A Review Article On Different Preparation Methods Of Microbubbles In Cancer And Hyperlipidemia Treatment

Ms.Apurva Shelar, Mr.Satish Mendake, Om Malpote, Pratiksha Mandalik, Kiran Marathe, Pranav Mendgule, Prathamesh Mengade

Department of Pharmaceutics, Sinhgad Institute of Pharmaceutical Science, Lonavala, Maharashtra, India.

ABSTRACT

Microbubbles are small spherical gas-filled bubbles and have emerged as a potential approach for targeted drug delivery and disease treatment. Microbubbles have special characteristics like slow buoyancy, large gas-liquid interfacial area, and high mass-transfer efficiency. Their small size and ability to be activated by ultrasound (US) make them ideal carriers for delivering medications to specific body parts. The microbubble has various applications in cardiovascular, gene therapy, diabetes, cancer, and hyperlipidemia treatment. This review consists of Constituents used in preparing the microbubble (shell, Gas core) and different methods used to formulate the microbubble. The challenges in the formulation of the microbubble are discussed.

INTRODUCTION

Microbubbles are easy to use in medical applications, particularly for drug delivery and imaging. With a gas-filled core and a stabilizing shell composed of biocompatible materials, microbubbles can be produced to enhance therapeutic efficacy and minimize adverse effects. Their unique characteristics—such as their large surface area and ultrasound responsiveness—make them great options for targeted drug delivery systems, especially in severe diseases like cancer therapy and the treatment of hyperlipidemia.[1]

The process of producing microbubbles includes several steps, such as choosing suitable gas cores and shell materials, which affect the stability and efficiency of the bubbles in biological conditions. These materials can range from proteins and lipids to synthetic polymers. Recent developments in manufacturing processes, such as coaxial electrohydrodynamic atomization, high shear emulsification, sonication, and microfluidic devices, have made it possible to precisely regulate the size and characteristics of microbubbles, which have promoted their use in difficult therapeutic applications.

The microbubble formulations face various challenges impacting their effectiveness. Some of these challenges include stability, size control, drug loading efficiency, and biocompatibility.[3] The most common applications of microbubbles are in the treatment of cancer and hyperlipidemia.

Cancer

A condition known as cancer occurs when specific body cells proliferate out of control and spread to other tissues in the body. Human cells often divide (grow and multiply) to form new cells when the body requires them and replace old or damaged cells. This natural process has been disrupted, leading to damaged or abnormal tissue proliferation and growth. Tumors are aggregations of tissue that can be formed by these cells. Both malignant and benign tumors are possible. Different types of cancer are Multiple Myeloma, Melanoma, Sarcoma, Leukemia, Lymphoma, Brain, and Spinal Cord Tumors.[4]

Hyperlipidemia

Hyperlipidemia can be defined as the elevation of lipid (cholesterol, fats, and triglycerides) levels in the blood. this develops the plaque inside the blood vessels and restricts the blood flow. This condition significantly increases the chance of major cardiovascular problems, including liver and renal dysfunction, heart disease, and stroke.

There are two types of Hyperlipidemia first type is primarily inherited and commonly genetic and the second type is caused by conditions such as obesity, thyroid disease, thyroid disease, alcoholism, certain medications, and chronic kidney disease. [5]

MICROBUBBLES CONSTITUENTS

Shells

The shell can be made of proteins, lipids, surfactants, biocompatible polymers, or a combination of these substances. Some examples of shell materials include:
Protein shell microbubbles are used in ultrasound imaging and drug delivery because they can form the shell with a gas core, especially with air or perfluorocarbon gases. The most commonly used protein shell microbubble is human serum albumin. Phospholipid shell microbubbles coated with lipids are used for biomedical imaging and drug delivery.
A surfactant shell as a coating can offer additional protection and prevent the microbubble from absorbing large molecules, for example, Polyethylene glycol (PEG).
Polyelectrolyte multilayer (PEM) shells represent a novel category of polymer-surfactant shell hybrids.[7]

Gas core

1. The most commonly used is air because it can diffuse quickly in water-based environments, air-filled microbubbles are often less stable, have a shorter half-life, and are less effective in vivo.
2. Perfluorocarbon gases, such as perfluorobutane and perfluorohexane, are frequently used because of their low blood solubility, which slows gas diffusion and improves microbubble stability.
3. Sulfur Hexafluoride (SF6) is used due to its high molecular weight and poor solubility, which promote stability, SF6 has been used extensively in contrast-enhanced ultrasound imaging.
4. Oxygen microbubbles filled with oxygen are being developed for potential applications in cancer treatment to address tumor hypoxia.
5. Nitrogen has been incorporated into some formulations, but it tends to dissolve more quickly than perfluorocarbons, which reduces its stability compared to gases like sulfur hexafluoride and perfluorobutane.[8]

