Nanosomes™ - used in DS Laboratories topical treatments.

Technical white paper

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A submicron technology, Nanosome , developed for DS Laboratories enhances the deposition of functional ingredients and sensory markers onto hair and skin from wash off applications. Nanosomes consists of solid hydrophobic submicron spheres having an average particle size of 0.1 micron to 1 micron, in the form of an aqueous dispersion. The submicron spheres also enhance the efficacy of these ingredients and prolong their release over an extended period of time onto the target site.

Nanosome submicron spheres have increased stability as compared to emulsion-based delivery systems, such as liposomes, and are more effectively dispersed than most suspension based systems. The enhanced stability of submicron spheres can be utilized to enhance stability of sensitive active ingredients and prolong product shelf life. Further, the substance to be delivered does not have to be soluble in the vehicle since it can be dispersed in the solid matrix. Nanosome also has a lower risk of reaction of substance to be delivered with the vehicle than in emulsion systems because the vehicle is a solid inert material.

The cosmetic industry offers a wide array of effective functional ingredients for skin and hair care applications that can be administered by a variety of products. However, these ingredients only work and provide the benefits if they reach the corresponding target sites at effective concentration. Most conventional wash-off skin and hair care products, such as body wash, shampoos, and conditioners, comprising treatment ingredients leave very little of the functional ingredients onto the hair and skin after rinsing. For these products to be effective the functional ingredients need to reach the target site.
In order to address this challenge DS Laboratories developed a proprietary line of submicron spheres, Nanosomes, to effectively deliver a broad range of ingredients and sensory markers onto skin, hair (Figure 1), and hair follicles, enhances their efficacy and prolongs their release over an extended period of time. Nanosomes consists of solid hydrophobic submicron spheres (Figure 1) having an average particle size of 0.1 micron to 1 micron, in the form of an aqueous dispersion.

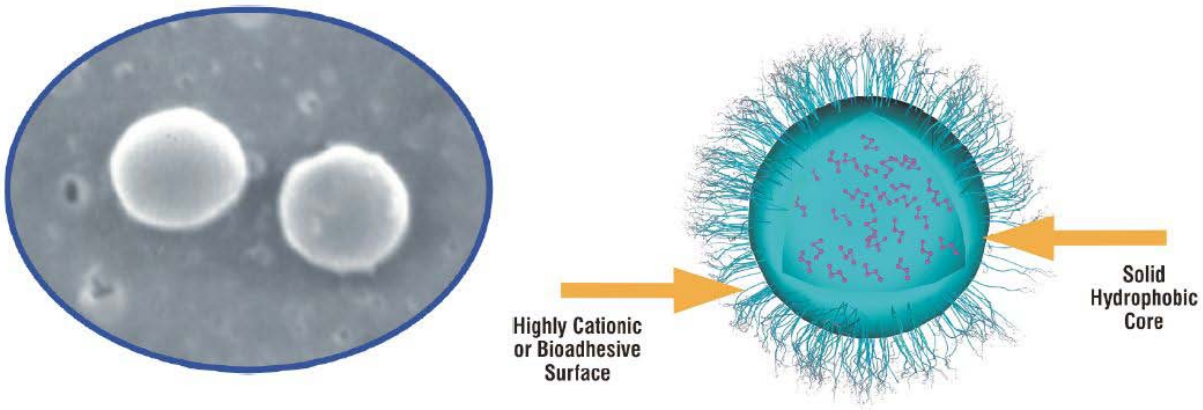

Figure 1: The left figure is a scanning electron microscope (SEM) image of the hydrophobic submicron spheres magnified 10,000 times. The right figure is a schematic illustration of Nanosomes.

The submicron spheres have high cationic charge density to improve their deposition onto the target site and prevent them from being washed off during the rinse process. The high cationic charge density of the submicron spheres is created by incorporating a cationic conditioning agent into the solid hydrophobic matrix of the submicron spheres in conjunction with a cationic "charge booster". In use, the highly cationic submicron spheres become associated with the proteinaceous portion of the hair, hair follicles, or skin. The hydrophobic matrix materials sustain the diffusion rate of the active ingredients and sensory markers through the spheres and enable the release of the active ingredients and sensory markers over an extended period of time, or during heat treatment such as blow-drying the hair.

