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# Fast and accurate peanut allergen detection with nanobead enhanced optical fiber SPR biosensor

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ABSTRACT

This paper is the first report of a fiber optic SPR biosensor with nanobead signal enhancement. We evaluated the system with a bioassay for the fast and accurate detection of peanut allergens in complex food matrices. Three approaches of an immunoassay to detect Ara h1 peanut allergens in chocolate candy bars were compared; a label-free assay, a secondary antibody sandwich assay and a nanobead enhanced assay. Although label-free detection is the most convenient, our results illustrate that functionalized nanobeads can offer a refined solution to improve the fiber SPR detection limit. By applying magnetite nanoparticles as a secondary label, the detection limit of the SPR bioassay for Ara h1 was improved by two orders of magnitude from 9 to 0.09 μg/mL. The super paramagnetic character of the nanoparticles ensured easy handling. The SPR fibers could be regenerated easily and one fiber could be reused for up to 35 times without loss of sensitivity. The results were benchmarked against a commercially available polyclonal ELISA kit. An excellent correlation was found between the Ara h1 concentrations obtained with the ELISA and the concentrations measured with the SPR fiber assay. In addition, with the SPR fiber we could measure the samples twice as fast as compared to the fastest ELISA protocol. Since the dipstick fiber has no need for microchannels that can become clogged, time consuming rinsing step could be avoided. The linear dynamic range of the presented sensor was between 0.1 and 2 μg/mL, which is considerably larger than the ELISA benchmark.

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## 1. Introduction

An increasing number of people is confronted with food hypersensitivity. At the very top of the food allergen list is the peanut. Although they have an eminent nutritional value, peanuts can be a serious threat towards sensitized individuals [1]. While other food-induced allergies, e.g. allergies triggered by milk or egg proteins, mainly affect children and disappear when growing up, peanut hypersensitivity tends to persist into adulthood [2]. Since no medical prophylactic treatment is available so far, the sensitive consumer should strictly avoid peanuts. The allergens are heat resistant and are conserved during food production and processing [3]. Hence, accurate and reliable product information is required, especially since the modern food industry frequently processes peanuts or peanut butter in products such as cookies, chocolate, cereal, crackers, and ice cream. Also, some products might contain these nuts inadvertently through contamination of raw materials or equipment used. Even a trace amount of peanut allergens has the potential to be life-threatening by inducing an anaphylactic shock [4]. Consequently, there is a need for sensitive biosensors that can detect low quantities of peanut allergens in complex food matrices and that can be implemented in food quality and safety laboratories.

The current reference method for detecting food allergens is an enzyme-linked immunosorbent assay (ELISA) [5,6]. Although ELISA has proven to be a sensitive and versatile bio-analytical assay, the technique is time-consuming, not reusable, rather expensive, and some steps are difficult to automate [7]. Polymerase chain reaction (PCR) techniques have frequently been reported as a promising alternative for ELISA [8]. While the sensitivity of the PCR methods may become superior, there is no fixed correlation between the amount of DNA and the amount of allergens, which may lead to false positive or false negative results. Therefore, immunoassays still remain the method of choice for the quantitative detection of low concentrations of peanut allergens in real food samples [2].

Surface plasmon resonance (SPR) is generally considered as one of the most suited methods to monitor biomolecular interactions and thus can be applied as an accurate allergen immunosensor [9]. Most commercial SPR systems (e.g. Biacore, GE Healthcare, Sweden; Spreeta, Sensata Technologies, Brazil) are fully automated, their sensor chips can be used for many samples and the SPR sen-

