Design, Formulation and Evaluation of Nanosponge-Based Novel Drug Delivery System

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Abstract
One of the escalating menaces to human health is invasive fungal infections. Predominant occurrence of these infections in the developed world can be ascribed to ever more aggressive immunosuppressive therapies. Vaginal candidiasis (VC) is a clinical condition which is prevalent in about 75% of women for at least once in their lifetime. About 80% of these cases are caused by the most pathogenic species of the genus Candida, i.e., Candida albicans. VC episodes are often extremely uncomfortable and painful, and vastly accompanied with irritation, itching, dysuria and incessant vaginal discharge. Regardless of advances in therapies, VC is considered as serious infection, significantly impacting patient's workability, performance, mental and emotional state in addition to clinical trauma. There is still room and enormous need for advancements in VC therapeutic strategies, for circumventing the diverse drug related toxicities, inefficacies and several allied tribulations.

Keywords: Vulvovaginal candidiasis, Clotrimazole, Quercetin, Cyclodextrins, Nanosponges, Intravaginal in situ gel.

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Introduction

A resurgent rise in investigation of new drug entities in the wake of recent clinical findings as well as greater healthcare expenses are demanding substitution of current drug deliveries and strategies. Current drug delivery scenario has become highly productive, and therefore, is fast developing. In order to optimize the therapy along with cost effectiveness, a colossal amount of research has been implemented towards developing advanced drug delivery systems.

Novel categories of pharmaceuticals and biopharmaceuticals are further boosting progression of drug delivery technology. However, delivering these novel entities through conventional existing systems is not efficient and feasible. Therefore, to address this muddle, more advance localized and targeted delivery systems are gaining more importance in recent times.

Vaginal candidiasis (VC) is a clinical condition which is prevalent in about 75% of women for at least once in their lifetime. About 80% of these cases are caused by the most pathogenic species of the genus Candida i.e. Candida albicans. C. albicans primarily exists as a dimorphic fungus that is propagated through its blastophore phenotype also known as 'blastoconidia'. It is capable to transforming into various forms such as hyphae, yeast and pseudohyphae upon recognition of environmental signals; which are important for its virulence. The outermost cell wall layer of Candida comprises mannoproteins with N-glycosylated polysaccharide and O-glycosylated oligosaccharide moieties. Both carbohydrate moieties have been shown to be vital in host-fungal interactions and virulence. The N-glycosylated polysaccharide has a comb-like structure with an α-1,6-linked backbone moiety and an oligomannose side chain chiefly consisting of α-1,2-, α-1,3-, and β-1,2-linked mannose residues with a trivial number of phosphate groups. Three types of β-1,2-linkage-containing manno-oligosaccharides are observed in mannan.

Among the recent findings are the nanosponges (NS); which are made up of microscopic particles containing cavities that are a few nanometers wide. These recently proposed nano-sized colloidal carriers specially designed for drug delivery can solubilize drugs that are insoluble in water, offer prolonged release, and by altering pharmacokinetic parameters they improve drug bioavailability. In the pharmaceutical field, cyclodextrins (CDs) are used to form inclusion

complexes with drug molecules in order to enhance the aqueous solubility of the drugs, mask their unwanted characteristics, reduce their side effects, and increase their photostability or aqueous stability. CDs have also been shown to control the release of certain active ingredients. A vital challenge in the vaginal drug delivery is patient's pliability during administration of dosage forms and following up with repeated-dose therapeutic regimen. Among the various conventional formulations, gels have critical advantages such as versatility, safety, high bioavailability and being economical. Gels are better tolerated by patients than any other dosage forms, and vaginal therapy can be considerably improved if the delivery system can hold the drug at the site of administration for an extended period of time. In situ gelling drug delivery systems release drugs in response to environmental signals as a result of stimulus-dependent changes in the rheological properties of the polymer platform. Hence, such systems can be formulated to deliver an adequate coverage of the vagina and to retain the formulation on the mucosal tissue. The thermoreversible behavior of Pluronics is due to the change in their micellar properties as a function of both concentration of the polymers and ambient temperature, and a reversible gelation may occur at physiological temperature. By using such systems, many benefits like high spreadability and ease of application at temperatures that are below sol-gel temperature and rheological structuring are offered to the vagina for topically administering therapeutic agents and therefore, vaginal retention at body temperature is enriched.

