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Designing of nanosized bioflavonoids using biodegradable polymeric nanoparticles by Plackett burman method

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ABSTRACT
In this present study, dual loaded flavono nanoparticulate systems have been developed for oral delivery of Naringin and Hesperidin to enhance its antioxidant and antidiabetic activities. The fabrication of Dual Loaded Flavono Nanoparticles by suitable method was optimized by Plackett Burman method. Optimization of the formulation requires proper designing of the experiments. For this reason, only in our current study, the placket burman method has been projected for the formulation of nanoparticles biodegradable polymers encompass bioflavonoid isolates for the antidiabetic activity. Ten critical parameters influencing the formulation has been selected and designed in Plackett Burman method of experimentation for 12 runs to assess independent variables influencing the result outcome. The results revealed that the 9th run shows the optimum particle size of 126.1 nm with zeta potential of 29.9 mV. Remarkably significant nanoparticles were obtained by exploiting the Plackett Burman method as designing tool.

Key Words: Naringin, Hesperidin, nanocarriers, optimization, design of experiment, Nanoprecipitation method.

INTRODUCTION

Fundamentally, utilization of factorial design for the optimization of a process allows testing of many factors concomitantly and prevents the use of an unwanted number of runs or trials, thus it prevents material wastage and time consumption. (Nahar *et al.* 2006; Fan *et al.*, 2012). Statistical design of experiment, is a perfect methodology to conduct and execute plan of experiments to extract the maximum amount of information with limited number of inputs. The most critical factors selected for the formulation along with the proper selection of design of experiment input a tool proves to be superior. DOE identifies optimal formulation conditions for these NPs provide understanding of the underlying relationship. Most commonly applied screening designs is the Plackett-Burman design (PBD) that evaluates larges number of factors and identify critical one in a minimal number of trials (Radhika *et al.*, 2010). The utilization of Plackett-Burman experimental design paves the way for the study of numerous factors in a systematic and logical way to select optimized runs. The important significance of Plackett-Burman design method is quicker screening obtained with minimum possible experimental runs (Moorthi *et al.*, 2012)

By and large, number of run needed to probe the main effects are equal to 2n or multiple of 4 in Plackett-Burman designs instead of 2 as in the relation of full -factorial designing (Moorthi *et al.*, 2013). PBD screening design with 12 experimental run was designed utilizing Expert Design® (Ver 9; Stat-Ease, Inc, United States of America).

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MATERIALS AND METHODS

Naringin and Hesperidin was purchased from Sigma Aldrich, Design of experiments used was obtained from Expert Design® (Ver 9; Statease, Inc, United States of America). By and large, number of run needed to scrutinize the main effects are equal to 2n or multiple of 4 in Plackett-Burman designs instead of 2 as in the relation of full -factorial designing (Moorthi *et al.*, 2013). PBD screening design with 12 experimental run was designed utilizing Expert Design® (Ver 9; Stat-Ease, Inc, USA).
The linear equation of this model is given as:
$Y=b0+b1X1+b2X2+b3X3+b4X4+b5X5+\cdots+bnX$n

Where, Y is the responses, b0 is stable and b1....bn are coefficient of factor X1, X2...Xn (representing the consequence of every factor are within − 1, + 1). 24

Nevertheless, the process parameters which includes polymer concentration (A), surfactant concentration (B), aqueous phase concentration (C) and organic phase concentration (D), duration of stirring (E), stirring speed (F), addition of organic or aqueous phase (G) and addition mode (H). Consequently, PLB design was exploited to optimize the procedure parameter at lesser (-) and upper (+) level. The nanoparticle parameters such as Average particle size (R1), particle surface area (R2) and Surface area (R3) are considered as reliant variable. Twelve investigational runs exploiting 8 self-regulating progression variables at superior and inferior niche were generated exploiting Expert Designing® Version 9.

Fabrication of bioflavonoid nanoparticles

Polymer (Eudragit E 100) was dispersed in organic solvent, which was mixed into the aqueous phase encompass surfactant exploiting mechanical agitator. NPs were created instinctively and converting the aqueous to faintly milky with blue shade. However, stirring was sustained to support reduction in particle and also to evaporate surplus solvent existing in the NPs. Though, the constraints like polydispersity index, particle size and zeta potential of the nanoparticles based on the procedure parameters such as

polymeric concentration, percentage of solvent, phase volumes, aqueous medium, surfactant concentration and magnetic agitating tenure. Therefore, PLB designing was exploited to standardize the progression parameters at lesser and superior niche. However, there are several methods available for the formation of NPs *viz.* desolvation method, dialysis method, ionic gelation method, nanoprecipitation method, direct solvent evaporation, salting out, supercritical fluid technology and spray drying (Ana *et al.*, 2011; Xie *et al.*, 2010) However, nanoprecipitation method is the most convenient & cost effective way to generate polymeric NPs.

