Research Article

Phytochemical Analysis

Received: 9 February 2016, Revised: 4 December 2016, Accepted: 5 December 2016 Published online in Wiley Online Library: 26 January 2017

(wileyonlinelibrary.com) DOI 10.1002/pca.2674

Synthesis of Quercetin-imprinted Polymer Spherical Particles with Improved Ability to Capture Quercetin Analogues

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ABSTRACT:

Introduction – Molecularly imprinted polymers (MIPs) are composed of specific cavities able to selectively recognise a template molecule. Used as chromatographic sorbents, MIPs may not trap related structures due to the high rigidity of their cross-linking.

Objective – To improve the capture of quercetin analogues by modulating the synthesis strategy for a quercetin-imprinted polymer (Qu MIP).

Methodology – An additional comonomer bearing a short oligoethylene glycol (OEG) unit was used to prepare a Qu MIP that was compared to a traditional one formulated in a similar fashion, but without the OEG-comonomer. The Qu MIPs were prepared in bead form through fluorocarbon suspension polymerisation. After solid phase extraction (SPE) assessment of their imprinted cavities, the MIPs were evaluated by HPLC for their recognition properties towards quercetin and other polyphenols, including flavonoids, phenolic acids and curcumin. The Qu MIPs were finally SPE-tested on a white onion extract.

Results – The incorporation of OEG units modulated the selectivity of the Qu MIP by improving the recognition of quercetin related structures (12–61% increase in the imprinting effect for distant analogues). It also allowed limiting or suppressing non-specific hydrophobic interactions (decrease of about 10% in the rate of quercetin retention on the non-imprinted polymer). The SPE application of the MIP to a white onion extract indicates its interest for the selective extraction of quercetin and its analogues.

Conclusion – The OEG-modified Qu MIP appears to be an attractive tool to discover new drug candidates from natural sources by extracting, amongst interfering compounds, structural analogues of quercetin.

Keywords: Quercetin; Curcumin; *Allium cepa* L.; MIPs; oligoethylene glycol

Introduction

Molecularly imprinted polymers (MIPs) are extensively cross-linked polymers composed of specific cavities designed upon a template molecule. As such, MIPs are able to selectively recognise a template and its more or less analogous molecules (Pardo *et al.*, 2012). Quercetin (**1**) (Fig. 1) is often chosen as a representative template based on its numerous biological activities, including reactive oxygen species (ROS)-scavenging activity and/or binding to specific proteins such as oxidative enzymes and transcriptional factors in signal transduction pathways. These properties form the basis for potential benefits to overall health and disease resistance. In fruits and vegetables, **1** is mostly present as glycoside species, which function as hydrophilic bioactive agents towards various ROS-generating systems and as precursors of more hydrophobic aglycones (Terao *et al.*, 2011); glycosides, aglycones and their microbial metabolites are absorbed, metabolised by enterocytes and hepatocytes and it is so far uncertain which of these compounds are biologically important. Quercetin-imprinted polymers (Qu MIPs) have consequently been investigated in a series of research projects mainly aimed at improving MIPs synthesis (Xie *et al.*, 2001a,b; Molinelli *et al.*, 2002; Weiss *et al.*, 2002; Zhou *et al.*, 2002; O'Mahony *et al.*, 2006; Yan *et al.*, 2006; He and Deng, 2007; Song *et al.*, 2009a; Song *et al.*, 2009b; Fan and Wang, 2010; Tian *et al.*, 2011; Castro López *et al.*, 2012; Pakade *et al.*, 2012; Yu *et al.*, 2012; Hong and Chen, 2013). A selective Qu MIP has previously been prepared using fluorocarbon suspension polymerisation which allowed confirmation of the chromatographic superiority of beaded MIPs compared to irregular particles prepared by bulk polymerisation (Pardo *et al.*, 2014a).

The selective extraction of structurally-related metabolites from plant material presents an attractive approach for the discovery of new lead compounds. Such quercetin-related compounds are not efficiently trapped by most of the previously developed Qu MIPs due to the high rigidity of their cross-linking (Molinelli *et al.*, 2002; Weiss *et al.*, 2002; O'Mahony *et al.*, 2006; Song *et al.*, 2009a; Yu *et al.*, 2012; Hong and Chen, 2013). Research has specifically focussed on drug discovery, with particular attention to the capability of MIPs for recognising analogues of the original template

