RESEARCH ARTICLE

Nanosponge Based Gastroretentive Drug Delivery System of 5-Fluorouracil for Gastric Cancer Targeting

Ihsan K. Jasim,[1] Alaa A. Abdulrasool,[2] Shaimaa N. Abd-Alhammid[2]

[1]*Babylon Health Directorate, Ministry of Health, Babylon, Iraq.*
[2]*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.*

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ABSTRACT

5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analog class widely used alone or in combination chemotherapy regimens However, it metabolizes so fast that the biological half-life is only 10–20 minutes. Cyclodextrin-based nanosponges (NS) are a novel class of cross-linked derivatives of cyclodextrins. They have been used to improved anticancer activity increase the solubility of drugs and control their release. Furthermore, X-ray diffraction (XRD) studies confirmed the interactions of 5-FU with NS. Also, X-ray diffraction (XRD) showed that the crystallinity of 5-FU decreased after loading into nanosponges (5-FUNS). The formula 5-FUNS2 had shown a highest encapsulation efficiency 46% w/w. The particle sizes of the loaded NS formulations were between (253.75–721 nm). The zeta potentials 5-FUNS2 was sufficiently high (-26.64 mV) to obtain a stable colloidal nanosuspension. The cytotoxicity studies on a gastric cancer cell line (MKN45) showed that the 5-FU nanosponges were more cytotoxic with lower IC_{50} than plain 5-FU after 72 hours of incubation. The floating nanosponge tablet prepared using HPMC.

Keywords: 5-Fluorouracil, β-cyclodextrin (β-CD), Diphenyl carbonate (DPC), MKN45, Nanosponges.

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INTRODUCTION

Gastric adenocarcinoma refers to tumors of the stomach that arise from the gastric mucosa (>90% of all gastric cancers). Gastric cancer is one of the most common malignant diseases with one of the highest mortality rates worldwide.[1]

In case of solid tumors cell division may be effectively ceased near the center, making chemotherapeutic agents insensitive to chemotherapy. Furthermore, chemotherapeutic agents often cannot penetrate and reach the core of solid tumors, failing to kill the cancerous cells.[2]

Nanosponges are a novel class of hyper-crosslinked polymer-based colloidal structures consisting of solid nanoparticles with colloidal and nanosized cavities. Nanosponges solubilize poorly water-soluble drugs, provide prolonged release, and improve drug bioavailability by modifying the pharmacokinetic parameters of active constituents.[3,4]

Regionally or locally administered chemotherapy allows For high drug concentrations at the tumor site while reducing systemic exposure and subsequent toxicity.[5] Furthermore, floating drug delivery systems offer several benefits such as prolonged gastric residence time (GRT) of dosage forms in the stomach up to several hours, increased therapeutic efficacy of drugs by improving drug absorption, and suitability for targeted delivery in the stomach. In addition, Gastroretentive drug delivery can enhance the controlled delivery of drugs by continuously releasing the drug for an extended period at the desired rate and to the desired absorption site until the drug is ultimately released from the dosage form.[6,7]

5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analog class widely used alone or in combination chemotherapy regimens. However, it metabolizes so fast that the biological half-life is only 10–20 minutes.[8,9] Other restrictions in using 5-FU are its irregular oral absorption as a result of metabolism by dihydropyrimidine dehydrogenase, toxic side effects on the bone marrow and gastrointestinal tract, and non-selective action against healthy cells.[10] On the other hand, to get an effective clinical blood drug concentration, people often choose to increase drug mass or administer the drug to patients continually or repeatedly, which enhances the toxic side effects of 5-FU. At present, only intravenous preparations of 5-FU are available in the market for clinical use. Intravenous administration of the drug has been reported to cause severe gastrointestinal, dermatological, hematological, cardiac, and neural side effects.[11] Most of these side effects are due to the exposure of the drug to unwanted sites. Severe systemic toxic effects and a short plasma half-life make it necessary that this drug be delivered by a local delivery system capable of providing a continuous sustained release.