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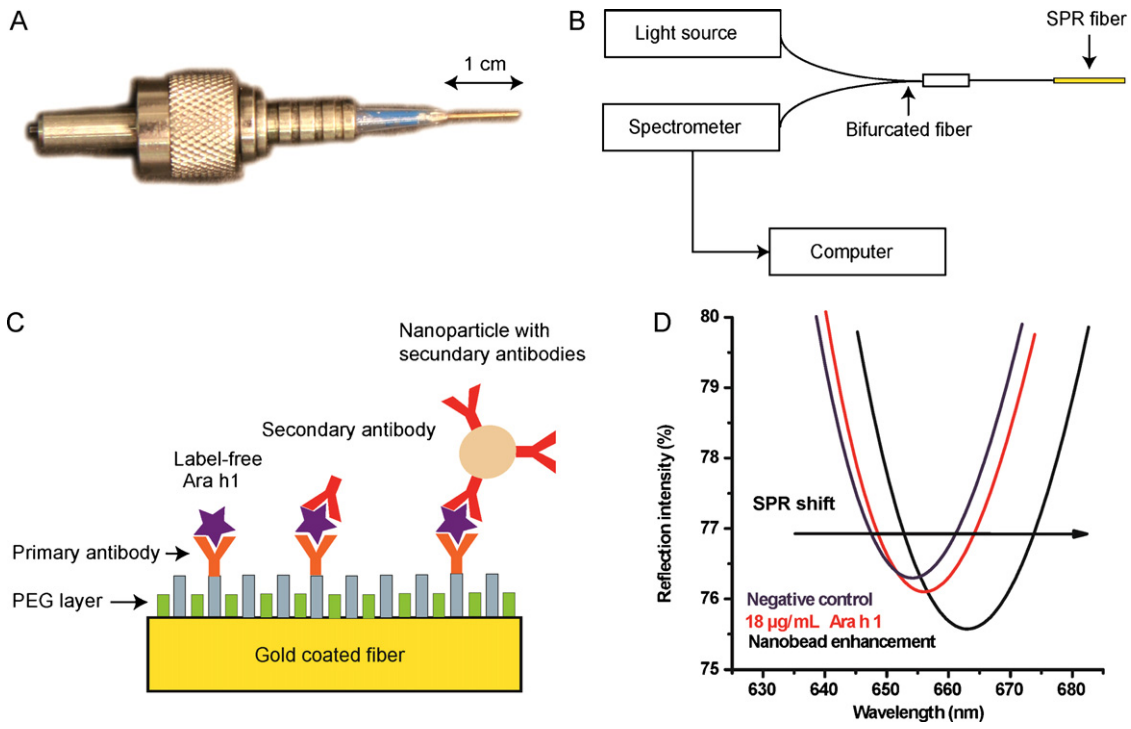


**Fig. 1.** (A) Fiber optic SPR probe; (B) schematic representation of the system setup; (C) overview of the Ara h1 immunoassay strategies on the fiber optic SPR biosensor; (D) the spectrum dips in PBS buffer after 10 min incubation of the SPR fiber in: a negative control sample (blue dip), a sample containing 18 μg/mL Ara h1 (red dip), a sample containing 18 μg/mL Ara h1 subsequently labeled with antibody linked nanobeads (black dip). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

sitivity is comparable with the most sensitive ELISA assays [10]. Nevertheless only a limited number of research labs are equipped with a SPR setup, since most commercial systems are expensive and bulky instruments with a prism based Otto or Kretchmann configuration. Optical fibers offer a more compact and affordable alternative to couple light and surface plasmons, however due to inherent characteristics of fiber optics, the sensitivity of such SPR system is limited compared to systems operating with a prism configuration [11–15]. Recently the SPR detection limits have been pushed towards impressive thresholds by using functionalized nanoparticles as a secondary label [16–22]. In the presented work this amplification principle is used for the first time on fiber optic SPR, improving the sensitivity towards to the detection level of ELISA and label-free prism based SPR instruments. The fiber optic platform is well suited for the combination with nano- or microbeads, since unlike many of the commercial available SPR systems there are no microfluidics involved, hence there is no clogging possible and there is no need for intensive and time consuming rinsing procedures.

The advanced sensitivity is demonstrated by tackling the peanut allergen detection challenge. We compared three approaches of a peanut allergen immunoassay on a SPR fiber; a label-free assay, a secondary antibody sandwich assay and a nanobead enhanced assay. For the latter, we coated magnetite nanoparticles with polyclonal antibodies against the major peanut allergen Ara h1. The super-paramagnetic character of the nanoparticles ensured easy handling. Ara h1 was chosen as peanut allergen biomarker, because it is the most abundant peanut allergen and it triggers a reaction in up to 95% of the patients [23]. The functionalized nanobeads were applied in a sandwich assay on an antibody coated SPR fiber. As such, they offer an elegant solution to improve the SPR detection limit and to deal with variable matrix effects. The results of the nanobead enhanced fiber optic assay were benchmarked to a commercially available ELISA kit.

