FORMULATION DEVELOPMENT AND CHARACTERIZATION OF QUERCETIN LOADED POLY CAPROLACTONE NANOPARTICLES FOR TUMORS

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Abstract:

Cancer is one of the dreadful diseases in the world, with poor prognosis. Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrapment in submicronic colloidal systems (nanoparticles). The rationale behind this approach is to increase antitumor efficacy, while reducing systemic side-effects. These nanoparticles are well known to show theranostic activity which further increases the therapeutic index. In this study we are developing antioxidant quercetin for anti-tumor effect. The quercetin nanoparticles are prepared using nanoprecipitation method with poly caprolactone

as polymer in which the nanoparticles are recovered. These nanoparticles are further optimized for the drug loading, characterized the morphology using SEM and drug-polymer interactions by FT-IR, DSC. This lipophilic drug has shown a maximum entrapment efficiency of 81% and the SEM images showed nanoparticles of different shapes. There showed no interactions indicating the stability of quercetin loaded poly caprolactone nanoparticles.

Key words: cancer, nanoparticles, theranostic, tumor cells, quercetin dihydrate.

Introduction: Cancer is a class of disease characterized by out-of-control cell growth. When this happens, cells begin to grow neglecting all growth control mechanisms resulting in cancer. It is a complex genetic disease caused primarily by environmental factors otherwise called ascarcinogens present in air, food, water, sunlight etc., Cancer is responsible for one in eight deaths worldwide[1]. It encompasses more than 100 distinct diseases with diverse risk factors and epidemiology which originate from most of the cell types and organs of the human body and which are characterized by relatively unrestrained proliferation of cells that can invade beyond normal tissue boundaries and metastasize to distant organs. It is not only confined to humans and animals; other living organisms can also get cancer. Cancer cells can break away from this original mass of cells, travel through the blood and lymph systems, and lodge in other organs where they can again repeat the uncontrolled growth cycle. This process of cancer cells leaving an area and growing in another body area is termed metastatic spread or metastatic disease.Nanoparticles applied as drug delivery systems are sub-micron sized particles (3-200 nm), devices, or systems that can be made using a variety of materials including polymers (polymericnanoparticles,micelles, or dendrimers), lipids (liposomes), viruses (viral nanoparticles), and organometallic compound or carbon nanotubes. Quercetin (Q) is a flavonoid and, to be more specific, a flavonol. It is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buck wheat and onions. quercetin forms the glycosides quercitrin and rutin together with rhamnose and rutinose respectively[2]. Quercetin classified as IARC group 3 (no evidence of carcinogenicity in humans).Quercetin is a powerful antioxidant. It is also believed to be a natural anti- cancer, and anti-inflammatory agent. Quercetin has been promoted as being effective against a wide variety of diseases.The aim of the present study is to design a novel formulation of nanoparticles for the treatment of cancer. This study involvesto design nanoparticles using PCL as polymer for

sustained release action, to optimize the formulation of nanoparticles,Characterization of Q loaded nanoparticles.The main objective of this study is to formulate Q loaded poly caprolactone nanoparticles for anti-cancer activity to have enhanced therapeutic activity[3].

Material & Methods: Quercetin dihydrate & poly caprolactone sample sigma Aldrich, Polyvinyl alcohol, Gelatin, Acetone, Tween 80, span 80 was found to be Himedia. Dichloromethane, poloxamer-188, ethanol was found to be Hayman.

Methodology:

Preparation of PCL Nanoparticles: In this the nanoparticles are prepared by solvent evaporation method. Initially a weighed quantity of poloxamer-188 is added to double distilled water with magnetic stirring. The solution was maintained at 50-60^0 C and the PCL was dissolved in acetone with mild sonication[4]. Then organic solution was added to the aqueous solution slowly using micro pipette to disperse the organic solution. Immediately on addition of PCL to aqueous solution it forms a bluish tinge which indicates the formation of nanoparticles. This solution is then stirred for about 2 hrs at the same temperature. Then the nanoparticles are recovered by centrifugation at low pressure above 10,000 rpm. The formed sediment is then lyophilized to form nanoparticles.

