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DESIGN AND *IN-VITRO* CHARACTERIZATION OF *CURCUMA LONGA* NANOGEL

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ABSTRACT

Nanogels based materials have high drug loading capacity, biocompatibility, and biodegradability which are the key points to design a drug delivery system effectively. An intense of this study was to design a *CURCUMA LONGA* ethanolic extract loaded Nanogel and to evaluate the *in-vitro* release condition of the drug from the dosage form. Three different formulation of Nanogels were formulated by altering the cholesterol and polyethylene glycol (PEG) concentration. The Nanogel were evaluated for the surface morphology characteristics by SEM studies, particle size, poly dispersibility index (PDI), surface charge by zeta potential, encapsulation efficiency and *in-vitro* drug release studies. From the studies it was concluded that nanogel was a versatile drug delivery system for delivering an herbal drug in efficient manner.

Key Words:- Nanogel, Curcumin, *in-vitro* drug, *Curcuma longa*.

INTRODUCTION

Curcumin is an extract of polyphenol from the herb of *Curcuma longa*. The chemical structure is shown as (1, 7-bis (4- hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione), which is insoluble in water. Curcumin is widely used as anti-inflammatory in traditional Chinese medicine (Ammon H & Wahl MA, 1991). It has been demonstrated that several types of biological and pharmacological activities are reported, including anti-inflammatory, anticancer and antioxidant properties (Shehzad A *et al*., 2010). However, prior studies have shown that due to its rapid systemic elimination the retention time of curcumin in body was limited and it shows less bioavailability (Yang KY *et al*., 2010).

To increase the bioavailability and retention time of curcumin in the body, various formulation techniques like formulation into lipid nanoparticle have been employed. For example, curcumin–phospholipid complex can extend the retention and bioavailability of curcumin in rat serum (Mythri RB *et al*., 2007).

Nanogels may be defined as nano-sized hydrogel systems which are highly cross linked systems in nature involving polymer systems which are either co-polymerized or monomers. Nanogels are useful in drug delivery due to their small size, which allows for easier cellular uptake as well as controlled properties and drug release. For example, Poly (N isopropylacrylamide) one of the most extensively studied polymers, which is cross linked through free radical, has been used for in controlled drug delivery in the forms of nanogel or microgels. Nanogels are typical formulations mainly of the size range of 100 nm, by varying solvent quality and branching the volume fraction can be altered variably to maintain a three dimensional structure (Mythri RB *et al*., 2007; Phillips MA *et al*., 2010).

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Nanogels are superior drug delivery system than others because

1. The particle size and surface properties can be manipulated to avoid rapid clearance by Phagocytic cells, allowing both passive and active drug targeting.
2. Controlled and sustained drug release at the target site, improving the therapeutic efficacy and reducing side effects. Drug loading is relatively high and may be achieved without chemical reactions; this is an important factor for preserving the drug activity.
3. Ability to reach the smallest capillary vessels, due to their tiny volume, and to penetrate the tissues either through the paracellular or the transcellular pathways.
4. Highly biocompatible and biodegradable (Phillips MA *et al*., 2010).

Disadvantages of Nanogels

a) Expensive technique to completely remove the solvent sand surfactants at the end of preparation process.
b) Surfactant or monomer traces may remain and can impart toxicity (Phillips MA *et al*., 2010).

MATERIALS AND METHODS

Dried rhizomes of *Curcuma longa* bought from local market were ground into fine powder using mechanical mixer. Then it was sieved into fine powder by sieve no 60. Cholesterol and PEG 4000 were taken as gift sample from Chem. Scientifics, Bangalore. All other solvents used in the formulation were belonging to analytical grade.

Nanogel preparation

The lipid formulation of cholesterol and PEG4000 (6: 1 molar ratio) in chloroform were evaporated under argon gas and the dried lipid film was hydrated with a solution containing the monomer, crude extract, and phosphate buffer (pH 7.4) for a minimum of 30 minutes. The solution was homogenized for 1 minute to remove any adhering lipid film and sonicated in a bath sonicator or 1 minute at room temperature to produce multilamellar vesicles (MLV). MLVs were then sonicated with a high speed probe sonicator for 2 minutes to produce small unilamellar vesicles as translucent solution. The formed nanogels were then purified by washing with formaldehyde to remove free monomers and crude extract. After purification, the nanogels were exposed to a handheld UV lamp at 365 nm wavelength for 5 minutes at room temperature. Extract concentrated Nanogel are measured and preserved in desiccator for further use (Phillips MA *et al*., 2010; Malmsten M *et al*., 2010; Hasegawa U *et al*., 2005; Wu W *et al*., 2010).

Nanogel characteristics

Particle size, polydispersity index and zeta potential:

The particle size and polydispersity index (PDI) of Nanogel were measured by dynamic light scattering method. The zeta potential of Nanogel was determined using a Malvern Zeta potential analyzer (Wu W *et al*., 2010).

Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was conducted to characterize the surface morphology of the Nanogels. The samples were mounted on alumina stubs using double adhesive tape, coated with gold in HUS-5GB vacuum evaporator. Then the sample was observed in Hitachi S-3000N SEM at an acceleration voltage of 10KV and a magnification of 5000X (Gong Y *et al*., 2009).