## 2. Materials and methods

### 2.1. Fiber optic SPR probes

Fig. 1A depicts an illustration of the fiber optic SPR probe. A detailed description of the fabrication of the fiber probe can be found in Pollet et al. [11]. In order to verify the response of different fibers after the production process, each fiber was calibrated based on a six point calibration curve of ethanol–water mixtures with a known refraction index. The average resolution of the sensors was approximately $5 \times 10^{-5}$ refraction index units (RIU).

Once calibrated, the gold surface of the sensor was rinsed with ethanol and linked with a 1 M mixed self-assembling monolayer (4:1 v/v) of polyethylene glycol and polyethylene glycol acid (Polypure, Oslo, Norway) during a 24-h incubation period. Polyclonal rabbit anti-Ara h1 antibodies (Indoor Biotechnologies Limited, Warminster, UK) were coupled to the glycol layer by a carbodiimide reaction. These antibodies are selected to bind to specific epitopes present on peanut allergen Ara h 1, however they also show a very limited amount of cross-reactivity for peanut allergens Ara h2 and Ara h6 when those are present at very high concentrations (<1 mg/mL).

The carboxyl groups on the fiber were first activated for 30 min with 0.4 M 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 0.1 M N-hydroxysulfosuccinimide (NHS) in a 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6). The fibers were then immersed for 1 h in MES buffer with 100 μg/mL polyclonal Ara h1 antibodies. Afterwards, the functionalized fibers were briefly washed with 1 M NaCl in 50 mM NaOH to remove all non-covalently bound antibodies and stored in a 10 mM phosphate buffered saline (PBS) buffer with 1% bovine serum albumin (BSA) and 0.05% Tween-20 at pH 7.4 (1% BSA PBS-T). The immobilization protocol could be monitored in real time based on the SPR response.

### 2.2. Apparatus and system setup

White light from a tungsten halogen light source (LS-1, Ocean Optics, Dunedin, USA) was guided through a bifurcated optical fiber in the SPR probes (Fig. 1B). The golden distal end face of the probe acted as a mirror and reflected the light back to a UV-VIS spectrophotometer (USB4000, Ocean Optics, Dunedin, USA). Based on a reference spectrum in air, the reflection spectrum of a typical solution showed a spectral resonance SPR-dip. A binding event on the gold layer causes a wavelength modulation of this SPR-dip, hence, based on the resonance wavelength shift, the surface chemistry on the gold layer was monitored in real time (Fig. 1C and D). In the resulting sensorgrams the SPR wavelength shift (nm) was plotted versus time.

Data were recorded with Spectrasuite (Ocean Optics, Dunedin, USA). Following spectrometer settings were used: integration time = 500 ms, average = 1 and boxcar = 10. Further processing was done using Matlab software (Mathworks, Natick, USA). The raw data were filtered with a Savitsky-Golay-filter. The resonance wavelength was finally calculated using the Minimum Hunt Method [15]. In this method, a second order polynomial was fit to the SPR dip to determine the local minimum. As a result of the different data processing steps, the impact of modal noise in the multimode fibers on the signal was minimized.

To ensure repeatable, fast and easy immobilization procedures and measurements, the setup was integrated into a fully automated system with a pre-programmed computer-controlled robot (Colinbus, Hulshout, Belgium). In the systems sample rack 96 PCR tubes of maximum 200 μL and four containers of 25 mL for the reaction and washing buffers could be placed.

### 2.3. Antibody linked nanoparticles

Super-paramagnetic nanoparticles were prepared using the forced hydrolysis method. First, 2.4 g of anhydrous $FeCl_3$ was dissolved in 10 mL ethylene glycol and 4 mL MilliQ water. Next, 37.5 mL of ethylene glycol and 25 mL of octylamine were mixed in a 100 mL round bottom flask and heated to 150 °C. After stirring, the $FeCl_3$ solution was added dropwise to the heated round bottom flask and further heated to reflux at 185 °C for 18 h. After cooling, the nanobeads were washed with acetone, precipitated using our homemade magnet and redispersed via sonication. This process was repeated four times. Next, the nanoparticles were dried in a vacuum oven. A typical synthesis yielded around 1 g of dried nanobeads. The nanoparticles were then silanized using a mixture of N-(trimethoxysilylpropyl) ethylenediamine triacetic acid trisodium salt and 3-[bis(2-hydroxyethyl)amino]propyl-triethoxysilane. The average size of the nanoparticles was measured to be $19.0 \pm 3.1$ nm based on images made with a scanning electron microscope (XL30 ESEM, Philips Electronics, The Netherlands). This was further verified based on images obtained with a transmission electron microscope (MPI KGF, Golm, Germany).

