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DEVELOPMENT OF DIOSMIN LOADED EUDRAGIT S100 POLYMERIC NANOPARTICLES: AN INVESTIGATION OF ANTIOXIDANT EFFECT

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ABSTRACT

In the presentstudy, diosmin loaded eudragit nanoparticles were developed by nanoprecipitation method and evaluated *in vitro* for FTIR, XRD, SEM, particle size, polydispersity index, zeta potential, drug entrapment, drug loading and release studies. Among the three different developed formulae (F1, F2 and F3), F1 showed an average particle size (212 nm), zeta potential (+32.7 mV), high entrapment of drug (54%), drug loading (1.88%). SEM imagesconfirmed that developed nanoparticles were spherical in shape with a smooth surface. *In vitro* release and anti-oxidant activity showed significant results.The results suggest that eudragit polymer based nanoparticle could be a potential option for diosminoral delivery.

Keywords: Polymer, nanparticle, drug entrapment, SEM, DPPH

INTRODUCTION

Biodegradable polymeric nanoparticles have been recognized as frequently used drug delivery carrier due to its better solubility, encapsulation efficiency, control release and less toxic properties **[1]**. Among the various polymers, Eudragithas been considered as an excellent and frequently used polymers to develop novel drug formulation due to various reasons like, biocompatible, biodegradable, modify the release of drugs and its non-toxic nature **[2, 3]**. A Literature search revealed that several dosage forms has been developed using eudragitpolymer **[4-6]**.

Diosmin (7-rhamnoglucoside 5, 7, 3'-trihydroxy-4'-methoxyfiavone) is the common active constituent of citrus plants

andbutchu leaves **[7]**. As one of the most extensively used flavanoid, it is generally recognized as strong antioxidant **[8-10]** potential anti-inflammatory, anti-cancer, anti-ulcer and improve venous tone by increasing the micro circulation **[11]**.The diosminis practically insoluble water. Thus the dissolution rate of diosmin is expected to limit its absorption from the gastrointestinal tract. An attempts to increase the oralbioavailability of the drug have therefore chiefly centered on particle size reduction. Increasing the rate and extent of dissolution ofdiosmin by nano sizinghas led directly to an increase oral bioavailability, which in turn enables dosage reduction. Polymeric nano-particle formation approach has been tried for enhancement of solubility and bioavailability of diosmin. Many approaches have been tried to enhance the bioavailability of diosmin by phytosomecarrier **[12]**, nanosuspension **[13]** and cyclodextrin complexation **[14]**.

Usual therapeutic dose of diosmin is very high (i.e, 500 mg twice daily), thus it is necessary to be administered frequently in order to maintain the therapeutic concentration. The purpose of this study to develop a diosmin loaded polymeric nanoparticle that could have potential to increase the bioavailability. The developed polymeric nanoparticle may be suitable to reduce the dose and frequency and hence reduce toxic or side effect of diosmin.

EXPERIMENTAL

Materials and Methods

Diosmin, Eudragit S100, Pluronic acid F68 were purchased were Fluka. HPLC grade methanol and water were purchased from sigma Aldrich. All chemicals and solvents used in this study were of analytical grade. Freshly prepared double distilled water was used throughout the work.

Preparation of Diosmin Nanoparticles

The nanoparticles of diosmin were prepared by nanoprecipitation method [4]. Briefly,10 mg of diosmin and eudragitS100 (100 mg) were dissolved in 2 mL of 1:1 mixture of ethanol and DMSO. This organic phase was slowly injected into 10 mL of an aqueous phase (i.e,1% PluronicacidF68 surfactant in distilled water). Mixture wassonicated for 30 minutes by probe-sonication (Ultrasonic processor, GEX 100) at 40% voltage efficiency. The formed nanoparticles was immediately stirred at 1000 rpm over ice bath for 2 hrs.The nanoparticle aggregate were obtained after evaporating organic solvent at 37^{o}C temperature for 4 h on a water bath. Polymeric nanoparticles were separated from aqueous phase by centrifugation at 15000 rpm for 30 min (Hettich, MIKRO-120, Tuttlingen, Germany). **Table 1**, represents the

composition of each of the prepared nanoparticles.