Microbubble structure
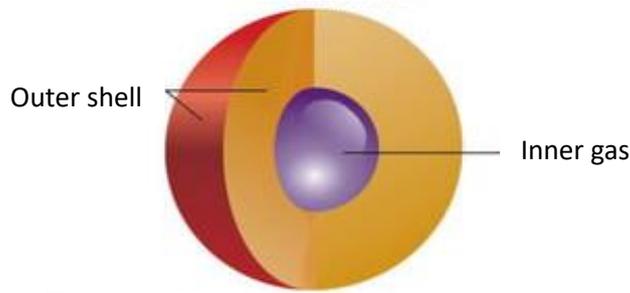
| Commonly used shell and gas in combination	
Stability coating (shell)	**Filling gas**
Phospholipid	Sulfur hexafluoride
Phospholipid	octafluropropane
Phospholipid	Air
Human serum-albumin	Air
Human serum-albumin	octafluropropane
Polymer protein bilayer	Air
Surfactant (PEG)	Dodocafluropentane

METHODS USED FOR FORMULATION OF MICROBUBBLES

Microbubbles are created by introducing a gas into a liquid medium using a variety of techniques, including Mechanical agitation, Sonication, and Microfluidic devices.

Ultrasonication Method

Sonication is the primary technique for creating microbubbles, where high-intensity ultrasound is used to disperse gas or liquid in a coating material suspension. Two mechanisms are believed to be responsible for this process. Initially, the gas or liquid is mixed to create a suspension of small droplets/bubbles that automatically absorb a coating of protein or surfactant on their surfaces. Furthermore, the elevated temperatures and pressures produced by inertial cavitation in the suspension cause a chemical alteration of the surface layer, enhancing its stability. For protein coatings, the reason for changes is the cross-linking caused by superoxide generated during water sonolysis; it has been observed that surfactant coatings undergo significant changes in structure, though the mechanisms are not fully understood. The size distribution of particles created is influenced by the frequency, power, and pulse regime of ultrasound, with fabrication methods being developed empirically due to the lack of a direct theoretical relationship between these variables. The range of sizes of microbubbles created through sonication is typically wide, so when planning to inject them intravenously, it is usually essential to separate and/or strain the suspension to eliminate bigger.[6]

Sonication is an inexpensive and straightforward technique, especially suited for minor manufacturing operations. It permits the formation of microbubbles without complicated equipment, making it a viable method in different research and therapeutic environments.[10]

Microbubbles prepared through sonication are commonly utilized in cancer diagnosis and treatment. They act as contrast agents in ultrasound imaging, improving the ability to see tumors. Microbubbles can be employed for medical treatment by improving the transportation of medications to cancerous cells either through ultrasound-induced release or by raising the permeability of nearby blood vessels.[9]
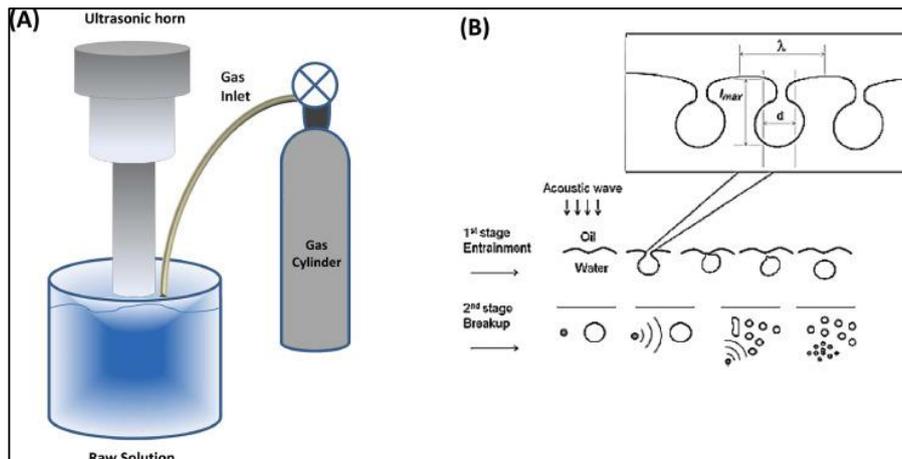
High Shear Emulsification

A second method that is commonly used, particularly for preparing polymer-coated microbubbles, is to emulsify the polymer (dissolved in a suitable solvent) in an aqueous suspension by high shear stirring, using another liquid that is immiscible with both the polymer and water as a stabilizer.

