

Diffusion Coefficient of Phosphate Ion in Citric Acid-Agarose Gel Used in Diffusive Gradient in Thin Films (DGT) Passive Sampler

Digwanggi Arum Tazkiyatu An-Nufuus¹, Barlah Rumhayati^{2*}, Qonitah Fardiyah², Diah Mardiana², Ulfa Andayani², Layta Dinira²

¹ Postgraduate Student Department of Chemistry, Brawijaya University, Malang, Indonesia.

² Department of Chemistry, Brawijaya University, Malang, Indonesia.

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Corresponding Author:

Barlah Rumhayati

rumhayati_barlah@gmail.com

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Abstract: In this research, the diffusion coefficient of phosphate ions in agarose-citric acid diffusive gel has been studied. The agarose-citric acid diffusion layer was prepared by mixing 1.5% agarose solution with the addition of citric acid solutions at various concentration. The diffusive gel was casted between two glass plates which were separated using a spacer of 0.1 cm thickness and clamped together in the three sides. Gel was formed for 15 minutes in an oven at 40°C. The diffusion coefficient then was calculated using Fick's First law formula. Gel that produced with addition of 0.45% citric acid absorbed more water during immersion in water for 3 hours for facilitating phosphate diffusion. Swelling degree of the agarose citric acid diffusive gels was not affected by the storing solution condition either in water or in NaNO₃ 0.1 M. Coefficient diffusion of phosphate in the agarose-citric acid diffusive gel was 1.009×10⁻⁷ cm²s⁻¹, less than the diffusion in the pure agarose gel 1.367×10⁻⁷ cm²s⁻¹. The diffusion is affected by the ionic strength of solution. Phosphate diffusion coefficient is higher in the phosphate solution with addition of 2 mgL⁻¹ nitrate. Increasing the ionic strength causes lower phosphate diffusion coefficient because the electrostatic attractive and repulsive force.

Keywords: Agarose; Citric Acid; Diffusion Coefficient; Diffusion Gel; Swelling

Introduction

One of the essential needs of all living things is water. As many as 2694 households experienced water quality, 19% of the water tasted bad, 19% smelled water, and 38% experienced water quality problems (Fransiska, 2022). In addition, one of the most polluted bays in the world, namely Jakarta Bay and Benoa Bay, have quite high levels of nitrogen and phosphorus (Damar et al., 2019; Suteja & Purwiyanto, 2018). Excess phosphates and nitrates have the potential to cause eutrophication (Suteja & Purwiyanto, 2018), most of which come from industrial discharges, household waste, and agricultural waste which accelerate the eutrophication process. Excessive phosphate in waters causes anoxic and also accelerates the growth of aquatic plants and algae which

pollute water intakes and waterways (Widyarani et al., 2022; Ngatia & Taylor, 2019).

Therefore, it is necessary to monitor water one of which is monitoring phosphate in water. Such monitoring can be done manually, with automatic sampling with laboratory analysis, and continuous in situ monitoring. Recently, passive in situ sampling using a passive sampler has been developed for phosphate monitoring. Passive samplers have many advantages, such as simple design, suitable for long periods of measurement, and have no mechanical noise. Passive sampling is driven by diffusion, allowing for the collection of solutes in water without the need for pumping. This method is suitable for monitoring phosphate and other solutes in water (Kalkhajeh et al., 2019).

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In this study, DGT was used as a passive sampler for monitoring phosphate. This method is based on the diffusion of the analyte between two compartments with different chemical potentials. This method is a combination of techniques for taking analytes, concentrating, and separating analytes at once in one sampling process. In the DGT technique, phosphate ions diffuse through a diffusive gel which is then bound to a binding gel containing a binder (Wahyuni & Saefumillah, 2019). When through the diffusive layer a linear concentration gradient is formed during the spread in the solution. During the measurement, the amount of analyte eluted from the binding gel (M) is correlated to the concentration of analyte in the sample (C), the diffusion coefficient of analyte passes through the diffusive gel (D) with the thickness of the diffusion layer (Δg), the deployment time (t), and the surface area of the gel exposing to the solution (A) (Cao et al., 2022), as shown in Eq. 1.

$$M = \frac{C D A t}{\Delta g} \quad (1)$$

As the analyte diffuses through the diffusive gel, the rate of diffusion can be determined by the diffusion coefficient of the analyte in the diffusive gel. For quantitative measurement, the diffusion coefficient (D) is a key parameter needed to calculate the time-weighted average concentration in passive sampling device of DGT (Bonnaud et al., 2021; Urik et al., 2020). The diffusion coefficient is a measure of the rate at which a substance diffuses through a medium and is influenced by various factors such as temperature, pH, and the type of diffusive gel used (Bonnaud et al., 2021; Urik et al., 2020).

Agarose hydrogel have a pore size of more than 20 nm (Li et al., 2019). For selective diffusion, adding of crosslinker to the diffusive gel can reduce the pore size of the agarose hydrogel. Citric acid can be used for cross-binding because it is biocompatible, soluble in water, and includes mild organic acids (Mariam Ninan et al., 2020; Nangare et al., 2021). Citric acid forms cyclic anhydride and has hydroxyl group that is in the adjacent polymer chain which leads to the formation of cross bonds (Mali, Dhawale, Dias, Dhane, & Ghorpade, 2018). Cross-binding temperature, cross-binding time, and crosslinker concentration can affect the performance of agarose-citric acid hydrogel in term of absorbing water.

As a diffusive layer in the DGT method, diffusion coefficient of analyte in the diffusive layer has to be determined firstly. The diffusive layer should be diffused analyte selectively. Therefore, the addition of crosslinker, such as citric acid, into agarose solution gel is aimed to reduce the hydrogel pore size so increasing the selectivity of the diffusive layer. In this present research, the agarose-citric acid diffusive hydrogel was

produced. The diffusion coefficient of phosphate in the diffusive gel has been determined in different phosphate bulk solutions condition at different ionic strength. In addition, the properties of agarose-citric acid hydrogel were also investigated including the gel swelling ratio, and gel reactivity.

