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Original Article

INFLUENCE OF ORGANIC SOLVENTS ON NANOPARTICLE FORMATION AND SURFACTANTS ON RELEASE BEHAVIOUR IN-VITRO USING COSTUNOLIDE AS MODEL ANTICANCER AGENT

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ABSTRACT

Objective: The objective of this study was to screen different solvents for optimizing nanoparticle preparation in terms of particle size,entrapment efficiency, and surfactants on release behaviour in-vitro.

Methods: Nanoparticles(NP's) of poly (d,l-lactic-co-glycolic acid) (PLGA),containing costunolide were prepared following modified oil-in-water single emulsion solvent evaporation technique using polyvinyl alcohol (PVA) and tween80 as stabilizers. NPs were characterized in terms of surface morphology, particle size and distribution, zeta potential, encapsulation efficiency, and costunolide release profile. Fourier transform infrared (FTIR) spectra and X-ray diffraction (XRD) were employed to determine any interactions between costunolide and polymer.

Results: Ethyl acetate (EA), acetone (ACE), and dichloromethane (DCM) were used as organic solvents individually. EA alone led to the formation of stable nanoparticles, while DCM and ACE when used alone as organic solvents failed to produce stable nanoparticles with the method used. NPs having mean diameter of 112.1±6.4 nm with low polydispersity index of 0.106±0.047 and zeta potential value -31.5±0.5 were obtained. By imaging with scanning electron microscopy (SEM), NPs having discrete, spherical morphology with smooth surface and low porosity was observed. Costunolide loaded NPs exhibited similar in- vitro release profile (Zero order) irrespective of stabilizer (TWEEN80 or PVA) used. However, the average release per day was higher in case of TWEEN80 compared to PVA formulation.

Conclusion: 8 formulations were evaluated and among them F8 was found to have good results. Formulation F8 with surfactant tween80 showed maximum in vitro costunolide release compared to formulation F2 with surfactant PVA. Formulation F1 and F2 shows better stability after 3months at room-temperature and 4^0C.

Keywords: Nanoparticles, PLGA, Costunolide, Modified O/W single emulsion solvent evaporation.

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm and prepared from natural or synthetic polymers. NPs have been extensively investigated in biomedical, biotechnological areas and especially, in drug delivery systems for drug targeting[1,2]. The advantages of targeted drug delivery to the specific site of body are in the therapy of several disease states such as anticancer treatment, gene therapy, viral disease,and bacterial infection in the specific body sites[3,4]. In recent years, biodegradable polymeric NPs have attracted considerable attention as potential drug delivery devices in view of their applications in the control release (CR) of drugs, their ability to target particular organs/tissues. PLGA is one of the most successfully used biodegradable polymers because its hydrolysis leads to metabolite monomers, lactic acid and glycolic acid. Because these two monomers are endogenous and easily metabolized in the body via Krebs cycle, thus, a minimal systemic toxicity, decrease in side effects and increase in the therapeutic benefits are associated with the use of PLGA for drug delivery[5].

Cancer is the leading cause of death and represents one of the most threatening diseases worldwide. Throughout the history of civilization, the human have relied on natural products as a primary source of medicine. Herbal medicines have been proven to be an important source of novel agents with a pharmaceutical potential. Many anticancer drugs in current use are either natural products or are derived from natural products. Herbal medicines, such as paclitaxel, camptothecin, vinca alkaloids, and etoposide hold great potential as promising agents for the treatment of cancer [6]. Natural products have traditionally provided a rich source of drugs for many diseases, including cancer and plants are an important source of novel natural products[7]. In 2008, of the 225 drugs being developed, 164 were of natural origin, with 108 being derived from plants, 25 from bacterial sources, 7 from fungal and 24 from animal sources. And, to throw some more numbers around, of the 108 plant-based drugs, 46 were in preclinical development, 14 in phase I, 41 in phase II, 5 in phase III and two had already reached preregistration stage [8]. David Newman and Gordon Cragg found that of 155 FDA-approved small molecule anti-cancer drugs, 47% were either natural products or directly derived there from.