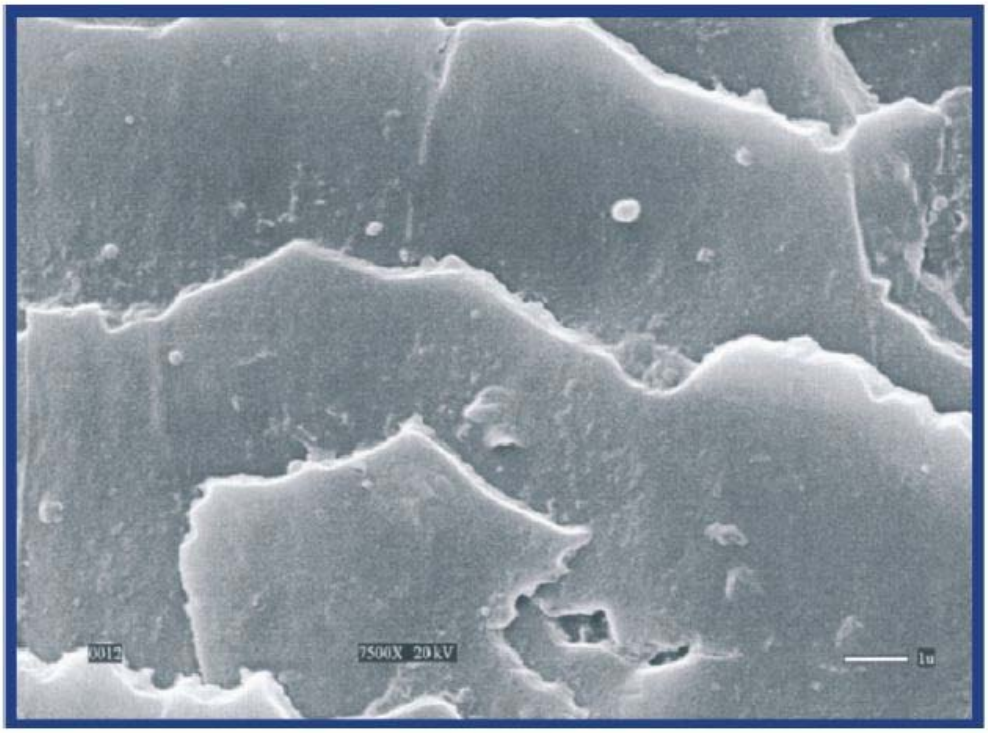

Figure 2: Scanning electron microscope (SEM) image of Nanosome on hair.

Submicron spheres and liposomes are competing systems for the controlled delivery of active ingredients. The major drawback associated with the use of liposomes as controlled delivery systems is their limited stability, both in terms of shelf life and after application onto the target site (Figure 3).

