Synthesis and Characterization of Hydrogel of Chitosan-Poly(N-Vinyl-2-Pyrrolidone) (PVP)-Alginate for Ibuprofen Release

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Received 4 August 2020; Accepted 31 October 2020

ABSTRACT

Hydrogels chitosan-poly-(N-vinyl-pyrrolidone)-alginate (Ch/PVP/Alg) have been synthesized with Ca²⁺, Zn²⁺ and formaldehyde as crosslinker. Hydrogels with ratio polymer 70:20:10 give a high swelling ratio and good network. The Ch/PVP/Alg/Ca has 463.73% swelling ratio and 80.59% gel. Ch/PVP/Alg/Zn has 489.21% swelling ratio and 81.67% gel. Ch/PVP/Alg crosslinked with formaldehyde result 488.03% swelling ratio and 85.34% gel. Dissolution test of hydrogels in pH 1.2 releases ibuprofen less than 30%. Whereas in the pH 7.4, the release of ibuprofen by hydrogels are relatively high. Ch/PVP/Alg/Ca reach up to 34.63% in 30 minutes and 40.86% for Ch/PVP/Alg/Zn. Meanwhile Ch/PVP/Alg/CH₂O can release 44.92% of ibuprofen in 30 minutes. The obtained hydrogel was characterized using infrared (FTIR) spectrophotometry, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM).

Keywords: chitosan, hydrogel, poly-(N-vinyl-pyrrolidone), alginate, swelling.

INTRODUCTION

Hydrogels are three dimensional cross-linked hydrophilic polymers. Hydrogels have an interesting behavior for their ability in absorbing and retaining water or biological fluid up to thousands of time of their dry weights even under certain pressure. Due to high water contents, porosity and soft consistency, hydrogels have been used for the design of controlled drug delivery systems especially for the hydrogels derived from the natural polymers [1-9]. Chitosan (Ch) is one of the important natural polymer-derived from the deacetylation of chitin. It consists of N-acetyl-D-glucosamine and D-glucosamine units. Chitosan has a good biocompatibility and biodegradability. Thus, it can be used as the main structure of hydrogels [10-11]. However, the flexibility of chitosan is not as good as its capability in absorbing fluid. This characteristic can be improved through blending, cross-linking and grafting with other materials. In this study we synthesized chitosan hydrogels using crosslinking method. In general, the cross-linked chitosan hydrogels can be extracted using four different ways; via physical cross-linking through a hydrophobic interaction, ionic interaction or hydrogen bonding, coordination with metal ions, chemical cross-linking between polymers and the cross-linker. The synthesis of chitosan hydrogel has been reported by several studies. For example, Zhou reported that chitosan hydrogel can be formed through electrostatic interaction between chitosan with Ca²⁺ [12]. Otherwise chitosan can generate hydrogel through physical interaction by adjusting pH value. Chitosan can be easily modified by Schiff base reaction with aldehyde groups such as glutaraldehyde, formaldehyde or acetaldehyde, due to the presence of hydroxyl groups and

The journal homepage www.jpacr.ub.ac.id
p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

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Amino groups distributed along the chitosan chain. The Schiff base reaction involving chitosan and aldehyde produce polymer networks [13].

Sodium alginate (Alg) is anionic natural polymer, extracted from brown seaweed such as Sargassum sp. and Tamerin sp. Alginat is a non-toxic biodegradable polymer contain various proportions of β-D-mannuronic acid (M block) and α-L-guluronic acid (G block) units. Alg is able to form three-dimensional networks with multivalent metal cations such as Ca$^{2+}$, Zn$^{2+}$ and Cu$^{2+}$ through carboxylic (-COO$^{-}$) groups in G block [14, 15]. Moreover, negative charges of Alg allow it to shrink in acidic pH and to swell in neutral or basic pH environment. Thus, the pH-sensitive characteristic allows the hydrogels obtained from Alg to be used as a drug release in gastric and intestinal environment.

