Title: UV-curable gels as topical nail medicines: in vivo residence, anti-fungal efficacy and influence of gel components on their properties

Corresponding Author: Dr. Sudaxshina Murdan, PhD

Corresponding Author's Institution: UCL School of Pharmacy

First Author: Laxmi V Kerai

Order of Authors: Laxmi V Kerai; Stephen Hilton; M Maugueret; B B Kazi; Jane Faull; Sanjib Bhakta; Sudaxshina Murdan, PhD

Abstract: UV-curable gels, used as nail cosmetics for their in vivo durability, were reported to be promising as topical nail medicines. Our first aim was thus to investigate whether such durability applies to drug-loaded formulations. This was found to be true. However, ethanol inclusion in the pharmaceutical formulation (to enable drug loading) reduced the in vivo residence. The second aim was therefore to determine any other effects of ethanol, and if ethanol could be avoided by the choice of monomers. Thus, three methacrylate monomers, ethyl methacrylate, isobornyl methacrylate and 2 -hydroxyethyl methacrylate (HEMA) were selected, and their influence on the formulation properties were determined.   Ethanol and the methacrylate monomer influenced some (but not all) of the formulation properties. The most significant was that HEMA could dissolve drug and enable the preparation of ethanol-free, drug-loaded formulations, which would benefit in vivo residence. The absence of ethanol reduced drug loading, release and ungual flux, but had no negative impact on the in vitro anti-fungal efficacy. Thus, judicious selection of gel components enabled the exclusion of ethanol. The long in vivo residence, little residual monomers, sufficient ungual permeation and in vitro anti-fungal activity of the gels indicate their potential as anti-onychomycotic topical medicines.

Cover Letter

International Journal of Pharmaceutics
10 August 2016

Dear Professor Siepmann,

On behalf of all the authors, I attach the **revised** manuscript for your consideration for publication.

Title: **UV-curable gels as topical nail medicines: in vivo residence, anti-fungal efficacy and influence of gel components on their properties**

Article type: original research paper

We have addressed your comment.

I look forward to hearing from you,
Yours sincerely,

Dr Sudaxshina Murdan
BPharm, PhD, MRPharmS
Reader in Pharmaceutics
UCL School of Pharmacy, London, UK
T +44-20 7753 5810
F +44-20 7753 5942
E s.murdan@ucl.ac.uk

IJP AUTHOR CHECKLIST

Dear Author,

It frequently happens that on receipt of an article for publication, we find that certain elements of the manuscript, or related information, is missing. This is regrettable of course since it means there will be a delay in processing the article while we obtain the missing details.

In order to avoid such delays in the publication of your article, if accepted, could you please run through the list of items below and make sure you have completed the items.

Overall Manuscript Details

- Is this the final revised version?   X
- Are all text pages present?   X
- Are the corresponding author's postal address, telephone and fax
  numbers complete on the manuscript?   X
- **Have you provided the corresponding author's e-mail address?**   X
- **Manuscript type – please check one of the following:**
  - Full-length article   X
  - Review article   ☐
  - Rapid Communication   ☐
  - Note   ☐
  - Letter to the Editor   ☐
  - Other   ☐
- **Manuscript section – paper to be published in:**
  - Pharmaceutical Nanotechnology section   ☐
  - Personalised Medicine section   ☐

Manuscript elements

- Short summary/abstract enclosed?   X
- 3-6 Keywords enclosed?   X
- Complete reference list enclosed?   X
- Is the reference list in the correct journal style?
- Are all references cited in the text present in the reference list?   X
- Are all **original** figures cited in the text enclosed?   X
  Electronic artwork format? --
- Are figure legends supplied?   X
- Are all figures numbered and orientation provided?   X
- Are any figures to be printed in colour?   x
  If yes, please list which figures here:-------------figures 1, 4-9
- If applicable, are you prepared to pay for reproduction in colour?   NO
- Are all tables cited in the text supplied?   X
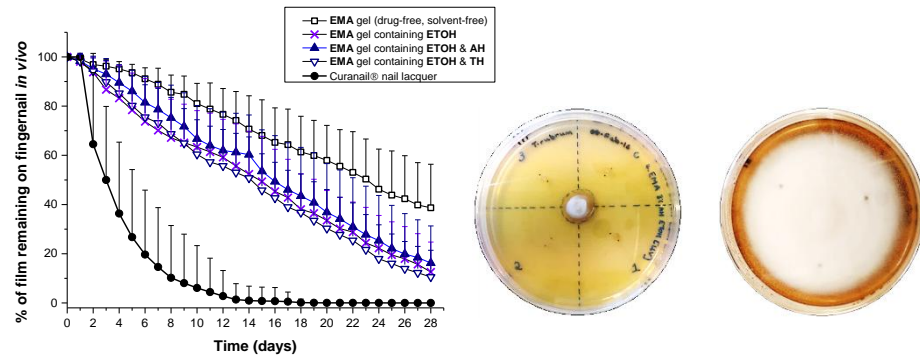
General

- Can you accept pdf proofs sent via e-mail?   X

Dear Dr. Murdan,

I will be pleased to accept your manuscript for publication in our journal, if you could please:

- adapt the structure of the abstract: We do not have sub-sections, such as "Results" and "Conclusion" in the abstract. It is one single paragraph.

Done. Sorry.

Long in vivo residence of UV-cured films (compared to nail lacquer control), and their in vitro anti-fungal efficacy, shown by the lack of fungal growth, compared to fungal growth in drug-free films

1   **UV-curable gels as topical nail medicines: in vivo residence, anti-fungal efficacy and**

2   **influence of gel components on their properties**

3

4   L V Kerai[1], S Hilton[1], M Maugueret[2], B B Kazi[2], J Faull[2], S Bhakta[2], S Murdan[1]*

5

6

7   [1]UCL School of Pharmacy, 29-39 Brunswick Square, London, WC1N 1AX, UK.

8   [2]Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck,

9   University of London, Malet Street, London, WC1E 7HX, UK.

10

11   *Corresponding author

12   T +44-207 753 5810

13   F +44-207-753-5942

14   E s.murdan@ucl.ac.uk

15

16

17

20   **Abstract**

21   UV-curable gels, used as nail cosmetics for their in vivo durability, were reported to be

22   promising as topical nail medicines. Our **first aim** was thus to investigate whether such

23   durability applies to drug-loaded formulations. This was found to be true. However, ethanol

24   inclusion in the pharmaceutical formulation (to enable drug loading) reduced the in vivo

25   residence. The **second aim** was therefore to determine any other effects of ethanol, and if

26   ethanol could be avoided by the choice of monomers. *Thus, t*hree methacrylate monomers,

27   ethyl methacrylate, isobornyl methacrylate and 2 -hydroxyethyl methacrylate (HEMA) were

28   selected, and their influence on the formulation properties were determined. Ethanol and

29   the methacrylate monomer influenced some (but not all) of the formulation properties. The

30   most significant was that HEMA could dissolve drug and enable the preparation of ethanol-

31   free, drug-loaded formulations, which would benefit in vivo residence. The absence of

32   ethanol reduced drug loading, release and ungual flux, but had no negative impact on the *in*

33   *vitro* anti-fungal efficacy. Thus, judicious selection of gel components enabled the exclusion

34   of ethanol. The long in vivo residence, little residual monomers, sufficient ungual

35   permeation and in vitro anti-fungal activity of the gels indicate their potential as anti-

36   onychomycotic topical medicines.

