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Galina A. Kryvshenko [a] , Pavel Yu. Apel [b] , Sergei S. Abramchuk [c] & Mikhail K. Beklemishev [a]
[a] Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia
[b] Flerov Laboratory of Nuclear Reactions, Joint Institute for Nuclear Research, Dubna, Russia
[c] Nesmeyanov Institute of Organoelement Compounds (INEOS), Russian Academy of Sciences, Moscow, Russia

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Galina A. Kryvshenko,[1] Pavel Yu. Apel,[2] Sergei S. Abramchuk,[3] and Mikhail K. Beklemishev[1]

[1]*Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia*
[2]*Flerov Laboratory of Nuclear Reactions, Joint Institute for Nuclear Research, Dubna, Russia*
[3]*Nesmeyanov Institute of Organoelement Compounds (INEOS), Russian Academy of Sciences, Moscow, Russia*

A polymer membrane imprinted with quercetin has been synthesized in the presence of nickel(II) acetate as a metal ion mediator. A mixture of methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), tetrahydrofuran (THF) – methanol (3:1 v/v) as a solvent and Darocur® 1173 (2-hydroxy-2-methyl-1-phenyl-propan-1-one) as a photoinitiator was polymerized on a track-etched membrane (pores 0.4 μm) used as a support. The polymer formed regular layers on both sides of the membrane and filled the pores. The highest initial flux of the template of $1.8 \cdot 10^{-7}$ mol · cm^{-2} · h^{-1} was observed for its diffusion from THF/acetate buffer (20:80, v/v) into THF. Membranes polymerized with nickel(II) were both more permeable and more selective. The maximum selectivity factors, calculated by using percent permeation, were 4.5 (quercetin/rutin) and 4.9 (quercetin/naringenin).

Keywords molecular imprinting; photopolymerization; preconcentration by membrane diffusion; quercetin; track-etched membranes

INTRODUCTION

Molecular imprinting has become a powerful tool of separation in analytical chemistry (1,2). Molecularly imprinted polymers (MIPs) are in most cases obtained by bulk polymerization, which, however, requires grinding and sieving of the obtained polymer block. In many cases it is advantageous to use molecularly imprinted membranes (MIMs) (3) that enable quasi-one-step separations based on selective membrane diffusion; MIMs are easy to obtain, as polymerization of thin films is fast and reproducible. However, the potential of MIMs as a means of selective separation of analytes is not yet fully disclosed.

A known method to create high-quality recognition sites is metal ion-mediated imprinting. It is based on the formation of a pre-polymerization complex by a template, a monomer, and a transition metal ion. Imprinting of transition metal complexes has been known since the 1950s, (4) while successful imprinting of an organic compound based on this approach was only reported in 1985 (5). Recently, metal ion-mediated imprinting has been applied to obtaining bulk-polymerized MIPs for bilirubin, (6) peptides, (7,8) proteins, (9) and small ions (formate, acetate) (10). However, this principle was rarely applied for obtaining imprinted membranes (11). The advantages of a metal ion-mediated approach are the possibility of obtaining imprints for the templates with internal hydrogen bonds (12) (such as quercetin containing two molecules of hydrogen-bound water that may block the hydroxy groups, preventing them from interacting with the functional monomer) and the possibility of using more polar polymerization solvents that are known to be more effective porogens (13,14), as the coordination bonds allow the metal ion to stabilize the pre-polymerization complex more efficiently than hydrogen and Van der Waals bonds. Various transition metals were used in obtaining MIPs and MIMs, among which Ni^{2+} was not extensively studied. When obtaining the polymers imprinted with a peptide, nickel(II) provided better rebinding properties than Mg^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, and Cu^{2+} (7). In this study we use nickel(II) acetate as a source of transition metal ions, unlike the prototype papers, where Zn^{2+} (11) and Cu^{2+} (15) were used.

To refine the techniques of obtaining imprinted membranes, it is desirable to use some extensively studied compounds as model templates. One of the most common bioflavonoids, quercetin was widely used for the preparation of selective MIPs in a number of papers, (13,17–21) including some research using the principle of metal ion-mediated imprinting (11,15,22). This compound, which may be regarded as a "standard template," was used in this study.

A weak spot of many MIPs is slow mass transfer (2). Diffusion may occur on the timescale of hours; for instance,

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Address correspondence to M. K. Beklemishev, Lomonosov Moscow State University, Department of Chemistry, 119991 GSP-1, Moscow, Russia. Tel.: +7(495) 939-5468. E-mail: mkb@analyt.chem.msu.ru

one of the most selective quercetin-imprinted membranes requires 30 h to only transport 2.3% of the template (11), which attracts interest to systems with more rapid diffusion. To accelerate template removal and its sorption-desorption in the analytical cycle, MIPs polymerized in bulk are usually ground to obtain particles sized at 10–40 μm. Similarly, imprinted membranes should be thin, preferably a few micrometers, which requires using a support. Track-etched membranes (TM) obtained by irradiation of polymer films by heavy ions with subsequent etching in hot alkali solutions (23) are promising as supports due to their durability, regularity of structure, and chemical stability. There are few examples of using TMs as supports for polymerization (16).

