Bioencapsulation Research Group

Bioencapsulation Innovations

March 2013

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- September 2013 : Best posters from XXI International conference on Bioencapsulation
- December 2013 : Enzyme encapsulation

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bi@bioencapsulation.net

EDITORIAL

MICROENCAPSULATION BY CHEMICAL METHODS : A SOLUTION FOR THE PAST OR FUTURE

Chemical microencapsulation methods are based on polymerisation or polycondensation mechanisms that may be implemented in a variety of different ways. Among them, interfacial and in situ polymerisation processes gained most scientific and industrial attention, and became an important alternative to coacervation microencapsulation processes (Figure 1).

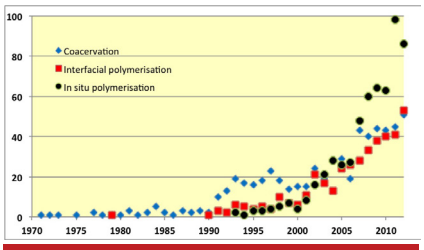

Figure 1. Scientific publications

Since the publication in 1959 by Morgan and his collaborators of a series of papers on interfacial polymerization, this technology has gained a great interest in the industry. Some years later, in situ polymerization technology was added, especially for technical applications (Figure 2). The volumes of production represent thousands of tons per year.

Most people associate the chemical methods to quite strong operating conditions. This is true when considering for example the polyamide capsules, where the pH higher than 12 and solvent like chloroform are needed to get a fast polymerization.

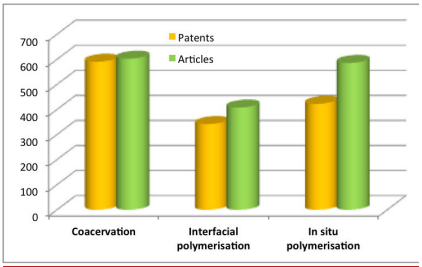

Figure 2. Patents vs Publications

These constraints could be overpassed. Work done by the group of Mrs Lévy in France, Kondo in Japan or Neufeld/Poncelet in Canada developed some approaches that allow to produce microcapsules in smoother conditions. Such capsules has been successfully applied in cosmetic applications. We may cite especially the development of the start-up Coletica in France, now part of BASF.

Similar story could be provided for the in situ polymerization or sol- gel methods. Their applications even extend to environment or biomedicine. However, the reputation of the chemical methods still suffers of their past image.

It is true also that many industrials are stuck with old chemistry, and innovations are developing slowly. Many questions remain. A simple one is what is the content of the pore in a polyamide membrane (Is it water ?). On the other hand, some technologies are still in the drawer, such as the continuous emulsification process.

In fact, the encapsulation by chemical methods remains the domain of few great companies, and very few small companies are to day in the business of producing microcapsules by chemical methods. We hope that the present newsletter will change the situation.

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ARTICLE

BARRETT K. GREEN
THE FATHER OF MICROENCAPSULATION

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INTRODUCTION

Barrett K. Green (September 11, 1906–August 29, 1997) was an American scientist, innovator, and industry pioneer who is best known as the inventor of microencapsulation, a term applied broadly to processes that create microcapsules of a payload material. Green was a long-time NCR employee (1933-1973), held 197 patents, and was highly respected and honored as both a scientist and as a leader in the development of practical, real-world products. Today, there are virtually thousands of controlled release products (and others) that are possible because of his ability to translate his science into practical, usable, real-world products. His story offers inspiration not only to the potential value of our current research efforts, but also to the broader scope of this value as it extends into many other areas of need.

The purpose of this article is to share information about Green's background, time at NCR, and the impact he has had on the controlled release industry.

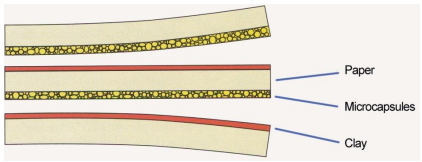
EARLY YEARS

Barrett Green developed a strong interest in science and chemistry during high school in the early 1920s. This interest took him to Cornell University in Ithaca, New York. Cornell was well-known (even in the early 20th century) for diversity in all fields of knowledge with an emphasis on both learning a discipline and applying it in the «real» world. Green focused his attention on colloids and colloid science in his undergraduate years and received a BS degree in chemistry in 1928. He continued his work in colloid chemistry an additional four years as a graduate student at Cornell. His keen interest in this area and Cornell's emphasis on applied technology formed a foundation for his inventive interests.

NCR

Barrett Green's career at NCR Corporation began as a research scientist (one of the first hired by NCR) in 1933, and ended in 1973 when he retired as Assistant Vice President of Central Research. During his long, celebrated career at NCR (previously known as National Cash Register), Green pioneered modern day coacervation techniques that led to the development of carbonless paper, scratch-and-sniff products, and time-released capsules among many other uses.

Carbonless paper—Green's major breakthrough product—emerged from research efforts extending from the late 1930s into the 1950s. By 1942, Green had developed a working method of microencapsulating ink and a prototype carbonless paper. Over the next decade, he refined his methods and scaled the process to production levels. He worked closely with Thomas Busch of Appleton Papers on the difficult process of applying the microcapsules to paper in a thin, flexible layer.



Carbonless paper had three layers: the paper; a film of acid-sensitive dye packaged in microcapsules; and a layer of acidic clay to develop the dye from transparent to dark blue or black. Pressure from a writing implement (pen or pencil) broke the microcapsules of dye on the underside of each sheet (except the last one); when the dye was released, it reacted with acidic layer on the surface of the next sheet. Considerable effort went into designing capsule walls that were sturdy enough to withstand processing but would rupture under the pressure of a pencil.

Scientists had long been intrigued by the possibilities of controlling the release of an active ingredient by encapsulating it. What was theoretically possible proved difficult in practice. Green's research, partly based on his studies as a graduate student at Cornell, involved enclosing a liquid in a solid. Essentially, he solved the pressure-triggered release problem by chemically «hardening» the outer layer of the capsule using gelatin. When gelatin is treated with a reactive chemical such as formaldehyde, chemical bonds form between the gelatin chains resulting is a three-dimensional network of cross-linked gelatin. Cross-linked gelatin is harder and less soluble than regular gelatin, yielding a tougher and more durable microcapsule.

NCR introduced the first successful carbonless paper to the business world in 1954. Green received a patent for microencapsulation on July 5, 1955.

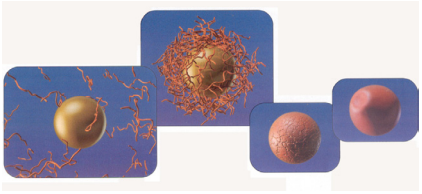
In an article written at the time of his retirement, Mr. Green reflected on his discovery.

«I can remember very well the day we found what we had been looking for with encapsulation and paper. We had developed a process earlier, but it wasn't good enough. We used an emulsion on the paper, and in a warm room, the emulsion melted and the paper was ruined.

«What we had visualized before we could actually do it, was to leave the oil in the clay, which was coated on the paper—leave it there isolated and insulated, but colorless.

«I remember the afternoon I applied the clay toluene test after I'd made some capsules by the coacervation process, and the test was successful. I knew right away that we had what we'd been looking for.»

As a result of Green's discovery, NCR (No Carbon Required) paper became a major cutting-edge product that was manufactured by NCR around the globe and was widely used by tens of thousands of customers. It provided a



much-improved business forms media at a time when the business forms industry was growing dramatically.

Prior to his discovery, no major products had been developed using the science of encapsulation. Again, Mr. Green reflected on his breakthrough.

«The science of encapsulation had been established, but no one had put it to work—to do a job. When I was a student at Cornell, the professors had very little to say about the idea of a liquid being dispersed in a solid.»

APPLIED TECHNOLOGY

A few years after NCR introduced carbonless paper, another first-of-its-kind product—based on Green's research—was delivered to the marketplace. Chester Carlson, an inventor, enlisted the aid of the Haloid Company of Rochester, New York to help commercialize his new copying process known as xerography. Xerography was a dry photocopy process that used toner consisting of microencapsulated dyes. The Xerox 914, released in 1959, was the first machine that faithfully produced copies of virtually any document without resorting to less desirable, messy wet processes.

An unexpected path that this versatile technology took was the development of fragrance ads used in advertising scented products. Commonly known as scratch-and-sniff, these «ads» consisted of small capsules filled with a solution—typically perfume. Scratching the surface ruptured the capsule and the scent was released.

The microencapsulation work of Barrett Green provided a foundation for applications in many diverse industries including pharmaceuticals, foods, cosmetics, nutritional supplements, personnel care, pet care, household, agricultural, detergents, paints, adhesives, and sealants. The real-world applications of Green's technology developed over a half century ago may be limited only by the imagination today.

RECOGNITION

Barrett K. Green was well-known and highly acclaimed for his work during his life. He was honored by his colleagues at NCR and other professionals; recognized by his community; received numerous awards for his research and product developments; and was inducted into the prestigious Engineering and Science Hall of Fame after his retirement.

During National Engineers Week in early 1965, Green was honored for his work in 1964 on the Photo Chromic Micro-Imaging concept. He was also acknowledged at that time as the author of the «Coacervation» section in the New Encyclopedia of Chemistry, as co-author of a paper entitled «New Principle of Emulsion Stabilization» presented to the American Chemical Society, and as co-author of a paper entitled «Chemical Switches» presented at the International Symposium on the Theory of Switching presented at Harvard University.

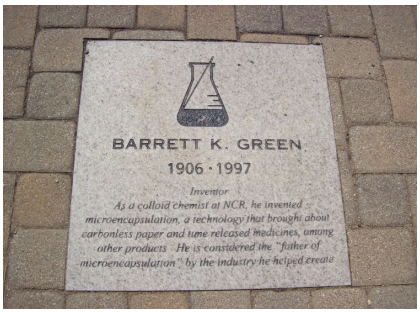
Later in 1965, Mr. Green and a fellow researcher from NCR, Lowell Schleicher, were acknowledged for their work in microencapsulation and colloid chemistry, receiving the «Modern Pioneers in Creative Industry» award from the National Association of Manufacturers (NAM).

On October 17, 1991, Barrett Green received one of his most prestigious awards from the Engineers Club of Dayton: He was enshrined into the Engineering and Science Hall of Fame as the «developer of the process of microencapsulation.» Others honored at that ceremony included Dr. Leland Clark, inventor of the heart-lung machine and Chester Carlson, the developer of xerography. Other well-known inductees include Orville and Wilbur Wright, Thomas Edison, Enrico Fermi, and Jonas Salk.

Green was also honored by the community in which he worked and lived with his name and accomplishments immortalized in granite on the Dayton Walk of Fame in 2004. Scientists, entertainers, philanthropists, corporate and business leaders, and others have been recognized on the Walk of Fame for their « . . . outstanding and enduring personal or professional contributions to the community, nation, and the world.» Green was honored as an «inventor» and acknowledged as the «father of microencapsulation.»

Perhaps Barrett Green's greatest legacy can be found in the hundreds of products that have been developed as a result of his work.

Green could easily be considered one of the original «green» thinkers. He believed in using resources to their best advantage. Over seven decades ago—with the invention of carbonless paper—he was «going green.»



Barry's Green's life was all about maximizing resources. About findings solutions to real-world problems. About turning pure science into economically viable and useful products.

MORE INFORMATION

More information on Barrett K. Green, coacervation, and microencapsulation can be found on the websites www.coacervation.net, www.microencapsulation.net, and www.controlled-release.com .

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The Ronald T. Dodge Company is a global supplier of microcapsules. It is a privately owned company founded in 1979. Dr. Ronald J. VeršiÐ is president, founder and Chief Scientific Officer. Products include fragrance inks and coatings, peroxide catalysts and custom products

ARTICLE

IN SITU POLYMERISATION MICROCAPSULES

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University of Ljubljana, Slovenia

IN SITU POLYMERISATION MICROENCAPSULATION

In situ polymerisation is one of the chemical microencapsulation processes, taking place in oil-in-water emulsions. The result is nicely smooth, spherical, reservoir-type microcapsules with transparent polymeric pressure-sensitive microcapsule walls (Figure 1).

Typical wall materials for *in situ* polymerisation are aminoplast resins, such as melamine-formaldehyde, urea-formaldehyde, urea-melamine-formaldehyde or resorcinol-modified melamine-formaldehyde polymers (Table 1).