Material & Methodology:

Sr. No.	Ingredient	Quantity
1	Clotrimazole (CTZ)	100 mg
2	β-cyclodextrin (β-CD) / HP-β-CD	500 mg
3	Carbonyldiimidazole (CDI)	300 mg
4	Dimethyl carbonate (DMC)	10 mL
5	Ethanol / Methanol	5 mL
6	Pluronic F-127 / F-68	0.5 g (0.5%w/v)
7	Triethanolamine	0.2 mL
8	Benzalkonium chloride	0.01 g
9	Distilled Water	q.s. to 100 mL
10	DMSO / Methanol	q.s.
11	0.1 N HCl	q.s.
12	Potassium Bromide (KBr)	q.s.
13	Trypan Blue	q.s.
14	Barium sulphate	Optional

As presented in the study design section, in the present research two different set of polymers and crosslinkers were used for the synthesis of CD NS as HP-β-CD with DMC (part a), and β-CD with CDI (part b). Subsequent to NS synthesis, two varied APIs, one from synthetic allopathic origin (Clotrimazole, CTZ) and one from natural/herbal origin (Quercetin, QCT) were loaded in the respective NS, followed by *in situ* gel formulation and lastly characterization and evaluation of the optimized formulations in great detail. Since flow of study and most of the characterization and evaluation techniques and/or parameters were same, methodology for both parts (part a, and part b) is discussed in common under this section.

Formulation of NS based intravaginal *in situ* gels

NS based thermosensitive and mucoadhesive intravaginal *in situ* gels were prepared to increase viscosity and to achieve a longer residence time in the vaginal cavity. Pluronic F-127 (PF-127) and Pluronic F-68 (PF-68) served as gelling agents and gel was prepared according to the classical 'cold method'. The PF-127 (18-22% w/w) and PF-68 (5-15% w/w) polymers were added into pre- cooled ultra-purified water (4°C) with continuous agitation. The solutions were then kept in a refrigerator overnight (4°C) to ensure complete polymer dissolution and pH was adjusted to 4.5 using triethanolamine. Benzalkonium chloride (0.01% w/v) was added as a preservative to the gel. Drug loaded NS (encapsulating drug equivalent to dose i.e. 2% w/w for CTZ and 2.5% w/w for QCT) were incorporated into the gel. Conventional gel was made in the same way; only instead of NS, free drug was incorporated. The prepared gels were finally packed in glass vials, and sealed until further use.

Evaluation and optimization of NS based intravaginal *in situ* gel

Gelation temperature

The gelation temperature of NS based thermosensitive *in situ* gel (5 ml) was determined by heating it in a thin-walled glass tube with an internal diameter of 10 mm, length of 82 mm, and thickness of 0.6 mm. The glass tube was placed in a temperature-controlled water bath for heating with a gentle shaking till the solution in it gets converted to gel. The temperature of water was increased constantly at the rate of 2 °C/5 min. The point, where no flow was observed when the test tube was inverted, was taken as the point of gel formation, and that temperature was noted as the gelation temperature.

Gelation time

Gelation time of NS based *in situ* gel was obtained by the tube inversion method. 5 ml NS based *in situ* gel was taken in a thin-walled glass tube having same geometry/specifications as mentioned above. The gelation time was measured at the respective gelation temperature noted earlier. The filled glass tube was immersed in the temperature-controlled water bath adjusted to the respective gelation temperature. At regular intervals, the test tube was taken out and inverted to observe the physical state of sample in it. The gelation time was noted by a flow or no-flow criterion with the inverted test tube, and the time taken by the system to gel i.e. flow to no flow was noted as the gelation time.

