Formation of Vesicles by the Depressurization of an Expanded Solution into Aqueous Media Process

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Introduction

Vesicles are self-assembled and self-organized structures with well-defined shape and size. They are used as carriers for different compounds including Active Pharmaceutical Ingredients (APIs) and imaging agents.

Liposomes are vesicles generated from natural and biocompatible phospholipids with an aqueous volume enclosed within a lipid membrane. The lipid membrane is composed of hydrophilic and hydrophobic domains that allow the encapsulation of both hydrophilic and hydrophobic compounds as shown in *Figure 1* [1]. Bilayer vesicles help decrease the unfavorable interactions between the bulk aqueous phase and the long hydrocarbon fatty acid chains. Liposomes have limited applications as drug carriers because the components are labile [2]. Polymersomes are synthetic polymer vesicles and possess more mechanical strength than liposomes. Polymersomes offer a wider range of chemical and physical properties, and are stable toward degradation over longer timescales [3]. Superparamagnetic Iron Oxide Nanoparticles (SPIONs) encapsulated by liposomes or polymersomes are called magnetoliposomes and magnetopolymersomes respectively and can be used for the targeted treatment of cancer and diagnostic imaging [4].

Conventional vesicle production techniques have drawbacks such as complex and time consuming procedures involving organic solvents. For liposomes, conventional methods can involve harsh conditions that result in denaturation of the lipids and drugs, and also cause poor drug encapsulation efficiency [2].

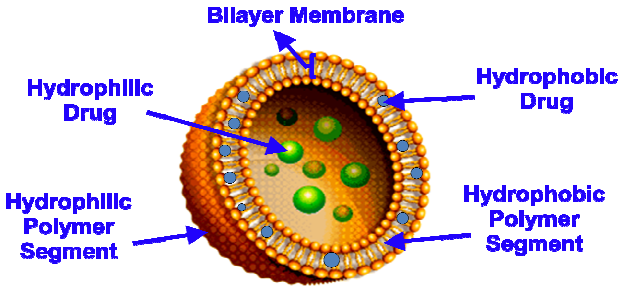


Figure 1: Structure of vesicle encapsulating both hydrophilic and hydrophobic drug compounds (Modified from Ref 1)

The use of organic solvents as required by most of the conventional production methods presents occupational and environmental issues and it is non-ideal for pharmaceutical applications. Therefore, a thorough removal of organic solvent has to be ensured for pharmaceutical

preparations [5]. Dense gas technology is an alternative production technique that provides a non-toxic, non-flammable, environmentally acceptable, and economical method for producing liposomes and polymersomes. Because of liquid-like density, gas-like diffusivity and viscosity, pressure-dependent solvent strength and no surface tension, dense gases are ideal for many different processes [6].

The Depressurization of an Expanded Solution into Aqueous Media (DESAM) is a dense gas technique designed to generate vesicles in a single-step process. In a DESAM process, dense gas is used to expand a liquid solution and then the gas expanded liquid is released into an aqueous medium via a nozzle. Vesicles are produced as the gas expanded lipid solution is released into the aqueous medium.

Materials and Methods

Dense gases:	Carbon Dioxide (CO_2) 99.5% from Linde
Liposomes production:	Cholesterol 99+% from Aldrich Chemical
	1,2-distearoyl-sn-glycero-3-phosphatidylcholine from Avanti
	Ethanol 100% v/v from BDH Analar and Merck Pty. Ltd.
Polymersomes production:	Dichloromethane from Asia Pacific Specialty Chemicals Limited
	PolyButadiene-block-PolyEthylene oxide from Polymer Source Inc. Mn: PBd(22000)-b-EO(12600); (PDI) Mw/Mn = 1.06 The average polymer chain consists of 406 units of Polybutadiene and 286 units of Polyethylene oxide.

Depressurization of an Expanded Solution into Aqueous Media (DESAM)

A solution of vesicle forming compounds was placed in a high pressure vessel (Vessel 1). The solution was expanded by introducing compressed carbon dioxide into the vessel. The compressed carbon dioxide was introduced from the bottom of the vessel and was sparged through a frit until the pressure reached 35 bars.

