"MICROENCAPSULATION OF ANTI-TUMOR, ANTIBIOTIC AND THROMBOLYTIC DRUGS IN MICROGRAVITY"

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ABSTRACT

Encapsulation of cytotoxic or labile drugs enables targeted delivery and sustained release kinetics that are not available with intravenous injection. A new liquid-liquid diffusion process has been developed for forming unique microcapsules that contain both aqueous and hydrocarbon soluble drugs. Microgravity experiments, on sounding rockets (1989-92) and Shuttle missions STS-52 (1992) and STS-56(1993) using an automated Materials Dispersion Apparatus, produced multi-lamellar microcapsules containing both cis-platinum (anti-tumor drug) and iodinated poppy seed oil (a radiocontrast medium), surrounded by a polyglyceride skin. Microcapsules formed with amoxicillin (antibiotic) or urokinase (a clot dissolving enzyme), co-encapsulated with IOD, are still intact after two years. Microcapsules were formed with the drug so concentrated that crystals formed inside. Multi-layered microspheres, with both hydrophobic and hydrophilic drug compartments, can enable diffusion of complimentary drugs from the same microcapsule, e.g. antibiotics and immuno-stimulants to treat resistant infections or multiple fibrinolytic drugs to dissolve emboli. Co-encapsulation of enough radio-contrast medium enables oncologists to monitor the delivery of anti-tumor microcapsules to target tumors using computerized tomography and radiography that track the distribution of microcapsules after release from the intra-arterial catheter. These microcapsules could have important applications in chemotherapy of certain liver, kidney, brain and other tumors.

BACKGROUND

Many cytotoxic or bioactive drugs and enzymes cannot be injected intravenously. Others can be injected, but are rapidly degraded before they reach the target tissue or they are cleared from the blood so quickly that their useful biological half-life is too short for good therapeutic value. Many drugs are insoluble in aqueous solutions and intravenous injection in hydrocarbon solvents is not well tolerated by patients. Encapsulation of drugs or biological therapeutics into liposomes or liquid microcapsules can enable delivery to target organs where the bioactive drug can be released directly to the target area by diffusion. Properly designed microcapsules can provide unique methods of direct delivery by parenteral injection, nasal inhalation and dermal administration for sustained release of important bioactive drugs [1]. The size and shape of the microcapsules is critical for the distribution and drug delivery in the tissues. Typically, microcapsules of 1-5 micron diameter are optimum for intravenous administration, whereas, 200-300 micron diameter microcapsules are used for intra-arterial delivery. Perfect microspheres are needed for maximum packing densities and maximum drug payload delivery to target organs or tumors. Anti-tumor liposomes containing doxorubicin [2] or muramyltripeptide [3] have already been studied extensively in clinical trials. The disadvantages of using conventional liposomes or microcapsules include manufacturing methods that require many batch process steps to: 1) form the liposomes, 2) remove unwanted organic solvents, detergents, and 3) to harvest the proper size micro-particles for optimum pharmacologic efficacy [4]. Also conventional liposomes often use natural lipids and lectins (from eggs, soybeans and other inexpensive sources) which attract certain phagocytic immune cells that rapidly remove the liposomes from the circulatory system before they arrive at the target tissue. This creates variable dose-responses which greatly complicates the pharmacokinetics and makes calculations of therapeutic doses very difficult [5]. New formulations of "stealth" liposomes have been made with lipids that are less detectable by immune cells in an attempt to avoid phagocytosis [6], however, new types of liposomes and microcapsules are needed to exploit the various unique applications of this type of drug delivery.
Conventional Methods