***In vitro* drug release studies**

The *in vitro* drug release studies were performed by using Franz diffusion cell (Perme Gear Inc., Bethlehem, PA) with donor chamber and water jacketed receptor chamber (20 ml) maintained at 37°C. Commercial semipermeable cellophane membrane (Fischer Scientific Co., London, England; pore size 0.45 µm) was used as permeation barrier; which was soaked overnight in SVF before the study. 1 g of gel was placed carefully on cellophane membrane; which was placed between the donor and receptor compartments. Receptor compartment contained 20 ml SVF, while donor compartment was empty and open to atmosphere. The contents of receptor section were maintained at 37±1 °C with continuous stirring at rate 25 rpm, on a magnetic stirrer. Aliquots (2 ml) were withdrawn at regular intervals from receptor compartment and equal volume of fresh receptor medium (37±5 °C) was replaced to maintain the sink condition. Withdrawn samples were analyzed by UV-spectrophotometry. The *in vitro* drug release data was fitted into various mathematical models to ascertain the mechanism of drug release from NS based gel system. The study was conducted in triplicate.

Check point analysis

The relationships between factors and response values of NS based intravaginal *in situ* gel were examined by construction of an overlay plot as well as desirability plot.

pH

The pH of prepared optimized NS gel was determined using a digital pH meter (Mettler Toledo MP 220, Greifensee, Switzerland) at 25 °C in triplicate.

Drug content

The drug content was determined by extracting 1 g of NS based *in situ* gel using methanol in 100 ml volumetric flask. After appropriate dilutions, samples were analyzed by UV-spectrophotometry (181,195).

Viscosity studies

Viscosity measurements of the formulated NS based *in situ* gel were carried out using Brookfield viscometer (DV-II, LV model, Brookfield, USA) using small volume adaptor with a thermo stated water jacket and SC4-18 spindle. The viscosity was measured (n=3) at three temperatures *viz.* at 4°C, 25°C and 37°C at different rotational speeds from 0.5-20 rpm with a torque of near to 100%. The samples were equilibrated for 10 min before the measurement; also the instrument was equipped with a temperature control unit (33,200).

Rheological studies

The rheological measurements of the optimized NS based intravaginal *in situ* gels were carried out by using MCR100 controlled stress rheometer (Paar Physica, Anton paar, Gmbh, Austria) equipped with the coaxial cylinders (CC 27). The radii ratio of coaxial cylinders was 1.08477. The rheometer was also equipped with an electric temperature controlled peltier system (TEZ-15P-C) for controlling the experimental temperature with an accuracy of 0.01 °C, and a circulating water bath (Viscotherm VT-2, Paar Physica, Anton paar, Gmbh, Austria) was also the part of the assembly used. The rheological parameter, shear stress (Pa), was measured linearly increasing up to a shear rate of 250 s^{-1} with 20 sec duration, and 25 shear stress shear rate data points were collected and analyzed by using universal software US200 (Paar Physica, Anton paar Gmbh, Austria) (12). The rheological measurements at three different temperatures viz. 4°C, 25°C, and 37°C were carried out corresponding to the storage, room, and the body temperature, respectively. All the measurements were done in triplicate by taking fresh sample for each measurement.

Spreadability studies

For a gel to meet the ideal qualities, one of the expected conditions is, it should possess good spreadability. This term is expressed to signify the extent of an area to which gel spreads readily on the site of application. The spreadability was determined using a method previously reported by Bachhav *et al*. (248). Accordingly, spreadability was evaluated by

placing 0.5 g gel within a premarked circle of 1 cm diameter on a glass plate with specifications of 5 mm thickness and 15 cm^2 area. Another glass plate with similar dimensions was placed over it; care has been taken to avoid entrapment of the air bubbles between the two slides. Weight of 500 g was kept on the upper glass plate for 5 min to spread the gel uniformly. The increase in the diameter because of the gel spreading was noted as an indicator of spreadability.

