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Encapsulation of Fluorescein in Polyacrylamide and Poly(ethylene glycol)methacrylate for pH Detection

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Abstract

pH sensors are now employed in a wide range of applications, including wastewater management, chemical industries, physiological processes in organisms and biotechnological such as monitoring bacterial growth. This study aims to investigate the entrapment of fluorescein in the different ratios of polyacrylamide and poly(ethylene glycol) methacrylate compounds which were then evaluated to detect pH changes. Three (3) different ratios of monomer to crosslinker were prepared (1:1, 5:3 and 3:1) through photo-polymerization which crosslinked by N,N'-methylenebisacrylamide (MBA) and initiated by a photo-initiator, diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO) under the ultraviolet (UV) light. Herein, fluorescein were added into the polymerization solution prior to photo-polymerization as fluorescein dye is one of the pH-sensitive probes. The ATR-FTIR spectra of the polymers show that the polymers were successfully synthesized which was indicated by the presence of functional groups of the polymers which are 3340.23cm-1 (N-H) and 1661.41cm-1 (C=O) for amide, 1090.58cm-1 (C-O) and 1455.24cm-1 (O-H) for glycol functional group. Next, for the leaching study of fluorescein, the absorption was observed to be higher at the first wash due to the presence of fluorescein which did not entrap into the polymer. The absorption of the sample decreased after a few washing, showing minimal leaching of fluorescein from the polymers. For pH testing, a set of pH solutions was prepared, ranging from pH 2 to pH 11 and it was observed that fluorescein entrapped in polyacrylamides started to fluoresce in the solution at the pH of 5 until 11 while fluorescein entrapped in poly(ethylene glycol) methacrylate started to fluoresce in the solution at the pH of 6 until 11. To conclude, the entrapment of fluorescein into polyacrylamide and poly(ethylene glycol) methacrylate was successful with the polymers showing pH changes at above pH 5 and 6, respectively.

Keywords: Encapsulated of fluorescein, photopolymerization, pH detection

1. Introduction

For the past few decades, polymer-based sensors have attracted a lot of attention. This statement is supported by the number of articles published on the subject and the amount of money spent on research in this field. The function of the sensor is widely known which is to provide information about the environment in which material is present. Polymers, being materials that can be modified or synthesized to perform certain functions, have found a lot of applications in the development of sensing devices. Polymers are the most often utilized materials in the building of sensors with varied applications [1]. The development of polymer-based pH sensors is one of the relevant research projects up till today as we acknowledge that pH is a crucial chemical and biological parameter.

The potential of hydrogen, or pH, is a chemical term that describes the acidity or basicity of a medium. pH is derived logarithmically from H+ ion concentration and is one of the most important parameters [2]. pH is often decisive in processes involving the availability or mobility of inorganic ions for organisms or in environmental compartments, wastewater management, the biotechnological or chemical industry, physiological processes in organisms, both vital and detrimental, or interactions between organisms and their environment, global ecological or intracellular regulatory scenarios are several among of the chemical and biological applications of pH sensors [3].

Chemical indicators such as litmus paper which change colour in response to the solution's pH, are the first method of pH monitoring. As we know, the litmus paper will turn to blue when added to the basic solution and the litmus paper will turn red when added to an acidic solution [4]. Other than that, the commonly used pH sensor is pH glass electrode, but it was stated that pH glass electrodes frequently show rather poor performance at both ends of the pH scale, particularly in the extremely alkaline zone and must be recalibrated on a regular basis [3]. Owing to this, optical pH sensors or optodes are suggested as a viable option that can operate through variations in pH caused by the changes in the optical properties of such as absorption, fluorescence or reflection. Fluorescence sensors have gained popularity in recent years due to their ease of use as well as excellent sensitivity and specificity. Technically, reversible protonation/deprotonation changes the spectrum or intensity of an implanted fluorophore.

Thus, the purpose of this research is to synthesize suitable dye encapsulated polymer via the photopolymerization method and will be evaluated for the capability of the polymer capsule in pH detection. The organic dye used is fluorescein which its strong fluorescence and sensitivity towards pH variations in the neutral domain [5].

