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ORIGINAL PAPER

Magnetic particles functionalized with PAMAM-dendrimers and antibodies: a new system for an ELISA method able to detect Ara h3/4 peanut allergen in foods

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Abstract An innovative enzyme-linked immunosorbent assay (ELISA) format based on antibody-coated magnetic micro-particles (MPs) for the sensitive detection of Ara h3/4 allergen in food is described. The immunosupport is suspended in the incubation solutions and the MPs with the captured allergen can be easily harvested on a magnet, separated from the solutions, and washed using an easy-to-use, fast and selective approach that allows its detection and quantification. Two differently coated MPs, ProteinA-Pn-b and MP-NH_2-PAMAM G 1.5 -Pn-b immunosupports, were tested. The functionalization of the MPs with PAMAM-sodium carboxylate dendrimers elicits a major stability on the immunoglobulin activity resulting in a threefold enhancement of the analytical sensitivity for the assay with respect to a ProteinA immobilization. Validation was carried out on two different matrices: corn flakes and biscuits. In the case of MP-NH_2-PAMAM G 1.5 -Pn-b immunosupport, limit of detection was found to be 0.2 mg peanuts/kg matrix in both matrices; the linear response range was demonstrated from 2.5 to 15 mg peanuts/kg matrix by performing statistical tests (homoscedasticity and Mandel fitting tests). Good accuracy and recovery (>80±2%) were obtained. Different food samples were tested and the results were compared with those obtained with a commercially available ELISA kit. The results obtained in this work demonstrated the applicability of the immunomagnetic ELISA methods on real samples and the possibility to perform the assay with significantly reduced reagent and sample consumption.

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Introduction

The recognition of hidden allergens in foods still represents a great analytical challenge to achieve a complete protection of the foods' final consumers. ELISA methods have been traditionally used for the analysis of hidden allergens in food [1, 2]. However, quantitative results are strongly dependent on the matrix tested leading to a lack of accuracy that mostly requires the use of a confirmatory method [3, 4].

Strategies based on sandwich immunoassay require the immobilization of the affinity probe on solid supports or substrates for the development of microarrays. Nevertheless, immobilization or conjugation procedures can adversely affect the integrity and the functionality of a biologically active molecule owing to the limitation of its conformational flexibility [5], thus giving rise to new challenges in the development of these systems. The conjugation of proteins or antibodies with macromolecules known as dendrimers [6, 7] has been reported to maintain the biological activity during immobilization procedures. Since their discovery in the early 1980s, dendrimers have been recognized as a new class of branched macromolecules, having an architecture and chemistry able to determine very different properties from the conventional linear polymers. Dendrimers are indeed polymeric nanostructures composed of multiple-branched monomers that radiate outward from a central core [8]. Dendrimers represent a really versatile class of molecules with different properties regarding solubility, monodispersity, and functionalization chemistry, with a peculiar multivalency that has determined the large application of these architectures in different fields, ranging from drug and

gene delivery, imaging, and use as bio-mimetic artificial proteins [9].

Many studies demonstrated that their binding with biologically active molecules like antibodies can positively improve the activity of the ligand and enhance sensitivity of the systems developed with these new conjugates [10-12]. This feature is especially desired when antibodies are used in a solid phase, for example when they are immobilized onto surfaces like microarray platforms [13, 14].

In order to develop an innovative ELISA strategy to detect hidden peanut allergens in foods, we investigated the effect of the immobilization on magnetic supports of PAMAM-sodium carboxylate dendrimers conjugated with monoclonal antibodies directed towards Ara h3/4 peanut allergen.

Magnetic particle-based ELISA represent an interesting immunoassay format based on the immobilization of the antibodies on nano- and micro-particles, as solid support [15-17]. Among the advantages, magnetic particles present a good surface coating, can be homogeneously dispersed in to the sample, and be rapidly isolated by a magnet. Our group had previously investigated the activation of magnetic particles functionalized with Protein A from *Staphylococcus aureus* with monoclonal antibodies directed towards Ara h3/4 peanut allergen for the selective capture of the allergen in food matrices. The selective enrichment obtained using these immunosupports was combined with a liquid chromatography–electrospray–tandem MS (LC-ESI-MS/MS) method for the determination of two specific biomarker peptides for Ara h3/4 allergen [4].

