ENCAPSULATION TECHNOLOGY

Hydrocapsules®: A New Method for Aqueous Drug Delivery

By: Ara Manukian and William Toreki, III, PhD

ABSTRACT

A new encapsulation technology developed by Analytical Research Systems, Inc. (ARS Inc., Gainesville, FL) with funding support from the USDA SBIR Program Office provides for a unique method of encapsulating a wide range of aqueous-based liquids with a cross-linked polymeric outer shell that can be used to deliver nutrients, vitamins, drugs, vaccines, and other chemical compounds. The method, originally developed for use in encapsulating aqueous-based solutions for entomological and agricultural applications, has the special capability of encapsulating live beneficial organisms, tissues, viruses, cells, bacteria, and fungi that need to be stored and delivered in aqueous solution. The liquid-filled capsules produced by this method are called Hydrocapsules® and have many potential applications in both veterinary and human pharmaceutical, medical, and dental sciences.

INTRODUCTION

The Hydrocapsule method allows for the formation of mononuclear microcapsules of the shell-core type that are produced by a patented process of simultaneously extruding an inner liquid core (encapsulant) material along with a continuous outer coating or layer of a polymerizable liquid (capsule shell), which is substantially immiscible with the inner liquid core, through concentrically aligned extrusion nozzles to form spherically layered bi-liquid droplets. These droplets are then subsequently exposed to energy input from high-intensity ultraviolet (UV) light, which causes the polymerization of the outer shell layer by the process of UV-initiated free-radical chain polymerization of functionalized pre-polymers and/or vinyl monomers. The resulting capsule shell material is a cross-linked hydrophobic elastomeric polymer network, which can have various physical and chemical properties depending on the formulation and application requirements. The capsules formed by this method are called Hydrocapsules, which implies that they have an aqueous liquid core surrounded by a thin hydrophobic polymer membrane; however, they are capable of containing a variety of liquid materials having a composition ranging from completely aqueous to completely non-aqueous, and typically range in size from a couple of hundred microns to several millimeters in size (Figures 1 & 2).

The capsule coatings produced with this Hydrocapsule method include a wide range of cross-linked polymers (many of which are FDA approved). These coatings can include a wide range of reactive or non-reactive components within the polymer matrix that can create a controlled or triggered release, swelling, or total breakdown of the capsule shell to deliver its contents. These release "mechanisms" can be designed into the polymer coating (shell) in such a way that it can react to changes in the surrounding environmental conditions to cause a breach of the coating, or in other instances, cause a transformation in the physical properties of the polymer coating that would allow for the diffusion or permeation of the contents through a softened or swollen shell. For pharmaceutical applications, some of these release mechanisms can include acidic or alkaline pH-sensitive triggers built into the polymer matrix.

Truly unique to this Hydrocapsule technology is the ability to encapsulate 100% water or other high aqueous-content mixtures that are not currently available in typical pharmaceutical capsule, softgel, or hard pill manufacturing. It should be noted that this process can equally encapsulate totally non-aqueous solutions, such as oils, other high-lipid concentration or emulsified liquid mixtures, sugar solutions, and alcohols with small amounts of water, which albeit, can be done by other types of industry-standard processes, such as the familiar "Softgel" technology used to encapsulate vitamin E, Omega-3 fish oils, and other oil-soluble drugs. However, these processes and are not suitable for encapsulating high concentrations of aqueous liquids, and unlike the"Softgels" and other similar products, Hydrocapsules can provide a stable capsule for long-term storage solution in the presence of high levels of external moisture (humidity) or water.[1]

HISTORY OF DEVELOPMENT

Encapsulation is commonly used to describe the process whereby an active ingredient is placed into a stabilized form in order to allow it to be conveniently

stored or protected from unfavorable conditions until needed. The active ingredient may be dispersed in a protective matrix, or it may be surrounded by a coating, a shell, or a membrane. The release of active ingredient(s) from the protected form may be rapid (such as by crushing or by ingestion), or gradual (such as by dissolution, diffusion, chemically triggered or controlled time-release, or biodegradation). In this manner, it is possible to maximize the effectiveness of the active ingredient by ensuring it is released at the proper time. This "controlled release" can also be made to occur over a programmed time interval (sustained release) or on demand (stimulated release).

