ORIGINAL ARTICLE

Aqueous-Core Nanocapsules of Lamivudine: Optimization by Design of Experiments

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Abstract

Aim: The prior objective of the current work was to optimize the surfactant concentration and type of polymer to achieve high entrapment efficiency, less particle size, and less drug release rate constant of aqueous core nanocapsules (ACNs) containing lamivudine. **Materials and Methods:** Modified multiple emulsion technique was used to prepare the nanocapsules with biodegradable polymers, namely, poly (lactide-co-glycolide) and polylactic acid; and the surfactant employed was Span 80. The experiments were designed by one factorial design that was repeated for every categoric factor under response surface methodology of design of experiments using Stat-Ease Design-Expert software. **Results:** The obtained results of response variable, namely, entrapment efficiency, particle size, and drug release rate constant were statistically treated by ANOVA for response surface quadratic model and found that influence of both factors on all the response variables was significant at $P < 0.05$. The optimized formulation was found to have the entrapment efficiency of 77.12%, particle size of 250.3 nm, and the drug release rate constant of 0.106 h^{-1}. **Conclusion:** The results indicated that the objective of this was achieved and ACNs technique was efficient to develop nanoparticles for water-soluble drugs with improved efficiency.

Key words: Aqueous core nanocapsules, biodegradable polymers, design of experiments, optimization

INTRODUCTION

The use of carriers for the drug delivery for several aspects such as targeted delivery of the drug,[1] to improve the bioavailability of the drug,[2] to cross blood-brain barrier,[3] and also to overcome the inhibitory actions of glycoprotein-P (P-gP) for permeation of drugs into the cells[4] has attained a remarkable interest in the field of carrier-mediated drug delivery systems in the recent years. Polymeric nanoparticles have achieved notable attention among various nanocarriers, namely, liposomes, niosomes, and solid lipid nanoparticles[5] due to their stability and aptness to incorporate a wide variety of drugs and biologicals. Nanoparticles can be manufactured using an extensive variety of either biodegradable or non-biodegradable natural and synthetic polymers. The biodegradable polymers do not deposit in the body, degrade into soluble and excretable form, thereby do not pose toxicity. Hence, employment of biodegradable polymers offers substantial advantage in the carrier-mediated drug delivery systems. The biodegradable polymeric nanoparticles can be administered through intravenous route effectively to control the spatial and temporal delivery of the incorporated drugs. They can be employed to deliver drugs at varied target sites[6] due to their submicron size and vast potentiality to modify their surface.

The most frequently used biodegradable polymers are Poly(lactide-co-glycolide) (PLGA) and polylactic acid (PLA) as their hydrolysis give simple monomers, namely, lactic acid and glycolic acid which are endogenous to the body so that they have little or no toxicity. PLGA and PLA polymers with carboxylic acid functions as end groups are obtained so that these can have higher negative zeta potential.[7,8] Nanoparticles prepared employing these polymers possess high negative zeta potential hence can be readily engulfed

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Received: 04-07-2019
Revised: 05-09-2019
Accepted: 20-09-2019

by macrophages and deliver them into the reticuloendothelial system (RES) rich organs such as spleen and liver.[9] For the delivery of drugs like lamivudine into liver in case of treatment of diseases such as hepatitis B (HBV), nanoparticles prepared employing these polymers can be used as passive targeting carriers. Lamivudine is an antiviral drug indicated in the treatment of HIV and chronic HBV. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and HBV. Lamivudine is phosphorylated to active metabolites that compete for incorporation into viral DNA.

Nanoprecipitation, emulsion solvent evaporation, salting out, polymerization, etc.,[10] are the different techniques used in the preparation of polymeric nanoparticles. The encapsulation efficiency of these methods is always one of the major drawbacks in case of water-soluble drugs like Lamivudine, as these drugs tend to leach out easily. One of the techniques reported to improve the encapsulation efficiency of such drugs is aqueous core nanocapsules (ACNs).[11] The distinctive liquid core which is optimized to have high solubility for the hydrophilic drugs helps in achieving high encapsulation efficiency of ACNs and entail less amount of polymer when compared to polymer nanospheres.

