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UV-curable gel formulations: Potential drug carriers for the topical treatment of nail diseases

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ABSTRACT

Nail diseases are common, cause significant distress and treatments are far from successful. Our aim was to investigate the potential of UV-curable gels – currently used as cosmetics – as topical drug carriers for their treatment. These formulations have a long residence on the nail, which is expected to increase patient compliance and the success of topical therapy. The gels are composed of the diurethane dimethacrylate, ethyl methacrylate, 2-hydroxy-2-methylpropiophenone, an antifungal drug (amorolfine HCl or terbinafine HCl) and an organic liquid (ethanol or NMP) as drug solvent. Following its application to a substrate and exposure to a UVA lamp for 2 min, the gel polymerises and forms a smooth, glossy and amorphous film, with negligible levels of residual monomers. No drug-polymer interactions were found and drug loading did not affect the film's properties, such as thickness, crystallinity and transition temperatures. In contrast, the organic solvent did influence the film's properties; NMP-containing films had lower glass transition temperatures, adhesion and water resistance than ethanol-based ones. Water-resistance being a desired property, ethanol-based formulations were investigated further for stability, drug release and ungual permeation. The films were stable under accelerated stability testing conditions. Compared to terbinafine, amorolfine was released to a greater extent, had a higher ungual flux, but a lower concentration in the nailplate. However, both drugs were present at considerably high levels in the nail when their MICs are taken into account. We thus conclude that UV-curable gels are promising candidates as topical nail medicines.

1. Introduction

The nail unit can be subject to numerous disorders, some of which, for example, onychomycosis (fungal nail infection) and psoriasis, can be extremely painful, recalcitrant to treatment (Arrese and Piérard, 2003), and result in psychosocial and occupational consequences, reducing a person's quality of life (Daniel, 2013; Scher, 1996). Onychomycosis affects approximately 14–18% of the general population (Baran et al., 2006; Murdan, 2002, 2008; Murdan, 2013), up to 25% of the geriatric and diabetic populations (Gupta et al., 1997, 1998; Piérard and Piérard-franchimont, 2005), and its occurrence is increasing. Current treatments include topical and oral antifungals. Oral therapy carries the inherent disadvantages of systemic adverse effects, drug interactions and contraindications, and is subject to a failure rate of 20%, as well as a relapse rate of 25% (Gupta, 2012; Roberts, 1999). Topical therapy, on the other hand, has a low success rate with currently available medicines, for example, 13–54% complete cure rates from amorolfine nail lacquer (Lauharanta, 1992; Paul et al., 2013; Reinel and Clarke, 1992), similarly low cure rates from ciclopirox nail lacquer (Gupta and Joseph, 2000), under 20% complete cure rate of efinaconazole solution (Tosti, 2013) and under 30% cure rate of tavaborole solution (Markham, 2014). Nail psoriasis affects approximately 1% of the population (Reich, 2009) and its treatment may involve repeated injections of corticosteroids into the nail folds, injections of biological agents such as adalimumab, photochemotherapy, topical or systemic treatment depending on the symptoms (Oram and Akkaya, 2013). The adverse effects of injections, photochemotherapy and systemic therapy, such as pain, radiation overexposure, systemic toxicity and drug interactions, make topical therapy very attractive; however topical management remains a challenge due to the low drug penetration through the nail plate.

Attempts to improve the efficacy of topical nail medicines are ongoing with the development of new drugs with optimised physiochemical properties and new drug carriers. Some of these are described in (Elsayed, 2015; Naumann et al., 2014). A number of the new formulations are water-soluble and need to be applied every

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day. Such frequent application relies on high patient compliance for therapeutic success. Given that patients' adherence to treatment, in general (Sabaté, 2003), and to topical nail therapy (Zhou et al., 2011) is low, we are investigating longer-lasting UV-curable gel formulations which could act as a drug-depot on the nail plate.

UV-curable gel formulations are currently used as nail cosmetics, where they are commonly known as UV gels (Barel et al., 2009; Draelos, 2010; Schoon, 2005). The formulations typically contain three components: a urethane methacrylate based monomer (at 75–85% w/w), (meth) acrylate based monomers (at 15–25% w/w) and a polymerisation photoinitiator (at 1–3% w/w). Following application of the formulation on the nail plate surface, the nail is placed under a UVA lamp for approximately 2 min. The UVA light initiates polymerisation and a glossy, cosmetically-acceptable polymeric film is formed on the nail plate. The film has a long residence on the nail plate and we propose that such a film could be used as a drug carrier to improve the topical therapy of nail diseases. The formulation would be applied and removed (when desired) by a healthcare professional (e.g. podiatrist) treating the patient.

The aim of the work presented in this paper was to investigate the pharmaceutical potential of such formulations. The objectives were to formulate and characterise such UV-curable gels, in terms of the formulation components, polymerisation, and the resulting film's properties, such as morphology, microstructure, thermal and viscoelastic properties, adhesivity, water sensitivity, stability and the loading, release and ungual (i.e. of the nail) permeation of loaded drug. Anti-fungal drugs were used as onychomycosis is the most common nail disease, although these formulations are expected to be suitable for other nail diseases.

UV gels have been widely used as cosmetics since 1982. Thus, they have a fairly long safety record, although a few cases of hypersensitivity and allergic reactions have been reported (Cravo et al., 2008; Erdmann et al., 2001; Fisher, 1990; Hemmer et al., 1996; Kanerva et al., 1996; Vázquez-Osorio et al., 2014). Such adverse reactions are caused by the monomers (rather than the polymer) and occur after several months of overexposure. The cured film does not pose a hazard (Draelos, 2010), and the adverse reactions can be avoided by applying the formulation to the nail plate only and avoiding the skin surrounding the nail plate. This is done by leaving a formulation-free margin at the nail folds (Draelos, 2010; Schoon, 2005). The use of UVA radiation to trigger polymerisation is not considered a risk factor. Recent research suggested that two hands (of a person) placed in a UV nail lamp for 10 min twice a month (as could happen for cosmetic use) is equivalent to that person spending an extra 2.7 min in sunlight every day for a month (Schoon et al., 2010). It has also been reported that a person could use a UV nail lamp for 2.8 h every day without any requirement for warning or protective measures (Dowdy and Sayre, 2013), and that the UVA radiation emitted by UVA nail lamps specifically designed for curing UV gels pose a low risk of skin cancer even when used weekly for over 250 years (Alina and Martin, 2013).

2. Materials and methods

2.1. Materials

Amorolfine HCl was purchased from Ranbaxy Research Laboratories (Haryana, India) and terbinafine HCl from AK Scientific (CA, USA). Diurethane dimethacrylate, ethyl methacrylate, 2-hydroxy-2-methylpropiophenone, absolute ethanol, methanol, 1-methyl-2-pyrrolidinone (NMP), propan-2-ol, triethylamine, phosphoric acid 85% wt. solution in water, trifluoroacetic acid and a dialysis tubing cellulose membrane (MW 10281) were purchased from Sigma–Aldrich (Dorset, UK). Acetonitrile HPLC gradient grade was purchased from Fisher Scientific (Hertfordshire, UK).

2.2. Methods

2.2.1. Determination of drug (amorolfine HCl and terbinafine HCl) solubility in monomers and in solvents (ethanol and NMP)

Saturation solubility studies were carried out to determine the solubilities of the antifungal drugs in the monomers and solvents used. The antifungals were added in excess to vials containing 2 ml of the monomers or solvents. The mixtures were left to stir on a magnetic stirrer placed in a water bath at 25 °C for 72 h. Subsequently, 1 ml of each sample was withdrawn from the vial using a syringe and passed through a 0.22 μm MILLEX® GP filter unit into an Eppendorf tube. The samples were then centrifuged using a bench centrifuge for 20 min at a speed of 13.2 × 1000 rpm. The supernatant for each sample was then collected and diluted by 50, 100 or 1000 times with ethanol (or NMP – for the solubility determination in NMP) and analysed by HPLC (described in Section 2.4.1).

2.2.2. Formulation preparation

A total of three types of formulations were prepared:

1. Drug-free, solvent-free formulations were prepared by mixing the two monomers (145.5 μl of ethyl methacrylate and diurethane dimethacrylate to one millilitre) with the photoinitiator (30 μl), and leaving the mixture to stir overnight, to produce a clear homogenous solution.
2. Drug-free, solvent-containing formulations were prepared by dissolving the monomers in a solvent (ethanol or NMP). To determine the optimal concentration of solvent, ethanol-containing formulations at concentrations of 25, 30, 35, 40, 45 and 50% v/v were prepared. Films were formed as described below and tested as described in Section 2.3. The optimal solvent concentration was found to be 25% v/v.
3. Formulations containing drug (at 1–5% w/v) and solvent (ethanol or NMP at 25% v/v) were prepared by first dissolving the drug in the chosen solvent and then adding the two monomers and photoinitiator to this drug solution. This mixture was left to stir overnight to produce a clear homogenous solution.

2.2.2.1. UV-curing of formulations. The formulation (30 μl) was applied using a pipette tip on a microscope glass slide (to an area of 15 mm × 15 mm) as a single layer. The glass slide was placed under a 36 Watt Cuccio Professional UVA nail lamp (purchased from Amazon, UK) for 2 min. This caused curing of the formulation and formation of a film. The surface of the film was wiped with propan-2-ol using a super absorbent 4 ply lint-free nail wipe to remove the oxygen inhibition layer, (an unreacted monomer layer). This revealed a glossy polymer film, which was then removed from the glass slide using a scalpel and used in characterisation studies described below.

2.2.3. Assessment of the polymerisation process and formation of the UV-cured film

2.2.3.1. Percentage mass conversion from monomer to cured polymer film. The percentage mass conversion from monomer mixture to polymer film was calculated using the following equation:

$$\text{Mass conversion}(\%) = \left(\frac{W_t}{W_o}\right) \times 100$$

where W_0 is the weight of the monomer mixture before curing and W_t is the weight of the UV-cured film.

