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Review

Lactoferrin, a unique molecule with diverse therapeutical and nanotechnological applications

Sally Sabra [a,*], Mona M. Agwa [b,*]

[a] Department of Biotechnology, Institute of Graduate studies and Research, Alexandria University, Alexandria 21526, Egypt
[b] Department of Chemistry of Natural and Microbial Products, Pharmaceutical and Drug Industries Research Division, National Research Centre, 33 El-Behooth St, Dokki, Giza 12311, Egypt

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ABSTRACT

Lactoferrin (LF) is a naturally glycoprotein with iron-binding properties and diverse biological applications including; antiviral, anti-inflammatory, antioxidant, anti-cancer and immune stimulating effects. In addition, LF was found to be an ideal nanocarrier for some hydrophobic therapeutics because of its active targeting potential due to overexpression of its receptor on the surface of many cells. Moreover, it was proven to be a good candidate for fabrication of nanocarriers to specifically deliver drugs in case of brain tumors owing to the capability of LF to cross the blood brain barrier (BBB). Consequently, it seems to be a promising molecule with multiple applications in the field of cancer therapy and nanomedicine.

Contents

1. Introduction

1.1. Lactoferrin: an overview

Lactoferrin (LF) is a cationic iron-binding glycoprotein with molecular weight of about 77–80 kDa that was first discovered in human milk

* Corresponding authors.
E-mail addresses: ssabra@uwo.ca, igsr.ssabra@alexu.edu.eg (S. Sabra), mona.m.agwa@alexu.edu.eg (M.M. Agwa).

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in the early 1960s. Later, it was reported to be found in other exocrine fluids such as blood, tears and saliva [1]. It is folded into two globular lobes connected by an α-helical bridge and stabilized by a number of disulfide bonds [2]. The main function of this protein is to control and regulate the concentration of free iron in biological fluids *via* its ability to either solubilize or sequester ferric ions (Fe^{3+}). This unique feature is the key reason behind its multifunctional properties including; antimicrobial, anticancer anti-inflammatory, antioxidant and immune stimulating properties [3,4]. LF either extracted from milk or produced by recombinant techniques in microorganisms is known to be widely added to many nutraceutical formulations including; infant formulas, cosmetics and food supplements owing to its abovementioned beneficial effects [5]. The level of LF in blood can also be used as biomarker of some inflammatory diseases such as septicemia or severe acute respiratory syndrome [6]. Also, LF in feces is considered as a non-invasive sensitive biomarker that can be used in the diagnosis and prediction of the severity of some intestinal inflammation diseases such as Crohn's disease [7] and chronic inflammatory bowel disease [8].

Generally, one LF molecule can typically binds two Fe^{3+} ions, which greatly depends on the synergistic binding of two HCO_3^- or CO_3^{2-}. Interestingly, it was found that *in vitro* iron release could be enhanced from LF molecule by lowering the pH or using chelating agents. Moreover, structure analysis of LF revealed that it is composed of 2 lobes. Each lobe includes 2 domains separated by a cleft that can co-bind $Fe3^+$ and CO_3^{2-}. The binding and the release of substrates is usually associated with conformational changes including opening and closing of the interdoamin cleft. This feature explains why lactoferrin bound to iron has a notable compact structure, in comparison to lactoferrin without iron. In addition, it was found that LF's α helix N terminal and an arginine sidechain are responsible for the formation of an anion-binding pocket. Anions are thought to bind to LF in order to neutralize the charges of the existing cationic groups before metal binding or they may be involved in stabilizing the protein metal co-ordination. More importantly, two carbohydrate attachment sites were discovered in LF molecule, one in each lobe. The first β-α-β unit (residues 1 to 40) includes about nine basic sidechains protruding from the surface (most near the C terminus of helix A) which seems to be a receptor binding moiety, responsible for receptor recognition of LF. In addition, studies conducted on LF receptors confirmed that carbohydrate molecules should be involved in the binding mechanisms [9].

Furthermore, LF was found to be able to maintain its iron binding capacity after its heating at temperatures ranged from 65 to 90 °C and ionic strength of about 0.01 or below. Upon increasing the temperature, partial precipitation of LF was observed with a notable decrease in its iron binding capacity. Interestingly, at pH 3.5, LF was able to resist heating at ionic strength up to 0.37, whereas, agglomeration was observed at ionic strength higher than 0.47, which revealed that the thermostability of LF is strongly dependent on both ionic strength and pH [10], which is mainly attributed to the basic nature of LF rich in lysine and arginine, so it carriers positively charge at pH below 7. The repulsive forces between these cations on the surface of LF prevent its agglomeration [10]. At acidic pH, a higher salt concentration is required to neutralize and precipitate LF due to the presence of greater amount of charges on the protein surface at this pH, which explains the enhanced resistance of LF to heat and salt treatments at low pH [10]. Another important factor that may influence the aggregation of LF is its iron saturation state. It was found that native and Apo- LF were more sensitive to heat treatment with greater tendency to form insoluble aggregates. On the other hand, iron saturated holo-LF was less aggregated and more soluble. This effect could be related to the compact structure of holo-LF which prevented its aggregation. Also, it was shown that iron saturation of LF could stabilize disulfide bonds between its lobes, especially at high temperature [11].

Nanocarriers are nanosized structures that can encapsulate drug (s) and precisely control their release when attached to diseased cells. Meanwhile, they are supposed to enhance the bioavailability and pharmacokinetic profile of the encapsulated drugs [12]. Many synthetic polymers utilized to fabricate nanocarriers, however they might exhibit toxic side effects [13]. As an alternative, naturally available polymers such as proteins or polysaccharides could be used to fabricate biocompatible and non-immunogenic nanocarriers [14]. As a matter of fact, LF has numerous advantages that motivate its intense involvement in the fabrication of nanocarriers. It is one of few proteins that possess net positive charge at physiological conditions (pI~8.0–8.5) [15]. This high pI value conferred LF to be positively charged over a wide range of pH [16]. In addition, LF is relatively stable in gastrointestinal tract and it has numerous intestinal receptors which facile the oral absorption and bioavailability of LF-based nanocarriers in the circulation, and hence can enhance the therapeutic efficacy of encapsulated active molecules within these nanoparticles [17].

Moreover, Tumor cells are widely known to be overexpressing LF receptors in order to fulfill the elevated nutritional demands of these highly proliferating cells [18]. Consequently, fabrication of nanocarriers from LF is considered an excellent strategy to attain active targeting [19]. In addition, LF-based nanocarriers were found to have a pH-dependent release profile. At acidic pH, faster drug release is observed, which could increase drug release in acidic sites such as tumor tissue microenvironment, and hence could enhance the therapeutic efficacy of the encapsulated hydrophobic active molecules [19]. In addition, LF receptors are overexpressed on the surface of the brain endothelial cells. Consequently, once LF attaches to its receptor on the cell surface, it will be easily able to pass across the BBB *via* receptor-mediated transcytosis. This privilege allowed the extensive application of LF as a promising molecule to develop smart nanocarriers that are actively targeted to the brain [20].

Based on all the aforementioned interesting advantages of LF as a nutraceutical molecule or for nanotechnological applications, it is important to address the most recent therapeutical and nanotechnological applications of LF. The aim of this review was to highlight some therapeutical benefits of LF as a nutraceutical molecule including; anti-bacterial, anti-viral, anti-inflammatory, anti-cancer and immune stimulating effects. Furthermore, fabrication of different actively targeted nanocarriers from LF will be discussed with special emphasis on nanoparticles, nanocomplexes and polymeric micelles.

2. Lactoferrin as a nutraceutical agent

2.1. Anti-bacterial and anti-viral effects

Lactoferrin is considered an important component of mammals' innate immune system. It has antimicrobial effect against diverse array of microorganisms including; bacteria, viruses and fungi. This effect is mostly due to the capability of this protein to bind or chelate iron, besides its cellular or even molecular interactions with both host and pathogens [5]. Concerning bacteria, LF affects many species of Gram- negative and Gram-positive pathogens [21]. For Gram- negative bacteria, Lf causes destabilization of their cell wall by its ability to influence the release of LPS *via* Ca^{+2} binding [22].Moreover, the potential of LF to chelate iron usually competes with pathogens iron acquisition systems, leading to the limitation of their growth. Another mechanism by which LF can exert its antimicrobial activity is its direct binding to microbes, which causes destabilization of cellular membranes of broad spectrum of bacteria [23]. In addition, it was reported that the glycan moiety of LF can attach to bacterial adhesins, causing a decrease in the bacterial ability to form biofilms [24]. Also, LF can inhibit the adhesion of some bacterial strains including; *S. mutans* and *P. aeruginosa* on surfaces such as contact lenses or hydroxyapatite [25]. Interestingly, LF was found to possess a proteolytic effect (serine- endopeptidase activity) with a much lower activity compared to trypsin. This proteolytic activity could be utilized to degrade some viral virulence factors or secreted proteins from some pathogenic bacterial [26].More importantly, adsorption of LF on host cells was proven to be strongly correlated to activation of the immune system through

nuclear targeting or activation of certain pathwys [27]. In case of viruses, LF was found to interfere with the binding ability of retroviruses to host cells. Bovine LF can also prohibit integrin-mediated internalization of adenovirus into host cells *via* its attachment to viral polypeptides III and IIIa [28]. LF can also bind to integrins and GAGs receptors at the surface of host cells. These receptors are essential for viruses to enter the host cells. As a result, binding of LF to these receptors may interfere with viruses attachment and thus might reduce infection of host cells [29].

2.2. Anti-inflammatory effects

It is well known that LF possess anti-inflammatory protective properties against microbial infections and non-infectious disorders such as arthritis, allergies and even cancer [30]. The protective anti-inflammatory effect of LF in case of microbial infection could be mainly attributed to the ability of LF to neutralize microbial molecules such as LPS *via* binding to its lipid A domain, preventing further interaction of LPS binding protein (LPB) with endotoxin, so as to block binding of LPS to membrane CD14 [31]. Moreover, LF has the ability to stimulate secretion of IL-10 (anti-inflammatory cytokine), besides, inhibiting synthesis of many pro-inflammatory cytokines including; IL-6, IL-8, IL-1b, and TNF-α [32]. In non-infectious diseases, LF exerts its protective effect mainly *via* suppressing the production of pro-inflammatory cytokines and stimulating production of anti-inflammatory cytokines. In case of lung and skin allergies, LF was found to be internalized into mast cells in order to interact with cathepsin G, chymase and tryptase which are three effective inflammatory proteases [33]. Another important function of lactoferrin is being an inhibitor for reactive oxygen species (ROS), which are highly produced by granulocytes during inflammation. Lactoferrin mainly inhibit the production of ROS and further lipid peroxidation by chelating iron which is essential for their production [34].

2.3. Immune stimulating effects

It was shown that oral administration of bovine LF (bLF) could stimulate both systemic and mucosal immune responses *in vivo* [35]. Moreover, it was proven that complexation of LF with monophosphoryl lipid A could be used as an efficient adjuvant to induce cellular and humoral immune responses [36]. The main mechanism by which this protein can induce immunity is stimulating the differentiation of T-lymphocytes, activation of phagocytes and maintaining Th1/Th2 cytokine balance [37]. In case of non-pathogenic conditions, LF can enhance the differentiation of T cells from their corresponding immature precursors *via* induction of CD4 antigen [38]. Additionally, oral administration of bLF can promote systemic host immunity *via* activation of intestinal transcription of some essential genes such as p40, IL-12 and NOD2 [30]. Furthermore, LF was found to activate macrophages *via* stimulation of TLR4-dependent and -independent signaling pathways [39], which can further induce production of IL-6 and CD40. Also, attachment of LF to macrophages was found to stimulate phagocytosis of pathogens (Fig. 1) [40].

2.4. Anti-cancer effects

LF has been known for its anti-tumor effect according to many *in vivo* studies, which suggest that oral administration of bLF could decrease chemically induced carcinogenesis in rodents with a notable cytotoxic and anti-metastatic activity against many cancer cell lines [41,42]. In particular, LF promotes its antitumor effect *via* multiple mechanisms, among which is induction of apoptosis in tumor tissue [5]. This protein can induce the activity of caspases (3 and 8), which are a family of proteases having a vital role in apoptosis, besides, induction of some members of pro-apoptotic protein such as Bcl-2 [43].On the other hand, some studies showed that the apoptotic effect of LF is strongly dependent on its concentration. In other words, *in vitro* studies on PC12 cells revealed that elevated concentration of LF can cause an activation of caspases-3 and -8 and a reduction in the expression of phosphorylated extracellular signal regulated kinases (ERK1/2), while low concentration can upregulate phosphorylated ERK1/2 [44]. Moreover, bLF-derived peptides were also found to exhibit a cytotoxic effect against many tumor types *in vitro* and *in vivo via* activation of caspase-3 signaling pathway, cell cycle arrest and induction of mitochondrial apoptosis through production of reactive oxygen species (ROS) [45]. In addition, pepsin-digested bLF was found to enhance apoptosis *via* activation of caspase-3 and stress activated protein kinases/c-Jun amino-terminal kinase (SAP/JNK) kinase in squamous carcinoma cells [46]. Another important mechanism by which LF can inhibit tumorigenesis is suppression of their cellular growth *via* modulating multiple pathways. In triple negative human breast cancer cell line (MDA-MB-231), LF was proved to inhibit the action of cyclin-dependent kinases (cdk2 and cdk4) activities, associated with an elevated expression level of P21 protein, which is a cdk inhibitor [47]. In another study, LF was also found to modulate p27/cyclin E pathway in head and neck cancer cells *in vitro*, which is a key regulatory pathway in cellular proliferation [48]. In addition, LF was proven to upregulate P53 which is a key tumor suppressor protein, besides, modulation in the activity of its two downstream target genes; mouse double minute (mdm2) and p21 (cyclin-dependent kinases inhibitor) [49]. Mdm2 serves as a common negative regulator of P53 that regulates its degradation *via* ubiquitination [50].

