Effects of pore forming agents on chitosan-graft-poly(N-vinylpyrrolidone) hydrogel properties for use as a matrix for floating drug delivery

E Budianto, M F Al-Shidqi and A H Cahyana
Department of Chemistry, University of Indonesia, Depok 16424 Indonesia

E-mail: emilb@ui.ac.id

Abstract. Eradicating H. pylori-based infection by using conventional oral dosage form of amoxicillin trihydrate finds difficulty to overcome rapid gastric retention time. Encapsulating amoxicillin trihydrate in floating drug delivery system may solve the problem. In this research, the floating drug delivery system of amoxicillin trihydrate encapsulated in floating chitosan-graft-poly(N-vinyl pyrrolidone) hydrogels containing CaCO₃ and NaHCO₃ as pore forming agents has been successfully prepared. Pore forming agents used was varied with the ratio of 10 to 25% pore forming agents to total mass of the used materials. The hydrogel were characterized using FTIR spectrophotometer and stereo microscope. As pore forming agents compositions increased, the porosity (%) and floating properties increased but followed by decrease in drug entrapment efficiency. Most of the floating hydrogels possessed floating ability longer than 180 min and the highest porosity was found in hydrogel containing 25% NaHCO₃. Hydrogel containing CaCO₃ showed sustained drug release profile than hydrogel containing NaHCO₃. However, the optimum formulation was achieved at composition of 10% NaHCO₃ with 57% of drug entrapped within the hydrogel and 43% drug released. The results of these studies show that NaHCO₃ is an effective pore forming agents for chitosan-graft-poly(N-vinyl pyrrolidone) hydrogel preparation as compare to CaCO₃.

1. Introduction

Helio bacter pylori (H. pylori) is one of the most frequent and persistent bacterial infections worldwide [1]. H. pylori is associated with peptic ulcer disease, gastric ulcers, mucosa-associated lymphoid tissue lymphoma and gastric cancer [2]. As stated in Indonesian Pharmacopoeia (2013), Amoxicillin is administered orally as trihydrate for the eradication of H. pylori in forms of tablets, capsules, or oral suspensions. Amoxicillin is known to have short plasma half-lives of 1 to 1.5 h [3]. Amoxicillin limitation of having short plasma half-lives cause amoxicillin should be administered by multiple dosing at regular interval. This conventional route of administration leads to a rapid rise and fall in drug concentrations within plasma which may fall outside the therapeutic range for significant time periods [4]. Several approaches have been recommended to overcome these limitation, one of them is the controlled drug delivery system. Nevertheless, conventional controlled drug delivery system still faces some kind of difficulties. One of such difficulties is the short gastric emptying time [5]. The short transit time means the drugs within oral controlled release dosage form are not completely released. This results in ineffective administered dose which is then accompanied by lesser bioavailability [6].
Approaches to increase the gastric residence time of drug formulation have been developed in recent years, including gastroretentive drug delivery system (GDDS). Floating drug delivery system (FDSS) is one of GDDS and it may be used for various drugs that are locally active in the stomach, possess narrow absorption window in gastrointestinal tract, degrade in colon, or are insoluble in alkaline pH [7-9]. In FDSS, while the system is floating on the gastric contents, the drug is released at a desired rate from the system [8]. By using amoxicillin in FDSS dosage form, the administered dose of amoxicillin can overcome the limited gastric transit time, thus increasing its bioavailability.

During the last two decades, natural and renewable polymers have attracted interest in researches related to drug delivery. Among various renewable polymers, chitosan has been evaluated as a potential polymer for drug administered orally [10]. Chitosan is natural, biocompatible, and biodegradable polymer prepared from partial deacetylation of chitin, the second prevalent form of polymerized carbon in nature [11,12]. In this research, chitosan-graft-poly(N-vinyl pyrrolidone) has been prepared and used as floating drug delivery system matrix with amoxicillin trihydrate as a model drug. The floating drug delivery system was prepared using two different pore forming agents, i.e. calcium carbonate (CaCO$_3$) and sodium bicarbonate (NaHCO$_3$). The effects of different pore forming agents and its efficacy toward the hydrogel matrix properties were examined.

