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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 50^0C 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 60^0C 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 (80^0C) 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 80^0C. 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 50^0C 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:PercentageyieldofSodiumAlginatedoxycyclinemicrospheres

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:DrugentrapmentefficiencyofHydroxypropylguarDoxycycline 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:Drugentrapmentefficiencyofsodiumalginatedoxycycline 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.

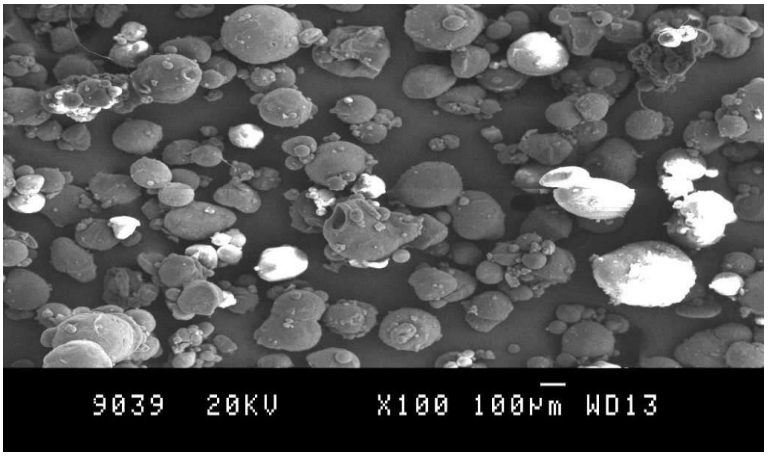


Fig:- SEM picture of chitosan microspheres (low magnification)

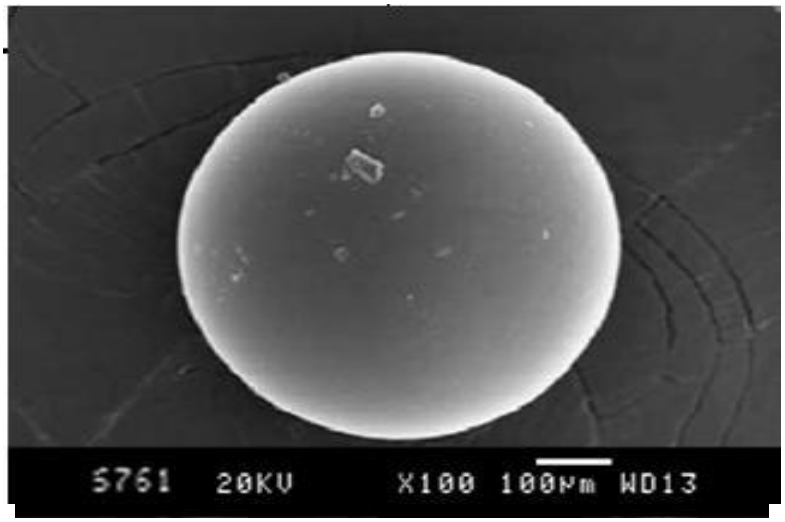


Fig. SEM picture of chitosan microspheres (high magnification)

Fig. SEM picture of HPG microspheres (low magnification)