Interestingly, expression of cytoplasmic delta LF isoform was found to induce cell cycle arrest *via* induction of Skp1 (S-phase kinase-associated protein) and Rb (retinoblastoma) genes [51]. It is worth mentioning that Skp1 is a member of SCF (Skp1/Cullin-1/F-box ubiquitin ligase) complex which is involved in the ubiquitination process of proteins leading to their subsequent degradation by the proteasome at the G1/S transition. It is speculated that this LF isoform may affect the half-life time of molecules involved in cell cycle regulation leading to their faster degradation [51]. In another study, it was shown that bLF could specifically inhibit the action of plasma membrane V-ATPase proton pump only in highly metastatic cancer cell lines such as osteosarcoma MG-63, breast cancer MDA-MB-231 and prostate PC-3 cell lines, while non-tumorigenic fibroblast BJ-5ta cells were found to be completely insensitive to bLF. Using proton transport inhibitors is emerging nowadays as a new cancer therapeutic approach, since cancer cells overexpress V-ATPase at the plasma membrane [52]. Inhibiting the activity of this proton pump leads to a reduction in the acidity of tumor microenvironment (TME), which as a consequent can decrease the growth and invasion of cancerous cells (Fig. 2) [42]. In another study, LF (500 μg/mL) was found to reduce cell viability in esophageal cancer cell line (KYSE) by 53% after 20 h without any cytotoxic effect on normal cells [53]. Moreover, oral administration of bLF (300 mg/kg of body weight) trice/week for 45 days in pulmonary tumor transgenic mice overexpressing hVEGF-A_{165} was found to inhibit the expression of hVEGF-A_{165} gene and block inflammation in the lung cells, suggesting the inhibitory effect of LF on angiogenesis [54]. In a combinational therapy approach, human LF (HLF) was co-administrated with temozolomide (TMZ), a conventional chemotherapeutic drug to examine its effect on human glioblastoma (GBM) cells. Results revealed an enhanced anti-tumor effect *in vitro* on human glioblastoma cell line and *in vivo* in nude mice (60 mg/kg/day) in terms of tumor size reduction, downregulation of cyclin D1 and D4 and cell cycle arrest at both G0/G1 and G2 phases [55].

3. Lactoferrin in nanotechnology

3.1. Lactoferrin nanoparticles

3.1.1. Lactoferrin nanoparticles prepared by sol-oil or (water-in-oil) emulsion method

Sol-oil or (water-in-oil) emulsion method is the most common method used for the fabrication of LF nanoparticles. It starts with

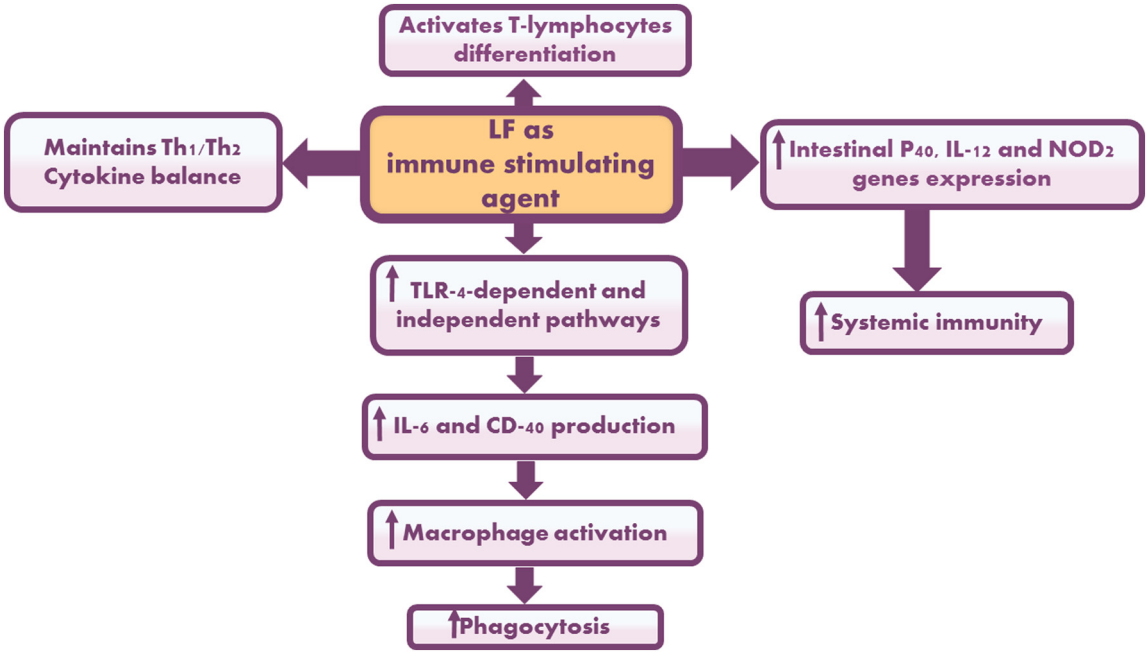

Fig. 1. A schematic diagram illustrating immunity stimulating mechanisms of lactoferrin.

preparation of aqueous solution of LF at the desired concentration followed by slow addition of this solution to olive oil at 4 °C under continuous dispersion. This mixture will be sonicated at 4 °C, then it will be snap frozen in liquid nitrogen. Afterwards, it will be slowly thawed in ice for about 4 h. Obtained nanoparticles will be collected by centrifugation, followed by washing with cold diethyl ether and finally it could be dispersed in appropriate medium (Fig. 3) [56]. Although doxorubicin (DOX) is an efficient cytotoxic anticancer drug, but it is reported to be extensively toxic to heart and spleen, besides its limited oral absorption. In order to overcome these drawbacks, DOX-loaded LF nanoparticles were prepared by sol-oil chemistry [57]. LF was selected to serve as the nanocarrier for DOX because its receptors are highly expressed on the surface of highly proliferating cells such as cancer cells (add reference here). DOX-loaded LF nanoparticles exhibited particle size of about 68 nm and encapsulation efficiency (EE %) of about 79%. In addition, drug-loaded formulation exhibited good physical stability with only 2.5–5% drug loss during a period of 3 months associated with very low hemolytic rate (~2%), which indicate their negligible erythrocyte membrane-damaging effect [57]. Oral administration of this formulation in hepatocellular carcinoma (HCC) bearing rats resulted in significantly lower number of neoplastic nodules in the liver of DOX-loaded LF nanoparticles treated group compared to either positive control group (receiving saline) or free DOX. In addition, the protein levels of p53 and cleaved caspase3 were elevated in the nanoformulation-treated group compared to both free DOX and untreated groups, indicating the antitumor efficacy of the fabricated nanoparticles as a result

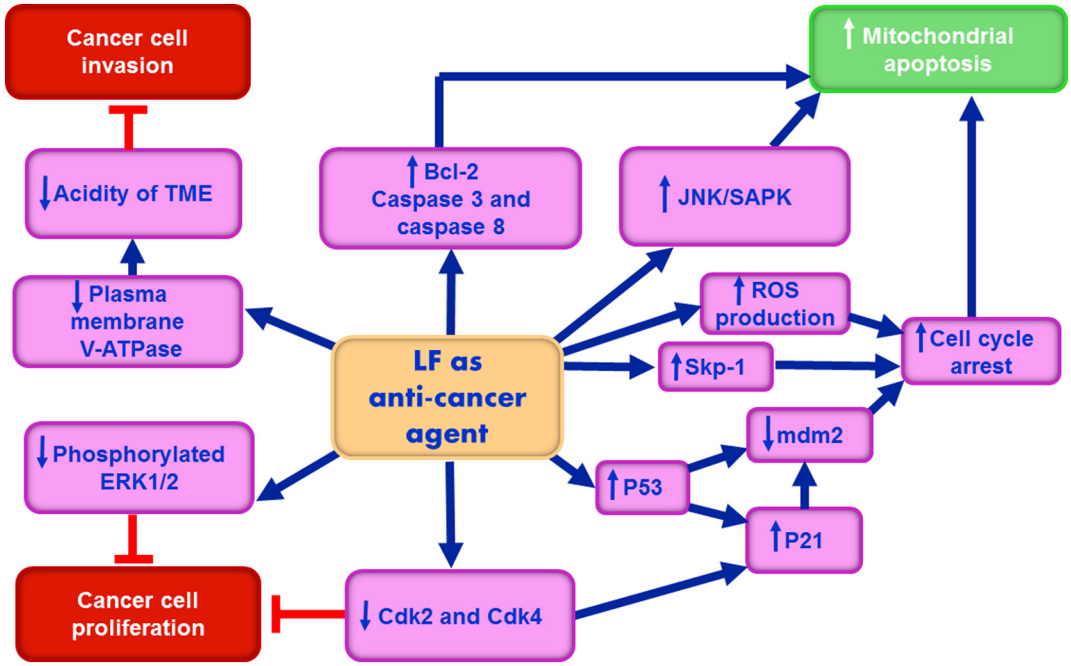

Fig. 2. A schematic diagram illustrating anti-cancer effects of lactoferrin.

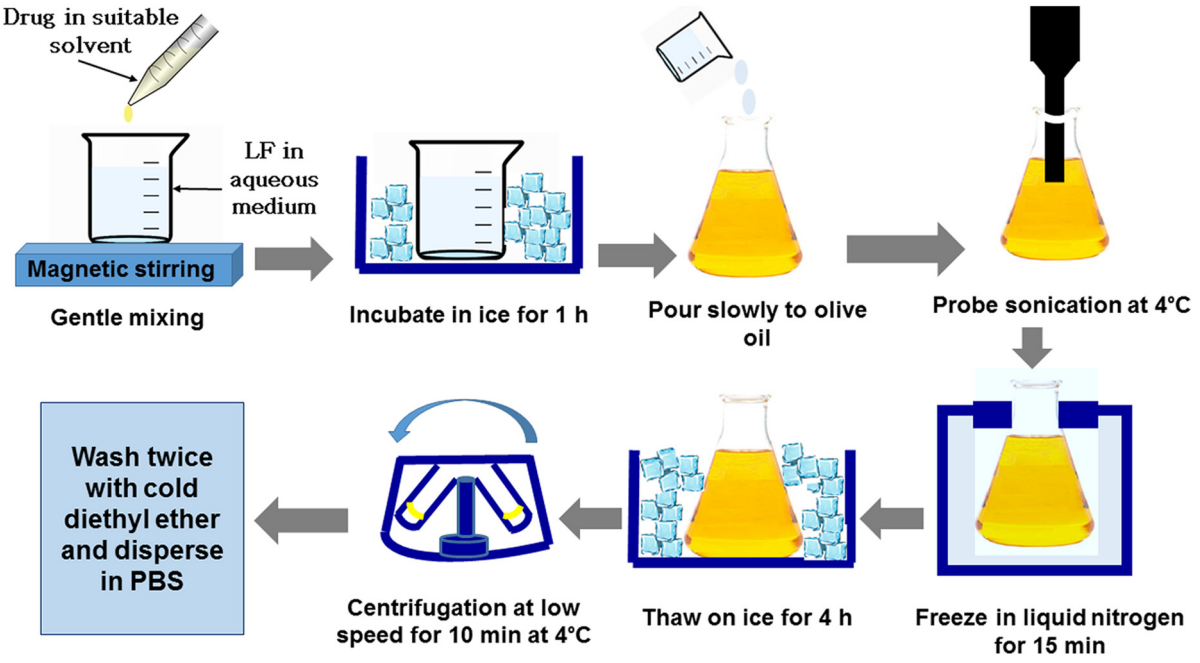

Fig. 3. Preparation of lactoferrin nanoparticles by sol-oil or (water-in-oi)l emulsion method.

of their better bioavailability and hepatic localization. Furthermore, oral administration of DOX-loaded LF nanoparticles did not show any symptoms of toxicity in terms of body weight loss, liver and kidney functions, which further confirmed the safety and biocompatibility of this nanoformulation [58].

In another study, LF nanoparticles were used to encapsulate the antiviral drug; Zidovudine. It is a potent antiviral drug with good bioavailability (50–75%), however, it causes bone marrow repression, neutropenia and organs toxicity. In order to overcome all these problems, nanoencapsulation of Zidovudine into LF nanoparticles was achieved by sol-oil chemistry method. Fabricated NPs were about 50–60 nm in size with 67% drug encapsulation efficiency and good physical stability at room temperature and 4 °C without any remarkable change in the particle size or the drug content. *In vitro* drug release study indicated that the maximum amount of drug was released at pH 5 with a biphasic release kinetics in which 60% of the drug was released in the first 4 h, followed by slower release up to 80% within 10 h then a limited release over 96 h. Interestingly, drug release in both simulated gastric and intestinal fluids was minimal indicating the stability of LF nanoparticles at extreme conditions. Oral administration of Zidovudine-loaded LF NPs showed that they exhibited anti-HIV-1 effect comparable to that of free drug. In addition, drug-loaded NPs displayed improved pharmacokinetic profile in comparison to free drug, associated with lower organs toxicities, which suggest this nanoformulation as a safe nanoplatform to enhance drug delivery [59].

Efavirenz (EFV) is a reverse transcriptase non-nucleoside inhibitor which is extensively utilized in the treatment of HIV infection. Despite its high efficacy, its administration is usually associated with low bioavailability and high toxicity levels which may restrict its use. Consequently, efavirenz-loaded LF nanoparticles were fabricated by sol-oil method in order to enhance oral delivery of the drug [60]. Fabricated drug-loaded LF nanoparticles displayed particle size of about 45–60 nm with 59%EE. Moreover, *in vitro* drug release studies revealed that maximum drug release was observed at pH 5, whereas drug release was minimal in both simulated gastric and intestinal fluids, which confirmed their stability in gastrointestinal environment, and hence their suitability for oral route of administration [60]. *In vitro* studies showed that the anti-HIV-1 activity of drug-loaded nanoparticles were 2 fold higher in comparison to free drug. Furthermore, efavirenz-loaded LF nanoparticles displayed enhanced pharmacokinetic profiles associated with minimized toxicity in comparison to free drug [60]. In another interesting approach, efavirenz and curcumin were co-loaded in LF nanoparticles prepared by sol-oil chemistry to be utilized as a multiple prevention technology (MPT) agent. In this formulation, efavirenz acted as an anti-HIV-1 drug and curcumin acted as anti-microbial spermicidal agent [61]. Dual drug-loaded LF nanoparticles demonstrated particle size of about 40–70 nm with EE% of 63 and 61 for curcumin and efavirenz, respectively. *In vitro* drug release study showed that the maximum release (>80%) was observed at pH 5, whereas, below pH 4 and above pH 6, only 10% of drug was released. This characteristic release profile of LF nanoparticles will offer slow release of both drugs in the vaginal lavage in its pH range (4–4.5), while higher drug release will occur at pH more than 4.5, a condition which is usually associated with viral infections in the vagina. In addition, these dual drug-loaded exhibited good physical stability for 20 days when incubated in PBS, pH 7.4 at 4 °C and 25 °C without any significant changes in their hydrodynamic particle size, EE% and zeta potential [61]. Upon application of this formulation as a vaginal lavage in rats, there was a remarkable increase in the amount of the delivered drugs in comparison to free drugs without any effect on the viability of the vaginal microflora, *Lactobacillus cripatus which is* believed to be the main gatekeeper of the vaginal ecosystem. In addition, dual drug-loaded LF nanoparticles showed better pharmacokinetic profiles in the vaginal lavage compared to free drugs associated with a notable decline in the toxicity levels in vaginal tissue. These results suggested that this triple action topical formulation could simultaneously act as anti-HIV-1, anti-bacterial with contraceptive effect [61].

