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Formulation and Characterization Of Qurecetin Perio Dontal Films For Local Delivery Of Antimicrobials

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ABSTRACT

A novel periodontal film for the treatment of periodontitis was developed by using herbal drug Quercetin, it is a plant flavanol from the flavonoid group of polyphenols. It is found in many fruits, vegetables, leaves, seeds and grains; capers and red onions. It has a bitter flavour and is used as an antimicrobial agent and effective against infecting microorganisms in the periodontal pocket. Calibration curves for Quercetin was developed in phosphate buffer P^H 6.6, FT-*IR* studies was performed, which revealed that no interaction between the selected drug and polymers. Differential scanning calorimetry was performed to the identification of various physical properties and thermal transitions of drug and the polymeric materials. Periodontal films were prepared by solvent casting technique using Eutragit and HPMC as polymers Dibutyl phthalate as plasticizers and PEG as surface active agent. The formulated periodontal films were evaluated for their folding endurance, percent moisture loss, surface pH, viscosity, thickness, uniformity of weight, content uniformity, and *in-vitro* release. Scanning electron microscopy was done to study the surface characteristics of the patch on placebo and optimized formulation F3 before dissolution and after dissolution. SEM analysis revealed that the drug was uniformly distributed in patch and drug was released by diffusion. Data of *In-vitro* release from the formulated periodontal films were fit to different equations and kinetic models to explain release kinetics. Kinetic models used were zero first-order equations and Higuchi models. The release mechanism was understood by fitting the data to Korsemeyer-Peppas model.

Keywords: Quercetin periodontal film, Periodontitis, Controlled release and Gingivitis.

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INTRODUCTION

Periodontal diseases is recognized as the major public health problem throughout the world. Daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can do occur in all age groups, ethnicities, races, genders and socioeconomic levels. Periodontal diseases, including gingivitis and periodontitis, are serious infections that left untreated can lead to tooth loss. The word periodontal literally means "around the tooth." Periodontal disease is a chronic bacterial infection that affects the gums and bone supporting the teeth. Periodontal disease can affect one tooth or many teeth. It begins when the bacteria in plaque (the sticky, colourless film that constantly forms on teeth) causes the gums to become inflamed. Periodontal diseases range from simple gum inflammation to serious disease those results in major damage to the soft tissue and bone that support the teeth. In the worst cases, teeth are lost [1]. Periodontitis is caused by microorganisms that adhere to and grow on the tooth's surfaces, along with an overly aggressive immune response against these microorganisms [2]. A diagnosis of periodontitis is established by inspecting the soft gum tissues around the teeth with a probe and x-ray films by visual analysis, to determine the amount of bone loss around the teeth. Specialists in the treatment of periodontitis are periodontists their field is known as “periodontology" or "periodontics". The main cause of periodontal disease is bacteria plaque, a sticky, colourless film that constantly forms on teeth. However, factors like smoking/ tobacco use, genetics, pregnancy and puberty, stress, medication, clenching or grinding teeth, diabetes and poor nutrition also lead to periodontal diseases. Periodontal pathogens grow only where atmosphere and nutrient composition are strictly conductive to their requirements and the disease once established, causes major changes in the periodontal microenvironment. The gingival crevicular fluid (GCF) flow occurs at extremely low levels in healthy gingival sulci but increases enormously to 3.5 ml/day or more. The most commonly grown anaerobic pathogenic bacteria are Actinobacillus actinomycetencomitans, Bacteroides gingivitis; Bacteroides melanogenic sub species intermedius, Porphyromonas gingivitis and Prevotella intermedia. Clinical signs such as bluish red thickened marginal gingiva, bluish red vertical zone from the gingival margin to the oral mucosa, gingival bleeding and localized pain are suggestive of the presence of periodontal pockets microorganisms [3,4,5.]

Figure 1: Representing the healthy gums, gingivitis, periodontal disease and periodontitis.

MATERIALS AND METHOD

Quercetin was purchased from Vasa lab Bangalore, HPMC, Eudrgit RL100, Dibutyl phthalate and Alcohol was obtained from laboratory.

METHODOLOGY

Preparation of standard stock solution of Quercetin in phosphate buffer P^H 6.6 [5]

Standard stock solution of Quercetin was prepared by dissolving accurately weighed 100mg of drug with little quantity of phosphate buffer P^H 6.6 in 100ml volumetric flask volume was made up to 100ml by using phosphate buffer P^H 6.6 to obtain the solution 1000µg/ml, from the standard stock solution 1ml was pipetted into 10ml volumetric flask and made up to the mark with phosphate buffer pH 6.6 the resulting solution containing 10µg/ml .The solutions were suitable diluted to get concentration range of 1µg/ml to 6µg/later the solutions scanned in between 200 – 410 nm using of phosphate buffer pH 6.6 as blank . Then the λ max was found to be 369 nm for Quercetin.

