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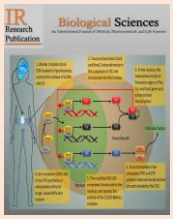

Solubility Enhancement of a Model Drug Quercetin by Nanotechnology Based Formulation Approach

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ABSTRACT

Approximately, 40% of drug candidates have poor water solubility and its oral delivery is frequently associated with implications of low bioavailability, hepatic first-pass metabolism, enzymatic degradation, high intra- and inter subject variability, and lack of dose proportionality. Interests in solid lipid nanoparticles (SLN) to improve the oral bioavailability of such drugs (PWSD) are well known and documented in the literature. The aim of the research is to prepare solid lipid nanoparticles of quercetin by emulsification-ultrasonication method and evaluate the optimized formulation for solubility enhancement. Different batches of SLNs were prepared by emulsification-ultrasonication methods by changing process parameters, and finally on the basis of nanoparticle size, polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency (EE), an optimum system was designed. The optimized formulation (Q-SLN9) demonstrated particle size, PDI, ZP, and EE; 327 nm, 0.118, 32.3 mV, and 57.9%, respectively. The optimized formulation has shown the highest zeta potential (-32.3 mV) confers its stability. In-vitro release of the drug showed significant improvement in the release of quercetin from SLN formulation as compared to plain drug. Furthermore, quercetin-loaded SLN was found to be stable at 4°C for 30 days of study period. The formulation was successfully prepared by the proposed method that can be used as a potential carrier for successful delivery of poorly water-soluble drugs associated with poor oral bioavailability.

Introduction

Oral route is one of the ideal route of administration due to numerous benefits including ease of administration and patient compliance. The pharmacological effects of drug administered orally depend on the involved mechanism from site of administration to the site of action (1). On the other hand, oral delivery of poorly water-soluble drugs present a big challenge due to low aqueous solubility. For such compounds, the absorption from the gastrointestinal (GI) lumen is dissolution rate limited (2), resulted in low and erratic bioavailability and hence making a big challenge for formulation development. Due to this biopharmaceutical drawbacks, many drugs fail to reach the market even though they exhibit potential

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pharmacodynamics activity. To achieve the desired plasma concentration to reach the therapeutic level, higher doses are required to be administered than actually needed. Hence, an appropriate formulation approaches are required to develop a suitable delivery system to enhance its solubility and bioavailability. Numerous approaches have been applied to enhance the solubility of such compounds by incorporating them into inert lipid-based formulations like emulsions, microemulsions, nanoemulsions, self-emulsifying/ microemulsifying and liposomal formulations (3-9).

Quercetin is an anti-cancer compound also demonstrated to possess a wide array of biological effects that are considered beneficial to health, including anti-oxidative, free radical scavenging and antiviral activities (10) abundantly found in citrus fruits and vegetables. However, its administration has been heavily hampered by its extreme water insolubility. Many serious problems are associated with the therapeutic use of this drug. This includes poor absorption and bioavailability upon oral administration, embolization of blood vessels from intravenous injection of the water-insoluble drug because of drug precipitation, and local tissue toxicity and low systemic drug bioavailability (11). In addition, it is chemically unstable, especially in aqueous alkaline medium (12) although acidic conditions can offer it some protection against degradation. The compound is also known to undergo extensive metabolism in the gut and the liver following absorption (13) and the resulting metabolites still retain some biological activity (14). All these problems lead to an extremely low oral bioavailability of quercetin (based on the unchanged quercetin) in human (15).

Nowadays, solid lipid nanoparticle (SLN) gaining a great attention for its capability to dissolve a large number of poorly water-soluble drugs and thus improved bioavailability (16). This nanocarrier is composed of high melting point biodegradable and biocompatible lipids stabilized by surfactants and exist in nanometer size (17,18). The main characteristic of SLN is that at physiological body temperature it remains in solid state (19). The advantage of SLN is the avoidance of organic solvent in formulation, and the lipids used in the manufacturing of SLN are significantly cheaper than synthetic polymers, also release of the drug can be controlled (20, 21). It can augment the advantages of liposomes and polymeric nanoparticles and can overcome the challenges associated with the oral delivery of bioactives that have low solubility, poor permeability, and P-glycoprotein-mediated efflux issues (22). However, SLN requires appropriate formulation technology to take these advantages since partitioning of the bioactives in the SLN may take place during the manufacturing process and/or storage period (23, 24). Therefore, the research aims to enhance the solubility of a poorly water-soluble drug (quercetin) using the emulsification-ultrasonication method, determine particle size, polydispersity index, Zeta potential, encapsulation efficiency, drug release, and stability over 30 days.

