Original Research Article

Glutaraldehyde-Crosslinked Chitosan-Pectin Nanoparticles as a Potential Carrier for Curcumin Delivery and Its In Vitro Release Study

Yosie Andriani[3*],Grasianto[1,2], Siswanta[1], Mudasir[1]

*Corresponding author:

Yosie Andriani

[1] Departement of Chemistry, Gajah Mada University (UGM), Jogyakarta, Indonesia
[2] Departement of Chemistry, Education and Teaching Faculty, Bengkulu University (UNIB), Bengkulu, Indonesia
[3] Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia

A b s t r a c t

Curcumin or diferuloylmethane as a naturally derived substance from herbal remedy and a good potential to posses many diseases are widely reported. However, Curcumin's poor aqueous solubility, low bioavalaibilty, and rapid degradation limiting clinical applicability. Nanoparticle encapsulation solves this problem and enables extended topical delivery of curcumin. There were several in vitro and invivo study of curcumin encapsulation, but different nano carriers were used for curcumin delivery, such as palmitat, formaldehyde, sodium tripolyphosphate and genipin croslinkings. Using a Glutaraldehyde-crosslinked chitosan-pectin nanoparticle for curcumin encapsulation to Increase the curcumin bioavailability in vitro was firstly described. The prepared nanoparticles were characterized by fourier transform infrared spectroscopy (FTIR), Transmission electron micrographs (TEM), Scanning electron microscopy (SEM) and X-ray diffraction (XRD). In vitro released kinetics study was adapted from Korsmeyer-peppas model. FTIR spectra revealed that nanoparticles were formed due to interaction between the carboxyl group of pectin and the prototonated amino groups of chitosan. Characterization of encapsulated curcumin using TEM was showed spherical morphology with size in around $\pm$ 40 nm. SEM revealed that no morphology changed between nanoparticles before and after the curcumin release, however the element composition of nanoparticles release has changed. Moreover, XRD analysis revealed the amorphous nature of the encapsulated curcumin. It was influenced by the concentration of polymer matrixs and glutaraldehyde. In vitro released kinetics study showed that the curcumin release from nanoparticle system tend to follow Korsmeyer-peppas model which had initial burst release followed by a steady state release. The release of curcumin was influenced by the concentration of polymer matrixs and glutaraldehyde. The present study concluded that glutaraldehyde-crosslinked chitosan-pectin nanoparticles are promising carrier for effective delivery of curcumin. Thus, curcumin as a potential drug to cure many diseases could be explored effectively for clinical applicability.

Keywords: nanoparticles; curcumin; glutaraldehyde-crosslinked; chitosan-pectin; kinetic study

Introduction

Curcumin (bis-R, -β-unsaturated β-diketone) is a hydrophobic polyphenol derived from the rhizome of the herb curcuma longa that has a wide spectrum of biological and pharmacological activities [1]. Curcumin is a potensial anti inflammatory agent with strong therapeutic potential against a variety of cancers. Curcumin has been shown to suppress transformation, proliferation, and metastasis of tumors [2]. In the other hand, curcumin has poorly properties such as low solubility and poor bioavalaibility like poor absorption, high rate of metabolism, rapid elimination and clearance from the body.

Recently, targeted and triggered drug delivery systems accompanied by nanoparticle technology have emerged as prominent solutions to the bioavailability of therapeutic agent. Moreover, the major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agent in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen [3]. Constraint in preparing nanoparticles is unstable and an easy aggregation of nanoparticles. Therefore, it needs formulation to prevent agregation and to improve nanoparticles stability. One of the ways to overcome this problem is by coating/blending with huge molecules such as polysaccaride or macromolecules. Nanoparticles delivery systems based on natural polymers are currently gaining interest to augment the systemic bioavailability of curcumin [4]. One of the natural polimers that can be used as drug delivery system of curcumin is chitosan. Chitosan is biodegradable, non-toxic, non-immunogenic and biocompatible [5]. Chitosan is polycationic polymer that have widely used in biomedic applications [6]. The positive charge is important in drug delivery system because it acts in muchoadhesion (adhesion to mucosal surface). Chitosan based drug delivery systems involves

complexation with ligands to form chitosan nanoparticles which can be used to encapsulate active compounds [7].

