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Synthesis and Evaluation of β-cyclodextrin Based Nanosponges of 5-Fluorouracil Using Ultrasound Assisted Method.

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Abstract

5-Fluorouracil (5-FU) is mostly used in the treatment of stomach cancer. After intravenous injection of 5-FU, it is rapidly distributed and eliminated with an apparent terminal half-life of 8-20 min. It is poorly absorbed after oral administration with an extremely variable bioavailability. Hence, this study has been made to synthesize 5-FU nanosponges (NS) to increase its accumulation in gastric tumors by the help of an enhanced permeability retention effect (EPR) and decrease its systemic side effects. On the other hand, 5-FU is sparingly soluble in water so its dissolution can be increased by incorporation in nanosponges as nanoparticles.

CD-nanosponges were prepared by crosslinking β-CD with diphenylcarbonate (DPC) using ultrasound assisted technique. 5-FU was incorporated with NS by freeze drying, and the phase solubility study, complexation efficiency (CE) entrapment efficiency were performed. Also, the particle morphology was studied using SEM and AFM. The in-vitro release of 5-FU from the prepared nanosponges was carried out in 0.1N HCl.

5-FU nanosponges particle size was in the nano size. The optimum formula showed a particle size of (405.46±30) nm, with a polydispersity index (PDI) (0.328±0.002) and a negative zeta potential (-18.75±1.8). Also the drug entrapment efficiency varied with the CD: DPC molar ratio from 15.6 % to 30%. The SEM and AFM showed crystalline and porous nature of the nanosponges. The in vitro drug release study of the selected formula 5-FUNS2 exhibited the fastest dissolution rate which is 56% in the first hr.

Different molar ratios of (cyclodextrin to crosslinker) (CD: DPC) has a proficient effect on complexation efficiency (CE), apparent stability constant (Kst) and entrapment efficiency of 5-FU. 5-FUNS2 with (1:4) molar ratio showed the best result of CE, Kst and entrapment efficiency. 5-FUNS2 gave a higher release rate than the 5-FU-βCD inclusion complex and 5-FU solution. Surface morphology of the prepared nanosponges by SEM, AFM indicate that nanosized and highly porous nanosponges was obtained. The overall results suggest that cyclodextrin nanosponges could be a promising 5-FU delivery system utilizing the suitable formula.

Keywords: 5-FU, nanosponges, ultrasound assisted method, βCD, DPC, SEM, AFM.

تحضير وتقييم جسيمات الإسفنجية النانوية لعقار ٥-فلوروبوراسيل باستخدام البيتا سايكلودكستيرين بمساعدة الموجات فوق الصوتية

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الخلاصه

٥ فلوروبوراسيل 5-FU يستخدم بشكل شائع في علاج سرطان المعدة . بعد حقنه في الوريد ، يتم توزيعه بسرعة ويتم التخلص منه مع عمر نصف تبلغ ٨-٢٠ دقيقة. يتم امتصاص ٥ فلوروبوراسيل بشكل ضعيف بعد تناوله عن طريق الفم مع توفر حيوي متغير للغاية. وبالتالي ، في هذه الدراسة قد تم تصنيع جسيمات إسفنجية من 5-FU لزيادة تراكم الدواء في أورام المعدة عن طريق المساعدة في تعزيز تأثير الاحتفاظ بالنفاذية (EPR)وتقليل الآثار الجانبية الناتجة عن انتشار الدواء في الجسم. أيضا ، ٥ فلوروبوراسيل شحيح الذوبان في الماء. يمكن زيادة معدل ذوبانه عن طريق تضمينه في إسفنجيات النانو-البيتا سيكلودكستيرين.

تم تصنيع جسيمات البيتا سيكلودكستيرين الإسفنجية النانوية عن طريق ربط باواصر تساهمية للبيتا سايكلودكسترين مع الكربونات ثنائية الفينول بمساعدة الموجات فوق الصوتية.تم دمج 5-FU داخل الجسيمات الإسفنجية النانوية بواسطة طريقة التجفيف بالتجميد وكذلك تم إنجاز دراسة طور الذوبانية و كفاءة تكوين المعقد (CE) و كفاءة حجز الدواء. كما تم قياس حجوم الجزيئات و جهد زيتا باستخدام محلل زيتا بلس. تمت دراسة شكل الجسيمات أيضًا باستخدام SEM و AFM و تم قياس تحرر الدواء داخل المختبر لـ 5-FU من الجسيمات الإسفنجية النانوية في محلول حامض الهيدروكلوريك المخفف بتركيز عياري ٠,١.

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حجم الجسيمات الإسفنجية النانوية لعقار 5-FU كان في حجم النانو ، الصيغة المثلى توضح حجم الجسيمات (٤٠٥,٤٦ ± ٣٠) نانومتر PDI,(0.002 ± 0.328) وجهد زيتا سالبة (-١٨,٧٥ ± ١,٨). تباينت معدل الدواء المحتجز مع النسبة المولية (CD: DPC) من ١٥,٦ ± ٢,٦٪ إلى ٣٠ ± ٢,٣٪. وأظهرت SEM و AFM الطبيعة البلورية والمثقبة للجسيمات الإسفنجية. أظهرت دراسة التحرر الدوائي للصيغة المثلى في المختبر أسرع معدل تحرر ٥٦ ٪ في الساعة الأولى.

النسب المولية المختلفة لـ (البيتا سيكلودكستيرين الى المكون للروابط التساهمية) (CD: DPC) لها تأثير كبير على معدل CE و Kst و كمية الدواء المحتجزة .الصيغة5-FUNS2 ذو نسبة المولية (١: ٤) يظهر أفضل نتيجة لـ CE، Kst ونسبة احتجاز الدواء. يوفر 5-FUNS2 معدل تحرير أعلى من (5-FU-βCD)ومحلول 5-FU .التشكل السطحي للإسفنجة النانوية المحضرة بواسطة SEM، AFM و توضح الحجم النانو ونسبة الثقوب العالية .

النتائج الإجمالية تشير إلى أن الجسيمات النانو للبيتا سيكلودكستيرين يمكن أن تكون نظام واعد لتسليم الدواء باستخدام الصيغة المناسبة.

الكلمات المفتاحية : ٥ فلوروويوراسيل ، الجسيمات النانوية الإسفنجية ، طريقة الموجات فوق الصوتية المساعدة ، βCD، DPC، SEM، AFM.

Introduction

Although chemotherapeutic agents can reduce tumor size and cancer remission and have a high potential to destroy cancer cells, they are not organ specific and can damage proliferative cells[1].