Preparation of quercetin loaded PCL Nanoparticles: The preparation of the quercetin loaded PCL nanoparticles in Table 1, follows the same method as that of empty nanoparticles along with addition of drug into organic solution with PCL. The formed quercetin nanoparticles are then recovered by centrifugation at low pressure and 10,000 rpm[5]. The different formulations of Q loaded PCL.

Table 1: Formulations of Quercetin- PCL Nanoparticle

Formul a-tion Code	Metho d of prepar a-tion of NPs	Stirrin g rpm/Te mp(°C) / Stirrin g time(hr s)	Stabiliz er	% of Stab ili-zer	Volum e of Stabili-zer in ml	Querce tin in mg	Poly caprol -actone in mg	Centrif -ugation (rpm)
N1	Emulsi on method	1000/ RT/ 2	Tween-80	0.2	25	2.5	50	10000
N2	Nano precipit -ation method		Gelatin					
N3			Poly vinyl alcohol					
N4			Poloxa mer-188					
N5								18000
N6						5		
N7						7.5		

Evaluation Of Quercetin Loaded Pcl Nanoparticles

Particle size: To confirm the formation of nanoparticles the nano suspension formed after the addition of organic phase to aqueous phase and stirring are then collected and analyzed by

Malvern Zeta Sizer. The nanoparticles after lyophilization are also analyzed for particle size analysis[6].

Polydispersity index: Polydispersity index is done for both nanosuspension both before and after lyophilization[7]. Polydispersity (non-uniform size distribution) was calculated by the following formula:

$$Polydispersity = (D_{0.9} - D_{0.1}) / D_{0.5}$$

Where $D_{0.9}$, $D_{0.5}$ and $D_{0.1}$ are the particle diameters determined at the 90th, 50th and 10th percentile of undersized particles, respectively. High polydispersity index value indicates the high level of non-uniformity and is used to characterize the nanoparticles as monodisperse, homogeneous and heterogenous systems[8].

Drug loading and encapsulation efficiency

Drug loading and Encapsulation efficiency:1 mg of nanoparticles was accurately weighed in 2ml eppendroff tubes and then vortexed with 200 µl of dichloro methane for 5 min. Then add 1800 µl of ethanol for precipitating PCL(as it is insoluble) and vortexed again for 10 min. Then the absorbances of the samples are measured using UV-Vis spectrophotometer at 370 nm. The absorbances obtained are then used for calculating the amount of Quercetin present in the nanoparticles[9]. These values are then used for calculating the % drug loading(DL) and entrapment efficiency(EE) using the formulae.

$$\%\ Drug\ loading = \frac{Weight\ of\ Quercetin\ in\ Nanoparticles}{Weight\ of\ Nanoparticles} * 100$$

$$\%\ Entrapment\ efficiency = \frac{\%\ Drug\ loading}{\%\ Theoritical\ loading} * 100$$

***In-vitro* release studies:**In this an accurately weighed quantity of nanoparticles are taken into a pretreated dialysis membrane. A small quantity of buffer is added into the dialysis bag along with nanoparticles and suspended in PBS at 37±1°C. Then aliquots of samples are collected at regular intervals and the same volume has to be replaced by the PBS to maintain sink conditions. Then the absorbances of the collected aliquots are measured at 370 nm using UV- Vis spectrophotometer[10].

Scanning electron microscopy: Scanning electron microscopy is an excellent tool for physical observation of morphological features of nanoparticles. It is helpful to examine microspheres shape and surface characteristics in order to correlate other determined characteristics such as surface area and bulk density. The nanoparticles were sprinkled on to one side of adhesive stub. The stub was then coated with conductive gold and was examined under scanning electron microscope for qualitative assessment of morphology of nanoparticles[11].

Drug - Excipient interaction & Polymorphism studies

Fourier transform infrared spectral (FT-IR) analysis:FT-IR spectra determine the positions and relative sizes of all the absorptions, or peaks, in the IR region. This is used to access the possible chemical interactions between the drug and other excipients in the formulation. Samples were analyzed by pressed pellet technique using KBr. The spectra of drug, polymer, empty nanoparticles and drug loaded nanoparticles are recorded[12].

