# An Enzyme Derivatized Polydimethylsiloxane (PDMS) Membrane for Use in Membrane Introduction Mass Spectrometry (MIMS)

A. Skye Creba, Alexandra N. E. Weissfloch, Erik T. Krogh, and Chris G. Gill

Applied Environmental Research Laboratories, Department of Chemistry, Malaspina University-College, Nanaimo, British Columbia, Canada

Membrane introduction mass spectrometry (MIMS) provides direct measurement of volatile and semivolatile analytes in condensed and gas-phase samples without sample preparation steps. Although MIMS has numerous advantages that include direct, on-line, real-time analysis with low detection limits, current applications of MIMS are predominantly limited to volatile and semivolatile analytes that permeate hydrophobic membranes (e.g., polydimethylsiloxane; PDMS). We report the first enzyme modified PDMS membrane for use with MIMS. This was achieved by immobilizing *Candida rugosa* lipase directly onto the surface of oxidized PDMS. These surface immobilized enzymes catalyze ester hydrolysis, releasing an alcohol product at the membrane interface that is readily detected. We have successfully used an enzyme modified membrane for the analysis and quantification of low-volatility and hydrophilic esters. We report the quantification of several carboxylic acid esters in dilute aqueous solutions, including a phthalate monoester carboxylate that is not readily detected by conventional MIMS. This new interface demonstrates the potential for extending the range and versatility of MIMS. (J Am Soc Mass Spectrom 2007, 18, 973–979) © 2007 American Society for Mass Spectrometry

The ability to detect and measure low concentrations of analytes, in real-time using online techniques without time consuming and/or expensive sample preparation steps has made membrane introduction mass spectrometry (MIMS) a valuable analytical strategy in a number of fields [1, 2]. In MIMS, a semipermeable membrane interface enriches hydrophobic analytes while simultaneously rejecting matrix components. The analyte is transferred directly from sample to the ion source of a mass spectrometer, allowing for excellent sensitivity (as low as parts-per-quadrillion in water for some analytes) without the need for any sample preconcentration [1–11]. The inherent sensitivity and selectivity of mass spectrometry (especially tandem MS techniques) combined with the use of semipermeable membranes to select for compounds of interest in raw, complex sample matrices makes MIMS ideal for monitoring dynamic chemical systems. The simplicity and speed of this system makes it well suited for gathering online data from reacting chemical streams, bioreactors, and other biological systems as well as rapid screening of environmental samples and pharmaceutical products [1, 2, 4, 12].

Address reprint requests to Dr. C. G. Gill, Applied Environmental Research Laboratories, Department of Chemistry, Malaspina University-College, 900 5th Street, Nanaimo, BC V9R 5S5, Canada. E-mail: gillc@mala.bc.ca

A variety of MIMS interface geometries and materials have been successfully employed including functionalized [13], ultra-thin [14], flat sheet [15], and hollow fiber [2, 12, 16, 17] membranes. In particular, capillary hollow fiber membranes (CHFMs) made of polydimethylsiloxane (PDMS) and arranged in a flow over configuration with pneumatically assisted carrier gas flowing through the lumen of the capillary (as presented by this work) have gained popularity [1, 2]. This robust system is capable of withstanding a wide range of temperature and chemical reagent variations, while effectively detecting volatile and semivolatile organic compounds (VOCs and SVOCs) in complex aqueous and air samples [18]. However, the mass transport of analyte across PDMS membrane (pervaporation) can be limited by any one (or a combination of) the following: partitioning of the analyte into the PDMS, diffusion of analyte through the PDMS and/or desorption of analyte into the carrier gas stream. Consequently, as analytes become more polar (less soluble in PDMS) and less volatile, their pervaporation across a PDMS interface becomes less favorable. A variety of thermal desorption techniques, where the membrane interface is heated during or after sampling, have been successful in facilitating the analysis of SVOCs that are difficult to detect with MIMS at ambient temperatures [6–8, 10, 11, 18–25]. Recently, our group has developed a coaxially heated hollow


1044-0305/07/$32.00
doi:10.1016/j.jasms.2007.02.005

Published online March 28, 2007
Received December 6, 2006
Revised January 31, 2007
Accepted February 3, 2007

fiber membrane interface that allows real-time analysis of many SVOCs [22] under ambient conditions. Although thermally assisted techniques increase analyte diffusion through the membrane and improve desorption into the carrier gas, they do little to improve (and often reduce) their partitioning from the sample into the PDMS. This on-line preconcentration establishes a concentration gradient that is responsible for mass transport, and is directly related to the analytical sensitivity of the technique. This limitation precludes the use of MIMS for many polar analytes, including a wide variety of important biomolecules such as esters, amides and peptides. We have addressed this limitation by derivatizing the outside of a PDMS capillary hollow fiber as a strategy to extend the use of MIMS to include molecules that are not otherwise detected.

