British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.2017.03

British Journal of Pharmacy

www.bjpharm.hud.ac.uk

Research Article

Nail lacquer films' surface energies and *in vitro* water-resistance and adhesion do not predict their *in vivo* residence

Sudaxshina Murdan*, Amani Bari, Suleman Ahmed, Basma Hossin, Laxmi Kerai

Department of Pharmaceutics, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

ARTICLE INFO

Received: 22/03/2017
Revised: 07/07/2017
Accepted: 18/07/2017
Published: XX/07/2017

*Corresponding author.
Tel.: +44 207 7553 5810
E-mail: s.murdan@ucl.ac.uk

KEYWORDS: Nail; Lacquer; *In-vitro in-vivo* correlations

ABSTRACT

The in vivo residence of nail lacquers (which are ideal topical drug carriers for the treatment of nail diseases) determines their frequency of application, and is thereby expected to influence patient adherence and success of treatment. Thus in vitro measurements to indicate lacquers' in vivo residence are routinely conducted during formulation development. However the literature on in vitro-in vivo correlations is severely limited. Thus, the aim of the work discussed in this paper was to investigate correlations between in vivo residence and in vitro film resistance to water, in vitro film adhesion and surface energy of lacquer films. In vivo measurements were conducted on fingernails in six volunteers. Seven commercially available nail lacquers were tested in commonly-used measurements. Correlations between in vivo residence and in vitro water resistance and adhesion were found to be extremely poor. The surface energies of the lacquer films (which were between 33 and 39 mJ/m^2) were also not predictive of in vivo residence. High density polyethylene (HDPE) sheet – whose surface energy was determined to be similar to that of the human nailplate – was found to be a suitable model for the nailplate (when investigating surface energy) and was used in a number of experiments.

INTRODUCTION

Nail diseases, for example, onychomycosis (fungal infections of the nail) and psoriasis are common, affecting approximately 14-18% and 1% of the general population respectively (Baran R et al., 2006; Murdan, 2002; Murdan, 2012; Reich, 2009) and demand for their treatment is increasing. For example, sales for the US dermatophytic onychomycosis market are estimated to grow at a compound annual growth rate of 20% for 2012-2022, which is expected to be partly driven by new efficacious topical medicines (PharmaPoint, 2013). Topical therapy for nail diseases is highly desirable compared to oral anti-fungal therapy and injectable anti-psoriatic therapy as it avoids their disadvantages such as adverse effects and drug interactions of oral anti-fungal therapy and the pain of injections. Thus, a number of formulations, such as lacquers, gels, films, patches are being investigated as topical nail medicines, as compiled in (Saner et al., 2014; Shivakumar et al., 2012).

Lacquers – nail varnish - are of special interest, due to their simplicity of application, widespread use as nail cosmetics and patient familiarity. Once applied on the nail plate, the lacquer solvents evaporate,

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producing a film from which the drug can be continuously released and permeate into the nail. Films which have a long in vivo residence on the nail plate would need less frequent lacquer application, which would reduce the cost of treatment, and possibly increase patient adherence, and thereby success of therapy. In addition to in vivo residence, nail lacquer formulations are tested for other properties including flow, brushability, drying qualities, film colour, gloss, thickness, hardness, flexibility, adhesion, mechanical and water resistance (Braunagel, 2005; Schlossman, 1981). The in vitro tests used - based on those employed in the technical lacquer industry – are said to be useful for screening purposes only, and that the preferred test is always an in-use test by human volunteers (Braunagel, 2005; Schlossman, 1981). Nonetheless, we have previously shown that water resistance tests could correctly rank formulations in terms of in vivo residence, while adhesion tests did not (Murdan et al., 2015). The latter study was conducted using pharmaceutical nail lacquers, to cater to the interest of pharmaceutical scientists. However only four lacquers were used due to the limited number of commercially available medicinal lacquers. Some of these lacquers were water-sensitive while others were more water-resistant. In addition, the pharmaceutical lacquers are fairly simple preparations compared to cosmetic ones, which are said to be among the most complex and difficult cosmetic products to formulate, comprising of 13 or more ingredients which can interact with one another (Renz HM in (Braunagel, 2005)). As pointed out by Murdan et al (2015) their findings of correlations between in vitro water-resistance and in vivo residence could have been partly predicted by their use of both water-sensitive and water-resistant lacquers in the study. This begs the question of whether the findings would apply if only fairly water-resistant nail lacquers had been tested.

The first aim of the work discussed in this paper was therefore to re-explore the in vitro-in vivo correlations between in vivo residence and in vitro water resistance and adhesion tests, using water-resistant nail lacquers. These in vitro tests were selected as they are commonly used in pharmaceutical ungual research e.g. (Kerai et al., 2015; Mididoddi and Repka, 2007; Shivakumar et al., 2010). The second aim was to determine whether film adhesion to the nail plate (and subsequently in vivo residence) could be predicted by the surface energies of the lacquer films in relation to that of the nail plate. Surface energy is known to influence adhesion; for good intrinsic adhesion, the surface energy of the adhesive must be equal to or less than that of the substrate (Venkatraman and Gale, 1998). Thus, theoretically, the surface energy of nail lacquer films should be equal to or less than 34.1 ± 5.5 mJ/m^2 – the surface energy of the human nail plate (Murdan et al., 2012).

To achieve these aims, seven commercially available cosmetic nail lacquers were tested. The in vivo residence of the lacquer films on fingernails was measured in six volunteers, while their surface energy, adhesion and resistance to water were measured in vitro in commonly-used laboratory tests. Subsequently, the potential of the in vitro tests to predict in vivo performance was evaluated.

