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PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION OF TIZANIDINE HYDROCHLORIDE NANOPARTICLES

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ABSTRACT
The commonly used methods for encapsulating hydrophilic molecules in nanoparticles (NPs) suffer from low encapsulation efficiency due to the drug rapidly being partitioned to the external aqueous phase. In the present study a new double emulsion solvent diffusion (DES-D) technique, resulted in particles with smaller size, lower size distribution and higher encapsulation efficiency. The utilization of partially water-miscible, class III organic solvent (ethyl acetate) enabled rapid diffusion through the aqueous phase during evaporation, creating regions of local supersaturation near the interface. Smaller NPs were formed following phase transformations and polymer aggregation at these regions. Physicochemical characterization of the nanoparticles was performed by measuring particle size, zeta potential, drug entrapment efficiency, FTIR study and in vitro drug release. Batch G4 with encapsulation efficiency (EE) of 54 ± 3.6 % and Zeta potential of -28.0 mV was selected as an optimized batch. The stability of the optimized batch G4 indicated that formulation was stable at different storage conditions. FTIR studies indicated that there was no chemical interaction between drug and polymer.

Keywords: *Tizanidine hydrochloride; double emulsion solvent diffusion (DES-D); Eudragit RS-100; Eudragit E-100; Ethyl Cellulose.*

INTRODUCTION

Tizanidine is an agonist at á2-adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons[1,2]. It acts mainly at the level of spinal cord and is used for the symptomatic relief of spasticity associated with multiple sclerosis or with spinal cord injury or diseases. Tizanidine undergoes extensive hepatic first pass metabolism in the liver (approximately 95% of dose) necessitating its frequent dosing. It is water soluble drug, and is poorly absorbed after oral administration (only 34-50 %). Eudragit RS-100 is insoluble at physiological pH values; therefore it has been utilized along with ethyl cellulose, another water insoluble polymer for the preparation of sustained-release formulations of the drug.

MATERIALS AND METHODS

Materials

Tizanidine hydrochloride was a generous gift sample from Endoc Pharma, Rajkot, Gujrat, India. Eudragit RS-100 and Eudragit E-100 were purchased from RÖhm Pharma GMBh, Germany. Poly vinyl alcohol (PVA) with molecular weight of 1,25000Da and ethyl cellulose (viscosity of 5 % w/w solution 18-24cp) were provided by CDH Labs, India. Pluronic® F-68 was procured from Himedia Labs Pvt. Ltd, Mumbai, India. Sodium hydrogen phosphate (dibasic), potassium dihydrogen phosphate were received from Merck, Germany. All other reagents used were of analytical grade. Milli Q double distilled water (DDW) water was used throughout the study. The compositions of all formulations are shown in Table 1.

Table 1: *Composition & Characterization of prepared nanoparticles*

Sr. No.	F. Code	Polymer(s)	Particle Size (nm)	PdI	%EE	Zeta Pot. (mV)
1	G1	EC	212	0.342	51.2±3.5	-26.9
2	G3	EC:RS (6:3)	273	0.415	51.6±2.9	-29.9
3	G4	EC:RS (5:4)	398	0.415	54.0±3.6	-28.0
4	G5	EC:E(6 3)	189	0.314	67.4±4.0	-20.7
5	G6	EC:E(5 4)	637	1.0	62.4±3.8	-22.2

RS=Eudragit RS 100, E=Eudragit E 100, EC=Ethyl Cellulose

Preparation of nanoparticles

TIZ- nanoparticles were prepared by the $W_1/O/W_2$ modified solvent evaporation method[3]. Briefly, 0.5 ml aqueous solution of drug was emulsified in 4.5 ml of ethyl acetate (EtAc) containing 2 % polymer by sonication over an ice-bath using a probe sonicator (Bandelin Electronic, Germany), at 20 W output for 120 s. The resulting primary emulsion was added to 10 ml of DDW containing 0.3% Pluronic F-68, and the mixture was sonicated for 120 s at 20 W output over an ice bath to form a double emulsion. Formation of a colloidal nanodispersion can be visualized by the bluish opalescence (Fig 1); this phenomenon is known as the Tyndall effect. It is a phenomenon, in which the scattering of light is caused by the dispersed colloidal

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particles[4].The organic solvents and excess of water were evaporated under reduced pressure. All the batches were prepared in triplicate. Nanoparticles were separated from the bulk suspension by centrifugation (Remi, Mumbai, India) at 10,000 rpm for 30 min. The supernatant was kept for drug assay and the precipitated nanoparticles were collected by filtration and washed with three portions of 30 ml of water and were re-dispersed in 5 ml of purified water before freeze-drying.

