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Research Article

IN VITRO DISSOLUTION STUDIES OF NIMESULIDE LOADED ETHYL CELLULOSE NANOPARTICLES BY SALTING OUT TECHNIQUE

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ABSTRACT

The aim of this study is to prepare nImesulide loaded Ethyl cellulose nanoparticles by salting out technique. The effect of drug and polymer concentrations upon particle size, entrapment efficiency, loading capacity, electrophoretic mobility, drug content and product yield was studied. Three Formulations were prepared by varying the concentration of drug and polymer. In Formulation 1 equal concentrations of drug and polymer were taken. In Formulation 2 the concentration of drug was exactly doubled to that of polymer. In Formulation 3 the concentration of polymer was exactly doubled to that of drug. The effect of drug and polymer concentrations upon particle size, drug content, entrapment efficiency, loading capacity, electrophoretic mobility and zeta potential was studied. Comparative study was made for the in vitro drug release profiles of all the three formulations. Ethyl cellulose was selected as polymer. Nimesulide was chosen as drug. On comparison, Formulation 1 was resulting particles in nano range. Drug content, entrapment efficiency and zeta potential values were found to be higher than other formulations indicating its stability. In vitro drug release study was conducted for a period of 92 hrs. In a time period of 92 hrs 18.08 % of the drug has been released from Formulation 1 indicating its sustained release nature. From the results it can be concluded that Formulation 1 can be considered as the best Formulation because of its small particle size, good stability and sustain release property for the preparation of ethyl cellulose nanoparticles.

Keywords: *Ethyl Cellulose (EC) Nemusilide (NM) Zetapotential, Scanning Electron Microscope (SEM) Photon Correlation Spectroscopy (PCS), Zetasizer (ZS)*

INTRODUCTION

Nanoparticles are sub nanosized colloidal structures made up of synthetic and semisynthetic polymers. Several methods exist for the preparation of nanoparticles from biodegradable polymers (Robinson JR and Lee VHL, 2005).

These includes: emulsification solvent evaporation, monomer emulsion polymerization, salting out, and nano precipitation. Depending on the preparation method drugs or antigens can either be entrapped in the polymer matrix, encapsulated in a liquid core, surrounded by a shell-like polymer membrane, or bound to the particle surface by adsorption (Hoffman *et al.*, 1983). For drug loading of nanoparticles, three major strategies can be employed: covalent attachment of the drug to the particle surface or to the polymer prior to preparation, adsorption of the drug to a preformed carrier system, and incorporation of the drug into the particle matrix during particle preparation.

Nanoparticle preparation using polymer precipitation methods (Le Thi Mai Hoa *et al.*, 2009; Patil, 2008). In these hydrophobic polymer and a hydrophobic drug is dissolved in an organic solvent followed by its dispersion in a continuous aqueous phase in which polymer is insoluble. The external phase also contains stabilizer (des Rieux and Fievez, 2006).

Depending upon solvent miscibility techniques they are designated as solvent extraction/evaporation method. The polymer precipitation occurs as consequence of the solvent extraction/evaporation at which can be brought by- a) Increasing the solubility of the organic solvent in the external medium by adding an alcohol (i.e. isopropranolol). b) By incorporating additional amount of water into the ultra emulsion. c)

By evaporation of organic solvent at room temperature or at accelerated temperature or by using vacuum. d) Using an organic solvent that is completely soluble in the continuous aqueous phase nanoprecipitation (Soppimath *et al.*, 2001).

Salting Out: It is one of the most commonly adopted methods to prepare nanoparticles. The method involves the incorporation of saturated aqueous solution of polyvinyl alcohol into an acetone solution of polymer under magnetic stirring to form an o/w emulsion.

The process differs from nanoprecipitation technique as in the latter the polymeric solution is completely miscible with the external aqueous medium. But in salting out technique, the miscibility of both phases is prevented by the saturation of external aqueous phase with PVA. The precipitate of polymer occurs when sufficient amount of water is added to external phase to allow complete diffusion of acetone from internal phase into aqueous phase17. This technique is suitable for drugs and polymers that are soluble in polar solvents such as acetone or ethanol (Chavanpatil *et al.*, 2007).

MATERIALS AND METHODS

Materials

Ethyl Cellulose Supplied by SD-Fine chemicals; Acetone Supplied by SD-Fine chemicals; Polyvinylalcohol supplied by Hi-Chem laboratories; Nemesulide Supplied by sigma laboratories; and Magnesium chloride Supplied by SD-Fine chemicals were used for the study.

Methodology

Ethyl Cellulose polymer and nemesulide is dissolved in acetone. Polyvinyl alcohol is dissolved in aqueous phase. Magnesium chloride is added to aqueous phase. The saturated aqueous solution of PVA was added to organic phase under magnetic stirring at 700 rpm. Stirring was continued until the solution becomes turbid. Finally, the nanoparticles were precipitated by adding sufficient quantity of water. The emulsion was centrifuged at 13,000 rpm for 30 minutes finally the particles are dried at room temperature. Three formulations were prepared by varying the concentration of the drug and polymer. In Formulation 1 equal concentrations of drug and polymer were taken (Galindo-Rodriguez *et al.*, 2004).

In Formulation 2 the concentration of drug was exactly doubled to that of polymer. In Formulation 3 the concentration of polymer was exactly doubled to that of drug. The effect of drug and polymer concentrations upon particle size, drug content, entrapment efficiency, loading capacity, electrophoretic mobility and zeta potential was studied. Comparative study was made for the in vitro drug release profiles of all the three formulations (Esmaeili *et al.*, 2008).

RESULTS AND DISCUSSION

The obtained formulations were evaluated for size, Product Yield, Drug Content, Entrapment Efficiency, Loading Capacity and Drug Release.

Scanning Electron Microscopy (SEM)

Morphological characterization of the nanoparticles was carried using scanning electron microscopy (SEM-S-3700N). For SEM the double – sided sticking tape, and coated with gold film (thickness 200nm) under the reduced pressure (0.001 torr) figure no 3, 4. The sample for the SEM analysis was prepared by sprinkling the nanoparticles on one side of double adhesive stub. The nanoparticles were viewed at an accelerating voltage of 15-20kv. The particles were found to be in nanorange (Quintanar-Guerrero, 1998).

