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Original Article

EFFECT OF POLYMERIC NANOPARTICLES OF CURCUMIN ON A549 CELL LINE

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ABSTRACT

Objective: Lung cancer cell line specially A549 is one of the most common malignant tumors. Curcumin is obtained from rhizomes of curcuma longa and does possess biological and pharmacological properties, among them anti-tumor activity is also one of the major findings. Here we try to developed a method for delivery of curcumin for cancer therapy.

Methods: Due to low water solubility and bioavailability curcumin delivery designed as polymeric nanoparticles and prepared by emulsification homogenization method.

Results: Characterization done by evaluating zeta size, potential, scanning electron microscopy. Release pattern also studied along with encapsulation and loading data. Nanoparticles were found to be spherical from morphological study and found to be below 200 nm. It does possess cytotoxic activity against lung cancer cell line and potent in comparison with its free from.

Conclusion: We can conclude that polymeric nanoparticles are suitable form to delivery for antitumor activity and have suitable anticancer ctivity.

Keywords: Curcumin, Scanning electron microscopy, Kinetic modelling, Cellular toxicity

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INTRODUCTION

Globally, Human lung cancer specially A549 cell line is one of the most familiar malignant tumors. Majority of lung cancer cases, basically from 75% to 85% are non-small cell lung cancer (NSCLC), and does lead to the highest death rate in so many countries [1, 2]. Essential treatment like chemotherapy and radiotherapy have severe side effects. Surgical strategy can sometimes probably miss metastases. So new formulation development of drug delivery is very important.

Curcumin (1, 7-bis (4-hydroxy–3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione) obtained from rhizomes of curcuma longa, manifest diverse biological and pharmacological properties [3, 4]. As it is not suitable with water (20 µg/ml) so its bioavailability and absorption are poor for biological applications [5, 6]. To improve the bioavailability of curcumin, various delivery modes have been developed such as nanoparticles, liposomes, microemulsion, vesicles, complexation with phospholipids and cyclodextrin based inclusion complexes using the unparallel potential of nanotechnology [7-9]. To deal with the bioavailability problem, various curcumin nano formulation has been made to make it effective in the target tissues. Among various types of nanocarriers, Poly (lactic-co-glycolic acid) (PLGA) [10, 11] is most used and recognized in drug delivery systems. PLGA nanoparticles for oral administration is a favorable approach for enhancing and controlling drug delivery [12]. Therefore, this study is foretold to explore the effect of free curcumin and curcumin loaded nanoparticles on lung cancer cell line.

MATERIALS AND METHODS

Materials

Curcumin, Poly (lactic-co-glycolic acid) (PLGA), Poly-vinyl alcohol (PVA) and methyl thiazole tetrazolium (MTT) were purchased from Sigma Aldrich. Acetone and Dimethyl Sulfoxide (DMSO) was obtained from Sisco Research Laboratories Pvt. Ltd. (SRL).

Preparation of PLGA-curcumin nanoparticles

PLGA and curcumin was dissolved in acetone with vortex and shaking. PVA solutions was taken in a beaker and drug and polymer mixer was added dropwise to the beaker with homogenization at 16000 RPM for 5 min at 4 °C. After that homogenized solution was filtered with 0.45-micron filter and centrifuged. After centrifugation again washing was done with double distilled water and lyophilized to collect [12].

Characterization of nanoparticles

Particle size and zeta potential

Nanoparticles were again dispersed in double distilled water and sonicated in bath sonicator and examined in zeta sizer using dynamic light scattering techniques [13].

Morphological characteristic by SEM

Lyophilized nanoparticles were dispersed in water and sampling done in a coverslip and air-dried for 2 h. After that it was examined in scanning electron microscopy (SEM) [14].

Standard curve preparation

Free curcumin was dissolved in acetone (1 mg/ml and prepared the stock. From the stock various working concentration were prepared like 10, 20, 30, 40, 50, 60, 70, 80 µg/ml. λ max was determined by scanning drug solution in the range of 200-800 nm in the UV-vis instrument. After that all the working solutions were scanned in the obtained λ max (fig. 1) [15].