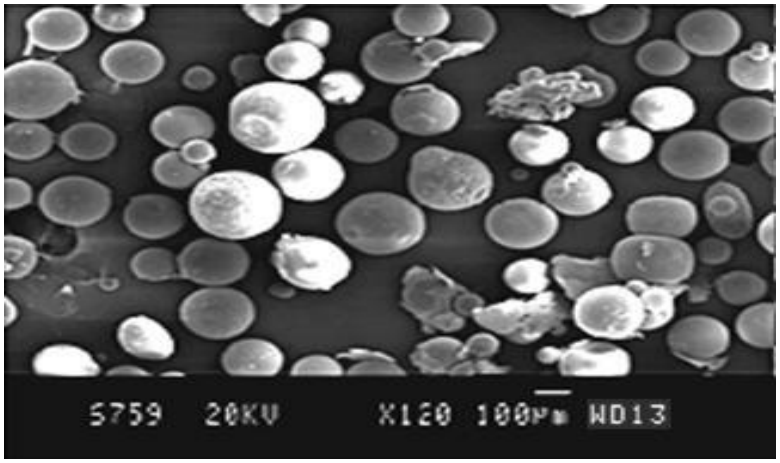


Fig. SEM picture of HPG microspheres (high magnification

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

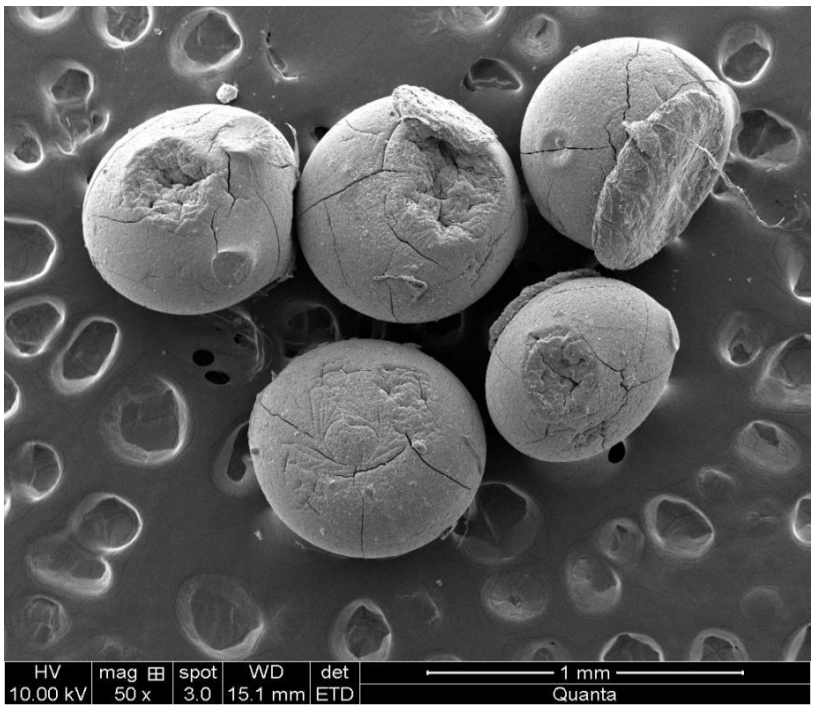
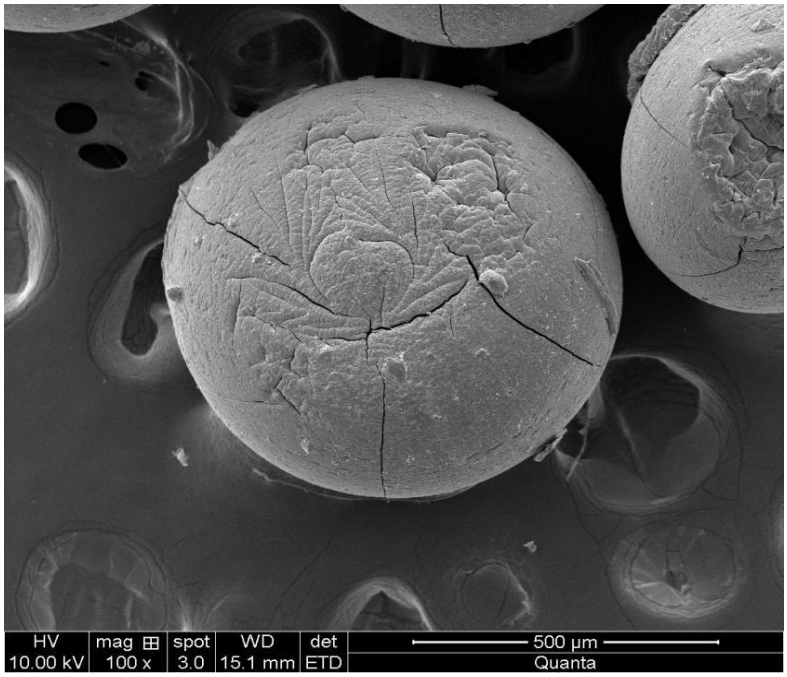