2.2.3.2. Fourier transform infrared (FT-IR) spectroscopy. Infrared spectroscopy was used to assess the polymer structure and the

influence of solvent and drug, if any. It was also used to determine the degree of conversion (DC) from monomer mixture to polymer film. Spectra of the uncured and cured formulations were obtained using the OPUS 7.0 software and recorded by a Bruker Alpha IR Spectrophotometer (Bruker Corporation, Germany), using 24 scans over the 400–4000 cm^{-1} range with background subtraction. The DC% was calculated from the ratio of the height of the absorbance peak of the aliphatic C=C bond (1636 cm^{-1}) relative to that of the carbonyl group (>C=O, 1702 cm^{-1}), used as an internal standard, using the following equation:

$$DC(\%) = \frac{(A_{1636}/A_{1702})_0 - (A_{1636}/A_{1702})_t}{(A_{1636}/A_{1702})_0} \times 100$$

where $(A_{1636}/A_{1702})_0$ and $(A_{1636}/A_{1702})_t$ are relative absorbance of C=C bonds to C=O before curing and after curing respectively.

2.3. Characterisation of UV-cured polymer film

Visually, the cured films were smooth, transparent and glossy. The films were characterised for thickness, microstructure, residual monomers, maximum drug-load, thermal properties, stability, drug release and ungual drug permeation. Stability, drug release and ungual drug permeation were only tested for ethanol-based formulations as the NMP-based ones were not resistant to water.

The thickness of the films produced was measured using a Sealey AK9635D 0–25 mm Digital External Micrometer (PVR Direct, UK). Each film was measured at three separate points and an average was taken. Precautions were taken to measure the thickness without compressing the polymer film.

Scanning electron microscopy (SEM) was used for imaging the surface of the polymer films produced. The samples were gold sputter coated (10 nm) and imaged using FEI Quanta 200F (Eindhoven, The Netherlands).

Maximum drug-load determinations were conducted using polarised light microscopy (PLM) and X-ray diffraction (XRD). To determine the maximum (soluble) drug-load in the films, the latter were examined by PLM for the absence/presence of drug crystals using a Nikon Microphot-FXA microscope (Nikon Corporation, Tokyo, Japan) and polarising filters. Images were taken using a Lumenera Infinity 2 digital camera (Lumenera Corporation, Ottawa, Canada) attached to the microscope. XRD was used: (i) to determine the crystallinity/amorphousness of the UV-cured films and (ii) to confirm the maximum (soluble) drug-load of the films determined by PLM. X-ray diffraction spectra of the antifungals and of the polymer films were obtained using a Rigaku MiniFlex 600 X-ray diffractometer (Rigaku Corporation, Tokyo, Japan) equipped with MiniFlex Guidance software. The samples were scanned over an angular range of 2–60°, with a step size of 0.02° and step duration of 0.5°/min. The generator voltage was set at 40 kV and the current at 15 mA. The data was analysed using OriginPro 9.0.

The exact drug-load in UV-cured polymer films was determined by ultrasonic extraction using a Transonic T460/H sonicator (Elma, Germany). Ten milligrams of each film was placed in a glass vial. Five millilitres of ethanol was added to the film and the vial was sonicated for up to 2 h. Subsequently the solvent was analysed by HPLC (as per Section 2.4.1). This method was optimised for the stated solvent volume and extraction time.

The levels of residual monomers in the polymer film were quantified by ultrasonic extraction. Immediately after curing, one gram of each film was placed in a glass vial and three millilitres of methanol was added. The mixture was sonicated for up to 2 h. Subsequently the solvent was analysed using GC (as per Section 2.4.1 to quantify the extracted residual monomers. The optimal duration and volume of extraction solvent were determined after a series of experiments.

Thermal gravimetric analysis (TGA) was used to determine the thermal stability of the polymer films. It was conducted using the Discovery TGA Model (TA Instruments–Waters LLC, Delaware, USA). Oxygen-free nitrogen gas with a flow rate of 25 ml/min was used. The sample mass was approximately 4 mg, and each sample was heated from 25 to 500 °C, with a heating rate of 10 °C/min. Data analysis was carried out with TA Instruments TRIOS V3.1.0.3538.

Differential scanning calorimeter (DSC). The thermal properties of the polymer films were also analysed by, using the Q2000 TA Instruments (Waters LLC, Delaware, USA), equipped with TA Universal Analysis 2000 software. Oxygen-free nitrogen gas with a purge rate of 50 ml/min was used. Approximately 8 mg of sample was contained within a T-zero pan following seal with a T-zero hermetic lid. Each sample was heated from −30 °C to 250 °C with a heating rate of 10 °C/min.

Dynamic mechanical analysis (DMA) was used to study the viscoelastic behaviour of the polymer films as a function of temperature. Films with an area of 15 mm × 6.5 mm (produced after curing 13 μl of the formulation) were prepared and analysed using the Q800 Dynamic Mechanical Analyser (TA Instruments–Waters LLC, Delaware, USA). The mode was set at DMA multi-frequency-strain using a film tension clamp. The purge gas used was nitrogen, and the heating range was from room temperature to 200 °C with a heating rate of 3 °C/min. The oscillating frequency was set at 1 Hz. A preload force (0.01 N) was applied to the sample prior to the dynamic oscillating force to prevent the film buckling and the force track was maintained at 125%. The data was collected using Advantage for Q Series Version 2.8.0.394, and the data analysis was carried out with TA Instruments Universal Analysis 2000. The glass transition temperature (Tg) was calculated as the tan δ peak.

UV-cured polymer film's adhesivity. The cross-cut test (adapted from ISO 2409:2013 – paints and varnished – cross-cut test) was used to assess the resistance of the polymer films to separation from a substrate (i.e. the films adhesion to the substrate) when a right-angle lattice pattern is cut into the film, penetrating through the substrate. A single layer of the UV-curable gel formulation (1.68 ml) was cured to a 70 × 180 mm area on the smooth surface of a high density polyethylene (HDPE) sheet (RS Components, UK) and was left to age for 24 h. Prior to testing, the thickness of the film was measured using a micrometre. A cross-hatch pattern was then cut manually into the polymer film using a scalpel (blade thickness of 0.38 mm, Swann–Morton, Sheffield, UK) by applying uniform pressure at a uniform cutting rate. A total of six parallel cuts were made in the direction of gel application, followed by six perpendicular cuts to form a lattice. The cuts made were 3 mm apart as the film thickness was between 121 and 250 μm. The loose film was then removed by brushing the substrate lightly with a soft brush several times backwards and forwards and several times forwards along each of the diagonals of the lattice pattern. This cross-hatch pattern was examined visually to assess the extent to which the polymer film had been removed off the polyethylene sheet. 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 ≥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. A high score therefore reflected poorer resistance of the film to removal from the substrate (i.e. poor adhesion).

Film's sensitivity to water. The water immersion test (adapted from ASTM D870 – standard practice for testing water resistance of coatings using Water Immersion) was used to determine the resistance of the cured films to water. The formulation (140 μl) was cured to a 15 mm by 70 mm area onto the smooth side of a HDPE sheet. The latter was then placed in a distilled water bath (Grants Instruments Ltd., Cambridge, England) at room temperature (25 °C) such that half the length of the film strip was immersed in water, while the other half was outside. At timed intervals, (initially every 10 min for 1 h, then hourly for 8 h and finally every 24 h for two days), the HDPE sheets were taken out of the water bath to observe the effect of water immersion on the polymer film. The film's sensitivity/resistance to water was scored as 0 for no change in film, 1 for ≤25% blister formation, 2 for >25≤50% blister formation, 3 for >50≤75% blister formation and 4 for complete film removal. Therefore a high score indicated high sensitivity to water. The extent of blister formation was reported since other factors such as colour, were not affected.

The stability of ethanol-based drug-loaded gel formulations, as well as that of the UV-cured films was tested over 3 months under accelerated testing conditions, i.e., 40 ± 2 °C and 75% RH ± 5% RH. This was conducted by placing the formulations and films in a desiccator containing a saturated solution of sodium chloride and placing this in a 40 °C oven. At timed intervals, the formulations were assessed for (i) visual appearance, (ii) drug concentration and (iii) the quality of the film produced (in terms of the presence of drug crystals and drug concentration) over time. The incubated polymer films were observed for (i) drug crystallisation and (ii) the drug concentration. Polarised light microscopy was used to visualise the presence of crystals in the film, if any. HPLC (as per Section 2.4.1) was used to determine whether the drug content in the gels and films was stable with time.

Drug release from films was measured in order to determine the profile of drug release from the UV-cured film, and whether this influences ungual drug permeation. The formulations tested were the 3% w/v amorolfine HCl UV-curable gel formulation, a commercially available amorolfine HCl 5% w/v nail lacquer (Curanail) for comparative purposes, and the 4% w/v terbinafine HCl UV-curable gel formulation. The study was carried out using Franz diffusion cells, and the drug-loaded films were supported on a cellulose membrane. The receptor fluid was a 0.1 M phosphate buffer at pH 5 as both amorolfine HCl and terbinafine HCl are stable at this pH (Hossin, 2014). Sink conditions were maintained throughout the duration of the study.

