ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Fabrication and Characterisation of Silymarin–Quercetin Loaded Polymeric Nanoparticles Using TPGS for Hepatic Drug Delivery

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Abstract

Objective: The aim of the present investigation was to enhance the hepatoprotective activity of silymarin and Quercetin by incorporating it in TPGS-PLGA nanoparticles (NPs) for passive targeted delivery, thereby prolonging its retention time. *Method:* Poly lactide-co-glycolide (PLGA) nanoparticles were prepared by modified spontaneous emulsification solvent diffusion (SESD) method. TPGS as an emulsifier and further as a matrix material blended with PLGA was used to enhance the encapsulation efficiency and improve the drug release profile of nanoparticles. Silymarin and Quercetin were used as model drugs which are having poor water solubility. *Result:* The surface morphology and size of the nanoparticles were studied by scanning electron microscopy (SEM). Drug encapsulation efficiency and in vitro drug release pattern of nanoparticles were determined using High Performance Liquid Chromatography (HPLC).The nanoparticles prepared in this study were spherical, with size range of 150–250 nm. It was shown that TPGS was a good emulsifier for producing nanoparticles of hydrophobic drugs like Silymarin and Quercetin and improving the encapsulation efficiency, drug loading and drug release profile of nanoparticles. *Conclusion:* This research suggests that the combined therapy system of Silymarin and Quercetin could be a better approach for liver targeted drug delivery.

Keywords: *Silymarin; Quercetin; Plga-tpgs nanoparticles; Encapsulation efficiency; In- vitro drug release; Liver targeted drug delivery.*

Introduction

Liver is an important organ for the maintenance of metabolic functions and detoxification of endogenous and exogenous substances like drugs and Xenobiotics. Drug induced liver injury is an unresolved problem and often limits drug therapy in clinical practice. Liver diseases like hepatitis B virus infections, liver cirrhosis and hepatocellular carcinoma are significant in health challenge worldwide due to lack of curative treatment options besides liver resection and transplantation [1].

Silybum marianum, widely known as milk thistle belongs to the family *Asteraceae* and is one of the oldest and profoundly researched plants for the treatment of liver and gallbladder disorders, including hepatitis, cirrhosis, jaundice and other toxin poisonings. Silymarin, the active component of this plant, is a standardized extract that contains 70 to 80 % silymarin flavonolignans like Silybin A&B, Isosilybin A &B, Silydianin, Silychristin and flavonoids like Taxifolin, Quercetin and the remaining 20-30% consisting of a chemically undefined fraction comprising of polymeric and oxidized polyphenolic compounds [2].This plant was used to safeguard and also regenerate the liver cells in various diseases affecting liver. It also has strong antioxidant action via free radical scavenging activity and inhibits lipid peroxidation.

It inhibits the entry of harmful chemicals like heavy metals, pesticides, alcohols, carbon tetra chloride etc into liver, thereby protecting the liver cells from further damage. It prevents the hepatotoxin's binding to receptor sites, protects hepatocyte membranes, enhances liver parenchyma regeneration and increases glutathione levels

[3].Quercetin is a plant derived aglycone form of flavonoid glycosides, Quercetin is chemically 3,3',4,5,7-penta-hydroxyflavone. It is a flavonol found in natural products, especially in apples and onions [4].It has been used as a nutritional supplement and is beneficial against a variety of diseases.

Some of the beneficial effects include antioxidant effects [5] free radical scavenging, cardiovascular protection, antiulcer, anticancer, antitumor, anti-allergy, antiviral, anti-inflammatory activities, gastro-protective, anti diabetic, anti hypertensive, immune modulatory, and anti-infective effects in addition to functioning as a hepatoprotective agent [6-8].

It has significant inhibitory effects on drug metabolizing enzymes. Flavonoids undergoes extensive metabolism prior to the entry into systemic circulation. Absorbed flavonoids will bind to albumin and will transport to the liver through the portal vein. Nanoparticles prepared using biodegradable copolymers are emerging as promising drug delivery system. Poly (D, L-lactide-co-glycolide) (PLGA), a biodegradable polymer is used most often in the literature for drug delivery and tissue engineering due to its good biocompatibility and biodegradability [9, 10].

The PLGA-based NPs could be applicable for delivery of hydrophilic or hydrophobic drugs. Tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble derivative of natural vitamin E with amphiphilic structure. It has been widely used as emulsifier, solubilizer, bioavailability enhancer of hydrophobic drugs, and colloid drug delivery vehicle [9].

TPGS-conjugated PLGA (PLGA-TPGS) nanoparticles have achieved high emulsification efficiency and high cellular uptake rate and its stability and safe to human bodies make it widely acceptable. Modification of PLGA NPs with TPGS can also enhance its biocompatibility [10, 11].

Our group has used TPGS as a novel kind of emulsifier in preparation of polymeric nanoparticles for drug formulation. The human health and safety aspects of TPGS along with its known antioxidant function and commercial availability make it potentially attractive molecular biomedical material for a nano carrier system [12]. The spontaneous emulsification solvent diffusion (SESD) has been mostly used for the preparation of biodegradable nanoparticles [13]. In this method, nano-sized particles of PLGA can be produced by pouring the polymeric organic solution into an aqueous phase with mechanical stirring. In this method a binary mixture of a water miscible organic solvent such as acetone and a water immiscible solvent such as dichloromethane as the solvent of the polymer is used for the preparation of nanoparticles.

The nanoparticles are then formed via an emulsification process followed by the eventual solvent evaporation process. In such process, a number of formulation parameters will affect the nature of the nanoparticles. The most substantial parameters are the quantity and type of the emulsifier.

The emulsifier stabilizes the dispersed-phase droplets formed during emulsification, inhibits coalescence of droplets and determines the particle size, size distribution, the morphological and the release properties of the nano particulate drug delivery system [14]. PLGA nanoparticles are usually prepared by using chemical emulsifiers such as poly vinyl alcohol (PVA). PVA have some disadvantages like low emulsification efficiency and difficulties to wash away in the formulation process.

