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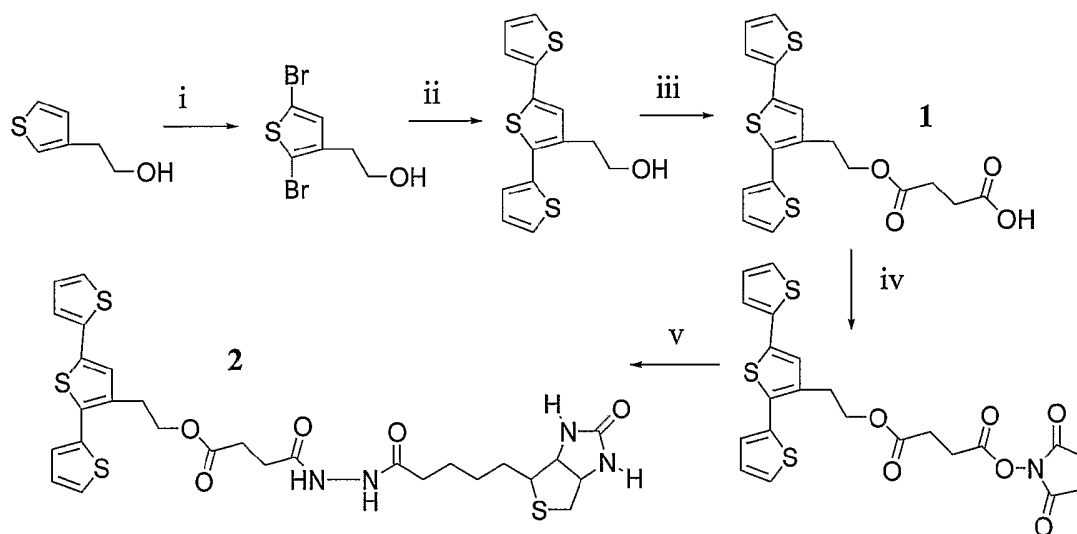
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(54) Title: POLYTHIOPHENE-BASED SENSORS



(57) Abstract: Disclosed are methods of producing defined polythiophene polymers for use as electrochemical sensors. Copolymerising receptor bearing monomers with varied proportions of underivatized monomers produces polymers with a defined density of receptor side-chains and improved consistency and sensitivity as sensors. Also disclosed are improved methods of polymerising regioregular polythiophene providing a regioregular array of receptors in a conducting polymer, suitable for use as a biosensor. Specific monomers and receptor moieties are also provided.

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Polythiophene-based sensors

Technical Field

The present invention is concerned with the production and use of polymers based on terthiophene compounds for sensing molecules in solution. Changes in the properties of conductive terthiophene polymers as a result of binding of ligands to specific receptor sites on sidechains form the basis for sensitive biosensors.

10 *Background of the Invention*

Many areas of biotechnology and medical diagnostics require the development of accurate and sensitive methods of detection of molecules of biological origin or interest in solution. Ideally, such methods should allow continuous monitoring using a responsive detection system giving a quantitative read-out. To this end, conductive polymers have been developed as part of electrochemical biosensor systems.

A number of potentially suitable conductive polymer systems are known, of which those based on polythiophenes are amongst the most promising. Polythiophenes bearing covalently-attached receptor sites have properties that make them
20 particularly desirable as sensor materials, and many sophisticated examples are now known (McQuade et al, 2000).

In principle, a number of physico-chemical principles may be used as a means of generating a quantitative response to binding of an analyte to the sensor system. Conductometric sensors function by detecting the change in conductivity of the polymers, bound to a suitable film. The resistance between two adjacent microelectrodes is measured as a function of the concentration of analyte. Alternatively, a potentiometric system may be used, in which changes in the system's redox potential in response to analyte binding are recorded. One advantage to this
30 approach is that it requires only one recording electrode, which may be incorporated in to the substrate film onto which the conducting polymers are bound. Colorimetric

methods (depending on changes in the polymer's absorption properties) and changes in fluorescence properties may also be used as the basis of detection techniques.

Polythiophenes are particularly suitable since they respond to binding events with measurable changes in their electrical, redox and optical properties. This is because they are semiconductors with a comparatively small bandgap, and they can be p-doped at modest positive potentials. Moreover, 3-functionalised thiophenes are readily prepared, polythiophenes are accessible both chemically and electrochemically (Roncali, 1999) and, unlike polypyrroles, polythiophenes are stable in both their neutral and p-doped states. The electrogeneration of polythiophene films from thiophenes often requires highly positive potentials, which may degrade delicate functional groups. However, this can be overcome by using bi- or terthiophenes as monomers, since their oxidation potentials are much less positive (Scheib and Bäuerle, 1999; Higgins *et al*, 2001) and, providing that appropriate substituents are employed, polymers with extended conjugation can thus be made.

Such systems may be used for the detection of a wide range of molecules, but are particularly suitable for specific detection of soluble ligands by cognate receptors. Sequence-specific binding and detection of nucleic acids by complementary single-stranded oligonucleotides linked to a biosensing apparatus is a particularly important application, but a wide variety of polypeptide receptors, antibodies or fragments thereof, or entirely synthetic chemical receptors have been used.

WO 03/024954 (Shim) discloses a 3'-carboxyl terthiophene compound and its polymerisation with the subsequent covalent attachment of a single-stranded oligonucleotide via the 3'-carboxyl side chain. However, such electrochemically polymerised chains are irregular and poorly-characterised, giving relatively poor and inconsistent performance as biosensors. In addition, polymerisation from pure functionalised monomers is expensive and unnecessary, since the resulting poorly defined high density of receptor-bearing sidechains is inefficient.

Accordingly, there remains a need for an efficient method of synthesising conducting receptor-bearing polymers to produce biosensors of increased consistency and sensitivity.

Summary of the Invention

The invention provides a novel compound, succinic acid mono-(2-[2,2';5',2'']terthiophen-3'-yl-ethyl) ester, and a functionalised conductive polythiophene polymer derived from this compound. Also provided is an improved method of synthesising derivatised polymers capable of specifically binding soluble ligands.

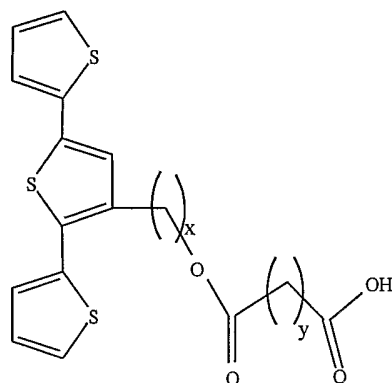
10 The method comprises the steps of covalently attaching a receptor moiety and suitable spacer to the carboxyl-terminated ester monomers, copolymerising such receptor-bearing monomers with varied proportions of underivatised monomers to produce defined polymers with a defined density of receptor side-chains.

In another aspect the invention provides an improved method of polymerising regioregular polythiophene, so as to produce a defined mixture of receptor-bearing and underivatised monomers so as to produce a regioregular array of receptors in a conducting polymer, attached to a solid substrate by solvent casting, suitable for use as a biosensor.

