CONVENTION APPLICATION FOR A PATENT

COMMONWEALTH OF AUSTRALIA
Patents Act 1952-1969

CONVENTION APPLICATION FOR A PATENT

We of Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA, England

hereby apply for the grant of a Patent for an invention entitled:

IMMOBILISATION OF HAPTENS AND MEASUREMENT

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered 8804669 for a patent or similar protection made in United Kingdom on 27th February 1988.

Our address for service is Messrs. Edwd. Waters & Sons, Patent Attorneys, 50 Queen Street, Melbourne, Victoria, Australia.

DATED this 27th day of February 1989

AMERSHAM INTERNATIONAL plc

by

Ian A. Scott
Registered Patent Attorney

To:
THE COMMISSIONER OF PATENTS.
**COMMONWEALTH OF AUSTRALIA**

**Patents Act 1952-69**

**DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT OR PATENT OF ADDITION**

In support of the Convention Application made by

Amersham International plc.

Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA, England.

(hereinafter referred to as the applicant) for a Patent

for the invention entitled:

**Immobilisation of Haptens and Measurement**

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1. Eustace Anthony Evans

of Amersham International plc, Amersham Place,

Little Chalfont, Buckinghamshire HP7 9NA, England.

do solemnly and sincerely declare as follows:

1. I am authorised by the applicant for the patent
to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was

made in Great Britain

on the 27th day of February 1988, by

Medical Research Council

on the [XXX] day of [XXX] in [XXX] by

---

3. John Corrie of 90 Hornbeam Spring, Knebworth, Hertfordshire,

SG3 6AY, England; Lynne Fairclough of 97 Heath Street, Hampstead, London,

NW3 6SS, England; Stephen Alexander Charles of 5 Eliot Close, Aylesbury,

Buckinghamshire HP19 3JB, England; Martin Francis Finlan of 7 Ballard Close, Aylesbury,

Buckinghamshire HP17 0DD, England.

are the actual inventors of the invention and the facts upon which the applicant
is entitled to make the application are as follows:

The applicant is the assignee of the inventors by virtue of

assignments from Messrs. Corrie and Fairclough dated

23 March 1988 and

As employer of Messrs. Charles and Finlan

by virtue of Sec. 34(1)(e)

4. The basic application referred to in paragraph 2 of this Declaration

was... the first application made in a Convention country in

respect of the invention the subject of the application.

DECLARED at Little Chalfont, Buckinghamshire HP7 9NA

this Eighth day of February 1989

Eustace Anthony Evans
1. An optically transparent material carrying a metal layer suitable for use in surface plasmon resonance spectrometry, wherein a metal surface has immobilised thereon spacer units to which haptens have previously been linked.

8. A method of assaying for an analyte in a sample, by the use of an optically transparent material carrying a metal layer suitable for use in surface plasmon resonance spectrometry, wherein a metal surface has immobilised thereon spacer units to which analyte or an analogue thereof have previously been bound, which method comprises bringing an antibody to the analyte, and the sample containing the analyte, into contact with the metal surface, and monitoring antibody binding to the immobilised analyte or analogue by means of surface plasmon resonance as indicative of the presence or the concentration of the analyte in the sample.

9. A method as claimed in claim 8, wherein the sample is brought into contact with the metal surface, on which the antibody has previously been reversibly bound to the immobilised analyte or analogue, displacement of antibody being monitored as indicative of the presence or the concentration of the analyte in the sample.
10. A method as claimed in claim 8 or claim 9, wherein the analyte is a hapten.
The following statement is a full description of this invention, including the best method of performing it known to us.
IMMOBILISATION OF HAPTENS AND MEASUREMENT

This invention concerns methods of assaying for analytes using the phenomenon of surface plasmon resonance (SPR). The method is applicable to analytes generally, but is likely to be of particular interest where the analyte is a hapten (a small molecule capable of being bound by antibody but not of itself immunogenic).

The phenomenon of SPR is well known and will not be described in detail. Briefly, the intensity of monochromatic plane-polarised light (conveniently obtained from a laser) reflected from the interface between an optically transparent material, e.g. glass, and metal depends on the refractive index of material in a thin layer, at most a few hundred nm thick, on the downstream side of the metal. Accordingly, by measuring changes in intensity of reflected light an indication can be obtained of changes in refractive index of material on the metal. The intensity of reflected light also varies with the angle of incidence, and reflectivity drops sharply to a minimum at a particular angle characteristic of the equipment. The metal surface is generally of silver, although this is not critical to the invention.

