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"INFLUENCE OF MICROENCAPSULATION TECHNIQUE AND POLYMER AMOUNT ON DESIGNING AND DEVELOPMENT OF LOSARTAN POTASSIUM MICROSPHERE"

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ABSTRACT

Microencapsulation of drugs into solid non-biodegradable polymeric microspheres by solvent evaporation technique remains challenging especially with those having low molecular weight and high hydrophilicity nature. This paper presents an efficient encapsulation protocol for this group of drugs, demonstrated using losartan potassium (LP) as a model compound which is encapsulated into Eudragit RS 100 (ERS) and Eudragit RL 100 (ERL) microspheres. The effects of polymer type and polymer/drug ratios on the size, surface morphology, encapsulation efficiency and the release characteristics of the microspheres were examined. The formulation containing drug/polymer ratio 1:5 (F2) in which the ratio between the two polymers (ERS:ERL) is 4:1 was found to be the most appropriate with respect to encapsulation At the optimum stirring speed of 750 rpm, best spherical shaped particles with good surface characteristics were found by SEM study, particles were distributed over the size range of 100-400 µm. This proposed method has been successfully used to prepare batches of microspheres having high encapsulation efficiencies.

KYEWORD: Polymer, Losartan Potassium, Microsphere, Microencapsulation.

INTRODUCTION

Drug delivery system is an interface between the patient and the drug. It may be a formulation of the drug to administer it for a therapeutic purpose or a device used to deliver

the drug. This distinction between the drug and the device is important, as it is the criterion for regulatory control of the delivery system by the drug or medicine control agency. If a device is introduced into the human body for purposes other than drug administration, such as therapeutic effect by a physical modality or a drug may be incorporated into the device for preventing complications resulting from the device, it is regulated strictly as a device. There is a wide spectrum between drugs and devices, and the allocation to one or the other category is decided on a case by case basis.

Losartan Potassium

Losartan potassium is the prototype of this new class of cardiovascular drug, and it offers the advantages of increased selectivity, specificity, and maintained blockade of the circulating and tissue renin-angiotensin system at the AT_1 receptor level without the adverse reactions associated with ACE inhibitors. Losartan has now been approved for use in the treatment of hypertension in Scandinavia, the UK, several European countries, and the USA.

Microencapsulation of drugs into solid non-biodegradable polymeric microspheres by solvent evaporation technique remains challenging especially with those having low molecular weight and high hydrophilicity nature. The objective and aim of this work is to present an efficient encapsulation protocol for this group of drugs, demonstrated using Losartan potassium (LP) as a model compound which is encapsulated into Eudragit RS 100 (ERS) and Eudragit RL 100 (ERL) microspheres.Parameters involved in the production and the formulation aspect were optimized to achieve the best protocol having controlled efficiency of encapsulation that is simple, safe, practical, and economical. This proposed method has successfully used to prepare batches of microspheres having different encapsulation efficiencies and its potential applications have been demonstrated accordingly.

MATERIAL AND METHOD

The losarten potassium pure drug was gathered as gift sample from Jubilant Organosys, Noida, UP, Eudragit RS & RL 100 obtained from Himedia Laboratories mumbai, other chemicals like Dichloromethane Extra Pure, Double Distilled Water, Polyvinyl alcohol (PVA), Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium Chloride, Concentrated Hydrochloric Acid were obtained from Merck (India) Limited, Mumbai.

Method of preparation of losartan potassium microspheres

Preparation of Phosphate Buffer pH 7.4 solution (IP-2007)

According to specification described in Indian pharmacopoeia an accurately weighed quantity of 2.38 gm of di-sodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8.0 gm of Sodium chloride was added to sufficient double distilled purified water and it was made up to a 1000 ml clear solution. The pH of the buffer was analyzed by digital pH meter (Hanna instruments pHep®, Model No.PHEP).