In recent years, considerable attention has been directed towards investigating the properties of PDMS [26–29]. The biocompatibility, chemical inertness, elasticity, gas permeability, and low-cost of this material have made it attractive for use in membrane technology, microfluidics [28], and bio-analytical applications [28, 30]. In attempts to tailor PDMS to specific needs, a diverse range of modification strategies have been explored, including the adsorption and covalent immobilization of polymers and biomolecules [28, 31], the introduction of active functional groups through polymer blending or copolymerization with a suitable monomer [32], grafting using a radical initiator, and surface oxidation to produce reactive silanol groups [26]. The presence of these reactive silanol groups on the surface increases material hydrophilicity and can also be exploited for further derivatization. Diaz-Quijada and Wayner have recently shown that PDMS treatment with ozone in the absence of photolysis results in effective oxidation [27]. While chemical modification of PDMS has been well documented, application of these modifications to MIMS work has been quite limited [33]. Xu et al. reported a derivatized cellulose membrane for use in MIMS that selectively adsorbed substituted benzaldehydes at high pH, thus preconcentrating them at the membrane surface [13]. Our work describes a MIMS membrane comprised of an immobilized hydrolytic enzyme on hydroxylated PDMS and reports the first enzyme derivatized membrane for use in MIMS. The immobilized enzyme catalyzes the hydrolysis of carboxylic acid esters (including those that are difficult to analyze with conventional MIMS), resulting in the production of readily detectable alcohol molecules.

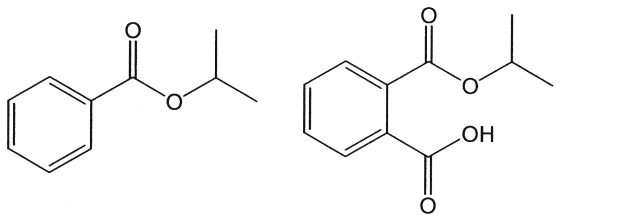
Enzyme use in analytical techniques, for example in biosensors, has been well documented [34, 35], as these catalysts allow for much greater reaction speed and specificity than could be otherwise achieved by chemical catalysis. In analytical systems, immobilized enzymes are advantageous over their soluble counterparts because of the increased stability that usually results from this process, allowing for easy manipulation and reuse [34]. Lipases are enzymes that hydrolyze a broad range of esters to produce alcohol and acid products with high enantio- and/or regioselectivity [36]. In natural systems, these enzymes play a digestive role in plants, animals, and microorganisms [36]. Lipases are now extensively used in organic chemistry to catalyze biotransformations, prepare enantiomerically pure pharmaceuticals, to protect and de-protect synthetic intermediates, and to modify natural lipids [36]. Lipases have also been successfully immobilized onto solid support carrier compounds such as silica, chitosan, and agarose for applications including microfluidics and fixed bed reactors [37–39].

For this work, we have covalently linked *Candida rugosa* lipase directly onto the outer surface of a derivatized PDMS capillary hollow fiber membrane coupled to a quadrupole ion trap mass spectrometer in a "flow-over" MIMS geometry. Lipases on the surface of the interface selectively bind and cleave esters, resulting in the release of the alcohol fragment for MIMS detection. The target analytes (Scheme **1**) isopropyl benzoate (IPBZ) and monoisopropylphthalic acid (MIPA) were chosen because of their poor analytical performance with unmodified membranes (especially MIPA, a charged ion at neutral pH, which is not detectable using isothermal, flow-over MIMS). While extending the analytical range of MIMS to include new analytes, this membrane modification embeds enzyme-substrate affinity into an on-line measurement strategy. This work reports the first use of an enzyme modified membrane for MIMS that demonstrates selective and sensitive real time measurement of low volatility esters.

## Experimental

### *Reagents*

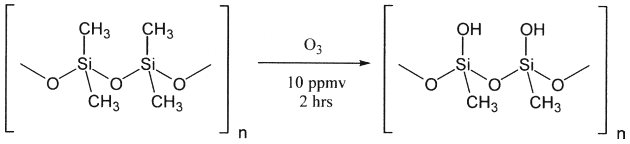
*Candida rugosa* lipase (1140 units activity/mg), cyanuric chloride, phthalic anhydride, isopropyl alcohol (IPA), and toluene (all ACS grade) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol, mono-basic and di-basic phosphate buffer, and hydrochloric acid were also ACS grade and obtained from Fisher Scientific (Ottawa, ON, Canada). IPBZ (Scheme **1**) was synthesized from benzoic acid and isopropyl alcohol (IPA) using simple acid-catalyzed ester synthesis chemistry [40]. MIPA (Scheme **1**) was synthesized from phthalic anhydride and IPA, following basic anhydride ester synthesis chemistry [41].




isopropyl benzoate (IPBZ) m/z 77, 105, 165

monoisopropylphthalic acid (MIPA) m/z 149

**Scheme 1**




**Scheme 2**

Target analytes were purified by recrystallization and analyzed by GC-MS (GC-Q; Thermo-Electron, San Jose, CA with a Restek Rtx-5MS column; Restek Chromatographic Specialties, Brockville, ON, Canada) to ensure purity before use. Ozone was produced at ~10 ppmv by passing ~2 L/min UHP oxygen (99.999% pure) through an Ultraviolet Products Inc. Ozone Generator (model 9487; San Gabriel, CA). Phosphate buffer was made with mono- and di-basic sodium phosphate using high purity deionized (DI) water (model MQ Synthesis A10; Millipore Corp., Billerica, MA). All MIMS samples were run in 0.06 M aqueous phosphate buffer with pH = 7.1 ± 0.1.

