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Preparation and Evaluation of Extended Release Ocular Inserts of Rebamipide for Local Effect Using Casting Technique

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Abstract

The aim of this study is to prepare and evaluate an extended release rebamipide ocular insert using hydroxypropyl methylcellulose as the main components of this drug delivery system for dry eye syndrome management, to have the advantage of both rebamipide inducing mucin secretion and hydroxypropyl methylcellulose lubricating properties. Solvent casting technique was used to prepare the inserts; the amino acid L-arginine was used to solubilize the drug, hydroxypropyl methylcellulose grades (HPMC E5 and K15M) and poly ethylene glycol 200 were used as excipients. The inserts were evaluated for their physical and mechanical properties, moisture loss% and absorption %, surface pH, and in-vitro drug release. The use of L-arginine exhibited an enhancement of rebamipide solubility in both deionized water and phosphate buffer (pH 7.4) by approximately 274 and 2.8 folds, respectively. The formulae showed uniform weight, thickness and drug content except for formula (F1) that composed mostly of HPMC E5: HPMC K15M at ratio of 1: 0.17 and had no plasticizer (poly ethylene glycol 200) in its composition; it showed haziness in its appearance and brittleness of the insert. While formula (F3) which contained mainly HPMC E5: HPMC K15M at ratio of 1: 0.4 with poly ethylene glycol 200 showed good physical and mechanical properties thus was selected for in vitro release and was compared to the marketed brand Mucosta® ophthalmic suspension unit dose 2%w/v; F3 showed significant larger T50% and T80% (p-values 0.034 and 0.015) compared to those of the reference marketed brand and similarity factor value was (f_2 = 37.27). The results of this study showed that rebamipide ocular inserts have good potential for futuristic rebamipide extended release ocular delivery system.

Keywords: Rebamipide, L-arginine, Hydroxypropyl methylcellulose, Ocular insert, Casting technique

تحضير و تقييم لاصق عيني لاطالة تحرير الريباميبايد للتأثير الموضعي باستخدام تقنية الصب

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الخلاصة

ان الهدف من هذه الدراسة هو تحضير وتقييم لاصق عيني لاطالة تحرير الريباميبايد باستخدام هايدروكسي بروبيل مثيل سولولوز كمكونات رئيسية في نظام توصيل دوائي لعلاج متلازمة العين الجافة ، وذلك للاستفادة من كل من خواص الريباميبايد الذي يحفز إفراز الميوسين وخواص الهايدروكسي بروبيل مثيل سولولوز المرطبة. استخدمت تقنية الصب باستخدام المذيب لتحضير اللواصق العينية ؛ و تم استخدام الحمض الأميني ل-أرجينين لإذابة الدواء. كما تم استخدام الهايدروكسي بروبيل مثيل سولولوز (E5 و K15M) والبولي إيثيلين جلايكول ٢٠٠ كسواغ. تم تقييم خصائص اللواصق الفيزيائية والميكانيكية ، ونسبة فقدان وامتصاص الرطوبة ، و الاس الهيدروجيني لسطح اللاصق ، وتحرير الدواء في المختبر. أظهر استخدام ل-أرجينين زيادة في ذوبانية الريباميبايد في كل من الماء منزوع الأيونات ومحلول الفوسفات المنظم(الرقم الهيدروجيني ٧,٤) بنحو ٢٧٤ و ٢,٨ مرة ، على التوالي. أظهرت الصيغ وزنًا وسُمكًا ومحتوىً متجانسًا باستثناء الصيغة (F1) التي تتكون في الغالب من الهايدروكسي بروبيل مثيل سولولوز (E5: K15M) بنسبة (١:٠,١٧) ولا تحتوي على ملدن (بولي إيثيلين جلايكول ٢٠٠) في تركيبها ؛ حيث أظهرت ضبابية في مظهرها و هشاشة في اللواصق . في حين أن الصيغة (F3) التي تحتوي بشكل أساسي على الهايدروكسي بروبيل مثيل سولولوز (E5: K15M) بنسبة (١:٠,٤) مع بولي إيثيلين جلايكول ٢٠٠ أظهرت خصائص فيزيائية وميكانيكية جيدة وبالتالي تم اختيارها لاختبار تحرير الدواء في المختبر وتمت مقارنتها بالعلامة التجارية ميوكوستا المعلق العيني ٢٪ وزن/حجم. أظهر F3 نسبة أكبر من T50٪ و T80٪ (القيمة الاحتمالية ٠,٠٣٤ و ٠,٠١٥) مقارنة بتلك الخاصة بالعلامة التجارية المسوقة وقيمة عامل التشابه (f2 =٣٧,٢٧). وأظهرت نتائج هذه الدراسة أن اللاصق العيني للريباميبايد لديه امكانات مستقبلية جيدة كنظام توصيل دوائي للعين يعمل على اطالة تحرير الدواء.

الكلمات المفتاحية: ريباميبايد, ل-ارجنين, هايدروكسي بروبيل مثيل سولولوز, اللاصق العيني, تقنية الصب .

Introduction

Dry eye syndrome is a multifactorial disease that affects the tears and ocular surface, and lead to various symptoms as visual disturbance, discomfort, gritty feeling and burning sensation; it may also cause tear film instability and possible defects of ocular surface and inflammation. [1] This disease may occur due to the decreased ability of lacrimal functional unit (lacrimal glands, ocular surface, and lacrimal drainage pathways) to produce sufficient amount of tear film components which results in a poor quality or/and low quantity of tear film [2,3,4,1] . Dry eye management includes patient's education, dietary and environmental changes, artificial tears, punctal plugs, topical and systemic medications; in addition to surgical intervention which can be a possible option. The choice of a suitable management is based on the etiology and severity of the syndrome [4,1,5] .

