Study the Effect of Polymer Types on Preparation and in-vitro Evaluation of β-sitosterol as a Topical Hydrogel

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ABSTRACT

Beta sitosterol is a non-cholesterol sterol, or neutral sterol applied locally for the treatments of acute and chronic ulcers of skin and mucous membranes as well as post operative wounds. In this work β –sitosterol was prepared as topical hydrogel by cold mechanical method using different types of gel-forming polymers such as chitosan (4%, 3%, and 2%) W/W, carbapol (1.5%, 1%, and 0.5%) , and poloxamer 407(30% and 25%)W/W. Physicochemical properties of all the prepared formulas were evaluated as a visual inspection, determination of pH, and spreadability, in addition to an *in-vitro* drug release.

The obtained results indicated that all the different concentrations of each polymer gave a percent of drug release profile inversely proportional with the polymer concentration. F8 which contain poloxamer 407 at 25% produced higher drug release than other formulas (100% β-sitosterol release withim 3 hrs).

Based on overall result, β-sitosterol can be successfully prepared as a topical hydrogel using 25% poloxamer as the best prepared formula.

Key words: hydrogel, β-sitosterol, chitosan, carbapol , poloxamer 407.

دراسة تأثير نوع البوليمرات على تحضيير وتقييم البيتاسايتوسترول كهلام مائي موضعي

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مفتاح البحث: هلام مائي موضعي, البيتاسايتوسترول, الكيتوسان, الكاربول, البوليكزامر 407.

الخلاصة

البيتاسايتوسترول هو ستيرول غير الكولسترول ، أو ستيرول محايد تطبيقه محليا لعلاج القرحة الحادة والمزمنة من الجلد والأغشية المخاطية وكذلك الجروح ما بعد العملية. في هذا العمل ، تم تحضير البيتاسايتوسترول على□كل هلام مائي موضعي باستخدام طريقة ميكانيكية باردة باستخدام أنواع مختلفة من البوليمرات المكونة للهلام مثل الكيتوسان (4٪ ، 3٪ ، 2٪) ، الكاربول (1.5٪ ، 1٪ ، و 0.5٪) ، و البوليكزامر 407 (30%, 25%) و مع تركيز مختلف لكل منهما. تم تقييم الخواص الفيزيائية الكيميائية لجميع الصيغ المعدة على أنها فحص بصري ، وتحديد درجة الحموضة ، وقابلية انتشار ، بالإضافة إلى إطلاق عقار في المختبر.

أ□ارت النتائج المتحصل عليها إلى أن جميع التراكيز المختلفة لكل بوليمر أعطت نسبة مئوية من صورة إطلاق الدواء تتناسب عكسياً مع تركيز البوليمر. أنتجت F8 التي تحتوي على البوليكزامر 407 بنسبة 25 ٪ الافراج عن المخدرات أعلى من الصيغ الأخرى (100 ٪ الافراج مع 3 ساعات).

بناء على النتيجة الكلية ، يمكن تحضير البيتاسايتوسترول بنجاح على□كل هلام مائي موضعي باستخدام البوليكزامر 407 25بتركيز ٪ باعتباره أفضل صيغة محضرة.

INTRODUCTION

Beta sitosterol is one of the most prevalent vegetable-derived phytosterols in the diet. It is structurally [1] related to cholesterol (figure1).

It appears to modulate the immune function, inflammation, and the pain levels by controlling the production of inflammatory cytokines [2-3].

Sitosterols are white, waxy powders with a characteristic odour. They are hydrophobic and soluble in alcohol.

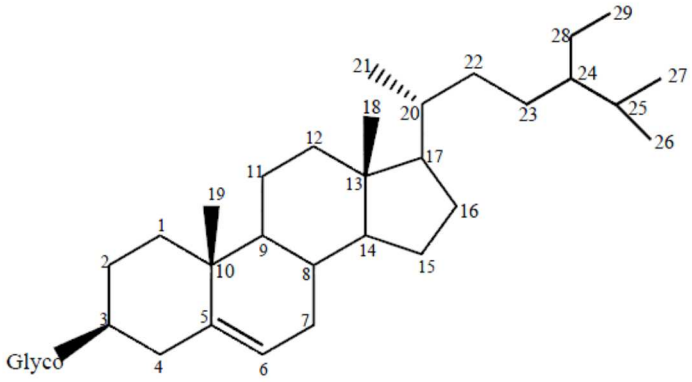

Figure 1: Structure of β-sitosterol [(1)]

Beta sitosterol is an active ingredient of wound healing ointment (MEBO). It can be used for the treatment of chemical burns, superficial (first degree) burns and adjunctive treatment for second and third degrees burns. It can also be used for the treatments of acute and chronic ulcers of skin and mucous membranes as well as post operative wounds [4-5].

