DOKUMEN KORESPODENSI

Nama Jurnal : **Pharmacy Education**
Judul : **Effect of heating temperature on citric acid-locust bean gum synthesis**
Publisher : International Pharmaceutical Federation (FIP)
Volume/Issue : 24(3)
Tahun : 2024
DOI : https://doi.org/10.46542/pe.2024.243.216221
Index : SJR(2023) 0.174
Penulis Pertama : Wuryanto Hadinugroho
Penulis Korespodensi : Wuryanto Hadinugroho
Tahapan & Lampiran :

No.	Tahapan	Waktu
1.	**Submission received to Pharmacy Education Journal** Email submission received to Pharmacy Education Journal Manuscript draft	**20-08-2023**
2.	**Revision request before peer review** Email revision request before peer review	**08-09-2023**
3.	**Submission manuscript draft revision before peer review** Manuscript draft of revision before peer review	
4.	**First revision request** Email first revision request Manuscript draft of first revision request from reviewer 1 Manuscript draft of first revision request from reviewer 2	**18-10-2023**
5.	**Submission of first revision to Pharmacy Education Journal** Manuscript draft of first revision to reviewer 1 & 2 Response to reviewers comments (first revision)	**30-10-2023**
6.	**Second revision request** Email second revision request from reviewer	**03-02-2024**
7.	**Submission of second revision to Pharmacy Education Journal** Manuscript draft of second revision Response to reviewers comments (second revision)	**06-02-2024**
8.	**Accepted for publication** Email accepted for publication	**15-02-2024**
9.	**Review proofreading request** Email review proof request	**26-02-2024**
10.	**Submission of corrections proofreading received** Final manuscript	**26-02-2024**
11.	**Article publish in Pharmacy Education Journal** Article publish in web of Pharmacy Education Journal https://pharmacyeducation.fip.org/pharmacyeducation/article/view/2736/1826	**01-05-2024**

Submission Received for the IGSCPS 2023 Publication in Pharmacy Education Journal

Dari: IGSCPS23 Scientific (igscps23.scientific@gmail.com)

Kepada: wuryanto.hadinugroho@ymail.com

Tanggal: Senin, 28 Agustus 2023 pukul 17.46 GMT+7

Dear Wuryanto Hadinugroho

Your manuscript entitled " Effect Of Heating Temperature On Citric Acid-Locust Bean Gum Synthesis " has been successfully submitted online and is presently being given full consideration for publication in the Pharmacy Education Journal. We will let you know the progress of your submission within 10 working days.

Thank you for submitting your manuscript to IGSCPS 2023, affiliated with Pharmacy Education Journal.

Kind regards
Dr. Chrismawan Ardianto
Assigned Editor, Pharmacy Education Journal

Pharmacy Education (2023) 23(2) XX - XX
https://doi.org/

SECTION NAME

RESEARCH ARTICLE

Effect Of Heating Temperature On Citric Acid-Locust Bean Gum Synthesis

Wuryanto Hadinugroho*, Stephanie Florencia Winarko, Echa Imanuela Sinta, Senny Yesery Esar, Jefri Prasetyo

Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia

Keywords
Locust bean gum
Citric acid
Esterification
Heating
Characterization

Correspondence
Wuryanto Hadinugroho
Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia
wuryanto.hadinugroho@ymail.com

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20oC, 40oC, 60oC, and 80oC for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20 oC, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80 oC, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm-1) and 60°C (1735.1 cm-1). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; Hadinugroho *et al*. 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) (Dey et al. 2013; Kaity et al. 2013; Alves et al. 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C-6 mannose and galactose atoms have the potential to bind to acidic compounds (Samavati *et al.*, 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al.*, 2001; Palit 2009; Dey *et al.*, 2013). CA's carboxylic group could replace the OH of the C-6 atom of mannose and galactose.

Hydrochloric acid (HCl) is often used as a polymer synthesis catalyst. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl also causes the release of OH at C-6 mannose and galactose (Colas 2005; Bhattacharya *et al.*, 2008)

Esterification requires energy to form a bond between the positive C atom of the carboxylic group and the O atom on C-6 LBG (Hadinugroho *et al.*, 2017, 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the presence of CA in LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity *(Hadinugroho et al.*, 2019; Hadinugroho *et al.*, 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.

The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20 °C, 40 °C, 60 °C, and 80 °C for 30 minutes. CA-LBG was characterized by viscosity, Fourier transform infrared spectroscopy (FTIR), UV spectrophotometer, pH, swelling index, and yield.

The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52×10^{-6} mol), which has been developed with hot water (100 mL), added CA (4.76×10^{-3} mol) and HCl (1.20×10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20 °C, 40 °C, 60 °C, and 80 °C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho et al. 2019, 2023; Hadinugroho, Wuryanto; Martodihardjo, Suwaldi; Fudholi, Achmad; Riyanto 2022).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Model LVDV-I Prime, AP6510416, Brookfield Engineering Labs, Inc., Middleboro, MA, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque < 10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR Perkin Elmer Spectrum Version 10.4.3.). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor can read UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140 °C to 170 °C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination can be seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. The atom reacts with the O atom on the C-6 atom of mannose and galactose from LBG. O atom from protonated OH ($^{+}OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group is a specific peak of CA-LBG, which is not visible on LBG

(Hadinugroho *et al*., 2017, 2019). Heating to a controlled temperature is needed as energy to bond between the positive C atoms of the carboxylate group and oxygen at the C-6 LBG position, thereby shortening the esterification time. Details of the experimental conditions are shown in Table I.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80 C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20-40 °C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40 °C was a pH value of 4.82 and at 60 °C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The wavelength of CA-LBG from heating to a temperature of 40 °C appears at 203.50 nm and a temperature of 60 °C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40 °C has 125-135 °C and 60 °C has 135-155 °C. The swelling index value of the CA-LBG synthesized at 40 °C was 20.37 and at 60 °C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40 °C was 39.76 % and at 60 °C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Table I: Details of mucilago and powder character of CA-LBG

Figure 1. CA-LBG esterification mechanism

Figure 2. CA-LBG infrared spectra at 40 °C and 60 °C

Figure 3. Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 3. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20 °C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40 °C and 60 oC can reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80 °C) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy can also break bonds between atoms in mannose, galactose, or

CA. High temperatures for a long time can damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilage's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed cannot trap the swelling solution because the bonds between the atoms have been broken.

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 3. The temperature of 60 °C produces the lowest pH of the mucilago. Heating to a temperature of 60 °C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80 °C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 -1743 cm^{-1}) (Hadinugroho *et al*. , 2017, 2019, 2023). On heating at 40°C and 60°C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) *(Krukowski et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125-155) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60 °C can form stronger bonds between atoms than the temperature of 40 °C at esterification. At 60 °C, it gives a lower yield than at 40 °C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40-60 °C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60 °C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). Polymer Grafting and Crosslinking. A John Wiley & Sons, Inc, Publication, Canada

Colas, A. (2005). Cow Corning Silicones : Preparation Properties and Performance. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications. InTech Publisher, Croatia

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation*, **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry*, **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis*, **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 879–1889

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [oC]	Heating time [minute]	Mucilago character		Powder character					
			Viscosity [cP]	pH	C=O [cm-1]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

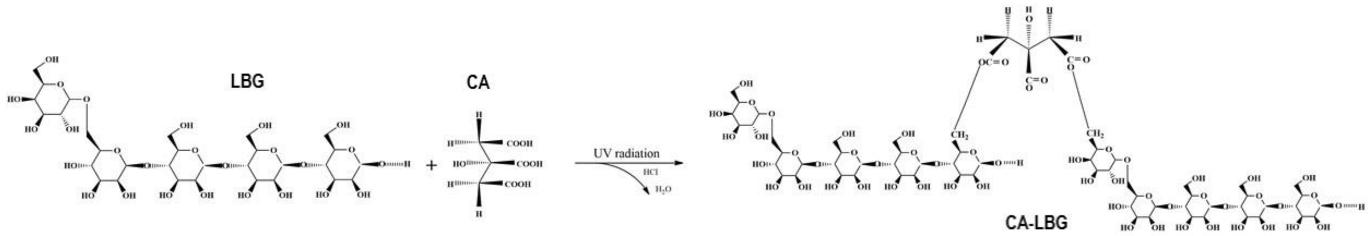


Figure 1. CA-LBG esterification mechanism

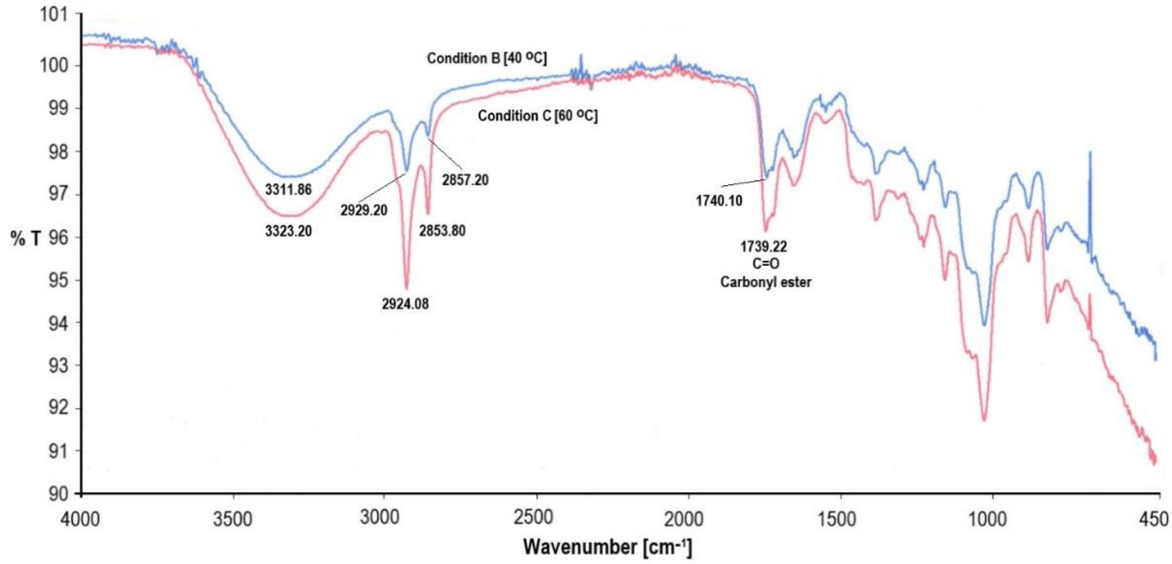
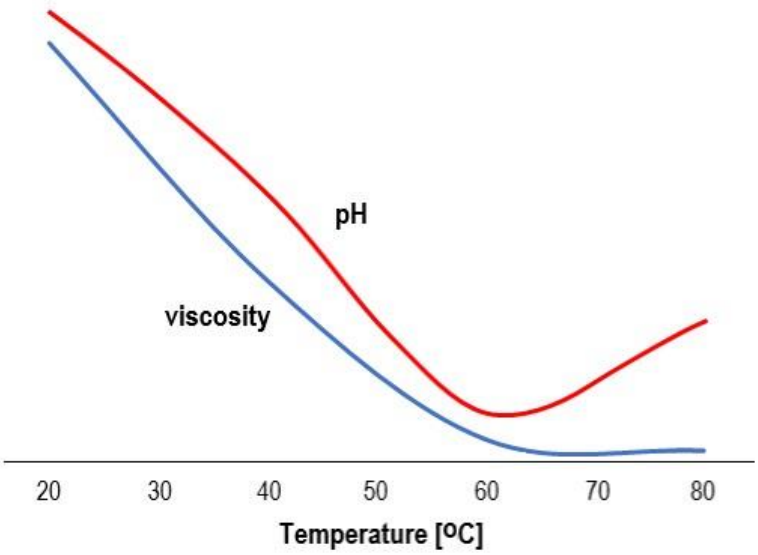