One of the major goals of cancer therapeutics is to kill cancer cells without damaging normal tissues. One way to achieve this is the use of molecularly targeted therapy combined with chemotherapy. Tissue and cell distribution of cancer therapeutic drugs can be controlled by the entrapment in sub-micron level (<1 μm) colloidal systems, in other words known as nanoparticles. Some of the desirable characteristics that are needed to deliver therapeutic agents to tumor cells include the ability to overcome drug resistance at the tumor and cellular levels and ensure an appropriate distribution, biotransformation, and clearance of the drug [2].

Conventional chemotherapeutic agents work by destroying rapid dividing cells, which is the main property of neoplastic cells. This is why chemotherapy also damages normal healthy cells that divide rapidly such as cells in the bone marrow, macrophages, digestive tract, and hair follicles [3].

Nanosponges are hyper-cross-linked cyclodextrins that can be obtained with α, β and γ cyclodextrins, either alone or as mixtures containing relevant amounts of linear dextrin, cross-linked with a suitable cross-linking molar ratio, by using an active carbonyl compound, e.g., diphenyl carbonate, by ultrasound-assisted synthesis. Thus, spherical nanosponges of submicron size of cyclodextrin are connected by nanochannels to form a cage-like structure. These nanosponges can be inclusion complex drug carriers[4].

Nanotechnology have been applied to improve drug delivery and to overcome some of the problems of drug delivery for cancer treatment [5]. Nanosponges are a novel class of hyper-cross linked polymer based colloidal structures consisting of solid nanoparticles with colloidal and nanosized cavities. Nanosponges solubilizes poorly water soluble drugs and provides a prolong release as well as improves the drug bioavailability by modifying the pharmacokinetic parameters of active constituents [6, 7].

5-Fluorouracil (5-FU) was most commonly used in the treatment of cancers of colon, breast, stomach and pancreas [8]. However, like other drugs used for chemotherapy, it affects the growth of normal body cells and often causes side effects such as hair loss, fatigue, birth defects, mouth sores, liver disease, and a temporary drop in bone marrow function [9].

After intravenous injection of 5-FU, it is rapidly distributed and eliminated with an apparent terminal half-life of 8-20 min with a pKa of 8, and 13 , LogP(-1) [10]. Also 5-FU is poorly absorbed after oral administration with an extremely variable bioavailability [11].

The aim of the study was synthesis and evaluation of 5-FU loaded nanosponges to enhance the dissolution rate of the sparingly soluble 5-FU. Also nanosponge increases its accumulation in gastric tumors by the help of an enhanced permeability retention effect (EPR) and decrease its systemic side effects. As it is intended to be formulated as floating tablet for local gastric cancer therapy in the future study.

Materials and Methods

Materials

5-Fluorouracil, βCD and diphenyl carbonate (DPC) were obtained from Hyper-chem Ltd Co. (Hangzhou, China). All other analytical reagents were of analytical grade.

Methods

Preparation of β-CD-nanosponges using ultrasound assisted method

Accurate amounts of βCD and diphenyl carbonate DPC were mixed in 100ml beaker at a different molar ratio as shown in Table (1). The beaker was then placed in an oil bath and heated to 90°C.Then the mixture was sonicated for 4 hours at 50% amplitude using ultrasound probe capable of supplying maximum power of 500 Watt at 20 kHz (Qsonica, USA). The reaction mixture is left to cool and the product obtained is broken up roughly. Numerous needle-shaped crystals of phenols can be seen on the clear surface of the beaker as shown in Figure (1) and part of the phenol developed contributes to agglomerating of the product [12].

Subsequently after cooling, the product was broken up roughly by mortar and repeatedly washed with an excess amount of distilled water (DW) through filtration by the Buchner funnel to remove unreacted βCD. An additional purification step consists of Soxhlet extraction in ethanol, which was performed for 24 hours to remove the unreacted DPC and phenol present as by-product of the reaction. Finally, the nanosponges (NS) were dried at room temperature to obtain a fine white powder [13-16].

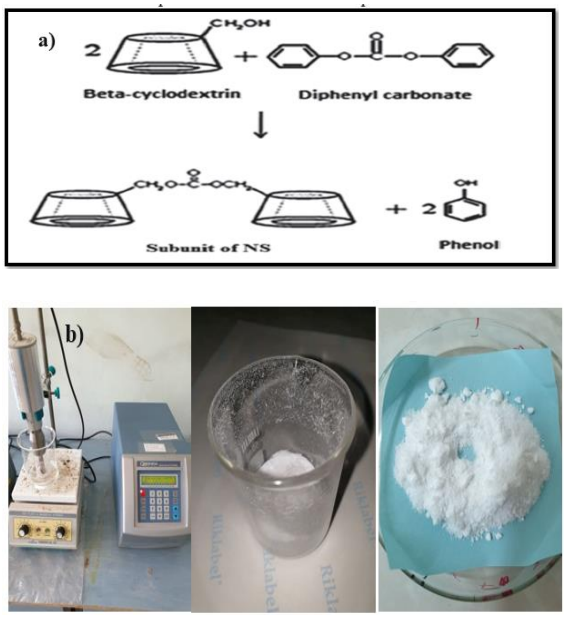

Figure1. a) Structural representation of reaction for preparation of NS, b) Ultrasound assisted method

Table1. The composition and molar ratios of CD:

Formulas codes***	β-CD:DPC** Molar ratio	β-CD (g)	DPC (g)
NS 1	1:2	2.27	0.856
NS 2	1:4	2.27	1.713
NS 3	1:6	2.27	2.57
NS 4	1:8	2.27	3.427
NS 5	1:10	2.27	4.28

DPC used to prepare βCD-NS*.

* β-CD β-cyclodextrin, DPC Dipenyl carbonate.**1 mole of β-CD =1.135 g, 1mole of DPC = 0.2142g.***NS1-NS5 plain NS

Preparation of 5-FU loaded nanosponges

5-Fluorouracil loaded NS were prepared by freeze drying method[17]. Briefly, the prepared βCD nanosponges at different CD:DPC molar ratios and an excess amount of 5-FU as a powder were mixed and the resultant mixture was suspended in 30 ml distilled water. Then, the mixture was sonicated for 10 min and stirred for 24 hours using a magnetic stirrer (Copley, Germany). The obtained aqueous suspension was centrifuged for 10 min at 2000 rpm to separate the uncomplexed drug as a deposit. The supernatant was then lyophilized by employing a lyophilizer (Copley, Germany) to get 5-FU loaded [18].