37

1. Introduction

Onychomycosis which is defined as fungal infection of the nail plate and/or nail bed, is a significant medical burden, affecting 10% of the general population, 20% of those over 60 years old, up to 50% of those over 70 years old and up to one-third of diabetics (Thomas et al., 2010) . The disease is often of long duration, is 'more than a cosmetic problem' (Scher, 1994), significantly affects patients' quality of life (Lubeck et al., 1993) and is recalcitrant to treatment (Arrese and Pierard, 2003; Daniel and Jellinek, 2010). Oral therapy with anti-fungal agents, such as terbinafine and itraconazole, is the treatment mainstay. However, drug efficacy is far from ideal, with pooled mycological cure rates in randomised controlled trials being 76 ±3% for terbinafine and 63% ±7% for itraconazole (Gupta et al., 2004) . The disadvantages of oral therapy, such as the potential for adverse systemic effects and drug interactions means that topical therapy is highly attractive, and a number of topical formulations have been tested, patented and a few have been marketed, compiled in, for example (Saner et al., 2014; Shivakumar et al., 2012) . Unfortunately, the success rates of topical therapy are even lower, as indicated in the medicines' patient information leaflets, and compiled in (Murdan, 2016) . A number of possible reasons for the low clinical cure of therapy have been proposed, such as inaccurate diagnosis, incorrect antifungal or delivery modality, presence of dormant fungal conidia (which can germinate and give rise to further infection), resistant fungal species, dermatophytoma (areas of the nail filled with debris of keratin and a large amount of fungi material which is more impermeable to drug), low patient compliance, and insufficient drug permeation into and through the nail plate for anti-fungal kill (Baran et al., 2005; Goodfield and Evans, 2000).

Insufficient drug in the nail could be due to the fact that the commercially available topical formulations do not have a continuous residence on the nail plate following application, and thus do not maximise the duration of ungual drug permeation. For example, the most recently marketed formulations Jublia®, Kerydin® and Onytec® are easily washed off, and the patient information leaflets recommend application prior to bedtime to enable ungual drug permeation to occur for at least the duration of sleep (AnacorPharmaceuticals, 2014; KakenPharmaceutical, 2014; Onytec). Loceryl® is meant for weekly application (Galderma,

68   2015). However, in vivo experiments have shown that the Loceryl film does not stay intact

69   on the nail for the duration of a whole week, at least on fingernails (Murdan et al., 2015).

70   Non-continuous residence of drug formulations on the nail could be compounded by poor

71   patient compliance. While the latter is not known, the extensive literature on (fairly low)

72   patient compliance in general, suggests that compliance to topical nail medicines is likely to

73   be lower than required.

74

75   Patient compliance could be improved by longer-lasting topical formulations and we have

76   previously proposed UV-curable gels as topical formulations which are likely to have long in

77   vivo residence on the nail (Kerai et al., 2015). In the work discussed in this paper, the **first**

78   **aim** was thus to investigate the in vivo residence of these formulations. During this study, it

79   was found that, while the UV-cured films have a fairly long in vivo residence, inclusion of

80   ethanol in the formulation (which enabled sufficient drug loading) reduced the in vivo

81   residence. The **second aim** was therefore to determine what other effects ethanol had, if

82   these effects were independent of the other gel components, and whether the inclusion of

83   ethanol could be avoided.

84

85   Cosmetic UV curable gel formulations typically consist of three components: i) a urethane

86   methacrylate, ii) a (meth)acrylate based monomer and iii) a polymerisation photoinitiator.

87   The pharmaceutical UV curable gel formulation reported previously consisted of these,

88   specifically, diurethane dimethacrylate (DUDMA), ethyl methacrylate (as the (meth)acrylate

89   based monomer) and 2-hydroxy-2-methylpropiophenone as the photoinitiator, as well as a

90   drug (amorolfine HCl or terbinafine HCl) and a solvent (ethanol). In order identify more

91   appropriate gel components (to avoid the use of ethanol), one could screen alternative

92   urethane-based methacrylates such as Di-HEMA trimethylhexyl dicarbomate,

93   photoinitiators such as 1-hydroxycyclohexyl phenyl ketone and different (meth)acrylate

94   based monomers. In this work, we decided to keep DUDMA as the backbone of the

95   formulation as it produces a strong film with exceptional abrasion resistance and durability,

96   and 2-hydroxy-2-methylpropiophenone as the photoinitiator, and to screen different

97   methacrylate based monomers. Thus, 2-hydroxyethyl methacrylate (HEMA) and isobornyl

98 methacrylate (IBOMA), in addition to ethyl methacrylate (EMA) were selected as the

99 methacrylate based monomers.   The influence of these monomers and of ethanol on the

100 gel and film properties were determined, and correlations among the different properties of

101 the films formed by photo-polymerisation were explored.

102

103 **2. Materials and Methods**

104 **2.1 Materials**

105 Amorolfine HCl was purchased from Ranbaxy Research Laboratories (Haryana, India) and

106 terbinafine HCl from AK Scientific (CA, USA). Diurethane dimethacrylate, ethyl methacrylate,

107 isobornyl methacrylate, 2-hydroxyethyl methacrylate, 2-hydroxy-2-methylpropiophenone,

108 absolute ethanol, methanol, propan-2-ol, sodium chloride, triethylamine, phosphoric acid

109 85% wt solution in water and trifluoroacetic acid and a dialysis tubing cellulose membrane

110 (MW 10281) were purchased from Sigma–Aldrich (Dorset, UK). Acetonitrile HPLC gradient

111 grade was purchased from Fisher Scientific (Hertfordshire, UK). A 36 Watt Cuccio

112 Professional UVA nail lamp was purchased from Amazon UK. Nail & Beauty Emporium lint-

113 free wipes (4 ply) were purchased from Just Beauty UK, an online retailer specialising in

114 professional beauty, hair and skin products. Sabouraud dextrose agar was purchased from

115 Oxoid Ltd (Basingstoke, UK). One dermatophyte strain, *Trichophyton rubrum* (CBS 118892),

116 was used. Curanail® nail lacquer was used as a control in some experiments and was

117 purchased from pharmacies in the UK. Human nail clippings (fingernails) were obtained

118 from healthy volunteers aged between 18 and 65 years (following ethics approval).

119

120 **2.2 Determination of drug (amorolfine HCl and terbinafine HCl) solubility in methacrylate**

121 **monomers**

122 The saturation solubility of the two drugs in EMA, HEMA and IBOMA was determined as

123 described in (Kerai et al., 2015) .

124

2.3 Preparation of UV gels and films

Drug-free gel formulations were prepared by mixing DUDMA with a methacrylate monomer (EMA or IBOMA or HEMA) at 85:15 or 75:25 (for HEMA only) v/v ratios, the photoinitiator at 3% v/v of the methacrylate mixture, with or without ethanol at 25% v/v of the methacrylate mixture, and leaving the mixture to stir overnight, which produced a clear homogenous solution. Drug-loaded formulations were prepared by first dissolving the drug in ethanol, and then adding the methacrylates and photoinitiator and leaving the mixture to stir overnight, which also produced a clear homogenous solution. To prepare the films, the gel mixture was applied on a microscope glass slide using a pipette tip (30 µl to an area of 15 mm x 15 mm as a single layer), which was then placed under the UVA lamp for two minutes. 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 characterised.

A curing time of 2 minutes was selected after experimenting with 0.5-5 minutes of UVA exposure, and measuring the gel blend to polymer film mass yield, the alkene-to-alkane degree of conversion (the route of polymerisation), and the levels of residual monomers in the polymer film. The measurements (Supplementary Data 1a) showed that a cure time of two minutes was sufficient for maximal mass yield and degree of conversion and for minimal residual monomers.

