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Formulation And Evaluation Of Doxycycline Proniosomal Gel For The Treatment Of Periodontitis

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Abstract

Objective:-The main motive of this research is to enhance stability and permeation of drug by incorporation of drug into a vesicle. It is also emphasis to improve its shelf-life.

Method: - Blank proniosomes and drug loaded with proniosomes by using coaceravation phase separation method.

Result: - The Doxycycline proniosomal gel at bottom and elevated conc. was found out to be 4.9 % and 70.56 % respectively. But main in- vitro study which based on comparison of Doxycycline proniosomal gel with marketed formulation. In-vitro release study of Doxycycline proniosomal gel increased with respect to the time. The present study claims that test drugs proniosomes of Doxycycline was shown more effective than the marketed formulation data.

Conclusion:- According to this study the effectiveness of the Doxycycline proniosomal gel were more effective than marketed formulation because of the vesicular drug delivery system and having natural potential to drug absorption ability of hydrophilic drug through the membrane and it will be cost effective than other formulation.

Keyboards: - proniosomes, Doxycycline, FTIR, drug content

INTRODUCTION

Periodontitis is an inflammatory disease of oral cavity. A large number of populations has affected by this disease worldwide, approximately 46 % of the total population affected by National Health and Nutrition Examination Survey report (NHANES)**(Mph et al. 2015).** The bacteria damage oral cavity and further leads to tooth decay. The nature of the periodontal disease is episodic. Bacterial infections are the major reasons for periodontitis, symptoms inclu des such as swelling and demolition of the equipment apparatus and then leads to tooth decay. Periodontitis is an immuno-inflammatory

condition which participates with tissue, surrounds and holds teeth in oral cavity. Implemented causative agents are such as actinobacillus action my cetemcomitans, and treponema denticola. **(Page et al. 2000)** The favourable area for periodontitis is periodontal within the gums where growths of periodontopathic organisms take place where the daily routine cleansing is not possible. This leads to the demolition of elastic tissue holding teeth and clinical attachment **(Arunachalam et al. 2017) [1].** Major factor responsible for inflammation are impaired cytokine profile and smoking**. (Chapple et al)** Other factors include age, tobacco use, genetics **(Kinane et al. 2005)** stress, and medications, clenching or grinding teeth, undernutrition and fatness also play important role in developing periodontitis. Often seen the acute periodontitis (gingivitis) is converted to chronic periodontitis disease over the period of time and head sign is inflammation in gum pockets. Bleeding when brushing on tooth and flossing, sometimes metallic taste occurs in the mouth, bad breath (halitosis) and lose teeth, more room appears between the teeth. **(Joshi et al. 2014) [20].** Scaling root planning is the non-surgical treatment used for cleaning beneath the oral cavity for periodontitis **(**Hefti AF**)**. If planning of scaling root treatment was failed in the cure of sign and symptoms of periodontitis activity then go for towards the surgical approaches in periodontitis. A large number of drugs cured the periodontitis like antibiotics (doxycycline, minocycline, tetracycline **(Yadav et al.2015)** antiseptics (chlorhexidine gluconate), analgesics (paracetamol, nimesulide, ibuprofen, ketoprofen, diclofenac) and topical anesthetics such as lidocaine **(Da Rocha junior 2015) [21].** In the human body, due to premature drug inactivation as well as elimination from the systemic circulation. Various drugs do not gain or touch the therapeutic level. In the conventional system of drug administration, sufficient quantity of concentration into systemic has been manifested and show a positive impact in curing in periodontitis but the disadvantage is that drug gain the active site and show the potential side effects to remain of the body, the drug has to do diluted 1000 times, so the drug directly administering to the intended to active site with minimum dose and reduce the issue through the locally applied antimicrobial therapy. For an increased time of period with very accurate control over release, the drug activation and excretion in systemic circulation which provide the frequent dosing in sustained drug delivery system and also reduce the adverse events, thereby increasing patient compliance and comfort as well **(Ahmed et al.2014) [22].** The most favoured antibiotics for the cure of this disease were tetracycline and penicillin. Actinobacillus action my cetemcomitans bacteria are responsible for periodontitis disease because of its ability to invade periodontal tissue over the periods of time **(Slots j a, 1999) [23].** Doxycycline is obtained from oxytetracycline and it's a semi-synthetic derivative of tetracycline **(Arunachalam et al. 2017).** Doxycycline has bacteriostatic activity and excellent activity against both Gram-positive and Gram-negative bacteria act through discourage the aerobic and anaerobic bacteria protein synthesis. Tissue penetration power is good of doxycycline and to be highly effective in infections of the bacteria over the other antibiotics **(doxycycline Cunha ba 1981) [24].** Being lipid soluble, Doxycycline has many advantages compared to the tetracycline and minocycline **(rajpoot et al.2017)** It can act locally at the place of activity of the desired rate claimed by the patient situation and it can easily penetrate into body **(hong ruan 2016) [25].** Marketed formulation of doxycycline is ATRIDOX GEL and it's approved by food and drug administration. **(ATRIDOX. FDA).** There was a need to grow a nanovesicular dosage form of doxycycline to reduce the undesirable effects and increase patient's compliance and to provide consistent drug levels in the body for the prolonged periods of time. The medicament in the vesicle form is one of the promising techniques such as the encapsulation of medicament. Overcome the undesirable effects associated with the marketed formulation. Proniosome, it's one of the most optimistic techniques for enhancing bioavailability at the target site for prolonged periods of time

