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REVIEW ARTICLE

Curcumin, Epigallocatechin Gallate, and Ursolic Acid-Based Formulations for Targeting Tumor Microenvironment in Oral Squamous Cell Carcinoma

Omkar Kokate, Neeta Rai, Narendra Vishwakarma, Aryan Vishwakarma, Viren Mandlecha, Piyush Jangid

Department of Pharmacy , School of Pharmacy, Vishwakarma University, Pune, Maharashtra, India

Abstract

Despite being a major public health problem, especially in developing nations, oral squamous cell carcinoma (OSCC) continues to pose great risks to global populations owing to its high frequency and poor survival prognosis, in addition to treatment failure. Chemotherapy, radiation, and surgical excision are examples of conventional methods that have adverse effects and even drug resistance. Recent literature reveals the importance of the tumor microenvironment (TME) in determining tumor progression, metastasis, and drug resistance. It can therefore serve as a new target in cancer therapy. This article reviews the use of bioactive phytochemicals as agents that modulate the TME and increase the efficiency of anticancer drugs in OSCC. These phytochemicals have various mechanisms of action; these include but are unrestricted to antioxidant, anti-inflammatory, apoptosis-inducing, and antiproliferation properties. The drawback in their clinical applications includes poor solubility, low bioavailability, and quick metabolism. Nanocarrier-based drug delivery techniques that have the potential of overcoming these weaknesses have been developed. Among these are nanoemulsion, liposome, nanogel, and polymer nanoparticles. They provide stability to the drug, increase its bioavailability, allow targeted delivery of drugs to tissues, and decrease the toxicity of such phytochemicals.

Key words: Anticancer therapy, curcumin, epigallocatechin gallate, oral squamous cell carcinoma, tumor microenvironment, ursolic acid

INTRODUCTION

Overview of oral squamous cell carcinoma (OSCC)

OSCC is the 6th most prevalent malignant disease. It accounts for 5% of all tumors worldwide. In India, it makes up 45% of all tumors. The 5-year survival rate is less than 50%. According to the Globocan report 2020 lip and oral cavity cancer cases, in India are 10.3%. Several risk factors, including tobacco use, alcohol consumption, and infections due to human papillomavirus and Epstein–Barr virus, amongst others, are responsible for the development of OSCC, all of which have a high prevalence in India. The treatment of OSCC typically requires a multimodal approach that includes radiation, surgery, and chemotherapy, either alone or in combination. The epidermal growth factor receptor monoclonal antibody cetuximab is a kind of targeted therapy that has increased overall survival and clinical efficacy, but it has also caused major side effects and the development of drug resistance. Thus the need for combination therapy to potentiate chemotherapics is the need of time.[1]

Challenges in current therapy

Photodynamic therapy (PDT), however, still has defects; for example, the poor performance of photosensitizers (PS), including non-specific targeting of tumor tissues and poor stability. Hematoporphyrin Derivative, the first-generation

Address for correspondence:
Omkar Kokate, School of Pharmacy, Vishwakarma University, Pune, Maharashtra, India.
Phone: 8602740665.
E-mail: neeta.rai@vupune.ac.in

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PS, has some therapeutic usefulness, but it still has drawbacks such as low targeting accuracy, poor penetration ability, and significant toxic side effects. The second-generation PS addresses many of those issues. A PS is anchored on the second half of a carrier, which is designed to target localization, while having some properties of altering the structure of the original PS for greater targeting toward their tissue sites and thus towards more effective PDT therapy. Clinical practice has made substantial use of blue light, which has a wavelength of about 630 nm. PDT can only efficiently treat superficial lesions due to its modest tissue penetration depth. The amount of oxygen in the tumor tissue also affects how well PDT works. Hypoxia, which is characterized by a low oxygen concentration in tumor tissue relative to normal tissue, is frequently seen in OSCC. This is due to the rapid proliferation of tumor, an imperfect vascular system, etc. Moreover, PDT itself also uses oxygen while it acts on the tumor tissue and intensifies the hypoxia issue. Hence, how to enhance the oxygen content of tumor tissue is one of the keys for promoting the PDT effect. PDT in the oral cavity has been reported to be influenced by environmental factors that are unique to the oral cavity. The oral cavity holds a lot of important, sensitive, and vulnerable structures. To avoid harm or complications, PDT must be careful to protect healthy tissues and organs. Adverse effects, including burns, edema, ulcers, bleeding, varying degrees of dysfunction, and others, might also manifest to some degree in the oral cavity following PDT treatment. Patients' oral function and quality of life are directly impacted by damage to the oral anatomical structure. Saliva in the mouth may lower PS concentrations, limit PDT efficiency, and cause light source irrigation. As of right now, OSCC's PDT therapy procedure lacks both set standards and procedures. Several factors influence the therapeutic response to PDT, including the type and dose of the PS, properties of the light source (type and fluence rate), and irradiation time. Exploring the PDT mechanism, since there is no standardized PDT protocol across different research centers and clinical institutions, the results obtained may not be as comparable or reproducible. On the other hand, the absence of standard procedure possibly makes patients feel apprehensive and ambiguous that impact on their decision to choose AND acceptance.[2]