On the basis of these results, we developed two ELISA formats. The first one was developed on magnetic particles presenting on their surface Protein A for antibody binding. To increase the antibody binding capacity of the solid supports and preserve the activity of the biological probe, a second format was generated immobilizing PAMAM-sodium carboxylate dendrimers on immunosupports. The terminal groups of the dendrimers were activated to bind immunoglobulins directed towards Ara h3/4. The antibodies thus immobilized were used to capture the hidden allergen in different food extracts and the formation of the immunocomplex was detected by using a second antibody directed towards peanut allergens and conjugated with horse-radish peroxidase. The addition of a chromogenic substrate determined a peculiar absorbance for the solution used for the quantification of the identified allergen. Hence, the developed sandwich assay represents a new kind of ELISA where antibodies are captured on magnetic materials suspended in solution instead of the plates traditionally used in these assays. Finally, the results were compared with a commercially available kit for the detection of peanut traces in foods.

Experimental

Materials

Phosphate buffer and triethanolamine were purchased from Carlo Erba (Milan, Italy). MES (2-morpholinoethansulfonic acid), EDC (*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride), NHS (*N*-hydroxy-succinimide), DMP (dimethyl pimelinediimidate dihydrocholride), Trizma® hydrochloride, hydroxylamine hydrochloride, Bradford reagent, bovine serum albumin (BSA), and Tween 20 were from Sigma Aldrich (St. Louis, MO, USA). All commercially available chemicals were of analytical grade and were used without further purification. Buffered solutions were obtained in Milli-Q water (Milli-Q element A10 System, Millipore, San Francisco, CA, USA).

PAMAM-sodium carboxylate generation 1.5 dendrimer, 1,6-diammino core was purchased from Sigma Aldrich: linear molecular formula $[NH_2(CH_2)_6NH_2]$:(G1.5); dendri-PAMAM $(NHCH_2CH_2COONa)_{16}$ and MW 2,990.67 Da. Generation 1.5 (G1.5) dendrimers present on their surfaces 16 sodium carboxylate groups, respectively. The dendrimers were supplied as a 10% (*w*/*v*) solution in methanol and were stored at −20 °C.

The homogeneity of the PAMAM-sodium carboxylate dendrimers G1.5 was evaluated using a quadrupole-time of flight Q-TOF Micro (Waters, Milford, MA, USA) mass spectrometer equipped with a pneumatically assisted ESI interface. A methanol solution of the PAMAM-dendrimers was infused in the mass spectrometer and full-scan mass spectra were acquired in the positive ion mode over the scan range *m*/*z* 90–4,000 using a step size of 0.1 Da.

Anti-Ara h3/4 (Pn-b) monoclonal immunoglobulins G were obtained using peanuts which were roasted in shell to prevent molding. Pn-b antibodies were kindly provided by the Leibniz-Center for Medicine and Biosciences at the Research Center Borstel (Borstel, Germany) and prepared as previously published [18]. The antibodies were purified using PURE-1A kit from Sigma Aldrich, aliquoted and stored at 4 °C. The purified antibodies were diluted upon usage in buffers suitable for the following reactions.

A commercial ELISA kit (Veratox® Quantitative Peanut Allergen Test) specific for peanut allergens was purchased from Neogen Corporation (Lansing, MI, USA), stored at 4 °C and used following the instructions given by the producers.

Samples

Roasted peanuts and different commercial breakfast cereal samples (corn flakes, dried fruits and cereal combination products, biscuits) were purchased at a local food store. The sample treatment and the ELISA method were developed

and validated using cereal flakes and biscuits free of peanuts. In all the other products used to test the method, peanuts were not present as ingredient and the labeling "may contain trace of nuts and soy" was reported.

Preparation of functionalized magnetic particles

Magnetizable polystyrene particles were purchased from Invitrogen Dynal (Oslo, Norway). Magnetic particles are commercially available with different activation chemistries on the surface. The analytical procedure was investigated on two different kinds of magnetic particles, i.e., magnetic particles functionalized with Protein A from *S. aureus* and magnetic particles activated with amino groups on the surface. Following the instructions given by the producers, both classes of particles were stored at 4 °C until use. All the reactions for the functionalization were performed at room temperature with slow tilt agitation, and the isolation of the particles was obtained using the Dynal®MPC™ magnet (Invitrogen Dynal).

