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Research Article

Mucoadhesive, Doxycycline-loaded Hydroxypropyl Guar Microspheres for the treatment of Periodontitis; Preparation and *In Vitro* Characterization

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ABSTRACT

The objective of the study was to develop a drug delivery system for localised controlled release of Doxycycline following insertion into and/or around the periodontal pocket which would ensure increased local drug concentration over an extended period of time with a subsequent decrease in the side effects associated with systemic administration. Hydroxypropyl Guar, a biodegradable polymer, was used in the preparation of microspheres by employing water in oil emulsification solvent evaporation technique. The formulations were evaluated for particle size, particle shape and surface morphology by Scanning Electron Microscopy, percentage yield, drug entrapment efficiency, *in vitro* mucoadhesion test, degree of swelling and *in vitro* drug diffusion through sheep buccal mucosa. The microspheres obtained were free flowing, spherical and had a mean particle size of 110.2 ± 1.13 µm. Increasing polymer concentration resulted in increased drug entrapment efficiency and increased particle size. Doxycycline Hydrochloride was entrapped into the microspheres with an efficiency of 74.7 ± 2.11 % to 83.9 ± 1.54 %. The prepared microspheres showed good mucoadhesion properties, swellability and sustained the release of the drug over a period of 8 h. The data obtained were analysed by fitment into various kinetic models; it was observed that the drug release was matrix diffusion controlled and the release mechanism was found to be non-Fickian. Stability studies were carried out on select formulations at $5^0C \pm 3^0C$, $25^0C \pm 2^0C$ / 60% RH ± 5% RH and $40^0C \pm 2^0C$ / 75% RH ± 5% RH for 90 days. The drug content was observed to be within permissible limits and there were no significant deviations in the *in vitro* mucoadhesion and *in vitro* drug diffusion characteristics.

INTRODUCTION

Periodontal disease is one of the world's most prevalent chronic diseases, which has been considered as a possible risk factor in other systemic diseases such as cardiovascular disease, including coronary heart disease and stroke and pre-term low birth weight infants, which can be characterized by a localized inflammatory response due to infection of periodontal pocket arising from the accumulation of periodontal plaque [1]. Periodontal disease is a collective term ascribed to several pathological conditions characterised by degeneration and inflammation of gums, periodontal ligaments, alveolar bone and dental cementum [2].

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Treatment of Periodontitis, with conventional root planning and scaling with the orally administered antimicrobial agents results in dose-related undesirable side effects [3]. Systemic administration of antibiotics have shown several drawbacks including: inadequate antibiotic concentration at the site of the periodontal pocket; a rapid decline of the plasma antibiotic concentration to sub-therapeutic levels; the development of microbial resistance due to sub-therapeutic drug levels and peak-plasma antibiotic concentrations which may be associated with various side effects, such as hypersensitivity, gastrointestinal intolerance and drug interactions with alcohol [4]. The efficacy of conventional antimicrobial formulations e.g. toothpastes and mouthwash is often reduced by the limited retention of the applied formulations within the oral cavity. Saliva flow, the swallowing reflex, mastication and speech can affect the dilution or dislodgement of a dosage form and

may lead to a rapid decline in the concentration of the active to sub-therapeutic levels. Thus, there is a need for the development of delivery systems that can bestow improved availability to active constituents whilst allowing reduced dosage frequency [5, 6].

In the recent years, local delivery of antimicrobial agents is becoming more prevalent since it leads to increased local drug concentration at the periodontal site to maintain an effective concentration of antibiotic; a decrease in superfluous distribution of the drug to other body organs with a subsequent decrease in side effects; a decrease in the amount of administered dose and hence a decrease in manufacturing costs; the maintenance of drug levels in a therapeutically desirable range over an extended period of time which may lead to improved patient compliance due to the reduction in the frequency of administration of doses and decreased side effects. A pre-requisite for drug delivery systems for localised periodontal therapy is therefore retention on the mucosal surface and controlled drug release at the site of action [7, 8].

Amongst the various approaches available to maximise the localised periodontal therapy and prolong the retention time of the dosage form in the periodontal pocket, the bioadhesive microsphere drug delivery system is an attractive concept that has the ability to control the rate of drug clearance from the oral cavity. The microspheres form a gel-like layer, which is cleared relatively slowly from the periodontal pocket, resulting in prolonged residence time of the drug formulation [9].