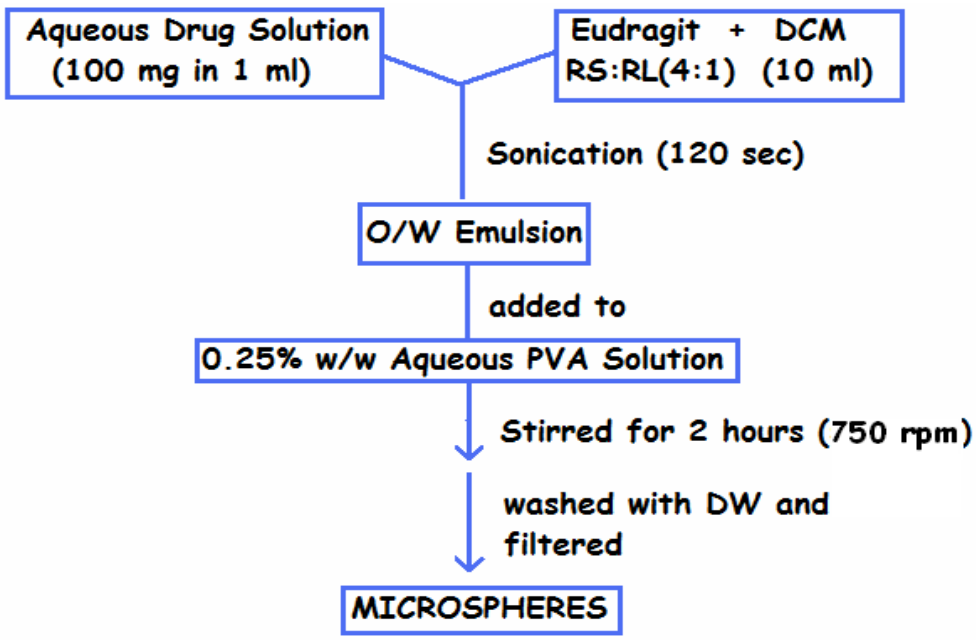
Preparation of 0.1M HCl solution (pH 1.2)

According to specification described in Indian pharmacopoeia an accurately measured quantity of 8.4 ml of concentrated hydrochloric acid was added to sufficient double distilled purified water and it was made up to a 1000 ml clear solution. The pH of the buffer was analyzed by digital pH meter (Hanna instruments pHep®, Model No.PHEP)

Preparation of stock solution

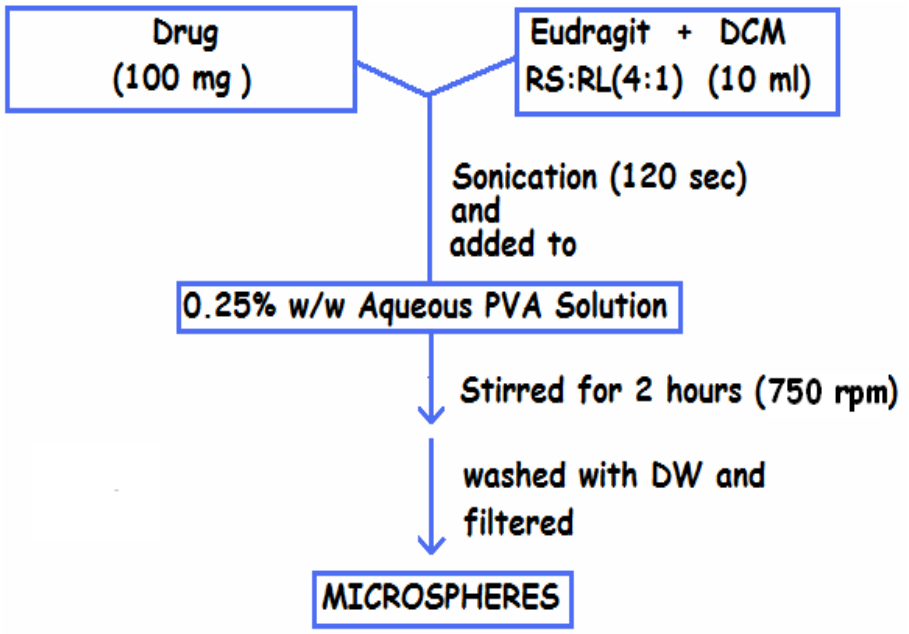
An accurately weighed quantity of 25mg of Losartan Potassium was taken in a clean, dry 250ml of volumetric flask. Then volume was made up to 250ml with phosphate buffer pH 7.4 and shaken vigorously to yield a clear solution of 100 mcg/ml concentration.