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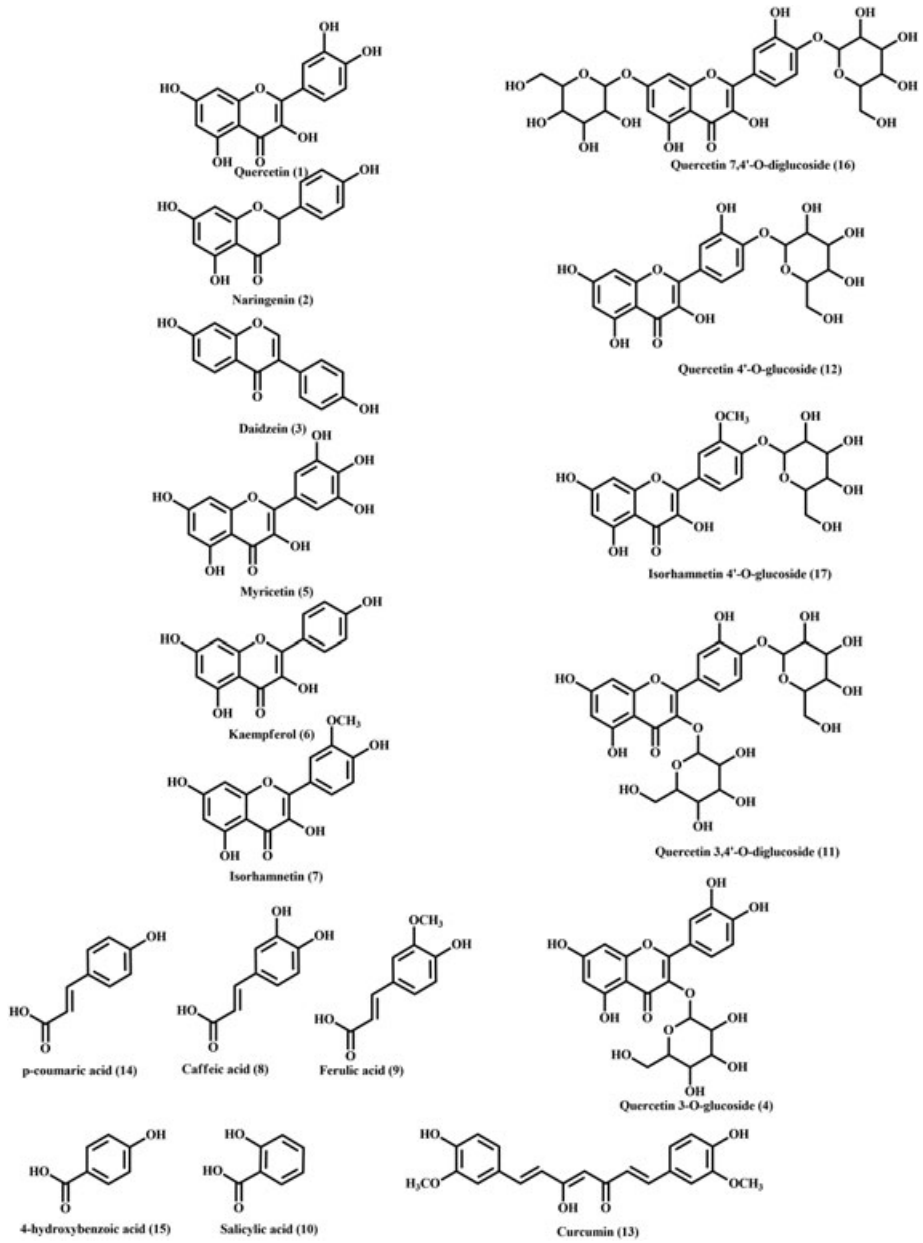

Figure 1. Chemical structures of quercetin and other investigated polyphenols.

molecules (Xie *et al*., 2001a,b; Zhu *et al*., 2003; Zhu and Xu, 2003; O'Connor *et al*., 2007; Huang *et al*., 2012). For example, Xie *et al*. (2001a, b) reported the extraction of related flavonoids from *Gingko* leaves using a Qu MIP. This Qu MIP was also applied to screen *Caragana jubata* (Pall.) Poir., a traditional Tibetan medicine, for related structures that may exhibit inhibitory activity towards the epidermal growth factor receptor (EGFR), a mediator hyperactivated in numerous tumours. The subsequent identification in *C. jubata* of two novel anti-cancer lead compounds demonstrates the feasibility of using a MIP to mine for structural analogues that may be useful for drug development (Zhu and Xu, 2003). A bulk-polymerisation Qu MIP that involved grafting of specific oligoethylene glycol (OEG) units on imprinted cavities, resulted in an increased flexibility of the imprinted cavities which improved the capture of template analogues (Pardo *et al*., 2014b). The present work focusses on the selectivity study of two Qu MIPs prepared by fluorocarbon suspension polymerisation: a control MIP, synthesised using acrylamide as functional monomer and ethylene glycol dimethacrylate (EDMA) as cross-linker; and a MA-OEG-MIP, synthesised in the same conditions but in the presence of a comonomer bearing a short OEG side chain [oligo(ethylene glycol) methyl ether methacrylate, MA-OEG]. The OEG segments of low degree of polymerisation aim to convey recognition of **1** but with enough deformability of the imprinted cavities for recognition of its analogues. The MIPs were evaluated as sorbents for solid phase extraction (SPE) and HPLC in order to confirm the presence of imprinted cavities and to evaluate their selectivity, respectively. Particles size and shape were characterised by scanning electron microscopy (SEM). The potential of this hybrid MIP strategy was tested by application to white onion (*Allium cepa* L.) extracts that are reported to contain high levels of **1** and its derivatives (Park and Lee, 1996).