## *Instrumental Parameters*

All data were collected using a quadrupole ion trap mass spectrometer with internal ionization (Saturn II; Varian, Walnut Creek, CA) equipped with an in-house constructed MIMS interface. The MIMS interface used here was fabricated by modifying a traditional flow-over interface previously described [3, 5, 22]. Briefly, the MIMS instrument consisted of a modified PDMS capillary hollow fiber membrane (10.0 cm, 0.94 mm o.d., 0.51 mm i.d., Silastic brand; Dow Corning, Midland, MI) mounted in a flow through cell constructed of 0.25 in. Swagelok (Supelco, Bellefonte, PA) connectors and stainless steel tubing. A controlled, low flow of helium gas (99.999% pure, 2.7 mL/min) was swept through the lumen of the CHFM. The exit helium flow was subsequently passed through a metal jet separator (model MJSC/HP5890, 15 mL/min jets; SGE, Austin, TX) and then to the mass spectrometer (base pressure 12 $\mu$Torr) via a heated transfer line (150 °C). The jet separator was backed (25 mTorr) by a mechanical roughing pump (model RV3; BOC Edwards, Mississauga, ON, Canada) equipped with an in-line molecular sieve trap (4 in. diameter, 13 Å sieve pore size; KJ Lesker Inc., Pittsburgh, PA). The MIMS interface was mounted entirely inside a retrofitted gas chromatograph oven (GOW-MAC Instrument Co., Bethlehem, PA). All samples were recirculated in a closed loop through the MIMS interface using a peristaltic pump (model 77200-62 Easy-Load II Masterflex; Cole-Parmer, Concord, ON, Canada, with LS 25 Viton pump tubing) at a flow rate of 375 mL/min to facilitate sample mixing and establish turbulent flow conditions at the membrane surface. A closed glass sample reservoir (1 L, septum equipped) was used for all experiments and was plumbed to the MIMS system and pump using 0.25 in. o.d. Teflon tubing (Cole-Palmer). Temperature control for the reservoir was accomplished using an isothermal water bath (model 66800/23; Precision Scientific Group, Chicago, IL).

## *Membrane Modifications*

The PDMS hollow fiber membrane was modified before installation in the MIMS system. Hydroxyl groups were formed on the surface of the PDMS membrane (Scheme **2**) as previously described by Diaz-Quijada and Wayner, through treatment with 10 ppmv ozone for 2 h [26, 30].

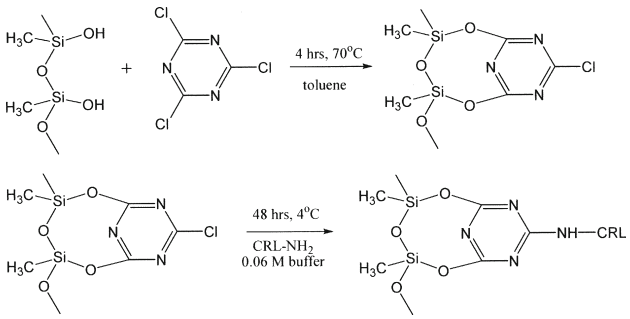
Hydroxylated PDMS membranes were mounted between two 10 cm long, 22 gauge stainless hypodermic tubes (in preparation for installation as MIMS interfaces) and were submerged in a reaction vessel containing 5% wt/vol solution of cyanuric chloride in toluene. The ends of the tubing were kept out of solution to eliminate contamination of the membrane interior. This system was refluxed for 4 h at 70 °C under nitrogen (Scheme **3**). Membranes were then rinsed three times with $10^{-4}$ N HCl to remove any unreacted cyanuric chloride and placed in a prepared enzyme solution containing 0.1% wt/vol *Candida rugosa* lipase (CRL) in 0.06 M pH 8.0 phosphate buffer. Membranes were stored in this solution for 48 h at 4 °C (Scheme **3**), after which they were washed three times with 0.06 M phosphate buffer (pH 8.0), then dried using nitrogen gas and stored at 4 °C [42–44] until used in the MIMS system (<8 wk for this work).

## *Free Enzyme Comparison Study*

To assess the performance of the immobilized enzyme, experiments were also conducted with enzyme free in solution. In these experiments, a PDMS membrane (no modifications) was used. The CRL enzyme was added to the sample reservoir to give a solution with 6.6 units of activity/mL (total activity = 7182 units).

## *Immobilized Lipase Thermal Stability*

The thermal stability of immobilized *Candida rugosa* lipase was evaluated by analyzing the enzymatic generation of IPA from 5 $\mu$M (800 ppb) MIPA with both the sample solution and the membrane at 25, 35, 45, and




**Scheme 3**

55 °C. The temperature of the membrane interface was regulated using the gas chromatograph oven, and the temperature of the solution was controlled using the isothermal water bath. In attempts to denature the immobilized *Candida rugosa* lipase, the membrane was incubated in the MIMS interface at 90 °C for 12 h in air, after which the activity was assessed by monitoring the enzymatic IPA production from MIPA with both the membrane and sample solution at 25 °C.

### *Calibration Standards*

Analyte calibrations were achieved by injecting precise volumes of stock IPBZ, MIPA, and IPA (separate experiments) into the sample reservoir to produce dilute standard solutions. These were recirculated through the MIMS interface in the same closed-loop experiment. In this manner, both analyte (when detectable) and hydrolysis products could be simultaneously quantified. As a calibration check, standard additions of appropriate analyte were also used to ensure run-to-run calibration reliability.

