The Influence of Conditioning Agent on Phosphate Diffusion Coefficient through Polyacrylamide and Agarose Gel

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ABSTRACT

Excess phosphate in natural water can cause algae to grow rapidly, and degenerate the quality of the ecosystem. Therefore, analyses or periodic observation of phosphate levels in the water is needed. One of the commonly used methods is diffusive gradient in thin films (DGT) technique. The DGT technique is based on the ability of analyte to diffuse through a gel, called diffusion coefficient. This research was conducted in order to study the effect of different storage solution for polyacrylamide and agarose gels to the phosphate diffusion coefficient through the gels. One step of the research was preparing the polyacrylamide and agarose gels. To observe the effect of different storage solutions, several gels were stored in distilled water gel while the others were stored in NaCl solution of 0.01 M. Phosphate diffusion coefficient was determined using Fick's Law and the analyses of phosphate concentration was using UV-Visible spectrophotometer. The results showed that phosphate diffusion coefficient was high when polyacrylamide and agarose gels had been stored in NaCl solution of 0.01 M.

Key words: phosphate, diffusion coefficient, polyacrylamide gels, agarose gels.

INTRODUCTION

Phosphate is a nutrient needed by living things, so excess phosphate in water can cause algae blooming that further even decreasing the water quality. However, excess phosphate in human body gives negative impact since it will lead to deposition of calcium phosphate onto the walls of blood vessels (vascular calcification) and increasing the risk of heart disease. Therefore, an reliable analysis method of phosphate concentration in the water is required.

The DGT (diffusive gradients in thin films) technique is type one of passive sampler that used for analyte separation, minimize damage, and contamination, so that the analysis of phosphate in the water will be more accurate. The analyte will diffuse through the hydrogel and then bind the adsorbent impregnated in the hydrogels which is called resin sink [1-3]. Analyte that is bound to the adsorbent is then generally eluted using an eluent, and the concentration is measured by using an appropriate analytical technique [4].

Hydrogels are used for its ability to limit the movement of solute other than analyte (like organic substances) so that the membrane is more selective. Based on the structure, hydrogels can be divided into two types, the homogeneous and the heterogeneous. The examples of homogeneous hydrogels are polyethylene oxide, polyacrylamide, and polyvinyl alcohol while the heterogeneous are calcium alginate hydrogel, agarose, and κ-carrageenan

[5]. The most commonly used hydrogel in DGT technique should have physically strong resistance [4]. The hydrogels that often used in the DGT technique are made of polyacrylamide (agarose cross-linked polyacrylamide and bis cross-linked polyacrylamide) and agarose.

Diffusive gel should be stored or conditioned in electrolytes such as $NaNO_3$ (0.01-0.1 M) before use [3]. The treatment is intended to prevent the diffusive gel surface becomes charged. If the diffusive gel surface is charged, either positive or negative, it interfere the diffusion of analyte. Therefore, in this study we focus on the ability of polyacrylamide and agarose hydrogel to act as a diffusive gel for phosphate. The experiments will be reported as diffusion coefficients and factors that affect the diffusion coefficient.

EXPERIMENT

Materials

Chemicals used in this study are of pro-analysis (pa) grades (Merck). The solids used include sodium chloride, potassium dihydrogen phosphate, bisacrylamide, agarose, ammonium heptamolybdate, stannous chloride, and ammonium persulphate. The other materials are liquids of acrylamide solution (40%), tetramethylethylenediamine (TEMED), sulfuric acid solution (95-97%), glycerol, and aquades.

Instrumentation

The instrumentation used in this study include heater (IKAMAG®RH), scales (Adventurer AR 2130), magnetic stirrer, pipette controller, micropipette (Accumax Pro), *syringe* (TERUMO ®), thermometer, chemistry laboratory glassware, Shimadzu 1601 UV-Visible spectrophotometer, and *diffusion cell*.

The diffusion cell has two compartments and an interconnection hole with diameter of 3.5 cm. Compartment A is for a feed phase (analyte) and compartment B is for the receiving phase (aquadest), each can hold up to 90 mL of liquid. Diffusive gel disc which has a diameter of 3 cm, both surfaces layered with a membrane filter, was put in an o-ring placed in the interconnection hole right between the two compartments as shown in Figure 1. Sample solutions are taken from sampler site located at the top of compartment A.