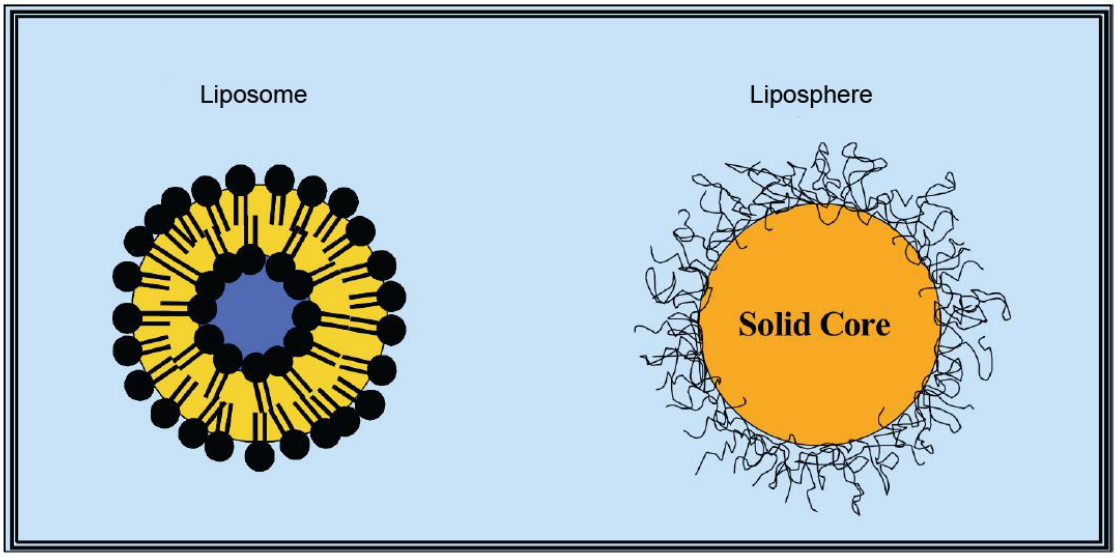

Figure 3: Schematic illustration of liposomes and solid hydrophobic submicron spheres, Nanosome .

The shelf life stability of liposomes in the product base depends on the interaction of the liposomes with the other base ingredients and the ability of the liposome lipid bi- or multi- layer structure to maintain its physical integrity in the product base. Formulating with liposomes can be quite challenging and procedures as well as raw materials must be considered carefully to avoid adverse effects on liposome stability. In general, liposomes should be added to a formulation below 40°C using low shear mixing. The addition of liposomes should also be the last step in the formulation's manufacturing process. Ethyl alcohol concentration should be kept below 5%, solvents should be kept below 10% and high levels of salts (>0.5%) should be avoided. Surfactants in general should also be avoided, but low levels (up to 1%) of non-ionic high HLB surfactants are usually well tolerated. The recommended storage temperature of most liposome formulations is 25°C. Another drawback associated with the use of liposomes as controlled delivery systems is the low payload of active ingredients. This limits the application of these systems to delivery of actives that are effective at very low levels.

Submicron spheres have increased stability as compared to emulsion-based delivery systems, such as liposomes, and are more effectively dispersed than most suspension based systems. The enhanced stability of submicron spheres can be utilized to enhance stability of sensitive active ingredients and prolong product shelf life. Further, the substance to be delivered does not have to be soluble in the vehicle since it can be dispersed in the solid matrix. Submicron spheres also have a lower risk of reaction of substance to be delivered with the vehicle than in emulsion systems because the vehicle is a solid inert material.

The solid hydrophobic inner core of the submicron spheres can be manipulated to sustain the diffusion rate of active ingredients and sensory markers through the spheres and enable the release of these actives over an extended period of time, or during heat treatment such as blow-drying the hair. Submicron spheres are also easier to prepare than structured vehicles such as liposomes, and are inherently more stable.

Submicron spheres provide the following benefits over liposomes:

- Enhanced shelf life stability in wide range of formulations and products
- Protect sensitive actives and reduce interaction with base ingredients
- Easy to process – Less sensitive to temperature and shearing rate
- Encapsulation of a wide range of actives in various physical form
- Solid inert materials, non-toxic
- Reduced irritation
- Sustain release of actives over an extended period of time
- Heat triggered controlled release

The performance of Nanosomes and its ability to effectively deliver functional ingredients and sensory markers, such as fragrances, onto hair from a wash-off application, such as shampoo, was determined by analytical method. Human hair tresses were treated with shampoo samples containing 1% free fragrance or 1% encapsulated fragrance and then evaluated by analytical methods (extraction of the hair followed by gas chromatograph (GC) mass spectroscopy (MS) analysis or by measuring the level of fragrance in the headspace of the samples followed by GC-MS analysis) as well as olfactory evaluation to determine fragrance intensity.

The amount of fragrance in the headspace of the tresses treated with a shampoo sample containing 1% encapsulated fragrance in Nanosomes after 24 hours was 10 times higher than that in the headspace of the sample treated with the shampoo containing the free fragrance (Figure 4)

