The Comparison of Swelling, Mucoadhesive, and Release of Ranitidine from Spherical Matrices of Alginate, Chitosan, Alginate-Chitosan, and Calcium Alginate-Chitosan

Anayanti Arianto[1]*, Hakim Bangun[1], Urip Harahap[1], and Syafruddin Ilyas[2]

[1]Faculty of Pharmacy, University of Sumatera Utara , Jl. Tridharma No. 5, Kampus USU, Medan, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Jl. Bioteknologi No. 1, Kampus USU, Medan, Indonesia

*Corres.author : anayantia@yahoo.com

Abstract: Alginate (Alg) is a polyanionic polymer whereas chitosan (Ch) is a polycationic polymer. The purpose of this study was to compare the swelling, mucoadhesive, and release of ranitidine HCl (RH) from the spherical matrices of Alg, Ch, Alg-Ch and calcium alginate-chitosan (Ca Alg-Ch). The spherical matrices containing RH were prepared by incorporating RH with sodium Alg and/or Ch with the aid of starch mucilage to form a compact mass. This compact mass was then molded to form spherical matrices with diameter of 8.8 mm. For the preparation of Ca Alg-Ch matrices, the Alg-Ch matrices were then immersed in calcium chloride solution. The release of RH and swelling of matrices were measured by using the USP paddle method in simulated gastric fluid (SGF). RH concentrations were determined spectrophotometrically at the wavelength of 224.6 nm. The mucoadhesive force was measured in rabbits stomachs using DuNouy tensiometer. The results showed that the release of RH from Alg-Ch matrices gave the most extended release, followed by matrices of Ca Alg-Ch and then Alg matrices, while Ch matrices did not give extended release of the drug. The Higuchi plot of the release of RH from spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch showed stright line with regressions coefficient (R^2) of 0.9470, 0.9638, 0.9932, and 0.9837, respectively. The drug release mechanism is anomalous or non-Fickian type. Alg-Ch matrices exhibited the greatest degree of swelling followed by Ca Alg-Ch and then Alg matrices, but Ch matrices eroded in SGF. Alg-Ch matrices showed the strongest mucoadhesive property followed by Ca Alg-Ch, Alg, and Ch matrices. It is concluded that the spherical of Alg-Ch matrices possess the highest swelling degree and mucoadhesive strength, and give the most extended of drug release in SGF than other matrices tested.
Keywords: Alginate, chitosan, alginate-chitosan, calcium alginate-chitosan, spherical matrices, ranitidine HCl, swelling, release, mucoadhesive.

Introduction

Alg is a polysaccharide produced from brown algae (Phaeophyceae) and bacteria. Alg is an anionic copolymer consisting of residue β-D-mannuronic acid and α-L- guluronic in bond 1,4.[1] Alg is commonly used in the form of sodium Alg which is soluble in water and when dissolved in a calcium chloride solution, then calcium Alg gel will immediately formed which is insoluble in water. It is reported, calcium ions chelate to polyguluronate sequences by inter-chain mechanism.[2] The gel formation properties between sodium Alg and calcium chloride solution is used in the preparation of Alg capsules matrix type that resistant to gastric acid and the drug is encapsulated in the matrix.[3] The most important advantage of sodium Alg as a matrix for the controlled release formulation is because it is biodegradable and biocompatible.[4] Ch, a chitin derivative, is the second most common biopolymer found in nature after cellulose. Ch is a weak base with pKa of residue D-

glucosamine about 6.2 to 7.0 and therefore is not soluble in neutral and alkaline pH but soluble in dilute acids to form a gel. Ch is non- toxic, biocompatible, easily biodegradable, and mucoadhesive.[5]

Alg and chitosan form electrostatic interaction between –COOH groups of Alg and NH_2 groups of Ch.[6] Previously, we studied the preparation and diffusion of urea, sodium salicylate, and bovine serum albumin through the Alg-membrane.[7]

For the purpose of drug encapsulation, researchers usually prepared the beads/microspheres /microparticles of Alg-Ch by dropping the sodium Alg solution containing drug into calcium chloride solution containing Ch. The preparation of beads/microspheres/microparticles by this method result in low entrapment efficiency of drug due to the release of drug during curing in calcium chloride solution, and also give the un-extended release of drug, especially for soluble and low molecular weight of drugs.[8-11]