A rapid method of obtaining polymer materials is photopolymerization that is especially convenient for synthesizing planar items such as membranes (24). Photoinitiation is convenient in obtaining MIPs with reactive templates, such as bioflavonoids, compounds that may be damaged due to oxidation by the components of a redox initiation system. Polymerization within the pores of track-etched membranes was performed (25), including the variant with photoinitiation (26), but it was not used for obtaining MIMs. 2,2′-Azobis(isobutyronitrile) (AIBN) is sometimes used to initiate photopolymerization (11,15), though this thermal initiator is not very efficient in the photochemical processes.

When the MIM has been obtained, separation conditions should be selected. What is critical is the composition of the rebinding solvent used in the membrane process. As polymerization normally occurs in nonaqueous media, the conformation of the binding sites is expected to be better preserved in the same polarity medium, which will be optimum for the recognition of the template (27). Accordingly, in most studies on sorption on MIPs and diffusion or filtration through MIMs organic solvents are employed, such as chloroform (28), methanol (29), or their mixtures (30,31). However, real-world samples may contain water, which generates interest in the studies of aqueous–organic media in diffusion. Membrane separation or sorption of bioflavonoids is feasible with the use of an aqueous–organic (5% of ethanol) (32) or even an entirely aqueous medium; the latter has been successfully used for separation of luteolin (33) and naringin (34,35).

The present study has been aimed at simplifying the polymerization protocol and increasing the membrane permeability without considerable sacrifice to the selectivity reached with the membrane (11) and sorbent (15) imprinted with quercetin. These goals have been achieved by use of a photoinitiator (Darocur 1173) more efficient than AIBN (11,15) and an appropriate support (a polyethylene terephthalate TM) for the polymerization mixture applied as a thin layer. In contrast to paper (11), we have shown the feasibility of separation of the bioflavonoid from an aqueous-organic rather than from a purely organic feed solution.

EXPERIMENTAL

Reagents and Materials

Methacrylic acid (MAA) (functional monomer) and ethylene glycol dimethacrylate (EGDMA) (cross-linker) were purchased from Sigma. Darocur® 1173 (BASF) was used as photoinitiator. Bioflavonoids quercetin, naringenin, and rutin (Sigma–Aldrich, Germany) were dissolved in 96.5% ethanol (Bryntsalov-A, Russia) to give 0.01 M solutions, that were stored at 4°C. Methanol, THF, acetic acid, sodium acetate, nickel diacetate, and sodium EDTA, reagent or analytical grade, were from Chimmed, Russia. All chemicals were used as received. Millipore water (specific resistance 18 MΩ · cm) was used to prepare aqueous solutions. Acetate buffer solutions of pH 4.0–6.5 were 0.5 M in total acetate. To obtain a pH between 7.2–9.5 without changing the nature of the buffer appropriate amounts of NaOH were added to 0.5 M sodium acetate solution. All pH values reported refer to initial pH of the aqueous buffer. For $pH > 7$, the pH values were measured with added bioflavonoid.

Polyethylene terephthalate (PETP) track-etched membranes (pore size 0.4 μm, pore density $9 \cdot 10^7$ cm^{-2}, thickness 12 μm) were obtained in the Joint Institute for Nuclear Research (Dubna, Russia) by irradiation with a beam of Krypton ions, subsequent sensitization with ultraviolet radiation and further etching with a warm NaOH solution, as described earlier (36). The pores were cylindrical in shape and oriented within an angle ranging from −30° to +30° to normal. Diffusion was studied using 2.5-cm membrane disks.

Instrumentation

Polymerization was initiated by a 15 W mercury UV lamp (Diac, Russia). UV-vis spectra were recorded with a spectrophotometer SF-102 (Akvilon, Russia). A polypropylene diffusion cell (Fig. 1) analogous to that used in ref (36). was employed: the membrane was fixed between two chambers filled with the feed (2.0 mL) and receiving solution (1.4 mL). The membrane exposed area was 3.8 cm^2.

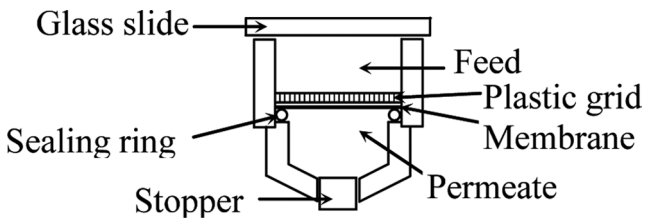

FIG. 1. Schematic diagram of the cell for diffusion experiments.