MATERIALS AND METHODS

Materials

The colourless cosmetic nail varnishes RIMMEL Lycra Pro 421®, RIMMEL 60 Seconds 740 ®, Maybelline Forever Strong Pro®, Collection 2000 Ice Cube ®, Barry M Base/Top Coat ®, Revlon Top Coat®, Nailed by Sleek® were purchased from various retail outlets in the UK. Colourless lacquers were chosen for volunteer acceptability. These water-insoluble cosmetic lacquers contained a large number of components, with some similarities and differences amongs them (Supplementary Table). For example, nitrocellulose was the primary film former in all, except for Collection 2000 which had cellulose acetate as the film former. The plasticiser, acetyl tributyl citrate, was present in five of the lacquers. UV absorbers were benzophoneone-1 (2 lacquers), benzophenone-3 (2 lacquers), octocrylene (1 lacquer) or etocrylene (1 lacquer). The solvents ethyl acetate, butyl acetate, isopropyl alcohol were present in all the lacquers.

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Scotch® Magic™ tape (25mm in width, 3M, UK) was purchased from stationery shops and Amazon UK®. High density polyethylene sheet (HDPE) was used as a model for the human nail in in vitro studies as its surface energy (reported to be 35 mJ/m² (Yao et al., 1993)) is fairly similar to that of the human nail, which was reported to be 34.1 ± 5.5 mJ/m² (Murdan et al., 2012). HDPE (4mm thick) was purchased from RS (Corby, UK) and cut into 180mm x 70mm plates. One side of the HDPE plate was smooth and shiny, the other less smooth and matt. The surface energy of both matt and shiny surfaces of the HDPE sheet was determined as described in Section 2.5, and found to be 40 mJ/m² (40.37 mJ/m² for the matt side and 39.64 mJ/m² for the shiny side). Due to the negligible difference between the surface energies of the matt and shiny surfaces, the smooth (shiny) side of the HDPE sheet was used in all the experiments in this study. The liquids used to measure surface energy of solid surfaces were glycerol (Ransom, UK), diiodomethane (Sigma, UK) and HPLC water (Sigma, UK).

In vivo residence of nail lacquer films on the fingernail

Following approval by the School of Pharmacy, University of London's ethics committee (REC/A/10/01), volunteers (6 females, aged 15-65 years) with healthy fingernails were recruited. A researcher than applied a nail lacquer to all the fingernails (which were clean and free from any other products) of the participants. Subsequently, the nails were visually observed daily by the same researcher to estimate the percentage of nail varnish film remaining on each of the fingernails. Estimation was facilitated by visually dividing the nail plate into quadrants which were then further divided into sub-quadrants. After 2 weeks, 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 the seven nail varnishes had been tested on all the volunteers. It was important to apply each nail lacquer to all the ten fingernails and then take an average, rather than applying different nail lacquers to the different fingernails, as the residence of nail lacquers differs on the different fingernails (for example, compare thumbs and middle fingers in Figure 1).

Fig 1. Photograph shows that the residence of the same nail varnish film (blue-coloured for visibility in this experiment) is highly variable on the different fingernails. Consequently, to compare nail lacquers, every nail lacquer should be applied to all the fingernails (rather than apply a different lacquer to a different nail).

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. The inherent subjectivity of visual estimation could have been further limited by blinding the researcher to the nature of the nail varnish or having two independent researchers estimating the in vivo residence. This was not conducted in the present study, but should be considered for future ones.

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" (ASTM, 2009). The nail lacquers were applied onto the smooth side of HDPE plates in strips (15mm x 70mm) and allowed to air-dry at room temperature for 20 minutes. The HDPE sheets were then placed in a distilled water bath at room temperature such that half the length of the lacquer strip was immersed in the water, while half was outside. 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 indicates high susceptibility to water.

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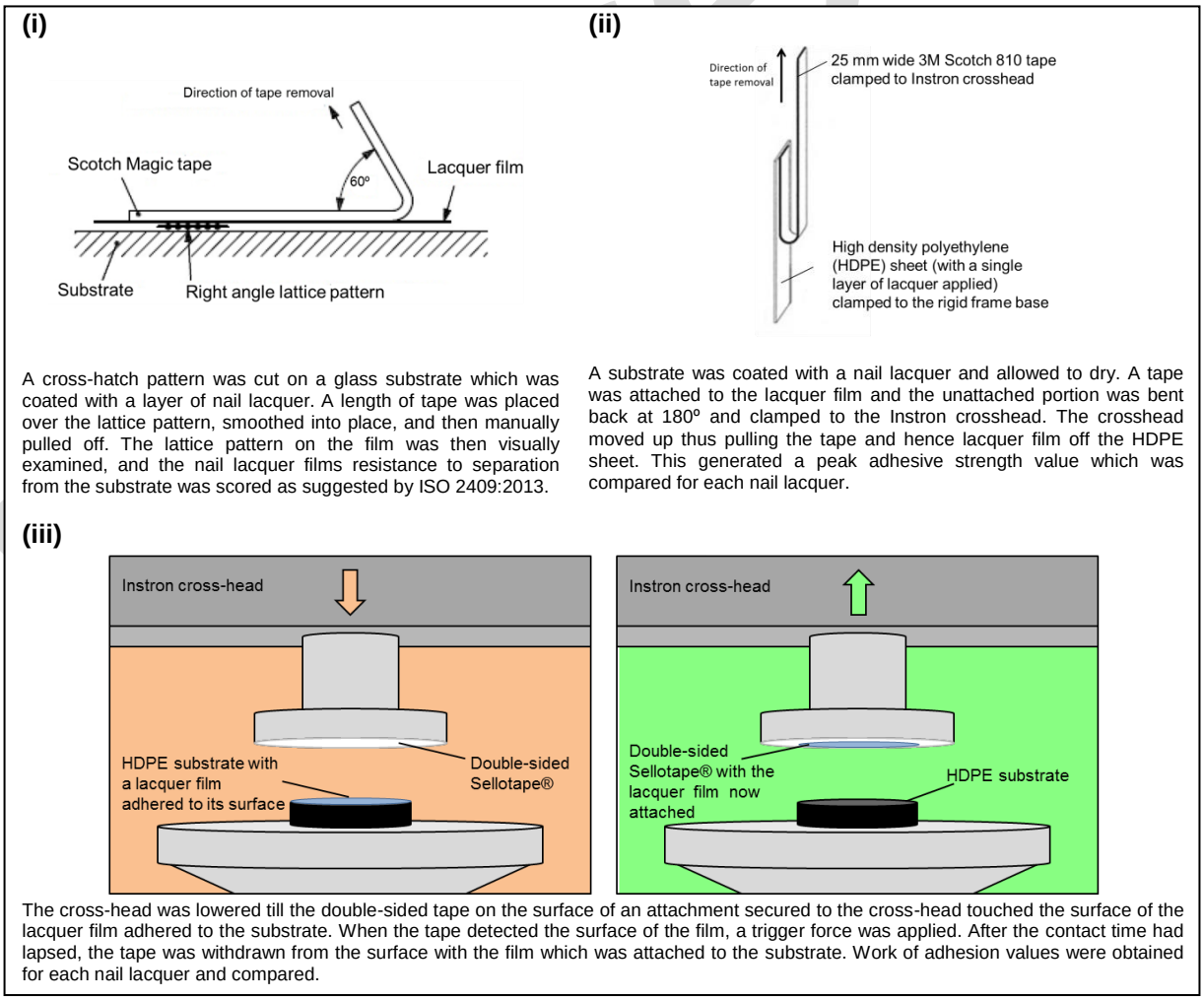
In vitro measurement of film adhesion

