

# Enhanced surface plasmon resonance inhibition test (ESPRIT) using latex particles\*

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**Abstract:** The pregnancy hormone human chorionic gonadotropin (hCG) was used as a model antigen to describe a new assay, the Enhanced Surface Plasmon Resonance Inhibition Test (ESPRIT). It was shown that the introduction of sub-micron latex particles instead of anti-antibodies in an enhancement step improved the sensitivity of the assay by a factor of 30. Latex particles are therefore considered to be versatile tools in the development of new immunochemical assays for the detection of any analyte using SPR immunosensors.

**Keywords:** Surface plasmon resonance, sub-micron latex particles, human chorionic gonadotropin, ESPRIT.

## 1. INTRODUCTION

Immunosensors based on surface plasmon resonance (SPR) are interesting because they offer a number of potential advantages over conventional immunoassay techniques (Liedberg *et al.*, 1983; Robinson, 1991). The detection is quick and in real-time when the immunochemical reaction takes place. Moreover, the system is easy to use and can readily be automated for decentralized testing.

The SPR immunosensor is based on changes in the reflection of laser light from a metal–liquid surface, caused by a change in refractive index. A real-time detection of binding of antigens to

immobilized antibodies on the surface is possible because the formation of an antigen–antibody complex changes the refractive index.

The SPR immunosensor is the most fully elaborated immunosensor available today. In 1990 Pharmacia Biosensor introduced the first SPR instrument on the market, the BIAcore (Biospecific Interaction Analysis, Fågerstam *et al.*, 1992). Since then, this SPR instrument has been used to monitor antibody–antigen interactions and to epitope-map antibody binding sites to antigens (Fågerstam *et al.*, 1990).

For covalent immobilization of biomolecules, BIAcore makes use of a hydrogel-modified surface consisting of a dextran layer of about 100 nm, in which the biospecific interactions take place (Johnsson *et al.*, 1991).

In our SPR immunosensor, biomolecules are simply adsorbed to the surface or to a sub-micron

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latex bead coating interface for increasing the specific coating surface (Schasfoort, 1990; Schasfoort & Van Benschop, 1990). This immobilization approach is compatible with the use of sub-micron latex particles as refractive index enhancers (Drake *et al.*, 1988). These latex particles are about the same size as the dextran layer of the BIAcore system and thus cannot penetrate into this matrix. Therefore, latex particles are less suitable for use in the Pharmacia BIAcore.

Latex particles are known to change the refractive index dramatically as a result of their large size relative to antigens or antibodies. Moreover, the diameter of the latex particles can be chosen to have about the same size as the penetration depth of the evanescent field of the SPR immunosensor. Latex particles are therefore considered to be useful intermediates between the biochemical domain and the physical domain of the SPR immunosensor.

The sensitivity of the SPR immunosensor normally depends on the molecular mass of the ligand to be detected. Small molecules (molecular mass < 5 kDa) cannot be detected directly, because binding to the surface hardly changes the refractive index. This paper presents a new assay for the detection of antigens, independently of their molecular mass, using the SPR immunosensor in combination with latex particles; the Enhanced SPR Inhibition Test (ESPRIT). The detection of the pregnancy hormone human chorionic gonadotropin (hCG) is used as a model system.

## 2. EXPERIMENTAL

### 2.1. Detection system

Surface plasmons can be excited in thin metal layers in different ways (Raether, 1977). The present SPR sensor (Fig. 1) makes use of the 'attenuated total reflection' method. A Kretschmann configuration (Daniels *et al.*, 1988) incorporates an adapted horizontally placed test slide holder for Balzers (BN 845 432-T) disposable glass slides, with a 50 nm gold layer evaporated on it (University of Twente, Department of Thin Films, The Netherlands).

p-Polarised laser light from a diode laser with an electronic control unit (DL25, wavelength 780 nm, Spindler & Hoyer) is reflected on the

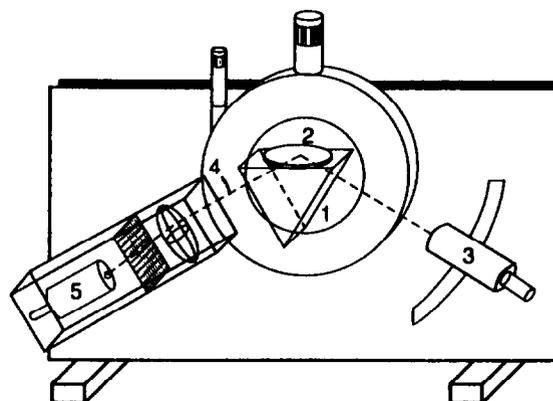


Fig. 1. The SPR apparatus in the Kretschmann configuration. A prism (1) is covered with a glass slide (2), coated with gold and a selective layer, and exposed to a sample fluid. p-Polarized laser light (3) is reflected on the gold-coated slide, inducing plasmons at a certain angle of incidence. Immunochemical reactions, can be detected by measuring the change in intensity of the reflected beam (4) with a photodiode (5) at a fixed angle of incidence.