Design and Formulation of Losartan Potassium loaded Microspheres by Double Emulsion Solvent Evaporation Technique ($W_1/O/W_2$ Method)



Schematic Representation of Preparation of Losartan potassium loaded microspheres by $W_1/O/W_2$ method

Design and Formulation of Losartan Potassium loaded Microspheres by Emulsion Solvent Evaporation Technique (O/W Method)



Schematic Representation of Preparation of Losartan potassium loaded microspheres by O/W method

Characterization Process of Losartan potassium Loaded Microspheres

a) % Yield Value of Microspheres

The prepared microspheres were assessed for the yield value. The batch was weighed after total drying and the yield % was calculated using the formula give below. Each batch was formulated in triplicate batches (n=3) to get a reproducible yield (Lim et al., 2000).

$$\% \text{ Yield} = \frac{\text{The amount of microspheres practically obtained (g)}}{\text{The theoretical amount (g)}} \text{ X } 100$$

b) Flow properties of prepared microspheres

Flow properties of prepared microspheres were determined by bulk density, tapped density, Carr's index and Hausner Ratio or Packing factor (Jain et al., 2005).

i) Determination of Bulk Density and Tapped Density

Accurately weighed quantities of prepared microspheres were carefully poured into the graduated cylinder (10ml). The initial volume was measured. The graduated cylinder was tapped for 100 times. After that the volume was measured.

ii) Carr's Index or Compressibility Index

$$\text{Carr's index} = \frac{\text{Tapped density - bulk density}}{\text{Tapped density}} \times 100$$

Grading of the powders for their Flow properties according to the Carr's index.

iii) Hausner Ratio

It indicates the flow properties of the microspheres and measured by the ratio of tapped density to bulk density.

$$\text{Hausner Ratio} = \frac{\text{Tappeddensity}}{\text{Bulk density}}$$

c) Drug Loading and Encapsulation efficiency

Microspheres were thoroughly triturated in a mortar with a pestle. An equivalent accurately weighed amount of 50 mg of powdered microspheres was extracted with 50 ml of 0.1 M HCl by extensive stirring for 24 h at 150 rpm. The solution was then filtered through a 0.45 micron membrane filter (Millipore Millex-HV); a sample of 1mL was withdrawn from this solution, diluted to 10 mL with 0.1 M HCl. The resulting acidic solution containing the extracted drug was clarified by centrifugation at 2000 g for 20 min and assayed using UV spectroscopy at 248.7 nm to find out the actual drug content of microspheres. The measured absorbance was converted to drug concentration using a standard curve for the known concentration of the drug in 0.1M HCl. The experiment was carried out in triplicate for each sample.

The % drug loading (Yang et al., 2003) was calculated using the following equation: Study was performed in triplicate (n=3) to get reproducible results.

$$\text{\% Drug loading} = \frac{\text{Amount of loaded drug}}{\text{weighed quantity of powder of microspheres}} \text{ X } 100$$

Encapsulation efficiency (Haznedar et al., 2003) was calculated using the following equation: Study was performed in triplicate (n=3) to get reproducible results.

$$\text{\% Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

d) Particle Size Distribution Study

The size distribution study was carried out using an optical microscope, and the mean particle size was calculated by measuring 300 particles with the help of a calibrated ocular micrometer. Calibration of the optical microscope was carried out using following procedure (Martin et al., 1993).

e) Scanning electron microscope analysis

The external and internal morphology of the microspheres were studied by scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling the powder on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan). SEM images are shown in Fig. 13-18 (Kılıcarslan et al., 2003).

f) Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra were taken on JASCO FT-IR (Model 4100, Japan) to confirm cross linking and to investigate chemical interactions between drug and polymer matrix. 2% of samples were crushed with KBr to get transparent pellets by applying a pressure of 6 Ton. FT-IR spectra of pure LP (Losartan potassium), pure polymers (ERS 100 & ERL 100) and Losartan potassium loaded microspheres prepared by both the techniques were scanned in the range between 500 and 4000 cm^{-1}. FT-IR spectra are shown in Fig. 19-27 (Chopra et al., 2007).

g) Thermal Analysis of Drug crystallinity (Differential Scanning Calorimetry)

Differential scanning calorimetry (DSC) thermograms were obtained by a Mettler Toledo DSC 822e Stare 202 System (Mettler Toledo, Switzerland) equipped with a thermal analysis automatic program. Aliquots of about 5mg of each sample were placed in an aluminium pan of 40µl capacity and 0.1mm thickness, press-sealed with a perforated aluminium cover of 0.1mm thickness.

RESULTS AND DISCUSSION

PREPARATION PROCESS OF THE LOSARTAN POTASSIUM LOADED MICROSPHERES

Losartan potassium (LP) is a potent, highly specific angiotensin II type 1 (AT_1) receptor antagonist with antihypertensive activity. It is readily absorbed from the gastrointestinal tract

with oral bioavailability of about 33% and a plasma elimination half-life ranging from 1.5 to 2.5 h. Administration of LP in a controlled release dosage form with an extended release over 12 h, would be more desirable as these characteristics would allow a rapid onset followed by rotracted anti-hypertensive effects by maintaining the plasma concentrations of the drug well above the therapeutic concentration.

BATCH YIELD OF MICROSPHERES

The % batch yield of all optimized batches is given in Table

Formuln Code	Drug Polymer Ratio (D:P)	Theoretical Amount of Microspheres (mg)	Practical Amount of Microspheres (mg)			% Yield
F 1 (W/O/W)	1:4	500	420	433	428	85.43 ± 1.34
F 2 (W/O/W)	1:5	600	486	485	488	81.09 ± 0.22
F 3 (W/O/W)	1:6	700	530	533	532	76.00 ± 0.17
F 4 (O/W)	1:4	500	372.5	385	381	75.96 ± 1.33
F 5 (O/W)	1:5	600	485.6	492	488	81.48 ± 0.56
F 6 (O/W)	1:6	700	523.7	536	528	75.65 ± 0.95

% Batch Yield of all optimized batches. Results are mean ± SD (n=3)

FLOW PROPERTIES OF OPTIMIZED MICROSPHERES

The results of Carr's Index of all optimized batches are given below in Table 8

Table 8: Carr's Index of all optimized batches.

Formulation code	Weight (gm)	Bulk volume	Tapped volume	Bulk density	Tapped density	Carr's Index (%)
F 1 (W/O/W)	2	1.5	1.2	1.33	1.66	19.87
F 2 (W/O/W)	2	1.6	1.3	1.25	1.53	18.85
F 3 (W/O/W)	2	1.8	1.4	1.11	1.42	22.43
F 4 (O/W)	2	1.1	0.8	1.81	2.5	27.6
F 5 (O/W)	2	1.6	1.2	1.25	1.66	25.10
F 6 (O/W)	2	1.4	1.1	1.42	1.81	28.04