Figure 2. CA-LBG infrared spectra at 40 °C and 60 °C



Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

Appendix A: XXX

Revision request before peer review

Dari: IGSCPS23 Scientific (igscps23.scientific@gmail.com)

Kepada: wuryanto.hadinugroho@ymail.com

Tanggal: Jumat, 8 September 2023 pukul 18.04 GMT+7

Dear Wuryanto Hadinugroho

Thank you again for submitting your manuscript entitled "Effect Of Heating Temperature On Citric Acid-Locust Bean Gum Synthesis" to the Pharmacy Education Journal.

After an initial check, we noted the following is missing or needs to be corrected (Several errors in your manuscript have been highlighted in red, while several things that are highlighted in yellow have been corrected by the editor's team):
1. Based on the result of the similarity check, your article has 5% from one source (file attached), which is above the requirement of not more than 3% similarity index from one source. We suggest you to rewrite/paraphrase some sentences to fulfill this requirement.
2. Please do not change the writing style contained in the PEJ file format:
a. Please write all of the authors' names and affiliation in the manuscript
b. Your article has more than 6 pages. Please make it short (5-6 pages, including the references).
c. Figures: All figures and charts should be mentioned in the text, and be of publishable quality. Images from any chart (excel) or diagram should be inserted in an editable image.
d. Tables: All tables and figures should be placed in their respective positions within the article.

Authors can access videos to assist with manuscript preparation via this link: https://bit.ly/PEJ_Manuscript_preparation

To revise your manuscript, Please rename your manuscript file (060_R1_title) and upload your revised manuscript via link: https://forms.gle/GmjBfw1jdz9wHNEH8
Please complete the requested amendments and re-submit your manuscript within 7 days. Don't hesitate to contact us if you have any questions or require any assistance.

Kind regards
Dr. Chrismawan Ardianto
Assigned Editor, Pharmacy Education Journal

Effect Of Heating Temperature On Citric Acid-Locust Bean Gum Synthesis.pdf
1.5MB

Manuscript_IGSCPS 2023_OP0120 - R.M. Wuryanto Hadinugroho, M.Sc., Apt.docx
241.8kB

Pharmacy Education (2023) 23(2) XX - XX
https://doi.org/

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Wuryanto Hadinugroho[1], Stephanie Florencia Winarko[1], Echa Imanuela Sinta[1], Senny Yesery Esar [1], Jefri Prasetyo[1]

1Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia

Keywords
Characterization
Citric acid
Esterification
Heating
Locust bean gum

Correspondence
Wuryanto Hadinugroho
Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia
wuryanto.hadinugroho@ymail.com

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20oC, 40oC, 60oC, and 80oC for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20 oC, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80 oC, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm-1) and 60°C (1735.1 cm-1). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) as monomers (Dey *et al.* 2013; Kaity *et al.* 2013; Alves *et al.* 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds. (Samavati *et al.*, 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al.*, 2001; Palit 2009; Dey *et al.*, 2013). CA's carboxylic group could replace the OH of the C6 atom of mannose and galactose.

Acid compounds such as hydrochloric acid (HCl) can act as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl can accelerate the release of the OH group on the C6 atom of monomers. (Colas 2005; Bhattacharya *et al.*, 2008)

Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al.*, 2017; 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity *(Hadinugroho et al.*, 2019; 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.

Synthesis of CA-LBG through the CA esterification process with LBG, HCl as the catalyst, and heat of water bath as the energy source. The temperatures used were 20 °C, 40 °C, 60 °C, and 80 °C for 30 minutes. CA-LBG was

characterized by viscosity, infrared (FTIR), UV spectrophotometer, pH, swelling index, and yield.
The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52 x 10^{-6} mol), which has been developed with hot water (100 mL), added CA (4.76 x 10^{-3} mol) and HCl (1.20 x 10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20 °C, 40 °C, 60 °C, and 80 °C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; 2022; 2023).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque < 10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor can read UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140 °C to 170 °C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination can be seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. O atom from protonated OH ($^{+}OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al.*, 2017, 2019). Heating at a controlled temperature is needed as the bond energy of the C atom is positive with the C6 atom of the LBG monomers, so the esterification time is shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80 °C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20-40 °C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40 °C was a pH value of 4.82 and at 60 °C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic. The profile of the relationship between temperature to viscosity and pH is presented in Figure 2.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40 °C appears at 203.50 nm and a temperature of 60 °C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40 °C has 125-135 °C and 60 °C has 135-155 °C. The swelling index value of the CA-LBG synthesized at 40 °C was 20.37 and at 60 °C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40 °C was 39.76 % and at 60 °C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20 °C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40 °C and 60 oC can reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80 °C) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy can also break bonds between atoms in mannose, galactose, or CA. High temperatures for a long time can damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilage's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed cannot trap the swelling solution because the bonds between the atoms have been broken.

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 2. The temperature of 60 °C produces the lowest pH of the mucilago. Heating to a temperature of 60 °C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80 °C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 - 1743 cm^{-1}) (Hadinugroho *et al.* , 2017; 2019; 2023). On heating at 40 °C and 60 °C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) *(Krukowski et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125-155) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60 °C can form stronger bonds between atoms than the temperature of 40 °C at esterification. At 60 °C, it gives a lower yield than at 40 °C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40-60 °C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60 °C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). *Polymer Grafting and Crosslinking*. A John Wiley & Sons, Inc, Publication.

Colas, A. (2005). *Cow Corning Silicones : Preparation Properties and Performance*. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). *Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. InTech Publisher.

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation,* **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry,* **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis,* **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 879–1889

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [oC]	Heating time [minute]	Mucilago character		Powder character						
			Viscosity [cP]	pH	C=O [cm-1]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

