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Synthesis of MIP Nanoparticles for α-Casein Detection using SPR as a Milk Allergen Sensor

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ABSTRACT: Food recalls due to undeclared allergens or contamination are costly to the food manufacturing industry worldwide. As the industry strives for better manufacturing efficiencies over a diverse range of food products, there is a need for the development of new analytical techniques to improve monitoring the presence of unintended food allergens during the food manufacturing process. In particular, the monitoring of wash samples from cleaning in place systems (CIP), used in the cleaning of food processing equipment, would allow for the effective removal of allergen containing ingredients in between food batches. Casein proteins constitute the biggest group of proteins in milk and hence are the most commonly milk protein allergen in food ingredients. As such, these proteins could present an ideal analyte for cleaning validation. In this work, molecularly imprinted polymer-nanoparticles (nanoMIPs) with high affinity towards bovine α-casein were synthesized using solid-phase imprinting method. The nanoMIPs were then characterized and incorporated into label free surface plasmon resonance (SPR) based sensor. The nanoMIPs demonstrated good binding affinity and selectivity towards α-casein (K_D ~10 x 10^{-9} M). This simple affinity sensor demonstrated the quantitative detection of α-casein achieving a detection limit of 127 ± 97.6 ng ml^{-1} (0.127 ppm) which is far superior to existing commercially available ELISA kits. Recoveries from spiked CIP waste water samples were within the acceptable range (87-120%). The reported sensor could allow food manufacturers to adequately monitor and manage food allergen risk in food processing environments while insuring that the food produced is safe for the consumer.

Food allergies remain a significant risk to public health with increasing rates of incidence within both infant and adult populations. A food allergy is a disorder as an adverse immune response towards certain dairy products[1]. The main mechanisms for an allergic reaction involve an IgE and non IgE mediated response to the presence of the milk protein allergen[2,3].

The increasing risk of milk protein allergens on public health is of particular concern to food manufacturers due to the increased risks of cross-contamination of allergen containing ingredients between food manufacturing lines and ensuring that food manufacturing equipment is allergen free. In addition, the required threshold dose that elicits an adverse reaction varies between the affected population[4]. This has prompted the US Food and Drug administration (FDA) to set out regulations that require food manufacturers introduce precautionary labeling onto all their food products[5,6]. In order to assure production line cleanliness, food manufacturer's place significant effort into validating the cleaning in place (CIP) processes. This can be supported through the use of quality control testing, typically done via swab testing of the food manufacturing equipment in between food batches to ensure that all surfaces are allergen free. The use of such testing is infrequent and requires the use of dedicated analytical labs which takes several days to complete. As such food manufacturers are looking to use simplified tests which are capable of on-line or at-line detection of food allergens within food manufacturing plants[7]. CIP systems are currently used for automated cleaning of the production lines. Within the CIP system on-line conductivity sensors can be deployed (with several such sensors being commercially available) these sensors allow for the conductivity of wash samples to be continually monitored as a measure of the levels of detergents in the circulating wash. However, these sensors give limited qualitative information on the quality of cleaning and no information on allergen residue levels. In 2005, Stephan *et al* demonstrated the feasibility of measuring residue allergens in CIP using a combination of Polymerase Chain Reaction (PCR), Bradford protein assay and Enzyme Linked Immunoassay (ELISA) techniques[8].

A number of analytical methods have been described in the literature for the detection of milk protein allergens. Immunological techniques such as ELISA and lateral flow assay (LFA) are the most widely used techniques for identifying and quantifying

milk protein allergens with several commercially available kits on the market[9,10]. They offer simple, rapid and cheap analysis but suffer from high variation in recoveries across different commercial kits and in different analysis conditions[11–13]. Hybrid liquid chromatography-mass spectrometry techniques have proven to be the bench mark technique for quantifying allergens in a number of processed foods displaying good sensitivity and accuracy[14–16]. Pilolli *et al* recently demonstrated the use of multiplex LC-MS/MS to identify several different allergens including casein demonstrating a limit of detection (LOD) of 7 $\mu g\ g^{-1}$ within the same cookie dough samples[17].