Measurement of Particle Size, Polydipersity Index and Zeta Potential

The mean particle size,polydispersity index and zeta potentialof the each formulations were determined by photon correlation spectroscopy using 90 Plus particle size analyzer, Brookhaven InstrumentsCorporation (Holtsville, New York, USA). The polymeric nanoparticle dispersions were diluted 1:200 with distilled waterand analysed at 25 °C with an angle of detection of 90°. Each measurement was done in triplicate.

Fourier Transform Infrared Spectroscopy (FT-IR)

The FTIR spectra of free diosmin, eudragit and nanoparticles (F1-F3) were recorded on the ALPHA FT-IR Spectrometer (OPTIK,USA) using the potassium bromide (KBr) disc technique. Samples equivalent to 3 mg of diosmin were mixed with crystalline potassium bromide(about 100 mg) in a clean glass pestle and mortar and were compressed to obtain a transparent pellet. Baseline was corrected and the samples were scanned against a blank KBr pellet background at a wave number rangingfrom 4000-400 cm^{-1}.

X-ray Diffraction Pattern

Powder X-ray diffraction (XRD) of free diosmin, eudragit and nanoparticles were obtained by using X-raydiffractometer (Altima IV Regaco, Japan). The scanningrate of spectra was 4°/min. The voltage/current used was 30 kV/25 mA and the target/filter (monochromator) was copper.

Scanning Electron Microscopic (SEM) Analysis

The images of the prepared nanoparticles were analyzed under the scanning electron microscope (JSM-6360LV Scanning Microscope; Jeol, Tokyo, Japan). Suspended nanoparticles were vortexed for 1 minute, and then one drop was spread on a slide and kept in a dessicator to dry. Dried sample was mounted on carbon tape and sputter-coated using a thin gold palladium layer under an argon atmosphere using a gold sputter module in a high-vacuum evaporator (JFC-1100 fine coat ion sputter; Jeol, Tokyo, Japan). The coated samples were then scanned and photomicrographs.

Determination of Entrapment Efficiency (EE) and Drug Loading (DL)

The content of diosminpolymeric particles was determined by a modified RP-HPLC method of El-Shafae& El-Domiatry, (2001) **[15]**. The mobile phase (methanol and water,45:55 v/v) was pumped at a flow rate of 0.6 ml/minute through a reversed-phase C18 (150 mm x 4.6 mm, with particle size 5 µm) column at room temperature. The injected

volume was 10 µl and the detection wavelength was 346 nm.

For entrapment efficiency of drug, the amount of diosmin present in the clear supernatant aftercentrifugation at 14000 rpm was determined and then subtracted from the total amount of diosmin added in the formulation **[16, 17]**.

The % drug loading (DL) was obtained by dividing that drug present in nanoparticle (wdnp) by the total weight of nanoparticles (Wnp) according to the following formulae:

$$\% DL = wdnp / Wnp \times 100$$

***In vitro* Release Studies**

In vitro release of nanoparticles was performed using biological shaker. Briefly, weighed amount of nanoparticle filled in empty capsule equivalent to 10 mg of diosmin. Each capsule were immersed into media containing 100 mL of 0.1 N HCl (pH 1.2) and put into biological shaker at a speed of 75 rpm and temperature, 37^{o} C. Each sample (2 mL) were withdrawn at a time interval of 0, 0.3, 1, 2, 4, 8 and 12hrours. The sample was filtered through 0.22 membrane filtered and analyzed by HPLC.

***In-vitro* Antioxidant Capacity**

Optimized nanoparticle (F1) wasevaluated for DPPH (1-1-diphenyl-2-picrylhydrazyl) radical scavenging activity according to the method of Blois **[18]**. Briefly, 0.1 mL of different concentration of free diosmin and nanoparticle (20-100 µg/ml) were added to 3mL of DPPH solution (0.3mM in methanol) as the free radical source. The mixture was shaken vigorously and kept for 45 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of nanoparticle was determined at 517 nm. A decrease in absorbance of the reaction mixture was an indication of higher free radical scavenging activity. Ascorbic acid was used as the positive control. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{Anti-oxidant activity (\%)} = \frac{A^0 - A^1}{A^0} \times 100$$

Where, A^{o} is the absorbance of the control, and A^{1} is the absorbance of standard sample.