Fourier Transforms Infrared Spectroscopy (FT-IR)

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and formulations were studied. The characteristic absorption peaks of Nimesulide were obtained at wave numbers 3284.32cm^{-1}, 2929.6cm^{-1}, 1489.10cm^{-1}, 1340cm^{-1}, 1247cm^{-1}, 1564.3cm^{-1}. The character absorption peaks of EC were obtained at 2976cm^{-1}, 2931cm^{1}, 3470 cm^{-1}. The peaks obtained in the

spectra's of each formulation correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

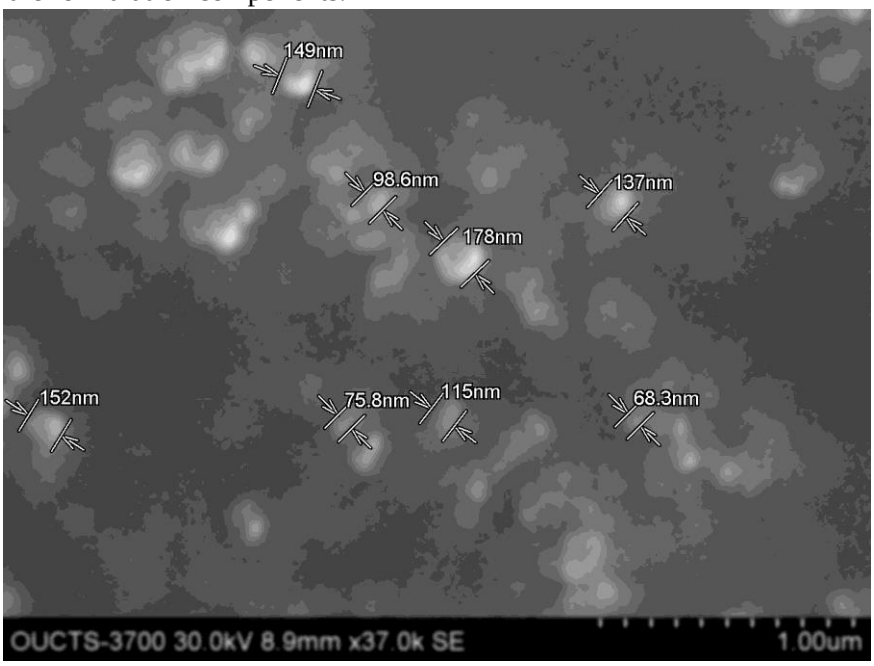

Figure: 1 SEM images of EC Formulation 1

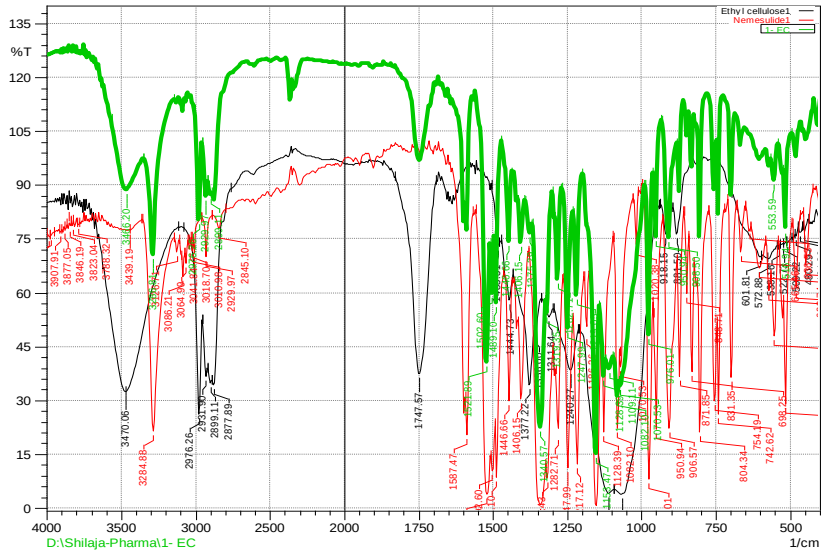

Figure: 2 FTIR Spectra of Formulation 1

Particle Size Analysis

Mean particle size of the nanoparticles was determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). Measurements were realized in triplicate at a 90º angle at 25ºC under suitable dilution conditions. Particle size distribution was expressed as mean diameter (nm) ± standard deviation and polydispersity index.

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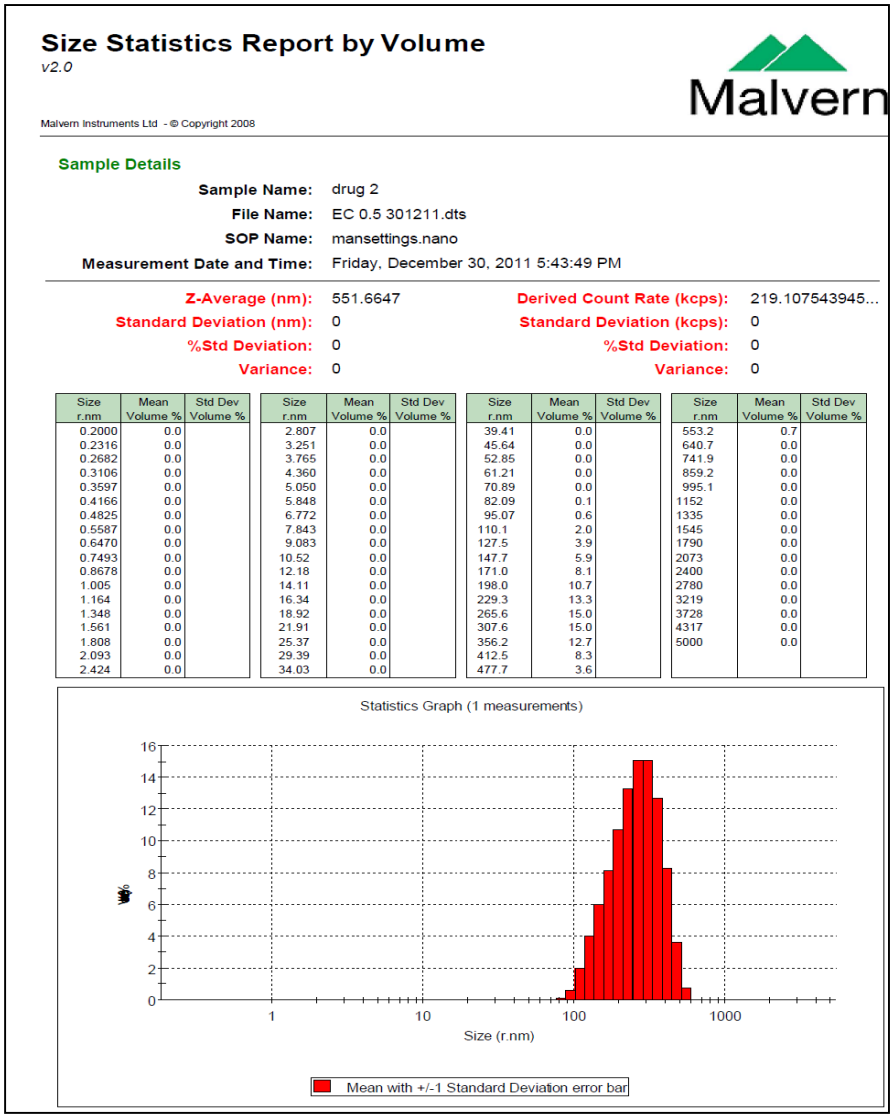
The Mean Particle diameters of Formulation 1, Formulation 2 and Formulation 3 were found to be 551.6 nm and 1753 nm and 995 nm respectively. From the results it was found Formulation 1 resulting particles in the nanorange when compared with other two formulations. On comparison Mean particle diameter of Formulation 1 was found to be in nanorange. This may be because of the molecular weight and concentration of the polymer which affects the size of the nanoparticles. Concentration of the polymer has opposite effects on nanoparticle size.

