Akreditasi LIPI Nomor : 395/D/2012
Tanggal 24 April 2012

SYNTHESIS AND IMMOBILIZATION OF CAPTOPRIL IN HYDROGEL POLYVINYL ALCOHOL-POLYVINYL PIRROLIDONE PREPARED BY FREEZING-THAW AND GAMMA RADIATION

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Received: 9 Januari 2013 *Revised: 30 April 2013* *Accepted: 13 Juni 2013*

ABSTRACT

SYNTHESIS AND IMMOBILIZATION OF CAPTOPRIL IN HYDROGEL POLYVINYL ALCOHOL-POLYVINYL PIRROLIDONE PREPARED BY FREEZING-THAW AND GAMMA RADIATION. In order to apply the hydrogel as a matrix for controlled drug release, the polyvinylalcohol (PVA)-polyvinyl pirrolidone (PVP) hydrogels have been synthesized and used for immobilization of captopril. The mixture of PVA-PVP solution containing captopril were freezing and thawing followed by gamma irradiation from Co-60 with the doses of 10 kGy, 20 kGy and 30 kGy. The water absorption and gel fraction of hydrogels were measured gravimetrically. The chemical changes of hydrogels were determined using Fourier Transform Infrared (FT-IR) spectrometer. The release of captopril were monitored using Ultra Violet-Visible (UV-Vis) spectrophotometer. It was found that with increasing cycled of freeze-thawing and irradiation dose up to 30 kGy, gel fraction and water absorption of hydrogels increase. FT-IR spectra demonstrated that there was strong intermolecular crosslinking between the PVA and PVP. The release rate of captopril from hydrogel increases with increasing irradiation dose. The release pattern indicates that the captopril released in medium were with zero order during most of the release period and generally with burst effect. The results suggest that crosslinked PVA-PVP hydrogels are suitable for controlled release of drug.

***Keywords*:** Drug released, Irradiation, Immobilization, Captopril, PVA-PVP, Hydrogel

ABSTRAK

SINTESIS DAN IMOBILISASI KAPTOPRIL PADA HIDROGEL POLIVINIL ALKOHOL-POLIVINIL PIRROLIDON HASIL PROSES BEKU-LELEH DAN IRADIASI GAMMA. Dalam upaya aplikasi hidrogel sebagai matriks untuk pelepasan obat terkendali, telah diakukan sintesis hidrogel polivinil alkohol (PVA)-polivinil pirrolidon (PVP) dan digunakan untuk imobilisasi kaptopril. Campuran larutan PVA-PVP yang mengandung kaptopril dibeku-leleh yang dilanjutkan iradiasi gamma dari sumber kobalt-60 dengan dosis 10 kGy, 20 kGy dan 30 kGy. Air terserap dan fraksi gel ditentukan secara gravimetri. Perubahan kimia hidrogel dianalisis menggunakan spektrometer *Fourier Transform Infrared* (*FT-IR*). Pelepasan kaptopril dari media uji diukur menggunakan spektrofotometer *Ultra Violet-Visible* (*UV-Vis*). Hasil penelitian menunjukkan bahwa dengan meningkatnya siklus beku-leleh dan dosis iradiasi hingga 30 kGy, fraksi gel dan air terserap hidrogel meningkat. Analisis pada spektrum FT-IR menunjukkan terjadinya ikatan silang antara PVA dan PVP. Laju pelepasan kaptopril dari hidrogel meningkat dengan meningkatnya dosis iradiasi. Pola pelepasan kaptopril dalam media sebagai orde nol yang sebagian besar bersifat sebagai *burst effect*. Disimpulkan bahwa PVA-PVP berikatan silang dapat digunakan sebagai matriks pelepasan obat terkendali.

***Kata kunci*:** Pelepasan obat, Iradiasi, Immobilisasi, Kaptopril, PVA-PVP, Hidrogel

INTRODUCTION

Hydrogels are three-dimensional, cross-linked networks of hydrophylic polymers. Hydrogels can be synthesized from any hydrophylic polymer, encompassing a wide range of chemical compositions

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ISSN : 1411-1098

and bulk physical properties. Furthermore, hydrogels can be formulated in a variety of physical forms, including slabs, microparticles, nanoparticles, coatings and films. As a result, hydrogels are commonly used in clinical practice and experimental medicine for a wide range of applications, including tissue engineering and regenerative medicine [1], diagnostics [2], cellular immobilization [3], separation of biomolecules or cells [4], and barrier materials to regulate biological adhesions [5].