2. Materials and Methods

2.1 Materials

Amoxicillin trihydrate API was kindly provided by PT. Dankos Laboratories Tbk, Chitosan (94.13 % deacetylation degrees, 25.20 cps viscosity) was purchased from PT. Biotech Surindo, N-vinyl pyrrolidone (analytical grade) was purchased from Sigma-Aldrich), calcium bicarbonate (CaCO$_3$) and sodium bicarbonate (NaHCO$_3$) (analytical grade) were purchased from Merck, acetic acid (glacial, analytical grade) purchased from Merck, N,N-dimethylbisacrilamide (NNMBA, analytical grade) was purchased from Sigma-Aldrich

2.2 Methods

Preparation of chitosan-graft-poly(N-vinyl pyrrolidone) hydrogel and chitosan-graft-poly(N-vinyl pyrrolidone) floating hydrogel matrix. An exact amount of 0.2 g dry chitosan was first dissolved in 15 mL of 2% acetic acid using a round bottom flask. Then, the flask was placed in a thermostated bath to initiate the polymerization at 60° C and followed by the addition of 0.1 g ammonium peroxide as initiator. A slow stream of nitrogen gas was passed continuously during the polymerization reaction and the reaction mixture was stirred for 10 min. 0.8 mL of N-vinyl pyrrolidone monomer and NNMBA crosslinker (2%) was added into the reaction mixture. The reaction was done after 180 min and
stopped by letting air into the flask. The reaction mixture was poured in a beaker and the grafted product and was neutralized to pH 8.0 by addition of 1 N NaOH. Then, 50 mL methanol was added to the hydrogel product while stirring. After complete dewatering for 8 h, the product was filtered, washed with fresh methanol, and dried at 50°C. To prepare floating hydrogel matrix, calcium carbonate with various composition were added to the hydrogel product when it reach pH 5. The same procedure was followed to prepare floating hydrogel with sodium bicarbonate. Table 1 list the various composition of pore forming agents added during hydrogel and floating matrix synthesis. The amoxicillin encapsulation was performed by using in-situ method. Drug solution was prepared by dissolving 500 mg amoxicillin trihydrate in 5 mL distilled water. The drug into the reaction mixture was added before the addition of crosslinker. The same variation of pore forming agents composition as indicated in Table 1 were used in the synthesis of amoxicillin trihydrate loaded floating hydrogel.

Table 1. Synthesis condition of chitosan-graft-poly(N-vinyl pirrolidone) hydrogel and chitosan-graft-poly(N-vinyl pirrolidone) floating hydrogel matrix containing CaCO₃ and NaHCO₃.

| Chitosan (g) | NVP (g) | APS (g) | MBA (g) | CaCO₃/ NaHCO₃ (g) | % PFA |
|-------------|---------|---------|---------|-------------------|-------|
| 0           | 0.2     | 0.834   | 0.1     | 0.0167            | 0     |
|             |         |         |         | 0.1808            | 10    |
|             |         |         |         | 0.214             | 15    |
|             |         |         |         | 0.2675            | 20    |
|             |         |         |         |                   | 25    |

Hydrogel physical characterization: porosity measurement of hydrogel and invitro buoyancy study. Wet hydrogels after being immersed in pH 1.2 solution were weighed after blotted. The dried hydrogels were also weighed before the hydrogels being immersed. The porosity was calculated based on the following equation:

$$ \frac{W_w \times \rho \times V}{\rho \times V} $$

Where W_w and W_d are mass of wet and dry hydrogel respectively;  $\rho$ is density of pH 1.2 solution, and V is volume of the hydrogel [13].

Floating properties of hydrogel were evaluated as floating lag time and floating time. Floating lag time can be defined as the time required for hydrogels to rise to the surface and float, while floating time is the length of time when hydrogel completely float on the surface of 5 mL pH 1.2 medium. The measurement was carried out in 37 ± 0.1 °C and was measured by visual observation.

Amoxicillin trihydrate entrapment efficiency. The weighed hydrogels were powdered and dissolved in 5 mL pH 1.2 solution, sonicated for 1 h, diluted further and analysed spectrophotometrically at 272 nm
using Shimadzu-2450 UVV spectrophotometer. In vitro release study. The loaded hydrogels were immersed in beaker containing 20 mL pH 1.2 solution and placed in a shaking waterbath at 37 ± 0.1 °C. At 10, 20, 30, 60, 120, and 180 min time interval, 1 mL of the sample was withdrawn, diluted, and analysed spectrophotometrically at 272 nm. Equal amount of fresh medium was replaced immediately after withdrawal of the test sample.