Table 1: Wall materials for *in situ* polymerisation

Microcapsule wall materials	
Melamine-formaldehyde polymer	[1 - 6]
Urea-formaldehyde polymer	[7 - 8]
Urea-melamine-formaldehyde polymer	[9]
Aminoplasts including polyamine moieties, polyols and substituted methylene moieties	[6]
Resorcinol-modified melamine-formaldehyde polymer	[10]
Ketimine – epoxy resin	[11]

Typically, the microencapsulation processes can start either directly from amine and aldehyde monomers, or from the precondensates. The condensation reaction in absence of water is thermally catalyzed and difficult to control. The condensation products are water soluble and suited for *in situ* polymerization, however, they are reactive and less suitable for industrial applications. These obstacles can be overcome by reacting the hydroxymethyl groups with lower alcohols to form alkoxymethyl compounds. Examples of etherification are tris(hydroxymethyl) melamine and hexa(hydroxymethyl) melamine (Figure 2), often used as precondensates in the *in situ* polymerisation microencapsulation

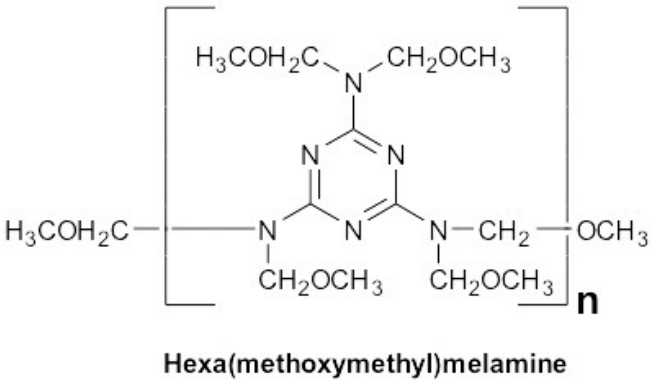

Figure 2. Hexa(methoxymethyl)melamine – HMMM, a typical precondensate for in situ polymerisation microencapsulation

Four main reaction types may occur in the polycondensation processes when amino-aldehyde precondensates are used for in situ polymerisation microencapsulation, resulting in the formation of: 1) methylene bridges and water, 2) methylolmethylenebisamide and water, 3) ether bridges and water, 4) methylene bridges and formaldehyde. The later contributes to residual formaldehyde in the aqueous suspension of microcapsules. Therefore, formaldehyde has to be removed by the addition of scavengers, such as urea, melamine, ammonia, or ammonium chloride.

In the in situ polymerisation microencapsulation process (Figure 3), all materials for the formation of microcapsule wall originate from the continuous aqueous phase of the oil-in-water emulsion system, and therefore have to be water soluble. Under ideal conditions, by change of pH and temperature all the mass of the wall material precipitates and distributes evenly over the surfaces of droplets in emulsion. To achieve better process control and improved mechanical properties of microcapsules, modifying agents / protective colloids are added, such as styrene-maleic acid anhydride copolymers, polyacrylic acid, or acrylamidopropylsulfonate and methacrylic acid/

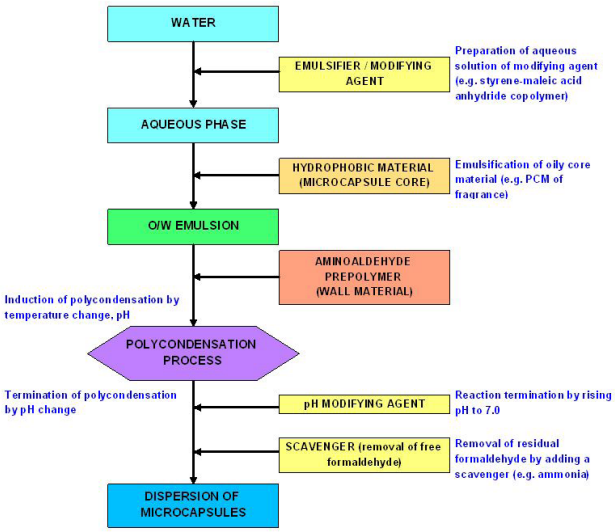

Figure 3. Synthesis of microcapsules by *in situ* polymerization process

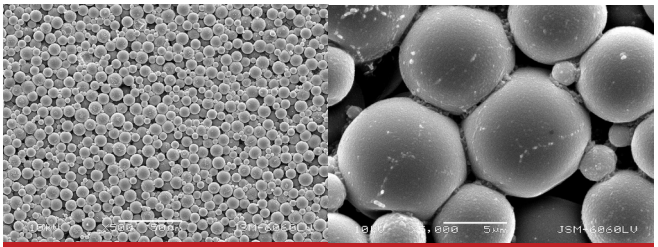

Figure 1. Spherical, reservoir-type microcapsules, produced by *in situ* polymerisation in oil-in-water emulsion (Scanning electron microscope - SEM, left 500x, right 5000x)

ARTICLE

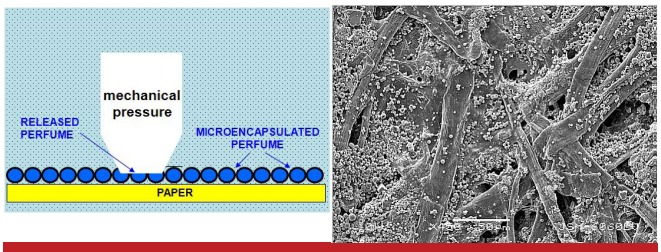

Figure 4. Application of microencapsulated fragrances in scratch-and-sniff papers: mode of action (left), and SEM photograph, 450x (right)

acrylic acid copolymers. At first, they serve as emulsifiers and emulsion stabilisers, and later enable the polymerisation to develop only at the surface of the emulsified microcapsule cores, and not throughout the whole aqueous phase.

PATENTS & COMMERCIAL PRODUCTS

Several industrial patents (3M, Aero, Agilent, Appleton Papers, BASF, Champion, Ciba, Eastman Codak, E-ink, Eternal Chemical Co, Fuji, Givaudan, Kanzaki, Koehler, Michubishi, Moore Business Forms, Motorola, NCR, Nippon Paper, Nippon Shokubai, Nordson, Procter & Gamble, Sipcam, Sol-Gel Technologies, Unichem, Xerox) claimed in situ microcapsule production and/or their innovative applications in various technical commercial products, including:

- Leuco dyes for pressure-sensitive copying papers and other colour recording materials [AU 66688/81, CA 2059851, DE 3447298, EP 0133352, EP 0219619, EP 0570209, SI 8411319, US 4675249, US 4997741],
- Fragrances and essential oils for textiles, papers and fabric softeners [EP 0782475, EP 1719554, US 4997741, US 8110284, WO 2009/100553, WO 2011/056904],
- Phase change materials for active accumulation and release of heat, incorporated in textiles, buildings, and electronic appliances [US 6207738, WO 2002/026911, US 20110081564],
- Pesticides, animal repellents and biocides [SI 23526, US 20120207844, WO 2010/124705],
- Enzymes in electrodes for biosensors [US 7312040],
- Fire retardants incorporated into plastics and textiles [US 3859151, US 20100285313],
- Adhesives and curing agents in self-healing materials [US 20040007784, US 7456233, US 7723405],
- Photosensitive materials [US 4962010, EP 0903629] ,
- Electronic ink for electrophoretic displays / electronic paper [WO 1999/010767, US 7875307, US 8174755].

IN SITU MICROCAPSULES IN PAPER PRODUCTS

Historically, *in situ* polymerization microcapsules have been widely used in large scale industrial production of pressure-sensitive copying papers, which used microencapsulated leuco dyes in combination with colour developers. With technological changes that replaced multiple-copy business forms with direct computer printouts, producers of carbonless copying papers searched for new specialised market niches. Commercial paper products with incorporated microencapsulated fragrances were invented (Figure 4), such as: scratch-and-sniff papers for advertising food and cosmetics, perfumed self-adhesive paper notes, and fragranced decorative papers.

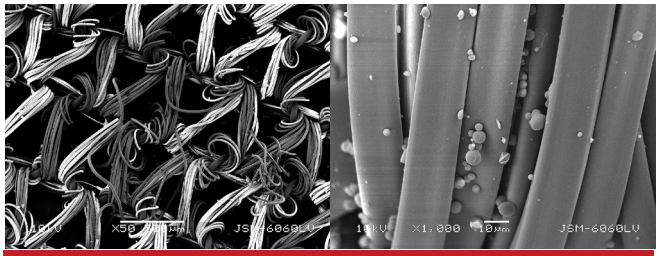

Figure 5. Nylon pantyhose textile with microencapsulated rose oil in pressure-sensitive microcapsules, produced by in situ polymerisation (SEM, left 50x, right 1000x)

IN SITU MICROCAPSULES IN TEXTILE PRODUCTS

Fragranced textiles represent another specialised family of products containing *in situ* microcapsules (Figures 5 - 7). Different techniques can be used for applications of microcapsules to textiles, such as immersion, impregnation with a transport of the textile through the basin, screen printing, or inclusion of microcapsules into the textile fibres during the spinning process. Applications of microencapsulated fragrances, perfumes and antimicrobial essential oils in woven and non-woven textiles expand from perfumed curtains, bed linen, shirts, socks and pantyhose to antimicrobial towels, shoe insoles, and textiles for seats used in public transportation. Other growing segments are microencapsulated phase change materials (PCMs) for active thermal control, and microencapsulated fragrances in fabric softeners. A special product niche is microencapsulated insect repellents for long-lasting impregnation of clothes, and animal repellents in agricultural textiles.

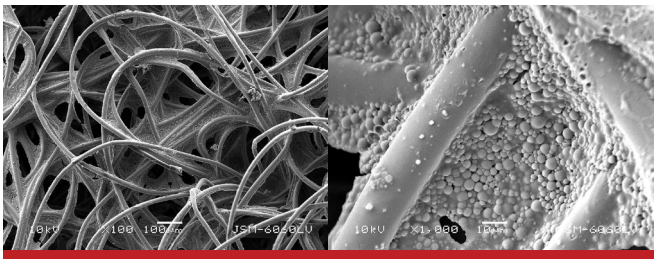

Figure 6. Non-woven textile for shoe insoles, impregnated with pressure-sensitive microcapsules, containing an antimicrobial composition. Essential oils are protected from oxidation until microcapsules open by mechanical pressure during walking (SEM, left 50x, right 1000x).

CONCLUSIONS

The *in situ* polymerization has been known and used for industrial production of microcapsules for half a century. The main constraint of the process is synthetic aminoaldehyde microcapsule wall, which limits the *in situ* microcapsules to technical products. Another well known drawback is residual formaldehyde in microcapsule suspension after the polyconden-

sation process. However, with the selection of process parameters and formaldehyde scavengers, the concentration of free formaldehyde can be minimised to meet the technical standards [4, 5, 12, 13]. In spite of these undesired characteristics, the in situ process results in numerous superior microcapsule characteristics, and for some applications the aminoaldehyde microcapsules remain irreplaceable. Their main advantages are: spherical reservoir-type shape with thin impermeable transparent walls (Figure 8), high chemical and thermal stability, high microcapsule resistance to harsh chemical environments (e.g. in detergents, softeners etc), good storage stability, high microencapsulation yields (≥99%), effective microencapsulation process control, controllable microcapsule size and size distribution, and good transferability of the in situ process to large-scale industrial production. In addition, wall permeability and mechanical characteristics can be regulated and adapted, to obtain tailor-suit pressure-sensitive or more elastic microcapsules with controlled diffusion, to support different release mechanisms of the products. Due to these characteristics, in situ polymerisation microencapsulation remains a popular and convenient industrial method for producing encapsulated formulations.

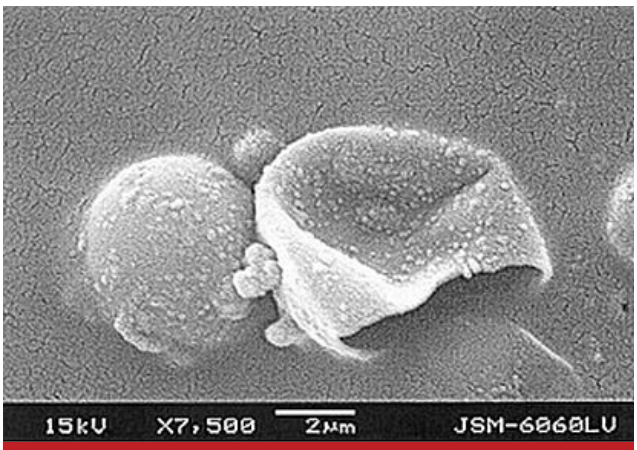

Figure 8. Spherical type pressure-sensitive microcapsules, produced by in situ polymerisation, after the release of encapsulated core material (SEM, 7500x)

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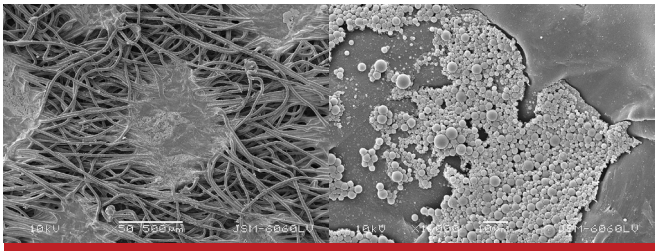

Figure 7. Non-woven textile handkerchief with microencapsulated decongestant eucalyptus oil (SEM, left 50x, right 1000x)

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Full professor at the Univeristy of Ljubljana, Faculty of Natural Sciences and Engineering, Head of the Department of Chemical Education and Informatics. She has been involved in university-industry cooperation research on microencapsulation technology and applications for 30 years. Other research domains include natural products chemistry, and scientific and technological informatics.