Film adhesion to a substrate was measured in vitro using three methods: (i) manual peel test (ii) peel test by texture analyser (iii) pull-off test by texture analyser, as detailed below, and as schematically shown in Figure 2.

In vitro indication of film adhesion by the manual tape test

This method was adapted from ISO 2409:2007 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 brush provided) onto scratch-resistant glass sheets over an area of 25mm x 130mm, and allowed to air-dry for 10 minutes. Subsequently, a cross-hatch 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. Using a soft brush, the panel was then lightly brushed, several times backwards and forwards along each of the diagonals of the lattice. A length of Scotch Magic tape (75mm) was then 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 (less than 5 minutes), the free end of the Scotch tape was grasped firmly and manually pulled steadily off the lacquer film at an angle of approximately 60°. The cross-hatch pattern on the lacquer film was visually examined to assess the extent to which the nail varnish had been removed off the glass plate by the Scotch tape. For each varnish, the experiment was repeated four times and the film's removal by the tape was scored as represented in Figure 3. A high score thus reflects poorer adhesion of the film to the glass substrate.



A cross-hatch pattern was cut on a glass substrate which was coated with a layer of nail lacquer. A length of tape was placed over the lattice pattern, smoothed into place, and then manually pulled off. The lattice pattern on the film was then visually examined, and the nail lacquer films resistance to separation from the substrate was scored as suggested by ISO 2409:2013.

A substrate was coated with a nail lacquer and allowed to dry. A tape was attached to the lacquer film and the unattached portion was bent back at 180° and clamped to the Instron crosshead. The crosshead moved up thus pulling the tape and hence lacquer film off the HDPE sheet. This generated a peak adhesive strength value which was compared for each nail lacquer.

The cross-head was lowered till the double-sided tape on the surface of an attachment secured to the cross-head touched the surface of the lacquer film adhered to the substrate. When the tape detected the surface of the film, a trigger force was applied. After the contact time had lapsed, the tape was withdrawn from the surface with the film which was attached to the substrate. Work of adhesion values were obtained for each nail lacquer and compared.

Fig. 1. (i) Manual tape adhesion test, (ii) 180 ^{0} texture analyser peel test and (iii) texture analyser pull-off test

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In vitro measurement of film adhesion by texture analyser (peel test)

The adhesion of nail lacquer films to the substrate (HDPE plate) was evaluated in terms of the peak adhesive strength using a 180o peel test on an Instron® materials testing system 5567 (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' (Lacombe, 2006). A nail lacquer (0.25g) was applied onto a defined area (130mmx30mm) of the smooth side of an HDPE plate using the brush provided, in a single coat and allowed to air-dry for 10 minutes 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 150mm 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 2kg steel roller was rolled over the Scotch tape on the lacquer film once to uniformly secure the Scotch tape to the lacquer film. 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 25mm/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 conditions adopted - such as 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 Peak Adhesive Strength (PAS) measurements (not shown).

In vitro measurement of film adhesion by texture analyser (pull-off test)

In addition to the peel test described above, the adhesion of films to a HDPE sheet was also determined by a pull-off test, using the Instron equipped with a 5 kg load cell (Instron®, UK). A lacquer (31.5 mg) was applied onto the smooth surface of a circular HDPE sheet (diameter 25mm), as a single layer using the brush provided, and allowed to dry for 20 minutes at room temperature. The HDPE sheet with the applied film was then secured to the base of the Instron equipment. Double-sided Sellotape® (Sticky Fixer Strip, 3M) was fixed to a circular Instron attachment (diameter of 50mm), which was secured to the Instron cross-head. The crosshead was lowered from a height of 35 centimetres at a speed of 1mm/sec until the Sellotape touched the surface of the lacquer film on the HDPE.

Appearance	Score and Description
	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 crosscut 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 ≥ 15 % but ≤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.