Particle size, zeta potential, drug content, and drug release rate constant[12] are the major physicochemical characteristics that influence the *in vivo* performance of the nanoparticles. The influence of formulation and process variables on these characteristics of nanoparticles is to be scrutinized so as to improve theirs *in vivo* performance. Extensive literature studies have shown that several studies have been reported in this regard enclosing priorly the influence of various formulation and process parameters such as polymer concentration, nature and composition of solvents, temperature, and speed of rotation. The influence of type of polymer, molecular weight of polymer, and surfactant concentration on performance of nanoparticles were reported in very few studies. Budhian *et al*.[13] studied the influence of ratio of lactic acid to glycolic acid in PLGA polymer. Sharma *et al*.[14] studied the effect of cosurfactant on characteristics of nanoparticles. Hence, there is a vast scope to study the influence of these formulation parameters to adjoin the new data to currently prevailing data that might serve the development of nanoparticles with surpassed performance. Nanoparticles are always desired to have high entrapment efficiency so that administered quantity can be reduced; less particle size so that they penetrate easily into the tissues, and less drug release rate so that drug release can be extended for long time. Hence, in the current work, we aimed to prepare ACNs of lamivudine by modified multiple emulsion technique and optimize the type of polymer, molecular weight of polymer, and the concentration of surfactant in the primary emulsion to have high entrapment efficiency, less particle size, and less drug release rate constant. In the current work, PLGA of two different molecular weight grades, namely, PLGA RG503H and PLGA RG502H, and PLA R203H were taken as polymers and the surfactant taken was Span 80. The experiments for this study were designed as per response surface one factorial design which was repeated for every categoric factor, and the obtained results were statistically analyzed using Stat-Ease Design-Expert software.

MATERIALS AND METHODS

Materials

Lamivudine was obtained from Hetero Drugs Ltd., Hyderabad, India. Poly (D, L lactic acid) (RESOMER R203H/PLA R203H) and Poly (D, L lactide-co-glycolide) (RESOMER RG503H/PLGA RG503H and RESOMER RG502H/PLGA RG502H) were obtained from Sigma-Aldrich (Mumbai, India). Polyvinyl alcohol was obtained from Merck Specialities Pvt., Ltd., Mumbai, India. Pluronic F-68, Span 80, and Chloroform were obtained from S. D Fine Chemicals (Mumbai, India). All other chemicals used were of reagent grade.

Fourier transform infrared spectroscopy (FT-IR)

The physicochemical compatibility of Lamivudine with PLA R203H, PLGA RG503H, and PLGA RG502H used in this study was determined by subjecting to IR spectral studies using FT-IR Spectrophotometer. The samples were prepared by mixing lamivudine with each polymer at the ratio at which they were used in the formulation and prepared as pellets with KBr using hydraulic press. These pellets were subjected to scanning under diffuse reflectance mode and spectra were recorded as mean of 16 scans per sample. The obtained pure drug spectrum was compared with the spectra of physical mixtures of the drug and polymers.

Preparation of lamivudine ACNs (Lamivudine ACNs)

Experimental design

In the present study, type of polymer and concentration of surfactant in primary emulsion (w/o) were taken as two factors where the concentration of Span 80 was the only numeric factor as the type of polymer is a categoric factor. Therefore, a one factorial design was chosen for the experimental design and was repeated for every categoric factor. Three levels of the numeric factor (A, surfactant in primary emulsion – 0%, 0.25%, and 0.5% v/v) were considered for a linear model and this design was repeated for every combination with categoric factor (B) which was also taken in three levels, i.e., 3 types of polymers, namely, PLGA RG503H, PLGA RG502H, and PLA R203H. The experimental runs with combinations of factors at different levels are given in Table 1. Entrapment efficiency, particle size, and drug release rate constant were opted as the response variables.

Preparation of lamivudine ACNs

Multiple emulsification method, after necessary modification of the method reported by Yadav *et al.*,[15] was used to prepare lamivudine nanocapsules. 80 mg of lamivudine was dissolved in 5 mL of aqueous phase comprising of distilled water with 1% w/v polyvinyl alcohol as a stabilizer. 120 mg of polymer was dissolved in 10 mL of chloroform containing Span 80 at a concentration of 0.0% or 0.25% or 0.5% v/v. Then, the aqueous phase was added dropwise to the organic phase with constant stirring for 30 min at 12,000 rpm to obtain primary emulsion of w/o type. The above primary emulsion was then added dropwise to 20 mL of 50% v/v aqueous glycerol containing 0.4% v/v of Pluronic F-68 as external phase under continuous stirring until the chloroform was evaporated to obtain ACNs of lamivudine. The ACNs were then recovered by centrifugation at 8,000 rpm and 4°C for 30 min, the pellet was collected and washed twice using distilled water so as to remove the unentrapped drug. The supernatant was analyzed to determine the unentrapped drug thereby entrapment efficiency was determined from it. The washed pellet was again dispersed in distilled water, and the dispersion was lyophilized for 24 h to produce freeze-dried nanocapsules which were stored in airtight containers.

Scanning electron microscopy (SEM)

SEM (ZEOL JSM–5610) was used to observe the shape and surface morphology of the lamivudine nanocapsules. The dispersion of nanocapsules was adequately diluted, and a small drop was mounted on the SEM sample stub using double-sided sticking tape, coated with gold film (thickness 200 nm) under reduced pressure (0.001 Torr) and pictures of the samples were taken.

