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Original Article

HESPERIDIN HYDROGEL FORMULATION USING PECTIN-CHITOSAN POLYMER COMBINATION

ANDHI FAHRURROJI[1], DEA THENDRIANI[2], HAFRIZAL RIZA[3]

[1]Departement of Pharmaceutical Technology, Pharmacy Subject, Faculty of Medicine, Tanjungpura University, [2]Undergraduate Program of Pharmacy Department Tanjungpura University, [3]Departement of Chemical Pharmacy, Pharmacy Subject, Faculty of Medicine, Tanjungpura University
Email: roji_apt@pharm.untan.ac.id

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ABSTRACT

Objective: Hesperidin, a flavonoid glycosides that have been proven to have therapeutic activity to some disease, one of them is colon disease; in addition of its efficacy,low solubility(<100 mg/l) makes hesperidin slightly absorbed,hence it needs a delivery system which could deliver hesperidin to its therapeutic target. This research aims to obtain an optimum formula for pectin polymer combination which can regulate *in vitro* hesperidin release.

Methods: Determination of optimum hydrogel formula uses Design Expert 7.0.0 with factorial method design, resulting in pectin-chitosan concentration formula plan-comparison, which are (P3%: C1%), (P3%: C2%), (P5%: C1%), (P5%: C2%) respectively. Hydrogel was obtained from a variety of formulas,then evaluation of the entrapment efficiency test, swelling index, *in vitro* drug release test, mucoadhesive strength were conducted.

Results: Optimum formula with: pectin: chitosan concentration comparison (5: %: 1%) have an entrapment efficiency of 96.658%; k (/hour) swelling index at pH 5.0, 6.8, and 7.4, was 34.917, 15.766, and 8.146 respectively; drug release at pH 5.0, 6.8, and a medium contained 2% rat *caecum* was 0.461, 20.116, and the mucoadhesive strength was 0.184 N/cm^2. Based on the test result using independent t-test sample, actual and prediction value from every test parameter produced by the optimum formula was not significantly different with p-value>0,05.

Conclusion: Combination of pectin-chitosan polymer in hydrogel mucoadhesive regulates hesperidin shesperidin *in vitro* release, with highest drug release in medium containing 2% rats *caecum* which releases 56% of active substance. Hesperidin hydrogel release mechanism follows Higuchi kinetics. The optimum hesperidin hydrogel formula is the formula with 5% of pectin and 1% of chitosan. Based on experimental data value which uses simplex lattice design, optimum hesperidin hydrogel formula has insignificant difference between observed and predicted value (p value>0.05).

Keywords: Hesperidin, Hydrogel, Chitosan, Colon, Pectin

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INTRODUCTION

Controlled drug delivery system is one of a method to control drug release in order to increase drug effectiveness; and commonly applied to the active substance with low solubility, one of them is *hesperidin*. it is a flavonoid *glycoside* which is isolated from citrus plant [1].

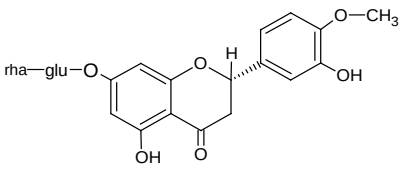

Fig. 1: Hesperidin structure [1]

Hesperidin has been proven to have anti-inflammation, antimicrobial, antioxidant, anti-hemorrhoid, and anticancer activity so it could be used in therapy of some colon related diseases such as hemorrhoid, chronic vein insufficiency, colon cancer, and ulcerative colitis [1-6], however, hesperidin has low solubility on digestive tract (<100 mg/l) as well as low bioavailability (<25%) [1, 7]. Therefore, the correct delivery system is required to increase the bioavailability and therapy effectiveness from *hesperidin*, one of them is by formulating it into a hydrogel.

Pectin is one of the hydrogel constituent polymer commonly used to deliver drugs to the *colon*. Previous research showed that ibuprofen release from hydrogel made from pectin decreases with the use of controlled pH dissolution medium, which indicates that pH controlled drug release has occurred [8]. Other research showed that hydrogel beads of pectin-zein protects indomethacin from upper gastrointestinal tract conditions and its release was controlled by pectin degradation with pectinase; however, pectin is highly soluble in water, which leads to the development of another polymer with low solubility in water, to produce a strong and effective gel for carrying drug which is entrapped in gastric fluid and small intestine [9-10]. One of the polymers that can be used to overcome this problem is chitosan, which its carboxylic group binds ionically with the chitosan amine group [11].

