

Traceability of Polyethylcyanoacrylate nanoparticles through the Stratum Corneum.

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ABSTRACT

In this work we follow the polyethylcyanoacrylate nanoparticles through human skin. Photoluminescence (PL) is used to quantify the amount of these nanocarriers into the layers of the skin stratum corneum (SC). To obtain samples of each layer of SC, tape stripping technique was conducted. Reproducible data were obtained, and the nanoparticles' distribution in each layer of the SC is presented. Possible applications are in the field of pharmaceutical development, especially concerning drug transport through the skin by nanoparticle carriers.

Keywords: transport phenomena, nanoparticles, polyethylcyanoacrylate, skin.

1 INTRODUCTION

One of the safest routes for therapeutic treatment is transdermal delivery but it offers certain difficulties; the most external skin layer is the stratum corneum, the first defense against external particles, microorganisms, chemicals, etc. The same task is assigned when a drug is delivered through this organ and not all the drugs can be administrated for this way. One of the strategies followed to accomplish with this goal is the use of nanocarriers, being the nanoparticles one of the most wanted among pharmaceutical nanoscientists because they improve dermal penetration [1]. The nanoparticles have some advantages over other kind of carriers, i.e. reproducible materials, more stability time, more amount of drug loaded, etc. One of the main options for transdermal delivery is the solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) [2]. Besides lipid nanoparticles, polymeric nanoparticles are very good option for transdermal delivery since they can be tailored made in different sizes and it is possible to modify surface polarity [3], [4] for improving skin penetration.

Nanoparticles as skin carriers are a topic not frequently found since they are difficult to follow into the skin [5], [6], [7]. There are lots of differences in the evaluation protocols, methods, etc. Moreover, in general, skin route is not considered as important as other routes. On the contrary, some authors consider it a very important route of penetration in occupational and consumer setting [8]. From the upper skin, nanoparticles can reach deeper skin regions

since they are able to show mechanical flexion [9]. In fact, nanoparticles can go from skin to lymph nodes becoming in a powerful carrier to be used for immunomodulation [10].

This work intends to quantify the amount of polyethylcyanoacrylate nanoparticles in the stratum corneum layers (SC). The way we use to evaluate these nanoparticles concentration in the skin stratum corneum is by mean of the technique called "Tape Stripping".

The first attempt to know the amount of nanoparticles in the SC was by mean of IR Spectroscopy and results were unexpected. The characteristic signal of the cyan group is 2220 cm^{-1} and 2260 cm^{-1} , when the tests were conducted there was no response. Also RAMAN Spectroscopy was conducted but no signal was found like in the IR Spectroscopy. It was decided to test a technique used to evaluate quantitatively other kind of nano structured materials called photoluminescence. This method, applied to quantify nanoparticles through different depths from the skin stratum corneum, is a novel technique and not reports in literature are found until we know.

First attempts to study mesoscopic systems formally began at the end of the seventies in the twenty century. The microscopic and the macroscopic approaches had not been useful to explain this scale. Nanoparticles and nanotubes are in this category. These kinds of systems are very interesting since they obey to different kind of rules since the bulk properties are neglected against the surface properties [11].

Due to the high surface energy, mesoscopic structures tend to self-assembling and they have very interesting properties. In colloids, nanostructures, pharmaceutical sciences, etc. these systems have reached a main role in the last years.

If we talk about mesoscopic systems, we must mention clusters. They are agglomerates of molecules or atoms from equal or different chemical nature. We think our system is formed from nanoparticles including clusters from nanometric sizes. In general, clusters have interesting electrical properties and that is why we can use photoluminescence to characterize them.

It is possible to excite clusters, neutral or charged, with light and study the radiation they then emit. Loss of energy by emission of radiation occurs for those clusters which have large band gaps in the bulk [12].

2 MATERIAL AND METHODS

2.1 Nanoparticles preparation.

First of all, it was prepared a batch of nanoparticles suspension of 70 to 110nm to perform the test. To produce polyethylcyanoacrylate nanoparticles, ethylcyanoacrylate from SIGMA ALDRICH, Pluronic F68 from SIGMA ALDRICH and hydrochloride acid (J.T. Baker) were employed. Particle size distributions were determined by a Submicron Particle Sizer N4 Plus, from COULTER.

2.2 Follow up of the transport of nanoparticles through the stratum corneum.

A glass chamber was placed on the volunteer arm and 3ml of nanoparticles suspension were put into the chamber. After that, the chamber was fixed to the arm by means of a stretching plastic film. We wait 30 min without movement and after that the chamber was removed and the arm zone was cleaned with a dry paper napkin.

Immediately after cleaning, a piece of tape (Tape 845 from 3M) about 5x3 cm was located and pressed to the arm zone where the nanoparticles suspension was applied. We took off the tape and the same procedure was repeated 14 more times.

