Development of Dual Drugs with Coated Microneedles

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Abstract *— Coated microneedles have been shown to deliver biopharmaceuticals and drugs into the skin in a minimally invasive manner. To increase patient acceptance, smaller needle diameters and lower insertion forces have been investigated to reduce the frequency of painful injections. Guided by these, microneedles have been developed to minimize pain and are more useful for vaccine administration. This research was to develop a process for coating two different drugs with microneedles, make uniform coatings, and establish that the mass ratio of the components in the coating solution is maintained when measured in microneedles. The results shows that the drugs when in a pure state and with high concentration the absorbance tends to be higher but the absorbance decreases proportionally with the concentration of each substance when the drugs are mixed in different concentrations. When using the coating solution at different concentrations, absorbance varied but the mass ratio remained the same..*

Key Terms *— Coating solution, Dip-coated method, Microneedles, Ratio*

PROJECT STATEMENT

The world is affected by various diseases that are treated with drugs injected with hypodermic needles. Since then, needles have become the most widely used medical device, with an estimated sixteen billion injections administered worldwide. Hypodermic injections cause the most common side effects are skin reactions at the site of the needle injection. These side effects can cause pain, irritation, swelling, and redness of the skin. To increase patient acceptance, it has been investigated that a smaller needle diameter and insertion force reduce pain considerably. Using these criteria, coated microneedles have been developed to minimize pain and achieve greater patient confidence. One of the most important parts is to know what concentration or drug is in the microneedles to deliver them into the skin. This research is generated for pediatric patients and to have side effects in the administration of the drug on the skin. In addition, to have drug delivery options and to observe the behavior of proteins and cells in the coating by using excipients to increase the viscosity of the product. This Investigation will demonstrate the law of conservation of matter with the coating solution of two different drugs in microneedles. The objective of this research was to determine that the mass ratio of the components in the coating solution is maintained and to confirm that it is conserved when measured in microneedles and that the quality attributes, product concentration, are maintained. The expected result of this research is to be able to observe and calculate the mass conservation during the coating of microneedles. In addition, to be able to maintain the concentration of the drug during the administration and that the parameters are met according to the established to maintain the stability of the drug.

Research Description

The coated microneedles have been demonstrated to deliver biopharmaceuticals and drugs to the skin in a minimally invasive manner. To increase patient acceptance, smaller needle diameters and lower insertion forces have been investigated to reduce the frequency of painful injections. Following this approach, coated microneedles have been developed to minimize pain and achieve greater patient confidence. One of

the most important parts is knowing what concentration or drug is in the microneedles to deliver to the skin. However, pain reduction must be balanced with the limitations of depth, volume and substance introduced into the skin due to the small dimensions of the needle. In addition, microneedles have been found to be more useful in the administration of vaccines and biopharmaceuticals such as insulin, as they are more effective, but are not suitable for all applications. The importance of this research is to develop a coating method using two different drugs with different viscosities and to be able to determine the mass conservation ratio to measure the efficiency of delivery to the skin and how the amount of concentration can be improved depending on the drug. The coating solution that is used to get a deposition of solution of uniform coating on the microneedle, is obtained using low concentrations of carboxymethylcellulose sodium salt and Lutrol F-68 NF as excipients to increase the viscosity and decrease the surface tension. We will be watching the amount of dual coating on the microneedles using two different substances as sulforhodamine-B and vitamin B2, which are substances fluorescents. With the instrument of fluorescence microscopy, we measure the amount of sulforhodamine-B or vitamin B2 in the microneedle to be able to determine the amount of drug that is coated on the microneedle. The coating of microneedles can be achieved using different techniques and using a different method. In particular, these methods provide opportunities for developing advanced drug delivery systems for personalized and tailored transdermal medicines.

Research Objectives

The objective of this research was to develop a simple process of microneedle coating of two different drugs, to make uniform coatings on microneedles and to establish the mass ratio of the components in the coating solution is maintained and to confirm that it is preserved when measured in the microneedles.

Research Contributions

The purpose of this research is to have another alternative for the administration of drugs that we produce, using microneedles and maintaining the law of conservation of mass, efficiency, efficacy, and quality without altering the standards of stability and concentration of the product. In addition, this research helps the company's goals to contribute to the patients to have administration options to reduce different side effects like pain, irritation, swelling and redness of the skin while maintaining the quality and efficiency of the product.

