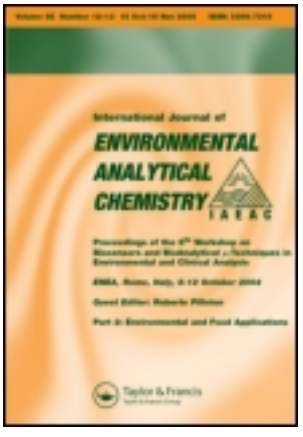

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Miniaturised flow-through PLM coupled with spectrophotometric detection for copper(II) speciation in fungicides†

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Miniaturised flow-through PLM coupled with spectrophotometric detection for copper(II) speciation in fungicides†

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Construction of a novel miniaturised Permeation Liquid Membrane (mPLM) set-up is described. A hydrophobic Celgard 2500 membrane with copper(II) carrier separates the source and receiving solutions flowing in the channels on two sides of the membrane. The set-up consists of two half-cells with channel width of 2 mm and depth variable between 100 and 500 μm made of poly(dimethylsiloxane) (PDMS) using a moulding technology. In contrast to most previous constructions, the new mPLM operates in full flow-through mode, in both source and receiving solutions. This allows for on-line and real-time coupling with the detection system, which significantly reduces the time of analysis. In the present set-up a UV/Vis spectrophotometric detection was applied for Cu(II) transport. For this purpose a composition of the receiving solution was optimised to allow for both free metal ion buffering, which is necessary for maintaining the driving force of the transport, and spectrophtometric detection. The applied UV/Vis detection method is linear in the range 2×10^{-6} M – 4×10^{-5} M ($R^2 = 0.996$) with a detection limit of 10^{-6} M in the volume of ca 350 μL (including the receiving channels, cuvette and tubing). The relative standard deviation does not exceed 5%. The transport capabilities of the mPLM set-up are demonstrated using four commercial copper-based fungicides: Nordox 75 WG, Miedzian 50 WP, Curzate 49.5 WP and Mag 50 WP.

Keywords: PLM; SLM; microfluidic; fungicides; azacrown ether; PDMS

1. Introduction

In view of the ever-increasing understanding of the pathways of both vital and toxic species internalisation by microorganisms, the need for reliable, general-purpose speciation tools constantly increases. With the advent of Biotic Ligand Model (BLM) [1] and Free Ion Activity Model (FIAM) [2], it has become obvious that the knowledge of a total concentration of the given species is not a sufficient estimate of their bioavailabilty. However, from the point of view of the latter, the elucidation of the exact structure of a plethora of species present in the sample is neither crucial nor feasible. For example, in the case of toxic metal ions, much more important is the knowledge of concentration of liposoluble species, free ions, and labile complexes. The latter might contribute to the flux through biological membranes, provided that their association/dissociation rate is comparable with the rate of diffusion transport in the vicinity of the membrane.

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Permeation Liquid Membranes (PLM), by some authors referred to as Supported Liquid Membranes (SLM), were applied for analytical purposes for the first time in 1986 [3]. The first successful reports on the use of this technique for trace metal ion transport date back to 1990s [4–6]. The speciation capabilities of PLM have been recently demonstrated both theoretically [7–10] and experimentally [11–16]. A PLM set-up consists of two aqueous phases (source and receiving) separated by a hydrophobic membrane. The latter contains an ion carrier, whose major role is to facilitate the transport of selected species through the membrane. Effectiveness of ion transport through the PLM is usually assayed in the receiving solution with some spectroscopic or electrochemical detection system, depending on the nature and concentration of the accumulating species. Thanks to the presence of the hydrophobic barrier separating two aqueous solutions, the PLM bares resemblance to biological membranes of living organisms and can be used as a model for the latter. Depending on the mode of employment, PLM can operate as equilibrium [16] or dynamic sensor [17]. In the latter PLM can serve as 'bioanalogical sensor'. Buffle *et al*. [8,14,17] pointed to several similarities between bioaccumulation in micro-organisms and carrier-assisted transport through the PLM membranes. In both processes, a diffusion-limited supply of metal ions is coupled to their consumption at a reactive interface (membrane).

One of the most often used speciation-oriented PLM systems is the one developed in Buffle's group, based on a mixture of alkylated azacrown ether and fatty acid as ionophore [24]. Such PLM has proven suitable for transporting Pb(II) [14,25], Cd(II) [26], Ni(II) [27] and Cu(II) [13,14], the latter being the most often studied. The detailed spectroscopic [28] and interfacial studies [29,30] revealed that both components of the mixed ionophore take part in the transport of Cu(II) ions.