These LF nanoparticles encapsulating anticancer or antiretroviral drugs have gained considerable importance for treating or preventing some sexually transmitted diseases such as AIDS and HIV either *via* oral or vaginal administration routes. Although these LF nanoparticles seemed to be promising in multiple prevention therapy, their health impacts are still to be examined. In order to investigate the possible systemic and reproductive toxicities of antiretroviral drug-loaded LF nanoparticles, three dual drug-loaded nanoparticles were selected for this purpose including; curcumin/efavirenz-loaded LF nanoparticles, curcumin/dapivirine-loaded LF nanoparticles and curcumin/tenofovir-loaded LF nanoparticles. These drug-loaded LF nanoparticles were

prepared by sol-oil method and fully characterized as previously mentioned [61]. Results indicated that administration of these drug-loaded LF nanoparticles *via* the vaginal route did not affect the reproductive performance or fertility of the examined rats and the postnatal development of their offspring was also normal. On the other hand, oral administration of the same drug-loaded LF nanoparticles in rats caused a significant reduction in their litter size, whereas their reproductive performance and postnatal development remained unchanged. These results suggested that administration of these dual drug-loaded LF nanoparticles through the vaginal route is preferred over the oral route. This might be due to the possible side effects of the utilized antiretroviral drugs [62].

In another study, zidovudine/efavirenz/lamivudine-loaded LF nanoparticles were prepared by sol-oil method [63]. Result revealed that triple drug-loaded LF nanoparticles exhibited particle size of about 67 nm with 5% drug loading (DL %) for each drug and EE% in the range of 58 to 71%, besides, enhanced hemocomptability with low hemolytic rate (~2%). pH-dependent release study demonstrated that the maximum amount of all encapsulated drugs were released at pH 5 in a biphasic drug release profile with 60% of the drugs were released up to 5 h and rest of encapsulated drugs were constantly released over the period of 24 h. On the other hand, only 20% of drugs were released after 24 h in pH 7.4, suggesting the ability of drug-loaded LF nanoparticles to resist drug release in simulated intestinal and gastric fluids, while most of the drugs (80–90%) will be released at the endosomal pH (pH 5) within the target tissue after successful delivery [63]. In addition, prepared nanoformulation displayed enhanced *in vitro* anti-HIV activity and improved *in vivo* drug bioavailability and pharmacokinetic profile compared to free drugs, associated with less tissue inflammation suggesting the multiple advantages of these triple drug-loaded LF nanoparticles as anti-HIV nanoplatform [63].

In another approach, carmustine-loaded LF nanoparticles were fabricated by sol-oil method. Carmustine is an antineoplastic drug which alkylates and crosslinks DNA resulting in disruption of its function, cell cycle arrest and apoptosis. Consequently, it is used as chemotherapeutic treatment regimen for many tumors including glioblastoma because of its ability to cross the BBB. On the contrary, this drug is extremely lipophilic which requires harsh conditions for complete solubilization, besides the occurrence of some negative side effects such as nausea, vomiting, low white blood cells count and pulmonary toxicity in case of high doses. In this context, carmustine-loaded LF nanoparticles were prepared with sol-oil method to enhance its therapeutic efficacy [64]. Fabricated nanoparticles were spherical with particle size ≤41 nm and EE% of about 43%. Moreover, pH-dependent drug release study demonstrated that the maximum amount of the drug was released at pH 5, which is the pH of the endocytotic vesicles involved in the cellular uptake of LF nanoparticles *via* its receptor-mediated endocytosis. This characteristic pH release profile will support minimal drug loss during systemic circulation, and hence reduce systemic toxicity. In addition, drug-loaded LF nanoparticles manifested enhanced *in vitro* cellular uptake and antiproliferative activity on C6 glioma cells compared to free drug. However, further *in vivo* studies are required to examine the anti-tumor efficacy and toxicity of this formulation [64].

Lenalidomide (LND) is another chemotherapeutic drug used in combination with radiotherapy to treat multiple myeloma cancer. It was reported that cancerous cells can develop resistance against this drug and in order to enhance its efficacy, higher doses might be used which indeed will cause unfavorable negative side effects. In an attempt to promote the therapeutic outcomes of LND, LF nanoparticles encapsulating LND were fabricated by sol-oil method [65]. Drug-loaded LF nanoparticles revealed particle size of about 120 nm and high EE% of about 60% associated with good physical stability at 4 °C without any remarkable change in either particle size or drug loading (DL%). Moreover, *in vitro* drug release studies showed that the release rate of LND was greatly dependent on the pH, with accelerated rate at pH 5 and 6. In addition, fabricated nanoparticles showed higher cellular uptake and 2.7 fold increase in the *in vitro* cytotoxicity on human ovarian cancer cell line (SK-OV-3) compared to free drug. However, further *in vivo* studies are required to confirm the anti-tumor efficacy of this formulation [65].

In an attempt to enhance the photodynamic therapy potential of the FDA-approved photosensitizer; Chlorine e6 (Ce6), it was loaded into non-toxic LF nanoparticles. Ce6-loaded LF nanoparticles were fabricated by water-in-oil emulsion method with particle size of about 80 nm and EE % of approximately 42% [66]. Lyophilized preparations could be stored for several months with good redispersion in aqueous media. Moreover, prepared nanoparticles exhibited higher Ce6 release at pH 5.5 (80%) in comparison to neutral pH 7.4 which exhibited % release (~20%) over 24 h. More importantly, encapsulation of Ce6 within LF nanoparticles resulted in a decrease (44 times) in the required amount of Ce6 to induce light-mediated cell death in MDA-MD 231 and SK-OV-3 cells in comparison to free Ce6. This significant decrease in IC_{50} value suggested the potential of using LF nanoparticles to enhance photodynamic therapy efficacy of some photosensitizers such as Ce6 [66]. Furthermore, LF nanoparticles were loaded with either 5-Fluorouracil (5-FU) or oxaliplatin to be utilized as s nanoplatform to treat colon adenocarcinoma. Drug-loaded LF nanoparticles were fabricated by water-in-oil method. Particle size of 5-FU-loaded LF nanoparticles and oxaliplatin-loaded LF nanoparticles were 60–90 nm and 65–100 nm, respectively, whereas, they exhibited EE% of about 48.6 and 51.3, respectively [67]. *In vitro* studies showed that both 5 FU- and oxaliplatin-loaded LF nanoparticles displayed enhanced antiproliferative activity in human colon adenocarcinoma (COLO-205) cell line in comparison to their counterpart free drugs. In addition, azoxymethane (AOM) carcinogen was utilized to induce aberrant crypt foci (ACF) in the colon of Wistar rats *via* intraperitoneal injection of 2 doses of AOM in the consecutive week with a concentration of 10 mg/kg body weight. These ACFs are further transformed into neoplastic lesions in the colon. Afterwards, drug-loaded LF nanoparticles and free drugs were injected intravenously into the induced rats for 4 weeks at concentration of 40 mg drug/kg body weight (1 dose/week). Results revealed that nanoformulations demonstrated enhanced anti-tumor effect compared to free drugs associated with lower systemic toxicity. This enhanced effect could be attributed to the improved pharmacokinetic profiles and better tissue biodistribution of drug-loaded LF nanoparticles when compared to free drugs, besides their enhanced cellular uptake due to the active targeting property of LF [67].

In an approach to sensitize the chemotherapeutical potential of temozolomide (TMZ), Aurora Kinase B (AKB) small interfering RNA-loaded LF nanoparticles were fabricated by water-in-oil emulsion method [56]. TMZ is the only approved drug for treatment of glioblastoma multiforme (GBM), which is a DNA methylating agent that can produce O6-methylguanine; a DNA adduct that can enhance cell cycle arrest and cell death. However, its efficacy is limited due to increased resistance by brain tumors against this drug. As a modulation, AKB-siRNA was used in combination with TMZ to inhibit gene expression of AKB. TEM analysis demonstrated the formation of spherical nanoparticle having particle size in the range of 50–70 nm with siRNA EE% of about 56%. Moreover, entrapped siRNA within LF nanoparticles was found to be intact and highly resistant to RNaseA, whereas, free siRNA was completely degraded when it was exposed to similar conditions (incubation with RNaseA for 1 h at 37 °C). Additionally, incubation of siRNA-loaded LF nanoparticles with serum containing contains nucleases and proteases did not cause any significant change in their particle size over a period of about 10 h at 37 °C, which confirmed stability in serum [56]. Fabricated nanoplatform either alone or in combination with TMZ demonstrated inhibitory action on the viability of glioma (GL261) cells *in vitro* due to silencing of AKB gene and cell cycle arrest. Furthermore, when siRNA-loaded LF nanoparticles were intravenously injected into GBM orthotopic mouse model alone or in combination with TMZ, there was a pronounced inhibition of tumor growth and about 2.5 fold increase in the survival rate. These results suggested that siRNA-loaded LF nanoparticles could inhibit tumor growth or it could be

combined with conventional chemotherapy to increase its efficacy with simultaneous decrease in the toxic side effects and resistance that may be acquired by many tumors upon repeated dosing [56].

In another study to enhance the anti-glioma effect of TMZ, it was encapsulated this time into LF nanoparticles by sol-oil method [20]. TMZ-loaded LF nanoparticles exhibited particle size in the range of 70 nm and EE% of about 42%, associated with enhanced stability for 2 months when incubated in PBS supplemented with 10% serum at 4 °C. Moreover, release of TMZ from LF nanoparticles was found to be induced (60–70%) in slightly acidic pH (5.5), while its release at physiological pH (7.4) was found to be reduced (10–2%). This effect is thought to be due to shrinkage of hydrodynamic particle size of LF nanoparticles from 160 to 80 nm, which might favour the drug release in the tumor acidic microenvironment. In addition, release kinetics of TMZ from LF nanoparticles at pH 5.5 exhibited a biphasic profile with initial burst release (60–70%) in the first 10 h, followed by sustained release (15–20%) till 72 h, whereas, release of TMZ at pH 7.4 was found to be minimal over 72 h [20]. Furthermore, *in vitro* studies on GL261 cell line demonstrated that LF nanoparticles showed better cellular uptake and internalization in brain tumor tissue compared to free LF, which caused an increase in the intracellular concentration of TMZ. It is worth mentioning that *in vivo* studies showed that systemic administration of TMZ-loaded LF nanoparticles demonstrated better pharmacokinetic profile and brain accumulation when compared to free drug. Moreover, there was a notable decline in tumor volume associated with higher apoptotic rate and enhanced survival in glioma bearing mice without any signs of organs toxicity. These results might confirm that LF nanoparticles are safe carriers for TMZ that can cross the BBB with enhanced active targeting towards brain tumors [20].

The cationic nature of LF could be utilized to form a complex with negatively charged DNA *via* electrostatic complexation. In this context, plasmid pGFPC1 (4.7 kb) encoding the green fluorescent protein was utilized as a cargo gene [68]. LF nanoparticles loaded with the plasmid were fabricated by sol-oil method. Prepared LF nanoparticles exhibited particle size in the range of 60 nm with low polydispersity index (~0.12) indicating the homogeneity of the preparation.

Moreover, prepared LF nanoparticles exhibited enhanced physical stability at 4 °C up to 10 weeks without any significant change in the particle size. Also, incubation of LF nanoparticles in DMEM culture media with 10% serum at 37 °C for 8 h did not cause any change in the particle size, which will lead to longer plasma level [68]. It is speculated that this improved stability could be related to the strong electrostatic interaction between positively charged LF and negatively charged DNA. In addition, LF is reported to have DNA binding domain which may aid further in DNA binding and formation of more compact DNA-LF nanocomplex [69]. Furthermore, EE% of pDNA in LF nanoparticles was found to be about 58% when estimated by SYBER GREEN assay. Also, these nanoparticle were proven to protect encapsulated DNA from degradation by DNase I treatment in comparison to free DNA which exhibited complete degradation [68]. Furthermore, *in vitro* studies showed that LF nanoparticles exhibited better gene delivery in LF receptor expressing cell lines including; hepatocellular carcinoma (HepG2) and mouse glioma (GL261) cell lines due to active targeting ability of LF nanoparticles. On the other hand, cellular uptake of LF nanoparticles by human lung epithelial (A549) cells was low due to lack of LF receptors on the surface of these cells. These results confirmed that LF nanoparticles could be utilized as a potent active targeting nanocarrier for efficient gene delivery in LF receptor expressing cells [68].

In a recent interesting study, Sodium 2-mercaptoethanesulfonate (MES)-decorated zidovudine loaded-LF nanoparticles were fabricated by water-in-oil emulsion sonochemical method in order to maintain the globular structure of the protein [70]. Prepared nanoparticles were conjugated with MES in order to embrace negative charge to the nanoparticles that imitate CCR5; the main chemokine co-receptor used by HIV-1 virus and Heparan sulfate which is the initial contact point of HIV-1 envelope to attach to the host cell surface [71]. HIV-1 entrance depends mainly on the interaction between gp120 protein which is the viral envelope protein and the co-receptor CCR5. Studies showed that gp120-CCR5 complex include conserved epitopes with overall positive charge located on variable loop (V3) of gp120 protein. These positive charges were found to play a key role in the electrostatic interaction with polyanion groups located in CCR5 [72]. On the other hand, it was reported that the virion particle should interact first with a cluster of the anionic polysaccharide; Heparan sulfate (HS) located on the host cell surface before its interaction with CCR5 co-receptor. This interaction is stimulated by V3 loop of the gp120 viral protein, so if compounds resembling HS were utilized, they can competitively bind to the V3 loop of gp120 protein, and hence can prevent binding of the virus to the surface of the host cell [73]. In this study, MES-decorated drug loaded LF nanoparticles served as a dual purpose nanoplatform since they can frustrate attaching and entry of the virus to the host cells and the loaded ART drug can inhibit the viral activity [70]. LF is known to include 34 cysteine resides with only two of them involved in the formation of disulfide bonds [74]. The other free cysteine residues were utilized to modify LF nanoparticles surface with MES to bind to viral V3 loop instead of HS. Morphological administration demonstrated that MES-decorated zidovudine-loaded LF nanoparticles were spherical in shape with particle size in the range of 55–75 nm and EE% of about 68%. Moreover, drug-loaded LF nanoparticles were found to be sensitive in acidic pH as maximum drug release was observed at pH 5. Results revealed that MES-decorated curcumin (as a fluorescent dye)-loaded LF nanoparticles exhibited better *in vitro* cellular localization in HIV-1 envelope surface expressing cell line (HL2/3) in comparison to curcumin-loaded LF nanoparticles. Moreover, MES-decorated zidovudine-loaded LF nanoparticles exhibited enhanced *in vitro* cytotoxic effect in SuoT1 cells compared to either free zidovudine or zidovudine-loaded LF nanoparticles (Table 1) [70].

