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Review Article

# NANOEMULSION AS A DRUG DELIVERY SYSTEM OF ANTICANCER DRUG

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**ABSTRACT**

Nanoemulsions are isotropic systems made up of nanoscale droplets (about 200 nm in size) created by combining two immiscible liquids with the aid of emulsifiers. They are often regarded as harmless excipients and are made to enhance the release of active medicinal compounds. Improving drug distribution to specific areas is the main goal of employing nanoemulsions in cancer treatment. In addition to increasing bioavailability, nanoemulsions reduce adverse effects on healthy cells by encasing medications in a closed structure. This is especially crucial because, in the absence of such formulations, different medications fall short of their intended targets. The study shows that by increasing the solubility and bioavailability of anticancer medications, nanoemulsions can greatly improve their delivery. This is important because a lot of anticancer medications have low solubility, which reduces their ability to effectively target cancer cells. Nanoemulsions have been shown to effectively target tumor cells while minimizing the impact on healthy tissues. This targeted approach helps overcome the common issue of multidrug resistance (MDR) seen in cancer treatments, as the nanoemulsions can be modified with specific ligands to focus on tumor cells. Targeting tumor cells and preventing MDR are two benefits of using nanoemulsions. Besides, hydrophilic and hydrophobic compounds can be encapsulated in nanoemulsions to satisfy a range of needs. Therefore, nanoemulsions are a promising new approach to cancer treatment. This review provides an overview of nanoemulsion in cancer therapeutics, aiming to highlight the current status of this technology.

**Keywords:** Nanoemulsion; Cancer; Targeted delivery; Cytotoxic agents; Nano-carriers.

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## INTRODUCTION

Nanoemulsions are colloidal dispersions that act as excellent medication carriers, especially for compounds with low solubility in water and are made with safe excipients [1,2]. These dosage forms are made up of a heterogeneous combination of nanometer-sized droplets suspended in another liquid, resulting in increased stability and solubility. The encapsulation process provides a shield that protects the medicine from decomposition, lengthening its half-life in the blood. Such dispersion is achieved with the use of emulsifying agents to stabilize the system [3]. Emulsifying agents possess amphiphilic properties; they have hydrophobic tails that preferentially associate with nonpolar liquids and polar heads that bind with polar liquids (Fig. 1). This unique architecture helps in decreasing interfacial tension between two immiscible phases leading to stabilization of nanoemulsion system (Fig. 2) [4,5].

Nanoemulsions have now become pivotal sites for therapy of cancer research, due to their significant properties for achieving suitable therapeutic outcomes. To achieve effective therapeutic outcomes, such key properties are a large surface area, surface charge, the possibility of a long circulation half-life, the ability to target en masse, and also for imaging purposes. With vascular tissues surrounding cancer cells, nanoemulsions can readily gather in those places; they are very small in size so that pass on through all kinds of biological barriers. Nanoemulsions can also be designed for certain functions that wanted to get in touch with particular cells. For instance, nanoemulsion may contain a blend of medical products and simultaneously one kind of drug; or it selectively targets special cells. The tumor microenvironment is composed of various components, including the extracellular matrix (ECM), fibroblasts, epithelial cells, immune cells, pericytes, adipocytes, glial cells (which are specialized cells within the central nervous system), proteins, endothelial cells, and the lymphatic system [6].

Tumor cell proliferation, structural support, migration, invasion, and metastasis all rely heavily on the ECM. Tumor cells have unique surface indicators, which are targets for therapeutic drugs. Tumors larger than 2.0 $mm^3$ encounter lower oxygen levels, hypoxia, and the formation of new arteries. In other words, oxygenated blood vessels must provide the necessary nutrients for a tumor to grow [7]. Initially, oxygen and nutrients are delivered through simple diffusion that would be something akin the process. If angiogenesis is inhibited, tumor cell proliferation can be effectively cut off. In contemporary pharmacological research, a variety of anti-angiogenic agents have emerged, such as bevacizumab (a neutralizing antibody targeting vascular endothelial growth factor [VEGF]), sorafenib (an inhibitor of the VEGF signaling cascade), sunitinib, and pazopanib. Nonetheless, the application of these angiogenesis inhibitors is often accompanied by significant toxicity, the development of resistance, and challenges in the effective distribution of the pharmaceutical agents. Nanoemulsions offer an innovative approach by encapsulating therapeutic compounds within their core, thereby mitigating toxicity and improving the delivery efficiency of these substances [8,9].

Glycolysis serves as the principal metabolic pathway for energy production in neoplastic cells. The microenvironment of the tumor exhibits an increased acidity due to the enzymatic conversion of pyruvate, a glycolytic byproduct, into lactate under conditions of limited oxygen availability. Lactate is subsequently extruded from the cell along with protons (H+) through a monocarboxylate transporter mechanism. Furthermore, there is an upregulation of carbonic anhydrase IX expression, which facilitates the conversion of carbon dioxide into bicarbonate in the hypoxic milieu. The relatively alkaline tumor cells then uptake this bicarbonate, establishing a concentration gradient between the intracellular and extracellular compartments. Lipids sensitive to pH fluctuations may play a significant role in this context. On exposure to acidic conditions, these lipids undergo alterations in their chemical properties, resulting in the release of their therapeutic payload, while remaining stable at the physiological pH of 7.4 [1].

The variations in the availability of oxygen, therapeutic compounds, and vital biomolecules within the tumor microenvironment can be

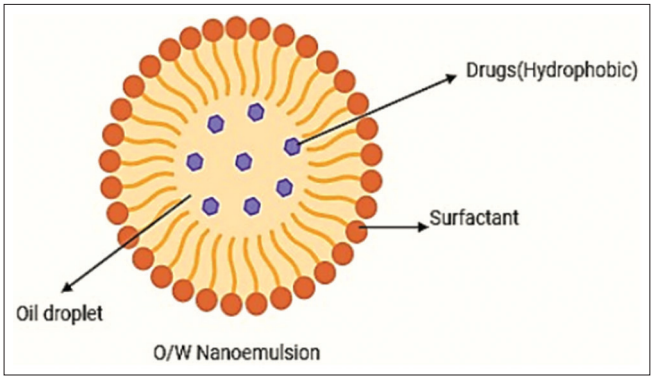


**Fig. 1: O/W nanoemulsion structure**

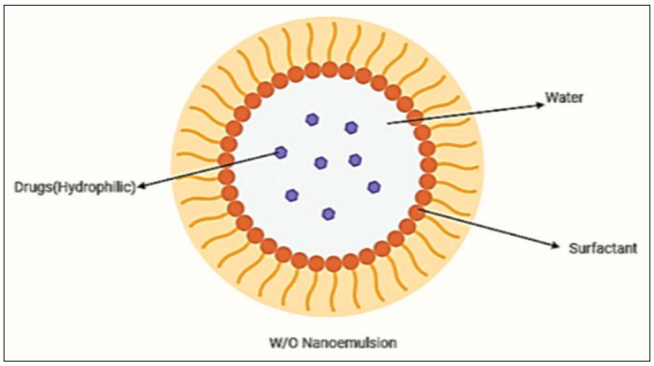


**Fig. 2: W/O nanoemulsion structure**

ascribed to the chaotic and diverse nature of the tumor stroma [10]. This phenomenon culminates in neovascularization and hypoxic conditions, both of which facilitate metastatic processes. Nevertheless, due to a diminished rate of clearance, instability within the lymphatic channels may extend the retention time of pharmacological agents. These elements work together to facilitate the occurrence referred to as enhanced permeability and retention (EPR), which is marked by inadequate lymphatic drainage and increased vascular permeability [11]. EPR is particularly advantageous for the administration of hydrophobic and macromolecular therapeutics, facilitating a therapeutic paradigm known as passive targeting. In this methodology, nanoemulsions with dimensions ranging from 20 nm to 100 nm are deemed optimal, as they are sufficiently large to evade swift renal elimination while concurrently being small enough to traverse endothelial barriers. However, the mononuclear phagocytic system (MPS) is predisposed to opsonize these particles due to their dimensional characteristics [12]. This issue may be mitigated through the encapsulation of nanoemulsions utilizing hydrophilic polymers. Moreover, the presence of negatively charged phosphatidylserine on the surfaces of tumor cells enhances the probability that positively charged particles will adhere to cancer cells for extended durations [13]. Passive targeting, nonetheless, lacks the capacity to distinguish between neoplastic and non-neoplastic tissues. By conjugating ligands to the exterior of nanoemulsions, active targeting offers a more advanced approach that enables the recognition of specific molecules present on neoplastic tissues [14]. This methodology enhances the efficacy of pharmaceutical delivery to neoplastic cells, encompassing various subtypes, by capitalizing on the unique microenvironment surrounding the neoplasm. Active targeting employs molecular linkages, including ligand-receptor and antigen-antibody interactions [15]. Targeting moieties possess the capability to bind to receptors that are overexpressed in neoplastic cells, such as prostate-specific membrane antigen, folate, transferrin, and epidermal growth factor receptors (EGFRs). This specialized delivery mechanism may encompass modifications of the surface to enhance responsiveness to extrinsic stimuli, mitigate adverse effects, and amplify cytotoxicity specific to tumors [16-19].