***In vitro* bioadhesion studies**

In vitro bioadhesive potential of drug loaded NS based *in situ* gel was evaluated and compared with marketed plain drug gel (Candid™-V gel, Glenmark Pharmaceuticals Ltd.), using method reported formerly by Bachhav *et al*. (248). In brief, an agar plate (1%, w/w) in citrate phosphate buffer (pH 4.5) was prepared and test sample (50 mg) was placed at the center of plate (Figure 12). After five minutes, plate was assembled with USP disintegration test apparatus (ED-2L, Electrolab, Mumbai, India) and ran in up-down motions in SVF (pH 4.5) at 37±1 °C. The motion of the instrument arm was such that the sample on plate was immersed at the lowest point into the solution and was taken out at the highest point of the solution. The residence time of test samples on plate was noted by keen visual observation.

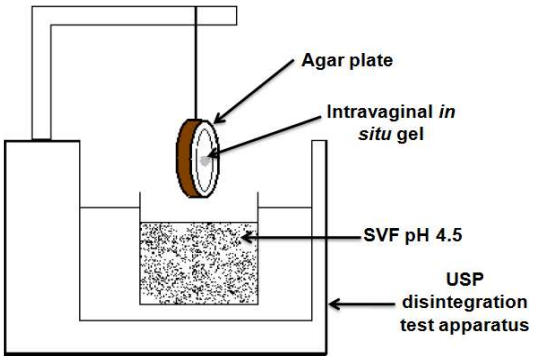


Figure: Diagrammatic presentation of assembled disintegration test apparatus used for *in vitro* bioadhesion study of intravaginal *in situ* gels

***In vitro* antifungal activity**

The evaluation of *in vitro* antifungal activity of developed NS based *in situ* gels was done against *Candida albicans* implying agar diffusion method *via* 'Cup- Plate' technique (30,252). Subcultures of *Candida albicans* MTCC183 strain 3102 were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The strains were inoculated onto sterile Sabouraud dextrose agar (SDA) plates followed by incubation at 35°C for 24 h. They were then sub-cultured on the same medium for next 24 h at 35°C. The fungal inocula were prepared by diluting the overnight culture with 0.9% NaCl to 5×10^{15} CFU/ml. To sterile Petri plates (sterilized in hot air oven at 160°C for 60 min) sterile SDA media (20 ml) containing chloramphenicol (50 µg/ml, for selective fungal growth and avoidance of bacterial contaminants) and seeded with inoculums (0.2 ml) were added aseptically, agitated for some time homogenous mixing and were allowed to solidify. Later, cups were bored in the solidified medium using a sterile cork borer (4 mm diameter). Sterile solutions of negative control (C), blank/placebo gel (B), plain drug marketed gel (M) and optimized NS based *in situ* gel (O or OO) in DMSO were added in respectively marked cups (0.1 ml of 0.1% w/v). The plates were kept aseptically at room temperature for about 2 h and then kept for incubation at 37°C for 24 h. The zone of inhibition (ZOI, diameter in mm) developed, if any, were then measured for the particular test sample by antibiotic zone finder. ZOI of the NS based *in situ* gels were then compared with marketed formulation. The solvent (DMSO) was implied as negative-control and entire study operations were carried out in aseptic conditions. Evaluation was done in triplicate.

RESULTS AND DISCUSSION OF CLOTRIMAZOLE-NS BASED INTRAVAGINAL *IN SITU* GEL

Characterization of active ingredient

Melting point

Melting point of CTZ was determined by using capillary method. It has been noted in the range of 147-148°C, while as per literature standard it is reported to be 147-149°C. As experimental values were in good agreement with official values, it could be concluded that procured CTZ was in pure state.