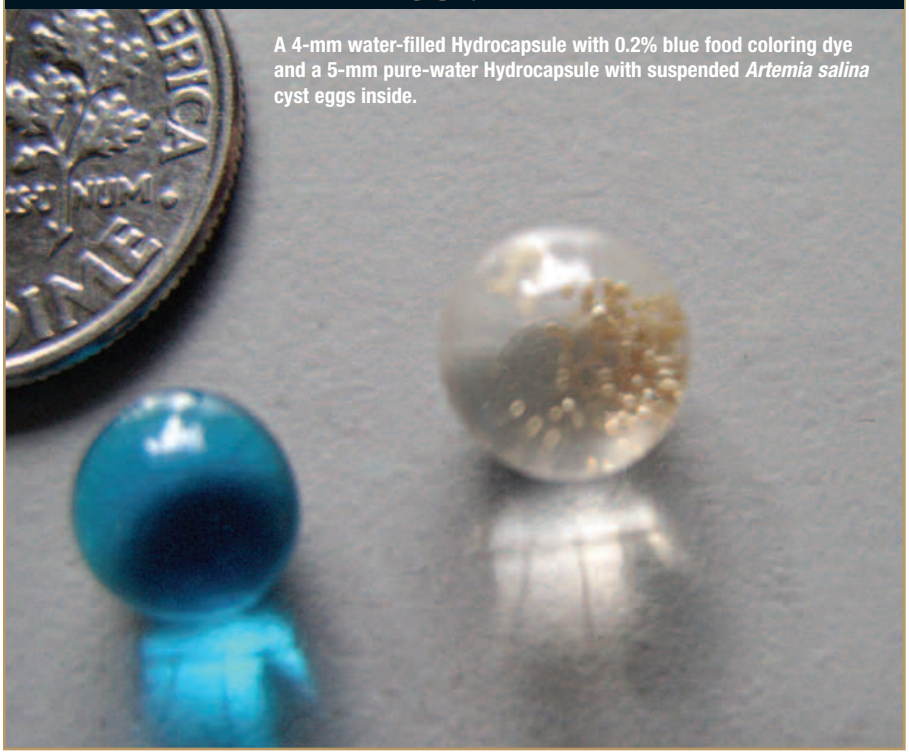
The term "microcapsule" has been used to describe small particles or beads, which range in size from less than one micron, up to several millimeters, which may contain a wide variety of active ingredients.[2-6] Microcapsules can be divided into two broad groups.

The first is Aggregate type microcapsules, which have the active ingredient dispersed uniformly throughout a continuous matrix. The matrix may be a solid dry polymer or a gel swollen with solvent. In the case where the gel is swollen with water, the term "hydrogel" is applied. Hydrogel encapsulation systems of this type are generally based on water-soluble polymers, such as alginate, gelatin, pectin, agar, gellan, or starch.[7]

The second is Mononuclear microcapsules, which consist of materials that show a true "shell-core" morphology. These are similar to an egg in that they have a solid outer shell or flexible membrane surrounding a core that may be a liquid, a solid, a gel, or a combination of any of these. Hydrocapsules fall into this second category.

Methods of producing microcapsules are the subject of numerous books and articles; however, the majority are simply not suitable for producing medium-to-large size (> 500 microns in diameter) mononuclear microcapsules with a true shell-core morphology and capable of containing an aqueous-based liquid core solution.[2-6,8,9] The method of "concentric extrusion" can be used to produce this type of microcapsule, in which two mutually immiscible liquids are simultaneously extruded through concentric

FIGURE 1



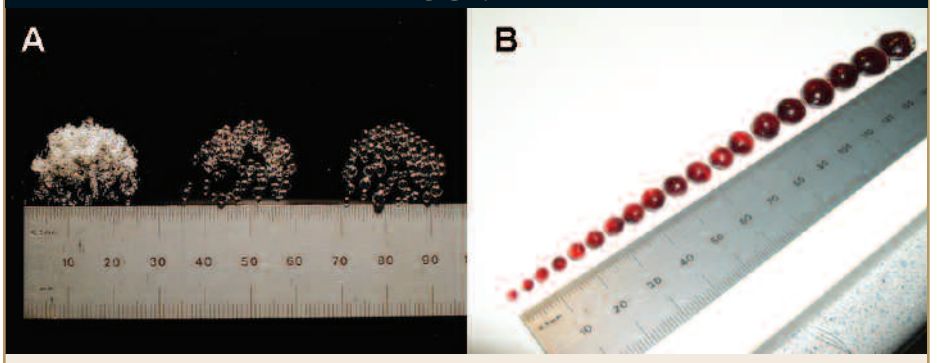
A 4-mm water-filled Hydrocapsule with 0.2% blue food coloring dye and a 5-mm pure-water Hydrocapsule with suspended *Artemia salina* cyst eggs inside.

