Micro- and Nano-encapsulation of Water- and Oil-soluble Actives for Cosmetic and Pharmaceutical Applications

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Abstract

Skin, hair and mucosal surfaces are useful targets for the delivery of active compounds, botanicals and, importantly, drugs. Encapsulation provides an invaluable tool to the cosmetic and/or pharmaceutical formulator, providing great flexibility in the choice of delivery mechanisms and excipients that can be used. Dispersions of solid lipid nanoparticles (SLNs) were prepared using biodegradable materials generally regarded as safe by a melt-emulsify-chill (MEC) method. Among the materials encapsulated in SLNs were hydrophobic active pharmaceutical ingredients, antiviral and fungicidal compounds, organic UV absorbers and fluorescent dyes. The loading, adhesion and intracellular localization of the SLNs were studied as a function of surface charge.

The MEC process can easily, and cheaply, produce a range of solid lipid nanoparticles (SLNs) - "smart colloids" - that offer flexibility in formulation, increased efficacy and decreased formulation complexity. The method is general and can be applied to many water- or oil- soluble cosmetic and pharmaceutical actives. Multiple actives can be co-encapsulated. The process is cost-effective, reproducible, robust and scalable. The bulk physical characteristics and surface chemical properties can be varied to allow systems to be custom designed to fit specific applications; use can be made

of this in the targeting of encapsulated actives, botanicals and therapeutics to skin and hair.

The non-specific nature of cellular uptake would suggest that SLNs produced via the MEC process are suitable candidates for delivering surface-attached actives into cells. The strong attachment and demonstrated entry into antigen presenting cells of the immune system illustrates the potential that these natural wax nanoparticles might have in vaccine formulations.

17.1 Introduction

Skin, hair and mucosal surfaces are useful targets for the delivery of active compounds, botanicals and, importantly, drugs. Encapsulation provides an invaluable tool to the cosmetic and/or pharmaceutical formulator, providing great flexibility in the choice of delivery mechanisms and excipients that can be used. For example, active pharmaceutical ingredients can be delivered in systems that would otherwise be unacceptable or hostile to them. Lipophilic (??) active compounds can be incorporated into aqueous dispersions without the need for a solvent. Conversely, water-soluble ingredients can be delivered in non-aqueous systems such as ointments. The matrix of the capsule can be chosen from a wide variety of materials (usually selected from a list of materials "generally regarded as safe" (GRAS) for pharmaceutical applications) to meet the needs of the application as well as regulatory demands, e.g., biodegradability. Multiple actives can also be delivered in the same particle; indeed, mutually chemically incompatible actives can be formulated together. A further way to exploit this technology is to encapsulate one ingredient that can facilitate, or stabilize, a second. By isolating problematic ingredients, formulation and processing issues are minimized; undesirable properties can be masked. This chapter is a brief review of traditional encapsulation methods that will be helpful as the basis for further discussing other encapsulation technologies.

17.2 Microencapsulation

Conventional microencapsulation can be described as surrounding either a liquid droplet or a solid particle core with a defined, solid shell (see **Figure 17.1**). It is used to deliver, protect, stabilize or control the release of the core. Ingredients so encapsulated include adhesives, drug substances, colors, fragrances, flavors, agricultural chemicals, solvents and oils. Especially in the cosmetic industry such capsules are designed to release the core by fracture of the shell. Although the earliest application by the cosmetic industry was probably for "scratch and sniff" fragrance samplers, the first commercial use

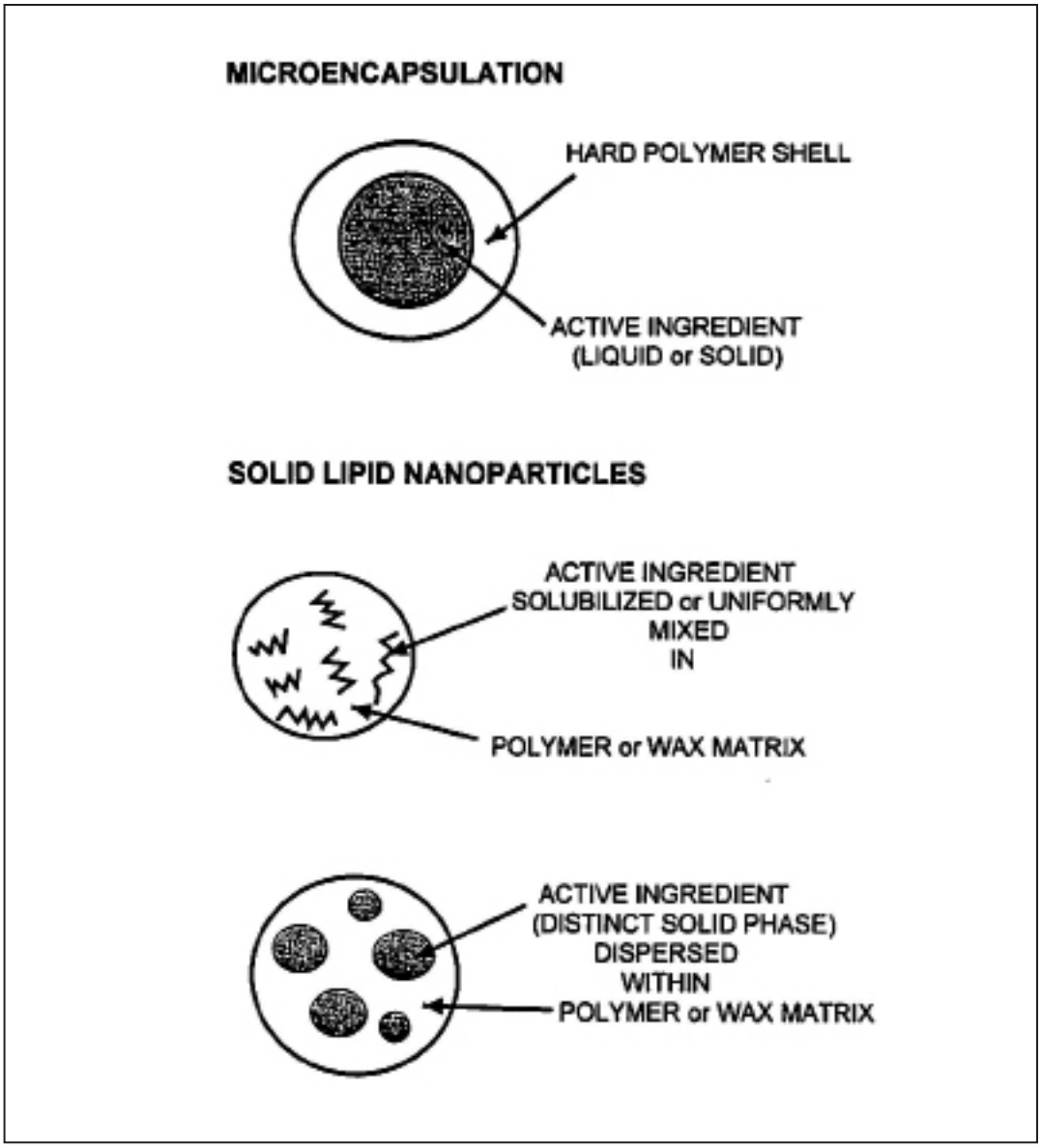


Figure 17.1. *Schematic comparing microcapsules with SLNs.*

of microencapsulation was in carbonless paper.[1,2]

Traditional microencapsulation capsules are typically larger than 1 μm in diameter, the normal range being 50–500 μm. Capsule size is determined, in part, by limitations of the methods used to produce them and by the fact that a large size is necessary for the capsule to be ruptured by either pressure or by shear of use. As capsule size decreases the capsules become less susceptible to the forces that can cause rupture of the shell. Such capsules are normally supplied in a dry powder form that, subsequently, often needs to be

incorporated (dispersed) into the cosmetic or pharmaceutical formulation.

17.3 Chemical Production

Microencapsulation capsules are produced by chemical or physical processes. The chemical methods used are categorized as coacervation, condensation or interfacial polymerization and generally involve dispersing the active core material in a non-solvent continuous phase to form an emulsion or dispersion (the continuous phase must be a non-solvent for the dispersed phase). The shell is created by a chemical reaction, and subsequent precipitation, of one or more shell-precursor materials that are dissolved in the continuous or discontinuous, phase at the interface of the core material and the continuous phase. It is possible to have different components of the shell dissolved in both phases.