In the present research, a new method without immersion the matrices in calcium chloride was performed to obtain the high entrapment efficiency and to produce extended release of drug. The product was called " Alg-Ch matrices". For the comparison, we also prepared the matrices with the immersion in calcium chloride solution and the product was called " Ca Alg-Ch matrices." RH was used as drug model. RH is a drug that commonly used to treat duodenal ulcer disease, gastric ulcer, gastric acid hypersecretion conditions, erosive esophagitis, and ulcers due to stress in critically ill patients. RH acts by reducing gastric acid secretion. The biological elimination half-life of RH is relatively short, ranges only 2.5-3 hours. RH is absorbed in the upper part of the small intestine and showed a low absolute bioavailability that is 50%.[12-13] Furthermore, the absorption will be reduced due to the decomposition and metabolism of ranitidine by microbes in the colon which causes the low bioavailability of RH.[14]

The purpose of this study was to prepare and to compare the swelling, mucoadhesive, and release of RH from spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch in SGF in order to obtain the gastroretentive drug delivery systems of drugs that are targeted to specific to the stomach and the release the drug is continuously and controlled so that would be beneficial to increase the efficacy of the drug. In this paper, the preparation, the swelling, mucoadhesive, and release properties of RH from Alg, Ch, Alg-Ch, and Ca Alg-Ch matrices in SGF will be discussed.

Experimental

Materials

Sodium Alg 500-600 cP were obtained from Wako Pure Chemical Industries, Ltd., Japan. Ch from Funakoshi Co., Ltd., Japan. RH HCl was obatined from PT. Mutifa, Medan. Hydrodrochloric acid, calcium chloride-dihydrate, and sodium chloride all were the product of Merck.

Preparation of spherical matrices

The formula for preparation ten spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch is showed in Table 1. Spherical matrices containing RH were prepared by incorporating sodium Alg, Ch, and RH in a mortar with the addition of starch mucilage as binding agent to form a compact mass. The compact mass was then divided in to ten parts and each part was molded to be spherical matrix with diameter of 8.8 mm.

For the preparation of Ca Alg-Ch spherical matrix, the spherical matrix of Alg-Ch was then immersed in 10 ml of 0.15 M calcium chloride solution for 35 minutes. Then, the spherical matrices obtained were dried at room temperature.

Table 1. The composition of ten spherical matrices containing RH

Formula	Ratio Alg to Ch	RH (mg)	Sodium Alg (mg)	Ch (mg)
F1	1 : 0	1680	1500	0
F2	0 : 1	1680	0	1500
F3	1 : 1	1680	750	750
F4	1 : 1	1680	750	750

Determination of drug content

For the spherical matrices of Alg, Ch, and Alg-Ch the drug content per spherical matrix was used the theoretical amount. But, for the Ca Alg-Ch matrices the amount of RH in per spherical matrix was obtained as a difference of theoretical amount and non entrapped amount. The non entrapped amount was determined from amount of RH in calcium solution used for curing the spherical matrix by using a UV spectrophotometer at 224.6 nm.

Determination of drug release

The release of RH from spherical matrix was tested using the USP paddle method dissolution tester at 50 rpm in the 900 ml medium of SGF (pH 1.2) at $37\pm0.5^{\circ}$C. Five milliliters of aliquot was withdrawn at predetermined time. The dissolution test was done for 10 hours. The medium was replenished with 5 ml of fresh SGF each time. RH concentrations were assayed by using UV spectrophotometer at 224.6 nm.

The dissolution data obtained were plotted as percent cumulative drug release versus square root of time according to Higuchi equation.

$$Q= Kt^{1/2} \tag{1}$$

Then, the release data were treated by the Rigers and Peppas equation.[15] The equation was treated logarithmically to determined the value of release exponent, n; the value of n is indicative of drug release mechanism.

$$\frac{Mt}{M\infty}= Kt^{n} \tag{2}$$

Determination of swelling or erosion

The swelling or erosion properties of spherical matrices were measured using USP dissolution tester at 50 rpm in the 900 ml medium of SGF (pH 1.2) at 37°C. After a selected time intervals, the spherical matrices were withdrawn and rolled on a tissue paper to remove excess water and weight. The swelling or erosion of matrices was determined based on the changed of weight and diameter of the matrices as

$$\text{Weight Change } (\%) = \frac{W2 - W1}{W1} \ x \ 100 \tag{3}$$

W_1: Initial weight of spherical matrix
W2: Weight of spherical matrix after immersion in the medium

In vitro evaluation of mucoadhesive strength

In vitro bioadhesion study was done using rabbit stomach by modification of the DuNouy tensiometer. The platinum-iridium ring of tensiometer was replaced by the spherical matrix that hanged to the arm of tensiometer. The experiment was done at 37°C. The stomachs tissue was used immediately for this study. The stomachs were rinsed with saline solution to replace the stomachs content. Before the measurement the spherical matrix and the fresh stomach was immersed in SGF (pH 1.2) for 15 minutes. The arm of tensiometer was lowered until the spherical matrix came in proper contact to the rabbit tissue and was kept as such for 15 minutes. Afterwards, the knob was move upward direction until spherical matrix completely detached from the tissue. During the test the stomachs was wetted by dropping of SGF. The force of mucoadhesion was determined in dyne/cm^2.