Experimental

Chemicals

Quercetin (**1**), naringenin (**2**), daidzein (**3**), quercetin 3-*O*-glucoside (**4**), myricetin (**5**), kaempferol (**6**), isorhamnetin (**7**), caffeic acid (**8**), ferulic acid (**9**), salicylic acid (**10**) (Fig. 1), β-carotene, oligo(ethylene glycol) methyl ether methacrylate [Mn = 300 g/mol, mean polymerisation degree =4–5 (MA-OEG (4–5)], methacrylic anhydride, Brij 35 [polyoxyethylene(23)lauryl ether], 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl methacrylate and EDMA were purchased from Sigma Aldrich (St Louis, MO, USA). Quercetin 3,4′-*O*-diglucoside (**11**) and quercetin 4′-*O*-glucoside (**12**) (Fig. 1) were obtained from Extrasynthese (Genay, France). Perfluoro-1,3-dimethylcyclohexane (PMC) was from Fluorochem (Hadfield, UK). Curcumin (**13**), *p*-coumaric acid (**14**) and 4-hydroxybenzoic acid (**15**) (Fig. 1) were obtained from Carl Roth (Karlsruhe, Germany). Acrylamide was purchased from Merck KGaA (Darmstadt, Germany). Azobisisobutyronitrile (AIBN) was obtained from Acros Organics (Geel, Belgium). Tetrahydrofuran (THF), acetone, acetonitrile and methanol (HPLC grade) and phosphoric acid (85%, analytical grade) were obtained from Chem-Lab (Zedelgem, Belgium). Acetic acid (100%) and chloroform were from VWR (Leuven, Belgium). Before use, EDMA, MA-OEG and THF were purified by passing through a column filled with basic alumina. Acrylamide was purified by recrystallisation from chloroform and AIBN by recrystallisation from methanol.

Preparation of molecularly imprinted polymers (MIPs)

The suspension polymerisation apparatus and the preparation of a perfluorinated surfactant have been previously described (Pardo *et al.*, 2014a). Briefly, methacryloyl-Brij 35 was prepared by reacting methacrylic anhydride with Brij 35. The perfluorinated graft copolymer surfactant was subsequently synthesised by copolymerisation of methacryloyl-Brij 35 with 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl methacrylate.

Synthesis of control MIP. A round bottom flask containing PMC (232.5 mL; 1062.4 mmol) with THF (5 mL) and the perfluorinated surfactant (290 mg) was immersed in an oil bath at 60 °C. The mixture was stirred until the surfactant has dissolved, sparged with nitrogen for 5 min, and then transferred through a capillary under nitrogen in a three-neck jacketed reaction vessel placed under nitrogen. Quercetin (**1**) (483.6 mg; 1.6 mmol) and acrylamide (796.1 mg; 11.2 mmol) were introduced into a round bottom flask and dissolved in THF (10.4 mL). After stirring for 30 min to let **1** and acrylamide form hydrogen bonds, EDMA (10466.0 mg; 52.8 mmol) and AIBN (656.8 mg; 4 mmol) were then added. After shaking for homogeneity, the solution was sparged with nitrogen for 5 min, transferred through a capillary under nitrogen to the reaction vessel containing the PMC and surfactant, stirred at 2000 rpm and purged with nitrogen for 5 min. Polymerisation was thermally initiated by stirring the solution at 600 rpm with the polymerisation vessel immersed in an oil bath at 60 °C. After 2 h, the polymer beads were separated by filtration on cellulose filter (Büchner funnel), washed with acetone and dried at 30 °C under vacuum. The filtrate was kept for redistillation and reuse of the PMC. Large polymer aggregates were removed by sieving the particles through a 45 μm sieve using a vibrating sieve AS200 digit (Retsch, Haan, Germany). To remove the template molecule, polymer particles were extracted with a mixture of methanol and acetic acid (9:1, *v/v*) in a Soxhlet extractor for 24 h (150 cycles). The extracted polymer particles were dried at 60 °C under vacuum during 16 h and stored in a desiccator for further use.

The corresponding blank polymer [Control NIP (non-imprinted polymer)], formulated in a similar fashion, without introduction of the template molecule, was produced, treated and studied in the same way as the MIP.

Synthesis of MA-OEG-MIP. The synthesis of MA-OEG-MIP was carried out in the same way as that of the control MIP, except that: (i) the amount of acrylamide was reduced (568.6 mg; 8 mmol) and (ii) the comonomer MA-OEG (0.9 mL; 3.2 mmol) was also added together with EDMA and AIBN (Fig. 2).

The corresponding blank polymer (MA-OEG-NIP), formulated in a similar fashion, without introduction of the template molecule, was produced, treated and studied in the same way as the MIP.

Characterisation of the polymer particles size and shape

The polymer particles <45 μm were characterised in terms of size and shape by SEM (SUB8020 SEM, Hitachi Ltd, Tokyo, Japan). Size bars are automatically calculated by the instrument at the selected magnification setting.