The augmented expression of multidrug transporters alongside alterations in apoptotic pathways constitutes the primary factors contributing to multidrug resistance (MDR) [20]. The increased activity of drug-efflux pumps belonging to the ATP-binding cassette (ABC) superfamily plays a crucial role in transporter-mediated MDR by effectively removing various anti-cancer drugs from the intracellular milieu. P-glycoprotein (P-gp), which is encoded by the ABC1 gene and is among the first ABC transporters to be characterized, has the ability to transport out drugs including vinblastine, colchicine, etoposide, and paclitaxel (PTX) [21, 22]. An increase in the expression levels of anti-apoptotic genes, such as Bcl-2 and nuclear factor-kappa B (NF-kB), is a significant characteristic linked to MDR concerning the apoptotic pathway [23]. Numerous approaches have been suggested to address MDR, such as the use of P-gp inhibitors, the downregulation of Bcl-2 and NF-kB expression, and the implementation of nanocarriers for both passive and active targeting [2]. Research has also demonstrated that a variety of ABC transporters are crucial in the emergence of drug resistance. Notably, essential human organs such as the central nervous system, lungs, liver, pancreas, stomach, intestines, kidneys, and several anatomical cellular barriers contain approximately 49 different ABC transporters [24].

As an additional strategy in chemotherapy, small interfering RNA (siRNA) molecules have been created to downregulate anti-apoptotic genes or decrease the production of MDR proteins. Finding appropriate vectors that can co-deliver siRNA and medications, however, is a major hurdle. Because they can overcome MDR when combined with Bcl-2 inhibitors or P-gp modulators, nanoemulsions offer a possible approach. Programed cell death is communicated through ceramides, a family of apoptotic chemicals generated in response to environmental stress. By overexpressing glucosylceramide synthase, which changes ceramide into its inert glycosylated form, certain MDR cells can avoid apoptosis. Ceramide can be efficiently delivered to tumor tissues by employing nanoemulsions for targeted distribution, which would improve therapeutic results and apoptotic effects [2,25].

The unique specificity and selectivity inherent in antigen–antibody interactions can be leveraged to enhance targeted drug delivery by linking nanoemulsions with antibodies or their fragments. According to studies, this type of conjugation makes it easier for cancer cells to absorb drug-loaded nanoemulsions, allowing for efficient drug administration. In addition, these nanocarrier–antibody complexes can be engineered to react to particular stimuli, improving their ability to precisely target cancerous areas [26]. An alternative to antibodies is aptamers, which are produced through a procedure known as SELEX. To build aptamers with a high affinity for certain targets, SELEX generates a library of single-stranded DNA and RNA molecules that can fold into secondary and tertiary structures [27]. Aptamers are smaller, non-immunogenic, simpler to make, and have a higher rate of tissue penetration than antibodies. Aptamers that specifically identify tumor cell receptors and biomarkers are made possible by SELEX. Folate receptors exhibit heightened expression in various types of cancers, encompassing malignancies of the brain, lung, pancreas, breast, ovary, cervix, endometrium, prostate, and colon [28-30]. Due to its high affinity for these receptors, folic acid is an often-utilized targeted drug [31]. The apical surface of polarized epithelia is where folate receptors are mostly located in healthy tissues [32]. The presence of folate receptors is generally elevated in instances of cancer metastasis. Folic acid serves as an ideal targeting moiety due to several advantageous properties, including its affordability, non-toxic nature, lack of immunogenic response, ease of conjugation with nanocarriers, strong binding affinity, and remarkable stability during storage and circulation [33]. Nanoemulsions can be combined with oligonucleotides for therapeutic applications. Nevertheless, the effectiveness of oligonucleotides is constrained by their intrinsic instability, brief half-life in biological fluids, and inadequate penetration into cells [34]. Changes such as substituting phosphorothioate linkages for phosphodiester bonds might increase stability and efficacy while guarding against enzymatic breakdown. These issues can also be resolved by conjugation with lipid

or plasmid DNA complexes, cationic liposomes, micelle polyelectrolyte complexes, polyethylene glycol (PEG), and pH-sensitive nanocarriers, which would increase the effectiveness of cancer treatment [35-38].

## NANOEMULSIONS – A CONCISE SUMMARY

### The formulation of the nanoemulsion

Nanoemulsions are defined as colloidal dispersions that consist of oil, surfactant, and an aqueous phase. The formulation's overall stability, physicochemical features, therapeutic payload, and particle size are all greatly impacted by the nanoemulsion core's characteristics [39,40]. The usage of long-chain triglycerides (LCTs) typically results in bigger particles with diameters of about 120 nm. On the other hand, smaller particles, usually about 40 nm, are produced by short-chain triglycerides (SCTs). A frequent LCT is soybean oil due to its high content of linoleic acid and other important C18 fatty acids. Medium-chain triglycerides derived from coconut oil can be utilized alone or in combination with LCTs to mitigate the risk of immunosuppressive side effects and inhibit lymphocyte activity [40,41]. The ultimate aspect of the formulation is influenced by the size of the particles. In general, larger particle sizes contribute to enhanced stability of the formulation. This particular characteristic serves as a countermeasure to issues such as Brownian motion, flocculation, and gravitational forces. Conversely, the high-water solubility of SCT oils facilitates the process of Ostwald ripening [42].

The ideal emulsifying agent must effectively stabilize the interface through steric or electrostatic interactions, rapidly adsorb at the boundary, and significantly reduce interfacial tension. Examples of amphiphilic compounds recognized as emulsifiers include surfactants such as Tween® 80, amphiphilic proteins like caseinate, phospholipids such as soy lecithin, modified starches classified as polysaccharides, and polymers like PEG [42]. PEG-modified nanoemulsions are especially useful for extending bloodstream circulation time and accomplishing precise targeting. The formulation can also be improved by adding ripening inhibitors, weighing agents, or texture modifiers [43,44]. To enhance stability and performance, the application of non-ionic surfactants such as sorbitan fatty acid esters (commonly referred to as Spans®) can be considered [45]. Temperature and pH-responsive materials can be utilized to develop nanoemulsions that react to external stimuli. The objective is to trigger a conformational alteration in the formulation, thereby enhancing the release rate of the payload [46,47].

### Characterization of nanoemulsion: Analysis of physical and chemical properties

Many physical and chemical characteristics affect how nanoemulsions behave, and one important factor is their average size. Furthermore, size distribution, as shown by the polydispersity index (PDI), is very important since determining the size range of nanoemulsions is crucial for evaluating their biological effects. Using dynamic light scattering, which uses the Brownian motion of colloidal particles to assess the diffusion coefficient based on light scattering, both parameters can be examined. Another crucial element is surface charge since it can change how cells react. It is essential to characterize the surface charge since it influences the stability of nanoemulsions and their electrostatic interactions. To measure surface charge accurately, a magnetic field must be applied [48]. The particles' electrophoretic movement follows Henry's equation (Equation 1).

$$U_e = 2\varepsilon z f(ka)/3\eta \quad (1)$$

Where z is the zeta potential, Ue is the electrophoretic mobility, ε is the dielectric constant, η is the viscosity of the dispersant, and f(ka) is the Henry function.