Particle sizing, polydispersity index (PDI) and zeta potential (mV) analysis

The pure (P), Naringin (Nr), Hesperidin (Ns) and Naringin-Hesperidin (Nr-Hs) polymeric nanoparticles were maintained at room temperature for 30 days, which were characterized for nanoparticles assessment (Yordonov *et al.*, 2012). About 1 ml of prepared plain and flavonoid loaded polymeric nanoparticles were diluted appropriately using distilled water, which was then taken individually in a zeta cell and measured nanoparticle assessment using Zetasizer (ZEN 3600, Malvern Instruments, Malvern, Worcestershire, UK). The experiments were executed in triplicate manner.

Surface morphology analysis

Prepared plain and flavonoid loaded polymeric nanoparticles were characterized for surface morphology using field emission scanning electron microscope (FESEM).

Drug content, encapsulation efficiency & drug loading estimation

Drug content of prepared flavonoid loaded polymeric nanoparticles was evaluated by performing assay using the established HPLC methods for Naringin and Hesperidin (Tzu *et al.*, 2008; Hector *et al.*, 2012). Encapsulation effectiveness and loading of drug were assessed by measuring the free Naringin and Hesperidin in the nanoformulations.

Prepared plain and flavonoid loaded NPs were centrifuged exploiting refrigerated centrifuges for forty-five minutes for 19,000 RPM, -20°C and above portion was removed and stored individually for further analysis (Ana *et al.*, 2012; Catarina *et al.*, 2006).

About one ml of above was assorted with one ml of methanol, which then vortexed for 5 minutes and filtered through 0.22 µm membrane. Estimated amount of unbound drug were denoted as W_{free}. The experiments were carried in triplicates. Efficiency of encapsulation (EE) and drug loading (DL) were estimated as follows

$$EE\ (\%) = \frac{Drug\ Content\ (W_{total}) - Drug\ in\ the\ supernatant\ (W_{free})}{Drug\ Content\ (W_{total})} \times 100$$

$$DL\ (\%) = \frac{Drug\ Content\ (W_{total}) - Drug\ in\ the\ supernatant\ (W_{free})}{Weight\ of\ the\ polymer\ used\ in\ the\ formulation\ (W_{polymer})} \times 100$$

Higher the particles in Brownian motion higher will be the stability of the nanoformulation. Since the likely charged particles, each other getting repelled against the weak vanders exists between particles due to dipole dipole interaction, because of gravitational force and hence, it prevents the agglomeration of particles altogether (Sergio *et al.*, 2004). The polymer concentration, duration of processing, concentration of surfactant, organic phase volume has inverse relationship with that of zeta potential.

RESULTS AND DISCUSSION

Physiochemical evaluation of the nanoparticles

Plain, Naringin, Hesperidin, Naringin-Hesperidin Nanoparticles encompassed polymeric nanoparticulates were estimated for Particle surface, Polydispersity index and zeta potential (mV). NPs prepared using stirring method were with an average particle size <200 nm, Polydispersity Index (*i.e.*, U <0.7) and Zeta potential >20mV.

Surface morphology of the prepared nanoparticles

Prepared Plain, Naringin, Hesperidin, and Naringin-Hesperidin loaded biopolymer nanoparticles were globular in character. Hence, all the nanoformulation encapsulated in the polymer milieu in circular shape for the basic function of nanoformulation, release of Naringin and bio-enhancers from the polymer matrix, transport of Nr and bio-enhancers in the body and internalization of Naringin and bio-enhancers by many folds than the free Naringin and bio-enhancers.

Drug content, encapsulation efficiency & drug loading estimation

The amount of Nr and Hs encapsulated in polymeric nanoparticles determines the effectiveness of prepared nanoformulations. Hence, drug content, encapsulation efficiency and drug loading estimated.

Drug content, EE & DL estimation

Prepared, Plain, Naringin, Hesperidin, Naringin-Hesperidin nanoparticles were carefully kept at room temp for a month time to estimate any agglomeration and after-formulation deprivation. Prepared, Plain, Naringin, Hesperidin, Naringin-Hesperidin nanoparticles were characterized for particle size, polydispersity index and zeta potential.