In Formulation 3 the concentration of the polymer was doubled so Particle size was observed to be increased when compared to Formulation 1. In Formulation 12 the drug concentration was doubled to that of polymer concentration which resulted in increased particle diameter. This was mainly due to greater amount of drug which results in more viscous dispersed phase. It makes mutual dispersion of the phases more difficult and results in origin of larger particles. So when drug and polymer were taken at equal concentrations particles were obtained in nanorange.



Size Statistics Report by Volume
v2.0

Malvern

Malvern Instruments Ltd - © Copyright 2008

Sample Details

Sample Name:	drug 2
File Name:	EC 0.5 301211.dts
SOP Name:	mansettings.nano
Measurement Date and Time:	Friday, December 30, 2011 5:43:49 PM

Z-Average (nm):	551.6647	**Derived Count Rate (kcps):**	219.107543945...
Standard Deviation (nm):	0	**Standard Deviation (kcps):**	0
%Std Deviation:	0	**%Std Deviation:**	0
Variance:	0	**Variance:**	0

Size r.nm	Mean Volume %	Std Dev Volume %	Size r.nm	Mean Volume %	Std Dev Volume %	Size r.nm	Mean Volume %	Std Dev Volume %	Size r.nm	Mean Volume %	Std Dev Volume %
0.2000	0.0		2.807	0.0		39.41	0.0		553.2	0.7	
0.2316	0.0		3.251	0.0		45.64	0.0		640.7	0.0	
0.2682	0.0		3.765	0.0		52.85	0.0		741.9	0.0	
0.3106	0.0		4.360	0.0		61.21	0.0		859.2	0.0	
0.3597	0.0		5.050	0.0		70.89	0.0		995.1	0.0	
0.4166	0.0		5.848	0.0		82.09	0.1		1152	0.0	
0.4825	0.0		6.772	0.0		95.07	0.6		1335	0.0	
0.5587	0.0		7.843	0.0		110.1	2.0		1545	0.0	
0.6470	0.0		9.083	0.0		127.5	3.9		1790	0.0	
0.7493	0.0		10.52	0.0		147.7	5.9		2073	0.0	
0.8678	0.0		12.18	0.0		171.0	8.1		2400	0.0	
1.005	0.0		14.11	0.0		198.0	10.7		2780	0.0	
1.164	0.0		16.34	0.0		229.3	13.3		3219	0.0	
1.348	0.0		18.92	0.0		265.6	15.0		3728	0.0	
1.561	0.0		21.91	0.0		307.6	15.0		4317	0.0	
1.808	0.0		25.37	0.0		356.2	12.7		5000	0.0	
2.093	0.0		29.39	0.0		412.5	8.3				
2.424	0.0		34.03	0.0		477.7	3.6				

Figure: 3 Particle Size Distribution Report of EC Formulation 1

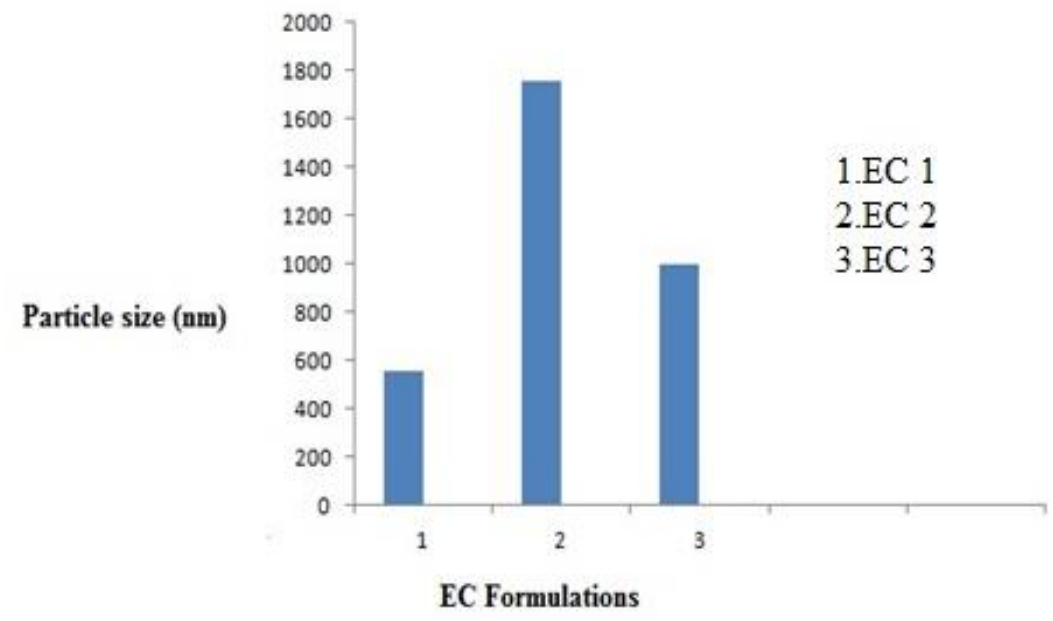


Figure: 4 Comparison of Particle Sizes of EC Formulations

Drug Content

Drug loaded nanoparticles were weighed, then grinded to fine powder and dissolved in a solvent in which the drug is completely soluble. It was subjected to stirring around 700 rpm for 3 hrs. Amount of drug in the supernatant was determined by UV-Spectrophotometric method.