Hydrogels are three dimensional ,hydrophilic ,polymeric ,crosslinked networks capable of absorbing large amounts of water or biological fluids (super absorbent) , the cross-linking facilitate the insolubility in water and provide required mechanical strength .

Hydrogels are highly biocompatible due to high water content and physiochemical similarity to the native extracellular matrix .The unique physical properties of hydrogels have particular interest in their use in drug delivery application, their highly porous structure can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogel for the aqueous environment in which they are swollen [6-8].

The objective of this study is to prepare β –sitosterol as topical hydrogel for inhibit inflammation, relieve pain, and improve healing by cold mechanical method using different types of gel-forming polymers with different concentration of each.

INGREDIENTS AND SUPPLIERS

β -sitosterol is supplied by Changdu biopurify phytochemica Ltd (China), Chitosan (Himedia labritory, India) Cabopol 940(Hi Media lab.,Ltd, Mumdai, India), Poloxamer 407 (Sigma, Germany), monobasic potassium phosphate (Fluka , Switzerland), tween 80 (Merk-Schuncherdt, Germany).

METHOD

Determination of β -sitosterol melting point

The melting point of β -sitosterol powder was measured using capillary tube method.

Differential Scanning Calorimetry (DSC) of β -sitosterol

Samples (3–5 mg) were placed in aluminum pan and heated in the DSC-60 (Shimadzu, Japan) at a constant rate of 10°C/min, in an atmosphere of nitrogen over a temperature up to300°C.

Fourier-transform Infrared Spectroscopy (FTIR) of β -sitosterol

It was performed using the infrared spectrophotometer (Lambda 7600, Australia). Samples of 2–3 mg were mixed with about 100 mg of dry potassium bromide powder and compressed into transparent discs then scanned over a wave range of 4000-400 cm-1 in FTIR instrument.

Calibration Curve of β -sitosterol

The required quantity of drug was dissolved in phosphate buffer pH7.4 containing 1% tween 80 to get a stock solution 1 mg/ml. From the stock solution, serial dilutions were made and absorbance of these dilute solutions was measured at 208 nm using double-beam ultraviolet (UV)/visible spectrophotometer (Cary, Australia).

Preparation of β -sitosterol hydrogel

Eight formulas of β -sitosterol hydrogel were prepared by cold mechanical method according to the table (1) using different concentrations of chitosan , carbopol 934, and plaxamer 407 as gelling forming agents .

F1-F3formulas which contain Chitosan were prepared by dispersing pre weight amount of polymer in sufficient quantity of distilled water with addition of 1 ml acetic acid, the mixtures were hand stirring for 10 minutes and put in an ultrasonic bath for additional 30 minutes to remove the entrapped air bubbles , the gels were allowed to swell for 48 hours at room temperature ,weighted amounts of β -sitosterol were added with continuous stirring ,then the final weight was completed to 100 g with distilled water .The formulas were placed in the refrigerator to complete the formation of hydrogel [9] .

F4-F6 that contain Carbopol were prepared by dispersing pre weight amount of polymers in sufficient quantity of distilled water (containing 1% tween80), the dispersion was homogenized using magnetic stirrer for 1 hour then left to 24 hours at room temperature for complete swelling of polymer, weighted amounts of β -sitosterol were added with continuous stirring and specific amount of tri-ethyl amine (TEA) was added. The final weight was completed to 100 g with distilled water [10].

Finally (F7-F8) were prepared by pre weighed amount of poloxamer 407was added to specified amount of distilled water, stirring for about 15 minutes and the solution was left in a refrigerator overnight. When the mixture became a clear solution, the drug and methyl paraben which dissolved in ethanol (10%) were thoroughly incorporated in the aqueous solution of poloxamer andmixed, and then complete the weight offormulas to final weight by water. Put the formula in the refrigerator to complete the formation of hydrogel [11].

