

## In vitro Evaluation of the Effect of Using Different Gelling agents on the Release of Erythromycin from a Nanocubosomal gel

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### Abstract:

Preparation of a topical gel for erythromycin using optimized nano-sized cubosome dispersion, which is changed into a gel to get nanocubosomal gel using different gelling agents. The optimum nanocubosomal dispersion

containing 4.4% glyceryl mono oleate (GMO) (as an oil phase) and 0.6% poloxamer 407 (as an emulsifying agent) was converted into a gel using different gelling agents including carbopol 974, carbopol 940, carbopol 934 and hydroxyl propyl methylcellulose (HPMC) at different ratios of (1:1 and 1:2). Each prepared gel was then evaluated in vitro to determine the homogeneity, consistency, spreadability, viscosity and drug release. The selected formula (FG6) showed best homogeneity, consistency and spreadability as well as it produced initial burst release of 60.3% (within 1 hour) followed by prolonged release of 96.3% continued for 4 hours. In addition, the optimum gel formula (FG6) has shown remarkable antibacterial activity, which was significantly higher than the marketed gel (ERYTHROMYCIN GEL ® 2%) against four different strains of bacteria (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*).

**Key words:** cubosomes, nanotechnology, erythromycin, poloxamer 407, GMO

### تقييم مفعول استخدام مواد جل مختلفة على إطلاق دواء الارثروميسين من الجل

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الخلاصة:

تحضير هلام موضعي للإريثروميسين باستخدام تشتت مجسمات بحجم النانو ، والذي يتحول إلى هلام للحصول على جل نانوكوبوسومي باستخدام عوامل التجلد المختلفة. الهدف من هذا العمل هو تحسين المشتت النانوي من خلال دراسة تأثير العوامل المختلفة. تم تحويل أفضل تشتت نانوكوبوسومي يحتوي على 4.4% من الأوليك و 0.6% من بولوكسامير 407 كعامل استحلاب و قد تم تحويله إلى جل باستخدام أنواع مختلفة و نسب مختلفة (1:1 و 1:2) من عوامل التجلد مثل الكاربوبول 934 و الكاربوبول 940 و الكاربوبول 974 و الهيدروكسي بروبيل ميثيل سليولوز و تم تقييم كل الجل الناتج من حيث التجانس ، الاتساق ، قابلية الانتشار ، اللزوجة وإطلاق الدواء و كانت اف جي 6 هي الأفضل في كل هذه المقاييس و كان إطلاق الدواء منها 60.3% خلال الساعة الأولى و استمر 4 ساعات إلى ان وصل الإطلاق 96.3% كما أظهرت الصيغة المثلى نشاطاً مضاداً للبكتيريا ، والذي كان أعلى بكثير من الجل الذي تم تسويقه لأربع سلالات مختلفة من البكتيريا. الكلمات المفتاحية: ارثروميسين، نانوتكنولوجيا، كيووسوم، جلايسريل احادي الاوليت، بولوكسامير.

## Introduction:

Topical application of active pharmaceutical ingredients offers an advantage over the oral and intravenous administration by avoiding the systemic action throughout the localization of drug in one area <sup>[1]</sup>. Various dermatological products can be applied to the skin, which may vary in their consistency. The most widely used dermatological preparations are the semisolids <sup>[2]</sup>. Topical preparations are used for their local effect at the site of application by permeation of a drug into underlying layer of skin or mucous membrane. The preparations used for their local action include antibiotics, antifungals, skin emollients and protectants. In addition, topical preparations may be formulated to impart sustained local contact with minimum or no systemic drug absorption <sup>[3]</sup>. One of the best dosage forms within the semisolid preparations is the gel, which is used widely both in pharmaceutical and cosmetics preparations <sup>[4,5]</sup>. Gels are coherent systems composed of two phases: the interior one is composed of a polymer producing a coherent three-dimensional structure, which fixes the second phase (exterior phase). Intermolecular forces join the molecules of these two phases so increasing the viscosity by decreasing the mobility of these molecules. The bonds joining the particles of the interior phase have a relatively steady structure can be instigated by swelling of solid polymers, or by lowering solubility of the polymer in the solution, an important group of gels used in pharmaceutical purposes are hydrophilic gels, or hydrogels, usually made of hydrophilic polymers, which under certain situations, polymer concentration and jellify <sup>[6]</sup>. Gels preparations in general provide faster drug release compared with conventional topical preparations due to elevated water content which provide better dissolution of the drug but due to the solubility problems, most of hydrophobic (lipophilic) drugs cannot be formulated directly as hydrogel <sup>[7, 8]</sup>. The aim of this work is to study the effect of using different gelling agents to convert the prepared optimized nano cubosomal dispersion prepared in our laboratory to a suitable gel that can give immediate release to give quick effectiveness followed by prolonged release for complete healing.

