Formulation, Characterization and Evaluation of Curcumin-loaded PLGA Nanospheres for Cancer Therapy

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Abstract. *Background: Among the potent anticancer agents, curcumin has been found to be very efficacious against many different types of cancer cells. However, the major disadvantage associated with the use of curcumin is its low systemic bioavailability when administered orally due to its poor aqueous solubility. Our present work investigated the efficiency of encapsulation of curcumin in poly (lactic-co-glycolic acid) (PLGA) nanospheres using solid/oil/water emulsion solvent evaporation method. Materials and Methods: The nanospheres were formulated and then characterized for percent yield, encapsulation efficiency, surface morphology, particle size, drug distribution studies, drug polymer interaction studies and in vitro drug release profiles. Results: Our studies showed the successful formation of smooth and spherical curcumin-loaded PLGA nanospheres, with an encapsulation efficiency of 90.88±0.14% . The particle size distribution showed a range of 35 nm to 100 nm, with the mean particle size being 45 nm. Evaluation of these curcumin-loaded nanospheres was carried out in prostate cancer cell lines. Results showed robust intracellular uptake of the nanospheres in the cells. Cell viability studies revealed that the curcumin-loaded nanospheres were able to exert a more pronounced effect on the cancer cells as compared to free curcumin. Conclusion: Our studies achieved successful formulation of curcumin loaded PLGA nanospheres, thus indicating that nanoparticle-based formulation of curcumin has high potential as an adjuvant therapy for clinical application in prostate cancer.*

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Curcumin has been used in traditional medicine for many centuries in countries such as India and China (1). It is chemically a yellow polyphenol, diferuloylmethane extracted from the rhizomes of turmeric (*Curcuma longa*). It posseses low intrinsic toxicity alongwith a wide range of pharmacological activities that include antitumor, anti-amyloid, antioxidant and anti-inflammatory properties. Previous investigations on curcumin have established that free curcumin induces cell cycle arrest and/or apoptosis and blocks nuclear factor kappa B (NF-κB) activation in various human cancer cell lines (2-11). Curcumin has been associated with regression of pre-malignant lesions of the bladder, soft palate, gastrointestinal tract, cervix and the skin, with treatment responses in established malignancy (12-16). The only factor that limits the use of free curcumin for cancer therapy is its poor solubility in water, which in turn limits its systemic bioavailability when administered orally. Polymeric nanoparticle-based drug delivery is being increasingly investigated as this delivery route is known to overcome many obstacles associated with the delivery of free drugs. PLGA was chosen for its biodegradable and biocompatible properties and its approval by the U.S. Food and Drug Administration.

In our present work, we formulated a delivery system where curcumin was encapsulated in PLGA nanospheres by solid-in-oil-in-water (s/o/w) solvent evaporation technique. The curcumin loaded nanospheres were then characterized for their percent yield and encapsulation efficiency, particle size, surface morphology. Curcumin distribution within the nanospheres and the existence of any drug-polymer interaction were investigated. Furthermore, the curcumin-PLGA nanospheres were evaluated for their *in vitro* release profiles, cellular uptake and effect on cell viability in prostate cancer cell lines (LNCaP, PC3 and DU145).

Materials and Methods

Materials. Poly(D,L-lactide-co-glycolide) 50:50; inherent viscosity 1.13 dl/g; mw 50,000 was purchased from Absorbable Polymers International (Pelham, AL, USA). Polyvinyl alcohol (PVA; mw 30,000-70,000), curcumin, chloroform, ethanol, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Nile red

were purchased from Sigma Aldrich (St. Louis, MO, USA). RPMI-1640 media, keratinocyte media and fetal bovine serum was obtained from Gibco, Invitrogen (Carlsbad, CA, USA). Nu Page® Nuclear extraction and electrophoretic mobility shift assay kits were obtained from Pierce®, USA and Panomics Inc, CA, USA. Gold antifade mounting agent with 4'-6-diamidino-2-phenylindole (DAPI) was purchased from Invitrogen. Double-distilled deionized water was used for all the experiments.

Solid/oil/water (s/o/w) technique for preparation of nanospheres. Curcumin loaded nanospheres were formulated using s/o/w emulsion technique (17). Briefly, 30 mg of the polymer, PLGA, were dissolved in chloroform. Free curcumin was added to the PLGA/chloroform solution and sonicated at 55 W for 1 minute in a Branson Sonifier model W-350 (Branson, Danbury, CT, USA) to produce the s/o primary emulsion. This emulsion was then added to a solution of 2% PVA and ethanol (1:1) and again sonicated at 55 W for 2 minutes to form the final s/o/w emulsion. The final s/o/w emulsion was then centrifuged at 15,000×g for 15 minutes to assist the removal of residual solvents. The nanospheres thus obtained were washed three times with deionized distilled water. They were then freeze dried and lyophilized for 24 hours on an ATR FD 3.0 system (ATR Inc., St. Louis, MO, USA). The nanoparticles were stored at 4°C until further use.

