

#### Available online at www.sciencerepository.org

### **Science Repository**



#### **Research Article**

## Exploring the unique anticancer properties of curcumin nanoparticles

Maha Nasr<sup>1,2\*</sup>, Areeg Awadallah<sup>2</sup>, Rawan Al-Karaki<sup>2</sup>, Faisal Madanat<sup>3</sup>, Leen Alsunna<sup>3</sup>, Khaleel Sami<sup>3</sup>, Zakaria Ibrahim<sup>3</sup>, Omar Makhlouf<sup>3</sup> and Rasha Almajali<sup>3</sup>

#### ARTICLEINFO

Article history:

Received: 14 May, 2019 Accepted: 6 June, 2019 Published: 28 June, 2019

Keywords: Curcumin microemulsion colon cancer lung cancer prostate cancer

#### ABSTRACT

The aim of the current work was to elucidate whether the encapsulation of curcumin in nanoparticles (of microemulsion type) would allow its exertion of anticancer activity on several cancer cell lines or not. Curcumin microemulsion was prepared using the water dilution method, in which oleic acid constituted the oily phase, and tween 20, ethanol constituted the surfactant and cosurfactant respectively. The microemulsion was tested for its particle size, charge, morphology using transmission electron microscopy, *in vitro* curcumin release, stability and anticancer activity on HCT-116 colon cancer line, A549 lung cancer cell line, and PC3 prostate cancer cell line. The safety of the formulation was tested on fibroblasts cell line. Results revealed that curcumin microemulsion was successfully prepared with a particle size of 11.45±0.21 nm, and a negative charge of -9.07 mV, and it was able to sustain the release of drug for 24 hours with a cumulative percent released of 66.72%. Its spherical morphology was confirmed using transmission electron microscopy. No significant changes in particle size or charge were observed upon storage, suggesting the physical stability of the microemulsion. Curcumin microemulsion was proven safe on the normal fibroblasts cell line, and it preserved the anticancer activity of curcumin against A549, HCT116, and PC3 cell lines. Results of this study delineate that microemulsion is a suitable nanodelivery system for curcumin, which needs to be tested on animal tumor models for verification of the anticancer properties *in vivo*.

© 2019 Maha Nasr. Hosting by Science Repository.

#### Introduction

Curcumin is considered a magical nutraceutical molecule, which has been found to exert anticancer activity mainly *via* apoptotic and antimetastatic mechanisms [1, 2]. Its efficacy was demonstrated in several cancer types, such as lung cancer, breast cancer, prostate cancer, colon cancer, in addition to others [3, 4]. Several nanoparticle types were reported to enhance or preserve the anticancer activity of drugs, owing to their ability to enhance cancer cell uptake by their nanometer size [5-8]. From the promising nanoparticle types are microemulsions, which are composed of oil, water and surfactant/cosurfactant mixture [9-11]. They differ from the conventional nanoemulsions is that they are

isotropic systems of small nanometer size (from 10-100 nm) which are formed spontaneously upon addition of an aqueous phase [12]. This small size is advantageous since it will allow better uptake by the cancer cells, allowing the drug to exert its cytotoxic action. A water-dilutable microemulsion based on oleic acid as oil and tween 20 as surfactant was developed by Deng et al., 2015, and till current date, its efficacy was only tested when loaded with antibiotics [13, 14]. Therefore, in the current manuscript, a very small sized microemulsion was prepared and loaded with curcumin, in order to test the potential of its use as an anticancer delivery system.

<sup>&</sup>lt;sup>1</sup>Department of Pharmaceutics and Industrial pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

<sup>&</sup>lt;sup>2</sup>Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Mutah University, Jordan

<sup>&</sup>lt;sup>3</sup>Faculty of Pharmacy, Mutah University, Jordan

<sup>\*</sup>Correspondence to: Maha Nasr, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Egypt; Tel:

<sup>+201003450279;</sup> E-mail: drmahanasr@pharma.asu.edu.eg

#### Materials and methods

#### Materials

Curcumin, oleic acid, tween 20, absolute ethanol, disodium hydrogen phosphate, potassium dihydrogen phosphate, glucose, DMSO, phosphate buffer saline, MTT dye and dialysis membrane (molecular weight cut off 12000-14000) were purchased from Sigma Aldrich Co., USA. Fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, penicillin, streptomycin and 0.25% trypsin-EDTA were purchased from Lonza, Belgium.