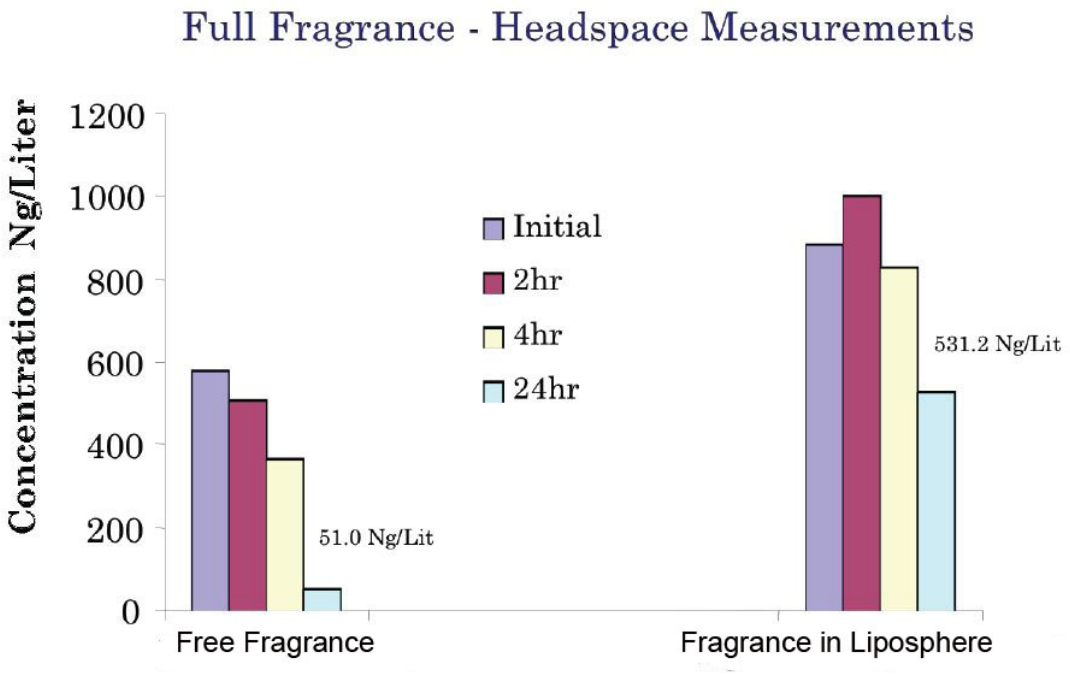

Figure 4: Performance of Nanosome from shampoo application. Headspace GC/MS data shows that hair treated with the fragrance encapsulated in Nanosomes releases 10 times more fragrance than the hair treated with the control sample comprising the free fragrance, after 24 hours.

The initial concentration of fragrance in the headspace of the hair tress treated with the shampoo sample comprising the fragrance encapsulated in Nanosomes was also higher than that of the control sample, indicating that Nanosome enhances the deposition of the fragrance on hair in addition to extending its release over a prolonged period of time.

The performance of Nanosome T and its ability to effectively deliver fragrances, onto hair from a wash-off application, such as hair conditioner, was also determined by analytical method. Human hair tresses were treated with conditioner samples containing 1% free fragrance or 1% encapsulated fragrance and then evaluated by analytical methods (extraction of the hair followed by gas chromatograph (GC) mass spectroscopy (MS) analysis or by measuring the level of fragrance in the headspace of the samples followed by GC-MS analysis) as well as olfactory evaluation to determine fragrance intensity.

The amount of fragrance in the headspace of the tresses treated with a conditioner sample containing 1% encapsulated fragrance in Nanosomes after 24 hours was more than 2 times higher than that in the headspace of the sample treated with the shampoo containing the free fragrance (Figure 5). The initial concentration of fragrance in the headspace of the hair tress treated with the conditioner sample comprising the fragrance encapsulated in Nanosomes was also 4 times higher than that of the control sample, indicating that Nanosome enhances the deposition of the fragrance on hair in addition to extending its release over a prolonged period of time.