The immunoassay of haptens by Surface Plasmon Resonance Spectrometry (SPRS) poses a particular problem because the haptens are, by definition of low molecular weight and therefore cause only very small changes in refractive index when they bind to or dissociate from an antibody-coated SPRS silver-coated surface.

This invention is also concerned with immobilisation of haptens, for use in such immunoassays. Difficulties arise when attempting to immobilise haptens on metal surfaces e.g. for use in immunoassays: although haptens
can be immobilised directly on a metal surface, the immobilised haptens may be unusable in immunoassays.

The present invention aims to overcome these difficulties.

The present invention therefore provides an optically transparent material carrying a metal layer suitable for use in surface plasmon resonance spectrometry, wherein a metal surface has immobilised thereon, spacer units to which haptens have previously been linked. Preferably there is included a metal surface carrying a macromolecular coating comprising macromolecular spacer units to which haptens have previously been covalently linked. Preferably the spacer units are protein molecules and the metal surface is gold or silver. Preferably corresponding antibodies are bound to the haptens.

The present invention also provides a method of making the above material which comprises linking haptens to spacer units to form conjugates and immobilising the conjugates on a metal surface of an optically transparent material carrying a metal layer, and thereafter applying a blocking agent to coat the metal surface. Preferably the blocking agent is a protein.

A method of assaying for an analyte in a sample, by the use of an optically transparent material carrying a metal layer suitable for use in surface plasmon resonance spectrometry, wherein a metal surface has immobilised thereon spacer units to which analyte or an analogue thereof have previously been bound, which method comprises bringing an antibody to the analyte, and the sample containing the analyte, into contact with the metal surface, and monitoring antibody binding to the immobilised analyte or analogue by means of surface plasmon resonance as indicative of the presence or the concentration of the analyte in the sample. Preferably the sample is brought into contact with the metal surface, on which the antibody has previously been reversibly bound to the immobilised analyte or analogue, displacement of antibody being monitored as indicative of the presence or the concentration of the analyte in the sample, and the
analyte is a hapten.

It is thought desirable that the protein has an excess of basic groups (i.e. at least one more basic than acidic amino acid residue), and preferred proteins include gamma globulin, thyroglobulin and methylated ovalbumin, with gamma globulin currently being the most preferred protein. It is believed that the presence of an excess of basic groups provides a beneficial effect by promoting adsorption of the protein to a metal, e.g. silver, surface. It is however, also thought that the presence of an excess of basic groups may have the undesirable effect of enhancing non-specific protein
binding, probably not at the metal surface but by association of non-specific proteins with the spacer protein. A degree of non-specific binding is tolerable and in any event can be reduced by use of a blocking material, e.g. by coating the immobilised hapten with material such as non-immune serum or gamma globulin.

The metal desirably comprises silver or gold, conveniently in the form of a layer e.g. deposited by evaporation on a carrier such as a glass slide.

This aspect of the invention is applicable to all haptens, including e.g. most drugs, steroid hormones, thyroxine.

It is found that by use of a suitable spacer unit, with the hapten indirectly immobilised near the metal surface, non-specific binding problems can be overcome. Although some non-specific binding may occur, as discussed above, a degree of non-specific binding is tolerable and dose dependent antibody binding can be obtained.

The present invention also provides a method of immobilising a hapten on a metal surface, comprising linking the hapten to a spacer unit to form a conjugate and immobilising the conjugate on the metal surface.

An immobilised hapten in accordance with this aspect of the invention finds application, inter alia, in immunoassays.

Hence in a further aspect the present invention provides an immunoassay method using hapten linked to a spacer unit and immobilised on a metal surface.

The method of the invention may be used for determining the presence in a sample of antibody to a particular hapten, by contacting immobilised hapten with the sample and determining whether hapten-antibody binding has occurred, although this possibility is unlikely to be of practical importance.

In a further aspect the invention provides an
immobilised hapten comprising a metal surface which has immobilised thereon spacer units to which haptens are linked, with corresponding antibody bound to the hapten.

These concepts lead to immunoassays of two kinds for analytes, preferably but not necessarily haptens.

Thus in a further aspect, the invention provides a method of assaying for an analyte in a sample, by the use of the analyte or an analogue thereof immobilised via spacer units on a metal surface, which method comprises bringing an antibody to the analyte, and the sample containing the analyte, into contact with the metal surface, and monitoring antibody binding to the immobilised analyte or analogue by means of surface plasmon resonance as indicative of the presence or the concentration of the analyte in the sample.