**Author for Correspondence: ihsan.aljanaby@yahoo.com*

This study aimed to formulate 5-FU as nanosponges to increase 5-FU activity against gastric cancer cells. Moreover, the nanosponges were incorporated into a floating tablet to retained and prolong and the release in the stomach for local gastric cancer targeting and reducing the systemic exposure and subsequent toxicity.

MATERIALS AND METHODS

5-Fluorouracil, β-CD, diphenyl carbonate (DPC), and HPMC (K4M and K15M) were obtained from Hyper-chem Ltd Co. (Hangzhou, China). All other analytical reagents were of analytical grade.

Preparation of CD-nanosponges

Nanosponges of βCD cross-linked with diphenyl carbonate (DPC) in ratios of 1:2, 1:4, 1:6, 1:8, 1:10 were synthesized by melting method, a previously reported procedure. Finely homogenized βCD and DPC were placed in a 100 mL beaker, gradually heated to 100°C, under magnetic stirring, and allowed to react for 5 hours. During the reaction, phenol crystals appeared at the neck of the flask repeatedly washed with an excess of distilled water through filtration by the Buchner funnel to remove unreacted βCD. An additional purification step, consisting of soxhlet extraction in ethanol, was performed for 24 hours to remove the unreacted DPC and phenol present as a by-product of the reaction. Finally, the NS were dried at room temperature to obtain a coarse powder. [12-14]

Preparation of 5-FU Loaded Nanosponges

5-Fluorouracil loaded NS's were prepared by freeze-drying method.[15] Briefly, the optimized formulations of nanosponges and excess 5-FU as powders were mixed and the resultant mixture suspended in distilled water (50 mL). Then, the mixture was sonicated for 10 minutes and stirred for 24 hours. The aqueous suspension obtained 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 to get 5-FU loaded NS depending upon the ratios of CD: cross-linker.[16]

Particle Size, Polydispersity Index Analysis, and Zeta Potential

Nanosponges sizes and polydispersity index were measured by dynamic light scattering using a 90 plus particle sizer PLUS (ZetaPlus Particle Sizing, NY, Software, Version 5.34). The samples were suitably diluted with water before measurements. Zeta potential measurements were also made using an additional electrode in the same instruments (Table 1).

Table 1: The composition of the prepared cyclodextrin nanosponges at a different molar ratio

No.	*Formulas codes*	*CD:DPC Molar ratio*	*β-CD (g)*	*DPC (g)*
1	NS 1	1:2	2.27	0.856
2	NS 2	1:4	2.27	1.712
3	NS 3	1:6	2.27	2.568
4	NS 4	1:8	2.27	3.424
5	NS 5	1:10	2.27	4.28

The particles' mean particle size and polydispersity index (PDI) were calculated in intensity using the cumulant analysis after averaging the three measurements.[15,17]

X-ray Powder Diffraction

The XPRD spectra of 5-FU and 5-FU loaded NS were recorded by Shimadzu XRD 6000 diffractometer using Cu (1.54060 A) as a radiation source. Data were collected over an angular range of 5 to 80°2θ at a step size of 0.02° and a time per step of 0.15 s.

Field Emission-scanning Electron Microscopy (FESEM)

FESEM analysis was significant for the determination of surface characteristics and morphology of the particle. The scanning electron microscope was operated at an acceleration voltage of 15 kV.[18]

Cytotoxicity Study

The human gastric adenocarcinoma cell line MKN45 and normal cell line WRL68 were obtained from the American Type Culture Collection (Rockville, MD, USA). MKN45 cells were grown as a monolayer culture in RPMI medium, at 37°C in 5% CO_2 humidified atmosphere. At the beginning of the experiments, cells in the exponential growth phase were removed from the flasks with 0.05% trypsin –0.02% EDTA solution. For these experiments, cells were seeded at a density of (1 × 104–1 × 106 cells/mL) in 96-well plates. The cells were allowed to attach for 72 hours, and the seeding medium was removed and replaced by the experimental medium. Cells were maintained for 72 hours in medium supplemented with increasing concentrations (from 12.5 to 400 μg/mL) of 5-FU and 5-FUNS2. All experiments were done two times, each condition being performed in triplicate. Cell viability was assessed by trypan blue exclusion assay. For cell experiments, NS and NS formulations were sterilized by autoclaving. 5-FU was solubilized in DW and then suitably diluted.[19]