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He has been involved in university-industry cooperation research on microencapsulation technology and applications, industrial R&D and production of microcapsules on laboratory and industrial scale for last six years. Recently he finished his doctoral dissertation at the University of Ljubljana, and is open for new opportunities with interest in R&D of microencapsulation and scientific and technological informatics.

CONFERENCES , WORKSHOPS ... CALENDAR

PROGRAM 2013

March

Workshop Powder Technologies and Analysing Techniques
March 7-8, 2013 - Arnhem, The Netherlands
http://www.ipdexperts.com

Fluid bed processing_
March 19-21, 2013 - Binzen, Germany
http://www.ttc-binzen.de/cm/index.php?id=467

Encapsulation – an industrial approach
March 25, 2013 - Copenhagen, Denmark
http://www.dti.dk/encapsulation-8211-an-industrial-approach/programme/32784.1

Powders for extrusion and extruded powders
March 27-28, 2013 – Sion, Switzerland
http://www.fbp-science.ch

9th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology
March 31 – April 3, 2014, Lisbon, Portugal
http://worldmeeting.org/

April

Drying in industrial applications
April 5, 2013 - Castre, France
http://www.apgi.org (French)

5th Training School on Microencapsulation
April 9-12, 2013 - Nantes, France
http://bioencapsulation.net/2013_Nantes (see page 29)

Granulation & Tabletting
April 16-18, 2013 - Binzen, Germany
http://www.ttc-binzen.de/cm/index.php?id=472

PROGRAM 2013

April

2nd Coating Workshop
April 17, 2013 - Lille, France
http://www.apgi.org/coating_WS

Diabetes : a call for action
April 23, 2013 – Harrogate - North Yorkshire - HG1 5LA UK
http://www.publicserviceevents.co.uk/programme/251/diabetes

May

EUCHIS 2013
International Conference of the European Chitin Society
5-8 May 2013, Porto, Portugal
http://www.skyros-congressos.pt/euchis2013

Drying, Coating and Agglomeration
May 22-24 2013 - Copenhagen, Denmark
http://powderinfonews.com/wp-content/uploads/2012/06/Fluid-Bed-Technology-20132.pdf

June

ESACT meeting
23-26 June 2013 – Lille, France
http://www.esact.org/index.aspx?p=NewsPage&NewsId=44

16th Industrial Symp. and 6th Trade Fair on Microencapsulation
June 25-27, 2013 - Madison, USA
http://bioencapsulation.net/2013_Madison (see page 29)

ESACT meeting
23-26 June 2013 – Lille, France
http://www.esact.org/index.aspx?p=NewsPage&NewsId=44

17th Gums & Stabilisers for the Food Industry Conference
June 25th -28th 2013 - Glyndwr University, Wrexham, UK
http://www.gumsandstabilisers.org

JuLy

7th Annual PSSRC Symp.
«Advanced Characterization methods for Solid Pharmaceutical Dosage Forms»
July 5, 2013, Lille, France
http://www.apgi.org/pssrc_2013/

Powders & Grains 2013
July 8-12, 2013 - Sydney, Australia
http://www.pg2013.unsw.edu.au

PROGRAM 2013

August

ISOPOW XII conference –
August 19 – 23, 2013 - Fiskebäckskil, Sweden
http://www.isopow.org/

21st International Conference on Bioencapsulation
August 28-30, 2013 - Berlin, Germany
http://bioencapsulation.net/2013_Berlin (see page 30)

September

19th International Symposium on Microencapsulation
September 09-11, 2013 Pamplona, Spain
http://www.symposiummicroencapsulation2013pamplona.com

3rd Conference on Innovation in Drug Delivery
Sept 22-25, 2013 - Pisa, Italy
http://www.apgi.org

Powder handling, quality control, and applied powder technology
Sept 26-27 2013 - Copenhagen, Denmark
http://powderinfonews.com/wp-content/uploads/2012/10/Course-flyer-20131.pdf

Delivery of Functionality in complex food systems
Sept 30-Oct 3 2013 - Haifa, Israel
http://DOF2013.org

October

Nano2013.pt
Oct 11, 2013 - Lisbon, Portugal
http://fcts.ulusofona.pt/index.php/eventos/simposios/details/64-Nano%202013

Pan Coating - TTC workshop 190
Oct 15-17, 2013 – Binzen, Germany
http://www.ttc-binzen.de/cm/index.php?id=511&L=0

ARTICLE

INTERFACIAL POLYMERIZATION VERSUS CROSS-LINKING MICROENCAPSULATION

Poncelet*, C. Perignon*# and G. Ongmayeb#

***Oniris and #Capsulae, Nantes France**

INTRODUCTION

Microencapsulation by chemical methods is largely developed in the industries, with productions up to several thousand tons per year. However, the technology suffers of the image of non-green technologies. The objective of this contribution is to compare the traditional approach of interfacial polymerization with interfacial cross-linking to form microcapsules, showing some advantages of the second method for applications where the encapsulated active are fragile and green technologies are required.

INTERFACIAL POLYMERISATION

In the interfacial polymerization technique the wall is formed from monomers that are dissolved in the two separate phases (oil and water phase) and they polymerize at the interface of the emulsion droplets. For example, monomers such as diamine can be dissolved in the water and the aqueous phase is dispersed in the oil phase. The second monomer that is oil-soluble, for example diacid chloride, is then added and reacts with the first monomer at the interface forming the wall material. Different types of polymers may be produced by selecting different monomers but most publications refer to polyamide membrane (Figure 1).

Morgan et al. (1959) demonstrated that the polyamide membrane grows in the organic phase. Initially, the reaction is very fast leading to dense membrane at the interface. Then the diamine has to transfer through this first layer. While not totally understood, the membrane grows while forming pores. The usual explanation is that some water are also transfer through the membrane, coalesce leading to water droplet in the membrane (Janssen and Nijenhuis, 1992). Figure 2 shows usual structure of the polyamide membrane. Figure 3 shows a diagram representing the membrane formation kinetics diffusion of the diamine from the aqueous phase to organic phase.

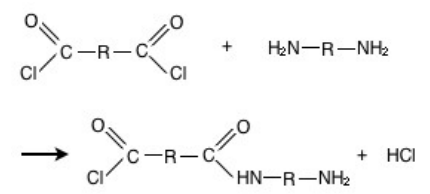

Figure 1. Polyamide reaction

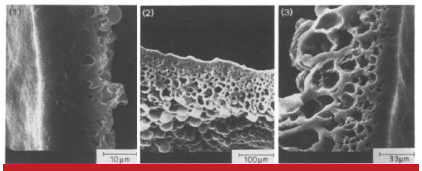

Figure 2. Polyamide membrane structure

Diamines (DA) exists in different acid-form:

$$H_2DA^{2+} \rightleftharpoons HDA^{+} + H^{+} \rightleftharpoons DA + 2\,H^{+}$$

Only the neutral diamine fraction φ_0 could be transferred in the organic phase. Neutral diamine fraction is strongly pH dependent (Figure 4). Lower pKa results in higher neutral diamine fraction at lower pH (Fig 4).

An equilibrium exists between the concentrations of the neutral diamine in the organic and aqueous phase, represented by the partition constant:

$$K_{o/a} = [DA]_o / [DA]_a$$

Where the index o and a refers respectively to concentration in the organic and aqueous phase at the interface. Figure 5 shows the impact of the number of carbons on the partition constant and the neutral linear diamine fraction.

Solvents are important parameters in the interfacial polymerization. More polar solvents gives high values of the partition constant of the hexamethylene diamine(Table 1) but are generally also more toxic (especially chloroform).

Assuming that the reaction between the diamine and the diacid chloride (DC) is very fast in regard to the diffusion of the diamine, the process kinetics is given by:

$$r = \frac{D}{\partial} K_{o/a}\ \varphi_0\ C_{DA}$$

where D is the diffusion coefficient in the membrane, the membrane thickness and C_{DA} the total concentration of diamine in the aqueous phase.

Table 1. Partition constant of the hexamethylene diamine in different solvants

Solvent	Ko/a
Cyclohexane	0.0055
Xylene	0.020
Dichloromethane	0.179
Chloroform/cyclohexane	0.290
Chloroform	1,43

While analysing last equation, one may conclude that a fast membrane formation requires a high pH (Figure 4) for high neutral diamine fraction and to select a polar solvent promoting the diamine transfer to the organic phase

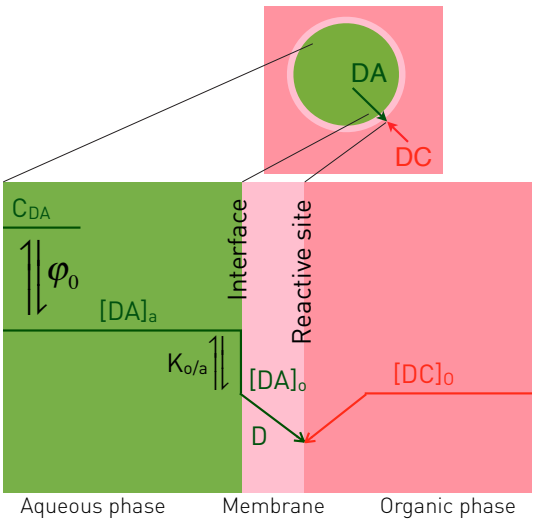

Figure 3. Interfacial polymerisation process

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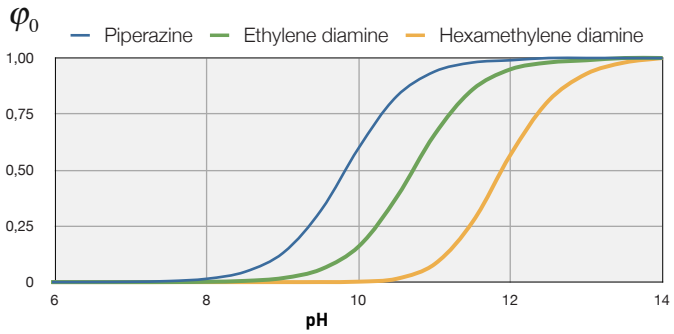

Figure 4. Neutral diamine fraction versus pH

(Table 1). Usually, polyamide microcapsules are produced at pH over 12 and using a mixture of chloroform/cyclohexane (1/4 v/v).

The selection of a diamine is a compromise between low pKa value, generally short carbon chain and high partition constant, i.e. long carbon chain (Figure 5). Often the hexamethylene diamine is selected as an optimum. Regarding the diacid chloride, aromatic one are more reactive than linear one. However, linear diacid chlorides give more flexibility to the membrane.

One drawback of the polyamide production is the release of hydrochloric acid (HCl). This may drop the pH, especially if the aqueous phase is the dispersed phase (then lower volume). While working at high pH, this effect is not very sensible but while using low pKa diamine and then low pH, one has to verify that the buffer capacity is high enough to avoid pH drop. Moreover, the local pH at the reaction site may be anyway lower than expected, reducing the reactivity of the diamine.

In conclusion, although microcapsules made by interfacial polymerisation have interesting properties, the production conditions are quite drastic (e.g. high pH) and lead to the use of toxic solvents (e.g. cyclohexane).

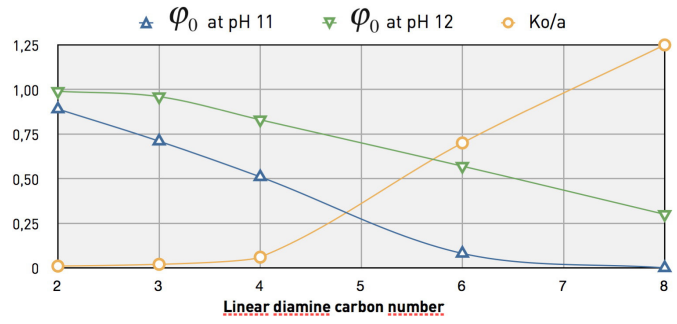

Figure 5. Impact on the linear dimine carbon number on the neutral fraction and partition constant

INTERFACIAL CROSS-LINKING

While contacting an aqueous phase containing a polymer with an organic phase containing a cross-linker, a membrane is formed. If the contact is done through an emulsion, it results in microcapsules with an aqueous or organic core depending which phase is dispersed in the continuous phase. This technology is still not largely spread in industry. The main development was linked to the French company, Coletica (today part of BASF) based on the work done by the group of Mrs Lévy (see page 22).These microcapsules have been essentially developed for cosmetic applications.

Different polymers have been used to form such capsules but may be divided mainly in three categories: proteins, polysaccharides and polyamines. All these polymers are insoluble in the organic phase and then the membrane is formed in the aqueous phase (Figure 6). The most usual cross-linkers are diacid chloride and diisocyanate which are only slightly soluble in water. The reaction is then quite slower then in the case of the interfacial polymerization. However, as we start from pre-polymer, limited number of cross-linking reactions is sufficient to get a membrane.

