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Active curcumin nanoparticles formed from a volatile microemulsion template

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Abstract

We report on biological performance of organic nanoparticles formed by a simple method based on rapid solvent removal from a volatile microemulsion. The particular focus of the study was on testing the suitability of the method for substances soluble in partially water-miscible organic solvents as well as on evaluating the therapeutic activity of the resultant nanoparticles. Curcumin was employed as a model for hydrophobic drug, and, as it is soluble in water-miscible organic solvents, it was successfully incorporated into a new cyclopentanone-water microemulsion system. During rapid solvent removal by spray-drying, the nanometric droplets of the microemulsion were converted into nanoparticles containing amorphous curcumin with the average size of 20.2 ± 3.4 nm, having ζ potential of -36.2 ± 1.8 mV. These nanoparticles were dispersible in water and retained the high loading of the active substance. The therapeutic activity of the resulting nanoparticles was demonstrated in a pancreatic cancer cell line Panc-1. The effective concentration for reducing the metabolic activity was found to be $11.5 \mu\text{M}$ for nanoparticles compared with $19.5 \mu\text{M}$ for free curcumin.

1. Introduction

Insufficient aqueous solubility of organic therapeutically-active substances presents a long-standing challenge in drug delivery. Numerous drug candidates routinely discovered through combinatorial screening programs do not reach clinical development stage, because they lack sufficient solubility in water^{1,2}. This significantly compromises their oral bioavailability and prevents the development of parenteral products³. Several chemical and formulative approaches are being exploited to solve the poor solubility problem; reduction of the particle size to nanometric range is one of them. This approach receives much attention lately since it enables increasing both saturation solubility and dissolution rate of the drug in water without altering its chemical structure. The resulting enhancement in

bioavailability typically leads to a higher biological potency. According to the Noyes-Whitney equation⁴ the dissolution rate of a solid particle in aqueous media is inversely proportional to the particle radius. Thus, diminishing particle size to nanoscale can significantly increase the dissolution rate. In addition, saturation solubility of particles with sizes below 1 μ m is also a function of the particle size according to the Ostwald-Freundlich relation.^{5,6} The saturation solubility is equilibrium between a dissolution process (governed by dissolution pressure) and a re-crystallization process. Nanosizing increases the dissolution pressure and shifts the equilibrium to increase the saturation solubility.⁷

Additional enhancement of solubility during the process of particle size reduction may be achieved when utilizing methods which lead to obtainment of partially or fully amorphous product. It is well known that amorphous materials are markedly more soluble than their crystalline counterparts.^{8,9} Provided that a sufficient stability of the final amorphous nanoparticles is obtained, additional solubility advantage may be gained over unprocessed crystalline material.

In this study we develop a novel, yet, simple method for preparing nanoparticles of organic compounds with poor aqueous solubility.¹⁰⁻¹³ This method is based on a rapid conversion of volatile oil-in-water microemulsions containing the insoluble substances, into water-dispersible powders, which are composed of nanoparticles, by direct solvent evaporation. The resultant nanoparticles do not contain lipid matrix and are not encapsulated in polymeric shell. Since the microemulsions are formed spontaneously and contain droplets smaller than 30nm, the new method requires a significantly lower energy investment to form nanoparticles compared with other techniques. The method is suitable for creating nanoparticles of hydrophobic drugs which are soluble in the inner droplets of the microemulsion. Hydrophobic volatile solvent is typically employed to dissolve the active compound and the resulting solution can be microemulsified in water by addition of proper amounts of solid surfactants and volatile co-solvents/co-surfactants. Spray-drying is a preferred technique for rapid simultaneous removal of solvents and water to prepare nanometric powder.

When the new method was first reported by us, we limited its applicability to producing organic nanoparticles which are soluble in water-immiscible organic solvents thus enabling formation of oil-in-water microemulsion.¹⁰⁻¹³

Since many drugs are not soluble in water-immiscible solvents, in the present study we aimed at applying the process for organic molecules that are soluble in water-miscible solvents (e.g., short-chain alcohols or ketones). The model substance employed here was curcumin. We found that by dissolving curcumin in an organic solvent which has limited miscibility in water and by utilizing this solvent at concentrations significantly above its aqueous solubility limit, a proper microemulsion system can be reached and can serve as the template for nanoparticle synthesis.

In addition to expanding the use of the investigated method for drug candidates which are soluble in partly water-miscible solvents, we also aimed to evaluate the extent to which the resultant nanoparticles preserve the therapeutic activity of the native substance.