Table (1) Composition of β –sitosterol Hydrogels Formulas (%w/w)

Formula No.	β – sitosterol	Chitosan	Carbopol 934	Poloxamer 407	Methyl-paraben	Distilled water (gm)
F 1	0.025	4	-	-	0.4	Up to 100
F2	0.025	3	-	-	0.4	Up to 100
F3	0.025	2	-	-	0.4	Up to 100
F4	0.025	-	1.5	-	0.4	Up to 100
F5	0.025	-	1	-	0.4	Up to 100
F6	0.025	-	0.5	-	0.4	Up to 100
F7	0.025	-	-	30	0.4	Up to 100
F8	0.025	-	-	25	0.4	Up to 100

Evaluation of Prepared Hydrogel

Visual examination

This examination considered a series of visual characteristics as consistency, homogeneity and clarity.

pH determination

The pH of all the prepared hydrogel was measured using pH-meter by putting the tip of electrode into the gel and after 2 minutes the result was recorded.

Swelling study

One gram sample from each formula was soaked into 5 ml of phosphate buffer pH7.4 and left for 1-3 hours , and then the excess buffer was removed and reweight the samples again[12] ,the following formula will be used to calculate the swelling ratio

Swelling ratio = (Ws –Wo / Wo) * 100

Ws weight of the swelling hydrogel at time t

Wo the initial weight of hydrogel

Spreadability measurement

Spreadability [13] was measured on the basis of slip and drag of gels. 1 g of hydrogel was placed in the centre of slide and spread over an area of 1 cm diameter .the gel was sandwiched between two slides by the application of 200 g weight. The spread diameter was recorded 5 minutes, and measured in cm.

In vitro dissolution test

10 g of gel (for each formula) was immersed in dissolution jar filled with 900 ml phosphate buffer solution (pH 7.4 containing 1% tween 80) at 37 ^{0} C using dissolution apparatus paddle type, the paddle was rotated at 50 rpm and samples of 5 ml were drawn at interval 30,60,120,180 minutes and replaced with equal volume of fresh phosphate buffer solution. The absorbance of each sample was measured by using the U V spectrophotometer.

Variable affecting release profile

Effect of polymer concentration on the in-vitro release

The effect of different concentrations of the used polymer on the release profile of the drug was studied; all formulas were subjected to this study.

Effect of different types of polymers on the release

One formula was selected from each polymer chitosan, carbapol 940 and poloxamer 407 (the highest release profile). Comparison between these formulas were achieved to show the effect of the polymer on the release.

RESULTS AND DISCUSSION

Determination of Melting Point

The melting point of **β –sitosterol** was found to be 140 ^{0} C as recorded in the reference .

DSC of β -sitosterol

The DSC thermogram of the drug in Figure 2 depicts a sharp endothermic peak at 140°C corresponding to the melting temperature of β -sitosterol. Such sharp endothermic peak signifies that drug used was in pure crystalline state.