The expansion of organic solutions by carbon dioxide can trigger the precipitation of solutes; hence preliminary studies have been conducted to identify the threshold pressure to avoid the precipitation of solutes in Vessel 1. Materials precipitation in Vessel 1 would not be delivered to Vessel 2 and therefore, they would not participate in vesicle production.

The gas expanded solution of vesicles was then delivered through a nozzle into Vessel 2 by a pressure gradient. The Vessel 2 contained deionized water heated to 55ºC, which is above the transition temperature of the phospholipids and the block copolymer and below the boiling point of the aqueous phase.

A flux of carbon dioxide was maintained between the vessels to keep the aqueous phase agitated and help to eliminate the solvent left in the aqueous phase. The aqueous phase was then immersed in icy water at around 4°C whilst still bubbling the CO_2. The flux of CO_2 is controlled by a valve and kept between 773.3L/min (measured at ambient conditions). The whole process is completed in 2 hours.

Inclusion of SPIONs

Superparamagnetic Iron Oxide Nanoparticles (SPIONs) were added and dispersed in an organic solvent. The resulting mixture was then used to solubilize the vesicle forming agents (either the phospholipids or the block copolymer). The SPION-solute solution was expanded with carbon dioxide below the threshold pressure of precipitation of the solutes (35 bars). The expanded SPION-solute solution entered Vessel 2, which was at atmospheric pressure.

Another technique for producing magnetoliposomes/magnetopolymersomes involved dispersing SPIONs in the deionized water in Vessel 2. The vesicle forming agents were dissolved in the organic solvent and placed in Vessel 1. After expansion with compressed carbon dioxide at 35 bars in Vessel 1, the solution of vesicle forming agents was pushed into Vessel 2.

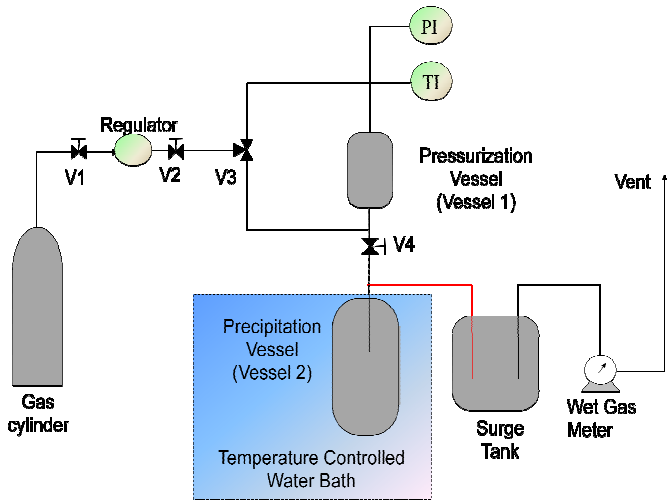


Figure 2: The schematic diagram of the DESAM system

Characterization of Vesicles Morphology

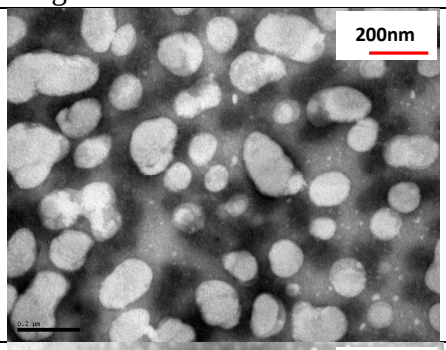
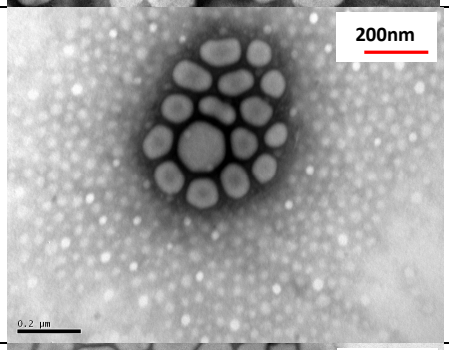
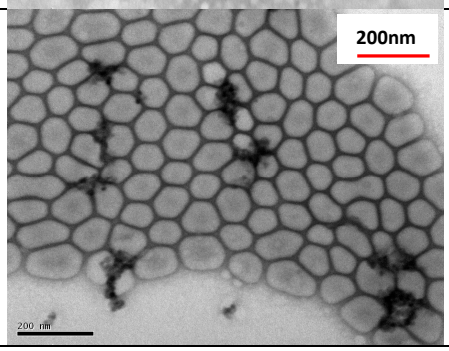
Transmission Electron Microscopy (JEOL 1400, 80–100kV accelerating voltage) was used with negative staining to investigate the shape and size of the produced vesicles.

