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Fluorocarbon nanostructures (PFOB-NEP) as camel milk lactoferrin and its anti-cancer effects on human breast cancer cell line MCF7

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Given the prevalence of cancer in the world, it is important to find new drugs for its treatment. Despite great advances in cancer treatment, a significant tendency for new anticancer agents due to the increased resistance of cancer cells to anticancer drugs is currently being expanded.

In this research the biological properties of lactoferrin (LF) from camel milk were studied and attempt was made to provide a way for preserving the three-dimensional structure of LF and increasing LF life resulting in preventing its denaturation by proteolysis *via* loading it on perfluorooctyl bromide nanoemulsion particles (PFOB-NEP). LF was extracted from camel milk by ion-exchange chromatography (carboxymethyl Sephadex C-50 resin) and its purity was confirmed by SDS-PAGE. Due to the biologically inert, chemically stable, non-biodegradable, nontoxic and nonvolatile properties of perfluorocarbon, PFOB-NEP was prepared using an oil-in-water emulsion method (O/W). LF was loaded onto the NEPs, then its characteristics and anti-cancer effects on human breast cancer cell line MCF7 were evaluated. According to the results, LF accession to NEPs was proved by circular dichroism and tryptophan fluorescence spectroscopy methods. The entrapment efficiency of LF into NEPs was about 91%. The mean diameter of the NEPs did not change after accession of LF (~ 100 nm) and the zeta potential of the NEPs was increased from –18.43 mV (without LF) to 21.61 mV (110.68μg/mL LF).

Keywords: Lactoferrin, Perfluorooctyl bromide (PFOB), Nanoemulsion Particles (NEP), Oil-in-water Emulsion, Camel Milk, Circular Dichroism (CD).

INTRODUCTION

Camel milk and cow milk are different in chemical composition, but in general camel milk contains all essential micronutrients that are found in cow milk including whey proteins such as LF and immunoglobulin [1]. Camel milk generally has white and opaque color, it has a sweet and sharp taste, but sometimes, depending on the eaten plant in the desert by the camel, it may be prone to salinity [2]. Because of the higher salt and lactose content of camel milk, its freezing point is lower than that of cow milk (between -0.57˚C and -0.61˚C). Camel milk is slightly higher in lactose content than cow milk. However, levels of potassium, magnesium, iron, copper, manganese, sodium and zinc are higher than in cow milk. Camel milk has anti-cancer, anti-diabetic, antimicrobial and hypoallergic properties for which LF, immunoglobulins, lysozyme or vitamin C play a major role among its compounds [3]. LF, also named lactotransferrin, is a glycoprotein of the transferrin family with a molecular weight of about 80 kDa [4]; the molecular weight of LF purified from camel milk was determined as 79.5 kDa and 75.3 kDa.

The concentration of camel milk LF (0.22 mg/mL) is about 2.44 times that of cow milk LF, and the concentration of camel milk colostrum LF was reported about 5.1 mg/mL on the second day after parturition, while its content in cow milk colostrum is about 0.5 mg/mL [4].

Camel milk LF concentration in the first week after parturition varied from 1.422 to 0.586 mg/mL [5,6].

This protein consists of a single polypeptide chain containing 703 amino acids that are folded into two globular lobes. These lobes, also called C – (carboxy) and N – (amino) terminal regions, are connected with an α-helix. Each lobe consists of two domains known as C1, C2, N1, and N2. The domains create one iron binding site on each lobe (Fig. 1) [7,8]. LF has the ability to combat bacteria (Gram+ and Gram−), yeast, fungi, viruses, and parasites [9]. Due to the wide distribution of LF in various tissues and the positive charge on its

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network, this protein is a multi-functional protein [10,11].

LF has been identified in blood, tears, saliva, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, urine, mucous secretions, secretions from exocrine glands and in specific granules of neutrophils [7,12,13]. A high amount of LF is present in milk and colostrum (7 g/L) [14,15]. LF is also found in fish and egg yolks [16].

LF is used in feed, food and pharmaceuticals. It exhibits anti-inflammatory and anticarcinogenic activities and has several enzymatic functions [17,18]. At higher concentrations of LF, it was found to act as a pro-oxidant [18].

