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by Dhena Ria Barleany Pros

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Chitosan-Graft-Poly (*Acrylic Acid*) Superabsorbent Hydrogel with Antimicrobial Activity

Dhena Ria Barleany[1]*, Intan Purnama Alim, Nida Rizkiyah, Utami Triana Lusi, Heri Heriyanto[1], Erizal Erizal[2]

Department of Chemical Engineering, Universitas Sultan Ageng Tirtayasa, Indonesia
[2]Centre for Application of Isotopes and Radiation, Indonesia

Abstract

Chitosan-graft-poly (acrylic acid) superabsorbent hydrogels by irradiation technique using ϒ-rays were carried out in this work. An important application of chitosan-graft-poly (acrylic acid) is the use as water absorbent. For hygiene products application, superabsorbent materials with antimicrobial activities are needed to prevent skin irritation. This research was developed to study the effects of chitosan's weight in the superabsorbent polymers on the water absorbency, gel content, and Equilibrium Degree of Swelling (EDS). The polymer products were also characterized by FT-IR and SEM. Antimicrobial activity of the hydrogel against Escherichia coli was further investigated. An amount of 0,75 g of chitosan in the reaction mixture resulted in a grafted polymer after ϒ-Rays irradiation with the best absorption (420 g g^{-1} in distilled water, 403,33 g g^{-1} in 0,009% NaCl solution, and 640 g g^{-1} in 0,9% urea solution). The gel content as high as 96,85% and EDS as high as 666,67 g g^{-1} of its dried weight were also obtained by using of 0,75 g of chitosan. The hydrogel was found to be effective against Escherichia coli.

Keywords: acrylic acid; antimicrobial; chitosan; Escherichia coli; superabsorbent

Correspondence should be addressed to dbarleany@yahoo.com

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INTRODUCTION

Superabsorbent hydrogels are hydrophilic crosslinked polymer that have great performances in water or other aqueous solution absorption [1]. The unique characteristics of hydrogel bring some interesting applications in environmental and agricultural field [2,3], biomedical [4,5], and hygiene products [6]. Absorbent hygiene products include material for baby care, sanitary pads, and adult pad diapers are an essential part for everyday life.

In the past few years, the majority of innovations in absorbent hygiene products prepared by using a combination of different materials and different technologies. The major components of diapers are inert polymers that are inherently safe, convenient, and effective in urine absorption under practical used conditions [7]. Diapers were a mixture of superabsorbent polymer, polyethylene, and polypropylene with the specific percentage of each component depended on the brand and type of diaper [8]. Polyacrylates have the ability to retain liquid in the diaper core, keeping it away from the baby's skin, even under pressure [7]. Sodium Polyacrylate can absorb up to 500 times their own weight in distilled water or aqueous liquid [8].

To reduce the occurrence of diapers dermatitis in users of disposable diapers with superabsorbent core, some improvements have been developed, included new materials that provide a cloth-like feel, breathable materials, to numerous comprehensive studies assessing safety aspects [7]. Used disposable diapers become the most critical environmental problems of modern societies [8,9] due to their non-degradable components. Superabsorbent products must contain non-toxic components in order to minimize inconvenient due to skin irritation.

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Acrylic acid based superabsorbent was not ecological friendly. It has to be grafted with natural materials to fit environmental requirements to produce degradable superabsorbent polymers. Chitosan is one of promising natural materials that has been widely studied due to its high performance on biodegradability and biocompatibility [10]. Chitosan also well known as antimicrobial agent [11,12]. Grafting of acrylic acid onto chitosan backbone may result superabsorbent resin for hygiene product with antimicrobial activity.

There are some grafting methods with different limitations. The use of chemical cross linkers brings some negative effects from toxic residues to the low conversion which cause high cost on the production process [10]. Synthesis of polymer network with no use of chemical catalyst is crucial especially for hygiene products and biomedical application. Some articles have been announced that ϒ-irradiation could became an interesting solution in the clean production of superabsorbent hydrogel [13,14,15].

In this present study, chitosan-graft-poly (acrylic acid) superabsorbent hydrogel was prepared by ϒ-ray irradiation with the total dose of 5 kGy. The functional groups of the obtained products were characterized. The gel content, swelling behaviors, and antimicrobial activity of the grafted polymers with respect to chitosan amount were evaluated.

MATERIALS AND METHODS

Materials

Analytical grade of acrylic acid (Merck, Darmstard, Germany), glacial acetic acid, and industrial grade of potassium hydroxide (KOH) were purchased from CV. Cahaya Kimia Indonesia. Chitosan was pharmaceutical grade with deacetillation degree of 85% purchased from PT. Biotech Surindo Indonesia. *Escherichia coli* and the growth media used for antimicrobial activity test of hydrogel product was purchased from Institut Pertanian Bogor Indonesia. Distilled water was used for preparation of the hydrogel and swelling measurement.