Preparation of 5-FU inclusion complexes

One formula of β-cyclodextrin inclusion complex with 5-FU (5-FU–βCD) was prepared. A weighted amount of 5-FU was finely suspended in a water solution containing an equimolar amount of βCD and 5-FU (1:1). The aqueous suspension was then stirred at room temperature in the dark place for 24 h. After centrifugation (5000 rpm, 10 min),(Labent, Germany) then the supernatant was freeze-dried[19].

Production yield of the prepared nanosponges

Production yield: The production yield can be determined by calculating initial weight of raw materials and final weight of nanosponges obtained [20]:

$$\textbf{Production yield} = \frac{\boldsymbol{Practical\ mass\ of\ nanosponges}}{\boldsymbol{Theoritical\ mass\ of\ nanosponge(polymer + crosslinker)}} \times \mathbf{100}$$

Encapsulation efficiency

Weighed amount of 5-FU-loaded nanosponges were dispersed in DW and sonicated for 10 min, then centrifuged at 15,000 rpm for 15 min (Copley, Germany) double cycle after that the supernatant was withdrawn, suitably diluted with distilled water and were subjected to UV spectroscopy for measuring the absorbance of the sample at the λmax of 5-FU (266 nm). With the help of absorbance, the concentration in the supernatant was determined by plotting the absorbance value against concentration in the standard curve. Furthermore, the total drug content of 5-FU was determined by dissolving a same amount of 5-FU loaded NS in methanol and sonicated for 10 min to destroy and break the complex to calculate the total amount of 5-FU present in the 5-FU loaded NS powder . The percentage encapsulation efficiency was calculated by following equation[21]:

$$\%\ \textbf{Encapsulation efficiency} = \frac{\boldsymbol{Drug_{encapsulated}}}{\boldsymbol{Drug_{total}}} * \mathbf{100}$$

Phase solubility studies

Phase solubility studies were carried out according to the Higuchi–Connors method[22]. An accurate amount of 5-FU (100 mg) was added to a series of aqueous solutions (5 mL) containing increasing concentrations of βCD-NS, from (9.5 to 12.7) mM and in βCD (1:1). The samples were stirred in the dark at room temperature for 5 days. After equilibration, the aqueous suspensions were centrifuged and the 5-FU content in the supernatant was determined by UV spectrophotometer at 266 nm.

The phase solubility diagram was constructed by plotting the total molar concentration of 5-FU against the molar concentration of βCD-NS. Stability constants (Kst) from the phase solubility diagram were calculated using the Equation (1):

$$\mathbf{Kst} = \frac{\mathbf{slope}}{S_0(1-slope)} \qquad (1)$$

Where, S_0 represents the solubility of 5-FU in the absence of CD. The slope was determined from the initial linear part of the concentration curves of 5-FU.

The complexation efficiency (CE) is the concentration ratio between cyclodextrin in a complex and free cyclodextrin, and it was calculated from the phase-solubility diagrams [15].

The complexation efficiency is calculated by the slope of the phase-solubility profile using equation 2, which is referred to as the complexation efficiency (CE) [23].

$$\mathrm{CE} = \mathrm{S_0K1{:}\,1} = \frac{{}^{\mathrm{D}}/_{\mathrm{CD}}}{\mathrm{CD}} = \frac{\mathrm{Slope}}{1-\mathrm{Slope}}$$

Since, the numerical value of CE is only dependent on the slope of the phase-solubility profile, less variation is usually observed in the CE values compared to the stability constant Kst value.

Characterization of the prepared 5-FU loaded nanosponges

Particle size, Polydispersity Index analysis (Dynamic light Scattering) and zeta potential

Nanosponges sizes and polydispersity index were measured by dynamic light scattering using a 90 plus particle sizer (ZetaPlus Particle Sizing, NY, Software, Version 5.34), The samples were suitably diluted with water prior to measurements. Zeta potential measurements were also made using an additional electrode in same instruments.

The mean hydrodynamic diameter (Dh) and polydispersity index (PI) of the particles were calculated in intensity using the cumulant analysis after averaging *the three measurements [17, 24]*.

Fourier Transform-Infrared Spectroscopy (FT-IR)

ATR–FTIR spectra of 5-FU, DPC, βCD, βCDNS and 5-FUNS were recorded on a IRAffinity-S1 Spectrum FT-IR (Shimadzu , Japan) in the region of 4000–650 cm^{-1}. It was performed, using a Shimadzu spectrophotometer, to confirm the formation of βCDNS and understand if there are interaction between drug and NS[15].

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (Tescan Mira3,France) was significant for determination of surface characteristics and size of the particle. Scanning electron microscope was operated at an acceleration voltage of 15 kV[25].

Atomic Force Microscopy (AFM)

A further in-depth morphological analysis was performed using an atomic force microscope (Angstrom Advanced Inc. AA3000) with a scanner of 3.1 µm with three piezo electrodes for three axes X, Y and Z in a noncontact mode. The sample suspensions (1% w/v) were prepared in distilled water and a drop was impregnated onto aluminum sheet (2 cm×2 cm). This was allowed to dry in a HEPA filter zone and the dried region was analyzed[26].

In-vitro release study of 5-FU nanosponges

In-vitro release study of 5-FU from 5-FU-βCD inclusion complex and the selected formula of 5-FUNS was performed by using an accurate amount of impregnated nanosponges equivalent to 100 mg 5-FU suspended in 5 ml of 0.1N HCl solution which was placed in the dialysis membrane (cut off 12,000 Da) and the samples were individually placed in dissolution vessel containing 900 ml of 0.1N HCl, maintained at 37 ± 0.5°C at 75 rpm using a paddle dissolution apparatus (USP Type II). . At various time intervals, aliquots of 5ml were withdrawn and replaced with the same volume of fresh dissolution medium to maintain the sink conditions and the withdrawn samples were analyzed by UV spectrophotometer (EMCLAB, Germany) at 266 nm[27, 28].

Statistical analysis

The results are reported as the mean±SD and statistical significance was determined using one-way analysis of variance (ANOVA) and Student's t-tests as appropriate. All experiments were performed in triplicate and values were expressed as the mean standard deviation SD. Values of $p < 0.05$ were considered statistically significant[29].

Results and Discussion

Production yield

The practical yield of nanosponges was found to be less for lower (β-CD: DPC) molar ratio (1:2). The practical yield increased with the increase in molar ratio up to 1:8, and at higher molar ratios (1:8 - 1:10), the yield was found to be almost the same for both molar ratio. This may be due to saturation of the reactive functional groups at higher concentration[30].the molar ratio significantly ($p<0.05$) affected the production yield of CD NS.