Morphology and structure characterization of matrices

The morphological of spherical matrix was examined by using SEM (Dekstop SEM Phenom Pro X) and the structure of matrix was characterized using TEM (Jeol JEM-1400).

Results nd Discussion

Preparation of large spherical matrices containing RH

In this work, the spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch containing RH were prepared which containing 168 mg RH per a spherical matrix, a usual dose of RH. The color of spherical matrices formed was yellow and the diameter was 8.8 mm. It was observed that the matrix of Alg-Ch was more rigid than the Alg or Ch matrices due to the ionic interaction between Alg and Ch. Ch matrix was fragile. Alg matrix was moist due to hygroscopic property of sodium Alg. The Ca Alg-Ch was the most rigid. The photograph of large spherical matix containing 168 mg RH is showed in Figure 1 and the spesifications of the matrices is listed in Table 2.

Table 2. Specifications of spherical matrices containing RH

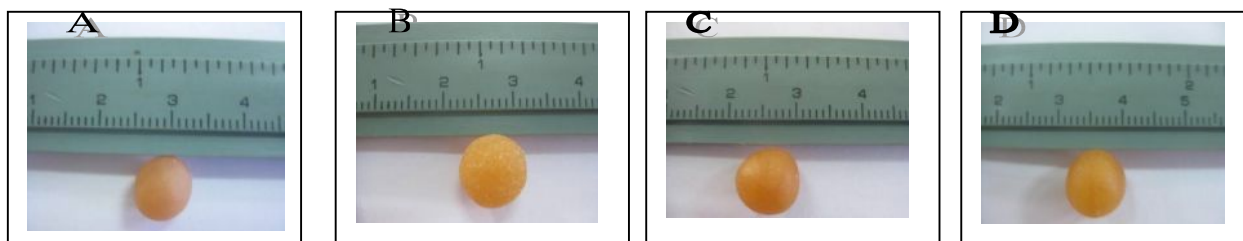| Formula	Name of spherical matrices	Weight (mg)	Diameter (mm)
F1	Alginate	371.3	8.80
F2	Chitosan	373.2	8.85
F3	Alginate-Chitosan	382.7	8.87
F4	Ca alginate-Chitosan	383.7	8.82

Figure 1. Photograph of spherical matrices containing RH
A (Alg matrix), B (Ch matrix), C (Alg-Ch matrix, and D (Ca Alg-Ch matrix)

Swelling of the spherical matrices

The comparison of swelling properties of spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch

As showed in Figure 2 and 3 the spherical Alg matrices only slightly swelled in SGF. As mentioned previously that sodium Alg was used to prepare Alg matrices, where in the medium of SGF the sodium Alg was immediately changed to alginic acid. Alginic acid is insoluble in SGF. The monomer of alginic acid is mannuronic acid and guluronic acid; the pka of mannuronic acid is 3.38 and the pka of guluronic acid is 3.65.[16] Therefore, alginic in SGF was mostly in unionized form. Therefore, the electrostatic repulsion between the carboxylate groups of alginic acid only slightly occurred, so that the matrices only slightly swelled in the SGF. On the other hand, Ch spherical matrices were eroded and dissolved in SGF due to Ch was soluble in acid solution. Ch containing amine ($-NH_2$), which to be protonated in the medium of pH 1.2 to form NH_3^+.

It was found that the spherical matrices that prepared from combination of Alg and Ch resulting the matrices that had different property with Alg and Ch matrices itself. The combination of Alg and Ch suppressed the erosion of Ch matrices. The Alg-Ch matrices exhibited the greatest degree of swelling (Figure 2 and 3). Alg and Ch can interact through the carboxylate groups of Alg and amine groups of Ch to form Alg-Ch complex. The swelling Alg-Ch matrices was thought to be due to the electrostatic repulsion between the positive charge of protonated amin groups, and the osmosis becaused of the high osmotic pressure of the matrix as a result of the protonization of amine groups of Ch in acid medium. The degree of swelling was lower in Ca Alg-Ch matrices than that of in Alg-Ch matrices. Alg and calcium ion formed calcium Alg, in this case, the chelation of guluronate residues to calcium ions formed the gel of calsium Alg.[2] Studied on the release of calcium ions from hard Alg capsules shell, it is found that 45.5% of calcium content of the capsules shell is difficult to release in SGF, but 52.5% is easily released.[17] This results correlate with the content of guluronate and mannuronate in Alg. The calcium guluronate was not prone to swelling and erosion of the matrices. The longer of curing time (immersion in the calsium chloride solution) of Alg-Ch matrices caused the decrease of the swelling degree of ALg-Ch matrices (the data was not shown in this paper).   .

