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Novel Multilayer Polymeric Inserts of Doxycycline for Treatment of Periodontal Diseases

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Abstract

- ***Objective:***

The objectives of this research was to formulate and evaluate novel multilayer chitosan inserts containing doxycycline hyclate for long term usage in periodontal diseases locally.

- ***Methods:***

This research was carried out to formulate three different polymer based (polymer used was chitostan) inserts that were uni, bi- and triple-layer inserts, and doxycycline hyclate as anti-microbial agents by means of solvent-casting method. The different layers of doxycycline loaded chitosan based inserts were tesed byFT- IR and DSC to evaluate the compatibilities of doxycycline hyclate and the chitosan and other additive present in this prepation. After compatibility study the physical parameters were examined. The physical properties included were difference in weight, thickness, durability, weight uniformity, % moisture absorbance, % moisture loss, swelling index, tensile strength, drug content uniformity, release pattern *in-vitro* antibacterial studies and stability and many more.

- ***Results and Discussion:***

The evaluated data of various physicochemical properties indicates that all the inserts had same amount of drug as per content uniformity test and exhibited more than 150 times endurance capability. The percentage moisture loss varied between 24.155 ± 7.35 and 40.43 ± 11.737. Tensile strength was found to be more in case of bi and triple layer of inserts as compared to single layer, it varied in the range 1.55± 0.0545to 4.543 ± 0.201 kg/mm^2. The drug pattern showed that the triple layer periodontal inserts exhibited controlled release drug pattern as compared to uni, bi layer inserts. The uni layer inserts exhibited the fasted release drug delivery pattern. The tripled layered drug loaded inserts continued their drug release for upto 17 days. The stability studies showed no signs of degradation, the degradation of the inserts were much less than the plain drug.

- ***Conclusion:***

The study indicates that physicochemical parameters shown by all the inserts were found satisfactory. Thus, the inserts prepared as tri-layer could showed better performences and keep an efficient amount of drug for the treatment of Periodontitis.

Keywords*: Chitosan, Inserts, Multilayer, Doxycycline Hyclate, Release Rate Antibacterial Activity.*

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I. INTRODUCTION

Periodontal diseases are the major public health issue all over the universe. Routine oral hygiene and cleanliness plays a very important role in maintaining healthy teeth along with the gums. Its occurrence is at any stages of life like childhood, young age, and elder ages irrespective of races, genders and economic condition of the peoples.

In broad sense this disease is associated with no. of different pathological conditions of periodontium like bacterial species, site of inflammations, its sign and

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symptoms etc. Gingivitis is a periodontal disease condition caused by shifting of gram + bacteria to gram gram –ve bacteria, which causes redness and swelling of gingiva whereas periodontitis is serious condition that causes detachment of ligament in the teeth.

Periodontal pocket formation with a sulcus of length up to 3mm and upto length 5mm is the common problem usual seen in the patient with periodontal diseases.[1]

➢ *Etiology:*

The main underlying etiology of this disease are bacterial plaque formation on to the tooth. Whereas other causes are smoking, chewing of tobacco products, genetic basis, occurrence of pregnancy and attaining of puberty, stressful life style, medicines, cleaning criteria, diabetes and nutritional deficiency etc. The pathogenic anaerobic bacteria responsible cause severity of these disease are namely *gingivalis, Bacteroides melaninogenicus sub species intermedius, Porphyromonas, and Prevotella intermedia*. [2, 3].

➢ *Pathogenesis:*

The underlying pathogenesis of periodontitis is the formation of microbial plaque with slightly inflamed gingival tissue. In healthy condition, the gingiva is having leukocyte infiltration, comprises of neutrophils, agranular leukocytes. Since it has a defense mechanism by phagocytosis of bacteria in gingival pocket because the bacteria in the pocket release a peptide called chemotactic. The bacteria in the pocket starts damaging the epithelial cells, which causes to release cytokines by epithelial cell.[4, 5, 6].

➢ *Microbiological aspect:*

Almost 400 species of bacteria are observed in the plaque specimen that formed in the peridontium. Out of which, only 11-22 species causes colonizations in periodontal tissuse and causes the damage to the epithelum tissue. For colonization the bacteria fisrt causes the attachment to tissue---------- Multiplicatio-------cause competation----------defense from host cell.

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The pocket sulcus provide optimum environment for microbial growth, their colonization in the subgingival site. Once they enters connective tissue, it has to face a numerous host defense cells like lymphocytes, neutrophils, macrophages but the bacterian can overcome bacteria host defense mechanisms. [4, 8, 9].

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➢ *Treatment:*

The main target in its treatment regimen is to minimize the inflamed tissue, kill the possible numbers of bacteria and demise the pockets depth and stoppage of bone resorption. The conventional methods of pocket demises is mechanical filling by removal of supra and mechanical plaque and root planning and curettage[11]. These causes recolonization of pathogen within 130-250 days.[12].

➢ *Antibiotic Therapy:*

The antibiotics applied in this diseases causes to reduce or kill bacteria that cannot be possible by conventional mechanical approaches. Antimicrobials employed as injection, ointment jelly, are generally Tetracycline's, imidazole derivatives, fluoroquinolones and manymore[14].

Antimicrobial administration parenterally is due to the severity of the disease and if it is more severe then it will go for systemic application to attain maximum concentration in the periodontal pocket to kill the pathogens.[15]. And if it is moderate to less stage of periodontitis then it will go for local application where centration of antimicrobial agents required to kill the bacteria is less. And attain less quality of drug at the site of action. Thus systemic administration exerts more quick and fast effects then the local application in periodontal disease.

But it has a limitation by systemic administration af antimicrobial drug due to occurrence of side effect than its beneficial effect if it is used for long term. So it is unacceptable to administer the antimicrobial agents by parenterally. These disadvantages can be markedly overcome by administering the antimicrobial agent locally. Due to less amount of drug, the local antimicrobial shows safer measure than other parenteral administration and produce less side effects also[16]

➢ *Local Delivery of drug:*

There are two categories of devices available.

- The topical delivery: This type of device releases its drug over prolonged periods in periodontal by initial increased release followed by decreased release of drug at the affected area.[19]
- The controlled delivery: This type of device is designed to release the drug over long duration of time at the affected site[20].

➢ *Periodontal Pocket Delivery:*

The outcome of Local Pocket drug delivery system is mainly depend on the attainment and retention of drug into the base of pocket at different drug concentration of antimicrobial agents to a prolong drug release pattern. Some techniques of drug administration: Drug Attainment[22] shown in Table 1:

Table 1 Different route of administration & effects in Periodontitis.

	Mouth wash	**Subgingival Irrigation**	**Parenteral application**	**Controlled Release**
Reaches affected area and potency	Very Less	Very Good	Good	Very good
Max. drugs conc.	Good	Very Good	Average	Excellent
Max. period of Treatment	Very less	Very less	Average	Excellent

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➢ *Topical Control Release Devices:*

There are various types of local controlled release devices for periodontal diseases designed to deliver desired amount of antimicrobial drugs in the periodontal pocket for upto 2-3 days by application of one device. They are as follows:

- *Reservoir type:*

This type of device is having a antimicrobial reservoir of diameter 2-4 mm long, 0.3 mm wide which is surrounded by a well designed membrane. This reservoir type device is placed in periodontal cavity/ pocket over a periods of 6- 7 days from where the drug is released in controlled manner. But disadvantages of this divices are in its rapidity in drug release, local irritation on the affected pocket, etc[23].

- *Monolithic devices:*

It is polymer based controlled released local device in which the drug to be applied is dispersed into a polymer matrix having biodegradable and absorbable properties. This device is having a diameter of 0-2 mm thick. The strip is applied to pocket for a period of 1-3 weeks with replacement of strip in a week. These polymer based matrix are biodegradable and bio absorbable after releasing the antimicrobial drug from the matrix. Some polymers matrix used in these type of devices are ethyl cellulose (EC), poly ethylene glycol (PEG), etc[23]

- *Melt fabrication:*

It is also controlled released local device which is prepared by squeezing the polymer between the roller. The roller is initially heated to ahigh temperature. A film is formed by melting the polymer[23].

- *Solvent casting:*

In these type of technique a viscous solution is prepared by dissolving the polymers in a suitable solvent which is then spread on non-sticky flat surface. After that the solvent evaporation process is followed. The resultant film is peeled from the surface.

- *Polymerization In situ*

Here polymerization are done within a desired location. Some examples of In Situ polymerization are Nano composite, lithium Polymer batteries. The release pattern of drug from the polymers depends on diffusion of drug through matrix.

➢ *Objectives*

The main objective of this study is to formulate polymer inserts which contain chitosan at numerous amount of the chemotherapeutic agen of Doxycycline for controlled release locally into periodontal pockets formed. Moreover, an investigational study is planned to elongate the drug release pattern over a few days by formulation of multilayer inserts (bi, tri layer).

➢ *The Objectives Follows here are,*

- Formulation of polymer (chitosan) unilayer insert with doxycycline hyclate conc. (10%, 20% and 30%).
- Development of doxycycline hyclate loaded chitosan bi and triple layer inserts.
- Evaluation physical parameters. The parameters are as follows.

✓ Weight differences
✓ Durability
✓ % Moisture Loss
✓ %Moisture absorbity.
✓ Content uniformity of drug in inserts.
✓ Tensile Strength
✓ DSC
✓ (FT-IR)
✓ Swelling factors

- Dissolution study.
- *In-vitro* antimicrobial property study.

II. MATERIAL AND METHODS:

A. Materials:

Table 2 Chemicals

Name
Doxycycline hyclate
Chitosan
Acetic acid
Potassium hydrogen ortho Phosphates
Sodium hydroxide,
Glutaraldehyde
Calcium chloride
Anhydrous aluminum chloride
Acetone

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Table 3 Instruments

Name
UV visible Spectrophotometer
Vortex mixer
Digital pH meter
Electronic weighing Balance
Tensile strength tester
Digital screw gauge
FT-IR
Levelled glass moulds

B. Methods:

➢ *Preformulation studies:*

It is the initial step in dosage form development of a subjected drug where physical and chemical characters of drug are studied indivisibly, and in combinations with additives and excipients. The physical properties includes solubility, density melting point, Particle size and surface area, polymorphism, hygroscopicity, compressibility, dug – excipient compatibility etc. The physical and chemical properties indicate the overall knowledge to formulate suitable dosage forms with greater extent of stability and bioequivalence, bioavailability, drug efficacy, ADME etc. which will help in mass production of drug dosage form. These properties of drug and combination of drug and excipients also indicate status of formulation design and molecular structural modification if needed.

