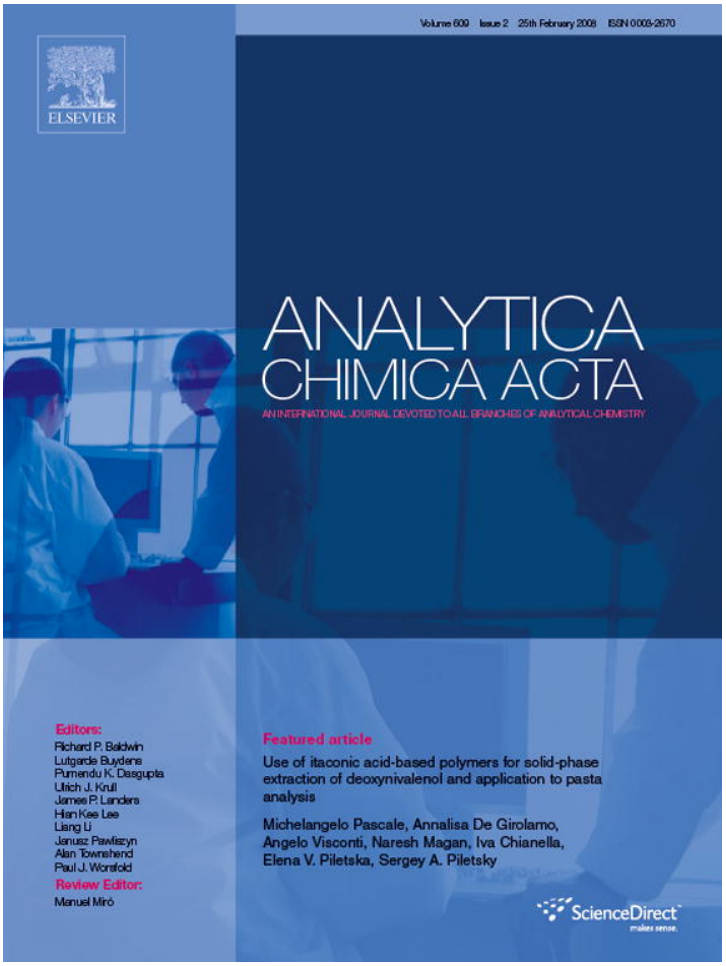

ANALYTICA CHIMICA ACTA 609 (2008) 131–138

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/aca

Use of itaconic acid-based polymers for solid-phase extraction of deoxynivalenol and application to pasta analysis

Michelangelo Pascale[a,*], *Annalisa De Girolamo*[a], *Angelo Visconti*[a], *Naresh Magan*[b], *Iva Chianella*[b], *Elena V. Piletska*[b], *Sergey A. Piletsky*[b]

[a] *Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via G. Amendola 122/O, 70126 Bari, Italy*

[b] *Cranfield Health, Cranfield University, Silsoe, Bedforshire MK45 4DT, UK*

ARTICLE INFO

Article history:
Received 29 October 2007
Received in revised form
20 December 2007
Accepted 2 January 2008
Published on line 10 January 2008

Keywords:
Deoxynivalenol
Molecular modelling
Itaconic acid
Computational design
Cross-linked sorbent
Solid-phase extraction

ABSTRACT

Molecular modelling and computational design were used to identify itaconic acid (IA) as a functional monomer with high affinity towards deoxynivalenol (DON), a *Fusarium*-toxin frequently occurring in cereals. IA-based polymers were photochemically synthesised in dimethyl formamide (porogen) using ethylenglycol dimethacrylate as cross-linker and 1,1′-azo-bis(cyclohexane carbonitrile) as initiator, and the relevant binding interactions with DON in solvents with different polarity were investigated. The performances of the non-imprinted IA-based polymer (blank polymer, BP) and the corresponding molecularly imprinted polymer (MIP) were compared using DON as a template. Both BP and MIP were able to bind about 90% DON either in toluene, water or water containing 5% polyethylene glycol. Non-imprinted polymers with different molar ratios of IA to cross-linker were evaluated as adsorbents for solid-phase extraction (SPE) clean-up and pre-concentration of DON from wheat and pasta samples prior to HPLC analysis. Samples were extracted with PBS/0.1 M EDTA solution and cleaned up through a cartridge containing blank IA-based polymer. The column was washed with PBS (pH 9.2) and the toxin was eluted with methanol and quantified by reversed-phase HPLC with UV detector ($\lambda = 220$ nm), using methanol:water:acetic acid (15:85:0.1, v/v/v) as the mobile phase. Effective removal of matrix interferences was observed only for pasta with DON recoveries higher than 70% (RSD < 7%, $n = 3$) at levels close to or higher than EU regulatory limit.

1. Introduction

Deoxynivalenol (DON), also known as vomitoxin, is a type-B trichothecene produced by several *Fusarium* species (mainly *F. graminearum* and *F. culmorum*) commonly found worldwide in cereals and derived products. Wheat and maize are the most frequently contaminated cereals [1,2]. The contamination of cereals and cereal-based products with DON might represent a real risk for human and animal health, due to its toxic effects (inhibition of DNA, RNA and protein synthesis, neurotoxicity, immunotoxicity, haematic and anorexic syndromes in mammals) [1,3]. Maximum permitted levels of DON in raw cereals and cereal-based products, ranging from 200 ng g^{-1} (processed cereal-based foods and baby foods for

* *Corresponding author*. Tel.: +39 080 5929362; fax: +39 080 5929374.
E-mail address: michelangelo.pascale@ispa.cnr.it (M. Pascale).
0003-2670/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2008.01.004

infant and young children) to 1750 ng g^{-1} (unprocessed durum wheat, oats and maize), have been recently established in the European Union [4,5].