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The swelling property of Alg-Ch matrices is suitable for the preparation gastroretentive formulation of RH. The swelling of matrices result in the increase of spherical matrices diameter, thereby it will be prevent the exit of matrices from stomach through the pylorus and consequently the matrix will be retained in stomach for longer period of time.

Erosion of Ch spherical matrices

The property of Ch matrices were different with Alg matrices, Alg-Ch matrices, and Ca Alg-Ch matrices. Spherical Ch matrices eroded and then dissolved in the SGF, as showed in Figure 2. The chitosan is poorly soluble in water. In acidic medium, protonation of the amine groups improves solubility. Thereby, the chitosan matrices soluble and caused the erosion of matrices.
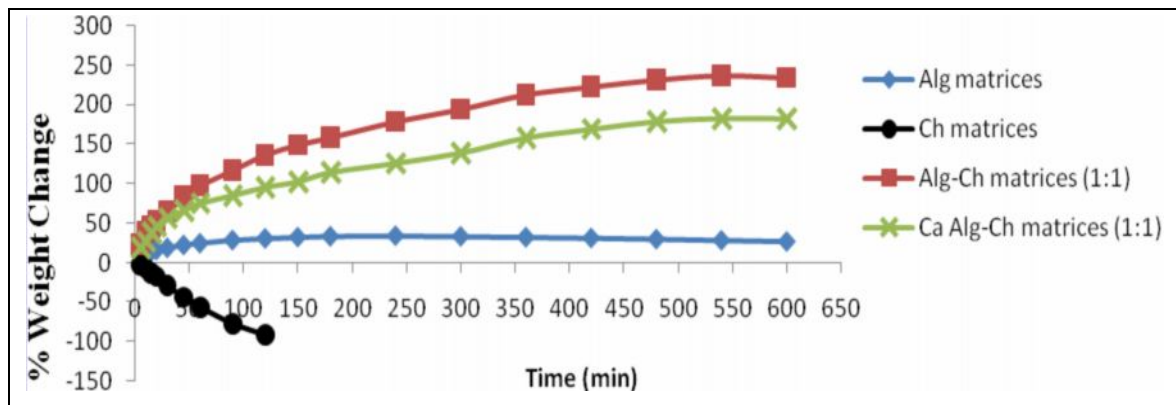

Figure 2. The comparison of swelling of spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch in the SGF at 37ºC showed as percent weight change versus time (n=3).
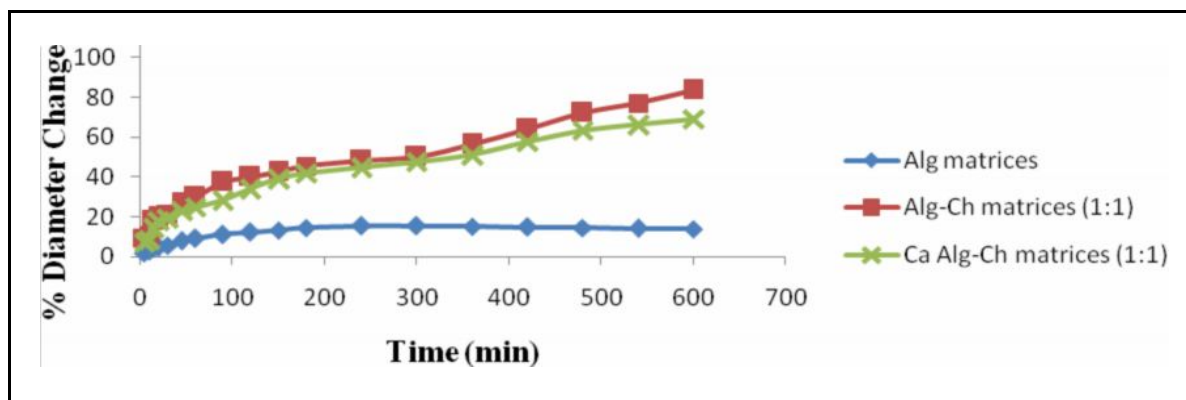

Figure 3. The comparison of swelling of spherical matrix of Alg, Ch, Alg-Ch, and Ca Alg-Ch in the SGF at 37ºC showed as percent weight change versus time (n=3).

