Polym. Bull. (2018) 75:4103–4115
https://doi.org/10.1007/s00289-017-2254-1

ORIGINAL PAPER

Microneedle-assisted transdermal delivery of acetylsalicylic acid (aspirin) from biopolymer films extracted from fish scales

O. Olatunji[1] · **M. Olubowale**[1] · **C. Okereke**[1]

Received: 16 May 2017 / Revised: 13 July 2017 / Accepted: 8 December 2017 /
Published online: 14 December 2017

Abstract This study investigates the transdermal release of aspirin (ASA) from films of hydrolyzed collagen obtained from fish scales. The patches were made with 0.2 g of ASA dissolved in 99% ethanol and 1.98 g of the extracted fish scale biopolymer (FSBP) dissolved in 10 mL distilled water which is then dried. Porcine skin is pre-treated with solid metal microneedles. ASA-loaded FSBP patch is then applied. The skin samples were placed on diffusion cells. Samples were taken at varying times: 5, 24, and 48 h and tested for the presence of ASA using UV spectrometry-based method introduced in this study. The results obtained showed that for all repeated study, no ASA was detected in the receiver compartment after 5 h. After 24 h up to 0.24 mg/mL ASA had released into the receiver compartment; and after 48 h, a concentration 0.74 mg/mL had been reached for skin samples treated with microneedles. ASA was not detected in the receiver compartment for skin samples that were not pre-treated with microneedles after 5 h. After 24 h, the concentration of 0.04 mg/mL was recorded, and at 48 h, concentration of 0.045 mg/mL was detected. This study presents for the first time transdermal drug delivery (TDD) films made of FSBP applied as drug delivery films for ASA and the use of microneedles to significantly enhance the release of ASA from the ASA-FSBP TDD films using the 'poke and patch' method. We also present a novel method for testing ASA in the TDD study using reaction with ferrous gluconate.

Keywords Microneedles · ASA · Drug delivery · Transdermal · Biopolymers · Acetyl salicylic acid

✉ O. Olatunji
Lolakinola@gmail.com

[1] Department of Chemical Engineering, University of Lagos, Akoka, Lagos, Nigeria

Abbreviations

ASA	Acetyl salicylic acid
TDD	Transdermal drug delivery
FSBP	Fish scale biopolymer
HPLC	High-pressure liquid chromatography
LC–MS	Liquid chromatography–mass spectrometry
GC–MS	Gas chromatography–mass spectrometry
FTIR	Fourier transform infrared spectrometry
FeGluco	Ferrous gluconate

Introduction

Acetyl salicylic acid, commonly referred to as ASA, has been described as the gold standard for pain relief, anti-inflammatory, and cardiovascular treatment [1]. Although oral administration is the most widely applied method for administering ASA, it is associated with gastrointestinal side effects and low bioavailability due to being susceptible to the first pass metabolism. Frequent dosing is, therefore, required to attain required pharmacokinetics of ASA in the body for effective treatment.

Transdermal drug delivery proffers several solutions to the limitations posed by oral drug delivery. These include avoidance of metabolism in the gastro intestinal tract, better compliance due to ease of use, and possibility of more controlled and sustained delivery. A number of transdermal patch formulations of ASA have been presented in the literature [1–3]. Many of which use a combination of natural and synthetic-based materials such as silicone-based adhesives, ethylene acetate, propylene glycol, and glycerol. These additives play roles such as provide adhesion, plasticize, and act as penetration enhancers. It is, therefore, desirable to have a transdermal system, which possesses all these attributes in a single material.

Fish scales are a rich source of collagen, which can be extracted through hydrolysis to yield hydrolyzed collagen. The fish scale biopolymer (FSBP) has intrinsic moisture activated adhesive property and elasticity, thus making it a potential candidate for transdermal drug delivery (TDD) application [4, 5].

Microneedles are micron-sized vertical projections with sharp tips erected on a flat substrate. The microneedles are fabricated, such that they are long enough to pierce the upper layer of the skin, the stratum corneum, and reach into the viable epidermis, which permits faster permeation of substances such as drugs. However, they are sufficiently short to not reach the lower layer of the skin where the pain receptors and blood vessels exist [6–8]. In such sense, they act as painless mechanical penetration enhancers.

