Adebileje and Amani, J Material Sci Eng 2018, 7:6
DOI: 10.4172/2169-0022.1000496

Journal of Material Sciences & Engineering

Research Article Open Access

Modelling Particle Size, Drug Loading and Release of BSA Encapsulated into PLGA Nanoparticles

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Abstract

Nanoparticle's (NPs) size, drug loading (DL) and drug release from NPs are critical physiochemical parameters to be considered while designing a polymeric carrier encapsulating therapeutic agents. In this study, bovine serum albumin (BSA) was chosen as a model protein to be encapsulated into poly (lactic-co-glycolic) acid (PLGA) NPs by double emulsion solvent evaporation method (DESE). Statistical Box-Behnken design (BBD) was used to investigate the effects and interactions of selected independent parameters of DESE (i.e. concentration of BSA (CBSA) and PVA (CPVA) as well as volume ratio (VR) between external aqueous phase and primary emulsion) on both particle size and DL. From the developed model, all the three parameters showed effects on size while only CBSA had a significant effect on DL. Based on the obtained model, mean (SD) of particle size, DL and entrapment efficiency of optimized samples were measured as 278.67 (9.29) (nm), 7.27 (0.02) (%w/w) and 86.6 (0.56) (%w/w) respectively. In-vitro release of BSA was determined and subsequently fitted into different models (i.e. zero order, Korsmeyer-Peppas and Higuchi) that describes the release behavior of therapeutic agents. A mean (SD) cumulative release of 44.20 (1.35)% of the encapsulated drug was determined during the first 24 h, Korsmeyer-Peppas model was found to be the best describing model for release from the carrier.

Keywords: PLGA NPs; BSA; Particle size; Drug loading; Drug release; Box Behnken design

Introduction

In controlled drug delivery and release of therapeutic agents encapsulated into polymeric carriers, particle size, drug loading (DL) and release of therapeutic agents are critical physiochemical parameters to be considered while designing a carrier system. Among several synthetic polymers, poly (lactic-co-glycolic) acid (PLGA) has been used extensively employed for encapsulation of therapeutic agents due to its biodegradability and biocompatibility.

Reviewing literature, several methods have been reported for preparing PLGA particles encapsulating therapeutic agents. These methods include emulsion/microemulsion polymerization, precipitation polymerization, emulsion diffusion, salting out, nanoprecipitation and emulsions solvent evaporation (single and double emulsion) [1,2]. Among these methods, water-in-oil-in-water (W/O/W) double emulsion solvent evaporation (DESE) has interesting potentials due to its ability to entrap hydrophilic drugs in hydrophobic carriers and also to provide sustained release of therapeutic agents [3-5]. However, an efficient approach of obtaining an optimum formulation with minimum particles size and maximum drug loaded PLGA particles have not been critically investigated.

From manufacturing point of view, physiochemical variables such as such as surface chemistry [6] and particle geometry [7,8] have been extensively studied to improve efficiency of the formulation e.g. by passive targeting. To improve efficacy of PLGA particles encapsulating hydrophilic drugs, not only the particle size (in nanometer range) needs to be considered, but also DL of NPs, which plays a critical role in amount of administered dosage, as well as the drug release profile need to be considered. PLGA NPs offer many advantages over micron-sized ones for drug delivery applications. These include efficient clearance of NPs after administration and their ability to penetrate through biological barriers [9,10]. Using preparations with higher DL is generally preferable due to less amount of inactive excipients added to the formulation. Such that, at higher DL values, lower number of particles are needed to deliver an equivalent dose of the drug. In recent studies, PLGA NPs prepared by emulsion/solvent evaporation have been reported to encapsulate up to 24% BSA (i.e. DL=24%) [11].

Several formulation/process parameters of DESE are expected to greatly affect physiochemical properties of prepared NPs. Parameters such as surfactant nature, viscosity, solvent nature, shear stress, additives, polymer/surfactant ratio, polymer properties, polymer concentration and primary phase/secondary phase ratios are among these formulation/ process parameters [2,9]. The relations of such parameters with themselves and with dependent parameters like size and DL are usually complicated and non-linear.

