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Research Article

Design and development of stealth liposomes for oral administration of poorly bioavailable drug

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ABSTRACT

The aim of the present study was to formulate Stealth Liposomes containing Curcumin which are formulated by combinations of Cholesterol, Distearoylphosphatidylcholine, hydrogenated soyphosphotidylcholine to treat rheumatoid arthritis. Turmeric and especially its most active compound curcumin have many scientifically-proven health benefits. It's a potent anti-inflammatory and help improve symptoms of Rheumatoid arthritis. Total 8 formulations were developed using Cholesterol, Distearoylphosphatidylcholine, hydrogenated soyphosphotidylcholine in various ratios by thin film hydration method & evaluated for Entrapment Efficiency, SEM, particle size and shape, FTIR studies, invitro dissolution studies. From the invitro studies we can say that as the cholesterol ratio increases, drug release rate is increased upto particular ratio. Among all the fomulations, F7 formulation shows best drug release of 92.62% by the end of 24 hours whereas all the other formulations did not release the drug more than F7. So F7 formulation was choosen as optimized formulation.

Keywords: Curcumin, Cholesterol, Distearoylphosphatidylcholine, hydrogenated soyphosphotidylcholine

INTRODUCTION

Traditional Medicine System

Traditional system of medication is one of the hundreds of years old practices and long serving partner to mankind in the battle against infection and in having a sound existence. Indigenous individuals have been utilizing the exceptional approach of their traditional system of medications for quite a long time and among the most eminent are the Chinese, Indian, African frameworks of drug. Traditional medicine refers to any ancient and cultural based healthcare practice differing from scientific medicine and is largely transmitted orally by communities of different culture (Karunamoorthi K, et al., 2012). The World Health Organization (WHO) sees that it is hard to assign one definition to the wide scope of attributes and components of traditional medicine, yet that a working definition is basic. It thus concludes that the traditional medicines "(include) diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques

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and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.

Traditional System of Medicines (Ravishankar, B Shukla, 2007)
Ayurveda, Unani, Homeopathy, Siddha

Current Status of Herbal Medicine
Currently more than 80% of the total population relies upon traditional and plant determined drug since, plants are a significant wellspring of medications and by and by about 25% of pharmaceutical medicines in the US contain at least one plant-derived ingredient. In the only remaining century, around 121 pharmaceutical products were defined dependent on the traditional knowledge acquired from different sources (Qazimajaz A, Molvikhurshid I., 2016),

Curcuma longa (Turmeric)
The name of the genus, Curcuma, is derived from the Sanskrit kuṅkuma, alluding to both turmeric and saffron, utilized in India since ancient times. Turmeric (Curcuma longa) is a blooming plant of the ginger family, Zingiberaceae, the roots of which are utilized in cooking. The plant is rhizomatous, herbaceous and lasting and is local to the Indian subcontinent and Southeast Asia and requires temperatures somewhere in the range of 20 and 30°C (68 and 86 F) and a considerable amount of rainfall to flourish.

Biological activities of curcumin

- Anti-inflammatory property
- Antioxidant properties
- It acts on lymphocytes
- It acts on platelet aggregation
- Curcumin as an anti-carcinogenic agent in humans
- Wound-healing properties
- Diabetes
- Antiviral properties

Turmeric in traditional system of medicine

- **Turmeric** is a natural antiseptic and antibacterial agent, useful in disinfecting cuts and burns.
- **Turmeric** when combined with cauliflower, it has shown to prevent prostate cancer and stop the growth of existing prostate cancer.