Antibacterial activity [19] of LF is effected by two mechanisms: (1) LF at the site of infection keeps out of reach iron which is a nutrient for the organism, thus LF shows a bacteriostatic effect; (2) direct interaction of LF with infectious microorganisms [20]. The peptide lactoferricin (LFcin) released from LF by pepsin proteolysis under acidic conditions exhibits higher antimicrobial activity than LF [21]. LFcinB displays *in vitro* cytotoxic activity *versus* various types of mouse and human cancer cell lines, including leukemia cells, fibrosarcoma cells, various carcinomas, and neuroblastoma cells [22,23]. LF is the only transferrin, which is able to maintain iron in a wide range of pH even if pH is too acidic. It is also resistant to proteolysis [17]. LF is able to stop the growth of cancer cells through the inhibition of cell proliferation by altering the expression or activity of regulatory proteins, and through activation of natural killer cells. It inhibits tumor growth through the activation of apoptosis (programmed cell death) [7,24]. LF can be used to increase the anti-cancer effect of anti-cancer drugs such as apotransferrin. Kishore Golla et al. evaluated the effect of doxorubicin loaded apotransferrin and LF nanoparticles (apodoxonano or lactodoxonano) in the drug orally for hepatocellular carcinoma treatment (HCC) in rats. Apodoxonano and lactodoxonano in comparison to doxo showed improved efficacy, bioavailability and safety [25]. LF is an iron-binding glycoprotein with antihypertensive properties [26]. It is available as a commercial extract from bovine milk and offers potential as a therapeutic intervention for preterm infants modulating infections and intestinal pathologies [27].

According to the research done about the effect of human, bovine, sheep and camel milk LF on the C hepatitis virus it was found that camel milk LF had the highest effect [28].

LF extracted from milk can be marketed as a food supplement. In addition, it has a great potential in the field of biomedicine. The structure of this protein as of other proteins is fragile, so a small change in the structure may reduce its activity. Lipid-based carriers which are produced by self-assembly have become favorite for a broad scientific and commercial usage as carriers for the delivery of peptides and proteins during the past few years [10,29]. A way to avoid denaturation by proteolysis and dilution effect is to trap proteins or peptides in nanopores (or coating) until this action causes a reduction in water activity and thus helps to their stability in storage [30]. Nanoliposomes may be fit for oral administration of LF and could be a useful approach for LF availability to tumor cells [31]. Its three-dimensional structure has been attempted *via* nanoencapsulation within lipid nanovesicles, integrating a multiple water-in-oil-in-water emulsion [10]. The production of biocompatible nanoemulsions can also help to it. Since perfluorocarbons (PFCs) are biologically inert, chemically stable, nonbiodegradable, nontoxic and nonvolatile, they can produce biocompatible nanoemulsions. Specifically, perfluorooctyl bromide (PFOB) is mostly used due to its low vapor pressure helping to reduce pulmonary emphysema [32-34]. PFOB is used as an ultrasound contrast agent [35]. The biological half-lives were estimated to be 12 days (PFOB) [36]. *In vivo* sensitivity for inflammation imaging was assessed using the ear clip injury model. The result of this test for PFOB provided 37%. In the present study, perfluorocarbon NEPs were formulated for LF loading, and the optimum nanostructure characteristics were found. The nanostructure particle size was analyzed and the particle size distribution, charge, as well as the surface morphology were determined. The obtained results showed the proper incorporation of LF into NEP.

MATERIALS AND METHOD

Bioactive protein: camel milk was obtained from Torkaman Sahra located in the northeast parts of Iran and stored at -20˚C until needed. The bioactive LF (purity: >85%) from bovine whey was purchased from Sigma-Aldrich (USA). Chemicals and biochemicals**:** perfluorooctyl bromide (PFOB) 99%, carboxymethyl Sephadex C-50 resin, soybean phosphatidylcholine (lecithin), dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE, assay: >99%) were purchased from Sigma-Aldrich (USA). Other chemicals and biochemicals including protein marker 26612 were purchased from Thermo