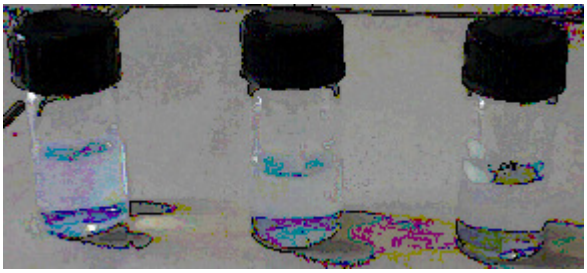


Fig 1: *Nanoparticle Dispersions showing bluish opalescence.*

Particle Morphology

Particle morphology was analyzed using Environmental Scanning Electron Microscope model FEI Quanta 200F with Oxford-EDS system IE 250 X Max 80, Netherlands as shown in Fig 2. The samples for SEM observations were coated with a thin gold coating.

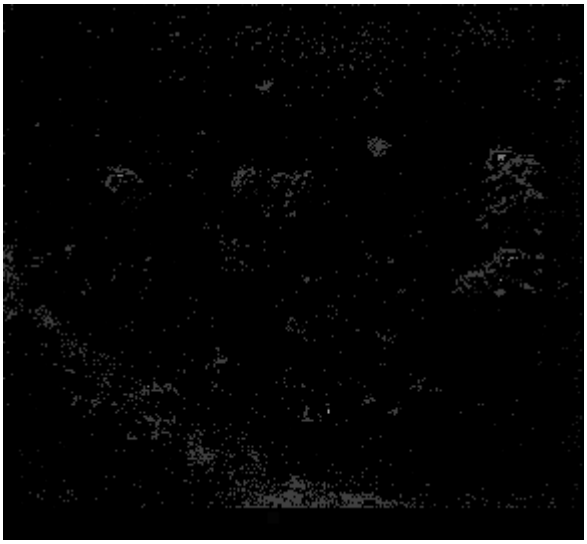


Fig 2: *SEM image of nanoparticles*

Particle size analysis and zeta potential measurement

The mean particle size for the formulations was determined by dynamic light scattering (DLS) using Nano particle sizer, model Zetasizer Nano ZS, Malvern, UK, using capillary folded cell (with knob). The reading was carried out at a 90^0 angle with respect to the incident beam. The zeta potential was also measured using disposable polystyrene cuvette with the help of same instrument. Deionized water was used as a dispersion medium during all measurements. The replicate analyses were carried out for each formulation The measurements are demonstrated in Fig 3 to Fig 12.

