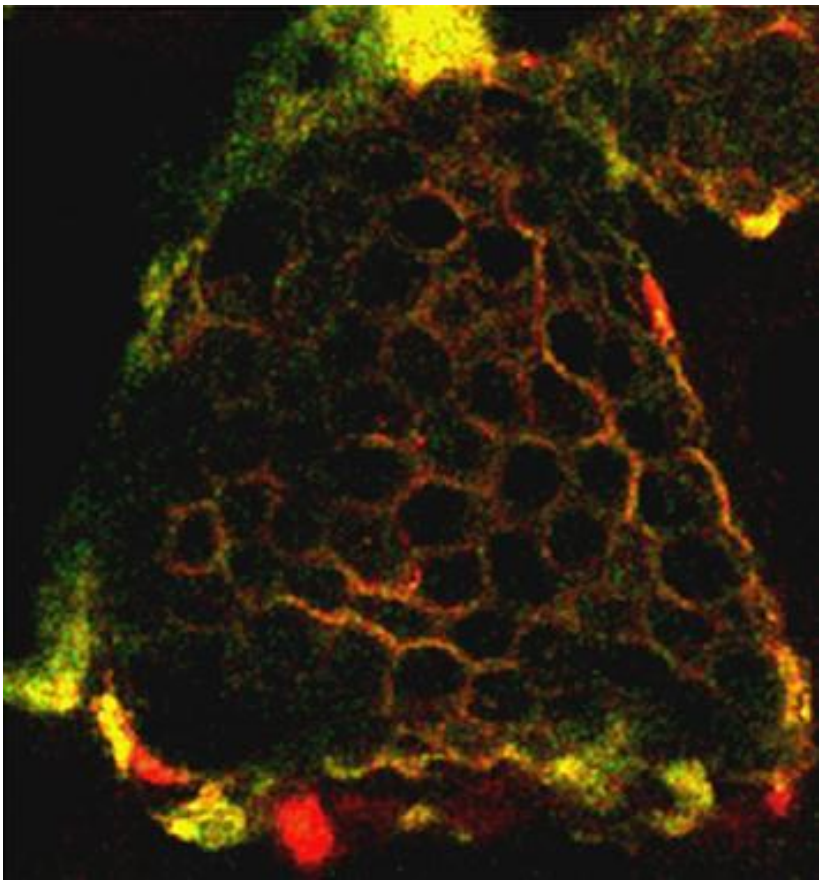
CHALMERS



Dermal Drug Delivery of Gold Nanoparticles in Cubic Phase

Bachelor of Science Thesis

ANDREAS ALBREKTSSON & MARIA JAPLIN

Department of Chemical and Biological Engineering
Division of Pharmaceutical Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden, 2012
Report No. 400

Report no. 400

Dermal Drug Delivery of Gold Nanoparticles in Cubic Phase

Andreas Albrektsson

Maria Japlin

Department of Chemical and Biological Engineering

CHALMERS UNIVERSITY OF TECHNOLOGY

Göteborg, Sweden 2012

Dermal Drug Delivery of Gold Nanoparticles in Cubic Phase

ANDREAS ALBREKTSSON
MARIA JAPLIN

© ANDREAS ALBREKTSSON & MARIA JAPLIN, 2012.

Technical report no. 400
Department of Chemical and Biological Engineering
Chalmers University of Technology
SE-412 96 Göteborg
Sweden
Telephone + 46 (0)31-772 1000

Cover: [Picture of skin exposed to gold nanoparticles in cubic formulation photographed using two-photon microscopy.]
Göteborg, Sweden 2012

Abstract

Reasons why medicinal formulations have been studied a lot over the last couple of years is due to the fact that skin formulations allow for a localized treatment and the possibility to abort the treatment quicker compared to oral administrated drugs. However a problematic issue when using dermal delivery routes is that our skin acts as a remarkable barrier preventing foreign compounds from entering our bodies. The main aim of this thesis is to study gold nanoparticles of three different sizes in cubic formulation, concerning how well it can diffuse into human skin. By performing different diffusion tests, on both the epidermis layer of the skin and full thickness skin, the formulation with gold nanoparticles might show uses for drug delivery purposes. The diffusion tests were performed either in water bath or by using iontophoresis.

Absorbance was measured on the receptor fluids from the diffusion cells and two-photon microscopy was used to analyse the full thickness skin samples. From the receptor fluids collected in the iontophoresis experiment there were indications that particles have made it through and that the smallest sized nanoparticles had passed through more effective than the largest. From the experiments where iontophoresis was not used no indication that gold nanoparticles have made it through the epidermis layer into the receptor fluids was found. The results from the two-photon microscopy of the full thickness skin samples were inconclusive as the gold nanoparticles were not able to be traced in the skin. With the analysis methods used in this thesis no indication that the cubic formulation increased the permeability of gold nanoparticles through the skin could be observed except for the experiments where iontophoresis was used.

The report is written in English.

Keywords: gold nanoparticles, cubic formulation, glyceryl monooleyl ether, human skin, iontophoresis, heat separation method, uv/vis, diffusion cells

Sammanfattning

Anledningar till att läkemedelsformuleringar har blivit uppmärksammade de senaste åren beror på faktumet att formuleringar som kan appliceras på huden medför en lokaliserad och lättavbruten behandling i jämförelse med piller. Problematik som uppstår med att använda formuleringar är att huden agerar som en väldigt bra barriär mot främmande ämnen. Det är därför viktigt att hitta formuleringar som lättare tar sig in i huden. Målet med denna rapport är att testa hudpermeabiliteten av guldnanopartiklar av tre olika storlekar i en kubisk formulering. Genom att utföra olika diffusionsexperiment, på både epidermislagret av huden och även fulltjock hud, så ska det påvisas om denna formulering med guldnanopartiklar uppvisar bra egenskaper för att användas som läkemedelsformulering. Diffusionstesterna genomfördes både i vattenbad eller genom att använda jontofores.

Absorbans mättes på receptorlösningarna från diffusionscellerna och tvåfotonmikroskopi användes för att analysera fullhudsproverna. Utifrån de insamlade receptorlösningarna från jontoforesexperimenten framkom indikationer att guldnanopartiklar hade tagit sig genom epidermislagret och att de minsta nanopartiklarna passerade igenom mer effektivt i jämförelse med de största. I experimenten där jontofores inte användes framkom inga indikationer på att guldnanopartiklar har tagit sig igenom epidermislagret in till receptorvätskorna. Resultaten från tvåfotonmikroskoperingen av fullhudsproven var ofullständiga då guldnanopartiklarna inte gick att spåra i hudproverna. Med de analysmetoder som används i denna rapport kunde inga indikationer på att den kubiska formuleringen skulle öka permeabiliteten genom hud påvisas förutom i experimentet där jontofores användes.

Rapporten är skriven på engelska.

Nyckelord: guldnanopartiklar, kubisk formulering, glyceryl monooleyleter, människohud, jontofores, värmesepareringsmetod, uv/vis, diffusionsceller

Acknowledgements

We would like to thank our supervisors Hanne Evenbratt and Stina Guldbrand for all the support, encouragement and interesting times during our bachelor thesis project. Additionally we would like to thank the department of Pharmaceutical Technology at Chalmers for allowing us to use their equipment and facilities. We would also like to thank Magnus Svensson for all the help with the UV/VIS instrument. Finally special thanks go to the other thesis project students at the department especially Mattias Andersson, Åsa Hermansson, Nina Khosravi and Sofie Olsson.

Table of contents

1 Introduction

A formulation can be a cream in which for example pharmaceuticals can be inserted and it is the formulation that transports the pharmaceuticals into the skin. This can be likened to a skin lotion. The importance of finding new formulations which have good permeability properties through human skin is very important, not least for the pharmaceutical industry, since a lot of medicinal creams and formulations are used today. The penetration of the skin is crucial in order to make sure that the drug can reach the bloodstream and begin to circulate within our bodies. In this project a certain cubic formulation, or liquid crystalline cubic phase, was to be investigated as a potential drug carrier system with gold nanoparticles. The cubic formulation is called cubic because of its structure. Other types of liquid crystalline structures are for instance hexagonal or lamellar.

1.1 Background

The cubic formulation used in this project was made by mixing glyceryl monooleyl ether (GME), propylene glycol (PG) and Milli-Q water. This results in a lipid based mixture where GME is the lipid used. This cubic formulation has previously shown promising permeability abilities through human skin [1-3].

Kuntsche et al. studied different lipid nanoparticle formulations, in respect to dermal drug delivery abilities, showing variations of corticosterone, a steroid hormone, permeability in the outermost layer of the skin by using fluorescence microscopy and thermal analysis. When they used nanoparticles in cubic phase an increased effect in skin permeation was noticed. The reason for this according to Kuntsche et al. is because the lipids in the skin might interact with the lipids in the formulation and therefore enhance penetration [3]. This increase in corticosterone permeability was only discovered when the cubic formulation was used.

A study by Bender et al. showed that due to the better skin permeability properties of the cubic phase, the cubic formulation needed less drug content than the commercial reference [2]. The results from these studies indicate that the cubic formulation seems promising when it comes to drug delivery through the skin.

Gold nanoparticles have shown great potential as drug carriers and also possess physical properties that give them good permeability through tissue [4]. Gold nanoparticles also show special interactions with light of different types, which make

them easy to detect [4]. Gold nanoparticles in combination with the cubic formulation has therefore potential to work as a good drug delivery system.

1.2 Purpose

The main purpose with this project is to investigate how effective the cubic formulation is when used for dermal delivery purposes. By performing different experiments with the formulation on human skin and by analysing the amount of gold nanoparticles that have passed through the skin, the aim is to find some answers to if the cubic formulation is successful for dermal delivery purposes. It is also of interest to find out how the different sizes of the gold nanoparticles affect the permeability through the skin. Another purpose with the project is to find a good heat separation method to separate the outermost layer of the skin, the epidermis, from the rest of the skin.

1.3 Scope

In this study different limitations had to be made due to time limitations and other aspects. Concerning the formulation it could only be studied on donated skin samples in diffusion cells and not in vivo. This thesis is meant to study whether or not the formulation can increase the amount of gold nanoparticles that can diffuse through the skin. The thesis does not focus on how the gold nanoparticles have entered the skin samples only if the particles have managed to enter. Additionally the skin samples used in this thesis had been frozen for a period of time and where not as fresh as they could have been.

2 Theory

In this section the theory of the project will be discussed. The structure of human skin, the basic principles of nanoparticles, information about gold nanoparticles and the cubic formulation, and descriptions of some methods that might need a deeper understanding will be discussed.

2.1 Human skin and its functions

The human skin has some remarkable ways of stopping foreign particles from entering our bodies and bloodstream [5]. The skin consists of three main layers, which are the epidermis, the dermis and the subcutaneous layer (see figure 1). The general functions of these different layers vary between them [6-7].

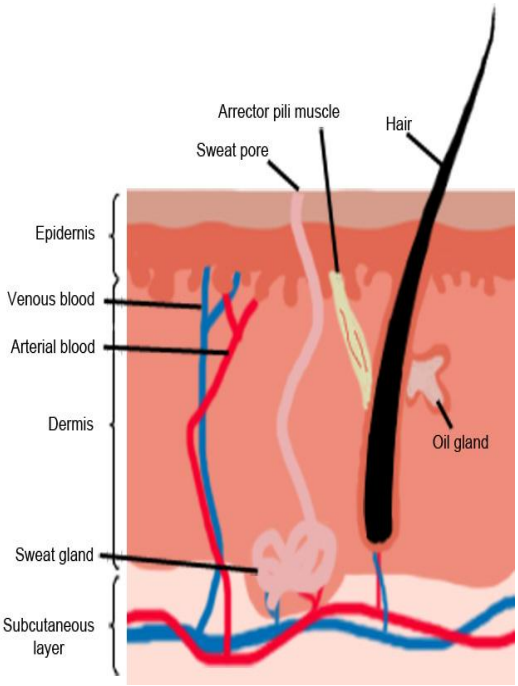


Figure 1 - A simplified picture of the different human skin layers and how they are built up [F1].

The deepest skin layer is called the subcutaneous layer. This layer contains fat cells for insulation and padding [6-7].

The dermis, which makes up for the biggest part of the three layers, is located between the subcutaneous and the epidermis layer (see figure 1). Dermis contains nerve cells, blood vesicles, sweet glands, and hair sacks [7]. The hair sacks are, in some cases, considered as potential weak spots in our skins defence against foreign substances [5].

The epidermis, the outermost part of the skin, consists of five different layers. These layers are from the inside and out: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and the outermost layer stratum corneum that consist of dead skin cells [7]. The cells in the stratum corneum have migrated from stratum basale and are called keratinocytes [8-9]. The dead skin on the surface falls off or is peeled off and is replaced by new cells.

The general function of the skin is to prevent foreign substances from entering the body, such as different chemicals and microorganisms. Other important roles our skin play is as a protective barrier against ultraviolet radiation, as protection against different temperatures, and as a barrier to prevent water loss [6-7]. Vitamin D is created in our

skin when it is exposed to sunlight and the skin also has a unique way of protecting itself and repairing damage caused to it.

All the different properties of our skin mentioned above give a general idea on why introducing a foreign substance, even really small molecules, can prove to be difficult. Another aspect that makes the skin so special is the fact that the thickness of the skin varies a lot depending on what part of the body that is being studied. Because of this it is important to make sure that the skin samples used during the experiments in this thesis come from the same body area. To test skin from as few patients as possible is also something that needs to be considered in the experiments to get valid results.

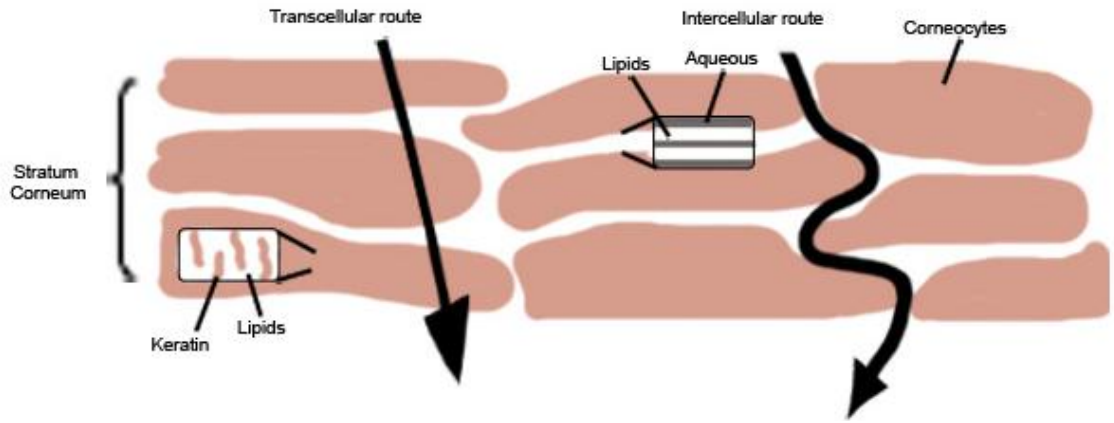


Figure 2 - A representation of the stratum corneum and its pathways [F2].

When studying how drugs or substances can be absorbed in the stratum corneum there are two important pathways that need to be considered. These two are the intercellular route and the transcellular route (see figure 2 above) [10]. These pathways are officially recognized while a potential third one, through our hair sacks and pores, is still being debated [5].

Epidermis is considered to be the most important barrier of the skin and if something has passed through this layer it has the potential to reach the rest of the body through the blood stream [5]. Because of this it was mostly the epidermis layer that was used in the following experiments.

The stratum corneum is often described as a brick-wall. While using this definition the corneocytes, the cells in stratum corneum, make up for the bricks and the intercellular lipids (see figure 2) are the mortar holding the wall together [10].

2.2 Nanoparticles

One definition of nanoparticles is that they are particles that range from sizes of 1 nm to about 100 nm [11]. These particles have become very interesting to scientists in many fields because of their special properties. They are described as having three major physical properties; they are highly mobile in the free state, they have a huge specific surface area, and they may exhibit quantum effects [11]. Of these three properties that nanoparticles possess, the quantum effects are the most interesting and relevant to this project. The quantum effects, the optical properties of nanoparticles, make them suitable to use with the detection methods used in this project.

Medicine, catalysis, engineering and environmental areas are some of the fields that have acknowledged nanotechnology. In our project we will focus on the special properties that concern the medicinal area. In this thesis gold nanoparticles will be used.

2.3 Gold nanoparticles

One type of nanoparticles that are interesting for medical purposes is gold nanoparticles, which will be used in this project. Gold nanoparticles have the properties of being biocompatible, chemically stabile, easily synthesized and have good permeability through tissue [12]. Gold nanoparticles also show special interactions, as luminescence, when irradiated with light of a frequency that matches their plasmon resonance frequency [31]. This frequency is dependent of the size of the nanoparticles, and if they have formed aggregates (several of them grouped in a cluster) the distance between them is believed to strongly contribute to the plasmon resonance. These features are crucial for detecting the particles with microscopy methods.

They can be used for medicinal purposes as drug carriers, in thermal therapy, as contrast agents and as sensors [4, 13]. When used for drug delivery the surface of the nanoparticles are designed to adsorb drug molecules [4] and the particle is then introduced into the cells. In the case of thermal therapy, the idea is to use controlled heating of the nanoparticles to manipulate the neighbouring tissue when the particles are placed in a target area. When the area is lit externally the gold particles absorb light and free electrons are excited and induce heat which is then spread to the surrounding tissue [4]. The same idea is applied when the particles are used as contrast agents.