Porous structure is one the unique physical properties of hydrogels that particular interest in drug delivery applications The porosity of hydrogels can easily be prepared by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network [6].. Indeed, the benefits of hydrogels for drug delivery may be largely pharmacokinetic-specifically that a depot formulation is created from which drugs slowly elute, maintaining a high local concentration of drug in the surrounding tissues over an extended period, although they can also be used for systemic delivery. Hydrogels are also generally highly biocompatible, as reflected in their successful use in the peritoneum and other sites in vivo.

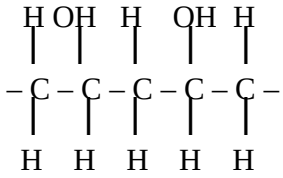

Figure 1. Chemical structure of Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is hydrophylic synthetic polymer with hydroxyl groups along its polymer chains (Figure 1), long utilized in the form of a hydrogel. The body tissue response to PVA gels has been found to be mild, but they are known to undergo calcification over time when in contact with body fluids and thay do not have optimum mechanical properties. Nevertheless PVA hydrogels are not of sufficient lubrication due to the strong action hydrogen bond formed in inner molecules. In order to improve the surface lubrication, poly(vinyl pirrolidone) (PVP) is another water soluble synthetic polymer (Figure 2) was compounded with PVA. However, the combination of PVA with PVP can be made medical grade of hydrogels. Freezing and thawing one of the best methods to improve the biomedical quality of poly(vinyl alcohol) (PVA)-polyvinylpirrolidone (PVP) blends with high biocompatibility, high-mechanical strength and good chemical stability [7,8]. Instead of the advantages of that method, freezing-thaw can also be applied for

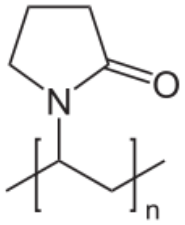

Figure 2. Chemical structure of poly(vinyl pirrolidone)

immobilization of drugs in PVA-PVP hydrogel matrix with combination using gamma rays irradiation process to enhance the strength, and sterility of hydrogels simultaneously. Besides, the effect irradiation on the drugs in the freeze state do not change significant, caused the drugs localized inside the densed hydrogel matrix with lower water content.

Captopril (Figure 3) is one of the most popular drugs used for treatment hypertension as an active inhibitor of angiotensin-converting enzyme (ACE), shows clinical effectiveness. However, the efficacy of captopril as a first choice of drug with antihypertensive action after oral dosing is limited from 6 h up to 8 h. Therefore, clinical use requires a dose of 37.5-75 mg to be taken 3 times daily [9]. Development of a sustained release dosage form of captopril have many advantages to patients such as; (1) decreasing the frequency of administration which lead to an improvement in patient compliance and as a result clinical efficiency would be improved; (2) it is expected that a minimization of fluctuations in the blood concentration of the drug will decrease the risk of side-effects [2].

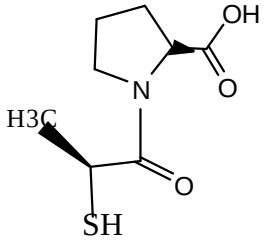

Figure 3. Chemical structure of captopril

On the based above descriptions, we immobilized captopril in PVA-PVP hydrogel matrix through freezing-thaw and followed by radiation processing simultaneously in the aim to prepare controlled drug release systems. The captopril release profile in the medium was monitored using UV-Vis spectrophotometer at 208 nm.

EXPERIMENTAL METHOD

Materials

Polyvinyl alcohol (PVA) with degree safonication of 99 % was produced by Kuraray. Polyvinyl pirrolidone (PVP) and Povidone 90 KF were obtained from BASF.

Captopril was produced by Merck. All other reagents were pro-analytical grade and used without purification.

