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An enzymatic flow analysis method for the determination of phosphatidylcholine in sediment pore waters and extracts

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Abstract

A sensitive and selective flow injection method for the determination of phosphatidylcholine (PC) in sediment pore waters and extracts is described. It involves the use of phospholipase C, alkaline phosphatase and choline oxidase co-immobilized on controlled pore glass in a packed column reactor. The final product of the enzymatic reaction of phosphatidylcholine is hydrogen peroxide, and this is detected by measuring the chemiluminescence emission resulting from cobalt(II) catalysed reaction with luminol. The flow injection method is rapid (30 injections/h), reproducible (1.4% R.S.D. at 3 μM PC, $n = 10$) with a detection limit of 0.14 μM (estimated from $3\sigma_{n-1}$ of the measured blank). A linear calibration response was obtained over a concentration range of 0.5–9 μM ($r = 0.999$). The method has been applied to the determination of phosphatidylcholine in sediment extracts and sediment pore waters.

Keywords: Phospholipase C; Alkaline phosphatase; Choline oxidase; Immobilized enzyme(s); Luminol

1. Introduction

Eutrophication of lakes and waterways is an increasing problem. Nitrogen and phosphorus are thought to be the most important of the nutrients responsible for eutrophication, as they are the limiting factor controlling productivity of aquatic plants and algae [1,2]. Phosphorus is most commonly the limiting nutrient for algal growth in freshwater environments [3].

Inorganic phosphorus in the form of orthophosphate has long been regarded as the most bioavailable form of phosphorus, and the focus of most environmental studies has been on this form. However, organic phosphorus species may also constitute a sizeable proportion of the total P; this organic phosphorus fraction includes sugar phosphates, nucleic acids, inositol phosphates and phospholipids which are derived from the decomposition of plants and animals [4]. While the majority of studies of the cycling and bioavailability of phosphorus in aquatic systems have focused on inorganic phosphorus, the potential algal bioavailability of organic phosphorus has been largely ignored, and in part, this is perhaps due to a lack of simple quantification methods.

One group of organic phosphorus species, the phospholipids, occurs mostly in cellular membranes [5,6] and in higher plants and animals phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant, whereas in soils phosphatidylcholine followed by phosphatidylserine (PS) and phosphatidylethanolamine [5] are the dominant species [7]. In waters, the phospholipid concentrations are generally low, e.g. eutrophic lake waters were found to contain 0.7–6.4 $\mu g\,l^{-1}$ as P [8], and this may reflect the rapid degradation of phospholipids that occurs within hours following cell death [9,10].

However, substantial phospholipid concentrations have been reported in marine coastal sediments (1000–5000 $\mu g\,g^{-1}$ of dry weight [11], and this raises the possibility that sediment pore waters may contain

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higher concentrations of phospholipids. If this is the case, diffusion of pore water into overlying waters may thus constitute an important and relatively labile source of dissolved organic phosphorus. The ability to detect and quantify phospholipids in pore waters, sediment extracts and overlying waters has been the driver for the work described here.

A number of methods have been published for phospholipid analysis by high performance liquid chromatography (HPLC), usually involving ultraviolet (UV) detection. The poor UV absorbance of saturated phospholipids, limitations in the choice of eluents with suitable spectral characteristics that would not interfere with phospholipid absorbance, combined with the low sensitivity and non-linear response obtained using light scattering detection [12] makes these methods unsuitable for phospholipid detection in environmental samples. Detection of phospholipids on thin layer chromatography has also been reported [13] but the procedure is laborious and time consuming. Therefore, there is a need for a more rapid and selective method for the quantification of phosphatidylcholine in environmental samples, and particularly in sediment pore waters and extracts.