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Rebamipide is a quinolone compound, its molecular formula is ($C_{19}H_{15}ClN_2O_4$) and it has a molecular weight (370.79 g/mole), the structure is illustrated in figure (1). It is classified as class IV according to the biopharmaceutical classification system (BCS).[6] Rebamipide acts pharmacologically by increasing mucus production and secretion, improving wound healing, and it has exhibited cytoprotective and anti-inflammatory properties. [1,7] The compound was first introduced, in 1990, for gastric ulcer treatment; then investigating rebamipide ability to induce mucin secretion from ocular cells, has led to its approval for dry eye treatment in 2011 and it was marketed, in 2012, as (Mucosta® ophthalmic suspension unit dose 2%) in Japan. [8,9]

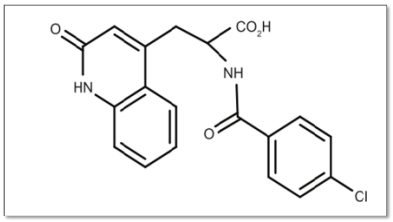

Figure (1) Chemical structure of rebamipide [9]

Ocular inserts are solid or semisolid sterile preparations that can be prepared with various shapes and sizes and should be suitable for ocular application; the inserts are usually placed into the cul-de-sac and less often in the upper fornix or on the cornea. The most important advantage is to increase the dosage form's contact time with the conjunctiva and it varies from few hours to days; when compared to solution which has contact time no longer than fifteen minutes [10], the inserts showed a great enhancement in residence time. [11,12]

Artificial tears and lubricants are the first-line management for dry eye syndrome because they are obtainable, non-invasive, and have minimum side-effects; they usually contain hydrophilic polymers in their composition. [4] Lubricating inserts were developed to substitute the artificial tear drops in order to overcome the frequent dosing problems of conventional eye drop. These inserts are generally composed of water soluble polymers as cellulose derivatives (for example hyroxypropyl cellulose and hydroxylpropyl methyl cellulose) and may also contain plasticizers. The plasticizers used in these inserts should be soluble in the lacrimal fluid as polyethylene glycol, glycerin, and propylene glycol; they may exist in the ocular inserts in a range from (1-40% by weight). [13]

The objective of this study is to prepare and evaluate an extended release rebamipide ocular insert using hydroxypropyl methylcellulose as the main components of this drug delivery system for dry eye syndrome management, to have the advantage of both rebamipide inducing mucin secretion and hydroxypropyl methylcellulose lubricating properties. Solvent casting technique was used to prepare the ocular inserts.

Materials and Methods

Materials

Rebamipide and hydroxypropyl methyl cellulose (HPMC) K15M were purchased from Shijiazhuang Aopharm medical technology Co. Ltd. (China). HPMC E5 and potassium dihydrogen phosphate monobasic (KH_2PO_4) were obtained from HiMedia laboratories Pvt. Ltd. (India). L-arginine was obtained from central drug house Ltd. (India). Polyethylene glycol (PEG 200) was supplied by Sinopharm chemical reagent Co. Ltd. (China). Sodium hydroxide was provided by Techno pharmchem (India). Potassium chloride was supplied by Merck group (Germany). Absolute ethanol was obtained from PanReac chemicals (Spain). Methanol was provided by Thomas Baker chemicals (India). Dichloromethane was supplied by Gainland chemical company (UK).

Methods

Characterization of rebamipide

Determination of melting point

Rebamipide melting point of was determined by using differential scanning calorimetry (DSC), a sample of three mg was heated from 25ºC to 350ºC at a rate of 10ºC/min in a sealed aluminum pan. The peak shown in the thermogram represents the drug's melting point.

Determination of λ_{max}

Stock solutions of rebamipide with a concentration of (0.2mg/ml) were prepared by dissolving 20 mg of the drug in up to 100 ml of phosphate buffer (pH 7.4), methanol and ethanol respectively. From these stock solutions, diluted solutions with concentrations of (0.009 mg/ml) for rebamipide in phosphate buffer and (0.008mg/ml) for rebamipide in both ethanol and methanol were prepared, and then UV spectrophotometer was used to determine rebamipide λ_{max} at 200-400 nm. [14]

Construction of calibration curve

Rebamipide calibration curve was constructed in three vehicles phosphate buffer (pH 7.4), methanol and ethanol by using diluted solutions of the drug with different concentrations (1, 2, 3, 4, 5, 6, 7, 8 and 9 µg/ml) prepared from a (0.2 mg/ml) stock solution. Samples were analyzed using UV-spectrophotometer at rebamipide λ_{max}. The recorded absorbance of each sample was plotted against its respective concentration.

Fourier transform infrared spectroscopy (FTIR)

Rebamipide FTIR spectrum was obtained by using pressed-disk technique. A small amount of drug was ground with KBr powder then the mixture was pressed into a disk. The FTIR spectroscopy was used to investigate the prepared disk at wave lengths range of 4000-400 cm^{-1} [15] .

Determination of rebamipide solubility

The solubility of rebamipide was determined in different solvents (deionized water, methanol, ethanol, and phosphate buffer (pH 7.4)). An excess amount of the drug was dissolved in 10 ml of solvent and shaken for 72 h at room temperature. The mixtures were centrifuged at 4000 rpm for ten minutes and then filtered with filter syringe (0.45 µm).[16,17] The filtrate was analyzed using UV-visible spectrophotometer at rebamipide λ_{max}, and then the concentration of rebamipide saturated solution was calculated.[18]

The solubilization effect of amino acid on rebamipide was determined by adding an equimolar amount of L-arginine in both deionized water and phosphate buffer (pH 7.4); the equimolar ratio of rebamipide to L-arginine is (1: 0.47).

Preparation of rebamipide inserts

Calculations of rebamipide insert size

Each insert has a surface area of 40 mm^2 that contains 2mg of rebamipide. The surface area of the inserts and their number in each batch was calculated by dividing the Petri-dish surface area over the insert's surface area as follow:

Surface area of petri-dish = 56 cm^2

Surface area of the rectangular insert = 5×8= 40 mm^2 =0.4 cm^2

Number of inserts/ batch = 56/0.4 = 140 insert

Total amount of rebamipide per each batch = 140 × 2= 280mg

Total amount of L-arginine per each batch = 140 × 0.95= 133 mg

Preparation of rebamipide solution for casting

To prepare homogenous liquid of the drug for casting, different solvent were used include deionized water, methanol, and ethanol. Also, trial to prepare rebamipide solution using equimolar quantity of amino acid (L-arginine) in water was studied.

Rebamipide was solubilized by using an equimolar amount of the amino acid L-arginine. A calculated amount of L-arginine (133 mg per batch) was dissolved in 5 ml of deionized water, and then an equimolar amount of rebamipide (280 mg per batch) was added with continuous stirring using a magnetic stirrer for five minutes.