Microcapsules prepared by coacervation rely on the fact that oppositely charged (natural and synthetic) polymers condense in solution and can attach to the surface of an emulsified oil droplet (see **Figure 17.2**). In coacervation encapsulation, a shell material precursor is normally dissolved in the continuous phase. Classic shell materials used are gelatin or Arabic gum dissolved in an aqueous continuous phase. Varying the conditions of the continuous phase, either by altering the pH or by adding a water-miscible solvent, changes the solubility of the dissolved shell material. This change in solubility causes the shell material to migrate to the surface of the dispersed core and to coat the non-continuous droplets. Another material is then added to chemically crosslink the shell material thus forming a hard shell around the active core.[3-5] The capsules can be as small as a few microns in diameter, or as large as a few millimeters. In this way, a wide range of encapsulated products can be produced where the encapsulation provides flavor-masking, payload degradation protection, or simply for the case where a liquid needs to be handled more as a solid (the dried capsules can be handled as a powder and the liquid contents evaporate only very slowly).

An alternative technique uses the phase separation of polymers in poor solvents to allow preparation of microcapsules from a wide range of hydrophobic polymers, such as poly(methylmethacrylate)—Plexiglass®.[6] Using this method, and utilizing polymers that change their properties with pH, microcapsules with pH-controlled release of model marker compounds can be prepared. Shown in **Figure 17.3** is an SEM of a microcapsule made with a shell of poly (4-vinylpyridine)—a material that swells at about or below pH 4. **Figure 17.4** shows the pH-dependent release kinetics for a marker compound dissolved within the core. The first order release of the encapsulated material was faster at a pH below the polymer swelling pH (solid

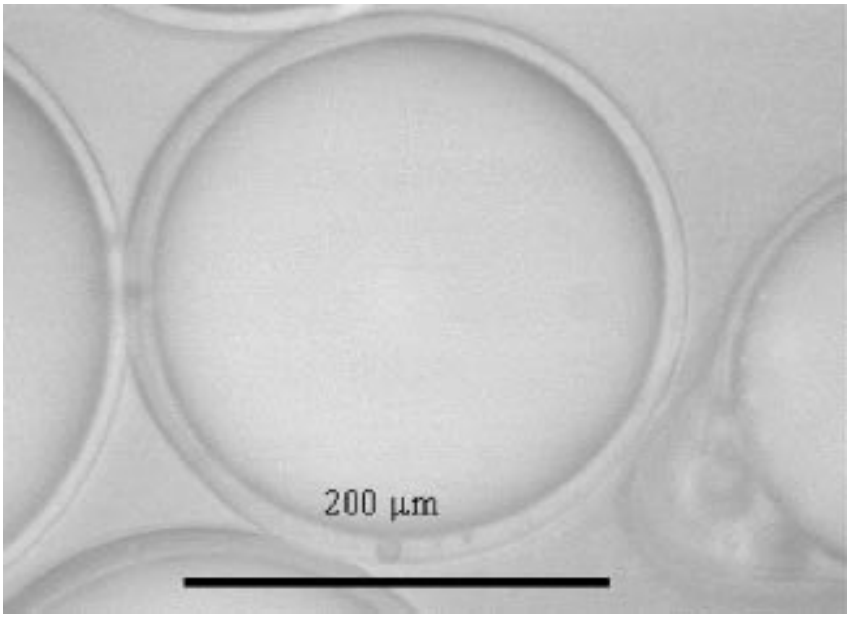


Figure 17.2. *Optical micrograph of gelatin-acacia microcapsule prepared by coacervation.*

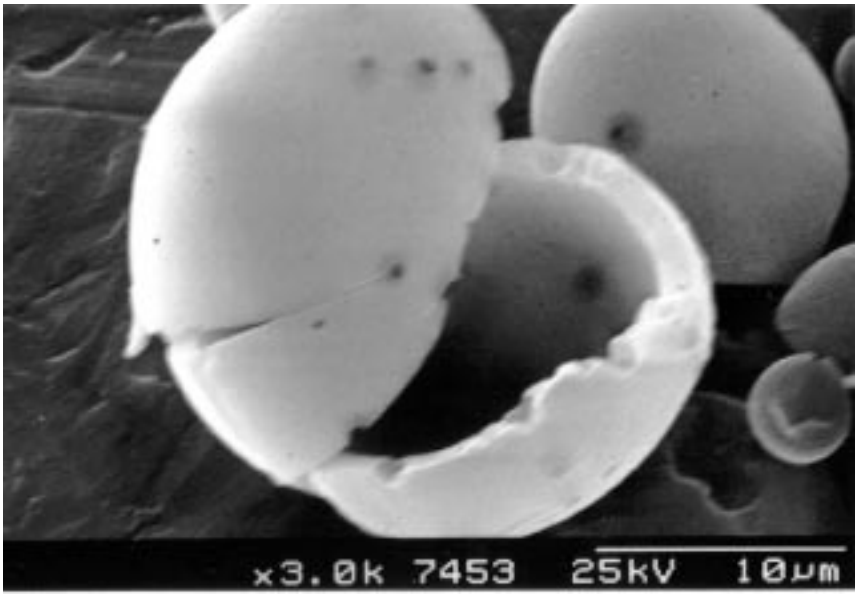


Figure 17.3. *SEM of fractured poly(4-vinylpyridine) microcapsule.*

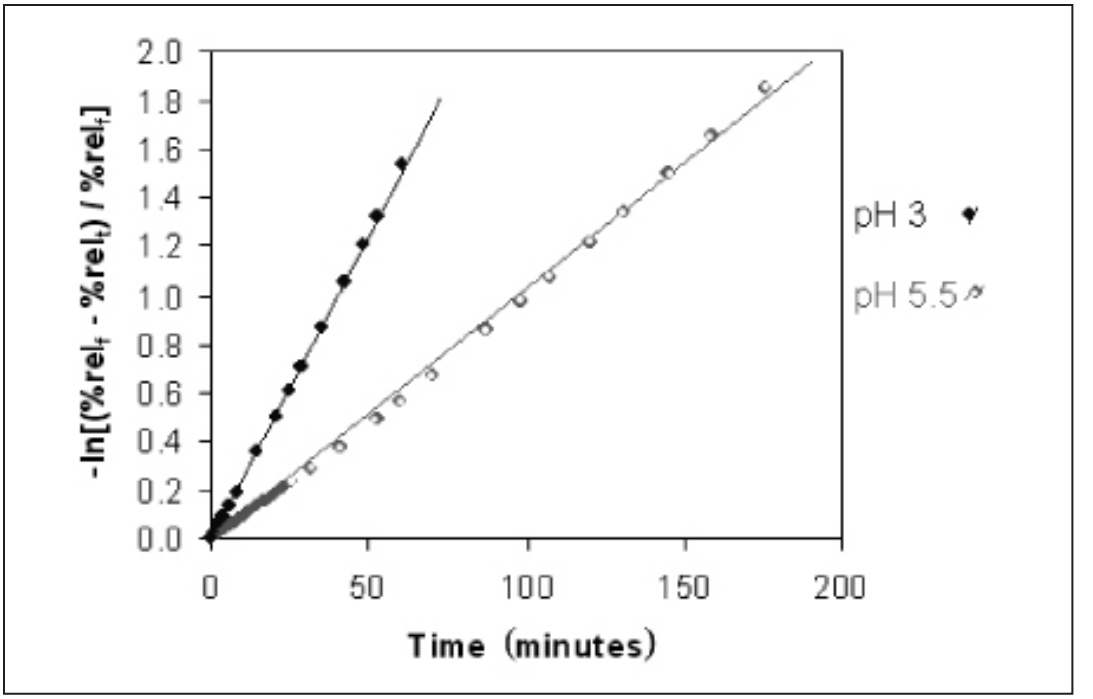


Figure 17.4. *pH dependent release kinetics from a PVPy microcapsule*

symbols).

Condensation and interfacial polymerization methods entail polymerization or cross-linking at the continuous/non-continuous interface of monomer or components dissolved in one or more phases.[7] The dispersion of capsules may be dried to provide a powder. Shell materials commonly used include polyesters, polyurethanes, polyureas, polycyanoacrylates and melamine-formaldehyde resins. Materials used to form, cross-link or harden the shell must be screened for any unwanted reaction with the core ingredients as well as for their toxicological potential; residual monomer content can pose a problem as is the presence of residual solvent.

17.4 Physical Production

These methods entail co-extrusion of a solution, melting the shell material over a droplet, or particle, of the core material, or spray-coating the shell over a solid particle where fluidized beds are often employed to ensure uniform coating of cores by shell forming solutions. The Wurster Process is the classic example, in which particles are fluidized in an air stream within a vertical chamber and coating material solution is injected from below into the fluidized particles. Such physical methods typically produce larger capsules, and are especially suited to particles with solid cores. For more

details on this process, see Chapter 18, "Encapsulation and Other Topical Delivery Systems".

