IJPSR (2012), Vol. 3, Issue 09 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 April, 2012; received in revised form 16 June, 2012; accepted 27 August, 2012

THE CONCEPT OF MICROBUBBLE AS A DRUG DELIVERY SYSTEM: AN OVERVIEW

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Keywords:
Microbubbles,
Biosurfactant-Stabilized Microbubbles,
diagnostic agents,
Ultrasound,
Drug delivery,
Gene delivery,
Ligands,
Contrasting agent

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ABSTRACT

Micro bubbles are small spherical type of bubble which consists of a gas, they are separated from each other, so they cannot agglomerates. Actually, they have size range in micrometers usually 1-100 micrometer. They are capable of penetrating even into the smallest blood capillaries & releasing drugs or genes, incorporated on their surface, under the action of ultrasound. Microbubbles in general have a wide variety of applications. They are used as a contrast agent in diagnosis. They are widely applicable in gene delivery due to its very important silent feature. Majorly main huge research is going on drug delivery by microbubble with ultra sound. Most of the physicians today prefer imaging with ultrasound in combination with microbubbles compared to other diagnostic techniques for low cost and rapidity.

INTRODUCTION [1, 2, 3]: Now a day, Micro bubble drug delivery system attracting researcher due to its wide scope and lots of application. They are now used in areas in advanced and conventional science and technologies like heat removal, energy conversion, cleaning and sterilization by shock waves etc. As a name suggests, we can predict meaning of micro bubble as bubbles with diameters less than several tens of micron.

Micro bubbles are small spherical type of bubble which consists of a gas; they are separated from each other, so they cannot agglomerates. Actually, they have size range in micrometers usually 1-100 micrometers.

Now a day, a lot of research is going on micro bubble. The micro bubbles, which mostly contain oxygen or air, can remain suspended in the water for an extended period. Gradually, the gas within the micro bubbles dissolves into the water and the bubbles disappear. Micro bubbles are applicable to a wide variety of field like medical field, Gene therapy etc.

In medical field used to scan the various organs of body and recently they are being proposed to be used as drug or gene carriers and also for treatment in cancer therapy. They are also used increase the aquaculture productivity, increase the hydroponic plant growth and improve the quality of water and used in sewage treatment.

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Properties of Microbubbles [1, 2]: The Properties of micro bubbles are as given below.

TABLE 1: PROPERTIES OF MICRO BUBBLES

Types	Sub Type with Example
Functional Properties	1. **Injectability:** They should be injectable. 2. **Ultrasound Scattering Efficiency:** They are used combination with ultrasound they should have ultrasound scattering efficiency. 3. **Biocompatibility:** They should be Biocompatible due to micro bubbles are interacting with the vital organs of the body.
Structural Properties	1. **Diameter**: should be between the ranges of 1-10 µM. 2. **Density & compressibility:** In Body, They are act as contrast agents because their density & compressibility difference between themselves & the surrounding body tissues to create acoustic impedance & to scatter ultrasound at a much higher intensity than the body tissues. 3. **Uniformity of shell thickness**

Components of Microbubbles [1, 2, 4, 5]: Micro bubbles comprise of basically 3 phases in which inner, middle and outer most phase. They are as following:

1) Innermost Gas Phase
2) Shell Material Enclosing the Gas Phase
3) Outermost Liquid or Aqueous Phase

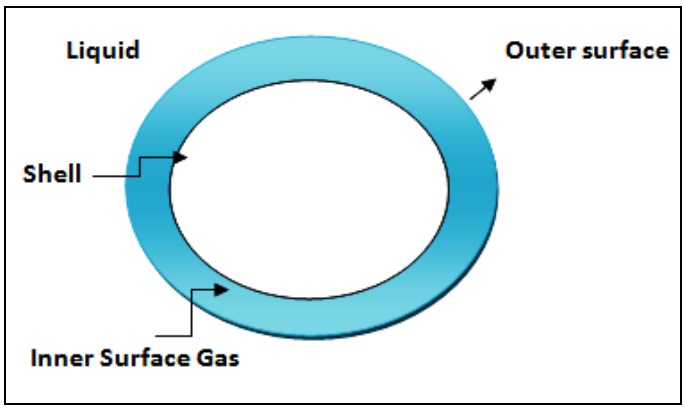

FIG. 1: COMPONENT OF MICROBUBBLES

1) **Inner most layer**: It is generally gas layer. The gas phase can be a single or a combination of gases can be used. Combination gases are used to cause differentials in partial pressure & to generate gas osmotic pressures which stabilize the bubbles. In combination of gas system there are two types of gas. Primary Gas known as First gas. Generally, it is air. Sometime nitrogen is also used as first gas. Secondary gas is a Gas Osmotic Agent. They are less soluble in blood and serum and they have sufficient partial or vapor pressure at the temperature to provide the desired osmotic effect.