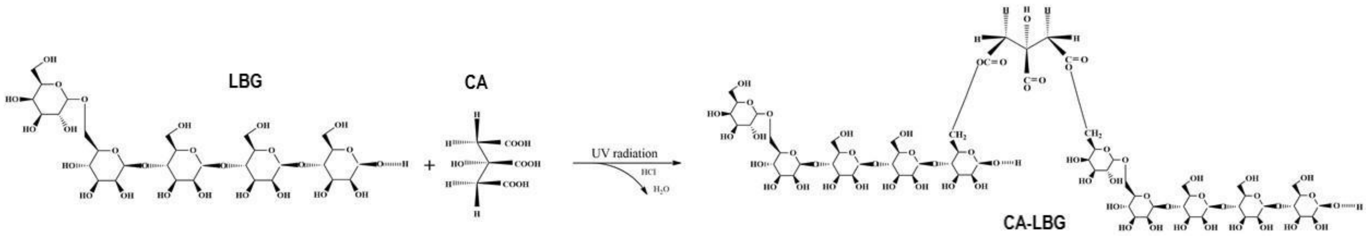


Figure 1: CA-LBG esterification mechanism

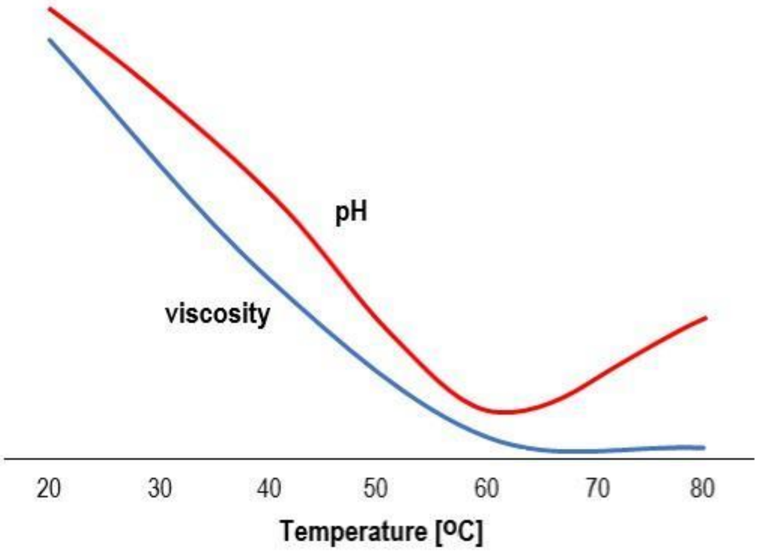


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

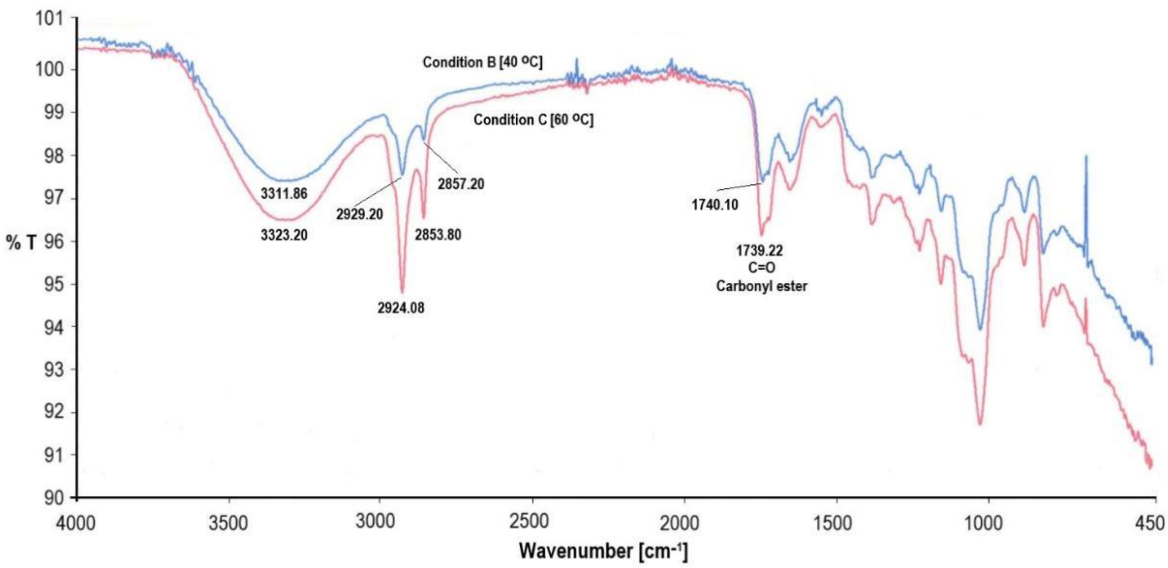


Figure 3: CA-LBG infrared spectra at 40 °C and 60 °C

Peer Review Evaluation

Dari: IGSCPS23 Scientific (igscps23.scientific@gmail.com)

Kepada: wuryanto.hadinugroho@ymail.com

Tanggal: Rabu, 18 Oktober 2023 pukul 08.16 GMT+7

Dear Wuryanto Hadinugroho

Thank you for submitting your manuscript ID 060 entitled "Effect Of Heating Temperature On Citric Acid-Locust Bean Gum Synthesis" to IGSCPS in collaboration with the Pharmacy Education Journal.

Your manuscript has been reviewed and requires minor modifications prior to acceptance. The comments of the reviewer(s) are included at the bottom of this letter. We invite you to respond to the reviewer(s)' comments and revise your manuscript. To revise your manuscript, Please rename your manuscript file (rev_title) and upload your revised manuscript via link: https://forms.gle/mACMT8n9GWz4CaxV8

The revised paper needs to be submitted within 2 weeks from now. Please submit the revised version of your manuscript along with a letter describing how you handled each reviewer's comment. Please highlight all changes or revisions in your manuscript file.
Please complete the requested amendments and re-submit your manuscript. Please contact us if you have any questions or require any assistance.

Kind regards
Dr. Chrismawan Ardianto
Assigned Editor, Pharmacy Education Journal

Reviewer(s)' Comments to Author:
Reviewer 1:
This research is presented simply and concisely, by determining the effect of temperature on the characterization of CA-LBG. Several things that need to be briefly explained in this research, as follows:
1. Consistency in writing and writing symbols correctly needs to be improved (highlighted in yellow).
2. Please write citations on the manuscript following the guidelines (highlighted in yellow).
3. In the discussion, a brief explanation can be added which proves that the results of the CA-LBG synthesis are pure and there is no longer a mixture of CA and LBG.

Reviewer 2:
The author describes the finding on the heating temperature of CA-LBG, the polymer for tablet excipient. The manuscript has been well prepared and may contribute significantly to the field. Several concerns needed to improve the manuscript as follows:
1. Figure 2 is better equipped with a Y-axis scale for each parameter
2. some language editing suggestions and typological editing for degree Celsius are needed (included in the edited version)

Reviewer 1_060_Effect of heating temperature on citric acid-locust bean gum synthesis.docx
131.5kB

Reviewer 2_060_Effect of heating temperature on citric acid-locust bean gum synthesis.docx
131.7kB

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Keywords
Characterization
Citric acid
Esterification
Heating
Locust bean gum

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20oC, 40oC, 60oC, and 80oC for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20 oC, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80 oC, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm-1) and 60°C (1735.1 cm-1). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) as monomers (Dey *et al*. 2013; Kaity *et al*. 2013; Alves *et al*. 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds. (Samavati *et al*., 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al*., 2001; Palit 2009; Dey *et al*., 2013). CA's carboxylic group could replace the OH of the C6 atom of mannose and galactose.
Acid compounds such as hydrochloric acid (HCl) can act as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl can accelerate the release of the OH group on the C6 atom of monomers. (Colas 2005; Bhattacharya *et al*., 2008)
Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al*., 2017; 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity *(Hadinugroho et al.*, 2019; 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.
Synthesis of CA-LBG through the CA esterification process with LBG, HCl as the catalyst, and heat of water bath as the energy source. The temperatures used were 20 °C, 40 °C, 60 °C, and 80 °C for 30 minutes. CA-LBG was characterized by viscosity, infrared (FTIR), UV spectrophotometer, pH, swelling index, and yield.
The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52 x 10^{-6} mol), which has been developed with hot water (100 mL), added CA (4.76 x 10^{-3} mol) and HCl (1.20 x 10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20 °C, 40 °C, 60 °C, and 80 °C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; 2022; 2023).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque < 10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor can read UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140 °C to 170 °C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination can be seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [oC]	Heating time [minute]	Mucilago character		Powder character					
			Viscosity [cP]	pH	C=O [cm-1]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

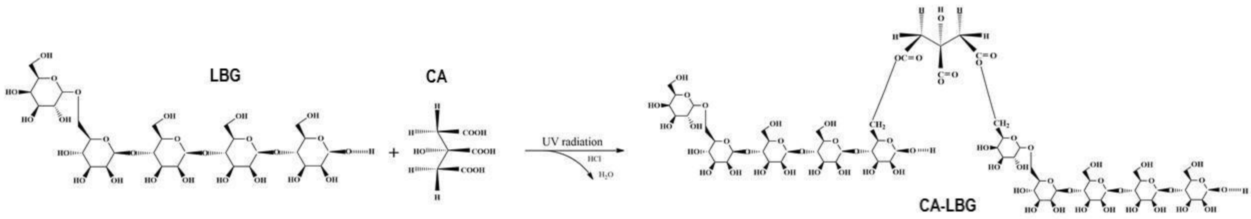


Figure 1: CA-LBG esterification mechanism

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. O atom from protonated OH ($^{+}OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al*., 2017, 2019). Heating at a controlled temperature is needed as the bond energy of the C atom is positive with the C6 atom of the LBG monomers, so the esterification time is shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80 °C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20-40 °C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40 °C was a pH value of 4.82 and at 60 °C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic. The profile of the relationship between temperature to viscosity and pH is presented in Figure 2.

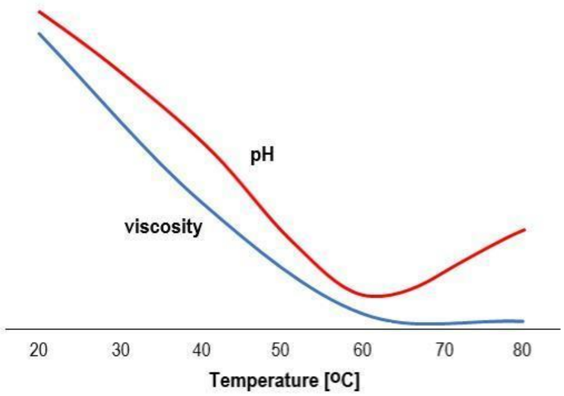


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40 °C appears at 203.50 nm and a temperature of 60 °C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40 °C has 125-135 °C and 60 °C has 135-155 °C. The swelling index value of the CA-LBG synthesized at 40 °C was 20.37 and at 60 °C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40 °C was 39.76 % and at 60 °C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20 °C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40 °C and 60 oC can reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80 oC) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy can also break bonds between atoms in mannose, galactose, or CA. High temperatures for a long time can damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilage's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed cannot trap the swelling solution because the bonds between the atoms have been broken.

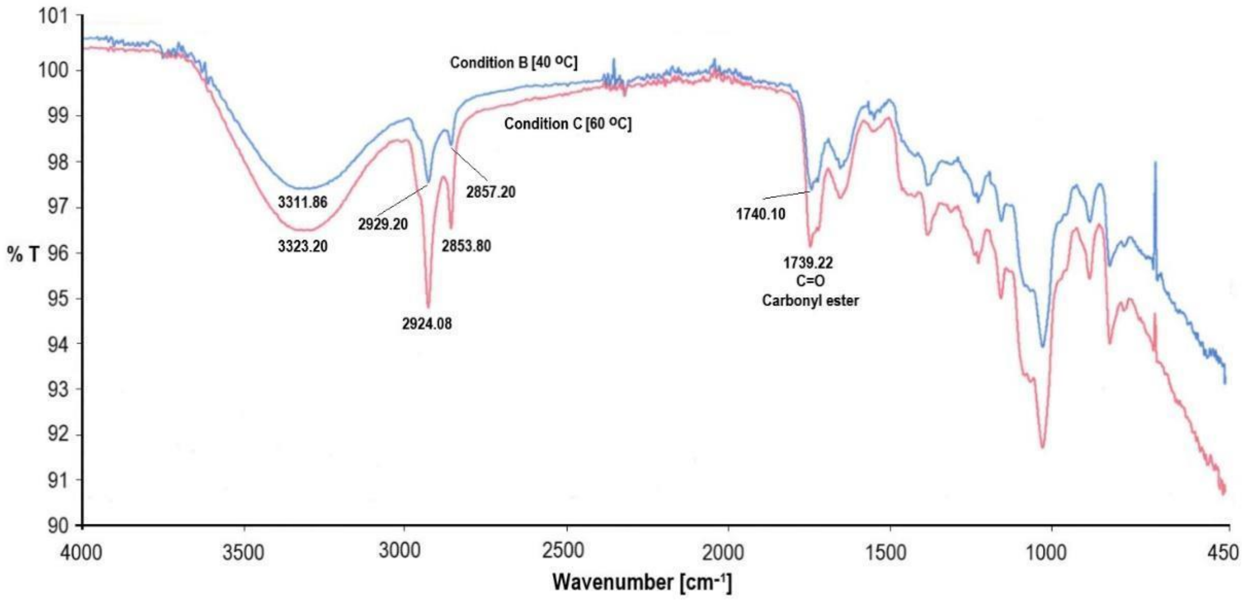


Figure 3: CA-LBG infrared spectra at 40 °C and 60 °C

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 2. The temperature of 60 °C produces the lowest pH of the mucilago. Heating to a temperature of 60 °C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80 °C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 - 1743 cm^{-1}) (Hadinugroho *et al.* , 2017; 2019; 2023). On heating at 40 °C and 60 °C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) *(Krukowski et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125-155) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60 °C can form stronger bonds between atoms than the temperature of 40 °C at esterification. At 60 °C, it gives a lower yield than at 40 °C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40-60 °C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60 °C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). *Polymer Grafting and Crosslinking*. A John Wiley & Sons, Inc, Publication.