Doxycycline is a semi-synthetic antibiotic from *Streptomyces* species and is a drug of choice in the treatment of periodontal diseases [10]. The present investigation was aimed to formulate site – specific controlled release of Doxycycline by preparing Hydroxypropyl Guar (HPG) microspheres for the treatment of periodontitis. Guar gum is the refined endosperm of Guar (*Cyamopsis tetragonalobus*) seed. HPG is made by reacting guar gum with propylene oxide[11]. HPG has been investigated for its gelling [12], viscosity enhancing[13], film forming[14], suspending[15] and mucoadhesive microsphere properties [16].

The purpose of this study is to investigate the suitability of HPG microspheres as localised mucoadhesive drug delivery system for periodontal pockets and also to study the influence of the process variables in the preparation of the microspheres.

MATERIALS AND METHODS

Doxycycline Hydrochloride (DH) and HPG were obtained as gift samples from Micro labs limited, Bangalore and Encore Polymers, Mumbai respectively. Liquid Paraffin (light & heavy) were purchased from Qualigens fine chemicals, Span 80 was purchased from Himedia Laboratories Private limited (Mumbai). All other reagents used were of analytical grade.

Preparation of Microspheres

Mucoadhesive HPG microspheres containing DH were prepared by water in oil emulsification solvent evaporation technique [16]. A 1 %w/v aqueous HPG dispersion was prepared using a magnetic stirrer. Pure DH was added to the aqueous polymeric dispersion and agitated for 15 min. The resultant dispersion was extruded through a syringe (Needle no. 20) into 100 ml of liquid paraffin (heavy and light 1:1 ratio) containing 0.5 %w/v span 80 as emulsifying agent. The aqueous phase was emulsified into the oily phase by agitating the system at a constant agitation speed of 2000 rpm. While stirring, the flask and its contents were heated to 80^0C. Stirring and heating were maintained for about 4.5 h until aqueous phase was completely removed by evaporation. The mineral oil was decanted and the microspheres obtained were washed three times with 100 ml aliquots of n-hexane, filtered through Whatman filter paper and then dried in a hot air oven at 50^0C for 2 h and preserved in a desiccator at room temperature.

Effect of process variables on microsphere properties

HPG microspheres were prepared with different drug to polymer ratio (0.5:3, 1:3, 1.5:3 and 2:3) at emulsifier concentrations of 0.2, 0.3, 0.4 and 0.5 % w/v, at temperatures of 60, 70, 80 and 90^0C, at agitation speeds of 1400, 1600, 1800 and 2000 rpm and with varying HPG to drug ratios of 1:1, 2:1, 3:1 and 4:1. The effect of process variables on the properties of the resulting microspheres is depicted in Table 1, 2 and 3.

Characterization of Microspheres

Percentage Yield

The practical percentage yield[17] was calculated from the weight of dried microspheres recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \text{ X } 100$$

Drug Entrapment Efficiency

Microspheres equivalent to 20 mg of DH were crushed in a glass mortar and pestle and the powdered microspheres were suspended in 100 ml of simulated salivary fluid pH 6.8. After 24 h, the solution was filtered, 1 ml of the filtrate was pipetted out and diluted to 25 ml and analyzed for the drug content using Elico SL- 159 UV Visible spectrophotometer at 365 nm[18].

The drug entrapment efficiency[19] was calculated using the following formula:

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

Particle Size Analysis

Particle size of the microspheres was determined by optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer. The particle diameters of more than 300 microspheres were measured randomly. The average particle size[19] was determined by using Edmondson's equation.

$$D_{mean} = \frac{\sum nd}{\sum n}$$

Where, n = Number of microspheres checked; d = Mean size range

Shape and Surface Morphology

The shape and surface characteristics[20] of the microspheres were evaluated by means of scanning electron microscopy (JEOL – JSM - 840A, Japan). The samples were prepared by gently sprinkling the microspheres on a double adhesive tape, which is stuck to an aluminium stub. The stubs were then coated with gold using a sputter coater (JEOL Fine coat JFC 1100E, ion sputtering device) under high vacuum and high voltage to achieve a film thickness of 30 nm. The samples were then imaged using a 20 KV electron beam.