Preparation of Superabsorbent Hydrogel

An amount of 15 mL acrylic acid and 5,6 g potassium acrylate were dissolved with distilled water and mixed well to form 100 mL neutralized acrylic acid solution. In a separate place, a pre-weight of chitosan in various amount (0,25; 0,75; 1,25; and 1,75 were dissolved in 20 mL of 2 % (w/w) acetic acid solution. Chitosan solution were then added into the neutralized acrylic acid solution and homogenized in a 250 mL reactor equipped with magnetic stirrer, and stirred for 10 min. The homogenized solution was packed in a 10 cm x 15 cm and thickness of 0,1 cm plastic container then ϒ-irradiated at constant total dose 5 kGy. The gel products were then dried in the oven at 60 C for 24 hours and grinded with the using of blender (Miyako, Indonesia). After grinding, the powdered superabsorbent with the average particle sizes between 250-400 µm was stored in the absence of moisture, heat, and light for further tests.

Gel Content and Swelling Measurements

An accurately weight of powdered hydrogel sample (0,1 g) was inserted into a tea bag and immersed in distilled water (200 mL) for 24 h at room temperature. The swelled hydrogel then was dried in a vacuum oven for 24 h at 60°C and weighed. The gel content was measured twice at room temperature according to a method of [16] and previous study of [15] using the following formula:

$$\text{Gel content (\%)} = \frac{W_1}{W_0} \text{ x } 100\ \% \quad (1)$$

where W_1= weight of dried gel after soaking (g) and W_0 = initial weight of gel (g)

The swelling capacity of gel was determined according to a tea bag method [16]. Powdered hydrogel (0,1 g) were immersed in distilled water (100mL) at room temperature. NaCl and urea were also used in this swelling study to find the hydrogel performance in the salt solutions. At 30 seconds time intervals, the samples were removed from the swelling medium and blotted on a filter prior to weighing to remove excess surface moisture. The swelling ratio was calculated according to the following expression:

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Swelling capacity $(g/g) = \frac{W_t - W_0}{W_0}$ (2)

where W_t = weight of the swollen gel at time t (g) and W_0 is the initial weight of the dried gel (g). The studies were carried out for 180 s.

Antibacterial Analysis

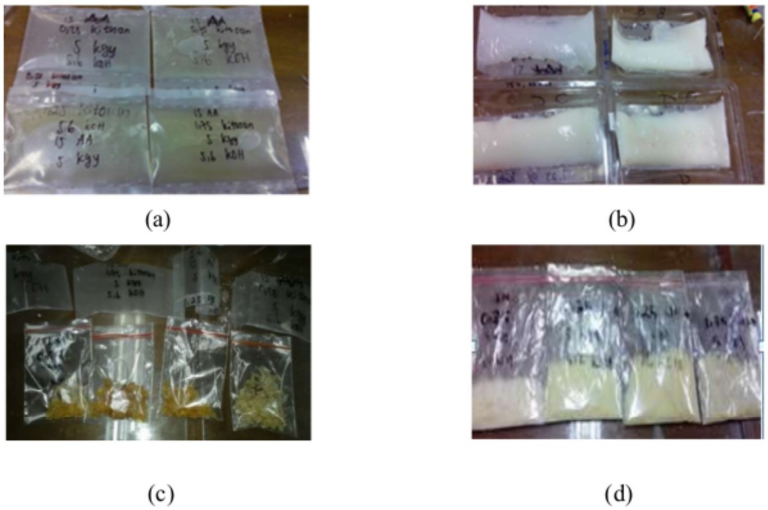
Evaluation of antibacterial activity of the hydrogel was prepared by using of ring method. The hydrogel at various compositions of chitosan were placed in Petri dishes with Escherichia coli and the Agar Nutrient inside. After 24 hours, the ring was taking off from Petri dish and the inhibition area was measured. Some procedures including preparation of agar, growth of Escherichia coli, micro test preparation, and inhibition zone analysis were done in this study.

Instrumental Analysis

Fourier Transform Infrared (FTIR) absorption spectra of samples were measured by means of Shimadzu IR Prestige-21 spectrometer model 800 series from 4000 to 500 cm^{-1} and recorded with DRS (Diffuse Reflectance Spectroscopy) system. The surface morphology of the gel was examined using Scanning Electron Microscopy (SEM) 515/RDAX PV 9900.