Colas, A. (2005). *Cow Corning Silicones : Preparation Properties and Performance*. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). *Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. InTech Publisher.

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation,* **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry,* **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis,* **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 879–1889

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Keywords
Characterization
Citric acid
Esterification
Heating
Locust bean gum

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20oC, 40oC, 60oC, and 80oC for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20 °C, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80 °C, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm-1) and 60°C (1735.1 cm-1). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) as monomers (Dey *et al*. 2013; Kaity *et al*. 2013; Alves *et al*. 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds (Samavati *et al*., 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al*., 2001; Palit 2009; Dey *et al*., 2013). CA's carboxylic group replaces the OH of the C6 atom of mannose and galactose.

Acid compounds such as hydrochloric acid (HCl) acts as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl accelerates the release of the OH group on the C6 atom of monomers. (Colas 2005; Bhattacharya *et al*., 2008)

Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al*., 2017; 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity *(Hadinugroho et al.*, 2019; 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.

Synthesis of CA-LBG through the CA esterification process with LBG, HCl as the catalyst, and heat of water bath as the energy source. The temperatures used were 20, 40, 60, and 80 ºC for 30 minutes. CA-LBG was characterized by viscosity, infrared (FTIR), UV spectrophotometer, pH, swelling index, and yield.

The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52 x 10^{-6} mol), which has been developed with hot water (100 mL), added CA (4.76 x 10^{-3} mol) and HCl (1.20 x 10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20 °C, 40 °C, 60 °C, and 80 °C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; 2022; 2023).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque < 10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor reads UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140 to 170 °C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination weref seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [oC]	Heating time [minute]	Mucilago character		Powder character					
			Viscosity [cP]	pH	C=O [cm-1]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

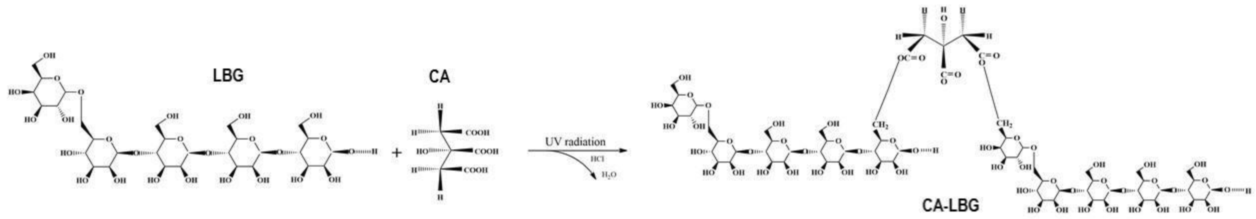


Figure 1: CA-LBG esterification mechanism

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. O atom from protonated OH ($^{+}OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al*., 2017, 2019). Heating at a controlled temperature is needed as the bond energy of the C atom is positive with the C6 atom of the LBG monomers, so the esterification time is shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80 °C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20-40 °C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40 °C was a pH value of 4.82 and at 60 °C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic. The profile of the relationship between temperature to viscosity and pH is presented in Figure 2.

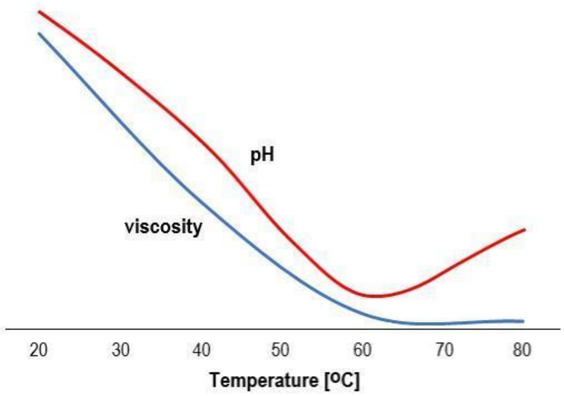


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40 °C appears at 203.50 nm and a temperature of 60 °C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40 °C has 125-135 °C and 60 °C has 135-155 °C. The swelling index value of the CA-LBG synthesized at 40 °C was 20.37 and at 60 °C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40 °C was 39.76 % and at 60 °C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20 °C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40 and 60 °C reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80 oC) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy may also break bonds between atoms in mannose, galactose, or CA. High temperatures for a long time may damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilage's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed may not trap the swelling solution because the bonds between the atoms have been broken.

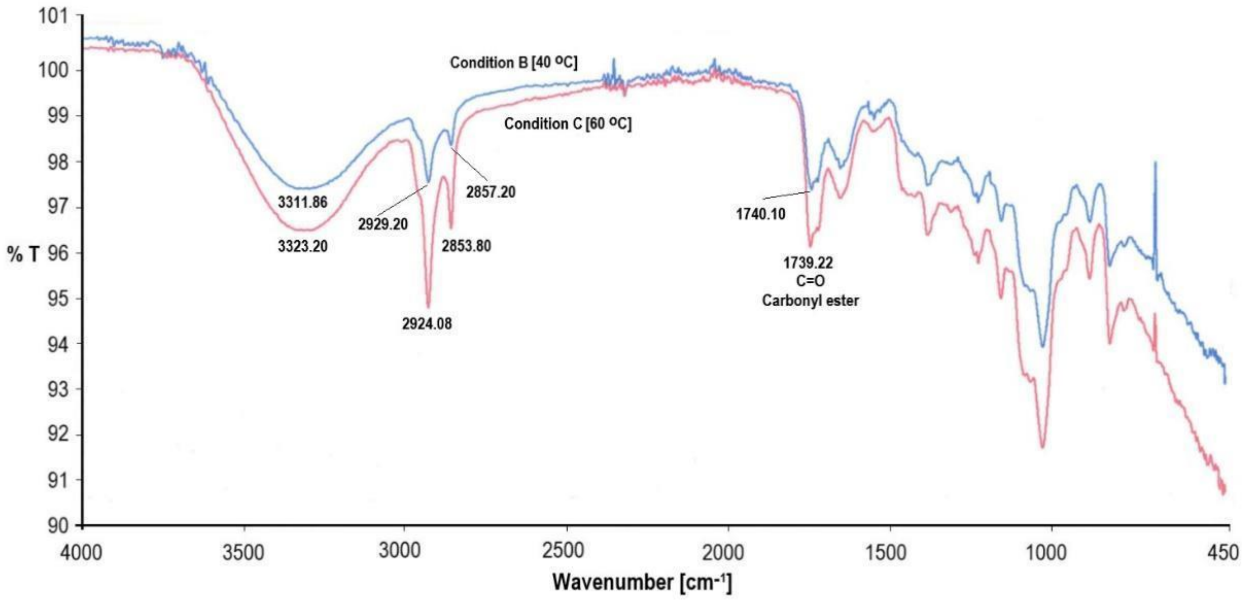


Figure 3: CA-LBG infrared spectra at 40 °C and 60 °C

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 2. The temperature of 60 °C produces the lowest pH of the mucilago. Heating to a temperature of 60 °C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80 °C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 - 1743 cm^{-1}) (Hadinugroho *et al.* , 2017; 2019; 2023). On heating at 40 °C and 60 °C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) *(Krukowski et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125-155) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60 °C may form stronger bonds between atoms than the temperature of 40 °C at esterification. At 60 °C, it gives a lower yield than at 40 °C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40-60 °C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60 °C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). *Polymer Grafting and Crosslinking*. A John Wiley & Sons, Inc, Publication.

Colas, A. (2005). *Cow Corning Silicones : Preparation Properties and Performance*. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). *Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. InTech Publisher.

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation,* **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry,* **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis,* **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 879–1889

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Wuryanto Hadinugroho, Stephanie Florencia Winarko, Echa Imanuela Sinta, Senny Yesery Esar, Jefri Prasetyo

Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia

Keywords

Characterization
Citric acid
Esterification
Heating
Locust bean gum

Correspondence

Wuryanto Hadinugroho
Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia
wuryanto.hadinugroho@ymail.com

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20°C, 40°C, 60°C, and 80°C for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20°C, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80°C, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm^{-1}) and 60°C (1735.1 cm^{-1}). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) as monomers (Dey *et al*. 2013; Kaity *et al*. 2013; Alves *et al*. 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds (Samavati *et al*., 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al*., 2001; Palit 2009; Dey *et al*., 2013). CA's carboxylic group replace the OH of the C6 atom of mannose and galactose.

Acid compounds such as hydrochloric acid (HCl) act as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl can accelerates the release of the OH group on the C6 atom of monomers. (Colas 2005; Bhattacharya *et al*., 2008)

Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al*., 2017; 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity (Hadinugroho *et al.*, 2019; 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.

Synthesis of CA-LBG through the CA esterification process with LBG, HCl as the catalyst, and heat of water bath as the energy source. The temperatures used were 20°C, 40°C, 60°C, and 80°C for 30 minutes. CA-LBG was characterized by viscosity, infrared (FTIR), UV spectrophotometer, pH, swelling index, and yield.