The same procedure was performed for 1h, 2hr and 3h of application of nanoparticles using the glass chamber.

Every tape obtained contained death cells, nanoparticles, lipids, etc. The more tapes we applied deeper corneum stratum we reached.

The nanoparticles amount determination in the tapes was conducted using photoluminescence.

Every factor was checked not to interfere in the final reading. Readings from tape, tape with skin and tape with nanoparticles and skin were conducted.

The measurements were made to room temperature in a system micro Raman (mark Dilor) model Labram, which has a laser of He-Ne that emits to 632.8 nm. The light was focused on the surface of the sample with an objective of 50 X that allowed to reduce the diameter of the laser beam to 2 microns. The equipment has an optical system that allows observing in a screen the region of the sample to analyze. With this device we took care to study similar regions of skin in each layer. This means, regions with a similar optical appearance as far as size and thickness. The obtained signal was separated in its spectral components by means of a diffraction grid of 1200 lines/mm and analyzed with the aid of a detector type CCD thermoelectrically cooled. In some cases the luminescence was so intense that there was necessity to use filters of neutral density to avoid saturation of the detector.

3 RESULTS AND DISCUSSION

As we mentioned, first of all the specificity of the test was done to be sure the signal was due to the nanoparticles and not for other substance present during the survey.

Preliminary readings were conducted in tape alone, tape with skin without treatment (**Figure 1**), tape with skin nanoparticles and tape with skin and nanoparticles previously to evaluate the tapes from the testing at different times and different depths.

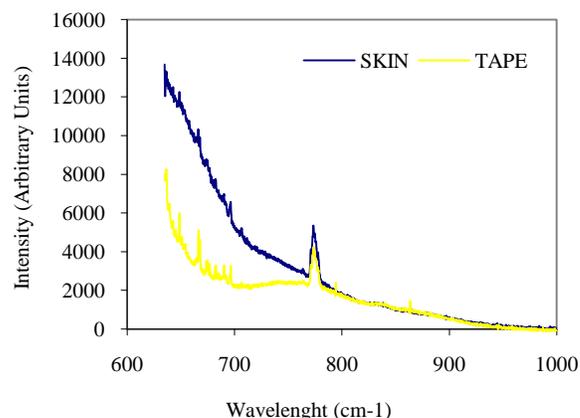


Figure 1. Photoluminescence of tape alone (TAPE), tape-skin (SKIN).

The results are clear and they show no interference from the tape or skin in the intensity produced by nanoparticles. As it is showed in the plot, the tape and the skin increase the nanoparticle signal but the total maximum peak is not affected.

The signal presented by nanoparticles in the tape without skin is very different from the signal of nanoparticles in the tape with skin (**Figure 2**). This could be explained due to an interaction of nanoparticles with melanin.

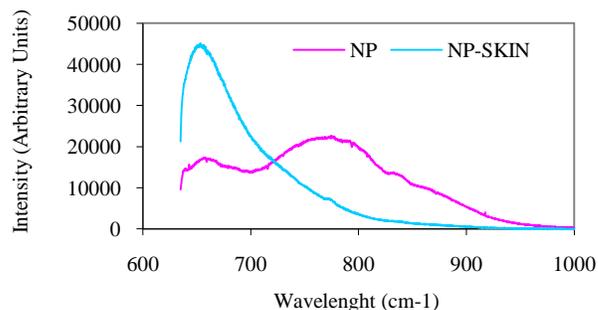


Figure 2. Photoluminescence of tape-nanoparticles (NP) and tape-skin-nanoparticles (NP-SKIN)

Non luminescent pigments and dyes exhibit colors because they absorb white light and reflect that part of the spectrum that is complementary to the absorbed light. A small fraction of the absorbed light is transformed into heat, but no appreciable radiation is produced. If, however, an appropriate luminescent pigment absorbs daylight in a special region of its spectrum, it can emit light of a color different from that of the reflected light. This is the result of electronic processes within the molecule of the dye or pigment by which even ultraviolet light can be transformed

to visible, for example, blue—light. These pigments are used in such diverse ways as in outdoor advertising, black light displays, and laundering. In the latter case, a residue of the “brightener” is left in the cloth, not only to reflect white light but also to convert ultraviolet light into blue light, thus offsetting any yellowness and reinforcing the white appearance. A non luminescent pigment as melanin could affect the signal of a part of the spectrum of nanoparticles. If the complex melanin-nanoparticle presents photoluminescence, the emitting signal could be in a different region of the spectrum far from the original signal.

Some insights were found in the literature; for instance, some pigments are used as indicators due to the interactions they have with quantum dots [13], there is a therapy based on an activation by mean of a laser of a chromophore present in a nanoparticle to localize cell damages [14], the nanoscale interaction of silica to fix chlorophyll as an electron carrier [15].