orifices in order to produce a biliquid column, with the core fluid on the inside. Under the influence of gravitational, surface tension, or other forces (centrifugal, pressure, etc.), this bi-liquid column fragments into discrete droplets having a shell/core morphology. The liquid outer shell is then made to undergo a physical/chemical change via various controlled mechanisms enabling the liquid core to have a specifically engineered shell ranging from elastomeric and/or permeable to completely hard and impervious to liquids. Hardening of the shell is generally effected by heating to remove a solvent or by cooling to solidify a molten shell material. The outer coating in these systems is often a molten wax or a solution of aqueous polymer, such as gelatin or alginate. The use of heat, to melt the shell material or to drive off solvent can be detrimental to sensitive core materials, such as protein solutions or suspensions of living organisms. Additionally, the use of solvent-based shell formulations can lead to undesirable contamination of the core material as well as health and safety concerns.

Aqueous-based shell formulations, such as gelatin, cannot be used in conjunction with aqueous core materials because phase incompatibility is a necessary prerequisite for formation of a shell/core morphology using this technique. Also, these types of shells are, by nature, easily affected by water and also very susceptible to dehydration. Another drawback of the existing liquid encapsulating techniques is that the physical and mechanical properties of the shell materials suitable for use in these approaches are limited. Waxes, for instance, have very poor elasticity and mechanical strength and also low melt viscosity, making production of very thin membranes impractical. Low molecular weight thermoplastic polymers are generally too brittle and lack the flexibility to give strong, thin-walled, individual capsules. Thin, flexible, and durable membranes are generally only associated with cross-linked elastomeric polymers, which are generally insoluble and will not melt into a flow-able liquid even at extreme temperatures.

The initial application that lead to the development of the Hydrocapsule technology was brought about by a need to encapsulate high-protein content, aqueous-based, artificial liquid nutrient diets by Dr. Patrick D. Greany at the USDA's Agriculture Research Services, Center for Medical and Veterinary Entomology (CMAVE) based in Gainesville, FL. The USDA needed to encapsulate these nutritional diets for the purposes of feeding beneficial entomophagous insects (good insects that eat pest insects) so they could be mass reared economically in large numbers so they could be subsequently released into agricultural settings for natural control of

FIGURE 2



Various-size (A & B) Hydrocapsules ranging from 200 microns to 10 mm in diameter.

phytophagous pest insects (plant-eating insects). This concept of releasing large numbers of beneficial insects to augment already-existing populations of beneficial insects is called Augmentative Biological Control (ABC), and is one of several Insect Pest Management (IPM) strategies being employed by the USDA to help decrease the usage of traditional chemical pesticides in agriculture. ARS submitted a Small Business Innovative Research (SBIR) Phase I grant proposal to the USDA and was funded in 1996. Subsequently, ARS was awarded an SBIR Phase II grant, and follow-up Phase III funding was provided by commercial partners to complete development of the Hydrocapsule technology. US and international patent applications (PCT) were filed in 2000, and the US Patent was awarded in 2004 (US Patent No. 6,780,507 B2).

THE HYDROCAPSULE PROCESS

The Hydrocapsule process comprises two critical steps: (1) the fluid-mechanical process of co-extruding two immiscible liquid streams (the outer shell and inner core liquids) to form a bi-liquid column and subsequent droplets; and (2) the chemical reaction to polymerize the outer liquid shell material to convert it to a solid coating that surrounds the liquid core.