Instrumentation

The chemical changes of hydrogels was characterized using Fourier Tranform Infrared (FT-IR) Spectrophotometer (Shimadzu, Prestige-21). Captopril released in the medium was monitored using UV-Vis Spectrophotometer (Shimadzu). Otoclaf was used for dissolving PVA powder. Irradiation sources from Co-60 was used to prepare hydrogels with dose rate of 7.5 kGy/h and calibrated with frieke dosimeter.

Procedure

Gel Fraction

The hydrogel samples in distilled water were taken into shaker incubator at room temperature to remove soluble fraction for 24 hours. The gels were dried to constant weight under vacuum to determine the insoluble fraction in the samples gravimetrically and was calculated as

$$\text{Gel Fraction} = W_g/W_o \times 100\ \% \quad \ldots\ldots\ldots\ldots \quad (1)$$

where:

W_g = The weight of dry gel after extraction
W_o = The initial weight of the gel

Water Absorption

The water absorption of hydrogels was determined by gravimetry method. The gels samples were dried in the oven at temperature of 60 °C up to constan weight. The dried samples were then immersed in distilled water at room temperature.The hydogels were periodically weighed after the excess surface water was removed with filter paper. The water absorption was calculated as

$$\text{Water absorption} = (W_1\text{-}W_o)/W_o \times 100\ \% \quad \ldots\ldots\ldots \quad (2)$$

where:

W_1 = The weight of swollen gel at time t
W_o = The weight of the dried gels.

FT-IR Characterization

Fourier transform-infra red (FT-IR) spectrophotometer was used to characterize the presence of specific chemical groups in the PVA-PVP hydrogels. FT-IR spectra were obtained over the range of 400-4000 cm^{-1} (Shimadzu IR-Prestige-21 spectrometer model 800 series). Samples were milled and mixed with dried KBr powder placed in a sampling cup, 20 scans were required at 2 cm^{-1} resolution with subtraction of the KBr background. FT-IR spectra were also obtained for pure PVA and PVP for comparison with the hydrogels.

Immobilization of Captopril

Eighteen grams of PVA and 12 g of PVP were poured into 200 mL aquadest. Afterward, the mixture was dissolved by autoclaving at 121 °C for 20 minutes, then the samples were taken out and left to cool at room temperature. The PVA-PVP solution was poured into 7 mL vials and 50 mg captopril, homogenized and then freezing at -15 °C for 16 hours and thaw at 25 °C for 8 hours 1 cycle process) respectively. The process of freezing-thaw were repeated up to 3 cycles. The mixtures were then irradiated using gamma rays from cobalt-60 at the doses of 10 kGy, 20 kGy and 30 kGy (dose rate 7.5 kGy/hours). The hydrogels were taken out from the vials, washed with water and soaked into 250 mL 0.01 N HCl solution in beaker glass and shaked in shaker incubator at 100 rpm at room tempeature. At a predetermined period of the in-vitro release experiment, 5 mL aliquots of the medium was removed from the beaker glass and the concentration of the captopril in that aliquots was measured by using a UV-Vis spectrophotometer (Shimadzu UV-Vis spectrophotometer and a standard calibration curve). A 5 mL fresh 0.01 N HCl solution was added back to beaker glass to maintain the same total solution volume. All release studies were carried out in triplicate. The results were presented in terms of cumulative release as a function of time.

RESULTS AND DISCUSSION

Gel Fraction

The effect of only freeze-thaw on the gel fraction of hydrogels are presented in Table 1. It can be seen that with increasing freeze-thaw from 1 cycle to 3 cycles in the preparation of hydrogel, the gel fraction of hydrogels increases from 65.71 % to 74.88 %. It is related with the decrease of molecular distances between PVA and PVP in the matrix in the form of physically crosslinked as a results of freeze-thaw. In addition, the gel fraction of hydrogels after freeze-thaw and followed by irradiation from 0 kGy to 30 kGy are increasing from 65.71 % to 91.17 %. It is indicated that crosslinking occurs between PVA and PVP.