Biosensors offer a viable alternative to traditional analytical techniques due to their comparable sensitivity, selectivity and potential for miniaturization and label free on-line detection that can potentially be used in food manufacturing environments[18–21]. A wide range of biosensors have been reported in the literature based on electrochemical, surface plasmon resonance (SPR), Quartz crystal microbalance (QCM) and optical transduction for the detection of allergens[22–24]. SPR has shown great potential as a biosensor in combination with antibodies based receptors for the detection of milk protein allergens demonstrating good sensitivity, low cost and easy fabrication[25,26]. However, biosensors which use antibody based receptors suffer from significant problems in terms of their limited shelf-lives and instability to testing conditions. The use of antibodies would easily denature under the harsh conditions of a CIP system. Therefore, there is interest in utilizing other types of receptors in biosensors for the detection of milk protein allergens such as aptamers or molecularly imprinted polymers[18,27]. Aptamers have been used in a number of SPR based biosensors but since their binding is also dependent on the temperature at which they were selected, the use of such biosensors would also not withstand the harsh conditions of a CIP system[28]. The use of molecularly imprinted polymer nanoparticles (nanoMIPs) in biosensors offer a number of advantages over other types of receptor due to their low cost, thermal stability and comparable binding kinetics. In addition, they can resist a wide pH range[29].

In the current study, we demonstrate the use of a nanoMIPs based SPR sensor for the detection of bovine α-casein protein residues in CIP wash samples. The sensor demonstrated sub ppm sensitivity, good selectivity and could detect spiked casein levels in CIP wash samples after protein precipitation and buffer exchange using G-25 SPE columns. To the best of our knowledge, this is the first reported synthesis of nanoMIPs specific for α-casein and could allow for the development of an on-line biosensor which could be incorporated into CIP procedures.

Experimental Section

Chemical and Reagents α-casein was used as the template for imprinting and was purchased from Sigma Aldrich. α-casein was made fresh and dissolved in 10 mM borate buffer pH 9.0 and diluted in 10 mM PBS buffer. All protein standards were standardized by measuring the absorbance at 280 nm. Glass beads were used as the medium for attaching the proteins as templates and were purchased from Potters; Spheriglass A glass 2429 CP-00. (3-Aminopropyl) trimethyoxysilane (APTMS) was mixed with (3%, v/v) toluene and used to functionalize the glass beads. Glutaraldehyde was dissolved in 10 mM PBS and used as a linker to attach the templates to the glass beads and was purchased from Sigma Aldrich. N-isopropyl acrylamide (NIPAm), N-tert-butylacrylamide (TBA), Acrylic acid (Acc), N-(3-Aminopropyl) methacrylamide HCl (APM), and N,N'-methylenebisacrylamide (BIS) were used as the monomers and cross-linker while Ammonium persulfate (APS) and N, N, N', N'- tetramethylethylenediamine (TEMED) were used as the initiator and catalyst respectively and were all purchased from Sigma Aldrich. Characterization of the nanoMIPs was performed using Dynamic light scattering (Malvern S-nanosizer) and transmission electron microscopy (TEM) (Phillips, CM20). All SPR experiments were performed on a Biacore T3000 (GE healthcare, USA). SIA sensor chips were purchased from GE Healthcare. 16-mercaptodecanoic acid (16-MUA) was purchased from Sigma Aldrich and 5 mM was dissolved in 10-50 ml of ethanol. N-hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), were purchased from Thermo Scientific and dissolved in water at a concentration of 0.1 M and 0.4 M respectively. A 50 $\mu g\ ml^{-1}$ bovine serum album dissolved in 10 mM PBS and 1M ethanolamine HCl were used to block the sensor surface and also purchased from Sigma Aldrich. CIP samples were collected from Unilever's dairy ice cream pilot plant at Colworth (Bedfordshire, UK). Samples from the cold wash cycle were aliquoted into 10 ml samples and stored at - 80 ^{o}C. G-25 spin columns were purchased from Fisher Scientific.

Preparation of templated glass beads. Casein was attached to glass beads using a method adapted from Poma and Canfarotta *et al*.[30–32]. Glass beads (60 g) were activated by boiling in 1M NaOH (30 ml) for 15 minutes. The resultant beads were washed with 8 x 200 ml DI water followed by washing with acetone (2 x 200 ml). The glass beads were then dried thoroughly in an oven. The glass beads were then incubated with 2 % (v/v) APTMS in anhydrous toluene (30 ml) for 24 hours in a closed container. The resultant beads were then washed with acetone (8 x 200 ml) followed by methanol (1 x 200 ml). The resultant amine functionalized glass beads were then mixed with 7 % (v/v) glutaraldehyde in PBS (30 ml) for 2 hours. The resultant glass beads were then washed several times with DI water (8 x 200 ml). The beads were quickly incubated with 5 $mg\ ml^{-1}$ of each protein in 10 mM PBS (30 ml) template overnight followed by an additional wash with water (8 x 200 ml). Vancomycin was used as the control template for the preparation of nanoMIPs against vancomycin which was used as the control for SPR experiments. This was prepared in the same manner as described for the protein templates. Templated glass beads were stored at 4^{o}C until further use.