Drug contents of Formulation 1, Formulation 2 and Formulation were found to be 41.693%, 25.16% and 28% respectively. From the results it was found that drug content of Formulation 1 was more when compared with other two formulations.

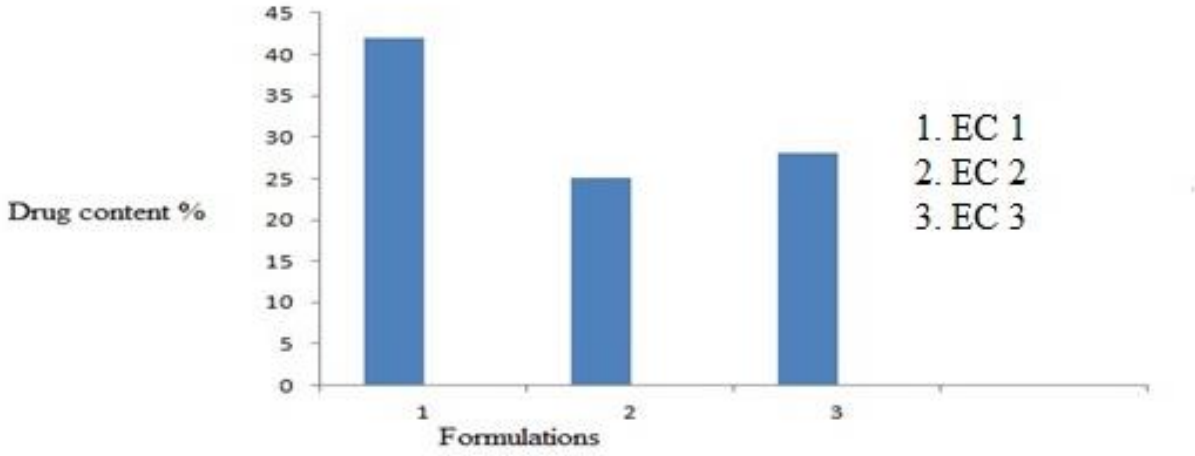


Figure: 5 Comparison of Drug Contents of EC Formulations

Encapsulation Efficiency (EE)

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (*w*) by UV- Spectrophotometry. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (*W*). Effectively, (*W*-*w*) will give the amount of drug entrapped in the pellet (Masareddy *et al.*, 2011). Then percentage entrapment is given by

$$\frac{(W - w)}{W} \times 100$$

Loading capacity was calculated by the Following equation

$$\frac{(W - w)}{\text{Nanoparticle weight}} \times 100$$

Entrapment efficiencies of Formulation 1, Formulation 2and Formulation 3 prepared by salting out technique were found to be 87.48% 95.65% and 87.2 % respectively. From the results it was found that entrapment efficiency of Formulation 2 was more when compared with other two formulations.

The lower encapsulation efficiencies obtained with the smaller particles could be explained by the longer surface area of smaller droplets for a given volume of organic phase. Hence, during the emulsification step, a more direct contact between internal and external phases occurred, resulting in a higher drug loss by diffusion towards the external medium.

Loading capacities of Formulation 1 Formulation 2 and Formulation 3 were found to be 18.3% 19.8 % and 17.44 % respectively. From the results it was found that loading capacity of Formulation 2 was more when compared with other two formulations.

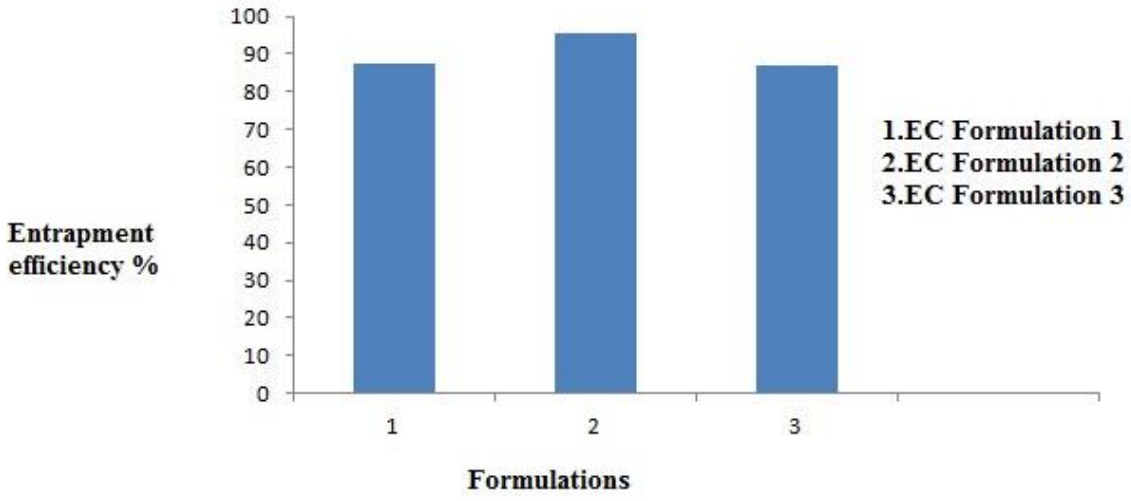


Figure: 6 Comparison of Entrapment Efficiencies of EC Formulations

Percentage Yield

The yields of the prepared nanoparticles were calculated. Nanoparticles dried at room temperature were weighed and the yield of nanoparticles was calculated using the formula.

Percent Yield = The amount of nanoparticles obtained (g) / The theoretical amount (g) x100

The Product yields of Formulation 1, Formulation 2 and Formulation 3 were found to be 38%, 66.6% and 24% respectively. From the results it was found that product yield of formulation 2 was more when compared with other two formulations.