Degree of Swelling

The Swellability[21] of microspheres in physiological media was determined by allowing the microspheres to swell in the simulated salivary fluid pH 6.8. 100 mg of accurately weighed microspheres were immersed in little excess of simulated salivary fluid pH 6.8 for 24 h and washed thoroughly with deionised water. The degree of swelling was arrived at using the following formula:

$$\propto = \frac{W_S - W_O}{W_O}$$

Where, α is the degree of swelling; Wo is the weight of microspheres before swelling and Ws is the weight of microspheres after swelling

***In Vitro* Mucoadhesion Studies**

The *in vitro* mucoadhesion study of microspheres was assessed using Falling liquid film technique [22].

A strip of sheep buccal mucosa was mounted on a glass slide and 50 mg of accurately weighed microspheres were sprinkled on the buccal mucosa [5]. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed on the stand at an angle of 45^0. Simulated salivary fluid of pH 6.8; previously warmed to 37 ± 0.5^0C was allowed to flow over the microspheres and membrane at the rate of 1 ml/min for 5 min with the help of a peristaltic pump. At the end of this process, the detached particles were collected and weighed. The % *in vitro* mucoadhesion was calculated using the following formula:

$$\% \text{ Mucoadhesion} = \frac{\text{Weight of sample} - \text{weight of detached particles}}{\text{Weight of sample}} \text{ X } 100$$

***In Vitro* Drug Diffusion Studies**

Preparation of buccal mucosa: Fresh sheep buccal mucosa was collected from a near by slaughter house. The buccal mucosa of sheep was separated from sub layer bony tissues and stored in distilled water containing few drops of Gentamycin injection. After complete removal of blood from mucosal surface, it was attached to the donor chamber tube.

In vitro drug diffusion study was done using Franz diffusion cell [8, 23]. The receptor compartment having a capacity of 100 ml filled with simulated salivary fluid of pH 6.8 was taken. The buccal mucosa was carefully cut with a scalpel and tied to the donor tube chamber and it was placed establishing contact with the diffusion medium in the recipient chamber. Microspheres equivalent to 20 mg of DH were spread on the sheep buccal mucosa. At hourly intervals, 1 ml of the diffusion sample was withdrawn with the help of a hypodermic syringe, diluted to 25 ml and absorbance was read at 365 nm. Each time, the sample withdrawn was replaced with 1 ml of pre-warmed simulated salivary fluid (pH 6.8) to maintain a constant volume of the receptor compartment vehicle.

Table 1: Effect of drug to polymer ratio on % drug entrapment efficiency and particle size of microspheres

Formulation code	Drug to polymer ratio	Emulsifier concentration (% w/v)	Temperature (^{0}C)	% Drug entrapment efficiency*	Particle size (μm)*
HD-1	0.5:2	0.5	80	78.7 ± 2.19	93.6 ± 3.04
HD-2	1:2	0.5	80	81.3 ± 1.63	112.6 ± 1.52
HD-3	1.5:2	0.5	80	73.4 ± 1.08	133.8 ± 3.11
HD-4	2:2	0.5	80	71.6 ± 2.54	174.5 ± 1.65

In all the formulations, agitation speed of 2000 rpm, was kept constant. *Data are expressed as mean ±SD., n=3

Table 2: Effect of emulsifier concentration, temperature and agitation speed on particle size of microspheres

Formulation code	Emulsifier concentration (% w/v)	Temperature (^{0}C)	Agitation speed (rpm)	Particle size (μm)*
HD-5	0.2	80	2000	191.5 ± 2.22
HD-6	0.3	80	2000	177.5 ± 2.57
HD-7	0.4	80	2000	162.1 ± 1.33
HD-8	0.5	80	2000	127.5 ± 2.50
HD-9	0.5	60	2000	169.5 ± 3.11
HD-10	0.5	70	2000	140.3 ± 2.85
HD-11	0.5	80	2000	128.5 ± 3.94
HD-12	0.5	90	2000	100.7 ± 2.77
HD-13	0.5	80	1400	316.7 ± 3.09
HD-14	0.5	80	1600	291.3 ± 3.19
HD-15	0.5	80	1800	200.5 ± 3.07
HD-16	0.5	80	2000	132.1 ± 1.99

In all the formulations, drug to polymer ratio of 1:2 was maintained constant. * data are expressed as mean ± SD., n=3

Table 3: Influence of polymer to drug ratio on % Yield, % Drug entrapment efficiency, particle size, degree of swelling and % mucoadhesion.