The shape of oil droplets is critical to influencing the formulation's stability. The lipophilic-hydrophilic characteristics of nanoemulsions have a major impact on drug loading, which directly affects encapsulation efficiency [1]. Optical microscopy, including sophisticated methods such as differential interference contrast and phase contrast techniques, frequently falls short in effectively analyzing nanoemulsions. To obtain significant insights into the morphology of these systems, alternative microscopic techniques are essential. Both transmission electron microscopy and scanning electron microscopy (SEM) have demonstrated their utility in examining the structural characteristics of nanoemulsions. In addition, SEM is capable of generating three-dimensional representations of the droplets, thereby offering valuable information [30,49].

The drug loading in nanoemulsions is a critical factor for formulations aimed at targeting specific tumor tissues with pharmaceuticals. Various methodologies have been developed to assess this parameter. One approach involves the separation of the free drug from the encapsulated drug, facilitating the evaluation of either the free drug (indirect entrapment efficiency [EE]) or the encapsulated drug by dissolving the nanoemulsion core in suitable organic solvents. Techniques such as filtration, filtration-centrifugation, ultracentrifugation, and the use of dialysis membranes are effective for isolating non-encapsulated drugs from nanoemulsions. Typically, the drug loading is quantified as a percentage (EE) or as the concentration of the drug within each nanoemulsion droplet [50-52].

### Advantages of nanoemulsion

The advantages of nanoemulsion are briefly mentioned in [Fig. 3].

### Stability studies

Stability assessments are essential for the characterization of nanoemulsions. One method to expedite stability evaluation is centrifugation, which accelerates the creaming phenomenon. Historically, the stability of these formulations has been determined by storing them at temperatures ranging from 4.0°C to 25.0°C for a duration of 3–6 months. During this period, monthly evaluations are conducted on critical parameters, including average droplet size, PDI, surface charge, and encapsulation efficiency. A lack of significant variation in these parameters throughout the storage period suggests that the nanoemulsion remains stable. In addition, the Food and Drug Administration (FDA) mandates that stability testing encompasses long-term, intermediate, and accelerated timeframes. To assess degradation products and overall stability, parameters such as peroxide value, anisidine value, and total oxidation value are employed. Moreover, the formulation's pH may be affected by oil oxidation processes, which can be monitored through these stability assessments [1].

### Nanoemulsion drug release

The mechanism of drug release exerts a significant influence on the bioavailability, absorption, and pharmacokinetics of a pharmaceutical agent. This phenomenon is commonly evaluated through the utilization of either Franz diffusion cells or the dialysis bag technique. In the dialysis bag method, the pharmaceutical sample is encapsulated within a dialysis bag, which is subsequently immersed in a buffer solution contained within a receiving chamber. This arrangement is maintained under continuous agitation at a temperature of 37°C to facilitate the diffusion of the pharmaceutical compound through the dialysis membrane into the adjacent buffer solution [53]. In contrast, Franz diffusion cells are characterized by the presence of two distinct chambers: A donor

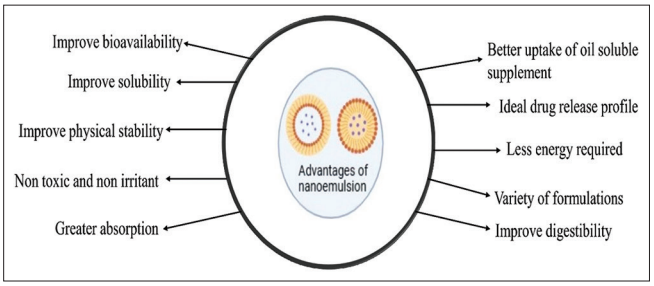


**Fig. 3: Advantages of nanoemulsion**

compartment and a receptor compartment. The nanoemulsion containing the pharmacological agent is introduced into the donor compartment, which is partitioned from the receptor chamber by means of a dialysis membrane. Typically, the receptor chamber is filled with a phosphate buffer solution to replicate physiological conditions. The medicine gradually diffuses from the donor compartment to the receptor chamber through the membrane. The amount of drug released is quantified by collecting samples from the receptor chamber at certain time intervals. This information is utilized to create a medication release profile, which aids in determining the kinetics and efficacy of the nanoemulsion formulation. Both approaches provide useful insights into formulation drug release behavior, which contributes to a better knowledge of their potential therapeutic efficacy. While the dialysis bag approach is simple and versatile, Franz diffusion cells provide more regulated and exact monitoring of drug diffusion [31].

## PREPARATION METHODOLOGY OF NANOEMULSIONS

The production of nanoemulsions encompasses two primary stages: thermal treatment and mechanical mixing, succeeded by homogenization. In the thermal treatment and mechanical mixing phase, the constituents are amalgamated at meticulously regulated temperatures and with appropriate agitation to ensure the dispersion achieves optimal uniformity. Subsequently, the emulsion undergoes homogenization through the application of shear force, which effectively diminishes particle size to the requisite minimum threshold. The resulting particles are stabilized by an emulsifier interfacial layer that encapsulates and segregates the lipophilic core from the aqueous medium. This emulsifier interfacial layer functions as a protective barrier and enhances stability through the action of repulsive forces. Depending on the specific emulsifier employed, these repulsive forces may manifest as electrostatic, steric, or electrosteric interactions, thereby ensuring that the nanoemulsion maintains stability and structural integrity over time [1].

High shear techniques employ high-pressure homogenizers, microfluidizers, and ultrasonicators to regulate particle dimensions. The characteristics of the resultant particles are influenced by several determinants, including the type of apparatus employed and operational variables such as energy input, duration of processing, thermal conditions, and formulation composition. Although high-energy techniques possess the advantage of scalability, they may not be suitable for thermally sensitive pharmaceuticals. In such instances, alternative low-energy strategies and temperature-regulated methods should be adopted, including self-emulsification phase transition and phase inversion [54,55].

### High-pressure homogenization

Nanoemulsions are produced through high-pressure homogenization (HPH), also known as piston homogenization, which is a precise and efficient method. This procedure entails the amalgamation of an aqueous phase endowed with an emulsifier and an organic phase, followed by the application of an ultraturrax to generate a coarse emulsion. Subsequently, the emulsion undergoes processing through the HPH process to further reduce the droplet size [56]. To produce the necessary nanoemulsion properties, the HPH process uses numerous cycles of homogenization at various pressures. During this process, multiple factors, including hydraulic pressure, turbulence, and cavitation, act synergistically to break down droplets into smaller pieces, resulting in finely distributed particles [1]. A key advantage of this technique is its flexibility, as it can be repeated multiple times to refine droplet size until optimal parameters are achieved, ensuring the production of stable, small-sized particles suitable for various applications [55,56].

### Microfluidization

The microfluidization methodology employs a proprietary microfluidizer apparatus equipped with a high-pressure positive displacement pump, which is capable of operating within a pressure spectrum of 500–20,000 psi. This pump propels the substance through an interaction chamber characterized by narrow micro-channels. As the substance traverses these channels and enters the impingement zone, substantial forces are exerted, resulting in the fragmentation of the material into exceptionally diminutive particles within the submicron dimension. To synthesize a nanoemulsion, it is requisite to initially amalgamate the aqueous and oily phases to create a coarse emulsion. Subsequently, this emulsion is conveyed to the microfluidizer for advanced processing. Through repeated passage through the interaction chamber, the droplet dimension is systematically reduced until the desired particle size is achieved. On completion of processing, the bulk emulsion undergoes filtration with nitrogen to eliminate any larger droplets, thus producing a homogeneous and stable nanoemulsion. This technological advancement is particularly suitable for the production of nanoemulsions at an industrial scale, as it facilitates precise regulation of particle size and formulation stability [57].

### Phase inversion temperature (PIT) technique

The PIT technique demonstrates a relationship between the attainment of small droplet sizes and the complete solubilization of oil within a bicontinuous microemulsion. This relationship is consistent irrespective of whether the initial phase equilibrium is classified as single-phase or multiphase. Nanoemulsions, characterized by their small droplet sizes, show improved stability against issues such as sedimentation and creaming, with Ostwald ripening recognized as the principal destabilization mechanism. In emulsions, phase inversion can occur through two primary mechanisms: Transitional inversion, which is induced by changes in factors such as temperature or electrolyte concentration that affect the hydrophile-lipophile balance (HLB) of the system, and catastrophic inversion, which occurs when the HLB of the surfactant is altered through the application of surfactant mixtures at a constant temperature. The PIT approach utilizes the temperature-dependent solubility of non-ionic surfactants, including polyethoxylated surfactants, to modify their affinities for both water and oil. As the temperature increases, these polyethoxylated surfactants become more lipophilic due to the dehydration of their polyoxyethylene groups, a characteristic essential for the formation of nanoemulsions within the PIT paradigm. At room temperature, a mixture of oil, water, and non-ionic surfactants results in an o/w macroemulsion, which contains oil-in-water microemulsions and excess oil, along with a surfactant monolayer that exhibits positive curvature. As heating progresses, surfactants become solubilized into the oily phase, leading to a transition from an o/w to a w/o emulsion, accompanied by the development of a surfactant monolayer with negative curvature. While heating is a critical component of this technique, it poses challenges for thermolabile compounds, such as tretinoin and peptides, due to the potential for degradation. This issue may be addressed through various strategies [58].