Importance of targeting tumor microenvironment (TME)

The TME might not only take part in immunosuppression, but also in angiogenesis, invasion, and metastasis. It may even contribute to tumor resistance to conventional therapies, including surgical, chemotherapy, and radiotherapy ones. Various types of mechanisms are responsible for tumor resistance. For instance, hypoxia may have an effect on radiotherapy, whereas desmoplasia may hinder chemotherapy; however, immunosuppression affects all these therapies in general, having the highest impact on immunotherapy. In addition, all mechanisms of tumor resistance in connection with TME factors may also serve as therapeutic targets, helping conventional therapies become more successful. At the same time, it is evident that conventional therapies are able to affect the microenvironment itself, making it unable to destroy tumors. Moreover, examination of the microenvironment may give an accurate prognosis and thus affect treatment plans, for example, selection of certain adjuvant therapies. Searching for predictive biomarkers will be easier in case of the TME, because the microenvironment is relatively stable compared to the tumor.[3]

Introduction of herbal bioactive compound

The phytochemicals present in plants have a significant biological impact since they are naturally occurring compounds having medicinal uses besides those associated with macronutrients and micronutrients. They are classified as secondary metabolites that exhibit various biological activities such as antioxidant, antimicrobial effects, detoxifying enzymes' regulation, immune system modulation, inhibition of platelet aggregating effects, hormones metabolism, and anticancer effect.

Phytochemicals are usually rated according to their role in the metabolic processes within the plant body into primary or secondary phytochemicals. More than 4,000 phytochemicals have been documented, while only 150 phytochemicals have been thoroughly analyzed. Carbohydrates, amino acids, nucleic acids, proteins, chlorophyll, purines, and pyrimidines are the main phytochemicals. Alkaloids, terpenes, flavonoids, lignans, steroids, curcumin, saponin, glucosides, and phenolic compounds are among the numerous secondary phytochemicals.[4]

TME IN OSCC

Overview of TME in OSCC

Immune cells, fibroblasts, vascular endothelial cells, and extracellular complexes make up the TME, which is intimately linked to the development of OSCC. The cells within the TME can generate complicated cellular networks through the secretion of RNA, protein, cytokine, and metabolic substances. In addition, the proliferation, invasion, metastasis, and angiogenesis of OSCC are all influenced by the interactions between cells inside TME. One of the most important components of OSCC's immunological microenvironment is T cells. Depending on their different surface markers and functions, T lymphocytes can be classified as regulatory T cells (Tregs), T helper (Th) cells, cytotoxic T lymphocytes (CTLs), mucosal-associated invariant T cells, natural killer (NK) cells, gd T cells, etc. CTLs have an immunologic role by controlling and eliminating tumor cells. Th cells also help immune cells eradicate harmful germs. NK cells and CD8+T cells also

exhibit the capacity for recognition and elimination of the tumor cells produced by Tregs which create an environment that is immunosuppressive and hence facilitate tumor progression. Evidence shows that suppression of SLC3A2 helps in immune escape and metastasis through reducing the capability of the immune system of T lymphocytes in OSCC. By modifying signaling pathways such as the VitD signaling route to control the production of Tregs and Th cells, the TME suppresses the immune system. In OSCC, CTL, and Th cell functions are compromised.[5]

TME role in OSCC progression

The TME is very essential in OSCC development. The components in the TME include various elements like the cancer cells themselves, stromal cells (including cancer-associated fibroblasts [CAFs] and endothelial cells), immune cells, and extracellular matrix (ECM).

Tumor growth and metastasis

The TME is very important in tumor development and metastasis niche formation. Interaction between the cancer cells and stromal cells, especially CAFs, increases tumor aggressiveness by promoting cell proliferation and invasion. CAFs produce different growth factors and cytokines that induce cell proliferation and ECM remodeling to provide an appropriate environment for tumor cell invasion.