Formulation of 5-FU NS Floating Tablets

To prepare 5-FU-nanosponge floating tablets, all the polymers and the active ingredients were passed through sieve no. 80 separately. Then the quantity of drug (5-FU) loaded nanosponges, polymer (HPMC E15, HPMC K4M, and HPMC K15M), effervescent agents, and excipients were accurately weighed and mixed using a glass mortar pestle. A manual punching machine was used to prepare the tablet with desired hardness. The direct Compression technique was employed for batch code from FNFT1 to FNFT3.[20]

Evaluation of Powder

The powder blend was evaluated for its angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio using reported standard optimized protocols.

Evaluation of 5-FU NS Floating Tablets

The 5-FU NS floating tablets were evaluated for their weight variations, friability, and hardness using reported standard optimized protocols (Table 2).

Drug Content

For the content uniformity test, ten tablets were weighed and powdered and a quantity of powder equivalent to 100 mg of

Table 2: Composition of different formulations of 5-FUNS2 floating tablets

Ingredients (mg)		*FNFT1*	*FNFT2*	*FNFT3*
5-FU pure powder		-	-	
Selected 5-FU nanosponge formula		Eq. to 100 mg 5-FU	Eq. to 100 mg 5-FU	Eq. to 100 mg 5-FU
HPMC	E15	-	-	150
	K4M	150	-	-
	K15M	-	150	-
Sodium bicarbonate		100	100	100
Citric acid		75	75	75
Microcrystalline cellulose		65	65	65
Magnesium stearate		10	10	10

5-FU was extracted into methanol. The drug content was determined by measuring the absorbance after appropriate dilution with DW. The drug content was determined using standard calibration curve. The mean percent drug content was calculated as an average of three determinations.[21]

Determination of Floating Lag Time (FLT) and Total Floating Time (TFT)

The floating lag time (FLT) is the time taken for a tablet to rise on a medium surface, and the total floating time (TFT) is the floating duration that a tablet remained on the surface. To determine the floating lag time, tablets ($n = 3$) were put on 100 mL of 0.1 N HCl in a beaker, and the time is required for a tablet to rise on the surface was measured. Then, the duration of each formulation that remained on the surface was determined as total floating.[22]

In-vitro Dissolution Studies of 5-FUNS Floating Tablet

The release rate of 5-FU from developed tablets was determined using USP dissolution testing apparatus II (Paddle type). To prevent sticking at vessel or paddle and improve the movement of dosage form, the method suggested is to keep paddle at the surface and not too deep inside dissolution medium.[23,24]

The dissolution test was performed using 900 mL 0.1N HCl, at 37 ± 0.5°C and 100 rpm. A sample (5 mL) of the solution was withdrawn from the dissolution apparatus hourly for 12 hours, and the samples were replaced with a fresh dissolution medium. The samples were passed through Whatman filter paper No. 41, after dilution, and the absorption of these solutions was measured at 266 nm.[25]

RESULTS AND DISCUSSION

Particle Size, Polydispersity Index (PDI), and Zeta Potential (ZP) Analysis

The dynamic light scattering (DLS) associated measurements were carried out after lyophilization. Table 3 show the particle size of the prepared 5-FU nanosponges were recorded to be smallest (253.75 ± 24 nm) and largest (721 ± 30 nm).

The overall sizes of NSs were found to be in the submicron range (<1 μm); thus, the increment in the NS particle size might be due to charge accelerated aggregation and molecular nature of relative CDs, in addition to aggregation during the drying process.[26] Overall, the size of NS with a CD-linker ratio of 1: 4 was smaller than the size of NS with a CD-linker ratio of 1: 6, possibly due to the system's cross-linking efficiency during the polymerization reaction.