An analogue of the analyte is a substance which competes with the analyte for binding to a specific binder, such as an antibody, therefor. Often the analogue will be arranged to be as near as possible or even completely identical to the analyte. The use in assays of analyte analogues is well known.

The order of addition of reagent is not critical. Preferably antibody is first added to the immobilised hapten or analogue, which is then washed to remove any unbound material. Then the sample is brought into contact with the metal surface, on which the antibody has previously been reversibly bound to the immobilised analyte or analogue, displacement of antibody being monitored as indicative of the presence or the concentration of the analyte in the sample. This arrangement has the advantage that bringing the sample into contact with the metal surface generates the SPR signal, no other reagent being required.

In a further aspect, the invention provides a method of assaying for an analyte in a sample, by the
use of a metal surface having immobilised thereon antibody to the analyte, which method comprises bringing a conjugate of the analyte or an analogue thereof, and the sample containing the analyte, into contact with the metal surface, and monitoring binding of the conjugate by means of surface plasmon resonance as indicative of the presence or concentration of the analyte in the sample.

The conjugate of the analyte or analogue should be with some substance of sufficient molecular weight to yield a significant SPR signal on the displacement of the conjugate by added free hapten, and may typically be a macromolecule such as a protein.

Again, the order of addition of reagents is not critical. Preferably conjugate is first reversibly bound to the immobilised antibody, and the sample thereafter brought into contact with the metal surface, displacement of the conjugate being monitored to assay the analyte.

These methods can be used qualitatively or quantitatively.

When producing antibodies to a hapten, the hapten is commonly linked to a larger unit using a bridge structure similar to the bridge structures mentioned above for linking a hapten to a protein spacer unit. For use in a particular immunoassay, different bridge structures should be used for the two purposes, to prevent recognition of the hapten-protein bridge structure by the antibodies. For example, where the hapten phenytoin is linked to lysozyme by use of glucuronyl bridge for immobilisation purposes, phenytoin-3-omega-valeryl-bovine serum albumin may be used as the immunogen against which antiserum is raised.

It is possible to use antibody fragments
incorporating the hapten binding site, such as Fab' or Fab fragments, in place of the entire antibody molecule, and references to antibodies should be construed as including such fragments.

References to antibodies should thus be construed as including modified antibodies.

Work carried out using SPR for refractive index detection indicates that the method is very sensitive and is capable of giving accurate results within a few seconds, with detection limits of below 1 ng/l.

The invention finds particular application in monitoring a wide range of analytes of clinical importance, typically having serum concentrations in the range $10^{-6}$ to $10^{-12}$ mol/l. In particular the invention is useful in monitoring serum levels of haptens including drugs such as theophylline, methotrexate, aminoglycoside antibiotics etc.

The invention will be further described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 illustrates schematically one embodiment of apparatus for carrying out the method of the invention, using surface plasmon resonance;

Figure 2 is a diagram showing two assay systems (a) and (b) according to the invention;

Figure 3 is a graph of reflectivity versus time showing results obtained for phenytoin using the apparatus of Figure 1;

Figure 4 is a graph of reflectivity versus time showing results obtained on theophylline using SPR equipment described in EPA 305109; and

Figure 5 is a graph of reaction rate against thyroxine concentration in an immunoassay of thyroxine using SPR.

Figure 6 is a graph of reflectivity versus time in
an assay for theophylline in which anti-theophylline antibodies were attached to the metal surface.

Detailed description of the drawings

The apparatus illustrated schematically uses the phenomenon of surface plasmon resonance (SPR) for measuring the refractive index of a layer absorbed on a thin metallic film.

The phenomenon of SPR is well known and will not be described in detail. Briefly, the intensity of monochromatic plane-polarised light (conveniently obtained from a laser) reflected from the interface between an optically transparent material, e.g. glass, and metal depends on the refractive index of material on the downstream side of the metal. Accordingly, by measuring changes in intensity of reflected light an indication can be obtained of changes in refractive index of material on the metal at a particular point of the metal. The intensity of reflected light also varies with the angle of incidence, and reflectivity drops sharply to a minimum at a particular angle X for any given set up. The apparatus is most sensitive when the angle of incidence Y of the light is more acute than the angle X, roughly half way along the linear part of the dip in SPR curve.