The need for miniaturisation of PLM has been recognised already in 1990s in the context of coupling the PLM with chromatographic techniques of detection [6,18,19]. Few microfluidic devices have been reported in the literature; they are mostly fabricated by micromachining [9,10] or based on a three-layer flow [20,21]. Unfortunately, miniaturisation of the transport module of PLM is not always accompanied by miniaturisation of the respective detection modules. In most previous set-ups, the receiving phase volume was maintained small at the cost of keeping it stagnant. Reports on successful on-line coupling of the detection system with the miniaturised PLM are, however, scarce [9,22,23]. This imposes serious limits on the construction of complete miniaturised and portable PLM set-ups for real field studies.

In this paper we report development of a new miniaturised PLM device (mPLM) made of poly(dimethylsiloxane) (PDMS). A soft moulding technology allowed for easy control of geometry of the flow-through channels, and high reproducibility thanks to the master replication technique. In contrast to most of the previously described miniaturised PLMs, the device operates in conjunction with the on-line flow-through spectrophotometric detection system. The new device was applied to study speciation of copper(II) ion in four commercial fungicides containing copper as an active component.

2. Experimental

2.1 *Chemicals*

The following commercial fungicides were tested: Nordox 75 WG (Nordox), Miedzian 50 WP (Organika Azot S.A.), Curzate 49.5 WP (Organika Azot S.A.) and Mag 50 WP (Agtrol International). Their composition and the content of Cu given by the producers

are collected in Table 1. Millipore water (18 MΩ cm^{-1} resistivity) was used for all preparations. $Cu(NO_3)_2 \cdot 5H_2O$, morpholinoethanosulphonic acid, (MES), 4-(pirydyl-2-azo)r-esorcinol, (PAR), cetyltrimethylammonium bromide, (CTAB), sodium dodecylsulphate (SDS), Brij-35 and Triton X-100 (all of puriss p.a. purity), were purchased from Fluka. Acetic acid and sodium acetate (puriss) were obtained from POCh (Poland). Kryptofix 22DD (for synthesis) was obtained from Merck. Toluene (puriss p.a.), Hexylbenzene (97%) and lauric acid (puriss p.a.) were obtained from Fluka. Poly(dimethylsiloxane), (Dow Corning Sylgard 184) was used for PDMS half-cells preparation.

The Celgard 2500 membrane support (25 μm thick, 45% porosity) was a generous gift from Celgard, LLC (USA). The organic phase of the membrane consisted of a mixture of Kryptofix 22DD and lauric acid in hexylbenzene. Concentration of both carriers (0.1 M) was the same as in original studies by Buffle *et al*. [24], the only difference being elimination of toluene from the organic phase, due to its incompatibility with PDMS.

All the glassware used for preparation of Cu(II) solutions was rinsed with diluted nitric acid (puriss p.a.), Fluka and Millipore water.

2.2 *Assembly of mPLM cell*

The mPLM set-up consists of two identical half-cells made of PDMS from the same master (symmetric pattern). The stamps were placed in a mould into which a degassed mixture of PDMS monomer with crosslinking agent was poured. The filled mould was cured for 4 h at 70°C, as described in [31]. Upon peeling the crosslinked PDMS block off from the mould, the half-cell with patterned channel was obtained. After freezing the block in liquid nitrogen the holes for connections with the tubing (Ismatec $\phi = 0.25$ mm and 1.02 mm) were drilled.

The stamps for half-cells with channels of 100 μm and higher were machined in poly(methyl methacrylate), PMMA or a commercial stamp rubber material. The total length of channels in all the cells was 31 cm, giving a contact area of 6.2 cm^2, comparable to that of a typical macroscopic PLM cell. More shallow channels (50 μm) were produced using a capillary film technique [32]. A dry Celgard 2500 membrane was placed between the two half-cells, which were then clamped with a PMMA holder and tightened using 4 screws (Figure 1). Prior to each measurement the membrane was soaked with the respective carrier solution in organic solvent through the connecting tubing. The tubing was subsequently washed with distilled water to remove the excess of organic solution, which was not retained by the membrane. The complete flow-cell was connected with a beaker containing the source phase and a flow-through spectroscopic cuvette (Hellma QS,

Table 1. Composition of the commercial fungicides (according to the producers' data).