But TPGS has been reported to have high emulsification efficiency several times higher than PVA [15]. It can also greatly improve the drug encapsulation efficiency, and enhance cellular uptake of nanoparticles and thus increase the cancer cell mortality [16]. The chemical structure of TPGS, similar to other amphiphile molecules contains lipophilic alkyl tail and hydrophilic polar head portion have an excellent emulsifying property.

While choosing an emulsifier for the preparation of particulate systems, several factors should be considered. It is crucial to use excipients that are either approved for human use or endogenous to the human body for the development of such particulate systems.

The effect of the emulsifier on different properties of nanoparticles like morphology, drug encapsulation, surface composition, drug release kinetics should be also considered during the selection [17, 18].The present work investigated the use of TPGS

as emulsifier and further on the possibility of applying TPGS as a matrix material blended with PLGA for the preparation of nanoparticles containing Silymarin and Quercetin as model drugs with different water solubility. The size of the colloidal carriers is a key factor for the biological fate of the nanoparticles. Nanoparticles smaller than 100nm can bypass the mononuclear phagocyte system macrophages uptake. Therefore the effect of TPGS on the size of nanoparticles was also investigated.

Materials and Methods

Materials

The Silymarin and TPGS were purchased from Sigma Aldrich, India. Poly Lactic-co-Glycolic Acid (PLGA) (75:25) was obtained as gift sample from Hindustan latex limited, Akkulam, Trivandrum. Quercetin was purchased from Sisco research laboratories, Mumbai. DCM and Ethanol (Analytical grade) were purchased from SD Fine, Nashik, Poly Vinyl Alcohol, Akshar Enterprises, Mumbai. All chemicals used in the study were of analytical grade and used without further purification. Deionized water was used throughout the experiment. The *in vitro* release measurement was carried out at pH 7.4 at 37^0C in phosphate buffer medium. All other chemicals used were of reagent grade.

Methods

Preparation of Silymarin-Quercetin-loaded PLGA-TPGS NPs (Sil- Qur PLGA-TPGS NPs)

Nanoparticles were prepared using spontaneous emulsification solvent diffusion method [19]. Briefly, known amounts of polymer and drug were added into the mixture of DCM/ Ethanol (1:1) and stirred for 15 minutes to ensure that all materials were dissolved. This solution of organic phase was slowly poured into an aqueous solution containing emulsifier using a high speed homogenizer at 14000 rpm for 5 min. stirring continued for the evaporation of the internal phase.

The polymer was then precipitated and the nanoparticles were isolated by using a centrifuge at 10000 RPM for 15 min and washed trice with deionized water. The suspension was then freeze-dried for 48 hr to obtain a fine powder of nanoparticles, which was then kept in a desiccator.

Saturation Solubility Studies

The saturation solubility studies were carried out for both the unprocessed pure drug and different batches of lyophilized nanosuspension.10mg of unprocessed pure drug and nanosuspension equivalent to 10 mg of silymarin were weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water.

The flasks were sealed and placed in rotary shaker for 24 hours at 37^0C and equivalent for 2 days. The samples were collected after the specified time interval and were filtered and analyzed. The samples were analyzed using UV spectrophotometer at 287nm [20].

Differential Scanning Calorimetry

Thermal properties of formulations were analyzed by differential scanning calorimetric analysis using Toledo-DSC II. To characterize the changes in internal structure, DSC analysis was carried out for pure drug, polymer and the lyophilized suspension. 5mg of sample is taken in the aluminium vial and kept in the instrument.

The sample was then heated from 20^0C to 200^0C at a heating rate of 10^0C/ min under a stream of nitrogen at flow rate of 50 ml/min. Enthalpy changes (ΔH) were calculated per peak to study the polymeric changes in the formulations [20].

Characterization of Nanoparticles

Fourier Transform Infra- Red Spectroscopy

FT- IR spectra were recorded on the sample prepared in KBr discs (2mg sample in 200mg KBr disks) using Shimadzu Fourier Transform Infra-Red spectrometer. The samples were scanned over a frequency range 4000-400 $cm^{-1.}$

Morphology

Scanning electron microscopy (SEM, LEO 435 VP, Eindhoven, Netherlands) was performed to study the surface morphology of Sil-Que PLGA-TPGS NPs. The formulations were gently sprinkled on a double adhesive tape stuck on an aluminium stub. Further, the stubs were coated with gold using a polaron sputter coater and the samples were examined at an acceleration voltage of 30 kV. The photomicrographs were taken at suitable magnification [21].

Particle Size and Polydispersity Index

Particle size and polydispersity index (PDI) of the Sil-Que PLGA-TPGS NPs were determined by Malvern particle size analyzer at 25^{0}C by diluting the sample with appropriate volume of deionized water.

Zeta potential

Malvern Zeta sizer was used to determine the zeta potential of the Sil-Que PLGA-TPGS NPs.

The instrument is a laser-based multiple angle particle electrophoresis analyzer. Sil-Que PLGA-TPGS NPs are dispersed in phosphate buffer saline (PBS, pH 7.4) and the zeta potential was determined.

Entrapment Efficiency

Two milliliters suspension of Sil-Que PLGA-TPGS NPsare taken in eppendorf tube and ultra-centrifuged at 30,000 rpm for 30 min. The supernatant was removed; settled pellets were dissolved in dimethyl sulfoxide, diluted with PBS (pH 7.4) and analyzed by high-performance liquid chromatography (HPLC) for direct estimation of drug content. The encapsulation efficacy was obtained as the mass ratio between the amount of each drug incorporated in nanoparticles and that used in the nanoparticles preparation.