In order to protect consumers from the exposure to this mycotoxin through the consumption of cereal-based food products, reliable analytical methods for rapid, sensitive and accurate determination of DON in cereals and cereal processed products are required. DON is commonly determined at ppb levels in these matrices by high performance liquid chromatographic (HPLC) methods with ultraviolet (UV) or diode array detector (DAD). However LC–MS/MS is becoming the technique of choice for the simultaneous determination of type-A and type-B trichothecenes, including DON. These (often expensive) methods require preliminary clean-up of extracts in order to obtain good sensitivity [6–9]. Among the commercially available columns, MycoSep® and immunoaffinity columns (IACs) are the most frequently used for DON rapid clean-up [10]. MycoSep® columns are solid-phase extraction (SPE) columns containing a variety of adsorbents (e.g. charcoal, celite, ion-exchange resins) in the syringe format. The major advantage in using these columns is the rapidity of clean-up due to the absence of rinsing steps, although purification of extracts is not always effective, depending on the matrix. On the contrary, IACs have several advantages including provision of cleaner extracts due to the specificity of the antibody, applicability also to complex matrices, good precision, accuracy and sensitivity of analytical method and limited use of organic solvents. The main limitations of the IACs are the limited stability in different solvents, the non-reusability and the high cost [11].

In the area of mycotoxin analysis there is an increasing demand for new and inexpensive materials with high affinity for the target analyte, to be used, for example, as the stationary phase in HPLC or as solid-phase extraction adsorbents. Molecularly imprinted polymers (MIPs) are cross-linked polymers synthesised by reaction of a monomer and a cross-linker in presence of the analyte (or mimic compounds) used as a template. After polymerisation, the analyte is removed leaving specific recognition sites inside the polymer. MIPs are cheap, easy to obtain and have high chemical stability shelf life should be better and long shelf-life. Imprinted polymers with affinity for the mycotoxins DON, zearalenone (ZEA), moniliformin (MON) and ochratoxin A (OTA) have been recently reported in the literature [12–16]. These polymers have been used as components of surface plasmon resonance or optical chemical sensors [17,18], as stationary phase in chromatographic applications or in SPE columns for sample clean-up [12,16,19–23]. In few cases, non-imprinted blank polymers (BPs, polymers synthesised without mycotoxin template) provided performances similar to those of MIPs [19,20]. Recently, Maier et al. reported for ochratoxin A analysis in red wines that BPs provide sample clean-up performances and recoveries similar to those obtained with an OTA-specific MIP [19]. Breton et al. showed that computationally designed BPs, based on monomers with high affinity to some photosynthesis-inhibiting herbicides, have binding properties similar to those of MIPs [24]. Molecular modelling and computational screening could therefore potentially eliminate the need of molecularly imprinting procedure in the synthesis of polymers with affinity towards specific molecules. The use of non-imprinted polymers with respect to MIPs has the advantage of avoiding the template during polymer synthesis and, therefore, no waste of toxic template is required and the risk of template leakage during the analysis is avoided. Nevertheless, BPs can compete with MIPs only through a good discrimination of appropriate monomers to interact with the target molecule. These findings lead us to explore the use of computationally designed BPs for DON affinity study and selective clean-up of sample extracts.

In this study, molecular modelling and computational design have been used to identify functional monomers capable of interacting with DON through electrostatic, hydrophobic, Van der Waals forces and dipole–dipole interactions. Itaconic acid (IA), or methylenesuccinic acid ($C_5H_6O_4$), an unsaturated diprotic acid with $pK_{a1} = 3.85$ and $pK_{a2} = 5.44$, showed the highest binding score energy for DON. Using DON as a template, ethylenglycol dimethacrylate as cross-linker and IA as monomer, MIPs and BPs were synthesised and tested for solid-phase extraction of DON from solvents of different polarity. The binding performance of the IA-based polymers was compared with those of "traditional" polymers for DON synthesised with methacrylic acid as monomer [16]. The use of IA-based BPs as adsorbents for SPE clean-up and pre-concentration of DON from wheat and pasta extracts prior to the HPLC analysis has been also evaluated.

2. Experimental

2.1. Reagents and materials

Deoxynivalenol (DON), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 1,1-azo-bis(cyclohexane carbonitrile), polyethylene glycol 8,000 (PEG), ethylendiaminotetracetic acid (EDTA) sodium salt and phosphate buffered saline (PBS) tablets were obtained from Sigma–Aldrich s.r.l. (Milan, Italy). Itaconic acid (IA) was from Acros Organics (Geel, Belgium). Acetonitrile, methanol, water, anhydrous dimethylformamide (DMF), acetone and hydrochloric acid (HCl) were purchased from Mallinckrodt Baker (Milan, Italy). MycoSep® #227 columns were from Romer Labs Inc. (Union, MO, USA); DONtest™ HPLC immunoaffinity columns were from VICAM (Watertown, MA, USA). All chemicals and solvents were ACS or HPLC grade.

2.2. Molecular modelling and computer simulation

The workstation used to simulate monomer–DON interactions was a Silicon Graphics Octane running the IRIX® 6.5 operating system (SGI, Sunnyvale, CA, USA). The workstation was configured with two 195 MHz reduced instruction set processors, 1 GB memory and a 18 GB fixed drive. This system was used to execute the software packages SYBYL 6.8 (Tripos, St. Louis, MO, USA). The virtual library consisted of 21 functional monomers (acid, basic and neutral molecules) able to interact with the template through non-covalent interactions and that can be polymerised through a radical mechanism [25].