17.5 Liposomes

Liposomes are a special case of microencapsulation. However, since the typical liposomal particle size is around 200 nm such systems should more rightly be termed sub-microcapsules or nanocapsules. Liposomes are self-assembled spherical micro-structures of lipids and lipid-like amphiphilic molecules that are arranged in single or multilayered closed bilayers in aqueous media; the active is trapped inside this fatty wall or the aqueous core. Since the wall material comprises triglycerides and phospholipids, when used in topical delivery liposomes can facilitate absorption of the active into the epidermis; the liposomes, themselves, do not appear to penetrate intact into the skin.[8] Although liposome technology has been around for about 40 years, it has suffered from problems of stability, poor mechanical integrity and low active concentrations.[9] Ever since the invention of liposomes, continuing efforts have been aimed at achieving a better understanding of lipid properties in order to gain better control over liposome formation and use. Their use in cosmetics has been extensively reviewed.[10] For more details, see Chapter 15, "Flexible Liposomes for Topical Applications in Cosmetics".

These issues have been addressed with the recent advent of the so-called "micellar nanoparticles"—multi-lamellar structures that are liposome-like.[11] Here, the outer layer encases the water containing the active pharmaceutical ingredient that allows delivery of the active to the interior of the epidermis.

17.6 Solid-Lipid Nanoparticles

Particulate lipid matrices, in the form of lipid pellets were originally developed for oral drug administration.[12] Solid-lipid nanospheres were initially produced by high pressure homogenization of melted or solid lipids.[13] Our work in this area began with the preparation and scale up of SLN's by a solvent-free process for the encapsulation of ethylhexyl methoxycinnamate (EHMC; Octinoxate) for dermal sunscreens.[14] Initially, the goal was to encapsulate the organic sunscreen in a benign matrix and effectively turn them into particulates in order to decrease the irritant/allergic sensitization potential. A low pressure proprietary melt-emulsify-chill (MEC) process was used (see **Figure 17.5**). This encapsulation method is general and can be applied to many water- or oil-soluble cosmetic and pharmaceutical actives. The process is cost-effective, reproducible, robust and scalable. For economic and technical (processing) reasons, it is very desirable to have a high solids-

content particle dispersion; stable, concentrated aqueous dispersions (up to 60% w/w) of SLN's can be prepared by the MEC method. The particle sizes range from 0.1–1 μm (see **Figure 17.6**); in the example shown the mean particle diameters were between 300 and 400 nm. The particle size and distribution is controlled by the rate of initial mixing, degree of shear and rate of cooling. These particles are a homogeneous mixture of matrix and active; the active can be present at concentrations as high as 30% by weight of the total dispersion. The matrix is typically a wax (or mixture of waxes) but may

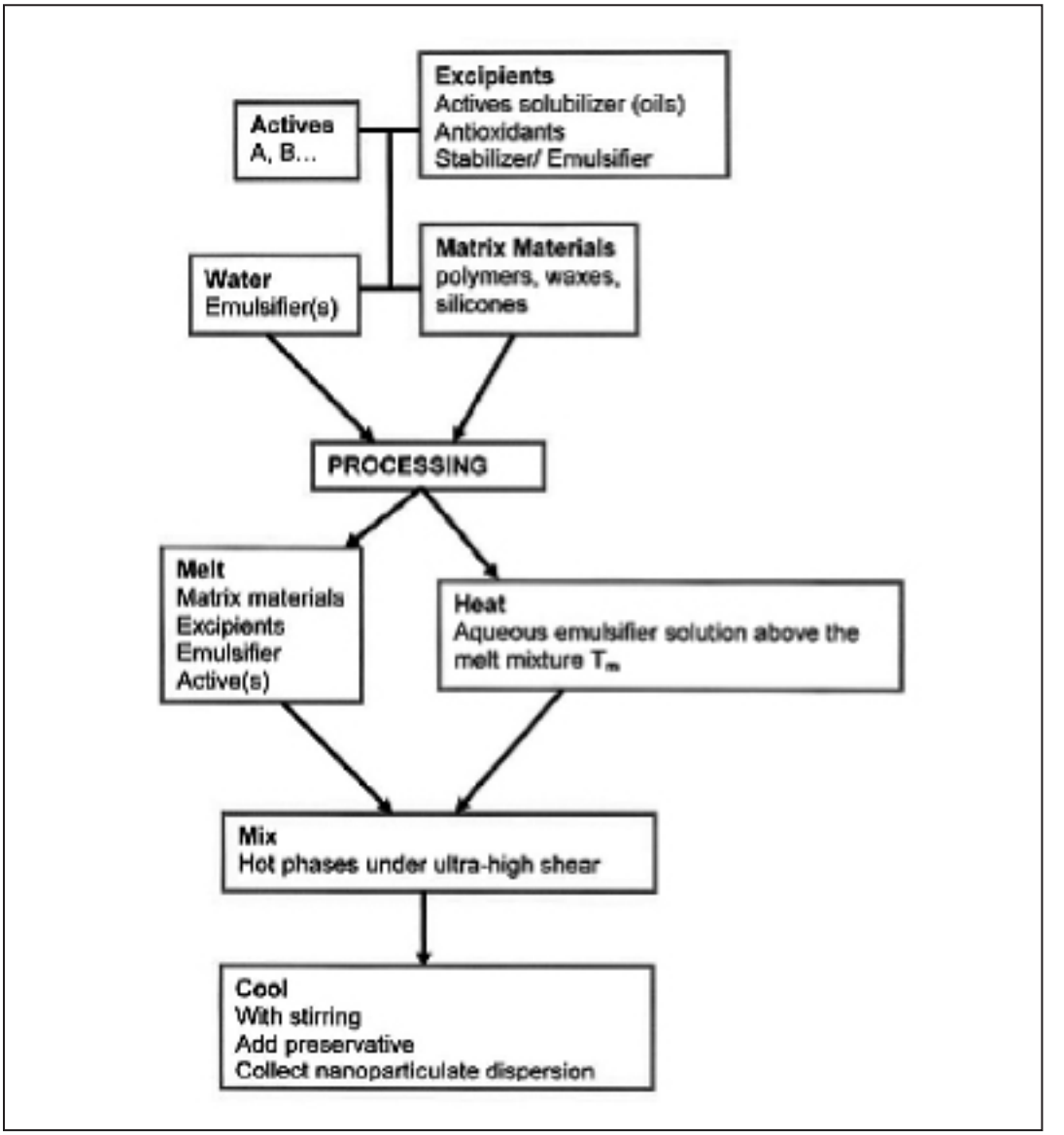


Figure 17.5. *Schematic of the Melt-Emulsify-Chill process.*

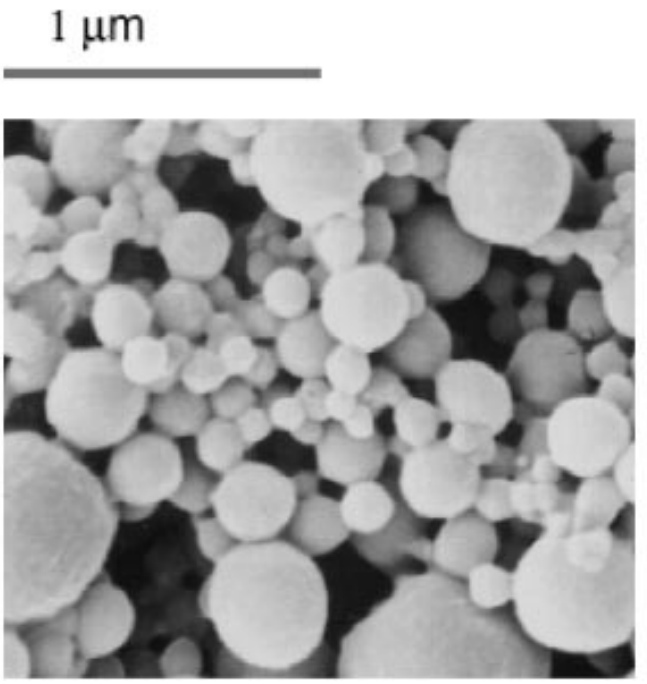


Figure 17.6. *SEM of a typical SLN. Note that the particles are spherical but polydisperse.*

Table 17.1. Controllable particle parameters using the Melt-Emulsify-Chill process

Bulk Physical Characteristics	**Surface Chemical Properties**
Melting point, hardness, density, solubility	Surface charge (anionic, nonionic, cationic)
Particle size and distribution	Hydrophobic/Hydrophilic Balance
Bioadhesion	Polarity

be an organic polymer or a silicone. A list of the controllable particle parameters are given in **Table 17.1**. In principle, the MEC process allows systems to be custom-designed to fit specific applications.