Hydrogel characterizations were conducted using Fourier transform infrared spectroscopy (FTIR) and Olympus SZX16 stereo microscope. FTIR analysis was performed by grounding and mixing hydrogel samples with KBr powder with ratio 1:9. The measurements were carried out within the range of 4000-400 cm⁻¹. Morphology of hydrogels was studied using stereomicroscope with 1x magnification and 10x zoom.

3. Results & Discussion

3.1 FTIR Characterization

Figure 1(a) and (b) illustrate the FTIR spectra of blank hydrogel and hydrogels containing CaCO₃ and NaHCO₃, respectively. The broad band showed at 3200-3650 cm⁻¹ is due to stretching vibration of -OH groups in chitosan while the band at 1598 cm⁻¹ is due to bending vibration of -NH group of chitosan. The presence of C=O stretching vibration band at 1674 cm⁻¹ and increase in band area of stretching vibration of C-N group at 1078 cm⁻¹ indicates that poly(N-vinyl pyrrolidone) chain has been successfully grafted to chitosan backbone. Addition of NaHCO₃ and CaCO₃ into chitosan-graft-poly(N-vinyl pyrrolidone) does not alter the existing functional group within the hydrogel matrix, for all characteristic bands of blank hydrogel are present in hydrogel containing both pore forming agents. The new bands appeared at 828 cm⁻¹ in CaCO₃ and 877 cm⁻¹ in NaHCO₃-containing hydrogels attributed to the carbonate peak which indicates pore forming agent present in hydrogel network. Meanwhile, another new band appeared at 2514 and 1793 cm⁻¹ in CaCO₃ containing hydrogel. These bands does not appeared in the NaHCO₃-containing hydrogels. The appearance of the new bands indicating that there is chemical interaction between the hydrogel matrix and CaCO₃. It is taught that the Calcium ion from CaCO₃ bind to the polymer structure of chitosan-graft-poly(N-vinyl pyrrolidone) hydrogel resulting in polymer-metal complex. The formation of complex through chelation of divalent cations with poly(N-vinyl pyrrolidone) may be through oxygen of poly(N-vinyl pyrrolidone) [16]. Figure 1(c) illustrates the FTIR spectra of blank hydrogel and floating hydrogel matrix loaded with amoxicillin trihydrate. The FTIR spectra shows one of the characteristic band of amoxicillin at 1515 cm⁻¹ indicating the bending vibration of N-H group. The band appeared at blank hydrogel, hydrogel containing NaHCO₃, and hydrogel containing CaCO₃ at 1520, 1517, and 1513 cm⁻¹, respectively. The bands show no significant shift from the original position in the spectrum of pure amoxicillin.
trihydrate at 1515 cm$^{-1}$ [17]. This shows no evidence for any chemical interaction between amoxicillin and hydrogel matrix and the pore forming agents [18]. The loaded amoxicillin trihydrate drug are binded to the hydrogel matrix though physical interactions between the functional groups of drug and the hydrogel itself.

3.2 Porosity Evaluation of Floating Hydrogel

During the immersion of hydrogel matrix within acidic medium, carbonate salts of pore forming agent react with the acid to produce carbon dioxide. The evolving gas permeates through the hydrogel leaving gas bubble or pores [19].
Porosity of floating hydrogels study was conducted to determine the effects of pore forming agents on pore structure of hydrogels. The results of the porosity study are shown in Figure 2. Blank chitosan-graft-poly(N-vinyl pyrrolidone) hydrogels possess very low porosity (1.4%). This shows that the hydrogel that is free from pore forming agent has a very compact and rigid internal structure. It was observed that the porosity of hydrogels increased as the concentration of pore forming agents increase from 10 to 25%. Hydrogels with 10% concentration of NaHCO₃ and CaCO₃ show low porosity which are 18 and 5%, respectively. At the highest concentration of pore forming agents, hydrogels with 25% of NaHCO₃ and 25% of CaCO₃ show higher porosity, where 47% and 36% for NaHCO₃ and CaCO₃ hydrogels, respectively. The results clearly shows that NaHCO₃ produced higher porosity of the hydrogels than CaCO₃.

| Table 2. The results of in vitro buoyancy study of chitosan-graft-poly(N-vinyl pyrrolidone) floating hydrogels |
|---------------------------------------------------------------|
| Pore forming agent | Floating lag time (min) | Floating time (min) |
|-------------------|-------------------------|---------------------|
| Control (0%)      | Not floating            | Not floating        |
| NaHCO₃            |                         |                     |
| 10%               | 34                      | >180                |
| 15%               | 20                      | >180                |
| 20%               | 6                       | <20                 |
| 25%               | 5                       | <20                 |
| CaCO₃             |                         |                     |
| 10%               | Not floating            | Not floating        |
| 15%               | 53                      | >180                |
| 20%               | 15                      | >180                |
| 25%               | 13                      | >180                |