Preparation of Imprinted and Non-Imprinted Membranes

The preparation of the most permeable membrane is described (membranes with different amounts of the reactants were obtained similarly). Precise weights of quercetin as template (10 mg; not added for the non-imprinted membrane) and nickel diacetate tetrahydrate (21 mg) were dissolved in the mixture of methanol (250 μL) and THF (500 μL), which required 5 min of shaking. Then 26 mg of MAA was added and the mixture was left for 15 min to allow for the formation of the pre-polymerization complex. After the addition of 70 mg of Darocur 1173 and 1000 mg of EGDMA and 5 min standing, a 200-μL portion of the mixture was dosed onto a track-etched membrane disk placed on a plastic grid. The excess of the mixture filtered under its weight through the membrane and the grid. After 30 sec, the membrane was pressed between two glass slides ($2.5 \times 6.0\,cm^2$) fastened with two clamps at the ends and placed for 1 hr under the UV lamp at a distance of 7 cm. The glass was partially transparent at >300 nm (absorbance of the slide at the mercury lamp band of 313 nm was about 1.0). The time required for polymerization was 1 hr, which is shorter than in other protocols (4 (11) or 12 hours (15)). (Note: In most procedures for obtaining MIMs, polymerization is carried out after deaeration or under inert gas atmosphere or under vacuum (11,28,32). Carrying out polymerization between the glass slides similar to (11,30) we found that the removal of oxygen from the mixture was unnecessary, most likely due to the slow rate of its income during polymerization. When the photopolymerization of the same mixture was attempted on an open surface, no polymer formed within a few hours).

The fact of polymerization could be confirmed by observing water filtration (an aqueous solution easily passes through a non-modified 0.4-μm-pore membrane under its own gravity, while the polymerized membranes did not let water filter even under applied external pressure).

After polymerization, the membrane was washed to remove the template, photoinitiator and metal salt by shaking with 2-mL portions of methanol: glacial acetic acid (9:1, v/v) during 15 min in a 25-mL beaker. The absorbance in the range of 250–400 nm decreased to the background absorption of the solvent after 3 washings. Then the membrane was washed by 2 mL of a 0.001 M solution of Na_2EDTA for 10 min to remove nickel and 3–5 min by ethanol to remove water. On the whole, washing operations required 45–60 min (we may note that frequently template is removed during hours or days (15,37)). When the obtained membranes are stored on air, the modification layer tends to peel off; thus, the membranes were stored in acetate buffer (pH 5.4, 0.5 M) at ambient temperature. To evaluate the reproducibility of preparation, the weight of the membranes synthesized on different days was controlled and diffusion studies performed.

Transmission Electron Microscopy (TEM)

The membrane was pressed in an incision of an epoxy resin piece and microtomed at room temperature in transverse direction with a diamond knife (Diatome Ltd, Switzerland) to obtain 100- or 300-nm-thick sections, that were picked up on copper TEM grids and imaged with a LEO 912 AB OMEGA microscope (Carl Zeiss, Germany).

Diffusion Experiments

Precise weights of bioflavonoids (quercetin, naringenin, and rutin) were dissolved in ethanol to give 0.01 M solutions that were stored at 4°C. Diffusion of individual bioflavonoids was studied separately. The membrane was assembled in the diffusion cell (Fig. 1), the lower chamber (volume 1.4 mL) was filled with a syringe by the receiving solution (normally, THF), and the chamber was stoppered from the bottom. The feed solution (2.0 mL), containing $5 \cdot 10^{-4}$ M of a bioflavonoid in an appropriate solvent (buffer solution, THF, or their mixture), was pipetted into the top chamber, thus starting the diffusion. The phases were not stirred. All experiments were carried out at room temperature (23–25°C). In a fixed time (usually 15 min), the receiving solution was withdrawn from the cell with a syringe and diluted with an equal volume of ethanol. Absorbance of the obtained solution at a wavelength corresponding to maximum absorption of the bioflavonoid was measured in a 1-cm cell; in some cases, the absorption spectrum in the range of 220–400 nm was recorded. Concentration of the bioflavonoid in the permeate was calculated knowing the molar absorption coefficient ε. Below are given the ε values ($L \cdot mol \cdot cm^{-1}$) for freshly prepared solutions of bioflavonoids in THF:ethanol (1:1, v/v): quercetin: 374 nm, $2.2 \cdot 10^4$, and 258 nm, $2.0 \cdot 10^4$; naringenin: 290 nm, $1.7 \cdot 10^4$; rutin: 358 nm, $1.1 \cdot 10^4$. Quercetin was quantified at 374 nm. Non-oxidized quercetin has two absorption bands of close intensity, located around 260 and 370 nm (38) (the precise position of the maxima depends on the nature of the solvent). The observed spectrum of the permeate also had maxima at 257 and 374 nm, which did not differ from the spectrum of quercetin in the same solvent, implying that no oxidation of quercetin occurred during diffusion.