To set up the Franz cell, a dialysis tubing cellulose membrane was cut into a circle with an area of 4.909 cm^2. A test formulation was then applied onto the surface of the cellulose membrane, covering a circular area of 0.9503 cm^2. This was allowed to dry, in the case of Curanail. For the UV-curable gel formulations, the cellulose membrane was placed under a UVA lamp and the formulation was cured for 2 min. The surface of the film produced was then wiped with propan-2-ol using a nail wipe. The cellulose support with the UV-cured or air-dried Curanail film was then placed between the donor and receptor compartments of a Franz diffusion cell, with the test films facing the donor side, and the compartments were clamped together. Subsequently, four ml of receptor fluid was added to the receptor compartment while ensuring that no air bubbles were introduced. The diffusion cells were left to stir on a magnetic stirrer placed in a water bath maintained at 32 °C. Samples of the receptor fluid were collected at pre-determined time intervals for 30 days. Half ml was collected via the receptor arm and replaced with 0.5 ml of fresh buffer at each sampling point. The samples were analysed by HPLC (as per Section 2.4.1) to determine the amount of drug released, and the cumulative% drug release over time was plotted. The UV-cured and Curanail films were also observed by polarised microscopy prior to the release study and at day 30 to determine if there was any drug crystallisation and precipitation out of the film during the release study.

In vitro drug permeation study was carried out in order to determine whether the polymer films produced show potential as topical ungual drug carriers. The formulations tested were the 3% w/v amorolfine HCl UV-curable gel formulation, the amorolfine HCl 5% w/v nail lacquer (Curanail) and the 4% w/v terbinafine HCl UV-curable gel formulation. The study was carried out using a modified Franz diffusion cell. Human nail clippings (fingernails) were obtained from healthy volunteers aged between 18 and 65 years, washed with water and soaked in distilled water for one hour prior to use. They were then cut to size (circular, with a diameter of 0.3 cm) and measured for thickness using a micrometre. A layer of the formulation (using 2 μl) was applied on the nail surface with a pipette tip and allowed to dry in the case of Curanail. For the UV-curable gel formulations, the formulation (2 μl) was applied on the nail surface and cured under an UVA lamp for 2 min. The surface of the film produced was then wiped with propan-2-ol using a nail wipe. The nail was placed in the donor compartment and fixed into place, such that when assembled with the receptor compartment, the under-surface of the nail alone was exposed to the receptor fluid. The area exposed was 0.025 cm^2. Subsequently, 900 μl of receptor fluid was added to the receptor compartment. The receptor fluid was 0.1 M phosphate buffer pH 5. The diffusion cell was assembled, covered with parafilm, and left to stir on a magnetic stirrer placed in a water bath at 32 °C. Samples of the receptor fluid were collected over 30 days by taking 50 μl of receptor fluid for analysis via the receptor arm and replacing it with 50 μl of fresh buffer. The samples were analysed by HPLC (as per Section 2.4.1) to determine the amount of drug permeated across the nail over time. Each experiment was repeated six times.

At the end of the permeation study, the nail clipping was removed from the donor compartment using forceps. The film on the nail surface was carefully lifted off the nail using a scalpel and placed in a vial containing 1 ml of ethanol. The mixture was ultrasonicated for 2 h and the solvent was analysed by HPLC to quantify the amount of drug remaining in the donor compartment. The nail plate was rinsed with distilled water and blotted dry with Kimwipes, before placing in a vial containing 1 ml of ethanol. This was ultrasonicated for 2 h and the solvent was analysed with HPLC while the nail clipping retrieved was placed in another vial containing 1 ml of ethanol for a further two hour sonication. This extraction procedure was repeated until no further drug was extracted. The total amount of drug extracted from the nail was then calculated.

Table 1
HPLC method for the quantification of amorolfine HCl and terbinafine HCl in samples.

Drug	Mobile Phase	Flow rate (ml/min)	Sample injection vol. (μl)	Wavelength (nm)	Retention time (min)
Amorolfine HCl	0.1% trifluoroacetic acid:acetonitrile (55:45 v/v)	1.0	20.0	220	5.8
Terbinafine HCl	0.012 M triethylamine + 0.020 M phosphoric acid:acetonitrile (65:35 v/v)	1.0	20.0	224	8.8

2.4. Analytical methods

2.4.1. High-performance liquid chromatography (HPLC)

The amount of amorolfine HCl and terbinafine HCl in samples was quantified by using a 1260 Infinity Agilent HPLC system equipped with an autosampler and a variable wavelength absorbance detector (Agilent Technologies, Germany). Elution was performed using a Luna C18 column (150 × 4.6 mm, 5 μm) at a temperature of 40 °C. Table 1 shows the HPLC method developed, which was validated for linearity, precision and accuracy (Hossin, 2014).

2.4.2. Gas chromatography (GC)

GC was conducted to determine the amount of diurethane dimethacrylate and ethyl methacrylate in samples using a 7890A GC System (Agilent, USA) equipped with a flame ionisation detector (FID) system. Chromatographic separation was achieved on a HP-5 column (30 m long × 320 μm inside diameter with 0.25 μm film thickness). The sample injection volume was 2 μl. The injector was in the split mode (100:1) and its temperature was maintained at 250 °C throughout the experiments. The column temperature was raised from 45 °C (hold 2 min) to 110 °C (hold 2 min) at a 10 °C/min heating rate to 280 °C (hold 2 min) at a 20 °C/min heating rate. The flow rate of carrier gas (N_2) was 1.5 ml/min. The detection was carried out by the FID with the temperature of 280 °C and the ratio of H_2/air at 25/250. Diurethane dimethacrylate and ethyl methacrylate were detected at 16.5 and 6.5 min respectively. The method developed was validated for linearity, precision and accuracy.

2.5. Statistical analyses

All the experiments described above were repeated three times (except for the in vitro drug permeation study which was repeated six times). Statistical calculations were conducted using SPSS 22. The two-sample *t*-test was carried out for testing significant differences in two data sets and the one-way analysis of variance (ANOVA) and post hoc Tukey were carried out for all statistical analyses involving more than two data sets. Repeated measures ANOVA and post hoc Tukey test were carried out to identify any statistically significant differences in release and permeation profiles of the antifungals from the polymer films over time.

3. Results and discussion

3.1. Components of the UV-curable gel formulations

A number of monomers and photoinitiators that are used in the cosmetic industry were considered and finally, diurethane dimethacrylate, ethyl methacrylate and 2-hydroxy-2-methylpropiophenone were chosen. Diurethane dimethacrylate was used as the backbone of the formulation due to its ability to produce a strong cross-linked polymer which imparts exceptional abrasion resistance and adhesion, and also due to its clarity, viscosity and inherent resistance to discoloration (Murray, 2012). Ethyl methacrylate was chosen for its ability to enhance film flexibility and as its safety has been extensively assessed and been found to be favourable (Panel, 2002). 2-Hydroxy-2-methylpropiophenone was chosen as the photoinitiator as it is capable of forming hard films, is useful for non-yellowing applications, and has good solvency properties (Segurola et al., 1999).

The drugs amorolfine HCl and terbinafine HCl were selected for their high potencies against onychomycosis-causing dermatophytes. Unfortunately the solubility of these drugs in the gel components was negligible. Both drugs were insoluble in diurethane dimethacrylate, while, in ethyl methacrylate, amorolfine HCl dissolved at 0.10 ± 0.01 mg/ml and terbinfine HCl dissolved at 0.66 ± 0.19 mg/ml at 25 °C. This poor drug solubility necessitated the inclusion of a solvent in the system to achieve a reasonable drug load. Two solvents – ethanol and NMP – were selected for their miscibility with the monomer blend and their solvency for the drugs. Ethanol dissolves amorolfine HCl at 119.5 ± 5.4 mg/ml and terbinafine HCl at 141.30 ± 1.7 mg/ml at 25 °C, while NMP dissolves amorolfine HCl at 56.2 ± 2.1 mg/ml and terbinafine HCl at 106.6 ± 1.3 mg/ml at 25 °C.

3.2. Proportions of the different gel components

The proportions of diurethane dimethacrylate, ethyl methacrylate and 2-hydroxy-2-methylpropiophenone were similar to those in cosmetic UV gels, with the ratio of diurethane dimethacrylate to ethyl methacrylate kept at 85:15% v/v and the photoinitiator concentration set at 3% v/v of the mixture of monomers and photoinitiator.

The amount of drug that could be loaded in the gel depended on the amount of solvent (ethanol/NMP) that could be incorporated. Formulations containing ethanol at concentrations 25–50% v/v produced films that were thinner and had a lower percentage mass conversion from monomer to polymer as the solvent concentration increased. Ethanol inclusion in the gel also increased the water-sensitivity of the UV-cured films (tested as described in Section 2.3) in a concentration dependent manner (Fig. 1). As the water-resistance of a nail preparation is critical to its residence on the nail (Murdan et al., 2015), a solvent concentration of 25% v/v was chosen as a compromise between the ability of the formulation to contain sufficient drug and to be water-resistant.

A high drug loading in a nail medicine is desired such that drug movement out of the preparation and into the nail along the thermodynamic activity is favoured. It was found that inclusion of ethanol (at 25% v/v) in a gel formulation allowed the loading of 3% w/v amorolfine HCl or 4% w/v terbinafine HCl. Meanwhile, inclusion of NMP, also at 25% v/v, allowed the inclusion of 1% w/v of amorolfine HCl or 1% w/v of terbinafine HCl. The lower drug load enabled by NMP reflects its lower solvency for the drugs as detailed in Section 3.1.

3.3. Preparation of UV-cured film

Drug-free gel formulations were prepared by mixing the two monomers and the photoinitiator, and leaving the mixture to stir overnight, which produced a clear homogenous solution. Drug-loaded formulations were prepared by first dissolving the drug in the chosen solvent and then adding the two monomers and photoinitiator to this drug solution and leaving the mixture to stir overnight. This also produced a clear homogenous solution.