As amine functions are largely more reactive than alcohol functions, especially at medium alkaline pH (~9), the formation of membrane is then easier with a protein than with polysaccharide. Selecting polyamines (such as poly-imines or chitosan (a natural polyglucosamine) with low pKa allows to work even at neutral or even slightly acid pH (Poncelet et al., 1991).

Generally, the dissolution of the cross-linker requires a slightly polar solvent. However, diamine has not to be transferred to organic phase, and low polarity may even favour the transfer of the cross-linker in the aqueous phase

The cross-linking of the polymer leads to a gel more than a dense membrane. Under wet conditions, the membrane is quite permeable. However, when drying oil core capsules, the membrane get dense and quite impermeable (Figure 7).

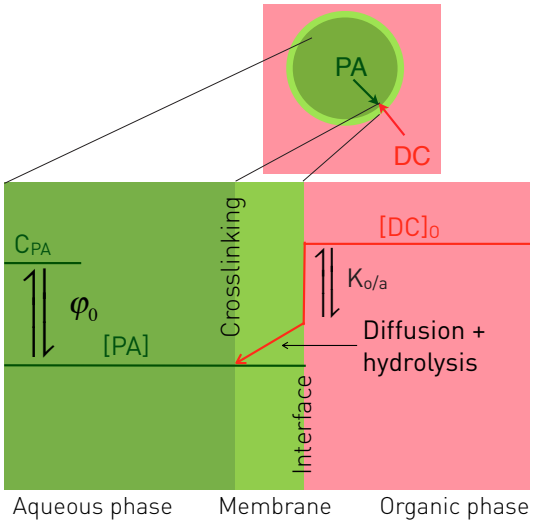

Figure 6. Polymer cross-linking process

Most cross-linker could react with water. It is then a competition between the polymer function and water. At the beginning the membrane is thin and cross-linker has to travel a short distance to reach amine polymer function. The membrane grows very fast. However, as the membrane thickness increases, the probability that the cross-linker react with water before to reach some free polymer function increases. High reactive cross-linker such as diacid chloride react too quickly with water leading to thin membrane while less reactive cross-linker such as di-isocyanate could migrate further from the interface to react with polymer function leading to thicker membrane (Ongmayeb, 2008). Membrane formation is slower (30 min) than through fast interfacial polymerisation (a few minutes).

ARTICLE

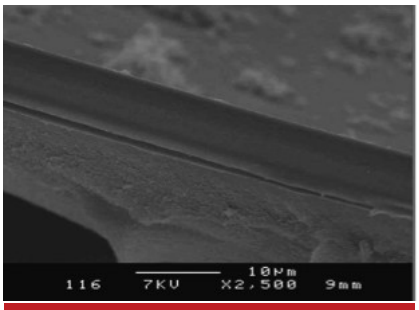

Figure 9. Cross-linked chitosan membrane structure

In conclusion, the interfacial polymer cross-linking allows forming microcapsules in mainly neutral pH, using low polar solvent (oil) and we are actually testing some cross-linker that may be considered as food grade. This technology has been successfully used for encapsulation of biocatalysts such enzymes or probiotics.

CONCLUSIONS

Interfacial cross-linking allows to produce capsules in softer conditions using green conditions. The encapsulation of fragile active molecules is then possible. Such capsules are biodegradable. The technology is more suitable for domains like cosmetics, food and feed and even medicine.

The objective of this article was to show that the polymerization is a technology that is largely spread in the industries (e.g. textile, agrochemical ...). But this reaction has some disadvantages (e.g. toxicity of solvents) and therefore does not always encapsulate sensitive actives (e.g. enzymes). Microcapsules obtained by interfacial crosslinking is an alternative which would form microcapsules in softer conditions, using green materials (e.g. proteins, chitosan),which can be used in others applications (e.g. cosmetics, food).

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http://bioencapsulation.net

http://capsulae.com

OPEN POSITION

agrosavfe
protecting and saving safely

AgroSavfe was recently established as a new spin-off company from VIB. AgroSavfe employs its proprietary AgrobodyTM technology platform to develop superior crop protection products, based on active ingredients with proven efficacy, in combination with AgrobodiesTM as formulation agents. AgrobodiesTM are derived from camelid antibodies and can be generated against virtually any target, to which they bind with high affinity and specificity. AgrobodiesTM are easy and cost-effective to manufacture and are intrinsically very stable. AgrobodiesTM directed against seeds, crops or particular structures thereof, crop produce or pests enable targeted delivery and retention of the active ingredient at or near its site of action. Targeted delivery and improved retention of AgrobodyTM-based crop protection products allow for reduced application dosage and for extended performance with reduced application frequencies. AgrobodyTM-based crop protection products are designed for superior characteristics over conventional crop protection products with respect to increased performance, improved sustainability and enhanced convenience for the grower and safety for the consumer.

To strengthen its current team AgroSavfe wishes to recruit

- a **Formulation R&D manager**, with extensive experience and expertise in R&D of agrochemicals preferably in an industrial environment.
- a **Head R&D**, with extensive experience and expertise in R&D of agrochemicals in an industrial environment. The Head R&D will report directly to the CEO and is expected to manage a multi-disciplinary team for the research, testing and development of AgrobodyTM-based crop protection products.

For more information and candidature :
AgroSavfe NV,
Technologiepark 4, B-9052 Ghent
Belgium
Phone: +32 (0)9 2610690
Email: info@agrosavfe.com
www: http://www.agrosavfe.com

International Chemical Company
willing to expand its presence in microencapsulation, is looking for the acquisition of a 1-15Mn USD revenue company, active in microencapsulation production for the feed, nutrition, textile, paper, cosmetics or specialty chemicals market in North America, Europe or Asia. Please do not hesitate to contact us for further discussion at interestinmicroencapsulation@yahoo.com if you could be interested in discussing this matter.

Distribution of TAGRA products in Asia

In October it was announced that DKSH and Tagra have entered into a strategic regional partnership covering China, India, Korea, Philippines, Thailand and Vietnam. DKSH has been appointed as a distributor for Tagra's range of encapsulated actives, oils and pigments.

More information:
http://www.dksh.com/htm/388/en/Distribution-agreement-across-Asia-between-DKSH-and-Tagra.htm?Id=375950

INDIA: CIPHET offers training courses for Food Science graduates

The Central Institute of Post-Harvest Engineering and Technology (CIPHET) [www.ciphet.in/] held a new 3 day course on February 5, announced in FnB news of India. The program covered microencapsulation methods for food and biotechnological applications including: two fluid nozzle systems, membrane emulsification, sonication and high-pressure homogenization for use in the fields of prebiotics, probiotics, aquaculture, feed, enzymes and other ingredients.

More information:
http://www.fnbnews.com/article/detnews.asp?articleid=33067&sectionid=13

The lifetime of biocides can be prolonged by polymeric encapsulation

Water-soluble biocides are prone to excessive leaching and high concentrations are therefore required in surface coatings for successful protection of a surface against biodeterioration. Sodium benzoate as a model water-soluble biocidal agent and Congo Red dye as a capsulation indicator were incorporated into branched polyethyleneimines capsules with molecular weights of 1300 and 5000 g/mol. Microscopic investigations verified that the Congo Red dye and sodium benzoate were entrapped within the capsules. The encapsulated water-soluble model biocide inhibited the growth of the decay fungi. The molecular weight of the encapsulated agent and the polyethyleneimine affected the release rate.

More information
http://www.european-coatings.com/European-Coatings/Home/Raw-Materials-Technologies/Raw-Materials/Additives/Encapsulation-to-prolong-lifetime-of-biocides

Patent: On-demand ultrasound-triggered drug delivery technology

In the US Columbia University, New York, has proposed an ultrasound drug delivery invention based on trapping microbubbles within a matrix encapsulated for example using liposomes which burst open on the application of ultrasound [US patent application 20130041311/A1]. This builds on prior art around the manufacture of gas filled microvesicles held by Bracco Suisse S.A. [US patent 8293214].

GLATT Times no. 33 - SPECIAL Pharmaceutial Services glatt.com

This issue of the Glatt international Times focuses on the Glatt Pharmaceutical Services. The business unit within the Glatt Group that is focused on the development and manufacture of solid dosage forms. The particular expertise is in the field of multi-particulate dosage forms.

More information:
http://www.glatt.com/cm/fileadmin/material/glatt-times_no33.pdf

Patent: Enteric coated microcapsules for functional food ingredients

Two related microencapsulation patent applications from Kraft were published last year; one was called Delivery of functional compounds [WO 2012/082631 A1] and the other Novel preparation of an enteric release system [WO 2012/087927 A1]. The novelty appears to lie around the modification of the functional ingredient to ensure a more efficient encapsulation process, producing a product with improved taste masking properties for example.

Patent: Light sensitive microcapsules

P&G have developed a light sensitive microencapsulation system based on azobenzene compounds for example 4,4'-bis(chlorocarbonyl)azobenzene. The release of fragrances, and active ingredients such as biocides, encapsulated using wall material comprising the appropriate azobenzene compounds can be triggered following exposure to infrared radiation, visible light or UV. The technology is covered in the international patent application WO/2013/022949/A1.

Stealth nanoparticles

An international team led by Ennio Tasciotti at the Department of Nanomedicine, The Methodist Hospital System Research Institute, Texas, and including researchers in the UK, Italy and USA have developed nanoparticles with a silicone core coated using the membranes extracted from active leukocytes and term the resulting structures «Leuko-Like Vectors» or LLVs. It is hoped that these particles can be used to deliver a therapeutic payload whilst the cloaking technology allows them to evade the immune system.

See their paper:
http://www.nature.com/nnano/journal/v8/n1/full/nnano.2012.212.html#/affil-auth

Gas-filled microvesicles for diagnostic and therapeutic use

New Patent: An interesting one from Bracco Suisse S.A. – with claims around the production and use of gas-filled microvesicles for use in therapeutics and diagnostics. The proposed products comprise three elements, one a phospholipid associated with the wall of a microvescicle, the second a targeting ligand and a third comprising at least two bis-sulphone groups.

See US patent 8293214:
http://www.uspto.gov/web/patents/patog/week43/OG/html/1383-4/US08293214-20121023.html

TO CONTRIBUTE, CONTACT

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ARTICLE

INORGANIC MICROENCAPSULATION

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Dow Corning, Belgium

HISTORY & INTELLECTUAL PROPERTY

From the first mechanical process in the late 1800s (1) to the first significant physico-chemical process in the mid 50s (2), organic materials have dominated microencapsulation and we can assume that this will last. However, today organic materials are no longer monopolizing the field. Indeed in the 90s researchers started to synthesize mesoporous inorganic materials from surfactant templated hydrolysis and condensation of metal alkoxides followed by high temperature calcinations (3). The purpose was to improve heterogeneous catalysis yields by protecting the metal catalyst in a colloid material allowing the free access of reactants and the release of the products. Before 1998, all patents related to the encapsulation of actives using the sol-gel process resulted in a monolith silicate matrix. The main issue with that approach is that the obtained doped monolithic material needs to be ground and consequently the active loses its protection. However this approach has been found useful for medical diagnostic devices (4).

The first use of surfactant templating to encapsulate active material with metal alkoxide precursors has been patented by Sol-Gel Technologies (5), a spin-off of the Hebrew University of Jerusalem. At the same time the Seiwa-Kasei company tried to encapsulate actives with peptide functionalized surface active alkoxysilanes (6). In 2002, both companies launched

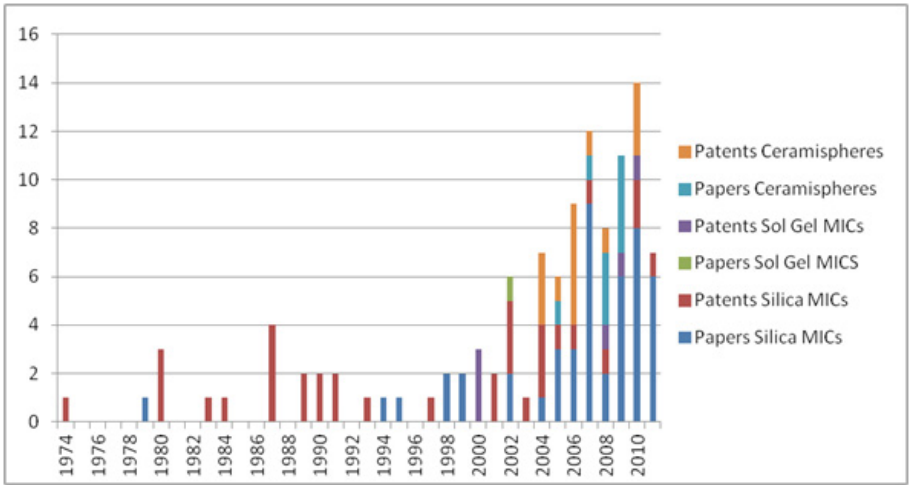

Figure 1. Number of hits as a function of time. Source SciFinder

organic sunscreens containing microcapsules to the market. Due to cost advantages as well as the ability to control the hydrolysis and condensation kinetics, metal alkoxides are by far the most used precursors for inorganic microencapsulation. Tracking patents and publications literature is made more complicated by the number of terms used to describe inorganic microencapsulation. However as shown in figure 1, the intellectual property and literature landscape is showing increasing activity that first started in industry and is now endorsed by academics.

