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Original research

To what extent do *in vitro* tests correctly predict the *in vivo* residence of nail lacquers on the nail plate?

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ABSTRACT

Pharmaceutical nail lacquers are used to topically treat nail fungal infections. The film's residence on the nail is an important factor, and in the laboratory, rapid *in vitro* adhesion and water resistance tests are often used to indicate their likely *in vivo* residence. However, the predictivity of such *in vitro* tests is unknown. The aim of this work was thus to determine whether, and to what extent, such *in vitro* tests can correctly predict the *in vivo* fate of nail lacquers. The *in vivo* residence of four commercially available nail lacquers (three pharmaceutical and one cosmetic) was determined in 16 volunteers. *In vitro*, the films' resistance to water, and their adhesion to a model nail plate was measured, and *in vitro-in vivo* correlations were explored. It was found that the *in vitro* films' resistance to water correctly predicted the *in vivo* residence of lacquers, while the adhesion tests did not.

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1. Introduction

The nail lacquer (varnish, enamel) is an obvious drug delivery vehicle [1] for the topical treatment of nail diseases, such as onychomycosis (fungal infections of the nail) and psoriasis, the two most common nail diseases, which affect approximately 14–18% and 1% of the population respectively [2–6]. Both diseases require long treatment durations – onychomycosis largely with oral antifungals and nail psoriasis with repeated steroid injections in the nail folds and a range of other medicines currently used in the treatment of skin psoriasis – and are difficult to cure. The adverse effects of oral and injectable therapies, such as systemic toxicity, drug interactions and pain, make topical therapy very attractive, and effective medicinal nail lacquers are seen as being ideal topical medicines for such nail diseases. As a consequence, a number of pharmaceutical nail lacquers, such as Penlac® (containing ciclopirox), Loceryl® (containing amorolfine), Onytec® (containing ciclopirox), Kerydin™ (containing tavaborole), Jublia® (containing efinaconazole) are commercially available for the topical treatment of onychomycosis. Much research into the formulation of pharmaceutical nail varnish is also ongoing, for example, thermogelling lacquers [7], bilayered lacquers [8], phase-separating lacquers [9], and those containing hydrosoluble polymers [10], econazole [11],

terbinafine [12], clotrimazole [13], clobetasol [14], natural products [15,16] and urea [17], among others.

Nail lacquers consist of solvents, a film forming polymer, other polymers called resins which improve the film's adhesion to the nail plate, plasticiser(s) and miscellaneous additives such as pigments in cosmetic varnish. Pharmaceutical nail lacquers also contain the drug. Once applied, the lacquer solvents evaporate, leaving a film on the nail plate. For pharmaceutical lacquers, the film acts as a drug depot, from which the drug can be continuously released and permeate into the nail. The duration of residence of the film on the nail plate is therefore critically important. A film with a long residence time would need less frequent lacquer application, which could in turn lead to increased patient compliance, improved treatment efficacy and reduced cost of treatment.

In research and development, the residence time of the film on the nail can be measured in volunteers, who would wear the nail varnish, and monitor its extent of residence until all of it came off naturally. However the potentially long duration of such *in vivo* studies means that faster *in vitro* indicative tests are necessary. Two such indicative tests are measurements of the adhesion of the film to a substrate (ideally a nail plate), and its resistance to water when applied on such a substrate. A film which strongly adheres to the nail plate and which is resistant to water is expected to have a long residence time. Adhesion of the film to a substrate, or its resistance to removal from the substrate, can be measured employing tests that are commonly used to assess paint coatings. For example, a nail lacquer is applied onto a glass slide and allowed to dry, after which

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a right-angle lattice pattern is cut into the film using a sharp blade. Subsequently, the film's resistance to removal from (or adhesion to) the substrate is visually scored. The film may be removed either by using a soft brush after cutting the lattice pattern or by applying an adhesive tape to the pattern, then pulling it off. In practice, this test is prone to operator-introduced bias, for example the force used to apply and remove the adhesive tape can vary inter- and intra-operator. The test can be rendered less subjective by using a texture analyser to pull the adhesive tape and measure the force needed to peel the lacquer film off the substrate. Alternatively, adhesion of nail lacquers' films to nails can also be measured by measuring the force needed to directly pull the films off nail clippings [8,18]. The water resistance test is conducted by applying a nail lacquer onto a substrate, allowing it to dry, then immersing the substrate (with the lacquer film) in water for a defined time, after which the amount of lacquer film lost from the substrate is quantified [19].