Gold nanoparticles can also be functional as sensors for registering the presence and concentration of substances. With different detection methods, using the optical properties of gold, different responses are achieved [4]. Because of gold's special interactions with light, they can be used for visualization purposes [4, 11-12]. Several of

these methods mentioned above have made progress in for instance cancer treatment [12].

There have been discussions about the allergy aspect and the toxicity of gold nanoparticles. Research concerning this has so far not shown any evidence of permanent damage to affected cells [4, 14].

Labouta H. I. et al. have made a study concerning the essential factors of gold nanoparticles penetration through human skin. Their results implied that the nanoparticles, which ranged from 6-15 nm, penetrate the skin mainly through the intercellular pathways, which have been mentioned in section 2.1. This was compared to how drug molecules make their way into the skin and they seem to use the same pathway. The complex nature of the skin is of great concern when trying to determine how the particles make their way through the skin and also how effective the permeability is through the skin. In the study mentioned above they found that their formulation, which was toluene and water, had a minimal effect on skin penetration of the gold nanoparticles and that the 6 nm particles showed better permeability than those that were 15 nm. The penetration was believed to depend primarily on the physical characteristics of the particles and the size of them. This is something that is interesting in this project as we are going to study the effects of penetration using a specific cubic formulation. Skin exposure time is also a parameter that was discussed. In the study made by Labouta H.I. et al., incubation time of at least 6 hours was needed to be able to study the results [22].

The gold nanoparticles are often in a colloidal form, which often means that they are dispersed in a fluid. The particles are in need of a stabilizing agent, which prevents them from aggregating. The stabilizer is linked to the surface of the gold nanoparticles and particles that have stabilizers around them repel each other due to steric effects. Colloidal stability is thereby provided [4]. Colloidal gold nanoparticles with polyethylene glycol as stabilizer will be used in this project.

2.4 The Cubic Formulation

The cubic formulation consists of a mixture of glyceryl monooleyl ether, propylene glycol and water [15]. A phase diagram of these three constituents that Engström et al. have constructed can be seen in figure 3.

To determine if a formulation is cubic it can be observed between crossed polarisers. If the sample does not contain any glowing parts, then it is cubic. If it does contain glowing parts then it contains hexagonal or laminar fragments. This has to do

with the structures of the different phases. The hexagonal and laminar phases have structures that turn the light and therefore these structures glow when they are placed between the crossed polarisers. The cubic structure does not have this ability. It is also, in some cases, possible to look at the viscosity of the sample to determine the structure. The cubic phase is solid and gel like while the laminar phase show less viscous properties [16]. The hexagonal phase is also highly viscous so in this case it is necessary to observe the sample between crossed polarisers.

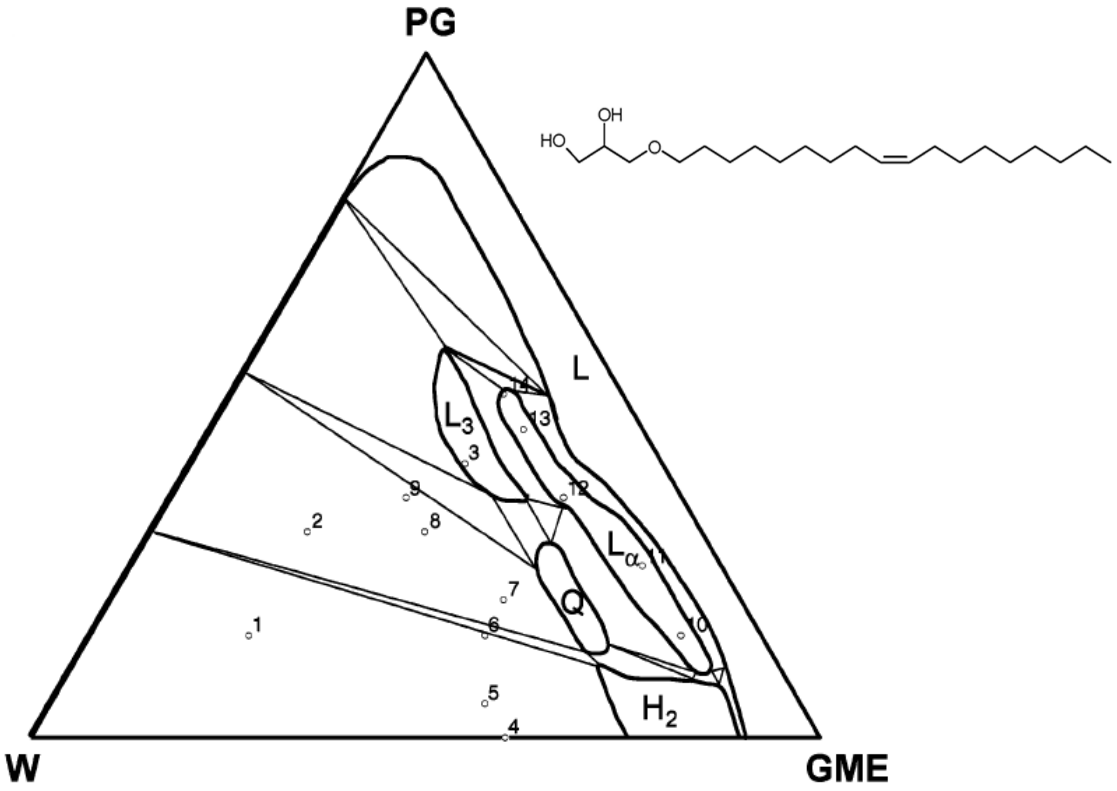


Figure 3 – Approximate phase diagram for the different phases that occur when mixing glyceryl monooleyl ether (GME), propylene glycol (PG), and water (W). Symbols: liquid phase, L; lamellar phase, L_α; reversed hexagonal phase, H_2; cubic phase, Q; sponge phase, L_3. The molecular structure of GME is shown to the right [F3].

The structure of the cubic phase is built up by small micelles which are regularly packed (see figure 4) [16]. In the cubic structure, channels are formed in which different particles can attach. It is believed that the lipids in the formulation and the lipids in stratum corneum are interacting. It is believed that this in combination with the structure of the cubic phase is advantageous when it comes to drug delivery [3].

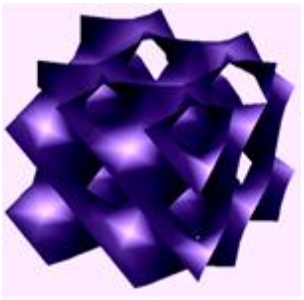


Figure 4 – Data simulation showing the structure of the cubic phase performed in MATLAB [F4].

2.5 Iontophoresis

The device that is used for iontophoresis consists of a DC power source and two electrode compartments [17]. The electrodes are placed so that a voltage difference is induced over the target area. By applying a charge over a membrane, which in this project is skin, the particles are drawn to the other side due to the opposite charge. This method makes it easier for hydrophilic and charged molecules to penetrate the skin and can also facilitate the delivery of nanoparticles as those are charged.

Because of the use of a voltage potential, it is important to consider that there might be competing ions in the formulation. The results might be affected by these ions [17]. Something that might also affect the iontophoresis tests is that different skin samples have different resistance and that the resistance seems to decrease with increased current [28]. A possible factor that makes the resistance lower is the voltage difference which affects the lipids in the skin [29]. The decreased resistance will facilitate the transport of charged particles. These changes are believed to be due to the channels that exist in the epidermis are being enlarged or that new channels are being created when iontophoresis is used [29].

In this project iontophoresis is used as a control method to evaluate if the effect of an outer influence can affect the permeability of gold nanoparticles in cubic formulation.

2.6 UV/VIS absorbance spectroscopy

UV/VIS absorbance spectroscopy is a method that uses the fact that different compounds absorb different wavelengths of light [30]. Depending on the colour of the sample it absorbs certain wavelengths of its complementary colour. In UV/VIS the wavelengths used are those in the visible and ultraviolet spectra [30]. By measuring the absorbance spectra of a compound it is possible to find the concentration that is present in a sample. Lambert-Beers law states that the absorbance is dependent on the molar absorptivity, the length of the cuvette and the concentration. The equation can be seen in equation 1.

$$A = \varepsilon * l * c \qquad (1)$$

where A is the absorbance, ε is the molar absorptivity ($L*mol^{-1}*cm^{-1}$), l is the length of the cuvette (cm) and c is the concentration of the solution (mol/dm^3) [30]. With this method it is possible to find absorbance peaks for the different gold nanoparticles used in these experiments and see if there are any particles that have passed through the skin samples after the tests.

2.7 Two-Photon Microscopy

The concept of fluorescence microscopy is to make fluorophores, fluorescent molecules, to fluoresce by sending photons that excite the molecules [23]. In confocal microscopy one high energy photon is sent to excite a fluorophore and in multiphoton microscopy more than one photon, with much less energy, is used to reach the same excitation state [23]. In figure 5 the absorbance of different constituents in our skin is shown. When using confocal microscopy wavelengths around 500-600 nm are used and it can be seen in the figure that the skin absorbs a lot of energy at this wavelength. When using multiphoton microscopy the wavelengths are higher, around 700-800, and it can be seen in the picture that the skin absorbs much less energy at these wavelengths. A low absorbance of the energy results in a higher transmittance, which leads to the possibility to take deeper images of the sample and cause less damage to it. This method is therefore preferred when looking at biological materials [23].

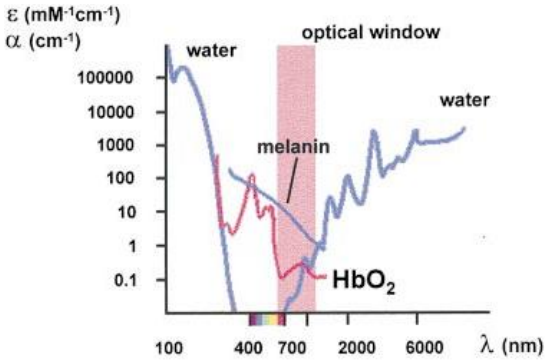


Figure 5 – The absorbance spectra of the most common constituents in human skin were the molecular exctinction coefficients for oxygenated haemoglobin (HbO2) and melanin and the absorption coefficient water are visualized [F5].

With multiphoton fluorescence microscopy it is possible to make 3D-images of a sample and by using this method it is possible to take pictures from the inside of the skin samples that will be used in this thesis. Two-photon microscopy is used in this project.

3 Materials and methods

In this section the different chemicals and machines that have been used in this thesis is presented. How the preparation of the ingredients and the human skin was executed and how the experiments were performed will also be described. Different abbreviations that will be used for methods and chemicals throughout this report are presented in the beginning of this section.

3.1 Chemicals and skin samples

Glyceryl monooleyl ether (95% purity) (GME) (Nikko Chemicals Co., LTD, Tokyo, Japan) and propylene glycol (PG) (Apoteket, Gothenburg, Sweden) was used to make the cubic formulation. The water used was of Milli-Q quality (18.2 MΩ.cm 25ºC, Millipore). The formulations were made in 4mL glass vials, which were provided with crimp caps. Gold nanoparticles (AuNP) were provided as colloidal gold with a diameter of 5, 20 and 40 nm. Each of the samples had a volume of 20 mL and contained 0.01% gold nanoparticles. The different concentrations were $5.0 * 10^{13}$AuNP/mL, $7.0 * 10^{11}$AuNP/mL, and $9.0 * 10^{10}$ AuNP/mL respectively [19]. The samples were purchased from Nanocs Inc. (Boston, USA) and the stabilizing agent was polyethylene glycol (PEG).

The human skin was provided by Sahlgrenska University Hospital (Gothenburg, Sweden) and the use of the skin has been ethically approved. All skin samples were from females that had undergone breast reduction surgery. The skin samples were prepared as 4 cm^2 squares and the samples used were from two different patients. The samples were stored in a freezer at -80ºC awaiting use, in order to keep the samples as fresh as possible.

Phosphate buffered saline (PBS) (Sigma-Aldrich, Co., St Louis, USA) in the heat separation method and was used as receptor fluid in the diffusion cells (Werner-Glas, Limhamn, Sweden) which contained 15 mL receptor fluid and was of Franz type. The receptor fluids were put in falcon tubes (Eppendorf, Hamburg, Germany) after the experiments. 2-methylbutane Reagent Plus >=99% (Sigma-Aldrich Inc., St. Louis, USA) and Tissue-Tek O.C.T Compound (Sakura Finetek Europe B.V., Alpheen aan den Rijn, the Netherlands) was used in the freezing process of the skin samples.

3.2 Machines

The ultrasonic bath used in the experiments was an Elma Transsonic 700 (Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany), the centrifuge was a Sigma

4K15 (DJB Labcare Ltd, Buckinghamshire, England) and the spectrophotometers used was a Cintra 40 (GBC Scientific Equipment Pty Ltd, Braeside, Australia) and PerkinElmer model Lambda 900 (PerkinElmer™ Instruments,Waltham, USA). The iontophoresis tests were performed with a Bio Rad model 1000/500 Constant Power Supply (Bio Rad Laboratories, Hercules, USA).

The multi-photon fluorescence microscopy images were acquired on a LSM 710 NLO (Carl Zeiss MicroImaging GmbH, Jena, Germany) microscope equipped with a Mai Tai DeepSee pumped femtosecond laser (Spectra-Physics, Mountain View, CA) as light source. The fluorescence emission was detected through a Plan-Apochromat 20x/1.0 water immersion lens (Carl Zeiss MicroImaging GmbH, Jena, Germany) via the descanned optical pathway. The fluorescence of the sample was detected by scanning at different depths; obtaining so called z-stacks (scan area: 512 × 512 pixels). For further detailed imaging, single images with higher resolution were obtained (1024 × 1024 pixels).

3.3 Preparations of ingredients

Before the nanoparticles could be used, the containers with the samples were put in an ultrasonic bath for approximately 1 minute. This was needed to make sure that the AuNP were separated evenly in the entire solution and not aggregated before use.

The PBS and Milli-Q water solution, which was used for the diffusion cell tests and the heat separation, was made by putting one PBS tablet for every 200 mL of Milli-Q water. All other ingredients were used without any further preparations.

3.4 Preparation of the cubic formulation

Samples were prepared in 4 mL vials. GME was taken from the refrigerator and melted in a vial using warm tap water with a temperature of approximately 55°C. The 4 mL vials were first placed on a scale and the different ingredients were weighed into the vials, using 1 mL sterile syringes. The vials were in total filled with about 1 gram each. The proportions of the ingredients were 62.7 mass % GME, 14.7 mass % PG, and 22.6 mass % Milli-Q water or AuNP solution. All the ingredients were assumed to have the same density as water. The AuNP solution was used without further dilution when the cubic formulations with AuNPs were made. Blank cubic samples were made without AuNPs. References were also made by taking the same amount of AuNPs as in the corresponding cubic phase sample and dilute it with Milli-Q water.

After the preparation of the samples the vials were provided with crimp caps to keep them under minimum exposure to the surroundings. The cubic formulation samples were centrifuged until they were well mixed and no colour differences could be observed. To achieve this, the samples were centrifuged upside down every other time. When the samples were upright they were centrifuged at 4500 rpm for 5 minutes at a time and when put upside down they were centrifuged at 3500 rpm for 5 minutes at a time. The samples where centrifuged at different velocities because the vials could not be placed as stable when they were centrifuged upside down.

To confirm that the samples had become cubic after preparation, the samples were observed between crossed polarisers.

3.5 Skin preparation

In this thesis an efficient heat separation method had to be implemented in order to separate the epidermis from the dermis skin layer. A thorough literature study was conducted and after discussing the various methods with our supervisors a suitable method for this thesis was developed. After studying the different articles from the literature study it became clear that the most common method used was to submerge the full thickness skin samples in different solutions with a temperature of approximately 60°C [21, 25-27]. In this thesis PBS was used as the solution in which the full thickness skin samples was submerged because PBS is frequently used when a solution that has similar properties to other solutions within our bodies is needed [24].

In order to study whether the time spent in the solution had any influence on the separation effect, three different samples were exposed to the solution for 30, 60 and 120 seconds respectively. 60 seconds was used in this thesis and after the exposure to the solution the epidermis layer was removed by using a scalpel and tweezers.

Before the separation of the skin layers excess fat could be trimmed off with a scalpel. The epidermis layer was handled with care, as it was important that it was not damaged. Even a tiny perforation in the skin samples can affect the results when studying diffusion through skin using diffusion cells.

3.6 Standard curves

Standard curves for the original AuNP solutions were made. When the absorbance was measured for a sample the results could easily be transferred to the standard curve and a concentration could be found.

The measurements were made as wavelength scans between 200-900 nm and the absorbance measurements of the original solutions can be found in Appendix A. Peaks for the three original solutions (5, 20, 40 nm) could be found at 514, 524 and 532 nm respectively. The original solution was diluted five times with known amounts of Milli-Q water and measurements were taken between every dilution. The maximum absorbance values from the peak that the respective size of AuNPs was taken from each measurement. From these absorbance values and the starting concentration of the original AuNP solution the concentrations could be calculated for the diluted original solution. These values should form a straight line from which unknown concentrations could be found after the absorbance of a sample has been measured. A quartz cuvette with the length of one centimetre was used and all standard curves were measured with the Perkin Elmer spectrophotometer.