Material and methods

Materials

Quercetin (MW: 320.23 g/mol) was obtained as a gift sample from Loba chemicals, Bangalore, India. Stearic acid (SA) and glyceryl monostearate (GMS) were generously supplied by SRL India. Tween 80 was purchased from Merck India Ltd. Dialysis membrane (molecular weight cutoff: 12-14 kDa) was purchased from HiMedia, Mumbai, India. The other chemicals were of analytical reagent grade.

Solubility study

The solubility of drugs in lipid is one of the most important factors that determine the drug loading capacity of SLNs. Hence, solubility of quercetin in different lipids was tested for maximum solubilization capacity. Due to the solid nature of lipids, an equilibrium solubility study is not possible and hence, an alternative method was used to identify the solid lipids having the best solubilization potential for drugs as reported in the literature (25, 26). In brief, a definite amount of the drug was accurately weighed and placed in a glass bottle screw-capped vial. All the lipids were heated separately above their melting point. 100 mg of lipid was added to the bottle and subjected to continuous stirring using a vortex mixer with temperature control (above the melting point of lipid). Further, lipid was added to the bottle in different proportions. Loss of transparency upon addition of lipid gives solubility of the drug in different solid lipids.

Preparation of solid lipid nanoparticle

Quercetin-loaded SLNs were prepared by emulsification–ultrasonication method as reported in the literature (27). Briefly, solid lipid having maximum solubility selected above was melted by heating at 80°C. The aqueous phase was prepared by dissolving the surfactant in double distilled water, and the lipid melt was dispersed in aqueous surfactant solutions at the same temperature using a

Table 1. Different batches of quercetin loaded SLNs for optimization.

Formulations	Lipid (mg)	Drug (mg)	Lipid: drug	Surfactant (mg)	Co-surfactant (mg)	Surfactant: co-surfactant
Q-SLN1	500	250	2:1	500	500	1:1
Q-SLN2	500	250	2:1	750	500	1.5:1
Q-SLN3	500	250	2:1	1000	500	2:1
Q-SLN4	1000	250	4:1	500	500	1:1
Q-SLN5	1000	250	4:1	750	500	1.5:1
Q-SLN6	1000	250	4:1	1000	500	2:1
Q-SLN7	1500	250	6:1	500	500	1:1
Q-SLN8	1500	250	6:1	750	500	1.5:1
Q-SLN9	1500	250	6:1	1000	500	2:1
Q-SLN10	2000	250	8:1	500	500	1:1
Q-SLN11	2000	250	8:1	750	500	1.5:1
Q-SLN12	2000	250	8:1	1000	500	2:1

high-speed homogenizer with a speed varying between 3,000-5,000 rpm. The resulting o/w nanoemulsion obtained was ultrasonicated using a probe sonicator and then was allowed to cool in an ice water bath which led to crystallization of the lipid and the formation of solid lipid nanoparticles. Different batches were prepared for optimization of formulation by changing the critical process variables as mentioned in Table 1.

Particle size and PDI measurement

The particle size and polydispersity index (PDI) of SLN was investigated by photon correlation spectroscopy (PCS) using Malvern Zetasizer at 25°C. The measurement was conducted at 90° detection angle. The nanoparticle dispersion was first appropriately diluted with double distilled water before analysis. The results obtained as z-average diameter (effective diameter) and the PDI, which is a parameter for estimating the particle size distribution (28).