SPE procedure for the assessment of MIPs and NIPs performances

A polyethylene frit (10 μm porosity) was placed on the bottom of an empty 8.0-mL polypropylene SPE cartridge (Grace Discovery Sciences, Deerfield, IL, USA) which was connected to an extraction mannifold (Waters, Milford, MA, USA). Dry polymer particles (MIP or NIP, 800 mg) were packed under vacuum pressure into the cartridge with methanol (8 mL). Polymer particles were then overlaid by a second frit which was gently tamped down with a plastic cone. To make sure that **1** used for MIP synthesis has been fully extracted from the polymer, the cartridge was washed with a mixture of methanol/acetic acid (9:1, *v/v*), until **1** could not be detected in the filtrate by UPLC analysis (washing volumes: 150 to 1000 mL), and then conditioned with THF (10 mL). The SPE cartridge was loaded with an aliquot (1 mL) of **1** solution (5.3 nmol/mL in THF), washed with THF (1.5 mL) and the captured **1** eluted with methanol (4.5 mL). The effluent liquids from the loading, washing and elution steps were separately collected, evaporated to dryness under nitrogen using a VLM evaporator (Bielefeld, Germany) and each redissolved in methanol (1 mL). These SPE eluents were analysed on a ACQUITY UPLC H-Class system (Waters) driven by the Empower 3 software and equipped with a quaternary solvent pump, an autosampler (set at 20 °C), a column oven and a photodiode array detector set at 365 nm. The ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.7 μm, Waters) was maintained at 25 °C. The mobile phase consisted of an isocratic mixture of acetonitrile and 0.1% (*v/v*) phosphoric acid aqueous solution (40:60, *v/v*). The flow rate was set at 0.37 mL/min and 4.2 μL of sample were injected. The procedure was performed in duplicate. The rate of retention was calculated using the equation:

$$\text{Rate of retention} = (Q_0 - Q)/Q_0$$

in which Q_0 is the amount of **1** initially loaded on the SPE cartridge and Q is the total amount of **1** recovered from the loading and washing steps. The imprinting factor (IF), defined as:

$$\text{IF} = \text{Rate of MIP retention}/\text{Rate of NIP retention}$$

corresponds to the ratio specific interactions vs. non-specific interactions.

HPLC selectivity study

The HPLC analyses were performed using a D-7000 HPLC System (Hitachi Ltd) with a L-7100 pump, a L-7400 UV detector and a L-7200 autosampler. Polymer particles (< 45 μm) were packed into stainless steel HPLC columns (150 mm × 4.6 mm i.d.) with methanol at 220 bar using an air-driven fluid pump (MAXIMATOR GSF60-NPT, Zorge, Germany). The prepared columns were equilibrated in the HPLC system with the mobile phase (THF) at a rate of 2 mL/min until a stable baseline was obtained. For analyses, the flow rate was 0.5 mL/min and the columns were maintained at room temperature. Samples were dissolved in THF and 20 μL, corresponding to 6.6 nmol analyte per gram of polymer, were injected. The void marker, β-carotene (6.6 nmol per gram of polymer), was also dissolved in THF and 20 μL were injected. The detection of **1**, quercetin glucosides, **5**, **6** and **8** was carried out at 365 nm. The detection of β-carotene, **7**, **2**, **3**, and **13** was carried out at 465 nm, 254 nm, 280 nm, 250 nm and 410 nm, respectively. The detection of **14**, **9**, **15** and **10** was carried out at 327 nm, 324 nm, 253 nm and 297 nm, respectively. Each analyte was injected independently and in

