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Kinetic Study of Chitosan-Alginate Biopolymeric Nanoparticles for the Controlled Release of Curcumin Diethyl Disuccinate

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Abstract

The aim of this study was to investigate the release profile, and evaluate the best fitted kinetic model and mechanism, of curcumin diethyl disuccinate (CDD) from chitosan-alginate biopolymeric nanoparticles (CANPs) in simulated gastrointestinal fluids (without enzymes) and simulated body fluid. The CDD-loaded CANPs (CDD-CANPs) were prepared by oil-in-water emulsification and ionotropic gelation under the previously reported optimal condition (3 mg/mL of CDD, 4.05% (w/v) of Tween™80 and a chitosan:alginate mass ratio of 0.05:1), which resulted in CDD-CANPs with a favorable particle size (327±14 nm), zeta potential (-27.3±0.2 mV), encapsulation efficiency (51.2±2.2%) and loading capacity (11.7±0.8%). The *in vitro* release of CDD from the CDD-CANPs in simulated gastrointestinal fluid at pH 1.2, 4.5 and 6.8, and simulated body fluid at pH 7.4, indicated that the release of CDD could be controlled, was sustained over at least 72 h and best fit the Korsmeyer-Peppas kinetic model with a Fickian diffusion mechanism. Therefore, CANPs have the potential to be used for the controlled release of CDD in the gastrointestinal tract and blood circulation.

Keywords: Controlled release; Kinetic; Nanoparticles; Alginate; Chitosan; Curcumin diethyl disuccinate

Introduction

Curcumin diethyl disuccinate (CDD) is an ester prodrug of curcumin and has previously been synthesized by succinylation of curcumin with ethyl succinyl chloride[(1,2)]. Compared to curcumin, CDD shows a better anti-colon cancer activity and is more stable in human plasma[(1)]. However, a fast release of CDD in simulated gastrointestinal tract conditions was observed, which could cause a sharp increase in the CDD concentration to reach toxic levels and so require frequent lower dose applications to avoid toxicity with the subsequent associated patient health, cost and compliance problems. Instead, the controlled release and sustained release of CDD could potentially be achieved using nanotechnology to increase the duration of effective therapeutic effect with reduced administration frequency and drug metabolism and toxicity effects. Accordingly, in this study chitosan-alginate biopolymeric nanoparticles (CANPs) were chosen for the encapsulation and controlled release of CDD due to their biodegradability, mucoadhesiveness, nontoxicity, biocompatibility and good film formation[(3-5)].

Alginate is an anionic polysaccharide extracted from brown algae consisting of α-L-guluronic acid and *β*-D-mannuronic acid linked by 1,4-glycosidic bonds[(6)]. On the other hand, chitosan, a cationic polysaccharide, is composed of *N*-acetyl-D-glucosamine and D-glucosamine units linked by *β*(1→4) glycosidic bonds[(7)].

The CANP system has been widely studied for the delivery of drugs and therapeutic reagents[(8-11)]. However, the use of CANPs for the controlled release of CDD has not been reported. Therefore, this study was undertaken to examine the *in vitro* release of CDD from CANPs in simulated gastrointestinal and body fluids, including the kinetics and mechanism of release.

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Materials and Methods

Materials

Sodium alginate (Mw = 80,000-120,000 g/mol and guluronic acid content = 0.39) was purchased from Sigma, St. Louis, MO, USA. Chitosan (Mw = 75,000 g/mol and DD = 85%) was provided by Marine Bio Resources Co., Ltd., Samut Sakorn, Thailand. Tween™ 80 was purchased from Thermo Fisher ACROS Organics™ (Geel, Belgium). The CDD was synthesized as previously reported[1]. Purified water was obtained by Milli-Q® water purifier (Millipore, France). Other chemicals were of analytical grade.

Preparation of CDD-loaded CANPs.