3.1.2. Lactoferrin nanoparticles prepared by nanoparticle albumin bound (NAB)-technology

This method depends mainly on presence of an oily phase (drug in water immiscible solvents) which is slowly added to the aqueous phase containing LF. An initial crude emulsion is formed by exposing this mixture to low speed homogenization shearing forces. Afterwards, a final emulsion could be formed by sonication of the initial emulsion for about 2 min. The final nanosuspension solution can be produced by removing the remaining organic solvent through rotary evaporator (Fig. 4A) [75]. Nanoparticle albumin-bound (NAB, nab™) paclitaxel (NAB-paclitaxel; Abraxane®, Abraxis BioScience, Los Angeles, CA, USA) was approved in 2006 by the FDA to be the first nanotechnology based chemotherapeutic agent that exhibited promising results in treating metastatic breast cancer in patients who failed in conventional combinational therapy [76]. Preparation of protein nanoparticles by NAB technology (a patent of Vivorx Pharmaceuticals Inc., patent No. 6652884 B2) has attracted great interest to fabricate anti-cancer drug delivery systems because of the excellent potential of this method to encapsulate highly lipophilic drugs into protein nanoparticles [77].

Gambogic acid (GA) is the main active component of *Garcinia hanburyi*. It possesses antioxidant, anti-inflammatory and neuroprotective effects. In addition, some studies demonstrated that GA could repress the growth of many cancer types. Intravenous injection of poorly water soluble GA is commonly performed by either conjugating it with L-arginine or by using Cremophor (EL) as a solubilizer, which is usually associated with allergy, ulceration and organs toxicity. In an attempt to develop oral nanoformulation of GA, LF nanoparticles were utilized for this purpose due to their proposed stability in gastrointestinal tract. They were fabricated by (NAB) technology which depends mainly on the fact that LF is cysteine-rich glycoprotein which have the capability to form nanoparticles by this technique depending on disulfide bounding and homogenization [78]. Drug-loaded LF nanoparticles exhibited particle size of about 100 nm with spherical and smooth morphology, besides EE% of about 92.3%. Moreover, loaded LF

Table 1
LF nanoparticles prepared by sol-oil or water-in-emulsion method.

Encapsulated drug(s)	Route of administration	Key features	References
DOX	Oral and intravenous	Enhanced anti-tumor effect in HCC-bearing rats without any significance toxicity.	[57,58]
Zidovudine	Oral	*In vivo* improved pharmacokinetic profile and enhanced anti- HIV-1 effect with lower toxicity.	[59]
Efavirenz	Oral	Enhanced *in vivo* anti-HIV-1 effect with minimized toxicity.	[60]
Efavirenz/CUR	vaginal lavage	Improved pharmacokinetic profiles, anti-HIV-1effect and spermicidal action with decline toxicity in vaginal tissue.	[61]
Efavirenz/CUR Dapivirine/CUR Tenofovir/CUR	Oral or as a vaginal lavage	Administration of these formulations *via* oral or vaginal routes did not affect animals' reproductive performance or offspring's postnatal development.	[62]
Zidovudine/ efavirenz /lamivudine	Oral	Improved *in vivo* drug bioavailability and pharmacokinetic profile compared to free drugs, associated with less tissue inflammation.	[63]
Carmustine	–	pH-dependent drug release profile with Increased *in vitro* cellular uptake and antitumor effect on C6 glioma cells.	[64]
Lenalidomide	–	Accelerated drug release at pH 5 and 6 with enhanced *in vitro* cytotoxicity and cellular uptake on human ovarian cancer cell line (SK-OV-3).	[65]
Chlorine e6	–	LF nanoparticles resulted in 44 times decrease in IC_{50} value required to cause death of SK-OV-3 and MDA-MD 231 cells *in vitro*.	[66]
Oxaliplatin or 5-FU	Intravenous	Enhanced *in vivo* anti-tumor effect associated with lower toxicity in colon cancer-induced animal model.	[67]
Aurora Kinase B (AKB) siRNA	Intravenous + free temozolomide	*In vivo* improved anti-tumor and 2.5 fold increase in survival rate in glioblastoma orthotopic mouse model.	[56]
Temozolomide	Intravenous	Better drug pharmacokinetic profile and brain accumulation with a notable decline in tumor volume and enhanced survival in glioma- bearing mice.	[20]
plasmid pGFPC1 (4.7 kb) encoding the green fluorescent protein gene	–	Enhanced gene delivery in cells expressing LF receptors.	[68]
zidovudine	–	MES-decorated LF nanoparticles exhibited better *in vitro* cellular localization in HIV-1 envelope surface expressing (HL2/3) cell line in comparison to LF nanoparticles.	[70]

nanoparticles demonstrated slow *in vitro* drug release after 24 h in both simulated gastric fluid (8.74%) and simulated intestinal fluid (15.4%), suggesting the suitability of these nanocarriers for oral drug administration. Results of *in situ* intestinal perfusion revealed that the absorption of GA-loaded LF nanoparticles was depending mainly on active transport *via* LF receptor-mediated transcytosis, whereas free GA exhibited passive diffusion absorption. More importantly, MTT assay showed that GA-loaded LF nanoparticles displayed nearly equal antiproliferative effect in comparison to arginine-conjugated GA on HepG2 human liver cancer *cell line*. Furthermore, oral administration of GA-loaded nanoparticles resulted in enhanced anti-tumor effect in S180 tumor-bearing mice in comparison to intravenous injection of arginine-conjugated GA, which indicates that LF nanoparticles could increase the oral absorption of hydrophobic drugs, while minimizing the toxicities associated with their intravenous injection [78]. Similarly, LF nanoparticles encapsulating oleanolic acid (OA) were prepared by NAB technology [79]. OA is a pentacyclic triterpenoid which is abundant in the plant kingdom [80]. It is known to have multiple pharmacological activities including; anti-inflammatory, antioxidant, antitumor and hepatoprotective effects [81]. On the other hand, its therapeutic efficacy is limited due to poor aqueous solubility, low membrane permeability and hence poor bioavailability [82]. OA-loaded LF nanoparticles prepared with a ratio of 1:6 (*w*/w %) of OA: LF displayed hydrodynamic particle size in the range of 202 nm, EE% of about 92.59% and zeta potential of about +27 mV, indicating their good colloidal stability. Unlike expected, *in vitro* drug release study revealed that drug-loaded LF nanoparticles exhibited rapid drug release with approximately 100% released within 20 min. These results could be related to the presence of highly dispersed Lf with its large surface area. In addition, interaction between LF and OA was found to cause a decrease in the surface tension of the drug, which resulted in the production of nanoparticles with good wettability associated with improved drug aqueous solubility. Oral administration of OA-loaded LF nanoparticles in Sprague Dawley rats resulted in significant enhancement in the bioavailability and pharmacokinetic profile of OA in comparison to its free form, which might be attributed to increased intestinal absorption and cellular uptake of prepared LF nanoparticles (Table 2) [79].

3.1.3. Lactoferrin nanoparticles prepared by thermal denaturation method

Another interesting application in which LF can be involved is the fabrication of nanovehicles encapsulating micronutrients such as iron to be utilized in food industry. Globular proteins such as LF was selected in this study to from nanoparticles because of its nutritional value and gelation capability. LF can form gels when exposed to thermal treatment, ionic strength or pH changes. Generally, thermal gelation of proteins starts with a heating step to denature the protein, followed by salt addition to induce protein aggregation (Fig. 4B). Among salts that could be involved in this method are sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$) and ferric chloride ($FeCl_3$) [83]. Another important factor which affect this process is the concentration of the utilized protein. Upon using high concentration of proteins, gels are formed while at lower concentration of proteins, nanoparticles are produced. After protein denaturation and polymerization *via* a heating step, a cooling step is required followed by addition of an appropriate salt to induce protein aggregation [84].

In this light, LF nanoparticles at concentration of 0.2% (*w*/*v*) were prepared *via* thermal gelation method at 75 °C and pH 7 for 20 min to entrap $FeCl_3$ (35 mM) [83]. LF was specifically selected in this study due to its high tendency to bind Fe^{3+} ions, besides being safe and biocompatible. When nanoparticles were prepared at pH 7 and 10, they exhibited particle size in the range of 107–110 nm with low PdI value and EE% of about 53.8%, whereas nanoparticles prepared at pH 4 exhibited particle size of about 383 nm. This big difference in the particle size may be related to occurrence of some conformational changes in the structure of LF under acidic conditions, which as result caused intense aggregation of LF and formation of larger particles [85]. Consequently, pH 7 was selected for further preparations. In addition, the temperature of the preparation was selected to be 75 °C because obtained sizes were around 100 nm without any significant turbidity or change in the PdI

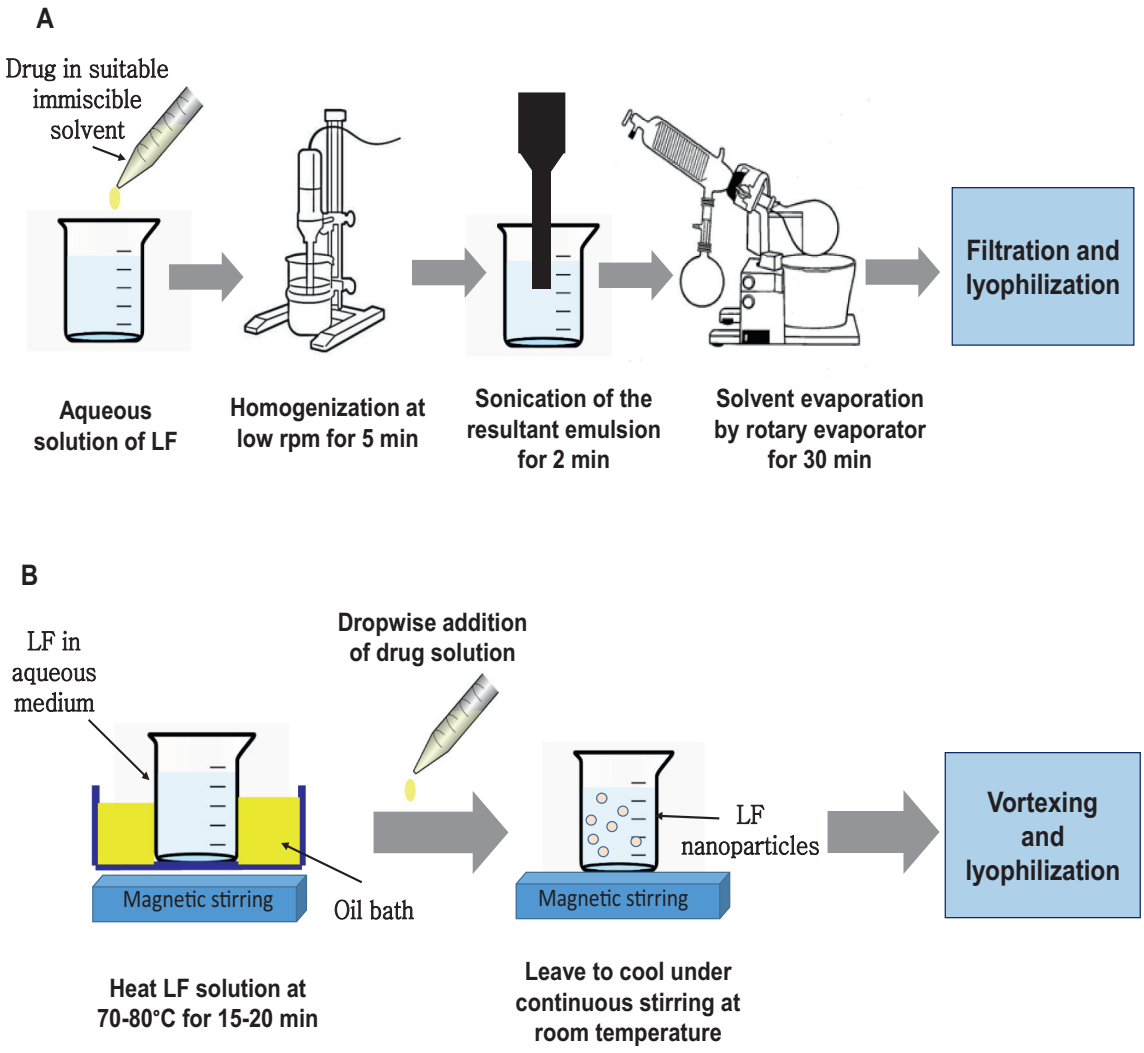

Fig. 4. Preparation of lactoferrin nanoparticles by NAB-technology (A) and thermal denaturation method (B).

values. On the other hand, there was significant increase in the turbidity above 80 °C. This notable increase in the turbidity could be attributed to protein aggregation induced by its thermal denaturation [85]. Addition of $FeCl_3$ at concentration of 35 mM during LF nanoparticles preparation did not cause any significant change in the particle size or PdI compared to unloaded nanoparticles. Fabricated nanoparticles demonstrated good stability at 4–60 °C and pH 2–11 for a period of about 10 weeks with no significant change in their particle size or PDI values, which suggest the feasibility of this nanosystem for iron delivery in food applications [83].

Cichoric acid (CA) is a natural polyphenolic compound which is found in edible plants. It was reported to have many beneficial biological effects such as antiviral, anti-inflammatory and antioxidant effects. Administration of CA as an oral formulation was found to be ineffective because of its instability in both aqueous media and the acidic environment of gastrointestinal tract, besides the possibility of degradation by the action of the enzymes located in that area. As a result, encapsulation of CA into a nanosytem could increase its efficacy. Regarding this, CA-loaded LF nanoparticles were prepared by pH-dependent thermal treatment. Results revealed that drug-loaded LF nanoparticles prepared at pH 7 and 95 °C exhibited the best stability and the smallest particle size in the range of 67.20 nm and EE% of about 97.87–99.87% [86]. Interestingly, thermograms obtained from differential scanning calorimetry demonstrated that encapsulation of CA into LF nanoparticles resulted in an increase in the thermal stability of LF, which might be due to the electrostatic and hydrophobic forces involved in the formation of CA-loaded nanoparticles. Furthermore, *in vitro* antioxidant studies showed that CA-loaded LF nanoparticles

Table 2
LF nanoparticles prepared by nanoparticle albumin bound (NAB) method.