2.4 In vitro characterisation of the gels and UV-cured films

The in vitro properties of the gels and of the films, such as gel viscosity, maximum drug loading, gel-to-film mass yield, degree of conversion and the films' appearance, microstructure, crystallinity, level of residual monomers, drug-polymer interactions, glass transition temperature, adhesion, water sensitivity, drug release and ungual permeation were determined as described in (Kerai et al., 2015) . The films' mass change with time was monitored over 28 days under the following testing conditions: 30°C ± 2°C and 50% RH ± 5%

6

154 RH, by placing the films in a desiccator containing a saturated solution of magnesium

155 nitrate and placing this in a 30°C oven. The film's mass was recorded on days 0, 1, 2, 3, 7, 14,

156 21 and 28.   The film thickness was measured using a Sealey AK9635D 0–25 mm Digital

157 External Micrometer (PVR Direct, Bristol, UK). Each film was measured at three separate

158 points and an average was taken, and each test was carried out in triplicate. Precautions

159 were taken to measure the thickness without compressing the polymer film. The uniformity

160 of thickness within each film and between films was calculated using the following formula:

$$\% \text{ Uniformity} = (1 - \frac{\text{standard deviation}}{\text{mean}}) \times 100$$

161

162 **2.5 *In vivo* fingernail residence of UV-cured polymer films**

163 Following approval by the UCL School of Pharmacy's ethics committee (Project 5337/002),

164 12 volunteers (aged between 18 – 65 years) with healthy fingernails were recruited. For

165 each volunteer, a single layer of the UV-curable gel formulation was applied on the five

166 fingernail plates of one hand using a pipette tip, leaving a formulation-free margin

167 (approximately 1-2 mm) near the nail plate perimeter, ensuring that skin contact was

168 avoided. This hand was then placed under a UVA nail lamp for two minutes. Subsequently,

169 the surface of the cured gel on each nail plate was wiped with propan-2-ol using a super

170 absorbent nail wipe to remove the oxygen inhibition layer, leaving behind a thin, smooth,

171 transparent film. The procedure was repeated on the other hand. The nails were visually

172 observed daily to estimate the percentage of UV-cured film remaining on the fingernails.

173 Estimation was facilitated by visually dividing the nail plate into quadrants which were then

174 further divided into sub-quadrants. Daily observation was continued until the UV-cured

175 films had completely dislodged from the nail plate or for up to 28 days, whichever was

176 sooner. After 28 days, the remaining film was removed using a wooden stick 10 minutes

177 following the application of a cotton pad soaked with acetone over the film. The experiment

178 was then repeated with a different formulation. A total of four UV-curable gel formulations

179 (which contained the monomer EMA, ± solvent and drug) were tested, and each

180 formulation was tested on a minimum of six volunteers.

181

2.6 In vivo occlusivity of UV-cured polymer films

The occlusivity of UV-cured films was assessed by measuring trans-onycheal water loss (TOWL) from the healthy fingernail plates of volunteers before and after the application of the gel formulations (which were applied as described in Section 2.5). Curanail® lacquer film was used as control, and was applied as instructed in the packaging (i.e. after filing and cleansing). Prior to TOWL measurements, the volunteers had rested for at least 60 minutes in the laboratory, and avoided contact with water. TOWL measurements were obtained using a Biox Aquaflux Model AF200 equipped with an *in vivo* nail cap (Biox Systems Ltd, London, UK). The finger was placed on a flat surface for support and the Aquaflux measurement head was placed on the centre of the nail plate. No pressure was applied as the weight of the measurement head itself ensured a good seal, and measurements were collected using AquaFlux version 4.8. Water vapour flux density versus time curves were recorded until a steady-state TOWL reading was obtained (between 90 - 120 seconds, with a mean of 10 points), and five repeats were obtained per formulation. All measurements were conducted in the same laboratory, where the ambient temperature and air humidity fluctuated between 22 - 26°C and 38 - 48% respectively. The % reduction in TOWL was calculated as follows:

$$\%\text{Reduction in TOWL} = \frac{\text{TOWL (before formulation application)} - \text{TOWL (after formulation application)}}{\text{TOWL (before formulation application)}} \times 100$$

A greater reduction in TOWL indicates higher occlusivity of the film.

2.7 In vitro antifungal efficacy of UV gels against *T. rubrum*

The dermatophyte *T. rubrum* was chosen as it is the most common causative organism of onychomycosis. The ability of the formulations to inhibit fungal growth was used as a measure to compare formulations. The classical disc diffusion method was adapted, where the 'disc' was replaced by a circular piece of nail clipping onto which a gel formulation had been cured, taking care to leave a formulation-free margin at the nail edge, such that the drug would have to be permeate into and through the nailplate to reach the agar gel. This

209 method was used as there is currently no standard in vitro method to assess the anti-fungal

210 efficacy of anti-onychomycotic formulations.

211

212 *Preparation of SDA plates:* A Sabouraud Dextrose Agar (SDA) solution (65 g SDA/L of distilled

213 water) was prepared, sterilised (by autoclaving at 121^0C for 15 minutes) and poured into

214 90mm petri dishes (25 ml of agar solution/dish) and allowed to solidify overnight.

215 *Preparation of test plates:* A cork borer (8mm-internal diameter, sterilised by dipping in

216 ethanol then flamed) was punched into the centre of a SDA plate (prepared above) to

217 remove a plug of agar, which was discarded. The cork borer was sterilised once more, and

218 used to punch out a *T. rubrum* plug from a fresh culture plate of *T. rubrum.* The fungal plug

219 was inserted into the slot created in the first SDA plate, and the latter was then incubated at

220 32^oC for three days to ensure that the fungal culture grew sufficiently and to identify (and

221 remove) defective plates, if any. During this time, the *T. rubrum* culture grew to 10 mm in

222 diameter.

223 *Preparation of nail clippings with drug formulations*: Human fingernail clippings were

224 washed with water, cut to size (circular, with a diameter of 3 mm) and autoclaved at 120^0C

225 for 20 minutes in order to sterilise them (autoclaving did not affect the nail properties -

226 indicated by the lack of change in solvent uptake). Two µl of a formulation was applied on

227 the nail surface, ensuring a small formulation-free perimeter at the edge of the nail surface.

228 For the control Curanail, the lacquer was allowed to air-dry. For the UV gels, the nail was

229 placed under an UVA lamp for 2 minutes, after which the surface of the film produced was

230 wiped with propan-2-ol using a nail wipe.

231 Details of the formulations tested are shown in Table 1. Drug-free counterparts of these

232 formulations were also tested to assess the influence, in any, of the UV-cured film itself

233 against *T. rubrum.* In addition, Curanail® nail lacquer (containing 5% w/v amorolfine HCl)

234 was tested as a positive control.

235 *Evaluation of anti-fungal efficacy:* Three days following the insertion of the fungal plug into

236 a prepared SDA plate, the latter was marked into four quadrants (which enabled the testing

237 of one test formulation on three nail clippings, and one drug-free formulation on a fourth

238 nail clipping per plate). Using a sterile tweezer, the prepared nail clippings were carefully

239 placed on the agar gel surface at a distance of 20 – 25 mm from the centre of the plate. This

240 distance was chosen to allow sufficient time for the drug to penetrate into and through the

241 nail into the agar gel, based on the ungual drug permeation results and *T. rubrum* growth

242 rates from preliminary studies. Thus, each plate contained a triplicate set of the same

243 formulation in addition to a drug-free control.

244 In addition to the test plates, two controls were set up: i) a formulation-free, nail-free

245 negative control was set up to monitor the growth of *T. rubrum,* and ii) a control plate

246 containing 3 equally-spaced nail clippings with drug-free UV-cured films on their surface,

247 with the clippings being placed at a distance of 20 – 25 mm from the centre of the plate.

248 The SDA plates were incubated at 32°C for as long as it took the *T. rubrum* culture to grow

249 over the entire SDA plate's surface (or when no further growth was detected). During this

250 period, the plates were observed daily to monitor the growth of *T. rubrum* and to monitor

251 the formation of a zone of inhibition, if any, around the nail clippings.