Table 2 shows the floating ability of the hydrogel matrix in pH 1.2 solution at 37 °C that stimulates the gastric fluid condition. Hydrogels containing pore forming agents shows the ability of floating. In contrast, hydrogels without pore forming agents could not float because their lower porosity, thus higher density, hindered their ability of float. The floating lag time of hydrogels increases with the addition of pore forming agents. This is due to the evolving of CO₂ gas produced from the effervescent reaction in hydrogel matrix which causes the hydrogel to float on the pH 1.2 solution in such faster time. When comparing CaCO₃ and NaHCO₃, floating lag time for hydrogels containing NaHCO₃ are
the shortest. It takes 5 min for hydrogels containing to 25% NaHCO₃ to reach the surface whereas for hydrogels containing 25% CaCO₃ are 13 min. Meanwhile, hydrogels containing 20% and 25% NaHCO₃ are only able to float less than 20 min and later dissolve. This is due to increase of pore forming agents which lead to decrease of the hydrogel strength as the hydrogels become highly porous and fragile at the higher concentration of pore forming agents [13,18].

3.3 Drug Entrapment Efficiency

The drug entrapment efficiency of the results show that as the concentration of pore forming agent increases from 10% to 25%, a decreased in drug entrapment efficiency are observed 57 to 23% for NaHCO₃ and 49 to 12% for CaCO₃. Hydrogels that do not contain any pore forming agents show high entrapment efficiency as compared to other hydrogels that loaded with pore forming agents. This may be due to the fact that hydrogels without pore forming agent have an extremely compact internal structure that are able to retain the drugs within its matrix [19].

![Figure 3. Drug entrapment efficiency of floating hydrogels with different concentration of sodium bicarbonate and calcium carbonate.](image)

On the other side, the decrease in drug entrapment observed in the addition of concentration of pore forming agents is possibly due to the higher porosity of the hydrogels. The less dense internal structure of the hydrogels which contain high amount of pore forming agents causing less physical interaction between the matrix and the drug resulting in decreased drug entrapment. Addition of pore forming agent tends to make hydrogels possess lower drug entrapment efficiency (<50%) and CaCO₃ tends to decrease the drug entrapment efficiency than NaHCO₃.

3.4 In Vitro Release Profile of Amoxicillin Trihydrate

The in vitro release profile of amoxicillin trihydrate from floating hydrogel matrixes which contain various concentration of NaHCO₃ and CaCO₃ are shown in Figure 4(a) and 4(b). The amount of amoxicillin release from hydrogels loaded with pore forming agents are higher than blank hydrogels. Lower drug release profile of blank hydrogels is due to the less porous nature of hydrogel network that
retain drugs more effectively. The rate of drug release is found to increase as the amount of CaCO$_3$ and NaHCO$_3$ increases.

![Figure 4](image)

**Figure 4.** The *in vitro* release of amoxicillin trihydrate from hydrogel containing CaCO$_3$(a) and NaHCO$_3$(b)

It is observed that hydrogels containing NaHCO$_3$ as pore forming agent showed higher drug release than hydrogels containing CaCO$_3$. At first 10 min, the release of amoxicillin from 25% of CaCO$_3$ and NaHCO$_3$ filled hydrogel are 3 and 7%, respectively. At both pore forming agents, increase in pore forming agents’ concentration is followed by gradual increases in amount of drug release. At t = 180 min, drug release for 25% CaCO$_3$ and 25% NaHCO$_3$ are 46 and 64%, respectively. It is also observed that different pore forming agent produces different pattern of drug release where hydrogels containing CaCO$_3$ shows slower and more controlled release than NaHCO$_3$. This is possibly due to the presence of smaller pore size in hydrogels containing CaCO$_3$ that able to control the drugs diffusion process from hydrogel matrix [10]. Meanwhile, hydrogels containing NaHCO$_3$ show fast drug release pattern at higher concentration (20 and 25%) and much slower and controlled release pattern at lower concentration of NaHCO$_3$ (10 and 15%). The fast release pattern at higher concentration is marked with sudden increase of the amount released drug between t = 10 min and t = 30 min. The fast release pattern is expected due to higher porosity of hydrogels causing drug diffusing quickly from the floating hydrogel matrix [20].
3.5 **Morphology of Hydrogels**

![Morphology of Hydrogels](image)

*Figure 5.* Morphology of blank hydrogel (a), hydrogel containing (b) 10% (c) 15% (d) 15% (e) 20% of NaHCO$_3$ and hydrogel containing (f) 10% (g) 15% (h) 20% (i) 25% of CaCO$_3$.

