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Preparation and *in vitro* evaluation of oxybutynin transdermal gel formulations

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ABSTRACT

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Oxybutynin (OXB) is one of the most used medications to treat urinary incontinence especially in older population. Because of the first pass metabolism which produce the active metabolite N-Desethyl-Oxybutynin the reason for adverse side effects especially dry mouth, topical gel formulations were developed using Carbopol ETD2020 (0.5%, 1%), Poloxamer 407 (2%) , Hydroxy Ethyl Cellulose HEC (1%, 2%), Hydroxy Propyl Methyl Cellulose HPMC (2%, 4%). These formulations were evaluated for drug content, pH determination, viscosity measurement at 25°C and 37°C, and for in-vitro drug release using Enhancer Cell, In order to reach the best formulation, which is effective, and can reduces the adverse side effects. Then a comparison was made with the brand product. Among all the prepared formulations, F1 and F2 formulations prepared by using 0.5% and 1% Carbopol ETD2020 respectively, were the best because they exhibited the best in-vitro drug release, and there were no statistical differences in the drug release from F1 and F2 formulations and the drug release from the brand product

Key words: Carbopol ETD2020, Oxybutynin, transdermal, Urinary disorders.

INTRODUCTION

Oxybutynin (OXB) is a tertiary amine that has anticholinergic and direct spasmolytic effects on the bladder smooth muscle. It is widely used in the treatment of various forms of urinary incontinence and overactive bladder. Particularly, Oxybutynin (OXB) effectively treats neurologically caused bladder disorders.(1)

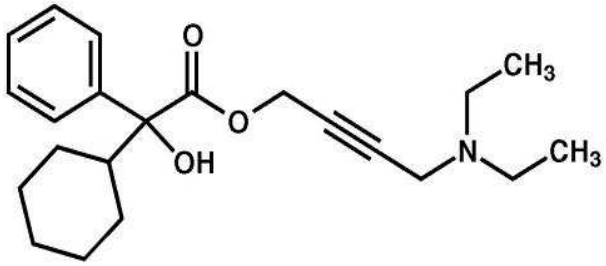


Figure (1) structural formula of oxybutynin

However, after oral administration of Oxybutynin (OXB), many patients discontinue its use because of unacceptable anticholinergic side effects such as dry mouth, dizziness, blurred vision, and constipation. In some cases, the adverse side effects are severe enough to persuade the patient to discontinue treatment.(2)These side effects have been associated with the presence of active metabolite of Oxybutynin, *N-desethyloxybutynin (N-DEO)*,which circulates in

concentrations approximately 4 to 10 times those of the parent compound. Presystemic metabolism of Oxybutynin (OXB) occurs primary because of extensive hepatic first-pass metabolism with a small contribution by intraluminal gastrointestinal metabolism, resulting in oral bioavailability of approximately 6% of the oral dose.(3)To reduce or even eliminate systemic anticholinergic adverse effects of Oxybutynin, novel anticholinergic agents and dosage forms have been currently developed that may avoid the hepatic first-pass metabolism so they exhibit the pharmacological effects. In fact, it has been shown that transdermal adhesive matrix patches of Oxybutynin (OXB) avoids that extensive presystemic metabolism, and directly introduces the drug into blood stream, and consequently enhances the bioavailability.(4) However, the skin irritation caused by the transdermal adhesive matrix patches remain to be a problem. Sometimes the irritation may discourage patients to discontinue the treatment, particularly for the long-term users.(5)Thus, the needs still remain for the improved formulations of Oxybutynin (OXB), which may significantly reduce the adverse side effects and skin irritation. Therefore, in the present study, gel formulations of Oxybutynin (OXB) using different gelling agents will be formed and evaluated for drug content, pH determination, Viscosity measurement at 25^{o}C and 37^{o}C, and for in-vitro drug release using Enhancer cell with cellulose acetate membrane (0.45 µm).Then a comparison between the prepared formulations and the brand product (Gelnique) was made in the same analytical conditions.