(kakar et at. 2009). Proniosome coated with the surfactant and it's a water-soluble carrier system which converted to niosome immediately by the support of water **(radha et al. 2010).** It is much uniform in size and very similar to conventional niosome. So proniosome may be locally acting drug delivery system for the cure of periodontitis. Proniosomes are unhydrated, free-flowing, produces a granular form product with the addition of aqueous solvent likewise water, to dissolve and produced a multiple lamellar niosomes, a form of suspension which was suitable for oral administration. Proniosomes has been proved to be for local delivery especially lipid-soluble compounds than the other vehicles such as niosomes, liposomes, transferosomes, and ethosomes. It has much more benefit by minimize the disadvantage of niosomal and liposomal drug delivery system and having the physical advantages as well such as the Drug targeting, Controlled release, Permeation enhancement of drugs, Low cost of formulation, Long shelf-life duration, Better attack by the drug at a specified organ follows the sustained manner, achieve enhancement of drug permeation. also, work on related to physical stability minimization issue such as the fusion of drug excipients, Leaking of API, Drug and all excipients aggregation Sedimentation on storage with additional advantages like its dry free-flowing form,Easy to transport, easily distribute in dosing, More stability during storage and sterilization. All in turn favor in Enhancing bioavailability and therapeutic efficacy **(Kaur et al. 2014**), Distribute, Easy to dosing, Easy to measuring and toxic with more stability during storage and free from the microorganism, Improve bioavailability,Therapeutic effect **(deepthi 2010) [26].**

Pre-formulation studies on drug and excipients

Solubility studies

The solubility of doxycycline in distilled water, oils (such as oleic acid, surfactants (span 80) and co-surfactant (Cholesterol, n-butanol) and inorganic medium (0.1 sodium hydroxide, 0.1 hydrochloric acids, petroleum spirit, etc) have been determined by U.V. visible spectrophotometer.

Procedure: Take 1 mg doxycycline and mix in 10 ml different solvents. Make a suitable dilution and filter out. Check its solubility by using UV visible spectrophotometer. **(Mokhtar et. al 2018)**

Measurement of drug melting point

Capillary fusion method use to check the melting point of doxycycline. In this process, take a small amount of drug in capillary tube and place it in melting point apparatus with a thermometer. Record the temperature where the drug crystal starts turning into liquid. Compare melting point with the literature value.

FTIR Characterization

Fourier-transform infrared spectroscopy is used to determine the drug-polymer interaction.

Procedure: 5.0 mg of Doxycycline (dried state) is mixed with 100 mg spectral grade of KBr. The mixture is pressed into the disc using hydraulic pressure. FTIR spectra of pellet were recorded in the range of 4000- 400 cm^{-1} range.