Data Treatment

Theoretically, diffusion proceeds until the concentrations in the feed and permeate equalize. We are presenting the diffusion data in the form of permeation P calculated as the percentage amount of the bioflavonoid in the permeate relative to its theoretical maximum:

$$P = c_{perm}/c_{eq} \times 100\%,$$

where c_{perm} stands for the concentration of the bioflavonoid in the receiving solution after a given diffusion time (usually 15 min) and c_{eq} is its theoretical (equilibrium) concentration in the permeate, calculated as $c_{feed} \cdot V_{feed}/(V_{feed} + V_{perm})$, where c_{feed} is the initial concentration of the bioflavonoid in the feed and V_{feed} and V_{perm} are the volumes of the feed and permeate. For most experiments, $c_{feed} = 5.0 \cdot 10^{-4}$ M, and hence $c_{eq} = 2.9 \cdot 10^{-4}$ M. The $2s$ confidence intervals and error bars on graphs have been calculated for 3 diffusion runs. Repeatability of permeation values for one membrane was within 8–21 relative %.

Selectivity of the separation of bioflavonoids A and B was characterized by the separation factor SF, determined as the ratio of the permeation values: $SF = P_A/P_B$. The imprinting factor that characterized the formation of the binding sites was calculated as the ratio of the permeations of the template across the imprinted and non-imprinted membrane: $IF = P_{im}/P_{non\text{-}im}$.

RESULTS AND DISCUSSION

Structure and Morphology of Polymerized Membranes

To conduct polymerization, a portion of the polymerization mixture was pipetted on a TM disk and its excess freely leaked through the 0.4-μm pores of the membrane. A part of the mixture retained on the support was photopolymerized, as a result of which the polymer films were formed on both sides of the support. TEM data (Fig. 2) show the two regular non-porous polymer layers, one on the top side of the support, 2.8 ± 0.2 μm thick, and the other one on the opposite side, 0.6 ± 0.1 μm thick (calculated by measuring the samples of one membrane in 5 different TEM images).

To verify the reproducibility of polymerization, the weight of membranes was measured. A blank TM disk weighs 6.8 mg, while the weight of a membrane after polymerization in optimum conditions is 11.2 ± 0.4 mg (averaged for 4 imprinted membranes obtained on different days), which implies that layer thickness is reasonably reproducible.

The pore structure of the TM is seriously damaged when a thin microtome is obtained (Fig. 2a). To keep this structure, an angular section was made (Fig. 2b), from which it can be seen that all pores are filled with the polymer. Visible voids are located along the direction of cutting; they could result from material deformation while microtoming. However, a more probable reason for the formation of the voids is shrinking of the mixture during polymerization. The actual degree of shrinking is 6–10% by volume, as it was evaluated by polymerizing the mixture in a capillary (0.5-mm wide, 5 cm long). The formation of the voids between the polymeric core and pore wall implies that the penetrant may pass across the TM within the solvent present in the voids.

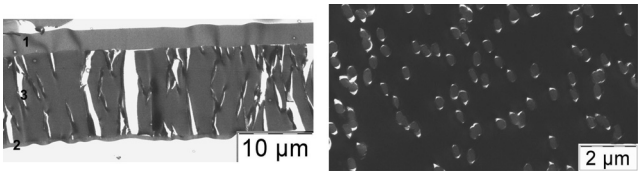

FIG. 2. TEM images of a quercetin-imprinted membrane: *a* – section along the pores of the TM support (perpendicular to TM surface), 100-nm-thick microtome. Upper and lower layers (1,2) are the polymer films formed during the imprinting procedure. Pore structure of the TM (3) has been destroyed in the course of microtoming; *b* – angular section (37° to TM surface), 300-nm-thick microtome. TM pores are filled with a substance of a different density. The direction of movement of the diamond knife coincides with the major axes of most ellipses.

Diffusion Rate

We have studied the diffusion of quercetin as template and two other common bioflavonoids, rutin and naringenin, as a function of time (Fig. 3). The kinetic curves level off approaching equilibrium state by about 1.5 hours that is close to the time ($\sim 10^2$ min) required for the quercetin-imprinted sorbents obtained in the presence of Cu^{2+} (15). As seen from Table 1, of six recent molecularly imprinted membranes for which diffusion rates can be estimated, four provide fluxes of $10^{-8} - 10^{-7}$ mol · cm^{-2} · h^{-1}, which is less than for the suggested membrane, while only two allow for the greater initial fluxes. This demonstrates that the suggested membrane belongs to the most permeable MIMs.

The selectivity studies have shown (Table 2) that separation factors reach their maxima at 15 min and do not significantly change thereafter. Assuming that selectivity has priority over recovery and with the time-saving purposes, this diffusion time was used for most of the further experiments.