In-vitro Drug Release Kinetics

The release of Silymarin and Quercetin entrapped in Sil-Que PLGA-TPGS NPs was

determined by dialysis tube diffusion technique. The prepared formulations (5 mL) were separately filled into the dialysis tube (MWCO 10 KDa; Hi Media, India), hermetically tied at both the ends and suspended in recipient media of 40 mL of PBS (pH 7.4) in different beakers under sink conditions, while maintaining study temperature at 37 ± 1^{0}C throughout.

At definite time intervals, samples were withdrawn and replaced with same volume of PBS. The samples were then analyzed by HPLC for drug content. Drug release data was normalized by converting drug concentration in solution to a percentage of the cumulative drug release.

Stability Study

Short-term stability studies (1 month) were conducted for lyophilized Sil-Que PLGA-TPGS NPs. The samples were stored in airtight vials in refrigerator (4^{0}C ± 2^{0}C) and at room temperature (25^{0}C ± 2^{0}C/60% RH ± 5% RH) (as per ICH guidelines).After 28 days, nanoparticles were analysed for two parameters namely entrapment efficiency and *in-vitro* release studies.

Results and Discussion

Saturation Solubility Studies

The solubility profile of nanoformulation increases dissolution velocity, saturation solubility and size reduction leading to increase in dissolution rate. The solubility of pure drug in different solvents is represented in Table 1&2.

Table 1: Solubility studies of Quercetin

Solvents	Solubility (mg/mL)
0.1N HCl	0.098
6.8pH buffer	0.312
7.4pH buffer	0.276
Methanol	0.976
Ethanol	1.025

Table 2: Solubility studies of Silymarin

Solvents	Solubility (mg/mL)
0.1N HCl	0.124
6.8pH buffer	0.569
7.4pH buffer	0.798
Methanol	2.024
Ethanol	1.916

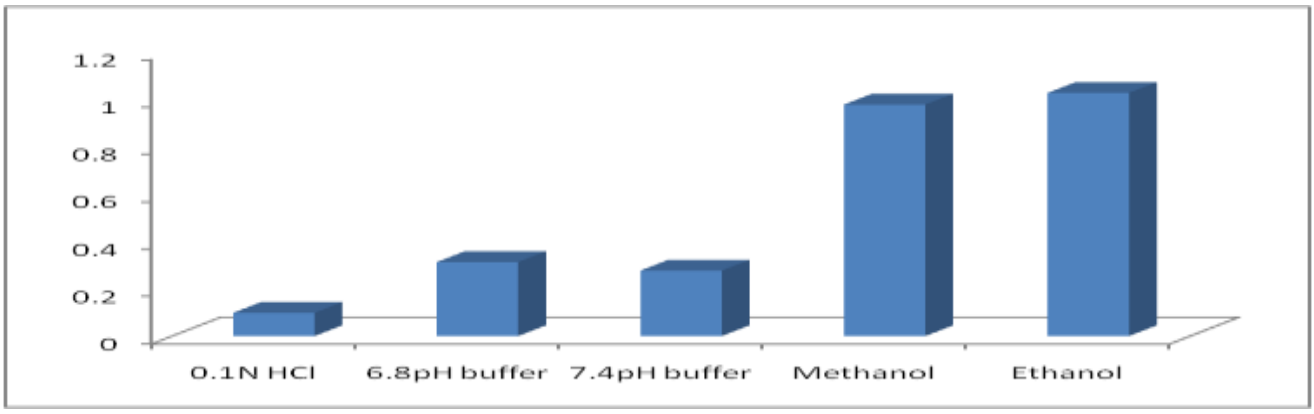

Fig. 1: Solubility profile –Quercetin

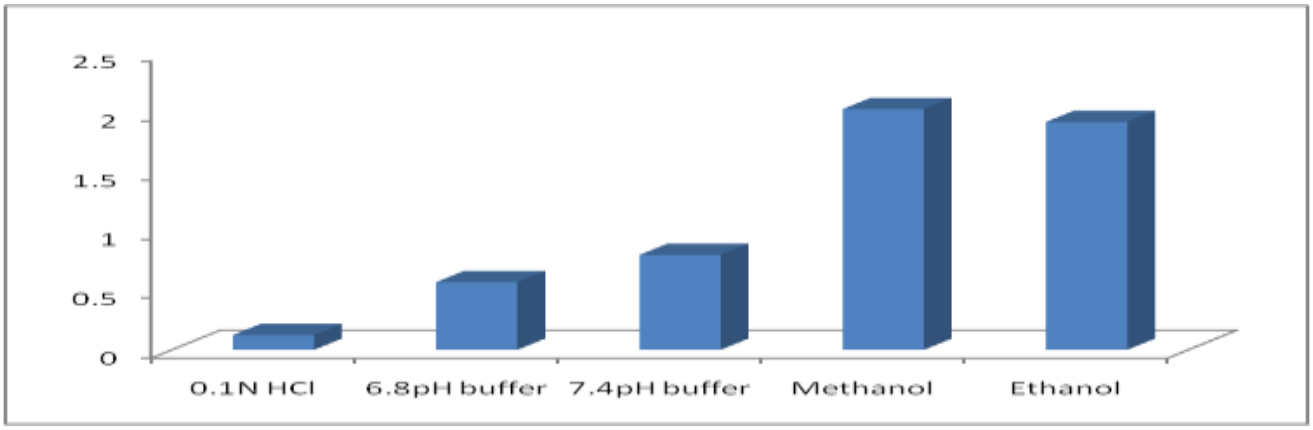

Fig.2: Solubility profile – Silymarin

Differential Scanning Colorimetry

Differential scanning colorimetry was used to elucidate the physical state of the drug within the system. In DSC thermo-gram of pure silymarin (Figure.3).

There was no important difference between the components during heating. From thermo-gram it was concluded that the drug and the surfactant do not interact with each other. The thermograms were represented in Fig.3 and 4.

