

Comparison of CIM discs and CPG glass as solid supports for bioanalytical columns used in allergen detection

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Received: 12 March 2010 / Revised: 10 June 2010 / Accepted: 13 June 2010
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Abstract This work presents a comparison of convective interaction media (CIM) and controlled pore glass (CPG) as solid supports for immunoglobulin antibodies used in bioanalytical detection of allergens in foodstuffs. A flow-injection manifold with highly sensitive thermal lens spectrometric detection was used for this purpose. Using beta-lactoglobulin, a milk allergen, as a model analyte, CIM disc supports had a higher linear range ($0.2\text{--}3.5\ \mu\text{g L}^{-1}$), better reproducibility (intra-day RSD=1%, inter-day RSD=10%), lower consumption of reagents, and better immunocolumn stability (1 month, over 240 injections of substrate), while providing comparable LODs ($0.1\ \mu\text{g L}^{-1}$). Application of CIM discs as solid supports in immunocolumns for allergen detection enables fast and sensitive screening of allergens in foodstuffs with sample throughput of up to eight samples per hour.

Keywords Bioanalytical columns · Controlled-pore glass (CPG) · Convective interaction media (CIM) · Allergen detection · Thermal lens spectrometry

Abbreviations

BLG β -Lactoglobulin
CIM Convective interaction media
CPG Controlled pore glass
TLS Thermal lens spectrometry
FIA Flow injection analysis
ELISA Enzyme linked immunosorbent assay

Introduction

Immunochemical methods have recently been recognised as a powerful tool for detection of various biomolecules in complex matrices. Their main advantages include minimum sample preparation, high specificity and sensitivity, low cost and low organic solvent consumption [1]. Optimum performance of immunoassays is achieved when only the reaction of a specific antibody with the target analyte is measured. All other non-specific interactions are interferences in a given analytical procedure or contribute to higher background of the assay. A common strategy for minimizing background effects from sample and assay components involves the use of solid phase-bound immunoreagents [2]. Moreover, the use of immobilized solid reagents instead of frequently unstable solutions results in time saving and often avoids the release of excess reagent as waste [3]. Therefore, immobilization of selective immunoglobulins on solid supports is widely used for biospecific detection. Immobilized proteins are more stable against heat, organic solvents, and pH and can be used repeatedly while they remain active [4]. Moreover, the immobilized antibodies can be used in flow-injection analysis. Flow immunomethods are potentially more reproducible than conventional immunoanalysis on microtiter plates. This is because by reusing the same antibodies, inhomogeneity variations are eliminated [5]. The coupling of a bioanalytical column with flow-injection analysis (FIA) results in highly reproducible and rapid methods with high specificity and low detection limits. However, supports for FIA systems should meet requirements such as little mass-transfer resistance, long term stability, and high flow-rate compatibility, and should be readily modifiable by stable immobilization of antibodies [6].

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The surface of the support on which the antibody is immobilized has an important role in retaining the tertiary structure of the protein, which strongly affects the activity and thermal stability of the immobilized protein. The choice of the support material is also of vital importance because different materials enable different accessibility of substrates to active sites of immobilized molecules. Furthermore, particle size and porosity affect mass-transfer resistance and diffusion of reagents [7].

In the past, controlled pore glass (CPG) was widely used as immobilization support for various biological molecules [2]. However, in the recent years, synthetic monoliths have gained importance as supports for covalent immobilization of enzymes [8]. CPG is known for high thermal stability and good resistance to acids, which makes it suitable for very harsh working conditions. In comparison with CPG, the structure of a monolithic convective interaction media (CIM) support is more compact and therefore easy to use. Characteristics such as flow-independent resolution and binding capacity, fast separation, and high binding capacity for large biomolecules makes CIM discs an interesting support in comparison to conventional materials [9]. Analytical techniques performed with CIM supports are fast, precise, and reliable [8]. Despite the widespread use of CPG and CIM as solid supports in chromatography, they are rarely used for immunological detection of food allergens. Mostly, these types of solid supports are used in microfluidic immunoaffinity separations using fluorescence as detection method [10].