Dynabeads®Protein A (diameter 2.8±0.2 μm, surface area 3-9 $m^2/10^9$ particles) covalently coupled with recombinant Protein A from *S. aureus* (MP-ProteinA) were used to directly immobilize the anti-Ara h3/4 immunoglobulins (Pn-b) in order to activate the particles for the selective capture of Ara h3/4 from food extracts. The functionalization protocol was modified from the method previously developed by Careri and co-workers [4]. Briefly, a Pn-b solution (21 μg/mL in phosphate buffer 100 mM, pH 8.0, 50 μL) was added to a test tube containing 100 μL of pre-washed MP-Protein A. Incubation was performed for 4 h at room temperature to allow the binding of the Fc portion of the immunoglobulin to Protein A. After the isolation of the immunocomplex, the particles were washed with 1 mL phosphate buffer 100 mM pH 8.0 and 500 μL triethanolamine 200 mM, pH 8.2. To ensure an efficient binding of the immunoglobulins to the particles, the washed particles were then incubated for 30 min with 500 μL of a DMP solution (5.4 mg/mL) in triethanolamine 200 mM pH 8.2. Cross-linking reaction was quenched adding 1 mL Tris–HCl solution 50 mM, pH 7.5 for 15 min. The particles were thus washed three times with 1 mL phosphate buffer 100 mM pH 8.0 with 0.02% Tween 20. To avoid aspecific binding, the functionalized particles were incubated for 1 h with BSA (1 mg/mL in phosphate buffer 100 mM, pH 8.0, 100 μl). After the incubation, the washing step with phosphate buffer and Tween 20 was repeated as reported above. The MP-ProteinA-Pn-b particles were ready to use.

Dynaparticles®M-270 Amine (diameter 2.8 μm, active chemical functionality 0.1–0.2 mmol/g) activated on the surface with a primary amino functionality on a short hydrophilic linker (MP-NH_2), were initially functionalized with PAMAM-dendrimers of the selected generation and then Pn-b immunoglobulins were covalently attached to the carboxy-terminal groups of the dendrimers. A 50-μL suspension of MP-NH_2 were washed three times with 500 μL MES 100 mM pH 5.0; the particles were isolated through the Dynal®MPC™ and the washing solution was discarded. A solution containing EDC 31 mM, NHS 78 mM and the PAMAM dendrimer 20 mM (final incubation volume 50 μL) was added to the test tube and incubated for 2 h. Amine groups on the surface of the particles were in this way linked to the carboxylic groups of the PAMAM dendrimer through the formation of a stable amide bond. The EDC/NHS reaction was then quenched by the addition of hydroxylamine (10 mM) for 15 min. The MP-NH_2-PAMAM particles were washed three times with 500 μL MES 100 mM, pH 5.0, incubated for 1 h with BSA (1 mg/mL in MES 100 mM, pH 5.0,50 μl) to block unreacted sites and washed three times with 100 μl MES 25 mM, pH 5.0. The functionalization of the particles with Pn-b was again achieved through the formation of a covalent amide bond between the carboxylic groups of the PAMAM dendrimer and the free amino groups of Pn-b immunoglobulins. The particles were hence incubated for 30 min with a solution containing EDC 130 mM, NHS 215 mM, MES 25 mM, pH 5.0 (50 μL), and 30 min with Pn-b (21 μg/mL in MES 25 mM, pH 5.0, 50 μL). The particles were blocked adding 100 μL Tris–HCl 50 mM, pH 7.5 for 15 min and washed twice with 500 μL MES 25 mM, pH 5.0. The MP-NH_2-PAMAM-Pn-b particles prepared in this way were ready to use.

