

HEALTH & BEAUTY 2008



Special Feature:

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GPCG 2 Isolator

Customer's desire for new products: cosmetic actives, skin delivery systems and therapeutic cosmetics

by Dr. Andrea Pahmeier

Cosmeceuticals, these "hybrids" or "marriages" between cosmetics and pharmaceuticals are the evolving group of new products for the coming decades, the fastest-growing segment of the natural personal care industry. Consumers are always interested in maintaining a youthful appearance, and as the global population's median age increases, this market is increasingly expanding¹. Addressing the rapidly expanding health and wellness awareness worldwide cosmeceuticals claim for function, for example to fight skin aging, itching and skin disorders. They arm customers with an easy to use tool to achieve healthy skin while neutraceuticals and functional foods support simultaneously from within. Furthermore, today's cosmeceuticals appear as first among equals in natural cosmetics, an area that led a niche life for many years associated with an image of "green freedom, selfmade, healthy but uncomfortable, eco-warrior".

Customer's desire

What initiated the difference for customers to favour natural products, to drive a niche market to a mega trend, what drives their desire for wellness?

According to Leo Nefiodow, Economist and one of the best known experts of the theory of the long waves and distinguished German expert on the information society, the sixth Kondratieff, the period of integral health, has already begun. The market economy knows regular fluctuations between upswing and downturn, boom and bust. In general more or less everybody is familiar with short- and medium-term fluctuations of the economy, lasting between 3-11 years. But the market economy also exhibits long-term fluctuations, with a period of 40-60 years. They are known as Kondratieff cycles. These long waves are triggered by landmark inventions, referred to as basic innovations. The first long-term cycle was carried by the invention of the steam engine and the boom of the textile industry. The second Kondratieff was the age of steel and public transport followed by the third, the period of electrical engineering and chemicals industries. The fourth Kondratieff was fuelled by petrochemicals, automobiles and individual mobility leading to mass

traffic and the culmination of the industrial society. Beginning in the 1950s, the world economy has been in the fifth Kondratieff cycle driven by the development and exploitation of information technology.

A detailed analysis by Nefiodow shows the health sector to be the bearer of the next long-term cycle. Its basic innovations will be psychosocial health and modern biotechnology². Nefiodow further explains that new concepts, strategies and approaches are needed that are designed not to repair disease but



This example shows an advertisement for a professional body peel off mask with unusual taste functioning as superior anti-cellulite agent. It is no longer a coffee, it is a body shaping cosmeceutical.

http://www.beauty-face-celle.de/wellness_gesicht.htm

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Another example for functional cosmetics: Cool Detox Face Mask helps purify, soothe and recharge chronically stressed skin, infusing it with a concentrated dose of nurturing and fortifying vitamins, minerals and stabilizing elements. Exhausted, damaged and depleted skin emerges soft, clear, fresh and glowing.

instead to produce and maintain health and well-being, and that take seriously the concept of humans as whole beings, the concepts of integral health – the new mega-market of the 21st Century.

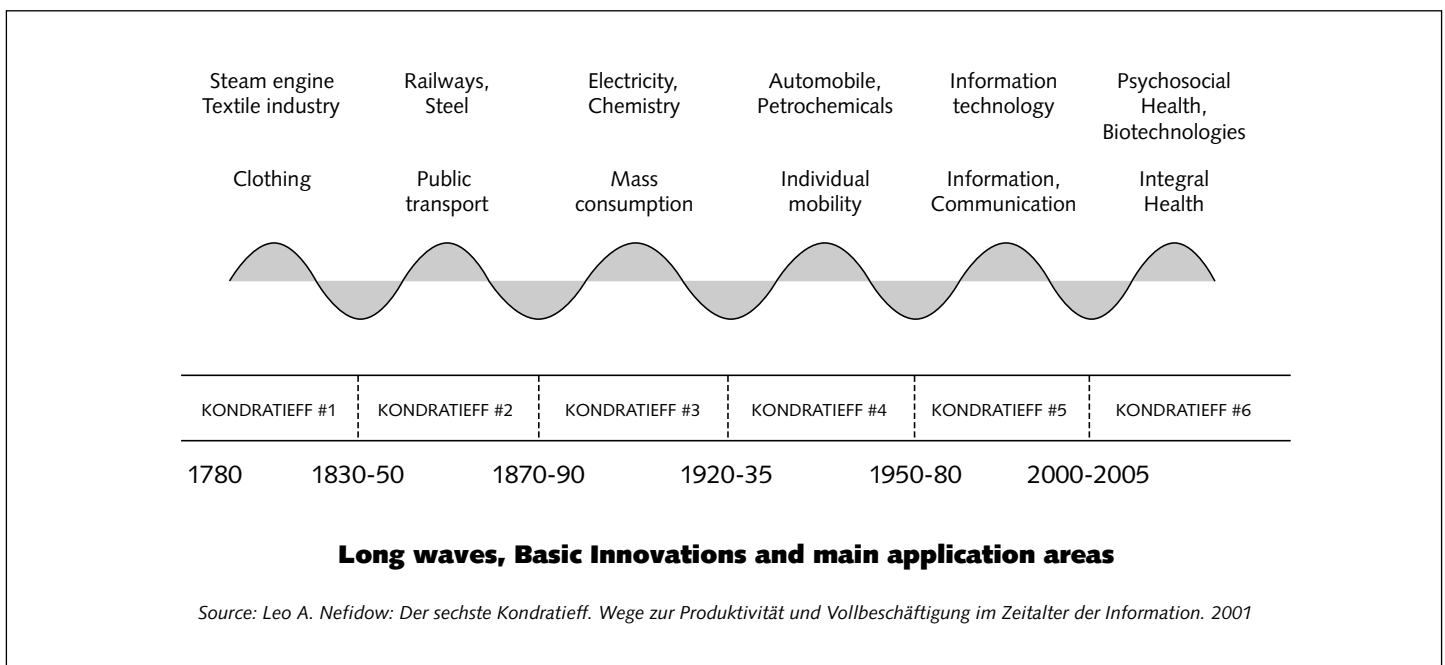
Along with Nefidow goes a scientific study conducted by Professor M. Hautzinger and Dr. P. Pössel at the Psychological Institute of Eberhard Karls University in Tübingen on the influences of cosmetics on health effects, which they had started in

2001³. They examined 60 female test persons who were shown before and after pictures of unmade-up and made-up women aged between 27 and 46. During stimulus presentation the reactions of the autonomous nervous system (electrodermal activity, heart rate change) and the neuroendocrine-immune system (cortisol, secretory immunoglobulin A – sIgA – in saliva) were monitored.

The sight of the pictures of made-up women triggered a more intense feeling of well-being amongst the test persons than those of unmade-up women. This manifested both in the subjective assessment of emotional state of mind (valence) as well as in the comparison of heart rate changes whilst looking at the pictures⁴.

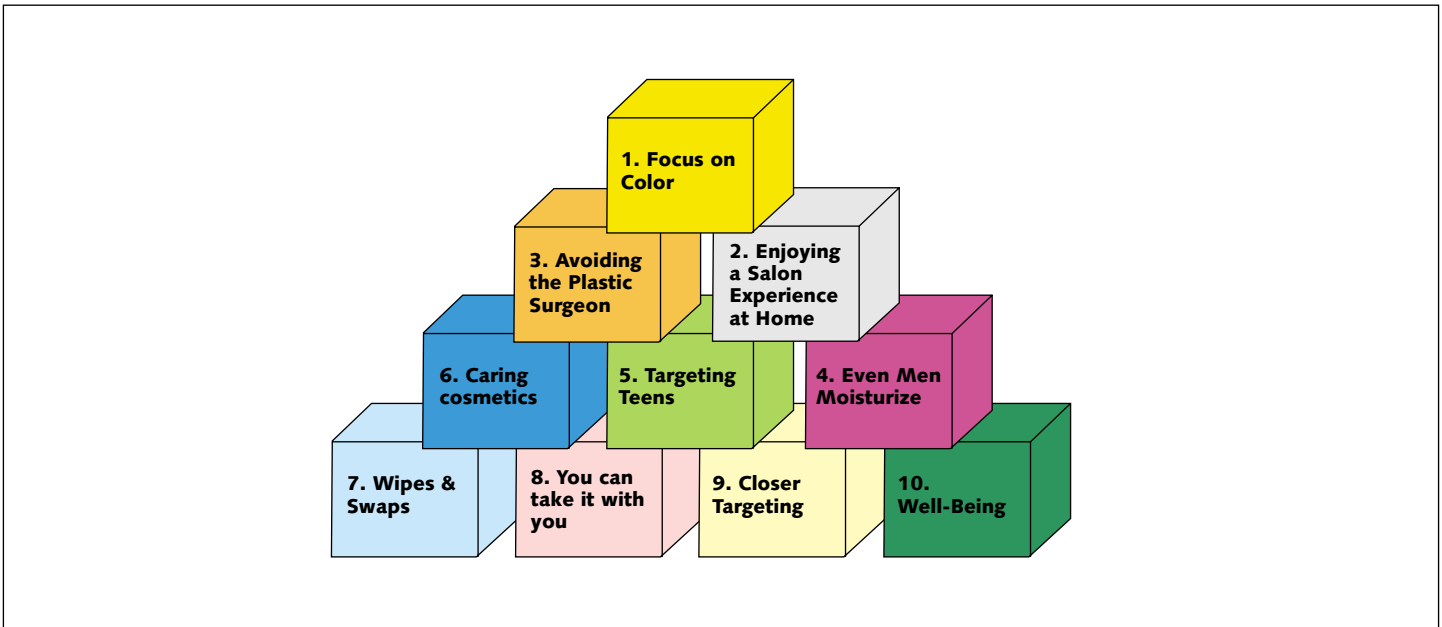
A poll report by Yankelovich Partners, Inc., for Work Your Image⁵ finds that nearly seven in ten Americans (69 percent) and more than eight in ten women say clothing, hair and makeup are very or extremely important for a woman on the job, and for her confidence. Large majorities say that a woman's appearance affects whether she is taken seriously, asked to represent her company at outside meetings, and considered for raises and promotions.

In 2005 Mintel, a leading supplier of competitive media, product and consumer intelligence released a list of the Top 10 beauty trends to watch over the next five years finding the "focus on colour" first place followed by "avoiding the plastic surgeon" on third and "well being" on place ten while "caring cosmetics" reached place six. With its topic "focus on colour" Mintel predicts an increase in cosmetic products that allow consumers to more easily customize results, along with more products that make it easier to make a choice in-store. There is expected growth of the spa-at-home concept (Enjoying a Salon Experience at Home), complete with head to toe treatments designed to nourish the body and mind, exfoliate and pamper. "Avoiding the Plastic Surgeon" counts on defying age through



The market economy also shows long-term fluctuations, with a period of 40-60 years. They are known as Kondratieff cycles. These long waves are triggered by landmark inventions, referred to as basic innovations.

Five Kondratieff cycles have come and gone since the late 18th century. The first long-term cycle was sparked by the invention of the steam engine and its use, especially in the textiles industry. The second Kondratieff was the great age of steel. The third was the product of the electrical engineering and chemicals industries. It was the first long-term cycle to profit from the practical application of scientific insights. The basic innovations that triggered the fourth Kondratieff were petrochemicals and automotive. This brought mass traffic onto roads and into the air and also marked the culmination of the industrial society. Since the 1950s, the world economy has been in the fifth Kondratieff cycle, which is driven by the development and exploitation of information technology.



Top 10 Beauty Trends by Mintel, January 2005

cosmetics while firming the skin, decreasing wrinkles and enhancing youthful characteristics, all without invasive or expensive surgery. Addressing men (men moisturize) and teens marketeers are obliged to focus on type specific packaging like masculine packaging and create new brands especially for the youth market or, as with "closer targeted" focus on diabetics, women's issues and smokers as more and more niche products hit the shelves. Most of these cosmetics to come will be cosmeceuticals, the long thought after combination of nature and science.

Cosmeceuticals

But what are cosmeceuticals? Is it a drug or a cosmetic or ..? Synonyms are quasi-drugs, therapeutic cosmetics, cosmetic drugs, active skin treatment, dermaceuticals to name a few. Like cosmetics, they are topically applied, but other than cosmetics they contain ingredients that influence the biological function of

the skin, improve appearance by delivering nutrients necessary for healthy skin.

To make a long story short: according to regulations (Food, Drug & Cosmetic Act) the Food and Drug Administration (FDA) does not recognize any such category as "cosmeceuticals" A product can be a drug, a cosmetic, or a combination of both, but the term "cosmeceutical" has no meaning under the law⁶.

The legal difference between a cosmetic and a drug is determined by a product's intended use. Different laws and regulations apply to each type of product.