To achieve an optimum formulation with minimum particle size and maximum DL, response surface methodology (RSM) as a classical modelling technique can be employed to design, investigate and model the parameters involved in encapsulation process. An approach of using central composite design as an RSM have been utilized to optimize the operational variables involved in Benzene Hydroxylation to Phenol [12]. Our previous work utilized Box-Behnken Design (BBD) as an RSM on W/O/W DESE, to modelled the effect of four independent parameters, namely, concentration of poly vinyl alcohol (PVA), PLGA, BSA as well as volume ratio (VR) between external aqueous phase to

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Received October 02, 2018; **Accepted** October 22, 2018; **Published** November 02, 2018

Citation: Adebileje T, Amani A (2018) Modelling Particle Size, Drug Loading and Release of BSA Encapsulated into PLGA Nanoparticles. J Material Sci Eng 7: 496. doi: 10.4172/2169-0022.1000496

first emulsion on only size of prepared nanoparticles [13]. Generally, RSM shows to be efficient for both investigation and optimization of experimental procedures.

The aim of this investigation was to use BBD to obtain an optimum formulation with minimum particle size and maximum DL of PLGA NPs encapsulating BSA as a model drug for hydrophilic therapeutic agents. Selected independents parameters studied were concentration of BSA (C_{BSA}), concentration of PVA (C_{PVA}), as an emulsifying agent, and VR between external aqueous phase (containing PVA) to first emulsion containing PLGA and BSA. Experiments were carried out at fixed amount of PLGA and processing parameters (e.g. stirring, sonication, and time for solvent evaporation) of W/O/W DESE. In vitro release of BSA from PLGA particles was subsequently investigated on replicates of optimum formulation between specific time intervals (1-24 hrs.). To understand the specific release mechanism of BSA from PLGA NPs, in vitro release data were then fitted into common models (such as Zero order, Korsmeyer-Peppas and Higuchi model) describing the release kinetics of therapeutic agents.

Materials and Methods

Materials

PLGA (50:50, Mw=50 kDa) was acquired from Shenzhen Esun Industrial Co., LTD (China). PVA was purchased from VAM and P.VAL Co. Ltd (Japan). BSA was purchased from Beijing Solarbio Science and Technology Co., Ltd (China). Organic solvent dichloromethane (DCM), Sodium Hydroxide (NaOH), Sodium dodecyl sulfate (SDS), Dimethyl sulfoxide (DMSO) were purchased from Merck Millipore (Germany).

Experimental design and model evaluation

A 3-factorial 3-level BBD was used to design and evaluate selected independent parameters on particle size and DL of PLGA NPs encapsulating BSA using STATISTICA™ Ver.12.0 software package (Stat Soft Inc., USA). At fixed amount of PLGA, C_{BSA} (%w/v), C_{PVA} (%w/v) and VR were considered as the three independent variables (Tables 1 and 2), when volume of primary emulsion was fixed at 5 ml. For each independent parameter, 3 different levels (the lowest, the highest and central values of the studied ranges) were selected as shown in Table 1. All other processing parameters of DESE such as PLGA concentration, sonication time, solvent evaporation time and centrifugation parameters were kept constant throughout the experiments. Model development was carried out by considering two-way linear-linear interactions at a centered and scaled polynomial of variables on particle size and DL, respectively. Replicates (n=3) of optimum formulation obtained from BBD model were subsequently subjected to in-vitro release studies.

Preparation of nanoparticles

PLGA NPs encapsulating BSA were prepared by W/O/W double emulsion with PVA as stabilizer at the external aqueous phase. Briefly, BSA (20 mg to 40 mg) was dissolved in 1 ml distilled water, then, emulsified with 4 ml DCM containing 55 mg PLGA, followed by sonication for 30 seconds. In the second emulsification step, the primary emulsion (5 ml) was added to the external aqueous solution (20 ml to 30 ml) containing different PVA concentrations to obtain a W/O/W double emulsion and sonicated (60 seconds) and stirred (4 hours) for complete evaporation of DCM. Particles of PLGA encapsulating BSA were then obtained through centrifugation (12,000 rpm for 30 minutes) and washed two times with distilled water to remove excess PVA and non-encapsulated drug. The particles were subsequently subjected to lyophilization for 48 hours.

Factors	Variables	−1	0	+1
F_1	BSA concentration (%w/v)	0.4	0.6	0.8
F_2	PVA concentration (%w/v)	0.1	0.2	0.3
F_3	Volume Ratio	4.0	5.0	6.0

Table 1: Designed Independent parameters with their lower level (–1), center value (0) and upper level (+1).

