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# DEVELOPMENT AND IN-VITRO EVALUATION OF CATECHIN LOADED ETHOSOMAL GEL FOR TOPICAL DELIVERY

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## ABSTRACT

Catechin is a polyphenol compound with antioxidant, anti-obesity and antiaging properties are formulated as a promising novel carrier in the form of ethosomes for topical drug delivery. In this study, catechin ethosomes are synthesized and then loaded into carbopol gel to make as a gel formulation. Ethosomes of catechin was formulated by using varying concentrations of catechin and cholestrerol, it was incorporated into carbapol 940 base gels to form ethosomal gel. The prepared ethosomal gels were also evaluated for particle size, viscosity, gel strength and spreadability. The Gel strength of the solution was found to be in the range of 93-140 seconds and spreadability was ranging from 27.13-78.63 gm.cm/sec. The viscosity obtained from various formulations was range between 2014 cps to 68385 cps under different rate of shear.

**Keywords:** Catechin ethosomal gel, Spreadability, Gel strength, Carbopol 940, Poloxamer 407

## INTRODUCTION

Catechin is a polyphenol which is predominantly present in green tea, guavas, red wine, apples and some other foods. [1] Green tea is considered as one of the foods that is rich in catechin content and it is widely consumed by many people nowadays. This is because green tea consists of several polyphenolic catechins which including epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), gallocatechin (GC) and epicatechin (EC). [2] Due to the presence of catechin polyphenols in green tea, this makes it beneficial for consumption and maintaining health as catechin possesses many health benefits such as antioxidant properties, antiaging effect, anti-obesity, anticarcinogenic (cell cycle arrest), anti-angiogenic (prevent formation of new blood vessel), antidiabetic, hypolipidemic and antiviral (Human papillomavirus, HIV) properties. [1,2,3] Regarding several advantages arise from catechin, it shows a great potential to be developed as a drug product for prevention and treatment of diseases. However, catechin is found not suitable for oral administration because it has very short half-life (approximately 1.25 hour), greater first-pass effect and poor oral bioavailability. Therefore, catechin has been extensively developed for topical application which was able to improve its bioavailability and enhance the drug delivery through skin layers by fabricating into liposome, ethosome and nanoemulsion gel. [1] Although liposomes and ethosomes are found to increase permeability of drug through stratum corneum barrier, but ethosomes can easily permeate through stratum corneum barrier to deeper layer of tissues as compared to liposomes. Liposomes are only managed to deliver the drugs to outer layers of skin which showed less therapeutic efficacy and permeability than ethosomes. [4] Hence, ethosomes are a better choice to encapsulate catechin for better outcome and performance.

Ethosomes are known as third generation lipid vesicle carriers which are mainly made up of phospholipids, high concentration (20-45%) of alcohol (ethanol or isopropyl alcohol) and water. [5,6,7] Ethosomes are soft and flexible vesicles that are extensively used as a choice for novel drug delivery as they can provide enhanced skin permeation, show high deformability and release drugs to deeper layers of skin. [8] Phospholipids are function as important vesicle forming component of ethosome, while ethanol is used as skin penetration enhancer to ease for penetration through skin barrier. [6,7] The functions of ethanol in ethosomal formulation including provision of flexible characteristics and stability to the vesicles, disruption of lipid structure of skin for easier penetration as well as improvement of drug loading. [7,8] Cholesterol is generally used to provide stability to the vesicle membrane with a concentration range from 0.1% to 1%. [8] Apart from that, ethosomes are widely selected by many researchers for studies of transdermal and topical drug delivery is due to several advantages of ethosomes. The advantages including enhanced permeation through skin, smaller in size, safe and non-toxic, non-invasive, improved stability, solubility and efficacy of many drugs. In addition, ethosomes are also suitable for encapsulation of large molecules of hydrophilic, lipophilic as well as

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amphiphilic drug molecules. [8] Ethosomes provide a number of applications not only in pharmaceutical field, but also in veterinary, cosmetic and nutraceutical markets. [7] In this study, catechin is formulated as ethosomal gel (semisolid form) for topical delivery which providing better patient compliance and improved stability.

## EXPERIMENTAL

*Materials:*

Catechin hydrate and cholesterol obtained from Sigma-Aldrich Co., Poloxamer 407 and carbopol 940 was purchased from Merck KGaA (Darmstadt, Germany)., Alcohol was purchased from R&M Chemical., All chemicals used in the formulation was of analytical grade.