In the first step, the process of co-extrusion involves ejecting two liquid streams from concentric nozzles under a force. In this manner, the liquid solution to be encapsulated and an immiscible shell-forming organic liquid are pushed simultaneously through concentric nozzles by force. The center nozzle carries the liquid material to be encapsulated, while the outer nozzle carries the coating precursor. The choice of orifice size will vary depending on the particular materials and final capsule size selected. The use of larger-diameter nozzles will generally result in the formation of larger Hydrocapsules. After emergence from the concentric nozzle, a series of concentric bi-liquid droplets is formed and then enter into a reaction zone (Figure 3). Inside this reaction zone, energy input from a high-intensity mercury lamp is used to supply UV light to catalyze, initiate, and promote the curing and free-radical chain polymerization of the vinyl monomers, oligomers, pre-polymers, and cross-linking agents, which are the typical components of an outer shell formulation. Under the influence of gravity, the bi-liquid stream will break-up into multiple smaller discrete droplets. This effluent enters into a column with a suspending medium that provides some buoyancy. The main purpose of the suspending medium (which can be a liquid or gas) is to slow the gravitational descent of the droplets, which increases the residence time in order to allow the polymerization, solidification, and/or cross-linking reactions to proceed to substantial completion, and aids in droplet separation.

The second step, polymerization of the hydrocapsule shell, is accomplished via free-radical chain polymerization of vinyl monomers utilizing photo-initiators, which rely on the absorbance of light energy in order to produce free radicals, which then initiate the polymerization of reactive vinyl groups present in the shell formulation. In this process, UV sensitive photo-initiators are used (such as benzophenone, benzoin ether, camphorquinone, and acyl phosphine oxide), which react within seconds. The concentration of photo-initiator used in the shell-forming liquids varies but is typically in the 0.1% to 2% weight range.

Selection of the proper shell components (formulation) is critical to completing the second step in the process. There are many shell-forming materials that are useful in making Hydrocapsules and can be selected from the broad class of vinyl compounds. These are compounds containing one or more polymerizable vinyl (-CH=CH_2) groups. These vinyl-containing shell-forming materials may be relatively low molecular weight compounds (< 200 amu), which are generally referred to as "monomers," or they may be larger molecules (> 200 amu), which are generally referred to as "reactive oligomers," "macromonomers," or "prepolymers." Thousands of such compounds are known, and there is a myriad of formulations, blends, and mixtures that can be useful. Typical low molecular weight monomers used in this process are methyl methacrylate (MMA), acrylic acid (AA), butyl acrylate (BA), hexyl acrylate (HA), and hydroxyethyl methacrylate (HEMA). Additional less-common acrylic monomers like long-chain alkyl acrylates and methacrylates (such as C_{12} - to C_{24}- acrylates), tetrahydrofuranyl acrylate, or caprolactone acrylate are used to impart useful properties to the shell formulation. Other commonly known vinyl monomers used are vinyl chloride, styrene, and vinyl acetate. Depending upon the application requirements of the shell, formulations can also include difunctional and multifunctional compounds (containing two or more vinyl units per molecule), such as divinyl benzene (DVB), ethylene glycol dimethacrylate (EGDMA), trimethyloyl triacrylate, and hexane diacrylate. Such polymers have desirable properties like good mechanical strength, elasticity, toughness, and flexibility.

Non-reactive components can also be incorporated into the shell formulations. These types of compounds do not react with the vinyl groups present, but instead are added to impart some type of desirable property to the shell-forming liquid (such as viscosity control) or to the final shell polymer (such as a plasticizing effect). Such compounds may be of any molecular weight. The use of non-reactive polymers in the shell formulation will result in a polymer blend or interpenetrating network

after the reactive vinyl components have undergone polymerization. Volatile components can also be added in order to facilitate processing or to modify the properties of the final shell materials. Other types of commonly used polymer additives, such as chain-transfer agents, antioxidants, anti-static compounds, UV stabilizers, dyes, and fillers can also be incorporated into the shell-forming fluids.