Std Order	(F_1) BSA (%w/v)	(F_2) PVA (%w/v)	(F_3) VR (ml)	Observed		Predicted		Residuals	
				SIZE (nm)	DL (%w/w)	SIZE (nm)	DL (%w/w)	SIZE (nm)	DL (%w/w)
1	0.4	0.1	5	423	4.90	425	5.12	-2	-0.22
2	0.8	0.1	5	507	5.60	492	5.99	16	-0.39
3	0.4	0.3	5	457	4.80	473	4.41	-16	0.39
4	0.8	0.3	5	382	6.04	380	5.82	2	0.22
5	0.4	0.2	4	406	4.20	396	5.10	10	-0.90
6	0.8	0.2	4	453	4.60	460	5.33	-7	-0.73
7	0.4	0.2	6	407	4.40	400	3.67	7	0.73
8	0.8	0.2	6	300	6.60	310	5.71	-10	0.90
9	0.6	0.1	4	388	9.50	396	8.38	-8	1.12
10	0.6	0.3	4	388	7.30	383	6.79	5	0.51
11	0.6	0.1	6	336	6.20	341	6.71	-5	-0.51
12	0.6	0.3	6	300	6.30	292	7.42	8	-1.12
13	0.6	0.2	5	329	6.60	316	6.50	13	0.10
14	0.6	0.2	5	291	7.20	316	6.50	-25	0.70
15	0.6	0.2	5	329	5.70	316	6.50	13	-0.80
Replicates of optimization and validation experiments									
R1	0.7	0.3	6	268	7.27	270	7.55	-2	-0.28
R2	0.7	0.3	6	283	7.25	270	7.55	13	-0.30
R3	0.7	0.3	6	285	7.28	270	7.55	15	-0.27

Table 2: Experimental design of independent parameters including observed, predicted and residual values of particle size and percentage DL.

Particle size analysis

Median hydrodynamic diameter (d50) of the NPs was analyzed with dynamic light scattering (DLS) (Scatteroscope-I, K-ONE LTD, South Korea).

Scanning electron microscopy

Sample was spread on metal stubs and gold coating was performed with an ion-sputtering device. Gold-coated samples were vacuum dried and examined. Under different magnifications, morphology, size and size distribution of optimized formulation (R1) was confirmed at a cross sectional view with a scanning electron microscope (SEM, Seron Technology, AIS-2100, Korea).

Protein content determination

Protein content in PLGA NPs was determined according to previously reported procedures to determine protein content in PLGA Microspheres [14]. Briefly, 15 mg of NPs was accurately weighed and put into a test tube to which 2 ml of DMSO (5%) was added and dissolved by occasional hand-shaking during a 1-h incubation. A 0.05 N NaOH solution of water containing 0.5% w/v SDS (8 ml) was then added to the corresponding test tube and gently mixed. After being allowed to stand at room temperature for 24 h, aliquots of the clear DMSO/NaOH/SDS solution were analyzed with UV-spectroscopy using Bradford protein assay. Blank, protein-free NPs were also subjected to the same procedure. The percentage DL was calculated as follows:

$$\text{Percentage DL} = \frac{\text{Amount of drugs in NPs}(\text{mg})}{\text{Amount of NPs}(\text{mg})} \times 100 \quad (1)$$

Protein release

40 mg of BSA-loaded PLGA NPs were dispersed in 10 mL of PBS (pH 7.4), and rotated on a rotary mixer (Fan Azma Gostar, Tehran, Iran) at 20 rpm and room temperature. Samples (2 mL) were withdrawn for centrifugation (15 min at 10,000 rpm and 5°C), and the supernatant was analyzed for drug content by Bradford assay with UV spectroscopy at a wavelength of 595 nm. The withdrawn volume was subsequently replaced with fresh release medium (PBS) between 1-24 hours. The percentage cumulative release of BSA at specific time intervals from PLGA NPs was subsequently fitted, using non-linear regression analysis, into common models describing the release kinetics of therapeutic agents such as zero-order model (eqn. (2)), Korsmeyer-Peppas (eqn. (3)) and Higuchi model eqn. (4)).

Zero order: It defines a linear relationship between the fractions of drug release versus time. A plot of fraction of drug release against time will be linear, if the release obeys zero order release kinetics.

$$Q = k.t + Q_0 \quad (2)$$

Korsmeyer-Peppas model: A semi-empirical equation describes the release mechanism of solute through polymer chains of particulate systems when the mechanism is a combination of Fickian (diffusion) and non-Fickian mechanisms (combined mechanism of pure diffusion and dissolution) [15,16].

$$Q = k.t^n \quad (3)$$

Where n is the value of diffusion exponent (n) which defines the release behavior of drugs. The value of n for a spherical system, <0.43 indicates Fickian release, 0.43<n<0.85 indicates non-Fickian release; n > 0.85 indicates case II release.