U.V. Spectroscopy of the drug

Procedure: Preparation of calibration curve of doxycycline was carried out using UV visible spectrophotometer with phosphate buffer at pH 6.8. 10 mg of doxycycline was diluted in 100 ml phosphate buffer at pH 6.8 to give a suitable concentration of 0.25-1 micro ml dilution. Doxycycline was analysed using 273 nm wavelengths in a UV visible spectrophotometer. Phosphate buffer (6.8) was used as a blank.

MATERIALS AND METHODS

Materials

Doxycycline was supplied as a generous gift sample by local Company (baddi, India). Span 80 (sorbitan monooleate) was purchased from Fischer Scientific (UP, India). Soya lecithin was received as a gift sample from and Fischer Scientific (UP, India). Oleic acid was purchased from Fischer Scientific (India). Cholesterol and nbutanol were purchased from Fischer Scientific (India), and Carbopol® 970P was received from Fischer Scientific (UP, India). Water was obtained by miet merrut. The available marketed product,atridox ® Gel (conventional formulation containing Doxycycline hcl), was procured from a local drug house.

Methods

Method of formulation development

Doxycycline and all excipient were weighing correctly and mixed in 0.5 ml butanol. Take a dry and wide mouth glass tube with tight closer to mix the solution. Next heat the glass tube for 5 mins at the temperature 65±3°C, then mixed phosphate buffer (aqueous phase 6.8) and heat on water bath for 2 minutes until a clear solution was observed. Now cool this mixture at the room temperature and after that store until the mixture converted into proniosome.

FTIR spectroscopy of proniosomes of Doxycycline

Procedure: Sufficient amount of proniosomal gel was mixed with 100 mg spectral grade of kbr and pressed into disc hydraulic pressure. FTIR spectra were recorded in the 4000-400 cm-1 range **(https://spectrabase.com/spectrum/46wtkZyLJIG)**

Percentage of Drug Entrapped

Drug content

First, thoroughly mix the formulation, then transfer 1 mL to a test tube and add 10 mL of phosphate buffer. Make an acceptable dilution. Using an instrument UV visible spectrophotometer, the assay was performed at 273 nm.

Straight line equation Y = 0.9728x-0.004

Correlation co-efficient r^2 = 0.9913

Encapsulation efficacy

Encapsulation efficacy is a term to describe the amount of the drug which is incorporated into the drug delivery system. It is generally express the percentage of drug bound to the drug

delivery system. For evaluating this parameter need to the separation of the free drug from the formulation. The encapsulation efficacy determined by this formula:

$$\text{Percent encapsulated} = \frac{[\text{Total drug}] - [\text{free drug}]^*}{[\text{Total drug}]} 100$$

Determination of particle size

In a small glass vial, taken 100 mg of doxycycline proniosome was hydrated in the 0.9 % NaCL solution (saline solution) with shaking for about 10 min and next to the observed under optical microscope. Usinga calibratedocular microscopy for measured the size of 50 vesicles and stagemicrometer as well. Vesiclesizeis calculated using this Equation:

$$\text{Size of each division} = \frac{\text{Number of divisions of stage micrometer}}{\text{Number of divisions of eye piece micrometer}} \times 10$$

Determination of the zeta potential

The potential of doxycycline proniosomes was measured by using malvern zeta potential.

Development of Doxycycline Proniosome Gel using carbopol 940 P

To formulate the doxycycline proniosomal gel, take the different concentration of carbopol 940 P and mixed in doxycycline proniosomes formulation.

pH determination

Take 1 ml of the doxycycline proniosomal gel from the all formulation samples. Gel disperse in 50 ml of distill water and record the pH using a digital pH meter **(Mohanty et al. 2018)**

Viscosity of doxycycline proniosome gel

The viscosity determination was performed by Brookfield viscometer (Brookfield R/S rheometer). Doxycycline proniosome gel was spun at 300 rpm for 20 seconds on a Brookfield viscometer plate. The readings were registered and an estimate was made. **(H.Priyanka et al. 2015).**

FTIR of Doxycycline proniosomal gel

Sufficient amount of doxycycline proniosomal gel was mixed with 100 mg spectral grade of kbr and pressed into disc hydrolic pressure. FTIR spectra were recorded in the 4000-400 cm^{-1} range.