DRUG -EXCIPIENT COMPATIBILITY STUDY [6]

Fourier Transform Infrared Spectroscopy:

FTIR studies were carried out for pure drug and polymer. 3mg of pure drug and 3mg of polymer HPMC Eudragit triturated were with 97 mg of KBR in a smooth mortar to obtain mixtures and then it was placed in sample holder of the instrument and scanned in IR spectroscopy between 400 – 4000 cm^{-1}. The obtained spectrum was investigated for any possible interaction between drug and the polymer. It was shown that there was no interaction.

Differential Scanning Calorimetric Studies: [7]

The compatibility and physical state of drugs inside the optimized formulation (F3) were determined by differential scanning calorimetric (DSC). Thermograms were used with a differential scanning calorimeter (Mettler, Toledo, 822e). The instrument was calibrated with 5 mg of indium at a heating rate of 10°C min^{-1}. The thermal behavior was studied by heating 2–10 mg of samples heating at a rate of 10°C min^{-1} from 25°C to 300°C in a hermetically sealed pan with a pinhole in the lid under a nitrogen purge of 20 ml $min.^{-1}$

Method of preparation of Quercetin periodontal films[8]

1. Quercetin was dissolved in polyethylene glycol and polymer HPMC and Eudragit was dissolved in alcohol at room temperature using magnetic stirrer in separate beaker and beaker containing drug solution was mixed with polymers solution.
2. Later dibutyl phthalate is added as plasticizer.
3. The solution was poured on a petri dish and dried at room temperature.
4. Films were removed from petri dish and were cut into specific size packed in aluminum foil and stored in desiccators for further evaluation.

Formulation containing Quercetin periodontal films

Table 1: Composition of Quercetin periodontal films.

Ingredients	F1	F2	F3	F4
Quercetin (mg)	100	100	100	100
HPMC K 15M(mg)	100	100	125	150
Eudrgit RL100 (mg)	-	75	100	125
Dibutyl phthalate(ml)	1	1	1	1
PEG 800 (ml)	5	5	5	5
Alcohol(ml)	10	10	10	10

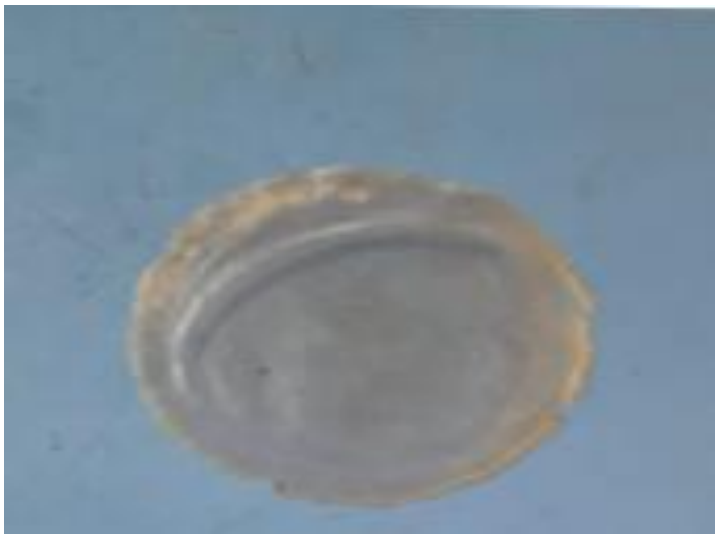

Figure 2: Representing periodontal film.

Physical characterization of periodontal films

Thickness and weight variation: [9]

The thickness of the films was measured by using a screw gauge. Three randomly selected films were measured by using a screw gauge, The individual values can be varied within ± 5% of the mean. selected films of each formulation having surface area 1 cm^2 were used. For determination of weight variation, 10 patches of each formulation were weighed separately on electronic balance and the average weight of the patch was calculated.

Surface pH: [10]

Periodontal patches were kept to swell for 1 hour on the surface of the agar plates, prepared by dissolving 2%(w/v) agar is distilled water under stirring and then pouring the solution into the Petri dish for gelling and solidify at room temperature, surface pH was measured by pH paper placed on the surface of the swollen patch, the recorded values or the mean of five determination. The P^H of films were evaluated by pH paper on agar plate and were found to be approximately pH 7. The surface pH of all the films were found to be neutral and hence no periodontal pocket irritation was expected

Folding endurance:[10]

It was determined by repeatedly folding the patch at the same place till it is broken or folded up to 300times, which is considered as an adequate to relieved good film properties. The patch was folded 300times at the same place without breaking gave the good folding endurance the test was done on all films for 5 times

Percentage moisture loss: [10]

Films were weighed individually and kept in a desiccator at room temperature containing calcium chloride. The films were weighed repeatedly until they showed a constant the percentage moisture loss was calculated using the following formula.

Percentage moisture loss = [(initial weight -final weight)/ initial weight] ×100

Viscosity: [6]

Solution containing drug, polymers and plasticizer with the same concentration as that of formulated films were prepared, these solutions were subjected for viscosity determination by Brookfield viscometer (L VDE-E model) attached to the Heli path spindle number 18. The viscosity was measured at 100rpm at room temperature. The recorded values were the mean of five determination.

