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Mathematical Analysis of Drug Release for Gastrointestinal Targeted Delivery Using β-Lactoglobulin Nanoparticle

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ABSTRACT

To answer challenge of targeted and controlled drug release in oral delivery various materials were studied by different methods. In the present paper, controlled metal based drug (Pd(II) complex) release manner of β‑Lactoglobulin (β-LG) nanoparticles was investigated using mathematical drug release model in order to design and production of a new oral drug delivery system for gastrointestinal (GI) tract. The β-LG nanoparticles containing Pd(II) complex were fabricated in the presence of low methoxyl pectin (LMP) at different conditions. Characterization of β-LG nanoparticles using dynamic light scattering (DLS) and atomic force microscopy (AFM) were performed. The *in vitro* drug release studies were carried out at 37 °C during 8 h in the simulation conditions of GI fluid. The obtained results were fitted in various kinetically release models. The Korsmeyer-Peppas model was evaluated the best describe of each simulation conditions such results indicated an anomalous diffusion manner for drug release. The release data were fitted to the Kopcha model; then, using statistically evaluation revealed that β-LG nanoparticles-LMP complex contain Pd(II) complex dramatically sensitive to pH. In addition, results indicated that for drug release from β-LG nanoparticles delivery system erosion is predominate. So, the erosion-controlled is drug release mechanism of this delivery system. We concluded that β-LG nanoparticles complex with LMP based on mathematical drug release model would be a targeted and practical promising device for GI drug delivery.

Keywords: β‑LG nanoparticle, Drug release, Gastrointestinal tract, Mathematical modelling, Kopcha model

INTRODUCTION

Over the past decades many efforts have been done to predict and create controlled vehicles for sustained released oral drug delivery based on mathematical model using various materials [1]. Polymers due to their properties rather than other materials were introduced as a suitable candidate to achieve this purpose [2]. Nevertheless, the targeted drug delivery by polymers and their controlled released manner are still remained as main questions whereas, there is no significant differences between them from release model point of view. On the other hand, the details of release profile study are ignored in many design drug delivery investigations. Recently, literature illustrated that Advanced Drug Delivery (ADD) using nanotechnology is the most probably solution for this problem. So, oral drug delivery as an active ADD was applied nanotechnology using mathematical release models to develop targeted delivery based on real quest [3,4]. Then, the polymers notice their properties using various methods of nanotechnology were manipulated to obtain complementary features with the target site [5]. Also, previous studies showed that the

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presence of extra layer is another way to achieve targeted delivery by nanotechnology so that it affects kinetics of drug release. The type of extra layer makes individual specificity for delivery system in complementary features with target site such considered release model can be controlled or made by it. The other important advantages of extra layer in the drug delivery system are enhancement in drug transportation yield, drug protection from fast release and solvent accessibility [6-8]. Furthermore, there are various mathematical release models that can describe drug transportation from carrier like diffusion or erosion mechanisms that act by Fick's or non-Fick low, respectively. Accordingly, these finding are lead to carrier shape illustration, evaluation of drug carrying ability and demonstration of carrier complementary features with target site. These results are accomplished with unique properties of materials in nanoscale that present in drug delivery [9-13].

In the current paper, we have investigated Pd(II) complex release from β-Lactoglobulin (β-LG) nanoparticles that complex with low methoxyl pectin (LMP) as an extra layer to determine mathematical release models in ADD. Previous studies have been shown among polymers β-LG as a milk whey carrier protein based on unique physico-chemical properties, high ability for carrying ligand and globular shape is introduced as a promising candidate for ADD [14-16]. β-LG is belonging to lipocalin superfamily with unique secondary structure contain nine anti-parallel β-stands (A-I) that make β-barrel as an active position for interaction with various ligands [17-19]. In addition, LMP as well as β-LG is resistant to acidic condition so that it illustrated that LMP can act as an extra layer in gastrointestinal (GI) tract drug delivery. Likewise, previously illustrated that β-LG nanoparticle complex with LMP can act as a suitable oral drug delivery [20].

Therefore, in this study we have tried to introduce GI drug release mechanism by mathematical release model in details and *in vitro* release profile of the synthesized drug delivery system in nanoscale at various pHs. Finally, the present investigation suggests the best drug release model as well as predominant release mechanism are critical parameters in the design and production of nanoscale drug delivery system for GI. Consequently, the creation of targeted oral drug delivery system seriously depending on mathematical release model.

MATERIALS AND METHODS

Materials

The protein β-LG isoform A from bovine milk with purity >90% and LMP were obtained from Sigma-Aldrich Chemical Co. Pd(II) complex (drug) was synthesized using previous method in our laboratory [17]. Other chemicals used in this study were of the highest purity analytical grade without further purification. The materials were dissolved in double distilled water.