Higuchi model: It defines a linear dependence of the active fraction released per unit of square root of time:

$$Q = k\sqrt{t} \quad (4)$$

Where, t is the release time, Q is the amount (%) of drug substance released at time t, Q_0 is the start value of Q, k is the release rate constant and n is the release exponent. BSA release rate constants and modelled parameters (Table 3) were calculated using an open source curve fitting software (KinetDS 3 rev 2010) developed in Lazarus RAD environment for description of cumulative dissolution curve by simple equations of mechanistic and empirical models applied to drug dissolution [17].

Results and Discussions

Table 3 represents the effect estimates of independent variables on observed size and DL with combined linear and quadratic effects of variables and their statistical p-values. The applied BBD within equally spaced level of each independent parameter sufficiently proves to fit a quadratic model with R^2 of 0.967 for particle size and 0.774 for DL. Mean Square (MS) residual reported in this model for size and DL were 390.783 and 1.444, corresponding to standard error of 19.768 for size and 1.202 for DL $\left(Standard\ error = \sqrt{(MS\ Residual)}\right)$. Standardized effect estimates and significant orders of parameters affecting both the particle size and DL were obtained through the Pareto charts (Figures 1 and 2). For prediction of size and DL, the regression coefficients of independent parameters and their interactions on size and DL were determined and fitted into the regression equations 5 and 6 with F_1, F_2 and F_3 representing C_{BSA}, C_{PVA}, and VR, respectively. Response surfaces were generated to study the effects and interactions among the independent parameters. Release model parameters of equations 2, 3 and 4 were also generated (Table 4).

Factor	Size			DL		
	R^2=0.967; MS Residual=390.783			R^2=0.732; MS Residual=1.444		
	Effect	Std error	p	Effect	Std error	p
Mean/Interc.	395.583	5.706	0.000	5.870	0.347	0.000
BSA (L)	-12.750	13.978	0.403	1.135	0.850	0.239
BSA (Q)	-82.208	10.288	0.000	1.770	0.625	0.037
PVA (L)	-31.750	13.978	0.072	-0.440	0.850	0.627
PVA (Q)	-43.708	10.288	0.008	-0.605	0.625	0.378
VR (L)	-73.000	13.978	0.003	-0.525	0.850	0.564
VR (Q)	7.041	10.287	0.524	-0.220	0.625	0.739
BSA (L) * PVA (L)	-79.500	19.768	0.010	0.270	1.202	0.831
BSA (L) * VR (L)	-77.000	19.768	0.011	0.900	1.202	0.488
PVA (L) * VR (L)	-18.000	19.768	0.404	1.150	1.202	0.383

Table 3: Effect estimate of selected composition parameters on size and DL (drug loading).

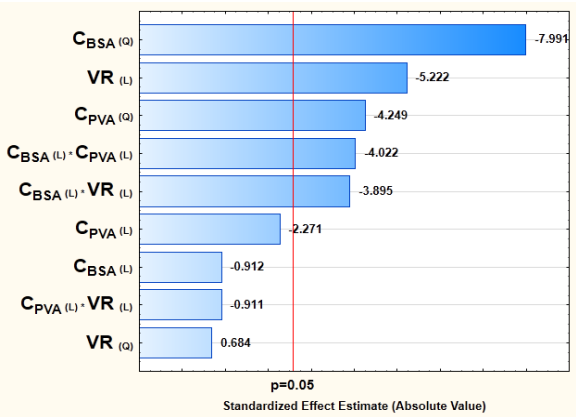

Figure 1: Pareto chart showing the significance order and absolute values of independent parameters effect's on size of PLGA NPs.

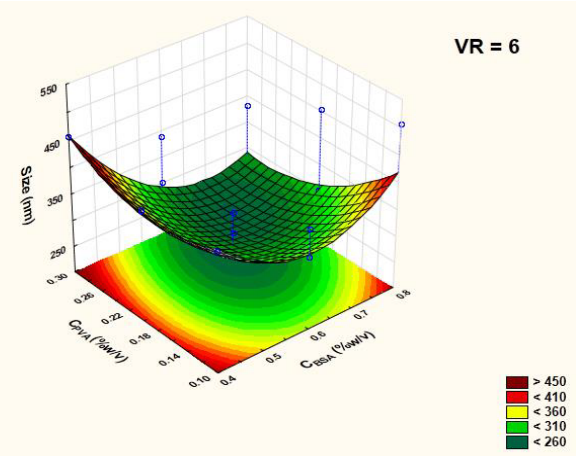

Figure 2: Interactions between C_{BSA} and C_{PVA} and their effects on particle size at a fixed VR.