Drug content uniformity of films: [6]

Patch of (1x1 cm^2) were taken from different areas of the films and placed in a 10ml of volumetric

flask and 10 ml of ethyl alcohol was added, the volumetric flask was kept a side until the patch gets completely dissolved. 1 ml of the solution was suitably diluted with pH 6.6 phosphate buffer. The absorbance of the solution was measured at 369 nm, the polymer solution without drug serves as the blank

Scanning Electron Microscopic Studies: [11]

The surface morphology of placebo, drug-loaded film and drug loaded film after dissolution of F3 was examined by scanning electron microscopy. The film sample was mounted on metal stubs with double-sided adhesive band then gold was sputtered on the specimen to ensure sufficient electrical conductivity. The images were taken using environmental mode and ET detector with 10 kV excitation

***In-vitro* drug release study:** [8]

As the P^H of the gingival fluid lies between 6.5-6.8 phosphate buffer of P^H 6.6 was used as the simulated gingival fluid and the films remains immobile in periodontal pocket. A static dissolution method was adopted for *in-vitro* drug release studies. Patches of known weight and dimension (1×1 cm ^{2}) were placed separately into small test tubes containing 1ml phosphate buffer P^H 6.6 the tubes were sealed and kept at 37º C for 24hrs. the buffer was drained off and replaced with 1ml of phosphate buffer P^H 6.6. Then the concentration of the drug in buffer were measured at 369nm. *In-vitro* drug release studies were carried out for 10 days.

Kinetic studies:[10,11]

To analyze the drug release kinetics and mechanism of drug release from the films, the *in vitro* dissolution studies data was fitted into zero order and first order.

Zero order kinetics:

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area of dose will not change and no equilibrium condition are obtained can be represented by following equation.

$Q_t=Q_0+K_0t$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution and K_0 is zero order release constant.

First order kinetics:

To study the first order rate kinetics the drug data were fitted in following equation.

$\text{Log } Q_t=\log Q_0+K_1t/2.303$

Where Q_t is the amount of drug released in time t,Q_0 is the initial amount of the drug in the formulation and K_1 is the first order release constant.

Higuchi model:

Higuchi developed several theoretical models to study the release of water soluble and partially soluble and in soluble drugs incorporated in semisolids and solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving has the diffusion media and the equation is $Q_t=K_{H.}\, t^{½}$

Where Q_t is the amount of drug released in time t , K_H is Higuchi constant.

Korysmeyer Peppas model

To study the mechanism of drug release, the release data was fitted to exponential equation (Kormeyer Peppas equation) which is often used to describe the drug release behaviour from excipients.

$M_t/M_a=Kt^n$

Where M_t/M_a=the fraction of drug released at time 't', K=constant incorporating the structure and geometrical characteristics of drug/excipients, N=diffusion exponent related to the mechanism of the release. Above equation can be simplified by applying log on the both sides

Log M_t/M_a=Log K +n log t

RESULTS AND DISCUSSION

Table 2: Calibration curve data of Quercetin in phosphate buffer P^H 6.6 at 369 nm

Concentration(µg/ml)	Absorbance(369nm)
0	0
1	0.189±0.234
2	0.38±0.104
3	0.597±0.256
4	0.762±0.435
5	0.857±0.267
6	0.976±0.202

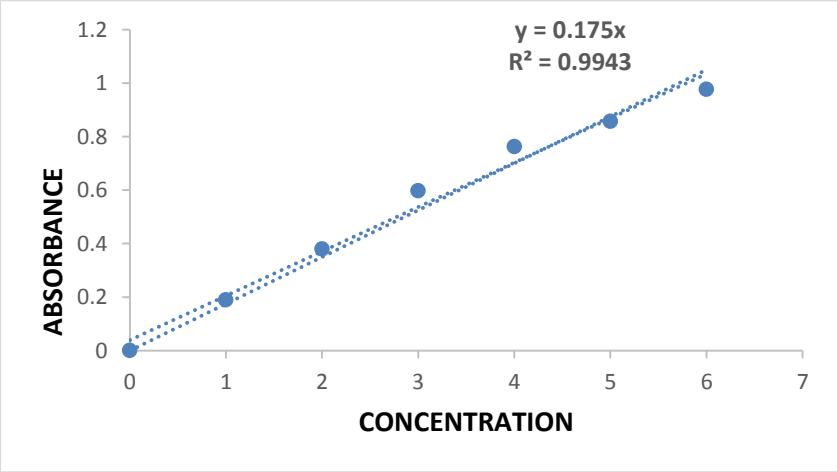

Figure 3: Standard graph of Quercetin in phosphate buffer pH 6.6

Standard graph was determined in phosphate buffer P^H 6.6**,** λ max of Quercetin in phosphate buffer P^H 6.6was found to be in 369nm with y=0.175x+ and R^2 = 0.9943

