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Formulation and *in-vitro* evaluation of controlled release delivery systems for anti-hyperlipidemic drug

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ABSTRACT

Dyslipidemia is one of the most common risk factors of cardiovascular diseases (CVD). Most of the lipid-lowering agents suffer from drawbacks because of low oral bioavailability due to hepatic metabolism. The purpose of this study is to develop a drug delivery system of polymeric nanoparticles (PNPs) for controlled release of an anti-hyperlipidemic drug belonging to the group of statins. The nanoparticles were developed by emulsion solvent evaporation method using PLGA or Eudragit RLPO® as a polymeric carrier; and polyvinyl alcohol (PVA) or Poloxamer 188 as an emulsifying agent. The goal is to investigate the effect of the amount of initial added drug and of the different types of polymers and surfactants on the percentage drug loaded in the nanoparticles (DL %) and on its release profile. It was found that the drug amount is an important factor affecting the DL %. Additionally, it was found that the polymer type is the significant factor affecting the release pattern of the drug. These formulation variables (factors) were optimized with full factorial design to select the formulation with optimum controlled drug release for future achievement of optimized prolonged and safe hypolipidimic effect.

Key words: Dyslipidemia, experimental design, nanoparticle, controlled release.

Introduction

Lipid-lowering agents also called hypolipidemic or anti-hyperlipidemic drugs play an essential role in the primary and secondary prevention of cardiovascular disease (CVD) and stroke and hence in controlling cardiovascular risks (Lardizabal and Deedwania, 2010; Punitha, 2011). Because cholesterol cannot be dissolved in the blood, it must be carried by molecules called lipoproteins in order to be transported from- and to the cells. Lipoproteins consist of an inner core of cholesterol and triglycerides (TG) and an outer layer of protein and enable cholesterol to move around the body. These lipids can be classified as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) (Dhaliya *et al.,* 2013).

Anti-hyperlipidemic drugs such as statins and fibrates (Pahan, 2006) reduce cardiovascular risks by suppressing the lipids in the body. Statins inhibit the biosynthesis of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and، consequently, suppress cholesterol biosynthesis (Pahan, 2006; Ioannis, 2010). Statins are the most efficient monotherapy to reduce TC, LDL and VLDL levels (Dhaliya *et al.,* 2013). Rosuvastatin (RSV) is the most potent statin for decreasing LDL levels (Olsson *et al.,* 2001; McKenney *et al.,* 2003). However, RSV, as other available statins, exhibits low bioavailability when administered orally as a result of first-pass metabolism (Lennernas, 2003; Martin *et al.,* 2003).

Polymeric nanoparticles (PNPs) are widely used as nanocarriers for drug delivery (Banik *et al.,* 2016). While initially mainly nonbiodegradable polymers such as polyacrylates were used (Turos *et al.,* 2017), there is now more emphasis on biodegradable polymers due to their biocompatibility and low toxicity. Biodegradable polymers include natural polymers such as alginate، chitosan, albumin, and gelatin in addition to synthetic polymers such as poly (ε-caprolactone) (PCL), poly (lactide) (PLA) and poly (lactide-co-glycolide) copolymers (PLGA) (Zhang *et al.,* 2013). PLGA is the most

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commonly used biodegradable polymer due to its biocompatibility and biodegradability, its suitability for controlled drug release as well as due to the fact that it is approved by FDA (Danhier *et al.,* 2012). In addition to PLGA, the non-biodegradable polymer, Eudragit RLPO®, is also of interest for the development of sustained release drug delivery systems. It is a copolymer of methyl acrylate, ethyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups (Gandhi *et al.,* 2014).

The aim of this study is to prepare PNPs using PLGA as well as Eudragit RLPO® and to study their effect on the release kinetics of RSV with the ultimate aim of improving its oral anti-hyperlipidemic effect.

