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Original Article

Formulation of gellified emulsions of Clotrimazole using essential oil from *Lavendula angustifolia* Miller

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ABSTRACT

Clotrimazole is a broad spectrum antifungal drug, widely used for topical treatment of *Tinea corporis*. It is available commercially as cream, powder, gel and vaginal pessary. However, its poor water solubility (0.49 µg/ml) presents a hindrance for its local availability and limits effective antifungal therapy. [3]In present study, an attempt has been made to enhance its efficacy and stability by formulating it as gellified emulsions (emulgels). Emulsions (o/w) of Clotrimazole were prepared using lavender oil and gellified using carbomer, carboxymethylcellulose sodium and xanthan gum. All the emulgels were white, homogeneous with good consistency and spreadability and possessed pH very close to that of normal skin (6-7). The formulations F4 and F7 which contained carboxymethylcellulose sodium and xanthan gum as gel base demonstrated fastest diffusion of Clotrimazole. Moreover, their antifungal efficacy (expressed as zone of inhibition) against *Trichophyton rubrum*, was greater than that of commercially available gel of Clotrimazole. The emulgels did not produce any skin irritation in treated rats and were stable when stored for 90 days at 25°C/60% (RH) and 40 ^{0}C /75 % RH

Keywords: Emulgels, Clotrimazole, Viscosity modifying polymers, Lavender oil, Topical delivery

INTRODUCTION

Clotrimazole (CTZ) is a broad spectrum antimycotic agent effective against several species of *Candida*, *Trichophyton*, *Microsporum*, *Epidermophyton*, and *Malassezia*. It is known to be very effective locally and presents no major side effects. CTZ is available as creams, gels, powders and vaginal pessaries for topical treatment. However, its poor water solubility (0.49 µg/ml) presents a hindrance for its local availability and limits effective antifungal therapy. [3] The interest in transparent gellified emulsions or emulgels has expanded due to their greater stability and superior solvent characteristics for water insoluble drugs like CTZ. They have faster and near complete release of drug from the vehicle to skin and therefore exhibit higher efficacy. Moreover, they are convenient to apply on hairy skin due to non-greasy nature and lack of residue upon application. [5, 6]Rao M. and co-workers have reported improved stability and permeation of Metronidazole from emulgels. [15] Khuller et al. have formulated emulgels of Mefenamic acid (NSAID) and reported greater analgesic and anti-inflammatory activity than commercially available gel of Diclofenac. [17] The objective of present study was to enhance antifungal efficacy and stability of CTZ by formulating as emulgels using different gelling agents and lavender oil as oil phase since it is reported to possess antifungal, anti-inflammatory and antiseptic activity. [7]

MATERIALS AND METHODS

***Materials*:** Clotrimazole (Nu-life Pharmaceuticals, Pune, India), carbomer 940, carboxymethyl cellulose sodium, xanthan gum, Span 20, lavender oil, propylene glycol (Research Lab Fine Chem. Industries, Mumbai) and *Trichophyton rubrum* (Microbial Type Culture Collection, Chandigarh, India, MTCC No. 296) were used. All other reagents and solvents used were of analytical grade.

Methods:

Compatibility of CTZ and formulation excipients: The compatibility of physical mixtures of CTZ and gelling polymers was assessed by FT-IR spectral analysis***:*** The spectra of the mixtures stored at for days were recorded using FTIR spectrophotometer (Shimadzu-8400 S, Japan). For this, KBr pellet

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method was used wherein the samples were dispersed with KBr and then compressed into a tablet (10mm diameter and 3 mm thickness) using a tablet press (Techno search) at 300 kg/cm for 1 min. The presence and position of peaks in the spectrum of pure CTZ and the individual excipient was compared with the peaks in spectra of blends of CTZ with individual excipients to detect any changes.