Utilization of chitosan as drug delivery has limitation in controlling drugs because of its hidrophilicity and solubility. This limitation can be solved by chemical modifications like combining with other polimers or crosslinker for improving biocompatibility [8]. Glutaraldehyde is one of the cross linked agents that is widely used for crosslinker because of its low cost production and great fixation. Glutaraldehyde can be used for cross linked agent to produce the matrixs that can control the release rate of drugs effectively [9]. Beside the use of cross linking, preparing chitosan based nanoparticles needs other polimers that can improve the stability of nanoparticles in order to control the curcumin release. Chitosan based polyelectrolyte complexes have been developed for oral, nasal or systemic administration of drugs and biodrugs. Curcumin prepared by loaded dextran sulphate-chitosan nanoparticles was done [10]. These nanoparticles were formed due to electrostatis interaction between negative charge of dextran sulphate and positive charge of chitosan and in this study, 70% of curcumin was released after 120 h. The polyanions that can be used for chitosan based polyelectrolyte complexes are tripolyphosphate, hyaluronic acid and pectin [11]. Pectin has the desirable stability under acidic conditions even at higher temperature which makes it an ideal candidate to be used in drug delivery system [12]. In this research, we synthesized chitosan-pectin cross linked by glutaraldehyde nanoparticles as nanocarier of curcumin. Chitosan-pectin nanoparticles were formed because of electrostatis interaction between chitosan and pectin on the nanoparticle surafce. The surface charge of nanoparticles can be adjusted by varying the concentration of the two polimers.

Electrostatic interaction between chitosan and pectin influenced the curcumin release from matrix system. The release rate of curcumin can be studied by using kinetic model approach. The use of kinetic model is very useful because this approach enables to predict the kinetics and mechanism of the drug release from matrixs before the release systems are implemented. More often, it allows the measurement of some important physical parameters, such as the drug diffusion coefficient and resorting to model fitting on experimental release data [13]. Several kinetic models such as the zero-order model equation; the first order equation, Higuchi's square-root equation; and the Korsmeyer-Peppas empirical equation have been proposed to describe the release characteristics of a drug from a polymer matrix because of their simplicity and applicability [14].

Experimental

Chemicals and reagents

Chitosan of medium molecular weight (degree of deacetylation, DD >75%), pectin (esterification degree, EE 65%) and curcumin pa were purchased from Sigma–Aldrich, distilled ethanol and other chemicals used were of analytical grade.

Preparation of curcumin loaded glutaraldehyde crosslinked chitosan-pectin nanoparticles

Chitosan solution of (0,7; 0,3; 0,1) wt% and pectin solution of (0,3; 0,7; 0,9) wt% were prepared in 1% acetic acid and 0,1% of curcumin was prepared in etanol. The combination of pectin and curcumin solutions was added to chitosan solution under vigorous stirring in a volume ratio of 1:1, then (0,5; 1,0; 1,5) w/v% glutaraldehyde was added to the mixed solution of chitosan-pectin cross. Nanoparticles were formed almost instantaneously. The suspension was allowed to stir for 4 h, and then the nanoparticles were separated from the suspension by centrifuging at 7,000 rpm for 60 min and washed twice in distilled water and re-suspended in PBS buffer.

Characterization

The mean size of the prepared nanoparticles was determined by TEM (JEM-1400 120 kV) and the morphology of the nanoparticles system was determined by SEM (SEM-EDS) (JEOL-JED 2300 Analysis Statio). An FTIR spectra was also recorded to study the potential interaction of functional-group constituents within the nanoparticle system, i.e. between curcumin and polymers using FTIR spectrophotometer (Shimadzhu Prestige21). To see the physical state of curcumin within the nanoparticles, XRD (Shimadzu S-600) analysis was used.