2. Methodology

2.1. Synthesis of dye encapsulated polymer

In the preparation of dye encapsulated polymer, 0.4 mL acrylamide was added into the beaker and followed by 0.4 mL of MBA. Then, 0.2 mL of TPO and 0.001 g of fluorescein also added into the beaker. The mixture was stirred and homogenized to undergo polymerization. The photopolymerization started when the mixture was exposed to UV light. The same steps were repeated using different ratios of monomer to the cross-linking agent. The whole procedure also was repeated by using poly(ethylene glycol) methacrylate following Table 1 below. After that, the dye encapsulated polymers were washed using distilled water and dried after being cured under UV light.

Table 1. Composition of dye encapsulated a) polyacrylamide and (b) poly(ethylene glycol) methacrylate

(a)

	Ratio of Acrylamide: MBA		
	1:1	**5:3**	**1:3**
Acrylamide (mL)	0.4	0.5	0.6
MBA (mL)	0.4	0.3	0.2
TPO (mL)	0.2	0.2	0.2
Fluorescein dye (g)	0.001	0.001	0.001

(b)

	Ratio of PEGMA: MBA		
	1:1	**5:3**	**1:3**
PEGMA (mL)	0.4	0.5	0.6
MBA (mL)	0.4	0.3	0.2
TPO (mL)	0.2	0.2	0.2
Fluorescein dye (g)	0.001	0.001	0.001

2.2. Characterization of dye encapsulated polymer

The polymer capsule was characterized by using attenuated total reflection infrared spectroscopy (ATR-FTIR). For ATR-FTIR, it requires the polymer capsules sample that has been prepared. Then, the sample will be applied to the crystal where some amount of infrared (IR) radiation penetrating through the crystal is absorbed by the sample. This absorbance was translated into the IR spectrum of the sample characterized. The clean and empty crystal is used for the collection of the background spectrum.

2.3. Leaching study

The fluorescein dye encapsulated went through a leaching study by immersing the polymers in the distilled water. Then, the water sample used from the sample was analyzed using UV-Vis spectroscopy every 30 minutes to record the absorption and the leaching was detected based on the spectrum at the wavelength of 470 nm.

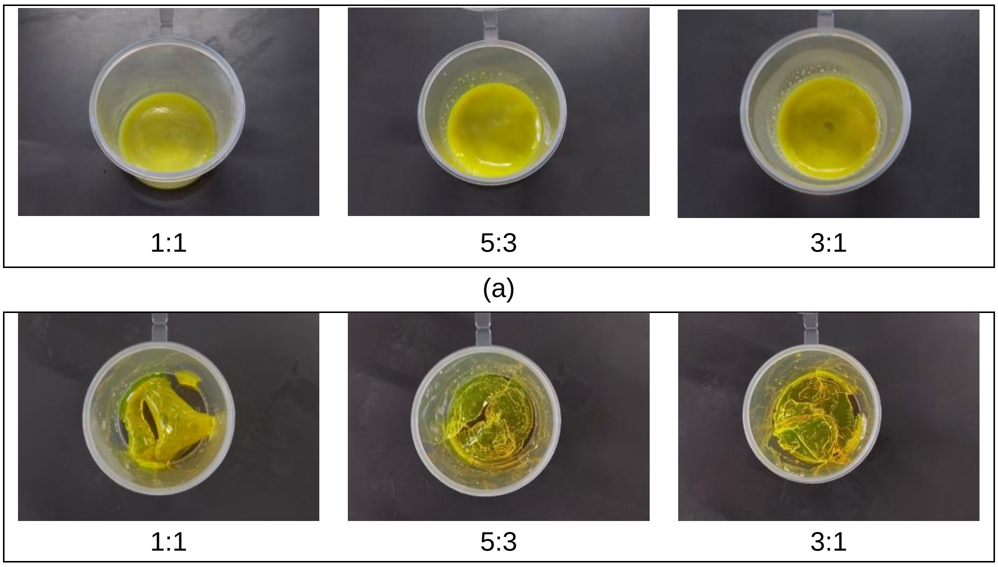
2.4. Application of Dye Encapsulated Polymer on the Detection of pH

The fluorescein dye encapsulated polymers were evaluated on the detection of pH. In this step, a set of pH solutions was prepared ranging from pH 2 to pH 11. The pH solutions were prepared by using distilled water where 1M of HCl and 1M of NaOH were added to change the pH value into acidic and basic solutions. The pH value of the solution was then measured by a pH meter. The intensity of the fluorescein was observed based on qualitative measurement in order to investigate the pH value where the fluorescent will emit.