Formulation of rebamipide inserts

Four formulas were prepared using solvent casting technique. They were simply prepared by mixing the components to form a matrix film, table (1). The polymer HPMC with two grades (E5 and K15M) were prepared as aqueous solutions with the concentrations (10%, and 2% w/v) respectively.

Homogenous polymer mixture was measured with a specified weight for each formula as stated in table (1); it was poured into the rebamipide/L-arginine solution, then PEG200 (35% of polymer weight) was added as a plasticizer with continuous stirring by magnetic stirrer for two hours, then the mixture was set aside for twelve hours to ensure air bubbles removal. The resultant solution was casted into a flat surface polystyrene petri-dish and left to dry at room temperature until complete solvent evaporation. [13, 19, 20] A dry film was obtained and cut into rectangular inserts (5 mm x 8 mm), packed in aluminum foil and stored at room temperature.

Table(1) Composition of formulas of matrix rebamipide ocular inserts

Formula	Rebamipide (mg)	L-arginine (mg)	HPMC E5 (mg)	HPMC K15M (mg)	PEG 200 (%)
F1	2	0.95	12	2	0
F2	2	0.95	12	2	35% w/w of polymer weight
F3	2	0.95	10	4	
F4	2	0.95	14	0	

Evaluation of rebamipide ocular inserts:

Physical appearance

Physical appearance of the insert's color, transparency and texture was observed and inspected visually [21] .

Weight uniformity

Five inserts were selected randomly from each batch (5mm x 8mm), they were weighed individually using a digital balance; the mean weight and standard deviation were calculated.[20] Batches with a variation of more than ±5% were rejected.[22]

Thickness measurements

Five inserts were selected randomly from each batch; the thickness of each insert was measured at five positions using a digital vernier caliper.[23] The mean thickness and standard deviation were calculated. Batches with a variation of more than ±5% were rejected.[22]

Content uniformity

Ten inserts from each batch were selected randomly. Each insert was dissolved in 50 ml of phosphate buffer (pH 7.4), and then the solution was filtered through 0.45µ filter syringe. One milliliter of the filtrate was diluted up to ten milliliters; the drug content was measured by analyzing the diluted solution with UV-visible spectrophotometer.[24,25] The mean and standard deviation were calculated. The dosage uniformity meets the requirements stated in the USP when the first ten units have an acceptance value less than or equal to 15% [26] .

Folding endurance

Folding endurance is done to evaluate the flexibility of the inserts; it is the number of folds that can be achieved without breaking the insert, when repeated folding at the same position is done. Folding endurance of three inserts of (5 × 16 mm) of each formulation was carried out; the mean value and the standard deviation were recorded.[24,27]

Moisture loss%

Moisture loss% was determined by placing pre-weighed inserts in a desiccator containing silica beads for 72 hr. at room temperature, then the inserts were weighed again and the mean moisture loss% was calculated using the equation[27]:

$$Moisture\ loss\% = \frac{Initial\ weight - final\ weight}{Initial\ weight} * 100$$

Moisture absorption%

Three inserts of the selected formulas were weighed and placed in a desiccator that contains a saturated solution of potassium chloride, that maintains relative humidity of about 84%[28], for 72 hr. at room temperature; then the inserts were reweighed and the moisture absorption% was calculated using the equation[27]:

$$Moisture\ absorption\% = \frac{Final\ weight - initial\ weight}{Initial\ weight} * 100$$

Surface pH

Surface pH of the inserts was determined by allowing the insert to swell for thirty minutes with 5 ml of de-ionized water in a glass vial, and then the electrode of a pH-meter was placed as close as possible to the insert surface and the pH was recorded. The process was done in triplicate.[19]

In-vitro drug release study

This study was performed using a modified diffusion cell shown in figure (2), which contains a receptor and a donor chambers; the receptor chamber was filled with 65 ml of phosphate buffer (pH 7.4) as the dissolution medium which was maintained at 34 ± 1º C [19,29], and the medium was stirred at 50 rpm using a magnetic stirrer.

The insert of the selected formula was placed on a dialysis tubing membrane (MW 12000- 14000 Dalton) which was mounted between the two chambers. A sample with a 2 ml volume was withdrawn with a syringe at specified time intervals (0.25, 0.5, 1, 2, 3, 4, 5, 6,7,8,9,10, 11 and 12 hrs.) which was replaced by a fresh medium to preserve the sink conditions. The sample was filtered by a filter syringe (0.45 µm), and then the filtrate was diluted and analyzed using UV- visible spectrophotometer at rebamipide λ_{max}. The cumulative drug release % of rebamipide was calculated.[30]

Four drops of Mucosta® ophthalmic suspension 2%w/v were used as the reference brand; these four drops are equivalent to 2 mg, the amount of the rebamipide in the ocular insert.

T50% and T80% (hr.) that denote for the time required for 50% and 80% of the drug to be released, respectively, were used to compare the rebamipide release from the selected formula with its release from marketed Mucosta® ophthalmic suspension unit dose 2%w/v, and these parameters were tested statistically using two-tailed Student's t-test; similarity factor (f_2) was also used to compare the drug release profiles; when two curves are identical ($f_2 = 100$), the FDA considers two release curves to be similar when $f_2 \geq 50$. This factor is calculated by the equation: [31]

$$f_2 = 50 \times log\left\{\left[1 + \frac{1}{n}\sum_{t=i}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

Where n is the number of time points, R_t and T_t are the drug% released values at time t from the two curves being compared.

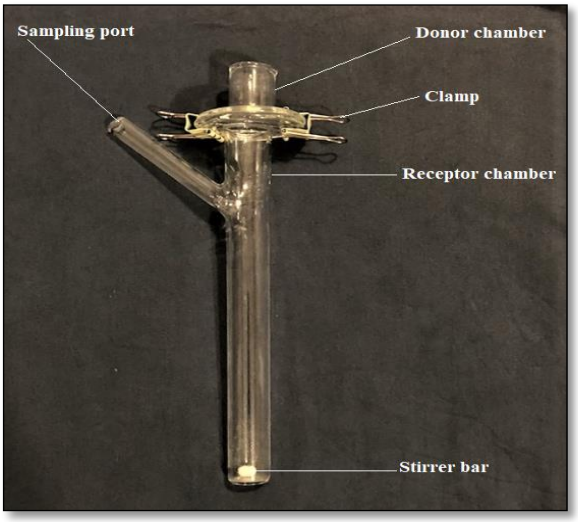

Figure (2) Modified diffusion cell used in the in-vitro drug release study

Compatibility study

This study was done to identify any possible interaction between rebamipide and the polymers used in the inserts preparation.