Saussurea lappa Clarke (Compositae) is indigenous to India and Pakistan, where it grows in the Himalayas at 2500–3500m elevations.Sesquiterpene lactones are the most common constituents of *Saussurea lappa*[9].Costunolide(6E,10E,11aR)6,10-dimethyl-3-methylidene-3a,4,5,8,9,11a-hexahydrocyclodeca[b]furan-2-one) is a sesquiterpene lactone.Costunolide was tested for in vitro cytotoxicity on different cancer cell lines by MTT assay. The cell lines used in this study were colo-205 (colo-cancer), A-431 (skin cancer), MCF-7 (breast cancer), and A-549 (lung cancer).All the cells were obtained from National Center for cellular Sciences (NCCS), Pune, India (http://www.atcc.org/pdf/30-1010k.pdf).Sesquiterpenes exert their antitumor activity by triggering apoptosis in human leukemia cells[10].

MATERIALS AND METHODS

Materials

The polymer studied was poly (d,l-lactide-coglycolide acid) (PLGA), with a copolymer ratio of dl-lactide to glycolide of 50:50 (*M*w 40,000–100,000 g/mol as indicated by the supplier, Sigma Aldrich CO,India). The surfactants used in the emulsification process was poly (vinyl alcohol)

(PVA) (87–89% hydrolysis degree and molecular mass 12,000–13,000 g/mol, Sigma Aldrich CO, India) and polysorbate80 or tween80 (molar mass 1310 g/mol, Sigma Aldrich CO, India). Acetonitrile (ACN; HPLC grade) and ethyl acetate (AR grade) were purchased from Rankem Fine Chemicals (New Delhi, India) while other organic solvents acetone (AR grade), chloroform (AR grade), and dichloromethane (AR grade) were purchased from Qualigens Fine Chemicals (Mumbai, India). As suspending medium, purified water (Milli-Q, Millipore Corporation, India) was used. The encapsulated agent was costunolide (isolated from the plant *Saussurea lappa*).

Table 1: Physical Properties of the Selected Solvents[11]

Solvent	Solubility in H2O at 250C	Interfacial Tension (dyne/cm)	Vapor Pressure (mmHg)	Viscosity (Cp)
Acetone	Miscible	6.8	185.23	0.32
Dichloromethane	1 in 50	28.3	348.75	0.44
Ethyl acetate	1 in 10	1.7	76	0.46-0.47

Interfacial Tension, Vapor Pressure and Viscosity values are at 20°C.

Calibration curve for Costunolide

The standard solution was prepared by dissolving 1mg of costunolide in 1ml of methanol. From this standard stock solution, serial dilutions of 10, 20, 40, 80, 160µg/ml were made and analyzed by using HPLC at 210nm.

Table 2: Calibration curve data of costunolide

Concentration(µg/ml)	Absorbance(nm)
10	0.03966
20	0.0764
40	0.1248
80	0.2489
160	0.47794

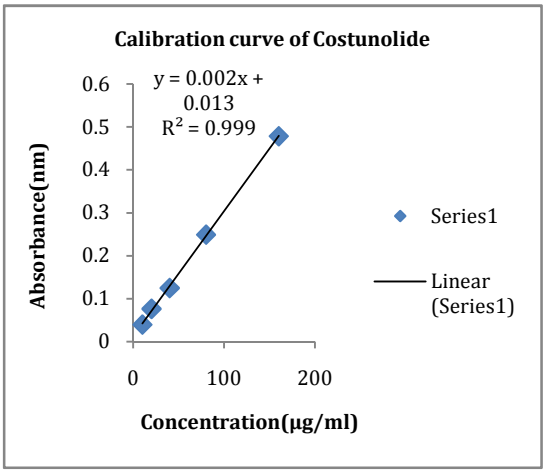

Fig. 1: Calibration curve of costunolide

Preparation of PLGA Nanoparticles containing Costunolide

The costunolide loaded nanoparticles were fabricated by a modified oil in water single emulsion solvent evaporation technique. Typically, a solution of 21mg of PLGA in 3.5ml of ethyl acetate containing costunolide (16.6% w/w of polymer), which was suitably stirred to ensure that all material was dissolved. An aqueous phase containing TWEEN80 as stabilizer (86mg of TWEEN80 in 10ml of Milli-Q-water; 0.86% w/v) which was homogenized for 1min by vortex. The organic phase 2ml was then added to 10ml of an aqueous phase. Then sonicated using a microtip probe sonicator set at 50W of energy output (XL 2002 Sonicator ultrasonic liquid processor) during 4min to produce the oil-in-water emulsion. The formed o/w emulsion was stirred at room temperature (22°C) by a magnetic stirrer at 800rpm for two-hours to evaporate organic solvent. The nanoparticles were recovered by ultracentrifugation (13,500rpm, 15min, Hitachi). The amount of non-entrapped costunolide in the supernatant was determined by HPLC. The nanoparticles were washed twice with water in order to remove the adsorbed costunolide. The washing solutions were eliminated by a further centrifugation as described above. The purified nanoparticles were freeze-dried.