## Materials and Methods:

### Materials

Erythromycin powder and poloxamer 407 was purchased from Sigma-Aldrich, Chemie GMBH, Germany, Glyceryl mono oleate was purchased from Hangzhou Hyper chemicals, China, Potassium phosphate monobasic, Carbopol 934, Carbopol 940 and Sodium hydroxide were purchased from Himedia, India, Carbopol 940 was purchased from Provizer pharma, India, HPMC was purchased from Fluka AG. Chem, Tri ethanolamine was purchased from Hopkins and Williams Ltd England. For all the experiments, deionized distilled water (DDW) was used.

### Preparation of Nanocubosomal Gel from Nanocubosomal Dispersion.

The optimized nanocubosomal dispersion prepared in our laboratory containing 4.4% GMO (as oil phase), 0.6% poloxamer 407 (as emulsifying agent) and 2% erythromycin (as a module drug). GMO and Poloxamer 407 were melted on a hot plate and then erythromycin was dispersed in the molten mixture. The molten mixture was then added drop by drop to the aqueous phase (phosphate buffer 7.4) at 70° C under mechanical stirring at 1500 rpm. The formulas were maintained under mechanical stirring for 2 hours and they were cooled to room temperature to solidify the lipid droplets. The formulas were sonicated for 10 minutes using probe ultra sonicator at 300 w in order to reduce the droplet size to the nano scale. All formulas were converted into gels using different gelling agents <sup>[9]</sup> (FG1-FG8) (Table 1) in different ratios (1:1, 1:2). This was accomplished by adding the gel base to the cubosomal dispersion in different ratios (1:1, 1:2) then mixed together using vortex mixer until smooth homogenous gel was obtained. Formulas were evaluated for their physical properties and drug release.

### Physical Appearance

All the prepared nanocubosomal gel formulations (FG1-FG8) were inspected visually for their homogeneity, color, grittiness, consistency and phase separation <sup>[10]</sup>.

### pH Determination

The pH of the nanocubosomal gel was measured to check if the pH of the formulas is suitable for skin application. The pH of all

nanocubosomal gel formulations (FG1-FG8) was determined using pH-meter and performed by positioning the tip of the electrode inside the nanocubosomal gel. After two minutes the reading was recorded <sup>[11]</sup>.

### Viscosity Studies

The viscosity of all formulas (FG1-FG8) was carried out with Brookfield digital viscometer using spindle number S-64. A specific weight of 100 g of the sample was put in a glass container and then the viscosity was measured at different rates (1, 1.5, 2, 2.5, 5, 10, 12, 20, 30, 50, 100 rpm), the sample temperature was maintained at 37 °C using a water bath. The viscosity was measured directly after 30 seconds <sup>[12]</sup>.

### Spreadability Studies

A sample of 0.5 g of each formula (FG1-FG8) was placed between two glass slides then a 0.5 Kg weight was applied and left for approximately 5 minutes when no further spreading was expected. Diameters of spread circles were marked and measured in centimeter (cm) and compared with the initial circle diameter (diameter of the spread circle that has been made without the use of the weight) <sup>[13, 14]</sup>.

### In Vitro Release of the Drug from the Prepared Nanocubosomal Gel Formula (FG1-FG8).

The release of erythromycin from the prepared nanocubosomal gel formulas (FG1-FG8) was done by using dialysis membrane (MWCO 2000 Da) <sup>[15]</sup>. Rotating paddle dissolution apparatus type II was used to measure the *in vitro* drug release from all prepared formulas. The sealed dialysis bag containing nanocubosomal gel formulas (equivalent to 60 mg erythromycin) was immersed in 250 mL <sup>[16]</sup> phosphate buffered (pH 7.4 dissolution media) <sup>[17]</sup> with a rotating speed of 50 rpm. The temperature of the medium was maintained at 37±0.5°C. Samples of 5 mL aliquots were withdrawn at suitable time periods (5,10,15,30,60,120,180 and 240 min) and immediately making the replacement with fresh dissolution medium. The drug content in the withdrawn sample was determined spectrophotometrically by using a UV-Visible spectrophotometer at the selected  $\lambda$  max 285 nm.