Zeta potential measurement

The charge present on the surface of nanoparticles in dispersion is indicated by value of zeta potential (ZP) and is one of the most important parameters to predict the physical stability of a colloidal dispersion system (29). Prior to the measurement, all samples were diluted using double distilled water. Electrophoretic mobility of nanoparticles was measured using a Malvern Zetasizer at field strength of 20 V cm^{-1} determined the zeta potential in mV.

Encapsulation efficiency (EE)

EE was calculated by comparing the difference between the initial amount of quercetin used in the formulation of SLN and the amount of free drug present in the supernatant with the initial amount of the drug used in the preparation of SLNs (30). Briefly, a little of freshly prepared Q-SLNs was centrifuged for 30 min at 5000 rpm. The resulting supernatant was collected, and the quantity of free quercetin was measured spectrophotometrically at 369 nm.

In-vitro release study

The in vitro release of quercetin from the nanoparticle was performed in triplicates in PBS buffer (pH 3.0 and pH 7.0) containing 0.5% w/v Tween 80. Nanoparticle dispersion (1 ml) was put into dialysis bag (MWCO 12–14 kDa) and placed in 20 ml of the PBS buffer with gentle shaking at 50 stroke/min in the water bath at 37°C temperature. An aliquot (0.5 ml) of the PBS buffer outside the dialysis solution was collected at designated time intervals of 0.5, 1, 2, 3, 4, 5, 6, and 12 h and compensated with an equal volume of fresh medium. The quantity of quercetin was measured spectrophotometrically at 369 nm. Quercetin dispersed in PBS was used as a control.

Stability studies

The optimized SLNs were stored at room temperature for 30 days, and average particle size, PDI, zeta potential, and EE were determined at 0, 15, and 30 days. Each sample was analyzed three times.

Statistical analysis

All of the data are represented as mean ± SD. One-way ANOVA was performed to determine the level of significance. A p-value of <0.05 was considered statistically significant.

Results and Discussion

Selection of lipid phase

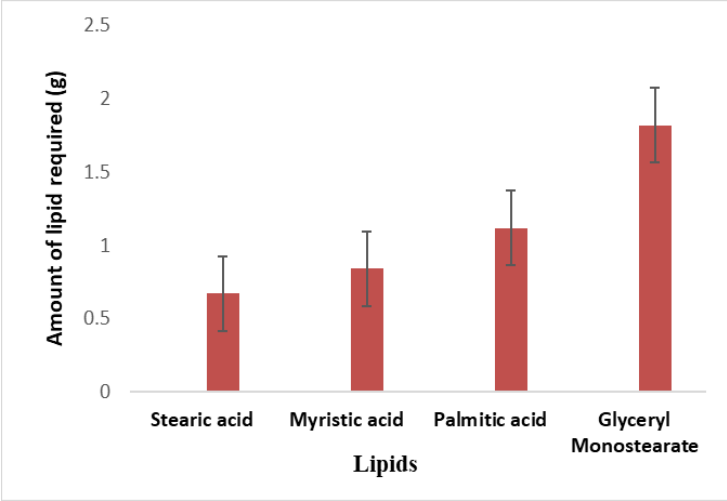

Figure 1. Solubility of quercetin in different lipids.

The quercetin loaded solid lipid nanoparticle (Q-SLN) formulation was optimized by varying the composition based on the lipid to drug ratio and surfactant to co-surfactant ratio. The average particle size, polydispersity index, zeta potential, and encapsulation efficiency of the formulations are given in Table 2. Increase in lipid to drug ratio from 2:1 to 8:1 showed a significant ($p < 0.05$) increase the particle size from 44 to 622 nm. This is because higher lipid content in the formulation requires high stabilizer concentrations to reduce the surface tension and facilitate particle size reduction. Among the formulations with the same lipid to drug ratios, the increase in surfactant content (Tween 80) reduced the particle size. Since, higher surfactant reduces interfacial tension more effectively, facilitates the homogenization of lipids in the aqueous phase and leads to smaller particles. Besides the Tween 80, its combination with the co surfactant, soy lecithin could have also helped to reduce the aggregation of the SLN and resulted in smaller particle size. Besides the particle size, encapsulation efficiency is one of the most important characteristics to have bioactive enriched SLN. An increase in the lipid to core ratio from 2:1 to 8:1 exhibited significant ($p < 0.05$) increase in encapsulation efficiency from 9 to 58%. Moreover, ratios at 6:1 and 8:1 had higher encapsulation efficiency due to higher lipid content that provides more space to incorporate the active compound and also reduces the escape of core compound to the external phase. However, beyond certain lipid to drug ratio (6:1), the encapsulation efficiency was not improved (in 8:1 ratio) much due to its limitations in the core accommodation (statistically insignificant). Moreover, increase in the surfactant ratio also increased the encapsulation efficiency and significantly ($p < 0.05$) in 6:1 lipid to drug ratio. Since,