Costunolide loaded (15-25%, w/w of polymer) nanoparticles were prepared by dissolving both; costunolide and polymer, in different organic solvents ethyl acetate (EA), acetone (ACE), and dichloromethane (DCM). The organic phase was then added to an aqueous phase containing PVA as stabilizer, keeping rest of the method same except probe sonication time. Sonication time was varied between 1 and 4min, increase in the sonication time leads to a reduction in the nanoparticle diameter.

Nanoparticles characterization

Particle Size and Zeta Potential

The size of nanoparticles was determined by Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) based on dynamic light scattering technique.

Zeta potential is an indicator of surface charge, which determines particle stability in dispersion.Zeta potential was also measured with Zetasizer using the principle of electrophoretic mobility under an electric field.

The polydispersity index (PDI) which is a dimensionless number indicating the width of the size distribution, having a value between 0 and 1 (0 being for monodispersed particles) was also obtained.

Scanning electron microscopy (SEM)

The shape and surface morphology of the nanoparticles was examined using scanning electron microscopy.An appropriate sample of nanoparticles was mounted on metal (aluminium) stubs, using double-sided adhesive carbon tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120s at 14mA under argon atmosphere for secondary electron emissive SEM and observed for morphology at an acceleration voltage of 15 kV.

Entrapment Efficiency

The percentage of costunolide encapsulated in the nanoparticles was determined by centrifuging the costunolide-loaded nanoparticles and separating the supernatant. The resulting pellet was washed twice with water and then costunolide content in the pellet was analyzed using validated HPLC method (direct method). This method was also used to determine the non-incorporated costunolide in the supernatant after the nanoparticles formation (indirect method). Waters high-performance liquid chromatography (HPLC) system consisting of 2998 Photodiode Array Detector and waters HR C_{18} 300 x 3.9 mm, 6µ column was used as stationary phase. Acetonitrile (ACN)**:** Water (60:40) was used as the mobile phase with a flow rate of 1ml/min. The injection volume was 20µL and retention time of costunolide was 8.2min. The detection wavelength (λ_{max}) for costunolide was 210nm. Standard solutions were made in methanol (HPLC grade).

The drug encapsulation efficiency was defined as the ratio of the mass of the encapsulated costunolide to the mass of the costunolide used for nanoparticles preparation using the following equation:

$$\text{Drug encapsulation efficiency} = \frac{\text{Amount of encapsulation costunolide}}{\text{Amount of costunolide used for nanoparticle preparation}} \times 100$$

Drug loading efficiency of costunolide loaded nanoparticles is calculated by the following equation:

$$\text{Drug loading efficiency} = \frac{\text{Amount of costunolide in nanoparticles}}{\text{Amount of costunolide loaded nanoparticles}} \times 100$$

In vitro costunolide release

In vitro release studies were carried out using vial method as reported by Danhier *et al* (2009) and Verger *et al* (1998). Nanoparticles containing 2.5-3.5mg equivalent costunolide was suspended in vial containing 10ml of pH 7·4 phosphate buffer with 0·3% tween-80 to improve solubility of costunolide. The vial was shaken horizontally using water bath shaker at 37ºC. In vitro costunolide release was assessed by intermittently sampling the vial (2ml) at predetermined time intervals (2, 4, 8, 16, 24, 32 and 48h) and was replaced with 2ml of fresh pH 7·4 phosphate buffer. The withdrawn sample was centrifuged at 5000rpm for 2min, supernatant was filtered through 0·45μm membrane filter and injected to HPLC system by using HPLC equipped with PDA detector at 210nm. The amount of costunolide released in each sample was determined using a calibration curve Fig. 4 and Fig. 5; the reported values are averages of three replicates (n = 3). Results of in vitro costunolide release studies obtained were tabulated in table 5 and table 6 and shown graphically as cumulative % of costunolide release vs time (Fig. 4 and Fig. 5).