The study about the role of chitosan as a coating to modify drug release has been conducted to amide pectin hydrogel [12]. Other research which uses pectin polymer in combination with alginate could float on water, 0.1N of HCl, and phosphate buffer, which concludes that increase in polymer concentration and crosslink forming time could increase polymerization, but higher pectin concentration lowers drug release up to 12 h, which is caused by the presence of pectinase enzyme [13]. Both drug solubility and drug insolubility in water inside a simulated intestinal condition was significantly reduced compared to pectin hydrogel without chitosan coating. Another research stated that hydrogel, which was formulated from the pectin-chitosan combination is proven to reduce vancomycin release in acidic condition, and increases drug release in a simulatedcolon condition [14]. Based on this research, the author conducts several experiments using hesperidin as an active substance which has low solubility in the digestive tract, but possess several pharmacological benefits which would be formulated into a hydrogel by using pectin-chitosan polymer, in order to maximize its potency in digestive tracts, especially colon.

MATERIALS AND METHODS

Materials

Materials used in this research including hesperidin obtained from Sigma-Aldrich, Batch Number: SLBJ1579V, chitosan (Biotech Surindo, Batch Number: 10A0215. F. HM. CHC), pectin, acetic acid (Merck), sodium acetate (Merck), sodium hydroxide, sodium dihydrogen phosphate, disodium hydrogen phosphate, zinc acetate, and distilled water.

Formulation of *hesperidin* hydrogel

Pectin solution was prepared by dissolving pectin in CO_2-free distilled water using a magnetic stirrer (300 rpm for 15 min) at room temperature (25 °C). Chitosan solution was prepared by mixing chitosan with 2% acetic acid (b/v), then stirred at 300 rpm until completely dissolved. A Zinc acetate solution was prepared by dissolving *zinc acetate* into chitosan solution and stirred with a magnetic stirrer until homogenous. Hydrogel is made through initial Hesperidin dispersion in pectin solution while stirring. Hesperidin-pectin solution, is slowly dripped into chitosan-zinc acetate mixture using 10 ml hypodermic syringe and stirred with a magnetic stirrer (Schott model D-55122 Mainz) at 300 rpm until hydrogel beads formed. The hydrogel is washed with CO_2-free distilled water and dried at room temperature for 48 h[15]. Dried hydrogel undergoes evaluation, including entrapment efficiency test, power test development, *in vitro* drug release test, and mucoadhesive strength test. Hydrogel formula design can be seen in table 1.

Table 1: Hesperidin hydrogel formula design

S. No.	Composition	F1	F2	F3	F4
1.	Hesperidin (mg)	50	50	50	50
2.	Pectin (%)	3	3	5	5
3.	Chitosan (%)	1	2	1	2
4.	*Zinc acetate* (%)	2	2	2	2

Pectin, chitosan, and *zinc acetate* solution used in every formula is 10 ml. The total volume on every formula is30 ml. Each of the formulation is made triplicate

Preparation of hesperidin standard curve

Hesperidin standard solution is made in the concentration of 100 ppm using acetate buffer of pH 5.0, pH 6.8 buffer, pH 7.4 buffer as the solvent (for drug test release), and in 0.3 M NaOH (for efficiency entrapment test) as presented in table 2. Afterwards, concentration series were made in 4, 10, 16, 22, 28 ppm for pH 5.0 acetate buffer and pH 6.8 phosphate buffer solvent, and concentration series of 8, 12, 16, 20, 24, 28 for pH 7.4 phosphate buffer solvent and concentration series of 12, 16, 22, 28, 34, 40 ppm for 0.2 M NaOH solvent. Solution series is analyzed using UV-Vis spectrophotometer at the maximum wavelength of hesperidin. Maximum wavelength is determined using UV-Vis spectrophotometer ranging from 200 to 400 nm [16]. The process of making standard solution concentration series is repeated for 6 times using the available standard solution. The best equation was used to calculate the drug level during *in vitro* drug release test [17].

The entrapment efficiency test

Dry hydrogels, which is equivalent to 50 mg of hesperidin is placed in 0.2 M NaOH solution and settled for 24 h. The solution is filtered using filter paper, and the filtration result is analyzed for hesperidin contents using UV-Vis spectrophotometer (Shimadzu type 2450®) at hesperidin maximum wavelength. The result showed the amount of hesperidin entrapped inside the hydrogel matrix. The entrapment efficiency is determined by equation (1) [18].

$$EE(\%) = \frac{\text{number of obtained drug}}{\text{total drug number}} \text{x } 100\% \dots\dots (1)$$

Swelling index

Swelling index (SI) is performed by preparing hydrogel from each formulation, in buffer solution of pH 5.0, pH 6.8, and pH 7.4. Each hydrogel is weighed at ±5.0mg, and placed in to the buffer solution. Sample buffer solution is removed at a set time interval and wet hydrogel mass was weighed. The Hydrogel swelling index is determined based on the equation below (2) [19-20].

$$SI\ (\%) = \left(\frac{Ws - Wd}{Wd}\right) \times 100\% \ \dots . (2)$$

For:

Ws: Swelling Hydrogel Weight

Wd: Dried Hydrogel Weight

SI: Swelling Index

In vitro drug release and determination of drug release mechanism

The *in vitro hesperidin* drug release from hydrogel uses USP apparatus 1 as a testing method. Dissolution medium is made using and a medium containing 2% of rat *caecum*. 900 ml of the medium is used for pH 5.0 buffer, and pH 6.8 buffer; 100 ml for medium containing 2% of rat *caecum*. *In vitro* drug release test was performed at 37±0,5 °C with stirring speed of 100 rpm. Drug release time in pH 5.0 buffer medium was observed for 4 h, pH 6.8 buffer for 5 h, and the medium contained 2% of rat *caecum* for 5 h. Sampling were done on min 15, 30, 45, 60; 90, 120, 180, 240 and 300, each sample is 3 ml in volume. The taken solution is immediately replaced by a certain amount of solution from the same medium at certain time interval. The absorbance of the sample is measured using UV-Vis spectrophotometer at a wavelength of 283,4 nm. In drug release test, drug release kinetic was set into zero, one, and Higuchi order, also into Korsmeyer-Peppas equation to observe the drug release mechanism. Korsmeyer-Peppas equation is shown below. (3)[21].

$$\text{Log } \%R = \log K + n \log t. \ (3)$$

For:

R: released drug amounts in every t

K: constant release rate

n: time power (showing the drug release mechanism)

Mucoadhesive strength

The hydrogel mucoadhesive strength assay is based on physical equilibrium. Equipment used in this procedure, including equal-arm balance in which a beaker containing pH 7 buffer solution is placed under the left balance disc. Fresh cow colon mucosa is used as a membrane and is attached to the mass using thread. The weight is then placed in a large beaker containing pH 7.4 buffer solution until the solution reached the upper surface of the mucosa. Hydrogel is attached at the bottom of the left balance disc and then the disc is slowly lowered until it made a contact with cow colon mucosa. A plastic container was placed in the right balance disc and water is added using a burette with a drop rate of 100 drops/minutes. Water addition is stopped when hydrogel separates from the cow colon mucosa. The mass of water that is required to release hydrogel from cow colon mucosa was calculated as the mucoadhesive strength in grams. Equation (4) and (5) is used to calculate the hydrogen mucoadhesive strength [22].

$$\text{Adhesion Ability (N)} = \frac{\text{Mucoadhesive Strength (g)}}{1000} \times 000a \text{.....(4)}$$

$$\text{Mucoadhesive Strength(N/cm2)} = \frac{\text{Adhesion Ability (N)}}{\text{surface area (cm2)}} \text{.....(5)}$$

RESULTS AND DISCUSSION

Measurements using UV-Vis spectrophotometer produced a maximum wavelength of 283,4 nm for hesperidin. Maximum wavelength data was used to create a standard curve in various mediums and concentration to obtain an equation,which will be used to measure the number of drug releases per unit after a certain time in the drug release test.

Based on the obtained results, absorbance in pH 5.0, pH 6.8, pH 7.4, and 0.2 MNaOH medium shows good linearity with r (linearity) value ≥0,99, where r value for equation 8, 9, 10, and 11 were 0.9999, 0.9997, 0.9996, and 0.9990 respectively. The result of accuracy measurement shows the recovery value within 98.64–100.8% for pH 5.0 and 98.11–104.8% for pH 6.8, within 98.75-101.47% for pH 7.4 and 96.53-105.43% for 0.2 M NaOH medium. This result proves that every concentration is in the range of 95%-105%. Precision measurement result shows RSD value which in the range of 0.481-1.937% for pH 5.0 and 0.684–1.788% for pH 6.8, also within 0.481–1.937% for pH 7.4 and 0.551-1.290% for 0.2 M NaOH medium. These results proved that the methods used in this research are valid because the precision value did not exceed 2% [23].

Entrapment efficiency (EE)

Results showed that an increase in pectin concentration resulted in increased entrapment efficiency (EE), where formula 3 and 4 were known to have higher EE, while formula 1 has the lowest EE. Pectin has a rapid gel-forming ability and high viscosity that led to stronger hydrogel matrix and produced optimum entrapment [24-27]. Comparison of the formula is shown in fig. 2.