For preparing fluorescent nanospheres, a 1 mg/ml aqueous stock solution of Nile red was prepared. From the stock solution, 10 μl of Nile red were added to a PLGA/chloroform solution and the formulation was carried out as described above. The labeled nanospheres were stored in the dark at 4°C until use in experimentation.

Optimization and characterization of nanospheres. The formulation procedure was varied in PVA concentration and sonication time to optimize the formulation. Characterization of the nanospheres is needed to ascertain their reproducibility in terms of *in vitro* and *in vivo* performances. For the determination of the optimal formulation parameters with respect to formulation of the curcumin-loaded PLGA nanospheres, percentage yield, percentage entrapment efficiency, particle size and surface morphology of the nanoparticles were determined.

Determination of percent yield and encapsulation efficiency: The dried nanospheres were weighed and the respective yields were calculated by using the following equation:

$$\%Yield = \frac{Weight\ of\ nanospheres\ obtained}{Weight\ of\ drug\ and\ polymer\ used\ for\ nanosphere\ preparation} \times 100$$

The encapsulation efficiency of the nanospheres was determined by analyzing the supernatant of the final emulsion once the nanospheres were removed from it by centrifugation at 15,000×g for 15 minutes. For the estimation of curcumin present in the supernatant, the absorbance was measured spectrophotometrically at 450 nm and the amount of drug present was calculated from calibration curves of concentration versus absorbance with known standards of the drug. The amount of the drug encapsulated and the % encapsulation in the nanospheres is given by:

$$Drug_{encapsulated} = Drug_{total} - Drug_{filtrate}$$

$$\%\ Encapsulation = \frac{Drug_{encapsulated}}{Drug_{total}} \times 100$$

Determination of particle size: Particle size analysis of the curcumin-loaded nanospheres was carried out using a Nanotrac system (Mircotrac, Inc., Montgomeryville, PA, USA). The lyophilized nanospheres were dispersed in aqueous buffer using an ultrasonic water-bath (Fisher Scientific, USA) for 2 minutes and then measured for particle size. The results were reported as the average of five runs with triplicate runs in each run.

Determination of surface morphology: The surface morphology of the nanospheres was studied using transmission electron microscopy, (TEM). A small quantity of aqueous solution of the lyophilized curcumin-loaded nanoparticles (1 mg/ml) was placed on a TEM grid surface with a filter paper (Whatman No. 1). One drop of 1% uranyl acetate was added to the surface of the carbon-coated grid. After 1 minute of incubation, excess fluid was removed and the grid surface was air dried at room temperature. It was then loaded into the transmission electron microscope (LEO EM910, Carl Zeiss SMT Inc, NY, USA) attached to a Gatan SC 1000 CCD camera.

Evaluation of drug distribution within the nanospheres. The distribution of curcumin within the nanospheres was examined by using a confocal laser scanning microscope (LSM 410, Carl Zeiss, USA). This technique allows visualization and characterization of structures not only on the surface, but also inside the particles, provided the material is sufficiently transparent and can be fluorescently labeled. Curcumin-loaded Nile red-labeled PLGA nanoparticles were used for this study. Since curcumin is naturally fluorescent in the visible green spectrum, no further labeling of curcumin was needed. A small drop of the curcumin-loaded fluorescently labeled nanospheres in water was mounted on a slide and visualized in the fluorescein isothiocyanate (FITC) (488 nm) channel. The laser was adjusted in the green/red fluorescence mode, which yielded two excitation wavelengths at 488 and 568 nm. Green and red fluorescence images were obtained from two separate channels and the final pictures were composed from a merge of red and green fluorescence to visualize the labeled structures.

Drug-polymer interaction studies. Differential scanning calorimetric (DSC) studies of the drug, polymer and nanospheres were carried out to define the physical state of the drug in this carrier and the possibility of any interaction between the drug and the polymer in the nanospheres. DSC curves were recorded on a scanning calorimeter equipped with a thermal analysis data system (Pyris Diamond; Perkin Elemer, USA). The instrument was calibrated using alumina powder as the standard. A small amount of nanosphere sample was placed in hermetically sealed aluminum pans and heated from 25°C to 700°C at a heat flow rate of 10°C/min under nitrogen spurge.