Monomers were: 1-vinylimidazole (VI), 2-vinylpyridine (2-VP), acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), 2-hydroxyethyl methacrylate (HEM), 4-vinylpyridine (4-VP), acrolein, acrylamide, acrylic acid (AA), acrylonitrile, allylamine, *p*-divinylbenzene, ethylene glycol dimethacrylate (EGDMA), urocanic acid ethyl ester (UAEE), itaconic acid (IA), *m*-divinylbenzene, *N*,*N*-methylenebisacrylamide (MBAA), methacrylic acid (MAA), styrene, urocanic acid (UA), *N*,*N*-diethylamino ethyl methacrylate (DEAEM) and trifluoromethylacrylic acid (TFMAA). These compounds represent monomers commonly employed in molecular imprinting studies [24,25,29]. The LEAPFROG™ algorithm was used to analyze binding interaction between monomers and DON using a dielectric constant (ε) of 78.4 (at 25 °C) to simulate water. One hundred thousand interactions were completed in approximately 5 h. Results were examined and the empirical binding score was ranked according to the binding interactions between the functional monomers and the template. The monomer giving the highest binding score was selected for polymer synthesis.

2.3. Synthesis of MIP and blank polymers

DON (5 mg, 16.9×10^{-3} mmol) was dissolved in 300 μL of anhydrous DMF and mixed with IA (13.2 mg, 10.1×10^{-2} mmol), EDGMA (200.1 mg, 1.01 mmol) and 1,1-azo-bis(cyclohexane carbonitrile) (4.3 mg) by vortex in a 2 mL glass vial with a removable screw cap and a TFE/SIL seal. Reaction mixture was purged with nitrogen for 2 min to remove oxygen, sealed and polymerised first by photo-initiation at 360 nm with UV lamp for 10 min (room temperature) and then thermally overnight at 80 °C. Blank (non-imprinted) polymers with different molar ratios of monomer to the cross-linker were also synthesised as described above, but in absence of template (i.e. DON). The resulting polymers were crushed, then ground by mortar and pester and wet-sieved in acetone through two sieves with aperture size of 105 and 45 μm, respectively. The collected fraction (105–45 μm) was dried in an oven at 60 °C for 24 h and used for packing SPE cartridges.

"Traditional" polymers (imprinted and non-imprinted), prepared with methacrilic acid (MAA) as functional monomer, were synthesised according to the procedure described by Weiss et al. [16].

The composition of the synthesised polymers is reported in Table 1.

2.4. Solid-phase extraction (SPE) experiments

Fifty milligrams (or 100 mg) of polymer particles (imprinted and non-imprinted) were packed into 1.5 mL SPE cartridges capped with fritted polyethylene diskettes at the bottom and at the top. Before their use, columns were conditioned with methanol (2 mL) followed by 0.1 M HCl/methanol solution (10 mL) and methanol (10 mL) at a flow rate of 2–3 drops per second.

The ability of MIP and blank polymers to bind DON in solvents with different polarity was tested by loading onto the cartridge (flow rate of about 1 drop per second) 0.5 mL of 20 μg mL^{-1} DON standard solutions in water, water–PEG (5%), acetonitrile–water (90:10, v/v), acetonitrile or toluene (corresponding to 10 μg of DON). The DON binding potential of polymers was established by loading different volumes of standard solutions (from 0.5 to 10 mL) containing 1.0 μg of DON in water or toluene (flow rate of about 1 drop per second). For these experiments, before loading DON solutions, the cartridges were also conditioned by passing 10 mL of solvent (or 5 mL in the case of toluene). The adsorbed DON was then recovered by eluting the column with 0.5 mL of methanol followed by 2 × 0.5 mL 0.1 M HCl/methanol. The eluted solutions were evaporated to dryness at ~50 °C under nitrogen and re-dissolved in 250 μL of acetonitrile:water (10:90, v/v) prior to HPLC analysis. In the case of water solutions (i.e. water, water–PEG and acetonitrile–water solutions) the eluates from DON standard solutions were previously lyophilized.

In order to evaluate the cartridge capacity, different amounts of DON (from 0.04 to 32 μg) were added to the column by loading 1.0 or 2.0 mL of DON aqueous solution at concentrations from 0.02 to 32 μg mL^{-1}.

All experiments were carried out with the same lot of SPE cartridges. Columns were regenerated by passing 10 mL 0.1 M HCl/methanol solution followed by 10 mL methanol at a flow rate of 2–3 drops per second.

The experiments were carried out in triplicate giving reproducible results with relative standard deviation (RSD) values ranging from 4% to 10%.

2.5. SPE clean-up of wheat and pasta extracts

DON-free wheat and pasta samples, spiked with DON at two different levels, were used in SPE clean-up experiments to explore the possibility of practical application of the designed

Table 1 – Composition of monomer mixture used for imprinted and non-imprinted (blank) polymers

Polymer	Template (DON)	Functional monomer	Cross-linker	Porogen	Molar ratio	Initiator (mg)
MIP-IA	5 mg (16.9×10^{-3} mmol)	IA, 13.2 mg (0.1 mmol)	EGDMA, 201.1 mg (1.0 mmol)	DMF, 290 μL	1:6:60[a]	4.3
BP1-IA	–	IA, 67.5 mg (0.5 mmol)	EGDMA, 1028.4 mg (5.2 mmol)	DMF, 1445 μL	1:10[b]	21.7
BP2-IA	–	IA, 131.7 mg (1.0 mmol)	EGDMA, 1003.3 mg (5.1 mmol)	DMF, 2890 μL	1:5[b]	43.2
BP3-IA	–	IA, 1235 mg (9.5 mmol)	EGDMA, 3763.3 mg (19.0 mmol)	DMF, 5270 μL	1:2[b]	100.2
MIP-MAA[c]	5 mg (16.9×10^{-3} mmol)	MAA, 17.4 mg (0.2 mmol)	EGDMA, 200 mg (1.0 mmol)	CH_3CN, 290 μL	1:12:60[a]	4.3
BP1-MAA[c]	–	MAA, 17.4 mg (0.2 mmol)	EGDMA, 200 mg (1.0 mmol)	CH_3CN, 290 μL	1:5[b]	4.3

[a] Template:monomer:cross-linker.
[b] Monomer:cross-linker.
[c] Polymers prepared according to [16].