In this work we have investigated the performance of CPG and CIM supports for bioanalytical columns used in immunological detection of allergens, with beta-lactoglobulin (BLG) as model compound. BLG is a food allergen, which is the major cause of allergy in small children [11]. Various detection methods have already been used for BLG detection varying from gold cluster-linked bioassay with detection limits of $100 \mu\text{g L}^{-1}$ and assay duration of 30 min, to immunometric assay with limits of detection as low as 30 ng L^{-1} and analysis time of 1 h [12–14]. The limitation of such methods is the high background, because of the presence of other food components in samples. Minute concentrations of allergens are therefore hidden in the food matrix and are difficult to detect [14]. For this reason chromatographic or FIA methods, in which the analyte is separated from the sample matrix are preferred. On the other hand, Levin and co-workers [15] reported a case study in which as little as $1.4 \mu\text{g}$ BLG triggered an allergic reaction. When calculated for 100 g or 100 mL of ingested food, this corresponds to a BLG concentration of $14 \mu\text{g L}^{-1}$. Therefore, methods which can overcome matrix problems and can detect quantities of allergens at $\mu\text{g L}^{-1}$ level are needed for fast and reliable screening of food products.

With the objective of selecting the most appropriate support for the development of novel FIA-based detection methods for food allergens, we compared the derivatisation of CIM discs and CPG support and their performance during detection of beta-lactoglobulin. The final objective was to achieve an improvement in LODs for the determination of beta-lactoglobulin, by lowering the unwanted background signals resulting from non-specific binding of secondary antibodies to the support material, or other matrix effects.

Experimental

Reagents and solutions

The phosphate buffer (0.1 mol L^{-1}) was prepared by dissolving 1.76 g sodium dihydrogen phosphate dihydrate (AppliChem, Darmstadt, Germany; min. 99%) and 5.98 g disodium hydrogen phosphate anhydrous (AppliChem) in 1 L double-deionized water. The pH was adjusted to 7.4 with 5 mol L^{-1} HCl (Merck, Darmstadt, Germany; 30%).

CPG glass beads (Sigma–Aldrich, St Louis, MO, USA) were obtained as granular material of 80–120 mesh size ($0.18\text{--}0.125 \text{ mm}$), mean pore diameter 22.6 pm , and surface area $94 \text{ m}^2 \text{ g}^{-1}$. CPG beads were pre-cleaned in 5% nitric acid (Fluka, Buchs, Switzerland; 65%). The (3-aminopropyl)triethoxysilane (Sigma–Aldrich; min. 98%) was prepared by diluting 1 mL pure substance in 9 mL deionized water, the pH was adjusted to 3.5 by addition of 5 mol L^{-1} HCl (Merck; 30%). The crosslinking reagent glutaraldehyde was prepared by diluting 5 mL glutaraldehyde (Merck; 50%) with 95 mL 0.1 mol L^{-1} phosphate buffer (pH 7.4).

Convective interaction media (CIM) discs (BIA Separations, Villach, Austria) are homogenous monolithic stationary phases with surface area of $7 \text{ m}^2 \text{ g}^{-1}$. The microCIM discs of 1.6 mg weight used for this research are small monolithic columns approximately 1 mm thick and 2 mm in diameter.

Sodium periodate solution was prepared by dissolving 0.5 g sodium metaperiodate (Sigma–Aldrich; min. 99%) in 10 mL dilute acetic acid (50:50, v/v; Fluka; min. 99.8%). The cyanoborohydride solution was prepared by dissolving 3 mg sodium cyanoborohydride (Acros Organics, Geel, Belgium; 95%) in 10 mL 0.1 mol L^{-1} phosphate buffer (pH 5.7). Sodium borohydride solution was prepared by dissolving 19 mg sodium borohydride (Acros Organics; 98%) in 10 mL 0.1 mol L^{-1} phosphate buffer (pH 7.4).

PBS buffer was prepared by diluting 25 mL 0.1 mol L^{-1} phosphate buffer (pH 7.4) to final volume of 250 mL and dissolving 2.2 g sodium chloride (Carlo Erba, Milano, Italy; 99%) and 0.05 g potassium chloride (Riedel-de Haën,

Buchs, Switzerland; 99%) in it. The glycine–HCl buffer was prepared by dissolving 1.876 g glycine (Sigma–Aldrich; 99%) in 250 mL deionized water. The pH was adjusted to 2.5 with 5 mol L⁻¹ HCl (Merck; 30%).

