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Original Article

SYNTHESIS AND CHARACTERIZATION OF POLY D-L LACTIDE (PLA) NANOPARTICLES FOR THE DELIVERY OF QUERCETIN

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ABSTRACT

Objectives: Synthesis and optimization of Poly D-L Lactide (PLA) nanoparticles for the delivery of an antioxidant molecule quercetin.

Methods: The quercetin was encapsulated by PLA nanoparticles by nanoprecipitation method. The average particle size and the electric charge for different formulations were measured by particle size and zeta potential analyzer. The quercetin loaded PLA nanoparticles (Q-PLAN) was characterized by differential scanning calorimetry & Fourier transform-Infra red spectroscopy, Scanning electron microscopy and Atomic force microscopy. The average drug content, encapsulation efficiency and drug release studies were carried out for different formulations of Q-PLAN. The antioxidant activity of the formulated Q-PLAN nanoparticles was tested using DPPH assay.

Results: The formulation F3 (Quercetin 75 mg: PLA 200 mg) was found to be optimized formulation based on particle size analysis, Zeta potential, drug content, encapsulation efficiency and drug release studies. The mean diameter and zeta potential of optimized Q-PLAN and PLA nanoparticles were found to be 242±20 nm, 185±10 nm and-22.5±1.5 mV,-20.5±1.0 mV. The F3 formulation showed encapsulation efficiency of 73.3% and 5.5±0.06 mg/ml of actual drug loading. The F3 formulations showed 99.7% of drug release. The optimized Q-PLAN showed better scavenging effects when compared to the free quercetin.

Conclusions: The poor aqueous solubility and stability of the antioxidant molecule quercetin have been improved by entrapping the quercetin molecules into the PLA nanoparticles.

Keywords: Quercetin, Nanoprecipitation, PLA nanoparticles, Antioxidant.

INTRODUCTION

Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone), one of the most widely distributed polyphenolic flavonoid in plants. It is abundantly found in berries with varying concentrations of 53 to 153 mg/Kg of the dry weight of the plant material [1]. The antioxidant activity of the molecule quercetin is higher than that of other antioxidants such as rutin, ascorbyl and trolox [2]. Quercetin exhibits the wide range of pharmacological properties, including antioxidant activity [3], anti-inflammatory activity [4], antiproliferative activity [5], and protective effects against oxidative damage [6]. Quercetin has been widely used in clinical treatments due to its multiple pharmacological activities. It has been hypothesized that these ameliorating effects of quercetin against toxicity was due to its wide range of mechanisms, including reducing oxidative stress by scavenging free radicals, reducing toxicities of xenobiotics and carcinogens by regulating gene expression and promoting cellular survival by modulating intracellular signals [7]. This is due to the singlet oxygen and the free hydroxyl groups present in the quercetin molecule [8]. However, the therapeutic use of quercetin is limited due to its poor aqueous solubility and instability in the physiological medium [9]. The quercetin is chemically unstable in aqueous medium, which may possibly involve the attack of hydroxyl ions on the C-ring of quercetin [10]. These properties of quercetin result in poor permeability, instability and extensive first past metabolism before reaching the systemic circulation [11]. Many scientists have tried to improve its solubility by adding non-polar solvents [12] and complexation with cyclodextrin and liposomes [13].

To overcome these problems, one of the effective methods is to encapsulate this molecule on suitable nanocarriers. There are various NPs systems are used to encapsulate the similar biomolecules [14-16]. However, biodegradable polymer acts as better carriers to encapsulate these molecules for therapeutic use [17-19]. Among the biodegradable polymeric nanoparticles PLA is widely used for encapsulation of many therapeutic drugs. PLA has the wide range of properties such as biodegradability, biocompatibility, high hydrophobicity, strong mechanical strength and slow drug release [20]. Advantages using biodegradable polymeric nanoparticles formulation includes, reduced systemic side effects, targeted and controlled drug release and high capability to cross various physiological barriers [21, 22]. Thus, for nanoencapsulation of quercetin, PLA has been employed as a ideal nanocarriers considering its nature and its properties [23, 24].