Method

Materials and Instrumentals

Materials used in this study were agarose powder for molecular biology (Himedia), citric acid (99.5% purity ACS reagent, Sigma), sodium chloride (Sigma), hydrochloric acid (Sigma), potassium dihydrogen phosphate (Sigma), glycerol (Sigma), Tin(II) Chloride Dihydrate (Sigma), Sulfuric Acid (Sigma), Ammonium Molybdate Tetrahydrate (Sigma), Sodium hydroxide (Sigma) and pure water. analytical class.

A glass tool set is used to prepare all solutions. The diffusive gel was casted using a glass mold with dimensions of 16x10 cm. The two glass molds are separated by a Teflon U-spacer (0.5 mm thick), binder clips, an oven (Memmert), an analytical balance used to measure the weight of the gel before and after swelling, a Diffusion cell with motor, and an O-ring. UV-vis spectrophotometer (Thermo Scientific Genesys) was used for phosphate analysis. FTIR (Shimadzu) was used for gel characterization.

Gel preparation

Approximately 45 mL of hot water was mixed with agarose powder in a 250 mL beaker to prepare a 0.75 gram agarose gel solution. The mixture was then stirred well until the agarose was completely dissolved. Then the solution was allowed to cool down to 60°C. While waiting for the agarose to cool, citric acid solutions 0.375; 0.300; 0.225; 0.150; 0.075; and 0.000 gram were prepared by dissolving citric acid in 5 mL of water. The citric acid solution was then poured into the agarose gel solution, stirred briefly, and poured into the gel mold as shown in Figure 1. The pre-casting gel was dried in an oven at 40°C for 15 minutes. After 15 minutes, the gel layer was cooled by allowing it to stand at room temperature for 1 hour. After that, the gel layer was pulled out of the mold and then cut into disks with a diameter size of approximately 3 cm.

Gel Properties

Measurement of Swelling Ratio

A disk of diffusive hydrogel layer should be swollen to a dimension stable in the storing solution before using as a diffusive layer in DGT passive sampler. Therefore, the agarose-citric acid gels that have been formed were observed for it's swelling ratio by soaking it in several storing solutions, *i.e.* distilled water, and 0.1 M sodium chloride solution for 3 hours. The mass of the

gel disk was weighed before and after being immersed in the media. The ratio of swelling can be calculated using Equation 2.

$$\text{Swelling ratio} = \frac{\text{Weight of Swollen gel}}{\text{Weight of dried gel}} \quad (2)$$

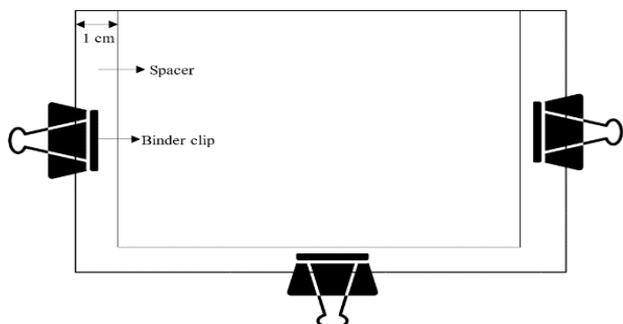


Figure 1. Gel mold 16 x 10 cm with teflon U-spacer (0.1 mm thick) and binder clips

Gel Reactivity

The diffusive gel should not adsorb the purposed analyte during measurement. For this reason, diffusive gel disks of crosslinked or natural agarose were immersed in solution containing 1 mgL⁻¹ of phosphate. The adsorption was conducted for 24 hours while shaking 100 rpm at room temperature using a shaker. After 24 hours, phosphate adsorbed on the diffusive gel was eluted using 15 mL of 0.3M H₂SO₄ solution by shaking with a shaker. Concentration of eluted phosphate then was detected using a UV-vis spectrophotometer.

Phosphate diffusion coefficient measurement

For this investigation, the diffusive gel disk 3 cm in diameter were placed in an o-ring as shown in Figure 2a which was made of acrylic and then placed between compartments A and B in a diffusion cell. Then the device is assembled as shown in Figure 2b. Compartment A was filled with solution (phosphate solution 1 mgL⁻¹; mixed solution A (Phosphate 1 mgL⁻¹ and NaNO₃ 2 mgL⁻¹); mixed solution B (phosphate 1 mgL⁻¹ and NaNO₃ 0.1M) while compartment B was filled with distilled water. Filling the solution in the compartment, is done simultaneously avoiding the gel diffusion out of the o-ring.

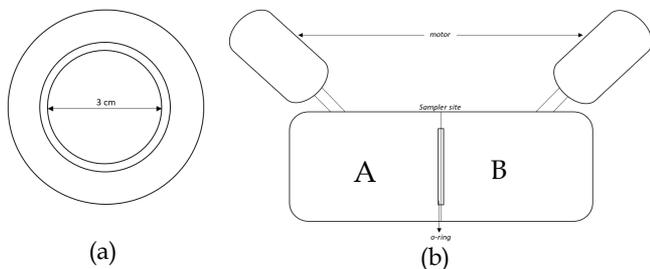


Figure 2. (a)O-ring; (b) Diffusion Cell

Both compartments were stirred using a motor (propeller) for 24 hours at speed of 100 rpm. 1 mL of phosphate solution was taken from compartment A from 1-7 hours.

Data Analysis

The diffusion coefficient of the analyte in diffusive gel layer can be determined by Fick's First law of diffusion Equation 3 (Hodges et al., 2023).

$$F = D \left(\frac{\partial C}{\partial x} \right) \quad (3)$$

F is the analyte flux through the gel (gcm⁻²s⁻¹), D is the diffusion coefficient (cm²s⁻¹), and (∂C/∂x) is the change in concentration across the gel. At a certain time, the ions in the analyte solution will diffuse from high concentration to low concentration by passing through the gel causing a change in concentration. If it is assumed that the displaced mass is small compared the initial mass, then the difference in concentration (C (gcm⁻³)) passing through a gel with a certain thickness (Δg(cm)), will be constant and consequently the flux will also be constant. The flux is transported mass (M) in grams, per unit area (A) in cm², per unit time (seconds).

$$F = \frac{M}{At} \quad (4)$$

The two equations are combined by assuming that C is constant, we get the equation regarding the mass of the analyte displaced.

$$M = \frac{DA \Delta C t}{\Delta g} \quad (5)$$

From this equation a graph is made between mass (M) and time (t), which will produce a linear line with a slope (α) of DACΔg⁻¹. If the values of A, Δg, and C are known, then the value of the diffusion coefficient, D, can be determined by Equation 6.