RESULTS AND DISCUSSION

Measurement of Particle Size, Polydispersity Index and Zeta Potential

Based on the physicochemical characterization such as mean particle size, polydispersity index, and zeta potential, the performance of nanoparticles recognized **[16]**. The measured parameters produced during study are being presented in **Table 2** and **Figure 1**. Particles size analysis showed a narrow range of variability in mean size (212 – 286 nm) and dispersion (PDI, 0.463 – 0.604). Narrow dispersions recognized

particles as a monodispers.At lowest concentration of surfactant (0.5%), nanoparticles showed optimum in size and zeta potential.

Zeta potential is an index of overall charge present on particles and how this is affected by changes in environmental factors such as pH, presence ofions, adsorption of proteins. Zeta potential above +30 mV lead to form a more stable nanoparticles due to repulsion between particles that prevent to aggregation **[19]**. Zeta potential of developed nanoparticles (F1-F3) was found in the range of +32.7 to +42.8 mV due to quaternary ammonium group present in Eudragit.

Particle Morphology

SEM images of diosminloaded nanoparticles were presented in **Figure 2**. It was confirmed from imagesthat developed nanoparticles were small spherical in shape without aggregation andsmooth surfaces in morphology. The particle size observed from SEM image strongly supports the result of particle size analyzer.

FTIR Spectra

Various peaks were observed in nanoparticles (F1-F3) with reduced intensitycorresponding to the absorption spectra to the diosmin in the region of 400-1300 cm^{-1}without any shift in peak position **(Figure 3)**. It could be understood, a shift and reduce in peak intensity probably due to the interaction of eudragit and diosmin **[20]**.

X-Ray Diffraction Pattern

X-ray diffraction studies are the useful tool to identify crystallinity and amorphicity nature of powders. XRD patterns of eudragit, diosmin and diosmin loaded eudragitnanoparticles (F1-F3) shown in **Figure 4**. XRD pattern of diosmin revealed several intense peaks (at 15.2◦, 19.2◦, 23.7◦, 37.1◦etc), however, these peaks were absent or reduced in peak intensity in the diosmin loaded eudragitnanoparticles.This indicates that the diosmin was encapsulated efficiently in the polymer **[21]**.

Determination of Entrapment Efficiency and Drug Loading

The eudragit polymer and pluronicacid F68 composition were found to affect the extent of diosmin entrapment in polymeric nanoparticles. Nanopartiles (F1) exhibited the highest entrapment efficiencies among all the three prepared formulae (F1-F3) with values equal to 54.19%. Loading efficiency of diosmin ranging from 1.88-288% were also observed at different concentration of surfactant used in the formulation. The concentration of pluronicacid F68 showed a little effect on the size, entrapment efficiency and loading efficiency. The optimum formula for the nanoparticles (F1) with regards to

surfactant used (0.5% w/v), the relatively small particle size (212±3.0 nm), polydispersity value of (0.463±0.023), and high encapsulation efficiency 54±1.2% **(Table 2)**.

***In-vitro* Release Study**

Release profile of diosminand loaded nanoparticles were shown in the **Figure 5**. A significant increase in release of drug from nanoparticles was observed when comparedwith free diosmin.The release behavior of drug from the eudragit polymer matrix showed a sustained release. The nanoparticles F1, F2 and F3 showed the release of 59.6 %, 51.1 % and 40.3 % respectively in12 hours at pH 1.2. The drug release in acidic condition is sustained due encapsulation. The nanoparticle F3 showed good release as compared to other nanoparticle and free diosmin.