The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to

provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52 x 10^{-6} mol), which has been swollen with hot distilled water (100 mL), added CA (4.76 x 10^{-3} mol) and HCl (1.20 x 10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20°C, 40°C, 60°C, and 80°C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; 2022; 2023).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque <10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor read UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140°C to 170°C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination can be seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [°C]	Heating time [minute]	Mucilago character		Powder character					
			Viscosity [cP]	pH	C=O [cm^{-1}]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

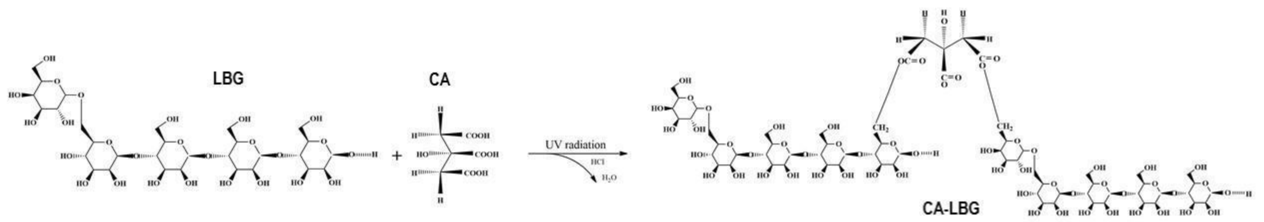


Figure 1: CA-LBG esterification mechanism

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. O atom from protonated OH ($^+OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al*., 2017, 2019). Heating at a controlled temperature is needed as the bond energy of the C atom is positive with the C6 atom of the LBG monomers, so the esterification time is shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80°C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20°C-40°C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40°C was a pH value of 4.82 and at 60°C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic. The profile of the relationship between temperature to viscosity and pH is presented in Figure 2.

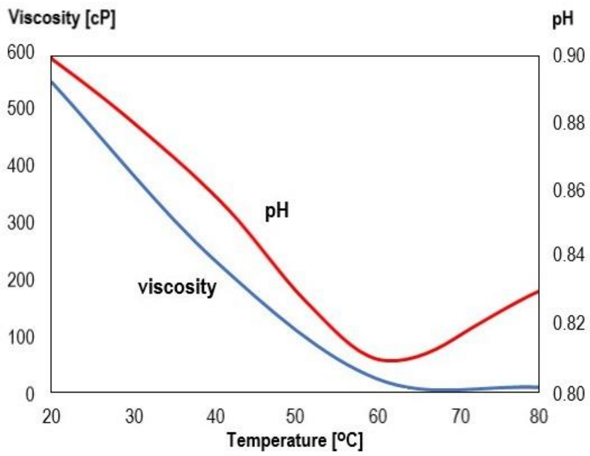


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40°C appears at 203.50 nm and a temperature of 60°C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40°C has 125°C-135°C and 60°C has 135°C-155°C. The swelling index value of the CA-LBG synthesized at 40°C was 20.37 and at 60°C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40°C was 39.76 % and at 60°C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20°C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40°C and 60°C reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80°C) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy may also break bonds between atoms in mannose, galactose, or

CA. High temperatures for a long time may damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilago's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed may not trap the swelling solution because the bonds between the atoms have been broken.

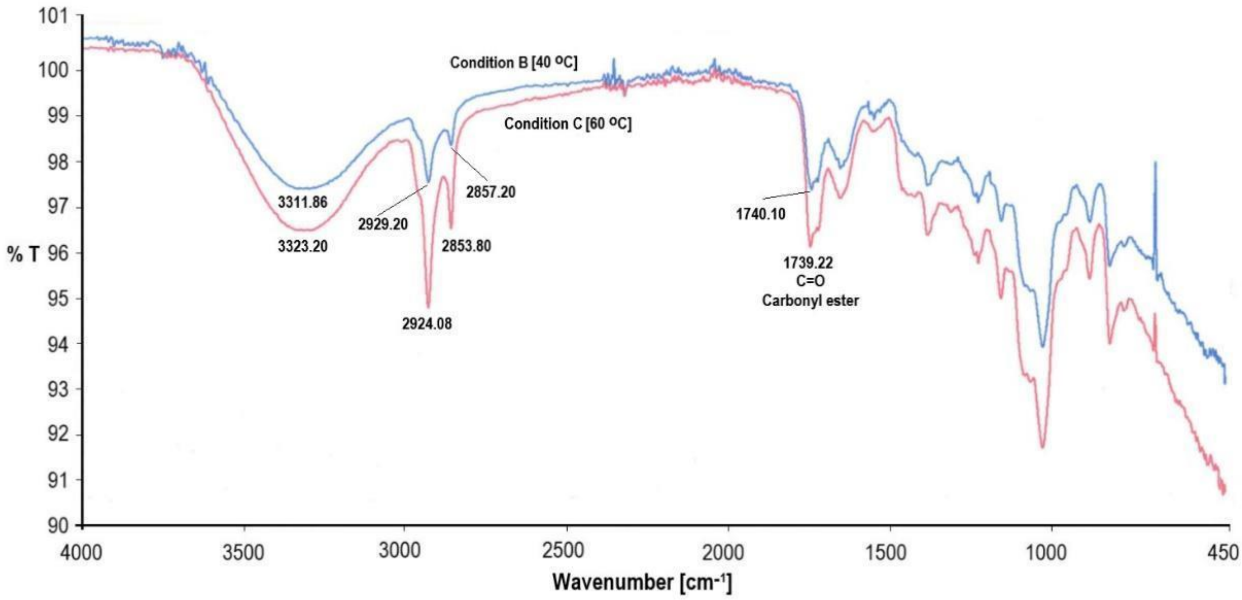


Figure 3: CA-LBG infrared spectra at 40°C and 60°C

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 2. The temperature of 60°C produces the lowest pH of the mucilago. Heating to a temperature of 60°C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80°C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The homogeneity of CA-LBG was evaluated based on the pH of CA-LBG and the pH value of the washing solution during manufacturing. The CA used in the synthesis has a pH of 2.02, while LBG has a pH of 5.85. CA-LBG has a pH of 4.82 & 4.88, which is between the pH of CA and LBG. In addition, repeated washing until pH 6.8-7.0 (pH acetone-distilled water) ensures that the CA-LBG precipitate is free of CA and that the LBG does not react. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 -1743 cm^{-1}) (Hadinugroho *et al.* , 2017; 2019; 2023). On heating at 40°C and 60°C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) (Krukowski *et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125°C-155°C) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60°C may form stronger bonds between atoms than the temperature of 40°C at esterification. At 60°C, it gives a lower yield than at

40°C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40°C-60°C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60°C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Surabaya Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). *Polymer Grafting and Crosslinking*. A John Wiley & Sons, Inc, Publication.

Colas, A. (2005). *Cow Corning Silicones : Preparation Properties and Performance*. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). *Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. InTech Publisher.

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation,* **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry,* **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis,* **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 1879–1889

Response to Reviewer comments

Comment of Reviewer 1

This research is presented simply and concisely, by determining the effect of temperature on the characterization of CA-LBG. Several things that need to be briefly explained in this research, as follows:

1. Consistency in writing and writing symbols correctly needs to be improved (highlighted in yellow).

   Response:

   Thank you for the comment and the suggestions. We have improved the consistency of writing °C symbols. In addition to the corrected yellow highlighted words, we wrote our corrections in blue ink on the manuscript.

2. Please write citations on the manuscript following the guidelines (highlighted in yellow).

   Response:

   Thank you for the comment and the suggestions. We have corrected the citation writing according to the instructions.

3. In the discussion, a brief explanation can be added which proves that the results of the CA-LBG synthesis are pure and there is no longer a mixture of CA and LBG.

   Response:

   Thank you for the comment and the suggestions. We often call homogeneous CA-LBG rather than pure CA-LBG because CA-LBG is an amorphous polymer (Hadinugroho et al., 2019). We have added an explanation regarding CA-LBG homogeneous evaluation in the discussion section written in blue ink. Here's the explanation:

   "The homogeneity of CA-LBG was evaluated based on the pH of CA-LBG and the pH value of the washing solution during manufacturing. The CA used in the synthesis has a pH of 2.02, while LBG has a pH of 5.85. CA-LBG has a pH of 4.82 & 4.88, which is between the pH of CA and LBG. In addition, repeated washing to pH 6.8–7.0 (pH of acetone-distilled water) ensured that the CA-LBG precipitate was free of CA and that the LBG did not react."

Comment of Reviewer 2

The author describes the finding on the heating temperature of CA-LBG, the polymer for tablet excipient. The manuscript has been well prepared and may contribute significantly to the field. Several concerns needed to improve the manuscript as follows:

1. Figure 2 is better equipped with a Y-axis scale for each parameter.

   Response:

   Thank you for the comment and the suggestions. We have added a Y-axis in Figure 2. Here is the Figure:

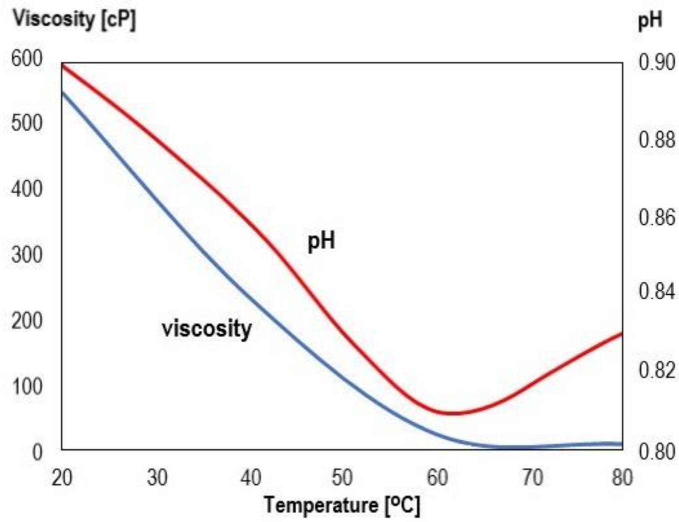


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

2. some language editing suggestions and typological editing for degree Celsius are needed (included in the edited version)

   Response:

   Thank you for the comment and the suggestions. We have corrected the sentences following the changes or additions of the given words (highlighted in yellow). We have also improved the typology and consistency of writing degrees Celsius (blue ink).

Editorial revision

Dari: IGSCPS23 Scientific (igscps23.scientific@gmail.com)

Kepada: wuryanto.hadinugroho@ymail.com

Tanggal: Sabtu, 3 Februari 2024 pukul 21.13 GMT+7

Dear Wuryanto Hadinugroho

Thank you for submitting your manuscript ID 060 entitled "Effect Of Heating Temperature On Citric Acid-Locust Bean Gum Synthesis" to IGSCPS in collaboration with the Pharmacy Education Journal.

We have now received the reviewers' opinions on your manuscript. I have also read your submission and the comments and I am pleased to inform you that there are some comments that the author should address.

The comments appear at the end of this mail.

The revised paper needs to be submitted within 1 week from now. Please submit the revised version of your manuscript along with a letter describing how you handled each comment. Please highlight all changes or revisions in your manuscript file (please do not use comment tools in office apps). Please rename your manuscript file (Edi_rev_ID060_title) and upload your revised manuscript via link: https://forms.gle/mACMT8n9GWz4CaxV8
Please complete the requested amendments and re-submit your manuscript. Please contact us if you have any questions or require any assistance.