The comparison of mucoadhesive force of the spherical matrices of Alg, Ch, Alg-Ch and Ca Alg-Ch

The force of mucoadhesive of the spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch in rat stomachs is showed in Table 3. The mucoadhesive force of spherical matrices of Alg-Ch > Ca Alg-Ch > Alg > Ch. The mucus lining of the stomach is rich in mucin, which contain an oligosaccharide chain with terminal sialic acid. Polyanions, especially polymer bearing carboxylic groups and high charge density, serve as powerful "ligands" for mucin and are called mucoadhesive polymers, for example Alg.[18] The formation of hydrogen-bonds among the fungtional groups of the polymer mucosa layer also plays an important role. In general, the stronger the hydrogen bond, the stronger the adhesion. The functional groups responsible for such kind of interaction are carboxyl, hydroxyl, and amine groups. Alg contains carboxyl and hydroxyl groups.

The mucoadhesive force of Ch matrices was found lower than that of the mucoadhesive force of Alg matrices. Alg matrices formed alginic acid gel in SGF, but Ch was soluble in gastric acid, thereby it mucoadhesive force was lower. The mucoadhesive property of Ch is expected to be dominated by the electrostatic attraction between the positive charged of Ch and negative charged of mucin (the negative charged of mucin is due to the ionization of sialic acid).[19] The mucoadhesive force of Alg-Ch matrices was higher than

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the mucoadhesive of Alg or Ch matrices. The mucoadhesive of Alg-Ch matrices was about the total of the mucoadhesive force of Alg matrices and Ch matrices. It means that on the Alg-Ch matrices the polymers   Alg and Ch both adhered to the mucosa layer of stomachs. The mucoadhesive force of Ca Alg-Ch was lower than that of Alg-Ch matrices. It was due to the binding of Alg to calcium ions. Bioadhesion offers several advantages such as longer gastrointestinal residence time and improves drug absorption and bioavailability.

Table 3. The mucoadhesive force of the spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch (n=3)

Formula	Name of spherical matrices	Mucoadhesive force (dyne/cm^2)
F-1	Alg	315.1 ± 7.2
F-2	Ch	187.2 ± 4.3
F-3	Alg-Ch (1:1)	512.7 ± 8.4
F-4	Ca Alg-Ch (1:1)	394.2 ± 9.0

Drug Release

The comparison of RH release from spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch

The comparison of RH release from Alg, Ch, Alg-Ch, and Ca Alg-Ch spherical matrices in SGF is showed in Figure 5. The release of RH was the most sustained from spherical matrices of Alg-Ch, followed from Ca Alg-Ch and then from Alg matrices. Ch matrices did not sustain the RH release, the drug release was fast. This result was due to Ch was soluble in hydrochloric acid solution so that the matrices eroded and dissolved in the medium, as showed in Figure 2.

The release of RH from Alg matrices was slower that that from Ch matrices. This result was due to sodium Alg was changed to alginic acid in the medium of SGF. Alginic acid was insoluble and retarded the drug release. The release of RH from the Alg-Ch matrices was slower than that from Alg and Ch matrices. The release of RH from Alg-Ch matrices was sustained for about 10 hours. This result was due to the formation of a polyelectrolyte complex as a result of the electrostatic interaction between carboxylate groups of Alg and amine groups of Ch. As a result of interactions it might caused the change the matrices properties like the increased of viscosity of the matrices, thereby the RH release was more sustained.