The use of silicone-based UV-curable elastomers as shell-forming components are particularly useful in making biocompatible capsules having favorable mechanical characteristics, environmentally benign properties, and desiccation resistance far superior to hydrogel-based polymers, such as alginate or gelatin (> 100X). Silicone polymers are commonly known to have, by far, the highest oxygen permeability of any class of synthetic polymer.[10-12] The oxygen permeability of silicone is 100 times that of polyethylene (PE). This is why it is particularly suited for applications such as gas-exchange membranes in heart-lung machines.[11] Many formulations are possible using reactive silicones blended with selected acrylic and urethane resins. Conversely, polymers like poly-vinyl chloride (PVC) or poly-ethylene terephthalate (PET) have very low oxygen permeability.[10,12]

CURRENT APPLICATIONS

The original application of this technology successfully demonstrated its first use in commercial applications to produce approximately 2- to 4-mm diameter hydrocapsules containing an aqueous-based liquid artificial nutritional diet used for the mass-rearing of beneficial insects that contained proteins, carbohydrates, and lipids, which were derived from processed animal livers along with added vitamins and antioxidants. The preparation of this and similar diets are described in detail in US Patent Nos. 5,799,607 and 6,129,935. A shell precursor solution was prepared by mixing a commercial aliphatic polyurethane acrylate composition (10 parts), a mixture of monofunctional acrylate monomers (15 parts: 50/50 caprolactone acrylate and tridecyl acrylate), a low viscosity aliphatic diacrylate oligomer (5 parts), a dialkyl phthalate plasticizer (10 parts), and a photo-initiator (1 part, benzoin isobutyl ether). The specific gravity of this mixture was measured and found to be approximately 1.04 g/cc. The capsule walls had an average thickness of about 50 microns and were generally soft and pliable such that the beneficial insects that were presented these hydrocapsules (*Podisus maculiventris and Diapetimorpha introita*) easily penetrated the shell and consumed the contents.

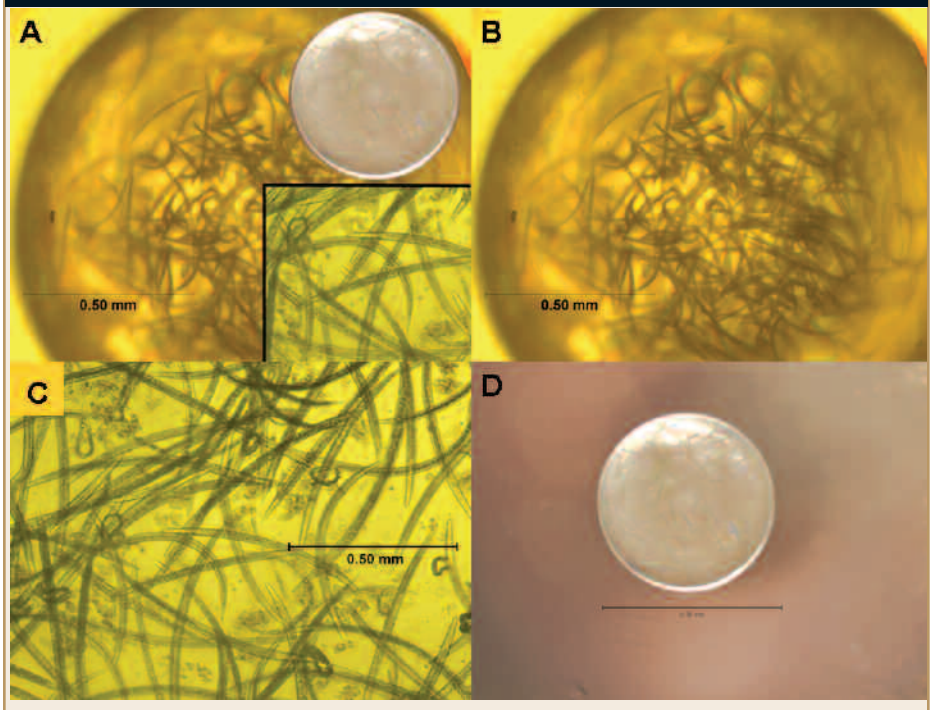
Subsequent work during this time was done on encapsulating and delivering attractant (bait) solutions, entomopathogenic nematodes, bacteria, and fungi for pest insect control. Production of hydrocapsules that contained an aqueous suspension of entomopathogenic nematodes (*Steinernema feltiae*) at a concentration of 2,000 AU/ml provided by a commercial supplier (BioLogic, Willow Hill, PA) were encapsulated in a solution of sucrose (40 g/L) and dextran (1 wt %) in de-ionized water (Figure 4). The specific gravity of this nematode suspension was measured and found to be approximately 1.008 g/cc. A shell precursor solution was prepared by mixing a commercial aliphatic polyurethane acrylate composition (6 parts), a mixture of monofunctional acrylate monomers (11 parts), an acrylate-functionalized silicone (6 parts), a dialkyl phthalate plasticizer (6 parts), and a photo-initiator (0.7 parts). Capsules were produced in a manner similar to that previously described. Microscopic examination of these capsules revealed they contained living nematodes. Capsule diameters ranged from approximately 2 to 4 mm. These capsules were stored in a loosely capped plastic vial in a refrigerator at approximately 5°C. After 9 months of continuous refrigerated and oxygenated storage, it was observed that the majority of the encapsulated organisms were still alive as evidenced by their swimming motions (active movement) when viewed under a 20X optical microscope.