CHEMISTRY

The hydrolysis and condensation of alkoxysilanes is part of sol-gel science and has been the topic of countless publications and text books illustrating the specificity and the complexity of the process (7, 8). However no paper can be found about the hydrolysis and condensation of alkoxysilane in O/W emulsions such that a shell is specifically built at the O/W interface.

In order to obtain the tightest shell material possible with an acceptable toxicological profile and encapsulation kinetic, tetraethylorthosilica (TEOS) is used as the main precursor. Blends of TEOS with other organoalkoxysilanes can be used as precursors to build organo- modified silica shells. The total conversion of TEOS into silica (SiO2) is sequentially obtained by hydrolysis (a) and condensation (b).

The use of this mild chemistry is delicate because the structure of the silica shell produced depends on many physical parameters like, tempera-

$$\text{(a)} \quad \text{TEOS} + 4\ H_2O \rightarrow Si(OH)_4 + 4\ C_2H_5OH$$
$$\text{(b)} \quad Si(OH)_4 \rightarrow SiO_2 + 2\ H_2O$$
$$\text{(a+b)} \quad \text{TEOS} + 2\ H_2O \rightarrow SiO_2 + 4\ C_2H_5OH$$

ture, pH, ionic strength etc...(7). The hydrolysis and condensation reactions described above are further complicated by the presence of a surfactant to template the silica shell as well as the presence of a dispersed oil in a large excess of water. The large excess of water is a reaction condition that is very rarely studied by the sol-gel research community. At the end of the process core-shell type microcap-

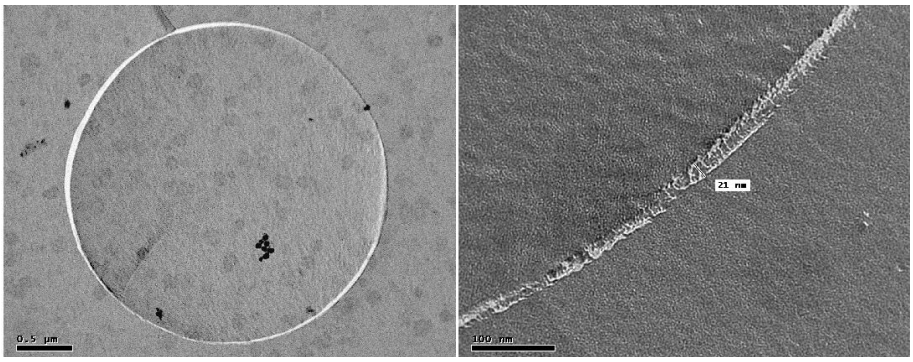

Figure 2: Cryo-TEM of ethylhexylmethoxycinnamate core / silica shell microcapsules

sules having payloads above 95% are obtained (Figure 2).

TRIGGERS FOR RELEASE

The interest of encapsulation technology not only depends on its ability to protect and control the delivery of actives but also on the different triggers one can use to release them. Silica and organo-modified silica have, in that respect, many advantages vs. organic shell materials.

One of the most interesting trigger to be used to release active is the drying of the microcapsule suspension (Figure 3). In that case we take advantage of the Laplace pressure, i.e. the pressure difference across a curved interface, that occurs between the microcapsules upon the evaporation of the continuous water/ethanol phase. The resulting stresses cause the microcapsules to break.

For spherical surface, Laplace Pressure:

For a typical microcapsule size of 3 µm, considering an amorphous silica shell surface tension of 330 mN/m

$$\Delta P = 2\,\gamma / R$$

(9), the pressure difference existing between both sides of the shell is 4.3 atm!

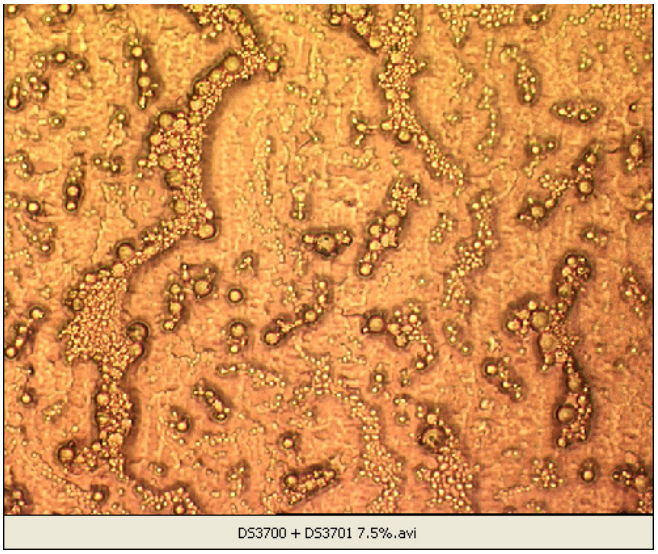

Figure 3: Drying of polydimethylsiloxane containing microcapsules in a 120 µm film on glass

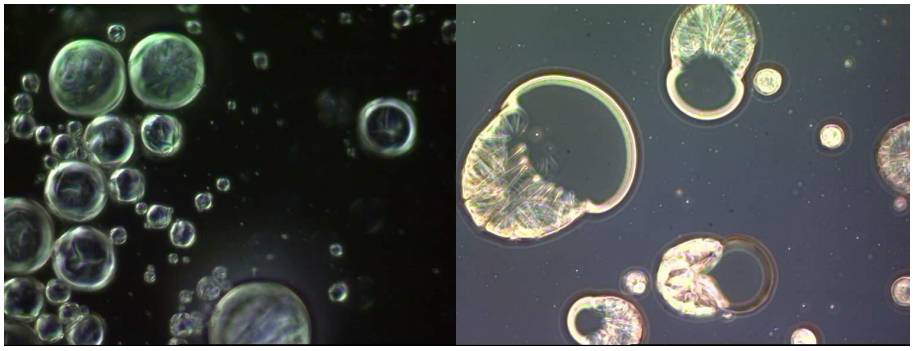

Figure 4: Vitamin A Palmitate containing microcapsules before (a) and after (b) glass slides compression (Average microcapsule size = 60 µm)

Since metal oxides in general and silica in particular have high Tg in the range of 520 – 600°C (10) they are not able to melt at low temperatures like waxes or low Tg organic polymers. However some strategies exist to rend microcapsules heat sensitive (11).

In colloidal systems at equilibrium such as microcapsule suspensions, chemical potentials always tend to equalize. Because of the chemical composition difference between each side of the microcapsule shells, the overall chemical potential must be compensated by the osmotic pressure. The later can be stronger than the mechanical resistance of the shell. Depending of the gyration radius of the active molecule and the silica shell porosity the later can be an impervious, semi-permeable or permeable membrane. The addition to the suspension of a good solvent or a small solute able to diffusion quickly through the shell will trigger zero order release of active.

Shear sensitivity of microcapsules is mainly correlated to their sizes and their mechanical strength. The later depends, amongst other parameters, on the payload, the viscosity of core material and the mechanical strength of the shell material. Therefore using shear as a trigger is easy providing large microcapsule sizes are acceptable in the application (Figure 4).

Other triggers like sonication, vacuum and silica dissolution at pH above 9 can be used to break the microcapsule (7).

INDUSTRIAL APPLICATIONS

Current industrial applications of inorganic encapsulation are multiple e.g. organic sunscreens for skin protection, benzoyl peroxide for acne treatment, phase change material for thermal isolation, yeast for improved fermentation yields, silicones for textiles water repellency and self-healing of cement (12).

In summary inorganic encapsulation can be of interest for many features:

- A broad microcapsule size distribution that only depends on the ability to emulsify the active.
- Mild encapsulation conditions (RT, pH) for volatile and labile substances.
- No chemical reaction between the encapsulant and the organic active to be encapsulated
- High encapsulation yield
- Useful for improved skin feel of greasy ingredients.
- Wide range of polar and apolar water insoluble actives.
- Zero order delivery or permanent encapsulation
- No formaldehyde & no glutaraldehyde and therefore can be used in aerosols.
- Suspension dosage form or powder by spray drying
- Very high payload (→ 95 %) and active content (up to 50 % in suspension) possible.

Despite a crowded patent landscape

(5, 6, 14) inorganic microencapsulation has great future in many industrial applications.

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ARTICLE

DOUBLE ENCAPSULATION: A SOLUTION TO ORAL PEPTIDE DELIVERY

A.Wawrezinieck (a), L. Danicher (a), S. Muller (b), Y. Frère(a)

(a) CNRS, Institut Charles Sadron (UPR022)

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INTRODUCTION

Drugs can be administered orally, intravenously, intramuscularly or sub- or transcutaneously. The oral way, the most physiologic and the most convenient for the patient, cannot be used for pharmaceuticals such as peptides or proteins. These drugs do not endure enzymatic attacks and extreme pH conditions encountered along gastrointestinal tract and their physicochemical properties (size, charge, hydrophilicity) interfere with their passage through intestinal barrier to circulation [1].

If previous studies have shown that encapsulation is the most suitable strategy to improve bioavailability of such pharmaceuticals [2], none of developed strategies is highly efficient. Only a small percentage of the drug (less than 5%) is able to reach systemic circulation [3], due essentially to the poor permeability of intestinal epithelium.

A pharmaceutical vector, based on "double encapsulation" (drug-containing nanoparticles are entrapped in beads) is expected to significantly increase peptide bioavailability [4-6]. Nanoparticles will protect the drug from degradation in intestinal fluid, facilitate its transport across intestinal epithelium and release them in systemic circulation. Beads will protect nanoparticles from degradation during their migration through mouth, esophagi and stomach and release them in the intestine.

In the present study, nanoparticles are obtained by complex coacervation from two biodegradable polyelectrolytes, namely sodium hyaluronate and chitosan hydrochloride [7], and do not yet contain a pharmaceutical. Beads are synthesized by ionotropic gelation [8-9] from sodium alginate with calcium ions. To our knowledge, there is no result published using polyelectrolyte nanoparticles encapsulation within alginate beads. It is thus necessary to verify that nanoparticles keep their integrity when they are encapsulated in alginate beads and that the presence of nanoparticles inside alginate beads does not significantly modify their properties. In this aim, alginate beads are synthesized in the presence or not of nanoparticles and their properties are compared. The presence of nanoparticles inside alginate beads is checked by fluorescence microscopy. The swelling of beads is measured in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated gastrointestinal transit (SGIT).

MATERIALS & METHODS

Materials

Alginic acid sodium salt (Alpha Aesar, Heysham, UK; low viscosity), hyaluronic acid sodium salt (HA; Fluka, Buchs, Switzerland; MW=1200kD), chitosan (CS; Fluka, Steinheim, Germany; MW=150kD, 85% deacetylation), poly(allylaminehydrochloride) (PAH; Sigma, MW=15kD) and other reagents of analytical grade were used without further purification. Phosphate-buffered saline (PBS) was prepared to reach a final composition corresponding to 27mM KCl, 137mM NaCl, pH 7.4.

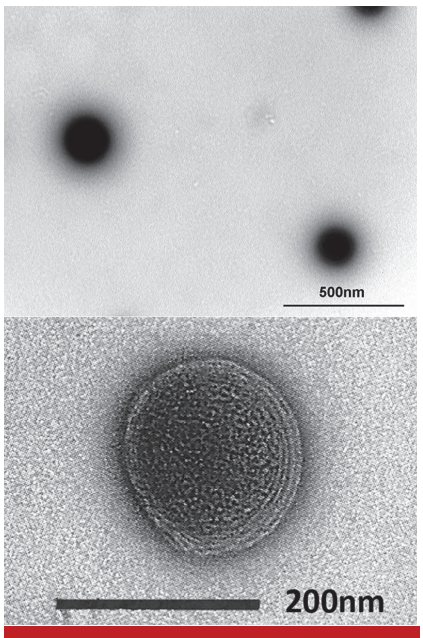

Figure 1. Photo of TEM observation of nanoparticles

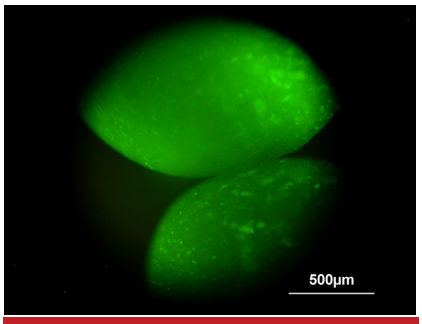

Figure 2. Photo of fluorescence microscopy observation of two alginate beads

Synthesis and characterization of nanoparticles

In this study, nanoparticles were synthesized by complex coacervation between two appositively charged polyelectrolytes, HA and CS, and were not loaded with any active compound. Loaded nanoparticles will be obtained by incorporating the drug in one of the polyelectrolyte solution prior the synthesis.