The synthesis of CDD-loaded CANPs (CDDCANPS) was performed under the previously reported optimal condition by oil-in-water (o/w) emulsification and ionotropic gelification[12]. Briefly, 1 mL of acetonic CDD solution (3 mg/mL) was added dropwise into 20 mL of alginate solution (0.6 mg/mL) containing 4.05% (w/v) Tween™ 80 using a syringe pump (NE 100, New Era, Pump System Inc., USA) at an addition rate of 20 mL/h and 4 mL of $CaCl_2$ solution (0.67 mg/mL) used as a crosslinking agent of alginate[13] was then added dropwise to the resulting o/w emulsion and continuously stirred at 1,000 rpm for 30 min. After sonication for 15 min using an ultrasonic bath (model CP 230, Crest Ultrasonic Corporation, NY, USA), chitosan solution (0.15 mg/mL) was added to the calcium-alginate pregel suspension to get a final chitosan: alginate mass ratio of 0.05:1, and stirred for a further 30 min. The obtained CDD-CANP suspension was equilibrated overnight in the dark at room temperature to allow the CDD-CANPs of a uniform size to form.

Characterization of CDD-CANPs

Particle size, polydispersity index (PDI) and zeta potential of CDD-CANPs were measured using a Zetasizer (model Nano-ZS, Malvern Instruments Ltd., UK). The morphology of the CDD-CANPs was observed using transmission electron microscopy (TEM) with a model H-9500 microscope (Hitachi High Technology America Inc., USA). The encapsulation efficiency and loading capacity of CDD in the CDD-CANPs were determined by measuring the residual CDD concentration in the supernatant of the CANP suspension after removal of CDD-CANPs by centrifugation at 45,000 rpm at 4°C for 45 min. The CDD concentration was then determined using UV-vis spectrophotometer at a wavelength of 405 nm. The encapsulation efficiency and loading capacity were then calculated from equations. (1) and (2), respectively:

$$\text{Encapsulation efficiency (\%)} = [(W_t - W_s)/W_t] \times 100 \quad (1)$$

$$\text{Loading capacity (\%)} = [(W_t - W_s)/W_{np}] \times 100 \quad (2)$$

where W_t is the total amount of CDD initially added in the formulation, W_s is the total amount of un-encapsulated CDD present in the supernatant and W_{np} is the total dry mass of CDD-CANPs[14].

In vitro release and kinetic studies.

The *in vitro* release and kinetic studies of CDD-CANPs were investigated by the dialysis method[15] using simulated fluids, without enzymes, containing 50% (v/v) of ethanol as the release medium with sink conditions for CDD. The simulated fluids includes simulated gastric fluid (SGF) at pH 1.2, simulated intestinal fluid (SIF) at pH 4.5 and pH 6.8, and simulated body fluid (SBF) at pH 7.4. The CDD-CANP suspension (20 mL) and free CDD at an equivalent concentration to that in the CDD-CANPs were loaded into separate dialysis bags (molecular weight cutoff of 35 kDa; Fisher Scientific, USA) and suspended in 150 mL of the respective release medium at 37°C with stirring at 150 rpm. At the indicated time intervals, 2 mL of the release medium was withdrawn (sampled) and immediately replaced with fresh release medium. The amount of CDD in the withdrawn samples was determined by UV-vis spectrophotometry at a wavelength of 405 nm. All experiments were performed in triplicate.

In order to evaluate the release kinetics of CDD-CANPs, the release data were fitted to the zero order, first order, Higuchi, Korsmeyer-Peppas and Hixon-Crowell kinetic models. The criteria for choosing the best fitted model were as follows: the R^2_{adjusted} value should be close to 1, the model selection criteria (MSC) should be high and the Akaike information criterion (AIC) should be low[16]. Data analysis was performed using the Excel add-in DDSolver program[17].

Results and Discussion

Characterization of the CDD-CANPs

The particle size of the CDD-CANPs was 327 ± 14 nm with a PDI of 0.43, indicating a narrow size distribution of the CDD-CANPs. From TEM image as shown in Figure 1 also confirmed that the CDD-CANPs were well dispersed as individual particles with a spherical shape. However, the particle size from TEM analysis was found smaller than that observed from dynamic light scattering (DLS) analysis using a Zetasizer. The reason of the difference in the particle size is that the TEM image was acquired on dry samples under vacuum and gave the true radius of the particles whereas the DLS profile was obtained in an aqueous solution and provided the hydrodynamic radius of the particles[18,19]. However, the common technique for particle size analysis is DLS because it is applicable for samples in the liquid phase[20].