Table 3: The characteristics of the prepared 5-FU nanosponges

Formulas No.	*Mean particle size ± SD (nm)**	*PDI*	*ZP (mV)*	*% Encapsulation efficiency*
5-FUNS 1	250 ± 20	1.003 ± 0.2	-	23 ± 3
5-FUNS 2	253.75 ± 24	0.245 ± 0.002	-26.64 ± 2.4	46 ± 3.6
5-FUNS 3	316 ± 28	0.584 ± 0.003	-22.06 ± 2.6	31 ± 2.6
5-FUNS 4	462.66 ± 27	0.387 ± 0.002	-	25.3 ± 3
5-FUNS 5	721 ± 30	0.426 ± 0.02	-	24 ± 2

On the other hand, zeta potential predicts the long-term stability of the nanoparticles.[27] Zeta potential was tested for 5-FU nanoformulations that have smaller particle sizes and lower PDI. The zeta potential of NS2 andNS3 was -26.64 ± 2.4 and -22.06 ± 2.6 mV, respectively.

The zeta potential of the optimized formulation was -26.64± 2.4 mV, which indicates high formulation stability. Negatively charged carrier systems provide high physical stability. It also offers a higher penetration through the lipoidal membrane and avoids non-specific cellular uptake.[28,29]

Percent Encapsulation Efficiency

The encapsulation efficiency values of the prepared CD nanosponges at different molar ratios were found to be between 23–46%. Furthermore, formula 5-FUNS2 shows the highest encapsulation efficiency value (46%). This may belong to the difference in crystallinity of the prepared nanosponges; thus, crystallinity plays a crucial role in the complexation with the drug.[30] Table 3 shows the encapsulation efficiency of the prepared NS. Moreover, the different 5-FU loading nanosponges showed that the degree of cross-linking affected the complexation capacity of NS. The porous matrix of nanosponges with cyclodextrin cavities besides the cross-linked network might favor the 5-FU complexation, either as inclusion or non-inclusion complex, due to the presence of various binding sites. XRD confirmed the interaction of 5-FU with the nanosponge structure.[31]

X-ray Powder Diffraction (XPRD)

The X-ray powder diffraction (XPRD) diffractogram of the 5-FU exhibits an intense sharp peak at 2(θ) of 28.75o, and some moderate sharp, intense diffractions at 2(θ) of 16.5°, 19.21°, 20.83°, 22.10°, 25.63°, 31.30°, 32.24°, 33.47°, and 59.59° due to its crystalline nature.32 The possible reduction in crystallinity of 5-FU in 5-FUNS2 may be due to complex formation with the NS,33 as shown in Figure 1.

Morphology Study

FESEM analysis revealed that nanosized particles with numerous pores on the surface. The lyophilization of 5-FUNS2 gives rise to fluffy mass powder showing highly porous structure as shown in FESEM Figure 2, of 5-FU loaded NS. These results are compatible with those obtained by Swaminathan S etal.[34]

Cytotoxicity Study

The MTT assay was indicated that the cytotoxic effect of 5-FU and 5-FUNS2 adverted that treating MKN45 cells at concentrations ranging from 12.5 to 400 µg/mL for 72 hours showed significant mortality in cell viability by increasing the concentration in a dose-dependent pattern that reached up to 53% killing and 35% for 5-FU and 5-FUNS2, respectively at 400 µg/mL with an IC50 of 165 µg/mL for 5-FU and 96.87 µg/mL for 5-FUNS2.

On the other hand, the 5-FU and 5-FUNS2 were applied against the normal morphological WRL68 cell line using the same range of concentrations. Only the higher concentration 400 µg/mL showed a significant inhibition rate between the drug and the selected 5-FU nanosponges formula(5-FUNS2) and the recorded IC50 values were 222.7 µg/mL and 215.3 µg/mL for 5-FU and FUNS, respectively, as shown in Figure 3.