Solid-phase synthesis of nanoMIPs. A solution containing the monomers was prepared by weighing out NIPAm (39 mg), BIS (2 mg) and APM (5.8 mg) and dissolved in water (98 ml). TBAm (33 mg) was dissolved in Ethanol (1 ml) and carefully added to the monomer solution. A 22 µl of acrylic acid was diluted in 1 ml and 100 µl was added to the monomer solution. The monomer solution was then made up to 100 ml with DI water. The resultant solution was degassed under vacuum, sonication for 15 minutes followed by bubbling nitrogen through the sample. The initiator and catalyst were prepared by dissolving 30 mg of APS in DI water (500 µl) and adding 30 µl of TEMED.

The templated glass beads were transferred to a column and the monomer solution was added. The polymerization was initiated by adding the solution containing APS and TEMED to the column. The polymerization was carried out at room temperature

overnight. Low affinity MIPs-NPs and unreacted monomers were washed off the beads with 3x30 ml aliquots of DI water. High affinity MIPs-NPs were incubated with 60 ^{o}C DI water (30 ml) for 30 minutes and eluted off. This was repeated a further two times. The MIPs-NPs were stored in solution at 4 ^{o}C until needed. Control MIPs were prepared in the same manner as described for the MIPs-NPs using the vancomycin templated beads.

Characterization of the nanoMIPs. A 10 ml aliquot of MIPs-NPs solution was dried using a freeze dryer to determine the yield of the MIPs-NPs synthesis and concentration of the stock solution. The size of the MIPs-NPs in solution was determined by Dynamic Light Scattering (DLS). A 10 ml solution of MIPs-NPs solution was reduced in volume using an Eppendorf vacuum dryer. The resultant solution was then filtered using a 1.2 µm diameter filter and sonicated. The same solution was also characterized using TEM by evaporating 10 µl of each sample onto a copper grid and immediately analyzing the sample.

The binding characteristics of each batch of nanoMIP were studied using an SPR binding assay. The sensor surface was prepared by incubating bare gold chips with 5 mM MUA in ethanol (10 ml) for 24 hours. Samples were degassed by sonication and the headspace of the container was filled with nitrogen. The self-assembly monolayer surface was then washed with water, then ethanol and finally dried using nitrogen. The chips were promptly docked into the machine and the instrument was primed three times prior to immobilization of the nanoMIPs and subsequent analysis. Immobilization of the nanoMIPs was performed by firstly setting the flow rate to 5 µl min^{-1} with 10 mM PBS and injecting a mixture of 0.1 M NHS and 0.4 M EDC (50 µl) to activate the carboxylic group on the SAM. A 50 µg ml^{-1} nanoMIPs from each batch was exchanged in MES buffer pH 6.0 (50 - 100 µl) and then injected. Blocking of the surface was performed by injecting 50 µg ml^{-1} BSA (50 µl) followed by 1M ethanolamine (50 µl). A control MIP was injected in the same manner as for the NanoMIPs with the exception that they were immobilized on spot 2 of the sensor chip. Binding assays were then performed by setting the flow rate to 10 µl min^{-1} with 10 mM PBS and injecting 0-1000 nM of α-casein protein (50 µl). The response was measured and the binding affinity parameters were determined using the Biacore analysis software and equilibrium affinity kinetic model.

Development of the α-casein nanoMIP sensor. For the development of the dose based assay, 500 µg ml^{-1} of nanoMIPs were immobilized in the same manner as described for the binding assay. Calibration curves were constructed by injecting 0 – 150 ppm of α-casein standard (50 µl) and measuring the response in a cumulative based assay where the lowest concentration was injected first and the sensor response was measured as the difference between the response of the baseline and the response just after the end point of the injection. The absolute response was then normalized by subtracting the reference channel response from the control nanoMIP to give the relative response. The responses at each concentration were plotted to form a non-linear calibration plot and the linear proportion was taken to extrapolate unknown samples and to determine the LOD. Specificity of the nanoMIPs was also determined by injecting 0.5 – 8 ppm of α-casein, BSA and BLG (50 µl) respectively on to the surface and measuring the response.