Fourier transform infrared spectroscopy (FTIR)

In addition to the FTIR spectrum of the pure rebamipide, the spectra of the pure polymer HPMC E5 and the prepared medicated insert of formula F3 were attained to detect the compatibility of the drug within the formulation. The procedure discussed previously in characterization of rebamipide section.

Differential scanning calorimetry (DSC)

To confirm the compatibility of rebamipide with the polymers, the DSC of the polymer HPMC E5, HPMC K15M and the prepared medicated insert F3 were studied according to procedure mentioned previously in the determination of melting point section, along with the DSC of the pure rebamipide.

Statistical analysis

The experimental data were conducted in triplicates unless otherwise stated, and expressed as means and their standard deviations (mean± SD). One-way analysis of variance (ANOVA) and two tailed Student's t-test were used to analyze the results of the study and asses the statistically significant differences in comparing means; p-values considered: significant at a level of ($P \leq 0.05$) and not significant at a level of ($p > 0.05$).

Results and Discussion

Characterization of rebamipide

Determination of melting point

DSC graph showed a peak at 307ºC figure (3), which is close to the values stated in the references. [16,17]

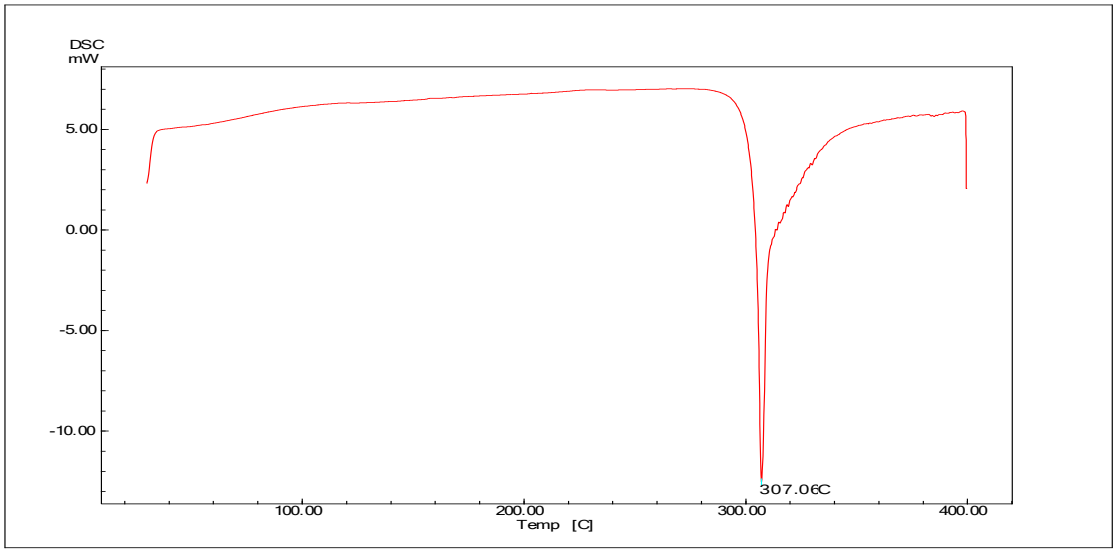

Figure (3) DSC thermogram of pure rebamipide powder

Determination of λ max

The diluted solution of (0.009 mg/ml) rebamipide in phosphate buffer (pH 7.4) and (0.008mg/ml) rebamipide in methanol and ethanol were scanned by UV-visible spectrophotometer at 200- 400 nm. The obtained spectrum of rebamipide in phosphate buffer (pH 7.4) had a λ_{max} at 227nm and in agreement with the value reported in reference.[14] while the spectra of both rebamipide in methanol and ethanol had the same λ_{max} at 229nm.

Construction of calibration curve

The calibration curve of rebamipide was constructed by plotting the absorbance of the diluted solutions of rebamipide in phosphate buffer (pH 7.4), methanol and ethanol against its respective concentration as shown in figure (4), figure (5) and figure (6), respectively. Straight lines with high coefficients of determination, (R2 =0.999), (R2 =0.9955) and (R2 =0.9948) respectively, were obtained; those indicate that the calibration curves of rebamipide obey Beer's law.

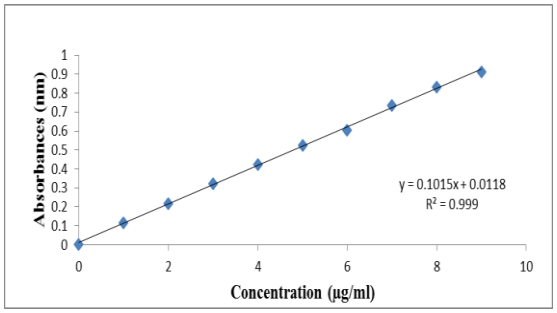

Figure (4) Calibration curve of rebamipide in phosphate buffer (pH 7.4)