### Selection of the Optimum

#### Nanocubosomal Gel Formula

The selection of the optimum formula (FG6) was done according to the results obtained from the spreadability test, consistency and drug release profile.

### Antimicrobial Test for the Optimum Formula

The *in vitro* antibacterial activity of erythromycin from the prepared nanocubosomal gel formula (FG6) was done by using Muller Hinton agar plate, which was prepared by dispersing 28 g of powder in 1 liter of sterile deionized water, then swirled to mix and sterilized by autoclaving at 15 lbs pressure and 121 °C temperature for 15 min and then cooled down to 47 °C. Following this, the prepared medium was poured in sterile plates under aseptic conditions and allowed to solidify at 25 °C. Accurately 0.1 mL of each bacterial suspension (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*) having a uniform turbidity (106 CFU/mL) was distributed gently over the surface of the medium with a sterile glass spreader. The wells on each plate were made aseptically with corn borer having 6 mm diameter. In each of these holes, sufficient quantity (to fill the wells on each plate) of nanocubosomal gel optimized formula, a carbopol gel base alone (as negative control) and the marketed erythromycin gel (ERYTHROMYCIN GEL ® 2%) as positive control, were placed with the help of a micropipette, then the plates were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. The test was performed in triplicate for each type of bacteria <sup>[18, 19]</sup>.

### Statistical Analysis

The results of all experiments are presented as samples mean  $\pm$  of standard deviation. The results were analyzed using one-way ANOVA at ( $p < 0.05$ ) being recorded as significant results.

### Results and Discussion:

#### Physical Appearance

All the prepared formulas (FG1-FG8) appeared as a white homogenous creamy gel without grittiness.

### PH Determination

The pH of the nanocubosomal gel formulations (FG1-FG8) was measured and the pH values of all prepared formulas ranged from (7.22-7.44) and this in agreement with skin requirements for topical preparations lying in the normal pH range of skin (pH 3-9) <sup>[20]</sup> to avoid skin irritation and it is high enough to decrease the ionization of the erythromycin (pKa 8.6) to enhance its penetration through the skin. These results are explained by the chemistry of the constituents of the formulas. In the case of carbopol base gel formulas, the pH (above 7) was achieved by neutralization of the gel base with triethanolamine (TEA) in order to achieve the required pH <sup>[21]</sup>. While the pH of the rest of the formulas, which contain HPMC gel base was similar to the pH range of the prepared nanocubosomal dispersion, which was in the range (6.67-7.51).

### Viscosity Studies

Viscosity studies are very important in deciding the drug release from the prepared nanocubosomal gel formulation <sup>[22]</sup>. All the nanocubosomal gel formulas (FG1-FG8) possessed a pseudo-plastic flow, as the shear stress was increased, the normally disarranged molecules of the gelling material were caused to align their long axes in the direction of flow. Such orientation reduced the internal resistance of the material and hence decreased the viscosity <sup>[23]</sup>. Generally, the formulas with the gelling agent to dispersion ratio of 1:1 (FG1, FG3, FG5 and FG7) have a lower viscosity in comparison to the formulas that have a gelling agent to dispersion ratio of 1:2 (FG2, FG4, FG6 and FG8). Formulas containing carbopol 974 (FG5-FG6) have higher viscosity than those containing carbopol 934 (FG1-FG2) and carbopol 940 formulas (FG3-FG4) because Carbopol 974 has a higher molecular weight cross linked polymers of acrylic acid, which when neutralized have the ability to absorb and retain water, resulting in a very viscous gel, in addition, all formulas containing carbopol have a higher viscosity in comparison to HPMC based formulas (FG7-FG8) <sup>[24]</sup>. HPMC based gel formulas have lower viscosity than carbopol based gel formulas (FG1-FG6) due to the higher hygroscopicity of cellulose derivatives as compared to carbopol formulas <sup>[25]</sup> as shown in Figure 1.

### Spreadability Studies

In general, the spreadability is an important characteristic for topical formulations which indicates that the formulas are easy to be spread by a small application of shear and it shows the behavior of a gel when it comes out from its tube. It was found that as the concentration of the poloxamer 407 was increased from 0.6% to 2.4% (FG1 to FG8) the viscosity of the gel was also increased and the spreadability was decreased from 3.3 cm to 2.5 cm. The spreadability of the nanocubosomal gel formulas (FG1-FG8) is related to the firmness, time of shear, rate produced upon smearing and the viscosity of the target site as well as the temperature of the formulation <sup>[26]</sup>. All the prepared formulations produced an acceptable spreadability in the range of (2.5-3.3 cm) and these results agree with the reported data <sup>[27]</sup>.