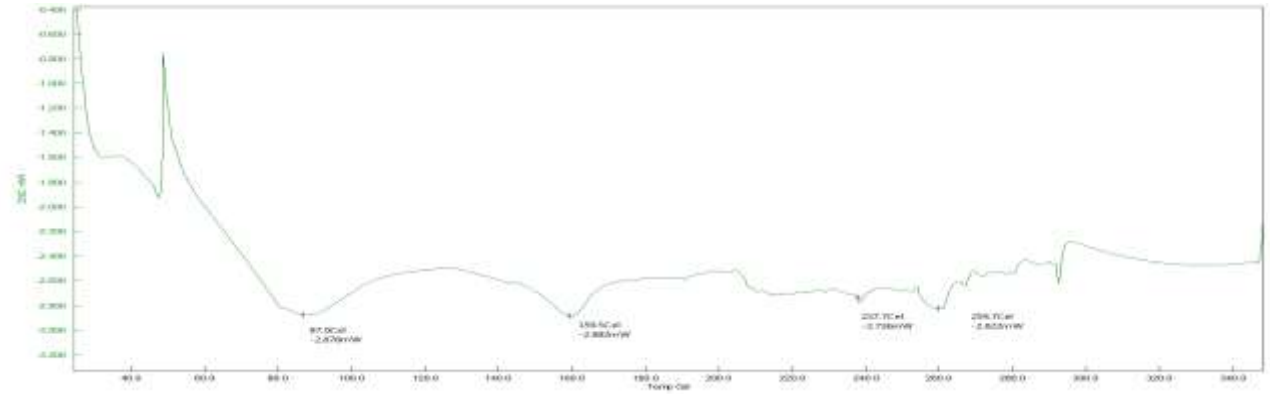

Fig. 3: DSC of Silymarin

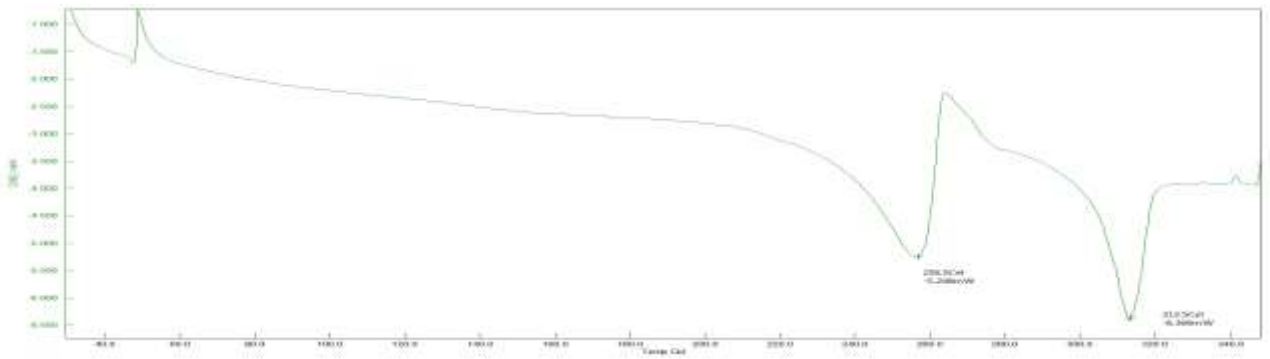

Fig. 4: DSC of Quercetin

Fourier Transform Infra Red Spectroscopy

The FT-IR analysis was used to evaluate the possible intermolecular interaction between Silymarin, Quercetin and the excipients. The IR spectra of final formulation showed all the characteristics peaks without any makeable change in their position after successfully preparing lyophilized Nano formulation, indicating that there is no chemical interaction between silymarin, Quercetin, TPGS and PLGA. The spectra are represented in Fig. 5, 6, 7.