Fourier transform infrared (FTIR) spectral studies

FTIR spectra of the polymer PLGA, costunolide, and Costunolide-loaded nanoparticles of formulation **F8** was obtained. In order to investigate the possible interaction between PLGA and Costunolide, Costunolide when treated with polymer PLGA. The scanning range was 400 to 4000 cm^{-1} and the resolution was 4 cm^{-1} using the KBr disk method.

Stability Studies

Stability is defined as "the capacity of the drug product to remain within specifications established to ensure its identity, strength, quality, and purity". The purpose of stability testing is to provide evidence on how the quality a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and to establish a re-test period for the drug substance or a shelf for the drug product.

X-ray diffraction study (X-RD)

The X-Ray diffraction patterns were obtained by using Siemen's D-5000 (Germany) with Cu Kα radiation and a crystal monochromator,voltage:45 kV and current 20 mA.

The diffraction patterns run at 5-10º/min in terms of 2θ angle. The graph was plotted in 2 theta angle Vs intensity count.

RESULTS AND DISCUSSION

Preparation and Characterization of Nanoparticles

Particle size is an important parameter as it has a direct relevance with the stability, cellular uptake, bio-distribution and costunolide release. The influence of organic solvents on particle size with TWEEN80 or PVA as stabilizers has been studied in table 3. From the obtained data it is very clear that the physical properties of the organic solvents strongly influence the nanoparticle preparations. Particle size was mainly found to be dependent on interfacial tension and viscosity of the solvent. EA alone led to the formation of stable nanoparticles, while DCM and ACE when used alone as organic solvents failed to produce stable nanoparticles with the method used.

Interfacial tension of EA is lowest (table 1) and therefore, were able to stabilize primary emulsion resulting in successful formation of smaller nanoparticles.

However, in case of DCM higher interfacial tension (table 1) as well as high viscosity could be the reason for the instability of primary emulsion.

On the other hand, stable primary emulsion was not formed with ACE due to its free miscibility with water (table 1).

Table 3: Effect of Solvents on Particle Size and Entrapment Efficiency Using PVA and TWEEN80 as Stabilizer

Formulations	Particle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Entrapment Efficiency (%)
F1- E.A: PLGA (1:5), PVA (1%W/V).	168.7±4.1	0.115±0.011	-1.64±0.3	-
F2- E.A: PLGA (1:6), PVA (0.5%W/V).	145.1±4.7	0.1±0.007	-1.66±0.4	-
	163.3±2.8	**0.101±0.010**	**-5.72±0.4**	**80.17±2.12**
F3- E.A: PLGA (1:4), PVA (1%W/V).	175.4±2.1	0.109±0.017	-2.01±0.2	-
F4- ACE: PLGA (1:6), PVA (0.76%W/V).	194.4±5.8	0.076±0.012	-6.17±0.4	-
F5- ACE: PLGA (1:5), PVA (0.86%W/V).	243.9±4.9	0.24±0.047	-5.03±0.2	-
F6- ACE: PLGA (1:4), PVA (0.9%W/V).	167.6±4.7	0.113±0.023	-5.38±0.3	-
F7- DCM: PLGA (1:6), PVA (0.6%W/V).	287.6±2.5	0.169±0.014	-0.797±0.2	-
	301.6±2.2	**0.189±0.018**	**-1.265±0.21**	**91.42±1.32**
F8- E.A: PLGA (1:6), TWEEN80(0.86%W/V).	101.9±5.3	0.1±0.033	-27.4±0.6	-
	112.1±6.4	**0.106±0.047**	**-31.5±0.5**	**74.28±1.69**

The choice of a particular method for encapsulation of costunolide in a colloidal carrier is most commonly determined by the solubility characteristics of the costunolide and polymer. Both,costunolide and polymer were readily soluble in ethyl acetate, acetone, and dichloromethane. The selection of an optimal formulation in the study was based on that which provided highest encapsulation efficiency, extreme uneffected particle size with better morphology (in terms of sphericity and discreteness), high zeta-potential value and better in-vitro release profile.