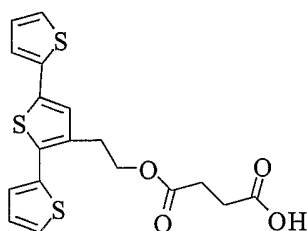
20 Preferred receptor moieties include single-stranded oligonucleotides, antibodies or functional antigen-binding fragments of antibodies, recombinant polypeptide receptor molecules, such as growth factor receptors, immunoglobulin superfamily receptors, integrins or lectins or functional ligand-binding fragments thereof. Non-peptide receptors based on carbohydrates may also be used. Alternatively, a high-affinity but non-specific synthetic receptor may be used, for example avidin or streptavidin, enabling detection of experimentally biotinylated molecules to be detected. This may be of use both as a means of detecting and purifying such biotinylated molecules but also as a model system for characterising and developing biosensor systems. Equally, a biotin-bearing receptor may be used to detect avidin or avidin-containing
30 molecules or complexes.

Accordingly, the invention provides a terthiophene derivative according to the following formula, wherein $x = 2$ to 10 and $y = 2$ to 10 :

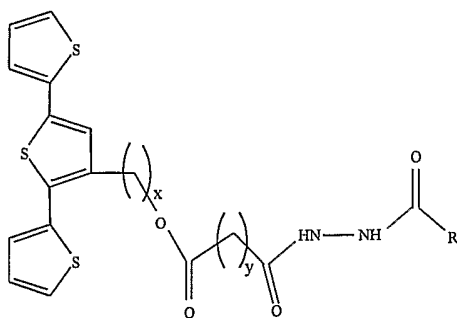
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Preferably, $x=2$ to 4, most preferably $x=2$, and $y=2$ to 4, most preferably $y=2$,
 wherein the terthiophene derivative is succinic acid mono-(2-[2,2';5',2'']terthiophen-3'-
 10 yl-ethyl) ester, as represented by the following formula:



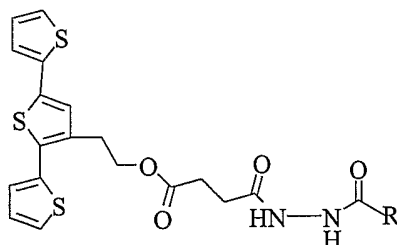
In another aspect, the invention provides a receptor-bearing terthiophene derivative
 according to the following formula, where R= a functional ligand-binding receptor
 moiety and wherein $x = 2$ to 10 and $y=2$ to 10:



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Preferably $x=2$ to 4 and most preferably $x=2$, and $y=2$ to 4, most preferably $y=2$, according to the following formula,



Preferably said functional ligand-binding receptor moiety is selected from the list consisting of single-stranded oligonucleotides, antibodies or functional antigen-binding fragments of antibodies, recombinant polypeptide receptor molecules or
10 functional ligand-binding fragments thereof, biotin, avidin and streptavidin.

It will be understood that, in principle, the functional ligand-binding receptor moiety may be linked to the polythiophene backbone of the polymer by a linker of variable length. Preferably, the linker contains from 5 to 20 in-chain atoms. The in-chain atoms may, by way of example, be C, O, N or S, e.g. C or O. Included is a class of formulations in which all the in-chain atoms are all carbon. In one class of compounds there are 0, 1, 2 or 3 in-chain atoms which are not carbon, e.g. 1 such non-carbon atom, and in a sub-class of compounds all these non-carbon in-chain atoms are oxygen. Typically, non-carbon atoms are directly bonded to carbon atoms
20 on either side of them in the chain, e.g. to form an ether linkage (typically $-\text{CH}_2-\text{O}-\text{CH}_2-$) in the case of oxygen being the non-carbon atom. L may be an alkylene group; in some instances it is an alkenylene group.

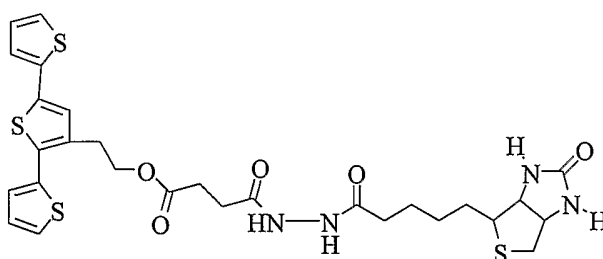
The in-chain bonds of the linker are typically double or single bonds; included is a class of compounds in which all the in-chain bonds are single bonds or are all single except one double bond. In many compounds the linker is unsubstituted; alternatively the linker may be substituted, for example each in-chain atom may have at least one substituent or, more frequently, the linker contains fewer substituent groups than in-chain atoms. In one class of compounds, no in-chain atom contains
30 more than one substituent. Included are compounds in which the linker has 1, 2 or 3 substituents, e.g. 1 substituent. As substituents there may be mentioned halogen

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(e.g. F or Cl), alkyl (e.g. 1C to 4C, for example methyl), alkoxy (e.g. 1C to 4C, for example methoxy), alkylthio (e.g. 1C to 4C) and haloalkyl (e.g. 1C to 4C, for example trifluoromethyl).

Exemplary linker groups are $-\text{CH}_2-$, $-(\text{CH}_2)_2-$, $-(\text{CH}_2)_3-$, $-(\text{CH}_2)_4-$, $-(\text{CH}_2)_5-$, $-(\text{CH}_2)_6-$, $-(\text{CH}_2)_7-$ and $-(\text{CH}_2)_8-$.

One preferred embodiment is a biotinylated terthiophene derivative according to the following formula:



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In another aspect, the invention provides a copolymer of 2,2'; 5', 2''-terthiophene and a receptor-bearing terthiophene derivative as described above. Preferably, the molar ratio of 2,2'; 5', 2''-terthiophene and the receptor-bearing terthiophene derivative monomers is between 50:1 and 2:1. More preferably, it is between 10:1 and 2:1. Most preferably it is 5:1.

In another aspect, the invention provides a regioregular copolymer of terthiophene derivatives as described above, in particular a copolymer of receptor-bearing and unfunctionalised terthiophene derivatives.

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Alternatively, the regioregular copolymer comprises at least different two receptor-bearing terthiophene derivatives. These may carry the same functional ligand-binding receptor moieties on different lengths of linker, which may give advantages in obtaining an optimal array of receptor moieties, or may carry different functional ligand-binding receptor moieties. Preferably the regioregular copolymer is a functionalised regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan 1-ol). More preferably it is biotinylated regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan 1-ol).

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Also provided is a method of preparing a receptor-bearing copolymer of 2, 2'; 5', 2''-terthiophene and a receptor-bearing terthiophene derivative as described above, comprising the steps of mixing the monomers and polymerising by repetitive scan cyclic voltammetry on an electrode substrate. Preferably the total monomer concentration is between 20mM and 1mM, more preferably between 10mM and 2mM, most preferably it is 5mM.