The bovine BLG A + B (L-0130, 90% PAGE) was obtained as a solution of 1.5 mg protein mL⁻¹ PBS. Affinity purified anti-bovine BLG A + B raised in rabbit (anti-BLG(rb)) were used as primary antibodies. The analyte was incubated before analysis with affinity purified anti-bovine BLG A + B raised in rabbit and labelled with HRP (anti-BLG(rb)-HRP). The immunoglobulins were purchased from Bethyl Labs (Montgomery, TX, USA).

The BLG A + B (18 300 g mol⁻¹) was purchased from Sigma–Aldrich as a lyophilized powder. BLG stock solution (250 ng mL⁻¹) was prepared by dissolving an appropriate amount of BLG powder in PBS buffer. Working standard solutions in the range 0.2 to 4.5 µg L⁻¹ were prepared fresh daily by further dilution of stock solution with PBS buffer.

The 33.29 µmol L⁻¹ OPD reagent for the HRP enzyme was freshly prepared by dissolving 3.6 mg OPD in 10 mL sodium acetate buffer. H₂O₂ (Merck; 30%, 2 µL) was added to 1 mL OPD solution before the detection step. The 0.05 mol L⁻¹ sodium acetate buffer was prepared by dissolving 26 mg anhydrous sodium acetate (Fluka; min 98.5%) in 250 mL water. The pH was adjusted to 4.5 by addition of 5 mol L⁻¹ acetic acid.

If not specified otherwise, the solutions were prepared in double deionized water.

Antibody immobilization on CPG

The CPG derivatisation was performed in batch mode. Primary antibodies were immobilized on the CPG surface in three steps: pre-cleaning of the CPG, aminoalkylation, and final immobilization of the antibodies. The procedure is described in detail elsewhere [16]. The derivatised CPG glass was stored in PBS buffer at 4 °C after immobilization. The immunocolumn was prepared by packing the derivatised CPG into a PEAK column (i.d. 2 mm, length 30 mm, made in-house).

Antibody immobilization on CIM

Immobilization of antibodies on CIM discs (BIA Separations) consists of three steps: oxidation of diol groups, antibody immobilization, and reduction of free binding groups on the solid support. It was performed according to a procedure described in the literature [17] which was slightly modified by performing it in a continuous flow. The immobilization reagents were pumped through the monolithic CIM disc at a flow rate of 0.1 mL min⁻¹ by use of an HPLC pump (Knauer, Berlin, Germany). The

oxidation of the support material was performed with 0.2 mol L⁻¹ NaIO₄ in acetic acid–water (50:50, v/v) for 60 min. After oxidation, washing for 10 min with 0.01 mol L⁻¹ phosphate buffer (pH 5.7) was carried out. The antibodies were added to CIM in solution containing 0.04 mol L⁻¹ NaCNBH₄ and were left to bind for 60 min. After immobilization the excess antibodies were washed with PBS buffer, pH 7.4, for 30 min. Reduction of unreacted binding sites was performed with 0.05 mol L⁻¹ NaBH₄ for 60 min. After reduction the CIM discs were washed with PBS buffer for an additional 30 min and stored at 4 °C. In comparison with the batch-mode procedure used by Puerta et al. [17], continuous flow immobilization is faster. Moreover, washing out of excess reagents is more efficient.

Evaluation of immobilization efficiency

The immobilization efficiency was estimated by checking protein content in immobilization buffer before and after the immobilization procedure. Because extensive washing was required to remove excess unbound antibodies, the Bradford (Sigma–Aldrich) method for protein quantification based on absorbance measurements was not sensitive enough for quantification of antibodies in buffer after immobilization. Therefore, proteins in immobilization buffer were precipitated and quantified by SDS-PAGE under reducing conditions (90 min at 100 V) on a polyacrylamide electrophoretic gel (15% resolving gel), based on comparison with BLG standards of different concentration. Protein bands were visualized by Coomassie brilliant blue stain (Riedel–de Haën). The antibody density (A per unit area) was calculated from the results obtained by SDS-PAGE electrophoresis and data obtained from the support producers.