Quercetin molecule has been successfully encapsulated in to biopolymer PLA nanoparticles [1], liposomes [25] and into chitosan nanoparticles [26]. In this study, an efficient method is used for preparation of PLA nanoparticles (PLAN). In order to produce small and low polydisperse NPs, the nanoprecipitation is one of the efficient and easiest methods. In this study, we have encapsulated the quercetin molecule into PLAN by nanoprecipitation method. The quercetin drug loaded PLAN were characterized by differential scanning calorimetric analysis (DSC), Fourier transform-infra red spectroscopy (FT-IR), scanning electron microscopy (SEM) and atomic force microscopy (AFM). The size and the zeta potential value for different formulations were measured. The drug content, entrapment efficiency (EE), *in vitro* drug release and antioxidant activity using DPPH assay was also been carried out to enhance its application in the pharmaceutical field. The encapsulated quercetin molecule showed higher efficient antioxidant activity, aqueous solubility and sustained drug release. Thus it is speculated that the aqueous stability of the quercetin can be improved by encapsulating the molecules into PLAN.

MATERIALS AND METHODS

Materials

Poly-d, l-lactide (PLA) (MW= 75, 000-120, 000), polyvinyl alcohol (PVA), Quercetin was purchased from Sigma-Aldrich and used as received. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich. All other solvents and chemicals were procured

from Sigma. Experimental solutions were prepared using water filtered through a Milli-Q water system (Millipore, Bedford, MA).

Preparation of quercetin loaded PLA nanoparticles (Q-PLAN)

Quercetin loaded PLA nanoparticles were prepared by the nanoprecipitaion method specified by Wu *et al.* (2008) [27] and Mehrotra & Pandit (2012) [28] with some modifications. In this method the Poly-d, l-lactide (PLA) (MW= 75, 000–120, 000) and quercetin were dissolved in acetone and homogenized using homogenizer (IkaLabortechnik) at 16, 000 X g for 5 min. The prepared solution was then added drop wise to 1% w/v aqueous polyvinyl alcohol (PVA) solution, by continuous homogenization for 20 min. Then, organic solvent was removed using the rotary evaporator (B-480 Buchi, Switzerland). The nanoparticles were separated by centrifugation at 13, 000Xg for 20 min at 10°C and washed three times with deionized water by centrifugation. The blank PLAN was prepared by following the same procedure by excluding the drug. Then the final samples were lyophilized (Martin Christ Gefriertrocknungsanlagen GmbH, 37507 Osterodeam Harz, Germany) and stored at 4 °C for further use. This synthesis was repeated as many times until the desired amount of samples was obtained. The details for formulation were given in table 1.

Table 1: Formulation of quercetin nanoparticles

S. No.	Ingredients	F-1	F-2	F-3	F-4	PLAN
1	Quercetin	25 mg	50 mg	75 mg	100 mg	-
2	PLA	200 mg	200 mg	200 mg	200 mg	200 mg
3	1% PVA	50 ml	50 ml	50 ml	50 ml	50 ml
4	Acetone	20 ml	20 ml	20 ml	20 ml	20 ml

Differential scanning calorimetric analysis (DSC) & fourier transform-infra red spectroscopy (FT-IR)

Differential scanning calorimetric (DSC) measurements were carried out using a SII Nanotechnology DSC 6200 thermal analyzer (Inkarp Instruments Pvt ltd, Japan) in a temperature range of 0°C-360°C at a heating rate of 5°C/min under nitrogen atmosphere. The samples were prepared by pressing them in a DSC aluminum pans and subjected to analysis. FT-IR spectra were recorded on Perkin Elmer 1 Fourier Transform Infrared Spectrophotometer. Samples were mixed with KBr, pressed into a disk and scanned from 400 to 4000 cm-1. DSC thermograms and FT-IR spectra for pure PLA, PVA, Quercetin, and formulated Q-PLAN were recorded.

Particle size analysis (PSA) and Zeta potential

The average size distribution of Q-PLAN was determined using Malvern zeta sizer (Malvern Instrument Inc, London, UK). The samples were diluted and uniformly dispersed with ultra purified water and the analysis was performed. The formulated Q-PLAN and PLAN were characterized with Zeta potential using Zeta Sizer. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted. The electrical charge on the nanoparticles was measured by particle electrophoresis (Zetasizer Zen Systems 3600, Malvern Instruments Ltd., UK) after they had been diluted in deionized water to avoid multiple scattering effects and then placed in a folded capillary cell (25 °C). Measurements were made in triplicate, and the results are shown as mean±standard error.

Determination of drug content

The whole of the freeze dried nanoparticles was redispersed in 10 ml of demineralised water. From these 2 ml of nanosusupension was freeze-dried. Then the dried nanoparticles were re dissolved in acetone. The obtained suspension was then centrifuged (Remi, Mumbai, India) at 3000 rpm for 2 min. The supernatant was diluted suitably with known concentration of phosphate buffer pH 7.4 and then analyzed using UV-Vis spectrophotometer at 422 nm. The drug content of the formulations was expressed as mg/ml of the nano suspension.