Enzymatic methods have been extensively reported for the determination of PC. In these methods, enzymes have been used in their soluble [14,15] or immobilized forms [16,17]. The use of enzymes in the their soluble form results in a method that is slower and more expensive compared to that when immobilized enzymes are employed. In a method developed by Kotsira and Clonis, two enzymes phospholipase D (PLD) and choline oxidase (ChO) and an indicator dye analogue, bromothymol blue–glutathione (BTB–GSH) conjugate, were co-immobilized on a gel [16]. Changes in the pH of the microenvironment of the immobilized enzymes results in a shift of the absorbance wavelength of the co-immobilized indicator dye conjugate (BTB–GSH). The change in the absorbance at λ_{max} is proportional to the PC concentration in the sample. However, this method is relatively slow, taking about 20 min before the change of absorbance can be measured. Furthermore, the immobilized system (PLD, ChO, and BTB–GSH conjugate) only has a lifetime of 2 weeks. Masoom et al. reported two methods for the determination of PC, both of which employed the use of immobilized enzyme reactors (IMERs) in flow injection systems with amperometric detection. The detection limits of these methods were 0.1 and 1.0 mM, respectively [17], which may have been due to the low conversion of PC by the enzymes, although this is unspecified.

In this paper, we describe the development of a selective enzymatic flow analysis method for the detection of phosphatidylcholine. This is based on the use of co-immobilized phospholipase C (PLC), alkaline phosphatase (AP) and choline oxidase (ChO) with detection of the chemiluminescence produced by the reaction between hydrogen peroxide and luminol, as shown in the enzymatic scheme in Fig. 1. The method is sufficiently sensitive to detect phosphatidylcholine in waters and sediment pore waters without preconcentration, and rapid when compared with liquid chromatographic techniques.

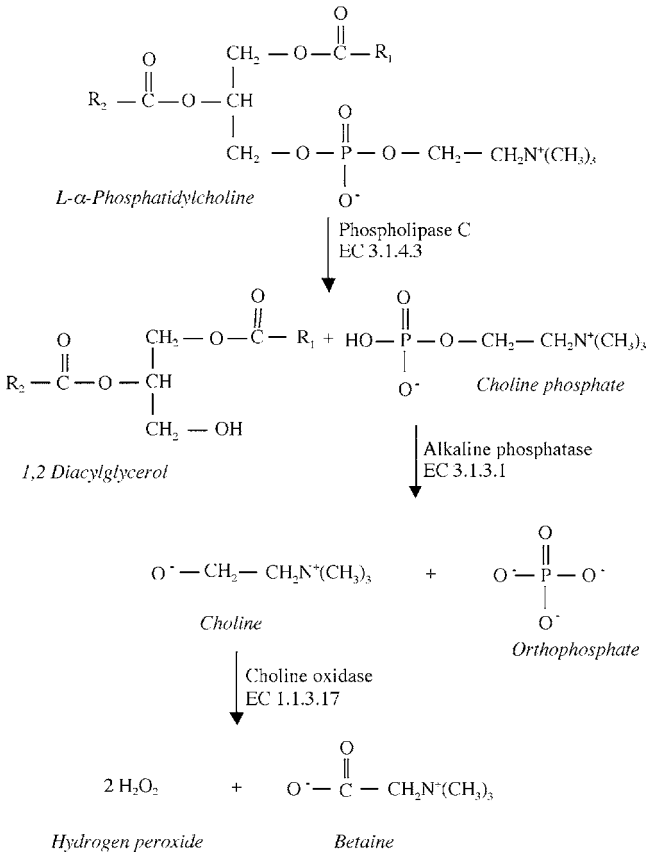

Fig. 1. Scheme for the enzymatic determination of PC using immobilized PLC, AP and ChO. Hydrogen peroxide produced from choline by ChO in the last step is detected using the chemiluminescence of luminol.

2. Experimental

2.1. Materials

The enzymes used were: AP (EC 3.1.3.1, from *Escherichia coli*, Sigma), ChO (EC 1.1.3.17, from *Alcaligenes* species, Sigma) and PLC (EC 3.1.4.3, from *Bacillus cereus*, Sigma). Enzymes were immobilized or co-immobilized onto aminopropyl-controlled pore glass (1273 Å, 125–177 μm particle size) obtained from CPG Inc. (NJ, USA).