Entrapment efficiency and loading capacity

The entrapment efficiency of curcumin within glutaraldehyde crosslinked chitosan-pectin nanoparticles was determined by spectrophotometry. A known quantity (5 mg) of nanoparticle product was dissolved into 100 ml of ethanol. The resulting solution was quantified spectrophotometrically (UV-Vis 1700) at a optimum wavelenght of 427 nm, which corresponds to the absorption peak of curcumin. Entrapment efficiency [EE] was calculated based on the ratio of amount of drug present in the nanoparticles to the amount of drug used in the loading process [10].

$$EE = \frac{Wt-Wf}{Wt} \qquad (1)$$
$$LC = \frac{Wt-Wf}{Wn} \qquad (2)$$

Where Wt is total amount of curcumin in the pellet; wf is mount of curcumin free in the supernatan and wn is amount of nanoparticles after freeze drying [15].

In vitro drug release studies (Korsmeyer-peppas model)

In vitro drug release profiles of curcumin from glutaraldehyde crosslinked chitosan-pectin nanoparticles were done by direct-dispersion method for 360 minutes. A known quantity of curcumin loaded nanoparticles was taken in the 100 mL of release media. The tubes were then incubated in an incubator shaker at 37 C. At

definite time intervals, 5 mL of solution was taken out and quantified spectrophotometerically at a wavelength of 427 nm.

Result and Discussion

Formation of curcumin loaded glutaraldehyde-crosslinked chitosan-pectin nanoparticles

The preparation of curcumin loaded glutaraldehyde-crosslinked chitosan-pectin nanoparticles was based on the electrostatic interaction between positive charge of amino group in chitosan and negative charge of carboxyl group in pectin on the nanoparticle surface. The surface charge of nanoparticles was adjusted by varying the polymer concentration. The charge of density has great effect on the electrostatic interaction and it greatly influenced by the pH value of solution. The range of pH 5-6 was chosen for the formulation in formation of curcumin loaded glutaraldehyde crosslinked chitosan-pectin nanoparticles because the amino group of chitosan has been optimum protonated in this range. Nanoparticles were obtained by mixing ethanolic curcumin and pectin solution to chitosan solution before cross lingked with glutaraldehida.

Spectra analysis

Figure 1 shows the spectra of pure curcumin, chitosan, pectin, curcumin loaded chitosan-pectin and curcumin loaded glutaraldehyde-crosslinked chitosan-pectin nanoparticles. In the spectrum of chitosan, the peak at 1597 cm^{-1} is observed and this is characteristic peak for amide II bending vibration of chitosan. Pectin has characteristic peak due to vibrational band of carboxyl group at number wave 1627 cm^{-1}. Spectra of curcumin loaded chitosan-pectin shows that there are changes in number wave of the amide II bending vibration at 1597 shifted to 1589 cm^{-1} while vibrational band of carboxyl group at 1627 cm^{-1} dissapeared. This indicates that there are interactions between the two groups that have opposite charge. In the spectra of curcumin loaded chitosan-pectin was also observed peaks in number wave 1519 and 1018 cm^{-1}. Those peaks indicates the –NH deformation of chitosan and the keto-enol tautomerism of curcumin respectively [10]. Spectra of curcumin loaded glutaraldehyde-crosslinked chitosan-pectin nanoparticles shows the peak in number wave 1627 cm^{-1}, indicating interaction between chitosan and glutaraldehyde formed imine bonding, C=N. FTIR spectra confirmed that the nanoparticles were formed due to interaction between the carboxyl group of pectin and the prototonated amino groups of chitosan.
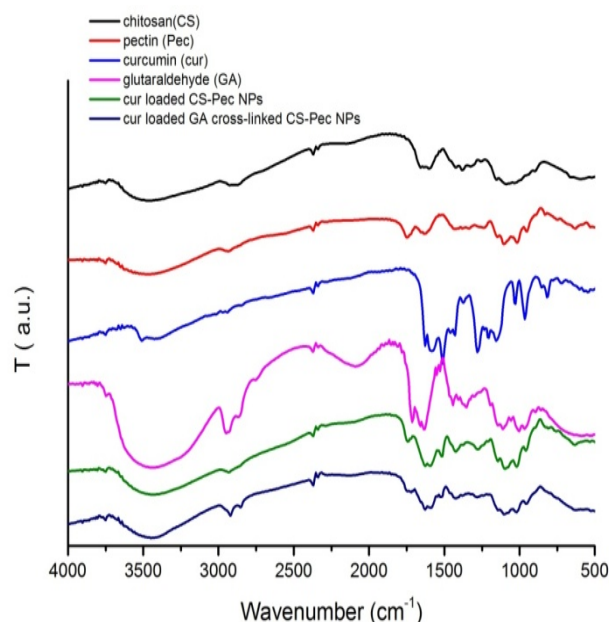