***In-vitro* Antioxidant Capacity**

DPPH is a stable free radical, decolorized in the presence of antioxidants. An odd electron present in DPPH is responsible for a visible deep purple color which shows absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant drug, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Results investigated in this study indicated different DPPH scavenging rate of tested diosmin and its nanoparticle. A decrease in color of DPPH radical due to the scavenging ability of tested tested nanoparticle. Optimized nanoparticle (F1) reached the highest anti-oxidant activity (80% at conc. of 100 µg/ml). A significant enhancement in antioxidant activity were observed as compared to free diosmin (54% at conc. of 100 µg/ml). As compared to standard (Ascorbic acid, 20-100 µg/ml), nanoparticle (F1) approaches the anti-oxidant activity of ascorbic acid **(Figure 6)**.

CONCLUSION

In this study, practically insoluble drug,diosmin was successfully incorporated into polymeric nanoparticles bynano precipitation method and the developed nanoparticles may be a suitable carrier for the possible oral delivery. The eudragit polymer selected in this study allowed to fabricate a nanometric size particles with a spherical morphology. A significant drug release and anti-oxidant activity were observed when diosmin encapsulated in nanoparticulate system with eudragit polymer. Hence, by using polymeric nanoparticle system reduction of high dose and dose frequency is possible.

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Conflict of Interest Statement

Author report no conflict of interest of this work. Author is responsible for content and the writing of the paper.

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Table 1: Composition of Prepared Nanoparticles

Code	Diosmin	Eudragit RS100 (mg)	Pluronic F68 (% w/v)
F1	**10**	**100**	**0.5**
F2	**10**	**100**	**1.0**
F3	**10**	**100**	**1.5**

Table 2: Preliminary Evaluation of Prepared Nanoparticles

Code	Mean diameter (nm)	Polydispersity Index	Zeta potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)
F1	**212±3.0**	**0.463±0.023**	**+32.7±0.48**	**54±0.4**	**2.88±0.3**
F2	**247±2.7**	**0.604±0.042**	**+37.0±1.02**	**51±0.8**	**2.43±0.6**
F3	**286±3.6**	**0.543±0.028**	**+42.8±0.91**	**49±1.1**	**1.88±0.8**