Release Model Parameters	REPLICATE 1			REPLICATE 2			REPLICATE 3		
	Zero Order	Korsmeyer-Peppas	Higuchi	Zero Order	Korsmeyer-Peppas	Higuchi	Zero Order	Korsmeyer-Peppas	Higuchi
R	0.782	0.953	0.697	0.829	0.964	0.735	0.824	0.960	0.729
RMSE	6.220	4.568	7.332	5.164	3.704	6.422	5.035	3.631	6.252
AIC	84.867	76.840	89.141	80.028	71.391	85.697	79.369	70.872	85.001
BIC	85.997	77.970	90.271	81.158	72.521	86.827	80.499	72.002	86.131
K	-	0.719	7.897	-	6.440	7.541	-	6.180	7.214
N	-	0.631	-	-	0.651	-	-	0.651	-

RMSE: Root mean square error, AIC: Akaike Information Criterion, BIC: Bayesian information criterion.

Table 4: Release Model parameters on Replicates of optimized formulations.

$$Y_{size} = 382.4 - 1138.1\,F_1 + 2055.2\,F_1^2 - 264.6F_2 + 4370.8\,F_2^2 + 167.4F_3 - 7F_3^2 - 1987.5\,F_1 \times F_2 - 192.5\,F_1 \times F_3 - 90\,F_2 \times F_3 \quad (5)$$

$$Y_{DL} = 11.85 + 43.34\,F_1 - 44.25\,F_1^2 - 59.20\,F_2 + 60.50\,F_2^2 - 4.96\,F_3 + 0.22\,F_3^2 + 6.75\,F_1 \times F_2 + 2.25\,F_1 \times F_3 + 5.75\,F_2 \times F_3 \quad (6)$$

Effect of independent parameters on particle size

In this section, the aim was to study the effects of the independent parameters on particle size. As shown in Figure 1, quadratic effect of C_{BSA} and C_{PVA}, linear effect of VR and linear interactions between C_{BSA} and C_{PVA} and that of C_{BSA} and VR were statistically significant. From Figure 2, particle size in general increases by increasing either C_{BSA} or C_{PVA}. However, for C_{BSA}, the size tends to decrease when C_{BSA} increases from 0.4 to ~0.6 (%w/v), followed by an increase when C_{BSA} exceeds 0.6. Above findings may also be confirmed from Figures 3 and 4.

Reviewing literature, complicated and contradictory findings have been documented from effect of C_{BSA} or C_{PVA} on particle size. For instance, a similar work observed an appreciable size reduction of PLGA NPs encapsulating BSA while increasing C_{BSA} (from 1.0 to 5.0

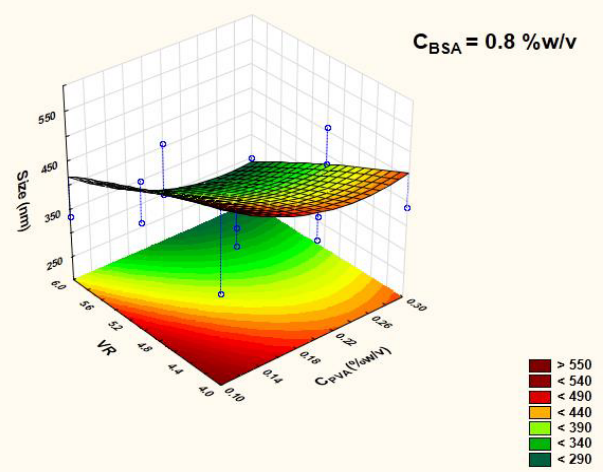

Figure 3: Interactions between C_{PVA} and VR and their effects on particle size at a fixed C_{BSA}.