10 In a further aspect, the invention provides a method of preparing a regioregular, receptor-bearing copolymer of 2, 2'; 5', 2''-terthiophene and the receptor-bearing terthiophene derivative as described above, comprising the steps of mixing the monomers at a molar ratio of between 100:1 and 2:1, ideally 5:1, to a total concentration of between 0.1 and 100 mM, ideally 5 mM, in an organic electrolyte solution, preferably 0.2 M tetraethylammonium tetrafluoroborate in acetonitrile, and polymerising by electrochemical potential cycling, preferably between 0 V and +1.15 V vs saturated calomel reference electrode, at a scan rate of between 1 and 1000 mV s⁻¹, preferably 10 mV s⁻¹. The number of repetitive scans (1 to 100) could be used as a control of film thickness. Preferably, 10 scans were employed. The polymerisation was typically stopped with the working electrode held at the negative limit.

20

Alternatively, by employing a suitably-functionalised 3-alkyl-2,5-dibromothiophene in concert with a 3-alkyl-2,5-dibromothiophene, and chemical polymerisation using a metal-diphosphine catalyst, the invention provides a method of preparing a regioregular poly-(3-alkylthiophene) copolymer in which a controlled number of alkyl substituents bear, at their terminal carbon, a substituent, to which receptors may subsequently be attached. The proportion of functionalised monomer in the polymer can be varied from 1 per 1000 thiophene rings, to 1 per 2 thiophene rings. Preferably, it is 1:100 – 1:5 rings. Most preferably, it is 1 per 20 thiophene rings.

30 In another aspect, the invention provides the use of a terthiophene derivative, a receptor-bearing terthiophene derivative, or a copolymer as described above as a sensor to detect the binding of an analyte. In one embodiment binding of an analyte is detected by mean of a change in electrochemical impedance. Alternatively binding of an analyte is detected by mean of a change in redox potential or by mean of a change in optical properties.

In another aspect the invention provides an apparatus comprising an electrochemical sensing copolymer as described above. In one embodiment, the binding of an analyte is detected by means of a change in the electroactivity of the copolymer. Where this change is a change in electrochemical impedance, the analyte may be dissolved in an aqueous buffer. This is advantageous for the analysis of a great many biological molecules and allows greater speed and convenience of processing. Detection methods based on potentiometric methods of measuring redox potentials require non-aqueous buffer systems (using, for example, acetonitrile). Alternatively, the binding of an analyte is detected by means of a change in the optical properties of the copolymer. Preferably this is a change in optical absorbance. Alternatively, it may be a change in fluorescence. These methods also have the advantage that the analyte may be in an aqueous buffer. More preferably, the apparatus comprises a functionalised regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan 1-ol). More preferably it comprises biotinylated regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan 1-ol).

Detailed Description of the Invention

The invention is described by means of the following examples and referring to the figures, in which:

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Figure 1 shows the reaction scheme for the synthesis of 'terthiophene 1' (the carboxyl-terminated ester of 3'-(2-hydroxyethyl)-2,2'; 5',2''-terthiophene) and 'terthiophene 2', its biotinylated derivative.

Figure 2 shows cyclic voltammograms (30 mV s^{-1}) of a 1:terthiophene copolymer film before and after incubation with avidin.

Figure 3 shows cyclic voltammograms (30 mV s^{-1}) of a 2:terthiophene copolymer film before and after incubation with avidin.

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Figure 4 shows electrochemical impedance spectra of a 2:terthiophene copolymer film at various applied potentials, prior to incubation with avidin.

Figure 5 (A) shows polymer 4 (Scheme 1) in 1:1 CHCl₃:dmsO; (B) after addition of 1 drop aqueous buffer containing excess BSA; (C) after addition of 1 drop aqueous buffer containing excess avidin.

Figure 6 Cyclic voltammograms of a drop-cast film of polymer 4 (Scheme 1) on a 0.13 cm² Pt disc electrode, cycled at 10 mV s⁻¹ in 0.1 M Et₄NBF₄/CH₃CN, (A) prior to exposure to avidin and (B) after incubation in 1 cm³ of a 0.1 M NaCl/10 mM edta buffer solution containing excess avidin. The polymer-modified electrode was washed gently with fresh buffer, dried in a stream of N₂, washed with CH₃CN and transferred back to the CH₃CN electrolyte prior to cycling.

Figure 7 shows *I/V* characteristics of a spin-coated film of polymer 4 (Scheme 1) prior to buffer exposure (full line), after exposure to BSA in 0.1 M aq. NaCl/10 mM edta buffer (broken line), and after exposure to avidin in buffer (crosses).

Figure 8 shows a simple solid-state device comprising polymer 4 (Scheme 1) spin-coated onto gold finger electrodes deposited onto a glass substrate

Example 1 Electrochemical preparation of biotinylated polythiophene copolymers

Terthiophenes 1 and 2 were prepared by the sequence shown in Figure 1. The 3'-(2-hydroxyethyl)-2,2':5',2''-terthiophene was prepared by a modified literature method (Scheib and Bäuerle, 1999; Higgins *et al*, 2001), and esterified with excess succinyl chloride to give 1 after workup. Terthiophene 1 was activated with 1,2,2,2-tetrachloroethyl-*N*-succinimidyl carbonate (Jaouadi *et al*, 1987), then reacted with commercially-available biotin hydrazide to give 2.

Copolymers of 1 with 2,2':5',2''-terthiophene, and of 2 with 2,2':5',2''-terthiophene, were grown by repetitive scan cyclic voltammetry (10 scans) on Pt disc electrodes, using a 5:1 mole ratio of 2,2':5',2''-terthiophene:1 (or 2), and a 5 mM total monomer concentration. The cyclic voltammograms of the two copolymers in pure 0.2 M TEAT / CH₃CN are shown in Figures 1 and 2. A SNIFTIRS spectrum of a neutral terthiophene: 2 copolymer film (on a gold electrode) showed bands at 3180 and 3120 cm⁻¹ (ν_{NH}), 1730 cm⁻¹ (sh; ester ν_{CO}), 1685 cm⁻¹ (hydrazido ν_{CO}) (Asorga *et al*, 1993)

and 1664 cm^{-1} (biotin ν_{CO}) (Sato and Machida, 1977) consistent with the presence of intact biotin moieties.

After electrochemical characterisation, modified electrodes were incubated in both buffer solution, and in buffer solution containing avidin. Exposure to buffer alone had negligible effect on the voltammetry of either copolymer film, when these were transferred back to 0.2 M TEAT / CH_3CN . Exposure of the terthiophene:2 copolymer film to avidin, in contrast, caused a dramatic change in film electroactivity, as shown in Figure 2. That this was due to specific avidin:biotin interaction, rather than non-specific protein adsorption to the rather hydrophobic polythiophene, was supported by the fact that the terthiophene:1 copolymer showed only a small change in its electroactivity after incubation with avidin (Figure 2). The change in electroactivity is consistent with the presence of an avidin adlayer which greatly slows the uptake of anions on electrooxidation of the polyterthiophene to its cationic (conducting) form, so that its rate is comparable with the voltammetric timescale. As the scan rate is decreased, the onset of oxidation occurs at less positive potentials, and the current maximum *increases*. At an intermediate scan rate (30 mV s^{-1} ; Figure 3), a nucleation loop is seen as the avidin-bound polymer is electro-oxidised.