2) **Shell Material**: The shell material covers the gas phase. Main important of this layer is it is act as a region in which drug molecule attached to shell layer which are used to achieving targeting various components of organ and tissue. It is useful for the elasticity or compressibility of micro bubbles. If more elastic, it has capacity to withstand before bursting and breaking up which increases the residence time of these bubbles in body. The material are used as a shell material like Proteins like albumin, Carbohydrates like galactose, Phospholipids like phosphotidyl-choline, phosphotidyl-ethanolamine etc., Bio-degradable polymers like polyvinyl alcohol, polycaprolactone etc.

3) **Aqueous or Liquid Phase:** The external, continuous liquid phase in which the bubble resides typically made up of surfactant or foaming agent. Surfactants suitable for use include any compound or composition that aids in the formation & maintenance of the bubble membrane by forming a layer at the interphase. The foaming agent or surfactant may be made up of a single component or any combination of compounds, such as in the case of co surfactants.

For example, Block copolymers are polyoxy-propylene, polyoxyethylene, sugar esters, fatty alcohols and Nonionic Surfactants are Polyoxyethylene polyoxypropylene copolymers. There are also various Anionic Surfactants Fatty acids having 12-24 carbon atoms (eg. Sodium Oleate) are used.

4) **Other Components**: The other components that may be incorporated in the formulation include viscosity modulators, air solubility modifiers, osmotic agents, stabilizers, chelators, buffers, salts& sugars can be added to fine tune the microbubble suspensions for maximum shelf life & contrast enhancement effectiveness. Such considerations as sterility, isotonicity & bio-compatibility may govern the use of such conventional additives to injectable compositions. These help in improving the property of a microbubble and make a stable formulation.

Preparation Method of Microbubbles [6-10]: The various methods that can be used for the preparation of these microbubbles are as follows:

1. Cross Linking Polymerization
2. Emulsion Solvent Evaporation
3. Atomization & Reconstitution
4. Sonication

1. **Cross Linking Polymerization:** A polymeric solution (2% aqueous solution of telechelic PVA) is vigorously stirred (at room temperature for 3 hrs at a pH of 2.5 by an Ultra Turrax T-25 at 8000 rpm equipped with a Teflon coated tip) so, formation of a fine foam of the polymer occurs which acts as a colloidal stabilizer as well as a bubble coating agent. The polymer is then cross linked, after cross linking microbubble float on the surface of the mixture. Floating microbubble are separated & extensively dialyzed against Milli Q water.

2. **Emulsion Solvent Evaporation:** In Preparation of microbubble there are two solutions are used. The First solution (aqueous solution) contains appropriate surfactant material which may be amphiphilic biopolymer such as gelatin, collagen, albumin or globulins. This becomes the outer continuous phase. The Second Solution contains mixture of two water immiscible organic liquids. One is volatile solvent for the polymer & the other is relatively nonvolatile nonsolvent for the polymer. Now Above second solution is mix with first solution to form emulsion. As solvents volatilizes, polymer conc. in the droplet increases to a point where it precipitates in the presence of the less volatile nonsolvent. This process forms a film of polymer at the surface of the emulsion droplet.

   As the process continues, an outer shell wall is formed which encapsulates an inner core of nonsolvent liquid. Once complete, the resulting microcapsules can then be retrieved, washed & formulated in a buffer system. After that drying, preferably by freeze-drying, removes both the nonsolvent organic liquid core & the water to yield air filled hollow microbubble.