#### Preparation of curcumin microemulsion

The curcumin-loaded microemulsion was prepared by water dilution, in which 50 mg curcumin was dispersed in a mixture composed of 4.1 ml tween 20, 0.28 ml oleic acid and 0.32 ml ethanol, and stirred using a magnetic stirrer [9, 10]. The mixture was titrated up to 10-gram water dropwise till the formation of an oil in water microemulsion loaded with curcumin.

## Determination of the particle size and charge of curcumin microemulsion

The particle size and charge of the prepared curcumin microemulsion was measured using the Zetasizer device (model ZS3600, Malvern, UK).

# Transmission electron microscopy of the prepared microemulsion

The prepared curcumin microemulsion was visualized using transmission electron microscopy without using a stain, after being dried on a carbon-coated grid (VERSA 3D, USA).

#### In vitro release of curcumin from the prepared microemulsion

The release of curcumin from the microemulsion was performed using membrane dialysis [15-17]. One ml of curcumin microemulsion was placed in a cylinder and attached to the shaft of USP dissolution apparatus (Pharma Test, Germany). The shaft was rotated at 50 rpm and 37°C, and the release medium was 200 ml phosphate buffer pH 7.4 containing 2% tween 20 to ensure sink conditions for curcumin. Three ml samples were taken from the release medium at definite time intervals (0.25, 0.5, 1, 2, 3, 4, 6, 24 hours), and the amount of curcumin released was measured spectrophotometrically at wavelength 424 nm (SPUV UV/VIS double beam spectrophotometer, SCO TECH, Germany).

#### Assessment of the storage stability of curcumin microemulsion

The properties of the Nano emulsion (particle size and charge) were remeasured after 3 months storage at room temperature, to assess the stability of the prepared formulation [5].

## Evaluation of the cytotoxicity of curcumin microemulsion in anticancer cell lines

The cell lines under investigation were human lung epithelial carcinoma

(A549) ATCC No. CCL-185, human prostate adenocarcinoma (PC-3) ATCC® CRL-1435, human colorectal carcinoma (HCT 116) ATCC No. CCL-247 and normal skin cells (fibroblasts). All cells were cultured in DMEM high glucose medium except for PC-3 cells which were grown in RPMI 1640 medium. Both media were supplemented with 10% heated fetal bovine serum, 1% of 2 Mm L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin. According to the cell's growth profile, cells were seeded with a density of 7000 cell/well, while fibroblast cells seeding density was 10000 cell/well. Cell viability was determined by trypan blue exclusion using a hemocytometer for the cytotoxicity assay, cells were washed three times with phosphate buffer saline (PBS). PBS was decanted and cells detached with 0.025% trypsin-EDTA. Media was added to a volume of 10 ml. The cell suspension was centrifuged at 1000 rpm for 10 minutes and the pellet was resuspended in 10 ml medium. After re-checking cellular viability, the cell suspension was diluted to provide the optimal seeding density and 100 µl of the cell suspension was placed in a 96 well plate. Cells were cultured at 37°C in a humidified atmosphere of 5% CO2. After 24 hours the cells were treated with different concentrations of curcumin microemulsion. Serial dilutions were made and 100 µL of each concentration was added to the cells and incubated for 72 hours. At the end of the exposure time, cell growth was analyzed using the MTT assay, in which 15 µl of MTT stock solution (5 mg/ml in sterile PBS pH 7.4) was added to each well. After the cells were incubated for 3 h in the presence of MTT, 100 µl of solubilizing stop solution was added to each well to solubilize the dark violet formazan crystals. The optical densities at 570 nm were then measured using a micro plate reader (Biotek, US). The results were expressed as a percentage of cell viability with respect to a control corresponding to untreated cells according to the following equation:

Viability 
$$\% = \frac{ODt}{ODc} \times 100\%$$

in which ODt and ODc is the optical density of the treated and untreated cells respectively. The  $IC_{50}$  value (the concentration causing 50% cellular death) was calculated.