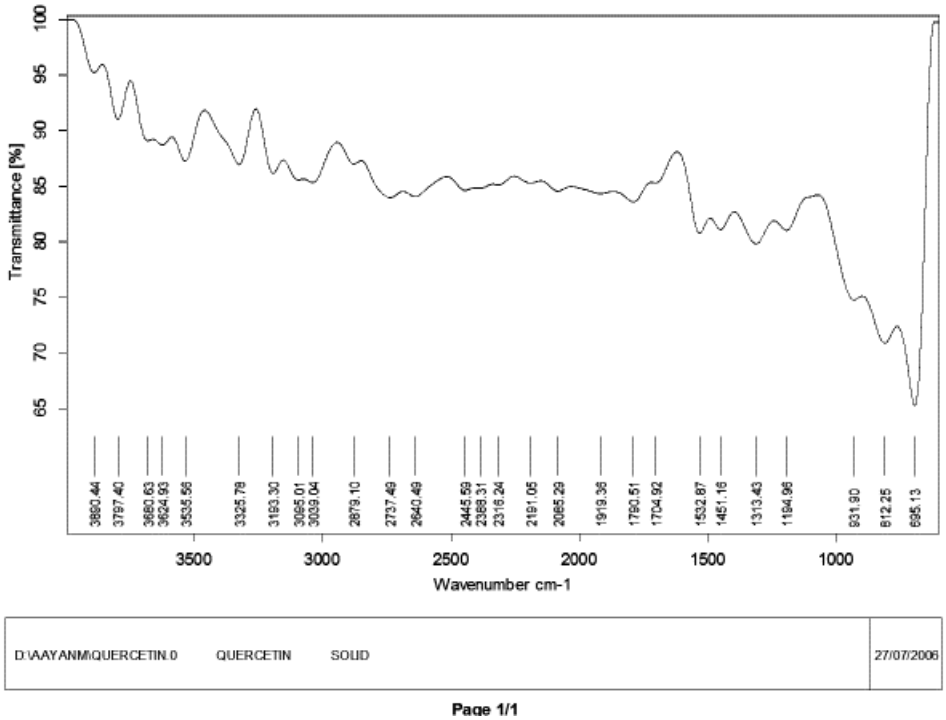

Figure 4: FTIR spectrum of Quercetin

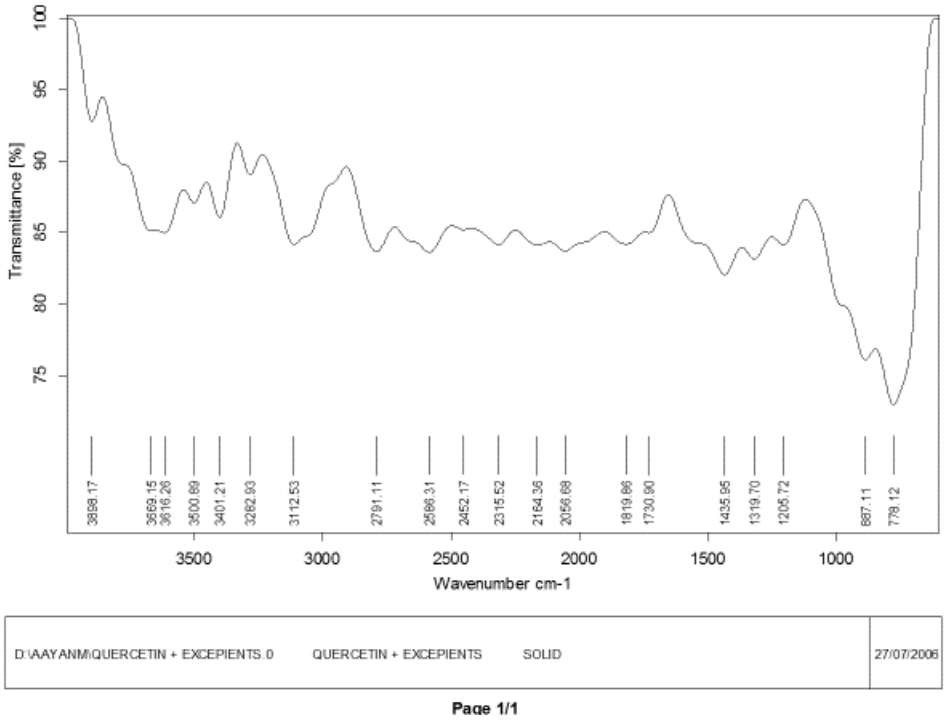

Figure 5: FTIR of Quercetin and polymer combination.

FTIR studies were carried out for pure drug and polymer, the pure drug showed OH group stretching at 3,400 cm^{-1} OH Bending at 1313 cm^{-1} C= O stretching at 1704.92 cm^{-1} Aryl

Ketone C=C stretching at 1532 cm $^{-1}$ C-O Stretching =1194.96 cm $^{-1}$ phenol and C-O bending out of plane =812 cm $^{-1}$ ·Same peaks were observed in the mixture of drug and polymers ,there was no change in the functional groups of the pure drug and polymer, hence there were no interaction between drug and polymer.