***Preparation of gellified emulsions/emulgels*:**
The detailed composition for the gellified emulsion formulations is given in **Table No. 1**.The formulations F1, F2 and F3 were prepared by dispersing carbomer 940 in purified water with continuous stirring (2000 rpm. for 5 min) while the formulations F4, F5 and F6 were prepared by dispersing sodium carboxy methyl cellulose (Na CMC) in purified water and formulations F7, F8 and F9 were prepared by dispersing xanthan gum in purified water. The oil phase of the o/w emulsion was prepared by dissolving Span 20 in lavender oil while the aqueous phase was prepared by dissolving Labrasol in purified water followed by addition of solution of CTZ, methyl and propyl paraben in propylene glycol. The two phases were separately heated to 65-70°C, before addition of the oily phase into the aqueous phase with continuous stirring. The emulsion was dispersed in the gel base (1:1) after cooling and was mixed with gentle stirring to from homogenous emulgels. [8]

EVALUATION OF EMULGELS

***Physical examination*:** The plain emulgels were examined for consistency, colour, homogeneity, grittiness and phase separation. [9]

***PH*:** The pH of emulgels was recorded using previously calibrated digital pH meter. For this, about 1gm of medicated emulgels was dispersed in 100 ml of distilled water and pH was recorded in triplicate. [10]

***Viscosity*:** The viscosity of emulgels was measured at 25°C-28^{0}C using a Brookfield viscometer (Brookfield LVDV, Spindle no. S64). For this, about 50 gm of sample was placed in the weighing bottle, the spindle was dipped into it and helipath was adjusted in such a way that neither it touched the bottom of container nor did it touch the walls of the container .The spindle was rotated at increasing and decreasing rpm. At each speed, the corresponding viscosity (Cps) and torque (%) values were noted. [11]

***Spreadability*:** Spreadability of emulgels was evaluated by apparatus suggested by Mutimer et al. (1956) which was suitably modified in the laboratory. The spreadability was measured on the basis of 'slip' and 'drag' characteristics of emulgels. For this, an excess of emulgels (about 2 gm) was sand witched between two glass slides. The air from the emulgels was expelled by placing 1 Kg weight on the top slide for 5 minutes. Excess of the emulgels was scrapped off from the edges of slides. The top plate was then subjected to pull (80 gms), with the help of string attached to the hook. The time (in seconds) required by the top slide to travel a fixed distance (7.5 cm) was noted. [12, 13]

***Content of CTZ*:** For this, the quantity of emulgels equivalent to 10 mg of CTZ was dissolved in 100 ml methanol. From this, 1 ml samples were withdrawn and diluted to 10 ml with methanol. These samples were analyzed spectrophotometrically at a wavelength of 260.4 nm and concentration of CTZ in each sample was estimated from previously prepared standard curve [14].

***Diffusion (in vitro) of CTZ through synthetic membrane*:** Release (*in vitro*) of CTZ from emulgels through synthetic membrane (porosity-2.4 nm, Mol. Wt: - 12000).was estimated using Franz diffusion cell. For this, the membrane was soaked in phosphate buffer (pH 7.4): methanol (6:4) for 9-12 hr. The quantity of emulgels equivalent to 10 mg of CTZ was spread uniformly over the membrane. Receptor compartment was filled with phosphate buffer (pH 7.4): methanol (6:4) which was maintained at 37±0.5°C and was stirred continuously using a magnetic bead, The diffusion cell was kept on a magnetic stirrer.. A similar blank set was run simultaneously as a control. Samples were withdrawn at suitable time intervals and replaced with equal amounts of fresh media. Samples were analyzed spectrophotometrically at 261 nm and the cumulative % release of CTZ was calculated. The difference between the absorbance of drug released and control solution was used as the actual reading in each case. [15]

***Skin irritation potential (in vivo)*:** The irritancy potential of experimental emulgel of CTZ was tested in healthy adult male albino Wistar rats (weighing g).The animals were grouped into four groups as follows;

Group I = No treatment control.
Group II = emulgel base (vehicle control)
Group III = Optimized emulgel formulation containing 1% w/w CTZ In Na CMC as gel base.
Group IV = Marketed gel formulation of CTZ (1%w/w)
Animals were randomly placed in cages upon receipt and then randomized according to the body

weights. Animals considered unsuitable because of outlaying body weights were excluded from the study. The backs of animal were carefully shaved using sterilized shaving blade. Circular areas of 2.54 cm (1 inch diameter) were marked on the back of each animal using marker ink, one spot on right side and one spot on left side of vertebral column. About 1gm of the product was applied on the marked spots using previously sterilized absorbent cotton wool. The treated skin areas were observed for toxic manifestations at pre-selected time intervals viz: 1 hr, 4 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs after application of product. The results were interpreted as numerical scores as follows for each animal.