Differential scanning calorimetry (DSC)

DSC thermographs were recorded to specify the physical state of lamivudine and polymers PLGA RG503H and PLA R203H inside the prepared nanocapsules. The sample was weighed, crimped to an aluminum pan and scanned in a nitrogen atmosphere at a temperature range from 20 to 250°C at the heating rate of 10°C/min. The thermographs of pure lamivudine, PLGA RG503H, and PLA R203H thus obtained were compared with those of lamivudine containing nanocapsules of PLGA RG503H and PLA R203H.

Entrapment efficiency and drug loading

The nanosuspension of lamivudine ACNs obtained after preparation and before lyophilization was subjected to centrifugation at 8,000 rpm and 4°C for 30 min. The solid pellet was separated and washed twice with distilled water to remove the unentrapped drug. The supernatant and washings were mixed and analyzed spectrophotometrically at a λ_{max} of 270 nm to determine the amount of drug unentrapped, which was subtracted from the amount of lamivudine taken for the preparation.[16] The entrapment efficiency and drug loading were obtained using formulae given below:

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of drug entrapped}}{\text{Theoretical amount of the drug in the nanocapsules}} \times 100$$

$$\text{Loading efficiency}(\%) = \frac{\text{Amount of drug entrapped}}{\text{Total amount of the drug and polymer in the nanocapsules}} \times 100$$

Particle size and zeta potential

Dynamic light scattering technique using Zetasizer Nano-ZS90 (Malvern Instruments, UK) was used to assess the particle size and zeta potential of the prepared lamivudine ACNs. The measurements were taken out at a fixed scattering angle of 90° by maintaining an equilibrated temperature of 25°C. The measurements were taken in triplicate for each sample after suitable dilution with distilled water.

In vitro drug release studies

In vitro release studies were carried out by dialysis bag method using dialysis membrane[17,18] (Dialysis Membrane-110; HiMedia Lab. Pvt., Ltd., Mumbai). The water suspended lamivudine ACNs were placed in a Gelatin bag, sealed at both ends and suspended into a receptor compartment containing 100 ml buffer solution (0.1N HCl) in a beaker. The medium in the beaker was maintained at a temperature of 37 ± 0.5°C and agitated continuously at 100 rpm using a magnetic stirrer. At various intervals of time, 2 mL of samples from the medium in the beaker were taken, and the volume was replaced with fresh buffer over a period of 24 h. The amount of drug released was quantified by analyzing the samples spectrophotmetrically at 270 nm after necessary dilutions. The obtained data were analyzed kinetically by zero-order and first-order models to determine the drug release kinetics and also by Higuchi's and Korsmeyer–Peppas models to ascertain the drug release mechanism.

Design of experiments validation and ANOVA

The concentration of surfactant was the numeric factor and the type of polymer was the categoric factor. Therefore, one factorial design linear model with one center point under response surface methodology was chosen which was repeated for all the three categoric factors that resulted in a total of 15 runs in this design. The lamivudine ACNs for the 15 runs were prepared and characterized for the selected responses, and the obtained results of the responses were analyzed by response surface quadratic model without any transformation. The design was validated by plotting the

predicted versus actual results of the responses and also by statistically treating the data using ANOVA of classical sum of squares–Type III for its fit into the selected quadratic model.

RESULTS AND DISCUSSION

FT–IR studies

The physical compatibility of drug and polymer was determined by employing I. R spectral studies. The IR spectra of pure lamivudine and the physical mixtures of drug and polymers are shown in Figure 1. The bands for characteristic groups of the pure lamivudine DF were observed at 3326.53 cm^{-1} due to amino group stretch; 1652.46 cm^{-1} due to the carbonyl group of cysteine ring; and 1286.25 cm^{-1} and 1159.79 cm^{-1} due to asymmetrical and symmetrical stretching of C-O-C of oxathiolane ring.[19] These bands were also observed at the identical positions in the spectra of physical mixtures of lamivudine with the polymers, namely, PLGA RG503H and PLA R203H. Hence, there was no incompatibility aroused between lamivudine and the polymers.

Preparation of lamivudine ACNs

Lamivudine ACNs were prepared by modified multiple emulsification technique as W/O/W emulsion. The aqueous solution of lamivudine was emulsified with chloroform solution of polymer to yield W/O emulsion, and this W/O emulsion was dispersed further in aqueous phase to get W/O/W emulsion. The final emulsion was kept under continuous stirring until chloroform had evaporated thus allowing the polymer to deposit on the innermost aqueous phase globules. Once the chloroform was evaporated completely, the polymer was rigidized and discrete nanosized capsules with small aqueous volume containing the drug as core and the rigid polymer as coat were formed which were termed as ACNs. The SEM studies specified that the obtained nanocapsules were almost spherical in shape, as shown in Figure 2. In this work, all the polymers taken, namely, PLGA RG503H, PLGA RG502H, and PLA R203H were hydrophobic in nature[20] so that the ACNs prepared from these polymers were possessing relatively hydrophobic surface which renders them to get attacked by opsonins, thus phagocytized easily by RES and hence the ACNs were carried to RES rich organs such as liver and spleen. Further, the obtained ACNs can have high negative zeta potential as the polymers taken have carboxylic functional groups on their outer surface. Hence, these characteristics can make ACNs suitable for passive targeting of drugs such as lamivudine into liver for the treatment diseases such as HBV.