The SLN is not a wall-and-core particle; the particles are stable to mechanical agitation as well as storage—unlike many liposomal systems. Release is accomplished via diffusion or particle breakdown. Diffusion is dependent on particle/surrounding medium interaction. Breakdown can be accomplished through exploitation of melting points. For example, an

active could be released in a matrix that melts or softens at body temperature or, an orally ingested drug could be encapsulated in a polymer that dissolves at stomach pH.

The MEC process has now been extended to the encapsulation of other hydrophobic and hydrophilic molecules, specifically to prepare biodegradable nanoparticles as carriers for drugs. We have investigated the attachment of a model antigen-tetanus toxoid (TTA) and the effect of surface charge on the adhesion of SLNs to cells and tissues. The TTA was electrostatically adsorbed to the surface of cationic SLNs. Cationic SLNs were also found to adsorb strongly to human hair shafts, making them useful for time-release of drugs from the hair, or to the surface of hair-bearing skin. SLN formulations containing encapsulated fluorochromes were efficiently internalized by antigen presenting cells of the immune system in vitro, making them interesting candidates as sub-unit vaccine carriers for mucosal vaccination.

17.7 Experimental Methods

Solid lipid nanoparticles were prepared by the MEC process as shown in **Figure 17.5**. In essence, a molten wax is dispersed into a hot aqueous emulsifier solution under controlled shear and then cooled to yield a stable dispersion of solid-lipid nanoparticles. In contrast to many other nanoparticulate systems under investigation for mucosal vaccines, no organic solvents are required in this process, and the nanoparticles are hydrolytically stable.[15-17] Fluorescent particles were prepared by encapsulating an oil-soluble fluorescent dye in the SLNs. Cationic, non-ionic and anionic emulsifiers were used to control the surface charge of SLNs.

17.7.1 SLN Characterization

The SLN particle size was determined by photon correlation spectroscopy using a Brookhaven BI90Plus (Brookhaven Instruments, Holtsville, NY, USA).[18] The zeta potential (a measure of surface electrical charge) of the SLNs was measured in 1 mM KCl by phase analysis light scattering using a Malvern Zetasizer NanoZS90 (Malvern Instruments, Malvern, Worcestershire, UK) and that for hair and skin by the technique of streaming potential using a SurPASS Electrokinetic Analyzer (Anton Paar, Richland, VA, USA).[19,20]

17.7.2 SLN bioadhesion

Commercially blended virgin brown hair, (DeMeo Brothers, New York, NY, USA) was washed with aqueous sodium dodecyl sulfate, rinsed with pure

water to remove residual anionic surfactant, then incubated in dilute SLN dispersions for 5 min and finally extensively rinsed in deionized water until no further change was seen in the measured value of the streaming potential.

17.7.3 SLN cell uptake

Immature dendritic cells (DC) were incubated with dilute dispersions of SLNs in culture medium at 37°C. Uptake was measured by flow cytometry (Beckman Dickinson, Franklin Lakes, NJ, USA) and cells with internalized fluorescent particles were imaged by confocal laser scanning microscopy (Leica Microsystems Ltd., Milton Keynes, Buckinghamshire, UK).

17.8 Results and Discussion

Table 17.2a. A simple sun product formulation (using SLN's) for SPF measurements

Phase A	
Deionized water	to 100.0
Carbomer (Carbopol 940), 2% solution	12.5
Propylene Glycol	2.5
Phase B	
Cyclomethicone	7.0
Octyldodecanol	5.0
Glycerol Stearate (and) PEG-100 Stearate	3.0
Stearic Acid	3.0
	1.3
Phase D	
SLN dispersion	qs to desired SPF
Phase E	
Propylene Glycol (and) diazolidinyl urea (and) Methyl paraben (and) Propyl paraben (Germaben II)	1.0

Table 17.2b. A simple sun product formulation preparation procedure.

Procedure

Heat Phase A to 77°C with propeller stirring. Heat Phase B to 75°C. Add Phase B to Phase A with constant stirring. Add Phase C. Cool to 45°C with constant stirring. Add Phases D and E.

Three SLN systems containing organic sunscreens (Octinoxate, Octinoxate/Benzophenone-3 and Octinoxate/Octocrylene) were formulated into a simple sun product formulation (see **Tables 17.2a** and **17.2b**) and the SPF values were determined both in-vitro—using an Optometrics SPF-290 (Optometrics, Ayer, MA, USA) with 3M Transpore Tape® as the substrate—and in-vivo (AMA Laboratories, New City, NY, USA). Three sun product formulations containing non-encapsulated active at the same concentrations were used as controls. The in-vivo data was obtained on five subjects using the approved FDA methodology.[21] The results are summarized in **Table 17.3** and clearly demonstrate a substantial increase in SPF when using encapsulated sunscreen active compared with the control products containing regular sunscreen active at the same levels. The exact reason for this initially rather unexpected result could arise from several factors. Importantly, the molecules of the organic sunscreen are well dispersed within the microcapsules and the microcapsules themselves form a more uniform film on the

Table 17.3. Enhancement in sun protection factor through encapsulation of organic sunscreens.

% Sunscreen	in vitro control	in vitro SLN	enhancement factor	in vitro control	in vivo SLN	enhancement factor
5% Octinoxate	6.5	19.8	3.0	5.9	16.7	2.8
5% Octinoxate 2.5% Benzophenone-3	10.0	21.5	2.1	11.4	24.0	2.1
2%Octinoxate 10% Octocrylene	6.3	18.6	2.9	8.8	16.7	1.9
Avg. SPF Enhancement Factor			**2.7**			**2.3**

skin (compared with the non-encapsulated material) thus improving the UV photon collection and absorption efficiency. Path length and additional scat-

tering of radiation also increase. Further, the encapsulation may also help to reduce photodegradation.

The SLN suspensions used in the study all had solid contents of 50% by weight. No change occurred in viscosity of a typical suspension after 12

Table 17.4. Stability of an SLN suspension: alone and in a formulation.

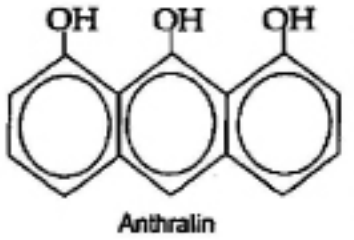
Measured Parameter	Time (months) 0	12
in vitro SPF value of sun product formulation containing 20% SLN (≡ 5% Octinoxate)	19.8	18.9
viscosity* of sun product formulation containing 20% SLN (≡ 5% Octinoxate)	40,800cP	41,250cP
viscosity** of SLN (25% Octinoxate) suspension alone	7.1 cP	8.0 cP

** Brookfield LV Viscometer, Spindle#4 @ 12RPM, 25°C*
*** Brookfield LV Viscometer, CP-40 @ 30RPM, 25°C*

months storage at room temperature (see **Table 17.4**). These SLNs are aqueous suspensions; as such they are designed to stay in the water phase of an emulsion. The addition of the SLN suspension into the sun product formulation does not adversely impact the product stability.

For the encapsulated sunscreens, since the primary goal was to minimize penetration of the active into the skin, a matrix was chosen that was chemically and physically compatible with the sunscreen active (e.g., Octinoxate); thermodynamically, the active has little incentive to diffuse out of the matrix (see also Chapter 6, "The Influence of Emollients on Skin Penetration from Emulsions"). To test this hypothesis, we encapsulated anthralin using the MEC process. Anthralin is an organic irritant and is an extremely potent inhibitor of mitosis but causes erythema and swelling in virtually every exposure. A 0.2% (active basis) emulsion was prepared containing the anthralin SLN and applied to the forearms of six subjects and occlusive patches were applied. The patches were removed after 12 hrs and the test sites were rated on erythema and swelling. The test subjects were also questioned regarding the degree of discomfort. Appropriate controls were run; blank SLN (i.e., no anthralin) alone, blank SLN plus non-encapsulated anthralin

Table 17.5. Active ingredient isolation from skin: Anthralin challenge test.



Anthralin

Anthralin is a drug used in severe psoriasis. It is usually applied in a 0.1% suspension and must often be discontinued because of severe irritation.

Subject SLN	Empty SLN	Anthralin + empty SLN	Anthralin alone	Anthralin
1	0 none	0 none	2+ itch/burn	3+ itch/burn
2	0 none	0 none	3+ itch/burn	3+ itch/burn/tender
3	0 none	0 none	3+ burn	3+ slight burn
4	0 none	0 none	3+ burn	3+ tender
5	0 none	0 none	2+ slight burn	2+ slight burn
6	0 none	0 none	3+ burn	3+ burn

Evaluation Keys

VISIBLE

0 = No visible reaction

1+ = Mild erythema

2+ = Well defined erythema, possible presence of barely perceptible edema

3+ = Erythema and edema

4+ = Erythema and edema with vesiculation and ulceration

and anthralin alone. The results (see **Table 17.5**) clearly demonstrate that anthralin was adequately encapsulated.