IA polymers. Recovery experiments were performed in triplicate at DON spiking levels of 2000 ng g^{-1} and 750 ng g^{-1}. Samples were extracted following the procedure reported by Lippolis et al. [26], with minor modifications. Twenty-five grams of ground samples were extracted with 100 mL water or PBS/0.1 M EDTA by blending at high speed for 2 min with a Sorvall Omnimixer (Sorvall Instruments, Norwalk, CT, USA). Extracts were centrifuged at 4500 rpm at 5 °C for 20 min in a Beckmann centrifuge (Allegra X 22 R), and the supernatant was filtered through glass microfibre filter (Whatman GF/A). SPE columns containing 100 mg of blank IA-based polymer (1:5 molar ratio of monomer to cross-linker) were conditioned with 5 mL PBS/0.1 M EDTA and 1 mL of the filtered extract was cleaned up on the SPE column at a flow rate of about 1 drop per second. The column was then washed with 2 mL PBS solution at pH 9.2 (adjusted with 0.1 M NaOH), and the toxin was eluted with 1 mL methanol. The eluted extract was evaporated under nitrogen stream at ca. 50 °C and reconstituted with 250 μL of the HPLC mobile phase. Reconstituted extracts were stored at 4 °C until HPLC analysis.

Extract clean-up based on MycoSep® and immunoaffinity columns were performed according to the protocol reported by Krska [27] and Visconti et al. [28], respectively.

2.6. HPLC analysis of DON

DON concentration was determined by HPLC using an Agilent 1100 Series HPLC system equipped with a UV diode-array detector set at 220 nm (Agilent Technologies, Palo Alto, CA, USA). The column was a Waters C18 Symmetry Shield, 150 mm × 4.6 mm, 5 μm (Waters, Milford, MA, USA) preceded by a 0.5 μm Rheodyne guard filter. The mobile phase was a mixture of acetonitrile:water (10:90, v/v) eluted at a flow rate of 1.0 mL min^{-1}. The detection limit was 0.01 μg DON (signal-to-noise ratio 3:1).

For wheat and pasta extracts, the reconstituted extracts were centrifuged (8000 rpm × 10 min, 5 °C) using 0.45 μm nylon Micro-Spin® filter tubes (Alltech, Deerfield, IL, USA) and 50 μL were injected into the HPLC/DAD (λ = 220 nm) system. The mobile phase was a mixture of methanol:water:acetic acid (15:85:0.1, v/v/v) at a flow rate 0.5 mL min^{-1}. The analytical column was a Phenomenex Synergi Hydro (150 mm × 3 mm i.d., 4 μm).

3. Results and discussion

3.1. Polymer design and solid-phase extraction (SPE) analysis

The use of molecular modelling and computational screening can potentially eliminate the need of molecularly imprinting procedure in the synthesis of polymers with affinity towards specific molecules [24]. This led us to test for deoxynivalenol (DON) binding affinity materials, which rely mainly on electrostatic or other interactions (hydrophobic, Van der Waals or dipole–dipole) of DON with blank polymer rather than DON-specific binding to molecularly imprinted polymer (MIP) imprinted cavities. A virtual library of 21 functional monomers was screened against the template (i.e. DON) minimised in water using the molecular modelling software patented by Piletsky et al. [29]. Monomers giving the highest binding energy represent candidates for polymer preparation because they form more likely stable complexes in water which is a common extraction solvent for DON [9]. Table 2 shows the binding energies of DON minimised in water with some of the screened monomers. Calculation of binding energies between template and monomers were performed according to the method described by Piletsky et al. [25]. Itaconic acid (IA) was the monomer giving the highest binding score (i.e. −29.05 kcal mol^{-1}) and was employed to synthesise IA-based polymers using ethylenglycol dimethacrylate (EGDMA) as cross-linker and 1,1′-azo-bis(cyclohexane carbonitrile) as initiator. DMF was used as porogen since it was able to dissolve IA, which is less soluble in traditional solvents (i.e. acetonitrile). In order to compare the performances of the blank polymer (BP) with molar ratio of monomer to the cross-linker of 1:10 (BP1-IA), a molecularly imprinted polymer (MIP), using DON as template, was synthesised under the same conditions. Based on the modelling results, six molecules of IA interacted with one molecule of DON (Fig. 1); therefore, a MIP using the template:monomer molar ratio of 1:6 was synthesised (MIP-IA). In addition, the DON binding performances of the new IA-based polymers (imprinted and non-imprinted) were compared with those of a recently reported "traditional" MIP for DON (and the relevant blank) that was based on photo-initiated polymerisation of methacrylic acid (MAA) in presence of EDGMA (cross-linking monomer) and acetonitrile (porogen) [16]. In order to assess the density of functional monomers providing multipoint interactions with the template (i.e. DON), additional IA-based blank polymers (BP2-IA and BP3-IA) were synthesised with different molar ratios of monomer to the cross-linker (Table 1) and compared with BP1-IA, MIP-IA and MAA-based polymers.

