



Influence of Drug/Polymer Ratio on the Encapsulation Efficiency of Highly Hydrophilic Drug

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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 as a model compound which is encapsulated into Eudragit RS 100 (ERS) and Eudragit RL 100 (ERL) microspheres. The microspheres were prepared using solvent evaporation technique with an ultimate aim of prolonging drug release. Six formulations were prepared using different drug/polymer ratio. 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 in which the ratio between the two polymers (ERS:ERL) is 4:1 was found to be the most appropriate with respect to encapsulation efficiency ($94.43\% \pm 0.277$), drug loading ($19.58\% \pm 0.417$), batch yield ($80.40\% \pm 1.712$), particle size (204.23 ± 8.438) and having highest correlation coefficient (0.9829) amongst all formulation thus providing a desired drug release characteristics. At the optimum stirring speed of 750 rpm, best spherical shaped particles with good surface characteristics were obtained, which were distributed over the size range of 120-270 μm . This proposed method has been successfully used to prepare batches of microspheres having high encapsulation efficiencies.

Key Words: Microencapsulation, Eudragit, Losartan potassium, Microspheres, Encapsulation efficiency.

INTRODUCTION

In the recent past, the development of targeted drug delivery systems¹ have received an increasing interest not only for a better treatment of specific local pathologies, but also for the systemic therapy of both conventional and labile molecules as well as a means of achieving chronotherapy for diseases like hypertension. The ability to deliver and have a controlled release of therapeutic agents at injured or targeted disease sites is an important aspect in drug development and regenerative medicine. Such system avoid unnecessary health side effects due to burst effect or overdose, ensuring optimum supply of drug that is required by the biological system for a prolonged period and cutting down wastage of expensive drugs. Encapsulating the active agents within a polymeric matrix microsphere is a good option to achieve the objective as the polymer can act as the rate-controlling membrane to obtain the desired controlled release^{2,3}. Besides, encapsulation minimizes the deactivation of drugs during the delivery process due to the protection by the polymer shell and this ensures sufficient amount of drug reaching the targeted area. Although, modern microencapsulation of bioactive substances still continues to be an important area and the effort mostly concentrates

on formulation and protocol optimization strategies. The solvent-evaporation method of microencapsulation involves the use of emulsification of a solution containing polymer and drug with an additional medium in which the drug and polymer cannot dissolve. The technique is relatively simple and has been used to prepare microspheres of a variety of compounds using several different polymeric materials. Various therapeutic drugs have been investigated and proven to portray such controlled release manner, which also include peptides and proteins⁴. In the solvent evaporation process, the polymer is dissolved in a suitable organic solvent and the medicament is dispersed or dissolved in this polymeric solution. The resultant solution or dispersion is then emulsified in an aqueous continuous phase to form discrete droplets. In order for the microspheres to form, the organic solvent must first diffuse into the aqueous phase and then evaporate at the water/ air interface. As solvent evaporation occurs, the microspheres harden and free flowing microspheres can be obtained after suitable filtration and drying⁵.

There are several formulation and process parameters that, when modified during the formulation of microspheres by solvent evaporation, may affect the properties of microspheres. The parameters in question include the aqueous solubility of

raw material and drug to be encapsulated, the type and concentration of the dispersing agent, the drug/polymer ratio and the stirring rate used to agitate the emulsion system formed during the manufacturing process.

Losartan potassium (LP) is an angiotensin II receptor (type AT₁) antagonist⁶. It is a non-peptide molecule, chemically described as 2-butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt. It is a white to off-white free-flowing crystalline powder with a molecular weight of 461.01, half life 1.5 to 2.5 h. It is freely soluble in water, soluble in alcohols and slightly soluble in common organic solvents, such as acetonitrile and methyl ethyl ketone.

Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic form. Eudragit RL 100 (ERL) and Eudragit RS 100 (ERS) are practically insoluble in aqueous media but they are permeable at the absorption site and both have pH independent release profile. The permeability of Eudragit RS and Eudragit RL in aqueous media is due to the presence of quaternary ammonium groups in their structure; Eudragit RL 100 has a greater proportion of these groups and as such is more permeable than Eudragit RS 100 (Eudragit data sheets)⁷.

The aim of this study is to prepare Eudragit microspheres containing highly hydrophilic LP to achieve better encapsulation efficiency and controlled drug release profile suitable for peroral administration. Firstly, we investigated some formulation variables (polymer type, drug: polymer ratio, stirring speed) to obtain spherical particles. Then, the yield of production, particle size distribution, encapsulation efficiency, surface properties and LP release rate from microspheres were investigated. The influences of formulation variables on the microsphere properties were examined and the microsphere formulations suitable to achieve our goal were determined.

EXPERIMENTAL

Losartan potassium (LP) was obtained from Jubilant Organosys Ltd., India as a free gift sample. Eudragit RS 100 (ERS) and Eudragit RL 100 (ERL) were obtained from Central Drug House (Pvt.) Ltd., Bombay, Magnesium stearate was obtained from HIMedia Pvt. Ltd., Mumbai and Liquid Paraffin was procured from Merck Ltd., Mumbai, India and all other chemicals were of reagent grade.

Preparation methodology of microspheres: Losartan potassium microspheres were prepared by solvent evaporation technique⁷⁻⁹. A total of six formulations were prepared taking different drug: polymer ratio. Concisely the polymers ERS and ERL were dissolved in 5 mL of acetone to get a clear solution. The drug losartan potassium and magnesium

stearate (30 mg) were added to this mixture and was stirred at the same speed for 0.5 h and then it was kept in the ultrasonic bath until dispersed completely¹⁰. The resulting dispersion was then poured into a 250 mL beaker containing 150 mL of light liquid paraffin¹¹ while stirring continuous with a mechanical stirrer at 700 rpm with a blade fitted with a four-blade 'butterfly' propeller with a diameter of 50 mm (Lab Digital Stirrer, Remi). Non-polar liquid paraffin was preferred as dispersing medium. Stirring was continued for 3 h till complete evaporation of acetone. Then the resulted microspheres were collected by filtration under vacuum, washed 4-5 times with 30 mL *n*-hexane¹² and dried at room temperature (25 °C) for 24 h to get free flowing microspheres. The different batch specifications of losartan potassium loaded microspheres are given in Table-1.

RESULTS AND DISCUSSION

Preparation process of the losartan potassium loaded microspheres with ERS and ERL: According to Arshady *et al.*¹³, various manufacturing parameters (apparatus design, type of stirrer, stirring speed, viscosity of emulsion phases and the stabilizer concentration) affect particle size. Here, the investigation is done on the effects of polymer concentration, thus the inner phase viscosity and the stirring speed of the system on particle formation and particle size, while keeping the other parameters constant. The O/W emulsion is produced by the agitation of two immiscible liquids. The drug substance is dispersed in solution of the polymer. Agitation of the system is continued until the solvent partitions into the aqueous phase and is removed by evaporation. This process results in hardened microspheres which contain the active moiety. Trials were made to prepare microspheres by solvent evaporation technique in the water phase using acetone/water and alcohol/water systems. Though many formulations were investigated but they did not prove to be satisfactory. Then acetone/liquid paraffin system was used and various formulations (F1, F2, F3, F4, F5 and F6) with different drug: polymer ratios were tried and effect of drug: polymer ratio, stirring speed (500, 750 and 1000 rpm) on the particle size of the microspheres were studied.