- To do so first we have determine kinetic release profile and drug- excipients compatibility status
- Hence, preformulation studies on obtained drug sample include physical tests and compatibility studies.

➢ *Compatibility studies*

- Drug- Excipients compatibility studies:

The selection of additive used in pharmaceutical formulation play an vital role to produce a suitable, biocompatible, pharmaceutically stable, therapeutically efficacious with less side effect possessing dosage form. This plays a crucial role in the administration and drug release pattern like controlled and prolong release of drug from the particular formulation. Therefore for suitable and pharmaceutically compatible additive and excipients two tools applied here are FTIR and DSC which can predict and after that assure the compatible additives.

➢ *FTIR Studies:*

- *Sample Preparation:*

For sample preparation Samples: potassium bromide = 1:100 and perform the titration with special precaution as if it cannot come in contact with the moisture because it can interference in the whole operation, Now the sample is subjected to about 300kg/cm2 pressure for convert it to suitable pellets. Then the peak taken by FTIR are compared with REFERENCE SOLUTION for any changes[64].

➢ *FTIR Spectra:*

A spectra obtained from FTIR to indicate the compatibility status by chemically between the drug and the excipient used. The spectra were taken at a wavelength of 4000 and 500 cm^{-1} separately for the drug and mixture and finally compared with the reference substances.

➢ *DSC study*

DSC is used to measure thermal characteristics of polymer matrix, analysis of chemical reactions and analysis of drug stability and investigating the melting properties of fats content in food materials. DSC instrumentation comprises of two pan namely pan for sample and pan for reference substances. The both the pan containing samples (5mg doxycline with chitosan) and references substances (empty pan) were heated upto 300^0c gradually from 20^0c/min. [65].

C. Analytical Methods:

➢ *Reagents Preparaton:*

- *Preparation of 1% v/v CH3COOH:*

Weighed accurate quantity of 1 ml of acetic acid conc. Then it transferd to 100 ml of distilled water, stirred it to perfectly dissolved to form a 1% v/v acetic acid solutions..

- *Preparation of 0.2M KH2PO4 solution:*

Accurately weighed 27.218 grams of KH2PO4 and transfer it to measuring cylinder containing small amount of distilled water, stirred it well with the help of a glass rod and finally adjusted the volume up to 1000 ml mark.

- *Preparation of 0.2 M NaOH solution:*

Accurately weighed 0.8 grams of NaOH and transfer it to measuring cylinder containing small amount of distilled water, stirred it well with the help of a glass rod and finally adjusted the volume up to 1000 ml mark.

- *Preparation of 6.6 pH phosphate buffer solution:*

Accurately measure 16.4 ml of 0.2 M NaOH solution and transfer it a 250ml measuring cylinder, add 50 ml of 0,2 M K2HPO4 solution,. Mixed it well and adjust the volume up to 200 ml mark. Finally pH 6.6 phosphate buffer is ready.

➢ *Preparation of Standard Stock Solution:*

Accurately weighed 100mg of doxycycline hyclate, transferred it to a 100ml volumetric flask containing a little 6.6 pH phosphate buffer in 1L volumetric flask. Mix it well

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and finally adjust the volume 100ml mark. The standard Stock solution is ready with the concentration of 1000µg/ml.

➢ *Wavelength Determination: UV Spectroscopy:*

➢ *UV Spectra for Doxycycline hyclate*

4ml of standard stock solution was taken with the help of pipette, transferred it to the 100ml of volumetric flask. Add gradually the with 6.6 pH phosphate buffer until the volume gained 100ml. Now the the solution having the concentration 40µg/ml. which is now subjected to UV scanning at the wavelength 200-400nm.

➢ *Calibration Curve of Doxycycline Hyclate in 6.6 pH Phosphate Buffer:*

Take appropriate quantities of standard stock solution with the help of pipette, transferred it to the different volumemetric flask. Add gradually the with 6.6 pH phosphate buffer until the volume gained 10ml. Now the the solution having the concentration 4 to 24 µg/ml. which is now subjected to uv scanning at the weavelenth 27 nm and absorbence was taken repetedly 6 times. For validation of calibration curve which is shown in Figure no.6.1. and the calibration curve form is shown in 6.2.

D. Inserts Preparation:

Chemotherapeutic l agent Doxycycline hyclate and polymer chitosan were subjected to solvent casting method to prepare antimicrobial insert. The polymer chitosan is dissolved in water, mix doxycycline with it. The mixture was then cast on the surface of mould to make a desired shape, now the solvent was evaporated by external heating.

➢ *Drug loaded chitosan inserts Preparation:*

Take small amount of chitosan in a conc. Of 2%w/v in beaker, Take 1% acetic acid in another beaker, chitosan is soaked in CH3COOH in the second beaker for the period of 24 hour, it was then filtered using muslin cloth as a filtering medium to remove undissolved part of chitosan. Add desired quantity of doxycycline in the solution, vortexed it for 2 hour to get a mixture of doxycycline and chitosan, apply the external heat to evaporate the air bubble to form film. The prepared film is casted on to the centered level glass mould and allow to dry it at 25 0C for one day, cut it to desired size, wrapped it with the help of aluminium foil[56].

➢ *Bi-layer inserts Preparation:*

Weighed 15 ml of 2% chitosan cast solution alone poured it into glass mould and it was allowed to dry at 30 ^{0}C, first layer is formed on the bi-layer membrane. In the second step take 20 ml of 30% drug containing chitosan solution it is then casted on the first layer of bi layered film and it was allowed to dry again at 30 ^{0}C. The insert was formed in dry condition. It was then kept in desiccators to absorb the moisture[56].

➢ *Preparation of tri-layer membrane:*

The bi layer insert is subjected to pour into glass mould And then 15 ml of 2% cast solution without drug was cast onto the previous membrane and it was allowed to dry to develop the tri layer membrane. It was then kept in the decicator for further use.

E. Physical Evaluation: Polymeric Inserts:

Evaluations of single, bi, tri layer inserts were evaluated for their physical characters like thickness, differences in wt., durability, tensile strength, equality of drug in inserts, *drug* release pattern, swelling factors, *in-vitro* chemotherapeutic activity and stability studies.

➢ *Thickness Measurement:*

A digital screw gauge instrument was used to observe the thickness of all prepared periodontal inserts at different area of the inserts and take its reading as 1,2,3,4. so on. Take the average of all the values taken which indicates the thickness of inserts[53].

➢ *Weight Variation:*

Took 5-6 pieces of insert from from a particular insert and weighed each and every piece separately with the help of digital balance. Calculated the mean value of all measured inserts and observed the standard deviation of weight variations[53]

➢ *Tensile Strength Measurement:*

Universal strength testing machine was employed to measure theTensile strength of the inserts

Fig 1 Digital Tensile Strength Measurement Instrument.

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➢ *% Drug Content:*

Weighed the prepared inserts and take the reading. It was dissolved in a little quantity of 1% acetic acid, a solution was formed, dilute it with phosphste buffer pH 6.6. This dilution was subjected to uv spectroscopy for the measurement of absorbence at a weavelength of 274 nm.[53]

➢ *% Moisture Absorption:*

Weighed the prepared insert, take the reading. in a decicator was made ready by placing saturated NH4Cl of 100 ml volume and maintained humidity as 79%. Now Placed the weighed inserts in the ready decicator and kept for 72 hours after that the insert is taken out and weighed again. The moisture absorption was evaluated by substracting initial wt reading from final wt reading and it was multiplied by 100, got a value which is then divided by initial wt reading. This value indicated thephysical stability or integrity of the inserts

➢ *% Moisture Loss:*

Weighed the prepared insert, take the reading. A desiccator was made ready by placing saturated CaCl2 of 100 ml volume and maintained humidity as 79%. Now placed the weighed inserts in the ready desiccator and kept for 72 hours after that the insert is taken out and weighed again. The moisture loss was evaluated by subtracting final wt reading from initial wt reading and it was multiplied by 100, got a value which is then divided by final wt reading. This value indicated the physical stability or integrity of the inserts

➢ *Swelling factor determination:*

Measured the size of drug loaded inserts with the help of a scale, placed them on to a filter paper and waited for a while. Took beaker, pour small amount of Phosphate buffer in it. In the second step the filter paper was immersed on to the beaker from the lower end of the filter paper. Placed the whole unit in a incubator maintaining the tempr. at 30^0C. Now took the wt reading of each insert again with the help of a scale. The size and wt increase was due swelling**.**

➢ *In-Vitro Antibacterial activity:*

Took two agar plate with drug loaded insert separately. In one of these plate incorporate the sample of bacteria *steptococuss mutans*. Took it for 2 days in a incubator, Now it was then transferred to second agar plate and again took it in incubator for another 2 days. Repeated the procedure until bacterial growth inhibition was observed. Measured the zone of inhibition.

➢ *In-vitro Release Studies:*

Since the pH of the gingival fluid lies between 6.5 – 6.8, phosphate buffer pH 6.6 was used as simulated gingival fluid for the dissolution studies and the inserts remains immobile in the periodontal pocket, a static dissolution model was adopted. A static dissolution method reported in the literature was adopted in this thesis. Sets of 3 inserts of known weight and dimension were placed separately into small test tubes containing 1.0 ml phosphate buffer, pH 6.6. The tubes were sealed and kept at 37°C ±1 for 24 hours. The buffer was then drained off and replaced with a fresh 1.0 ml phosphate buffer pH 6.6. The concentration of drug in the buffer was measured at 274nm .The procedure was continued for consecutive days[53].