Cosmetics are defined by their intended use as "articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body...for cleansing, beautifying, promoting attractiveness, or altering the appearance"⁷ Among the products included in this definition are skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, shampoos, permanent waves,

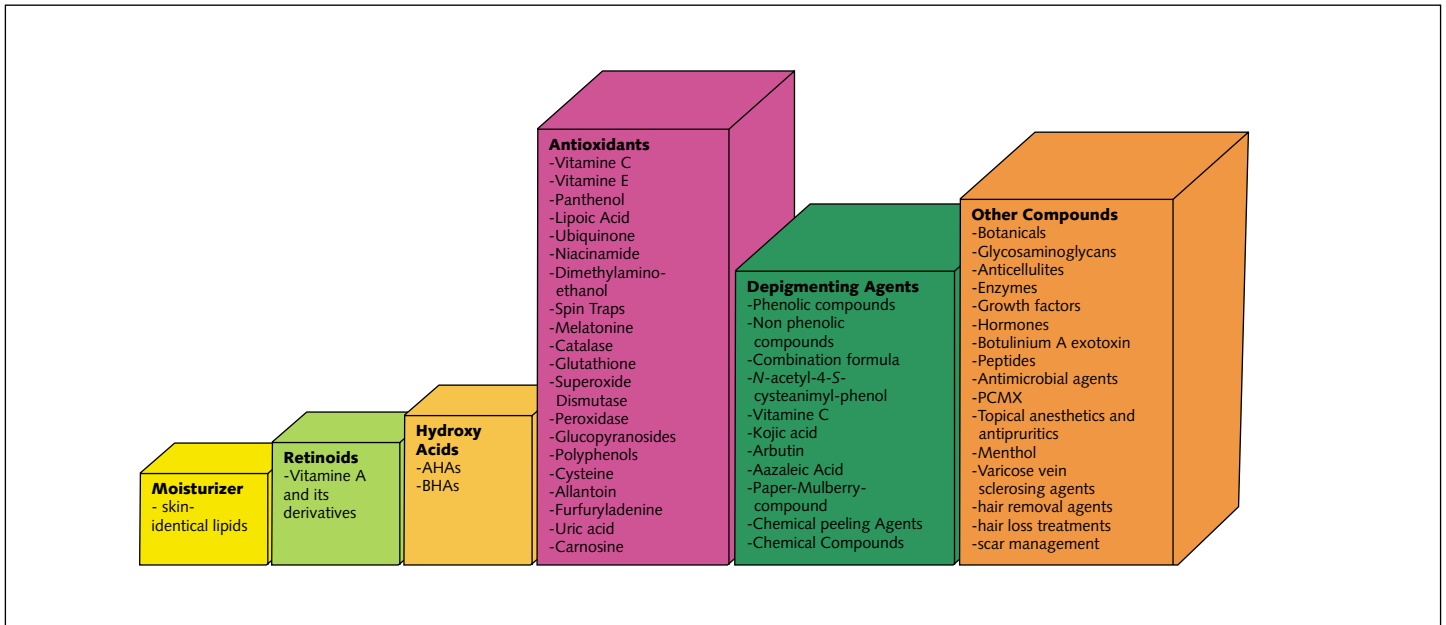


Green Ginseng Soap



Green and White Ginseng Soap and Ginseng Body Oil.

Soap and body oil with ginseng exhibited on the International Green Week, Berlin 2007



Active Ingredients

hair colors, toothpastes, and deodorants, as well as any material intended for use as a component of a cosmetic product.

The FD&C Act defines drugs by their intended use, as "(A) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease..and (B) articles (other than food) intended to affect the structure or any function of the body of man or other animals"⁸.

Some products meet the definitions of both cosmetics and drugs. For example, a shampoo is a cosmetic because its intended use is to cleanse the hair. An antidandruff treatment is a drug because its intended use is to treat dandruff. Consequently, an antidandruff shampoo is both a cosmetic and a drug. Among other cosmetic/drug combinations are toothpastes that contain fluoride, deodorants that are also antiperspirants, and moisturizers and make-up marketed with sun-protection claims. Such products must comply with the requirements for both cosmetics and drugs⁹.



Degussa Innovation Award 2006: With the help of TEGOSPHERE®, sensitive components in cosmetics can be effectively protected and brought on to the skin in optimal fashion, http://www.degussa.com/degussa/en/innovations/r_d_awards/innovation_award/sivara_process/

Active ingredients

Other than regulatory concerns customers, patients and doctors view cosmeceuticals as a significant increase in armament in improving the treatment of skin conditions. However, claims of effectiveness lack convincing evidence, thus the industry is challenged to provide convincing evidence of the effectiveness of these compounds.

Such active ingredients for cosmeceuticals are moisturizer, retinoids, hydroxy acids, antioxidants, depigmenting agents and other compounds like enzymes, hormones, DNA, peptides or glycosaminoglycans to name a few.

Retinoids are possibly the most prevalent cosmeceuticals in the market. Retinoids are vitamin A derivatives present in all living organisms either as preformed vitamin A or as carotenoids. Vitamin A (retinol) is the prototype of all other retinoids and is necessary for proper growth, bone development, and integrity of mucosal and epithelial surfaces.

Vitamin A and its derivatives have 2 main functions: they act as antioxidants, and they activate specific genes and proteins and furthermore induce epidermal thickening, increase mitoses, differentiate keratinocytes, and reduce the number of sebocytes. The dermis shows increased amounts of glycosaminoglycans (GAGs) and anchoring fibrils. Structural changes underlying the cosmetic benefits include correction of epidermal atrophy, deposition of new collagen, generation of new vessels, and enhancement of mitogenesis.

Hydroxy acids are likely the second most available cosmeceutical, and in low concentrations, they are found in mass-marketed cosmetic formulation and have been shown to decrease the signs of aging.

Looking at antioxidants one finds a rich variety of compounds including Vitamin C and E as well as panthenol, the alcohol analog of Vitamin B-5, lipoic acid, ubiquinone, niacinamide, melatonin, uric acid or allantoin. Antioxidants counteract the harmful effects as skin is frequently exposed to a constant assault of endogenous and exogenous damaging agents and intervene at different levels in the protective process.

Viewing active ingredients one has to mention botanicals, now part of every product in the market from cosmetics to soft drinks. Avocado, banana, lemon, and other similar botanicals are listed on thousands of labels. They exert their purported

effects through the mechanisms of antioxidants, hydroxy acids, and other unclear properties. Examples of botanicals include chamomile, which inhibits the release of histamine and has anti-inflammatory properties, and ginseng, which stimulates the biosynthesis of proteins, RNA, and lipids. Ginkgo biloba extract was found to locally induce SOD and to catalase enzyme activity in the epidermis after topical application as well as to systemically increase the activity of both enzymes in the liver, the heart, and kidneys. Curcumin found in curry has anti-inflammatory activity by inhibiting leukotriene formation, inhibiting platelet aggregation, and stabilizing neutrophilic lysosomal membranes. Glycyrrhizin found in licorice roots inhibits proinflammatory activities of prostaglandins and leukotrienes. Capsaicin inhibits substance P, a peptide transmitter of the inflammatory process. Aloe vera has been shown to accelerate wound healing and to protect and soothe the skin¹⁰.

The upcoming proximity of medicine and cosmetics is clearly expressed in a news ticker from the *Ärztezeitung* of November 2006¹¹, a daily news for medical doctors, saying that Estée Lauder, the US cosmetic company is planning to set up a hospital complex at Tempelhof Airport in Berlin.

Skin delivery systems

Finding active ingredients to be the core of novel cosmetics the question arises on how to transport actives into deeper skin layers and what functions are to expect. Screening potential delivery systems one sees him or herself faced with an armada of possibilities ranging from encapsulation with microcapsules to adhesive patch delivery via silicones, liposomes, foams, emulsions, structured systems based on intelligent polymers or sugar or...specific ingredient delivery and starch based systems¹². The question to answer is which of these systems should be applied and whether or not active ingredients surpass the skin surface for effectiveness.

The following example attempts to depict the effects of delivery systems on human skin. An *ex vivo* study in viable human skin for the effects of vitamin E and its bioconversion was performed by Baschong et al.¹³. They tested for various delivery and application systems for comparable amounts of vitamin E acetate (i) in oil (Mygliol-812N), (ii) surfactantsolubilized in water, (iii) encapsulated in liposomes, or (iv) encapsulated in Nanotopes™. Nanotopes are a ultra-small unilamellar carrier system for cosmetic actives. Findings were that vitamin E acetate in Mygliol deposited exclusively on the surface and in the stratum corneum. In contrast, solubilized or encapsulated vitamin E acetate deposited also in the underlying skin. Nanotopes performed best, followed by liposomes and solubilized vitamin E acetate. Non-occlusive application favored deposition in the skin relative to occlusive application. Conversion of vitamin E acetate to vitamin E was not observed on the skin surface or in the horny layers, while in the underlying skin up to 50% of the vitamin E total was deacetylated.

Another example from Degussa¹⁴ shows the importance of encapsulation of a cosmetic active ingredient in microscopically small particles based on a polymer to protect sensitive active ingredients in cosmetic preparations against atmospheric oxygen and UV radiation, which decompose them so they cannot be easily and selectively released on the skin.

When these microparticles come into contact with the skin, they become permeable, and the active ingredient is released onto the skin, where it produces the desired effect. The amino groups of the polymer are responsible for this release mechanism. Due to the natural acid mantle of the skin, whose pH is

about five, these groups become protonated. As a result, the polymer dissolves partially, the permeability of the polymer shell increases and the active ingredient is released. The first cosmetic ingredient on which the patented release system was tested was retinol (vitamin A).

These two examples may highlight the importance of novel delivery systems designed to facilitate the use of the eternal "fountain of youth" and other functional actives whose time has come. Delivery systems are the central idea uniting science and marketing in a rapidly growing global market eager for products that really work.

1 Cosmeceuticals, Schwartz, R.A., Centurion, S.A., Solis, C.S., 05.09.2006 http://www.emedicine.com/derm/topic509.htm#section%7Eauthor_information

2 Leo A. Nefiodow: *Der sechste Kondratieff. Wege zur Produktivität und Vollbeschäftigung im Zeitalter der Information.* (The Sixth Kondratieff: Paths to Productivity and Full Employment in the Information Age; Rhein-Sieg Verlag; Sankt Augustin; Fifth Edition 2001

3 P. Pössel, S. Ahrens and M. Hautzinger, *International Journal of Cosmetic Science*, 2005, 27, 343–349.

4 http://www.ikw.org/pages/prodgr_details.php?info_id=242&headline=informationen

5 http://www.careerexposure.com/resources/resources_502.jsp

6 Is It a Cosmetic, a Drug, or Both? (or Is It Soap?) U. S. Food and Drug Administration Center for Food Safety and Applied Nutrition Office of Cosmetics and Colors, July 8, 2002

7 FD&C Act, sec. 201(i).

8 FD&C Act, sec. 201(g)(1)]

9 Is It a Cosmetic, a Drug, or Both? (or Is It Soap?) U. S. Food and Drug Administration Center for Food Safety and Applied Nutrition Office of Cosmetics and Colors, July 8, 2002

10 Cosmeceuticals, Schwartz, R.A., Centurion, S.A., Solis, C.S., 05.09.2006 http://www.emedicine.com/derm/topic509.htm#section%7Eauthor_information

11 Kosmetikkonzern plant Klinik in Berlin-Tempelhof, *Ärzte Zeitung*, 16.11.2006

BERLIN (dpa). Der US-Kosmetik-Konzern Estée Lauder will im Flughafen Berlin-Tempelhof eine Klinik einrichten. Berlins Regierender Bürgermeister Klaus Wowereit (SPD) habe bereits ernsthafte Gespräche mit dem US-Konzern geführt, berichtet das "Handelsblatt".

Der US-Konzern wolle den Airport auch als Landeplatz für die Flüge mit Patienten und Geschäftsleuten nutzen. Der Flughafen Tempelhof soll im Herbst 2007 offiziell geschlossen werden. Sowohl die US-Zentrale des Unternehmens als auch die deutsche Vertretung von Estée Lauder wollten dies nicht bestätigen.

12 Meyer R. Rosen, *Delivery System Handbook for Personal Care and Cosmetic Products: Technology, Applications and Formulations*, 2005, William Andrew Publishing

13 W. Baschong, C. Artmann, D. Hueglin, and J. Roeding, M. E. Mueller, *Vitamine E*, *Journal of Cosmetic Science* 52, 155-161 (May/June 2001)

14 First Skin-Activated pH-Dependent Microcapsule Delivery System for Cosmetics

http://www.degussa.com/degussa/en/innovations/r_d_awards/innovation_award/sivara_process/

Dr. Andrea Pahmeier obtained her PhD as a biologist at the Biochemical Department, Justus Liebig University Giessen. She is a cell culture specialist in tissue engineering, regenerative medicine and polymer application. 2004 she founded ticoché Cosmeceuticals and in 2007 her latest custom product design, a variety of cosmeceutical Ginseng care products, received the Brandenburg Agricultural Marketing Award.



Fundamentals and pharmaceutical applications of near-infrared spectroscopy

by I. Antal, Á. Z. Dávid

Abstract

In recent years, near-infrared spectroscopy has gained growing interest within the pharmaceutical industry for both quality control and process monitoring. Extending the with diffuse reflectance option, pharmaceutical applications of near-infrared spectroscopy offers the nondestructive instrumental analysis even for intact solid samples (for in-process and end-product control) and gives both chemical and physical information in development or production. Nowadays NIR is used in both off-line and on-line applications in different pharmaceutical industrial process control applications. This paper reviews the history and development of near infrared (NIR) spectroscopy mainly from a pharmaceutical point of view.

The near-infrared region

The electromagnetic (EM) spectrum (Figure 1.) covers the range from corpuscular gamma rays to radio radiation. Near infrared (NIR), as defined by the International Union of Pure and Applied Chemistry (IUPAC) [2] is the radiation range of the electromagnetic spectrum, extending from 780 – 2 500 nm (12 800 – 4 000 cm^{-1}). The absorption in the NIR region arises from overtones and combinations of the fundamental mid-infrared bands. Thus in a wider sense, NIR spectroscopy (NIRS) deals including wavelengths between 700 - 3000 nm, near the red of the visible spectrum and near the beginning of fundamental infrared stretches of organic compounds.

