Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/za4ark25

MICRONEEDLE-MEDIATED DELIVERY OF LEVALBUTEROL FOR PEDIATRIC ASTHMA: FORMULATION DEVELOPMENT AND PHARMACOKINETIC EVALUATION

Venkatesh K, Komala M*

*Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai - 600117, Tamilnadu, India.

***Corresponding Author:** Komala M
*E-mail: komala.sps@vistas.ac.in

Abstract
It is challenging to manage pediatric asthma due to the frequent administration of medications and the difficulty of using conventional inhalers by young children. Our study explores microneedle-mediated delivery as a novel method for administering levalbuterol, a β2-adrenergic agonist commonly used to treat pediatric asthma. There are many advantages to transdermal drug delivery, but there are also some drawbacks. Certain drugs cannot penetrate the skin barrier effectively, so transdermal delivery may not be feasible. A number of factors can also affect the permeability of the skin, such as thickness, hydration, hair follicles, and sweat glands. There are a number of medications that can be administered via transdermal delivery. Medication compliance is improved and convenience is provided. Asthma patients treated with microneedle-mediated levalbuterol demonstrated feasibility and effectiveness. The results of this study suggest that microneedle technology has the potential to be a safe, effective, and patient-friendly alternative to traditional inhalers for the delivery of asthma medication to pediatric patients.

Keywords: Pediatric, Asthma, Levalbuterol, Microneedle

Introduction

Recently, there has been renewed interest in developing novel drug delivery systems for existing drug molecules [1]. Development of a novel delivery system for existing drug molecules not only improves the efficacy and safety of the drug, but also improves patient compliance and achieves a significant therapeutic benefit [2, 3].

TDDS development involves a multidisciplinary approach that encompasses a wide range of fundamental feasibility studies ranging from the selection of the right drug molecule to the demonstration of sufficient drug flux in both an ex vivo and in vivo model [4]. The next step is to fabricate a drug delivery system that meets all the stringent requirements that are specific to the drug molecule (physicochemical, stability factors), the patient, the manufacturer (scale-up and manufacturing capability), as well as the economy [5].

Anatomy of Skin:

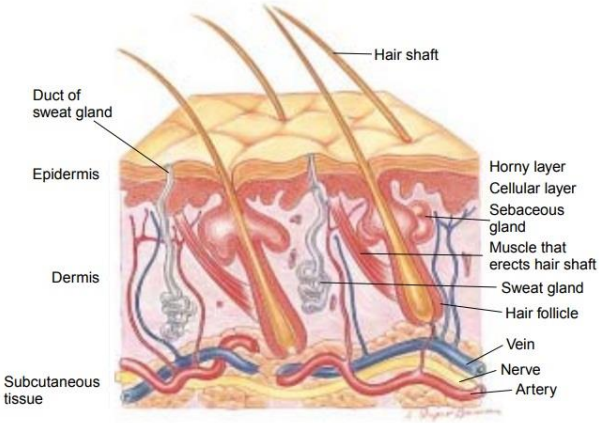

Figure 1: Anatomy of skin

The Epidermis:
A layer of squamous epithelium known as the epidermis covers the entire outer surface of the body, constantly self-renewing and stratified [6, 7]. The epidermis is made up primarily of two parts: the living and viable cells in the malpighian layer (viable epidermis) and the dead cells in the stratum corneum, commonly called the horny layer.

Dermis:
A dermis is a layer of skin located just beneath the epidermis. It is a thick layer ranging from 3 to 5 mm thick and is composed of a matrix of connective tissues, which contains blood vessels, lymphatic vessels, and nerve fibers. It is essential for the body's temperature to be regulated by an adequate supply of blood to the skin. The skin receives nutrients and oxygen from the blood, while waste products and toxins are removed from it by the liver. Approximately 0.2 mm of the skin surface is covered by capillaries, which provide the sink conditions for most molecules that are able to penetrate the skin barrier and penetrate deeper.