0 = no visible reaction.
1 = mild erythema.
2 = intense erythema.
3 = intense erythema with edema.
4 = intense erythema with edema and vesicular erosion.

The scores for treatment group and control group animals were then compared. [16]

Antifungal efficacy: For this, agar well diffusion method was used. . The fungus *Trichophyton rubrum* was grown on the Emmon's modified Sabouraud's agar medium (sterilized by autoclaving at 121^0C, 15 lbs for 15 minutes). The fungal cultures were transferred into test tubes containing 10 ml of sterile saline (0.9%). A glass spreader was moistened using this suspension and Emmons modified Sabourauds agar plates were streaked with it so as to cover the entire surfaces. Using sterile cork borer, the wells (5 mm) were bored into the medium. The appropriate quantities of test samples were added into each well. The plates are incubated at 28^0C for 4- 5 days and the dimensions of zones of inhibition were recorded using digital vernier calipers. [17]

Accelerated stability: The prepared CTZ gellified emulsions were packed in aluminum tubes (5 grams) and stored at 25°C/60% RH and 40°C/75% RH for a period of 3 months. Samples were withdrawn at time intervals of 15 days and evaluated for appearance, pH, rheological properties, drug content and drug release. [18]

RESULTS AND DISCUSSION

***Drug excipients interaction*:**
***FT-IR Analysis*:** IR spectra of physical mixtures of CTZ with gelling polymers revealed retention of all major peaks corresponding to functional groups of CTZ (3059, 1433, 1210, 1113, 1080, 1040, 903, 824, 720 cm-)(**Fig. 1**). This is suggestive of probable absence of any major interaction of drug with any of excipients.

***Physical characteristics of* gellified emulsions:** The emulgels were white, smooth creamy semisolids with a homogeneous consistency and glossy appearance. No phase separation was noticed. (**Table 2)**

***pH*:** pH of emulgels ranged from 6.1±0.1 to 6.7±0.01. This pH is close to the normal pH range of the skin. There was no significant change in pH values as a function of time for all formulations.

***Viscosity*:** Viscosities of emulgels at both low and high shear rates were affected by type and concentration of gelling agent. The viscosity increased as concentration of polymer increased. Among of them the carbomer 940 has higher viscosity than carboxy methyl cellulose sodium and xanthan gum. This may attributed to variation in shape and dimensions of crystallites of different polymers and their ordering in the three dimensional structure within the resulting network where the emulsion phase is held by adsorption, capillary and molecular interaction mechanisms. [19] All the emulgels demonstrated a shear-thinning behavior (Fig**. 2).**

***Spreadability*:** The emulgels could easily spread by small amount of shear. Their spreadability decreased with the increase in the concentration of the polymer (**Fig. 3**). The formulations containing carboxy methyl cellulose sodium gel base possessed higher spreadability than the formulations containing xanthan gum and carbomer gel bases.

Contents of CTZ: The CTZ contents of emulgels were in the range 96.6 to 98.7 % (**Fig. 4)**

***Release (in vitro) of CTZ from emulgels*:** The release of CTZ from emulgels was influenced by composition of the gel base. Thus emulgels prepared with sodium CMC facilitated faster diffusion of CTZ than the emulgels containing xanthan gum followed by those containing carbomer 940. The progressive increase in the amount of drug diffused through membrane from emulgels is attributed to gradual decrease in the viscosity due to decrease in concentration of gelling polymer (**Fig. 5)**. Mura et al. reported similar findings where the release of mention drug candidate from different hydrophilic ointment bases was inversely related to their viscosity. When drug diffusion is the rate-limiting step, the viscosity of the matrix is a significant factor that controls the rate of drug release from where matrix system is appearing in semisolids. [20]

***Skin irritation potential*:** The healthy rats treated with selected emulgel formulations (those containing carboxymethylcellulse sodium and xanthan gum as gel base) demonstrated no symptoms of allergy viz: inflammation, redness or irritation over the period of 72 hr.

***Antifungal efficacy*:** The antifungal efficacy expressed as zone of inhibition (mm) of *Trichophyton rubrum* (most prominent species causing *Tinea corporis* infection of skin, hair and nail) possessed considerable antifungal activity for placebo emulgels prepared Na CMC and xanthan gum as a gel base and lavender oil as disperse phase. The emulgels prepared with Clotrimazole possessed significant antifungal activity. Moreover, their antifungal efficacy was greater than the commercially available product of CTZ. **Fig. 6**.