### Solvent displacement method for preparing nanoemulsion

The solvent displacement method, which is derived from the nanoprecipitation process employed in the fabrication of polymeric nanoparticles, offers a simple strategy for the spontaneous formation of nanoemulsions. This technique involves dissolving the oily phase in water-miscible organic solvents such as acetone, ethanol, or ethyl methyl ketone. When this organic phase is added to an aqueous solution that contains a surfactant, spontaneous nanoemulsions are generated as the organic solvent rapidly disperses into the aqueous environment [59]. The organic solvent is then removed using processes like vacuum evaporation. This approach works well at room temperature and requires only basic stirring, giving it a promising choice in pharmaceutical research for producing nanoemulsions, particularly for parenteral applications. However, there are some significant downsides. The use of organic solvents, such as acetone, requires additional stages for removal, complicating the process. Furthermore, getting the necessary droplet size frequently necessitates a high solvent-to-oil ratio, which can be limiting in certain circumstances. While solvent removal is controllable at the laboratory size, it presents substantial hurdles when scaling up, affecting the method's reproducibility and viability for industrial applications [30]. As a result, while the solvent displacement approach is useful for small-

scale production, its scalability and dependence on organic solvents remain significant problems.

**Phase inversion composition technique (self-nanoemulsification method)**

This methodology facilitates the formation of nanoemulsions at ambient temperature, eliminating the necessity for organic solvents or elevated temperatures. To achieve kinetically stable nanoemulsions characterized by small droplet sizes (approximately 50 nm), one must carefully incorporate a water phase into a surfactant solution dispersed in oil while maintaining a constant temperature [60]. This technique, referred to as spontaneous nanoemulsification, is influenced by the phase transitions that take place during the emulsification process. Such transitions often involve the formation of lamellar liquid crystalline phases or D-type bicontinuous microemulsions. Although the nanoemulsions produced through this method do not exhibit thermodynamic stability, they possess significant kinetic energy, allowing them to maintain long-term colloidal stability. Consequently, they are applicable in a diverse array of fields, notwithstanding their fundamental thermodynamic constraints [30].

**Ultrasonic emulsification**

This method is notably effective in minimizing droplet size. An ultrasonic generator introduces energy into a mixture through the use of an ultrasonic probe. This process utilizes piezoelectric quartz crystals. The device operates on an alternating current supply, enabling it to contract and expand in response to the electrical current. Fluctuations in voltage lead to mechanical vibrations. A sonicator agitates the liquid, resulting in cavitation, which facilitates the reduction of droplets to microscopic dimensions. Cavitation is characterized by the formation and subsequent collapse of droplets [61].

**Low-energy emulsification**

Emulsions are formed through low-energy emulsification techniques, including phase inversion, solvent replacement, and spontaneous emulsification. The phase inversion method relies on the interactions between the components and their response to temperature changes. Critical to this process are the variations in temperature while maintaining a constant composition, or conversely, altering the composition through temperature manipulation. At ambient temperature, a mixture of water, oil, and a non-ionic surfactant exhibits positive curvature. Rapid fluctuations in temperature during this process can facilitate the development of a stable emulsion by encouraging phase inversion. This technique not only yields a stable emulsion but also minimizes the risk of droplet coalescence, leading to a more uniform and consistent product. Therefore, meticulous control of both temperature and composition is essential for maintaining emulsion stability [62].

**Spontaneous emulsification method**

The Spontaneous Emulsification Method is a prevalent low-energy approach for the formulation of nanoemulsions, particularly in sectors that necessitate small droplet dimensions, such as pharmaceuticals, cosmetics, and food products. Nanoemulsions produced through this technique generally exhibit droplet sizes ranging from 10 nm to 200 nm, which contribute to enhanced stability, increased bioavailability, and optimized delivery characteristics. The process involves three key steps: (a) Preparing a uniform organic solution by mixing oily and lipophilic surfactants with hydrophilic surfactants in an aqueous medium; (b) forming an oil-in-water emulsion by gradually introducing the organic phase into the aqueous phase while maintaining continuous magnetic stirring; and (c) finalizing the process by evaporating the aqueous phase under reduced pressure [63].

## METABOLISM

Nanoemulsions need to withstand the challenges posed by both the MPS and renal clearance mechanisms when delivered systemically to effectively target tumor tissues. The MPS acts as a biological barrier, comprising phagocytic cells that can capture nanoemulsions [64]. The nanoemulsion, once in circulation, engages with numerous blood constituents, including erythrocytes, opsonins, monocytes, platelets, leukocytes, dendritic cells, tissue macrophages, liver Kupffer cells [65], lymph node cells, and splenic B cells. Given that erythrocytes account for the bulk of blood cells, nanoemulsions are expected to interact with them, resulting in hemolysis and macrophage clearance [66]. Furthermore, the longer half-life of nanoemulsions increases the possibility of interactions with blood cells, which could lead to thrombogenic events such as blood vessel obstruction [67]. Opsonins have the potential to adhere to the surfaces of nanoemulsions, which can enhance the uptake by macrophages while simultaneously reducing the efficacy of drug delivery to the intended site. Furthermore, this activation of immune cells may lead to adverse reactions such as anaphylaxis, allergic responses, or hypersensitivity [66]. The nanoformulation's size, charge, and surface qualities all determine how it interacts with erythrocytes. Larger cationic or anionic particles are more likely to be phagocytosed, with cationic particles being particularly prone to causing erythrocyte damage and hemolysis [67,68]. To reduce opsonization, nanoemulsions are frequently surface modified with PEG, poloxamer, or poloxamine, which form a "steric shield" surrounding the particles [68-70]. The nanoemulsions are taken up by cells through phagocytosis, macropinocytosis, or endocytosis, where they accumulate in lysosomes, vacuoles, or the cytoplasm. The blood-brain barrier offers a substantial difficulty to medication administration in formulations targeting brain tumors, but it can be overcome by utilizing nanoemulsions tailored to target receptors at the site of action [71]. In terms of metabolism, the liver plays an important role in nanoemulsion clearance. Nanoparticles that are internalized by hepatocytes are eliminated through the biliary system, while those taken up by Kupffer cells undergo phagocytosis, subsequent degradation, and eventual excretion. The kidneys significantly contribute to the metabolism of nanoemulsions through their filtration processes. Specifically, particles with a size <6 nm are excreted, whereas larger particles are returned to the circulatory system [72,73].

## NANOEMULSION IN THE FIELD OF DRUG DELIVERY

Nanotechnology has significantly enhanced the safety and effectiveness of cancer therapies by creating advanced drug delivery systems, such as nanocarriers. Due to their small dimensions, these carriers can exploit the EPR effect to passively target tumor sites. In addition, they facilitate active targeting through receptor-mediated uptake, allowing for the selective distribution of therapeutics to specific cell types and organs. These innovative systems enable controlled drug release, improve drug stability, and address the solubility challenges associated with hydrophobic compounds. Among the various nanostructured delivery systems, polymeric nanoparticles, nanostructured lipid carriers, liposomes, and nanoemulsions have emerged as promising options for the administration of cancer treatments [74-77].

Inorganic nanoparticles, despite extensive research, exhibit limitations regarding their safety and biocompatibility. Recent investigations have addressed these challenges by modifying the particle surfaces with biocompatible substances; however, additional research is necessary to develop more effective coatings and delivery methods. Conversely, nanoemulsions present several unique benefits, notably their composition from biocompatible, generally recognized as safe (GRAS) materials, along with their ease of scalability and production. Similar to other nanosystems, nanoemulsions possess a high capacity for encapsulating hydrophobic drugs, enhanced physicochemical stability, improved bioavailability, and more consistent pharmacokinetics with reduced interindividual variability [78-80].