## Results and Discussion

### *Isopropyl Benzoate*

In two separate experiments, a 4 $\mu$M (650 ppb) solution of IPBZ was re-circulated over either a CRL modified PDMS membrane or a PDMS (control) membrane installed in the MIMS system. In either case, both IPBZ ($m/z$ = 77,105, 165) and IPA ($m/z$ = 45) were simultaneously monitored. The results show (Figure 1d), that the ester is slow to permeate the unmodified control membrane, with a signal delay of 4.9 min and a $t_{10}$-$t_{90}$ rise-time of 40 min, severely compromising real-time quantitation of IPBZ by MIMS operated in this mode. Furthermore, no appreciable signal is observed for IPA in this control experiment (Figure 1c). In the CRL-membrane experiment, the IPBZ signal is atypical of SVOCs on unmodified PDMS (Figure 1b). There is a longer signal delay time (8.8 min) and the signal shows a rapid increase that eventually stabilizes instead of a more characteristic sinusoidal shape (Figure 1d). Additionally, we observe an almost immediate onset for the IPA signal, which rises rapidly to a steady-state signal equivalent to 40 $\mu$M IPA, 10-fold higher than the concentration of the bulk ester (Figure 1a). This data provides evidence that the immobilized *Candida rugosa* lipase catalyzes the release of IPA at the membrane interface and allows for measurement of IPBZ with much shorter duty cycles by detecting the hydrolyzed alcohol instead of the parent ester (calibration for IPBZ from 0.65 to 9.7 $\mu$M based upon IPA signal is linear; signal ($m/z$ 45) = 13.4 ($\mu$M IPBZ) + 3.18, $r^2$ = 0.994; data not shown). The delayed onset of the IPBZ signal for the CRL-PDMS versus PDMS membrane (3.9 min longer, Figure 1b versus d) is consistent with enzymatic activity at the membrane surface. Enzymatic binding and subsequent hydrolysis of IPBZ slows the initial build up of the ester within the PDMS membrane itself, lengthening the delay time between sample introduction and the pervaporation of IPBZ. We interpret this as a surface binding phenomenon since it is only after all enzyme active sites are occupied that excess IPBZ itself can permeate the membrane. Furthermore, the steady-state signal for IPA in these experiments translates into a 10-fold concentration enhancement over the bulk ester concentration due to the high local concentration of alcohol as a result of its release directly on the membrane surface. As shown in Figure 1, a greater concentration of IPA appears to be produced (~40 $\mu$M) than the concentration of IPBZ substrate supplied to the enzyme (4 $\mu$M), corresponding to an apparent 10-fold enrichment. Xu et al. documented similar enhanced transport and detection with their affinity MIMS where analyte was released directly at the solution-membrane interface [13].

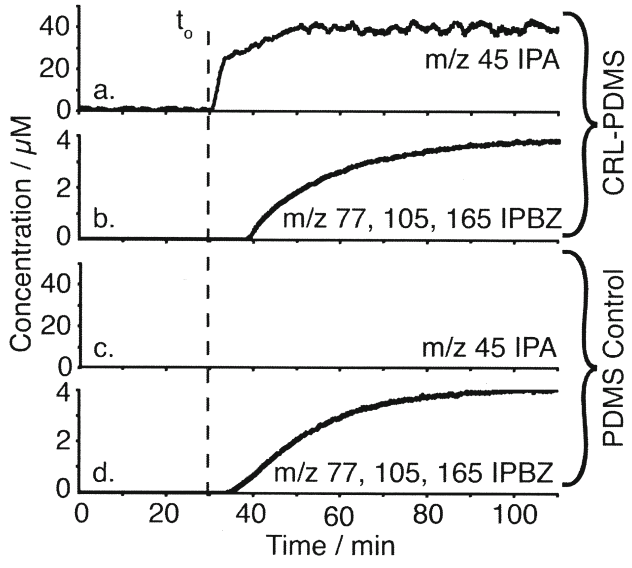



**Figure 1.** Comparison of a *Candida rugosa* modified PDMS membrane (CRL-PDMS) versus a PDMS control membrane for the analysis of IPBZ. At $t_o$, IPBZ standard was spiked into the sample reservoir, yielding a final concentration of 4 $\mu$M (650 ppb). The detection of IPA for the CRL-PDMS membrane indicates the successful enzyme mediated hydrolysis of IPBZ at the membrane surface.

### *Monoisopropylphthalic Acid*

To better assess the performance of the new enzyme derivatized membrane interface, MIPA was employed as a target analyte. As a control, a 3.1 $\mu$M (470 ppb) MIPA solution was analyzed using an unmodified PDMS membrane (Figure 2). No signal for MIPA ($m/z$ = 149) was observed up to concentrations of 66 $\mu$M (10 ppm), presumably because of the reduced permeation and low volatility imparted by the carboxylate ion moiety [45]. In addition, no IPA was observed in this control, confirming that any alcohol signal is the result of lipase activity. When a 3.1 $\mu$M MIPA solution was