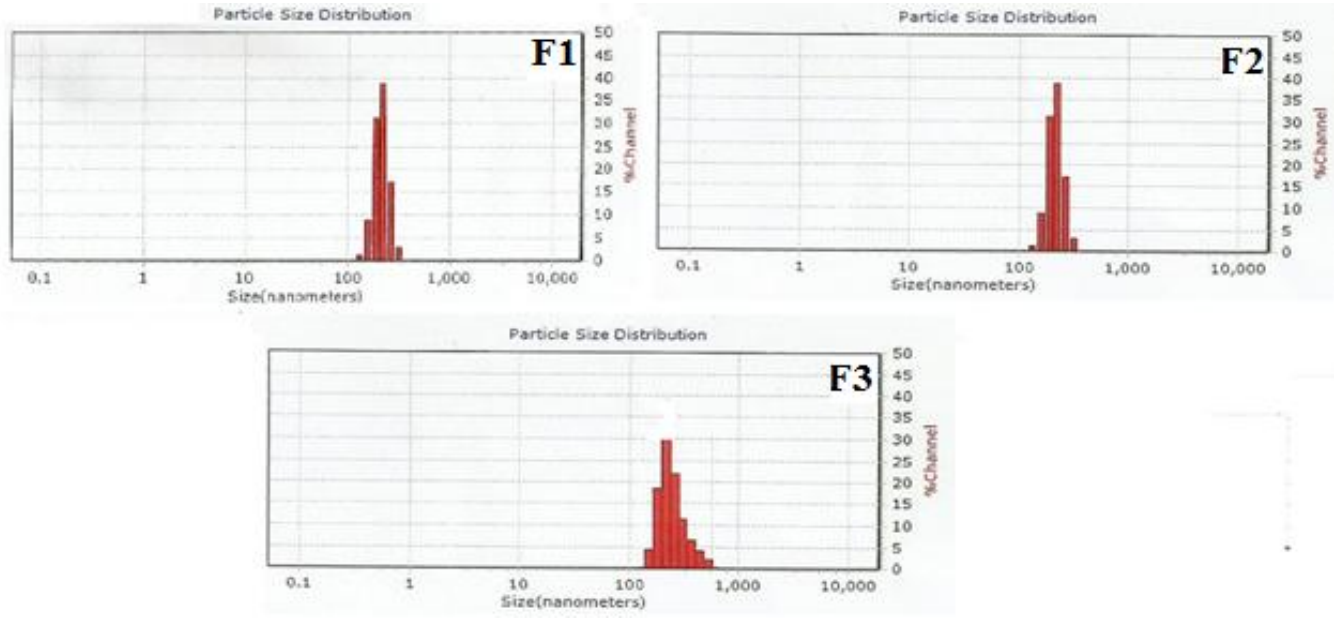

Figure 1: Particle Characteristics Measured by Particle Size Analyzer

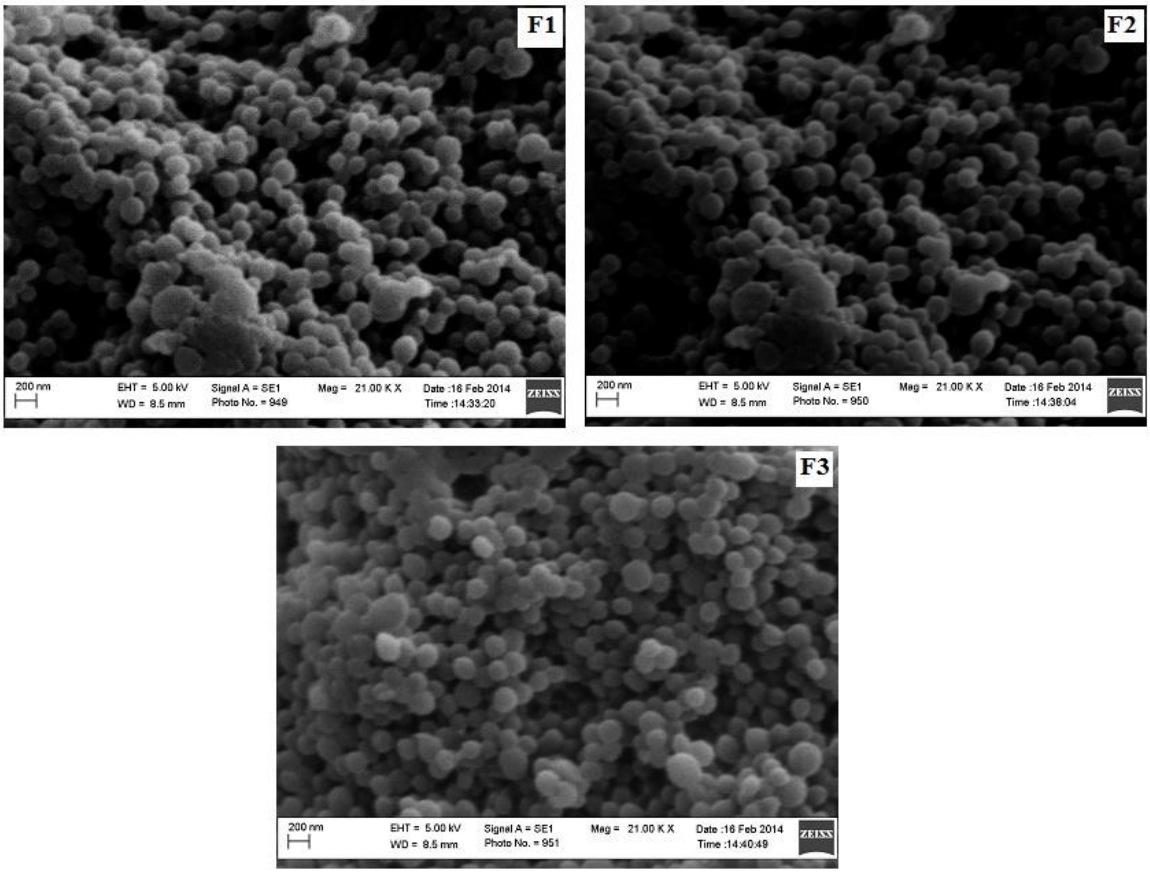

Figure 2: SEM Images of Prepared Nanoparticles

