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# Design and *Invitro* Evaluation of Mucoadhesive Microspheres containing Amodel Antibacterial agent for Periodontitis

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## Abstract

In the present work, mucoadhesive microspheres of Chitosan, Hydroxypropyl Guar and Sodium alginate were formulated to deliver Doxycycline monohydrate to oral cavity infections(periodontitis).The present investigation involves formulation and evaluation of mucoadhesive microspheres with doxycycline monohydrate as model drug for prolongation of drug release time. The microsphere formulations were prepared by using three different polymers (Chitosan, Hydroxypropyl Guar and sodium alginate), DOSS and Span 80 were used as emulsifiers; Calcium chloride as a cross linking agent.The ratio of Polymer to drug for each polymer were varied in the microsphere preparation and then they were evaluated for % yield, % drug entrapment efficiency,particle size analysis, *in vitro* mucoadhesion tests, degree of swelling, morphological study by SEM and *In – vitro* drug diffusion profile.Further the analysis of release mechanism was carried out by fitting the drug diffusion data to various kinetic equations like, Zero order, First order Korsmeyer- Peppas, Higuchi (matrix) and Hixson Crowell equations and from the values so obtained, the best fit model were arrived at

The results obtained have been discussed in the chapter 6. Results of FT-IR revealed that there was no chemical interaction between the drug and the polymer used. The obtained microspheres were spherical, free flowing and had a particle size ideal for oral cavity delivery. The prepared microspheres had good mucoadhesiveness and revealed good degree of swelling. The release pattern of the formulations was observed to be biphasic characterized by initial burst effect followed by a slow release. The kinetic model fitting data shows that the release of drug from the microspheres follow Higuchi (matrix) model. From the above the results CDX3, HDX2 and SDX2 were found to be best formulations for the oral delivery of doxycycline monohydrate that complied with all the parameters. However, *in – vivo* experiments need to be carried out to know the absorption pattern and bioavailability of drug from the microspheres and thus enabling us to establish *in vitro – in vivo*correlation.

**Keywords:** Microsphere, Amodel, Bacteria

## Introduction

Infections of the oral cavity may result from the activity of the commensal oral flora. These include dental caries, abscesses, periodontal infections and gingivitis and actinomycosis.There are also infections of the oral cavity that are caused by primary pathogens. These include cold sores caused by herpes simplex virus, oral thrush caused by the fungus Candida albicans and other Candida species, and lesions associated with syphilis, caused by Treponema pallidum.Bioadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1–1000μm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site achieved by anchoring plant lectins, bacterial adhesins and antibodies, etc. on the surface of the microspheres. Bioadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, oral cavity, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localised as well as systemic controlled release of drugs. Application of bioadhesive microspheres to the mucosal tissues of ocular cavity, oral cavity, gastric and colonic epithelium is used for administration of drugs for localised action. Prolonged release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance. The latter advantage can also be obtained for the drugs administered intranasally due to the reduction in mucociliary clearance of drugs adhering to nasal mucosa. Microspheres prepared with bioadhesive and bioerodible polymers undergo selective uptake by the M cells of Peyer patches in gastrointestinal(GI)mucosa.Thisuptakemechanismhasbeenusedforthedeliveryofproteinandpeptidedrugs,antigensforvaccinationandplasmidDNAforgenetherapy.The concept of a non-invasive single shot vaccine, by means of mucosal immunization, offers controlled release of antigens and thus forms another exquisite application of bioadhesive microspheres.

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## Material and Method

### Preparation of mucoadhesive microspheres ofchitosaN

### Preliminary studies

The preliminary studies were carried out by preparing various batches of microspheres with different process parameters in an effort to optimize the formulations for obtaining microspheres with proper physical characteristics and of particle size ranging from which are ideal for oral cavity.The following are the process variables which were studied to standardize the method for preparation of the microspheres.

- Amount of cross-linking agent(Glutaraldehyde)
- Cross-linkingtime
- Concentration of surfactant(DOSS)
- Stirringspeed

### Effect of amount of cross-linking agent(Glutaraldehyde)

Four different batches namely CD1 – CD4 were formulated with varying the amount of cross-linking agent (Glutaraldehyde) from 1ml - 4ml respectively while other conditions such as Cross-linking time (3hours), Concentration of surfactant (DOSS) (0.2%w/v) and Stirring speed (1800rpm) constant. The obtained microspheres were evaluated for % drug entrapment efficiency, % mucoadhesion and physical characteristics.

**Table: Effect of amount of Cross-linkingagent on % Drug entrapment efficiency, % Particle size and Physical characteristics**

| Batch no | Amount of cross-linking agent | % Drug Entrapment Efficiency | Particle size in μm | Physical Characteristic |
|---|---|---|---|---|
| CD1 | 1ml | 45.1 | 47.6 | Irregular |
| CD2 | 2ml | 54.8 | 56.7 | Slightly irregular |

| | | | | |
|---|---|---|---|---|
| CD3 | 3ml | 72.3 | 64.2 | Slightly irregular |
| CD4 | 4ml | 82.4 | 72.9 | Spherical, free flowing |

**Effect of cross-linkingtime**

The time for cross-linking reaction was varied from 1hour – 3hours. Three sets of formulations were prepared while keeping other process variables such as amount of cross-linking agent (4ml), Concentration of surfactant (DOSS) (0.2%w/v) and Stirring speed (1800rpm) constant. The formulations were designated as CD5, CD6, and CD7 with varying cross-linking time of 1hr, 2hrs and 3hrs respectively. The obtained microspheres were evaluated for particle size, % drug entrapment efficiency, % mucoadhesion.

**Table: Effect of Cross-linking time on Particle size and% Drug entrapmentefficiency**

| Batch no | cross-linking time (hours) | Particle size in µm | % Drug Entrapment Efficiency |
|---|---|---|---|
| CD5 | 1 | 72.3 | 47.8 |
| CD6 | 2 | 78.1 | 64.7 |
| CD7 | 3 | 82.8 | 78.9 |

**Effect of concentration of surfactant**

Three different formulations namely CD8, CD9 and CD10 were prepared by varying the surfactant (DOSS) concentration from 0.1%, 0.15% and 0.2% w/v respectively, while keeping all other process variable like cross-linking agent (4ml), cross-linking time (3 hours) and Stirring speed (1800rpm) constant. The prepared microspheres were evaluated for particlesize.