Therapeutic resistance

In addition, the TME provides considerable help in therapy resistance, especially in OSCC. There are several mechanisms involved in therapy resistance, especially conventional therapies such as chemotherapy and radiotherapy. CAFs and myeloid-derived suppressor cells (MDSCs) present in the TME create a microenvironment that favors therapy resistance. CAFs can produce factors responsible for drug resistance by activating survival pathways in cancer cells, thereby reducing their sensitivity to chemotherapy treatment.[6]

OVERVIEW OF SELECTED PHYTOCHEMICALS

Curcumin

Curcumin was identified as diferuloylmethane (curcumin) in 1910, but it was first isolated from turmeric in 1815. Curcumin is a crystalline orange-yellow compound used as an additive and food coloring. Curcumin dissolves readily in polar and non-polar organic solvents as well as extremely acidic solvents like glacial acetic acid, despite being weakly soluble in water with an acidic or neutral pH range. Curcumin has a chemical formula of C21H20O6 and a weight of 368.38 Da. Its melting point is 183°C. Animal models, such as mice, rats, or dogs, have been used in the majority of curcuminoid research.[7]

Curcumin protective role in cancer

The generation of free radicals and harmful metabolites through oxidative stress is a key factor in the initial phase of the development of cancer. Antioxidants can aid in the prevention of cancer initiation. Since curcumin traps free radicals, it could contribute significantly in inhibiting cancer initiation. *In vitro* and preclinical trials have shown that curcumin inhibits oxidative-induced DNA damage, for instance, ionizing radiation, by limiting free radicals and active oxygen species. The nuclear factor kappa B (NF-kB) contributes to the synthesis of nitric oxide synthase and oxidative stress, thus causing cancer. Curcumin prevents cancer initiation by suppressing the formation of NF-kB. Curcumin was proven effective in liver enzymes, cytochrome P450, that play an indispensable role in toxin oxidation and detoxification; curcumin also inhibits phase I enzymes that participate in generating harmful metabolites and carcinogenic substances. On the other hand, curcumin activates phase II enzymes that aid in detoxifying harmful metabolites. By inhibiting phase I enzymes and activating phase II enzymes, curcumin prevents tumor development and expansion.[8]

Epigallocatechin gallate (EGCG)

EGCG is a polyphenolic catechin extracted from leaves of *Camellia sinensis* (Green Tea). ECGC is the major bioactive catechin component found in *C. sinensis* leaves, accounting for more than 40% of total catechins, and possesses multiple biological activities such as antioxidative, anti-inflammatory, antibacterial and antineoplastic activities. EGCG possesses a chemical structure consisting of a flavan-3-ol backbone having hydroxyl groups attached to it and hence is able to scavenge and neutralize free radicals. The molecular formula and molecular mass of EGCG are C22H18O11 and 458.38 g/mol, respectively. EGCG exists in the form of colorless to pale yellow crystals and is highly water soluble, although its high instability makes it less bioavailable. It was initially discovered as a beneficial compound due to its existence in green tea and has since been receiving extensive attention for its anticancer activity in breast, prostatic, hepatic, and cervical cancer cells.[9]

EGCG protective role in cancer

The effectiveness of using EGCG to treat OSCC showed that the cancer cells' vitality was decreased. The cell cycle study results also showed that EGCG caused cell cycle arrest in the G1 phase, which enhanced the amount of apoptotic cells and the activities of caspase-7 and caspase-3 in comparison to controls. By preventing cell viability and triggering CAR apoptosis and autophagy, this catechin clearly demonstrated

anticancer action. In addition, EGCG enhanced the expression of proteins such as Bax, cleaved caspase-9, and cleaved caspase-3 in the cancer cells, as well as the activity of caspase-3 and caspase-9. It is noteworthy that this chemical treatment reduced the expression of multidrug resistance genes and proteins. According to reports, EGCG, the main component of green tea, significantly inhibits the activation of indoleamine 2,3-dioxygenase, which is activated in cancer cell lines when gamma-interferon is present. Furthermore, EGCG prevents indoleamine 2,3-dioxygenase from being induced at the transcriptional stage. Because EGCG inhibits Met phosphorylation, which in turn prevents cell invasion, proliferation, and production of matrix metalloproteinase (MMP-9) and 2, it has been shown to be useful in preventing the formation and invasion of oral cancer *in vitro*. In an oral squamous cell line, a recent study has clarified the molecular mechanism of action behind this catechin's antimetastatic activity. The outcome was a total prevention of cell invasion because MMP-2 expression was downregulated.[10]