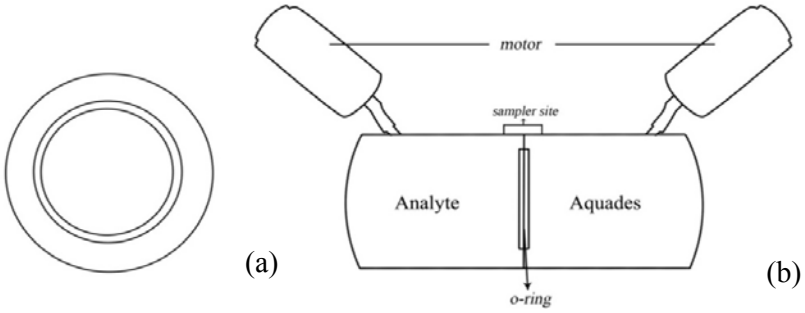

Figure 1. (a) O-ring; (b) Diffusion cell. Motor was used to stir the liquid in the diffusion cell at the fixed time.

Preparation of Gel Solution

Bis-polyacrylamide 1 (BPA1) gel solution was prepared from 18.75 mL of 40% acrylamide solution added with 0.15 grams of bisacrylamide. This suspension was stirred

until the bisacrylamide dissolved completely. Solution was diluted with distilled water up to 50 mL in a volumetric flask.

Bis-polyacrylamide 2 (BPA2) gel solution was prepared from 12.5 mL of 40% acrylamide solution added with one gram of bisacrylamide. This suspension was stirred until the bisacrylamide dissolved completely. Solution was diluted with distilled water up to 100 mL in a volumetric flask.

Preparation of Diffusive Gel

To prepare BPA 1 and 2 gels each of 10 mL BPA 1 and 2 gel solution was added with 70 µL ammonium persulphate solution and 20 µL TEMED. The solution was stirred using a magnetic stirrer until homogeneous. The solutions were then drawn using a pipette and placed into a mold, and aged at room temperature for at least 1.5 hours.

To prepare agarose gel 0.15 g of agarose was put in the beaker and then added with 10 mL of hot distilled water. If the agarose didn't dissolve completely, the beaker was covered with a watch glass and heated until the agarose dissolve completely. The gel solution was then drawn using a pipette and placed into a mold, and aged at room temperature to form a gel.

Each of those formed gels was washed with distilled water two to three times and then soaked in distilled water. The gel's length, width, and thick were measured, before and after immersion. The gels were stored in distilled water and the 0.01 M NaCl before used.

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The gel that stored in distilled water was cut into a circle with a diameter of 3 cm. Both surfaces of each gel were layered with a membrane filter. The gel was then placed in the o-ring, and fixed between the two compartments. Compartment A was filled with a solution of analyte (phosphate, 100 ppm) and compartment B was filled with distilled water. The solutions in both compartments were stirred using an electromotor for an hour, and every 10 minutes the one in compartment A was drawn 1 mL. The concentration of the analyte was analyzed with spectrophotometer. The temperature of the solution before and after the experiment was measured. The same procedure was carried out on polyacrylamide and agarose gels that were stored in a solution of 0.01 M NaCl.

RESULT AND DISCUSSION

Properties of the Gel

Table 1 shows the properties of the formed gels stored in distilled water and 0.01 M NaCl. Expansion factors were calculated by comparing the volume of the gel before and after immersed in the storage solution. Porosity is the ratio of empty space in the gel filled by water as compared to the total volume of the gel. Porosity determination was developed from Cwirko and Carbonell methods [6]. Although the structure and chemical formula of polyacrylamide and agarose gels have been widely known, the ability of the gel as a diffusive gel cannot be predicted [3]. Polyacrylamide gels tend to swell when immersed in water, however, the expansion factor will differ depending on the concentration of monomer and cross-linker.

The expansion factor of BPA1 gel decreases when it is stored in a solution of 0.01 M NaCl. The differences of both expansion factors (gels stored in distilled water and 0.01 M NaCl) may be caused by the osmotic pressure difference between the two storage solutions.