Despite the use of such tests for characterising cosmetic and pharmaceutical nail lacquers, currently there is, to the authors' knowledge, no literature showing the extent to which such *in vitro* tests correctly predict the *in vivo* residence time of nail lacquers. The aim of the work described in this paper was therefore to explore the *in vitro-in vivo* correlations, if any.

Four commercially available nail lacquers (three pharmaceutical and one cosmetic) were used. The *in vivo* residence of the lacquer films on the finger and toe nails was measured in sixteen volunteers, while their adhesion and resistance to water were measured *in vitro*.

Adhesion of the film was measured both manually, using a general test method for paints and varnishes (ISO 2409), and by texture analyser to determine whether removal of operator-introduced variability would improve the test's predictivity. It was not practical to use human nailplates for these tests due to their small size and scarcity, and therefore models - high density polyethylene (HDPE) and glass - were used. HDPE was used as its surface energy (35 mJ/m^2) [20] is similar to that of the nail plate ($34.1 \pm 5.5 \text{ mJ/m}^2$ [21], surface energy being known to influence adhesion [22]. Glass was used as it is a commonly used substrate to test adhesion of paints and lacquers. *In vitro* water resistivity of the lacquer film was also conducted using HDPE as a substrate.

2. Materials and methods

2.1. Materials

The colourless pharmaceutical lacquers Curanail[®], Ciclopoli and Nailon[™] were purchased from pharmacies in England, Germany and India respectively, while the colourless cosmetic nail varnish REVLON Quick Dry Top Coat was purchased from a retail outlet. Colourless lacquers were chosen for volunteer acceptability. Scotch[®] Magic[™] tape (25 mm in width, 3 M, UK) was purchased from stationery shops and Amazon UK[®]. High density polyethylene sheet (4 mm thick) was purchased from RS (Corby, UK) and cut into 180mm × 70 mm plates. Glass microscope slides were obtained from VWR (Leuven, Belgium).

2.2. Methods

2.2.1. *In vivo* residence of nail lacquer films on the finger and toe nails

The *in vivo* research followed the tenets of the Declaration of Helsinki promulgated in 1964. Following approval by the UCL School of Pharmacy's ethics committee, 16 volunteers (four males, twelve females, aged 15–65 years) with healthy fingernails and toe nails were recruited and informed consent was obtained. A nail lacquer was applied to all the fingernails and toe nails of the

participants by the researcher. Subsequently, the nails were visually observed daily by the researcher to estimate the percentage of nail lacquer film remaining on the fingernails and toe nails. Estimation was facilitated by visually dividing the nail plate into quadrants which were then further divided into sub-quadrants. After 30 days, any remaining nail varnish was removed with a nail varnish remover, the nails were cleaned, and the experiment was repeated using a different nail varnish. This was repeated until all four nail varnishes had been tested on all the volunteers. It was important to apply each nail lacquer to all the ten fingernails and all the ten toe nails and then take an average, rather than applying different nail lacquers to the different fingernails, as the rate at which nail lacquers come off differs on the different fingernails. The inherent subjectivity of visual estimation was limited by the fact that the same researcher applied all the nail varnishes and performed all the estimations.

