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Research paper

Self-adhesive thin films for topical delivery of 5-aminolevulinic acid

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Abstract

Self-adhesive thin-films have been developed as a topical delivery system for 5-aminolevulinic acid (ALA). The thin films are suitable for use during the photodynamic therapy of epithelial skin tumors. They are composed of a combination of the lipophilic polymer Eudragit NE and the lipophilic plasticiser acetyl tributyl citrate (ATBC). Because of its hydrophilicity, ALA forms suspension systems within these thin films, as evidenced by light microscopy. ALA release measured using Franz cells is very rapid from a Eudragit NE thin film loaded with 10% w/w ALA (200 μg ALA after 2.5 h), and even higher when ATBC is included. A Eudragit NE/ATBC (1: 2) thin film loaded with 20% w/w ALA releases 2000 μg ALA after 3.5 h. Combined release/permeation of ALA through excised membranes of human stratum corneum plus epidermis yielded fluxes of 50–100 μg ALA within 5 h for the Eudragit NE/ATBC (1: 2) thin film. The ATBC acts as a permeation enhancer for ALA. Scanning electron microscopy of the thin film surface shows protruding ALA particles which rapidly dissolve on contact with an aqueous medium. This surface dissolution mechanism is the cause of the rapid ALA release and hence also the high skin permeation in vitro. The mechanical properties of the thin films were also briefly examined. Adhesive strength increases with higher ATBC loading and decreases with higher ALA loading. Internal cohesion decreases with greater ATBC loading and increases with higher ALA loading. As part of this project, an improved derivatisation assay for gradient HPLC of ALA with 9Fluorenylmethyloxycarbonylchloride is also presented.

Keywords: Aminolevulinic acid; Thin film; Plaster; Rapid release; Skin Permeation

1. Introduction

The topical application of 5-aminolevulinic acid (ALA) during the photodynamic therapy (PDT) of epithelial skin tumors greatly reduces the unpleasant side effects occurring after intravenous application of photosensitisors [1]. It is current practised to disperse crystalline ALA powder in an ointment or cream base, which is then applied as a thin film to the skin area to be treated [2]. This is covered with an occlusive dressing and the ALA allowed to permeate into the skin tissues for, typically, 3–6 h before commencing laser therapy [3]. This practice has a number of disadvantages. First, ALA dimerises rapidly in an aqueous environment at pHs above approx. 5.5 [4] and looses its PDT activity. Although there are no reliable, quantitative data available in the literature, the shelf life of ALA ointments and creams used during PDT is limited to two weeks. Secondly, the ALA ointment/cream is not localised on an exact, well-defined skin area, since it melts on contact with the skin surface (approx. 33°C) and spreads over adjacent healthy skin areas. This causes unnecessary damage to healthy skin tissue on laser treatment. Thirdly, the use of an ointment/cream does not control the rate of ALA release to the skin surface, and hence uptake into the skin. Indeed, it is not known which ALA release and skin permeation rates would provide the best photodynamic effects [5]. Since a systemic effect of ALA is not required, it can be assumed that a high ALA release rate would promote high skin permeation and therefore be advantageous for therapy.

We have investigated the use of a self-adhesive topical thin film for ALA which avoids these disadvantages of ALA ointments/creams. Initial experiments proved the utility of lipophilic polymers, such as Eudragit NE, as a carrier matrix for ALA, and which can be made self-adhesive (turned into a pressure sensitive adhesive, PSA) by addition of the plasticiser acetyl tributyl citrate. The use of such a PSA allows the simple architecture of a single polymer/plasticiser/ALA-layer (drug-in-adhesive type), combined with a backing film and a release liner [6]. The ALA shows an unexpectedly rapid release out of this PSA thin film which is particularly propitious for use during PDT. We present here a study of

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the ALA release data obtained from the thin films, and also of combined release and permeation data through a contiguous membrane of excised human stratum corneum. The effects of ALA loading and concentration of plasticiser on release and stratum corneum permeation are shown, and a mechanism for the rapid ALA release is proposed. Additionally, the influence of these two variables on the adhesive properties of the PSA-thin films are briefly demonstrated. At the beginning of this work it was necessary to select a suitable HPLC assay for ALA in aqueous solution. We chose from the literature a derivatisation procedure with ^{9}Fluorenylmethyloxycarbonylchloride (FMOC) [7] which, after extensive modification and improvement, proved to be much more robust and stable than the standard orthopthaldialdehyde (OPA) assay [8]. The results presented in this paper demonstrate the utility of the PSA-thin film as a means of providing a convenient, effective ALA delivery to the epidermis during PDT.

2. Materials and methods

2.1. Materials

Crystalline 5-aminolevulinic acid (ALA) was obtained from Medac GmbH (D-Wedel). Eudragit NE is an ethylacrylate/methylmethacrylate co-polymer and was obtained as a 30% w/w aqueous dispersion from Röhm (D-Darmstadt). Acetyltributyl citrate (ATBC) was used as received from Jungbunzlauer (D-Ladenburg) and acetone analysis grade from Merck (D-Darmstadt). FMOC and the other substances used for the ALA assay were obtained from Sigma Chemicals (D-Munich). The backing film Melinex 813 was obtained from Tricon (D-Freiburg), as well as the release liner, a siliconized foil. Water was double-distilled from an all-glass apparatus.