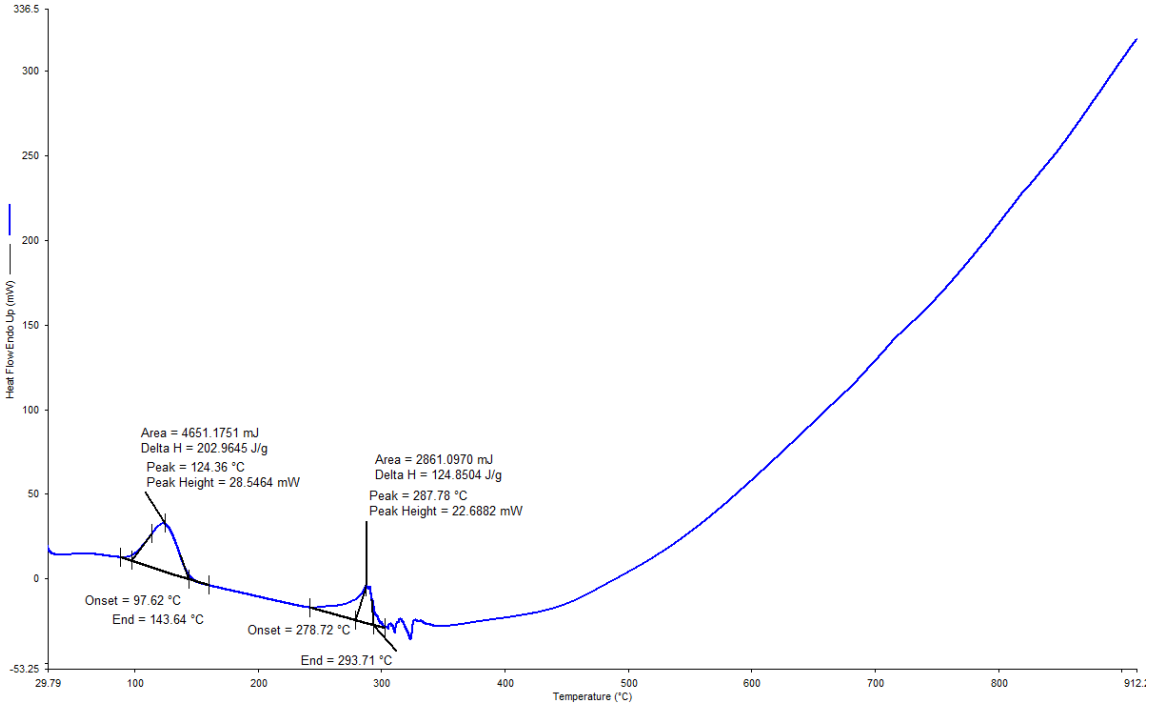

Figure 6: DSC of Pure Quercetin

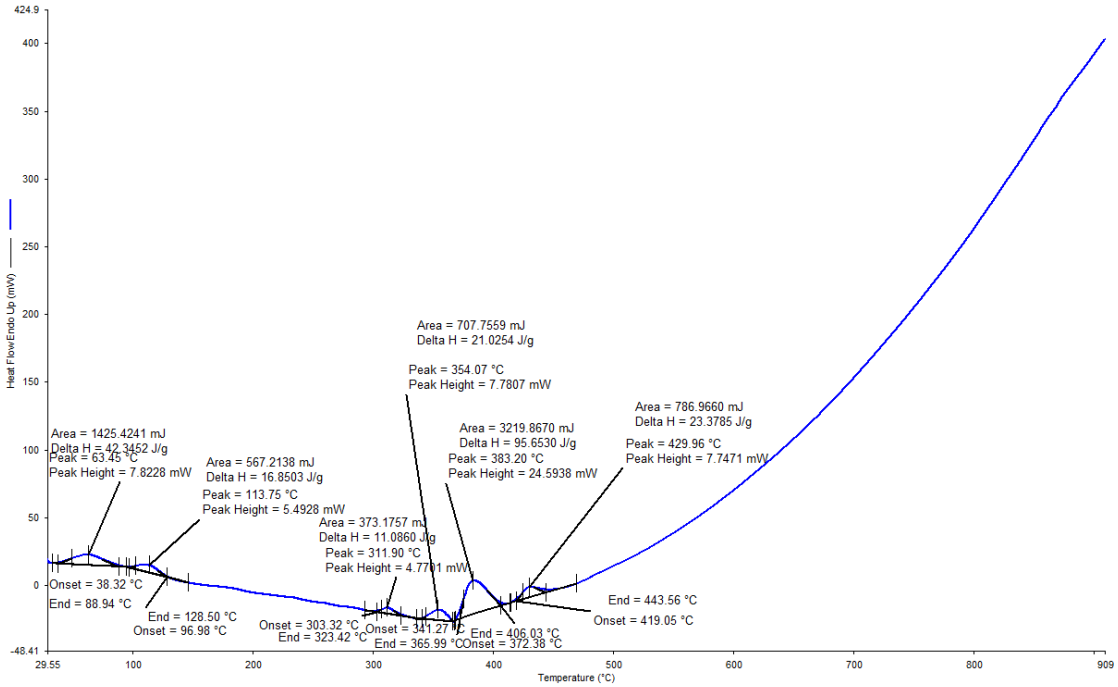

Figure 7: DSC of Pure Quercetin HPMC and Eudragit

DSC studies showed endothermic peak of pure Quercetin at 288º C which is attributed to melting point of Quercetin and one peak at 120 º C associated with water molecule loss. With the

