Azhar Int J Pharm Med Sci 2022; Vol 2 (2):60-69
Research Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Formulation and evaluation of HPMC topical gel of Ectoine

Sammar A. Bayoumi[1*], Aya M. Dawaba[2], Zeinab A. Zalat[3], Amal A Ammar[2]

[1]Department of Pharmaceutics and pharmaceutical technology, Faculty of pharmacy, Heliopolis University, Cairo, Egypt.
[2]Department of Pharmaceutics and pharmaceutical technology, Faculty of pharmacy (Girls), Al Azhar University, Cairo, Egypt.
[3]Department of Clinical Pharmacy, Faculty of pharmacy (Girls), Al Azhar University, Cairo, Egypt.
***** Correspondence: sammar.bayoumi@hotmail.com

Article history: Received 06/09/2021 Revised 07/11/2021 Accepted 20/11/2021

Abstract: The objective of this study is to formulate and evaluate a topical gel containing Ectoine for further use in treating skin diseases. Three gel formulations were prepared using 1.5%, 2% and 3% of gelling agent HPMC K15M and these formulae were evaluated for their physical appearance, pH, spreadability, viscosity, in vitro release profile, freeze thaw cycle and ex vivo release. The preformulation studies for both Ectoine and HPMC were applied using DSC and FTIR. HPLC analysis of the natural compound was conducted to detected concentration of Ectoine in formulated gels. Formulated gels were homogenous, had good consistency, spreadability but unstable when subjected to high temperature. Among the three formulae, H1 showed better release (91.4%) characteristics than other concentrations. The kinetic data of the release investigated that Ectoine release from all examined gel followed the diffusion mechanism except H1 was first order. When ex vivo was applied to the selected formula it showed good release through rat skin (85%) after 8 hrs.

Keywords: Ectoine; Topical drug delivery; HPMC K15M; Ex vivo.

1. INTRODUCTION

Ectoine is a natural, water soluble, osmoprotectant, amino acid compound which is produced by many bacterial cells as a return to uncontrolled environmental conditions and osmotic stress. Although, it never interacts with natural processes inside cell. It was found to have many uses especially in cosmetics due to its hydration effect. Ectoine has the ability to stabilize enzyme, protect human skin, can be used as anti-inflammatory, and can be utilized in neurodegenerative diseases because of its inhibitory effect(1).

This active drug is found in commercial products with different concentrations as in nasal spray, eye drops, lozenges, Mouth and Throat Spray, Inhalation solution, cream, and ear spray. Nasal Spray is used for treatment and prevention of allergic rhinitis symptoms. Eye drops can be used for treatment and prevention of allergic conjunctivitis symptoms. Lozenges are used for treatment and prevention of allergic symptoms in mouth and throat that can be caused by airborne allergens also, in treatment and prevention of common cold symptoms, dry mouth and throat, and hoarseness. Mouth and Throat Spray for treatment of dry and irritated epithelia in mouth and throat. Inhalation solution for supportive treatment to reduce inflammation of the airways in case of bronchitis, asthma or Chronic Obstructive Pulmonary Disease (COPD). Cream for symptomatic treatment, relief of skin redness, itching experienced with various types of inflammatory dermatoses and prevention of psoriasis. Ear spray for treatment of symptoms caused by non-infectious inflammation of the outer ear, for prevention of otitis externa and earwax plugs, and for eased clearing of the outer ear canal (2-4).

Different uses for Ectoine were found, but also new mode of action for this drug have been discovered. Ectoine has a whitening property, it can inhibit melanogenesis and has no cytotoxic effects(5).

Bioactivity of the drug can be improved with reducing its side effects, enhancing its penetration, ease of administration, increase patient compliance and enhance the therapeutic efficacy by using it topically(6). Delivery of drug through the skin is a targeted and an effective treatment for dermatological diseases. This drug delivery route has been popular because it prevents gastrointestinal irritation and metabolic degradation that are caused due to oral administration (7, 8). One of the most usable topical dosage form is gel.

For various reason gel provide proper drug delivery as they are less greasy, easily spreadable, and easy to remove, emollient, thixotropic, and are soluble in water. Topical formulations is explained as

Cite this article: Bayoumi S., Dawaba A., Zalat Z., Ammar A. Formulation and evaluation of HPMC topical gel of Ectoine. Azhar International Journal of Pharmaceutical and Medical Sciences, 2022; 2(2):60-69. doi:**10.21608/AIJPMS.2022.94549.1092**

DOI : 10.21608/AIJPMS.2022.94549.1092 https://aijpms.journals.ekb.eg/

the release of the drug from the formula followed by penetration via the skin to reach tissue or cell. Many factors can affect the drug release from topical formula, as physical and chemical properties of the polymer or active ingredient [9-11]. As the skin is the most attainable organ of body so, it has become the main source for topical administration. Topical Medicated products utilized on the mucous membrane or skin have many advantage as they either promote or renovate an essential function of a skin, also it could pharmacologically change a behavior in the underlined tissues[12].