Formulation code	Polymer to drug ratio	% Yield	% Drug entrapment efficiency*	Particle size (μm)*	Degree of swelling*	% Mucoadhesion
HDM-1	1:1	74.35	74.7 ± 2.11	92.6 ±0.63	0.816 ± 0.012	76.83 ± 0.116
HDM-2	2:1	79.41	83.9 ± 1.54	110.2 ± 1.13	0.942 ± 0.017	79.22 ± 0.095
HDM-3	3:1	84.48	81.5 ± 1.22	131.8 ± 2.44	1.013 ± 0.008	81.23 ± 0.194
HDM-4	4:1	86.64	76.7 ± 1.06	158.1 ± 1.28	1.141 ± 0.014	83.64 ± 0.110

In all the formulations, emulsifier concentration of 0.5% w/v, temperature of 80^{0}C and agitation speed of 2000 rpm was maintained constant. *Data are expressed as mean ±SD. n = 3

Table 4: *In vitro* release data fitting into various mathematical models

		HDM-1	HDM-2	HDM-3	HDM-4
Zero order	R	0.8542	0.8926	0.8886	0.9008
	k	0.0046	0.0040	0.0039	0.0040
First order	R	0.8531	0.8922	0.8876	0.9013
	k	0.0000	0.0000	0.0000	0.0000
Matrix	R	0.9936	0.9947	0.9922	0.9927
	k	0.0111	0.0097	0.0093	0.0097
Hixon-Crowell	R	0.8536	0.8938	0.8891	0.8999
	k	0.0000	0.0000	0.0000	0.0000
Peppas	R	0.9920	0.9904	0.9879	0.9876
	k	0.0120	0.0101	0.0096	0.0099
	n	0.4490	0.4694	0.4754	0.4281

In Vitro Drug Release Kinetics

For understanding the mechanism of drug release and release rate kinetics[24] of the drug from the dosage form, the data obtained was analysed with software (PCP - Disso V2.08) equipped with zero order, first order, Higuchi matrix and Krosmeyer – Peppas model kinetics. By analyzing the R values, the best fit model was arrived at.

Stability studies

Stability studies were carried out at $5^0C \pm 3^0C$, $25^0C \pm 2^0C$ / 60% RH ± 5% RH and $40^0C \pm 2^0C$ / 75% RH ± 5% RH for three months using Programmable environmental test chambers[25] (REMI Instruments ltd.). The selected formulations were packed in amber coloured glass containers and closed with air tight closures and stored for 90 days. Samples were analyzed at the end of 30, 60 and 90 days and they were evaluated for % Drug entrapment efficiency, *in vitro* mucoadhesion test and *in vitro* drug diffusion studies.

RESULTS AND DISCUSSION

Doxycycline loaded mucoadhesive microspheres of HPG were prepared by water in oil solvent evaporation technique. Preliminary trials were carried out to optimize the process of preparation. Batches HD-1 to HD-16 were prepared to study the effect of drug to polymer ratio, emulsifier concentration, temperature and agitation speed on the % drug entrapment efficiency and Particle size.

As the drug concentration was raised from 0.5:2 to 2:2, it was observed that the particle size increased, whereas, entrapment efficiency decreased in the same concentration range. The increase in particle size could be attributed to the increased drug content of the emulsion droplet at higher drug concentration. The decrease in entrapment efficiency with increase in drug concentration could be related to the increased extent of drug diffusion to the external phase due to greater flux at higher drug content during the emulsification and microsphere formation process. Hence, further trials were carried out with a drug to polymer ratio of 1:2.