All reagents used were of analytical grade and were as following: adenosine-5′-monophosphate hexahydrate (Sigma), anhydrous sodium carbonate (BDH); cobalt(II) chloride (BDH); disodium adenosine-5′-diphosphate (Sigma); disodium adenosine-5′-triphosphate (Sigma); disodium D-glucose-6-phosphate hydrate (Sigma); β-nicotinamide adenine dinucleotide (reduced form; Sigma); disodium phenyl phosphate (Sigma); glutaraldehyde (Ajax Chemicals; 25% solution); hydrochloric acid (BDH); hydrogen peroxide (BDH; 30% w/v); magnesium–potassium phytic acid

(Sigma); *t*-octylphenoxypolyethoxyethanol (Sigma); L-α-phosphatidylcholine (from frozen egg yolk, Sigma); L-α-phosphatidylethanolamine (from egg yolk, Sigma); L-α-phosphatidyl-L-serine (from Bovine brain, Sigma); *o*-phosphorylethanolamine (Sigma); potassium dihydrogen orthophosphate (BDH); potassium permanganate (BDH); sodium 5-amino-2,3-dihydro-1,4-phthalazinedione (Sigma); sodium hydroxide (BDH); sodium oxalate (BDH); sulfuric acid (BDH); trisodium inosine-5′-triphosphate (Sigma); trisodium phosphonoformic acid hexahydrate (Sigma); zinc chloride (BDH).

2.2. Immobilization procedure

The enzymes were immobilised on aminopropyl-controlled pore glass according to established procedures [18]. CPG beads (0.2 g) were placed in a stoppered vessel and 3 ml of 5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.0) was added to it. The beads were filtered and washed thoroughly with water after the vessel had been rotated end-over-end for 2 h at room temperature. A known number of units of enzyme in 1.0 ml phosphate buffer (0.1 M, pH 6.0) were mixed with the beads and left overnight at 4 °C. After completion of the immobilisation reaction, the beads were washed with phosphate buffer (0.1 M, pH 6.0) then packed and stored in a reactor made from cast acrylic rod (Machined Plastic Products Pty. Ltd.) with an internal diameter of 3 mm and a length of 22 mm (internal volume = 155 μl). The reactor was packed by pipetting the immobilised enzyme suspension in buffer into the reactor, while creating a vacuum at the bottom using a syringe. The packed reactor was plugged with nylon mesh (20 μm; Swiss Screens) at both ends to retain the immobilised enzyme beads. When not in use, the reactor was stored in 0.1 M sodium carbonate buffer, pH 7.5, at 4 °C.

2.3. Flow injection analysis system

The FIA system used comprised an electrically actuated rotary injection valve (Rheodyne 5041) and two peristaltic pumps (Ismatec CA-5E), with 0.5 mm internal diameter PTFE tubing used for reaction coils and injection loops (Fig. 2). A custom-made chemiluminescence detector consisting of a Hamamatsu compact photomultiplier (H5783) with stabilised high voltage power supply and amplification system housed in a light-tight box was used to detect hydrogen peroxide. The flow cell consisted of a Teflon gasket sandwiched between a stainless steel backing plate and a Vitreosil window held in a compression frame [19]. Flow through the cell was defined by an elliptical aperture cut in the Teflon gasket, the flow cell had a total volume of 110 ± 2 μl. Data were collected using a MacLab/200 system (AD Instruments Pty. Ltd., Australia). Cobalt(II) chloride was used as the catalyst for the luminol and hydrogen peroxide chemiluminescence reaction [19,20] and was pre-mixed on-line with luminol solution before merging with the carrier/sample at a T-piece within the detector enclosure and passing through the flow cell situated immediately in front of the photomultiplier window. The distance from the merging point to the flow cell was 2 cm. The optimal concentrations and pH of luminol and $CoCl_2$ reagents recommended by Fan et al. for the detection of hydrogen peroxide were used [19].