gold layer. At specific angles of incidence of the laser light, electronic oscillations, called plasmons, are generated in the gold film and the intensity of the reflected beam is diminished. These plasmons cause a non-propagating evanescent field, which penetrates from the gold surface into the sample fluid on top of it to a depth of about half the wavelength (400 nm). Local refractive index changes, produced by binding of antibodies to immobilized antigens, disturb this field and the plasmons arise at a different angle of incidence. This angle shift can be detected by measuring a calibrated change in intensity of the reflected beam at a fixed angle of incidence with a pin diode (EDT pin-10DP 128-1, United Detector Technology/Te Lintelo Systems BV).

To prevent the influence of light from the surroundings, the laser is modulated by an oscillator from a lock-in amplifier (PARC 5209, single phase, EG&G, Princeton Applied Research). The SPR signal is stored on the diskette of a digital oscilloscope (Nicolet 310) and computed in VU-Point (version 1.28, S-Cubed). SPR signals are presented as an angle shift in millidegrees.

## 2.2. Antigens and antibodies

Human chorionic gonadotropin (hCG), sheep polyclonal IgG antibodies to hCG (anti-hCG) and hCG-coated latex (diameter 238 nm) were kindly donated by Flemming GmbH. Unconjugated rabbit anti-sheep IgG antibodies (anti-antibodies) were obtained from Dakopatts.

## 2.3. Coating procedure

HCG was coated on gold-deposited slides by incubating 320 nM hCG in phosphate-buffered saline (PBS, pH 7.4) for 10 min. The slides were washed three times with PBS, blocked with gelatin (0.1% in PBS) for 5 min and finally washed twice with dilution buffer (0.1% Tween 20 and 0.1% gelatin in PBS).

As negative controls, gold-deposited slides were coated with bovine serum albumin (BSA) instead of hCG, according to the same procedure.

## 2.4. Enhanced SPR inhibition test (ESPRIT)

ESPRIT consists of two steps: an inhibition step (Fig. 2) and an enhancement step (Fig. 3).

### 2.4.1. Inhibition step

In the inhibition step (Fig. 2), samples with increasing concentrations of free hCG (0–150 nM) were mixed with a fixed amount of anti-hCG in dilution buffer and incubated on hCG-coated slides for 30 min, under thorough mixing. In this step, binding of anti-hCG to the hCG coat is inhibited by free hCG. When there is no free hCG present, anti-hCG will bind to the hCG coat, causing a strong SPR signal. However, at

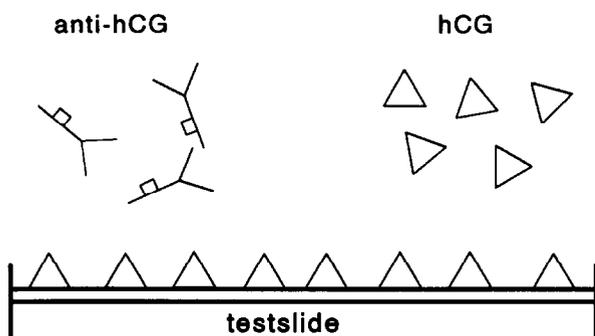


Fig. 2. Principle of the inhibition step. Binding of anti-hCG in solution to hCG, coated on a gold-deposited slide, is inhibited by free hCG.

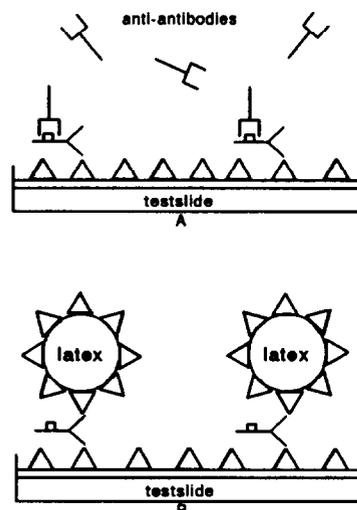


Fig. 3. Principle of the enhancement step. Binding of the enhancement ligand to anti-hCG, immobilized during the inhibition step on a gold-deposited slide coated with hCG. A: anti-antibodies as enhancement ligand; B: hCG-coated latex as enhancement ligand.

high concentrations of free hCG, less anti-hCG will bind to the coat and consequently a weaker SPR signal will be obtained.