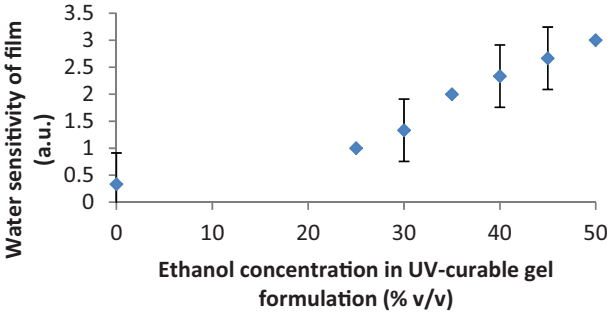

Fig. 1. Water sensitivity (at 48 h incubation) of films produced by UV-curable gel formulations containing between 0 and 50% v/v ethanol. Mean ± standard deviation are shown, $n = 3$. Where no error bars are shown, the standard deviation was zero.

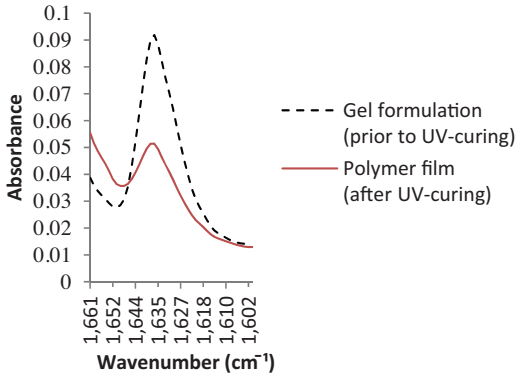

Fig. 2. FT-IR spectra of solvent-free and drug-free monomer formulation and the resulting polymer film after UV-curing.

Table 2
Percentage degree of conversion (DC) from monomers to polymer. Means ± standard deviation are shown, $n = 3$.

Formulation	DC (%)
Solvent-free and drug-free gel	51.3 ± 4.5
Drug-free gel containing ethanol	61.3 ± 4.5
Gel containing ethanol and 3% w/v Amorolfine HCl	59.5 ± 3.6
Gel containing ethanol and 4% w/v Terbinafine HCl	62.7 ± 3.4
Drug-free gel containing NMP	63.3 ± 4.7
Gel containing NMP and 1% w/v Amorolfine HCl	60.2 ± 5.6
Gel containing NMP and 1% w/v Terbinafine HCl	59.7 ± 5.0

To prepare a UV-cured film, the gel formulation was applied on a glass slide and exposed to a UVA lamp. Exposure of the gel formulation to UVA light initiated polymerisation to form a diurethane dimethacrylate/ethyl methacrylate copolymer, where the original C=C alkene bonds in the acrylate moieties of the monomers were converted to alkane ones (See Supplementary data for proposed reaction pathway). Polymerisation and the conversion of C=C to saturated bonds was confirmed by FT-IR (Fig. 2), where the C=C stretching related absorption band at 1636 cm^{-1} were much weaker upon UV-curing.

The existence of a small C=C peak in the UV-cured film (Fig. 2) indicates that conversion of the monomers to the polymer was not complete. This is due to the fact that UV-curing was conducted under ambient conditions. Atmospheric oxygen is known to inhibit polymerisation; thus as unreacted layer – called the oxygen inhibition layer (Draelos, 2010; Schoon, 2005) – is formed on the surface of the polymer film. As practised in the cosmetic industry, this unpolymerised layer was removed by wiping the film surface with a super absorbent 4 ply lint-free nail wipe soaked in propan-2-ol. Wiping the surface resulted in the removal of 10–15% of the mass of the film first formed upon UV exposure.

Table 4
Thickness of UV-cured polymer films. Means ± standard deviation are shown, $n = 3$.

Formulation	Film thickness (μm)
Solvent-free and drug-free gel	211.1 ± 6.0
Drug-free gel containing ethanol	161.1 ± 7.8
Gel containing ethanol and 3% w/v Amorolfine HCl	163.3 ± 8.7
Gel containing ethanol and 4% w/v Terbinafine HCl	164.4 ± 7.3
Drug-free gel containing NMP	165.6 ± 7.3
Gel containing NMP and 1% w/v Amorolfine HCl	167.8 ± 8.3
Gel containing NMP and 1% w/v Terbinafine HCl	166.7 ± 7.1

3.4. Degree of conversion and amount of residual monomers in cured polymer film

Following the removal of the oxygen inhibition layer, the polymer film was re-examined by FT-IR to measure the extent of polymerisation within the bulk of the film. The% degree of conversion – using the change in alkene group peak – was found to be between 50 and 63% (Table 2). Such a degree of conversion reflects values (43–73%) reported for photo-activated dental composites (Halvorson et al., 2003). The presence of a solvent or of a drug did not cause any significant changes in the percentage degree of conversion.

To determine whether the less than 100% degree of conversion shown in Table 2 was due to the presence of unreacted monomers or the presence of unreacted groups within reacted monomers, the level of monomers in polymer films was quantified following their extraction. The amount of diurethane dimethacrylate and ethyl methacrylate monomers in the polymer films were found to be extremely low, especially in films produced from solvent-containing gels (Table 3). This low level indicates that the ≈60% degree of conversion shown in Table 2 is due to the presence of unreacted groups within the polymer, rather than unreacted monomers.

It seems that a significant proportion of the methacrylate groups of the diurethane dimethacrylate monomer are not involved in the polymerisation reaction. This could be due to restricted mobility of some of the side chains in the viscous polymerising mixture. The presence of solvent in the formulation resulted in a small increase, (although this was not statistically significant), in the degree of conversion (Table 2) and a significant decrease (ANOVA, $p < 0.05$) in the amount of unreacted monomers (Table 3). It seems that the solvent increased polymerisation, possibly due to a reduction in the viscosity of the polymerising mixture (from 0.4 Pas for solvent-free formulations to 0.02 Pas for ethanol- and NMP-containing formulations) and a subsequent increase in the mobility of the chains.

The negligible amounts of monomers in drug-containing UV-cured films (Table 3) show that such UV-curable formulations can be used as topical nail medicines, especially if a formulation-free margin is left around the nail to avoid contact with the skin, as the monomers (but not the polymer) have the potential of causing allergic contact dermatitis (Zondlo Fiume, 2002).

Table 3
Concentration of residual monomers in the UV-cured polymer films. Mean levels of monomers in film ± standard deviation are shown, $n = 3$.

Formulation	Diurethane dimethacrylate (% w/w)	Ethyl methacrylate (% w/w)
Solvent-free and drug-free gel	2.9 ± 0.04	0.1 ± 0.002
Drug-free gel containing ethanol	0.7 ± 0.03	0.003 ± 0.0001
Gel containing ethanol and 3% w/v Amorolfine HCl	0.7 ± 0.03	0.003 ± 0.00008
Gel containing ethanol and 4% w/v Terbinafine HCl	0.7 ± 0.03	0.005 ± 0.002
Drug-free gel containing NMP	0.4 ± 0.009	0.003 ± 0.0001
Gel containing NMP and 1% w/v Amorolfine HCl	0.4 ± 0.01	0.003 ± 0.00008
Gel containing NMP and 1% w/v Terbinafine HCl	0.3 ± 0.008	0.002 ± 0.00001