III. RESULT AND DISCUSSION

A. *Preformulation Studies*

The studies were performed for drugs and polymer.

➢ *Melting Point determination:*

Electronic Melting point apparatus was employed to determine the melting point of drug and it was found that the melting point of the subject drug was **201^0- 202^0C** $\pm 2^0$C which is very nearer to its standard value, implies the purity of the drugs.

B. *Experimental Methods*

➢ *Determination of λ max:*

In UV spectrophotometer absorbance was taken for the drug doxycycline hyclate with prepared buffer phosphate pH 6.6 in wavelength of 200-400nmm. The λ max found is given in fig. 2 The spectra obtained in uv spectoscopy for the drug was measured at wavelength 200-400 nm. And it was seen that the λ max obtained at wavelength 274 nm. And it is shown in figure no.2.

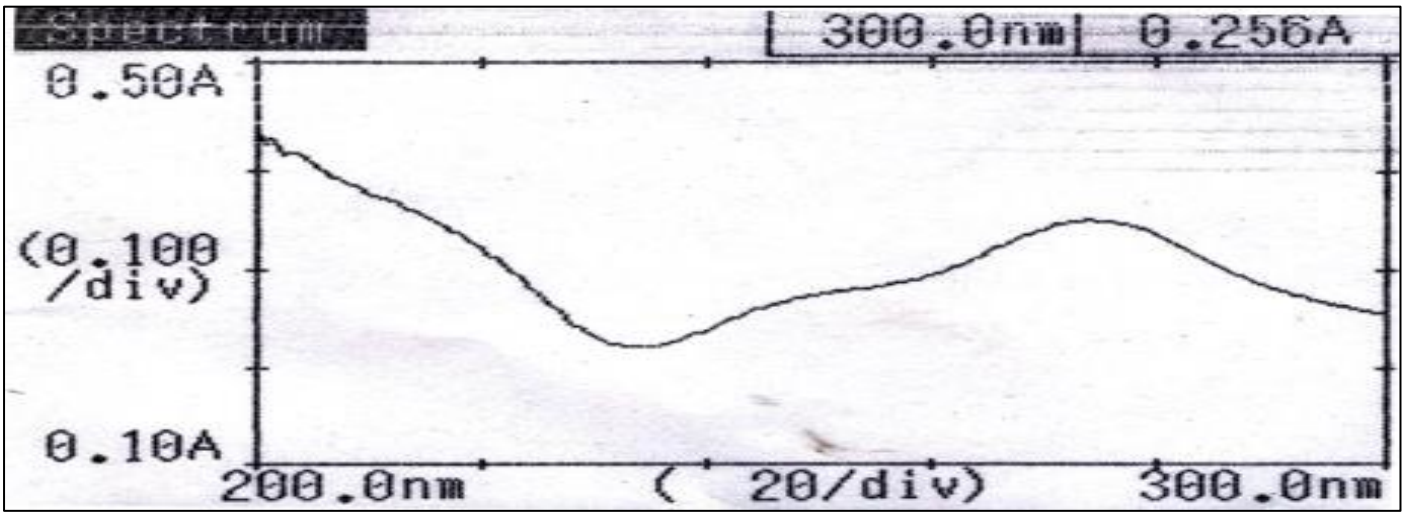

Fig 2 Wavelength, λ max of Doxycycline Hyclate.

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➢ *Calibration Curve of Doxycycline hyclate:*

The standard solutions contains the drug concentration of 4- 24 µg/ml in phosphate buffer pH 6.6. Fig. number 2 visualize calibration curve for DCL with the correlation coefficient of 0.997. The uniformity of drug in inserts and its release pattern from th inserts study are given on the basis of calibration curve. The absorbance taken at λ max 274 nm.

Table 4 In Phosphate buffer solution pH 6.6: The calibration Data of Doxycycline hyclate

CONCENTRATIONs (µg/ml)	**ABSORBANCEs* AM ±SD**
4	0.158 ± 0.003
8	0.264 ± 0.005
12	0.415 ± 0.006
16	0.525 ± 0.002
20	0.686± 0.027
24	0.777 ± 0.008

Each value is an average of six replications* R^2=0.997

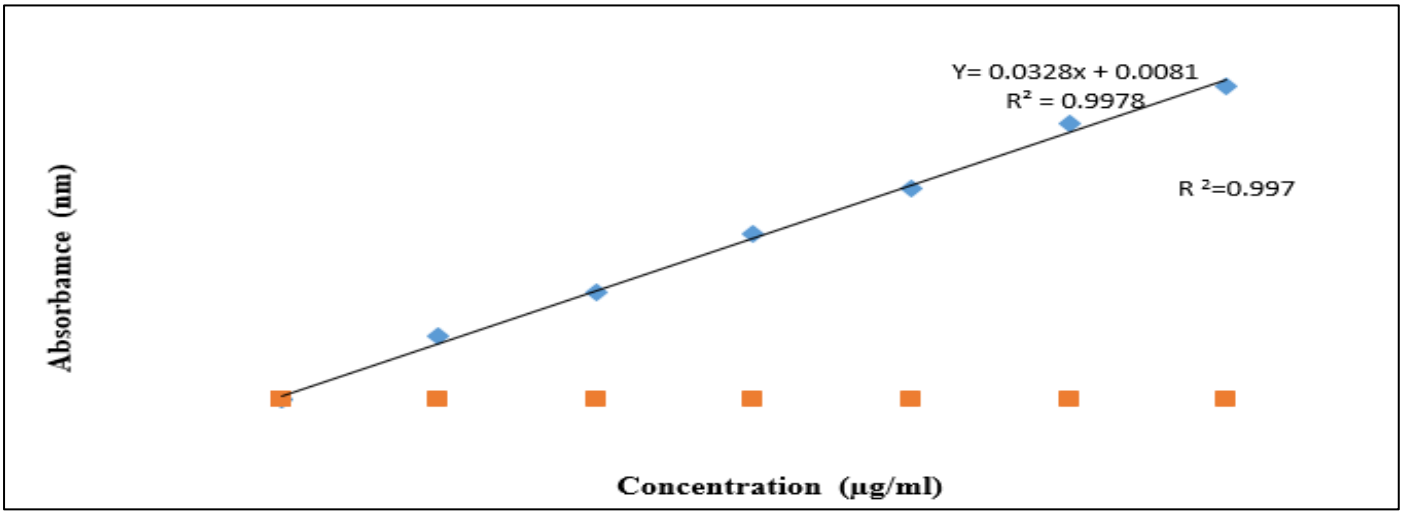

Fig 3 Calibration Curve of Doxycycline in buffer Phosphate pH 6.6.

C. Drug Excipient Compatibility Studies:

➢ *Study of FT-IR Spectra:*

The drug excipient study were done separately by using FT-IR instrument using potassium bromide disc. The peak obtained for drug and entire excipient in the formulation were correlated. It was found that the peak match, hence the drug and excipients were compatible to one anothers. The below shown the IR spectal behavior. The IR spectra clarified the peaks for DCL shown in fig below, the polymer (chitosan) and its prepation components are given in the tab. 4

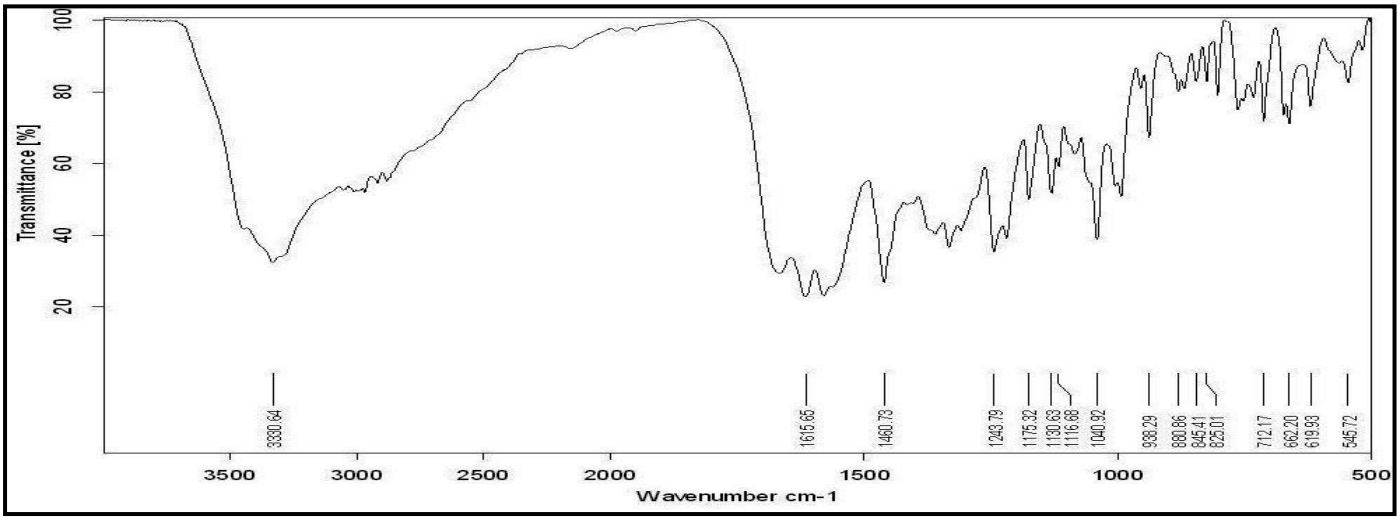

Fig 4 FT-IR of Doxycycline.