FIA–TLS set-up

Measurements related to BLG determination were performed on an FIA-immuno system coupled to a thermal lens spectrometer (TLS) as detector. The TLS spectrometer was a pump/probe dual-beam instrument built in-house and its detailed description and operating characteristics can be found elsewhere [18]. It consisted of an excitation Argon-ion laser operating at 476 nm emission line (100 mW) (Coherent CR4, Moorpark, CA, USA) and a 2-mW (632.8 nm) probe helium–neon laser (Uniphase 1103P).

For FIA we used an HPLC Smartline 1000 pump purchased from Knauer and two injection valves from Rheodyne (San Francisco, USA). The immuno-column contained derivatised CIM or CPG supports with immobilized primary antibodies. The tested flow rates of the carrier buffer for determination of HRP activity ranged from 0.3 to 0.5 mL min⁻¹. The samples were injected through the

system at a flow rate of 0.1 mL min^{-1} for 80 s. Washing of the bioanalytical column was performed at a flow rate of 0.4 mL min^{-1} for 60 s.

Analytical procedure

HRP conjugated IgG secondary antibodies were added to the sample or standard solution (dilution of antibodies 1:10 000) before the analysis. Antibody–sample mixture (100 μL) was injected into the FIA system. Beta-lactoglobulin was specifically adsorbed in the immunocolumn with the co-bound labelled antibodies. The activity of retained HRP conjugated IgG was detected by injecting 10 μL OPD solution with added H_2O_2 . The formation of the enzymatic product was measured with TLS. After each assay the bound analyte was washed off the column by use of Gly–HCl buffer. The FIA system was operated at room temperature ($25 \pm 2^\circ\text{C}$).

Stability of the bioanalytical column

Long-term stability of the bioanalytical column was determined by daily monitoring of the response of the system to sample standard solutions by comparison of the slopes of calibration plots. The experiments were performed according to the procedure described in the previous section.

Linearity, LOD, and LOQ evaluation

The calibration plots were obtained by least-square analysis based on analytical signals from BLG standard solutions of five concentrations ($0.5\text{--}4.5 \mu\text{g L}^{-1}$) corresponding to 50–450 pg of injected BLG, which were injected into the FIA-ELISA TLS system in triplicate.

In order to estimate the limits of detection (LOD) and quantification (LOQ), the blank sample was injected six times and the standard deviation of the blank was calculated (SD(bl)). The LOD was then calculated on the basis of $S/N=3$ from the slopes of the calibration lines ($\text{LOD}=3\text{SD}(\text{bl})/\text{slope}$).

The intra-day and inter-day reproducibility of the method were evaluated by determining the RSD of responses to a selected standard ($1 \mu\text{g L}^{-1}$) determined within the same day at least in triplicate, and on different days during this research.

Results and discussion

As part of method development, we tested the suitability of CPG and CIM supports for immobilization of immunoglobulin. Evaluation of the efficiency of binding of antibodies to the support showed that the amounts of

antibodies bound in a single immunocolumn differed by approximately 50% in favour of CIM disk supports. However, when also taking into account the specific surface area of the supports, much larger difference between CIM and CPG supports becomes evident, which is reflected in 350 times higher density of antibodies on the CIM support (Table 1).

When testing the performance of immunocolumns we observed the appearance of high blank signals when no beta-lactoglobulin was present in the sample. A possible explanation for the origin of the blank signal is non-specifically bound secondary antibodies, which are added to the sample as detection antibodies. As reported in the literature, the retention of free secondary antibodies in the bioanalytical column occurs because of non-specific protein–protein and protein–support interaction [2]. We observed the unwanted background signals on both support materials. To confirm the origin of the blank signal we compared signals obtained with OPD before any sample injection on the column and the signal obtained with OPD after the injection of a sample containing secondary antibodies, but without beta-lactoglobulin (blank).