Subcutaneous:
The subcutaneous layer, or hypodermis, is the deepest layer of the skin, located beneath the dermis. It consists primarily of fat tissue, connective tissue, and blood vessels. The subcutaneous layer helps regulate body temperature, stores energy, and provides cushioning and insulation for the body.

Microneedles
Microneedles [mns] are a new method of drug delivery that was introduced by the FDA to the public in 1998. During the past few years, the field of drug delivery research has been extensively studying how to deliver proteins, deoxyribonucleic acid (DNA), genes, antibodies, and vaccines to the human body efficiently and safely [8, 9].

Advantages of microneedle drug delivery
- It is possible to administer large molecules,
- The active pharmaceutical ingredient can be administered in a painless manner,
- It is avoided that first-pass metabolism occurs,
- The injection site heals faster than if it were injected with a hypodermic needle.

Disadvantages of microneedle drug delivery:
- A hypodermic needle may be less accurate when it comes to dosage accuracy,
- The veins may collapse as a result of repeated injections,

▪ There is a possibility that the tip of the microneedle may break off when the patch is removed and remain in the skin,
▪ It is possible to give a small amount of drug by bolus (less than 1 mg) and
▪ Microneedles can be obstructed by compressed dermal tissue [10 - 12].

METHODOLOGY

Materials

Unsaturated polyester resin and peroxides, Polyvinyl alcohol, Polyvinyl pyrrolidone K-30, Levalbuterol.

Methods

Preformulation Study

Description:

Color, odor, and appearance of Levalbuterol were observed

A standard curve for the development of levalbuterol

Preparation of standard calibration curve

Weighted Levalbuterol working standards were transferred to volumetric flasks containing 100 ml of water. The stock solution was prepared by adding approximately 50 ml of pH 7 to the amount of 250 μg/ml and shaking to dissolve it completely. UV-visible spectrophotometer measurements were performed at 276 nm using SIPB pH 7 as a blank when measuring UV-visible spectrophotometer. Using the absorbance concentration as a reference, calibration curves were plotted and obtained.

Compatibility study of drug excipients

Fourier transform infra red spectroscopy:

The compatibility between an excipient and a drug must be ensured prior to formulation. A confirmation must be made that the drug does not react with polymers and excipients under experimental conditions in order to ensure that the formulation does not suffer any adverse effects. As part of the investigation into the compatibility between drugs and excipients, FTIR spectral analysis was conducted. Levalbuterol's IR spectrum was recorded using a Fourier transform infra red spectrophotometer. Using Levalbuterol and the polymer mixtures polyvinyl alcohol (PVA) and polyvinylpyrrolidone (1:1), a mixed solution of Levalbuterol plus polyvinyl alcohol and polyvinylpyrrolidone was prepared. We measured the FTIR spectrum of a mixture containing a drug and a polymer using fourier transform infrared spectroscopy. We prepared samples by hydrostatically pressing KBr pellets with 5.2 N cm-2 for 3 minutes. If the spectrum was scanned over the frequency range of 4000 - 500 cm-1 and the resulting spectrums were compared, it is possible that any spectral changes might have been detected.

Formulation studies

The preparation of the cavity for the microneedle mold consists of the following steps:

- The microneedle moulds are prepared by mixing unsaturated polyester resin with peroxide.
- This type of mould is produced by using a microneedle mould developed by 3M.
- As a first step, it was necessary to take a mixture of hardener and resin (1:100) material and properly mix it together.
- It was necessary to pierce the surface of the array with the microneedle tip of the 3M microneedle in order to obtain the array mold.
- Upon preparing the mold arrays, it was dried for 24 hours at room temperature for the formation of hard, solid moulds after it had been prepared for 24 hours at room temperature.