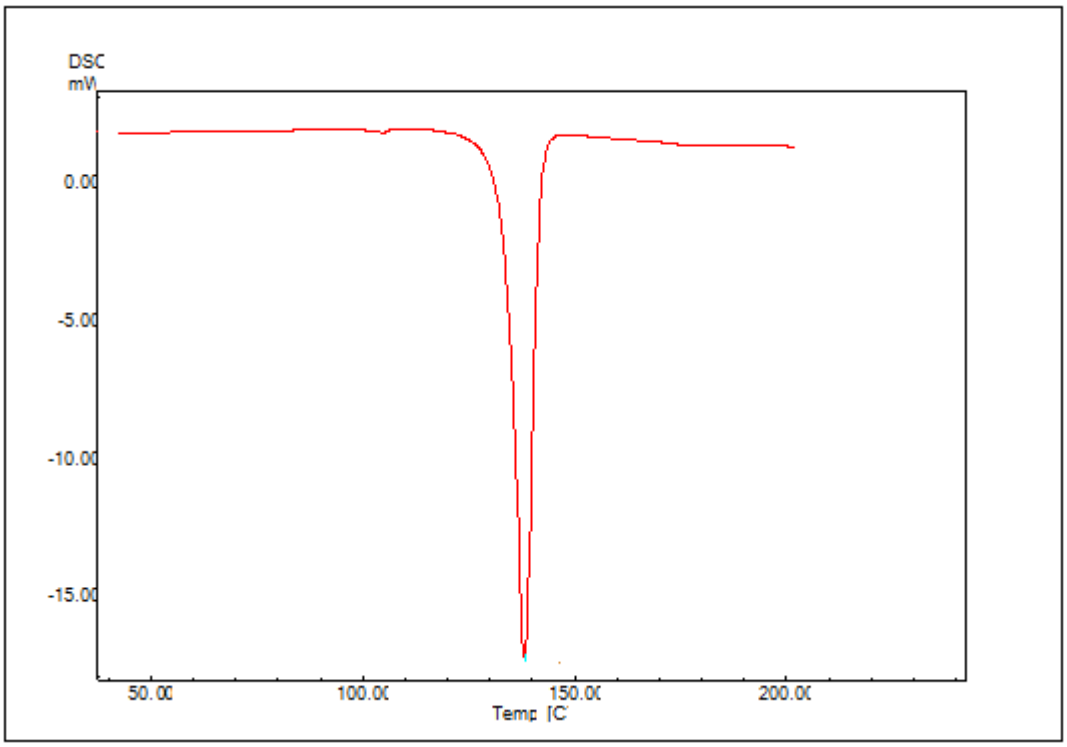

Figure (2): DSC thermogram of β –sitosterol

Fourier-transform Infrared Spectroscopy (FTIR) of β -sitosterol

The FTIR spectrum of β –sitosterol was illustrated in Figure 3 shows characteristic absorption peaks at3570.36 – 3186.51 cm^{-1} that is characteristic of O-H stretching , 2864.39 cm-1 is due aliphatics or C-H stretching or (CH_3), 1585.54 cm^{-1} due to double (C=C) stretching, 1016.52cm^{-1} due to (C-O). Other absorption frequencies include

3838.47cm^{-1} due to combination of absorption and 2353.23cm^{-1} due to overtone of the absorption, at 1269.2 cm^{-1} is a bending frequency for cyclic $(CH_2)_n$. These absorption frequencies resemble the absorption frequencies observed for β-sitosterol as resembled published data [14].

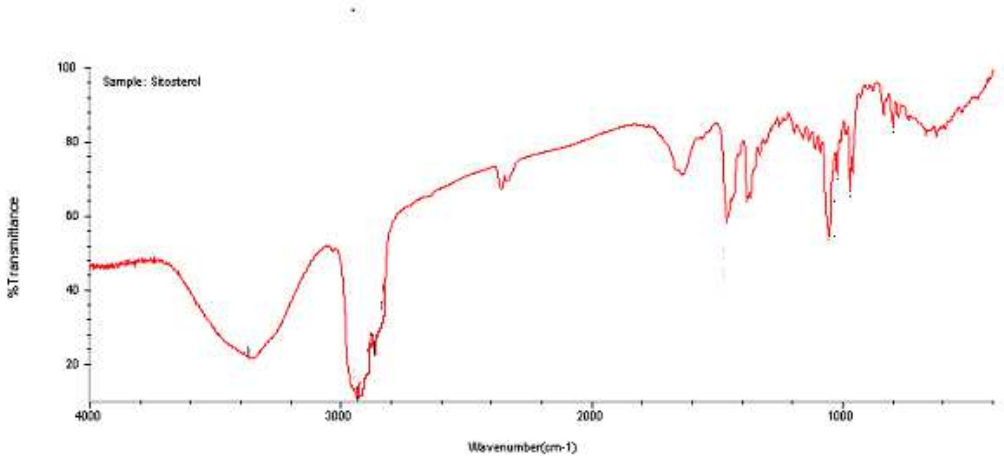

Figure 3 FTIR spectrum of β -sitosterol

Calibration Curve of β -sitosterol

The calibration curve of β -sitosterol in phosphate buffer solution pH7.4 containing 1% tween 80 (Figure 4) was found to be linear in the concentration range 1–5 µg /ml. The Beer's law was verified from the calibration curve by plotting a graph of concentration versus absorbance, the plot shown in Figure 4. Regression analysis showed very good correlation, the results obtained which indicate that the curves obey Beer–Lambert's law within the concentrations used [15].