2.2.2. *In vitro* nail lacquer film resistance/susceptibility to water

This test was adapted from ASTM (American Society for Testing and Materials) D870:2009, "Standard Practice for Testing Water Resistance of coatings using Water Immersion" [23]. The nail lacquers (0.07 g) were applied onto the smooth side of HDPE plates in strips (15mm × 70 mm) and allowed to air-dry at room temperature for 20 min. The HDPE sheets were then placed in a distilled water bath at room temperature such that half the length of the lacquer strip (35 mm) was immersed in the water, while half (35 mm) remained outside (to serve as control). At timed intervals, the HDPE plates were taken out of the water to observe the influence of water immersion on the lacquer films. The experiment was repeated five times, and the film's susceptibility to water was scored as follows: 0 = no change in film; 1 = film becomes slightly white/translucent; 2 = film becomes white/opaque; 3 = film blisters/is removed off the substrate. Thus a high score indicated high susceptibility to water.

2.2.3. *In vitro* measurement of film adhesion by texture analyser

The adhesion of nail lacquer films to the substrate (HDPE plate) was evaluated in terms of the peak adhesive strength (PAS) using a 180° peel test on an Instron[®] materials testing system (Instron[®], UK) at ambient temperature. A peel test was used to measure adhesion as it is said to be a semiquantitative measure of the coating adhesion to the substrate, which can be used for ranking coatings. A nail lacquer (0.25 g) was applied onto a defined area (130mm × 30 mm) of the smooth side of an HDPE plate using the brush/stick provided, in a single coat and allowed to air-dry for 10 min at room temperature. The strength of adhesion between the lacquer film and the polyethylene sheet was then determined by measuring the force needed to peel the lacquer film off the HDPE sheet, using a tape, as it was not possible to peel off the lacquer film on its own. Thus, a length of Scotch[®] Magic[™] tape was applied onto the surface of the lacquer film strip, leaving an excess of 150 mm tape tab unattached to the lacquer film. Intimate contact between the lacquer film and the Scotch tape was made, ensuring that no air bubbles were trapped under the tape, and a 2 kg steel roller was rolled over the Scotch tape on the lacquer film once to uniformly secure the Scotch tape to the lacquer film. Following this, the unattached part of the Scotch tape was then bent back on itself at an angle of 180°, and clamped to the Instron tester. The polyethylene sheet was also clamped, so that the Scotch tape could be peeled away from the HDPE sheet at a peel angle of 180°. Peeling was conducted at a speed of 25 mm/min and load versus extension curves were obtained, from which the peak adhesive strengths were calculated. For each nail lacquer film, the experiment was repeated ten times. The experimental set-up is schematically shown in Fig. 1. The experimental conditions adopted - such as

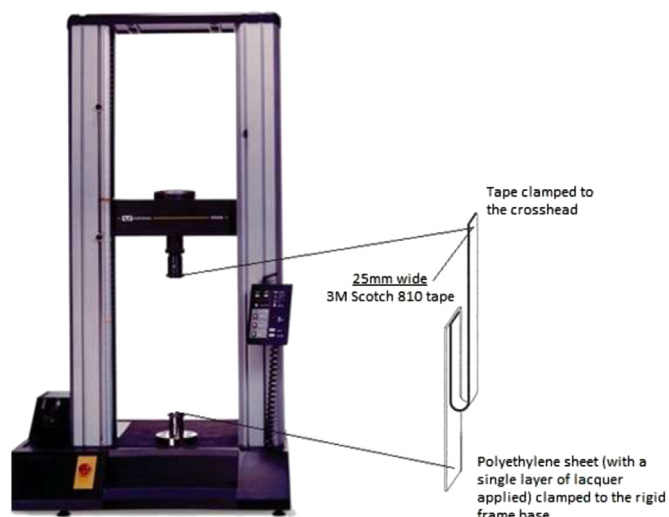


Fig. 1. Instron set up for 180° peel test. The crosshead moved up at a speed maintained at 25 mm/min thus pulling the tape and hence lacquer off the polyethylene sheet clamped to the rigid frame base.

amount of lacquer, drying time, smooth/matt side of HDPE plate, width of Scotch tape, rate of peeling – were those that were found to be optimal following tests to determine their influence on the quality of PAS measurements (data not shown).