In addition to those, poly (N-vinyl-2-pyrrolidone) (PVP) is an aprotic polar, a water-soluble linear polymer and a nontoxic polymer. So, it can increase the water affinity of the polymers [6, 16]. PVP has been used to produce hydrogels and has been applied in the pharmaceutical industry as drug release such as PVP-film for release enrofloxacin [17]. The combination of PVP and chitosan or chitosan and alginate had been used in several studies for a controlled release of drug such as paracetamol and lysozyme [14, 15]. However, there is no previous works about the combination of chitosan, PVP and sodium alginate in the drug delivery field.

In this work, we design hydrogels chitosan, poly-(N-vinyl-2-pyrrolidone) and alginate through physical, ionic and chemical interaction, to be used as new drug delivery material. It is expected that chitosan, PVP and alginate will form cross-linking interaction and increase drug release ability. The characteristic of hydrogels is then improved by using the various cross-linkers (e.g. Ca$^{2+}$, Zn$^{2+}$ and formaldehyde). The obtained hydrogels undergo a physical characterization by using Fourier transform infrared (FTIR) spectrophotometry, thermal analysis (DSC) and morphological analysis (SEM).

**EXPERIMENT**

**Chemicals and instrumentation**

Chitosan (Ch) with deacetylation degree of 85% and molecular weight of about $1 \times 10^4$ is obtained from BATAN. Poly-(N-vinyl-2-pyrrolidone) K30 (PVP) and sodium alginate from Sigma Aldrich. The other chemical materials such as formaldehyde, calcium chloride (CaCl$_2$), zinc chloride (ZnCl$_2$), ibuprofen and acetic acid glacial are obtained from Merck, Germany. All chemical reagents are in analytical grade and all solutions are prepared with distilled water.

Instruments used for research include analytical balance (AND) is used to observe gel fraction and swelling ratio of hydrogels. The film of Ch/PVP/Alg hydrogels are characterized by spectrometer Fourier transform infra red (FTIR, IR prestige-21), differential scanning calorimetric (DSC 60-A Shimadzu), and scanning electron microscope (SEM Jeol JSM 6510 LA).

**Preparation of Ch/PVP/Alg hydrogels**

Chitosan (2.0 g), PVP and alginate (0.2 – 10.5 g) are dissolved in 90 mL acetic acid solution (1%, v/v) at room temperature. The cross-linker, formaldehyde (0.1 M)/ CaCl$_2$ (0.5 M)/ ZnCl$_2$ (0.5 M), is directly added to the solution. The mixture is continuously stirred using mechanical stirrer for 3 h allowing the crosslinking reaction to complete. The hydrogels are filtered out and washed with distilled water to neutral pH. Finally, the hydrogel products are dried to reach constant weight at 40ºC.
Hydrogels swelling test

The hydrogels film (0.01 g) is placed into a sieve pouch and soaked in distilled water to swell until a suitable period. The sample is carried out from water and removed the excess water in the surface with filter paper. The weight of the swollen hydrogels is measured. The swelling ratio (Q, g/g %) is calculated following equation below.

\[ Q(\text{g/g} \%) = \frac{W_s - W_d}{W_d} \times 100\% \]  

(1)

Where \( W_s \) is the weight of the swollen hydrogels and \( W_d \) is the weight of the dry hydrogels.

Determination of Gel Fraction

The hydrogels film (0.01 g) is extracted by acetic acid solution for 24 h and then dried to a constant weight. The gel fraction is calculated following equation below.

\[ G(\%) = \frac{W_e}{W_o} \times 100\% \]  

(2)

Where \( G \% \) is gel fraction percentage, \( W_e \) is the weight of the dry hydrogels after extraction and \( W_o \) is the weight of the dry hydrogels before extraction.