Experimental design

In the current study, one factorial design that was repeated for every categoric factor was employed as among the two factors selected one is a numeric factor (surfactant concentration, A) and another

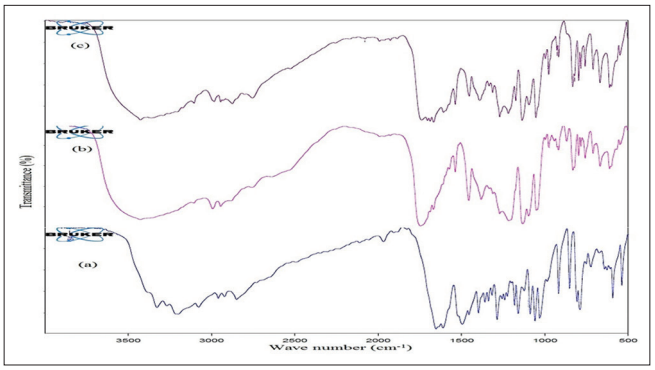

Figure 1: FT–IR spectra of (a) lamivudine; (b) physical mixture of lamivudine and PLA R203H; (c) physical mixture of lamivudine and PLGA RG503H

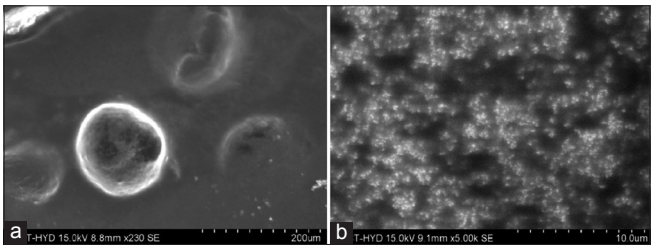

Figure 2: SEM images indicating (a) ACNs in almost spherical shape and (b) small and discrete lamivudine ACNs of formulation F3

is categoric factor (type of polymer, B). ACNs were prepared at all the combinations of the factor levels and characterized for the responses, namely, entrapment efficiency, particle size, and drug release rate constant. The obtained responses were analyzed by response surface quadratic model. The equations thus obtained for the responses in terms of coded factors:

$$EE = 71.50 + 3.92*A + 2.80*B[1] - 2.64*B[2] + 0.083*AB[1] + 0.68*AB[2] - 1.23*A^2$$

$$\text{Partice size} = 248.63 - 8.60*A + 7.57*B[1] - 9.21*B[2] - 1.28*AB[1] + 0.075*AB[2] + 4.02*A^2$$

$$k = 0.11 - 0.005*A - 0.004*B[1] + 0.008*B[2] - 0.0006*AB[1] - 0.001*AB[2] + 0.0008*A^2$$

where, EE = Entrapment efficiency; k = drug release rate constant; A = surfactant concentration; B[1] and B[2] are coefficients of multi-level categoric factor.

DSC

The physical state of the drug in the prepared ACNs was examined by DSC analysis. DSC spectra were taken for pure lamivudine; pure polymers PLA R203H and PLGA RG503H, and ACNs of lamivudine prepared with these polymers. The spectra are shown in Figure 3. The pure drug spectra have shown a sharp endothermic peak at 180.5°C representing to its melting point temperature. However, this peak was not

observed in the spectra of lamivudine ACNs with either of the polymers, which inferred that the drug was not in crystalline state in the ACNs, but it might be either in molecular dispersion or amorphous form.[21] This might be because of the way of incorporation of lamivudine in its aqueous solution form into the nanocapsules while preparation.

The pure spectra of PLA R203H are shown an endothermic peak at 54.6°C analogous to its glass transition temperature (T_g), and an exothermic peak at 101.8°C that resembles the re-crystallization of PLA and later another endothermic peak at 154.4°C, which specified melting of the PLA.[22] However, the spectra of lamivudine ACNs with PLA are shown an initial endothermic peak at 60.3°C corresponding to the T_g of PLA indicating an increase in T_g which might be attributed to its rapid solidification during the process. The absence of further re-crystallization and melting peaks might be due to the solidification of PLA into amorphous form. The increase in T_g was also observed in case of PLGA as the endotherm of its pure spectra was observed at 47.5°C whereas this was seen at 52.3°C in the spectra of ACNs with PLGA.[23]