Increasing the surfactant concentration from 0.2 to 0.5% w/v exhibited a reversal in trend between particle sizes. Microspheres fabricated with 0.2% w/v Span 80 showed the largest particle size while those fabricated with 0.5% w/v showed lowest particle size. When the surfactant is added in small concentrations, it may not have been able to cover the entire droplet surface. Thus some of the droplets would tend to aggregate till the surface area was decreased to such a point that the available amount of surfactant was able to coat the entire surface of the agglomerate and form a stable emulsion resulting in a larger microparticle size. When the concentration of emulsifier is increased, it will allow the emulsion to stabilize to a greater interfacial surface area, thus leading to smaller particle size. Based on these observations concentration of emulsifier was optimized to 0.5% w/v.

Increase in the temperature from 60^0C to 90^0C led to a decrease in the mean particle size. However, further increase in temperature above 90^0C did not produce any significant change in mean particle size. Increase in temperature from 60^0C to 90^0C increases the degree of congealing or rigidization of the polymer, which ultimately results in shrinking of the particles, leading to

decrease in particle size. Hence for the final formulation design, a temperature of 80^0C was optimized.

The results were in general agreement with the general theory of microspheres that the particle size of microspheres prepared at 2000 rpm were smaller than those prepared at 1400, 1600 and 1800 rpm. Since the microspheres obtained at 2000 rpm were in the size range of 100-170 µm, which are suitable for periodontal pocket delivery, 2000 rpm was chosen to obtain microspheres.

It was observed that as the polymer to drug ratio increases, the product yield also increases. The low percentage yield in some formulations may be due to microspheres lost during the washing process. The percentage yield was found to be in the range of 74.35 to 86.64%. % Drug entrapment efficiency of DH ranged from 74.7 ± 2.11 % to 83.9 ± 1.54 % for HPG microspheres. Increase in the polymer concentration resulted in increased viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency.

The prepared microspheres were in a size range suitable for periodontal pocket drug delivery. The mean microsphere size increased with increasing polymer concentration due to a significant increase in the viscosity, thus leading to an increased aqueous droplet size leading to an increase in the size of the microsphere. HPG-DH microspheres in the size range of 92.6 ± 0.63 µm to 158.1 ± 1.28 µm were obtained.

The photographs of the optimized formulation (HDM-2) taken by scanning electron microscope are depicted in the Figure 1 and 2. The SEM photographs revealed that the microspheres were discrete and spherical in shape with a rough outer surface morphology which could be because of the surface association of the drug with the polymer. The pores on microsphere surface could help in drug release by diffusion mechanism.

Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 3 that with an increase in polymer concentration, the degree of swelling also increases ranging from 0.816 ± 0.012 to 1.141 ± 0.014. Thus we can say that amount of polymer directly affects the degree of swelling.
As the polymer to drug ratio is increased, HPG microspheres exhibited an increase in % mucoadhesion ranging from 76.83 ± 0.116 to 83.64 ± 0.110; the results of *in vitro* mucoadhesion test are compiled in Table 3.

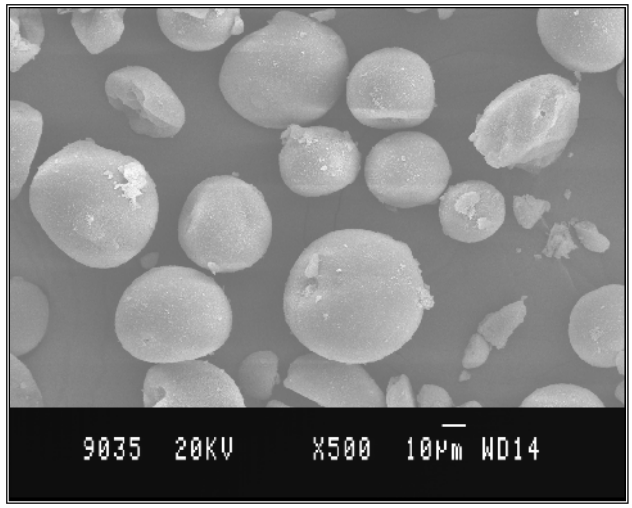

Figure 1: SEM microphotograph of optimized formulation HDM-2 (Low magnification)