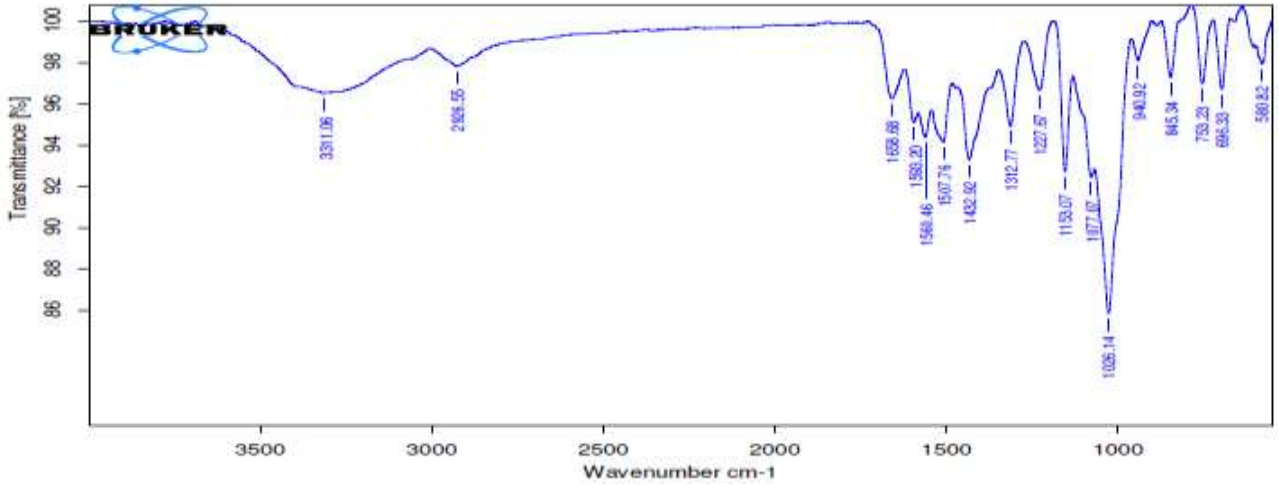

Fig. 5: FTIR-Silymarin

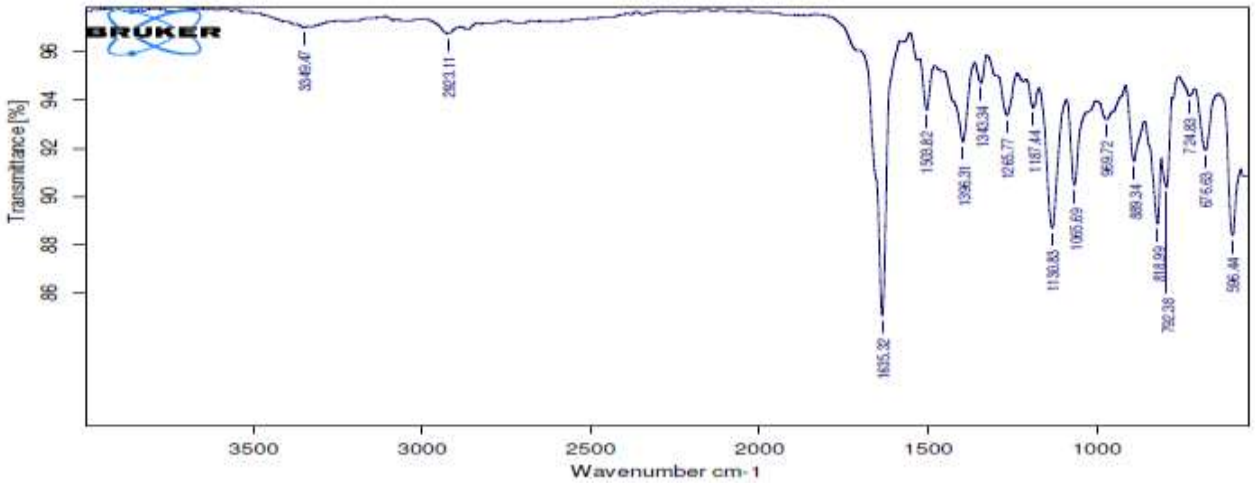

Fig. 6: FTIR- Quercetin

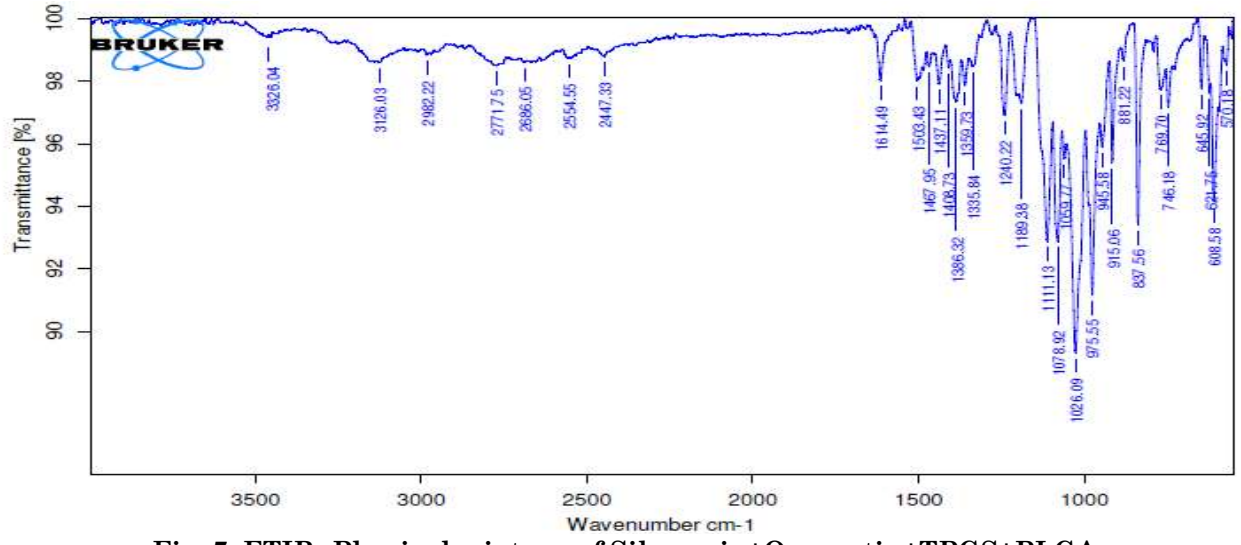

Fig. 7: FTIR- Physical mixture of Silymarin+Quercetin+TPGS+PLGA

Characterization of nanoparticles

Entrapment efficiency

Based on the yield and entrapement efficiency data, F13 formulation was found to be the better one compared to other formulations. Percentage entrapment efficiency of optimized Sil-Que PLGA-TPGS

NPs was found to be 90.24 (Silymarin) and 92.56 % (Quercetin).

Table 3: Entrapment efficiency & Yield

Formulation code	Yield	Entrapment efficiency (%)	
		Silymarin	Quercetin
F1	72.63	91.62	90.18
F2	80.25	90.05	91.02
F3	79.24	92.16	89.22
F4	70.23	92.32	89.04
F5	65.95	90.95	90.29
F6	76.34	91.26	90.1
F7	83.12	93.65	90.55
F8	86.95	93.18	91.28
F9	82.32	89.32	88.42
F10	80.24	83.26	82.34
F11	83.61	88.95	89.04
F12	86.32	93.12	91.02
F13	90.24	90.24	92.96
F14	88.02	85.25	87.24
F15	89.24	89.35	86.46

Morphology

SEM images (Fig.8 & 9) confirmed the spherical nature of Sil-Que PLGA-TPGS NPs. The images also proved that the size of nanoparticles.