In a control experiment, the same step was performed on slides coated with gelatin instead of hCG.

### 2.4.2. Enhancement step

This step (Fig. 3) was introduced to enhance the SPR signal from the inhibition step. Two enhancement ligands were used: unconjugated rabbit anti-sheep IgG antibodies ('anti-antibodies', 0.3% in dilution buffer, Fig. 3A) and hCG-coated latex (0.4% w/w in dilution buffer, Fig. 3B). Binding of the enhancement ligand to anti-hCG causes an enhanced SPR signal, due to the high concentrations of ligand used and to the size of the latex particles. The slides were washed twice with dilution buffer before the enhancement ligand was added. The SPR signal was recorded directly.

## 3. RESULTS

Figure 4 shows the SPR signals recorded during ESPRIT.

Curve 1 of Fig. 4 shows that the SPR signal of the inhibition step alone was very weak (100 millidegrees after 10 min), despite the very high

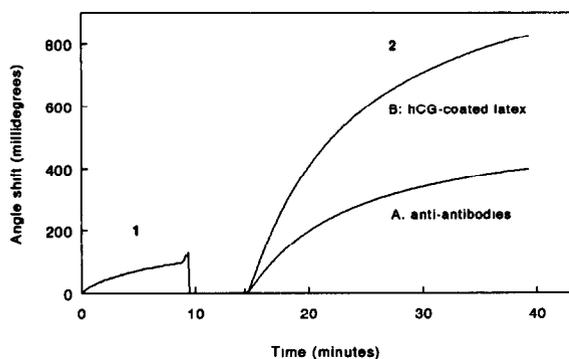


Fig. 4. SPR signals of ESPRIT. 1: inhibition step of 500 nM anti-hCG without free hCG and without mixing; followed by 2: enhancement step with anti-antibodies (A) or hCG-coated latex (B).

concentration of anti-hCG used. This signal could be enhanced by introducing an enhancement ligand such as anti-antibodies of hCG-coated latex (curves 2A and B). The inhibition signal was enhanced by a factor of 3 by anti-antibodies, while hCG-coated latex enhanced the signal by a factor of 6.

The signals were highly specific: control experiments with BSA-coated slides did not give any signal (data not shown).

To optimize the enhancement step, different amounts of anti-hCG were used in the inhibition step in the absence of free hCG. The enhanced signals appeared to depend on the concentration of anti-hCG used (Fig. 5). As can be seen in

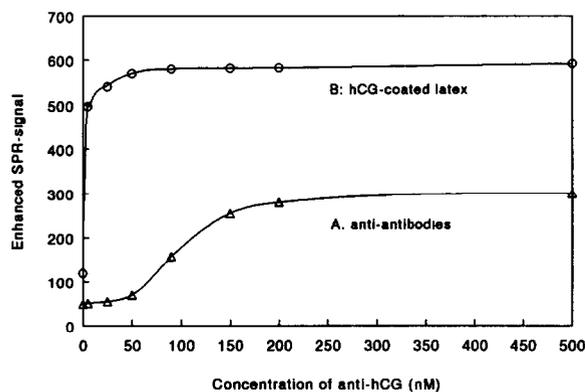


Fig. 5. Enhanced SPR signals (angle shift in millidegrees after performing the enhancement step for 10 min) as a function of anti-hCG concentration during the inhibition step without free hCG. Comparison of enhancement ligands anti-antibodies (A) and hCG-coated latex (B).

curve A of Fig. 5, when anti-antibodies were used as the enhancement ligand, low concentrations of anti-hCG resulted in weak SPR signals, while concentrations above 150 nM resulted in an almost saturated signal. On the other hand, even at low concentrations of anti-hCG (5 nM), hCG-coated latex induced very strong, almost saturated SPR signals (Fig. 5, curve B). For example, at a concentration level as low as 5 nM anti-hCG, the SPR signals of the two enhancement ligands differed by a factor of 10 (50 versus 500 millidegrees). Moreover, this hCG-coated latex signal at 5 nM anti-hCG was almost twice as high as the saturated signal produced by anti-antibodies at 200 nM anti-hCG (280 millidegrees).

In brief, when hCG-coated latex is used instead of anti-antibodies, a 30 times lower concentration of anti-hCG can be used in the inhibition step (5 nM instead of 150 nM), while a saturated signal is still obtained. Therefore a 30 times lower concentration of hCG can be detected with this assay; thus the sensitivity of ESPRIT is increased by a factor of 30.