Encapsulated drug(s)	Route of administration	Key features	References
Gambogic acid	Oral	Enhanced anti-tumor effect in murine sarcoma (S180) tumor-bearing mice in comparison to intravenous injection of arginine-conjugated gambogic acid.	[78]
Oleanolic acid	Oral	Improved pharmacokinetic profile and intestinal absorption resulted in enhanced bioavailability in Sprague Dawley rats.	[79]

exhibited better antioxidant potential in comparison to free CA, which might be attributed to the protective effect aided by encapsulation of CA into LF nanoparticles [86].

In another study, dual non cytotoxic drugs (simvastatin and fenretinide) were co-encapsulated into LF nanoparticles prepared by green thermal denaturation method without any harsh solvents or crosslinkers [87]. Simply, aqueous solution of LF was stirred in an oil bath for only 10 min at 80 °C, then each drug ethanolic solution was dropwise added. After that, oil bath was removed and the mixture was stirred till reaching room temperature. Drug-encapsulated LF nanoparticles can be further purified *via* gel filtration chromatography using a Sephadex G50 column to remove unentrapped drugs [87]. Simvastatin is generally used to lower cholesterol level, but recent studies showed that it could be beneficial in treating brain tumors as it can remodel tumor microenvironment (TME) and tumor associated macrophages (TAM) [88,89]. Fenretinide is a synthetic retinoid derivative without any remarkable toxicity [90]. *In vitro* studies showed that it has anti-tumor effect mainly *via* pro-apoptotic mechanisms and extensive generation of reactive oxygen species (ROS) [91], besides its potential to suppress the pro-tumor M2-phenotype of TAM [92]. Despite of all the previously mentioned antitumor mechanisms of both drugs, their application to manage glioma *in vivo* have been limited due to their weak passage across the BBB and uptake by brain tissue [87]. LF was selected to be the nanocarrier in this study because it is well recognized by low-density lipoprotein receptor-related protein 1 (LRP-1) which is highly expressed on both tumor vascular endothelial cells and glioma cells, besides the ability of LF-based nanocarriers to cross the BBB [93]. After penetrating the BBB, it is important to enhance intra-brain penetration to increase drug delivery. In order to fulfill this, a cell-penetrating peptide TAT was attached to D-α-tocopheryl polyethylene glycol succinate (TPGS) to form an amphiphilic structure (TPGS-TAT), which was further attached to LF nanoparticles to increase their penetrating potential [87]. Dual drug-loaded TPGS-TAT LF nanoparticles demonstrated EE% of about 52.8% for fenretinide and 35.3% in case of simvastatin with particle size in the range of 177 nm. Moreover, they exhibited good physical stability with slight increase in their particle size over the period of 24 h with a sustained release profile [87]. In addition, dual loaded-TPGS-TAT-LF nanoparticles exhibited inhibitory effect on U87 glioma cell line mainly *via* suppressing Ras/Raf/p-Erk signaling pathway in comparison to either free drug combination or dual loaded-LF nanoparticles without decoration with cell penetrating peptide. More importantly, dual loaded-TPGS-TAT-LF nanoparticles after intravenous administration showed enhanced anti-glioma effect in both orthotopic and subcutaneous glioma animal models in comparison to free drugs combination or undecorated dual loaded-LF nanoparticles due to TAM repolarization from the M2 (pro-tumor) phenotype to M1 (antitumor) phenotype [87].

In a similar recent approach, LF nanoparticles were prepared by green thermal denaturation method, but this time they were co-encapsulated with shikonin (SHK) and JQ [94]. SHK is a natural hydrophobic naphthoquinone which is the major active constituent isolated from the dried root of the Chinese herb (*Lithospermum erythrorhizon*). It has multiple pharmacological activities including; generation of ROS, inhibition of pyruvate kinase-M2 (PKM2) and NF-κB-regulated downstream signaling molecules [95]. Moreover, SHK can stimulate immunogenic cell death (ICD) *via* triggering antitumor immunity and regulating tumor immune microenvironment (TIME) [96]. It can also repress glucose metabolism in cancerous cells, which might limit tumor proliferation [97]. JQ1 is a bromodomain and extra-terminal motif (BET) inhibitor that can decrease the expression of programmed death-ligand 1(PD-L1) on tumor cells, tumor-associated dendritic cells (DC) and TAM, which might help cytotoxic T lymphocytes (CTL) to perform their function [98]. Combinational therapy of SHK and JQ1 is thought to consolidate the ability of immune normalization as a new strategy which is preferred over immune enhancement strategy that sometimes may cause some immune-related adverse effects [99]. Moreover, some previous studies reported that mannose receptors (MR) are highly expressed on both colon cancerous cells and TAM2 [100]. Consequently, mannosylated (SHK/JQ) loaded-LF nanoparticles were fabricated to dual target both cancer cells and TAM by MR (*via* mannose) and LRP-1 (*via* LF). Mannosylated (SHK/JQ) loaded-LF nanoparticles exhibited particle size in the range of 150 nm with EE % of 48.58 and 85.76% for SHK and JQ1, respectively. Moreover, these nanoparticles demonstrated good physical stability when incubated for 24 h in PBS, pH 7.4 supplemented with 10% goat serum, besides a sustained *in vitro* drug release profile [94]. *In vitro* and *in vivo* experiments revealed that mannosylated (SHK/JQ) loaded-LF nanoparticles exhibited a superior antitumor effect in comparison to free drug combination of dual drug-loaded LF nanoparticles. This enhanced effect could be attributed to enhanced ability of dual active targeted nanoformulation to simultaneously affect diverse integrated molecules in the immune microenvironment, which further confirmed that inhibiting tumor metabolism (through inhibiting glucose metabolism and lactate production) and modulating immunity (through TAM repolarization) might be a potential therapeutic strategy with better outcomes (Table 3) [94].

3.2. Lactoferrin-based nanocomplexes

Lactoferrin can also be involved in the fabrication of polyelectrolyte complexes (PECs) nanocarriers. Synthesis of this class of nanocarreris is based on utilization of 2 oppositely charged molecules such as positively charged proteins and negatively charged natural polysaccharides. Polyelectrolyte complexes-based nanocarriers are likely to be stabilized by the strong electrostatic interaction between cationic proteins and anionic polysaccharides. This stabilization may also enhance the stability of the encapsulated active components. In this study, polyelectrolyte complex nanoparticles were synthesized by electrostatic interaction between heat-denatured positively charged LF and negatively charged pectin. They were mixed at mass ration (1:1) and concentration 1 mg/mL at pH 4.5. Curcumin was then encapsulated into this nanocomplex platform probably *via* binding to the hydrophobic pouches located in the denatured protein and they were stabilized by hydrogen bond and vander Waals interactions [101]. Prepared nanocomplex encapsulating curcumin demonstrated particle size in the range of 208 nm with EE% of about 85%. Cumulative curcumin release from LF/pectin nanocomplex was 67.18% and 78.36% at pH 7.4 and 4.5, respectively indicating a faster *in vitro* drug release at acidic pH which might be attributed to decreased electrostatic interaction between LF and pectin. In addition, there was a notable enhanced *in vitro*

Table 3
LF nanoparticles prepared by thermal denaturation method.

Encapsulated drug(s)	Route of administration	Key features	References
$FeCl_3$	–	Good stability at 4–60° and pH 2–11 for 10 weeks.	[83]
Cichoric acid	–	Loaded LF nanoparticles exhibited better *in vitro* antioxidant potential compared to free form.	[86]
Simvastatin/fenretinide	Intravenous	Dual-loaded LF nanoparticles attached to a cell-penetrating peptide (TAT) exhibited enhanced anti-glioma effect in both orthotopic and subcutaneous glioma animal models with repolarization from M2 to M1 phenotype.	[87]
Shikonin/JQ1	Intravenous	Mannosylated dual loaded-LF nanoparticles exhibited *in vivo* a superior antitumor effect compared to free drugs combination of naked dual drug-loaded LF nanoparticles.	[94]

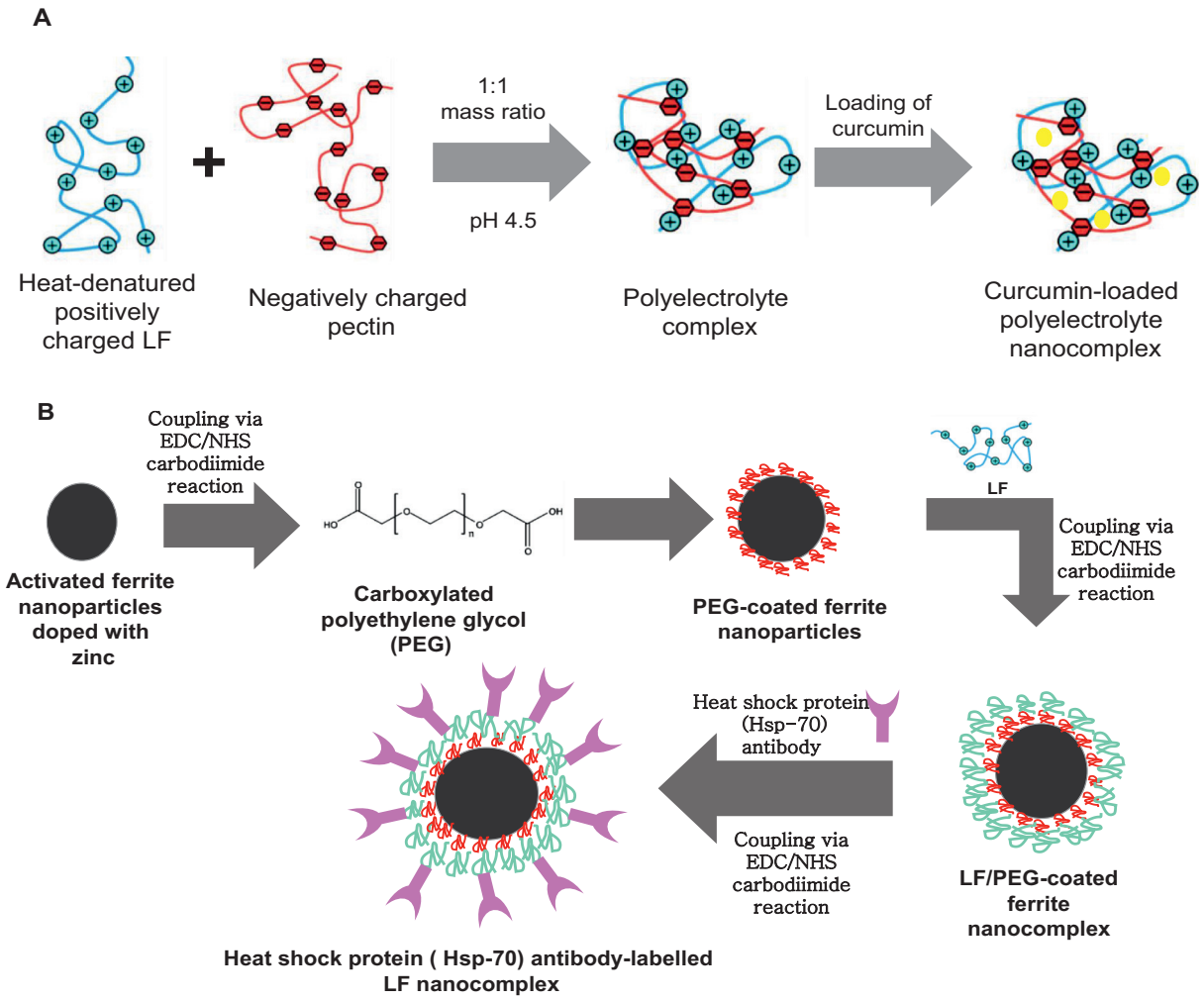

Fig. 5. A schematic illustration showing preparation of LF-based polyelectrolyte nanocomplex (A) and LF-based nanocomplex as a magnetic resonance imaging (MRI) contrast agent (B).

antioxidant activity in comparison to free curcumin. The ease of this preparation might suggest their feasibility as a food grade delivery system (Fig. 5A) [101].

LF was also involved in the synthesis of nanocomplex-based magnetic resonance imaging (MRI) contrast agents [102]. Firstly, ferrite nanoparticles doped with zinc (ZF) of particle size in the range of 24 nm were fabricated using chemical hydrothermal method in order to utilize them as MRI contrast agent. Zinc was selected as the dopant in this formulation owing to its ability to enhance the magnetic property of the prepared nanoparticles *via* increasing their saturation magnetization (108.4 emu/g) compared to ferrite nanoparticles without zinc. Secondly, Spherical nanocomplexes were fabricated *via* EDC/NHS carbodiimide conjugation reaction between LF and activated ZF nanoparticles. Finally, LF-ZF nanocomplexes were reactivated once again with EDC/NHS conjugation reaction to covalently attach heat shock protein antibody (Hsp-70) to it. Synthesis of Hsp-70 (LF-PEG-ZF) was accomplished by the same way except that carboxylated polyethylene glycol (PEG) was conjugated to ZF nanoparticles before its conjugation to LF (Fig. 5B) and their hydrodynamic particle size was found to be 224.8 nm with PdI of about 0.398 [102]. Fabricated nanocomplexes were labelled with Hsp-70 antibodies in order to enhance their targeting to atherosclerotic inflammatory site. LF-ZF represented the inside core of synthesized nanocomplexes, in which ZF nanoparticles were utilized as MRI contrast agent and LF was involved in the fabrication of this nanoplatform because of its known targeting and therapeutic potential against atherosclerosis, besides its anti-inflammatory and immunomodulatory effects *via* activation of T lymphocytes [103]. In addition, PEG was implicated in the synthesis of Hsp-70 (LF-PEG-ZF) in order to form stable hydrophilic nanocarriers with minimum non-specific protein binding that might cause aggregation upon *in vivo* injection [104]. Moreover, Hsps' are temporary expressed in atherosclerotic sites, thus Hsp-labelled nanocarriers could be a good choice for early

Table 4
LF-based nanocomplexes.