2.2. Quantitative determination of ALA using FMOC-derivatisation and fluorescence HPLC

FMOC-reagent was freshly prepared from 200 mg FMOC in 100 ml acetone. Acetic acid buffer of pH 4.2 was prepared from 3 ml concentrated acetic acid and 1 ml triethylamine, made up to 1 l with freshly distilled water. The pH was adjusted by addition of either 1 N HCl or 1 N NaOH accordingly. A borate buffer of pH 7.7 was prepared by mixing 53 ml of the following solution A with 47 ml of O.IN HCl. Solution A was prepared from 12.4 g boric acid in 100 ml NaOH made up to 1 l with distilled water. For the derivatisation reaction 10–100 μl samples containing ALA were added to 100 ml of the borate buffer and 700–1000 μl of the FMOC-reagent and mixed well. After 45 s the derivatisation reaction is complete. The ALA/FMOC complex was than stabilised by adding 100 ml of the acetic acid buffer [7].

The HPLC analysis was performed using a Perkin Elmer Liquid Chromatography series 200 LC pump, fluorescence Detector LC 240, and Auto Sampler ISS 200. The stationary phase was a 250 × 4 mm RP18 column (Merck, D-Darmstadt) containing LiChroCart 2504/LiChrospher 100 packing. The mobile phase was acetate buffer pH 4.2/acetonitrile (60:40), and the running time for each sample was 25 min. Between samples the volume was washed-out using pure acetonitrile for 5 min. Fluorescence detection was performed with excitation at $\lambda = 263$ nm and emission at $\lambda = 313$ nm.

2.3. Preparation of thin films containing ALA

Crystalline ALA powder was lightly ground in a pestle and mortar and then fractionated in an air jet sieve (Alpine Hosokawa). The Eudragit NE dispersion was freeze-dried as described before [9] to yield a fine, white powder. The required amount was then dissolved in acetone with stirring at 30 rpm using a propeller stirrer, avoiding hereby inclusion of air bubbles. The corresponding amount of ATBC was then added, with further stirring. Subsequently, the ALA powder fraction under consideration was dispersed in this viscous Eudragit NE/ATBC acetonic solution. The resulting dispersion was then cast as a thin film on the Melinex backing film using a purpose-built casting block of standard construction. A wet film-thickness of 500 μm produced after drying for 45 min at 60°C a final thin film of 200–250 μm thickness. The release liner was then applied to the top release surface of the thin film, and individual round plasters of 1 cm diameter punched out of the film. The thin films (plasters) were then individually heat-sealed in Al coated polyethylene foil (Tricon).

2.4. Measuring ALA release rate from thin films

A single ALA-thin film was placed with its release surface facing downwards in a glass Franz cell (1 cm internal diameter) and the acceptor compartment filled with pH 5.0 borate buffer. The release of ALA was measured at 33°C ±0.5°C by removing 100 μl samples of the acceptor solution at regular time intervals and replacing these with blank buffer. The ALA content of each sample was determined using the FMOC fluorescence method described above. The result of each experiment was expressed as a release profile of mass of ALA released, $m_r(t)$, versus time, t.

2.5. Measuring combined release and permeation of ALA through excised human skin

Membranes of human stratum corneum plus epidermis (SCE) were obtained from excised whole thorax skin by immersion for 2 min in water at 60°C [10]. The SCE membranes were stored in aluminium foil at 5°C after drying at 25% relative humidity. A Franz cell was first filled with pH 5 buffer and an SCE membrane placed across the ground glass joint. An ALA-thin film was carefully placed over the SCE membrane and the Franz cell clamped together. 100 μl samples of the acceptor solution were removed at regular time intervals and their ALA contents

determined using the FMOC fluorescence method described above. The sample volumes were replaced with blank buffer. The result of each experiment was expressed as a combined release/permeation profile of mass of ALA in the acceptor solution, $m_{rp}(t)$, versus time, t.

2.6. Measuring mechanical properties of ALA/PSA-thin films

The adhesive strength of the PSA-thin films was determined using the 'Bioadhesion of Single Coated Pressure Sensitive Tapes at 180° Angle' Test published by the Pressure Sensitive Tape Counsel (PSTC). A PSA-thin film of length 400 mm was applied to a cleaned stainless steel plate of dimensions $2 \times 50 \times 200$ mm and placed in a standard tensile tester (Zwick, type Z 050, 1 kN). A length of 25 mm was pulled downwards as an angle of 180° from the plate at a speed of 12″/min and the adhesive strength (N/cm) measured. PSA-thin films were examined containing either increasing concentrations of ATBC or ALA, and the results expressed as plots of adhesive strength versus film loading with the respective material.

The internal adhesion of the PSA-thin films was determined according to the 'Holding Power of Pressure Sensitive Tape' Test of the PSTC. Each test film was sandwiched between two stainless steel plates of dimensions 1×5 cm. After 10 min holding at 25°C, the sandwiched sample was placed in a test stand according to PSTC Method 7, and a 250 gm weight applied. The time [s] necessary to cause cold flow of the PSA thin-film and complete separation from the test plate was determined in dependence of increasing ALA or ATBC loading of the thin film.