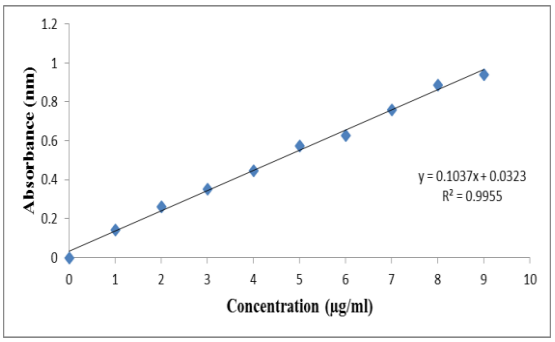

Figure (5) Calibration curve of rebamipide in methanol

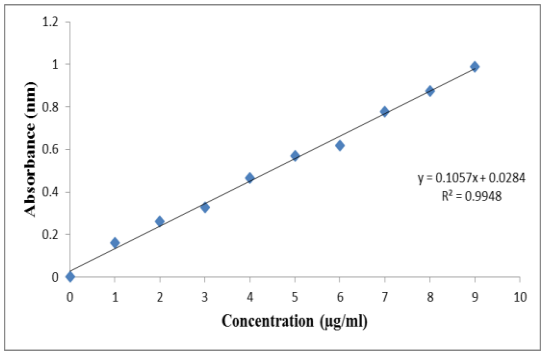

Figure (6) Calibration curve of rebamipide in ethanol

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of pure rebamipide figure (7) was compared to the reference FTIR spectrum of rebamipide reported in Japanese Pharmacopoeia.[7] The characteristic peaks of FTIR spectrum of pure rebamipide are illustrated in figure (7), at wave numbers (in cm^{-1}): 685 for out of plane N-H wagging of amide (800-666), 758 for out of plane N-H wagging of lactam (800-700), 872, 843, and 832 for out of plane C-H bending bands of aromatic ring (900-675), 1235, 1183, and 1092 for in-plane C-H bending of aromatic ring (1300-1000), 1273 for C-O stretching of carboxylic acid (1320-1210), 1420 for C-C stretching of aromatic ring (1500- 1400), 1539 for N-H bending of amide band II (1650-1515), 1642 for C=O stretching of amide band I (1680-1630), 1725 for C=O stretching of carboxylic acid (1730-1700), 3060 for C-H stretching of aromatic ring (3100-3000), 3088 for O-H stretching of carboxylic acid (3300-2500), 3271 for N-H stretching of amide (3300-3060). [15] These interpretations indicated the drug purity.

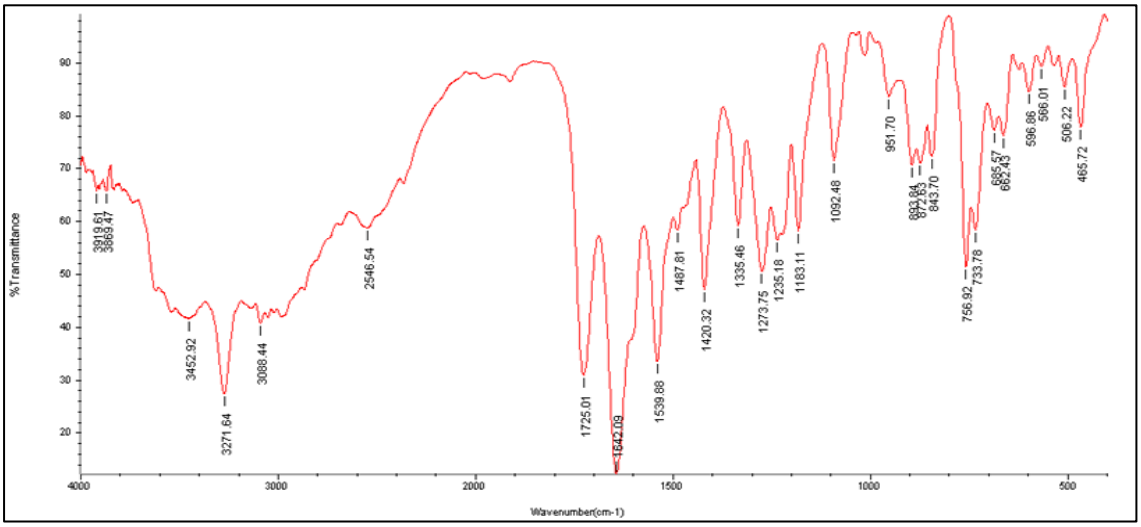

Figure (7) FTIR spectrum of pure rebamipide powder.

Determination of rebamipide solubility

The solubility of rebamipide was determined in different solvents at room temperature as shown in table (2).

Table (2) Solubility of rebamipide in several solvents

Solvent	Solubility (mg/ml)
Deionized water	0.045± 0.011
Methanol	1.41± 0.076
Ethanol	0.83± 0.038
Phosphate buffer (pH 7.4)	3.88± 0.25

These results confirmed the poor solubility of rebamipide in both aqueous and organic solvents and they are in agreement with the references. [7, 17, 16] The poor solubility can be explained by the high lipophilic nature of the drug. [32] Rebamipide solubility in phosphate buffer (pH 7.4) was slightly higher than its solubility in deionized water and the other organic solvents used; this result can be explained by acidic properties of the drug (pKa = 3.38), thus its solubility is pH dependent. [32]

Finding a way to solubilize the drug is essential in this study in order to form a homogenous solution suitable for casting, and the solvent should be highly evaporative to obtain a dry film with minimal residual solvent, thus from the results and the literature survey it was concluded that it is not possible to solubilize the drug with solvent or solvent system.

After the failure of the trials to solubilize the rebamipide with the solvents, thus enhancing rebamipide solubility by using amino acid was considered and an excess of equimolar amounts of rebamipide and L-arginine (1:0.47) were added in both deionized water and phosphate buffer (pH 7.4) as shown in table (3). [33, 34]

Table (3) Solubility of rebamipide in presence of L-arginine

Solvent	Solubility (mg/ml)
Deionized water	12.33 ± 1.136
Phosphate buffer (pH 7.4)	11.01 ± 1.911

The addition of L-arginine enhanced the solubility of rebamipide in both deionized water and phosphate buffer (pH 7.4) by approximately 274 and 2.8 folds respectively. This can be explained by the formation of rebamipide arginine salt.[34]

Preparation of rebamipide inserts

Preparation of rebamipide solution for casting

In solvent casting technique, the drug is preferred to be in solution to ensure its even distribution throughout the casted film, and since the drug is intended for ophthalmic use then it should have a particle size less than ten µm, to be an acceptable ophthalmic preparations and avoid irritation of the eye.[35] Rebamipide's poor solubility in many solvents, makes it difficult to formulate; thus L-arginine was added to solubilize the drug and to prepare a homogenous solution as discussed in previous section.

Formulation of rebamipide inserts

HPMC E5 was used in all four formulae as a film forming polymer (primary polymer), while HPMC K15M which is a higher molecular weight grade of HPMC was used in (F1-F3) as a secondary polymer in order to optimize the rebamipide ocular inserts properties and retard the drug release, PEG 200 was used as a plasticizer in (F2-F4). The inserts dimensions are shown in figure (8).

Figure (8) Photograph showing the rebamipide ocular insert's dimensions in millimeters (5mm x 8mm)

Evaluation of rebamipide ocular inserts

Physical appearance

The physical appearances of the inserts are shown in figure (9), while the detailed texture, transparency and color of each formulation are stated in table (4). Formula F1 had a hazy appearance and a rough texture compared to the other formulas, which points out the major effect of the plasticizer in the inserts appearance.