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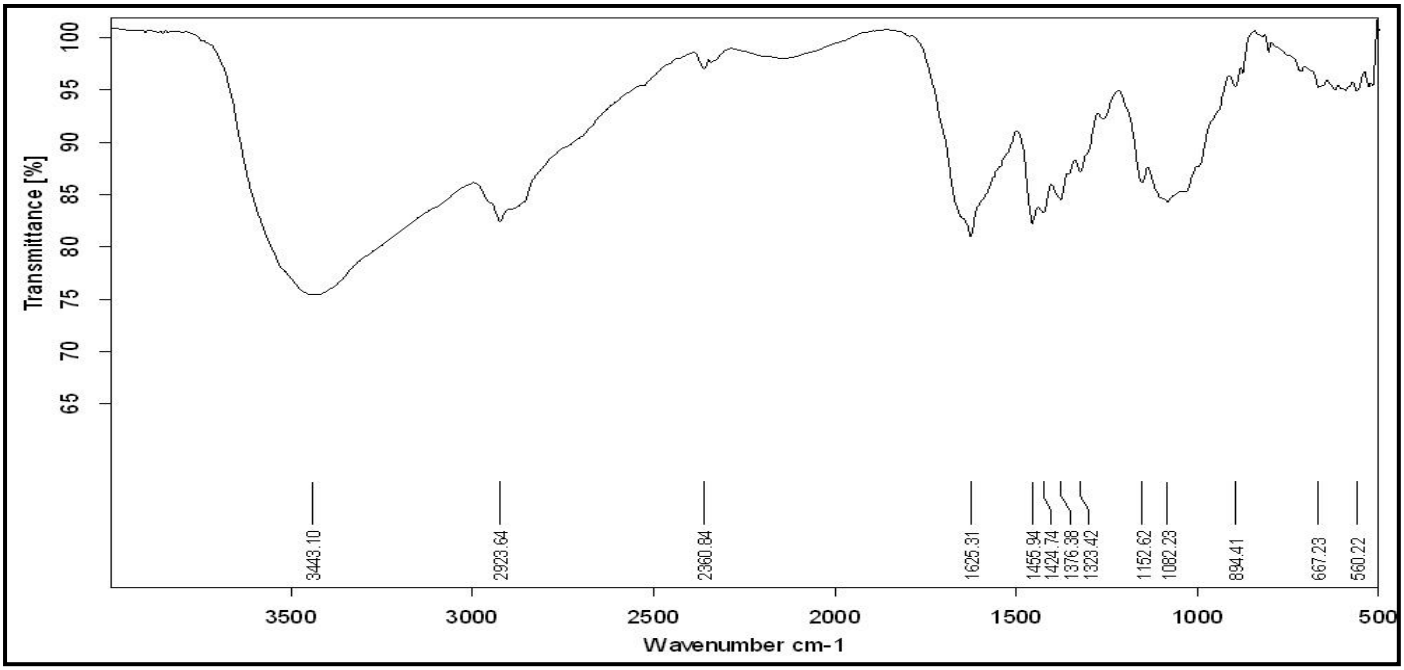

Fig 5 FT-IR Spectra of Polymer Chitosan.

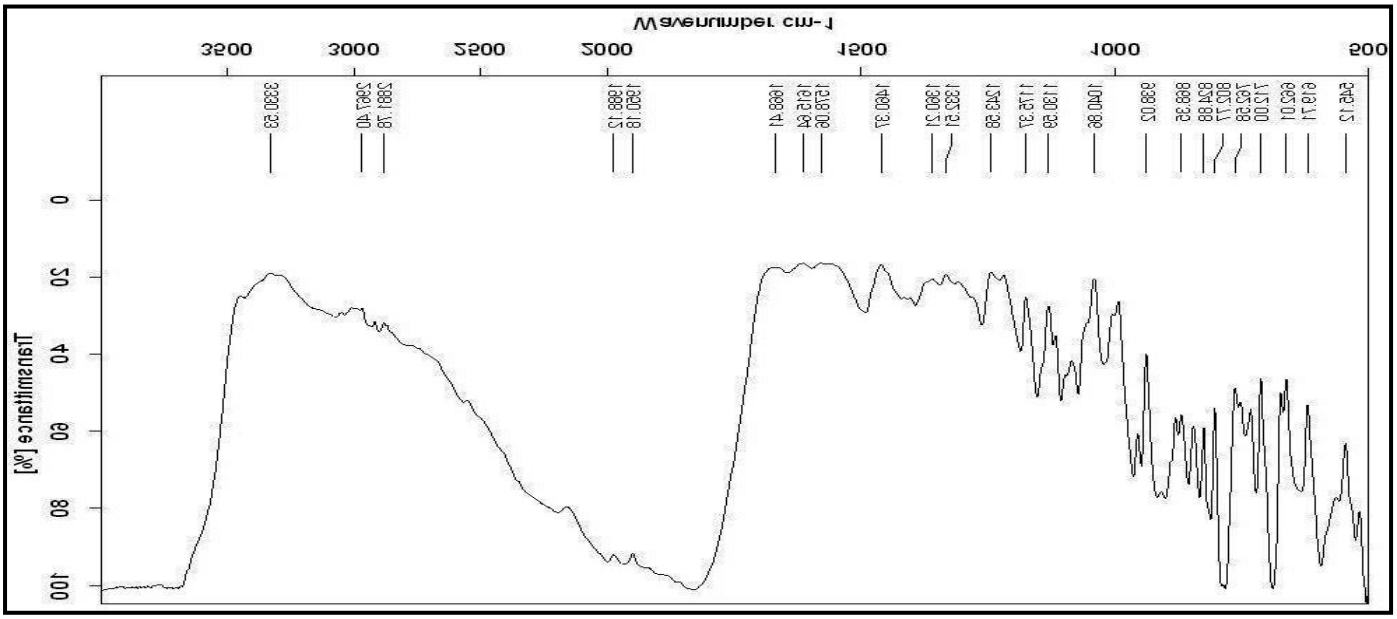

Fig 6 FT-IR Spectra for whole Preparation.

Table 5 Data for FT-IR Spectrum of Doxycycline alone and Polymer Chitosan Peaks of Functional Group (Cm^{-1}).

FORMULATION	C=O Stretching cm^{-1}	Ar-CH Stretching cm^{-1}	-OH stretching cm^{-1}	N-H bending cm^{-1}	-OH bending cm^{-1}	C-N Stretching cm^{-1}	C-C-C-O Stretching cm^{-1}
DCL	1614.65	2901.21	3330.12	1570.22	1460.75	1243.80	1175.33
DCL+ Chito-san	1614.64	2966	3330.37	1578.09	1460.36	1243.59	1175.42

➢ *Differential Scanning Calorimetry*

This study were evaluated chemical interaction status of a formulation by preparing thermogram for drug and formulation separately. A endothermic peak was for in case of absence of chemical interaction between drug and formulation and chemical shift was observed in case of chemical interaction between drug ang formulations.

The DSC thermogram of doxycycline and its whole additive are shown in fig7,8,9. The drug Doxycycline was showing clear endotherm at 218.69C which is very closer to its actual melting point. The IR spectrum also showed the almost same result that, there was no interaction between drug and excipients used.

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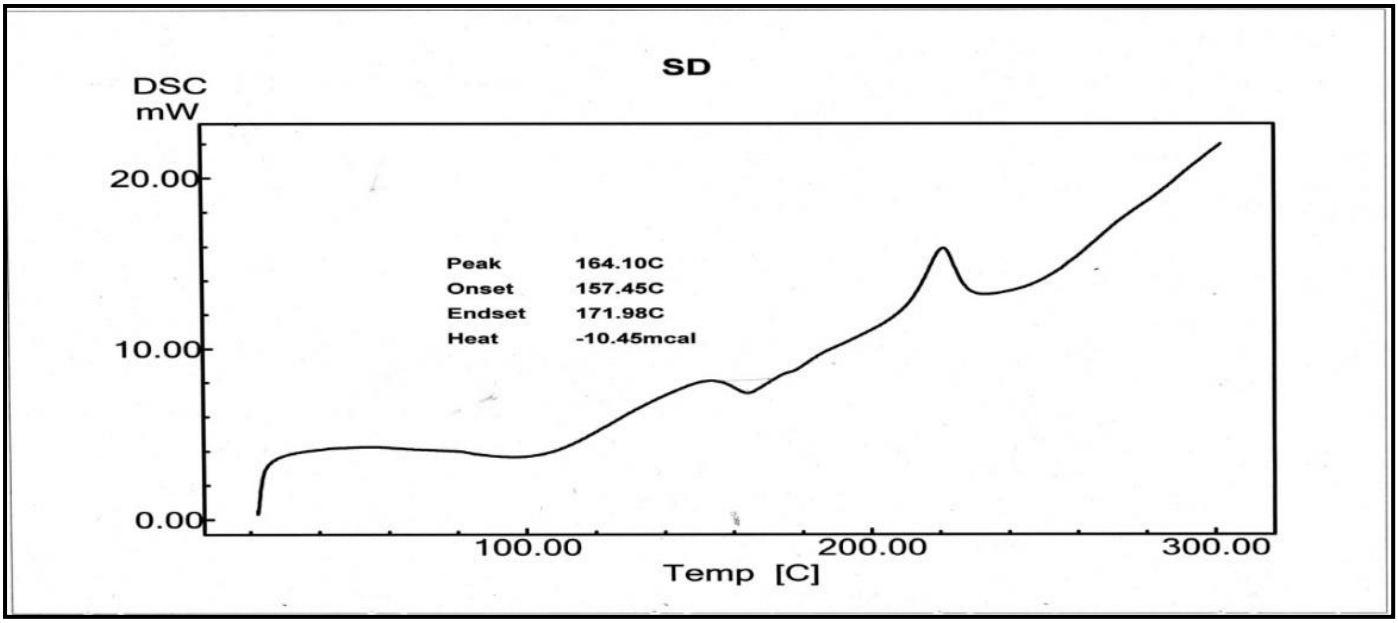

Fig 7 DSC Thermogram of Doxycycline Hyclate Drug.