Fig.:- SEM picture of Sodium Alginate microspheres (high magnification)



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

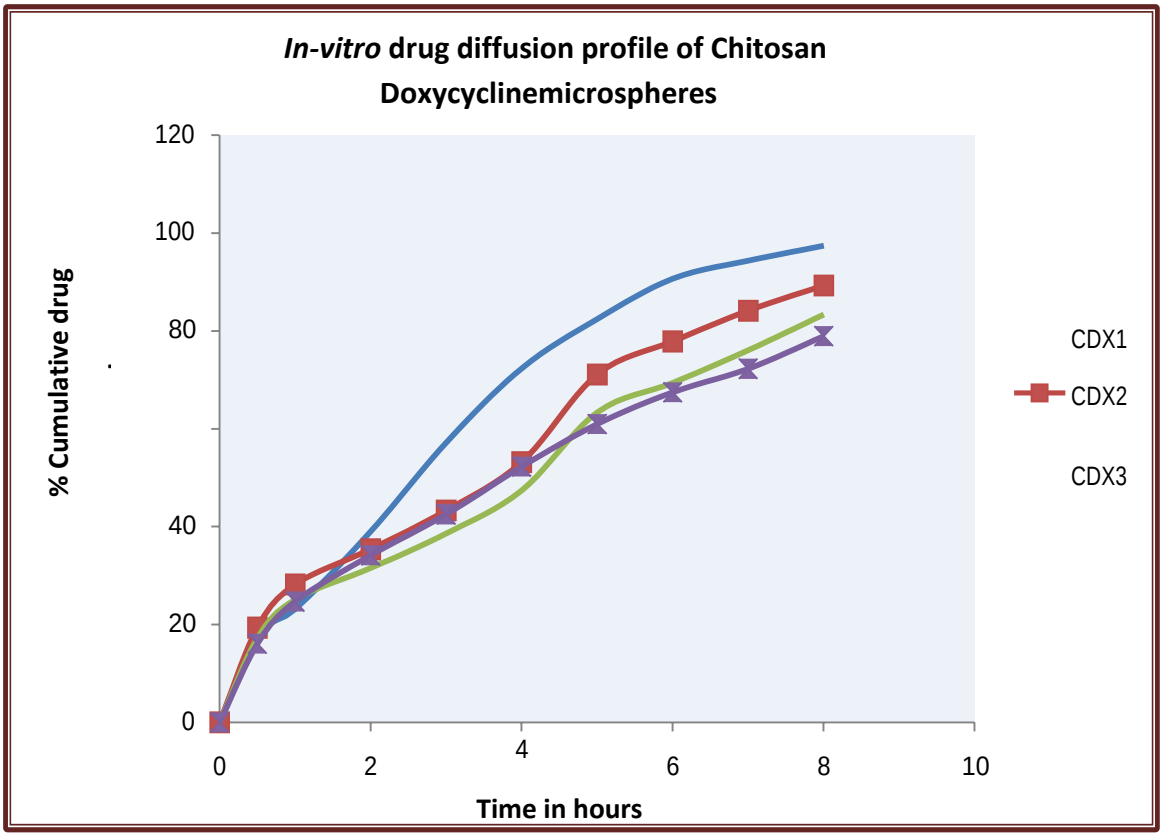

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Table: In-Vitro drug diffusion data of Hydroxypropyl guar Doxycyline Microspheres
Dose of DOXYCYCLINE: 20mg Volume withdrawn: 1ml ,Volume made upto: 25ml