	MAG 50 WP	CURZATE 49.5 WP	MIEDZIAN 50 WP	NORDOX 75 WG
Copper content (%)	50	45	50	75
Copper source	$Cu(OH)_2$ 76.7%	$3Cu(OH)_2 \cdot CuCl_2$ 78%	$3Cu(OH)_2 \cdot CuCl_2$ 87%	Cu_2O 86.2%
Other components	n.a.	Cymoxanil – 4.5%	Surface active components	Dispersants – 13.8%

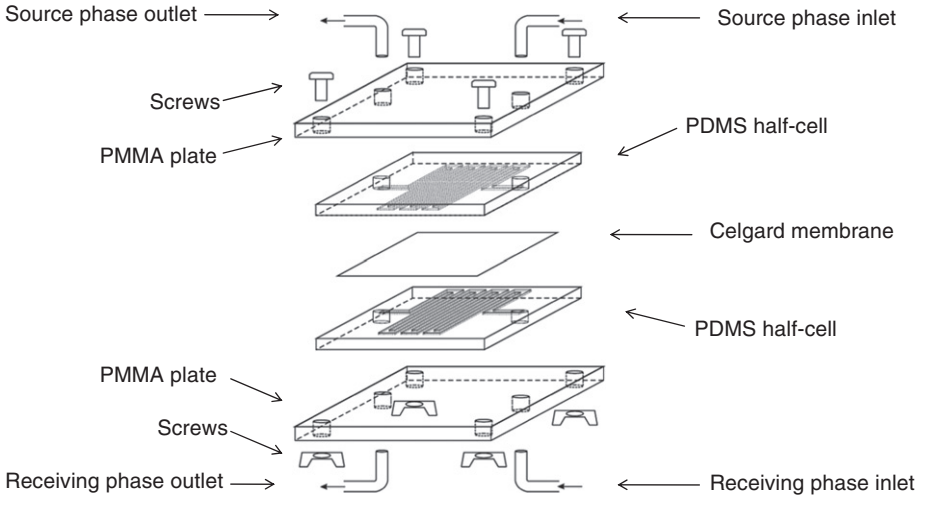

Figure 1. Scheme of the mPLM cell.

1 cm optical path). The diameter and length of the tubing were chosen to obtain the total volume circulating in the receiving compartment $V_r = 351.3\,\mu L$. Peristaltic pump (Ismatec MCP) was used to circulate the solutions through both halves of the mPLM set-up.

2.3 *Experimental procedure*

PLM preconcentration was followed spectrophotometrically by analysing absorption at 508 nm, characteristic for the PAR-Cu(II) complex. For the studies on transport of Cu(II)-based fungicides, given amounts of each fungicide, corresponding to 10^{-4} mole, 10^{-3} mole and 10^{-2} mole of Cu were weighted and dispersed in 1 L of distilled water and left to equilibrate for at least 2 days. The suspensions were then filtered through the Millipore 0.45 μm syringe filters. The total concentration of Cu(II) was measured spectrophotometrically (Zeiss Spectral S 600) after reaction with PAR in acetate buffer (pH 6.0), and the activity of free Cu(II) was measured with Cu(II)-selective electrode (Metrohm) in acetate buffer solution (0.01 M) at pH 6.

SEM pictures were obtained with Hitachi TM-1000 microscope. Speciation calculations were performed with CHEAQS Pro 2008.1 (http://home.tiscali.nl/cheaqs/index.html).

3. Results

3.1 *Theoretical background*

Detailed derivation and discussion of the governing equations for studying speciation with PLM are out of the scope of the present paper and can be found elsewhere [13,14,33]. Here only the major points, relevant for Cu(II) speciation in miniaturised PLM system will be discussed.

Provided that no depletion occurs in the source phase ($c_s^0 = c_s^e = c_s$, where superscripts 0 and e refer to the initial and equilibrium situation, respectively), the equilibrium concentration of Cu(II) in the receiving solution, c_r^e, is proportional to the free [Cu(II)]

concentration in the source, $c_r^e = \alpha_r[\mathrm{Cu(II)}]_s$, with α_r being the degree of complexation in the receiving phase (ratio of total ion concentration to the concentration of its free form). In practice the volume of the source and receiving solutions should fulfil the condition $V_s \gg V_r$ [7], which can be achieved e.g. by minimising the size of the receiving compartment. If this is the case, depletion in the source phase during transport is negligible and measurement of the free Cu(II) concentration is possible.