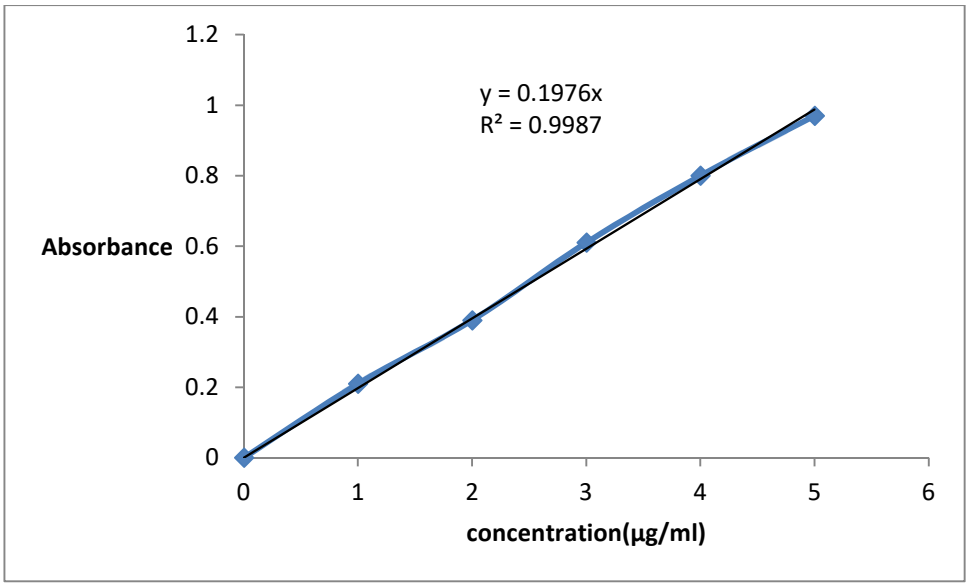

Figure 4: calibration curve of β -sitosterol in phosphate buffer pH 7.4 solution containing 1% tween 80

Evaluation of β -sitosterol hydrogel :

The visual examination of prepared gel indicated the homogenicity of all formulas, no phase separation, with semisolid texture and white to pale white smooth gel. pH value, Swelling ratio after 1 hr., Spread-ability cm. of all formulas were shown in the table (1). Swelling of the polymer depends on the concentration of the polymer, degree of cross- linking, ionic strength and presence of water [16]. Increasing in cross- linker concentration shows a direct negative effect due to tighter structure [17]
(F7-F8) being contain neutral gel have no swelling appearance may be due to full hydration of the polymer [18-19].

All the prepared hydrogels using different concentrations of different polymers were spreadable on the tissue surface as show in table (1). Spreadibilty of prepared gels was decreased as the polymer concentration increased [20]. F1 was not subjected for further investigations due to its unaccepted Spreadability results.

Table (1): pH values, Swelling Ratio, and Spreadability of Prepared Formulas

Formula no.	pH values	Swelling ratio after 1 hr.	Spread-ability(cm)\
F 1	5.3±0.4	-	-
F 2	5±052	70%±1%	4.5±0.25
F 3	4.58±0.72	50%±11%	7±1
F 4	7.1±0.4	100%±14%	3±1
F 5	7.2±0.45	90%±9%	4±0.5
F 6	8±0.81	50%±11%	6.5±0.75
F7	7.1±0.1	-	0.4±0.02
F8	7.0±0.08	-	0.6± 0.04

Effect of polymer concentration on the in-vitro release

It was found in the formulas (F2, F3) that the amount of drug release from chitosan hydrogel was decreased with increasing polymer concentration as in the figure (5).