Characterization of Vesicles Size Distribution

Brookhaven ZetaPlus was used to examine the particle size distribution of the population of vesicles by Photon Correlation Spectroscopy. Each sample was tested at room temperature.

Results

Transmission Electron Microscopy (TEM)

Sample	Image	Description
1		Figure 3: Liposomes produced by the DESAM process Morphology of vesicles: Spherical
2		Figure 4: Polymersomes produced by the DESAM process Morphology of vesicles: Spherical, Prolate
3		Figure 5: Magnetopolymersomes produced by the DESAM technique Morphology of vesicles: Spherical, Prolate Observation: The SPIONs were not encapsulated within the inner domain of the vesicles, but within the polymeric domains.

An experiment was conducted to test the responsiveness of the magnetopolymersomes to the magnetic field. A magnet was placed next to a vial containing magnetopolymersomes overnight (Figure 6). The vial content was initially cloudy and brownish for the presence of SPIONs within the vesicle suspension (Figure 6a). The vesicles containing the SPIONs were attracted to the magnet on the side wall of the vial leaving the bulk of the suspension clear (Figure 6b) which indicates that the magnetopolymersomes responded to the magnetic field.

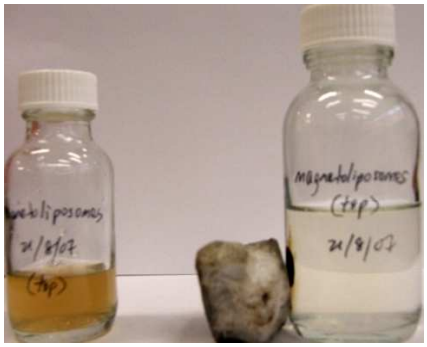


Figure 6: Responsiveness of Magnetopolymersomes to a magnetic field.

Photon Correlation Spectroscopy (PCS)

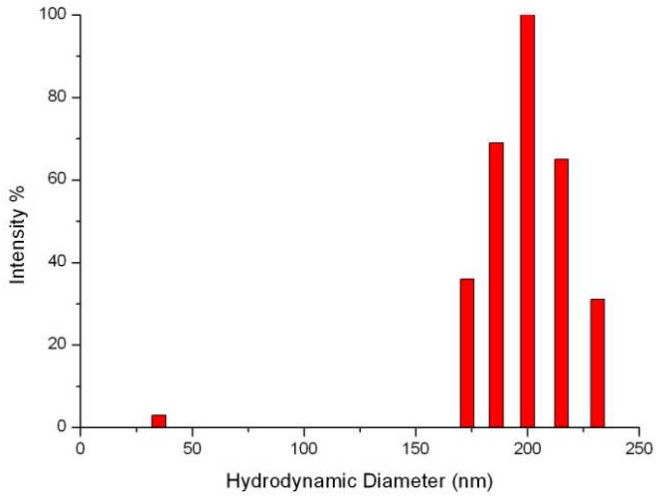


Figure 9: Graph of Intensity against Hydrodynamic Diameter for Polymersomes produced by the DESAM process (Sample 2)

Effective diameter: 185.6nm

Dynamic Light Scattering results are shown in Figure 9. By comparing the results obtained from TEM and PCS, a small difference can be observed. If small vesicles are present in the samples that are close to the lower detection limit of the instrument, PCS may show a deceptive size distribution [7]. PCS therefore provides accurate results for small vesicles analysis only if they are present in large populations. For instance, the polymersomes from TEM were observed to fall within size range of 50 to200nm with a prevalence of 100nm, but from PCS, an effective diameter of 185.6nm was obtained.

Conclusion

The DESAM technique is a non-complicated and fast method of producing both liposomes and polymersomes vesicles in suspension. SPIONs can be encapsulated within the polymer membranes.

Reference

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