Using the same formulations and procedures, an encapsulation of a commercial bacterial pesticide formulation (Thuricide^{R} HPC, purchased from Home Depot),which is essentially a suspension of the entomopathogenic bacterium *Bacillus thuringiensis kurstaki* (otherwise known as BT), was also performed. The activity of this suspension was listed at 4,000 IU/mg. The capsule shell formulation was similar to the one described earlier. Capsules with an average diameter of approximately 3 mm were obtained. A sample of the encapsulated

FIGURE 3

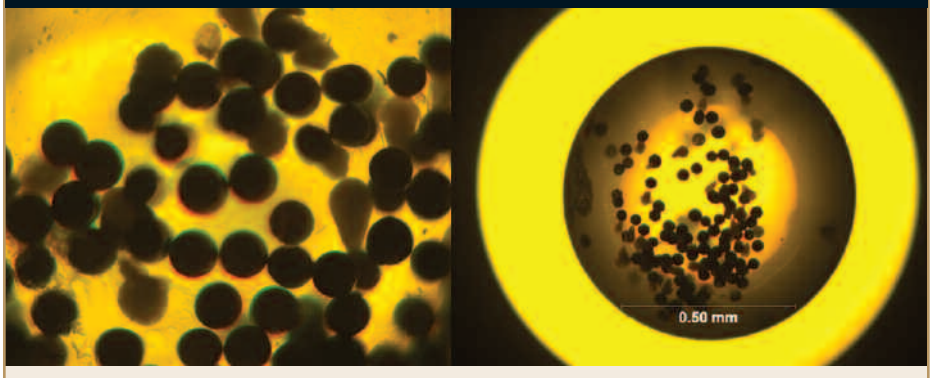
Hydrocapsule Encapsulation Machine (HEM) utilizing a liquid-liquid co-extrusion nozzle system in conjunction with a UV photo-chemical exposure chamber.

FIGURE 4



Various-size pictures (A through D) of a 2-mm Hydrocapsule containing live beneficial nematodes (*Steinernema feltiae*) at a concentration of 2,000 AU/ml in water-sucrose, which was encapsulated using an oxygen-permeable silicone containing cross-linked polymer.

FIGURE 5



A 1.5-mm water-filled Hydrocapsule with suspended *Artemia salina* cyst eggs inside

material was subsequently opened and cultured on agar in a Petri dish. After several days, extensive colonization of the Petri dish by BT was observed and verified.

Additional development was done encapsulating various biological components, such as animal blood products and tissue. To demonstrate the ability of larger particles to pass through the co-extrusion nozzles, a solution of Artemia salina (brine shrimp eggs) was made and successfully encapsulated (Figures 1 & 5). Utilization of pH-sensitive polymer formulations for coating and delivery of additional entomopathogens (such as viruses and fungi) have shown promising results in initial testing by government and academic laboratories and are currently proprietary.

FUTURE PHARMACEUTICAL APPLICATIONS

The use of this technology has much broader application potential in the fields of veterinary and human medical and pharmaceutical science than originally developed. Currently, new investigations are being conducted for using Hydrocapsules to deliver essential nutrients, drugs, and vaccines to farm-reared fish in large-scale aquaculture. The unique ability of Hydrocapsules to encapsulate aqueous solutions also allows its use for delivering active ingredients in an aquatic environment. Methods of release currently being employed are based on pH-reactive coatings to allow the capsule to remain intact in water (pH 6 to 8) until ingested, and then pass through the stomach region of a fish, where the stomach acid causes a triggering of the polymer coating to begin breaking down over a predetermine time interval (based on coating thickness and formulation chemistry) and ultimately deliver its contents into the lower digestive tract of a fish. These types of reactions can be acid or alkali triggered. The formulations and mechanisms currently under development for aquaculture drug delivery have direct application to human and other animal pharma.