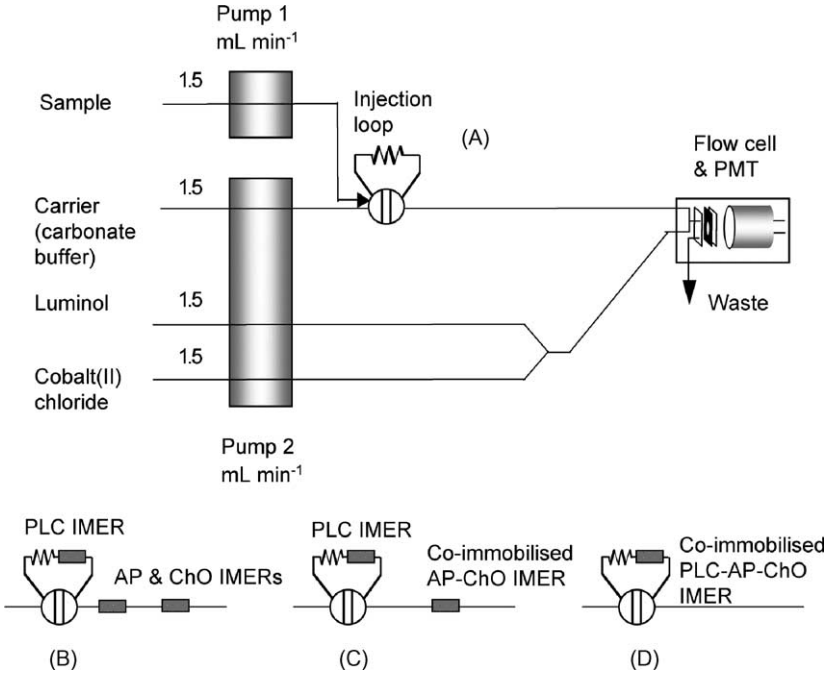

Fig. 2. Schematic diagram of the FIA manifold used for measurement of (A) hydrogen peroxide and phosphatidylcholine with three combinations of immobilization and placement for the enzyme reactors (B–D).

Phospholipase C (100 U), 40 U of alkaline phosphatase and 40 U of choline oxidase were either immobilised separately or co-immobilized on aminopropyl-controlled pore glass using the procedures described above, and packed in reactors. The activities of the separately immobilized and co-immobilized enzymes were compared by placing the appropriate reactor in the FIA manifold shown in Fig. 2(B)–(D) and calculating the conversion of a 370 μM PC sample prepare in 0.1 M sodium carbonate buffer, pH 7.5. The carrier used was 0.1 M sodium carbonate buffer, at pH 7.5, that contained 10 μM $ZnCl_2$ [21–23] and 0.3% (v/v) Triton X-100 [24,25].

Stopped-flow experiments were performed by placing the enzyme reactor, in the injection loop of the FIA manifold and switching off pump 1 for variable time periods.

2.4. Determination of optimum conditions

To determine the optimum pH and concentrations of $ZnCl_2$ and Triton X-100 for the co-immobilized enzymes, 0.1 M sodium carbonate buffers varying in pH and concentrations of $ZnCl_2$ and Triton X-100 were prepared and used as the carrier and also for preparing PC and standard hydrogen peroxide solutions. The co-immobilized PLC–AP–ChO reactor was placed in the injection loop of the FIA manifold shown in Fig. 2(D) and each solution was stopped in the reactor for 1 min. The % conversion of PC was calculated using standard hydrogen peroxide calibration graphs. Standard hydrogen peroxide and PC solutions were prepared using the carrier buffer. Therefore, the chances of any changes occurring in the emitted chemiluminescence as a result of altering the pH or $ZnCl_2$ and Triton X-100 concentrations were eliminated.

2.5. Sample collection and analysis

Samples for this work were collected from sediment core experiments being undertaken on contaminated sediments obtained from the Yarra River, south-east Australia. Intact sediment cores with overlying water were used to create environmental conditions similar to those from which they were collected. During the 17-day incubation of the sediment cores, only small samples could be withdrawn, and this provided only sufficient sample for duplicate analyses to be performed.