EXPERIMENTAL SECTION

Materials:

Oxybutynin chloride was obtained from Chempi Fine Chemicals-India. Hydroxypropylmethyl cellulose HPMC USP substitution type 2208was supplied from BASF global chemical-Germany. Hydroxyethyl cellulose HEC QP52000H, Propylene Glycol were obtained from Hutong Global-China. Carbopol ETD2020, Picric Acid were supplied from Shanghi Hope Chem-China. While Poloxamer407, Ethanol, Diisopropanolamine, Anhydrous Sodium Acetate, Glacial Acetic Acid, Monosodium Phosphate, Sodium Phosphate Di Basic, Chloroform were obtained from Merck-Germany. All chemicals are of analytical grades.

Methods:

Preparation of Oxybutynin Gel Formulations:

Oxybutynin concentration was 9% w/w in all the prepared formulations. Oxybutynin gels were prepared by dissolving an accurately weighed amount of Oxybutynin powder in water. In another beaker, a mixture of ethanol and propylene glycol was prepared. Then the mixture of ethanol and propylene glycol was added to the Oxybutynin solution using magnetic stirring bar. CarbopolETD2020 gels (0.5& 1% w/w), were prepared by dispersing the specified amount of Carbopol ETD2020, on drug, ethanol, propylene glycol and water mixture prepared as previously mentioned using magnetic stirring bar. The dispersion was left overnight to ensure complete swelling of the polymer. Carbopol gels were spontaneously formed by the addition of Diisopropanolamine dropwise until neutralization. For Hydroxypropylmethyl cellulose HPMC, and Hydroxyethyl cellulose HEC gel preparation, The specified amounts of the gelling polymers; HEC (1&2% w/w), HPMC (2&4% w/w), were added slowly to the drug, ethanol, propylene glycol and water mixture and allowed to soak overnight for Complete polymer solvation. The mixture was continuously stirred to get the required gels. For Poloxamer407 gels (2% w/w) preparation, the specified amount of poloxamer407 were added to the drug, ethanol, propylene glycol and water mixture. The mixture was continuously stirred using magnetic stirring bar to get the required gels, a clear transparent gel was obtained when the solution was left at room temperature. Table (1) shows the composition of the prepared formulations.

Table (1) composition of the prepared gel formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7
Oxybutynin chloride	9	9	9	9	9	9	9
Propylene glycol	15	15	15	15	15	15	15
Car ETD 2020	0.5	1					
Diisopropanolamine	0.5	1.2					
Poloxamer407			2				
HEC				1	2		
HPMC						2	4
Ethanol	10	10	10	10	10	10	10
Distilled water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

UV spectrophotometric method for quantitatively estimation of Oxybutynin(OXB):
UV spectrometric method was based on ion-pair complexation of picric acid with tertiary-amine group of OXB to form an UV active complex (picrate). Which was extracted to chloroform. In the analytical procedure, picric acid solution (PAS) was prepared; this solution consisted of 100 mg picric acid, 14.5 mg of anhydrous sodium acetate and 20 ml of glacial acetic acid in 480 ml of demineralized water. To each standard/sample solution (15 ml), 5 ml of picric acid solution (PAS) and 10 ml of chloroform were added in 60 ml separators and shaken for 2 min on a mechanical shaker operated at 50 motions/min. Chloroform layer was collected and scanned for UV spectrum in the range 200–500 nm on UV spectrophotometer. λ max was traced and calibration curve of absorbance versus concentration of standard solutions was constructed. The picrate complex showed quantifiable absorbance at 344 nm.(6)

Method validation:
The proposed method was validated for drug linearity, and precision. For Linearity determination, six standard solutions with concentrations evenly distributed across the range 10–110% on drug assay in the dissolution medium was established. (7) Precision was assessed using six replicates of solutions with 100% of drug concentration. The results were expressed as RSD%. (8)

Physical appearance, pH and actual drug content:
The prepared formulations were inspected visually for their color and homogeneity. The pH of the prepared medicated gel formulations was determined directly after preparation using a Sartorius PB-11, (Germany). The drug content was determined by dissolving accurately weighed 1 g of each prepared formulation in phosphate buffer pH 5.5 using magnetic stirrer in order to get complete solubility of the drug. These solutions were filtered through0.45 µm filters and analyzed by using UV-spectrophotometer at 344 nm.