Drug loading and encapsulation

Weight amount of nanoparticles were dissolved in acetone and scanned in UV-vis instruments to get the absorbance and the obtained absorbance value was put in the standard curve to get the loading and encapsulation by following equation [16].

$$\text{Drug Loading (\%)} = \frac{Drug\ Amount\ (mg)}{Weight\ of\ Nanoparticles\ (mg)} \times 10$$

$$\text{Encapsulation Efficiency (\%)} = \frac{Drug\ Amount\ in\ Nanoparticles\ (mg)}{Initially\ Added\ Drug\ (mg)} \times 100$$

Drug release

Weight amount of nanoparticles were taken with phosphate buffer solution of 10 ml. Then the solution was kept in a magnetic stirrer and sample withdrawn at particular time interval and to maintain

sink condition the same amount of phosphate buffer was added after every withdrawn. Withdrawn samples were centrifuged at 3000 RPM for 3 min and the supernatant was collected and observed in a UV-vis spectrophotometer for drug release [17].

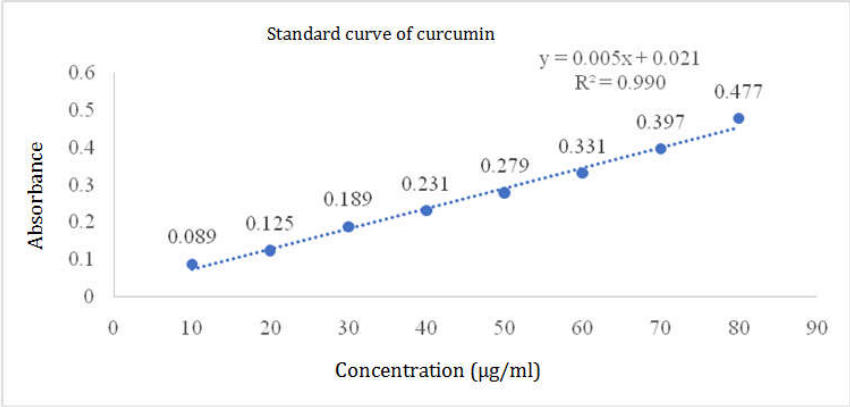

Fig. 1: Standard curve of curcumin in acetone by UV-vis method

Kinetic modeling

From the drug release data various kinetic model was developed like zero order, first order, higuchi model and korsemeyer-peppas model. These models were then used to determine the release pattern of the nanoparticles.

Cellular toxicity study on A549 cell line

A549 cell line was subcultured in T-25 flask and when it was about 80% confluent cells were seeded in 96 well plate in a density of 1×10^{-3} and incubate for 12 h. After that cells were washed with phosphate buffer saline (PBS) and treatment were added in terms of free drug and nanoparticles at a range of concentration ranging from 2µM to 40µM. Then again incubate for 24 h. 48 h. and 72 h. After incubation wells were washed with PBS and methylthiazol tetrazolium (MTT) solutions (4 mg/0.8 ml) were added and incubate for 3 h. After that DMSO were added to all wells including control and blank and kept in a shaker for 30 min and fluorescence intensity was determined at 570 nm [18].

Statistical analysis

All results were expressed in mean±standard deviation (SD). Analysis were done by Origin 8 pro software. For significance P value was taken as $P<0.05$.

RESULTS AND DISCUSSION

Preparation and characterization of nanoparticles

Nanoparticles were prepared using high pressure homogenization method using a laboratory-grade homogenizer. After that, particle size and zeta potential were determined and found to be 148.71±2.71 nm and-0.9±1.72 mV, respectively (fig. 2 and fig. 3). Particle size was found to be suitable as they were below 200 nm so it is suitable for cellular uptake and greater biodistribution. It is only possible for nanoparticles below 200 nm size range to passive transport into RBC [19]. Even we can opt for tumor or brain targetting along with intravenous or intramuscular pathway. Poly dispersity index (PDI) was found to be 171.21±3.42. PDI was found to be below 0.7 and so particles were not accumulated with each other. They were monodisperse. Our size range was below 0.2 and this is acceptable for polymeric nanoparticles [20]. Drug loading and encapsulation efficiency were determined using UV-vis spectrophotometer and found to be 11.62±0.61 % and 5.21±0.23 % respectively. SEM was done to see the morphological characteristic of the prepared nanoparticles and the morphology was found to be spherical and average particle size was under 200 nm range (fig. 4) (table 1).