Fig. 3. *Scoring of the surface of cross-cut area from which flaking has occurred. Adapted from ISO (International Organisation for Standardisation) 2409:2013, "Paints and varnishes – Cross-cut test" BSI Standards Limited, 2013.*

When the tape detected the surface of the film, a trigger force of 20 N was applied for 30 seconds. After the contact time had lapsed, the Instron crosshead was moved upwards at a pre-set speed of 0.5 mm/s. This resulted in removal of the lacquer film from the HDPE substrate by the Sellotape, and

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the force needed to remove the film was recorded as peak adhesive force (PAF). The energy at break (i.e. work of adhesion) was also determined from the force deflection profiles. The two parameters were interpreted using Instron Bluehill software.

Measurement of the surface energy of nail lacquer films

The surface energy of the lacquer films (adhering to a HDPE plate) was estimated from contact angle measurements of liquids (water, diiodomethane and glycerol) on the lacquer films and subsequent computation using the Lifshitz-van der Waals/acid-base (LW-AB) approach. The latter method was employed as it is currently one of the most commonly used computations and it has been successfully applied in many instances (Van Oss, 2006; van Oss et al., 1987, 1988a; van Oss et al., 1988b). Measurements were conducted in a laboratory where the room temperature ranged from 20 to 26 °C and the relative humidity from 19 to 29%. High density polyethylene plates were thoroughly cleaned by wiping with acetone, and then rinsing with distilled water and allowed to dry completely. A nail lacquer was then applied to the smooth side of the HDPE plate, using the brush provided, to produce an even film. The lacquer film was allowed to air-dry for ten minutes at room temperature. To measure the contact angles that liquid droplets make on the film surface, a goniometer (FTA 1000, First Ten Angstroms, Portsmouth, VA, USA) was used. A liquid droplet (15µL) was dispensed from a Gilmont micrometer syringe (Cole-Parmer Instrument Co. Ltd, London, UK) fitted to a 20 gauge blunt needle onto the solid film surface. The liquid droplet was allowed to stabilize on the film surface, all the while video recording the droplet. For each video, a series of droplet images were analysed, and the triple point at the intersection of the liquid, solid and vapour phases on both sides of each image was manually identified. The contact angles were calculated and averaged for the two sides on each video image. Subsequently, contact angle versus time was plotted to enable visualization of the stable contact angles, which were then averaged to obtain a mean contact angle. For each liquid, the contact angles of five droplets were measured on each lacquer film. The means were then used to calculate the film's surface energy with the goniometer software. The same procedure was used on clean, unlacquered HDPE sheets to determine the latter's surface energy.

Statistical analyses

Repeated measures ANOVA, and post hoc Tukey was conducted to determine whether there were differences in the in vivo residence (Figure 4) and in vitro water resistance (Figure 5) of the seven nail lacquers over the experimental time. One-way ANOVA and post hoc Tukey was conducted to test for differences in the peak adhesive strengths, work of adhesion, and in the scores for the manual tape tests (Table 1). When there were significant differences among lacquers, the appropriate greater than (>) or less than (<) signs were used. When there was no significant differences among lacquers, this was represented by ≈. SPSS 22 was used for all statistical calculations. The number of replicates in the different in vitro and in vivo tests varied, depending on the estimated variability (which was estimated to be high in the in vivo experiments), practical considerations and the ability to measure statistical significance.

RESULTS AND DISCUSSION

In vivo residence of lacquers on the fingernail

The mean in vivo residence – defined as the percentage varnish film remaining on the nail with time - of the lacquers over a two-week period is shown in Figure 4. The in vivo residence of a nail varnish is expected to be governed by the wearer's activities such as manual work, swimming, etc. Thus variability in residence of the same nail varnish in different wearers is expected and is shown by the error bars. Despite the variability, it can be seen that some nail lacquers are significantly longer-lasting than others (repeated measures ANOVA, $p<0.05$). The order of in vivo residence, from least to greatest, determined by post hoc Tukey tests, was: Maybelline < Rimmel Lycra Pro < Rimmel 60 Seconds < Barry M ≈ Revlon ≈ Collection 2000 ≈ Nailed by Sleek. Of the seven lacquers studied, the formulations of Barry M, Revlon, Collection 2000 and Nailed by Sleek seem to be the most optimal with respect to long in vivo residence.

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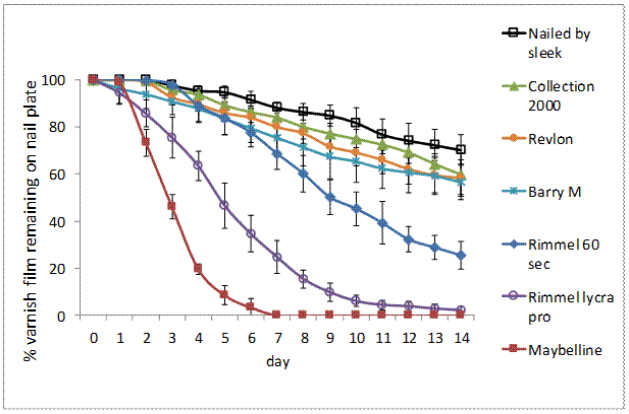

Fig. 4. *In vivo residence profile of 7 commercially available nail lacquers on the 10 fingernails in 6 volunteers. Means ± SD are shown; n=60.*

In vitro lacquer film susceptibility/resistance to water

The lacquer films' susceptibility/resistance to water over the experimental period were significantly different (Figure 5, Repeated measures ANOVA, $p<0.05$). The nail varnish films showed a gradual deterioration over time, except for Revlon which was not damaged at all, even after 24 hours immersion in water. The order of water resistance, from least to greatest, as shown by post hoc Tukey tests, was: Collection 2000 < Maybelline ≈ Nailed by Sleek ≈ Rimmel Lycra Pro ≈ Barry M ≈ Rimmel 60 seconds < Revlon.