Peanut allergens extraction

Peanuts and peanut-containing food extracts were prepared by adding 10 mL of phosphate buffer 100 mM, pH 8.0 to 1 g of ground sample. Proteins were extracted following a procedure previously developed [19]. Briefly, samples were shacked with a magnetic stirrer for 2 h at 60 °C, then the extract was centrifuged (9500 g, 20 min) and filtered on 0.2 μm nylon filters before treatment with the magnetic particles. In the case of Veratox® Quantitative Peanut Allergen Test analysis, peanut-containing food extracts were prepared following the instructions given by the producers. All the extracts were aliquoted and stored at −20 °C.

Sample analysis by ELISA

Due to the limited availability of commercial antibodies directed towards food allergens, the anti-peanut residues HRP-antibody conjugates supplied with the Veratox® Quantitative Peanut Allergen Test were used for the development of the ELISA method. The substrate KBlue Substrate® (a stabilized solution containing 3,5,3′,5′-tetramethylbenzidine and H_2O_2), the Stop solution and the Wash solution (diluted

following the instructions given by the producers) supplied with the test were used for the development of the analytical strategy in the same quantities specified on the protocol.

Sample extracts (800 μL) were incubated for 3 h at room temperature either with MP-ProteinA-Pn-b or MP-NH_2-PAMAM-Pn-b particles. After the capture of the Ara h3/4 allergen by the immobilized Pn-b immunoglobulins, the particles were extensively washed three times with 1 mL washing solution. The particles were isolated using the Dynal®MPC™ magnet and were resuspended in 100 μL of anti-peanut allergen HRP-antibody conjugate and incubated for 10 min. The washing step was repeated as above. The particles were then incubated with 100 μL KBlue Substrate® for 10 min. The particles were isolated on the magnet and the supernatant was transferred to a new test tube. The Stop solution (100 μL) was added to block the peroxidase reaction. The Veratox® Quantitative Peanut Allergen Test was performed in traditional coated wells supplied with the test following the procedure suggested by the producers. The absorbance of the solution was measured at 650 nm using the UV–visible spectrometer Lambda 25 (PerkinElmer, Waltham, MA, USA) with quartz cuvettes (0.1 cm light path, Hellma Italia, Milan, Italy).

Magnetic particle-ELISA method validation

Validation of the whole analytical procedure was performed on fortified samples of corn flakes and biscuits. For this purpose, the samples were fortified with different amounts of ground peanut, mixed and treated with the functionalized particles after protein extraction and analyzed with the ELISA procedures developed. The detection limits (LOD) were calculated as the concentration of peanuts corresponding to the three times the ratio between the standard deviation of the blank and the slope of the regression curve. The limit of quantification (LOQ) was calculated as the concentration of peanuts corresponding to the five times the ratio between the standard deviation of the blank and the slope of the regression curve. The matrix-matched calibration curve was obtained by analyzing the corn flakes and biscuit extracts fortified with different peanut amounts in the 2.5–15 mg peanut/kg matrix range and treated applying the whole analytical procedure. Linear regression analysis was done using Microsoft Excel. In order to satisfy basic requirements such as homoscedasticity and linearity, the Bartlett test and linearity test (Mandel's fitting test) were performed at the 95% confidence level. Precision was calculated in terms of intra-day repeatability as R.S.D.% on two concentration levels (2.5 and 15 mg peanut/kg matrix) by analyzing five independent sample extracts for each level. Trueness was evaluated by evaluating the calibration function of the fundamental analytical procedure (i.e., peanut aqueous extract diluted at different concentration levels) and the matrix-matched calibration curves in the 2.5–15 mg peanuts/kg matrix range. The matrix-matched calibration curves were obtained by spiking matrix extracts (i.e., cornflakes and biscuits) with different concentration of aqueous peanut extracts (five concentration levels: 2.5, 5, 7.5, 10, and 15 mg peanuts/kg matrix). The slopes of the calibration curves were compared by performing a *t* test at the 95% confidence level.

Recovery was calculated at two different concentration values (5 and 15 mg peanut/kg matrix, $n=3$) for both matrices by adding known amounts of ground peanut to the sample before extraction.

Results and discussion

Development and validation of the immunomagnetic Protein A ELISA method

In this study, we immobilized Pn-b antibodies on magnetic particles to isolate and capture Ara h3/4 allergen in food extracts and the innovative immunosupports were used as the solid phase for the development of a new format of ELISA assay. Selectivity of the monoclonal antibodies (Pn-b) used in this work had been previously verified in term of cross-reactivity with different food ingredients using ELISA-ICP-MS [19, 20].