3. Results and Discussion

3.1. Synthesis of dye encapsulated polymer

Polyacrylamide and poly(ethylene glycol) methacrylate with fluorescein encapsulated were synthesized by the photopolymerization method. In this study, MBA was employed as a crosslinker, TPO as a photoinitiator and the polymer was prepared in an aqueous solution with the aid of UV light. The polyacrylamide and poly(ethylene glycol) methacrylate were prepared with three (3) different ratios of monomer to crosslinker, which were 1:1, 5:3 and 3:1. From Figure 1, all the three samples of fluorescein encapsulated polyacrylamide and poly(ethylene glycol) methacrylate were polymerized when exposed to the UV light within one minute and three minutes respectively. The texture of dye encapsulated polyacrylamide is much more delicate than dye encapsulated poly(ethylene glycol) methacrylate as they were brittle when polymerized under UV light. Hence, polyacrylamide is more robust and suitable for fluorescein encapsulation under UV photopolymerization



(a)

(b)

Figure 1. The texture of fluorescein encapsulated (a) polyacrylamide and (b) poly(ethylene glycol) methacrylate

3.2. Characterization of Dye Encapsulated Polymer by ATR-FTIR

The dye encapsulated polymers were characterized by attenuated total reflection infrared spectroscopy (ATR-FTIR) in order to confirm the presence of functional. Different functional groups absorb at different wavenumbers. Figure 2 shows the spectrum for polyacrylamide with fluorescein encapsulated with the absorption band at 3340.23 cm^{-1} which is attributed to the vibration of N-H groups from the acrylamide and MBA. The absorption bands at 1661.41 cm^{-1} and 1609.99 cm^{-1} correspond to the stretching and deformation vibrations of the C=O and N-H bond from the amide group. In Figure 3, the ATR-FTIR spectrum of poly(ethylene glycol) methacrylate also shows the absorption band at 3390.04 cm^{-1} which corresponded to the vibration of N-H groups from the crosslinking agent. Then, the absorption bands at 1090.58 cm^{-1} and 1455.24 cm^{-1} attributed to the vibration of C-O and O-H respectively. From the absorption bands that appeared in the spectrum, it can be concluded that the acrylamide and poly(ethylene glycol) methacrylate were polymerized.