BACKGROUND

The world is being impacted by various diseases which are treated with medicine injected using hypodermic needles. The hypodermic needle was invented independently by Charles Gabriel Pravaz in France and by Alexander Wood in England in 1853 [1]. Since then, needles have become the medical device most commonly used, with an estimated 16 billion injections administered worldwide [1]. Hypodermic injections cause the side effects most common are skin reactions at the injection site of the needle [2]. These side effects can cause pain, irritation, swelling and redness of the skin. Despite the effects that they can cause to the skin, the hypodermic needles are effective, the pain, the anxiety, the phobia of needles, and the difficulty of use have made them widely unpopular for both children and adults [3,4]. Using these criteria, coated microneedles have been developed microneedle to be able to minimize the pain and to gain greater acceptance of the patient. However, the reduction of pain must be balanced with the limitations of depth, volume, and substance introduced to the skin by the small dimensions of the needle. In addition, it has been found most useful of the microneedle in the delivery of vaccines and biopharmaceuticals such as insulin since they are more efficient but are not suitable for all applications. The first microneedles were made of silicon since they have been made from various

materials such as metal, polymer, glass, and ceramic. They have also been designed in different shapes and sizes (Figure 1), as needed for different applications. In general, the microneedles can be classified as drug-coated microneedles, dissolving microneedles, and hollow microneedles. Drug-coated microneedles typically use a water-soluble formulation as it dissolves in the skin. While the dissolving microneedles can be made entirely of water-soluble polymer and in this way, the microneedles dissolve or degrade in the skin, transferring the entire drug into the skin.

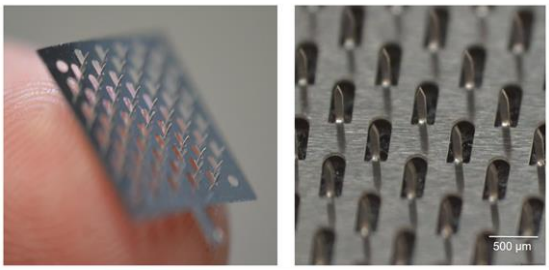

Figure 1
Microneedles Design for Multi-concentration Development

In addition, hollow microneedles are useful for liquid solutions for diffusion into the skin through the needle orifice. These different types of needles can benefit in different areas such as the application of the drug in the microneedle or the process that is carried out to supply the drug to the skin. There are a variety of processes for the coating of the microneedles. Most methods involve immersing the microneedles using an aqueous solution and a high viscosity in order to maintain the maximum amount of the drug. These methods include dip coating, gas jet drying and spray coating. The dip coating process is the easiest procedure for coating the microneedles since they are first dipped into the formulation and then removed. The application of dip coating uses similar dimensions of the microneedles. In addition, one of the most used techniques for the coating is layer by layer applying directly the drug. Jet drying gas is the slow drying process associated with the immersion coating process which is limited especially for curved microneedles. It has as drug coating solution a wet surface reducing and varying the generated dose. Another method used is spraying the microneedles using an aqueous solution often formulated to have increased viscosity to retain more of the formulation on the microneedles during drying. The coating by this method by spraying passes through three stages automation, deposition, and coalescence. This process is used for the millimetric size of the microneedles. These methods require a coating solution which must be soluble in water and for a rapid dissolution in the skin, which is an aqueous environment. In addition, the solvent must be safe for human use and should not damage the coated drugs in the microneedles. The substances used for these methods according to the application of the microneedle to increase the dispersion to facilitate the coating of the microneedles are Lutrol F-68, Tween 20 and Poloxamer [4]. To increase the viscosity can be used carboxymethylcellulose sodium salt, methylcellulose, and glycerol these substances do not alter to the drug. The microneedle coating formulations usually consider the controlled wetting and spreading of drug solution on the microneedle substrate is critical to obtain uniform coating on the microneedle surface. The microneedles were previously coated by dipping patches in a liquid solution for several hours to ensure a full coat on their surface. However, this simple procedure has several drawbacks such as waste and loss of drugs. The micro precision method where currently, there are different ways of delivering the drugs using microneedle. One of them is to coat the drug over the microneedle and insert it into the skin. Microneedles coated are more attractive for the provision of fast and it can store the drug in a solid phase. The coating solution that is used to get a deposition of solution of uniform coating on the microneedle, is obtained using low concentrations of carboxymethylcellulose sodium salt and Lutrol F-68 NF as excipients to increase the viscosity and decrease the surface tension. We will be watching the amount of dual coating on the microneedles using two different substances as the sulforhodamine-B and vitamin B2, which are substances fluorescents. With the instrument of

fluorescence microscopy, we measure the amount of sulforhodamine-B or vitamin B2 in the microneedle to be able to determine the amount of drug that is coated on the microneedle.