Scientific (USA). HCl (37%), NaCl, monosodium phosphate, acrylamide, bisacrylamide, Tris, TEMED, ammonium persulfate (APS), sodium dodecyl sulfate (SDS), ethanol, methanol tetramethylbenzidine (TMB), acetic acid, silver nitrate, sodium thiosulfate, formaldehyde, sodium carbonate, sulfuric acid, hydrogen peroxide, dimethyl sulfoxide (DMSO), citric acid, bovine serum albumin (BSA), coomassie brilliant blue (C.Blue) G250 were purchased from Merck (Germany). Analytical equipment: all spectrophotometric readings were carried out using quartz cuvettes on an UV-VIS spectrophotometer, Shimadzu (Kyoto, Japan). Upper speed vacuum centrifuge (Ultracentrifuge) model VS-30000i of VISION SCIENTIFIC CO. LTD was used. Micro-spin/ FUGE/ VOTEX KIAGEN (Molecular Biology Company), magnetic stirrer (yellow MAGHS7), pH meter of Metrohm, Switzerland (827 pH lab) were used. Eppendorf centrifuge tubes (5415D) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of Sigma PS 2000-2 (USA) were used. Cation exchange chromatography column (10×3 cm) was used. Orbital shaker (FINEPCR SH30t) was used. The zeta potential and particle size of the nanoemulsion particles were determined on a ZetaCompact (CAD Instruments, France) and a particle size analyzer (VASCO - 1, France), respectively. SEM analysis of nanoemulsions was carried out on a scanning electron microscope (LEO, model VP 1450, Germany). Also, ultrasound (XL2020-010, USA), ultraturax (IKA, Model T25, Germany), circular dichroism (CD) spectrometer (J-815, CD spectrometer, Japan), fluorescence intensity (model 2500, Hitachi, Japan) were used for analytical assays.

Isolation and purification of LF from camel milk

Fat and casein were isolated from camel milk by centrifuge at 4500 g at 4˚C for 15 min (two times) and ultracentrifuge at 30000 g for 30 min at 25˚C (two times), then filtered by Whatman filter paper No. 7. LF was extracted from whey (pH 6.8) by ion-exchange chromatography (carboxymethyl Sephadex C-50 resin) [37,38]. Whey was passed through the CM column for 30 min (two times) in order to make a connection between the whey proteins and CM. After a designated time, whey was removed from the column. The unabsorbed proteins were eluted from the column with sodium phosphate buffer (10 mM, pH = 6.8). Then the column was washed with NaCl solutions of increasing molarity (0.3 and 1.2 mol/L) in a stepwise procedure. The protein solution was desalinated by dialysis where the dialysis bag was surrounded with sucrose or polyethylene glycol (PEG)[30].

SDS PAGE analysis

Extracted LF and purchased LF standard were compared using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE separates proteins conforming to their molecular weight, based on their different rates of departure through a sieving matrix (a gel) under the influence of an applied electrical field. In this method, silver staining was used to detect the band of acrylamide gel containing the protein.

SDS-PAGE shows a 80 kDa band confirming the existence of lactoperoxidase.

Gel composition is as follows: (for a 5 mL stacking 15% gel: deionized water (DDW) (2.975 mL); Tris-HCl 0.5 M, pH 6.8 (1.25 mL); SDS 10% w/v (0.05 mL); acrylamide/bis-acrylamide (30%/0.8% w/v) (0.67 mL); APS 10% w/v (0.05 mL); TEMED (0.005 mL) and for a 10 ml separating 15% gel: DDW (2.2 mL); Tris-HCl 1.5 M, pH 8.8 (2.6 mL); SDS 10% w/v (0.1 mL); acrylamide/bis-acrylamide (30%/0.8% w/v) (5 mL); APS 10% w/v (100 µL); TEMED (10 µL)). The running buffer contains: SDS 0.5 g; Tris-HCl 1.2 g; glycine 7.2 g to a final volume of 500 mL with DDW; the loading buffer contains: SDS 0.6 g; beta-mercaptoethanol 200 µL; glycerol 6 mL; Tris-HCl 1.5M, pH 6.8 (3 mL); bromophenol blue 0.005 g, at 120V and 90 minutes.

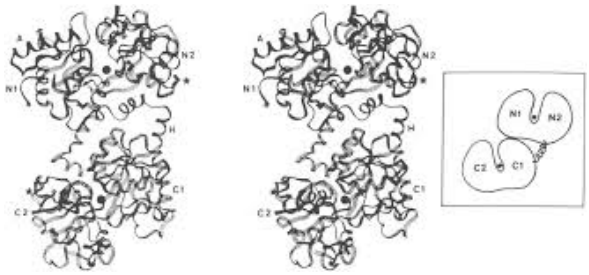


Fig. 1. Schematic diagram of the three-dimensional structure of the LF molecule. LF is lactoferrin