1. Polymer solution preparation:

As shown in Table 1, a polymer solution was prepared. A specified amount of PVP was added to distilled water that was heated to 900 degrees Celsius. A magnetic stirrer was used to stir the

mixture, and then PVA was added and stirred again in a magnetic stirrer for a further 20 minutes. At this stage, the solution was left at room temperature until the polymer had been dissolved in the solution.

2. The preparation of drug-loaded microneedle patches consists of the following steps:

- There are a number of steps involved in the preparation of the polymer solution.
- A small quantity of PVP was added to the distilled water after it had been heated to 900°C in order to prepare the solution.
- With a magnetic stirring device, PVA was added, and then the magnetic stirring device was used to stir the PVA in for 10 minutes, after which the PVA was added and stirred for another 10 minutes.
- After the polymer has been dissolved in the solution, the solution was kept at room temperature for a long period of time.
- It was decided to take 6 ml of the polymer solution that had been prepared, and to this 6 ml was added 20 mg of weight levalbuterol and thoroughly mixed into the polymer solution.
- After the mixture was injected drop by drop into the mold cavities, it was allowed to dry overnight at room temperature, before being peeled from the mould and used as a microneedle patch containing the drug.

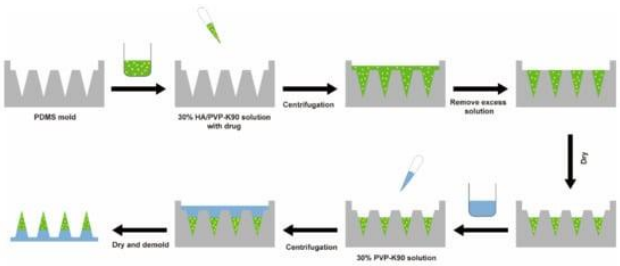

Figure 2: A schematic of the fabrication process of dissolving microneedle patches

EVALUATION:

1. Physical examination of patches

The patches were examined visually for smoothness, brittleness, transparentness, stickyness, flexibility, and homogeneity.

2. Weight variation

A digital scale was used to measure weight uniformity between three patches from each formulation. Three patches of 1.5 cm2 were weighed in order to determine how the weight varies between formulations.

3. Folding endurance

The folding endurance of a polymer film 1 x 1 cm was evaluated by folding it repeatedly until it broke. Using a strip of 1 x 1 cm, we removed the adhesive film from the patch's center and edge. The test was conducted with three patches randomly selected from each formulation.

4. Percentage moisture content

Each formulation was evaluated based on its moisture content percentage. From each patch, a film measuring 1 x 1 cm was taken. A digital weight balance was used to weigh each of these films individually. A desiccator containing silica beads containing these polymeric films was placed at 25°C with labelled Petri dishes. In order to achieve a constant weight, the films were weighed repeatedly. The following formula was used to calculate the percentage moisture content.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

5. Percentage moisture uptake

The percentage of moisture absorbed by various formulations was determined. Transdermal films measuring 1 x 1 cm were cut from each patch. Each film was weighed individually using a digital weighing balance. These films were placed in Petri dishes labeled with their relative humidity (RH) at 25°C with 84% relative humidity (RH). It was necessary to continuously weigh the transdermal films until a constant weight was achieved. A percentage was calculated for moisture uptake using the formula below:

$$\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

6. Scanning electron microscopy

Using scanning electron microscopy (Quanta 200 F), the surface morphology of the microneedle patch was examined to assess the array formation and sharpness of the needles.

7. Drug content

Several dilutions of the microneedle patch were performed in 0.1N HCl buffer pH 7, and then the drug content of the patch was determined by UV visible spectrophotometry after the patch had been diluted.

8. In vitro drug release

The in vitro drug release studies were conducted using a Franz diffusion cell equipped with a receptor compartment. Microneedle films were placed in between the donor and receptor compartments with a membrane acting as a diffusion barrier. There was also added to the receptor compartment of the diffusion cell a buffer containing 0.1N HCl pH 7. The entire assembly was fixed on a hot plate magnetic stirrer and the solution was continuously stirred with magnetic beads using the hot plate magnetic stirrer, and the temperature was maintained at 32° plus 0.5°C, since the skin temperature is 32°C. The samples were analyzed for drug content at different time intervals using a UV spectrophotometer at 276 nm. Each time samples were withdrawn, the receptor phase was refreshed with 0.1N HCl buffer.