Novel Drug Delivery System
Clinical medicine possesses an extremely broad range of drug molecules currently in use, and new drugs are added to the list every year. One of the main goals of any treatment employing xenobiotics is to increase the therapeutic index of the drug while minimizing its side-effects. The clinical utility of most conventional chemotherapeutics is limited either by the inability to deliver therapeutic drug concentrations to the target tissues or by severe and harmful toxic effects on normal organs and tissues. Different approaches have been attempted to overcome these problems by providing "selective" delivery to the affected area; the ideal solution would be to target the drug only to those organs, tissues, or cells affected by the disease. Selected carriers, such as molecular conjugates and colloidal particulates, can be suitable for this purpose. Colloidal particulates result from physical incorporation of the drug into a particulate colloidal system such as liposomes, niosomes, micro- and nano-spheres, erythrocytes, and polymeric and reverse micelles. Among these carriers, liposomes have been most studied. Their attraction lies in their composition, which makes them biocompatible and biodegradable. They consist of an aqueous core entrapped by one or more bilayers composed of natural or synthetic lipids. Liposomes composed of natural phospholipids are biologically inert and weakly immunogenic, and they possess low intrinsic toxicity. Further, drugs with different lipophilicities can be encapsulated into liposomes: strongly lipophilic drugs are entrapped almost completely in the lipid bilayer, strongly hydrophilic drugs are located exclusively in the aqueous compartment, and drugs with intermediate easily partition between the lipid and aqueous phases, both in the bilayer and in the aqueous core (Lasic DD, et.al 1993, Allen TM. et.al 1994)

Concept of Stealth liposomes: -
As stated by Yuanpeng Zhang, In World war II NAZI German's bombed the British command centers and they were helpless as their RADAR failed to detect the Nazi's bombers. These bombers were stealth bombers which were designed by Germans, were made up of special materials and unique design. As the allies failed to tackle the stealth bombers they were helpless and so the command centers were destroyed to bits…the same concept were used in long circulatory liposomes. We need such a newly designed drug delivery system which delivers the killer bombs deep inside tumor cells and destroy them, just like the stealth bombers did or was intended to. Camouflaging the liposomes so as to fool phagocytes into ignoring them thus became a key objective of pharmaceutical chemists, hence The result of their efforts was a process known as PEGylation, i.e. Covering outer side of liposome with polymer, To phagocytes, this molecular "cloak" of water of hydration makes the PEGylated liposomes look like little watery bubbles rather than something edible, so they tend to leave them alone (Allen TM. et.al., 1994, Gabizon AA. Et.al., 2001).

MATERIALS AND METHOD

Turmeric, Distearoylphosphatidyl choline, Soya lecithin, Cholesterol, obtained from BMR Chemicals, Hyd, India. Chloroform, Methanol was procured from Narmada Chemicals, Hyd. Polyvinylpyrrolidone procured from Rankem, India.

Method of Preparation of Curcumin Loaded Stealth Liposomes: (Amelia P, 2015, Mohamed M, 2016)

Accurate amount of Curcumin, Phospholipid and cholesterol were dissolved in little quantity of chloroform:Methanol (2:1) in a round bottom flask. The solvent was allowed to evaporate to form a thin film of lipid on the wall of flask. Lipid film was then hydrated with phosphate buffer of pH 7.4 by mechanical shaker for 1 h at Room temperature. The flask was rotated again at same speed and temperature as before, for the removal of lipid film from the walls and dispersion to from liposomes. To this suspension of conventional liposomes, 1 ml of 10 % w/v of PVP was added slowly under 100 r/min rotation for the preparation of stealth liposomes. Both the suspensions were then allowed to stand for 2- 3 h for the complete swelling. Each batch was prepared similarly and was stored in the refrigerator in a suitable container.

Table 1: A Composition of curcumin loaded stealth liposomes

Ingredients (mg)	F1	F2	F3	F4
Curcumin	100	100	100	100
Distearoylphosphatidyl choline	400	400	400	400
Soya lecithin	-	-	-	-
Cholesterol	50	100	150	200
Chloroform:Methanol (2:1)	Q.S	Q.S	Q.S	Q.S
Phosphate buffer	10	10	10	10
PVP (mL)	1	1	1	1

Table 2: B Composition of curcumin loaded stealth liposomes

Ingredients (mg)	F5	F6	F7	F8
Curcumin	100	100	100	100
Distearoylphosphatidyl choline	-	-	-	-
Soya lecithin	400	400	400	400
Cholesterol	50	100	150	200
Chloroform:Methanol (2:1)	Q.S	Q.S	Q.S	Q.S
Phosphate buffer	10	10	10	10
PVP (mL)	1	1	1	1

Evaluation Studies: Zhengui Y, et.al, 2010, Okhil KN, et.al, 2013, Mali D, 2013, Narashimhan LR, et.al, 2012)

Entrapment efficiency:

The percentage drug entrapped was determined by centrifugation. Liposomal suspension was placed in the centrifugal tube and it was balanced on the other side with an equivalent weight. The centrifugation was carried out 1000 r/min for 60 min. The supernatant was removed and the concentration of the supernatant was determined spectrophotometrically at 421 nm. The percentage of drug entrapment was calculated using the equation,

$$\text{Percentage Drug Entrapment} = \frac{\text{Total Drug} - \text{Drug in supernatant}}{\text{Total Drug}} \times 100$$

Scanning electron microscopy:

The average size and size distribution of curcumin loaded stealth liposomes were determined by SEM, in which the samples were mounted rigidly on the surface of a bronze specimen holder called a specimen stub using a double sided adhesive tape and coated with an ultrathin coating of electrically-conducting material, gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation with gold and observed under suitable magnification.

FTIR Studies:

Infrared spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixture and as a tool in establishment of the structure. The infrared data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the polymers. Infrared spectra of extract and polymers, alone and in physical mixtures were taken. Then it was investigated for any possible interaction between polymer and the drug.

Particle size and shape

The size of particles and their distribution were determined using Zetasizer (Nano ZS Malvern Instruments, UK) using a process called dynamic light scattering (DLS). Samples were examined to determine the mean particle size, size distribution, and polydispersity index (PDI). This technique measures the time-dependent fluctuations in the intensity of scattered light, which occurs because the particles are under Brownian motion. Analysis of these intensity fluctuations enables the determination of the diffusion coefficient of the particles, which are converted into the size distribution.

Zeta potential:
There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge. First, by the adsorption of ions present in the solution. Second, by the ionization of functional groups on the particle's surface. Third, due to the difference in dielectric constant between the particle and the medium. Attention should be paid to the formation of electric double layer at the solid-liquid interface. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases.

As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions. The zeta potential cannot be measured directly; however, it can be calculated using theoretical models and from experimentally determined electrophoretic mobility data. The theory is based on electrophoresis and can be expressed as:

$$\mu = \zeta\varepsilon/\eta$$

Where (μ) is the electrophoretic mobility,(ε) is the electric permittivity of the liquid,(η)
Is the viscosity and (ζ) us the zeta potential.

***In-vitro* drug diffusion**
The release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 250 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±0.5ºC. Dialysis membrane was taken and one end of the membrane was sealed. 20mg weight equivalent Curcumin loaded stealth liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 5ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium. The Aliquots was measured for the amount of the drug by using UV spectrophotometer at 421nm.

Kinetic studies:
To analyze the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order, Higuchi matrix, Peppas models. Based on the r-value, the best-fit model was selected.

RESULTS AND DISCUSSION

Evaluation studies:
Particle size analysis:

Measurement Results

Date	:	Friday, March 13, 2020 6:13:37 PM
Measurement Type	:	Particle Size
Sample Name	:	F7
Scattering Angle	:	90
Temperature of the holder	:	25.3 °C
T% before meas.	:	15494
Viscosity of the dispersion medium	:	0.890 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	1338 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	179.5 nm	538.2 nm	22.9 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	179.5 nm	538.2 nm	22.9 nm

Cumulant Operations

Z-Average	:	5.6 nm
PI	:	2.206

Molecular weight measurement

Molecular weight	:	---
Mark-Houwink-Sakurada parameters	:	---

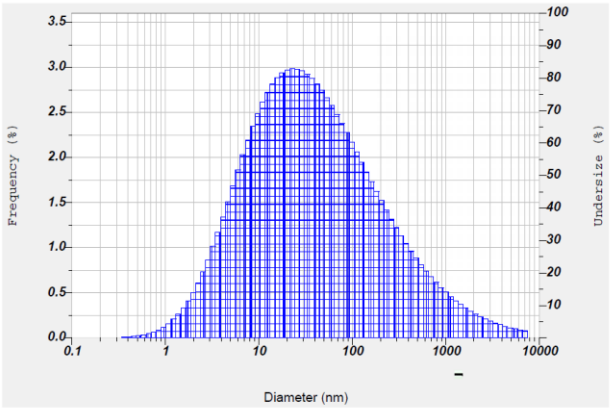

Fig 1: Particle size analysis
Discussion: Average particle size of curcumin loaded stealth liposomes of optimized formulations (F7) was found to be 179.5 nm.