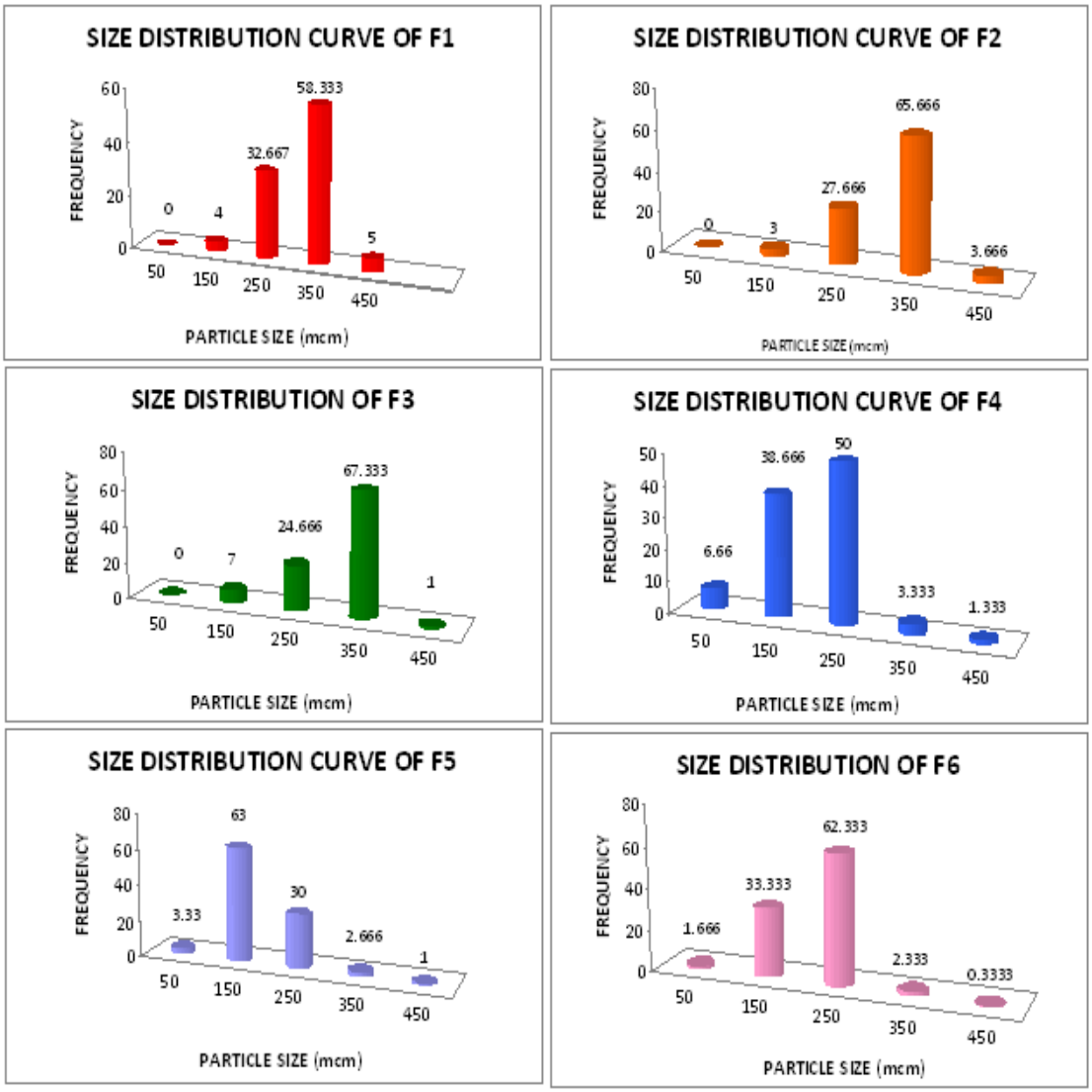
The results of Hausner's Ratio of all optimized batches are given below in Table 9

Table 9: Hausner's Ratio of all optimized batches

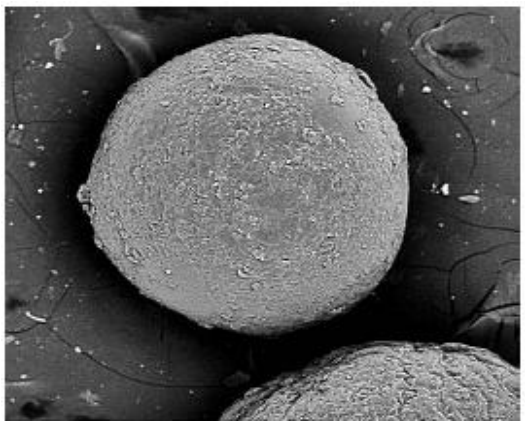
Formulation code	Weight (gm)	Bulk volume	Tapped volume	Bulk density	Tapped density	Hausner's Ratio
F 1 (W/O/W)	2	1.5	1.2	1.33	1.66	1.24
F 2 (W/O/W)	2	1.6	1.3	1.25	1.53	1.22
F 3 (W/O/W)	2	1.8	1.4	1.11	1.42	1.27
F 4 (O/W)	2	1.1	0.8	1.81	2.5	1.38
F 5 (O/W)	2	1.6	1.2	1.25	1.66	1.32
F 6 (O/W)	2	1.4	1.1	1.42	1.81	1.27