The relase of RH from Ca Alg-Ch was faster than that from ALg-Ch matrices, but the difference was not significant statistically ($p > 0.05$) which calculated based on the difference of the area of under curve (AUC) of Figure 5. As mentioned above, in the preparation of Ca Alg-Ch, the Alg-Ch matrices were immersed in the calcium chloride solution to form the Ca Alg-Ch matrices. The release of RH from Ca Alg-Ch matrices was faster than that of from Alg-Ch matrices might be due to the decrease of the viscosity of the matrices.
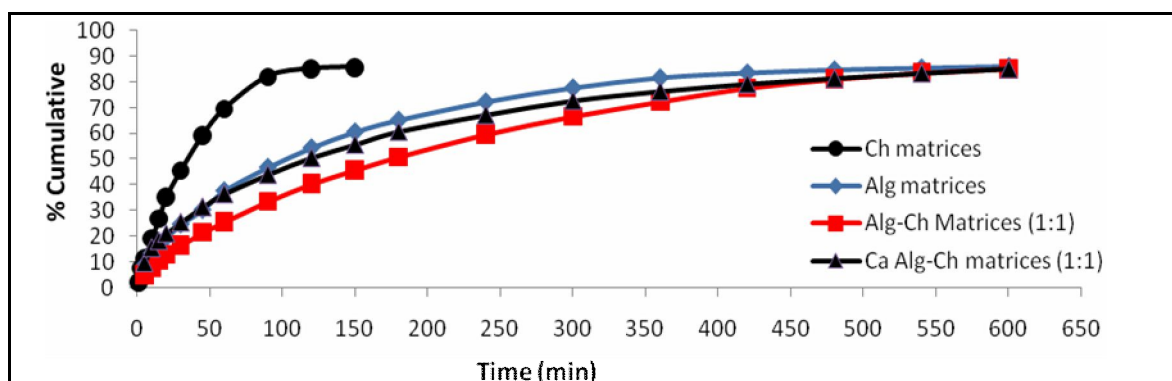

Figure 5. The comparison of RH release from spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch in SGF at 37°C (n=3).

Drug release kinetics

The drug release data were analyzed for determination of the kinetics and mechanism of drug release. For Higuchi model, the data were treated with Equation (1). It was found that the release of RH from the

matrices to the SGF was dependent on square root of time. When the percent cumulative of drug was plotted versus the square root of time, a linear relationship was observed with the regression coefficient close to 1 (Figure 6 and Table 3). The most approach to 1 of the regression coefficient was the regression coefficient of the release of drug from Alg-Ch matrices (R^2=0.9942), and followed by Ca Alg-Ch matrices (R^2=0.9726), Ch matrices (R^2=0.9638), and Alg matrices (R^2=0,9470).

In controlled or sustained release formulations, diffusion, swelling, and erosion are the three most important rate controlling mechanism. The drug release from the polymeric system is mostly by diffusion and is best described by Fickian diffusion. But, in the case of formulations containing swelling polymers, other processes in addition to diffusion play an important role in exploring the drug release mechanism. These processes include relaxation of polymer chains, imbibitions of water causing the polymers to swell.[20] Therefore, the release data were further treated by Eq. (2) given by Ritger and Peppas, or also called the Power low.[15] The release mechanism of RH from the matrices was characterized by determination of n value. For the spheres when n takes the value 0.43 indicates Fickian diffusion and for the value 0.43 < n < 0.85 indicates the anomalous transport drug release. Value of n 0.85 indicates the Case II transport.[20] The Korsmeyer-Peppas plot of the release of RH from the matrices is showed in Figure 7. To determination of exponent n the portion of the release curve the M_t/M_∞ < 0.6 was used. The n values obtained are listed in Table 3. The n value was obtained from the slope of the plot of percent cumulative versus log of time (Figure 7). The value of n with regression coefficients of all formulations is shown in Table 3. For all formulations the value of n was found in ranged of 0.5009 – 0.6737 indicating anomalous transport. The n value of Ch matrices was 0.6737, the highest among the other formulations was due to the erosion property of Ch matrices in the SGF as shown in Figure 2. The value of n of spherical Alg matrices was 0.5939 was due to Alg slightly swelled in SGF. The n value of spherical Alg-Ch was 0.6571, it was higher than n value of Alg matrices due to the swelling property of Alg-Ch. The comparison of the kinetic parameters release of RH from the different matrices based on zero order, first order, Higuchi model is also listed in Table 3.
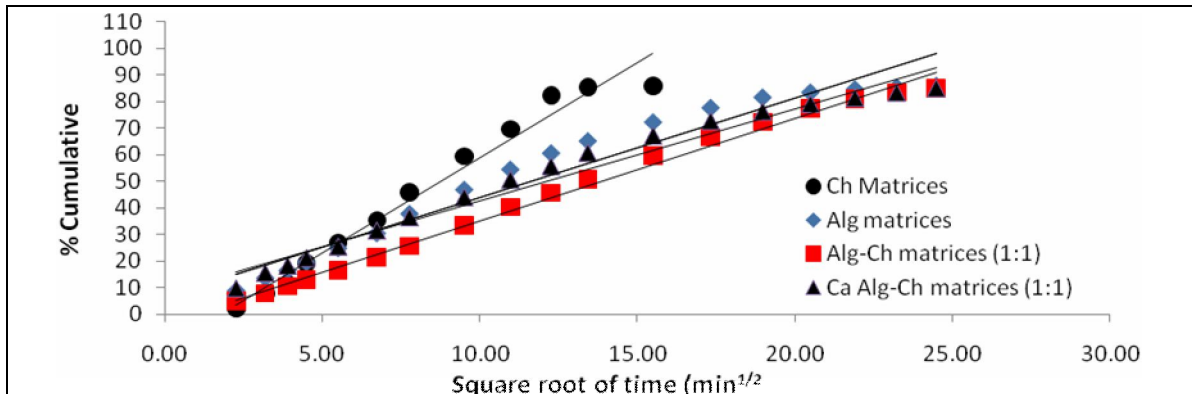

