Preparation and investigation of substance with polymer based nanoparticles

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Abstract

Topical drug administration is very important delivery system to localize drug anywhere in the body through skin, ophthalmic, vaginal and rectal routes. The challenge of developing a topical product stems from many requirements like product stability, skin penetration and cosmetic acceptability. There are many topical drug delivery systems to overcome such problems like liposomes, cubosomes, niosomes, ethosomes and catezomes as nanoparticles. Since erythromycin is a potent antibacterial drug but still its topical usage needs further optimization. There is a wide range of pharmaceutical dosage forms available for topical drug - delivery systems. The particles of the dispersed phase form an interlaced three-dimensional network due to the interaction between the polymer and the liquid dispersion medium. That showed that F5 gave 26.15% drug release within 1 h and the release increasingly continued for the next 2 h (48.56%), which was better than other formulas, and this could be due to the presence of high amount of poloxamer 407 in F5.

Keywords: *Erythromycin, Cubosome, GMO, Poloxamer 407.*

Introduction

Topical dosage form

Most topical pharmaceuticals are designed to have a local effect, thus preparations are made to keep the drugs in touch with the skin for as long as possible while limiting its systemic absorption.. Drug such as antiseptics, antifungal agent, skin emollients are applied to the skin for their local action. Avoidance of first pass metabolism is the main advantage of topical drug delivery system, Other benefits of topical preparations include avoiding the hazards and inconveniences of intravenous medication and the many circumstances of absorption, such as pH changes, the presence of various enzymes, and the time it takes for the stomach to empty(1).

In topical preparations, drug release rates are determined by the physiochemical characteristics of the carrier and the medication. (2, 3). In a topical medication delivery system, the drug is applied to the skin, where it diffuses out of the delivery system and is absorbed by the skin at the site of action. (4). Therefore, increasing the drug's release rate from the dose form might enhance percutaneous absorption. (5).

Structured liquid crystalline particles called cubosomes are made up of certain ratios of amphiphilic lipids. It is made up of lipid bilayers that are folded into honeycomb

structures in three dimensions and separated into two internal aqueous channels that are accessible to different bioactive substances including chemical medicines, peptides, and proteins(6). Because of their potential for controlled release via functionalization and other features like thermodynamic stability, bio-adhesion, and encapsulation of hydrophilic, hydrophobic, and amphiphilic substances, cubosomes are seen as promising vehicles for various routes of administration. (7). Self-assembly of lipids with a low molecular weight results in cubosomes. Due to their highest continuous interface and high interface to volume ratio, these lipids show great promise as potential adsorbents and host-guest applications. (8).

Erythromycin is a widely used antibiotic for treatment of several bacterial infections such as respiratory tract, skin, soft tissue, and urogenital infections (9).It is produced by fermentation using bacteria Saccharopolyspora erythraea, with the titre typically varying from 8-10 g/L (10).

Erythromycin is macrolide antibiotic mixture created by Streptomyces erythreus. The antibiotic erythromycin dissolves in alcohol and just slightly in water (11) and is typically used to the skin to treat conditions including acne, rosacea, skin infections, soft tissue inflammation, and inflammation of the gums and eyelids. Bacteriostatic activity is more prominent against Gram (+) bacteria. (12). Most topical commercial formulations of erythromycin consist of either high alcohol content solutions (52–92%) or petrolatum as well as mineral oil ointments. Local dryness, oiliness, and desquamation are only some of the documented side effects that may be attributable to the ingredients used in these formulations. (13, 14).this studies aim to invitro evaluation of the effect of polymers on release of erythromycin from nano cubosomal dispersion formulas. Optimizing the preparation through studying the effect of different characters and evaluating the antimicrobial activity of the all formulas.

Materials and Methods:

Materials

Erythromycin powder and poloxamer 407, Glyceryl mono oleate, HPMC, Carbopol was purchased from China, Potassium phosphate monobasic ,Tri ethanolamine was purchased from LOBA chem India , and Sodium hydroxide were purchased from SDFCL, India, For all the experiments, deionized distilled water (DDW) was used.