PARTICLE SIZE DISTRIBUTION STUDY

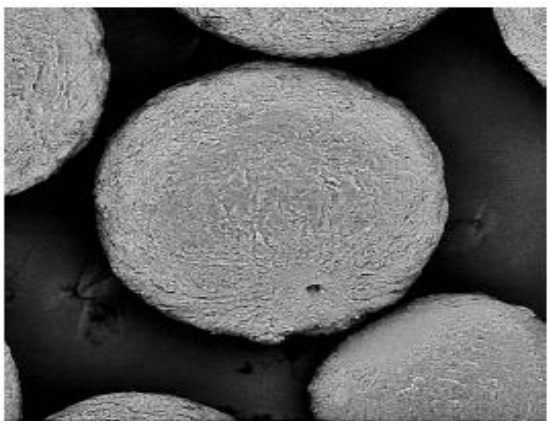
Size- frequency distribution curve of all optimized batches is given in Fig.12



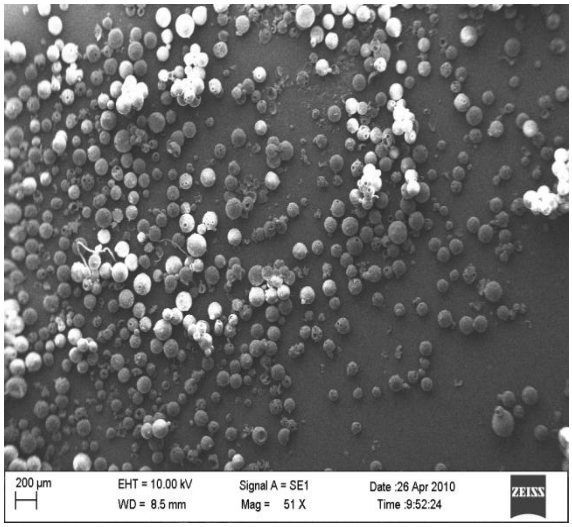
SURFACE MORPHOLOGY STUDY



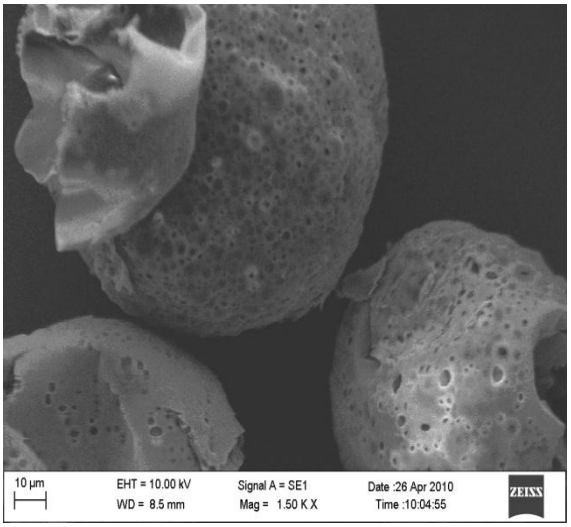
SEM micrograph of optimized formulation F2