2.2.4. Manual *in vitro* measurement of film adhesion

This method was adapted from ISO 2409 (2007&2013) which describes a method of assessing the resistance of paint coatings to separation from substrates when a right-angle lattice pattern is cut into the coating. A nail lacquer (0.25 g) was applied (using the applicator/brush provided in the pack) onto i) HDPE or ii) scratch-resistant glass sheets over an area of 25 mm × 130 mm, and allowed to air-dry for 10 min. Subsequently, a right-angle lattice pattern was cut into the lacquer film using a scalpel (blade thickness of 0.38 mm, Swann-Morton, Sheffield, UK). Six parallel cuts at 1 mm spacing were made in the direction of nail varnish application, followed by six perpendicular cuts (also at 1 mm spacing) to form a lattice. The resistance of the lacquer film to removal from the substrate was then measured in two ways (shown in Fig. 2): i) any loose film was removed by brushing the substrate lightly with a soft brush or ii) a length (75 mm) of Scotch Magic tape was placed over

the lattice pattern, parallel to one set of cuts, and smoothed over firmly with a finger to ensure good contact, leaving a piece of free (unadhered) tape tab. After a few minutes (<5 min), the free end of the Scotch tape was grasped firmly and manually pulled steadily off the lacquer film at an angle of approximately 60°.

In both cases, the cross-hatch pattern on the lacquer film was then visually examined to assess the extent to which the nail varnish had been removed off the glass plate by brushing or the Scotch tape. For each varnish, the experiment was repeated five times and the film's removal was scored as follows: 0 = lattice is totally unaffected; 1 = some small flakes of film are detached at the intersections of the cuts with less than 5% of the lattice area being affected; 2 = the film has flaked along the edges and/or at the intersections of the cuts with a cross cut area greater than 5% but less than 15% being affected; 3 = the film has flaked along the edges of the cuts partly or wholly in large ribbons, and/or on different parts of the squares, with a cross-cut area $\geq 15\%$ but $\leq 35\%$ being affected; 4 = the film has flaked along the edges of the cuts in large ribbons or some squares have detached partly or wholly with a cross-cut area $>35\%$ but $<65\%$ being affected; 5 = any degree of flaking or detachment that cannot be classified under 4. A higher score thus reflects poorer resistance of the film to removal from (or adhesion to) the substrate. Fig. 3 schematically shows the scoring patterns. The two substrates (HDPE and glass) and the two removal methods (brushing and tape application and removal) were used in these experiments to investigate their potential influences on the test results. The four experimental conditions will be denoted glass-brush, glass-tape, HDPE-brush and HDPE-tape henceforth.

2.2.5. Statistical analyses

Repeated measures ANOVA was conducted to determine whether there were differences in the *in vivo* residence and *in vitro* water resistance of the four nail lacquers over the experimental time. One-way ANOVA was conducted to test for differences in the peak adhesive strengths measured by the texture analyser. Two-way ANOVA was used to analyse the results from the manual adhesion tests. SPSS 21 was used for all statistical calculations.

3. Results and discussion

3.1. *In vivo* residence of the lacquers on the fingernail

The mean *in vivo* residence of the lacquers on finger and toe nails over the 30-day period is shown in Figs. 4–5. The *in vivo*