The illustrated apparatus comprises a glass prism 10 to which is attached a glass microscope slide 12 covered on one side by a thin (about 50 to 60nm thick) film 14 (not shown to scale) of silver deposited on the slide by evaporation. A hapten is indirectly immobilised near to the surface of the silver film, as will be described below. A laser light source 16, e.g. a Uniphase Model 1108P He-Ne laser is located on the prism side of the slide, with a light detector 18 located to receive light reflected from the glass-silver interface. The apparatus is adjusted so that
the angle of incidence of the light has a value \( Y \) at which sensitivity is maximised. The intensity of reflected light received by detector 18 is monitored while the immobilised hapten is contacted with a sample to be analysed.

Figure 2 shows two alternative assay systems in diagrammatic form. In 2(a), the hapten 20 is immobilised to the silver surface 22 used for SPRS detection, and binds the corresponding antibody 24. Introduction of free hapten 26 (whose concentration it is wished to determine) displaces antibody by competing with surface bound hapten. This displacement of antibody from the surface is detected as an SPRS signal. In 2(b), the antibody 24 is bound to the surface 22 and binds a conjugate 28 of the hapten 20 and (typically) a protein 30 of sufficient molecular weight to yield a significant SPRS signal on displacement of the conjugate by added free hapten.

The assays in Examples 1 to 3 were carried out using the apparatus of Figure 1. The assays in Examples 4 to 6 were carried out using the improved equipment described in EPA 305109.

Example 1

Phenytoin linked to glucuronide bridge, as described by J.A. Hinds, C.F. Pincombe, S. Smith and P. Duffy in J. Immunol. Methods, 80 239-53 (1985), was coupled to rabbit gamma globulin using the mixed anhydride technique, as described by B.F. Erlanger, F. Borek, S.M. Beiser and S. Leibermann in J. Biol. Chem. 234, 1090-4 (1959) to produce a phenytoin-glucuronide-rabbit gamma globulin conjugate.

A glass microscope slide covered on one side by a thin (50-60 nm) film of silver was immersed for 30-45 minutes in a 1 \( \mu \)mol/l solution of the conjugate in buffer (10 mmol/l sodium phosphate, pH 7.5). The
gamma globulin adsorbed to the silver and indirectly immobilised the phenytoin near to the surface. The slide was then immersed in a solution of 5 μmol/l rabbit gamma globulin in phosphate-buffered saline for 30 minutes. The gamma globulin forms a blocking coating and acts to minimise non-specific binding. The slide was then rinsed once in buffer, then immersed for 16 hours in a solution of phenytoin antiserum (raised in a rabbit against a phenytoin-3-(omega-valeryl)-bovine serum albumin conjugate, essentially as described by C.E. Cook, J. F. Kepler and H.D. Christensen in Res. Commun. Chem. Pathol. Pharmacol., 5, 767 (1973)) diluted 1/800 in buffer containing ovalbumin. This results in binding of antibody to the immobilised phenytoin. The slide was then rinsed 4 times in buffer containing 0.05% Tween 20 and a further time in buffer without Tween 20, resulting in a removal of unbound material, and the non-silvered surface was cleaned by wiping with a tissue moistened with isopropanol.

The surface plasmon resonance properties of the slide (i.e. reflectance versus angle of incidence, and reflectance at fixed angle versus time) were then examined essentially as described by B. Liedberg, C. Nylander and I. Lundestroem in Sensors and Actuators, 4, 299-304 (1983). Thus reflectance versus time was measured with the coated face of the slide initially exposed to buffer, and then following injection into a flow cell of a solution of phenytoin in buffer. The presence of phenytoin causes dose-dependent displacement of antibody from the slide, and hence a decrease in refractive index of the biolayer attached to the metal surface, as shown by the decrease in reflectivity. Typical results are shown in Figure 3, where the points indicated by arrows represent
injection of phosphate buffered saline containing 0, 1 or 5 µg/l phenytoin. The initial slope after injection measured and the values related to hapten concentration.

Because the injection of buffer or hapten sometimes causes small spikes in the reflectivity traces, measurement of the initial slope of the reflectivity vs time curves is taken over a 1 minute period commencing 30 seconds after the injection. Results expressed in this way are set out in the Table below, together with results from Example 2.