Method:

Preparation of Nano cubosomal Dispersion formula:

Modifications to the emulsification technique were used to create cubosomes. Mixing and melting 2 g of Glyceryl monooleate and 0.1 g of poloxamer 407 polymer in a water bath at 60°C is a brief summary of the process.

Erythromycin at a concentration of 0.25 g was added to the mixture and thoroughly dissolved with vigorous stirring. Preheated 95% distilled water (up to 70°C) was slowly added to this solution while stirring constantly. The finished formulation was allowed to rest at room temperature for a day after the addition of all of the water in order to achieve equilibrium.

During the course of storage, a two-phase system was discovered. Two hours of stirring at 1500 rpm at room temperature disrupted this. To produce cubosomes, the whole system was homogenized at 60°C at 1500 rpm for 1 minute. In order to preserve the cubosomes for future study, we placed them in glass vials that had been flushed with nitrogen. To improve

the process and formulation, many factors were held constant while the others were varied (15, 16, 17, 18).

1. Physical appearance

Nanocubosomal formulations (F1-F5) were visually evaluated for color, homogeneity and consistency, as well as phase separation (19).

2. PH determination

Using a digital pH meter, we immersed its electrode in a beaker containing 10 mL of each formulation (F1–F5) of nano cubosomal dispersion and recorded the findings two minutes later (20).

4. Separation test

In order to ensure the dispersions' physical stability, this test is crucial. Each nanocubosomal dispersion formula (F1-F5) was cooled by centrifugation at (10,000 rpm) for 30 minutes to test its resistance to separation (21).

5. Drug content

A clear solution was obtained after sonicating for 30 min with 1 mL of each nano cubosomal dispersion formula (F1, F5) added to a volumetric flask (100 mL) and 70 mL methanol appended. After getting the volume of the solution to 100 mL with methanol, after rupturing cubosomes with Triton X 100, the residual drug entrapped in cubosomes was measured (22).

D.C. = wt. of drug entrapped /wt. of cubosome *100%

6. Entrapment efficiency

Centrifugation was used to evaluate the entrapment efficiency of the obtained cubosomal formulations. Cubosomes were centrifuged for 10 min at 14000 rpm in a temperature-controlled environment. By using H2SO4 method, we were able to separate and determine the concentration of un-entrapped erythromycin in the supernatant. The quantity of erythromycin entrapped in cubosomes (%EE) was calculated using the entrapment efficiency with equation (23):

%EE = [Total drug] - [Estimated drug]/ [Total drug] x 100

7. Conductivity test:

The nature of created nanocubosomal dispersion formulations (F1-F5) was determined by measuring their electrical conductivity. If the exterior continuous phase is aqueous, then it is o/w (high conducting), but if the internal dispersion phase is aqueous, then it is w/o (poorly conducting) (not conducting).

The electrical conductivity (σ) was measured by placing the upright probe of a conduct meter into 10 ml of the produced formula in a beaker at room temperature; the meter's readings are displayed in µS/cm. (24).

8. Dilution test:

To ensure the physical stability of nanocubosomal dispersion formulations, a dilution or dispersibility test was performed (F1-F5). 1 ml of each formula (F1–F5) was diluted to 50 ml, one 100 ml, and 500 ml in distilled water at temperature of 37 ° c with constant stirring at 50 rpm, and the results were inspected visually for clarity, turbidity, as well as phase separation (25).

9. In vitro release

Erythromycin was released from nano cubosomal dispersion formulations (F1-F5) by passing them over a dialysis membrane (MWCO 7000 Da). Measurements of in vitro drug release from all formulations were made

using a rotating paddle of dissolution equipment of type 2(equivalent to 120 mg erythromycin).

The sealed dialysis bag including formulae was submerged at 50 rpm in a dissolving medium comprising 500 ml of phosphate buffered (pH 7.4) solution. The medium temperature was preconditioned and preserved at 34±0.5°C.