Encapsulated drug(s)	Composition of the nanocomplex	Route of administration	Key features	References
Curcumin	heat-denatured LF and pectin depending on electrostatic interaction	–	High encapsulation efficiency with enhanced *in vitro* antioxidant activity compared to free drug.	[101]
Ferrite magnetic nanoparticles doped with zinc	LF and heat shock protein antibody (Hsp-70) conjugated to PEG- magnetic core *via* EDC/NHS reaction	Intravenous	Enhanced *in vivo* MRI and CT contrast upon injection of the nanocomplex suggesting its use in early diagnosis of atherosclerosis	[102]

diagnosis of atherosclerosis [105]. *In vitro* cellular uptake studies revealed that fabricated nanocomplexes demonstrated enhanced cellular uptake after 2 h in both human monocytic cell line (THP-1) and immortalized human T lymphocyte Jurkat cells. Interestingly, *ex vivo* results in *Psammomys obesus* rat model showed that MRI and computed tomography (CT) contrast scans of animals injected with nanocmplexes displayed higher T2 (1.7 fold) contrast compared to control animals. Histological investigation indicated that there was a significant accumulation of Hsp-70 labelled nanocomplexes at the atherosclerotic sites, which suggested that these nanocomplexes could be involved in the early diagnosis and treatment of atherosclerosis and other cardiovascular disorders (Table 4) [102].

3.3. Lactoferrin-based micelles

Micelles are a group of molecules composed of hydrophobic and hydrophilic segments. They can easily self-assemble in aqueous media with the hydrophobic core directed inside and the hydrophilic corona directed towards the outer aqueous medium. Micelles have been widely used in drug delivery due to their unique features such as low critical micelle concentration (CMC), enhanced physical stability and prolonged circulation in comparison to other types of nanocarriers. Moreover, micelles are easily fabricated with a potential to synthesize diverse core/shell structures [106]. Many surfactants and synthetic *co*-polymers are known to form micelles, but these micelles are usually toxic and unstable at physiological conditions [107]. As an alternative, natural proteins such as lactoferrin could be utilized instead of these synthetic polymers to fabricate micelles because of their known low immunogenicity, high biocompatibility and possibility of active targeting [108].

An amphiphilic co-polymer was fabricated from LF and zein (corn protein) using EDC/NHS carbodiimide reaction [19]. This amphiphilic co-polymer was found to form micelles at low CMC of about 0.01 mg/mL, associated with enhanced physical stability. Afterwards, rapamycin and wogonin were physically entrapped into these micelles, followed by crosslinking with 8% glutaraldehyde. Results revealed that the dual-loaded crosslinked micelles exhibited hydrodynamic particle size of about 212 nm with increased *in vitro* cytotoxicity on MCF-7 human breast cancer cell line. In addition, cellular uptake was also induced due to the active targeting capability of LF *via* its binding to LF receptors which are highly expressed on the surface of cancerous cells [18]. Moreover, *in vivo* anticancer efficacy study in Ehrlich ascites tumor animal model revealed a superior effect for these dual-loaded crosslinked micelles in terms of suppression of tumor volume, reduction in CD1, VEGF, MAPK and p-AKT expression levels [19]. In another approach, the same micelles were used to develop dasatinib-loaded magnetic micelles [109]. First, magnetite (Fe_3O_4) nanoparticles were fabricated using co-precipitation method, then these magnetic nanoparticles were coated with oleic acid to minimize their high tendency to aggregate. Dasatinib and oleic acid-coated magnetic nanoparticles were then co-encapsulated into zein hydrophobic core by evaporation/sonication method. The newly developed nanoplatform exhibited good superparamagnetic property with enhanced *in vitro* serum stability and hemocmpatability. Moreover, these magnetic micelles displayed enhanced *in vitro* cytotoxicity on MDA-MB-231 human breast cancer cell line in the presence of an external magnetic field with reduced *in vitro* cellular migration and p-c-Src protein expression level and in the presence of an external magnetic field due to dual targeting property of magnetic NPs and LF hydrophilic shell [109]. In a combined nano-in-microparticle approach, zein-LF micelles encapsulating dasatinib were further encapsulated in sodium alginate spheroids *via* an on-chip microfluidic method. Morphological investigations revealed successful encapsulation of the micelles within alginate spheroids. In addition, this fabricated drug delivery system displayed a sustained drug release with improved *in vitro* cytotoxic effect against MDA-MB-231human breast cancer cell line, suggesting the potential of utilizing nano-in-microparticles as efficient drug delivery systems [110].

In a recent study, LF was utilized to develop new amphiphilic micelles to be used as actively targeted micelles to alleviate or treat Alzheimer's disease. LF was covalently attached to conjugated linoleic acid (CLA) by EDC coupling reaction, then CLA was loaded into the hydrophobic core of the micelles by using solvent evaporation method in order to enhance the delivery of CLA to the CNS [111]. Fabricated CLA-based micelles exhibited particle size of about 53 nm with sustained *in vitro* drug release at pH 6.8 and enhanced hemocompatibility [111]. In addition, *in vivo* studies revealed better biodistribution of rhodamine-labelled micelles in the brain compared to free dye, which confirmed the better ability of these micelles to cross the BBB. Moreover, drug-loaded LF-CLA micelles demonstrated improved cognitive abilities with reduced oxidative stress, inflammation and apoptosis in the brain tissue of aluminum chloride Alzheimer's-induced animal model. These results suggested that LF-CLA micelles could serve as a promising CNS drug delivery system (Table 5) [111].

3.4. Nanocarriers to encapsulate lactoferrin

It is thought that nanoencapsulation of active molecules into nanocarriers could enhance their efficacy. In this study, biocompatible constituents were utilized to fabricate a nanosystem to encapsulate iron saturated bovine LF. In this light, a nanoplatform was developed to encapsulate LF, in which calcium phosphate nanocores were used to adsorb either LF or paclitaxel (Taxol®) onto their surfaces *via* electrostatic interaction, then these nanocores were coated with chitosan. Afterwards, these nanocarriers were further encapsulated in alginate gel to protect this nanoplatform from degradation in the GI tract. When this formulation was supplemented orally in both treatment and prevention human colon cancer xenograft animal model, it showed significant enhanced efficiency in either treatment or prevention of colon cancer in comparison to paclitaxel. The results of this study suggested that nanocarriers loaded with LF may contribute in the treatment or prevention of human colon cancer [112]. In another attempt, LF was

Table 5
LF-based micelles.

Encapsulated drug(s)	Composition of the micelles	Route of administration	Key features	References
Rapamycin/wogonin	LF and zein conjugated by EDC/NHS coupling reaction	Intravenous	Enhanced anticancer effect in Ehrlich ascites tumor animal model.	[19]
Dasatininb/oleic acid-coated magnetite nanoparticles	LF and zein conjugated by EDC/NHS coupling reaction	–	Improved *in vitro* cytotoxicity on MDA-MB-231 human breast cancer cells associated with reduced cellular migration in the presence of an external magnetic field.	[109]
Dasatinib	LF and zein conjugated by EDC/NHS coupling reaction, encapsulated in alginate spheroids	–	sustained drug release with increased *in vitro* cytotoxic effect against MDA-MB-231human breast cancer cell line	[110]
Conjugated linoleic acid	LF and Conjugated linoleic acid	Oral	Enhanced *in vivo* biodistrbution in the brain tissue associated with improved anti-Alzheimer effect	[111]

encapsulated into lipid nanocarriers by employing water-in-oil-in water (W/O/W) emulsion technique, in which, LF was dissolved in the inner aqueous phase, then it was dispersed in melted middle Softisan lipid with homogenization at 8000 rpm for 10 min, then final W/O/W emulsion was obtained *via* subsequent homogenization of W/O dispersion in Lutrol F68™ (poloxamer 188) or Lutrol F127 ™ [113]. This method of emulsion preparation is preferred over simple oil-in-water emulsion especially for encapsulation of active molecules because they can encapsulate both hydrophobic and hydrophilic molecules, besides their enhanced ability in controlling the release profile of encapsulated active components [114]. Results revealed that presence of electrolyte with low ionic strength and utilization of low concentration of LF yielded the most stable nanoformulation with particle size from 100 to 200 nm and zeta potential of about −14.6 mV. Moreover, Optimized formulation encapsulating LF exhibited a comparable antimicrobial effect compared to free LF, suggesting that it could be applied in the preparation of elixirs for oral hygiene without any harmful side effects [113].

4. Conclusion and perspectives

Nowadays, utilization of nutraceuticals is emerging as a promising strategy for diverse biomedical applications because of their relative abundance, safety, ease of administration and biocompatibility. There was a great interest in exploring the possibility of utilizing LF for different medical purposes especially in the field of nanomedicine. LF is also a potent anti-viral, immune modulatory and an anti-cancer alternative with integrated inhibitory pathways similar to the effect of chemotherapeutic drugs, but with no side effects. As a result, it could be administrated in combination with conventional therapies so as to minimize the administrated doses and enhance their therapeutic efficacy. In addition, fabrication of LF-based nanocarriers was proven to extensively enhance the therapeutic potential of the encapsulated active molecules. In this review, we have discussed the most recent methods of preparation of LF-based nanocarriers including; nanoparticles, nanocomplexes and polymeric micelles. Moreover, different biomedical applications of these nanocarriers were also highlighted.

In general, all methods utilized for preparation of LF nanoparitcles have advantages over other types of nanoparticles as their method of preparation is non-destructive and no corsslinkers such as glutaraldehyde are included in the procedure which may further affect their *in vivo* safety [115].

LF nanoparticles are prepared by sol-oil method, NAB-technology or thermal denaturation method. Sol-oil method is the most commonly used method to prepare LF nanoparticles. In this method, LF protein and active components to be encapsulated are dissolved together in a suitable aqueous medium, then mixed with olive oil, emulsified *via* homogenization, snap frozen in liquid nitrogen, then thawed on ice for 4 h and finally prepared nanoparticles are washed with cold diethyl ether to get rid of the oil [61]. It is though that preparation of LF nanoparticles without using any chemical harsh treatments may preserve its native conformation which is a critical issue for efficient receptor recognition [56]. Moreover, it doesn't include any heating steps or chemical modifications either for the drug or even the protein itself, which is not the case in other preparation methods such as protein coacervation method [116]. Most solid state characterizations confirmed that LF nanoparticles prepared by this method restored the conformation of the natural native protein, besides being highly reproducible regarding particle size and entrapment efficiency as reported earlier [20,59]. NAB-technology is a well-known method to prepare protein nanoparticles depending mainly on the presence of drug in water immiscible solvents which is slowly added to the aqueous phase containing LF. An emulsion is formed by homogenization and sonication, then final formulation could be obtained by removing remaining organic solvent [75]. Preparation of optimized drug-loaded protein nanoparticles by this method was found to be greatly dependent on the ratio between the drug and the utilized protein, pH of the preparation, power and duration of homogenization [79]. Regarding thermal denaturation method, it starts with a heating step at 70–80 °C for 15–20 min in an oil bath to denature the protein, followed by dropwise addition of drug solution and finally leaving this mixture to cool under continuous stirring at room temperature to allow formation of drug-loaded LF nanoparticles [87,94].

Taken all together, sol-oil method is reproducible and suitable to encapsulate both hydrophobic and hydrophilic drugs, but it includes a 4 h thawing step and washing steps with diethyl ether which may relatively consume time in comparison to other methods of preparation. In case of NAB-technology method, only drugs soluble in immiscible organic solvents could be utilized, so this method will not be suitable to encapsulate hydrophilic drugs or drugs soluble in water miscible organic solvents. Although thermal denaturation method is rapid and simple, there are many parameters that were found to extensively affect the particle size and the stability of the prepared nanoparticles including; concentration of the protein, pH of the reaction, temperature of denaturation and duration of heating. In order to prepare an optimized reproducible formulation, all the previous parameters must be studied and controlled all at once, which will be very difficult in a scaling-up approach for industrial production.

LF nanoparticles prepared by all the above mentioned methods have common advantages including; their particle size are in the range of 50–70 nm which can avoid renal clearance, and hence will exert extended blood circulation leading at the end to enhanced therapeutic index [117]. It was also notable that most of the prepared drug-loaded LF nanoparticles exhibited their maximum drug release at pH 5, which might be attributed to change in the orientation of LF molecules leading to the release of encapsulated drug present in the inner cavities of the protein [60]. In the future, it is expected to examine the clinical potential of LF combinational therapy in the treatment of various caner types [118]. Moreover, LF-based nanocarriers were proven to be easily prepared by simple methods with superior active targeting potential towards tumor tissue, especially brain tumors. Additionally, administration of LF-based nanocarriers encapsulating hydrophobic drug(s) could be achieved by multiple routes including; oral, nasal, intravenous and even vaginal route with great potential to replace many available conventional formulations including organic solvents or surfactants because of its safety and better therapeutical outcomes.

References

[1] J.M. Steijns, A. Van Hooijdonk, Occurrence, structure, biochemical properties and technological characteristics of lactoferrin, Br. J. Nutr. 84 (S1) (2000) 11–17.
[2] P.F. Levay, M. Viljoen, Lactoferrin: a general review, Haematologica 80 (3) (1995) 252–267.
[3] M.C. Harmsen, P.J. Swart, M.-P.d. Béthune, R. Pauwels, E.D. Clercq, T.B. The, D.K. Meijer, Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vitro, J. Infect. Dis. 172 (2) (1995) 380–388.
[4] B. Lönnerdal, S. Iyer, Lactoferrin: molecular structure and biological function, Annu. Rev. Nutr. 15 (1) (1995) 93–110.
[5] D. Legrand, A. Pierce, E. Elass, M. Carpentier, C. Mariller, J. Mazurier, Lactoferrin structure and functions, Bioactive Components of Milk, Springer 2008, pp. 163–194.
[6] R. Reghunathan, M. Jayapal, L.-Y. Hsu, H.-H. Chng, D. Tai, B.P. Leung, A.J. Melendez, Expression profile of immune response genes in patients with severe acute respiratory syndrome, BMC Immunol. 6 (1) (2005) 2.
[7] S. Buderus, J. Boone, D. Lyerly, M.J. Lentze, Fecal lactoferrin: a new parameter to monitor infliximab therapy, Dig. Dis. Sci. 49 (6) (2004) 1036–1039.
[8] S. Kane, W. Sandborn, P. Rufo, A. Zholudev, J. Boone, D. Lyerly, M. Camilleri, S. Hanauer, Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation, Am. J. Gastroenterol. 98 (6) (2003) 1309–1314.
[9] B.F. Anderson, H.M. Baker, G.E. Norris, D.W. Rice, E.N. Baker, Structure of human lactoferrin: crystallographic structure analysis and refinement at 2·8 Å resolution, J. Mol. Biol. 209 (4) (1989) 711–734.
[10] H. Kawakami, M. Tanaka, K. Tatsumi, S.i. Dosako, Effects of ionic strength and pH on the thermostability of lactoferrin, Int. Dairy J. 2 (5) (1992) 287–298.
[11] G. Brisson, M. Britten, Y. Pouliot, Heat-induced aggregation of bovine lactoferrin at neutral pH: effect of iron saturation, Int. Dairy J. 17 (6) (2007) 617–624.
[12] E. Blanco, T. Sangai, S. Wu, A. Hsiao, G.U. Ruiz-Esparza, C.A. Gonzalez-Delgado, F.E. Cara, S. Granados-Principal, K.W. Evans, A. Akcakanat, Colocalized delivery of rapamycin and paclitaxel to tumors enhances synergistic targeting of the PI3K/Akt/mTOR pathway, Mol. Ther. 22 (7) (2014) 1310–1319.