admixture of Quercetin and polymers mixture sharp endothermic peak was observed at 311 º C showing the melting point of Quercetin small peaks at 113 º C as the melting of Eudragit at broad peak at 383.34 º C due degradation of drug .Hence DSC study suggested that Quercetin was in amorphous form in physical admixture. Differential scanning calorimetry study conformed physical properties and thermal transitions of drug and the polymeric materials was negligible.

Table 3: Physical characterization of periodontal films

Formulation	Thickness(mm)	Weight variation(mg)	Surface pH
F 1	0.14±0.12	0.613±0.11	Neutral
F 2	0.16±0.24	0.670±0.21	Neutral
F 3	0.18±0.25	0.708±0.24	Neutral
F 4	0.2±0.23	0.766±0.25	Neutral

Thickness uniformity of films was carried the average thickness was in the range of 0.14±0.12 to 0.2±0.23mm. Uniformity of weight of films were carried the values were found to be varying from range of 0.613±0.11 to 0.766±0.25 mg The surface P^H of all the films were evaluated by P^H paper on the agar plate and were found to be approximately 7. The surface P^H of all the films were found to neutral and hence no periodontal pocket irritation was expected.

Table 4: Physical characterization of periodontal films

Formulation	Folding endurance	Percentage moisture loss(%)	Viscosity(cps)	Drug content uniformity
F 1	285.2±0.22	11.03	12.16±0.253	95.748
F 2	288.2±0.24	10.83	13.8±0.568	92.091
F 3	288.6±0.12	13.12	15.65±0.921	99.012
F 4	288.2±0.31	12.78	14.12±0.15	96.179

The folding endurance of the films were > 288times. It means all the formulations have good folding endurance. percentage moisture loss was carried out on all the prepared films, it was in the range of 10.83-13.12 . F 3showed 13.12 maximum moisture loss these may be due to the more water vapour permeability of these polymers. Viscosities of the films were measured it was in the range of 12.16±0.0253- 15.65±0.921. F 3 had the highest of 15.65±0.921cps these may be due the completely solubility of the polymer in the solvents. The drug content uniformity was carried out, the drug content was analysed at 369 nm, all the formulations showed the drug loading more than 90% indicating much of the drug was not lost and uniformly dispersed.

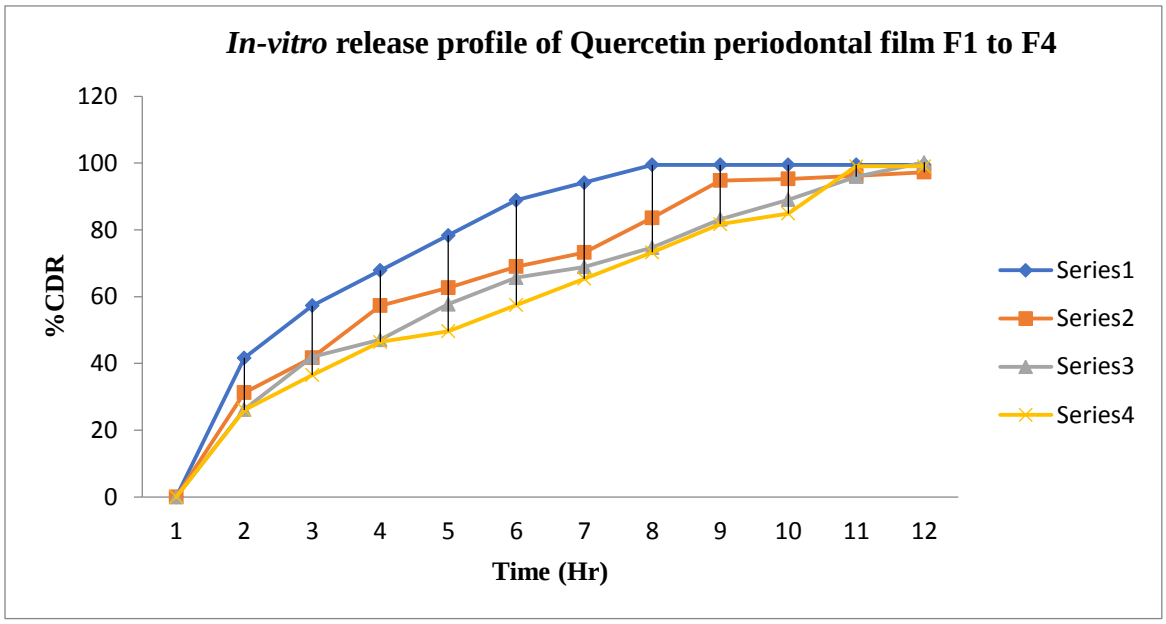

Figure 8: Comparative *In-vitro* release profile of Quercetin periodontal film F1 to F4 in phosphate buffer P^H 6.6

In vitro drug release was studied for 10days and the drug release was in the range of 26.204-99.928%. F3 and F4 showed the release for 10days, all the formulations released the drug above 92.928%.