Table 1. Gel fraction (%) of PVA-PVP hydrogels prepared by freeze-thaw and irradiaton

Dose (kGy)	Process (cycle)		
	1	2	3
0	65.71	68.32	74.88
10	81.16	82.81	83.63
20	86.27	86.61	87.09
30	87.59	89.43	91.17

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Water Absorption

The water absorption gives an indication of the hydrophobicity or hydrophylicity of polymers. This was evaluated using Eq.(2), and the results are shown in Figure 4(a), 4(b), 4(c) and 4(d). From Figure 4(a), it can be seen that water absorption of hydrogels prepared by freeze-thaw alone increased with increasing freeze-thaw cycles up to 3 cycles. In contrary, this phenomenon actually uncommon occurs for the hydrogel contained PVA alone, in which with increasing cycled freeze-thaw, the water absorption of hydrogels decreased [10]. However, it is attributted from not all PVP molecules effectively entrapped in the hydogel during freeze-thaw, and when the hydrogels are exposured to medium, the water absorption increase [11].

The effect of freeze-thaw and irradiation doses on the water absorption of hydrogels are presented in Figure 4(b), 4(c) and 4(d). For 10 kGy, it can be seen that water absorption of hydrogels are increased gradually as a function of time of immersing. But, water absorption decreased with increasing freeze-thaw cycles. The results suggested that the degree of crosslinking of hydrogels increased and water diffusion restricted. With increasing irradiation doses up to 30 kGy, the water absorption of all hydrogels increase. It is atributtable from PVP as hydrophylic site not incorporated into the PVA network out of the gel (non irradiation) and with increasing irradiaton dose crosslinks between PVA and PVP increased [11].

In-Vitro Captopril Release from PVA-PVP Hydrogels

The effect of time on the cumulative amounts of captopril released from PVA-PVP hydrogels prepared by freeze-thaw up to 3 cycles without irradiation treatment (control) are shown in Figure 5a. It can be seen that all hydrogels exhibite a slower rate of release and show an initial burst release of captopril. During this burst release within the first 4 hours, the cumulative captopril release was 40 % for 1 cycle, 45 % for 2 cycles and 50 % for 3 cycles freeze-thaw process. Beyond the first burst period, the release rate increases probably because captopril located near the densed surface of the hydrogels. Although all the hydrogels exhibited very similar release profiles, their release rates and extends are different. The cumulative captopril release during the 24th study period was 63 % for 1 cycle, 68 % for 2 cycles and 72 % for 3 cycles freeze-thaw process.

The observed initial burst release is probably due to those captopril that were located near the hydrogel surface. Since the concentration gradient is the driving force for captopril diffusion, high captopril concentration gradient between the hydrogel surface and the release medium during the very early stage of contact leads to higher initial