Table2. Production yield percent of the prepared CD NS , data are expressed as Mean ± SD, n =3, standard deviation (SD).

Formulas No.*	Theoretical weight (g)	Production yield (g)±SD	Production yield %
NS 1	2.702	1.85±0.19	48.1
NS 2	3.13	3.18±0.3	63.9
NS 3	3.559	4±0.1	70.2
NS 4	3.987	4.72±0.2	72.7
NS 5	4.414	5.64±0.3	74.8

***NS1-NS5= plain nanosponges.**

Phase solubility study

Phase solubility studies were conducted for all the prepared nanosponges and their respective nativeβ-cyclodextrin in DW. The solubility of 5-FU was found to be about (0.02 M) in the absence of β-cyclodextrins nanosponges. Also, the molar concentration of NS was determined by calculated the molecular weight of NS according to the chemical formula that was mentioned in Figure (1). The phase solubility diagram revealed that the solubility of 5-FU increased linearly as a function of increasing cyclodextrin nanosponges concentration indicating the phase solubility profile obtained was an "A-type" diagram, according to the Higuchi and Connors classification[22] .

Cyclodextrin-based nanosponges showed superior complexing ability than natural cyclodextrins towards many molecules. The parent βCD complex shows lower complexation efficiency(CE) and apparent stability constant (Kst) (0.22) and (10±0.5 M^{-1}) respectively ,as shown in Table (3).These values were lower than that obtained by βCD nanosponges. This gave significant ($p<0.05$) differences of CE and Kst values between the prepared CDNS and the parent CD. This is due to the carbonate linkage which was added to the primary hydroxyl groups of the parent βCD unit. Thus, the drug molecules could be included inside the nanocavities of βCD and due to the cross-linking further interactions of the guest molecules with more βCD units might be thought. Moreover, the presence of the cross-linked network might also form nanochannels in the NS structure for the polymer mesh[16]. Figures (2a) and (2b) shows the phase solubility diagram of 5-FU with the prepared NS and βCD, respectively. The slopes of the curves of complexes were lower than one, demonstrating the formation of 1:1 inclusion complex.

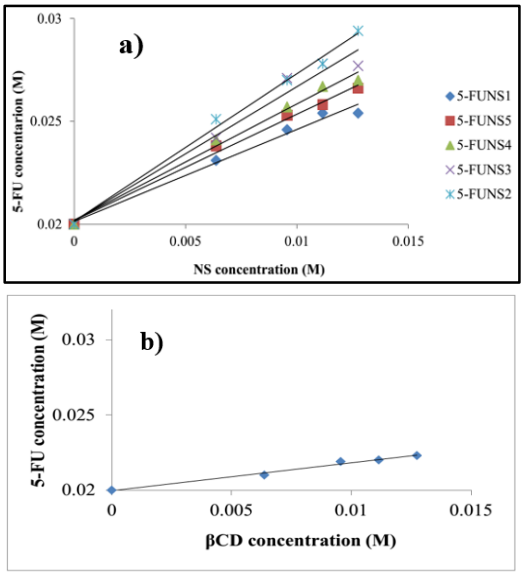

Figure 2.Phase solubility diagram of 5-FU included in ; a) NS, b)βCD

Table3. Different parameters of phase-solubility studies of 5-FU with the prepared NS and βCD in distilled water, at 25 °C.

Formulas codes	Phase solubility study*				
	Slope (M^{-1})	Intercept S_o (M) (5-FU solubility)	R^2	Kst (M^{-1}) mean ± SD*	Complexation efficiency (CE)
5-FUNS 1	0.3856	0.02	0.936	30.2±1.6	0.63
5-FUNS 2	0.6511	0.02	0.9817	89.5±3.6	1.87
5-FUNS 3	0.5811	0.02	0.8699	66.3±2.5	1.39
5-FUNS 4	0.4502	0.0199	0.9725	41.1±1.4	0.81
5-FUNS 5	0.4358	0.02	0.9702	37.2±3.1	0.77
5-FU-βCD Complex	0.18	0.02	0.987	10.7±1.2	0.22

***Data are expressed as mean ± SD, n =3, standard deviation (SD).**

Encapsulation efficiency

Among different types of nanosponges, the encapsulation efficiency of β-CDNS for 5-FU was observed to be higher in 5-FUNS2 (β-$CDNS_{(1:4)}$) as much as 30±2.3% w/w followed by 5-FUNS3(β-$CDNS_{(1:6)}$) 25±2% and NS5 (β-$CDNS_{(1:10)}$) 22±2 % as shown in Table 4.

The results revealed that the degree of cross-linking affected the encapsulation efficiency of nanosponges with a significant difference ($p<0.05$) between 5-FUNS1 and 5-FUNS2. It was found that at 1:2 molar ratio, the degree of cross-linking may be low, resulting in insufficient nanochannels for the guest complexation; thus 5-FU might not be encapsulated in higher amounts.

Particle size, polydispersity index analysis and zeta potential

The dynamic light scattering (DLS) related measurements were carried out after lyophilization. Table 5 illustrates the particle size values of the prepared 5-FU nanosponges of βCD-NS in which the smallest value was (256.3±24nm) and the largest one was (846.83±51nm). The overall sizes of NS found in the submicron range (<1μm) might be due to charge accelerated aggregation and molecular nature of relative CDs, resulting in a size increment. The increased size may be due to the aggregation during the drying process [31].

Zeta potential predicts the long term stability of the nanosized formulations[32]. Zeta potential as a measure of surface charge was tested for 5-FU nanoformulations that have small particle size and lower PDI (5-FUNS2 and 5-FUNS3). The results of zeta potential obtained are presented in Table 4.

Table 4. Characterization of 5-FU nanosponges, (mean±SD) *n=3*

Formula code	CD:DPC	Particle size ± SD (nm)*	PDI	ZP (mV)	Encapsulation efficiency
5-FUNS 1	1:2	545.45±23	0.492±0.01	-	15.6±2.6
5-FUNS 2	1:4	405.46±30	0.328±0.002	-18.75±1.8	30±2.3
5-FUNS 3	1:6	435.43±18	0.464±0.02	-16.1±1.2	25±2
5-FUNS 4	1:8	846.83±51	0.359±0.01	-	19±1.2
5-FUNS 5	1:10	256.3±24	1.711±0.1	-	22±1.7

On the basis of particles size, polydispersity index, zeta potential and encapsulation efficiency formulas 5-FUNS2 was chosen as the optimized formula for the preparation of nanosponges.