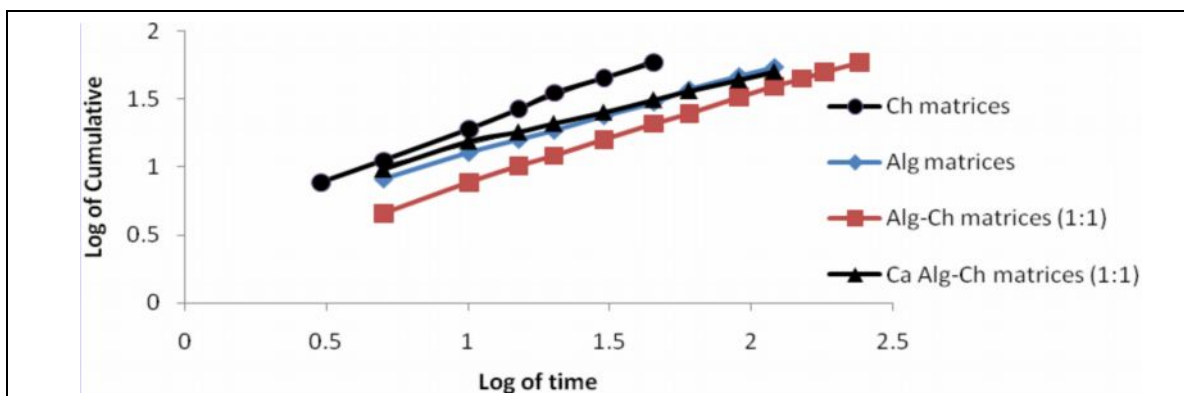**Figure 6. Higuchi plot of RH release from spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch in SGF at 37°C.**

Figure 7. Korsmeyer-Peppas plot of RH from spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch in SGF at 37°C.

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Table 3. The comparison of the kinetic parameters release of RH from the different matrices based on zero order, first order, Higuchi model, and Krosmayer-Peppas model plots.

Formula	Name of spherical matrices	Zero order	First order	Higuchi model	Korsmeyer Peppas model	
		R^2	R^2	R^2	R^2	n
F-1	Alg	0.8157	0.6406	0.9470	**0.9781**	0.5939
F-2	Ch	0.8450	0.5481	0.9638	**0.9633**	0.6737
F-3	Alg-Ch (1:1)	0.9218	0.7071	0.9932	**0.9942**	0.6571
F-4	Ca Alg-Ch (1:1)	0.8620	0.6830	0.9726	**0.9889**	0.5009

Morphology and structure characterization of spherical matrixes

Figure 8 shows the scanning electron microscopy (SEM) image of spherical matrix of ALg, Ch, Alg-Ch, and Ca Alg-Ch without RH. The surface of all spherical matrices were not so smooth. The surface of Alg matrix was smoother than that of Ch matrix. Alg spherical matrix was prepared using sodium Alg. Sodium Alg is water soluble and starch mucillage containing 5 % water, thereby the smooth surface was obtained. On the other hand, Ch was insoluble in water caused the surface of Ch matrix was not smooth. The surface of Alg-Ch was smoother than the surface of Ch matrix. Ca Alg-Ch matrix was also smoother than that of Ch matrix.
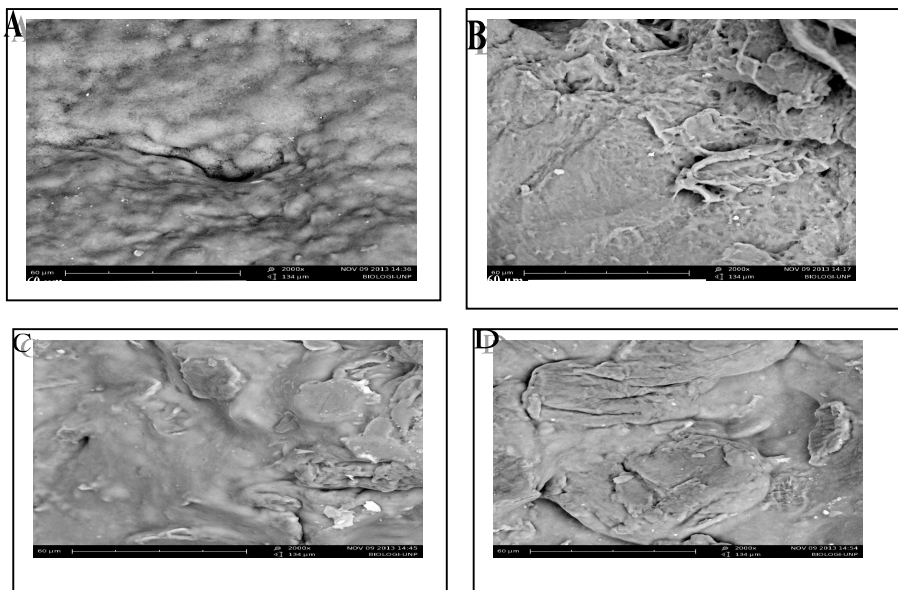