9. In vitro release kinetics

Zero order equation

A zero-order release kinetics can be derived by plotting cumulative percent of drug released (versus) time (hours). A formulation with a zero-order release profile is ideal for achieving long-lasting pharmacological effects.

$C = K_o t$ Equation 1

Where,

Ko = Zero order constant in conc. / time

t = Time in hours

First order equation

A logarithmic graph showing cumulative drug remaining percentage (versus) time was plotted.

$\text{Log } C = \log Co - Kt/2.303$ Equation 2

Where,

Co = Initial drug concentration

K = First order constant

t = Time in hours.

Higuchi Kinetics

The graph was plotted as % cumulative drug remaining (vs) square root of time.

$Q = Kt^{1/2}$ Equation 3

Where,

K = Constant reflecting design variable system (Differential rate constant)

t = Time in hours.

The drug release rate is inversely proportional to the square root of time.

Korsmeyer – Peppas equation

To evaluate the mechanism of drug release, it was further plotted in Peppas equation as log cumulative % of drug released (vs) log time.

$M_t/M_\alpha = Kt^n$ Equation 4

Where,

M_t/M_α= Fraction of drug released at time t

t = Release time

K = Kinetics constant (Incorporating structural and geometric characteristics of the formulation)

n = Diffusional exponent indicative of the mechanism of drug release.

Hixson and Crowell erosion equation

$Q^{1/3} - Q^{1/3} = K\ t$ Equation 5

Where,

Qt = Amount of drug released at time t

Qo = Initial amount of drug

K_{HC} = Rate constant for Hixson Crowell equation

RESULTS AND DISCUSSION

Preformulation studies:

Calibration curve in Levalbuterol

The linearity of the calibration curve was estimated by plotting absorbance (nm) versus concentration (g/ml) (y). A calibration curve was prepared by measuring absorbance at 276 nm. Figure 4 shows graphical representations of the statistics evaluation parameters listed.

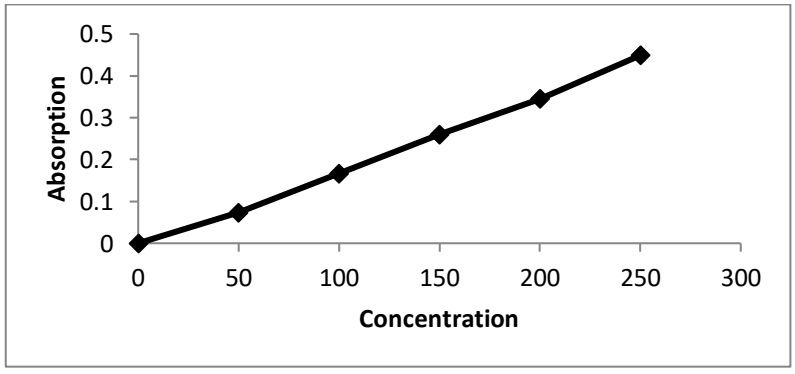

Figure 3: Calibration curve of Levalbuterol using pH 7

Drug excipient compatibility study:
FTIR spectroscopy:

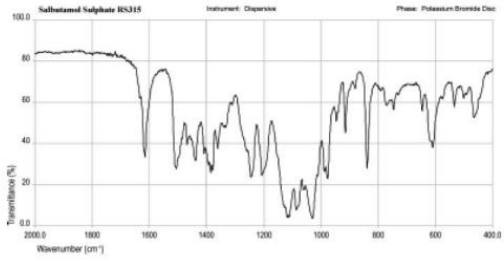

Figure 4: FTIR spectrum of Levalbuterol

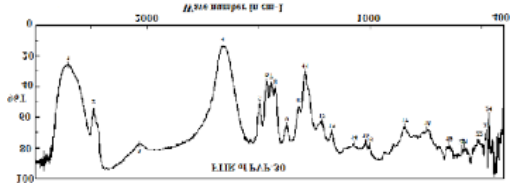

Figure 5: FTIR of Polyvinyl pyrrolidone

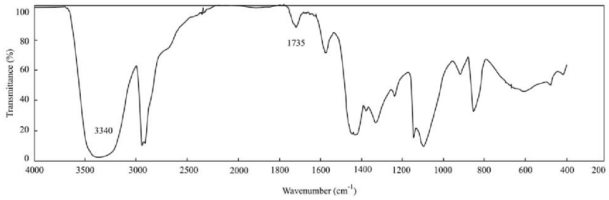

Figure 6: FTIR of Polyvinyl alcohol

Compared to the standard peaks, all characteristic peaks can be seen in the result, which is different from standard peaks. A significant change in the spectrum of drugs was not observed in the amount of drugs available.

Evaluation:
Transdermal patch evaluation parameters

Table 1: Weight variation

Formulation	Weight Variation mg/1.5 cm^2
F1	32.5 ±4.56
F2	33.7 ±4.05
F3	34.3 ±6.7
F4	34.7 ±2.44
F5	35.3 ±2.34

As a result of the evaluation, we found that there were deviations of 3-4 mg between the weights of the patches ranging from 32 to 35 mg. This was within the permissible range when it comes to the weight variations of a conventional transdermal patch.

Table 2: Folding endurance

Formulation	Folding Endurance
F1	> 200
F2	> 200
F3	> 200
F4	> 200
F4	> 200

Across all formulations of transdermal patches, the endurance of the patches was determined to be more than 200 fold.

Table 3: Moisture content, Moisture Uptake

Formulation	Moisture Content (%)	Moisture Uptake (%)
F1	3.44	7.64
F2	4.34	7.68
F3	4.61	6.12
F4	7.82	6.39
F5	7.21	6.34

There was a range of moisture content in all formulations ranging from 3.44 - 7.21%.
The moisture uptake of all formulations was found to be adequate, ranging from 6.34 to 7.68%.

Table 4: Drug content

Formulation	Drug Content (%)
F1	97.7
F2	98.4
F3	96.4
F4	97.9
F5	97.8

In these formulations, there was an active ingredient content ranging from 96.4 to 98.4%.
Table 1 presents the weight variation over 1.5 cm2 measured with a digital weight balance for each formulation.
The folding endurance of each patch was determined by folding 1x1 cm of each patch repeatedly for a period of five minutes, as shown in Table 2.
In Table 3, we show the percentage moisture content of the formulation patches after they had been weighed before, when they had been kept in a desiccator for two months, and then after they had been weighed again when they reached the same weight as they were when they were first weighed.
In the humidity chamber, 1 inch by 1 inch samples of each formulation were taken and weighed at constant weights.
To determine the presence of drugs in each formulation, UV spectrophotometry at 272nm was used. The values are shown in Table 4.

Scanning electron microscopy:
A scanning electron microscope was used to examine the surface morphology of the microneedle patch shown in Figures 7-9.

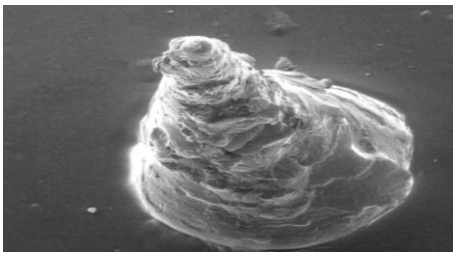

Figure 7: Scanning electron microscopy image of microneedle for analysis of its sharpness

Figure 8: Measurement of the space between two needles by scanning electron microscopy