**Table: Effect of Concentration of surfactant on Particle size**

| Batch no | Concentration of surfactant % w/v | Amount of Cross-linking agent | Cross-linking time (hours) | Stirring speed (rpm) | Particle size in |
|---|---|---|---|---|---|
| CD8 | 0.1 | 4ml | 3 | 1800 | 147.8 |
| CD9 | 0.15 | 4ml | 3 | 1800 | 92.8 |
| CD10 | 0.2 | 4ml | 3 | 1800 | 78.4 |

**Effect of stirringspeed**

The speed of the propeller was varied to get the particle size suitable for oral cavity. Four batches of microspheres were prepared namely CD11, CD12, CD13 and CD14 with a stirring speed of 1000, 1200, 1500 and 18000rpm respectively. The other process variables like cross-linking agent (4ml), cross-linking time (3 hours) and Concentration of surfactant (DOSS) (0.2%w/v) constant. The prepared microspheres were evaluated for particle size.

**Table : Effect of Stirring speed on Particle size**

| Batch no | Stirring speed (rpm) | Drug to polymer ratio | Amount of Cross-linking agent | Cross-linking time (hours) | Particle Size in µm |
|---|---|---|---|---|---|
| CD11 | 1000 | 1 : 2 | 4ml | 3 | 124.3 |
| CD12 | 1200 | 1 : 2 | 4ml | 3 | 109.4 |
| CD13 | 1500 | 1 : 2 | 4ml | 3 | 93.4 |
| CD14 | 1800 | 1 : 2 | 4ml | 3 | 75.4 |

**Formulation design**

Based on the results of preliminary investigation, the different process parameters like cross linking agent, cross-linking time, concentration of surfactant and stirring speed were optimized and final formulations were designed by varying polymer to drug ratio as mentioned in Table .

**Method**

The chitosan solution was prepared in 5% aqueous acetic acid in which the drug was dispersed. The resultant mixture was extruded through a syringe (no. 20) in 100ml of liquid paraffin (heavy and light, 1:1 ratio) containing 0.2%w/v dioctyl sodium sulfosuccinate and stirring was performed using a propeller at 1800rpm. After 2minutes, 4ml of Glutaraldehyde saturated toluene was added into the dispersion. Then at the end of 15minutes, 4ml of 25% aqueous Glutaraldehyde was added drop by drop and stirring was continued for 3hours. The microspheres thus obtained were filtered and washed several times with hexane to remove traces of oil. They were then washed with plenty of ice cold water to remove the acetic acid and Glutaraldehyde. The microspheres were then dried in an air oven at $50^0$C and stored in desiccators at roomtemperature.

**Table : Formulation design by varying polymer to drug ratio**

| Formulation code | Drug to Polymer ratio | Amount of cross-linking agent | Cross-linking time | Concentration of surfactant (% w/v) | Stirring speed (rpm) |
|---|---|---|---|---|---|
| CDX1 | 1:1 | 4ml | 3 hours | 0.2%w/v | 1800 |
| CDX2 | 1:2 | 4ml | 3 hours | 0.2%w/v | 1800 |
| CDX3 | 1:3 | 4ml | 3 hours | 0.2%w/v | 1800 |
| CDX4 | 1:4 | 4ml | 3 hours | 0.2%w/v | 1800 |

**Preparation of mucoadhesive microspheres of sodium alginate**

**Preliminary studies**

The preliminary studies were carried out by preparing various batches of microspheres with different process parameters in an effort to optimize the formulations for obtaining microspheres with proper physical characteristics and of particle size ranging from which are ideal for oralcavity.The following are the process variables which were studied to standardize the method for preparation of the microspheres.

- Effect of different cross linkingagent
- Effect of concentration of cross linkingagent

**Effect of different cross linkingagent**

Three batches of microspheres were prepared namely SD1, SD2 and SD3 with three different cross linking agent calcium chloride, barium chloride and aluminium sulphate with stirring speed of 300rpm respectively. The other process variables like concentration of cross linking agent (5.0%w/v) and rpm (300) was kept Constant. The prepared microspheres were evaluated for particlesize.

**Table: Effectofdifferentcrosslinkingagenton%drugentrapment efficiencyandparticlesize**

| Batchno | Different cross linking agent | Concentration of cross linking agent % w/v | % Drug Entrapment Efficiency | Particle Size in µm |
|---|---|---|---|---|
| SD1 | $Cacl_2$ | 5% | 78.5 | 580.4 |
| SD2 | $Bacl_2$ | 5% | 67.3 | 630.7 |
| SD3 | $Al_2(so_4)_3$ | 5% | 58.2 | 680.2 |

**Effect of concentration of cross linkingagent**

Four different formulations namely SD4, SD5, SD6 and SD7 were prepared by varying the cross linking agent (calcium chloride) concentration from 2.5%, 5.0%, 7.5% and 10% w/v respectively, while keeping all other process variable like Stirring speed (300rpm) and drug to polymer ratio constant. The prepared microspheres were evaluated for particlesize.

**Table: Formulations with varying concentration of cross linking agent**

| Batch no | Concentration of cross linking agent % w/v | Stirring speed (rpm) | % Drug Entrapment Efficiency | Particle size in µm |
|---|---|---|---|---|
| SD4 | 2.5 | 300 | 45.6 | 560.8 |
| SD5 | 5.0 | 300 | 76.7 | 640.3 |
| SD6 | 7.5 | 300 | 68.5 | 720.5 |
| SD7 | 10.0 | 300 | 55.4 | 840.4 |

**Formulation design**

Based on the results of preliminary investigation, the different process parameters like cross linking agent and concentration of cross linking agent were optimized and final formulations were designed by varying polymer to drug ratio as mentioned in Table .

**Method**

The alginate solution comprissing 1-4% w/v sodium alginate were prepared by initially dissolving the polymer in deionised water using gentle heat, being stirred magnetically.Oncompletesolution,anaccurateweighedquantityofdrugwasadded.The dispersions were sonicated for 30mins to remove any air bubbles that may have been formed during stirring. The sodium alginate-drug dispersion(25ml) were added drop wise via a 26 guage hypodermic needle fitted with a 10ml syringe into 50ml of 5% cross linking agent calcium chloride being stirred at 300rpm. The formed alginate microspheres were further allowed to stir in the solution of cross linking agents for an additional one hr, then the solution was decanted and the microspheres were thereafter dried at $60^0$C for 2 hrs in an oven.

**Table:-Formulation design with varying polymer to drug ratio**

| Formulation code | Drug to polymer ratio | Concentration of Crosslinking agent ( $cacl_2$) (%w/v) | Stirring speed (rpm) |
|---|---|---|---|
| SDX1 | 1:1 | 5%w/v | 300 |
| SDX2 | 1:2 | 5%w/v | 300 |
| SDX3 | 1:3 | 5%w/v | 300 |
| SDX4 | 1:4 | 5%w/v | 300 |

**Preparation of mucoadhesive microspheres of hydroxyl propyl guar**

**Preliminary studies**

The preliminary studies were carried out by preparing various batches of microspheres with different process parameters in an effort to optimize the formulations for obtaining microspheres with proper physical characteristics and of particle size ranging from which are ideal for oralcavity.The following are the process variables which were studied to standardize the method for preparation of the microspheres.