Drug- excipients compatibility studies

The spectrum of 5-FU shows characteristic absorption bands in the region between 1656 and 1723 cm^{-1} correlated to the C=C, C=N, C=O, while the region at1247–1425 cm^{-1} was assigned at the vibration of the substituted pyrimidine. The bands at 470, 551, 642, 749, and 813 cm^{-1}, as well as those between 2407 and 3100 cm^{-1} are due to the aromatic ring[33], as shown in Figure(3).

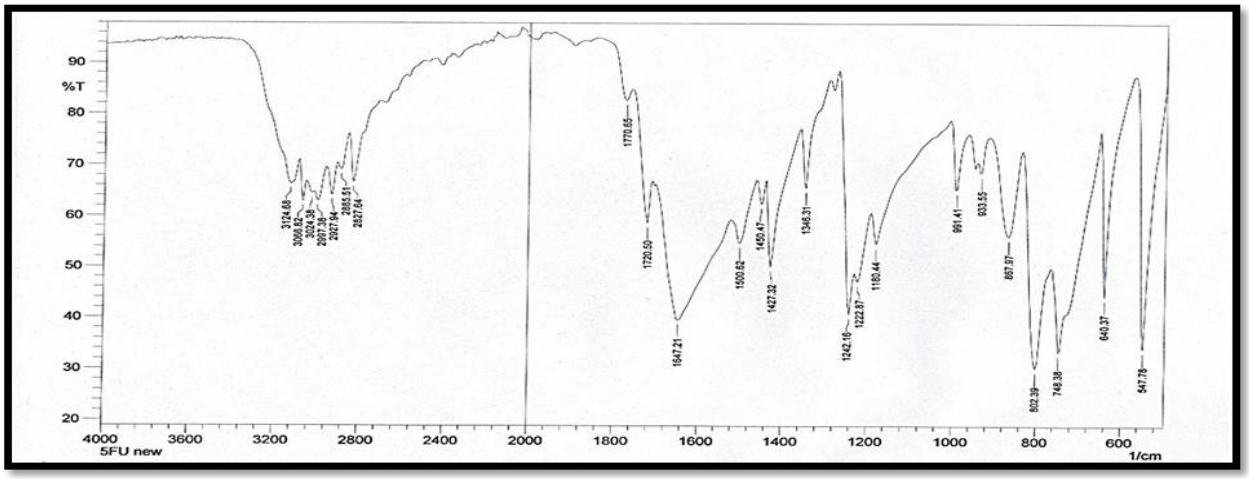

Figure 3. FTIR of 5-FU

The appearance of the new peak of the carbonyl (C=O) group at 1751 cm^{-1} in NS spectra confirmed the successful cross-linking of relative βCDs by DPC in various ratios[31]. The 5-FU characteristic peaks were broadened or shifted in the formulations suggesting definite interactions between 5-FU and NS[16].

The peaks correlated to the aromatic ring for the drug alone are weakened in the spectra of 5-FU loaded NS and some bands in the region between 2407 and 3100 cm^{-1} correlated to the aromatic ring result disappeared. These changes suggest the formation of the inclusion complexes[34], as shown in Fgure(7).