Additional medical/pharmaceutical applications include the ability to deliver beneficial organisms, tissues, cells, and bacteria. There is the potential need to replenish beneficial bacteria in the stomach and mouth after patient exposure to long-term treatments with antibiotics after surgical or dental procedures or after serious infections. The ability of delivering aqueous-stored anti-viral agents, antimicrobial, or aqueous-based anti-cancer treatments through oral ingestion by animals and humans is possible. There is also the possible use of delivering these same agents in combination with a topical ointment or external treatment application in which the capsules can be mechanically ruptured by direct application or rubbing of an infected area. The Hydrocapsule process allows for the encapsulation of many such agents for any of these applications and others, without the use of direct heat, extreme pressures, or solvent processes that could degrade these agents or volatile compounds or cause the breakdown or denaturing of proteins, amino acids, or lipids.

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BIOGRAPHIES

Mr. Ara Manukian is the current CEO of ARS, inc. and has served as the Director of Engineering for the past 12 years, and has been responsible for management of all engineering projects for the company. Mr. Manukian, a co-pi and co-inventor of the Hydrocapsule technology, is a systems design engineer specializing in the development of electronic instrumentation and computer and PLC-based control systems, specializing in electrical and mechanical interfacing and fluid flow, pressure, and temperature control. He has been responsible for over 500 engineering contract projects during the past 12 years for several academic, government, military, and private industry laboratories, working on projects ranging from design of avionic systems, space-flight hardware, analytical instruments, material processing, and chemical plant reactors. He has co-authored 20 scientific papers and several technical articles, contributed to 4 books, and has 4 US Patents. Previously, Mr. Manukian worked for 5 years as a systems engineer at the USDA-ARS, CMAVE laboratory in Gainesville, FL, in the Chemistry Research Group. In that position, he was responsible for the development of a wide range of automated volatile collection systems, bioassay systems, and analytical instrument and chemical analysis methods development for semio-chemical research. Mr. Manukian was a collaborating investigator on 10 research grants during that 5-year period and received 4 USDA Merit Awards for Outstanding Performance. Prior to working with the USDA, Mr. Manukian was employed as a research assistant in several positions under grants from NASA, University Space Research Association (USRA), Florida Space Grant Consortium, Florida Space Foundation, and Florida Challenger Astronaut Memorial Foundation. Research under these grants covered topics related to developing systems for growing crop plants in space and mathematical modeling of dynamic control systems used in a spaced-based Closed Environmental Life Support System (CELSS).

Dr. William Toreki III is a Senior Research Polymer Chemist with ARS, inc. and was a co-principle investigator and co-inventor of the Hydrocapsule technology development from 1996-2000. Dr. Toreki has worked with ARS since 1996 and was key to the formulation chemistry development for Hydrocapsules and is currently consulting with ARS on several polymer-chemistry-related new product applications. Dr. Toreki is currently employed as the Chief Polymer Chemist for Quick-Med Technologies, another company also based in Gainesville, FL, which develops polymer-based systems for advanced wound care, cosmetic, medical, and military markets. Dr. Toreki has extensive research experience in various applications of polymer and silicone chemistry and has been recently focused on incorporating biologically active compounds and antimicrobial agents into various polymer systems and cellulose fibers for entomological, agricultural, biomedical, and wound-care research. He has been involved with microencapsulation, biomaterials research, and polymer fiber development for over 20 years and previously worked as a materials research scientist with the biomaterials research group in the Dept. of Material Science and Engineering at the University of Florida in addition to being a court-qualified expert witness in the field of polymer and silicone chemistry in several states. Dr. Toreki has additionally consulted to numerous companies in these same fields during the past 15 years, and has been involved in over 100 new product development projects and currently has 11 issued US Patents and 9 Patents pending, in addition to co-authoring over 20 publications. He has received an Outstanding Service Merit Award from ARS, as well as received the DuPont Excellence in Teaching Award.