Table 1 Solvent and matrix-matched calibration curves established in corn flakes and biscuits extracts (fortified with peanut prior to extraction in the 2.5–15 mg/kg range) using the ProteinA-Pn-b and MP-NH_2-PAMAM G 1.5 -Pn-b ELISA methods

	Homoscedasticity test p ($\alpha=0.05$)[a]	Mandel test	$b_1 \pm s_{b1}$ [a]	$b_0 \pm s_{b0}$ [a]	r^2 ($n=10$)
ProteinA-Pn-b ELISA					
Solvent	0.06	0.09	0.010±0.005	0.020±0.003	0.987
Corn flakes	0.06	0.08	0.013±0.004	0.072±0.003	0.914
Biscuits	0.18	0.12	0.012±0.003	0.033±0.002	0.991
MP-NH_2-PAMAM G 1.5 -Pn-b ELISA					
Solvent	0.16	0.15	0.032±0.005	0.041±0.004	0.937
Corn flakes	0.13	0.08	0.029±0.004	0.034±0.003	0.978
Biscuits	0.09	0.07	0.030±0.002	0.043±0.002	0.968

Calibration function: $y=b_1x+b_0$

[a] Confidence level 95%.

Table 2 Extraction recovery obtained by using the ProteinA-Pn-b and MP-NH_2-PAMAM G 1.5 -Pn-b ELISA methods on corn flakes and biscuits matrices fortified at two different concentration levels

	Recovery % (n=3)	
	5mg/kg	15mg/kg
ProteinA-Pn-b ELISA		
Corn flakes	80±2	95±7
Biscuits	94±3	93±9
MP-NH_2-PAMAM G 1.5 -Pn-b ELISA		
Corn flakes	84±5	84±5
Biscuits	114±6	114±6

The first attempt to develop the new ELISA format was made using magnetic particles coated with Protein A from *S. aureus*. The concentration of the capture antibodies, the sample volume and incubation time have been optimized in a previous work [4].

The performance of the developed ELISA strategy was evaluated in terms of detection and quantitation limits, linearity, accuracy (trueness and precision) on peanut aqueous solution and matrices (i.e., corn flakes and biscuits) and recovery.

Using external calibration good linearity of the method was established over about two orders of magnitude for both solvent and matrices. Linearity was demonstrated by verifying homoscedasticity over the range tested (Bartlett test) and by applying the Mandel fitting test (Table 1; Fig. 3b). The LOD and LOQ resulted 0.8 and 2.4 mg peanut/kg matrix.

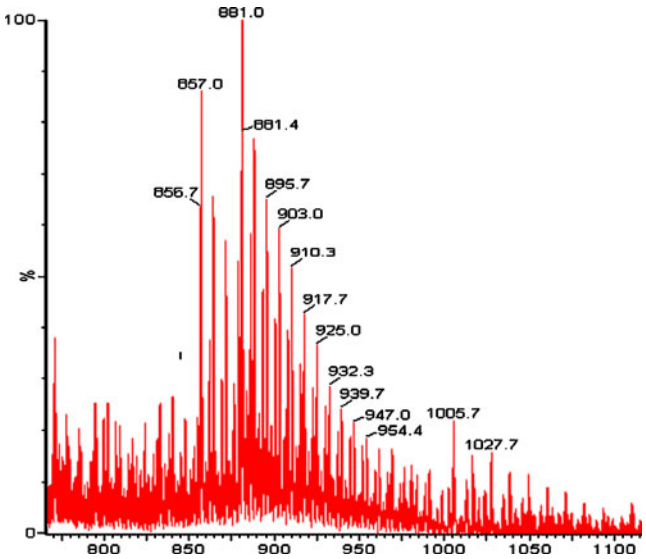

Fig. 1 ESI-Q-TOF-MS full-scan mass spectrum of PAMAM-sodium carboxylate dendrimer (+3 charge state), ethylenediamine core, generation 1.5. Molecular Formula [$NH_2(CH_2)_6NH_2$]; dendriPAMAM($NHCH_2CH_2COONa)_{16}$. Molecular weight 2934.56

As for trueness, the comparison of the fundamental calibration curve with the matrix-matched calibration curves evidenced the absence of systematic proportional errors (paired t test>0.05). Thus, absence of matrix effects allows an accurate determination of peanut residues in the products tested using peanut aqueous solutions.