$$D_{\text{salt}} = \frac{\alpha \Delta g}{CA} \quad (6)$$

The value of the diffusion coefficient calculated from the equation is the salt diffusion coefficient. Diffusion coefficients such as phosphate are referred to as self-diffusion coefficients. The self-diffusion coefficient value depends on the salt diffusion coefficient as in the following equation.

$$D_{\text{cation}} = (9.55 + 0,409T) \times 10^{-6} \quad (7)$$

$$D_{\text{anion}} = \frac{D_{\text{salt}}(|Z_1|C_1 D_{\text{cation}} + |Z_2|C_2 D_{\text{anion}})}{D_{\text{cation}}(|Z_1|C_1 + |Z_2|C_2)} \quad (8)$$

$$\log D_{\text{anion}} = \frac{1,37023 (T - 25) + 8,36 \times 10^{-4} (T - 25)^2}{109 + T} + \log \frac{D_{25}(273 + T)}{298} \quad (9)$$

D_{cation} and D_{anion} are self-diffusion coefficients for cations and anions. Z_1 and Z_2 are the ionic charge, C_1 and C_2 are the concentrations of cations and anions. The diffusion coefficient of phosphate at given temperature can be calculated based on the Equation 8. Meanwhile, the diffusion coefficient of phosphate at 25°C can be calculated using Equation 9.

Result and Discussion

Gel properties

Selected agarose-citric acid diffusion gels were subjected to FTIR characterization to determine the functional groups in the gel and see whether the gel was cross-linked or not. According to the structure of agarose and citric acid in Figure 3, there is no C=O bond in agarose. The FTIR spectra are shown in Figure 4 for the crosslinked hydrogel and pure agarose gels. The 0.45% citric acid-agarose hydrogel has a peak at 1730 cm^{-1} which does not exist in the spectra of pure agarose hydrogel.

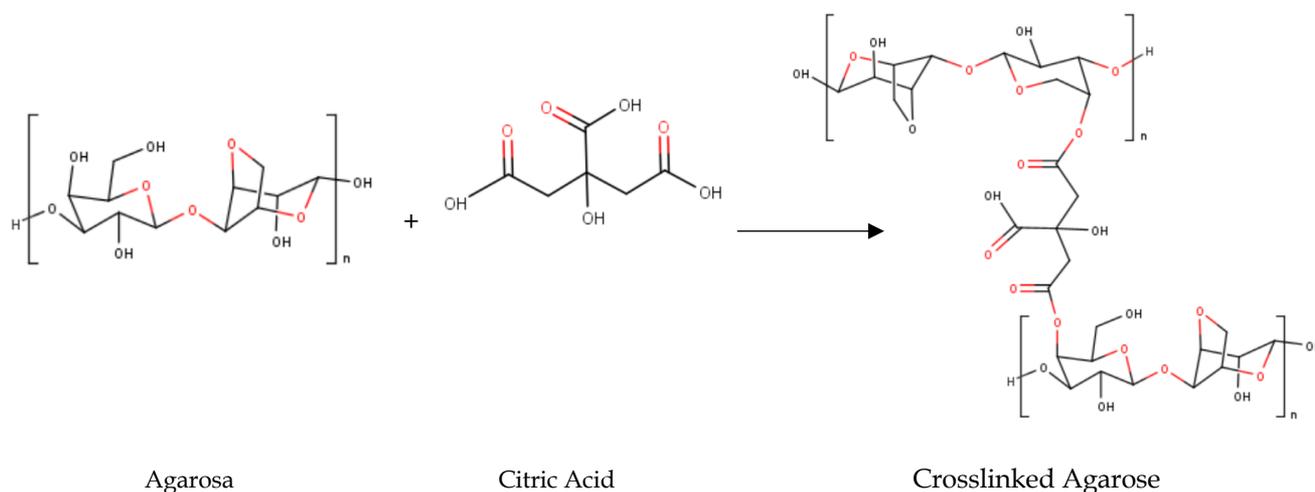


Figure 3. Agarose/citric acid crosslinking reaction

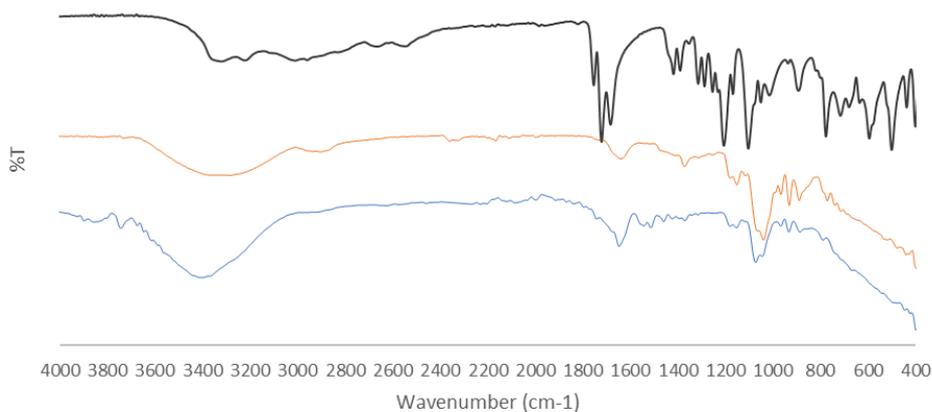


Figure 4. IR spectrum of – 0.45% Citric Acid crosslinked agarose, – pure agarose, – citric Acid

The wavelength of 1730 cm^{-1} refers to ester functional group which occur when citric acid reacts with agarose by esterification reaction (Figure 3). The esterification reaction that occurs is slow because this study did not use a catalyst. Esterification reaction between agarose and citric acid produced the

crosslinked citric acid agarose that can be identified from the high intensity of ester peak. This ester peak is not shown in pure agarose spectra.

Crosslinker serves as a link between monomers with other monomers in hydrogel, one of which is citric acid. Citric acid forms a cyclic anhydride and esterifies

the hydroxyl groups present on adjacent polymer chains leading to the formation of crosslinks (Mali et al., 2018). The crosslinker concentration can affect the amount of water adsorbed by the hydrogel. Agarose hydrogel without crosslinker had a fairly large pore size of more than 20 nm. The purpose of adding a crosslinker is to reduce the hydrogel pore size that the ions will be more diffusive selectively. So, in our study we used 0.00; 0.15; 0.30; 0.45; 0.60; and 0.75% of the agarose used in pure water.