The morphology study of floating hydrogels was studied using stereo microscope and the results are illustrated by Figure 5. The hydrogels were studied after being used in drug release test. In general, the study shows that the hydrogel surfaces are eroded. The acidic condition of pH 1.2 solution causes protonation of amine groups in chitosan. The protonation leads to repulsion in the polymer chains and dissociation of intramolecular hydrogen bonding [21]. Hydrogels with NaHCO$_3$ as pore forming agent are more susceptible to surface erosion, shown by more translucent surface seen at Figure 5(a)-5(b). This may due at higher concentration of NaHCO$_3$, the high porosity and fragile nature of hydrogels facilitate the erosion process. The more intact structure of CaCO$_3$ containing hydrogels as can be seen at Figure 5(f)-5(g) can be attributed to the lower porosity of CaCO$_3$ containing hydrogels. In addition, change in chemical properties of floating CaCO$_3$-containing hydrogels as explained in Section 3.1 may also explained the more resistant nature of hydrogels towards surface erosion in pH 1.2 solution.
4. Conclusion
At the same amount of pore forming agent concentrations, NaHCO$_3$ and CaCO$_3$ affects the properties of floating chitosan-graft-poly(N-vinyl pyrrolidone) hydrogels to different extent. CaCO$_3$ can alter the physical properties of hydrogel more significantly than NaHCO$_3$ as seen from the morphology study results and the chemical properties. Addition of pore forming agent at different concentrations increase the buoyancy property and porosity of the hydrogel matrixes but lowers the drug entrapment efficiency. Overall, on the buoyancy study, drug entrapment efficiency, and drug release pattern, it was demonstrated that NaHCO$_3$ at 10% concentration is the most effective pore forming agent for floating drug delivery system employing chitosan-graft-poly(N-vinyl pyrrolidone) as the matrix.

Acknowledgment
We would like to thank the University of Indonesia for supporting this study through Hibah Publikasi Internasional Terindeks untuk Tugas Akhir Mahasiswa No. 2011/UN2.R12/HKP.005.00/2016 and the Department of Chemistry University of Indonesia for the additional support.

References
[1] Tonkic A 2012 Helicobacter 17 1-8.
[2] Bardonnet PL et al 2006 J. Control. Release 111 11–18.
[3] Martindale 2009 The Complete Drug Reference ed Sweetman SC 36 (London: Pharmaceutical Press).
[4] Siepmann 2012 J Fundamentals and Applications of Controlled Release Drug Delivery ed (New York City: Springer) 21.
[5] Arora S et al 2005 AAPS Pharm. Sci. Tech. 6 372–90.
[6] Patil J M et al 2006 J. Sci. Ind. Res. (India) 65 11-21.
[7] Narang N 2010 Int. J. App. Pharm. 3 1-7.
[8] Singh B N and Kim K H 1999 J. Control. Release 63 235-259.
[9] Pahwa R et al 2012 Expert Opin. Drug Deliv. 9 525-539.
[10] Sokker H H et al 2008 Carbohydr. Polym. 75 222-229.
[11] Zargar V et al 2015 Chem. Bio. Eng. Rev 2 1-24.
[12] Bhattarai N et al 2010 Adv. Drug Deliv. Rev 62 83-99.
[13] Selvakumaran S et al 2016 Carbohydr. Polym. 135 207-214.
[14] Sahasathian T et al 2010 Arch. Pharm. Res. 33 889-899.
[15] Srivastava A et al 2010 Carbohydr. Polym. 80 790-798.
[16] Anasuya K V et al 2014 Indian J. Adv. Chem. Sci. 2 12-15.
[17] Songsurang K et al 2011 AAPS Pharm. Sci. Tech. 12 35-45.
[18] Choi B Y et al 2002 Int. J. Pharm. 239 81-91.
[19] Jassem N A and Rajab N A 2012 Karbala J. Pharm. Sci. 4 166-176.
[20] Krishnan V et al 2010 Trends Biomater. Artif. Org. 24 (3) 139-145.
[21] Mirzaei E B et al 2013 Polym-Plast Technol. 52 1147-53.