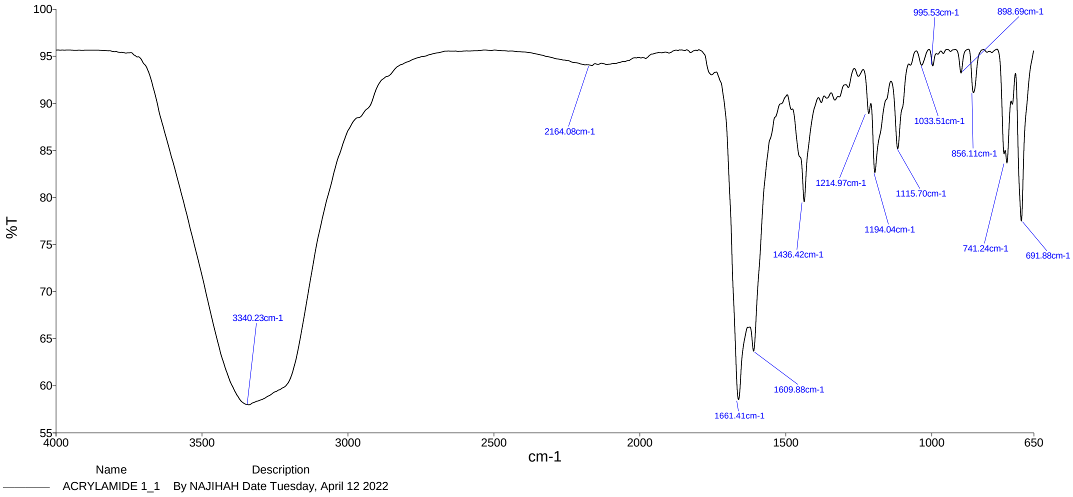


Figure 2. ATR-FTIR spectrum for fluorescein encapsulated polyacrylamide

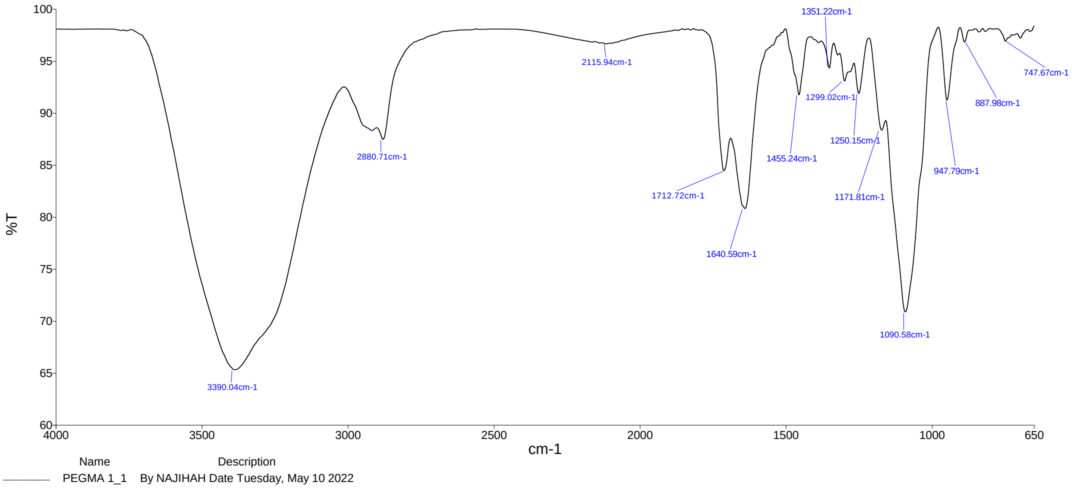


Figure 3. ATR-FTIR spectrum for fluorescein encapsulated poly(ethylene glycol) methacrylate

3.3. Leaching Study

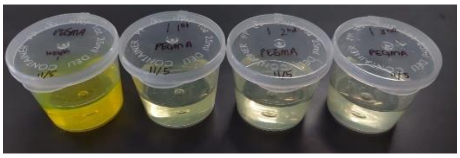
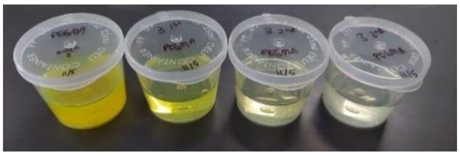
Dye leaching was evaluated by soaking the fluorescein encapsulated polyacrylamide and poly(ethylene glycol) methacrylate in distilled water for a period of time. The polymers were previously washed with distilled water and then were soaked for two hours and absorbance was collected at 30 minutes intervals. Table 2 below shows the leaching test done on three samples of fluorescein encapsulated polyacrylamide and poly(ethylene glycol) methacrylate. Then, the absorbance of the water sample was monitored using UV-Vis spectroscopy as shown in Figure 4 and 5.

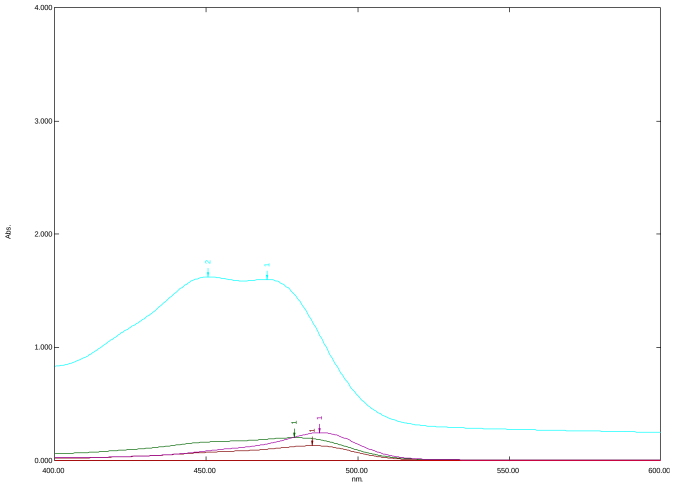
Table 2. Leaching test of fluorescein encapsulated (a) polyacrylamide and (b) poly(ethylene glycol) methacrylate