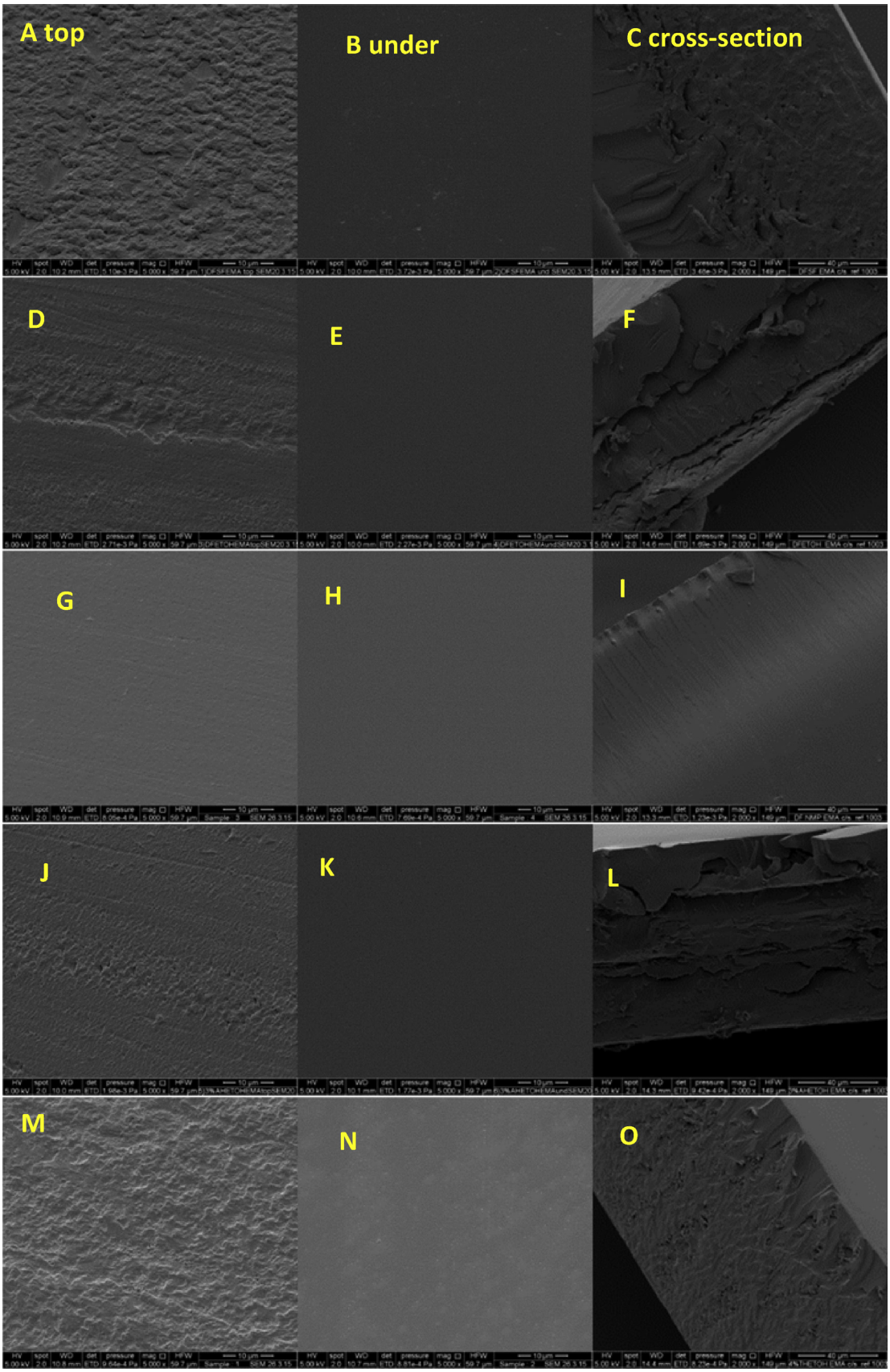

Fig. 3. Scanning electron micrographs of: drug-free and solvent-free film (A–C); drug-free film with ethanol (D–F); drug-free film with NMP (G–I); film with ethanol and amorolfine (J–L); film with ethanol and terbinafine (M–O). A, D, G, J, M – top surfaces (i.e. exposed to UV light); B, E, H, K, N – under surfaces (i.e. in contact with the support); C, F, I, L, O – cross-sectional surfaces.

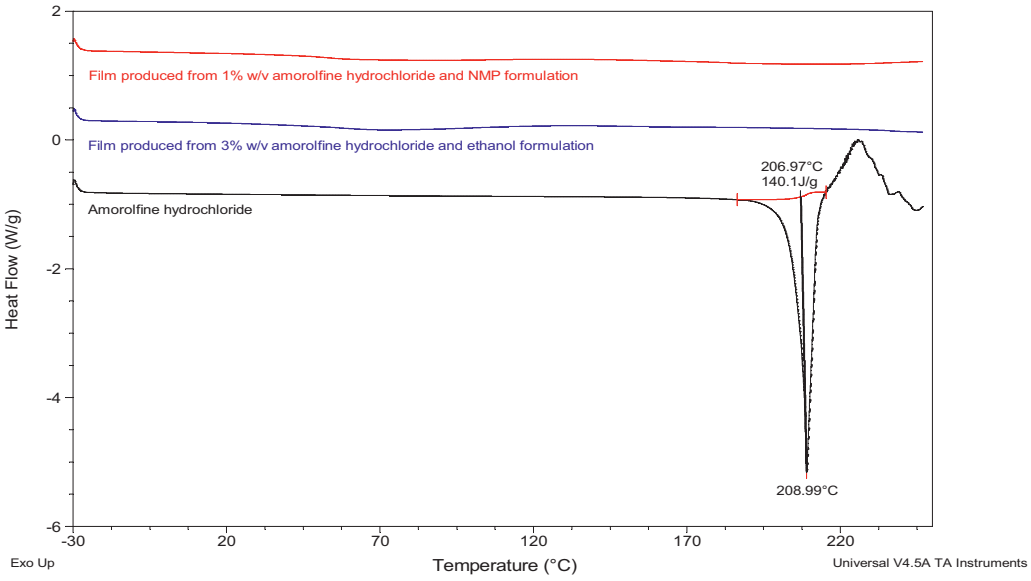

Fig. 4. DSC curves of amorolfine HCl and of amorolfine HCl loaded diurethane dimethacrylate/ ethyl methacrylate copolymer films prepared from formulations containing ethanol or NMP.

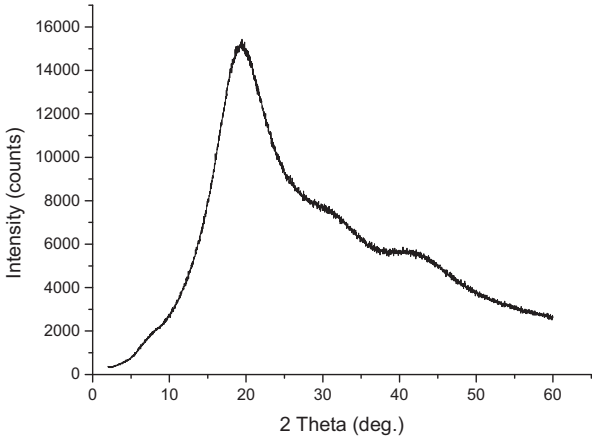

Fig. 5. Polarised light micrograph and corresponding XRD pattern of amorolfine HCl and ethanol loaded film at the concentration at which it is saturated.

3.5. Morphology, thickness and microstructure of UV-cured films

Drug-free, solvent-free UV-cured films were transparent, visually smooth and about 200 μm thick (Table 4). Films produced when formulations contained a solvent were significantly ($p < 0.05$) thinner at about 165 μm due to the lower monomer content in the mixture (Table 4).

Scanning electron microscopy (Fig. 3A–O) of UV-cured films with/without solvent and with/without drug revealed a generally rough surface of the side that was exposed to UV light and a much smoother surface where the film had been formed in contact with the support. The latter provides a certain containment for the flow of the fluid during polymerisation and the smooth film surface reflects the smooth surface of the support. The cross-sectional views show a fairly dense film interior. Inclusion of the solvent ethanol in the gel formulation seems to have no influence on the film microstructure, while inclusion of NMP results in films with smoother top and cross-sectional surfaces. NMP seems to influence polymerisation to a greater extent than ethanol, probably due to its higher boiling point of 204 °C (vs 78 °C for ethanol). While much of the ethanol originally present in the gel formulation is expected to evaporate off during UV curing, NMP is likely to remain in the film and influence the polymer formed. To investigate this further, UV-cured films were stored under ambient conditions (22 ± 2 °C and 50 ± 5% RH) and weighed over time. While the films formed from solvent-free formulations showed negligible change in mass over 28 days, those formed with ethanol-containing formulations lost 1 ± 0.4%, 3 ± 1% and 3.5 ± 0.8% of their mass at 24 h, 14 days and 28 days respectively, and those formed with NMP-containing formulations lost 7±0.1%, 11±0.1% and 12±0.04% of their mass at the same respective times. The differences in mass loss were statistically significant ($p < 0.05$). The mass loss is thought to be due to solvent loss from the film and shows that more NMP (compared to ethanol) remains in the film upon UV curing.

Inclusion of the drug amorolfine HCl or terbinafine HCl had no influence on film thickness ($p > 0.05$) or on its microstructure. The amount of drug included in the formulation was below the saturation limit, i.e. the drugs were molecularly dispersed in the formulation, and was fairly low. It seems that such a low drug load caused no visible changes in the film's microstructure.

Table 5
Mass yield and drug yield of formulations after UV-curing and removal of oxygen inhibition layer. Means ± standard deviation are shown, $n = 3$. NA = not applicable.

Solvent in formulation	Drug-free formulation	Film containing amorolfine HCl		Film containing terbinafine HCl	
	Mass yield	Mass yield	Drug yield	Mass yield	Drug yield
None	90.7 ± 0.3	NA	NA	NA	NA
Ethanol	85.1 ± 1.6	85.9 ± 1.9	87.1 ± 1.3	86.5 ± 1.7	87.1 ± 0.9
NMP	85.8 ± 1.3	85.9 ± 0.5	87.2 ± 2.2	85.5 ± 1.5	87.1 ± 2.7

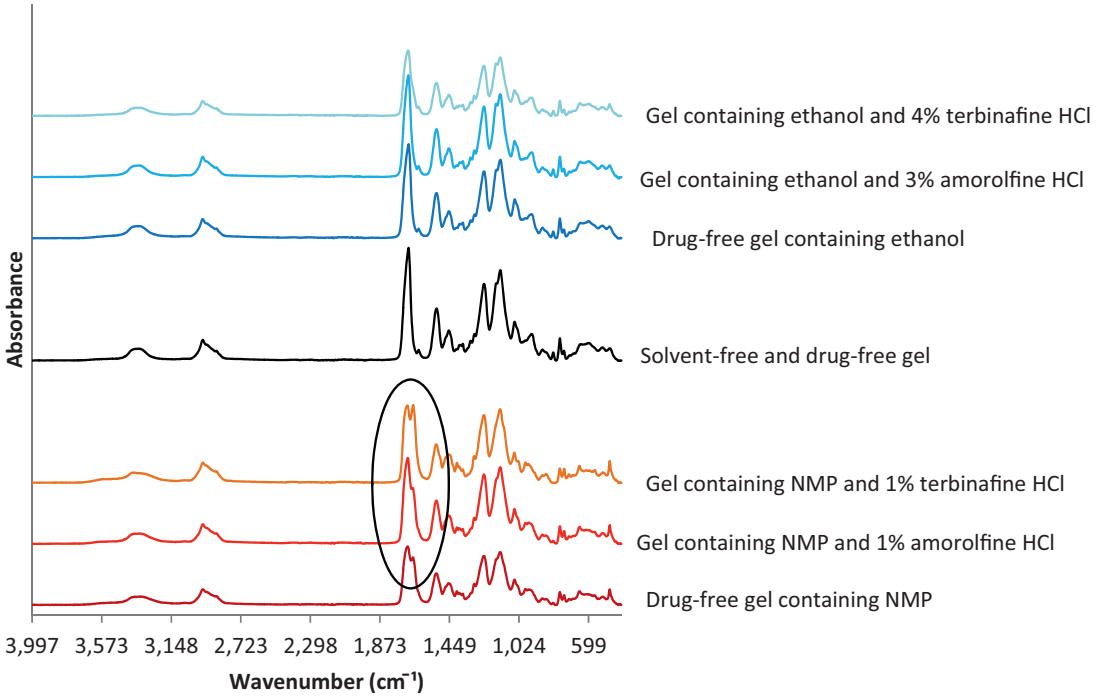

Fig. 6. FT-IR spectra of films produced from the UV-curable gels (±solvent and ±drug). The absorption bands circled relate to the C=O stretch of NMP's five ring lactam.