FT-IR spectroscopy:

FT-IR spectrum of drug sample depicted all the characteristic IR peaks corresponding to functional groups of CTZ as reported in the literature) confirming the purity of drug.

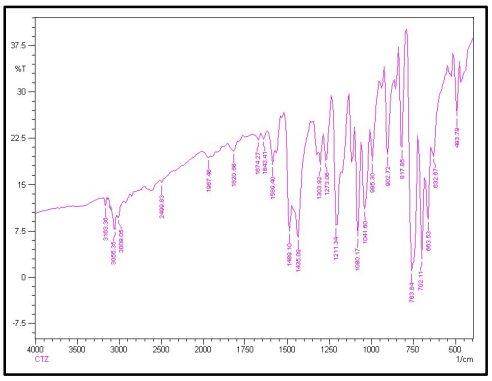


Figure : FT-IR spectrum of Clotrimazole

Thermal analysis (DSC)

CTZ was subjected to thermal analysis implying DSC technique. The obtained DSC thermogram is presented in Figure 14. DSC analysis of pure CTZ depicted a gradual enthalpy change and reproduced the sharp endothermic peak at 146.99°C corresponding to its melting point. No other peaks were observed, confirming purity of CTZ sample procured.

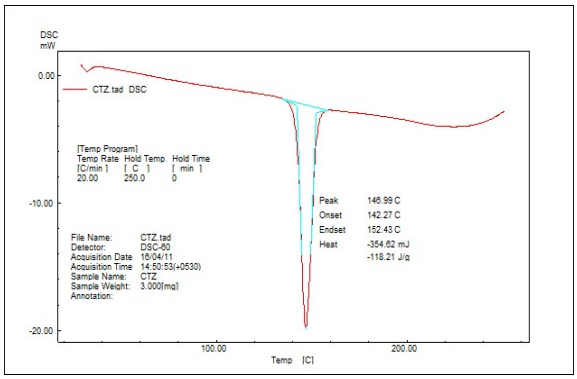


Figure: DSC thermogram of Clotrimazole

Table: Absorbance values for different concentrations of Clotrimazole in simulated vaginal fluid pH 4.5

Sr. No.	Concentration of CTZ (µg/ml)	Absorbance*
1.	0	0
2.	50	0.132±0.014
3.	100	0.239±0.021
4	150	0.337±0.016
5	200	0.463±0.024
6	250	0.561±0.015
7	300	0.659±0.023
8	350	0.783±0.027

*Mean±SD

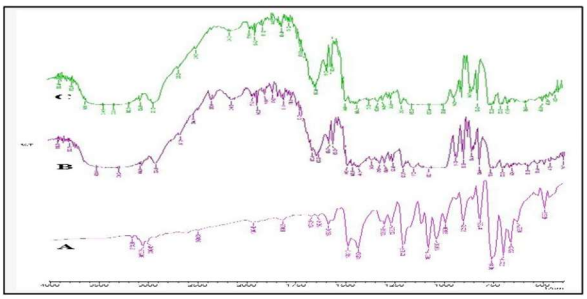


Figure: Overlain FT-IR spectra of (A) Clotrimazole, (B) hydroxypropyl-β-cyclodextrin and (C) physical mixture.

Scanning electron microscopy

The size and surface morphology of the optimized NS were further investigated using SEM analysis. NS were uniformly found to be roughly spherical in shape with a spongy nature (186,248). SEM images (Figure 32) showed that the formed NS were having numerous fine surface voids, probably as a result of solvent diffusion. No particulate aggregation was noticed, and results were in good agreement with particle size analysis results. Also no residual, intact crystals of CTZ were observed on the NS surface, establishing the fact that the NS matrix was entirely formed by CTZ and HP-β-CD.