Table 2. Particle size, Polydispersity index, Zeta potential and Encapsulation efficiency of different SLNs.

Formulation	Particle size	Polydispersity index	Zeta potential	Encapsulation efficiency
Q-SLN1	65 ± 6	0.421 ± 0.012	21.5 ± 0.25	9.5 ± 0.53
Q-SLN2	55 ± 2	0.396 ± 0.014	23.4 ± 0.21	12.5 ± 0.75
Q-SLN3	44 ± 11	0.364 ± 0.011	24.3 ± 0.30	13.4 ± 0.84
Q-SLN4	255 ± 7	0.378 ± 0.010	23.5 ± 0.25	26.5 ± 1.43
Q-SLN5	209 ± 4	0.342 ± 0.011	25.4 ± 0.21	34.6 ± 0.67
Q-SLN6	177 ± 8	0.334 ± 0.013	28.3 ± 0.30	39.6 ± 0.43
Q-SLN7	421 ± 12	0.254 ± 0.012	25.5 ± 0.25	43.8 ± 0.61
Q-SLN8	378 ± 9	0.218 ± 0.015	26.4 ± 0.21	51.6 ± 0.87
Q-SLN9	327 ± 7	0.118 ± 0.014	32.3 ± 0.30	57.9 ± 1.32
Q-SLN10	622 ± 4	0.324 ± 0.012	24.5 ± 0.25	53.5 ± 1.26
Q-SLN11	576 ± 6	0.333 ± 0.010	23.4 ± 0.21	56.4 ± 0.76
Q-SLN12	509 ± 12	0.289 ± 0.011	27.3 ± 0.30	57.8 ± 0.82

For formulation development, the excipients used should be pharmaceutically acceptable and generally regarded as safe. The solubility of drug in lipid is one of the important factor for evaluating the encapsulation efficiency of SLNs. It is expected that high lipid solubility will result in high encapsulation efficiency. Solubility was recorded in four lipids namely; Stearic acid, Palmitic acid, Myristic acid and Glyceryl monostearate. Results from the solubility studies tested in 4 lipids indicated that Stearic acid showed the highest solubilization capacity followed by Myristic acid (Figure 1). The amount of Palmitic acid (1.12 ± 0.08 g) and Glyceryl monostearate (1.82 ± 0.22 g) required to solubilize 10 mg quercetin was significantly higher than stearic acid (0.84 ± 0.03 g) and Myristic acid (0.67 ± 0.02 g). This study indicated that the quercetin loading capacity of stearic acid and Myristic acid might be more than Palmitic acid and Glyceryl monostearate.