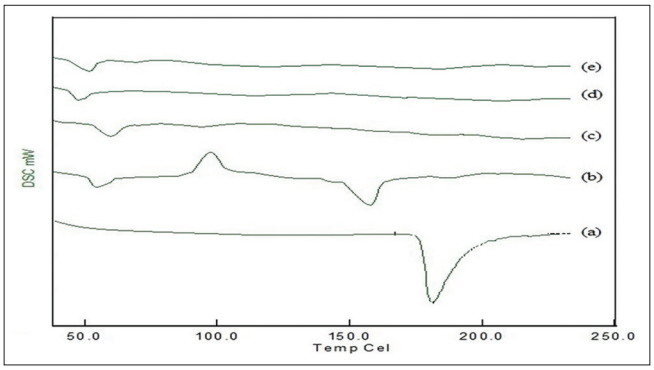

Figure 3: DSC spectra of (a) lamivudine; (b) PLA R203H; (c) lamivudine–PLA R203H ACNs; (d) PLGA RG503H; (e) lamivudine PLGA RG503H ACNs

Entrapment efficiency

Lamivudine ACNs obtained from all the formulations were found to have the entrapment efficiency in the range of 62.57–77.32% (given in Table 1). Influence of the two factors on entrapment efficiency is depicted in Figure 4(a). The entrapment efficiency was found to be high in ACNs of PLGA RG503H and comparatively less in ACNs of PLA R203H and further decreased in ACNs of PLGA RG502H. It can be inferred from these results that the entrapment efficiency was directly related to the molecular weight of the polymer. Due to its high viscosity, the polymer with high molecular weight might restrict the escape of the drug encapsulated in it, thus ensuring high entrapment efficiency.[24] Besides the low viscosity, the smaller particle size in case of low molecular weight polymer might also responsible for low entrapment efficiency as the small particles have high surface area and thereby more chance for the drug to escape from the nanocapsules.[25] Higher the hydrophobicity of polymer greater, the resistance for escape of encapsulated drug into outer aqueous medium indicates that hydrophobicity of the polymer can also influence the entrapment efficiency. A fascinating finding was observed in this work that though PLA is more hydrophobic than PLGA, high entrapment efficiency in case of PLGA RG503H inferred that the effect of molecular weight of the polymer might have dominated the effect of hydrophobicity. This effect might be attributed

Table 1: Different runs of the experiment indicating a combination of the factors according to the design and the obtained results of lamivudine ACNs

Formulation code	Concentration of surfactant (%w/v)	Type of polymer	Entrapment efficiency (%)	Loading efficiency (%)	Particle size (nm)	Zeta potential (mV)	Release rate constant (hr^{-1})
F1	0	PLGA RG503H	68.51±3.61	26.72±1.41	271.8±8.4	−26.5	0.116±0.002
$F1_R$	0	PLGA RG503H	69.73±1.85	27.19±0.72	268.3±6.7	−24.9	0.118±0.004
F2	0.25	PLGA RG503H	74.06±4.07	28.88±1.58	256.4±10.1	−25.3	0.112±0.003
F3	0.5	PLGA RG503H	77.32±5.11	30.15±1.99	249.4±5.3	−26.4	0.107±0.002
$F3_R$	0.5	PLGA RG503H	76.92±2.35	30.00±0.92	251.2±7.5	−25.8	0.106±0.005
F4	0	PLGA RG502H	63.30±1.79	24.69±0.7	253.4±5.9	−22.8	0.129±0.006
$F4_R$	0	PLGA RG502H	62.57±3.41	24.40±1.33	251.1±3.7	−23.1	0.131±0.004
F5	0.25	PLGA RG502H	69.24±4.06	27.00±1.58	238.3±6.2	−21.9	0.122±0.007
F6	0.5	PLGA RG502H	72.52±5.03	28.28±1.96	234.3±7.4	−23.6	0.119±0.005
$F6_R$	0.5	PLGA RG502H	71.74±2.89	27.98±1.13	236.1±4.9	−22.5	0.118±0.001
F7	0	PLA R203H	66.52±3.31	25.94±1.29	262.2±11.4	−19.1	0.114±0.003
$F7_R$	0	PLA R203H	67.46±1.94	26.31±0.76	260.7±5.4	−21.8	0.113±0.003
F8	0.25	PLA R203H	71.20±3.58	27.77±1.4	251.2±4.7	−20.9	0.110±0.002
F9	0.5	PLA R203H	73.56±2.88	28.69±1.12	245.8±8.1	−20.6	0.108±0.004
$F9_R$	0.5	PLA R203H	73.03±1.67	28.48±0.65	247.5±7.6	−19.9	0.107±0.003