Preparation of Drug Delivery System for GI in Nanoscale

To obtain oral drug delivery system based β-LG in nanoscale, solutions were prepared at three pHs of 3, 4.5 and 7. Hence, to preparing β-LG nanoparticles contain Pd(II) complex molar ratio 1:1 of β-LG and drug, respectively, taking into account previous study [21] was used. On the other hand, to adding extra layer on β-LG nanoparticle containing drug, LMP with final concentration of 0.025 (%Wt) was prepared. All solutions for nanoparticles synthesis were made by sodium phosphate with concentration of 10 mM and were shaken at ambient temperature.

β-LG Nanoparticle Characterization Studies

Size and zeta potential measurements of β-LG nanoparticle-LMP complex contain drug were performed using Dynamic Light Scattering (DLS) (Brookhaven Instruments Corporation, USA). Shape determination was carried out using Atomic Force Microscopy (AFM) (Veeco Instruments). AFM measurements were performed taking into account the best DLS data. All evaluations were taken at the temperature of 25 °C.

In vitro Release Studies

To determine targeting release mechanism for oral drug delivery the Souder and Ellenbogen method [22] was used using a dialysis bag (MW cutoff 10 kD). For each samples 2 ml of β-LG nanoparticle-LMP complex contain Pd(II) complex, 3 times, was injected into dialysis bag and then were shaken with a rotation speed of 30 rpm during 8 h at

37 ± 1 °C. Hence, the *in vitro* release studies in the presence of the best nanoparticle characterization were performed based on gastrointestinal tract fluid simulation. The simulated fluids were selected in four region pHs of 1.2, 4.5, 7.5 and 7 so that each represent simulated gastric fluid, simulated gastric and upper intestinal fluid, simulated intestinal fluid and simulated colonic fluid, respectively. The amount of evaluation of Pd(II) complex release from β-LG nanoparticle-LMP complex in dissolution medium was carried out using the Shimadzu-3100 double beam spectrophotometer at a wavelength of 220 nm for Pd(II) complex. To evaluate *in vitro* release study the obtain data were analyzed using zero order model, first order model, Higuchi model, Korsmeyer-Peppas and Kopcha model as release mathematical equations. In addition, the Kopcha model fitting results statistically were investigated using ANOVA.

RESULTS AND DISCUSSION

DLS and AFM Results

To characterize size and zeta potential of β-LG nanoparticle-LMP complex containing Pd(II) complex at various pHs, DLS measurements were performed at 25 °C. The obtained data were summarized in Table 1 and Fig. 1A. Hence, the particle size and charge surface of β-LG nanoparticle-LMP complex contain Pd(II) complex were investigated to make a targeted release manner. DLS results illustrated that the lowest size of β-LG nanoparticle-LMP complex contain drug with colloidal stability was made at pH 4.5 due to this point is close to isoelectric point of β-LG (pI = 5.2 [23]). At this pH, the charge profile of β-LG is closely balanced. Nevertheless, there are restrict positively charge areas of β-LG contain drug that can bind to anionic LMP. This is due to possible physical interaction between β-LG and LMP whereas the level of repulsive forces for β-LG are lower than attractive forces. Along with β-LG trend to self-association at pH 4.5 that is due to charge profile. At pHs 3 and 7, β-LG is strongly cationic and anionic, respectively [5] so that presence of strong repulsive forces were inhibited of self-association and suitable complex formation. In the other words, nature of β-LG and ionic strength are effective on β-LG nanoparticle formation. On the other hand, charge surface profile results show that LMP is effective on charge distribution of β-LG nanoparticles. Results indicated that zeta potential at various pHs goes to net charge. Zeta potential results were shown in Table 1. According to what was mentioned above there is no balanced for physical forces at pHs 3 and 7. Therefore, results of zeta potential revealed that colloidal stability of β-LG nanoparticle-LMP complex contain Pd(II) complex with solubility and homogeneity size distribution at pH 4.5. These findings indicated that the level of pH and ionic strength are the most factors to determine size of β-LG nanoparticles.