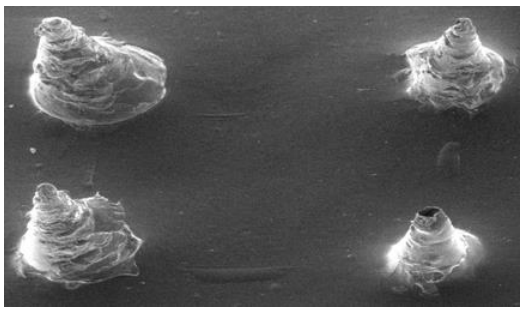

Figure 9: Microneedle array and tip sharpness analysis using scanning electron microscopy

Using scanning electron microscopy (SEM), a SEM image was generated of the freshly fabricated microneedle so that the morphology and dimensions of the microneedle could be studied. From the result, we can see that the needle is sharp and that the array has formed as a result of the needle's sharpness. It is shown in the image that a transdermal microneedle patch is prepared with a sharp needle. In the process of peeling the microneedle mold cavity, one needle tip breaks as a result of excessive peeling force. There is no doubt that the needle is mechanically strong; however, it penetrates the stratum corneum and delivers the drug directly into the bloodstream despite its strength.

In vitro release studies:

Table 5: In vitro release studies values of formulations F1 – F5

TIME (h)	F1	F2	F3	F4	F5
1	17.6	35.3	35.11	30.05	30.06
2	38.20	64.1	52.3	55.01	54.67
3	73.35	80.02	86.15	81.02	80.89
4	97.2	97.6	93.04	90.13	89.92

It can be concluded that all formulations have good release properties (i.e. >90%). Among the formulations F1 and F2, it was found that the drug was released between 96% and 97%, whereas formulations F3 and F4 were found to be released between 91% and 96%. It has been concluded, therefore, that the release of the drug from transdermal patches is delayed if the PVA content of the patches is increased.

Zero order release:

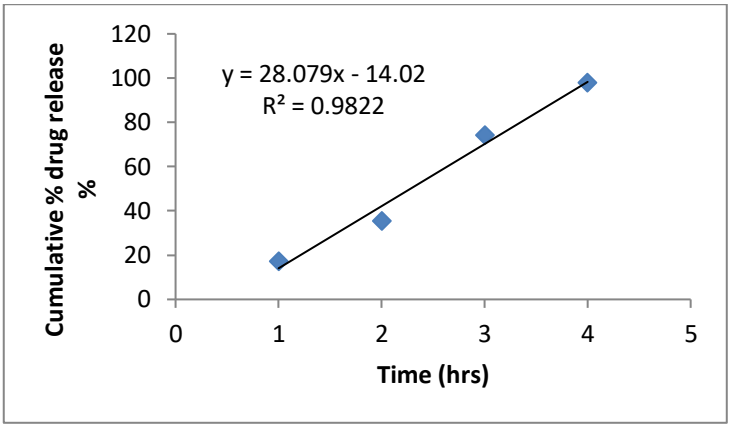

Figure 10: Zero order release

First order release:

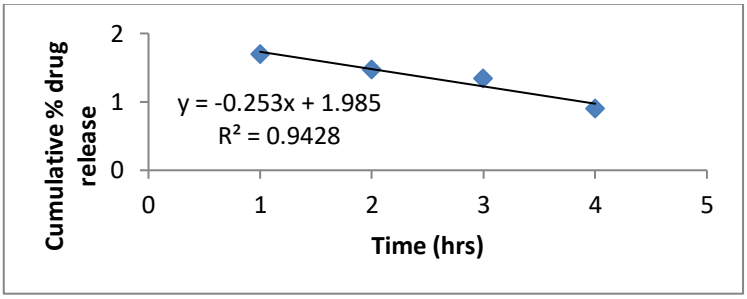

Figure 11: First order release

Higuchi:

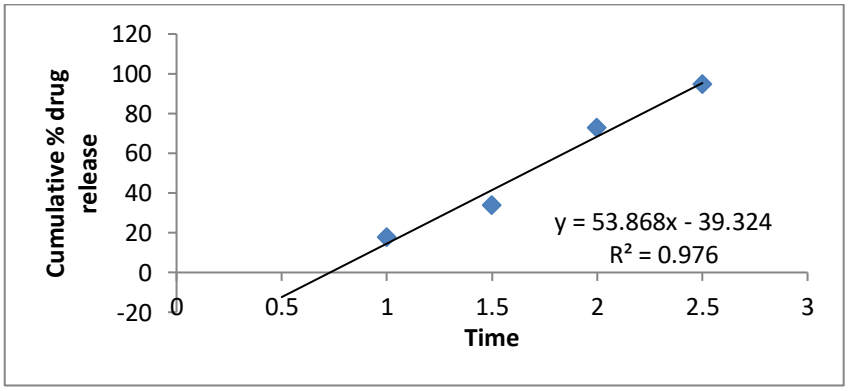

Figure 12: Higuchi percentage drug release

Koarsmeyer Peppas:

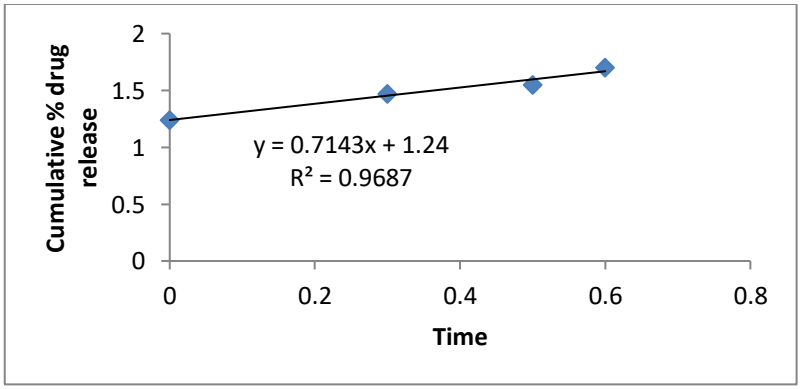

Figure 13: Koarsmeyer Peppas log of percentage drug release

Hixson Crowell:

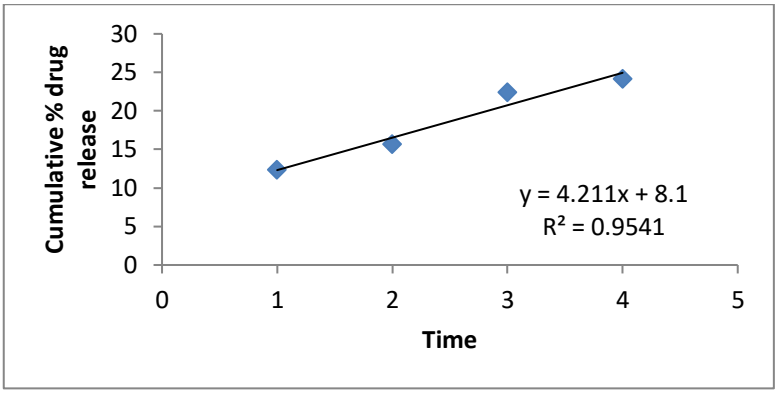

Figure 14: Hixson Crowell cube root of percentage drug release

The R2 values were determined for zero-order plots (0.9822), first-order plots (0.9428), Higuchi plots (0.976), Koarsmeyer Peppas plots (0.9687), and Hixson Crowell plots (0.9541) [13 - 15].

CONCLUSION

In conclusion, microneedle transdermal patches are a novel and innovative means of delivering drugs through the skin that may improve therapeutic outcomes, improve patient adherence, and increase the range of medications that can be administered through the skin. Microneedle technology in healthcare holds the potential of being fully realized with the expansion of research and development in this field.