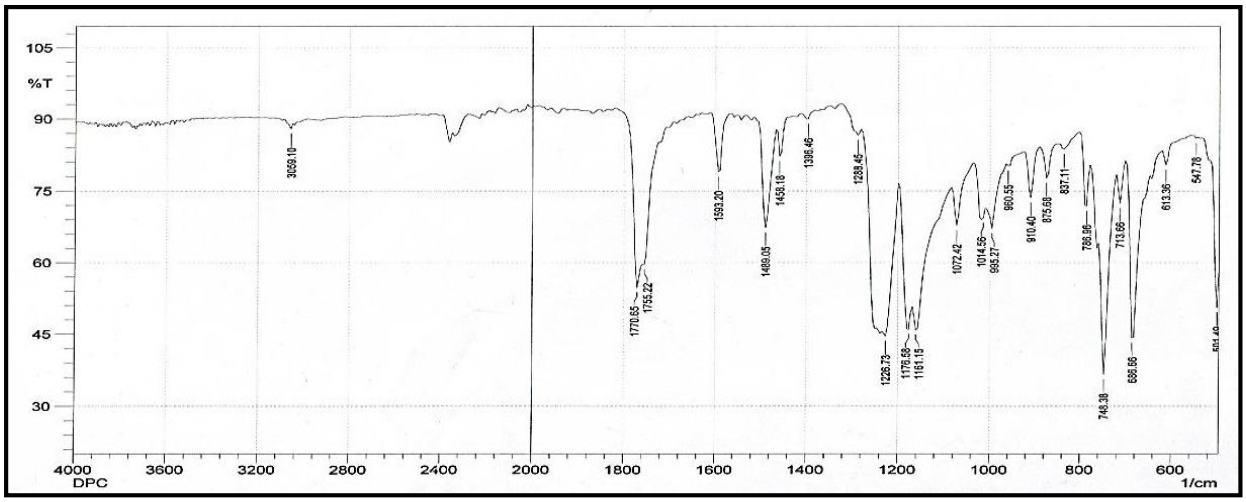

Figure 4. FTIR of di-phenyl carbonate

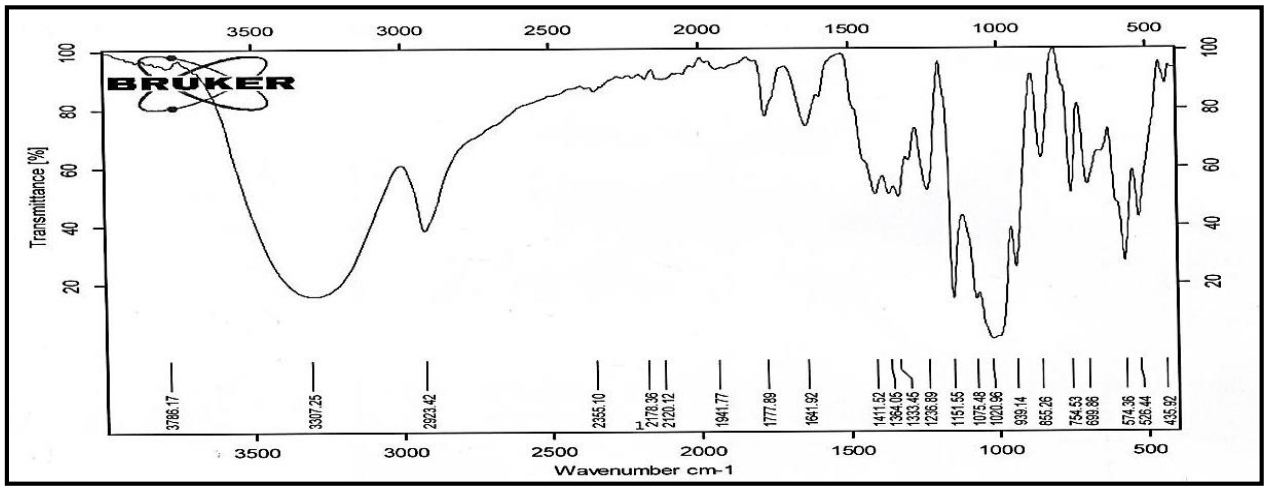

Figure 5:FTIR of β-cyclodextrin

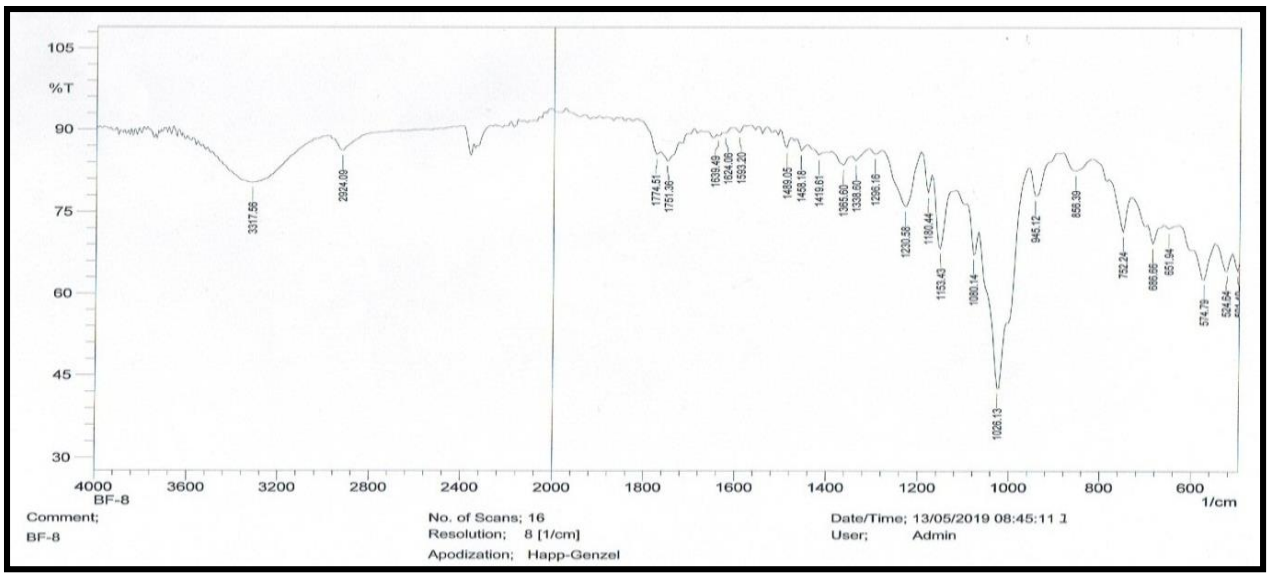

Figure 6. FTIR of plain NS (1:4)

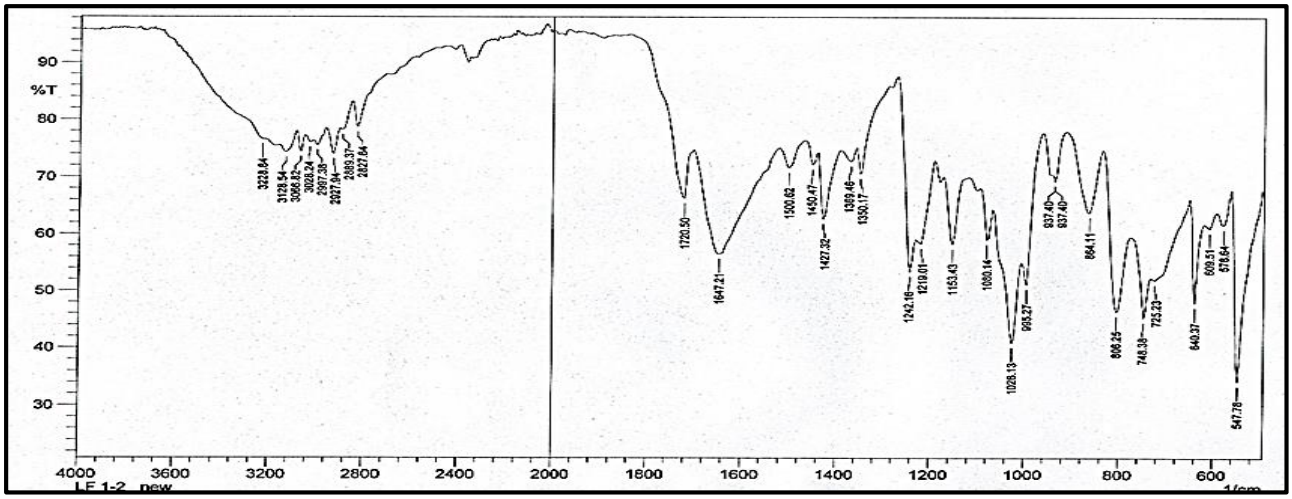

Figure 7. FTIR of 5-FUNS

Morphology studies

AFM has been employed to Figure molecular structure of β-CD NS in the distilled water and examine their mechanical assets. The spherical crystalline NS presented the spectacular crystal planes with ordinary height of less than 400 nm. The SEM images of the plain βCD nanosponges were shown in Figure 8. SEM analysis revealed that nanosized particles with numerous pores on its surface.