The method precision assessed at 2.5 and 15 mg peanut/kg matrix concentration levels was between 7 and 15% (n=5). Excellent results in terms of recovery were calculated at both the concentrations tested (Table 2).

Even though these results suggest good method performance, taking into account that immobilization of Abs on a solid support could be affected by wrong orientation and diminished immunoreactivity, in order to improve the biological activity of the capture antibodies, we tried to develop a

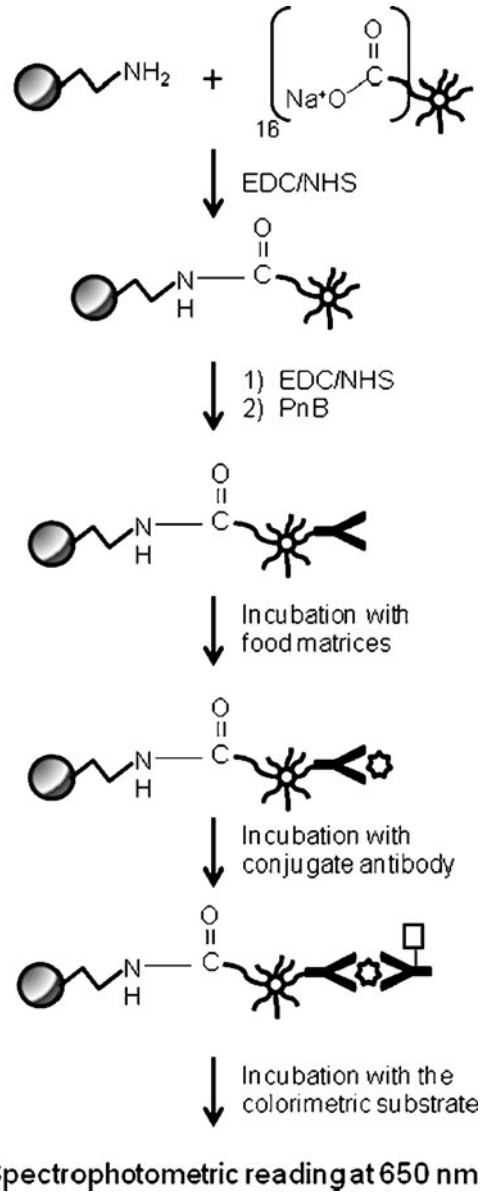

Fig. 2 Scheme of the MP-NH_2-PAMAM functionalization protocol

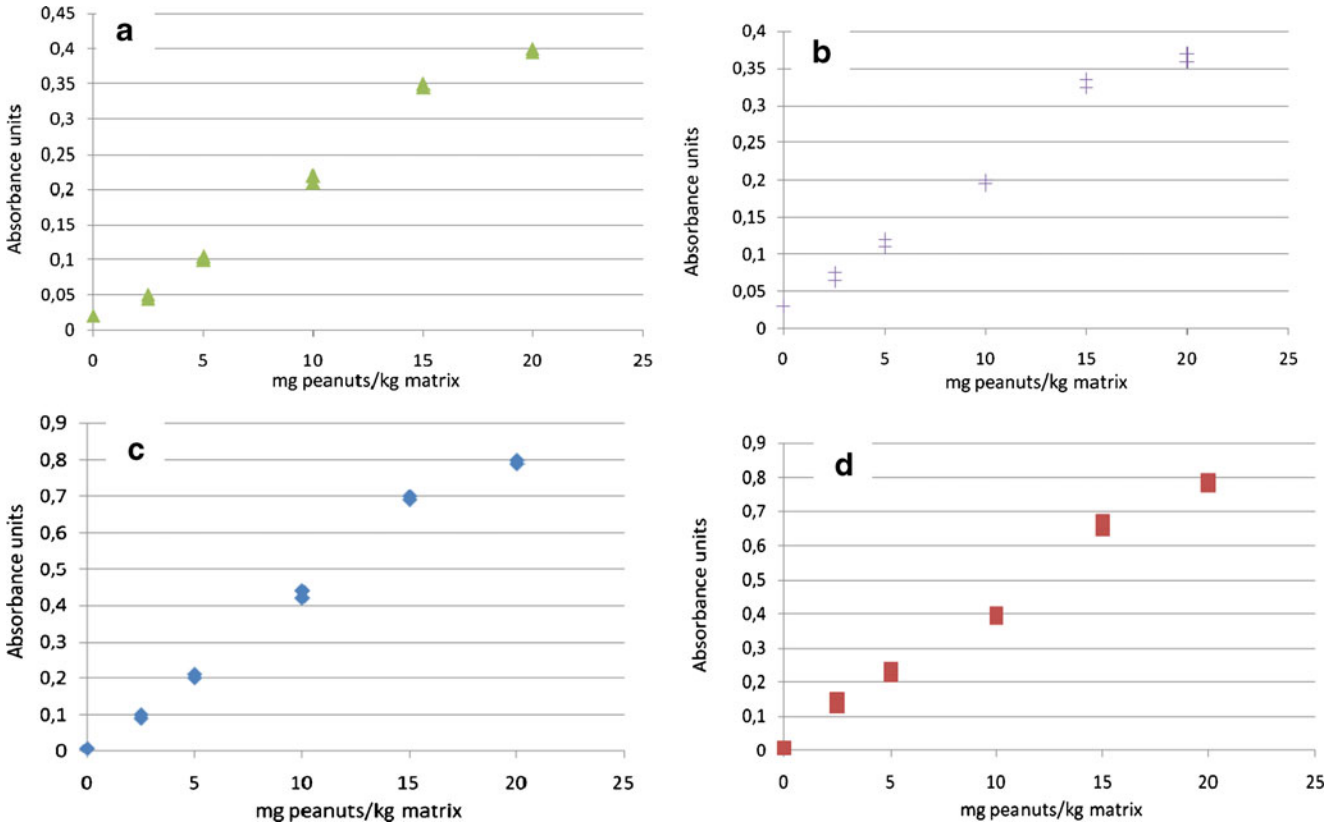

Fig. 3 Curves dose–response established in corn flakes and biscuits extracts, respectively, (fortified with peanut prior to extraction in the 2.5–20 mg/kg range) using the (**a**, **b**) ProteinA-Pn-b and (**c**, **d**) MP-NH_2-PAMAM G 1.5 -Pn-b ELISA methods

second ELISA method. For this purpose, magnetic particles functionalized with $–NH_2$ end groups able to covalently bound PAMAM-sodium carboxylate dendrimers were used.

Development and validation of the immunomagnetic PAMAM dendrimer ELISA method

PAMAM-sodium carboxylate dendrimers were chosen for their high solubility in aqueous solutions that makes them suitable reagents to be used in combination with biological molecules.