In Vitro Release Studies using dialysis Membrane

Drug release from the proniosomal gel was evaluated by using dialysis method. First, take the dialysis membrane in distilled water on the water bath for 24 hours at the 30 oC for removing the preservatives. In vitro release of doxycycline proniosomal gel using Franz diffusion cell with phosphate buffer (pH 6.8) and adjust the temperature at 37oC with 50 rpm as well. After

complete, this process adds 2 ml of doxycycline proniosomal gel on the upper layer of the apparatus. At every hrs interval, samples were collected from apparatus and replaced by 3ml of the dissolution medium each time. Samples were assayed for drug content by U.V visible spectrophotometer at the 273 nm and repeated for each sample and readings were recorded.

Ex-Vivo Permeation and Retention Studies

Firstly arrange and collect the porcine buccal tissue for performing the Ex-Vivo Permeation and Retention Studies. To perform the permeation study, buccal tissue was chosen because this tissue has similarity to human buccal epithelium in terms of non-keratinized morphology. After killing the animal, buccal tissue was collected from a slaughterhouse and was stored in Krebs buffer (6.8) solution and used within 3 hours after killing of the animal. Mucosal membrane from the porcine buccal tissue was separated from the main connective tissue through the scalpel and surgical scissors.

Drug Permeation Studies

Take harvested buccal membrane from the solution and mount it between the compartments of Franz diffusion cell apparatus. Then it was placed in 5 ml of doxycycline proniosomal gel on the top part of donor compartment which was filled with phosphate buffer (pH 6.8). After that receptor or receiver compartment adjust the temperature at the 37±1°C and maintain stirred at 600 rpm with the help of a magnetic bar. For further analysis, take 1 ml of aliquots from receptor medium and samples will be withdrawn continuously from the medium at regular time interval such as 5,8,10,12,24,48,72 hrs respectively. After that, all samples were assayed by the U.V. spectrophotometer.

Comparison in-vitro release data from the literature value of marketed atridox formulation

The in-vitro release of doxycycline proniosomal gel compared (different concentration) with atridox formulation which was a marketed formulation. (Ranbaxy Laboratories Ltd) (Heba A. Gad et.al 2008)

Stability studies

The stability of the doxycycline proniosomal gel was evaluated by using a stability chamber. Ten batches were formulated and were kept in vials which covered with a close tight lid and stored at 2-8oC, room temperature, and 45oC for 3 months. The samples were evaluated for the change color of formulation and formulation pH. The formulated samples were visually verified at specific time interval such as over a one month period and observed several factors such as aggregation, flocculation, and loss of solvent as well.

RESULTS AND DISCUSSION

Solubility studies of Doxycycline

The solubility of Doxycycline was determined in distilled water and different oils including oleic acid, surfactants (span 80), co- surfactant (Cholesterol, n-butanol). And also solubility was determined in inorganic medium (0.1 sodium hydroxide, 0.1 hydrochloric acids, and petroleum spirit) by using U.V visible spectrophotometer. Doxycycline shows the maximum solubility. slightly soluble in n-butane and diluted hydrochloric acid. Sparingly soluble in diluted sodium

hydroxide, practically insoluble in petroleum spirit. Following that, a comparison of Doxycycline solubility to the literature value of the Doxycycline shows the evidence of Doxycycline.

Melting point of drug

Melting point of Doxycycline as compared to the value found in the literature. The result shows evidence of Doxycycline. Melting point was 210 °C.