Optimization of quercetin loaded solid lipid nanoparticles

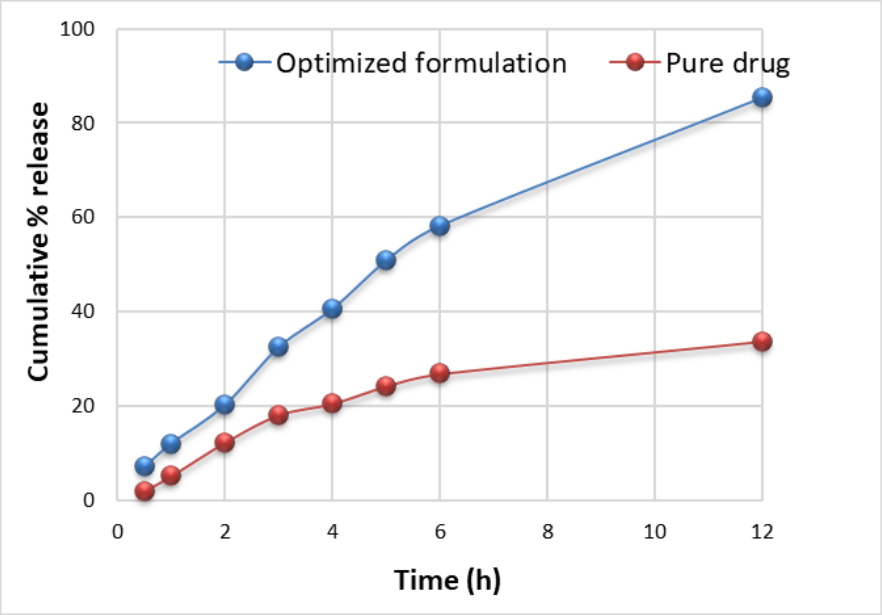

Figure 2. In-vitro release of quercetin from optimized formulation and pure drug in PBS buffer at pH 3.0.

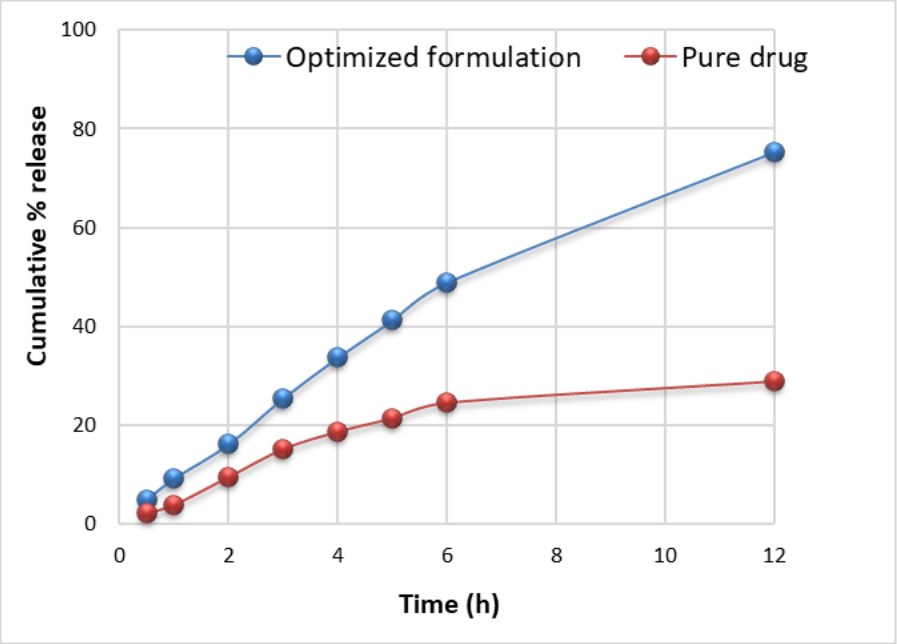

Figure 3. In-vitro release of quercetin from optimized formulation and pure drug in PBS buffer at pH 7.0.

high concentration of Tween 80 increase the thickness of hydrophilic coating that helps to disperse the hydrophilic compound. The lipid to drug ratio of 6:1 and 8:1 with surfactant to co-surfactant ratio of 2:1 gave the highest encapsulation efficiency of around 58%. Based on the higher encapsulation efficiency and lower particle size, formulation Q-SLN9 (6:1, lipid: drug ratio with 2:1 surfactant: co-surfactant ratio) which had particle size around 327 nm and encapsulation efficiency of 57.9% was considered as the best formulation. Moreover, formulation Q-SLN9 demonstrated lower polydispersity index and the highest zeta potential indicating a narrow size distribution of nanoparticles and stable formulation towards aggregation.