Much more work is needed to really determine why nanoparticles signal with presence of skin changes the profile of some part of the plot in comparison with no presence of skin in the tape.

It was tested the proportionality of the signal to be sure intensity was proportional to the nanoparticles concentration (Figure 3).

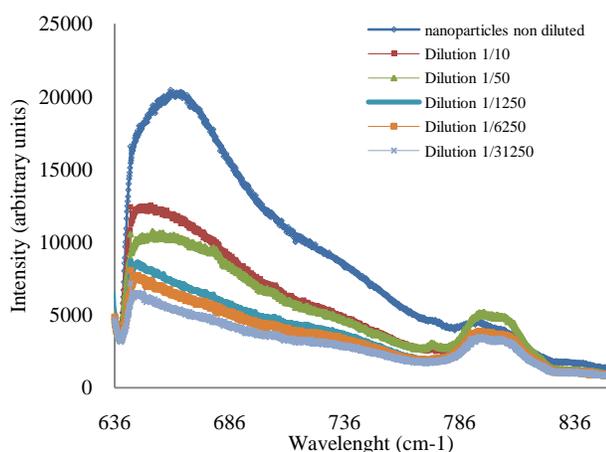


Figure 3. Test to evaluate the proportionality of intensity and amount of polyethylcyanoacrylate nanoparticles.

Tape stripping was done at different times (Figure 4). As we can see the results are very interesting. The nanoparticles are distributed among the stratum corneum layers very different depending on the time. For instance, it is notorious that at 30 min the highest nanoparticles concentration is in the first layers, this is due to they have not reached the quantity to diffuse to deeper layers. After one hour the distribution of the nanoparticles is different because they occupy the totality of the internal space and only the size distribution plays an important role in the distribution.

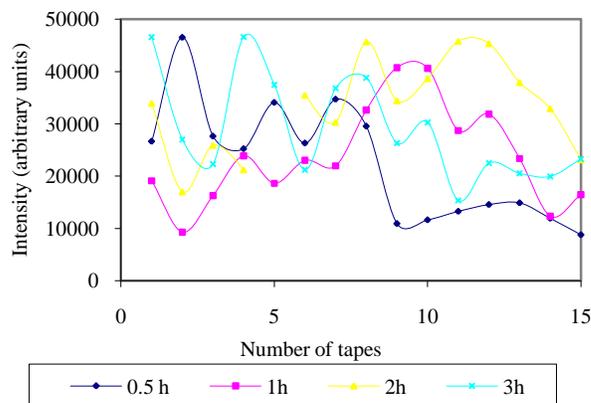


Figure 4. Amount of nanoparticles into the stratum corneum layers. The number of tapes indicates the nanoparticles diffusion; the bigger number indicates the deeper distance.

The two hours plot shows an expected tendency because is logical that when times goes by, the nanoparticles have been displaced to deeper layers but when three hours test was done, the profile broke the tendency and the highest nanoparticles amount is located in the middle layers. We do not have a suitable explanation for three hours. However, we know that in the skin exist some mechanisms to take off estrange objects as a protection for the body. It is something that we need to study more. In other paper our group published [16], we propose a mathematical model to explain this behavior but biologically spoken the answer is more complicated.

Maximum intensity is almost the same for all the times. This is not expected, but explainable. This behavior seems like only some determined quantity had been administrated during the test with no excess of nanoparticles and a constant supply was given to the skin. We think saturation is reached in the first layer in a short time due to a tapping of the diffusion routes in the skin, and then the nanoparticles entering into the skin only take a place and diffuse depending on the particle size. Also it is worth to mention that we are evaluating only 15 cm² approximately and may be the radial diffusion is playing an important role to find a maximum in the testing skin area.

4 CONCLUSIONS

It is notorious that this new tool to follow polymeric nanoparticles through the skin could be very useful to develop new drug delivery systems. The skin is the biggest organ in the body and it has a lot surface to apply drugs, for that reason, it is important to know how the transport of these drugs is. Nanoparticles are novel drug delivery systems that can help to the physicians to better attend the health needs. These nanoparticles have a lot of advantages because they avoid the immune system, increase the stability of drugs and can be used as carriers to deliver

drugs in the target increasing the specificity and as a consequence the side effects decrease.

As it was mentioned, this is the first time photoluminescence is used for tracking the transport of nanoparticles through the stratum corneum. There are a lot of illnesses in the skin and the study of the action of drugs in this organ is not very extended. Skin is the first barrier to the environment and it is important to know how drugs, toxic agents, etc. can penetrate its defenses and may be reach the general blood. The knowledge of the transport can help to understand the mechanisms of entrance for estrange agents and increase or decrease the amount of those substances depending of what the body needs.

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