Viscosity estimation:
The viscosity of the prepared gel formulations was determined using Brookfield DV-II ultra-programmable rheometer. The viscosity was measured at temperature 25ºC and 37ºC to study the effect of temperature on the gel viscosity.

In vitro drug release studies:
The in vitro release of Oxybutynin (OXB) from prepared formulations was studied using Enhancer cell, which is a Teflon cell with adjustable volume and a screw cap to retain the skin or artificial membrane. The paddle over disk method (USP apparatus 5) with Enhancer cell was used in this study, the vessels of the dissolution tester was filled with 900 ml phosphate buffer of pH (5-6) reflecting physiological skin conditions. For the same reason, the medium temperature was typically set at 32°C, and 100rpm was the typical agitation rate. A one-gram sample of each prepared formulation was accurately weighed and placed in the Enhancer cell with a semi permeable cellulose acetate membrane (previously immersed in phosphate buffer pH 5.5 for 24 hours). The study was carried out for 6 hours, Samples of 5 ml were withdrawn at 30 min interval and absorbance was measured spectrophotometrically at 344 nm. The volume of each sample was replaced by the same volume of fresh buffer (kept at the same temperature) to maintain constant volume. (9)

Analysis of the release data:
The release mechanisms of Oxybutynin from gel formulations prepared in this study were elucidated by fitting the in-vitro drug release data to some kinetic models. Regression analysis was adopted to compute the constants and correlation of data (r^2).(10,11,12)

Zero order kinetics:
$Q = k_* t$
Where Q is the cumulative drug release at time t, k is the zero order release constant, and t is the time(hours).

First order kinetics:
$\text{Log } Q_t = \text{Log } Q_o - k_* t/2.303$

Where Q_o is the initial amount of the active drug, $Q_t$is the released amount of the active drug at time t, k is the first order release constant, and t is the time (hours).

Hixson-Crowell kinetics:
$Q_O^{1/3} - Q_t^{1/3} = K_{h.c*}\ t$
Where Q_O is the initial amount of the active drug, Q_t is the released amount of the active drug at time t, $K_{h.c}$is the Hixson-Crowell release constant, and t is the time (hours).

Higuchi kinetics:
$Q = k_{h*}t^{1/2}$
Where Q is the amount of drug released at time t, $k_h$is the Higuchi release rate constant, and t is the time (hours).

Korsmeyerpeppas equation:
$F = Mt/M = k_{*}t^{n}$
Where Mt/M is the fraction of released drug at time t, n is the release exponent.

n value is indicative for the drug release mechanism; If $n \leq 0.5$ it is a fickian diffusion mechanism, $0.5 < n < 1$ it is a non-fickian mechanism (anomalous diffusion), and if $n = 1$ means that the release mechanism from the formulation follows a zero order mechanism (case-2 relaxation). In case of $n > 1$, it indicates a super case-2 transport. Anomalous diffusion or non-fickian diffusion refers to combination of both diffusion and erosion controlled release rate while case-2 relaxation and super case-2 transport refer to erosion of the polymeric chain.

Statistical analysis:
All studies were performed in triplicate, and the values were expressed as mean ± S.D. The data were analyzed by Student T-test at a significance level of 0.05.

RESULTS AND DISCUSSION

Method validation (linearity):
Linearity of the method was observed in expected concentration range 0.01-0.11 mg/ml (10–110%) in pH 5.5 phosphate buffer, as seen in figure(2). Statistical analysis of the calibration curve was done. Correlation coefficient (r^2 = 0.997) shows the validity of Beer's law. Indicating functional linearity between the concentration and the absorbance.