Materials and Methods

Materials:

Rosuvastatin (RSV) was kindly donated from Marcyrl Pharmaceutical Industries - Egypt. Poly (lactic-co-glycolic acid) (PLGA; 50:50, inherent viscosity 0.2 dl/g) was kindly provided from PURAC BIOMATERIALS, The Netherlands. Eudragit RLPO® (EUD) was purchased from Röhm GmbH & Co KG, Germany. Polyvinyl alcohol (PVA), Poloxamer 188 (Polox) and dialysis tubing cellulose membrane (flat width 25 mm, molecular weight cut-off 14,000 Dalton) were purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

Methods:

Preparation of polymeric nanoparticles:

Nanoparticles were prepared by oil in water (O/W) emulsion solvent evaporation method using PVA or Polox as emulsifying agent in the external aqueous phase. The drug and the polymer were dissolved in a water-immiscible solvent, methylene chloride (DCM), representing the inner oil phase. Then the oil phase was poured drop wise onto the aqueous phase and homogenized for 5 minutes at 26000 rpm using high shear homogenizer (Silent Crusher Homogenizer, Heidolph) to form O/W nanoemulsion. Afterwards, the DCM was evaporated for 10 min. at 35 °C using rotary vacuum evaporator (Rotary Evaporator, Heidolph), and the resulting nanoparticle suspension was centrifuged twice at 8,000 rpm for 1 h using a cooling centrifuge (Refrigerated Large Capacity Centrifuge, Union 32R) at 4 °C, to remove the non-encapsulated drug.

Experimental design:

2^3 full factorial design was generated to optimize the following formulation variables (factors) at selected levels; X_1: drug content at two levels (10 and 20 mg), X_2: polymer type at two levels (PLGA and EUD RLPO®), and X_3: surfactant type at two levels (PVA and Polox). The dependent variables (responses) were; Y_1: % drug loading (DL %), Y_2: % drug released (R %) at 2 h and Y_3: % drug released (R %) at 8 h. The different factors and responses are listed in table 1 and the 2^3 full factorial design of the experiments is presented in table 2.

One way analysis of variance (ANOVA) was used to determine the statistically significant effects of the single factors and of their interactions.

Table 1: Factors and responses of 2^3 full factorial design

Independent variables (Factors)	Levels	
	-1	+1
X_1: Drug amount (mg)	10	20
X_2: Polymer type	PLGA	EUD
X_2: Surfactant type	PVA	Polox
Dependent variables (Responses)	Constraints	
Y_1: DL %	Maximize	
Y_2: $R_{(2h)}$ %	Minimize	
Y_3: $R_{(8h)}$ %	Maximize	

Table 2: Design table of all experimental combinations of 2^3 factorial design

Formulation ID	Drug content (mg), X_1	Polymer type, X_2	Surfactant type (1% w/v), X_3
F1	10	PLGA	PVA
F2	20		
F3	10		Polox
F4	20		
F5	10	EUD	PVA
F6	20		
F7	10		Polox
F8	20		

Determination of encapsulation efficiency (EE) and drug loading (DL):

The drug EE % was determined from the ratio of the amount of encapsulated drug to the amount of drug initially added. The encapsulated drug amount was determined by subtracting the drug amount present in the supernatant after centrifugation from the amount of drug initially added. The drug concentration in the supernatant was measured spectrophotometrically at 241 nm using UV–VIS spectrophotometer (Shimadzu, model 2401/PC, Japan).

EE (%) = (Encapsulated drug / Added drug) X 100

DL (%) = W_D/W_{NP} X100

Where W_D is the weight of the drug in the nanoparticles and W_{NP} is the weight of the nanoparticles.

In-vitro release study:

The drug release from nanoparticles was determined by the dialysis-bag method. The study was performed using dissolution test apparatus (USP Drug Dissolution Apparatus II, paddle type, Hanson-SR8 Plus, USA) using pH 6.8 phosphate buffer as the release medium. The paddles were operated at 50 rpm and temperature was maintained at 37 ± 0.5 ºC throughout the experiment. At fixed intervals, 5mL of the release medium were withdrawn and replaced by fresh medium to maintain sink condition. The amount of drug released was measured at 241 nm by the UV–VIS spectrophotometer. The release study for each formulation was conducted in triplicate.

In-vitro drug release data were fitted to the different kinetic models such as zero order, first order, Higuchi equation and Korsmeyer–Peppas equation. Regression analysis of Q vs. t (zero order), log Q vs. t (first order), Q vs. square root of t (Higuchi), log% Q vs. log% t (Korsmeyer–Peppas), where Q is the amount of drug released at time t, was performed to obtain the correlation coefficients R^2 (Thakkar *et al.,* 2009).

Transmission Electron Microscopy (TEM) and particle size (PS) analysis:

The particle size and morphology of the optimized formulation were examined by TEM (model JEM-1230, Jeol, Tokyo, Japan), where the nanoparticle suspensions were diluted with water (1:10). A drop of diluted sample was deposited directly on grid, stained with 1% aqueous solution of phosphotungstic acid and observed after drying at suitable magnification.