After fabrication, prepared Plain, Naringin, Hesperidin, Naringin-Hesperidin nanoparticles matters were kept in room at a specified temperature for one month. However, there was no aggregation. Plain polymeric nanoparticles have shown an average particle size of 129. 2 nm with PDI of 0.1841 and zeta potential of 27.3 mV. Naringin polymeric nanoparticles has shown an average particle size of 83.12 nm with PDI of 0.270 and zeta potential of 9.93 mV. Hesperidin polymeric nanoparticles have shown an average particle size of 102.3 nm with PDI of 0.361 and zeta potential of 13.80 mV. Naringin-Hesperidin polymeric nanoparticles have shown an average particle size of 91.20 nm with Polydispersity index of 0.306 and zeta potential of 15.3 mV. Off all the prepared nanoparticles the order of particle size is FE1<FE3<FE2<PL. Off all the prepared nanoparticles the order of Polydispersity index is PL<FE1<FE3<FE2. Of all the prepared nanoparticles, the order of Zeta potential is PL>FE3>FE2>FE1.

Table 1: Optimization process parameters at lower and higher levels.

Code	Variables	Levels	
		Lower (-)	Higher (+)
A	Naringin	50 mg	55 mg
B	Hesperidin	50 mg	55 mg
C	Polymer concentration	100 mg	250 mg
D	Surfactant concentration	15 mg	20 mg
E	Aqueous Phase	10 ml	20 ml
F	Organic phase	10 ml	15 ml
G	Duration of stirring	30 min	60 min
H	Speed of stirring	500 rpm	1000 rpm
I	Addition type	Org to Aqueous	Aqueous to org
J	Addition mode	All at once	By syringe

Table 2: Scheme of fabrication of flavonoid loaded nanoparticles by Plackett-Burman method.

Trials	Naringin (mg)	Hesperidin (mg)	Polymer (mg)	Surfactant (mg)	Aqueous Phase (ml)	Organic Phase (ml)	Stirring rate (min)	Stirring Speed (rpm)	Addition mode	Addition Method
1	50	55	250	50	20	15	60	500	O to A	All at once
2	50	50	250	50	20	15	30	1000	A to O	By syringe
3	55	55	100	100	20	15	30	500	O to A	By syringe
4	55	50	100	50	20	10	60	1000	O to A	By syringe
5	50	50	100	100	15	15	60	500	A to O	By syringe
6	50	55	100	100	20	10	60	1000	A to O	All at once
7	50	55	250	100	15	10	30	1000	O to A	By syringe
8	55	55	250	50	15	10	60	500	A to O	By syringe
9	55	50	250	100	15	15	60	1000	O to A	All at once
10	55	55	100	50	15	15	30	1000	A to O	All at once
11	55	50	250	100	20	10	30	500	A to O	All at once
12	50	50	100	50	15	10	30	500	O to A	All at once

O -→ A = Organic phase to aqueous phase
A-→ O = Aqueous phase to organic phase

Table 3: Characterization of prepared nanoparticles.

Runs	Average Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
1	735.3	0.599	0.200
2	174.9	0.091	21.9
3	290.0	0.270	13.9
4	883.0	0.741	1.30
5	1200.0	0.871	2.71
6	181.9	0.136	18.9
7	950.8	0.852	2.19
8	183.1	0.320	10.9
9	126.1	0.180	29.9
10	669.8	0.652	0.359
11	376.5	0.359	-0.056
12	207.9	0.300	9.13

Table 4: ANOVA table for Average particle size (Z)

Parameters	Numerical value
P value	< 0.0001
P value summary	***
P < 0.05	Yes
Number of groups	12
F Value	13580
R Squared	0.9998

ANOVA Table

	Sum of Square	Difference	Mean of Square
Treatment (between columns)	4616000	11	419600
Residual (within columns)	741.4	24	30.89
Total	4617000	35	-

(P < 0.05) are considered significant

Table 5: ANOVA table for Polydispersity index (PI)

Parameters	Numerical value
P value	< 0.0001
P value summary	***
P < 0.05	Yes
Number of groups	12
F Value	35850
R Squared	0.9999

ANOVA Table

	Sum of Square	Difference	Mean of Square
Treatment (between columns)	2.596	11	0.2360
Residual (within columns)	0.000158	24	0.000006583
Total	2.596	35	-

(P < 0.05) are considered significant

Table 6: ANOVA table for Zeta potential (mV)