Figure 1: FTIR spectrums of chitosan, pectin, curcumin, glutaraldehyde, curcumin loaded chitosan-pectin nano particles and curcumin loaded glutaraldehyde-crosslinked chitosan-pectin nanoparticles.

Morphology analysis

Characterization of nanoparticles using TEM (Figure 2) was revealed that nanoparticles have spherical morphology with distribution diameter size by 10-59nm. Highest frequency of distribution was obtained around ±30-39nm. Moreover, Figure 3 shows that morphology of nanoparticles has no significantly changed before (a) and after (b) the curcumin release, however the element composition of nanoparticles before and after curcumin release has changed (Table 1).
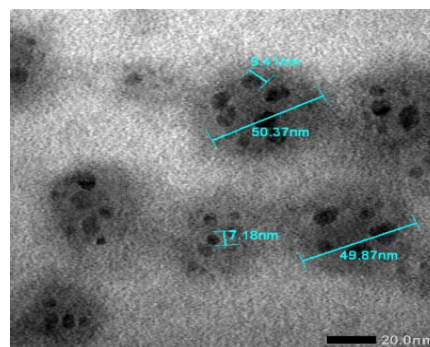

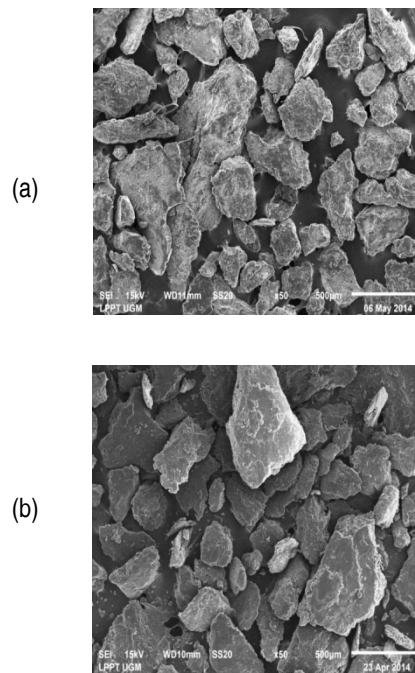**Figure 2:** TEM image of nanoparticles size distribution of particles

Figure 3: SEM image before curcumin release (a) SEM image after the curcumin released (b

Table 1: The comparasion of element composition of nanoparticles before and after the curcumin release.

Element	Content of elements (%)		
	Theoritic (%)	Before release	After release
C	56.54	49.74	20.45
N	1.36	19.38	13.86
O	34.57	30.36	52.37

Physical state analysis

The XRD can be used to investigate physical state of curcumin in the matrix of glutaraldehyde crosslinked chitosan-pectin nanoparticles. If the curcumin crystall are formed inside the nanoparticles matrix, it will cause curcumin elution from the nanoparticles system hindered and drug release profile will be irregular [10]. Based on XRD property of curcumin (Fig. 4), result shows that a number of peaks were observed in the 2Ɵ range of 10-30, indicating its crystalline state, but in nanoparticles of curcumin loaded glutaraldehyde-crosslinked chitosan-pectin, there were no such crystalline peaks. These data confirm that curcumin has amorphous state in nanoparticles system.