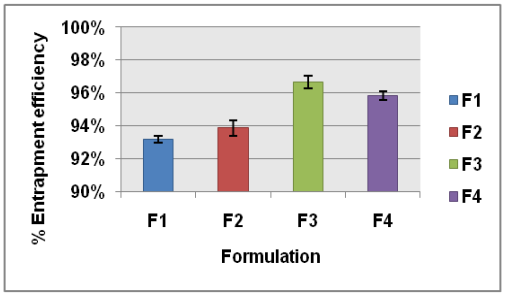

Fig. 2: Result of entrapment efficiency in 0.2 M NaOH. (n= 3; mean value±SD)

The results shows that the analyzed data were distributed normally. This means that the standard deviation of the actual response value which separates the EE with value prediction is insignificant. EE response data showed a normal conformity model against assumptions of ANOVA. The lowest EE was 92.95% and the highest EE was 96.99%. Factorial equations for the response shown in equation 6 EE.

$$EP = 90{,}17+0{,}75A-0{,}23B+0{,}31AB. \ldots\ldots (6)$$

NB: A=Variation of Pectin

B= variation of chitosan

Based on equation 12, the coefficients of A and AB is positive, which means that EE will rise along with increasing concentration of pectin and interaction between the two polymers pectin-Chitosan.

Swelling index

The swelling index test aims to determine the swelling time associated with the hydrogel ability to regulate drug release within its polymer matrix. The test result shows the characteristic of hydrogel swell in pH 5.0, 6.8, and 7.4 buffer medium, the duration of swelling is positively related to hydrogel swelling ability, longer swelling time will increase the swelling percentage.

Hesperidin hydrogel swelling index

The test result showed that in pH 5.0 hydrogel swells slowly compared to pH 6.8 and 7.4 medium (fig. 3), it can be seen in fig. 3 that formula 2 has the highest swelling percentage in pH 5.0 medium, where chitosan concentration that is used in both formulas (formula 2 and formula 4) were higher (2%). It is shown that in pH 5.0 chitosan concentration affected swelling percentages. Chitosan with pKa ranging from 6.3–6.5 is easily soluble at low pH, where generally at low pH protonation of primer amine group (NH_3^+) in chitosan will occur, while pectin is unionized, so repelling force takes place inside the chitosan structure which causes significant swelling in formula with higher chitosan concentration (formula 2 and 4)[2].

Hydrogel swelling percentage at pH 6.8 is higher than swelling percentage at pH 5.9 acetate buffer for every formula, with formula 3 having the highest percentage of swelling, while formula 2 has the lowest swelling percentage (fig. 3). Pectin, with its characteristic as a weak acid (pKa 3–4) with the carboxylic group (-COO-) tends to ionize at higher pH. This causes a repelling force among the carboxylate group in pectin leading to the increase of hydrogel swelling power [11]. The highest swelling percentage was achieved in pH 6.8 medium after 5 h, at the 6th hour, swelling percentage begin to decrease until 8th hour. Fig. 3 also shows a higher percentage of swelling, which increases in formula with higher pectin concentration, where the highest swelling percentage was achieved by formula 3 and the lowest was formula 2.

The higher percentage of swelling than those at pH 5.0 and 6.8 was achieved by the sample in pH 7.4 medium, increase in swelling percentage along with swelling time is observed, where the maximum value is recorded at the 5 h, shown in fig. 5. Fig. 5 shows the highest swelling percentage in the formula with higher pectin concentration, which is formula 3 and 4, and the lowest from formula 2. The degraded hydrogel swelling percentage (after 6 hours) in pH 6.8 and 7.4 medium is related to the erosion in the hydrogel matrix. Fig. 3 shows that swelling percentage on F3 was higher in pH 6.8 and 7.4 phosphate buffer compared to other formulas. Swelling percentage reached its maximum on the 5th hour of the test.

This could be caused by erosion that occurred when significant swelling of hydrogel matrix loosens the matrix tissue, and after prolonged time pores forms on the matrix surface, causes in more solution entering the hydrogel. When maximum swelling occurs, there is the possibility of the drug leaking out while the matrix erodes slowly. Therefore, at the 6th, 7th, and 8th hour the swelling percentage in pH 6.8 and 7.4 medium decreases [11, 28]. The result shows that the distribution of pH 5.0 SI data that is analyzed is distributed normally. This means that the standard deviation of the actual response value that separates SI pH 5.0 with value prediction is insignificant. pH 5.0 SI response data distributed normally, which shows the conformity of the model against assumptions of ANOVA on the response of pH 5.0 SI. pH 5.0 model lowest SI was 34.804 and the highest was 50.011. Factorial equations for pH 5.0 SI response is shown in equation 7.

$$IP\ pH5{,}0=23{,}91-0{,}32A+14{,}74B-0{,}42AB. \ldots\ldots\ldots (7)$$

NB: A= Variation of Pectin

B= variation chitosan

Based on equations 13,coefficient A and AB is negative, which means that the pH 5.0 SI will decreases along with the increased concentration of the polymer, pectin, and the increasing interaction between the Chitosan and pectin polymer. Coefficient B has positive value, which means that pH 5.0 SI will rise by increasing the concentration of Chitosan. At pH 5.0, protonation of primary amine cluster (-NH_3^+) would occur on Chitosan, while pectin will not be ionized, so that the occurrence in the framework of Chitosan repelling led to significant development in a formula with high concentration of Chitosan (formula 2 and 4) [29].