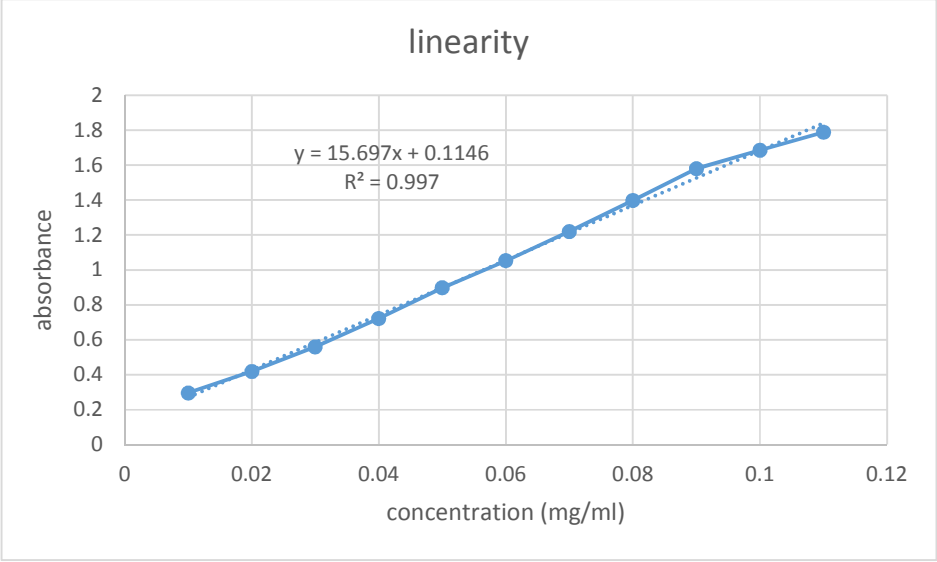


Figure (2) linearity determination of analytical procedure

Precision:
Precision is demonstrated by RSD% of six replicates of 0.1 mg/ml (100%) solutions. The relative standard deviation is 0.94 that proves the precision of the analytical method. As shown in table(2).

Table(*2*) precision of analytical procedure

No. of assay	Concentration(mcg/ml)
1	33.703
2	33.272
3	33.641
4	33.949
5	34.196
6	33.58
Average	33.724
RSD%	0.94

Physical appearance, pH and actual drug content:

All the tested formulations were homogenous. Formulations prepared with carbopol and poloxamer were much clear and transparent. Table (3) shows that the pH range of the prepared formulations is 5.8-6.8, which is safe to use on the human skin without causing irritation. Also, Table (3) shows that the Drug content range is 98.9–100.6%, which indicates the homogeneity in composition between the different prepared formulations.

Table (*3*) pH values and drug content of the prepared formulations

Formulation	pH	Drug Content %
F1	6.5±0.264	99.4 ± 0.02 %
F2	6.8±0.173	98.9 ± 0.03 %
F3	5.9±0.360	100.6 ± 0.01 %
F4	6±0.115	100.2 ± 0.05 %
F5	6.5±0.321	99.8 ± 0.02 %
F6	6.5±0.289	99.5 ± 0.1 %
F7	5.8±0.305	99 ± 0.01 %

Viscosity determination:

The changes in viscosity of the prepared gel formulations of Oxybutynin (OXB)due to the differences in type of the gelling agents, concentration of the gelling agents and the temperature are illustrated in table (4).

Table(4) viscosity of the prepared formulations at 25^{o}C and 37^{o}C

Formulation	Viscosity (cps) at 25^{o}C	Viscosity (cps) at 37^{o}C
F1	15167±12	13475±14
F2	20671±25	18923±18
F3	37365±74	39784±61
F4	9278±112	7677±22
F5	28500±109	23204±85
F6	5244±33	4932±50
F7	22683±41	19452±167

The viscosity has an inverse relationship with the temperature in all prepared gel formulations except for poloxamer formulations, as the viscosity increases in F3 formulations from 37365±74 cps at 25^{o}C to 39784±61 cps at 37^{o}C. That is because the poloxamer polymers consist of polyoxyethylene (POE) and polyoxypropylene (POP) units, representing hydrophilic and hydrophobic parts respectively. When the polymer is in cold water, hydrogen bonding keeps the hydrophobic portions of the poloxamer separate. When the temperature is increased, the hydrogen bonding is disrupted, and hydrophobic interactions cause a gel to be formed and viscosity to be increased. (13,14)Similar observations were obtained by Fetih. (15)

The viscosity increases upon increasing the polymer concentration for all the used polymers. Meshali et al.(16) reported that the increase in gel-viscosity with increasing HPMC concentration was due to the formation of dense network by entanglement or attraction between HPMC molecules through hydrogen bonds or van-der Waal forces. These forces increase as increasing the polymer concentration leading to aggregation that is manifested by increasing viscosity.