Finally, different concentrations of free hCG were measured in buffers with ESPRIT. When anti-antibodies were used as the enhancement ligand, signals of all four samples tested were below 50 millidegrees (data not shown). In contrast, when hCG-coated latex was used as the enhancement ligand, 5 nM hCG reduced the SPR signal from 600 to 400 millidegrees (Fig. 6). This result clearly demonstrates the increased sensitivity of ESPRIT resulting from the use of latex particles. Concentrations in the range of 5–10 nM hCG could be detected quantitatively.

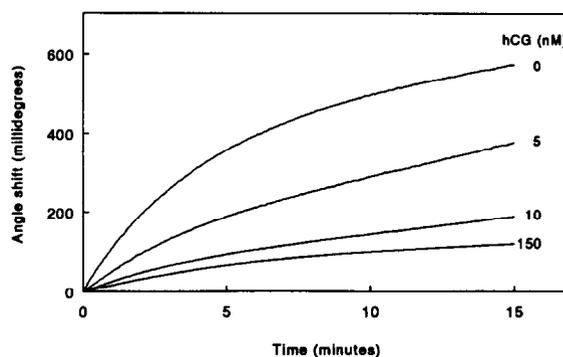


Fig. 6. Enhanced SPR signals of ESPRIT by using hCG-coated latex after inhibition steps with 5 nM anti-hCG, mixed with different concentrations of free hCG.

Concentrations exceeding 10 nM resulted in a very weak (strongly inhibited) signal.

The dynamic detection range of the assay could be chosen by altering the concentration of anti-hCG used in the inhibition step. For example, when 25 nM anti-hCG was used, the detection range was 25–50 nM hCG.

In this study, hCG was used as a model antigen. Further work, especially in relation to haptens, which are normally beyond the reach of SPR, is in progress. The versatility of latex particles for a wide range of analytes will have a large impact on future SPR immunosensor applications.

#### 4. DISCUSSION

Latex particles seem to cause two effects in ESPRIT, compared to anti-antibodies: the signals produced are at least twice as high, and, secondly, a saturated signal is obtained at a 30 times lower concentration of anti-hCG (5 nM instead of 150 nM).

These effects can possibly be explained by the fact that hCG-coated latex (with a diameter of 238 nm) is about 20 times the size of an anti-antibody (dimension about 10 nm). Therefore, one latex particle occupies the same surface area as 400 antibodies.

As one latex particle (with a mass of  $10^{-14}$  g) weighs 50 times as much as 400 antibodies (with a total mass of some  $2 \times 10^{-16}$  g), binding of latex will cause a larger effect on the evanescent field than binding of 400 antibodies on the same surface area. So, theoretically, one should expect a 50 times higher signal (50 times more mass change on the same surface area). The most obvious reason for the lower signal increase we obtained is that latex binds less efficiently than free anti-antibody. Another reason is the fact that most of the mass of the latex particle is at a distance of 117 nm (the radius of the particle) from the metal-liquid interface, where the evanescent field is less sensitive for refractive index changes than at a distance of about 10 nm (the dimension of an anti-antibody).

As latex occupies a 400 times larger surface area than antibodies, the surface does not have to be fully occupied by anti-hCG to obtain a saturated latex coverage. Theoretically, only one anti-hCG is necessary on the surface to bind one 400 times larger latex particle. This means that

even with an anti-hCG coverage of 0.25% (1/400) it is possible to achieve a latex coverage of 100%. The coverage of anti-antibodies, on the contrary, is maximally the coverage of anti-hCG, because they have the same dimensions.

Therefore, by using hCG-coated latex instead of anti-antibodies, a saturated signal is obtained at lower anti-hCG concentrations. Experimentally, we found saturation of latex at a 30 times lower concentration of anti-hCG. This means that only 3% (1/30) of the available anti-hCG binding sites on the hCG coat has to be occupied by anti-hCG to reach a saturated latex coverage, while a saturated anti-antibody coverage needs 100% occupation of the available anti-hCG binding sites.

#### 5. CONCLUSIONS

This paper clearly demonstrates the advantages of the application of latex particles to SPR immunosensors. It was possible to detect a model antigen hCG, independently of its molecular weight, with a new SPR assay: the Enhanced SPR Inhibition Test (ESPRIT). By applying latex particles instead of anti-antibodies in an enhancement step, the sensitivity of the assay was improved by a factor of 30. The detection limit of the test was in the nanomolar range.

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