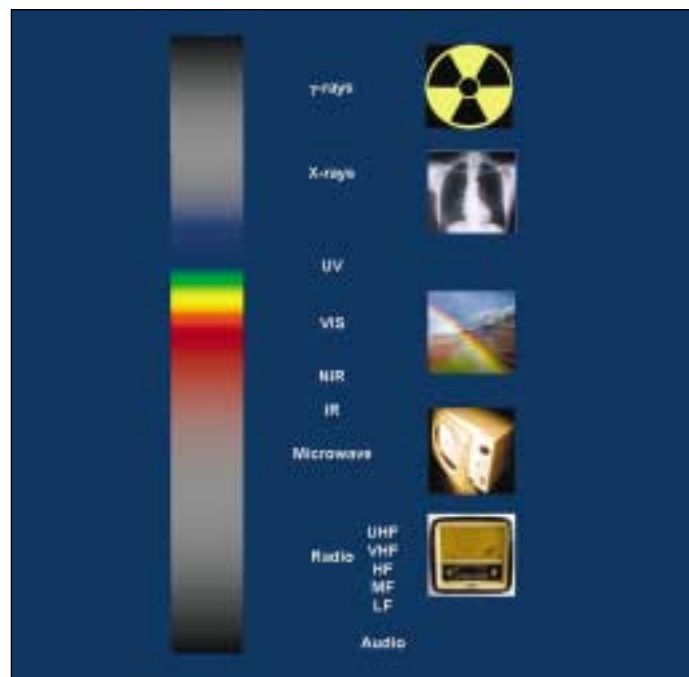


Figure 1: The electromagnetic spectrum

History of NIR spectroscopy

According to the famous experiment of *Isaac Newton* in 1665, the white light composes of all the visible colors in the electromagnetic spectrum, and the prism merely separates them. This breakthrough in optics excited the imagination of scientists to develop modern spectroscopy.

In 1880 *William Herschel* published his experiment on the heating effect of sunlight projecting a rainbow on to a bench by the aid of a prism (Figure 2.) [1]. The temperature increased as the thermometers were moved from violet to red and reached a maximum beyond the red end of the visible spectrum [2]. Herschel concluded that the sunlight contains more than just the colors, he referred the "invisible light" as "radiant heat" and the "thermometrical spectrum". Herschel's discovery was a milestone to the rest of the electromagnetic spectrum.

In 1821 *Thomas Johann Seebeck* discovered that when any conductor (such as a metal) is subjected to a thermal gradient, it will generate a voltage. This is now known as the thermoelectric effect, which opened up the possibility to compose thermocouple detectors. Eight years later, in 1829, *Niépce* and *Daguerre* invents the photographic plate, which turns out to be sensitive to NIR radiation. It was left to *Ampere*, in 1835, to demonstrate that NIR had the same optical characteristics as visible light and conclude that they were the same phenomenon, by using thermocouple detectors. Later on in the century *Abney* and *Festing* recorded the spectra of organic liquids in the 1 – 1,2 mm range in 1881 and recognized atomic grouping and the importance of hydrogen bonds in the NIR region

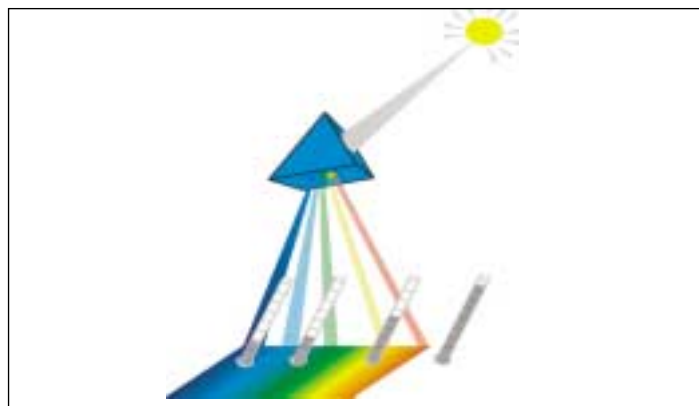


Figure 2: Heating effect of light discovered by Herschel

Inspired by their work, *W.W. Coblentz* constructed a spectrometer which was highly sensitive to both vibration and thermal disturbances. *Coblentz* had to leave the room in which the instrument was set up, after each step in the rotation of the prism. It took one whole day to record a single spectrum, but he ultimately recorded the spectra of several hundred compounds in the 1 – 15 μm wavelength region. Using his instrument he was the first to verify *Planck's Law*, and was also the first to show that different atomic and molecular groupings absorb characteristic wavelength "fingerprints" in the infrared region [3].

Following, in the first half of the twentieth century, the spectral database have been broadly extended by many contributors of NIR and IR spectroscopy. Though while infrared spectroscopy had moved away from being a scientific curiosity, it was used very little, since suitable spectrometers did not exist. Over half a century has passed, before infrared spectroscopy became a tool for routine chemical analysis, and almost two-third of a century was required for NIR measurements making their debut in everyday laboratory work.

Possibly the first quantitative NIR measurement was done by *F.E. Fowle* in 1912, by determining atmospheric moisture in the Mount Wilson observatory.

In the 1930s lead sulphide (PbS) was studied as a compound semiconductor for heat-sensing and the Second World War stimulated its development as an infrared detector. Twenty years later, in the 1950s, it turned out that it is very sensitive for the 1 – 2.5 μm wavelength region, and thus it became a commercially available NIR detector.

In 1931 *Kubelka* and *Munk* published their theory on diffuse light scattering which still serves as a background for measurements of solid materials [4].

Analytical use of NIRS have developed more slowly than applications in the visible and infrared range.

The lack of accurate calibration techniques and computational hardware and methods were a setback of NIR spectroscopy. Beside the construction of low cost NIR instruments with high signal-to-noise ratio, advances in mathematical and statistical analysis were necessary for industrial use. In the 1960s, *Karl Norris* from the U.S. Department of Agriculture did pioneer work in the field of NIR analysis and introduced it into practice for agricultural and food products [5]. Development of chemometrics and appropriate calibration models were a prerequisite for pharmaceutical applications from the 1980s [6,7].

Principles of NIR spectroscopy

NIR spectra are primarily the consequences of overtones and combinations of the many fundamental absorption bands of the mid and far infrared regions. The overtones are anharmonic, which makes NIR spectra complex and overlapping. Due to energy considerations, most of the overtones found in the NIR spectrum arise from the X–H stretching modes (O–H, C–H, S–H and N–H) [8]. Being quantum mechanically forbidden transitions, the overtones represent a 10 to 1000 times weaker band, than the fundamental mid-IR vibrational bands.

The absorption in the infrared region is a result of molecular vibrational and rotational states. The background of vibrational spectroscopy is the concept that atom-to-atom bonds within molecules vibrate with frequencies that may be described by the laws of physics and are, therefore, subject to calculation. When the material coming into contact with the given radiation, absorbs energy, it will be excited to a higher energy level, thus the difference in the energetic state of the material may be described by quantum mechanical calculations. Assuming, that the band energies arising within a molecule from the vibration of a diatomic harmonic oscillator, and obey Hooke's Law, the lowest or fundamental frequencies may be roughly calculated by the following equation:

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad (1)$$

where ν is the vibrational frequency, k is the classic force constant and μ is the reduced mass of the two atoms. This theory works well in case of the fundamental frequencies of diatomic molecules, and gives comparable results for calculation of the average value for two-atom stretch of polyatomic molecules. However in real molecules, the electron sucking-, or donating effect of nearby atoms can significantly influence the bond strength and length, thus the fundamental frequency of X-H bonds as well. By using quantum physical calculations the fundamental transition states of a molecule can be calculated:

$$E(\nu_1, \nu_2, \nu_3) = \sum_{i=1}^{3N-6} (\nu_i + 1/2) h\nu \quad (2)$$

$$(\nu_1, \nu_2, \nu_3, \dots = 0, 1, 2, \dots)$$

where E is the energy level of the different ($\nu_1, \nu_2, \nu_3, \dots$) vibrational states and $h\nu$ is the quantum term. Since fundamental transitions between the ground and excited energetic states are only allowed by selection rules, whenever the molecule reaches a vibrational state above the fundamental, it is called overtone. Transition to a state, where the fundamental quantum levels are 1 in both direction, gives combination bands. By calculation, overtones and combinations are not allowed states, but do appear as weak bands due to anharmonicity or Fermi resonance.

In practice, the so called ideal harmonic oscillator has limits. This can be best explained with the so called spring model, where atoms of a molecule are imagined as being attached to each other with springs. In this way the oscillation of the molecules can be among bonds: stretching, or it can be among bond angles: vibration. These different types of oscillations are graphically shown in Figure 4.

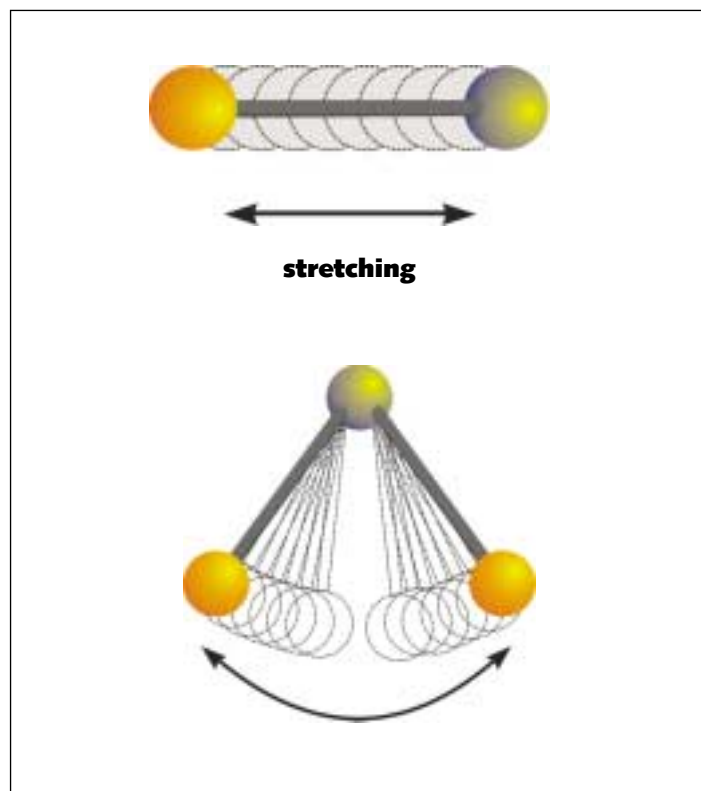


Figure 4: Stretching and vibration model of molecules.

However in an ideal case, as the atoms close in on each other, real compression forces are fighting against the spring, while on the other end, if stretching is too much, the spring loses its capacity to return to its coil form. This means that in real molecules the electron clouds between the two bonding atoms mean a resistance against the nuclei closing in too much, while on the other hand, if the energy with which the molecule will stretch reaches the amount of the dissociation energy, the bond will break up. To reach a state, where the bond breaks is always easier than to compress the atoms of the molecule. The barrier for decreasing distances among two neighboring nuclei

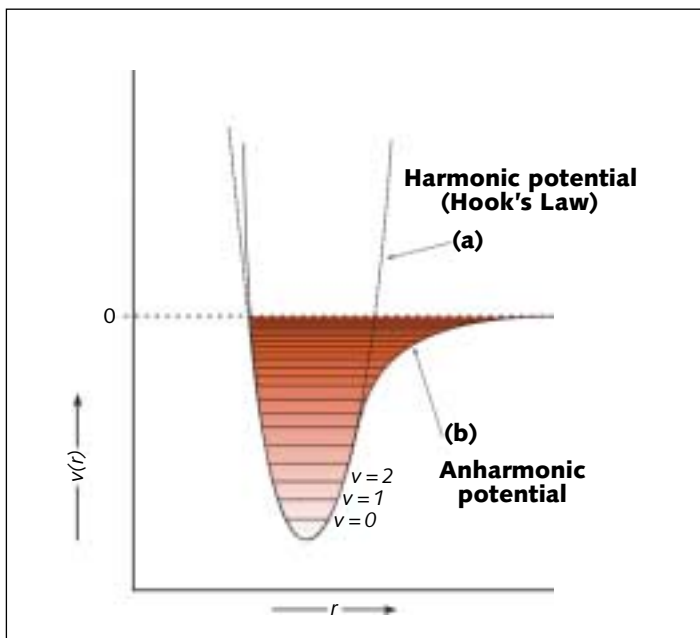


Figure 5: The energy diagram of molecule's vibrational model showing an (a) ideal diatomic or (b) anharmonic diatomic oscillator.

increases at a rapid rate, while at the other direction, stretching slowly approaches zero, which is shown in Figure 5. As it is apparent from the graphic representation, energy levels are not equal in an anharmonic oscillator. In practice, anharmonicity of the first overtone of a fundamental band occurring at 3500 nm would be at 3500/2 plus a small shift to longer wavelengths, placing the signal between 1785 to 1925 nm.

NIR instrumentation

The components of the instrument include a light source, monochromator, sample interface, and detector [9]. The parts of NIR instruments are basically similar to the UV/Visible spectrophotometers but they usually allow both transmittance and reflectance measuring modes depending on the positioning of the sample and the detector.

The light source in most cases is a tungsten halogen lamp, however spectrophotometers equipped with NIR emitting diodes are also available. The monochromator can either be some light filter, grating or prism.

The recordable wavelength range depends on the detector type, which usually includes silicon, lead sulfide (PbS) or indium gallium arsenide (InGaAs). Silicon detectors are fast with a low noise and sensitive between 200-1000 nm. The most widely used detector for NIR consists of the photoconductor PbS, which is slower, but it is useful between 1000-2500 nm with still satisfactory signal-to-noise ratio. InGaAs detectors are more expensive since they combine the speed and size characteristics of the silicon detector in the 800-1700 nm range. Additional extended wavelength options are available for them to achieve the wavelength range of the PbS detector.

The sample presentation interface may involve cuvettes or flow cells for liquids or specifically designed quartz sample holders for semi-solids and solids.

The appropriate NIR measuring mode depends on the optical properties of the sample. Transparent materials and turbid liquids or semi-solids are usually measured for transmittance (diffuse transmittance). For solid materials, a special sample interface is needed, where the diffuse reflection accessory is designed so the diffusely reflected energy is optimized and the regular reflected component is minimized. Therefore the diffuse reflectance option requires an integrating sphere, whose interior is coated with an almost non-absorbing material such as MgO or BaSO₄. Since light reflects back among a semi-sphere, only small portion of it crosses the detector window. Therefore the integrating sphere is needed to collect the scattered and diffusely reflected light, and will guide it towards the detector. In a typical single-beam instrument a monochromatic light beam irradiates the sample surface at 0° and the detector is mounted at 45° to the sample surface. The reference light intensity is obtained by replacing the sample with a reflectance standard.