Next, the magnetic nanoparticles were functionalized through a carbodiimide reaction with polyclonal Ara h1 antibodies (Indoor Biotechnologies Limited, Warminster, UK). To ensure a maximum number of antibodies linked to the beads, a slightly different protocol was used compared to the immobilization of antibodies on the gold surface of the fibers. First, 25 μL of a 10 mg/mL nanobeads stock solution was dissolved in 475 μL of a 100 mM MES buffer (pH 6). The carboxyl surface of the nanoparticles was washed 3 times by dissolving, magnetically capturing and decanting the nanoparticles in MES buffer. Next, the carboxyl groups of the cleaned nanobeads were activated by adding 20 μL of 0.03 M EDC in 480 μL MES. After 10 min 1 μL of 2 mg/mL of antibodies was added for bioconjugation. Finally, the functionalized nanoparticles were washed three times and stored in 1% BSA PBS-T in a concentration of 2 mg/mL.

### 2.4. Sample preparation

Mars™ candy bars with a dense chocolate matrix produced by Mars Inc (McLean, VA, USA), were purchased at the local supermarket and used to evaluate the biosensor. As labeled, this type of chocolate bar may inadvertently contain allergens, but all evaluated samples responded negative on the ELISA and the SPR test.

Chocolate is generally considered as a difficult matrix for the extraction of proteins mainly due to the high concentration of phenolic compounds, such as tannins, which have an affinity towards proteins [21]. Not only do they block the allergens, phenolic compounds can also bind to the antibodies [26]. The chocolate bar samples were first powdered in liquid nitrogen. Then, 1 g of skimmed milk powder (Nestlé, Belgium) was added to 1 g of sample to bind the phenolic compounds of the chocolate. The mixture was then extracted for 10 min in 20 mL of a preheated (60 °C) TRIS–HCl buffer (pH 8.2) [4,23–27]. Before extraction, some of the samples were spiked with known concentrations of Ara h1. Subsequently, the samples were centrifuged at $2500 \times g$ at 4 °C to precipitate the bound phenolic compounds [4]. The supernatant was used in both the standardized ELISA and the fiber optic SPR biosensing assays.

### 2.5. ELISA protocol

The commercially available ELISA kit 'Ridascreen fast' (r-Biopharm, Darmstadt, Germany), which comprised polyclonal antibodies directed against peanut was used as a benchmark. This kit is especially designed for a fast ELISA protocol and can be carried out within 45 min. The main steps of the protocol were executed as described in the manufacturer's manual. The final color reaction was measured at 450 nm (Spectramax M2e, Molecular Devices, Silicon Valley, CA, USA) (Supporting information).

### 2.6. SPR assay protocol

Similar to the ELISA technique, we used 100 μL of the supernatant samples for each SPR assay. A standard protocol consisted out of six steps, and was performed within 20 min. First, the fibers were placed in the PBS buffer for 3 min to stabilize and to acquire a baseline signal. Next, the fibers were dipped for 5 min into the samples. The sensors were then rinsed to remove all non-bound molecules and transferred to the vial with nanobeads for 10 min to amplify the SPR signals. After each measurement the sensors were regenerated with a 2 min acid treatment in a glycin buffer at pH 1.7. The rinsing and regeneration procedures were enhanced by vigorously moving the sensor head up and down. Afterwards, the sensor was stored in the 1% BSA PBS-T buffer. One vial of 100 μL antibody functionalized nanoparticles (2 mg/mL) was prepared for a series of ten measurements. One in ten samples was a negative control sample (blank sample).