At predefined time intervals (appropriate times), 5 ml aliquots are extracted and promptly replaced with new dissolution media. The drug concentration was measured spectrophotometrically at 485 nm by using H2SO4 method (26,27)

Result and discussion:

1. Physical appearance

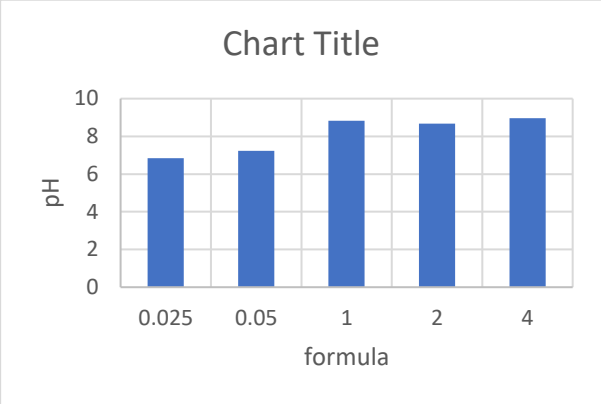
All the formulas (F1-F5) appeared as a white homogenous milky solution without any aggregates.

2. PH determination test:

The pH of the nano cubosomes formulations (F1-F5) was measured using pH meter. The pH values were ranged (6.85-8.97) as shown in figure (1), and indicated that erythromycin at PH more than 7 that mean erythromycin was unentrapped while at PH less or equal than 7 that mean erythromycin was entrapped.

Formula	Formula	PH
F1	20: 0.025	6.85
F2	20: 0.05	7.24
F3	20: 0.1	8.82
F4	20: 0.2	8.67
F5	20: 0.4	8.97

Figure (1): PH of the prepared cubosomal dispersion formulas (F1-F5)



3. Separation test

All five of the nano cubosomal dispersion formulae that were developed showed excellent stability following centrifugation, with no sedimentation, creaming, or phase separation being detected. This showed that the dispersion droplets' thermal motion (the Brownian motion) was stronger than the extrinsic forces of centrifugation and/or gravity. As shown in figure (2).

Figure (2): the prepared nano cubosomal dispersion formulas (F1-F5)

4. Conductivity test:

The electrical conductivity (σ) of nanocubosomal dispersions defined as a measurement of materials ability to conform the transport of an electric charge and it was measured to determine the nature or type of the external phase of dispersion and to detect

the phase inversion phenomena. Conductivity measurement depends on the higher conductivity of the water compared to the oil and give high values in o/w dispersions where water is the external phase. Table ( ) showed the results of conductivity test using conductmeter pen. The results indicated that all the prepared nanocubosomal dispersion formulas were o/w type since high conductivity (20 -140.1 µs/cm). The higher conductivity is due to a large percentage of water, which allows higher freedom for mobility of ions.

Table (1): Conductivity measurements of nano cubosomal dispersion formulas (F1-F5) measured by conductometer pen.

Formula code	Concentra. code	σ(µs/cm)
F1	20: 0.025	20 -21.5
F2	20: 0.05	29.4
F3	20: 0.1	130.1
F4	20: 0.2	135.1
F5	20: 0.4	140.1

5. Dilution test:

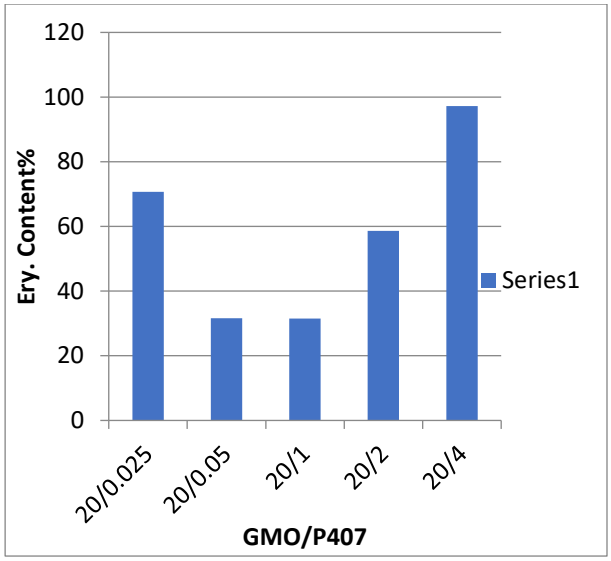
The dilution test is crucial for determining if the dispersion is oil-in-water (o/w) or water-in-oil (w/o). The addition of extra continuous phase (water) will not lead to breaking or separation of the emulsion if it is of the o/w type. There was no breaking or separation observed when water was added to any of the five formulations for nanocubosomal dispersion (F1-F5), suggesting that all five are of the o/w type.