Statistical analysis:

All measured data were expressed as mean ± standard deviation (SD). The statistical analyses were conducted using one way analysis of variance (ANOVA) test for P value = 0.05 using SPSS Software.

Results and Discussion

Preparation of polymeric nanoparticles:

In this investigation, nanoparticles (NPs) were successfully prepared by O/W emulsion solvent evaporation method. The emulsions formed were stable after few hours of emulsification and neither phase separation nor polymer aggregation was observed.

Determination of encapsulation efficiency (EE) and drug loading (DL):

The encapsulation efficiencies (EE %) of the prepared NPs were shown in table 3 and Fig. 1. The EE values of NPs prepared with PLGA were within the range 21.42 % (F4) to 28.06 (F1); while the EE of NPs prepared with Eudragit were ranged from 22.36 (F8) to 32.25 % (F5). It was shown that there was a decrease in EE when the initial amount of RSV was increased from 10 to 20 mg. This observation might be attributed to the effect of the concentration gradient of the drug between the dispersed and the aqueous phase, as the diffusion of the drug into the aqueous phase during the particle-hardening step is mainly dependent on the concentration gradient. Higher drug concentration gradient led to higher drug loss and lower EE. This result was in agreement with other published reports (Choi *et al.,* 2011). On the other hand, increasing the drug amount resulted in a subsequent increase in the % drug loaded in NPs (DL %) which was within the range 4.72 ± 0.48 % (F3) to 9.69 ± 0.54 % (F6) (see Fig. 2).

Generally, the EE values of NPs prepared with Eudragit were slightly higher than those prepared with PLGA but the difference was not statistically significant (P>0.05). The percent of drug loaded into the nanoparticles (DL %) was similar with both types of polymer.

It was observed that the surfactant type has an effect on the EE, where PVA led to higher EE than Polox. This might be attributed to the higher hydrophilic-lipophilic balance (HLB) of Polox. It was reported in previous studies that the encapsulation efficiency depended on the HLB of the surfactants and lower EE was observed for surfactants with higher HLB (Taymouri *et al.,* 2016). When HLB of the surfactant in the external aqueous phase increased, the drug might diffuse out from the internal oil phase and solubilize in the external aqueous phase resulting in lower EE.

Table 3: Encapsulation efficiency (EE) of PLGA and Eudragit RLPO® drug-loaded nanoparticles

Formulation ID	EE (%) (Mean ± SD, n=3)	Drug loading (%) (Mean ± SD, n=3)
F1	28.06 ± 1.03	5.31 ± 0.20
F2	25.87 ± 0.85	9.38 ± 0.31
F3	24.78 ± 0.76	4.72 ± 0.48
F4	21.42 ± 1.15	7.89 ± 0.70
F5	32.25 ± 0.92	6.06 ± 0.43
F6	26.83 ± 1.53	9.69 ± 0.54
F7	27.91 ± 1.26	5.29 ± 0.37
F8	22.36 ± 0.98	8.21 ±0.35