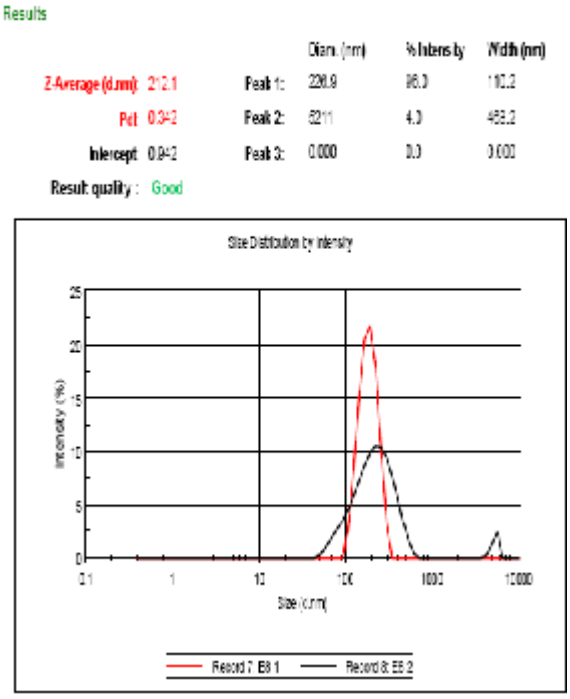


Fig. 3: *Particle size of Batch G1*

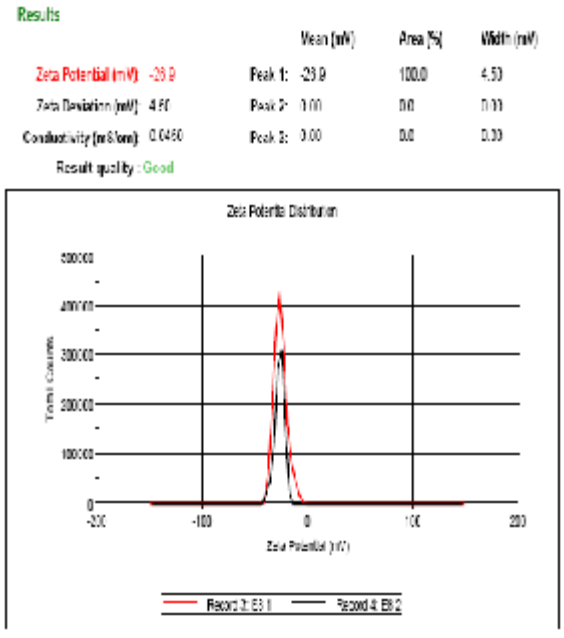


Fig. 4: *Zeta Potential of Batch G1*

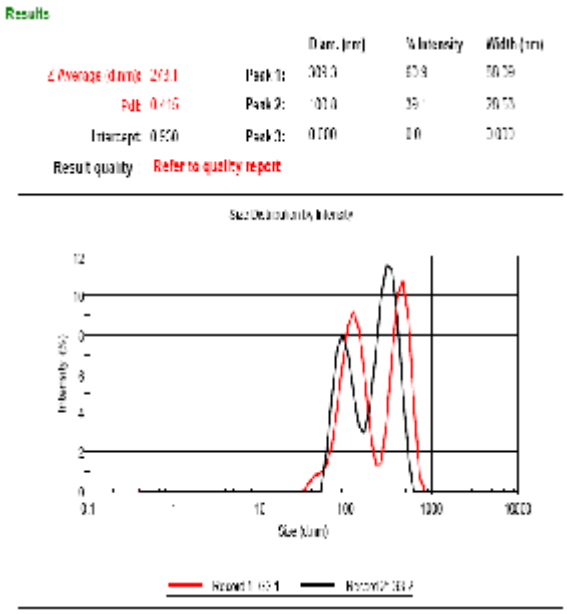


Fig. 5: *Particle size of Batch G3*

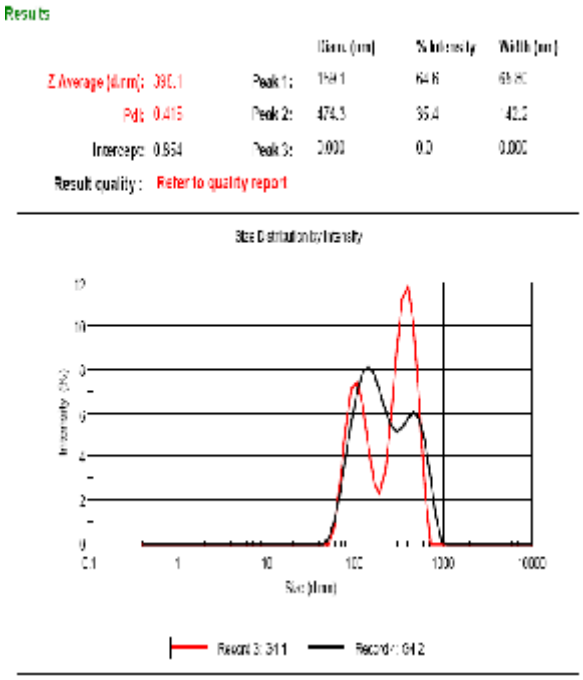


Fig. 7: *Particle size of Batch G4*

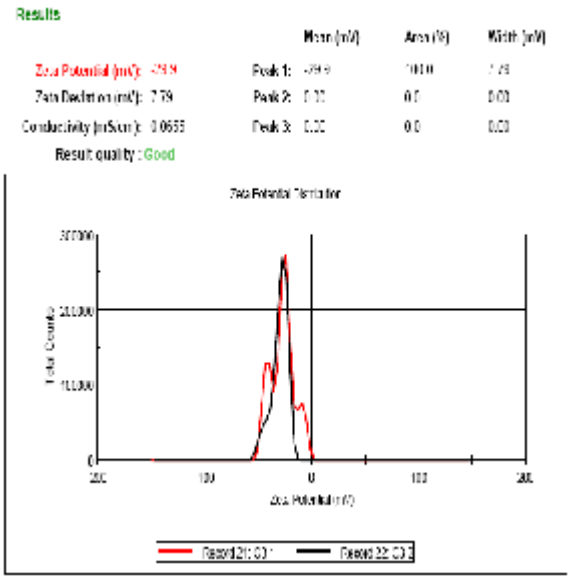


Fig. 6: *Zeta Potential of Batch G3*

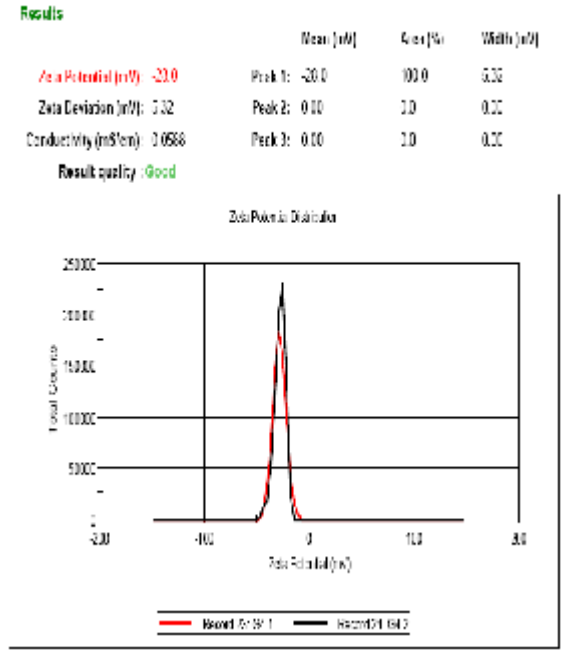


Fig. 8: *Zeta Potential of Batch G4*

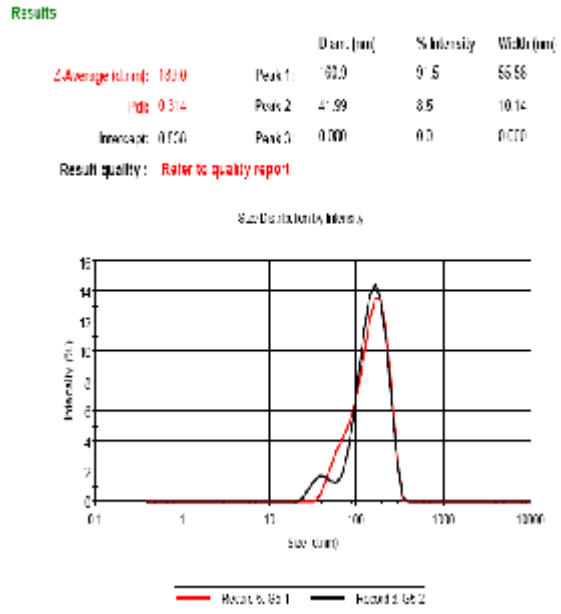


Fig. 9: *Particle size of Batch G5*

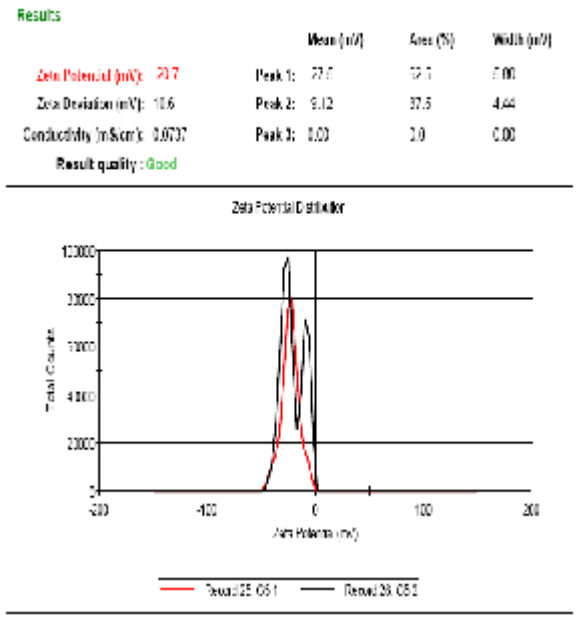


Fig. 10: *Zeta Potential of Batch G5*

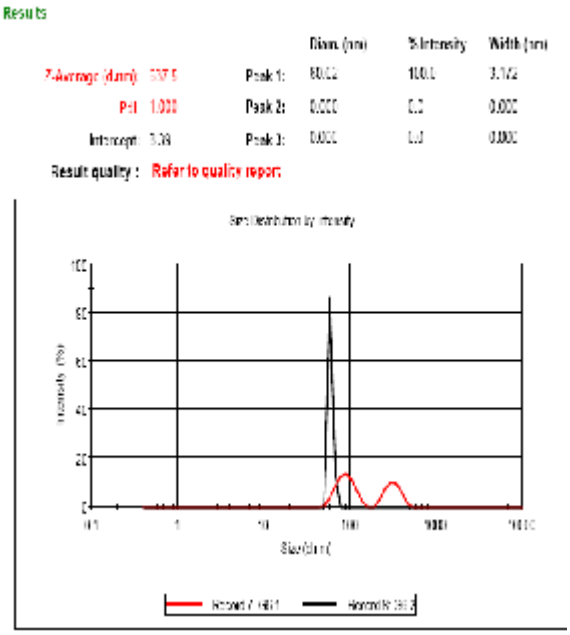