Coloring gel preparation: 1- Put the gel in a fixative solution and put it on a shaker with moderate speed for 30 min (water 20 mL, acetic acid 5 mL, methanol 25 mL); 2- Put the gel in methanol solution while shaking for 15 min (twice); 3- Put the gel in Hypo solution while shaking for 2 min (sodium thiosulfate 20% w/v 200 µL to a final volume of 50 mL with DDW) then wash the gel for 2 min three times with DDW; 4- Put the gel in a staining solution (silver nitrate 0.13 g, formaldehyde 100 µL to a final volume of 50 mL with DDW) in the dark for 10 min then wash again

the gel for 2 min three times with DDW; 5- Put the gel in a developing solution (sodium carbonate 3.4 g, formaldehyde 1400 µL, sodium thiosulfate 20% w/v 800 µL to a final volume of 50 mL with DDW) until the appearance of a protein band, drain it and put the gel in the fixative solution.

For the detection of lactoperoxidase the TMB (3, 5, 3′, 5′ - tetramethylbenzidine) color reaction was used in the presence of hydrogen peroxide and the purity of LF was confirmed. Briefly: 1- Prepare a solution of TMB (dissolve 5 mg of TMB powder in 1 ml DMSO in a water bath at 45°C and add 300 µL of it to 700 µL of sodium phosphate buffer (pH = 6)). Add 2-100 µL of the sample to 100 µL of TMB solution and 10 µL hydrogen peroxide 30% and incubate at 37˚C for 5 min, then read the absorbance at 450 nm using a spectrophotometer. LF concentration was determined by coomassie dye binding or Bradford assay [39]. In summary, 50 µL of sample was added to 2.5 ml of Bradford solution (100 mg C. blue G250 dissolved in 50 mL ethanol 96%, then 100 ml of 85% phosphoric acid was added and the volume was brought to 1 L with DDW). Using BSA standard solution and measuring absorbance at 595 nm, the absorption curve was drawn with Excel 2013 and the concentration of LF was found.

100 mg Blue Kumasi LF was lyophilized and stored at -20˚C in the dark until needed.

Preparation of NEP stabilized emulsions

Pre-emulsions were prepared as follows : 2% W / V surfactant (including 98% mol egg lecithin and 2% mol DPPE) and 20% V/V PFOB in DDW were stirred by a magnetic stirrer [40] for 10 min at 5000 rpm. Homogenization was carried out using an Ultra-Turex stirrer for 10 min at 12000 rpm within water-ice solution. To obtain nano-sized particles and uniform emulsions duplicate stirring in an ultrasonic stirrer for 10 min followed. The formulations prepared with each of these lipids showed a milky and uniform appearance [41].

Addition of LF to NEPs

After the preparation of NEPs, LF (selected amounts in saline phosphate buffer of 110, 55, 27.5 µg/mL, respectively) was added to the preformed NEPs, and the residual free (unbound) LF was washed out by centrifugation at 1000 rpm for 10 min. The amount of unbound LF was determined by measuring the tryptophan fluorescence and Bradford method [39]. Control NEPs (without LF) were stored for 52 days under the same conditions [22].

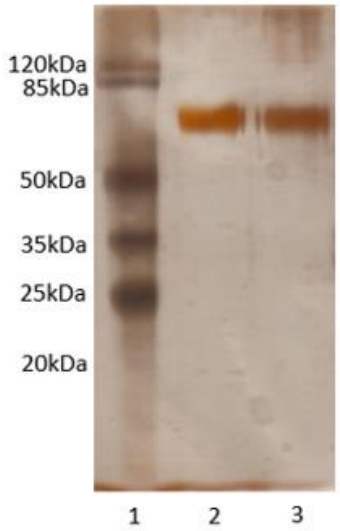


Fig. 2. SDS PAGE analysis of LF on a 15% gel with 120V and 90 min of fractions obtained in ultrafiltrated (purified) whey by ion-exchange column using saline gradient. Lane 1, 2, and 3 represent protein markers, elution peak with 0.7 mol/L NaCl and LF standard, respectively.

Entrapment efficiency (EE):

The loading efficiency was determined by centrifugation of NEPs. Following the addition of LF to NEPs, the residual free (unbound) LF was washed out by centrifugation at 1000 rpm for 10 min. The protein content of the supernatant was measured by tryptophan fluorescence and Bradford method [39] and the total amount of LF was determined spectrophotometrically.

Entrapment efficiency (in percentage) was calculated using Eq.1.