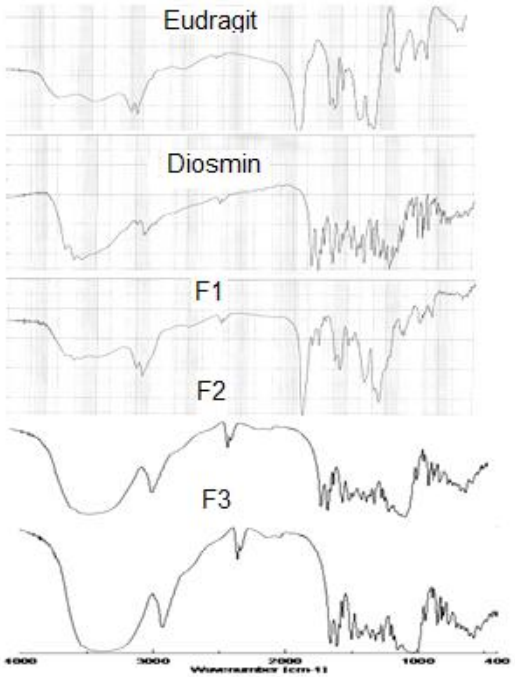

Figure 3: FT-IR Spectra of Eudragit, Diosmin and its Nanoparticles

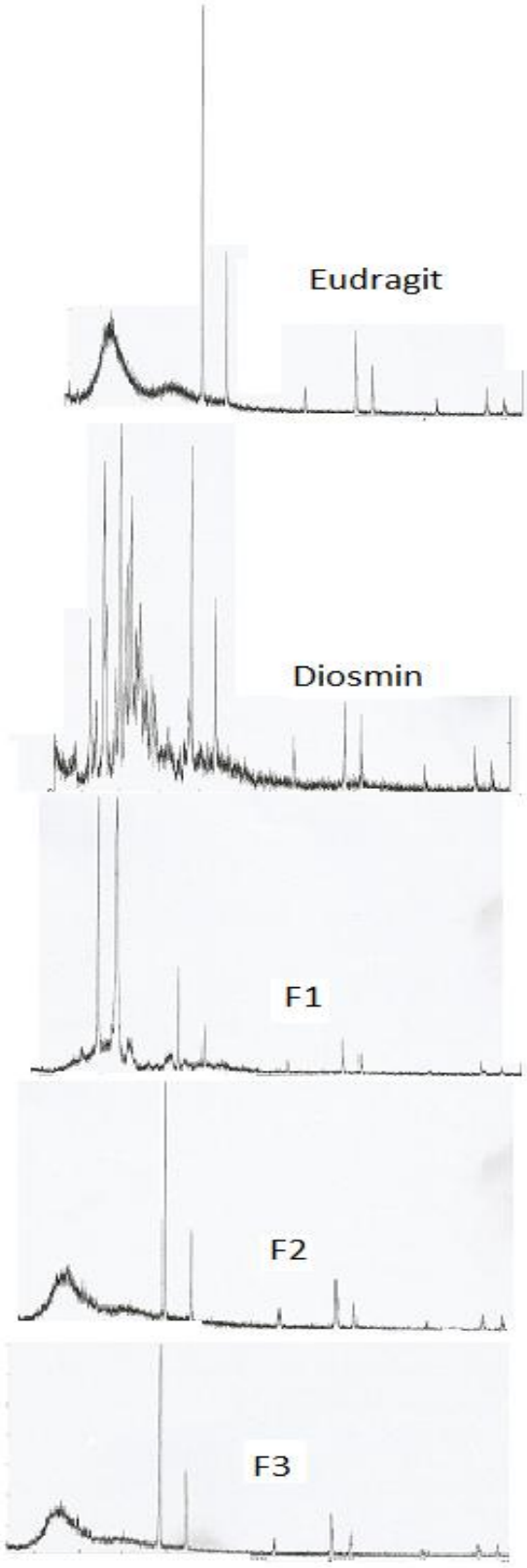

Figure 4: X-Ray Diffraction Pattern Eudragit, Diosmin and its Nanoparticles