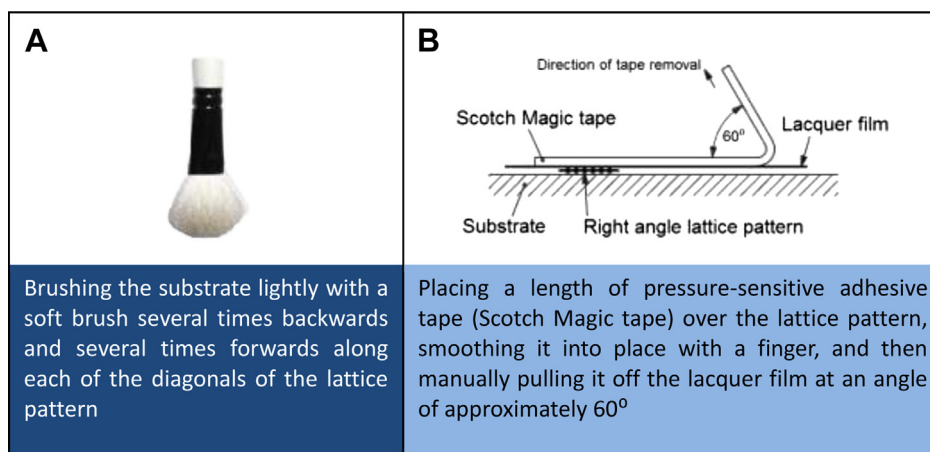


Fig. 2. Methods of loose film removal. Adapted from ISO (International Organisation for Standardisation) 2409:2013, "Paints and varnishes – Cross-cut test" BSI Standards Limited, 2013.

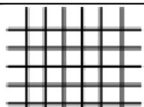
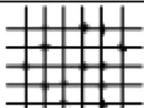




Score	Appearance of surface of cross-cut area from which flaking has occurred
0	
1	
2	
3	
4	
5	

Fig. 3. Scoring of cross-cut test results. Adapted from ISO (International Organisation for Standardisation) 2409:2013, "Paints and varnishes – Cross-cut test" BSI Standards Limited, 2013.

residence of a nail varnish is expected to be governed by the wearer's activities such as manual work, swimming, etc. Thus variability in the residence of the same nail varnish in different wearers is expected and is seen by the error bars in Figs. 4–5.

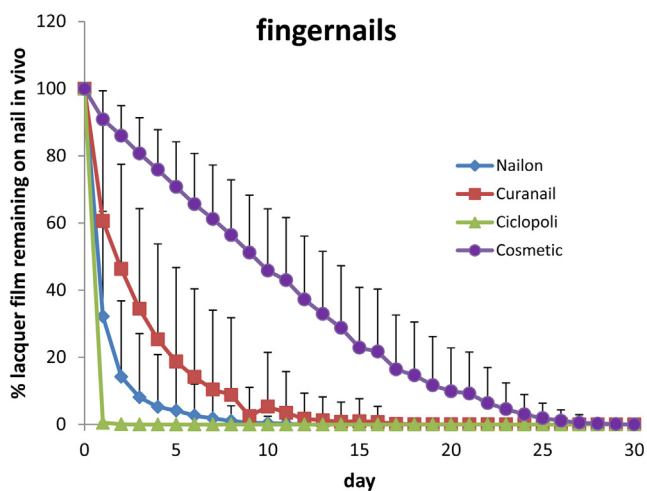


Fig. 4. The *in vivo* residence profile of four commercially available nail lacquers on the ten fingernails in sixteen volunteers. Means \pm sd are shown; $n = 160$. Data points with no error bars are those where sd was zero.

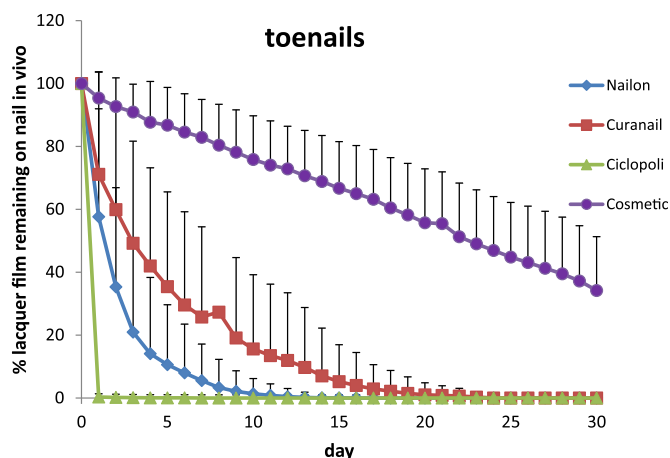


Fig. 5. The *in vivo* residence profile of four commercially available nail lacquers on the ten toe nails in sixteen volunteers. Means \pm sd are shown; $n = 160$. Data points with no error bars are those where sd was zero.