The ability of the sensor to detect α-casein in cleaning in place wash samples was assessed by spiking CIP wash samples with 0.5 – 8 ppm of α-casein. G-25 spin columns were equilibrated with 10 mM PBS buffer. A 0.5 ml of each spiked sample was loaded onto the column and the columns were centrifuged for 2 minutes at 1000 g^{-1}. Samples were then injected onto the sensor and the responses measured at a 10 µl min^{-1} flow rate.

Results and Discussion

Preparation of templated glass beads. The glass beads were functionalized with an amine group to allow for the template to be attached to the beads. The amine functionalized beads were characterized by using a simple colorimetric test. 2, 4, 6-trinitrobenzene sulfonic acid (TNBSA) (5% w/v) was diluted to 0.05% in PBS buffer. Upon the addition of TNBSA, the beads turned a bright yellow color confirming the presence of the amine group on the beads. On addition of glutaraldehyde the yellow color disappears suggesting the formation of the Schiff base. A 0.5 mg ml^{-1} of protein was incubated with the glass beads overnight. The amount of protein that was successfully immobilized onto the beads was determined by a BCA assay. The concentration of protein solution was measured before and after incubation with the glass beads and the amount of protein that was determined to be was between 2.0 - 2.2 n mol g^{-1}. The presence of the template on the beads was also confirmed by adding BCA reagent to an aliquot of beads.

Synthesis and characterization of nanoMIPs. As previously mentioned, nanoMIPs were synthesized using a solid phase based synthesis method. Concentrations of nanoMIP stock solutions were determined using an equation described by Hoshino *et al*[33].

The size of the nanoMIPs was determined by DLS. The 10x dilution of nanoMIPs was essential for obtaining an accurate reading upon the nanosizer instrument. The hydrodynamic radii, PDI and general nanoMIP yields in terms of % w/w are shown in **Table 1**.

Table 1: Physical dimensions and yields of different batches of nanoMIPs using Dynamic light scattering.

Batch	Diameter (nm)	PDI	(% w/w) Yield of nanoMIPs
1	235 ± 6.92	0.276 ± 0.076	39.6
2	457 ± 5.45	0.430 ± 0.054	32.9
3	276 ± 9.08	0.275 ± 0.027	34.2

Variations in the size of the nanoparticles were observed between some batches. The reason for this is that this is a batch method and time and environment of the synthesis has to be controlled for each step to ensure uniformity of the produced particles. Filtration can be used to remove large and very small particles from synthesized particles batch. Therefore, batches displaying <300 nm and a PDI of less than 0.3 were analyzed

for the SPR binding assays. This requirement was also essential for ensuring that the nanoMIPs would not block the microfluidic system of the SPR.

TEM images of the nanoMIPs were taken on a Phillips CM20 transmission electron microscope. A typical image of the nanoMIP for α-casein is shown in **Figure 1.** TEM images showed a similar size of nanoparticle as observed for DLS experiments suggesting shrinkage is linked to time of analysis.

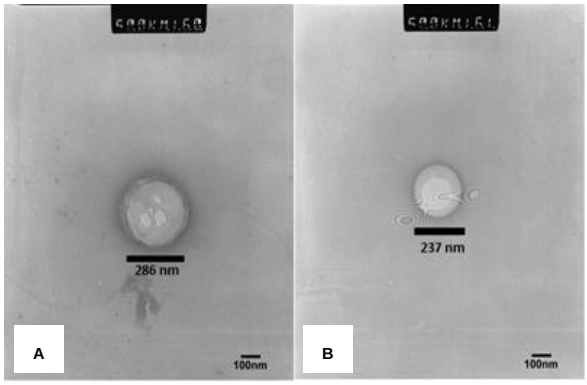

Figure 1: TEM image of α- casein nanoMIPs taken on a Phillips CM20. (A), Batch 3, (B) Batch 1.

The binding affinities of the nanoMIPs were measured using the SPR biosensor. 50 μg ml^{-1} of nanoMIPs were immobilized onto the SPR sensor chip and the flow rate was set at 10 μl min^{-1}. Different concentrations of casein were injected onto the surface. The relative response was measured and the binding affinities for batches 1, 2 and 3 were determined using the affinity equilibrium model. The response was normalized by subtracting the control channel which contained the control nanoMIPs. The binding affinities (K_D) of the three batches were determined to be 8.85 x 10^{-9} M, 10.8 x 10^{-9} M and 11.7 x 10^{-9} M respectively suggesting that nanoMIPs with high affinity were synthesized. The reported binding affinities are comparable to those expected of antibodies. The binding affinities were determined using Equilibrium analysis binding model and it must be noted that the nanoMIPs and protein are assumed to have a 1 to 1 binding stoichiometry. However, due to the lack of methods currently available, these NanoMIPs could demonstrate multiple binding stoichiometries due to the large differences in size between the nanoMIP and the protein. In addition the immobilization of the nanoMIPs rather than the protein analyte allows for the accurate determination of the K_D from a known concentration of protein rather than an estimated concentration of nanoMIPs.