- Effect of drugConcentration
- Effect of concentration ofsurfactant
- Effect of Stirringspeed

**Effect of drugconcentration**

Four different formulations namely HD1, HD2, HD3 and HD4 were prepared by varying the Drug to polymer ratio from 0.5:2, 1:2, 1.5:2 and 2:2 respectively, while keeping all other process variable like Concentration of emulsifier (0.5%w/v) and Stirring speed (2000rpm) constant. The prepared microspheres were evaluated for particle size and drug entrapment efficiency.

**Table: Effect of Drug to polymer ratio on Particle size and % Drug entrapment efficiency**

| Batch no. | Drug to polymer ratio | Concentration of emulsifier (% w/v) | Stirring speed (rpm) | Particle size (µm) | % Drug entrapment efficiency |
|---|---|---|---|---|---|
| HD1 | 0.5:2 | 0.5 | 2000 | 364.3 | 78.7 |
| HD2 | 1:2 | 0.5 | 2000 | 440.4 | 80.3 |

| | | | | | |
|---|---|---|---|---|---|
| HD3 | 1.5:2 | 0.5 | 2000 | 570.6 | 74.6 |
| HD4 | 2:2 | 0.5 | 2000 | 610.9 | 69.19 |

**Effect of concentration ofsurfactant**

Four different formulations namely HD1, HD2, HD3 and HD4 were prepared by varying the surfactant (span 80) concentration from 0.2%, 0.3%, 0.4% and 0.5% w/v respectively, while keeping all other process variable like Stirring speed (2000rpm) and drug to polymrer ratio constant. The prepared microspheres were evaluated for particlesize.

**Table: Effect of Concentration of emulsifier on Particle size**

| Batch no | Concentration of surfactant % w/v | Drug to polymer ratio | Stirring speed (rpm) | Particle size in µm |
|---|---|---|---|---|
| HD5 | 0.2 | 1:2 | 2000 | 620.8 |
| HD6 | 0.3 | 1:2 | 2000 | 523.6 |
| HD7 | 0.4 | 1:2 | 2000 | 430.6 |
| HD8 | 0.5 | 1:2 | 2000 | 390.4 |

**Effect of stirringspeed**

The speed of the propeller was varied to get the particle size suitable for nasal delivery. Four batches of microspheres were prepared namely HD5, HD6, HD7 and HD8withastirringspeedof1400,1600,1800and2000rpmrespectively.Theother

Process variables like concentration of emulsifier (0.5%w/v) and temperature ($80^0$C) were kept Constant. The prepared microspheres were evaluated for particle size.

**Table: Effect of Stirring speed on Particle size**

| Batch no | Stirring speed (rpm) | Drug to polymer ratio | Concentration of surfactant % w/v | Particle size in µm |
|---|---|---|---|---|
| HD9 | 1400 | 1:2 | 0.2 | 621.6 |
| HD10 | 1600 | 1:2 | 0.2 | 540.2 |
| HD11 | 1800 | 1:2 | 0.2 | 486.4 |
| HD12 | 2000 | 1:2 | 0.2 | 420.7 |

**Formulation design**

Based on the results of preliminary investigation, the different process parameters like concentration of surfactant and stirring speed were optimized and final formulations were designed by varying polymer to drug ratio as mentioned in Table.

**Method**

A 1%w/v aqueous hydroxyl propyl guar solution was prepared using a magnetic stirrer. Pure amlodipine besylate was added to the aqueous polymeric solution and stirred for 15minutes. The resultant dispersion was poured into 100ml of liquid paraffin containing 0.5%w/v of span 80 as emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system at a constant speed of 2000rpm. While stirring, the flask and its contents were heated to $80^0$C. Stirring and heating weremaintainedfor4.5hoursuntilaqueousphasewascompletelyremovedbyevaporation. The light mineral oil was decanted and the collected microspheres were washed three times with 100ml aliquots of hexane, filtered through whatman filter paper and then dried in an oven at $50^0$C for 2hours and stored in a desiccator at room temperature.

**Table: Formulation design with varying polymer to drug ratio**

| Formulation code | Drug to polymer ratio | Concentration of emulsifier (% w/v) | Stirring speed (rpm) |
|---|---|---|---|
| HDX1 | 1:1 | 0.5 | 2000 |
| HDX2 | 1:2 | 0.5 | 2000 |
| HDX3 | 1:3 | 0.5 | 2000 |
| HDX4 | 1:4 | 0.5 | 2000 |

**Evaluation and characterisation of the prepared microspheres**

**Percentage yield**

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to microspheres lost during the washing process. A 100% yield could not be achieved principally due to adhesion of microspheres to the stirring rod of the homogenizer. The percentage yield was found to be in the range of 82.25 to 95.12% for chitosan microspheres, 78.62 to 89.75% for Hydroxypropyl Guar microspheres and 74.35 to 86.64% for sodium alginate microspheres.

**Table: Percentage yield of Chitosan Doxycycline microspheres**

| Formulation code | CDX1 | CDX2 | CDX3 | CDX4 |
|---|---|---|---|---|
| % Yield | 82.25 | 86.67 | 93.42 | 95.12 |

**Table: Percentage yield of Hydroxypropyl Guar Doxycycline microspheres**

| Formulation code | HDX1 | HDX2 | HDX3 | HDX4 |
|---|---|---|---|---|
| % Yield | 78.62 | 83.47 | 85.22 | 89.75 |

**Table: Percentage yield of Sodium Alginate doxycycline microspheres**

| Formulation code | SDX1 | SDX2 | SDX3 | SDX4 |
|---|---|---|---|---|
| % Yield | 74.35 | 79.41 | 84.48 | 86.64 |

**Drug entrapment efficiency**

% Drug entrapment efficiency of doxycycline monohydrate ranged from 66.9 to 84.3% for chitosan microspheres, 64.7 to 80.4% for Hydroxypropyl Guar microspheres and 67.3 to 81.3% for sodium alginate microspheres.