There have been recent studies into delivering drugs using microneedles [9]. One area of research interest is in the development of drug delivery systems from fully biocompatible, biodegradable, and readily available material. Researchers have explored use of naturally occurring materials such as silk fibroin for microneedle-

assisted drug delivery [10]. Fish scales are readily available biomaterials that presently exist as unused byproducts from fish processing generally regarded as waste. The ability to convert this readily available resource into a biomedical product contributes towards addressing the challenge of waste management and value addition to aquatic resource. Furthermore, the widely used techniques for testing transdermal permeation such as HPLC and GCMS are most often inaccessible in basic laboratories particularly in developing countries. For the global advancement of research in this field, it is important to develop methods that require less expensive techniques for analysis.

In this study, we, therefore, explore a novel method for delivery of ASA using FSBP films in combination with solid microneedles using the "poke and patch" method [6]. In addition to this, an alternative method for testing for ASA using ferrous gluconate as an inexpensive method and safer option to ferric chloride is presented [11, 12].

Methodology

Materials

Croaker fish scales were obtained from the fish market at mile 12 Lagos, Nigeria, hydrochloric acid, sodium hydroxide, isopropyl alcohol, and ethanol were all reagent grade obtained from Finlab, Lagos, Nigeria. Porcine skin was obtained from the ear of freshly slaughtered pigs obtained from a blucher at Odo market, in Lagos, Nigeria. The solid metal microneedles used were AdminPatch purchased from (AdminMed, NanoBiosciences LLC, California, USA). The microneedles are 1400 μm-long solid metal microneedles produced from medical grade stainless steel. The arrays contain 31 microneedles on a 1 cm^2 base. ASA tablets used are 75 mg dispersible ASA tablets from TEVA purchased from YEM-YEM Pharmacy, University of Lagos, Nigeria.

Extraction of ASA from aspirin tablets

For this study, we extract ASA from commercial pharmaceutical ASA tablets. This is mainly due to availability as pure ASA can easily be obtained over the counter. The ASA tablets contain other materials like binders that must be isolated. The following procedure is used to isolate the ASA from other components present in the tablet.

100 mL of isopropanol is added to 100 tablets of ASA in a conical flask. The mixture is placed under continuous stirring at 50 °C under reflux to prevent evaporation of the isopropanol. This is done for 45 min until the ASA dissolves into the solvent leaving behind the other contaminants forming a cloudy solution. The mixture was filtered using a Whatmann filter paper to recover the dissolved ASA. The beaker containing the ASA in isopropanol solution was placed in a cooling bath and precipitated using ice-cold distilled water. The precipitate formed was then filtered out using filter paper. To purify this precipitate, about 300 mL of

isopropanol was added and heated briefly to dissolve the precipitate, which was then filtered and dried to obtain the purified acetylsalicylic acid.

The ASA used was a 75 mg dispersible ASA tablet, having an average weight of 150.8 g.

100 tablets of ASA were used, meaning that there is 7500 mg of acetylsalicylic acid for the 100 tablets present. After extraction, dry weight of ASA was 5323.5 mg. The yield is calculated, as shown in the following equation:

$$\text{Aspirin yield} = \frac{\text{Dry mass of ASA extracted}}{\text{Dry mass of ASA before extraction}} \times 100. \quad (1)$$

Preparation of TDD films

The TDD films used consisted of ASA, FSBP, ethanol, and water combined in the quantities listed in Table 1. ASA extracted from the oral tablets is dissolved in ethanol at room temperature. Dry FSBP is mixed with water, heated on a hot plate, with constant stirring at 45 °C until FSBP has dissolved in water. The ASA dissolved in ethanol was then mixed with the FSBP dissolved in water, mixing for a further 10 min to form a uniform mix. The viscous fluid is then spread on a flat surface and left to dry for 24 h.