A monodisperse dendrimer with good homogeneity in the number of ramifications and unique molecular weight is required for the development of a precise and accurate analytical strategy. The ESI-MS measurements on PAMAM-dendrimers G1.5 demonstrated a narrow mass distribution and thus the suitability of the dendrimer to be used for the MP coating (Fig. 1).

Table 3 Quantitative analysis of peanut in different commercial foods by using the ProteinA-Pn-b, MP-NH_2-PAMAM G 1.5 -Pn-b immunosupports and the Veratox® Quantitative Peanut Allergen Test (n=3)

Sample	ProteinA-Pn-b ELISA	MP-NH_2-PAMAM G 1.5 -Pn-b ELISA	Veratox® Quantitative Peanut Allergen ELISA test
Muesli 1	21.9±0.2	23±1	22±2
Muesli 2	–	–	–
Muesli 3	15.2±0.8	14.6±0.4	19±2
Muesli 4	–	–	–
Muesli 5	–	–	–
Biscuits 1	–	–	–
Biscuits 2	–	–	–
Biscuits 3	9±2	11.4±0.6	12.9±1.5
Biscuits 4	–	–	–

– not detected

The chosen dendrimers were attached to MPs using a molar excess of EDC and NHS. The formation of an amine-reactive intermediate on the carboxylic terminal groups of the dendrimers resulted in the formation of a covalent amidic bond with the amino groups present on the surface of the particles. Addition of NHS was necessary to stabilize the intermediate in the aqueous solution avoiding hydrolysis side reactions [14]. The same kind of activating chemistry was used to link the free amino groups of immunoglobulins to the terminal groups of PAMAM-dendrimers. PAMAM-sodium carboxylate dendrimers G 1.5 presenting 16 carboxylate groups on the surface was used.