Nanoemulsions can be delivered by a variety of methods. When administered orally, they can protect medication molecules against destruction in the stomach and intestines while avoiding first-pass metabolism. Nanoemulsions are as stable as liposomes, ethosomes, or microspheres, but with improved solubility and absorption of weakly bioavailable substances. In an *in vivo* investigation comparing

lipid nanoparticles and nanoemulsions, nanoemulsions demonstrated considerably longer retention time in the brain. Further research into skin penetration indicated that, whereas solid lipid nanoparticles mostly release medications in the outer skin layers, liposomes and nanoemulsions enter deeper skin layers. However, nanostructured lipid carriers provide better protection for photosensitive medicines than nanoemulsions [81-83].

A lipid-based nanoemulsion delivered through nebulization for the treatment of lung cancer has shown efficacy in solubilizing substantial quantities of hydrophobic pharmaceuticals, while simultaneously enhancing resistance to hydrolysis and enzymatic breakdown [84].

## NANOEMULSIONS IN CANCER TREATMENT

### Nanoemulsions for various types of cancer

Nanotechnology has demonstrated its efficacy as a viable approach for cancer therapy, prompting researchers to concentrate their efforts on the treatment of various cancer types [1].

### Nanoemulsion for leukemia treatment

Cancer remains one of the leading causes of mortality globally, with leukemia representing a significant proportion of cancer-related fatalities among children. In this regard, nanoemulsions have emerged as biocompatible delivery systems that encapsulate therapeutic agents, thereby enhancing their efficacy while minimizing adverse side effects. Extensive research has been conducted on lipid-based nanoemulsions as a promising method for drug delivery in cancer therapy. Moura *et al*. developed lipid nanoemulsions capable of binding to low-density lipoprotein (LDL) receptors, effectively targeting tissues that overexpress these receptors, such as tumors. They encapsulated methotrexate within these nanoemulsions for the treatment of leukemia and conducted *in vitro* evaluations. The results demonstrated significantly higher cellular uptake of the nanoemulsions compared to the free drug, leading to enhanced cytotoxicity against tumor cells [85]. Winter *et al*. similarly developed nanoemulsions that encapsulate chalcones for the treatment of leukemia, investigating their efficacy through both *in vitro* and *in vivo* studies. The results indicated that these nanoemulsions induced apoptosis in cancer cells during *in vitro* experiments and exhibited anti-leukemic effects comparable to those of free chalcones. Notably, free chalcones were found to be more toxic to VERO cells than their nanoemulsion counterparts. *In vivo* assessments produced analogous outcomes, revealing that free chalcones resulted in reduced weight gain, liver damage due to oxidative stress, and a heightened inflammatory response, all of which were less severe in the presence of chalcone-loaded nanoemulsions [86].

### Nanoemulsion for melanoma treatment

Melanoma represents the most lethal form of cutaneous malignancy, constituting over 80% of fatalities associated with skin cancer. A primary challenge in the management of melanoma lies in the suboptimal response rates to currently available therapeutic modalities, attributable to the limited efficacy of chemotherapeutic agents, and the inherent resistance exhibited by melanoma cells. Conventional interventions for advanced and metastatic melanoma often yield unsatisfactory outcomes, characterized by pronounced adverse effects and diminished overall survival probabilities. Consequently, novel approaches, such as nanotechnology-derived nanoemulsions, are under exploration to enhance therapeutic effectiveness. Kretxer and associates developed lipid-based nanoemulsions encapsulating paclitaxel aimed at selectively targeting LDL receptors. These nanoemulsions successfully mitigated drug-induced toxicity while augmenting anticancer efficacy. In addition, the combination of simvastatin and paclitaxel nanoemulsions was tested in melanoma mice. Compared to free paclitaxel, this combination greatly boosted anti-tumoral effects. Statins are thought to increase LDL receptor expression, allowing nanoemulsions to enter the body through these receptors [87]. Other researchers have encapsulated cholesterol derivatives, inclusive of 7-ketocholesterol, within lipid-core nanoemulsions and subsequently investigated their effects *in vivo* utilizing a murine melanoma model. The application of these nanoemulsions resulted in a reduction of tumor size exceeding 50%, an augmentation of the necrotic area, and a diminution of intratumoral vascular structures. *In vitro* analyses substantiated that tumor cells internalized these nanoemulsions through LDL receptor-mediated endocytosis. Unexpectedly, a singular administration of cholesterol-enriched nanoemulsions induced apoptosis in 10% of melanoma cells [88].

Monge-Fuentes and associates employed an innovative methodology that synergistically combined photodynamic therapy with acai oil encapsulated in a nanoemulsion. This nanoemulsion functioned as a photosensitizer in both *in vitro* and *in vivo* experimental settings. The application of this treatment to NIH/3T3 normal cells and B16F10 melanoma cell lines resulted in a significant 85% cytotoxicity in the melanoma cells, while the normal cells exhibited sustained viability. Moreover, tumor-bearing C57BL/6 mice subjected to the acai oil nanoemulsion treatment revealed an extraordinary 82% diminution in tumor volume [89].

### Nanoemulsion as a therapeutic approach for lung cancer

Lung carcinoma is a highly intrusive, fast-spreading, and malignant disease, responsible for a significant proportion of cancer-related deaths globally. It is the most prevalent type of tumor, comprising various subtypes with unique biological and clinical characteristics [90]. Paclitaxel (PTX) is an extensively utilized chemotherapeutic agent that exhibits efficacy against lung, breast, pancreatic, and ovarian carcinomas. Its mechanism of action entails the disruption of microtubule disassembly during mitosis, leading to programmed cell death, mitotic inhibition, and decreased cellular functionality [91]. Nevertheless, due to the limited aqueous solubility of PTX, pharmaceutical formulations such as Taxol®, which incorporates Cremophor-EL® and ethanol, have been created [92]. Regrettably, the presence of Cremophor-EL® poses significant toxicity risks, thereby highlighting the necessity for alternative targeted delivery methodologies [93]. Hyaluronic acid (HA) has been explored as a potential targeting ligand for the administration of PTX. HA possesses a negative charge and exhibits a specific binding affinity for cluster of differentiation 44 (CD44), a biomarker that is frequently overexpressed in various neoplasms [94]. A nanoemulsion carrier encapsulating paclitaxel (PTX) and HA, referred to as HA-complexed PTX nanoemulsions (HPNs), was developed to evaluate its therapeutic effectiveness against CD44-expressing neoplasms within a non-small cell lung carcinoma cell line (NCI-H460). HPNs exhibited favorable physicochemical properties, characterized by a particle size conducive to prolonged half-life, a zeta potential that promotes formulation stability, a low PDI indicative of uniformity, and a desirable spherical morphology. Assessments of tumor mass indicated that both PTX nanoemulsions and HPNs markedly inhibited neoplasm proliferation. Nevertheless, the incorporation of HA in HPNs enhanced therapeutic efficacy due to its targeted action. Evaluations of body weight showed no statistically significant discrepancies among the groups treated with PTX nanoemulsions and HPNs, suggesting that these formulations possess a reduced cytotoxicity towards healthy tissues [95].

Chang and associates conducted a comprehensive investigation into the anticancer properties of curcuminoid extracts derived from *Curcuma longa* Linnaeus. They formulated nanoemulsions and meticulously examined the underlying mechanisms associated with their anticancer efficacy against lung carcinoma cells. Both the curcuminoid extract and the nanoemulsion interventions were observed to impede the progression of the cell cycle of lung cancer cells specifically in the G2/M phase; however, the precise biological mechanisms involved may exhibit distinct characteristics. H460 cells demonstrated a heightened sensitivity to apoptosis in comparison to A549 cells when exposed to either the curcuminoid extract or the nanoemulsion treatment [96].

### Nanoemulsion as a therapeutic approach for breast cancer

Breast carcinoma represents 23% of all incident malignancies identified in the female population globally, and it contributes to 13.7% of all

mortality attributable to neoplastic diseases. Current chemotherapeutic drugs' efficiency is frequently limited by their low concentration in tumors compared to other organs, resulting in greater toxicity and lower therapeutic efficacy. To overcome these obstacles, a variety of initiatives for improving breast cancer treatment have emerged. Nanoemulsions generated from natural chemicals offer a promising approach to breast cancer treatment. For example, Periasamy *et al*. created a nanoemulsion with *Nigella sativa* L. essential oil that showed anticancer efficacy *in vitro* against Michigan Cancer Foundation-7 MCF-7 breast cancer cells by triggering apoptosis. This nanoemulsion has the potential to be a delivery mechanism for active medicines, improving breast cancer therapy outcomes [97].