By appropriate choice of emulsifiers, it is also possible to create aqueous nanoparticles in an oil-based emulsion. Such an emulsion can be used to encapsulate, for example, water-soluble materials such as L-ascorbic acid (vitamin C). L-ascorbic acid is considered one of the most important water-soluble anti-oxidants, providing photoprotective capabilities by inhibiting UVA and UVB radiation-induced damage by neutralizing the oxygen-free radicals in the skin and it prevents UV immunosuppression.[21] Vitamin C also stimulates collagen synthesis and has anti-inflammatory properties.

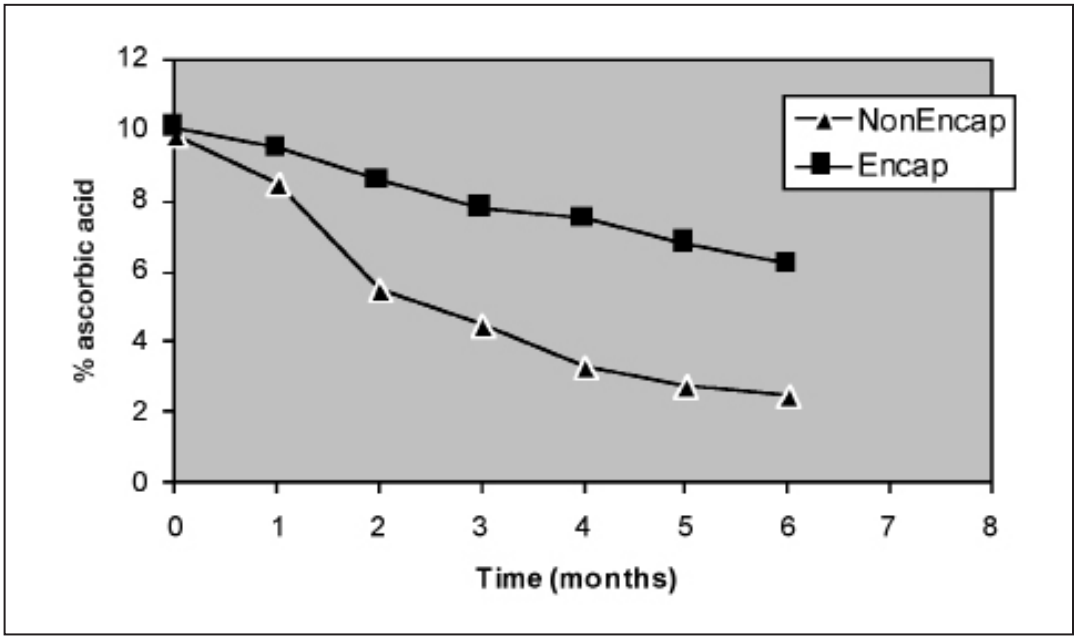


Figure 17.7. *The stability at 45°C of encapsulated ascorbic acid versus non-encapsulated material. Note: assay for ascorbic acid was by HPLC.*

However, it readily degrades in the presence of moisture, oxygen and light. Here, not only does encapsulation provide a convenient formulation vehicle, but it also enhanced the stability of the encapsulated payload, as shown in **Figure 17.7** for data taken at an elevated temperature of 45°C.

Another unstable cosmetic active, hydroquinone, is used in some countries for the gradual lightening of hyperpigmented skin conditions such as acne spots, freckles, age spots, and other unwanted areas of melanin hyperpigmentation that may arise from exposure to the sun, during pregnancy, and from the use of oral contraceptives.[22] Hydroquinone arrests the pro-

duction of melanin through tyrosinase inhibition.[23] Hydroquinone rapidly darkens in the presence of moisture and oxygen rendering formulations cosmetically and aesthetically unacceptable. **Figures 17.8** and **17.8b** illustrate the benefit of encapsulation in deterring these unwanted reactions. Whereas

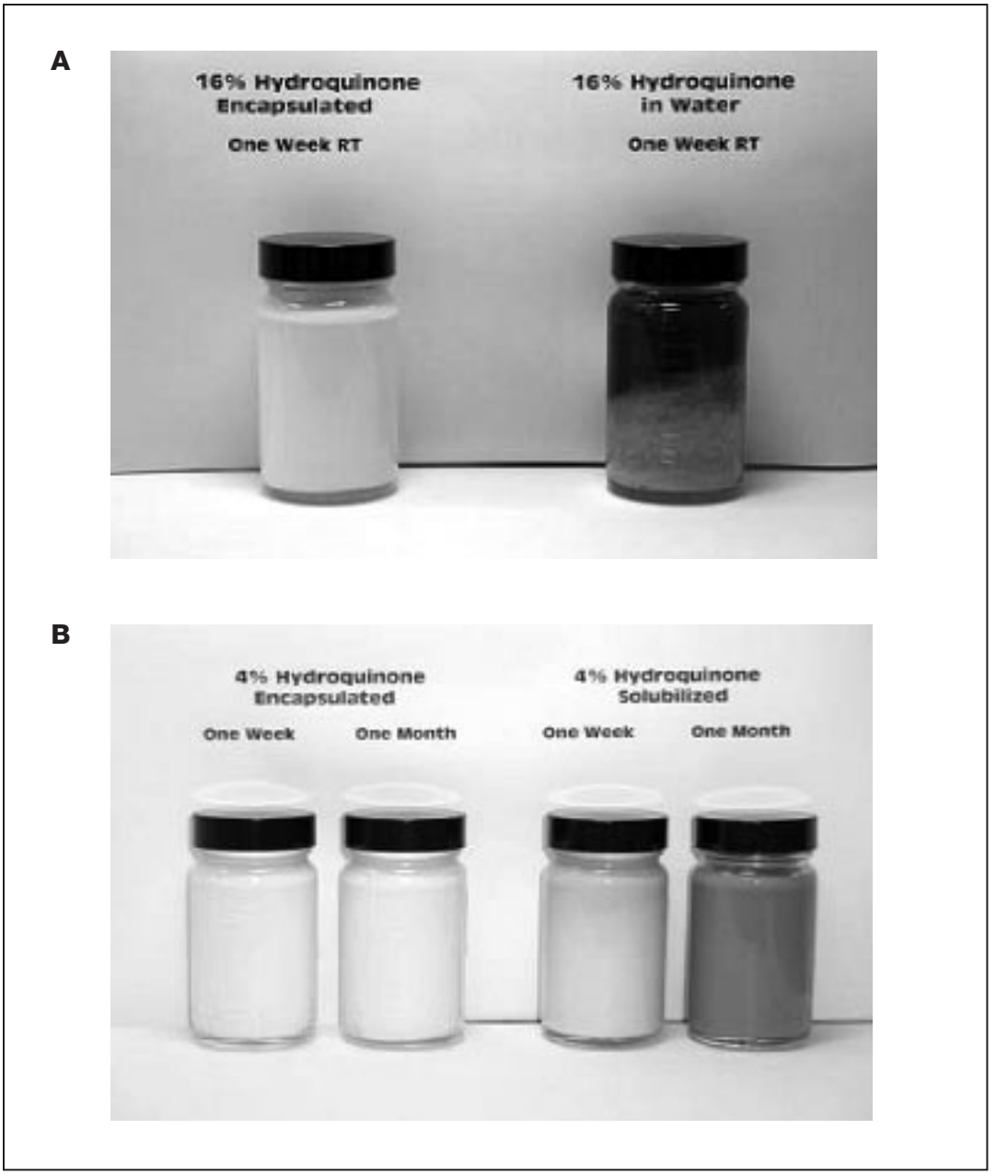


Figure 17.8a. *Color fastness of encapsulated hydroquinone. Note: the sedimentation of the non-encapsulated hydroquinone and the dark color of the supernatant.*
Figure 17.8b. *Color stability of encapsulated hydroquinone in a skin lightening formulation.*

16% hydroquinone is a slurry that rapidly settles leaving a dark brown supernatant, encapsulation in an SLN results in a stable suspension that, when formulated (at 4% hydroquinone) into a skin lightening lotion, also provides enhanced stability to light.