Fig. 11: *Particle size of Batch G6*

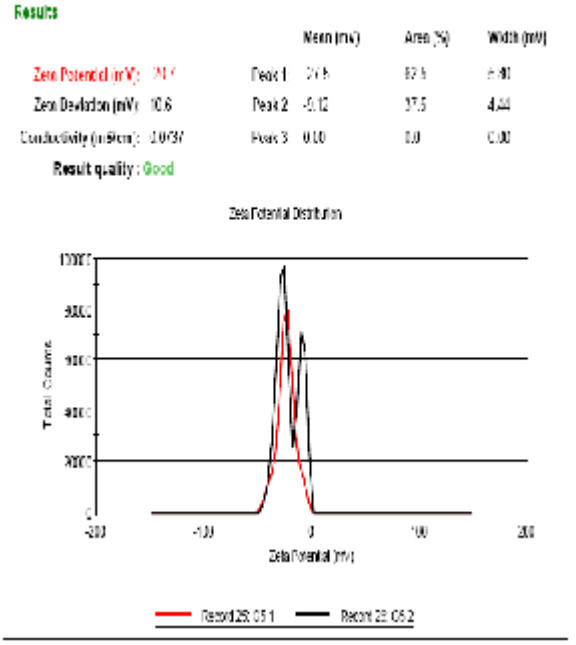
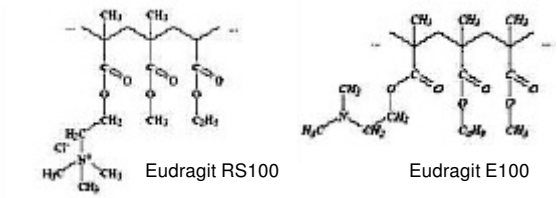


Fig. 12: *Zeta Potential of Batch G6*

The chemical structures of Eudragits are shown below in the Fig. 13



Eudragit RS100 Eudragit E100

Fig. 13: *Chemical structures of Eudragit polymers* [5,6]

Drug Entrapment Efficiency

After preparing the fresh nanosuspensions, it was centrifuged and the free drug present in the supernatant was analyzed by UV-Visible spectrophotomer1700 (Shimadzu) using a calibration curve. The calibration curve was constructed by measuring the absorbance of solutions of different concentrations of drug at 319.5 nm. By subtracting the amount of drug in supernatant form