**Table: Drug entrapment efficiency of Chitosan Doxycycline microspheres**

| Formulation code | Absorbance | | | Average absorbance | Drug content (mg) | % Drug entrapment efficiency |
|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | | | |
| CDX1 | 0.159 | 0.161 | 0.163 | 0.161 | 13.39 | 66.9 |
| CDX2 | 0.169 | 0.171 | 0.174 | 0.171 | 14.86 | 74.3 |
| CDX3 | 0.191 | 0.194 | 0.199 | 0.194 | 16.68 | 84.3 |
| CDX4 | 0.174 | 0.171 | 0.177 | 0.174 | 15.08 | 75.7 |

**Table: Drug entrapment efficiency of Hydroxypropyl guar Doxycycline microspheres**

| Formulation code | Absorbance | | | Average absorbance | Drug content (mg) | % Drug entrapment efficiency |
|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | | | |
| HDX1 | 0.154 | 0.146 | 0.149 | 0.149 | 12.94 | 64.7 |
| HDX2 | 0.179 | 0.174 | 0.175 | 0.177 | 15.34 | 76.7 |
| HDX3 | 0.181 | 0.189 | 0.186 | 0.185 | 16.08 | 80.4 |
| HDX4 | 0.180 | 0.176 | 0.184 | 0.181 | 15.64 | 78.2 |

**Table: Drug entrapment efficiency of sodium alginate doxycycline microspheres**

| Formulation code | Absorbance | | | Average absorbance | Drug content (mg) | % Drug entrapment efficiency |
|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | | | |
| SDX1 | 0.157 | 0.153 | 0.156 | 0.155 | 13.46 | 67.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SDX2 | 0.189 | 0.185 | 0.188 | 0.187 | 16.26 | 81.3 |
| SDX3 | 0.179 | 0.185 | 0.182 | 0.181 | 15.74 | 78.7 |
| SDX4 | 0.169 | 0.167 | 0.164 | 0.167 | 14.51 | 72.5 |

**Particle size analysis**

The prepared microspheres were in a size range suitable for oral delivery. The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased emulsion droplet size and finally a higher microspheres size. Chitosan doxycycline microspheres had a size range of 45.8µm to 94.5µm, Hydroxypropyl Guar doxycycline microspheres exhibited a size range between 443.7µm to 493.8µm and sodium alginate Amlodipine microspheres had a size range of 660.4µm to 734.6µm.

| BATCH | Average Particle size |
|---|---|
| CDX1 | 45.8 µm |
| CDX2 | 48.9µm |
| CDX3 | 88.1µm |
| CDX4 | 94.5µm |
| HDX1 | 443.7µm |
| HDX2 | 475.2µm |
| HDX3 | 484.5µm |
| HDX4 | 493.8µm |
| SDX1 | 660.4µm |
| SDX2 | 682.2µm |
| SDX3 | 720.8µm |
| SDX4 | 734.6µm |

**Shape and surface morphology**

Morphology of the microspheres was investigated by Scanning electron microscopy. The photographs of the optimized formulations taken by scanning electron microscope are shown in the figure.The results of SEM revealed that the microspheres of chitosan (CDX3) were discrete and spherical in shape with a rough outer surface morphology which might be due to surface associated drug and cross-linking of the polymer with Glutaraldehyde. Microspheres of Hydroxypropyl Guar (HDX2) and Sodium alginate (SDX2)werespherical and their surface was smooth, giving them a good appearance.

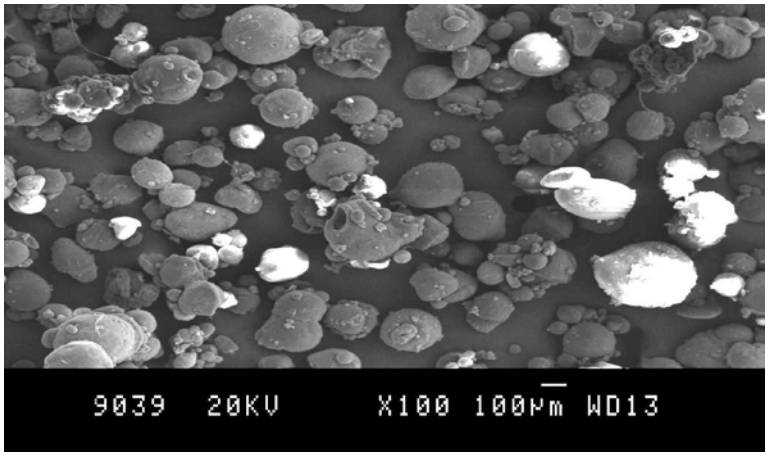


**Fig:- SEM picture of chitosan microspheres (low magnification)**

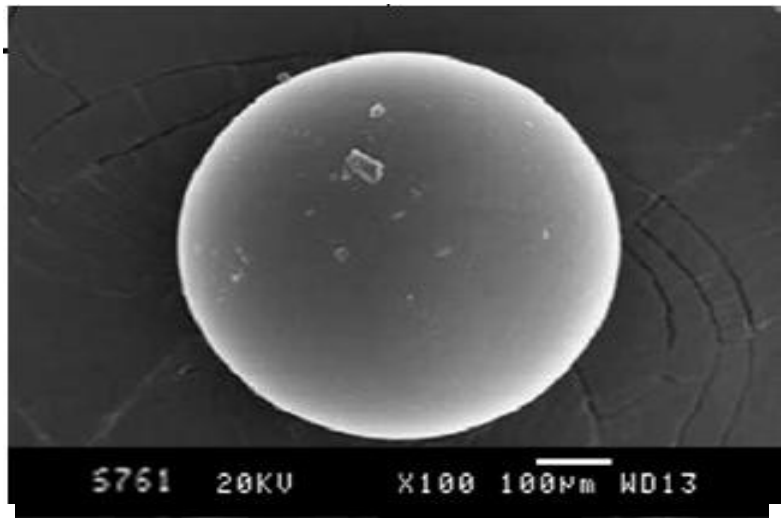


**Fig. SEM picture of chitosan microspheres (high magnification)**

**Fig. SEM picture of HPG microspheres (low magnification)**

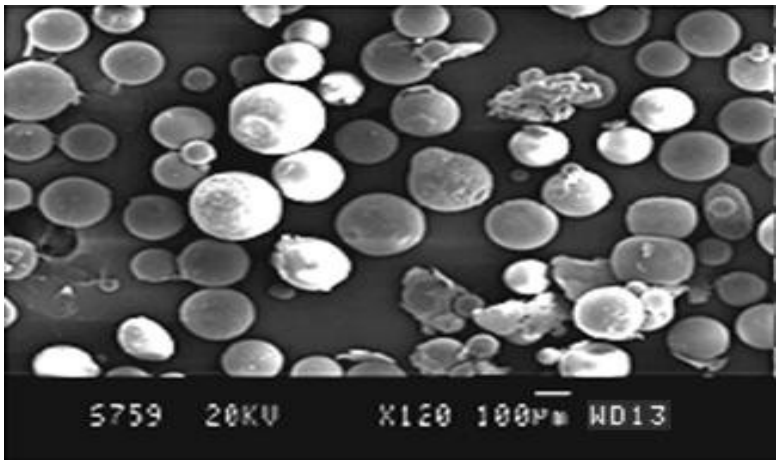


**Fig. SEM picture of HPG microspheres (high magnification**

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**Fig.:- SEM picture of Sodium Alginate microspheres (low magnification)**