Effect of the dispersing agent and dispersing medium on loaded microspheres: Magnesium stearate was added to the formulation as a droplet stabilizer to overcome the problem of droplet coalescence during solvent evaporation¹³. The use of magnesium stearate as a dispersion agent decreased the interfacial tension between the lipophilic and hydrophilic phases of the emulsion and further simplified the formation of microspheres. As the solvent evaporated, the viscosity of the individual droplets increased and highly viscous droplets

TABLE-1
BATCH SPECIFICATION OF LOSARTAN POTASSIUM LOADED MICROSPHERES

Formulation code	Drug: Polymer	Amount of drug taken (mg)	Amount of polymer taken (mg)		Magnesium stearate (mg)	Acetone (mL)	Liquid paraffin (mL)	<i>n</i> -Hexane (mL)
			ERS	ERL				
F1	1:1	100	50	50	30	5	150	15
F2	1:2	100	100	100	30	5	150	15
F3	1:3	100	150	150	30	5	150	15
F4	1:4	100	320	80	30	5	150	15
F5	1:5	100	400	100	30	5	150	15
F6	1:6	100	480	120	30	5	150	15

were observed to coalesce at a faster rate than they could be separated. Magnesium stearate formed a thin film around the droplets and thereby reducing the extent of coalescence, before hardening of the particles, on collision of the droplets. The resultant microspheres were free-flowing and the use of magnesium stearate was deemed effective.

Liquid paraffin was selected as a bulk or outer phase, since losartan potassium and Eudragit RS/RL are only very slightly soluble in liquid paraffin. Acetone has a dielectric constant of 20.7 and was therefore chosen as the dispersed or inner phase, since solvents with dielectric constants between 10 and 40 show poor miscibility with liquid paraffin^{14,15}.

Effect of the stirring speed on microparticles size and morphology loaded microspheres: During processing, it was found that when the stirring speed was kept at 500 rpm, the shapes of particles were found to be irregular for all formulations because the stirring speed was not fast enough to disperse the inner phase in outer phase and a huge coalesced mass was obtained. This is due in part to inadequate agitation of the media to disperse the inner phase in discrete droplets within the bulk phase. At stirring speeds above 1000 rpm, the turbulence caused frothing and adhesion of the microparticles to the container walls and propeller blade surfaces, resulting in high shear and a smaller size of the dispersed droplets.

When stirring speed was raised to 750 rpm the best spherical particles with good surface characteristics were obtained with all formulations which was exhibited in the scanning electron micrograph (Figs. 1-3).

The mean particle size was determined for all the microspheres. The particle size was distributed over the range of 120-270 μm . An overall increase in mean particle size of

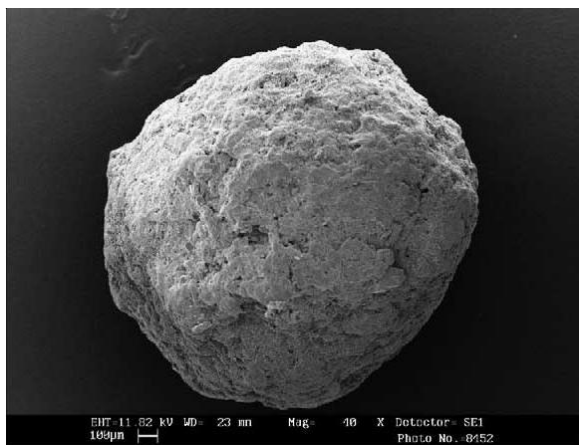


Fig. 1. SEM of prepared microsphere without drug

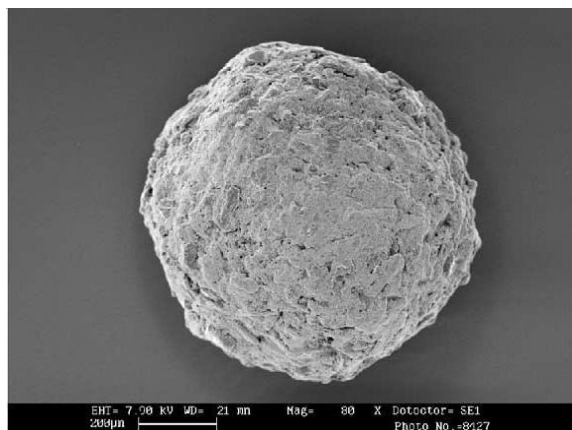


Fig. 2. SEM of prepared microsphere with drug



Fig. 3. Cross-sectional view of microsphere after dissolution

microspheres was observed with a definite increase in the amount of polymer in the formulation (Table-2), keeping the drug concentration and the solvent volume constant. This may be due to the fact that, an increase in polymer concentration in the inner phase lead to an increase in inner phase viscosity and droplet size, which in turn resulted in the formation of larger sized particles.