In order to obtain emulsified systems, the addition of energy is a fundamental step. To verify the influence of this factor on nanoparticle diameter, sonication time was varied between 1 and 4min. The results are presented in table 4**.** From the results obtained, it can be concluded that the increase in the sonication time leads to a reduction in the nanoparticle diameter of the formulations **F7, F4, F2** and **F8** respectively. With the larger time of sonication (4min), the high energy released in the process leads to a rapid dispersion of polymeric organic phase as nanodroplets of small size. The emulsification can be considered one of the most important

steps of the process, because an insufficient dispersion of phases results in large particles with wide size distribution. The final size of the nanoparticles depends on the globule size throughout the emulsification process. A reduction of the emulsion globule size allows the formation of smaller nanoparticles. Our results are in accordance with those observed by other authors (Quintanar-Guerrero et al., 1996; Kwon et al., 2001).

Table 4: Influence of sonication time on nanoparticle diameter of the Formulations F7, F4, F2 and F8

Formulations	Sonication time(min)	Particle diameter(nm)
F7	1	287.6±2.5
F4	2	194.4±5.8
F2	3	145.1±4.7
F8	4	101.9±5.3

Zeta potential is an indicator of surface charge, which determines particle stability in dispersion. Zeta potential was also measured with Zetasizer using the principle of electrophoretic mobility under an electric field. Zeta potential values, either positive or negative, should be high in order to ensure stability and avoid aggregation of the particles. The TWEEN80 resulted in high zeta potential value while PVA imparted a low zeta potential value (table 3).

The shape and surface morphology of costunolide loaded nanoparticle formulation **F2** and **F8** was confirmed by SEM analysis (Fig. 2 and Fig. 3).

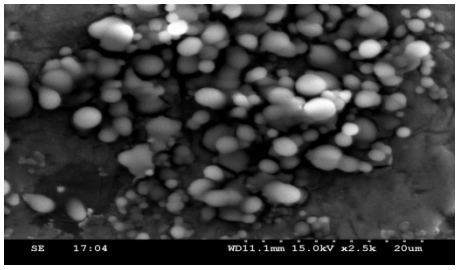

Fig. 2: SEM analysis of formulation F2

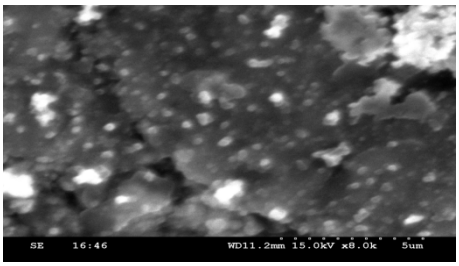

Fig. 3: SEM analysis of formulation F8

The successful entrapment of costunolide within the nanoparticles depends on many factors;

(a) low solubility of costunolide in the aqueous phase;

(b) a fast rate of precipitation/solidification of the polymer in the organic phase which in turn depends on high aqueous phase solubility, high vapor pressure of solvent, and low viscosity of the internal phase; and

(c) high solid-state solubility of costunolide in the polymer.

Like particle size, entrapment efficiency was also found to be strongly dependent on the physical properties of the solvents. The costunolide entrapment efficiency using EA and TWEEN80 was 74.28±1.69% (table 3). Although EA has maximum water solubility, but low vapor pressure might have led to slow precipitation of polymer resulting in moderate entrapment compared to DCM. Solubility of DCM in water is low, but vapor pressure is very high; therefore, DCM rapidly diffused into water and evaporated out resulting in fast precipitation of polymer without giving much time for costunolide molecules to partition into aqueous phase, leading to high entrapment with DCM (table 3).

On the other hand, PVA when used as stabilizer led to higher entrapment to that obtained for the TWEEN80 particles (table 3), which could be due to poor solubility of costunolide in PVA to that of TWEEN80 that could have prevented the loss of costunolide during nanoparticle preparation with PVA.Thus, apart from the physical properties of solvents,costunolide solubility in external phase also plays an important role in determining the entrapment efficiency when emulsion techniques are used.

In Vitro Costunolide Release Studies

The release of drug from the biodegradable particles occurs through several mechanisms such

as: (i) desorption of the surface-bound/adsorbed drug;

(ii) disintegration;

(iii) diffusion through the particle matrix;

(iv) diffusion through the polymer wall, in case drug is encapsulated in the core;

(v) surface and bulk degradation; and

(vi) a combined degradation/diffusion process.