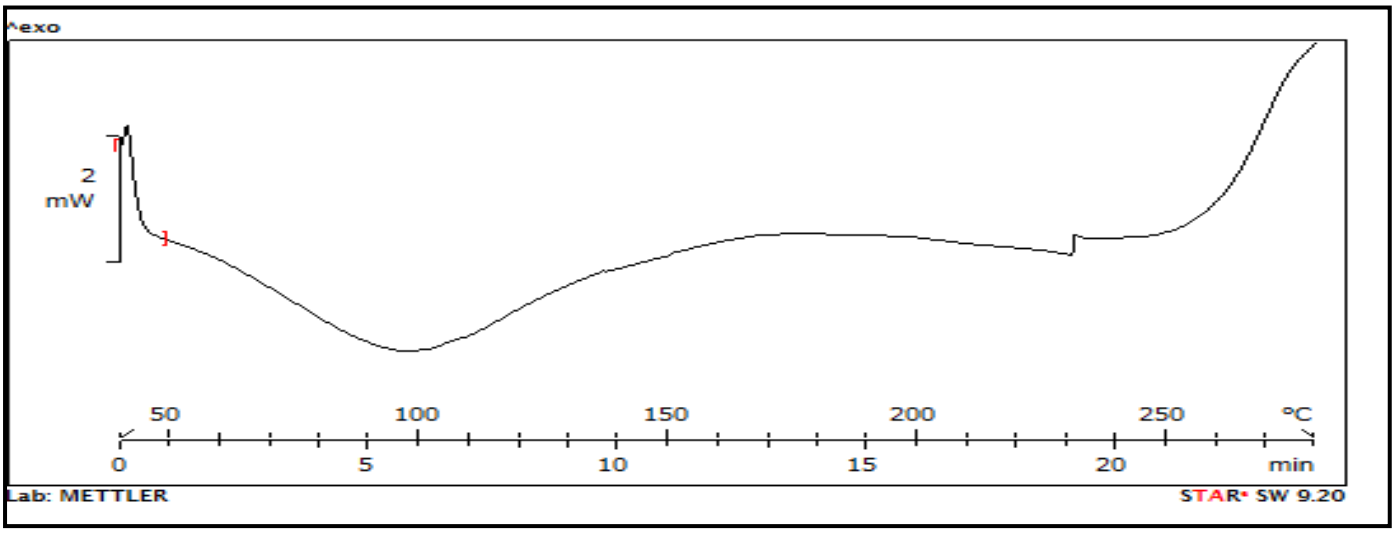

Fig 8 DSC Thermogram of Chitosan.

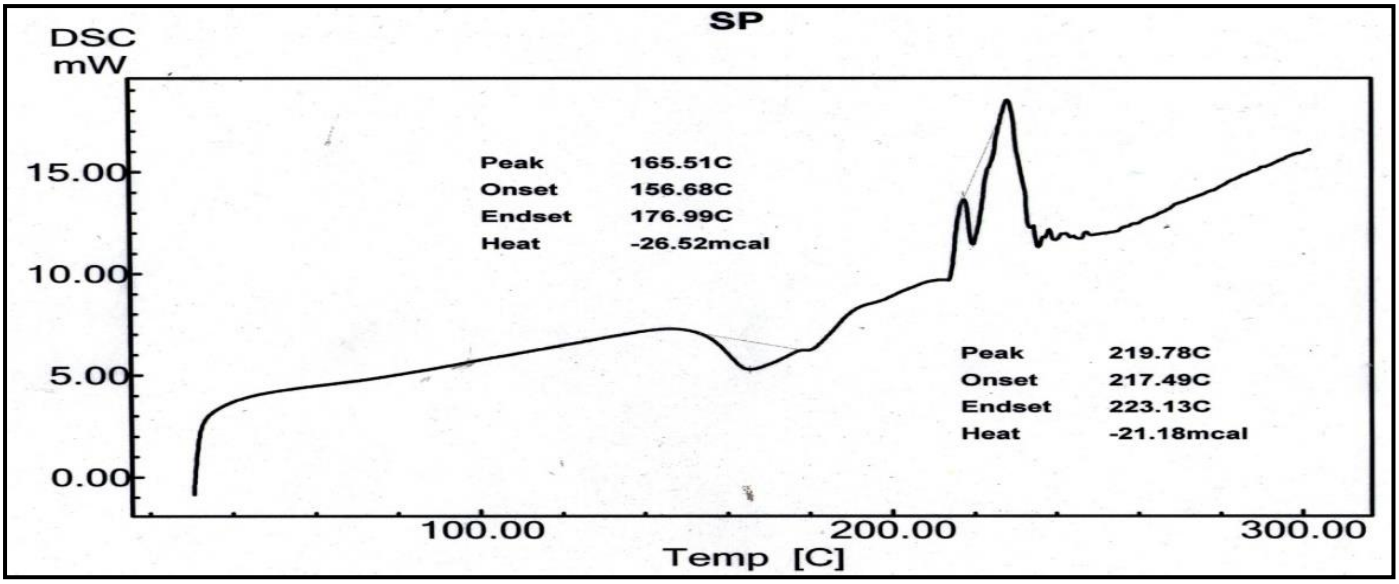

Fig 9 DSC Thermogram of whole preparation.

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➢ *Preparation of Drug Loaded Chitosan Inserts:*

At 2% conc. of polymer of chitosan produced suitable insert and the drug added to the polymer was 30%w/w because to get optimum formulation which could be conveniently removed from insert die.

➢ *Evaluation: Physical characteristics of drug Loaded Polymer Inserts:*

- *Physical appearance:*

Both drug and polymer combination used for formulation of periodontal inserts showed good film properties and reproducibility. The fabricated inserts were thin, flexible, elastic, smooth and non transparent. Photography of doxycycline hyclate was shown in the figure no.6.6.

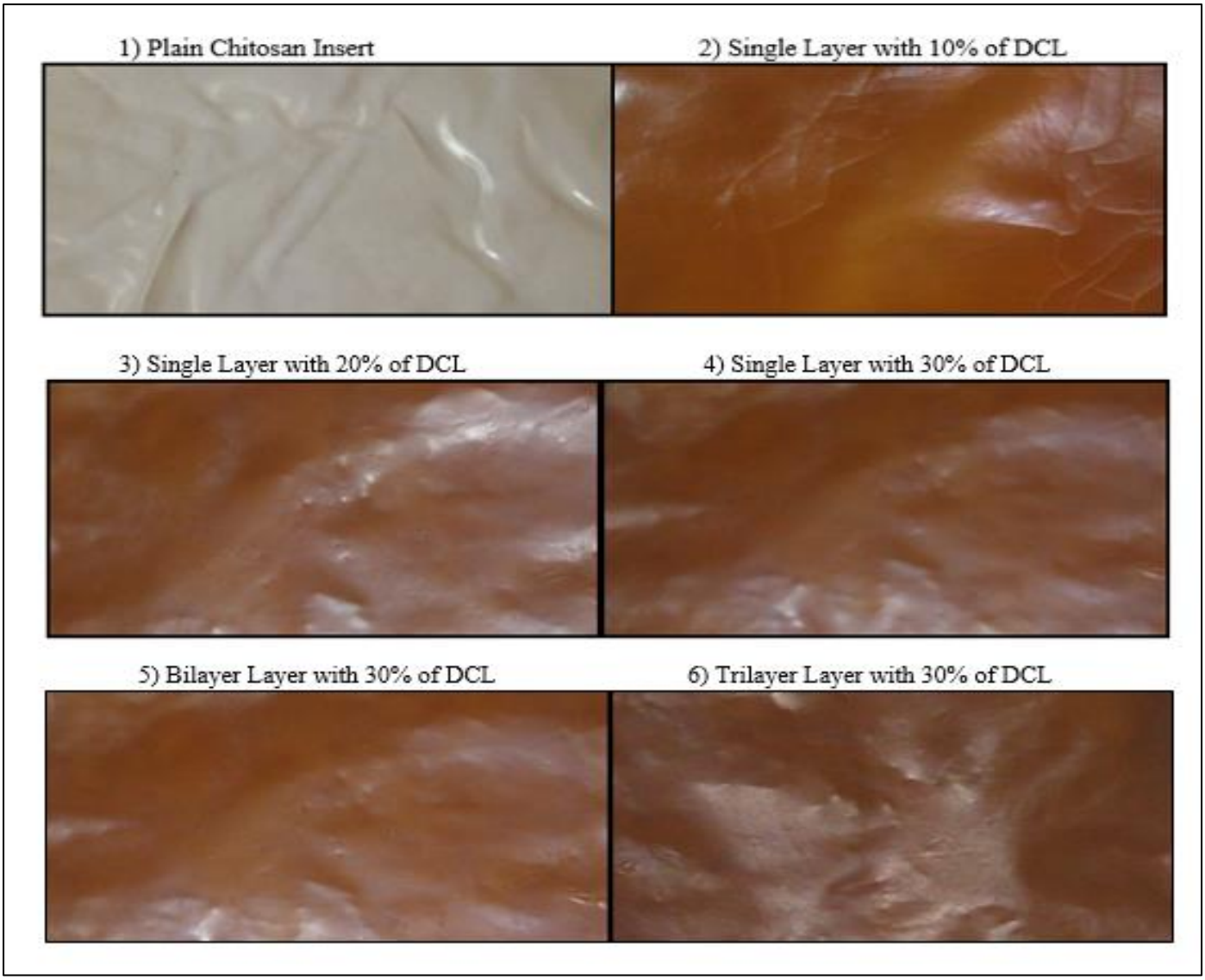

Fig 10 Visual of Doxycycline Hyclate loaded Inserts.

➢ *Thickness Measurement:*

The thickness of mono, bi and triple layered inserts increased respectively. It was checked by taking the length of at 5-7 site of every insets, took the average value of these. Thus check that how these value were deviated. The shown in tab.5 told the absence of deviation.

➢ *Uniformity in Wt.*

This test was performed by taking wt. of each insert and took average value. Now substract the indivisual value from this average value. It was seen the wt. of insert increased with increased in drug content. The tab. 5 showed the wt. uniformity of the insert.

➢ *Folding endurance:*

It was observed that the folding endurance increased with decreased in drug drug content in the inserts and vice versa. Thus it could be clarified that triple layered inserts had less endurance the mono and bi layered insert. The tab 5 showed the result of endurance.

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Table 6 Physical Parameters of Doxycycline loaded Inserts.