3.7 Dermal delivery experiments using Franz cells

In this section of the report the main skin experiments will be described. The experiments were divided into three parts; *Epidermis experiments*, *Iontophoresis experiments* and *Full thickness skin experiments*. In the *Epidermis*- and *Iontophoresis experiments* the purpose was to analyses the receptor fluids. In the *Full thickness experiments* the purpose was to analyse and visualise if and how far into the full thickness skin samples the cubic formulation has the ability to take the nanoparticles with two-photon microscopy. When referring to the reference of a sample it is the corresponding AuNP diluted in Milli-Q water to get the same concentration as in the cubic formulation sample.

3.7.1 Epidermis experiments

Experiments with the epidermis layer were performed in Franz diffusion cells (see figure 6). Five tests was performed simultaneously, three with the cubic formulation and AuNP, one with reference from the corresponding AuNP and one blank cubic formulation without nanoparticles. The tests were made according to table 1.

Table 1 – Table of the six test sessions that were made in the Epidermis section. The numbers represent how many of each sample that were made at the different sessions. 5 h and 21 h stands for how many hours the sessions were. 5, 20, and 40 nm cubic represents the AuNP in cubic phase. AuNP + H_20 represent the corresponding reference solution. Blank stands for the blank cubic phase without AuNP.

	5nm cubic	20nm cubic	40nm cubic	AuNP+H20	Blank
5h	3			1	1
5h		3		1	1
5h			3	1	1
21h	3			1	1
21h		3		1	1
21h			3	1	1

Diffusion cells were prepared by placing a stir bar in the receptor chamber and filling it with PBS solution. The PBS solution was filled up so that it had contact with the membrane, which in these experiments was the epidermis layer of human skin.

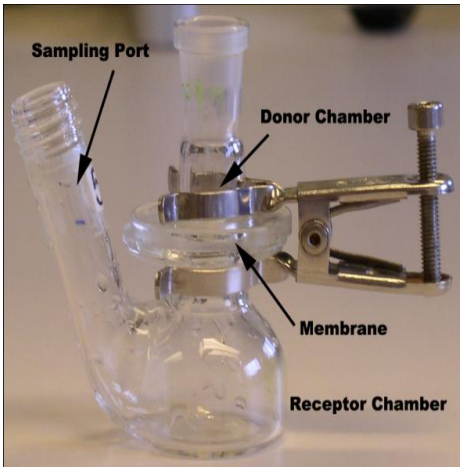


Figure 6 - The assembly of a diffusion cell.

When the heat separation method was done and the epidermis layer was separated from the dermis layer of the skin, epidermis was placed, with the outside up, on a glove covered finger. The cubic phase was carefully placed on top of epidermis with a spatula. It was then placed in a prepared diffusion cell with the formulation upwards. The sample with the reference solution, which was first placed in an ultrasonic bath, was prepared by putting epidermis in the diffusion cell, with the stratum corneum facing upwards, and then dripping approximately 0.3 mL of the reference into the donor chamber. The diffusion cell was sealed with a clamp and the donor chamber and the sampling port were covered to prevent evaporation.

The diffusion cells were put in a water bath after they were checked for air bubbles. By tilting the diffusion cells the bubbles could be forced to the sampling port. This was done to make sure that there was always contact between the skin sample and the PBS solution. The cells were checked for bubbles after 15 minutes and then every hour if there were any left. The temperature in the water bath was 34°C to simulate the temperature that the skin normally has.

The diffusion was stopped after 5 or 21 hours and the diffusion cells were taken out of the water bath. The diffusion cells were carefully taken apart and the excess

formulation was carefully removed from the epidermis with a Kleenex tissue. The excess reference solution was decanted out before the diffusion cell was disassembled.

A biopsy of the skin was placed in a small plastic mould which was partially filled with Tissue-Tek O.C.T compound. After placing the skin piece in the mould additional Tissue-Tek was added so that the whole mould was filled (see figure 7). The mould was then submerged in to a mixture of 30 mL of 2-methylbutane and 2-3 small spoons of dry ice to freeze the sample. The container with 2-methylbutane and dry ice was refilled when needed. It was important that the mixture of 2-methylbutane and dry ice did not come in contact with the sample and that the stratum corneum was facing upwards. The samples were then covered with Parafilm and aluminium foil and put in a freezer at -80°C.

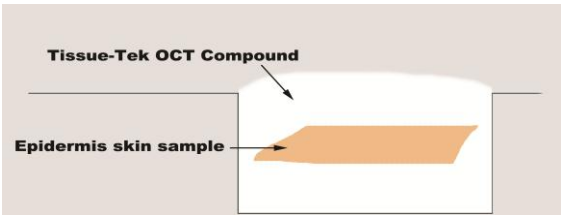


Figure 7 – Sketch of the mould in which the skin samples were frozen.

The receptor fluids from the different samples was put in separate falcon tubes except for the receptor fluid from one of the samples with AuNP in cubic phase which was instead allowed to dry on a watch glass.

Absorbance measurements were performed on all the receptor fluids. The dried samples were dissolved in 1.5 mL PBS solution before measuring. The measurements were made as wavelength scans between 200-900 nm and 1 cm plastic cuvettes were used. The Cintra 40 spectrophotometer was used for these analyses.

3.7.2 Iontophoresis experiments

Iontophoresis was used as a method to see if the outer influence from the voltage difference affected the permeability of the AuNPs compared to the experiments performed in section 3.7.1. The epidermis layer of the skin was used and the skin samples were prepared in the same manner as mentioned in section 3.5. The epidermis was then prepared with formulation and reference in diffusion cells filled with PBS in the same way as in the *Epidermis experiments* in section 3.7.1 (see figure 8). The tests were performed according to table 2.

Table 2 – Table of the three test sessions that were made in the Iontophoresis section. The lines represent which tests that were made at the different sessions. 2 h stands for how many hours the sessions were. 5 and 40 nm cubic represents the AuNP in cubic phase. AuNP + H_20 represent the corresponding reference solution. In the last session one test of each reference solution was made.

	5nm cubic	40nm cubic	AuNP+H20
2 h	2		
2 h		2	
2 h			1 5nm + 1 40nm

Gold nanoparticles have a negative surface charge [18], so the negative electrode was placed in contact with the cubic formulation or reference solution. It was important that the electrode did not come in contact with the skin as this could damage the skin and therefore alter the results of the experiment. The positive electrode was placed in contact with the PBS solution (see figure 8). When all the connections were properly in place the iontophoresis power supply was set to 5 V DC.

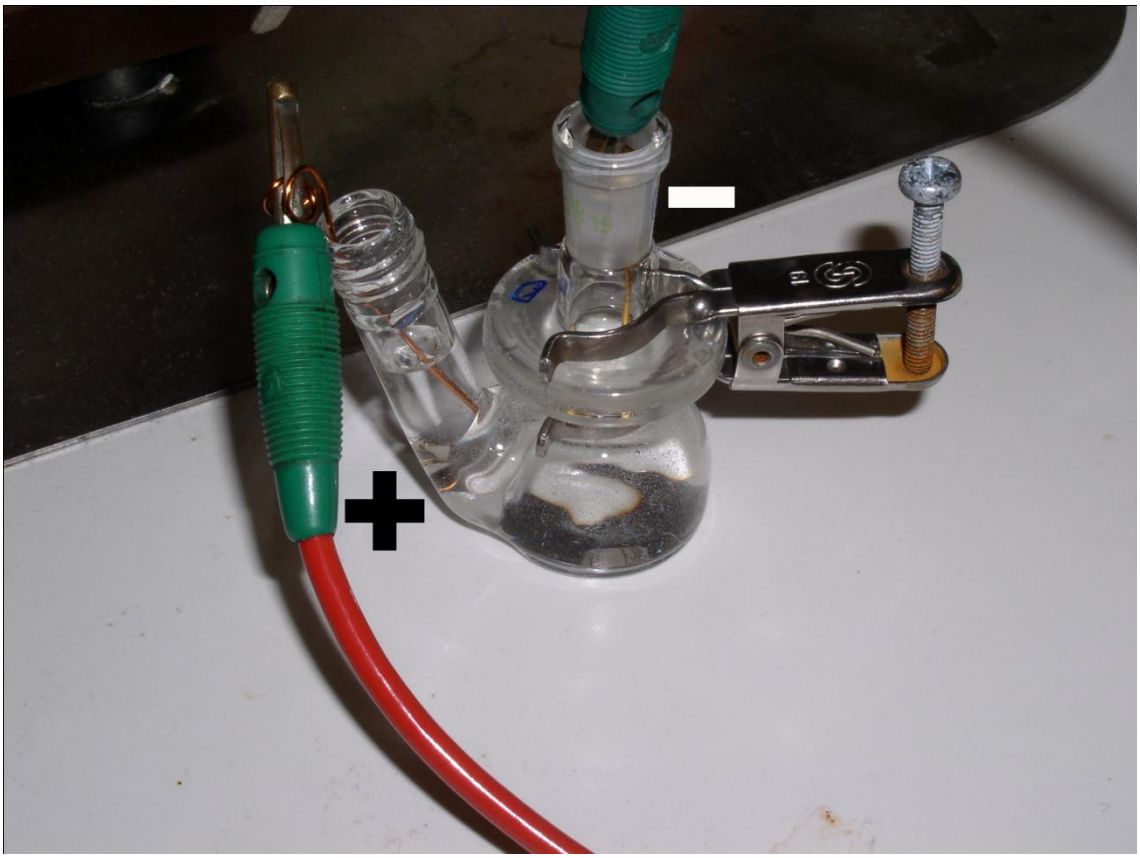


Figure 8 - A picture of how the iontophoresis equipment was setup during the experiment.

The iontophoresis was stopped after 2 hours and the skin samples were removed and deep-frozen by using the same method mentioned previously in section 3.7.1. The receptor fluids were collected in falcon tubes for absorbance measurements. Absorbance measurements were performed on all the receptor fluids after they had been sonicated

and were taken two weeks after the experiment. All the receptor fluids were then dried out on watch glasses. When completely dried they were dissolved with 1.5 mL PBS solution and absorbance measurements were performed again. The measurements were made as wavelength scans between 200-900 nm and a quartz cuvette with the length of 1 centimetre was used. The PerkinElmer spectrophotometer was used for these measurements and skin samples from a different patient than in the *Epidermis experiments* tests were used.

3.7.3 Full thickness skin experiments

In these tests full thickness skin samples were used, hence the epidermis was not separated from the rest of the skin. The skin samples were thawed in room temperature and excess fat was removed from the dermis side of the samples. 1 mL of the original 40 nm AuNP solution was centrifuged (at 2750 g for 90 min) and the cubic formulation was made from the pellet that was formed. This was done to make the concentration higher ($1.5 * 10^{11}$ nanoparticles/mL). Five diffusion cells were then prepared with formulation and reference in diffusion cells filled with PBS and tested in a water bath in the same manner as the *Epidermis experiments* above (section 3.7.1). Two cells contained 5 nm AuNP in cubic formulation respectively 40 nm AuNP in cubic formulation and two contained the corresponding references. One diffusion cell with blank cubic formulation was also prepared. The diffusion cells were placed in the water bath, 34°C, for approximately 22 hours.

When the diffusion experiment was stopped and the skin samples were taken out of the diffusion cells, excess formulation was carefully removed. Biopsies of the exposed skin were punched out from the skin samples. Double-sided tape was prepared by folding the tape so that it had the same height as the skin samples. A hole was then punched out in the tape, before attaching it to a cover glass. The skin piece was then placed in the hole of the double-sided tape with the epidermis facing down towards the cover glass. The skin sample was then carefully pressed down, so that the epidermis was in good contact with the cover glass. A microscope slide could then be attached to the cover glass using the double-sided tape. The skin samples were stored in room temperature for 6 hours before the two-photon microscopy. The skin samples were from the same patient as in the *Iontophoresis experiments* section.

4 Results and discussion

In this section the results from the experiments will be presented and discussed.

4.1 The cubic formulations

The cubic formulations containing 62.7 mass % GME, 14.7 mass % PG and 22.6 mass % Milli-Q/AuNPs were successfully created. This was confirmed by observing the cubic formulations between crossed polarisers and also by studying the viscosity of the formulations. In our case the cubic formulations did not light up between the crossed polarisers and had a solid gel like appearance. These characteristics indicate that the formulations were indeed cubic. From this it can be concluded that the assumption used in this thesis that the constituents had the same density as water was valid.

4.2 Heat separation

The heat separation method that was developed in this thesis resulted in a fast and easy way to separate the epidermis and the dermis layer of the skin. The decision to put the thawed skin samples in a stirred Milli-Q water and PBS solution at 60ºC for ~60 seconds proved to be effective. When exposing the skin samples to the PBS-solution for 30 seconds it did not result in an easy separation and when exposing the skin sample for 120 seconds sample it became too shrivelled up. After 60 seconds in the PBS solution the epidermis could easily be removed from the rest of the skin sample with the use of a scalpel and tweezers. Therefore in this thesis, it was decided to use this heat separation method as our standard method to separate the epidermis from the dermis. The usage of a Milli-Q and PBS solution seemed to work well and did not damage the skin. The temperature and time that the skin was exposed to the warm water solution appeared to enhance a harmless and easy removal of the epidermis layer, which did not tear or fall to pieces.

4.3 Absorbance measurements results

The standard curves and absorbance measurements that were performed on the receptor fluids from the *Epidermis* and *Iontophoresis experiments* will be presented.

4.3.1 Standard curve results

In this section the results of the standard curves for the original gold nanoparticles solutions will be displayed. The norms of residuals that can be seen under each respective graph indicate that the standard curves were good as they are close to zero.

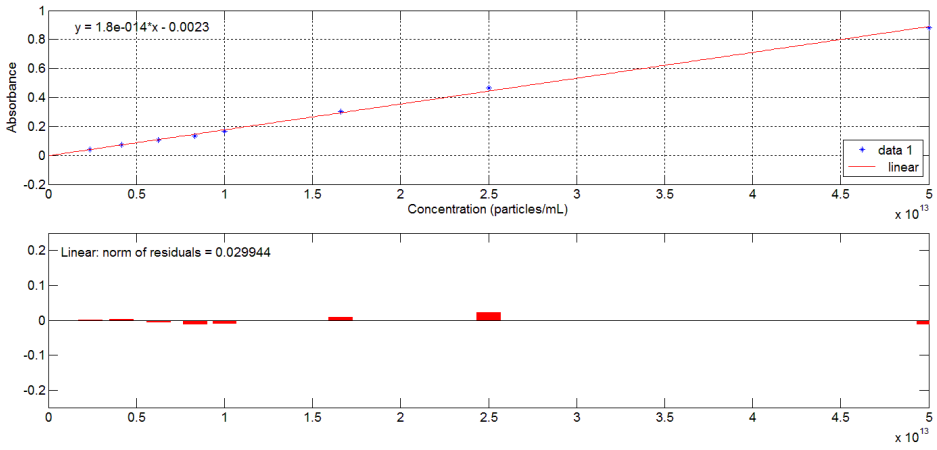


Figure 9 – The top graph shows the standard curve for the 5 nm gold nanoparticles. The bottom graph shows the residuals in order to get an estimate concerning how well the line is fitted to our dilutions.

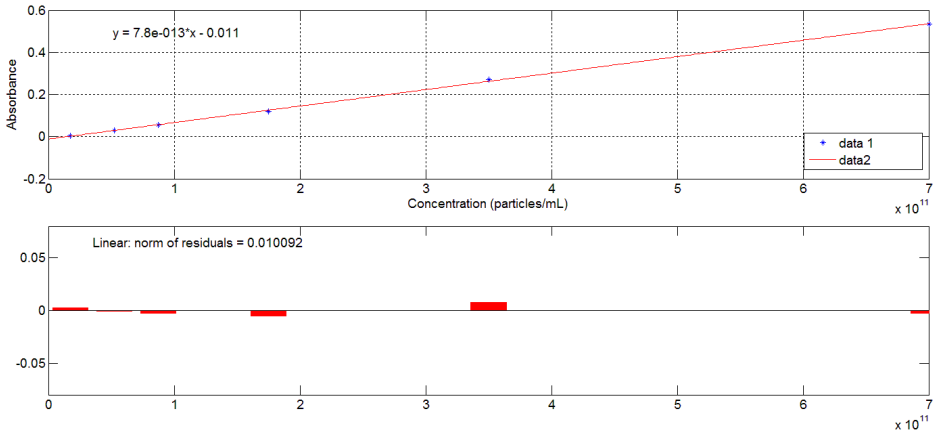


Figure 10 – The top graph shows the standard curve for the 20 nm gold nanoparticles. The bottom graph shows the residuals in order to get an estimate concerning how well the line is fitted to our dilutions.