$ZnCl_2$ (10 μM) and 0.2% (v/v) of Triton X-100 were added to the sample prior to injection into the FIA manifold that included the co-immobilised PLC–AP–ChO IMER with the optimised conditions. To account for any hydrogen peroxide already present in the samples, a background signal was obtained by injection of the samples and quantified against standard hydrogen peroxide solutions, with the IMER removed from the manifold.

Sediment segments from the core reactor were sliced at the desired depth intervals under a nitrogen atmosphere. The interstitial (pore) water was extracted from the sediment samples by centrifuging (Beckman; Avanti^{TM} 30 centrifuge) the sediment at 7000 rpm for 10 min, at 23 °C, and filtered using 0.8 and 0.2 μm syringe filters in series. The samples were also passed through Sep-Pak® silica cartridges (Waters Associates) with a pore size of 125 Å and a particle size range of 55–105 μm to remove the hydrophilic components that were possibly complexing with the catalyst (Co^{2+}). The required volume of sample (1–2 ml) was passed through the cartridge, which was then rinsed with deionized water. Each sample was subsequently treated so that it contained 0.2% (v/v) of Triton X-100 and 10 μM of $ZnCl_2$.

In order to extract phospholipids from the sediment samples, after the interstitial water was removed, the sediments were air-dried, crushed and sieved using a 1 mm mesh size nylon sieve. To compare two different extraction methods, two 1 g sub-samples were weighed from each sample and were treated differently. Ten millilitres of a 20% (v/v) methanol/water solution was added to one set of the sub-samples and 10 ml of a 0.2% (v/v) Triton X-100/water solution was added to the other set. The sub-samples were rotated end-over-end using a vertical centrifuge (R.S.M. 6 Ratek Instruments) for 24 h at room temperature. The sediments were separated from the extracted solution by centrifuging (Z 230 A Hermle) for 5 min at 4000 rpm, then the supernatant solutions were filtered using 0.8 and 0.2 μm syringe filters in series and 0.2% (v/v) of Triton X-100 and 10 μM of $ZnCl_2$ were added to each sample.

3. Results and discussion

3.1. The effect of co-immobilization on the activities of the enzymes

Immobilization of more than one enzyme on the same matrix may influence the activities of the individual enzymes.

Table 1
Effect of the use of different combinations of immobilized and co-immobilized enzymes on conversion of phosphatidylcholine

IMER configuration	Fig. 2 configuration	Mean conversion of PC (%)	σ_{n-1} ($n = 4$)
Separately immobilized PLC, AP and ChO	B	0.06	0.01
Co-immobilized AP and ChO	C		
Uninterrupted flow		0.60	0.02
With 1 min stop-flow		0.86	0.01
Co-immobilized PLC, AP and ChO	D		
Uninterrupted flow		1.30	0.02
With 1 min stop-flow		12.30	0.10

Error bars are $\pm 1\sigma_{n-1}$ for $n = 4$.

For example, it has been observed in certain cases that co-immobilization of enzymes resulted in an increase in activity [26]. The effect of co-immobilization on AP and ChO, as well as on PLC, AP and ChO was investigated (Fig. 2(B)–(D)). The activities of AP and ChO were observed to increase approximately ten-fold when they were co-immobilized compared with the situation where separately immobilized AP and ChO were used. A similar effect has also been reported by Masoom [27]. However, when all three enzymes were co-immobilized, there was an increase in the total activity by a factor of 20 (Table 1). To our knowledge, this effect has not been reported previously. The increase in the activity may be due the availability of a higher concentration of substrate (produced by the first enzyme) in the immediate proximity of the active site of the second enzyme compared to the separately immobilized enzymes.