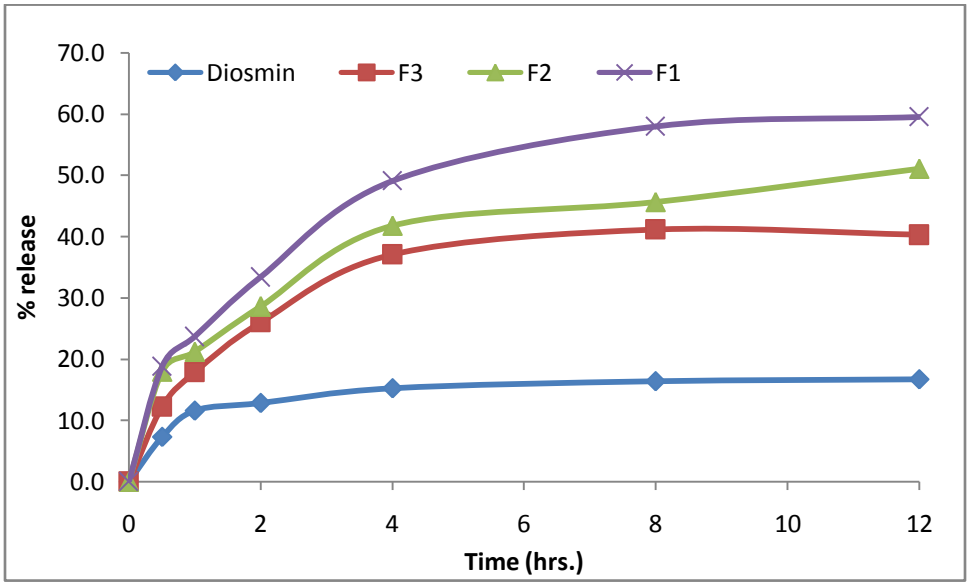

Figure 5: *In vitro* Release Studies of Diosmin and its Nanoparticles

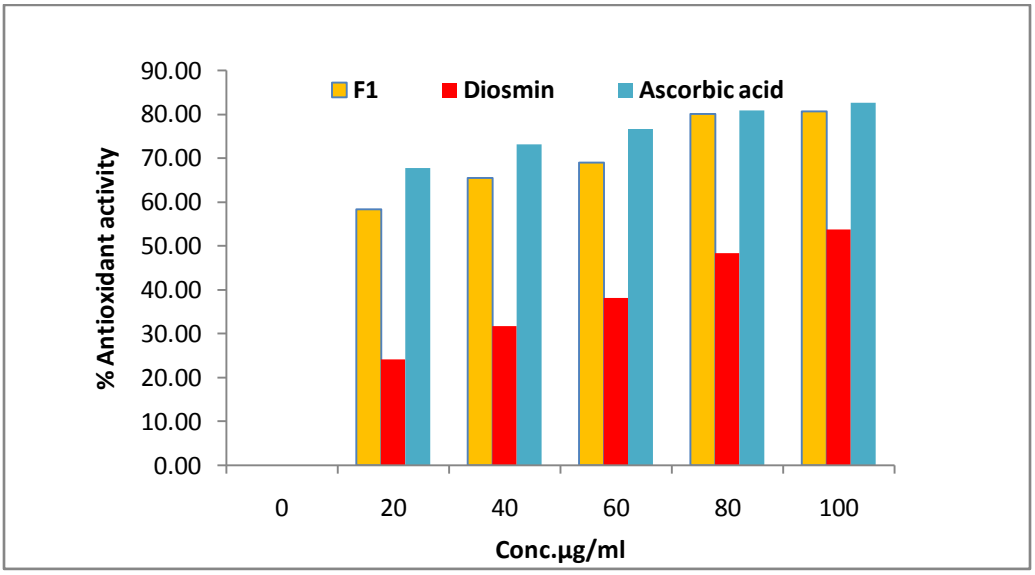

Figure 6: Anti-oxidant Activity of Diosmin and Optimized Nanoparticle (F3)