Electrochemical impedance spectra (EIS) confirmed this picture. The EIS of the terthiophene:1 copolymer, recorded at 0.1 V intervals from + 0.40 – + 0.90 V, were almost identical before and after avidin incubation. The oxidised form of the polymer showed a 45° line at high frequencies, due to Warburg impedance caused by ionic diffusion, and a near-vertical line at lower frequency, where the film behaves as a capacitor. This behaviour can be fitted within the classical dual rail transmission line circuit proposed for the analysis of conducting polymer impedance spectra (Albery *et al*, 1989; Ren and Pickup, 1993; Ren and Pickup, 2001). As the potential is made less positive, a semicircle appears in the high frequency domain, due to charge transfer capacitance in parallel with charge transfer resistance. The EIS of the terthiophene:2 copolymer before avidin incubation (Figure 4) were similar, but with somewhat higher ionic and electronic resistances at a given potential, possibly owing to the bulkier side-chain. However, after avidin incubation, the film exhibits a complete loss of electronic conductivity, even at positive potentials (+0.8 V), and its EIS could not be fitted to any reasonable model for a conducting polymer. We have also prepared terthiophene:2 copolymer films on a Pt microdisk electrode ($20\text{ }\mu\text{m}$

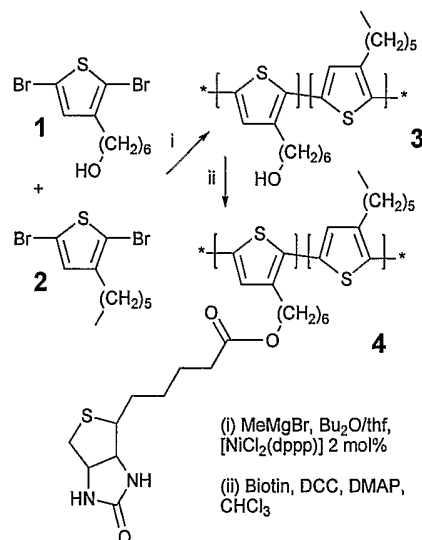
diameter), and these show an even more drastic change in redox behaviour on avidin binding.

Example 2 Chemically-coupled regioregular biotinylated polyalkylthiophenes

In addition to the electrochemically-fabricated biotinylated polythiophene, we have also prepared a soluble, biotinylated regioregular polyalkylthiophene derivative, using chemical coupling methodology. Head-to-tail regioregular polyalkylthiophenes (Loewe *et al*, 1999; Loewe *et al*, 2001) have been shown to have particularly high conductivity when doped, they are highly solvato- and thermochromic (McCullough, 1998; Yamamoto *et al*, 1998), and they give unusually high field effect mobility when used as the semiconductor in field effect transistors (Sirringhaus *et al*, 1999); this is improved further by doping with electron acceptors (Sedghi *et al*, 1999). The latter property has been related to the observed self-assembly of the polymers, during solvent casting, into a 2-dimensional structure in which the regularly-placed alkyl groups of neighbouring polymer chains are able to intercalate, and efficient π - π stacking between neighbouring polythiophene chains occurs in the remaining dimension, facilitating carrier transport from one chain to another. The synthesis of functionalised versions of these polymers, for sensing and related applications, is therefore of great interest. Examples published to date either involve post-
10 polymerisation functionalization of poly(ω -bromoalkyl)thiophenes (Zhai *et al*, 2003), or Stille coupling of appropriate functionalised (and protected) thiophenes or 2,2'-bithiophenes (Reitzel *et al*, 2000).
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We have prepared a regioregular copolymer of 6-thiophen-3-yl-hexan-1-ol with 3-hexylthiophene, using the McCullough Grignard metathesis route (Loewe *et al*, 2001), from the corresponding 2,5-dibromothiophene derivatives **1** and **2** (Scheme 1).

Scheme 1



Preparation of regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan-

1-ol), **3**:—Monomer **1** (1.0 g, 2.9 mmol), monomer **2** (4.76 g, 14.6 mmol) and anhydrous thf (50 cm³) were placed in a 100 cm³ flame-dried round bottomed flask. Methylmagnesium bromide (17.5 cm³ of a 1.0 M solution in Bu₂O; 17.5 mmol) was added, and the solution was stirred for 5 min., and then heated to reflux for 2 h. After this time, the solution was cooled to room temperature, and [NiCl₂(dppp)] (0.19 g, 2 mol%) was added in one portion. More thf (20 cm³) was added, and the mixture was again brought to reflux. After 16 h., it was allowed to cool to room temperature, and was then quenched by pouring into MeOH (400 cm³). The precipitated polymer was filtered into a Soxhlet thimble, and sequentially extracted with MeOH, hexanes, then CHCl₃. The CHCl₃ fraction was evaporated to dryness, and characterised. Yield 2.04 g, 69%. Microanalyses: Found: C = 70.85, H = 8.12 %. Calc. for copolymer **3** (1:8.5 **1**:**2**): C = 71.51, H = 8.40 %. ¹H NMR (CDCl₃): δ = 7.00 (s, head-to-tail thienyl H), 3.67 (t, CH₂OH), 2.82 (t, CH₂thienyl), 1.7–1.2 (m's, —CH₂—), 0.94 (t, —CH₃). ¹³C{¹H} NMR (CDCl₃): δ = 140.3, 134.2, 130.9, 129.0 (thienyl C), 63.4 (CH₂OH), 33.2, 32.3, 30.9, 29.9, 29.8, 29.6, 29.3, 26.0, 23.1 (various —CH₂—), 14.5 (—CH₃). GPC (PL-ELS 1000; PS calibration, thf, 1 cm³ min^{−1}, 40 °C): M_w 17,500, M_n 14,330, PD 1.22.

Biotin functionalisation of regioregular poly-(3-hexylthiophene)-copoly-(thiophen-3-yl)-hexan-1-ol) to give **4**: Polymer **3** (0.100 g; ca 0.063 mmol –OH equiv.) was dissolved in dry CHCl_3 (15 cm^3) and DCC (0.143 g, 0.69 mmol), DMAP (0.02 g, 0.16 mmol) and biotin (0.100 g, 0.41 mmol) were added. The mixture was stirred at room temperature for 48 hours, and was then partitioned between CHCl_3 and water (4 x 50 cm^3). The CHCl_3 fractions were combined, dried over MgSO_4 , filtered and the solvent removed to yield the polymer as a purple solid. The polymer was Soxhlet extracted with methanol for 16 h. ^1H NMR (CDCl_3 250 MHz): δ 7.00 (s, 1H, H4), 4.47, 4.29 (m's, 1H each, biotin –CHCH–), 4.10 (t, 2H, $\text{CH}_2\text{OC(O)}$), 3.67 (overlapping m, unreacted CH_2OH , and biotin CHS), 3.51 (m, 2H, biotin CH_2S), 2.80 (CH_2 thienyl), 2.30 (t, 2H, $\text{CH}_2\text{C(O)}$), 1.7-1.2 (m's), 0.94 (t, CH_3). The reaction is not quantitative presumably owing to the poor solubility of biotin in CHCl_3 , but reactions conducted in CHCl_3/dmf mixtures were completely unsuccessful.