Stability: The formulations F4 and F7 were found to be stable upon storage for three months. No change was observed in their appearance; homogeneity, pH, and drug content (**Table No.3**)

CONCLUSION

From the results it can be concluded that gellified emulsions are a good option for incorporating hydrophobic drugs in hydrophilic gel bases. The gellified emulsions of CTZ prepared using either carbomer940 or carboxy methyl cellulose sodium or xanthan gum possessed acceptable physical properties, pH, drug content, viscosity. , Gellified emulsions containing carboxy methyl cellulose sodium demonstrated superior drug release than those prepared using xanthan gum, or carbomers 940. The inclusion of lavender oil has supplemented antifungal action of CTZ against *Trichophyton rubrum.* Stability studies has revealed no significant difference in characteristics of emulgels on storage

Acknowledgement: The authors would like to thank Nu-life Pharmaceuticals Ltd., Pune for the gift sample of Clotrimazole and Accurate Analytical Laboratory, Pune for technical assistance for microbial testing.

Table No. 1: Formulation of emulgels of CTZ.

Name of ingredient (% w/w)	Formulation code								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Clotrimazole	1	1	1	1	1	1	1	1	1
Carbopol 940	1	1.25	1.5						
Na CMC				3	3.5	4			
Xanthan gum							2	2.5	3
Lavender oil	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Span 20	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Labrasol	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Propylene glycol	5	5	5	5	5	5	5	5	5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Purified water (q.s.) to make	100	100	100	100	100	100	100	100	100

Table No. 2: Physical properties of emulgel formulations

Formulation code	Color	Phase separation	Homogeneity	Consistency	Grittiness
F1	White	No	Excellent	Excellent	-
F2	White	No	Excellent	Excellent	-
F3	White	No	Excellent	Excellent	-
F4	White	No	Good	Good	-
F5	White	No	Good	Good	-
F6	White	No	Good	Good	-
F7	White	No	Good	Good	-
F8	White	No	Good	Good	-
F9	White	No	Good	Good	-

Table No. 3: Stability data of emulgels

Formulation code	Day	Color		Homogeneity		pH		% Drug content	
		4 °C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
F4	0	White	White	+++	+++	6.31	6.31	99	99
	30	White	White	+++	+++	6 .26	6.26	98	97
	60	White	White	+++	+++	6.22	6.21	96	96
	90	White	White	+++	+++	6.20	6.18	94	94
F7	0	White	White	+++	+++	6.56	6.56	100	100
	30	White	White	+++	+++	6.50	6.50	100	99
	60	White	White	+++	+++	6.40	6.38	98	99
	90	White	White	+++	+++	6.36	6.37	96	97