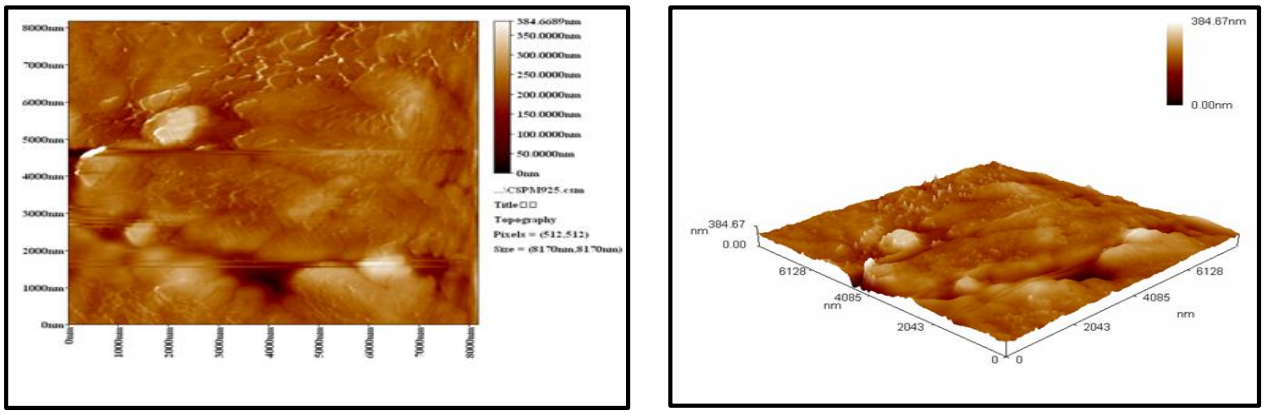

Figure 8. The AFM of βCD nanosponges.

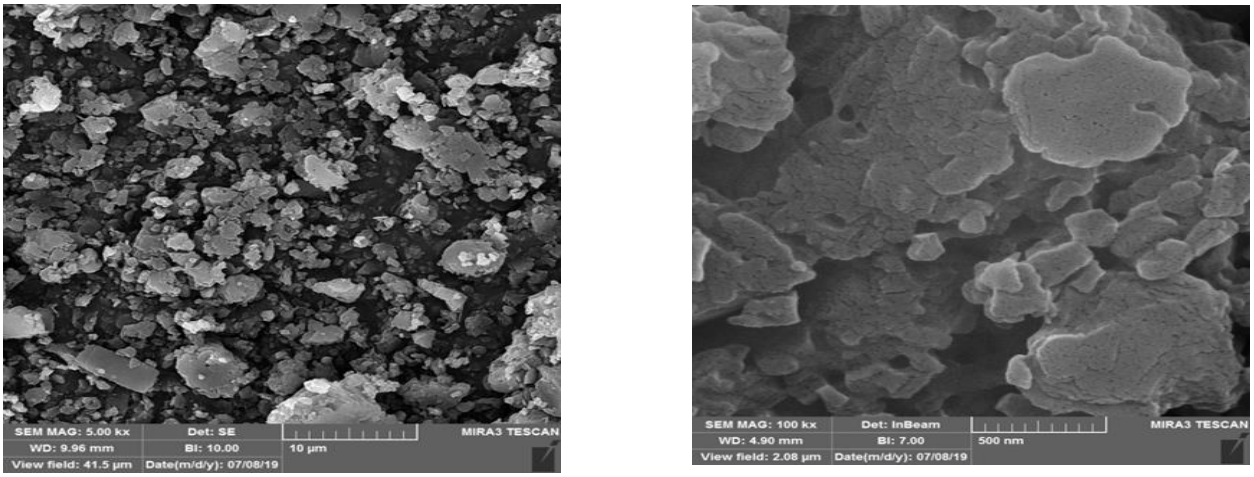

Figure 9. The FE-SEM of βCD nanosponges

***In-vitro* release study**

Drug release was performed in 0.1 N HCl. The 5-FU cumulative percent release of 5-FNS2 (Fig. 4a) showed a burst effect at the end of first hour. This fast release (56%) of the active ingredient was a result of increase in solubilization of the drug. After the first hour, the drug was released in a controlled manner indicating encapsulation of 5-FU in the nanostructures.

5-FU release from 5-FUNS2 was found to be higher than 5-FU-βCD complex(1:1) as compared to 5-FU solution as mentioned previously, this is belong to the carbonate linkage which was added to the primary hydroxyl groups of the parent CD unit. Thus, the drug molecules could be included inside the nanocavities of CD and due to the cross-linking further interactions of the guest molecules with more CD units might be thought. Moreover, the presence of the cross-linked network might also form nanochannels in the NS structure of the polymer mesh. This peculiar structural organization might be responsible for the increased solubilization and protection capacities of NS in comparison with the parent CD [16].

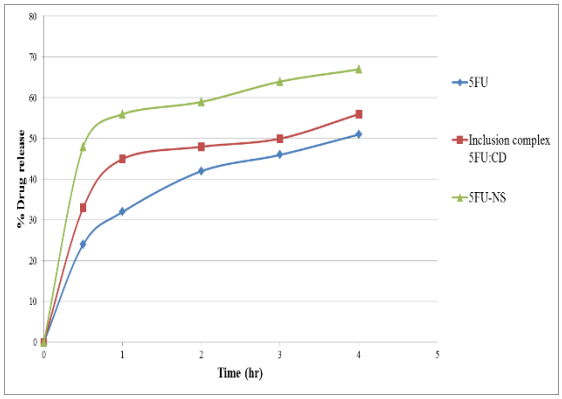

Figure 10. 5-FU release from 5-FUNS2, 5-FUβCD complex and 5-FU solution in 0.1N HCl

Conclusion

Different molar ratios of (cyclodextrin to crosslinker) have a proficient effect on CE, Kst and entrapment efficiency of 5-FU. 5-FUNS2 with (1:4) molar ratio shows the best result of CE, Kst and entrapment efficiency. 5-FUNS2 gave higher release rate than 5-FU-βCD inclusion complex and 5-FU solution.

Surface morphology of the prepared nanosponges by SEM, AFM and indicated nanosized and highly porous nanosponges. The overall results suggest that cyclodextrin nanosponge could be a promising 5-FU delivery system utilizing the suitable formula.

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References

1. Nabavizadeh F, Fanaei H, Imani A, Vahedian J, Amoli FA, Ghorbi J, et al. Evaluation of nanocarrier targeted drug delivery of capecitabine-pamam dendrimer complex in a mice colorectal cancer model. Acta Medica Iranica. 2016:485-93.
2. S.L.Craig VBJ. Animal models in cancer nanotechnology. Nanotechnology in Cancer. 2017:45-69.
3. Zhao G, Rodriguez BL. Molecular targeting of liposomal nanoparticles to tumor microenvironment. International journal of nanomedicine. 2013;8:61.
4. Trotta F, Zanetti M, Cavalli R. Cyclodextrin-based nanosponges as drug carriers. Beilstein journal of organic chemistry. 2012;8(1):2091-9.
5. Pathak P, Katiyar V. Multi-functional nanoparticles and their role in cancer drug delivery–a review. Virus. 2007;30:100.