Encapsulation efficiency (EE) and loading capacity (LC) of nanoparticles

Polymer concentration (chitosan and pectin) and glutaraldehyde concentration has effects on encapsulation efficiency and loading capacity of nanoparticles (Table 2).
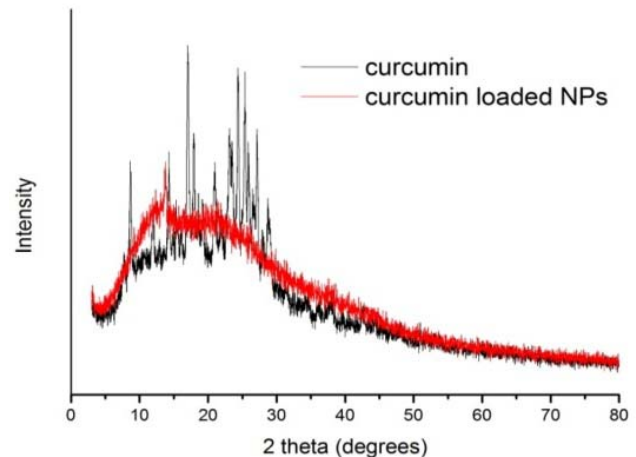

Figure 4: XRD pattern of curcumin (black) and curcumin loaded glutaraldehyde-crosslinked chitosan-pectin nanoparticles (red).

Tabel 2. Encapsulation efficiency and loading capacity of nanoparticles.

Concentration of Chitosan (%)	Concentration of pectin (%)	Concentration of GA (%)	EE	LC
0.1	0.3	1.5	94.7	21.05
0.3	0.3	1.5	77.7	16.7
0.7	0.3	1.5	43.5	4.34
0.7	0.7	1.5	95.4	9.74
0.7	0.9	1.5	55.4	7.59
0.7	0.3	1.0	24.0	6.30
0.7	0.3	0.5	29.8	7.65
0.7	0.3	0.0	67.8	23.8

The glutaraldehyde-crosslinked chitosan-pectin nanoparticles has %EE and %LC of nanoparticles lower than that of the non-crosslinked nanoparticles. It is caused by the formation of an imine covalent bonding between glutaraldehyde and chitosan, which consequently makes nanoparticles more rigid and decreases the free volume space within the nanoparticles, thus decreasing the %EE and %LC. Generally, the higher of the chitosan concentration in the nanoparticles system, the lower the %EE and %LC because the imina covalent bondings produced becomes higher. On the

contrary, the higher of the pectin concentration, the lower of the %EE and %LC because it makes the glutaraldehyde cross linking to chitosan less effective.

In vitro release study of curcumin from glutaraldehyde cross linked chitosan-pectin nanoparticles