### *In vitro* Release of Drug from the Prepared Nano Cubosomal Gel Formulas (FG1-FG8)

The effect of the gelling agents on the release of erythromycin from nanocubosomal gel formulas (FG1-FG8) is shown in (Figure 2). There was no significant increase in the release of the erythromycin ( $p < 0.05$ ) after 4 h from nanocubosomal gel formulas (FG1, FG3, FG5 and FG7) with the ratio of gelling agent to nanocubosomal dispersion ratio is 1:1 in comparison to nanocubosomal gel formulas (FG2, FG4, FG6 and FG8) with the gelling agent to nanocubosomal dispersion ratio is 1:2. It was observed that there was a significant increase ( $p < 0.05$ ) in the percentage of erythromycin released after 4 h from FG5 and FG6 formulas in comparison with other formulas (FG1, FG2, FG3, FG4, FG7 and FG8). These observations may be due to the low viscosity of carbopol 934; carbopol 940 and HPMC based formulas in comparison to carbopol 974 based formulas. These results agreed with the reported data where carbopol 974 usually used as a thickener for aqueous or hydro alcoholic gels <sup>[28]</sup> as shown in Figure 2. Since FG6 showed satisfactory drug release after 1 h (60.3%) and a high percentage of release after 4 h (96.3%) as well as to optimum spreading coefficient, consistency, viscosity and other physical properties, it was selected as the optimum formula for erythromycin nanocubosomal gel and subjected to further

investigation.

### Antimicrobial Test for the Optimum Formula

The selected formula (FG6) exhibited excellent antibacterial activity against (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*) in comparison with the results with marketed erythromycin gel (ERYTHROMYCIN GEL ® 2%). All the diameters of the inhibition zones (including the size of the cork borer) are illustrated in (Table 2). Figure 3 illustrates the inhibition zones of FG6, the marketed erythromycin gel as positive control (C+) and the gel base alone as negative control (C-) against the four types of bacteria. The inhibition zones of FG6 are significantly bigger in diameter than that of the marketed gel ( $p < 0.05$ ). Indicating that cubosomes

loaded with the drug have a greater antibacterial activity <sup>[29]</sup>.

### Conclusions

This work has shown a successful method for preparing gel using different gelling agents. Nanocubosomal gel (FG6) which containing 4.4% GMO as an oil phase, 0.6% poloxamer 407 as an emulsifying agent and 1% carbopol 974 at a ratio of 1:2 as a gelling agent was selected to be the optimized formula. This formula has excellent consistency, spreading properties and the highest percentage of drug release after four hours. In addition, it shows high antibacterial activity against (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) in comparison with the marketed gel.

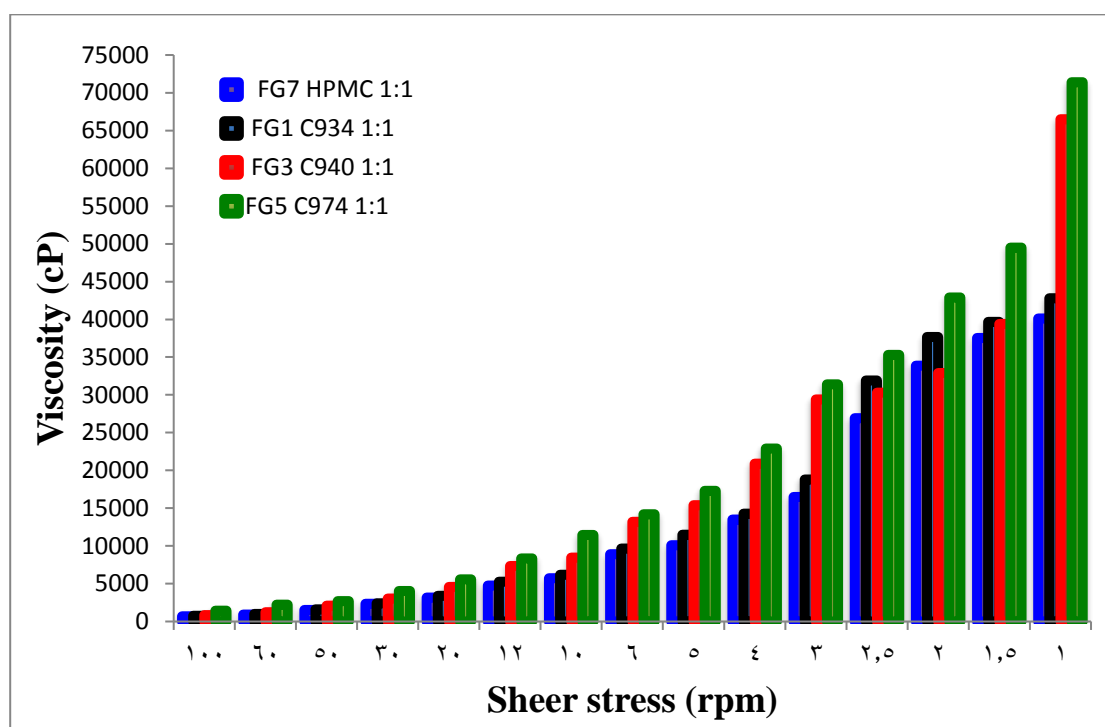
**Table (1): Composition of Nanocubosomal Gel Formulas Prepared Using Nanocubosomal Dispersion**