By taking samples for the analysis, cuvettes, flow cells, and reflectance sample holders can be used for at-line or off-line measurements. However, it is possible to measure on-line or in-line with automatic sample transfer or in situ measurements.

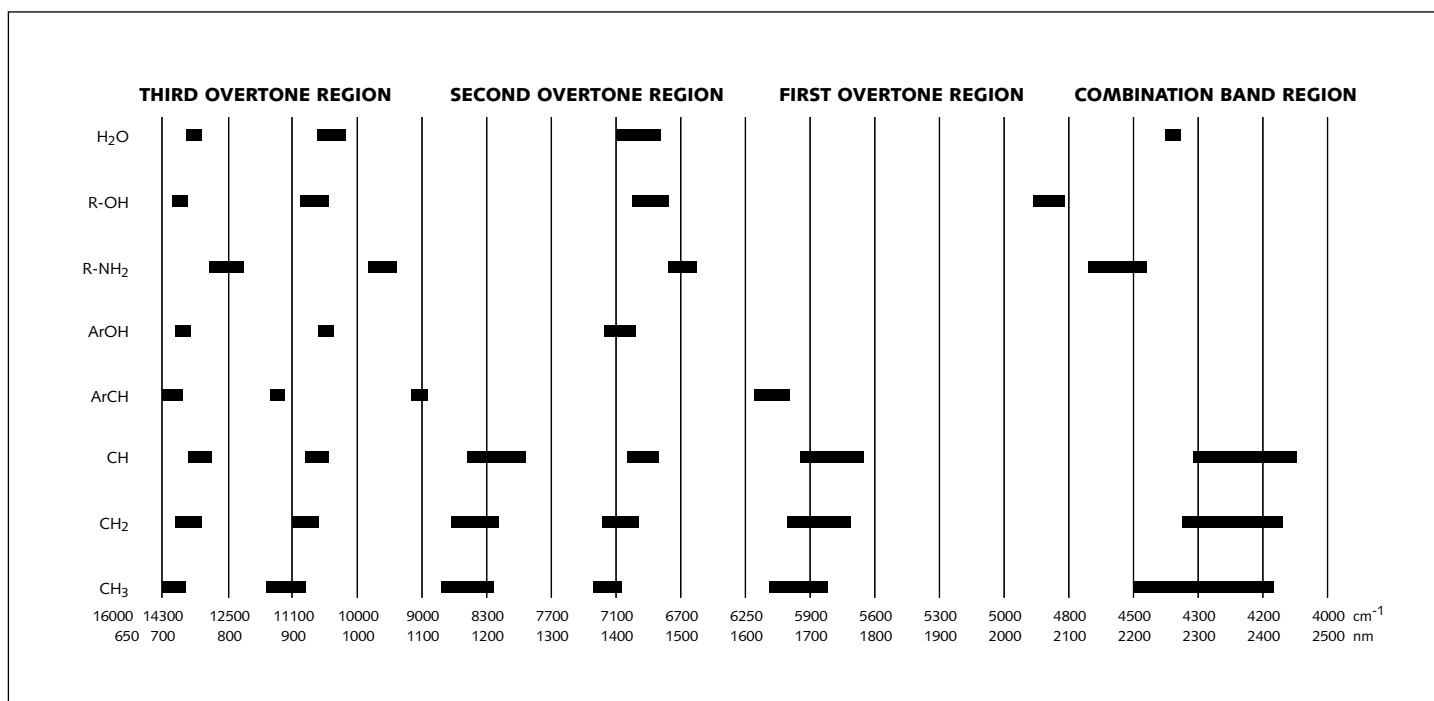


Figure 3: Characteristic NIR absorption bands

Powerful in situ measurements can be performed with a probe inserted into the sample (e.g. powder or even dissolution medium) and coupled to a spectrophotometer through fiber optics. The light is transferred from the instrument radiation source to the probe through an optical fiber and it carries back to the detector after reflection.

Fundamental considerations and diffuse reflectance

According to the origin of absorption, NIRS may serve for routine service in the qualitative and quantitative analyses of any organic compounds comprising C-H, N-H, S-H and/or O-H groups. Numerous other elementary bond combinations are also likely to generate NIR absorption peaks. Figure 3 demonstrates characteristic absorption bands related to the overtone and combination bands of the fundamental vibrations occurring in the mid-IR region. The majority of near-infrared spectra include methyl C-H stretching vibrations, methylene C-H stretching vibrations, aromatic C-H stretching vibrations, and O-H stretching vibrations. Minor but still important spectral features include methoxy C-H stretching, carbonyl associated C-H stretching; N-H from primary amides, secondary amides and secondary, and tertiary amines, as well as from amine salts. In addition, NIRS is very sensitive to measure water and moisture content due to O-H vibrations.

The apparent disadvantage of NIR measurement, namely the extremely weak absorption bands occurring in this region of the electromagnetic spectrum make this technique applicable in the direct measurement of solid samples. The nondestructive analysis with NIRS is based on the capability of reflectance measurement mode.

The reflectance (R%) can be interpreted according to the following formula:

$$R\% = \frac{I_R}{I_0} \times 100 \quad (2)$$

where I_R is the intensity of the diffusely reflected light collected by the integrating sphere, and I_0 is the intensity of the incident light. The absorbance can be interpreted as the logarithm of the reciprocal reflectance analogous to transmittance measurements:

$$A = \log \frac{1}{R} \quad (3)$$

However when measuring the amount of light reflected from a rough surface, special considerations have to be taken into account. This is foreseeable when we look at light reflecting from a completely matte surface. The reflected radiation is everywhere of the same intensity, no matter what the angle of observation or what the angle of incidence is. This observation led Lambert [10] to the first mathematical description of diffuse reflection using the cosine law:

$$\frac{dI_r / df}{d\omega} = \frac{CS_0}{\pi} \cos \alpha \cos \beta = B \cos \beta \quad (4)$$

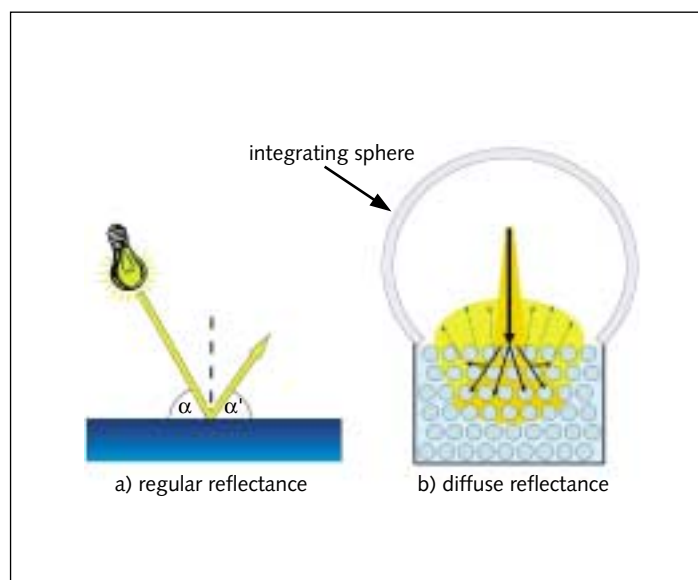


Figure 6: Scheme for reflectance and the task for integrating sphere

where I_r is the reflected radiation flux, in an f (cm²) area. ω is a solid angle in steradians, α is the angle of incident radiation, β is the angle of observation and S_0 is the intensity of the irradiation in W/cm² for normal incidence, B is the radiation density, or surface brightness in W/cm²sr and the constant C is the fraction of the incident radiation flux that is remitted. Due to absorption, C is generally less than 1. This equation is also known as the Lambert-cosine law.

During the XX. century many scientists have worked in the field of light scattering [11,12], such as *Rayleigh*, *Gans*, *Born*, *Mie*. The difficulty in describing diffuse reflectance lies in the difference between regular and diffuse reflection properties (Figure 6.). While in regular reflection the whole amount of the incident radiation reflects back with a well defined angle, in diffuse reflectance the light penetrates the material up to some mm, where it is partially scattered on the surface of particles, as well as being partially absorbed. Also reflection can be hampered due to the same particles. To overcome the mathematical problem *Schuster* made an attempt to find simpler solutions to the radiation transfer equations. In his model he used different vectors for the light being incident or being remitted by the sample. The boundary of Schuster's work is that it is applicable to single particles only.

Kubelka and *Munk* [4] made new assumptions in the derivation of a simplified solution to the radiation transfer equation. In their solution only the ratio of the absorption (K) and scattering (S) coefficients of the material, derived from *Kortüm's* work [12], are measured by the reflection, and not their absolute values. Thus the so-called Kubelka-Munk-equation is:

$$f(R_\infty) = \frac{K}{S} = \frac{(1 - 2R_\infty + R_\infty^2)}{2R_\infty} = \frac{(1 - R_\infty)^2}{2R_\infty} \quad (5)$$

where R_∞ is the reflection measured, represents the whole multiparticulate sample, and not just single particles. Since the simplified solution obtained by Kubelka and Munk contains only two constants which makes the equation well testable, and also since many scientists have developed similar models, which either can be converted to, or initiated from the Kubelka-Munk-equation, it is the most widely accepted, tested and used description of diffuse reflectance measurements.

S depends on the number, size, shape, and refractivity of particles, while K depends on the absorbing species and the wavelength. The relationship is a limiting equation like Beer's Law and can be applied with the following most important assumptions [13]:

- the distribution of the scattered light is uniform and specular reflection is ignored,
- the randomly distributed particles are smaller than the sample layer,
- and the particles are much larger than the wavelength of the illuminated light (to ensure the independence of the S from wavelength).

Nevertheless, diffuse reflectance measurement provides NIRS to give both chemical and physical information on the sample materials.

Chemometrics and spectral processing

Beside the developments in NIR optics and instrumentation, the improved computer hardware and chemometrics led to a wide application of NIRS in the pharmaceutical industry [14]. Use of chemometrics [15] is required for both qualitative or quantitative NIR analysis because the spectra contain multivariate information due to overlaps of chemicals in the NIR region as well as they are influenced by physical characteristics (e.g. particle size), too.

The mathematical treatments of data used include reduction of noise (smoothing), light scattering correction through Multiplicative Scatter Correction (MSC) and Standard Normal Variate Transformation (SNV), correcting pathlength differences and baseline effects with normalization [2]. First and second derivatives of spectra can be applied to improve the resolution of overlapping bands and to reduce baseline shifts. Since derivatives amplify the spectral noise, Taylor or Savitzky Golay smoothing algorithms are usually combined with derivation [16].

For qualitative interpretation of the spectra, sample properties have to represent a product identity or a product quality by similarity matching ("good" or "bad"). Cluster analysis (e.g. principal component analysis) serves for "non-supervised" methods, where a priori knowledge is not present. Discriminant analysis is applied to build classification rules when the group structure of the training set is known and classification is needed for new or unknown samples to be compared to the most probable subgroup.

To construct spectral libraries the algorithms involve Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), Soft Independent Modelling of Class (SIMCA), as well as the so called "K-nearest neighbours" (KNN).

A calibration process is required to build spectral libraries expressing the similarity with a correlation coefficient, such as the spectral match value (SMV), or the measure of the distance from the reference using calculations on the basis of Euclidian or Mahalanobis distance [17]. The conformity index (CI) can be calculated based on the distance method for qualitative testing (identification) of raw materials and/or products.

The choice of the small number of appropriate wavelengths may accelerate the analysis. Recording spectra at d -wavelength in a d -dimensional space, the distance between clusters of spectral points in the space can be used to discriminate the materials.

The quantile BEAST (Bootstrap Error Adjusted Single-sample Technique) can also be applied to detect samples that contain a component present in the training set.

For quantitative analysis, multivariate regression methods such as multiple linear regression (MLR), principal component analysis/principal component regression (PCA/PCR) and partial least-squares (PLS) regression are applied during calibrations and estimations [18].

Pharmaceutical applications of NIRS

Although NIRS has been used for more than 40 years in the agricultural and food industry, this technique has slowly been accepted within the pharmaceutical industry [19]. The Food and Drug Administration first approved the use of NIRS for release testing of a bulk drug in the case of ampicillin trihydrate in 1992 [20].

Actually, NIR diffuse reflectance spectroscopy offers useful and well interpretable results without destructive preparation of samples to study both chemical composition and physical properties of raw materials, in-process samples or end-products [21].

Significant benefits of NIRS are that it saves time and materials in comparison to many more conventional analytical methods because:

- chemical information is available on the molecules,
- relationships between physical characteristics and spectral data can be established,
- multicomponent analysis can be performed simultaneously,
- analysis times under 1 minute are possible,
- no sample preparation is needed,
- nondestructive analysis is possible,
- no reagents are used, hence the method is cost-saving and environment friendly.

Today a general chapter is included in major pharmacopoeias (e.g. Eur. Ph. and USP), which refer to instrument control and verification (wavelength scale, repeatability, photometric noise) as well as to the establishment of a spectral reference library.

TESTING OF RAW MATERIALS

Identification

NIRS is capable of the chemical identification of raw materials in real-time without the need for sample preparation. Identification involves the recording of the spectrum of an unknown sample and the comparison of its spectrum to a reference one. A decision is made on the identity of the unknown applying a spectral reference library approach.

In 1982 Rose first showed that several structurally similar penicillins could be identified by NIRS and two years later Ciurczak introduced a method based on Mahalanobis distance algorithm for the identification of raw materials [5,22]. Since then, many papers have reported on the applicability of NIR based qualification of both active ingredients and excipients. NIRS can extract information also from polymer packaging materials due to its extreme sensitivity to hydrogen bonding.