252

253 **2.8 Statistical analysis**

254 All the experiments described above were repeated three times (except for the *in vitro* drug

255 permeation study which was repeated six times, in vivo residence studies where n=60 (10

256 fingernails x 6 volunteers) and in vivo TOWL measurements where n=5). Statistical

257 calculations were conducted using SPSS 22. Repeated measures ANOVA and post hoc Tukey

258 tests were performed on the drug release, ungual permeation and in vivo residence profiles

259 over time. When n ≥5, Students t-tests were performed to compare two data sets while

260 ANOVA, with post hoc Tukey were conducted to compare more than two data sets.   Non-

261 parametric tests were used when n=3; specifically, Mann-Whitney to compare two groups,

262 and Kruskal-Wallis, followed by Nemenyi's tests to compare more than two groups.

263

264 **3. Results and Discussion**

265 **3.1 In vivo residence of UV-cured films**

266   The in vivo residence of UV-cured films, and of Curanail nail lacquer (used as a commercially

267   available control) is shown in Figure 1. It can be seen that the UV-cured films have a much

268   longer residence on the nail compared to Curanail (p<0.05). Four weeks after gel

269   application and film formation, a considerable proportion of the film remained on the nails.

270   This shows that the UV-cured films have promise as long-term topical nail medicines,

271   continuously allowing drug to permeate into the nail, and thereby improve the success of

272   topical nail medicines. A longer residence on the nail could also improve patient compliance

273   as less frequent drug application will be needed.

274

275   The longer residence of the ethanol-free UV-cured formulation compared to ethanol-

276   containing ones ($p<0.05$) indicates that ethanol-free pharmaceutical formulations would be

277   advantageous for nail medicines. The presence of ethanol had enabled drug loading in the

278   formulations and had influenced some, but not all of the formulation properties

279   investigated (Kerai et al., 2015) . In order to further explore the influence of ethanol on the

280   formulation properties and determine whether such influence was independent of the

281   nature of the other gel components, and evaluate the possibility of developing ethanol-free

282   formulations, two additional methacrylate-based monomers (in addition to ethyl

283   methacrylate) were selected and their formulations were characterised as discussed below.

284

285   **3.2 Selection of the nature and proportion of the methacrylate monomers in the gel**

286   In contrast to cosmetic UV gels examined, which contain a mixture of acrylate- and

287   methacrylate-based monomers, only methacrylate-based monomers were included in our

288   pharmaceutical formulations due to their lower toxicity and lower sensitising capacity

289   (Clemmensen, 1984; Rietschel et al., 2008; Yoshii, 1997), which render them more

290   favourable for nail products (Schoon, 2010). Of the possible methacrylate-based monomers,

291   ethyl methacrylate (EMA), hydroxyethyl methacrylate (HEMA) and isobornyl methacrylate

292   (IBOMA) were selected due to their unique structures (Figure 2), which could differently

293   impact the resulting films' properties, and which would, in turn, enable the exploration of

294   correlations among the formulation properties. EMA was expected to enhance film

295   flexibility, IBOMA – with its cyclic group - could affect the resulting film's mechanical

296   strength and glass transition temperature while HEMA – with its hydroxyl group – could

297   affect the hydrophilic/hydrophobic character of the resulting film. The three monomers

298   also have different solvencies for the two drugs being tested (Table 2), and the higher

299   solvency of HEMA towards the drugs enables the development of ethanol-free gels.

300

301   The ratio of diurethane dimethacrylate (DUDMA) to methacrylate monomer (EMA or HEMA

302   or IBOMA) in the gel formulations were generally kept at 85:15 % v/v as in (Kerai et al.,

303   2015) , except for the ethanol-free HEMA-based formulations. As HEMA could dissolve the

304   drugs to some extent (Table 2), it was possible to produce drug-loaded gels without ethanol.

305   In this case, the proportion of HEMA was increased to enable sufficient drug loading. Thus

306   HEMA-containing formulations were also produced at a DUDMA:HEMA ratio of 75:25 %v/v.

307

308   The photoinitiator (2-hydroxy-2-methylpropiophenone) was kept at 3% v/v of the

309   methacrylate mixture as this was found to be sufficient for maximal gel-to-polymer degree

310   of conversion and mass yield and minimal levels of residual monomers (supplementary data

311   1b). Ethanol was included at a concentration of 25% v/v as this concentration increased the

312   formulations' drug loading and did not compromise the films' water-resistance (Kerai et al.,

313   2015). Ethanol-free formulations of HEMA were also produced.

314

315   **3.3 Influence of gel components on its properties**

316

317   **3.3.1   Gel viscosity:** The viscosities of the gels (± ethanol and ± drug) are shown in the

318   Supplementary Data 2. In ethanol-free gels, the nature and proportion of the methacrylate

319   monomer had a direct influence on viscosity ($p < 0.05$), which was between 162 mPas and

320   672 mPas. Inclusion of ethanol (whose viscosity is 1 mPas) considerably reduced the gel and

321   all ethanol-containing gels (with/without drug) had similar viscosities of 15-25 mPas

322   ($p > 0.05$). Inclusion of the very low viscosity ethanol overrides any influence of the

323 methacrylate monomer on gel viscosity. Gel viscosity is an important parameter: too high

324 viscosity will cause difficulty during gel application, while insufficient viscosity will result in

325 the gel spreading from the nail plate into the nail folds. Excessive gel spreading is

326 undesirable to avoid contact between the gel (containing potentially allergenic and

327 sensitising **monomers**) and the skin. The viscosities of these gels are deemed to be

328 appropriate as the gel could be applied to the nail in volunteers, without any problems

329 (Section 2.5).

330

331 **3.3.2 Drug loading and stability in gels:** Drug loading in a gel (Table 3) depended on the

332 drug's solubility in ethanol and in the methacrylate-based monomer, the drugs being

333 insoluble in DUDMA (Kerai et al., 2015). Thus, gels containing HEMA and ethanol dissolved

334 the most drug due to the greater drug solvency of HEMA. Terbinafine loading was higher

335 than that of amorolfine, due to its greater solubility in ethanol (Kerai et al., 2015) . Over a

336 period of six months, all the drug-loaded gels (stored under accelerated stability conditions)

337 showed no change in colour, no visible signs of drug precipitation, and no change in drug

338 concentration. The UV-curable gel formulations were therefore considered stable and

339 suitable for further development.

340

341 **3.3.3 Polymerisation, degree of conversion and levels of residual monomers:** Exposure of

342 the gels to UVA initiates polymerisation between DUDMA and the methacrylate-based

343 monomer (EMA or IBOMA or HEMA), where the original alkene bonds in the acrylate

344 moieties are converted to alkane ones. The degree of alkene-to-alkane conversion (DC) was

345 between 56% and 68% (Supplementary data 3a). Ethanol inclusion in the gels led to

346 significantly higher ($p<0.05$) degrees of conversion, possibly due to the lower gel viscosities,

347 which could have enabled increased reaction among the more mobile reactants. In contrast,

348 the nature of the methacrylate-based monomer (EMA, IBOMA, HEMA) and the presence of

349 drug did not influence DC ($p>0.05$).

350

351 Very low levels of residual monomers (≤1% DUDMA, ≤ 0.005% EMA, ≤0.004% IBOMA and

352 ≤0.005% HEMA) were found in drug-containing films (Supplementary data 3b), which

353 indicates that the much less than 100% alkene-to-alkane degrees of conversion seen are

354 due to the presence of unreacted **alkene groups** within the polymer, rather than **unreacted**

355 **monomers**. It appears that **most** of the methacrylate **monomers** in the UV gel are involved

356 in the polymerisation process, while a large number of the alkene **groups** are not. The very

357 low levels of residual monomers make the films attractive drug carriers, as the potential for

358 allergic reactions (which is linked to monomers) is reduced.