Provided the polymer solvent is sufficiently volatile, it will begin to evaporate, causing the polymer to precipitate onto the surface Typical size distributions of microbubbles prepared from a phospholipid suspension via sonication, CEHDA, and a microfluidic T-junction device of the droplets to form coated liquid filled microspheres. The microspheres are washed to remove excess solvent and then freeze-dried to produce gas-filled shells. In some cases, the liquid filling may only be partially removed if the microspheres are to be used therapeutically. It has been observed by Bohmer et al. that the composition of the polymer is extremely important in determining the structure (porosity) of the coating and whether or not it is possible to remove sufficient liquid for the microbubbles to be acoustically active. As might be expected, the homogeneity and porosity of the shell also appear to be important in determining the acoustic pressure required for fragmentation and hence drug delivery. Once again, the microbubble size distribution depends on that of the droplets in the initial emulsion and upon any fragmentation or coalescence of the microspheres during subsequent processing. Additional filtration may also be required when the size is citical.[6]

Emulsification allows for precise control over microbubble size and surface properties by adjusting the surfactants, viscosity, and flow rates during production. It also results in the production of stable microbubbles, which is ideal for applications that require consistent and durable contrast agents or delivery vehicles.[11]

This method is used in large-scale clinical producing microbubbles for medical imaging, such as ultrasound contrast agents, or for therapeutic applications like targeted drug delivery. The fine control over bubble size makes it ideal for high-precision tasks in clinical settings.[6]

Microfluidic devices

Microfluidic devices allow accurate manipulation of microbubble creation by regulating the flow of gas and liquid in small channels, making it possible to produce bubbles using a "pinch-off" process. This method creates uniform bubbles of the same size by closely managing the interface between the gas and liquid. Flow-focusing and T-junction devices are the two primary types of microfluidic devices utilized for microbubble generation. Soft lithography is utilized to make flow-focusing instruments, which are efficient in producing almost uniform microbubbles. Researchers have successfully created phospholipid-coated microbubbles that are around 5 µm in size and have a lifespan of 10 minutes, as well as nitrogen-filled bubbles with a diameter of 1.5 µm that remain stable for up to 9 hours. Nevertheless, flow-focusing devices with narrow channels (~7 µm) need to be operated in controlled environments such as clean rooms, and they necessitate high surfactant levels and low liquid viscosities to minimize pressure and subsequently decrease production rates. Multiple devices are frequently utilized to increase output. Devices at a T-junction, constructed with capillaries in a

polymer block, present a cheaper option. These units can operate without the need for a sterile environment and generate bigger bubbles ranging from 30 to 100 micrometers. T-junction devices can modify bubble sizes by changing capillary spacing, endure greater pressures, resulting in a wider range of bubble sizes and increased yields. Even though they are capable of creating bubbles as tiny as 10 μm, additional research is necessary to consistently produce even smaller bubbles. The amount of microbubbles produced, which is important for both research and commercial uses, is determined by the rate of production and the volume of coating material. CEHDA, a similar technology, improves precision of yield by guaranteeing full gas encapsulation. Flow-focusing and T-junction microfluidic devices offer precise control for producing microbubbles, each catering to specific needs for bubble size, stability, and production conditions..
[6]

Coaxial electrohydrodynamic atomization (CEHDA)

A new method for creating microbubbles that build on conventional electrohydrodynamic atomization is coaxial electrohydrodynamic atomization (CEHDA). In CEHDA, syringe pumps are used to feed two fluids through needles that are positioned coaxially. To create a coaxial jet that traps gas within a liquid coating, a high electrical potential is supplied between the needles, with a grounded electrode below. The homogeneous microbubbles produced by this process can be adjusted by varying the applied voltage, gas and liquid flow rates, and other factors. These variables affect the three different flow modes that provide control over bubble size and homogeneity: bubble dripping, coning, and microbubbling.[6]

According to experimental data, bubble diameter is considerably reduced when the gas flow rate is decreased while the liquid flow rate is maintained. The jet diameter and bubble size are further reduced by increasing the voltage. For example, CEHDA has produced phospholipid-coated microbubbles that are approximately 6.6 μm in diameter and stable for more than 2.5 hours at ambient temperature. In addition, the technology has produced liquid-filled capsules and bubbles coated with polymers in a single step, which gives it an edge over microfluidic and emulsification techniques.