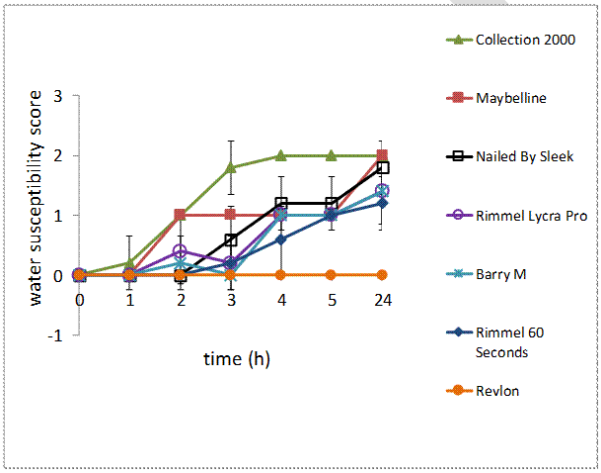

Fig. 5. *Water susceptibility profiles of nail lacquers when the films were incubated in a water bath over 24 hours. Mean ± SD are shown; n=5.*

***In-vitro* lacquer film adhesion**

The results from the three in vitro adhesion measurements – lacquer removal score by manual tape test, peak adhesive strength from texture analyser peel test and work of adhesion from texture analyser pull-off test - are shown in Table 1. No result for the texture analyser peel test is shown for Collection 2000, as the latter could not be peeled off the HDPE sheet, despite numerous attempts, using different tapes and peeling rates. Thus, it can be said that Collection 2000 had the greatest adhesion to HDPE compared to the other lacquers. For each of the three tests, the lacquers were compared using ANOVA and post hoc Tukey, and significant differences were seen among some, but not all, of the lacquers tested. Thus, the order of peak adhesive strength (texture analyser peel test), from least to greatest, was Rimmel 60 Seconds ≈ Revlon ≈ Nailed by Sleek ≈ Barry M ≈ Maybelline < Rimmel Lycra Pro < Collection 2000.

The order of work of adhesion (texture analyser pull-off test), from least to greatest, was Rimmel 60 Seconds ≈ Revlon ≈ Nailed by Sleek ≈ Barry M ≈ Collection 2000 ≈ Rimmel Lycra Pro < Maybelline.

The order of lacquer removal (manual tape test), from least to greatest was: Nailed by Sleek ≈ Revlon < Maybelline ≈ Barry M ≈ Rimmel 60 seconds ≈ Collection 2000 ≈ Rimmel Lycra Pro.

It can be seen that the seven lacquers are ranked differently, from best to worst, in terms of strength of adhesion by the three adhesion tests. The latter tests were employed in this study as they have previously been used for the characterisation of topical nail formulations. For example, the pull-off test has been used in Mididoddi and Repka, 2007; Shivakumar et al., 2010), while the Instron peel test has been used in (Kerai et al., 2015 and the manual tape test is often used for cosmetic nail lacquers (Braunagel, 2005; Schlossman, 1981).

To our knowledge, this is the first time the three tests have been used to compare the same set of nail lacquers, and the first time that different adhesion tests been found to give different rankings to nail lacquers. The variable results given by the tests may be due to a variety of factors, such as different substrates onto which the varnish was painted (glass/HDPE), different materials used to peel the varnish (Scotch tape/double-sided sticky-tape) off the substrates, methods of pulling the varnish film off the substrate (manual/mechanical), and amount and method of contact between the lacquer film,

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substrate and material used to peel the film. Our results confirm the caution urged by (Braunagel, 2005; Schlossman, 1981) to view such in vitro tests as useful for screening purposes only.

3.4 In vitro-in vivo correlations

Simple in vitro tests are generally used in formulation laboratories to rapidly indicate the potential in vivo residence of prepared lacquers. To determine the predictivity of such in vitro adhesion and water resistance tests, the in vivo residence was plotted as a function of water susceptibility and adhesion (Figure 6). As can be seen, some of the trendlines had the expected directions, for example, increase in water susceptibility (Fig. 6a) and in manual removal of the lacquer film by a tape (Fig. 6b) correlated with reduction in in vivo residence. In contrast, and against expectations, increase in the peak adhesive force and work required to remove the lacquer off the substrate was associated with decreasing in vivo residence (Fig. 6c-d). All the in vitro-in vivo curve fits were extremely poor, and the Pearson correlation coefficients were statistically insignificant ($p>0.05$). Thus, the in vivo-in vitro correlations for all the adhesion and water-resistance tests were found to be negligible.

It might be said that the in vitro tests are only indicative and should only be used to rank different nail lacquer formulations in terms of quality in order to enable the formulation scientist to choose the best and/or eliminate the worst formulations. Thus, to investigate whether ranking the formulations would be useful in predicting in vivo residence of nail lacquers, the in vitro and in vivo data was ranked (determined by post ANOVA Tukey; Table 2). It can be seen that in vitro and in vivo rank orders are not strictly maintained. For example, Barry M - one of the longest-lasting varnishes in vivo – does not have the highest water resistance or adhesion. Maybelline has the lowest in vivo residence, but the highest adhesion (by Instron pull-off test).

