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Polymer Coated Polymeric (PCP) microneedles for sampling of drugs and biomarkers from tissues

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ABSTRACT

The Polymer Coated Polymeric (PCP) microneedles were fabricated using PVP K30 in the core and ethyl cellulose in the coating. The PCP microneedles do not disintegrate in the tissue upon insertion and rather stays intact and allows diffusion of drugs and analytes across the membrane both inward and outward. In this project the potential use of PCP microneedles for sampling analytes from the dermal tissue was explored. The amount of analyte sampled depended on the concentration in the tissue, physicochemical properties of the analyte and duration of insertion of the array in the tissue. Further, an advanced type of PCP microneedle array was fabricated by entrapping absorbent beads in the core microneedles. The adsorbent enabled the PCP microneedles to recover significantly higher amount of analyte from the tissue.

1. Introduction

Microneedles are micron size needles prepared in the form an array for the delivery of drugs and biologicals into the tissues (Panda et al., 2022; Singh et al., 2013). Polymeric microneedles are intended for disintegration in the tissue rapidly or slowly for immediate and controlled release of therapeutic agents. Coated polymeric microneedles are novel approach in controlled release of active ingredient, in which the microneedles are coated with a semipermeable polymeric layer which will allow only the active ingredient to diffuse out of the microneedles without allowing the polymer construct to dissipate in the tissue (Ingrole and Gill, 2019).

The tissue fluids are a milieu of biochemicals that is in homoeostasis with the systemic circulation. Generally, any imbalance in the biochemical constituents in the tissue or systemic circulation is reflected in the interstitial fluid (Roumelioti et al., 2018; Dermatokinetics of Therapeutic 2022). Therefore, aspirating the extracellular fluid from the tissues or sampling its constituents if of significant interest to from the perspective of developing novel diagnostic techniques and identifying biomarkers the content from the tissue extracellular fluid to diagnose systemic disorders and potential diseases (Dermatokinetics of Therapeutic 2022). Most of the methods available for such purposes are invasive and traumatic. Therefore, there is need for exploring noninvasive and minimally invasive techniques of sampling contents from the tissues that do not cause any trauma to the patient (Hailu et al., 2020). Another application of such sampling technique is in the field of therapeutic drug monitoring and in determining the locoregional kinetics of drugs following topical or systemic delivery too (Kiang et al., 2017; Caffarel-Salvador et al., 2015).

In this project, the use of Polymer Coated Polymeric (PCP) microneedles as a potential minimally invasive technique of sampling contents form the tissue was explored. Further, the adsorbent embedded PCP microneedles were fabricated to improve the extraction efficiency of PCP microneedles. The feasibility of using PCP microneedles for sampling analytes was investigated using some model small molecules porcine skin.