Drug/polymer ratio, 1:1 (w/w) resulted in poor quality of microspheres. In this formulation, both the polymers, ERS and ERL were taken in equal amount. These were irregularly shaped, not flowing and presented with lots of indentation. Microspheres were perfectly formed when the polymer concentration was increased to ratios between 1:2 and 1:6 (w/w) with respect to the drug concentration, as can be seen in Fig. 2. Discrete, spherical and uniform microspheres were obtained with a 1:5 (w/w) drug/polymer ratio for both the ERS and

TABLE-2
EFFECT OF FEEDING POLYMER ON MEAN PARTICLE SIZE, % YIELD VALUE,
DRUG LOADING, ENCAPSULATION EFFICIENCY OF MICROSPHERES

Formulation code	Drug: Polymer	Amount of drug taken (mg)	Amount of polymer taken (mg)		Mean particle size (μm)	Yield value (%)	Drug loading (%)	Encapsulation efficiency (%)
			ERS	ERL				
F1	1:1	100	50	50	123.53 \pm 06.372	65.21 \pm 1.826	52.36 \pm 1.038	68.27 \pm 0.581
F2	1:2	100	100	100	132.76 \pm 11.807	76.25 \pm 2.696	23.97 \pm 1.141	54.77 \pm 0.743
F3	1:3	100	150	150	167.40 \pm 13.079	51.36 \pm 1.097	17.37 \pm 0.497	35.67 \pm 0.456
F4	1:4	100	320	80	198.16 \pm 13.046	77.43 \pm 2.802	20.78 \pm 0.781	80.40 \pm 0.347
F5	1:5	100	400	100	204.23 \pm 08.438	80.40 \pm 1.712	19.58 \pm 0.417	94.43 \pm 0.277
F6	1:6	100	480	120	222.53 \pm 15.302	83.71 \pm 2.045	16.29 \pm 0.440	95.38 \pm 0.305

Values are average of three readings \pm standard deviation (n = 3).

ERL polymers in the ratio of 4:1. SEM was performed to confirm whether microspheres had been formed. The use of SEM is important for establishing the encapsulating ability of different polymers, since the degree of porosity may be observed and therefore, encapsulation ability of the polymers can be established in a qualitative manner.

Batch yield, encapsulation efficiency and drug loading of loaded microspheres: Yield and encapsulation efficiency remained high at all drug loadings. As polymer content increased, the encapsulation efficiency slightly decreased as seen with the trends for the formulations, F1, F2 and F3 (Table-2). This can be due to the fact that an increase in polymer content led to an enhancement of the concentration gradient between the emulsion droplets and the continuous phase; as a result increasing the amount of drug partitioning into the continuous phase. Also the fact that the ratio within the polymer ERS and ERL remained 1:1 (ERS:ERL) for F1, F2 and F3 and the polymer ERL being more permeable than ERS due to presence of more quaternary ammonium groups has led to insufficient encapsulation of drug in polymeric matrix. The high encapsulation efficiencies of formulations, F4, F5, F6 in which the ratio between the polymer was kept at 4:1 (ERS: ERL) was found to be remarkably high (F5- 94.43 % \pm 0.277) as an increase in the ERS concentration has led to an increase in the viscosity of the internal phase. When the viscosity of the internal phase is increased, the efficiency of the stirring is reduced and large size microparticles were formed. The increased viscosity as polymer levels increase can impede drug mobility in the droplets and this was observed as an increase in encapsulation efficiency at high ERS levels. High ERS levels may also lead to rapid polymer precipitation on the droplet surface and rapid microparticle solidification, resulting in the hindering of drug diffusion, effectively trapping it in the particle. These suggested that the present method was suitable for the preparation of microspheres of a highly water-soluble drug, such as losartan potassium. The drug loading was decreased proportionately with increase in amount of added polymer for the formulations, F1, F2 and F3. The same pattern was also observed for next three formulations F4, F5, F6.