Results & Discussion:

Optimization of method of preparation for Q loaded PCL nanoparticles: The formulation N1 is prepared by using emulsion method in which emulsion is formed and stabilized by using surfactants. The preparation procedure includes the organic solution which contains poly caprolactone, span-80 and quercetin. This organic solution is then added into aqueous solution containing Tween-80 as stabilizer. The next formulation N2 is prepared by nanoprecipitation method with different stabilizers. In this method organic solution containing poly caprolactone, quercetin is added to aqueous phase with stabilizers. The formed nanoparticles in both formulations are then centrifuged at 10,000 rpm at 4^0 C and then lyophilized.In this the nanoparticles in N1 formulation have failed to recover because of the surfactants which formed a slurry but in N2 formulation the nanoparticles are recovered. So, nanoprecipitation method is best suited for the formation of quercetin nanoparticles using PCL as polymer. Therefore, further optimization of stabilizers in nanoprecipitation method was done.

Optimization of Stabilizers in Nano precipitation method

Characterization of nanosuspensions: In this different stabilizer's gelatin, poly vinyl alcohol, poloxamer-188 of similar concentration are used in nanoprecipitation method. The formed

nanosuspensions were then stirred for 2 hrs and then are analyzed for particle size and zeta potential.The particle size analysis of the nanoparticle suspension using gelatin, poly vinyl alcohol, poloxamer-188 i.e., n3, n4, n5 respectively. The zeta potentials of the formulations are also measured. From reports the particle sizes and zeta potentials of gelatin, PVA stabilizers are better compared to that of poloxamer-188. For these suspensions the poly dispersity indexes are also measured to determine the uniformity of nanoparticles. Thepoly dispersity index of poloxamer-188 was found to be lesser than the other two indicating higher uniformity of particles Table 2.

Table 2: Evaluation of nanoparticle formulations

Formulation Code	Polydispersity index	% drug loading
n1	**0.64**	**---**
n2	**0.15**	**---**
n3	**0.69**	**---**
N4	**1**	**68%**
N5	**1**	**75.6%**
N6	**1**	**84%**
N7	**1**	**80.3%**

Optimization of centrifugation rpm for recovery of product: The nanosuspensions are then centrifuged at 10,000 rpm and 18,000 rpm for N4 and N5 formulations respectively. The formulations are then evaluated for particle size and zeta potentials.It has shown that 18,000 rpm for centrifugation produced nanoparticles of much uniform size because of the higher rpm that collects the smaller sized nanoparticles. This also further has enhanced the % yield of nanoparticles. This polydispersity index in table 8.1 indicates the homogeneous distribution of nanoparticles in the suspensions when measured for particle size.The formed nanoparticles are then centrifuged and lyophilized at-54^{0}C and 1 pas pressure for 3 days for the removal of aqueous solution. The dried nanoparticles are recovered and observed. The nanoparticles formed

by using PVA, gelatin stabilizers have formed film like structures failing to recover the nanoparticles. The nanoparticles of poloxamer-188 are powdery and are recovered properly.

So, the poloxamer-188 stabilizer was selected for formulating the quercetin nanoparticles by nanoprecipitation method. The formed nanoparticles also easily got redispersed in water.

Optimization of drug loading: In N5 formulation initially 2.5 mg of Quercetin was loaded into PCL nanoparticles (1:20). Then the nanoparticles are then evaluated for entrapment efficiency. The fomulations N6, N7 are then loaded with higher % of Quercetin. The formulation N6 was loaded with 5mg (2:50) of Quercetin into the PCL nanoparticles and evaluated. The formulation N7 was also loaded with higher amount of Quercetin (3:50) and then evaluated.The entrapment efficiency of the N6 formulation was found to be 84% and so it is then used for further studies. The N6 formulation was then evaluated for drug excipient interaction and polymorphism characterization.

***In vitro* release studies:**Based on the paticle size and entrapment efficiency formulation F6 was selected for *in vitro* release studies. The quercetin release profiles was measured in phosphated buffer saline(PBS) of pH 7.4 at 37^0c. the drug release profiles were shown in the figure 1. The formulation (N6) initially showed burst release for 24 hrs and then a constant release was observed. Initial burst release of quercetin were shown in figure 2 from nanoparticles may be attributed due to the heterogeneous quercetin distribution: Quercetin that are either loosely associated with the surface or embedded in the surface layer are responsible for the burst release Table 3.