6. Drug content

The drug content of all nano cubosomal dispersion formulas (F1-F5) results are in consistent with the requirements, indicating high adequacy of the preparation method and high content uniformity of the prepared formulas, where the prepared nano cubosomal dispersions have a drug content (97.21%) and this agreed with the acceptable range according to the USP (85-115%) (114), as shown in figure (3) table (2).

Formula	Concent. Formula	Drug content
F1	20/0.025	70.68437473
F2	20/0.05	31.60593527
F3	20/1	31.48955792
F4	20/2	58.67566451
F5	20/4	97.21543723

Figure (3): The drug content of the nano cubosomal dispersion formulas (F1-F5).

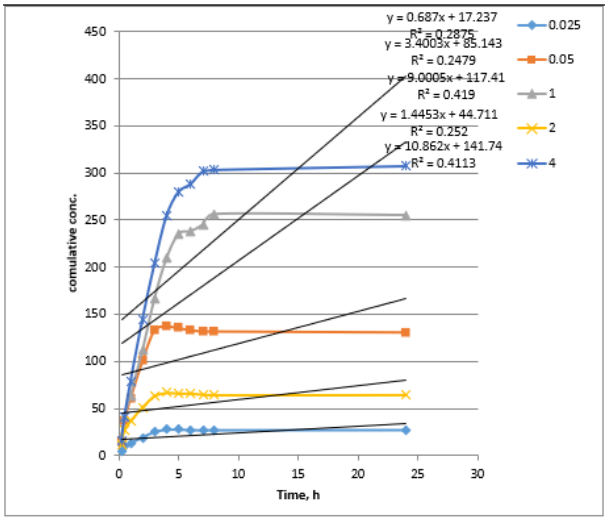


7. In-vitro dissolution test:

Erythromycin based nanocubosomal dispersions (F1-F5) was prepared with a different proportion of Poloxamer 407 and they were subjected to in vitro drug release studies at phosphate buffer (pH 7.4) using rotating paddle dissolution apparatus type II. The cumulative percentage of erythromycin at different time intervals for each nano cubosomal dispersion is shown in figure (4). The result of cumulative drug release showed that different Poloxamer 407 content had a

profound effect on drug release. The result showed that F5 gave 26.15% drug release within 1 h and the release increasingly continued for the next 2 h (48.56%), which was better than other formulas, and this could be due to the presence of high amount of poloxamer 407 in F5 (0.4 g).

Figure (4): (A) accumulative concent. Of In-vitro release profile of erythromycin from (F1-F5) formulas (B) accumulative percent of In-vitro release profile of erythromycin from F1-F5 formulas (C) higuchi comulative release % (D) first order kinetic (E) Krosmyer-pepas (F) hixon-crowell



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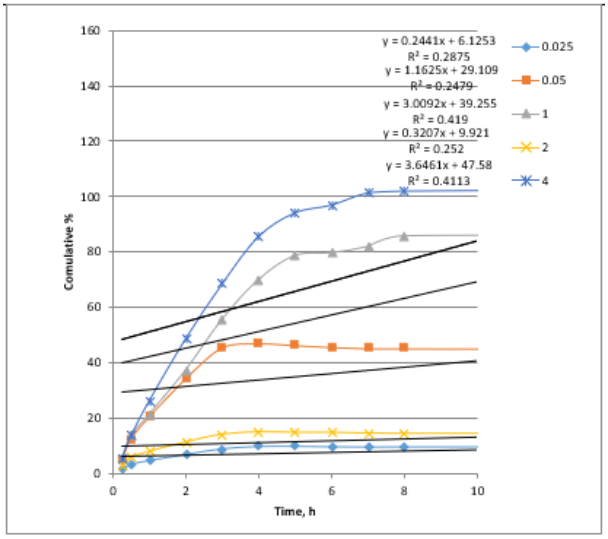