(a)

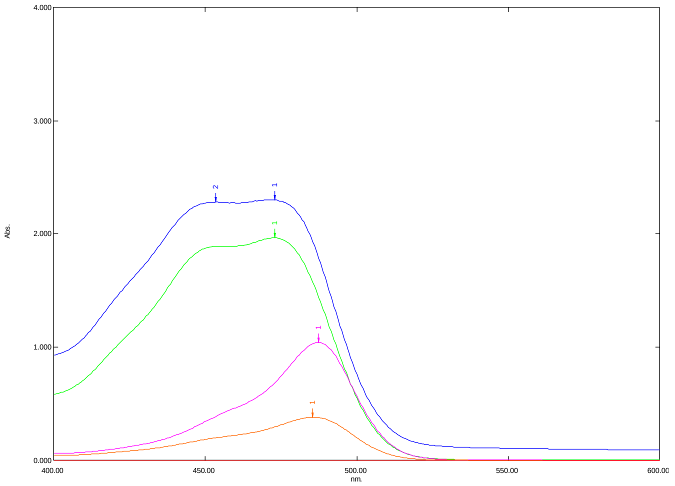
Sample	**Ratio Acrylamide:MBA**	**Leaching test**
1	1:1	
2	5:3	
3	3:1	

(b)

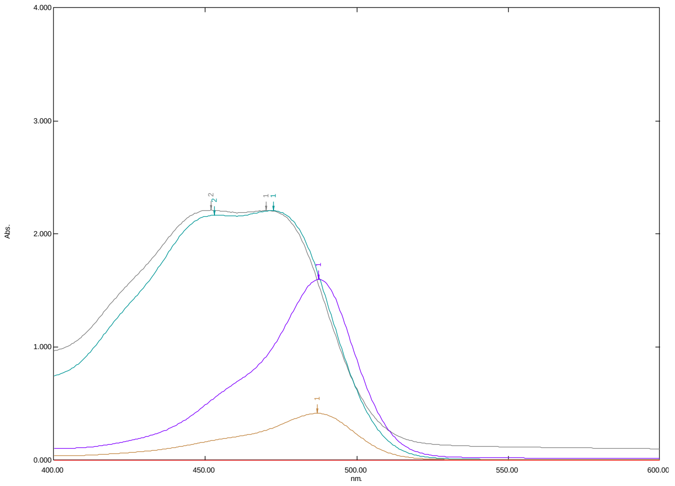
Sample	**Ratio PEGMA:MBA**	**Leaching test**
1	1:1	
2	5:3	
3	3:1	