Evaluation of curcumin release from nanospheres. Curcumin-loaded PLGA nanospheres were evaluated for their *in vitro* release kinetics of curcumin. Since free curcumin is insoluble in water, free curcumin released in aqueous buffer can easily be quantified after its separation. A known quantity of lyophilized PLGA nanospheres (100 mg) encapsulating curcumin was dispersed in 10 ml phosphate buffer, pH 7.4. The solution was divided into microcentrifuge tubes (500 μl each). The samples were kept in an orbital shaker (Cellstar, USA) maintained at 37°C±0.5°C stirring at 50 rpm. At predetermined intervals of time, the solution was centrifuged at 3000 rpm for 10 minutes to separate the released (pelleted)
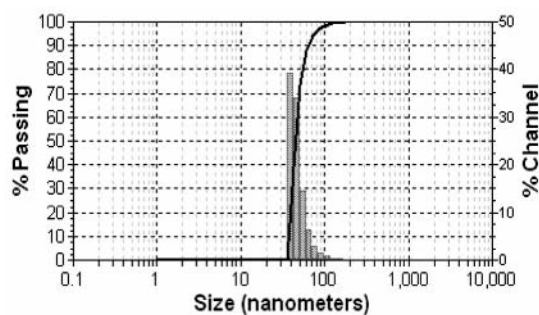
Figure 1. *Particle size distribution of curcumin-loaded PLGA nanospheres: The mean particle diameter was found to be 45 nm.*

Table I. *Effect of process variables on the particle size of the PLGA nanospheres.*

Sonication time (min)	Particle size (nm)	
	1% PVA	2% PVA
0.5	460.1	390.5
1.0	297.4	232.8
1.5	246.7	168.2
2.0	87.3	45.0

curcumin from the loaded nanospheres. The released curcumin was redissolved in 1 ml of ethanol and the absorbance was measured spectrophotometrically at 450 nm. The concentration of the released curcumin was then calculated using a previously prepared standard curve of curcumin in ethanol.

Intracellular nanosphere uptake studies. Prostate cancer cell lines, LNCaP, PC3 and DU-145, (ATCC, Manassas, VA, USA) were grown under standard cell culture conditions in RPMI-1640 media supplemented with 10% FBS and 1% penicillin/streptomycin. Cellular uptake of curcumin-loaded Nile red-labeled nanospheres was determined using a fluorescence microscope (Olympus-Provis, AX70) attached to an Olympus camera (DP70 Digital). For these experiments, cells were placed on a cover slip in a 6-well tissue culture plate and incubated at 37°C until they reached sub-confluent levels. The cells were then exposed to 100 μg/ml concentrations of curcumin-loaded labeled nanoparticles. At time points of 30 minutes, 1 hour and 3 hours, cells were viewed under the microscope to determine maximum uptake.

Cell viability assay in cancer cell lines. To determine the effect of curcumin-loaded nanospheres on cell growth, cell viability (MTT) assay was carried out with prostate cancer (LNCaP, PC3 and DU-145) cell lines. Non tumorogenic cell line, PWR1E, was used to establish the relative action of the curcumin nanospheres on the cell viability of normal cells. The inhibition in cell growth was measured by the MTT assay. For this assay, ~2000 cells/well were plated in a 96-well plate and were treated with 0, 10, 15, 20 and 30 μM concentrations of free curcumin and equivalent doses of curcumin-loaded nanospheres. The assay was terminated after 72 hours and relative growth inhibition compared to control cells was measured. All experiments were set up in triplicates and repeated twice for statistical analysis. Results were expressed as mean±S.D.

Effect of curcumin nanoparticles on NF-κB activity. NF-κB is a ubiquitous transcription factor and it plays a critical role in the cells of the immune system, the host defense against disease. It controls the expression of various cytokines and the major histocompatibility complex gene. Curcumin has been shown to target and inhibit activated NF-κB to restrict tumor cell growth in various cancer types (18-20). In order to demonstrate the similarity of the effect of curcumin loaded nanospheres to that of curcumin, an EMSA was carried out to demonstrate the DNA-binding ability of NF-κB in the absence (control) and the presence of curcumin and curcumin-loaded PLGA nanospheres. For this experiment, LNCaP cells (2×10^6 cells/ml) were treated with curcumin-loaded nanospheres for 12 hours. Untreated cells were kept as control. Nuclear extracts of both treated and untreated cells were prepared using Nu Page® Nuclear extraction kit (Pierce®, USA). The assays were performed by incubating 5 μg of nuclear extract with poly d(I-C) and binding buffer. This solution was then incubated with NF-κB probe at 15ºC for 30 minutes in a thermal cycler. A competition assay was also performed alongside wherein the above solution was incubated with cold NF-κB probe before being incubated with NF-κB probe. This resulted in the cold NF-κB probe binding with the NF-κB probe, thereby rendering it inactive for binding to NF-κB. All the samples (DNA-protein complexes) were then resolved on a 6.0% non denaturing polyacrylamide gel using Tris-borate-EDTA (TBE) buffer. After electrophoresis, the proteins were transferred to a Pall Biodyne B nylon membrane (Pall Corporation, PA, USA) using an electroblotting device for 30 minutes at 300 mA. The oligotides on the membrane were fixed by UV crosslinking. The oligotides were then blocked, treated with streptavidin-horseradish peroxidase (HRP) and a chemiluminescence agent and finally detected by chemiluminescence using the FluorChem Imager (Alpha Innotech Corp., CA, USA).