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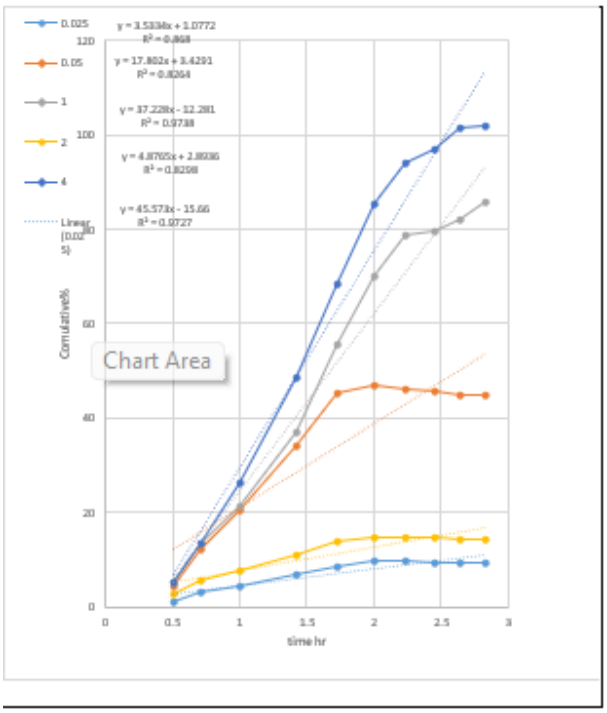

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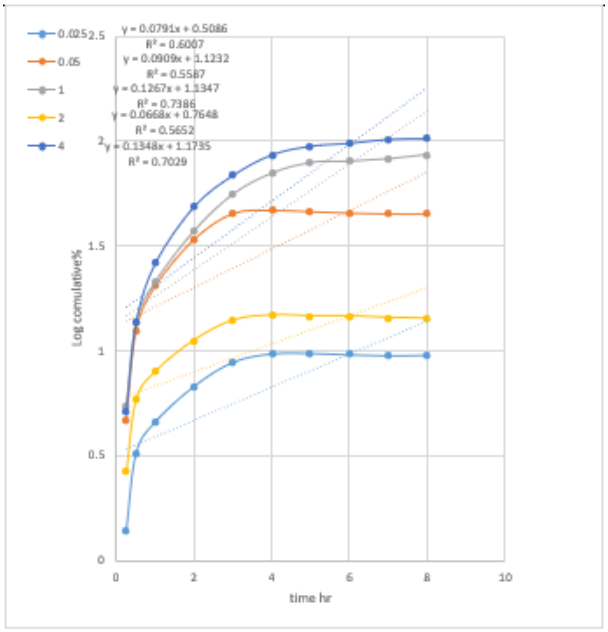

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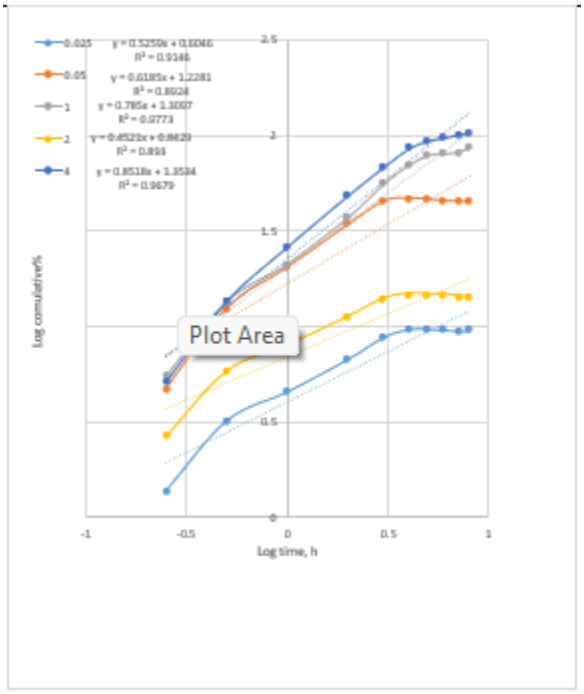