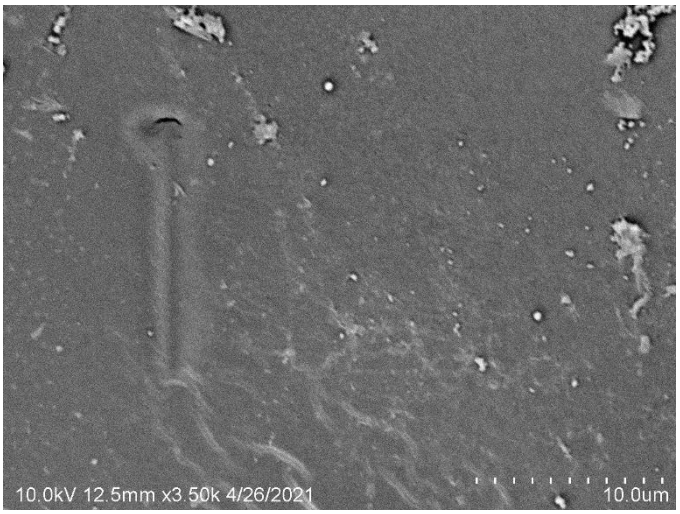

Figure 9: SEM of Placebo

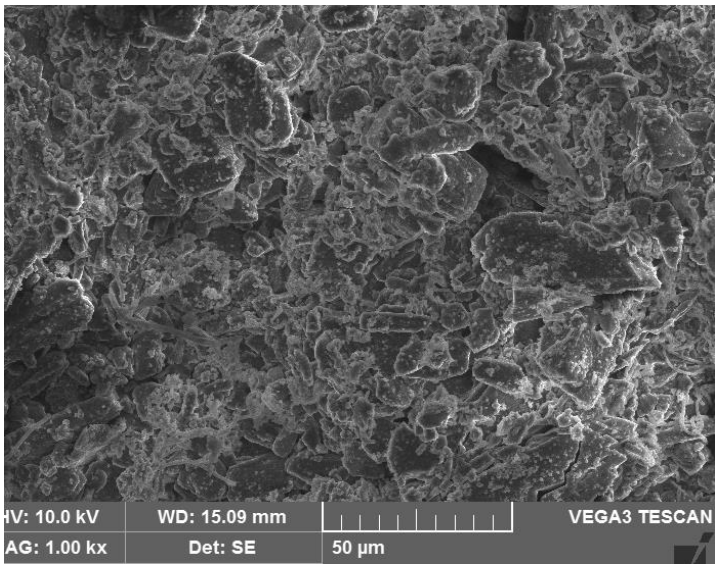

Figure 10: SEM of Quercetin drug loaded film F3

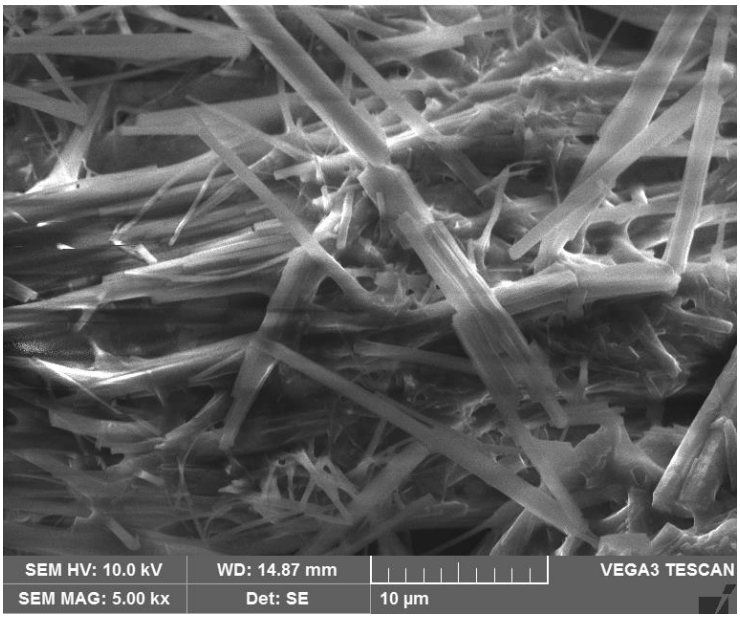

Figure 11: SEM of Quercetin drug loaded film F3 after dissolution

Surface Electron Microscopy revealed that the drugs appeared as white specks on the surface of the carrier matrix in case of optimized film (Figure 10). The placebo film showed no such specks (Figure 9) Surface characteristics of film from which drug had been released was also studied and it showed pore formation in the polymer matrix indicating release of the drug (Figure 11) SEM indicated that the films had a smooth surface prior to drug release while after release surface irregularities were evident. Some irregular pores were also seen. Formation of pores in the formulation of drug loaded film indicated that the release of Quercetin started with dissolution of the drugs and subsequently followed diffusion through the pores.