359

360

361 **3.4 Influence of gel components on the properties of the UV-cured films**

362

363 **3.4.1 Film macro-and micro- structure, amorphicity, FTIR**: All the UV-cured films

364 (irrespective of monomer nature, and presence or absence of ethanol and of drug) were

365 visually smooth, transparent, had uniform thickness (≥95%), were amorphous, and were

366 aesthetically acceptable as topical drug carriers. Scanning electron microscopy revealed a

367 generally rough film surface on the side that was exposed to UV light, a much smoother

368 surface where the film had been formed in contact with the support and a fairly dense film

369 cross-section (Figure 3). Film thickness was about 200 μm, with no influence of drug or of

370 nature of the monomer ($p>0.05$). Inclusion of ethanol significantly ($p<0.05$) reduced film

371 thickness to about 165 μm, due to the formulations' lower methacrylate content. FT-IR of

372 the films showed no drug-polymer interactions.

373

374 **3.4.2 Drug loading and stability:** Drug in the film was in the dissolved state, and its

375 concentration in the film was similar to that in the gel shown in Table 3 ($p>0.05$). Upon

376 storage of the films for 28 days under accelerated stability conditions, the drug levels

377 remained the same, and the drug remained dissolved for at least two weeks, with a few

378 drug crystals appearing in week 3. There were no differences among films with/without

379 ethanol, with/without drug and containing the different methacrylate-based monomers

380 (Supplementary data 4).

381

382 **3.4.3 Glass transition temperatures of films.** The films produced from ethanol-free

383 formulations showed two transition temperatures (Table 4). It is thought that the lower Tg

384 value is the polymer's Tg caused by UV curing of the gel mixture, and that the second Tg is

385 related to a thermal cure which occurred during the DMA measurement. The UV-cure

386 related Tg is influenced by the nature of the methacrylate-based monomer, with EMA-

387 based gel having higher Tg than HEMA-based one, which in turn had higher Tg than IBOMA-

388 based one ($p < 0.05$). Such a difference in gel Tgs could be due to their different viscosities,

389 which were 367 mPas, 598 mPas and 672mPas for EMA- , HEMA- and IBOMA- based gels

390 respectively. The negative correlation between gel viscosity and polymer Tg can be

391 explained by the fact that, in a reaction mixture with lower viscosity, reactants are likely to

392 be more mobile, which would increase the likelihood of polymerisation and of cross-linking

393 and which would in turn increase the resulting polymer's Tg.

394 Inclusion of ethanol in the formulation had a major influence on the films' glass transition

395 temperatures, resulting in films with only one Tg, and increasing the UV-cure Tg ($p < 0.05$).

396 The higher Tgs reflect the greater extent of polymerisation (shown by higher degrees of

397 conversion and lower levels of residual monomers in Supplementary Data 3b) in ethanol-

398 containing formulations and could also be a result of these gels' much lower viscosities.

399

400 **3.4.4 In vitro adhesion and water sensitivity of films:** In contrast to the in vivo situation

401 where the presence of ethanol reduced the in vivo residence of the films (Figure 1), the

402 three in vitro tests used (Instron pull-off, the cross-cut and water sensitivity) showed no

403 influence of ethanol, or of the nature and concentration of the methacrylate monomer on

404 film adhesion and water sensitivity ($p > 0.05$, Supplementary data 5). It seems that DUDMA

405 (present at a much higher content than the methacrylate monomer in the film) is the main

406 factor responsible for the adhesion and water-resistance properties of the films in vitro.

407

3.4.5 In vitro drug release: Drug release profiles (Figures 4-5) of all the UV-cured films (containing the maximum amount of drug that could be loaded in the dissolved state in each film) showed a burst release over the first 24 hours, followed by a slower phase, followed by a plateau. Examination of the films at the end of the release study showed negligible change in film mass over the study duration and no drug precipitation. Fitting of the release data using zero order, first order and Higuchi models showed the best fit to be the Higuchi model ($r^2 \geq 0.8$), indicating drug release to be diffusion-controlled through the film matrix following the burst release phase.

For both drugs, release was incomplete over the 30-day experiment as shown when % drug release was plotted against time (Supplementary data 6). The amorolfine-containing films released about 46% of their drug load, while the terbinafine-containing films released about 26% of their drug load during the 30-day study. The reason(s) for the greater amorolfine release is as yet unclear. The respective amorolfine- and terbinafine- loaded gels have similar reactants-to-film mass conversion, alkene-to-alkane degree of conversion, film thickness, glass transition temperatures, water sensitivity and dense internal structure. It is possible that terbinafine binds to a greater extent to the polymer film compared to amorolfine, which would hinder its release.

In addition to the nature of the drug, the nature of the methacrylate-based monomer also has an impact on the extent of drug release, via its influence on drug solvency, and consequently, on drug loading in the film. Films produced using EMA and IBOMA (both non-solvents for the drugs) contained the same drug loadings (3% amorolfine or 4% terbinafine), which was solely due to the presence of the ethanol. In contrast, films produced using HEMA (a solvent for the drugs) could have a certain drug loading in the absence of ethanol (2% of amorolfine or of terbinafine), and the drug loading was considerably increased in the presence of ethanol (to 4% amorolfine or 6% terbinafine). As the release studies were conducted using films containing the maximum drug loading, the drug concentrations in the films varied from 2% to 4% for amorolfine, and from 2% to 6% for terbinafine. For both drugs, the greatest drug release at the end of the experiment was obtained from the films

438   containing the highest drug concentration, i.e. the film containing HEMA and ethanol

439   (p<0.05, Figures 4-5). Drug concentration in the film is thus an important parameter

440   governing the extent of drug release. Both the nature of the methacrylate-based monomer

441   and the inclusion of ethanol influenced drug release, via their influence on drug

442   concentration in the film.

443

444   When drug release from the control Curanail lacquer film was examined , it was found that

445   like the UV-cured films, the Curanail lacquer film containing amorolfine also showed a burst

446   of drug release (Supplementary data 6a). In contrast to the UV-cured films however, the

447   Curanail polymer film released most of its drug load, possibly due to the fact that the film

448   disintegrated during the 30-day study.

449

450   **3.4.6 In vitro ungual drug permeation:** The ungual drug permeation profiles and the

451   permeation parameters are shown in Figures 6-7 and Table 5. Mass balance calculations are

452   shown in Supplementary Data 7.   The fairly lengthy lag times in Table 5 are typical of ungual

453   permeation (McAuley et al., 2016) and are due to the poor permeability of the nailplate. The

454   latter is also evidenced by the very small percentage (maximum of 6%) of applied drug

455   which permeated into and through the nail (Supplementary Data 7).

456

457   Examination of the ungual permeation profiles shows significant influence of the drug

458   nature and concentration, with amorolfine and the higher drug loadings resulting in greater

459   ungual permeation (p<0.05). Thus, amorolfine-containing formulations showed significantly

460   higher steady-state flux and permeability coefficient ($p<0.05$) compared to the respective

461   terbinafine-containing ones. This reflects the greater amorolfine released (discussed in

462   section 3.4.5), and thereby its greater availability for ungual permeation. An additional

463   cause for the higher amorolfine permeation could be its lower affinity to nail keratin,

464   compared to that of terbinafine (Tatsumi et al., 2002). Drug-keratin affinity could also

465   explain the similar drug-in-nail concentrations at the end of the permeation experiments

466   ($p>0.05$; Table 5) despite the lower terbinafine release from the films.

467

468 For both drugs, drug loading in the film was the maximum possible (while keeping the drug

469 dissolved) and was related to the nature of the methacrylate-based monomer, and the

470 presence of ethanol as discussed in section 3.4.5. From Figures 6-7, it can be seen that as

471 drug concentration in the film increased, drug permeation increased. This reflects the

472 greater amounts of drug released at the highest drug loadings (section 3.4.5) and

473 consequently, greater drug availability for ungual permeation. Thus, the choice of the

474 monomer and addition of ethanol had important consequences on ungual drug permeation,

475 with the addition of ethanol to HEMA-based formulations significantly increasing drug

476 loading and flux ($p<0.05$).