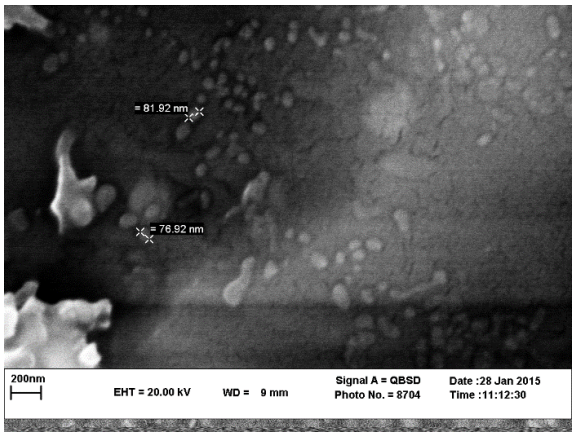
$$EE\% = \frac{(C_t - C_f)}{C_t} * 100 \quad (1)$$

where C_f is the amount of free LF and C_t is the total amount of LF present in 500 µL of NEPs [31].

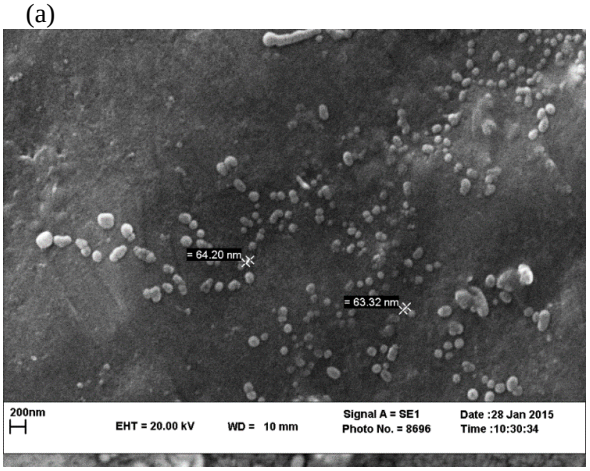
Scanning electron microscopy analyses

The scanning electron microscopy (SEM) produces a variety of signals at the solid samples surface by using a focused beam of high-energy electrons. The signals which are obtained by the interaction of electrons with the solid sample give information on the morphology, chemical composition, crystalline structure and orientation of the solid sample. Coating of samples is required to enable or improve the imaging of samples. Samples of NEPs were coated with a thin layer of conducting material (gold/palladium (Au/Pd)). Magnifications of NEPs before and after the accession of LF are shown in Fig. 3, where the shape structure of the particles is spherical. The scanning electron microphotographs of the exposed

surface of LF binding to nanoemulsions (see Fig. 3a and 3b) show the matrix structure developed during the manufacturing process and the highly homogeneous distribution of nanoemulsions diameters.



(a)



(b)

Fig. 3. Scanning electron microscopy analysis of the PFOB-NEP using 100 000 magnification (a) and inserted LF into PFOB-NEP using 50 000 magnification (b). PFOB-NEP are perfluorooctyl bromide nanoemulsion particles.

Determination of particle size

Particle size analysis is a part of particle science and it is a patented innovative technology. Particle size distribution is an important physical property for determining the behavior and nature of particles. The principles of measurement are based on dynamic light scattering (DLS) of backscattered light. DLS (also known as photon correlation spectroscopy or quasi-elastic light scattering) is a technique in physics that performs measurements in dark which can be used to characterize the size distribution profile of small particles in suspension or emulsion or of polymers which exist in the solution [42]. This method is also used to check the behavior of complex fluids such as concentrated polymer solutions. A laser is used as monochromatic light source that is shot through a polarizer into the sample. The scattered light then goes through a second polarizer where it is collected by a photomultiplier and the resulting image is projected onto a screen [43].

Determination of zeta potential

Zeta potential shows the surface charge of particles in emulsions and suspensions so that the measurement of zeta potential serves for predicting formulation stability and interactions between particles. Optimization processing is an important and easy way to control quality. Zeta potential is the electric potential that dominates in the incision plan of a particle, which is at a tiny distance from its level, as a consequence of the measuring of the mobility distribution of a scissoring of charged particles that are under an electric field [44]. The mobility is the speed of a particle per unit electric field. In another definition, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. Methods of measuring a particle's zeta potential are dependent on the nature of both the particle and the suspension or emulsion formulation.