Ursolic acid (UA)

UA, also known as 3β-Hydroxy-urs-12-ene-28-oic acid, is a pentacyclic triterpenoid molecule that prefers oil. UA, a white powder with a molecular weight of 456.68 g/mole, was first identified in 1920 in the wax layer of apple leaves. UA is primarily found in some plant species and foods, such as apple, cranberry, prune, and bilberry. It is often extracted from plant leaves, stem bark, and fruit peel. Historically, UA was traditionally used for its health benefits by virtue of its herbal extract formulation in medicines. Since UA is readily available in common edible plants, it is perceived to possess extremely low or no toxicity to human health. Due to the fast advancements in the study of traditional medicines, numerous pharmacological and physiological effects of UA have been documented, which include its antioxidant, anti-inflammatory, antidiabetic, antibacterial, and antitumor properties. Moreover, UA has been utilized in protecting the human body from cancer.[11]

UA role in prevention of cancer

It has been reported that UA can display its anticancer properties through induction of apoptosis and inhibition of migration and invasion. In addition, it has been noted that UA can target certain signaling pathways that are important in the development of pancreatic cancer. For instance, it has been shown that UA can prevent the activation of NF-κB, a transcription factor that is important for the development of cancer and inflammation. As a result, it can lower pro-inflammatory and pro-survival gene expression. In addition, UA affects the PI3K/Akt/mTOR signaling pathway, which is dysregulated in pancreatic cancer and increases cell survival and proliferation. Preclinical trials on animal models have displayed favorable results regarding the effectiveness of UA as an anticancer agent in pancreatic cancers; decreases growth of tumor and better survival outcomes were obtained. There is a need for further research to explore the full potentials of UA for treating pancreatic cancers. More clinical trials are required to assess the effectiveness of UA as monotherapy as well as a combination therapy with conventional treatments.[12]

NEED FOR ADVANCED FORMULATION

Natural products are one of the best drug discovery agents, particularly when used in anticancer drugs. Natural products face a number of issues during their delivery in the body due to low solubility, poor absorption, improper size, instability, poor permeability, and first-pass effect. Therefore, it becomes important to investigate new delivery routes of natural products to make them useful for treatment. Nanotechnology is the most sophisticated technology through which cancer can be treated without any harm to healthy cells, only targeting cancer cells. Therefore, the current study discusses the advances made in recent years in developing new strategies of delivery of active natural products, which increase the availability, efficacy, and safety of the natural products in treating and preventing oral cancer. Some of the suggested strategies include liposomes, microemulsion, solid lipid nanoparticles (NPs), polymeric NPs, liquid crystals, and polymer-stabilized liquid crystals to overcome some problems in herbal medicine, food supplements, and essential oils formulations. Biotechnological strategies may provide solutions to improve the bioavailability and bioactivity of herbal medicine formulations, since various drugs' pharmacokinetics and pharmacodynamics have been modified using nanotechnology. There have been some methods adopted to achieve the objectives, which include changing the particle size, surface modification, and adding a polymer coating to increase the stability of the formulation.[13]

TYPES OF FORMULATION FOR DELIVERY

Nanoemulsion

These formulations will be safe and will not irritate the skin, as well as being suitable for use on the skin. The presence of nano-sized droplets in the drug formulation will affect the transportation properties, which is important for targeted and sustained drug delivery. Due to their small sizes, there will be some sediments but no creaming. With the small droplet sizes, the gravitational forces will be small, and thus Brownian movement might be enough to counteract these gravitational forces. In the gastrointestinal tract, peptide medications will hydrolyze enzymatically. Peptide medications can be administered by nanoemulsions. They boost absorption at cheap energy costs since they are made up of tiny droplets with large surface areas. The toxicity of oil-soluble medications can be tested using the cell culture approach. To increase the drug's bioavailability, vesicles can

take the place of liposomes. These vesicles can be found in lotions, liquids, sprays, and foams, among other forms. This formulation just has to contain biocompatible surfactants in order to be administered orally, which is beneficial for both humans and animals because it does not harm tissues.[14]