Parameters	Numerical value
P value	< 0.0001
P value summary	***
P < 0.05	Yes
Number of groups	12
F Value	10690
R Squared	0.9998

ANOVA Table

	Sum of Square	Difference	Mean of Square
Treatment (between columns)	3306	11	300.5
Residual (within columns)	0.6746	24	0.02811
Total	3307	35	-

(P < 0.05) are considered significant

Table 7: Observed & Predicted value of size, PDI and zeta potential

Run	Average Particle Size		Polydispersity Index		Zeta Potential	
	O	P	O	P	O	P
1	735.3	732.2	0.599	0.599	0.200	0.198
2	174.9	176.5	0.091	0.091	21.9	22.3
3	290.0	289.3	0.270	0.270	13.9	13.9
4	883.0	880.4	0.741	0.741	1.30	1.52
5	1200.0	1215	0.871	0.871	2.71	2.23
6	181.9	182.0	0.136	0.136	18.9	17.6
7	950.8	952.3	0.852	0.852	2.19	2.34
8	183.1	180.6	0.320	0.320	10.9	9.56
9	126.1	123.5	0.180	0.180	29.9	30.7
10	669.8	665.7	0.652	0.652	0.359	0.349
11	376.5	374.2	0.359	0.359	-0.056	-0.072
12	207.9	204.6	0.300	0.300	9.13	8.53

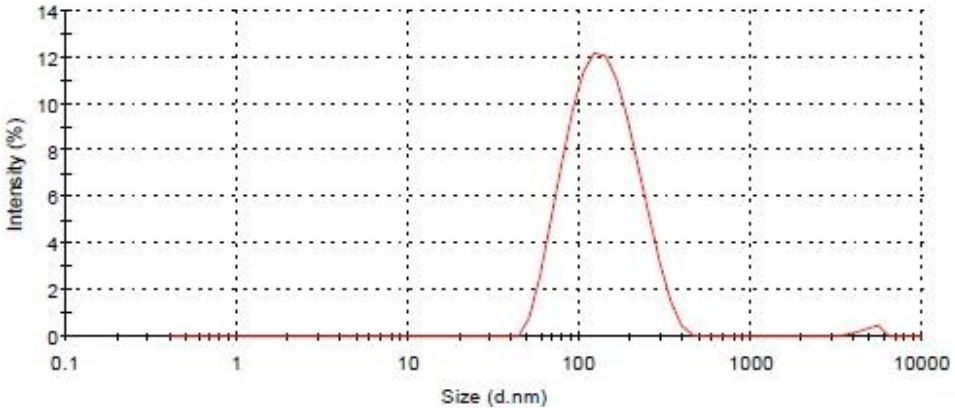
O- Observed value, P- Predicted value.

Table 8: Average particles size, polydispersity index and zeta potential of prepared plain and flavono loaded polymeric nanoparticles

Formulation Code	Average particle size (nm)	Polydispersity Index	Zeta Potential (mV)
PL	129.20±0.176	0.185±0.0003	28.0±0.0577
FE 1	83.12±0.0796	0.270±0.001	9.93±0.554
FE2	102.30±1.506	0.361±0.0026	13.80±0.723
FE3	91.20±1.537	0.306±0.002	15.03±0.441

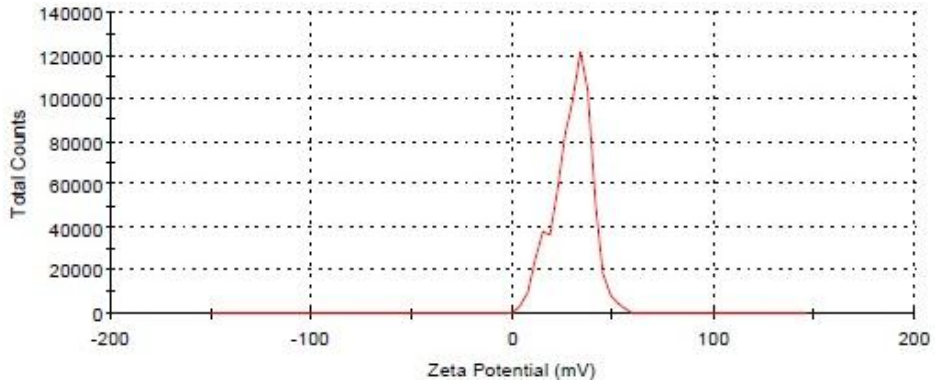
Table 9: Optimized formulation for flavonoid loaded nanoparticles.