Formulation studies for polymer and drug were made to determine any interaction between polymer and Ectoine. Different analytical techniques like Fourier transform spectrometers (FTIR) and Differential scanning calorimetry (DSC) are used to determine if there is interaction between drug and excipient. Such tests have been shown to be capable of rapidly detecting physical and chemical incompatibilities [13, 14]. DSC is a thermodynamically tool that measures the heat energy uptake that occur within a sample when there is change in temperature either increase or decrease. The calorimetry is particularly used to monitor the changes of phase transitions. A biochemical reactions, that can be described as a single molecular transition of a molecule from one phase to another can also be determined by DSC. Thermal transition temperatures (T_t; melting points) are also inspected in solution, solid, or mixed phases such as suspensions[15, 16]. On the other hand, Fourier transform spectrometers (FTIR)is a tool that is used to obtain infrared spectrum of emission, absorption, and photoconductivity of solid, liquid, and gas. It is also used to detect various functional groups. FTIR spectrum is measured between 4000 and 400 cm^{-1}.

Various techniques can be used to quantitatively detect Ectoine, utilizing HPLC and qualitatively analysis by NMR [17]. But until this time, there is no specific effective HPLC method that can be used for screening and quantifying Ectoine. So, a method that can determine a numerous quantity of isolates along with an acceptable range of accuracy and reproducibility rapidly became essential. For excellent separation of low polarity substances RP-HPLC is used. On the other hand, it should be considered that RP-HPLC would not give enough separation for highly polar substances. For Highly polar substance high aqueous mobile phase is needed to yield enough retention by the reverse phase. For Ectoine determination a high qualified RP-HPLC chromatographic column is essential due to considerable reduction in the lifetime of the chromatographic columns[18]. Polar embedded stationary phase methods have been progressed using amides. This phase 'wettability' has been raised by the polar functional group found at the surface, that will decrease phase collapse, which will make this phase harmonious with mobile phase of up to 100% water content. Moreover, the polar functional group provides sharp peak shape for high polar and strong basic compounds with various selectivity than the traditional C18 columns[19].

The study objective was to formulate Ectoine topical gel utilizing hydroxypropylmethylcellulose (HPMC K15M) to enhance the skin penetration of Ectoine for use in many skin diseases. HPMC K15M was selected due to its good swelling properties, accommodation of high levels of drug and nontoxic nature.

2. MATERIALS AND METHODS

2.1 Materials

From Biotop Company (In Germany) Ectoine was procured. Hydroxypropylmethylcellulose (HPMC K15M) was Supplied by Eipico Pharma (Egypt). Cellulose membrane Spectra Pore (12,000 - 14,000 molecular weight cut-off) was procured from Chemical Company Sigma.

2.2 Preparation of gels containing Ectoine

For Formulation H1, H2 and H3 specific amount of Ectoine for each formula was weighed. Then Ectoine was dissolved in weighted amount of distilled water (solution A). Specific quantity of HPMC K15M was weighted to give the needed concentrations (1.5%, 2 and 3%), then the powder was added gradually to three quarter of hot distilled water (60°C) and stirred until dissolved. Stirring was stopped when a clear solution, with no lumps was observed. The rest of water was cooled and added to solution with mixing till smooth homogenous gel was made (solution B). The gel was left overnight in refrigerator for complete gel dispersion after that solution A was added on the gel, then mixed together till complete homogeneity. The final weight was 100g [20].

Table 1. Gel formulations containing Ectoine

Formula Code	HPMC K15M	¾ of total water volume	HPMC K15M	Stirring till homogenous	5ml water
H1	1.5%	▲	1.5gm	√	*
H2	2%	▲	2gm	√	*
H3	3%	▲	3gm	√	*

▲ *Ectoine 7% was added in each formula. Hot distilled water.

2.3 Differential scanning calorimetry and fourier transform spectrometers (FTIR)

In this study to test for compatibility DSC apparatus {DSC822e Mettler-Toledo differential scanning calorimeter (Mettler-Toledo International Inc., Columbus, OH, USA)} and Fourier transform infrared spectroscopy (1600 series, Perkin-Elmer Corporation, Norwalk, USA) in transmittance mode were used. The samples were first prepared, 1mg from each Ectoine and HPMC was weighted, and then a mixture of both Ectoine and HPMC was prepared consisting of 1mg from each. Then the samples were placed in aluminum pans under dynamic atmosphere of N2 (flow rate of 50 ml min-1) and heating rate of 10 min-1 in the temperature range from 25 to 450 ºC. Tests were carried out individually with Ectoine, polymer and previously prepared physical mixtures to test DSC. Moreover, to ensure compatibility FTIR was also used. The spectra were obtained in the spectral area 400-4000 cm-1, with a resolution 0.5 cm-1 at room temperature and heat treated at 240 and 260 ºC [21].