Curcumin is a natural product, mainly composed of diferuloylmethane (principle curcuminoid of the spice turmeric (Figure 1)), known for its wide range of beneficial properties. Its anti-inflammatory,^{14,15} antimicrobial,^{15–16} anti-androgenic,¹⁷ anti neoplastic,^{18–28} anti-angiogenic,²⁹ chemopreventive,^{19,30–32} hepatoprotective,³³ and hypoglycemic³⁴ effects have been reported. It was established that curcumin is safe even at very high doses,^{14,31} and, at the same time, possesses reasonable anti-proliferative effect on cancer cells.^{18–27} Despite its known therapeutic activity and general safety, curcumin is not approved as therapeutic agent mainly due to its poor oral bioavailability,³⁵ which most probably results from its low water solubility, 0.6 µg/ml.³⁶ Therefore, curcumin presents an excellent model substance for the conversion to nanoparticles by the novel method.

To evaluate that the biological activity of curcumin is preserved, here we tested the biological activity of curcumin nanoparticles (C-NP) formed by the volatile microemulsion method, and compared it to the biological activity of free curcumin dissolved in dimethylsulfoxide (C-DMSO).

2. Materials and methods

2.1. Materials

Curcumin from *Curcuma Longa* (containing a mixture of curcumenoids with at least 65 wt% diferuloylmethane by HPLC), cyclopentanone and ammonium glycyrrhizinate (AG) were obtained from Sigma-Aldrich (Israel), soybean phosphatidylcholine (SbPC) (at least 92% purity, Lipoid S75) was supplied by Lipoid (Germany), and dipotassium glycyrrhizinate (DG) was obtained from TCI (Japan). Thiazolyl Blue Tetrazolium Bromide (MTT) was purchased from Sigma-Aldrich (USA). PANC-1 cell line was from ATCC (USA), DRAQ7TM was purchased from Biostatus (UK).

2.2. Methods

The process of conversion of curcumin to nanoparticles is schematically shown in Figure 2.

2.2.1. Microemulsion formation—The microemulsion was prepared by dissolving curcumin in cyclopentanone and adding surfactants to the resultant solution as to create an oil phase at the first step. Next, the cyclopentanone solution was added to water. The exact concentrations of components used for the microemulsion formation are summarized in Table 1. The mixture was then allowed to equilibrate at 25°C until a transparent isotropic system was formed. As a control, system without surfactants was prepared, which resulted in immediate phase separation.

2.2.2. Converting microemulsion to nanoparticles—To remove the organic solvent and water at once, the microemulsion was spray dried using a Mini-laboratory Spray Dryer B-290 equipped with inert loop dehumidifier B-296 (Buchi, Flawil, Switzerland). Process conditions were: air inlet temperature 110°C, drying chamber temperature (outlet temperature) 60°C, liquid introduction rate 5 ml/min, spray flow rate 414 normliter/hour, aspirator rate 35 m³/hour, nitrogen pressure 6 atmospheres.

2.2.3. X-ray diffraction (XRD)—X-ray powder diffraction measurements were performed using the D8 Advance diffractometer (Bruker AXS, Karlsruhe, Germany) with a goniometer radius of 217.5 mm, Göbel Mirror parallel-beam optics, 2° Sollers slits and 0.2 mm receiving slit. Standard sample holders were carefully filled with the samples. The specimen weight was approximately 0.5 gram. XRD patterns within the range of 5° to 50° 2 θ were recorded at room temperature using CuK α radiation ($\lambda=1.5418$ Å) with the following measurement conditions: tube voltage of 40 kV, tube current of 40 mA, step-scan mode with a step size of 0.02° 2 θ and counting time of 1 s/step. The diffraction pattern of the final powder was compared to the diffraction pattern of the raw crystalline curcumin and to the diffraction pattern of the physical mixture of all powder components mixed at the same proportions as in the final powder. In order to obtain a proper physical mixture all powder components were thoroughly ground with mortar and pestle.

2.2.4. Powder dispersion in water—Powders obtained at the end of the spray drying process were dispersed (0.1–10 wt%) in deionized water or phosphate buffer saline, (PBS), 0.01M, pH 7.4. The samples were vortexed for 30 seconds to ensure homogenous dispersion.