## FORMULATION OF CATECHIN ETHOSOMES

Preparation of catechin ethosomes gel as shown in scheme 1. The various formulations containing different concentrations of catechin and cholesterol as shown in table 1

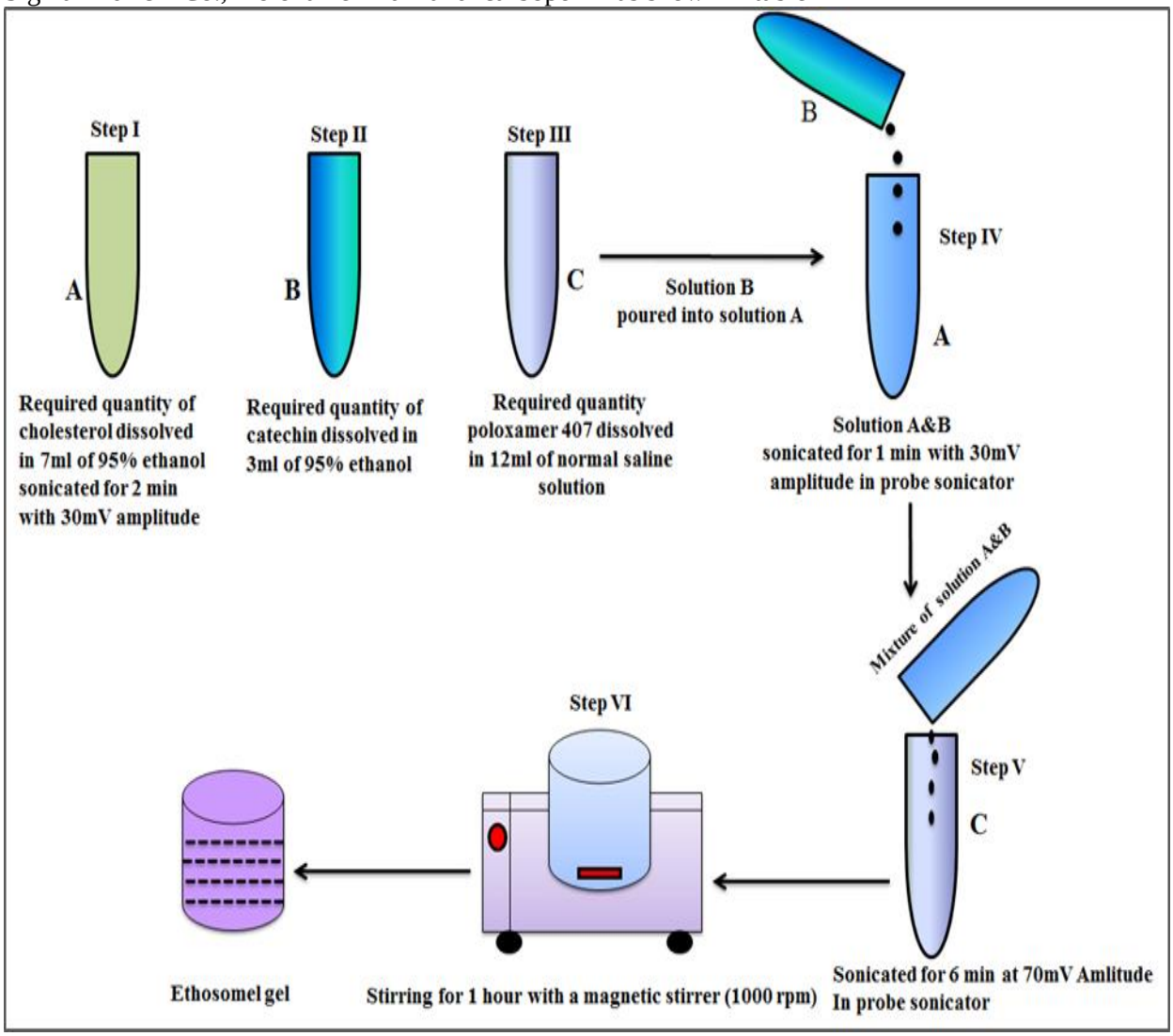


**Figure 1: Scheme of catechin loaded ethosomal gel**

**Table 1: Formulations of Catechin ethosomes**

| Formulation | Catechin (mg) | Cholestrerol (mg) | Ethanol (ml) | Normal saline (ml) | Poloxamer 407 (mg) |
|---|---|---|---|---|---|
| 1 | 15.4 | 200 | 10 | 12 | 150 |
| 2 | 30.8 | 200 | 10 | 12 | 150 |
| 3 | 46.2 | 200 | 10 | 12 | 150 |
| 4 | 15.4 | 100 | 10 | 12 | 150 |
| 5 | 15.4 | 300 | 10 | 12 | 150 |

**FORMULATION OF CATECHIN BASED ETHOSOMAL GEL**

Various ratio of gelling agent (0.5%, 0.75%, 1.0 %, 1.25 % and 1.5 % w/v) namely, carbopol 940 were added to form a ethosomal gel. Gelling agent was dispersed slowly in require ml of the ethosomes with the help of magnetic stirrer. The suitable gelling agent was selected on the basis of compatibility with ethosomes structure, feel and ease of spreadability.

**EVALUATION OF ETHOSOMAL GEL**

*Particle size:*

The average globule size of the ethosomes was determined by Anton Paar-Litesizer 500, Malaysia.

*Determination of Viscosities:*

The viscosities of various formulations were determined by using DV-II + Pro Viscometer (Brookfield). The unit of viscosity was measured in Centipoise.

*Measurement of Gel Strength:*

A sample of 50gm of ethosomal gel was placed in a 50 ml graduated cylinder. The apparatus for measuring gel strength (weighing 15.7 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the catechin ethosomal gel was determined by the time (seconds) where the apparatus took to sink 20 cm down through the prepared gel. [9]

*Determination of Spreadability:*