FSBP extraction

FSBP was extracted from fish scales using method described in the previous studies [13]. The fish scales were cleaned with tap water to get out any form of visible dirt and dried under the sun after the pre-cleaning. 390 g of dried fish scales was treated with 0.1 M of sodium hydroxide (pH 12) for an hour. The treated fish scales are then rinsed with tap water till the pH dropped down to about 7.5; after which it was further treated with 10% hydrochloric acid with a ratio of 1:10 water for an hour to remove minerals. This also was rinsed with tap water until the pH dropped back to about 7.5. 500 mL of water and the treated fish scales were put in a stainless steel closed vessel, and temperature was monitored until it got to 80 °C where it was then kept constant. The hydrolysis process was carried out for 8 h. Following the hydrolysis, the system was allowed to cool and the solid was separated from the liquid using a sieve. The liquid filtrate was centrifuged at 2500 rpm (Corning LSE compact centrifuge 6758, Sigma-Aldrich), for 15 min to remove any form of solid residue that might be in the fluid. The centrifuged fluid is transferred into a beaker on a hot plate and the water allowed to evaporate at 80 °C until the fluid becomes

Table 1 Data sheet of drug matrix

Component	Amount
Ethanol	4 mL (99.86%)
Aspirin	0.2 g
FSBP	2 g
Water	5 mL

very viscous. The FSBP is then poured unto a flat surface and left for 48 h to solidify.

FSBP yield

The yield of FSBP from the croaker fish scale is measured as a ratio of dry mass of FSBP extracted relative to the total dry mass of fish scales used. This is represented by Eq. (2) using the formula below:

$$\text{Percentage yield} = \frac{\text{Dry mass of FSBP extracted}}{\text{Dry mass of Fish scale before extraction}} \times 100. \quad (2)$$

Moisture content analysis

0.5 g of prepared FSBP patch was weighed individually and dried in an oven at 180 °C. The film was weighed every 10 min until the weight remained constant. The percentage moisture content was determined using the following equation [14]:

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100. \quad (3)$$

Microneedles insertion test

It is important that the microneedles pierce into the skin and create microconduits for the drug to permeate through the stratum corneum into the viable epidermis where it can permeate much faster into the dermis where it is absorbed into the blood (in this case modeled by the receiver compartment of the permeation cell). To test that microneedles being used (AdminPatch) is effectively piercing the skin, repeated skin insertions were carried out. Porcine skin sample was cut into approximately 2 cm by 2 cm samples. The skin is treated with the AdminMed solid metal microneedle patch (AdminMed, NanoBiosciences LLC, California, USA) by manually pressing against the skin. Methylene blue solution is then applied to the pierced region using a pipette. The skin is then observed visually for sign of piercing indicated by a deeper blue staining in the pierced region.

Drug diffusion studies

Drug diffusion studies were carried out in a permeation cell (Permegear, NJ, USA). Porcine skin with the subcutaneous tissue and the dermis removed using a blade was used as the skin model. A 1.5 cm^2 sample of porcine skin was placed on a flat base. The microneedles were applied 12 times in all cases to increase the number of holes created. Approximately 1 cm^2 samples of prepared ASA-loaded transdermal film were attached to the skin on the stratum corneum end. The films were cut, such that each patch weighed about 0.1 g. The receiver compartment of the diffusion cell is filled with distilled water and a magnetic stir bar inserted. Distilled water is used for

the purpose of this study to allow reaction of ferrous gluconate and ASA. The skin with patch attached is then mounted on the donor end of the receiver compartment. The assembly was placed on the inbuilt magnetic stirrers installed in the permeation cell system. The temperature of the system was maintained at 37 ± 1 °C using circulating water bath. 2 mL of receptor fluid was withdrawn at regular intervals of times (5, 24 and 48 h) and replaced with equal volume of fresh distilled water [15].

The ASA concentration in the receiver compartment is corrected for sample removal using the following equation:

$$C_n^1 = C_n \frac{V_t}{V_t - V_s} . \frac{C_{n-1}^1}{C_{n-1}}, \tag{4}$$

where C_n^1 and C_n are the corrected and measured ASA concentrations, respectively. V_t is the total volume of the receiver compartment and V_s is the volume of the sample. C_{n-1}^1 and C_{n-1} are the corrected and measured drug concentrations before sampling [8, 16].