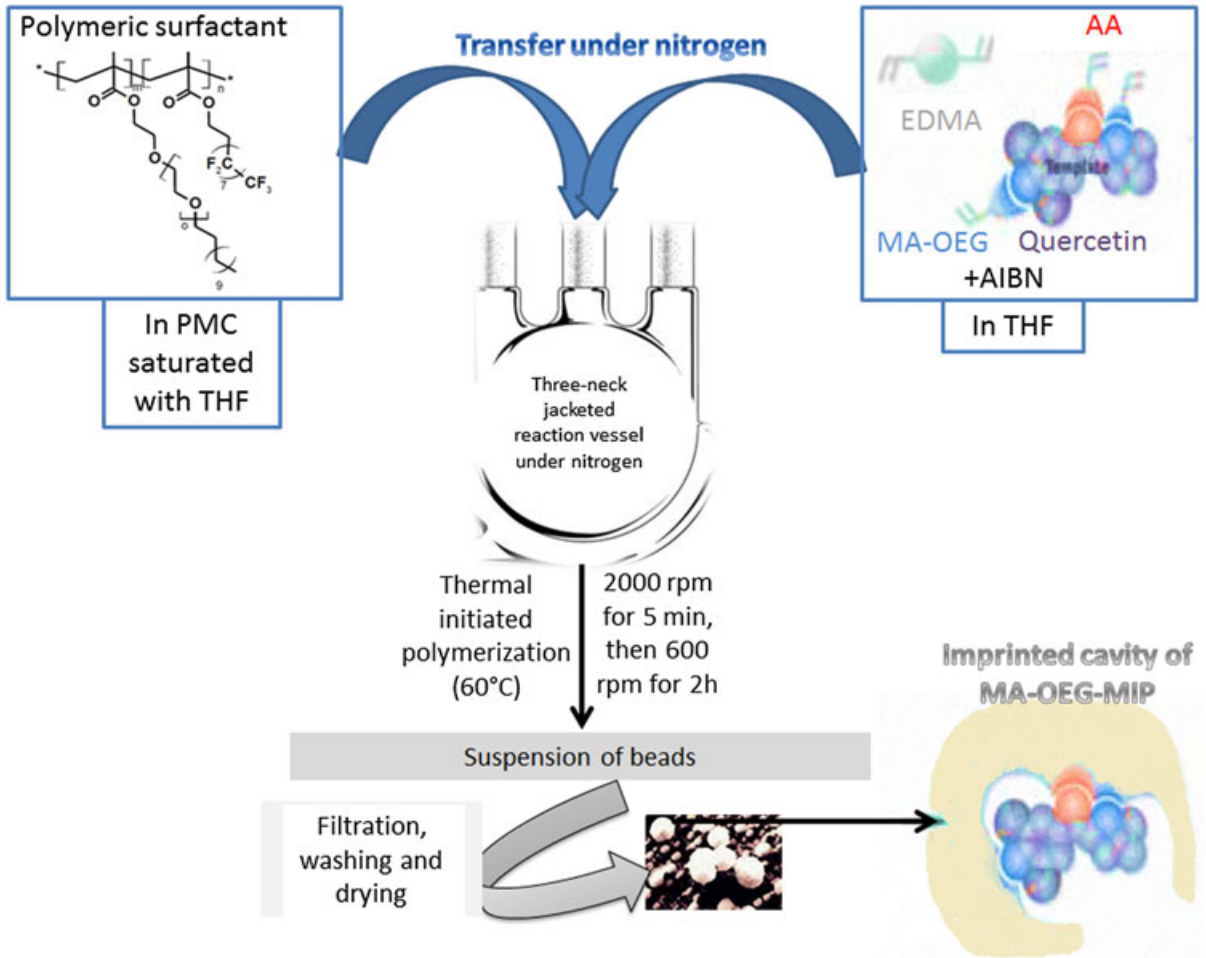

Figure 2. Schematic graph of the preparation of MA-OEG-MIP. [Colour figure can be viewed at wileyonlinelibrary.com]

triplicate. All measurements were performed on both the imprinted polymers (MIPs) and the corresponding blank polymers (NIPs). The capacity factors (k') of the prepared polymers were calculated using the equation:

$$k' = (t_R - t_0)/t_0$$

where t_R is the retention time of an analyte, and t_0, the time to elute the void marker. The imprinting effect (IE) was defined by the equation:

$$IE = k'_{MIP}/k'_{NIP}$$

where k'_{MIP} is the capacity factor of the MIP, and k'_{NIP} is that of the corresponding NIP.

MIP-SPE procedure applied to an *Allium cepa* L. bulb extract

Six onion bulbs (white onions; origin, Peru) were bought in a Delhaize supermarket (Belgium), hand peeled, cut into pieces, weighed (707 g) and left to dry at room temperature for a week. The dry material (76 g) was ground to a powder. This bulb powder (25 g) was macerated in the dark for 24 h with methanol/water (50:50; *v*/*v*) (300 mL). The extracting solvent was then drained off, and the powder was rinsed with 700 mL methanol/water. The combined extracts were concentrated using a rotary evaporator (30 °C) to a syrup, sonicated (20 min; 130 Watt) with methanol (30 mL) and centrifuged. The supernatant, collected by aspiration, was transferred equally into five glass vessels, evaporated under nitrogen, frozen and lyophilised using a Heto Power Dry LL1500 Freeze dryer (Thermo Scientific, Zellik, Belgium). Each recovered residue was sonicated (20 min; 130 W) with THF (25 mL) and centrifuged. Each supernatant was collected by aspiration and evaporated under nitrogen to dryness. An aliquot (92.4 mg) of the dried residue was dissolved in THF (100.0 mL, 0.924 mg/mL). The MIP-SPE and NIP-SPE cartridges (800 mg of polymer) were conditioned with THF (10 mL) and loaded with an aliquot (1 mL) of the sample solution. The loaded columns were washed with THF (1.5 mL) and the captured components eluted with methanol (4.5 mL). The collected liquids were evaporated to dryness and reconstituted in methanol (1 mL). The initial sample and collected fractions were analysed using UPLC. The ACQUITY UPLC BEH Shield RP18 column (2.1 mm × 100 mm, 1.7 μm, Waters) was maintained at 40 °C. The mobile phase was a gradient between A (0.2% formic acid in water) and B (methanol): 0–0.74 min: 100:0, 4.21–5.95 min: 85:15, 7.68–12.89 min: 45–55, 13.24–14.64 min: 0:100 (washing step), 14.97–16.72 min: 100:0 (conditioning step). The flow-rate was maintained at 0.4 mL/min and 4.2 μL of sample were injected.

Results and discussion

Structural characterisation of polymers

Based on previous data (Mayes and Mosbach, 1996; Ansell and Mosbach, 1997; Pardo *et al.*, 2014a), the fluorocarbon suspension polymerisation method was chosen as an efficient, faster and less laborious alternative to the bulk polymerisation method. A high yield of regularly shaped polymer beads of about 0.9 to 20 μm in diameter was obtained by fluorocarbon suspension polymerisation (Fig. 3), indicating promising perspectives for their use in chromatography. From the SEM micrographs acquired at magnification 25000 (Fig. 3), the control polymers appear more