CEHDA has therapeutic promise, particularly when it comes to combining numerous coaxial liquid streams to create multi-layered microbubbles in a single operation. This makes it possible to attach targeting agents to microbubbles and precisely control drug dosage, both of which are useful features for medicinal applications.
[13]
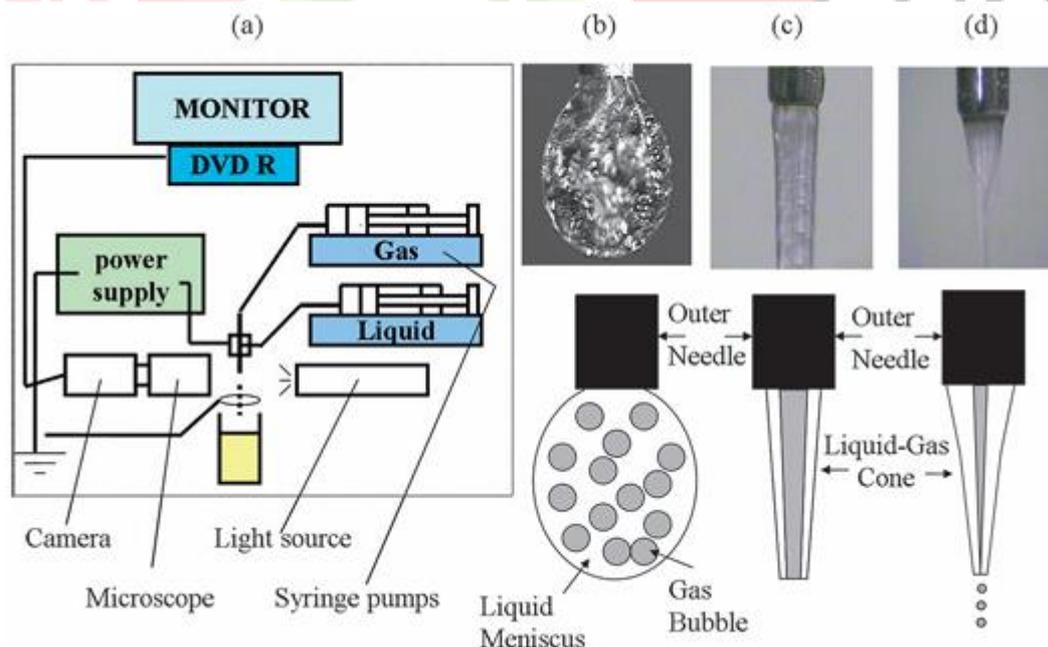
MICROBUBBLE FORMULATION FOR CANCER THERAPY

Drug-Loaded Microbubbles

Chemotherapeutic chemicals are incorporated into or onto microbubbles using a variety of techniques: Based on phospholipids Microbubbles: It is possible to embed chemotherapeutic drugs, such as doxorubicin, into the phospholipid shell of MBs. Drug stability is ensured until US-triggered release at the tumor site thanks to these interactions, which are sustained by hydrophobic and electrostatic forces.[14]

Polymeric Microbubbles: For multifunctional purposes, microbubbles can also be designed using materials like poly(lactic-co-glycolic acid) (PLGA) which encapsulate drugs and diagnostic agents, providing dual-mode imaging and targeted delivery.[15]

Janus Conjugates: These microbubbles utilize amphiphilic conjugates to improve drug-loading capacity, enhancing tumor accumulation of chemotherapeutics like camptothecin and floxuridine.[16]

Ultrasound-Triggered Drug Release

The localized release of drugs from microbubbles is facilitated by ultrasound-targeted microbubble destruction (UTMD). The process involves:

Focused Ultrasound: Ultrasound waves are used to burst microbubbles at tumor sites, enhancing the permeability of cell membranes, a phenomenon known as *sonoporation*. This allows for increased uptake of chemotherapeutics at the site of interest while minimizing systemic toxicity.[18]

Blood-Brain Barrier Opening: In glioma treatment, focused ultrasound with microbubbles has been shown to temporarily open the blood-brain barrier (BBB), enhancing drug delivery to brain tumors and improving therapeutic efficacy. [17]