Table 5: Showing the Zero order, First order and Higuchi model of Quercetin patch from F1-F4

Film code	Zero order	First order	Higuchi model	Korsmeyer Peppas model
F1	y=6.5978x+56.643 R^2= 0.3021	y=-0.190x+2.179 R^2=0.9654	y =39.261x-13.24 R^2=0.9169	R^2=0.9982 n=0.4545
F 2	y=5.4557x+62.639 R^2=0.4034	y=0.1406x+2.2063 R^2=0.9115	y=30.428x+0.9035 R^2=0.9952	R^2=0.9884 n=0.5145
F3	y=4.8703x+64.242 R^2=0.3474	y=-0.115x+2.0557 R^2=0.906	y=28.705x-0.6426 R^2=0.9971	R^2=0.9925 n=0.5343
F4	y=4.8556x+66.697 R^2 =0.337	y=0.0854x+1.9985 R^2=0.9738	y=28.459x-2.9845 R^2=0.9874	R^2=0.9902 n=0.5376

The release mechanism was not sufficient to predict drug release, hence the values were plotted in zero and first order kinetics curves were having straight line and different slopes as the R^2 values were higher in First order kinetics the mechanism of the drug release from the periodontal film followed First order kinetics, the data was fitted into Higuchi's as the R^2 values were in the range of 0.9169-0.9874 which showed the release followed diffusion rate-controlled mechanisms. Korsmeyer-Peppas is a simple model which describes drug release from a polymeric system. In Korsmeyer-Peppas model as the value of n is in the range of 0.4545-0.537 which indicates that the drug release followed Fickian diffusion.

CONCULSION

In the present research work periodontal patch of Quercetin was prepared by solvent casting method using HPMC and Eudragit as polymers. The patches were checked for various physicochemical parameters. The best formulation was selected using this parameters, dissolution data and content uniformity of F3 showed maximum of 99. 928% of drug release in 10 days and excellent drug content uniformity of 99.012% and hence considered best among the prepared formulations, F3 formulations followed diffusion rate controlled mechanism and Fickian diffusion

REFERENCES:

1. Steinberg A, Friedman M. Sustained release drug delivery for local treatment of dental diseases, Drug delivery devices, Fundamental and application. New York: Tyles P Marcel Dekker Inc; 1998:492-515
2. Goodson JM Offenbacher S, Farr DH, Hogon PE Dunham. Periodontal disease treatment by local drug delivery. J Per 1985;56(5):265-72
3. Prabhushankar GL, Gopalkrishna B, Manjunath KM. Formulation and evaluation of Levofloxacin dental films for periodontitis. Int J Pharm Sci 2010;1(2):1-13

4. Samal HB, Das IJ, Patra N, Murthy PN. Design and development of dental film containing Aloe vera for the treatment of human periodontal disease. Asi J. PharmTech 2015;5(4):273-80
5. Jothi V, Vijay KT, Vasudev B, Giliyar SB. Antimicrobial effect of Anacardiumoccidentale leaf extract against pathogens causing periodontal disease. Adv biosci Biotec2013;(4):15-18.
6. Sathesh babu PR, Manoj k, Prabhushankar GL. Formulation and *In-vitro* evaluation of Periodontal films containing Metronidazole. Int J PharTech Res 2010; 2(4): 2188-93
7. Amal HEK, Lubna YA, Ibrahim AA . Micromatricial Metronidazole Benzonate film as a local mucoadhesive delivery system for treatment of periodontal diseases AAPS Pharm SciTech 2007;8(3)1-11
8. . Himansu B, Itishree JD, Niranjan P, Sreenivas SA. Design and *In vitro* evaluation of Curcumin dental films for the treatment of periodontitis. Asi J Pha2017;11(3): 80-7
9. . *Vaishali P, Sushita D Ajit M. Formulation and evaluation of dental films for the treatment of periodontal diseases. Int J Pha Res 2016:7(2) ;41-56*
10. . *Ahmed MG, Harish NM, Charyulu RN, Prabhu P.* Formulation of chitosan-based Ciprofloxacin and Diclofenac film for periodontitis therapy. Tro J of Phar Res2009 ;8 (1): 33-41
11. . Sajid AM, Javed A, Alka A, Sarfaraz AM. Formulation and characterization of dental film containing Ofloxacin. J App Phar Sci 2012;2(11):114-9.