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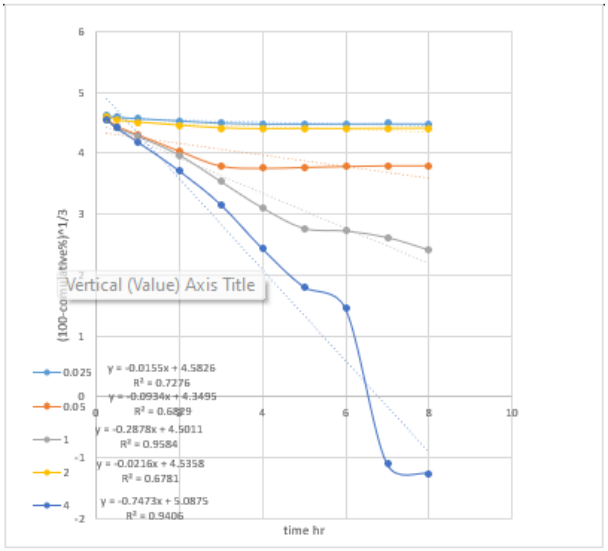
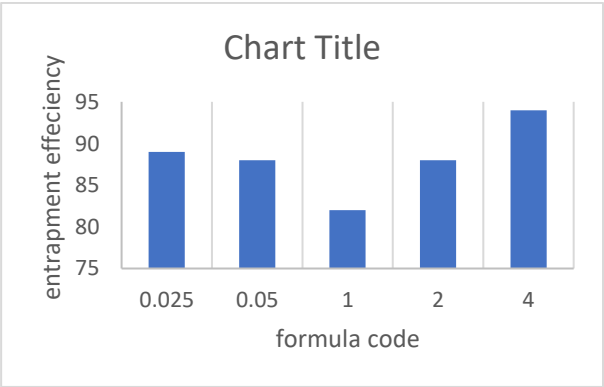


F

8. Entrapment efficiency

The efficiency of erythromycin nano cubosomal dispersion formulations for trapping is depicted graphically in figure (5). Due to the high entrapment efficiency, it can be concluded that the method used to manufacture the nanoparticles is effective and practical. Due to the high solubility of the drug (erythromycin) in the lipid part of the dispersion, the entrapment efficiency of (F5) is greater than the entrapment efficiency of (F1-F4) due to the presence of a high percent of lipid content (p407).

Figure (5): Entrapment efficiency of erythromycin nano cubosomal dispersion formulas.