The purpose of adding a crosslinker is to reduce the hydrogel pore size so that the ions will be more diffusive selectively. So, in our study we used 0.00; 0.15; 0.30; 0.45; 0.60; and 0.75% of the agarose used in pure water. Its should be clear and concise. The discussion should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

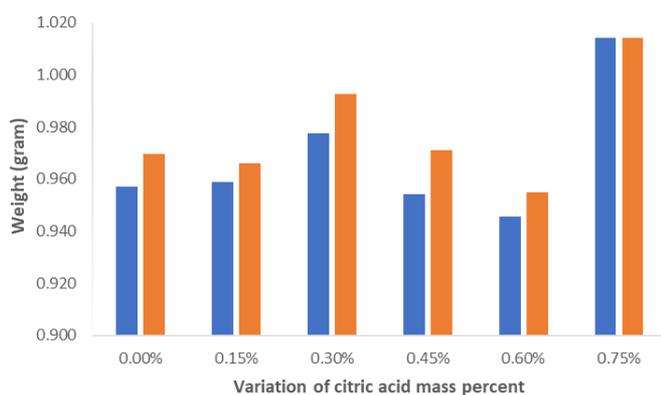


Figure 5. Hydrogel weight before swelling, and after swelling 3 hours in distilled water.

As shown in Figure 5, each hydrogel with varying masses experienced water absorption except for 0.75% citric acid-agarose hydrogel. The higher the cross-linker mass, the stiffer and less flexible the hydrogel so could not absorb water for facilitating diffusion. It can be seen that agarose hydrogels with 0.00%, 0.30%, and 0.45% citric acid were able to absorb water optimally compared to other hydrogels. In the 0.00% gel, the initial weight was 0.957 and the weight after 3 hours of soaking was 0.970, resulting in a swelling ratio of 1.013. The 0.30% gel had an initial weight of 0.978 and a weight after 3 hours of soaking of 0.992, resulting in a swelling ratio of 1.015. Meanwhile, the 0.45% gel had an initial weight of 0.954 and a weight after 3 hours of soaking of 0.971, resulting in a swelling ratio of 1.018. Among these three gels, the 0.45% gel was able to absorb the most water, as indicated by its higher swelling ratio. Therefore, the 0.45% citric acid-agarose hydrogel was chosen to be used as the diffusive layer.

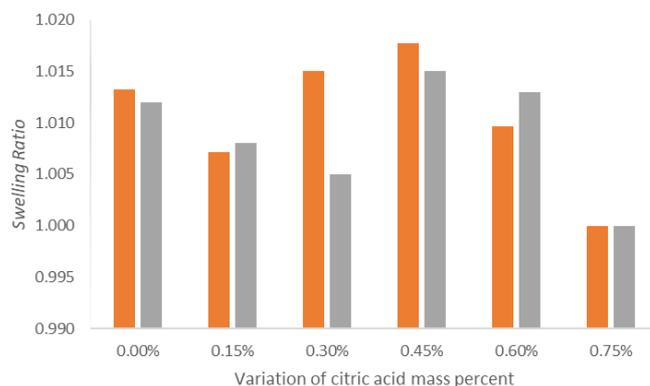


Figure 6. Swelling ratio of agarose-citric acid in different storing solution (distilled water NaCl 0.1M).

Figure 6 shows the nature of the formed gel which was then stored in distilled water and 0.1M NaCl in terms of swelling ratio. It can be observed that the gel at 0.75% concentration did not undergo any changes in swelling, which can be attributed to the increased amount of crosslinker used resulting in a denser gel that absorbs less water or solution. Meanwhile, the gels at 0.00%, 0.30%, and 0.45% showed higher swelling when soaked in water. This could occur due to the difference in osmotic pressure between 0.1M NaCl and water. Hydrogels are polymeric networks capable of absorbing and retaining a large amount of water. When the hydrogel is soaked in water, water enters the hydrogel network through osmosis, causing the hydrogel to expand and swell due to the amount of water absorbed (Fayer, 2023). However, when the hydrogel is soaked in 0.1M NaCl, it was found to have lower swelling than when soaked in water. This happens because of the difference in osmotic pressure between the NaCl solution and water inside the hydrogel. Osmotic pressure drives solvent molecules into the tissue, increasing tissue volume. Swelling stops when there is no pressure difference or it has reached equilibrium (Vervoort, n.d., 2006). This is because 0.1M NaCl solution has greater osmotic pressure than pure water. On the other hand, for gels at 0.15% and 0.60%, soaking in 0.1M NaCl resulted in higher swelling compared to soaking in water. This could be due to other factors affecting the hydrogel swelling besides osmotic pressure, such as temperature, soaking time, or the concentration of the hydrogel used.

Gel reactivity

A diffusive layer should not adsorb the targeted ions. In this study, we investigated the reactivity of citric acid crosslinked agarose towards phosphate (PO₄⁻). As shown in Table 1, the hydrogel could adsorb phosphate as much as 9.8% in pure agarose gel, and 5.9% in crosslinked hydrogel. This result is in accordance with other research which shows that citric acid crosslinked agarose hydrogel cannot adsorb ions. The addition of

crosslinker actually reduces the adsorption because the crosslinker strengthens the interaction between polymer chains in the hydrogel, reducing the available space for water molecules and ions in the tissue (Nasution et al., 2022). The more crosslinker used, the more crosslinks are formed, and the polymer network becomes denser and more stable. Therefore, the amount of phosphate absorbed in this study is lower in citric acid crosslinked hydrogel than in pure agarose hydrogel. There are studies that suggest crosslinkers are used to make hydrogels, which are hydrophilic polymer structures that have a high capacity to absorb water and solutions. The chemical association of polymer chains with the added crosslinker affects the physical properties of the polymer depending on the degree of crosslinking. The more crosslinker added, the less water and solution are absorbed because the crosslinker prevents the complete dissolution of the polymer in water (Xu et al., 2022).