**Example 2**

Progesterone. The 11α-glucuronyl derivative of progesterone, prepared as described by J.E.T. Corrie, W.M. Hunter and J.S. Macpherson, Clin. Chem., 27, anhydride technique of Erlanger et al (op. cit.) was used to produce a progesterone-glucuronyl-rabbit gamma globulin conjugate. Other details exactly as for theophylline, except that the progesterone conjugate was coated onto the slide at a concentration of 1 µmol/l, and the antiserum (raised in a rabbit against a progesterone-11α-hemisuccinyl-bovine serum albumin conjugate as described by K.K. Dighe and W.M. Hunter, Biochem., J. 143, 219 (1974)) was used at a dilution of 1 in 400. The Table shows the results for progesterone doses of 0, 12.5 and 50 ng/ml in PBS.

<table>
<thead>
<tr>
<th>Example</th>
<th>Hapten</th>
<th>Concentration</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenytoin</td>
<td>5µg/ml</td>
<td>-0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1µg/ml</td>
<td>-0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>-0.14</td>
</tr>
<tr>
<td>2</td>
<td>Progesterone</td>
<td>50ng/ml</td>
<td>-0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5ng/ml</td>
<td>-0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>-0.13</td>
</tr>
</tbody>
</table>
Example 3

Other spacers. Theophylline-7-propionic acid, prepared as described above, was conjugated to ovine fibrinogen, equine haemoglobin and lysozyme, all by the technique of Erlanger et al (op. cit.). Each conjugate, together with the theophylline-7-propionyl-rabbit gamma globulin conjugate described above, was coated onto separate silvered glass slides using the conditions described above (i.e. 1 μmol/l in phosphate buffer for 45 min). Blocking of residual binding sites on the metal surface was effected by immersion for 30 minutes in 1 in 200 diluted normal rabbit serum in 10 mmol/l sodium phosphate, pH 7.4. The slides were then incubated with the theophylline antiserum and washed as described above and the SPR properties determined in response to doses of theophylline at 0, 1 and 10 μg/ml. Initial slopes (% reflectivity change/min) of the reflectivity vs. time curves were as follows:

<table>
<thead>
<tr>
<th>Theophylline Protein Spacer</th>
<th>Dose (μg/ml)</th>
<th>Fibrinogen</th>
<th>Gamma Globulin</th>
<th>Haemoglobin</th>
<th>Lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>0.05</td>
<td>0.05</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1.06</td>
<td>0.97</td>
<td>0.91</td>
<td>0.46</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>1.57</td>
<td>2.04</td>
<td>1.66</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Example 4

Theophylline. The derivative theophylline-7-propionic acid, prepared as described by T. Nishikawa, M. Saito and H. Kubo, Chem. Pharm. Bull., 27, 893-8 (1979), was coupled with rabbit gamma globulin using the mixed anhydride technique of Erlanger et al (1959) (as in Example 1) to produce a theophylline-7-propionyl-rabbit gamma globulin conjugate.
A glass microscope slide covered on one side by a thin (50-60 nm) film of silver was immersed for 30-45 min in an 8 μmol/l solution of the conjugate in buffer (10 mmol/l sodium phosphate, pH 7.4). The coated slide was then immersed for 30 min in a solution of 5μmol/l rabbit gamma globulin solution in the same buffer to block residual binding sites on the metal surface. The slide was then incubated overnight in a solution of theophylline antiserum (raised in a rabbit against a theophylline-8-butyryl-bovine serum albumium conjugate, essentially as described by T. Nishikawa, M. Saito and H. Kubo, Chem. Pharm. Bull, 32, 4951-7 (1984) diluted 1 in 500 in a buffer (50 mmol/l sodium phosphate/0.154 mol/l sodium chloride, pH 7.4, hereafter called PBS) which also contained 0.1% ovalbumin. The slide was then rinsed twice in PBS buffer containing 0.05% Tween 20, and twice in PBS, and stored until use in PBS. For use, the non-silvered surface was cleaned with isopropanol and the SPR properties of the slide determined before and after exposure to theophylline. Typical results are shown in Figure 4.

Example 5

Thyroxine. Tri-iodothyronoine (T3) linked to disuccinimidyl suberate (DSS) was coupled to human gamma globulin to produce a T3-DSS-human gamma globulin conjugate.