RESULTS AND DISCUSSIONS

In this study, the grafted superabsorbent hydrogel was synthesized from ϒ-irradiated of chitosan and 50% partially neutralized of acrylic acid. Fig. 1 shows the general product performances before irradiation procedure, after irradiation, after drying, and after grinding process.



(a) (b) (c) (d)

Fig. 1: The Superabsorbent Appearances (a) Reactant Solution, (b) Gamma-Irradiated, (c) Dried Polymer, (d) Polymer Powder

Gel Content Study

Value of gel content describe the percentage of raw material that was converted to gel form during the irradiation process. It also presents the process efficiency which depends on the sensitivity of raw material to gamma spectra. The effects of chitosan to the value of gel content are shown in Fig. 2.

The highest gel content obtained from this study was 96,85% and resulted from the reactant composition of 0,75 g of chitosan. The use of chitosan at higher quantity (1,25 and 1,75 g) cause the decreasing of gel content. It can be explained by [17] that the increasing of weight ratio of acrylic acid to chitosan can cause more acrylic acid molecules to be grafted onto the

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backbones of chitosan. In contrast, the excess of chitosan in the reactant composition caused the polymer density decreased and as a result, gel concentration of the product also decreased.

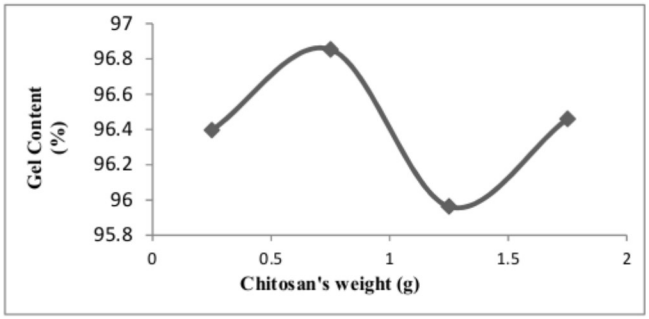

Fig. 2: Effect of Chitosan's Weight to The Gel Content of Grafted Polymer

Swelling Behavior

Swelling ratio shows the superabsorbent ability to absorb water or other solution. The water swelling study of chitosan-graft-poly (acrylic acid) superabsorbent hydrogel from this experiment is illustrated in Fig. 3. Examination was performed during the time intervals of 30, 60, 90, 120, 150, and 180 seconds, in various quantities of chitosan in the reactant mixture (0,25; 0,5; 1,25; and 1,75 g). The data shows that the increasing of time intervals results the increasing of swelling ratio of hydrogel. The longer the time period of immersing, the more water were absorbed into the polymer matrix. The addition of chitosan in the reactant composition also cause the increasing of water swelling ratio. The water absorbency was getting higher, but at certain point (when chitosan up to 0,75 g chitosan) it was decreasing with the increasing of chitosan concentration. This is due to the excess of chitosan which can act as IPN (Interpenetrating Network), fill in the polymer matrix and cause some difficulties for water to penetrate [14]. The highest swelling capacity of 420 (g water/g dry hydrogel) was reached at 120 seconds, resulted from product which was synthesize with addition of 0,75g of chitosan.

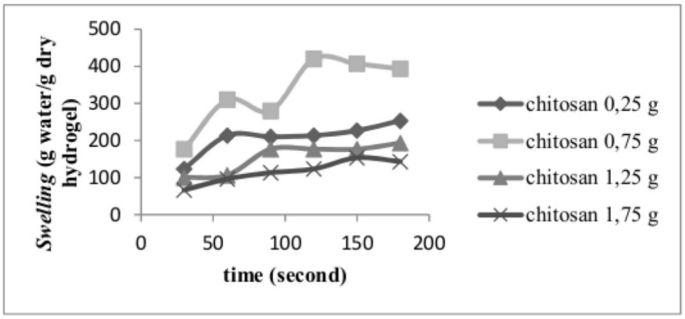

Fig. 3: Effect of Chitosan's Weight to The Water Swelling Ratio

Study of swelling capacity of hydrogel was also evaluated to urea and salt solution for 1 hour of time immersion. Based on the data from previous researcher [18], the acceptable swelling ratio of superabsorbent polymer was 20-40 g urine/g dry polymer. Urea and NaCl solution were varied 0,009; 0,09; and 0,9% of concentration (m/v). Concentration of 0,009% represents the urine of new born babies, concentration of 0,09% represents babies to kids from 6 months – 5 years, and 0,9% represents the adults.