In vitro release studies:
Release of Oxybutynin (OXB) from carbopol gel formulations was inversely related with the polymer concentration. Formulation F1 prepared with carbopol (0.5% w/w) showed a higher Oxybutynin (OXB) release than formulation F2 prepared with carbopol 1% w/w (Fig.3), but the differences were not significant (P = 0.05).

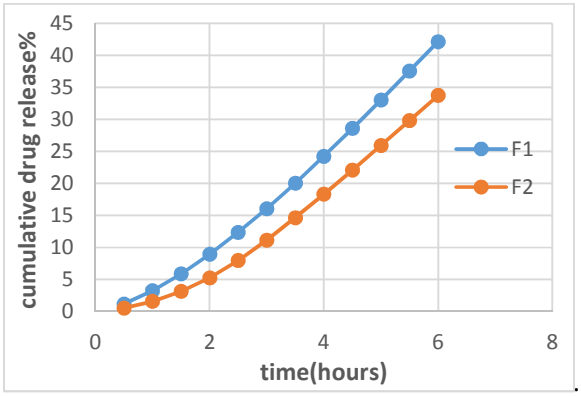
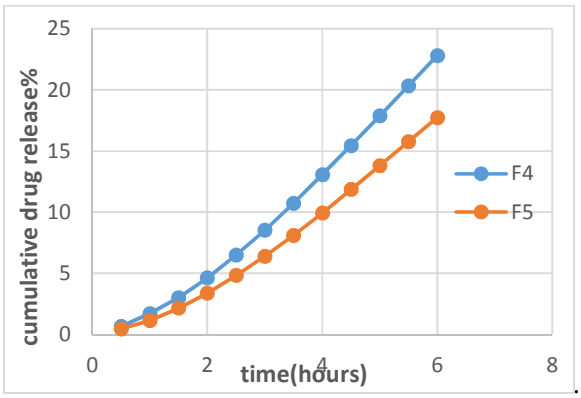


Figure (3) cumulative drug release of OXB from F1&F2 in phosphate buffer pH=5.5

The increase in carbopol concentration will increase the crosslink density which increase the tortuosity of the gel from which the drug release occur within the hydrogel network. These findings are in agreement with the data obtained by Macedo et al,(17)who studied the effect of increasing Carbopol concentration from 1 % to 2 % w/w. He found that the increase in polymer concentration had no significant effect on tolmetin release from gel formulations.

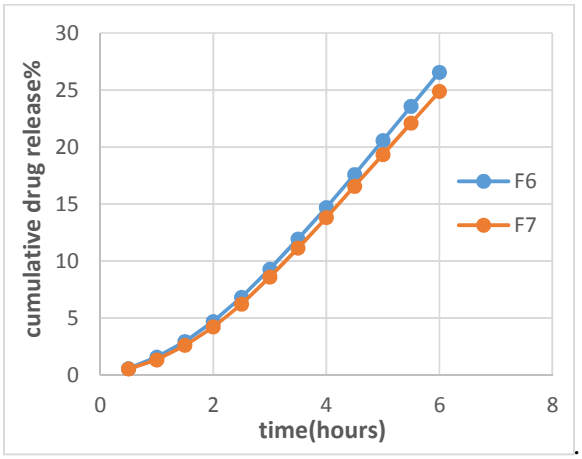
The structure of carbopol plays an important role in drug release, the main barrier for drug release from the aqueous Carbopol polymer gels is a mechanical layer formed by the random network of the polymer molecules that bind and entrap the surrounding water, and this aqueous phase may be the region for drug diffusion from the gel.

The percent of Oxybutynin (OXB) released from HEC gel formulations was found to decrease insignificantly (p= 0.05) with increasing the polymer concentration from 1% to 2% (Fig. 4).