Formula Code	Gelling Agent	Ratio (Dispersion: gel)
FG1	Carbopol 934	1:1
FG2	Carbopol 934	1:2
FG3	Carbopol 940	1:1
FG4	Carbopol 940	1:2
FG5	Carbopol 974	1:1
FG6	Carbopol 974	1:2
FG7	HPMC	1:1
FG8	HPMC	1:2

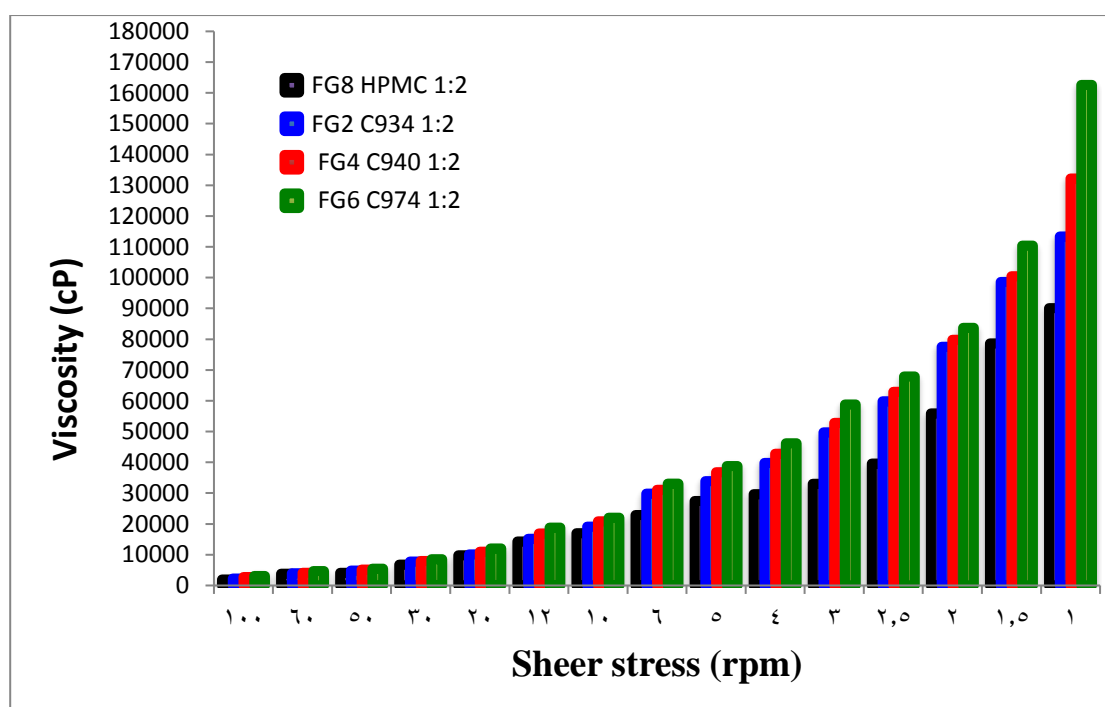
**Table (2): Antimicrobial Inhibition Zone Diameter of FG6, Marketed Erythromycin Gel (C+) and Carbopol Gel Base (C-). (Values are Mean  $\pm$ SD) (N=3)**