Characterizations

The FTIR spectrum is obtained from the film sample on FTIR spectrometer in wavelength region 400-4000 cm\(^{-1}\) (FTIR, IR prestige-21). The surface morphologies of the sample are examined by a scanning electron microscope (SEM Jeol JSM 6510 LA) after coating the sample with gold film. Differential scanning calorimetric measurements are performed in DSC 60-A Shimadzu, with heating rate 10\(^\circ\)C min\(^{-1}\) from 30 \(^\circ\)C to 500 \(^\circ\)C.

![Figure 1. Proposed reaction of the formation of Ch/PVP/Alg hydrogel](image)

RESULT AND DISCUSSION

Reaction mechanism

When the chitosan (Ch), PVP and alginate (Alg) is blended in acetic acid solution with the present of cross-linker, formations of network between -COO- group of Alg with ionic cross-linker (Ca\(^{2+}\), Zn\(^{2+}\)) and produce molecular interaction among each polymers chain. Its form physical hydrogels through ionic interaction between positive charge of chitosan and negative charge of alginate, and hydrogen bonding between -OH group of chitosan and C=O groups of PVP or between –COO\(^{-}\) groups from alginate and NH\(_2\) groups of chitosan. Chitosan
network is also produced by crosslinking reaction of chitosan with formaldehyde. The chitosan is easily crosslinked by the formation of Schiff base between amino group of chitosan and carbonyl group of formaldehyde. The reaction is formed by formation of the imine bonds (C=N) [13]. According to the proposed reaction, a linier PVP interact with chitosan and alginate network through hydrogen bonding to form Ch/PVP/Alg (Figure 1).

**FTIR spectral analysis**

FTIR spectral analysis is used to provide information about the formation of network between chitosan, PVP, and alginate. The FTIR spectra of chitosan (Ch), PVP, alginate, and Ch/PVP/Alg hydrogels film using different kinds of cross-linker are depicted in Figure 2. Chitosan has characteristic absorption bands at 3291 cm\(^{-1}\) belonging to hydroxyl and amino group stretching. Absorption band at 1585 cm\(^{-1}\) is bending vibration of amino group. Meanwhile the absorption band at 1646 cm\(^{-1}\) is carbonyl stretching vibration of remaining acetamide group in chitosan.

![Figure 2](image)

**Figure 2.** FTIR spectra of (a) PVP (b) chitosan, (c) alginate, (d) Ch/PVP/Alg, (e) Ch/PVP/Alg/Ca, (f) Ch/PVP/Alg/CH\(_2\)O, and (g) Ch/PVP/Alg/Zn

The spectra of PVP have characteristic absorption band at 1644 cm\(^{-1}\) for carbonyl stretching vibration (C=O) and the absorption band at 1297 cm\(^{-1}\) is stretching vibration of C-N group. The alginate showed broad band at 3231 cm\(^{-1}\) correlated to O-H stretching. Sharp band at 1593 cm\(^{-1}\) correspond to -COO\(^{-}\) asymmetric stretching vibration, and 1404 cm\(^{-1}\) belong to the -COO\(^{-}\) symmetric stretching vibration. The peaks of C-O stretching found at 1023 cm\(^{-1}\), respectively. The characteristic peaks of Ch/PVP/Alg blended film are shifted from those of the original chitosan, PVP, and alginate. This result proves that molecular interactions and molecular compatibility occur among each polymer chains of chitosan, PVP, and alginate. The broad band at 3241 cm\(^{-1}\) correspond to O-H the stretching band from the combination between
chitosan and alginate. Absorption band at 1643 cm\(^{-1}\) and 1541 cm\(^{-1}\) relate to –CO stretching of PVP and the remaining acetamide group in chitosan and –COO- groups of alginites. A significant absorption peak from Ch/PVP/Alg/CH\(_2\)O hydrogel occur at 1566 cm\(^{-1}\) indicates the formation of Schiff base (imine bonds, C=N) after the crosslinking reaction between amino groups in the chitosan and aldehyde group in formaldehyde. Ionic interaction between Ca\(^{2+}\), Zn\(^{2+}\) ions and the carboxylate ion of alginate are shown by the shifted of –COO- groups of the alginate from 1593 cm\(^{-1}\) to lower wavenumber at 1540 cm\(^{-1}\) (Ch/PVP/Alg/Ca\(^{2+}\)) and at 1541 cm\(^{-1}\) (Ch/PVP/Alg/Zn\(^{2+}\)). Those peaks area also show the interaction between Zn\(^{2+}\) ion with amino group of chitosan through the formation of coordination bond. This bond is also found between Ca\(^{2+}\), Zn\(^{2+}\) with –OH group and –NH\(_2\) group of chitosan and –OH group of alginites. This is showed by the shifted peak from 3291 cm\(^{-1}\) (–OH, -NH\(_2\) of chitosan) and 3231 cm\(^{-1}\) (–OH of alginate) to 3248 cm\(^{-1}\) (Ch/PVP/Alg/Ca\(^{2+}\)) and 3241 cm\(^{-1}\) (Ch/PVP/Alg/Zn\(^{2+}\)). These finding proved that interaction between Ca\(^{2+}\), Zn\(^{2+}\) and functional groups of polymers occurring, and this result is also in agreement to the previous reports [14, 15, 20].