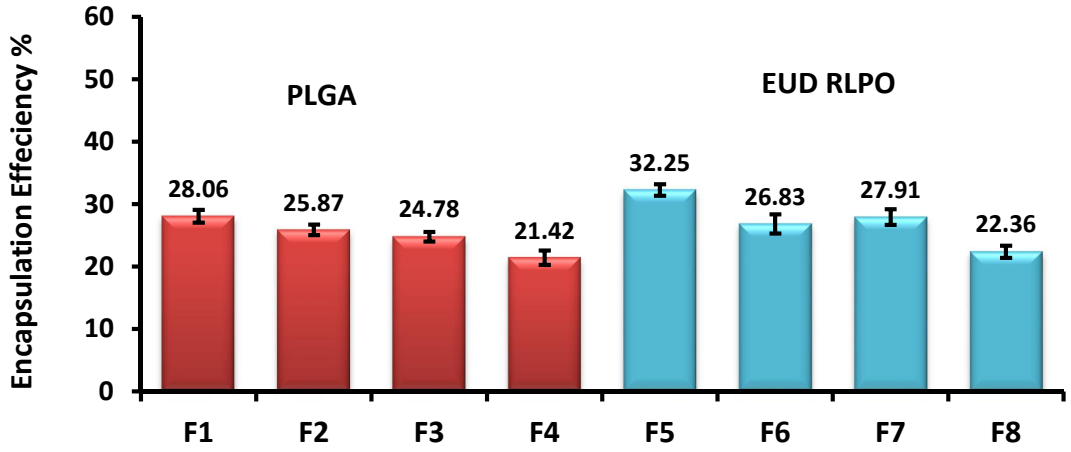


Fig. 1: Encapsulation efficiency (EE %) of the prepared nanoparticles

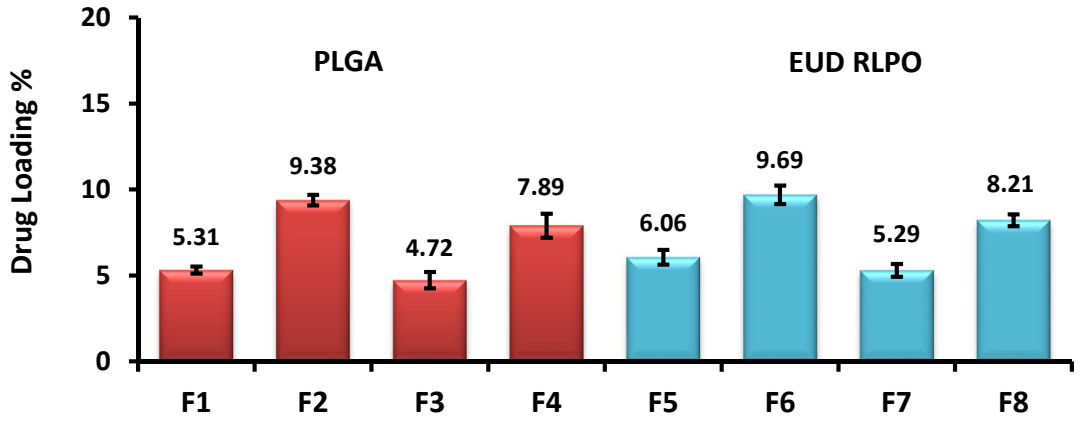


Fig. 2: Drug loading (DL %) of the prepared nanoparticles

In-vitro release study:

The cumulative percentages of drug released after 8 h, $R_{(8h)}$ %, for NPs prepared with PLGA and Eudragit were presented in tables 4 and 5, respectively, and the release profiles were illustrated in Fig. 3. It was observed that the drug amount influences the release behavior, where NPs with higher drug content exhibited enhanced release profiles than those with lower drug content due to higher concentration gradient. When the drug amount increased from 10 to 20 mg, the $R_{(8h)}$ % increased from 48.57 % (F1) to 51.65 % (F2) and from 52.68 (F5) % to 54.91 (F6) for NPs prepared with PLGA and EUD, respectively.

It was also observed from the results in tables 4 and 5 that the polymer type exhibited significant effect on the release pattern of the NPs. Although there was no significant difference in the cumulative percent drug released after 8 h, the dissolution profiles of NPs prepared with Eudragit, unlike those prepared with PLGA, exhibited a burst effect within the initial 2-3 h. This may be due to the presence of weakly bound drug on the NPs surface. The PLGA NPs exhibited slower release rate without the initial burst release event. This can be attributed to the fact that the drug is released from PLGA by diffusion followed by polymer degradation. This finding is of importance, because potential side effects can be triggered if too much drug is released over a short time.

Table 4: Cumulative percent drug released from PLGA loaded nanoparticles

Time (h)	Cumulative percent drug released (Mean ± SD, n=3)			
	F1	F2	F3	F4
0.5	4.95 ± 1.49	5.40 ± 1.02	5.11 ± 1.31	8.33 ± 1.82
1	10.72 ± 2.20	10.99 ± 0.68	10.81 ± 1.85	11.82 ± 2.38
2	19.54 ± 2.28	19.81 ± 0.91	17.24 ± 2.36	21.96 ± 2.03
3	24.56 ± 1.31	28.50 ± 1.42	23.13 ± 1.83	25.20 ± 1.93
4	29.10 ± 1.44	34.03 ± 1.31	29.82 ± 1.84	32.46 ± 2.62
5	35.57 ± 1.71	38.91 ± 1.32	34.20 ± 0.08	38.13 ± 1.57
6	40.00 ± 0.81	43.42 ± 2.04	38.19 ± 1.96	39.80 ± 1.45
7	44.20 ± 0.91	47.56 ±1.17	41.37 ± 1.69	44.31 ± 0.29
8	48.57 ± 0.90	51.65 ± 1.51	44.37 ± 1.44	46.16 ± 0.67