FTIR of drug

FTIR spectral analysis was shown that it contains a wide variety of carbon-carbon bond and methylene with carbon-fluorine. Some aromatic and aliphatic ring based substances were found

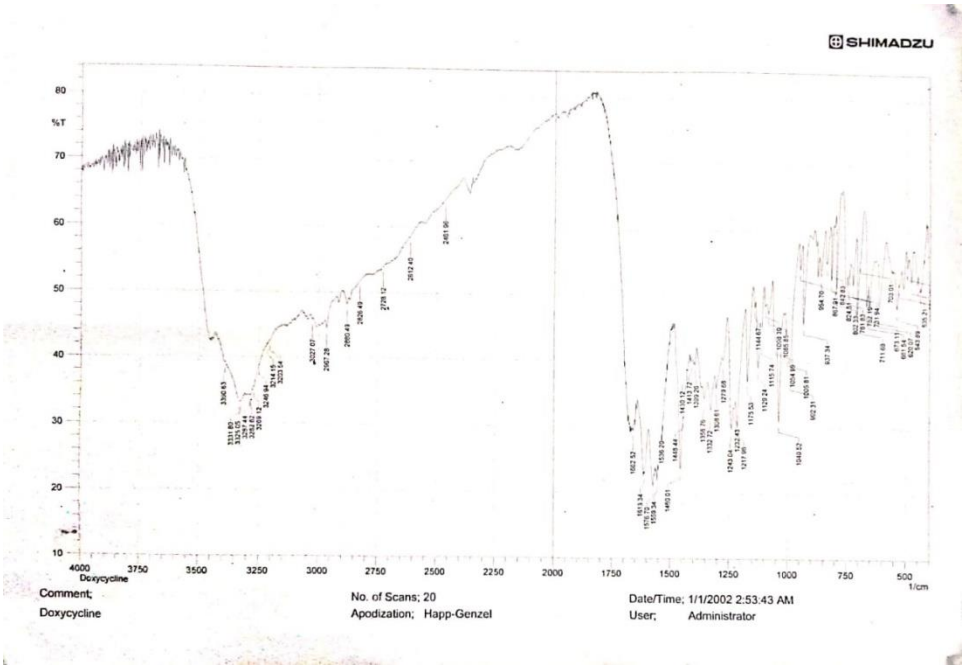

Figure 1- FTIR Spectra of the Doxycycline

U.V. Spectroscopy of the drug

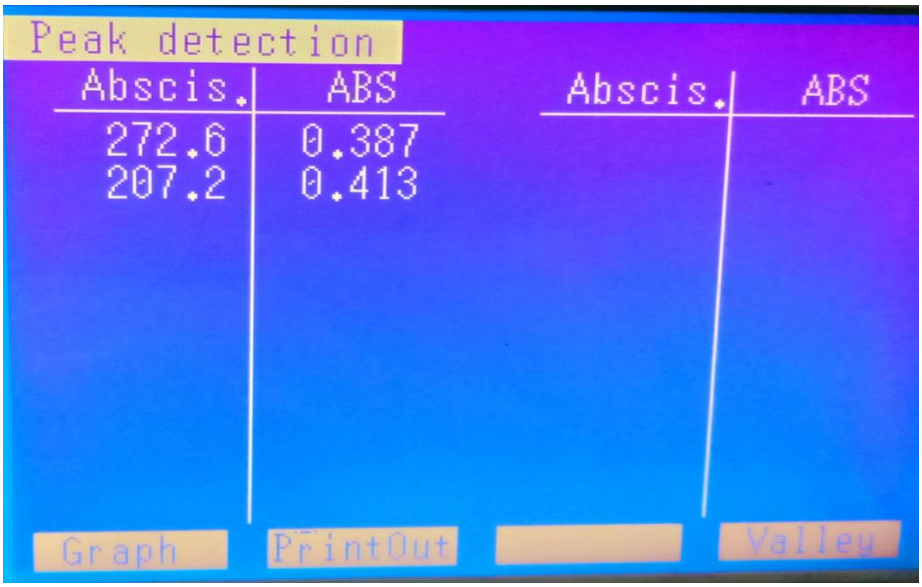

Figure 2- U.V. Spectroscopy of the Doxycycline

The U.V. Peak was 273nm and according to our spectra, it falls down the peak 272.6 which indicating the confirmation of the doxycycline.

Preparation of doxycycline proniosomes:-

First, doxycycline drug and all excipient were weighted correctly and then mixed in 0.5 ml butanol. Take a dry and wide mouth glass tube with tight closer to mix the solution. Next, heat the test tube for 5 mins at temperature 65±3°C, mix phosphate buffer (aqueous phase 6.8) and re on water bath for 2 minutes until a clear solution observe. Afterwards allow to cool this mixture at the room temperature before storing until the mixture converted into proniosomes.