2.4. Evaluation of Polymer based gel containing Ectoine

The formulated Ectoine gel was evaluated for the following parameters:

2.4.1 Analysis using HPLC

A developed green method, established on polar RP-HPLC was used for quantifying and screening Ectoine from gel formulae prepared according to ICH guidelines and Chinese Pharmacopoeia [22]. HPLC system model 1100 (Agilent Technologies, USA), with aquaternary pump, an autosampler, a variable wavelength detector and a chromatographic column of HiQsil C18 HS column (5µm particle size, 250 x 4.6mm) were used. The samples were prepared first before analysis, 1gm from each formula was weighted in volumetric 100 ml flask, then completed with phosphate buffer pH (6.4). All conical flask were placed in sonicator and left for 2 hr to ensure complete dissolving of gel. 1ml from each sample was withdrawn using micropipette then filtered using 0.45µm filter syringe. The concentration of Ectoine was determined with microgram/ml. The retention time of each formula was in range between 3.4-4 min until a sharp peak was shown. Injection time was fixed for 5 min and UV wave length at 210 nm. The mobile phase used was HPLC distilled water to obtain adequate retention by reverse phase. Then the analysis was applied using a reverse phase technique by the use of C18 column.

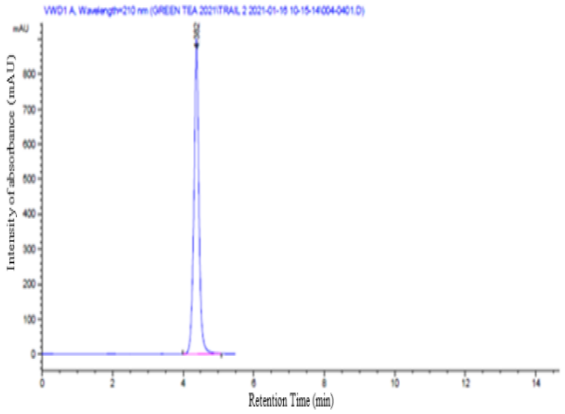

Figure 1. Chromatogram of Ectoine, revealing its concentration in gel.

2.4.2. Preparation of calibration curve

Calibration curve for the estimation of Ectoine content in the dissolution medium: To prepare a standard solution, 50 mg of Ectoine was measured in an Electrical Balance ViBRA HT (Antielectrostatic, Japan) and dissolved in 50 ml of freshly prepared phosphate buffer to produce a solution of 1000 µg / ml. Working standard solutions with varying strength (70, 140, 210, 280, 350 and 420 µg /ml) were freshly prepared by suitable dilution from the stock solution with phosphate buffer. Then absorbance of the solutions was measured at 210 nm using HPLC. A plot was constructed showing concentration at X-axis and absorbance at Y- axis.

2.4.3. Visual examination

After being left overnight all medicated gel have undergone visually inspection for color, homogeneity and presence of cluster [23].

2.4.4. pH determination

Gels pH was measured utilizing BioBase pH meter model PHS-3BW (China). The readings were taken in triplicate.

2.4.5. Determination of spreadability

One gram from each formula was weighted on one of glass slides and then the other slide was added followed by introducing of pressure utilizing specific weight. The pressure was applied for 5 minutes, or until no more spreading was inspected. A circle shape was formulated followed by pressure applied, the diameters were measured in cm. The values obtained were then taken as explanation for spreadability of gels. The test was applied three times[24].

2.4.6. Determination of viscosity

The viscosity is measured to investigate the rheological of formulations. Viscometer Brookfield model (RVF), Brookfield engineering laboratories, INC. Stoughton, MA.02072, U.S.A with spindle 7 was used. Viscosity are measured three times and average is taken into consideration[25].

2.4.7. Determination of drug content

One gram from each formula was weighted and allowed to dissolve in 100ml of prepared phosphate buffer with a pH of 6.4. To ensure complete solubility of Ectoine the volumetric flasks were added in the shaker for 2hrs. After complete solubility of Ectoine, filtration was done using Millipore filter (0.45µm). After suitable dilution drug concentration was measured using the previously mentioned HPLC method.

2.4.8. Ectoine gel in vitro release study

To investigate release rate of Ectoine from the three prepared gels, vitro release study was applied for each formula. Dissolution test apparatus was used for the experiment to be proceeded. The apparatus shafts were rotated at 100 rpm. The medium in which the release of Ectoine occur was freshly prepared phosphate buffer pH 6.4. 1gm of each prepared gel was injected in Spectra Pore dialysis cellulose membrane tube that has been soaked overnight in the phosphate buffer medium with pH 6.4, then the tube was tied from both ends and attached to the paddle of a dissolution apparatus. Then Paddles were allowed to sink in the dissolution vessel containing 150ml of phosphate buffer, and maintained at 32ºC ± 1ºC[26, 27]. At specified time intervals a certain amount from each sample were taken and replaced by an equal amount from the freshly prepared phosphate buffer to stabilize sink condition. Filtration of each sample was done using a Millipore filter (0.45µm). Then HPLC was used to assay samples at λmax 210nm. The previously constructed calibration curve was utilized to determine the concentration of drug. The experiment was done in triplicate. In vitro release data were recorded for 24 hr. Mathematical models were done to combine dissolution profiles with the mechanisms of drug release from the drug delivery system[28].