3.2. The effect of using a stopped-flow FIA on the conversion of PC

Table 1 shows that even with co-immobilization of all three enzymes, only 1.30% conversion of PC was achieved under conditions of uninterrupted flow. To investigate whether longer contact time between the substrate and co-immobilized enzymes had an effect on the conversion of the substrate, a number of stopped-flow experiments were performed. Maximum substrate conversion was achieved after a 3 min stopped-flow period as shown in Fig. 3. The conversion of PC decreased after a period of 3 min, which is most likely due to inhibition of AP [21,22,28,29] because of phosphate product competition with phosphoester substrates for the active sites of the enzyme [30]. A 1 min stopped-flow time was subsequently used as a compromise between the % conversion and sample throughput, and for the co-immobilized enzymes gave a % conversion in excess of 12% (Table 1).

The low conversion of PC observed in Fig. 3 occurred most probably because the concentration of PC used in this experiment was 370 μM, which is somewhat lower than most of the reported K_m values for these PCs [31,32] and thus the rate of reaction is expected to be lower in this concentration range. However, when coupled with a highly sensitive chemiluminescence detection system, this lower conversion efficiency is not problematic.

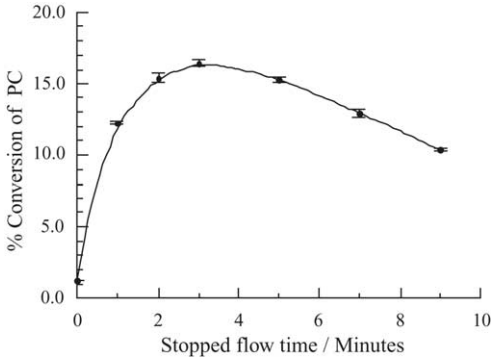

Fig. 3. The effect of stopping the flow in the co-immobilized PLC–AP–ChO IMER on the % conversion of PC (error bars are $\pm 1\sigma_{n-1}$ for $n = 4$).

3.3. The effect of pH and concentrations of $ZnCl_2$ and Triton X-100 on the activity of the enzymes

Both PLC (from *B. cereus*) and AP (from *E. coli*) are zinc metalloenzymes and their activities can be temporarily inhibited and restored by the removal and subsequent addition of zinc ions at the active sites [21–23]. To study this effect, zinc chloride was added to the buffer. Chloride has been reported to inhibit PLC in the mM range, possibly due to chloride interacting with the Zn^{2+} ions in the enzyme [33]. Thus the buffer used had a much lower concentration of chloride than the inhibitory concentration. Under these conditions, the optimum concentration of $ZnCl_2$ was found to be 10 μM (Fig. 4). At higher zinc concentrations the % conversion of PC decreased, an effect that has also been reported by Masoom et al. [17].

The activity of PLC also depends on the physical state of the substrate [24,34]. PLC functions best above the critical micelle concentration of phospholipids and the activity is greater for micellar phospholipids than monomeric forms [24,25]. In the presence of a surfactant, phospholipids are solubilized into mixed micelles, which are the most frequently utilised substrate form [24]. The mixed micelles that are formed offer the advantage that the physical state of the phospholipid can be controlled, and in excess surfactant the micellar structure should be similar regardless of the type of phospholipid. While surfactants often inhibit enzymes, non-ionic surfactants are generally less inhibitory [24,35]. The most commonly used surfactant is the uncharged Triton X-100 [34] and in the experiments described, this was added to the reaction media at concentrations greater than its CMC ($CMC_{Triton\ X\text{-}100} \approx 0.3$ mM) [35–37] to solubilize PC into mixed micelles. The concentration of Triton X-100 was altered in the buffer (pH 8.6, containing $ZnCl_2$) used as the carrier and the optimum concentration of Triton X-100 was found to be 0.2% (v/v) (Fig. 5). This value (0.2% v/v) corresponds to the reported molar ratio of 10:1 (Triton

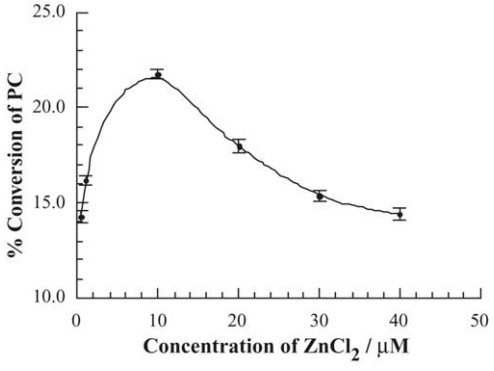

Fig. 4. The effect of $ZnCl_2$ concentration on the % conversion of PC by the co-immobilized PLC–AP–ChO IMER (error bars represent $\pm 1\sigma_{n-1}$ for $n = 4$).