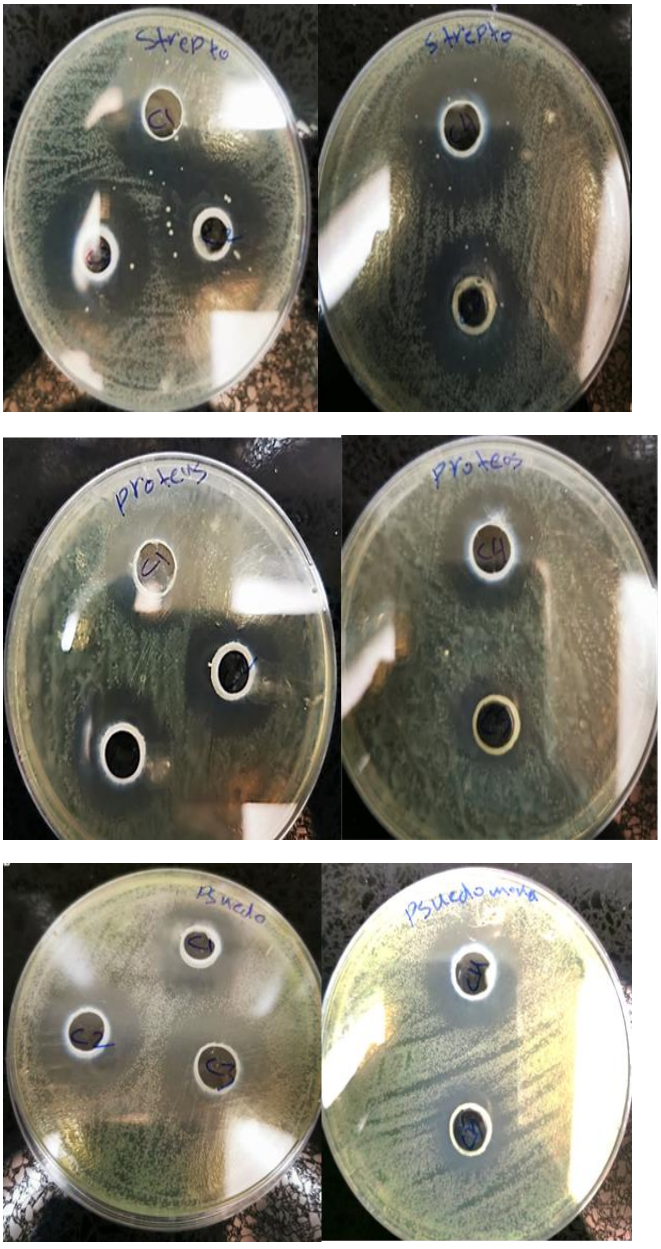
9. Antimicrobial test for the optimum formula F (1-5):

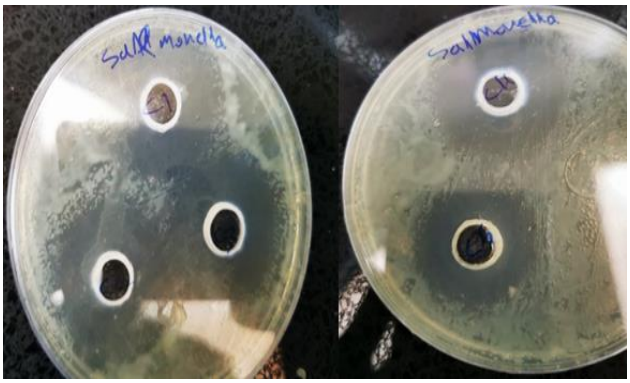
The formulas (F1-5) exhibited remarkable antibacterial activity against salmonella typhi , proteus vulgaris, streptococcus pyogenes and Pseudomonas aeruginosa as compared the results. All the diameters of inhibition zones (including the size of the cork borer) are illustrated in Table (3). Figure (6) illustrates the inhibition zones of F (1-5). The inhibition zones of F3 is significantly bigger in diameter than that of the other nanocubosomal dispersion formulas indicating that cubosomes loaded with the drug enhanced the penetration of the drug through the skin barrier and the bacterial cells wall and improved the antibacterial effect of erythromycin significantly.

Table 3. Antimicrobial inhibition zone diameter of F (1-5)

Formula code	Inhibition zone in (mm)	*Streptococcus*	*Proteus*	*Salmonella*	*P. aeruginosa*
F1		23	18	28	17
F2		20	15	22	19
F3		25	14	29	21
F4		28	20	25	15
F5		21	10	21	10

Figure (6): Photographs of zones of inhibition of F1=C1, F2=C2, F3=C3, F4=C4, F5=C5 against (A) Salmonella (B) proteus (C) Pseudomonas aeruginosa and (D) streptococcus.