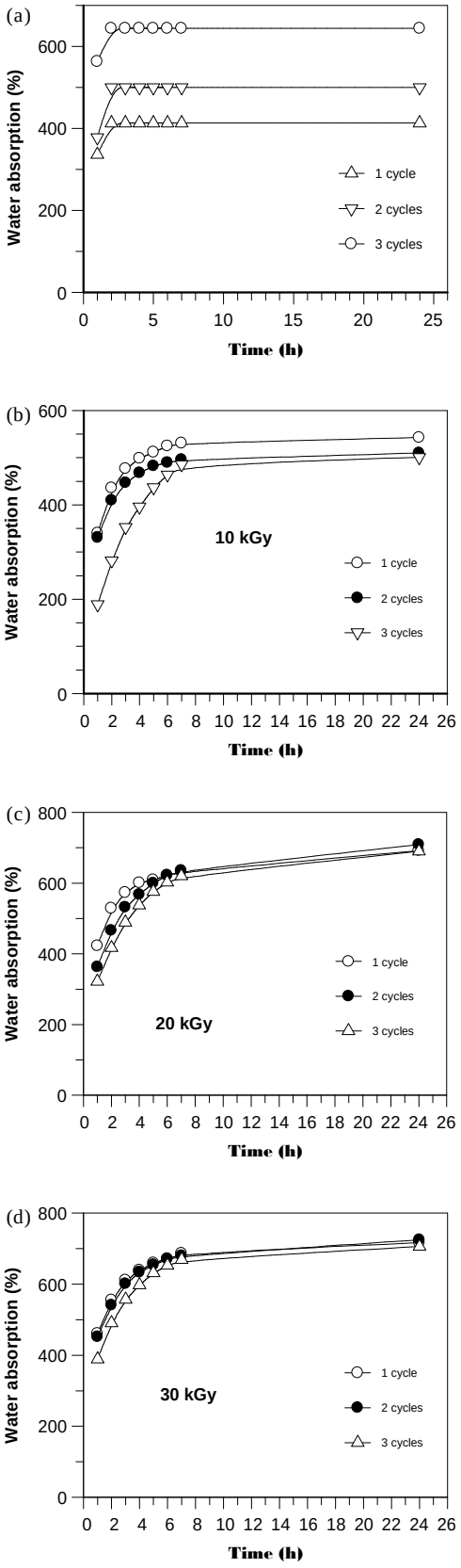

Figure 4. The effect of time of immersing on the water absorption of PVA-PVP hydrogels prepared by combination of freeze-thaw from 1 cycle up to 3 cycles and irradiation at the doses of : (a). 0 kGy (control), (b). 10 kGy, (c). 20 kGy and (d). 30 kGy

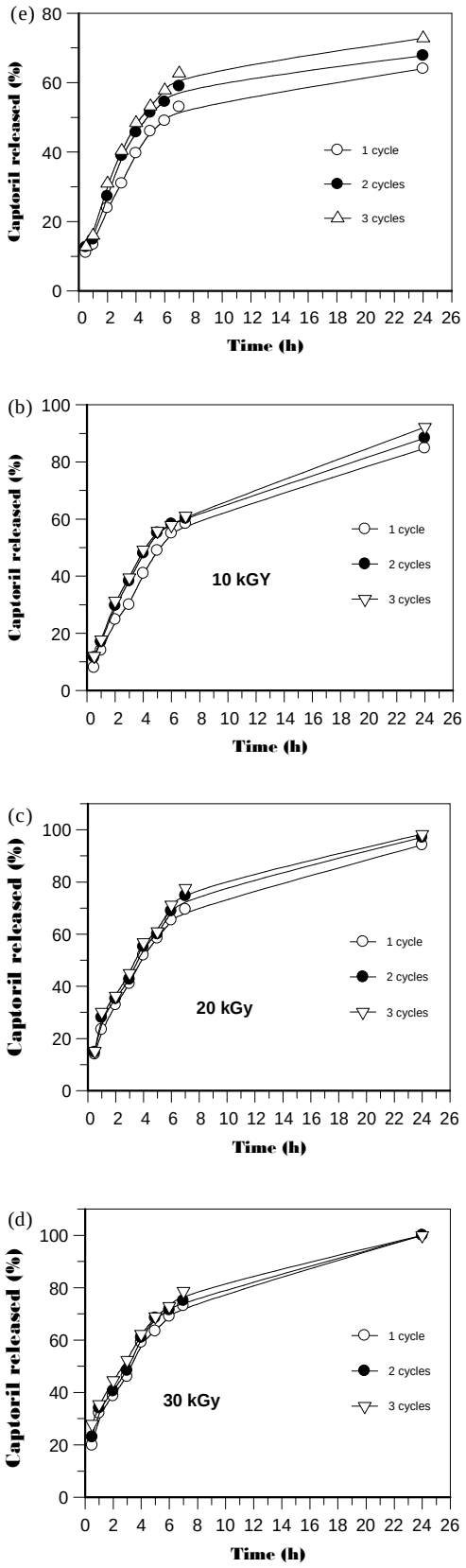

Figure 5. Cumulative amounts of captopril released from the PVA-PVP hydrogels prepared by freeze-thaw up to 3 cycles and irradiated at different irradiation doses of : (a). 0 kGy (control), (b). 10 kGy, (c). 20 kGy and (d). 30 kGy

burst and fast release rate. Those captopril located near and at surface could be released immediately from the hydrogel to the surrounding medium as soon as the hydrogel was placed into medium.

The release of captopril from different polymers microsphere such as chitosan, ethyl cellulose and sodium alginate has also been studied [12]. They found that the captopril released during the 8 h study period was 59 % for chitosan, 62 % for ethyl cellulose, and 66 % for sodium alginate.