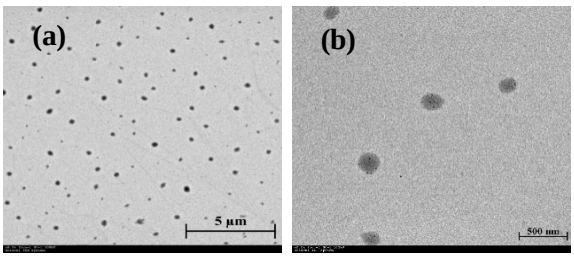

Figure 1. TEM image of CDD-CANPs: (a) overview image (Magnification 10,000X); (b) zoomed-in image (Magnification 60,000X).

The other important parameter is zeta potential which indicates the stability of the colloidal systems. Wu et al.[21] suggested that the zeta potential above ± 20 mV indicated a good stability. In this study, the zeta potential of -27.3 ± 0.2 mV was observed for the prepared CDD-CANPs, suggesting its good stability. The encapsulation efficiency and loading capacity were 51.2 ± 2.2% and 11.7 ± 0.8%, respectively. Together, these results were compatible with those reported previously[12], indicating the reproducibility of this preparation method for CDD-CANPs designed and optimized by Box-Behnken design (BBD) and response surface methodology (RSM), respectively. The RSM is an effective tool for optimization in which a response of interest is affected by several factors[22]. The BBD is the most popular design among all designs in RSM because it requires fewer runs in 3 factors and 3 levels experimental design than all other designs in RSM[23].

In vitro release study.

The cumulative release profiles of CDD from CANPs in the SGF pH 1.2, SIF pH 4.5 and pH 6.8 (without enzymes) and SBF pH 7.4 are shown in Figure 2. The free CDD was initially released very fast (> 50% in 6 h) and almost 80% was released within 24 h in all four simulated fluids, with sustained release of the last 20%. In contrast, the CDD was released at a slower rate from the CDD-CANPs with sustained release being observed after 72 h in all four simulated fluids. With this formulation CANPs, the CDD was released faster in an acidic condition compared to that in a near neutral (pH 6.8) and alkaline (pH 7.4) condition. For example, 51%, 59%, 38% and 24% of the CDD was released after 24 h from the CDD-CANPs at a pH of 1.2, 4.5, 6.8 and 7.4, respectively. This is possibly due to an increased solubility of chitosan in the acidic environment. At an acidic pH, chitosan was dissolved from the CANP matrix leading to the matrix erosion and rapid release of CDD. In contrary, at neutral or alkaline pH, chitosan is not swell and soluble and that therefore limits the release of the CDD from the matrix[24]. Gonçalves et al.[25] suggested that the drug release mechanism from nanoparticles (NPs) could occur by desorption, diffusion or degradation of the NPs. In this study, the release kinetics and mechanism of CDD release from CDD-CANPs were evaluated by fitting the data to the zero order, first order, Higuchi, Korsmeyer-Peppas and Hixon-Crowell mathematical models (Table 1). From the criteria for choosing the best fitted model (see methods), the release of CDD from CDD CANPs was best fitted by the Korsmeyer-Peppas model for all four simulated media (Table 1). This mathematical model, also known as the "Power Law", is a generalized model of the Higuchi equation that is used to explain drug delivery mechanisms where dissolution or erosion of the matrix occur. This model has been frequently used to describe the drug release from polymer systems and various pharmaceutical modified release dosage forms[26–28]. In the selected Korsmeyer-Peppas model, the value of n characterizes the drug release mechanism as follows: $n \leq 0.45$ (Fickian diffusion mechanism), $0.45 < n < 0.89$ (non-Fickian transport), $n = 0.89$ (Case II (relaxational) transport) and $n > 0.89$ (super case II transport)[29]. Given that the value in this study was in the range of 0.38 - 0.49, it suggests that Fickian diffusion was the controlling factor in CDD release from the CDD-CANP.