Kind regards
Dr. Chrismawan Ardianto
Assigned Editor, Pharmacy Education Journal

Comments:
1. The short title (in the top right corner of the manuscript) must be written with a maximum of 50 characters.
2. Please send us the final version of your manuscript included with the author's name and affiliation.

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Wuryanto Hadinugroho, Stephanie Florencia Winarko, Echa Imanuela Sinta, Senny Yesery Esar, Jefri Prasetyo

Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia

Keywords
Characterization
Citric acid
Esterification
Heating
Locust bean gum

Correspondence
Wuryanto Hadinugroho
Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia
wuryanto.hadinugroho@ymail.com

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20ºC, 40ºC, 60ºC, and 80ºC for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20ºC, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80ºC, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm^{-1}) and 60°C (1735.1 cm^{-1}). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) as monomers (Dey *et al*. 2013; Kaity *et al*. 2013; Alves *et al*. 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds (Samavati *et al*., 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al*., 2001; Palit 2009; Dey *et al*., 2013). CA's carboxylic group replace the OH of the C6 atom of mannose and galactose.

Acid compounds such as hydrochloric acid (HCl) act as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl can accelerates the release of the OH group on the C6 atom of monomers. (Colas 2005; Bhattacharya *et al*., 2008)

Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al*., 2017; 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity (Hadinugroho *et al.*, 2019; 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.

Synthesis of CA-LBG through the CA esterification process with LBG, HCl as the catalyst, and heat of water bath as the energy source. The temperatures used were 20ºC, 40ºC, 60ºC, and 80ºC for 30 minutes. CA-LBG was characterized by viscosity, infrared (FTIR), UV spectrophotometer, pH, swelling index, and yield.

The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to

provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52×10^{-6} mol), which has been swollen with hot distilled water (100 mL), added CA (4.76×10^{-3} mol) and HCl (1.20×10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20°C, 40°C, 60°C, and 80°C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; 2022; 2023).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque <10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor read UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140°C to 170°C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination can be seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [°C]	Heating time [minute]	Mucilago character		Powder character					
			Viscosity [cP]	pH	C=O [cm^{-1}]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

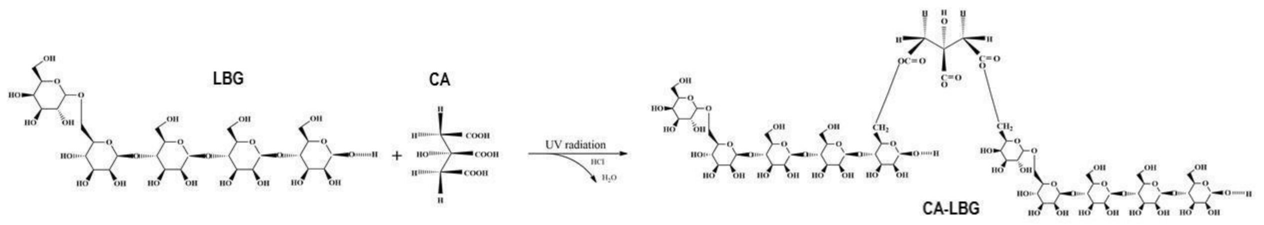


Figure 1: CA-LBG esterification mechanism

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. O atom from protonated OH ($^+OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al*., 2017, 2019). Heating at a controlled temperature is needed as the bond energy of the C atom is positive with the C6 atom of the LBG monomers, so the esterification time is shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80°C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20°C-40°C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40°C was a pH value of 4.82 and at 60°C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic. The profile of the relationship between temperature to viscosity and pH is presented in Figure 2.

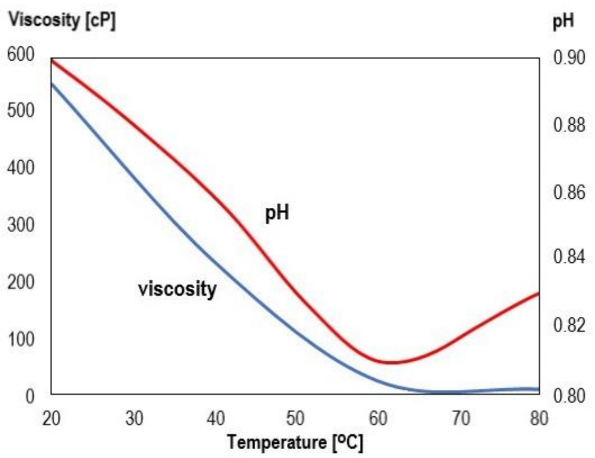


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40°C appears at 203.50 nm and a temperature of 60°C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40°C has 125°C-135°C and 60°C has 135°C-155°C. The swelling index value of the CA-LBG synthesized at 40°C was 20.37 and at 60°C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40°C was 39.76 % and at 60°C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20°C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40°C and 60°C reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80°C) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy may also break bonds between atoms in mannose, galactose, or

CA. High temperatures for a long time may damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilago's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed may not trap the swelling solution because the bonds between the atoms have been broken.

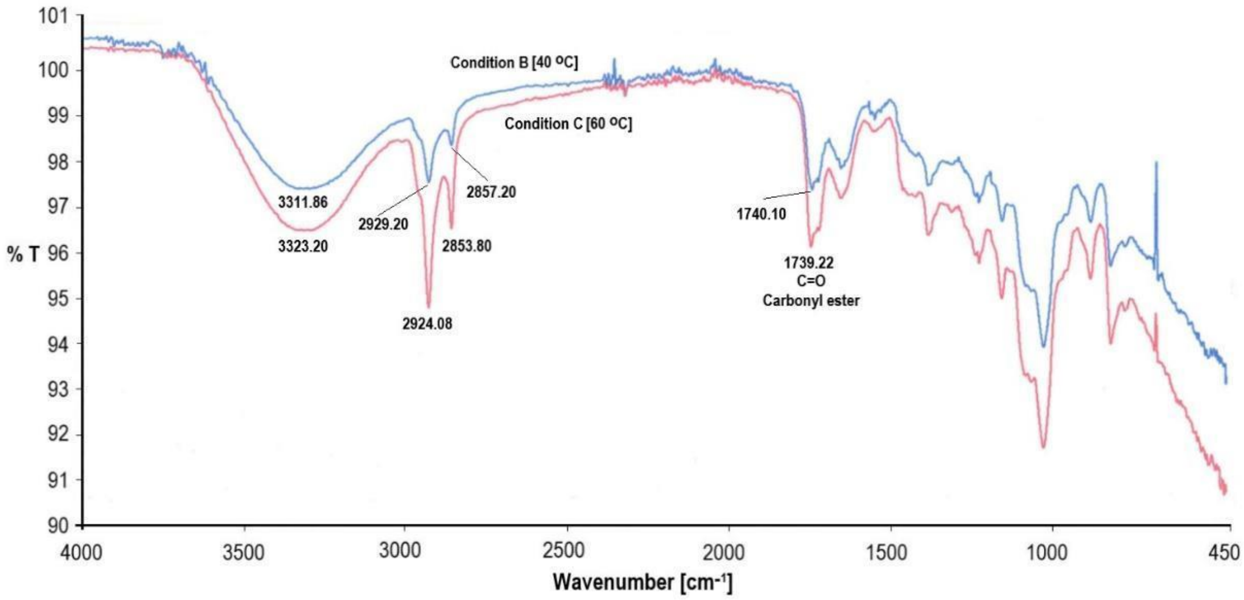


Figure 3: CA-LBG infrared spectra at 40°C and 60°C

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 2. The temperature of 60°C produces the lowest pH of the mucilago. Heating to a temperature of 60°C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80°C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The homogeneity of CA-LBG was evaluated based on the pH of CA-LBG and the pH value of the washing solution during manufacturing. The CA used in the synthesis has a pH of 2.02, while LBG has a pH of 5.85. CA-LBG has a pH of 4.82 & 4.88, which is between the pH of CA and LBG. In addition, repeated washing until pH 6.8-7.0 (pH acetone-distilled water) ensures that the CA-LBG precipitate is free of CA and that the LBG does not react. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 -1743 cm^{-1}) (Hadinugroho *et al.* , 2017; 2019; 2023). On heating at 40°C and 60°C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) (Krukowski *et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125°C-155°C) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60°C may form stronger bonds between atoms than the temperature of 40°C at esterification. At 60°C, it gives a lower yield than at

40°C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40°C-60°C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60°C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Surabaya Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). *Polymer Grafting and Crosslinking*. A John Wiley & Sons, Inc, Publication.

Colas, A. (2005). *Cow Corning Silicones : Preparation Properties and Performance*. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). *Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. InTech Publisher.

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation,* **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry,* **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis,* **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 1879–1889

Response to Reviewer comments

Comment of Reviewer 1

1. The short title (in the top right corner of the manuscript) must be written with a maximum of 50 characters.

   Response:

   Thank you for the comment and the suggestions. We have corrected the short title, which was originally **"Effect of heating temperature on citric acid-locust bean gum synthesis"** (**70** characters), to **"Effect of heating temperature on CA-LBG synthesis"** (**49** characters). Improvements were made with a green highlighter on the manuscript.

2. Please send us the final version of your manuscript included with the author's name and affiliation.

   Response:

   Thank you for the comment and the suggestions. We have added the name of the author, co-author, corresponding author, affiliation, and email. This addition is given a green highlighter on the manuscript.

Gmail

Wuryanto Hadinugroho <wuryanto.hadinugroho@gmail.com>

Manuscript acceptance for the IGSCPS 2023 Publication in Pharmacy Education Journal

1 pesan

IGSCPS23 Scientific <igscps23.scientific@gmail.com> 15 Februari 2024 pukul 16.06
Kepada: wuryanto.hadinugroho@gmail.com

Dear Wuryanto Hadinugroho,

Your manuscript ID 060 entitled "Effect of heating temperature on citric acid-locust bean gum synthesis" has been accepted and will proceed to publication in the Pharmacy Education Journal in collaboration with the International Graduate Student Conference on Pharmaceutical Sciences (IGSCPS 2023) hosted by the Faculty of Pharmacy Universitas Airlangga Indonesia.

We congratulate you for your achievement. The technical details of the publication will be provided subsequently.