Solid state interaction studies: In order to confirm the interaction of Eudragit polymers with losartan potassium and thus, its role in the release mechanism, samples were analyzed by IR spectroscopy. FTIR spectra of the drug, polymers and the optimized formulation were recorded in range of 4000-500 cm^{-1} . The spectrum of pure losartan potassium showed strong absorption bands attributable to hydroxy and (C=N-) stretching at 3684 and 1574 cm^{-1} , respectively, as well as the absorption band of -CH group at 2949 cm^{-1} , -Cl bands at 760 cm^{-1} . Eudragit RS 100 showed the characteristic band of the carboxylic groups at 1725 cm^{-1} (C=O ester vibration at 1725 cm^{-1}), absorption bands at 1157 cm^{-1} for C-O-C ether linkages, 1256 cm^{-1} for -CH₂ in plane bending vibrations, 1256 cm^{-1} also for tertiary N absorption band. ERL showed the characteristic band of the carboxylic groups at 1641 cm^{-1} (C=O ester vibration at 1641 cm^{-1}), absorption bands at 1106 cm^{-1} for C-O-C ether linkages, 3487 cm^{-1} absorption band for -NH₂ and 1256 cm^{-1} peak for tertiary N absorption band. In the case of the losartan potassium loaded ERS/ERL microspheres the

peaks corresponding to the hydroxy, imine (C=N) groups of drug and the carbonyl groups of ester present in Eudragit remained at 3682, 1571 and 1729 cm^{-1} , respectively and also the C-O-C ether linkages of polymer were retained in the optimized formulation showing the absorption peaks at 1156 cm^{-1} . Hence it was a clear indication that no interaction occurred involving the hydroxy, imine (C=N) group of drug, ERS and ERL which is indicative by presence of all the absorption peaks in the optimized formulation F5.

Thermal analysis of drug crystallinity: DSC was used to examine thermal behaviour of pure drug (losartan potassium), ERL, ERS, physical mixture of losartan potassium and polymers, formulation (F2 and F5) were presented in Fig. 4a-4e, respectively. A sharp endothermic peak corresponding to the melting of crystalline drug was found at 241.83 °C (Fig. 4a). For pure polymer ERL and ERS the thermal transition at 73.80 and 59.98 °C was attributed to the glass transition temperature (T_g) of polymers (Fig. 4b and 4c). T_g of the polymer was observed at 85.39 and 79.4 °C while the endotherm 115.78 °C was determined as the m.p. of magnesium stearate, dispersing agent in microspheres of F2 and F5 formulation. No endothermic peak confirming crystalline drug was present as shown in thermograms of the optimized formulation F5. Hence, it can be concluded that the drug is found in amorphous state rather than a phase separated crystalline phase. The absence of losartan potassium crystalline peak, which should have been expected at approximately 241.83 °C, proved that losartan potassium was in amorphous state in this formulation, F5.

Control of drug-release behaviour of microspheres: Examination of the release profiles reveals that drug release was generally faster for losartan potassium microspheres produced with the high concentration of ERL polymer despite the apparent similarity in particle size of the microspheres. Eudragit RS 100 and Eudragit RL 100 are copolymers of partial esters of acrylic and methacrylic acids containing low amounts of quaternary ammonium groups, *ca.* 5 and 10 % for ERS and ERL, respectively. The ERS polymer is water-insoluble and drug delivery systems prepared from it show pH-independent sustained drug release, attributed to the quaternary ammonium groups. The quaternary ammonium groups in the ERS and ERL chemical structures play an important role in controlling drug release because they relate to water uptake followed by the swelling of the polymers. This is most likely because the number of quaternary ammonium groups of ERS is lower than that of ERL, which renders ERS less permeable.