Despite the error bars, it can be seen that some nail lacquers are considerably longer-lasting than others, and statistically significant differences among the four lacquers (repeated measures ANOVA, $p < 0.05$) were observed. For both finger and toe nails, the order of *in vivo* residence, from least to greatest, as determined by post hoc Tukey tests, was: Ciclopoli < Nailon < Curanail < Cosmetic lacquer.

Residence on the hands was of shorter duration than on the feet for Nailon, Curanail and Cosmetic lacquers ($p < 0.05$); this can be explained by the facts that the hands are much more exposed to abrasions during daily activities and are washed much more frequently than the feet. Ciclopoli's extremely fast loss from both hands and feet resulted in no difference between residence on hands and feet ($p > 0.05$). There was no difference in residence between the left and right hand or foot sides, for any of the four nail varnishes ($p > 0.05$).

The cosmetic nail lacquer is considerably longer-lasting than all three pharmaceutical lacquers. This is most likely due to its different composition; it contains nitrocellulose as the primary film former, acrylates copolymer as the resin (whose role is to promote adhesion of the film) and triacetin as the plasticiser, in addition to solvents and a multitude of other excipients. Nitrocellulose in combination with a resin and a plasticiser has been used in cosmetic nail lacquers for decades to produce long-lasting nail varnish. In contrast, the pharmaceutical nail lacquers have different film-forming polymers and far fewer excipients. Curanail contains the film forming polymer Eudragit RL100, the plasticiser glycerol triacetate, the drug amorolfine and solvents (butyl acetate, ethyl acetate and ethanol). Ciclopoli contains the water-soluble polymer hydroxypropylchitosan, the drug ciclopirox, solvents (ethyl acetate, alcohol, water) and cetylstearyl alcohol. Nailon also contains the drug ciclopirox, however it was not possible to find the nature of the excipients.

The very short residence of the pharmaceutical nail lacquers Ciclopoli and Nailon explain their daily application regimen [product patient information leaflet], with Ciclopoli's PIL informing patients not to wash their hands/feet for 6 h following application. It seems that the manufacturer expects the films to be removed after the first washing(s), and a contact time of 6 h to give sufficient drug permeation every day. In contrast to Ciclopoli and Nailon, Curanail is intended to be applied once weekly [product patient information leaflet]. From Figs. 4–5, it can be seen that much of the lacquer film (and hence drug) is lost from the nail plate within 3–4 days of application. This could be partly responsible for the relatively low success rate of Curanail in the treatment of

onychomycosis; a recent study reported a complete cure rate of only 13% in 71 patients with toenail onychomycosis who applied amorolfine lacquer twice weekly for a period of 36 weeks [24]. Daily (rather than weekly) application or reformulation of this lacquer to improve its residence on the nail, such that a drug-loaded film is present on the nail plate surface, especially on the fungally-infected parts, at all times, is therefore likely to improve its success rate in the topical treatment of onychomycosis.

3.2. *In vitro* lacquer film susceptibility/resistance to water

The lacquer films' susceptibility/resistance to water over the experimental period is shown in Fig. 6. The profiles are significantly different from one another (repeated measures ANOVA, $p < 0.05$), with the order of water resistance, from least to greatest being:

Ciclopoli < Nailon < Curanail < Cosmetic lacquer (post hoc Tukey test, $p < 0.05$). This is the same order as that seen for the *in vivo* studies (Section 3.1). Although the *in vitro* test is fairly extreme – in practice, hands and feet are not in prolonged contact with water for days – the fact that it accurately reflects the *in vivo* rank order shows that it can be a useful tool in the laboratory for rapid selection of the best lacquer formulation when a long *in vivo* residence is desired.