Kind regards
Dr. Chrismawan Ardianto
Assigned Editor, Pharmacy Education Journal

Gmail **Wuryanto Hadinugroho <wuryanto.hadinugroho@gmail.com>**

[PE] [Action to authors] Proofreading request

4 pesan

Oluwatosin Akinwotu via Pharmacy Education <noreply@pharmacyeducation.fip.org> 26 Februari 2024 pukul 02.52

Balas Ke: Oluwatosin Akinwotu <tosinakinwotu@gmail.com>
Kepada: Wuryanto Hadinugroho <wuryanto.hadinugroho@gmail.com>

Dear Wuryanto Hadinugroho,

The proofreading stage is an opportunity to ensure that all information contained on the created PDF is accurate and correct, as changes post-publication can only be made in line with our Article Corrections, Replacement, Retractions & Removal Policy [https://pharmacyeducation.fip.org/pharmacyeducation/about].

You may download the PDF galley that requires proofreading in the "copyedited" section. Blue and red edits are minor corrections made by the copyeditors. You may add your comments as notes to the PDF or send them here inserted in a message reply.

We also need your response to the comments

Kindly provide the missing references.

Please ignore the format of the table/galley at the moment. After this process, we will send you a clean galley for your further approval.

We look forward to hearing from you soon,

Submission URL: https://pharmacyeducation.fip.org/pharmacyeducation/authorDashboard/submission/2736

Thank you,

Best wishes,

Tosin (Copywriter, PEJ-Team)

Pharmacy Education

Wuryanto Hadinugroho <wuryanto.hadinugroho@gmail.com> 1 Maret 2024 pukul 03.34

Kepada: Oluwatosin Akinwotu <tosinakinwotu@gmail.com>

Dear Mr./Mrs./Ms. Tosin
Copywriters,
Pharmacy Education journal-Team

Thank you for your email. Please advise how I can get a PDF proofreading of my manuscript. I've tried logging in via the link address emailed, but the sentence "You don't currently have access to that stage of the workflow" appears.
Thank you for your attention and cooperation

Yours sincerely,
Wuryanto Hadinugroho
[Kutipan teks disembunyikan]

---

toswots <tosinakinwotu@gmail.com> 1 Maret 2024 pukul 13.12

Kepada: Wuryanto Hadinugroho <wuryanto.hadinugroho@gmail.com>

Dear Wuryanto Hadinugroho,
I am sorry to know about your experience with the PEJ website. I am sure it will be rectified. Do not hesitate to communicate with me while we wait for the solution with the PEJ system. Kindly find the documents attached.

Tosin Akinwotu

[Kutipan teks disembunyikan]

2 lampiran

2736 Hadinugroho_proofread_version.pdf
1021K

2736 Hadinugroho_proofread_clean_version.pdf
1035K

Wuryanto Hadinugroho <wuryanto.hadinugroho@gmail.com> 4 Maret 2024 pukul 03.12
Kepada: toswots <tosinakinwotu@gmail.com>

Dear Tosin Akinwotu
Copywriters,
Pharmacy Education journal-Team

Thank you for the proofreading email. The author has read and corrected it according to your suggestions. The author has added a reference (yellow highlighter) in the side notes section of the manuscript. The author has also clarified the sentences (red highlighter) in the side notes section of the manuscript. The author needs to inform us that the account in the side notes of the manuscript is "Pak Wur Punya," which is the author's identity, "Wuryanto Hadinugroho."
If anything else needs to be corrected or clarified, please get in touch with this email again.
Thank you for your attention and cooperation.

Yours sincerely,
Wuryanto Hadinugroho
[Kutipan teks disembunyikan]

Rev_2736 Hadinugroho_proofread_version.pdf
1483K

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Wuryanto Hadinugroho, Stephanie Florencia Winarko, Echa Imanuela Sinta, Senny Yesery Esar, Jefri Prasetyo

Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia

Keywords
Characterization
Citric acid
Esterification
Heating
Locust bean gum

Correspondence
Wuryanto Hadinugroho
Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Kalisari Selatan no. 1 Pakuwon City, Surabaya, Indonesia
wuryanto.hadinugroho@ymail.com

Abstract

Background: Synthesis of citric acid-locust bean gum (CA-LBG) has been carried out previously using UV light as an energy source. This study used the heating method as an alternative energy source because it is simple and affordable. **Objective:** To determine the effect of temperature on the characterization of CA-LBG. **Method:** The synthesis of CA-LBG through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20ºC, 40ºC, 60ºC, and 80ºC for 30 minutes. CA-LBG was characterized chemically and physically. **Result:** At 20ºC, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80ºC, the viscosity of the mucilago was very low, so it was difficult to precipitate and denaturation. The temperatures reported for further characterization were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm^{-1}) and 60°C (1735.1 cm^{-1}). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%. **Conclusion:** The higher temperature tends to increase the UV wavelength, Mp, pH, swelling index, and decrease the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrix (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, Leguminosae family. LBG contains mannose and galactose (4:1) as monomers (Dey *et al*. 2013; Kaity *et al*. 2013; Alves *et al*. 2016). The advantages of LBG are inert, safe, non-toxic, biocompatible, biodegradable and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds (Samavati *et al*., 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al*., 2001; Palit 2009; Dey *et al*., 2013). CA's carboxylic group replace the OH of the C6 atom of mannose and galactose.

Acid compounds such as hydrochloric acid (HCl) act as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl can accelerates the release of the OH group on the C6 atom of monomers. (Colas 2005; Bhattacharya *et al*., 2008)

Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al*., 2017; 2019). Previous CA-LBG synthesis studies reported esterification using UV radiation as an energy source. The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity (Hadinugroho *et al.*, 2019; 2022). The problem in previous studies is that UV radiation requires a long time to synthesise CA-LBG. The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago with high viscosity inhibits the extraction of CA-LBG separation with distilled water. This research is expected to provide information regarding the effect of temperature on the characteristics of the CA-LBG mucilago mass.

Synthesis of CA-LBG through the CA esterification process with LBG, HCl as the catalyst, and heat of water bath as the energy source. The temperatures used were 20ºC, 40ºC, 60ºC, and 80ºC for 30 minutes. CA-LBG was characterized by viscosity, infrared (FTIR), UV spectrophotometer, pH, swelling index, and yield.

The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to

provide opportunities for industrial-scale production of CA-LBG and utilization of CA-LBG in various fields.

Methods

CA-LBG Synthesis

LBG (4.52×10^{-6} mol), which has been swollen with hot distilled water (100 mL), added CA (4.76×10^{-3} mol) and HCl (1.20×10^{-2} mol), stirred until homogeneous. Mucilago CA-LBG was heated in a water bath at the design temperature (20°C, 40°C, 60°C, and 80°C) for 30 minutes. Mucilago CA-LBG was precipitated with acetone and washed repeatedly with acetone water (1 : 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and characterized. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; 2022; 2023).

Viscosity

Mucilago CA-LBG is poured into a 200 mL beaker glass. The spindle was mounted and speed-regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle is inserted into the CA-LBG mucilago up to the limit mark, and press the power button. The spindle rotates until a stable viscosity and torque <10% are obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder is placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). Turn the stick to press until the infrared spectra appear clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analyzed for functional groups.

UV spectrophotometer

CA-LBG powder (50 mg) dissolved with distilled water (10 mL) in volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor read UV spectra and wavelengths (λ).

Melting temperature

CA-LBG powder was filled into the capillary tube (± 2 mm) and placed in the melting point holder. Inspection temperature from 140°C to 170°C (1°C per minute). CA-LBG melting point (Mp) display on melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter has been calibrated at pH 4, 7, and 10. Clean and dry the electrode of the pH meter. The electrode is immersed in the CA-LBG filtrate. The results of the examination can be seen on the monitor of the pH meter.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50mL) was poured and stirred until it swelled. Weigh the filter paper with the swollen CA-LBG. The swelling index is the difference between CA-LBG swelling with the initial powder multiplied by 100% (Gulrez *et al.*, 2011).

Yield

The synthesized CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [°C]	Heating time [minute]	Mucilago character		Powder character					
			Viscosity [cP]	pH	C=O [cm^{-1}]	λ [nm]	Mp [°C]	pH	Swelling index [%]	Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

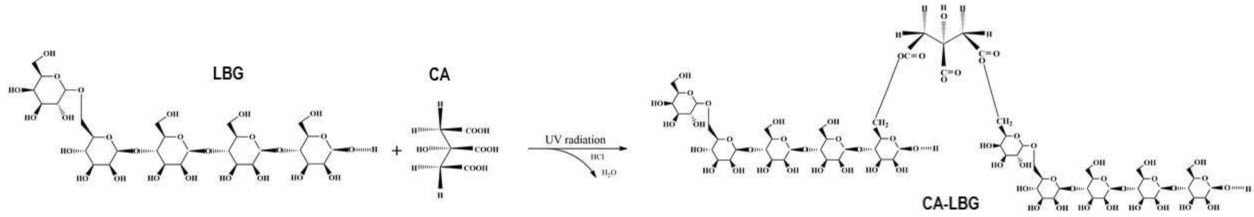


Figure 1: CA-LBG esterification mechanism

Results

The esterification mechanism begins with the citric acid carboxylic group protonated to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. O atom from protonated OH ($^+OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al*., 2017, 2019). Heating at a controlled temperature is needed as the bond energy of the C atom is positive with the C6 atom of the LBG monomers, so the esterification time is shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ± 4.03. CA-LBG viscosity with the influence of temperature 20-80°C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; and 2.52 cP. The pH of mucilago at a synthesis temperature of 20°C-40°C shows a pH < 1 as confirmation that the mucilago condition before heating is acidic. The pH of the CA-LBG synthesized at 40°C was a pH value of 4.82 and at 60°C a pH value was 4.88. CA-LBG pH values from both synthesis heating temperatures are weakly acidic. The profile of the relationship between temperature to viscosity and pH is presented in Figure 2.

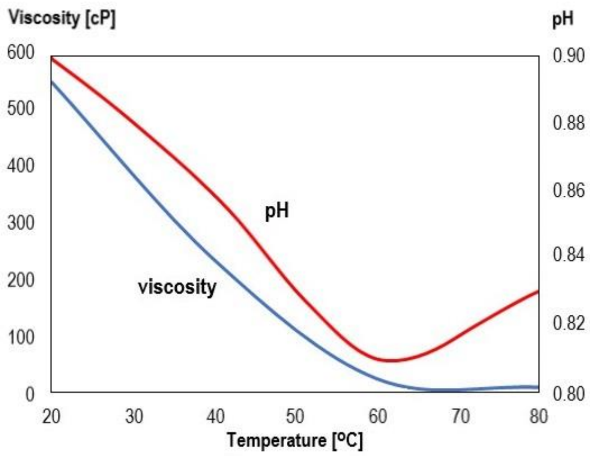


Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago.