Based on an analysis described in the work cited above [14], when $\alpha_r \gg 1$, the flux resistance in the receiving phase is negligible with respect to that in the source and membrane phases, and the following equation describes the preconcentration factor in the absence of inert complexes, $F(t)$:

$$F(t) = \left(\frac{t - t_{lag}}{\alpha_s h_r \left(\frac{\delta_s}{D_s \alpha_s} + \frac{\ell}{D_m^{MC} K_D} \right)} \right) \tag{1}$$

where t is time, t_{lag} – is the lag time (i.e. the time necessary for reaching the constant flux through the membrane, h_r is the depth of the receiving channel, α_s, δ_s, D_s are the degree of complexation, source diffusion layer thickness and the diffusion coefficient, respectively, all in the source phase. D_m^{MC} and K_D are diffusion coefficient of the complex MC in the membrane, and ditribution coefficient of Cu(II) ($=[\mathrm{Cu}]_m/[\mathrm{Cu}]_{aq}$), respectively. l is the membrane thickness.

Total Permeability (P_T) and initial flux ($J^0 = P_T \cdot c_s^0$), through the PLM, which provides access to speciation studies, can be calculated from the slope of the $F(t)$ curve, assuming that the source phase is not depleted in the initial phase of transport ($t \to 0$) [10]:

$$P_T = \frac{V_R}{A} \cdot \frac{\mathrm{d}F(t)}{\mathrm{d}t} \tag{2}$$

where V_R and A are the volume of the receiving phase and membrane surface area, respectively.

The main aim of this study was to develop a PLM system with low (microlitres) volume of the receiving phase that could be easily coupled to a spectrophotometric detector. In contrast to typically used PLM detection systems, like AAS or ICP-MS, spectrophotometric detection can be easily miniaturised, or even integrated with the PLM cell. It should be mentioned that the low detection limit offered by AAS or ICP-MS is not advantageous from the point of view of the present system, due to relatively high concentrations of Cu(II) in the source solutions. In this respect, the low detection limit of UV/Vis techniques (10^{-6} M) is acceptable for speciation studies in commercial fungicides. The possibility of spectrophotometric real-time monitoring of Cu(II) concentration in the receiving solution significantly shortens the time required for recording the preconcentration curve, $F(t)$ (where $F = c_r/c_s^0$). This is a great advantage with respect to most other constructions, where sampling is required prior to Cu(II) determination and each point in the $F(t)$ must be acquired in a separate run [13].

The molar extinction coefficient of free Cu(II) in the UV/Vis range is too low for monitoring of the Cu(II) accumulation in real time ($\varepsilon \cong 10\,\mathrm{M^{-1}cm^{-1}}$ at 750 nm), thus the receiving solution should contain a spectrophotometric reagent capable of complexing Cu(II) ions. Functioning of PLM requires that the concentration of free metal ions in the receiving solution be as low as possible, in order to maintain the concentration gradient constant and high. Therefore, such reagent should also play a role of free Cu(II)

concentration buffer. 4-(pirydyl-2-azo)resorcinol (PAR) was chosen in this study, for the high ε value of its complex with Cu(II) ($3 \cdot 10^4\,M^{-1}\,cm^{-1}$ at 500 nm), high hydrophilicity, and suitable dissociation constants (pK_1 (H_3L^+) = 3.1, pK_2 (H_2L) = 5.6, pK_3 (HL^-) = 11.9) [34,35]. At the pH value chosen for the current PLM studies (pH 6), PAR molecule exists mostly in a HL^- form. This should help to keep PAR in the receiving phase and hinder its passage through the hydrophobic membrane towards the source solution. At 25°C and ionic strength of 0.1 M, the logarithm of the stability constants for Cu(II)-PAR complexes, $\log K_{Cu\text{-}PAR} = 14.8$, and $\log K_{Cu(PAR)2} = 9.1$ [34].

Thickness of the Nernst diffusion layer in the source compartment (Equation (1)) can be controlled in a wide range by varying either the flow rate through the source channel or depth of the channel. This provides a very convenient way of changing lability of the complexes present in the source phase. Under planar diffusion conditions, the latter is directly proportional to the diffusion layer thickness, δ [17]. The total volume of the receiving phase (351.3 μL) is a sum of the channel volume, the volume of spectrophotometric cuvette with the connections (20 μL) and the tubing. In order to maintain a constant volume of receiving phase throughout the experiments with different channel depths, the volume of connecting tubing can be adjusted by varying its length and diameter. With the source solution volume, $V_s = 100$ ml, the condition $V_s \gg V_r$ is well fulfilled in the present set-up.

The average thickness of the diffusion layer (δ) in both source and receiving flowing phases can be varied either by choosing different channel depths and/or by changing the flow rate through the two phases, according to Equation (3) [10]:

$$\bar{\delta} = \frac{1.4327 \cdot D^{\frac{1}{3}} \cdot d^{\frac{1}{3}} \cdot h^{\frac{2}{3}} \cdot L^{\frac{1}{3}}}{V_f} \tag{3}$$

where D is the diffusion coefficient [$cm^2\,s^{-1}$], d – the channel half-width [cm], h – its half-height [cm] and L – its length [cm]. V_f is the volume flow rate [$cm^3\,s^{-1}$].