3. Results and discussion

3.1. FMOC-derivatisation analysis of ALA

The standard amino acid assay involving derivatisation with orthopthalaldehyde (OPA) is neither robust nor convenient [8]. Although FMOC-derivatives of primary and secondary amino acids are very stable and show high fluorescence intensity [7], a 20–200 times excess of FMOC-reagent over the amino acid is necessary to achieve complete derivatisation. The fluorescence spectra of FMOC and the FMOC-derivative are, however, very similar, making it necessary to separate the underivatised FMOC and its hydrolysis products from the FMOC/amino acid-derivative using a complicated, time consuming pre-column extraction [7]. We overcame this major disadvantage by using a gradient wash-out of the mobile phase after each sample. Fig. 1 illustrates the good separation of the FMOC/ALA-complex from the peaks attributable to underivatised FMOC and its hydrolysis products. The FMOC/ALA-complex has a retention time of 9.3 min, compared with approx. 11 min for the first of the FMOC-related substances, namely FMOC-OH. The gradient then washes out the remaining underivatised FMOC and its hydrolysis products. The FMOC/ALA-complex is stable and showed no change in its fluorescence chromatogram after 1 week's storage at room temperature with exposure to daylight. In contrast, the OPA/ALA-complex is unstable, resulting in decreasing fluorescence intensity with time and an inconvenient technique [8]. The calibration curve of the FMOC/ALA-complex (not shown) is linear down to <1 ng ALA per sample. This good detection sensitivity was the same in

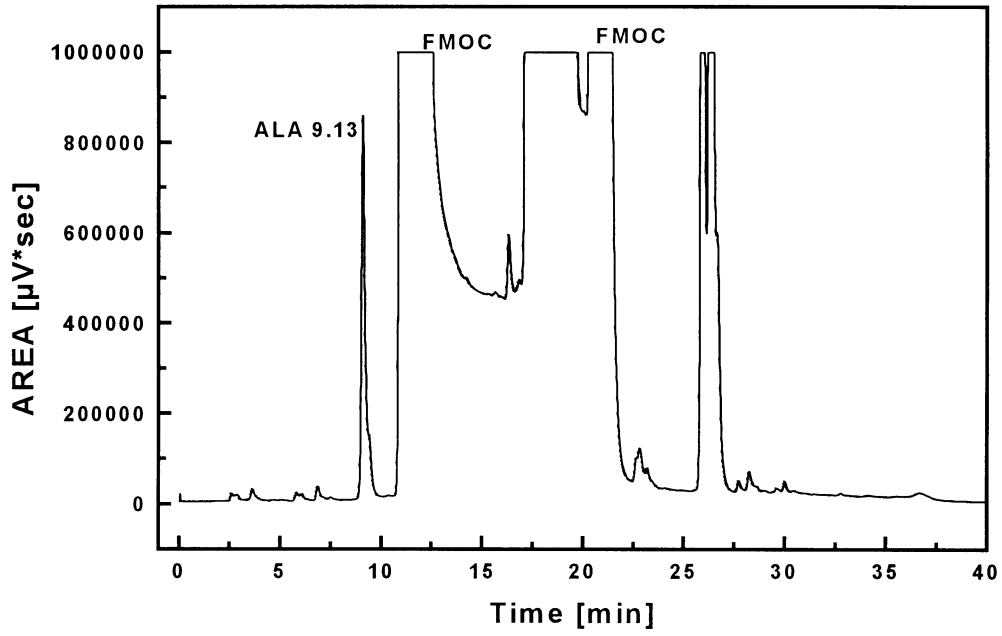

Fig. 1. FMOC-derivatisation HPLC chromatogram obtained for sample of 9 ng of 5-amino levulinic acid (ALA). The ALA elutes after approx. 9.3 min, and a clear separation is obtained from the excess FMOC reagent and its hydrolysis products. The mobile phase gradient was as follows: 0–25 min, acetate buffer/acetonitrile (60: 40); 26–30 min, acetate buffer/acetonitrile (0: 100); 31–40 min, acetate buffer/acetonitrile (60: 40).

the samples withdrawn from the Franz cells during skin experiments which are contaminated with various SCE components. The latter also did not affect the accuracy of separation of the FMOC/ALA peak at 9.3 min in the chromatogram.

3.2. Release of ALA from thin films

Fig. 2a shows the release profiles of ALA from pure Eudragit NE thin-films (not containing ATBC, therefore not PSAs) having 10% w/w loading of different fractions of milled ALA powder. This loading gives 2 mg ALA per film of thickness 200 μm and diameter 1 cm. There is no difference between the amounts of ALA released, $m_r(t)$, with the various fractions during the time frame up to 3 h of relevance for clinical application during PDT. The thin-films show a remarkably rapid initial ALA release. After 2.5 h the release profiles in Fig. 2a give values for $m_r(2.5)/m_0$ of approx. 0.1, equal to 200 μg ALA, or 10% of their total ALA loading (2 mg per film). This rapid release occurs because the ALA/Eudragit NE thin-films are suspension systems. The highly hydrophilic ALA (octanol/water-partition coefficient = 0.03) is poorly soluble in the lipophilic polymer. When viewed under the polarising light microscope (Fig. 3a for 20% w/w ALA film loading of the 90–160 μm fraction) the ALA crystals can be seen embedded in the anisotropic Eudragit NE matrix. Even with ≤1% w/w ALA film loading, suspended ALA crystals could still be observed. After removal from the Franz cell at the conclusion of a release experiment (Fig. 3b) the thin-film with 20% w/w ALA loading now shows loss of most of its ALA crystals, and the polymer matrix has a slightly milky appearance indicative of water uptake [9].