Table 3: *In-vitro* drug release of quercetin from nanoparticles

Time (hrs)	Abs	Conc (µg/ml)	Vol of SF(ml)	Conc. of SF(µg)	Conc. in TV (µg)	Cumulative Concentration(µg)	% Quercetin Release
0	0	0	2	0	0	0	0
0.25	0	0	2	0	0	0	0
0.5	0	0	2	0	0	0	0
1	0.0061	0.123232	2	0.246465	4.929292929	4.929293	1.040264415
1.5	0.0016	0.032323	2	0.064646	1.292929293	1.539394	0.324869461

2	0.0036	0.072727	2	0.145455	2.909090909	3.220202	0.679582573
4	0.0132	0.266667	2	0.533333	10.66666667	11.12323	2.347416339
7	0.0105	0.212121	2	0.424242	8.484848485	9.474747	1.999524633
10	0.0158	0.319192	2	0.638384	12.76767677	14.18182	2.992891882
19	0.0276	0.557576	2	1.115152	22.3030303	24.35556	5.13992942
24	0.0374	0.755556	2	1.511111	30.22222222	33.3899	7.046512396
30	0.0818	1.652525	4	6.610101	66.1010101	70.7798	14.93717378
42	0.1372	2.771717	4	11.08687	110.8686869	122.1576	25.77979862
54	0.1638	3.309091	4	13.23636	132.3636364	154.7394	32.65577587
66	0.1676	3.385859	4	13.54343	135.4343434	171.0465	36.09717519
90	0.2263	4.571717	4	18.28687	182.8686869	232.0242	48.96575761
114	0.2022	4.084848	4	16.33939	163.3939394	230.8364	48.71507094
174	0.1874	3.785859	4	15.14343	151.4343434	235.2162	49.63937145
234	0.1774	3.583838	4	14.33535	143.3535354	242.2788	51.12984866
294	0.1662	3.357576	4	13.4303	134.3030303	247.5636	52.24514854