Table 2 – Binding energies of DON minimised in water

Monomer	Binding energy (kcal mol^{-1})
Itaconic acid	−29.05
Urocanic acid	−28.04
N,*N*-Methylenebisacrylamide	−27.14
2-Hydroxyethyl methacrylate	−26.64
Acrylamide	−26.22
Allylamine	−25.16
Methacrylic acid	−15.97
Trifluoromethylacrylic acid	−14.75
1-Vinylimidazole	−14.75

All polymers were used as adsorbent materials in the preparation of solid-phase extraction cartridges for evaluating their affinity towards DON in solvents of different polarity (toluene, acetonitrile and water-based solutions) and their possible use for clean-up and pre-concentration of the toxin prior to HPLC analysis. Water and water–PEG were selected because they are commonly used as extraction solvents of DON from cereals in several analytical protocols [8,9]. Polymer binding of DON in water, water–PEG and toluene was high (range 75–98%), as compared to the other tested solvents (Table 3). No DON was found in water and toluene eluates from loading toxin solutions. More than 95% of the adsorbed DON was recovered with 0.5 mL of methanol. The successive

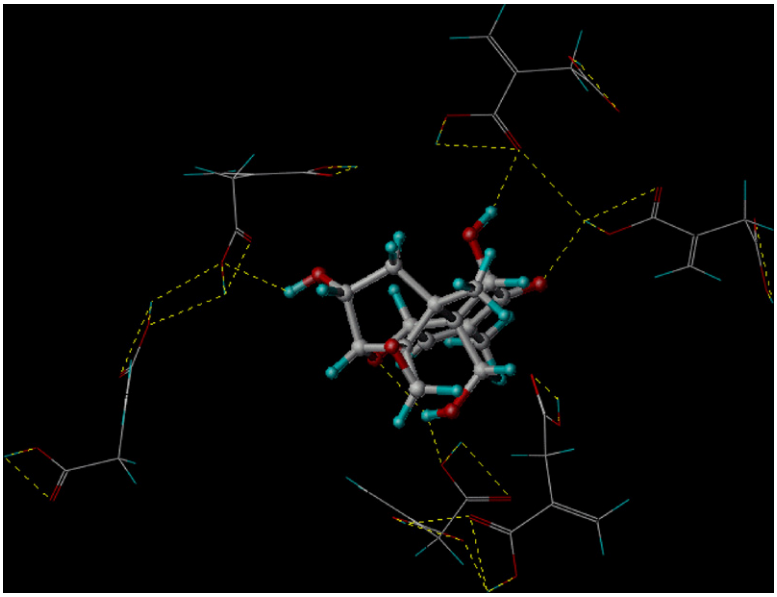

Fig. 1 – Molecular representation of interactions between itaconic acid (IA) and deoxynivalenol (DON), minimised in water.

elutions with 2 × 0.5 mL of 0.1 M HCl/methanol recovered the remaining toxin. Additional experiments showed that DON bound to polymers was completely desorbed also after elution with 1 mL methanol. No significant differences ($P < 0.05$) in DON binding affinity were observed between both IA- or MAA-based blank polymers (BP1-IA, BP1-MAA) and MIPs (MIP-IA, MIP-MAA) in all tested solvents with the exception of MIP-MAA that showed a higher affinity than BP1-MAA in water–PEG and acetonitrile (DON binding of 85% vs. 75% and 41% vs. 31%, respectively). Similar imprinting recognition properties were found also by Weiss et al. using acetonitrile as the mobile phase in high-performance liquid chromatography experiments using MAA-based polymers as stationary phase [16]. In our experiments, a significant loss in DON binding to the polymers was observed in acetonitrile or acetonitrile/water with respect to water and toluene (Table 3). This behaviour could be explained by a loss of both electrostatic and hydrophobic interactions due to the high solubility of DON in acetonitrile.

Capacity testing for polymers BP1-IA, MIP-IA, BP1-MAA and MIP-MAA showed that both imprinted and non-imprinted polymers were able to fully bind DON (DON binding higher than 95%) at all tested concentrations (up to 32.0 μg DON).

The binding properties of the polymers depended on the volume of DON water solution loaded onto the SPE column (Fig. 2). Different volumes containing the same amount of DON (i.e. 1.0 μg) were loaded onto the column. In particular, when small volumes (up to 2.0 mL of DON solutions) were loaded, the non-adsorbed DON amount ranged from 0.2% to 12% and from 0.4% to 24% for IA- and MAA-based polymers, respectively. When 4.0 mL of DON solution were loaded on columns, the performances of IA-based polymers were still good showing a loss of 22–23% DON, whereas MAA-based polymers gave a higher loss (56–59%). By increasing the volume of DON solution loaded on column, i.e. 5, 8 and 10 mL, the amount of non-adsorbed DON increased from 25% to 77% and from 59% to 92% for IA- and MAA-based polymers, respectively. The whole amount of DON non-adsorbed by the polymers was found in

Table 3 – Recoveries of deoxynivalenol (DON) after elution with different solvents from solid-phase extraction columns packed with IA- and MAA-based polymers

	DON recovery (%)									
	Water		Toluene		Water–PEG (5%)		Acetonitrile		Acetonitrile–water (90:10)	
	Eluate 1[a]	Eluate 2[b]	Eluate 1	Eluate 2	Eluate 1	Eluate 2	Eluate 1	Eluate 2	Eluate 1	Eluate 2
MIP-IA	n.d.[c]	97	n.d.	98	1	93	60	40	74	30
BP1-IA	n.d.	95	n.d.	94	3	94	59	41	73	31
MIP-MAA	n.d.	97	n.d.	97	10	85	62	41	79	21
BP1-MAA	2	93	n.d.	95	22	75	68	31	82	22

[a] Eluate 1: eluate from loading of DON standard solution.
[b] Eluate 2: methanolic eluate.
[c] n.d.: Not detected (<0.01 μg DON).