the initial amount of drug, EE was calculated. The EE values are shown in Fig 14.The indirect method is suitable for determining entrapment efficiency of nanosuspensions when fairly high concentration of free drug is present in the supernatant after centrifugation [7].

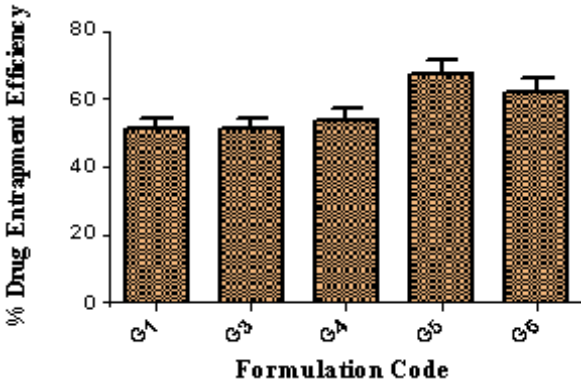


Fig. 14: *Drug Entrapment Efficiency of different formulations*

Infrared spectroscopy

Infrared spectroscopy was used to study the interactions between the drug and the polymers. Infrared absorption spectra of drug and nanoparticles in the wavelength region 4500 cm^{-1} to 500 cm^{-1} were recorded using a Fourier transform IR spectrometer (Shimadzu) and demonstrated in Fig 15 & Fig 16.