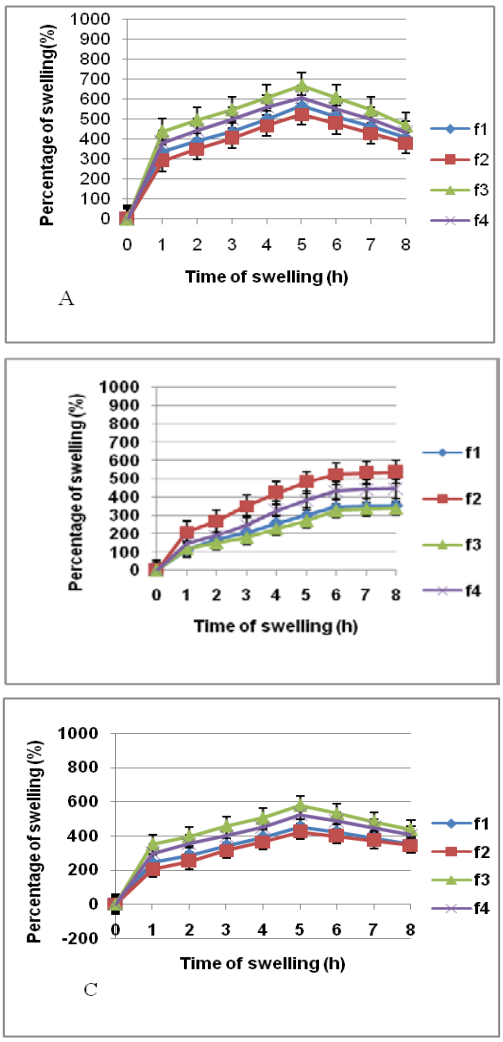

Fig. 3. Result of swelling Index in different buffer pH. Note: A: Acetic buffer (pH 5.0); B: Phosphate buffer (pH 6.8); C: **Phosphate buffer (pH 7.4). (n= 3; mean Value±SD)**

In vitro drug release

In vitro drug release was performed in three conditions, pH 5.0 represents *gastric* condition after a meal, pH 6.8 represents gut condition after a meal, and medium containing *caecum* represents *colon* condition.

Drug release in acetic buffer pH 5.0, phosphate buffer pH 6.8, and medium contained 2% rat

The test in pH 5.0 medium indicates the drug release was less than 2% in every formula during a4-hour testing, even within the first hour, only≤ 1% drug release for every formula was attained. The highest release occurs in formula 2 with the release rate reaching 1.814% in a 4-hour test.

Lower drug release at low pH was consistent with studies conducted by previous researchers which concludes that pectin-chitosan combination could decrease drug release in acidic conditions [10-12]. Drug release in pH 5.0 medium is shown in fig. 4.

Testing in medium pH 6.8 shown different release rates. The highest release was achieved by formula 3 with 20.11% of release, while the lowest release is formula 1, with 8.37% for 5 h of testing.

The drug release percentage was quite high in pH 6.8 medium, possibly because, besides swelling, after 6 h the hydrogel also eroded [11, 28], as indicated by the previous test result which is a decrease in swelling percentage after entering the 6th hour. Drug release at pH 6.8 medium is shown in fig. 4.

Testing on medium containing 2% of rat *caecum* shows increased drug release was compared to two other mediums, where the percentage of drug release is between 27–56% in 5 h testing. Based on fig. 4, it is known that the highest percentage of drug release was achieved by formula 3 and 4 which were 53% and 56% respectively after 5 h testing. Formula 3 and 4 were the formula with largest pectin concentration.

The increased percentage of drug release in the medium containing rat *caecum* in comparison to two other mediums were consistent with the previous study which has been done by previous researchers [30-31]. The study showed that *pectinolytic* or *pectinase* enzymes in medium containing rat *caecum* degrades pectin in the hydrogel matrix and broke the polymer chain, causing more pores to form on the matrix surface, making the hydrogel matrix more permeable for hesperidin [11, 22].

In fig 4, drug release in medium containing 2 % of rat caecum tends to be constant. This is because of hydration and pectin swelling produces viscous layer in gel layer, which results in slower drug release [32].