2.2.5. Particle/droplets size distribution measurements—The size distributions of particles after dispersion in water (0.1 wt%) and of microemulsion droplets before spray drying were measured at room temperature by dynamic light scattering (DLS) using a Nano-ZS Zetasizer (Malvern, UK). The samples were not filtrated prior to measurement. The instrument is equipped with 633 nm laser, and the light scattering is detected at 173° by a back scattering technology (NIBS, Non-Invasive Back-Scatter). The measurements were performed in triplicate.

2.2.6. Zeta (ζ) potential measurements—Z-potential measurements were performed at 25°C using a Nano-ZS Zetasizer (Malvern, UK). The dry powder was dispersed 0.1 wt% in 10mM NaCl solution. The measurements were performed in triplicate.

2.2.7. Curcumin concentration in nanoparticles—The concentration of curcumin present as C-NP below 0.7 μ m was determined after filtrating the dispersions (0.1 wt %) through a 0.7 μ m glass microfiber filter at various time intervals, immediately prior to the quantification. The particles showed a low adsorption to this membrane and therefore it was chosen for the experiment. The filtrate was diluted 20 times with 95 wt% ethanol to dissolve the curcumin, and its concentration was determined by UV spectrophotometer (UV–visible spectrophotometer Cary 100, Varian, Palo Alto, CA, USA) in triplicates. A standard absorbance-concentration calibration curve was attained for curcumin prior to this experiment, and it was found that the absorbance of curcumin in ethanol at 428 nm wavelength was linearly dependent on the concentration of curcumin at the range of 0.5–15 μ g/g. No correction was made in this experiment for curcumin possibly adsorbed onto filter during the filtration procedure.

2.2.8. Control experiments—Two control experiments were performed to validate the necessity of the initial microemulsion formation: solubilization of curcumin within surfactant micelles, and a formation of solid solution instead of nanoparticles. Possible

solubilization was evaluated by dispersing raw curcumin in water with surfactants at concentrations similar to those of the components in 0.1% water dispersion of the powder (curcumin 0.0143 wt%, ammonium glycyrrhizinate 0.0238 wt%, soybean phosphatidylcholine 0.0143 wt%, dipotassium glycyrrhizinate 0.0476 wt% and water 99.9 wt%). The mixture was stirred for 48 hours at room temperature, followed by filtration and measuring the concentration of dissolved/solubilized curcumin by UV spectroscopy as above. The possibility of solid solution formation was evaluated by mixing curcumin and all the surfactants in cyclopentanone at concentrations similar to those of the initial microemulsion composition (curcumin 3 wt%, ammonium glycyrrhizinate 5 wt%, soybean phosphatidylcholine 3 wt%, dipotassium glycyrrhizinate 10 wt% in pure cyclopentanone), followed by evaporation of the solvent by heating to 60°C. The resultant solid was dispersed in water at concentration of 0.1 wt%, stirred for 5 minutes and the dissolved/solubilized curcumin was determined as described above.

2.2.9. Evaluation of biological efficiency of nanoparticles—To verify that curcumin preserves its therapeutic activity in nanoparticles, its metabolic activity and cytotoxic efficiency were evaluated in pancreatic cancer cell line, PANC-1. Viability/proliferation of PANC-1 cells was tested in vitro by a colorimetric analysis for measuring the activity of cellular enzymes (MTT assay) and the intactness of the cell membrane (DRAQ7 signal) as the function of curcumin concentration.

Briefly, for the MTT assay, cells were seeded at 2000 cells/well in a 96 well microtiter plate and allowed to attach overnight at 37°C. The cells were then treated with two-fold serial dilutions of C-DMSO or C-NP starting from 100 µM to 12.5 µM concentrations in 200 µL media. At the 48 and 72 h timepoints, the cells were washed and incubated with 500 µg/mL of MTT for 2 h. After noticing the formation of purple formazan crystals, 200 µL of dimethylsulfoxide (DMSO) was added and constantly shaken for 10 min to dissolve the crystals. The absorbance was read at 590 nm with a reference filter of 630 nm on a Synergy H4 Hybrid Multi-Mode Microplate Reader (Biotek Instruments, Inc. USA).