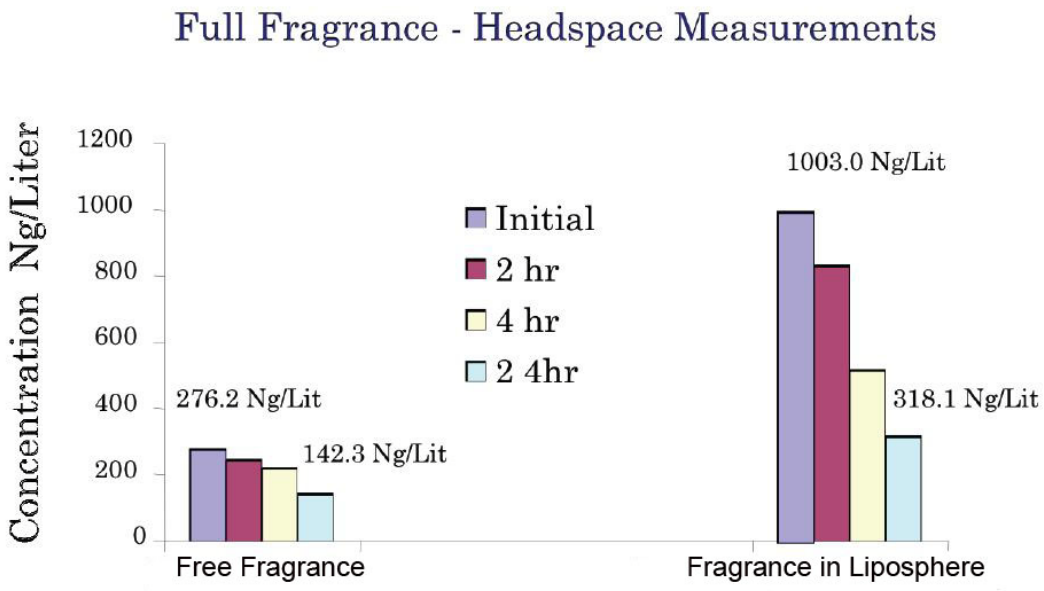

Figure 5: Performance of Nanosome from conditioner application. Headspace GC/MS data shows that hair treated with the fragrance encapsulated in Nanosome releases 4 times more fragrance than the hair treated with the control sample comprising the free fragrance.

A model fragrance ingredient, lilial, was also used to evaluate the performance on Nanosomes. Human hair swatches were treated with a conditioner comprising 1% encapsulated lilial in Nanosome and a control sample comprising 1% neat lilial. The

hair swatches were extracted and analyzed by gas chromatograph (GC) mass spectroscopy (MS) analysis or by measuring the level of fragrance that has been deposited on the hair.

The amount of lilial extracted from wet human hair swatches treated with the encapsulated ingredient was 10 times higher than that on the hair swatches treated with the control sample (Figure 6). Nanosomes also sustain prolonged the release of the fragrance over an extended period of time and can be utilized to provide fragrance "burst" upon blow-drying the hair.