Moisture content determination

Water has the strongest absorption of light in the NIR region, therefore NIRS serves as an alternative to traditional moisture testing methods [23]. The calibration of the NIR method to determine moisture content requires Karl-Fisher titration, differential scanning calorimetry (DSC) or thermogravimetry (TGA) for the various types of water. Moisture content or hydration number of raw materials as well as intermediate and end-products (e.g. freeze-dried samples) can be investigated.

Characterization of polymorphism

NIRS is capable to identify the crystalline forms and assay the crystalline and/or amorphous content of drugs (e.g. caffeine, theophylline, miokamycin, sulfathiazol) and excipients (e.g. lactose monohydrate, mannitol, sucrose) as an alternative to traditional techniques of X-ray powder diffraction or DSC [24,25].

Quantitative assay

Excellent results were reported in the control of primary components or in mixtures for the active ingredients [6]. Beside many types of detectors, NIRS can be successfully combined with chromatography. Quantification of spots and identification of degradation products can be performed easily [26,27].

QUALITY CONTROL OF PHARMACEUTICAL DOSAGE FORMS

Quick nondestructive identification of active ingredients and excipients in whole tablets even through the blister packaging were carried out.

A recent study reported the use of NIR spectroscopy to evaluate 48 different formulations of furosemide tablets and the chemometric algorithms elucidated relationships between spectroscopic measurements and quality attributes such as tablet hardness, moisture analysis and endpoint dissolution testing [28]. The NIR spectra were suitable to discriminate between formulations based on filler composition, lubricant concentration, hardness, and binder concentration, too.

The applications covered are from identification and assay to physical and biopharmaceutical parameters, such as characterization of particle size, hardness, coating thickness and dissolution rate.

Drennen and co-workers were among the first who reported the test of tablet hardness by NIRS. They observed a baseline shifting to higher absorbance values due to an increase in tablet hardness.

In case of capsules, moisture content, drug dissolution as well as cross-linking of gelatin were characterized by NIR method [29]. Results demonstrated that the spectral range between 1800 and 2500 nm is suitable for hard gelatin capsule shell identification and qualification purposes [30].

NIR feasibility studies focused on shell crosslinking and plasticizer content of soft capsules [31].

Noninvasive test of freeze-dried products through the glass vial were performed with NIRS for moisture determination, porosity, particle size and formulation changes [32,33].

MONITORING AND UNDERSTANDING MANUFACTURING PROCESSES

In recent years, process analytical technology (PAT) has become a dominant issue in pharmaceutical manufacturing [34,35]. PAT focuses on the principles of building quality into the product and process as well as continuous process improvement. The PAT initiative promotes the use of techniques that enable the monitoring of critical process parameters and more importantly to enable process understanding and ultimately process optimization. A few examples of PAT tools and strategies are as follows:

- at-line, in-line, or on-line measurement of process quality and performance attributes,
- chemometric approaches such as multivariate statistical and pattern recognition methods.
- real-time data and information management systems for process control.

Continuous monitoring of quality is essential for PAT, which requires real-time measurements on the system as well as a model to predict product characteristics.

NIR diffuse reflectance method among spectroscopic techniques enable rapid, nondestructive analysis of samples and can be employed at a number of points in the pharmaceutical development and manufacturing process for timely measurements [36]. The power of NIRS for PAT is based on its speed and non-destructive nature which allows the analysis of intact dosage forms or intermediate forms (e.g. granules, pellets) for chemical and physical information. NIRS is fast becoming an important technique for analysis used in the validation of industrial pharmaceutical processes, because the spectra can be measured directly on samples without any pretreatment. Timely measurements during production are carried out by NIRS off-line or at-line, but there are successful examples for on-line and in-line modes, too [37].

Blending of solids

The determination of powder blend homogeneity as in-process control plays a crucial role in the manufacture of solid pharmaceutical dosage forms. The results of the traditional analytical methods show only the quantity of the active drug after destructive sample preparation. Usually the homogeneity study is typically a labor-intensive process involving the removal of samples from predefined mixer locations, extraction of the active drug from the sample matrix, and analysis. Diffuse Reflectance Spectroscopy (DRS) is suitable for the analysis of complex matrices rapidly, non-destructively and without using organic solvents, and it offers substantial advantages over traditional wet chemical techniques.

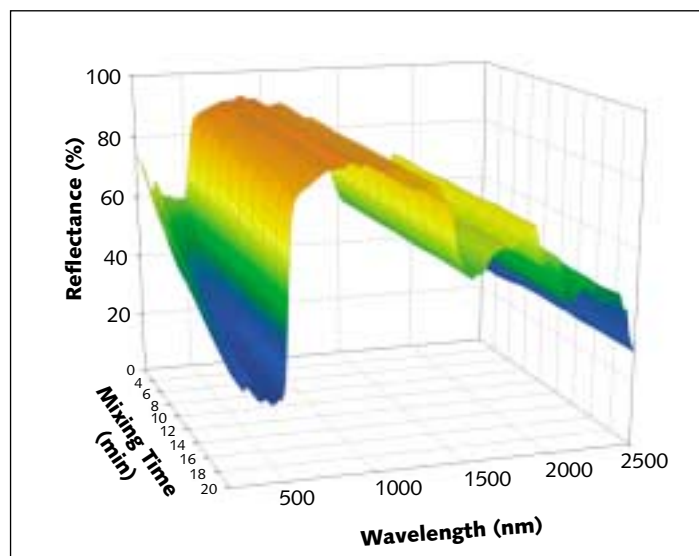


Figure 7: Changes of diffuse reflectance spectra as a function of mixing time.

NIR fiber-optic method was applied placing the probe in the axis of rotation of a tumble blender for real on-line stop-start measurements at different times of the blending process [38].

During the blending of riboflavine as a low-dose drug with lactose in a planetary mixer, the apparent disadvantage of the reflectance analysis, namely the underestimation of the active ingredient concentration (4% riboflavine in lactose) due to the inhomogeneous distribution of riboflavine particles was temporal [39]. The direct detection of optimal mixing time as endpoint was ensured through a characteristic, stable reflectance spectrum (Figure 7).

Wetting and drying during granulation

Since NIRS is highly sensitive to O–H vibrations, monitoring of moisture levels is a feasible option to determine end-points of wetting and drying processes directly instead of using traditional moisture analysis e.g. Karl-Fisher titration or loss on drying measurements. A proper calibration model enables NIRS for the in-process control of wet granulation processes. Several processes involving NIRS were described in the literature, including fluid-bed and high-shear granulation as well as microwave and vacuum drying [40,41]. Results suggest that NIR spectroscopy may be applicable to process monitoring of wet granulation in a high-shear-mixer, also in cases where monitoring of impeller torque is difficult to apply [42]. Due to characteristic wavelength of O–H band, it is enough to record the spectral values at fixed-wavelengths (e.g. 960, 1200, 1450 and 1920 nm-s) to ensure speed for timely measurements on-line. Figure 8 shows the typical changes of reflectance spectra of microcrystalline cellulose based pellets as a function of drying time.

In situ measurement with a fiber optic probe can be applied also to follow the freeze-drying process [43]

Interaction between the binder material and substrate can be observed as well as distribution migration of active ingredients [44].

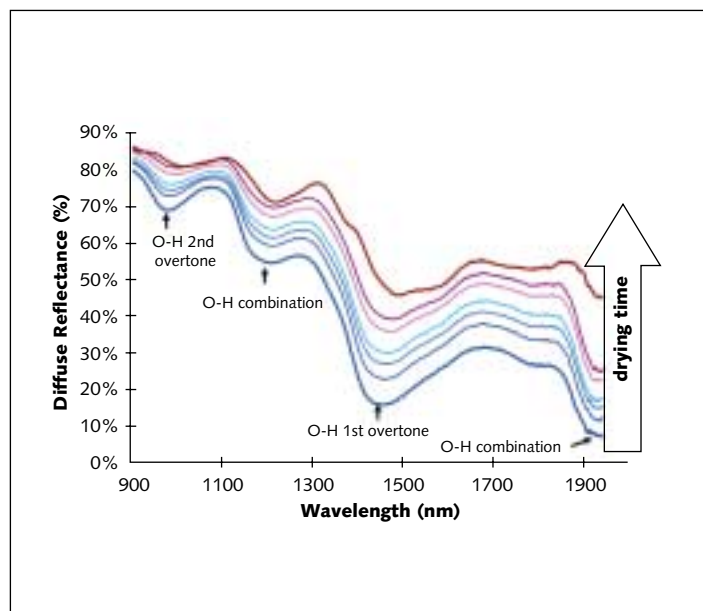


Figure 8: Changes in reflectance spectra during drying time

Pellet agglomeration

NIRS during the production of pellet spheres may serve as an alternative approach for monitoring control of agglomeration considering optimal wetting, as well as for the determination of the end-point in order to increase the final product yield [45]. If a phase exists with moisture equilibrium exists during the wet pelletization in a fluid-bed [46] or high-shear mixer [47], the changes of reflectance spectra can monitor the increase in particle size, too. The detection of particle formation relates to the Kubelka-Munk theory [48], where scattering is influenced by the particle size (Figure 9).

Film-coating

If mathematical relationship can be established between the reflectance and dissolution rate depending both on coating thickness, the dissolution profile of coated dosage form can be predicted from spectral values measured during the coating process. Beside the extent of coating, the NIR spectra were sensi-

ve to plasticizer content, too [49]. On the basis of several reports, the application of diffuse reflectance measurements can be recommended as a useful in-process tool to control and understand the parameters influencing the coating effectiveness [50]. Figure 10 shows example for the effect of the amount of coating polymer on the diffuse reflectance spectra of pellets coated with Eudragit L30D.

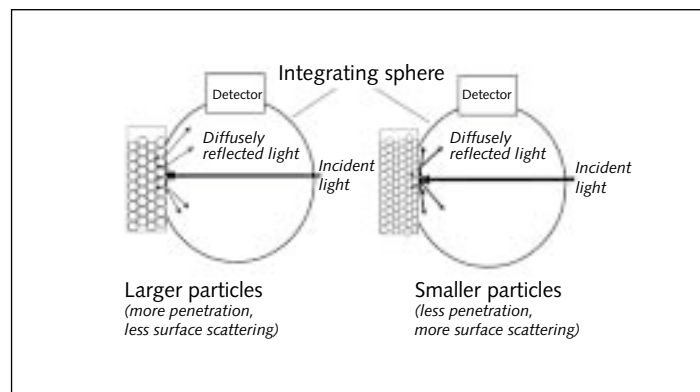


Figure 9: Difference in the diffuse reflectance depending on particle size of pellets

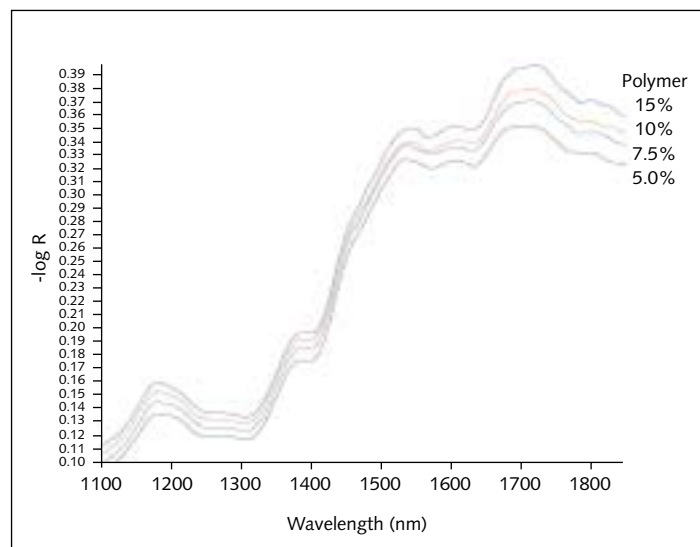


Figure 10: Effect of the amount of coating polymer on the diffuse reflectance of pellets

Conclusions

Even though that NIR techniques have widely been used in agricultural, food and beverages, paint, textile and other industries, it was only in the 1990s that NIR instrumentation and measuring techniques have reached a state, where its pharmaceutical application was accepted by the authorities. By the end of the 20th century, the development burst in computer hardware and software have made the use of NIR in the at-line, in-line and on-line applications possible in reliable and reproducible way. In the last couple of years the introduction of process analytical technology (PAT) to understand and control industrial processes gave again increase for the applications of NIR spectroscopy. Its greatest advantage is the possibility to measure solid samples without the any sample preparation directly, and also it bears the possibility to collect both physical and chemical spectral information. In the future, the knowledge obtainable with NIR imaging combined with microscopy for mapping of dosage forms will open a new field of research, where still are many possibilities awaiting us.

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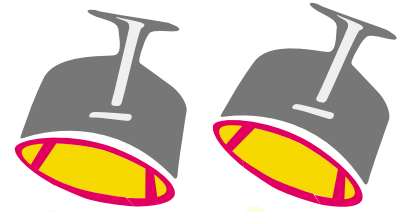


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SPOTLIGHTS



Glatt Technology Center obtains FDA approval

In the beginning of last December two inspectors from the American health authorities FDA (Food and Drug Administration) conducted a pre-approval inspection at the premises of the Binzen Glatt Technology Centre. They investigated intensely the general localities, the pharmaceutical production area and the analytical laboratories. Their special inspection focus was on the GMP-conform documentation.

The inspection resulted in four minor findings only, such entitling us to now call the **Binzen Technology Centre "FDA approved"**. For us, this presents a major milestone in the relatively young history of our Technology Centre, as we now better qualify as a competent partner not only for the American pharmaceutical companies but for all companies interested in the US market. Moreover, also for domestic markets a manufacturing place qualified by the FDA always recommends itself as a top address for the outsourcing of

- pharmaceutical development
- manufacturing of clinical samples
- product launch supply
- contract manufacturing

Since mid 2006 we are furthermore manufacturing a pharmaceutical product for the US market which has been developed in a co-operation between Glatt Air Techniques in Ramsey and our Technology Centre in Binzen.