Inserts	Thickness (mm)AM ± SD	Folding Endurance	Weight Uniformity (mg)
CP	0.102±0.0155	330±2.481989	0.9±0.208145
DSL-10%	0.144±0.047	282±15.7193	1.0± 0.20568
DSL-20%	0.172 ±0.273	261.75±14.50	1.5 ±0.3358
DSL-30%	0.198 ±0.022	202.75±18.431	2.2 ±0.0456
DBL-30%	0.361 ±0.013	188±9.12867	2.9 ±0.3047
DTL-30%	0.457±0.0033	156.25±9.846	3.9 ±0.8789

➢ *%Moisture Loss:*

The hygroscopic compound mostly absorb moisture and stay as most moisture content compound in a formulation. This played a important role in retaining the physical and mechanical status of a insert. Similarly moisture loss was a important in this aspect of physical and mechanical status. The more was the moisture loss, the more would be drug conent the periodontal inserts. The table no.6.showed that the preparations which contained more quantity of drug showed more quantity of moisture loss.

Table 7 %Moisture Loss of Doxycycline Inserts.

INSERTS	MOISTURE LOSS (%) AM ± SD
CP	23.175±7.363
DSL-10%	23.758 ±12.387
DSL-20%	24.328±12.7465
DSL-30%	24.397± 6.4457
DBL-30%	30.467±12.845
DTL-30%	39.453±11.744

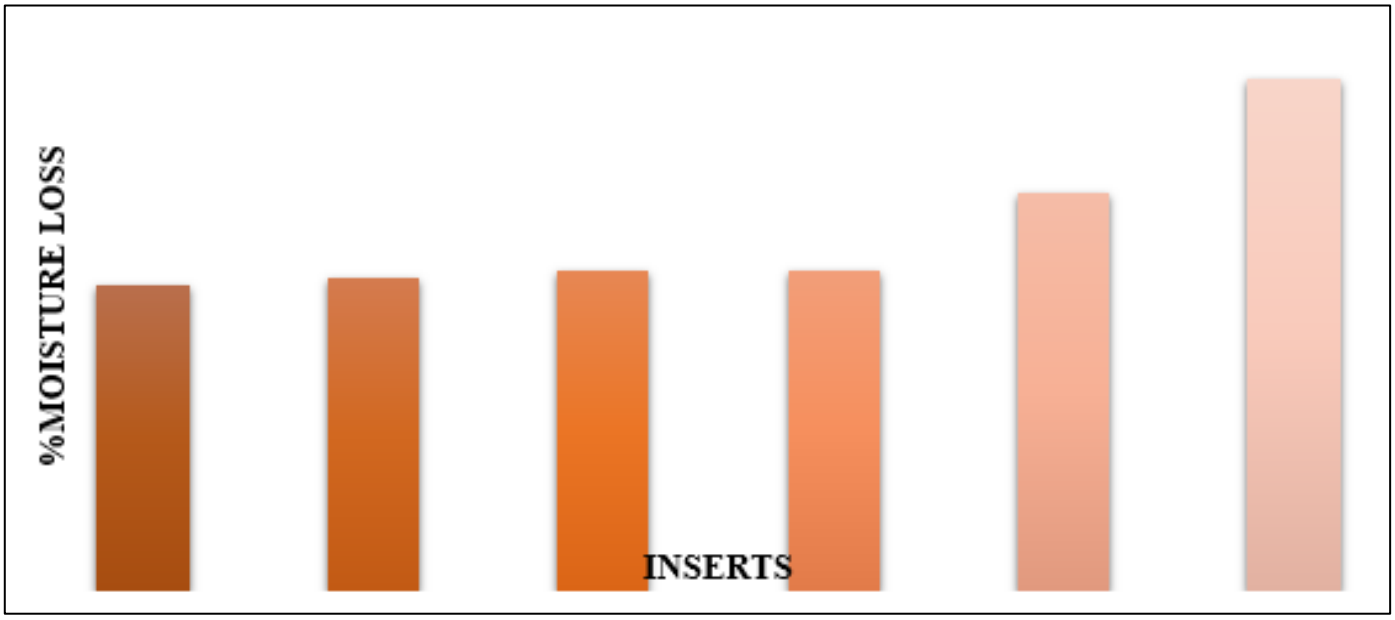

Fig 11 Graphical representation of %Moisture Loss of Doxycycline loaded Inserts.

➢ *Percentage Moisture Absorption:*

The hygroscopic compound mostly absorb moisture and stay as the most moisture content compound in a formulation. This played a important role in retaining the physical and mechanical status of a insert. Similarly moisture loss was a important in this aspect of physical and mechanical status. The more was the moisture absorption, the more would be drug conent the periodontal inserts which might impact on release pattern of drug. The table no.7 showed that the formulations containing maximum amount of drug

Table 8 %Moisture Absorption of Doxycycline loaded Inserts.

INSERTs	MOISTURE ADSORBENCEs (%) AM ± SD
CP	29.35± 16.0387
DSL-10%	29.43±5.5355
DSL-20%	33.048±20.167
DSL-30%	33.526±5.565
DBL-30%	37.393±11.348
DTL-30%	41.97 ±13.634

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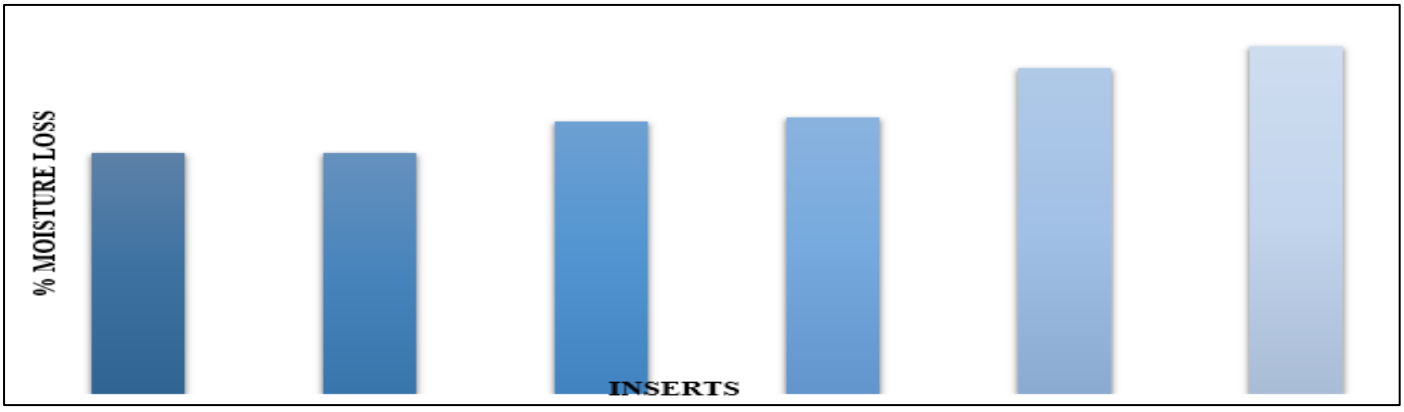

Fig 12 Graphical representation of % Moisture Absorption of Doxycycline loaded Inserts.

➢ *Water Uptake and Swelling Behavior:*

The more was the polymer content in the formulation and more would be swelling behavior, Since Single layer of insert contained less quantity of polymers than bi and tri layers insert, the tri and bilayer insert absorbed and uptaken more amount of water which showed more swelling behavior of insert. The table 8 showed swelling behavior of the inserts.

Table 9 Swelling behavioral data of Doxycycline Inserts.

INSERT CODE	H2O ABSORBED AND SWELLING BEHAVIOUR	
	INITIAL WEIGHT (mg) (0hr)	FINAL WEIGHT (mg) (2hrs)
	5.4	5.8
	5.6	7.4
	5.2	5.9
	4.7	6.8
	5.6	5.6
	6.2	8.5

➢ *Tensile Strength:*

The individual determinations of tensile strength would be performed for dummy and drug loaded inserts. It was shown that the drug loaded insert was having more tensile strength than the polymer loaded inserts. The table 9 showed the result:

Table 10 Tensile Strength of Doxycycline loaded Insert.

INSERTS CODE	TENSILE STRENGTH (Kg/sq.mm.) AM ± SD
CP	1.55± 0.0545
DSL-10	2.240±0.123
DSL-20	2.418±0.134
DSL-30	2.910±0.123
DBL-30	3.270±0.123
DTL-30	4.643 ± 0.201

*Each value was an average of three determinations.

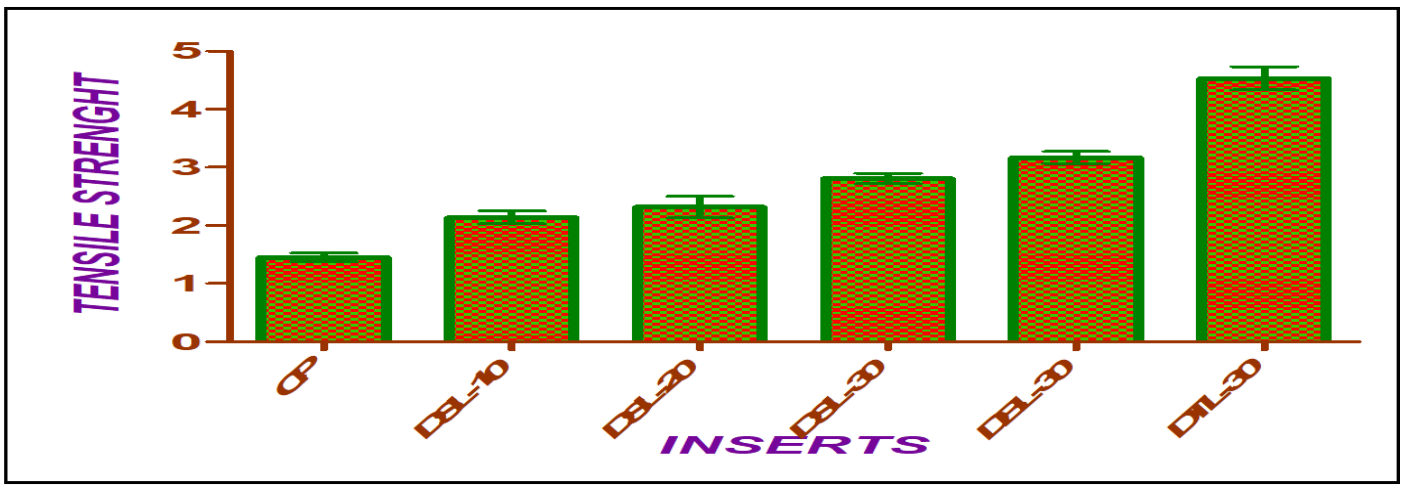

Fig 13 Graph for Tensile Strength of Doxycycline Inserts.