In-vitro drug release

The release of drug from SLN was studied by the dialysis bag diffusion technique. The release rate of quercetin from the formulation and its appearance in the dissolution media was governed by the partition coefficients of the drug between the lipid phase and aqueous environment in the dialysis bag and also by the diffusion of the drug across the membrane. The dialysis bag retained the nanoparticles and enabled the diffusion of the drug immediately into receiver compartment. Percent cumulative drug release in the release versus time was plotted to demonstrate the drug release pattern (Results are shown in Figure 2 and 3). In-vitro release of quercetin from the formulation was performed with PBS (pH 3 and 7). SLN dispersion showed faster release profile compared to pure quercetin powder. Release of quercetin from optimized formulation was significantly higher ($p<0.05$) compared to pure quercetin powder.

Stability study

Results obtained from stability study of SLN stored at room temperate are summarized in Table 3. After 30 days storage at room temperature, size of SLN increased in the range from 327 ± 6 to 329 ± 3 nm (i.e. 0.61 % increment in the particle size). The slight increment in particle size of SLN after 30 days was seen, possibly due to the partial aggregation brought by the minimization of high surface-to-volume ratios. Encapsulation efficiencies of drug loaded-SLN were decreased by 1.8 after 30 days of storage. Transitions of lipid from metastable forms to stable form might occur slowly on storage due to small particle size. Therefore, the solid lipid nanoparticles

Table 3. Particle size, PDI and EE of quercetin loaded SLN after 0, 15, 30 days.

Days	Optimized formulation		
	Particle size (nm)	PDI	EE
0	327 ± 6	0.118 ± 0.012	57.9 ± 2.35
15	328 ± 5	0.120 ± 0.013	56.7 ± 2.42
30	329 ± 3	0.123 ± 0.015	56..1 ± 1.72

All values reported are mean ± SD (n = 3).

prepared by the emulsification-ultrasonication method showed excellent storage stability (Results are shown in Table 3).

Conclusion

The study successfully developed solid lipid nanoparticles loaded with quercetin was developed successfully. Nanoparticles were successfully developed by optimizing the formulation variables like lipid, surfactant and co-surfactant concentration. The result demonstrated that the particle size, polydispersity index, zeta potential, and encapsulation efficiency are significantly affected by these variables. Based on lower particle size, polydispersity index and highest zeta potential, encapsulation efficiency, Q-SLN9 was considered the best formulation. The drug release study exhibited enhanced release of quercetin from SLN as compared to plain drug. SLNs were stable at 4 °C for 30 days of the study period. Hence, the findings concluded that the developed SLNs can be used as a potential carrier for improved delivery of poorly water-soluble drugs like quercetin.

Contribution of authors

Conceptualization and methodology (Mohammad Akhlaquer Rahman), data curation, formal analysis, investigation and visualization (Bashayer Alsofyani, Reem Sari Alayli, Maram Sari Alayli, Teaf Salah Alkhaldi, Ghadi Hammad Altalhi).

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Conflict of Interest

The authors declare no conflict of interest.

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None

References

1. Klueglich M, Ring A, Scheuerer S, Trommeshauser D, Schuijt C, Liepold B, et al. Ibuprofen extrudate, a novel, rapidly dissolving ibuprofen formulation: relative bioavailability compared to ibuprofen lysinate and regular ibuprofen, and food effect on all formulations J. Clin. Pharmacol. 2005;42:1055-1061.
2. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 1995;12:413-420.
3. Hiroshi A, Mikio T, Masahiro H. The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water-soluble compounds. Int. J. Pharm. 2005;305:61-74.
4. Sheikh S, Faiyaz S, Sushma T, Farhan JA, Roop RK, Mushir A. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur. J. Pharm. Sci. 2007;66:227-243.
5. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm. Res. 1992;9:87-93.
6. Li P, Ghosh A, Wagner RF, Krill S, Joshi YM, Serajuddin ATM. Effect of combined use of nonionic surfactant on formation of oil-in-water microemulsions. Int. J. Pharm. 2005;288:27-34.
7. Kararli TT, Needham TE, Griffin M, Schoenhard G, Ferro LJ, Alcorn L. Oral delivery of a renin inhibitor compound using emulsion formulations. Pharm. Res. 1992;9:888-893.
8. Nakano M. Places of emulsions in drug delivery. Adv. Drug Deliv. Rev. 2000;45:1-4.
9. Schwendener RA, Schott H. Lipophilic 1-beta-D-arabinofuranosyl cytosine derivatives in liposomal formulations for oral and parenteral antileukemic therapy in the murine L1210 leukemia model. J. Cancer Res. Clin. Oncol. 1996;122:723-726.
10. Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol. 1995;33:1061-80.