Fig. 4 describe the swelling capacity of hydrogel in NaCl and urea solutions. The highest swelling ratio of 403,33 g NaCl solution/g dry hydrogel was obtained at NaCl concentration of

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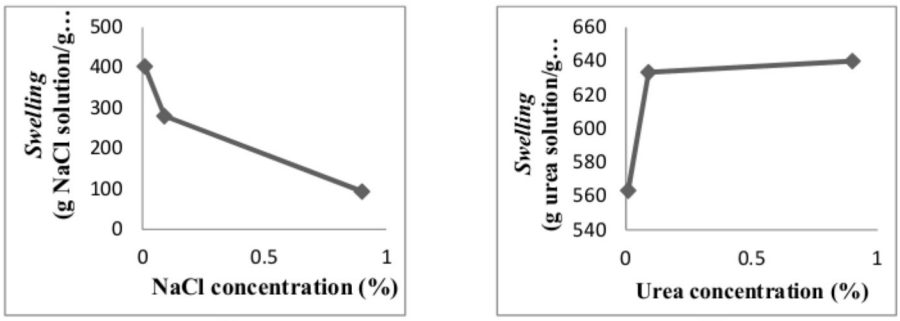
0,009% (Fig. 4a). Higher concentration of NaCl solution caused the decreasing of swelling ratio [19]. It has been reported before [20] that the ions concentration difference in the water could influence the osmotic pressure. The osmotic pressure of hydrogel in the NaCl solution would be low due to the existence of Na^+ and Cl^- ions. The negative charge of (COO^-) from the polymer interacted with Na^+, filled in the network and as consequence reduced the absorbency.



(a) (b)

Fig. 4: Swelling Behavior of the Hydrogel in (a) NaCl Solution, (b) Urea Solution

The different result was obtained at 0,9% of urea solution concentration (Fig. 4b). In urea swelling study, higher concentration of urea promoted greater solution absorbency. The highest swelling ratio in urea solution resulted from this study was 640 g urea solution/g dry hydrogel. Previous article [21] explained that urea has the NH_2 side which is hydrophilic and possible to interact with water. This is the reason why the swelling ratio of hydrogel in the urea solution smaller than in the pure water.

Equilibrium Degree of Swelling (EDS) Study

Chitosan has NH_2 and OH functional groups which will form the hydrogen bound with water molecules and enhance water absorbency of hydrogel product. Water penetration into hydrogel matrix occurs continually until the equilibrium condition is reached. This equilibrium condition is called Equilibrium Degree of Swelling (EDS) [20]. EDS value shows the maximum swelling capacity of hydrogel at a certain period of time.

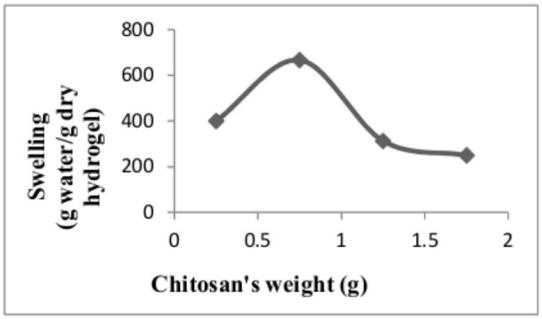


Fig.5: Effect of Chitosan's Weight to The EDS

In this research, 24 hours of time immersion was performed to evaluate the maximum capacity of water absorbency of the hydrogel (Fig. 5). Addition of 0,75 g chitosan resulted the maximum value of EDS (666,67 g water/g dry hydrogel). This huge capacity of water absorbency is fited with SNI16-6363-2000 requirement and suitable for sanitary napkins application [22].

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FTIR Study

This analysis was observed to find the functional groups that may be formed as an effect of ϒ-irradiation reaction. Fig. 5c shows the spectra of non-irradiated acrylic acid which contained –OH observed at 2500 cm^{-1} and C=C observed at 1750 cm^{-1}. Spectra of non-irradiated chitosan was illustrated in Fig. 5b, the band observed at 3500 cm^{-1} can be attributed to –NH and –OH observed at 2500 cm^{-1}. Fig. 5a describes the sprectra of chitosan-graft-poly (acrylic acid). The band observed at 3500 cm^{-1} is due to stretching of –NH groups of chitosan, the –OH or –OK groups at 2500 cm^{-1} represented acrylate salt. The C-H groups stretching was shown by the band observed at 1350 cm^{-1}, meanwhile C=C functional grups did not appear in the spectrum of produced hydrogel. This fact prooved that chitosan and neutralized acrylic acid were succesfully crosslinked, and there was no acrylic acid in the form of homopolymer in the polymer product.