Figure(4) cumulative drug release of OXB from F4& F5 in phosphate buffer pH=5.5

Figure (5) shows that Oxybutynin(OXB) release from HPMC formulations decreases when the concentration of the gelling agent increases, This is because as the proportion of these polymers in the matrix increased, there was an increase in the amount of water uptake and proportionally greater swelling leading to a thicker gel layer with longer diffusional paths.(18)



Figure(5) cumulative drug release of OXB from F6 &F7 in phosphate buffer pH=5.5

The percent of Oxybutynin (OXB) release from HPMC gel formulations didn't differ significantly (p = 0.05) upon changing the concentration of the polymer from 2 to 4 % w/w in spite of the increase in viscosity, as shown in figure (4). while, Songkro et al. (19) has found that increasing HPMC concentration from 4% to 10% w/w decreases the percent of nicotinamide released from the prepared gel formulations. So the results in this study may be explained by the low percent modification in the polymer concentration.

The results show that HPMC gel formulations exhibit higher drug release than HEC gel formulations. This result may be due to the low viscosity of HPMC gel formulations comparing to HEC gel formulations, and the greater hydrophilicity of HPMC. Cheong et al.(20) reported that the HPMC molecules are giant macromolecules compared to drug and water molecules. They are composed of hundreds of chain segments in random coils held tightly by hydrogen bonding. HPMC being a hydrophilic has a great affinity for water so when the polymer chain comes in contact with water, polymer-water interaction replaces the polymer-polymer attraction.

In general, the inverse relation between polymer concentration and Oxybutynin (OXB) release is in agreement with lauffer molecular diffusion theory of polymer gels. (21)The theory states that the diffusion of a solute is inversely proportional to the volume fraction occupied by the gel-forming agent.

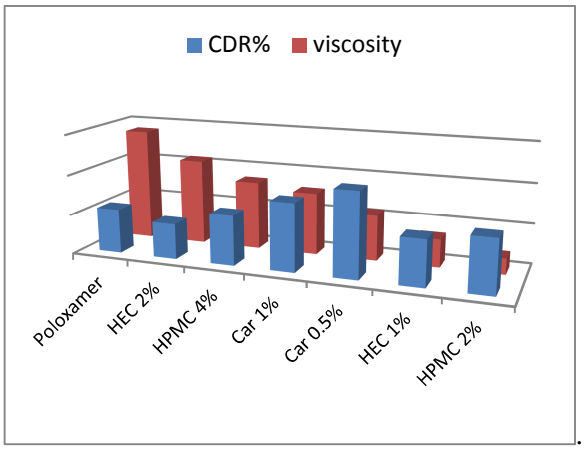


Figure (4) the relation between viscosity and CDR% from the prepared formulations

Figure (6) exhibits the relation between viscosity and cumulative drug release CDR % of Oxybutynin (OXB) from all the prepared formulations, It is obvious that they do not follow the expected behavior in which - when the viscosity of the gel increases then the drug release will decrease, so this lack of correlation indicates that the

viscosity is not the only factor that affects the drug release, and release mostly depends also on another factor which is the nature of the polymer.

These findings are in agreement with data of El Gendy et al.(22)who found a significant difference in flubiprofen release from Carbopol and Pluronic gels indicating that the drug release is influenced by the nature of each individual polymer. In addition, this result is in agreement with Patel et al.(23)

These observations are confirmed by the relationship of log viscosity and the percent of Oxybutynin (OXB) released after 6 hours from the prepared formulations. It was appeared that there is a weak correlation between the log viscosity and the percent of Oxybutynin (OXB) released from the prepared formulations. On the contrary, there was a good relationship between log viscosity and the drug release form F1&F2&F3 prepared by using carbopol 0.5%, carbopol1%, and poloxamer 2%, respectively (r^2 = 0.992), as seen in figure 7, this means that the drug release from the gel formulations prepared by using carbopol and poloxamer can be predicted through viscosity of these formulations. This result is in agreement with those obtained by Songkro et al. who stated that there is a high correlation between the log viscosity and cumulative drug release of nicotinamide from the prepared gels (r2 =0.7289).