11. Torchilin VP. "Micellar Nanocarriers: Pharmaceutical Perspectives", Pharm Res. 2007;24(1):1-11.
12. Makris DP, Rossiter JT. 2000. Quercetin and rutin (quercetin 3-O- rhamnosylglucoside) thermal degradation in aqueous media under alkaline conditions. In: Buttriss J, Saltmarsh M, editors. Functional foods II: Claims and evidence. Cambridge, UK: Royal Society of Chemistry. pp 216–238.
13. Chen X, Zuo Z, Chow AHL, Chow MSS. 2001. Low bioavailability of quercetin in rats: Contributions by the gut and liver. AAPS PharmSci 3(S1). Abstract R5177. Available from: http://www. aapspharmsci.org/.
14. Manach C, Morand C, Crespy V, Demigne C, Taxier O, Regerat F, Remesy C. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett. 1998;426:331-36.
15. Kim M, Sedykh A, Chakravarti SK, Saiakhov RD, Zhau H. Critical Evaluation of Human Oral Bioavailability for Pharmaceutical Drugs by Using Various Cheminformatics Approaches. Pharm Res. 2014;31(4):1002-1014.
16. Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47(2):165-96.
17. Kakkar Thukral D, Dumoga S, Mishra AK. Solid lipid nanoparticles: promising therapeutic nanocarriers for drug delivery. Curr Drug Deliv. 2014;11(6):771-91
18. Q-y X, Wang M, Chen F, Gong T, Y-l J, Z-r Z. Lung-targeting delivery of dexamethasone acetate loaded solid lipid nanoparticles. Arch Pharm Res. 2007;30(4):519-25.
19. Muller RH, Keck CM. Challenges and solutions for the delivery of biotech drugs-a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol. 2004;113(1):151-70.
20. Mukherjee S, Ray S, Thakur R. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian J Pharm Sci. 2009;71(4):349.
21. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN/NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm. 2009;366(1):170-84.
22. Abdelbary G, Fahmy RH. Diazepam-loaded solid lipid nanoparticles: design and characterization. AAPS PharmSciTech. 2009;10(1):211 -9.
23. Kalepu S, Manthina M, Padavala V. Oral lipid-based drug delivery systems: An overview. Acta Pharm Sin B. 2013;3(6):361-72.
24. Aditya N, Macedo AS, Doktorovova S, Souto EB, Kim S, Chang P-S, et al. Development and evaluation of lipid nanocarriers for quercetin delivery: a comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE). LWT-Food Sci Technol. 2014;59(1):115-21.
25. Joshi M, Patravale V. Formulation and evaluation of nanostructured lipid carrier (NLC)-based gel of valdecoxib. Drug Dev Ind Pharm. 2006;32:911-18.
26. Helgason T, Awad TS, Kristbergsson K, McClements DJ, Weiss J. Effect of surfactant surface coverage on formation of solid lipid nanoparticles (SLN). J Colloid Interface Sci. 2009;334:75-81.
27. Chen X, Zuo Z, Chow AHL, Chow MSS. 2001. Low bioavailability of quercetin in rats: Contributions by the gut and liver. AAPS PharmSci 3(S1).
28. Robhash KS, Keon WK, Hoo-Kyun C. Preparation and characterization of solid lipid nanoparticles loaded with doxorubicin. Eur J Pharm Sci. 2009;37:508-13.
29. Pradhan M, Singh D, Singh MR. Influence of selected variables on fabrication of triamcinolone acetonide loaded solid lipid nanoparticles for topical treatment of dermal disorders. Artif Cells Nanomed Biotechnol. 2016;44(1): 392-400.
30. Eskandani M, Nazemiyeh H. Self-reporter shikonin-act-loaded solid lipid nanoparticle: Formulation, physicochemical characterization and geno/cytotoxicity evaluation. Eur J Pharm Sci. 2014;59:49