Targeting Strategies

Targeted microbubbles are developed to improve therapeutic efficacy by using ligands or antibodies that direct MBs to specific cancer cells:

VEGF-targeting MBs: These microbubbles are functionalized with ligands like VEGF, which bind to overexpressed receptors on tumor cells, ensuring precise targeting and enhanced drug delivery.[17]

Ligand-Modified Lipid Microbubbles: Modifications to the lipid shell of MBs with targeting moieties ensure specificity to tumor cells, reducing off-target effects. [6]

Examples of Microbubble-Based Cancer Therapies

1. Doxorubicin-Loaded Microbubbles: These have demonstrated enhanced anti-tumor activity compared to free doxorubicin, particularly when combined with ultrasound.[15]
2. Taxol-Loaded Microbubbles: Lipid-coated microbubbles have shown significant tumor inhibition when loaded with Taxol, reducing tumor progression in preclinical models.[19]
3. Gene Therapy Microbubbles: Ultrasound-stimulated MBs are also being explored for gene therapy, offering a non-invasive approach for targeted gene delivery.[20]
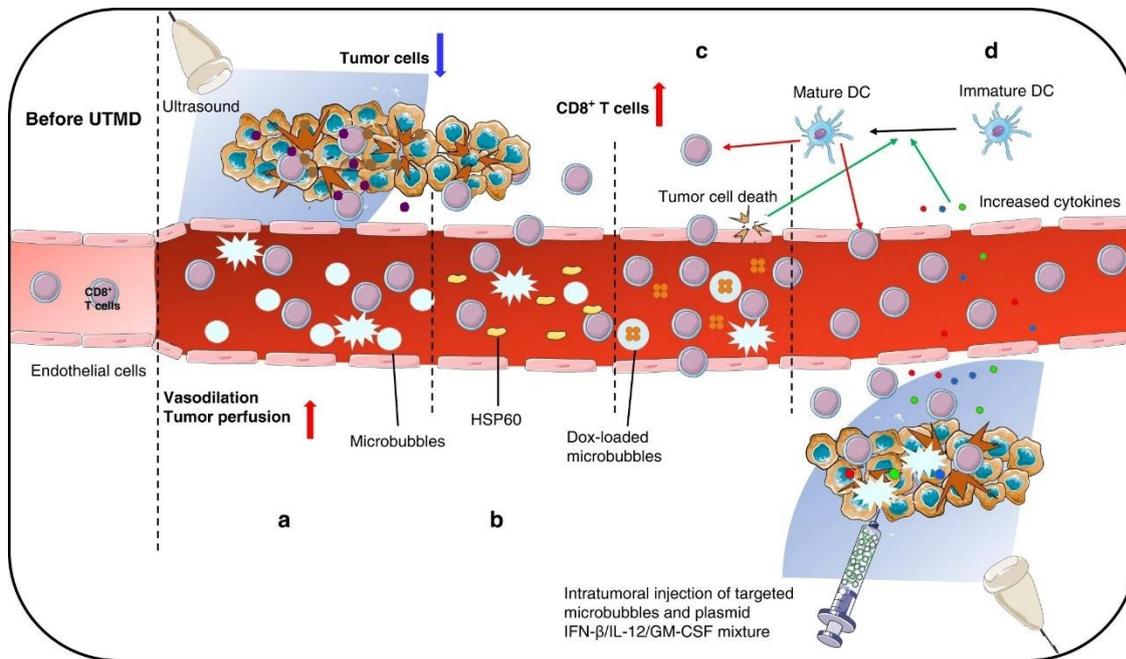
MICROBUBBLE FORMULATION FOR HYPERLIPIDEMIA TREATMENT

To administer lipid-lowering medications like statins, microbubble compositions that target arterial plaques particularly are being determined as a possible therapeutic method for hyperlipidemia. A list of the main elements of using microbubbles to treat hyperlipidemia is given below:

1. Lipid-lowering agents in the form of therapeutic microbubbles

Statins and other cholesterol-lowering medications can be encapsulated in the lipid shells of microbubbles or affixed to their surface. The objective is to administer these medications to hyperlipidemia patients' arterial plaque-deposition sites. This method lessens systemic negative effects while improving localized drug delivery. For instance, liposome-based microbubbles that carry statins or other anti-lipidemic drugs present a viable approach to non-invasive plaque-targeted treatment. [25]

2. Targeted Microbubbles for Plaque

To improve the specificity of medication delivery to arterial plaques, targeting molecules, such as ligands or antibodies, can be attached to the surfaces of microbubbles. For instance, low-density lipoprotein (LDL) receptor-targeted microbubbles can be employed to improve the therapeutic action on atherosclerotic plaques by delivering medications to regions with high cholesterol deposition.