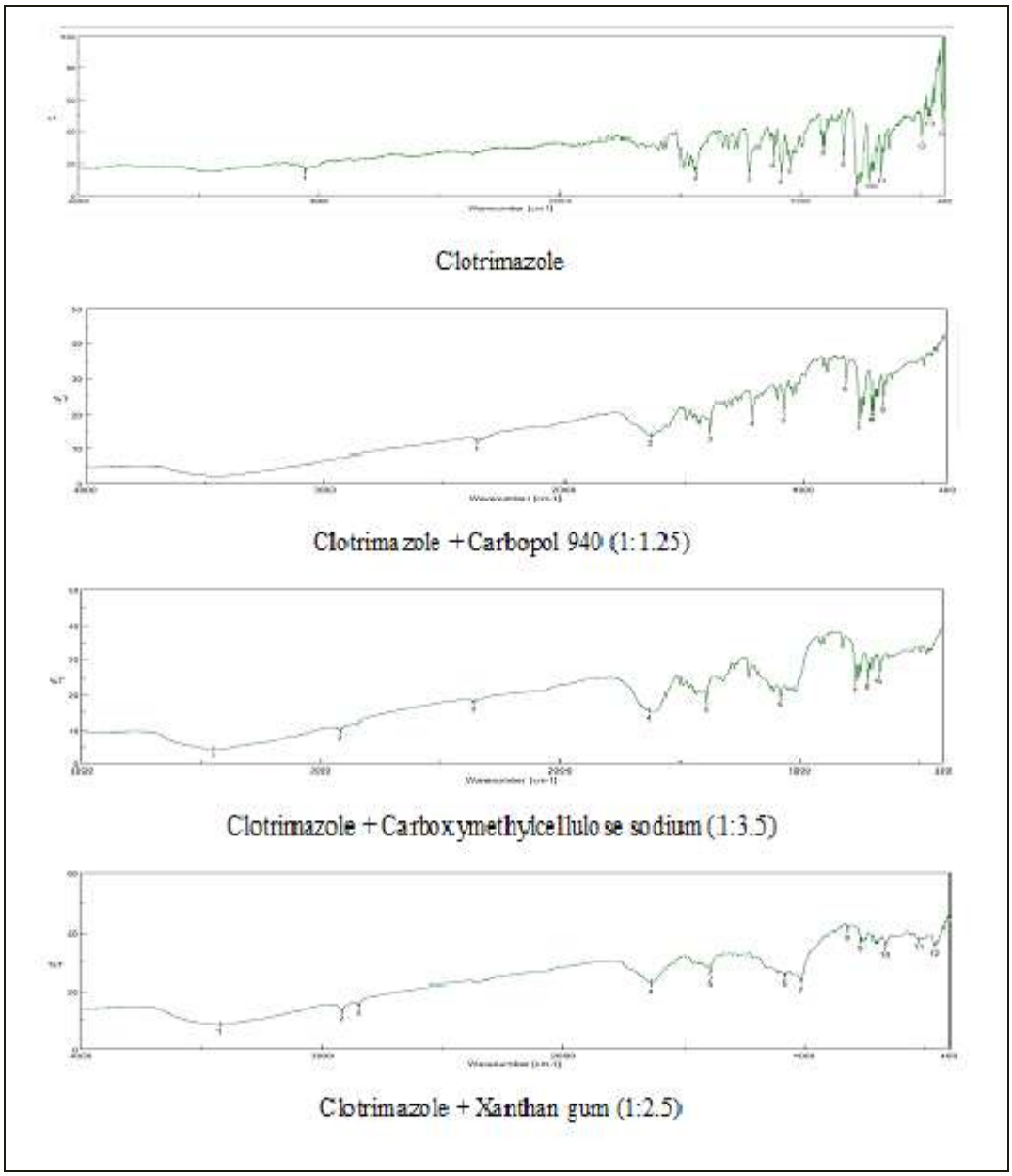

Fig.1: IR spectra of Clotrimazole and its physical mixture with excipients

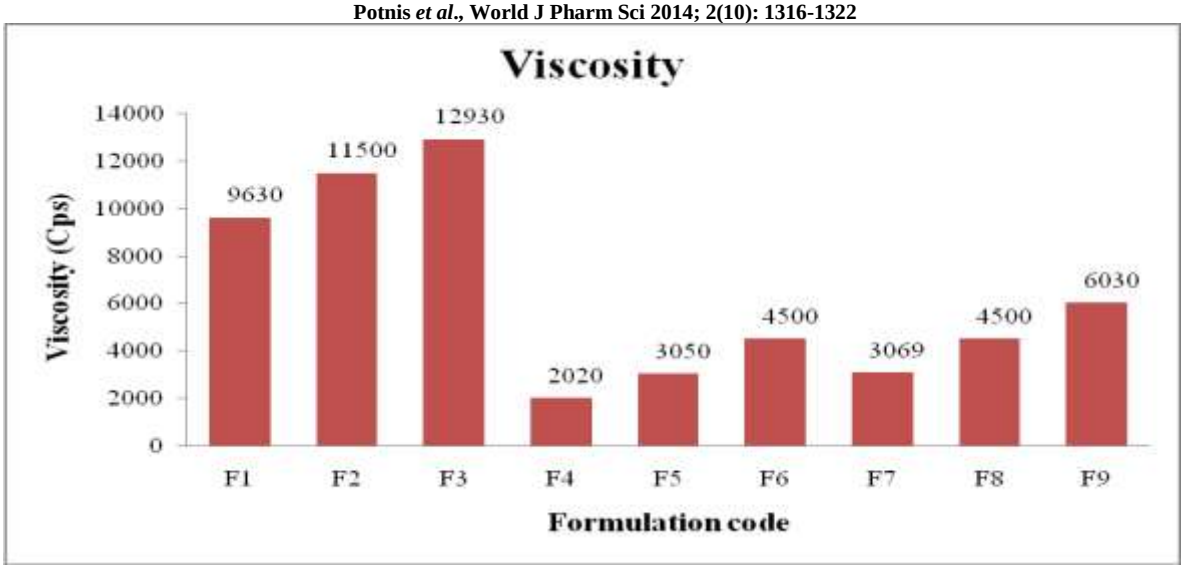

Fig. 2: Viscosity of different emulgel formulations F1- F9

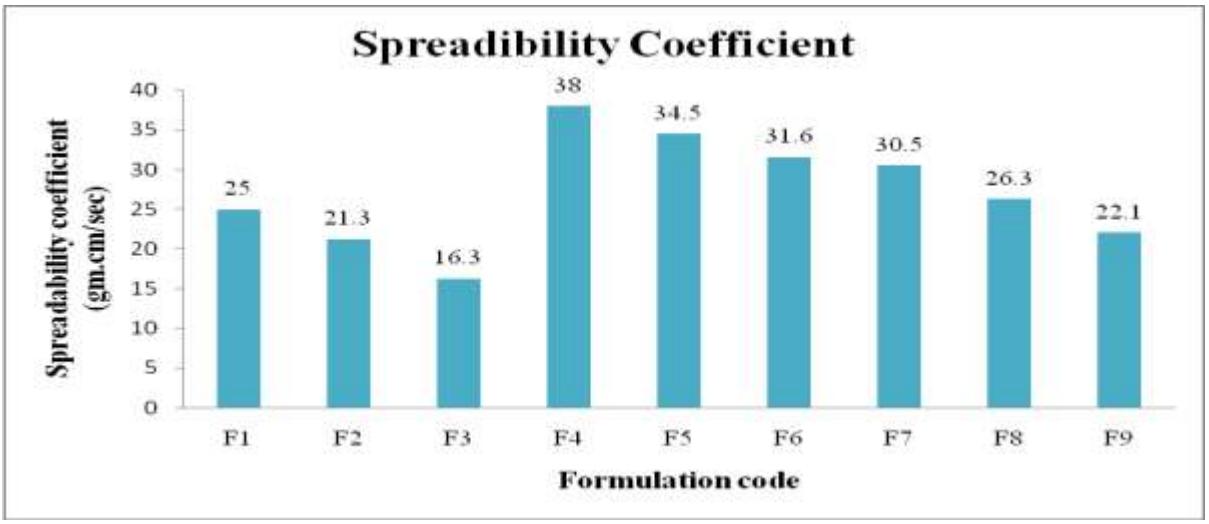