In an attempt to improve predictivity of the in vitro tests, the in vitro ranks were combined given that in vivo residence of a lacquer film is not only a function of its adhesion to the nail plate, but also of its simultaneous resistance to water. The ranks for the different in vitro tests for each nail lacquer (shown in

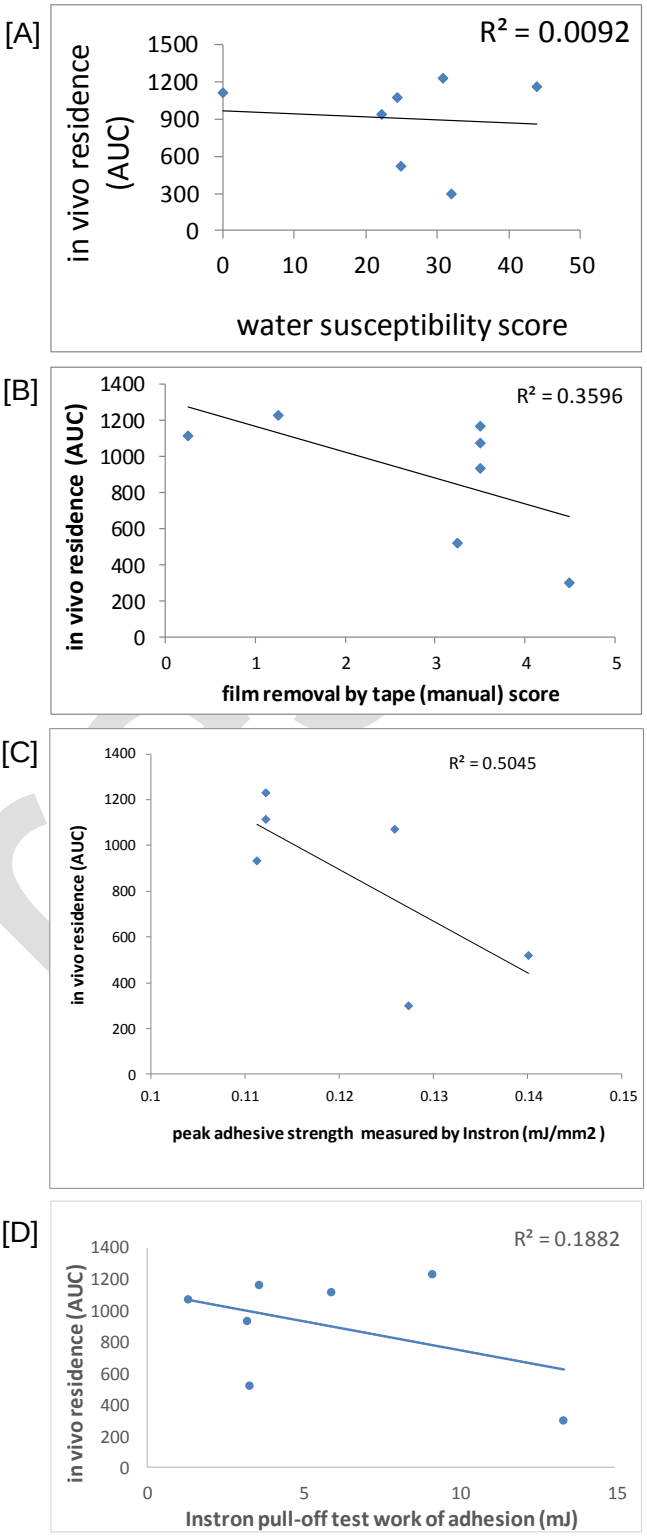

Fig.6. Area under the curve (means) for different lacquers. The Influence on the in vivo residence of nail lacquers of [A] water susceptibility of films [B] film adhesion to a substrate (measured manually), greater film removal score ≡ lower adhesion, [C] film adhesion to a substrate (measured by Instron peel test), [D] film adhesion to a substrate (measured by Instron pull-off test).

Table 2) were then multiplied to give a composite rank (Table 3) to each nail lacquer; multiplication was chosen for its advantages (Tofallis, 2014). It can

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be seen that combining the different in vitro tests does not seem to improve the predictivity of the in vitro tests, in terms of accurately ranking the different nail lacquers. For example, Collection 2000 was ranked last (i.e. the worst formulation) from the product of in vitro tests, yet, it was among the longest-lasting formulations in vivo. The inability of obtaining in vitro-in vivo correlations by ranking the formulations reflects the lack of correlations seen in Figure 6, and confirms the negligible predictivity of in vitro tests towards in vivo residence of lacquers.

The lack of in vitro-in vivo correlation could be due to a number of reasons. The in vitro tests were perhaps too simple, did not reflect the in vivo situation sufficiently, for example, nail plate models were used instead of nail plates, test conditions were extreme (e.g. in water resistance tests and only tested one aspect at a time, e.g. adhesion or water-resistance, while in practice, multiple factors would be operating simultaneously. The use of cadaver nails might improve correlations, although cadaver nails are very expensive and scarce. The use of a more biological model e.g. bovine hoof membrane (whose surface energy is similar to that of human nail (Murdan et al., 2012) might also improve correlations.

Table 1. Indicators of adhesion between lacquer film and the substrate. The peak adhesive force data (from the pull-off test) are not shown, but they followed a similar trend to the work of adhesion. Means ±SD are shown.