PLGA is known to undergo bulk degradation. This process is characterized by random scission of ester bonds in the polymer backbone proceeding homogenously throughout the matrix. The acidic (lactic acid and glycolic acid) monomers and oligomers thus formed further catalyze the degradation of the parent polymer, a process known as autocatalysis.

The amount of costunolide released in each formulation F2 and F8 was determined using a calibration curve; the reported values are averages of three replicates (n = 3). Results of *in vitro* costunolide release studies obtained in each formulation F2 and F8 were tabulated (table 5&6) and shown graphically as cumulative % of costunolide release vs time respectively (Fig. 4&5).

This can be explained on the basis of stabilizer nature. PVA is a swellable, hydrophilic macromolecule and it is possible that PVA present on the surface could form a hydrogel barrier to the release of costunolide resulting in slower release in case of PVA stabilized nanoparticles. Also, particle size and entrapment efficiency are important parameters that could effect the degradation rate of the polymer matrix. With an increase in particle size, surface area/volume ratio decreases leading to decreased buffer penetration and slower escape of costunolide. This may be another reason for the comparatively slower release of costunolide from PVA stabilized nanoparticles as compared to TWEEN80 stabilized ones.

Table 5: In vitro costunolide release studies of formulation F2

Time(hrs)	Cumulative % of Costunolide release	Amount of costunolide released (mg)
0	0	0
2	7.41±0.006	0.20
4	13.33±0.012	0.37
8	19.66±0.001	0.55
16	34.45±0.033	0.96

24	47.13±0.044	1.32
32	61.58±0.069	1.72
48	86.13±0.074	2.40

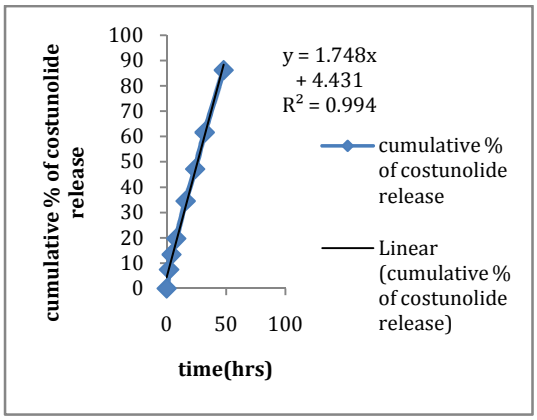

Fig. 4: Cumulative % of costunolide release vs time (hrs) of formulation F2

Table 6: In vitro costunolide release studies of formulation F8

Time(hrs)	Cumulative % of costunolide release	Amount of costunolide released (mg)
0	0	0
2	8.01±0.002	0.20
4	14.02±0.041	0.36
8	20.12±0.085	0.52
16	35.69±0.017	0.92
24	49.01±0.004	1.27
32	63.11±0.011	1.64
48	91.12±0.015	2.36