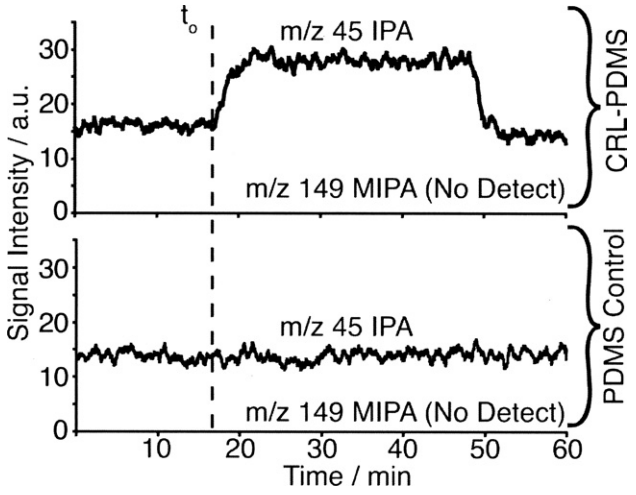



**Figure 2.** Comparison of a *Candida rugosa* modified PDMS membrane (CRL-PDMS) versus a PDMS control membrane for the analysis of MIPA. At $t_o$, MIPA solution was added to give a final concentration of 3.1 $\mu$M (470 ppb). While MIPA is not detected in either case, IPA produced by enzymatic hydrolysis at the membrane surface is readily monitored and can be used to quantify the presence of MIPA.

analyzed using the CRL-PDMS membrane, an immediate IPA signal appears (Figure 2). Thus, on-membrane hydrolysis allows the ready detection of MIPA via monitoring the IPA signal. The IPA signal obtained from the enzymatic hydrolysis of 3.1 $\mu$M MIPA substrate is equivalent to an apparent IPA concentration of 11.3 $\mu$M (Figure 2), representing a 3.6-fold enrichment for the enzymatic hydrolysis of MIPA. These results lend further support to a high, localized concentration enhancement for enzymatic cleavage products at the MIMS interface. When a 6.1 $\mu$M (930 ppb) MIPA solution is analyzed using either a CRL-PDMS membrane or a PDMS membrane with CRL free in solution (Figure 3), there is substantially more IPA signal generated by the CRL-PDMS membrane. In the free enzyme experiment, the measured IPA concentration is 1.8 $\mu$M (less than the initial MIPA concentration) suggesting a 30% conversion in solution. However, for the CRL-PDMS membrane, we observe an IPA signal equivalent to an apparent concentration of 30 $\mu$M, a 4.8-fold enrichment over the concentration of MIPA. Although we have not quantified the immobilized CRL surface coverage, clearly the immobilized enzyme methodology offers several advantages including no sample preparation and an order of magnitude improved sensitivity over the free enzyme experiment (at the tested activity). Calibration data can be readily obtained using the enzyme-derivatized membrane that allows MIPA quantitation via monitoring the IPA signal at *m/z* 45 (Figure 4). The exponential fit for this data (1.5 to 15.3 $\mu$M MIPA) can be simplified to a linear relationship at lower concentrations (e.g., 1.5 to 6.1 $\mu$M), consistent with the results observed for IPBZ above. In summary, the results obtained from the analysis of MIPA confirm that a membrane interface with *Candida rugosa* lipase immobilized onto the surface enables the analysis of analytes previously not amenable to conventional MIMS.

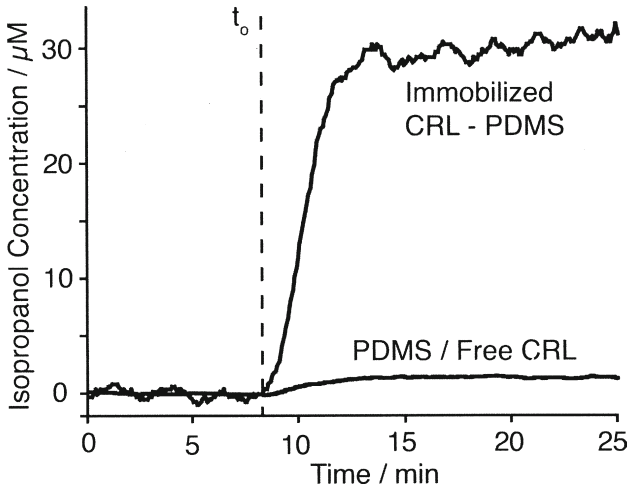



**Figure 3.** Comparison of MIPA detection using both the CRL-PDMS membrane and a PDMS membrane combined with CRL free in solution. Enzymatic activity was assessed by monitoring the production of IPA (*m/z* 45) from 6.1 $\mu$M (930 ppb) MIPA at 25 °C, introduced at $t_o$ in both experiments. Substantial sensitivity enhancement is observed for the CRL-PDMS membrane.