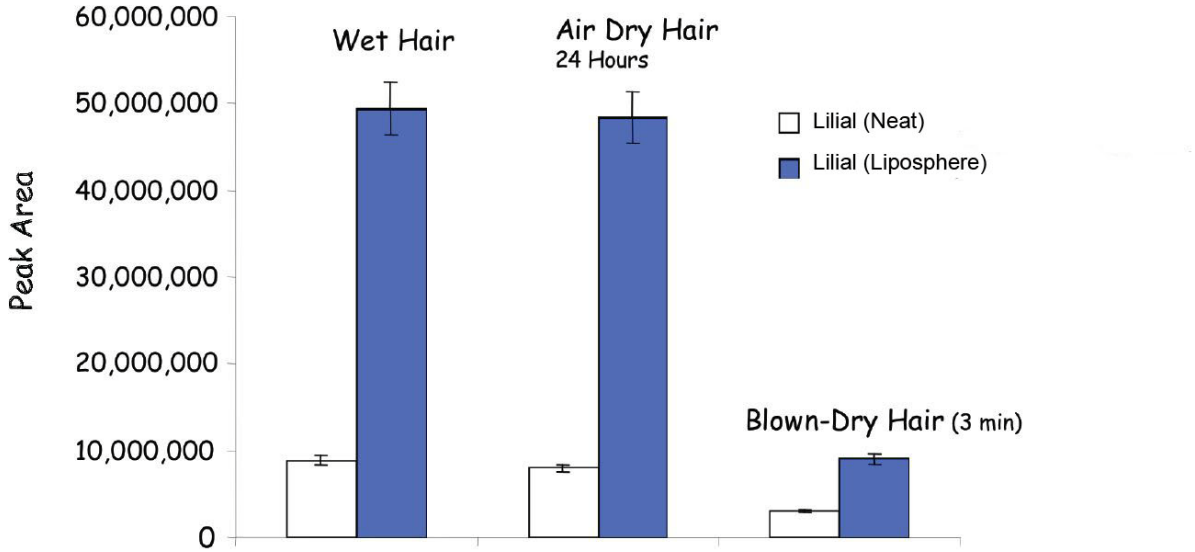

Figure 6: Performance of Nanosome from conditioner application. Extraction followed by GC/MS analysis shows that hair treated with the fragrance encapsulated in Nanosome deposits 10 times more lilial than the hair treated with the control sample comprising the free lilial.

To summarize, Nanosome has the following benefits:

- Enhanced stability of ingredients
- Prolonged product shelf life
- Enhanced bioavailability and efficacy of ingredients
- Product benefits last longer due to extended release of ingredients
- Precise local targeting of ingredients from wash-off products
- Heat triggered release upon blow-drying the hair
- Bioadhesive
- Reduced reapplication occurrences and improved treatment effect
- Competitive edge, point of differentiation

What is Nanosome Minoxidil?

A topical application of Minoxidil has been clinically proven to re-grow and stop thinning hair. A number of *in vitro* studies on skin and hair follicle cells show that Minoxidil stimulates cell proliferation, inhibits collagen synthesis, and stimulates vascular endothelial growth factor and prostaglandin synthesis.

Since Minoxidil is not water-soluble, it is formulated in products by dissolving it in propylene glycol or alcohol. As a result, the most common side effects of these products include skin irritation and itchy scalp. Nanosome Minoxidil is a controlled delivery system that contains Minoxidil in the form of a water dispersion. This system can be incorporated into a water-based product and is therefore, less irritating to the scalp as it releases the Minoxidil over a prolonged period of time. The Nanosome technology consists of solid hydrophobic sub micron spheres comprising cationic charge groups on their surface that further enhance the deposition of the Minoxidil onto the scalp from wash off applications, such as shampoos and conditioners.

- Reduced skin irritation - Water dispersion that is gentle to the skin
- Extended release of Minoxidil for a prolonged period of time
- Enhanced deposition from wash off applications
- Enhanced penetration into the scalp
- Easy to formulate into water based formulations
- Small particle size – Ideal for spray applications

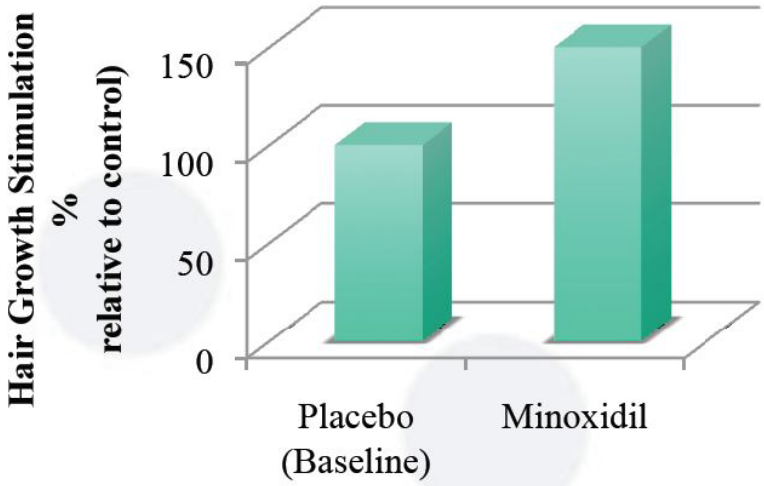

Figure 1: Efficacy of Minoxidil.

Clinical studies have shown (Figure 1) that Minoxidil stimulates hair growth in men on the back of the head. In women, Minoxidil can increase hair growth in the forehead areas. Minoxidil is in a class of drugs called hair growth stimulants.

If hair growth is going to occur with the use of Minoxidil, it usually occurs after the medicine has been used for several months and lasts only as long as the medicine continues to be used. Hair loss will begin again within a few months after Minoxidil treatment is stopped. In the U.S., this medicine is available without a prescription. In Canada, this medicine is available only with a doctor's prescription.