Compared to all the other formulations, % amount of dissolved drug was higher for F1, F2 and F3 formulation which has the least polymer amount (ERS:ERL= 1:1). This situation is due to the fact that scarcity of polymer augmented release of the drug and also the thin polymer wall of microspheres as diffusion path led the drug to be easily released in the dissolution medium. The % amount of dissolved drugs of F1, F2 and F3 were not significantly different after the fourth hours. But it is found that, % amount of dissolved drug for F4, F5 and F6 which has the highest polymer amount (ERS: ERL = 4:1) were less than the other formulations. It can be seen from the deeper cracks on the surface of microspheres (Fig. 3) that as the polymer amount increases the matrix wall of microspheres becomes

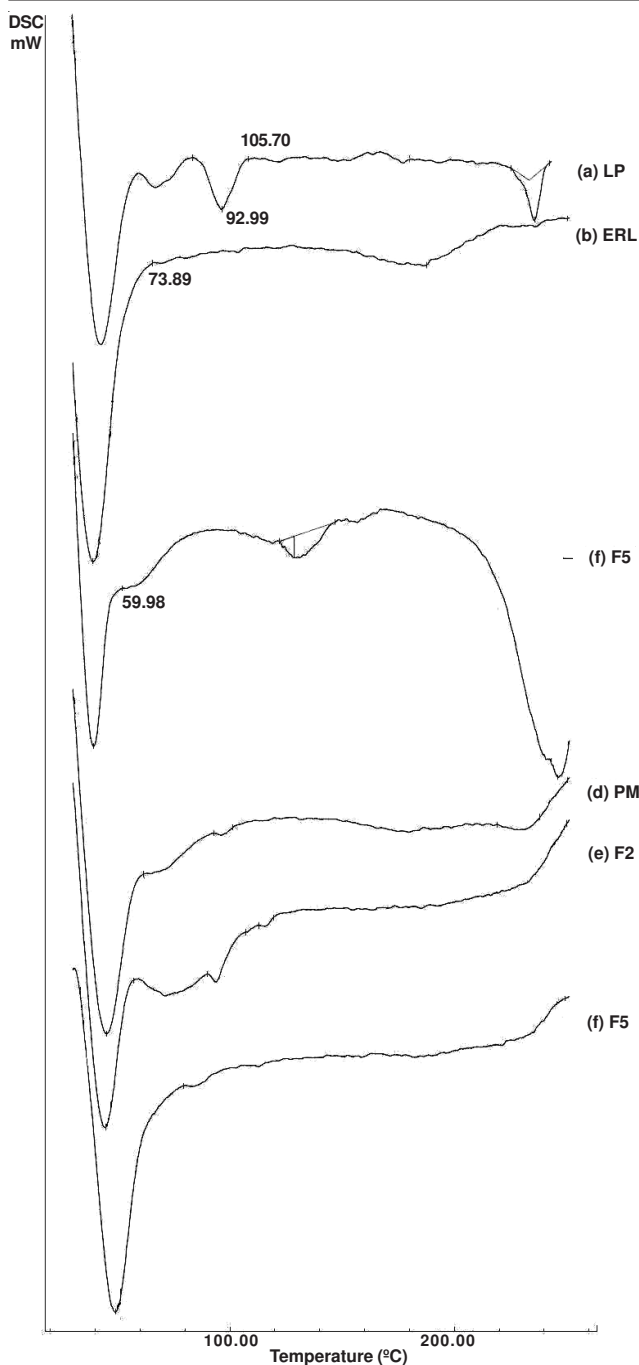


Fig. 4. DSC thermograms (a) LP - losartan potassium (b) ERL - Eudragit RL 100 (c) ERS - Eudragit RS 100 (d) PM-Physical mixture of LP, ERI, ERS and magnesium stearate (e) F2-microspheres with drug:polymer ratio, 1:2 and (f) F5-microsphere with drug:polymer ratio 1:5