Determination of percentage entrapment efficiency

The entrapment efficiency (EE) is also known as Association Efficiency. The weighed amount drug loaded nanoparticles are centrifuged at a high speed cooling centrifuge of 16000Xg for 40 min and the supernatant is assayed for free drug concentration by spectrophotometer. EE was calculated as follows:

$$EE\% = \big((\text{Total amount of drug added} - \text{Non bound drug}) / (\text{Total amount of drug added})\big) \times 100$$

***In vitro* release studies**

The *in vitro* release profile of the quercetin from the PLAN was studied by using dialysis technique. In the donor compartment nano suspension containing the known concentration of drug was placed and in the receptor compartment buffer was placed and constantly agitated using a magnetic stirrer at 37 °C. Samples were withdrawn from the receptor compartment for estimation of released drug and replaced with the same volume of buffer. The experiment was carried out in triplicate and the values were reported as mean value±SD.

Scanning electron microscopy (SEM) analysis

The morphology of formulated PLAN and Q-PLAN were determined by SEM. The equipment used was Quanta 200 FEG Scanning electron microscope (FEI Quanta FEG 200). The NPs were dispersed in water and drop coated on aluminium stub using double sided carbon tape. The sample was then coated with gold sputter coating unit at 10 Pa vacuum for 10 S (SC7620, Japan). The typical acceleration potential used was 30 kV and the image was captured at the desired magnification.

Atomic force microscope (AFM) analysis

PLAN and Q-PLAN were characterized using Atomic force microscope (XEI 70, Park system, Korea). Analyses were carried out by running the machine in the non-contact tapping mode. The samples were drop coated on glass substrates. The nanoparticles were characterized by observing the patterns that appeared on the surface topography and analyzing the AFM data. The topological 3D image was obtained in tapping mode at a resonance frequency of 272.98 kHz.

Determination of antioxidant activity of free quercetin and Q-PLAN using DPPH assay

The free radical scavenging (antioxidant) activity for the free quercetin, Q-PLAN and PLAN was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay according to method reported by Kumar *et al.* (2009) [29]. An aliquot of 0.5 ml of different concentration of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.

$$\text{Percentage (\%) of inhibition} = \big((\text{absorbance of control} - \text{absorbance of sample}) / (\text{absorbance of control})\big) \times 100$$

RESULTS AND DISCUSSION

Synthesis and encapsulation of quercetin in PLA nanoparticles

Quercetin molecule is hydrophobic in nature. The antioxidant molecule quercetin has several beneficial effects in pharmaceutical field, the encapsulation of this molecule is necessary. Thus to entrap this quercetin molecule, the biodegradable polymer PLA was found to be the best used for nanoparticles synthesis.

The nano precipitation was the method used for the preparation of Q-PLAN. This method was specifically used to produce small and low polydisperse NPs population. This is one of the best and the easiest methods for producing NPs population. In this process the particle formation is spontaneous; it is due to polymer precipitated in the aqueous environment. The Marangoni effect explains the process of nano precipitation; solvent flow, diffusion and surface tension at the interference of organic solvent and the aqueous phase cause turbulences, which leads to the formation of small droplets containing the polymer.

Later, as the solvent diffuses out form the droplets, the polymer precipitates [30]. Finally the organic solvent is evaporated with the help of rotary evaporator. This method yields very good encapsulation of functional quercetin.

Differential scanning calorimetric (DSC) analysis

DSC is an important technique to analyze the polymer-drug interactions and also it has previously been used to show the dispersity of the molecules [31]. In order to understand the thermal properties of quercetin, PLA, PVA and the formulated Q-PLAN, DSC measurements were conducted. The phase transition analyses of quercetin, PLA, PVA and the formulated Q-PLAN are shown in fig. 1. The DSC curves for quercetin showed two endothermic transitions at 103°C and 313 °C corresponding to dehydration and melting of quercetin, respectively [32, 33]. The DSC curves of the PLA showed two different endothermic transitions at 42 °C and 48 °C, which corresponds to the melting temperature of the polymer. The DSC curves of PVA showed an endothermic glass transition peak at 222°C. The Q-PLAN showed DSC profiles that exhibited a series of thermal transitions that corresponds to those observed for PLA, PVA and quercetin alone. For Q-PLAN the DSC curves showed no shift in thermal transitions corresponding to pure quercetin but showed a slight shift in endothermic peaks corresponds to the melting temperature of the PLA. These results indicate that quercetin is in a crystalline state within the polymeric matrix of PLAN. Our results are found to be similar to that of other reports [34-36]. The major peaks of quercetin remained unaltered when combined with PVA and PLA. Thus it can conclude that the Q-PLAN posses physical and chemical stability within the polymer, thus retaining its biological activity.