Results

Percent yield, encapsulation efficiency and particle size analysis. In this nanosphere formulation process, the PVA solution acts as the stabilizer and the ethanol acts as the non-solvent. The effect of process variables such as PVA concentration and sonication time on particle size is given in Table I. The formulation was optimized at 2% PVA concentration. Sonication time of 2 minutes resulted in the smallest range of particle size, with optimum entrapment. The yield of the optimum batch of nanospheres was found to be high at 92.01±1.13%. The corresponding encapsulation efficiency of curcumin in the nanospheres was found to be 90.88±0.14%. The particle size of the nanospheres was determined by dynamic light scattering. Figure 1 depicts a narrow size distribution for the optimized batch of PLGA nanospheres ranging from 35 nm to 100 nm, with the mean particle size being 45 nm.
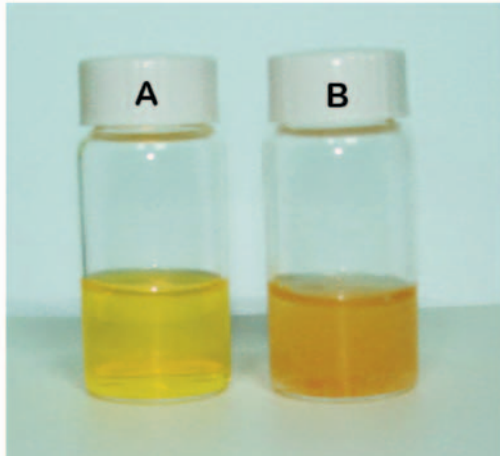
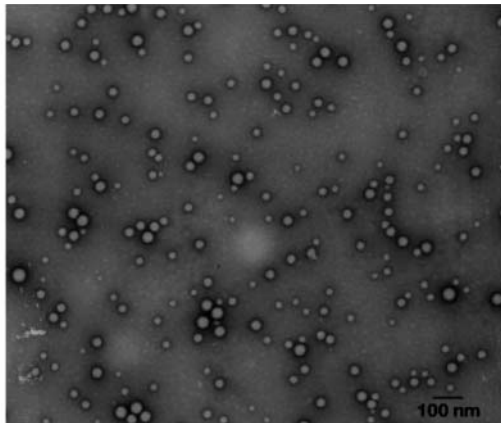Figure 2. *A, Curcumin-loaded PLGA nanospheres in water, and B, free curcumin in water.*

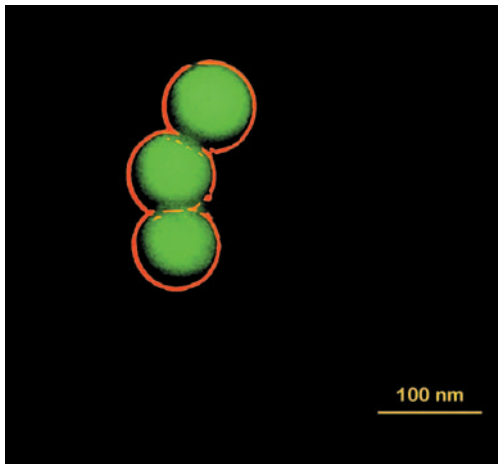Figure 3. *Transmission electron microscopy scan of curcumin-loaded PLGA nanospheres.*

Figure 4. *Confocal microscopy scan illustrating the encapsulation and distribution of curcumin (green fluorescence) within the PLGA nanospheres.*
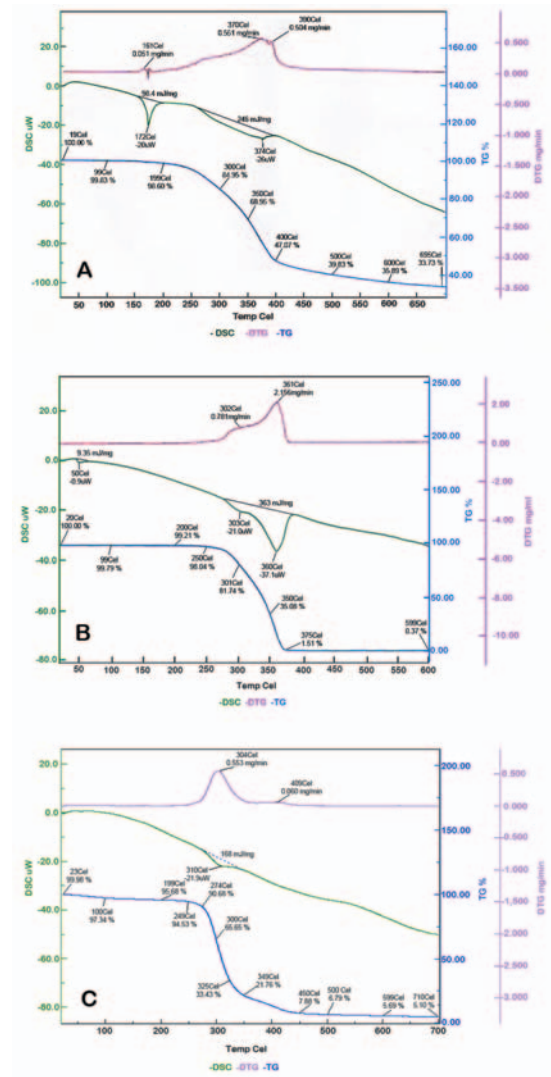