In vitro drug release of curcumin from glutaraldehyde cross linked chitosan-pectin nanoparticles was investigated by using dissolution method and the release profile is shown in Figure 5.
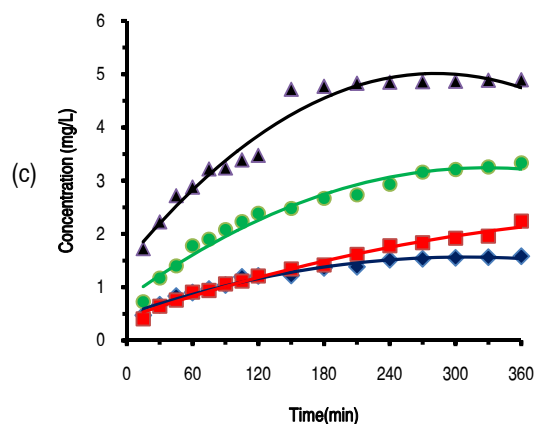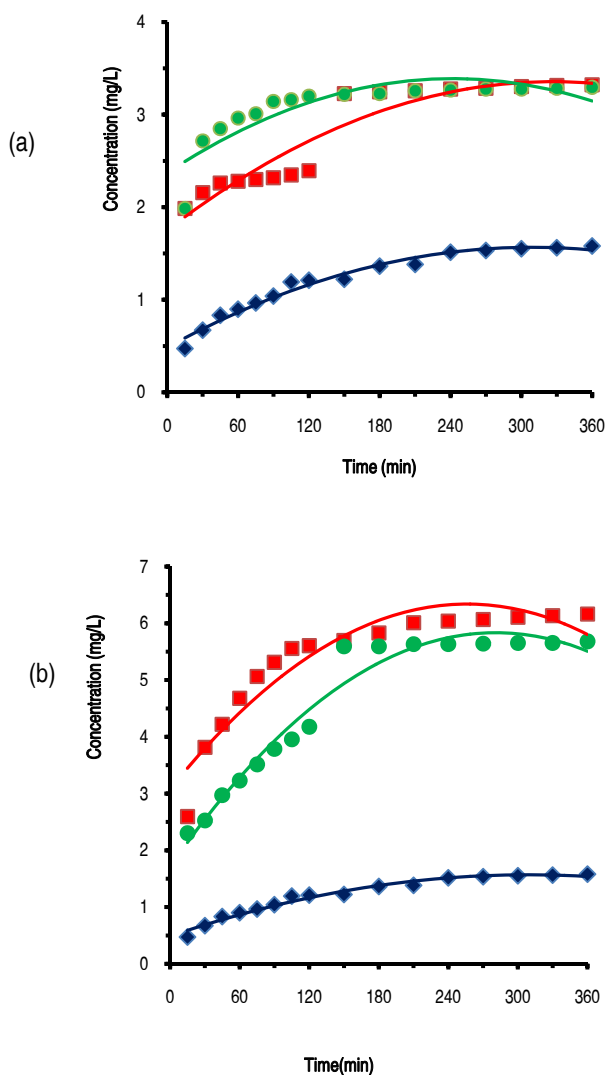
(c)

(a)

(b)

Figure 5: In vitro release of curcumin in the variation of chitosan concentration (= 0,7%, = 0,3%, □ = 0,1%) (A) the variation of pectin concentration (= 0,3%, □ = 0,7%, = 0,9%) (B) the variation of glutaraldehyde concentration (= 1,5 %, □ = 1 %, = 0,5 %, = without glutaraldehyde) (C)

The drug profile shows that there is initial burst release in the first 120 minute continued by a steady state release of curcumin. The initial burst release is due to dissolution of curcumin which is adsorbed on the nanoparticles surface and drug entrapped near the surface because the dissolution rate of the polymer near the surface is high, the amount of curcumin release is also high [10]. It is abvious that the release of curcumin is influenced by the concentration of polymers (chitosan and pectin) and glutaraldehyde. The higher the pectin concentration, the faster the curcumin release in the dissolution media. It is caused by increasing of pectin concentration make increasing of protonazed amino groups of chitosan because of the solution acidity. The higher the chitosan and glutaraldehyde concentration, the slower the release of curcumin due to the more chitosan cross linked by glutaraldehide that make bonding net more crowded.

Kinetic evaluation of curcumin from glutaraldehyde-crosslinked chitosan-pectin nanoparticles was studied by kinetic modelling of zero order, first order, Higuchi model and Korsmeyer-Peppas (KP) models (Table 3). The nanoparticles of curcumin loaded glutaraldehyde-crosslinked chitosan-pectin tend to follow Korsmeyer-Peppas model. From the TEM and SEM image, it is revealed that nanoparticles have spherical form (Fig. 2). Korsmeyer-Peppas equations describe the release of a drug from a carrier of a thin planar geometry, equivalent equations for release from thick slabs, cylinders, and spheres [16-17].