Current methods of forming liposomes are based on mechanisms that certain phospholipids exhibit by arranging themselves into bilayers when they are dispersed in an excess of water. Above the main transition temperature, which can vary from -20 to +90 °C (depending on the nature of the phospholipid), these bilayers spontaneously form microspheres that entrap an aqueous core. Drugs that are soluble in organic solvents are usually limited to those that bind inside the hydrophobic region of the liposome bilayer. However, most drugs are dissolved in the aqueous phase which is entrapped when the liposome forms and the drug is either incorporated into the aqueous core or electrostatically binds to the polar regions of the bilayer. Some drugs are insoluble and are not associated with the bilayer therefore these have very low encapsulation efficiencies. Table 1 summarizes the major classes of drugs that are used in liposome formulations. Major difficulties with commercial preparation of microcapsules often involves density-driven phase separation of the immiscible carrier fluids, esp. when forming water/oil emulsions or trying to encapsulate multiple drugs. This limits the yield and often results in microparticles that are not spherical nor uniform in size, thereby limiting the packing density (and drug payload delivered) when the microcapsules arrive at the target tissues.

Table 1. Classes of drugs used in liposome formulations.

<table>
<thead>
<tr>
<th>CLASS OF DRUG</th>
<th>LOCATION IN LIPOSOME</th>
<th>ENCAPSULATION EFFICIENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-soluble, non-bi-layer interacting</td>
<td>Aqueous phase, usually inner core</td>
<td>High</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>Bound inside hydrophobic region of bi-layer</td>
<td>Low</td>
</tr>
<tr>
<td>Bi-layer associated - electrostatic interaction</td>
<td>Outer or inner polar regions of bi-layer</td>
<td>Low</td>
</tr>
<tr>
<td>Neither water-soluble, nor bi-layer associated</td>
<td>Suspended in aqueous or hydrocarbon phase</td>
<td>Very Low</td>
</tr>
</tbody>
</table>

Microcapsule formation by liquid-liquid dispersion of aqueous drugs and organic solvents typically produces water-in-oil (W/O) type liposomes, which then require the organic solvent to be removed (evaporated) to form reverse-phase evaporation vesicles (rev) or stable pluri-lamellar vesicles (splv). Multi-lamellar vesicles (mlv) are rarely formed by these methods, but usually require film casting with organic solvents, hydration and sizing using filtration through inert membrane filters [4]. Methods of forming multi-layered microencapsules often require emulsification of the aqueous phase into organic carrier solutions by shear, bubbling or sonication. Sophisticated, multi-step emulsion technology is required and yields of uniform type and size are often very low.

Liquid microemulsions also are being developed as drug delivery systems, especially for drugs that are poorly soluble in aqueous carriers. A microemulsion typically contains droplets in the range of 0.1 - 1 μm in diameter and is characterized by very fluid and dynamic micelles which are formed by sequential mixing one immiscible phase with another using surfactants and co-surfactants [7]. Typically, surfactants that produce water-in-oil (W/O) have a hydrophilic-lipophilic balance (HLB) rating of 3 to 6, while those that produce oil-in-water (O/W) microemulsions have an HLB of 8 to 18. The surfactants can be non-ionic, ionic, or amphoterically and often medium chain alcohols are added as the co-surfactant in the last step in achieving the final microemulsion.