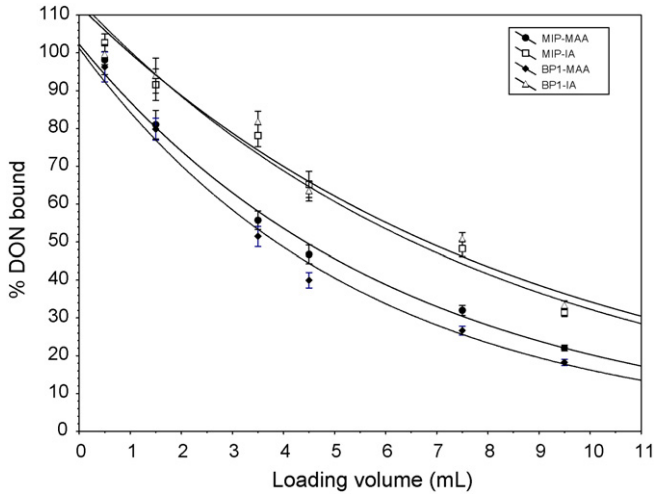

Fig. 2 – Deoxynivalenol (DON) bound (%) vs. volume (mL) of DON water solution loaded onto SPE columns packed with 50 mg polymers. Curves fit the equation $y = a \times \exp(-b \times x)$ (BP1-MAA: $a = 108.5$, $b = 0.180$, $r = 0.9967$; MIP-MAA: $a = 109.5$, $b = 0.160$, $r = 0.9979$; BP1-IA: $a = 114.4$, $b = 0.113$, $r = 0.9793$; MIP-IA: $a = 116.9$, $b = 0.121$, $r = 0.9883$). Data represent the mean (±1S.D.) of three replicated experiments.

the eluate from the loading step. Exponential equations of the type $y = a \times \exp(-b \times x)$ fitted the experimental data with coefficients of correlation higher than 0.9793 (Fig. 2).

Concerning re-binding experiments performed in toluene, no DON was detected in eluates from loading DON solution of both IA- and MAA-based polymers packed columns, independently from the loaded volume of toluene solutions (up to 10 mL). One hundred percent of DON loaded on column was retained by polymers; this clearly indicates an important role of the electrostatic interaction in DON binding to the polymer.

No significant differences ($P < 0.05$) in DON binding were observed between BPs and MIPs for both IA- and MAA-based polymers, although a major affinity of the IA-based polymers towards DON in water was observed with respect to the MAA-based polymers (Fig. 2). Therefore, the identification by computational approach of itaconic acid as a high affinity monomer for DON has been shown to be effective for designing polymers that can be used for SPE of DON.

New IA-based blank polymers (see Table 1) with different IA:cross-linker molar ratios, i.e. 1:2 (BP3-IA), 1:5 (BP2-IA) and 1:10 (BP1-IA), were synthesised to be used as sorbents in solid-phase extraction of DON prior to the HPLC analysis. Under the experimental conditions used, the BP containing the higher percentage of IA (BP3-IA) surprisingly showed less affinity towards DON. Average recovery of DON was 56% (CV = 20%, $n = 3$) when 2 mL of a water solution 2.0 $\mu g\,mL^{-1}$ DON were loaded onto SPE columns containing 100 mg of BP3-IA, whereas recoveries of 89% and 86% (CV < 4%, $n = 3$) were found with BPs having IA:cross-linker molar ratios of 1:5 (BP2-IA) and 1:10 (BP1-IA), respectively. The possible explanation for this could be in the need here for a particular balance between electrostatic interactions provided by IA and hydrophobic interactions provided by cross-linker backbone. The polymer containing high concentration of IA could be too polar for effective DON binding. Therefore, BP2-IA was selected for recovery experiments of DON from raw wheat and pasta samples.

3.2. IA-based polymers for SPE clean-up of wheat and pasta extracts

Water and phosphate buffered saline (PBS) were used as extraction solvents because they have been shown to be efficient extraction solvents of DON from wheat and wheat-based products [9,26]. SPE columns packed with 100 mg of BP2-IA were tested for the purification of wheat and pasta extracts prior to DON determination by HPLC. Based on recovery values, PBS resulted as the best extraction solvent. Different volumes (up to 4 mL) of several washing solvents (water, water:methanol 99:1, PBS pH 7.2, PBS pH 9.2) were tested to minimise the interactions between interfering compounds and polymer. Most interfering compounds were eluted with PBS (pH 9.2) washing step (2 mL), although minor interfering peaks were still observed close to DON retention time. The addition of EDTA to the extraction solvent provided cleaner extracts, reducing significantly both noise and number of interfering peaks in the HPLC chromatogram. Fig. 3 shows the chromatograms of a "blank" pasta sample (lower line) and the same sample spiked with DON at level of 750 $ng\,g^{-1}$ (i.e. the EU regulatory limit) after clean-up with SPE column containing BP2-IA. The peak purity was estimated by using a photodiode-array detector. Average recoveries of 80% (CV = 7%, $n = 3$) and 73% (CV = 6%, $n = 3$) were obtained for pasta sample spiked with DON at 2000 $ng\,g^{-1}$ and 750 $ng\,g^{-1}$, respectively. The detection limit was 80 $ng\,g^{-1}$, based on a signal-to-noise ratio of 3. The use of a mixture of methanol:water:acetic acid as the mobile phase improved appreciably the peak resolution. This mobile phase has been showed to provide a good separation of the DON peak from other interfering compounds when complex matrices are analyzed [30].