Results

A 69% yield of the CHCl_3 soluble fraction of **3** was obtained, which had >97% head-to-tail regioregularity, measured by comparing the major thienyl– CH_2 triplet resonance at $\delta = 2.82$ p.p.m. with the minor multiplet (due to non-head-to-tail thienyl– CH_2 moieties) centred at 2.54 p.p.m (McCullough, 1998). Integration of the CH_2OH resonance and comparison with the thienyl– CH_2 resonance gave a monomer ratio in the polymer of 1:8.5 **1**:**2**; it is possible that polymer chains richer in **1** were more soluble in the other solvents used in the Soxhlet purification procedure. Biotin was grafted to copolymer **3** using dicyclohexylcarbodiimide (DCC)-mediated, 4-(dimethylamino)pyridine (DMAP)-catalysed coupling in CHCl_3 . The biotin-functionalised copolymer, **4**, was purified by washing with water, followed by Soxhlet extraction with MeOH overnight, to remove excess reagents and dicyclohexylurea. Integration of the $-\text{CH}_2\text{OC(O)}-$ ($\delta = 4.10$ p.p.m.) and residual $-\text{CH}_2\text{OH}$ resonances in the ^1H NMR spectrum of **4** indicated that ca. 40% of the –OH groups had been esterified by biotin. Both **3** and **4**, dissolved in 1:1 $\text{CHCl}_3:\text{dmso}$ (1 mg in 2 cm^3 , equivalent to 2.7×10^{-7} mol. biotin in the case of **4**), gave purple solutions with λ_{max} for the $\pi - \pi^*$ absorption at 515 nm, and shoulders at 555 and 600 nm. This is characteristic of these regioregular polymers in 'poor' solvents, in which a degree of polymer self-assembly (and consequent $\pi - \pi$ stacking) takes place (Yamamoto *et al*, 1998); the solvent system was chosen for compatibility with both the polymer, and avidin in aqueous buffer. On addition of 1.5×10^{-9} moles of avidin (dissolved in 0.1

cm³ 0.1 M NaCl/10 mM EDTA aqueous buffer) to the polymers, virtually no change in λ_{max} was observed for polymer **3**, but a gradual change (over 15 min.) to a yellow colour (λ_{max} for the $\pi - \pi^*$ absorption at 472 nm; no vibronic splitting) occurred for **4**. Furthermore, addition of bovine serum albumin (BSA; 10⁻⁸ mol. in 0.1 cm³ buffer) to the solution of **4** also caused almost no change, illustrated in Figure 5.

This is consistent with specific binding of avidin to the biotin moieties of **4**, causing a dissociation of interpolymer π - π stacking, and significant thiophene-thiophene inter-ring torsion, increasing the energy of the $\pi - \pi^*$ transition. This result is comparable with that for the poly(3-alkyl-4-alkoxy)thiophene derivative already described (Bernier *et al*, 2002).

We have also shown that polymer **4** (Scheme 1) can be used to fabricate both electrochemical and solid-state avidin detectors. Figure 6 shows cyclic voltammograms of a drop-cast film of polymer **4** on a Pt disc electrode in 0.1 M Et₄NBF₄/CH₃CN, before and after exposure to 10⁻⁹ moles of avidin in 1 mL of aqueous buffer. The large decrease and positive shift in the oxidation wave after exposure to avidin is consistent with the blocking of ion ingress/egress on polymer switching by the protein. No significant change in the electrochemistry of a film of **4** was observed after exposure to 10⁻⁸ moles of BSA in 1 cm³ buffer, indicating that the specific biotin:avidin interaction was responsible for the electrochemical change. The minimum amount of avidin sufficient to cause a detectable shift and diminution of the redox wave of **4** was 5 x 10⁻¹⁴ moles, using an electrode of area 0.13 cm².

Example 3 A solid-state sensor device using biotinylated polyalkylthiophene

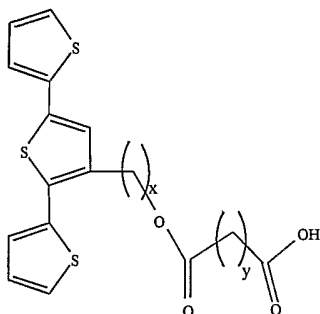
A simple solid-state device was fabricated by spin-coating ca. 100 nm of polymer **4** (Scheme 1) onto two gold finger electrodes (0.1 x 1 cm; 0.1 cm apart) deposited onto a hexamethyldisilazene-treated glass substrate (Figure 8). Figure 7 shows the effect of avidin exposure on the *I/V* characteristics (shown as log *I*; the polymers obeyed Ohm's law). While exposure of the polymer to BSA (10⁻⁸ moles in 1 cm³ buffer) causes a very small change in conductivity, exposure of this device to excess avidin (10⁻⁹ moles in 1 cm³ buffer) causes a lowering of conductivity by nearly four orders of magnitude.

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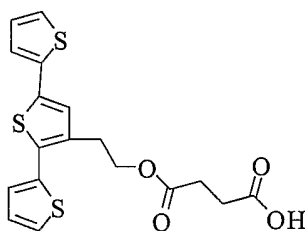
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Claims

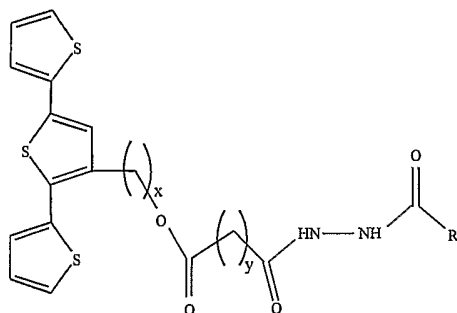
1. A terthiophene derivative according to the following formula, wherein $x=2$ to 10 and $y=2$ to 10:



2. Succinic acid mono-(2-[2,2';5',2'']terthiophen-3'-yl-ethyl) ester according to claim 1, as represented by the following formula:

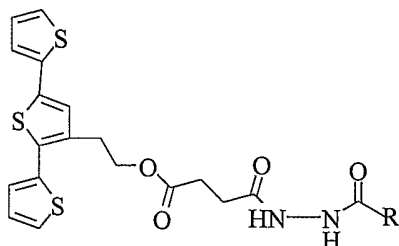


3. A receptor-bearing terthiophene derivative according to the following formula, wherein R= a functional ligand-binding receptor moiety and wherein $x=2$ to 10 and $y=2$ to 10:

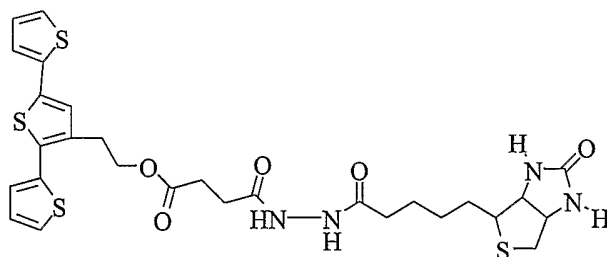


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4. A receptor-bearing terthiophene derivative according to claim 3 and as represented by the following formula:



5. A receptor-bearing terthiophene derivative according to claim 4, wherein the functional ligand-binding moiety is biotin, as represented by the following formula:



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6. A copolymer comprising a receptor-bearing terthiophene derivative, said receptor-bearing terthiophene derivative comprising a functional ligand-binding receptor moiety attached by a linker of 5 to 20 in-chain atoms.
7. A copolymer according to claim 6, comprising 3'-(2-hydroxyethyl)-2,2'; 5', 2''-terthiophene and a receptor-bearing terthiophene derivative according to any of claims 3 to 5.
8. The copolymer of claim 7, wherein the molar ratio of 3'-(2-hydroxyethyl)-2,2'; 5', 2''-terthiophene and the receptor-bearing terthiophene derivative monomers is between 100:1 and 2:1
9. The copolymer of claim 8 wherein the molar ratio of 2,2'; 5', 2''-terthiophene and the receptor-bearing terthiophene derivative monomers is 5:1.
10. A regioregular copolymer according to any of claims 6 to 9.

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11. A regioregular copolymer comprising a terthiophene derivative according to claim 1 and a receptor-bearing terthiophene derivative according to claim 3.
12. A regioregular copolymer comprising at least two receptor-bearing terthiophene derivatives according to claim 3.
13. A regioregular copolymer according to claim 12 being functionalised regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan 1-ol).
- 10 14. A regioregular copolymer according to claim 13 being biotinylated regioregular poly-(3-hexylthiophene)-copoly-({thiophen-3-yl}-hexan 1-ol).
15. Method of preparing a regioregular, receptor-bearing copolymer of 2, 2'; 5', 2''-terthiophene and receptor-bearing terthiophene derivative of any of claims 3 to 5, comprising the steps of mixing the monomers at a 5:1 molar ratio at a total monomer concentration of 5mM and polymerising by repetitive scan cyclic voltammetry on an electrode substrate.
- 20 16. Apparatus comprising an electrochemical sensing copolymer according to any of claims 6 to 14
17. Apparatus according claim 16 comprising the copolymer of claim 14.
18. Apparatus according to either of claims 16 or 17 in which binding of an analyte is detected by means of a change in the electrochemical impedance of the copolymer.
19. Apparatus according to either of claims 16 or 17 in which binding of an analyte is
30 detected by means of a change in the redox potential of the copolymer.
20. Apparatus according to claim 16 in which binding of an analyte is detected by means of a change in the optical properties of the copolymer.

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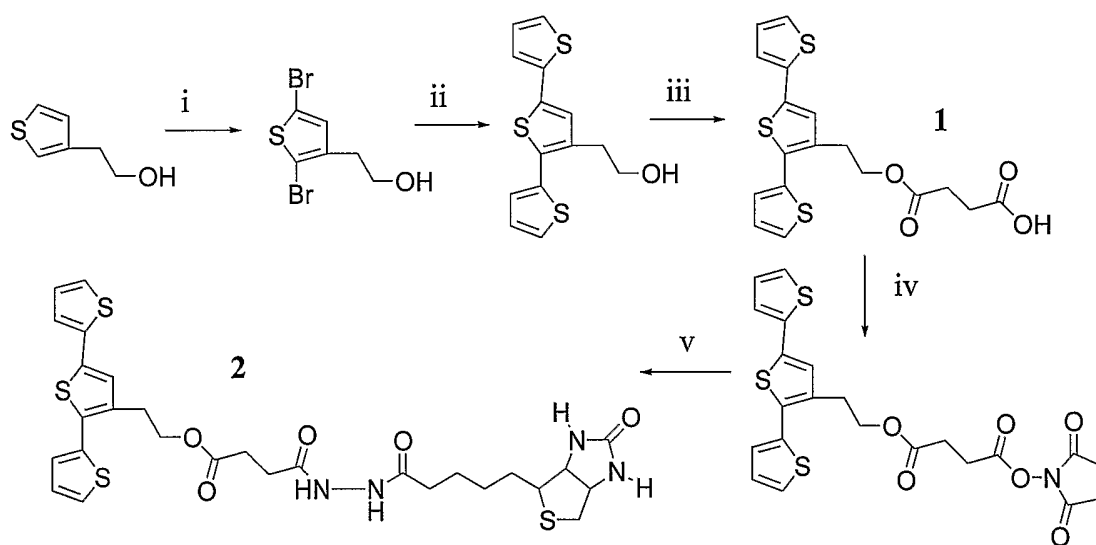
21. Use of a terthiophene derivative according to claim 1 or claim 2, a receptor-bearing terthiophene derivative according to any of claims 3 to 5, or a copolymer according to any of claims 6 to 14 as a sensor to detect the binding of an analyte.
22. Use according to claim 21 wherein binding of an analyte is detected by mean of a change in electrochemical impedance.
23. Use according to claim 21 wherein binding of an analyte is detected by mean of a change in redox potential.

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24. Use according to claim 21 wherein binding of an analyte is detected by mean of a change in optical properties.

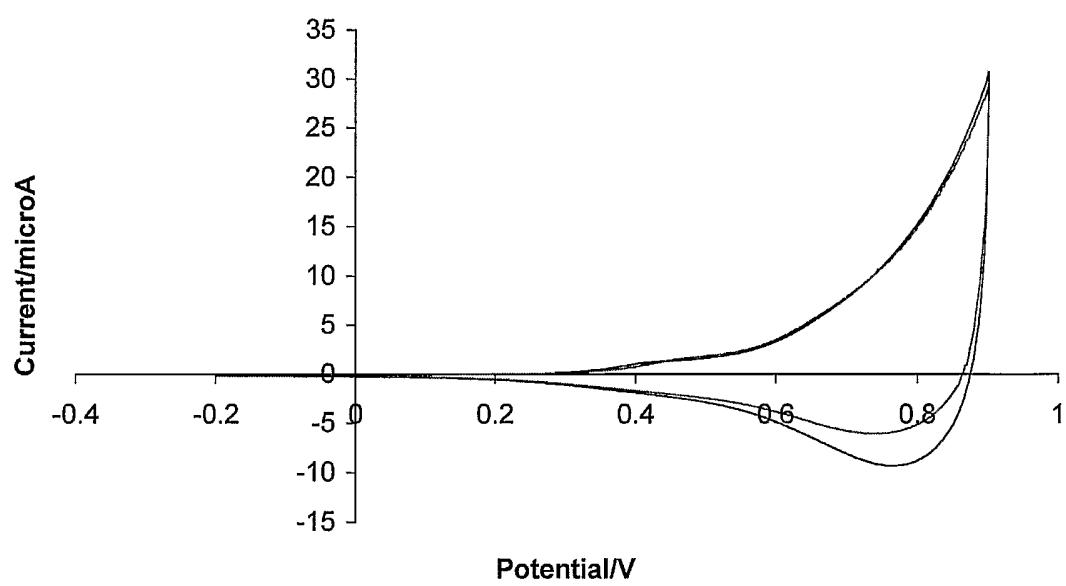
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Figure 1