477

478 The ungual permeation of amorolfine from the control Curanail was similar to that from the

479 'best' UV-cured film ($p>0.05$ for the permeation curve, permeability coefficients, diffusion

480 coefficients and drug-in-nail at day 30). Curanail® nail lacquer however showed a slightly

481 greater steady-state flux ($p<0.05$) probably due to its higher drug-load (32% w/w in the film

482 vs. 4% w/w) and much greater drug release (shown in Supplementary Data 6a). However,

483 the UV-cured film displayed a shorter lag time possibly due to its greater occlusivity

484 compared to the lacquer film (Fig. 9), which could increase nail hydration, which is known to

485 enhance permeation (Gunt and Kasting, 2007; Gunt et al., 2007).

486

487 **3.4.7 In vitro anti-fungal efficacy:** The in vitro anti-fungal efficacy of the formulations was

488 measured using an anti-fungal whole cell phenotypic assay, that we adapted from the

489 classical disc diffusion method, in our laboratories. In the negative controls (i.e. where a

490 drug-free formulation was cured onto a nail clipping), the *T. rubrum* culture grew over the

491 entire plate's surface (Table 6). In contrast, no *T. rubrum* growth was observed when drug-

492 loaded formulations had been cured on the nail clippings. This shows that sufficient anti-

493 fungal drug was released from the UV-cured film, permeated into the nailplate, and

494 subsequently permeated out of the nail plate and into the agar gel to inhibit fungal growth.

495 The UV-cured gels are thus promising topical carriers for anti-onychomycotic drugs.

496 All the gels tested showed complete inhibition of fungal growth (example shown in Table 6),

497 and no difference could be discerned among the formulations (in Supplementary data 7).

498 This could be related to the fact that the amounts of drug present in the nail and that had

499 permeated through the nail plate were fairly similar for all the formulations (as shown in

500 Figures 6-7). In these anti-fungal assays, the ethanol-free gels with the lowest drug load was

501 as good as the ethanol-containing gels with the highest drug load. It seems that the

502 increased drug loading afforded by the inclusion of ethanol in the gels, which results in

503 increased flux was not necessary for anti-fungal kill in this in vitro set-up. Optimisation of

504 the in vitro anti-fungal methodology is needed and it might enable discrimination among

505 the formulations or confirm anti-fungal equivalence despite different drug loadings, release

506 and ungual permeation.

507

508 **3.4.8 In vivo occlusivity of the UV-cured films:** The UV-cured films reduced trans-onycheal

509 water loss (TOWL) by about 40% (Figure 8), and were more occlusive than the control

510 Curanail lacquer film. Occlusion is desirable, as a reduction in TOWL has been hypothesised

511 to hyper-hydrate the nail plate (Marty, 1995; Spruit, 1971), which is in turn beneficial for

512 ungual drug permeation (Gunt and Kasting, 2007; Gunt et al., 2007). A second benefit of

513 increased nail plate hydration in the treatment of onychomycosis is that hydration could

514 induce the germination of drug-resistant fungal spores into drug-susceptible hyphae, which

515 would enable fungi eradication (Flagothier et al., 2005). From Figure 8, it can be seen that

516 ethanol or drug or the nature of the methacrylate monomer in the UV-cured films did not

517 have any influence on the films' occlusivity (p>0.05). This indicates that DUDMA (present in

518 the film at a much higher content than the methacrylate monomer) is the main factor

519 responsible for the occlusivity of the films.

520

521 **4. Conclusions**

522 Drug-loaded diurethane dimethacrylate-based UV gels, with or without ethanol, and

523 containing one of three different monomers (EMA, IBOMA or HEMA) were produced and

524 characterised to determine the influence of the monomers, ethanol and drugs on the gel

525 and resulting films' properties, and to explore correlations among the films' characteristics.

526 The drugs – amorolfine HCl and terbinafine HCl – had negligible influence on the films'

527 properties, except for the extent of their release and ungual flux, with amorolfine release

528 from the film and ungual flux being much higher compared to that of terbinafine. The major

529 influence of ethanol was its ability to increase drug loading, reduce gel viscosity, increase

530 polymerisation and alter the films' Tg. The greater drug loading enabled by ethanol resulted

531 in higher drug release and ungual flux. However, ethanol inclusion had a negative impact on

532 the film's in vivo residence, which shows the importance of screening the other

533 methacrylate monomers in order to identify suitable ones.   The major difference among the

534 monomers was the fact that HEMA dissolves the drugs to some extent and enabled

535 sufficient drug loading, such that ethanol-free formulations could be developed. All the

536 formulations inhibited anti-fungal growth to the same extent i.e. fully, and differences

537 among the formulations regarding anti-fungal efficacy could not be discerned in the tests

538 used i.e there was no influence of methacrylate monomer nature, ethanol inclusion and

539 drug concentration on anti-fungal activity. The UV gels' anti-fungal activities and long in

540 vivo residence further show the promise of these formulations as nail medicines.

541

546 **References**

547 AnacorPharmaceuticals, 2014. Kerydin Highlights of Prescribing Information, US.
548 Arrese, J.E., Pierard, G.E., 2003. Treatment failures and relapses in onychomycosis: A stubborn
549 clinical problem. Dermatology 207, 255-260.
550 Baran, R., Gupta, A.K., Pierard, G.E., 2005. Pharmacotherapy of onychomycosis. Expert Opinion on
551 Pharmacotherapy 6, 609-624.
552 Clemmensen, S., 1984. Cross-Reaction Patterns in Guinea Pigs Sensitised to Acrylic Monomers. 7,
553 527-540.
554 Daniel, C.R., Jellinek, N.J., 2010. Commentary: The illusory tinea unguium cure. J. Am. Acad.
555 Dermatol. 62, 415-417.
556 Flagothier, C., Pierard-Franchimont, C., Pierard, G.E., 2005. New insights into the effect of amorolfine
557 nail lacquer. Mycoses 48, 91-94.
558 Galderma, 2015. Loceryl amorolfine 5% w/w Medicated Nail Lacquer, p. Loceryl Package Leaflet.