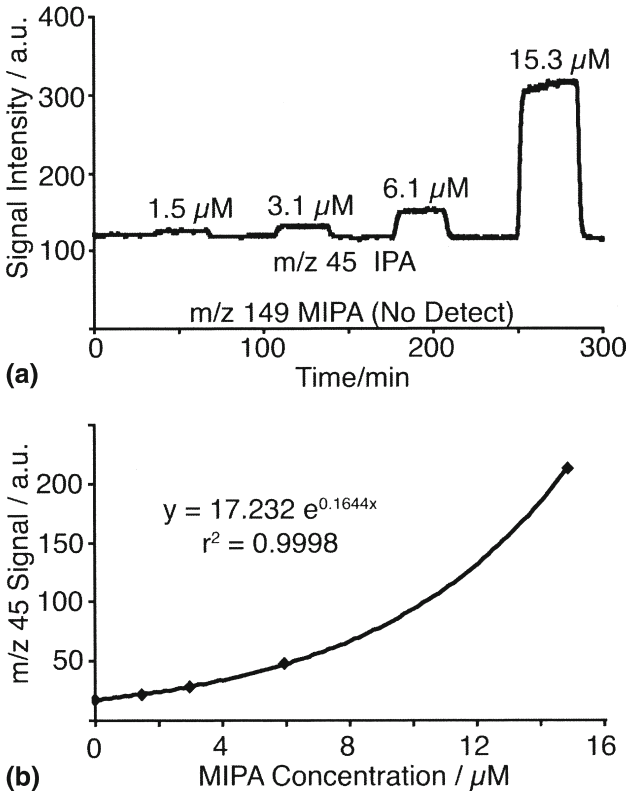



**Figure 4.** Calibration curve for MIPA. (**a**) An ion chromatogram for IPA produced by enzymatic hydrolysis of MIPA used to generate the calibration curve for MIPA (**b**) at concentrations: 1.5 $\mu$M (230 ppb), 3.1 $\mu$M (470 ppb), 6.1 $\mu$M (930 ppb), and 15.3 $\mu$M (2.3 ppm).

### *Immobilized Lipase Thermal Stability*

*Candida rugosa* lipase proved to be quite thermally stable when covalently immobilized on the PDMS membrane. Initial testing of lipase activity after heating it in aqueous buffer solution was conducted at temperatures up to 55 °C. Lipase activity, monitored by the production of IPA from MIPA, remained constant at all temperatures measured. This is consistent with previous studies that have reported immobilized lipase activity°at°comparable°temperatures°[46].°Further°denaturation of the immobilized *Candida rugosa* lipase was attempted in situ to assess thermal stability. After heating to 90 °C in air for 12 h and subsequently monitoring enzymatic hydrolysis of MIPA (at 25 °C), the time evolution of the IPA signal and its steady-state level remain unchanged, indicating thermal stability of the immobilized enzyme under conditions that are known to denature the majority of free proteins°[47].

## Conclusions

We have successfully demonstrated the use of an enzyme modified PDMS membrane for use in MIMS. Covalently linked lipase enzymes catalyze the hydrolysis of ester functional groups, releasing alcohols that can be subsequently detected by MIMS. We have applied this to the detection and quantification of two polar hydrophilic esters, IPBZ and MIPA, which were not previously amenable to conventional MIMS systems (particularly MIPA). Enzyme functionalized membranes are a logical extension of the simplicity, sensitivity and selectivity inherent in MIMS techniques. Although this work represents a specific enzyme-analyte system for a relatively nonselective lipase, we believe that it establishes the feasibility of using immobilized enzymes in MIMS applications. This strategy can be expanded to provide for greater membrane selectivity based upon specific enzyme-substrate affinities. There are a number of potential applications for enzyme modified MIMS interfaces that may arise from the enzymological and biological work reviewed by Lloyd°et°al.°[12].°We°have°not°yet°explored°potential complications arising from the analysis of mixtures of esters, such as competitive inhibition and multiple esters producing the same hydrolysis product. This will be an important area of future investigation by our group. We are currently exploring new applications of this interface, examining its potential for the detection of biomolecules as well as studying the fundamental properties of enzyme modified membranes. We believe that the delay time for the onset of IPBZ ester signal observed with CRL-modified membranes is a measure of enzyme surface coverage and may be an important application of time resolved MIMS data to the field of polymer surface science.

## Acknowledgments

The authors acknowledge and thank the Natural Sciences and Engineering Research Council of Canada for funding this research under the Discovery Grant and Undergraduate Student Research Award programs as well as the Canada Foundation for Innovation, the British Columbia Knowledge Development Fund, the Science Council of British Columbia, and Malaspina University-College for infrastructure funding to establish the Applied Environmental Research Laboratories.