	Indication of adhesion		
	Peak Adhesive Strength (N/mm) Instron Peel Test (n=10)	**Work of Adhesion** (mJ) Instron Pull-off test (n=3)	**Lacquer removal score** Manual adhesion test (n=4)
Nailed by sleek	0.120 ± 0.003	9.1 ± 1.8	1.3 ± 0.5
Collection 2000	ND	3.6 ± 1.5	3.5 ± 0.6
Revlon	0.112 ± 0.003	5.9 ± 1.0	0.3 ± 0.5
Barry M	0.126 ± 0.005	1.3 ± 0.4	3.5 ± 0.6
Rimmel 60 seconds	0.111 ± 0.004	3.2 ± 1.6	3.5 ± 0.6
Rimmel Lycra Pro	0.140 ± 0.008	3.3 ± 1.1	3.3 ± 0.5
Maybelline	0.127 ± 0.004	13.3 ± 2.2	4.5 ± 0.6

Table 2. Ranking of nail lacquers, from best (1) to worst, in each in vitro test

Rank	**In-vivo**	***In-vitro***			
	Residence over 2 weeks	**Water resistance**	**Adhesion (Instron peel test)**	**Adhesion (Instron pull-off test)**	**Adhesion (manual test)**
1	Nailed by Sleek Collection 2000 Revlon Barry M	Revlon	Collection 2000	Maybelline	Revlon Nailed by Sleek
2	Rimmel 60 sec	Rimmel 60 sec Barry M Rimmel Lycr Pro Nailed by Sleek Maybelline	Rimmel Lycra Pro	Nailed by Sleek Collection 2000 Revlon Barry M Rimmel 60 sec Rimmel Lycr Pro	Rimmel Ly Pro Collection2000 Rimmel 60 sec Barry M Maybelline
3	Rimmel Lycra Pro	Collection 2000	Maybelline Barry M Nailed by Sleek Revlon Rimmel 60 sec		
4	Maybelline				

https://doi.org/10.5920/bjpharm.2017.03

Table 3. Ranking of nail lacquers, from best (1) to worst, using the composite rank from the different in vitro experiments.

Rank	In vivo	In vitro
	Residence over 2 weeks	Water resistance x Instron peel test x Instron pull-off test x manual adhesion test
1	Revlon Nailed by Sleek Barry M Collection 2000	Revlon
2	Rimmel 60 seconds	Nailed by Sleek
3	Rimmel Lycra Pro	Maybelline
4	Maybelline	Rimmel Lycra Pro
5		Barry M
6		Rimmel 60 seconds
7		Collection 2000

The in vitro tests also need more sophistication and a greater understanding of the factors which influence peeling of nail lacquer films, although an argument against this would be the desirability of simple, quick, easy to use and inexpensive tests.

This study confirms the poor predictivity of in vitro adhesion tests reported by (Murdan et al., 2015). However, our findings about the poor predictivity of in vitro water resistance tests are in contrast to those reported previously (Murdan et al., 2015). This is likely due to the fact that in this study, all the nail lacquers used were cosmetic lacquers which were fairly water-insoluble, while the pharmaceutical lacquers used in Murdan et al 2015 had greater water-solubility. Water-solubility of nail lacquers seems to be the first critical factor; strong adhesion will not lead to a long in vivo residence if the nail lacquer film is washed off as soon as one washes their hands/feet.

Influence of film surface energy on its adhesion

The second aim of the work was to determine whether film adhesion could be predicted by their surface energies. The surface energy values of the seven nail varnish films and of the HDPE sheet are shown in Table 4. The HDPE sheet's surface energy was found to be 40 mJ/m^2; this is slightly higher than literature values of 35 mJ/m^2 (Yao et al., 1993), which could be due to different HDPE samples, sources and different methods for the estimation of surface energy. The HDPE sheet's surface energy was similar to that of the nail plate, reported to be 34.1 ± 5.5 mJ/m^2 (Murdan et al., 2012). HDPE was therefore an adequate model for the nail plate in these experiments.

Table 4. *The surface energies of high density polyethylene sheet and of lacquer films, computed through mean contact angle determined for each liquid on each solid surface using five different droplets.*

Nail lacquer film or HDPE	Total surface energy (mJ/m^2)
RIMMEL Lycra Pro	33.12
Collection 2000	34.86
RIMMEL 60 Seconds	36.76
Barry M	37.24
Nailed By Sleek	37.27
Revlon	38.06
Maybelline Forever	38.82
High density polyethylene sheet	40.37

All the films' surface energies are between 33 and 39 mJ/m^2. The lacquer films adhered fairly well to the high density polyethylene sheet. This was not surprising given that the surface energy of all the varnishes were below that of the HDPE plate and the fact that for good intrinsic adhesion, the surface energy of the adhesive must be equal to or less than that of the substrate (Venkatraman and Gale, 1998). What is not clear from the literature is whether a greater difference between the surface energies of the adhesive and that of the substrate leads to greater adhesion. In order to investigate this, the lacquers' surface energies were plotted against the peak adhesive force determined in the peel tests. An inverse relationship between surface energy of the

https://doi.org/10.5920/bjpharm.2017.03

film and the force needed to peel it off the substrate can be seen (Fig. 7). However, the correlation is low and statistically insignificant ($p>0.05$) i.e. a greater difference between the surface energies of the lacquer film and that of the substrate does not in fact lead to greater adhesion.

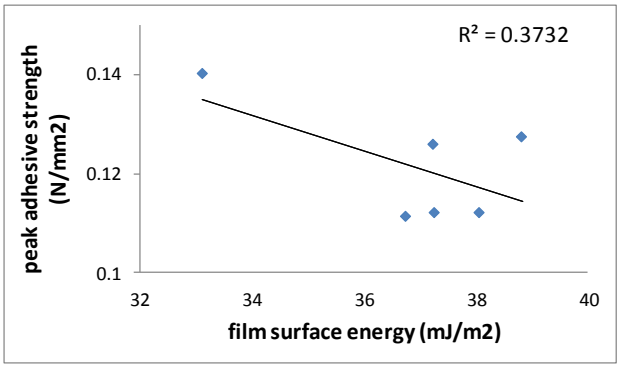

Fig.7. *Influence of film surface energy on its adhesion to a substrate.*

The nail lacquers also adhered well to the nail plate in vivo when first applied. Again, this was not surprising given that the lacquer films' surface energies are similar to that of the nail plate, (34.1 ± 5.5 mJ/m^2). When the film's surface energy was plotted against in vivo residence, a poor fit and a statistically insignificant Pearson correlation ($p>0.05$) was obtained (Fig. 8). That is, the film's surface energy cannot be used to predict in vivo residence.