thicker. The formation of a thicker matrix wall leads to slower dissolution rate of drug caused by longer diffusion path. According to the dissolution tests conducted by USP I method, F5 and F6 formulations gave the lowest dissolution rate. Since losartan potassium has weak acidic properties, its solubility varies in parts of gastrointestinal tract with different pH values. Dissolution tests were performed separately in two dissolution media with different pH values (1.2 and 7.4) for 12 h by USP I apparatus it is seen that the dissolution profiles were identical at these pH values. After 2 h in 0.1 M HCl, the release of losartan potassium was restricted to less than 31 % for F5.

When the pH increased, a rapid losartan potassium release was observed from the microparticles prepared at the entire range of polymer concentrations as seen in Fig. 5.

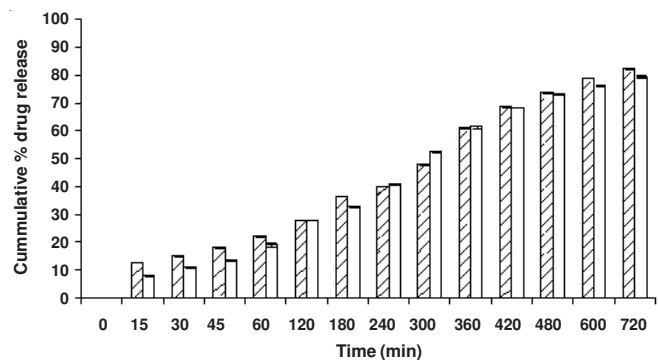
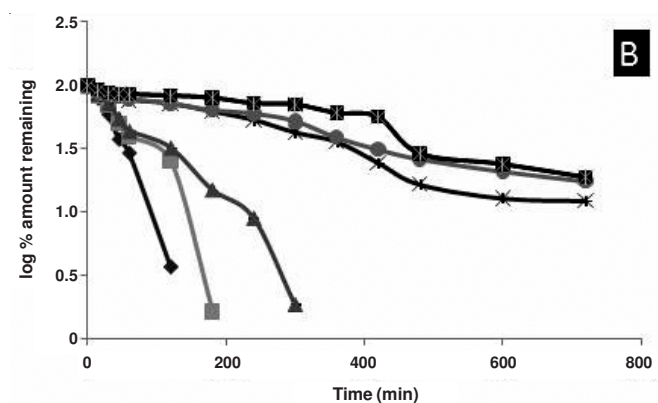
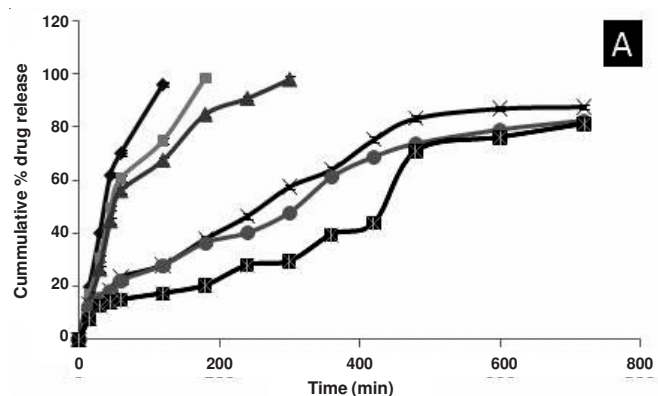


Fig. 5. Bar graph showing comparison between the cumulative drug release of losartan potassium from F5 formulation with two different dissolution medium \square PBS (pH 7.4) and \square HCl (pH 1.2)

Considering the gastrointestinal transit time, release profiles from F1, F2 and F3 seems to be too fast for controlled release. To decrease the release rate ERS and ERL were mixed at different amounts for three microsphere formulations (F4, F5 and F6) in a ratio of 4:1. Losartan potassium dissolution rates from these microspheres are shown in Fig. 6. The amount of losartan potassium released in 12 h was between 83-87 % for these three formulations. Release rates decreased as the amount of ERS increased considerably.