Development of the α casein nanoMIP sensor. The nanoMIPs were immobilized onto the surface of the gold SPR chip. The SAM was formed using MUA followed by amine coupling reaction using NHS and EDC. As the nanoMIP contains a primary amine groups, it is possible to attach these nanoMIPs in the same manner as antibodies. In order to obtain the highest degree of immobilization of the nanoMIPs onto the gold SPR chip, the nanoMIP stock solution was concentrated down and exchanged in 10 mM MES pH 6.0 buffer using an Eppendorf concentrator. The concentration was determined for each batch from the stock solution. The nanoMIPs were exchanged in MES buffer to encourage electrostatic interactions between the amine groups on the nanoMIPs with the carboxylic group on the SAM. The exact charge on the nanoMIP is currently unknown as there are no methods available for measuring the isoelectronic point of the nanoMIP. The extent of immobilization was measured as the difference in response from the base line to the point just after the injection of 1 M ethanolamine. Responses of >1000 RU were deemed acceptable for further analysis. NanoMIPs are several times larger than the protein and as such there was concern about whether the size difference would cause sensitivity issues. A concentration of nanoMIPs roughly 10 times the concentration of those used in the kinetic experiments was injected for the sensor design.

The chip surface was then blocked with injections of 50 μl BSA (50 μg ml^{-1}) and 1 M ethanolamine. The control MIP (vancomycin MIP) was immobilized in exactly the same manner using spot 2 on the sensor chip and gave a similar degree of immobilization as seen for the MIP. A typical immobilization sensorgram is shown in (**Figure 2A).**

The nanoMIPs appear to be able to undergo amine coupling with the sensor surface in the same manner as observed for other types of receptors such as antibodies and DNA. However, since the nanoMIP contains a number of different functional groups, there is the possibility that nanoMIPs are physically adsorbed to the surface. However, no leaching of nanoMIPs was observed during analysis. The use of BSA as a blocking agent was effective in preventing non-specific absorption of α-casein and in the final sensor design, other methods could be used such as Poly vinyl alcohol. To further confirm the coverage of the sensor surface with the NanoMIPs, an AFM was taken of a 10 μm area of a chip which was immobilized with nanoMIPs **(Figure 2B-D)**. The AFM confirmed that there is good coverage of the dried nanoMIPs on the surface. However, it was observed that there was also some aggregation of the nanoMIPs on the sensor surface.

For the dose based assays, 0 – 150 ppm of α-casein (100 μl) were injected over the surface of both the casein nanoMIPs channel and the control channel (Vancomycin nanoMIP). Sensorgrams were generated by setting the flow rate to 25 μl min^{-1} with the flow buffer and injecting α-casein at different concentrations in accumulative based assays starting from the lowest concentration to an ever increasing protein concentration (**Figure 3A**). The sensorgram shows a higher response to the nanoMIPs when compared to the control but a response from the control MIP is the matrix effect of the sample including non-specific binding. Nevertheless, the difference in signal between the nanoMIPs and the control MIP is still relatively high. An accumulative based approach was used for all experiments due to the fact that injections of the regeneration buffer after every injection of protein standard did not remove all the protein adequately (i.e. the signal did not return to the baseline). The use of much harsher regeneration conditions could be used in future. In addition, the nanoMIPs contain N-isopropylacrylamide which is a thermally responsive monomer. This monomer allows the nanoMIP to swell at different temperatures causing the release of the analyte from the nanoMIP receptor. This property could be exploited to allow the released of the protein and regenerate the sensor surface. However, due to the limitations of the SPR machines we are using, we were unable to test whether we could regenerate the sensor surface using temperature changes and such changes in temperature could also adversely affect the baseline response due to the changes in refractive index.