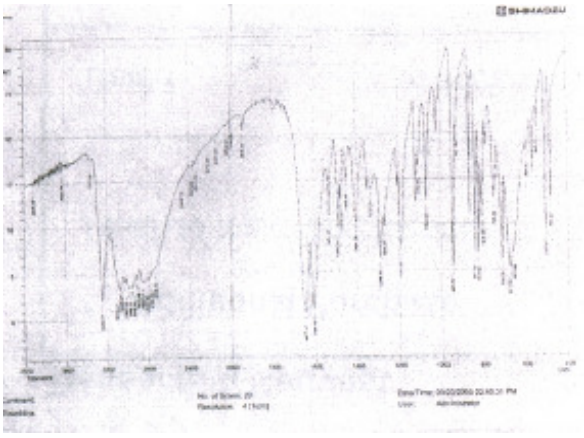


Fig. 15: *IR spectra of Drug*

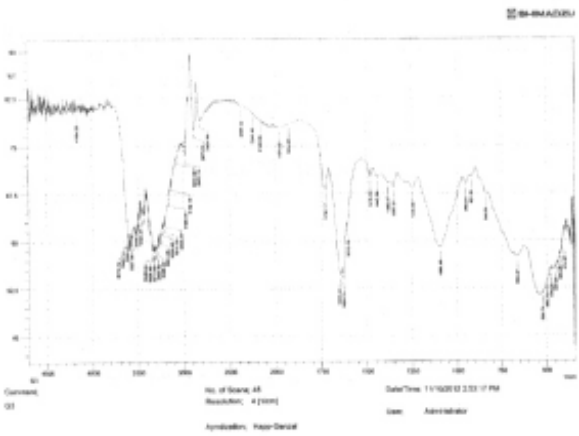


Fig. 16: *IR spectra of Batch G4*

In vitro release studies

As formulations G5 and G6 showed zeta potential values outside the region of stability (i. e., ± 30 mV) hence the batches G1, G3 and G4 were selected for in vitro release studies. In vitro release of the drug from the polymeric nanoparticles was studied for the selected batches using dialysis membrane[8] (Himedia, Mol wt cut off 12,000 Da). Phosphate buffer pH 7.4 was used as dissolution medium[9]. It was maintained at 37±1 °C & stirred at 50 rpm. Dialysis membrane soaked overnight in dissolution medium was tied at one end of a glass cylinder open at both ends. Nanoparticles containing known amount of drug were suspended in 5 ml phosphate buffer (pH 7.4) and poured into cylindrical donor compartment which was suspended in 150 ml dissolution medium in such a way that membrane just contacts the medium. At scheduled time intervals, 2 ml samples were withdrawn, centrifuged (10,000 rpm for 10 min) and the supernatant were removed. Volumes of fresh dissolution medium equilibrated at 37°C equal to that withdrawn were immediately added. The amount of drug released was determined by UV-Visible spectrophotomer1700 (Shimadzu) at 319.5nm. The tests were performed with 3 parallel runs; the values reported are mean values of the 3 runs as shown in Fig 17.

Stability study

The stability of drug loaded nanoparticles was evaluated in terms of drug content for the batch G4. The stability of nanoparticles was evaluated in phosphate buffer (pH 7.4). Nanoparticles formulation was incubated at 5-8°C and 37 ± 1°C for a period of 30 days. After specified time intervals, the suspension was centrifuged at 10,000 rpm for 30 min, supernatant was removed and detected by UV-Vis spectrophotometer at 319.5 nm.

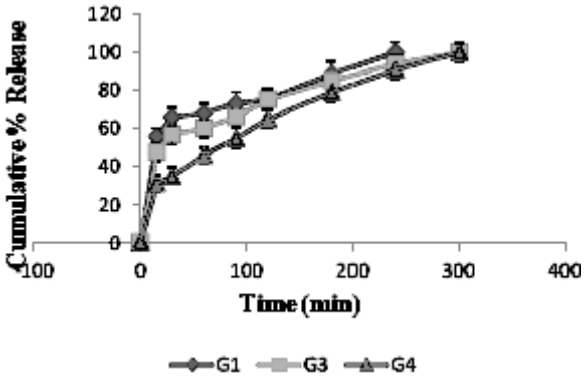


Fig. 17: *In vitro release studies of batches G1, G3 and G4*

Statistical Analysis

The statistical analysis for the determination of differences in the measured properties was accomplished using one-way analysis of variance (ANOVA) followed by Tukey's tests. Differences were termed significant with a $p < 0.05$.

RESULTS & DISCUSSION

Particle Morphology

Particles were in nano range and of spherical in shape.

Particle size analysis and zeta potential

Average particle diameter of the different nanoparticle batches is shown in Table1.The batch G1containing ethyl cellulose alone showed average particle diameter of 212 nm. For the particles prepared using ethyl cellulose with Eudragits on increasing the ratio of Eudragit RS 100 (batches G3 & G4) or Eudragit E 100 (batches G5 & G6) the nanoparticle size was increased. The particle size data showed that prepared nanoparticles were of submicron size and of small polydispersity, as it can be seen, the mean PdI values for the drug loaded formulation varied in the range of 0.314 to 0.415 except for the batch B5.