Sr. No	Particulars	Parameters
1	Trials No	9
2	Nr	50 mg
3	Hs	50 mg
4	Polymer Concentration	250 mg
5	Surfactant Concentration	100 mg
6	Aqueous Phase Concentration	15 ml
7	Organic Phase Concentration	15 ml
8	Duration of Stirring	60 min
9	Speed of Stirring	1000 rpm
10	Addition of Organic or Aqueous Phase	0rganic to aqueous phase
11	Addition Mode	All at once



	Z-Average (126.1)	PdI (0.180)	Intercept (0.962)
	Peak-1	Peak-2	Peak-3
Size (nm)	145.9	4983	0.000
Percentage Intensity (%)	98.99	0.99	0.00
Width (nm)	64.92	617.5	0.000

Figure 1: Particle size distribution of Run-9



	Zeta Potential (mV) (29.9)	Zeta Deviation (9.5)	Conductivity(0.0302)
	Peak-1	Peak-2	Peak-3
Mean (mV)	31.9	14.9	0.00
Percentage Area (%)	84.1	15.9	0.0
Width (mV)	7.36	3.99	0.00

Figure 2: Zetapotential (mV) distribution of Run-9

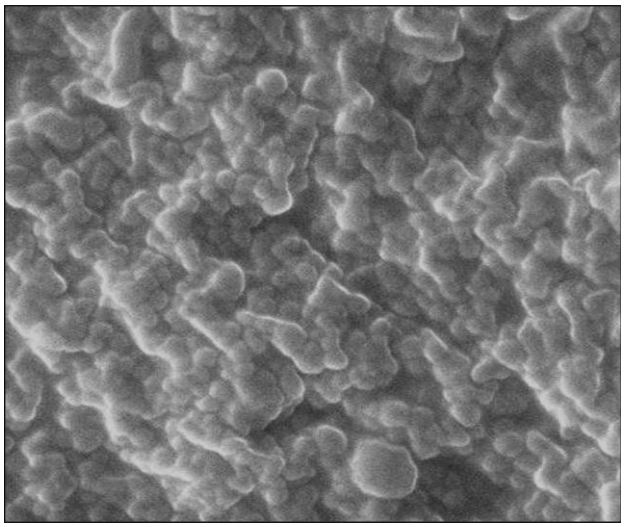


Figure 3: FESEM of Prepared Nanoparticles (Trial-9)