Table 5: Cumulative percent drug released from Eudragit RLPO loaded nanoparticles

Time (h)	Cumulative percentage drug released (Mean ± SD, n=3)			
	F5	F6	F7	F8
0.5	11.50 ± .60	17.92 ± 1.46	11.91 ± 1.17	13.26 ± 1.92
1	23.59 ± 2.48	26.32 ± 1.41	22.52 ± 1.02	23.96 ± 1.35
2	33.64 ± 1.65	36.84 ± 1.53	30.01 ± 0.92	37.26 ± 1.89
3	39.74 ± 2.01	44.11 ± 1.74	39.18 ± 1.78	42.35 ± 1.86
4	44.84 ± 0.87	48.00 ± 2.02	42.88 ± 1.88	45.05 ± 1.02
5	46.34 ± 2.07	50.54 ± 1.95	45.62 ± 1.35	49.43 ± 2.34
6	48.98 ± 1.57	52.81 ± 2.46	46.28 ± 0.94	51.04 ± 1.72
7	50.06 ± 1.64	54.10 ± 1.43	48.51 ± 2.50	51.93 ± 2.21
8	52.68 ± 2.04	54.91 ± 0.93	49.41 ± 2.13	52.60 ± 1.81

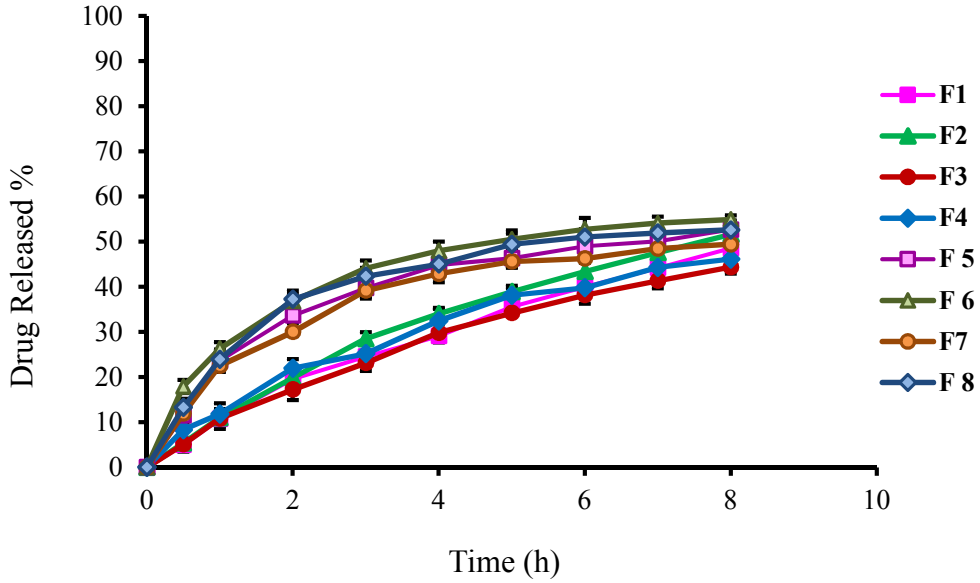


Fig. 3: Release profile of RSV from PLGA and Eudragit nanoparticles in phosphate buffer (pH=6.8)

The *in-vitro* release data were fitted to kinetic models such as zero order, first order, Higuchi and Korsmeyer-Peppas equations. The correlation coefficients (R^2) and the exponent of the Korsmeyer-Peppas model are shown in table 6. It can be seen that the release kinetics of formulations F1-F4 were best fitted with Higuchi equation ($R^2 \rightarrow 0.992$ - 0999) and had, n values well above 0.5, indicating a non-Fickian transport of the drug. On the other hand the Eudragit formulations F5 - F8 exhibited poorer fit for both Higuchi and Korsmeyer-Peppas models due to the more pronounced burst effect ($R^2 \rightarrow 0.916$ - 0.962). The diffusional exponent n was equal or lower than 0.5 indicating that the drug release was close to pure Fickian diffusion.