5-FU nanosponges were formulated as a floating tablet to retained and sustained drug release in the stomach for regional or local administration of chemotherapy to get a high drug concentration at the tumor site, and further reducing systemic exposure and subsequent toxicity

Physical Characterization

The hardness of various batches of prepared formulations (4.3 ± 2.5 – 4.5 ± 2.2 kg /cm^2) and Friability (0.31 ± 0.01 – 0.41 ± 0.2%) indicates that the tablets have sufficient strength to withstand physical abrasion. The drug content assessments were found uniform among different batches of 5-FU nanosponges floating tablets, ranging between 87 ± 2% and 98 ± 1.1%. Furthermore, tablets of all batches pass the weight variation test as per the limits prescribed in USP (Table 2).

In-vitro Floating Study

It was found that the formula FNFT1 containing HPMC K4M fails to maintain the tablet integrity for 24 hours, whereas FNFT2 containing HPMC K15M, because of its higher viscosity, maintains tablet integrity for more than 24 hours, which is an essential requirement for gastroretentive system design to have sustained release for 24 hours.

In this study, water penetration into tablets with low viscosity HPMC K4M was slow, causing delayed gel formation and subsequent increase in the floating lag time and decreased total floating duration (< 10 hours) compared to the tablets prepared with K15M. FNFT2 showed the best floating lag time of 15 ± 3 seconds. Furthermore, when the E-series of HPMC (E15) in formula FNFT3 was tried, it was found that HPMC E15 gave comparable results to the K-series of HPMC (K15M) for formula FNFT2.