Circular dichroism (CD) spectroscopy

Circular dichroism (CD) is dichroism involving circularly polarized light, i.e., differential absorption of left- and right-handed light. CD spectroscopy is an important technique in structural biology for examining the folding, conformational changes, and especially the secondary structures of proteins. Herein, CD was used to confirm the incorporation of LF in the nanoemulsions. This interaction produces a CD signal at the plasmon resonance frequency of the chiral protein−NEP complex.

Fluorescence spectroscopy

For the analysis of sample fluorescence, fluorescence spectroscopy (also known as fluorometry or spectrofluorometry) is used which is a type of electromagnetic spectroscopy. An emission map is measured by recording the emission spectra resulting from a range of excitation wavelengths and combining them all together. The intrinsic tryptophan fluorescence (TRP19) in LF (excitation, 280 nm; emission, 355 nm) was used to study the nature of the LF interaction with the lipid monolayer of perfluorocarbon NEPs. Fluorescence emission spectra show the quenching of endogenous

tryptophan fluorescence of LF upon insertion into the lipid monolayer of NEPs.

The effects of nanoparticles prepared in breast cancer MCF7 cells lines

The *MCF-7* breast cancer cell line was obtained from Razi Institute, Ministry of Jahad-e-Keshavarzi, Mashhad, Iran. The cells were thawed to revive and recover. The recovered cells were transferred into culture flasks and were incubated with 5% carbon dioxide at 37 ° C. To investigate the effect of nanoparticles containing LF in the growth of cancer cells, three different concentrations were used. For this purpose, a day before adding the nanoparticles, the cells were transferred into 96-well plates with 200 ml of medium. After adding the nanoparticles, the cell-containing plates were incubated with 5% carbon dioxide at 37 ° C. After 24 hours the survival rate of the cells was controlled with MTT assay *in vitro*.

RESULTS AND DISCUSSION

SDS PAGE analysis

The fraction in NaCl solution (0.7mol/L) was identified as LF by SDS-PAGE analysis on a 15% gel (Fig. 2, Lane 2) and TMB color reaction was used to confirm the purity of LF. SDS-PAGE results showed (Fig. 2, Lane 2) that the identified fraction in the NaCl solution had higher purity. Following electrophoresis, the gels were stained using the silver nitrate method [45]. Silver staining of proteins on a gel increases the sensitivity by a factor of 10-100.

The comparison between line 2 (isolated protein) and line 3 (standard LF) shows that the molecular weight of the protein is 80 kDa. The amount of LF or lactoperoxidase was determined by the coloring reaction of tetramethylbenzidin (TMB) test at a later stage.

Entrapment efficiency (EE)

By measuring the amount of free (unbound) LF after centrifugation and comparison with LF present in 500 µL of NEPs by tryptophan fluorescence and Bradford method according to equation 1, the entrapment efficiency was obtained (91%).

Scanning electron microscopy analyses

Magnifications of PFOB-NEP and inserted LF into lipid bilayer nanoemulsions of 100 000 and 50 000 are depicted in Fig. 3a, 3b, respectively. The scanning electron microphotographs of the exposed surface of PFOB-NEP and inserted LF into PFOB-NEP showed that the nanoemulsions structure remained unchanged during the manufacturing process and the highly homogeneous distribution of nanoemulsion diameters and the morphology after reunification of LF in the spherical particles have not changed.

Particle size analysis and determination of zeta potential

The mean particle diameter and the zeta potential were monitored by increasing the concentration of LF (Fig.4). No changes were observed in the mean diameter (∼100 nm) of the PFC NEPs after addition of LF, but since LF is a cationic protein, as expected, by increasing the concentration of LF, the zeta potential of the NEPs increased from –18.43 mV (without LF) to 21.61 mV (110.68µg/mL LF).

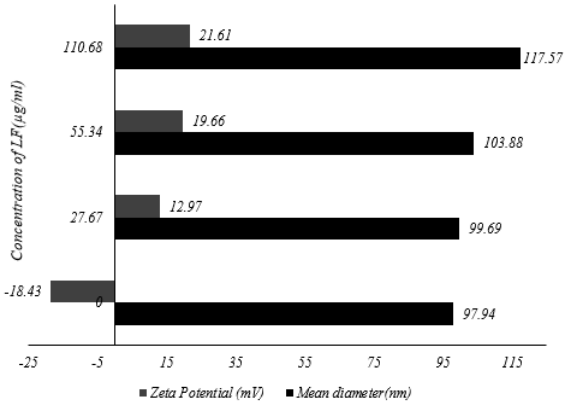


Fig. 4. Mean hydrodynamic diameter and zeta potential of NEPs before and after incorporation of LF.