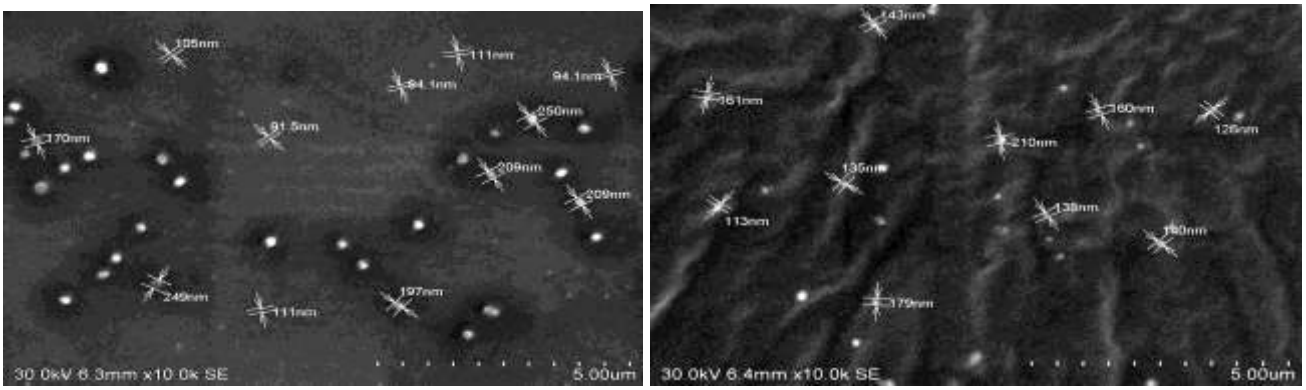

Fig. 8 & 9: SEM Images of Optimised formulation (F13)

Particle size and polydispersity index

The size of Sil-Que PLGA-TPGS NPs was found to be 128.1± 1 nm with PDI 0.102 (Fig.10).

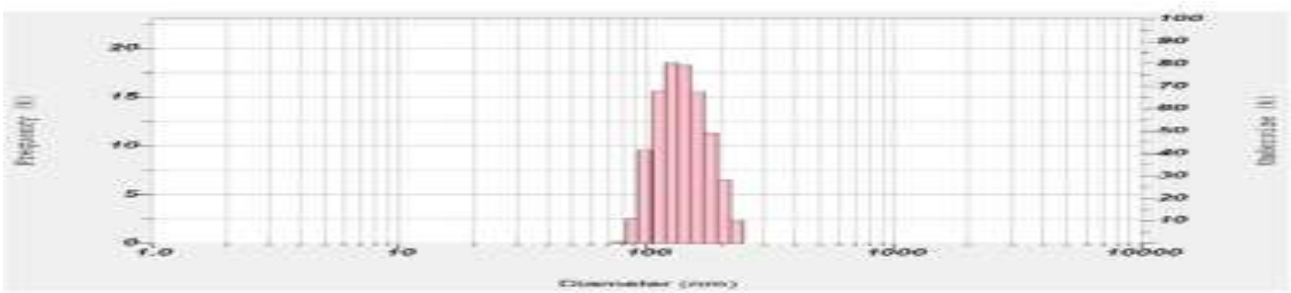

Fig.10: Particle size distribution data

Zeta potential

The zeta potential of Sil-Que PLGA-TPGS NPs was -53.8 mV (Fig.11). Zeta potential of Sil-Que PLGA-TPGS NPs was negative due to terminal carboxylic functionalities of the PLGA used in the formulation.

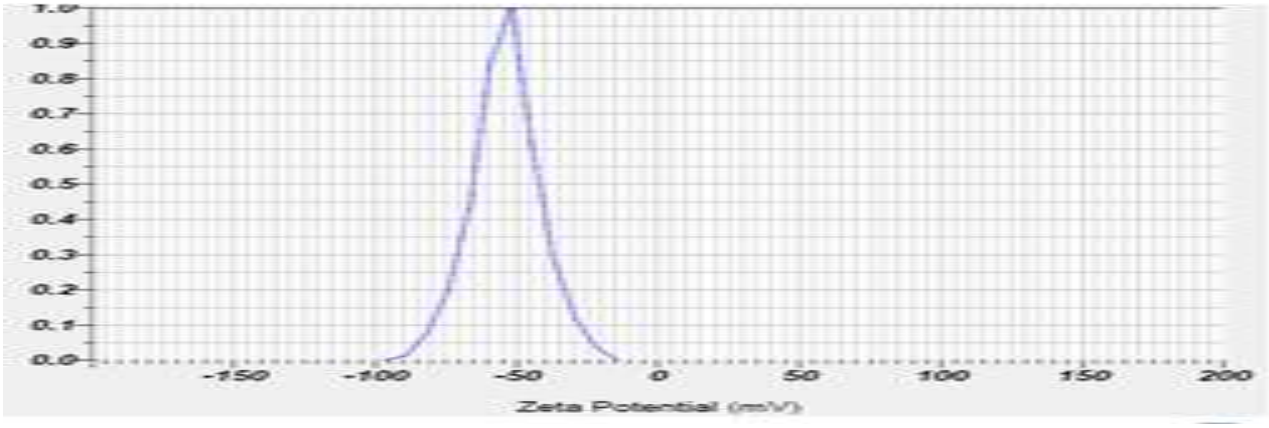

Fig.11: Zeta potential measurement

***In vitro* Drug Release Studies**

In *vitro* studies, Sil-Que PLGA-TPGS NPs exhibited an initial 50% release at 5th hr. This may be due to burst release of drug absorbed at the surface or present in the outermost layer just beneath the surface of nanoparticles. Subsequently the drug release followed a characteristic sustained pattern until the end of 12 hr. The cumulative drug released over 12 hr from optimised formulation; Sil-Que PLGA-TPGS NPs was 93.16 % (Silymarin) and 92.01% (Quercetin) (Figure 12 & 13).