Drug content

First, thoroughly mix the formulation, then took 1 ml in a test tube and makeup with 10 ml of phosphate buffer and Make suitable dilution. The assay was performed at the 273 nm by using an instrument UV visible spectrophotometer.

Straight line equation Y = 0.9728x-0.004

Correlation co-efficient r^2 = 0.9913

S. No.	Formulation Type	Drug conc.	Surfactant Type	surfactant (ml) %	Soya Lecithin (mg) %	Cholesterol (mg) %	Oleic acid (ml) %	Butanal (ml)	Water (ml)	Total amount (%)

1	DPG 1	50 mg	Span 40	64.5 %	35 %	10 %	8 %	0.5	0.5	118.5 %
2	DPG 2	50 mg	Span 40	30%	24 %	5 %	2 %	0.5	0.5	62 %
3	DPG 3	50 mg	Span 40	25 %	25 %	6 %	2 %	0.5	0.5	59 %
4	DPG 4	50 mg	Span 40	20 %	28 %	4 %	2 %	0.5	0.5	55 %
5	DPG 5	50 mg	Span 40	15 %	28 %	5 %	7 %	0.5	0.5	56 %
6	DPG 6	50 mg	Span 40	17 %	27 %	4 %	2 %	0.5	0.5	51 %
7	DPG 7	50 mg	Span 40	40 %	27 %	8 %	2 %	0.5	0.5	78 %
8	DPG 8	50 mg	Span 40	60 %	31 %	9 %	5 %	0.5	0.5	106 %
9	DPG 9	50 mg	Span 40	56 %	27 %	8 %	4 %	0.5	0.5	96 %
10	DPG10	50 mg	Span 40	52 %	27 %	5%	6 %	0.5	0.5	91 %

Table: 1. various concentration of the content excipients

U.V visible spectroscopy was used to determine the drug content. According to estimates, the drug content was found to be in the range of 2.18-2.87.

Entrapment efficiency of Doxycycline

Entrapment efficiency was measured by the help of using U.V. visible spectroscopy. The EE% of Doxycycline pronio somes were found in the range of 99.82- 99.96%. 99.96 % was higher EE% then the EE% of all formulations.

Malvern Zetasizer and zeta potential

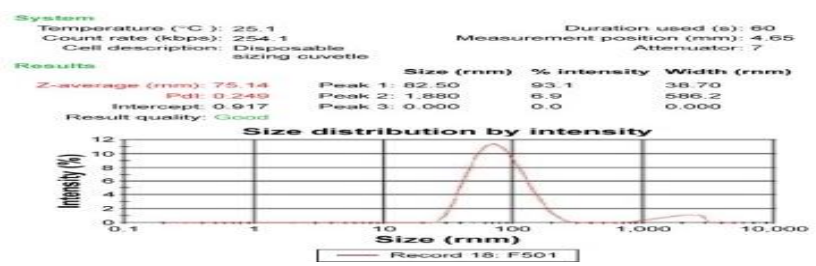

Figure: 3. Zetasizer ranges of Doxycycline proniosomes

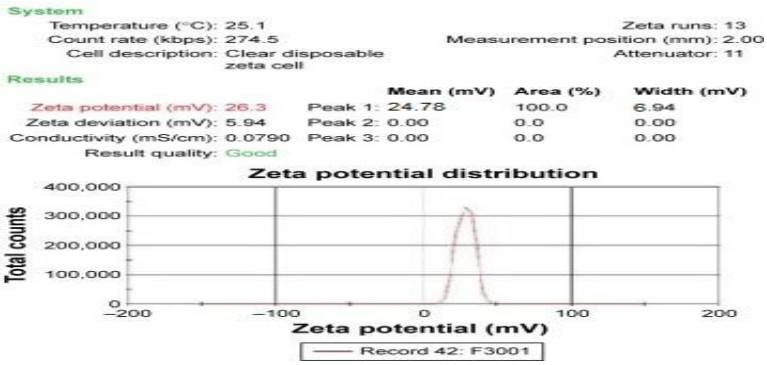

Figure: 4. Malvern Zeta Potential ranges of doxyclcline proniosomes

Doxycycline proniosomes sample were found in range 150-225 um of some droplets but big proniosome were available in nano range measured by Malvern Zetasizer. The first region has a radius 82.50 nm and a width of 38.70 nm with a maximum range of 93.1 % pronisome and According to the Malvern Zeta Potential, samples was mean potential was 26.3mV and with the width of 6.94mV. First peak mean was 24.78.