Table 1. Adsorption of agarose-citric acid diffusion gels by phosphate ions

Gel		Adsorbed phosphate
Phosphate 1 mgL ⁻¹	0.00%	9.8 %
	0.45%	5.9%

As illustrated in Figure 7, if the gel only carries a non-moving negative charge, there will be an imbalance of electro-neutrality during concentration homogenization. To achieve electro-neutrality, ion migration is required through the redistribution of heterogenous concentrations induced in the solution, causing anions and cations to move/diffuse back and forth until equilibrium is reached, corresponding to a Donnan equilibrium.

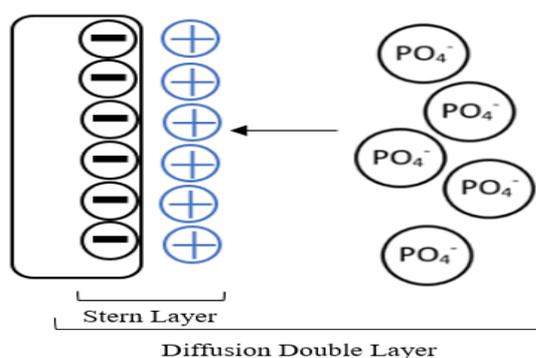


Figure 7. Illustration of the increase in the diffusion coefficient in the presence of cations (■K⁺)

Figure 7 explains that there are positive charges in the Stern layer, which are K⁺ in the phosphate solution (KHPO₄), that help the negative charge (PO₄⁻) in the diffusion double layer to quickly enter the hydrogel. However, if cross-linking occurs, the negative charge in the hydrogel decreases, resulting in fewer bound

positive charges and fewer phosphates entering. This illustration is similar to the Gouy-Chapman model. DGT does not fully utilize this model for measuring ion diffusion in thin polymer layers. However, the principles of the Gouy-Chapman model are used to explain how charged ions in electrolyte solutions can move and diffuse through thin polymer layers (Dourado, 2022).

Positively charged hydrogel membranes can achieve high rejection of solutes with positive charge (Jin et al., 2022), the positive and negative charges in a porous hydrogel are nearly balanced (Tanikawa et al., 2023), and a hydrophilic and positively charged hydrogel network is promising for the uptake and prolonged release of negatively charged guest molecules (Blöhbaum et al., 2019).

Determination of the Diffusion Coefficient of Phosphate through Diffusive Gel Agarose-Citric Acid

In water environment, nitrate and phosphate are nutrients for algae growth. Therefore, we investigated diffusion of phosphate with and without the presence of nitrate ions at various concentration. It will induce different ionic strength of solution and affect the diffusion of phosphate.

The diffusion coefficient is expressed either at the same temperature directly measured or as a percentage value in water. The initial conditions of the hydrogel and the composition of the solution (carrier solution) used will affect the reproducibility of diffusion measurements. Various carrier solutions are used for thorough tests when measuring the diffusion coefficient in agarose diffusion gels. In this study, 1 mgL⁻¹ phosphate solution was used, mixed solution of 1 mgL⁻¹ phosphate; nitrate 2 mg L⁻¹ (1:2), and a mixed solution of phosphate 1 mgL⁻¹; nitrate 0.1 M. If the units are generalized, 2 mgL⁻¹ nitrate equals 23.5 × 10⁻⁶ and 1 mgL⁻¹ phosphate equals 7.35 × 10⁻¹ M.

First we consider the effect of adding nitrate to the solution. If you compare the addition of NaNO₃ (1 mgL⁻¹ (23.5 × 10⁻⁶ M) and 0.1 M) to the mixed solution, it can also affect the results of measurements and calculations of phosphate concentrations. Where high nitrate concentrations have an effect that causes a negative bias in phosphate measurements where the results of phosphate concentrations will be lower than they should be According to Galceran (2021), the diffusion coefficient can decrease due to the low surface concentration when it is measured from solution to resin, while the diffusion coefficient in the gel itself does not change (Galceran et al., 2021).

Diffusion is defined as the random movement of molecules from an area of high concentration to an area of lower concentration (Reinke et al., 2019). The diffusion coefficient here uses a diffusion gel that has an optimum gel, which is 0.45% citric acid hydrogel by comparing the

gel whitout a crosslinker, which is 0.00% citric acid hydrogel with pure water as the storage solution.

Table 2 shows the diffusion coefficient through pure agarose gel and agarose-citric acid gel in various solutions. Based on Table 2, each gel without citric acid crosslinking resulted in a high phosphate diffusion coefficient compared to the crosslinked ones. As previously explained, this is probably due to the pore size of the agarose gel being larger than 20 nm, allowing phosphate ions to easily pass through the diffusion gel. The greatest diffusion coefficient of phosphate, which is 1.867×10^{-7} , was obtained by the diffusion gel of 0.00% citric acid in a mixed solution of 1 mgL⁻¹ phosphate; nitrates 2 mgL⁻¹ (1:2). The difference in the magnitude of the diffusion coefficient can be different for each ion, depending on the ions moving in the solution. If we review the comparison between the two nitrate concentrations used, the greater the nitrate concentration, the smaller the phosphate diffusion coefficient. This happens because, according to Debye-Hückel, the higher the ionic strength of the solution, the lower the ion activity coefficient (Tivant, Turq, Drifford, Magdelenat, & Menez, 1983).

Additionally, as illustrated in Figure 8, the higher the concentration, the more positive charges cover the surface of the hydrogel, not only from K⁺ but also from Na⁺ that are bound to the negative charges on the gel. This results in fewer phosphate ions that can diffuse through the diffusion gel, leading to a lower phosphate diffusion coefficient.