NEW METHODS FOR MICROENCAPSULATION OF DRUGS

In the past few years, new methods have been developed for preparing microspheres specifically designed for intravascular delivery. The objective is to make the appropriate size microspheres and co-encapsulate both immiscible phases into microspheres that can withstand the shear forces of intravascular injection and flow through the blood vessels until they are entrapped at the target site. The outer lipid bilayer or microsphere matrix must also be designed for drug diffusion, at appropriate release rates, once the microcapsule has reached the target tissue. One of the authors (Mosier) had invented a new method of preparing microspheres by liquid encapsulation and solid-phase entrapment wherein the water-soluble drug is dispersed in a solid matrix material [8]. This method involves dissolving the aqueous drug and the matrix material in an organic solvent, in which they are mutually soluble, then dispersing this mixture in a second organic solvent to form an emulsion that is stable enough for intravascular injection.
Dispersion of finely divided solids in liquids or formation of microemulsions including dense materials can be achieved with similar techniques, however, successful formulations depend upon the interparticle behavior in the suspensions. For example, when finely divided Cis-platinum (anti-tumor drug), in saline solution, is dispersed in alcohol solution containing a lipid and surfactant, dense microspheres will form when the mixture is shaken in a reciprocating motion. These experiments illustrate a well known phenomenon wherein a second liquid phase (such as water) causes the solid to be preferentially wet therein causing sticky collisions of the solid owning to the interfacial tension between the two liquid phases, thereby causing dense microspheres to be readily formed. The sedimentation rate of the microspheres was found to be quite high compared with the original suspension. This phenomenon does not occur when Cis-platinum is dispersed in alcohol alone, which suggests that the hydrophilic nature of the diamino,dichloro, Cis-platinum complex in the hydrophobic polyglyceride solution causes spontaneous emulsification. These experiments are unique and offer interesting technical possibilities for separation of suspended solids, such as Cis-platinum from liquid carriers that have appropriate hydrophilic or hydrophobic characteristics.

Production of multi-layered microspheres with alternate hydrophobic and hydrophilic drug compartments opens up the possibility of developing multiple-therapy microcapsules which can allow sequential diffusion of two or more drugs out of the same microcapsules once they arrive at the target tissues. In some applications, the organic phase can include a tracer compound or radiocontrast medium to provide the additional advantage of real-time imaging of the microcapsules with computerized tomography (CT) scanning as they are released from the catheter and travel to the target tissue. Practical applications of immiscible, liquid-liquid microcapsule formation are limited, however, because of density-driven phase separation and stratification into horizontal layers resulting in the necessity to use multi-step batch processing with solvent evaporation phases and mechanical mixing, which often is not practical.

Gravity-dependent restrictions in our two step liquid-liquid spontaneous microencapsulation process led to the design of several microgravity experiments to explore the utility of this process when density-driven phenomena were eliminated. The microgravity flight experiments have led to the development of a new liquid-liquid microencapsulation process that involves use of surfactants and co-surfactants in the aqueous phase and co-surfactant alcohols in the organic phase, which also contains high molecular weight lipids that can form a tough outer "skin" on the final microcapsules. In microgravity a single step dispersion produced unique multi-lamellar microcapsules containing various aqueous drugs co-encapsulated with iodinated poppy seed oil (a radiocontrast medium with a sp. gravity =1.35). Subsequent ground control experiments also produced some of these unique microcapsules and illustrated that the 1-g process could be improved to yield useable microcapsules by using different formulations or electrostatic deposition to add the polymeric, outer coating.

**MICROGRAVITY EXPERIMENTS**

A collaborative research project was developed by the Institute for Research, Inc. and the Johnson Space Center starting in July 1987. The basic formulations and simplified liquid-liquid dispersion methods were developed in 1988 and 1989. Microencapsulation related experiments were attempted on eight space missions beginning in April 1989 with the Consort-1 sounding rocket using the Materials Dispersion Apparatus (MDA) mini-lab developed by Instrumentation Technology Associates, Inc. The sounding rocket flights produced only 6.5 minutes of microgravity conditions, but this was adequate to form the unique microcapsules in a single step. Experiments on the Space Shuttle permitted 10 minute dispersion times followed by curing of the outer polyglyceride skin for eight days under microgravity conditions. A summary of these experiments is shown in Table 2. New formulations were tested on Shuttle STS-52, using only aqueous-soluble drugs, polymers and surfactants, and on STS-56 using alcohols as co-surfactants.