#### Statistical analysis

Measurements were done in triplicate and reported as mean $\pm$ S.D. T-test was performed using Graphpad® Instat software, at significance of P $\leq$ 0.05. The IC $_{50}$  values were calculated using Graphpad Prism software (San Diego, CA. USA)

#### Results

## Measurement of particle size and charge of curcumin microemulsion

As shown in (Figure 1), curcumin microemulsion displayed a small particle size of  $11.45\pm0.21$  nm and a charge of -9.07 mV.

#### TEM examination of the curcumin microoemulsion

As shown in (Figure 2), the curcumin microemulsion displayed homogenous non-aggregated spherical droplets, concurring with the small particle size obtained with the Zetasizer.

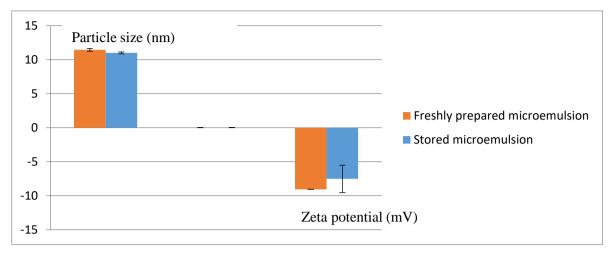


Figure 1: The particle size and charge of curcumin microemulsion freshly prepared and after storage for 3 months.

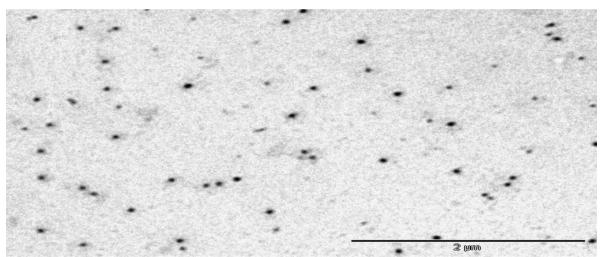


Figure 2: TEM micrograph of curcumin microemulsion

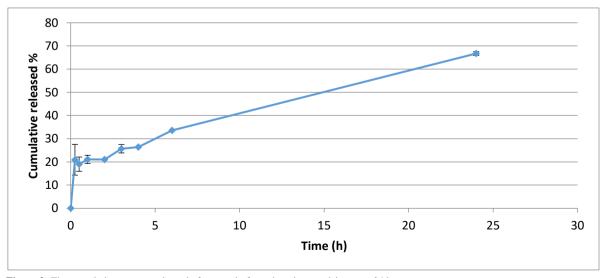


Figure 3: The cumulative percent released of curcumin from the microemulsion over 24 hours.

Stability of curcumin microemulsion

As shown in (Figure 1), no significant changes occurred to the particle size or charge of the prepared curcumin microemulsion after storage for 3 months (P>0.05).

#### In vitro release of curcumin from the microemulsion

As displayed in (Figure 3), a sustained release of curcumin was achieved over 24 hours from the microemulsion, reaching a percentage of 66.72%±0.83 after 24 hours.

# Evaluation of cytotoxicity of curcumin microemulsion in A549, PC3 and HCT-116 cancer cell lines as well as the normal fibroblasts cell line

The cytotoxicity of curcumin microemulsion was tested in fibroblasts cell line to assess the safety of the formulation. No IC50 value was obtained in case of fibroblasts and cells displayed high viability percentages at all tested concentrations. Regarding the cancer cell lines, (Table 1) shows the IC50 values for curcumin obtained for the lung cancer A549, prostate cancer PC3, and colon cancer HCT 116 cell lines, compared with the values reported in the literature [18-20]. No statistical significance was found in the IC50 values of the curcumin and the curcumin microemulsion (P>0.05).