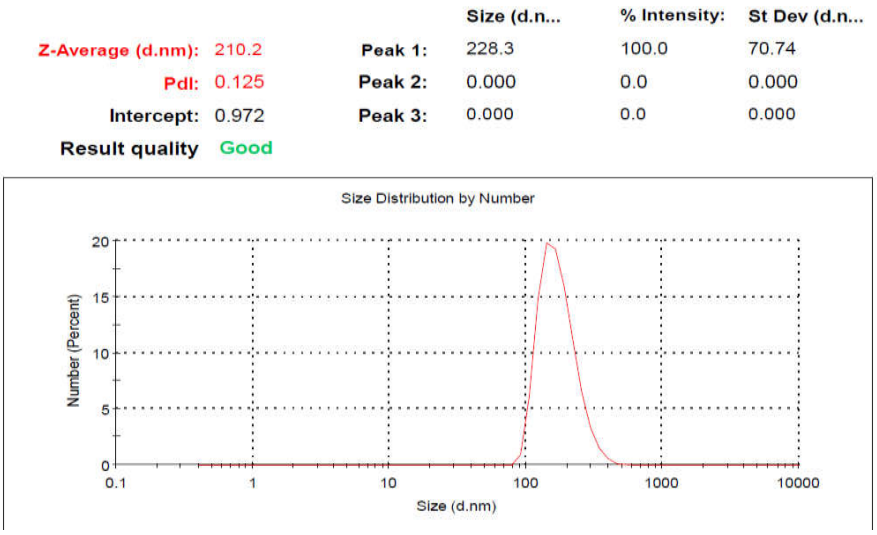

Fig. 2: Particle size images by dynamic light scattering technique of curcumin nanoparticle

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -8.87	Peak 1:	-8.87	100.0	2.74
Zeta Deviation (mV): 2.74	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 1.42	Peak 3:	0.00	0.0	0.00
Result quality Good				