## 3. Results and discussion

### 3.1. ELISA reference measurements for the peanut allergen Ara h1

The standard ELISA calibration curve that came with the assay kit is based on a dilution series of a peanut extract. The sample concentrations are accordingly expressed in parts per million (ppm). The data of the calibration curve was experimentally verified and confirmed (Fig. S1). Because this calibration curve does not provide any quantitative data of the actual allergen exposure, we reevaluated the polyclonal ELISA kit with a dose–response experiment of

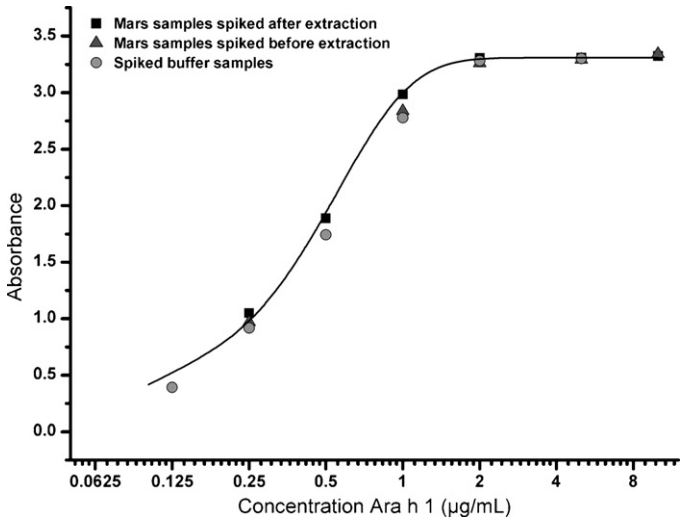


**Fig. 2.** Dose–response curve and calibration curve of the peanut ELISA kit based on dilution series of Ara h1. The squares represent extraction buffer spiked with Ara h1, the triangles represent chocolate bar sample extractions spiked with Ara h1 after the extraction protocol, dots represent chocolate bar sample extraction spiked with Ara h1 before the extraction process.

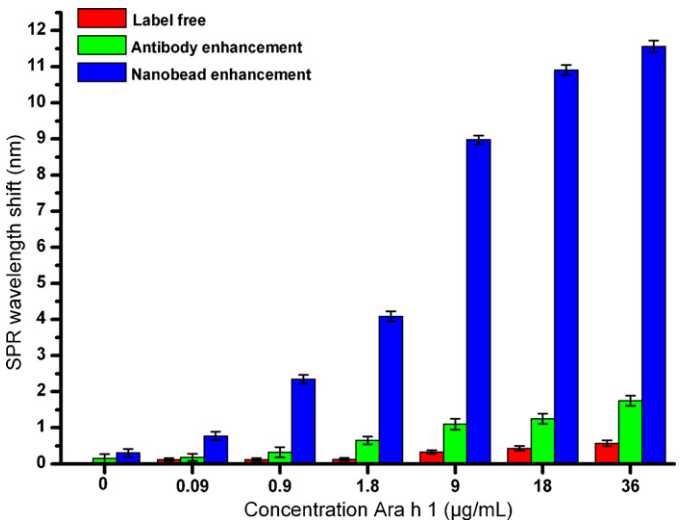


**Fig. 3.** Comparison of the three presented immunoassays on the fiber optic SPR sensor for different Ara h1 concentrations. Red bars: the label free assay, green bars: secondary antibody sandwich assay and blue bars: the nanobead enhanced assay. The error bars indicate standard deviations ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

spiked samples with a known concentration of Ara h1, expressed in microgram Ara h1 per milliliter extraction buffer (Fig. 2).

To verify the extraction procedure and the possible interference of the chocolate matrix, three types of dose–response curves were compared.

A first curve was made based on a dilution series of Ara h1 in TRIS–HCl extraction buffer. A second curve was made in an identical way as the first, but the allergen was added to the supernatant of an extracted chocolate sample. There was no loss of sensitivity observed when measured in the sample matrix. A third curve was made with chocolate samples spiked with Ara h1 before the extraction. As shown in Fig. 2 all dose–response points show a nearly perfect overlap. Hence, the extraction procedure was considered as successful in the evaluated concentration range. For concentrations higher than 1 μg/mL the signal saturated. Based on a signal-to-noise ratio of 3, the limit of detection was determined to be 0.10 μg/mL.