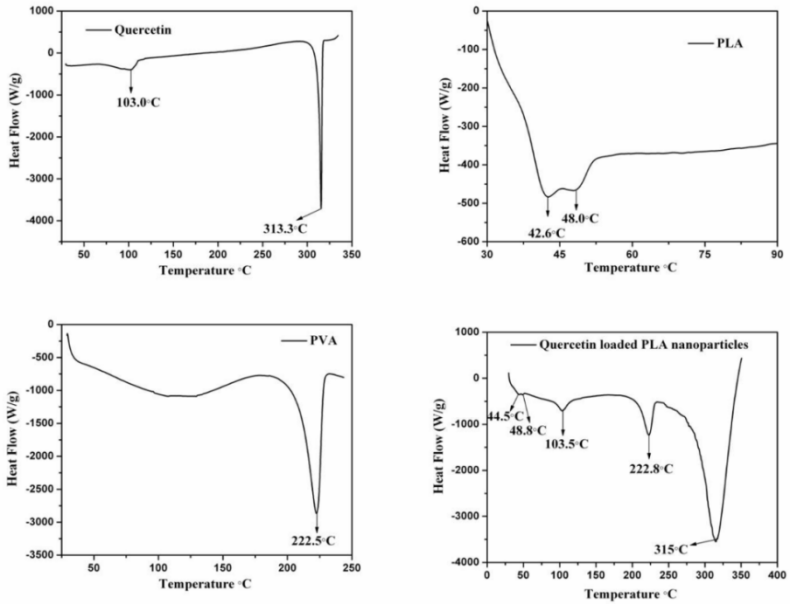

Fig. 1: DSC thermograms of free quercetin, PLA, PVA and Q-PLAN

Fourier transform infra red (FT-IR) analysis

FT-IR analysis is one of the efficient tools for the quick and efficient identification of encapsulated chemical molecules. Information on the nature of the molecular interactions within the solid matrix of the NPs was obtained using FT-IR analysis. The FT-IR spectra of pure quercetin, PLA, PVA and Q-plan were shown in fig. 2 and the corresponding peaks assignment were listed in table 2. The FT-IR spectrum of Q-PLAN showed similar peaks corresponding to those for quercetin, PLA and PVA alone. The major characteristic peaks of quercetin as (1100-1700 cm^{-1}) and-OH phenolic bending (1365 cm^{-1}) are present in free and in formulated Q-plan. PLA characteristic peaks of C=O stretching (1653 cm^{-1}) and C-CO-C stretching (1148 cm^{-1}) appeared in the Q-plan and also in pure PLA. The presence of quercetin characteristic peaks on the Q-plan indicates the quercetin encapsulation within plan. The functional group present in the free quercetin was also present in the formulated Q-plan without any alterations. Our results are supported by the findings of other authors, similar kind of FT-IR spectrum was observed for nanoencapsulation of Q-plan by Kumari *et al.* (2010) [1]. The major peaks of quercetin were remained unaltered when combined with PLA and PVA. From the above interpretations, we confirm the encapsulation of quercetin within the plan.