As evidenced by the TEM data (Fig. 2a), the thickness of polymer films on the top and bottom sides of the

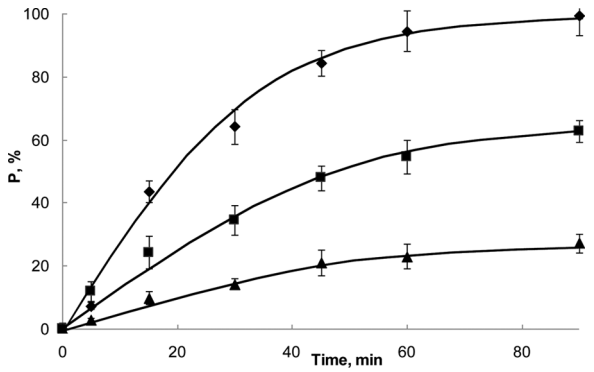

FIG. 3. Kinetic curves of diffusion of bioflavonoids (◆ – quercetin, ■ – naringenin, ▲ – rutin) across the quercetin-imprinted membrane. Polymerization conditions: MAA:quercetin = 10:1; nickel:quercetin = 3:1 (mol/mol), THF:methanol = 2:1 (v/v). Diffusion conditions: feed, THF:0.5 M acetate buffer, pH 5.4 (20:80, v/v), $5 \cdot 10^{-4}$ M quercetin; receiving phase, THF; $t = 15$ min.

TABLE 1
Maximum diffusion fluxes across molecularly imprinted membranes

Membrane composition/support	Penetrant, its initial concentration, M	Maximum flux observed, $mol \cdot cm^{-2} \cdot h^{-1}$	Reference
4-Vinylpyridine–EGDMA (stand alone)	Quercetin, $2 \cdot 10^{-3}$	$1.6 \cdot 10^{-8}$	(11)
MAA–EGDMA/cellulose acetate	6-Benzyladenine, $3 \cdot 10^{-3}$	$6.1 \cdot 10^{-8}$	(39)
MAA–(hydroxymethyl)propane trimethacrylate/poly(tetrafluoroethylene)	Trimethoprim, $5 \cdot 10^{-4}$	$\sim 1 \cdot 10^{-7}$	(40)
MAA–1,1,1-(trishydroxymethyl)propane trimethacrylate, composite membrane	Carboxybenzoyl-*L*-tyrosine, $2 \cdot 10^{-3}$	$1.2 \cdot 10^{-7}$	(33)
MAA–EGDMA/PETP	Quercetin, $5 \cdot 10^{-4}$	$1.8 \cdot 10^{-7}$	This study
Acrylic acid–EGDMA/polyamide-6	*trans*-2-Phenyl-1-cyclohexanol, $7 \cdot 10^{-3}$	$7 \cdot 10^{-7}$	(41)
Aminopropyl triethoxysilane–tetraethoxysilane	Luteolin, $2 \cdot 10^{-3}$	$4.2 \cdot 10^{-6}$	(42)

**Notes*. References to the publications of 1990-s can be found in paper (3); the fluxes do not exceed $2 \cdot 10^{-8}$ mol $\cdot$ cm$^{-2} \cdot$ h^{-1}. Many other MIMs have only been characterized in sorption or filtration experiments (15,32,34,35,43), in which cases the diffusion flux data are unavailable.

membrane is different, which is a direct consequence of the polymerization protocol used. However, no signs of anisotropic permeability have been found, as the diffusion rates are equal independently of the membrane position (Table 3).

Quercetin is transported more rapidly than other bioflavonoids, which is understandable: adsorption of the template on selective binding sites raises its quasi-equilibrium concentration within the membrane and hence improves both its outflow from and inflow to the membrane. This implies that the template will diffuse faster than the non-imprinted species.

However, it is supposed that the polymerized layers should cause some diffusional resistance, but the transport of the template across the imprinted membrane is even more rapid than across a bare support (Table 3). To make this possible, the template diffusion rate within the imprinted polymer or within the pores must be faster than that within the pores of the original track-etched membrane. In the literature, much higher permeability compared with the blank membranes was observed for a number of free-standing MIMs (3). Transport rates were paradoxically increased in the TMs whose pores were filled with anionic polyelectrolyte Nafion (44). A model describing the phenomenon suggested the existence of a narrow solvent layer between the pore wall and polymer core, acting as a ''swift lane'' for the diffusing low-molecular species (45). In case the voids described in section 3.1 actually existed in the working membrane, they could serve as ''lanes,'' providing rapid transport.

The diffusion rates commented in this paragraph are dependent on the polymerization procedure used to modify the membrane and by diffusion conditions, which are discussed below.