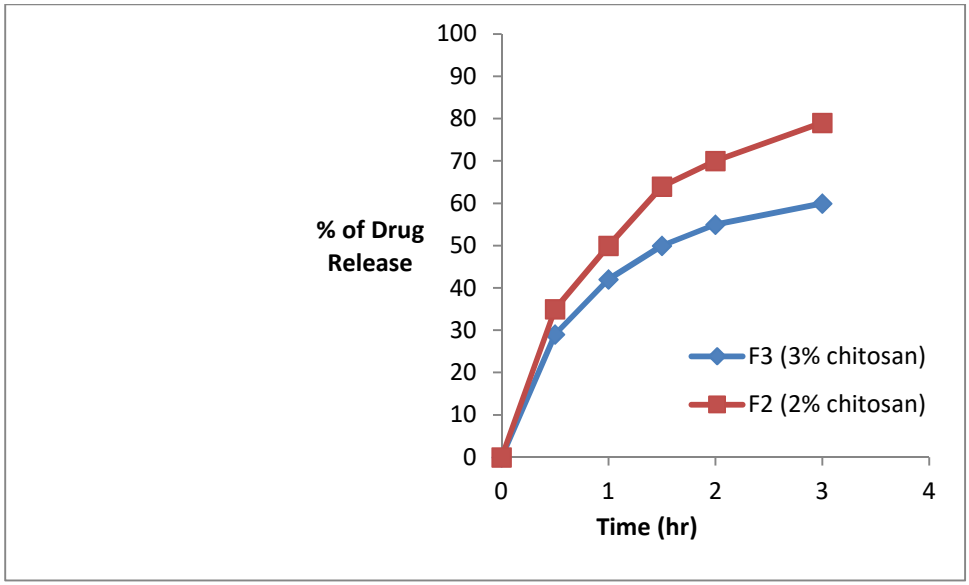

Figure (5): Effect of different chitosan concentrations on th β -sitosterol release in phosphate buffer pH 7.4 at 37 ^{0}C.

Also the amount of drug release from carbopol 934 hydrogel (F4, F5, F6) was decreased with increasing concentration of polymer as demonstrated in figure 6.

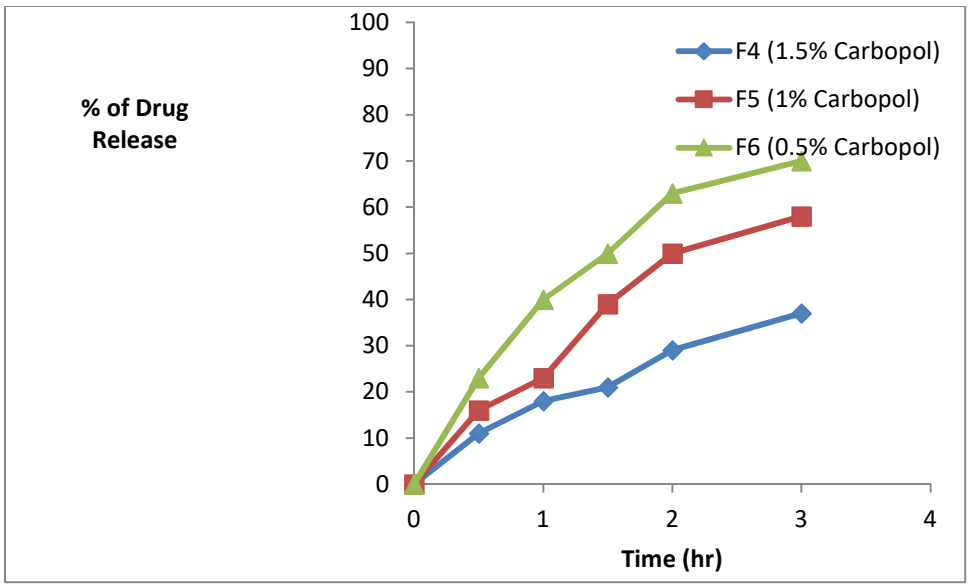

Figure (6): Effect of different carbopol concentrations on the β -sitosterol in phosphate buffer pH 7.4 at 37 ^{0}C.

The obtained result (Figure 7) showed that the increase in polyxamer 407 concentration led to decrease the release rate (F7- F8). It is hypothesized that the drug is released by diffusion through the extra micellar water channels of the hydrogels matrix, and higher concentration of polyxamer 407 causes smaller size of water channels [21-22].