Fig. 3: Spreading coefficient of emulgels formulations F1-F9

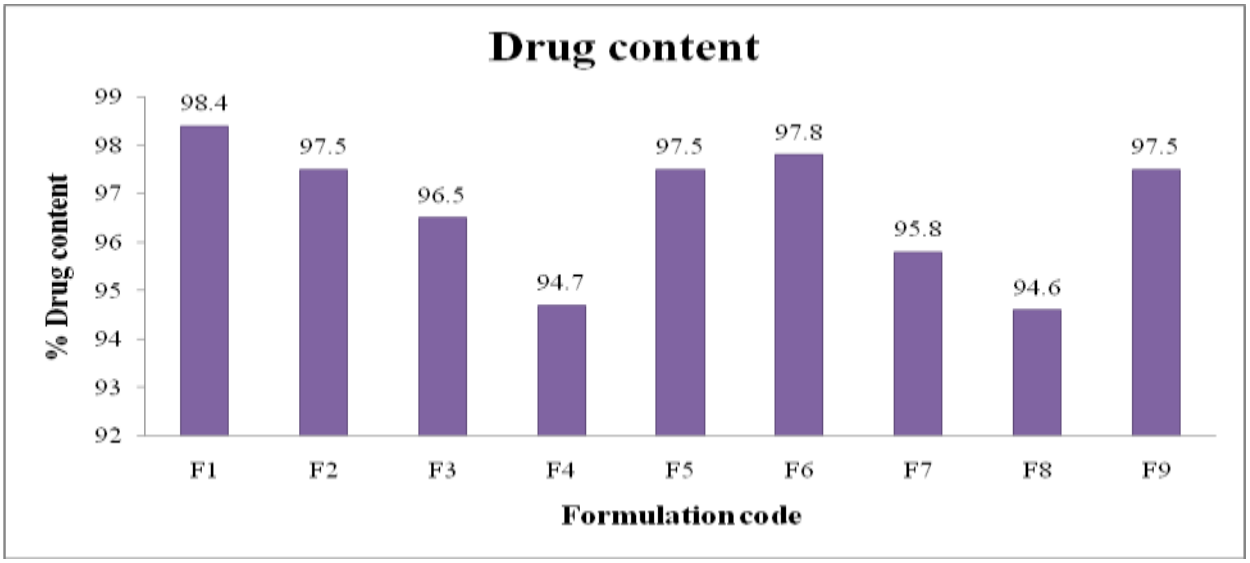

Fig.4: Contents of CTZ in emulgel formulations F1-F9.

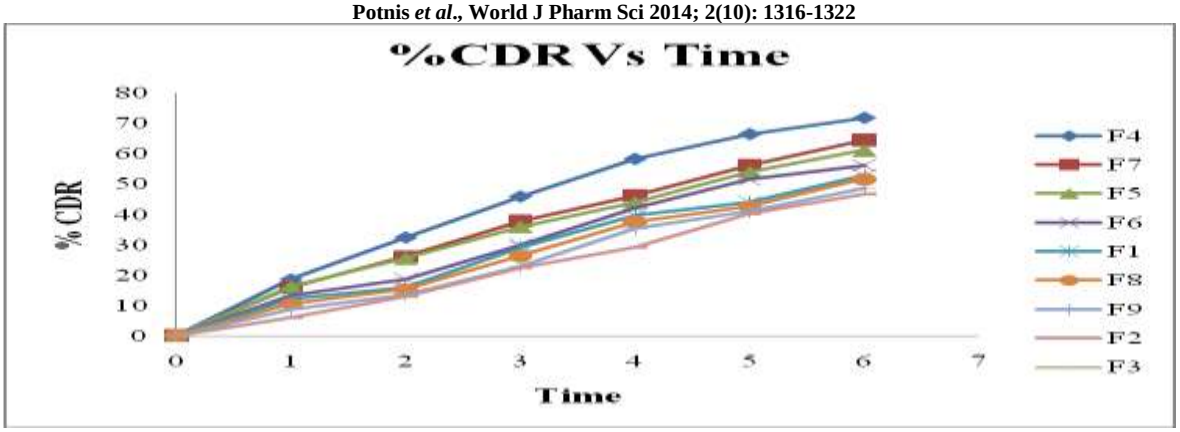

Fig. 5: Cumulative % release profile (*in vitro*) of CTZ from emulgel formulations F1 – F9