### 3.2. Ara h1 immunoassay strategies on the fiber optic SPR biosensor

The SPR wavelength shift of the sensor is related not only to the number of bound target molecules, but also to their weight. As we previously reported, a 1 nm SPR wavelength shift recorded by the fiber sensor represents a change of 28.6 ng/cm$^2$ in the molecular coverage [11]. Hence, the higher the molecular weight, the stronger the SPR wavelength shift, for an equal amount of bound molecules. Nevertheless, the binding of larger molecules can be influenced by steric hindrance and diffusion limitations. In the search for low limits of detection and accurate quantification of the peanut allergen Ara h1, we evaluated three different approaches (Fig. 1C).

### 3.3. Label-free assay

The binding of the first layer of antibodies to the activated mixed PEG layer resulted in an average shift of 7.0 ± 0.5 nm. The maximum binding capacity of the Ara h1 proteins at equilibrium (after 15 min, for concentrations >50 μg/mL) was 1.4 ± 0.4 nm. Considering their relative difference in weight (the molecular mass of the antibody is assumed to be 150 kDa, the mass of the Ara h1 protein 63 kDa), approximately 48% of the immobilized antibodies were able to capture an allergen (Table 1). This seems to be in accordance with the fact that the antibodies were not bound with a specific orientation. The label-free response for different Ara h1 concentrations is illustrated in the red bars of Fig. 3. Based on a signal to noise ratio of 3, the limit of detection for the label-free SPR assay was estimated to be 9 μg/mL.

### 3.4. Secondary antibody sandwich assay

As can be perceived from the green bars in Fig. 3, a secondary antibody sandwich based assay significantly enhanced the sensitivity of the sensor. The maximum SPR wavelength shift at equilibrium was 2.6 ± 0.3 nm. This indicates that up to 78% of the bound allergens could be captured in the formation of an antibody sandwich (Table 1). The detection limit of the polyclonal antibody sandwich assay was estimated to be 0.21 μg/mL. This is almost 35 times lower than the same assay without application of a secondary immuno-tag. Therefore, even though a label-free assay might be more convenient, a hefty label can significantly enhance the sensitivity of the SPR sensor.

**Table 1**
Overview table of the maximum binding capacity for the different layers of the sandwich immunoassay.

| | Maximum binding capacity | | | |
|---|---|---|---|---|
| | MW (kDa) | Shift (nm) | ng/cm$^2$ | nmol/cm$^2$ |
| Primary antibody | 150 | 7.0 ± 0.5 | 20 ± 1.4 × 10 | 1.3 ± 0.10 |
| Ara h1 allergen | 63 | 1.4 ± 0.4 | 40 ± 11 | 0.64 ± 0.18 |
| Secondary antibody | 150 | 2.6 ± 0.3 | 74 ± 8.6 | 0.50 ± 0.06 |
| Functionalized nanoparticle | 11,280 | 21 ± 1.0 | 60 ± 2.8 × 10 | 0.05 ± 0.01 |

The average weight sensitivity of the fiber optic SPR sensor was 1 nm shift per 28.6 ng/cm$^2$.

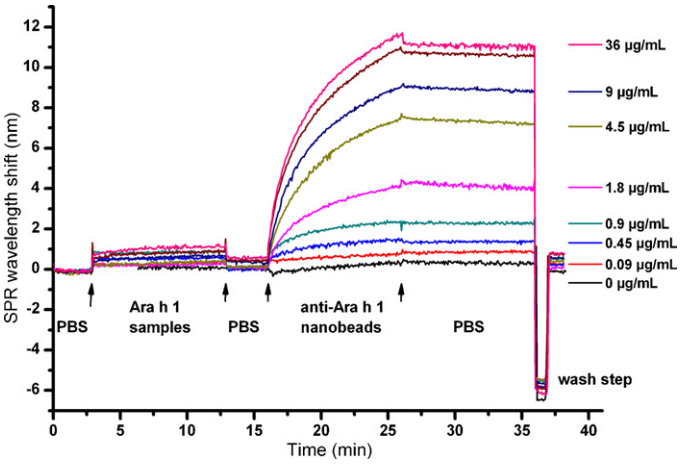


**Fig. 4.** SPR sensorgrams illustrating the binding of different concentrations of Ara h1, followed by an amplification step with nanoparticles functionalized with polyclonal antibodies. First the fiber was put for 3 min in the main buffer to stabilize and to acquire a baseline signal. Next, the fiber was dipped for 10 min in one of the samples, rinsed and placed again for 3 min in PBS buffer. Finally, the fiber was transferred to the vial with nanobeads to amplify the signal for 10 min. Desorption of the beads was monitored during 10 min of incubation in the PBS buffer. After each measurement the sensor was regenerated with a 2 min acid treatment.