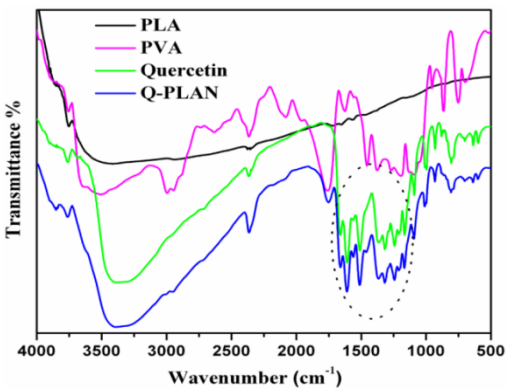

Fig. 2: FT-IR spectra of free quercetin, PLA, PVA and Q-PLAN

Table 2: FT-IR spectral peaks assignment of free quercetin, PLA, PVA and Q-PLAN

PLA (cm^{-1})	PVA (cm^{-1})	Quercetin (cm^{-1})	Q-PLAN (cm^{-1})	Assignment
3758	3760	3767	3769	O-H stretching vibration of phenol
3446	3509	3393	3409	O-H stretching vibration of phenol
-	3002	-	2941	C-H aromatic ring stretch
1720	1767	-	1756	C=O stretching vibrations of ketones
1653	1629	1662	1662	C=O Aryl ketonic stretch
-	-	1611	1610	C---C Aromatic ring stretch
1565	-	-	1562	C=C aromatic stretch
-	-	1514	1510	C=O aromatic stretch
-	1458	-	1455	C=C aromatic stretch
-	1384	1365	1372	O-H bending of phenols
-	-	1319	1316	C-H bond in Aromatic hydrocarbon
-	-	1247	1242	C-O stretch of Aryl ether
-	1199	1213	1211	C-O stretch of phenol
1148	-	1169	1167	C-CO-C stretch and bending in ketone
-	1085	1094	1093	C-O stretching of Phenols
-	-	999	1010	C-O stretching of Phenols
-	955	931	932	C-H bending of aromatic hydrocarbons
-	867	881	880	C-H bending of aromatic hydrocarbons
-	-	806	808	C-H bending of aromatic hydrocarbons
-	696	705	704	C-H bending of aromatic hydrocarbons
-	-	640	638	C-H bending of aromatic hydrocarbons
-	-	599	599	C-H bending of aromatic hydrocarbons

Size distribution and stability of various formulations of Q-PLAN and plain PLAN

The various formulations of Q-PLAN and PLAN were prepared by the nanoprecipitation method. The prepared formulations were studied for particle size distribution and electrical properties. Particle size distribution is an important parameter towards development of suitable nanomedicines for therapeutic purposes. It determines *in vivo* drug release behavior, biological fate, toxicity and the targeting ability of NPs after administration. Also they can also influence the drug loading, drug release and stability of drug inside NPs [37]. The particle size distributions of various formulations are shown in table 3 and the corresponding spectra is shown in fig. 3 which indicated the uniform size distribution of Q-PLAN. The formulations (F1-F4) show average size distribution of 220±30, 235±24, 242±20 and 250±18 nm respectively and the plain PLAN showed average size distribution about 185±10 nm. Zeta potential, i.e., the surface charge can greatly influence the stability of NPs in suspension through the electrostatic repulsion between the particles. The zeta potential values of the PLAN and Q-PLAN are shown in table 3. The zeta potential values for different formulations (F1-F4) of Q-PLAN showed-21.5±2.2 mV,-22.4±1.8 mV,-22.5±1.5 mV,-22.5±1.4 mV and-20.5±1.0 mV and PLAN showed-10.5±1.0 mV respectively.

Table 3: Mean particle size and Zeta potential values of various formulations of Q-PLAN

Formulations	Average particle size (nm)	Zeta potential (mV)
F1	220±30	-21.5±2.2
F2	235±24	-22.4±1.8
F3	242±20	-22.5±1.5
F4	250±18	-22.5±1.4
PLAN	185±10	-20.5±1.0

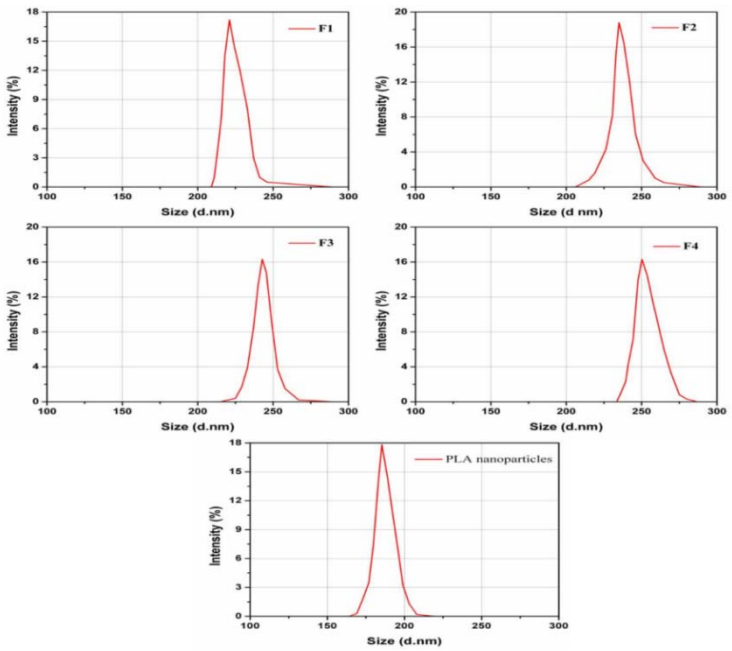

Fig. 3: PSA spectra for different Q-PLAN formulations (F1, F2, F3 and F4) and PLAN