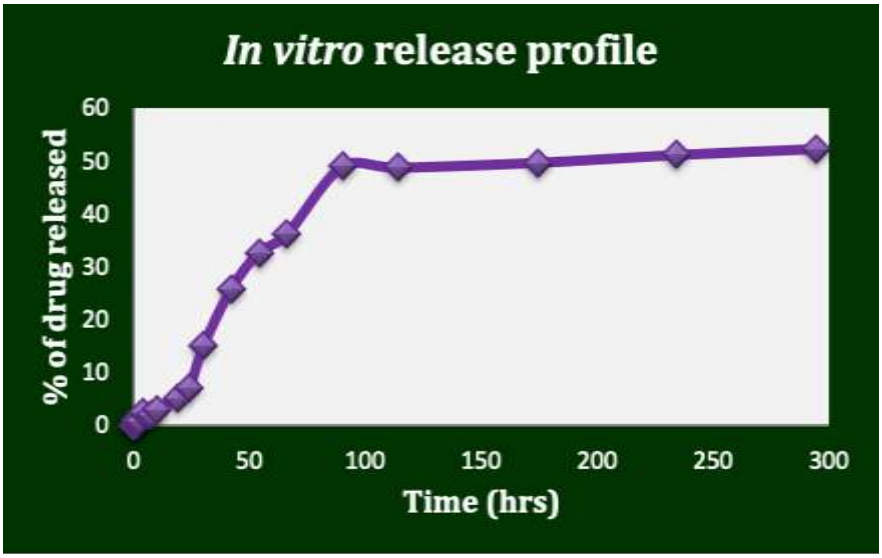

Figure 1: *In vitro* release profile

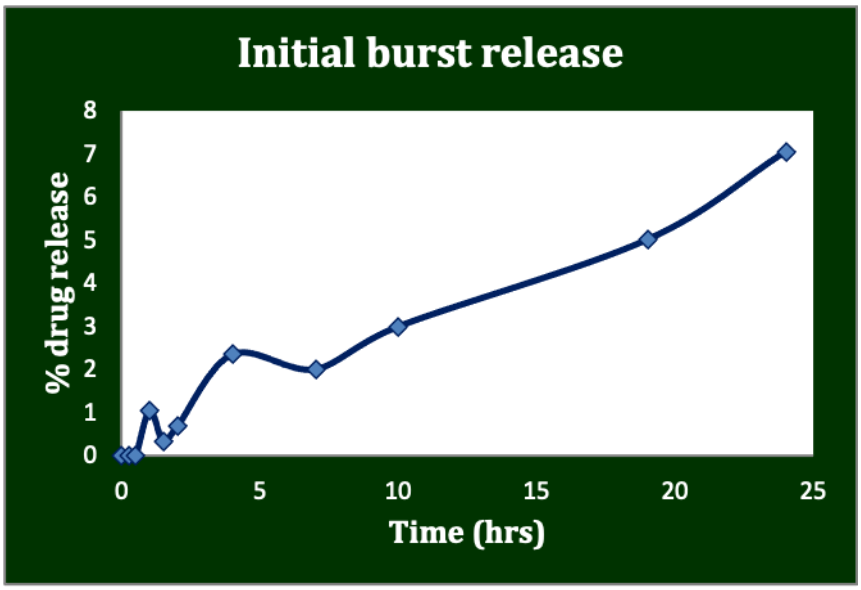

Figure 2: Initial burst release of quercetin

Surface morphology: The surface morphology of the nano particles prepared by using poloxamer- 188 as stabilizer are centrifuged at 18,000 rpm and lyophilized Figure 3. These nanoparticles are then analyzed for surface morphology using Scanning electron microscope, and found that they are spherical, rod shaped with heterogenous morphology Figure 4.

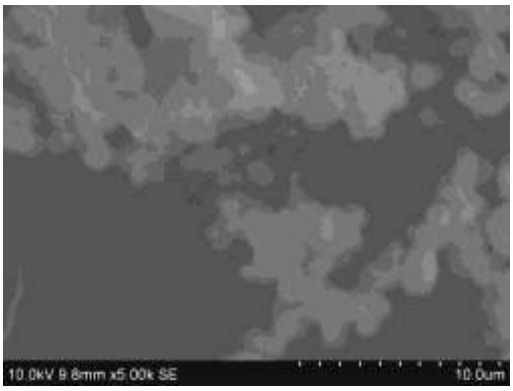

Figure 3: Surface morphology of empty PCL nanoparticles

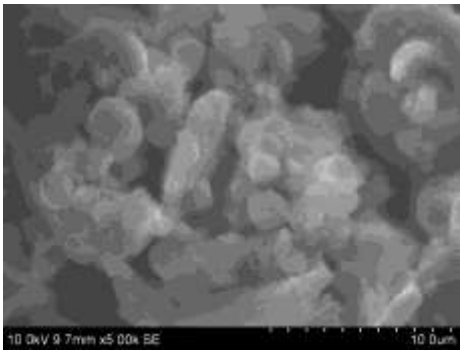

Figure 4: Surface morphology of Q loaded PCL nanoparticle

Drug excipient interaction and polymorphism characterization

FT-IR spectra: The optimized formulation N6 is then characterized by FT-IR spectra obtained could confirm the chemical stability of quercetin in the nanoparticles. FT-IR spectra of quercetin, PCL, empty PCL nanoparticles and quercetin loaded PCL nanoparticles are shown in Figures 5, 6, 7, 8 respectively. Quercetin shows characteristic aromatic bending and stretching around 1100 and 1600 cm^{-1}, -OH phenolic bending around 1200 and 1400 cm^{-1}. FTIR spectrum of PCL shows prominent peaks at 1730 and 3440 cm^{-1}, which corresponds to the –CO (stretching) and –OH (bending) groups. The peaks at 2868 and 2947 cm^{-1} are related to the C–H bond of saturated carbons. The FTIR analysis of empty PCL nanoparticles shows similar characteristic peaks as that of plain PCL. The FTIR spectrum of quercetin loaded PCL nanoparticles shows additional peaks due to quercetin in the blend matrix.Some bands of quercetin are not prominent in drug-loaded nanoparticles since these are identical to those of placebo nanoparticles and appear at almost the same wavenumber. This spectral analysis showed the quercetin stability in the PCL blend. This study also confirms the polymer stability in the processing conditions.