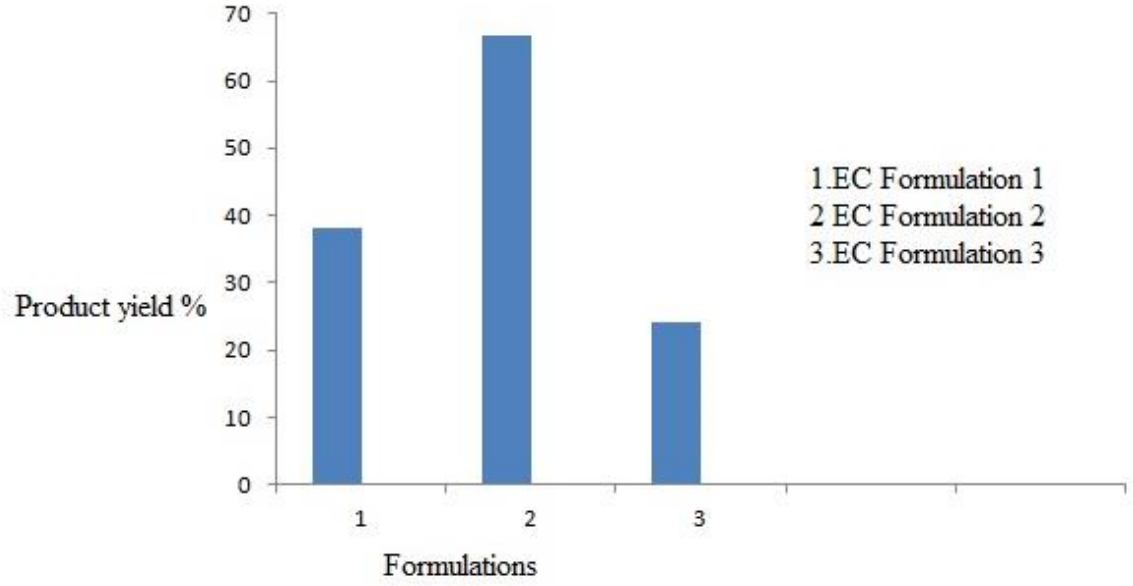


Figure: 7 Comparison of Product Yields of EC Formulations

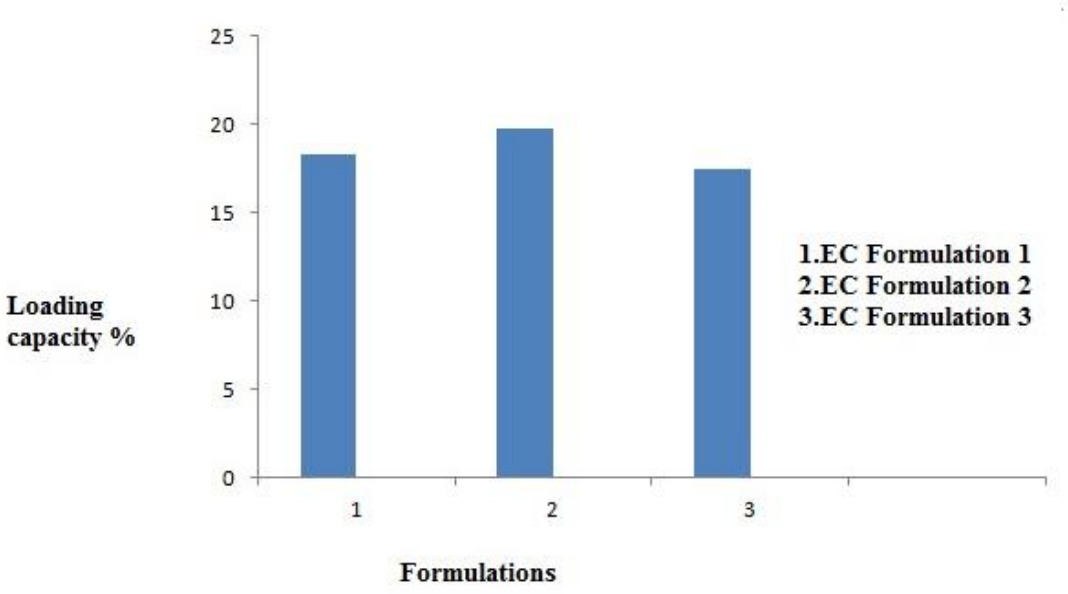


Figure: 8 Comparison of Loading Capacities of EC Formulations

Zeta Potential Measurement

Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) in triplicate to determine the surface charge and the potential physical stability of the nanosystem.

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Zeta potential of nanoparticles was measured in aqueous dispersion. Measurements were realized in triplicate at a 120º angle at 25ºC (Suri and Singh, 2007).

Electrophoretic mobility values of Formulation 1, Formulation 2 and Formulation 3 were found to be -1.672, -1.111 and -1.592 respectively. The Zeta potential values were found to be -21.3, -14.2 and -20.6 respectively. From the results it was found that electrophoretic mobility value and zeta potential value of Formulation 1 was higher when compared with other two formulations indicating more stability. Zeta potential is a measure of the charge of the particle, as such the larger the absolute value of the zeta potential the larger the amount of charge of the surface. In a sense, the zeta potential represents an index for particle stability. For the case of charged particles, as the zeta potential increases, the repulsive interactions will be larger leading to the formation of more stable particles with a more uniform size distribution. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of ± 20 mV. This stability is important in preventing aggregation. Among three EC Formulations particle size of Formulation 1 was less. Hence, zeta potential value was higher indicating greater stability.