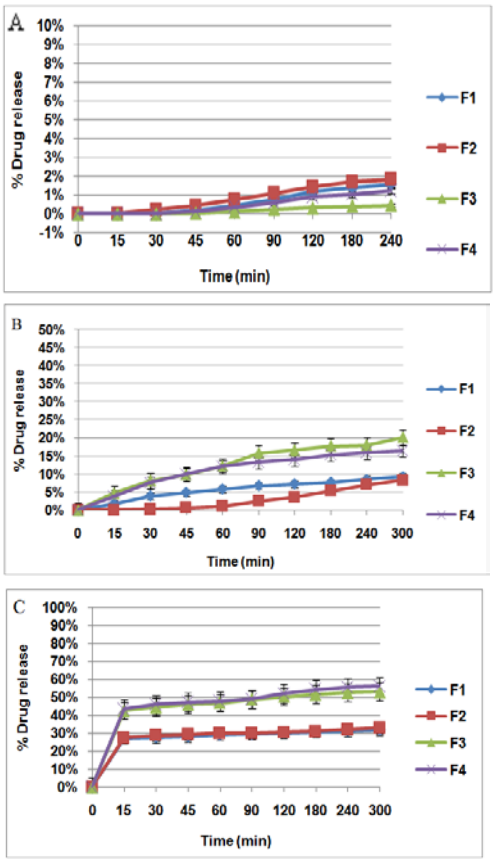

Fig. 4: Drug release on various pH. A: Drug release in acetic buffer (pH 5.0); B: Drug release in Phosphate buffer (pH 6.8); C: Drug Release in Medium Containing 2% of Rat *Caecum*.(n_a=3, mean value±SD)

The result of the drug release test is then used to observe the drug release kinetic profile against time in pH 5.0, pH 6.8 medium, and medium containing 2% of rat *caecum*. The result of drug release parameters can be seen in table 2.

Measurement result shows that *hesperidin* release from the hydrogel matrix in medium pH 5.0, pH 6.8 and medium containing 2% of rat caecum is controlled by Higuchi kinetic release. This is based on the value of r^2 in table 2 which shows that formula 1 to 4 releases the drug based on Higuchi kinetic in each medium. Higuchi kinetic describes that drug release is affected by drug diffusion through matrix pores. The drug release is shown at table 3.

Drug release exponent (n) in Korsmeyer-Peppas equation describes the drug release mechanism which happens to the preparation of test medium. Hesperidin hydrogel with pectin-chitosan polymer combination yield a n value around 1.30–1.60 in the pH 5.0 medium, the values were categorized as n>1 (transport super case 2), and it showed that drug release mechanism in the preparation is controlled by relaxation ability or matrix swelling. On the contrary, hesperidin hydrogel with pectin-chitosan polymer combination gave n value in the range of 0.43–1.43 in pH 6.8 medium, which describes the drug release mechanism in the preparation was a combined mechanism of *Fick diffusion* and transport super case 2.

Table 2: Hesperidin drug release parameter from hydrogel (n_a=3, mean value±SD)

Acetic buffer pH 5						
Formula	**Zero Order**		**First Order**		**Higuchi**	
	k(min^{-1})	**r^2**	**k(min^{-1})**	**r^2**	**k(min^{-1})**	**r^2**
F1	0.007±0.00	0.885±0.00	0.010±0.00	0.700±0.09	0.161±0.01	0.940±0.01
F2	0.008±0.00	0.881±0.03	0.009±0.00	0.709±0.06	0.167±0.02	0.949±0.02
F3	0.002±0.00	0.895±0.03	0.012±0.00	0.634±0.12	0.048±0.02	0.945±0.01
F4	0.005±0.00	0.890±0.01	0.011±0.00	0.653±0.10	0.121±0.00	0.944±0.01
Phosphate buffer pH 6.8						
Formula	**Zero Order**		**First Order**		**Higuchi**	
	k(min^{-1})	**r^2**	**k(min^{-1})**	**r^2**	**k(min^{-1})**	**r^2**
F1	0.021±0.00	0.768±0.06	0.004±0.00	0.583±0.04	0.494±0.05	0.876±0.03
F2	0.031±0.00	0.982±0.01	0.011±0.00	0.813±0.06	0.711±0.10	0.984±0.01
F3	0.046±0.00	0.771±0.02	0.004±0.00	0.637±0.02	1.064±0.04	0.889±0.01
F4	0.035±0.00	0.700±0.04	0.035±0.00	0.700±0.04	0.837±0.07	0.836±0.03
Medium contained 2% rat *caecum*						
Formula	**Zero Order**		**First Order**		**Higuchi**	
	k(min^{-1})	**r^2**	**k(min^{-1})**	**r^2**	**k(min^{-1})**	**r^2**
F1	0.015±0.00	0.892±0.01	0.001±0.00	0.881±0.01	0.337±0.04	0.961±0.00
F2	0.016±0.00	0.892±0.01	0.001±0.00	0.877±0.00	0.358±0.03	0.944±0.02
F3	0.035±0.00	0.847±0.00	0.001±0.00	0.828±0.00	0.789±0.00	0.946±0.00
F4	0.043±0.00	0.910±0.01	0.001±0.00	0.894±0.01	0.959±0.04	0.974±0.00

Abbreviations: n_a= amount of data, SD= deviation standard, k= drug release constant, r^2 = coefficient of determination

Thus, drug release occurs through dissolution medium in the hydrogel matrix, under influence of matrix swelling. The n value in the range of 0.0052–0.0087 were observed in the caecum medium, which describes drug release mechanism in the preparation is controlled by *Fick* diffusion; when the medium dissolution penetrated into the hydrogel matrix, the three dimension hydrogel network is relaxed, thus the hydrogel will swell until medium which enters the matrix could carry the drug out through the pores of the hydrogel matrix by diffusion [33-34].