Figure 5. *Differential scanning calorimetric curves of free curcumin (A) PLGA polymer (B) and curcumin-loaded PLGA nanospheres (C).*
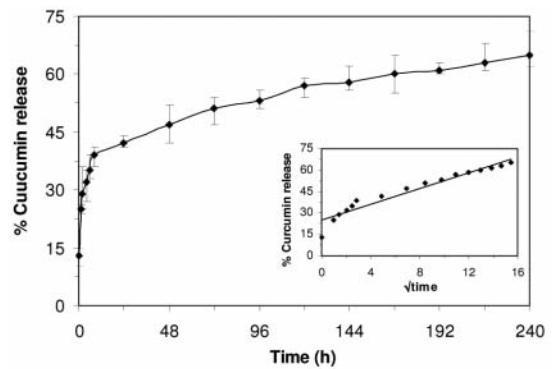

Figure 6. *In vitro profile of release curcumin from PLGA nanospheres in PBS (pH 7.4) over a period of 10 days. All release assays were performed in triplicate, and the mean±standard deviations are shown. The inset shows the Higuchi plot indicating the controlled release of curcumin from the PLGA nanospheres.*
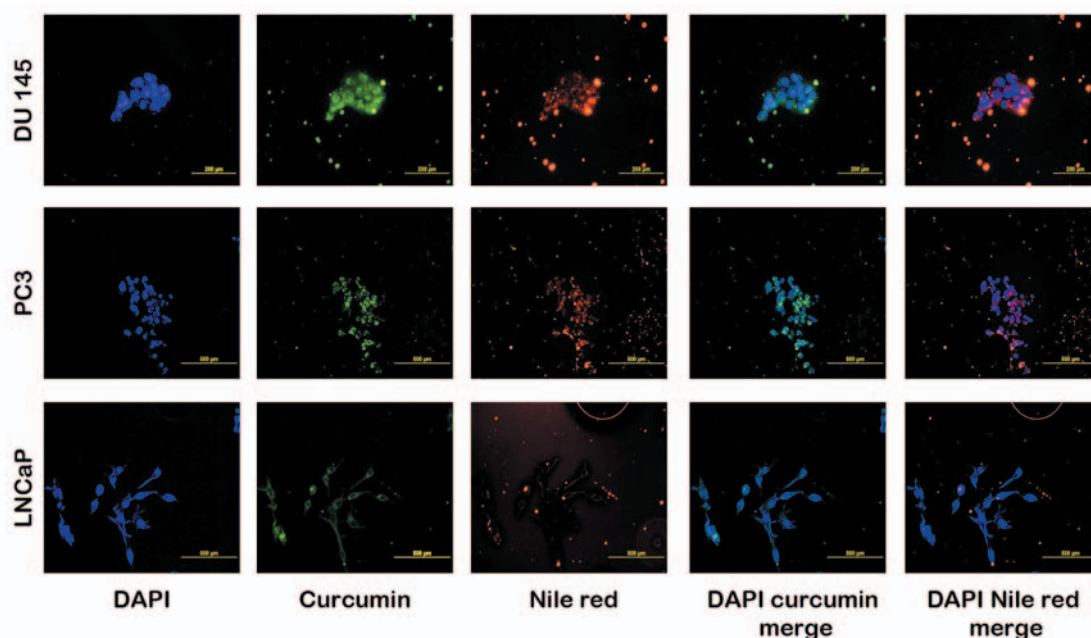
Figure 7. *Confocal images of different cell lines incubated with curcumin-loaded nile red-labeled PLGA nanospheres: cell nuclei stained with DAPI, cells showing curcumin in PLGA nanospheres, cells showing nile red-labeled PLGA nanospheres, merged image of DAPI and curcumin in PLGA nanospheres, and merged image of DAPI and nile red-labeled nanospheres.*

In addition, with this formulation procedure, the curcumin-loaded PLGA nanospheres prepared were completely dispersed in aqueous media with no aggregates as opposed to free curcumin which exhibits poor aqueous solubility (Figure 2).

Surface morphology of nanospheres. The surface morphology of the nanospheres encapsulating curcumin, prepared by the s/o/w emulsion technique, was determined by TEM. Figure 3 illustrates a TEM scan showing the formation of spherical and smooth nanospheres. The scan also reveals that the particles have a more or less uniform size distribution and low polydispersity as represented in Figure 1.

Drug distribution of curcumin within nanospheres. Figure 4 shows three nanospheres with curcumin, appearing green entrapped inside the PLGA nanospheres. The polymer shell of these nanospheres appears red as it was labeled with Nile red during the formulation process. The drug, curcumin, can be seen to be uniformly distributed within the nanospheres. This is a critical parameter that would help in uniform drug release from the nanospheres once they are administered *in vivo*.