2. Materials and method

BASF generously provided Polyvinyl Pyrrolidone (PVP K30), New

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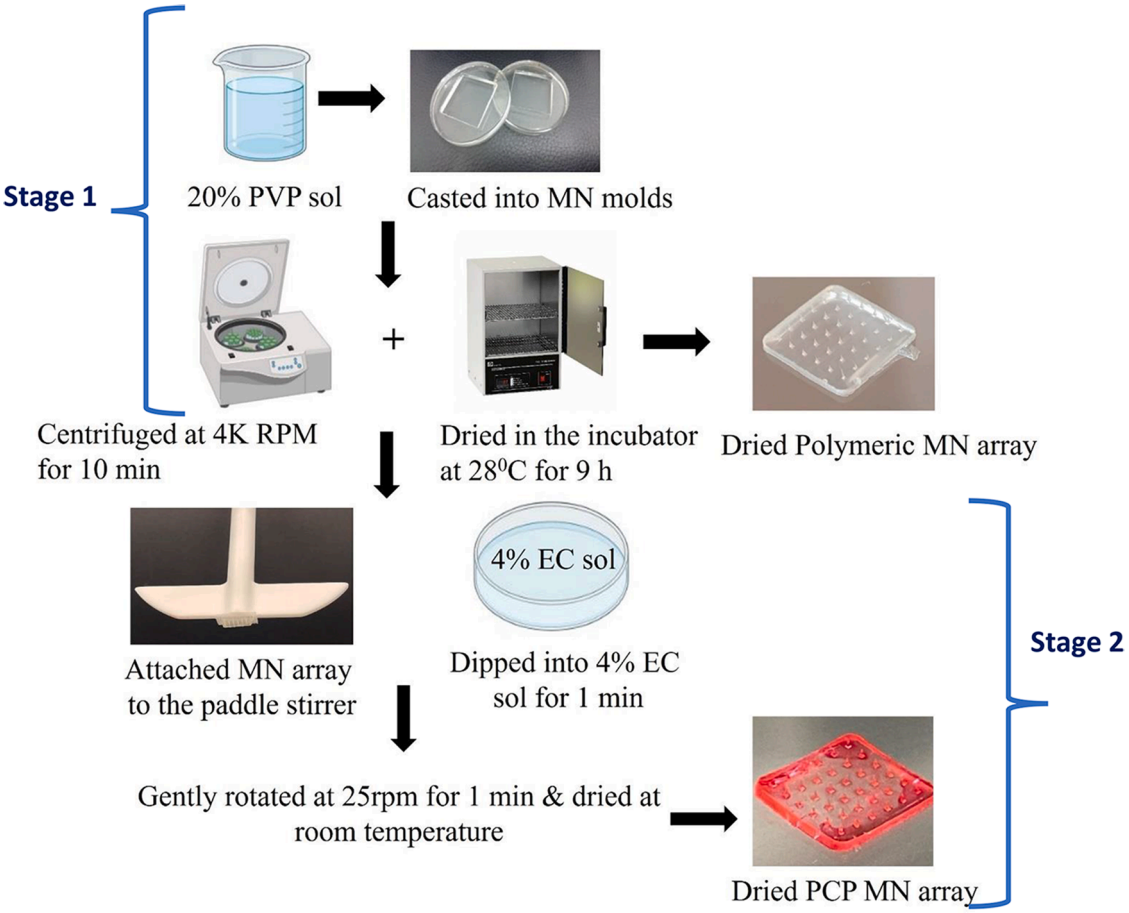

Fig. 1. Flowchart of fabrication of PCP microneedles in two stages. The first stage was preparation of an array of core microneedles. The second stage involved coating of core microneedles using a polymer. Nile red was incorporated in the coating solution for microscopic resolution. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

York, USA. Nile Red was procured from TCI Chemicals, Portland, USA. Toluene and Ethyl cellulose were purchased from Aqualon, Wilmington, USA. Carboxen was bought from Sigma Aldrich, St. Louis, USA. The carboxen was passed through mesh 120 was used for fabrication. Porcine ear skin was obtained from the local slaughterhouse.

2.1. Preparation of PCP microneedles

The microneedles were prepared using the mold casting technique (Maurya et al., 2018; A. Maurya et al., 2019). A PDMS (poly dimethyl siloxane) template was used to fabricate the polymeric microneedles. The PDMS silicone molds were composed of a 6 × 6 conical array. PVP K30 (20% w/v) was dissolved in deionized water to prepare the casting solution. The silicone molds were filled with 0.2 mL of casting solution and centrifuged at 4000 rpm for 10 min to fill the mold cavities with the solution. 0.3 mL of the PVP K30 solution was poured into the molds to form the complete microneedle array. The microneedle molds were dried in an incubator at 28 °C for 9 h. Dip-and-spin coating was used to coat the prepared polymeric microneedles. In toluene, a 4% w/v ethyl cellulose solution and 0.001% of nile red was dissolved to coat on microneedles made with PVP K30 (20% w/v). The microneedle array was attached to the paddle shaft with an adhesive tape, dipped in the coating liquid, and revolved at 25 rpm for 2 min. The coated PCP microneedles were stored in a desiccator until further research. The entire process of fabricating the PCP microneedles is shown in Fig. 1.

2.2. Preparation of adsorbent embedded PCP microneedles

PVP K-30 (20% w/v) was dissolved in deionized water to prepare the casting solution. Carboxen was added into the casting solution and vortexed for 15 s. 0.2 mL of the casting solution containing carboxen was poured into the PDMS molds and centrifuged at 4000 rpm for 10 min to push the casting solution containing carboxen into the microneedle mold cavities. 0.3 mL of the PVP K30 solution without the carboxen was placed into the molds as a backing platform to complete the microneedle array. The molds were placed in the incubator at 28 °C for 9 h. The microneedle was attached to the back of the paddle shaft with the help of an adhesive tape. The microneedle was dipped into the ethyl cellulose coating solution and rotated at 25 rpm for 2 min. The carboxen loaded PCP microneedles were dried and stored in the desiccator until further research.