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40°C appears at 203.50 nm and a temperature of 60°C appears at 204.50 nm in the ultraviolet wavelength range.

The melting point of CA-LBG produced at 40°C has 125°C-135°C and 60°C has 135°C-155°C. The swelling index value of the CA-LBG synthesized at 40°C was 20.37 and at 60°C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesized at 40°C was 39.76 % and at 60°C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment shows that temperature has excellent potential as an energy source for CA esterification with LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the C atom has the potential to bind to the C positive from CA of CA. The energy produced at low temperatures (20°C) is not strong enough to reduce the bond strength between atoms in mannose and galactose, so the decrease in mucilago viscosity is insignificant. The energy produced at 40°C and 60°C reduce the bond strength between atoms in mannose and galactose to provide a greater chance of bonding between O atoms and the C positive of CA. The presence of CA in LBG decreases the ability of mannose and galactose to trap the swelling solution. This ability shows the specific character of CA-LBG. The energy at high temperature (80°C) is powerful to reduce the bond strength between atoms in mannose and galactose so that the O atom has a very large opportunity to bind to the C positive of CA. This enormous energy may also break bonds between atoms in mannose, galactose, or

CA. High temperatures for a long time may damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilago's final viscosity determines the CA-LBG deposition's success. Mucilago, which is too viscous, is difficult to precipitate and tends to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Mucilago that is too runny is difficult to precipitate because the bonds between atoms are broken when mucilago interacts with acetone and water. This condition is because the CA-LBG formed may not trap the swelling solution because the bonds between the atoms have been broken.

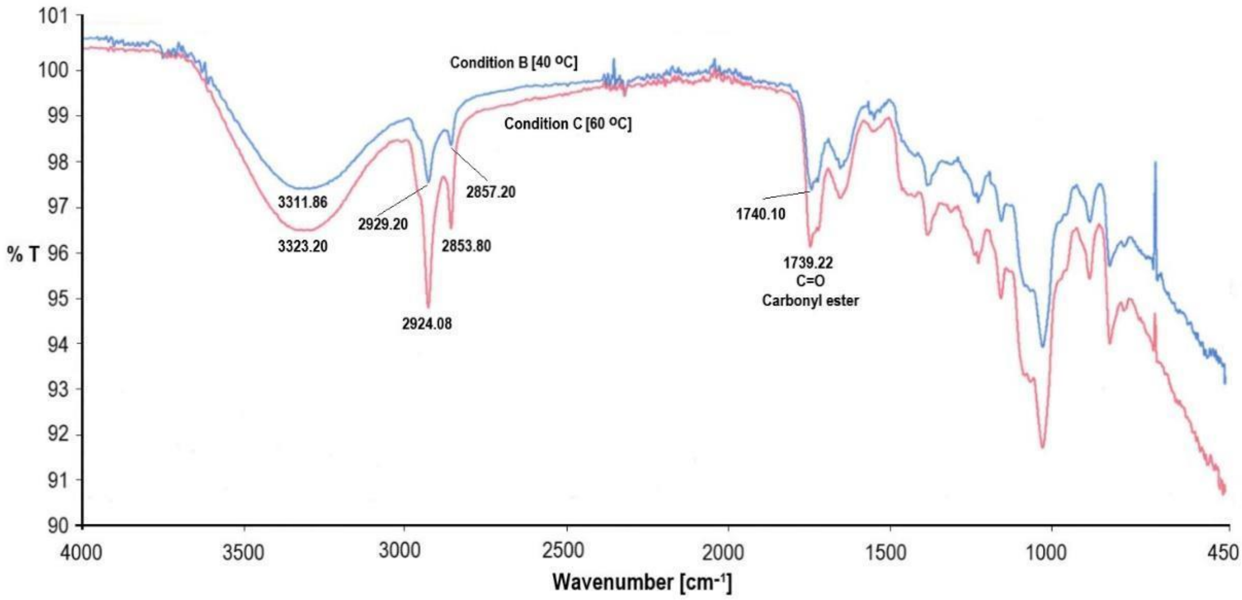


Figure 3: CA-LBG infrared spectra at 40°C and 60°C

The heating temperature of the mucilago can change the pH which is presented in Table 1 and Figure 2. The temperature of 60°C produces the lowest pH of the mucilago. Heating to a temperature of 60°C causes the solubility of CA to be higher and there is stretching of the bond between the mannose and galactose atoms so that the distribution of dissolved CA is more homogeneous. This condition will give a more acidic pH value. At 80°C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent characterization was only carried out on mucilago, which could be deposited and produced CA-LBG. The homogeneity of CA-LBG was evaluated based on the pH of CA-LBG and the pH value of the washing solution during manufacturing. The CA used in the synthesis has a pH of 2.02, while LBG has a pH of 5.85. CA-LBG has a pH of 4.82 & 4.88, which is between the pH of CA and LBG. In addition, repeated washing until pH 6.8-7.0 (pH acetone-distilled water) ensures that the CA-LBG precipitate is free of CA and that the LBG does not react. The infrared spectra show the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification and CA-LBG. In previous studies, the C=O group appeared at 1735 -1743 cm^{-1}) (Hadinugroho *et al*. , 2017; 2019; 2023). On heating at 40°C and 60°C, the spectra were clear, smooth, and without damage. This shows that the two temperatures do not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) is lower than that of galactomannan (205 nm) (Matsuda *et al*., 2016) and CA (210 nm) (Krukowski *et al*., 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

Mp CA-LBG (125°C-155°C) between LBG and CA because CA-LBG undergoes decomposition. The higher the heating temperature, the Mp tends to increase due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the pH tends to increase.

The swelling index parameter shows the strength of the atomic bonds in CA-LBG that can withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature of 60°C may form stronger bonds between atoms than the temperature of 40°C at esterification. At 60°C, it gives a lower yield than at

40°C. This is because less CA is tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40°C-60°C is the suitable heating temperature for esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60°C is the highest, which decreases the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, swelling index, and decrease the yield of CA-LBG synthesis.

Acknowledgement

The authors thank the Widya Mandala Catholic University Research and Community Service Institute in Surabaya for providing a research grant (7417/WM01/N/2022). The authors would like to thank the Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, for the laboratory facilities used in this study.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

Widya Mandala Surabaya Catholic University.

References

Alves, A. D., Cavaco, J. S., Guerreiro F, Lourenço, J. P., Rosa D. C., A. M., Grenha, A. (2016). Inhalable antitubercular therapy mediated by locust bean gum microparticles. *Molecules*, **21**(6), 1–22. https://doi.org/10.3390/molecules21060702

Bhattacharya, A., Rawlins, J W., Ray, P. (2008). *Polymer Grafting and Crosslinking*. A John Wiley & Sons, Inc, Publication.

Colas, A. (2005). *Cow Corning Silicones : Preparation Properties and Performance*. Midland, USA

Dey, P., Maiti, S., Sa, B. (2013). Novel etherified locust bean gum-alginate hydrogels for controlled release of glipizide. Journal of Biomaterials Science, Polymer Edition, **24**(6), 663–683. https://doi.org/10.1080/09205063.2012.703950

Gulrez, S. K. H., Al-Assaf, S, Phillips, G. O. (2011). *Hydrogels: Methods of Preparation, Characterisation and Applications. In: Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. InTech Publisher.

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2022). Preparation of Citric Acid-Locust Bean Gum (CA-LBG) for the Disintegrating Agent of Tablet Dosage Forms. *Journal of Pharmaceutical Innovation,* **17**, 1160–1175. https://doi.org/10.1007/s12247-021-09591-0

Hadinugroho W, Martodihardjo S, Fudholi A, Riyanto, S., Prasetyo, J. (2023). Hydroxypropyl Methylcellulose as Hydrogel Matrix and Citric Acid-Locust Bean Gum as Negative Matrix for Controlled Release Tablet. *ACS Omega*, **8**(8), 7767-7778. https://doi.org/10.1021/acsomega.2c07432

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2019). Esterification of citric acid with locust bean gum. Heliyon, **5**(8), e02337. https://doi.org/10.1016/j.heliyon.2019.e02337

Hadinugroho, W., Martodihardjo, S., Fudholi, A., Riyanto, S. (2017). Study of a catalyst of citric acid crosslinking on locust bean gum. *Journal of Chemical Technology and Metallurgy*, **52**(6), 1086–1091

Kaity, S., Isaac, J., Kumar, P. M., Ghosh, A. (2013). Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery. *Carbohydrate Polymers*, **98**(1), 1083–1094. https://doi.org/10.1016/j.carbpol.2013.07.037

Karadag, E., Saraydin, D., Sahiner, N., Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science - Pure and Applied Chemistry,* **38 A**(11), 1105–1121. https://doi.org/10.1081/MA-100107132

Krukowski, S., Karasiewicz, M., Kolodziejski, W. (2017). Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations. *Journal of Food and Drug Analysis,* **25**(3), 717–722. https://doi.org/10.1016/j.jfda.2017.01.009

Matsuda, Y., Sugiura, F., Okumura, K., Tasaka, S. (2016). Renaturation behavior of xanthan with high molar mass and wide molar mass distribution. *Polymer Journal*, **48**(5), 653–658. https://doi.org/10.1038/pj.2015.128

Palit, S. R. (2009) Some recent results of end group analysis of dye techniques. *Pure and Applied Chemistry*, **12**(1), 451–462. https://doi.org/10.1351/pac196612010451

Samavati, V., Razavi, S. H., Rezaei, K. A., Aminifar, M. (2007). Intrinsic viscosity of locust bean gum and sweeteners mixture in dilute solutions. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **6**(3), 1879–1889

Pharmacy Education (2024) 24(3) 216 - 221
https://doi.org/10.46542/pe.2024.243.216221

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

Wuryanto Hadinugroho, Stephanie Florencia Winarko, Echa Imanuela Sinta, Senny Yesery Esar, Jefri Prasetyo
Faculty of Pharmacy, Widya Mandala Surabaya Catholic University, Surabaya, Indonesia