The exploration of localized administration in conjunction with the formulation of C6 ceramide nanoemulsions has been rigorously examined as a potential methodology for the treatment of breast cancer. This innovative approach is designed to specifically target malignant and pre-tumor lesions, thereby mitigating systemic adverse effects through the mechanism of targeted drug delivery. Investigators engineered bioadhesive nanoemulsions encapsulating C6 ceramide and modified the surface characteristics utilizing chitosan. The nanoencapsulation of C6 ceramide resulted in a significant reduction of the concentration required to achieve a 50% decrease in MCF-7 cell viability (EC50), exhibiting a 4.5-fold enhancement in efficacy relative to the drug in its aqueous solution. In addition, tributyrin, a pro-drug of butyric acid, was incorporated into the oil phase of the nanoemulsion, leading to a notable 2.6-fold decrease in the requisite concentration. When administered through intraductal routes, the nanoemulsion demonstrated an extended drug localization within mammary tissue, surpassing 120 h in comparison to the conventional solution form. In a parallel investigation, Natesan and associates employed chitosan to formulate nanoemulsions containing camptothecin. Their developed formulations exhibited superior performance relative to the unencapsulated drug in both *in vitro* and *in vivo* experimental assessments [98,99].

**Nanoemulsion as a therapeutic approach for colon cancer**

Colon cancer accounts for a significant proportion of cancer-related deaths worldwide. This category encompasses various subtypes, including familial adenomatous polyposis, hereditary non-polyposis colorectal cancer, spontaneous colon cancer, and colitis-associated cancer. Treatment modalities for colon cancer typically involve surgical intervention, herbal remedies, immunotherapy, radiation therapy, and/or chemotherapy. Nevertheless, the survival rate tends to decline approximately 5 years post-surgery, primarily due to issues such as metastasis and recurrence, indicating that the tumor itself is not the sole contributor to mortality. The process of epithelial-mesenchymal transition plays a crucial role in enhancing cancer invasion and migration, as it facilitates the transformation of epithelial cells into mesenchymal cells, leading to structural changes that promote improved adhesion and motility [100-102].

Lycopene (LP), a chemical found in tomatoes, has various health benefits, including protection against chronic diseases, anti-proliferative effects on leukemia and colon cancer cells, and the ability to cause cell cycle arrest in specific tumor cells [103]. These characteristics make it an appealing candidate for cancer treatment; yet, its limited stability and bioavailability pose substantial hurdles. To address this, researchers created a nanoemulsion formulation with LP to improve its stability and therapeutic potential. The formulation also incorporates gold nanoparticles (AN), which serve a dual purpose as both a drug delivery system and a platform for functionalization with cell receptor ligands, facilitating targeted delivery to particular cell types. Nevertheless, high concentrations of AN may induce migration of human fibroblast cells, posing a risk of adverse effects. This risk can be mitigated by integrating liposomes, polymeric substances, or other lipid-based entities, including those derived from LP, into the formulation [104-106].

The formulation's oil phase consists of oil containing LP, while the water phase is comprised of an aqueous solution of AN, with Tween 80® serving as the emulsifier. This nanoemulsion was evaluated on the human colon cancer cell line HT-29, revealing its effectiveness in comparison to treatments with AN alone, LP alone, and their combination. The toxicity of AN is influenced by its particle size, with smaller nanoparticles exhibiting more pronounced effects on HT-29 cells. An increase in LP concentration within the formulation correlates with a higher occurrence of early apoptotic cells. Both the combination treatment and the nanoemulsion formulation significantly elevate the numbers of early apoptotic, late apoptotic, and necrotic cells. The application of nanoemulsions has been shown to induce apoptosis and necrosis in these cells. While the emulsifier does not exert a significant direct effect on the cells, it plays a crucial role in stabilizing the nanoemulsion. Nanoemulsions containing moderate levels of AN and LP reduce the expression of procaspases 3 and 8, as well as Bcl-2, which are indicators of tumor survival, while enhancing the expression of Bax and PARP-1, which are associated with apoptosis. These alterations highlight the apoptotic effects of the nanoemulsion on cancer cells [107].

**Nanoemulsion for prostate cancer (PrC) therapy**

PrC mortality rates have increased in the past 10 years, with 70% of men who have undergone treatment experiencing recurrence and advancing to a stage that is no longer amenable to treatment [108]. Cancer stem cells (CSCs), often referred to as tumor-initiating cells, are critical contributors to the processes of tumor growth, metastasis, and the development of resistance to therapies. Research indicates that cancer cells characterized by CSC markers, such as CD133 and CD44, not only exhibit resistance to pharmacological treatments but also have the capacity to proliferate following therapeutic interventions. The mechanisms underlying drug resistance in CSCs are multifaceted and may include the overexpression of drug efflux transporters, the activation of anti-apoptotic signaling pathways, the inhibition of apoptotic mechanisms, and an enhanced capability for detecting and repairing DNA damage. Collectively, these factors enhance the resilience and longevity of CSCs in the context of conventional treatment approaches [109,110].

PrC therapy presents a substantial problem because treatments often target rapidly proliferating cancer cells while ignoring subpopulations such as CSCs. Furthermore, the development of anti-PrC medications frequently relies on cell lines with large passage numbers for preclinical investigations, which might result in genomic and epigenomic modifications that do not match the original tumor [111]. To address this challenge, the research team utilizes the PPT2 cell line, derived from a PrC patient and characterized by a notably low passage number. This characteristic preserves the cells in a more immature and stem-like state. The PPT2 cells exhibit the expression of genes associated with anti-apoptotic signaling and drug resistance, rendering them a valuable model for evaluating treatments aimed at targeting CSCs [112].

Abraxane® is a formulation of paclitaxel designed to enhance its solubility through the use of human serum albumin-bound nanoparticles. Despite this advancement, paclitaxel encounters challenges when addressing MDR cancer cells. In contrast, SBT-1214, a novel taxoid, has shown effectiveness in targeting drug-resistant cancer cells [110]. This compound has the potential to be conjugated with docosahexaenoic acid (DHA), a naturally occurring polyunsaturated fatty acid known for its strong binding affinity to human serum albumin, the principal transporter in the bloodstream. This interaction facilitates the targeted delivery of the therapeutic agent to cancer cells. When paclitaxel was administered in conjunction with DHA, there was only a minimal reduction in the levels of P-gp and ABC transporters [113]. The nanoemulsion DHA-SBT-1214 developed for this study comprises phospholipids and fish oil. The drug's strong affinity for fish oil enhances its encapsulation process. This nanoemulsion is designed to target the CSC-initiated PPT2 cell line, utilizing the EPR effect to promote apoptosis in cancer cells [110].

The application of patient-derived CSC-enriched PPT2 cells can facilitate the creation of therapies aimed at the cells that initiate

tumors. The combination of DHA and SBT-1214 enhances the duration of the formulation's presence in the bloodstream. By encapsulating the conjugated hydrophobic drug within a nanoemulsion formulation, effective delivery is achieved. Consequently, surface modification with PEG prolongs the circulation time of the drug, leading to increased accumulation through the EPR effect. Effective cellular uptake indicates that the nanoemulsion formulation is capable of delivering its therapeutic payload more efficiently than the conventional drug solution [110].

**Nanoemulsion as a therapeutic approach for ovarian cancer**

Platinum-based chemotherapeutic agents are extensively employed in the management of various cancers, with carboplatin and cisplatin – both of which incorporate platinum – demonstrating superior efficacy compared to alternative therapies for ovarian carcinoma [114]. Platinum compounds operate by inducing intra- and inter-strand crosslinks that compromise the integrity of DNA. A significant issue associated with platinum-based treatments is their cytotoxicity to normal cells, which can adversely affect non-malignant tissues. In addition, cancer cells may acquire resistance to platinum through various mechanisms, including the upregulation of membrane transporters, the activation of detoxifying enzymes, or enhancements in DNA repair pathways. Consequently, it is essential for ovarian cancer treatment to assess the sensitivity of the tumor to platinum agents, determining whether it is platinum-sensitive or platinum-resistant [115,116].