For the DRAQ7 assay, 50,000 cells were seeded on 12 well plates and allowed to attach overnight at 37°C. The cells were treated with two-fold serial dilutions of C-DMSO or C-NP starting from 40 µM to 10 µM concentrations in 2 mL of fresh media, along with 20 µL of DRAQ7. DRAQ7 is a far-red fluorescent dye that binds the A-T rich regions of DNA of only dead and/or membrane permeabilized cells. Cytometric analysis was performed using an ImageXpress, a high content screening system (Molecular Devices, USA). Images (20x) from 36 different fields, located in the center of each well, were obtained with no spacing between fields. Images were captured at three wavelengths; the DRAQ7 and the curcumin were captured with an excitation and emission of 633/655nm and 358/461nm respectively and the brightfield images were obtained using transmitted white light. The mean fluorescence intensity per unit area from the curcumin channel was quantified using MetaXpress software (Molecular Devices, USA) as it can be assumed that mean pixel intensity is proportional to curcumin dose. Uptake of curcumin made it possible to segment over 250 cells per group. Mean pixel intensity per cell was plotted for C-DMSO and C-NP. The quantification of the total number of DRAQ7 signals was also performed using MetaXpress software. Cell death was quantified as the total number of DRAQ7 signals at

each time point. There was between 8000 and 11000 cells in total occupying the area being imaged in each well. The biological activity of C-NP was compared with the activity of C-DMSO at the same curcumin concentrations. C-NP were dispersed in water, while the raw curcumin was dissolved in DMSO prior to introduction to cell medium. In any given experiment, the final concentrations of DMSO and water added to the cell culture medium were kept below 0.1% and 5 % v/v respectively. It was experimentally proven that at these low concentrations DMSO and sterile water do not exhibit any antiproliferative action on the cells.

3. Results and discussion

3.1. Microemulsion formation

At the first stage, a volatile solvent, capable of dissolving curcumin and creating oil-in-water microemulsion with water had to be found. Cyclopentanone was the solvent that was proved suitable for this goal. It is a cyclic volatile ketone, which has a limited solubility in water, about 15 wt% at room temperature. Curcumin is readily soluble in cyclopentanone (above 20 wt %) and when the solvent is added to water above its solubility limit, phase separation instantly occurs.

We aimed to create oil-in-water microemulsion by employing pharmaceutically acceptable, GRAS listed (generally regarded as safe), natural amphiphiles in this system. It was found that using a combination of soybean phosphatidylcholine, ammonium glycyrrhizinate, and dipotassium glycyrrhizinate enabled spontaneous emulsification. These microemulsions were stable, isotropic and visually transparent and were created upon equilibration of all components at room temperature. Compositions that remained transparent and homogeneous after 24 hours were recognized as microemulsions and one of them was chosen for loading with curcumin. Table 1 summarizes the composition of the microemulsion. It should be noted that although the solubility of curcumin in cyclopentanone is significantly higher than 9.1 wt% (as employed in the initial microemulsion), the partial solubility of the solvent in water and the presence of amphiphiles decrease the concentration of cyclopentanone available for dissolving the model substance.

SbPC is a widely used surfactant approved for various pharmaceutical applications. AG and DG are ammonium and dipotassium salt (respectively) of glycyrrhizic acid. Glycyrrhizic acid is a natural triterpenoid glycoside extracted from a licorice root. Dipotassium salt is fairly hydrophilic and water-soluble, while ammonium salt is amphiphilic and sparingly soluble in both water and cyclopentanone, thus preferably positioned at the interface between them.

The average droplet size of the microemulsion loaded with curcumin as measured by DLS was 4.4 ± 0.2 nm (Figure 3). The relatively small microemulsion droplet size can be attributed to a partial solubility of the solvent in the aqueous phase.

3.2. Nanoparticle formation and characterization

Curcumin loaded microemulsion was rapidly converted into a dry, free-flowing powder by spray drying. The concentration of the curcumin in this powder was 14.3 wt% and the exact

composition of the powder is given in Table 1. XRD measurements indicated that curcumin is present in its amorphous form, since no peaks of crystalline curcumin were detected (Figure 4). It is interesting to note that the curcumin remained in its amorphous form when the powder was stored at room temperature for over a year. We assume that the amorphous character of curcumin in the powder can be attributed to both the described process and the presence of surfactants in the system, which can act as crystallization inhibitors. For example, we have previously reported on crystallization inhibition properties of ammonium glycyrrhizinate.³⁷