2.3. Sampling of contents form skin tissue

2.3.1. Skin tissue preparation

The freshly excised pig ear skin was freshly obtained from the local abattoir and cut into small piece and stored at −80O C until use. On the day of experimentation, the skin was removed from the freezer and thawed and mounted on the diffusion cells. Phosphate buffered saline was placed in both receiver and donor compartments. The electrical resistivity was measured using LCR meter (BK solution, NY). The TEER less than 10 kΩ cm^2 were not used in the study. After measurements, the skin was unmounted, wiped with tissue paper.

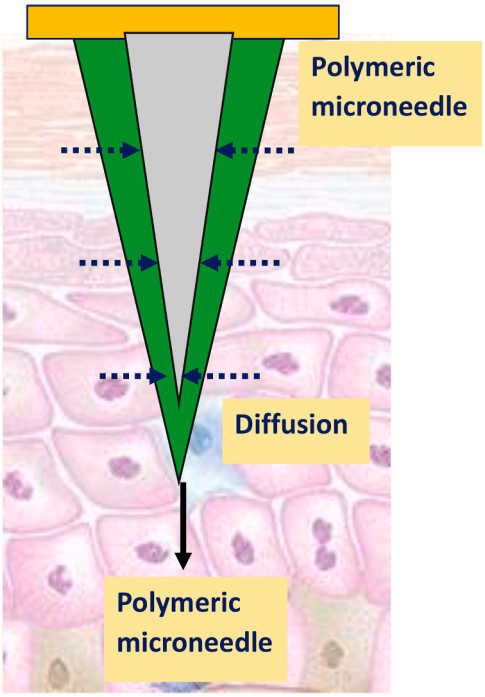

Fig. 2. Schematic representation of the process of sampling of contents from the tissue using PCP microneedles.

2.3.2. Sampling of markers from the skin

The pig ear skin was saturated with marker molecules by placing it in contact with different concentration solution of the marker molecule in the donor and receiver of a Franz diffusion cell (Abhijeet Maurya et al., 2019). The Franz diffusion cells are of 1 cm^2 area and 8 mL receiver compartment fluid. After overnight equilibration, the solutions were removed from the donor and receiver compartments, the skin samples were washed on both the surfaces with 1:1 methanol water to get rid of the adhering content. One part of the skin was biopsy sampled and subjected to analysis to know the total amount of drug in the skin. The microneedle array was inserted into the tissue for different durations. The PCP microneedle was retracted from the tissue and amount of marker molecule in the PCP microneedle was determined by disintegrating and dissolving the entire microneedles in deionized water. The solution was centrifuged, and the supernatant was subjected to analysis by HPLC.

2.4. Analysis of drugs

The HPLC system (Waters, MA) consisted of a chromatographic pump (Waters 1525), an autosampler (Waters 717 plus), a UV detector (Waters 2487).

Analysis of prednisolone sodium phosphate samples was carried out on a Symmetry® C18 column (4.6 × 150 mm) and the mobile phase was prepared by mixing 250 mL of isopropanol with 2 mL H_3PO_4 and diluting with water to 900 mL. The pH of the solvent was adjusted to 3.0 with 1.0 M NaOH and then diluted to 1000 mL with water and the flow rate was set to 1 mL/min (Sammeta and Murthy, 2009). The detection was at 242 nm and the linearity range was between 10 and 1000 ng/mL (R^2=0.99).

Nicotine was quantified using Waters XBridge™ C18 5 μm (4.6 × 250 mm) column was used. The HPLC conditions were developed and standardized (flow rate 1 mL/min, with isocratic mobile phase of 50:50 v/v, methanol, and 10 mM phosphate buffer, λ_{max} = 260 nm). For bioanalytical evaluation, calibration curves were prepared in plasma samples using acetonitrile as a solvent and the linearity was (R^2 > 0.99) evaluated over the concentration range 1–1000 ng/mL (Panda et al., 2021). Sodium fluorescein was quantified by Fluorescence spectroscopy at 520 nm emission wavelength and 494 nm excitation wavelength (Bruyn et al., 2011).