The functional performance of NPs based delivery systems depends on the physicochemical properties of the NPs, such as size and charge [38, 39]. Therefore we measured the mean particle diameter and zeta-potential of the polymeric NPs produced in this study. Relatively, with an increase in drug concentration, the size of the formulated Q-PLAN increased proportionally. Molecular weight, concentrations of polymer, surfactant and the drug used, are the major factors that affect the final size of the particles [40, 41]. In this experiment, the higher molecular weight of PLA was used for encapsulating the drug. Apparently the higher molecular weight of the PLA used in this study increased the viscosity of the internal phase, leading to a decreased net shear stress, thus producing larger NPs with increasing in drug concentration.

The electrical charge for formulated plain PLAN and Q-PLAN was found to be negative at pH 7, which may be attributed to the presence of ionized carboxyl groups on the PLA matrix [42]. Differences in zeta potential values were also observed between PLAN and Q-PLAN. The increase in the negative zeta potential of the Q-PLAN, when compared with the PLAN may be complimented by the acidic OH group of the flavonoid group of quercetin. The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the particles [43]. The results indicate that the presence of quercetin alters the electrical charge of the polymeric NPs. However, the presence of flavonoid within PLAN may not affect the stability of the NPs in aqueous dispersions. Some authors have indicated that with zeta values ≈+30 mV or ≈-30 mV are enough for repulsion forces to avoid particle aggregation [44, 45]. Therefore, the zeta potential values for the PLAN and Q-PLAN obtained in this study indicate that they show strong electrostatic repulsion force that prevents particle aggregation. The small particle size as well as uniform size distribution of Q-PLAN is suitable for the development of nanomedicines.

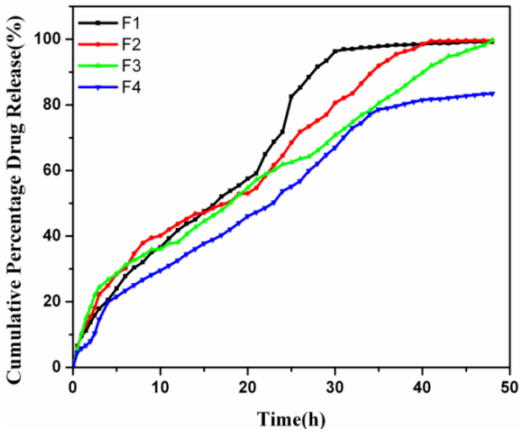

Fig. 4: Percentage of drug released from various formulations of Q-PLAN (F1-F4)

Table 4: Drug content and entrapment efficiency of various Q-PLAN formulations (F1-F4). *Results of triplicate trials±S. E. M (mg/ml)

Formulations	Average drug content (mg/ml)*	Average entrapment efficiency (%)
F1	1.5±0.03	60.0
F2	3.3±0.05	66.0
F3	5.5±0.06	73.3
F4	7.3±0.05	73.0

Drug content, encapsulation efficiency and release studies for Q-PLAN

The average drug content and average EE are listed in table 4. The formulations F1, F2, F3 and F4 showed average drug content of 1.5±0.03, 3.3±0.05 and 5.5±0.06 and 7.3±0.05 mg/ml. The average EE was observed about 60.0%, 66.6%, 73.3% and 73.0% corresponding to the formulations of F1, F2, F3 and F4. The percentage of *in vitro* drug release was shown in fig. 4. The formulations F1, F2, F3 and F4 showed an average release of 27.8, 30.2, 31.2 and 23.4 within 0-6 h. There was about 71.7%, 64.5%, 61.9% and 53.7% corresponding to the formulations of F1, F2, F3 and F4 was observed at 24 h. At 48 h there was about 99.2%, 99.6% 99.7% and 83.4% of drug release was observed for the corresponding formulations of F1, F2, F3 and F4 respectively.