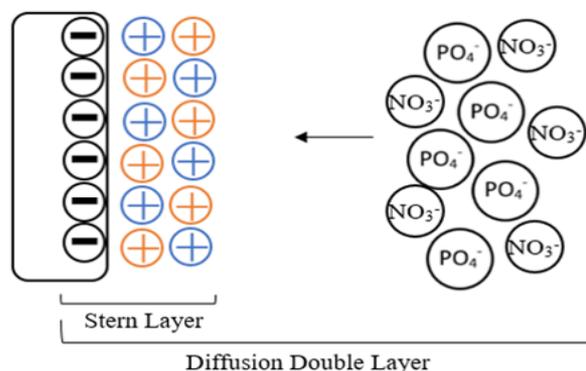
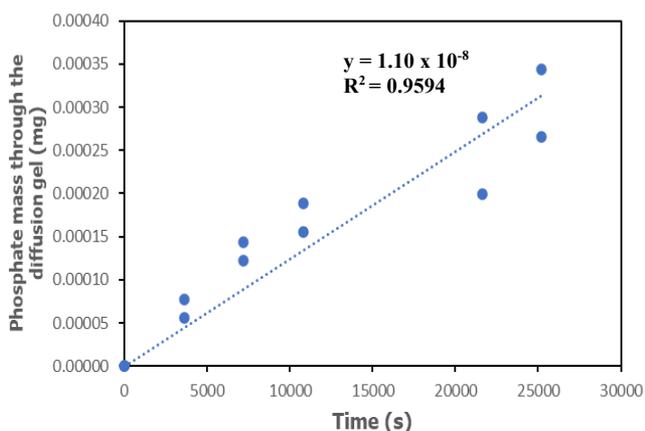


Figure 8. Illustration of the increase in the diffusion coefficient in the presence of cations with nitrate. (■K⁺ and ■Na⁺)

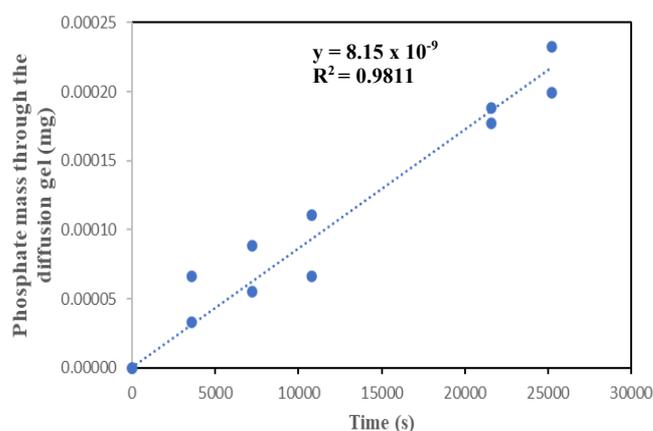
Table 2. Phosphate diffusion coefficient through agarose gel with various mass percent and different solutions.

Solution	Crosslinked Hydrogel (%) [*]	Solution temperature (°C)	Gel surface area (A, cm ²)	Diffusive gel thickness (Δg, cm)	Slope	D _{salt} (cm ² s ⁻¹)	D(k) (cm ² s ⁻¹)	D ₂₅ phosphate (cm ² s ⁻¹)
Phosphate (1mgL ⁻¹)	0.00	26.2	7.065	0.13	1.10×10^{-8}	2.78×10^{-7}	2.027×10^{-5}	$1.337 \times 10^{-7} \pm 5.453 \times 10^{-10}$
	0.45	26.2	7.065	0.13	8.15×10^{-9}	2.10×10^{-7}	2.027×10^{-5}	$1.009 \times 10^{-7} \pm 1.748 \times 10^{-10}$
a mixed solution A	0.00	26.3	7.065	0.13	1.15×10^{-8}	3.87×10^{-7}	2.031×10^{-5}	$1.867 \times 10^{-7} \pm 7.618 \times 10^{-10}$
	0.45	26.2	7.065	0.13	7.08×10^{-9}	2.67×10^{-7}	2.027×10^{-5}	$1.285 \times 10^{-7} \pm 2.114 \times 10^{-10}$
a mixed solution B	0.00	26.3	7.065	0.13	7.00×10^{-9}	2.87×10^{-7}	2.031×10^{-5}	$1.367 \times 10^{-7} \pm 2.838 \times 10^{-10}$
	0.45	26.3	7.065	0.13	6.14×10^{-9}	2.15×10^{-7}	2.031×10^{-5}	$1.020 \times 10^{-7} \pm 5.181 \times 10^{-10}$

*% w/w; a mixed solution A: phosphate 1 mgL⁻¹ and nitrate 2 mgL⁻¹; a mixed solution B: phosphate 1 mgL⁻¹; nitrate 0.1 M;



(a)



(b)