Figure 5(b), 5(c) and 5(d) exhibits the cumulative amounts of captopril released from the PVA-PVP hydrogel prepared by combining the freeze-thawing and irradiation. The release profiles at the doses of 10 kGy, 20 kGy and 30 kGy are similar to those control, but the amounts of captopril release at 4 h of study period were higher than those control for all PVA-PVP hydrogels prepared by gamma irradiation, especially for 3 cycles repeated freeze-thaw. For examples, 10 kGy sample had 49 %, 20 kGy had 57 %, and for 30 kGy had 62 %. It is suggested that with increasing irradiation dose, the crosslink between PVA and PVP increased and surface lubricative properties of PVA-PVP hydrogels were improved and stable [11].

FT-IR Characterization

FT-IR spectrum of pure PVA and pure PVP are given in Figure 6. The major peak assign to PVA, PVP and PVA-PVP are summarized in Table 2. It can be seen that the spectrum of PVA-PVP hydrogel (non irradiation) consisted of the functional groups present in all two pure components. O-H (stretch) and CH_2 stretch in pure PVA, CH_2 stretch, contribution from C=O and –C in pure PVP was clearly observed in the FT-IR spectrum for non irradiated PVA-PVP hydrogel (Figure 6). In contrary, as results of PVA blended with PVP, the peak height of OH and C=O was decreased and widespread when compared with the similar peaks contributed from each polymer. It is indicated that a hydrogen bonded occur as intermolecular interaction between the carbonyl oxygen on a PVP chain and a hydroxyl group along a PVA chain as shown in Figure 7 [13,14].

Table 2. Major peaks present in FT-IR spectrum of PVA and PVP

Absorption (cm^{-1})	Functional groups
PVA	
2989-3766	OH stretch
2931	CH_2 strecth
2840	CH strecth
1718,1469	C=O
PVP	
3661	OH strecth (probable)
2931/2864/2738	CH and CH stretch
1703	contribution from C-O and N-C
1493/1469/1440	CH deformation of cyclic CH_2 groups
1289/1270	amide III band (C-N stretch)
754	amide V band or CH2 rock
647	amide IV band

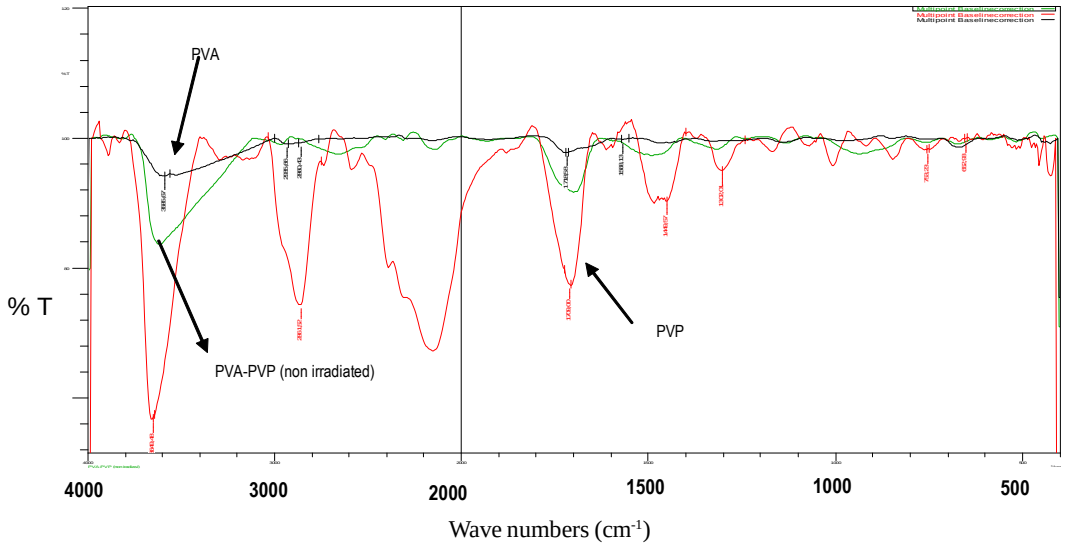

Figure 6. FT-IR spectrum of (a). PVA, (b). PVP and (c). PVA-PVP (non irradiation) hydrogel produced by freeze-thaw