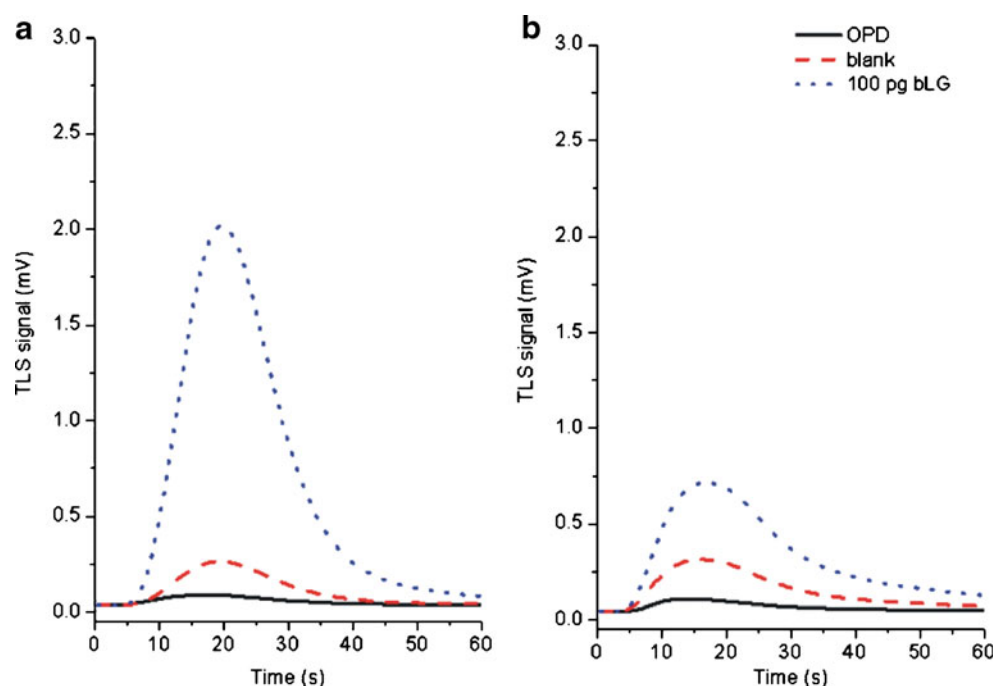
The differences in the response are shown in Fig. 1. On this figure it is clearly shown that the unwanted blank signal is mainly caused by non-specific retention of antibodies. Although the absorbance spectrum of OPD measured on a spectrophotometer (HP-8453 Agilent Technologies, Waldbronn, Germany) showed no absorbance at the excitation wavelength used, we observed increases of the TLS signal after the injection of OPD in the flow injection system (solid line). However, the difference between the blank signal (dashed line) and signal from OPD reagent passing through the system before injecting the HRP labelled secondary antibodies (solid line) is substantial. The blank signal is five times higher than the signal from OPD alone. As shown in Fig. 2a, the reagent itself produces a response in the detection system, which is otherwise hidden in the blank signal. The higher the reagent concentration the higher the contribution to the blank (Fig. 2a). Therefore we were searching for the concentration of reagent in which the ratio between the analytical signal and the background signal from OPD was the highest for the given experimental conditions.

Table 1 Estimated values for immobilised primary antibodies

Support	Immobilized antibodies per column (μg)	Antibody per unit area ($\mu\text{g m}^{-2}$) ^a
CIM	55	4900
CPG	35	13

^a The antibody per unit area was calculated from the data provided by the suppliers as given in Experimental for each support, and from experimental evaluation of the amount of antibody immobilized on the support.

Fig. 1 Signal achieved on CPG support (a) and on CIM support (b)



As calculated from experimental data in Fig. 2b, the ratio of analytical signal to signal from OPD varied only by approximately 10% for OPD concentrations 140–180 mg L⁻¹. Therefore all further experiments were performed using an OPD concentration of 180 mg L⁻¹.

In Fig. 3 there is a representation of acquired signals which are typical for evaluation of BLG with the method described in this paper. Most importantly, a substantial difference between the unwanted background signals (signals from 150 s to 350 s) and the signal confirming the presence of the allergen (signals from 600 s to 850 s) is shown. It is also clearly evident that the signals arising from the presence of the allergen in the sample are higher, and

enable reliable detection of beta-lactoglobulin, even though the limits of detection are hindered because of the high background signal.

As seen in Fig. 4, the calibration curves depend strongly on the support material and on the flow rate in the stage of OPD injection. In the case of CIM discs the calibration curves show good linearity, with higher slopes at lower flow-rates. This could be explained simply by the fact that the contact time between the OPD reagent and the HRP-labelled secondary antibodies is longer at the lower flow rate. In the case of CPG, the slopes of calibration curves are, in general, ten times higher and, in addition, show deviations from linearity at higher analyte concentrations.