For this specific product we are applying one of our innovative and proprietary pelletising technologies.



The FDA inspectors with the Glatt-team



A philosopher amongst engineers?

by Christian Augustin

The Glatt company is working since quite a while and with considerable success, based on its general process technological know-how, on the development of new material classes, i.e. cellular materials. This development is done in cooperation with numerous partners, such as universities, research institutes and industrial companies. The basic idea is to coat polystyrene spheres with a suspension. Thereafter, the spheres are removed again by low temperature pyrolysis, where as the outer shell formed by the suspension layer is sintered or calcined at higher temperatures, such producing a hollow sphere the characteristics of which can be selected via the sphere diameter, the thickness of the layer, the sintering temperature and other parameters. This technique is usually used to produce hollow spheres made from metal or ceramic powders.

These new materials can be used for a wide range of applications, such as light engineering, for sound absorption or as catalysts. For the marketing of these new products one has to detect suitable applications amongst a huge number of potential customers and field of applications, as potential users have first to be convinced of the benefits. Strategic marketing must hence find and explore the most promising applications. Unfortunately, there are no standard recipes for the latter, such forcing the Glatt company to develop their own new approaches.

During my study of philosophy and history, I did gain knowledge about this problem. Not intending to focus my the-

sis on literature only, I was quite keen to practically test my theories on innovation and invention research from a philosophical point of view and did discuss this option with Wolfgang Hungerbach from the Glatt company.

Initially the Glatt management was quite sceptical about the potential benefit of employing a philosopher, with untechnical thinking patterns for the market development of the above described new products. However, historians and philosophers have something in common with all other faculties: To handle the complexity of this world. The specific methods may differ, but the basic concept of approach is the same like the one from engineers, chemists or pharmacists. Like the engineer, who has to select a few materials from thousands for the construction of a functioning machine, the chemist has to check numerous options before he finds the final formula.

An engineer achieves the reduction of complexity by engineering know-how and calculation methods, a pharmacist by



Fig. 1: New material structures made from differently sized metallic hollow spheres.

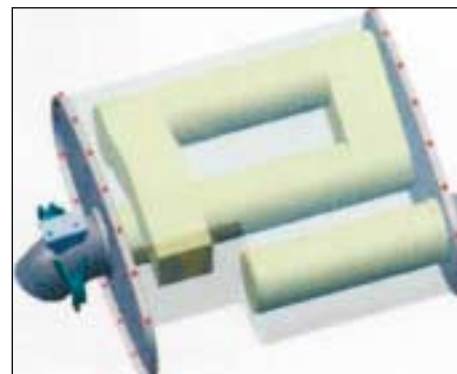


Fig. 2: Hollow spheres, integrated in the car exhaust pipe, can be used as silencers, optionally with catalyst effect. They are temperature resistant and easy to handle.

means of chemical and biological know-how and methods, a historian by evaluating archives and selection and interpretation of data, and a philosopher by language analysis.

Hence, why should one in some cases not use the specific philosophical and historical methods to solve alien problems of complexity reduction in order to find out if philosophical and historical theories do apply elsewhere, e.g. for the application and development for new cellular materials. The question is if the function

Forthcoming Events

BASF Symposium - Pan Coating -

WEEK 12, MARCH 2007
Sheraton Heliopolis, Cairo, Egypt

Powtech Nürnberg, Germany

27-29 MARCH 2007
Please visit our stand at the
Powtech, hall 11
stand no. 11-105 and 111

3rd FIP PHARMACEUTICAL SCIENCES WORLD CONGRESS

22-25 APRIL 2007
RAI Congress Center
Amsterdam, Netherlands

TTC Workshop No. 116 "Granulation and Tableting"

24-26 APRIL 2007
Binzen, Germany

3rd International Granulation Workshop Sheffield

27- 29 JUNE 2007
Tapton Hall of Residence,
the University of Sheffield,
United Kingdom

TTC Workshop No. 120 "Quality assurance by process understanding"

18-20 SEPTEMBER 2007
Binzen, Germany

CPhI Worldwide 2007

02-04 OCTOBER 2007
Fiera Milano Rho Exhibition Centre,
Milano, Italy

of machines can be described with a philosophical theory about social systems (e.g. Niklas Luhmann theory) or if a marketing problem can not be solved by means of a philosophical approach.

The aim is always the reduction of complexity. If a marketing problem can not be solved with scientific marketing tools, why not try chemical methods, psychological knowledge or even historical and philosophical theories?

Many other faculties meanwhile use such a holistic approach, often described as trans- or interdisciplinary.

Background of this assumption is, that the history of science can be described as the history of separating disciplines from the philosophy. Initially mathematics did belong to the faculty of philosophy, just like chemistry and psychology. Only with specialization and a massive expansion of knowledge the individual branches had

to develop into own faculties. Immanuel Kant (1724-1804) did not only teach philosophy at the university of Königsberg, but also law, mathematics, physics and biology. Quite an impossible thought today. How strongly natural scientists understood themselves as philosophers is also demonstrated by Isaac Newton (1643-1727) by his trendsetting publication "Philosophiæ naturalis principia mathematica" in 1686.

Today, philosophy is not too far away from the thinking strategies of other disciplines and can contribute ideas, methods and theories collected during centuries, for the manifold explanation of this world, be it mathematically, biologically, chemically, phenomenologically, ontologically, historical-genetically etc., to its previous sections, such reflecting the latter.

For my thesis I will systematically explore, if and to which extent philosophical and historical theories can be used for process and product innovation and will practically test them during the application tests for cellular materials (i.e. hollow spheres) of the Glatt company. Wolfgang Hungerbach will support my work and introduce me to the marketing strategies of the company, to assist my effort to produce with my thesis a quantifiable value for Glatt.

First projects have been initiated, such as contacts to the technical universities of Dresden, Kaiserslautern and Karlsruhe, via the alumni network of the "Studienstiftung des Deutschen Volkes", which have already lead to a diploma work and a cooperation with a major machine building company.

Presently, we are evaluating with our partners, which projects will be the most promising. Again, this has to be chosen from a large number of potential projects. Furthermore, I will work with an innovative Dresden group (please see <http://www.innozellmet.de>) to detect further applications for hollow spheres, i.e. as sound attenuators for small combustion motors, as equipment components or as light weight construction material, e.g. in combination with epoxy resins.

Most likely, the heterogeneous catalysis will be the biggest and most promising market for us, as environmental technology is gaining global importance. The ever increasing demand for energy and the uncertainties on the oil market will lead to potential shortages, such motivating the global players to look out for alternatives to the also politically unpredictable oil supply. One option would be

to not produce petrol and plastics from oil, but from natural gas, oil sand or coal. All relevant processes will necessitate the use of efficient and low price catalysts and create a potentially huge market opportunity. At present, for large scale applications 6000 different catalysts are imperatively used. Market estimations are running up to 10-15 billion Euro.

Tightening international regulatory issues will further boost the use of catalyst for all kind of chemical processes and enhance the use of innovative technologies to reduce the cost for the usually quite expensive catalysts.

Hollow spheres ideally qualify for this purpose, at they allow to minimize the content of catalysts to the mere quantity required for the chemical reaction.

For expensive metals hollow spheres present an economical alternative to solid spheres or layers on inert nuclei. Hollow spheres can be pneumatically filled into large reactors, due to their ideal shape and low friability, sometimes leading to significantly extended operation times.

All presently available results and cooperations with reputed research institutes are very promising. For this year we are already expecting first contract manufacturing orders from catalyst producing companies.



Fig. 3: Solid catalyst sphere Left: Unused catalyst Right: Used catalyst. The photo shows clearly, that only the outer darker shell has been reactive. The yellow core was not used. Consequently, a hollow sphere saves material and can save cost and increase reactivity due to the possibility to "engineer" the surface layer.



Christian Augustin Borne 1981, is a Ph.D. student working with the Glatt company. He has already won several prestigious national awards and focuses his work on theory and history of science and innovation and invention research. christian.augustin@glatt.com

Our cooperation partners

The GPCG 2 Isolator

Research and development with highly active substances

by Klaus Gröschel

The application of highly active substances in the pharmaceutical industry is gaining in importance. Thus, technical specifications must fulfil high demands.

Throughout the world, strict legal requirements stipulate that employers must safeguard the health of employees but it is practically impossible to guarantee absolute safety when active pharmaceutical ingredients are used. A certain extend of exposure is inevitable and must be knowingly accepted.

Limit values at the workplace are either regulated by national authorities or, if not existing, by the company itself under awareness of the product's toxicity, such defining a maximum daily exposure level.

Common limit value regulations are the OEL (Occupational Exposure Limit) and the DEL (Design Exposure Limit). The OEL defines an average during a period of 8 hours. The DEL narrows the average down to shorter individual steps.

In pharmaceutical process engineering these limit values have a serious effect on the techniques. This primarily affects the product handling, i.e. the charging and discharging of a machine.

As a matter of principle, fluid bed machines have the advantage of working at negative pressures such actively avoiding that any dust is emitted during operation. During charging and discharging, however, the machine and consequently the product are handled openly. In case of working with highly active substances inherent protection must be considered – including all restrictions which are necessary.

Over the years, unique seal and valve systems have been developed for production scale machines which enable a low contamination operation. The designs of all critical components are always product-specific. This is a small problem for production scale equipment because the machines are normally only used for one or a few known products.

Unlike the production scale, the machine technology must provide flexibility with maximum safety in the field of research and development where new and unknown materials are developed.

The *GPCG 2 Isolator* is based on the familiar laboratory scale device *GPCG 2 LabSystem*. It is therefore ensured that all basic factors and process parameters are comparable and scalable with standard processes.

To ensure maximum flexibility for active substances Glatt has decided to offer an isolator concept, thereby allowing an OEL level of up to $1 \mu\text{g}/\text{m}^3$.

Essential for the quality of an isolator is the continuity of the safety concept, in particular the actual isolator technology as well as the fluid bed machinery. The product can be charged via RTP-ports (Rapid Transfer Port) or – if required – air locks. As an alternative, a tubing system can also be used. When using RTP-ports or tubing systems for product discharge, cleaning of the locks is not required.

Handling is of great importance to the operator. All components must be well accessible and visually perusable. For these purposes a Mock-Up was built by Glatt to locate the optimal positions of all operation and display systems.

The simple charging and discharging of the product bowl is practically identical to the *GPCG 2 LabSystem*. The product bowl can be moved sideways and tilted by 180° to discharge material from the bowl.

The fluid bed process itself does not differ from the standard. Whether in developer's mode or fully automatic sequence of operation; the modern touch-screen control provides optimal ergonomic comfort for the operator as well as quickest possible access to all adjustable parameters – essential for developers. The controls for the isolator and the fluid bed unit are integrated into one operation terminal.

A good isolator concept must prove its value when changing the product. All contaminated parts must either be easy to clean or able to be sealed for contamination-free discharge. The machine tower is cleaned automatically from the HEPA inlet air filter to HEPA exhaust air filter by a built-in WIP System. Cleaning nozzles are situated inside the isolator box. The exterior of the machine tower as well as the complete isolator can therefore be cleaned comfortably.

It is needless to say that essential details such as smoothed and easy-to clean corners, polished and slightly sloped surfaces were emphasized. Even the peristaltic pump heads were modified according to the definition of the project: the drive unit is sealed off outside of the isolator and the pump head can be removed for cleaning by means of a bayonet coupling.

A particular highlight is the push/push-filter technology which is used in the inlet and exhaust air of the isolator as well as in the exhaust air side of the machine tower. When changing a filter, a "clean" filter is inserted, thus pushing the contaminated filter into the interior of the isolator. From there the filter can safely be bagged, sealed and discharged via a tubing system (endless tube). The unique filter change principle and the gasket of the filter itself guarantee that there is no danger of contamination to environment while changing filters.

Of course, the machine can also be used for non-toxic substances with opened isolator hatches. There are no differences in comparison to conventional laboratory systems when operating in this mode.

Glatt offers the *GPCG 2 Isolator* in dust- or solvent-explosion-proof design. All laboratory scale process inserts of the conventional *GPCG 2 LabSystem* for drying, granulation and particle coating are available. Even the Plug&Play-concept was implemented consistently. All parts have been integrated into the shapely design of the housing.



Klaus Gröschel passed his engineering exams in 1980 and is working for the company Glatt since, originally on various engineering tasks and for a few years as manager of the Binzen sales office.



Understanding nanoscale drug delivery systems

by Udo Bakowsky* and Johannes Sitterberg

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Introduction

The development of new drugs with short biological half-life and highly active drugs with potentially dangerous side effects has led to an increased interest in techniques to deliver these drugs to their desired site of action. New high affinity ligands often lack the biopharmaceutical properties that are necessary to use the compound as a drug, e.g., solubility or permeability. In addition, the interaction between the administered drug and the human body may still result in short circulation time, due to rapid clearance by the reticulo-endothelial system or fast metabolism. These novel drug molecules demand new formulations which protect the drug, as well as the host and are able to direct the drug to the destined organ. One possibility to overcome these problems is the development of nanoscale carriers for various applications, such for a nasal or pulmonary administration.

Controlled drug delivery offers multiple options for the optimisation of drug action by adjustments of the release rate, design of pro-drugs and the alterations in the accumulation of the drugs at their desired site of action (drug targeting). Drug targeting can be achieved by incorporation of the drug into polymeric particles or liposomes, use of solid lipid nanoparticles, surfactant- or lipid-modified hydrogels or systems based on biodegradable nanoparticles. Additionally to these rather unspecific controlled release systems, the affinity towards the site of action (bioadhesion) can be further enhanced by modification of the surfaces with target-seeking moieties, such as lectins, antibodies, peptides, carbohydrates or invasion factors [1-13]. In this rapidly growing area of research, special attention has been paid to the physicochemical characterisation of colloidal drug carrier systems. This includes the determination of the size distribution, charge density, calorimetry, time dependence of the drug release and analysis of the adhesive properties using e.g., quartz crystal microbalance or surface plasmon spectroscopy.