Nanosome Minoxidil has an average particle size of about 1 micron and can therefore penetrate into the hair follicles, that are about 3 microns, to effectively deliver the active onto the target site where it is needed to enhance hair growth (Figure 2). The hydrophobic matrix of the spheres retain the Minoxidil, thereby enabling the release of the active over an extended period of time.

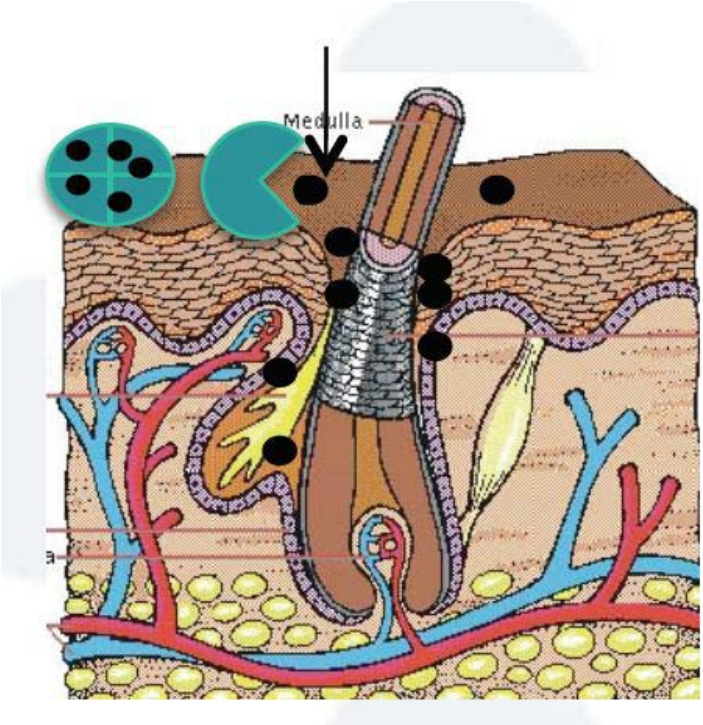

Figure 2: Schematic illustration of the skin.

The sub-micron spheres comprising the Minoxidil have a high cationic charge on their surface. In leave-on and wash-off applications the spheres become associated with the proteinaceous portion of the skin or hair, and retain the Minoxidil there, thereby improving the deposition of Minoxidil onto the scalp and preventing it from being washed off during rinsing.

Nanosome Minoxidil Penetrates Deeper Into the Scalp

The ability of Nanosome Minoxidil to penetrate the scalp was studied by comparing a lotion comprising 6% of free Minoxidil to one containing Nanosome Minoxidil. and an encapsulated Minoxidil, The method used to determine Minoxidil penetration was a skin extraction technique for HPLC analysis (Figure 3). Using an extraction apparatus (circular bulb 15.5 cm_2 in area), we marked the application area on the forearm (inner arm) with a ballpoint pen. This step was repeated on the other arm, designating one arm for the control sample and the other for the test sample. A measured amount of the product, about 0.250 grams, was applied on the target area using gloves to prevent cross-contamination. The samples were left on the target site for 1 minute. The area was rinsed with 100 ml water and tapped dry with paper

towel. A 3 mL disposable syringe was filled with 1.5 mL of ethanol and the ethanol was placed into the skin extraction apparatus and carefully inverted onto the application area, tightly holding the bulb in place and swivel for 30 seconds. The ethanol fraction was collected into a labeled glass jar. The extraction step was repeated for a total of three times per marked area on the arm. The three ethanol fractions from the same arm area were combined into one jar for deposition studies and release kinetics, or each ethanol fraction could be run separately for penetration studies. The three ethanol fractions were analyzed by HPLC (Agilent 1100 Series HPLC with quaternary pump, degasser, DAD detector, and auto-sampler).