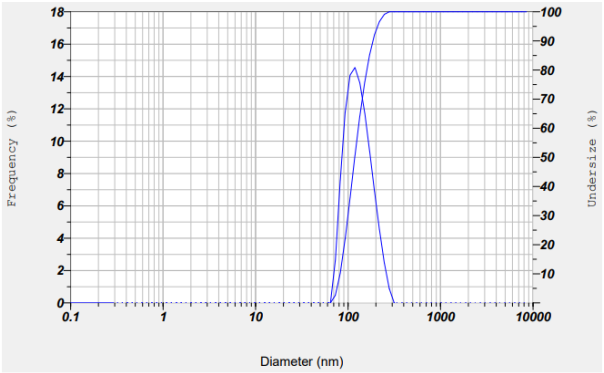
10. Zeta potential, Particle size and PDI determination

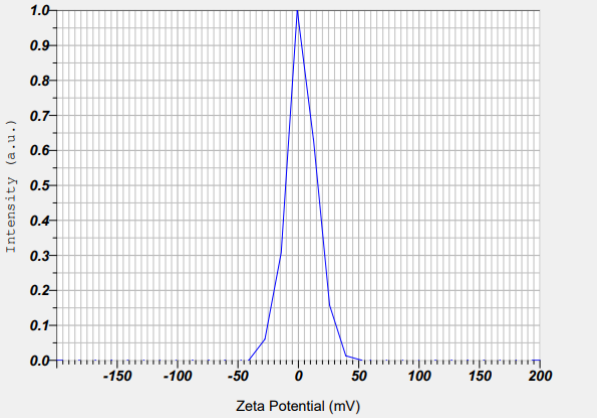
Particle size measurement was carried out to confirm that all particles of the dispersion are in nanometer size range as shown in figure (7). The effective diameter of the measured cubosomal dispersions is shown in (Table (4)). The effective diameter of all cubosomal dispersions was in the nanometer range (average particle size values ranged from 360.6 nm to 133.4 nm), with a polydispersity index of < 1, low value of polydispersity index is considered to be desirable for uniform distribution and homogeneity of nano-sized particles within the preparation. While pdI value > 0.7 to less than 1 is considered to have broad distribution of particle size (28) The zeta potential of all formulas are comparably low, ranging between (0.6 to 2.7 mV), which could be due to the absence of charge in cubosomal dispersion ingredients, this is mainly because of the use of water instead of buffer in preparation and use non-ionic surfactant (poloxamer 407) and the presence of fatty acid (oleic acid oil) which generally made the surface charge of the particle positive, the effect of zeta potential on the stability of nanoparticle was explained by rule of thumb. This rule states that values of zeta potential in the range (≤-30mV) to (≥+30mV) indicate that there is good stability and values in the range (≤ -60mV) to (≥ +60mV) indicate that there is an excellent stability in the formulation. For nonionic surfactant (poloxamer 407) that acts by steric stabilization, the value of zeta potential of 20mV or even lower provides an efficient stabilization due to that the non-ionic surfactant (poloxamer 407) provides a good steric stability for maintaining the stability of single layer nano dispersion(29).

Table (4): PdI, ʒ-potential and effective diameter, of prepared nano cubosomal dispersion formulas.

Formulation code	Observed pdI	ʒ-potential (mV)	Effective diameter in (nm)
F1(0.025)	0.772	1.4	360.6
F2(0.05)	0.219	1.0	186.4
F3(0.1)	0.274	1.3	102.0
F4(0.2)	1.135	0.6	461.2
F5(0.4)	0.602	2.7	133.4

Figure7: F5 (0.4) zeta potential and partical size.





11. Shape and Surface Morphology of nanaocubosomal dispersion formula:

The surface morphology of the cubosomes was determined using scanning electron microscopy (SEM). It was observed that the obtained cubosomes have a smooth surface and cubic in shape

Conclusion:

This work has shown a successful method for preparing nanocubosomal dispersion formula using different polymers. Nanocubosomal dispersion formula which containing 2 gm GMO as an oil phase, 0.4 gm poloxamer 407 as an emulsifying agent and contain 0.25 gm of erythromycin powder was selected to be the optimized formula. This formula has excellent consistency, spreading properties and the highest percentage of drug release after each hours.

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