3.2 *Construction of mPLM*

Transport of Cu(II) in PLM with Kryptofix 22DD/lauric acid as carrier requires counter-transport of Na(I) ions from the receiving to the source solution. For this reason. acetate buffer was chosen to maintain pH in this solution and to serve as a rich source of Na(I) in the receiving solution.

Unfortunately, in practice either PAR or its complex with Cu(II) at pH 6 crosses the PLM membrane and is found in the source solution after few minutes, zeroing the driving force for Cu(II) transport. Romero *et al.* [36] reported previously on the use of PAR for spectrophotometric detection of Cu(II) using hollow fibre membrane. In their study, however, in order to maximise the enrichment factor and avoid passage of PAR, the pH of the receiving phase was significantly increased. In the present work, a surfactant was added at concentration above the cmc to form micelles in the receiving phase to prevent the PAR transport without inducing any pH gradients. Thanks to high affinity of PAR to the micellar phase [37], the copper(II) complexation takes place inside the micelles, not in the bulk of aqueous solution and PAR leakage through the membrane is minimised. Four different surfactants were tested: two neutral (Triton X-100 and Brij-35), one cationic (cetyltrimethylammonium bromide, CTAB), and one anionic (sodium dodecylammoniumsulphate, SDS), at three different concentrations: 0.2%, 2% and 10%.

The best results were found for SDS, even though Agnihotri *et al.* [38] found that anionic surfactants inhibit Cu(II) complexation with structurally similar ligand, PAN. Almost complete blocking of PAR transport to the source solution was obtained for 2% w/w SDS, and this concentration was used throughout the present study. PAR concentration close to the solubility limit ($2 \cdot 10^{-4}$ M) was chosen for the present study, giving the degree of Cu(II) complexation in the receiving phase $\alpha_r = 10^{4.3}$. The present set-up fulfils then the $\alpha \gg 1$ condition, described in the theoretical background section.

The microfluidic cell as described in the experimental part was constructed with channel dimensions of (width × depth) $2 \times 0.1\,mm^2$, $2 \times 0.2\,mm^2$, $2 \times 0.5\,mm^2$ (Figure 2) and the total channel length of 31 cm. This allowed for maximising the area of contact between the two aqueous phases and the membrane, while keeping the receiving phase volume at minimum. At the expense of a slight increase of the receiving phase volume (the minimum volume for the 100 μm deep channels = 62 μL + 20 μL cuvette + tubing), the latter phase can be circulated, in contrast to other miniaturised PLMs [9,10].

It should be mentioned here that mechanical properties of PDMS did not allow for use of more shallow channels of large width. Upon assembly of the half-cells with 50 μm deep channels, the latter were blocked due to collapsing of the channel walls of two contacting half-cells. Unfortunately, the efforts to prevent the channel collapse by placing the circular pillars of 300 μm in diameter inside the channels were unsuccessful. The minimum thickness of the PDMS half-cells proving a sufficient mechanical resistance for the set-up was found to be 4 mm.

Half-cells with three different channel depths were produced and tested: 100 μm, 200 μm and 500 μm, giving the channel volume of 62, 124 and 310 μL, respectively.

In the experimental set-up used for further tests in this study ($h = 200\,\mu m$, $0.0021\,cm^3\,s^{-1} < V_f < 0.0862\,cm^3\,s^{-1}$ (source) and $0.0002\,cm^3\,s^{-1} < V_f < 0.0072\,cm^3\,s^{-1}$ (receiving)), the diffusion layer thickness is calculated to vary in the range $45\,\mu m < \delta_{source} < 150\,\mu m$. In the receiving solution, even for the highest available flow rate, the $\delta_{receiving}$ remains the same as if it was stagnant ($\delta_r = h_r/2 = 100\,\mu m$).