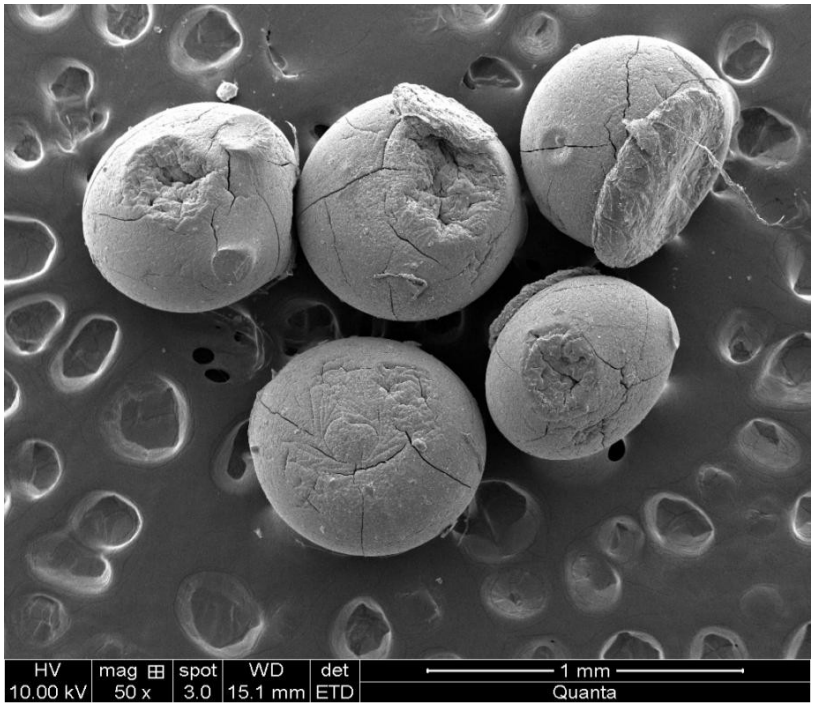
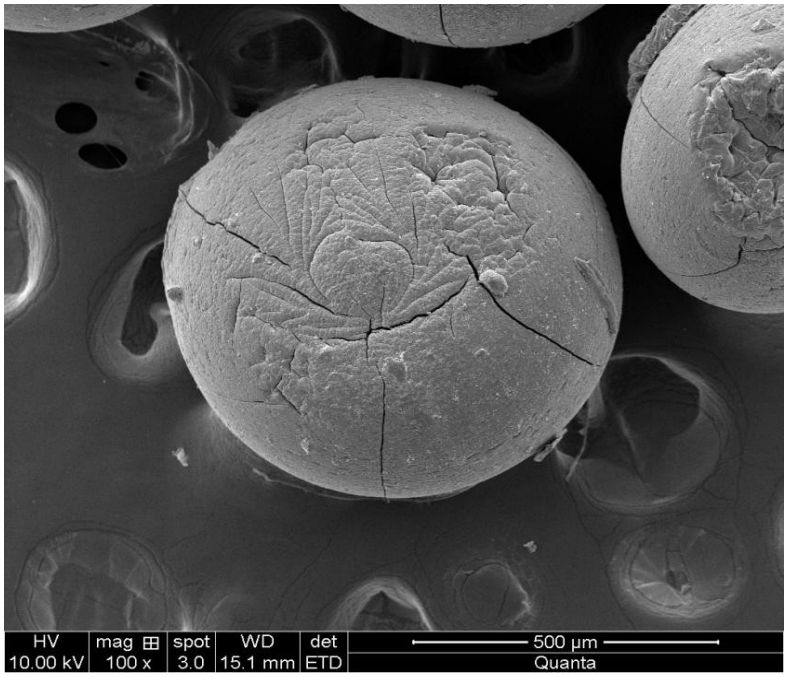



**Fig.:- SEM picture of Sodium Alginate microspheres (high magnification)**



**Degree ofswelling**

The degree of swelling is expressed as the percentage of water in the hydrogel at any instant during swelling. As the polymer to drug ratio increased, the degree of swelling increased from 0.7985 ± 0.013 to 1.1607 ± 0.014 for chitosan microspheres, 0.8162 ± 0.014 to 1.1457 ± 0.009
for Hydroxypropyl Guar microspheres and 0.8678 ± 0.013 to 1.1484 ± 0.006 for Hydroxypropyl Guar microspheres.

**Table: Degree of swelling of Chitosan Doxycyline microspheres**

| Formulation code | Degree of Swelling | | | Average Swellability | ± SEM |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | | |
| CDX1 | 0.7241 | 0.8436 | 0.8279 | 0.7985 | 0.0132 |
| CDX2 | 0.9462 | 0.8473 | 0.9542 | 0.9159 | 0.0149 |
| CDX3 | 0.9543 | 1.0243 | 0.9739 | 0.9841 | 0.0086 |
| CDX4 | 1.1256 | 1.1873 | 1.1693 | 1.1607 | 0.0140 |

**Table: Degree of swelling of Hydroxypropyl Guar Doxycycline microspheres**

| Formulation code | Degree of Swelling | | | Average Swellability | ± SEM |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | | |
| HDX1 | 0.7642 | 0.7781 | 0.9063 | 0.8162 | 0.014 |
| HDX2 | 0.9420 | 0.9832 | 0.9011 | 0.9421 | 0.0068 |
| HDX3 | 0.9756 | 0.9931 | 0.9867 | 0.9851 | 0.0078 |
| HDX4 | 1.1135 | 1.1452 | 1.1786 | 1.1457 | 0.0095 |

**Table: Degree of swelling of Sodium Alginate Doxycycline microspheres**

| Formulation code | Degree of Swelling | | | Average Swellability | ± SEM |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | | |
| SDX1 | 0.8134 | 0.8247 | 0.9654 | 0.8678 | 0.0131 |
| SDX2 | 0.9465 | 0.9693 | 0.9883 | 0.9662 | 0.0116 |
| SDX3 | 0.9971 | 0.9882 | 0.9981 | 0.9944 | 0.0064 |
| SDX4 | 1.1461 | 1.1272 | 1.1721 | 1.1484 | 0.0060 |

***In-vitro* mucoadhesion test**

As the polymer to drug ratio increased, Chitosan microspheres exhibited % mucoadhesion ranging from 78.75 ± 0.05 to 84.50 ± 0.21, Hydroxypropyl Guarmicrospheres exhibited % mucoadhesion ranging from 76.85 ± 0.12 to 81.40 ± 0.17 and sodium alginate microspheres in the range of 78.70 ± 0.16 to 83.70 ± 0.05.