Different quantities of Pn-b antibodies (0.5, 1.2, and 13 mg) to coat the PAMAM functionalized particles were tested. The functionalization of the system MP-NH_2-PAMAM with the lowest Pn-b quantity resulted in an inefficient binding capability of the immobilized affinity reagents for the capture of Ara h3/4 hidden allergen giving low absorbance values at 650 nm. By contrast, there was no difference in the curves obtained with the highest antibody quantity with respect to 1.2 mg Pn-b. Both the quantities tested gave the same trend in the absorbance at 650 nm suggesting the saturation of the PAMAM-dendrimers binding sites or a loss in biological activity due to steric hindrance when too many antibodies are bound to the solid phase. On the basis of these findings, we decided to investigate the system functionalized with the intermediate antibody amount (1.2 mg). The final protocol developed is presented in Fig. 2.

Initially, the ELISA experiments were carried out both in the absence or in the presence of PAMAM-dendrimers G1.5, without the functionalization with Pn-b. The results showed a baseline absorbance at 650 nm suggesting the absence of aspecific binding.

A good linearity for the developed ELISA method was established using aqueous peanut extracts in the 2.5 to 15 mg/L concentration range (Table 1). In this case, by using the dendrimer coated MPs the slope resulted approximately threefold higher than that obtained with the Protein A coated MPs (Fig. 3b). This result can be explained with an increased immunoreactivity of the antibodies when they are bound to dendrimers and immobilized on a solid phase.

Linearity of the matrix-matched calibration curves was demonstrated in the same range reported for the previous method (Table 1). The LOD and LOQ resulted 0.2 and 0.9 mg/kg matrix for both matrices, evidencing that the use of dendrimer support allows to obtain a threefold increase of sensitivity with respect the Protein A-based method. As for trueness, the slopes obtained for both matrices using peanut aqueous solutions as control were not significantly different (paired t test>0.05) suggesting the absence of systematic matrix effects or bias. Excellent precision in terms of intra-day repeatability was calculated providing RSD% in the 3.4–10% (n=5) range. Good recovery ranging from 84±5% to 114±6% were obtained as reported on Table 2.

Sample analysis

Different commercial samples of cereals and biscuits were analyzed by using the ProteinA-Pn-b and MP-NH_2-PAMAM G 1.5 -Pn-b immunosupports. The results were then compared with those obtained with the Veratox® Quantitative Peanut Allergen Test from Neogen Corporation (Table 3). This commercially available kit is useful for the analysis of peanut residues in food matrices in the 2.5 and 25 mg peanut/kg matrix range with a LOD value of 2.5 ppm as described in the protocol supplied by the producers. Among the samples investigated, residues of peanuts were detected and quantified in two cereal samples (dried fruits and cereal mix). Even if in one of the positive samples the Veratox® test provided a slightly higher peanut concentration value, in general the ELISA methods provided comparable results in terms of qualitative and quantitative analysis, proving the applicability of the proposed methods to the detection of hidden peanut allergens in these matrices.

Conclusions

In this work, we developed a new ELISA format based on antibody-dendrimer-conjugated MPs. The immunosupport is suspended in the incubation solutions instead of a traditional plate and the major advantage of this format is that the magnetic particles with the captured allergens can be easily harvested on a magnet, separated from the solutions, and washed with an easy-to-use, fast, and selective approach that allow its detection and quantification.

In addition, the functionalization of the MPs with PAMAM-sodium carboxylate dendrimers G 1.5 elicit a major stability on the immunoglobulin activity resulting in an enhancement of the analytical sensitivity for the assay with respect to a traditional immobilization.

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