Table 6: Correlation coefficient values for different kinetic models

Formulation ID	Zero order model (R^2)	1st order model (R^2)	Higuchi model (R^2)	Korsmeyer-Peppas model	
				(R^2)	N
F1	0.982	0.820	0.997	0.989	0.79
F2	0.970	0.812	0.999	0.990	0.80
F3	0.973	0.825	0.998	0.991	0.76
F4	0.961	0.850	0.992	0.992	0.64
F5	0.838	0.686	0.938	0.933	0.51
F6	0.847	0.750	0.945	0.962	0.44
F7	0.837	0.708	0.937	0.947	0.49
F 8	0.806	0.677	0.916	0.933	0.48

Evaluation of results of the experimental design:

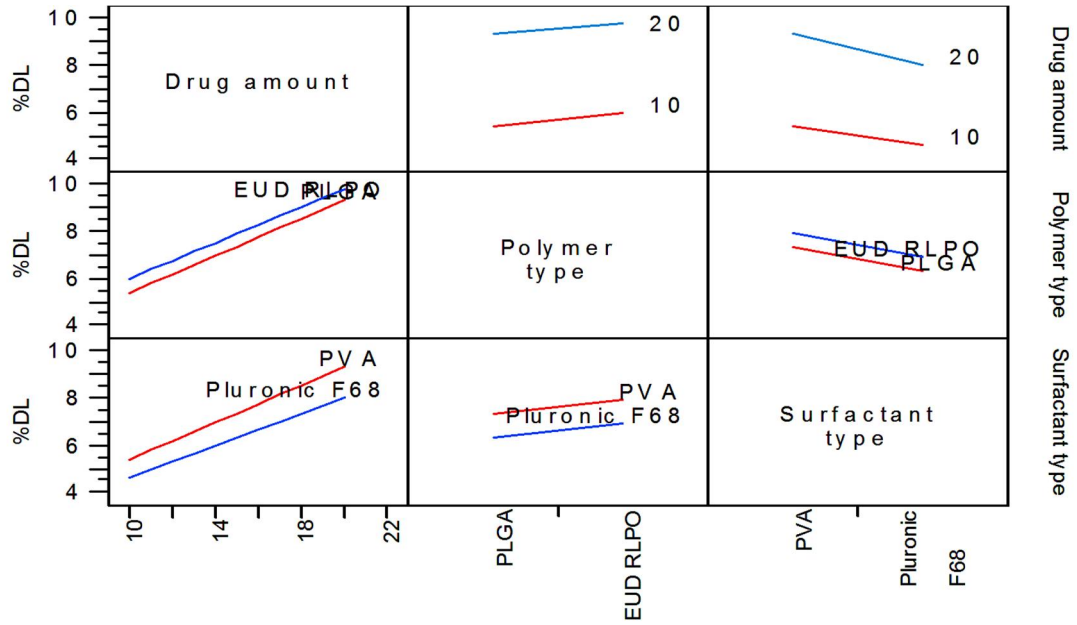
Three dependent variables (responses) were used for the evaluation of the effects of the independent variables (factors). They are DL %, $R_{(2h)}$ % and $R_{(8h)}$ %, representing the percentage drug loading and the percentage drug released at 2 and 8 h, respectively. The results of the statistical evaluation of the factorial design fitting the data to a generalized linear model are given in table 7, showing the values of the estimated effects of both the single factors as well as their interactions for each response. Also, shown in table 7 are the P-values of all the effects, a P-value smaller than 0.05 indicating a statistically significant result. It is evident that the effects of the drug amount [X_1] and the surfactant type [X_3] on the DL % [Y_1] are statistically significant ($P < 0.05$), whereby the drug amount has the strongest effect which is positive for 20 mg. However, the effect of polymer type [X_2] is statistically insignificant ($P > 0.05$). The effect of variables interactions are statistically significant for (Drug amount*Surfactant type) as shown in Fig. 4A.

Table 7: Parameter Estimates for independent variables

Response	Term	Estimate	P-value Prob >F
DL % [Y_1]	X_1: Drug amount (20)	1.72	0.0088*
	X_2: Polymer type (PLGA)	-0.24	0.0618
	X_3: Surfactant type (PVA)	0.54	0.0279*
	Drug amount *Surfactant type (PVA)	0.13	0.0367*
$R_{(2h)}$ % [Y_2]	X_1: Drug amount (20)	1.93	0.0165*
	X_2: Polymer type (PLGA)	-7.4	0.0043*
	X_3: Surfactant type (PVA)	0.42	0.0754
	Drug amount *Polymer type (PLGA)	-0.68	0.0466*
	Drug amount *Surfactant type (PVA)	-1.06	0.0299*
$R_{(8h)}$ % [Y_3]	X_1: Drug amount (20)	1.29	0.1370
	X_2: Polymer type (PLGA)	-2.36	0.0756
	X_3: Surfactant type (PVA)	1.91	0.0931