The following procedure was used. Negatively charged polyelectrolyte solution was prepared by dissolving 40mg HA in 50mL of distilled water to obtain a concentration of 800µg/mL and positively charged polyelectrolyte solution by dissolving 17mg CS in 50mL HCl (0.006N, pH 3) to obtain a final concentration of 340µg/mL. Both solutions were kept overnight on a 3D-shaker to complete polymers dissolution. Using a syringe, 5mL CS solution was then added to 5mL HA solution under constant magnetic stirring. When synthesis was complete, aggregates were removed from the suspension by filtration through a Millipore device (3µm filter) and the nanoparticles isolated from free polymer

chains by three successive cycles of centrifugation (2370g, 90min).

Zeta-sizer (Malvern 3000HS, Worcestershire, UK) measurements reveals nanoparticles with a size between 200 and 300nm, a polydispersity index of 0.01 and a zeta potential of -25mV. Transmission electron microscopy observations (TEM; Phillips CM12 operating at 120kV, Amsterdam, The Netherlands) confirm the size given by the zeta-sizer and show that nanoparticles have a spherical morphology (figure 1).

Synthesis of labelled nanoparticles

In order to assess their presence inside alginate beads, nanoparticles were labelled with a fluorescein-labelled poly(allylaminehydrochloride) polyelectrolyte (PAH-FITC). This label was obtained by adding fluorescein isothiocyanate (FITC; 5mL, 2mg/mL in DMSO) to PAH (125mL, 2mg/mL, pH 10). The mixture was allowed to react 5h at room temperature under gentle stirring. PAH-FITC was purified by dialysis (MWCO 4000-5000) and freeze-dried. Nanoparticles were then dispersed in a PAH-FITC solution to be coated with a fluorescent layer and the suspension was centrifuged (2370g, 90min) three times to remove free PAH-FITC chains.

Synthesis and characterization of alginate beads

Sodium alginate was dissolved in distilled water in various concentrations (0.5-3% w/v). Polymer solution was deaerated by centrifugation (2370g, 2min) before to be extruded dropwise into a CaCl2 solution through a 27-gauge syringe needle at a constant flow rate of 20mL/h. Once formed, beads were cured in a CaCl2 solution overnight and then isolated by filtration. They were washed three times with distilled water to remove unbound calcium cations and stored at 4°C in a 0.005% (w/v) CaCl2 solution to prevent gel degradation.

The average bead diameter in hydrated state is determined by optical microscopy by measuring the size of at least 25 beads.

Nanoparticles encapsulation in alginate beads

Nanoparticles or labelled nanoparticles were redispersed in a 2% (w/v) HA solution to get a final alginate concentration of 1% (w/v). This suspension was extruded as described above.

In the case of labelled nanoparticles, the experiment was carried out under cover to prevent them from light and resulting beads were immediately observed with a microscope (Omicron Twin Snom, HBO 100 lamp; Zeiss, Oberkochen, Germany).

Beads stability in simulated gastric and intestinal fluids

Hydrated alginate beads were incubated for 4h in media that mimic either the gastric fluid (SGF; HCl, pH 1) or the intestinal fluid (SIF; NaCl 9g/L, pH 8). The beads incubated in the SGF were then transferred in PBS (pH 7.4) for 2h.

The swelling and the disintegration of both loaded and unloaded beads are observed and compared using an optical microscope (TopView 1000, Motic).

RESULTS & DISCUSSION

This work is done for determining if nanoparticles obtained by coacervation can be encapsulated inside alginate beads synthesized by ionotropic gelation, and if their presence inside the alginate matrix has a significant effect on the characteristics of resulting beads. The first step is designed to prove that nanoparticles are efficiently encapsulated inside alginate beads. The second step consists in determining synthesis conditions of alginate

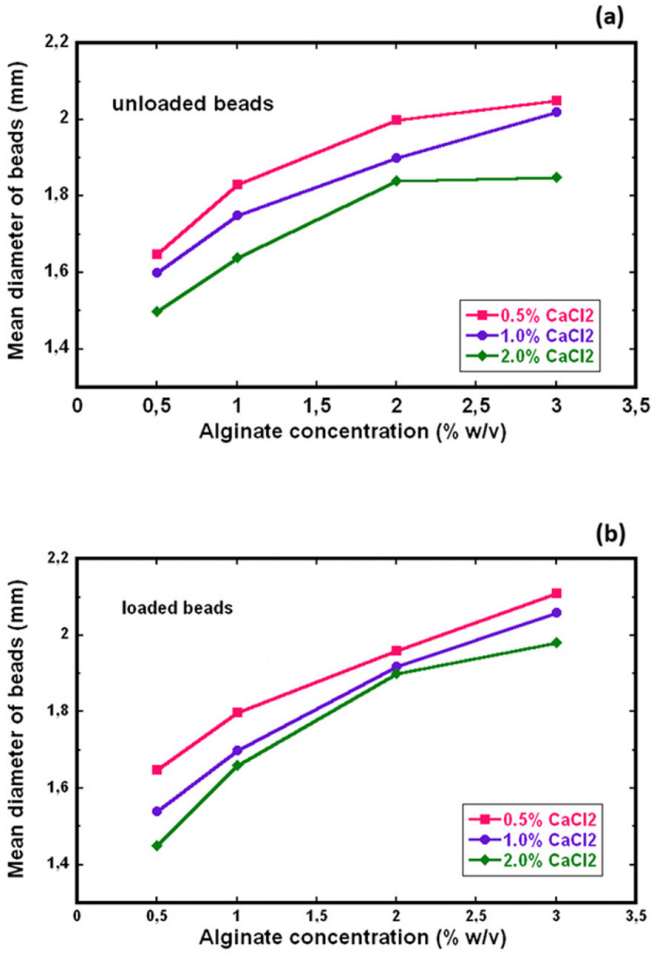

Figure 3. Influence of concentration of sodium alginate solution on size of beads obtained from different concentrations of calcium chloride bath; (a) unloaded beads; (b) loaded beads

ARTICLE

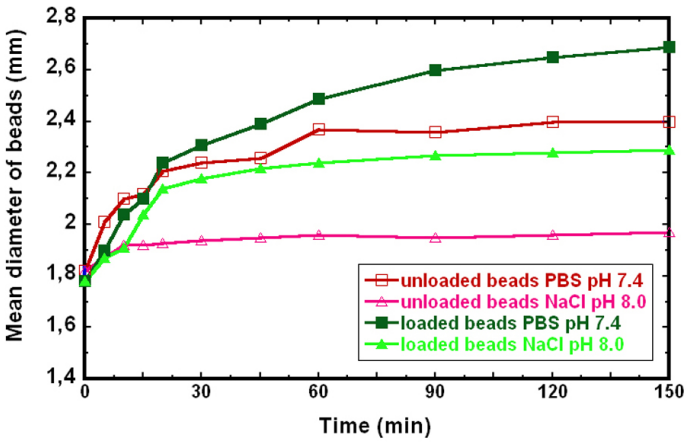

Figure 4. Influence of incubation time in SIF and in a commun in-vitro model on size of loaded and unloaded beads

beads with or without polyelectrolyte nanoparticles. In the third step, the stability of alginate beads containing nanoparticles (loaded beads) is studied in SGF and SIF and compared with alginate beads synthesized without nanoparticles (unloaded beads).

Nanoparticles encapsulation

A suspension of nanoparticles coated with a fluorescent layer is dispersed in the dark in an alginate solution (1.0%; w/v). The dispersion is dripped into a CaCl2 bath (0.5%; w/v). The resulting alginate beads are collected and, without washing, immediately observed by fluorescence microscopy (figure 2).

The data show that the fluorescence is confined inside beads. Some fluorescent aggregates are visible; they were formed during the dispersion of positively charged FITC-PAH nanoparticles within alginate solution. This indicates clearly that virtually all fluorescent nanoparticles are encapsulated in alginate beads and that there is no significant loss of nanoparticles during the ionotropic gelation. Thus it is possible to encapsulate polyelectrolyte nanoparticles in alginate beads and this encapsulation is almost complete.

For experiments described below, unmodified nanoparticles (with no fluorescent probe) are encapsulated in alginate beads.

Alginate beads synthesis conditions

The influence of different synthesis parameters (CaCl2 concentration, sodium alginate concentration and beads incubation time in CaCl2) on size and morphology of beads obtained with or without nanoparticles are studied (figure 3).

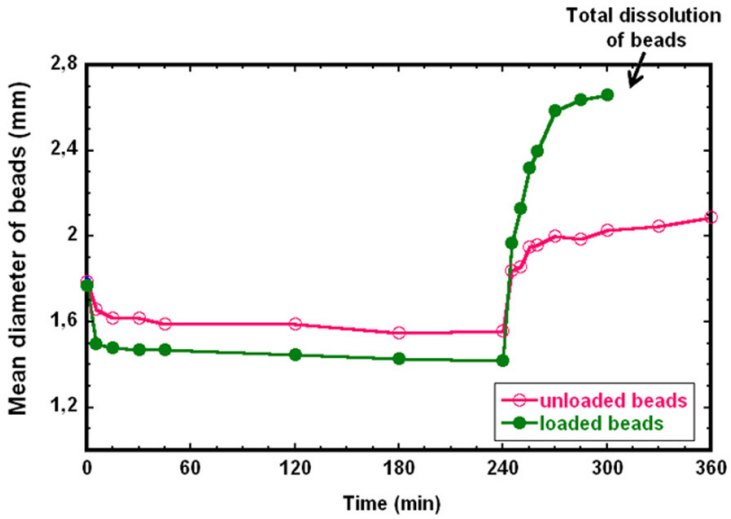

Figure 5. Influence of incubation time in SGIT on size of loaded and unloaded beads

The data show i) that CaCl2 solution concentration has to be kept over 0.25% (w/v) to produce firm and well-defined beads. Below this value, the number of calcium cations in the solution is too low to efficiently cross-link alginate chains, leading to beads aggregation; ii) that the minimum alginate concentration required to obtain stable and cohesive beads is 1.0% (w/v). At 0.5% (w/v), distorted beads with an irregular surface are produced, displaying low cohesion. Above 3.0% (w/v), the solution is too viscous and difficult to extrude through the syringe; iii) that depending on CaCl2 and alginate concentrations, the average size of unloaded (figure 3a) or loaded (figure 3b) beads lies between 1.5 and 2.0mm; iv) that the incubation time in CaCl2 gelling bath has a great influence on average size of alginate beads. The higher the beads incubation time, the higher the amount of calcium cations inside beads and the smaller the alginate beads size.

Very similar curves are obtained for unloaded (figure 3a) and loaded beads (figure 3b). The presence of nanoparticles inside alginate matrix does not affect dramatically those results.

In the following experiments, alginate concentration has been fixed to 1.0% (w/v) and CaCl2 concentration to 0.5% (w/v). The incubation time in CaCl2 bath is overnight.

Alginate beads characteristics

As future application, these beads will transport a pharmaceutical administered via the oral route. These beads have first to be resistant to gastric fluid, to protect encapsulated nanocapsules and second, to be degraded in the intestinal fluid to deliver nanoparticles in the intestine. The knowledge of loaded alginate beads behaviour in these two fluids is required.

Alginate beads behaviour in SIF and in a common in-vitro model

Stability experiments were conducted in a SIF, namely pH-adjusted saline solution (NaCl 9g/L, pH 8) (NaCl-8.0) or in a common in vitro model used to mimic intestinal fluid [10] namely PBS pH 7.4 (PBS-7.4). All loaded and unloaded beads swell in both media (figure 4).

The swelling degree is higher in PBS-7.4. Phosphate anions have chelating properties [11]. At neutral pH, the affinity of calcium cations for phosphate anions is higher than for carboxylic groups. Hence, calcium cations are captured by phosphate ions and progressively displaced from beads, leading to the weakening of bead structure. Furthermore, loaded beads exhibited a higher swelling degree (figure 4) than unloaded one. This reveals that the presence of nanoparticles inside the gel decreases its strength and cohesion. No sign of erosion of unloaded and loaded beads is noticed by optical microscopy.

Alginate beads behaviour in SGF and in SGIT

To mimic their way through gastrointestinal tract, alginate beads are incubated in SGF (HCl, pH 1) during 4h, and then transferred in PBS-7.4. The mean diameter of beads is evaluated for different incubation times in SGF (figure 5).

In SGF, the mean diameter of unloaded and loaded beads decreases. As alginate is not soluble at very low pH, it precipitates decreasing the size of beads and preventing the release of nanoparticles [12]. As no visible alteration is observed by optical microscopy, it can be concluded that beads will survive in the harsh environment of stomach while potentially protecting nanoparticles from degradation.

After 4h in SGF, beads are transferred in PBS-7.4. Both nanoparticles exhibit swelling. After 1h incubation, loaded beads are totally dissolved. Considering their future application as pharmaceutical vector, this feature is highly interesting. The nanoparticles will not only be protected from the acidic environment of the stomach but will be also rapidly released in the intestine, thus prolonging their contact time with the intestinal epithelium.