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

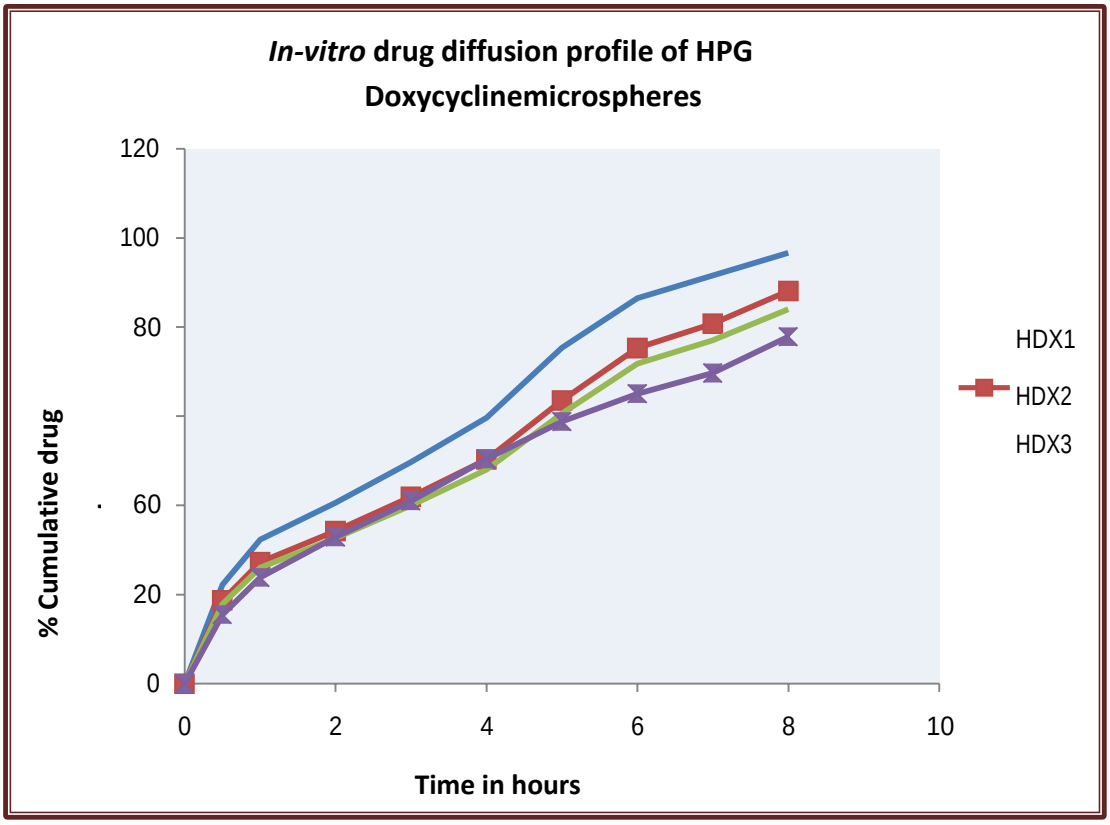

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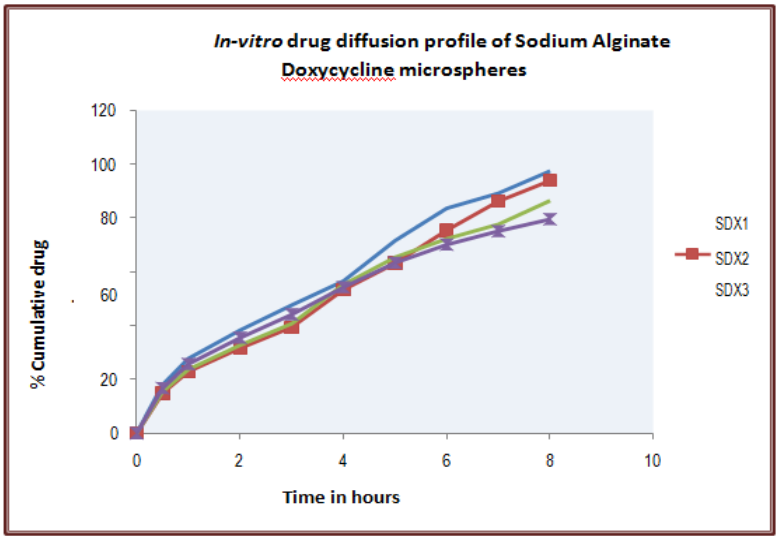
Table: In-Vitro drug diffusion data of Sodium alginate Doxycyline Microspheres
Dose of DOXYCYCLINE: 20mg Volume withdrawn: 1ml , Volume made upto: 25ml

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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