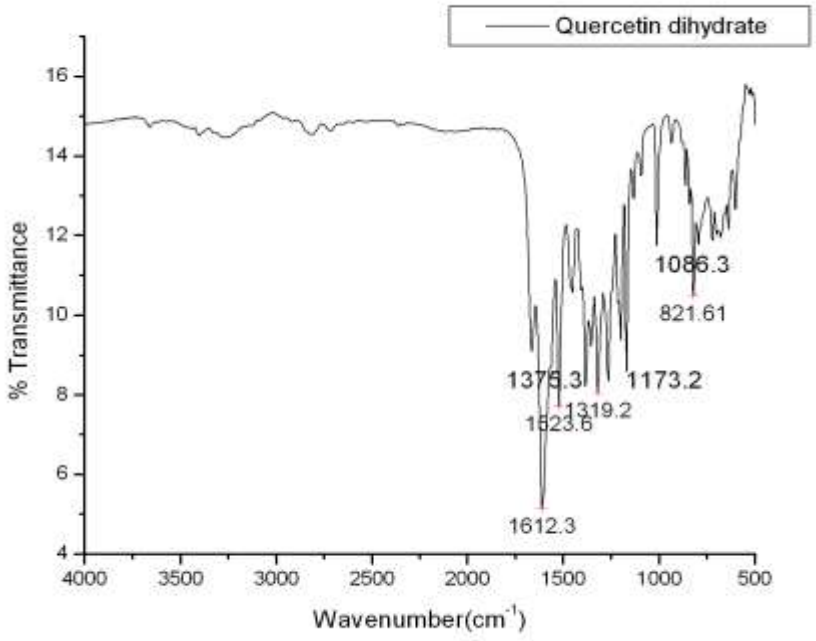

Figure 5: FT-IR spectra of Quercetin dihydrate

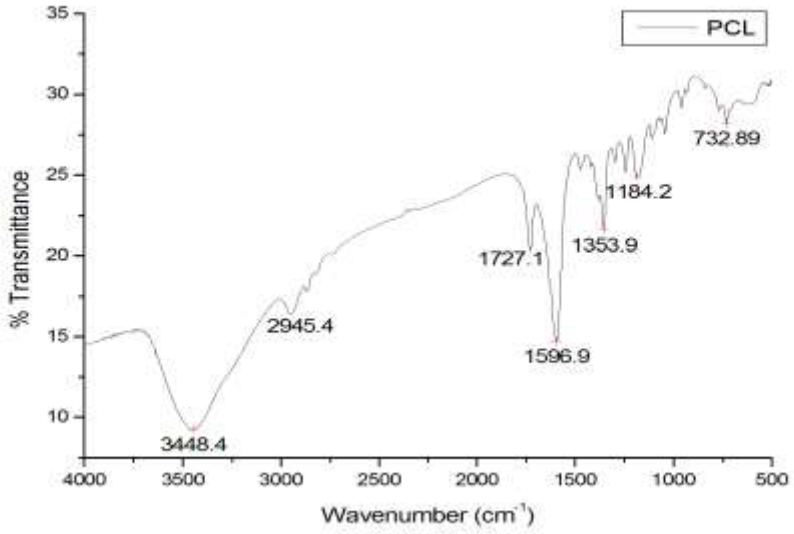

Figure 6: FT-IR spectra of poly caprolactone

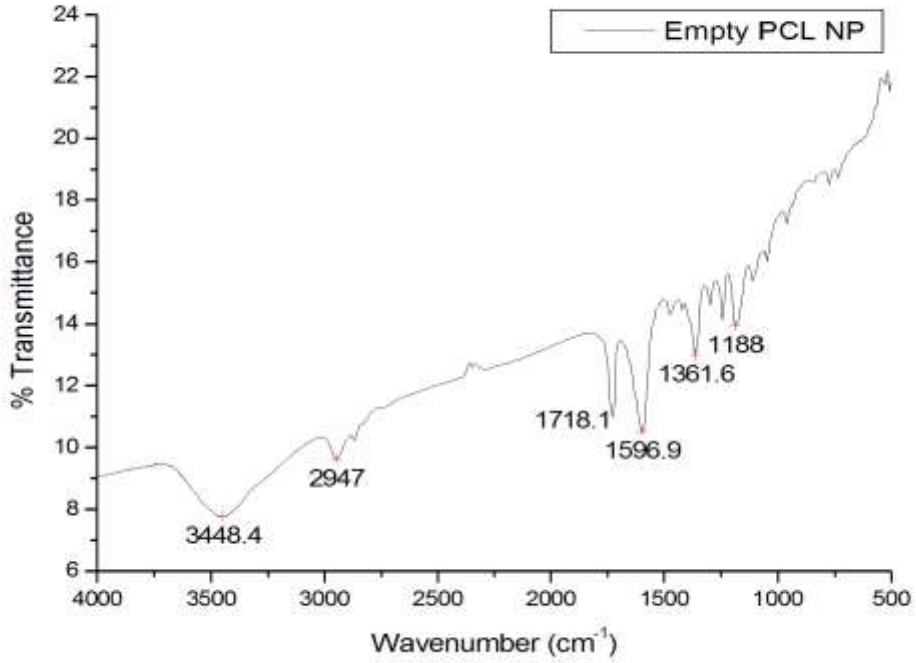

Figure 7: FT-IR spectra of empty polycaprolactone nanoparticles

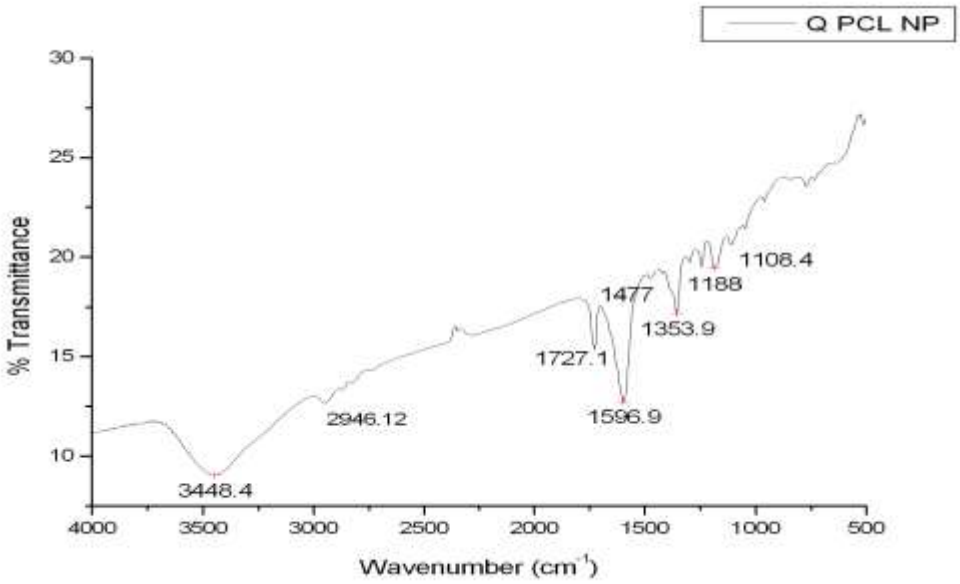

Figure 8: FT-IR spectra of Q loaded polycaprolactone nanoparticles

CONCLUSION:

In this study we have prepared the Quercetin nanoparticles for sustained release to show anti-tumor effect are designed and optimized. The nanoparticles prepared by nanoprecipitation method showed product recovery. The stabilizers gelatin,PVA,poloxamer-188 used during the formulation are then optimized. The nanoparticles formed using poloxamer- 188 as stabilizer are found well lyophilized. These nanoparticles are then evaluated for the drug loading efficiency and higher drug loading formulation (N6) was found. This optimized formulation is then characterized and reported.

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