It was found that the powder was easily dispersible in water (up to 10 wt %) forming a transparent stable dispersion (at concentration more than 4 orders of magnitude higher than the intrinsic solubility of curcumin in water). The average particle size in this dispersion was 20.2 ± 3.4 nm. The dispersion was stable at ambient conditions for at least three hours without any detected growth in particle size. A typical DLS measurement of particle size distribution is shown in Figure 3 and is compared to a typical size distribution of microemulsion droplets. As we already reported in the past, the increase in particle size in the final dispersion compared with the initial microemulsion droplet size may be attributed to a transitional change in microemulsion structure and droplet aggregation during solvent removal. Although the evaporation process is rapid, the in-process compositional changes may eventually bring about a significant amphiphile rearrangement at the last stages of solvent evaporation which may result in the formation of structures larger than the initial microemulsion droplets. An example for such compositional change that can cause a partial collapse of the microemulsion structure is a rapid decrease in cyclopentanone concentration, which, in our case, acts both as a solvent and as the co-solvent required for microemulsion formation^{11,12}. However, it is noteworthy that despite this increase in size, the average diameter of curcumin particles obtained by this method is still extremely small, well below 100nm.

For quantitative determination of the fraction of curcumin which is present as C-NP, the powder was dispersed 0.1 wt% in water and the resultant dispersion was filtered through a $0.7 \mu\text{m}$ glass-fiber filter. From measurements of curcumin concentration in the filtrate it was found that nearly 100 wt% of the dispersed curcumin is present in particles smaller than $0.7 \mu\text{m}$.

The stability of C-NP in water was evaluated by measuring the curcumin concentration in the filtrate at various time intervals after dispersing the powder (Figure 5).

As shown, the C-NP suspension is quite stable, and after 3 hours, more than 90 wt% of curcumin is still present as submicron particles.

Z-potential of the particles in aqueous dispersion (pH 5.5) was -36.2 ± 1.8 mV, and remained unchanged when the dispersion was stored at room temperature for several hours. This result was expected, since the surfactants are negatively charged at this pH (SbPC has isoelectric point of 4.15,³⁸ while the dissociation constants (pKa values) of at least two carboxylic groups of glycyrrhizic acid are below 5).^{39,40} The resultant ζ -potential is

sufficiently high to provide electrostatic stabilization to the particles and should be attributed to the adsorption of the surfactants onto particle surface.

Also, it was found from UV absorption measurements that curcumin within the nanoparticles did not undergo rapid chemical degradation in the aqueous medium. Curcumin degradation leads to several products, which have a distinctive absorbance at 280nm, but not at 410–430nm region (wavelength range typical for curcumin, depending on solvent).^{41,42} We did not observe a rapid decrease in UV absorbance intensity at 428 nm. Although a relatively high resistivity of the drug towards the degradation is expected at this slightly acidic pH, this exceptional stability is probably a result of curcumin presence in nanoparticulate form.^{17,43–45} A slow decrease in curcumin concentration detected in the filtrate may be partially explained by chemical decomposition of the free curcumin dissolved from nanoparticles.^{17,41–45}

C-NP powder was also dispersed in Phosphate Buffered Saline, 0.01M, pH 7.4. The powder was easily dispersible in PBS to form a transparent dispersion. The size of the particles in this case was larger, 60.7 ± 1.8 nm, while ζ -potential was less negative, -29.5 ± 1.3 mV. The initially clear dispersion was not as stable as in water and particle size increase was detected within 1 hour. In addition to a lower electrostatic stability of the particles at this saline environment, a much faster chemical degradation of curcumin is expected when it dissolves from the particles at this more alkaline medium.^{41,42}

3.3. Control experiments

To verify that curcumin is not simply solubilized in micellar solution of surfactants, the unprocessed curcumin was dispersed in water with the surfactants at concentrations similar to those in 0.1 wt% aqueous dispersion of the powder, and the mixture was stirred for 48 hours at room temperature. The presence of solubilized curcumin was detected by the filtration experiment, as above. It was found that only about 19 wt% of the active substance was solubilized in this micellar solution.

To prove that the increased solubility of curcumin is not merely due to a formation of solid solution with surfactants, all powder components were dispersed in cyclopentanone. The solvent was evaporated and the solid mass obtained at the end of evaporation was not easily dispersible 0.1 wt% in water. It formed a turbid dispersion at which very rapid sedimentation of curcumin occurred with the cease of stirring. In this system, about 24 wt% of curcumin was solubilized.

It can be therefore concluded that the microemulsion formation is a necessary step for converting curcumin into water-dispersible nanoparticles.