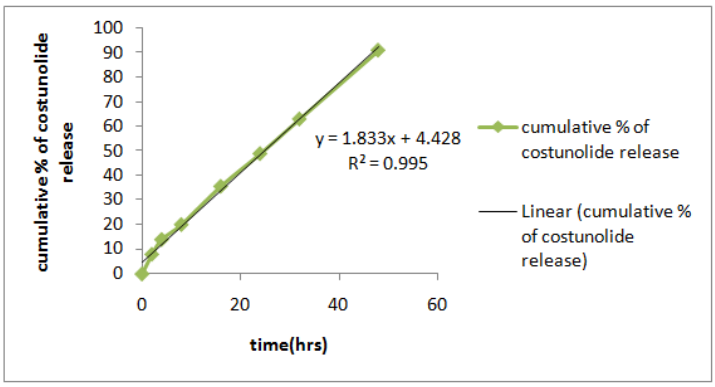

Fig. 5: Cumulative % of costunolide release vs time (hrs) of formulation F8

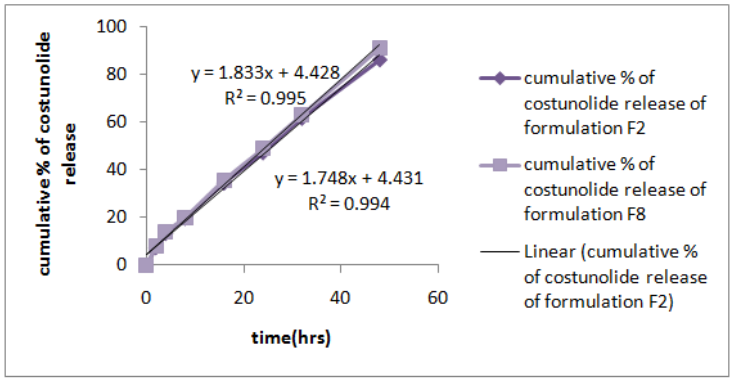

Fig. 6: Comparison of cumulative % of costunolide release vs time(hrs) of formulation F2 and F8

Table 7: In vitro costunolide release kinetics of formulation F2

Dissolution Models	Regression Coefficient (R^2)	Slope (m)
Zero Order	0.994	1.748
First Order	0.990	-0.040
Hixson Crowell	0.934	-0.015
Korsmeyer Peppas	0.921	0.997
Higuchi	0.955	12.44

Table 8: In vitro costunolide release kinetics of formulation F8

Dissolution Models	Regression Coefficient (R^2)	Slope (m)
Zero Order	0.995	1.833
First Order	0.988	-0.044
Hixson Crowell	0.943	-0.015
Korsmeyer Peppas	0.914	0.999
Higuchi	0.950	13.01

In Korsemeyer–Peppas model in order to find out "m"(slope) value,which describes the costunolide release mechanism.The formulation **F2** and **F8** showed release profile (slope) has a value of (m>0.5), which indicates a zero order release controlled by non Fickian diffusion.

Fourier transform infrared (FTIR) spectral studies:

FTIR spectra of the costunolide (Fig.7) and Costunolide-loaded nanoparticles (Fig.8) of formulation **F8** was investigated.The following characteristic peaks were observed with costunolide as well as the formulation **F8** containing costunolide-loaded nanoparticles, hence it shall be confirmed that interactions do not exist between the costunolide and polymer (Fig.7&8).

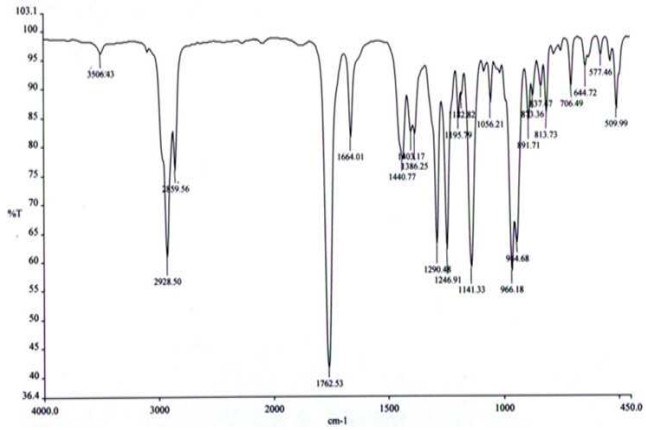

Fig. 7: FTIR spectra of the costunolide

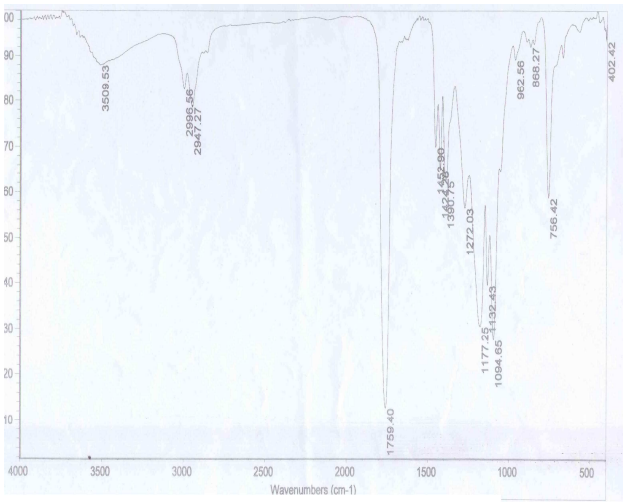

Fig. 8: FTIR spectra of the costunolide-loaded nanoparticles

Stability Study

The stability study of optimized formulation was carried out as per ICH (International Conference on Harmonization) guidelines at room-temperature and 4°C for three months. After stability studies particle size of formulation **F1** and **F2** was measured at room temperature and 4°C.