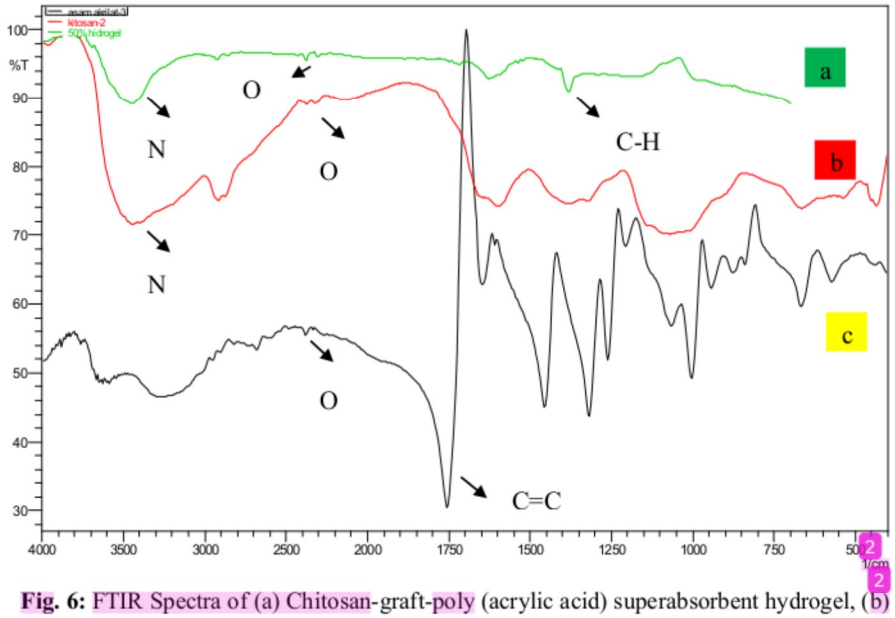

Fig. 6: FTIR Spectra of (a) Chitosan-graft-poly (acrylic acid) superabsorbent hydrogel, (b) Chitosan, (c) Acrylic Acid

Antimicrobial Study

The resulted graft polymer was antimicrobial tested against *Escherechia coli.* After 24 hours of incubation, the inhibition activity of hydrogel is shown in Fig. 7. The use of 0,75 g of chitosan as a backbone for the graft polymer, and at the same time act as an antimicrobial agent resulted 1,5 cm of inhibition area. This is re best inhibition area compared with the other chitosan weight. The use of 0,25; 1,25; and 1,75 g of chitosan resulted inhibition areas 1,3; 1,25, and 1,2 cm.

Up to 0,75 g of chitosan promoted high viscousity of hydrogel and caused poor diffusion in the area of inhiition. The effectivity of chitosan as an antimicrobial agent has been proofen by some researchers [11,23]. The antimicrobial activity of chitosan was explained through a mechanism that involved inhibition of microorganism metabolism and moreover caused the death. The other mechanism claimed that the positive charge of chitosan has the ability to interact with the DNA from microbia and inhibits the formation of RNA and protein.

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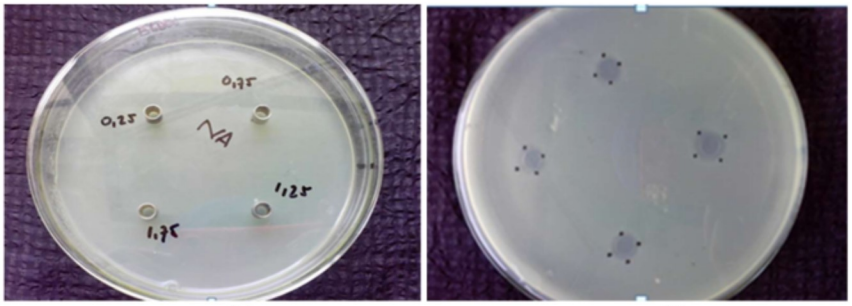

(a) *(b)*

Fig. 7: Antimicrobial analysis against Escherechia coli

CONCLUSIONS

Superabsorbent hydrogel could be made by grafting of synthetic and natural hydrophilic polymers. Chitosan-graft-poly (acrylic acid) resulted in this study has a light-yellow colour and good physical appearance. An amount of 0,75 of chitosan in the reaction mixture resulted a grafted polymer after ϒ-rays irradiation with the best absorption (420 g g^{-1} in distilled water, 403,33 g g^{-1} in 0,009% NaCl solution, and 640 g g^{-1} in 0,9% urea solution). The gel content as high as 96,85% and EDS as high as 666,67 g g^{-1} of its dried weight were also obtained by using of 0,75 g of chitosan. The hydrogel was found to be effective against *Escherechia coli*, and fited with SNI16-6363-2000.

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