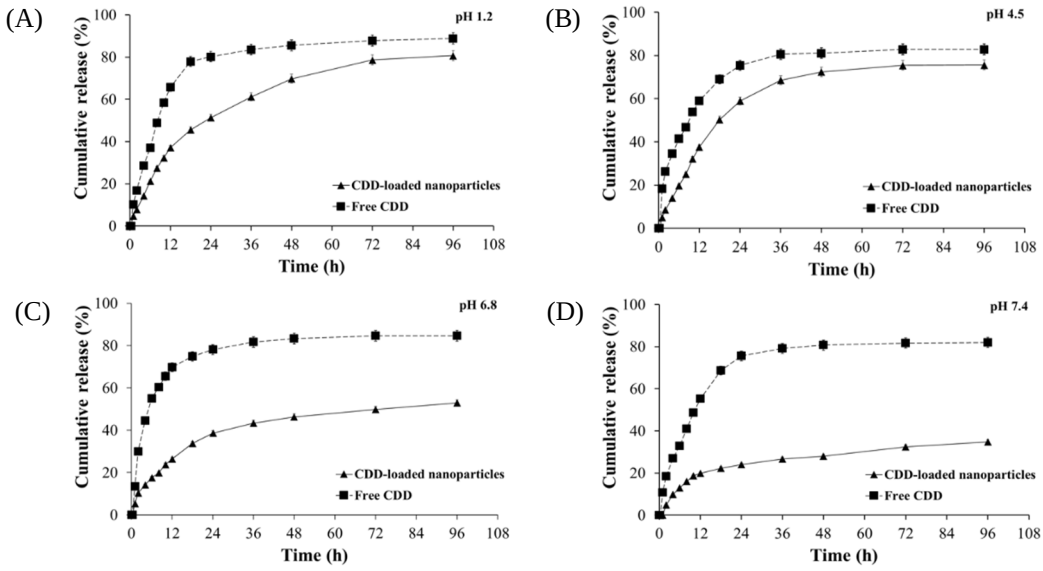

Figure 2. *In vitro release* profiles of CDD from the CDD-CANPs in simulated gastrointestinal fluids (without enzymes) and body fluid. (A) SGF pH 1.2, (B) SIF pH 4.5, (C) SIF pH 6.8 and (D) SBF pH7.4. Data are shown as the mean ± SD, derived from three independent repeats.

Table 1 Mathematical kinetic models used to describe release characteristics of CDD from CDD-CANPs in simulated gastrointestinal fluids (without enzymes) and simulated body fluid.

Model	Media	Parameter	$R^2_{adjusted}$	AIC	MSC
Zero order ($F = k_0.t$)	SGF	k_0 = 1.156	0.6500 ± 0.17	135.92 ± 0.18	0.61 ± 0.22
	$SIF_{4.5}$	k_0 = 1.252	0.6856 ± 0.31	137.21 ± 0.26	0.74 ± 0.72
	$SIF_{6.8}$	k_0 = 0.731	0.3455 ± 0.14	130.32 ± 0.58	-0.14 ± 0.63
	SBF	k_0 = 0.496	0.4239 ± 0.26	115.98 ± 0.35	-0.16 ± 0.81
First order ($F = 100.\ e^{-k_1 t}$)	SGF	k_1 = 0.029	0.9592 ± 0.71	101.52 ± 0.11	2.76 ± 0.42
	$SIF_{4.5}$	k_1 = 0.034	0.9869 ± 0.22	86.33 ± 0.43	3.92 ± 0.27
	$SIF_{6.8}$	k_1 = 0.013	0.6204 ± 0.72	121.61 ± 0.27	0.41 ± 0.59
	SBF	k_1 = 0.007	0.5628 ± 0.39	111.56 ± 0.54	0.11 ± 0.73
Higuchi ($F = k_H.t^{0.5}$)	SGF	k_H = 9.383	0.9694 ± 0.35	96.93 ± 0.46	3.05 ± 0.73
	$SIF_{4.5}$	k_H = 10.967	0.9663 ± 0.64	101.46 ± 0.76	2.98 ± 0.11
	$SIF_{6.8}$	k_H = 6.177	0.8977 ± 0.73	100.63 ± 0.19	1.72 ± 0.66
	SBF	k_H = 4.148	0.9157 ± 0.84	85.22 ± 0.15	1.76 ± 0.82
Korsmeyer-Peppas* ($F = k_{KP}.t^n$)	SGF	k_{KP} = 10.267, n = 0.476	0.9868 ± 0.18	98.25 ± 0.55	2.97 ± 0.62
	$SIF_{4.5}$	k_{KP} = 10.217, n = 0.497	0.9670 ± 0.83	103.45 ± 0.55	2.85 ± 0.71
	$SIF_{6.8}$	k_{KP} = 9.625, n = 0.382	0.9365 ± 0.53	93.90 ± 0.39	2.14 ± 0.34
	SBF	k_{KP} = 6.134, n = 0.396	0.9431 ± 0.18	79.83 ± 0.71	2.11 ± 0.56
Hixon-Crowell ($F = 100.[1-(1-k_{HC}.t)^3]$)	SGF	k_{HC} = 0.008	0.9166 ± 0.72	112.97 ± 0.38	2.05 ± 0.11
	$SIF_{4.5}$	k_{HC} = 0.010	0.9634 ± 0.39	102.81 ± 0.85	2.89 ± 0.48
	$SIF_{6.8}$	k_{HC} = 0.004	0.5359 ± 0.11	124.82 ± 0.47	0.21 ± 0.29
	SBF	k_{HC} = 0.002	0.5182 ± 0.23	113.12 ± 0.59	0.02 ± 0.17