In a classical PLM set-up, the membrane is soaked with the organic solution of the carrier prior to the cell assembly. To simplify operation, in the present set-up a new procedure was developed. When the cell is tightly screwed, the hexylbenzene solution of the carrier (Kryptofix 22DD + lauric acid) is passed through the source channel to impregnate the membrane. This allows for minimising the amount of expensive carrier, since the membrane is impregnated only in the parts, through which the transport will take place. The process of membrane soaking can be easily followed with a naked eye through

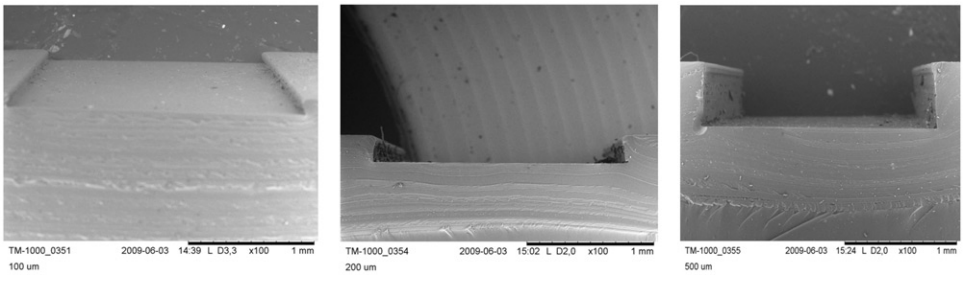

Figure 2. SEM photographs of microchannels in PDMS half-cells (100 μm, 200 μm, 500 μm depth).

the transparent building blocks of the cell (PDMS half-cells and a PMMA holder, see Figure 1). The excess of organic solution is then removed by rinsing the channel with distilled water. Next, the channels are filled with the respective solutions prior to starting the UV/Vis spectra acquisition:

source: copper(II) solution (e.g. fungicide);

receiving: PAR in acetate buffer pH 6.0.

Changes of copper(II) concentrations in the receiving solutions, allowing for calculating the preconcentration factor (F), are followed by acquiring the UV/Vis spectra in the range 300–800 nm with help of a flow-through spectroscopic cuvette. It should be stressed that during the routine operation of mPLM, single point absorbance measurements can easily replace the acquisition of the whole spectra. Prior to passing the fungicides, the set-up was tested with $Cu(NO_3)_2$ solution of 10^{-4} M buffered with MES at pH 6. From the slope of the $F(t)$ curve the permeability, $P_T = 2.3 \cdot 10^{-4} \pm 0.1 \cdot 10^{-4}$ cm s^{-1}. The calibration curve is linear in the range 2×10^{-6} M – 4×10^{-5} M ($R^2 = 0.996$) with a detection limit of 10^{-6} M. The relative standard deviation does not exceed 5%.

3.3 *Characterisation of fungicides*

Copper fungicides have been used in French vineyards already since 1885, mostly in the form of so called *Bordeaux mixture* ($Ca(OH)_2 + CuSO_4$ in different proportions). Different copper-based fungicides are still used in large quantities against e.g. coffee berry disease, vine downy mildew, etc. Even though their use is being limited or even banned in some countries, large amounts of different copper compounds have already been applied to the soil and will remain there for many years due to low mobility of divalent copper in the soil. While most fungicides contain copper(II) in a form of simple well dissociated species, some additives as well as the soil components may interact with Cu(II), drastically changing its bioavailability. For example cymoxanil [39], which is mixed with copper oxychloride in Curzate 49.5 WP contains an acetamide moiety, which is capable of coordinating copper(II) ions. The presence of organic matter and microorganisms in the soil further changes copper(II) speciation when sprayed on plants. Due to copper toxicity at high concentrations, intensive application of fungicides may affect living organisms [40], either through direct phytotoxic effects on the plant, or indirectly, e.g. by reducing the earthworm abundance [41].

All fungicides used for this study are aimed for spraying in the form suspensions in water. The fungicide concentration in the spraying suspensions suggested by the producers varies from species to species, typical value being few g L^{-1} corresponding to ca. 10^{-2} M of Cu(II). In order to mimic the real-life conditions, the fungicides were suspended in unbuffered water. The pH of the supernatant solutions measured after filtration varied between 6.8 and 7.8. Surprisingly, the total concentration of dissolved copper(II) in supernatant solutions increased with the concentration of fungicide in the suspension, despite all the solutions being equilibrated with the solid phase for at least two days. The results for suspensions corresponding to total Cu concentration of 10^{-2} M are presented in Table 2, together with the concentrations of free Cu(II) measured with copper(II)-selective potentiometric electrode. One of the fungicides (Nordox) contains Cu(I) in a form of fine particles of Cu_2O as an active component. Nevertheless, upon suspending in water, apparently Cu(I) oxidises to large extent, giving the highest concentration of both total and free copper(II) ions in the supernatant among all the

Table 2. Concentrations of free ([Cu(II)]) and total ($Cu(II)_{Total}$) copper(II) in the supernatant of 10^{-2} M solutions of fungicides, the corresponding degree of complexation ($\alpha = Cu_{Total}/[Cu(II)]$), dissolved Cu(II) fraction and PLM initial flux (J^0) and permeability (P).