TABLE 2
Characteristics of the quercetin-imprinted membrane measured at various diffusion times

Diffusion time, min	Imprinting factor	Separation factor: Quercetin/naringenin	Separation factor: Quercetin/rutin
5	6.3	0.6	2.6
15	9.3	1.8	4.4
30	9.0	1.7	4.1
45	8.8	1.8	4.0
60	8.1	1.7	4.1
90	7.8	1.5	3.6

**Note*. For conditions, see Fig. 3.

TABLE 3
Permeation of quercetin (% of equilibrium concentration) across the imprinted and non-imprinted membranes (diffusion time 15 min, for other conditions see Fig. 3)

Membrane side facing the feed	Non-imprinted membrane	Quercetin-imprinted membrane
Top side*	6 ± 2	44 ± 4
Bottom side*	5 ± 2	43 ± 3

**According to the position of the membrane during polymerization.

Notes. Permeation across initial track-etched TM under these conditions was $29 \pm 3\%$. Data have been obtained form 5 diffusion runs, and uncertainties calculated for the significance level of 0.95.

TABLE 4
Effect of nickel salt introduced into the polymerization mixture on the characteristics of the obtained quercetin-imprinted membrane

Membrane, polymerized	Permeation, % of equilibrium concentration			Imprinting factor	Separation factor	
	Quercetin	Rutin	Naringenin		Quercetin/rutin	Quercetin/naringenin
without Ni(II)	14	9	22	2.0	1.7	0.7
with Ni(II)	44	10	24	9.2	4.4	1.8

*Note. For conditions, see Fig. 3.

Polymerization Conditions: Effect of Nickel Salt

The properties of MIMs polymerized with and without nickel(II) (Table 4) show that the metal ion affects both the permeability and the selectivity: the membrane obtained with nickel is more permeable to the template and other bioflavonoids, and the separation factors are also higher for this membrane. The imprinting factor for this membrane is also 4.6 times higher. The obtained results imply that nickel ion probably coordinates the template and monomer even in the presence of such a polar polymerization solvent as THF, promoting the formation of efficient binding sites.

The effect of the amount of nickel in the polymerization mixture was also studied (Fig. 4). Maximum permeability and imprinting factor are observed for metal: template ratio of 3:1 in the polymerization mixture. Metal-to-template ratio does not significantly affect the selectivity, though maximum separation factors are reached for the same 3:1 value (for MAA: quercetin ratio of 10:1, they are: SF(quercetin/naringenin) = 1.8 and SF(quercetin/rutin) = 4.4). Higher and lower Ni(II) content lead to membranes with lower selectivity (data not shown). Existence of an optimum metal-to-template ratio can be understood supposing that low amount of metal in the mixture does not bind all the template (leading to difficulties in obtaining imprints, especially in a polar solvent), while an insufficient amount of the template lowers the number of recognition sites.

It is important to stress that the membranes polymerized with nickel do not contain the metal at the time the diffusion is conducted. The necessity to remove the metal for the membrane to be efficient was shown in a number of papers (6,9). Accordingly, we washed out nickel(II) by 0.001 M EDTA after polymerization. Absence of trace metal in the membrane has been confirmed by determination of nickel (46) after hydrolyzing the membrane with hot alkali. So, most experiments were conducted with metal-free membranes.

The question that has to be answered is what will happen if the metal is introduced in the feed solution. If during polymerization the metal ion has formed a complex with both template and monomer, as shown in Fig. 5, adding Ni(II) into the feed should promote the formation of the same type structure, or in other words, facilitate template binding. As known from literature, for some membranes polymerized with a transition metal salt, the metal introduced into the feed really accelerates the diffusion of the template, (11) but in some other systems it does not (15). Our experiments show (Table 5) that addition of nickel into the feed does not improve the transport of quercetin

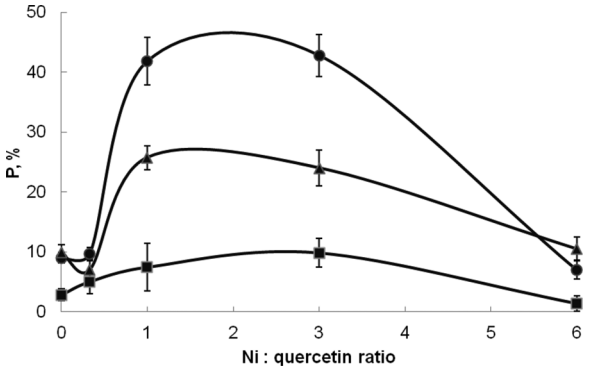

FIG. 4. Permeation of the bioflavonoids (% of equilibrium concentration) across the quercetin-imprinted membranes polymerized with various metal-to-template ratios (mol/mol). ● – quercetin, ▼ – naringenin, ■ – rutin. For the conditions, see Fig. 3.

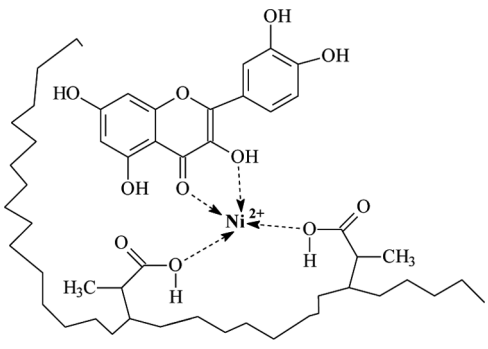

FIG. 5. A possible scheme of interaction of template, monomer and metal ion in metal ion-mediated imprinting. Suggested in paper (15) for copper(II) complexes.