The advancement of nanomedicine has permitted the creation of formulations aimed at addressing toxicity and resistance [117]. However, overcoming these hurdles is difficult, especially given the lipophilicity of platinum complexes. Nanoemulsions provide a solution by increasing the distribution and efficacy of platinum-based medicines. These nanoemulsions may encapsulate huge amounts of hydrophobic medicines and have particular ligands on their surface, allowing for targeted administration [118]. In this research, a nanoemulsion was developed incorporating myrisplastin, a novel platinum-based therapeutic agent, alongside C6-ceramide, which serves as a pro-apoptotic compound. In addition, the formulation includes an EGFR-binding peptide functioning as a surface ligand, as well as gadolinium utilized as an imaging agent. The primary objective of this study is to investigate the effects of this formulation on ovarian cancer cell lines SKOV3, A2780, and A2780CP [116].

A cytotoxicity assessment demonstrated that SKOV3 cells, characterized by the expression of the EGFR, exhibit resistance to cisplatin, with an inhibitory concentration ($IC_{50}$) measured at 18 μM. In contrast, the encapsulation of myrisplatin resulted in a marked increase in cytotoxicity for both targeted and non-targeted nanoemulsions. Notably, the targeted nanoemulsions displayed a toxicity level that was double that of their non-targeted counterparts. The most pronounced enhancement in cytotoxicity was observed when ceramide was also encapsulated, suggesting a synergistic effect. Specifically, the targeted nanoemulsion containing both ceramide and myrisplatin demonstrated an efficacy that was 50.5 times greater than that of cisplatin. In addition, the A2780 and A2780CP cell lines, which lack EGFR expression, exhibited greater toxicity in response to myrisplatin compared to cisplatin [116].

Zheng *et al*. developed nanoemulsions based on Vitamin E that encapsulate paclitaxel, which have the ability to modulate the expression of Bax and BCL-2, proteins associated with tumor drug resistance, and inhibit the transport activity of P-gp [119]. These formulations were tested on the A2780 human ovarian cancer cell line, known for its resistance to paclitaxel [120]. The results indicated that the combination of PTX with the nanoemulsions enhanced antiproliferative effects while reducing mitochondrial potential. The authors suggest that the use of multifunctional nanoemulsions derived from Vitamin E for the delivery of anticancer agents may represent an effective strategy to address MDR in cancer treatment [119,120].

**NANOEMULSIONS AS A STRATEGY TO OVERCOME MULTIDRUG – RESISTANT**

MDR tumors present a significant obstacle to effective cancer treatment and, in conjunction with metastasis, are considered primary factors in cancer-related mortality (Fig. 4). The heightened expression of multifunctional efflux transporters belonging to the ABC gene family is recognized as a critical factor in the development of MDR in tumor cells [22]. ABC transporters utilize the energy derived from ATP hydrolysis to facilitate the efflux of a diverse array of endogenous substances. This includes proteins, lipids, metabolic byproducts, and pharmaceutical agents, particularly cytotoxic antibiotics [24].

The ABC transporter family comprises a wide array of proteins that play a significant role in the development of MDR against various anticancer therapies. P-gp, which is also referred to as MDR-1 or ABCB1 and is encoded by the ABCB1 gene, was the inaugural ABC transporter identified. This protein has the ability to transport out drugs such as vinblastine, colchicine, etoposide, and paclitaxel [22,24]. A variety of significant MDR transporters exist, including ABCA2, which is encoded by the ABCA2 gene and confers resistance to estramustine. The ABCC1 gene is responsible for encoding MRP1, a transporter that is prominently expressed in cancerous cells and facilitates resistance to various chemotherapeutic agents, including doxorubicin, vincristine, etoposide, colchicine, camptothecin, and methotrexate. In addition, MRP2, another transporter within this family, imparts resistance to vinblastine, cisplatin, doxorubicin, and methotrexate. This transporter is located on the membranes of polarized cells, such as those found in the kidney, liver, and intestinal epithelium. The ABCC3 gene encodes MRP3, which transports organic anions and medicines such as methotrexate and etoposide [22,24]. The ABCC4 gene encodes MRP4, which facilitates the efflux of methotrexate, 6-mercaptopurine, and 6-thioguanine (6-TG). In rare situations, a single medication may act as a substrate for numerous ABC transporters. MRP5 transports 6-mercaptopurine and 6-TG, whereas MRP6, MRP1, and MRP3 efflux etoposide. The ABCC11 gene encodes MRP8, which mediates resistance to 5-fluorouracil. The ABCG2 gene is responsible for encoding the MXR/BCRP proteins, which are associated with multixenobiotic resistance and breast cancer resistance. These proteins confer resistance to various therapeutic agents, including mitoxantrone, topotecan, doxorubicin, daunorubicin, CPT-11, imatinib, and methotrexate [22].

The results of this research indicate that oncologists are progressively seeking innovative pharmacological agents to target and inhibit the hyperactive ABC transporters, with the aim of enhancing the effectiveness of chemotherapy. A number of inhibitors and modulators

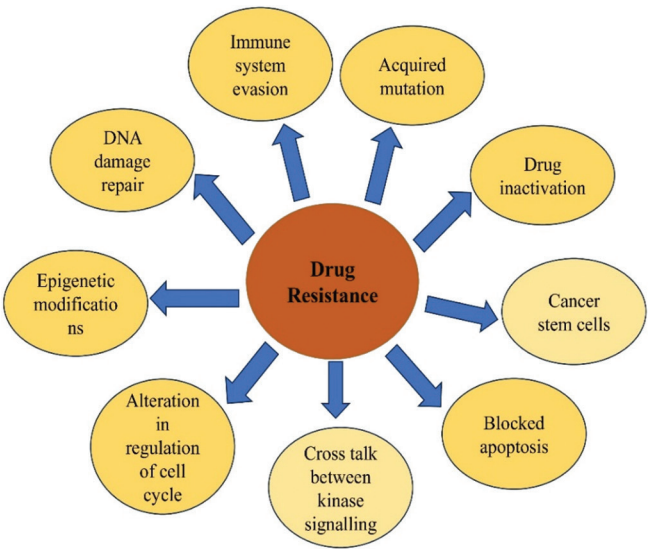


**Fig. 4: Various causes of anticancer drug resistance**

of ABC transporters, particularly those incorporated into functionalized nanoemulsions, have been explored as potential treatments for cancer-related MDR [24].

Ganta and associates developed folate-functionalized nanoemulsions designed to deliver docetaxel specifically to ovarian cancer cells, effectively addressing the issue of MDR associated with the drug [121]. The incorporation of folate in these nanoemulsions is a deliberate choice, given that the folate receptor is minimally expressed in most normal tissues while being significantly overexpressed in various cancers, such as epithelial ovarian carcinoma. The researchers investigated the expression of P-gp and found that endocytosis mediated by the folate receptor can circumvent the MDR mechanisms present in ovarian cancer cell lines. Consequently, the folate-targeted nanoemulsions successfully facilitated the delivery of docetaxel through receptor-mediated endocytosis, leading to enhanced cytotoxic effects that effectively counteract ABC transporter-mediated resistance [122]. Taxanes such as paclitaxel and docetaxel have been increasingly important in the treatment of breast cancer; yet, overcoming MDR remains a serious hurdle [122]. Meng *et al*. employed baicalein to inhibit P-gp and promote oxidative stress as a strategy to address this problem [122]. This approach aims to enhance the sensitivity of cells to paclitaxel by elevating the levels of reactive oxygen species (ROS) and glutathione (GSH), both of which are critical for cellular redox balance. The researchers found that by encapsulating paclitaxel and baicalein within nanoemulsions, there was a significant increase in ROS levels, a reduction in cellular GSH, and an enhancement of caspase-3 activity in MCF-7/tax cells. Importantly, an *in vivo* study on anticancer efficacy revealed that the baicalein-paclitaxel nanoemulsions exhibited substantially greater antitumor activity compared to other formulations of paclitaxel. These findings suggest that this co-delivery method could serve as a promising strategy for combination therapy aimed at overcoming MDR [123]. Zheng *et al*. investigated a novel technique to combat paclitaxel's MDR by creating nanoemulsions that modulated the expression levels of Bax and Bcl-2 while simultaneously decreasing P-gp transport function. They used a Vitamin E derivative called TPGS, which is renowned as a powerful surfactant and P-gp inhibitor capable of reversing MDR in cancer cells [119]. Vitamin E alters the connections between Bcl-xL and Bax, activating Bax and promoting mitochondrial-centered apoptotic cell death [124]. As a result, Vitamin E-based nanoemulsions containing paclitaxel were found to be acceptable for investigations on paclitaxel-resistant human ovarian cancer cells [119].