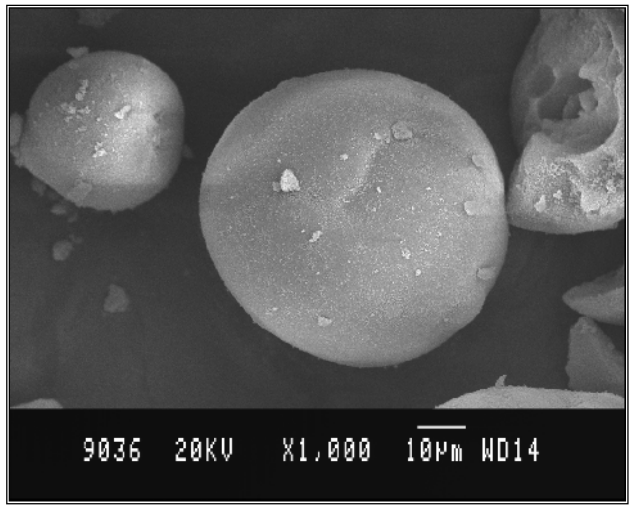

Figure 2: SEM microphotograph of optimized formulation HDM-2 (High magnification)

The *in vitro* diffusion of DH from the prepared microspheres exhibited a biphasic mechanism. The release of DH from the microspheres was characterized by an initial phase of burst effect due to the presence of drug particles on the surface of the microspheres followed by a second phase of moderate release. The initial burst effect is a desired effect to achieve initial therapeutic plasma concentration of the drug.