**The rank of order of mucoadhesion is Chitosan > sodium alginate > HPG.**

**Table: % Mucoadhesion of Chitosan Doxycycline microspheres**

| Formulation code | % Mucoadhesion | | Average % Mucoadhesion | ± SEM |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | | |
| CDX1 | 78.8 | 78.7 | 78.75 | 0.056 |
| CDX2 | 79.9 | 80.2 | 80.10 | 0.115 |
| CDX3 | 82.1 | 82.2 | 82.15 | 0.200 |
| CDX4 | 84.4 | 84.6 | 84.50 | 0.210 |

**Table: % Mucoadhesion Hydroxypropyl Guar Doxycycline microspheres**

| Formulation code | % Mucoadhesion | | Average % Mucoadhesion | ± SEM |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | | |
| HDX1 | 76.9 | 76.8 | 76.85 | 0.123 |
| HDX2 | 78.2 | 78.8 | 78.50 | 0.396 |

| | | | | |
|---|---|---|---|---|
| HDX3 | 79.5 | 79.6 | 79.55 | 0.221 |
| HDX4 | 81.6 | 81.2 | 81.40 | 0.176 |

**Table: % Mucoadhesion of sodium alginate Doxycycline microspheres**

| Formulation code | % Mucoadhesion | | Average % Mucoadhesion | ± SEM |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | | |
| SDX1 | 78.6 | 78.8 | 78.70 | 0.166 |
| SDX2 | 79.6 | 79.8 | 79.70 | 0.066 |
| SDX3 | 80.4 | 81.6 | 81.50 | 0.115 |
| SDX4 | 83.8 | 83.6 | 83.70 | 0.056 |

**Table: % Yield, % Drug entrapment efficiency, Particle size, Degree of swelling and % mucoadhesion of Chitosan Doxycycline microspheres**

| Formulation code | % Yield | % Drug entrapment efficiency | Particle size (µm) | Degree of Swelling | % Mucoadhesion |
|---|---|---|---|---|---|
| CDX1 | 82.25 | 66.9 | 45.8 | 0.7985 | 78.71 |
| CDX2 | 86.67 | 74.3 | 48.9 | 0.9159 | 80.06 |
| CDX3 | 93.42 | 84.3 | 88.1 | 0.9841 | 82.13 |
| CDX4 | 95.12 | 75.7 | 94.5 | 1.1607 | 84.56 |

**Table: % Yield, % Drug entrapment efficiency, Particle size, Degree of swelling and % mucoadhesion of HPG Doxycycline microspheres**

| Formulation code | % Yield | % Drug entrapment efficiency | Particle size (µm) | Degree of Swelling | % Mucoadhesion |
|---|---|---|---|---|---|
| HDX1 | 74.35 | 64.7 | 443.7 | 0.8162 | 76.83 |
| HDX2 | 79.41 | 76.7 | 475.2 | 0.9421 | 78.43 |
| HDX3 | 84.48 | 80.4 | 484.5 | 0.9851 | 79.51 |
| HDX4 | 86.64 | 78.2 | 493.8 | 1.1457 | 81.01 |

**Table: % Yield, % Drug entrapment efficiency, Particle size, Degree of swelling and % mucoadhesion of Sodium Alginate Doxycycline microspheres**

| Formulation code | % Yield | % Drug entrapment efficiency | Particle size (µm) | Degree of Swelling | % Mucoadhesion |
|---|---|---|---|---|---|
| SDX1 | 78.62 | 67.3 | 660.4 | 0.8678 | 78.63 |
| SDX2 | 83.47 | 81.3 | 682.2 | 0.9662 | 79.26 |
| SDX3 | 85.22 | 78.7 | 720.8 | 0.9944 | 81.06 |
| SDX4 | 89.75 | 72.5 | 734.6 | 1.1484 | 83.83 |

***In-vitro* drug diffusion studies**

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As the polymer to drug ratio was increased, the formulations CDX1 – CDX4 showed% CDR of 97.44 - 78.96%, formulations HDX1-HDX4 showed a % CDR of 96.67- 77.87% and SDX1-SDX4 showed a % CDR of 97.47- 79.58% at the end of 8 hours. The results obtained in the *in-vitro* drug diffusion studies are tabulated in Table andFigure.

**Table: In-Vitro drug diffusion data of Chitosan Doxycyline MicrospheresDose of DOXYCYCLINE: 20mg Volume withdrawn: 1mlVolume made upto: 25ml**

| Time (Hours) | % Cumulative Drug Release | | | |
|---|---|---|---|---|
| | CDX1 | CDX2 | CDX3 | CDX4 |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 18.11 | 19.34 | 17.23 | 16.08 |
| 1 | 23.15 | 28.17 | 25.10 | 24.73 |
| 2 | 39.02 | 35.37 | 31.51 | 34.14 |
| 3 | 57.21 | 43.29 | 38.57 | 42.53 |
| 4 | 72.38 | 53.17 | 47.37 | 52.27 |
| 5 | 82.47 | 71.09 | 63.33 | 60.99 |
| 6 | 90.71 | 77.85 | 69.36 | 67.48 |
| 7 | 94.39 | 84.12 | 76.02 | 72.29 |
| 8 | 97.44 | 89.27 | 83.32 | 78.96 |