![Figure 3](image_url)

**Figure 3.** DSC curve of (a) chitosan, (b) PVP, (c) alginate, and (d) hydrogel.

**DSC Analysis**

Changing of the thermal characteristic of the hydrogels after the incorporation PVP and alginate is investigated by DSC. The thermogram of chitosan, PVP, alginate and Ch/PVP/Alg hydrogels, the endothermic and exothermic peaks are shown in Figure 3. The thermograms are similar for natural Ch, PVP, Alg and hydrogels. For chitosan (Figure 3a), the endothermic peak at 169 °C corresponds to the glass transition temperature (T\(\text{g}\)) of chitosan. It is influenced by deacetylation degree of chitosan [18]. The increasing of deacetylation degree of chitosan raises the flexibility of amorphous region and cause the decreasing of glass transition temperature. The exothermic peak at 313 °C is attributed to the chemical degradation of chitosan. The chemical degradation is the decomposition process of each amine group of chitosan at 295 °C. Meanwhile, the endothermic peak at 179 °C (Figure 3b) shows the glass transition temperature of PVP that is relate to the previous studies [19]. It was observed that T\(\text{g}\) of PVP lies on 174 °C. Another exothermic peak at 474 °C relates to the chemical degradation of PVP. Alginate shows endothermic peak at 169 °C that relate to the glass temperature of alginate (Figure 3c).
The single endothermic peak (162 °C, 168 °C, 173 °C and 169 °C) in the DSC curve of hydrogels indicates glass transition of new material (Figure 3d). While the appearance of an exothermic peak at 460 °C, which is similar to the characteristic peak of PVP, indicates that the linear PVP chains are not covalently bonded with chitosan network. PVP and Alg are incorporated in the network through hydrogen bonds between hydroxyl and amine group of chitosan. Hence, according to the change in DSC thermogram of hydrogels, it can be concluded that Ch/PVP/Alg hydrogels structure is formed by interaction between polymers with cross-linker and molecular interaction among of each polymers chain, Ch, PVP and Alg.

**Morphology analysis**

The comparison of morphology between the chitosan film formed by chitosan solution in acetic acid (Figure 4) and dry film hydrogels of Ch/PVP/Alg are shown in Figure 5. In short, the surface of hydrogels composed of Ch/PVP/Alg is rougher than that in chitosan film (Figure 4). Chitosan film shows a very flat surface and a tiny wrinkle in several spots. It is predicted that he roughness of hydrogel synthesized from Ch/PVP/Alg is due to the incorporated PVP and Alg in polymers network. The combination of PVP and Alg may improve the surface and network structure of hydrogels. However, it also improves the water absorption (hydrophilic) capability. That sharpening the hydrogel surface more wrinkle created.