AFM analysis was done for evaluations of morphological characterization and particle size distribution of β-LG nanoparticle-LMP complex contain Pd(II) complex taking into account the best result of DLS. Therefore, the sample at pH 4.5 was selected for AFM measurement whereas this pH was optimal for β-LG nanoparticle synthesis. As it can be seen in Fig. 1B, the β-LG nanoparticle-LMP complex contains drug population were well separated and distributed homogeneity so that were remained intact. AFM results show that β-LG nanoparticle-LMP complex contain drug shape were spherical. AFM data were in good agreement with DLS results whereas illustrated that the size of β-LG nanoparticles-LMP complex contain Pd(II) complex were approximately 183 nm. Size, shape and surface charge characteristics are known as important complementary features with targeted site for GI targeted oral drug delivery individually in colorectal cancer. This fact is due to the vessel pores of the LS174T human colon adenocarcinomas are between 400 and 600 nm [24]. Moreover, there is a specific charge profile due to M cell and enterocytes at colorectal cancer area [25]. Therefore, it can be concluded β-LG nanoparticle-LMP complex is a vehicle with complementary properties for GI so that was made targeted based on colorectal cancer physical features.

Drug Release

To demonstrate nanoparticle ability for presence in GI oral delivery another important property that must be considered is the mechanism of drug release. Hence, *in vitro* estimation rather than *in vivo* study reveals drug release pattern in details [26]. For this purpose, based on the best result of DLS, *in vitro* drug release from β-LG nanoparticle-LMP complex was carried out at simulated GI fluids. The *in*

Table 1. Size and Zeta Potential Characterizations of β-LG Nanoparticle-LMP Complex Contain Pd(II) Complex

pH	Size (nm)	Zeta potential (mV)
3.0	328 ± 8	7.81 ± 0.3
4.5	183 ± 5	-7.90 ± 0.1
7.0	451 ± 6	-10.3 ± 0.5

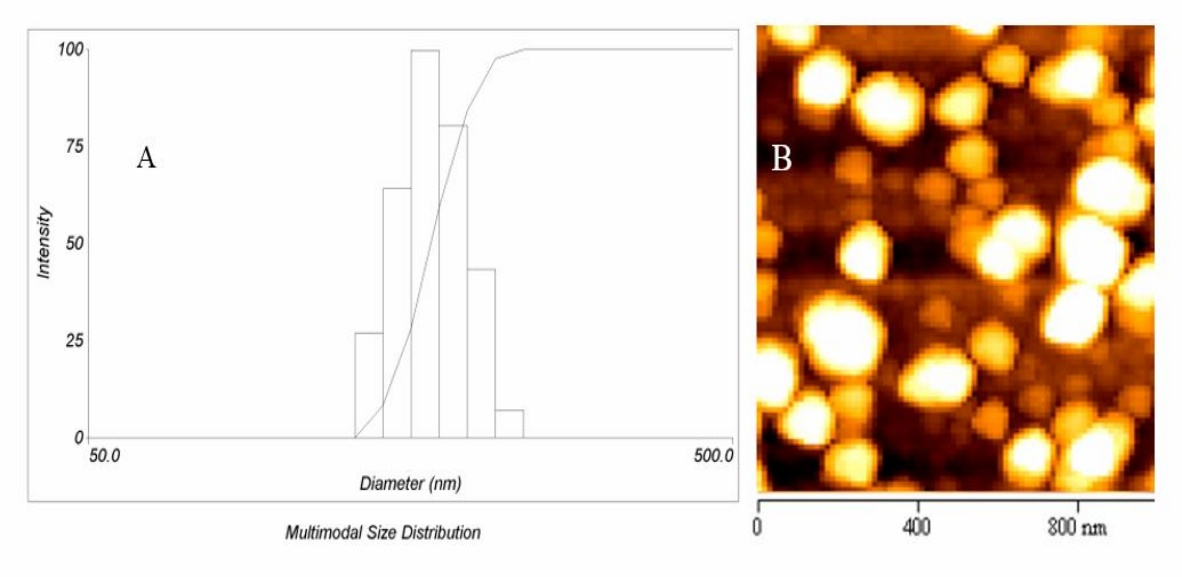

Fig. 1. Representative of particle size distribution using DLS (A) and AFM image (B) of β-LG nanoparticles-LMP complex containing Pd(II) complex at pH 4.5.

vitro drug release profile during 8 h at 37 °C was shown in Fig. 2. The obtained release profile results in various simulated GI condition show that the maximum Pd(II) complex release of β-LG nanoparticle-LMP complex occurred at pH 7.5 which describes simulated intestinal fluid condition. For practical demonstration of β-LG nanoparticle-LMP complex ability to presence in GI oral delivery, release profile kinetics of Pd(II) complex was also investigated by fitting into mathematical kinetics models. The best release model with taking into account the correlation coefficient (R^2) was obtained. The release kinetics models of zero order, first order [27], Higuchi [28] and Korsmeyer-Peppas [29] were selected and by following mathematical equations were used (Eq. (1-4)):