The zeta potential measurements showed negatively-charged particle surfaces, varying from -20.7 to -29.9 mV. The zeta potential values didn't vary significantly from the batch G1 in case of batches G3 and G4 as Eudragit RS100 is a neutral polymer. However, the formulations with Eudragit E100 (G5and G6) showed less negative zeta values due to cationic nature of this copolymer. Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particles and its value can be pertained to the stability of colloidal dispersion .Thus, the formulations G1, G3 and G4 with zeta potential values -26.9, -29.9, -28.0 respectively were acceptable and favoring a good stability. However, the formulations G5 and G6 were found to be unstable with zeta values of -20.7 and -22.2 mV respectively. The zeta potential of all formulations is shown in Table 1.

Drug Entrapment Efficiency

Encapsulation efficiencies (EE) of the nanoparticles are reported in Fig 12. Despite the good aqueous solubility of tizanidine hydrochloride, favoring the leakage of the drug into the external aqueous phase, entrapment efficiencies were rather high. It is assumed that drug is localized at the interfaces. Therefore a considerable amount of drug is supposed to be adsorbed at the outer surface. In addition, the elimination of the organic solvent under reduced pressure favors its fast evaporation followed by the polymer precipitation, thus reducing the movement of the drug to the external phase. It is evident from Table 1 that the encapsulation efficiency was affected by the different polymer ratio. The formulations containing Eudragit E 100 with ethyl cellulose showed comparatively higher EE (67.4%, 62.4% for batches G5 & G6 respectively) than the formulations containing Eudragit RS100 with ethyl cellulose (51.6%, 54.0% for batches G3 & G4 respectively). This could be due to hydrophilic nature of both the drug and the polymer Eudragit E 100.

FTIR Spectra

FTIR study (Table 2) showed that characteristic peaks of pristine drug tizanidine hydrochloride[10]; such as peaks for aromatic C-H stretch at 3074.32 cm^{-1}, C-N stretch at 1197.06 & 837.05 cm^{-1}, ring bending at 709.76 cm^{-1} , N-H stretch at 3245.97 cm^{-1} have appeared in the spectra of nanoparticles without any markable change in the position. It indicated that there was no chemical interaction between drug and polymer. Also, the position of strong stretching vibration of the carbonyl moiety of ester groups of polymer Eudragit RS100 at1726.17 cm^{-1} [11] was not much changed. The peaks corresponding to the amino groups of polymer have also been identified previously [12] at 2820 cm^{-1}. Any change in the position of these peaks was not observed when tizanidine hydrochloride was incorporated in the nanoparticles (Table 3).

Table 2: *FTIR Spectra of Drug*

Type of Vibration	Characteristic Absorption (cm^{-1})	Observed Peak (cm^{-1})
Secondary amine N-H stretch	3100-3600	3245.97
Aromatic C-H stretch	Just above 3000 (3000-3100)	3074.32
Secondary amine N-H bending	1900	1938.39
C=C aromatic ring stretch	Occurs in pairs at 1600 & 1475	1605 & 1485.09
Secondary amine C-N stretch	1100-1300 & 700-900	1197.06 & 837.05
Aromatic C-Cl stretch	1035-1100	1088.49
Ring bending	Strong peak near 700	709.76

Table 3: *FTIR Spectra of Formulation*

Type of Vibration	Characteristic Absorption (cm^{-1})	Observed Peak (cm^{-1})
Aliphatic ester carbonyl C=O stretch	1725-1750	1726.17
C-O stretch	Two or more bands between 1000-1300	~1245.93
Aromatic Overtone	2360	2368.42

In vitro release studies

One of the most important applications of polymeric nanoparticles is the sustained and controlled delivery of drugs. Various factors such as solubility of drug, desorption, drug diffusion, particle matrix degradation

or erosion can affect drug release. Batches G1, G3 and G4 were selected for in vitro drug release studies. An initial burst release of 56%, 48% and 31 % respectively were observed and the release was completed within 5 hr. Two possible mechanisms can be proposed for the initial burst release from nanoparticles. First, the nanoparticles can contain a larger proportion of drug at the surface of the nanoparticles in comparison to the interior of the particles. Such an uneven distribution could arise from diffusion of the small molecules to the particle surface during the particle preparation and drying process. When such particles are immersed in dissolution medium, the drug at the surface is immediately released, and only further control of drug release is due to the polymers. Second, if the dissolution medium can penetrate to some extents to the polymer matrix, the drug molecules at and close to the surface will dissolve [13,14].

Kinetic modeling:

The results of in vitro drug release study of batch G4 were fitted with various kinetic equations (Fig 18 to Fig 21) like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time) and Korsmeyer-Peppas model (log cumulative percentage drug release *vs.* log time). Various regression equations are given in Table 4.