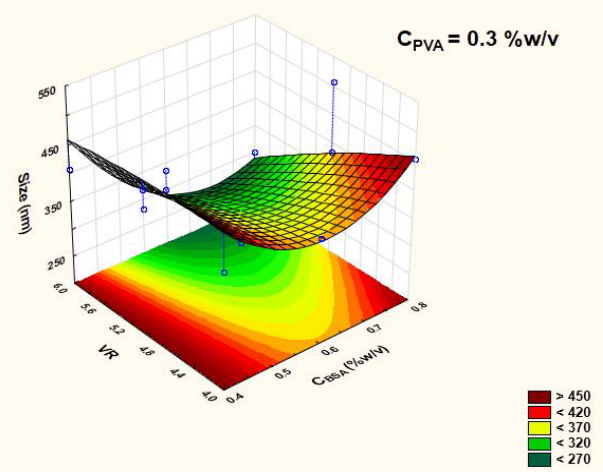

Figure 4: Interactions between C_{BSA} and VR and their effects on particle size at a fixed C_{PVA}.

w/v%) in the primary emulsion [18]. Also, increasing C_{PVA} has been observed to reduce the size of PLGA particles [11,19,20]. while in another work, an increase in C_{PVA} concentration, was observed to first decrease, then, gradually increase mean diameter of PLGA NPs [21]. Reduction in particle size at higher C_{PVA} is probably due to stabilizing activity of PVA which tends to reduce the surface area of emulsion droplets and promotes both formation and stability of smaller particles [11]. However, the findings obtained in our work are arguably due to the effect of viscosity: Increasing C_{PVA} and/or C_{BSA} increases the viscosity of the preparation which in turn leads to decreasing the effect of applied shear stress on the particles to efficiently break down the emulsion droplets and produce smaller sizes. Therefore, larger particles are expected to be prepared [13]. The transient effect of C_{BSA} on particle size (i.e. reverse effect on particle size when C_{BSA} is 0.4-0.6 (%w/v)) is probably due to specific amount of BSA which is required to achieve particle stability. We believe a minimum amount of BSA should be met to provide appropriate intra-particle interactions and stabilize the nanoparticles.

Also, from Figures 3 and 4, VR shows a reverse effect on size. In contrast to our study, in a previous work, the size of PLGA particles was reported to increase when increasing the volume of external aqueous phase of double emulsion. It was suggested that shear stress applied was probably ineffective at high volume of external phase [22]. Formation of smaller particles as a function of higher VR values could be attributed to an efficient dispersion/stability of primary emulsion in external aqueous phase when compared to a formulations with lower VR values.

Effect of independent parameters on drug loading

In the present study on DL, the aim was to study the effect of independent parameters on DL. Majority of independent parameters showed insignificant effects on DL, except a quadratic effect from C_{BSA} (Pareto chart and effect estimates in Figure 5 and Table 3). From Figures 6-8, C_{BSA} values of ~0.7 (%w/v) are required to obtain maximum DL, while effect of other two parameters is negligible.

Increase in DL has been observed when increasing protein concentration at the inner aqueous phase of DESE [23]. Obtaining maximum DL at higher C_{BSA} is expected due to direct addition of BSA into the formulation. However, the subsequent reduction of DL at C_{BSA}

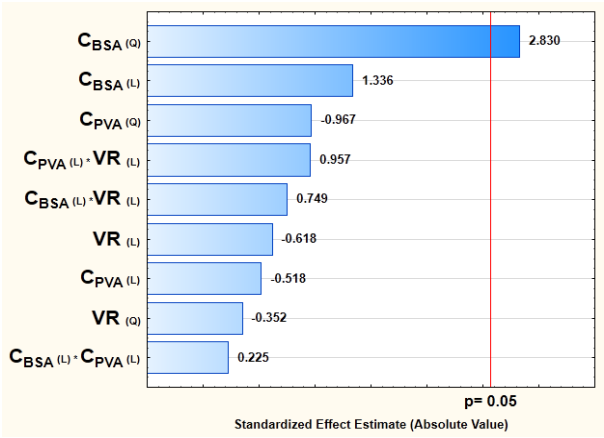

Figure 5: Pareto chart showing the significance order and absolute values of independent parameters effect's on DL of PLGA NPs.

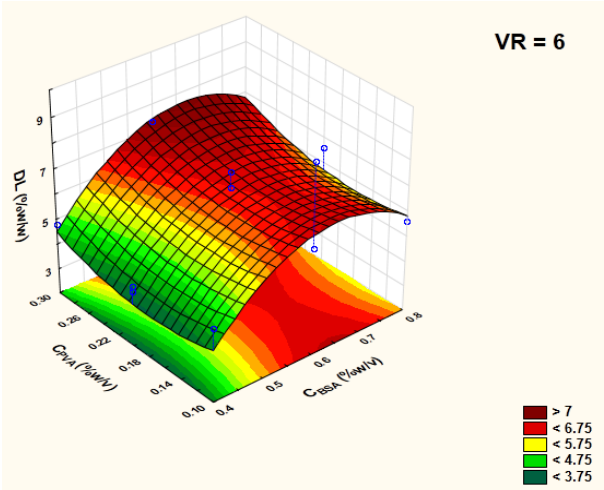

Figure 6: Interactions between C_{BSA} and C_{PVA} and their effects on drug loading at a fixed VR.