CONCLUSION

This work offers for the first time convincing evidence that nanoparticles obtained by complex coacervation between CS and HA can be encapsulated in alginate beads synthesized by ionotropic gelation with calcium ions and that they are protected in a simulated gastric media and release in a simulated intestinal media. It has also shown that the alginate beads properties are not significantly modified by the presence of nanoparticles. In fact, loaded beads still resist to an incubation time of 4h in an acidic solution (pH 1) displaying only a slight shrinkage but no degradation and, when transferred in PBS-7.4, they exhibit a high degree of swelling before their dissolution.

Thus, this alginate vehicle should ensure the protection of polyelectrolyte nanoparticles containing pharmaceuticals during their transit through the stomach and release them in the intestine.

In the future, research should consider the internal structure of the pharmaceutical vector and the release conditions of nanoparticles.

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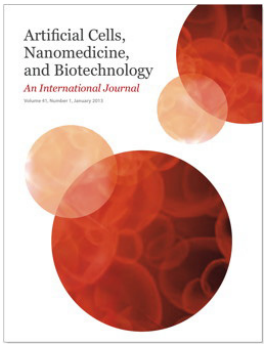

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ARTICLE

MICROCAPSULES MADE OF CHEMICALLY CROSS-LINKED PROTEINS

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INTRODUCTION

Easily available and often biocompatible, biopolymers play an increasing role among the materials available to constitute the frame of microparticles. Among natural proteins, serum albumin is a readily available, non-toxic, non-antigenic (if of human origin), biocompatible and biodegradable polymer. Furthermore, its binding properties towards various xenobiotics, and its solubilizing properties towards hydrophobic compounds, are well known. A protein, like albumin, can be very useful in encapsulation processes, due to physicochemical properties like high solubility in water, low viscosity in solution, interfacial properties, denaturation upon heating. Furthermore, the functional groups of proteins are available for chemical modifications needed for encapsulation by chemical methods. Serum albumin is then widely used to prepare microparticles [1].

A protein has to be cross-linked or stabilized using various methods in order to achieve sustained or controlled release properties. This paper presents a panel of different technologies developed for the preparation of microparticles from proteins, based on chemical processes.

CROSSLINKING USING ALDEHYDES

Several simple dialdehydes can be used to form protein cross-links [2]. The most extensively used reagent is glutaraldehyde. The reaction involves amino groups on the protein through Schiff bases and the product formed is irreversible. Glutaraldehyde has been found to form polymers in solution, at neutral or slightly alkaline pH, and presumably it is the unsaturated polymer that cross-links the amino groups of the protein, creating a network of cross-linked protein (figure 1).

Formaldehyde also can be used as a cross-linker, forming bridges between two protein molecules by a two-step reaction [3]. Some examples can be found in the literature [3].

For the preparation of covalently cross-linked protein microspheres using glutaraldehyde, the emulsion-cross-linking method described by Lee and co-workers is often cited as a reference [4]. The drug is dissolved into an aqueous solution of protein. The solution is emulsified in a hydrophobic phase. An aqueous solution of glutaraldehyde is added to the emulsion in order to start the cross-linking reaction. The meeting and fusion of glutaraldehyde aqueous droplets with the ones containing the protein is a statistical phenomenon which is not easy to control. Modified procedures can be found in the literature to facilitate the diffusion of the cross-linking reagent through the organic phase.

Another technique using glutaraldehyde cross-linking of albumin involves a spray-drying step to produce the particles. In this method, explored by D'Souza and co-workers, the cross-linking step can be carried out after the spray-drying step, or the glutaraldehyde solution can be mixed to the albumin-drug solution just before atomization. This method has been applied to the encapsulation of many drugs to obtain a slow release of the drug [5].

Glutaraldehyde can also be used to prepare cross-linked serum albumin hollow microcapsules. The method involves precipitation of the protein onto a spherical inorganic support, cross-linking using glutaraldehyde, and re-dissolution of the sacrificial core [6].

But although glutaraldehyde was for a long time the preferred chemical agent for cross-linking of proteins, today its use for health looks very doubtful for toxicological reasons.

CROSSLINKING USING RADICAL COPOLYMERIZATION

Covalent cross-links can be created between protein molecules using radical chemistry. In the radical copolymerization method, the protein is first derivatized by introducing unsaturated groups onto it, for example by acylation with methacrylic anhydride. The derivatized protein is then employed as a macromer, associated to N,N-

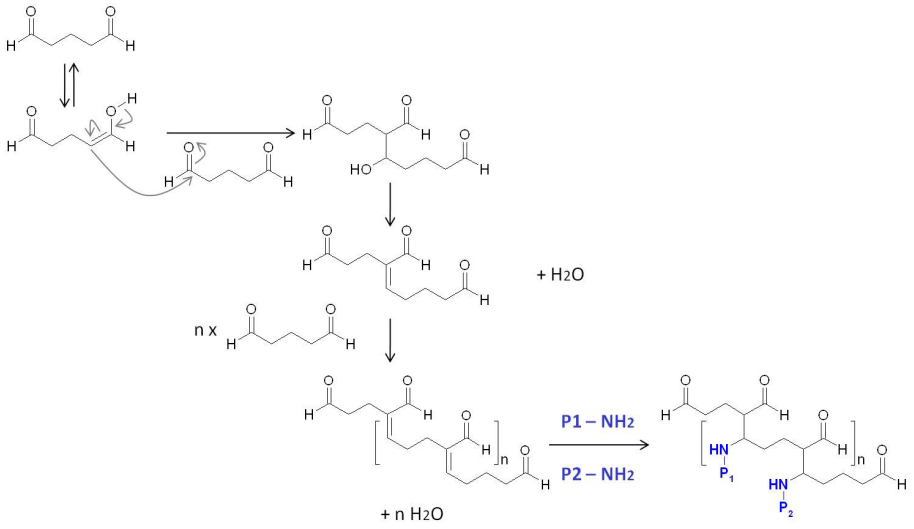

Figure 1. Polymerization of glutaraldehyde and its cross-linking reaction with proteins.

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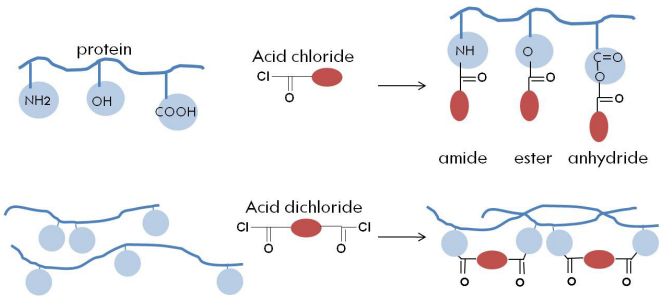

Figure 2. Acylation of functional groups of a protein by acid chlorides (up), and application to the cross-linking of proteins by acid dichlorides (bottom).

dimethylacrylamide (DMAA) for the preparation of microparticles by a radical polymerization mechanism. The resulting particles present a good stability due to the covalent nature of the created bonds. PH-sensitive or thermo-sensitive microspheres could be prepared by the same method by introducing stimuli-sensitive monomers (sodium methacrylate or N-isopropylacrylamide) in the medium, leading to the controlled release of drugs as a function of pH or temperature of the release medium [7]. However the radical copolymerization uses toxic monomers.

INTERFACIAL CROSSLINKING

For the preparation of microcapsules, interfacial cross-linking of proteins was intensively studied by Lévy and co-workers. Besides the free amino groups of lysine residues, easily acylated by the cross-linking agent into amides, proteins bear hydroxyl and carboxyl residues, which acylation lead to the formation of ester and anhydride bonds, respectively (figure 2), and take part in the membrane [8].

The method (Figure 3) involves the emulsification of a buffered protein solution in an organic phase. An organic solution of acid dichloride, like terephthaloyl chloride (TC), is added to the emulsion. The reaction is stopped by dilution. The size distribution of the microcapsules is controlled by the stirring speed and surfactant concentration, and the degree of cross-linking can be tuned by adjusting the reaction pH and time, and the cross-linking agent concentration [9]. The method has been applied to the preparation of chelating microcapsules with iron-binding properties, by treating the particles with alkaline hydroxylamine, thus creating chelating hydroxamate moieties on the membrane, from the ester and anhydride bonds [10]. The semi-permeable cross-linked protein membrane of albumin microcapsules has been used to obtain a prolonged release of drugs from cross-linked cyclodextrin microcapsules encapsulated in these microcapsules [11]. Serum albumin cross-linked microcapsules (figure 4) can be used for the controlled local release of growth factors [12].

The elastic properties of these particles were assessed by a novel method involving the microfluidic technology. The method consists in determining the deformation profiles of the microcapsules in microfluidic circuits and

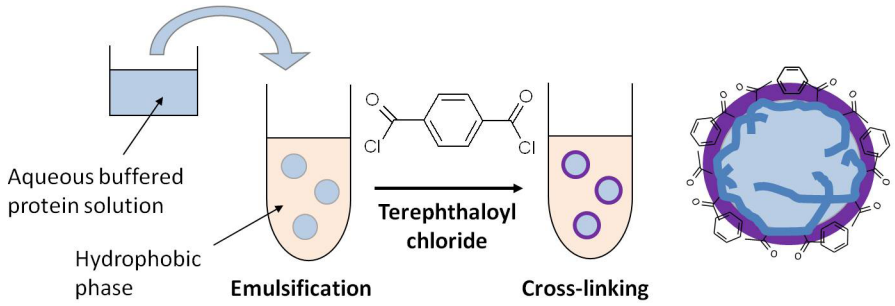

Figure 3. Preparation of protein microcapsules with the emulsification-cross-linking method.

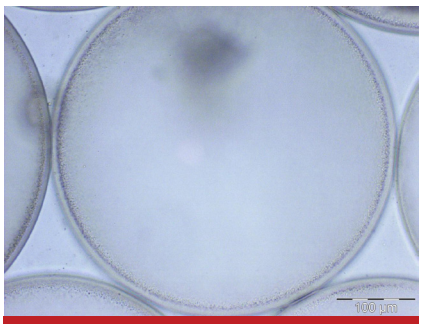

Figure 4. Optical microphotograph of serum albumin microcapsules cross-linked with TC.

comparing the experimental profiles with theoretical ones obtained from mathematical modeling. The values obtained with microcapsules prepared varying the cross-linking reaction conditions correlated well with the values of cross-linking degrees of the membranes obtained from a chemical assay [13].

ZERO-LENGTH CROSS-LINKING

By activating a chemical group on the protein, which will react with another functional group, cross-linking of proteins can be achieved without cross-linking reagents, producing a zero-length cross-linking [2]. Microspheres for temporary arterial embolization have been produced in an emulsion system by zero-length cross-linking of human serum albumin [14]. The dispersed aqueous phase contained a mixture of albumin and carbodiimide for the activation of carboxyl groups of the protein and further reaction with amino groups to form in situ a network linked through amide bonds.

Another zero-length cross-linking method, which requires the presence of two biopolymers, is based on a transacylation reaction between a polysaccharidic ester, like propylene glycol alginate, and a protein. The carboxyl groups of the polysaccharide are activated in the form of esters, and the transacylation reaction, starting upon alkalization, produces amide bonds between the two biopolymers (figure 5). A thermostable gel is obtained, consisting of a covalent network produced in mild conditions without any toxic reactant.

The reaction has been adapted to mi-

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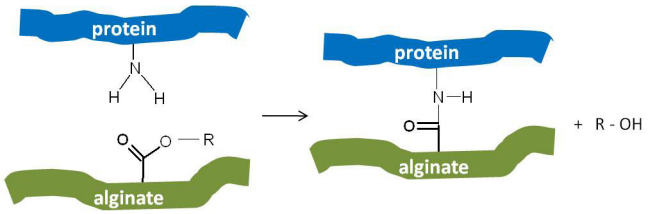

Figure 5. Transacylation reaction between a protein and an ester of alginic acid.

croencapsulation by Edwards-Lévy and co-workers [15]. Stable and biodegradable membranes with controllable thicknesses and interesting mechanical resistance could be created around hydrogel beads [16-18](Figure 6). The membranes are formed of a hydrophilic network constituted of a protein directly bound to alginate through amide bonds.

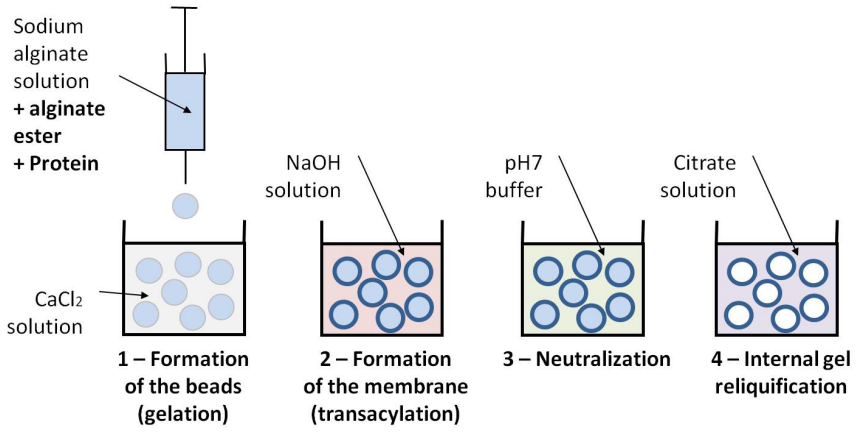

Figure 6. Preparation of membrane-coated calcium alginate beads using the transacylation reaction.