3. Results and discussion

The coated microneedles consist of a core made up of water-soluble polymer PVP K30. The core microneedles were coated with a suitable water insoluble polymer that forms a semipermeable layer as shown in the Fig. 2. The hypothesis is that when the PCP microneedles are inserted into the tissue, the inner core polymer being water soluble and the coat being hydrophilic and semipermeable, (but insoluble in physiological medium), will be able to trigger the diffusion of contents from the skin extracellular fluid into the microneedle array. The microneedle can be retracted from the skin at predetermined time point and measure the amount of drug or analyte extracted. This principle is analogous to micro-dialysis process in which the micro-dialysis probe is semipermeable and stays in contact with the extracellular fluid. The drug or analytes will diffuse across the semipermeable membrane into the perfusion medium circulating through the probe by passive diffusion. The perfusion fluid is collected eventually at different time intervals and analyzed to obtain the real time concentration of drug in the tissue fluid (Nguyen et al., 2018). Multiple PCP microneedles would be required is one needs to sample analytes at different time points. Whereas microdialysis can sample analytes continuously. However, micro-dialysis is an invasive and traumatic process. The use of PCP microneedles is minimally invasive and painless technique of sampling analytes.

In the microdialysis technique, the amount of analyte recovered could be improved by reducing the perfusion rate and increasing the volume of the perfusion fluid (Stenken et al., 2001). The recovery could also be improved by altering the probe dimensions. Likewise, in case of PCP microneedles, one can envision that the recovery of samples could be potentially enhanced by modifying the dimensions of the needle, coating material, concentration of the polymer in the coating solution and other design associated factors.

On the other hand, the rate of diffusion and extent of diffusion of drug generally depends on the unbound drug concentration in the tissue fluid besides its molecular size and physicochemical properties (Sammeta and Murthy, 2009; Panda et al., 2021). Therefore, the amount of drug sampled is a fraction of free or pharmacologically active drug concentration only in the tissue as the membrane is size selective similar to microdialysis probe.

Generally, the free drug concentration in the tissue is challenging to determine (Bruyn et al., 2011). Therefore, the extent of binding of drugs to the serum protein albumin is often considered as a surrogate for practical purposes. The objective of the research was to investigate the feasibility of using PCP microneedles for the sampling of analytes or drugs from tissues. Therefore, to assess the feasibility of the sampling technique, three model molecules were chosen. The model molecules are of different physicochemical properties (sodium fluorescein (log P = 0.1), prednisolone sodium phosphate (log P = 1.15) and nicotine (log P = 1.17). In addition, the model molecules also represent molecules of high (sodium fluorescein 80%), moderate (prednisolone phosphate (60%) and low protein (nicotine 5%) binding category (Nguyen et al., 2018; Stenken et al., 2001; Ungerstedt, 1991). The other main reason for selecting these molecules as model for this study is also because the extent of protein binding remains consistent across a broad range of concentration (Drug Distribution 2022).

3.1. PCP microneedles

The PCP microneedles were prepared in two stages. In the first stage, the core microneedle array was prepared using PVP-K30. The concentration of casting solution was 20% w/v. The microneedles were uniform in dimensions (Length 566.14± 3.53 μm and two-dimensional area 7.53±2.34 μm^2). The microneedle array was further coated with ethyl

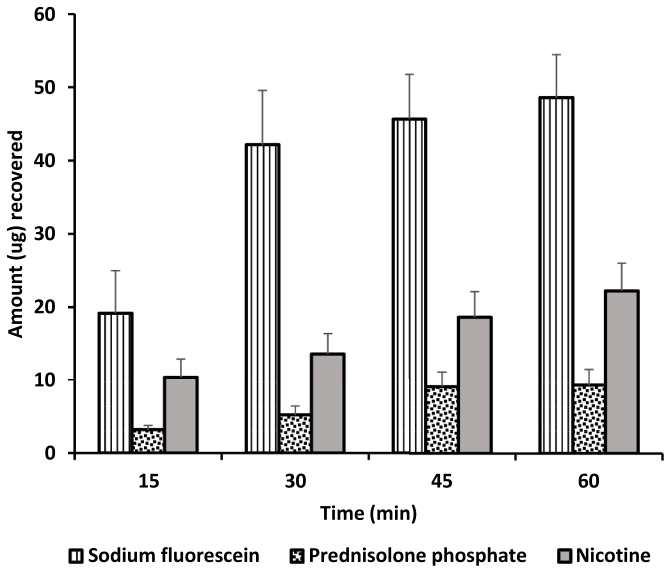

Fig. 3. Amount of analytes sampled from the skin using PCP microneedles after different durations of exposure.