3.4. Biological effects of C-NP in pancreatic cancer cells

To evaluate that the therapeutic potential of curcumin in the C-NP is preserved, metabolic activity and membrane intactness of Panc-1 pancreatic cancer cells were assessed in vitro by MTT and DRAQ7 assays, respectively, as a function of curcumin concentration. In these experiments the performance of C-NP was compared to that of the free curcumin dissolved in DMSO (C-DMSO) to enable introduction of this hydrophobic compound to the aqueous

cell medium. It should be emphasized that the nanometric powder was dispersed in aqueous medium without the addition of any organic solvent. The metabolic activity of cancer cells after 48 and 72 hours incubation with curcumin was measured by MTT assay, and is presented in Figure 6. While at higher concentrations, both C-DMSO and C-NP are similarly effective at reducing the metabolic activity of the cells, at lower concentrations such as 12.5 and 25 μM , C-NP are markedly more effective, especially at 12.5 μM . Consequently, the IC_{50} was 19.5 μM for C-DMSO and 11.5 μM for C-NP. Higher potency of C-NP may be explained by their increased stability in cell medium.⁴⁶ While the nanoparticles were easily dispersible and stable in aqueous medium, C-DMSO dissolved in water-miscible solvent would be much more prone to crystallization, particle growth and eventual precipitation through solvent displacement process during DMSO mixing with water. It was previously shown, that highly water dispersible curcumin nanoparticles may be more effective against various types of cancer cells compared with native curcumin.^{17,43} Curcumin has an established activity in inducing apoptosis/ inhibiting proliferation in numerous types of cancer cells by various mechanisms.³⁰

Our results with curcumin (40 μM) accumulation in cells at 48 h are given in Figure 7a. Image quantification of the pixel intensity shows that both C-DMSO and C-NP have similar concentration in pancreatic cancer cells. DRAQ7 signal quantification in cells treated with both C-DMSO and C-NP follows similar trends. It is noteworthy, that DRAQ7 stains only cells that have a compromised cell membrane, while MTT test detects much earlier observed impairment of metabolic activity/mitochondrial function. Anticancer activities of curcumin in pancreatic cell lines have been attributed mostly to its mitochondriotoxic effects.⁴⁷ Also, apoptosis induced by curcumin is known to be caused through the mitochondrial pathway.⁴⁸ Our results suggest that at low doses ($\sim 10 \mu\text{M}$) C-NP are more effective at reducing the metabolic activity of pancreatic cancer cells (as evidenced by the results of MTT assay) when compared to C-DMSO, however the cell membrane integrity (evaluated by DRAQ7 permeability) does not seem to be compromised at these doses (data not shown). At higher doses ($>20 \mu\text{M}$) both C-NP and C-DMSO are equally effective at reducing the metabolic activity and cell membrane permeability. Our data is in accordance with the previously reported capability of curcumin to suppress growth of pancreatic cancer cell lines.^{20,47,49,50} C-NP increases curcumin dispersibility in aqueous solution whilst maintaining its anticancer properties.

4. Conclusions

In this study we report on utilizing volatile microemulsions with partly soluble solvents for obtaining nanoparticles of poorly water-soluble drugs. Curcumin was employed as a model substance and was incorporated into cyclopentanone-in-water microemulsion formed by natural, pharmaceutically acceptable surfactants. The microemulsion was rapidly converted into nanometric powder by spray drying. The resultant powder was lipid / polymer free, contained amorphous curcumin and was easily dispersible in water (up to 10 wt%) to form nanoparticles with average size of $20.2 \pm 3.4 \text{ nm}$ and ζ potential of $-36.2 \pm 1.8 \text{ mV}$. By filtration experiment, it was found that in freshly prepared aqueous dispersion about 100wt% curcumin was present as nanoparticles. These particles are more effective at reducing the metabolic activity and retaining the cytotoxic effect as demonstrated on pancreatic cancer

cell line, Panc-1, when compared with C-DMSO. The higher potency of C-NP may be explained by their increased solubility and stability in the cell medium. We expect that the approach described in this report would enable formation of nanoparticles of other water insoluble drugs and, as the result, augmenting their activity. Powders of nanoparticles prepared by the described method may be either injected after dispersal in water or incorporated into various oral dosage forms.