This can be explained by the fact that in vivo residence of a lacquer film is not only a function of its adhesion to the nail plate, but also its resistance to water. A film might adhere very strongly to the nail, but if it is easily washed off, its residence will be low.

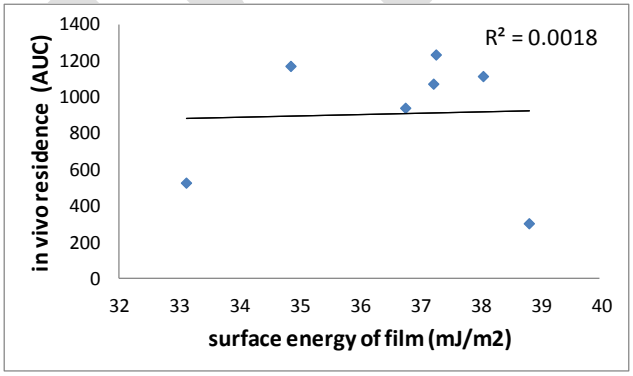

Fig. 8. *Influence of film surface energy on the in vivo residence of nail lacquers.*

CONCLUSIONS

We conclude that correlations between in vivo residence of nail lacquers and commonly-used in vitro water resistance and film adhesion tests are poor. The simple in vitro tests did not correctly predict even the ranks of best to worst nail lacquers, in terms of in vivo residence.

The surface energies of a number of cosmetic lacquer films were found to be around 33 and 39 mJ/m^2. Such films adhered well to nails in vivo; however, the surface energy of lacquer films does not predict their in vivo residence, given that while surface energy influences adhesion, in vivo residence of a lacquer film is also dependent on its water-resistance in practice. High density polyethylene sheet was found to have a similar surface energy to the human nail plate, although it must be remembered that HDPE does not reflect human nail in many aspects, for example, in surface roughness which is also likely to affect adhesion.

Our findings confirm the view that nail lacquers should always be evaluated by in-use tests in human volunteers. The findings will be applicable to pharmaceutical lacquers and other formulations that are being developed as topical nail medicines.

ACKNOWLEDGEMENTS

We are extremely grateful to all the volunteers who participated in this study, and to The School of Pharmacy, University of London, (now UCL School of Pharmacy) for funding this work.

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British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.2017.03

SUPPLEMENTARY MATERIAL

Table S1: *Composition of nail lacquers used in this study.*

Formulation	Maybelline Forever Strong Pro	RIMMEL Lycra Pro 421	Collection 2000 Ice Cube	RIMMEL 60 Seconds 740	Barry M Base/Top Coat	Nailed by Sleek	Revlon topcoat
Primary Film Former	Nitrocellulose	Nitrocellulose	Cellulose acetate	Nitrocellulose	Nitrocellulose	Nitrocellulose	Nitrocellulose
Secondary Film Former/ Modifying Resin	Tosylamide/epoxy resin, Adipic acid/neopentyl glycol/trimellitic anhydride copolymer, Acrylates copolymer Phthalic anhydride/glycerin/glycidyl deconate copolymer	Polyvinyl butyral, Polybutylene glycol/mdi copolymer, Adipic acid/neopentyl glycol/trimellitic anhydride copolymer	Butyrate sucrose benzoate, phthalic anhydride/trimellitic anhydride/glycols copolymer	tosylamide/epoxy resin/acrylates copolymer, adipic acid/neopentyl glycol/trimellitic anhydride copolymer, Polyvinyl butyral	Polyvinyl butyral, adipic acid/neopentyl glycol/trimellitic anhydride copolymer	Phthalic anhydride/ trimellitic anhydride/glycolcopolymer, styrene acrylates copolymer	Acrylates copolymer
Plasticizer	Triphenyl phosphate, Ethyl tosylamide, Acetyl tributyl citrate	Acetyl tributyl citrate	Camphor	Acetyl tributyl citrate, trimethylpentanediyl dibenzoate	Acetyl tributyl citrate, trimethylpentanediyl dibenzoate	Acetyl tributyl citrate	Tiacetin
Solvent	Ethyl acetate, Butyl acetate, Isopropyl alcohol, Propyl acetate	Ethyl acetate, Butyl acetate, IsoPropyl alcohol, MEK, ethyl pyrrolidone	Ethyl acetate, Butyl acetate, IsoPropyl alcohol	Ethyl acetate, Butyl acetate, IsoPropyl alcohol, N-butyl alcohol	Ethyl acetate, Butyl acetate, IsoPropyl alcohol	Ethyl acetate, Butyl acetate, IsoPropyl alcohol	Ethyl acetate, Butyl acetate, IsoPropyl alcohol, N-butyl alcohol
Diluent		Heptane					
Miscellaneous Additives	Benzophenone-1 (UV absorber), Aqua, Ferrous gluconate, Calcium pentothenate, Silica(surfactant)	Benzophenone-3 (UV absorber), Citral, Litsea cubeba fruit oil	Octocrylene (UV absorber)	Benzophenone-1 (uv absorber), aqua/water, trimethylsiloxysilicate, dimethicone, phosphoric acid, Silica(surfactant), stearalkonium bentonite, Polyethylene, quaternium-18 bentonite, corallina officinalis	Benzophenone-3 (UV absorber)	Silica (Surfactant), lecithin (wetting agent) Stearalkonium hectorite(suspending agent)	Etocrylene (UV absorber), dimethicone, tribenzoin