## References

1. Ketola, R. A.; Kotiaho, T.; Cisper, M. E.; Allen, T. M. Environmental Applications of Membrane Introduction Mass Spectrometry. *J. Mass Spectrom.* **2002,** *37*(5), 457–476.
2. Johnson, R. C.; Cooks, R. G.; Allen, T. M.; Cisper, M. E.; Hemberger, P. H. Membrane Introduction Mass Spectrometry: Trends and Applications. *Mass Spectrom. Rev.* **2000,** *19*(1), 1–37.
3. Cisper, M. E.; Gill, C. G.; Townsend, L. E.; Hemberger, P. H. On-Line Detection of Volatile Organic Compounds in Air at Parts-Per-Trillion Levels by Membrane Introduction Mass Spectrometry. *Anal. Chem.* **1995,** *67*(8), 1413–1417.
4. Nelson, J. H. L.; Krogh, E. T.; Gill, C. G.; Friesen, D. A. Monitoring the $TiO_2$-Photocatalyzed Destruction of Aqueous Environmental Contaminants at Parts-per-Trillion Levels Using Membrane-Introduction Mass Spectrometry (MIMS). *J. Environ. Sci. Health Part A* **2004,** *39*(9), 2307–2317.
5. Soni, M.; Bauer, S.; Amy, J. W.; Wong, P.; Cooks, R. G. Direct Determination of Organic Compounds in Water at Parts-per-Quadrillion Levels by Membrane Introduction Mass Spectrometry. *Anal. Chem.* **1995,** *67*(8), 1409–1412.
6. Allen, T. M.; Cisper, M. E.; Hemberger, P. H.; Wilkerson, C. W., Jr. Simultaneous Detection of Volatile, Semivolatile Organic Compounds, and Organometallic Compounds in Both Air and Water Matrices by Using Membrane Introduction Mass Spectrometry. *Int. J. Mass Spectrom.* **2001,** *212*(1/3), 197–204.
7. Aggerholm, T.; Lauritsen, F. R. Direct Detection of Polyaromatic Hydrocarbons, Estrogenic Compounds and Pesticides in Water Using Desorption Chemical Ionization Membrane Inlet Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2001,** *15*(19), 1826–31.
8. Creaser, C. S.; Weston, D. J. In-Membrane Preconcentration/Membrane Inlet Mass Spectrometry of Volatile and Semivolatile Organic Compounds. *Anal. Chem.* **2000,** *72*(13), 2730–2736.
9. LaPack, M. A.; Tou, J. C.; Enke, C. G. Membrane Mass Spectrometry for the Direct Trace Analysis of Volatile Organic Compounds in Air and Water. *Anal. Chem.* **1990,** *62*(13), 1265–1271.
10. Lauritsen, F. R.; Mendes, M. A.; Aggerholm, T. Direct Detection of Large Fat-Soluble Biomolecules in Solution Using Membrane Inlet Mass Spectrometry and Desorption Chemical Ionization. *Analyst* **2000,** *125*(1), 211–215.
11. Mendes, M. A.; Eberlin, M. N. Trace Level Analysis of VOCs and Semi-VOCs in Aqueous Solution Using a Direct Insertion Membrane Probe and Trap Release Membrane Introduction Mass Spectrometry. *Analyst* **2000,** *125*(1), 21–24.
12. Lloyd, D.; Thomas, K. L.; Cowie, G.; Tammam, J. D.; Williams, A. G. Direct Interface of Chemistry to Microbiological Systems: Membrane Inlet Mass Spectrometry. *J. Microbiol. Methods* **2002,** *48*(2/3), 289–302.
13. Xu, C.; Patrick, J. S.; Cooks, R. G. Affinity Membrane Introduction Mass Spectrometry. *Anal. Chem.* **1995,** *67*(4), 724–728.
14. Cisper, M. E.; Hemberger, P. H. The Direct Analysis of Semivolatile Organic Compounds by Membrane Introduction Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1997,** *11*(13), 1449–1453.
15. Maden, A. J.; Hayward, M. J. Sheet Materials for Use as Membranes in Membrane Introduction Mass Spectrometry. *Anal. Chem.* **1996,** *68*(10), 1805–1811.
16. LaPack, M. A.; Tou, J. C.; Enke, C. G. Membrane Extraction Mass Spectrometry for the On-Line Analysis of Gas and Liquid Process Streams. *Anal. Chem.* **1991,** *63*(15), 1631–1637.
17. Westover, L. B.; Tou, J. C.; Mark, J. H. Novel Mass Spectrometric Sampling Device. Hollow Fiber Probe. *Anal. Chem.* **1974,** *46*(4), 568–571.
18. Riter, L. S.; Takats, Z.; Charles, L.; Cooks, R. G. High Surface Area Membrane Introduction Mass Spectrometry for Analysis of Volatile and Semivolatile Organic Compounds in Air. *Rapid Commun. Mass Spectrom.* **2001,** *15*(17), 1520–1524.
19. Lauritsen, F. R.; Ketola, R. A. Quantitative Determination of Semivolatile Organic Compounds in Solution Using Trap-and-Release Membrane Inlet Mass Spectrometry. *Anal. Chem.* **1997,** *69*(23), 4917–4922.
20. Riter, L. S.; Takats, Z.; Cooks, R. G. Single-Sided Membrane Introduction Mass Spectrometry for On-Line Determination of Semivolatile Organic Compounds in Air. *Analyst* **2001,** *126*(11), 1980–1984.
21. Leth, M.; Lauritsen, F. R. A Fully Integrated Trap-Membrane Inlet Mass Spectrometry System for the Measurement of Semivolatile Organic Compounds in Aqueous Solution. *Rapid Commun. Mass Spectrom.* **1995,** *9*(7), 591–596.