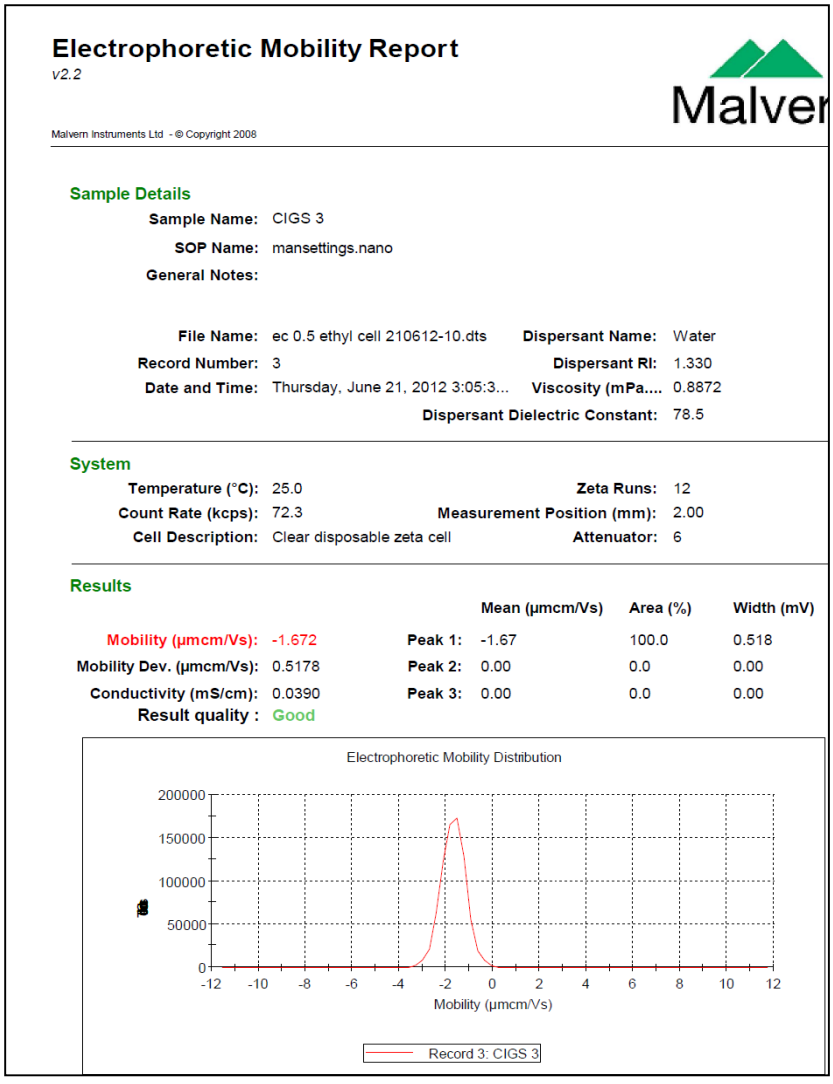

Figure: 9 Electrophoretic Mobility Report of EC Formulation 1

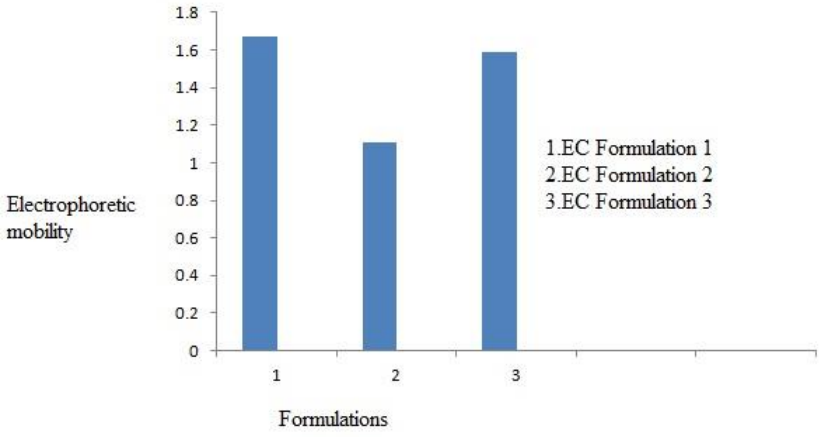

Figure: 10 Comparison of Electrophoretic Mobilities of EC Formulations

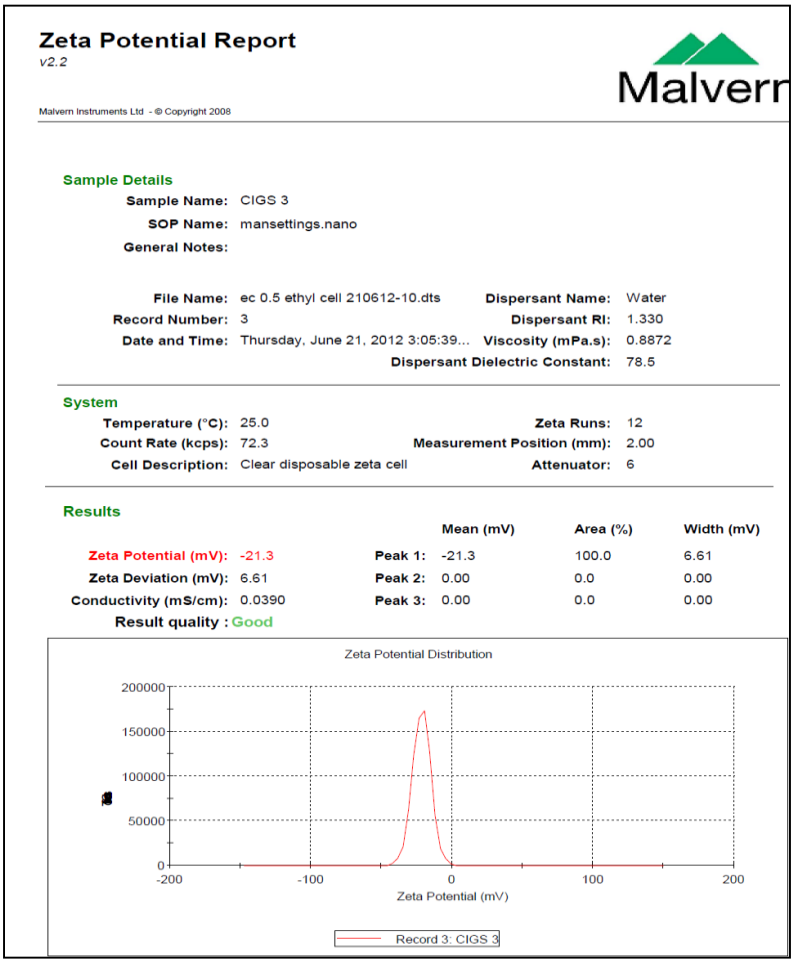

Figure: 11 Zeta Potential Report of EC Formulation 1

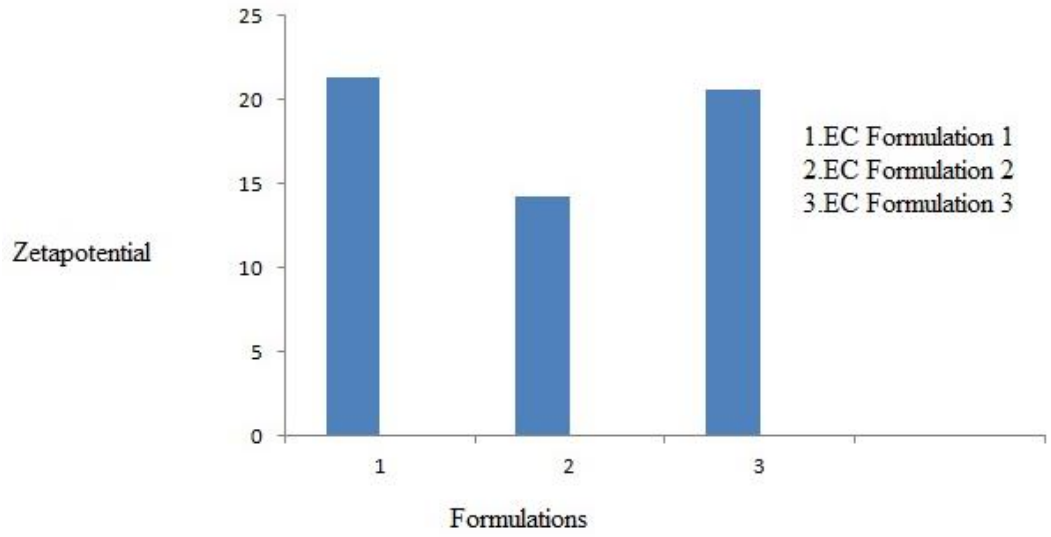

Figure: 12 Comparison of Zeta Potential Values of EC Formulations

Drug Release Studies

Drug release studies were performed by means of orbitary shaker. Drug release from polymeric nanoparticles was determined as follows. A known amount of nanoparticles was transferred to a conical flask and 50 ml of the Phosphate buffer pH 7 was added to the tube. The temperature and rotation were adjusted to 37^0C and 90 rpm, respectively. At predetermined time of 0.5, 2, 4, 6, 8, 10, 12, and 24, 36, 48 hours 5mL of sample was removed and ultra centrifuged at 15, 000 × *r* for 60 minutes, and 5ml of the supernatant were replaced by fresh medium. The samples were further analyzed using UV Spectrophotometer.

This experiment was continued for a period of 92 hrs. In all EC Formulations the drug release was slow, spread extended over period of several days. In a time period of 92 hrs 18.3%, 16.75% and 17.42 % of drug has been released from EC Formulation 1, Formulation 2 and Formulation 3 respectively. EC Formulation 1 was showing maximum drug release among all the three formulations. An important phenomenon observed here is that as larger the amount of drug present in nanoparticles then more quickly the release occurred, and the particles with little quantity of drug exhibited a release in a more sustained fashion. On comparison because of the higher drug content in EC Formulation 1 it was showing maximum drug release. Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Mean diameter of EC Formulation 1 was less when compared with other formulations. Smaller particles have longer surface area, hence drug release also more in EC Formulation 1. The curve Fitting data revealed that the release followed First order kinetics and Higuchis and Peppas plots stated Fickian diffusion controlled in all the three formulations.

Conclusions

Nimesulide loaded EC Formulations were prepared by salting out technique. Three formulations were prepared by varying the concentration of drug and polymer. When drug and polymer were taken at equal concentrations best nano formulations were obtained with increased stability and good sustained release property. In a time period of 92 hrs 18 % of the drug has been release from this formulation. From the results it can be concluded that Formulation 1 can be considered as best formulation for the preparation of ethyl cellulose nanoparticles. From the results it can be concluded that best nano formulation can be obtained by taking equal concentrations of drug and polymer.