**Fig.:- Comparison of *In-Vitro* drug diffusion profile of Chitosan Doxycycline microspheres**

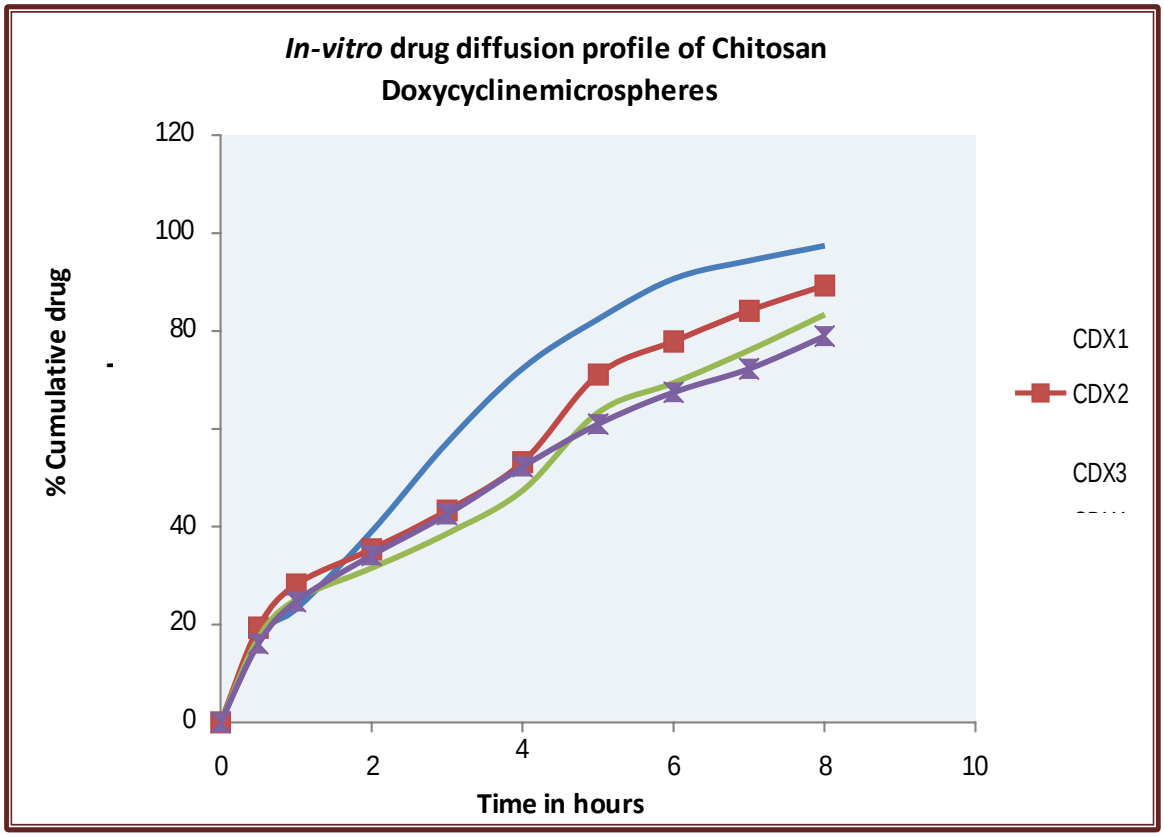

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**Table: In-Vitro drug diffusion data of Hydroxypropyl guar Doxycyline Microspheres**
**Dose of DOXYCYCLINE: 20mg Volume withdrawn: 1ml ,Volume made upto: 25ml**

| Time (Hours) | % Cumulative Drug Release | | | |
|---|---|---|---|---|
| | HDX1 | HDX2 | HDX3 | HDX4 |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 22.20 | 18.73 | 17.87 | 15.50 |
| 1 | 32.35 | 27.29 | 26.04 | 23.84 |
| 2 | 40.61 | 34.26 | 32.68 | 32.91 |
| 3 | 49.71 | 41.94 | 40.01 | 41.01 |
| 4 | 59.67 | 50.34 | 48.02 | 50.40 |
| 5 | 75.36 | 63.57 | 60.65 | 58.81 |
| 6 | 86.48 | 75.29 | 71.82 | 65.06 |
| 7 | 91.54 | 80.77 | 77.06 | 69.70 |
| 8 | 96.67 | 88.10 | 84.04 | 77.85 |

**Fig.:- Comparison of *In-Vitro* drug diffusion profile of HPG Doxycycline microspheres**

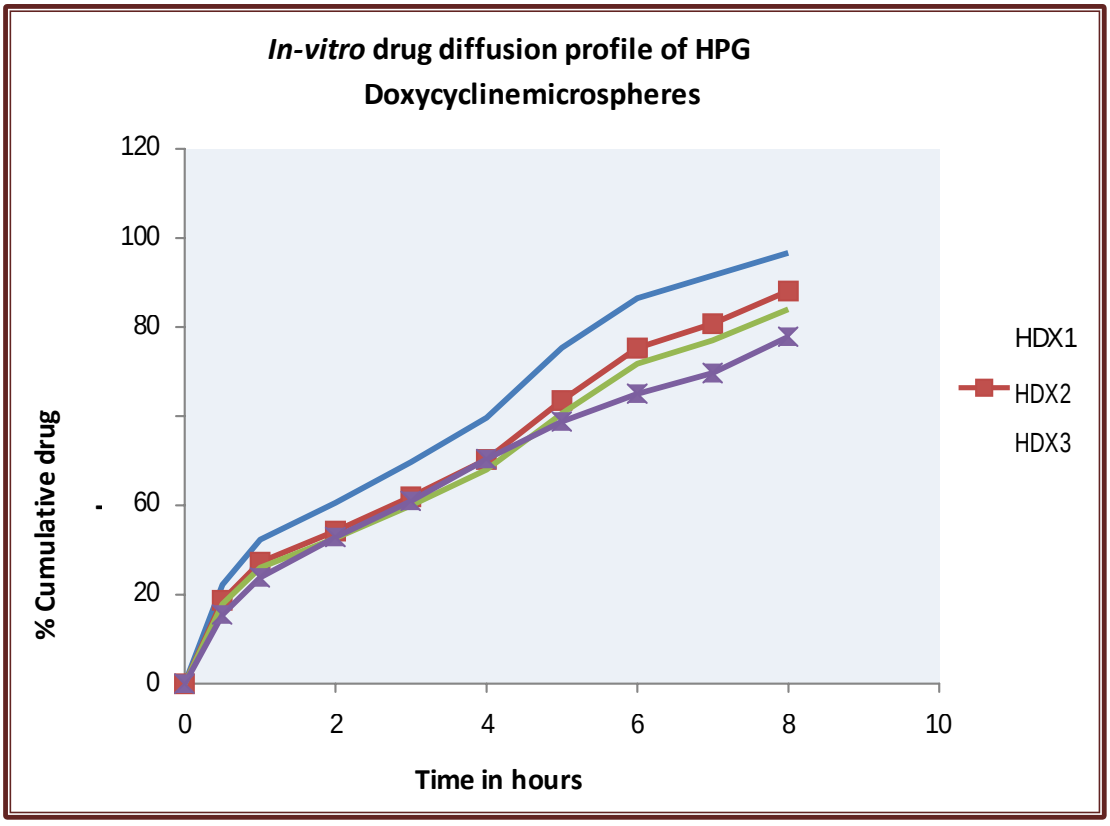

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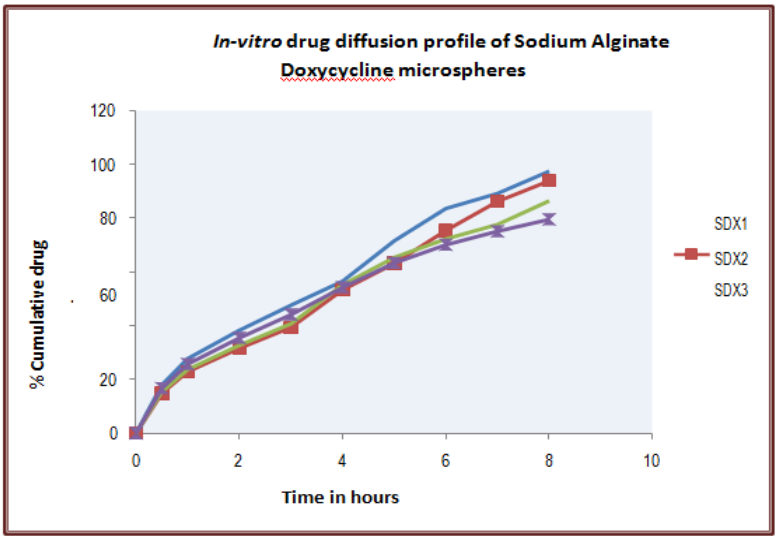
**Table: In-Vitro drug diffusion data of Sodium alginate Doxycyline Microspheres**
**Dose of DOXYCYCLINE: 20mg Volume withdrawn: 1ml , Volume made upto: 25ml**