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➢ *Drug Content Uniformity:*

The type and concentration of drug in the periodontal insert plays important role in content uniformity of drug in periodontal insert. The table 10 showed the variation in drug conc. in different insert.

Table 11 Content Uniformity of Doxycycline loaded Insert.

Inserts Code	Drug Content* (µg)	Theoretical Drug Loading (µg)	% Drug Loading
CS	----	----	---
DSL-10	108.810± 0.05567	108.3103	99.637
DSL-20	217.562± 0.6543	217.2587	98.632
DSL-30	275.062± 0.345	327.126	84.524
DBL-30	274.135± 0.0623	327.126	84.242
DTL-30	273.279± 0.0234	327.124	83.981

➢ *Drug Release PATTERN:*

The phosphate buffer of pH 6.6 was resemble to gingival fluid which is having the pH rang of 6.5 to 6.8. So this buffer solution was used for dissolution studies of insert to determine the bio availability, bio equivalence pattern, onset of action time for the insert..

➢ *Release of DCL from Chitosan Inserts:*

This operation was conducted for continuous 7 days schedule to determine the release pattern of the drug from the single layer insert. It was seen that on 1 st day there would more release rateand from 2nd and 3rd day the release rate would be much less because following controlled release pattern. The cumu. % release of drug was found to be 97.84%, 92.091 % and 93.310 % for uni layered inserts of 10%, 20% and 30% of DCL respectively.

➢ *Release Pattern of Doxycycline Hyclate from Bilayer and Triplelayer:*

The release pattern of unilayered doxycycline loaded insert showed the imidiate release whereas bi and triple layered drug loaded inserts initialy release at faster rate but after 7 days they would showed controlled release behavior and last for 18 days. The tab. 14 and tab 15 showed the data in detail. The cumulative % release of drug from single layer insert was about 90-95% on the 8th day. However the release of drug from bilayer and trilayer insert was about 90% on 13th and 17th day.

Table 12 Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-10%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug release (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.404	5	62.565	62.565	58.1022	44.837
02	0.137	5	18.373	81.933	76.0734	25.947
03	0.076	5	9.843	91.784	85.1364	16.834
04	0.054	5	7.404	98.185	91.1453	95.049
05	0.027	5	3.185	100.376	93.1743	8.8237
06	0.025	5	3.034	100.403	95.0584	6.942
07	0.026	5	3.33	104.745	97.235	4.765
08	0.016	3	0.652	105.406	97.834	4.1614

- Each value is an average of three Replication, Temperature maintained =37° C

Table 13 Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-20%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.356	10	106.938	106.935	49.937	52.089
02	0.414	5	64.435	170.375	79.293	22.787
03	0.106	5	15.377	184.744	85.845	15.154
04	0.045	5	5.846	189.595	88.0865	13.914
05	0.034	5	4.124	192.715	89.547	12.474
06	0.026	5	3.655	195.376	90.759	11.246
07	0.032	3	2.784	197.153	91.577	10.422
08	0.022	3	0.935	198.094	92.007	9.993

- Each value is an average of three replication* Temperature maintained 37° C

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Table 14 Static Dissolution-Time Profile for Chitosan Inserts Containing Doxycyline-30%.

Time (days)	Absorbance	Dilution factor	Conc. of drug released (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.498	10	153.55	153.507	56.64	45.354
02	0.456	5	70.066	222.566	81.846	20.155
03	0.124	5	18.814	240.377	88.345	13.656
04	0.054	5	8.346	247.715	91.026	10.976
05	0.022	5	3.184	249.907	91.826	10.185
06	0.024	5	2.716	251.626	92.445	9.556
07	0.013	5	2.245	252.876	92.904	9.097
08	0.022	3	0.845	253.714	93.215	8.796

- Each value is an average of three replication* Temperature maintained 37° C

Table 15 Static Dissolution-Time Profile for Chitosan Bilayer Inserts Containing Doxycyline-30%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.467	10	143.125	143.123	52.404	47.606
02	0.364	5	55.316	198.435	72.653	27.345
03	0.164	5	24.210	222.653	81.514	18.485
04	0.064	5	8.909	231.565	84.775	15.226
05	0.036	5	4.379	235.933	86.386	13.625
06	0.037	5	2.966	238.906	87.465	12.536
07	0.026	5	2.1867	241.095	88.266	11.736
08	0.037	3	2.066	243.159	89.027	10.976
09	0.034	3	1.784	244.933	89.677	10.323
10	0.025	3	1.6874	246.623	90.296	9.704
11	0.024	3	1.124	247.745	90.703	9.293
12	0.023	3	0.845	248.586	91.015	8.983
13	0.013	3	0.655	249.246	91.255	8.735

- Each value is an average of three replication* Temperature maintained 37° C

Table 16 Static Dissolution-Time Profile for Chitosan Tri Layer Inserts Containing Doxycyline-30%.

Time (days)	Absorbance*	Dilution factor	Conc. of drug released (µg) AM ± SD	Cumulative release (µg)	% conc. of drug released 'a'	% conc. of drug unreleased
01	0.364	10	111.03	111.006	41.397	60.603
02	0.375	5	57.872	167.873	62.285	39.714
03	0.166	5	25.064	191.935	71.126	30.875
04	0.131	5	19.594	210.56	77.959	24.046
05	0.070	5	10.0623	219.595	81.280	20.716
06	0.044	5	6.010	224.593	83.119	18.884
07	0.036	5	3.752	228.344	84.498	16.506
08	0.028	5	3.503	230.845	85.416	16.588
09	0.026	5	2.185	233.029	86.218	15.787
10	0.024	5	2.874	234.909	86.907	15.096
11	0.027	3	2.501	236.408	87.456	14.548
12	0.026	3	2.316	237.716	87.935	14.065
13	0.023	3	2.124	238.847	88.355	13.646
14	0.022	3	0.936	239.778	88.697	13.304
15	0.020	3	0.755	240.487	88.956	13.043
16	0.018	3	0.563	241.046	88.997	13.004
17	0.016	3	0.374	241.427	89.298	12.701

- Each value is an average of three replication* Temperature maintained 37° C,

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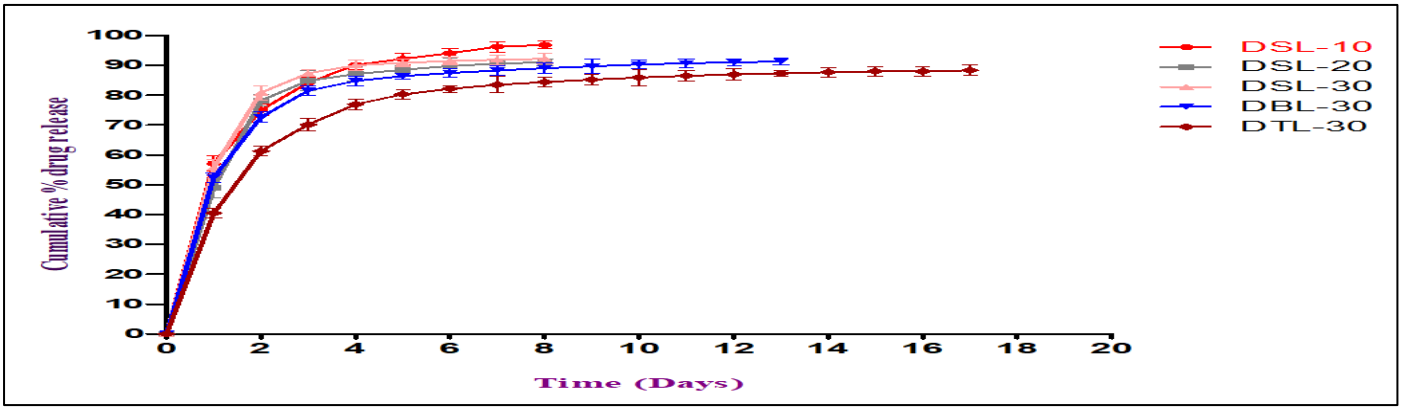

Fig 14 Cumulative Percentage Release of Doxycycline Hyclate from Chitosan Inserts.

➢ *Release kinetics:*

A linear relationship was obtained by the produced release pattern of the drug doxycycline hyclate, which was subjected to regressional analysis

➢ *Dependent-model method:*

The data of release pattern of different inserts were the plotted in different kinetic model

➢ *First Order-*

- *Log of % Cumulative Drug Release VS. Time*

Best Fit line for first order equation for chitosan inserts containing doxycycline.