We think that the rapid ALA release out of these thin films is a result of surface dissolution of the suspended ALA crystals. It is unlikely to be a result of diffusional release, as in the case where drug solubility in the polymer is good. Moderately lipophilic drugs showing good solubility in Eudragit NE have low diffusivities in this polymer. Clenbutenol, for example, has a diffusivity of 4×10^{-11} cm^2/h in a Eudragit NE thin-film (200 μm thickness), and only <1% of the total dissolved drug loading is released after 5 h diffusion [9]. This is an order of magnitude less than that seen here with the ALA/Eudragit NE thin films. Fig. 2b shows the effect of increased ALA film-loading on its release out of the pure Eudragit NE thin-films (without ATBC). Higher ALA loading produces increased release rate and hence larger amounts of ALA released at any time point. Indeed there is a linear relation between% w/w ALA film-loading and the value of m_r(2.5 h). Although this would be the case for diffusion-controlled release [11], we think that the high observed release rates prohibit a diffusion-controlled mechanism, and can best be explained by surface dissolution of ALA crystals. Evidence to corroborate this idea is offered by the SEM in Fig. 3c. It shows the release surface of a freshly-prepared Eudragit NE thin film,

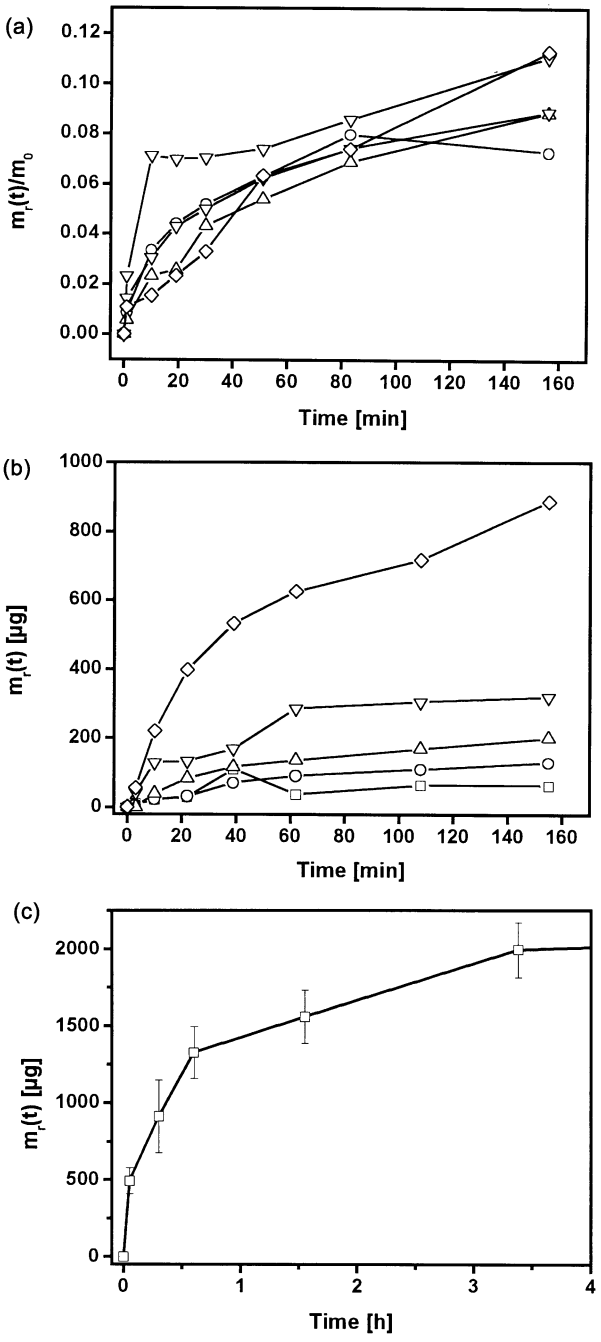

Fig. 2. Release profiles for 5-aminolevulinic acid (ALA) out of Eudragit NE-thin films of 200–250 μm thickness. (a) Effect of ALA sieve fraction on release profile. (□) <32 μm; (○) 32–63 μm; (△) 63–90 μm; (▽) 90–160 μm; (◇) >160 μm. All thin-films were loaded with 10% w/w ALA, = 2 mg ALA/film. The 90–160 μm sieve fraction was selected for all subsequent experiments. The fines (<32 μm) were difficult to deaggregate in the acetone/polymer solution during film production, whereas the crystals >160 μm sedimented. (b) Influence of ALA loading of thin films on release profile for 90–160 μm sieve fraction. (□) 1% w/w; (○) 5% w/w; (△) 10% w/w; (▽) 20% w/w; (◇) 60% w/w. (c) Influence of added acetyltributyl citrate (ATBC) on release profile of ALA. The PSA-thin film contains Eudragit NE/ATBC (1: 2) loaded with 20% w/w ALA of the 90–160 μm sieve fraction. Each PSA-thin film contained therefore a total loading of 4 mg ALA, of which approx. 2 mg has been released after 3.5 h ($n = 4$).