**Sounding rocket experiments**

Initial experiments on Consort-1 and -3 were used to determine the effective mixing and diffusion kinetics in the MDAs. The first drug encapsulation experiments were attempted on the Joust-1 rocket, but the rocket was destroyed shortly after launch. The next attempt was on the Shuttle STS-43 mission, however, the experiment fluids were removed after a launch delay and concern about possible internal leakage of the alcohol solutions resulted in a decision to not reload the MDAs with the microencapsulation fluids. The first successful microencapsulation of drugs in microgravity was conducted on the Consort-4 mission in November 1991. The unique microcapsules were recovered and analysed by microscopic image analysis. Mono-dispersed fluorescent beads were included as internal size standards and fluorescent labels were used to determine the distribution of drug in the various fluid compartments. Additional experiments, conducted on Consort-5 in September 1992, confirmed the capabilities of the new method for forming multi-lamellar microcapsules with alternating layers of hydrophilic and hydrophobic drugs.
Table 2. MED Flight Experiments Summary:

<table>
<thead>
<tr>
<th>MISSION</th>
<th>DATE</th>
<th>EXPERIMENTS</th>
<th>MATERIALS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consort 1</td>
<td>April 1989</td>
<td>Protein Diffusion</td>
<td>Urokinase &amp; antibodies</td>
<td>diffusion rates established</td>
</tr>
<tr>
<td>Consort 3</td>
<td>March 1990</td>
<td>Diffusion Kinetics</td>
<td>Urokinase &amp; Myoglobin</td>
<td>kinetics verified</td>
</tr>
<tr>
<td>JOUST -1,</td>
<td>June 1991</td>
<td>Lipid Emulsions ^a</td>
<td>Urokinase &amp; antibodies</td>
<td>None - Launch aborted</td>
</tr>
<tr>
<td>STS-43</td>
<td>July 1991</td>
<td>Lipid Emulsions ^a</td>
<td>Strept-avadin, Urokinase &amp; Cis-Platinum</td>
<td>Expt. removed after 1st launch scrub</td>
</tr>
<tr>
<td>Consort-4</td>
<td>Nov. 1991</td>
<td>Microencapsulation of Drugs ^a ^b</td>
<td>Cis-Platinum, Amoxicillin Urokinase &amp; Strept-avadin</td>
<td>multi-lamellar vesicles w/ alternating hydrophilic &amp; hydrophobic layers</td>
</tr>
<tr>
<td>Consort-5</td>
<td>Sept. 1992</td>
<td>Microencapsulation of Drugs ^a ^b</td>
<td>Cis-Platinum, Amoxicillin &amp; Urokinase</td>
<td>multi-lamellar vesicles w/ alternating hydrophilic &amp; hydrophobic layers</td>
</tr>
<tr>
<td>STS-52</td>
<td>Oct. 1992</td>
<td>Microencapsulation of Drugs ^a ^b (aqueous polymers only)</td>
<td>Cis-Platinum, Amoxicillin &amp; Urokinase</td>
<td>multi-lamellar vesicles crystals within microcaps</td>
</tr>
<tr>
<td>STS-56</td>
<td>April 1993</td>
<td>Microencapsulation of Drugs ^a ^b (alcohol co-surfactants)</td>
<td>Cis-Platinum, Amoxicillin &amp; Urokinase</td>
<td>multi-lamellar vesicles crystals within microcaps</td>
</tr>
</tbody>
</table>

^a Fluorescent labels included  ^b Fluorescent beads included

Microcapsules formed in 36 microgravity mini-experiments on sounding rockets used liquid-liquid dispersion of aqueous drug solutions, surfactant, and polyethylene glycol dispersed in alcoholic co-surfactant solutions containing soluble polyglycerides. Microcapsules of both oil/water and polymer/water/oil were recovered from the Consort flights. These experiments produced multi-lamellar liquid microspheres (concentric spheres within spheres) comprised of three or more, alternating immiscible layers. Image analysis of the microcapsules was made possible by co-encapsulation of standard size fluorescent beads. Microcapsules were formed in the ranges of 1-15 µ, 40-50 µ, 110-130 µ and 160-230 µ diameters. Digital analysis of phase contrast and fluorescent images taken with a fluorescent microscope also confirmed that the aqueous-soluble drugs were often encapsulated within the inner aqueous core and the outermost aqueous shell of the microcapsules. This typical distribution is illustrated in Figure 1, which is a composite of a transmitted light photomicrograph and a fluorescent photomicrograph (lower right) of the same multi-lamellar microcapsule. The polyglyceride skin is clearly shown in the normal-light photomicrograph (upper left).