For the visualisation of the nano-carriers, scanning force microscopy and electron microscopy could be used.

Visualisation of drug delivery systems

The visualisation of surface morphology allows an understanding of physical, chemical and biological phenomena. For many years, optical microscopy has been used as a tool to produce images of surfaces. The resolution that can be achieved by traditional light microscopes is limited to approximately one micrometer by the Nyquist relation, and measurements in the z-direction are not possible. Developed in the 1940's, the next most widely used instrument for investigating surface morphology has been the scanning electron microscope (SEM). With this technique, only the near surface of samples can be visualised. Similar to an analysis by optical microscopy, SEM only measures in the x and y dimension of a sample and insight into the z-direction can not be obtained. With today's SEM, resolution is limited to about five nanometres, owing to the properties of the electromagnetic lenses. However, this resolution can only be achieved under vacuum conditions. Furthermore, a rather laborious sample preparation is often required for SEM, as it may include steps, such as freeze drying, staining or metal coating. Scanning force microscopy (SFM), also known as atomic force microscopy (AFM), was developed by Binnig and co-workers in 1986 [14]. SFM allows the imaging of conducting, as well as non-conducting samples. High lateral and vertical resolutions can be achieved in vacuum, in air and even on liquid-covered surfaces. Measurements under physiological conditions are also possible. In addition, with this microscopic approach, it is not only possible to analyse the topography of a sample, but also other physical properties, including friction forces, softness and viscoelasticity, and the charge density on a nanometre scale. With the optimal choice of equipment,

resolutions in the range between 0.1 nm and 2.0 nm for the x-y dimension, and less than 0.1 nm for the z-dimension can routinely be obtained. All these options have made SFM a very useful tool in many biological, medical or pharmaceutical applications. Large objects, such as whole microparticles can be imaged, as well as smaller structures in a nanometre range.

The principle of scanning force microscopy

The main element of every SFM is the tip-cantilever system (figure 1). The cantilever can be made from different materials, usually silicon or siliconnitride. The choice of material depends on the specific application. A sharp tip is mounted on one end of a 100 to 500 μm long lever. The geometry of this tip is crucial, as it

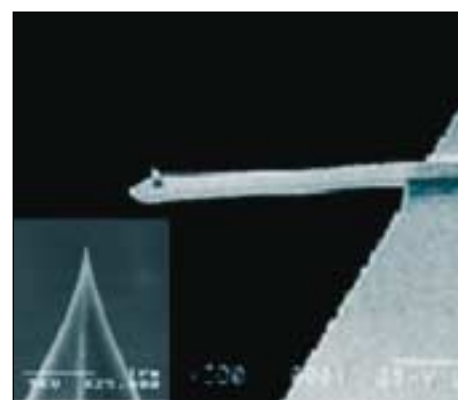
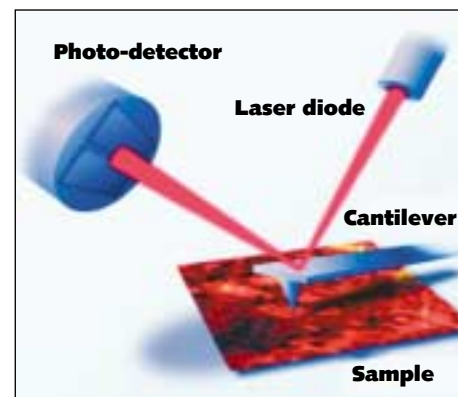


Figure 1: Schematic of a scanning force microscope (SFM). The cantilever / tip system is the "heart" of the SFM and determines the resolution and the quality of the measurements.

represents one of the major parameters that determine the resolution of a measurement. The highest resolution can be achieved with a tip that tapers off in a single atom. Imaging of a conventional SFM tip reveals it to be spherically shaped with a diameter approximately 5 nm.

In order to be moved over the sample surface, the tip-cantilever system is integrated into a stylus profilometer. Such equipment has been used in the optics industry for many years. In essence, a stylus profilometer is a piezoelectric scanner that can generate movements of an accuracy and magnitude required to generate topographic images with a resolution of some nanometres. The movement of the piezoelectric elements can be controlled by the voltage that is applied across its electrodes. Depending on the design of the SFM, the scanner is used either to move the sample underneath the cantilever or to move the cantilever over the sample. This feature of the equipment is the limiting factor for the maximum scanning field. Hence, distances of about 125 microns in the x-y direction and 10 microns in z-direction can be covered. This is sufficient for many but not all technological or biological purposes. When the cantilever is moved over a structured surface, the attractive and repulsive forces between tip and surface will change. These forces are measured by sensing the deflections of the cantilever. Deflections of the cantilever can be detected by a variety of methods, such as electron interferometry, optical deflection or capacity methods. In SFM, a number of different scanning modes can be distinguished that differ primarily in the type of interaction between tip and sample that is measured, but also, at least in some cases, in the way the tip is moved across the surface. The most relevant techniques for technological and biological applications are contact SFM, TappingMode™ SFM, lateral force microscopy and force spectroscopy.

Scanning force microscopy techniques

When using the contact technique, the tip is always in contact with the surface and the cantilever scans line by line across the sample. Accordingly, the topography of the sample results in deflections of the cantilever which are detected and amplified in order to produce an image. This image shows a map of tip-

sample interactions resulting from the inter-atomic repulsive forces between these two surfaces. Especially, when scanning soft material, the surface can be damaged and the possibility of monitoring artefacts has to be taken into account.

TappingMode™ or intermitted contact imaging was a key advancement in SFM of soft, adhesive or fragile samples, i.e., most biological materials. For tapping mode, the cantilever has to be oscillated, close to its own resonance frequency. This can usually be achieved with a piezoelectric element. The oscillating tip is then moved toward the surface until it senses or “taps” the surface. This reduction in oscillation amplitude is used to identify and measure surface features.

Force spectroscopy can be used to measure forces between the tip and the sample surface and to generate force-distance curves. This type of operations can be divided into scanning techniques, lateral force microscopy and force spectroscopy, and relies on the measurement of forces at different points of the sample, which thus allows one to construct force vs. distance curves. By sensing the deflection of the cantilever, it is also possible to monitor frictional interactions between the tip and the sample. The contrast of SFM images generally depend the mechanical properties of the surface and the probe, such as adhesiveness and elasticity. Studies on the interactive forces between single molecules have contributed significantly to our understanding of major processes in nature. For example, the binding force of complementary molecules (ligand-receptor pairs and drug-substrate pairs) can be characterised by interpretation of force distance curves in the pico-newton range.

Sample preparation

A proper sample preparation and the selection of a suitable substrate for the specific sample are crucial for high resolution and artefact-free scanning force microscopy. The substrate for high resolution imaging have to be flat and smooth, and the surface roughness has to be substantially below the size of objects under investigation. Substrate materials that have been successfully used in SFM include: freshly cleaned surfaces of highly orientated pyrolytic graphite (HOPG), mica, evaporated or single crystal gold, glass slides and silicon wafers, as known from the production of

electronic hardware. These surfaces can be further modified by chemical reactions, in order to change their hydrophobicity, charge and charge density, surface ion concentration and other parameters. It is important, that the interaction between the sample and the substrate surface is not too strong. Otherwise, the morphology of the sample might be changed dramatically during sample preparation. On the other hand, the interaction has to be strong enough to prevent dislocation of the sample during the scanning process. The size of the sample is limited to 10 x 10 cm in x-y direction and about 1 cm in height. Various methods are available for sample preparation. A very convenient procedure involves the evaporation of a droplet of the sample that has been dissolved or suspended in buffer. A few

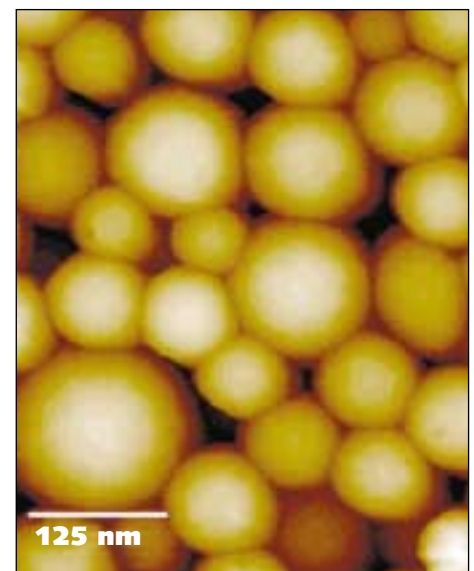
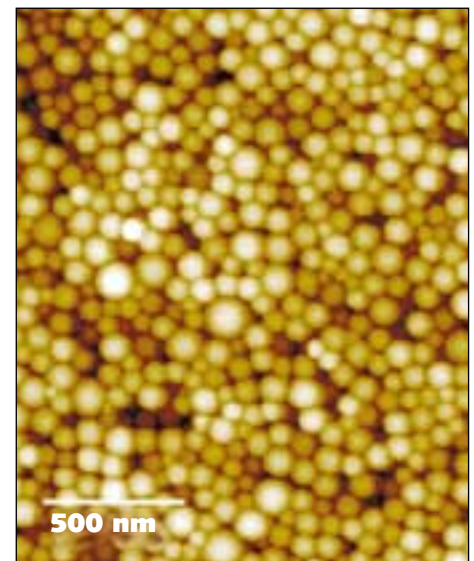


Figure 2: SFM images of biodegradable 125 nm-sized PLGA/chitosan nanoparticles prepared according to M.N.V.R. Kumar [1,2] visualized with TappingMode™ (Digital Instruments, Nanoscope IV Bioscope) in air.

micro litres are simply pipetted onto the substrate which is then dried in the air. It is also possible to place the sample as a dry power onto the substrate, but in this case, the sample has to be homogeneous distributed over the surface. In general, no fixation or staining of the sample is needed.

Examples

Since a number of years, our group's efforts are devoted to the development and the physicochemical characterisation of nanoscale drug carrier systems, particularly for dermal and pulmonary application. We focused our scientific interest on controlled release drug delivery systems based on biodegradable poly-

mers and lipid carrier, such as liposomes or solid lipid nanoparticles (SLN). These drug vehicles can be used to deliver drugs with a short biological half-life (e.g., peptide drugs, proteins or nucleic acids) and highly potent drugs with potentially dangerous side effects (e.g., immunosuppressants, glucocorticoids). We characterised these systems intensively by SFM, analysing their size, shape, surface morphology, viscoelastic properties and erosion kinetics.

Nanoparticles

Techniques for the preparation of monodisperse polymer colloids received greater attention during 1980s. The use of monodisperse particles makes it possi-

ble to give sharp, reliable and reproducible results for their respective applications. Emphasis should be on the uniformity and size, as well as the chemistry and morphology of the particles. Biodegradable poly-lactide-co-glycolide (PLGA) / chitosan nanoparticles can be prepared by the most widely used solvent replacement technique at sharp size distributions between 190 and 780 nm (i.e., 190 nm, 220 nm, 350 nm, 450 nm, 600 nm and 780 nm) [1,2]. Surfactants, such as poly(vinyl alcohol) (PVA) can be used to stabilise the dispersed particles. From the SFM investigations, it appears that the obtained nanospheres were uniform in size and spherical in shape with smooth surfaces (figure 2). High resolution

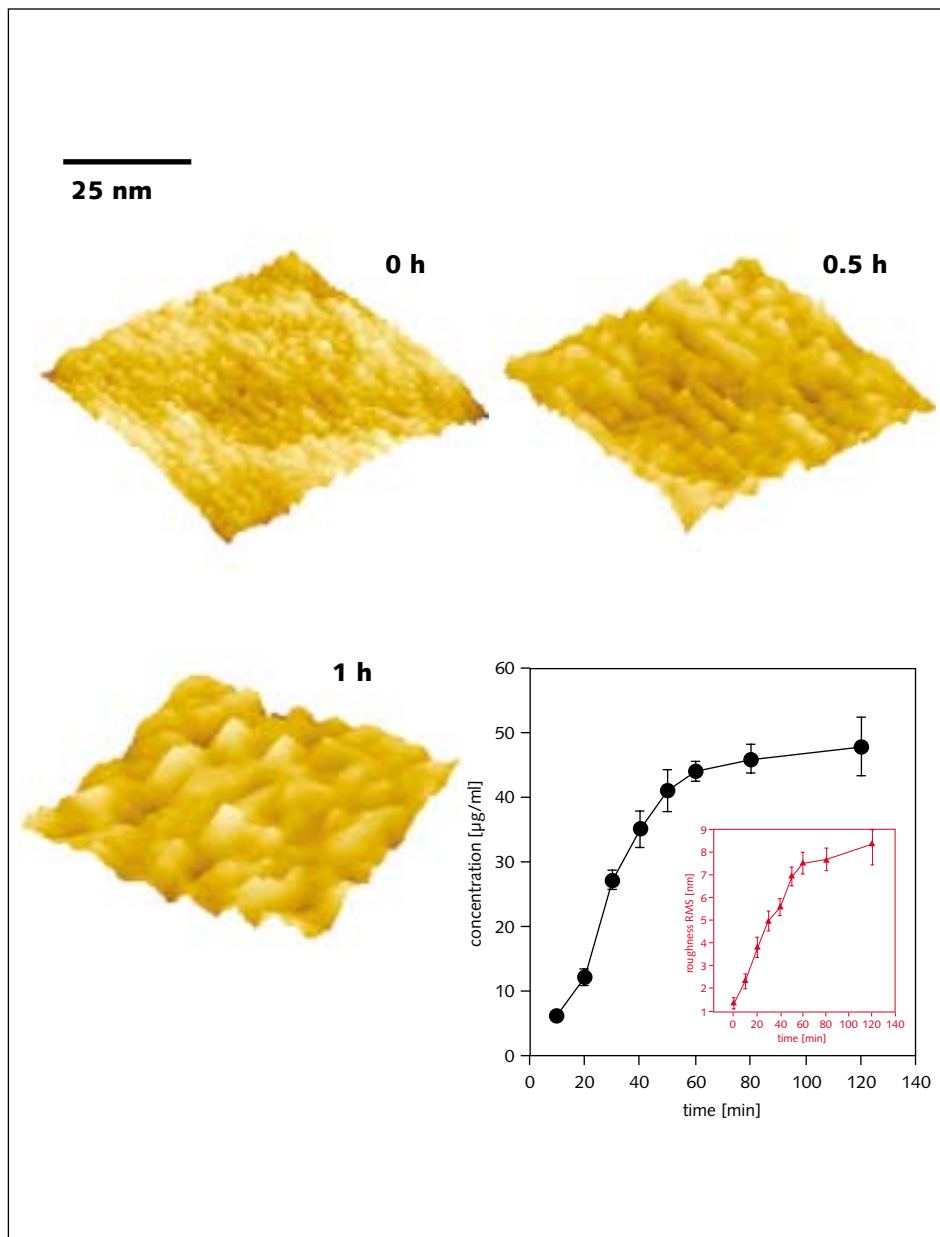


Figure 3: Erosion and drug release. The surface erosion of PLGA / chitosan / dexamethason nanoparticles could be analysed in buffer suspension. The surface roughness increased with increasing time as shown in the SFM images. The drug release The calculated change in surface roughness was proportional to the drug releases (diagram). Nanowizard (JPK instruments) measurements in contact modus.