(a) 1:1

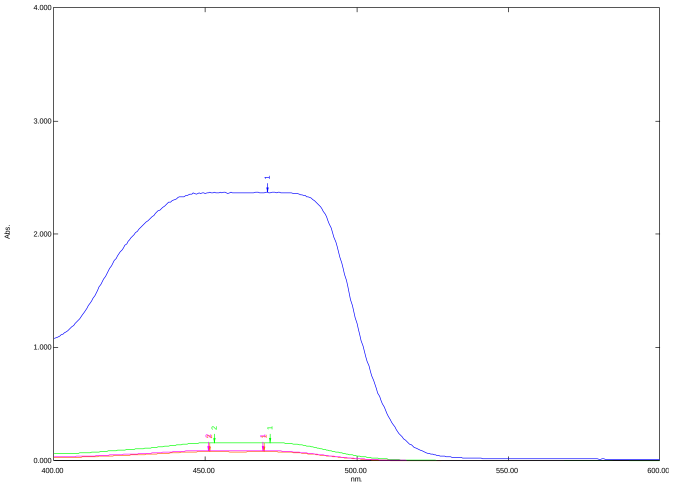


(b) 5:3

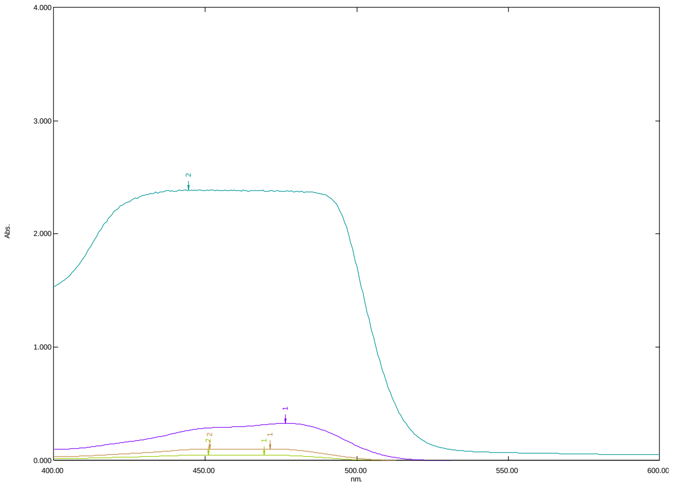


(c) 3:1

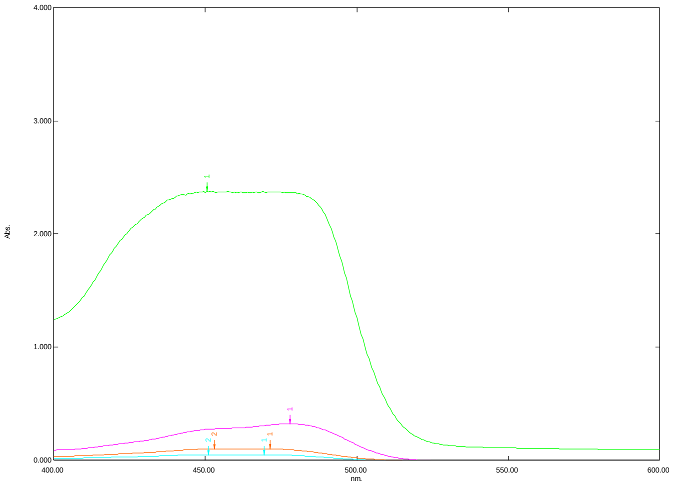
Figure 4. UV-Vis spectra of leaching test on fluorescein encapsulated polyacrylamide



(a) 1:1



(b) 5:3



(c) 3:1

Figure 5. UV-Vis spectra of leaching test on fluorescein encapsulated poly(ethylene glycol) methacrylate

The intensity of fluorescein dye at the first wash was higher for every sample tested as shown in Table 2. For polyacrylamide samples, the colour of fluorescein dye for ratio 1:1 started to show minimal leaching of fluorescein at the second wash and above while for 5:3 and 3:1, they show maximum leaching of fluorescein until the third wash. However, it can be seen that the leaching test for poly(ethylene glycol) methacrylate gives a less amount of leaching starting from the second wash. It was confirmed in Figure 6 below where the absorbance of fluorescein at the wavelength of 470 nm for both dye encapsulated polyacrylamide and poly(ethylene glycol) methacrylate.