REFERENCES

1. Himanshi Tanwar, Ruchika Sachdeva. A review of transdermal drug delivery system. Int J Pharm Sci Res. 2016; 7(6): 2274-2290.
2. Sonia Dhiman,Thakur Gujarath Singh, Ashik Kumar Rehni.Transdermal patches: A recent approach to new drug delivery system. Int J Pharm Pharm Sci. 2011; 3(5): 26- 34.
3. Tajashree Waghule, Gautam Singhvia, Sunil Kumar Dubeya, Murali Monohar Pandeya, Gaurav Guptab, Mahaveer Singh et al. Microneedle: A smart approach and increasing potential for transdermal drug delivery system. Biomed Pharmacother. 2019; 109: 1249-158.
4. Pablo Serrano-Castaneda, José Juan Escobar-Chávez, Isabel Marlen Rodríguez- Cruz,Luz María Melgoza-Contreras1, Jessica Martínez-Hernández e. Microneedles as enhancer of drug absorption through the skin and applications in medicine and cosmetology. J Pharm Pharm Sci. 2018; 21: 73-93.
5. Valsa Remony Manoj, Hima Manoj. Review on transdermal microneedle based drug delivery. Asian J Pharm Clin Res. 2019; 12(1): 18-29.
6. Hiep X.Nguyen, Behnam Dasht Bozorga , Yujin Kima , Alena Wieberb, Gudrun Birkb, Dieter Lubdab,et al., Polyvinyl acohol microneedle: Fabrication, characterisation and application for transdermal drug delivery of doxorubicin. Eur J Pharm Biopharm. 2018; 129(88): 103-113.
7. Yanqi Ye, Jicheng Yu, Di Wen, Anna R. Kahkoska, Zhen Gu. Polymeric microneedle for protein delivery. Adv Drug Deliv Rev. 2018; 18: 300-323.
8. Maelisoa TC McCrudden, Ahlam Zaid Alkilani, Cian M McCrudden, Emma McAlister A, Helen O McCarthy A, A David Woolfson , Ryan F Donnelly. Design and physiochemical characterisation of novel dissolving polymeric microneedle arrays of transdermal delivery of high dose, low molecular weight drugs. J Control Release. 2014; 180: 71-80.
9. Min Wang, Lianzhe Hub and Chenjie Xu . Recent advance in the design of polymeric microneedles for transdermal drug delivery and biosensing. RSC.LIC/LOC. 2017;10:1039.
10. Lanke SSS, CS Kolli , JG Stroma, AK Banga. Enhanced transdermal delivery of low molecular weight heparin by barrier perturbation. Int J Pharm. 2019; 365: 26-33.

11. Leone M, J Monkare, JA Bouwstra1, G Kersten. Dissolving microneedle patches for dermal vaccination. Pharm Res. 2017; 34(11): 2223-2240.
12. Mary-Carmel Kearney, Ester Caffarel-Salvador, Steven J Fallows, Helen O McCarthy, Ryan F Donnelly. Microneedle mediated delivery of donezepil:potential for improve treatment option in Alzheimers disease. Eur J Pharm Biopharm. 2016; 103: 43-50.
13. Chi Lee, Jheng-Siou, Meng-Tsan Tsai, Kai-Che Lin. Fabrication of novel partial dissolving polymer microneedle patch for transdermal delivery. J Mater Chem. 2014; 12(48): 2-30.
14. Yukako Ito, Aiko Murakami, Tomohiro Maeda, Nobuyuki Sugioka, Kanji Takada. Evaluation of self dissolving needle containing low molecular weight heparin in rat. Int J Pharm. 2007; 349: 124-129.
15. Shubhmita Bhatnagar, Neha Gajanan Bankar, Mrunal Vijay Kulkarni, Venkata Vamsi Krishna Venuganti. Dissolvable microneedle patch containing doxorubicin and docetaxel is effective in 4T1 xenografted breast cancer mouse model. Int J Pharm. 2019; 10: 263-275.