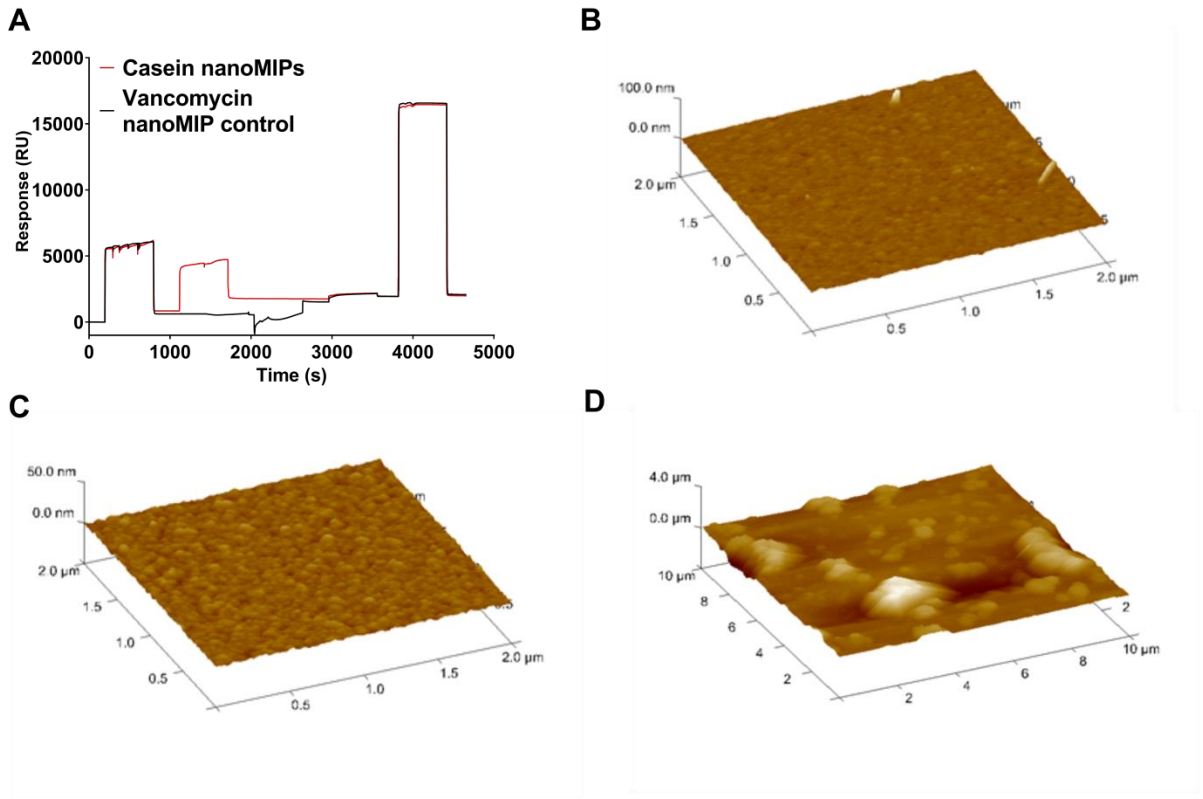

Figure 2: (A) SPR sensorgram showing the immobilization of casein nanoMIPs and the control nanoMIP on the sensor surface.AFM of (B) bare gold, (C) SAM monolayer and (D) Covalently attached nanoMIPs.

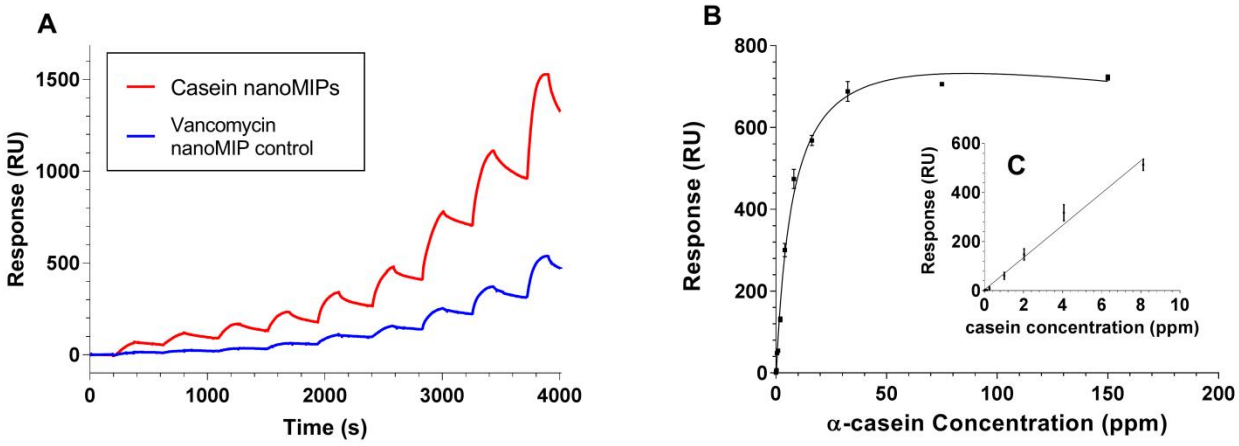

Figure 3: (A) Typical sensorgram for a nanoMIP based biosensor synthesized from the bead based method; (B) Non-linear (r^2: 0.9934) and (C) linear calibration (r^2: 0.9779) plot for the α-casein nanoMIP based biosensor (n =3).