**P-Values less than 0.05 indicate model terms are significant.*

[A]



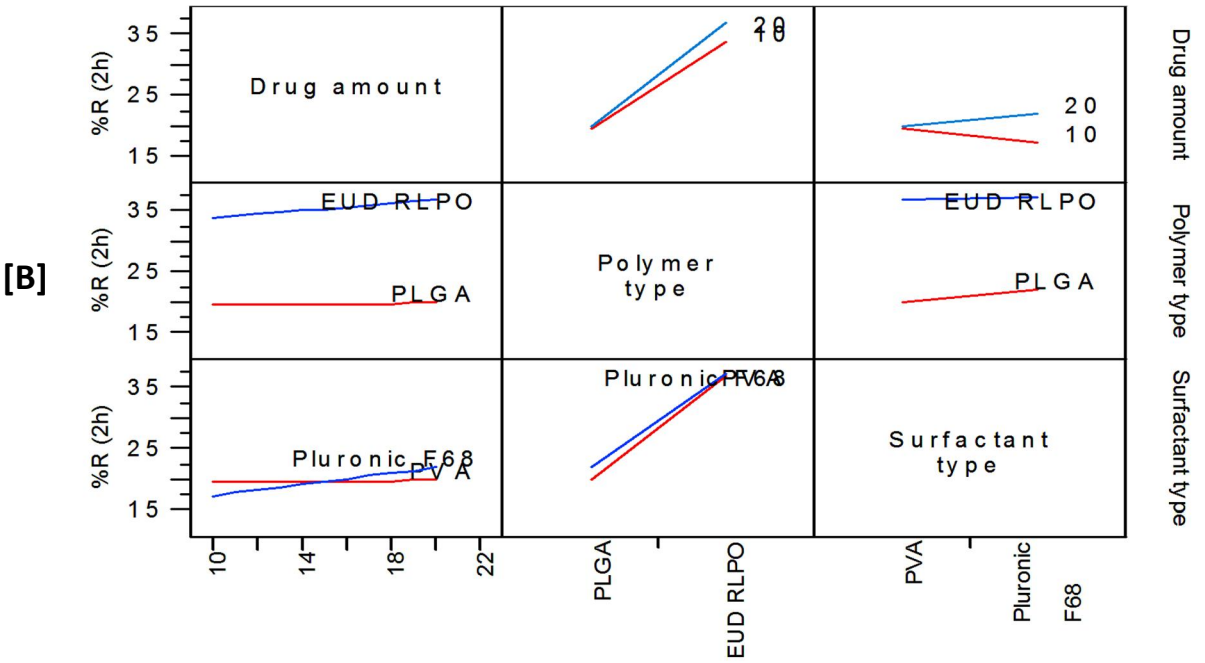


Fig. 4: Interaction Profiles for [A] DL % and [B] $R_{(2h)}$ %

For the drug released % at 2 h ($R_{(2h)}$ %) [Y_2], the effects of all factors are statistically significant except the effect of surfactant type [X_3] which is insignificant ($p>0.05$). The polymer type has the strongest effect which is negative for PLGA. The effect of variables interactions are statistically significant for (Drug amount*Polymer type) and (Drug amount*Surfactant type) as shown in Fig. 4B.

The estimates of the effects of all the factors on the percent drug released after 8 h ($R_{(8h)}$ %) [Y_3] are statistically insignificant, which is also supported by the release profiles (see Fig. 3).

The aim of the study is to use the estimates of the experimental design to obtain an optimal formulation for the nanoparticles with high % drug loading and low initial burst effect in order to minimize the hepatic metabolism of RSV. The prediction profiler shown in Fig. 5 was used to predict the optimum formula which is F2 composed of 20mg drug, PLGA as polymer and PVA as surfactant.