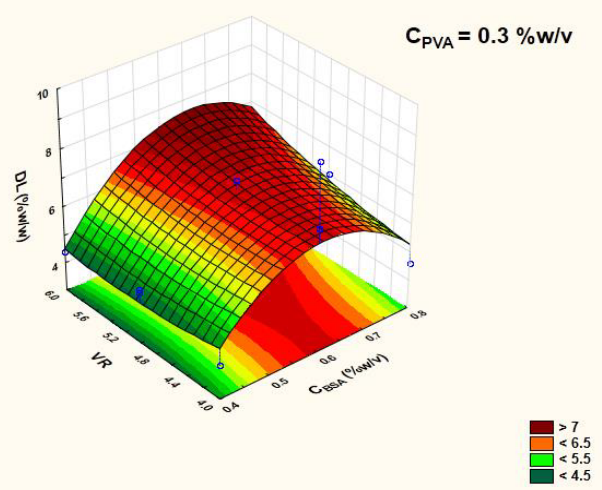

Figure 7: Interactions between C_{BSA} and VR and their effects on drug loading at a fixed C_{PVA}.

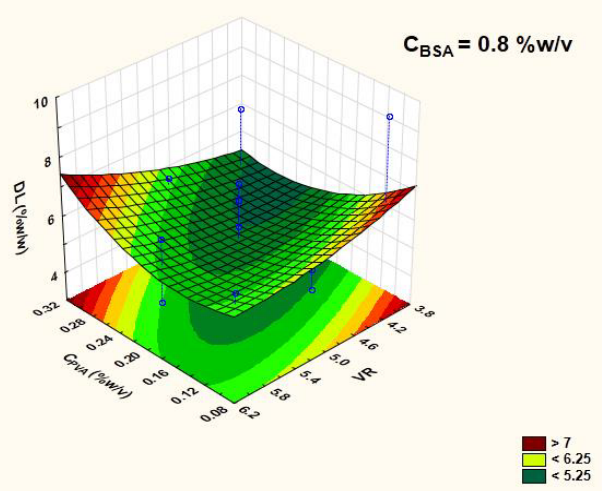

Figure 8: Interactions between C_{PVA} and VR and their effects on drug loading at a fixed C_{BSA}.

>0.7 (%w/v) is probably due to hydrophobic-hydrophilic interactions between PLGA and BSA, such that, repulsion of BSA might occur when increasing BSA during its encapsulation into PLGA matrix.

Optimization and validation

Using the software option for optimization to obtain an optimum sample with minimum particle size and maximum DL, optimum values for input variables were suggested as 0.7 (%w/v), 0.3 (%w/v) and 6 for C_{BSA}, C_{PVA}, and VR, respectively. The predicted size and DL from equation 5 and equation 6 were 270 nm and 7.55 (%w/w) respectively. To validate the model, three replicates on optimum independent parameters were experimentally prepared (Table 2) and particle size and DL produced were measured as mean (SD) 278.7 (9.29) (nm) and 7.27 (0.02) (%w/w), respectively. The results show capability of the model to predict the optimum size and DL of PLGA particles encapsulating BSA.

Furthermore, the optimum sample showed mean (SD) entrapment efficacy of 86.6 (0.56) (%w/w), an appreciable value which could be interesting for drug delivery purposes. SEM image of the optimized sample spherical shapes for the NPs with average particle size of 256 nm (Figure 9).

Release of BSA from PLGA NPs

In the present study, within 24 hours, replicates of optimized formulation were observed to follow a similar percentage cumulative release (SD) of 44.20 (1.35)% of the initial BSA encapsulated into PLGA NPs (Figure 10). To study the release mechanism of BSA from PLGA NPs, data obtained from in vitro release studies were fitted into equations 3, 4 and 5. From corresponding correlation coefficient (r), root mean square error (RMSE), Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), Korsmeyer-Peppas model was chosen as the best model fitting the release profile of BSA from PLGA NPs (Table 4). Mean (SD) Korsmeyer-Peppas n-value of PLGA NPs encapsulating BSA was 0.64 (0.01) for the replicate formulations. This indicates that release of BSA follows anomalous non-Fickian transport from PLGA NPs, and one could suggest that release of BSA is related to combination of both diffusion and dissolution processes [16].