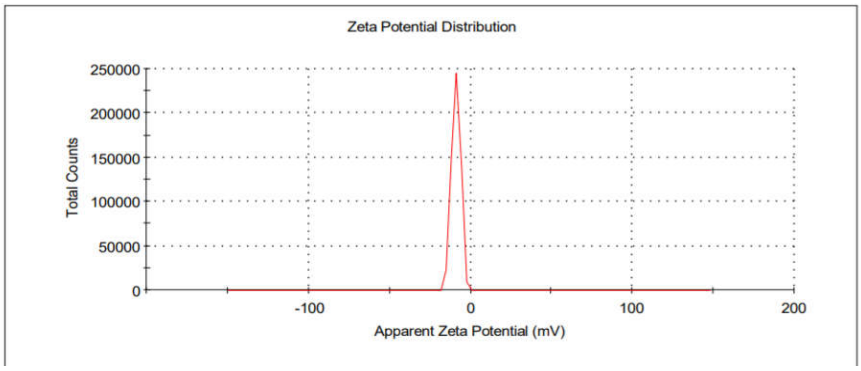

Fig. 3: Zeta potential of prepared nanoparticles

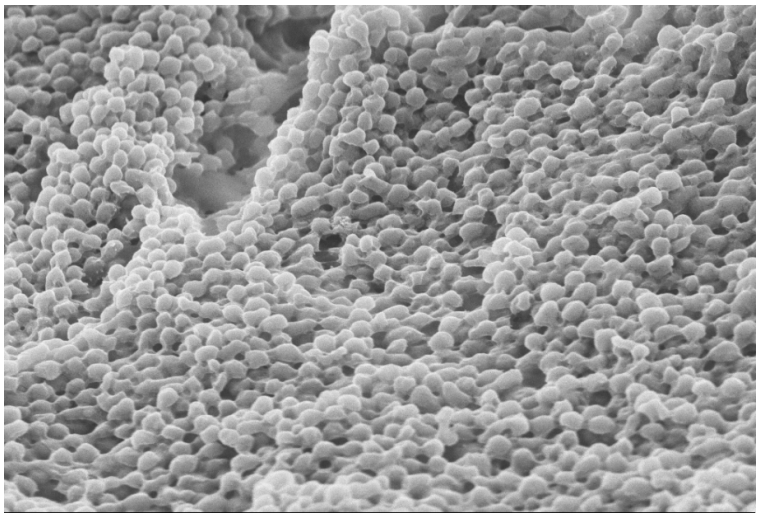

Fig. 4: SEM image for morphological study of nanoparticles

Table 1: Physicochemical characterizations of nanoparticles*

Size (nm)	Potential (mV)	Poly dispersity index	Encapsulation efficacy %	Drug loading %
148.71±2.71	-0.9±1.72	0.171±3.42	5.21±0.23	11.62±0.61

*Data are presented as mean±standard deviation (SD) (n = 3)

Drug release

Release of the drug was observed using the UV-vis method and for the first 2 h. burst release was found and it was approximately 25%. After that stable release occur and up to 7 d it was observed and found to be release in steady state manner. So, it can be concluded as biphasic, firstly burst release and then prolonged release, which is most suitable for polymeric nanoparticle [21].

Kinetic modelling

From the obtained drug release kinetic data model was developed like first order, zero order, higuchi and korsemeyer peppas. R^2, n and $|k_1|k_2|$ were determined to see which type of release pattern nanoparticles were following. R^2values of higuchi model were closed to 1 and from that we can conclude that it was following diffusion control release pattern (table 2) (table 3).

Table 2: Release condition for empirical model

Release condition	Zero order release			First Order		Higuchi model		
	K_0	C_0	R^2	K_f	R^2	K_H	C_H	R^2
pH 7.4	9.73	3.46	0.6471	0.293	0.7421	14.73	4.6	0.9988

Table 3: Release condition for non-empirical model

Release condition	Korsemeyer-peppas			Peppas-sahlin			
	K_{KP}	n	R^2	K_1	K_2	R^2	$\|K_1\|/\|K_2\|$
pH 7.4	14.23	0.31	0.9871	17.92	5.32	0.9989	6.05

Cellular toxicity study

Cellular toxicity study was done on A549 cell line. IC_{50} was obtained for free drug and polymeric nanoparticles. The IC_{50} values for free drug were found to be 11.07±0.03 µM, 9.13±0.07 µM and 7.42±0.05 µM for 24, 48 and 72 h. respectively. IC_{50} values for nanoparticles were 7.32±0.71 µM, 5.27±0.31 µM and 3.21±0.02 µM for 24, 48, and 72 h. respectively (table 4). It can be seen that nanoparticles were more effective on lung cancer cell line than the free drug. So, nanoparticles get more potency than the free drug [22].

Table 4: A549 IC_{50} values for 24, 48 and 72 h*

Sample	A549 IC_{50}		
	24 h	48 h	72 h
Free curcumin	11.07±0.03	9.13±0.07	7.42±0.05
Nanoparticles	7.32±0.71	5.27±0.31	3.21±0.02

*Significance level p<0.05, ** significance level p<0.01 Free Drug vs Nanoparticles

CONCLUSION

So, in this study, we basically developed the polymeric nanoparticles of curcumin and compared it cytotoxicity with its free from. Characterization and release pattern were also studied and release was found to be suitable for prolong delivery with diffusion control. The cytotoxicity was found to be more for nanoparticles on 24, 48 and 72 h. compared to free curcumin according to IC_{50} values. So, it can be concluded that polymeric nanoparticles of curcumin could be a potential delivery system for cancer therapy.

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CONFLICT OF INTERESTS

Declared none

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