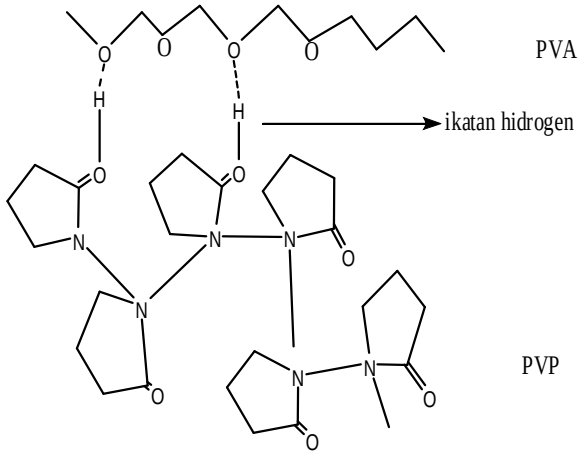

Figure 7. Interchain hydrogen bonding within a PVA/PVP blend occurs between carbonyl groups on PVP and hydroxyl groups on PVA

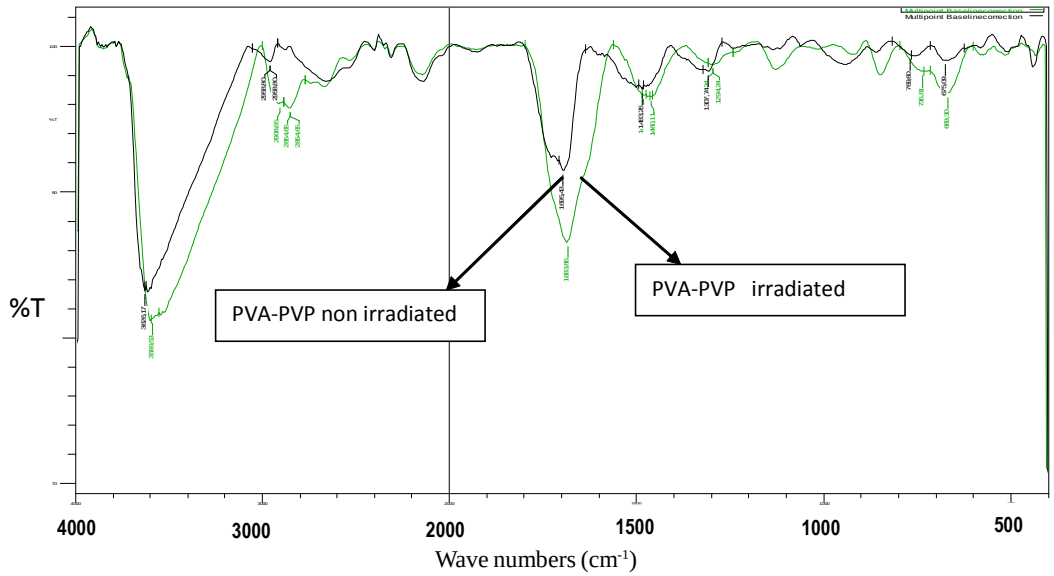

Figure 8. FT-IR spctrum of PVA-PVP hydrogel (non irradiated) and PVA-PVP hydrogel (freeze-thaw and irradiated)

When the spectrum of PVA-PVP (non irradiation) was compared with the irradiated hydrogel (Figure 8) there was no significant difference in peak pattern, but there was an increased in the peak height and shifted of position of OH and C=O towards lower frequencies. It is indicated that crosslink occurred between PVA and PVP as the the effect of irradiation [11].

CONCLUSION

The crosslinked PVA-PVP hydrogel was synthesized through combination of freeze-thaw and gamma irradiation. The number of cycle of freeze-thaw and irradiation dose had an direct effect on gel fraction of PVA-PVP hydrogels and inverse effect on their water absorption.The release of captopril results correlated with the water absorption results. The release of drug from hydrogel is found to be dependent on the irradiation dose. As the irradiation dose increases, drug release increased. The results suggest that PVA-PVP crosslinked hydrogels are suitable for controlled release of drug.

ACKNOWLEDGMENT

Author gratefully acknowledged to colleagues at irradiation facilities PATIR-BATAN which has been providing the samples irradiation and to Prof. Ir. Sugiarto for his valuable comments and discussions.

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