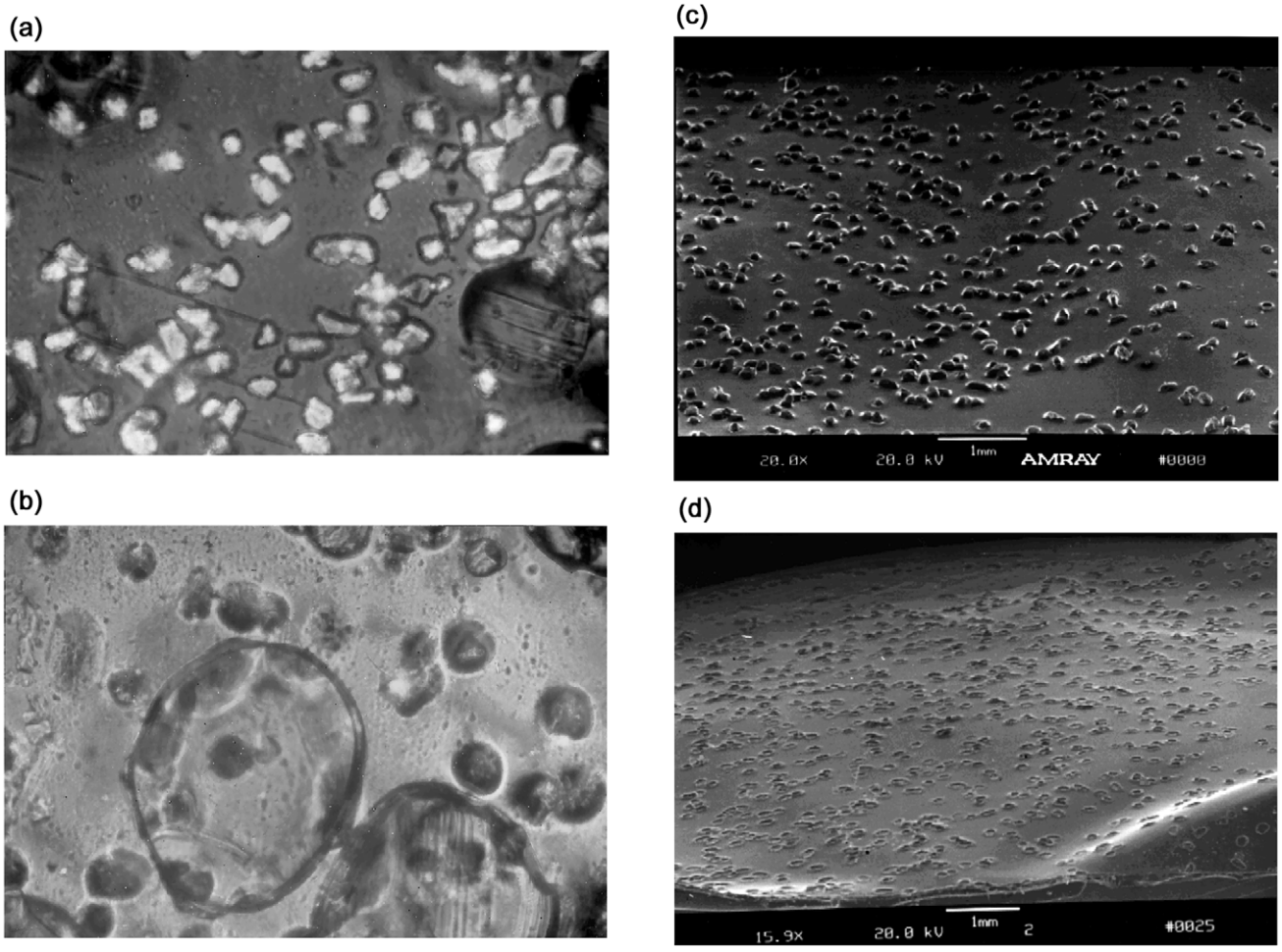

Fig. 3. (a) Eudragit NE/ATBC (1: 2) thin film loaded with 20% w/w 5-aminolevulinic acid (ALA) of sieve fraction 90–160 μm viewed under polarising light microscope. The film is 250 μm thick and was photographed immediately after its preparation. (b) The same thin film viewed after completion of a release experiment at $t = 24$ h. It is evident that most of the anisotropic ALA crystals originally present in the thin film have disappeared during the release experiment. (c) Scanning electron micrographs (SEM) of release surface of Eudragit NE thin film, clearly showing ALA particles protruding slightly through the surface. The crystals are 90–160 μm in diameter and the film thickness is 200–250 μm. (d) The same film viewed after 1 min contact of the release surface with water. The protrusions of the ALA particles are no longer visible, indicating dissolution of the ALA particles exposed to the liquid.

where the presence of ALA particles (50% w/w ALA loading to accentuate effect) in the release surface of the polymer film is evident. The 90–160 μm ALA particles protrude slightly through the surface of the 200–250 μm thick polymer film. The numerous protrusions appear to be coated with the polymer, but this is an artefact of the Au sputtering procedure used to prepare the samples. After 1 min contact with the acceptor medium in a Franz cell the protruding ALA particles are no longer visible in the release surface of the polymer film (Fig. 3d). It is therefore likely that the extremely rapid release of ALA from the thin films is a result of surface dissolution of the ALA particles projecting through the release surface of the film. The fortuitous insolubility of ALA within the lipophilic polymer/plasticiser combination produces a suspension system, whose dimensions further ensure direct contact between the 90–160 μm ALA particles protruding through the surface of the 200–250 μm thin film, and the release medium.

ALA thin films prepared from Eudragit NE can be made self-adhesive by the addition of ATBC. This increases the release rate of ALA, as illustrated in Fig. 2c for a Eudragit NE/ATBC (1: 2) PSA-thin film containing 20% w/w ALA loading of the 90–160 μm fraction. The release of ALA is now very rapid indeed; after 3.5 h almost 2000 μg ALA have been released, equivalent to approx. 50% of the total ALA film-loading (4 mg). Recall that the thin film containing the same ALA loading (20% w/w) but no ATBC released 350 μg ALA after 2.5 h (Fig. 2b) equivalent to 9% of its ALA loading. The appearance of the Eudragit NE/ATBC (1: 2) thin films under polarising light microscopy is the same as that for ATBC-free thin films. The ATBC has therefore no evident influence on ALA solubility in the polymer films. The more rapid release may be because the ATBC accelerates the passage of water through the thin film by increasing the latter's elasticity, but this remains an unanswered question.