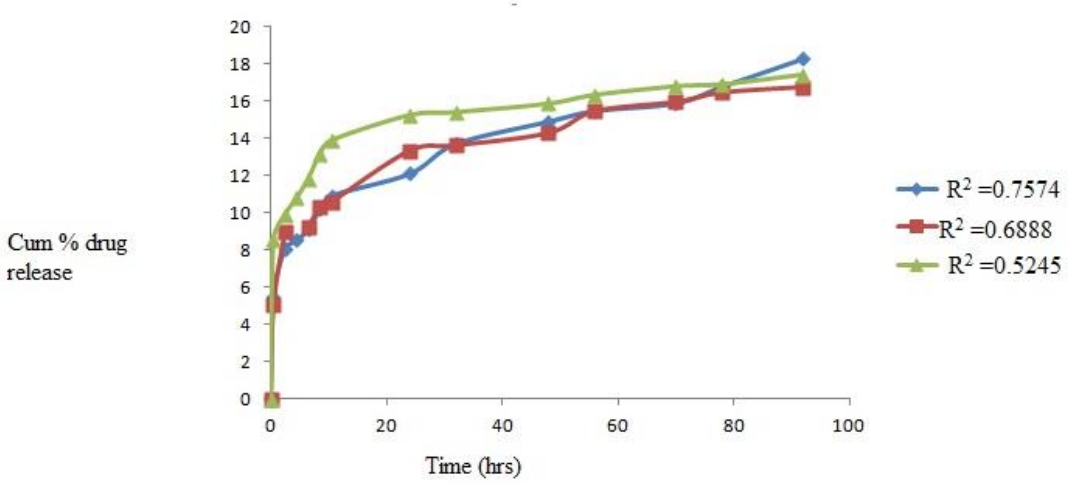

Figure: 13 Comparison of in Vitro Drug Release Pattern of EC Formulations

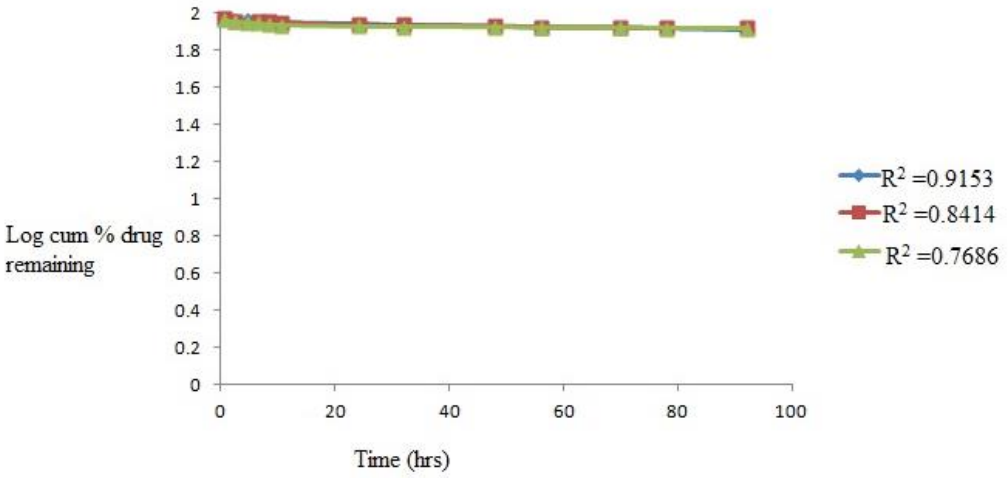

Figure: 14 Comparison of First Order Release Pattern of EC Formulations

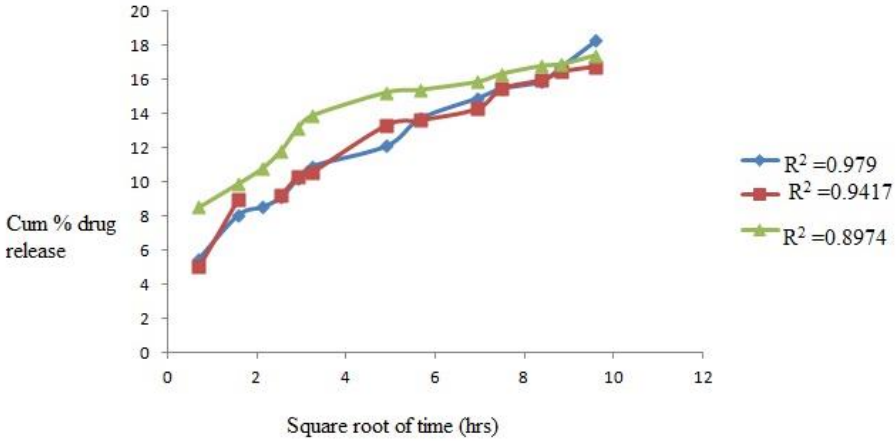

Figure: 15 Comparision of Higuchis Square Root Time Dependent Plots of EC Formulations

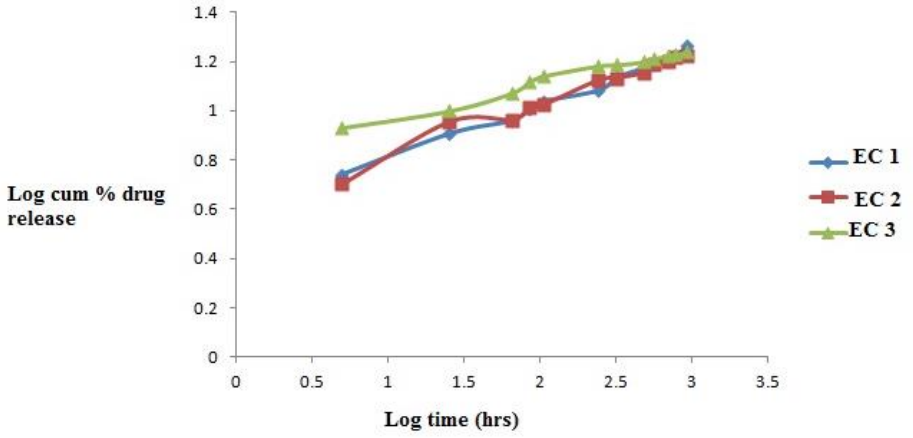

Figure: 16 Comparison of Peppas Double Log Plots of EC Formulations

REFERENCES

Robinson JR and Lee VHL (2005). Controlled Drug Delivery, fundamental and Applications 2nd edition, Revised and expanded [New York: Informa Health care] 9-19.

Vyas SP and Khar RK (2002). Controlled drug delivery, Concepts and advances, 1st edition, [Vallabh Prakashan, New Delhi]**1-4** 55-90.

Hoffman F, Pressman JH and Code CF (1983). "Controlled entry of orally administered drugs: Physiological considerations", *Drug Development and Industrial Pharmacy* **9** 1077-1085.

des Rieux A and Fievez V (2006). Nanoparticles as potential oral delivery systems of proteins and vaccines:A mechanistic approach. *Journal of Controlled Release* **116** 1–27.

Soppimath KS, Aminabhavi TM, Kulkarni AR and Rudzinski WE (2001). Biodegradable Polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release* **70** 1–20.

Das S and Suresh PK (2011). Design of Eudragit RL 100 nanoparticles by Nanoprecipitation method for ocular drug delivery Nanomedicine: *Nanotechnology*, *Biology and Medicine* **7**(2) 242-247.

Le Thi Mai Hoa, Nguyen Tai Chi *et al.*, (2009). Preparation of drug nanoparticles by emulsion evaporation method. *Journal of Physics: Conference Series* **187**(1) 012047.

Patil VB (2008). *Nanosuspensions: A Novel Approach in Drug Delivery*, 2008. Available: http://www.Pharmainfo.net **6**(2).

Chavanpatil MD *et al.*, (2007). Polymer-surfactant nanoparticles for sustained release of water-soluble drugs. *Journal of Pharmaceutical Sciences* **96**(12) 3379-89.

Galindo-Rodriguez SA, Allemann E, Fessi H and Doelker E (2005). Polymeric nanoparticles for oral delivery of drugs and vaccines: a critical evaluation of in vivo studies, *Critical Reviews in Therapeutic Drug Carrier Systems* **22** 419–464.

1923. "The solvent properties of Acetone" R f Ramler, *Industrial and Engineering Chemistry* **15**(7) 717-720.

Susan B, Maryadele JO Neil (1997). An Encyclopedia of chemical, drugs and Biological In: *The Merck Index*, 12th edition, (Merck Research Laboratories. Division of Merck and co Inc. Whitehouse Station, NJ: Merck & Co.) **196**(3828).

Galindo-Rodriguez S, Allemann E, Fessi H and Doelker E (2004). Physicochemical Parameters Associated with Nanoparticle Formation in the Salting-out, Emulsification-Diffusion and Nanoprecipitation Methods, *Pharmaceutical Research* **21**(8).

Esmaeili F, Atyabi F and Dinarvand R (2008). Preparation and characterization of Estradiol -loaded PLGA nanoparticles using homogenization-solvent diffusion method, *DARU* **16**(4).

Murakami H, Kobayashi M, Takeuchi H *et al.*, (1999). Preparation of poly (DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *International Journal of Pharmaceutics* **187**(2) 143-52.
Mitra S and Maitra A (2002). Nanoparticle carriers in drug delivery and targeting. *Proceeding of the Indian National Science Academy* (PINSA) B 68 **4** 349-360.
Mohanraj VJ and Chen Y (2006). Nanoparticles- AReview. *Tropical Journal of Pharmaceutical Research* **5**(1) 561-573.
Suri SS and Singh B (2007). Nanotechnology-based drug delivery systems: *Journal of Occupational Medicine and Toxicology* **2**(16) 1-6.
Masareddy RS, Sutar RS and Yellanki SK (2011). Nano drug delivery systems - a review *International Journal of Pharma Sciences and Research* **2**(2) 203-216.
Quintanar-Guerrero D (1998). Preparation and characterization of nanocapsules from preformed polymers by a new process based on emulsification-diffusion technique. *Pharmaceutical Research* **15**(7) 10656-1062.
Duncan R (2003). The dawning era of polymer therapeutics. *Nature Reviews* **2** 347–360.