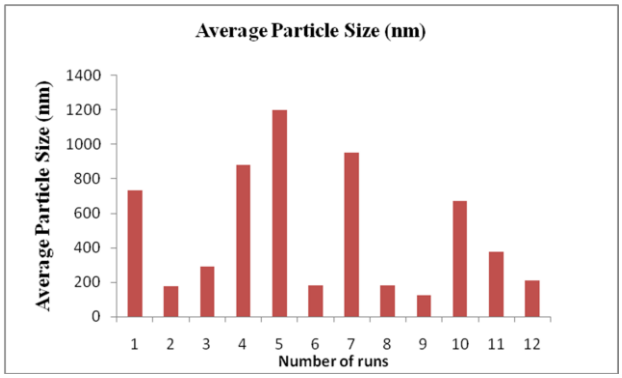


Figure 4: Pattern of Average particle size of 12 runs

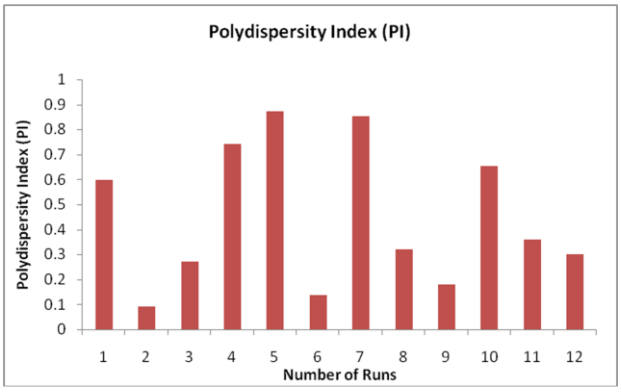


Figure 5: Pattern of Polydispersity Index of 12 runs

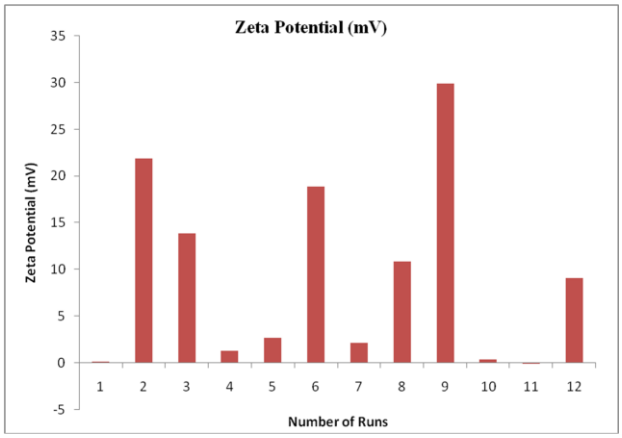


Figure 6: Pattern of Zeta Potential of 12 runs

CONCLUSION

Both single loaded and dual loaded nanoparticle were successfully prepared by the nanoprecipitation method. The design of experiment tool Plackett burman method has significantly given the 12 runs for assessing the critical parameters affecting the experimental outcome. The critical parameters influencing the resulting nanoparticles formulation were identified by the designing tool. Based on the optimized trial out of 12 runs in placket burman design, the final formulation of Naringin and hesperidin single loaded, and Naringin-Hesperidin dual loaded nanoparticles were prepared. From the present investigation, it is concluded that the bioflavonoid nanoformulation fabrication using the placket burman as the identification of critical parameters, was more successful. It may significantly enhance and improve the antidiabetic and antioxidant activity for the treatment of diabetes mellitus.

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REFERENCES

Ana, C.P., Michelle, F., Karen, C.D., Igor, P., Maria, F.D., (2011). Femandes, D.S., Preparation and characterization of polymeric nanoparticles loaded with luteolin, by using factorial design. International journal of drug delivery, 3(6), 683-698.

Ana, G.L., Jaime, L., Francisco, M.G., Miguel, A.V., Ana, L.A., Simon, B.H., (2012). Characterization and antiproliferative activity of Nobiletin-loaded chitosan Nanoparticles. Journal of Nanomaterials, 1(2), 1-7.

Catarina, P.R., Ronald, J.N., Antonio, J.R., Francisco, V., (2006). Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanotechnology biology and medicine, 2(1), 8-21.

Fan, W., Yan, W., Xu, Z., Ni, H., (2012). Formation of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique. Colloid Surface B, 90(1), 21-27. [DOI]

Hector, P., David, Q., Juang, D.F., Camila, M.M., Etelvino, H.B., Luis, A.G., (2012). Antioxidant effect of Quercetin and catechin encapsulated into PLGA nanoparticles. Journal of nanomaterials, 1(2), 1-12.

Moorthi, C., Kathiresan, K., (2013). Application of Plackett-Burman factorial design in the development of curcumin loaded Eudragit E 100 nanoparticles. Nano Biomed Eng, 5(1), 28-33. [DOI]

Moorthi, C., Kiran, K., Manavalan, R., Kathiresan, K., (2012). Preparation and characterization of curcumin-piperine dual drug loaded nanoparticles. Asian pacific journal of tropical medicine, 2(3), 841-848.

Nahar, M., Dutta, T., Murugesan, S., Asthana, A., Mishra, D., Rajkumar, V., Tare, M., Saraf, S., Jain, N.K., (2006). Functional polymeric nanoparticles: an efficient and promising tool for active delivery of bioactives. Crit Rev Ther Drug Carrier Syst, 23(4), 259-318. [DOI]

Radhika, R., Piramanayagam, S., (2010). Preparation and characterization of biopolymeric nanoparticles used in drug delivery. Indian Journal of Biochemistry and Biophysics, 47(1), 56-59.

Sergio, G.R., Eric, A., Hatem, F., Eric, D., (2004). Physiochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion and nanoprecipitation methods. Pharmaceutical research, 21(3), 1428-1439.

Tzu, H.W., Feng, L.Y., Liang, T.L., Tong, R.T., Chun, C.L., Thau, M.C., (2008). Preparation, physiochemical characterization and antioxidant effects of quercetin nanoparticles. International journal of pharmaceutics, 346(3), 160-168.

Xie, H., Smith, J.W., (2010). Fabrication of PLGA nanoparticles with a fluidic nanoprecipitation system, J. Nanobiotechnology, 8(18), 1-7. [DOI]

Yordanov, G.G., Dushkin, C.D., (2012). Preparation of poly (butylcyanoacrylate) drug carriers by nanoprecipitation using a pre-synthesized polymer and different stabilizers. Colloid Polym Sci, 288(9), 1019-1026. [DOI]