In addition to that, the ionic interaction between Ca$^{2+}$, Zn$^{2+}$ ions and –COO-, and also the complexation of Ca$^{2+}$, Zn$^{2+}$ ions with -OH groups of alginate, and the complexation of Ca$^{2+}$, Zn$^{2+}$ ions with -OH and -NH$_2$ groups of chitosan also affect the surface of hydrogel to be more surge (Figure 5a-d). This result is related with the higher ability of hydrogel in absorbing water. Hydrogel of Ch/PVP/Alg/CH$_2$O shows regular rough surface because the network is formed through chemical reaction between carbonyl group of formaldehyde and amino groups of chitosan.

![SEM micrographs of chitosan film](image)

**Figure 4.** SEM micrographs of chitosan film

**Swelling behaviour of hydrogels**

The swelling behavior of hydrogels depends on the nature of polymer, and also by the existing number of hydrophobic/hydrophilic groups of polymers, the nature of crosslinker and the crosslinking density (gel fraction). Gel fraction indicates the percentage of the polymer crosslinked. If gel fraction of hydrogels is higher, then there will be a little space for water to enter into the polymer. Thus, the swelling ratio of polymer decreases. In this work, we are...
investigated the effects of the amounts of polymers on the swelling behavior and the gel fraction of hydrogels Ch/PVP/Alg.

**Figure 5.** SEM micrographs of CH/PVP/Alg/Ca (a), Ch/PVP/Alg/Zn (b), Ch/PVP/Alg/CH₂O and Ch/PVP/Alg (d).

In Figure 6 is depicted of the percentage of swelling and gel fraction for Ch/PVP/Alg hydrogels. It shows that the swelling and gel fraction are affected by the nature of polymer. The incorporation of a hydrophilic polymer such as PVP and Alg increase swelling and water uptake. The hydrophilicity of additional polymer affects the ability of hydrogels in swelling and diffusion. The portion of water bound to the polymer matrix increase with the improving of the hydrophilicity, and thus decrease the volume of free water and its movability. Conversely, increase hydrophobicity by addition polymer forms the hydrophobic regions, which is able to block the water absorbed to the hydrogel, and as result decreases the swelling ratio [16].

The optimum swelling ratio (Q%) of hydrogel with Ca²⁺ and Zn²⁺ crosslinker in each composition of polymers is shown in Figure 6. The Ca²⁺ and Zn²⁺ ions form ionic interaction with carboxyl (-COO-) group of alginates. The Zn²⁺ ion also produces coordination bond with –OH group of alginates, –OH and –NH₂ group of chitosan. This interaction produces a higher gel fraction (G %) in hydrogel. The swelling ratio (Q%) of Ch/PVP/Alg with Ca²⁺, Zn²⁺ and CH₂O crosslinker, is still high because the addition of 20% (w/w) of hydrophilic polymer, PVP.
In vitro drug release studies

The release study apply ibuprofen as a model. Ibuprofen is an anti-inflammation of non-steroids drug [20], for prevention of lung-vascular injury [21], inhibit the spinal cord injury [22]. Thus, ibuprofen commercially available and easy to be accessed by public. In this study, Ibuprofen (IBU) was used as a solubled model drug to investigate drug release behavior of the Cs/PVP/Alg hydrogels. In order to investigate the effect of pH on cumulative release profiles of drug, the percentage cumulative release of ibuprofen was measured under acidic (pH 1.2) and neutral media (pH 7.4). The result is summarized in Table 1.