Ferrous gluconate preparation

Two 300 mg tablets of colored ferrous gluconate (YEM-YEM Pharmacy, University of Lagos, Lagos, Nigeria) were swirled in 10 mL of distilled water till the red coloring came off and just the brownish black ferrous gluconate that was left. The ferrous gluconate tablets were then left to dry. Dry weight was taken and found to be 0.4168 g. A 200 mL solution of ferrous gluconate was made giving a concentration of 0.00432 mol/L of ferrous gluconate solution.

UV spectrometry test for ASA

Standards were prepared using ferrous gluconate solution only as the blank and varying concentrations of ASA were then added to the same solution of ferrous gluconate giving a standard curve with concentration against absorbance.

The 2 mL samples withdrawn from the receptor compartment of the diffusion cells were put in a test tube, and 3 mL ferrous gluconate solution is then mixed with the 2 mL solution. These were placed in a water bath at 95 °C for a period of 6 h. A color change from light brown to reddish blue indicated the presence of ASA. This is quantified using UV spectrometry using the Beer Lambert's rule.

Protein test

A burette test was carried out on the extracted biopolymer and on the prepared patch. The burette test was quantified using UV spectrometry. Analytical grade gelatin from porcine skin was used as standard (Sigma-Aldrich, Supplied by Bristol Scientific, Lagos, Nigeria). The protein test on the prepared patch serves to confirm that the FSBP was still present in the patch and not reacted with the ASA to form a new compound.

FTIR analysis

The ASA extracted and the films formulated are characterized using Fourier transform infrared spectrometry. A diamond crystal ATR spectrometer accessory attached to the Agilent Cary 630 FTIR at a wavelength between 500 and 4000 cm^{-1}. The process involved the samples which cut into thin films of 1 cm × 1 cm were cut and placed on the sample port. The port was then closed to allow the probe make contact with the sample. The sample is scanned to obtain a spectrum plot of transmittance against wavelength.

Statistical analysis

A student *t* test was carried out on the results obtained. The experiment was repeated five times and the best three were chosen for analysis. *p* value was obtained to obtain the level of significance.

Results

The composition of the transdermal patch is listed in Table 1, showing each component in % w/w. The values of the yield, moisture content, and protein concentration of FSBP and ASA obtained from this study are summarized in Table 2.

385 g of croaker fish scale was used in the extraction of FSBP. After the process and FSBP was dried, the dry weight of FSBP was 93.79 g, giving FSBP yield of 24.36%.

During extraction of ASA from the tablets, there is possibility of losing some of the ASA during transfer, filtration, and precipitation. This accounts for the yield of 70.98% obtained relative to the 75 mg per 1 g contained within the tablet according to manufacturer's label.

The moisture content of the FSBP is relevant to know how much of the dry mass of the patch is FSBP and ASA. Using the oven drying method, a moisture content of 22.3% was obtained for the FSBP extracted. The FSBP extracted is kept in zip lock bags to maintain the moisture content. The samples were, however, used within a day of drying, such that there was a little chance of moisture variation during storage.

Table 2 Yield, moisture, and protein content of extracts

Property	Value (% w/w)
FSBP yield	24
Aspirin yield from tablet	71
FSBP moisture content	22.3
FSBP protein concentration	73

A burette test indicated a protein concentration of 73% in the extract. From the previous studies [4], we can assume that the other 5% unaccounted for is Ash content.

Protein test carried out on the transdermal film indicted each patch prepared contained about 68% protein by weight. We, therefore, know that the patch contains FSBP, ASA, and moisture; the unaccounted content can be attributed to slight material loss during preparation and moisture content. ASA test was also carried out on the patch confirming the presence of ASA up to 5% w/w in the patch after preparation.