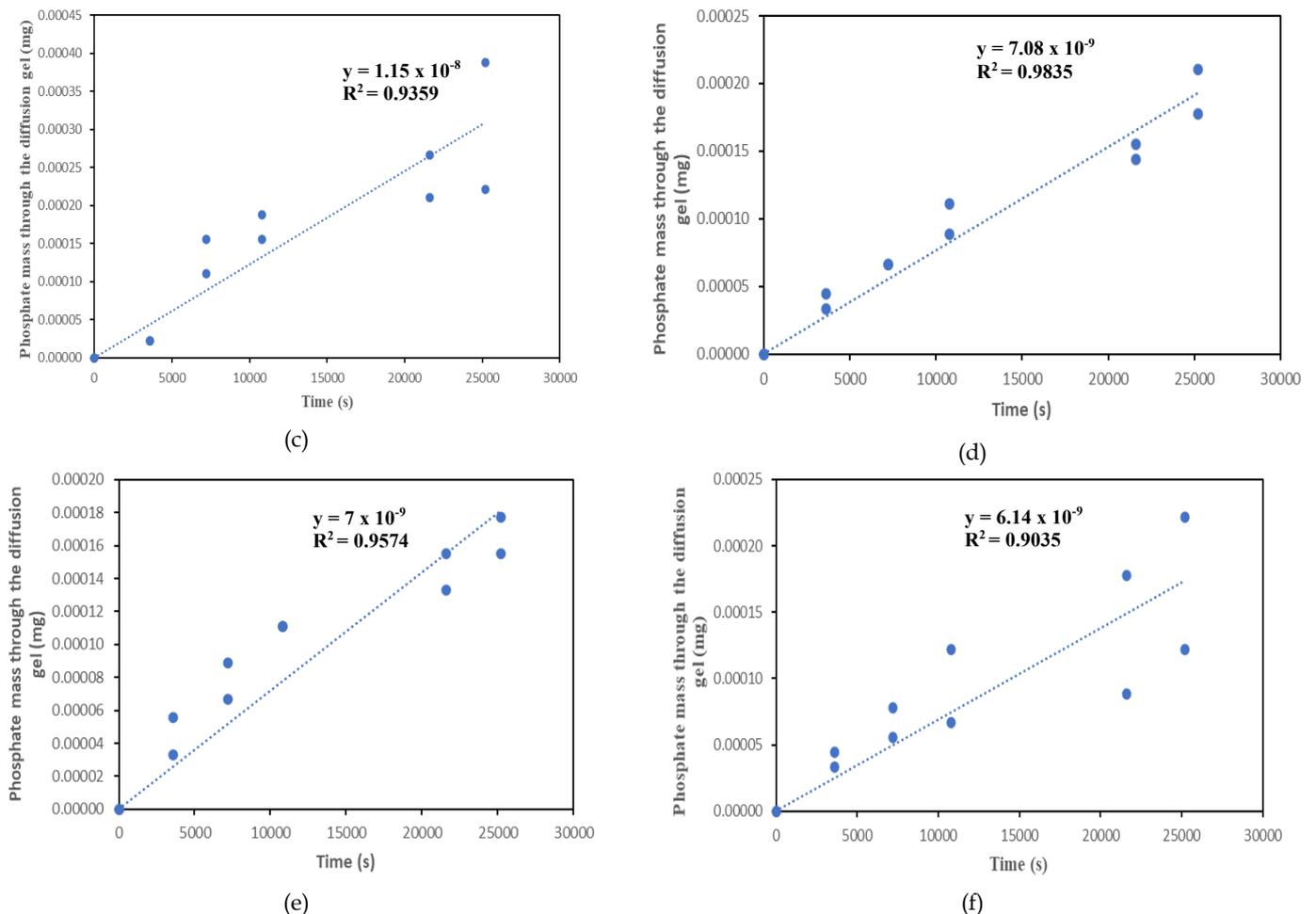


Figure 9. Determination of the phosphate diffusion coefficient of the slope resulting from 0.00% citric acid hydrogel in 1 mgL⁻¹ phosphate solution (a) 0.45% citric acid in 1 mgL⁻¹ phosphate solution (b) hydrogel 0.00% citric acid in mixed solution (2 mgL⁻¹ NaNO₃) (c) 0.45% citric acid in mixed solution (NaNO₃ 2 mgL⁻¹) (d) 0.00% citric acid in mixed solution (0.1M NaNO₃) (e) 0.45% citric acid in mixed solution (0.1M NaNO₃) (f).

In addition, the large number of water molecules is inversely proportional to the number of ions at low ionic strength (Wang & Alvarado, 2018). The ionic strength can affect the diffusion coefficient because a high ionic strength can reduce the diffusion rate of ions in solution (Giorgi et al., 2019). High ionic strength can cause electrostatic attractive and repulsive forces, which can slow down the movement of ions. The diffusion coefficient decreases as the ionic strength increases. This has been demonstrated in Table 2. Phosphate all analyses had a linear correlation with time (Figure 9). The slope of each linear equation is obtained and used to calculate the diffusion coefficient according to equations 5 to 9.

Conclusion

The characteristics of agarose-bound diffusion gel and non-crosslinked agarose used in DGT were investigated. The crosslinked agarose diffusion gel with citric acid was confirmed by FTIR characterization

showing the presence of carboxylate groups on agarose-citric acid gel (0.45%) at a wavelength of 1700-1600 cm⁻¹. Crosslinked agarose diffusion gel with citric acid was better at 0.45% citric acid mass, which had the highest water absorption capacity. The obtained diffusion coefficient for the agarose diffusion gel was 1.009×10^{-7} in a 1 mgL⁻¹ phosphate solution. Various solutions resulted in 1.285×10^{-7} for phosphate with 2 mgL⁻¹ nitrate and 1.020×10^{-7} for phosphate with 0.1 M nitrate. The increase in the diffusion coefficient of phosphate is influenced by ionic strength. The higher the ionic strength, the lower the diffusion coefficient of phosphate due to the electrostatic attraction that can hinder the movement of phosphate ions.

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Author Contribution

All authors had contribution on conducting part of research, writing up, editing, and lay outting manuscripts. Conceptualization of research comes from the Environmental Analytical Group of Discussion with members are Barlah, Qonitah, Diah, Ulfa, and Layta. There authors had also contribute on developing methods of analysis, writing original draft preparation of manuscript, analyzing and validating data. Digwanggi contributed on doing investigation on the laboratory and lay outting labels and graphs.

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Conflicts of Interest

The authors declare no conflict of interest.