Transmission electron microscopy (TEM): In brief, the Nanogel solution was placed drop wise onto a 400 mesh copper grid coated with carbon. About 15 min after nanoparticle deposition, the grid was tapped with filter paper to remove excess water and stained using a solution of phosphotungsten acid (2%, w/v) for 20 min. After the stained sample was allowed to air dry, TEM samples were obtained (Gong Y *et al*., 2009).

Entrapment efficiency: Entrapment efficiency estimated the amount of curcumin encapsulated in Nanogel. The Nanogel was diluted with buffer solution and then it was centrifuged at 5000 rpm for 10 min. After centrifugation, the supernatant was removed and 1 ml of acetonitrile was added to the settled nanoparticle and then treated with sonication for 5 min to leach out the drug from the viscous gel. The amount of curcumin in Nanogel was analyzed by HPLC system. The % entrapment efficiency was given by (Wu W *et al*., 2010).

(Curcumin encapsulated/Curcumin total) × 100

Size measurement of Nanogels

A cuvette was filled with approximately nanogel solution and fresh DI water (in the ratio of 1:3) was taken and then placed into a Malvern zeta sizer in order to record the average particle size, polydispersity, and standard deviation. Furthermore, SEM and TEM were used to visualize curcumin nanogels to observe the morphology of these nanogels (Hasegawa U *et al*., 2005; Wu W *et al*., 2010; Gong Y *et al*., 2009).

***In-vitro* drug release study:** Release of curcumin from the Nanogel was performed by the dialysis membrane method. First, 1 ml of Nanogel solution (equivalent to

0.25mg of curcumin) was transferred in dialysis bags. The bags were suspended in 200 ml of 0.9% normal saline contain 50% v/v of ethanol at 37°C in a shaking water bath at 100 rpm . At selected time intervals, 200 ml of normal saline sample was collected and replaced by an equal volume of fresh medium. The curcumin content of normal saline was analyzed by an HPLC system. From the area of the peak concentration of the curcumin was calculated (Sun H *et al*., 2005; Modi A Megha & Unnikrishnan Unnma, 2013).

RESULTS AND DISCUSSION

The properties of all the three formulation of nanogel containing curcumin were shown in Table 1. The particle size, PDI, zeta potential and encapsulation efficiency of Nanogel was found to be in the range of 455.9 to 542.8 nm, 0.323 to 0.395, −12.46 to -15.26 mV and 75.45 ± 2.08 to 82.94 ± 2.54%, respectively. The average particle size distribution and poly dispersibility index (PDI) result showed that the formulated nanogel particles were in narrow size distribution of Nanogel. The results of all the formulation indicated that the size of particles present in Nanogel is consistent with low PDI. The morphology of Nanogel recorded from SEM was irregular and spherical in shape with smooth surface that shown in Fig. 1. In the formulation system design, it is important that a drug should be readily released from Nanogel because restricted release of a drug from its formulation materials will interfere with the drug availability and reduce the drug efficacy (Aggarwal BB & Harikumar KB, 2009; Pandey MK *et al*., 2010; Kang MJ *et al*., 2009). Drug release from Curcumin nanogel shows multiple release patterns. It shows initial burst release of curcumin followed by zero order release pattern. The initial burst release is regulated by diffusion of drug from the surface of the nanogel i.e., free drug from the surface was released efficiently and also through the pore associated with drug and polymer and then followed by zero order release are controlled by polymer erosion mechanism. *In-vitro* release pattern of Nanogel (mean ± SD, n = 3) exhibit a diffusion-controlled release mechanism. Initially, a burst release occurred in the first 2 hrs with 24.52 ± 2.4% of the curcumin released from the Nanogel. Following this, cumulative sustained release was obtained, i.e., 82.4 ± 3.54% of curcumin released from Nanogel released upto 12 hours. The sustained release profile of curcumin from nanogel was consistent with a Peppas diffusion equation ($r^2 = 0.95$).

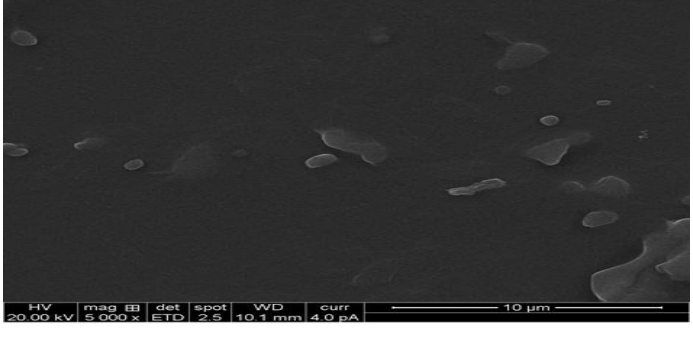
Table 1. Particle Characteristics of Nanogel

Formulation	N1	N2	N3
Mean Particle size (nm)	542.8	672.3	455.9
Poly Dispersibility Index	0.346	0.395	0.323
Zeta Potential (mV)	-15.26	-12.46	-13.54

Table 2. Entrapment of Nanogel formulation

Formulation code	Entrapment efficiency
N1	75.45 ± 2.08
N2	82.94 ± 2.54
N3	79.94 ± 2.62

Figure 1. SEM studies - Nanogel



CONCLUSION

From the results and discussion it shows that the sustained release of curcumin from nanogel was prepared by using phospholipids shows a good diffusion controlled release drug delivery system. Sustained release of the drug in a delivery system is an important property which was closely related with pharmacokinetic and pharmacodynamic parameters. Thus it may conclude that nanogel was a versatile drug delivery system for delivering herbal drugs in efficient manner.

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