The potential of plaque-specific gene therapy for hyperlipidemia has been demonstrated by studies that found that microbubbles containing plasmid DNA targeting the LDL receptor enhanced LDL receptor expression in HepG2 cells [25].

3. Delivery of Drugs Assisted by Ultrasound

Lipid-lowering substances can be released from microbubbles right at the location of arterial plaques in response to ultrasound. This process called ultrasound-targeted microbubble destruction (UTMD), allows for localized drug delivery by causing the microbubbles to burst at the target spot due to concentrated ultrasound.[26]

4. Examples of Microbubble-Based Applications in Hyperlipidemia

LDL Receptor Gene Delivery: A potential approach to treating hyperlipidemia is the use of microbubbles to transfer genes that cause the liver cells to express more LDL receptors. In one investigation, hepatocytes' expression of the LDL receptor gene was significantly increased by ultrasound-guided microbubble destruction.

Targeted Drug Delivery to Plaques: research on microbubbles containing medications that lower cholesterol have demonstrated that ultrasound-triggered drug release can successfully decrease plaque size, albeit the majority of these research have been conducted on animal models or in the early phases.[25]

CHALLENGES IN MICROBUBBLE FORMULATION AND PREPARATION

Microbubble formulations face several challenges that limit their therapeutic potential in cancer treatment. Below are some of the major issues encountered in their design and application:

1. Stability Issues

It can be difficult to keep microbubbles (MBs) stable in vivo, particularly for extended periods of time. The functionality of MBs is affected by the bubble collapse or size reduction caused by the gas core's resistance to diffusion.

Gas diffusion causes lipid-shelled MBs to be unstable. Although they can extend their circulation period, attempts to stabilize these bubbles—such as by employing complex phospholipid compositions—still have limitations.[21]

Because of their smaller size, nanobubbles provide better stability and longer systemic circulation. But striking a balance between stability and size is still challenging.[22]

2. Size Control

Controlling the size of MBs is crucial for both diagnostic imaging and therapeutic delivery. Uniform size distribution ensures consistent ultrasound response and optimized drug release:

Microfluidic fabrication techniques are used to achieve precise control over MB size, with uniform bubbles being more effective for both imaging and drug delivery. Size-controlled MBs (under 5 μm) exhibit enhanced ultrasound contrast and targeted delivery efficiency.[23]

Size variation can impact the effectiveness of ultrasound-targeted drug release. Smaller MBs tend to extravasate more easily into tumor tissues, while larger MBs are better suited for enhanced imaging and immediate release mechanisms. [24]

3. Drug Loading Efficiency

Loading chemotherapeutic agents onto or into MBs is a delicate process. Achieving high drug loading while ensuring controlled release is challenging due to limitations in encapsulation techniques:

Low encapsulation efficiency is often encountered, especially for hydrophilic drugs. Techniques like polymer-based loading or using novel lipid compositions have been explored to increase loading capacity without compromising the MB's stability

Strategies like using three-phase microbubble fabrication allow for higher yields and efficient encapsulation of chemotherapeutics like doxorubicin [23].

4. Biocompatibility and Safety

Biocompatibility is a crucial factor, especially for repeated MB applications:

Lipid-shelled MBs generally show good biocompatibility, but repeated use raises concerns about potential immunogenicity or toxicity due to the accumulation of shell materials in the body.[20]

Clearance from the body can be a challenge, as larger MBs may be trapped in the microvasculature, leading to potential blockages. The development of biodegradable microbubbles that can be easily cleared by the liver or kidneys is essential for improving their safety profile.

CONCLUSION

When combined with ultrasound, microbubbles are particularly useful for targeted drug delivery, improving efficiency and minimizing adverse effects. In Cancer therapy, they enhance tumor targeting and treatment effectiveness by improving the delivery of chemotherapeutic drugs. Using microbubbles to deliver lipid-lowering medicines directly to arterial plaques can improve the treatment of hyperlipidemia locally and decrease systemic adverse effects. In the preparation of microbubbles, a variety of techniques are used, including sonication, high-shear emulsification, and microfluidic devices. Each technique has unique benefits for a given application.

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