**Best fitted release kinetic model for CDD-CANPs. F is the fraction (%) of drug released in time t; k_0 is the zero-order release constant; k_1 is the first-order release constant; k_H is the Higuchi release constant; k_{KP} is the release constant incorporating structural and geometric characteristics of the drug-dosage form; n is the diffusional exponent indicating the drug-release mechanism; k_{HC} is the Hixon-Crowell release constant [17]. Data are shown as the mean ± SD, derived from three independent samples.*

Conclusions

The results of the *in vitro* release studies demonstrated that encapsulation of CDD by CANPs improved the subsequent sustained release of CDD. The Korsmeyer-Peppas kinetic model best fitted the data to describe the CDD release mechanism, and supported that CDD was released by Fickian diffusion.

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References

1. Wichitnithad, W., Nimmannit, U., Wacharasindhu, S., and Rojsitthisak, P. (2011). Synthesis characterization and biological evaluation of succinate prodrugs of curcuminoids for colon cancer treatment. *Molecules*. **16(2):** 1888-1900.

2. Ratnatilaka Na Bhuket, P., Niwattisaiwong, N., Limpikirati, P., Khemawoot, P., Towiwat, P., Ongpipattanakul, B., and Rojsitthisak, P. (2016). Simultaneous determination of curcumin diethyl disuccinate and itsactive metabolite curcumin in rat plasma by LC-MS/MS: Application of esterase inhibitors in the stabilization of an ester-containing prodrug. *J. Chromatogr B.* **1033-1034:** 301-310.

3. Li, P., Dai, Y., Zhang, J., Wang, A., and Wei, Q. (2008). Chitosan-alginate nanoparticles as a novel drug delivery system for nifedipine. Int *J. Biomed Sci.* **4(3):** 221-228.

4. Bhunchu, S., and Rojsitthisak, P. (2014). Biopolymeric alginate-chitosan nanoparticles as drug delivery carriers for cancer therapy. *Pharmazie*. **69(8):** 563-570.

5. Katuwavial, N.P., Perera, A.D.L.C., Samarakoon, S.R., Soysa, P., Karunaratne, V., Amaratunga, G.A.J., and Karunaratne, D.N. (2016). Chitosan-Alginate Nanoparticle System Efficiently Delivers Doxorubicin to MCF-7 Cells. *J. Nanomater*. ***2016:*** Article ID 3178904, 12 pages.

6. Szekalska, M., Puciłowska, A., Szymańska, E., Ciosek, P., and Winnicka, K., (2016). Alginate: Current use and future perspectives in pharmaceutical and biomedical applications. *J. Polym. Sci.* **2016:** Article ID 7697031, 17 pages.

7. Lertsutthiwong, P., Rojsitthisak, P., and Nimmannit, U. (2009). Preparation ofturmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. *Mater Sci Eng C*. **29(3):** 856-860.

8. Motwani, S.K., Chopra, S., Talegaonkar, S., Kohli, K., Ahmad, F.J., and Khar, RK. (2008). Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and in vitro characterisation. Eur. *J Pharm Biopharm*. **68(3):** 513-525.

9. Zohri, M., Alavidjeh, M.S., Mirdamadi, S.S., Behmadi, H., Nasrsima, S.M.H., Gonbaki, S. E., Ardestani, M. S., and Arabzadeh, A.J. (2013). Nisin-loaded chitosan/alginate nanoparticles: A hopeful hybrid biopreservative. *J. Food Safety*. **33(1):** 40-49.

10. Balaji, RA., Raghunathan, S., and Revathy, R.. (2015). Levofloxacin: formulation and in vitro evaluation of alginate and chitosan nanospheres. *Egypt Pharm J.* **14(1):** 30-35.