**Table 1:** IC50 values (μg/ml) for curcumin versus curcumin microemulsion on A549, PC3 and HCT116 cell lines

	Curcumin	Curcumin
	microemulsion	
A549 cells	4.38±3.54	6.69±0.08
HCT 116 cells	7.61±0.13	3.25±2.73
PC3 cells	158.77±84.93	140.06±40.62

#### Discussion

Curcumin microemulsion was successfully prepared using the water dilution method. Owing to the surfactant and cosurfactant content of the microemulsion, it was reported to exhibit very small particle size. The negative charge exhibited by the microemulsion could be ascribed to the presence of ethanol and oleic acid [21, 22]. Neither the particle size nor the charge was changed after 3 months storage, which indicated the stability of the microemulsion system. Curcumin microemulsion displayed sustained release for curcumin over a period of 24 hours. This property is of particular benefit in the treatment of cancer, since a reservoir of curcumin can be created inside the cancer cells after endocytosis of the microemulsion, which would be released over time.

When tested on fibroblasts, curcumin microemulsion demonstrated considerable safety, as concluded from the high viability percentage for the cells at all tested concentrations. As seen from the IC50 results, the curcumin microemulsion preserved the anticancer activity of the curcumin on the lung, prostate and colon cancer cell lines, suggesting its suitability for further animal experimentation. Encapsulation of drugs within nanoparticles was reported to enhance the entry of drugs within tumor tissues *in vivo* by the enhanced retention and permeation effect (EPR) [8]. Curcumin was reported to exert its anticancer effects by different mechanisms, such as the inhibition of the STAT3 and NF-kB signaling pathways which play important roles in the cancer development and progression, leading to apoptotic and anti-angiogenic actions on cancer cells [23]. The encapsulation of curcumin in microemulsion nanoparticles sustained its release and preserved its cytotoxic activity on cell lines, which delineates the prepared

formulation as a promising cancer treatment modality.

#### Conclusion

A current research focus is the exploration of the properties of natural molecules for treatment of serious diseases such as cancer. In the current study, novel nanoparticulate system was prepared and characterized to create a suitable delivery means for curcumin. Futuristic work will include testing the prepared formulation on tumor bearing animal models to validate its therapeutic efficacy *in vivo*.

#### **Declaration of interest**

This work was supported by King Abdullah II Fund for development. Research grant No. 15/2019.

#### REFERENCES

- Wilken R, Veena MS, Wang MB, Srivatsan ES (2015) Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer* 10: 12. [Crossref]
- Panda AK, Chakraborty D, Sarkar I, Khan T, Sa G (2017) New insights into therapeutic activity and anticancer properties of curcumin. *J Exp Pharmacol* 9: 31-45. [Crossref]
- Ye MX, Li Y, Yin H, Zhang J (2012) Curcumin: updated molecular mechanisms and intervention targets in human lung cancer. *Int J Mol Sci* 13: 3959-3978. [Crossref]
- Waghela BN, Sharma A, Dhumale S, Pandey SM, Pathak C (2015) Curcumin conjugated with PLGA potentiates sustainability, antiproliferative activity and apoptosis in human colon carcinoma cells. PLoS One 10: e0117526. [Crossref]
- Aldalaen S, El-Gogary RI, Nasr M (2019) Fabrication of rosuvastatinloaded polymeric nanocapsules: a promising modality for treating hepatic cancer delineated by apoptotic and cell cycle arrest assessment. *Drug Dev Ind Pharm* 45: 55-62. [Crossref]
- Fadel M, Kassab K, Abd El Fadeel DA, Nasr M, El Ghoubary NM (2018) Comparative enhancement of curcumin cytotoxic photodynamic activity by nanoliposomes and gold nanoparticles with pharmacological appraisal in Hep G2 cancer cells and Erlich solid tumor model. *Drug Dev Ind Pharm* 44: 1809-1816. [Crossref]
- Said-Elbahr R, Nasr M, Alhnan MA, Taha I, Sammour O (2016) Nebulizable colloidal nanoparticles co-encapsulating a COX-2 inhibitor and a herbal compound for treatment of lung cancer. Eur J Pharm Biopharm 103: 1-12. [Crossref]
- Ramzy L, Nasr M, Metwally AA, Awad GAS (2017) Cancer nanotheranostics: A review of the role of conjugated ligands for overexpressed receptors. Eur J Pharm Sci 104: 273-292. [Crossref]
- Nasr M, Abdel-Hamid S, Moftah NH, Fadel M, Alyoussef AA (2017) Jojoba oil soft colloidal nanocarrier of a synthetic retinoid: preparation, characterization and clinical efficacy in psoriatic patients. *Curr Drug Deliv* 14: 426-432. [Crossref]
- Nasr M, Abdel-Hamid S (2016) Optimizing the dermal accumulation of a tazarotene microemulsion using skin deposition modeling. *Drug Dev Ind Pharm* 42: 636-643. [Crossref]