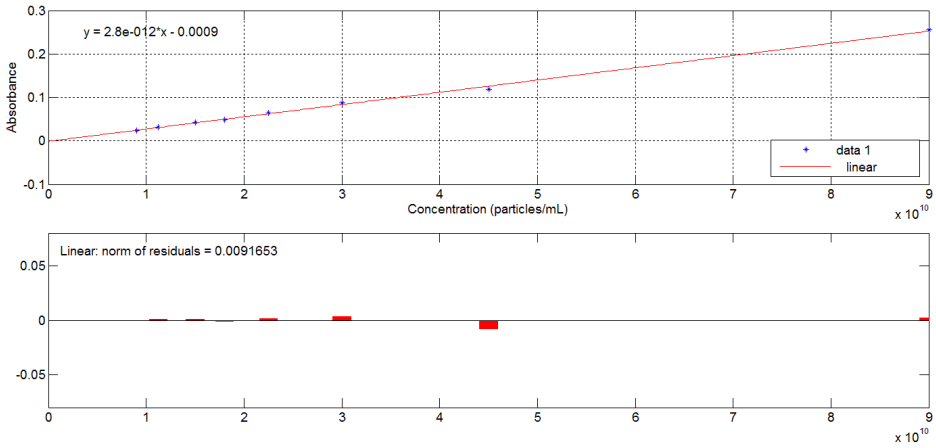


Figure 11 – The top graph shows the standard curve for the 40 nm gold nanoparticles. The bottom graph shows the residuals in order to get an estimate concerning how well the line is fitted to our dilutions.

4.3.2 Epidermis absorbance measurements

The results from the *Epidermis experiments* showed that AuNPs had not made it through the epidermis layer in the diffusion cells. In figure 12 the results from the absorbance measurements of the receptor fluids from the 21 hour tests with 5 nm AuNPs are shown. This graph is representative for all the measurements that were made in section 3.7.1. The graph from the original 5 nm AuNP solution is also shown in figure 12. When comparing the graph from the original solution to the graphs from the experiment no indications that AuNPs had diffused through the skin sample can be seen. The remaining absorbance graphs are displayed in appendix B (graphs B1-B30).

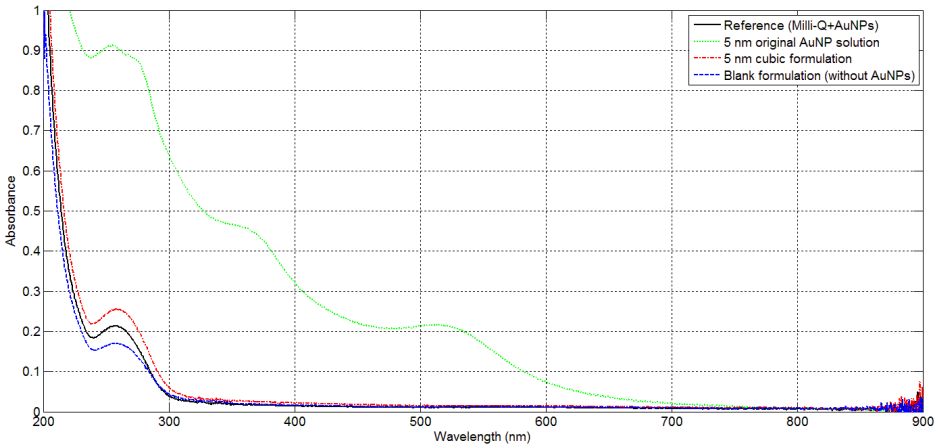


Figure 12 - Absorbance spectrum for the receptor fluids from the 21 hour test using 5 nm AuNPs. In the graph the original 5 nm AuNP solution is the green ····· line, the reference is the solid black line, the 5 nm cubic formulation is the red -·-·-·- line and the blank formulation is the blue --------- line.

No differences can be observed between the different sizes of AuNP in cubic phase, the time difference between the tests or when comparing the loaded cubic phases

with the reference or the blank cubic phase. These results indicate that the AuNPs have stayed in the epidermis skin sample or that they have not penetrated at all.

If there were in fact particles that have made it through the epidermis samples they might have become so diluted in the receptor chambers that their concentration was below the detection limit of the spectrophotometer, which was at the absorbance of 0.02. Another problematic issue that was thought to affect the results was the concentration of the original AuNP solution, which contained 0.01% gold nanoparticle solution. The low concentration of the nanoparticles and the large amount of receptor fluid that had to be used might have diluted the samples to a point where they could not be detected in the spectrophotometer.

4.3.3 Iontophoresis absorbance measurements

After the iontophoresis tests were done the receptor fluids from the 5 nm cubic tests showed a big colour difference between them. On the 5 nm cubic receptor fluid that was coloured there was foam on top of the skin, which is believed to be evidence that water had come through the skin. The fluid had become yellow in contrast to the transparent fluids from the other samples. During the two weeks that the receptor fluids was stored awaiting analysis the other 5 nm cubic receptor fluid had also gotten a more yellow colour and visible aggregates was seen in the two 5 nm cubic fluids.

Results from the absorbance measurements of the dried out receptor fluids from the *Iontophoresis experiments* can be seen in figure 13. In this figure the 5 nm cubic graph is from the 5nm cubic receptor fluid that was not visibly coloured after the experiment. The graph indicates transmission through the skin samples during the tests with 5 and 40 nm AuNPs in cubic formulation. When comparing the tests with cubic formulation to the 5 nm AuNP reference graph a clear difference in absorbance is noticed and this indicate that the AuNPs in cubic formulation have passed through more successfully.

A peak around 600 nm can be observed in the 5 nm cubic graph, but considering that the peak do not correspond to the 514 nm peak for the original 5 nm gold nanoparticles (see graph A1 in Appendix A) this is an indication that the particles have aggregated. Another indication of aggregation was that the graphs for the cubic samples do not start at zero, which suggests that particles that give responses to all wavelengths in the scan have formed. All other graphs from the absorbance measurements from the iontophoresis tests can be found in Appendix B (figure B31-B42).

In the figure it can be seen that the absorbance was much higher for the 5 nm AuNPs than the 40 nm AuNPs, which might suggest that the 5 nm AuNPs penetrate the skin easier than the 40 nm AuNPs.

It cannot be excluded that the skin samples in these test were damaged and therefore give misleading results, because so few tests were made using iontophoresis. More correct results might also have been obtained if the iontophoresis samples would have been analysed sooner after the experiment as differences in colour and visible aggregates had formed after two weeks. On the other hand, the aggregates must come from particles that must have been transmitted. It is also unknown how the resistance of the different skin samples affected the results.

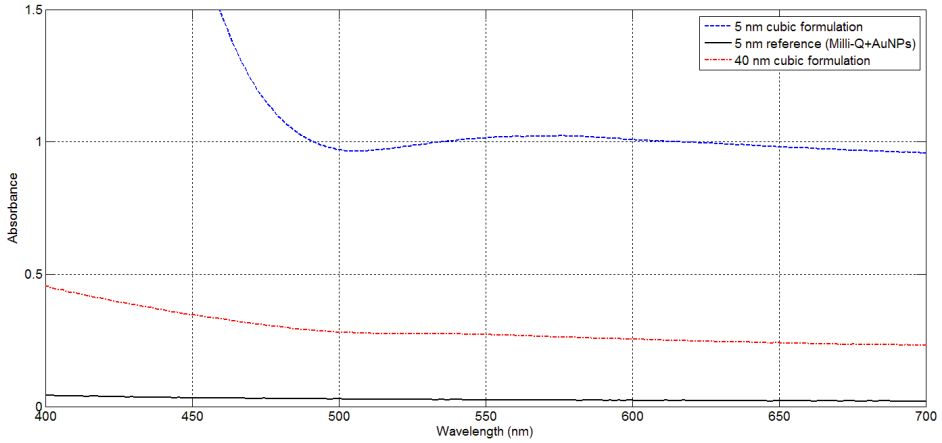


Figure 13 – Absorbance spectrum of the receptor fluids collected in the iontophoresis test. In this graph the blue ------- line show the 5 nm cubic formulation, the solid black line show the 5 nm reference and the red -·-·-·- line show the 40 nm cubic formulation. The receptor fluid was dried out on a watch glass and then dissolved in 1.5 mL PBS.

4.4 Two-photon microscopy results

Two-photon microscopy was performed on the full thickness skin samples. From the images produced from the microscopy method, aggregates of nanoparticles on the surface of the skin could be observed, which was probably due to formulation left from after the experiments (see figure 14). There was no sign of particles deeper into the skin. This could be due to that no particles had made it deeper into the skin or that the microscope could not detect the gold nanoparticles when they are separated, which might have to do with that the cubic phase enhances the separation distance between them. The images from the cubic phases were more blurred than the corresponding reference with Milli-Q water which confirms the separation theory and also made the analysis more difficult.

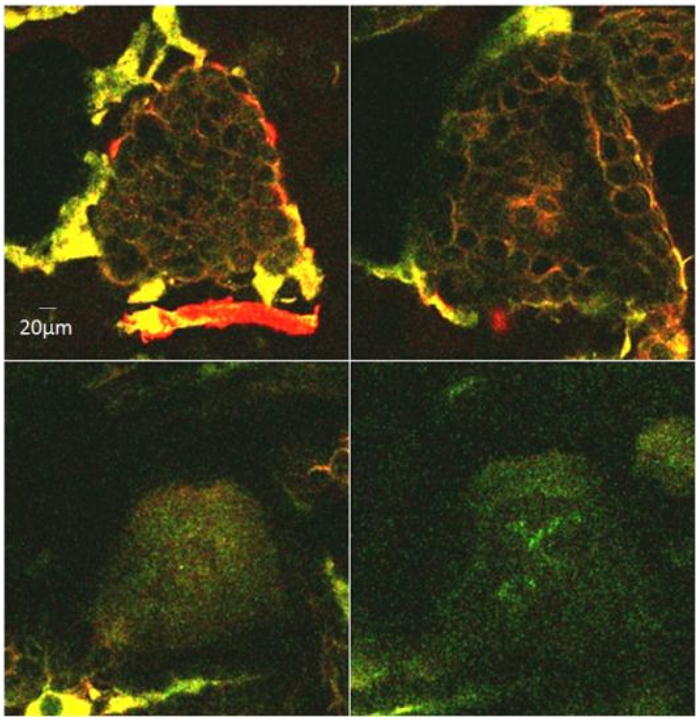


Figure 14 - Four pictures taken with two-photon microscopy on skin exposed to cubic formulation with AuNPs. The picture in the top left corner was from the skin surface and the picture in the bottom right corner was from about 142 µm into the skin. The green parts are the skins own autofluorescence, the red is probably the aggregated AuNP.

5 Conclusion

It can be concluded that AuNPs in cubic formulation have diffused through the epidermis layer in the diffusion cells when iontophoresis was used, in contrast to the AuNP reference solutions using iontophoresis and the tests without iontophoresis.

The results from the two-photon microscopy showed that the AuNPs could not be detected when they are too far away from each other as in the cubic formulation. However, this indicates that the formulation holds the AuNPs away from each other and that they do not aggregate in the formulation.

It can be concluded that the heat separation method that was developed was very effective when it came to separate the epidermis layer from the dermis layer of the skin. The method proved to be both fast and harmless to the epidermis.

The formulations that were created became cubic which concludes that the recipe for the formulation proved to be correct.

6 Outlook

Some work still remains with the experiments that have been performed in this thesis. For example the frozen tissue samples from the epidermis and iontophoresis experiments still need to be analysed.

Additional tests using iontophoresis have to be done in order to find a valid answer to if the size matters in terms of permeability properties of the different gold nanoparticles and to get a better view of how the voltage difference affects the skin. Another interesting thing to look at is the fact that the receptor fluid in some cases changed colour. Because of this, analyses of the receptor fluids that have aged for different time periods could prove interesting. However, further test still needs to be conducted to verify this.

The experiments in the diffusion cells might be improved by testing smaller diffusion cells or by having gold nanoparticles with higher concentration.

The question whether the nanoparticles really can be detected if they are separated further away than a certain distance needs to be answered and another detection method than two-photon microscopy have to be found.

7 Reference list

[1]: Bender J., et al. (2007). Lipid cubic phases in topical drug delivery: Visualization of skin distribution using two-photon microscopy. *Journal of Controlled Release*, vol. 129, issue 3, pp. 163-169. DOI: 10.1016/j.jconrel.2008.04.020

[2]: Bender J., et al. (2005). Lipid cubic phases for improved topical drug delivery in photodynamic therapy. *Journal of Controlled Release,* vol. 106, issue 3, pp. 350-360. DOI: 10.1016/j.jconrel.2005.05.010

[3]: Kuntsche J., et al. (2007). Interaction of lipid nanoparticles with human epidermis and an organotypic cell culture model. International Journal of Pharmaceutics, vol. 354, issue 1-2, pp. 180-195. DOI: 10.1016/j.ijpharm.2007.08.028

[4]: Sperling, R.A., et al. (2008) Biological applications of gold nanoparticles. *Chemical Society Review*, vol. 37, issue 9, pp. 1896-1908. DOI: 10.1039/B712170A

[5]: Birgerson, B., Sterner, O., Zimerson, E. (2009) *Kemiska Hälsorisker - Toxikologi i kemiskt perspektiv.* Malmö: Liber AB.

[6]: Hud. (2012) In Nationalencyklopedin. http://www.ne.se/lang/hud?i_h_word=m%C3%A4nniskans%20hud (2012-03-14).

[7]: Human skin. (2012) In Encyclopaedia Britannica Online. http://www.britannica.com/EBchecked/topic/547591/human-skin (2012-03-14).

[8]: Dermis. (2012) In About.com. http://dermatology.about.com/od/glossaryd/g/dermis.htm (2012-03-14).

[9]: Koster, M. I. (2009), Making an Epidermis. Annals of the New York Academy of Sciences, 1170: 7–10.

[10]: Marjukka Suhonen T., et al. (1999). Chemical enhancement of percuteaneous absorbtion in relation to stratum corneum strucural alterations. *Journal of controlled Release*, vol. 59, issue 2, pp. 149-161. DOI: 10.1016/s0168-3659(98)00187-4

[11]: Robson, P., Jarvie H., King S., (2012). Nanoparticle. In Encyclopaedia Britannica Online. http://www.britannica.com/EBchecked/topic/1109065/nanoparticle (2012-03-14)

[12]: Kyaw Khaing Oo, M. (2011) Multifunctional Gold Nanoparticles for Cancer Therapy. *Nanobiomaterials Handbook*, red. B. Shitarhaman, pp. 1-24. Boca Raton: Taylor and Francis Group

[13]: Jain S. et al. (2011). Gold nanoparticles as novel agents for cancer therapy. *British Journal of Radiology*, vol. 85, pp. 101-113. DOI: 10.1259/bjr/59448833

[14]: Miranova T. et al. (2010). Gold nanoparticles cellular toxicity and recovery. *Nanotechnology 2010: Bio sensors, Instruments, Medical, Environment and energy,* vol. 3, pp. 199-201. ISBN:978-1-4398-3415-2

[15]: Engström S. Wadsten-Hindrichsen P, Hernius B. (2007). Cubic, Sponge, and Lameller Phases in the Glyceryl Monooleyl Ether – Propylene Glycol – Water System. *Langmuir*, vol. 23, pp.10020-10025. DOI**:** 10.1021/la701217b

[16]: Holmberg, K., et al. (2002). Phase behaviour of concentrated surfactant systems. *Surfactants and Polymers in Aqueous solution*. 2nd edition. West Sussex: John Wiley & Sons Ltd. ISBN: 0-471-49883-1

[17]: Kalia Y. N., (2003). Iontophoretic drug delivery. *Advanced Drug Delivery Reviews*, vol. 56, pp. 619-658. DOI: 10.1016/j.addr.2003.10.026

[18]: Cheng K. L., (2006). The negative charge of nanoparticles. *Microchemical Journal,* vol. 82, issue 1, pp. 119-120. DOI: 10.1016/j.microc.2005.11.002

[19]: Nanocs Inc. (2011) Gold nanoparticles in Aqueous solution. http://www.nanocs.com/gold_nanoparticle.htm (2012-05-14).