3.6. Drug load in UV-cured films

As previously mentioned, ethanol-containing gels (prior to curing) could incorporate a maximum of 3% w/v amorolfine HCl or 4% w/v terbinafine HCl, while NMP-containing gels could contain 1% w/v of either amorolfine HCl or terbinafine HCl in the dissolved form. Upon UV-curing, the drug remained in the dissolved state and no crystals were observed by polarised light microscopy. The absence of drug crystals in the UV-cured film was confirmed by DSC (which showed an absence of endothermic peaks related to the drugs' melting point), polarised light microscopy and by XRD which also showed the films (both drug-loaded and control) to be amorphous (Figs. 4–5 for amorolfine). The DSC and XRD curves and polarised light micrograph for terbinafine-loaded films were comparable to those in Figs. 4–5, and are not shown.

The UV-cured polymer films were expected to contain a lower drug load compared to the uncured gel, due to the removal of the oxygen-inhibition layer following curing. This was indeed the case. Amorolfine was present at 2.6% (vs 3% prior to curing) and at 0.9% (vs 1% prior to curing) in films produced from gels containing ethanol and NMP respectively,

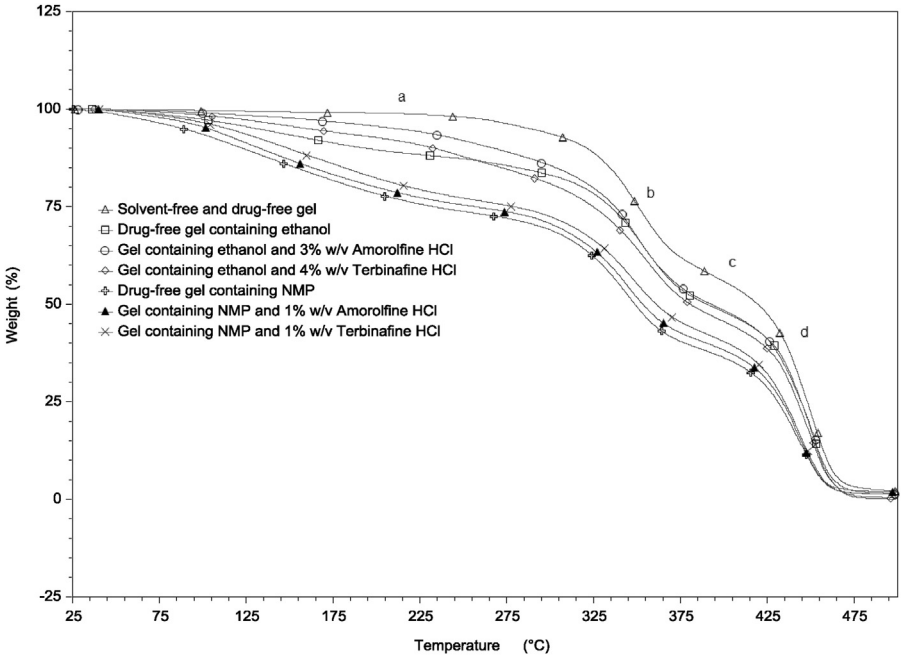

Fig. 7. TGA profiles of UV-cured polymer films produced from gels (±solvent and drug).

Table 6
Tg values of UV-cured polymer films.

Formulation	Tg (°C)
Solvent-free and drug-free gel	85.7 ± 1.2 and 146.0 ± 2.5
Drug-free gel containing ethanol	112.5 ± 1.8
Gel containing ethanol and 3% w/v Amorolfine HCl	109.2 ± 1.1
Gel containing ethanol and 4% w/v Terbinafine HCl	109.6 ± 0.8
Drug-free gel containing NMP	95.1 ± 1.5
Gel containing NMP and 1% w/v Amorolfine HCl	94.8 ± 2.0
Gel containing NMP and 1% w/v Terbinafine HCl	95.0 ± 1.8

while terbinafine was present at 3.5% (vs 4% prior to curing) and 0.9 % (vs 1% prior to curing) in the corresponding films. The percentage drug loss reflects the percentage polymer film loss upon removal of the oxygen inhibition layer (Table 5). The fact that the film and drug losses are almost the same indicates that there was no drug migration to the film surface or interior during UV curing.

The FT-IR spectra of the drug-free and drug-loaded polymer films showed no shifts in the characteristic bands (Fig. 6), indicating the absence of drug-polymer interactions in the film. This bodes well for drug release from film and ungual drug permeation.

Interestingly, the FT-IR spectra of the films produced from the NMP containing formulations show an absorption band between 1670 and 1710 cm^{-1} relating to the C=O stretch of NMP's five ring lactam, suggesting that NMP becomes incorporated into the polymer upon curing. Presence of NMP in the film could be due to its high boiling point (204.3 °C) such that the solvent does not evaporate off upon curing.

3.7. Thermal properties of films

The thermal properties of the films were determined using TGA and DMA and the influence (if any) of solvent and drug incorporation on the film's glass transition temperature and degradation were evaluated.

The TGA curves for the polymer films (with and without solvent and drug) are shown in Fig. 7. The profiles seem to consist of four phases, a–d, and are similar to those reported by Kunwong et al. (2011) for UV-cured coatings based on urethane acrylate oligomers. The first phase relates to the loss of volatile materials from the film as the latter is heated. Films produced from solvent-containing formulations showed a greater loss in mass than the control drug-free, solvent-free film during phase a ($p < 0.05$). This indicates that some of the solvent which remained in the film upon UV-curing was being lost as the film was heated. NMP-containing films showed a greater mass loss than ethanol-containing ones and a transition at around 200 °C. This is linked to the greater amount of residual solvent in these films (as discussed in Section 3.5) and to NMP's boiling point of 204 °C. Phases b–d in the profiles are related to polymer decomposition via the degradation of polymer side-chains, followed by scission and depolymerisation, and finally complete de-crosslinking and thermal degradation, as suggested for similar films by other researchers (Chattopadhyay and Webster, 2009).

Dynamic Mechanical Analysis (DMA) Tg values are shown in Table 6. The DMA curves for the solvent-free films showed two transitions at 86 °C and at 146 °C. This indicates two types of polymer chains/ areas in the film, with one containing more cross-links (and hence showing a higher Tg) than the other. The greater cross-linking would have occurred when both methacrylate groups on the diurethane dimethacrylate monomer would have

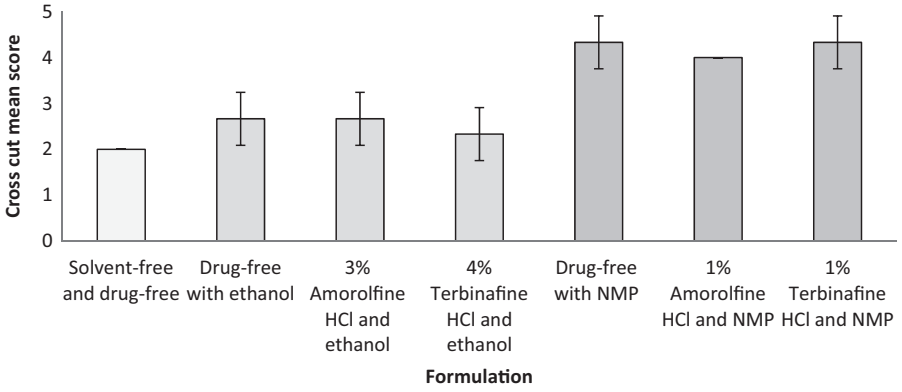

Fig. 8. Cross-cut scores for films produced from gels (±solvent and drug). Means ± standard deviation are shown, $n = 3$. Where there are no error bars, standard deviation is zero.

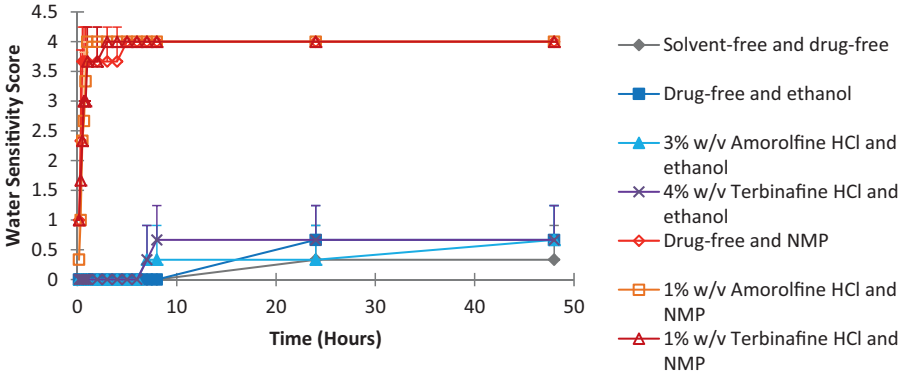

Fig. 9. Water sensitivity score of UV-cured films (±solvent and drug). Means ± standard deviation are shown, $n = 3$.

reacted during polymerisation. Inclusion of ethanol or NMP in the gel resulted in films with one Tg only – which was around the mid-point of the two values seen in the solvent-free films. It was mentioned earlier that inclusion of a solvent considerably decreased the viscosity of the gel. This could have resulted in greater mobility of the reactants and consequently a more homogenous polymerisation process and thereby a more homogenous type of cross-linked polymer film with one Tg. Interestingly, films formed from NMP-containing gels have lower (paired *t*-test, $p < 0.05$) Tg values than ethanol-containing ones. The greater amount of residual solvent in the NMP-based film (as discussed in Section 3.5 and evidenced in Fig. 7) seems to result in a plasticising effect on the UV-cured polymer film, such that Tg is lowered. In contrast, the presence of the drugs in the films does not cause any significant changes in the film Tg, possibly due to their low concentrations.