3. **Atomization & Reconstitution:** This is a very interested technique it form microbubble. In this method, a surfactant solution is atomizing into a heated gas so formation of porous spheres occurs. Primary modifier gas is enclosed into it. Then packaged into a vial and filled with the second gas or gas osmotic agent and finally seal it upon reconstitution with sterile saline solution. The primary modifier gas diffuses out & the secondary gas diffuses in, resulting in size reduction. The microbubble forms.

4. **Sonication:** A vial containing a surfactant solution & gas in headspace of the vial can be sonicated through a thin membrane**.** Due to Sonication microbubble forms and delivers to patient. This is a very small and easy method to prepare microbubble.

Characterization of Microbubbles [2, 11]:

TABLE 2: CHARACTERIZATION OF MICROBUBBLES

Characteristic	Evaluation Method
Diameter & Size Distribution:	It can be determined by Laser light Scattering, Scanning Electron Microscopy, and Transmission Electron Microscopy.
Shell Thickness:	It is determined by coating the shell with a fluorescent dye like Red Nile, this is then determined by Fluorescent Microscopy against a dark background.
Microbubble Concentration	It is determined by counting the no. of microbubbles per ml by using the Coulter Counter Machine.
Air Content by densitometry	The content of air encapsulated within the microbubbles in the suspension samples is measured by oscillation U-tube densitometry with a DMA-58.

Biosurfactant-Stabilized Microbubbles [12, 15, 16]: The lack of well-established generation and characterization methods and the lack of understanding of microbubble properties limit the application of microbubble technology shows that properties of microbubbles depend on their generation method and the surfactant used. Therefore, selection of a suitable method and suitable surfactant is important for the application of microbubble.

Feng et al., [13] shows that microbubble stability increases with surfactant concentration due to increases in the viscosity of the solution, the viscoelasticity and mechanical strength of the interfacial film, and electrostatic repulsion. The effect of surfactant concentration is more pronounced at higher pH than at lower ph.

Pattle [14] characterized microbubbles prepared with lung extracts and found that the bubbles initially shrank but then maintained a constant size (12 µm). The microbubbles remained unbroken even after several washes with distilled water but disappeared in air-free water. This indicated that the bubbles were stabilized by a layer of solid, water-insoluble substance (later identified as PS) and freely permeable to air.

Now a day applications of biosurfactant-based microbubbles are mainly on ultrasound diagnosis and therapy and remediation or bio-remediation of contaminants. Ultrasound-induced microbubble destruction thus provides a promising therapeutic approach for targeted treatments.

In Ultrasound mediated drug delivery technology, ultrasound and microbubbles that are partially filled with cancer drugs. Microbubbles are to be injected into the patient's bloodstream and carried to the site of a tumor. The arrival of the microbubbles at the tumor can be monitored with ultrasound imaging.

Upon arrival of the microbubbles in the tumor, they will then be ruptured with a focused ultrasound pulse to release the drugs. As the drugs are only released at the site of the tumor, the patient's total body exposure to them could be limited, which for certain types of cancer could help to reduce the unpleasant side effects of chemotherapy.

Classification of Non-Viral Vehicles and Ultrasound Microbubbles [18]:

TABLE 3: CLASSIFICATION OF NON-VIRAL VEHICLES AND ULTRASOUND MICROBUBBLES

Non-viral vehicles	Inside	Shell	Mean size
Nanoparticles	---	Latex	10-20 nm
Micelles	Surrounding solvent	Phospholipid particle	10-100 nm
Liposomes	Surrounding solvent	Phospholipid bilayer	~100 nm
Microbubbles:			
1. First-generation Echovista[a]	Air	Galactose matrix	~2 um
2. Second-generation Albunexb[b]	Air	Galactose matrix	1.5-6 µm
3. Third-generation Optisona[a]	Perfluoropropane	Human albumin	2-4.5 µm
4. Sonovuea[a]	Sulphur hexafluoride	Human albumin	2-5 µm
5. PESDA	Perfluorobutane	Phospholipid monolayer	2-5 µm
6. Quantison	Air	Sonicated albumin	2-5 µm
7. Definity	Perfluoropropane	Dried albumin	2.5 µm
		Phospholipid monolayer	

[a]Licensed for clinical use. [b]No longer commercially available.