References

- Blöbbaum, J., Paulus, I., Pöppler, A. C., Tessmar, J., & Groll, J. (2019). Influence of charged groups on the cross-linking efficiency and release of guest molecules from thiol-ene cross-linked poly (2-oxazoline) hydrogels. *Journal of Materials Chemistry B*, 7, 1782-1794. <https://doi.org/10.1039/C8TB02575D>
- Bonnaud, B., Miege, C., Daval, A., Fauvelle, V., & Mazzella, N. (2021). Determination of diffusion coefficients in agarose and polyacrylamide gels for 112 organic chemicals for passive sampling by organic Diffusive Gradients in Thin Films (o-DGT). *Environmental Science and Pollution Research*, 29, 25799-25809. <https://doi.org/10.1007/s11356-021-17563-7>.
- Cao, H., Bu, Q., Li, Q., Gao, X., Xie, H., Gong, W., Wang, X., Yang, L., & Tang, J. (2022). Development and applications of diffusive gradients in thin films for monitoring pharmaceuticals in surface waters. *Environmental Pollution*, 311, 119979. <https://doi.org/10.1016/j.envpol.2022.119979>
- Damar, A., Hesse, K.-J., Colijn, F., & Vitner, Y. (2019). The eutrophication states of the Indonesian sea large marine ecosystem: Jakarta Bay, 2001-2013. *Deep Sea Research Part II: Topical Studies in Oceanography*, 163, 72-86. <https://doi.org/10.1016/j.dsr2.2019.05.012>
- Dourado, A. H. B. (2022). Electric Double Layer: The Good, the Bad, and the Beauty. *Electrochemistry*, 3(4), 789-808. <https://doi.org/10.3390/electrochem3040052>
- Fayer, A. (2023) *Perspective Chapter: Hydrogel Draw Agent Desalination Systems-Outlook*. In TechOpen. <https://doi.org/10.5772/intechopen.110666>
- Galceran, J., Gao, Y., Puy, J., Leermakers, M., Rey-Castro, C., Zhou, C., & Baeyens, W. (2021). Speciation of Inorganic Compounds in Aquatic Systems Using Diffusive Gradients in Thin-Films: A Review. *Frontiers in Chemistry*, 9, 624511. <https://doi.org/10.3389/fchem.2021.624511>
- Giorgi, F., Coglitore, D., Curran, J. M., Gilliland, D., Macko, P., Whelan, M., Worth, A., & Patterson, E.A. (2019). The influence of inter-particle forces on diffusion at the nanoscale. *Scientific Reports*, 9, 12689. <https://doi.org/10.1038/s41598-019-48754-5>
- Hodges, S. D., Wahman, D. G., Hauptert, L. M., Pham, H. T., Bozarth, M. K., Howland, M. B., & Fairey, J. L. (2023). Non-Steady-State Fickian Diffusion Models Decrease the Estimated Gel Layer Diffusion Coefficient Uncertainty for Diffusive Gradients in Thin-Films Passive Samplers. *Environmental Science and Technology*, 57(26), 9793-9801. <https://doi.org/10.1021/acs.est.3c01861>
- Jin, P., Mattelaer, V., Yuan, S., Bassyouni, M., Simoens, K., Zhang, X., Ceysens, F., Bernaerts, K., Dewil, R., & Bruggen, B. V. D. (2022). Hydrogel supported positively charged ultrathin polyamide layer with antimicrobial properties via Ag modification. *Separation and Purification Technology*, 284, 120295. <https://doi.org/10.1016/j.seppur.2021.120295>
- Kalkhajeh, Y. K., Amiri, B. J., Huang, B., Khalyani, A. H., Hu, W., Gao, H., & Thompson, M. L. (2019). Methods for sample collection, storage, and analysis of freshwater phosphorus. *Water*, 11(9), 1889. <https://doi.org/10.3390/w11091889>
- Li, C., Ding, S., Yang, L., Wang, Y., Ren, M., Chen, M., ... Lichtfouse, E. (2019). Diffusive gradients in thin films: Devices, materials and applications. *Environmental Chemistry Letters*, 17(2), 801-831. <https://doi.org/10.1007/s10311-018-00839-9>
- Mali, K. K., Dhawale, S. C., Dias, R. J., Dhane, N. S., & Ghorpade, V. S. (2018). Citric Acid Crosslinked Carboxymethyl Cellulose-based Composite Hydrogel Films for Drug Delivery. *Indian Journal of Pharmaceutical Sciences*, 80(4). <https://doi.org/10.4172/pharmaceutical-sciences.1000405>
- Mariam Ninan, C., Ajay, A., Ramaswamy, K. P., Thomas, A. V., & Bertron, A. (2020). A critical review on the effect of organic acids on cement-based materials. *IOP Conference Series: Earth and Environmental Science*, 491(1), 012045. <https://doi.org/10.1088/1755-1315/491/1/012045>
- Nangare, S., Vispute, Y., Tade, R., Dugam, S., & Patil, P. (2021). Pharmaceutical applications of citric acid. *Future Journal of Pharmaceutical Sciences*, 7(1), 54. <https://doi.org/10.1186/s43094-021-00203-9>
- Nasution, H., Harahap, H., Dalimunthe, N. F., Ginting, M. H. S., Jaafar, M., Tan, O. O. H., Aruan, H. K., & Herfananda, A. L. (2022). Hydrogel and Effects of Crosslinking Agent on Cellulose-Based Hydrogels: A Review. *Gels*, 8(9), 568. <https://doi.org/10.3390/gels8090568>

- Ngatia, L., & Taylor, R. (2019). *Phosphorus Eutrophication and Mitigation Strategies*. In T. Zhang. IntechOpen. <https://doi.org/10.5772/intechopen.79173>
- Reinke, N. B., Kynn, M., & Parkinson, A. L. (2019). Conceptual Understanding of Osmosis and Diffusion by Australian First-year Biology Student. *International Journal of Innovation in Science and Mathematics Education*, 27(9), 17-33. <https://doi.org/10.30722/IJISME.27.09.002>
- Suteja, Y., & Purwiyanto, A. I. S. (2018). Nitrate and phosphate from rivers as mitigation of eutrophication in Benoa bay, Bali-Indonesia. *IOP Conference Series: Earth and Environmental Science*, 162, 012021. <https://doi.org/10.1088/1755-1315/162/1/012021>
- Tanikawa, S., Ebisu, Y., Sedlačík, T., Semba, S., Nonoyama, T., Kurokawa, T., ... & Tanaka, S. (2023). Engineering of an electrically charged hydrogel implanted into a traumatic brain injury model for stepwise neuronal tissue reconstruction. *Scientific reports*, 13(1), 2233. <https://doi.org/10.1038/s41598-023-28870-z>
- Urik, J., Paschke, A., & Vrana, B. (2020). Diffusion coefficients of polar organic compounds in agarose hydrogel and water and their use for estimating uptake in passive samplers. *Chemosphere*, 249, 126183. <https://doi.org/10.1016/j.chemosphere.2020.126183>
- Wahyuni, W., & Saefumillah, A. (2019). Development study of Diffusibe Gradient in Thin Film (DGT) method with binding agent Fe-Ca complex for phosphate measurement. *IOP Conf. Series: Materials Science and Engineering*, 496, 012007. <https://doi.org/10.1088/1757-899X/496/1/012007>
- Wang, H., & Alvarado, V. (2018). Ionic strength-dependent pre-asymptotic diffusion coefficient distribution in porous media—Determination through the pulsed field gradient technique. *Journal of Natural Gas Science and Engineering*, 49, 250–259. <https://doi.org/10.1016/j.jngse.2017.10.013>
- Widyarani., Wulan, D. R., Hamidah, U., Komarulzaman, A., Rosmalina, R. T., & Sintawardani, N. (2022). Domestic wastewater in Indonesia: generation, characteristics and treatment. *Environmental Science and Pollution Research*, 29(22), 32397-32414. <https://doi.org/10.1007/s11356-022-19057-6>
- Xu, J., Kim, K. O., & Yoon, K. J. (2022). Effect of Cross-Linker Length on the Absorption Characteristics of the Sodium Salt of Cross-Linked Polyaspartic Acid. *Polimers*, 14(11), 2244. <https://doi.org/10.3390/polym14112244>