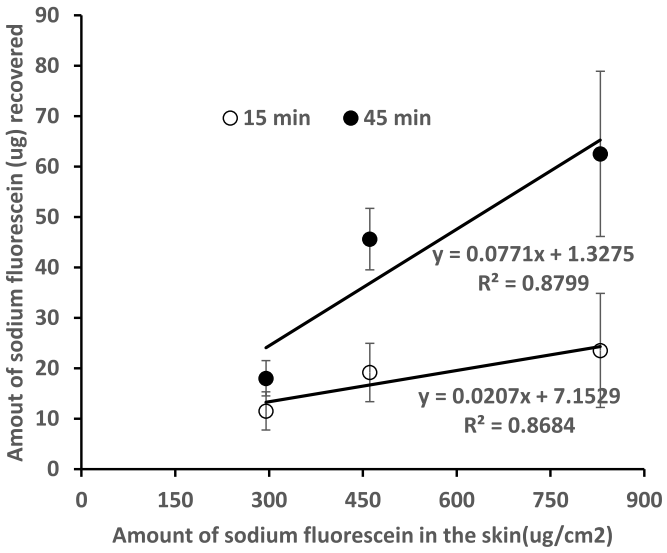

Fig. 4. The amount of sodium fluorescein sampled at different equilibrium dermal levels.

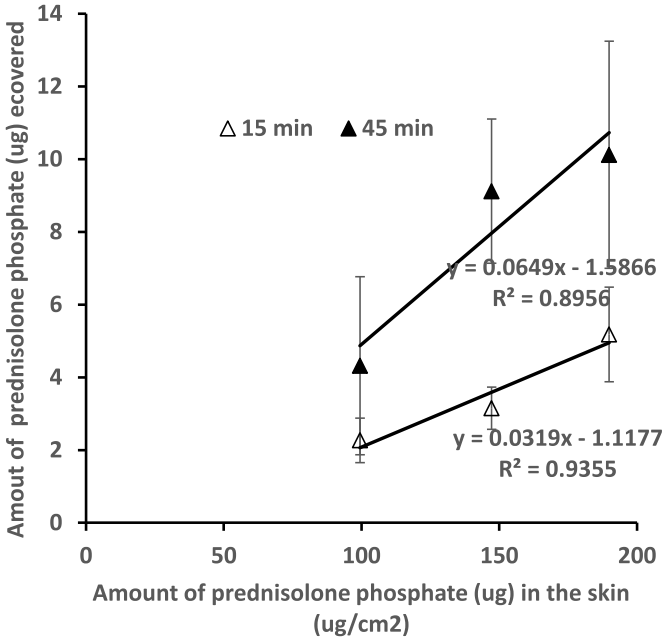

Fig. 5. Amount of prednisolone sodium phosphate sampled at different equilibrium dermal levels.

cellulose 4% w/v coating solution by dip-and-spin technique. The coating was uniform, and the thickness was about 100 μm. The mechanical strength of the uncoated and coated microneedles was 0.221 ±0.027 N/mm and 0.211±0.017 N/mm respectively.

The method of coating was optimized after several trial-and-error experiments. The coating uniformity was confirmed by performing a challenge test. In brief, Fluorescein isothiocyanate (FITC)-albumin was incorporated in the core microneedles and after coating, the microneedles were dipped in buffer medium for 6 h. The buffer was withdrawn and analyzed for fluorescence content. The PCP microneedles that lead to detectable fluoresce in the buffer was considered defective. No fluorescence in the buffer medium was the target to ensure coating integrity. The process variables and coating formulation variables were modified until an intact coating was achieved.

3.2. Sampling using PCP microneedles

The PCP microneedles were manually applied on the skin with uniform force by the same person each time to minimize the variability. Application of PCP microneedles increased the TEWL of skin from 7.2 ± 1.7 g/m^2 h to 15.23±1.55 indicating the perturbation of the physical barrier of the skin.

Duration of application: The PCP microneedles were applied for different durations. After predetermined duration of application, the PCP microneedles were retracted and analyzed for the content of analyte present in them. All the three analytes were recovered in significant amount from the skin tissue. The amount of analyte that was extracted from the skin tissue depends on the duration of insertion of the array in the tissue. However, beyond a threshold duration, the amount of analyte recovered from the tissue did not increase significantly. This is likely because the core microneedles were saturated with the analyte which diminished the concentration gradient eventually leading to mitigation of the diffusion process across the semipermeable coating membrane. In case of all the analytes, the amount of drug recovered increased significantly from 15 to 30 min. But thereafter, there was no significant difference in the amount of analyte recovered (Fig. 3).