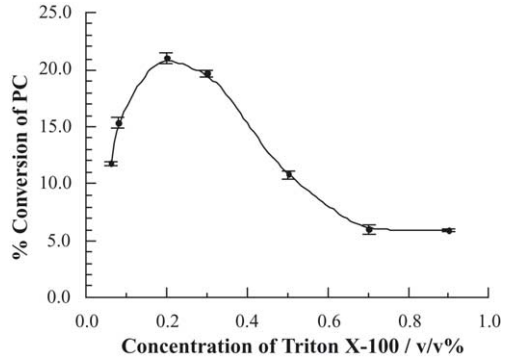

Fig. 5. The effect of Triton X-100 concentration on the % conversion of PC by the co-immobilized PLC–AP–ChO IMER (error bars represent $\pm 1\sigma_{n-1}$ for $n = 4$).

X-100/phosphatidylcholine) required for the formation of mixed micelles with a size range similar to pure Triton X-100 micelles [37], and it appears to be the preferred substrate size for PLC. At concentrations higher than 0.2% (v/v) the % conversion of PC decreased, which is probably as a result of competitive inhibition of PLC by Triton X-100 [38,39].

The pH optima of the enzymes reported by the manufacturer are 7.3 for PLC, 10.4 for AP and 8.0 for ChO, respectively at 37 °C [39]. Fig. 6 shows that there is an optimum pH for the co-immobilized enzymes at 8.6, with another smaller maximum at pH 7.6. These two peaks may arise because of the use of three enzymes all with different pH optima. The maximum point of the first peak represents the pH at which PLC shows its optimum performance, while the second peak at pH 8.6 represents the best pH compromise in obtaining the highest % conversion of PC to hydrogen peroxide via AP and ChO. These values may also reflect some pH optimum shift as a result of the covalent immobilization reaction.

3.4. Selectivity and evaluation of the analytical performance

The selectivity of this enzymatic FIA system in detection of PC was examined by injection of standard PC and a number of other organic phosphate compounds, as listed in the experimental section, into the FIA system. Of all the organic phosphate compounds injected only PC was detected, and this high selectivity is directly attributable to the combined use of ChO and chemiluminescence detection (Fig. 1).

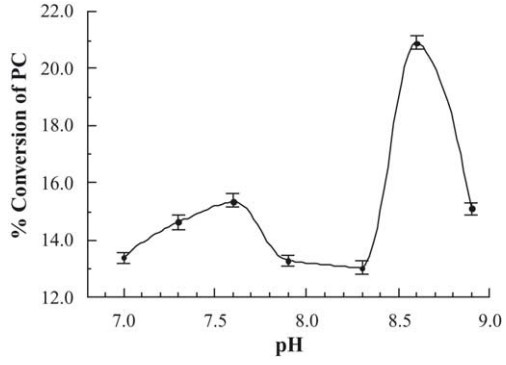

Fig. 6. The effect of pH on the % conversion of PC by the co-immobilized PLC–AP–ChO IMER (error bars represent $\pm 1\ \sigma_{n-1}$ for $n = 4$).

The method developed showed excellent linearity ($r = 0.999$) within the tested PC standard range of 0.5–9.0 μM. The limit of detection, the minimum concentration which can be distinguished from the blank with a statistical probability of 99%, i.e. signal to noise ratio of 3:1 [40], was 0.14 μM. Reproducibility of the technique was determined by performing 10 replicate injections of 3.0 μM PC, and was 1.4% R.S.D. Sample throughput was estimated to be 30 injections/h. Spike recovery tests on sediment interstitial water samples containing 0.1–1.4 μM PC were in the range of 65–85%. The co-immobilzed IMERs showed great operational and storage stability; one IMER used over a period of more than 3 months for the analysis of more than 300 samples showed no measurable decrease in activity.