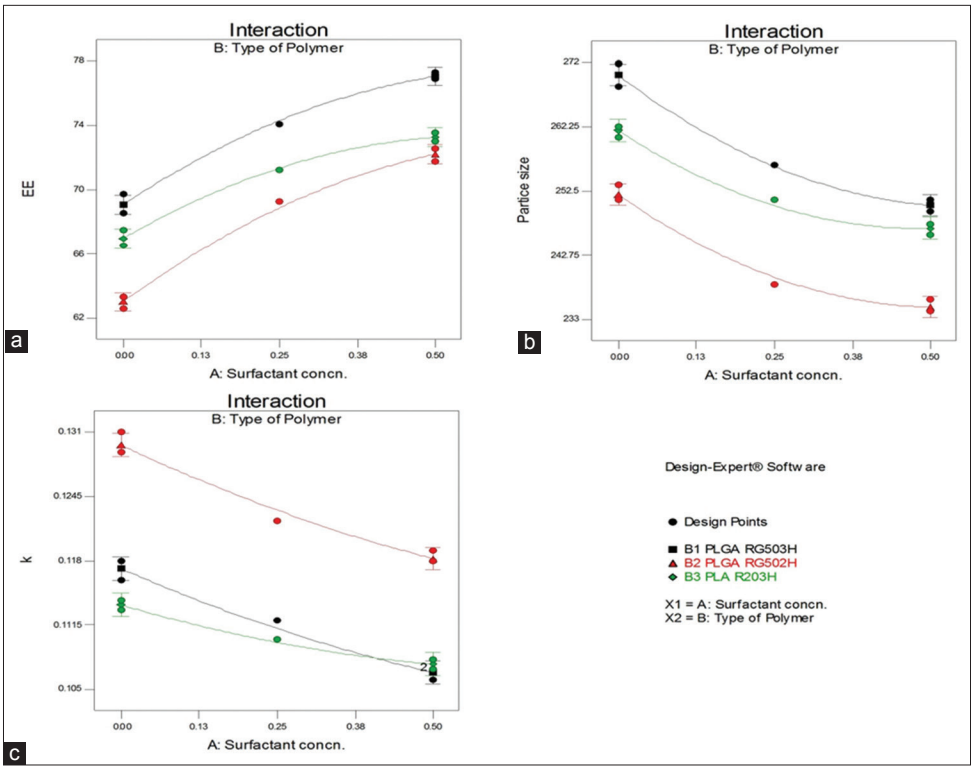

Figure 4: Interaction plots showing the influence of surfactant concentration in primary emulsion and type of polymer on (a) entrapment efficiency; (b) particle size; (c) release rate constant

to the high aqueous solubility of lamivudine that minimized the effect of polymer hydrophobicity. The increase in concentration of Span 80, a hydrophobic surfactant, in the primary emulsion (W/O) resulted in increase in the entrapment efficiency. As the Span 80 was employed in primary emulsion (W/O), it might deposit over the surface of the aqueous globules along with the polymer and also might improve the interaction between the aqueous and organic phases of emulsion containing the drug and polymer, respectively. Therefore, on increase in the concentration of Span 80, the nanocapsules became more hydrophobic and improved interaction between lamivudine and polymer so that the leakage of lamivudine into outer aqueous medium in the final W/O/W emulsion was minimized resulting in increased entrapment efficiency. The influence of these two factors on entrapment efficiency was significant at $P < 0.05$ by ANOVA of response surface quadratic model (given in Table 2).

The maximum entrapment efficiency observed in this study was 77.32%. Some studies have been reported on the enhancement of entrapment efficiency of hydrophilic drugs into nanoparticles. Dordelmann *et al.*[26] developed poly (D, L-lactide-co-glycolide acid) (PLGA) nanocapsules by W/O/W emulsion solvent evaporation technique for loading nucleic acids and achieved an encapsulation efficiency of 52% using calcium phosphate in the innermost phase. Kashi *et al.*[27] prepared PLGA nanoparticles by solid/oil/water ion pairing method to incorporate minocycline and reported a maximum entrapment efficiency of 29.9%. Peltonen *et al.*[28] worked on by modified nanoprecipitation method to load sodium cromoglycate into polylactide nanoparticles and reported a maximum entrapment efficiency of 70%. Henceforth, it can be inferred that ACNs developed in our work by modified multiple emulsion technique is a considerable alternative technique to improve the encapsulation efficiency of nanoparticles for hydrophilic drugs.