The performances of BP2-IA column for purification and pre-concentration of DON from pasta extracts was compared with those of MycoSep® #227 column, a multifunctional col-

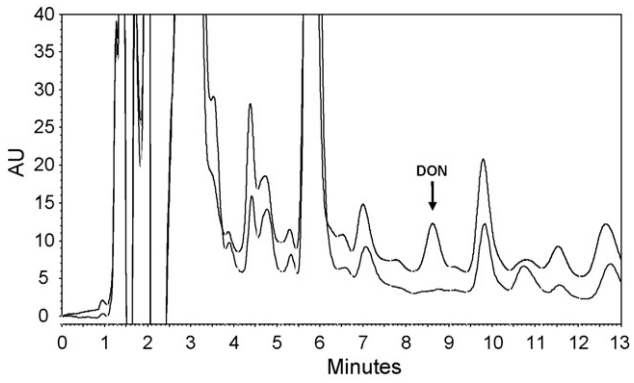

Fig. 3 – Chromatograms of a "blank" pasta sample (lower line) and the same sample spiked with DON at level of 750 $ng\,g^{-1}$ after extraction with PBS/EDTA solution and clean-up with SPE columns containing 100 mg BP2-IA (upper line).

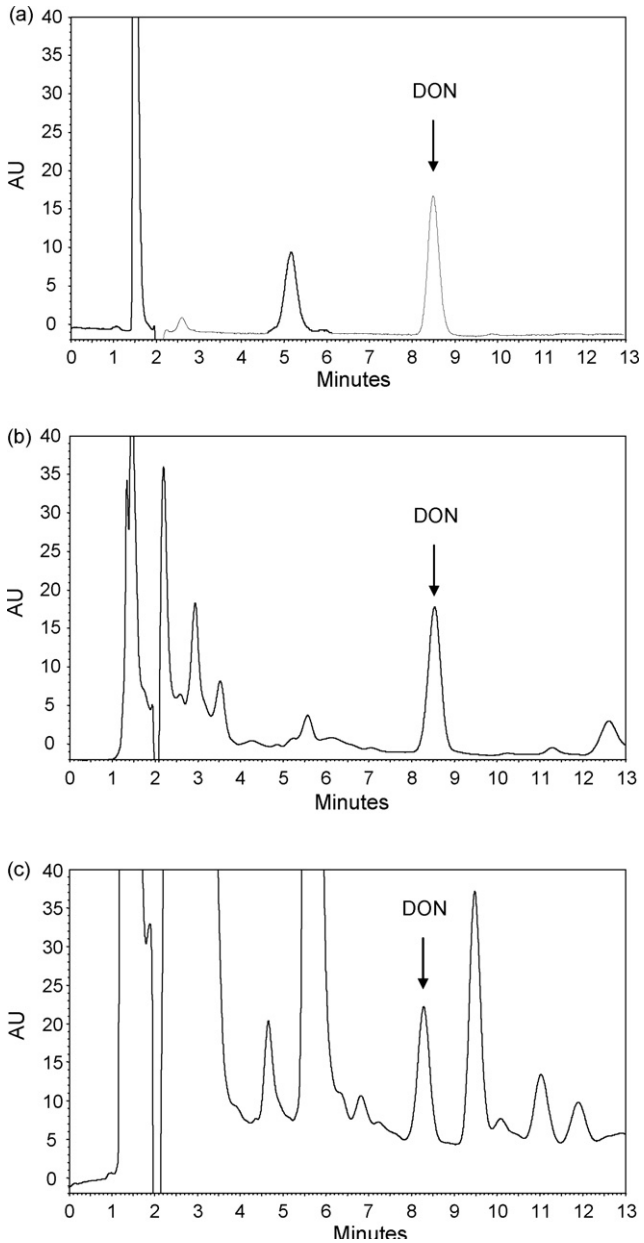

Fig. 4 – Chromatograms of a pasta sample spiked with DON at level of 2000 ng g^{-1} after extract clean-up with (a) immunoaffinity column, (b) MycoSep® column and (c) IA-based SPE column.

umn commonly used for purification of cereal extract in the HPLC analysis of trichothecenes, including DON [10] and with those of immunoaffinity column, the most used for DON determination in food commodities [8,9,11]. Although cleaner chromatograms were observed with both MycoSep® and immunoaffinity columns (Fig. 4), the polymeric column allowed comparable, sensitive and reliable quantification of DON with good recoveries and reproducibility of results. In addition, the polymeric columns have the advantage to be much cheaper than the above mentioned columns, are easy to obtain and resist to different chemical environments (pH, ionic strength, solvent composition) and temperatures.

The blank polymer, under the optimised experimental conditions allowed effective removal of matrix interfering compounds during the loading and washing steps for pasta but not for wheat extracts. In the present study all efforts to improve clean-up of wheat extract for DON determination failed in the case of BP2-IA.

4. Conclusions

This study evidences that molecular modelling and computational design can be used to select functional monomers to be used in the synthesis of cross-linked polymers with high affinity for deoxynivalenol (DON), excluding the need of molecular imprinting. The high cost of such template in MIP development can be, therefore, overcome. This approach has been recently used for affinity studies of computationally designed polymers with herbicides [24] and could be successfully applied for the analysis of many other chemicals in food and the environment.