The average drug content and EE for Q-PLAN were calculated. The formulation F3 and F4 showed better average drug content and also showed better average EE when compared to the other formulations. A parallel increase in the drug content and the EE of the prepared NPs were observed with increase in drug concentration. Although there is a parallel increase in the drug content with increase in the concentration of quercetin, the EE of the formulated NPs has not increased beyond F3 (75 mg: 200 mg) formulations. This may be attributed due to the unaltered polymer concentration which might have caused the saturation of the polymeric micelles during the formulation process. This is due to the nanoprecipitation method used for the nanoencapsulation of this molecule. The rapid solidification by nanoprecipitation method enabled the drug to be entrapped rapidly and preventing its diffusion in to the outer phase [46]. The reason is that a high viscosity holds back the shear forces of solution, and avoids the leakage of the drug [47].

Michailova *et al.* (2010) [48] observed that drug to polymer feed weight ratio influences the EE, irrespective of the solvent used; this was due to the sufficient amount of drug in the system, which promoted the formation of the micellar structure during the process of incorporation. Our study showed similar results, as when we increased the drug ratio by keeping the polymer constant, EE was increased. Biodegradable NPs like PLA, PLGA, etc. have been reported for the encapsulation of wide range of therapeutic molecules such as taxol and ellagic acid [49] with the highest affinity. Drug content and EE depends on the solid-state drug solubility in matrix material or polymer. It is related to the polymer composition, molecular weight, drug polymer interaction and the presence of end functional groups [50, 51].

The release of drug from polymeric NPs was studied by the dialysis-bag diffusion technique. This is a method commonly used and highly suitable method to study the release of drugs from colloidal suspensions [46]. The rate of drug release and its appearance in the outer dissolution medium is governed by the partition coefficient of the drug, between the polymer and the aqueous environment, and by the diffusion of the drug across the membrane as well [52]. The *in vitro* release studies are important, to know the adsorption or encapsulation of quercetin in the PLAN. When compared to F1 and F4 formulations, 30.2% and 31.2% of burst release were observed in formulations F2 and F3 within 0 to 6 h. This was normally attributed to the fraction of quercetin which was adsorbed close to the surface of the NPs [53]. Upon addition of the NPs to the external medium, this fraction of quercetin was diffused rapidly into the surrounding liquid. This shows the rapid initial burst release profiles.

At 24 h F2 and F3 formulations of Q-PLAN showed slow and sustained release. Whereas, F1 formulation showed very rapid release and F4 formulations showed very slow release when compared to the F2 and F3 formulations. In 40 h, the formulations F1 and F2 showed drug release percentage up to 99% where as F3 and F4 showed only 89.7% and 81.5% release. The F1 and F2 formulations showed rapid release and 99% of the drug got released within 40 h. Likely, the formulations F3 and F4 showed slow and sustained release and the drug release reached up to 48 h. The formulation F4 showed slow and sustained release but showed only 81.5% of drug release; whereas the formulation F3 showed maximum drug release up to 99%. From this we can say that the formulation F3 showed rapid burst release in the initial stage and also showed slow and sustained release up to 48 h.

The release profile of Q-PLAN revealed that the drug release is in the controlled manner. The maximum release of quercetin was obtained

in F3 formulations up to 48 h. The slower and sustained release of quercetin from F3 formulation may be attributed to diffusion of the quercetin from the core of the polymer NPs. The drug loaded by nanoprecipitation method has a relatively small burst effect and better-sustained release characteristics [27]. Such controlled release of quercetin favors the development of quercetin based nanomedicines. Being amenable to surface modification, Q-PLAN can also be used for the targeted delivery. In this study, the formulation F3 showed better EE and drug release. Hence the formulation F3 was considered to be the optimized formulation, which can serve as an effective carrier for the delivery of quercetin. Thus we used this F3 formulation for further studies.

Surface analysis of plan and Q-PLAN

The SEM and AFM analysis of both the formulated plain PLAN and Q-PLAN are shown in fig. 5 and 6SEM images of PLAN and formulated Q-PLANreveals that NPs are smooth and spherical. The similar shaped NPs with bright and smooth surface have been observed in AFM images. These results agree with the studies reported by Kumari *et al.* (2010) [1], Pool *et al.* (2012) [54]. The SEM and AFM images showed uniform size distribution of small spherical shaped particles. This spherical shaped Q-PLAN is suitable for the development of nanomedicines.