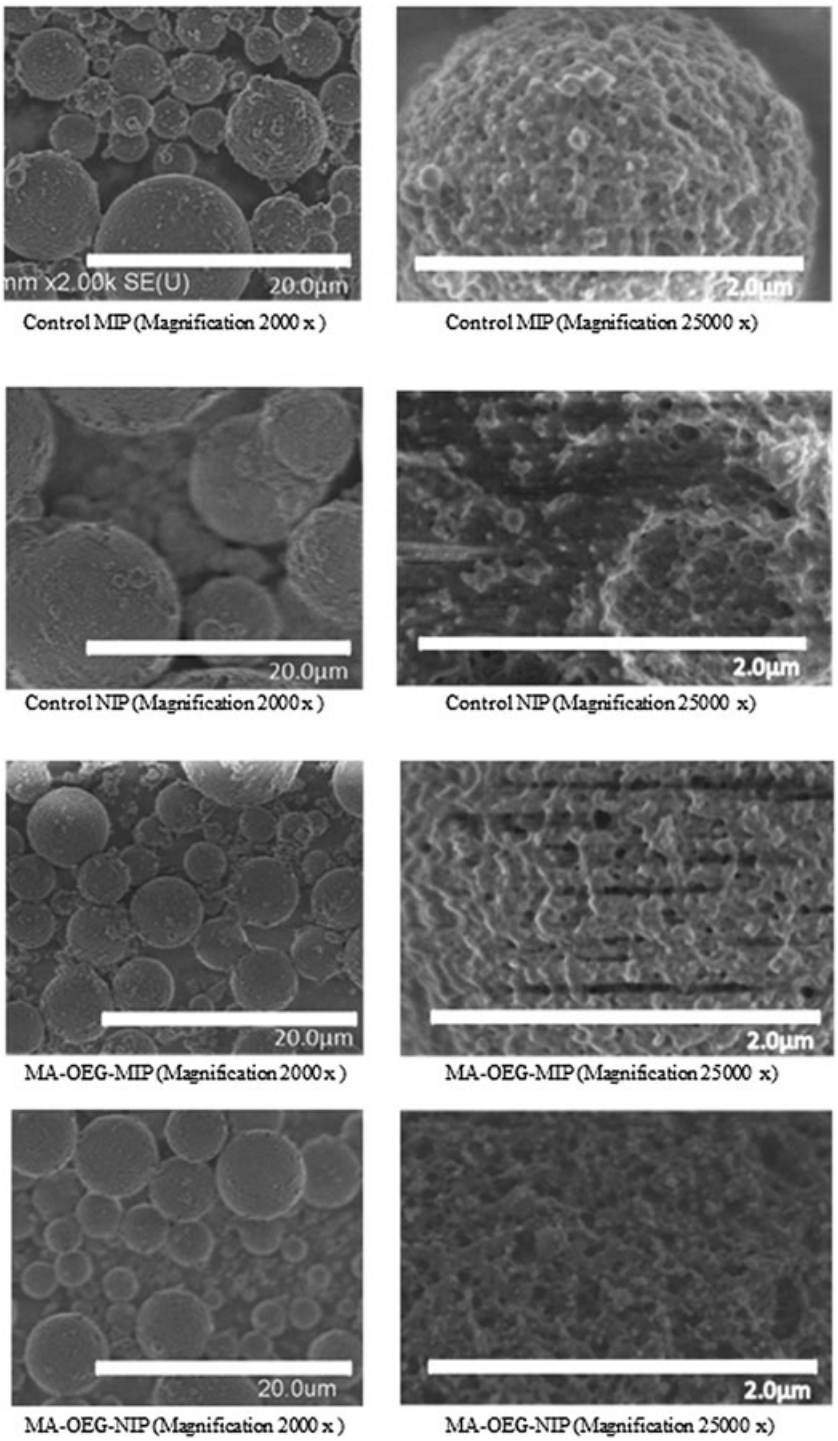

Figure 3. Micrographs of particles obtained by scanning electron microscopy (SEM).

compact than the MA-OEG-polymers. The solvation of OEG chains during synthesis probably brought a swelling to the MA-OEG-polymers that made them more relaxed than the control polymers.

SPE assessment of MIPs and NIPs

Differences between MIPs and NIPs. The performances of Control MIP and MA-OEG-MIP relative to their corresponding NIPs (Control NIP and MA-OEG-NIP) were assessed by SPE. In such rebinding experiments, the polarity of the washing solvent is crucial to disrupt non-specific interactions with the polymeric network and to favour specific hydrogen bonds within imprinted cavities.

Confirming the presence in MIPs of imprinted sites structurally and chemically complementary to **1** (Table 1), virtually no **1** was retained by the NIP-SPE whereas, about 50% of **1** was retained on the corresponding MIP cartridges (Tables 1 and 2; $p = 0.0004$). This is reflected by the observed high IF values. These data indicate that part of **1** applied to the column is not retained; indeed, THF, a weak acceptor of hydrogen bonds, is able to disrupt both van der Waals interactions and a fraction of hydrogen bonds (Sahu and

Table 1. Performances of MIP-SPE sorbents to recognise the quercetin template (precision computed from eight determinations in duplicate: 10.67%)

Polymer	Test 1			Test 2		
	Rate of retention (%)	Imprinting factor (IF)	Elution recovery (%)	Rate of retention (%)	Imprinting factor (IF)	Elution recovery (%)[b]
Control polymers						
Control MIP	49.2	4.69	30.5	56.7	3.22	28.1
Control NIP	10.5		0	17.6		8.1
MA-OEG polymers						
MA-OEG-MIP	40.3	$>>$ (n.c.)[a]	24.7	43.8	5.2	30.3
MA-OEG-NIP	0		0	8.4		0.5

[a]Not calculated.
[b]Percentage of the total of quercetin that was initially added to the column.