Liposomes

They are an efficient drug delivery system for herbal extracts since they are biodegradable and enhance the paracellular and transcellular transfer of medications. Phospholipids with hydrophilic and lipophilic components make up liposomes, which are drug delivery vehicles. They are self-assembling molecules that resemble the functions of the cell. There are two groups of liposomes, namely the polar group and the non-polar group. Liposomes are classified based on their lipid bilayers to be either multilamellar (>400 nm) or unilamellar (<20 nm). The multilamellar liposomes contain concentric phospholipid bilayers, while the unilamellar liposomes contain phospholipid bilayers enclosing the aqueous phase. Liposome delivery methods can be divided into four generations based on how they are used: standard liposomes, stimulus-responsive liposomes, stealth liposomes, ligand-targeted liposomes, and theranostic liposomes (which include transferosomes and niosomes). Liposomes have medicinal value in addition to their imaging capabilities. Therapeutic medicines can react to both internal and exterior stimuli thanks to controlled release, precise targeting, and biomarkers. Therapeutic medicines are added to either the hydrophilic core or the lipophilic bilayer to create theranostic liposomes.[15]

Nanogel

The nanogel is referred to as a NP (10–100 nm) that has been produced by the formation of the hydrogel through either chemical or physical linking of polymers. The cross-linking properties of the nanogels make it an excellent platform for the delivery and storage of the drug. There have been indications that it may be a good way of releasing active drugs in order to avoid the immunogenic property.[16]

Nanocapsule

It is a nanovesicular colloid formulation characterized by its unique core-shell design, whereby the drug is entrapped inside the reservoir or cavity protected by a polymeric shell or membrane. The core can enclose the drug in either liquid form, where it forms an oily or aqueous core, the solid state, or as molecules. The main factors influencing NC, particularly the release mechanism, are the core-shell structure and formulation. In addition, depending on the manufacturing method and component ingredients, the formulation may be hydrophobic or lipophilic. Changing the oral bioavailability of weakly hydrophilic active chemicals is the main goal of this formulation's development. As suggested by various scholars, other benefits of using NC as a drug carrier are the high drug loading capacity due to the enhanced solubility of the drug within the core, less polymer loading than other systems, drug protection from degrading agents such as pH and light by virtue of being enclosed within the polymeric shell, and the decreased likelihood of tissue irritation owing to the polymeric shell.[17]

Polymeric NPs

Polymer NPs consist of biodegradable and biocompatible polymers, thus providing an effective and controlled form of drug delivery system. Polymer NPs can be used as a drug delivery strategy since they can be targeted. Polymer NPs are colloids that function as vectors for drug delivery by ensuring delivery at specific sites. These types of particles have the potential of enhancing solubility of the components, reducing dose of the drug used, and increasing the efficacy of active compounds compared to conventional drug delivery systems. NPs have various benefits in the bloodstream as they are non-toxic, non-thrombogenic, non-immunogenic, and non-inflammatory.[18]

A comparative overview of advanced phytochemical-loaded nanocarrier formulation for targeting the TME in OSCC is presented in Table 1.

MECHANISM OF TME TARGETING BY FORMULATION

Numerous factors, either directly or indirectly, contribute to the development of tumors, including interleukins' (IL) dysregulation of inflammation, transforming growth factor- and IL-10's impairment of the host immune response, angiogenesis brought on by high vascular endothelial growth factor levels, and the overexpression of anti-apoptotic molecules like bcl-2 and X-IAP, which reduce apoptosis. The TME surrounding the tumor site involves a variety of immune effectors, and these immune effectors typically lose their anticancer effectiveness. In addition to losing their capacity to halt tumor growth, they also promote the growth of tumors. These cells include macrophages, MDSC, and regulatory T cells. Tumor development results from these cells' survival in the TME. The other component of TME is tumor-infiltrating lymphocytes (TIL); however, the role of TIL in tumor development is still debatable. However, according to recent clinical findings, TILs are considered to play a part in tumor development, particularly in cases of breast cancer and colorectal cancer.[19]

Passive targeting

Passive targeting is achieved through the uptake of the nanocarrier molecules via the passage of the molecules

Table 1: Types of Phytochemical-Based Nanocarrier Systems, Their Characteristics, and Potential Applications in Oral Squamous Cell Carcinoma (OSCC) and Tumor Microenvironment (TME) Targeting