An excess of ethosomal gel sample was applied at the center of one glass slide. Two glass slides were compressed by placing 1kg weight on the upper slide and left for five minutes. A thread was tied on the upper slide and the other end of the thread was tied to a beaker. Around 76 g of weight was added to the beaker. The time taken in which the upper glass slide moved over to the lower glass slide in a fixed distance of 5 cm was noted down. [9] Spreadability was measured by a formula as followed: S = ML/T , where,

M= weight tide to upper slide (g)

L= distance moved on the glass slide (cm)

T= time taken (s)

*In vitro diffusion study:*

The in vitro permeation study was carried out by using modified Franz diffusion cell with egg membrane. The study was performed with acetate buffer solution (pH 7.4). 1 ml of ethosomal gel without separation of the non-entrapped drug was placed on the upper side of skin in donor compartment. The temperature of the assembly was maintained at 37±2º. Samples were withdrawn after every predetermine minutes from the receptor media through the sampling tube and at the same time, same amount of fresh receptor media was added to make sink condition. Withdrawn samples were analyzed for catechin constant using UV spectrophotometer.

*Fourier transform infrared spectroscopy (FT-IR):*

FT-IR spectrum of catechin (pure), cholesterol, poloxamer 407 and physical mixture of catechin, cholesterol and Poloxamer 407 were recorded on a Perkin Elmer, Germany. FT-IR spectrometer with 3 scans in the scan range of 10000–370 cm−1.[10]

**RESULTS AND DISCUSSION**

Ethosomal gel formulations of SEM image analysis, nano-sized (235–322 nm) spherical or near-spherical-shaped lipidic vesicular structures are clearly observed figure 2. Appearances of microscopic image are shown in figure 3.