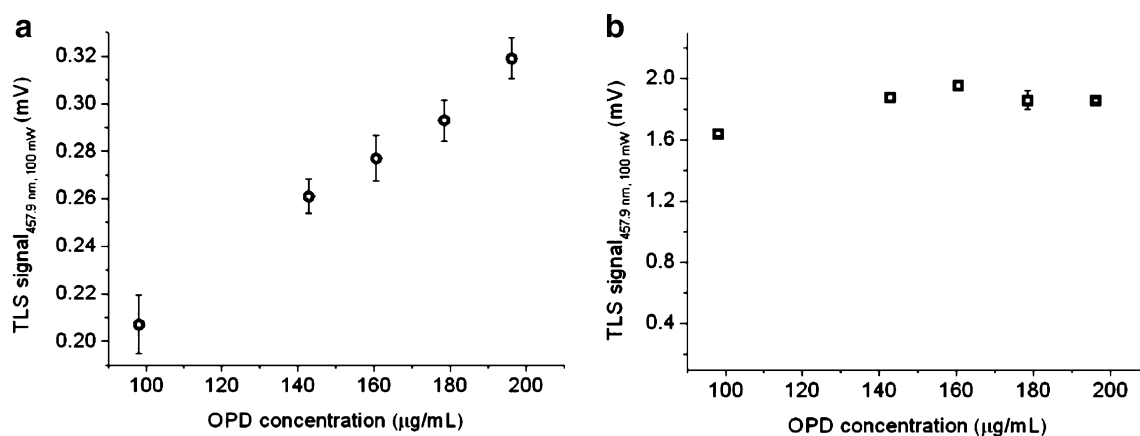


Fig. 2 Effect of OPD on signal intensity in an FIA system with detection by TLS spectrometry. Higher concentrations of OPD lead to a stronger signal even though the analyte is not present (a). Signals

achieved at different OPD concentrations from 100–200 mg L⁻¹ for a constant concentration of BLG (10 μg L⁻¹) are depicted in (b)

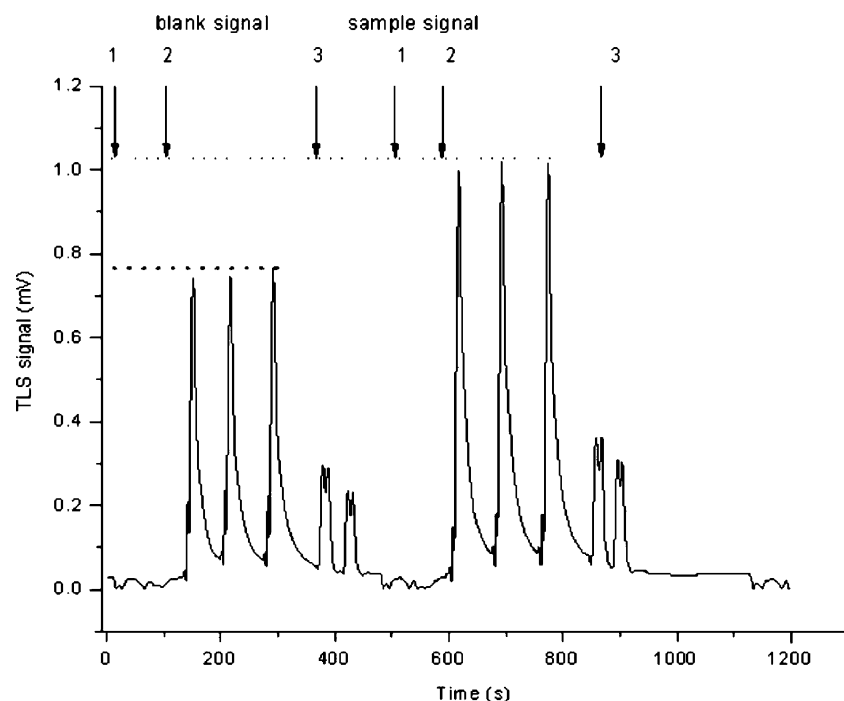


Fig. 3 A typical time sequence of acquired signals during detection of BLG ($1.2 \mu\text{g L}^{-1}$) on CPG: 1, sample injection; 2, reagent injection (three consecutive times); 3, washing buffer injection (duplicate injection)