Table 2. Precision of the SPE procedure (rates of retention); two-way analysis of variance (ANOVA) with repetition; two fixed factors: (1) presence of MA-OEG monomer during polymerisation (control polymers/MA-OEG polymers); (2) presence of template during polymerisation (MIP/NIP) (duplicate analyses)

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (*F*)	Probability[a]	RSD% (within-day)	RSD% (total)
Presence of MA-OEG monomer during polymerisation	215.28	1	215.28	9.09	0.0394*	17.2	17.2
Presence of template during polymerisation	2945.28	1	2945.28	124.36	0.0004*		
Interaction	0.55	1	0.55	0.02	0.8861[NS]		
Residual (error)	94.74	4	23.68				
Total	3255.85	7					

[a]NS, non-significant;
* significant.
Note: %RSD, relative standard deviation.

Lee, 2007; Pardo *et al.*, 2014b). Quercetin (**1**) was eluted from both MIPs by methanol with a recovery of about 30% of the applied **1** (Table 1). Although methanol is a protic and polar solvent able to break hydrogen bonds between **1** and MIP cavities (Caro *et al.*, 2003; Dong *et al.*, 2005; Claude *et al.*, 2008; Lopez *et al.*, 2011), it is also a solvent favouring hydrophobic interactions between **1** and polymeric networks (Pardo *et al.*, 2014a). This methanol property may explain that a part of **1** (typically 19%) was not eluted from the MIP cavities.

Influence of the OEG chains. The introduction of OEG chains in the polymer structure was observed to improve the IF value of **1** (Tables 1). Indeed, these OEG segments increase the hydrophilicity of the polymer network reducing the proportion of non-specific hydrophobic interactions that retain **1** non-selectively on the network.

HPLC evaluation of MIPs selectivity

HPLC analyses on MIP and NIP columns allowed determining the *k'* and IE values for a series of natural polyphenols (Table 3). The control MIP exhibits the strongest affinity towards the template molecule and its close analogues, **2**, **4**, **5**, **6**, **7**, **11** and **12**. More distant analogues of **1**, i.e. **3**, **8**, **9**, **10**, **14** and **15** interacted less with the imprinted cavities and were probably mainly retained within the polymer network by non-specific interactions. By contrast the OEG units in the OEG methyl ether-grafted polymer (MA-OEG-MIP) elicited a slight drop-off in the IE values for the distant analogues to approach the IE value of **1**. This effect was not manifested for the non-analogue polyphenol (**13**) that has a significantly higher molecular dimension (19 Å) than **1** (12 Å) (Pardo *et al.*, 2014a) and is therefore not complementary to the imprinted cavities of the Qu MIPs.

Table 3 also indicates that the introduction of OEG segments tends to decrease the capacity factors of the MIP. As such segments result in increased hydrophilicity, the analytes are less retained by non-specific hydrophobic interactions, crossing faster through the polymer network.

These observations allowed to conclude that the OEG (4–5) ponytails: (i) afford a good compromise between flexibility and rigidity, improving the capture of analogues more distant from the template; and (ii) limit the non-specific hydrophobic interactions, which confirms data obtained with **1** (Table 1).

Application to a complex matrix

The performances of the synthesised MIPs were evaluated on a complex matrix, a white onion methanol/water extract. The raw extract was subjected to the SPE protocol, both on the control polymers (MIP and NIP) and the MA-OEG polymers (MIP and NIP). According to published data (Park and Lee, 1996; Lindahl

Table 3. HPLC recognition performance of MIPs (vs. blank polymers) towards quercetin and other polyphenols (RSD < 5%[a]; $n = 3$)

Compound	Control polymers			MA-OEG-polymers		
	k'_{MIP}	k'_{NIP}	IE	k'_{MIP}	k'_{NIP}	IE
Template molecule						
Quercetin (**1**)	0.40	0.12	3.41	0.19	0.11	1.79
Close analogues of quercetin						
Quercetin 3-*O*-glucoside (**4**)	0.35	0.08	4.60	0.19	0.07	2.55
Quercetin 4′-*O*-glucoside (**17**)	0.40	0.07	6.05	0.20	0.07	2.95
Quercetin 3,4′-*O*-diglucoside (**11**)	0.58	0.05	12.15	0.40	0.04	9.04
Myricetin (**5**)	0.29	0.08	3.85	0.13	0.08	1.67
Kaempferol (**6**)	0,39	0,16	2.46	0.22	0.13	1.71
Isorhamnetin (**7**)	0.26	0.13	2.00	0.14	0.10	1.35
Naringenin (**2**)	0.33	0.22	1.54	0.24	0.18	1.32
Distant analogues of quercetin						
Daidzein (**3**)	0.45	0.32	1.38	0.41	0.26	1.59
Caffeic acid (**8**)	0.51	0.38	1.35	0.48	0.29	1.67
p-Coumaric acid (**14**)	0.52	0.45	1.14	0.51	0.32	1.57
Ferulic acid (**9**)	0.42	0.37	1.14	0.35	0.27	1.28
4-Hydroxybenzoic acid (**15**)	0.56	0.54	1.1	0.64	0.39	1.65
Salicylic acid (**10**)	0.39	0.47	0.8	0.37	0.29	1.29
Non-analogue of quercetin						
Curcumin (**13**)	0.13	0.08	1.67	0.06	0.08	0.81

[a]Overall precision of the experiment computed as in the previous table by ANOVA.

et al., 2010; Pérez-Gregorio *et al.*, 2010), and comparison with the retention times and UV spectra of reference compounds, free **1** and five flavonol glycosides were identified in the white onion extract as quercetin 7,4′-O-diglucoside (**16**), **11**, **4**, **12** and isorhamnetin 4′-O-glucoside (**17**) (Fig. 1). For each identified polyphenol, the elution recoveries were calculated as the ratio of peak areas obtained before and after MIP extraction.