22. Thompson, A. J.; Creba, A. S.; Ferguson, R. M.; Krogh, E. T.; Gill, C. G. A Coaxially Heated Membrane Introduction Mass Spectrometry Interface for the Rapid and Sensitive On-Line Measurement of Volatile and Semivolatile Organic Contaminants in Air and Water at Parts-per-Trillion Levels. *Rapid Commun. Mass Spectrom.* **2006,** *20*(13), 2000–2008.
23. Bocchini, P.; Pozzi, R.; Andalo, C.; Galletti, G. C. Experimental Upgrades of Membrane Introduction Mass Spectrometry for Water and Air Analysis. *Anal. Chem.* **2001,** *73*(16), 3824–3827.
24. Soni, M. H.; Callahan, J. H.; McElvany, S. W. Laser Desorption-Membrane Introduction Mass Spectrometry. *Anal. Chem.* **1998,** *70*(15), 3103–3113.
25. Creaser, C. S.; Lamarca, D. G.; dos Santos, L. M.; New, A. P.; James, P. A. A Universal Temperature Controlled Membrane Interface for the Analysis of Volatile and Semivolatile Organic Compounds. *Analyst* **2003,** *128*(9), 1150–1156.
26. Graubner, V. M.; Jordan, R.; Nuyken, O.; Schnyder, B.; Lippert, T.; Kotz, R.; Wokaun, A. Photochemical Modification of Cross-Linked Poly(dimethylsiloxane) by Irradiation at 172 nm. *Macromolecules* **2003,** *37*(16), 5936–5943.
27. Diaz-Quijada, G. A.; Wayner, D. D. M. A Simple Approach to Micropatterning and Surface Modification of Poly(dimethylsiloxane). *Langmuir* **2004,** *20*(22), 9607–9611.
28. Linder, V.; Verpoorte, E.; Thormann, W.; de Rooij, N. F.; Sigrist, H. Surface Biopassivation of Replicated Poly(dimethylsiloxane) Microfluidic Channels and Application to Heterogeneous Immunoreaction with On-Chip Fluorescence Detection. *Anal. Chem.* **2001,** *73*(17), 4181–4189.
29. Boscaini, E.; Alexander, M. L.; Prazeller, P.; Mark, T. D. Investigation of Fundamental Physical Properties of a Polydimethylsiloxane (PDMS) Membrane Using a Proton Transfer Reaction Mass Spectrometer (PTRMS). *Int. J. Mass Spectrom.* **2004,** *239*(2/3), 179–186.
30. Diaz-Quijada, G. A.; Wayner, D. D. M. *Covalent Immobilization and Detection of Antibodies in Polymeric Microfluidic chips. Proceedings of the 87th Canadian Society of Chemistry Conference*; London, Ontario, Canada, 2004.
31. Barker, S. L.; Tarlov, M. J.; Canavan, H.; Hickman, J. J.; Locascio, L. E. Plastic Microfluidic Devices Modified with Polyelectrolyte Multilayers. *Anal. Chem.* **2000,** *72*(20), 4899–4903.
32. Okaniwa, M.; Ohta, Y. Novel Emulsion Graft Copolymerization onto the Silylmethyl Group of Poly(dimethylsiloxane). *J. Polymer. Sci. Part A. Polymer. Chem.* **1997,** *35*(13), 2607–2617.
33. Makamba, H.; Kim, J. H.; Lim, K.; Park, N.; Hahn, J. H. Surface Modification of Poly(Dimethylsiloxane) Microchannels. *Electrophoresis* **2003,** *24*(21), 3607–3619.
34. Carr, P. W.; Bowers, L. D. *Immobilized Enzymes in Analytical and Clinical Chemistry: Fundamentals and Applications*; John Wiley and Sons: New York, 1980, pp. 1, 48.
35. Pearson, J. E.; Gill, A.; Vagdama, P. Analytical Aspects of Biosensors. *Ann. Clin. Biochem.* **2000,** *37*(2), 119–145.
36. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformation*; Wiley-VCH: Weinheim, 1999.
37. Balcao, V. M.; Paiva, A. L.; Malcata, F. X. Bioreactors with Immobilized Lipases: State of the Art. *Enzyme Microbiol. Technol.* **1996,** *18*(6), 392–416.
38. Chiou, S. H.; Wu, W. T. Immobilization of *Candida rugosa* Lipase on Chitosan with Activation of the Hydroxyl groups. *Biomaterials* **2004,** *25*(2), 197–204.
39. Reis-Costa, L.; Soares, A. M.; Franca, S. C.; Trevisan, H. C. Immobilization of Lipases and Assay in Continuous Fixed Bed Reactors. *Protein Pept. Lett.* **2003,** *10*(6), 619–628.
40. Vogel, A. I. *Practical Organic Chemistry, 3rd ed.*; Lowe and Brydone Ltd.: London, 1972, p. 379.
41. Smith, M. B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*; Wiley-InterScience: New York, 2001, p. 483.
42. Moreno, J. M.; Hernaiz, M. J.; Sanchez-Montero, J. M.; Sinisterra, J. V.; Bustos, M. T.; Sanchez, M. E.; Bello, J. F. Covalent Immobilization of Pure Lipases A and B from *Candida rugosa*. *J. Mol. Catal. B Enzym.* **1997,** *2*, 177–184.
43. Mateo, C.; Abian, O.; Fernandez-Lafuente, R.; Guisan, J. M. Reversible Enzyme Immobilization via a Very Strong and Nondistorting Ionic Adsorption on Support-Polyethylenimine Composites. *Biotechnol. Bioeng.* **2000,** *68*(1), 98–105.
44. Han, H. S.; Yang, S. L.; Yeh, H. Y.; Lin, J. C.; Wu, H. L.; Shi, G. Y. Studies of a Novel Human Thrombodulin Immobilized Substrate: Surface Characterization and Anticoagulation Activity Evaluation. *J. Biomater. Sci. Polym. Ed.* **2001,** *12*(10), 1075–1089.
45. Hansen, K. F.; Lauritsen, F. R.; Degn, H. An On-Line Sampling System for Fermentation Monitoring Using Membrane Inlet Mass Spectrometry (MIMS): Application to Phenoxyacetic Acid Monitoring in Penicillin Fermentation. *Biotechnol. Bioeng.* **2004,** *44*(3), 347–353.
46. Soares, C. M.; De Castro, H. F.; De Moares, F. F.; Zanin, G. M. Characterization and Utilization of *Candida rugosa* Lipase on Controlled Pore Silica. *Appl. Biochem. Biotechnol.* **1999,** *79(1/3)*, 745–758.
47. Voet, D.; Voet, J. G.; Pratt, C. W. *Fundamentals of Biochemistry*; John Wiley and Sons: New York, 2002; p. 151.