Table 3: Drug dissolution profile based on Korsmeyer-Peppas equation

Acetic buffer (pH 5)			
Formulation	k(min^{-1})	r^2	N
F1	1.306±0.23	0.866±0.07	1.306±0.23
F2	1.030±0.17	0.910±0.04	1.031±0.17
F3	1.606±0.71	0.801±0.09	1.606±0.71
F4	1.516±0.71	0.824±0.09	1.516±0.70
Phosphate buffer (pH 6.8)			
Formulation	k(min^{-1})	r^2	N
F1	0.483±0.02	0.870±0.03	1.191±0.59
F2	1.439±0.24	0.970±0.02	1.439±0.24
F3	0.454±0.02	0.921±0.01	0.454±0.02
F4	0.435±0.05	0.857±0.01	0.435±0.05
Medium contained 2% rat *caecum*			
Formulation	k(min^{-1})	r^2	N
F1	0.053±0.00	0.964±0.00	0.052±0.00
F2	0.055±0.00	0.954±0.02	0.054±0.00
F3	0.078±0.00	0.988±0.00	0.077±0.00
F4	0.089±0.00	0.976±0.00	0.087±0.00

Abbreviations: n_a= amount of data, SD = standard deviation, k = drug release constants, r^2 = coefficient of determination, n = exponent of drug release, (n_a=3,meanvalue±SD)

Mucoadhesive strength

Mucoadhesive strength aims to determine the ability of hydrogel in sticking to the colon mucosa after swelling process. The result (fig. 5) shows that increased chitosan concentration led to the increased mucoadhesive strength of hydrogel, in which formula 2 and 4 with a higher level of chitosan concentrations (2%) had the highest-mucoadhesive strength.

Previous research which analyzes chitosan effect on rat colon mucosa showed that increased chitosan concentration will increase chitosan tendency to attach to mucosal tissue [34].

This ability to attachment occurs as a result of an electrostatic force between cationic chitosan with anionic mucous glycoprotein (sialic acid) and negative cell surface [35].

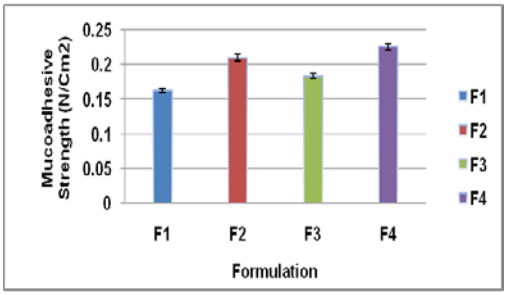

Fig. 5: Result of mucoadhesive strength test

The result shows that the distribution of the mucoadhesive strength data is distributed normally. This means that the standard deviation of the actual response value that separates mucoadhesive strength with predicted value is significant. Data of mucoadhesive strength data conforms against the assumption from ANOVA in response to mucoadhesive strength. The lowest mucoadhesive strength was 0.160 N/cm2 and the highest was 0.230 N/cm$^{2.}$ Factorial equations for mucoadhesive strength response is shown in equation 8.

Mucoadhesive Strength=-0.0774+0,01286A+0,0547B–(2.55985.10^{-3})AB. . . . (8)

NB: A= Variation of Pectin

B= variation chitosan

Based on equation 19, it can be seen that coefficient A, and B has positive value, which means that mucoadhesive strength will rises, by increasing the concentration of the polymer: Chitosan and pectin. Through equations, it can be noted that the value of coefficient B (Chitosan) is greater than coefficient A (pectin), this indicates that Chitosan has a greater influence in increasing the mucoadhesive strength, compared to pectin. Cationic chitosan, when interacting with mucous glycoprotein which is anionic, sustains the electrostatic force, thereby increasing the strength of the mucoadhesive [36]. The results showed the formula with the highest concentration of chitosan (formula 2 and 4) has the highest mucoadhesive strength.