SEM micrograph of optimized formulation F5



SEM micrograph of Clusters of Microspheres

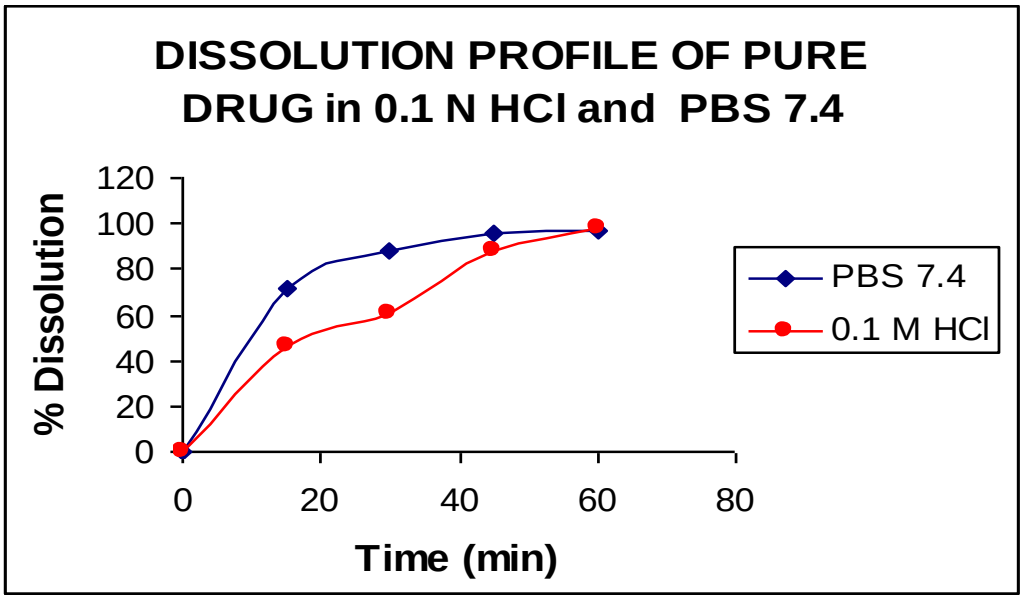


SEM micrograph of Microspheres after Dissolution

IN-VITRO RELEASE STUDY

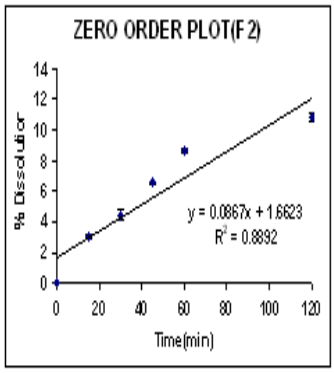
***In vitro* release study of pure drug in 0.1 M HCl & PBS 7.4.**

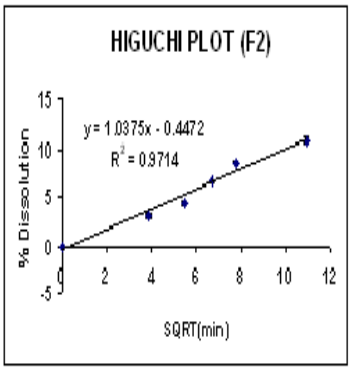
TIME (min)	SQRT	in 0.1 M HCl		PBS 7.4	
		Cummulative Amount Released (mg)	% Dissolution	Cummulative Amount Released (mg)	% Dissolution
0	0	0	0	0	0
15	3.872983	4.618812	46.18812	7.201969	72.01969
30	5.477226	6.099917	60.99917	8.81698	88.1698
45	6.708204	8.836634	88.36634	9.540741	95.40741
60	7.745967	9.776485	97.76485	9.733877	97.33877

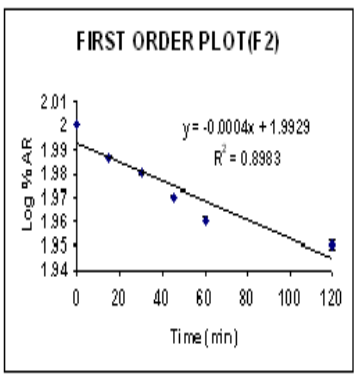


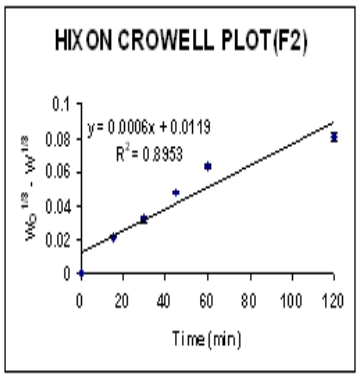
F2 (Results are Mean±SD)