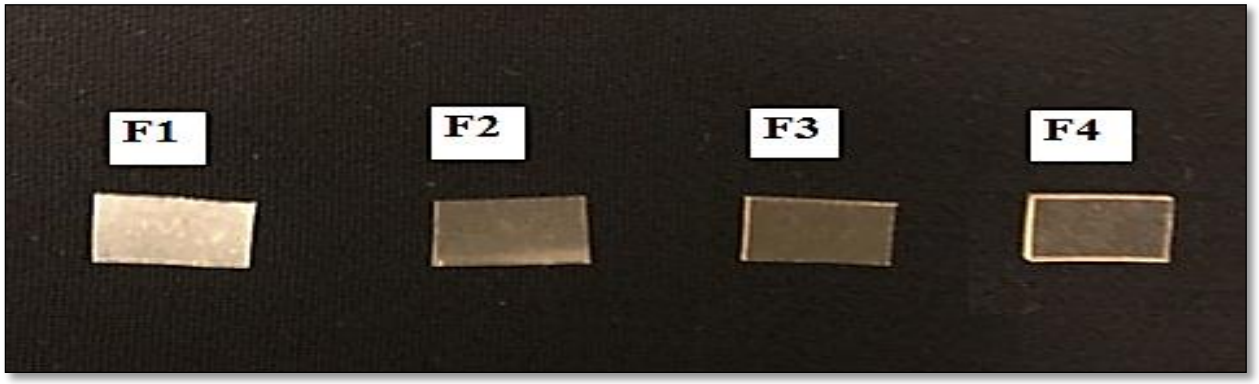

Figure (9) Photograph showing the physical appearance of the prepared rebamipide ocular inserts

Table (4) Physical appearance description of rebamipide inserts

Formula	Texture	Transparency	color
F1	Rough	Hazy	Colorless
F2	Smooth	Clear	Colorless
F3	Smooth	Clear	Colorless
F4	Smooth	Clear	Colorless

All the inserts of formulae (F2-F4) had good physical appearances, clear, colorless and smooth. While F1 which lacks PEG200 in its composition had dramatic change in its physical appearance; the ocular inserts were rough and hazy, because the plasticizer can affect the polymer properties as its optical clarity which might be the reason for the haziness; [36] PEG200 may associate with enhancement of rebamipide solubility in the film according to the reference [16], hence its absence may result in precipitating out of the drug when the solvent evaporated and gave the rough texture to the film.

Weight uniformity

The mean weights of the samples of all formulae were ranged between (16.23±1.39 mg – 20.30±0.92 mg) as shown in table (5).

Thickness measurement

The mean thickness of all formulae ranged between (0.31±0.030 mm- 0.40±0.012 mm) as showed in table (5).

Drug content uniformity

The prepared inserts of all formulae had drug content means ranged between (1.84±0.14 mg - 2.16±0.17 mg); the results are presented in table (5). According to USP dosage uniformity criteria the acceptable range would be (1.7mg -2.3 mg).[26]

The inserts of formulae (F2-F4) had good uniformity parameters and the results were within the acceptable criteria; except for formula F1 that had variation of more than 5% from its weight and thickness means and it also showed an effect on content uniformity; although the drug content mean was within the acceptable range but some of the samples had drug content out of the acceptable range which are apparent from its standard deviation value. This suggests that the process of casting and evaporation did not result in a uniform film; the absence of the plasticizer in F1 composition may affect its pour-ability.

Folding endurance

The results of folding endurance are shown in table (5). F1 was very brittle due to the absence of the plasticizer in its composition, which ensures the importance of the plasticizer presence in formulating the inserts. The plasticizer role can be explained by its improvement of the flexibility of the polymers by lowering their glass transition temperature degree (Tg). The small plasticizer molecules occupy the intermolecular spaces among the polymer chains leading to the reduction of the secondary forces between them that lead to the reduction of the required energy for polymer molecular motion and hence, the polymer flexibility is enhanced.[36] On the other hand, F4 which composed of only one grade of HPMC E5 with lower molecular weight had significantly lower folding endurance compared to F2 (p-value 0.004) and compared to F3 (p-value <0.001); while F3 that had HPMC E5: K15M ratio of (1: 0.4) showed significant (p-value <0.001) increase in folding endurance compared to F2 which had HPMC E5: K15M ratio of (1: 0.17)

Table (5) Physical parameters of rebamipide inserts

Formula	Weight (mg)	Thickness (mm)	Content (mg)	Folding endurance
F1	16.23±1.39	0.31±0.030	2.16±0.17	Very brittle
F2	19.63±0.58	0.39±0.006	1.95±0.11	77.67±6.314
F3	19.47±0.53	0.38±0.007	1.86±0.06	288.00±15.18
F4	20.30±0.92	0.40±0.012	1.84±0.14	57.00±11.37

Moisture loss%

Moisture loss% values of all formulas are shown in table (6), the values are within the range of (3.31±2.17%- 6.76±1.81%). The plasticizer and polymer ratio showed no effect on moisture loss% on the ocular inserts. In F4 which had only HPMC E5 in its polymer composition showed significantly higher moisture loss% compared to formulae F2 and F3 with (p-values 0.028 and 0.014) respectively, this indicates that the presence of higher viscosity grade of HPMC K15M in the composition of these formulae hindered the water evaporation and kept the moisture within the insert, and since water is a natural plasticizer then the ocular insert flexibility will be maintained. [36]

Moisture absorption%

The results of moisture absorption% of all formulas are shown in table (6), the values ranged between (16.43± 2.75 % - 22.68± 6.53 %). There was a significant difference (p-value 0.036) between F1 and F2, which indicates that the plasticizer

addition in F2 has increased the moisture absorption%. This increment may be due to the hygroscopic nature of the polymer PEG which under certain values of relative humidity, has the ability to absorb water from the surrounding air and experiences the phenomenon of deliquescence.[37] There were no significant differences in moisture absorption% of the formulas in which the HPMC ratio or grade had been changed, thus these factors have no effect on the moisture absorption % of inserts.

Surface pH

Surface pH of the inserts are shown in table (6), and found to be between (7.52±0.05 - 6.93 ±0.04); this range of values would not change the pH value of the tears[38], since tears have pH value equal to 7.4 with some buffering capacity.