3.8. Film adhesion to substrate

The mean cross-cut score of the polymer films is shown in Fig. 8. A high score means lower adhesion (or resistance to separation from a substrate). The greatest adhesion was shown by the films produced from the drug-free, solvent-free formulation. Presence of ethanol (with and without antifungal drugs) in the formulation caused no statistically significant change in film adhesion ($p > 0.05$). In contrast, presence of NMP (with and without antifungal drugs) significantly reduced the adhesivity ($p < 0.05$). As discussed earlier, more NMP (than ethanol) remains in the film upon gel curing. The presence of a greater amount of solvent causes a larger change in the properties of the NMP-containing films resulting in a significant decrease in film adhesion to the substrate. The incorporation of either amorolfine HCl or terbinafine HCl in the formulation did not affect the films cross-cut score ($p > 0.05$); possibly due to the small percentage of drug in the film.

3.9. Film sensitivity to water

The water sensitivity of the UV-cured films are shown in Fig. 9. It can be seen that films produced by the NMP-containing formulations are extremely water sensitive; by 5 h the films had detached from the substrate. NMP is miscible in water (Jouyban et al., 2010) and as it is present at a considerable level in the film, contact with water increases the latter's water sensitivity. In contrast, solvent-free and drug-free films had the lowest water sensitivity. This was expected as urethane methacrylate based films are known for water resistance. Inclusion of ethanol caused no significant change ($p < 0.05$) in water sensitivity. Like NMP, ethanol is miscible in water and therefore one could have expected the water sensitivity of ethanol-and NMP-containing films to be comparable. A possible explanation for the greater water sensitivity displayed by NMP containing films is that the large planar nonpolar region of NMP intercalates among polymer chains forming hydrophobic interactions with the polymer, whereas most of the ethanol evaporates off during and after curing due to its lower boiling point. The incorporation of either amorolfine HCl or terbinafine HCl in the formulation did not affect the films' water sensitivity, possibly due to the very low drug-load. A low water sensitivity of films produced from ethanol and drug containing gels indicates that these could be used as long-term topical nail medicines, water sensitivity of films being a good predictor of their in vivo residence (Murdan et al., 2015). The high water sensitivity of NMP-containing films means that these films would not remain on the nail in vivo for prolonged periods. These films were therefore not included in the work described in the following sections.

3.10. Stability

Both the gel formulations and the UV-cured films were stored under accelerated stability conditions and assessed for any changes

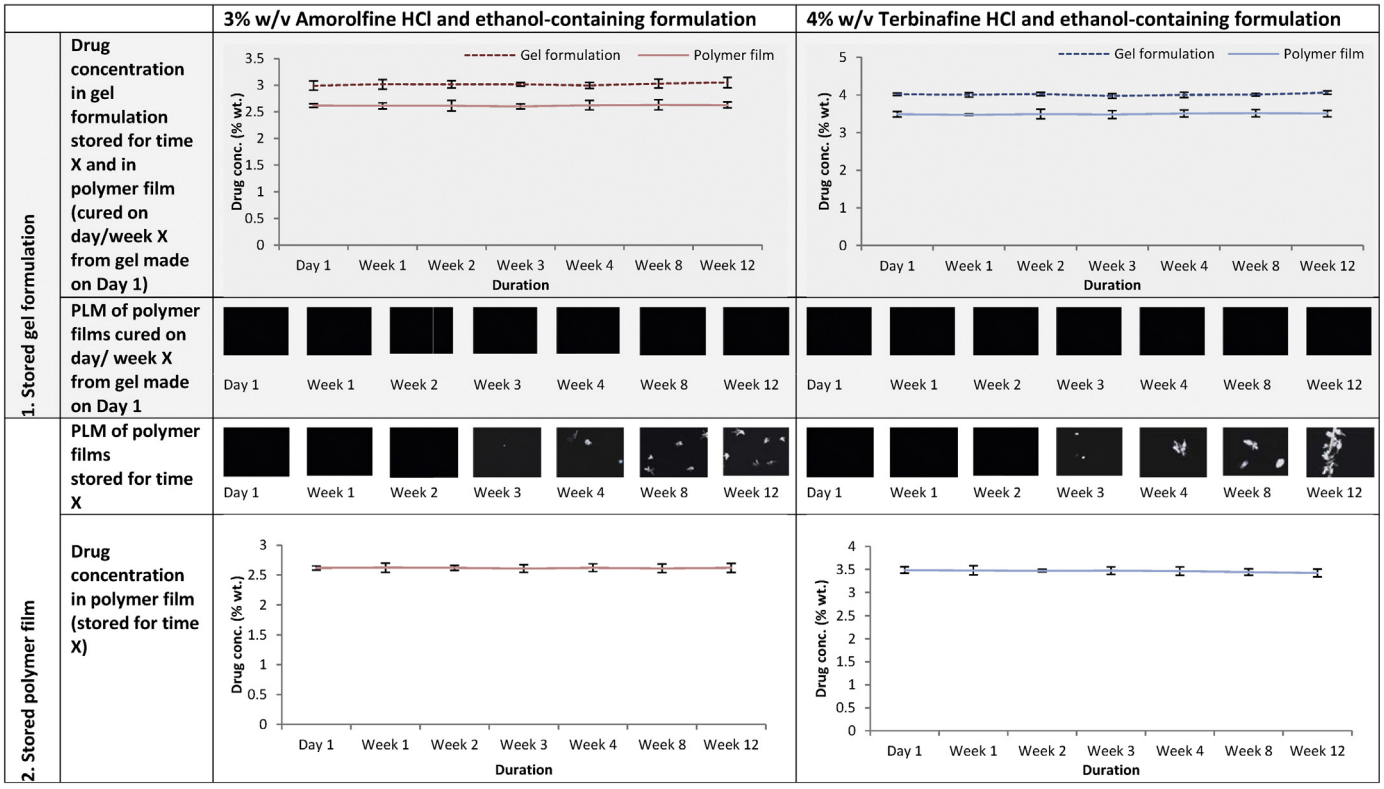

Fig. 10. Stability of stored: (1) Gel formulation – drug concentration and the quality of the film produced (in terms of drug concentration and the presence of drug crystals) over time and (2) Polymer film – drug crystallisation and drug concentration over time. Mean ± standard deviation are shown for drug concentrations, $n = 3$.

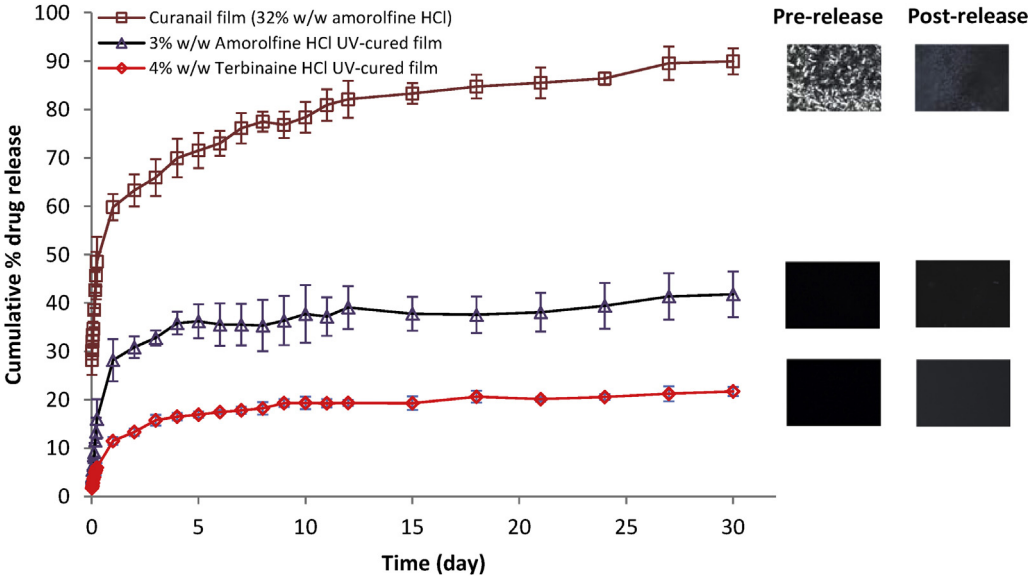

Fig. 11. Cumulative% drug release from the UV-cured films and Curanail. Means ± standard deviation are shown, $n = 3$. Polarised light microscopy images (pre- and post-release) are shown alongside their corresponding release profiles.

in their appearance and drug concentration over time. In addition, the gel formulations were also tested at time intervals during storage for the quality of the films produced upon UV-curing, in terms of drug state (amorphous/crystalline) and load. Over a period of three months the drug-loaded gel formulations showed no changes in colour (not shown), consistently produced polymer films that were free of drug crystals and showed no significant changes ($p > 0.05$) in drug concentration in the gel as well as in the polymer films produced (Fig. 10). The UV-curable gel formulations are therefore stable for three months. In contrast, stored polymer films show a change in the state of the drug, drug crystals being visible from week 3 (Fig. 10). Drug precipitation in the films could be due to the loss of residual ethanol solvent from the polymer film over time.

3.11. Drug release

The drug release profiles from the UV-cured polymer films are shown in Fig. 11, along with that of Curanail. The latter is a commercially available nail lacquer containing amorolfine HCl at 5% w/v. Upon evaporation of the solvent, a polymer film containing drug at 31.5 ± 0.8% w/w is formed (measured by dissolving the Curanail film in ethanol and quantifying by HPLC). This film was used as a comparison in these release studies.