[21]: Kligman, A., Cristophers, E. (1963), Preparation of Isolated Sheets of Human Stratum Corneum. *Arch Dermatol*, vol. 88, issue 6, pp. 702-705. DOI: 10.1001/archderm.1963.01590240026005

[22]: Labouta, H.I., et al. (2011) Gold nanoparticles Penetration and Reduced Metabolism in Human skin by Toluene. *Pharmaceutical Research*, vol. 28, issue 11, pp. 2931-2944. DOI: 10.1007/s11095-011-0561-z

[23]: König, K. (2000) Multiphoton microscopy in life sciences. *Journal of Microscopy*, vol. 200, issue 2, pp. 83-104. DOI: 10.1046/j.1365-2818.2000.00738.x

[24]: Du, S., et al. (2012) Aggregation and adhesion of gold nanoparticles in phosphate buffered saline. *Journal of nanoparticle research*, vol 14, issue 3. DOI: 10.1007/s11051-012-0758-z

[25]: Trost, A., et al. (2007) Rapid, high-quality and epidermal-specific isolation of RNA from human skin. *Experimental Dermatology*, vol. 16, issue 3, pp. 185-190. DOI: 10.1111/j.1600-0625.2006.00534.x

[26]: Macdiarmid, J., Wilson, J.B. (2001) Separation of Epidermal Tissue from Underlying Dermis and Primary Keratinocyte Culture. *Epstein-Barr Virus Protocols*, vol. 174, issue 8, pp. 401-410. DOI: 10.1385/1-59259-227-9:401

[27]: Lee, G., Parlicharla, R. (1986) An Examination of Excised Skin Tissues used for In Vitro Membrane Permeation Studies. *Pharmaceutical Research*, vol. 3, issue 6, pp. 356-359. DOI: 10.1023/A:1016488108213
[28]: Kasting, G.B., Bowman, L.A. (1990) DC Eletrical Properties of Frozen, Exised Human Skin. *Pharmaeutical Research*, vol. 7, issue 2, pp. 134-143. DOI: 10.1023/A:1015820600672
[29]: Jadoul, A., et al. (1999) Effects of iontophoresis and electroporation on stratum corneum –Review of the biological studies. *Advanced Drug Delivery Reviews*, vol. 35, issue 1, pp. 89-105. DOI: 10.1016/S0169-409X(98)00065-9
[30]: Harris, D.C. (2010) *Quantitative Chemical Analysis*. Eight Edition. New York: W. H. Freeman and Company.
[31]: Lance Kelly, K. et al. (2003) The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dieletric Environment. *The Journal of Physical Chemistry*, vol. 107, issue 3, pp. 668-677. DOI: 10.1021/jp026731y

Figure References

[F1]: The image is reproduced from: dermis: human skin. [Art]. *Encyclopaedia Britannica Online*. Retrieved 14 March 2012, from http://www.britannica.com/EBchecked/media/2027/Section-through-human-skin-and-underlying-structures
[F2]: The image is reproduced from: Marjukka Suhonen T., et al. (1999) [Art]. Chemical enhancement of percuteaneous absorbtion in relation to stratum corneum strucural alterations. *Journal of controlled Release*, vol. 59, issue 2, pp. 149-161. Retrieved 16 March 2012, from http://ars.sciencedirect.com/content/image/1-s2.0-S0168365998001874-gr1.gif
[F3]: Reprinted with permission from Engström S., Wadsten-Hindrichsen P, Hernius B. (2007) [Art]. Cubic, Sponge, and Lameller Phases in the Glyceryl Monooleyl Ether – Propylene Glycol – Water System. *Langmuir*, vol. 23, pp.10020-10025. DOI**:** 10.1021/la701217b. Copyright (2012) American Chemical Society.
[F4]: Hanne Evenbratt. [Art]. Visualization study of dermal delivery of new nanoparticle drugs – does size really matter? Retrieved 18 May 2012, from http://www.chalmers.se/chem/EN/education/undergraduate-programmes/degree-project/visualization-

study/downloadFile/attachedFile_f0/MasterThesisNanoparticles.pdf?nocache=1316758855.61

[F5]: König K. (2001) [Art]. Multiphoton microscopy in life sciences. *Journal of Microscopy*, vol. 200, issue 2, pp. 83-104. DOI: 10.1046/j.1365-2818.2000.00738.x

8 Appendix A – Absorbance spectrums of the 5, 20 and 40 nm original solutions.

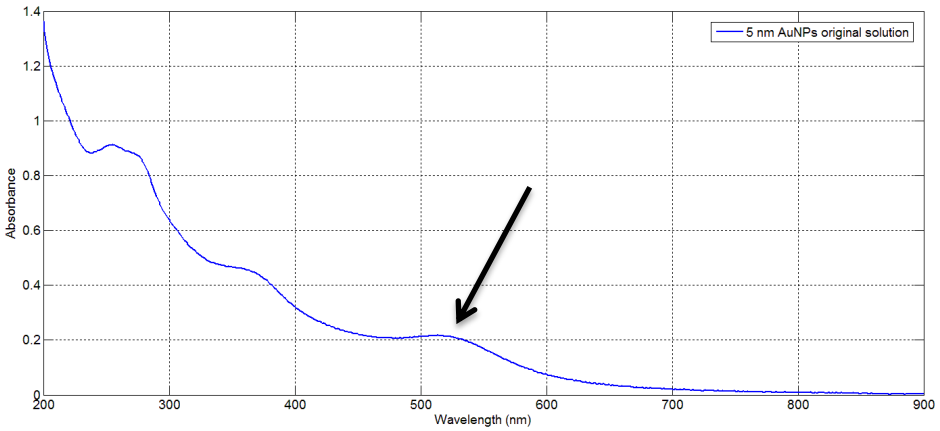


Figure A1 – Absorbance spectrum for the 5 nm AuNP original solution. From this graph a peak at 514 nm can be observed.

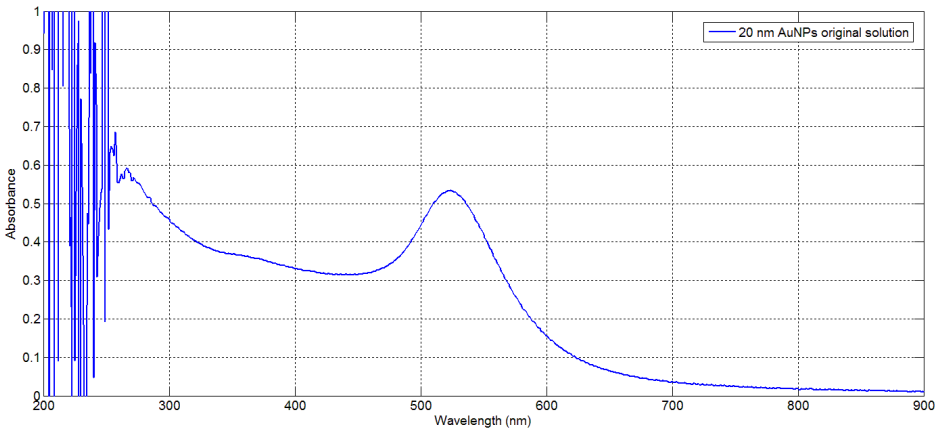


Figure A2 – Absorbance spectrum for the 20 nm AuNP original solution. From this graph a peak at 524 nm can be observed. The distortion in the graph between 200-280 nm is believed to be temporary interferences.

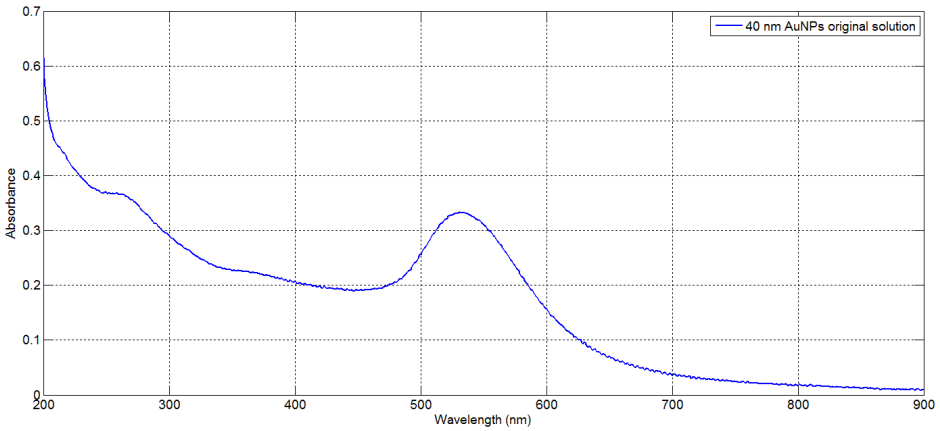


Figure A3 – Absorbance spectrum for the 40 nm AuNP original solution. From this graph a peak at 532 nm can be observed.

9 Appendix B – Absorbance measurements

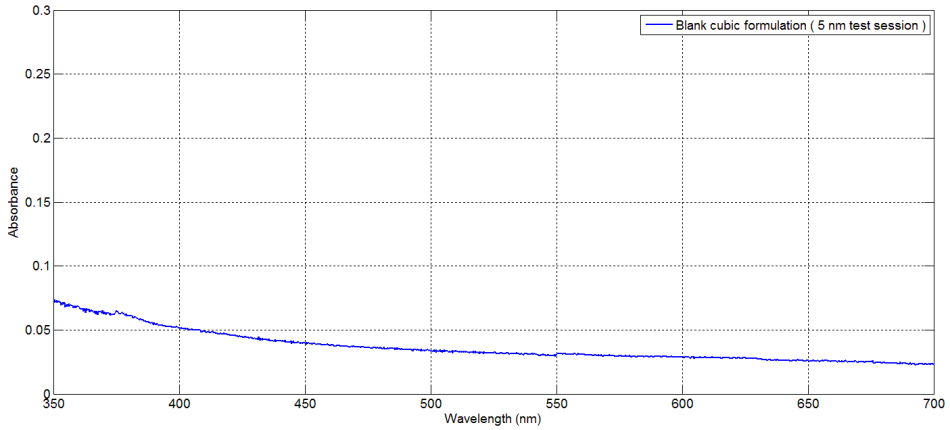


Figure B1 – Absorbance spectrum from the receptor fluid in the diffusion cell test from the blank cubic formulation (without AuNPs, 5 hour test session). Epidermis skin was used in this experiment.

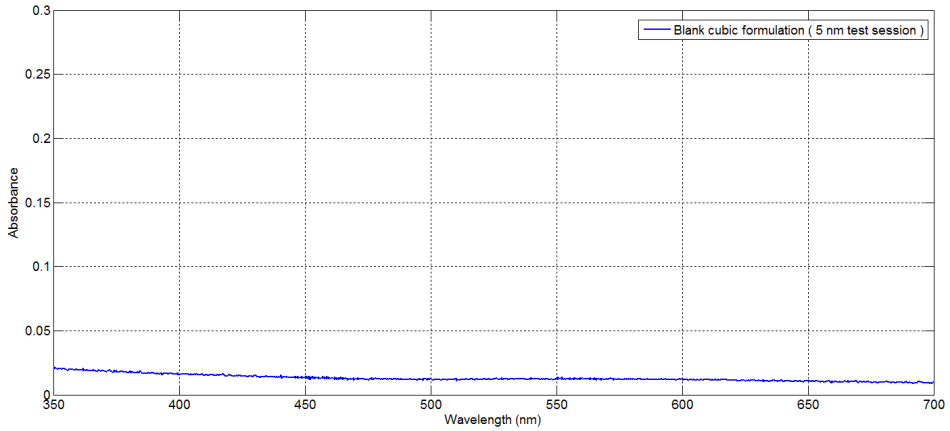


Figure B2 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the blank cubic formulation (without AuNPs, 21 hour test session). Epidermis skin was used in this experiment.

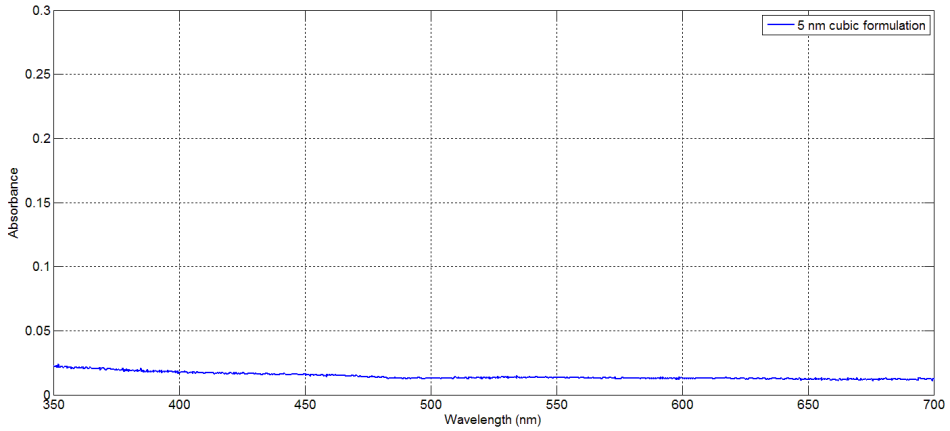


Figure B3 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 5 nm cubic formulation (5 hour test session). Epidermis skin was used in this experiment.

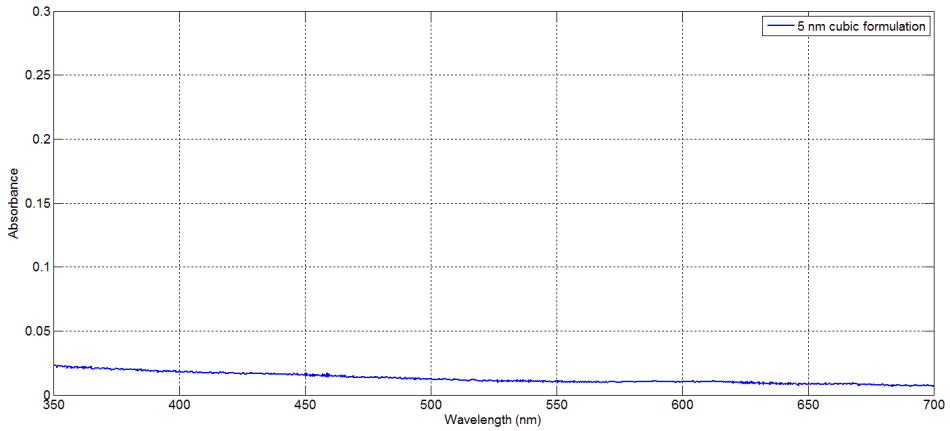


Figure B4 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 5 nm cubic formulation (21 hour test session). Epidermis skin was used in this experiment.

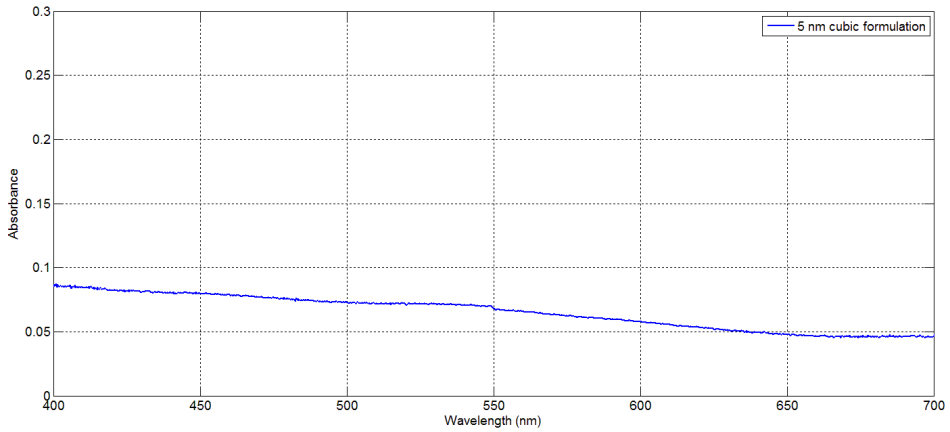


Figure B5 - Absorbance spectrum from the receptor fluid in the diffusion cell test. The 5 nm cubic formulation sample was dried out on a watch glass after the 5 hours of diffusion. The dried out sample was then dissolved in 1.5 mL PBS and this solution was analysed using UV/VIS. Epidermis skin was used in this experiment.

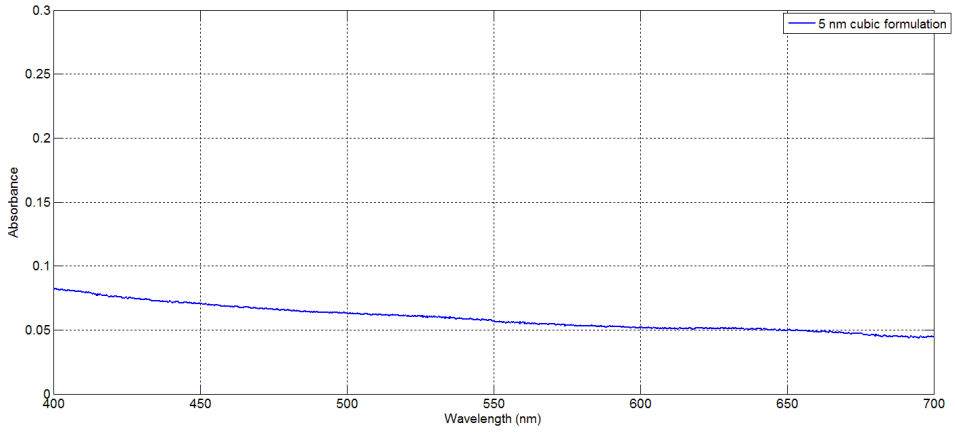


Figure B6 - Absorbance spectrum from the receptor fluid in the diffusion cell test. The 5 nm cubic formulation sample was dried out on a watch glass after the 21 hours of diffusion. The dried out sample was then dissolved in 2.5 mL PBS and this solution was analysed using UV/VIS. Epidermis skin was used in this experiment.

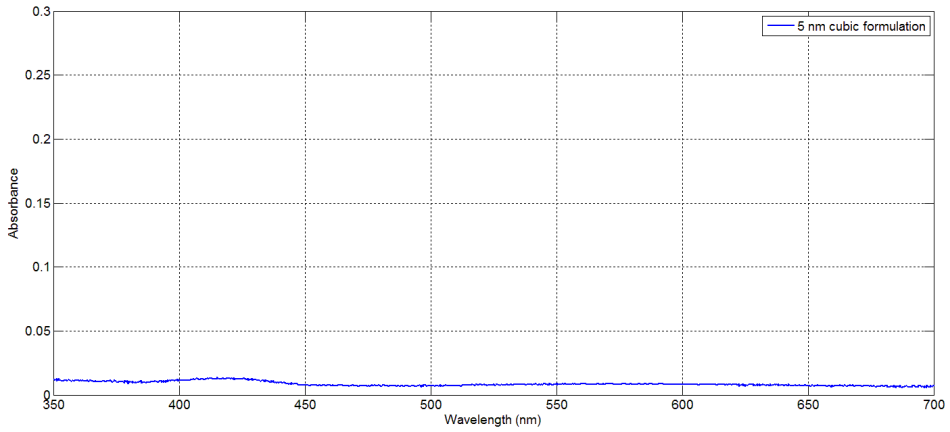


Figure B7 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 5 nm cubic formulation (5 hour test session). Epidermis skin was used in this experiment.