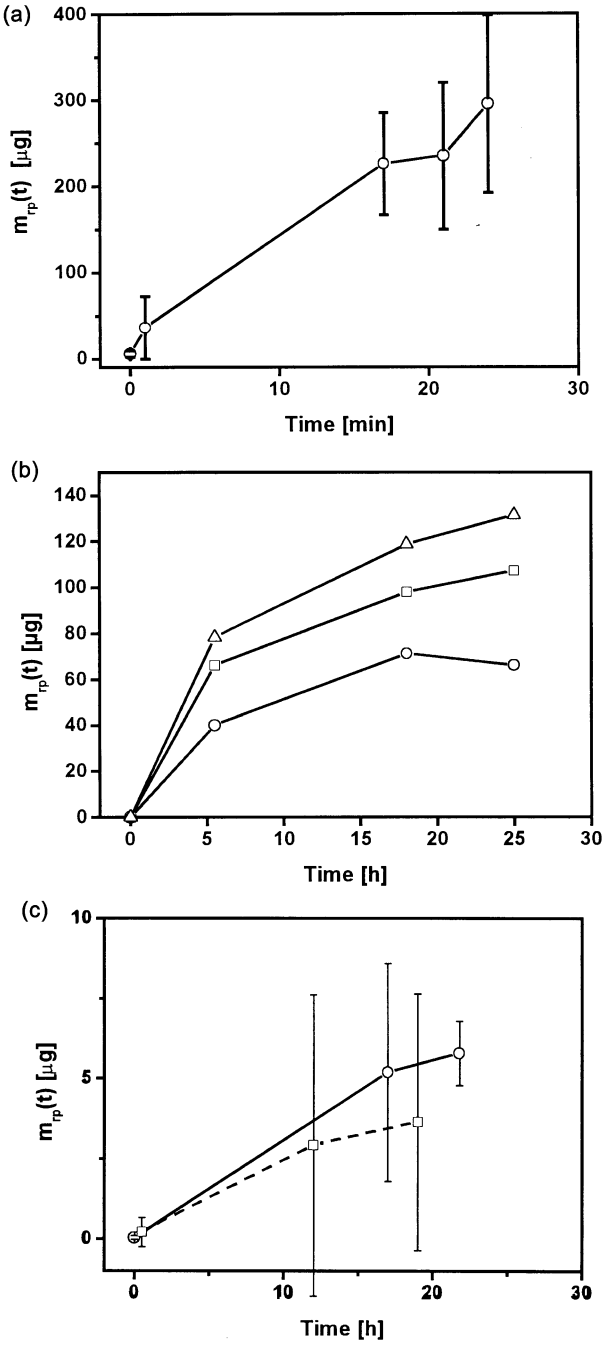

Fig. 4. Release/permeation profiles of 5-aminolevulinic acid (ALA) through membranes of excised human stratum corneum plus epidermis (SCE). (a) Release/permeation profile of ALA from a Eudragit NE/ATBC (1: 2) PSA-thin film with 20% w/w ALA loading of the 90–160 μm sieve fraction. Plots of amount of ALA present in acceptor solution, $m_{rp}(t)$, versus time, t, for $n = 4$. (b) Release/permeation profiles of ALA from Eudragit NE/ATBC PSA thin-films of varying polymer/plasticiser ratio: (○) 1: 0.5, (□) 1: 1, and (△) 1: 2. Thin films contained 20% w/w ALA. (c) Release/permeation profile of ALA from psoralon lipophilic cream with 10% w/w ALA loading (○), and 10% hydroxyethyl cellulose gel with 10% w/w ALA loading (□).The slope, $\Delta m_{rp}(t)/\Delta t|_{0-20\ h}$, with Psoralon = 0.3 μg/cm^2/h and with hydroxyethyl cellulose gel = 0.25 μg/cm^2/h.

3.3. Combined release and permeation of ALA through membranes of excised human SCE

In our previous paper [8] we presented the permeation properties of ALA from aqueous solution through membranes of excised human SCE using side-by-side diffusion cells and an infinite dose technique. From a 10% w/w ALA solution in water a flux of 1–2 μg/cm^2/h was measured during the initial 10 h of measurement. The permeation of ALA through SCE from the Eudragit NE/ATBC (1: 2) PSA-thin film with 20% ALA loading is much greater, as shown in Fig. 4a over a 24 h period. There is no discernable lag time, and after 5 h between 50 and almost 100 μg of ALA (interpolated) have passed the SCE-membrane into the acceptor compartment. The flux is approx. 12 μg ALA/h, a value 5–10 times greater than that obtained from the aqueous solution of ALA (8). This unusually rapid permeation can only be a consequence of the very high release-rate of ALA out of the Eudragit NE/ATBC (1:2) PSA-thin film. We imagine that this results in a high local concentration of ALA at the interface between the thin film and the outer surface of the SC membrane. Additionally, there is an evident enhancing effect of ATBC on the permeation of ALA through the SCE membrane. Fig. 4b illustrates that increasing amounts of ATBC in the Eudragit NE thin films produce more rapid permeation of ALA through the SCE membranes. Even after 5 h, a change in the weight ratio Eudragit NE/ATBC from (1:0.5) to (1:2) doubles the amount of ALA present in the acceptor from 40 μg to >80 μg. The amounts of ATBC appearing in the acceptor solution are, however, small (Table 1) being in each case only approx. 0.1% of the total amount of ATBC present in the film. This is certainly a result of the low water solubility of the lipophilic ATBC. We conclude that the presence of ATBC in the polymer film, necessary to produce a PSA, thus fortuitously also leads to enhanced permeation of ALA through the SCE membrane.