Zero order model:

$$M_t = M_0 + k_0 t \quad (1)$$

First order model:

$$\log M_t = \log M_0 + \frac{k_1 t}{2.303} \quad (2)$$

Higuchi model:

$$M_t = k_H \sqrt{t} \quad (3)$$

Korsmeyer-Peppas:

$$\frac{M_t}{M_\infty} = k_{KP} t^n \quad (4)$$

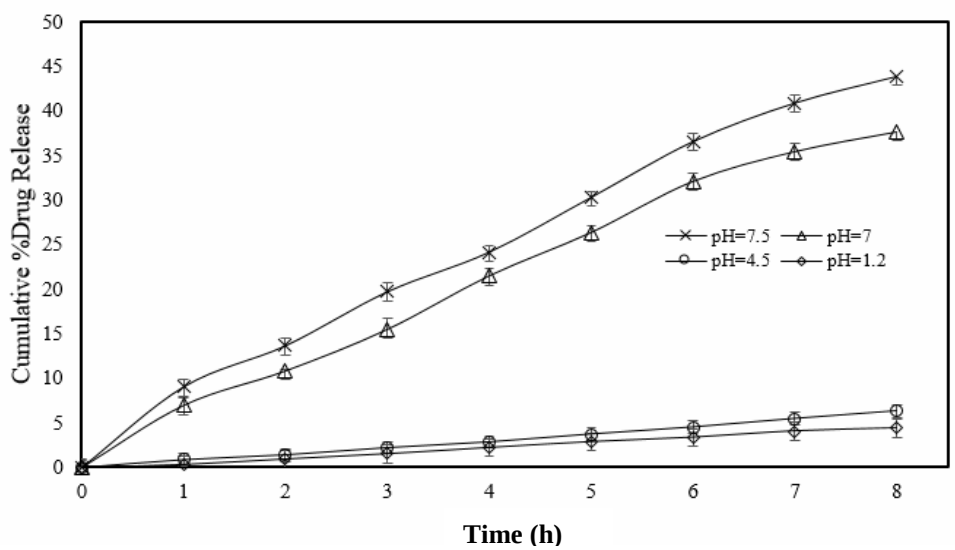

Fig. 2. Cumulative amount of Pd(II) complex released from β-LG nanoparticle-LMP complex during 10 h.

Table 2. Correlation Coefficients (R^2) Obtained from Modeling Pd(II) Complex Release from β-LG Nanoparticle-LMP Complex through Release Kinetic Models in Simulated GI Fluids

pH	Zero order	First order	Higuchi	Korsmeyer-Peppas
1.2	0.97	0.91	0.93	0.99
4.5	0.95	0.89	0.88	0.99
7.0	0.99	0.98	0.97	0.99
7.5	0.97	0.96	0.93	0.99

where M_0, M_t and M_∞ are the amount of drug dissolved at time zero, amount of drug dissolved at time t, and the amount of drug dissolved at time infinity, respectively. The kinetic constants of release model are described by k_0, k_1, k_H and k_{KP} in zero order, first order, Higuchi and Korsmeyer-Peppas models, respectively. Also, n as release exponent in Korsmeyer-Peppas model is used to characterize release mechanism. The value of n represent Fickian diffusion, anomalous (non-Fickian) diffusion (*i.e.* by both diffusion and erosion), case II transport (zero order (time-independent) release) and super case II transport by $n = 0.5$, $0.5 < n < 1$, $n = 1$ and $n > 1$, respectively [29,30]. Table 2 shows the release data fitting in various kinetics models. The best correlation coefficient values were obtained from fitting data in Korsmeyer-Peppas equation. Hence, drug release from β-LG nanoparticle-LMP complex is described by release kinetics models of Korsmeyer-Peppas so that the values of n were obtained between $0.5 < n < 1$ in simulation conditions. Therefore, drug release follows anomalous non-Fickian pattern. Therefore, to demonstrate diffusion and erosion contribution the Kopcha model was used (Eq. (5))

$$M_t = A\sqrt{t} + Bt \quad (5)$$

In the Kopcha model, A and B represent the diffusion and the erosion terms, respectively. According to previous studies, when $A/B = 1$, $A/B < 1$ and $A/B > 1$ contribution of A and B is indicated so that diffusion and erosion are equal,

Table 3. The Release Exponent Factor of Korsmeyer-Peppas Model and Kopcha Release Model Fitting Results

pH	Korsmeyer-Peppas	Kopcha			
	n	R^2	A	B	A/B
1.2	0.91	0.98	0.02	0.122	0.16
4.5	0.97	0.96	0.06	0.303	0.21
7.0	0.85	0.98	0.17	0.713	0.25
7.5	0.79	0.99	0.26	0.834	0.31