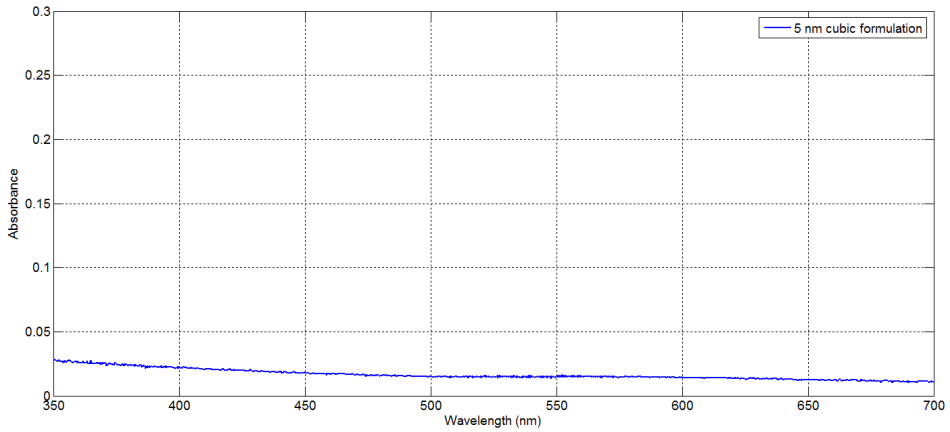


Figure B8 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 5 nm cubic formulation (21 hour test session). Epidermis skin was used in this experiment.

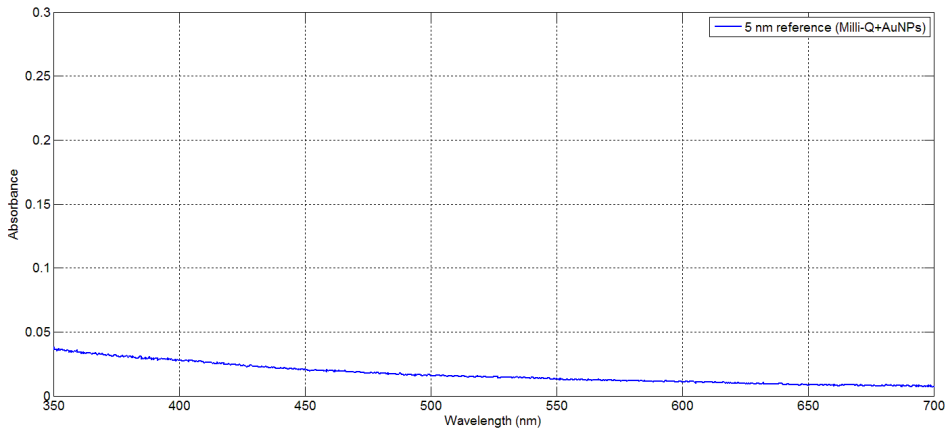


Figure B9 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 5 nm reference (21 hour test session). Epidermis skin was used in this experiment.

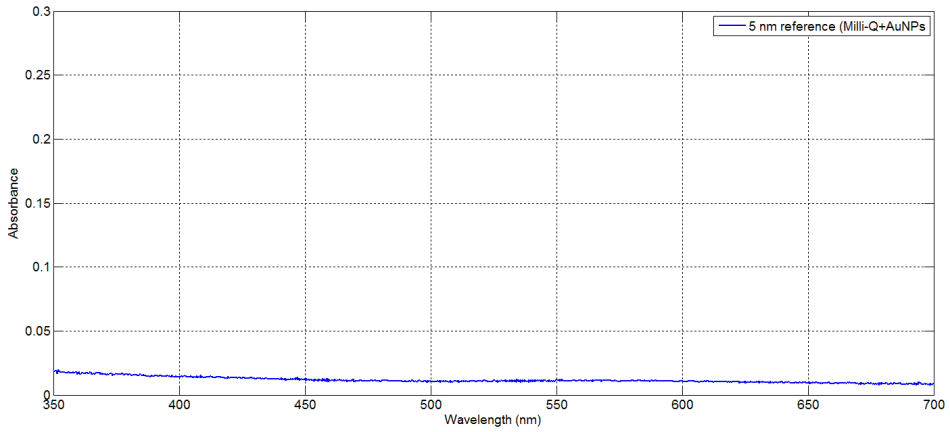


Figure B10 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 5 nm reference (21 hour test session). Epidermis skin was used in this experiment.

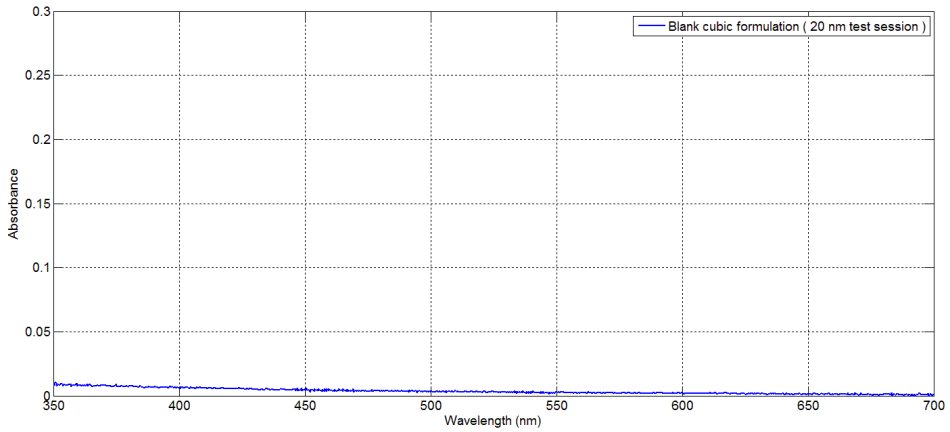


Figure B11 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the blank cubic formulation (without AuNPs, 5 hour test session). Epidermis skin was used in this experiment.

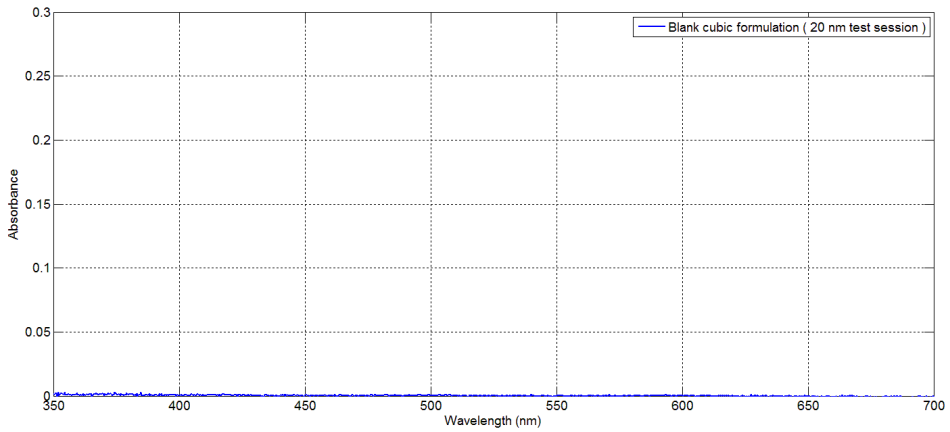


Figure B12 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the blank cubic formulation (without AuNPs, 21 hour test session). Epidermis skin was used in this experiment.

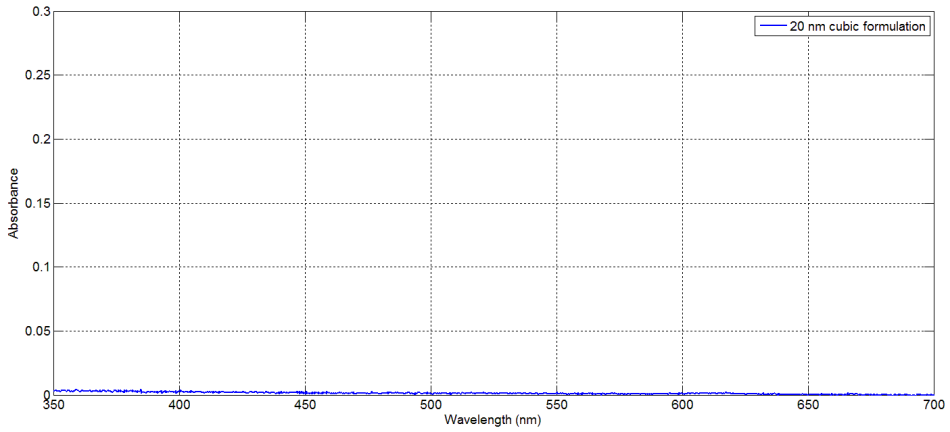


Figure B13 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 20 nm cubic formulation (5 hour test session). Epidermis skin was used in this experiment.

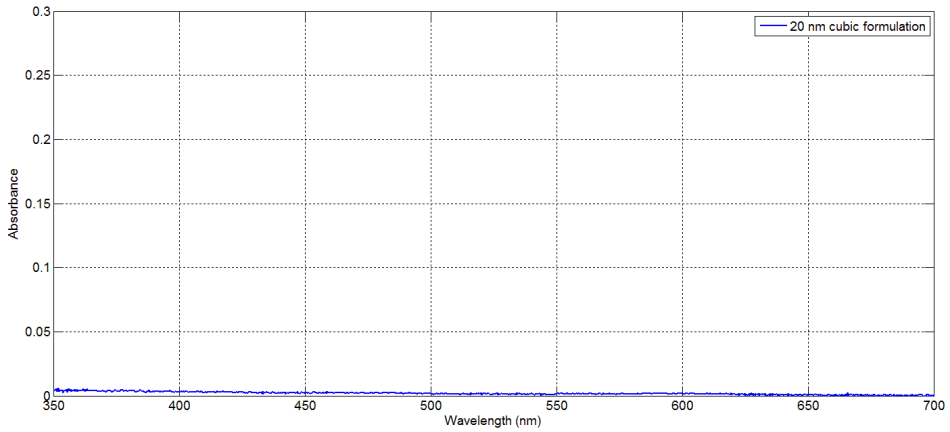


Figure B14 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 20 nm cubic formulation (21 hour test session). Epidermis skin was used in this experiment.

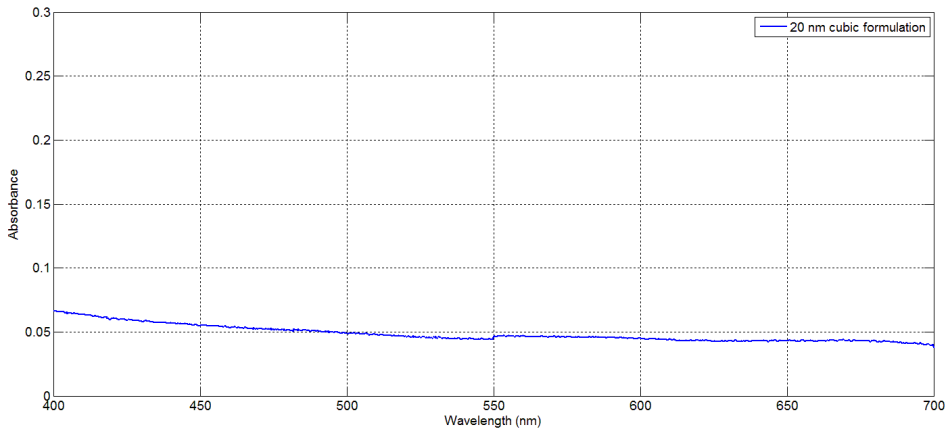


Figure B15 - Absorbance spectrum from the receptor fluid in the diffusion cell test. The 20 nm cubic formulation sample was dried out on a watch glass after the 5 hours of diffusion. The dried out sample was then dissolved in 1.5 mL PBS and this solution was analysed using UV/VIS. Epidermis skin was used in this experiment.

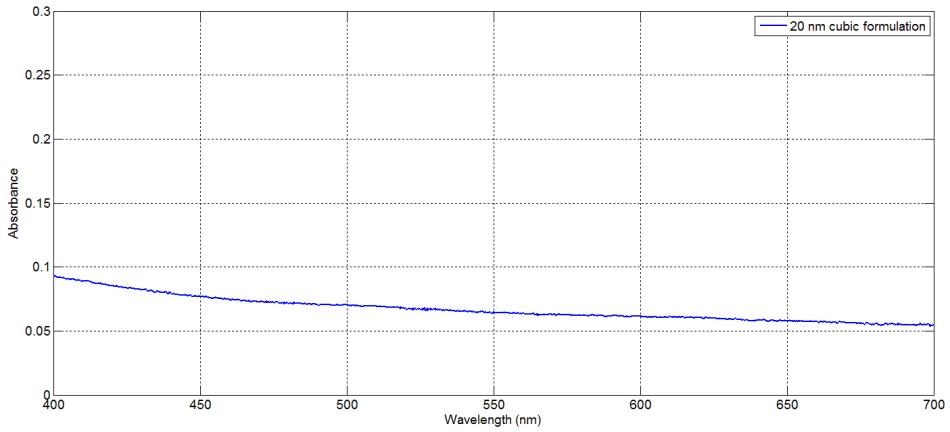


Figure B16 - Absorbance spectrum from the receptor fluid in the diffusion cell test. The 20 nm cubic formulation sample was dried out on a watch glass after the 21 hours of diffusion. The dried out sample was then dissolved in 1.5 mL PBS and this solution was analysed using UV/VIS. Epidermis skin was used in this experiment.

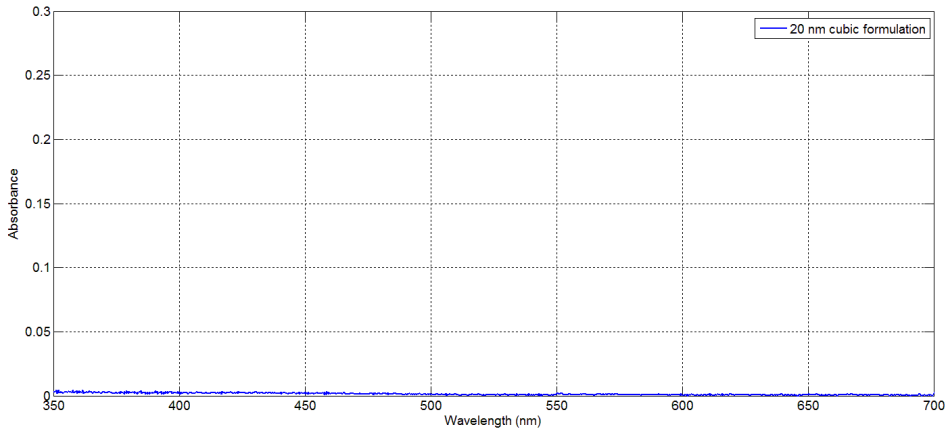


Figure B17 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 20 nm cubic formulation (5 hour test session). Epidermis skin was used in this experiment.

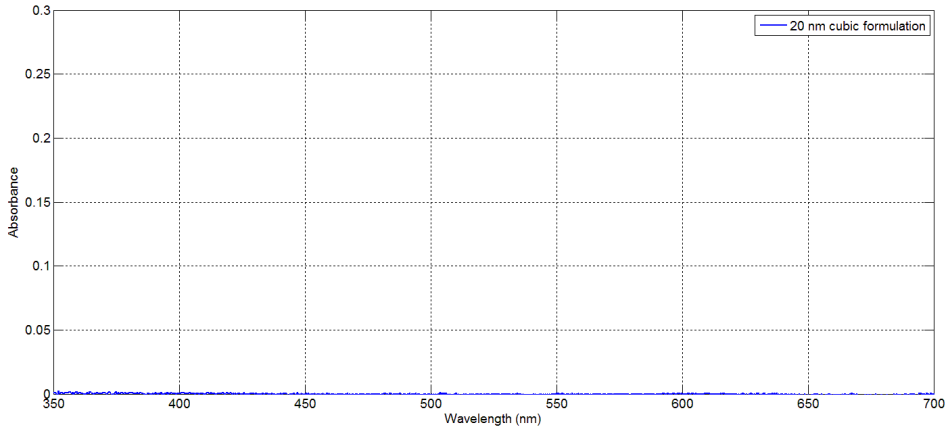


Figure B18 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 20 nm cubic formulation (21 hour test session). Epidermis skin was used in this experiment.

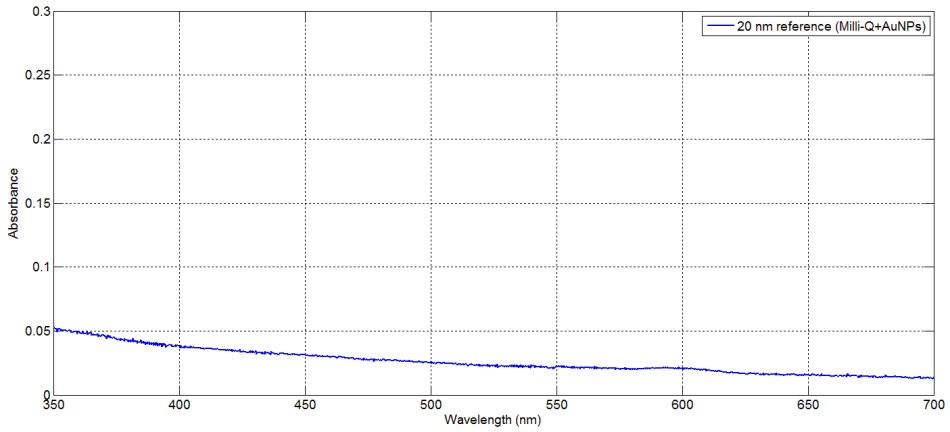


Figure B19 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 20 nm reference (5 hour test session). Epidermis skin was used in this experiment.