METHODOLOGY

The methodology used in this investigation is presented in the next paragraphs.

- Microneedle Fabrication - The 2D microneedle array with 50 microneedles were made from 50 μm-thick stainless-steel sheets. The individual microneedles were curved manually to make them perpendicular to the metal sheet. Each individual microneedle has to be 90° to the metal surface.
- Coating Solution - The microneedles were coated using micro-precision dip coating in order to transfer the amount of drug to be used. It comprised in an automated stage controlled by an x-y linear computer with the Math-Lab program where the microneedles were placed. The coating solution was housed in an orifice in which the microneedles were immersed through the motion control of the x-y stage. The coating solution was composed of 2%(w/v) carboxymethylcellulose sodium salt, 1% (w/v) Lutrol F-68 NF and a model drug. Use two different solutions to be determined as solution A and B. The solution A was composed of coating solution and 1% sulforhodamine B where is the fluorescent substance. The solution B was composing of coating solution and 1% vitamin B2. These two solutions A and B were mixed to result in the double drug to coat the microneedles. Use of solution A 25 μL while solution B is used 25 μL and the coating solution used 50 μL to have 100 μL of the solution to be used to coat the microneedles.
- Micro-Precision Coating - Ten patches of microneedle were coated using micro-precision dip coating to transfer the amount of drug. The amount of layer by layer in the microneedles was five. After coating the microneedle with the solution was introduced into a falcon where was 3ml of water. Sulforhodamine B and vitamin B2 are water soluble and then mixed for ten seconds. When the coating solution was mixed with water, 800 μL were taken with a pipette to analyze the sample.
- A fluorescence microscopy is used to analyze and determine the amount of dual drug found in the microneedles. This equipment is intended to measure the absorbance of each substance since it has a wavelength determined by the emission and excitation of the compound. The wavelength of sulforhodamine B is 565 excitation and 585 of emission. The wavelength of vitamin B2 is 448 excitation and 525 of emission this helps to be able to determine the amount of substance that can be found in the microneedles.
- Standard curve of Vitamin B2 - The standard curves are made to determine the amount of coating found in the microneedles. A stock solution was prepared from 1.0 $mg\ mL$ of the two major drugs used as sulforhodamine B and vitamin B2. Five different solutions will be prepared with different concentrations starting from the stock solution. The following solutions are 500 μL, 250 μL, 125 μL and 62.5 μL then each solution is completed up to 1000 μL with water.
- Standard curve of Sulforhodamine B - The standard curves are made to determine the amount of coating found in the microneedles. A stock solution was prepared from 1.0 $mg\ mL$ of the two major drugs used as sulforhodamine B and vitamin B2. Five different solutions will be prepared with different concentrations starting from the stock solution. The following solutions are 6.25 μL, 12.50 μL, 25 μL, 50 μL and 100 μL then each solution is completed up to 1000 μL with water.
- Coating of sulforhodamine B and vitamin B2 on some patch - Different concentrations of sulforhodamine B and vitamin B2 were mixed

to calculate the ratio of each substance. Sulforhodamine B concentrations are 90 ng/mL, 70 ng/mL, 60 ng/mL and 30ng/mL. These concentrations in the Figure 2 were mixed with vitamin B2 at the fallowing concentrations 50 ng/mL, 150 ng/mL, 200 ng/mL and 350 ng/mL. When preparing each solution and combining different concentrations, the coating solution was made to be placed in the microneedles.

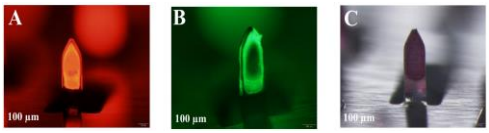

Figure 2
Microneedles with Multi-concentration

RESULTS AND DISCUSSION

The following results were obtained from methodology execution. Two standard curves were made for two different drugs to be used for the coating solution. This standard curve determines the amount of concentration found in the microneedles. The fluorescence spectrophotometer was used to measure the absorbance of each substance. Absorbance is defined as the logarithmic relationship between the intensity of light incident on a sample and the intensity of that same light that is transmitted through that sample. The absorbance measures the excitation and emission of each substance since they are different. In Figure 3 we can observe the standard curve of sulforhodamine B that has a linear behavior. The higher the concentration the greater the absorbance. In Figure 4 we observe an increase in absorbance with a linear trend.