Formula Code	Inhibiti on zone in (mm)	S. Aureus	K. Pneum oniae	E. Coli	P. Aeruginosa
FG6		19	16	40	27
C+ (marketed ery. gel)		9	4	18	15
C- (carbopol gel base)		0	0	0	0



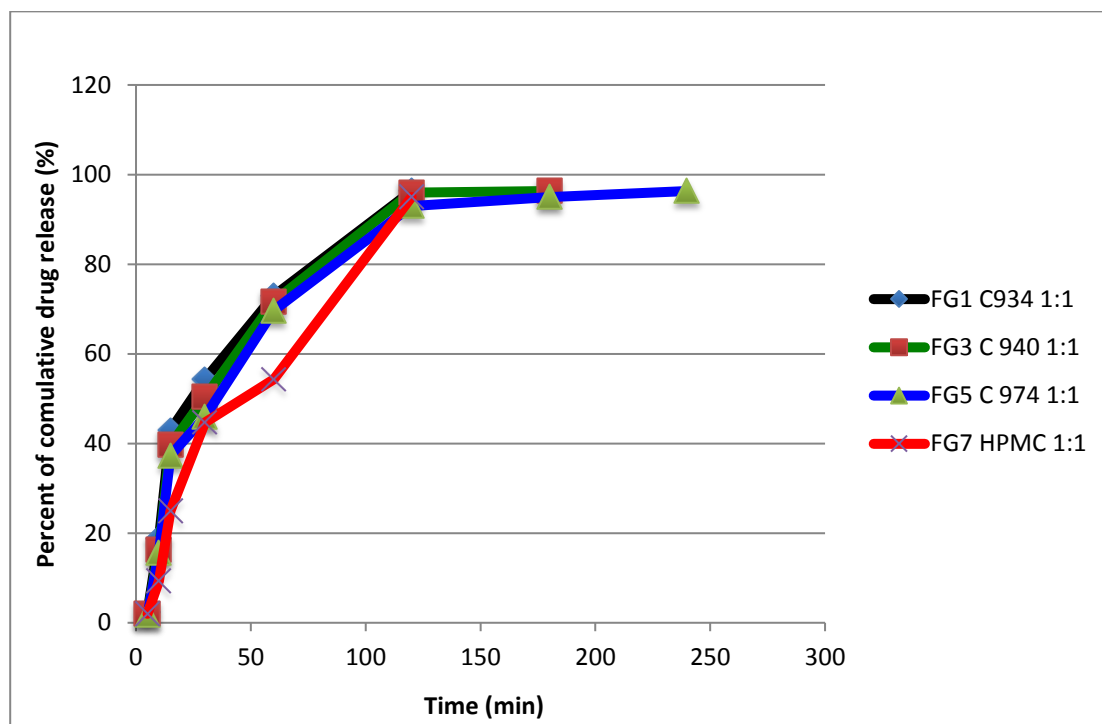


(A)

