its enantiomer, (R)-propranolol. As seen from the figure, the chemical sensing polymer (MISP 3) displays very high chiral selectivity when used in an aqueous solvent, since the cross-reactivity from (R)-propranolol is less than 5%.

CLAIMS

1. A molecularly imprinted polymer having specific binding sites, which polymer comprises at least one component for energy transfer located in proximity to said binding sites.

2. A molecularly imprinted polymer according to claim 1, wherein said component for proximity energy transfer is chemically incorporated into the polymer.

3. A molecularly imprinted polymer according to claim 1, wherein said component for proximity energy transfer is bound to the surface of the polymer.

4. A molecularly imprinted polymer according to any one of claims 1-3, wherein said component is a scintillator.

5. A molecularly imprinted polymer according to claim 4, wherein said scintillator comprises a reactive group.

6. A molecularly imprinted polymer according to claim 5, wherein said reactive group comprises at least one C=C bond.

7. A molecularly imprinted polymer according to claim 5 or 6, wherein the scintillator is 2,5-diphenyloxazole or a derivative thereof.

8. A molecularly imprinted polymer according to claim 5, wherein said reactive group comprises at least one of the groups -COOH, -CHO, -OH and $-NH_2$.

9. A molecularly imprinted polymer according to any one of claims 1-8, which polymer is an organic polymer comprising as a main component at least one polymer chosen from the group comprising polyacrylate, polystyrene, polyurethane, polyanaline and polyamide.

10. A molecularly imprinted polymer according to any one of claims 1-8, which polymer is an inorganic polymer obtained from alkoxides of silicon, aluminum or titanium.

11. A molecularly imprinted polymer according to any one of claims 1-10, which polymer has a configuration chosen from the group comprising monolith, irregular particles, thin films, membranes, microspheres and beads.

12. A molecularly imprinted polymer according to any one of claims 1-11, which polymer has been polymerised in situ within wells of a microtitre plate or on a microchip.

13. A molecularly imprinted polymer according to any one of claims 1-12, which polymer comprises two scintillators.

14. A molecularly imprinted polymer according to any one of claims 1-13, which polymer comprises an aromatic substance, which assists in exciting the component for proximity energy transfer.

15. A molecularly imprinted polymer according to any one of claims 1-14, in which the distance between the component for proximity energy transfer and the binding sites of the polymer is within the range 0-50 μm.

16. A method of preparing a molecularly imprinted polymer having specific binding sites, comprising

incorporation into and/or conjugation with the polymer of a component for proximity energy transfer.

17. A method according to claim 16, whereby said method comprises the steps:

copolymerisation of functional monomers in the prescence of at least one template molecule using conventional polymerisation techniques, which monomers comprise at least one component for proximity energy transfer; and

removal of the template molecule.

18. A method according to claim 16, whereby said method comprises the steps:

copolymerisation of functional monomers and at least one reactive monomer carrying an optionally protected, reactive group in the prescence of at least one template molecule using conventional tecniques;

chemical conjugation of the reactive group of the polymer and a reactive group of the component for proximity energy transfer; and

removal, before or after said conjugation, of the template molecule.

19. A method according to claims 16-18, whereby the component used for proximity energy transfer is a scintillator.

20. A method according to claim 19, whereby the scintillator comprises at least one C=C bond.

21. A method according to claims 19 or 20, whereby the scintillator is 2,5-diphenyloxazole or a derivative thereof.

22. A method according to any of claims 19-21, whereby the reactive group of the scintillator is chosen from the group comprising -COOH, -CHO, -OH and $-NH_2$.

23. A method according to any one of claims 18-22, whereby the reactive group of the monomer is chosen from the group comprising -COOH, -CHO, -OH and $-NH_2$.

24. A method according to any one of claims 16-23, whereby the polymerisation is performed so that a polymer with a configuration chosen from the group comprising monolith, irregular particles, thin films, membranes, microspheres and beads is obtained.

25. A method according to any one of claims 16-23, whereby the polymerisation is performed in situ within the wells of a microtitre plate or on a microchip.

26. A method according to any one of claims 16-24, whereby two different scintillators are in use.

27. A method according to any one of claims 16-26, whereby an aromatic substance, which assists in exciting the component for proximity energy transfer, is incorporated into the polymer.

28. A method according to any one of claims 16-27, whereby the incorporation of the second scintillator and/or the aromatic substance is obtained by physical absorption or by chemical linkage.

29. Use of a molecularly imprinted polymer according to any one of claims 1-15 or prepared according to any one of claims 16-28 in a proximity scintillation assay.

30. Use of a molecularly imprinted polymer according to any one of claims 1-15 or prepared according to any one of claims 16-28 for screening of combinatorial libraries.

31. Use of a molecularly imprinted polymer according to any one of claims 1-15 or prepared according to any one of claims 16-28 for use in sensors.

32. Use of a molecularly imprinted polymer according to any one of claims 1-15 or prepared according to any one of claims 16-28 for in situ monitoring of radioactive metabolites or enzymatic reactions.

33. Use according to claim 29-32, whereby an imaging system is used for quantifying the fluorescence signal.

34. Use according to any one of claims 29-33, whereby the imaging system is a Charge Coupled Device (a CCD camera).

35. Use according to claim 29-32, in which arrays of photomultiplier tubes are used for scintillation counting.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/00268

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/531, G01N 21/64 // C08F 220/18, C08F 12/08, C08G 18/00, C08G 73/00, C08G 69/00

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)

IPC7: G01N, C08F, C08G

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

SE,DK,FI,NO classes as above

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

EPO INTERNAL, WPI-DATA, CHEM.ABS-DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biofunctional Membr., (Proc.Int.Conf.) 1996, Michael Jay et al: "Molecular Recognition of Photoimprinted Surfaces", pages 211-222 --	1-35
X	Tetrahedron Letters, volume 41, 2000, Daniel L. Rathbone et al: "Molecular recognition by fluorescent imprinted polymers", pages 123-126 --	1-35
X	Anal.Chem. volume 70, 1998, Petra Turkewitsch et al: "Fluorescent Functional Recognition Sites through Molecular Imprinting. A Polymer-Based Fluorescent Chemosensor for Aqueous cAMP", pages 2025-2030 --	1-35

[X] Further documents are listed in the continuation of Box C. [X] 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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2002	24 -06- 2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Monika Bohlin/Els Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/00268

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Bioorganic Chemistry, volume 27, 1999, Yuan Liao et al: "Building Fluorescent Sensors by Template Polymerization: The Preparation of a Fluorescent Sensor for L-Tryptophan", pages 463-476 --	1-35
X	Organic Letters, Volume 1, No. 8, 1999, Wei Wang et al: "Building Fluorescent Sensors by Template Polymerization: The Preparation of a Fluorescent Sensor for D-Fructose", pages 1209-1212 --	1-35
A	WO 0066790 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 9 November 2000 (09.11.00) --	1-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/05/02

International application No.
PCT/SE 02/00268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0066790 A1	09/11/00	AU 4705800 A	17/11/00
		EP 1097242 A	09/05/01