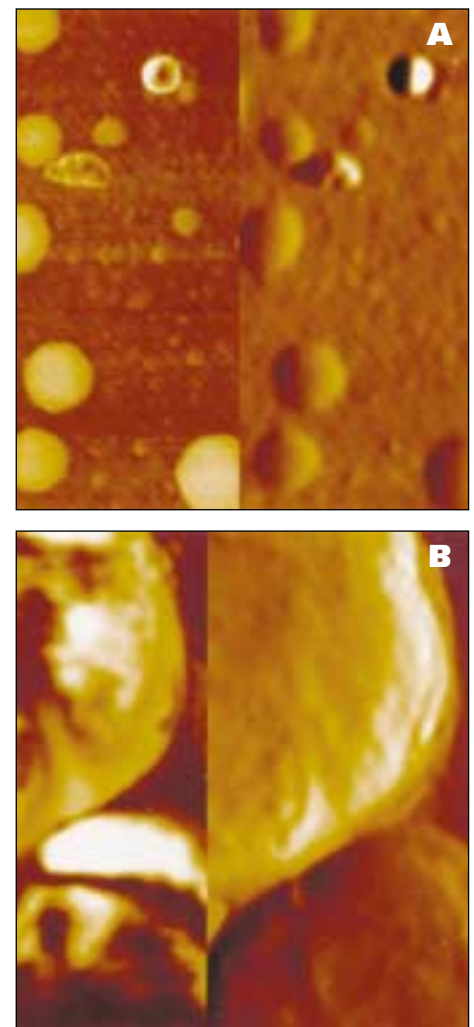


Figure 4: Phase contrast imaging. With the SFM the material properties could be visualized. A) PLGA / chitosan / dexamethason nanoparticle, the drug is homogeneously distributed within the nanoparticles, the particles show the same bright colour, B) poly[(vinyl-3-(diethylamino)propylcarbamate-co-(vinyl acetate)-co-(vinyl alcohol)]-graft-poly(L-lactic acid) / insulin nanoparticle (size 200 nm), the insulin is inhomogeneous distributed (bright areas) [9]. Left phase contrast image / right height image, dark areas are softer than bright areas.

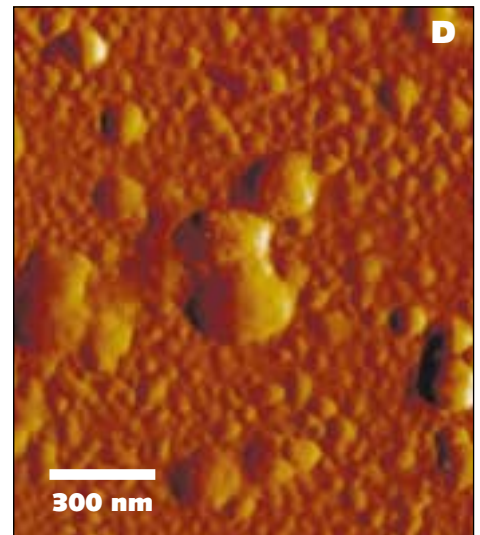
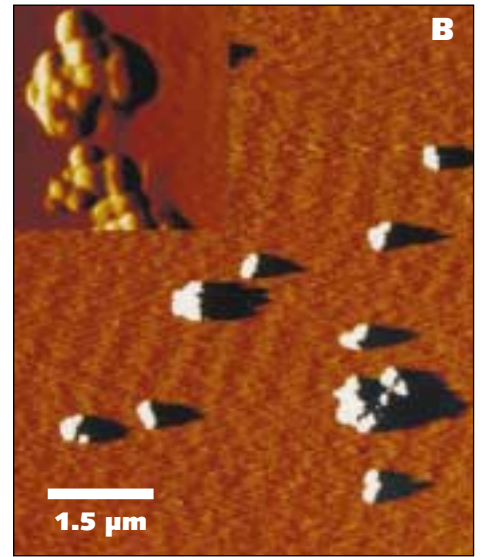
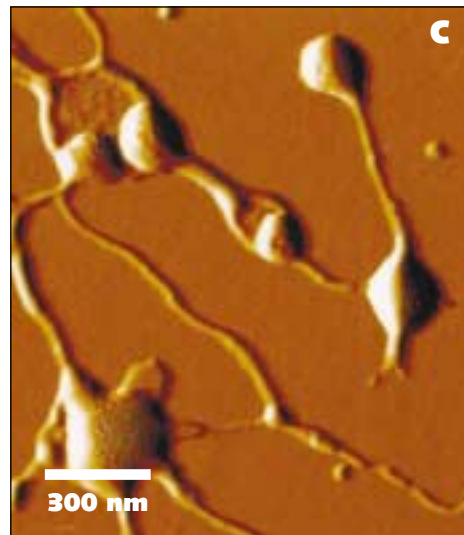
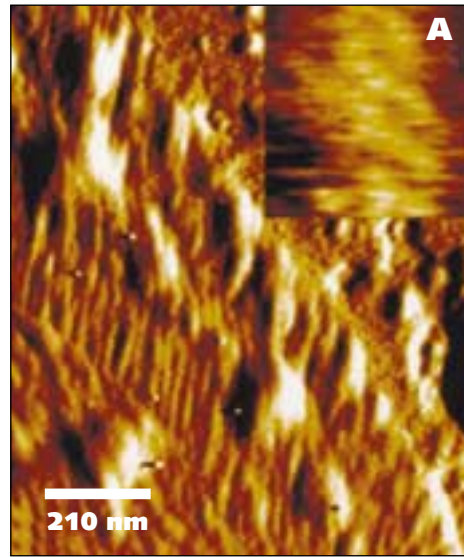
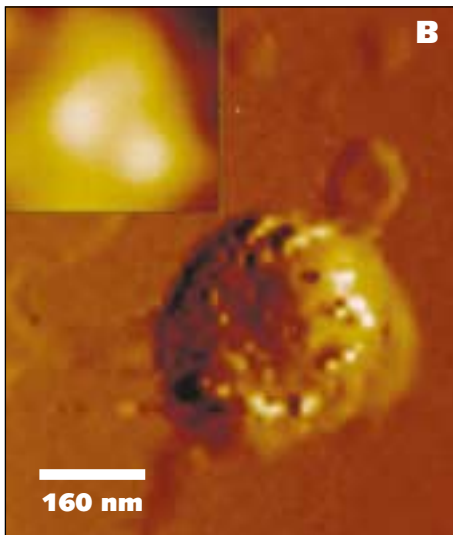
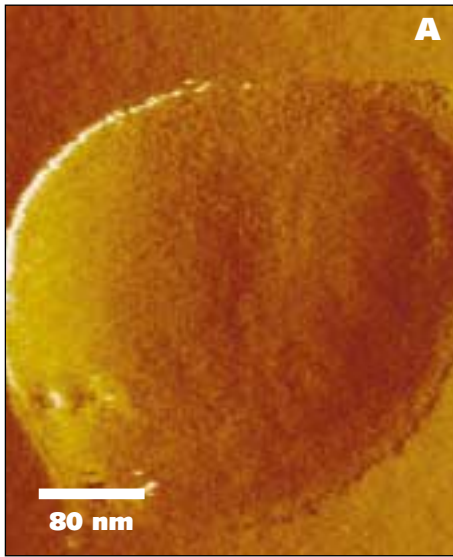


Figure 5: Typical morphology of phosphatidylcholine PC/cholesterol/PE liposomes. (A) unmodified, (B) after covalent binding of immunoglobulin (IgG) molecules to the liposome surface according to Bendas et al. [4]. The vesicles were adsorbed to silicon wafers via self assembly and imaged in buffer in TappingMode™ (Digital Instruments, Nanoscope IIIa). The insert in (B) shows a single IgG molecule (dimension 25 x 25 nm).

Figure 6: Visualisation of the structure of DNA and various gene transfection systems. A.) Ordered strands of DNA. The DNA fully hydrated and in a supercoiled organization. A higher magnification is given in the insert (dimension 10 nm x 10 nm) [13]. B) Nanoplexes formed between positively charged silica nanoparticles and plasmid DNA (pCMVbeta). The size of the complexes is between 200 nm and 300 nm [11]. The DNA is protected by surrounding particles. The insert (dimension 250 nm x 250 nm) shows the structure of a single complex. C.) The complexes shown here are the results of a self assembly of SAINT2 and DNA (pCMVbeta). The DNA was covered by the lipid, some not condensed plasmids could be visualized [13]. D.) Typical morphology of PEI/DNA complexes. The mean diameter of the complexes is in the range of 100 nm to 200 nm [9].

imaging of the particle surface gives an idea about the sub-surface morphology of the nanoparticle. When the particles were placed in water, surface erosion processes could be followed by repeatedly scanning the surface. The calculated change in surface roughness was proportional to the erosion, and in this case, to the drug releases (figure 3). The erosion is dependent on the pH of the dispersion medium, the temperature and other physiological factors, and this can be simulated in a time-resolved manner with the SFM technique. The knowledge of drug distribution inside the nanoparticles is important for the understanding of the drug release. The phase mode permits to

give a general statement about the material properties (viscoelastic properties) of the sample and opens the possibility to visualise the drug distribution within the particles. This can be seen by the different brightness in the SFM image. The images in figure 4 show a PLGA / chitosan / dexamethason nanoparticle. The model drug is homogeneously distributed. The figure 4 also shows a poly[(vinyl-3-(diethylamino)-propylcarbamate-co-(vinyl acetate)-co-(vinyl alcohol)]-graft-poly(L-lactic acid) / insulin nanoparticle with a mingled internal structure. In the latter case, the drug forms clustered domains within the particle [3].

Liposomes

The use of soft liposomes as drug vehicles has been explored extensively, because of their low immunogenicity and toxicity. To obtain a targeting effect, specific ligands can be easily attached to the liposome surfaces. Among several options, the coupling of antibodies is very popular, resulting in immuno-liposomes. In addition to specific targeting, the concept of sterical stabilisation has been developed (long circulation liposomes). The latter can be achieved by coating of the liposome surface with amphipathic polyethylene glycole (PEG) derivatives [4-7]. Large unilamellar vesicles were prepared by extruding multilamellar vesicles

eleven times through a poly-carbonate membrane (pore size 200 nm). The basic composition of the liposomes was soy PC/cholesterol 2:1 (molar ratio), containing different amounts of activated protein anchor lipids (1.5, 2.5, 5, 7.5 mol% cyanur-PEG-DSPE, cyanur-DSPE or PEG-DSPE₂₀₀₀, respectively). An anti-E-selectin antibody was covalently bound to the liposome via the amine-reactive cyanur-groups on the liposome surface. Two different types of liposomes produced are illustrated in figure 5. The surface of an unmodified liposome is smooth and soft. In the case of the immuno-liposome, the antibody on the liposome surface can be visualised. The major problem when measuring liposomes by SFM is their tendency to spread to the substrate surface, especially when the liposomes are analysed under physiological conditions.

DNA and DNA complexes

Probably the most important biopolymer is DNA, which is also reflected by the fact that nucleic acid is the most frequently investigated "polymer" by SFM. In recent years, most SFM studies of DNA have been performed in the context of artificial gene delivery systems. This context includes naturally occurring complexes of DNA with other biomolecules, as well as fully synthetic gene transfection systems. The latter include DNA complexes based on positively charged lipids, liposomes, proteins, polymers and nanoparticles (figure 6). SFM has been used to study the influence of polymer chain length and molecular weight on the size and shape of polyethylenimine (PEI)/DNA complexes [8,9] and to study the change in the structure of such complexes from globular to toroidal, after the addition of hydrophilic PEG-blocks to the polymer. Indeed, SFM has proven very useful for the characterisation of DNA complexes, including those that are assembled with lipids, organic and inorganic nanoparticles or artificial virus capsids, as shown in figure 6 [10-13].

Conclusions

Scanning probe microscopy provides a new method for the imaging of biological and technological objects. This method has a number of advantages over established imaging techniques, in so far, as it (i) is non-destructive, (ii) allows examination of samples under "natural" conditions, (iii) involves fast and convenient

sample preparation procedures and (iv) provides a direct online image generation. In addition, SFM is not limited to obtaining images of the topography of the sample; it also allows collecting data on the mechanical and elastic properties and on specific surface properties, such as adhesiveness. In the future, the development of new techniques in scanning probe microscopy and their combination with existing methods, such as fluorescence microscopy, will further extend the potential of this exciting technology.

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