The difference in sensitivities obtained on the two tested support materials can also be explained on the basis of different contact times. It should be emphasized that different physical characteristics, for example compactness and porosity of CIM and CPG supports required different volumes of columns where the supports were placed. The column for CIM discs has a small volume of only $3.4 \mu\text{L}$ whereas the volume of the column which was filled in-house by CPG can vary up to $100 \mu\text{L}$ and in this particular case was $63 \mu\text{L}$. This different volumes causes also differences in contact times between the HRP-labelled antibodies and the reagent used to detect their presence. In the case of the CPG columns the calculated contact time

is 7.5 s at the flow rate of 0.5 mL min^{-1} . In the case of CIM discs the contact time for the reaction is shortened to only 0.4 s at the same flow rate.

To explain the deviations from linearity for CPG, the porosity of the support, evident also from large specific surface and consequently low surface density of primary antibodies, and the diffusion of the OPD reagent into the pores, have to be considered. As described in the Experimental section, the pre-incubated solution of the analyte is injected on to the column at slower flow rates in order to allow time for the analyte to interact with the immobilized antibodies. A certain amount of analyte is retained by the primary antibodies on the surface of the

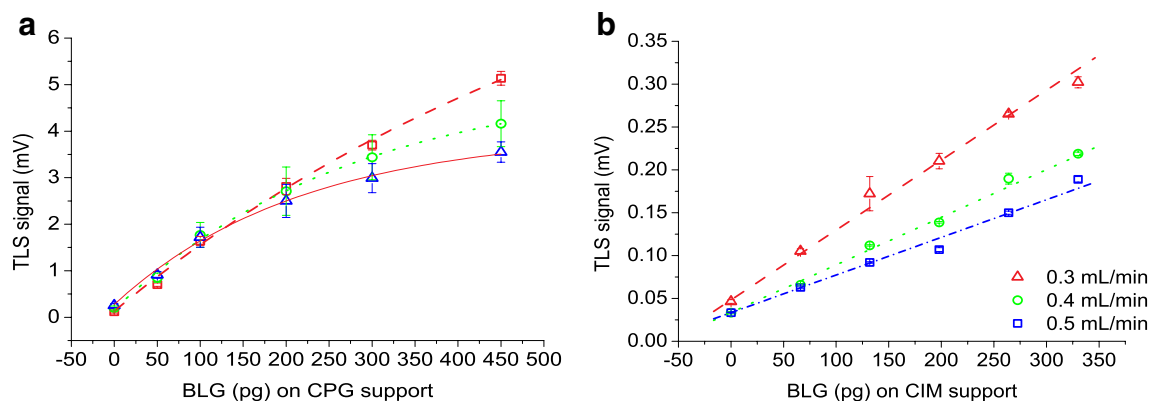


Fig. 4 Calibration curves obtained on CPG (a) and CIM (b) supports, at different flow rates of OPD ($0.3, 0.4$, and 0.5 mL min^{-1})

glass beads. However, when the surface sites are all occupied, the rest of analyte can also diffuse into the pores. Therefore, at higher concentrations the amount of analyte inside the pores is proportional to the amount of the analyte which was not retained on the surface. On the other hand, the OPD reagent is injected on to the column at higher flow rates in order to limit the broadening of the signal due to diffusion. The higher the flow rate, less OPD reagent reaches the HRP in the pores and, therefore, the formation of the enzymatic product is mainly the consequence of HRP enzyme retained on the surface of the glass beads, which leads to relatively lower analytical signals at higher BLG concentrations.

This effect could not be observed for the CIM discs. The CIM discs are composed by interconnecting pores, forming a network of channels. According to Podgornik and co-workers [19], the carrier buffer and the reagents are transported through such channels by convection which makes all active sites (i.e. all HRP) equally accessible to the OPD.

It shall be pointed out, that polyclonal antibodies do not bind to one specific site of BLG, but can bind to different sites of protein. This can result in disturbed binding of pre-incubated BLG to immobilized primary antibodies. On the other hand, the sensitivity of the method can be affected by the binding of more than one antibody to a single molecule of BLG. All these effects can, however, be controlled or kept to a minimum, by maintaining the experimental conditions as constant as possible. It is therefore assumed that the use of polyclonal antibodies does not affect the reproducibility of the described method, nor the observed differences between CPG and CIM supports, because the flow rate for injection of pre-incubated BLG was always constant at 0.1 mL min^{-1} .