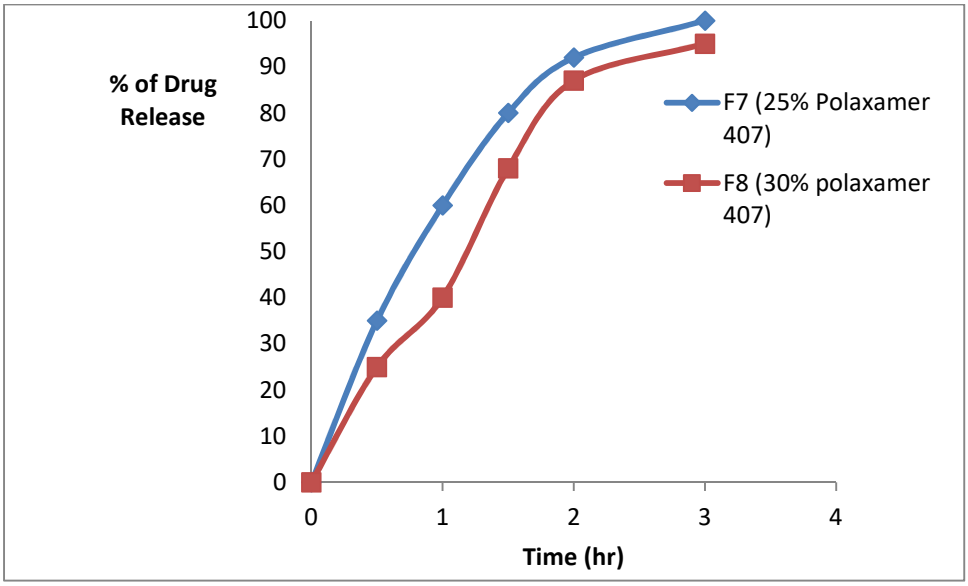

Figure (7): Effect of different polaxamer 407concentrations on the release of β -sitosterol in phosphate buffer pH 7.4

Effect of polymer types on *in vitro* release of β -sitosterol

Three formulas of different types of polymers which showd best release profile were chosen for this study and illustrated in figure (8).

Poloxamer presented as complete dissolution in three hours, and this feature can be used to prepare pharmaceutical formulation. The amount of β -sitosterol released, was found in the following order: poloxamer407 > chitosan > carbopol 943.

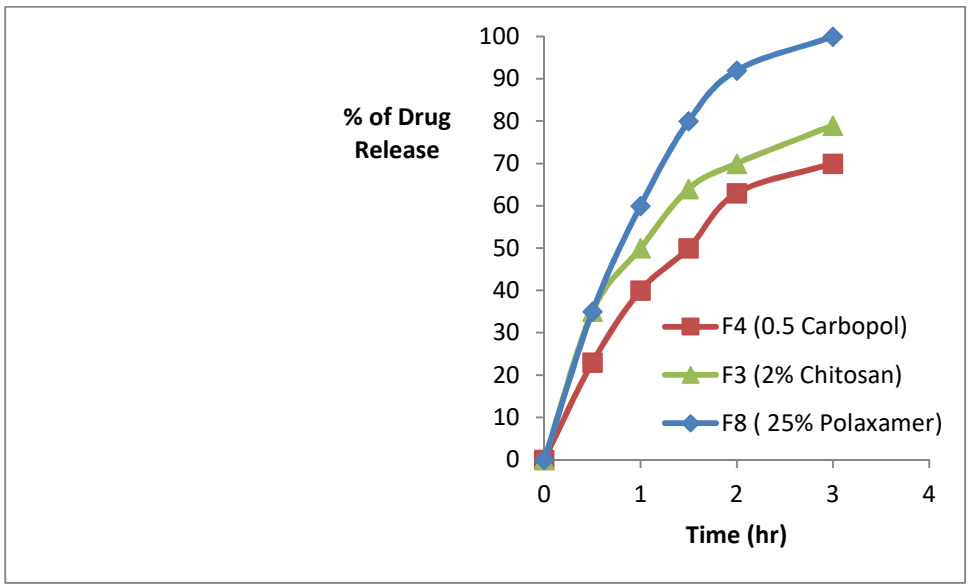

Figure (8): Effect of different polymers typed on the release of β -sitosterol in phosphate buffer pH 7.4

CONCLUSION

Based on overall results, β -sitosterol can be successfully prepared as topical hydrogel using poloxamer 407 of 25% (F8) as hydrogel forming polymer.

REFERENCES

1- The Merck index, Merck & Co., Inc, USA, 14th ed. 2006.

2- Gupta M.B., Nath R., Srivastava N., Shanker K., Kishor K., Bhargava K.P (1980). Anti-inflammatory and antipyretic activities of *β*-sitosterol. Planta Med 39(2):157-63.