Drug-polymer interaction studies. The DSC curves of PLGA, curcumin and curcumin-loaded PLGA nanospheres are shown in Figure 5A-C. The pure drug curcumin (Figure 5A) gives rise to a sharp peak that corresponds to melting at 172˚C, indicating its crystalline nature. A broad peak is observed due to the thermal decomposition of the drug, with maximum temperatures around 400˚C. The pure PLGA polymer exhibits a small peak at 50˚C, referring to the relaxation peak that follows the glass transition (Figure 5B). No distinct melting point was observed because PLGA is amorphous in nature. The two peaks at 303˚C and 360˚C are related to the thermal decomposition of the polymer. The DSC curves of pure PLGA show that the polymer is thermally stable upto 250˚C. It can be observed from Figure 5C that the microencapsulation process did not affect the polymer structure: the pure polymer had the same value for thermal decomposition as that of curcumin-containing particles. The DSC study did not detect any crystalline drug material in the curcumin-loaded nanosphere sample as the sharp peak of curcumin was absent. Thus, the drug incorporated into the nanospheres was in an amorphous or disordered-crystalline phase of molecular dispersion or solid solution state within the polymer matrix.

In vitro profile of curcumin release from nanospheres. The release kinetics of curcumin from the PLGA nanospheres was studied for 10 days. Drug release from PLGA nanospheres usually occurs in a biphasic manner, with an initial burst phase followed by a diffusion-controlled slower drug release phase. In our studies, an initial burst phase corresponding to about 10-13% was observed within 1 hour due to the drug desorption and release from the nanosphere
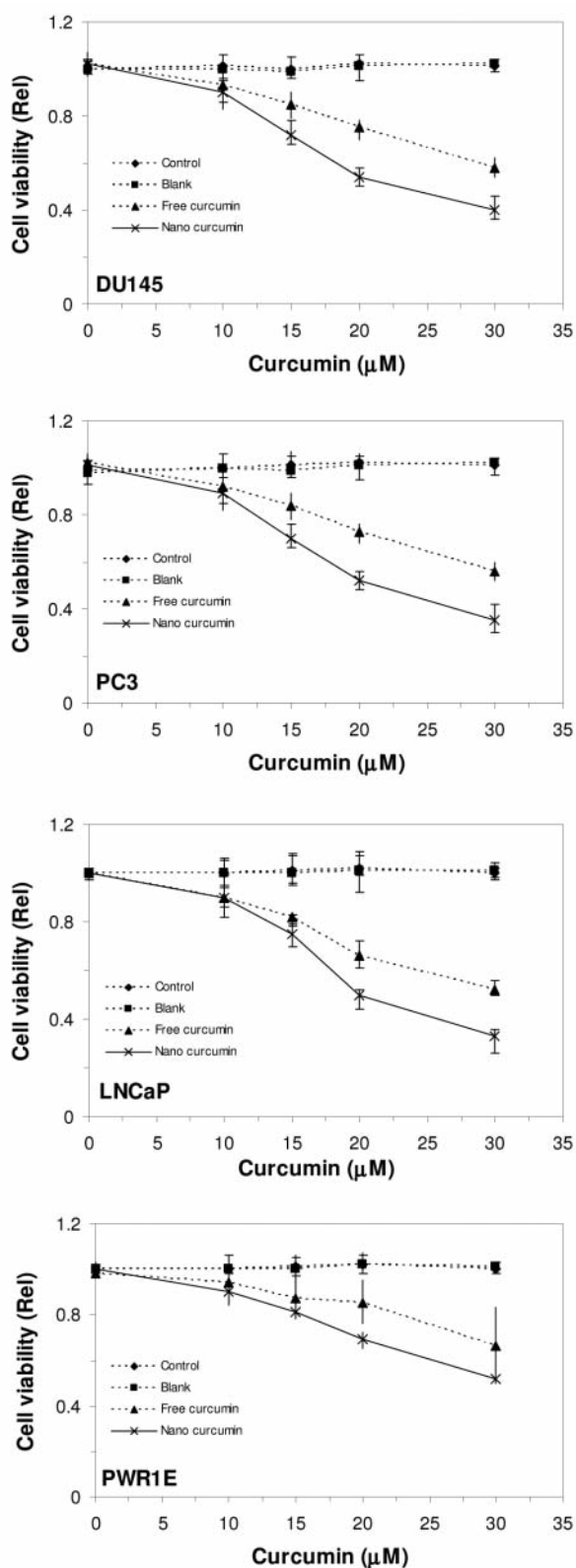
Figure 9. *Electromobility shift assay: Lane 1: control untreated LNCaP cells; Lane 2: LNCaP cells treated with free curcumin; Lane 3: LNCaP cells treated with curcumin-loaded PLGA nanospheres; Lane 4: Competition assay with cold NF-κB probe. EMSA shows marked decrease in NF-κB-DNA binding due to inhibition in action by curcumin-loaded PLGA nanospheres.*