Figure 8. Scanning electron microscopy (SEM) image of spherical matrix: (A) Alg (2000x), (B) Ch (2000x), (C) Alg-Ch (1:1) (2000x), and (D) Ca Alg-Ch (1:1) (2000x).

Then, the transmission electron microscopy (TEM) image of the sperical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch without RH is showed in Figure 9. The particle of Alg matrix was seen to be spherical, but the particles of Ch, Alg-Ch, and Ca Alg-Ch were irregular. The particle size of Alg-Ch matrix and Ca Alg-Ch matrix were smaller that of particle size of Alg matrix and Ch matrix.

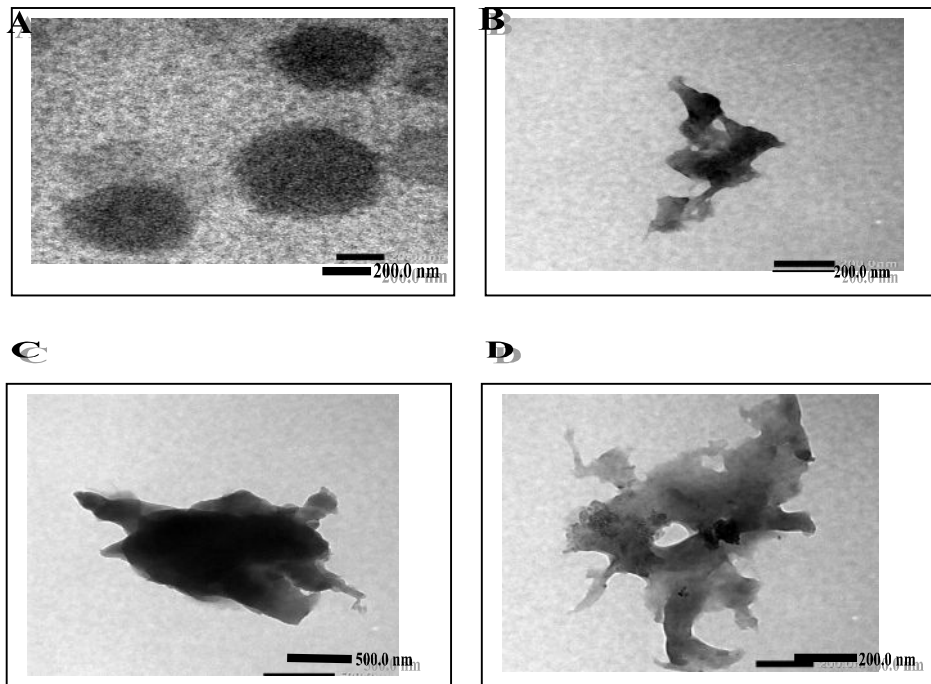**Anayanti Arianto** *et al* /Int.J. PharmTech Res.2014,6(7),pp 2054-2063.

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Figure 9. Transmission electron microscopy (TEM) image of the sperical matrix: (A) Alg matrix, (B) Ch matrix, (C) Alg-Ch matrix, and (D) Ca Alg-Ch matrix.

Conclusions

This study shows that the properties of spherical matrices of Alg, Ch, Alg-Ch, and Ca Alg-Ch are different in degree of swelling, mucoadhesive force, and drug release rate in medium of SGF. For the purpose of the preparation of gastroretentive drug delivery systems the spherical matrices Alg-Ch is suspected to be more suitable than that of Ca Alg-Ch matrices. However, more research will be necessary to obtain the suitable ratio of Alg to Ch for the preparation of an effective gastroretentive drug delivery system of RH.

Acknowledgements

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