TABLE 5
Permeation of quercetin (% of equilibrium concentration) across the membranes depending on the presence of nickel(II) in the feed solution

Composition of the feed	Membrane	
	Imprinted	Non-imprinted
Quercetin only	44 ± 4	6 ± 2
Quercetin and $Ni(CH_3COO)_2$*	39 ± 3	7 ± 1
Quercetin–nickel complex**	23 ± 2	3 ± 1

Notes. Feed: THF:0.5 M acetate buffer, pH 5.4 (20:80, v/v); initial concentrations: $5 \cdot 10^{-4}$ M quercetin, $1.5 \cdot 10^{-3}$ M $Ni(CH_3COO)_2$; receiving phase: THF; $t = 15$ min; $n = 3$, $P = 0.95$. For polymerization conditions, see Fig. 3.

*Mixed directly before diffusion.

**Quercetin and $Ni(CH_3COO)_2$ solutions were mixed 15 min before diffusion.

(formation of Ni(II)–quercetin complex even retards diffusion). As it has been found in separate experiments, the metal does not cause sorption of quercetin on the MIM either (data not shown). This implies that the presence of metal in this system does not promote template binding. We have to conclude that either the actual structure of the pre-polymerization complex differs from the scheme depicted in Fig. 5, or the mechanism of the facilitated diffusion of the templates across such membranes is quite different from the generally accepted one. In any case, the reasons for the efficiency of metal ion-mediated imprinting require further investigation.

Effect of Monomer-to-template Ratio

The monomer-to-template ratio is supposed to affect the number and quality of the binding sites (47). If polymerization is conducted in the presence of a transition metal, the selected functional monomer must form a pre-polymerization complex with both the template and metal ion. Such monomers as vinyl imidazol, (48) 4-vinylpyridine (11) and MAA (7) were successfully used in metal-mediated imprinting. MAA was also used in imprinting of quercetin (20).

We obtained a number of membranes with varied MAA: quercetin ratio and studied their selectivity towards quercetin (Table 6). As seen from Table 6, lack of monomer is detrimental for the permeability, which agrees with the idea that the monomer-template complex is not very stable, and thus an excess of monomer is needed. On the other hand, its large excess also leads to a gradual decrease in permeation, probably due to the decrease in the number of binding sites.

Monomer-to-template ratio has a significant effect on the membrane selectivity and imprinting factors (Table 6). Maximum imprinting factor (IF = 22) is reached at the MAA:quercetin ratio of 50, which coincides with the maximum selectivity quercetin/naringenin (SF = 4.9). At the same time, maximum selectivity quercetin/rutin is observed at a different monomer-to-template ratio (10:1), which, in its turn, coincides with the maximum permeability to quercetin. This implies that there is no general optimum, and the ratio of monomer to template should be selected depending on the current task. In choosing other parameters of this study, we aimed at maximum selectivity in quercetin/rutin pair.

Maximum separation factors reached with the obtained membranes (4.9 for the pair quercetin/naringenin and 4.5 for quercetin/rutin, Table 6) are close to those obtained by Wang's group in membrane (11) and sorbent (15) studies (about 7 for quercetin/naringenin (11,15); 5.4 (11) and 9.5 (15) for quercetin/rutin).

The diffusion results did not significantly change for repeated polymerizations; for example, the P values for the membranes most permeable to quercetin (with MAA: quercetin = 10:1) obtained on 3 different days were equal to (%): 42 ± 4, 37 ± 5 and 45 ± 6, which gives an average value of 41 ± 4. That allowed us to obtain consistent data in case of membrane rupture or damage.

TABLE 6
Effect of monomer-to-template ratio on the characteristics of the quercetin-imprinted membranes

MAA:quercetin ratio (mol/mol)	Permeation, % of equilibrium concentration			Imprinting factor	Separation factor	
	Quercetin	Rutin	Naringenin		Quercetin/rutin	Quercetin/naringenin
5	19 ± 2	10 ± 1	27 ± 4	6	1.8	0.7
10	$\mathbf{42 \pm 4}$	9 ± 1	24 ± 5	8	**4.5**	1.8
20	28 ± 3	11 ± 1	10 ± 2	14	2.5	3.2
50	31 ± 1	13 ± 1	7 ± 1	**22**	2.3	**4.9**
75	27 ± 4	12 ± 2	6 ± 1	9	2.1	**4.9**
100	22 ± 2	11 ± 2	7 ± 1	3	1.9	3.2

Notes. For other polymerization and diffusion conditions, see Fig. 3. Average values and uncertainties were calculated from 3 parallel diffusion runs ($P = 0.95$).