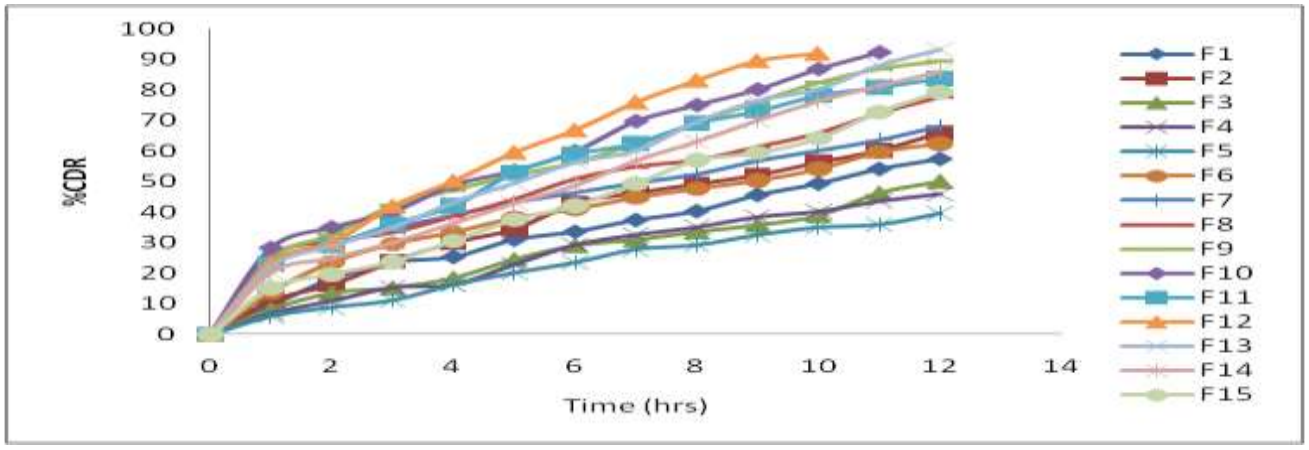

Fig.12: In-vitro drug release –Silymarin

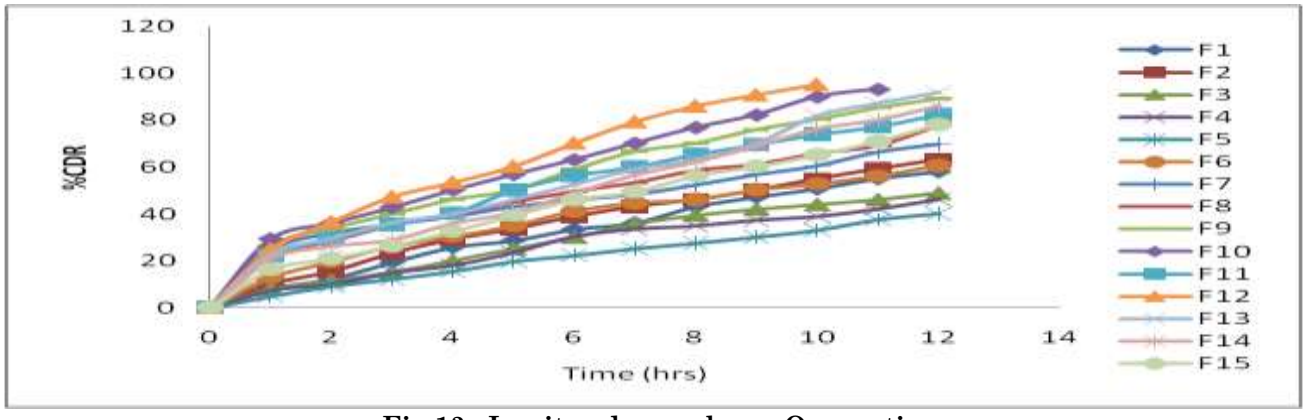

Fig.13: In-vitro drug release-Quercetin

Stability Study Effect of Temperature and Humidity

Short-term stability studies (1 month) were conducted for lyophilized Sil-Que PLGA-TPGS NPs. The samples were stored in airtight vials in refrigerator (4^{0}C ± 2^{0}C) and at room temperature (25^{0}C ± 2^{0}C/60% RH ± 5% RH) (as per ICH guidelines). Two parameters namely entrapment efficiency

and *in-vitro* release studies were carried out. It is found that nanoparticles stored at room temperature are not stable whereas the ones stored at 4^0C is stable .The results are given in Table 4. NPs at room temperature showed decrease in the entrapment efficiency and different release pattern. After 28 days of storing the NPs, stability testing showed the entrapment efficiency and drug release to be within standard limits.

Table 4: Effect of temperature and humidity on optimised formulation (F13)

Temperature	Parameters	0 days	7 days	14 days	28 days
4 ±2^0 C	Entrapment efficiency (Silymarin)	90.24	89.88	88.64	87.56
	Entrapment efficiency (Quercetin)	92.96	92.12	91.54	90.24
	%drug release(12^{th}hour) (Silymarin)	93.16	92.15	91.90	90.55
	%drug release(12^{th}hour) (Quercetin)	92.04	91.44	89.45	88.40
At room temperature	Entrapment efficiency (Silymarin)	90.24	88.85	86.64	84.56
	Entrapment efficiency (Quercetin)	92.96	90.12	88.54	86.24
	%Drug release(12^{th} hour) (Silymarin)	93.16	91.05	90.90	88.55
	%Drug release(12^{th} hour) (Quercetin)	92.04	90.44	89.45	87.40

Conclusion

In this paper, we developed a new combination therapy system for liver that was prepared by Silymarin- Quercetin loaded PLGA-TPGS NPs. Silymarin promotes liver cell regeneration and repairs necrotic cells. Quercetin, a known antioxidant, will enhance the curative effect of Silymarin. Since both the drugs are poorly soluble in nature, thus shows poor bioavailability.