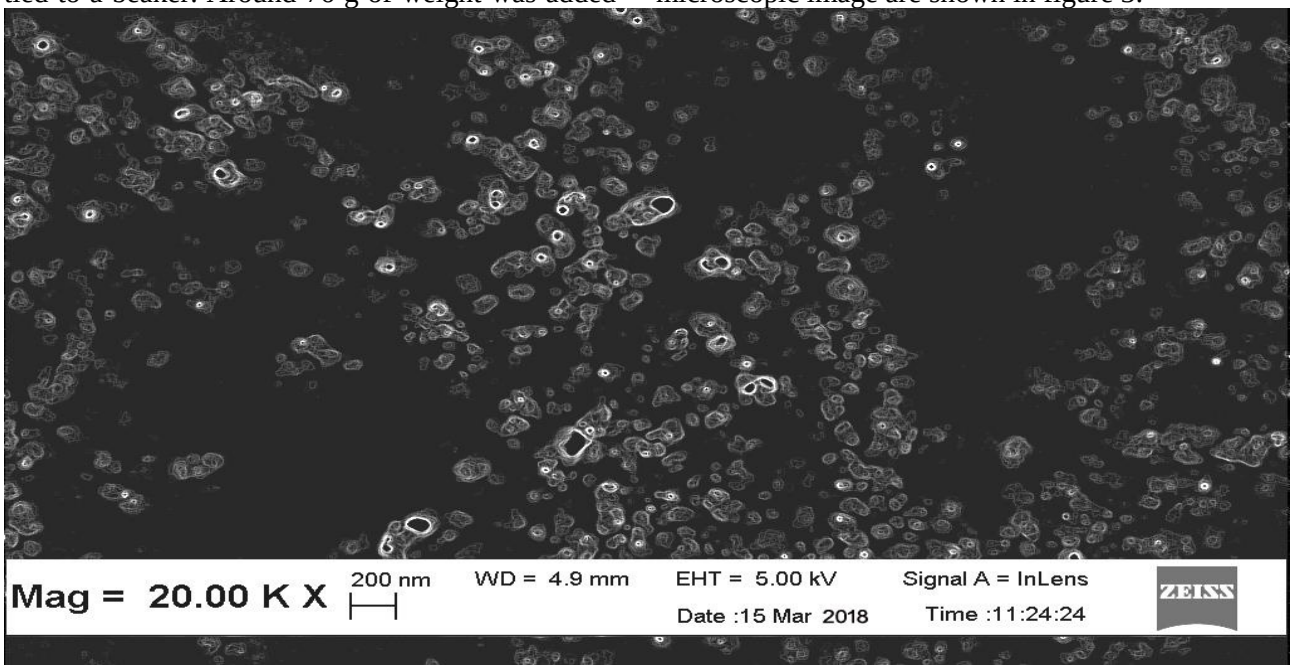


**Figure 2: SEM image of ethosomes**

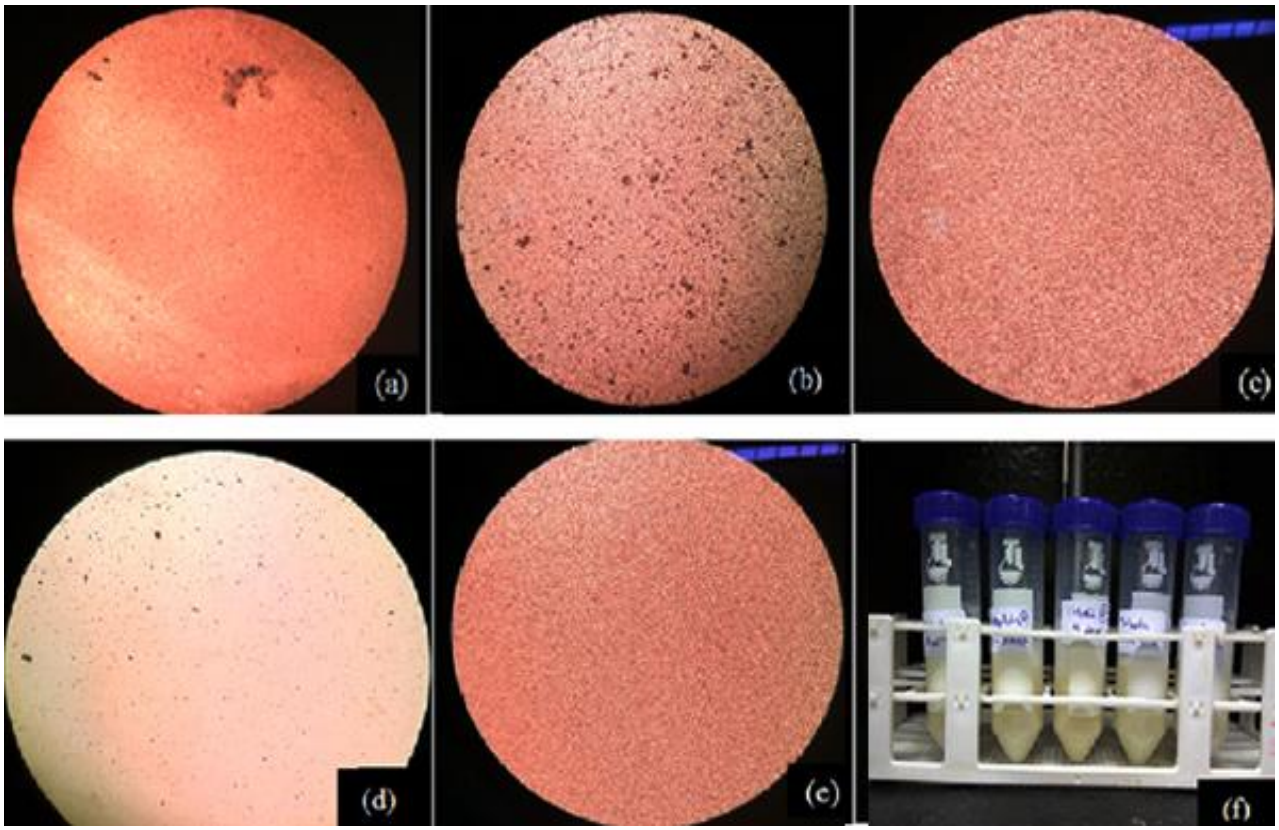


**Figure 3: a,b,c, d Microscopic images of catechin ethosomes (f) various formulation of catechin ethosomes.**

**Table 2: Characteristics of catechin ethosomal gel**

| Formulation Code | pH | Particle size (nm) | Spreadability (gm.cm/sec) | Gel strength seconds |
|---|---|---|---|---|
| C1 | 6.99 | 287 | 78.63 | 110 |
| C2 | 7.02 | 289 | 27.13 | 115 |
| C3 | 7.00 | 288 | 60.67 | 125 |
| C4 | 7.04 | 235 | 38.25 | 93 |
| C5 | 6.93 | 322 | 55.04 | 140 |

The reological properties of various catechin ethosomal gel were determined at different shear rates. As the rpm increased, the viscosity of prepared solution and gel decreased (Fig 4). Also the increase in concentration of carbopol 940 to nano carrier caused an increase in viscosity of the gel. The formulation C5 having the maximum concentration of carbopol 940 (1.5 w/v) showed higher viscosity in ethosomal gel forms. It is also found that the formulation C5 having higher concentration of polymer is a poor candidate for topical gel formulation.

The spreadability were performed for all the formulations in order to determine the therapeutic efficacy of gels as uniform spreadability can enhance the absorption and therapeutic action. [11] The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability of optimized formulation C1, C3, C4 and C5 showed (38.25-78.63 gm.cm/sec) and was found to be more as compared to C2 formulations. This indicates spreadability of ethosomal gel system containing catechin having 0.75% of carbopol 940 was good as compared with high concentration of polymer.

Gel strength is important because strong gels will support a much higher pressure than weak gels. The formulations C1 to C5 (93 to 140 sec) exhibited good gel strength among all C code formulation which may be due to increase in concentration of gelling agent. The results for various formulations of gel strength are demonstrated in Table 2 and Figure 4.

FT-IR analysis was carried out to ensure there was no occurrence of chemical interaction between the drug and cholesterol in the formulation as shown in figure 5.