2.4.9. In vitro kinetics of Ectoine

The data obtained from in vitro test were analyzed using four kinetics models:

$$Q_t = Q + K_0 t \quad (29)$$

Where Q_0 is the primary amount of drug in solution, Q_t is quantity of drug dissolved in specific time, and K_0 is elimination rate constant of zero order.

$$\text{Log } C - \log C_0 = - Kt/ 2.303 \quad (30)$$

Where k is rate constant of first order, C_0 is the primary drug concentration, and Time (t)

$$1/C - 1/C_0 = k_2 t \quad (31)$$

Where C_0 and C are the concentration, k_2 is rate constant of second order, and Time (t)

$$f_t = Q = A \sqrt{D(2C-C_s) C_s t} \quad (32)$$

Where D is diffusion coefficient of the drug molecules in substance, Q is amount of drug released in specific time (t), C is drug primary concentration and C_s is solubility of drug in the media.

2.4.10. Freeze-Thaw Cycle

This test was applied for 12 days. For each cycle, the sample was left in a specific temperature for 24 hours. The temperature in refrigerator was 4 ± 2∘C and 40∘C in the incubator. The physicochemical properties including pH, viscosity, homogeneity and spreadability of each gel sample was performed before and after the 12 days [33].

2.4.11. Ectoine gel ex vivo release study

The selected formula was then subjected to ex vivo release study using Franz diffusion cell. Skin of male rats was used to apply test. An approval has been taken from the ethical committee of Al Azhar University to conduct this research study with ethical approval number 52. The abdominal skin of rat was removed after been shaved. After preparing of skin, it was fixed on Franz cells that was previously filled with phosphate buffer pH (6.4)[34]. The receptor compartment was filled with phosphate buffer stirred at 60 RPM and maintained at 37 ± 0.5°C. Whereas, 1 gm of gel formulation was placed on the skin which was tied to one side of the donor compartment. Release studies were carried out for 24 hr and samples were withdrawn from receptor side at regular intervals and replaced with same volume from fresh media. Samples withdrawn were subjected to filtration to remove any impurities using Millipore filter (0.45µm). Then they were analyzed by developed and validated RP-HPLC method[35].

2.4.12. Statistical analysis

Results were expressed as the mean ± SD for all experiments done. Significance in the difference of the means was determined by the student's t-test. Statistics was shown to be significant with a $P<0.05$ value.

2. RESULTS AND DISCUSSION

3.1. Fourier transform spectrometers (FTIR) and Differential Scanning calorimetry

Ectoine was characterized by DSC analysis, to determine the nature of the drug and to detect any change in state or possible interaction between Ectoine and polymer. Thermograms obtained for pure Ectoine, HPMC K15M, and mixed formulation are shown in figure 2. The DSC of Ectoine showed a sharp endothermic peak at 345.25°C and HPMC showed a sharp endothermic peak at 337.48°C as shown in figure 2, similarity in results were shown by Ching Mien Oh et al and Elena Golovina et al [21], [36].Thermograms of the mixed formulation did not demonstrate any considerable shift in the endothermic peak, which means that no interaction had occurred between polymer and Ectoine. For IR spectra of Ectoine shown in figure 3 contained broad overlapping bands in the hydrogen stretching region N-H (1612.2cm^{-1}), C-H (2860.88cm^{-1}) and a set of narrow lines in the finger print region. The OH stretching vibration band around 3195.47cm^{-1} similarity was shown by Elena Golovina et al [36]. HPMC K15M exhibit a broad peak at 3461.6 cm−1, respectively, indicative of intermolecular hydrogen bonding of the OH group [37]. No overlapping was observed in the mixture.

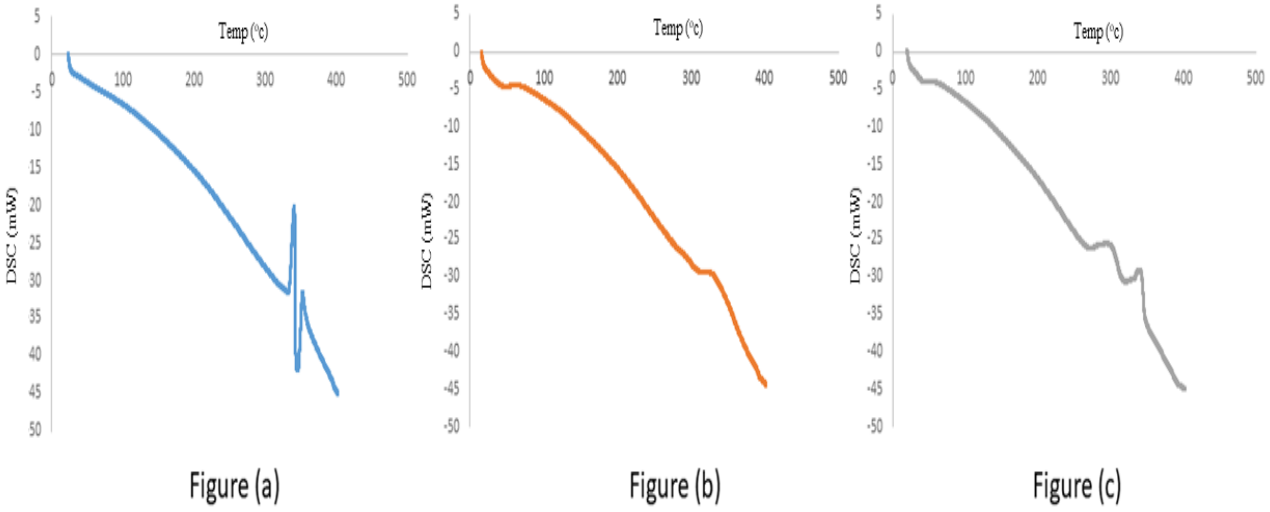

Figure 2. DSC thermogram, Figure (a) represents Ectoine thermogram, Figure (b) represents HPMC K15M thermogram and Figure (c) represents mixture between Ectoine and HPMC K15M