FTIR analysis

To study the chemical structure of the ASA extracted and the interaction between the ASA and the FSBP, FTIR analysis is carried out on the ASA and the FSBP extracted separately and then when combined. The graphs are shown in Fig. 1a–c. The FTIR graph for FSBP and ASA shows typical peaks as obtained in the previous literature [13, 17]. In the composite film containing ASA and FSBP, the main peaks for the FSBP are still present in the ASA-FSBP sample. Although the typical peaks for ASA are not clearly seen due to the lower concentration. Nonetheless, at wavelength between 859 and 663 cm^{-1}, the peaks for the aromatic group out of plane bending for ASA are present. Thus, indicating that the ASA still, to a significant extent, maintains its chemical structure within the transdermal film.

Microneedle insertion test

Figure 2 shows the skin sample after piercing with microneedles and applying methylene blue staining to allow holes to be visualized. Thus, confirming that the microneedles are able to penetrate the stratum corneum layer of the skin. Here, the microneedles have been applied with thumb pressure. Studies have shown that the force applied to microneedles has an effect on the permeation rate [18]. Here, the pressure applied is estimated to be about 80 kN/m^2 care was taken to ensure that the same pressure was applied by the same person for every test. In the practical application, it is desired that users should be able to apply the microneedles by hand. For this study, we consider the slight variation in the force applied due to natural human variation to be negligible.

Skin permeation studies

Running a spectrum scan of the standard (Fig. 3), it was found that peak absorption occurred at a wavelength of 532 nm for the ASA–FeGluco complex formed. Similar peaks have been observed for ASA–Ferrous complex [11]. The infinite peak seen at around 300 represents the formation of salicylic acid. The peak of 532 nm is, therefore, used for the study. Figure 4 shows the color of the ASA–FeGluco complex formed.

The diffusion studies were carried out repeatedly for skin samples treated with metal solid microneedles and with intact skin without microneedles pre-treatment.

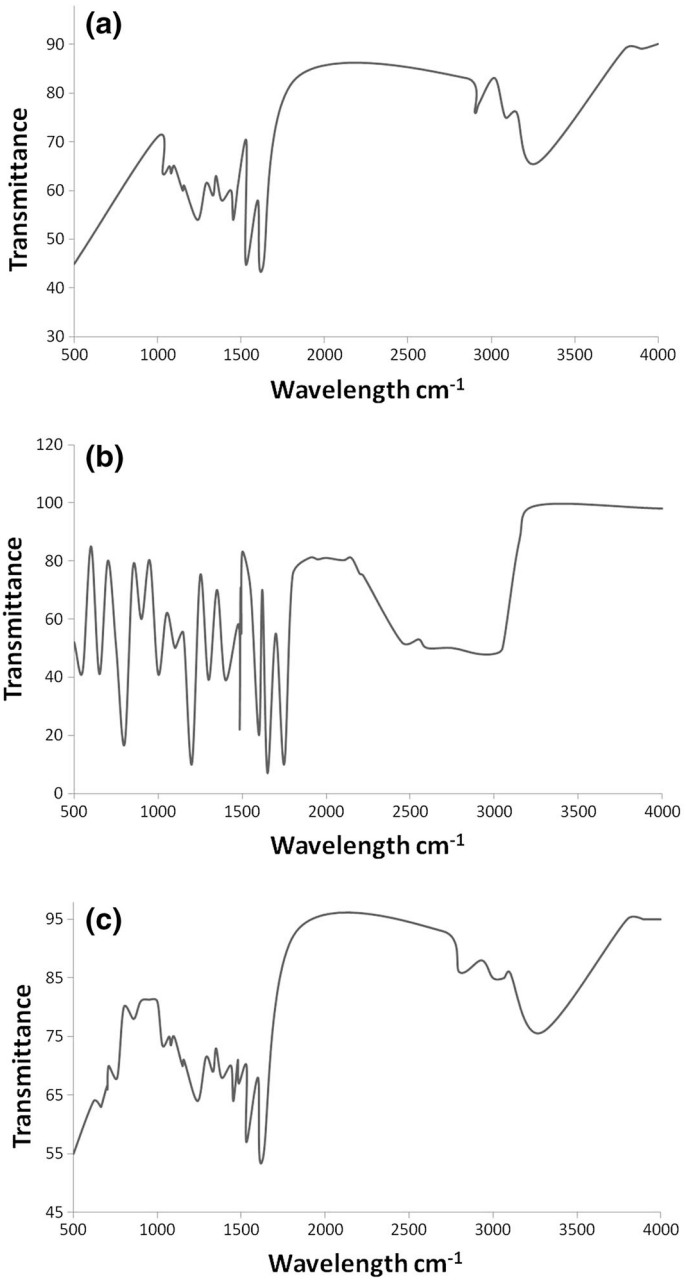

Fig. 1 **a** FTIR spectrum of fish scale biopolymer c, **b** FTIR spectrum of extracted aspirin, and **c** FTIR spectrum of FSBP–ASA composite film