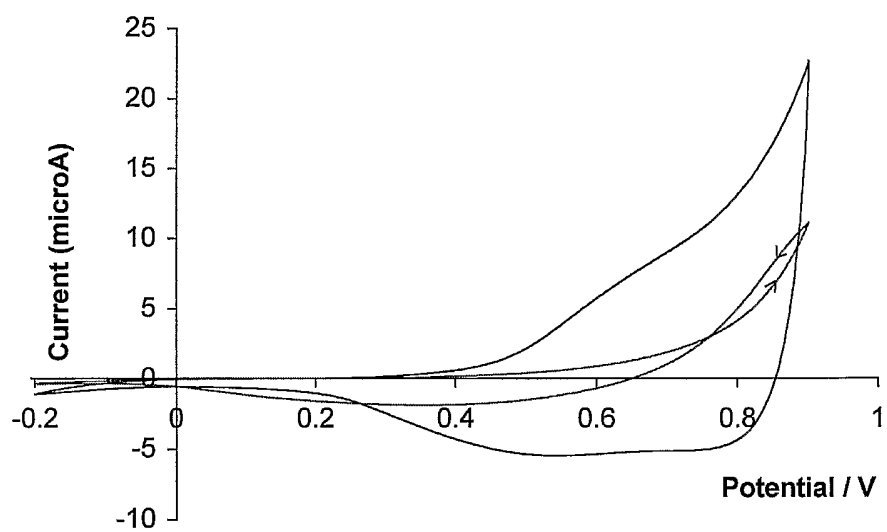
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Figure 2



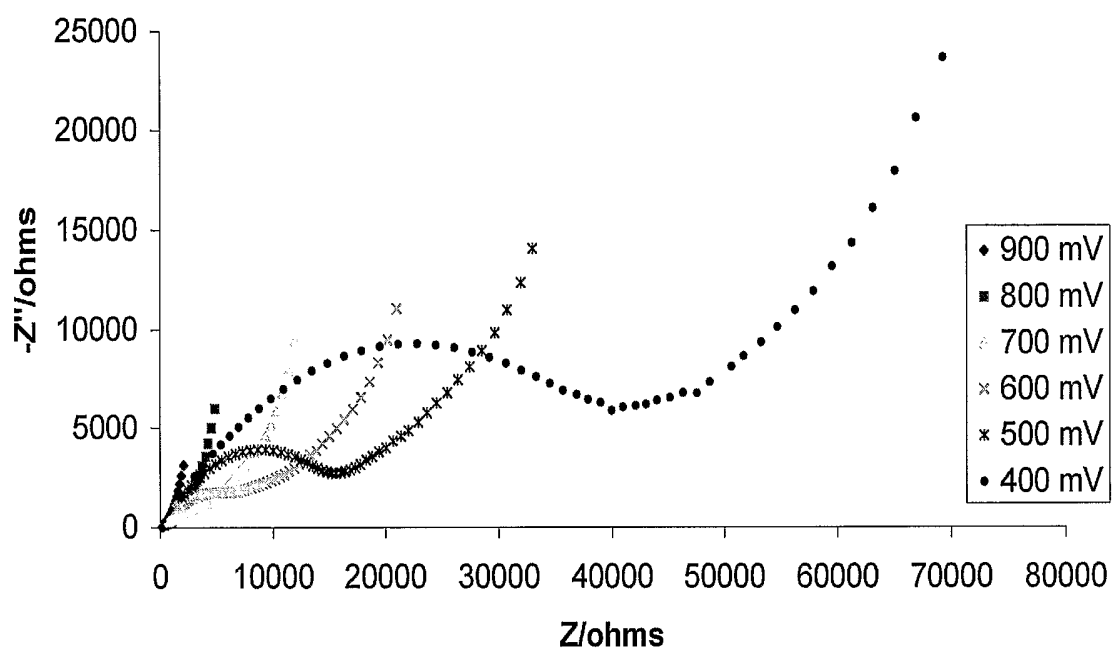
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Figure 3



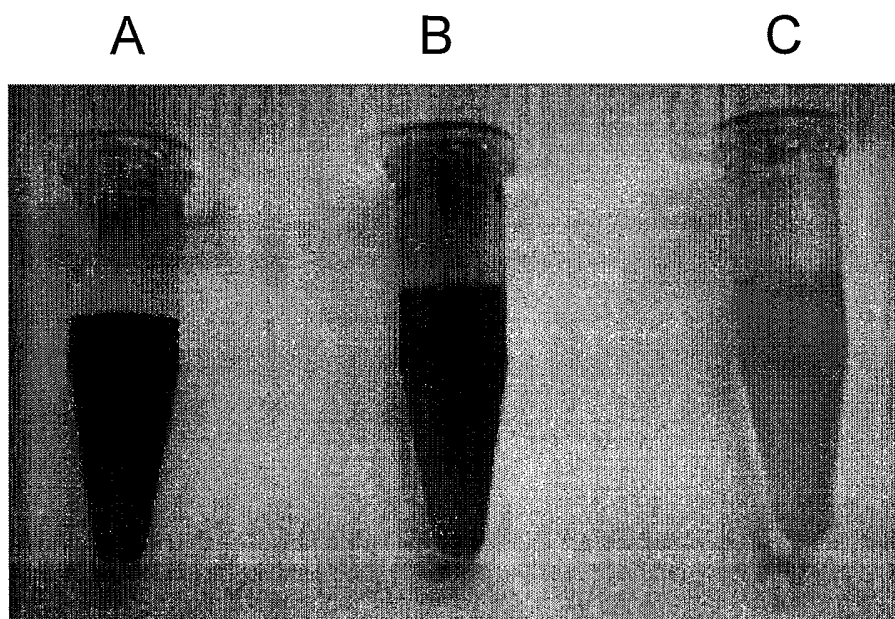
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Figure 4