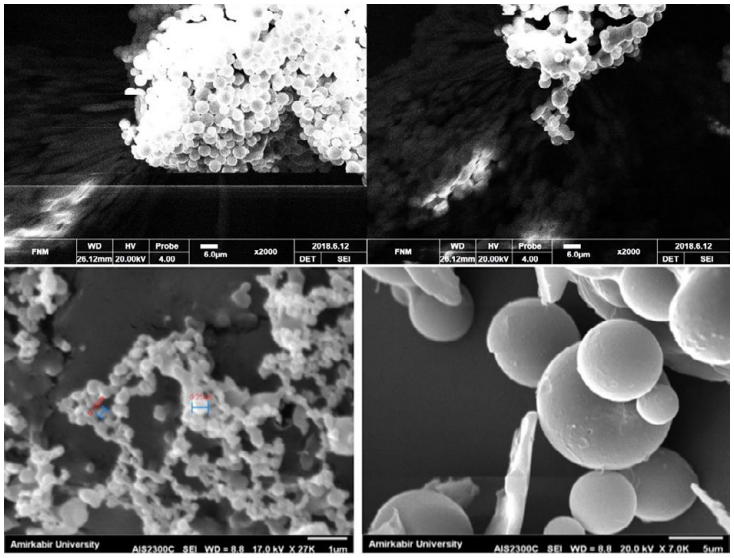

Figure 9: SEM image of optimum Replicate 1 of PLGA particles encapsulating BSA at different Resolution.

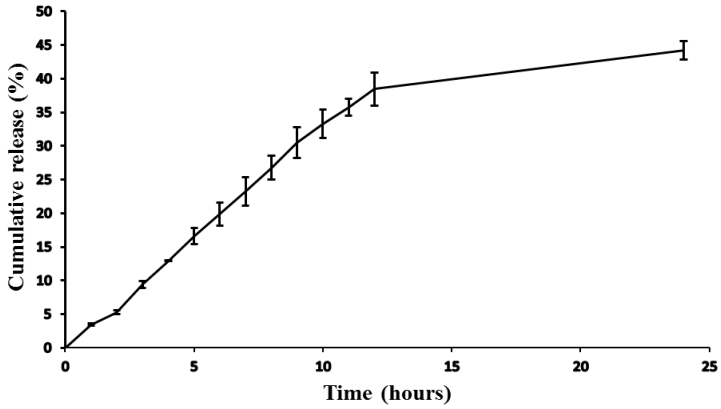

Figure 10: Percentage cumulative release of BSA from PLGA NPs.

In a work, burst release less than 20% was reported, followed by a sustained release over a period of 19 days and it was suggested that the release was due to either simple diffusion or both diffusion and erosion of microsphere erosion [24]. Reviewing literature, the release rate pattern of drugs from PLGA is often unpredictable. Factors such as molar ratio of lactic and glycolic acid groups, molecular weight of the polymer, degree of crystallinity (Tg), pH of release media, drug type (hydrophobic/hydrophilic) and percentage drug loaded are known to influence release process of drugs [1]. Release of protein from PLGA often displays several phases. Usually, a burst release from protein from particle surface is observed, (in some cases up to 60% of the initial protein loaded) [25]. This is then followed by a lag phase when little or no release of encapsulated protein occurs due to slow matrix desorption [26]. In recent studies, diffusion and dissolution have been reported to be the main mechanisms of drugs released from PLGA NPs [27].

Conclusion

Using BBD as a statistical response surface methodology, this study demonstrated the ability to model and achieve optimum selected independent parameters producing minimum particle size with maximum drug loading (DL) of PLGA NPs encapsulating BSA. Optimum independent parameters (i.e. 0.7%w/v, 0.3%w/v and 6 for C_{BSA}, C_{PVA}, and VR, respectively) produced mean (SD) particle size and DL of 278.67 (9.29) (nm) and 7.27 (0.02) (%w/w), respectively. From the model, all the three independent parameters played a role in determining the size, while only C_{BSA} affected DL. It was suggested that viscosity of the preparation and stability of the emulsion droplets result in appreciable size and drug loading. Furthermore, controlled release of BSA from PLGA particles was fitted to Korsmeyer-Peppas release model. Conclusively, this approach represents an efficient means of designing drug-loaded PLGA NPs using statistical design of experiments for investigating the effects of parameters involved in production process.

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