Physicochemical nature of the analyte: The amount of analyte recovered is likely to be determined by the physicochemical nature of the analyte. For example, the ratio of amount of drug recovered to the total amount of drug present in the skin tissue was found to decrease with the increase in the tendency of the molecules to bind to protein (Fig. 3). The percent content recovered from the skin was in the following decreasing order nicotine>prednisolone phosphate>sodium fluorescein. This is likely due to the difference in the binding properties of the analytes to the tissue proteins. It is likely that the nicotine is least bound to tissue proteins and the proteins in the tissue fluids, compared to prednisolone and fluorescein.

Drug concentration in the tissue: It is likely that the diffusion process depends on the concentration of the analyte in the tissue. It is necessary that the amount of analytes sampled varies proportionately with the concentration of unbound analyte in the skin tissue so that contents recovered reflects the real time content of analyte in the tissue fluid. Therefore, to assess the validity of PCP microneedle approach of sampling analytes, the skin was equilibrated with different concentration solutions of the three APIs to generate different amount of drug levels. Following which the PCP microneedles were applied on the tissue and the analytes were sampled. Two time-durations were considered,

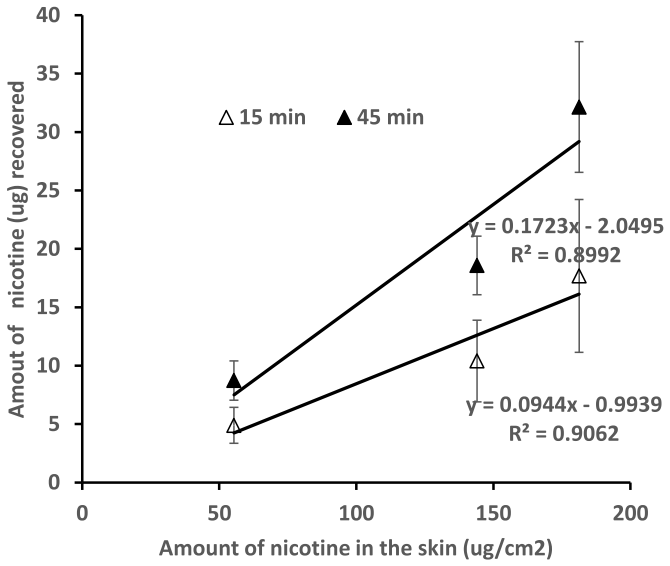

Fig. 6. The amount of nicotine sampled at different equilibrium dermal levels.

15 min and 45 min.

There was a linear relationship between the amounts of analytes recovered at different concentrations versus the amount of analyte in the skin tissue (Figs. 4-6). For any given analyte, the slope of the linear plot at 15 min sampling time was about two-fold lesser than that at 45 min indicating that optimizing the duration of exposure of PCP microneedles could significantly improve the recovery efficiency and lead to better discrimination of differences in drug concentration in the skin tissue.

3.3. Adsorbent loaded PCP microneedles

Adsorbent loaded Polymer Coated Polymeric (APCP) microneedles was fabricated with an objective to enable the PCP microneedles to recover significantly higher amounts of analytes. In principle the adsorbent in the APCP microneedles would adsorb the analyte and does not allow the core polymer to saturate. The carboxen is a very potent material used in solid phase microextraction of materials for analysis by GC–MS. It can adsorb a plethora of materials due to huge number of active centers present on it. The same material was used for extraction of alkaloids, steroids, and organic volatile compounds.

The adsorbent was suspended in the casting solution uniformLy and casted immediately without allowing them to settle down in the container. The adsorbent particles were size separated by sieving so that the particle size range is 50–150 μm. Lesser than 50 μm led to localization of the adsorbent at the tip of the microneedles and the tip chipped off. The beads larger than 150 μm would not be able to penetrate deeper into the mold and got entrapped at the base of the microneedles closer to the baseplate leading to rupture of microneedles or detachment of individual needles from the baseplate. The adsorbent beads of 50–150 μm could occupy the mid region of the microneedle mold and get entirely covered with the polymer to be able to stay intact and attain symmetric morphometrics. The microneedles were then coated with ethyl cellulose similar to that discussed previously. The figure a show an array of PCP microneedles with one or two adhesive beads embedded in them. About 25–30 beads were embedded per array (Fig. 7). An enlarged picture of a PCP microneedle with the adsorbent beads is shown in the figure.

The PCP microneedles embedded with adsorbent was subjected to sampling of the three analytes. The duration of sampling was 15 min. The results of amount of analytes recovered from the skin tissue is shown in the Fig. 8.