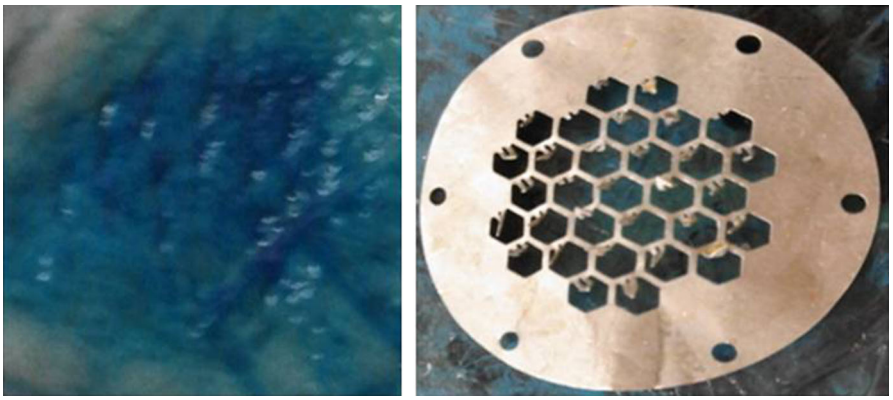

Fig. 2 **a** Porcine skin with methylene blue showing microneedle piercing and **b** admin patch microneedle used

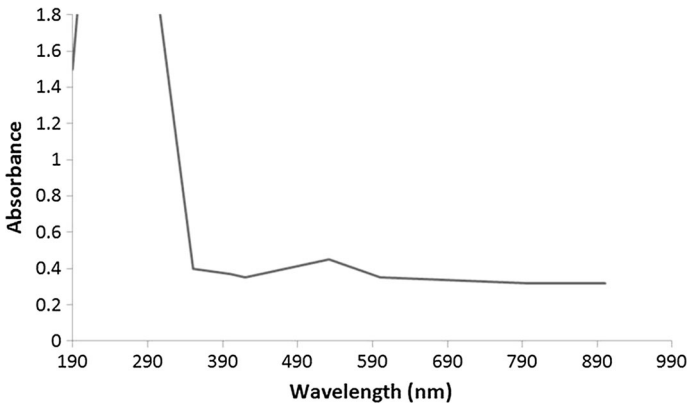

Fig. 3 Spectrum scan of ASA–FeGluco complexes showing the peak at 523 nm

These results are presented in Fig. 5. The error bars indicate the standard error for each sample.

For the first 5 h, in both cases, no ASA was detected in the receiver compartment. This is likely due to the time required for the ASA to permeate into the skin which is the barrier between the drug loaded patch and the receiver compartment. There is likely a small amount of ASA permeating through, however, is below the detection limit of this method. In preparing the standards, the minimal concentration that was detectable was 0.02 mg/mL.

After 24 h, the concentration in the receiver compartment rose to 0.24 mg/mL in the sample treated with microneedles, while in the sample that was not treated with microneedles, there was a much lower average reading of 0.04 mg/mL.