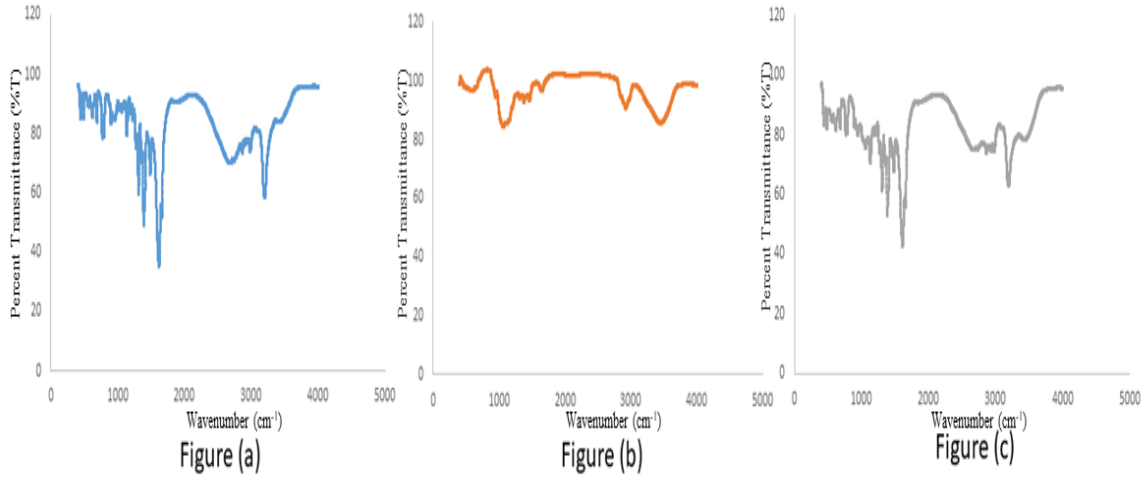

Figure 3. IR of Ectoine and polymer, Figure (a) represents Ectoine, Figure (b) represents HPMC K15M and Figure (c) represents mixture between Ectoine and HPMC K15M.

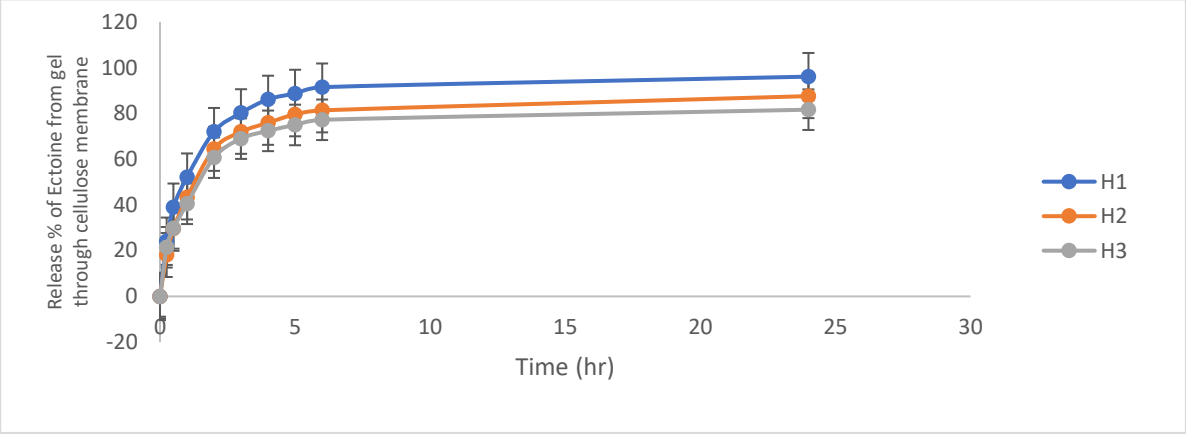

Figure 4. Release pattern of HPMC K15M different concentrations.

3.2. Evaluation of Ectoine gel

3.2.1. Visual examination

The prepared gels were examined visually for their color. All preparations were clear, transparent and homogenous with absence of cluster.

3.2.2. pH

Values of pH for all prepared gels were in range 6.75-6.88 which means that skin irritation will not be present.

3.2.3. Spreadability

The diameter of the circle when measured had range value 8.1-6.5 cm, which investigate that all the prepared gels had spread by low amount of stress. These valued explains that increasing the concentration of HPMC K15M will always be associated with a decrease in the spreadability as expressed by the low diameter of the spreaded circle. Similarity was shown by S. P. N. Kumara et al [38].

3.2.4. Drug content

Following the test of determining the drug content using HPLC for all formulation it was found that the results are in the official limits. The drug content estimated that Ectoine was equally and homogenously distributed throughout the gel.

Table 2. Physical characters of gels, pH, Spreadability, viscosity and drug content.