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References

1. Lipinski CA. *Am Pharm Rev.* 2002; 5:82–85.
2. Merisko-Liversidge EM, Liversidge GG. *Toxicol Pathol.* 2008; 36:43–48. [PubMed: 18337220]
3. Zhu XM, Yuan J, Leung KCF, Lee SF, Sham KWY, Cheng CHK, Au DWT, Teng GJ, Ahujaa AT, Wang YXJ. *Nanoscale.* 2012; 4:5744–5754. [PubMed: 22895638]
4. Noyes AA, Whitney WR. *J Am Chem Soc.* 1897; 19:930–934.
5. Knapp LF. *Trans Faraday Soc.* 1922; 17:457–465.
6. Simonelli AP, Mehta SC, Higuchi WI. *J Pharm Sci.* 1970; 59(5):633–638. [PubMed: 5446418]
7. Keck CM, Muller RH. *Eur J Pharm Biopharm.* 2006; 62(1):3–16. [PubMed: 16129588]
8. Hancock BC, Parks M. *Pharm Res.* 2000; 17(4):397–404. [PubMed: 10870982]
9. Yu L. *Adv Drug Deliv Rev.* 2001; 48(1):27–42. [PubMed: 11325475]
10. Magdassi, S.; Nativi, H.; Margulis-Goshen, K. Organic nanoparticles obtained from microemulsions by solvent evaporation, international patent application number. PCT/IL2007/001136
11. Margulis-Goshen K, Kesselman E, Danino D, Magdassi S. *Int J Pharm.* 2010; 393:230–237. [PubMed: 20403417]
12. Margulis-Goshen K, Silva BFB, Marques EF, Magdassi S. *Soft Matter.* 2011; 7:9359–9365.
13. Margulis-Goshen K, Magdassi S. *Nanomedicine.* 2009; 5:274–281. [PubMed: 19268272]
14. Chainani-Wu N. *J Altern Complement Med.* 2003; 9(1):161–168. [PubMed: 12676044]
15. Kumar A, Ahuja A, Ali J, Baboota S. *Crit Rev Ther Drug Carrier Syst.* 2010; 27(4):279–312. [PubMed: 20932240]
16. De R, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB, Mukhopadhyay AK. *Antimicrob Agents Chemother.* 2009; 53 (4):1592–1597. [PubMed: 19204190]
17. Dev S, Prabhakaran P, Filgueira L, Iyer KS, Raston CL. *Nanoscale.* 2012; 4:2575–2579. [PubMed: 22193941]
18. Aggarwal BB, Kumar A, Bharti AC. *Anticancer Res.* 2003; 23(1A):363–398. [PubMed: 12680238]
19. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M, Diederich M. *Cancer Lett.* 2005; 223(2):181–190. [PubMed: 15896452]
20. Wang Z, Zhang Y, Banerjee S, Li Y, Sarkar FH. *Cancer.* 2006; 106(11):2503–2513. [PubMed: 16628653]
21. Mehta K, Pantazis P, McQueen T, Aggarwal BB. *Anti-Cancer Drugs.* 1997; 8:470–481. [PubMed: 9215611]
22. Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. *Oncogene.* 2001; 20:7597–7609. [PubMed: 11753638]
23. Elattar TM, Virji AS. *Anticancer Res.* 2000; 20:1733–1738. [PubMed: 10928101]
24. Hanif R, Qiao L, Schiff SJ, Rigas B. *J Lab Clin Med.* 1997; 130:576–584. [PubMed: 9422331]