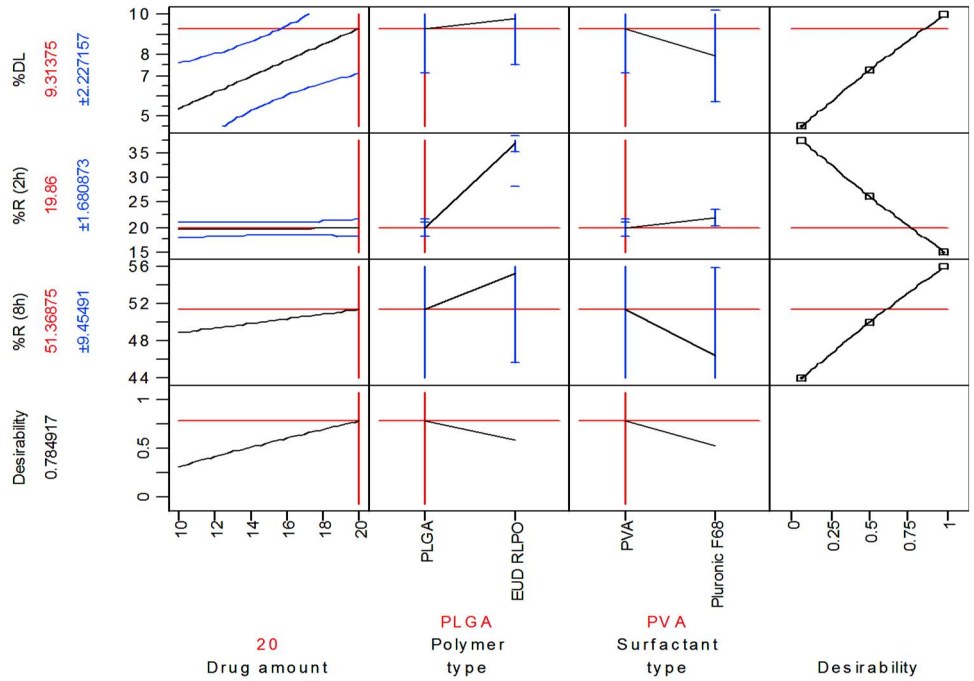


Fig. 5: Prediction Profiler for the prepared NPs

Transmission Electron Microscopy (TEM) and particle size (PS):

The morphology of the optimized formulation F2 is illustrated in the TEM images in Fig. 6. NPs appeared almost spherical in shape, well dispersed and with mean particle size of 243.3 ± 15.28 nm.

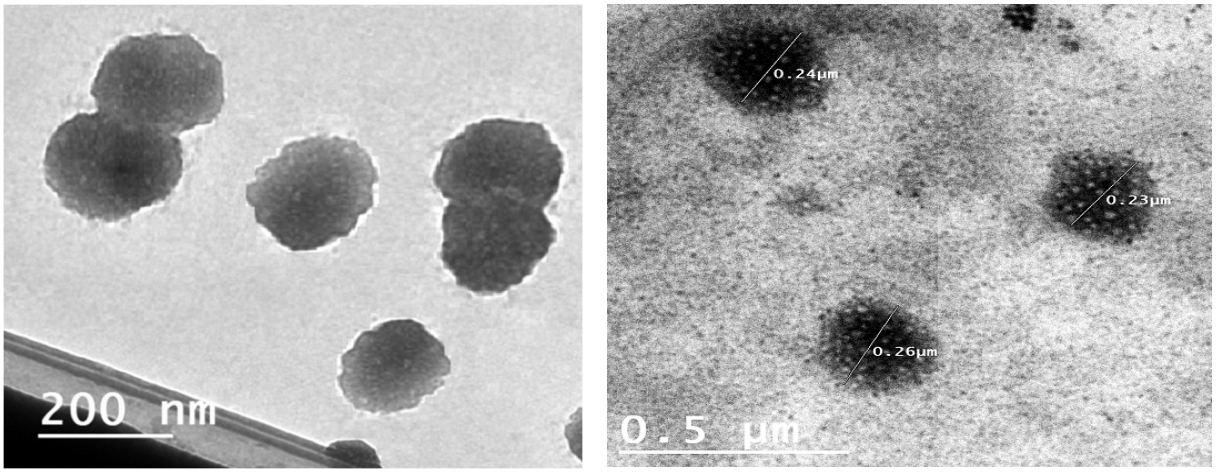


Fig. 6: Transmission electron micrographs of F2

Conclusion

PNPs were prepared by the emulsion solvent evaporation method. By investigating the effect of different variables on the DL %, it was found that the drug amount has the highest effect. Additionally, the type of polymer strongly influenced the release pattern especially after 2 h whereas no significant difference was observed for the drug released % after 8 h. The optimum formula predicted by the experimental design is composed of 20mg drug, PLGA as polymer and PVA as surfactant. TEM micrographs of the optimized formula show that the NPs are spherical and well dispersed.

Conflicts of interest

There are no conflicts of interest

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