A glass microscope slide covered on one side by a thin (approx 56 nm) film of silver was exposed for 5 minutes to a 1 μmol/l solution of the conjugate in buffer (10 mmol/l sodium phosphate, pH7.4). The gamma globulin adsorbed to the silver surface and indirectly immobilised the T3 near to the surface. After washing with buffer, the slide was exposed for 5 minutes to a 5 μmol/l solution of horse gamma globulin in buffer.
The gamma globulin forms a blocking coating and acts to minimise non-specific binding. After washing with buffer and then PBS, the slide was exposed for a further 5 minutes to a 1 µmol/l solution of monoclonal anti-thyroxine (anti-T4) in PBS. This results in binding of the anti-T4 to the immobilised T3 (the anti-T4 exhibits approx 10% cross reactivity with T3). Unbound material was removed by rinsing in PBS.

Figure 5 shows the initial rate of change in reflectivity for different doses of added T4 in PBS + 0.5% bovine serum albumin + 0.2% anilino naphthalene sulfonic acid.

Example 6

Theophylline. A glass microscope slide covered on one side by a thin (approx 56 nm) film of silver was exposed for 5 minutes to a 1 µmol/l solution of monoclonal anti-theophylline in buffer (10 mmol/l sodium phosphate, pH7.4). After washing with buffer, the slide was exposed for 5 minutes to a 5 µmol/l solution of rabbit gamma globulin in buffer. The gamma globulin forms a blocking coating and acts to minimise non-specific binding. After washing with buffer and then PBS, the slide was exposed for a further 5 minutes to a 1 µmol/l solution of a theophylline-rabbit gamma globulin conjugate (prepared as described in Example 4) in PBS + 0.1% ovalbumin. Unbound material was removed by rinsing in PBS.

Figure 6 shows the SPR response of the slide when exposed to 0 and 25 µg/ml theophylline in PBS.
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An optically transparent material carrying a metal layer suitable for use in surface plasmon resonance spectrometry, wherein a metal surface has immobilised thereon spacer units to which haptens have previously been linked.

2. A material as claimed in claim 1, comprising a metal surface carrying a macromolecular coating comprising macromolecular spacer units to which haptens have previously been covalently linked.

3. A material as claimed in claim 1 or claim 2, wherein the spacer units are protein molecules.

4. A material as claimed in any one of claims 1 to 3, wherein the metal surface is gold or silver.

5. A material as claimed in any one of claims 1 to 4, wherein corresponding antibodies are bound to the haptens.

6. A method of making the material of any one of claims 1 to 5, which method comprises linking haptens to spacer units to form conjugates and immobilising the conjugates on a metal surface of an optically transparent material carrying a metal layer, and thereafter applying a blocking agent to coat the metal surface.

7. A method as claimed in Claim 6, wherein the blocking agent is a protein.

8. A method of assaying for an analyte in a sample, by the use of an optically transparent material carrying a metal layer suitable for use in surface plasmon resonance spectrometry, wherein a metal surface has immobilised thereon spacer units to which analyte or an analogue thereof have previously been bound, which method comprises bringing an antibody to the analyte, and the sample containing the analyte, into contact with the metal surface, and monitoring antibody binding to the immobilised analyte or analogue by means of surface plasmon resonance as indicative of the
presence or the concentration of the analyte in the sample.

9. A method as claimed in claim 8, wherein the sample is brought into contact with the metal surface, on which the antibody has previously been reversibly bound to the immobilised analyte or analogue, displacement of antibody being monitored as indicative of the presence or the concentration of the analyte in the sample.

10. A method as claimed in claim 8 or claim 9, wherein the analyte is a hapten.

DATED this 8th day of August 1990

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IAS:JJC
**Fig. 2** DISPLACEMENT IMMUNOASSAY

(a) FIXED HAPTEN

(b) FIXED ANTIBODY

ANTIBODY

PROTEIN

ANTIBODY

CONJUGATE ANTIBODY

ANALYTE

ANALYTE ANALOGUE

SILVER LAYER

[Diagram showing the displacement immunoassay processes (a) and (b)]
Fig. 3
0; 0.2; 1.0; 25 μg/ml THEOPHYLLINE

SPR - plot versus time

0 μg/ml

0.2 μg/ml

1.0 μg/ml

25 μg/ml

TIME(SECONDS)

REFLECTIVITY %

0.83

330.83

38.00

46.00

Fig. 4
REACTION RATE (ref/sec x 10^-3)

IMMUNOASSAY OF THYROXINE USING SPR

Fig. 5

THYROXINE (nmol/l)
0 AND 25 µg/ml THEOPHYLLINE

SPR - plot versus time

Fig. 6

TIME (SECONDS)