That the surface of the nanoparticles can also be tailored to give them specific properties is readily demonstrated for the case of the surface electrical charge on the particles. All particles dispersed in water carry an electric charge.[24] The sign and magnitude of that charge significantly influences the properties of the bulk dispersion, and also the interaction of the particles with other surfaces. Although the surface charge is not readily determined directly, the fact that electrically charged particles move in an electric field allows their "electrophoretic mobility" to be measured, and this measurement can be converted into an electrical potential at the surface, called the zeta-potential.[25] By choosing appropriate levels of a charge modifying director (CMD) during the encapsulation process, the resulting particles can be charged negatively (negative zeta potential) or positively (positive zeta

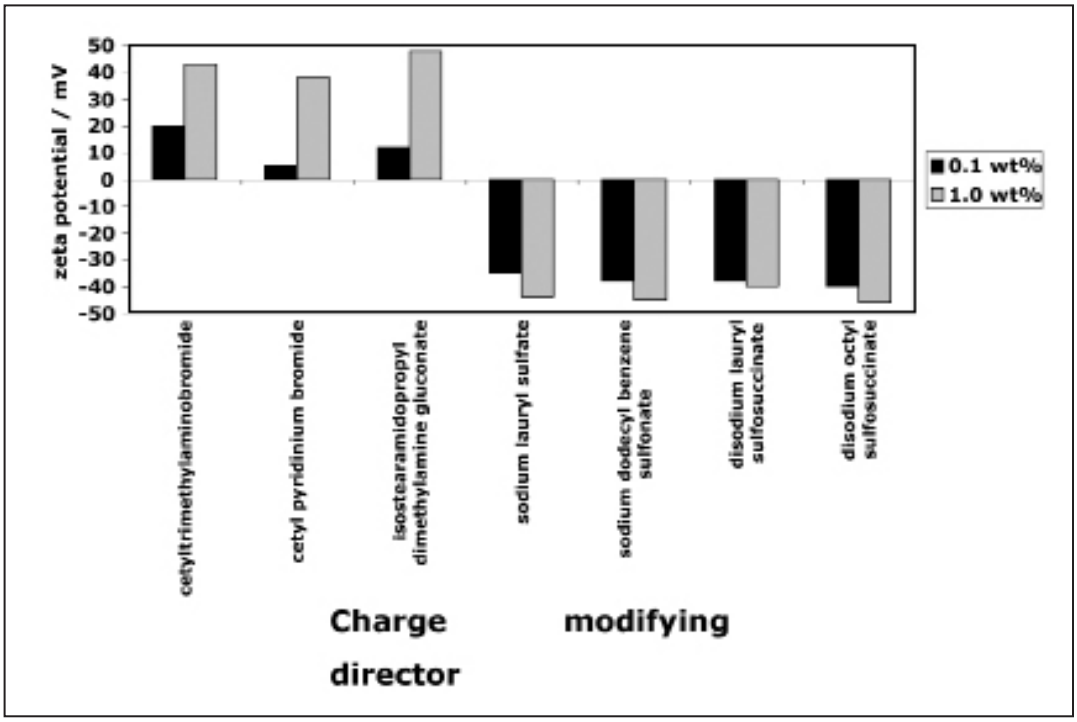


Figure 17.9. *Effect of charge modifying director (CMD) on the zeta potential of an SLN*

potential), with high or low magnitude (see **Figure 17.9**). The ability to create particles having an anionic, cationic or non-ionic nature simplifies the

formulator's job by making it easier to incorporate the particles into new or existing formulations.

Practical use can be made of such surface charge control, as for example in the case of formulations of actives directed to the scalp or hair. Both skin and hair carry a net negative charge.[26] Positively charged particles have a tendency to attach strongly to the follicles of clean hair, especially near their base, close to the scalp. A sample of clean hair was exposed to three types

Table 17.6. Cationic SLNs for hair treatment.

CMD description	Zeta potential (mV)
Particle A	
CMD: isostearamido propyldimethyl ammonogluconate	+17
Particle B	
CMD: dimethylditallow ammonium chloride	+48
Particle C	
CMD: cationic (amino functional) silicone polymer	+60

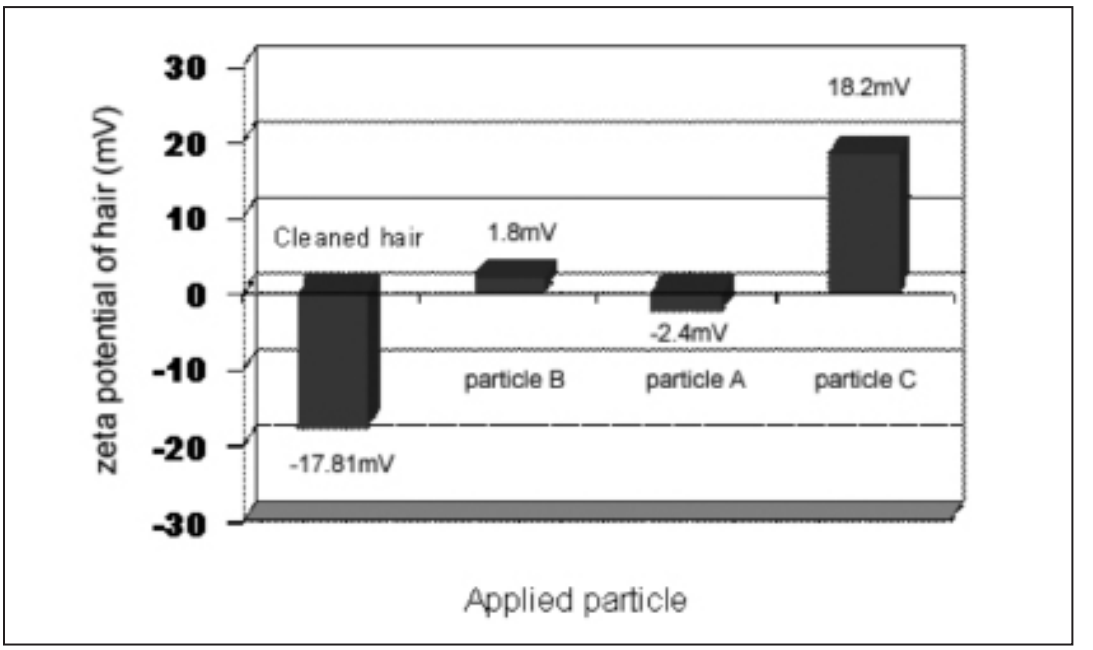


Figure 17.10. Effect of adsorption of cationic SLN particles onto hair.

of particles with different positive surface charges; the particles contained different CMDs (see **Table 17.6**). The zeta potential of the treated hair was measured before and after particle-exposure to determine the efficiency of particle attachment to the follicles. The results are shown in **Figure 17.10**.

It is clear that particles with positive charge attach strongly, and in the cases of particles B and C, the hair was so covered in attached particles that the follicle surface charge was reversed from negative to positive. The positive charge (+48mV) of the SLN particle B was engineered using a commercial cationic quaternary surfactant used in hair conditioner formulations and was therefore expected to be quite substantive. The largest effect was seen with the SLN particle C in which the positive charge (+60mV) arises from cationic groups along the backbone of an amino-functional silicone polymer. The least effect was obtained with particle B that had the smallest positive zeta potential (+17mV). Here, the CMD was a simple amphoteric and though it reduced the charge on the hair, it was not capable of reversing the sign. In part this result may be because the pH of the challenge solution was close to neutral.

Use can be made of this type of system in the targeting of encapsulated therapeutics to the base of the follicle, close to the scalp (for skin disorders) or for encapsulated biocides to kill head lice for example. Inclusion of a sunscreen active could help prevent hair damage through sun exposure because of drying and loss of elasticity and also reduce fading of color treatments.

17.9 Biodegradable SLNs for Pharmaceutical and Medical Applications

Vaccines produced from the isolated protein "sub-units" of a virus vaccines or gene therapy—the treatment of genetic disorders by inserting healthy DNA within the nucleus of cells with the faulty genetic material—or the emerging field of "RNA-interference" ("gene-silencing") share one challenge. That challenge is delivering what is a water-soluble active (or actives) across the fatty membrane of the cell, intact, and to the correct intracellular compartment, from where it can be processed, or released depending on the mode of action. In devising strategies to accomplish this outcome, the fact that the antigen-presenting cells of the immune system are designed to internalize particulate materials, that have particle sizes of less than 1 μm, means that, for example, attaching the sub-unit proteins of a vaccine to such a "nanoparticle" is a logical approach. Further, all such "actives", e.g., proteins, DNA, dsRNA, and siRNA, share a feature that may allow them to be delivered by a common approach: they are all anionic (negatively

charged) in aqueous solution. By using biodegradable, non-toxic, natural waxes (with materials selected from the list of chemicals generally regarded as safe), nanoparticles were prepared using the MEC process. Various cell types were incubated with such SLNs having positive, neutral and negative surface charge in vitro. Irrespective of the surface charge all SLN types indiscriminately entered the cells with which they were incubated, including THP-1 (promonocytes), peripheral blood dendritic cells, Raji (B-cells), PM-1 (T-cells), and endocervical epithelial cells. **Figures 17.11a** and **17.11b** show confocal laser scanning microscopy images of cells with internalized particles; dendritic cells containing green fluorescent nanoparticles and a