Multilamellar microspheres were also formed which contained relatively large amounts of IPO in discrete lamellae or vesicles. Figure 2 (left side) shows a microcapsule heavily loaded with IPO, which often comprised up to 38% of the total volume. Often small hemispheres of IPO were also found clinging to the outer surface of the large inner (aqueous) sphere or adhered to the outer polymer skin of the microcapsule as also shown in Figure 2 (upper center). Microcapsules formed by almost all of the formulations survived 15+g accelerations, severe vibrations and turbulent mixing, during the reentry of the experiment capsule, and have remained intact for two years after recovery from space. These multi-layered microcapsules are similar to liquid-filled, thin-skinned, micro-balloons which are flexible enough to be manipulated on a microscope slide without collapse. The microcapsules formed in just 6.5 minutes of micro-g retain their spherical shape and appear tough enough to survive the extensive physical manipulations required for sizing, final preparation and storage of parenteral suspensions, and the fluid shear encountered after intravascular injection. Augmented mixing, under microgravity condition, using finely dispersed iron particles drawn through the immiscible interface by a small magnetic field was also used to form microcapsules containing hydrophilic drugs, microbeads, and hydrophobic components.
Also we have formed very unusual structures (multiple small spheres of aqueous-soluble drug) distributed within multi-lamellar o/w/o microspheres, wherein the aqueous spheroids are arranged in an annular ring that appears fixed in a plane within the innermost sphere (not shown). These ring structures remain intact when the microcapsules are "rolled around" on the microscope slide.

**Space Shuttle Experiments**

Microencapsulation experiments on Consort 4 and Consort 5 used mixtures of aqueous-soluble drugs, IPO, C3-C8 alcohols and polyglycerides that are insoluble in aqueous solutions. Experiments on STS-52 co-encapsulated Cis-Platinum with IPO by forming liposomes from water-soluble polymers using special formulations of aqueous, non-alcoholic solvents. Polyvinyl pyrrolidone (PVP) and a commercial lecithin (Centrolex-F™) were used to form the liposome-type multi-lamellar microcapsules at 20 °C. Fluorescent beads and fluorescent labeled were co-encapsulated with the drugs to permit drug-distribution measurements, within the various vesicles and lamellae, using fluorescence microscopy and digital image analysis at the Johnson Space Center. The final microcapsules were suspended and recovered in either aqueous solutions, IPO or mineral oil. The microcapsules formed by these formulations were similar to those made using alcohol-soluble polyglycerides, however, without the hydrocarbon-soluble polyglyceride skin these microcapsules were more fragile.

Another unique type of microcapsule was formed during these experiments that was characterized by drug crystals formed within the inner aqueous core of the multi-lamellar microspheres. Figure 3 shows an example of a microcapsule which is packed (approximately) 65% of the aqueous compartment with crystals of Cis-platinum, anti-tumor drug. Figure 4 shows two microcapsules containing crystals of amoxicillin that were formed in the STS-52 experiments. These illustrate that aqueous-soluble drugs can be encapsulated at very high concentrations near the solubility limit of the drug. After the microcapsules are formed the drug can become further concentrated to form large crystals which can be more stable than the dissolved drug during prolonged storage.