IA-based polymers have been shown to have a high affinity towards DON in both apolar (toluene) and polar (water) solvents, with exception of acetonitrile showing a significant loss in DON binding. The non-imprinted polymer has been used as a low-cost material for clean-up and pre-concentration of DON from pasta allowing HPLC determination of the toxin at levels close to EU regulatory limit. The use of IA-based polymers in SPE application for clean-up of raw cereal extracts needs further investigations.

Acknowledgements

This work has been supported by the Italian Ministry of Education, University and Research, MIUR (DL 297/99, 27 July 1999), Project no. 12792 “SINSIAF”. MP would like to thank the National Research Council (Rome, Italy) for receiving the grant within the N.A.T.O.-CNR “Senior Fellowship” Programme 2001.

REFERENCES

[1] R.A. Canady, R.D. Coker, S.K. Egan, R. Krska, T. Kuiper-Goodman, M. Olsen, J. Pestka, S. Resnik, J. Schlatter, Safety evaluation of certain mycotoxins in food, FAO Food and Nutrition Paper 74, WHO Food Additives Series 47, WHO, Geneva, Switzerland, 2001, p. 419.
[2] R. Schothorst, H. van Egmond, Toxicol. Lett. 153 (2004) 133.
[3] B.A. Rotter, D.B. Prelusky, J.J. Pestka, J. Toxicol. Environ. Health 48 (1996) 1.
[4] Commission regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Off. J. Eur. Union L 364 (2006) 5.
[5] Commission regulation (EC) No. 1126/2007 of 28 September 2007 amending Regulation (EC) No. 1881/2006 setting maximum levels ofor certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products, Off. J. Eur. Union L 255 (2007) 14.
[6] S. Biselli, C. Hummert, Food Addit. Contam. 22 (2005) 752.
[7] V.M.T. Lattanzio, M. Solfrizzo, S. Powers, A. Visconti, Rapid Commun. Mass Spectrom. 21 (2007) 3253.
[8] L.M. Cahill, C.K. Scott, B.T. McAlice, C.S. Ramsey, R. Prioli, B. Kohn, J. Chromatogr. A 859 (1999) 23.

[9] S.J. MacDonald, D. Chan, P. Brereton, A. Damant, R. Wood, J. AOAC Int. 88 (2005) 1197.
[10] R. Krska, S. Baumgartner, R. Josephs, Fresen. J. Anal. Chem. 371 (2001) 285.
[11] M. Pascale, A. Visconti, in: D. Barug, D. Bhatnagar, H.P. van Egmond, J.P. van der Kamp, W.A. van Osenbruggen, A. Visconti (Eds.), The Mycotoxin Factbook, Wageningen Academic Publishers, The Netherlands, 2006, p. 269.
[12] M. Appell, D.F. Kendra, E.K. Kin, C.M. Maragos, Food Addit. Contam. 24 (2007) 43.
[13] C. Baggiani, G. Giraudi, A. Vanni, Bioseparation 10 (2002) 389.
[14] J. Jodlbauer, N.M. Maier, W. Lindner, J. Chromatogr. A 945 (2002) 45.
[15] J.L. Urraca, M. Marazuela, E.R. Merino, G. Orellana, M.C. Moreno-Bondi, J. Chromatogr. A 1116 (2006) 127.
[16] R. Weiss, M. Freudenschuss, R. Krska, B. Mizaikoff, Food Addit. Contam. 20 (2003) 386.
[17] F. Navarro-Villoslada, J.L. Urraca, M.C. Moreno-Bondi, G. Orellana, Sens. Actuators B 121 (2007) 67.
[18] J.C.C. Yu, E.P.C. Lai, React. Funct. Polym. 63 (2005) 171.
[19] N.M. Maier, G. Buttinger, S. Welhartizki, E. Gavioli, W. Lindner, J. Chromatogr. B 804 (2004) 103.
[20] N.W. Turner, E.V. Piletska, K. Karim, M. Whitcombe, M. Malecha, N. Magan, C. Baggiani, S.A. Piletsky, Biosens. Bioelectron. 20 (2004) 1060.
[21] J.L. Urraca, M. Marazuela, M.C. Moreno-Bondi, Anal. Bioanal. Chem. 385 (2006) 1155.
[22] J.C.C. Yu, S. Krushkova, E.P.C. Lai, E. Dabek-Zlotorzynska, Anal. Bioanal. Chem. 382 (2005) 1534.
[23] S.N. Zhou, E.P.C. Lai, J.D. Miller, Anal. Bioanal. Chem. 378 (2004) 1903.
[24] F. Breton, R. Rouillon, E.V. Piletska, K. Karim, A. Guerreiro, I. Chianella, S.A. Piletsky, Biosens. Bioelectron. 22 (2007) 1948.
[25] S.A. Piletsky, K. Karim, E.V. Piletska, C.J. Day, K.W. Freebairn, C. Legge, A.P.F. Turner, Analyst 126 (2001) 1826.
[26] V. Lippolis, M. Pascale, A. Visconti, J. Food Prot. 69 (2006) 2712.
[27] R. Krska, J. Chromatogr. A 815 (1998) 49.
[28] A. Visconti, E.M. Haidukowski, M. Pascale, M. Silvestri, Toxicol. Lett. 153 (2004) 181.
[29] S.A. Piletsky, R.M. Day, B. Chen, S. Subrahmanyam, O. Piletska, A.P.F. Turner, (2000) PCT/GB01/00324.
[30] J. Stroka, M. Derbyshire, C. Mischke, M. Ambrosio, K. Kroeger, I. Arranz, E. Sizoo, H. van Egmond, J. AOAC Int. 89 (2006) 1012.