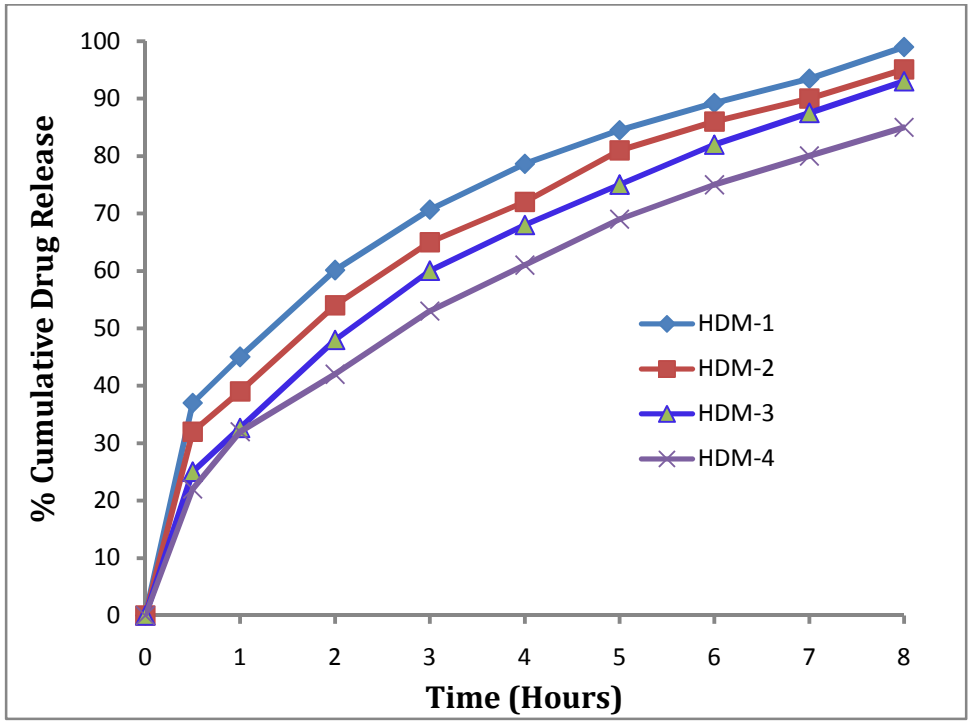

Figure 3: *In vitro* drug diffusion profile from HPG microspheres

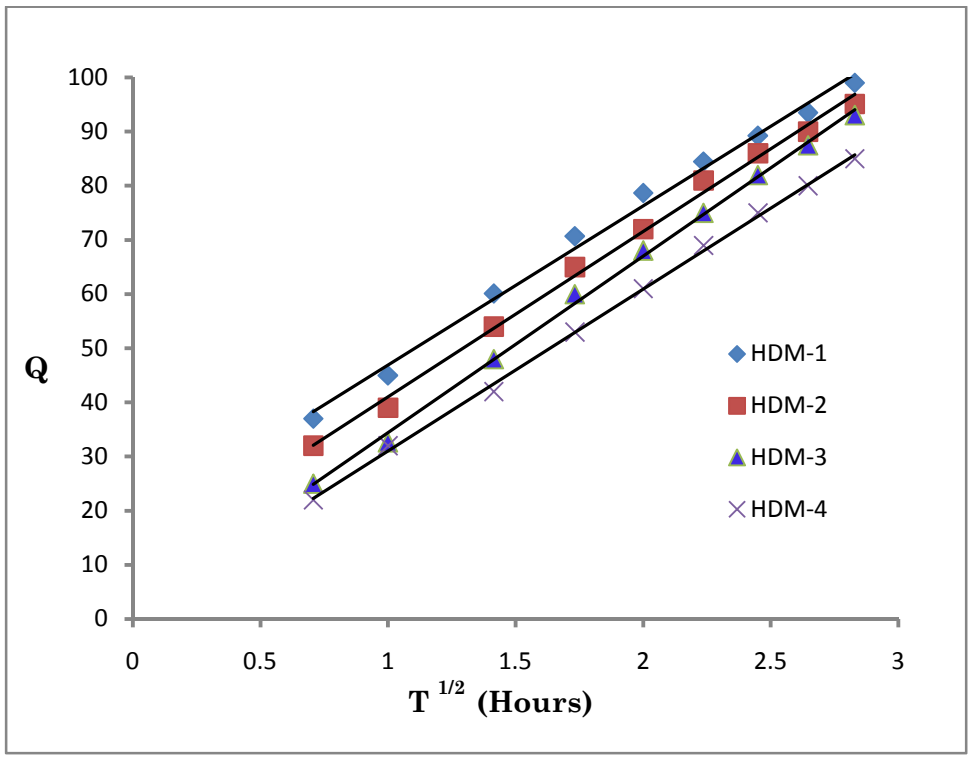

Figure 4: Higuchi Plot

The initial burst effect was considerably reduced with increase in polymer concentration. The increase in the polymer concentration resulting in better entrapment efficiency could be the reason for the observed decrease in burst effect. As the polymer to drug ratio (HDM-1 to HDM-4) was increased, the extent of drug release decreased from 99.00 – 85.15%. A significant decrease in the rate and extent of drug release is attributed to the increase in density of polymer matrix that results in increased diffusion path length which the drug molecules have to traverse. The release of the drug has been controlled by swelling control release mechanism. Additionally, the larger particle size at higher polymer concentration also restricts the total surface area thus resulting in slower drug release over a span of 8 h. The comparative *in vitro* drug diffusion profile from the HPG microspheres is depicted in Figure 3.

The drug release kinetic data is compiled in Table 4. In all the cases, the R values of Higuchi matrix model were close to 1. The diffusion coefficient (n) values ranged between 0.4490 to 0.4754. Since the R values of Higuchi matrix were close to 1, the drug release follows matrix diffusion kinetics and the plot shown in Figure 4 revealed linearity; hence it was concluded that diffusion was the main mechanism of drug release from the mucoadhesive microspheres. Further, the observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows non-Fickian transport mechanism.

The stability data showed that there was no change in the appearance of the microspheres indicating that the formulations were stable at different conditions of storage. It was observed that there was slight reduction in the drug content of the microspheres which were stored at 40^0C ± 2^0C / 75% RH ± 5% RH at the end of 90 days and no significant change in drug content were observed for formulations stored at room temperature and at 5^0C ± 3^0C. The extent of mucoadhesion of the formulations did not show any significant change after the microspheres were subjected stability studies. *In vitro* drug diffusion studies for all the four formulations were carried out at the end of 90 days and did not reveal any significant change in drug release from all the formulations. Thus, we may conclude that, the drug does not undergo degradation on storage.

CONCLUSION

The limitations of systemic therapy with the antibiotics have evoked an interest in the development of localised drug delivery systems that can provide an effective antibiotic concentration at the periodontal site with minimal side effects. The water in oil solvent evaporation technique for obtaining HPG microspheres has proved to be a useful tool in the preparation of microspheres. By virtue of prolonged drug residence at the site of absorption, improved bioavailability and therapeutic efficacy can be achieved in contrast to systemic administration of antibiotics. The results indicate that the formulation of Doxycycline loaded HPG microspheres could be utilised as a potential drug delivery system to periodontal pocket.

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