In 1993 modifications to the MDA flight hardware enabled drug encapsulation experiments to be conducted on STS-56 at 20 °C. using alcohol solvents for the polyglycerides. Cis-platinum, amoxicillin and urokinase were each co-encapsulated with IPO, radio-contrast medium, using mixtures of aqueous-soluble polymers, dextran, C3-C6 alcohols (co-surfactants), and polyglycerides that are insoluble in aqueous solutions. These microcapsules were tougher than those formed on STS-52 using only water soluble polymers. The STS-56 experiments again produced multi-lamellar liquid microspheres (multiple concentric spheres within spheres) comprised of alternating immiscible layers. Using fluorescent 6.4 micron beads and image analysis, it was found that the most interesting microcapsules were formed in the range of 10-15 μ, 40-50 μ, 50-100 μ and 160-230 μ diameters. More microcapsules were formed containing crystals of cis-platinum or amoxicillin which were formed after encapsulation. Several microcapsules were formed that contained a single large cubic crystal of Cis-platinum which so completely filled the inner sphere that only about 15% of the inner volume remained as a liquid. One encapsulated, cubic Cis-platinum crystal was measured at 48 μ across within a 57 μ diameter microcapsule. (data not shown). After formation, some of the microspheres were dispersed in an external oil phase (either IPO or mineral oil) and allowed to cure for eight days before return to Earth.

Our space experiments have shown that formation of multilamellar, alternating-phase microspheres can be controlled by proper timed-sequence exposures of the immiscible phases using special solvent formulations and surfactants. Once formed these microcapsules remain spherical due to the predominant surface tension of the internal phases. High molecular weight polymers and polyglycerides can be included to form flexible, permeable "skins" around the liquid microcapsules as they are created by phase partitioning mechanisms. These experiments clearly demonstrated the capability to use liquid-liquid diffusion mixing to form unique microcapsules containing hydrophilic and hydrophobic drugs under microgravity conditions. The flexible microcapsules, formed under microgravity conditions, have more uniform size distributions than those formed in 1-g, largely due to the absence of thermal convection and instabilities that occur at the immiscible interfaces. The microgravity experiments illustrate the feasibility of co-encapsulating aqueous-soluble drugs, hydrocarbon-soluble drugs and oil-based contrast media within a lipid-soluble, polyglyceride, outer film which cures rapidly enough to be impervious to oil or hydrocarbon resolubilization. These methods allow the formation and harvesting of unique microcapsules which are durable enough to be removed from the external solvent without disruption or destruction of the internal phases. These new microcapsules have several advantages over conventional liposomes that are designed for intravascular injection.

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1 Centrolex-F™ is a lecithin produced by U.S. Soya, Inc.
Other Microencapsulation Flight Experiments

Previously, aerosol dispersion of agarose-type gels, dissolved in aqueous vehicles, were used to encapsulate pancreatic cells during short periods of weightlessness (25 seconds) during parabolic flights of the NASA KC-135, Zero-g aircraft. These were commercial pilot studies conducted by the Canadian Aerospace Ltd. and NASA [9]. A joint microencapsulation program was planned to expand these experiments to use electrophoretic separations to harvest only those microcapsules containing the pancreatic cells so that animal trials could be conducted to treat diabetes by transplantation of the encapsulated cells that produce insulin.

Microencapsulation in Space (MIS) experiments were also conducted on Shuttle mission STS-53 in December 1993 under a joint program between the Department of the Army's Space Technology and Research Office and Dr. T. Tice, Southern Research Institute, Birmingham, AL., using a traditional approach to aerosol dispersion of ampicillin. The main objectives of the experiments were to compare formation and curing of the liposomes in an electrostatic field vs. no electrostatic field, without having the liposomes sediment to the floor of the test chamber. The results presented in testimony to the U.S. Congress in April 1993 indicate that more uniform liposomes were formed in microgravity as compared to the same process on Earth [10].

Flight Hardware Description

The microencapsulation experiments described in this paper were conducted using ITA's automated laboratory, the Materials Dispersion Apparatus (MDA). The MDA is a compact device capable of bringing into contact and mixing up to 100 separate samples of multiple fluids and/or solids at precisely timed intervals. The samples mix either by simple liquid-liquid diffusion or magnetic mixing techniques. The device also can be used to grow protein crystals by three different methods and to cast polymer membranes in the absence of thermal convection.