Table (6)Rebamipide insert moisture loss%, absorption% and surface pH evaluation

Formula	Moisture loss%	Moisture absorption%	Surface pH
F1	3.89±1.94	16.43±2.75	7.52±0.05
F2	3.74±2.25	22.68±6.53	7.22±0.07
F3	3.31±2.17	17.79±5.12	6.99±0.06
F4	6.76±1.81	17.30±1.82	6.93±0.04

From the results of the evaluation of the physical and mechanical properties of rebamipide ocular inserts, F3 showed the best results and was selected for further evaluation.

In-vitro release study

The in-vitro drug release profiles of selected formula F3 and the reference are shown in figure (10), and their T50% and T80% (hr.) are listed in table (7).

Formula F3 which contains HPMC E5: K15M (1:0.4) and PEG200 (35%w/w of total polymer weight) showed significantly larger T50% and T80% compared to the Mucosta® ophthalmic suspension (p-values 0.034 and 0.015) respectively; the similarity factor between then was (f_2 =37.27). These results indicate that the prepared rebamipide ocular insert (F3) release profile was different from that of the reference and had noticeable drug release retarding effect. This result supports the objective of this study to extend the drug release from the insert compared to available marketed dosage form.

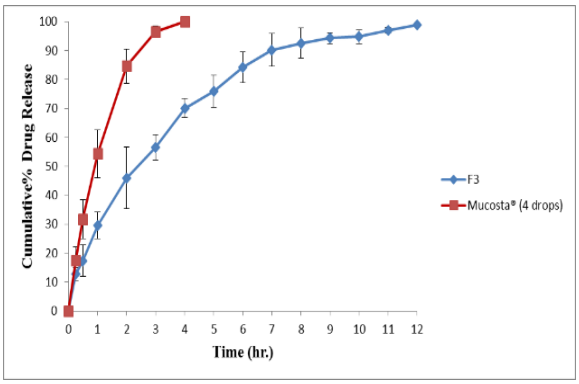

Figure (10) Cumulative% release profile of rebamipide in phosphate buffer (pH 7.4) at 34ºC from F3 and reference

Table (7) Drug release parameters from the insert F3 and mucosta® suspension (4drops)

Formula	T50% (hr.)	T80% (hr.)
F3	2.30	5.53
Reference Mucosta®	0.90	1.87

Compatibility studies

Fourier transform infra-red spectroscopy (FTIR)

The FTIR spectra of the pure rebamipide figure (7), polymer HPMC E5 and formula F3 are shown in figure (11) and figure (12) respectively. The characteristic peaks of FTIR spectrum of rebamipide are illustrated in figure (12), at wave numbers (in cm^{-1}): 1115 for in-plane C-H bending of aromatic ring (1300-1000), 1316 for C-O stretching of carboxylic acid (1320-1210), 1412 for C-C stretching of aromatic ring (1500- 1400), 1644 C=O stretching of amide band I (1680-1630) ; this indicate that there is no interaction between rebamipide and the excipients.

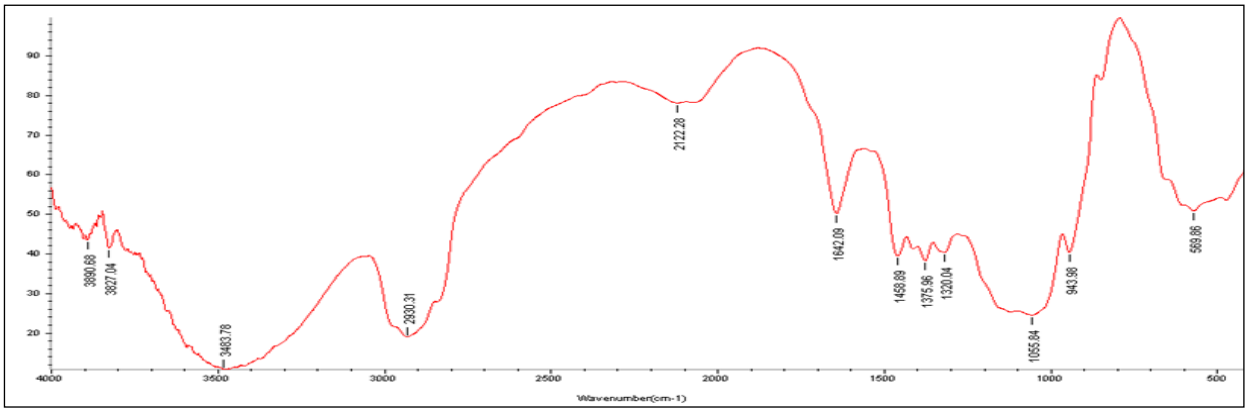

Figure (11) FTIR spectrum of pure HPMC E5 powder

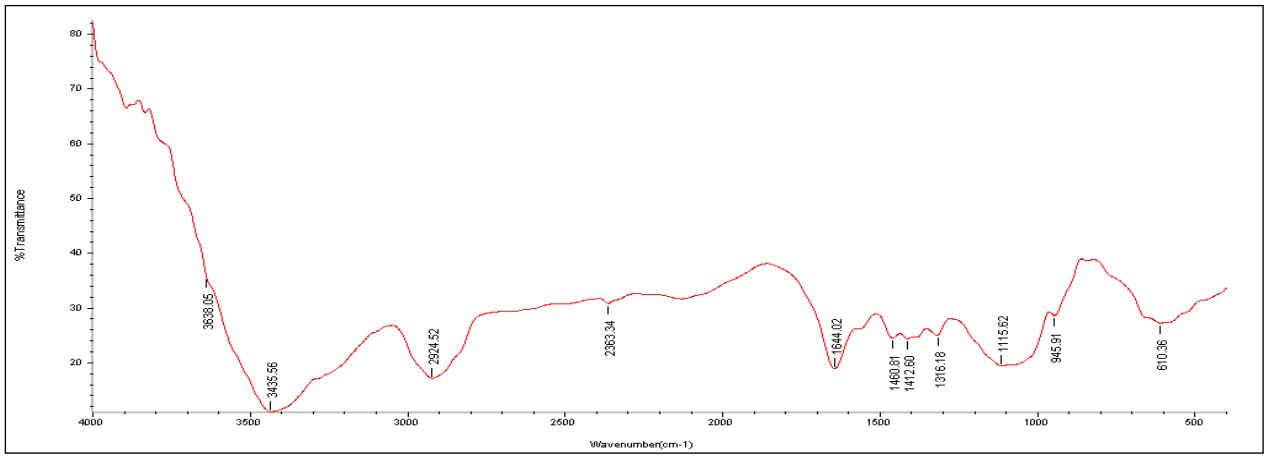

Figure (12) FTIR spectrum of the selected rebamipide ocular insert F3

Differential scanning calorimetry (DSC)

DSC thermogram image of the pure rebamipide was shown in figure (3); DSC images of polymers used in the selected formula HPMC E5 and HPMC K15M; and for the selected formula F3 shown in figure (13), figure (14) and figure (15) respectively.