At 48 h, the concentration in the receiver compartment increased to 0.74 mg/mL in the sample treated with microneedles and a reading of 0.045 mg/mL was obtained for the intact skin sample. The results presented here is typical values from

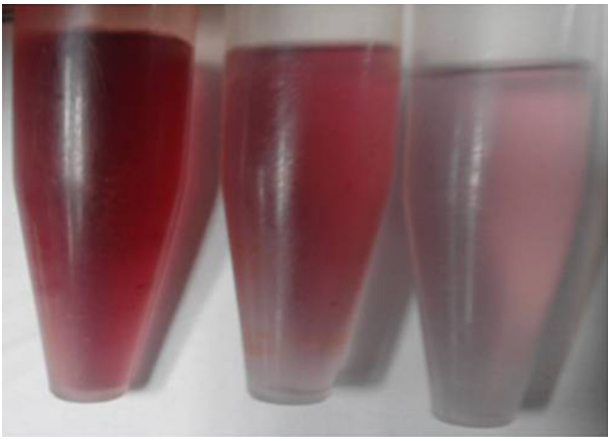

Fig. 4 ASA–FeGluco complexes formed at varying ASA concentrations

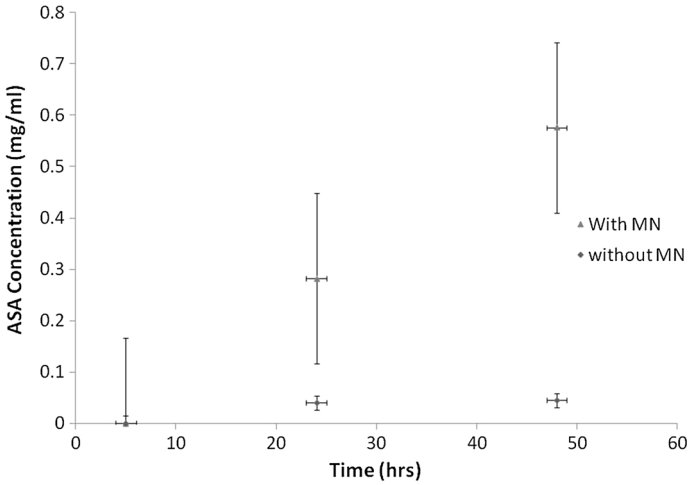

Fig. 5 ASA concentrations in receiver compartment over time

several repeated experiments with triplicates selected for analysis. Student's *t* test gave a *p* value of 0.23 indicating a 23% probability that the difference between the concentrations obtained with the samples with intact skin that obtained with MN treatment is due to chance. Although this is fairly high considering a usually accepted p value < 0.05; however, *t* test carried out within the same sample groups gave much higher p values of > 0.8.

Discussion

The transdermal films presented in this study have particular advantage of being made from 100% biodegradable material, which has moisture, activated adhesive property. The previous studies that explored transdermal delivery of ASA include use of additional components to provide adhesive properties and penetration enhancement [1, 3].

The ASA concentrations reached in the receiver compartment are relatively low compared to those typically recorded in serum where concentrations could reach 0.003 mg/mL in an adult human [19]. After 48 h, the percentage ASA absorbed was 1.85 and 0.6% after 24 h. This is low compared to 30–40% ASA absorbed for Duro-Tak ASA transdermal patch after 24 h [3]. The concentration in blood could potentially be increased by increasing the concentration of the ASA in the TDD film or increasing the surface area where it is applied. Further studies will investigate optimizing formulation to increase the release rate.

The reaction between ferrous gluconate and ASA has previously been studied with the aim of investigating drug interaction in the body [11]. This has now been applied here as a means of measuring the concentration of ASA in TDD studies. Albeit having a lower sensitivity to other methods such as HPLC and LC–MS, this is of particular significance as it provides a low cost and easily quantified technique for TDD studies of ASA and is worth exploring.

Conclusion

The findings of this study shows substantial release of ASA from transdermal patches made from fish scale biopolymer. These patches are particularly attractive for their moisture activated self-adhesive property requiring no additional material to give adhesive property and made from a completely biodegradable polymer from food waste, fish scales. Furthermore, the results show that a lipophilic drug such as ASA can be loaded unto fish scale biopolymer patch and released into the skin. We have also presented a method for carrying out TDD studies for ASA using ferrous gluconate as a less hazardous option to other ferric compounds such as ferric chloride. The results from this study provide evidence that FSBP films have potential application as transdermal ASA delivery systems.

Acknowledgements Funding was provided by the International Foundation for Science (Grant no. F/5544-1).

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