From Table 3, we can conclude that the optimum composition of curcumin from glutaraldehyde cross linked chitosan-pectin nanoparticles is chitosan 0.7 %, pectin 0.3 % and glutaraldehyde 1.0 % which has the lowest rate constants (K) e.g. 3.435 menit$^{-0.503}$. If we convert to %w/w chitosan, pectin and glutaraldehyde, then the optimum percentage would be 15.59; 6.68 and 75.50% respectively. [18] reviewed that several clinical trial of

the curcumin in treating many diseses have used in high doses (until 3600mg/day). Usage of the highly bioavailable developed nanoparticulate formulation of curcumin may bring about reduction in dose and improvement in efficacy of curcumin for clinical medicine [19]. Curcumin nano formulations have been developed for preclinical studies on cancer [20-21], inflammation, wound healing, and antibacterial agent [21]. However different nano carriers were used for curcumin delivery, such as palmitat, formaldehyde and sodium tripolyphosphate croslinkings [22].

Table 3. Kinetics model of nanoparticles curcumin loaded glutaraldehyde crosslinked chitosan-pectin nanoparticles

Variation of Concentration							
	CS 0.7%	CS 0.3%	CS 0.1%	Pec 0.7%	Pec 0.9%	GA 1.0%	GA 0.5%
Zero order							
R^2	0.867	0.0653	0.799	0.819	0.473	0.971	0.888
K	0.131	0.087	0.146	0.089	0.054	0.147	0.173
First order							
R^2	0.940	0.761	0.819	0.823	0.633	0.983	0.985
K	4.3×10^{-4}	4.3×10^{-4}	4.3×10^{-4}	3.9×10^{-4}	4.3×10^{-4}	4.3×10^{-4}	8.6×10^{-4}
Higuchi model							
R^2	0.957	0.802	0.891	0.861	0.627	0.991	0.970
K	3.300	2.318	3.689	2.174	1.492	3.551	4.322
K-P model							
R^2	0.977	0.882	0.939	0.840	0.756	0.994	0.972
K	8.851	9.998	12.189	21.777	45.289	3.436	6.607
n	0.424	0.422	0.300	0.197	0.120	0.503	0.574

*Several clinical trials have determined

Conclusion

Nanoparticles of curcumin loaded glutaraldehyde-crosslinked chitosan-pectin has been succesfully prepared by ionic gelation. The prepared nanoparticles have a spherical form with average diameter of around 40 nm. Curcumin in nanoparticles system has amorphous state, which was confirmed from the XRD data. Curcumin release from nanoparticles tends to follow Korsmeyer-peppas model as shown by the highest liniearity value (R^2) compared to the other models. Concentration of polymer matrixs and glutaraldehyde significantly influences the encapsulation efficiency and loading capacity of nanoparticles and the curcumin release. These results clearly demonstrate that glutaraldehyde-crosslinked chitosan-pectin nanoparticles can be used as promising carrier for effective delivery of hydropobic drugs like curcumin.

Acknowledgement

References

[1]. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: Problems and Promises. Mol Pharm 2007;6: 807-8.

[2]. Shishodia S, Chaturvedi MM, Aggarwal BB. Role of Curcumin in Cancer Therapy. Curr Probl Cancer 2007;31:243-305.

[3]. Mohanraj VJ and Chen Y. Nanoparticles – A Review. Trop J Pharma Res 2006; 5:561-573.

[4]. Liu J, Xu L, Liu C, Zhang D, Wang S, Deng Z, Lou W, Xu H, Bai Q, Ma J. Preparation and Characterization of Cationic Curcumin Nanoparticles for Improvement of Cellular Uptake. Carbohydr Polym 2012;90:16-22.