Figure 8. *Cell viability (MTT) assay of curcumin-loaded PLGA nanospheres in LNCaP, PC3, DU145 and PWR1E cells. All assays were performed in triplicate, and the mean±standard deviations are shown.*
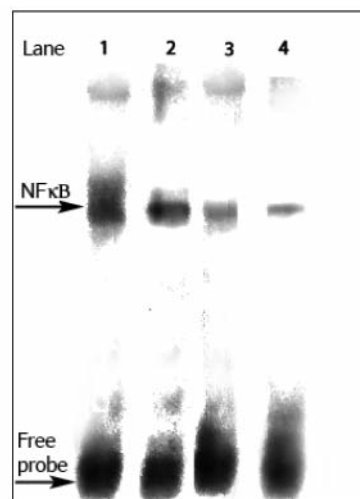
surface. A sustained curcumin release to a total of about 65% was found for the nanospheres over the entire period of study, as depicted in the graph shown in Figure 6. The inset in the Figure represents a linear Higuchi plot, which illustrates controlled release of curcumin from the PLGA nanospheres.

Intracellular uptake studies. Having determined the formulation and characterization of the curcumin-loaded nanospheres, we investigated the ability of these nanospheres to be endocytosed by cells. Figure 7 illustrates an entire panel of the fluorescence microscope images of different cancer cell lines, DU145, PC3 and LNCaP incubated with curcumin-loaded Nile red-labeled PLGA nanospheres for three hours. Our results depict robust uptake of the nanospheres in all three prostate cancer cell lines. Control cells without any curcumin or nanosphere treatment did not show any fluorescence (data not shown). The cells incubated with curcumin-loaded Nile red-labeled nanospheres exhibited either red (due to Nile red) or green (due to curcumin) fluorescence, depending upon the excitation wavelength following rapid internalization and accumulation of curcumin nanospheres by the cancer cells.

Cell viability assay. Cell viability (MTT) assays were performed using equivalent dosages of free curcumin, curcumin-loaded PLGA nanoparticles and blank PLGA nanoparticles. Untreated cells served as controls. The assay was terminated at 72 hours and colorimetric determination

of cell viability was performed. The results of the MTT cell viability assay on prostate cancer cell lines, LNCaP, PC3 and DU145, and a non tumorigenic cell line, PWR1E, are shown in Figure 8. The IC_{50} of curcumin-loaded PLGA nanoparticles was found to be between 20 μM to 22.5 μM while that of free curcumin ranged from 32 μM to 34 μM among all the cancer cell lines. This accounts for almost 35% reduction in the IC_{50} value with curcumin-loaded nanoparticles. The assay demonstrated that curcumin-loaded PLGA nanospheres were more effective in arresting cell growth as compared to that shown by free curcumin. The cell viability assay of PWR1E cells showed that the IC_{50} of curcumin-loaded PLGA nanoparticles was 31 μM and that of free curcumin was 37 μM. Comparing the IC_{50} values between non tumorogenic cell line and prostate cancer cell lines, we see a lower IC_{50} value for the cancer cells than normal cells for both free curcumin and curcumin-loaded PLGA nanoparticles. Furthermore, fewer viable prostate cancer cells were seen for the same concentration of curcumin-loaded nanospheres. This indicates that curcumin preferentially induces apoptosis in highly proliferating cells, and death is more pronounced in cancer cells than in normal cells.

Effect of curcumin nanoparticles on NF-κB activity. Electrophoretic mobility shift assay (EMSA) was carried out to determine the DNA-binding ability of NF-κB in LNCaP cells before and after treatment with curcumin and curcumin-loaded nanospheres. The results, as seen in Figure 9, show that curcumin-loaded PLGA nanospheres were indeed able to inhibit NF-κB function to a greater degree as compared to free curcumin, as depicted with fainter bands of NF-κB (lane 3 as compared to lane 2), which essentially represents absence of NF-κB binding of the kappa-binding oligonucleotide.

Discussion

Numerous investigations have been conducted at developing more efficient systems for drug delivery. The most important challenge in the successful formulation of polymeric drug delivery systems involves preparing carrier systems which are capable of encapsulating the desired drug within its structure and then deliver the drug to the cancerous tissues in its active form. The formulation of synthesizing drug loaded polymeric nanoparticles involves preparation and characterization of drug encapsulated nanoparticles. The fate of a drug after administration *in vivo* depends primarily on the physicochemical properties of the drug and on its chemical structure (21), therefore physicochemical characterization of drug loaded nanoparticle system becomes essential. Particle size is an important parameter as it can directly affect the physical stability, cellular uptake, biodistribution and drug