TIME (min)	SQRT	% Dissolution	Log % Amount Remaining (Log%AR)	Hixon ($W_0^{1/3} - W^{1/3}$)
0	0	0	2	0
15	3.8729	3.0198 ± 0.1495	1.9866 ±0.00066	0.0219 ± 0.0010
30	5.4772	4.4227 ± 0.2893	1.9803 ±0.0013	0.0322 ± 0.0021
45	6.7082	6.5759 ± 0.1073	1.9704 ± 0.00049	0.0482 ± 0.0008
60	7.7459	8.5825±0.1746	1.9610± 0.0008	0.0634 ± 0.0013
120	10.954	10.7782 ± 0.2783	1.9504 ± 0.0013	0.0803 ± 0.0021



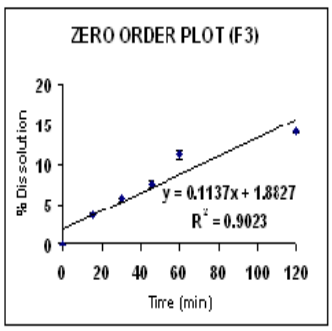


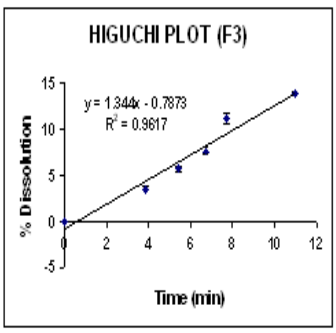


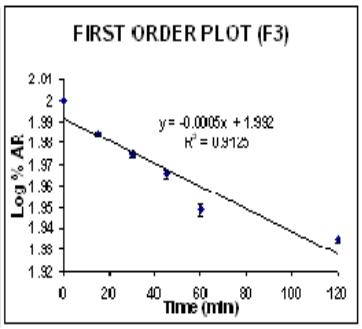


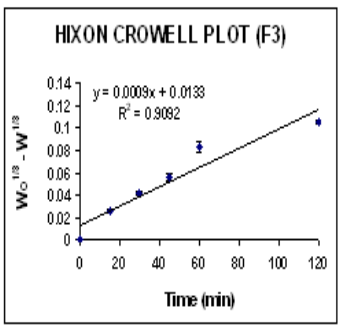
F3 (Results are Mean±SD)

TIME (min)	SQRT	% Dissolution	Log % Amount remaining Log%AR)	Hixon ($W_0^{1/3} - W^{1/3}$)
0	0	0	2	0
15	3.8729	3.5643 ± 0.1782	1.9842 ± 0.0008	0.0259 ± 0.0013
30	5.4772	5.6633 ± 0.2533	1.9746 ± 0.0011	0.0414 ± 0.0018
45	6.7082	7.6254 ± 0.4199	1.9655 ± 0.0019	0.0562 ± 0.0031
60	7.7459	11.172 ± 0.6020	1.9485 ± 0.0029	0.0834 ± 0.0046
120	10.954	13.9667 ± 0.1360	1.9346 ± 0.00068	0.1053 ± 0.0010









CONCLUSION

In conclusion, LP loaded ERS/ERL microspheres was formulated by using two emulsification techniques ($W_1/O/W_2$ and O/W) and six different formulation were formulated using different drug polymer ratio. Influence of both the formulation and process parameters in formulation of LP loaded microspheres was studied with respect to the size, size distribution, yield, entrapment efficiency and drug loading. Physical state analysis (crystalline

or amorphous) and *in-vitro* characterization was carried out to evaluate the release characteristics of the drug from microspheres with respect to the pure drug in two different medias.

Different test was performed and flow property for microspheres was found good for F2 and F3 formulation which is F2-18.85 & F3-22.43.

Drug entrapment value was found maximum for F6-89.1 ± 1.26.

The mean particle size was determined for all the microsphers the particle size was distributed over the range for 100-400µm.

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