Non-linear calibration plots were generated by measuring the relative responses between the baseline and a point 30 seconds after the end of the injection (**Figure 3B**). The analyte signal was normalized by subtracting the relative response from the control nanoMIP reference channel. The normalized response was then plotted against the concentration of the protein standard to give a non-linear curve. The linear proportion of the graph before the point of saturation was replotted and is shown in **Figure 3C.** The LOD (limit of detection) of the sensor in standard buffer conditions was determined by calculating 3 times the slandered deviation of the intercept and converting it into a protein concentration from the gradient. An LOD of 127 ± 97.6 ng ml^{-1} (0.127 ppm) which was well below the specified 2 µg ml^{-1} (2 ppm) required for allergen detection levels was determined. The sensor demonstrated good reproducibility.

The selectivity of the sensor towards α-casein was assessed by injecting 0.5 - 8 ppm and measuring the relative responses of α-casein, β-lactoglobulin and BSA over the sensor surface with the relative responses shown in **Figure 4A.** Responses measured for α-

casein at high concentrations were observed to be at much bigger than the responses observed for BSA and BLG at the same concentrations. However, at the lowest concentration (0.5 ppm), the differences between the responses measured for all proteins were low. However, due to the fact that α-casein is a major constituent of cows' milk protein making up 82% of proteins in milk and contrasting this with the relative amounts of whey and serum proteins (18%), the cross reactivity of BLG and BSA are negligible at this level[34]. The responses measured for both BSA and BLG showed no correlation between concentration and SPR response. Therefore, the sensor demonstrates good selectivity towards casein over the linear range of concentrations tested.

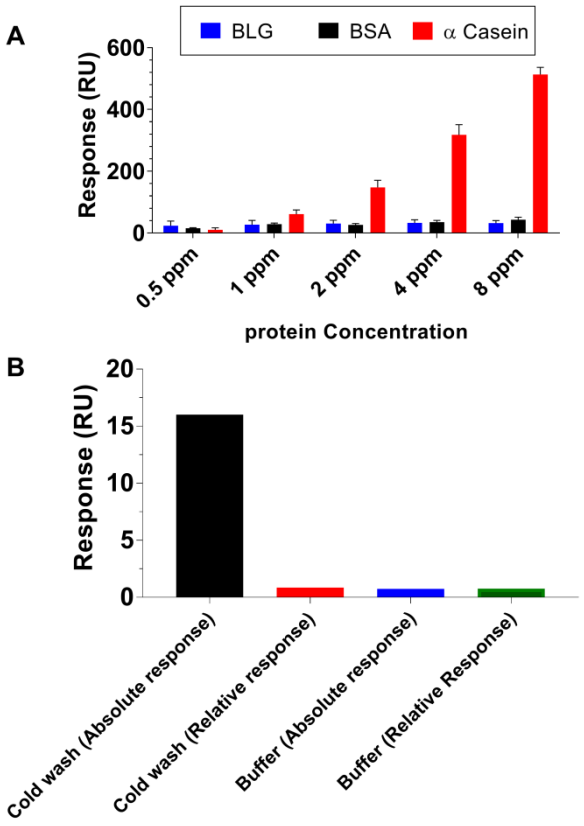

Figure 4: (A) The responses of α-casein, β-lactoglobulin and BSA when injected at 0.5-8 ppm for a nanoMIP based sensor (n=3) and (B) the relative baseline responses of the cold wash after gel filtration.

Sample recoveries in cleaning in place (CIP) samples. To determine the performance of the sensor in measuring casein levels within cleaning in place cold wash samples which are defined as the final wash samples completed at room temperature in the CIP cleaning procedure. Samples of waste product were collected from the dairy ice cream pilot plant from Unilever at Colworth. Samples were aliquoted and immediately stored at -80 ^{o}C prior to analysis.

The sensor performance with real samples was assessed next by again spiking each CIP sample with 0.5 – 8 ppm of casein. Initially, the proteins were exchanged into borate buffer by precipitating the protein out with acid and washing the sample before reconstituting the protein into borate buffer.