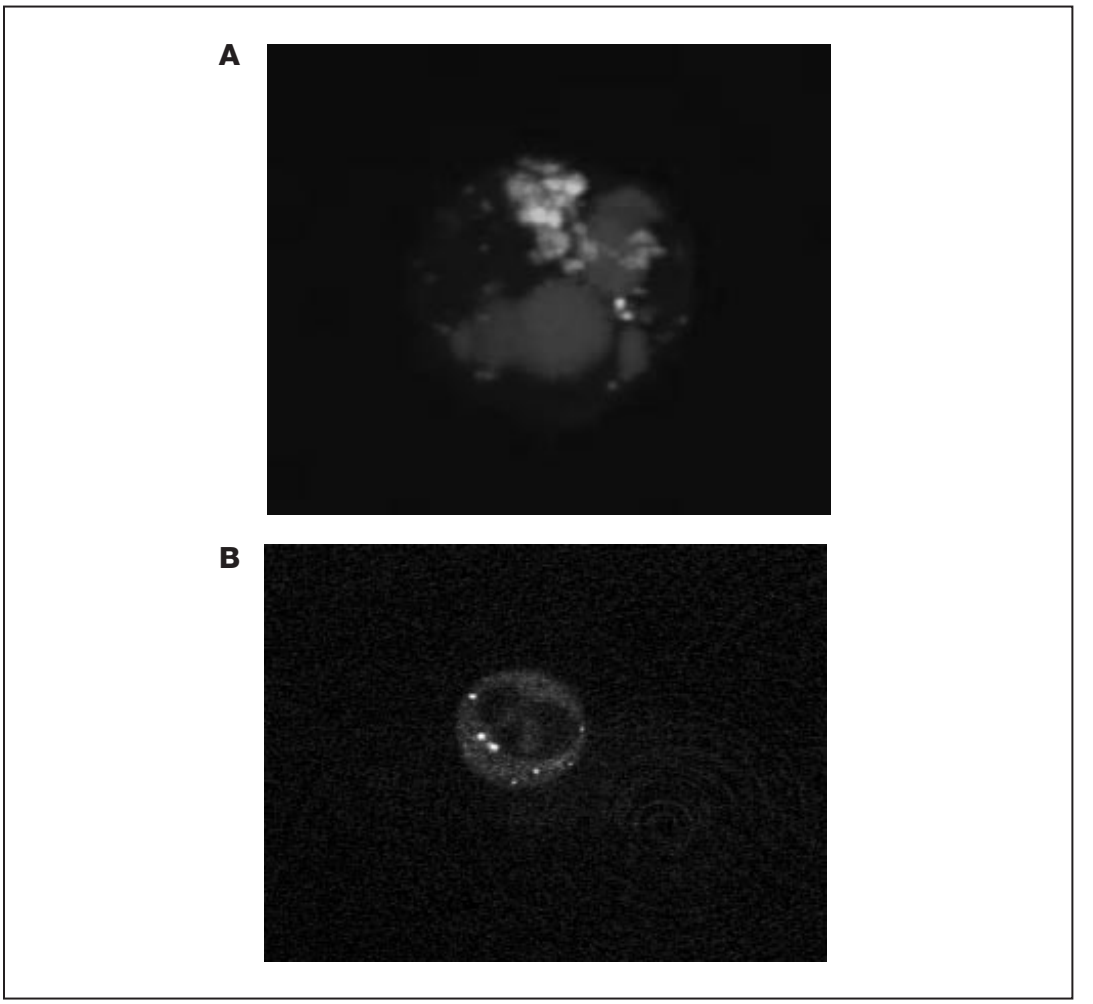


Figure 17.11a. *Confocal images of cells containing internalized SLNs. Dendritic cell containing green fluorescent SLNs with the nuclei stained blue.*
Figure 17.11b. *Confocal images of cells containing internalized SLNs. THP-1 cell with internalized particles shown as bright red spots within the fainter red cytoplasm*

THP-1 cell with internalized particles shown as bright green spots within the cytoplasm, respectively. Thus, the non-specific nature of the cellular uptake would suggest that such SLNs are suitable candidates for delivering surface-attached actives into cells.

The attachment of negatively charged active molecules to a positively charged surface should decrease the magnitude of the surface charge of the particle and, with sufficient adsorption, should reverse the sign of the particle charge. This reversal was confirmed, using tetanus toxoid antigen (TTA) as the (negative) active, by following the zeta potential of a cationic SLN as function of amount of active attached. TTA was used because it is found in commercial vaccine formulations. The results showed that very high attachment of the TTA can be achieved (up to 0.45 mg of antigen was attached to 0.75 mg of SLN); the zeta potential of the SLN changes from +64mV for the naked particle to –11mV following adsorption of the TTA (see **Figure**

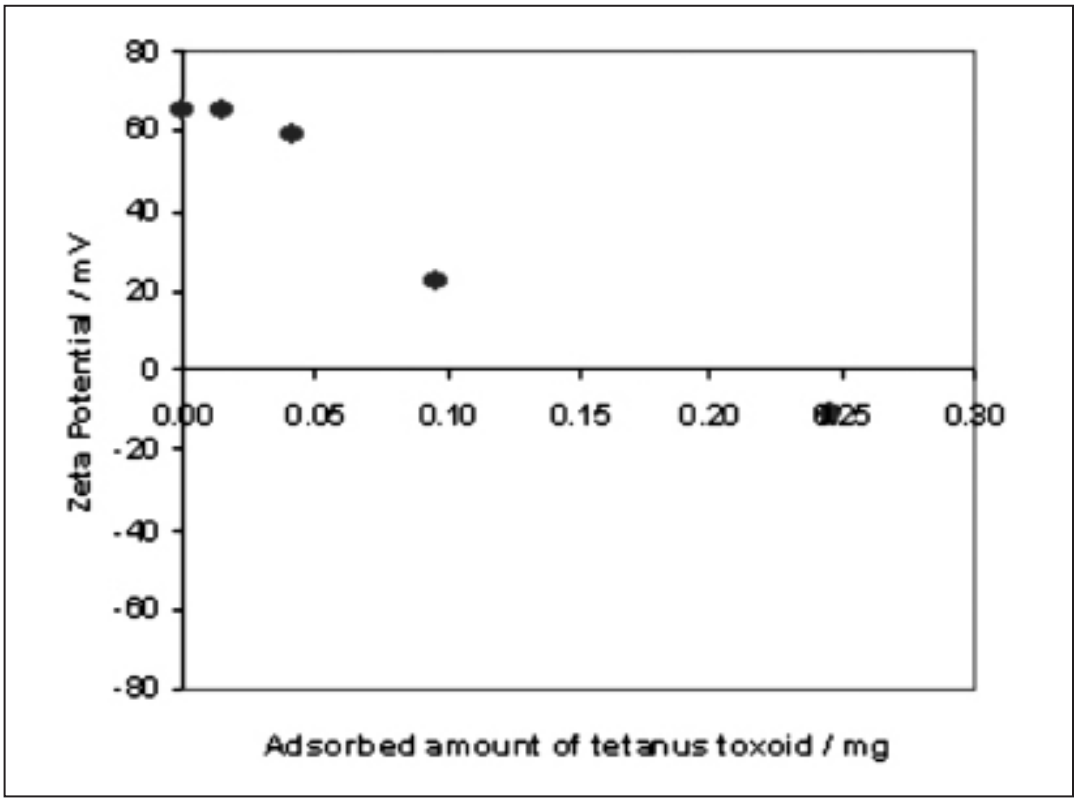


Figure 17.12. *Effect of adsorption of tetanus toxoid on the zeta potential of a cationic SLN*

17.12). The strong attachment, and demonstrated entry into antigen presenting cells of the immune system (such as the dendritic cells shown in **Figure**

17.11), illustrates the potential that these natural wax nanoparticles might have in vaccine formulations.