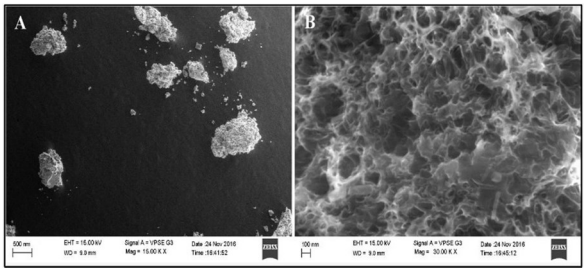


Figure : SEM images of (A) the CTZ loaded HP-b-CD NS sample and (B) magnified surface of CTZ loaded HP-b-CD NS sample

RESULTS AND DISCUSSION OF QUERCETIN-NS BASED INTRAVAGINAL *IN SITU* GEL

Melting point

Melting point of QCT determined by using the capillary method was noted in 322-323°C range, while as per literature standard it is reported to be 324-325°C. As experimental values were in good agreement with official values, it could be concluded that procured QCT was in pure state.

FT-IR spectroscopy

FT-IR spectrum of QCT depicted all characteristic IR peaks corresponding to functional groups of QCT as reported in the literature confirming the purity of drug.

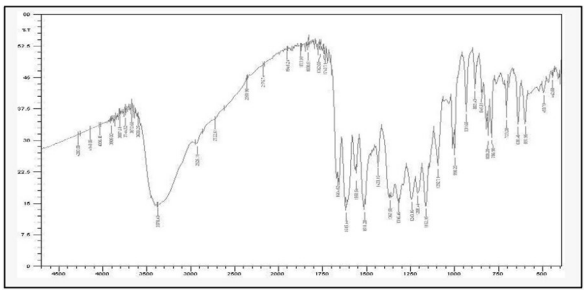


Figure : FT-IR spectrum of Quercetin

DSC analysis

Pure QCT was subjected to thermal analysis implying DSC technique. The traced DSC thermogram is presented in Figure 51. DSC analysis depicted a gradual enthalpy change, producing the sharp endothermic peak at the temperature 323.49°C corresponding to its melting point. No other peaks were observed, confirming purity of QCT sample procured.

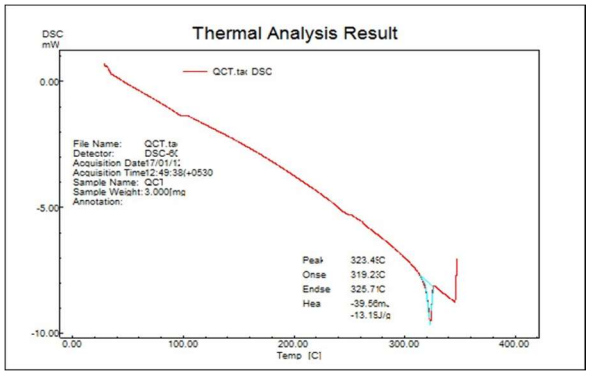
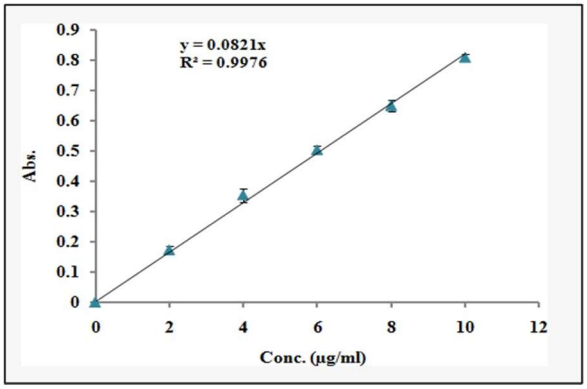


Figure: DSC thermogram of Quercetin

Table : Absorbance values for different concentrations of Quercetin in simulated vaginal fluid pH 4.5

Sr. No.	Concentration of QCT (µg/ml)	Absorbance*
1.	0	0
2.	2	0.173±0.011
3.	4	0.354±0.023
4.	6	0.503±0.013
5.	8	0.649±0.019
6.	10	0.808±0.012