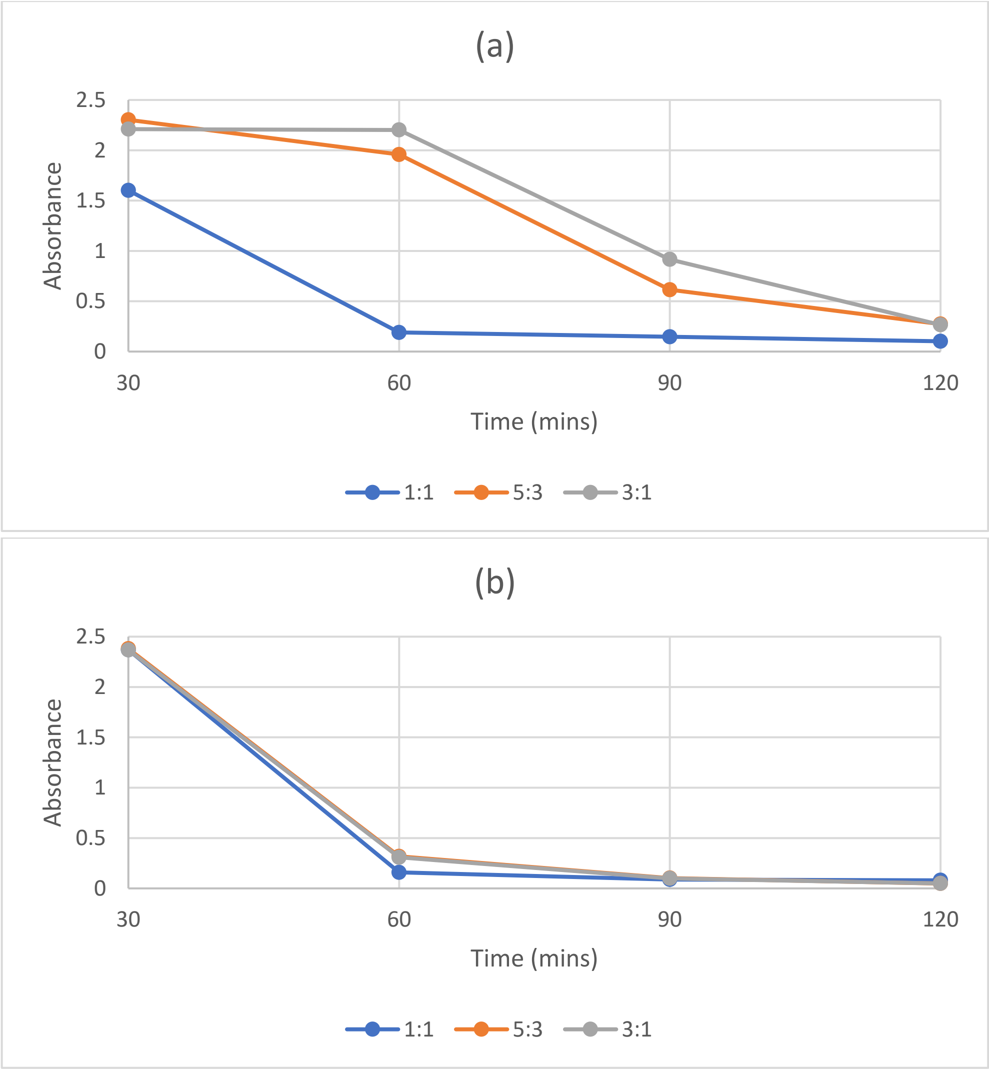


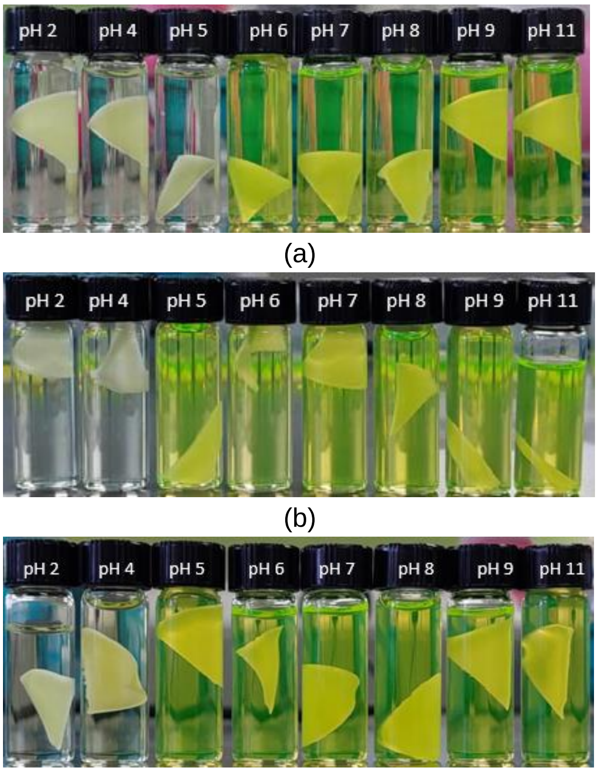
Figure 6. Graph of Absorbance against time for leaching test on (a) polyacrylamide and (b) poly(ethylene glycol) methacrylate

3.4. Detection of pH by Dye Encapsulated Polymer

In the application for detection of pH, the fluorescein dye encapsulated polymers, polyacrylamide and poly(ethylene glycol) methacrylate were immersed in a set of pH solutions ranging from pH 2 to pH 11. From the observation in Figure 7 and 8, fluorescein entrapped in the polyacrylamide began to fluoresce in the solution at the pH 5 until pH 11 while fluorescein entrapped in the poly(ethylene glycol) methacrylate began to fluoresce in the solution at the pH 6 until pH 11.