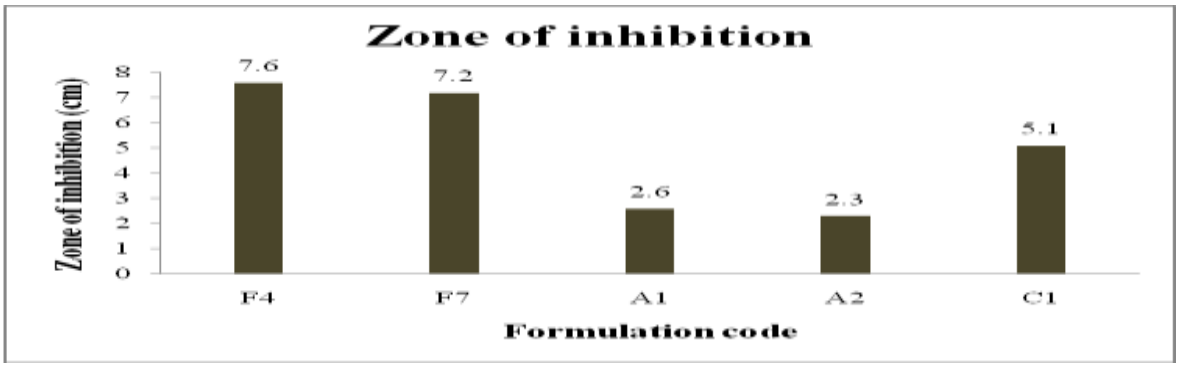

Fig.6: Zone of inhibition of *Trichophyton rubrum* by CTZ in optimized emulgel formulations (Note: A1-Na CMC placebo emulgel, A2- Xanthan gum Placebo emulgel, C1- Marketed product)

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