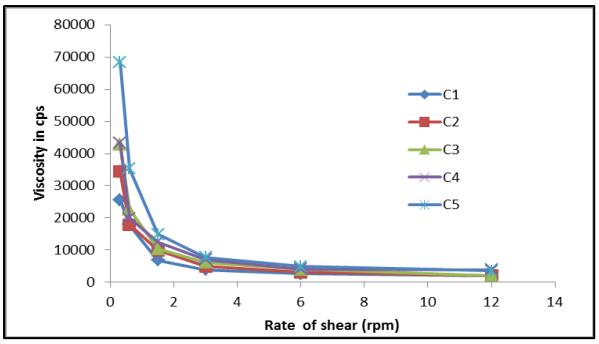


**Figure 4: Viscosity of catechin ethosomal gel**

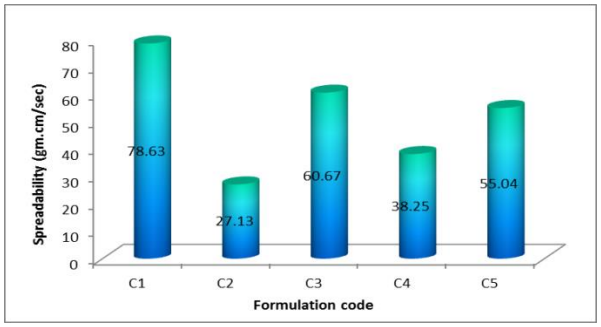


**Figure 3: Spreadability of catechin ethosomal gel**

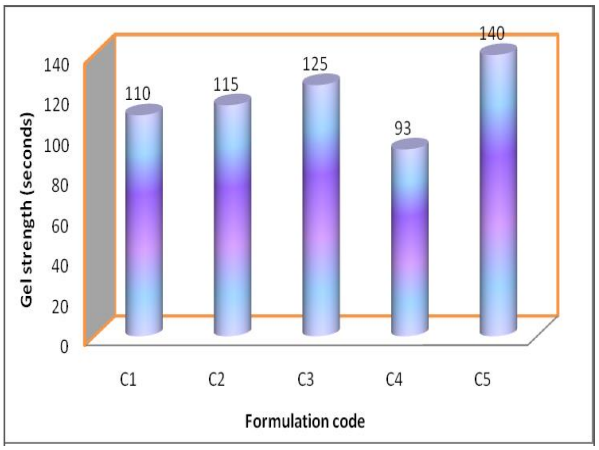


**Figure 4: Gel strength of catechin ethosomal gel**

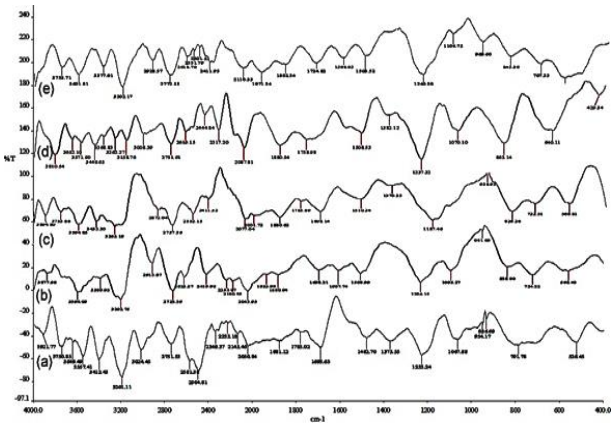


**Figure 5: (A) Catechin , (B) Cholesterol, (C) Poloxamer 407, (D) Cholesterol + Poloxamer 407, (E) Catechin + Cholesterol + Poloxamer 407**

The in vitro diffusion profile of formulation contain catechin from ethosomal gel containing different concentration of (carbapol 940) were conducted in diffusion medium pH 7.4. The formulations C4 and C5 released 82.1% and 75.2 % of drug respectively at 540 min. The formulations C1 containing lower concentration of polymer (0.5%) released 60.1% as shown in figure 6.

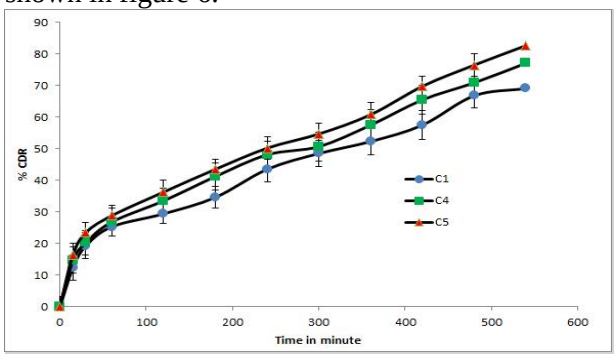


**Figure 6: % of CDR for C1,C4 and C5 formulations**

## CONCLUSION:

Topical drug delivery by application of ethosomal gel have found several advantages in drug formulations as ethosomal gel are having the ability to retain on skin to enhance skin penetration to deeper layers of tissue and thus, improve drug therapeutic efficacy. As a result, ethosomal gels are recently widely used as a novel drug delivery in topical form for treatment of various diseases in order to improve patient compliance and avoid first-pass metabolism prior to oral administration. Few parameters of gel formulation such as spreadability, viscosity and gel strength are studied to evaluate the catechin ethosomal gel.

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