Formulation type	Approx. size range	Suitable phytochemical	Main advantage	Role in OSCC/ TME	Example application
Nanoemulsion	20–200 nm	Curcumin, EGCG, ursolic acid	Improve solubility, permeability, bioavailability.	Enhanced penetration, passive targeting, improve uptake in tumor tissue	Curcumin nanoemulsion for oral cancer delivery
Liposome	50–400 nm	Curcumin, EGCG	Biocompatible, dual loading of hydrophilic and lipophilic drugs and targeted delivery.	Can be surface-modified for active targeting in OSCC	EGCG/curcumin liposomal delivery
Nanogel	20–200 nm	Curcumin, EGCG, ursolic acid	Sustained release, mucoadhesion, local retention.	Useful for local oral cavity delivery and prolonged contact with lesion	Mucoadhesive nanogel for oral lesion targeting
Nanocapsule	50–500 nm	Curcumin, EGCG, ursolic acid	Drug protection, controlled release, improved stability.	Protect phytochemical and improves delivery to tumor site	Herbal nanocapsule system
Polymeric nanoparticle	10–1000 nm	Curcumin, EGCG, ursolic acid	Controlled release, site specific delivery, improve efficacy.	Useful for targeted and sustained delivery in TME	Curcumin polymeric nanoparticle system

EGCG: Epigallocatechin gallate, TME: Tumor microenvironment, OSCC: Oral squamous cell carcinoma

through the leaky tumor fenestrated capillaries into the tumor interstitium and tumor cells by convection or diffusion. The movement of molecules in fluids is known as convection. When the net filtration rate is zero, convection should be the primary means of moving big molecules through big pores. However, for molecules of lower molecular weights like oxygen molecules, their transportation is mostly through diffusion. Diffusion is the mechanism by which molecules pass through a concentration gradient in a cell membrane without requiring cellular energy. Due to the tumor interstitium's hypertension, convection through it is ineffective.

Active targeting

Targeting ligands are conjugated to the surface of nanocarriers for interaction with receptors expressed at the target sites. The selection of targeting ligands is based on the presence of a specific type of receptor that is highly expressed by cancerous cells or tumor blood vessels and does not occur on healthy cells. In addition, the selected targeting receptors must express uniformly in all target cells. Monoclonal antibodies and antibody fragments or non-antibody targeting ligands (peptidic or otherwise) are the two categories of targeting ligands. Tumor penetration is influenced by ligand binding affinity via the "binding-site barrier." Strong ligand-receptor interaction is necessary for targets where cells can readily interact due to the fluid environment, such as tumor blood arteries.[20]

MODULATION OF TME

The TME, containing immunosuppressive cells and soluble factors, abnormal vasculature, and ECM, displays incredible resistance to current immune-targeted therapies. Recent developments in the field of nanotechnology and biomedical engineering provide exciting potential for the local (tumors) and systemic (lymph nodes) modulation of the TME by transfer of immunomodulators to restore the cancer immunity cycle. The application of NPs in immunotherapy approaches, alone or combined with chemotherapy, gene therapy, phototherapy, and radiotherapy, has revolutionized cancer treatment. This review outlines *in vivo* studies with different immunotherapy agents as related to the NP type, delivery method, and immune control.[21]

NANOEMULSION BASED DELIVERY

Overview of nanoemulsion

Those emulsions having a mean diameter between 50 and 1000 nm are known as nano-emulsions (NEs). Mini-emulsion and submicron emulsion, which can be defined as emulsions with an average diameter of 100–500 nm, are the most commonly used names that may be regarded as synonymous among the numerous varieties of NEs. NEs are two-phase colloidal dispersions that use cosurfactant

and surfactant in specific ratios to create an oil phase and an aqueous phase. The functional characteristics of NEs are largely determined by the characteristics of both phases and surfactants. To formulate NEs, non-polar substances including essential oils, mineral oils, free fatty acids, and other lipophilic nutrients are taken into consideration as an oil phase.

Types of nanoemulsion

NEs come in four different varieties, including: Water-in-oil (w/o), Water-in-oil-in-water, Oil-in-water (o/w), and Oil-in-water-in-oil. These types can change as the emulsions' constituent parts are altered.[22]

Advantages of nanoemulsion

- Large surface area and high free energy, nanoemulsions are an excellent means of transportation
- Nanoemulsions are not toxic and do not irritate, so you can put them on your skin and mucous membranes without any issues
- You can take nanoemulsions by mouth if they have helpers called surfactants that are safe for your body
- Nanoemulsions do not hurt human cells or animal cells so they are good for people and animals to use for therapy
- Nanoemulsions help get oil- supplements into cells in a lab, which helps the cells grow and makes it easier to study the effects of oil-soluble medicines
- Nanoemulsions can be used of liposomes and vesicles, and you can even make special liquid crystals around the nanoemulsion drops
- Because nanoemulsions so small they can get through the rough parts of your skin, which helps the good stuff get in
- Making nanoemulsions is the step in making nanocapsules and nanospheres using special methods like nanoprecipitation and interfacial polycondensation, and nanoemulsions are really important for this process.[23]

Formulation component

Oil phase

Medium-chain triglycerides, propylene glycol monocaprylate ester, isopropyl myristate, castor oil.