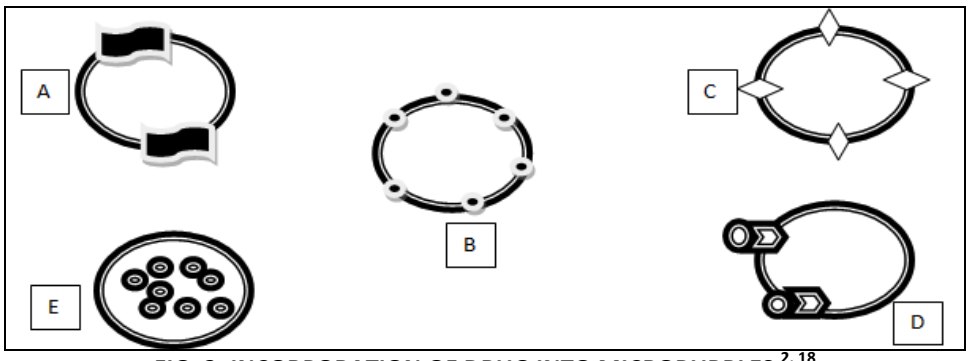

FIG. 2: INCORPORATION OF DRUG INTO MICROBUBBLES [2, 18]

Application of Microbubbles:

1. **For Diagnosis [2, 5, 19]:** They are used as contrast agents because they are elastic and compressible &undergo compression and creating an acoustic impedance mismatch between biological tissues and fluids. These are used as diagnostic aids for:

    A. Organ Edge Delineation

    B. Blood Volume and Perfusion

    C. Inflammation

    D. Cancer

    E. Liver

    F. Also used to scan the tumors arising in the body.

    G. Used for imaging the gall bladder stone.

2. **Drug Delivery [2, 6, 18]:** Two factors consider during drug delivery are as follows:

    1) Incorporation of drug into these Microbubbles

    2) Drug release from these Microbubbles

For Incorporation of drug into Microbubbles methods which are used they are shown in **fig. 2**. Method A shows Drugs can also be attached to the shell of the microbubble. Method B shows if the microbubble is made up of multiple layers it can also be incorporated within the various layers of these Microbubbles. Method C shows Drug molecules can also be incorporated within the bubble membrane or shell material of the microbubble. Method D shows drug can also be attached to the microbubble surface via a ligand. Method E shows Drug molecules can be incorporated within the bubble.

In Drug release from Microbubbles, bursting or breakup of the microbubble on application of ultrasound occurs called cavitation. On cavitation the body fluids start creating acoustic cavitation. When microbubble oscillate gives raise small eddies therefore rise of micro streaming or micro jets resulting in increase in permeability of the cell membrane & facilitating drug transfer across the membrane.

Sometimes the Microbubbles may also be phagocytosed by the cell membrane resulting in drug release. There is an also another mechanism which shows that fusion of the phospholipid microbubble with the phospholipid bilayer of cell membrane resulting in delivery of the drug or genes directly into the cytoplasm of the cell membrane. This is the proposed mechanism for gene delivery.

3. **Gene Delivery [21]:** Microbubbles also used in gene delivery due to it contains following features;

    - Microbubbles are metabolically inert
    - When injected into the body they do not produce any immune response
    - Also the gene encapsulated or attached to the microbubble is carried to its target without getting digested by the various enzymes.

This Feature will significantly useful in gene delivery. Charged drugs can be stabilized in or onto the surfaces of microbubble by virtue of electro-static interactions lipid-coated micro bubbles to bind DNA The gene is released when ultrasound energy cavitates the microbubble.

CONCLUSION: The application of microbubble with ultrasound which gives synergistic effect for drug or DNA delivery. Different approaches to non-viral gene and drug delivery are being explored, and much has been learned from viruses that have evolved into extremely efficient infection mechanisms.

The use of targeted microbubble has challenging therapeutic options, not only in CVS disease but also in treatment of inflammatory and malignant disease.

In the coming years; this technique needs further development to make it available for clinical application, imaging microscopy, offer excellent opportunities to study *in vitro* this process at the (sub)cellular level in real-time, thereby creating the possibility of visualizing the interaction of fluorescent-labeled Microbubbles and myocardial or endothelial cells under ultrasound pressure.

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How to cite this article:
Katariya HB: The Concept of Microbubble as a Drug Delivery System: An Overview. *Int J Pharm Res Sci*. 3(9); 3058-3063.