Table 9: Shows particle size of formulation F1 and F2 after stability studies.

Formulation	4°C	Room temperature
F1	170.7±4.1nm	172.7±3.2nm
F2	164.3±2.8nm	166.3±1.9nm

X-ray diffraction study (X-RD)

X-Ray diffraction is a means of identifying crystalline compounds.The X-Ray diffraction spectrum of costunolide and formulation **F8** were determined using X-Ray Diffractometer

apparatus:Siemen'sD-5000(Germany).Fig.9&10 report the XRD diffractogram of costunolide and formulation **F8** respectively. The XRD scan of costunolide showed intense peaks of crystallinity. Diffractogram of costunolide showed high intensity peaks between 2θ of 9-20^0 values demonstrating the crystalline nature of drug. The XRD pattern of formulation **F8** exhibited less intense peaks compared to costunolide. As the area under the peaks is very small it was concluded that the obtained sample was mostly amorphous rather than crystalline nature.

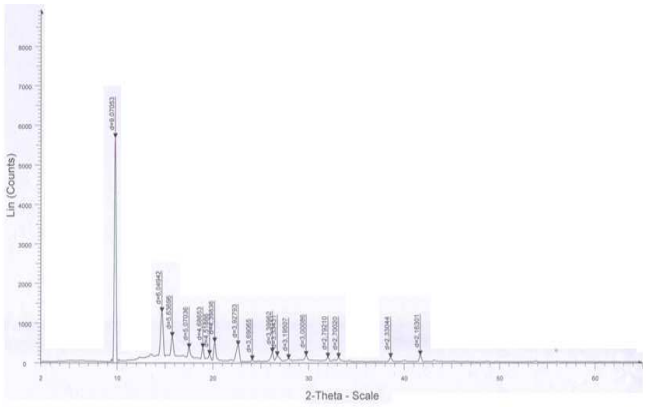

Fig. 9: X-ray diffraction studies of the costunolide

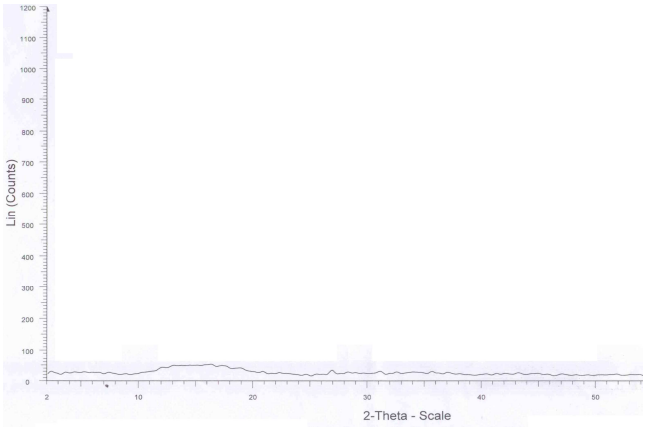

Fig. 10: X-ray diffraction studies of the Formulation F8

CONCLUSION

The study was accomplished by formulating a nanoparticulate delivery system for costunolide using PLGA with controlled release by modified oil-in-water single emulsion solvent evaporation technique.

The present investigation suggests that organic solvents play a significant role in nanoparticle formation. Physical properties of the organic solvents strongly effect the particle size and entrapment efficiency.

On preliminary screening,different formulations were developed with various ratios of polymer and different surfactants. It revealed that formulation F2 and F7 with that polymer concentration and surfactant (PVA) had good entrapment efficiency compared to surfactant tween80.

Formulation F8 with surfactant tween80 showed maximum in vitro costunolide release compared to formulation F2 with surfactant PVA.

8 formulations were evaluated and among them F8 was found to have good results. Formulation F8 showed maximum in vitro costunolide release in 48 hours diffusion study, better entrapment efficiency, particle size (nm), and zeta-potential (mV).

Formulation F1 and F2 shows better stability after 3months at room-temperature and 4^{0}C.

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