A burst of drug release from all three films was found in the first 24 h, due to release of drug found at the surface of the films. Subsequently, drug release slowed and plateaued by day 10. Curanail film showed almost complete drug release, possibly due to its high water-sensitivity (Murdan et al., 2015) such that the film largely dissolved during the experiment and released the drug. In contrast, UV-cured films only released part of the incorporated drug, with terbinafine release being significantly less than that of amorolfine ($p < 0.05$). The relatively low drug release from UV-cured films indicates a high affinity of the drugs for the UV-cured films. It is also possible that the UV-cured films are highly cross-linked, and that the pore sizes are such that diffusion of the drug molecules through the film is limited. It is unclear why terbinafine release is almost half that of amorolfine; the two drugs

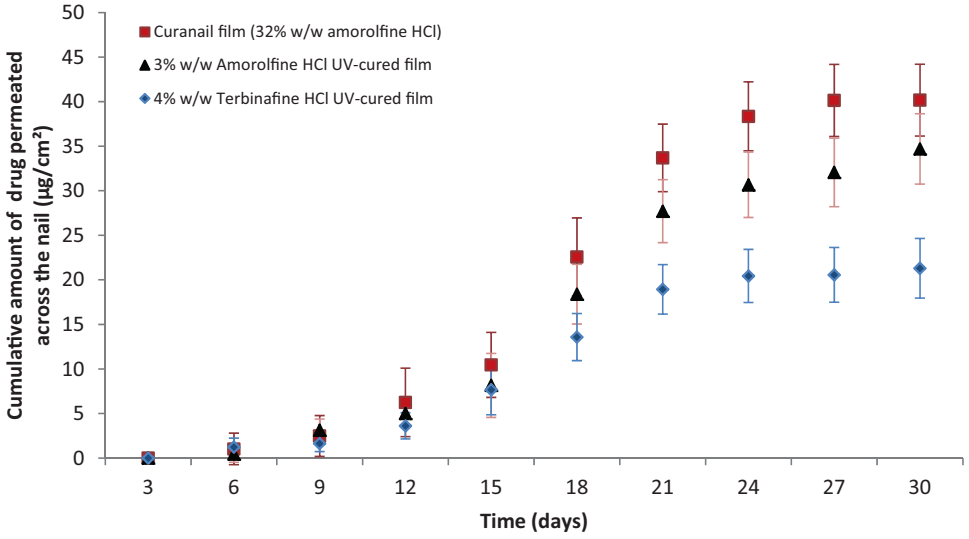

Fig. 12. Cumulative amount of drug permeated across the nail from the UV-cured and Curanail films. Mean ± standard deviation are shown, $n = 6$.

Table 7
Lag time, steady-state flux, permeability coefficient, diffusion coefficient and amount of drug in nail clippings. Means ± standard deviation are shown, $n = 6$. Statistical differences were determined using the *t*-test.

Formulation	Lag time (day)	Steady-state flux ($\mu g/cm^2$/day)	Permeability coefficient$\times 10^{-5}$ (cm/day)	Diffusion coefficient $\times 10^{-5}$ (cm^2/day)	Drug in nail clipping ($\mu g/cm^2$)
Curanail film (32% wt. Amorolfine HCl)	10.4 ± 1.3	2.9 ± 0.2	5.8 ± 0.3	2.5 ± 0.5	61.1 ± 14.8
3% wt. Amorolfine HCl UV-cured film	10.4 ± 0.9	2.4 ± 0.2	7.9 ± 0.7	2.7 ± 0.8	37.5 ± 17.6
4% wt. Terbinafine HCl UV-cured film	9.5 ± 1.2	1.5 ± 0.1	3.7 ± 0.3	1.6 ± 0.4	71.1 ± 8.0
Statistical difference between Curanail and amorolfine UV-cured film?	No ($p > 0.05$)	Yes ($p < 0.05$)	Yes ($p < 0.05$)	No ($p > 0.05$)	Yes ($p < 0.05$)
Statistical difference between amorolfine and terbinafine UV-cured film?	No ($p > 0.05$)	Yes ($p < 0.05$)	Yes ($p < 0.05$)	Yes ($p < 0.05$)	Yes ($p < 0.05$)

Table 8
Mass balance in permeation experiment at day 30. Means ± standard deviations are shown, $n = 6$.

Formulation	Percentage drug recovered			
	Receptor (i.e. permeated)	Nail clipping	Donor (i.e. film remaining on the nail plate surface)	Total
Curanail film	1.0 ± 0.1	1.6 ± 0.4	96.9 ± 1.4	99.5 ± 1.3
UV-cured film with amorolfine	1.7 ± 0.2	1.8 ± 0.9	96.4 ± 1.4	99.9 ± 1.4
UV-cured film with terbinafine	0.8 ± 0.1	2.6 ± 0.3	96.4 ± 1.1	99.8 ± 1.1

have similar logP values (5.8 and 5.3 for amorolfine HCl and terbinafine HCl respectively) and molecular weights (353.97 and 327.89 Da for amorolfine HCl and terbinafine HCl respectively), and sink conditions were maintained for both drugs during the study. A low drug release could occur if terbinafine was incorporated in the polymer structure upon UV-curing. Terbinafine does have the potential to take part in the polymerisation process due to its alkene group. However we do not think this occurred as most of the drug included in the gel mixture could be extracted from the UV-cured film (just as amorolfine HCl could be). Polarised light micrographs of the UV-cured films (pre- and post-release study) are similar for the two drugs and show no obvious change during the release study and do not offer any clues to the lower terbinafine release. In contrast to the UV-cured films, the Curanail film contained a much greater drug-load at 32% w/w. Such a high drug-load caused amorolfine HCl precipitation into crystals (shown in the pre-release polarised light micrograph in Fig. 11).

3.12. Ungual drug permeation

The ungual drug permeation profiles from the polymer films are shown in Fig. 12, while the calculated lag times, study-state flux, apparent permeability coefficients, effective diffusion coefficients and amount of drug in nail clippings are shown in Table 7 and the mass balance calculations in Table 8.

The ungual permeation profiles are significantly different from one another ($p < 0.05$), but the rank order of the formulations is the same as the release profiles, i.e. Curanail > amorolfine HCl-loaded UV-cured film > terbinafine HCl-loaded UV-cured film. This indicates that drug release from the films may be a rate-limiting step to ungual drug permeation. At the same time, similar lag times (approximately 10 days) for all three films show the critical role played by the nail plate barrier to ungual drug permeation.

From Table 7, it can be seen that terbinafine HCl-containing UV-cured films showed a lower drug flux compared to amorolfine HCl-containing ones ($p < 0.05$), but achieved greater drug concentrations in the nail ($p < 0.05$). The lower drug flux could be due to a lower terbinafine release by the film (shown in Fig. 11) and/or due to greater terbinafine-nail keratin affinity which could have resulted in the greater terbinafine concentration in the nail. Terbinafine has previously been shown to bind more strongly to keratin compared to amorolfine (Tatsumi et al., 2002). While drug binding to keratin has been correlated to a reduction in a drug's antifungal potency (Tatsumi et al., 2002), it could also result in the creation of a drug reservoir in the nail, which could be beneficial over the longer term. The nail keratin would then act as a drug depot releasing drug over time to (i) kill the fungus and any newly-germinating fungal spore residing in the nail plate and (ii) permeate into the nail bed to exert anti-fungal action there.

The comparator – Curanail – achieved higher drug flux as well as higher drug concentration in the nail clipping compared to the amorolfine-loaded UV-cured film ($p < 0.05$, Table 7). This could be due to its higher drug-load (32% w/w vs 3% w/w), hence a greater drug concentration gradient between the donor and the receptor phases, and a greater amount of drug released (seen in Fig. 11). Whether this difference in drug flux and drug concentration in nail translates to greater anti-onychomycotic efficacy remains to be seen in vitro and in vivo. Compared to Curanail's in vivo residence of a few days (Murdan et al., 2015), the UV gel is expected to have a much longer residence on the nail in vivo, like its cosmetic counterparts. The long residence would mean less frequent drug application, which could result in greater patient compliance and thereby higher success of therapy.

Table 8 shows that for both Curanail and the UV-cured films, most of the drug remains in the film and a very small proportion enters the nail plate and the receptor medium. This very low ungual permeation reflects previous reports in vitro and in man. For example, vanHoogdalem et al. (1997) showed that less than 0.2% of the applied dose permeated fingernails after 6 weeks in a human volunteer study. However, despite the low percentage of applied drug which permeates into the nail, the drug levels achieved in the nailplate (shown in Table 7) are considerable when the drugs' minimum inhibitory concentrations (MICs) are taken into account. The MICs of amorolfine and terbinafine have been reported to be 0.12–0.5 μg/ml and 0.004–0.06 μg/ml respectively against *Trichophyton rubrum,* the most common cause of onychomycosis (Tamura et al., 2014). Although these MIC values determined in vitro in broth, are not expected to be directly applicable to MICs in the nailplate, they do indicate that the drug levels achieved in the nail are more than sufficient to cause fungal kill. Thus, we can conclude that the UV-cured films are promising candidates for the topical treatment of onychomycosis.

4. Conclusions

UV-curable gels show promise as topical ungual drug carriers. Anti-onychomycotic drugs can be incorporated within these gels, in the presence of a suitable solvent. Ethanol-containing formulations have favourable properties such as high stability and water resistance. The drug is slowly released from the film and permeates into and through the nailplate. This is the first report of the potential of UV-curable gels – commonly used as cosmetics – as topical ungual drug carriers. Such formulations are used as cosmetics for their long residence on the nail. It is expected that the drug-loaded formulations will also have a long residence on the nail, allowing drug permeation into the nail over a long time. This is expected to improve topical therapy of nail diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2015.07.020.

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