6. Shringirishi M, Prajapati SK, Mahor A, Alok S, Yadav P, Verma A. Nanosponges: a potential nanocarrier for novel drug delivery-a review Asian Pacific Journal of Tropical Disease. 2014;4:S519-S26.

7. Panda S, Vijayalakshmi S, Pattnaik S, Swain RP. Nanosponges: A Novel Carrier For Targeted Drug Delivery. International Journal Of Pharmatech Research. 2015;8:213-24.

8. Zhu L, Shen G-J, Ding S-Q, Hua X. Determination of 5-Fluorouracil in 5-Fluorouracil Injection and Human Serum by HPLC. Journal of Food & Drug Analysis. 2012;20(4).

9. Aydin R, Pulat M. 5-Fluorouracil encapsulated chitosan nanoparticles for pH-stimulated drug delivery: evaluation of controlled release kinetics. Journal of Nanomaterials. 2012;2012:42.

10. Moffat AC, Osselton MD, Widdop B, Watts J. Clarke's analysis of drugs and poisons: Pharmaceutical press London; 2011.

11. Zankhana S, Shikha M, Mahendra S, Mukesh G. Design and development of 5-Fluorouracil loaded biodegradable microspheres. Int J Ayurveda & Pharm. 2010;1(1):160-8.

12. Patel P, Deshpande A. Patent review on cyclodextrin based nanosponges prepared by different methods: physicochemical characterization, factors influencing formation and applications. World J Pharm Sci. 2014;2(4):380-5.

13. Rao MR, Bhingole RC. Nanosponge-based pediatric-controlled release dry suspension of Gabapentin for reconstitution. Drug development and industrial pharmacy. 2015;41(12):2029-36.

14. Kumar P, Hematheerthani N, Vijaya Ratna J, Saikishore V. Design and characterization of Miconazole nitrate loaded nanosponges containing vaginal gels. Int J Pharm Ana Res. 2016;5(3):410-7.

15. Argenziano M, Haimhoffer A, Bastiancich C, Jicsinszky L, Caldera F, Trotta F, et al. In Vitro Enhanced Skin Permeation and Retention of Imiquimod Loaded in β-Cyclodextrin Nanosponge Hydrogel. Pharmaceutics. 2019;11(3):138.

16. Swaminathan S, Pastero L, Serpe L, Trotta F, Vavia P, Aquilano D, et al. Cyclodextrin-based nanosponges encapsulating camptothecin: physicochemical characterization, stability and cytotoxicity. European Journal of Pharmaceutics and Biopharmaceutics. 2010;74(2):193-201.

17. Ansari KA, Vavia PR, Trotta F, Cavalli R. Cyclodextrin-based nanosponges for delivery of resveratrol: in vitro characterization,stability, cytotoxicity and permeation study. AAPS Pharmscitech. 2011;12(1):279-86.

18. Shringirishi M, Mahor A, Gupta R, Prajapati SK, Bansal K, Kesharwani P. Fabrication and characterization of nifedipine loaded β-cyclodextrin nanosponges: An in vitro and in vivo evaluation. Journal of Drug Delivery Science and Technology. 2017;41:344-50.

19. Argenziano M, AH, CB, LJ, , Caldera F, et al. In Vitro Enhanced Skin Permeation and Retention ofImiquimod Loaded in -CyclodextrinNanosponge Hydrogel. Pharmaceutics 2019;11(138):1-17.

20. Trotta F, Cavalli R, Tumiatti W, Zerbinati O, Roggero C, Vallero R. Ultrasound-assisted synthesis of cyclodextrin-based nanosponges. Google Patents; 2008.

21. Connors K, Higuchi T. Phase solubility techniques. Adv Anal Chem Instrum. 1965;4(2).

22. Loftsson T, Másson M, Sigurjónsdóttir JF. Methods to enhance the complexation efficiency of cyclodextrins. STP pharma sciences. 1999;9(3):237-42.

23. Sherje AP, Dravyakar BR, Kadam D, Jadhav M. Cyclodextrin-based nanosponges: a critical review. Carbohydrate polymers. 2017;173:37-49.

24. Jyoti Pandey AS. Formulation and Evaluation of Nanosponge Based Controlled Release Topical Gel Preparation of Ketoconazole. International Journal of Pharmacy and Pharmaceutical reserach. 2018;12(3):367-82.

25. Swaminathan S, Vavia PR, Trotta F, Cavalli R. Nanosponges encapsulating dexamethasone for ocular delivery: formulation design, physicochemical characterization, safety and corneal permeability assessment. Journal of biomedical nanotechnology. 2013;9(6):998-1007.

26. Deshmukh K, Tanwar YS, Sharma S, Shende P, Cavalli R. Functionalized nanosponges for controlled antibacterial and antihypocalcemic actions. Biomedicine & Pharmacotherapy. 2016;84:485-94.

27. Dora CP, Trotta F, Kushwah V, Devasari N, Singh C, Suresh S, et al. Potential of erlotinib cyclodextrin nanosponge complex to enhance solubility, dissolution rate, in vitro cytotoxicity and oral bioavailability. Carbohydrate polymers. 2016;137:339-49.

28. Khoder M, Gbormoi HK, Ryan A, Karam A, Alany RG. Potential use of the Maillard reaction for pharmaceutical applications: gastric and intestinal controlled release alginate-albumin beads. Pharmaceutics. 2019;11(2):83.

29. Anandam S, Selvamuthukumar S. Fabrication of cyclodextrin nanosponges for quercetin delivery: physicochemical characterization, photostability, and antioxidant effects. Journal of materials science. 2014;49(23):8140-53.
30. Singh V, Xu J, Wu L, Liu B, Guo T, Guo Z, et al. Ordered and disordered cyclodextrin nanosponges with diverse physicochemical properties. RSC Advances. 2017;7(38):23759-64.
31. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2). Tropical Journal of Pharmaceutical Research. 2013;12(2):265-73.
32. Wang L-L, Zheng W-S, Chen S-H, Han Y-X, Jiang J-D. Development of rectal delivered thermo-reversible gelling film encapsulating a 5-fluorouracil hydroxypropyl-β-cyclodextrin complex. Carbohydrate polymers. 2016;137:9-18.
33. Di Donato C, Lavorgna M, Fattorusso R, Isernia C, Isidori M, Malgieri G, et al. Alpha-and beta-cyclodextrin inclusion complexes with 5-fluorouracil: characterization and cytotoxic activity evaluation. Molecules. 2016;21(12):1644.