- Nasr M (2016) Development of an optimized hyaluronic acid-based lipidic nanoemulsion co-encapsulating two polyphenols for nose to brain delivery. *Drug Deliv* 23: 1444-1452. [Crossref]
- Ramez SA, Soliman MM, Fadel M, Nour El-Deen F, Nasr M et al. (2018) Novel methotrexate soft nanocarrier/fractional erbium YAG laser combination for clinical treatment of plaque psoriasis. *Artif Cells Nanomed Biotechnol* 46: 996-1002. [Crossref]
- Deng L, Que F, Wei H, Xu G, Dong X et al. (2015) Solubilization of tea seed oil in a food-grade water-dilutable microemulsion. *PLoS One* 10: e0127291. [Crossref]
- Mohamed MA, Nasr M, Elkhatib WF, Eltayeb WN (2018) In vitro evaluation of antimicrobial activity and cytotoxicity of different nanobiotics targeting multidrug resistant and biofilm forming Staphylococci. *Biomed Res Int* 2018: 7658238. [Crossref]
- Nasr M, Mansour S, Mortada ND, Elshamy AA (2008) Vesicular aceclofenac systems: a comparative study between liposomes and niosomes. *J Microencapsul* 25: 499-512. [Crossref]
- Nasr M, Mansour S, Mortada ND, El Shamy AA (2008) Lipospheres as carriers for topical delivery of aceclofenac: preparation, characterization and in vivo evaluation. AAPS PharmSciTech 9: 154-162. [Crossref]
- Ashraf O, Nasr M, Nebsen M, Said AMA, Sammour O (2018) In vitro stabilization and in vivo improvement of ocular pharmacokinetics of

- the multi-therapeutic agent baicalin: Delineating the most suitable vesicular systems. *Int J Pharm* 539: 83-94. [Crossref]
- Wu J, Cai Z, Wei X, Chen M, Ying S et al. (2015) Anti-lung cancer activity of the curcumin analog JZ534 in vitro. *Biomed Res Int* 2015: 504529. [Crossref]
- Adahoun MA, Al-Akhras MH, Jaafar MS, Bououdina M (2017)
  Enhanced anti-cancer and antimicrobial activities of curcumin nanoparticles. Atrif Cells Nanomed Biotechnol 45: 98-107. [Crossref]
- Cen L, Hutzen B, Ball S, DeAngelis S, Chen C-L et al. (2009) New structural analogues of curcumin exhibit potent growth suppressive activity in human colorectal carcinoma cells. *BMC Cancer* 9: 99.
   [Crossref]
- Bseiso EA, Nasr M, Sammour OA, Abd El Gawad NA (2016) Novel nail penetration enhancer containing vesicles "nPEVs" for treatment of onychomycosis. *Drug Deliv* 23: 2813-2819. [Crossref]
- Bsieso EA, Nasr M, Moftah NH, Sammour OA, Abd El Gawad NA (2015) Could nanovesicles containing a penetration enhancer clinically improve the therapeutic outcome in skin fungal diseases? Nanomedicine (Lond) 10: 2017-2031. [Crossref]
- Vallianou NG, Evangelopoulos A, Schizas N, Kazazis C (2015)
  Potential anticancer properties and mechanisms of action of curcumin.
  Anticancer Res 35: 645-651. [Crossref]