3.5. Application of the FIA method to determination of phosphatidylcholine in waters, interstitial waters and sediment extracts

Sediment interstitial waters were collected from sediment at different depths and were analysed using the optimized FIA system. Negative peaks were observed, which were thought to be due to the presence of some interfering substances (e.g. natural organic matter) in the samples, which reduced the free concentration of the Co^{2+} catalyst through complexation [41]. This was overcome by passing the samples through Sep-Pak® cartridges. Positive peaks were observed after removing interfering ions present in the samples using the polar silica sorbent.

Phospholipids were extracted from the same sediment samples from which the interstitial waters were taken. The extraction was performed using two different extractants, Triton X-100/water and methanol/water (Table 2).

As expected from the higher abundance of bacteria in sediments (typically 10^9 bacteria ml^{-1} of sediment compared with 10^5 to 10^6 bacterial ml^{-1} of water column [42]), greater concentrations of phosphatidylcholine were found in the sediments and their interstitial waters than in the overlying water. Greater concentrations of phosphatidylcholine were extracted from the sediments using a Triton X-100/water solvent compared to using methanol/water (Table 2). A linear plot of the extraction efficiency of methanol/water versus that of Triton X-100/water gave the regression equation: $[PC]_{MeOH} = 0.766[PC]_{Triton\ X\text{-}100} - 0.051$, $r = 0.969$, ($n = 7$), and this is clearly due to the higher solubility of phosphatidylcholine in Triton X-100 compared to methanol. A paired *t*-test [40] performed on data for the two extraction methods suggests that they are significantly different at the 99.9% confidence level ($t = 8.34$, $n = 7$).

Subsequent application of this method to the analysis of sediment interstitial waters (Gippsland Lakes south-east Australia) before and after 7-day incubations showed that the

Table 2
Phosphatidylcholine concentrations measured in sediment interstitial water and sediment extracts

Sediment depth (cm)	Phosphatidylcholine concentration (μM)		
	Pore water concentration	Sediment extract (methanol/water extraction)	Sediment extract (Triton X-100/water extraction)
1–2	1.31, 1.38	0.73, 0.78	1.15, 1.19
2–3	1.01, 1.04	1.04, 1.09	1.53, 1.56
3–4	0.81, 0.85	1.18, 1.22	1.61, 1.66
4–5	0.17, 0.18	1.26, 1.30	1.68, 1.76
5–7.5	0.30, 0.32	1.40, 1.45	1.71, 1.77
7.5–10	1.14, 1.20	1.97, 2.03	2.72, 2.78
10–12.5	0.38, 0.41	1.32, 1.37	1.66, 1.70

phosphatidylcholine concentration increased from an initial value of 0.40 μM (12 $\mu g\,l^{-1}$ P) to 0.76 μM (24 $\mu g\,l^{-1}$ P) [oxic incubation] and 1.08 μM (33 $\mu g\,l^{-1}$ P) [anoxic incubation]. Such relatively large concentrations of this organic P moiety in the sediment pore water suggests that phospholipid sediment release may comprise an important source of bioavailable P, and serves to illustrate the value of developing new tools for the measurement of phosphorus species in environmental matrices.

4. Conclusions

A method is described for the determination of phosphatidylcholine that involves the use of co-immobilized phospholipase C, alkaline phosphatase and choline oxidase in a flow injection system in concert with chemiluminescent detection of product hydrogen peroxide. Compared with chromatographic techniques, the method is rapid, requires no preconcentration, and only minimal sample cleanup. Extraction of phosphatidylcholine from sediments with the surfactant Triton X-100 at a concentration near the critical micelle concentration was shown to be more efficient than extraction with methanol. The proposed method has been successfully applied to the determination of PC in sediments, sediment pore and overlying waters, and should prove to be a useful tool in the speciation of organic phosphorus in aquatic ecosystems.

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