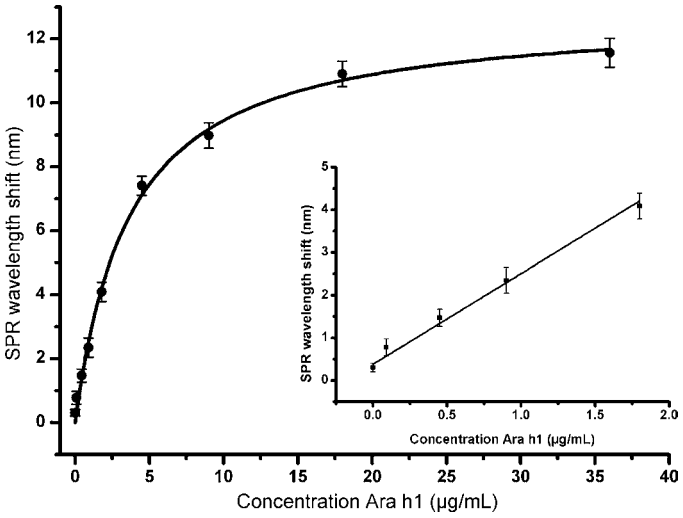


**Fig. 5.** Dose–response and calibration curve of Ara h1 based on the results obtained by a nanobead enhanced SPR sensor. The results were collected after 5 min of allergen binding and 10 min of nanobead amplification. The inset figure shows the linear calibration curve. The error bars indicate standard deviations ($n = 3$).

### 3.5. Nanobead enhanced assay

In the last approach we immobilized the Ara h1 antibodies on the super-paramagnetic nanoparticles and applied them as a secondary label to amplify the SPR signal (Fig. 1C and D). A diameter of 19 nm was shown to be the most ideal size for the nanobeads in this assay. Particles larger than 60 nm settled down too rapidly, resulting in fewer successful binding reactions and a decreased sensitivity during the experiments. Recently, Piletska and Piletsky described the relation between particle dimensions and their binding affinity [28]. Taking into account steric hindrance, diffusion limitations and the fact that the SPR shift is proportional to the amount of bound mass, they suggested the use of 50–100 nm silica particles to enhance SPR signals. Because the magnetite nanobeads used in our assay have a higher density as compared to the silica beads, we could use particles with smaller dimensions, diminishing the impact of steric hindrance and diffusion limitation.

With our SPR system we were able to monitor the different protocol steps of the bioassay in real time. This is demonstrated in Fig. 4, where we combined the sensorgrams from a series of measurements with different Ara h1 concentrations. In each measurement cycle the fiber was first incubated for 10 min in the allergen spiked samples, subsequently the signal was enhanced by antibody coated nanoparticles. As can be noticed in Fig. 4, the binding process of the nanobeads did not achieve an equilibrium for Ara h1 concentrations higher than 2 µg/mL. In order to reach the point of equilibrium for these high concentrations, it was necessary to extend the signal amplification step to 45 min. Our kinetic studies revealed that in contrast to the slow nanoparticle binding process, the allergen–antibody binding was much faster and was in equilibrium after only 5 min. This difference will probably be related to diffusion limitations, electrostatic repulsion and steric hindrance. Based on this observation we further used 5 min of incubation for the Ara h1 binding, and 10 min for the nanoparticle amplification step. The resulting dose–response curve is shown in Fig. 5. The detection limit is in the same order as the detection limit of the ELISA kit (0.1 µg/mL).

Based on the linear part of the data from the dose–response curve a calibration curve was made (inset of Fig. 5). For the Ara h1 concentrations in this region, the binding process of the nanobeads got to the point of full equilibrium within the timeframe of the used protocol. The detection limit of the nanobead enhanced assay was estimated at 0.09 µg/mL. Hence, the sensitivity was improved by two orders of magnitude, as compared to the label-free assay.

Applying the suggested time protocol, the nanobead enhanced SPR fiber assay could be carried out within 20 min. In comparison, the execution of the ELISA protocol was done in about 45 min. The extra time necessary for ELISA can be explained by the time consuming rinsing steps and the extra step for the enzyme reaction.