Hence we developed a nanoparticulate drug delivery system. Sil-Que NPs were prepared by modified spontaneous solvent evaporation method using TPGS as emulsifier and PLGA as polymer. Sil-Que PLGA-TPGS NPs shows approximately 128.1 nm in diameter and achieved high Drug loading and Entrapement Efficiency.

The *in vitro* experiments demonstrated that the combination of Silymarin and Quercetin achieved more effective drug release pattern. Further study is planned to assess the hepatoprotective action of Silymarin and Quercetin nanoparticles with superior cell regeneration effects when compared with single drug treatment.

For the first time, in this study, the advantages of Silymarin and Quercetin combination therapy system as nanoparticle for improved hepato targeted drug delivery is reported..

Acknowledgment

The authors are thankful to the R&D, CSIR – National institute for interdisciplinary Science and Technology, Trivandrum for the performing the analytical work and R&D, Hindustan latex limited (HLL), Akkulam, Trivandrum for providing the polymer for the study. The authors sincerely thank the Principal and Management, The Dale view College of Pharmacy and Research Centre, Trivandrum, for providing an opportunity to carry out this research work.

References

1. Shamama Javed, Kanchan Kohli, Mushir Ali. Reassessing bioavailability of silymarin, alternative medicine review. 2011; 16(3):239-46.
2. Dixit N, Baboota S, Kohli K, et al. Silymarin: A review of pharmacological aspects and bioavailability enhancement

approaches. Indian J Pharmacolgy. 2008; 39: 172-179.

3. S Jeganath, S Palanichamy, S H Seyed Mohamed Buhary, P Solai Raj, C Prabhu, Thanga Thrupathi. Formulation and evaluation of silymarin controlled release tablets. Research J Pharm and Tech. 2010; 3(3):927-30.
4. Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the netherlands. Nutr Cancer. 1993; 20: 2129.
5. Mokhtar, Sahar, Marwa, Laila. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat; Food and chemical toxicology: An international journal published for the British Industrial Biological Research Association. 2010; 48(11):3246-61.
6. Alexander Victor Anand David 1, Radhakrishnan Arulmoli 1, Subramani Parasuraman 2. Overviews of biological importance of quercetin: A bioactive flavonoid. 2016; 10(20):84-89.
7. Yukio, Akifumi, Shigeru, Tadashi, Seiichiro, et.al Radical-scavenging and anti-inflammatory activity of quercetin and related compounds and their combinations against RAW264.7 Cells stimulated with porphyromonas gingivalis fimbriae. Relationships between Anti-inflammatory activity and quantum chemical parameters, food and chemical toxicology: An International Journal Published for the British Industrial Biological Research Association. 2013; 48(11):3246-61.
8. Hernández-Ortega LD, Alcántar Díaz BE, Ruiz Corro LA, Sandoval Rodriguez A, BuenoTopete M, Armendariz Borunda J, Salazar Montes AM. Quercetin improves hepatic fibrosis reducing hepatic stellate cells and regulating profibrogenic/anti-fibrogenic molecules balance. J Gastroenterol Hepatol. 2012; 27:1865-72.
9. Gao J, Kou G, Chen H, et al. Treatment of hepatocellular carcinoma in mice with PE38KDEL type I mutant-loaded poly(lactic-co-glycolic acid) nanoparticles conjugated with humanized SM5-1 F(ab') fragments. Mol Cancer Ther. 2008; 7(10):3399-07.
10. Chen H, Gao J, Lu Y, et al. Preparation and characterization of PE38KDEL-loaded anti-HER2 nanoparticles for targeted cancer therapy. J Control Release. 2008; 128(3):209-16.
11. Guo Y, Luo J, Tan S, Otieno BO, Zhang Z. The applications of Vitamin E tpgs in drug delivery. Eur J Pharm Sci. 2013; 49(2):175-86.
12. Ghosh S, Kar N, Bera T. Oleanolic acid loaded poly-lactic co-glycolic acid-vitamin E TPGS nanoparticles for the treatment of leishmania donovani infected visceral leishmaniasis. Int J Biol Macromol. 2016; 93(Pt A):961-70.
13. Wang YY, Zhang DD, Kong YY, et al. CS/PAA@TPGS/PLGA nanoparticles with intracellular pH-sensitive sequential release for delivering drug to the nucleus of MDR cells. Colloids Surf B Bio interfaces. 2016; 145:716-27.
14. Zhu X, Zeng X, Zhang X, et al. The effects of quercetin-loaded PLGATPGS nanoparticles on ultraviolet B-induced skin damages in vivo. Nanomedicine. 2016; 12(3):623-32.
15. T. Niwa, H. Takeuchi, T. Hino, N. Kunou, Y. Kawashima. L-lactide/glycolide copolymer (PLGA) nanospheres with nafarelin acetate prepared by a novel spontaneous emulsification solvent diffusion method. J. Pharm. Sci., 1994; 83: 727.
16. Hashida M, Hirabayashi H, Nishikawa M, Takakura Y. Targeted delivery of drugs and proteins to the liver via receptor mediated endocytosis. J Control Release. 1997; 46:129-137.
17. Mu.L S.S. Feng. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. J. Control. Rel. 2003; 86: 33.
18. PRMu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin ETPGS. J Control Release 2003; 86:33-48.
19. Farnaz Esmaeili, Fatemeh Atyabi. Rassoul Dinarv and Preparation of PLGA nanoparticles using TPGS in the spontaneous emulsification solvent

diffusion method, Journal of Experimental Nanoscience, 2007; 2(3): 183-92.

20. Sumathi R, Tamizharasi S, Gopinath K, Sivakumar T. Formulation, characterization and in-vitro release study of silymarin nanosuspension. Indo American Journal of pharmaceutical sciences. 2017; 4 (01):85-94.
21. Saminathan C, P Venkatesan, S Madhusudhan. Selection of better method for the preparation of nanoparticles by applying analytic hierarchy process. Research J Pharm Tech. 2019; 12(11):5320-24.