[13] K. Saravanakumar, X. Hu, D.M. Ali, M.-H. Wang, Emerging strategies in stimuli-responsive nanocarriers as the drug delivery system for enhanced cancer therapy, Curr. Pharm. Des. 25 (24) (2019) 2609–2625.
[14] H. Rostamabadi, S.R. Falsafi, S.M. Jafari, Starch-based nanocarriers as cutting-edge natural cargos for nutraceutical delivery, Trends Food Sci. Technol. 88 (2019) 397–415.
[15] M.C. Wahlgren, T. Arnebrant, M.A. Paulsson, The adsorption from solutions of β-lactoglobulin mixed with lactoferrin or lysozyme onto silica and methylated silica surfaces, J. Colloid Interface Sci. 158 (1) (1993) 46–53.
[16] S. Gonzalez-Chavez, Lactoferrin: structure, function and applications, Int. J. Antimicrob. Agents 33 (2009) 301–308.
[17] R. Brines, J. Brock, The effect of trypsin and chymotrypsin on the in vitro antimicrobial and iron-binding properties of lactoferrin in human milk and bovine colostrum: unusual resistance of human apolactoferrin to proteolytic digestion, Biochimica et Biophysica Acta (BBA)-General Subjects 759 (3) (1983) 229–235.
[18] K. Golla, C. Bhaskar, F. Ahmed, A.K. Kondapi, A target-specific oral formulation of doxorubicin-protein nanoparticles: efficacy and safety in hepatocellular cancer, J. Cancer 4 (8) (2013) 644–652.
[19] S.A. Sabra, A.O. Elzoghby, S.A. Sheweita, M. Haroun, M.W. Helmy, M.A. Eldemellawy, Y. Xia, D. Goodale, A.L. Allan, S. Rohani, Self-assembled amphiphilic zein-lactoferrin micelles for tumor targeted co-delivery of rapamycin and wogonin to breast cancer, Eur. J. Pharm. Biopharm. 128 (2018) 156–169.
[20] S. Kumari, S.M. Ahsan, J.M. Kumar, A.K. Kondapi, N.M. Rao, Overcoming blood brain barrier with a dual purpose Temozolomide loaded Lactoferrin nanoparticles for combating glioma (SERP-17-12433), Sci. Rep. 7 (1) (2017) 1–13.
[21] P. Valenti, G. Antonini, Lactoferrin, Cell. Mol. Life Sci. 62 (22) (2005) 2576.
[22] P. Rossi, F. Giansanti, A. Boffi, M. Ajello, P. Valenti, E. Chiancone, G. Antonini, Ca2+ binding to bovine lactoferrin enhances protein stability and influences the release of bacterial lipopolysaccharide, Biochem. Cell Biol. 80 (1) (2002) 41–48.
[23] J.M. Ling, A.B. Schryvers, Perspectives on interactions between lactoferrin and bacteria, Biochem. Cell Biol. 84 (3) (2006) 275–281.
[24] E.J. Dial, L.M. Lichtenberger, Effect of lactoferrin on Helicobacter felis induced gastritis, Biochem. Cell Biol. 80 (1) (2002) 113–117.
[25] T.J. Williams, R.P. Schneider, M.D. Willcox, The effect of protein-coated contact lenses on the adhesion and viability of gram negative bacteria, Curr. Eye Res. 27 (4) (2003) 227–235.
[26] H.F. Gomez, T.J. Ochoa, L.G. Carlin, T.G. Cleary, Human lactoferrin impairs virulence of Shigella flexneri, J. Infect. Dis. 187 (1) (2003) 87–95.
[27] K. Shimizu, H. Matsuzawa, K. Okada, S. Tazume, S. Dosako, Y. Kawasaki, K. Hashimoto, Y. Koga, Lactoferrin-mediated protection of the host from murine cytomegalovirus infection by a T-cell-dependent augmentation of natural killer cell activity, Arch. Virol. 141 (10) (1996) 1875–1889.
[28] A. Pietrantoni, A.M. Di Biase, A. Tinari, M. Marchetti, P. Valenti, L. Seganti, F. Superti, Bovine lactoferrin inhibits adenovirus infection by interacting with viral structural polypeptides, Antimicrob. Agents Chemother. 47 (8) (2003) 2688–2691.
[29] K. Hara, M. Ikeda, S. Saito, S. Matsumoto, K. Numata, N. Kato, K. Tanaka, H. Sekihara, Lactoferrin inhibits hepatitis B virus infection in cultured human hepatocytes, Hepatol. Res. 24 (3) (2002) 228–235.
[30] K. Yamauchi, H. Wakabayashi, K. Shin, M. Takase, Bovine lactoferrin: benefits and mechanism of action against infections, Biochem. Cell Biol. 84 (3) (2006) 291–296.
[31] B.E. Britigan, T.S. Lewis, M. Waldschmidt, M.L. McCormick, A.M. Krieg, Lactoferrin binds CpG-containing oligonucleotides and inhibits their immunostimulatory effects on human B cells, J. Immunol. 167 (5) (2001) 2921–2928.
[32] D. Legrand, E. Elass, M. Carpentier, J. Mazurier, Lactoferrin, Cell. Mol. Life Sci. 62 (22) (2005) 2549.
[33] S. He, A.R. McEuen, S.A. Blewett, P. Li, M.G. Buckley, P. Leufkens, A.F. Walls, The inhibition of mast cell activation by neutrophil lactoferrin: uptake by mast cells and interaction with tryptase, chymase and cathepsin G, Biochem. Pharmacol. 65 (6) (2003) 1007–1015.
[34] M. Konishi, M. Iwasa, K. Yamauchi, R. Sugimoto, N. Fujita, Y. Kobayashi, S. Watanabe, S. Teraguchi, Y. Adachi, M. Kaito, Lactoferrin inhibits lipid peroxidation in patients with chronic hepatitis C, Hepatol. Res. 36 (1) (2006) 27–32.
[35] R.M. Sfeir, M. Dubarry, P.N. Boyaka, M. Rautureau, D. Tome, The mode of oral bovine lactoferrin administration influences mucosal and systemic immune responses in mice, J. Nutr. 134 (2) (2004) 403–409.
[36] G. Chodaczek, M. Zimecki, J. Lukasiewicz, C. Lugowski, A complex of lactoferrin with monophosphoryl lipid A is an efficient adjuvant of the humoral and cellular immune response in mice, Med. Microbiol. Immunol. 195 (4) (2006) 207–216.
[37] R. Fischer, H. Debbabi, M. Dubarry, P. Boyaka, D. Tome, Regulation of physiological and pathological Th1 and Th2 responses by lactoferrin, Biochem. Cell Biol. 84 (3) (2006) 303–311.
[38] I. Dhennin-Duthille, M. Masson, E. Damiens, C. Fillebeen, G. Spik, J. Mazurier, Lactoferrin upregulates the expression of CD4 antigen through the stimulation of the mitogen-activated protein kinase in the human lymphoblastic T Jurkat cell line, J. Cell. Biochem. 79 (4) (2000) 583–593.
[39] C.S. Curran, K.P. Demick, J.M. Mansfield, Lactoferrin activates macrophages via TLR4-dependent and-independent signaling pathways, Cell. Immunol. 242 (1) (2006) 23–30.
[40] K. Kai, K.i. Komine, Y. Komine, T. Kuroishi, T. Kozutsumi, J. Kobayashi, M. Ohta, H. Kitamura, K. Kumagai, Lactoferrin stimulates a Staphylococcus aureus killing activity of bovine phagocytes in the mammary gland, Microbiol. Immunol. 46 (3) (2002) 187–194.
[41] H. Tsuda, Y. Ohshima, H. Nomoto, K.-I. Fujita, E. Matsuda, M. Iigo, N. Takasuka, M.A. Moore, Cancer prevention by natural compounds, Drug metabolism and pharmacokinetics 19 (4) (2004) 245–263.
[42] J.P. Guedes, C.S. Pereira, L.R. Rodrigues, M. Côrte-Real, Bovine milk lactoferrin selectively kills highly metastatic prostate cancer PC-3 and osteosarcoma MG-63 cells in vitro, Front. Oncol. 8 (2018) 200.
[43] K.V.P.C. Mohan, H. Devaraj, D. Prathiba, Y. Hara, S. Nagini, Antiproliferative and apoptosis inducing effect of lactoferrin and black tea polyphenol combination on hamster buccal pouch carcinogenesis, Biochimica et Biophysica Acta (BBA)-General Subjects 1760 (10) (2006) 1536–1544.
[44] T.-Y. Lin, S.-H. Chiou, M. Chen, C.-D. Kuo, Human lactoferrin exerts bi-directional actions on PC12 cell survival via ERK1/2 pathway, Biochem. Biophys. Res. Commun. 337 (1) (2005) 330–336.
[45] J.S. Mader, J. Salsman, D.M. Conrad, D.W. Hoskin, Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines, Mol. Cancer Ther. 4 (4) (2005) 612–624.
[46] T. Sakai, Y. Banno, Y. Kato, Y. Nozawa, M. Kawaguchi, Pepsin-digested bovine lactoferrin induces apoptotic cell death with JNK/SAPK activation in oral cancer cells, J. Pharmacol. Sci. 98 (1) (2005) 41–48.
[47] E. Damiens, I. El Yazidi, J. Mazurier, I. Duthille, G. Spik, Y. Boilly-Marer, Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells, J. Cell. Biochem. 74 (3) (1999) 486–498.
[48] Y. Xiao, C.L. Monitto, K.M. Minhas, D. Sidransky, Lactoferrin down-regulates G1 cyclin-dependent kinases during growth arrest of head and neck cancer cells, Clin. Cancer Res. 10 (24) (2004) 8683–8686.
[49] S. Oh, C. Pyo, Y. Kim, S. Choi, Neutrophil lactoferrin upregulates the human p53 gene through induction of NF-kappaB activation cascade, Oncogene 23 (50) (2004) 8282–8291.
[50] C. Zhang, F. Zhang, Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities, Protein & cell 6 (2) (2015) 88–100.
[51] C. Mariller, M. Benaïssa, S. Hardivillé, M. Breton, G. Pradelle, J. Mazurier, A. Pierce, Human delta-lactoferrin is a transcription factor that enhances Skp1 (S-phase kinase-associated protein) gene expression, FEBS J. 274 (8) (2007) 2038–2053.
[52] C. McGuire, K. Cotter, L. Stransky, M. Forgac, Regulation of V-ATPase assembly and function of V-ATPases in tumor cell invasiveness, Biochimica et Biophysica Acta (BBA)-Bioenergetics 1857 (8) (2016) 1213–1218.
[53] M.A. Farziyan, F. Moradian, A.R. Rafiei, Anticancer effect of bovine lactoferrin on human esophagus cancer cell line, Research in Molecular Medicine 4 (1) (2016) 18–23.
[54] Y.-T. Tung, H.-L. Chen, C.-C. Yen, P.-Y. Lee, H.-C. Tsai, M.-F. Lin, C.-M. Chen, Bovine lactoferrin inhibits lung cancer growth through suppression of both inflammation and expression of vascular endothelial growth factor, J. Dairy Sci. 96 (4) (2013) 2095–2106.
[55] A. Arcella, M.A. Oliva, S. Staffieri, S. Aalberti, G. Grillea, M. Madonna, M. Bartolo, L. Pavone, F. Giangaspero, G. Cantore, In vitro and in vivo effect of human lactoferrin on glioblastoma growth, J. Neurosurg. 123 (4) (2015) 1026–1035.
[56] S. Kumari, D. Bhattacharya, N. Rangaraj, S. Chakarvarty, A.K. Kondapi, N.M. Rao, Aurora kinase B siRNA-loaded lactoferrin nanoparticles potentiate the efficacy of temozolomide in treating glioblastoma, Nanomedicine 13 (20) (2018) 2579–2596.
[57] K. Golla, B. Cherukuvada, F. Ahmed, A.K. Kondapi, Efficacy, safety and anticancer activity of protein nanoparticle-based delivery of doxorubicin through intravenous administration in rats, PLoS One 7 (12) (2012).
[58] K. Golla, F.A. Cherukuvada Bhaskar, A.K. Kondapi, A target-specific oral formulation of doxorubicin-protein nanoparticles: efficacy and safety in hepato-cellular cancer, J. Cancer 4 (8) (2013) 644–652.
[59] P. Kumar, Y.S. Lakshmi, C. Bhaskar, K. Golla, A.K. Kondapi, Improved safety, bioavailability and pharmacokinetics of zidovudine through lactoferrin nanoparticles during oral administration in rats, PLoS One 10 (10) (2015).
[60] P. Kumar, Y. Lakshmi, A. Kondapi, An oral formulation of efavirenz-loaded lactoferrin nanoparticles with improved biodistribution and pharmacokinetic profile, HIV medicine 18 (7) (2017) 452–462.
[61] Y.S. Lakshmi, P. Kumar, G. Kishore, C. Bhaskar, A.K. Kondapi, Triple combination MPT vaginal microbicide using curcumin and efavirenz loaded lactoferrin nanoparticles, Sci. Rep. 6 (1) (2016) 1–13.
[62] L. Madugulla, A.R. Ravula, A.K. Kondapi, S. Yenugu, Evaluation of the reproductive toxicity of antiretroviral drug loaded lactoferrin nanoparticles, Syst Biol Reprod Med 65 (3) (2019) 205–213.
[63] P. Kumar, Y.S. Lakshmi, A.K. Kondapi, Triple drug combination of zidovudine, efavirenz and lamivudine loaded lactoferrin nanoparticles: an effective nano first-line regimen for HIV therapy, Pharm. Res. 34 (2) (2017) 257–268.
[64] H. Athmakur, A.K. Kondapi, Carmustine loaded lactoferrin nanoparticles demonstrates an enhanced antiproliferative activity against glioblastoma in vitro, Int J App Pharm 10 (6) (2018) 234–241.
[65] S.A. Mallina, R. Sundararajan, Lenalidomide loaded lactoferrin nanoparticle for controlled delivery and enhanced therapeutic efficacy, Research Journal of Pharmacy and Technology 11 (9) (2018) 4010–4014.
[66] M.G. Adimoolam, A. Vijayalakshmi, M.R. Nalam, M.V. Sunkara, Chlorin e6 loaded lactoferrin nanoparticles for enhanced photodynamic therapy, J. Mater. Chem. B 5 (46) (2017) 9189–9196.
[67] F. Ahmed, S. Kumari, A.K. Kondapi, Evaluation of antiproliferative activity, safety and biodistribution of oxaliplatin and 5-fluorouracil loaded Lactoferrin nanoparticles for the management of colon adenocarcinoma: an in vitro and an in vivo study, Pharm. Res. 35 (9) (2018) 1–17.
[68] S. Kumari, A.K. Kondapi, Receptor-mediated targeted delivery of DNA using Lactoferrin nanoparticles, Int. J. Biol. Macromol. 108 (2018) 401–407.
[69] M. Elfinger, C. Maucksch, C. Rudolph, Characterization of lactoferrin as a targeting ligand for nonviral gene delivery to airway epithelial cells, Biomaterials 28 (23) (2007) 3448–3455.