	H1	H2	H3
Color (Clear)	√	√	√
Homogeneity (Homogeneous)	√	√	√
Texture (No grittiness)	√	√	√
pH	6.76±0.01	6.87±0.01	6.69±0.01
Spreadability cm	8.1±0.1	7.9±0.1	6.56±0.1
Viscosity (Cp)	13,000±0.05	16,000±0.05	54,000±0.05
Drug content %	81.5±0.1	86.2±0.1	84.5±0.1

3.2.5. Ectoine gel in vitro release examination

Release profile of Ectoine topical gels were represented in Figure 3, to detect the change in polymer concentration on the release of Ectoine. It was observed that the release of Ectoine differ from 96.06 to 81.6% after 24 hr according to concentration of polymer. The release of Ectoine from different formulae can be graded in the following inclined order: H3>H2>H1. The drug release decreases with increasing HPMC K15M concentrations, which could be due to the increase in the HPMC

concentration that led to an increase in the gel viscosity, and therefore decreasing the rate of penetration of dissolution medium. Moreover, it can be due to entrapment of the drug by the HPMC K15M molecules. In addition, the chain structure in the microstructure of polymer was found to have high density with increasing in concentration of polymer and this limits the drug movement space. Similar results were obtained by Uddin et al in their study [39]. They observed that the increase in the HPMC amount, led to a decrease in the release of tramadol from the tablets.

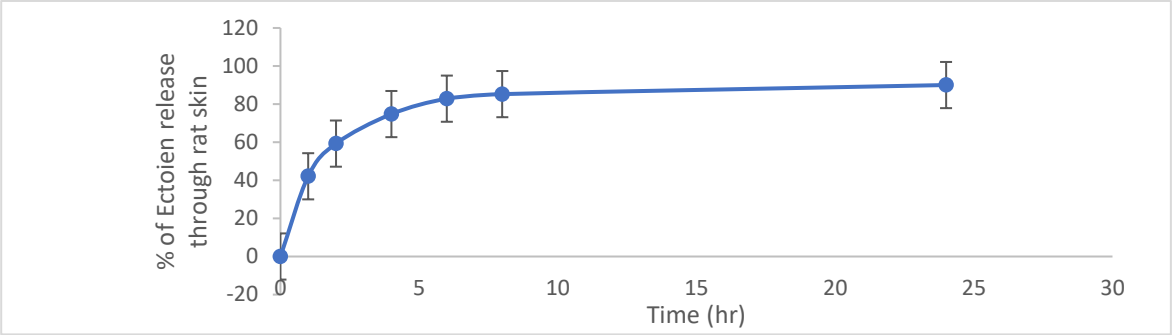

Figure 5. Ex vivo release study of Ectoine from H1.

3.2.6. In vitro release kinetic parameters of Ectoine

Many kinetic models that could calculate drug release cumulative % vs. time (Zero order), log of drug remaining % cumulative vs. time (First order) and diffusion were used to investigate a typical kinetic model for explaining the diffusion release results. Following the utilizing of these models it has been specified that release of Ectoine gels obey diffusion transport. Therefore, the release of Ectoine from gels is controlled by swelling of HPMC K15M molecules.

Table 3. Kinetics of in vitro release of HPMC K15M different concentrations.

Formula	r	K	t1/2	Release Model
H1	0.98729	0.37559	1.84509	First
H2	0.969022	32.89859	2.309858	Diffusion
H3	0.973312	29.72874	2.828701	Diffusion

3.2.7. Freeze-Thaw Cycle

According to previous studies that were done the best formula was selected from the three prepared according to physical characters, pH, viscosity, spreadability, drug content and in vitro release study which was H1. This formula was subjected to freeze thaw cycle test for 6 cycles. The gels was first tested for pH, Spreadability and viscosity. The values were 6.7, 8.1cm and 13,000cp. At the end of test the values became 6.6, 8.9cm and 12000cp. These results revealed that the formula was affected by differences in temperature.

3.2.8. Ectoine gel ex vivo release study

As previously mentioned data H1 formula was the selected for further test. The data obtained from release test revealed the effect of polymer on Ectoine release to phosphate buffer medium through the skin. It is shown that the release of Ectoine through skin was not affected that much when compared to in vitro study, after 24 hr the release date was 90%. This means that the polymer doesn't trap Ectoine in its molecules.

5. CONCLUSIONS

According to the results obtained we can determine that a promising formula was formulated using HPMC K15M polymer. In which this new formulated dosage form can be used in treatment of different skin conditions. Gel as known is the most preferable dosage form for patient as it is easy to apply and to remove, less greasy. H1 was selected as best formulation based on the physical characters, pH, drug content and release pattern from polymeric matrix. The drug content percentage was found to be within the range of 81.5±0.1 to 86.2±0.1. Moreover, the release data of Ectoine in vitro release study from this polymer especially in H1 formula was 96%, this means that low concentration of HPMC K15M didn't affect the release of Ectoine. Also, according to membrane release study applied it was found that the drug release reached 90% after 24 hr. All these information can reveal that this polymer can allow sustain release of Ectoine into the skin. By consolidating sustain drug release, viscosity, spreadability and physical character, Ectoine (1.5% HPMC K15M gel) was selected as optimum formulation. On the other hand, as investigated from Freeze thaw cycle test that viscosity of gel was

slightly affected by change in temperature so, it is better to preserve the gel in low temperature.