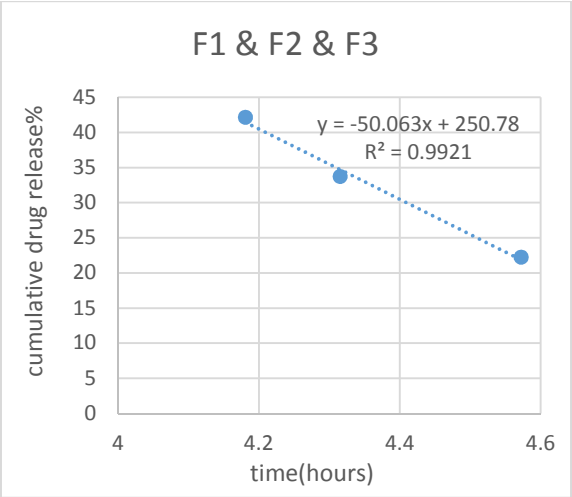


Figure (5) relationship between log viscosity and the cumulative drug release form F1 & F2 & F3

Previous results exhibit that the drug release from gel formulations prepared with different gelling agents depends on the nature and concentration of the gelling agents, and the drug release can be altered by changing either the nature, or the concentration of the gelling agents. Which means that an appropriate choice of the polymer used in preparing gel formulations is very important for achieving the desired drug release profile.

Analysis of the release data:

After in-vitro release study of Oxybutynin (OXB) from the prepared formulations, the data have been fitted to different kinetic models, in order to elucidate the release mechanisms of Oxybutynin from the prepared gel formulations, by determine the coefficient of regressionr^2 and the release exponent (n) of korsmeyer-Peppas equation.

The in-vitro release data of Oxybutynin (OXB) from all the prepared gel formulations are best fitted to Zero order kinetics, this means that the drug release from gel formulations is controlled by gel dissolution rather than the drug diffusion. This result is in good agreement with El-Houssieny(24), Moore et al. (25) , Paavola et al. (26) and Wang et al. (27) who found that the release of the studied drugs from poloxamer gels follows Zero order process and the drug release is controlled by gel dissolution rather than by drug diffusion. In addition, the release exponent (n) of korsmeyer-Peppas equation was found to be > 0.5 and < 1. This indicates that non-fickian diffusion or Anomalous diffusion controls the drug release from the gels, which means that the release rate is controlled by both; erosion and diffusion mechanisms.

Statistical analysis:

In vitro release study of Oxybutynin (OXB) from the brand product (Gelnique) was done in the same analytical conditions, and the obtained data was compared to the release data of Oxybutynin (OXB) from the prepared formulations. The in-vitro release data of Oxybutynin (OXB) from the brand product (Gelnique) have been fitted to different kinetic models, and it was appeared to be in favor of Zero order kinetic, this means that the drug release is controlled by gel dissolution rather than the drug diffusion. Moreover, when the drug release data from the brand product was fitted to korsmeyer-Peppas equation to compute the (n) value it was 0.663 ($1< n < 0.5$), which means that the release rate from the studied formulation is always controlled by erosion and diffusion mechanisms.