The Control MIP selectively recognises **1** from its related compounds present in the extract (Fig. 4). An excellent MIP/NIP selectivity was obtained for **1** with elution recoveries of 3.5 and 41.6% for the NIP and MIP, respectively. On the contrary, the flavonol glycosides were not specifically retained by the MIP since they present low and similar elution recoveries on both MIP and NIP cartridges. These compounds were quantitatively eliminated

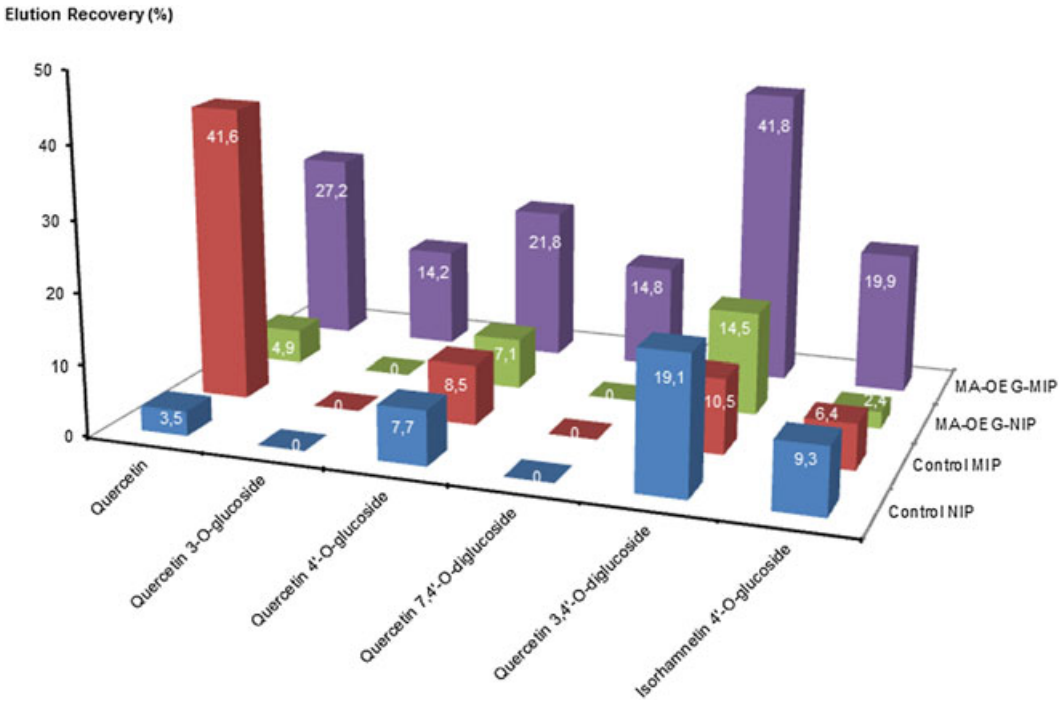

Figure 4. Recoveries measured on solid phase extraction (SPE) columns packed with Control NIP, Control MIP, MA-OEG-NIP and MA-OEG-MIP. Sample solution: *Allium cepa* raw methanol/water extract (924 μg/800 mg polymer). [Colour figure can be viewed at wileyonlinelibrary.com]

during the SPE loading and washing steps. However, the HPLC analysis of the flavonol glycosides, injected independently on the Control MIP column, indicates some extent of recognition by the polymer (Table 3). These contradictory results can tentatively be explained by a phenomenon of competition for the imprinted cavities between **1** and its substituted analogues when they are simultaneously applied to the SPE cartridge.

However, SPE columns prepared with the newly synthesised MA-OEG-MIP correctly recognise the flavonol glycosides (Fig. 4). Indeed, an excellent MIP/NIP selectivity was obtained for **1** and its analogues.

These results are in line with our SPE and HPLC data and confirm that the OEG (4–5) ponytails allow improving the capture of template analogues by a good compromise between flexibility and rigidity.

Acknowledgements

Antonelle Pardo gratefully acknowledges the fellowship granted to her by UMONS. Authors thank Paul Suvarov of the Electricity Department at the University of Mons for his support during the packing of the HPLC columns, Mr Hissam Sekfali and Dr Philippe N. Okusa at the University of Mons for their support in the analysis of *Allium cepa* L. and Mrs Laetitia Verdy for her technical support. CIRMAP is grateful to the "Belgian Federal Government Office Policy of Science (SSTC)" for general support in the frame of the PAI-7/05, the European Commission and the Wallonia Region (FEDER Programme) and OPTI^{2}MAT programme of excellence. The laboratory of Pharmaceutical Analysis expresses its gratitude to FNRS (Fonds National de la Recherche) for support via the FRSM Grant 3.4614.11. Part of this work is supported by the F.R.S.-FNRS and the Science Policy Office of the Belgian Federal Government (IAP 7/05).

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