An osmotic pressure between the solvent outside and inside the gel is generated because of the initially high polymer concentration in the network. This osmotic pressure drives solvent molecules into the network, which makes an increase of the network volume. Gel will stop expanding when there is no longer pressure difference (already reached equilibrium) [7]. The distilled water or 0.01 M NaCl drives into the gel until there is no difference between the pressure inside and outside the gel. Since the 0.01 M NaCl solution has a greater osmotic pressure than distilled water, the equilibrium pressure outside and inside the gel achieved faster so that BPA1 gel stored in 0.01 M NaCl does not expand as much as stored in distilled water.

Table 1. Polyacrylamide and agarose gel properties

Gels	Color	Expansion Factor (distilled water)	Expansion Factor (0.01 M NaCl)	Porosity (distilled water)	Porosity (0.01 M NaCl)	Texture
BPA1	Colorless	1.675	1.07	0.403	0.065	Robust
BPA2	White	1	1	0	0	Easily torn
AGE	Turbid	1	1	0	0	Robust

Information: BPA1 gel is polyacrylamide gel made from 15% acrylamide as the monomer and 0.3% bis-acrylamide as the cross-linker, while BPA 2 gel is made from 5% acrylamide and 1% bis-acrylamide. AGE is an agarose gel with monomer concentration of 1.5%.

Compared with BPA1 gel, the expansion factor of BPA2 and AGE gels stored in 0.01 M NaCl solution did not change when both are stored in distilled water. It was consistent with the porosity data of both gel. This means the functional groups present in the polymer networks on both gels no longer able to bind water from the storage solution so that the gels do not swell. Although the BPA2 gels have the cross-linker concentrations higher than that of BPA1 gels, the initial concentration of its monomer is lower, only 5%. Low acrylamide monomer concentration is likely producing shorter polymer chains so that the network in the gel is less robust when compared with BPA1. In the steps after, the gels used were BPA1 and AGE. BPA2 gel was not studied further because it was very fragile.

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Table 2 shows the diffusion coefficient of phosphate through gels after they are stored in different type of storage solution. Based on the data in Table 2, in general, both these storage solutions gave the highest diffusion coefficient of phosphate when agarose gel was used as the diffusive gel. This is probably due to agarose gel has pore size >20 nm, larger than polyacrylamide gel pore size >2 nm [3], thus allowing phosphate ions more easily pass through the gel. The largest diffusion coefficient (10.50×10^{-7} cm^2/s) is given by agarose gel.

The phosphate diffusion coefficients are better, i.e. higher, if BPA1 and AGE gel were previously stored in 0.01 M NaCl. The electrolyte solution seems to reduce the occurrence of the junction potential effect by providing sufficient conductivity to prevent the accumulation of charge on the surface of the gel [3]. Junction potential effects arise when the two solutions with different concentrations made a contact. The solution that contains higher concentration of ions will tend to diffuse towards the solution with a lower concentration [8]. The rate of diffusion of each ion will be different, depending on the movement of ions in solution.

The movement of ions in solution is affected by the mass. If the cations have a lower mass than the anions, it will diffuse faster than anions. The cation filling up the solution with lower concentration, leaving the previous solution full of negative charge, so that both surfaces of the gels will form a layer of electrical double layer, which is a negatively charged and the other part positively charged [8]. The negative charged layer will repel the negative charged ions, i.e. phosphate ions, so that the phosphate becomes more difficult to diffuse through the BPA1 and AGE gel that stored in distilled water.

Table 2. Phosphate diffusion coefficient through gel with a different storage solution

Conditioning Agent	Gels	Diffusion Coefficient (cm^2/s) (25 ^oC)
Distilled Water	BPA1	4.94×10^{-7}
	AGE	8.49×10^{-7}
NaCl (0.01 M)	BPA1	5.23×10^{-7}
	AGE	10.5×10^{-7}

CONCLUSION

Based on the results of research and discussion, it can be concluded that the type of storage solution affects the value of phosphate diffusion coefficient through polyacrylamide and agarose gels. The phosphate diffusion coefficients were high when polyacrylamide and agarose gels were stored in a solution of 0.01 M NaCl.

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