559 Goodfield, M.J.D., Evans, E.G.V., 2000. Combined treatment with surgery and short duration oral
560 antifungal therapy in patients with limited dermatophyte toenail infection. Journal of Dermatological
561 Treatment 11, 259-262.
562 Gunt, H.B., Kasting, G.B., 2007. Effect of Hydration on the Permeation of Ketoconazole Through
563 Human Nail Plate In Vitro. European Journal of Pharmaceutical Sciences 32, 254-260.
564 Gunt, H.B., Miller, M.A., Kasting, G.B., 2007. Water Diffusivity in Human Nail Plate. Journal of
565 Pharmaceutical Sciences 96, 3352-3362.
566 Gupta, A.K., Ryder, J.E., Johnson, A.M., 2004. Cumulative meta-analysis of systemic antifungal agents
567 for the treatment of onychomycosis. Br. J. Dermatol. 150, 537-544.
568 KakenPharmaceutical, 2014. Jublia Highlights of Prescribing Information, US.
569 Kerai, L.V., Hilton, S., Murdan, S., 2015. UV-curable gel formulations: Potential drug carriers for the
570 topical treatment of nail diseases. Int. J. Pharm. 492, 177-190.
571 Lubeck, D.P., Patrick, D.L., McNulty, P., Fifer, S.K., Birnbaum, J., 1993. Quality-of-life of persons with
572 onychomycosis. Qual. Life Res. 2, 341-348.
573 Marty, J.-P.L., 1995. Amorolfine Nail Lacquer: A Novel Formulation. Journal of the European
574 Academy of Dermatology and Venereology 4, S17-S21.
575 McAuley, W.J., Jones, S.A., Traynor, M.J., Guesne, S., Murdan, S., Brown, M.B., 2016. An
576 Investigation of how Fungal Infection influences Drug Penetration through Onychomycosis Patient's
577 Nail Plates. European Journal of Pharmaceutics and Biopharmaceutics 102, 178-184.
578 Murdan, S., 2016. Nail disorders in older people, and aspects of their pharmaceutical treatment. Int.
579 J. Pharm., in press.
580 Murdan, S., Kerai, L., Hossin, B., 2015. To what extent do in vitro tests correctly predict the in vivo
581 residence of nail lacquers on the nail plate? Journal of Drug Delivery Science and Technology 25, 23-
582 28.
583 Onytec, Package leaflet: Information for the user Onytec 80 mg/g medicated nail lacquer, Ireland, p.
584 Patient Information Leaflet.
585 Rietschel, R.L., Fowler, J.F., Fisher, A.A., 2008. Medical Devices, Implants, and Equipment, in:
586 Rietschel, R.L., Fowler, J.F., Fisher, A.A. (Eds.), Fisher's Contact Dermatitis 6. BC Decker Inc., Ontario,
587 pp. 370-392.
588 Saner, M.V., Kulkarni, A.D., Pardeshi, C.V., 2014. Insights into drug delivery across the nail plate
589 barrier. J. Drug Target. 22, 769-789.
590 Scher, R.K., 1994. ONYCHOMYCOSIS IS MORE THAN A COSMETIC PROBLEM. Br. J. Dermatol. 130, 15-
591 15.
592 Schoon, D., 2010. Cosmetic Prostheses as Artificial Nail Enhancements. Blackwell Publishing Ltd.,
593 Oxford.
594 Shivakumar, H.N., Juluri, A., Desai, B.G., Murthy, S.N., 2012. Ungual and Transungual drug delivery.
595 Drug Dev. Ind. Pharm. 38, 901-911.
596 Spruit, D., 1971. Measurement of Water Vapor Loss Through Human Nail In Vivo. Journal of
597 Investigative Dermatology 56, 359-361.
598 Tatsumi, Y., Yokoo, M., Senda, H., Kakehi, K., 2002. Therapeutic Efficacy of Topically Applied KP-103
599 against Experimental Tinea Unguium in Guinea Pigs in Comparison with Amorolfine and Terbinafine.
600 Antimicrobial Agents and Chemotherapy 46, 3797-3801.
601 Thomas, J., Jacobson, G.A., Narkowicz, C.K., Peterson, G.M., Burnet, H., Sharpe, C., 2010. Toenail
602 onychomycosis: an important global disease burden. J. Clin. Pharm. Ther. 35, 497-519.
603 Yoshii, E., 1997. Cytotoxic Effects of Acrylates and Methacrylates: Relationships of Monomer
604 Structures and Cytotoxicity. Journal of Biomedical Materials Research 37, 517-524.

605

606

Figure legends

608 Figure 1 *In vivo* residence of UV-cured films (± ethanol and ± drug) on the ten fingernails in
609 six volunteers. Curanail lacquer (containing amorolfine HCl) was used as a commercially
610 available control. Abbreviations: ETOH – ethanol; AH – amorolfine HCl; TH – terbinafine HCl.
611 Means and standard deviations are shown, n=60.

612 Figure 2: Chemical structures and viscosities of the different methacrylate-based monomers

613 Figure 3: Scanning electron micrographs of the top surface (i.e. exposed to UV light), under
614 surface (i.e. in contact with the support), and cross-sectional surface of a drug-free, ethanol-
615 free UV-cured film (DUDMA:HEMA 85:15). Films with the other methacrylate monomers,
616 and ± ethanol and ± drug (amorolfine HCl or terbinafine HCl) had similar micrographs.

617 Figure 4: Cumulative amount of drug release from the amorolfine HCl loaded UV-cured films.
618 Means and standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; ETOH,
619 ethanol.

620 Figure 5: Cumulative amount of drug release from the terbinafine HCl loaded UV-cured.
621 Means and standard deviations are shown, n=3. Abbreviations: TH, terbinafine HCl; ETOH,
622 ethanol.

Figure 6: Cumulative amount of amorolfine HCl permeated across the nail with time from
the UV-cured and Curanail® films, and the % of drug permeated across the nail and
remaining in the nail at day 30. Means and standard deviations are shown, n=6.
Abbreviations: AH, amorolfine HCl; ETOH, ethanol.

Figure 7: Cumulative amount of terbinafine HCl permeated across the nail with time from
the UV-cured films, and the % of drug permeated across the nail and remaining in the nail at
day 30. Means and standard deviations are shown, n=6. Abbreviations: TH, terbinafine HCl;
ETOH, ethanol.

Figure 8: Reduction of TOWL by the different UV-cured films and the Curanail lacquer film
control. Means and standard deviations are shown. n=5

Table(s)

Table 1: Table shows details of test formulations used for anti-fungal efficacy assessment. In addition, drug-free counterparts of these and Curanail were used as controls.

Nature of monomer	Ethanol concentration	Drug nature and concentration
EMA	25%	Amorolfine HCl; 3%
IBOMA	25%	Amorolfine HCl; 3%
HEMA	25%	Amorolfine HCl; 4%
HEMA	None	Amorolfine HCl; 2%
HEMA	25%	Terbinafine HCl; 6%

Table 2 Solubilities of amorolfine HCl and terbinafine HCl in the three methacrylate monomers; means and standard deviations are shown, n=3.

	Solubility (mg/ml)		
	EMA	**IBOMA**	**HEMA**
Amorolfine HCl	0.10 ± 0.01	0.19 ± 0.02	209.3 ± 9.4
Terbinafine HCl	0.66 ± 0.19	0.12 ± 0.02	229.0 ± 9.8

Table 3 Theoretical and actual drug-load in UV-curable gel formulations. AH- amorolfine HCl, TH- terbinafine hydrochloride. N=3. Sd=0 for all. The theoretical (based on drug solubility in the gel components) and actual (determined by the absence/presence of drug crystals in the formulation) drug loadings were fairly similar for all the gels except for ethanol-free HEMA-containing gels, where the actual drug-load was much lower than anticipated, due to drug precipitation upon mixing with DUDMA and the photoinitiator.

Solvent	Methacrylate-based monomer	Drug	Expected Drug load (% w/v)	Actual drug load (% w/v)
Ethanol (25% v/v)	EMA	AH	3.0	3
		TH	3.5	4
	IBOMA	AH	3.0	3
		TH	3.5	4
	HEMA	AH	5.2	4
		TH	6.0	6
None	HEMA	AH	5.1	2
		TH	5.6	2
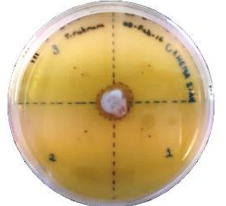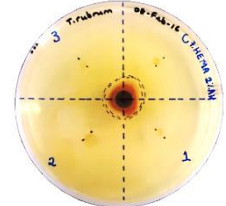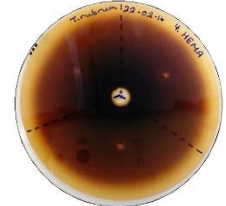
Table 4: Transition temperatures (Tg) of UV-cured films. Means ± standard deviations are shown, n=3.