Surfactant/co-surfactant

Polyoxy-35-castor oil, diethylene glycol monoethyl ether.

Co-solvent

Glycerol triacetate.

Aqueous phase

Distilled water.[24]

PREPARATION METHOD OF NANOEMULSION

People have come up with a ways to make nanoemulsion. The main goals of making nanoemulsion are to get the size just right between 100 nm and 600 nm and to make sure it is stable. To make a nanoemulsion system, you need a lot of energy. You can get this energy from machines or from the ingredients themselves. Here are some methods that people use to make nanoemulsion.

High pressure homogenizer

This method involves putting a lot of pressure on a mixture of oil, water, and special helpers called surfactants or co-surfactants. You need a machine called a homogenizer to do this. There are some issues with using a homogenizer, like it can be hard to make a lot of nanoemulsion, and the ingredients can get damaged. Furthermore, it can get very hot. With this method, you can only make a certain kind of nanoemulsion, where the oil is mixed with water. The oil is <20% of the mixture. You cannot make hard nanoemulsions with really small droplets, like <200 nm using nanoemulsion preparation and the high pressure homogenizer method, for nanoemulsion preparation.[25]

Phase inversion method

It is through this process that fine dispersion can be achieved through chemical energy derived from the changes in phase along the process of emulsification. Such changes in phase are brought about by composition changes at constant temperature or temperature changes at constant composition using the phase inversion temperature (PIT), discovered by Setia *et al*. through the changes in the solubility of polyoxyethylene-type surfactant with temperature. It is at higher temperatures that the surfactant becomes lipophilic due to dehydration of the polymer chain, and at lower temperatures, where the surfactant has a pronounced spontaneous positive curvature that leads to the formation of an oil-swollen micelles solution phase.

Sonication method

One other excellent way of making nanoemulsion would be through the process of sonication. It is through this process that the size of drops of the emulsion and even microemulsion is reduced. However, such a process cannot produce large amounts of nanoemulsion, but small amounts only.[26]

Low energy method

The low energy process involves the use of Nanoemulsions that are produced by means of the chemical energy present within the system or the chemical potential energy of the

individual elements. It is thought that the chemical energy released through the formation of an emulsion is the driving force behind the low energy process. This is due to a change in the spontaneous curvature of the surfactants from being positive to negative (w/o) or vice versa (o/w). The low energy process consists of microemulsion dilution, D phase emulsification, phase inversion composition, PIT, and spontaneous emulsification. By reducing the rate of agitation (1600 rpm), the low energy process becomes more energy efficient. Generally speaking, the low-energy process can be divided into two forms, namely, isothermal and thermal. The former is applicable to bioactive substances that are highly sensitive to heat. D phase emulsification, microemulsion dilution, phase inversion composition, and spontaneous emulsification are the main elements of the isothermal process.[27]

CHARACTERIZATION AND EVALUATION OF NANOEMULSION

Determination of particle size and polydispersity index

These two parameters of the nanoemulsion formulations were ascertained using the Brookhaven Corp. 90 Plus particle size analyzer system. These tests were carried out to guarantee the created compositions' consistency and stability. This device uses room-temperature dispersed light at a 90° angle. In a cuvette, the light was focused on the precise volume of the nanoemulsion. To get the mean values, the analysis tests were run 6 times.

Zeta potential determination

The Malvern Zetasizer Nano ZS90 was used for this test. Since the zeta potential is dependent on the electrophoretic mobility measurement, the unit of measurement is micrometers per second. Any particle is stable if its zeta potential is either <30 mV or more than + 30 mV.

pH determination

All created formulations were measured for pH using a calibrated pH meter, which was submerged in 30 mL of each formulation.[28]

Refractive index

The isotropic character of the formulation is indicated by the refractive index, which is the net value of the components of the nanoemulsion. It is a method for determining the formulation's transparency as well as the sample's thermodynamic stability. It is ascertained by placing a sample drop on a slide and using a refractometer to compare it with water that has a RI of 1.33. Formulation is transparent if the system's RI can be compared to that of water. A refractometer was used to calculate the refractive index.