Particle size

Lamivudine ACNs obtained from all the formulations were in size range of 234.3–271.8 nm (shown in Table 1). Effect of the two factors on the particle size is illustrated in Figure 4(b). On increase in the surfactant concentration, the particle size was found to be reduced. In an emulsion, high amount of surfactant along sufficient energy of dispersion can result in a fine emulsion with good stability as the high interfacial free energy because of the small globule size (high interfacial area) in the fine emulsion can be reduced by the surfactant due to its ability to deposit at the interface. Therefore, higher amount of surfactant results an emulsion with smaller particle size and good stability. These results related to those reported by Rehfeld[29] for benzene-in-water emulsions; and also with those reported by Gupta *et al.*[30] for solid lipid nanoparticles. The particle size of ACNs prepared with PLGA RG503H was found to be high, and it was decreased in the order of PLA R203H and PLGA RG502H. This might be due to the molecular weight of the polymers which are 24,000–38,000; 18,000–28,000; and 7000–17000 Daltons for PLGA RG503H, PLA R203H, and

Table 2: Response surface quadratic model ANOVA test results of the three responses

Response	Source	SS[a]	Df[b]	MSS[c]	F value	*P* Value	Inference[d]
Particle size (nm)	Model	265.97	6	44.33	157.57	<0.0001	Significant
	A[e]	184.08	1	184.08	654.36	<0.0001	Significant
	B[f]	74.01	2	37.00	131.54	<0.0001	Significant
	AB	4.22	2	2.11	7.50	0.0147	Significant
	A^2	3.66	1	3.66	13.01	0.0069	Significant
	Residual	2.25	8	0.28			---
	Lack of Fit	0.27	2	0.14	0.41	0.6780	Not significant
EE[g] (%)	Model	1662.45	6	277.08	128.11	<0.0001	Significant
	A	887.52	1	887.52	410.36	<0.0001	Significant
	B	723.93	2	361.96	167.36	<0.0001	Significant
	AB	12.29	2	6.14	2.84	0.1169	Not significant
	A^2	38.72	1	38.72	17.90	0.0029	Significant
	Residual	17.30	8	2.16			---
	Lack of Fit	2.72	2	1.36	0.56	0.5984	Not significant
k^h (h^{-1})	Model	8.23×10^{-4}	6	1.37×10^{-4}	127.06	<0.0001	Significant
	A	2.61×10^{-4}	1	2.61×10^{-4}	242.16	<0.0001	Significant
	B	5.42×10^{-4}	2	2.71×10^{-4}	251.37	<0.0001	Significant
	AB	1.72×10^{-5}	2	8.58×10^{-6}	7.95	0.0125	Significant
	A^2	1.67×10^{-6}	1	1.67×10^{-6}	1.54	0.2492	Not significant
	Residual	8.63×10^{-6}	8	1.08×10^{-6}			----
	Lack of Fit	2.63×10^{-6}	2	1.32×10^{-6}	1.32	0.3357	Not significant

PLGA RG502H, respectively. As the viscosity of a polymer is directly related to its molecular weight, under similar experimental conditions, a lower molecular weight polymer solution could be dispersed into finer globules in external medium than a higher molecular weight polymer solution and hence, the particle size was less in case of PLGA RG502H and increased in the order of molecular weight.[31] The effect of concentration of surfactant and type of polymer on the particle size ACNs were found to be statistically significant at $P < 0.05$ by ANOVA for response surface quadratic model (shown in Table 2).

Zeta potential

Lamivudine ACNs obtained from all the formulations were found to have zeta potential in the range of −19.1–-26.5 mV (shown in Table 1). The average zeta potential was found to be -25.8 mV in case of lamivudine ACNs of PLGA RG503H; -22.8 mV in case of ACNs of PLGA RG502H and -20.5 mV in case of ACNs of PLA R203H. All three selected polymers possess free carboxylic acids as the end groups, and this might be the reason behind the negative zeta potentials.[7,8] These results inferred that there was no influence of surfactant concentration, but the type of polymer had influenced the zeta potential. This might be due to the phenomenon of zeta potential that it depends on the nature of materials which present on the mantle of the nanocapsules. High negative zeta potential is important to be cleared from the blood circulation and to reach the liver rapidly on I. V administration of nanocapsules and as well higher the zeta potential greater the physical stability in the nanosuspension.

Drug release studies

The drug release rate constant values of lamivudine ACNs of all formulations were found to be varied in the range of 0.106–0.131 h^{-1} and shown in Table 1. Effect of both factors was illustrated in Figure 4(c). ACNs prepared with PLGA RG502H released the drug at a faster rate than from ACNs with other two polymers. This might be attributed to the low molecular weight of PLGA RG502H polymer among the three because lower the molecular weight of PLA or PLGA polymer, faster the degradation or erosion thereby the drug encapsulated in it released at a higher rate.[32] An interesting finding was observed in case of the other two polymers that the release rate constant was found to be lesser in case of PLA R203H than PLGA RG503H at lower levels of surfactant concentration. Although the molecular weight of the former polymer is slightly lesser, its higher hydrophobicity might cause drug release slower. However, at the highest level of surfactant (Span 80) concentration, the lipophilic surfactant might impart sufficient hydrophobicity to the ACNs of PLGA RG503H and hence exhibited lesser