*Mean±SD, *n*=3



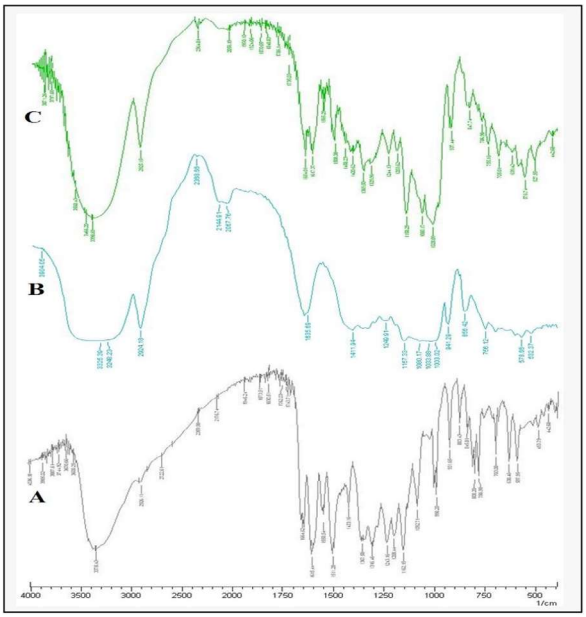


Figure: Overlain FT-IR spectra of (A) Quercetin, (B) β-cyclodextrin and (C) physical mixture

Scanning electron microscopy (SEM)

The size and surface morphology of the optimized NS were further investigated using SEM analysis. NS were unvaryingly found to be roughly spherical

in shape with a spongy nature. SEM images (Figure 69) showed that the formed NS were having numerous fine surface voids and no particulate aggregation was noticed, and the results were in good agreement with particle size analysis results. Also no residual, intact crystals of QCT were observed on the NS surface, establishing the fact that the NS matrix was entirely formed by QCT and β-CD.

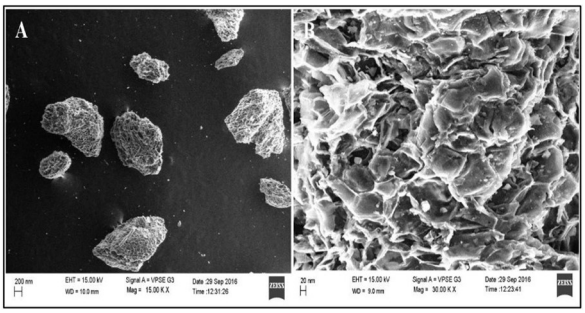


Figure: SEM images of (A) the QCT loaded β-CD NS sample and (B) magnified surface of QCT loaded β-CD NS sample.

Conclusion:

The design, formulation, and evaluation of a nanosponge-based novel drug delivery system represent a promising approach for improving the therapeutic performance of pharmaceutical agents. Nanosponges provide a versatile and efficient carrier system capable of encapsulating a wide range of drug molecules, thereby enhancing their solubility, stability, and controlled release characteristics. Through appropriate formulation and optimization, nanosponges can improve drug bioavailability, reduce dosing frequency, minimize adverse effects, and enhance patient compliance.

Comprehensive evaluation of the developed formulation, including physicochemical characterization, drug loading, particle size analysis, zeta potential, entrapment efficiency, in vitro drug release, and stability studies, confirms the suitability of the nanosponge system as an effective drug delivery platform. The results indicate that the optimized nanosponge formulation possesses desirable properties such as high encapsulation efficiency, sustained drug release, and satisfactory stability.

Overall, nanosponge-based drug delivery systems offer significant potential for overcoming the limitations associated with conventional dosage forms. Continued research, including in vivo evaluation and clinical investigations, is expected to further establish their safety, efficacy, and commercial applicability. Therefore, nanosponge technology represents a valuable advancement in the field of novel drug delivery systems with broad applications in pharmaceutical and biomedical research.

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