Correlation coefficients of different mathematical models are shown in Table-3.



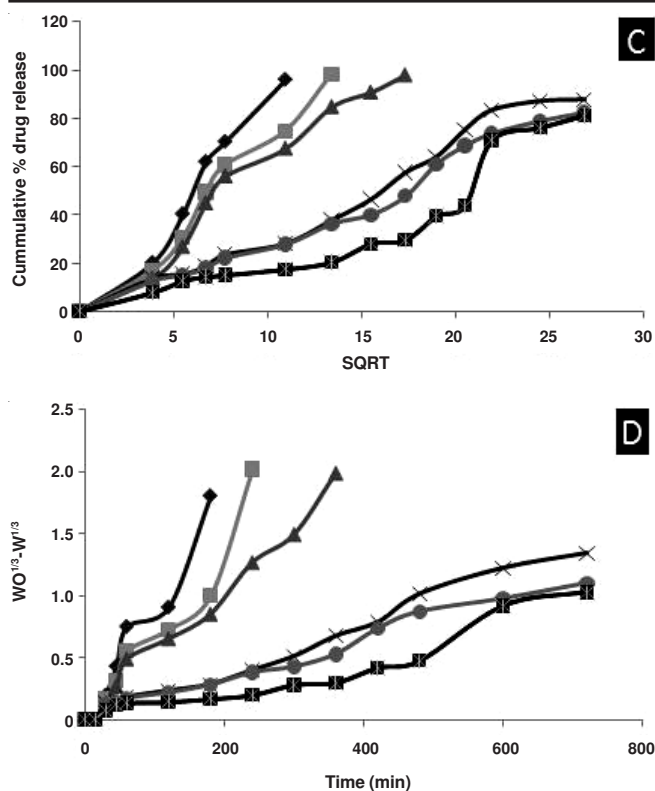


Fig. 6. Dissolution graph (n = 3) (A) Zero order model (B) First order model (C) Higuchi model and (D) Hixon-Crowell model comparing six formulations

TABLE-3
CORRELATION COEFFICIENTS OF DIFFERENT
MATHEMATICAL MODELS FOR LOSARTAN
POTASSIUM LOADED MICROSPHERES

Formulation code	Zero-Order	First-Order	Higuchi	Hixon-crowell
F1	0.8993	0.9798	0.9667	0.9970
F2	0.9008	0.8987	0.9728	0.9640
F3	0.8604	0.9621	0.9691	0.9807
F4	0.9394	0.9701	0.9754	0.9719
F5	0.9479	0.9829	0.9780	0.9794
F6	0.9484	0.8971	0.8723	0.9194

Conclusion

This work has successfully demonstrated a direct microencapsulation for low molecular weight and highly water-soluble compounds *via* solvent evaporation technique, represented by the model molecule of losartan potassium. Various engineering factors affecting the microencapsulation process of losartan potassium were revealed and discussed accordingly. Key finding in this work was the consideration of having a higher concen-

tration of polymer in the stirring solution during the production process that ensures effective microencapsulation with high encapsulation efficiencies. Drug/polymer ratio and stirring speed of the system were important to obtain spherical particles with smooth surfaces. The yields of preparation and encapsulation efficiencies were very high for all microspheres obtained. Losartan potassium release rates from microspheres were dependent on the type of polymer used and ratio between the polymers. The drug release profile aimed for p.o. administration could be obtained by adding ERS to ERL and changing the ratio of these polymers. Controlled release without initial peak levels achieved with these microsphere formulations can reduce dosing frequency, decrease side effects and improve patient compliance. Further optimizations and refinements for sure can facilitate the development to utilize the microspheres in real clinical application.

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