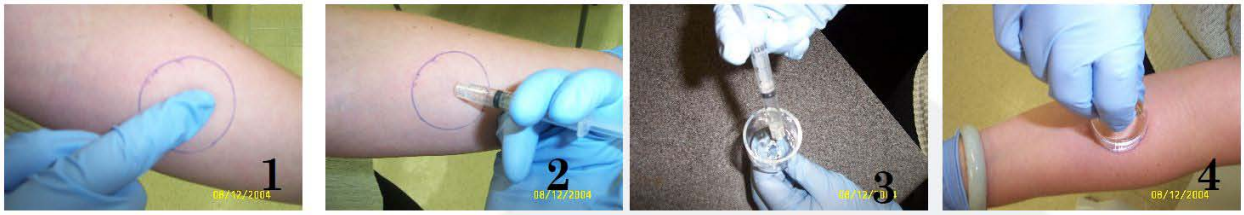

Figure 3: Skin extraction technique

Extraction studies show that Nanosome Minoxidil penetrates deeper the skin layers compared to the control sample (free Minoxidil). For Nanosome Minoxidil, more Minoxidil was extracted in the fourth extraction (deeper layer) than in the first extraction from the top layer of the skin. The opposite was observed for the free Minoxidil.

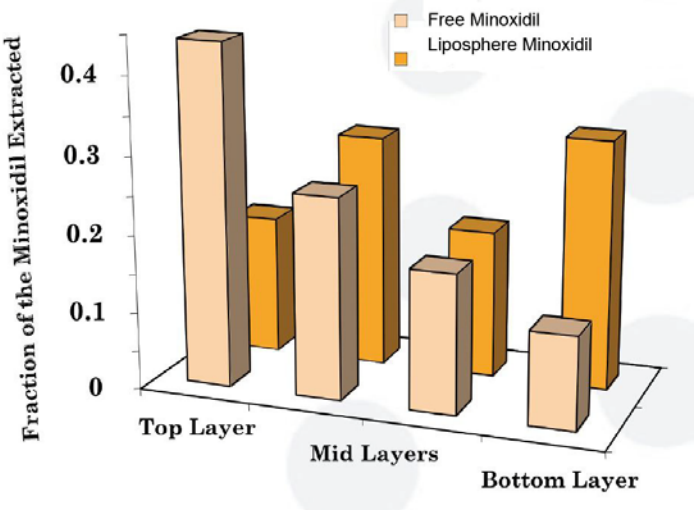

Figure 4: Penetration of free and encapsulated Minoxidil into the skin from a lotion application

Figure 5 shows that leave on products formulated with Nanosome Minoxidil retains more Minoxidil on the scalp and for longer period, compared to products formulated with free Minoxidil.

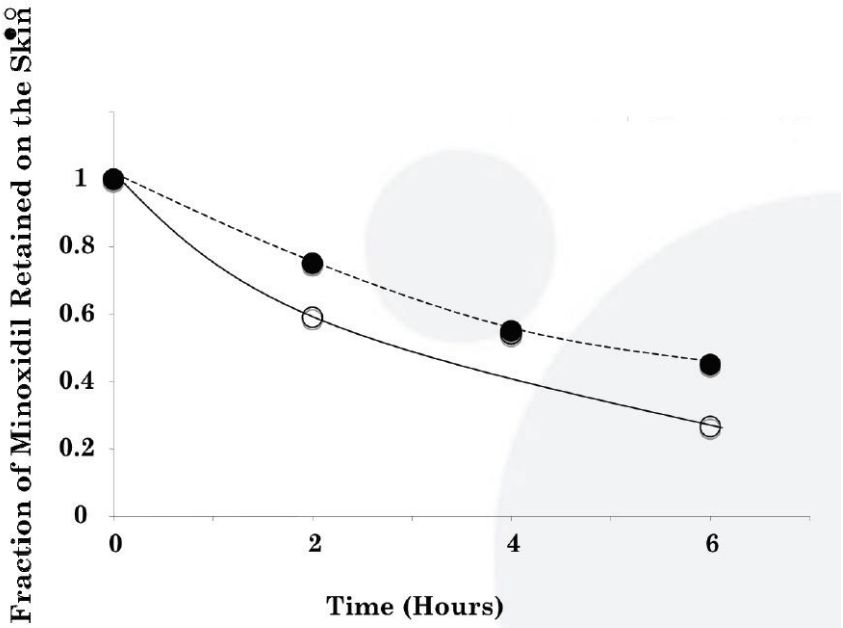

Figure 5: Release kinetics of free and encapsulated Minoxidil