3- Klippel K.F, Hiltl D.M, Schipp B. (1997). A multicentric, placebo-controlled, double-blind clinical trial of beta-sitosterol (phytosterol) for the treatment 133 of benign prostatic hyperplasia. German BPH-Phyto Study Group. Br J Urol .80(3):427-32.

4- Berges, R. R., Kassen, A., and Senge, T.(2000). Treatment of symptomatic benign prostatic hyperplasia with beta-sitosterol: an 18-month follow-up. BJU.Int. 85(7):842-846.

5-Lina Du, Tong, Yiguang Jin ,Junwei Jia , Yangpu Liu , Chang Su , Shanjiang Yu, and Xin Li(2012). A multifunctional in situ–forming hydrogel for wound healing. Intetnational journal of tissue repiar and regeneration, 20(6):904-910.

6- Gupta, P., Vermani, K. & Garg, S.(2002). Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discovery Today. 7(10): 569-579.

7-Amin, S. , Rajabnezhad, S. & Kohli, K.(2009) Hydrogels as potential drug delivery systems. Scientific Research and Essay. 3 (11): 1175-1183.

8-Swapnil S.(2014) Microemulsion based gel system, a noval approach for topical drug delivery. ntetnational journal of advance pharmaceutical science. 5(1):1777-1779.

9- Julia H. (2012). Potential of chitosan-based delivery in wound therapy.Journal of functionl biomaterial.3:39-44.

10-Sushil, R., Vaibhav, U., Santosh, B., Avinash, G., Sunil, K.& Shrishail, P.(2012) .Comparative evaluation of zidovudine ioaded hydrogels and emulgels .Research J. Pharm. and Tech. 5(1) : 41-45 .

11- Schmolka, I.R. (1972).Preparation and properties of pluronic pF–127 gels for the treatment of burns. J. Biomed. Mater. Res. 6: 571-582 .

12- Maswadeh, H.M., Kanaan, R.A., Aljarbou, A.H.& Al-Hanbali, O.A. (2010).Effect of different biological membranes on in vitro bioadhesion property". Drug Invention Today. 2(2):155-159.

13- Kirt K. (2013). Formulation and evaluation of topical hydrogel of mometasone furoate using different polymers. International journal of pharmaceutics science. 2(1):99-

14- Arjun, P., Jha, S., Murthy, P.N., Manik, M., and Sharone,(2010) . solation and Characterization of β – Sitosterol from the leaves of Hygrophila spinosa. International Journal of Pharma Science & Research. 1(2): 95 -100.

15- Manojkumar H., and Vijay S.(2013) Simultaneous estimation of Beta Sitosterol and Palmitic Acid from Methanolic extract of Caralluma Adscedens Var Fimbriata by UV Spetrophotometry.

Research journal of pharmaceutical, biological and chemical sciences . 4 (3): 226-230.

16- Kumar, L.& Verma, R. (2010). In vitro evaluation of topical gel prepared using natural polymer. International Journal of Drug Delivery. (2):58-63.

17- Hiremath,J.N. & Vishalakshi, B. (2012). Effect of Crosslinking on swelling behavior of IPN hydrogels of Guar Gum &Polyacrylamide ". Der Pharma Chemica. (3):946-955.

18- Singh, S., Jain, S., Muthu, M.S., Tiwari, S. & Tilak, R. (2008). Preparation and evaluation of buccal bioadhesive films containing clotrimazole. AAPS Pharm Sci Tech. 9(2): 660-667 .

19- Shilpa,K. & Poddar, S.S. (2010). Design of mucoadhesive vaginal metronidazole films. Acta Pharmaceutica Sciencia. (52):181-189.

20- Garg, A., Aggarwal, D.& Garg, S. (2002). Spreading of semisolid formulation". Pharm Tech. 9:89-105.

21- Huang,H.O.,Sokoloski,F.C. & Sheu, M.T.(1994). The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system. J. Pharm. Pharmacol. 46:636–642.

22- Hani,U.& Shivakumar, H.G. (2013). Development of miconazole nitrate thermosensitive bioadhesive vaignal gel for vaginal candidiasis. American Journal of Advanced Drug Delivery . 1(3): 358-368.