The solution (A) is composed of 2% (w / v) carboxymethylcellulose sodium salt, 1% (w/v) Lutrol F-68 NF and sulforhodamine B. Solution (B) is composed of 2% (w/v) carboxymethylcellulose sodium salt, 1% (w/v) Lutrol F-68 NF and vitamin B2. Sulforhodamine B and vitamin B2 drugs were prepared at different concentrations to mix and calculate the amount of mass of each in the microneedles. As Table 1 shows the different concentrations that were used for solution A and solution B with their respective amounts of drug for the microneedles

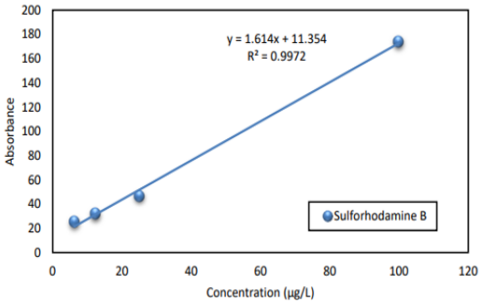

Figure 3
Standard curve Sulforhodamine B with Different Concentratiosns

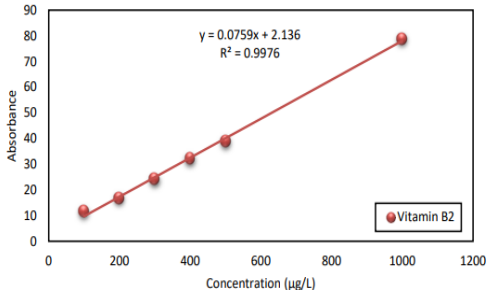

Figure 4
Standard curve vitamin B2 with different Concentrations

Table 1
Concentration of Solution A and B for the Solution Coating of the Microneedle

Sulforhodamine B (ng/mL)	Vitamin B2 (ng/mL)
90	50
70	150
60	200
30	350

It is observed that the drugs of sulforhodamine B and vitamin B2 when they are in their pure state and with high concentration the absorbance tends to become greater. When mixing A and B the absorbance decreases proportionally with the concentration of each substance. In the absorbance graph (Figure 5) it is observed that when the two drugs are mixed the concentration of sulforhodamine B 90 ng/ml with an absorbance of 98.634 and the concentration of vitamin B2 50 ng/ml with an absorbance of 8.693 having a higher sulforhodamine B concentration the absorbance is much greater, but at Decrease the concentration low the absorbance. To calculate the concentration of the drugs it is necessary to establish the emission

and the excitation of the substance. These substances have very similar numbers in the emission, and this can cause some discrepancy in the mass that is in the microneedles of the drugs. In the absorbance graph (Figure 6) the higher the concentration the greater the absorbance. By mixing the two drugs in the same solution the concentrations decrease from vitamin B2 and the absorbance decreases proportionally.

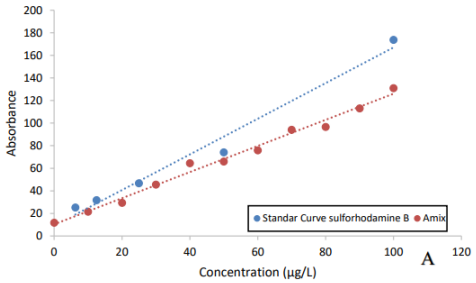

Figure 5

Mixing of the Concentration compared with the Standard Curve Sulforhodamine B Mixture

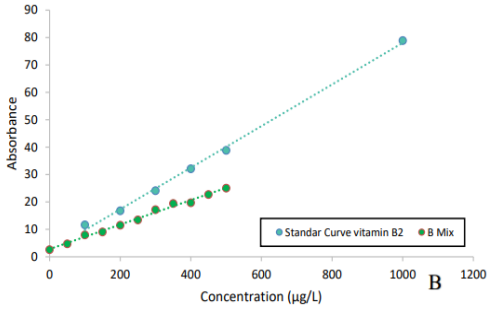

Figure 6

Mixing of the Concentration compared with the Standard Curve Vitamin 2 mixture

In the absorbance graph (Figure 7) the mixture between sulforhodamine B and vitamin B2 can be observed in the graph. The theoretical and experimental values of the ratio of the solutions with different concentrations using the coating solution for the microneedles (Table 2). These values were acquired between the division of the concentration of sulforhodamina B and Vitamin B2. The ratio was calculated with five different microneedles with different concentrations. The concentration of each substance is µg / L, the behavior of the graph of the mixture is linear. The tendency of sulforhodamine B is that the greater the concentration in the mixture the greater the absorbance. The tendency of vitamin B2 is that the higher the concentration in the mixture the greater the absorbance, but the lower the emission of this drug. This causes sulforhodamine B to interfere with the absorbance emitted by vitamin B2 in the mixture.