Table 4. Estimation of pH Relation with Kopcha Fitting Results by ANOVA at 0.01 P-level and Correlation Coefficients (R^2)

pH	A		B		A/B	
	R^2	P-value	R^2	P-value	R^2	P-value
	0.98	0.004	0.98	0.005	0.98	0.003

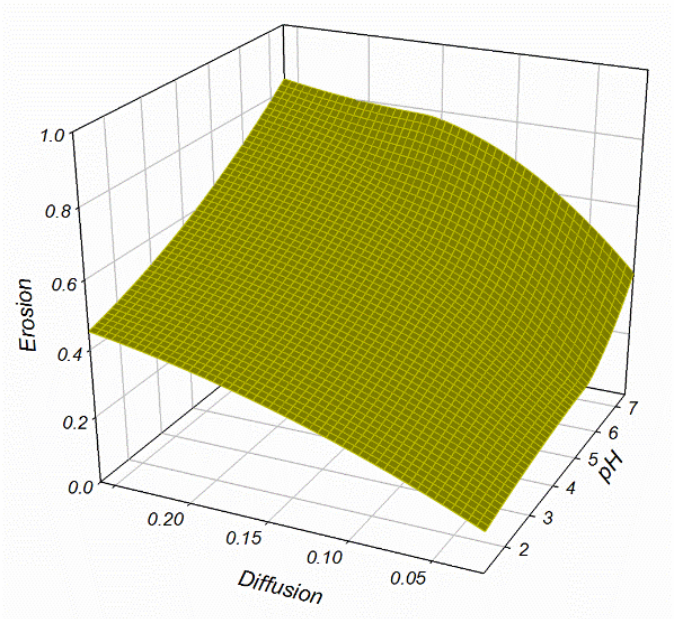

Fig. 3. 3D graph of diffusion and erosion changes at various pHs.

erosion predominates over diffusion and diffusion predominates over erosion, respectively [29-32]. The results of data fitting in Kopcha model are shown in Table 3 and Fig. 3. The correlation coefficients of Kopcha model in simulated conditions confirmed that there is a contribution of erosion and diffusion such non-Fickian pattern illustrated in drug release from β-LG nanoparticle-LMP complex. As it can be seen in Fig. 3 with increasing pH value, the ratio of A/B is increased; nevertheless, its value is lower than 1. In other words, Fig. 3 and values of A/B ratio in Table 3 demonstrate that erosion is predominate in drug release pattern from β-LG nanoparticle-LMP complex. Among simulation conditions, the obtained release from data fitting in kinetics models revealed that the best results are occurred at pHs 7 and 7.5 which represent simulated intestinal fluid and simulated colonic fluid, respectively. On the other, the release profile in Fig. 2 showed there is no significant drug release in pHs 1.2 and 4.5 that present simulated gastric fluid and simulated gastric and upper intestinal fluid, respectively. So, it can be concluded that there is a relation between pH and drug release from β-LG nanoparticle-LMP complex. Hence, the Kopcha model parameters relation with pH were investigated using correlation coefficient and ANOVA with a p-value smaller than 0.01. The obtained results indicated that there is direct relation between pH and Kopcha model parameters. These findings illustrated that β-LG nanoparticle-LMP complex dramatically sensitive to pH. Furthermore, statistical analyzing shows that with pH increasing the probability of erosion is increased .These findings are well in agreement with previous studies that proposed at pHs 7 and 7.5 due to alkaline condition LMP is degraded [33]. Also, β-LG structure is resistance to acidic condition and changed in alkaline condition [34]. Therefore, drug release from β-LG nanoparticle-LMP complex delivery system is following erosion-controlled mechanism such slowly diffusion occurred during it. Overall, mathematically release models findings represented that β-LG nanoparticle-LMP complex is significantly sensitive to pH and targeted for alkaline condition of GI.

CONCLUSIONS

In this study, we successfully suggested a controlled release oral drug delivery for GI in nanoscale based β-LG-LMP complex. We propose β-LG nanoparticle-LMP complex containing drug is targeted vehicle for colorectal cancer oral delivery whereas; there are complementary features between them from size point of view, specially. The release pattern of drug from our formulation is strongly sensitive to pH so that at pH 7 and 7.5, there is dramatically drug release. Mathematically release model indicated that it due to non-Fickian mechanism. On the other hand, the Kopcha release kinetics model revealed that in this event erosion is predominate. In other words, erosion contribution in non-Fickian mechanism is controlled drug release so that there is a slowly drug diffusion from delivery system. Consequently, these findings strongly suggested that our formulation is well characterized for controlled release oral drug delivery of colorectal area.

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