Incorporation of adsorbent did not improve the recovery of hydrophilic molecule sodium fluorescein. However, the recovery of lipophilic molecules prednisolone phosphate and nicotine were enhanced by six and three-fold respectively. The results clearly demonstrated that the adsorbent embedded PCP microneedles could be one of the approaches to enhance the amounts of analytes sampled from the tissues,

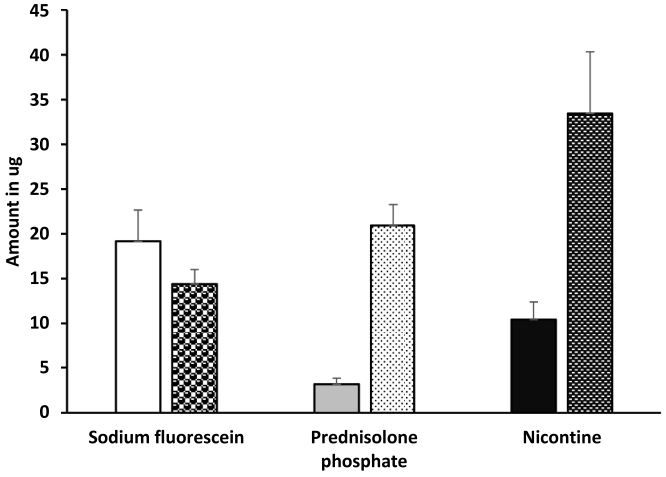

Fig. 8. Amount of analytes sampled using PCP microneedles and Adsorbent embedded PCP microneedles from the skin tissue respectively.

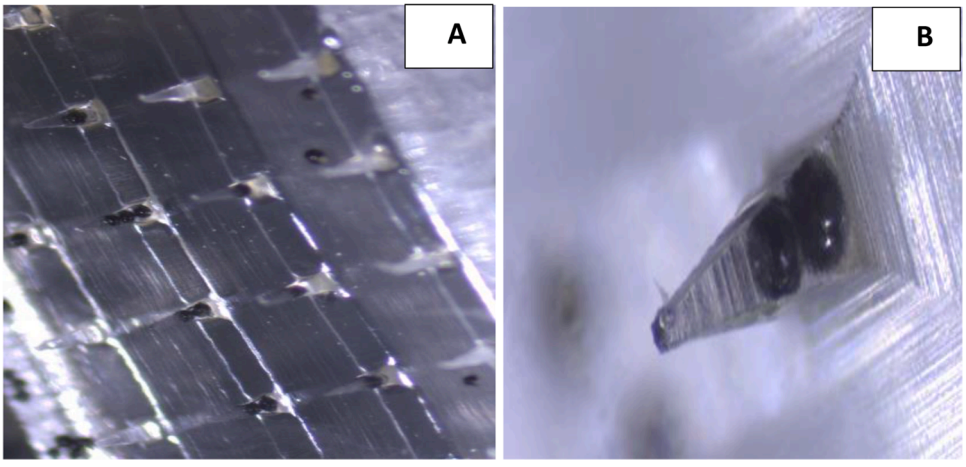

Fig. 7. Bright field microscopic images of the adsorbent entrapped PCP microneedles. The left side picture (7A) shows the entire array, and the right picture (7B) is an enlarged single PCP microneedle.

particularly the lipophilic molecules.

There are a number of approaches reported on sampling of drugs from the skin. Typically, the amount of drug in the stratum corneum is sampled by tape stripping. Tape stripping although is a great method of sampling substrates from the skin, it is not harmonized and is also affected by a number of variables such as tape material, adhesivity of the tape, the mode of application and stripping the tape (23–26). There are other methods that the researchers explored such as ultrasound medicated sampling and electroporation medicated sampling. There are reports of sampling glucose, biomarkers, drugs, and other materials (27–30). Iontophoresis has been a relatively successful method of sampling glucose and other analytes (31–33). PCP microneedles offer the advantage of a simple design, easy application and easy retraction. The limitations of PCP microneedles could be its ability to accommodate limited amount of content due to its size and variability due to difference in insertion depths in the tissues. PCP microneedles would be useful in sampling analytes form mucosal tissues as well.

4. Conclusions

The PCP microneedles were successfully fabricated and its potential application in sampling analytes form the tissue was explored. The analytes of physicochemical nature could be sampled form the tissue and the amount of analyte sampled was proportional to the concentration of analyte in the tissue. The PCP microneedles were modified by incorporating adsorbent beads into the core microneedles which enhanced the extent of recovery of lipophilic analytes.