Table 1. Percentage of (%) cumulative release of hydrogels formulation in pH 1.2 and 7.4

| Time (min) | Control | Ch/PVP/Alg/Ca | Ch/PVP/Alg/Zn | Ch/PVP/Alg/CHO | Ch/PVP/Alg |
|-----------|---------|---------------|---------------|----------------|-------------|
|           | pH 1.2  | pH 7.4        | pH 1.2        | pH 7.4         | pH 1.2      | pH 7.4      | pH 1.2 | pH 7.4 |
| 30        | 26.96   | 43.73         | 17.19         | 40.86          | 15.98       | 34.63       | 15.49  | 44.92  | 17.30  | 46.30  |
| 60        | 26.96   | 44.17         | 21.07         | 43.96          | 21.14       | 41.91       | 18.38  | 46.85  | 19.88  | 48.27  |
| 120       | 27.40   | 45.15         | 24.03         | 45.92          | 26.02       | 44.32       | 23.10  | 48.94  | 23.72  | 50.53  |
| 180       | 27.80   | 46.27         | 30.12         | 48.33          | 27.85       | 47.04       | 26.26  | 50.15  | 25.04  | 51.17  |
| 300       | 30.36   | 48.35         | 31.17         | 49.60          | 30.78       | 50.89       | 29.18  | 50.95  | 28.31  | 54.62  |

In overall, the cumulative release of ibuprofen in all sample and in both pH conditions give augmentation value with addition of time. The highest augmentation is resulted from polymer Ch/PVP/Alg at neutral pH for 300 minutes evaluation. Conversely, the lowest ibuprofen release is provided from polymer Ch/PVP/Alg/CHO at acidic media (pH 1.2) by releasing ibuprofen 15.49%. In the same time, controls give a moderate value in between 26.96% and 30.36% after 30 minute until 300 minute evaluation.
The detailed pattern of ibuprofen release in both pH conditions is visualized in Figure 7. The pattern of ibuprofen release for condition pH 1.2, indicate all sample have similar release improvement with increase of time. Compared to the control, it give no improvement with the time. However, under condition pH 7.4, a lesser improvement provided from Ch/PVP/Alg/Ca and Ch/PVP/Alg/Zn hydrogel. Meanwhile, the control, Ch/PVP/Alg/CH₂O and Ch/PPVP/Alg give a higher level improvement release of ibuprofen.

![Figure 7](image)

**Figure 7.** % Cumulative IBU release of hydrogels in pH 1.2 (a) and pH 7.4 (b)

In the control, ibuprofen no hydrogels is released more rapidly at pH 7.4 than at pH 1.2. Ibuprofen requires more than 5 h to be dissolved or released by 50 wt%. Its low solubility in an acidic medium (pKa value at 4.91). On the other hand, all hydrogels release 50 wt% of ibuprofen in 5 h and lesser for Ch/PVP/Alg/CH₂O (3 h) and Ch/PVP/Alg (2 h). This result
suggest, that, the hydrogels film can be apply for neutral to basic medium. Conversely, the hydrogels give a slow release profile in pH 1.2 and pH 7.4, and hydrogel of Ch/PVP/Alg/Ca and Ch/PVP/Alg/Zn provide a more effective as a slow release drug carrier at pH 7.4 compared to hydrogel created with formaldehyde as cross-linker and blended hydrogel (Ch/PVP/Alg).

CONCLUSION

A new specific drug delivery hydrogel based on combination of chitosan, poly (N-vinyl-2-pyrrolidone) and alginate was successfully developed using Ca\(^{2+}\), Zn\(^{2+}\) and CH\(_2\)O as cross-linker. The thermal behaviour, swelling capabilities and surface morphologies of Ch/PVP/Alg hydrogel indicate improvement. The optimal condition of the synthesized hydrogel is reached at the composition Ch/PVP/Alg about 70:20:10. This condition allows the hydrogel to absorb water around 445-448\%, and is able to release ibuprofen between 49.6\% and 54.62\% in neutral conditions (pH 7.4). This result opens potency application for Ch/PVP/Alg hydrogels as a slow release for ibuprofen in drug delivery system.

CONFLICT OF INTEREST

Authors declare that the manuscript published has no any competing interest.

ACKNOWLEDGMENT

This research was funded by Center of Industrial Education and Training, The Ministry of Industry, Republic of Indonesia.