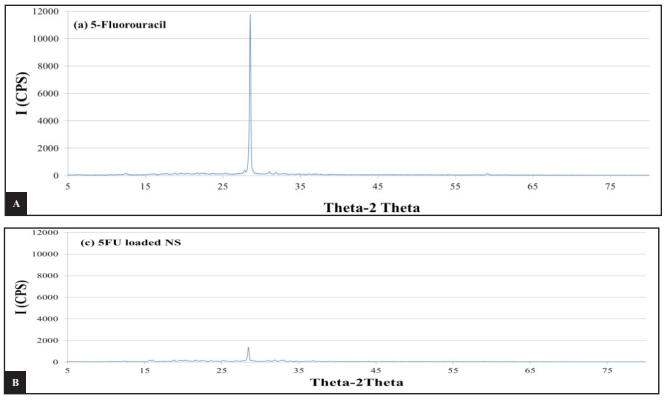

Figure 1: XRD patterns of; a) 5-FU and b) 5-FU loaded NS

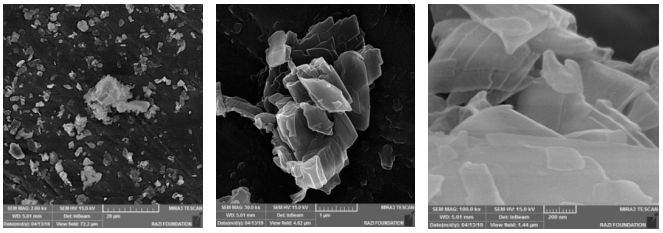

Figure 2: FE-Scanning electron microscopy of 5-FUNS2

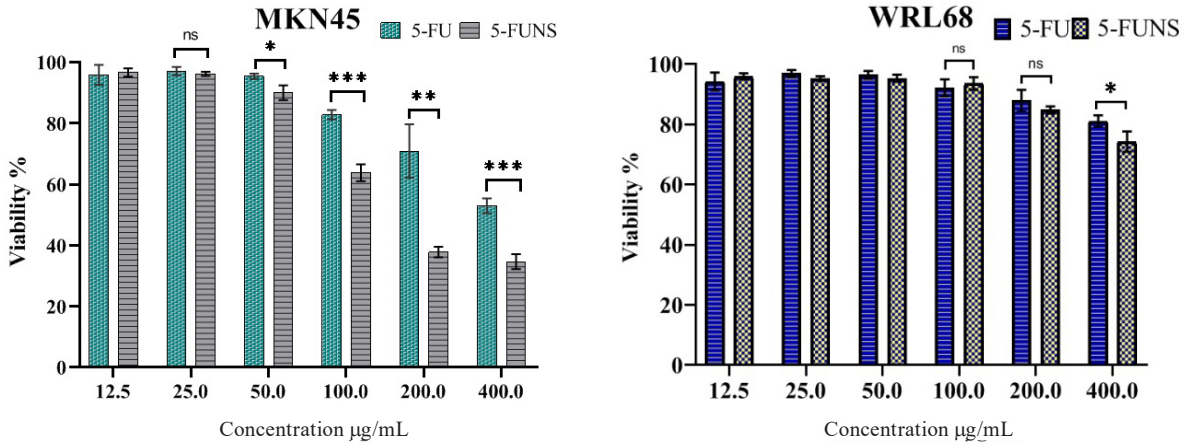

Figure 3: Cytotoxic effect of 5-FU and 5-FUNS2 on; a) MKN45, b) WRL68 cells after 72 hours incubation at 37ºC (p consider significant at P* <0.05, P**<0.01 and P***<0.001), ns = non-significant

Table 6: Physical characterization of the 5-FUNS floating tablet

Formula No.	*Weight (mg)*	*Hardness (kg/cm3)*	*Friability (%)*	*5-FU content (%)*	*FLT* (sec)*	*TFT** (h)*
FNFT 1	596 ± 9.6	4.3 ±0.3	0.31 ± 0.01	98 ± 1.1	35 ± 2	10 ± 1.5
FNFT 2	601 ± 8	4.7 ± 0.3	0.38 ± 0.03	95 ± 1.2	15 ± 3	>24
FNFT 3	599 ± 10	4.8 ± 0.8	0.41 ± 0.2	93 ± 2	10 ± 7	>24

All data were expressed in (mean±SD), *TFT=total floating time, **FLT=floating lag time.

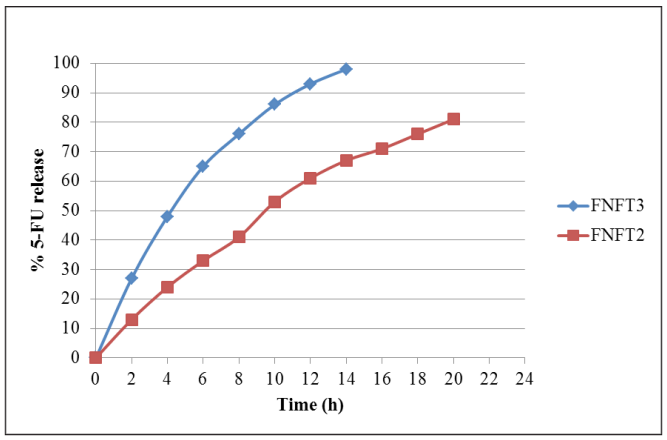

Figure4: The release profile of 5-FU from floating tablets FNFT2 and FNFT3

In-vitro 5-FU Release Study

Three formulations of 5-FU floating tablet were prepared. FNFT1, FNFT2 and FNFT3 was prepared using drug-loaded NS, as shown in Table 2. FNFT1 prepared using HPMC K4M shows low total floating time and is not included in this release study. Furthermore, 81% of 5-FU was released from FNFT2 within 20 hours, and 86% release was observed at 10 hours FROM FNFT3 as shown in Figure 4. Therefore, formula FNFT2 was chosen as an optimized formula for sustained release of 5-FU from nanosponges floating tablet.

CONCLUSION

We have successfully prepared a new generation of floating tablets using NS as a key formulation ingredient. With this finding, the advantages of porous nanosponges materials can be further integrated into floating tablets with fine control over the sustainable release of 5-FU. Our success has paved the way for formulation improvement in localized drug release. Furthermore, 5-FU loaded nanosponges show a higher activity against cancer cells MKN45, floating tablet of 5-FUNS exhibits a good floating behavior and sustained release. The overall results suggest that 5-FU nanosponges floating tablet could be a promising 5-FU delivery system utilizing the suitable formula.

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