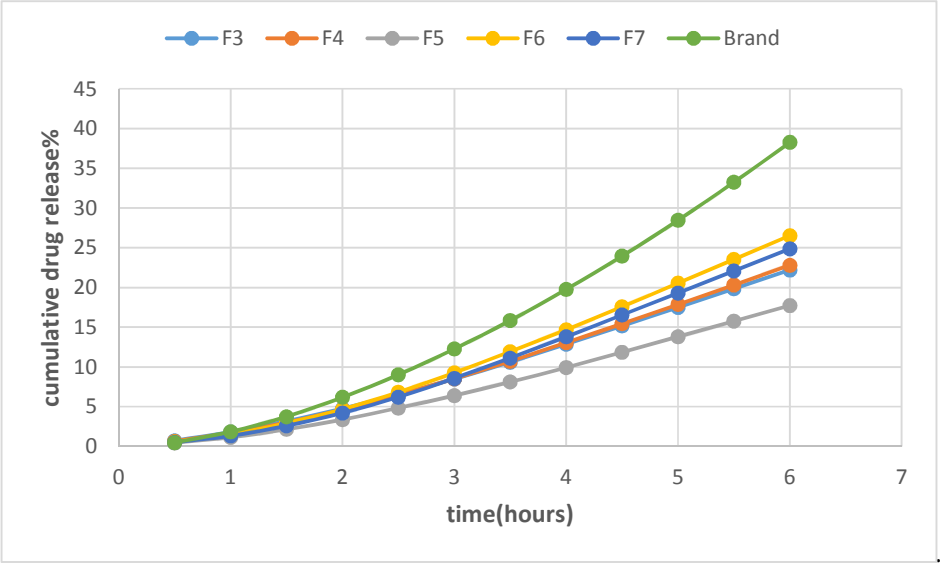


Figure (6) cumulative drug release of OXB from F3, F4, F5, F6, F7 and Brand product

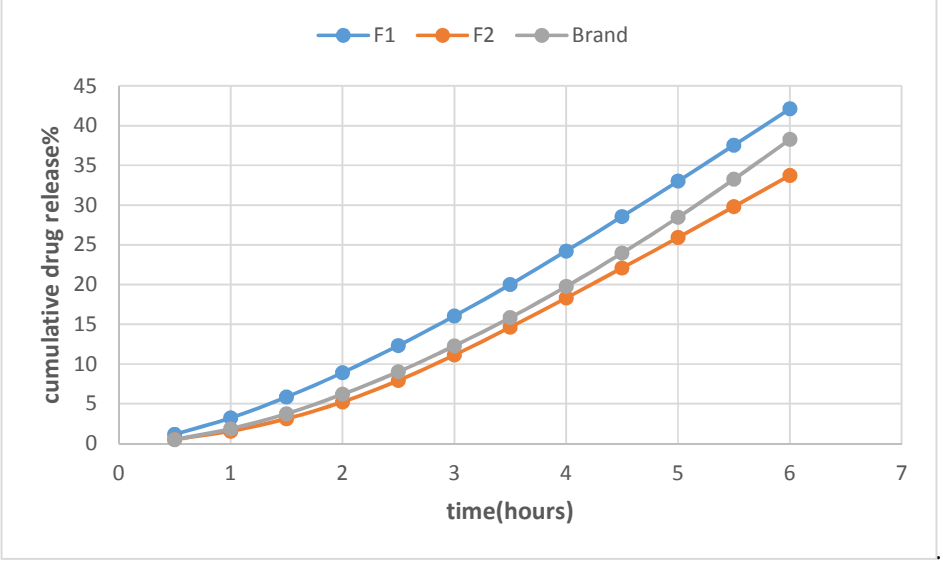


Figure (7) cumulative drug release of OXB from F1, F2, and Brand product

The brand product (Gelnique) showed a significantly ($p = 0.05$) higher Oxybutynin(OXB) release than F3, F4, F5, F6, and F7(Fig.8).While, there were no statistical differences ($p = 0.05$) in the percentage of drug release of OXB from the brand product and from F1(carbopol 0.5%), and F2(carbopol 1%) formulations at all-time intervals, (fig.9). All of that indicate to the in vitro bioequivalent between those formulations (F1, F2, and the brand product).

CONCLUSION

* All prepared gel formulations have good physical characteristics.
* In vitro drug release from gel formulations prepared with different gelling agents depends on the nature, and the concentration of the gelling agent. Which means that an appropriate choice of the polymer used in preparing gel formulation is very important in achieving the desired drug release profile.
* F1 and F2 formulations prepared by using 0.5% and 1% Carbopol ETD2020 respectively, were the best because they exhibited the highest in-vitro drug release of OXB after 6 hours.
* The in-vitro release data of OXB from the prepared gel formulations and from the brand product (Gelnique) are in favor of Zero order kinetics
* There were no statistical differences (p = 0.05) in the drug release from F1, F2 formulations prepared with carbopol ETD 0.5%, 1% respectively and the drug release from the brand product (Gelnique) at all time-intervals. Besides, The drug release from F1, F2 and brand product (Gelnique) was in favor of Zero order kinetics. Which indicate to the in vitro bioequivalence between these formulations.

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