Funding: Self-funding

Acknowledgments: The Author is thankful for Ahmed S. Mancy for his help in pharmacokinetic and statistical part.

Conflicts of Interest: The authors declare no competing interests.

Ethical Statement: An approval has been taken from the ethical committee of Al Azhar University to conduct this research study with ethical approval number 52.

Author Contribution: Amal Ammar: Design of the work; supervision, and Final approval of the version to be published, **Zeinab Alkasaby**: Design of the work; supervision, and Final approval of the version to be published, **Aya Mohamed**: Design of the work; supervision, and Final approval of the version to be published, **Sammar Ashraf Bayoumi**: Performed the research in the lab, data acquisition, data analysis, and writing it as part of her master's degree thesis.

List of Abbreviations: FTIR: fourier transform spectrometers.
DSC: differential scanning calorimetry.
HPMC: hydroxypropyl methyl cellulose.
COPD: chronic obstructive pulmonary disease.
NMR: nuclear magnetic resonance.
HPLC: high performance liquid chromatography.
RP-HPLC: reverse phase high performance liquid chromatography.

REFERENCES

1. Bownik A, Stępniewska Z. Ectoine as a promising protective agent in humans and animals. Arh Hig Rada Toksikol. 2016;67(4):260-5.

2. Müller D, Lindemann T, Shah-Hosseini K, Scherner O, Knop M, Bilstein A, et al. Efficacy and tolerability of an ectoine mouth and throat spray compared with those of saline lozenges in the treatment of acute pharyngitis and/or laryngitis: a prospective, controlled, observational clinical trial. European Archives of Oto-Rhino-Laryngology. 2016;273.

3. Dao V-A, Overhagen S, Bilstein A, Kolot C, Sonnemann U, Mösges R. Ectoine lozenges in the treatment of acute viral pharyngitis: a prospective, active-controlled clinical study. European Archives of Oto-Rhino-Laryngology. 2019;276.

4. Casale M, Moffa A, Carbone S, Fraccaroli F, Costantino A, Sabatino L, et al. Topical Ectoine: A Promising Molecule in the Upper Airways Inflammation—A Systematic Review. BioMed Research International. 2019;2019:7150942.

5. Chung YC, Kim M-J, Kang EY, Kim YB, Kim BS, Park S-M, et al. Anti-Melanogenic Effects of Hydroxyectoine via MITF Inhibition by JNK, p38, and AKT Pathways in B16F10 Melanoma Cells. Natural Product Communications. 2019;14(6):1934578X19858523.

6. VIJAYAKUMAR M.R. AHSA, ARUN K. Formulation and evaluation of diclofenac potassium ethosomes. International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2(4).

7. Kikwai L, Babu RJ, Prado R, Kolot A, Armstrong CA, Ansel JC, et al. In vitro and in vivo evaluation of topical formulations of spantide II. AAPS PharmSciTech. 2005;6(4):E565-E72.

8. Behl CR, Char H, Patel SB, Mehta DB, Piemontese D, Malick AW. In Vivo and In Vitro Skin Uptake and Permeation Studies. In: Shah VP, Maibach HI, editors. Topical Drug Bioavailability, Bioequivalence, and Penetration. Boston, MA: Springer US; 1993. p. 225-59.

9. Kalia YN, Naik A, Garrison J, Guy RH. Iontophoretic drug delivery. Advanced drug delivery reviews. 2004;56(5):619-58.

10. Dey S, Mazumder B, R P. Enhance percutaneous permeability of acyclovir by DMSO from topical gel formulation. International Journal Pharmaceutical Science and Drug Research. 2009;1.

11. Patel J, Patel B, Banwait H, Parmar K, Patel MJIJDDR. Formulation and evaluation of topical aceclofenac gel using different gelling agent. International Journal of Drug Development and Research. 2011;3(1):156-64.

12. Gupta A, Mishra A, Singh A, Gupta V, Bansal P. Formulation and evaluation of topical gel of diclofenac sodium using different polymers. Drug Invention Today. 2010;2:250-53.

13. Schick C. Differential scanning calorimetry (DSC) of semicrystalline polymers. Analytical and bioanalytical chemistry. 2009;395(6):1589-611.

14. Pani NR, Nath LK, Acharya S, Bhuniya B. Application of DSC, IST, and FTIR study in the compatibility testing of nateglinide with different pharmaceutical excipients. Journal of Thermal Analysis and Calorimetry. 2012;108(1):219-26.

15. Van Holde KE, Johnson WC, Ho PS. Principles of physical biochemistry2006.

16. Cooper A, Nutley M, Wadood AJP-liH, calorimetry. Differential scanning microcalorimetry. Protein-ligand interactions: hydrodynamics and calorimetry2000. p. 287-318.

17. Li H, Zhang Q, Wang XL, Ma XY, Lin KF, Liu YD, et al. Biodegradation of benzene homologues in contaminated sediment of the East China Sea. Bioresource technology. 2012;124:129-36.