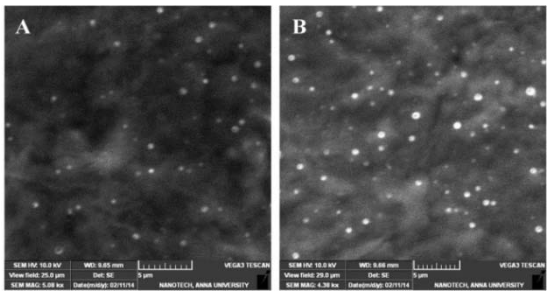

Fig. 5: SEM images of (A) PLAN and (B) Q-PLAN (F3)

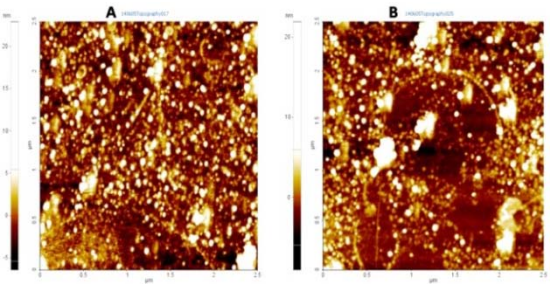

Fig. 6: AFM micrographs of PLAN and Q-PLAN (F3)

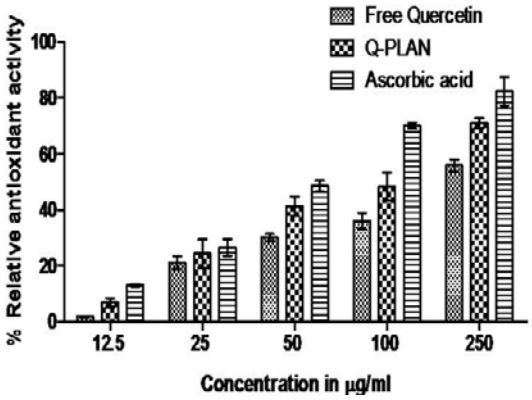

Fig. 7: Free radical scavenging activity of free quercetin and Q-PLAN compared with ascorbic acid standard (Determined using DPPH assay)

Free radicals scavenging activity using DPPH assay

The free radicals scavenging activity of free quercetin and formulated Q-PLAN were determined by DPPH assay. The scavenging activity of free quercetin and Q-PLAN was with respect to the standard molecule (ascorbic acid) was shown in fig. 7. The antioxidant activity for PLAN was also tested and found that, no scavenging activity.

Free radicals are generated by the normal metabolic process or from other exogenous factors which are capable of initiating the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. These generated free radicals are scavenged by a group of compounds called antioxidants. The toxicity and the most of the disease conditions are associated with generation of free radicals. Flavonoids are the potential candidates to reduce the lipid peroxidation and treat the diseases. The antioxidant activity of any compound is determined by its ability to scavenge the DPPH, which has capability to oxidize the molecules by acting as hydrogen ion acceptor. Thus the antioxidant activity of free quercetin and Q-PLAN with respect to standard molecule (ascorbic acid) was calculated.

The Q-PLAN showed better antioxidant effect than free quercetin. This is due to the quercetin molecules dispersed in PLAN, which are protected from molecular oxygen that could be produced during the incubation process. Therefore, during an incubation period the encapsulated quercetin were released and scavenged the free radicals, as opposed to the case of free quercetin, which are degraded in an incubation process, therefore decreasing their scavenging activity. The scavenging ability of Q-PLAN relied on the concentration of NPs as well as the encapsulation efficiency. These results indicate that the nanoencapsulated quercetin maintained its full antioxidant activity and showed better scavenging activity than free quercetin. The previous studies have also showed that the nanoencapsulation of the molecule quercetin have improved antioxidant activity *in vitro* [27, 55].

CONCLUSION

Quercetin was successfully encapsulated in PLAN using nanoprecipitation method. The formulation F3 (Quercetin 75 mg: PLA 200 mg) was found to be the best formulation based on its morphology, encapsulation efficiency and drug release studies. The morphology of both the PLAN and Q-PLAN showed spherical shaped particles. The PSA revealed the average mean diameter of the optimized Q-PLAN and PLAN was about 242±20 nm, 185±10 nm and its zeta potential values was found to be-22.5±1.5 mV,-20.5±1.0 mV. The encapsulation efficiency of and the average drug content of the formulation F3 was found to be 73.3% and 5.5±0.06 mg/ml. The optimized formulation F3 showed 99.7% of drug release. The scavenging activity of Q-PLAN showed better scavenging effects when compared to the free quercetin. Antioxidant activity assay revealed that the functional activity of quercetin was retained after nanoencapsulation. The shape, small size, high encapsulation efficiency and sustained slow release makes Q-PLAN a suitable drug for treatment against diseases and also paves away for further development of nanomedicines.

CONFLICT OF INTERESTS

Declared None

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