The DSC thermogram of selected formula (F3) of rebamipide ocular insert did not show a noticeable peak; this may be due to the dilution effect of mixing small amount of rebamipide within the formula with higher proportions of polymers and other excipient.

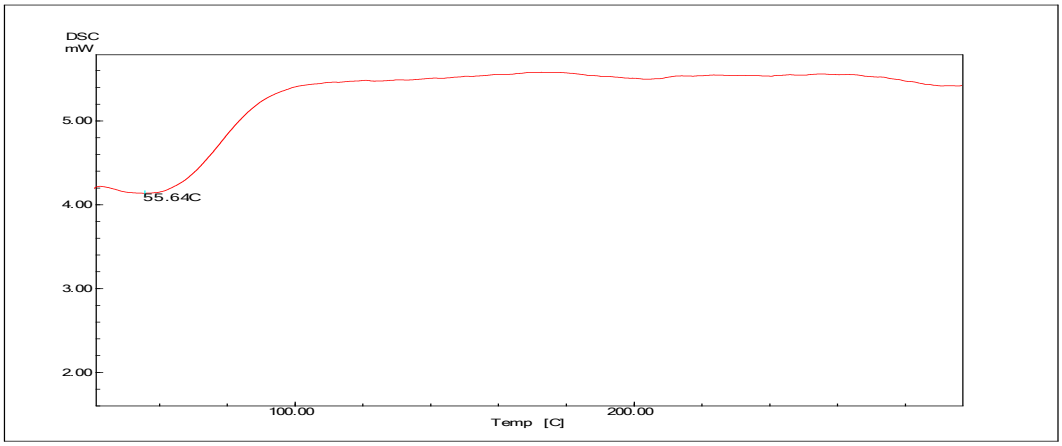

Figure (13) DSC thermogram of HPMC E5

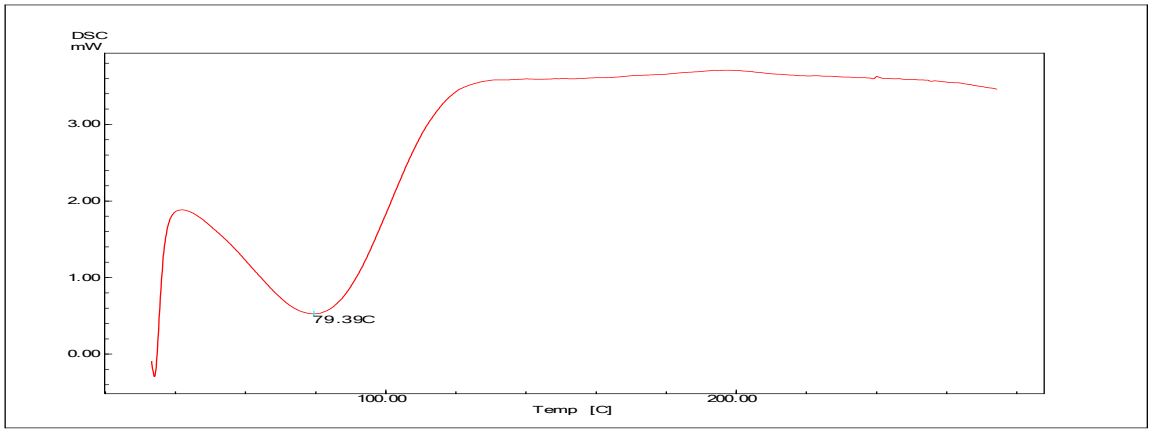

Figure (14) DSC thermogram of HPMC K15M

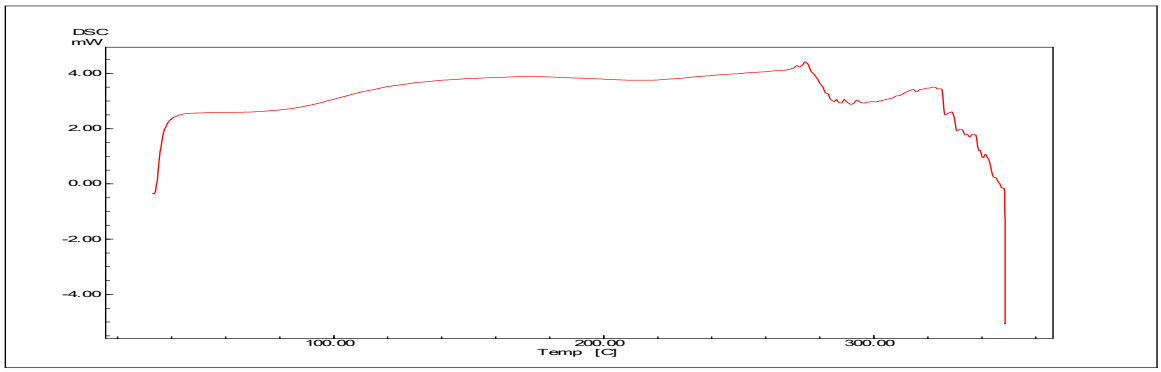

Figure (15) DSC thermogram of selected rebamipide ocular insert F3

Conclusion

Based on the results obtained from the study, it was concluded that rebamipide ocular inserts have good potential for futuristic rebamipide extended release ocular delivery system for dry eye treatment.

Few points are noticed from the experimental work that should be considered during rebamipide insert formulation; the enhancement of rebamipide solubility by adding L-arginine to the formulation, the importance of using plasticizer in the insert composition to improve physical and mechanical characteristics of the insert, the incorporation of higher molecular weight polymer (HPMC K15M) lead to improving the inserts flexibility and extended the drug release.

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