Fig. 4c shows by way of comparison with the polymeric thin films, the release/permeation profiles for ALA out of semi-solid bases, Psoralon lipophilic cream and a 10% aqueous hydroxyethyl cellulose gel, both containing 10% w/w ALA. After 5 h the bases deliver approx. 2 μg ALA through the SCE membrane (interpolated from the profile), only a tenth part of that from the Eudragit NE/ATBC (1:2) PSA-thin film containing 20% w/w ALA shown in Fig. 4a. The permeation rates out of the two bases as determined

Table 1
Measured amounts of ATBC in acceptor solutions of Franz cells at the conclusion (after 24 h) of a combined release/permeation experiment

Film loading with ATBC [Eudragit NE/ATBC]	Mass of ATBC recovered in acceptor [μg] ($n = 4$)
1:0.5	9.0 ± 2.0
1:1	18.0 ± 2.5
1:2	17.0 ± 3.0

from the slope $\Delta m(t)/\delta t|_{t=0\text{-}20\ h}$ are also lower than with a 10% aqueous solution of ALA, being approx. 0.2–0.3 μg/cm^2/h compared with 1 μg/cm^2/h [8]. The Eudragit NE/ATBC (1:2) PSA-thin film is therefore greatly superior to the topical bases regarding delivery of ALA through the SCE membranes. We attribute this to the surface dissolution release mechanism of ALA out of the thin film proposed above.

3.4. Adhesive strength and internal cohesion of PSA thin films

Pure Eudragit NE-thin films are not per se self-adhesive and need to be rendered so by incorporation of ATBC. With greater amount of ATBC in the thin film, the adhesive strength increases and reaches a maximum value of approx. 2.5 N/cm for the combination Eudragit NE/ATBC of 1: 2 (Fig. 5a). There is little information available about the amounts of plasticiser necessary to make PSAs from Eudragit polymers. The hydrophilic Eudragit E having a polymer/ATBC weight ratio of 1: 0.5 gives an 'acceptable' adhesive strength of 1 N/cm [12]. With Eudragit NE Fig. 5a shows this value is reached with the combination polymer/ATBC of 1: 1 by weight, but the thin film was still not sufficiently 'tacky' to adhere firmly to skin in vivo. The high proportions of ATBC required to produce good adhesive strength cause, however, a low internal cohesion of the thin film (Fig. 5b). As will be seen presently, this effect is counterbalanced by the presence of ALA. Film loading with ALA decreases the adhesive power of a Eudragit NE/ATBC (1: 2.25) PSA-thin film (Fig. 5c), as has been previously shown for other PSA-drug combinations. The Eudragit NE/ATBC (1: 2) PSA-thin film containing 20% ALA had, however, excellent adhesion to human skin in vivo up to 24 h and could be removed at this time without residue remaining attached to the skin. ALA increases the internal cohesion of the PSA-thin film (Fig. 5d) which evidently partly compensates for the detrimental effects of the ATBC seen in Fig. 5b. It is the