25. Siwak DR, Shishodia S, Aggarwal BB, Kuzrock R. *Cancer*. 2005; 104:879–890. [PubMed: 16007726]
26. LoTempio MM, Veena MS, Steele HL, Ramamurthy B, Ramalingam TS, Cohen AN, Chakrabarti R, Srivatsan ES, Wang MB. *Clin Cancer Res*. 2005; 11:6994–7002. [PubMed: 16203793]
27. Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, Armaiz-Pena GN, Kamat AA, Spannuth WA, Gershenson DM, Lutgendorf SK, Aggarwal BB, Sood AK. *Clin Cancer Res*. 2007; 13:3423–3430. [PubMed: 17545551]
28. Mangalathillam S, Rejinold NS, Nair A, Lakshmanan VK, Nair SV, Jayakumar R. *Nanoscale*. 2012; 4:239–250. [PubMed: 22080352]
29. Gou ML, Men K, Shi HS, Xiang ML, Zhang J, Song J, Long JL, Wan Y, Luo F, Zhao X, Qian ZY. *Nanoscale*. 2011; 3:1558–1567. [PubMed: 21283869]
30. Wilken R, Veena MS, Wang MB, Srivatsan ES. *Mol Cancer*. 2011; 10:12–31. [PubMed: 21299897]
31. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YT, Tsai CC, Hsieh CY. *Anticancer Res*. 2001; 21(4B):2895–2900. [PubMed: 11712783]
32. Rao CV, Rivenson A, Simi B, Reddy BS. *Cancer Res*. 1995; 55:259–266. [PubMed: 7812955]
33. Singh R, Sharma P. *Toxicol Int*. 2011; 18(2):124–129. [PubMed: 21976817]
34. Eshrat MH, Hussain A. *Indian J Clin Biochem*. 2002; 17:33–43. [PubMed: 23105348]
35. Singh DK, Jagannathan R, Khandelwal P, Abraham PM, Poddar P. *Nanoscale*. 2013; 5:1882–1893. [PubMed: 23348618]
36. Kurien BT, Singh A, Matsumoto H, Scofield RH. *Assay Drug Dev Technol*. 2007; 5(4):567–576. [PubMed: 17767425]
37. Margulis-Goshen K, Weitman M, Major DT, Magdassi S. *J Pharm Sci*. 2011; 100(10):4390–4400. [PubMed: 21630277]
38. Spornath A, Aserin A, Ziserman L, Danino D, Garti N. *J Controlled Release*. 2007; 119:279–290.
39. Koide M, Ukawa J, Tagaki W, Tamagaki S. *J Am Oil Chem Soc*. 1997; 74:49–54.
40. Zeng CX, Hu Q. *Indian J Chem*. 2008; 47:71–74.
41. Wang YJ, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY, Lin JK. *J Pharmaceut Biomed*. 1997; 15:1867–1876.
42. Basnet P, Skalko-Basnet N. *Molecules*. 2011; 16:4567–4598. [PubMed: 21642934]
43. Mohanty C, Sahoo SK. *Biomaterials*. 2010; 31:6597–6611. [PubMed: 20553984]
44. Tonnesen HH. *Pharmazie*. 2002; 57:820–824. [PubMed: 12561244]
45. Manju S, Sreenivasan K. *J Colloid Interface Sci*. 2011; 359:318–325. [PubMed: 21492865]
46. Meker S, Margulis-Goshen K, Weiss E, Magdassi S, Tshuva E. *Angew Chem Int Ed*. 2012; 51(42):10515–10517.
47. Jutooru I, Chadalapaka G, Lei P, Safe S. *J Biol Chem*. 2010; 285(33):25332–25344. [PubMed: 20538607]
48. Anto RJ, Mukhopadhyay A, Denning K, Aggarwal BB. *Carcinogenesis*. 2002; 2:143–150. [PubMed: 11756235]
49. Youns M, Fathy G. *J Cell Biochem*. 2013; 114(12):2654–2665. [PubMed: 23794119]
50. Swamy MV, Citineni B, Patlolla JM, Mohammed A, Zhang Y, Rao CV. *Nutr Cancer*. 2008; 60 (1): 81–89. [PubMed: 19003584]

Fig. 1.
Curcumin (diferuloylmethane) structure.

Fig. 2.

Schematic presentation of conversion of curcumin to nanoparticles by the investigated method.

Fig. 3.

Typical DLS measurement of particle size distribution (by volume) in the microemulsion (droplet size) compared with that of the dispersed C-NP.

Fig. 4. X-ray diffraction patterns for powder of C-NP (black, 1), physical mixture of all powder components (red, 2), crystalline curcumin (blue, 3).

Fig. 5.
Fraction of submicron sized curcumin in aqueous dispersion at various time intervals after the dispersal of C-NP in water.

Fig. 6.

Panc-1 cell line viability after incubation with C-NP and C-DMSO for 48 and 72 hours.

Fig. 7.

Effect of C-DMSO and C-NP on the cell viability of Panc-1 cells. (A) Fluorescence image (pseudo-colored DAPI signal) of cells dosed with 40 μ M C-DMSO and C-NP at 48 h and quantification of mean fluorescence intensity per unit area of cell. ($n > 250$) (B)(i): brightfield and fluorescence images (DRAQ7 signal) of cells dosed with 40 μ M C-DMSO and C-NP after 0, 24 and 48 h of exposure. (B)(ii) and (B)(iii): the total number of DRAQ7 positive cells over time.

Table 1

Compositions of the initial microemulsion and the final nanoparticles

Composition	Curcumin	Cyclopentanone	SbPC	AG	DG	H ₂ O
Microemulsion	3 wt%	30 wt%	3 wt%	5 wt%	10 wt%	49 wt%
C-NP	14.3 wt%		14.3 wt%	23.8 wt%	47.6 wt%	