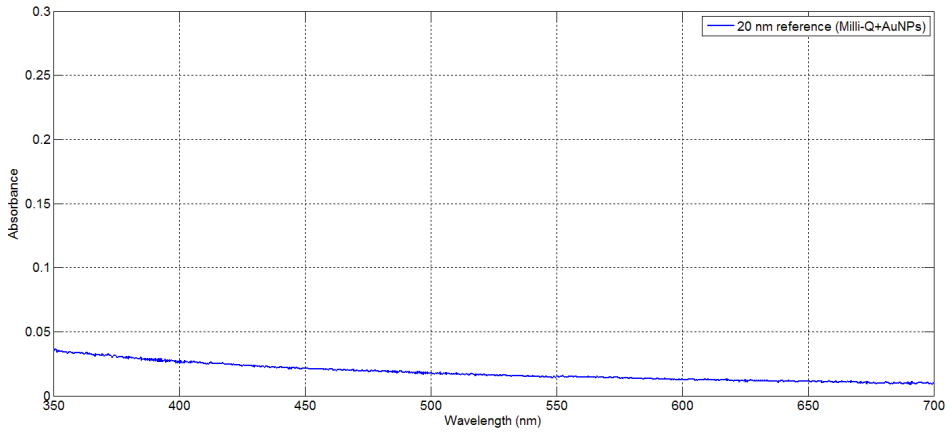


Figure B20 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 20 nm reference (21 hour test session). Epidermis skin was used in this experiment.

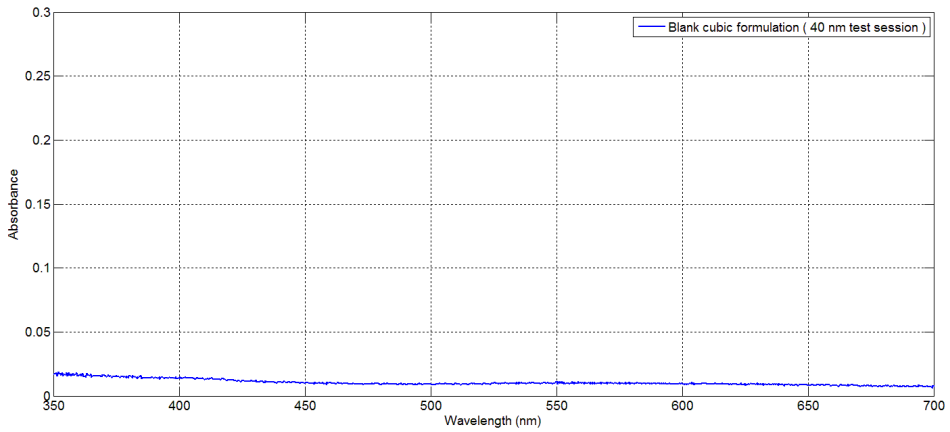


Figure B21 - Absorbance spectrum from the receptor fluid in the diffusion cell from the blank cubic formulation (without AuNPs, 5 hour test session). Epidermis skin was used in this experiment.

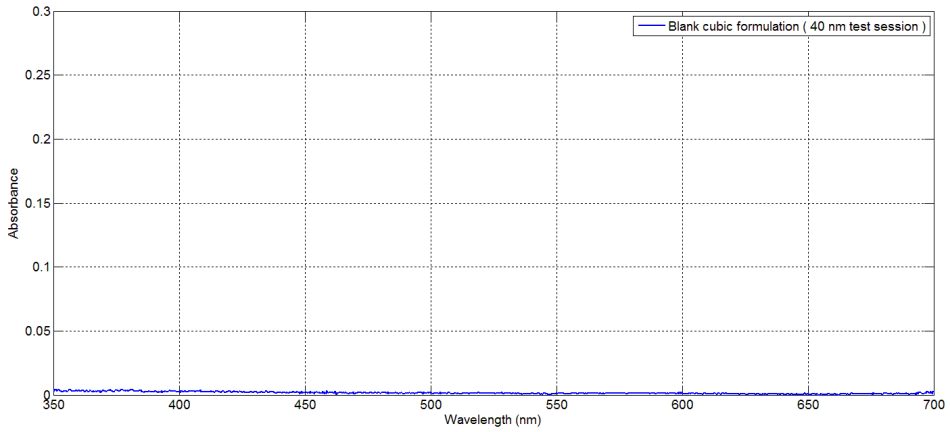


Figure B22 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the blank cubic formulation (without AuNPs, 21 hour tests session). Epidermis skin was used in this experiment.

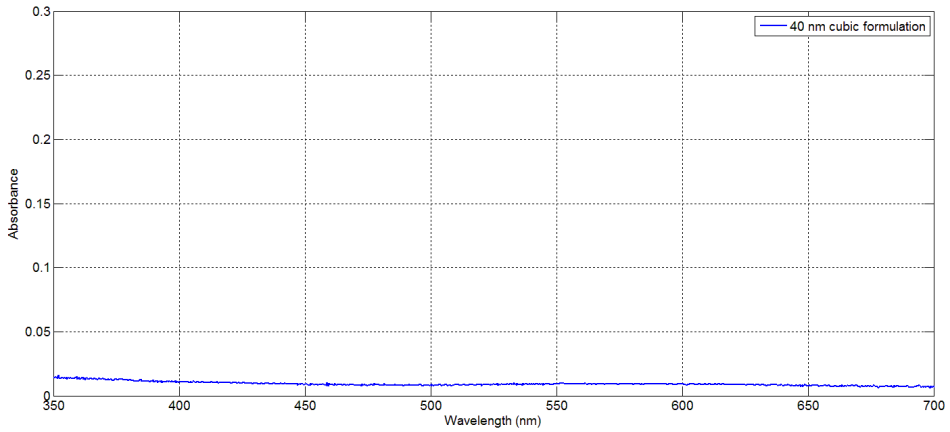


Figure B23 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 40 nm cubic formulation (5 hour test session). Epidermis skin was used in this experiment.

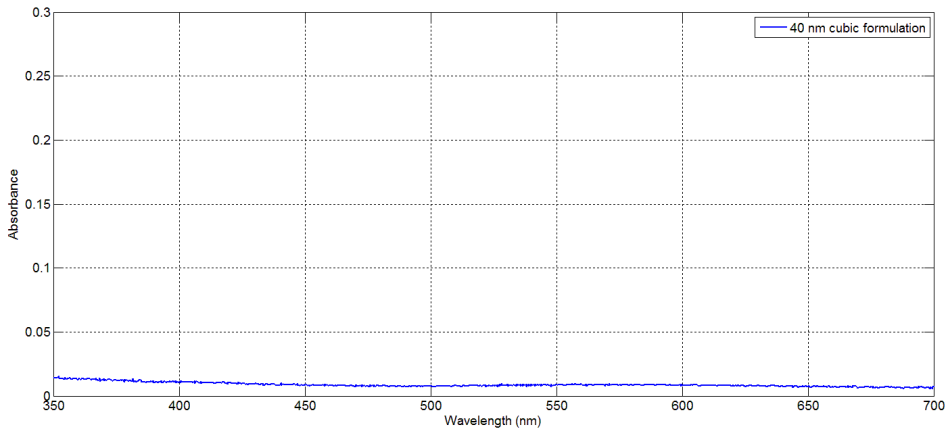


Figure B24 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 40 nm cubic formulation (21 hour test session). Epidermis skin was used in this experiment.

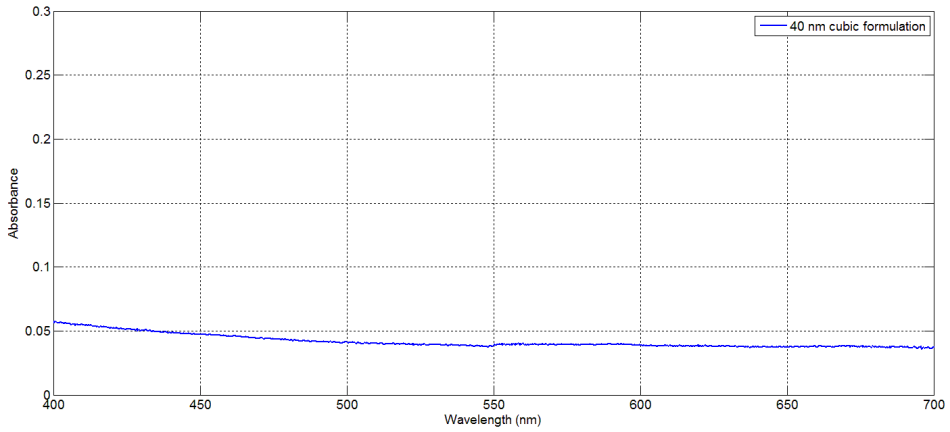


Figure B25 - Absorbance spectrum from the receptor fluid in the diffusion cell test. The 40 nm cubic formulation sample was dried out on a watch glass after the 5 hours of diffusion. The dried out sample was then dissolved in 1.5 mL PBS and this solution was analysed using UV/VIS. Epidermis skin was used in this experiment.

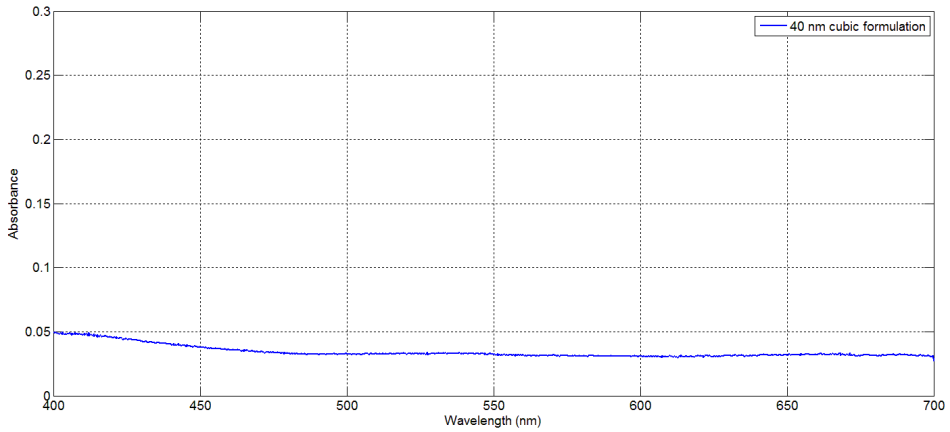


Figure B26 - Absorbance spectrum from the receptor fluid in the diffusion cell test. The 40 nm cubic formulation sample was dried out on a watch glass after the 21 hours of diffusion. The dried out sample was then dissolved in 1.5 mL PBS and this solution was analysed using UV/VIS. Epidermis skin was used in this experiment.

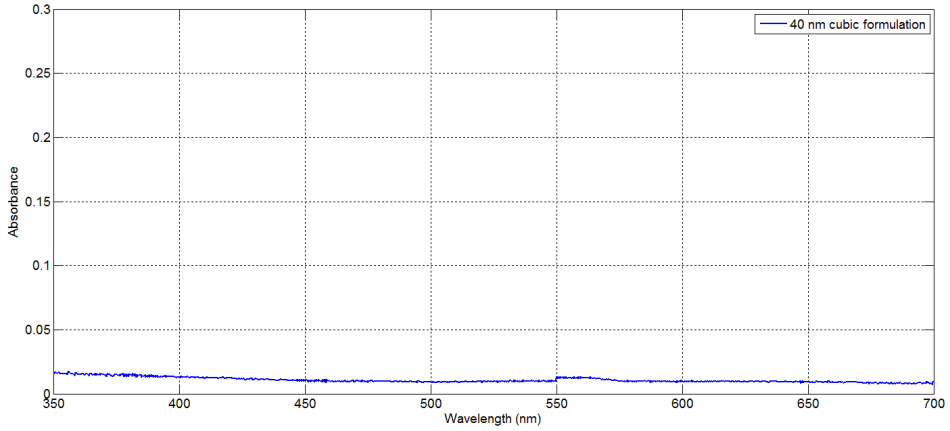


Figure B27 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 40 nm cubic formulation (5 hour test session). Epidermis skin was used in this experiment.

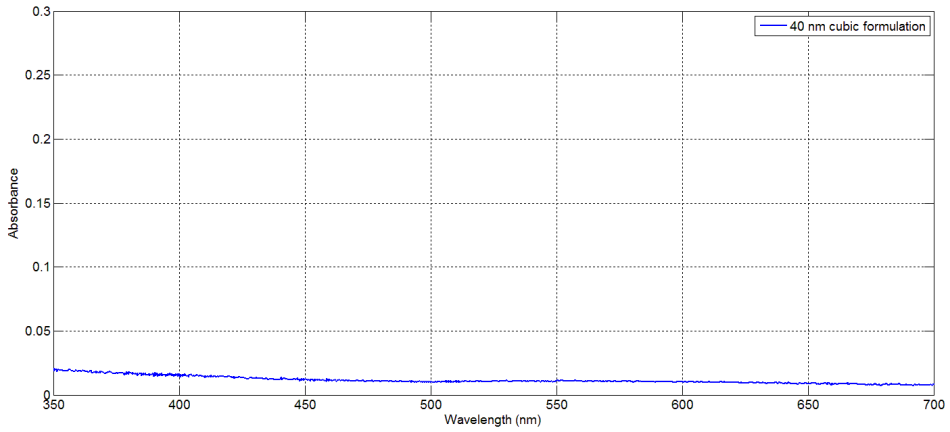


Figure B28 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 40 nm cubic formulation (21 hour test session). Epidermis skin was used in this experiment.

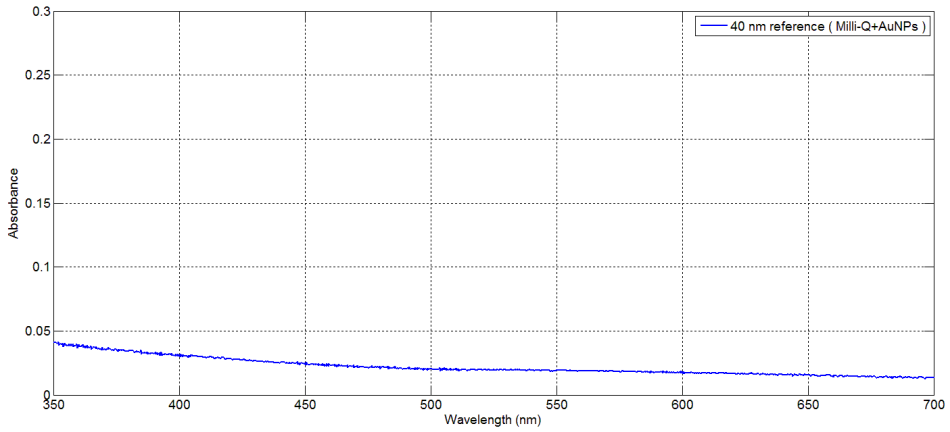


Figure B29 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 40 nm reference (5 hour test session). Epidermis skin was used in this experiment.

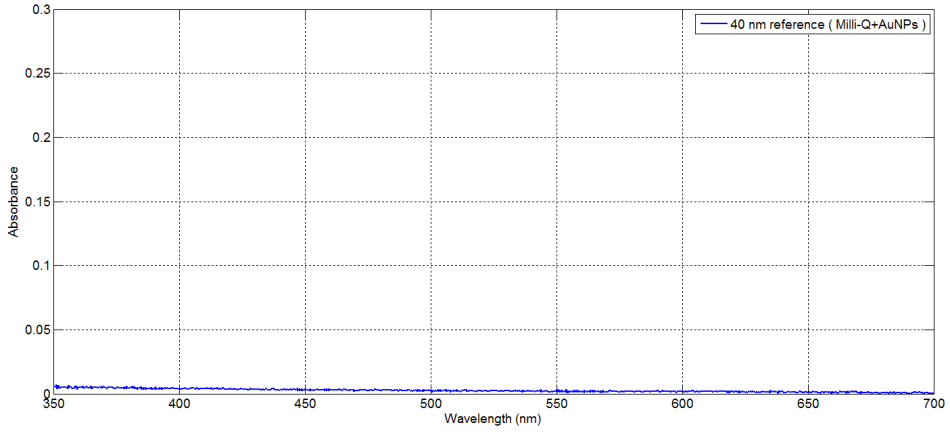


Figure B30 - Absorbance spectrum from the receptor fluid in the diffusion cell test from the 40 nm reference (21 hour test session). Epidermis skin was used in this experiment.