The strength of the electrostatic interaction between a negatively charged active and a positively charged SLN may affect the release of the active on arrival at the target cell. We have already seen how this reaction can be controlled by the CMD chemical type (see **Figure 17.9**), but it can also be controlled, for a given type, by the level of CMD. Control of the surface charge of nanoparticles made from fatty alcohol waxes was investigated by using different ratios (100:1, 1000:1 and 10,000:1) of wax to CMD (see **Figure 17.13**). The ability to reduce the zeta potential (surface charge) of the nanoparticles could mean that the strength of the binding of the attached active can be controlled, and this could translate to control of the release of

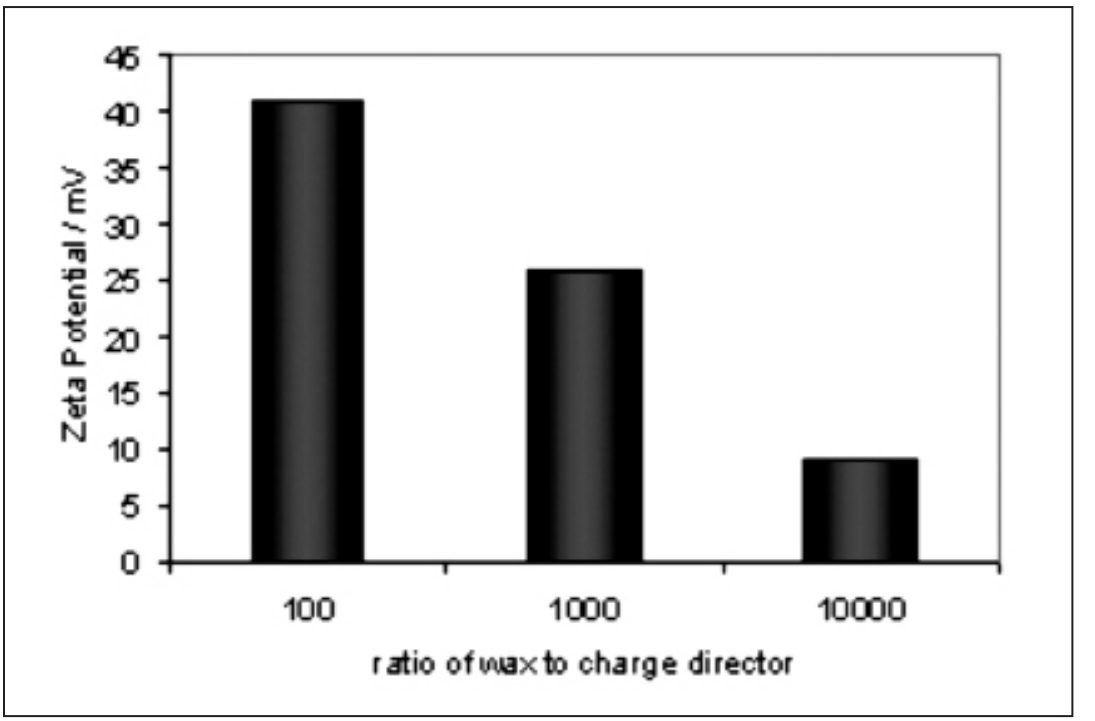


Figure 17.13. *Effect of wax: CMD ratio on the nanoparticle zeta potential.*

active from the nanoparticles once inside the cell—a crucial requirement for DNA, siRNA and dsRNA delivery, suggesting that the efficacy of a formulation can be tested for its therapeutic effect as the binding-strength of active-to-nanoparticle is varied.

17.10 Conclusions

The MEC process can easily, and cheaply, produce a range of solid lipid nanoparticles (SLNs) —"smart colloids"—that offer flexibility in formulation, increased efficacy and decreased formulation complexity. The method is general and can be applied to many water- or oil-soluble cosmetic and pharmaceutical actives. Multiple actives can be co-encapsulated. The process is cost-effective, reproducible, robust and scalable. The bulk physical characteristics and surface chemical properties can be varied to allow systems to be custom designed to fit specific applications; use can be made of this in the targeting of encapsulated actives, botanicals and therapeutics to skin and hair. The non-specific nature of the cellular uptake would suggest that SLNs produced via the MEC process are suitable candidates for delivering surface-attached actives into cells. The strong attachment and demonstrated entry into antigen presenting cells of the immune system (such as the dendritic cells shown in **Figure 17.11**) illustrates the potential that these natural wax nanoparticles might have in vaccine formulations.

References

1. US Pat. 2,712,507, Pressure sensitive record material, BK Green (1955).
2. US Patent 2,800,457, BK Green and L Schleicher (1957).
3. CG De Kruif, F Weinbreck and R de Vries, Complex coacervation of proteins and anionic polysaccharides, *Curr Opin Coll Interf Sci* 9 340–349 (2004).
4. L Baruch and M Machluf, Alginate-chitosan complex coacervation for cell encapsulation: Effect on mechanical properties and on long-term viability, *Biopolymers* 82 570-579 (2006).
5. GF Palmieri, D Lauri, S Martelli and P Wehrle, Methoxybutropate microencapsulation by gelatin-acacia complex coacervation, *Drug Devel Indust Pharm* 25 399–407(1999).
6. A Loxley and B Vincent, Preparation of poly(methylmethacrylate) microcapsules with liquid cores, *J Coll Interf Sci* 208 49–62 (1998).
7. T Whateley, Microcapsules: Preparation by interfacial polymerization and interfacial complexation and their applications. in: S Benita (Ed.), *Microencapsulation: Methods and Industrial Practices*, Marcel Dekker Inc., New York, NY, USA, 349-375 (1996).
8. HE Hofland, JA Bouwstra, H Boddé, F Spies and HE Junginger, Interactions between liposomes and human stratum corneum in vitro: Freeze fracture electron microscopical visualization and small angle X-ray scattering studies, *Br J Dermatol* 132 853-866 (1995).
9. AD Bangham, MM Standish and JC Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, *J Mol Biol* 13 238-252 (1965).
10. VS Kulkarni, D Aust, J Wilmott and J Hayward, "Liposomes in cosmetics: An overview of production, characterization and applications." In: M Schlossman (Ed.), *The Chemistry and Manufacture of Cosmetics, Vol. III 3rd edition*, Allured Publishing,

Carol Stream, IL, USA, Chapter 35, 571–596 (2000).
11. RW Lee, *private communication*; Novavax Technical Briefing Document.
12. EU Patent 0167825, Lipid nanopellets as carrier system for drugs for oral administration, P Speiser (1990).
13. EU Patent 0605497, Medication vehicles made of solid lipid particles (solid lipid nanospheres – SLN), RH Müller and JS Lucks (1996).
14. D Fairhurst and MA Mitchnick, Sub-micron encapsulation of organic sunscreens, *Cosmet Toilet*, 110 (9) 47–50 (1995).
15. J Kazzaz, J Neidleman, M Singh, G Ott and DT O'Hagan, Novel anionic microparticles are a potent adjuvant for the induction of cytotoxic T lymphocytes against recombinant p55 gag from HIV-1, *J Control Rel* 67 347–356 (2000).
16. J Chesko, J Kazzaz, M Ugozzoli, DT O'Hagan and M Singh, An investigation of the factors controlling the adsorption of protein antigens to anionic PLG microparticles, *J Pharm Sci* 94 2510–2519 (2005).
17. M Singh, J Kazzaz, M Ugozzoli, P Malyala, J Chesko and DT O'Hagan, Polylactide-co-glycolide microparticles with surface adsorbed antigens as vaccine delivery systems, *Curr Drug Del* 3 115–120 (2006).
18. BB Weiner, "Particle sizing using photon correlation spectroscopy." In: H Barth (Ed.), *Modern Methods of Particle Sizing*, Wiley-Interscience, NY, USA, 93-116 (1984).
19. WW Tscharnuter, F McNeil-Watson and D Fairhurst, A new instrument for the measurement of very small electrophoretic mobilities using phase analysis light scattering, ACS Symposium Series 693 Chapter 23 327–340 (1998).
20. D Fairhurst and V Ribitsch, Zeta potential measurements of irregular shape solid materials, ACS Symposium Series, 472 Chapter 22, 337–353 (1991).
21. Anonymous, Sunscreen drug products for over-the-counter human use; Final Monograph, Federal Register, May 21st, 21CFR Part 352 (1999).
22. J Murray, D Darr, J Reich and S Pinnell, Topical vitamin C treatment reduces UVB radiation-induced erythema in human skin, Abstract #340, *J Invest Dermatol* 96 587 (1991).
23. VJ Hearing and RA King, "Determinants of skin color: melanocytes and melanisation", in: N Levine (Ed.), *Pigmentation and Pigmentary Disorders*, CRC Press, Ann Arbor, USA, 4–18 (1993).
24. O-S Lee and EJ Kim, Skin lightening, *Cosmet Toilet*, 110 (10) 51–56 (1995).
25. RJ Hunter, *Zeta Potential in Colloid Science*, Academic Press, NY, USA (1981).
26. DJ Shaw, *Electrophoresis*, Academic Press, London, UK (1969).
27. ED Goddard and PS Leung, Elektrische Eigenshaften der Haut und des Haares, *Parfümerie und Kosmetik*, 68 546–561 (1987).