| Time (Hours) | % Cumulative Drug Release | | | |
|---|---|---|---|---|
| | SDX1 | SDX2 | SDX3 | SDX4 |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 18.01 | 14.91 | 15.40 | 16.71 |
| 1 | 27.70 | 22.93 | 23.69 | 25.70 |
| 2 | 38.25 | 31.66 | 32.71 | 35.48 |
| 3 | 47.65 | 39.44 | 40.75 | 44.20 |
| 4 | 56.56 | 53.44 | 55.21 | 54.32 |
| 5 | 71.63 | 63.29 | 65.38 | 63.39 |
| 6 | 83.63 | 75.51 | 72.30 | 70.13 |
| 7 | 89.18 | 86.30 | 77.63 | 75.13 |
| 8 | 97.47 | 93.95 | 86.45 | 79.58 |

**Fig.:- Comparison of *In-Vitro* drug diffusion profile of Sodium Alginate Doxycycline Microspheres**



**In-vitro drug release kinetics**

**Table : Data for analysis of drug release mechanism from Mucoadhesive microsphere formulations**

| Formulation code | Zero order | | First order | | Matrix | | Peppas | | Hixson-Crowell | | Parameters for korsmeyer - peppas equation | | Best fit model |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | K | R | K | R | K | R | K | R | K | n | k | |
| CDX1 | 0.9744 | 5.4827 | 0.9849 | -0.0668 | 0.9557 | 12.895 | 0,9912 | 5.7810 | 0.9819 | -0.0208 | 0.9949 | 5.7810 | Peppas |
| CDX2 | 0.8830 | 0.0042 | 0.8830 | 0.0000 | 0.9915 | 0.0102 | 0.9876 | 0.0106 | 0.8830 | 0.0000 | 0.4733 | 0.0106 | Matrix |
| CDX3 | 0.9008 | 0.0038 | 0.9008 | 0.0000 | 0.9925 | 0.0091 | 0.9876 | 0.0093 | 0.9008 | 0.0000 | 0.4821 | 0.0093 | Matrix |
| CDX4 | 0.9181 | 0.0045 | 0.9181 | 0.0000 | 0.9963 | 0.0109 | 0.9950 | 0.0106 | 0.9181 | 0.0000 | 0.5104 | 0.0106 | Matrix |
| HDX1 | 0.8542 | 0.0046 | 0.8542 | 0.0000 | 0.9936 | 0.0111 | 0.9920 | 0.0120 | 0.8542 | 0.0000 | 0.4490 | 0.0120 | Matrix |
| HDX2 | 0.8926 | 0.0040 | 0.8926 | 0.0000 | 0.9947 | 0.0097 | 0.9904 | 0.0101 | 0.8926 | 0.0000 | 0.4694 | 0.0101 | Matrix |
| HDX3 | 0.8886 | 0.0039 | 0.8886 | 0.0000 | 0.9922 | 0.0093 | 0.9879 | 0.0096 | 0.8886 | 0.0000 | 0.4754 | 0.0096 | Matrix |
| HDX4 | 0.9008 | 0.0040 | 0.9008 | 0.0000 | 0.9927 | 0.0097 | 0.9876 | 0.0099 | 0.9008 | 0.0000 | 0.4281 | 0.0099 | Matrix |
| SDX1 | 0.8720 | 0.0045 | 0.8721 | 0.0000 | 0.9918 | 0.0110 | 0.9885 | 0.0115 | 0.8271 | 0.0000 | 0.4651 | 0.0115 | Matrix |
| SDX2 | 0.9391 | 0.0050 | 0.9392 | 0.0000 | 0.9858 | 0.0119 | 0.9822 | 0.0114 | 0.9392 | 0.0000 | 0.5152 | 0.0114 | Matrix |
| SDX3 | 0.8872 | 0.0039 | 0.8873 | 0.0000 | 0.9926 | 0.0095 | 0.9883 | 0.0098 | 0.8873 | 0.0000 | 0.4729 | 0.0098 | Matrix |
| SDX4 | 0.8693 | 0.0042 | 0.8693 | 0.0000 | 0.9907 | 0.0102 | 0.9877 | 0.0107 | 0.8693 | 0.0000 | 0.4657 | 0.0107 | Matrix |

## Conclusion

In the present work, mucoadhesive microspheres of Chitosan, Hydroxypropyl Guar and Sodium alginate were formulated to deliver Doxycycline monohydrate to oral cavity infections(periodontitis).Details regarding the preparation and evaluation of the formulations have been discussed in the previous chapters. From the study following conclusions could be drawn:-

The results of this investigation indicate that Emulsion cross-linking; Water in oil emulsification solvent evaporation technique and ionic cross linking technique can be successfully employed to fabricate doxycycline monohydrate - loaded Chitosan, HPG and Sodium alginate microspheres respectively.

Micromeritic studies revealed that the mean particle size of the prepared microspheres was in the size range of 50 - 750µm and are suitable for oral cavity administration.

SEM analysis of the microspheres revealed that all the prepared microspheres were discrete, spherical in shape and had ideal surface morphology.

Increase in the polymer concentration led to an increase in % Yield, % Drug entrapment efficiency, Particle size, Degree of swelling and % Mucoadhesion

The *in-vitro* mucoadhesive study demonstrated that chitosan adhered to the mucus to a greater extent than the Sodium alginate and Hydroxypropyl Guar.

The *in-vitro* drug diffusion decreased with increase in the polymer concentration. The drug diffusion was characterized by an initial phase of higher release followed by a second phase of moderate release.

Analysis of drug release mechanism showed that the drug release followed Fickian diffusion and the best fit model was found to be Higuchimatrix.

Based on the results of evaluation tests CDX3, HDX2 and SDX2 were concluded as best formulations for oral cavity infections.

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