Experiments were conducted on the sounding rocket flights during approximately 6.5 minutes of microgravity and two Space Shuttle missions which lasted 8 days. The fluid volumes were 500 microliters per sample. Approximately 8-10 microencapsulation experiments were conducted on each flight. The MDA's consist of an upper and a lower block that contain chambers for each sample fluid. The blocks are misaligned at launch so that the chambers are not in contact with each other. Upon activation in microgravity, the blocks are moved to align the chambers so that the fluids can mix by liquid to liquid diffusion. For some experiments, a single-step, magnetic-mixing technique was utilized to accelerate the process. Some of the experiments were conducted with a single-step fluid mixing, and some were done with a two-step fluid mixing technique which allows diffusion of a third fluid or sample into the mixture of the first two fluids while still in the microgravity environment. Figure 5 shows four MDA Minilabs ready for experiments to be conducted on the Consort sounding rocket flights. Two of these MDA units were used in each sounding rocket and four of the MDA units were utilized in the space shuttle missions. Figure 6 shows the integrated package ready for a Shuttle flight. The MDAs are completely automated system with commands being given by an onboard process controller when used on the sounding rockets. When the MDA units are flown in the Space Shuttle, a controller activated by the crew provides commands to process the samples. In addition, the MDA minilabs contain a manual back-up mechanism for astronaut intervention if the controller fails. As a result of the space experiments conducted in the MDAs a new microencapsulation apparatus is now being developed by NASA-JSC, IRI and ITA to exploit this technology on Earth and for conducting new flight experiments dedicated to microencapsulation of drugs under microgravity conditions.

DISCUSSION

Commercially oriented space experiments have shown that microgravity can permit the encapsulation of drugs into unique microcapsules by liquid-liquid dispersion and spontaneous emulsification using alcohol/ water/ oil mixtures, appropriate surfactants, and co-surfactants [11-14]. Spontaneous formation of multi-lamellar, microcapsules containing alternating layers of aqueous and hydrophobic solvent compartments is strongly dependent on the interfacial tension and the amount of mixing between immiscible liquid phases. On Earth this process is limited by gravity-dependent, density-driven separation of the immiscible liquids into stratified horizontal layers. In microgravity, this process is largely dependent on the surface-free energies of the different liquids, but independent of density-driven convection or bouyant phase separation. Hydrocarbon soluble, high molecular weight polymers have been included in the formulations to form flexible, permeable "skins" around the liquid microcapsules as they are created by phase partitioning mechanisms. The microcapsules can be formed and cured without deformation by contact with container walls. This offers new possibilities for using electric fields to form the microspheres or deposit coatings.
Co-encapsulation of an aqueous-soluble, anti-tumor drug (Cis-platinum) and a radio-contrast medium (IPO), in microgravity, has produced a unique drug delivery system that can be visualized by radiologic or computerized tomography scanning to insure that the cytotoxic drug is delivered directly to the target tumor. Multi-layered microcapsules have been developed which can provide a new intravascular delivery system for targeted tissues and sequential, sustained release of multiple anti-tumor drugs. This method has formed perfect microspheres and more uniform sizes, which can provide maximum packing densities and maximum drug delivery to target organs or tumors.

The capability to obtain larger, more uniform size microcapsules opens up more possibilities to treat highly vascular tumors (liver and kidney) with chemoembolization. Therein, the microcapsules (100-200 μ) are injected via an arterial catheter to form artificial emboli which block the blood supply to the tumor. The reduced blood volume, that flows past the tumor, becomes loaded with the antitumor drug that diffuses out of the microcapsules, thus increasing the chemotherapeutic dose to the tumor cells. The use of multiple drugs within the same microcapsule can provide an opportunity to design microcapsules specifically for chemoembolization treatments. Multiple-drug microcapsules also could be used to deliver first a chemotherapeutic drug which would kill tumor cells, and then an immuno-adjuvant (tumor necrosis factor) or immunological stimulant (e.g. Interferon-γ) that would enhance the patient's immune response to the tumor.