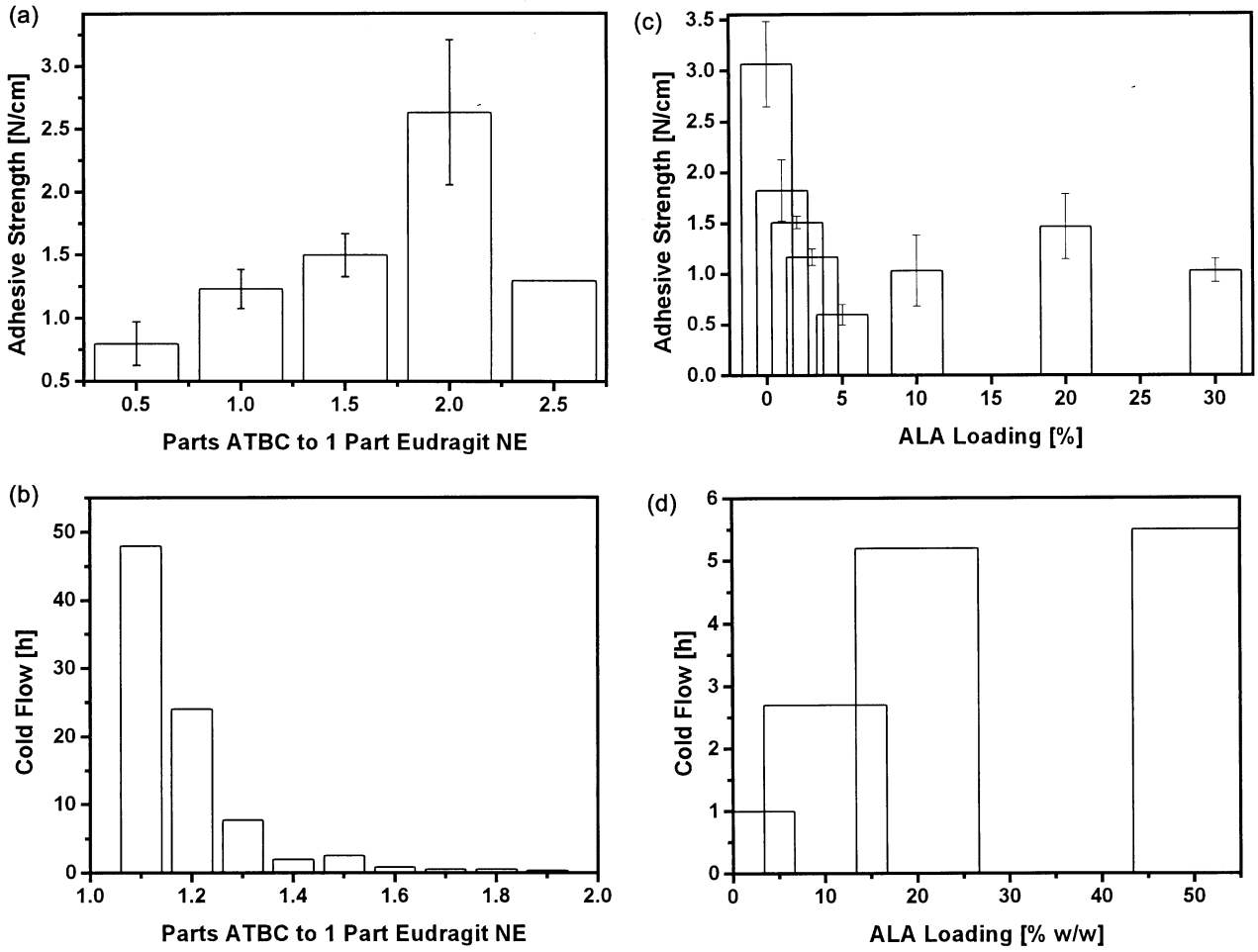

Fig. 5. Mechanical properties of thin films. (a) Influence of ATBC loading of Eudragit NE-thin films on adhesive strength measured by 180° Peal Test. All films contained 10% w/w 5-aminolevulinic acid (ALA). In all cases $n = 4$ and measurements were performed on a stainless steel surface. (b) Influence of ATBC loading on internal adhesion (cold flow) of Eudragit NE-thin films containing 10% w/w ALA. Measurements performed using Cold Flow Test, with $n = 4$. (c) Influence of ALA loading of Eudragit NE/ATBC (1: 2.5) PSA-thin films on adhesive strength ($n = 4$). (d) Influence of ALA loading on internal adhesion (Cold Flow) of Eudragit NE/ATBC (1: 2.25) PSA-thin films ($n = 4$).

presence of ALA crystals within the polymer film that is responsible for this effect, which is the opposite to that seen with dissolved drug.

4. Conclusions

The selection of a lipophilic polymer/plasticiser combination for the PSA-thin film produces a suspension system when loaded with ≥1% w/w ALA. In contact with an aqueous acceptor medium the ALA particles (Ø 90–160 μm) which protrude through the release surface of the film (200–250 μm thickness) can dissolve directly. The result is an extremely rapid release of ALA from the PSA-thin films, e.g. 50% of the total ALA loading within 3.5 h (2 mg from 4 mg) for the Eudragit NE/ATBC (1: 2) PSA-thin film. We found the same effect with other lipophilic PSAs, for example with the Duro Tacs (results not shown), confirming the importance of the proposed surface dissolution release mechanism. High levels of ATBC are necessary to give good adhesive strength of Eudragit NE, without being overtly detrimental to internal cohesion in the presence of ALA. These also promote SC permeation of ALA. As a result, high doses of ALA can be delivered through SCE-membranes from the PSA-thin films, e.g. 50–100 μg of ALA per cm^2 within 5 h for the above mentioned system. During PDT a short application time of a topical carrier for ALA would be propitious. The high permeation rates of ALA observed here with the Eudragit NE/ATBC thin films are therefore very promising. It should be borne in mind that the SC above epithelial skin tumors is frequently in a damaged condition [3], which could further increase ALA uptake into the epidermis. In our next paper on this theme [13] we present the first clinical fluorescence data obtained with the PSA-thin films for ALA.

References

[1] T. Dougherty, K. Weißhaupt, D. Boyle, Photodynamic Therapy, in: S. Hellmanns, S. Roseburg (Eds.), Principles and Practices of Oncology, Philadelphia, Lippincott, 1985.
[2] R.M. Szeimies, T. Sassy, M. Landthaler, Penetration potency of topically applied 5-aminolevulinic acid for photodynamic therapy of basal cell carcinoma, Photochem. Photobiol. 59 (1994) 73–76.
[3] F. Cairnduff, M. Stringer, E. Hudson, D. Ashand, S. Brown, Superficial photodynamic therapy with topical 5-aminolevulinic acid for superficial primary and secondary skin cancer, Br. J. Cancer 69 (1994) 605–608.
[4] A. Bunke, O. Zerbe, H. Schmid, G. Burmeister, H.P. Merkle, B. Grander, Degradation mechanism and stability of 5-aminolevulinic acid, J. Pharm. Sci. 89 (2000) 1335–1341.
[5] F. DeRosa, M. Bentley, Photodynamic therapy of skin cancers: sensitises, clinical studies and future directives, Pharm. Res. 17 (2000) 1447–1455.
[6] T. Peterson, S. Wick, C. Ko, Design, Development, Manufacturing and Testing of Transdermal Drug Delivery Systems, in: T. Gosh, W. Pfister, S. Yum (Eds.), Transdermal and Topical Drug Delivery Systems, Interpharm Press, Buffalo Grove, IL, USA, 1997.
[7] I. Betner, P. Földi, New automated amino acid analysis by HPLC precolumn derivatization with fluorenylmethyloxycarbonylchloride, Chromatographia 22 (1986) 381–387.
[8] E. Bretschko, R.M. Szeimies, M. Landthaler, G. Lee, Topical 5-aminolevulinic acid for photodynamic therapy of basal cell carcinoma. Evaluation of stratum corneum permeability in vitro, J. Contr. Release 42 (1996) 203–208.
[9] A. Göpferich, G. Lee, Measurement of drug diffusivity in stratum corneum membranes and a polyacrylate matrix, Int. J. Pharm. 71 (1991) 245–253.
[10] A. Kligman, E. Christophers, Preparation of isolated sheets of human stratum corneum, Arch. Dermatol 88 (1963) 63–66.
[11] J. Crank, The Mathematics of Diffusion, Oxford University Press, London, 1974, pp. 49–53.
[12] Röhm Pharma Polymer, personal communication (2000).
[13] S. Lieb, G. Lee, R.M. Szeimies, Measurements of epidermal fluorescence after application of a topical plaster system for 5-amino levulinic acid, Arch Dermatol. (2001) submitted for publication.