The maximum SPR wavelength shift induced at equilibrium was $21 \pm 1.0$ nm. The molecular weight of a spherical 19 nm diameter magnetite ($5.15\ g/cm^3$) nanoparticle coated with one antibody is approximately 11280 kDa. Hence, if we assume a linear relation between the increased weight and the signal enhancement, almost 9% of the allergens captured by the antibodies on the fiber are sandwiched by a secondary antibody immobilized to a nanobead (Table 1).

After measuring, the SPR fibers could be regenerated within 2 min. This is a major advantage compared to microfluidic SPR systems which usually require long washing steps, certainly when nano- or microbeads are being used [22]. The sensors were regenerated for 35 cycles without any significant loss of sensitivity (Supplementary information).

The non-specific binding of the nanobeads was prevented by the repulsive character of the mixed PEG layer. Furthermore, the sensor's binding surface is vertically positioned, hence no particles can simply settle down on the gold layer of the sensor.

Based on the calibration curves of the ELISA and nanobead enhanced SPR fiber assay, we compared the assays with a new set of samples. An excellent correlation was found between both immunoassays (Fig. 6).

In further research we will further exploit the superparamagnetic character of the nanobeads. When the beads are coated with monoclonal antibodies, they can also be applied to purify and concentrate antigens from different extracts of complex sample matrices. With this technique, even lower detection limits might be pursued by concentrating the allergens before measuring. However, this step should not be considered trivial, as in contrast to

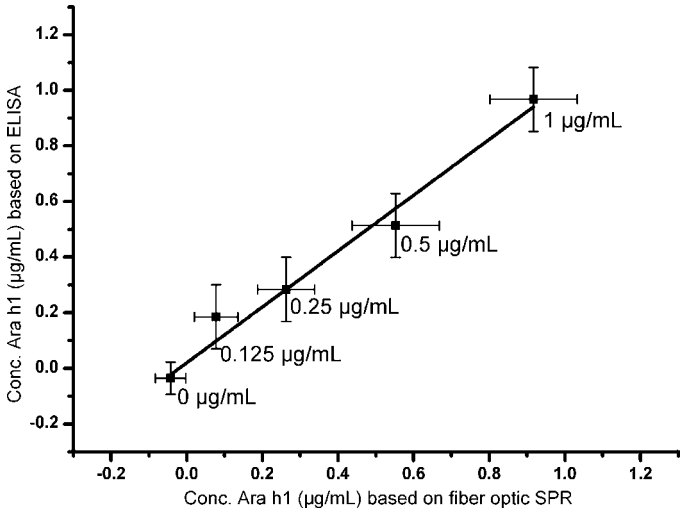


**Fig. 6.** A correlation between the results of the ELISA kit and the results of the nanobead enhanced SPR fiber for different Ara h1 concentrations. One set of spiked chocolate samples was prepared for both experiments. The error bars represent the standard error of the mean based on three constitutive measurements of the same set of samples.

the presented assay, both the number of correctly oriented bound antibodies per particle and the quantity of particles used per sample will become of major importance.

## 4. Conclusions

It has been demonstrated how antibody linked nanobeads can be effectively used to amplify fiber optic SPR signals. We successfully employed this combination in a bioassay to detect Ara h1 peanut allergens in a complex sample matrix such as chocolate candy bars. The biosensor detection limit for Ara h1 was improved by two orders of magnitude, from 9 μg/mL for the label-free assay over 0.21 μg/mL for an antibody sandwich assay to 0.09 μg/mL for the nanobead enhanced assay. The SPR fibers could be regenerated in 2 min with an acidic buffer. Negative control samples proved that non-specific interactions were well controlled. The sensor was benchmarked against a commercially available polyclonal ELISA kit. The detection limit of the SPR fiber was found to be comparable to the detection limit of the ELISA kit (0.1 μg/mL), but the dynamic range of the SPR sensor was considerably larger. The ELISA system saturated for 1 μg/mL Ara h1 whereas the fiber based assay had a linear response until 2 μg/mL. In addition, the time necessary to carry out the analysis was remarkable reduced. Using the SPR fiber, we could measure the samples twice as fast as compared to the ELISA protocol. A last important advantage of the setup is that all components are suitable for miniaturization. We strongly believe that when made portable the system could be useful in a broad range of applications.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.11.032.

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