Fungicide	$Cu(II)_{Total}$ [M]	[Cu(II)] [M]	α	$Cu(II)_{dissolved}$ [%]	J^0 [mol (cm^2 s^{-1})]	P [cm s^{-1}]
MIEDZIAN 50 WP	$5.3 \cdot 10^{-5}$	$1.7 \cdot 10^{-5}$	3.1	0.5	$5.0 \cdot 10^{-12}$	$9.6 \cdot 10^{-5}$
MAG 50 WP	$4.1 \cdot 10^{-5}$	$2.3 \cdot 10^{-5}$	1.8	0.4	$4.1 \cdot 10^{-12}$	$1.1 \cdot 10^{-4}$
CURZATE 49.5 WP	$2.3 \cdot 10^{-4}$	$2.2 \cdot 10^{-5}$	10.5	2.3	$5.3 \cdot 10^{-12}$	$2.3 \cdot 10^{-5}$
NORDOX 75 WG	$2.2 \cdot 10^{-4}$	$6.7 \cdot 10^{-5}$	3.3	2.2	$6.8 \cdot 10^{-12}$	$3.3 \cdot 10^{-5}$

studied fungicides. In addition, according to the producer data, some of the Cu_2O particles are below 0.45 μm and could remain in the filtered supernatants, biasing the results.

The single-component fungicides, based on either copper oxychloride or copper hydroxide (Mag 50 WP and Miedzian 50 WP, respectively), are in simple equilibrium with the aqueous phase, governed by their solubility product. From the mass balance using the data from Table 2, it can be concluded that only about 0.5% of the available copper(II) is present in their supernatant solution, compared with >2% for the other two products (Curzate 49.5 WP and Nordox 75 WG), where additional equilibria (redox, complexation) are present. As a result, both Mag 50 WP and Miedzian 50 WP produce comparable total and free copper(II) concentrations in the respective supernatants.

The two fungicides containing copper oxychloride of a general formula $3Cu(OH)_2 \cdot CuCl_2$ (Miedzian 50 WP and Curzate 49.5 WP) show very distinct behaviour: even though the free metal ion concentrations are comparable, the total Cu(II) concentration is four times higher for Curzate than for Miedzian 50 WP. The former product contains cymoxanil (1-(2-cyano-2-methoxy aminoethyl)-3-methylurea), which may participate in Cu(II) complexation thus increasing its total content, while keeping the effective free metal ion concentration low (free metal ion buffering). In addition, it should be mentioned here that the results of potentiometric measurements with Cu(II)-selective ISE for Curzate 49.5 WP are not fully reliable. Probably due to the presence of additional components, large signal drifts were observed during each measurement. The values after 2.5 hours were taken as the equilibrium ones, but in fact the potential readings were not yet stable.

3.4 *Transport of fungicides through the mPLM*

Filtered solutions of the four commercial fungicides were passed through the mPLM. The flow rate (V_f (receiving) $= 0.0037\,cm^3 s^{-1}$) was set by the volume of the tubing linking the receiving phase with the cuvette (67 μL), and the expected frequency of UV/Vis spectra acquisition ($0.05\,s^{-1}$) to allow for unbiased determination of Cu(II) in the receiving phase, $c_r(t)$. For the $2 \times 0.2\,mm^2$ channel the diffusion layer thickness, $\delta = 124\,\mu m$. The transport results are shown in Figure 3 in a form of time evolution of Cu(II) concentration in the receiving phase and preconcentration factor, $F(t)$ (where $F = c_r/c_s^0$). The lag time ($t_{lag} \approx 120\,s$) observed in all the curves originates from the geometry of the flow system. Since interfacial transfer processes and transport through the membrane are fast for Cu(II) [10], the major contribution to t_{lag} is the hydrodynamic transport in the tubing.