3.3. *In vitro* lacquer film adhesion

3.3.1. Measured by texture analyser

The peak adhesive strengths (PAS) of the lacquer films are shown in Fig. 7. Curanail had the lowest adhesive strength (ANOVA, post-hoc Tukey, $p < 0.05$), while the other three lacquers had statistically similar PAS values ($p > 0.05$). Thus, the order of PAS, from least to greatest, as shown by post hoc Tukey tests, was Curanail < Nailon \approx Ciclopoli \approx Cosmetic lacquer. This order does not reflect that of the *in vivo* residence (Section 3.1).

3.3.2. Measured by the manual ISO 2409 test

The lacquer films' adhesion or propensity to separation from the substrate (glass or HDPE), when removed either by a brush or an adhered tape, is shown in Fig. 8. Two-way ANOVA, followed by post-hoc Tukey, were conducted to explore the influence of lacquer nature and removal method on the removal scores. It was found that:

- i) The HDPE-tape and glass-brush methods could not discriminate among the four lacquers, and statistically similar removal scores

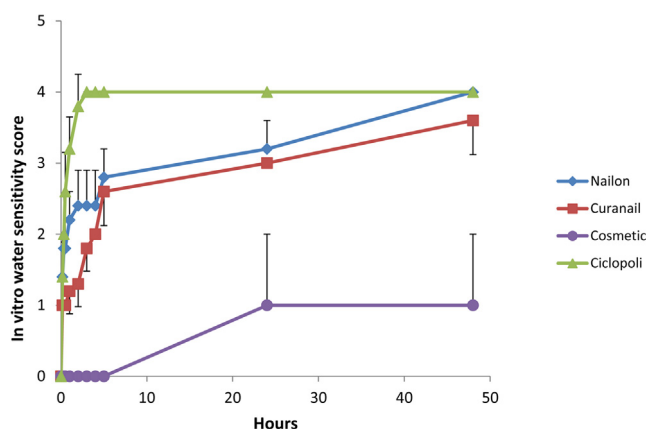


Fig. 6. Water susceptibility profiles of nail lacquers. Mean \pm sd are shown; $n = 5$. Data points with no error bars are those where sd was zero.

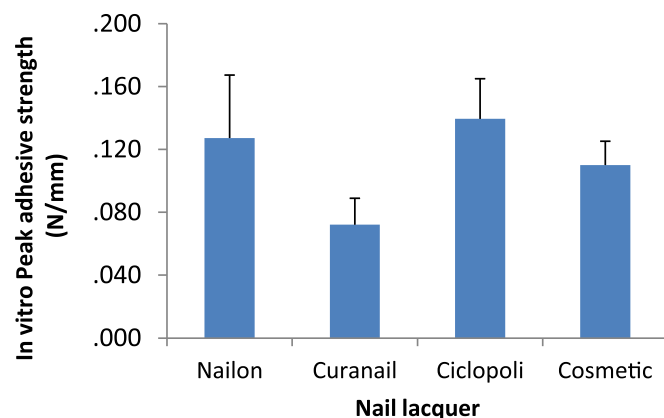


Fig. 7. Peak adhesive strengths of nail lacquers measured in peel tests using a texture analyser. Mean \pm sd are shown; $n = 10$.

were found for all four lacquers ($p > 0.05$). While the HDPE-tape method removed most/all of the lacquer, the glass-brush method removed very little of any of the lacquers.

- ii) In contrast, the glass-tape and HDPE-brush methods could differentiate among the four lacquers ($p < 0.05$). In addition, these two methods gave similar removal scores for the four nail lacquers (Fig. 8). Curanail and the cosmetic lacquer were statistically similar to each other ($p > 0.05$); Ciclopoli and Nailon were statistically similar to each other ($p > 0.05$) but different to Curanail and the cosmetic lacquer ($p < 0.05$).