18. Harishchandra RK, Sachan AK, Kerth A, Lentzen G, Neuhaus T, Galla HJ. Compatible solutes: ectoine and hydroxyectoine improve functional nanostructures in artificial lung surfactants. Biochimica et biophysica acta. 2011;1808(12):2830-40.

19. Maldaner L, Collins CH, Jardim ICJQN. Fases estacionárias modernas para cromatografia líquida de alta eficiência em fase reversa. 2010;33(7):1559-68.

20. Al-Saidan SM, Krishnaiah YS, Chandrasekhar DV, Lalla JK, Rama B, Jayaram B, et al. Formulation of an HPMC gel drug reservoir system with ethanol-water as a solvent system and limonene as a penetration enhancer for enhancing in vitro transdermal delivery of nicorandil. Skin pharmacology and physiology. 2004;17(6):310-20.

21. Gaber DA. Nanoparticles of Lovastatin: Design, Optimization and in vivo Evaluation. International journal of nanomedicine. 2020;15:4225-36.

22. Verma S, Verma RK, Sahoo D, Srivastava SK. Reverse-phase HPLC method for the quantification of two antihyperglycemic glycolipids in Oplismenus burmannii. Biomedical chromatography : BMC. 2015;29(11):1675-81.

23. Bachhav YG, Patravale VB. Microemulsion based vaginal gel of fluconazole: formulation, in vitro and in vivo evaluation. International journal of pharmaceutics. 2009;365(1-2):175-9.

24. Ashara K, Paun J, Soniwala M, Chavada J, Mori N. Micro-Emulsion Based Emulgel: A Novel Topical Drug Delivery System. Asian Pacific Journal of Tropical Disease. 2014;4:S27–S32.

25. Ashara KC, Paun JS, Soniwala MM, Chavada JR, Mori NM. Micro-emulsion based emulgel: a novel topical drug delivery system. Asian Pacific Journal of Tropical Disease. 2014;4:S27-S32.

26. Siewert M, Dressman J, Brown CK, Shah VP. FIP/AAPS guidelines to dissolution/in vitro release testing of novel/special dosage forms. AAPS PharmSciTech. 2003;4(1):E7.

27. El-Houssieny BM, Hamouda HM. Formulation and evaluation of clotrimazole from pluronic F127 gels. Drug discoveries & therapeutics. 2010;4(1):33-43.

28. Abdelmalak NS, El-Menshawe SFJIJPPS. A new topical fluconazole microsponge loaded hydrogel: preparation and characterization. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(1):460-8.

29. Carstensen JT. Modeling and Data Treatment in the Pharmaceutical Sciences: CRC Press; 1996.

30. Hintz RJ, Johnson KC. The effect of particle size distribution on dissolution rate and oral absorption. International journal of pharmaceutics. 1989;51(1):9-17.

31. Wen WY, Johnson DR, Dole M. Second-order diffusion-controlled reaction. Decay of allyl free radicals in irradiated polyethylene. The Journal of Physical Chemistry. 1974;78(18):1798-804.

32. Ozturk SS, Palsson BO, Donohoe B, Dressman JB. Kinetics of release from enteric-coated tablets. Pharmaceutical research. 1988;5(9):550-65.

33. Dantas MG, Reis SA, Damasceno CM, Rolim LA, Rolim-Neto PJ, Carvalho FO, et al. Development and Evaluation of Stability of a Gel Formulation Containing the Monoterpene

Borneol.TheScientificWorldJournal.2016;2016: 7394685.

34. Ahmad N, Ahmad R, Mohammed Buheazaha T, Salman AlHomoud H, Al-Nasif HA, Sarafroz M. A comparative ex vivo permeation evaluation of a novel 5-Fluorocuracil nanoemulsion-gel by topically applied in the different excised rat, goat, and cow skin. Saudi Journal of Biological Sciences. 2020;27(4):1024-40.

35. Barse R, Kokare C, Tagalpallewar A. Influence of hydroxypropylmethylcellulose and poloxamer composite on developed ophthalmic in situ gel: Ex vivo and in vivo characterization. Journal of Drug Delivery Science and Technology. 2016;33.

36. Tanne C, Golovina E, Hoekstra F, Meffert A, Galinski E. Glass-forming property of hydroxyectoine is the cause of its superior function as a desiccation protectant. Frontiers in microbiology. 2014;5:150.

37. Oh CM, Heng PW, Chan LW. Influence of Hydroxypropyl Methylcellulose on Metronidazole Crystallinity in Spray-Congealed Polyethylene Glycol Microparticles and Its Impact with Various Additives on Metronidazole Release. AAPS PharmSciTech. 2015;16(6):1357-67.

38. Manna S, Lakshmi U, Racharla M, Sinha P, Kanthal Lk, Kumar S N P. Bioadhesive HPMC Gel Containing Gelatin Nanoparticles for Intravaginal Delivery of Tenofovir. Journal of Applied Pharmaceutical Science. 2016;6:22-9.

39. Rehman Au, Khan GM, Shah KU, Wahab A, Khan A. In Vitro Drug Release Kinetics of Tramadol HCl-Ethocel Matrix Tablets and Studying the Effect of Co-Excipients on the Release Pattern. Latin American Journal of Pharmacy. 2013;32:1131-8.