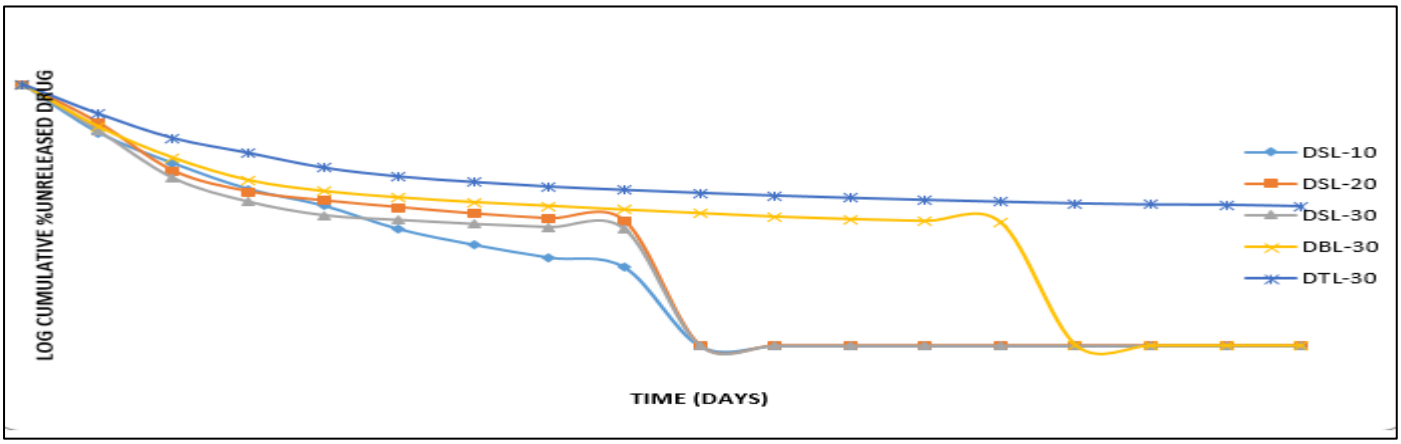

Fig 15 First Order Equation for Chitosan Inserts Containing Doxycycline hyclate

➢ *Higuchi Equation*

% Cumulative Drug Release Vs. Square Root of Time

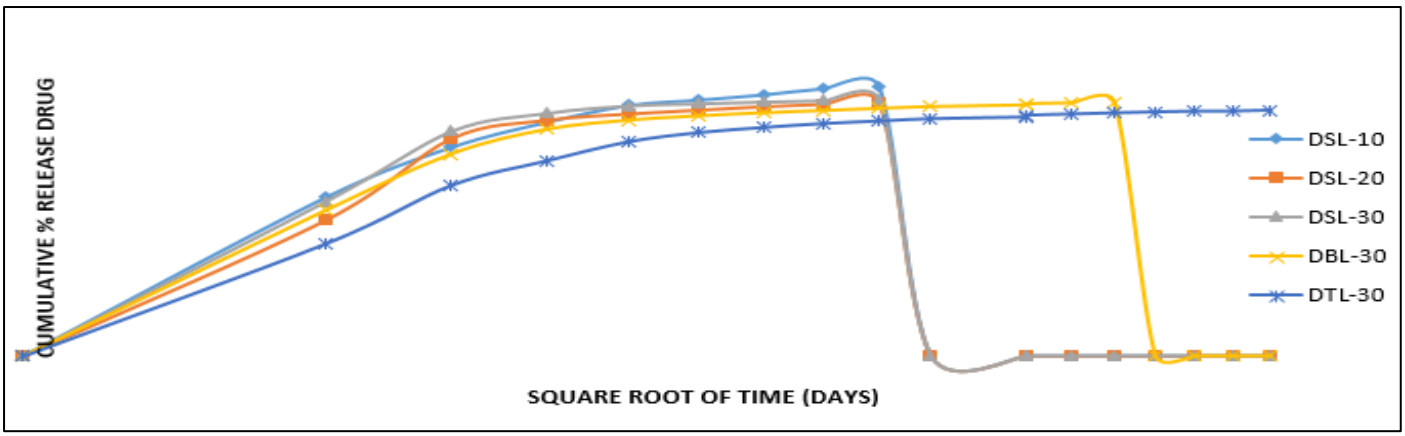

Fig 16 Higuchi Equation for Chitosan Inserts Containing Doxycycline hyclate

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➢ *Korsemeyer Peppas Equation*
Log of % Cumulative Drug Release Vs. Log of Time

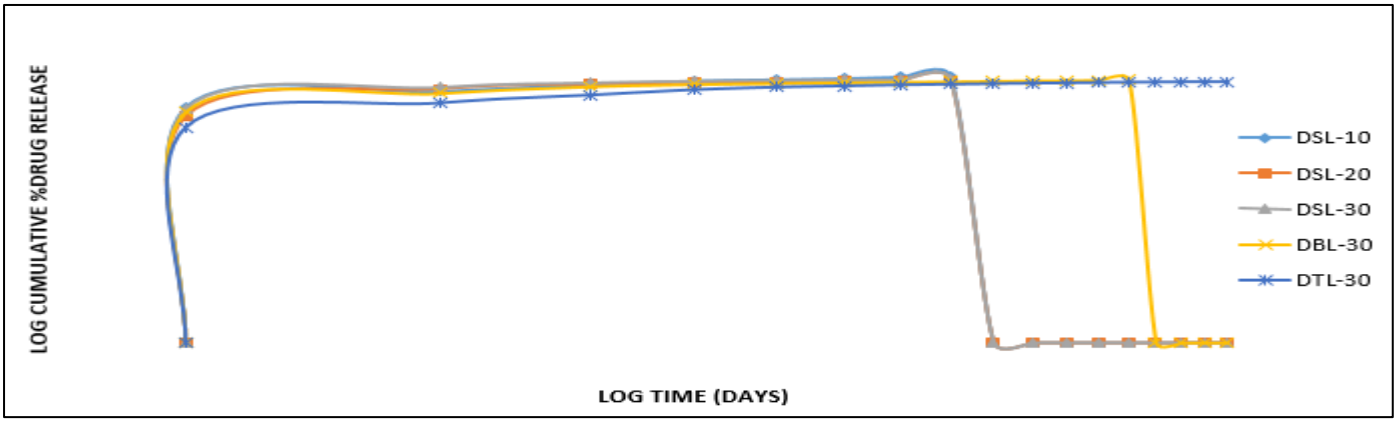

Fig 17 Korsemeyer Peppas Equation for Chitosan Inserts Containing doxycycline hyclate.

The results obtaining by *In-vitro* release studies were plotted in different kinetic models as follows.

Table 17 Data of Kinetic modeling of Doxycycline loaded polymer Inserts

INSERTs	ZERO ORDER	FIRST ORDER	HUGUCHI MATRIX	KORSEMEYER PEPPAS	
	R^2	R^2	R^2	R^2	N
DSL-10	0.558	0.953	0.888	0.416	0.938
DSL-20	0.597	0.807	0.848	0.423	0786
DSL-30	0.558	0.767	0.824	0.403	0.788
DBL-30	0.478	0.744	0.739	0.389	0.812
DTL-30	0.514	0.751	0.765	0.422	0.823

➢ *In-Vitro Antibacterial Activity:*
In Table no. 17. it was observed that single layer periodontal insert exihibited fast release pattern but antibacterial activity last for short span of time, but it was observed that bi and tri layered periodontal insert exihibited slow antibacterial action but lasted for long duration. The zone of inhibition values of DCL.

Table 18 In vitro antibacterial activity data of Doxycycline hyclate.

Days	Diameter of inhibition of growth area of *S.mutans* (mm)				
	DSL-10	DSL-20	DsL-30	DBL-30	DTL-30
1	17	21	27	24	21
2	15	18	23	19	18
4	11	14	17	15	14
8	8	11	12	13	12
11	-	-	-	11	9
14	-	-	-	8	8
17	-	-	-	8	6

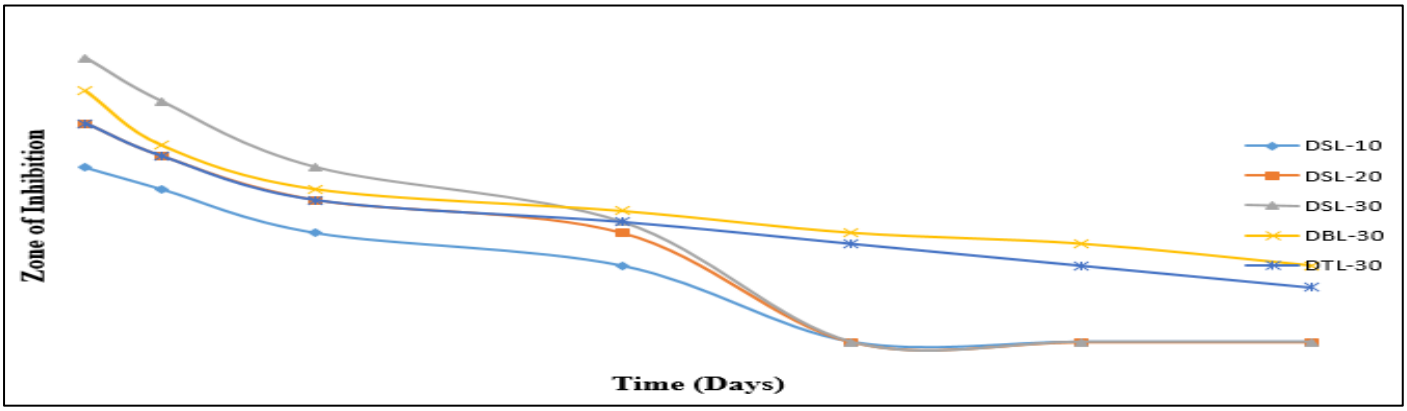

Fig 18 Zone of inhibition of Doxycycline Hyclate.

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➢ *Stability Study:*

Table 19 Stability Studies for Drug Content at various temperatures for Chitosan Inserts Containing Doxycycline Hyclate.

Insert code	Initial drug conc. (μg)	30 Days	60 Days	90 Days
		A.F	A.F	A.F
DSL10	109.810	108.804	108.785	106.701
DSL-20	214.562	215.563	217.533	214.489
DSL-30	275.062	276.056	276.023	275.988
DBL-30	274.135	274.134	274.101	275.027
DTL-30	273.279	275.276	275.251	274.145

Each value is an average of 3 determinations. A.F = Packed in aluminum foil. Drug content uniformity in the inserts revealed that the inserts was fully stable in characters.

IV. CONCLUSIONS

From the above study it can be concluded that the periodontal insert if prepared by means of solvent casting methods in single, bi and tri layered it can exhibit good Physical stability like hardness, content uniformity etc. no chemical interaction among polymer chitosan and drug Doxycycline hyclate, good release pattern as in case bi ad tri layered doxycycline loaded inserts than single layer, good dissolution pattern and bioavailability, and good antibacterial activity. It can be concluded that these inserts can be used upto three weeks after its manufacturing. In the sac of performance this project will be fruitful in future prospect**.**

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