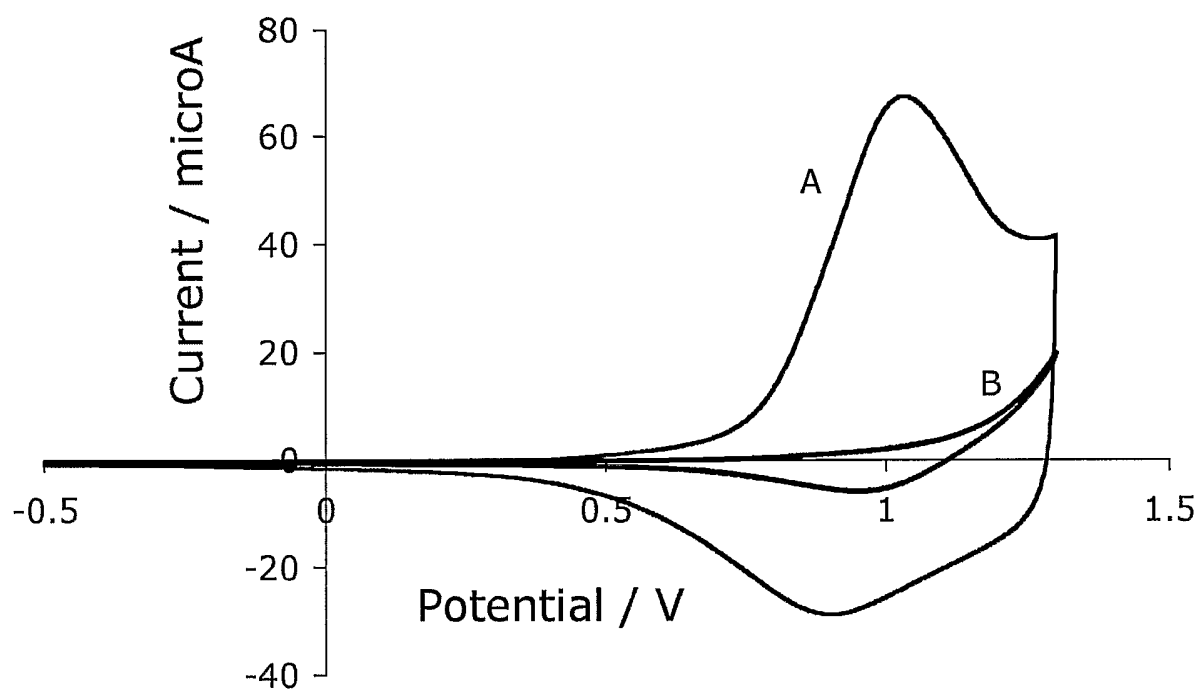
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Figure 5



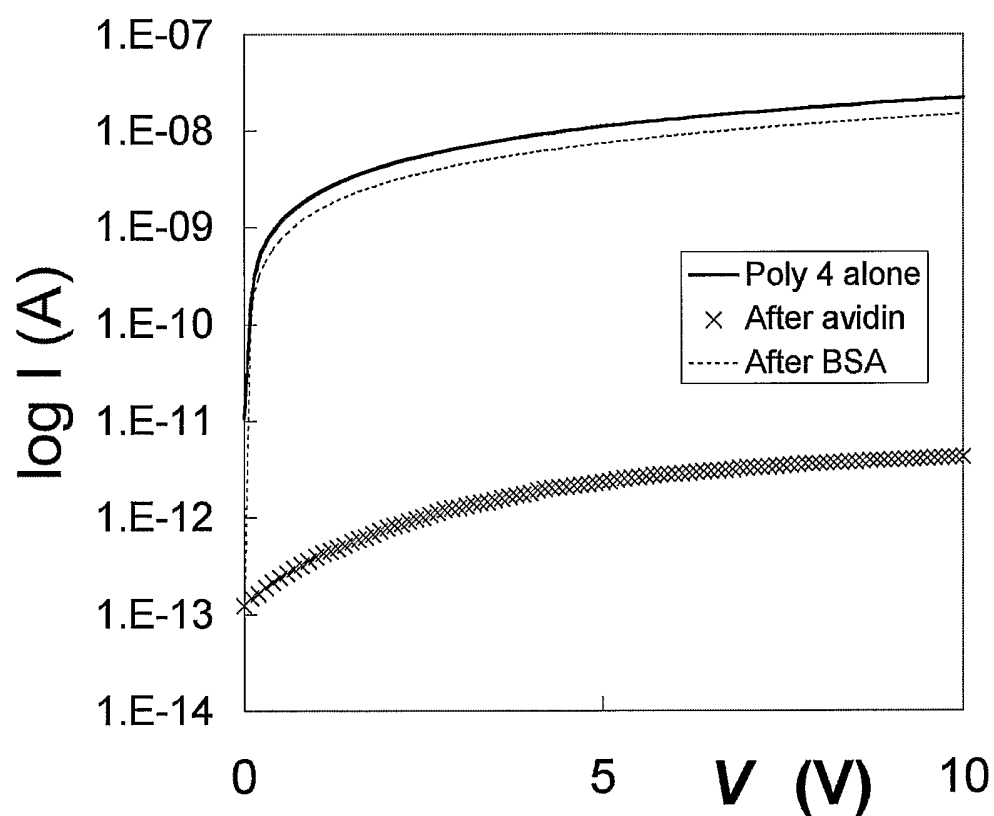
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Figure 6



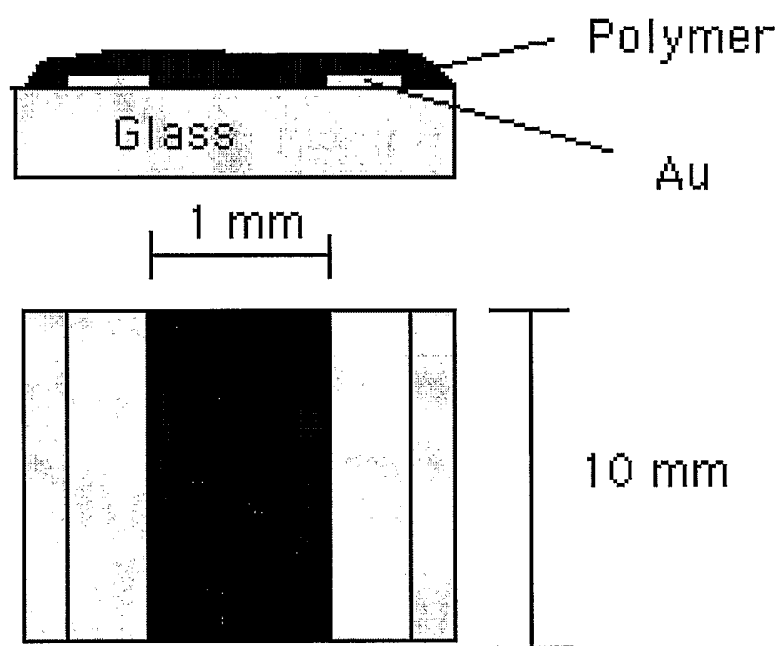
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Figure 7



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Figure 8



INTERNATIONAL SEARCH REPORT

International Application No
/GB2004/003616

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D409/14

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 C07D

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

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

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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P,X	US 2003/195330 A1 (GROENENDAAL LAMBERTUS ET AL) 16 October 2003 (2003-10-16) claims 1-8 ----- -/-	6

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

10 December 2004

Date of mailing of the international search report

22/12/2004

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INTERNATIONAL SEARCH REPORT

International Application No

/GB2004/003616

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

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