REFERENCES

[1] Aldana, A.A., Gonzalez, A., Strumia, M.C., and Martinelli, M., *Mater. Chem. Phys.*, 2012, 134(1), 317–324.
[2] Abdel-Mohsen, A.M., Aly, A.S., Hrdina, R., Montaser, A.S., and Hebeish, A., *J. Polym. Environ.*, 2011, 19(4), 1005-1012.
[3] Matricardi, P., Di Meo, C., Coviello, T., Hennink, W.E., and Alhaique, F., *Adv. Drug Deliv. Rev.*, 2013, 65(9), 1172-1187.
[4] Sahoo, S., Sasmal, A., Nanda, R., Phani, A.R., and Nayak, P.L., *Carbohydr. Polym.*, 2010, 79(1), 106-113.
[5] Li, G., Guo, L., Chang, X., and Yang, M., *Int. J. Biol. Macromol.*, 2012, 50(4), 899-904.
[6] Yu, C., Yun-fei, L., Hui-min, T., and Jian-xian, J., *Carbohydr. Polym.*, 2009, 75, 287–292.
[7] Chang, C., Duan, B., Cai, J., and Zhang, L., *Eur. Polym. J.*, 2010, 46(1), 92–100.
[8] Deligkaris, K., Tadele, T.S., Olthuis, W., and Van den Berg, A., *Sens. Actuators B. Chem.*, 2010, 147(2), 765–774.
[9] Zhang, L., Bai, X., Tian, H., Zhong, L., Ma, C., Zhou, Y., Chen, S., and Li, D., *Carbohydr. Polym.*, 2012, 89(4), 1060-1066.
[10] Li, Q., Yang, D., Ma, G., Xu, Q., Chen, X., Lu, F., and Nie, J., *Int. J. Biol Macromol.*, 2009, 44(2), 121-127.
[11] Spagnol, C., Rodrigues, F.H., Pereira, A.G., Fajardo, A.R., Rubira, A.F., and Muniz, E.C., *Carbohydr Polym.*, 2012, 87(3), 2038–2045.
[12] Zhou, Y., Li, H., Liu, J., Xu, Y., Wang, Y., Ren, H., and Li, X., *Polym Adv Technol.*, 2019, 30(1), 143–152.
[13] Marsano, E., Bianchi, E., Vicini, S., Companino, L., Sionkowka, A., Skopińska, J., and Wiśniewski, W., *Polym.*, 2005, 46(5), 1595-1600.
[14] Treenate, P., and Monvisade, P., *Int. J. Biol. Macromol.*, 2017, 99, 71-78.
[15] Wu, T., Huang, J., Jiang, Y., Hu, Y., Ye, X., Liu, D., and Chen, J., *Food Chem*, 2017, 240, 361-369.
[16] Wang, W., and Wang, A., *Carbohydr. Polym*, 2010, 80(4), 1028–1036.
[17] Kumar, G.P., Phani, A.R., Prasad, R.G.S.V., Sanganal, J.S., Manali, N., Gupta, R., Rashmi, N., Prabhakara, G.S., Salins, C.P., Sandeep, K., Raju, D.B., *Int. J. Pharm*, 2014, 471(1-2), 146-152.
[18] Guinesi, L.S., and Cavalheiro, E.T.G., *Thermochim. Acta*, 2006, 444(2), 128-133.
[19] Lewandowska, K., *Thermochim. Acta*, 2011, 517(1-2), 90-97.
[20] Hao, H., Wang, G. and Sun, J., *Drug Met. Rev.*, 2005, 37(1), 215-234.
[21] Perlman, M.B., Johnson, A.R.N.O.L.D. and Malik, A.B., *Am. J. Physiol. Heart Circ. Physiol.*, 1987, 252(3), H605-H614.
[22] Pire, L.R., Lopes, C.D., Salvador, D., Rocha, D.N. and Pego, A.P., *J. Mater. Sci. Mater. Med.*, 2017, 28(10), 157.