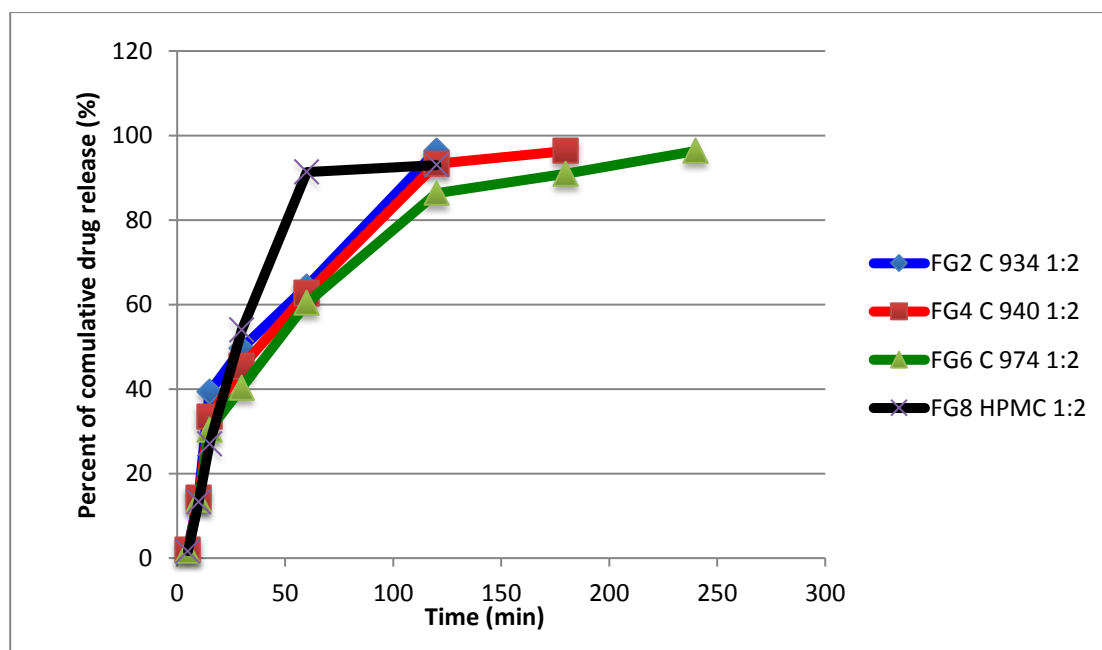


(B)

**Figure (1): (A) The viscosity of the prepared formulas (FG1, FG3, FG5, FG7) at different speed rates, (B) The viscosity of the prepared formulas (FG2, FG4, FG6, FG8) at different speed rates, (values are mean  $\pm$ SD) (n=3)**

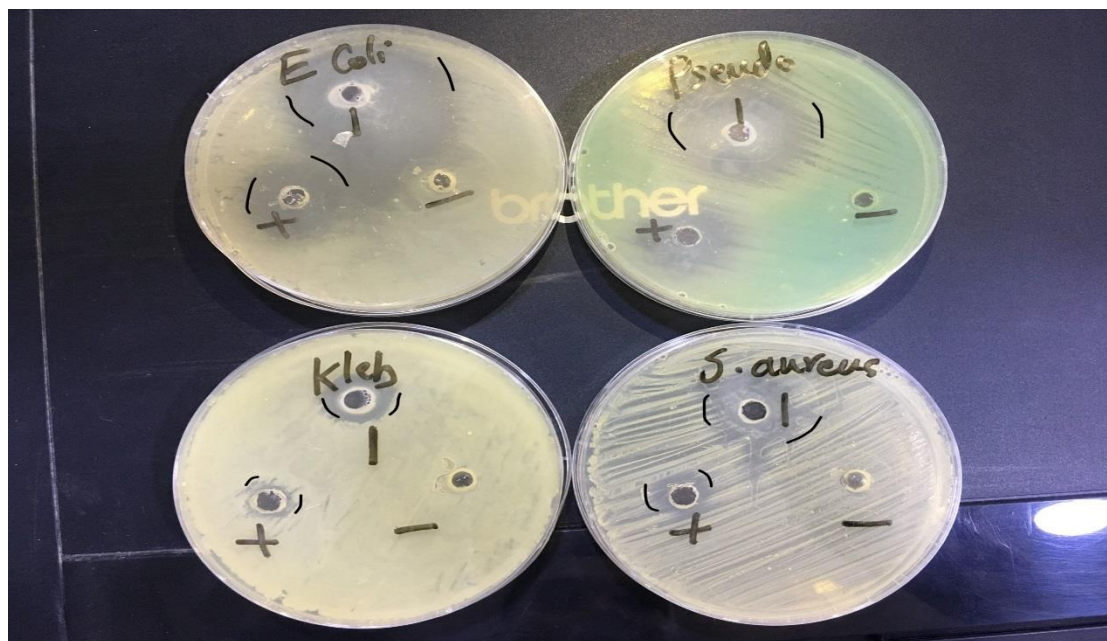


(A)



(B)

**Figure (2): (A) In-vitro Release Profile of Erythromycin from (FG1, FG3, FG5, FG7) Formulas (Values Represent Mean ±SD) (n=3) in Phosphate Buffer Solution (pH 7.4) at 37±1 °C, (B) In-Vitro Release Profile of Erythromycin from FG2, FG4, FG6, FG8 Formulas (Values Represent Mean ±SD) (n=3) in Phosphate Buffer Solution (pH 7.4) at 37±1 °C**



**Figure (3): Images of Zones of Inhibition of FG6, C+ and C- against (A) Staphylococcus Aureus, (B) Klebsiella Pneumoniae (C) Pseudomonas Aeruginosa and (D) Escherichia coli.**

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