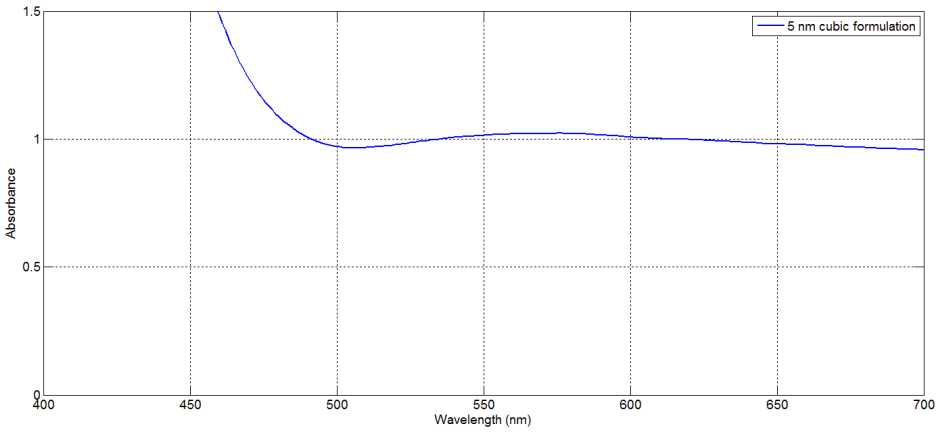


Figure B31 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was dried on a watch glass and then 1.5 mL of PBS was added to dissolve the crystals. This figure shows the 5 nm cubic formulation.

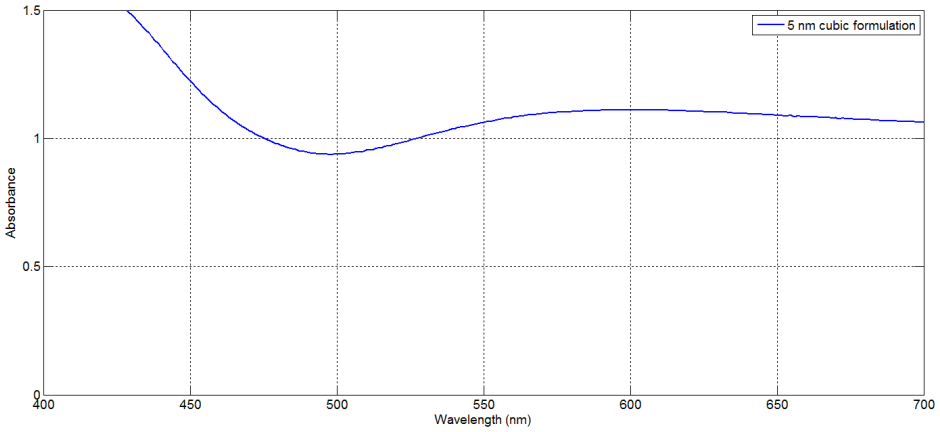


Figure B32 – Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was dried on a watch glass and then 1.5 mL of PBS was added to dissolve the crystals. This figure shows the 5 nm cubic formulation.

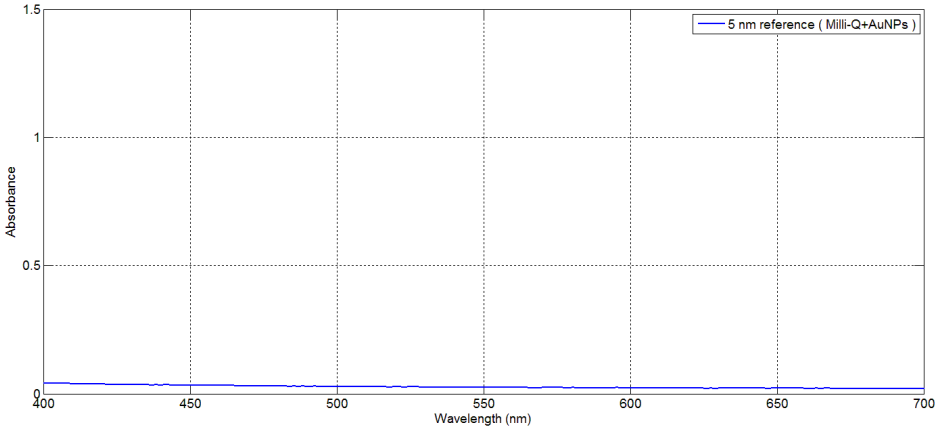


Figure B33 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was dried on a watch glass and then 1.5 mL of PBS was added to dissolve the crystals. This figure shows the 5 nm reference (Milli-Q+AuNPs).

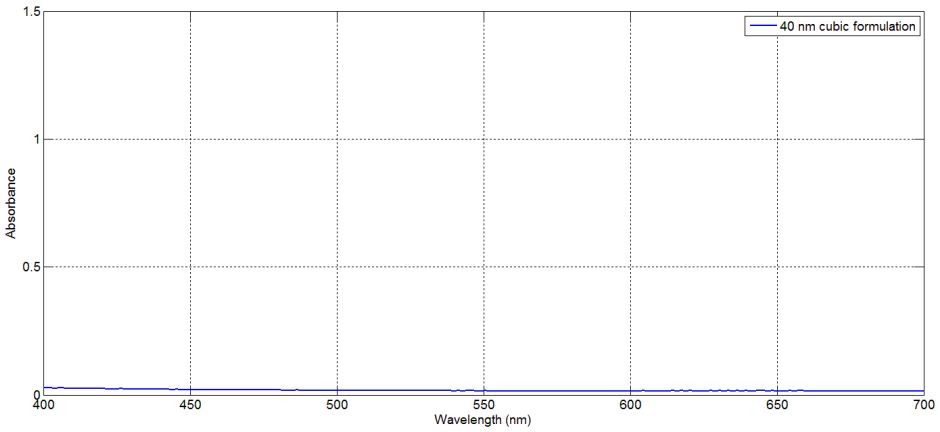


Figure B34 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was dried on a watch glass and then 1.5 mL of PBS was added to dissolve the crystals. This figure shows the 40 nm cubic formulation.

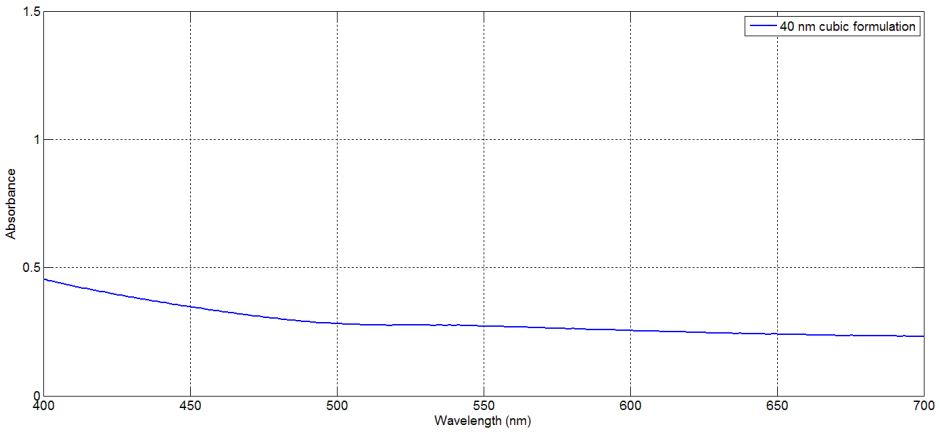


Figure B35 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was dried on a watch glass and then 1.5 mL of PBS was added to dissolve the crystals. This figure shows the 40 nm cubic formulation.

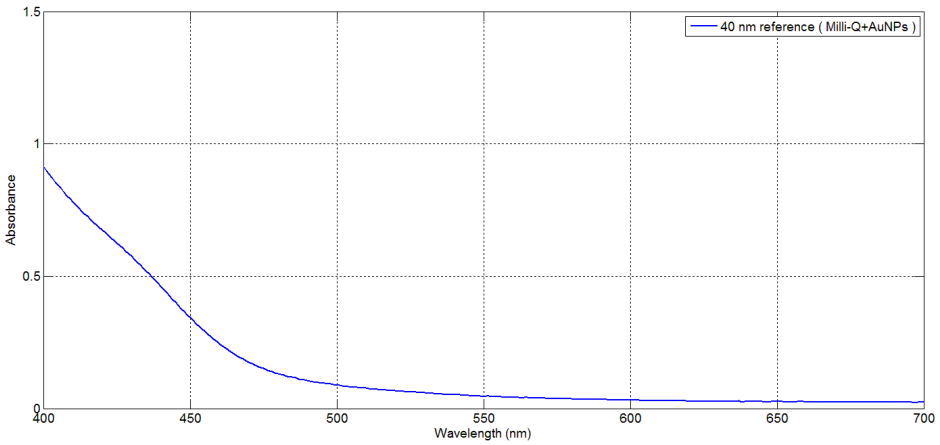


Figure B36 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was dried on a watch glass and then 1.5 mL of PBS was added to dissolve the crystals. This figure shows the 40 nm reference (Milli-Q+AuNPs).

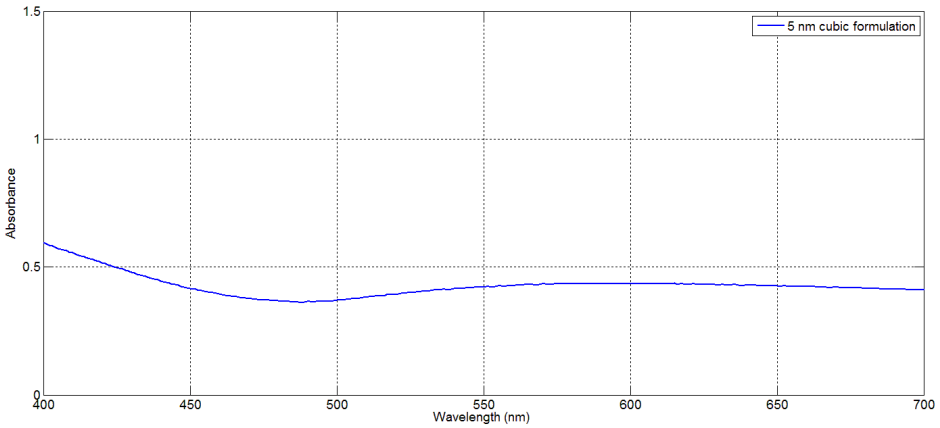


Figure B37 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was measured in an UV/VIS instrument directly after the experiment was completed. This sample was not dried out on a watch glass. This figure shows the 5 nm cubic formulation.

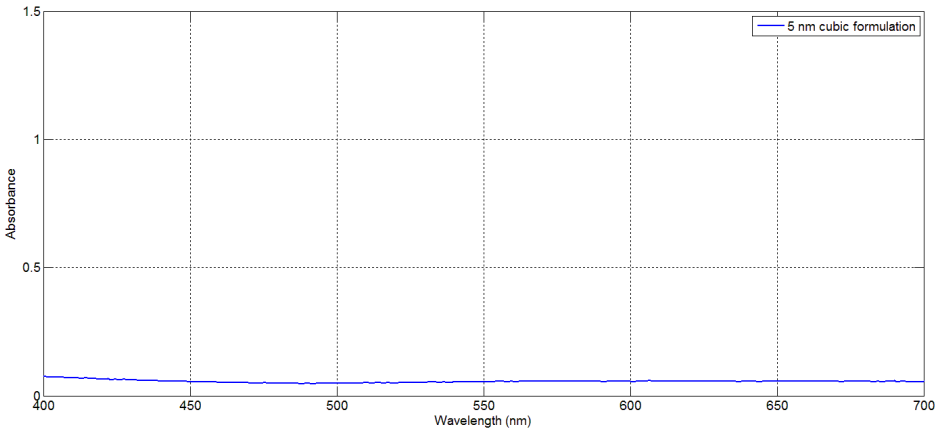


Figure B38 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was measured in an UV/VIS instrument directly after the experiment was completed. This sample was not dried out on a watch glass. This figure shows the 5 nm cubic formulation.

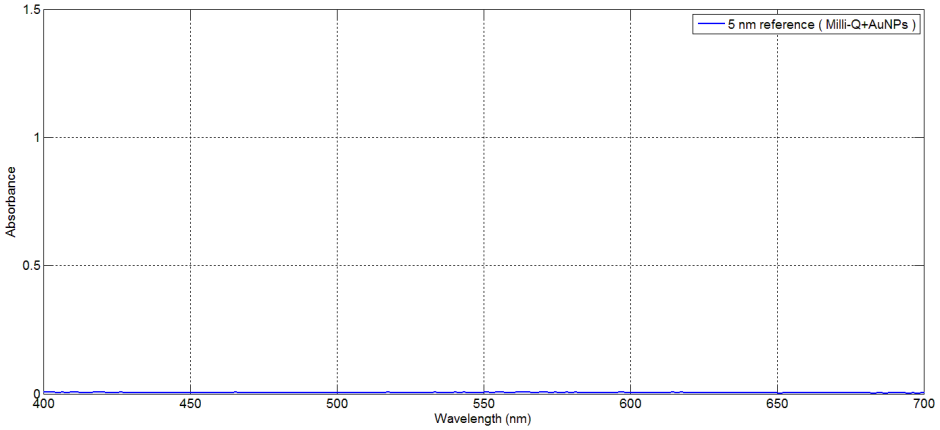


Figure B39 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was measured in an UV/VIS instrument directly after the experiment was completed. This sample was not dried out on a watch glass. This figure shows the 5 nm reference (Milli-Q+AuNPs).

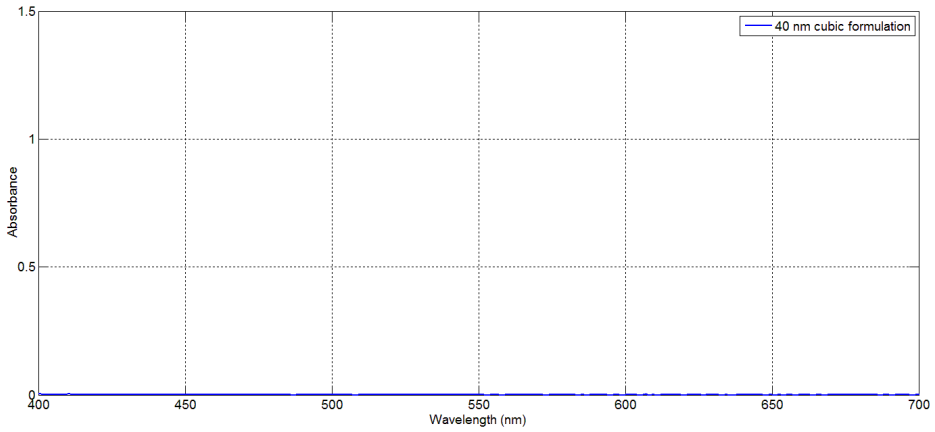


Figure B40 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was measured in an UV/VIS instrument directly after the experiment was completed. This sample was not dried out on a watch glass. This figure shows the 40 nm cubic formulation.

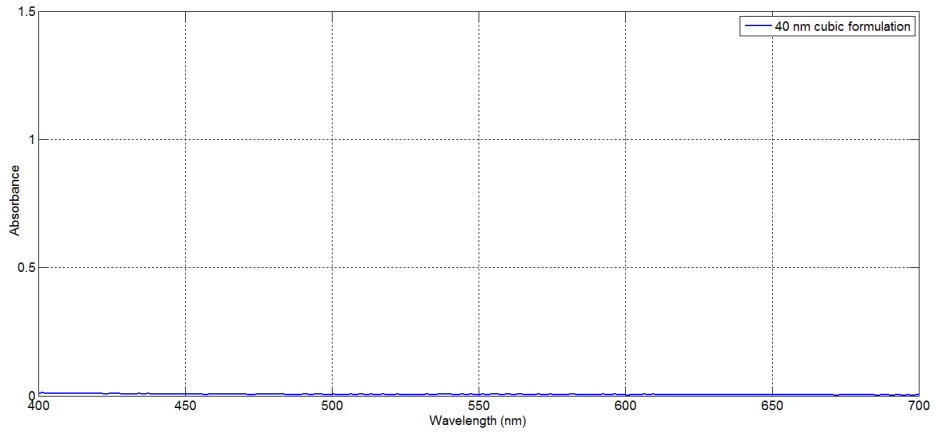


Figure B41 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was measured in an UV/VIS instrument directly after the experiment was completed. This sample was not dried out on a watch glass. This figure shows the 40 nm cubic formulation.

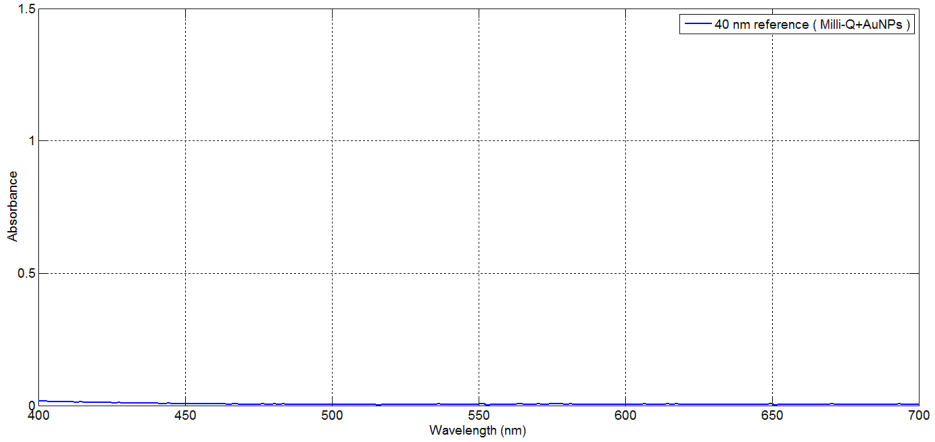


Figure B42 - Absorbance spectrum of the receptor fluid from the iontophoresis experiment. The receptor fluid was measured in an UV/VIS instrument directly after the experiment was completed. This sample was not dried out on a watch glass. This figure shows the 40 nm reference (Milli-Q+AuNPs).