From this, we conclude that HDPE-tape and glass-brush methods should not be used to test the adhesion/resistance to removal of nail lacquers *in vitro* as they are not discriminatory. Using methods HDPE-brush or glass-tape, the order of resistance to separation from a substrate was, from least to greatest, Nailon \approx Ciclopoli < Cosmetic \approx Curanail. This order does not reflect that shown when peak adhesive strengths were tested by texture analyser (Section 3.3.1) or the order of *in vivo* residence (Section 3.1).

3.4. *In vitro-in vivo* correlations

A perfect correlation was found between the *in vivo* residence rank order and the water-sensitivity test results, i.e. the latter test could have correctly predicted the *in vivo* residence of the four nail lacquers used in this study. In contrast, the results of the two

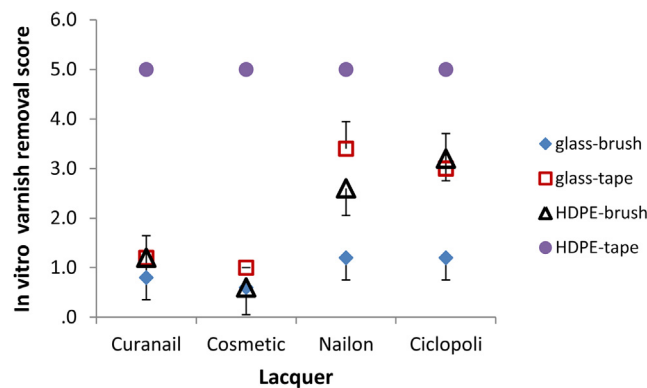


Fig. 8. Nail lacquer removal scores, measured manually. High scores relate to greater removal of nail lacquer films i.e. lower adhesion. Mean \pm sd are shown; $n = 5$. Data points with no error bars are those where sd was zero.

adhesion methods (manual or texture analyser) correlated neither with each other nor with the *in vivo* residence ones. The adhesion tests could not therefore have correctly predicted the *in vivo* residence of nail lacquers. From this study using only four lacquers (due to the limited number of pharmaceutical nail lacquers that are commercially available), water sensitivity of the lacquer films seems to be a more important factor (than adhesion to a substrate) influencing the *in vivo* residence of nail lacquers.

Adhesion tests could still have a role in predicting the *in vivo* residence of nail lacquers for example, if all the nail lacquers had similar water resistances. Another study is needed, where only water-resistant lacquer films are used, to determine, i) whether the strength of adhesion between the lacquer film and the substrate is related to *in vivo* residence and, ii) whether the manual or the texture analyser tests are more predictive of *in vivo* residence. The manual test is the one that is most commonly used in the characterisation of nail lacquers, however, this test is quite operator-dependent. Improvements in the texture analyser peel tests, for example, with the use of nails as substrates for lacquer application, and more sophisticated peel tests, with the inclusion of parameters such as the film's tensile strength (which is likely to influence its resistance to chipping and flaking due to everyday mechanical hazards of wear) could increase the predictivity of these tests.

4. Conclusions

In this study where water-sensitive and water-resistant nail lacquers were used, the water-sensitivity test was found to be able to correctly predict the *in vivo* residence of these lacquers. While it can be predicted that water-sensitive films will have a lower *in vivo* residence than water-insensitive films, this is, to our knowledge, the first report to show experimental correlation between the extent of water-sensitivity of nail lacquer films and their *in vivo* residence. Meanwhile, the adhesion tests could not predict the *in vivo* residence. It is possible that adhesion tests have a predictive role when water-resistant nail lacquers, such as most cosmetic lacquers, are being formulated. Our studies showed the importance of the substrate (glass/plastic) and lacquer removal method (brush/tape) for tests that can be discriminative among different lacquers.

Conflict of interest

The authors report no declarations of interest.

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