release from the nanoparticles (21). Our formulation of curcumin-loaded PLGA nanoparticles by using the s/o/w technique resulted in particles with high curcumin encapsulation (~91%) with a size range of 35-100 nm. Other studies have reported different curcumin formulations such as micellar aggregates of cross-linked and random copolymers of *N*-isopropylacrylamide (NIPAAM), with *N*-vinyl-2-pyrrolidone (VP) in a particle size range of 50 nm to 500 nm for curcumin nanoparticles and poly(ethyleneglycol) monoacrylate (PEG-A) (9). Self-assembling methoxy poly(ethylene glycol)–palmitate curcumin nanocarrier and micelles of poly(ethylene oxide)-b-poly(e-caprolactone) were also reported to have a similar particle size range (22-23). Morphology of the nanoparticle in terms of surface and sphericity (as shown by TEM study) plays a critical role in their *in vitro* and *in vivo* release characteristics.

Characterization of the physiochemical properties of the drug encapsulated within the nanoparticles may possibly reveal useful information for the feasibility of using nanoparticles for cancer therapy (24). All fluorescent compounds can be seen and identified by confocal microscopy at light microscopic resolution. Unlike conventional light microscopy, confocal microscopy also enables us to qualitatively determine localization as well as the amount of the inner drug phase without having to first establish complex analytical procedures. DSC analysis helped in determining the chemical integrity of curcumin and its interaction with the polymer and confirmed that the drug retained its properties even when encapsulated inside nanoparticles (21). *In vitro* drug release experiments were carried out to measure the release of encapsulated curcumin in buffer. The rapid initial release is attributed to the drug which is adsorbed or weakly bound to the surface area of the nanoparticles, than to the drug incorporated inside nanoparticles (25). A much slower sustained release of curcumin (~65%) is observed over a prolonged period of 10 days from the nanoparticles. Other studies have reported a release of ~40% curcumin in 24 hours with co-polymeric micellar aggregates of NIPAAM and VP (9).

Intracellular uptake of curcumin-loaded Nile red-labeled PLGA nanoparticles in DU145, PC3 and LNCaP cell lines were investigated by fluorescence microscopy. We observed that these nanoparticles were actively taken up by all the cancer cells as seen by red (due to Nile red-labeled particles) and green (due to curcumin present within the particles) fluorescence within the cells. Other studies have shown green fluorescence by curcumin micelles (nanocurcumin) in BxPC3 cells (9) and similar uptake characteristics of curcumin nanocarriers prepared from methoxy polyethylene glycol palmitate in HeLa cell lines (21). Further, to investigate the therapeutic potential of our formulation, prostate cancer cell lines, DU145, PC3 and LNCaP, and a non tumorigenic cell line, PWR1E, were treated with free curcumin, curcumin-

loaded PLGA nanoparticles and blank nanoparticles at different concentrations (0-30 µM) for 72 hours and cell proliferation was measured by a standard MTT assay. The results of the MTT assay confirmed that curcumin-loaded PLGA nanoparticles exhibited a lower IC50 value in comparison to free curcumin in all cancer cell lines studied. Other studies of curcumin formulations such as micellar aggregates of cross-linked and random copolymers of NIPAAM, with VP and PEG-A and self-assembling methoxy poly(ethylene glycol)–palmitate curcumin nanocarrier have shown to exhibit similar growth inhibition to that of free curcumin (9, 22). Furthermore, it was observed that when cell viability was compared between the non tumorigenic cell line and prostate cancer cell lines, fewer viable prostate cancer cells were seen for the same concentration of curcumin-loaded nanospheres. This may be explained by the fact that curcumin preferentially induces apoptosis in highly proliferating cells, with death being more pronounced in cancer cells than in normal cells (12). This phenomenon is amplified by using curcumin-loaded nanoparticles, resulting in increased preferential cytotoxicity to cancer cells. The effect of curcumin-loaded PLGA nanoparticles on NF-κB activity in cancer cells was demonstrated by EMSA. The results indicated that LNCaP cells treated with curcumin-loaded PLGA nanoparticles showed a distinct decrease in NF-κB activity, possibly due to the absence of NF-κB binding to the kappa oligonucleotide. Therefore, this curcumin-loaded PLGA nanoparticle formulation might be a better therapeutic approach than free curcumin for treating prostate cancer.

Conclusion

In this study, curcumin-loaded PLGA nanospheres were prepared by using a s/o/w emulsion solvent evaporation technique. Our studies showed that smooth, spherical PLGA nanospheres were formed and exhibited high yield and drug entrapment efficiency, with a narrow size range of 35 nm to 100 nm and mean particle diameter of 45 nm. The *in vitro* curcumin release studies from the nanospheres showed that curcumin was released in a sustained manner over a prolonged period of time. Intracellular uptake and cell viability assays also demonstrated efficient uptake and action of the curcumin nanospheres in prostate cancer cell lines. It is therefore concluded that PLGA nanospheres are capable of delivering curcumin over a prolonged period achieving a sustained delivery of curcumin, thus making it a potential candidate for cancer therapy.

Acknowledgements

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