Circular dichroism (CD) spectroscopy

CD spectroscopy played an important role in these studies. CD spectroscopy was used to study the structure of NEP free of LF and upon interaction with LF. As Fig. 5 shows, the nanoparticles without LF give no peak, while after accession LF creates a negative peak at a wavelength of 202. The differences in the CD spectra are indicative of the LF incorporation upon interaction with NEP.

Tryptophan fluorescence

Typically LF undergoes a blue shift (or a change in emission from 330 to 325 nm) upon insertion into lipid bilayers due to the hydrophobic membrane environment. The fluorescence of NEP associated LF was completely quenched (Fig. 6a). Fluorescence emission spectra show the quenching of endogenous tryptophan fluorescence of LF (110.68 µg/mLof LF) upon insertion into the lipid monolayer of NEPs with a PFOB core. Because tryptophan fluorescence quenching is not generally

a feature of peptide interaction with membranes, the special role of the PFOB core was tested as a mechanism for the observed fluorescence quenching upon integration into NEPs. Fig. 6b shows the percentage quenching of tryptophan fluorescence by free LF and LF with perfluorocarbon cores.

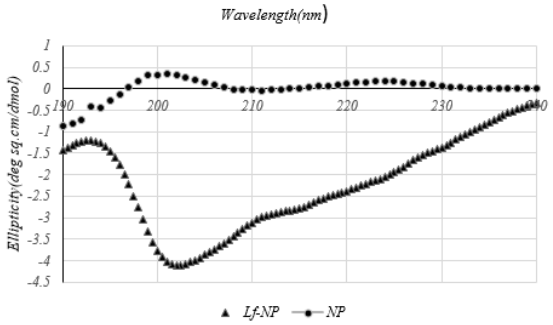
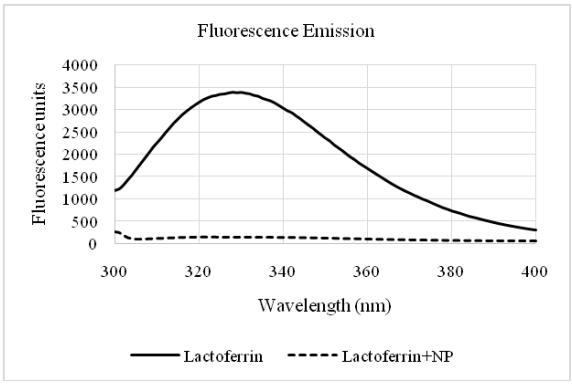
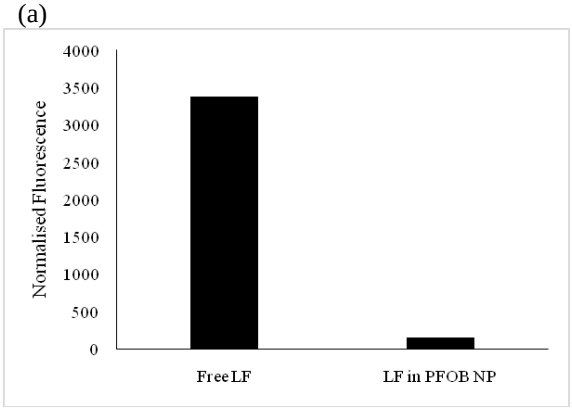


Fig. 5. Far-UV CD spectra of NEPs and LF in monolayered PFOB NEPs at 25˚C. CD is circular dichroism



(a)



(b)

Fig. 6. Interaction of LF tryptophan with PFC NEPs (a) Percentage quenching of tryptophan fluorescence with free LF and LF with perfluorocarbon cores; (b) PFC in perfluorocarbons.