Viscosity

It is a crucial feature of nanoemulsion. Viscosity, or the friction that occurs inside fluids, is the resistance to fluid flow. The Brookfield viscometer is the most widely used tool for measuring viscosity. The resulting nanoemulsion's viscosity was measured using the Brookfield DV-II+ Pro viscometer at 25°C without dilution by averaging three data points at a certain shear rate. After the mixture was in the beaker for 5 min, spindle measurements were taken at 0.5, 1, 2.5, and 5 rpm for 12 weeks at room temperature and sustained temperature. This implies that the viscosity of nanoemulsions increases with decreasing storage temperature. The dial that came with the viscometer was read and noted.

Entrapment efficiency

The calculation of the percentage encapsulation efficiency was done by measuring the amount of free or unencapsulated drugs present in the formulations. This is undesirable since it changes the characteristics of the drug molecule's release mechanism. The amount of drug encapsulated in relation to the total weight of the formulation was calculated using an equation after extracting the drug from the nanoemulsion formulation.

EE is equal to (amount of drug added − free (unentrapped) drug)/(amount of drug added) times 100.[29]

In vitro diffusion studies

Using a cellophane membrane and a Franz diffusion cell, the produced nanoemulsions' diffusion studies are carried out. Five milliliters of the nanoemulsion sample are put inside a cellophane membrane, and the dissolution medium is 250 mL of 25% methanolic phosphate buffer (pH 7.4). The temperature used for the diffusion experiments is 37 ± 1°C. Five milliliters of each sample were removed at intervals of 1, 2, 3, 4, 5, 6, 7, and 8 h in order to maintain sink conditions. After that, an equivalent volume of fresh dissolving liquid was added to each sample. An ultraviolet spectrophotometer set at 271 nm is used to analyze samples for drug content.[30]

TOXICITY AND SAFETY EVALUATION

In vivo bioavailability study

The improved formulations were evaluated *in vivo* in mature Wistar rats (200–250 g) of both sexes. The animals were kept in groups of six (n6) and had unrestricted access to food and

water. The ISF College of Pharmacy's Institutional Animal Ethics Committee-approved study protocol was adhered to. The investigations were carried out in accordance with the standards set forth by the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. Oral administration of the formulations (plain medicine and nanoemulsions) was done at a dose of 4 mg/kg body weight. A polyethylene cannula was placed into the jugular vein 5 days before to the commencement of the trials, and ketamine was used to put the rats to sleep.

The cannula was exteriorized in the dorsal region of the neck. The 0.2 mL blood samples were collected in heparinized Eppendorfs at 0.5, 1, 2, 4, and 8 h, and they were centrifuged at 5000 rpm for 10 min. Following separation, the plasma was stored at 20°C in a freezer until further analysis. Rat plasma samples were analyzed using a validated UV/VIS spectrophotometric technique after 45 mg of artemether was added to each Eppendorf. The drug plasma concentration measurements were computed using the calibration curve.[31]

FUTURE PERSPECTIVES AND CHALLENGES

Since green biomarkers are readily available, safe, and naturally occurring food components, their introduction in OSCC may be one of the most original and groundbreaking ideas in oral cancer research. Despite encouraging results on the possible use of green biomarkers in OSCC treatment, a number of obstacles must be overcome. Research is required to assess each green biomarker's bioavailability information, long-term toxicity, and enhanced permeability-retention effects. Before starting ongoing clinical trials using these biomarkers, biosafety with Food and Drug Administration approval should be taken into account. Future studies should demonstrate the green biomarkers' beneficial effects for providing maximum therapeutic potentials and reduced adverse effects by evaluating their level of efficiency using particular parameters such as pharmacokinetics, distribution, and bioavailability. A special consideration for studying the release profile index of the biomarkers needs to be identified as a research gap. Future studies on the relationship between green biomarkers and Immunohistochemistry indicators can provide precise ideas on the effectiveness of oral carcinogen detoxification and its advantages for early intervention.[32]

CONCLUSION

Conclusively, the development of treatments that target the TME is a revolutionary way of achieving efficient therapeutic intervention in OSCC cases. Phytochemicals such as curcumin, EGCG, and UA have been shown to have considerable anti-cancer properties based on their capability of modulating the cancer-associated signaling pathways.

Unfortunately, several challenges associated with these compounds, such as low solubility, instability, and poor bioavailability, prevent their clinical implementation. The incorporation of nano-carrier-based drug delivery systems like nanoemulsion technology offers the most practical approach for overcoming the mentioned limitations and enables both active and passive targeting methods. While preclinical results show positive effects of nano-formulated phytochemicals, future research studies will need to focus on establishing the safety and effectiveness of these compounds in clinical practice.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable. This manuscript is a review article and does not involve any studies with human participants or animals performed by the authors.

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