The results observed showed that fluorescent intensity was affected by the pH value of the solution as fluorescein responded differently. This is because of the various forms of fluorescein at the different pH values; cation, neutral molecule, monoanion and dianion. The cation form is the predominant species in the strongly acidic solution. Then, fluorescein is in the neutral form at the pH of 2 until 4. The monoanionic form of fluorescein appears at the pH range from 4.3 to 6.4 when the solution becomes more basic while the dianion form of fluorescein appears at the solution with a pH over 6.5.

However, it can be observed that the fluorescence intensity for polyacrylamide shows a uniform from pH 5 to pH 11 while it can be seen that the fluorescence intensity increases from pH 6 to pH 11 for polyethylene glycol methacrylate as it should show an increase in the intensity of the fluorescent. In order to determine the acidity and basicity of the solution, the absorption of the fluorescence can be measured by using fluorescence spectroscopy where it will show the low absorption of fluorescence in acidic solution and high absorption of fluorescence in basic solution.

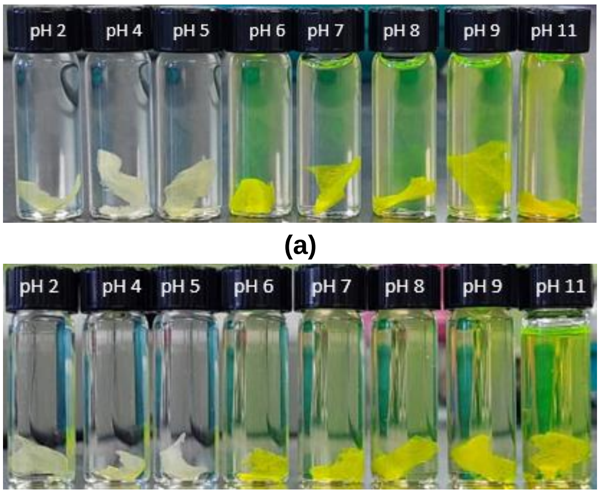


(a)

(b)

(c)

Figure 7 Detection of pH for fluorescein encapsulated polyacrylamide with ratio acrylamide: MBA (a) 1:1, (b) 5:3, (c) 3:1



(a)

(b)

(c)

Figure 8 Detection of pH for fluorescein encapsulated poly(ethylene glycol) methacrylate with ratio PEGMA: MBA (a) 1:1, (b) 5:3, (c) 3:1

Conclusion

Polyacrylamide and poly(ethylene glycol) methacrylate encapsulated with fluorescein dye were successfully synthesized via photopolymerization with three different ratios of monomer to crosslinker (1:1, 5:3 and 3:1). The polyacrylamide and poly(ethylene glycol) methacrylate were characterized by ATR-FTIR to identify the functional groups and the spectra show the presence of absorption bands at 3340.23 cm^{-1} (N-H) and 1661.42 cm^{-1} (C=O) for amide, 1090.58 cm^{-1} (C-O) and 1455.24 cm^{-1} (O-H) for glycol functional group indicated that the polymers were successfully synthesized. Then, the leaching test was conducted on the dye encapsulated polymers where the polymers were soaked in the distilled water in order to investigate the robustness of the fluorescein entrapment. The absorbance of fluorescein was recorded by UV-Vis every 30 minutes and the result shows that the absorption was observed to be higher at the first wash due to the presence of fluorescein which did not entrap into the polymer. However, the absorption of the sample decreased after a few washings showing minimal leaching of fluorescein from the polymers. Lastly, the application of the fluorescein dye encapsulated polymer was performed by pH testing where it was observed that fluorescein entrapped in polyacrylamides started to fluoresce in the solution at the pH of 5 until 11 while fluorescein entrapped in poly(ethylene glycol) methacrylate started to fluoresce in the solution at the pH of 6 until 11. In conclusion, the entrapment of fluorescein into polyacrylamide and poly(ethylene glycol) methacrylate was successful with the polymers showing pH changes above pH 5 and pH 6, respectively.

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