In this research we investigated also the changes in activity of immunocolumn with time in order to determine the stability of immobilized antibodies. Both derivatized supports were stored in PBS buffer at 4°C , when they were not used in experimental work, which was carried out at room temperature without any cooling of the carrier buffer. Both materials were stored at 4°C for more than a month without showing any decrease in activity of the bound antibodies. However, CIM discs performed better. The CIM immunocolumn retained its activity for a longer period than the CPG support (results shown in Table 2).

Use of the immunocolumn during experiments was also tested. We observed that antibodies immobilized on the CIM support retain their activity after a larger number of analysed samples. For each sample there were at least three injections of reagent. The antibodies on CIM support resist at least 240 reagent injections, which is sufficient for analysis of approximately 80 samples. In comparison, CPG bonded antibodies lost their activity toward pre-incubated allergen rapidly after 150 reagent injection (results shown in Table 2).

The supports acted differently also regarding antibodies binding. After immobilization of the primary antibodies, we evaluated the efficiency of the immobilization processes. We discovered that more antibodies bind to CIM discs (results shown in Table 2). However, the capacity of CIM discs is not comparable with the quantity of antibodies immobilized, suggesting that the orientation (accessibility) of such proteins is mainly unfavourable for detection of the allergen BLG. Most probably, the problem would be avoided by using a spacer between the proteins and the support. However those results were not proved, because such immobilization would take more time and the spacer would alter the mechanical properties of the CIM support. Anyway

Table 2 Comparison of performances

Parameters	Performances	
	CPG	CIM
LOD ($\mu\text{g L}^{-1}$)	0.1	0.1
Sensitivity ($\text{mV L } \mu\text{g}^{-1}$)	104	8
R^2	0.989	0.998
Linear range ($\mu\text{g L}^{-1}$)	0.2–1.5	0.2–3.5
RSD (%) inter-day	15	10
RSD (%) intra-day	4	1
Analysis time for three consecutive injections of the substrate (min)	8	7
Column volume (μL)	62.8	3.4
Immobilisation time (optimised) (h)	10 (batch)	5 (dynamic)
Antibody density (approximate) ($\mu\text{g m}^{-2}$)	~13	> $4.9 \cdot 10^3$
Stability	150 injections of OPD	240 injections of OPD
Storage period (stored in PBS, 4°C)	1 month	1 month
Eluent consumption per sample (mL)	2	1.7

the unfavourable orientation of immobilized antibodies negatively affects the binding capacity of the column.

Conclusions

Comparison of CPG glass and CIM discs as support materials for immunoglobulin antibodies in bioanalytical detection of BLG revealed that CIM discs offer better performance regarding linear range, reproducibility, and immunocolumn stability, whereas estimated LOD values do not differ significantly. Moreover, CPG derivatisation is a more time-consuming procedure and the additional packing step results in loss of material compared with the CIM immobilisation procedure. Use of CIM discs also results in lower eluent consumption and, therefore, less waste liquid. Therefore, CIM discs should be the support of choice for the described TLS detection of beta-lactoglobulin and other allergens in food products.

We demonstrated that non-specific background signals arise from both supports as a result of improper non-specific binding of primary antibodies, and increase the LOD of the method for BLG, which was estimated to be $0.1 \mu\text{g L}^{-1}$ (3.3 pmol L^{-1}) for both supports. Detection of beta-lactoglobulin at concentrations substantially below those triggering allergic reactions ($14 \mu\text{g L}^{-1}$ for 100 g ingested food) can therefore be performed with a sample throughput of up to eight samples per hour.

Further improvements of the specificity, reproducibility, and sensitivity of the method are possible by using monoclonal antibodies to eliminate eventual disturbances in binding between pre-incubated BLG and primary antibodies, which might arise from multiple site binding of currently used polyclonal antibodies.

Acknowledgment This work was financially supported by the Slovenian Research Agency, through research program P1-0034 and a young researcher's grant to Ana Čevdek. Financial support for equipment was obtained from the EU and the Municipality of Nova Gorica through structural funds contract C1536-08S330265. The

authors are also grateful to BIA Separations, for their kind donation of diol CIM micro discs, and to Dr Aleš Podgornik for inspiring discussions.

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