[70] J. Senapathi, A. Bommakanti, S. Mallepalli, S. Mukhopadhyay, A.K. Kondapi, Sulfonate modified Lactoferrin nanoparticles as drug carriers with dual activity against HIV-1, Colloids Surf. B: Biointerfaces 191 (2020), 110979. .
[71] D. Baram-Pinto, S. Shukla, M. Richman, A. Gedanken, S. Rahimipour, R. Sarid, Surface-modified protein nanospheres as potential antiviral agents, Chem. Commun. 48 (67) (2012) 8359–8361.
[72] A. Albanese, P.S. Tang, W.C. Chan, The effect of nanoparticle size, shape, and surface chemistry on biological systems, Annu. Rev. Biomed. Eng. 14 (2012) 1–16.
[73] S. Honary, F. Zahir, Effect of zeta potential on the properties of nano-drug delivery systems-a review (part 1), Trop. J. Pharm. Res. 12 (2) (2013) 255–264.
[74] S.A. Moore, B.F. Anderson, C.R. Groom, M. Haridas, E.N. Baker, Three-dimensional structure of diferric bovine lactoferrin at 2.8 Å resolution, J. Mol. Biol. 274 (2) (1997) 222–236.
[75] Q. Fu, J. Sun, W. Zhang, X. Sui, Z. Yan, Z. He, Nanoparticle albumin-bound (NAB) technology is a promising method for anti-cancer drug delivery, Recent patents on anti-cancer drug discovery 4 (3) (2009) 262–272.
[76] N. Desai, V. Trieu, Z. Yao, L. Louie, S. Ci, A. Yang, C. Tao, T. De, B. Beals, D. Dykes, Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel, Clin. Cancer Res. 12 (4) (2006) 1317–1324.
[77] J. Cortes, C. Saura, Nanoparticle albumin-bound (nab™)-paclitaxel: improving efficacy and tolerability by targeted drug delivery in metastatic breast cancer, Eur. J. Cancer Suppl. 8 (1) (2010) 1–10.
[78] Z.-H. Zhang, X.-P. Wang, W.Y. Ayman, W.L. Munyendo, H.-X. Lv, J.-P. Zhou, Studies on lactoferrin nanoparticles of gambogic acid for oral delivery, Drug delivery 20 (2) (2013) 86–93.
[79] X. Xia, Preparation, characterization, and in vitro/vivo studies of oleanolic acid-loaded lactoferrin nanoparticles, Drug Design, Development and Therapy 11 (2017) 1417–1427.
[80] E.O. Fukushima, H. Seki, K. Ohyama, E. Ono, N. Umemoto, M. Mizutani, K. Saito, T. Muranaka, CYP716A subfamily members are multifunctional oxidases in triterpenoid biosynthesis, Plant Cell Physiol. 52 (12) (2011) 2050–2061.
[81] J.J. Patel, S.R. Acharya, N.S. Acharya, Clerodendrum serratum (L.) Moon.–A review on traditional uses, phytochemistry and pharmacological activities, J. Ethnopharmacol. 154 (2) (2014) 268–285.
[82] N.R. Parikh, A. Mandal, D. Bhatia, K.S. Siveen, G. Sethi, A. Bishayee, Oleanane triterpenoids in the prevention and therapy of breast cancer: current evidence and future perspectives, Phytochem. Rev. 13 (4) (2014) 793–810.
[83] J.T. Martins, S.F. Santos, A.I. Bourbon, A.C. Pinheiro, Á. González-Fernández, L.M. Pastrana, M.A. Cerqueira, A.A. Vicente, Lactoferrin-based nanoparticles as a vehicle for iron in food applications–development and release profile, Food Res. Int. 90 (2016) 16–24.
[84] G. Remondetto, P. Paquin, M. Subirade, Cold gelation of β-lactoglobulin in the presence of Iron, J. Food Sci. 67 (2) (2002) 586–595.
[85] C. Bengoechea, I. Peinado, D.J. McClements, Formation of protein nanoparticles by controlled heat treatment of lactoferrin: factors affecting particle characteristics, Food Hydrocoll. 25 (5) (2011) 1354–1360.
[86] J. Li, C. Zhao, L. Wei, X. Li, F. Liu, M. Zhang, X. Liu, Y. Wang, Preservation of cichoric acid antioxidant properties loaded in heat treated lactoferrin nanoparticles, Molecules 23 (10) (2018) 2678.
[87] X. Mo, Z. Zheng, Y. He, H. Zhong, X. Kang, M. Shi, T. Liu, Z. Jiao, Y. Huang, Antiglioma via regulating oxidative stress and remodeling tumor-associated macrophage using lactoferrin-mediated biomimetic codelivery of simvastatin/fenretinide, J. Control. Release 287 (2018) 12–23.
[88] M.C. Alupei, E. Licarete, L. Patras, M. Banciu, Liposomal simvastatin inhibits tumor growth via targeting tumor-associated macrophages-mediated oxidative stress, Cancer Lett. 356 (2) (2015) 946–952.
[89] E. Mira, L. Carmona-Rodríguez, M. Tardáguila, I. Azcoitia, A. González-Martín, L. Almonacid, J. Casas, G. Fabriás, S. Mañes, A lovastatin-elicited genetic program inhibits M2 macrophage polarization and enhances T cell infiltration into spontaneous mouse mammary tumors, Oncotarget 4 (12) (2013) 2288–2301.
[90] E. Ulukaya, E.J. Wood, Fenretinide and its relation to cancer, Cancer Treat. Rev. 25 (4) (1999) 229–235.
[91] V. Appierto, P. Tiberio, M.G. Villani, E. Cavadini, F. Formelli, PLAB induction in fenretinide-induced apoptosis of ovarian cancer cells occurs via a ROS-dependent mechanism involving ER stress and JNK activation, Carcinogenesis 30 (5) (2009) 824–831.
[92] T. Lin, P. Zhao, Y. Jiang, Y. Tang, H. Jin, Z. Pan, H. He, V.C. Yang, Y. Huang, Blood–brain-barrier-penetrating albumin nanoparticles for biomimetic drug delivery via albumin-binding protein pathways for antiglioma therapy, ACS Nano 10 (11) (2016) 9999–10012.
[93] Z. Su, L. Xing, Y. Chen, Y. Xu, F. Yang, C. Zhang, Q. Ping, Y. Xiao, Lactoferrin-modified poly (ethylene glycol)-grafted BSA nanoparticles as a dual-targeting carrier for treating brain gliomas, Mol. Pharm. 11 (6) (2014) 1823–1834.
[94] H. Wang, Y. Tang, Y. Fang, M. Zhang, H. Wang, Z. He, B. Wang, Q. Xu, Y. Huang, Reprogramming tumor immune microenvironment (TIME) and metabolism via biomimetic targeting codelivery of Shikonin/JQ1, Nano Lett. 19 (5) (2019) 2935–2944.
[95] I. Andújar, J.L. Ríos, R.M. Giner, M.C. Recio, Pharmacological properties of shikonin–a review of literature since 2002, Planta Med. 79 (18) (2013) 1685–1697.
[96] T.-J. Lin, H.-T. Lin, W.-T. Chang, P.-W. Hsiao, S.-Y. Yin, N.-S. Yang, Shikonin-enhanced cell immunogenicity of tumor vaccine is mediated by the differential effects of DAMP components, Mol. Cancer 14 (1) (2015) 174.
[97] K. Vališ, P. Talacko, V. Grobárová, J. Černý, P. Novák, Shikonin regulates C-MYC and GLUT1 expression through the MST1-YAP1-TEAD1 axis, Exp. Cell Res. 349 (2) (2016) 273–281.
[98] H. Zhu, F. Bengsch, N. Svoronos, M.R. Rutkowski, B.G. Bitler, M.J. Allegrezza, Y. Yokoyama, A.V. Kossenkov, J.E. Bradner, J.R. Conejo-Garcia, BET bromodomain inhibition promotes anti-tumor immunity by suppressing PD-L1 expression, Cell Rep. 16 (11) (2016) 2829–2837.
[99] M.F. Sanmamed, L. Chen, A paradigm shift in cancer immunotherapy: from enhancement to normalization, Cell 175 (2) (2018) 313–326.
[100] P. Zhao, W. Yin, A. Wu, Y. Tang, J. Wang, Z. Pan, T. Lin, M. Zhang, B. Chen, Y. Duan, Dual-targeting to cancer cells and M2 macrophages via biomimetic delivery of mannosylated albumin nanoparticles for drug-resistant cancer therapy, Adv. Funct. Mater. 27 (44) (2017) 1700403.
[101] J.-K. Yan, W.-Y. Qiu, Y.-Y. Wang, J.-Y. Wu, Biocompatible polyelectrolyte complex nanoparticles from lactoferrin and pectin as potential vehicles for antioxidative curcumin, J. Agric. Food Chem. 65 (28) (2017) 5720–5730.
[102] R. Chaudhary, K. Roy, R.K. Kanwar, K. Walder, J.R. Kanwar, Engineered atherosclerosis-specific zinc ferrite nanocomplex-based MRI contrast agents, Journal of Nanobiotechnology 1 (14) (2016) 1–17.
[103] J.R. Kanwar, K. Roy, Y. Patel, S.-F. Zhou, M.R. Singh, D. Singh, M. Nasir, R. Sehgal, A. Sehgal, R.S. Singh, Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions, Molecules 20 (6) (2015) 9703–9731.
[104] M. Lee, S.W. Kim, Polyethylene glycol-conjugated copolymers for plasmid DNA delivery, Pharm. Res. 22 (1) (2005) 1–10.
[105] R.K. Kanwar, J.R. Kanwar, D. Wang, D.J. Ormrod, G.W. Krissansen, Temporal expression of heat shock proteins 60 and 70 at lesion-prone sites during atherogenesis in ApoE-deficient mice, Arterioscler. Thromb. Vasc. Biol. 21 (12) (2001) 1991–1997.
[106] Z. Liu, Y. Wang, N. Zhang, Micelle-like nanoassemblies based on polymer–drug conjugates as an emerging platform for drug delivery, Expert opinion on drug delivery 9 (7) (2012) 805–822.
[107] V.P. Torchilin, Micellar nanocarriers: pharmaceutical perspectives, Pharm. Res. 24 (1) (2007) 1–16.
[108] S. Sabra, M. Abdelmoneem, M. Abdelwakil, M.M. Taha, D. Anwar, R. Mohamed, S. Khattab, A. Bekhit, K. Elkhodairy, M. Freag, Self-assembled nanocarriers based on amphiphilic natural polymers for anti-cancer drug delivery applications, Curr. Pharm. Des. 23 (35) (2017) 5213–5229.
[109] S.A. Sabra, S.A. Sheweita, M. Haroun, D. Ragab, M.A. Eldemellawy, Y. Xia, D. Goodale, A.L. Allan, A.O. Elzoghby, S. Rohani, Magnetically guided self-assembled protein micelles for enhanced delivery of dasatinib to human triple-negative breast cancer cells, J. Pharm. Sci. 108 (5) (2019) 1713–1725.
[110] D. Ragab, S. Sabra, Y. Xia, D. Goodale, A.L. Allan, S. Rohani, On-chip preparation of Amphiphilic nanomicelles–in–sodium alginate spheroids as a novel platform against triple-negative human breast cancer cells: fabrication, study of microfluidics flow hydrodynamics and proof of concept for anticancer and drug delivery applications, J. Pharm. Sci. 108 (11) (2019) 3528–3539.
[111] M.M. Agwa, D.A. Abdelmonsif, S.N. Khattab, S. Sabra, Self- assembled lactoferrin-conjugated linoleic acid micelles as an orally active targeted nanoplatform for Alzheimer's disease, Int. J. Biol. Macromol. 162 (2020) 246–261.
[112] J.R. Kanwar, G. Mahidhara, R.K. Kanwar, Novel alginate-enclosed chitosan–calcium phosphate-loaded iron-saturated bovine lactoferrin nanocarriers for oral delivery in colon cancer therapy, Nanomedicine 7 (10) (2012) 1521–1550.
[113] V.M. Balcão, C.I. Costa, C.M. Matos, C.G. Moutinho, M. Amorim, M.E. Pintado, A.P. Gomes, M.M. Vila, J.A. Teixeira, Nanoencapsulation of bovine lactoferrin for food and biopharmaceutical applications, Food Hydrocoll. 32 (2) (2013) 425–431.
[114] P.D. Marcato, N. Durán, New aspects of nanopharmaceutical delivery systems, J. Nanosci. Nanotechnol. 8 (5) (2008) 2216–2229.
[115] L. QI, In vitro effects of chitosan nanoparticles on proliferation of human gastric carcinoma cell line MGC803 cells, World J. Gastroenterol. 11 (2005) 5136–5141.
[116] D. Shome, N. Poddar, V. Sharma, U. Sheorey, G.B. Maru, A. Ingle, R. Sarin, S. Banavali, R. Dikshit, V. Jain, Does a nanomolecule of carboplatin injected periocularly help in attaining higher intravitreal concentrations? Invest. Ophthalmol. Vis. Sci. 50 (12) (2009) 5896–5900.
[117] H.S. Choi, W. Liu, P. Misra, E. Tanaka, J.P. Zimmer, B.I. Ipe, M.G. Bawendi, J.V. Frangioni, Renal clearance of quantum dots, Nat. Biotechnol. 25 (10) (2007) 1165–1170.
[118] A. Cutone, L. Rosa, G. Ianiro, M.S. Lepanto, B. di Patti, M. Carmela, P. Valenti, G. Musci, Lactoferrin's anti-cancer properties: safety, selectivity, and wide range of action, Biomolecules 10 (3) (2020) 456.