Scanning electron microscopy:

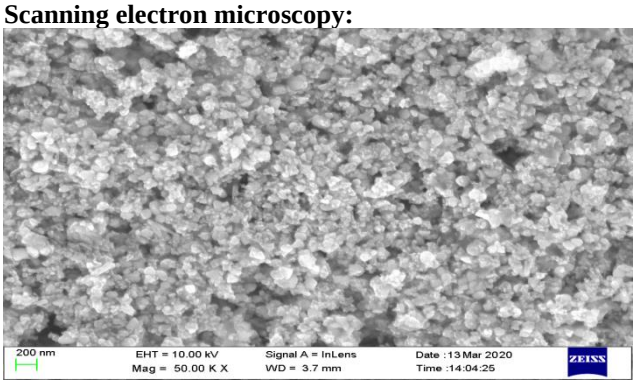

Fig 2: SEM analysis of Curcumin loaded stealth liposomes of optimized formulation (F7)
Discussion: SEM analysis revealed that Nanosized spherical particles with numerous pores on surface.

Entrapment efficiency: -The entrapment efficacy of the formulated stealth liposomes was found to be in the range of 76.32±1.02%-96.13±0.94% respectively.

Table 3: Entapment efficiency

Formulation code	Entrapment efficacy ± S.D
F1	93.16±0.54
F2	95.42±1.34
F3	76.32±1.02
F4	87.42±0.89
F5	96.13±0.94
F6	95.24±0.65
F7	91.16±1.04
F8	86.31±0.31

Discussion: Formulation F3 & F5 were obtained good results in entrapment efficiency with 76.32±1.02 and 96.13±0.94.

In-vitro drug release studies:

Table 4: In-vitro drug release data of formulation (F1-F4)

Time (hrs)	Cummulative Percentage Drug release			
	F1	F2	F3	F4
0	0	0	0	0
2	12.64± 0.12	16.34± 0.69	23.06± 0.02	29.34± 0.16
4	26.34± 0.35	29.31± 0.45	39.64± 0.34	35.62± 0.21
6	32.05± 0.64	41.05± 0.31	46.21± 0.68	40.15± 0.18
8	43.16± 0.78	49.37± 0.02	56.32± 0.75	46.31± 0.52
10	51.05± 0.52	56.28± 0.74	62.25± 0.24	54.13± 0.36
12	59.67± 0.02	61.79± 0.52	69.73± 0.01	61.08± 0.02
16	65.24± 0.69	73.16± 0.31	75.32± 0.36	69.31± 0.21
20	70.32± 0.35	85.39± 0.46	82.04± 0.29	75.32± 0.64
24	79.34± 1.01	92.34± 0.12	86.31± 0.25	79.31± 0.39

Table 5: In-vitro drug release data of formulation (F5-F8)

Time (hrs)	Cummulative Percentage Drug release			
	F5	F6	F7	F8
0	0	0	0	0
2	22.04± 0.42	16.32± 0.36	13.06±0 .26	20.31± 0.26
4	39.64± 0.36	29.34± 0.13	22.18±0 .42	36.12± 0.41
6	52.03± 0.15	39.25± 0.02	36.01±0 .86	46.31± 0.53
8	63.13± 0.78	53.12± 0.69	49.21±0 .312	54.05± 0.52
10	72.02± 0.96	61.05± 0.78	58.15±0 .21	59.36± 0.14
12	78.14± 0.32	72.32± 0.15	71.02±0 .56	65.13± 0.26
16	82.05± 0.02	81.09± 0.34	79.02±0 .93	71.05± 0.37
20	86.31± 0.64	86.32± 0.26	85.31±0 .78	82.31± 0.14
24	89.52± 0.12	92.15± 0.36	97.02±0 .24	87.26± 0.26

Discussion: From the above invitro studies we can say that as the cholesterol ratio increases, drug release rate is increased upto particular ratio. Among all the fomulations, F7 formulation shows best drug release of 97.02% by the end of 24 hours where as all the other formulations did not release the drug more than F7.