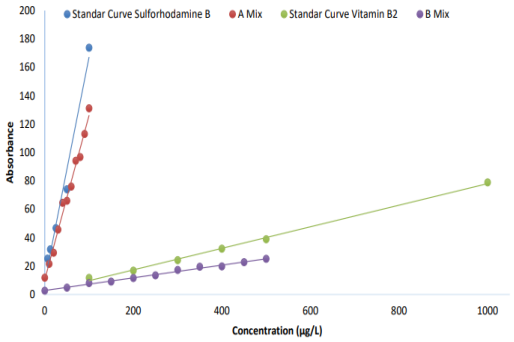

Figure 7

Mixture of each substance

Theorical Ratio (A/B)	Average Experimental Ratio (A/B)
1.8	1.856
0.466	0.578
0.3	0.336
0.085	0.0868

Table 2

Theoretical and experimental values of the ratio

After calculating the experimental ratio concentration value using the spectrophotometer, the amount of substance in each microneedle was identified with a microscope. Each microneedle patch contains fifty-seven inserted layers of five drugs. The substances with their respective colors can be identified by using the Olympus SZX12 stereo microscope to capture the photos. Each microneedle patch was coated with the solution using the micro-precision mechanism as shown in (Figure 8) to have homogenization of each solution. In the (Figure 9) shows the sulforhodamine B coating solution in the microneedle patch with a concentration of 90 ng/mL. In addition, the sulforhodamine B solution can be seen to be transferred around the entire microneedle so that it can be effectively transferred. Vitamin B2 coating in 2-dimensional microneedles without patch

(Figure 10) also shows the behavior and the coating solution that helps the viscocity to maintain the correct concentration in each microneedle. In addition, as shown in (Figure 11) is the coating of sulforhodamine B and vitamin B2 in 2-dimensional microneedle patches. In image A there is a mixture of 90 ng/mL of sulforhodamine B and 50 ng/mL of vitamin B2. It can also be observed how the sulforhodamine coating solution is calculated the amount of vitamin B2 in the microneedle and the difference.

Figure 8
Micro-precision Coating Equipment with Microneedles

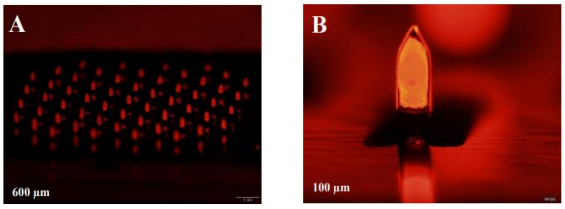

Figure 9
Sulforhodamine B Coating in 2-dimensional Microneedles with a Concentration of 90 ng/ml.

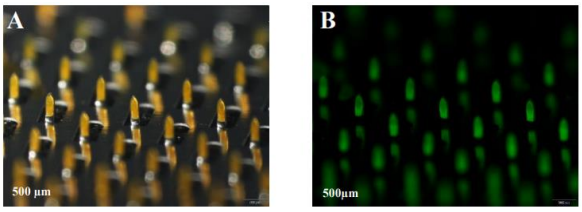

Figure 10
Vitamin B2 Coating in 2-dimensional Microneedles

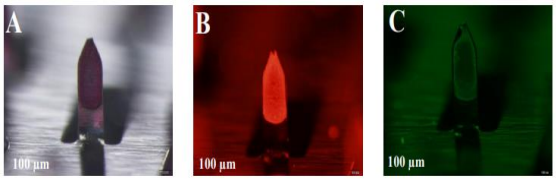

Figure 11
Sulforhodamine B and Vitamin B2 Coating Microneedles

CONCLUSION

In a world with a high incidence of diseases, where patients regularly require drugs, it is important to ensure the effectiveness of the drug. The coated microneedles have been developed to minimize pain and achieve greater patient confidence. Needles have become the most widely used medical device, with an estimated 16 million injections administered worldwide. One of the most important parts is to know how much concentration or drug is in the microneedles to supply them to the skin. Because the concentration is a determinant factor for the efficiency of the treatment with microneedles, the value for the solution of Coating and pure substances under study. It is observed that the drugs sulforodamine B and vitamin B2 when they are in pure state and with high concentration the absorbance tends to be greater. As the coating solution was used at different concentrations, the absorbance was identified as varying, but the mass ratio remained the same. The results of the research demonstrated the law of conservation of matter using absorbance as the main indicator through experimentation. For future work, will be investigating larger molecules. It will be experimented with sulforhodamine DNA and proteins for the administration of the drugs to different animals in the mucosal area.

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