However, there would be considerable difficulty incorporating this type of sample preparation into any CIP sensor. Therefore, gel filtration was chosen due to the possibility that this technology can be incorporated into an on-line sensor. G25 spin columns were equilibrated with the flow buffer. The protein was then loaded onto the column and spun down. The G25 spin column would separate small molecule containments from the protein which elute straight through the column. The spin column method does not require any dilution and therefore the collected samples were injected straight onto the sensor. The absolute and relative responses of the biosensor for the unspiked sample showed minimal interference from the sample matrix (**Figure 4B**). The response of each protein spiked sample was measured using an accumulative based assay and compared against the calibration plot. Percentage recoveries from most of the spiked CIP wash samples were shown to be within the 80 - 120 % range and with a % RSD below 5 (**Table 2**). For the sample spiked with 0.5 ppm, a percentage recovery of 120% was just on the borderline of acceptable validation and suggests trace matrix effects of the wash sample.

Table 2: Percentage recoveries of cold wash CIP samples spiked with casein (n =3).

Spiked protein concentration (ppm)	Measured protein concentration (ppm)	Recovery (%)	% RSD
0.5	0.60	120	1.79
4	3.51	87.5	5.16
8	7.59	94.9	2.24

These results demonstrate the feasibility of our biosensor in terms of detecting α-casein in CIP waste samples. The matrix effects of the samples have been adequately removed using the gel filtration. This suggests that gel filtration could be used as an effective sample preparation technique in the analysis of protein samples in biosensors.

Conclusion

NanoMIPs for α-casein were successfully synthesized and characterized using the solid phase synthesis. The protein was attached to the glass beads via an amine coupling reaction and was verified using colorimetric methods. NanoMIPs were synthesized with a secondary monomer containing a primary amine group which were eluted with adequate yields and diameters. The measured binding affinities of the nanoMIPs were found to be in the nanomolar range. The sensor was successfully fabricated by attaching the nanoMIPs and control MIP as the receptors to their respective spots using an amine coupling protocol. The resultant sensor demonstrated an LOD of 127 ± 97.6 ng ml^{-1} (0.127 ppm). The reproducibility of protein injections was also found to be adequate for the sensor performance. The selectivity of the sensor was determined by measuring the response of different milk proteins from 0.5 to 8 ppm and the MIP based sensor demonstrated excellent selectivity towards α-casein. The ability of the sensor to detect proteins in the CIP cold wash samples were also demonstrated by spiking the samples with the protein standard and sample recoveries were found to be within the 80 -120% range. This suggests that gel filtration could be used as an effective sample preparation tool in the

analysis of proteins in waste samples. The sensor could allow for food manufacturers to monitor levels of unintended milk protein allergens that could be present within food manufacturing processes in near real-time, and could be a useful tool in managing the risk(s) of allergen cross-contamination. Currently we are testing the feasibility of using temperature related elution to regenerate the sensor surface and developing MIP based biosensors for other milk protein targets.

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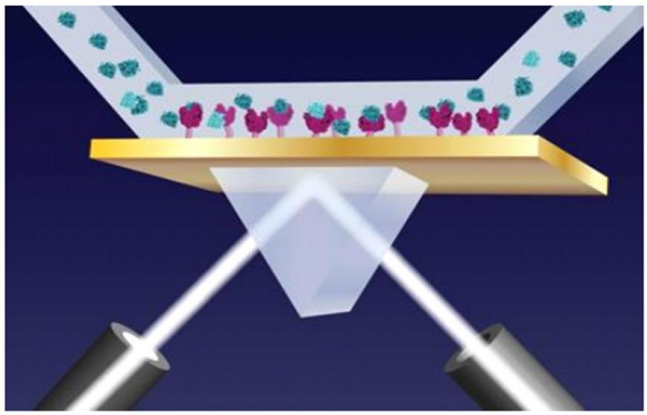
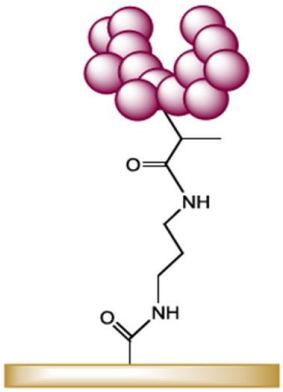
TOC Graphic



Surface Plasmon Resonance Sensor
for α-Casein Detection



Sensor Chip Interfaced
with NanoMIPs