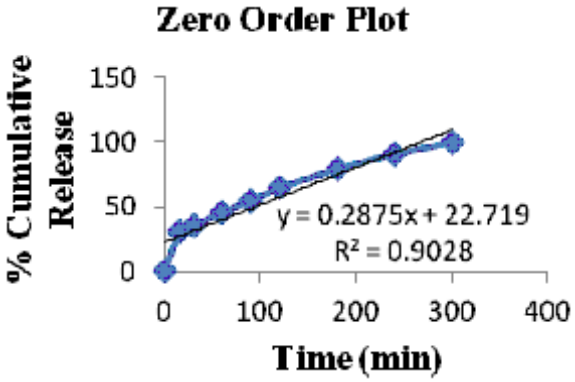


Fig. 18: *Zero Order Plot*

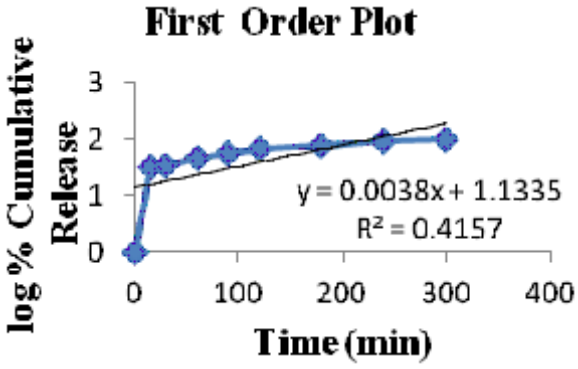


Fig. 19: *First Order Plot*

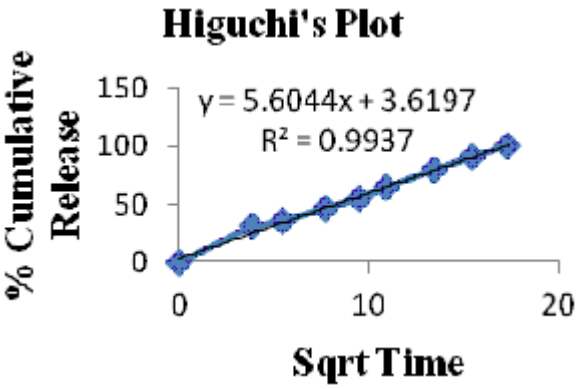


Fig. 20: *Higuchi's Plot*

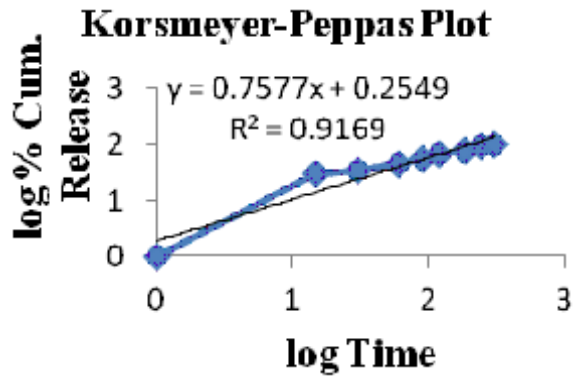


Fig. 21: *Korsmeyer-Peppas Plot*

Table 4: *Regression Equations of In Vitro Release of Batch G4*

Release Model	Regression Equation	Correlation coefficient (R^2)	Slope
Zero order	y = 0.2875x + 22.719	R^2 = 0.9028	0.2875 ± 0.03565
First order	y = 0.0038x + 1.1335	R^2 = 0.4157	0.003767 ± 0.001688
Higuchi's model	y = 5.6044x + 3.6197	R^2 = 0.9937	5.604 ± 0.1652
Korsmeyer-Peppas	y = 0.7577x + 0.2549	R^2 = 0.9169	0.7577 ± 0.08622

It was found that the in-vitro drug release of nanoparticles was best explained by Higuchi's model as the plots showed highest linearity. The correlation coefficient (R^2) was found 0.9937 for the selected batch G4. The slope n < 0.89 indicates anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled-drug release.

Stability studies

Stability study shows no remarkable change in the EE of batch G4. This indicated that formulation was stable at different storage conditions (Table 5).

Table 5: *Stability Studies for optimized batch G4*

Temperature (°C)	% Entrapment Efficiency
5-8°C	52 ± 4.0
37 ± 1°	53.4 ± 3.6

CONCLUSION

This study confirms that the double emulsion solvent diffusion (DES-D) technique is suitable for the preparation of hydrophilic drug Tizanidine hydrochloride nanoparticles with high encapsulation efficiency. This study shows that polymethacrylic acid copolymers (Eudragit®) along with ethyl acetate nanoparticles could be a useful carrier for Tizanidine hydrochloride sustained release formulation.

ACKNOWLEDGMENTS

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