11. Loquercio, A., Castell-Perez, E., Gomes, C., and Moreira, RG. (2015). Preparation of chitosan-alginate nanoparticles for trans-cinnamaldehyde entrapment. *J Food Sci*. **80(10):** N2305-2315.

12. Bhunchu, S., Rojsitthisak, P., and Rojsitthisak, P. (2015). Response surface methodology to optimize the preparation of chitosan/ alginate nanoparticles containing curcumin diethyl disuccinate. *Adv Mater Res.* **1119:** 398-402.

13. Lee, KY., and Mooney, DJ. (2013). Alginate: properties and biomedical applications. *Prog Polym Sci.* **37(1):** 106-126.

14. Debrassi, A., Bürger, C., Rodrigues, CA., Nedelko, N., Ślawska Waniewska, A., Dłuewski, P., Sobczak, K., and Greneche, J.M. (2011). Synthesis, characterization and in vitro drug release of magnetic N-benzyl-O-carboxymethyl chitosan nanoparticles loaded with indomethacin. *Acta Biomater.* **7(8):**3078-3085.

15. Chereddy, K.K., Coco, R., Memvanga, P.B., Ucakar, B., DesRieux, A., Vandermeulen, G., and Préat, V. (2013). Combined effect of PLGA and curcumin on wound healing activity. *J Control Release.* **171(2):** 208-215.

16. Yuksel, N., Kanik, AE., and Baykara, T. (2000). Comparison of in vitro dissolution profiles by ANOVA-based, model dependent and independent methods. Int. *J Pharm.* **209(1-2):** 57-67.

17. Zhang, Y., Huo, M., Zhou, J., Zou , A., Li, W., Yao, C., and Xie, S. (2010). DD Solver: An add-in program for modeling and comparison of drug dissolution profiles. *AAPS J.* **12(3):** 263-271.

18. Wang, B., Yu, X-C., Xu, S-F., and Xu, M. (2015). Paclitaxel and etoposide co-loaded polymeric nanoparticles for the effective combination therapy against human osteosarcoma. *J. Nanobiotechnol.* **13:** 22.

19. Lim, J., Yeap, S., Che, H., and Low, S. (2013). Characterization of magnetic nanoparticle by dynamic light scattering. *Nanoscale Res Lett.* **8(1):** 381.

20. Segets, D. (2016). Analysis of particle size distributions of quantum dots: From theory to application. *KONA Powder Part J.* **2016(33):** 48-62.

21. Wu, L., Zhang, J., and Watanabe, W. (2011). Physical and chemical stability of drug nanoparticles. *Adv Drug Deliv Rev.* **63(6):** 456-469.

22. Yeniay, Ö. (2014). Comparative study of algorithms for response surface optimization. *Math Comput Appl.* **19(1):** 93-104.

23. Trivedi, D., Karri, VVSR., Spandana, AKM., and Kuppusam, G. (2015). Design of Experiments: Optimization and applications in pharmaceutical nano-technology. *Chem Sci Rev Lett.* **4(13):** 109-120.

24. Wang, F., Yang, S., Yuan, J., Gao, Q., and Huang, C. (2016). Effective method of chitosan-coated alginate nanoparticles for target drug delivery applications. *J Biomater Appl.* **31(1):** 3-12.

25. Gonçalves, C., Pereira, P., and Gama, M. (2010). Self-assembled hydrogel nanoparticles for drug delivery A*pplications. Materials*. **3(2):** 1420-1460.

26. Yarce, C., Echeverri, J., Palacio, M., Rivera, C., and Salamanca, C. (2016). Relationship between surface properties and *In Vitro* drug release from a compressed matrix containing an amphiphilic polymer material. *Pharmaceuticals*. **9(3):** 34.

27. Costa, P., Sousa Lobo, J.M. (2001). Modeling and comparison of dissolution profiles. *Eur J Pharm Sci.* **13(2):** 123-133.

28. Ramteke, KH., Dighe, PA., kharat, AR., Patil, SV. (2014). Mathematical models of drug dissolution: A review. *Sch Acad J Pharm*. **3(5):** 388-396.

29. Dash, S., Murthy, P. N., Nath, L., and Chowdhury, P., (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm.* **67(3):** 217-223.