Data analysis result

The response test result data was processed using Design Expert 7.0.0 trial program, with a simplex Lattice design. The program will predict the best combination from components which optimizes pectin and chitosan variations. The optimum formula that is suggested by the design was 5% pectin: 1% chitosan. Desirability values obtained for these predictions is of 0.785 which means optimum formula will yield a product with parameters or the most optimum response and liking was amounted to 78.5%.

The value of the desirability of approaching 1 indicates that the actual response value will have great possibilities for significant value not unlike the response prediction results. This value is strongly influenced by the complexity of the components, the range used in the component, the number of components and response, as well as targets to be achieved in obtaining optimum formula. The image of the curve desirability can be seen in fig. 6.

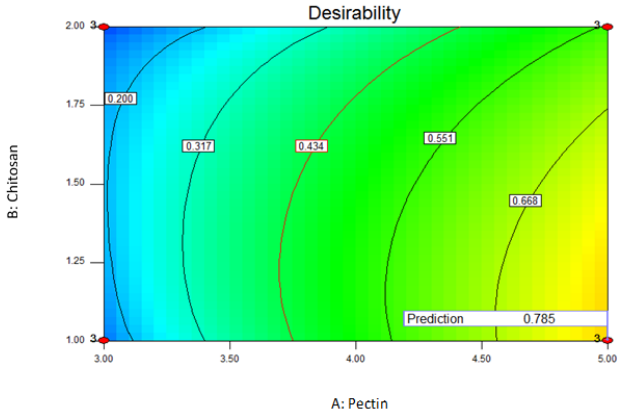

Fig. 6: Optimum hydrogel formula desirability curve

The curve above shows the tendency of desirability for the optimum formula in each comparison of pectin and chitosan. The lowest (0) and the highest value (1) were marked with the color blue to yellow-orange. Based on these curves,it can be observed that the formula with a comparison of 5% pectin: 1% Chitosantends to be inside the orange-coloured area.

The lower concentration of pectin will results in the lower desirability of the formula, in which a comparison of 4% pectin: 1.5% chitosan would only yield 55% of desirability.

Table 4: Result and analysis of optimum formulation parameter

Parameter	Prediction result	95%PI*low*	95%PI*high*	Observation result	Significance
EE	96.656	95.76	97.55	96.658±0.38	p>0.0
SI pH5.0	34.949	33.98	35.92	34.917±0.15	p>0.05
SIpH6.8	15.773	15.04	16.49	15.766±0.26	p>0.05
SI pH 7.4	8.150	7.18	9.12	8.1460±0.32	p>0.05
kpH5.0	0.463	0.10	0.82	0.4610±0.14	p>0.05
kpH6.8	20.107	17.82	22.41	20.116±0.39	p>0.05
k*Caecum*	52.963	51.26	54.65	52.955±0.63	p>0.05
MS	0.183	0.17	0.19	0.1840±0.00	p>0.05

(n=3, mean value±SD)Abbreviations: n=number of data; =average; SD=standard deviation; EE=Entrapment Efficiency; SI= Swelling Index; k=drug release constant; PI=prediction interval; MS = Mucoadhesive strength

Based on the result which was analyzed using independent t-test sample, it shows that actual value and prediction value from every test parameter produced by optimum formula were not significantly different due to p-value>0.05. This indicates that the suggested equations by factorial design program method can predict the values that results in optimum hydrogel formula.

CONCLUSION

In the present study, the authors are formulating a hesperidin hydrogel using chitosan-pectin combination polymer. The study design used was a factorial design which results in 4 variations of chitosan-pectin concentration. The combination of pectin-chitosan polymer was observed in several physicochemical traits, which are entrapment efficiency, swelling index, mucoadhesive strength and drug release. Based on the observation, it is concluded that higher entrapment efficiency was achieved by formula F3 with the highest pectin concentration, which is 96.65%. Highest mucoadhesive strength was achieved by formula 4, which has the highest chitosan-pectin concentration. In the mucoadhesive state, hydrogel matrix can control *in vitro* release of hesperidin, with the highest drug release rates achieved by the formula which have the highest pectin concentration in medium containing 2% of rat *caecum,* releases 56% of the drug. *Hesperidin* containing hydrogel release mechanism follows Higuchi's kinetics. Optimum hydrogel *hesperidin* formula

was obtained by comparing pectin and chitosan concentration, which is 5% (pectin) and 1% (chitosan) respectively. Based on experimental design data, the optimum hydrogel *hesperidin* formula had insignificant response value between observed and predicted value (p value>0.05). Based on this study which uses hesperidin as the active substance, it can be concluded that adding a combination of chitosan-pectin could control drug releases in the target organ, which is a colon.

AUTHORS CONTRIBUTION

1. First author: Conducts formula design and physicochemical traits test.
2. The second author: Conducts drug release test and an analysis of drug release
3. The third author: Analyzes the formula and verifies the analysis method

CONFLICTS OF INTERESTS

All authors have none to declare

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