TABLE 7
Effect of THF: methanol ratio in the polymerization mixture on the characteristics of the quercetin-imprinted membranes

THF:methanol ratio (v/v)		Permeation, % of equilibrium concentration			Imprinting factor	Separation factor	
		Quercetin	Rutin	Naringenin		Quercetin/rutin	Quercetin/naringenin
3:1	imprinted	39	11	24	7	3.5	1.6
	non-imprinted	5.6	4.8	6.8			
2:1	imprinted	43	8.6	21	9	5.0	2.0
	non-imprinted	4.6	0.8	13			
1:3	imprinted	17	7.1	14	4	2.4	1.2
	non-imprinted	4.2	5.2	5.8			

*Note. For other conditions, see Fig. 3.

Composition of the Porogenic Solvent

The nature of the porogenic solvent affects the energy of non-covalent bonding between the template and monomer, which influences the selectivity of the obtained binding sites (1,2,49). At the same time, the solvent must provide homogeneity of the polymerization mixture. For obtaining the imprints of quercetin by polymerization of acrylamide-EGDMA mixture, THF was found (19) to be the best solvent (the others were 1,4-dioxane, acetone, and acetonitrile). THF with additions of methanol was successfully used in the synthesis of a quercetin-imprinted membrane using 4-vinylpyridine as monomer (11). THF-methanol mixtures were also used in this study.

As can be seen from Table 7, a higher fraction of methanol (THF:methanol, 1:3) results in slower and less selective transport of the template, probably due to the destabilizing effect of the polar medium on the monomer–template interactions (27). Smaller contents of methanol lead to more permeable and selective membranes. Amounts of methanol lower than 25% vol. could not be used because of low solubility of quercetin in THF. In other experiments, 2:1 ratio of these solvents was used as providing maximum separation factors.

TABLE 8
Effect of the composition of the feed on the permeability of the quercetin-imprinted membrane

Content of buffer solution in THF – buffer mixture (% vol.)	Imprinting factor	Separation factor	
		Quercetin/rutin	Quercetin/naringenin
0	4	1.1	0.5
20	3	0.6	0.2
40	4	1.5	0.9
60	7	2.1	1.1
80	9	4.4	1.8
100	7	2.9	0.8

*Note. For other conditions, see Fig. 3.

Diffusion Conditions: Composition of the Feed

Generally, the synthesis of MIPs is conducted in non-aqueous solvents, and the obtained sorbents and membranes are supposed to be efficient in organic media. Polar environments weaken polar interactions of the template with the binding site and promote less specific hydrophobic interactions, thus impeding molecular recognition (27). The addition of an organic solvent to water is favorable, as it reduces non-specific bonding (50). Sometimes membrane diffusion from aqueous-organic and aqueous media is selective (39,42). Aqueous-containing solutions may be especially useful in the determination of bioflavonoids, which stimulated us to study an aqueous buffer as a possible component of the feed. Another constituent of the feed is an organic solvent; it is more favorable to use the same solvent as employed on the polymerization stage, as it would increase the chance for the binding sites to accept the right conformation.

As the feed, we used the mixtures of an acetate buffer solution with THF employed as polymerization solvent; neat THF was used as receiving solution. Maximum permeability for the template is observed for 20% of THF and 80% of acetate buffer. The same composition of the feed is optimum in terms of imprinting factor and selectivity (Table 8).

The pH value of the buffer has no dramatic effect on the permeability. The permeation is maximum around pH 9.2 and 5.4 (data not shown). Maximum selectivity of diffusion is reached at pH 5.4, for which reason this pH value was used in all other experiments. These results confirm the regularity known for MAA-based MIPs, for which optimum pH of template binding frequently lies between 5 and 6 (27). This pH value is close to pK_a of carboxylic groups and thus provides comparable quantities of –COOH and $–COO^-$ moieties, which may favor the formation of

H-bonds with the analytes through oxygen atoms and carboxylates, respectively.

CONCLUSION

In this study we have obtained a highly permeable and selective quercetin-imprinted membrane by a rapid and simple polymerization procedure. The technique used for obtaining the membrane provides certain advantages over the previous papers: we have shortened the polymerization time and avoided the stage of degassing the polymerization mixture; minimization of the amount of the mixture polymerized on a track-etched support results in the formation of thin selective layers with easily accessible binding sites, which ensures fast removal of the template and its rapid transport in diffusion experiments. The suggested polymerization protocol improves the permeability compared to paper (11), not seriously altering the separation efficiency. The results of this study also demonstrate that diffusion of quercetin across an imprinted membrane may be successfully performed from an aqueous–organic into an organic solution.

Further improvement of the selectivity of metal-ion mediated MIMs is a challenging task. Varying the nature of the metal ion may become a powerful tool of adjusting the binding sites selectivity. A correct choice of the combination of a transition metal, template, and monomer may help in the development of highly permeable and at the same time selective MIMs; however, the problem lies in the fact that the mechanism of action of metal-ion mediators and causes of their favorable effect are not completely understood. More research should be focused on this issue.

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