In Vitro Release Studies using dialysis Membrane

Time (hrs)	DPG 1 (mg %)	Cumulative amount of drug	DPG 2 (mg %)	Cumulative amount of drug	DPG 3 (mg %)	Cumulative amount of drug
5	4.9	4.9	4.12	4.12	4.12	4.12
8	7.8	12.7	7.21	11.33	10.11	14.23
10	9.7	22.4	8.77	20.1	14.19	28.32
12	11.22	33.62	12.31	32.41	16.91	45.23
24	23.52	57.14	21.66	54.07	33.49	78.72
48	47.12	104.26	45.55	99.59	45	123.72
72	70.56	174.82	69.85	169.41	55.09	178.76

Table: 2. In Vitro Release Studies by using dialysis Membrane

Comparison between doxycycline proniosomal gels (different concentration) with atridox

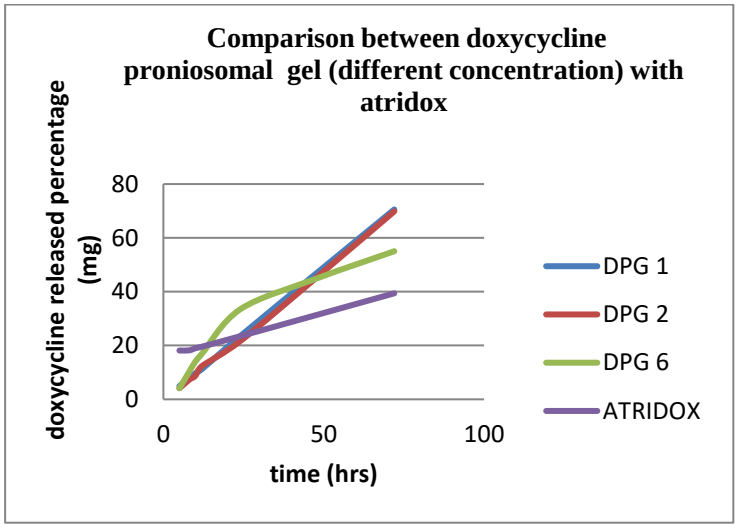

Figure: 5. Comparison between doxycycline proniosomal gels (different concentration) with atridox

Stability study of doxycycline proniosomal gel

Parameters	Changes during storage								
	Within 15 days			Within 1 month			Within 3 month		
	2-8°C	RT	45°C	2-8°C	RT	45°C	2-8°C	RT	45°C
Drug remaining	100%	100%	100%	100%	100%	100%	100%	100%	100%
pH	7	7	7	7	7	7	7	7	7
observation	Stable , no change								

Table: 3. Stability study of doxycycline proniosomal gel

According to the stability study of the formulation, the result shows that there was no improvement in the drug content, pH and coloration over the course of 3 months accelerated stability study.

Conclusion: In the present studies preniosomal gel formulations of Doxycycline were prepared and evaluated for in vitro parameters like drug release, drug loading, and particle size and stability studies. Doxycycline gel loaded proniosomes was found to be more effective then marketed product Atridox. (Ranbaxy Laboratories Ltd). Permeation of hydrophilic drug was more pronounced when formulated in preniosomal form made from cholesterol, soya lecithin. Proniosomes gel further enhances encapsulation efficiency of doxycycline and delayed its release. In this fashion, it may decrease dosing frequency. Present study also demonstrated that doxycycline proniosomal gel minimized its physical stability. It has been concluded that the doxycycline proniosomal gel was more effective and more successful than the marketed product atridox.

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