The particularly mild conditions required for the preparation of the capsules adapted very well to bioencapsulation (figure 7), and the covalent membrane showed a better stability as compared with the polyionic alginate-polylysine membrane classically used. The encapsulation of several cell types showed a high preservation of cell viability and functionality [19, 20].

Using this method, calcium alginate microspheres can be stabilized by encapsulation in a polysaccharide-protein covalent membrane. These particles were shown to release a bioactive peptide by an ion-exchange mechanism [21-22].

Microparticles constituted of the polysaccharide-protein covalent network without calcium alginate gel have also been prepared, by starting the transacylation in an emulsion system where the dispersed aqueous phase contained the two biopolymers (figure 8) [22]. This gentle procedure leads to stable, biocompatible and biodegradable microparticles, with promising properties for the encapsulation of fragile biological molecules like growth factors.

Furthermore, if the constitutive protein in the crosslinked network with alginate is an enzyme, the resulting particles retain an important proportion of the initial enzymatic activity.

CONCLUSION

Creating a covalent network ensures a good stability of protein microparticles, but the chemistry has to be carefully chosen in order to guarantee a perfect safety for biomedical applications. From the use of toxic aldehydes to the very mild conditions of the transacylation method, improvements have been made in the biocompatibility of the particles. Covalently-crosslinked protein microparticles are now promising tools for the protection and delivery of active ingredients, and also for the encapsulation of various types of living cells.

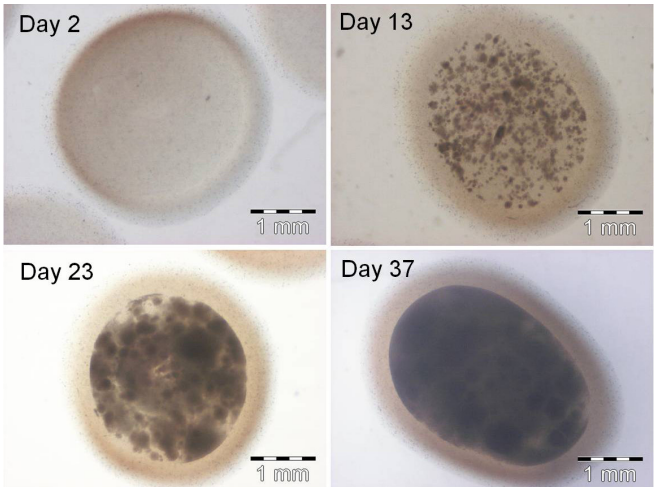

Figure 7. albumin-alginate coated beads containing the Jurkat cell line after various times in culture medium at 37°C [23]..

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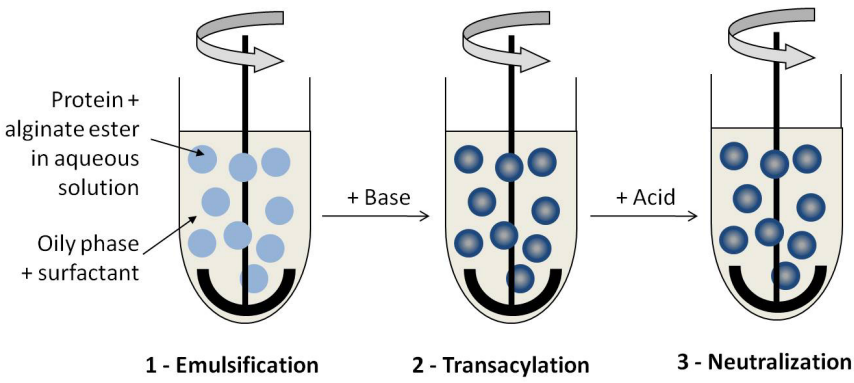

Figure 8. Preparation of albumin-alginate covalent microspheres by emulsion-transacylation

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ARTICLE

NOVEL DOUBLE-SHELL MICROCAPSULES

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FROM SINGLE-SHELL TO DOUBLE-SHELL MICROCAPSULES

In the last decade, there has been an increased interest in the development of new commercial products containing microcapsules with a low cost for cosmetic, food, pharmaceutical and detergent markets, in which the encapsulated active molecule (fragrances, flavours, drugs, dyes, bleaches, etc.) can be protected from environmental agents for a long period of time without affecting its main properties and have a well-controlled release profile. Single-shell microcapsules can be developed from organic or inorganic materials. Organic materials such as melamine formaldehyde were used to encapsulate perfume oil by in situ polymerization due to the possibility of increasing the oil shelf life and targeted delivery. The microcapsules presented excellent resistance to acid and alkaline, good mechanical strength and low production cost. However, they exhibited a certain leakage of oil in aqueous solution [1]. Among inorganic materials, silica and CaCO3 have been studied for encapsulation due to their biocompatible and biodegradable nature. They were demonstrated to form single-shell microcapsules containing water-soluble biomacromolecules (bovine serum album, duplex DNA, drugs, enzymes, proteins, etc), which showed high resistance to impact and deformation. These single-shell microcapsules were developed using different techniques: a w/o/w interfacial reaction method was used both for producing silica microcapsules [2] and CaCO3 microcapsules [3], sol-gel process for silica microcapsules [4] and layer-by-layer adsorption of polyelectrolytes into porous CaCO3 microparticles to form CaCO3 microcapsules [5, 6]. Basically any bio-molecule larger than the pores of microcapsules could be encapsulated, and they were not released from the microcapsule unless their shell was destructed. However, the shell of silica or CaCO3 microcapsules had a high porosity, which limits their application to encapsulate small molecules in liquid. Double-shell microcapsules are seen as a feasible system to overcome these limitations and to offer a broad range of applications. The double shell can be formed from a single polymer, for example poly(methacrylic acid) double-shell hollow microspheres via a combined inorganic sol-gel process and polymerization reaction [7]. These microspheres with different degrees of cross-linking allowed hierarchical pH-response when they were used in drug delivery systems for the controlled or sustained release and represent an overall improvement over the conventional single-shell microspheres. Inorganic silica double-shell hollow microspheres were obtained by making silica/poly(methacrylic acid) hybrid microcapsules, followed by calcinations of the polymer layer [7]. These microspheres opened the possibility of their use as microreactors for confined reactions. Microcapsules with polymer (polyurea) as an inner layer formed by interfacial polymerization and resin (urea formaldehyde) as outer layer by in-situ polymerization were developed. These microcapsules enhanced the protection of encapsulated oils and presented higher thermal stability than single layered polyurea ones [8]. In some cases, the formation of the second layer increased the mechanical stability at high temperatures, for example double-shell melamine formaldehyde microcapsules with phase-change materials as core obtained by a two-step prepolymer addition method had potential applications in energy fields [9]. With the same phase change materials as core, the shell compactness and resistance to permeation of the double-shell melamine formaldehyde microcapsules were increased using a two-step coacervation of the prepolymer aided by a hydrolyzed copolymer of styrene and maleic anhydride [10]. Another application of microcapsules with double-shell structure is in the flame retardant field. Fire stability of flame retardants has been improved remarkably by their encapsulation in double-shell melamine formaldehyde-epoxy microcapsules prepared by in situ polymerization [11]. Moreover, multilayer organic-inorganic microcapsules have been used for enzyme immobilization. Very recently, Wang et al. reported a development of core-shell microcapsules with ultrathin alginate/protamine/silica hybrid membranes through a co-extrusion minifluidic approach and a biosilicification method for immobilization of a model enzyme laccase [12]. The immobilized enzyme had significantly higher thermal, pH and storage stabilities than the free enzyme.

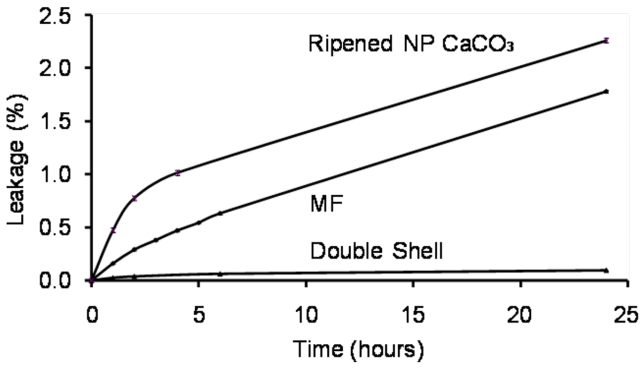

Figure 1. Percentage leakage of the core oil from the melamine formaldehyde, ripened nanoparticulate CaCO3 and double-shell composite microcapsules over 24 hours [13].

DOUBLE-SHELL ORGANIC-INORGANIC COMPOSITE MICROCAPSULES

In our group, we used for the first time melamine formaldehyde-CaCO3 composite materials to produce double-shell microcapsules with a core of perfume [13]. In order to compare the

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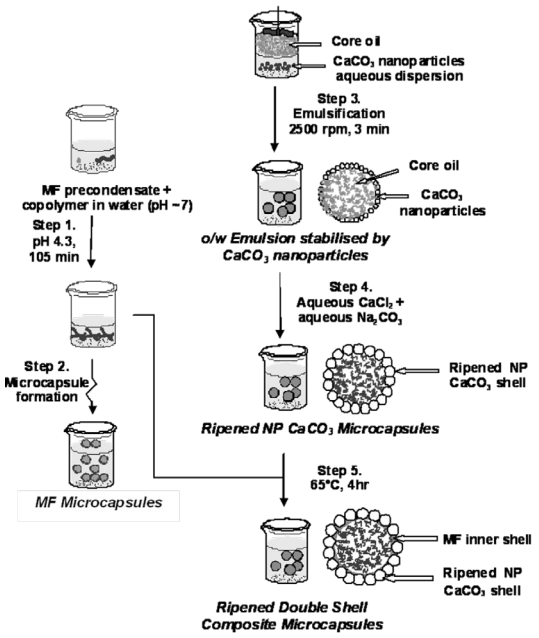

Scheme 1. Schematic representation of the melamine formaldehyde, ripened nanoparticulate CaCO3 and double-shell microcapsules [13]

properties of the new microcapsules, 3 types of microcapsules were synthesized. First, single-shell melamine formaldehyde microcapsules were obtained by in-situ polymerization of a solution of melamine formaldehyde and copolymer (poly(acrylamide-acrylic acid, sodium salt)) [1]. The second type was the ripened nanoparticulate CaCO3 microcapsules (Scheme 1). The third type was double-shell nanocomposite microcapsules prepared by adding the pre-crosslinked melamine formaldehyde/copolymer (poly(acrylamide-acrylic acid, sodium salt)) to the ripened CaCO3 microcapsule dispersion, followed by its migration through the gaps of ripened CaCO3 nanoparticles and reaction at the oil-water interface to form the melamine formaldehyde polymer inner shell. It was found by gas chromatography that the double-shell microcapsules presented a higher protection of perfume from leakage than the other two types of microcapsules (Figure 1).

CONCLUSIONS

The new double-shell nanocomposite microcapsules presented above have great potential applications as carriers of small molecules in cosmetic, homecare, nutraceutical and pharmaceutical products due to their low production cost and to the possible release mechanisms based on pH modification and/or mechanical fracture. The research conducted in our lab is aiming to prepare single and double-shell microcapsules with various industrial applications and to understand the relationship between performance, structure and properties of new microcapsules.

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Sorin N. Sauca obtained his PhD degree in Chemical Engineering from University of Basque Country, San Sebastián, Spain, working on waterborne olefin-acrylic polymers prepared by catalytic polymerization. Since 2011, he has been working as a Marie Curie Research Fellow in the group of Prof. Zhibing Zhang at the University of Birmingham, UK. His project is funded by European Union, and aims for novel encapsulation of active molecules for detergent applications in collaboration with Procter & Gamble.

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Professor Zhibing Zhang has built up an international reputation for developing original work on micromanipulation studies of biological and non-biological materials and formulation of particulate products for pharmaceutical, nutraceutical, human care and fabric care applications based on micro/bioencapsulation. He is a leader of the Group of Micromanipulation and Deputy Head of the School of Chemical Engineering at Birmingham, which won the Queen's Anniversary Prize 2011. He has authored or co-authored 142 refereed academic papers and 174 other publications, and made a number of invited presentations at international conferences or meetings.

Bioencapsulation Research Group

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Bioencapsulation Research Group is a non-profit association promoting networking and research in the encapsulation technology of bioactives. It organises academic conferences and industrial symposiums, publishes newsletters and manages a website.

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