CRediT authorship contribution statement

Deeksha Jakka: Investigation, Conceptualization. **A.V. Matadh:** Investigation, Conceptualization. **H.N. Shivakumar:** Project administration, Formal analysis. **Howard Maibach:** Project administration. **S. Narasimha Murthy:** Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bruyn, T.D., Fattah, S., Stieger, B., Augustijns, P., Annaert, P., 2011. Sodium fluorescein is a probe substrate for hepatic drug transport mediated by OATP1B1 and OATP1B3. J. Pharm. Sci. 100 (11), 5018–5030.

Caffarel-Salvador, E., et al., 2015. Hydrogel-forming microneedle arrays allow detection of drugs and glucose in vivo–Potential for use in diagnosis and therapeutic drug monitoring. PLoS One 10 (12), e0145644.

Dermatokinetics of Therapeutic Agents - Google Books. https://books.google.com/books?hl=en&lr=&id=zFWYEPGP9NkC&oi=fnd&pg=PA149&dq=Sampling+Substrates+by+Skin+Permeabilization&ots=B-xX83Dz5T&sig=P9KQBZ-t6GWvxq2BSXW33qaadZs#v=onepage&q=Sampling Substrates by Skin Permeabilization&f=false (accessed Jan. 08, 2022).

Drug Distribution to Tissues- Clinical Pharmacology- Merck Manuals Professional Edition, https://www.merckmanuals.com/professional/clinicalpharmacology/pharmacokinetics/drug-distribution-to-tissues (accessed Jan. 07, 2022).

Hailu, R., et al., 2020. Minimally invasive tissue sampling in preterm deaths–A validation study. Glob. Pediatric Health 7.

Ingrole, R.S.J., Gill, H.S., 2019. Microneedle coating methods–A review with a perspective. J. Pharmacol. Exp. Ther. 370 (3), 555.

Kiang, T.K.L., Ranamukhaarachchi, S.A., Ensom, M.H.H., 2017. Revolutionizing therapeutic drug monitoring with the use of interstitial fluid and microneedles technology. Pharmaceutics 9 (4).

Maurya, A., Nanjappa, S.H., Honnavar, S., Salwa, M., Murthy, S.N., 2018. Rapidly dissolving microneedle patches for transdermal iron replenishment therapy. J. Pharm. Sci. 107 (6), 1642–1647.

Maurya, A., Rangappa, S., Bae, J., Dhawan, T., Ajjarapu, S.S., Murthy, S.N., 2019a. Evaluation of soluble fentanyl microneedles for loco-regional anti-nociceptive activity. Int. J. Pharm. 564, 485–491.

xvii Maurya, Abhijeet, Rangappa, Srinath, Bae, Jungeun, Dhawan, Trisha, Ajjarapu, Sankar Srinivas, Narasimha Murthy, S., 2019b. Evaluation of soluble fentanyl microneedles for loco-regional anti-nociceptive activity. Int. J. Pharm. 564, 485–491.

Nguyen, H.X., Ashana, P., Arup, B.S., Banga, A.K., 2018. Qualitative and quantitative analysis of lateral diffusion of drugs in human skin. Int. J. Pharm. S0378517318302308.

Panda, A., Sharma, P.K., Shivakumar, H.N., Repka, M.A., Murthy, S.N., 2021. Nicotine loaded dissolving microneedles for nicotine replacement therapy. J. Drug Deliv. Sci. Technol. 61, 102300.

Panda, A., Matadh, V.A., Suresh, S., Shivakumar, H.N., Murthy, S.N., 2022. Non-dermal applications of microneedle drug delivery systems. Drug Deliv. Transl. Res. 12 (1), 67–78.

Roumelioti, M.E., et al., 2018. Fluid balance concepts in medicine–Principles and practice. World J. Nephrol. 7 (1), 1.

Sammeta, S.M., Murthy, S.N., 2009. ChilDrive–A technique of combining regional cutaneous hypothermia with iontophoresis for the delivery of drugs to synovial fluid. Pharm. Res. 26 (11), 2535–2540.

Singh, T.R.R., Mcmillan, H., Mooney, K., Alkilani, A.Z., Donnelly, R.F., 2013. Microneedles for drug delivery and monitoring. Microfluid. Devices Biomed. Appl. 185–230.

Stenken, J.A., Chen, R., Yuan, X., 2001. Influence of geometry and equilibrium chemistry on relative recovery during enhanced microdialysis. Anal. Chim. Acta 436 (1), 21–29.

Ungerstedt, U., 1991. Microdialysis-priniciples and applications for studies in animals and man. J. Intern. Med. 230, 365–373.