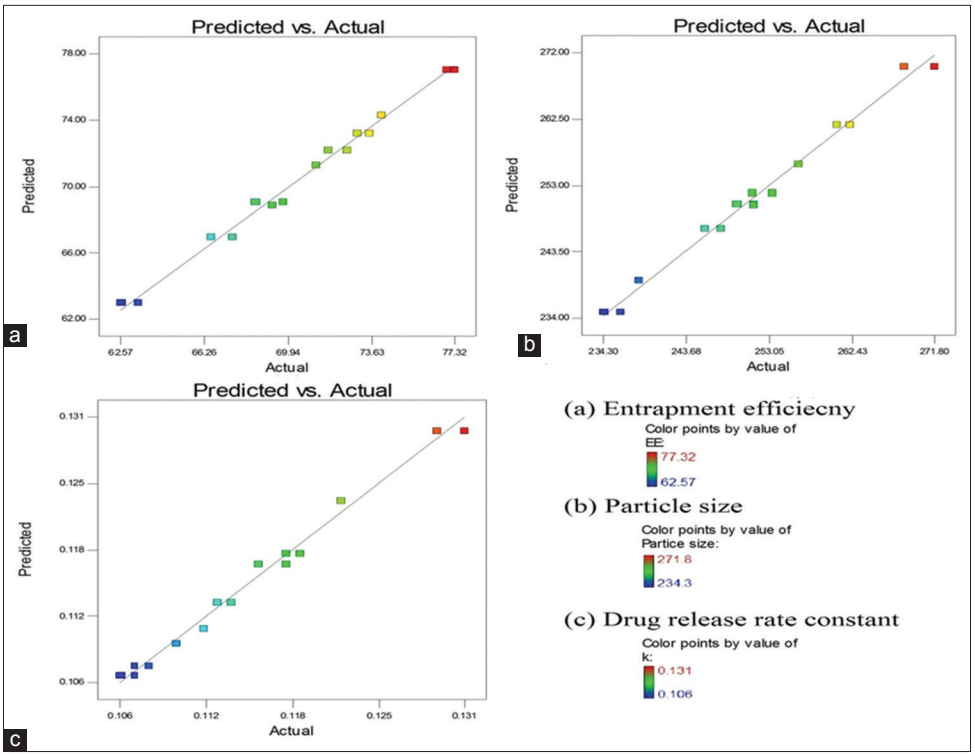

Figure 5: Predicted versus actual plots for the responses (a) entrapment efficiency; (b) particle size; (c) drug release rate constant

release rate constant due to its higher molecular weight than the ACNs of PLA R203H. The release rate constant in case of ACNs of any polymer was found to be decreased on increase in the concentration of Span 80 in the primary W/O emulsion, because with the increase in surfactant concentration, the affinity between both the phases might be increased, which could have resulted in strong association of the polymer in the outer organic phase with the surface of the internal aqueous phase globules on evaporation of the organic solvent and also the stability of the emulsion could be increased.[33] Hence, the ACNs prepared at high concentration of Span 80 have controlled the release of lamivudine to a higher extent. The effect of both the factors was significant at $P < 0.05$ (shown in Table 2). The drug release from ACNs of all formulations exhibited first-order kinetics and non-Fickian diffusion mechanism.

DoE validation and ANOVA

The results of the response variables were plotted between the predicted versus actual values and depicted in Figure 5. The proximate conformity between the predicted values by the design with the actual values obtained from the runs of experiment specified that the selected response surface quadratic model was appropriate for the design employed in the current study. This was further justified by the results of ANOVA (given in Tables 2) that the model F value was significant and lack of fit F value was insignificant at $P < 0.05$ in case of every response.

CONCLUSION

Nanoparticles with superior quality characteristics such as high entrapment efficiency, less particle size, and prolonged drug release can be developed only when the detailed influence of various formulation and process variables is known. Hence, this present work was designed with the prior objective of elucidating the effect of two formulation variables, namely, type of polymer and the concentration of surfactant in primary emulsion whose influence was not much reported on potential *in vitro* characteristics of nanoparticles. It was inferred from the detailed analysis of the results of the current study that the type of polymer and concentration of surfactant were found to have significant individual and interaction effects on entrapment efficiency, particle size, and drug release rate constant of nanocapsules. Hence, these formulation variables can be effectively optimized to attain the ACNs with desired characteristics. In this study, lamivudine ACNs with entrapment efficiency of 77.12%, particle size of 250.3 nm, and drug release rate constant of 0.106 h^{-1} were obtained as best formulation. Further, it was also inferred from this work that the entrapment efficiency of nanoparticles for hydrophilic drugs could be enhanced by ACNs technique.

ACKNOWLEDGMENT

The authors are acknowledged to the authorities of Jawaharlal Nehru Technological University Kakinada, Kakinada and

Acharya Nagarjuna University, Guntur, for their constant support and encouragement throughout the work.

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Source of Support: Nil. **Conflict of Interest:** None declared.