[5]. Dutta PK, Dutta J, Tripathi VS. Chitin and Chitosan: Chemistry, Properties and Applications. J Sci Ind Res 2004;63: 20-31.

[6]. Ilium L, Farraj NF, Davis SS. Chitosan as Novel Nasal Delivery System for Peptides Drugs. Pharm Res 1994;11: 1186-9.

[7]. Morris GA, Kok MS, Harding SE and Adam GA. Polysaccharide Drug Delivery Systems Based on Pectin and Chitosan. Biotechnol Genet Eng Rev 2010;27:257-284.

[8]. Chandur VK, Badiger AM, Shambashiva RKRS. Characterizing Formulations Containing Derivatized Chitosan With Polymer Blending. Int J

Res Pharm Chem 2011; 4(1): 950-967.

[9]. Gupta KC and Jabrail FH. Glutaraldehyde and Glyoxal Cross-Linked Chitosan Microspheres for Controlled Delivery of Centchroman. Carbohydr Res 2006; 341:744–756.

[10]. Anitha A, Deepagan VG, Rani D, Menon D, Nair SV, Jayakumar R. Preparation Characterization, in Vitro Drug Release and Biological Studies of Curcumin Loaded Dextran Sulphate–Chitosan Nanoparticles. Carbohydr Polym 2011;84;1158–1164.

[11]. Luppi B, Zecchi V, Bigucci F, Abruzzo A, Corace G, Teresa Cerchiara T. Freeze-Dried Chitosan/ Pectin Nasal Inserts for Antipsychotic Drug Delivery. Eur J Pharm Biopharm 2010;75:381–7.

[12]. Mishra RK, Banthia AK, and Majeed A. Pectin Based Formulations for Biomedical Applications: a review. Asian J Pharm Clin Res 2012;5:1-7.

[13]. Dash S, Murthy PD, Nath L, Chowdhury P. Kinetic Modeling on Drug Release from Controlled Drug Delivery Systems. Acta Poloniae Pharm Drug Res 2010;3(67):217-223.

[14]. Ravi PR, Ganga S, Saha RN. Design and Study of Lamivudine Oral Controlled Release Tablets. AAPS Pharm Sci Tech 2007;4(8):1-9.

[15]. Li J, Feng L, Fan L, Zha Y, Guo L, Zhang Q, Chen J, Phang Z, Wang Y, Jiang X, Yang VC, Wen L. Targeting the brain with PEG-PLGA nanoparticles modified with phage-displayed peptides. Biomaterials(2011). 32: 4943–50.

[16]. Siepmann J, Peppas NA. Higuchi equation: Derivation, Applications, Use and Misuse. *Int J Pharm* 2011; 418: 6– 12.

[17]. Siepmann J, Peppas NA. Modeling of Drug Release from Delivery Systems Based on Hydroxypropyl Methylcellulose (HPMC). Adv Drug Deliv Rev 2012;64: 163–174.

[18]. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. Biochem. Pharmacol. 2008;75:787–809.

[19]. Shaikha J, Ankolab DD, Beniwal V, Singha D, Ravi Kumarb MNV. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci 2009;37: 223–230.

[20]. Sahu A, Bora U, Kasoju N, Goswami P. Synthesis of novel biodegradable and self-assembling methoxy poly(ethylene glycol)–palmitate nanocarrier for curcumin delivery to cancer cells. Acta Biomater 2008;4:1752–1761.

[21]. Krausz AE, Adler BL, Cabral V, Navati M, Doerner J, Charafeddine RA, Chandra D, Liang H, Gunther L, Clendaniel A, Harper S, Friedman JM, Nosanchuk,JD Friedman AJ. Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent. Nanomedicine: NBM 2015;11:195–206.

[22]. Tahtat D, Mahlous M, Benamer S, Khodjaa AN, Oussedik-Oumehdi H, Laraba-Djebarib F. Oral delivery of insulin from alginate/chitosan crosslinked by Glutaraldehyde. In J Biol Macromol 2013;58:160– 168.