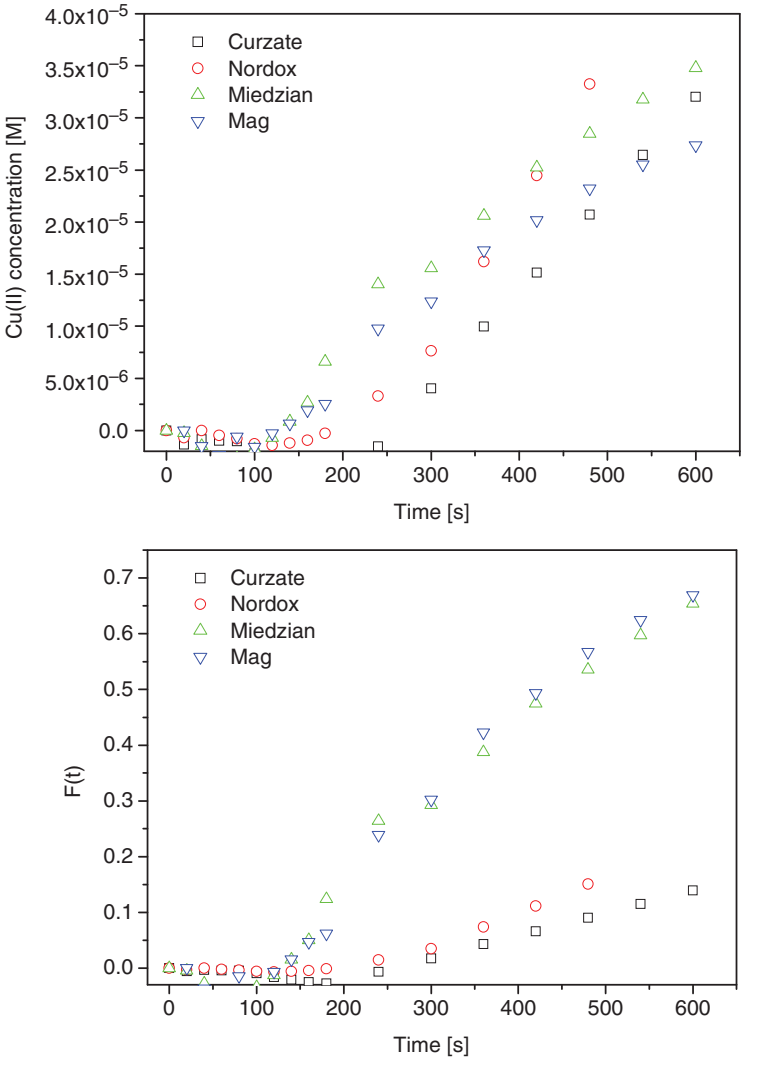

Figure 3. Time dependence of total copper(II) concentration and preconcentration factor (F) in the receiving phase during transport of fungicides in mPLM, determined spectrophotometrically.

The measured value is in very good agreement with the time estimated from the total volume of the receiving phase and the volume flow rate (100 s).

Analysis of the initial flux, J^0, of Cu(II) for all four fungicides using the newly developed mPLM allows for calculating P_T using Equation (2) (Table 2). In all cases P_T is smaller than that obtained with a solution of $Cu(NO_3)_2$ ($P_T = 2.3 \cdot 10^{-4} \pm 0.1 \cdot 10^{-4}$ cm s^{-1}), suggesting that important part of copper(II) in the fungicides solutions remains bound. The ratio of permeabilities in the presence and absence of fungicides is in good agreement with the degree of complexation calculated on the basis of the free ion activities measured with ISEs (Table 2). The permeability is the highest for the two fungicides, which produce the lowest total Cu(II) concentration in the supernatant solution ((Mag 50 WP and

Miedzian 50 WP). According to Salaun and Buffle [9], the initial flux through the PLM is directly proportional to the free metal ion concentration in either of the two cases: (1) if the diffusion in the membrane is limiting (much slower than in the source solution), or (2) if the diffusion in the source solution is limiting (much slower than in the membrane) and the complexes are inert. In fact, the J^0 values are very similar for all fungicides, except Nordox (Table 2). The latter, producing significantly higher [Cu(II)], gives the highest J^0. Despite different compositions of the studied fungicides, in all cases the overall flux was proportional to the concentration of free Cu(II), confirming the usefulness of mPLM in the study of copper(II) speciation in fungicides.

4. Conclusions

A versatile platform for building miniaturised PLM device (mPLM) with adjustable speciation capabilities was described. The master replication technology of PDMS allows for easy control over the microchannel geometry and high reproducibility. This, in combination with variable volume flow rate, provides convenient means of controlling the diffusion layer thickness, which is very useful in speciation studies. Both source and receiving solutions are circulating, which allows for performing a real-time spectrophotometric detection of transported copper(II) ions permeating through the membrane to the receiving phase. The optical transparency of materials used (PDMS and PMMA) enables easy visual inspection of the constructed mPLM, and opens the possibility of integrating the detection system within the microchannel structure in the future. This significantly reduces the time of acquiring the whole F(t) curve, which can now be gathered in a single run.

The results concerning the fungicides presented in this paper are only preliminary and are shown only as a proof of concept for studying copper(II) speciation with the new mPLM set-up. More detailed information can be obtained by varying the lability of the copper-containing complexes. Only this would allow for drawing more far-reaching conclusions of potential bioavailability of Cu(II) from fungicides.

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