MTT test results in breast cancer MCF7 cell lines

Cell viability was determined as a ratio of absorbance ($A_{570\ nm}$) in treated cells relative to absorbance in control cells (DMSO) ($A_{570\ nm}$). The IC_{50} was defined as the concentration of sample needed to reduce 50% of absorbance relative to the vehicle (DMSO)-treated control (Fig. 7).

$$Cell\ viability\ (\%) = \frac{A570nm(Sample)}{A570nm\ \ (Control\ DMSO)} \times 100$$

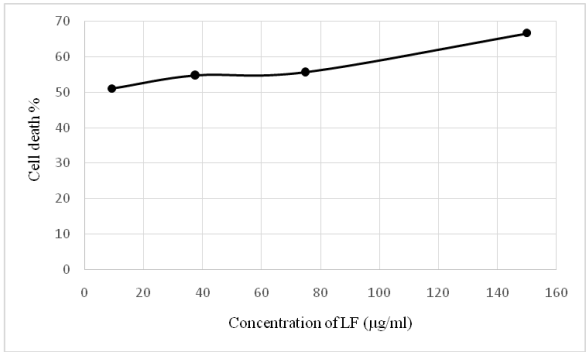


Fig. 7. Cell death % with concentration of LF in PFOB NEPs.

CONCLUSIONS

Due to the unique properties of LFs such as antimicrobial activity, immunomodulatory, and even antineoplastic properties, LF seems to have great potential in practical medicine. A lot of research has been done on it of late. Nevertheless, more research still needs to be carried out in order to obtain a better understanding of its activity and interactions and to enable the full and safe utilization of this glycoprotein. As already mentioned, due to the sensitivity of LF to proteolysis, a lot of research has been done on the use of alginate and carrageenan nanoparticles as carriers.

The purpose of this research was to design, formulate, characterize, and assess the advantage of PFOB particles as nanovehicles for LF delivery in pharmaceutical applications, e.g., the use of this combination on breast cancer cells. In this study LF was isolated from camel milk using ion-exchange chromatography.

Although LF is resistant to pH changes, to avoid breaking LF and to retain its biological activity against factors such as sonication, stirring and heat, LF was added after the formation of NEPs.

Effective connection between the NEPs and LF is mediated by electrostatic interactions between the negatively charged lipid monolayer of NEPs and the positively charged LF surface. These interactions were proved by CD and tryptophan fluorescence.

SEM analysis (Fig. 3a and 3b) of PFOB NEPs illustrates that their structural integrity is preserved even after addition of LF. The very high surface tension of PFOB NEPs, as well as the hydrophobic and lipophobic perfluorocarbon core prevent

disintegration of the NEP after LF insertion and may actually contribute to the stability and controlled release of LF from PFOB NEPs. The results showed that perfluorocarbon NEPs can be used to deliver cytolytic peptides and proteins.

The results of MTT assay showed that LF loaded on PFOB NEPs causes the death of cancer cells and with increasing LF concentration, the percentage of dead cells increased.

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ФЛУОРОВЪГЛЕРОДНИ НАНО-СТРУКТУРИ (PFOB-NEP) КЪМ ЛАКТОФЕРИН ОТ КАМИЛСКО МЛЯКО И НЕГОВИЯТ ПРОТИВО-ТУМОРЕН ЕФЕКТ ВЪРХУ КЛЕТЪЧНА ЛИНИЯ MCF7 НА РАК НА ГЪРДАТА

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(Резюме)

Поради разпространението на рака в световен мащаб е важно да се търсят нови лекарства за неговото лечение. Въпреки големия напредък, разширеното разработване на нови лекарства се обуславя от резистентността на раковите клетки към известните противоракови средства.

В настоящото изследване са изследвани биологичните свойства на лактоферин (LF) от камилско мляко и е направен опит да се запази неговата тримерна структура и да се повиши живота на LF, като се предотврати денатурирането му от протеолиза. Това се постига чрез добавяне на нано-емулсия с частици от перфлуоро-октил бромид (PFOB-NEP). LF се извлича от камилско мляко чрез йонообменна хроматография (карбоксиметилова Sephadex C-50 смола), а неговата чистота бе потвърдена чрез SDS-PAGE.

Перфлуоровъглеродът е биологически инертен, химически стабилен, нетоксичен и нелетлив, PFOB-NEP се приготвят от емулсия масло/вода (O/W). LF се натоварва върху нано-частиците, а след това се определят неговите характеристики и противо-туморния ефект върху клетъчна линия MCF7 на рак на гърдата. Достъпът на LF до нано=частиците се доказва чрез кръгов дихроизъм и флуоресцентно спектроскопски методи. Ефективността на включване на LF в наночастиците беше около 91%. Средният диаметър на нано-частиците не се променя след включването на (~ 100 nm) а ζ-потенциалът на нано-частиците нараства от –18.43 mV (без LF) до 21.61 mV (110.68μg/mL LF).