## NANOEMULSIONS FOR NANOTHERAGNOSTICS

Nanotheragnostics represents a novel approach that integrates the imaging and diagnostic functionalities of nanotechnology. The primary objective of this approach is to engineer nanoscale entities capable of executing both therapeutic and diagnostic functions. This methodology encompasses a range of nanotechnological techniques, particularly focusing on nanoemulsions, with a significant emphasis on the treatment and diagnosis of cancer. Recent progress in this field has facilitated the characterization of specific tumors, the prediction of interactions between nanoemulsions and tumor cells, and the formulation of personalized nanomedicines aimed at targeted therapeutic interventions [125].

In this framework, Fernandes and associates developed perfluorohexane nanoemulsions as innovative modalities for drug delivery and as contrast agents for *in vivo* ultrasound and photoacoustic imaging of cancer. When contrasted with conventional optical techniques, these nanoemulsions demonstrate enhanced tissue penetration and superior spatial resolution. This approach presents a non-invasive option for cancer imaging and treatment, thereby reducing the need for more invasive procedures and offering significant advantages for patient care [52].

Wu *et al*. employed a comparable approach by developing magnetic nanoemulsion hydrogels designed to promote the regression of tumors through the application of ferrofluid-based magnetic hyperthermia in cancer therapy [126]. Patel *et al*. have innovatively formulated nanoemulsions that incorporate three distinct platinum-based chemotherapeutic agents: Dimyrisplatin, dipalmiplatin, and distearyplatin. These nanoemulsions, composed of fatty acids, exhibit a specific affinity for the folate receptor $\alpha$, which is frequently overexpressed in various cancers, including ovarian cancer. This targeted approach utilizes receptor-mediated endocytosis to effectively bypass mechanisms of resistance present at the cell surface. In a related study, Roberts and his team employed sonophore molecules to conduct multi-spectral optoacoustic tomography for cancer detection. By integrating near-infrared dyes into their nanoemulsions, they successfully identified tumors *in vivo*, thereby avoiding the need for invasive procedures [127].

## LIMITATIONS OF NANOEMULSIONS

Nanoemulsions have tremendous potential for delivering medications to cancer cells because they have been shown to be safe and capable of targeting specific tissues, increasing treatment efficacy while limiting toxicity. However, no nanoemulsion-based formulation has achieved FDA approval. Several crucial aspects play an important part in determining the performance of such systems, as they may have limitations [2].

The formulation process for drugs may necessitate high temperatures and pressures, which vary depending on the drug and excipients. As a result, not all beginning materials are suitable for various manufacturing processes. In such circumstances, it may be essential to create an appropriate production technique or optimize an existing one, which might be time-consuming. It is critical to ensure that sensitive medications can be produced in big quantities. The multiple factors required in creating multifunctional nanoemulsions for large-scale manufacturing can make it especially difficult. To determine an appropriate procedure for a certain nanoformulation, it is critical to examine material safety, scalability, and all areas of quality control [1,2].

The behavior of nanoemulsion systems, their constituents, and there *in vivo* metabolism must be thoroughly examined. Each chemical has various properties, including unique metabolic pathways. Absorption, distribution, and excretion all have a substantial impact on drug efficacy and safety, necessitating ongoing monitoring [2]. Targeting nanoemulsions for cancer medication delivery remains a significant issue, with research indicating that just 0.7% of drug doses supplied by nanotechnological techniques reach solid tumors. Metabolism is critical in this process because only nanoemulsions capable of escaping the MPS and overcoming renal clearance barriers can effectively interact with tumor tissues [64].

When a new substance is introduced into a formulation, its long-term stability and safety must be thoroughly assessed. The dilemma stems from the limits of the models employed to measure toxicity, which frequently lack the dynamic interactions found in human tissues under real physiological settings. Typically, research begins with cellular models or animal species that differ significantly from humans in terms of features and metabolism. Furthermore, individual responses can vary greatly depending on gender, ethnicity, age, environmental influences, and other factors. These differences hamper the process of translating findings from *in vitro* or *in vivo* experiments into viable real-world therapies [1].

The primary constraint of nanoemulsions in the context of cancer drug delivery stems from the limited clinical applicability of these formulations [64].

## FUTURE PERSPECTIVES

Nanoemulsions are drug delivery systems capable of encapsulating both hydrophilic and hydrophobic molecules, specifically designed to meet diverse requirements [2].

The primary challenge for the future development of nanoemulsions is to discover new mechanisms that will increase their efficiency and distinguish them from existing drug delivery systems. Ongoing research must consider the drug's interactions with other components in the system, the consequences of production processes, and the drug's stability. Furthermore, understanding how nanoemulsions interact with target cells remains a critical area of research, with a focus on novel strategies for enhancing drug release and uptake. Another intriguing avenue to pursue is the investigation of various administration routes for nanoemulsions containing cancer medications. The overarching goal is to gain fresh insights into nanoformulation, paving the way for new possibilities in anticancer drug delivery [1].

The application of nanoemulsions as imaging agents is gaining traction, offering real-time monitoring of cancer while minimizing tissue damage and invasiveness. Conventional imaging methods, such as X-ray tomography, magnetic resonance imaging, and ultrasound, typically rely on labeling a targeted nanoemulsion with a radioactive isotope or a fluorophore.

Vaccine carriers targeting tumors using nanoemulsion formulations are also in advanced phases of research. As previously stated, nanoemulsions can effectively transfer macromolecules, such as antigens, to elicit a specific immune response. These methodologies enable extended circulation durations and promote the absorption by cells that possess the corresponding antibody for the antigen present on their surface, or the other way around, leading to highly targeted interactions [2].

## CONCLUSION

Nanoemulsions represent a promising and innovative approach in the domain of oncological therapeutics. Their hydrophobic core facilitates the encapsulation of lipophilic pharmaceutical agents, effectively overcoming a significant obstacle in cancer treatment by augmenting drug solubility and bioavailability. The incorporation of emulsifying agents and GRAS excipients contributes to the formulation of a stable and secure product. Due to their diminutive particle size, nanoemulsions are capable of prolonged circulation, thereby enhancing drug retention and therapeutic efficacy.

Nanoemulsions present a considerable advantage over traditional pharmaceutical carriers due to their capacity to be meticulously engineered for the specific targeting of neoplastic cells while circumventing the challenges posed by MDR. This represents a noteworthy advancement in oncological treatment, as one of the most formidable challenges is the pronounced toxicity of numerous anticancer agents to non-malignant cells and tissues, coupled with the propensity of cancer cells to develop resistance mechanisms. The phenomenon of passive targeting, which utilizes the EPR effect observed in neoplastic tissues, provides a foundational mechanism for drug delivery. Nevertheless, active targeting further amplifies these benefits by integrating the EPR effect with specialized targeting strategies aimed at malignant cells.

Multifunctional nanoemulsions confer supplementary advantages by encapsulating or adhering various substances to their surfaces, which can impede MDR mechanisms. The illustrations provided within this manuscript elucidate diverse methodologies for the fabrication of emulsions aimed at achieving superior therapeutic results across an array of oncological conditions. Nevertheless, all of these benefits become moot if the production processes and metabolic pathways of the pharmaceutical agent and its excipients are not meticulously examined. These issues continue to represent the principal obstacles in the development of nanoemulsions. For such formulations to advance through the entirety of clinical trial phases and secure regulatory approval, every variable must be scrupulously analyzed. Pioneering strategies are essential to formulate anticancer therapies that possess both safety and efficacy. The formulation of such preparations is of paramount importance in oncological treatment, as this intricate ailment accounts for a considerable fraction of mortalities, and no effective therapeutic solution has been identified to date.

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## AUTHORS' CONTRIBUTIONS

SOUMIK MAITY: Writing – original draft, and Editing. INDRANIL BANERJEE: Conceptualization, Reviewing, Editing, and Supervision.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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