Multiple-drug microcapsules can also be used to deliver combinations of chemotherapeutic drugs to tumors that are located in privileged sites, such as brain tumors. An example would be the simultaneous delivery of different types of drugs, e.g. Diaziquone and Cis-platinum, to brain tumors via the carotide artery [15]. Multi-layered microcapsules could also be used to treat deep infections that are resistant to systemic antibiotics. In these applications, one or more antibiotics could be sequentially delivered or a immuno-stimulating cytokine (Interleukin-1) could be delivered to the site of the infection. Multi-layered microcapsules can be designed to protect active forms of urokinase and other thrombolytic enzymes until they are delivered and entrapped at the local site of a blood clot, where therapeutic doses of the enzyme can diffuse out to dissolve the unwanted embolism. These immiscible-liquid diffusion methods also could be used for encapsulating certain labile drugs to make microcapsules for special purpose drug delivery systems, esp. those designed to deliver drugs via the nasal or buccal mucosa or via inhalation directly to the lungs. Examples include protected delivery of mucolytic DNase for sustained release treatment of cystic fibrosis [16] and anti-trypsin for patients with deficiencies in the lung epithelium [17].

Our Earth-based research is now directed towards elimination of certain specific limitations of the liquid-liquid dispersion method of forming multi-lamellar microcapsules by modification of organic co-surfactants to improve the efficiency of initial microcapsule formation, characterization of those microparticles, then addition of an electrostatic coating step to create the final polymer skin in a more traditional fashion. These microcapsules will then be characterized and compared with those formed in microgravity. Another series of microgravity experiments is planned for the CMIX-3 payload now targeted to fly on Shuttle STS-67 mission in the fall of 1994. The data will be used to develop a new microencapsulation system that can be used for future commercial and microgravity encapsulation research. A joint Patent Disclosure has been prepared by NASA-JSC and the Institute for Research (IRI) to enable future commercial applications of this new microencapsulation technology.

**SUMMARY**

A new method of forming multi-lamellar microcapsules has been developed with the aid of microgravity experiments that eliminate the density-driven portions of phase separations that occur with immiscible liquids having different densities. Experiments on four different space missions produced unique microcapsules of Cis-platinum (antitumor drug), amoxicillin (antibiotic) and urokinase (fibrinolytic enzyme) that were co-encapsulated with iodinated poppy seed oil, which is a radio-contrast medium. Microcapsules also were formed with crystals of Cis-platinum or amoxicillin inside the aqueous liquid core. Two different solvent systems were used, based on either aqueous-soluble phospholipids and polymers or a system using aqueous-soluble drugs dispersed in alcohol solutions containing polyglycerides which form a tough outer skin around the liquid phases of the microcapsules. The formation of microcapsules with alternating hydrophilic and hydrophobic layers offers new advantages for drug delivery systems that are designed specifically for intravascular administration. Microcapsules can now be designed for chemoembolization and multiple-drug chemotherapy of vascularized tumors. Microcapsules containing the radiocontrast medium can be monitored by radiologists during intravascular administration. Other medical applications include multiple-drug therapy of resistant infections, blood clots and inhalation delivery of labile proteins to lung tissues.
REFERENCES

10. Dr. Thomas Tice, Southern Research Institute, Birmingham, AL. Congressional Testimony, U. S. House of Representatives, Committee on Science, Space and Technology, Subcommittee on Space, April 9, 1993.
Figure 1. Multi-lamellar microcapsules

Figure 2. Microcapsules containing IPO.

Figure 3. Cis-platinum crystals in microcapsules.

Figure 4. Amoxicillin crystals in microcapsules
Figure 5. Four units of Materials Dispersion Apparatus (MDA) automated mini-lab configured for sounding rocket flights. Photo courtesy of ITA, Inc.

Figure 6. MDAs configured for the CMIX payload that flew in the Shuttle Middeck on STS-52 and STS-56 missions. Photo courtesy of ITA, Inc.