Because F7 formulation having hydrogenated soya phosphatidyl choline with cholesterol at 3:1 ratio shows better drug release then the other concentrations.

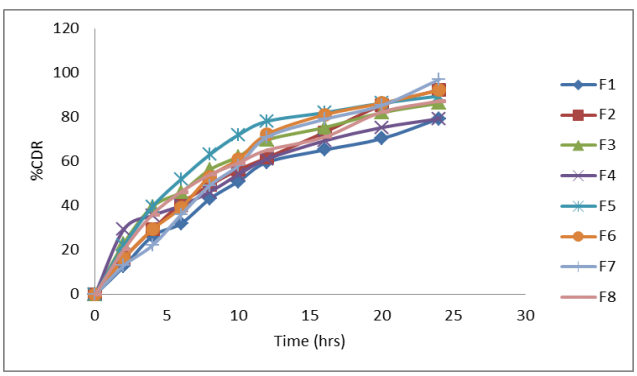

Fig 3: In-vitro drug release for all the formulations (F1-F8)

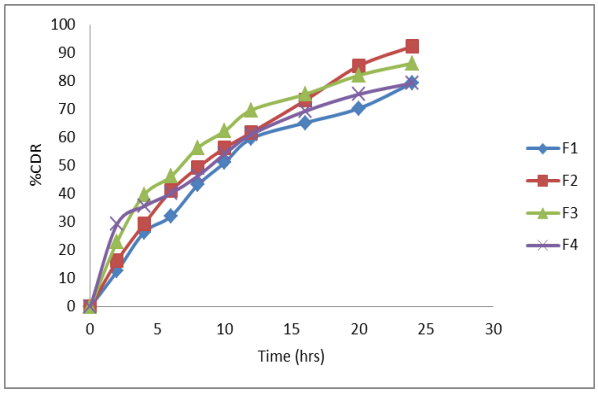

Fig 4: In-vitro drug release of formulations F1-F4

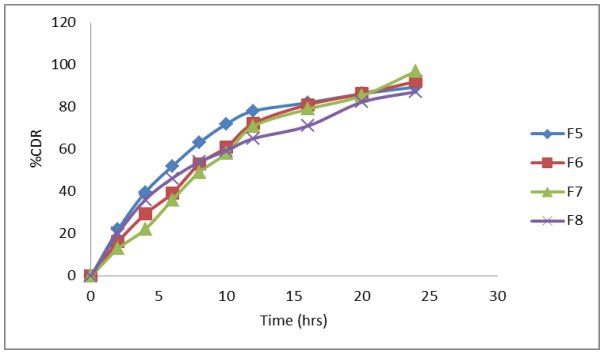

Fig 5: In-vitro drug release of formulations F5-F8

Drug release kinetics:

Table 6: Drug release kinetics data of optimized formulation (F7)

Time (hrs)	% CDR	%Drug Remaining	Square Root Time
0	0	100	0
2	13.06	86.94	1.414
4	22.18	77.82	2
6	36.01	63.99	2.449
8	49.21	50.79	2.828
10	58.15	41.85	3.162
12	71.02	28.98	3.464
16	79.02	20.98	4
20	85.31	14.69	4.472
24	97.02	2.98	4.899

Table 3.2.1-B: Drug release kinetics data of optimized formulation (F7)

Time (hrs)	Log Cummulative % Drug Remaining	Log Time	Log % CDR
0	2	0	0
2	1.939	0.301	1.116
4	1.891	0.602	1.346
6	1.806	0.778	1.556
8	1.706	0.903	1.692
10	1.622	1	1.765
12	1.462	1.079	1.851
16	1.322	1.204	1.898
20	1.167	1.301	1.931
24	0.474	1.380	1.987

Zero order:

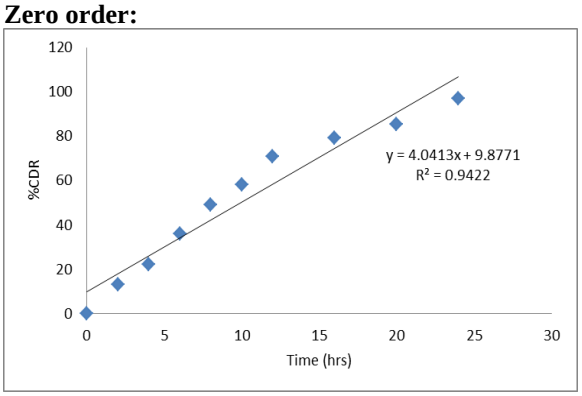

Fig 6: Zero order graph of optimized formulation

First order:

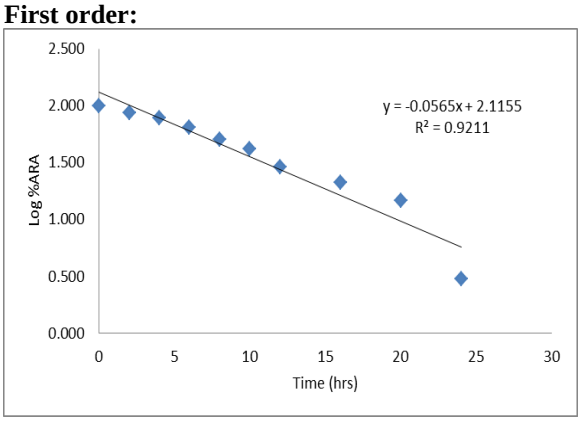

Fig 7: First order graph of optimized formulation

Higuchi plot:

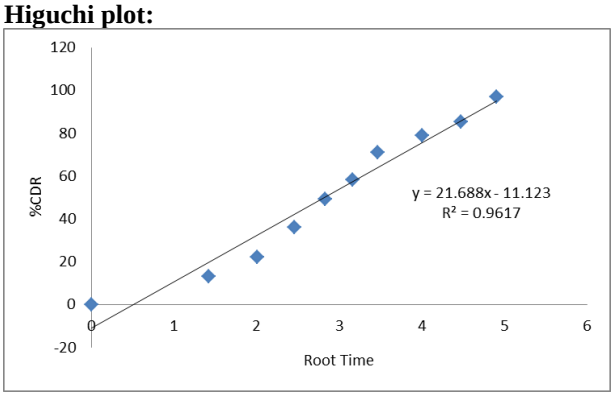

Fig 8: Higuchi graph of optimized formulation

Peppas plot:

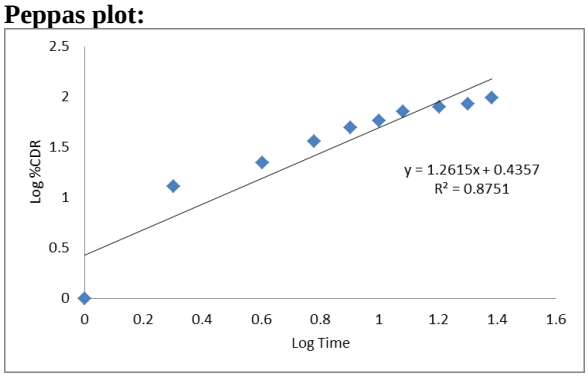

Fig 9: Peppas graph of optimized formulation

DISCUSSION

The invitro dissolution data for best formulation F7 were fitted in different kinetic models i.e, zero order, first order, Higuchi and korsemeyer-peppas equation. Optimized formulation F7 shows R^2 value 0.942. As its value nearer to the '1' it is conformed as it follows the First order release. The mechanism of drug release is further confirmed by the korsmeyer and peppas plot, if n = 0.45 it is called Case I or Fickian diffusion, 0.45 < n < 0.89 is for anomalous behavior or non-Fickian transport, n = 0.89 for case II transport and n > 0.89 for Super case II transport.

CONCLUSION

Stealth liposomes containing curcumin was developed by using combination of cholesterol, Distearorylphosphatidyl choline, soya lecithin & PVP to treat rheumatoid arthritis. FTIR spectroscopic studies designate that are no drug-excepient interaction Average PS of curcumin loaded stealth liposomes of optimized formulation (F6) was found to be 179.5 nm. Zeta potential value for the optimized formulation (F7) was found to be within the acceptable limits.

From the above invitro studies we can say that as the cholesterol ratio increases, drug release rate is increased upto particular ratio. Among all the fomulations, F7 formulation shows best drug release of 97.02% by the end of 24 hours where as all the other formulations did not release the drug more than F7.

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