Tg values of UV-cured films produced from formulations containing						
Excipients	**DUDMA**	**Solvent Ethanol**	**Drug**	**EMA**	**IBOMA**	**HEMA**
Formulations	DUDMA 85 % v/v : diluent monomer 15 % v/v	None	None	85.7 ± 1.2 and 146.0 ± 2.5	67.2 ± 2.4 and 164.0 ± 0.2	74.4 ± 2.5 and 147.2 ± 0.3
		25% v/v	None	112.5 ± 1.8	139.2 ± 9.0	98.1 ± 2.3
			AH	109.2 ± 1.1	137.2 ± 0.02	99.2 ± 1.8
			TH	109.6 ± 0.8	133.4 ± 3.6	94.7 ± 4.8
		None	None	95.1 ± 1.5	NF	NF
			AH	94.8 ± 2.0	NF	NF
			TH	95.0 ± 1.8	NF	NF
	DUDMA 75 % v/v : diluent monomer 25 v/v	None	None	NF	NF	81.8 ± 0.5 and 142.6 ± 0.4
			AH	NF	NF	92.0 ± 0.9 and 138.9 ± 0.5
			TH	NF	NF	92.4 ± 0.4 and 133.2 ± 0.2
Tg of homopolymers (°C)	-	-	-	65	110	57

Table 5 Ungual permeation parameters. Lag time, steady-state flux, permeability coefficient, diffusion coefficient and amount of drug in nail clippings. Means and standard deviations are shown, n=6. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

	Amorolfine HCl (AH)						Terbinafine HCl (TH)					
	% in formula-tion (% w/v)	Lag time (day)	Steady-state flux ($\mu g/cm^2/day$)	Permeability coefficient x 10^{-5} (cm/day)	Diffusion coefficient x 10^{-5} (cm^2/day)	Drug in nail clipping (mg/cm^3)	% in formula-tion (% w/v)	Lag time (day)	Steady-state flux ($\mu g/cm^2/day$)	Permeability coefficient x 10^{-5} (cm/day)	Diffusion coefficient x 10^{-5} (cm^2/day)	Drug in nail clipping (mg/cm^3)
Curanail® nail lacquer	5	10.4 ± 1.3	2.9 ± 0.2	5.8 ± 0.3	2.5 ± 0.5	0.6 ± 0.2	NA	NA	NA	NA	NA	NA
DUDMA & EMA gel containing ETOH & AH or TH	3	10.4 ± 0.9	2.4 ± 0.2	7.9 ± 0.7	2.7 ± 0.8	0.3 ± 0.2	4	9.5 ± 1.2	1.5 ± 0.1	3.7 ± 0.3	1.6 ± 0.4	0.9 ± 0.2
DUDMA & IBOMA gel containing ETOH & AH or TH	3	10.5 ± 0.6	2.3 ± 0.3	7.8 ± 1.0	2.2 ± 0.4	0.4 ± 0.1	4	8.3 ± 0.8	1.4 ± 0.3	3.4 ± 0.8	3.1 ± 0.9	0.4 ± 0.1
DUDMA & HEMA gel containing ETOH & AH or TH	4	7.1 ± 1.7	2.2 ± 0.3	5.5 ± 0.7	3.5 ± 1.4	0.6 ± 0.1	6	7.1 ± 1.0	1.6 ± 0.2	2.6 ± 0.4	3.0 ± 0.8	0.7 ± 0.2
DUDMA & HEMA gel containing AH or TH	2	10.0 ± 1.9	1.4 ± 0.1	7.0 ± 0.7	3.2 ± 0.4	0.3 ± 0.2	2	8.9 ± 1.0	0.7 ± 0.1	3.5 ± 0.7	2.6 ± 0.9	0.3 ± 0.1

Table 6 Photographic images (at day 30) of *T. rubrum* inoculated SDA plates containing nail clippings with drug-free and drug-loaded formulations cured on the surface. An example, using the ethanol-free HEMA containing formulation is shown. All the other formulations showed similar anti-fungal activity and the images are shown in the Supplementary file.

Formulation applied on nail clipping surface	*T. rubrum* inoculated SDA plates containing nail clippings with drug-loaded formulations cured on the surface (n=3) and the corresponding drug-free formulation cured on the surface (C)		*T. rubrum* inoculated SDA plates containing nail clippings with drug-free formulations cured on the surface (n=3)	
	Top surface	**Under surface**	**Top surface**	**Under surface**
None (nail clipping-free & formulation-free control)				
DUDMA & HEMA gel ± 2% w/v AH				
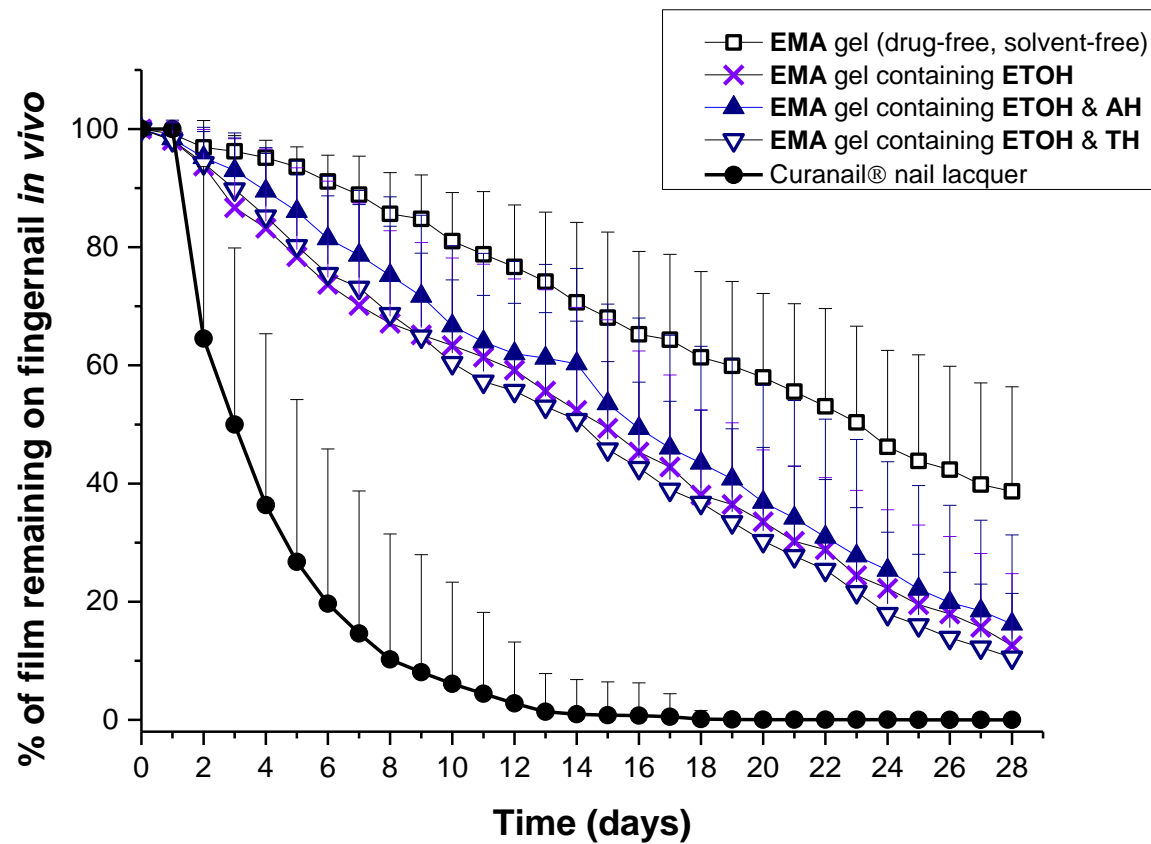
Figure 1 *In vivo* residence of UV-cured films (± ethanol and ± drug) on the ten fingernails in six volunteers; n=60, means and standard deviations are shown. Curanail lacquer (containing amorolfine HCl) was used as a commercially available control. Abbreviations: ETOH – ethanol; AH – amorolfine HCl; TH – terbinafine HCl.
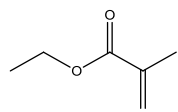
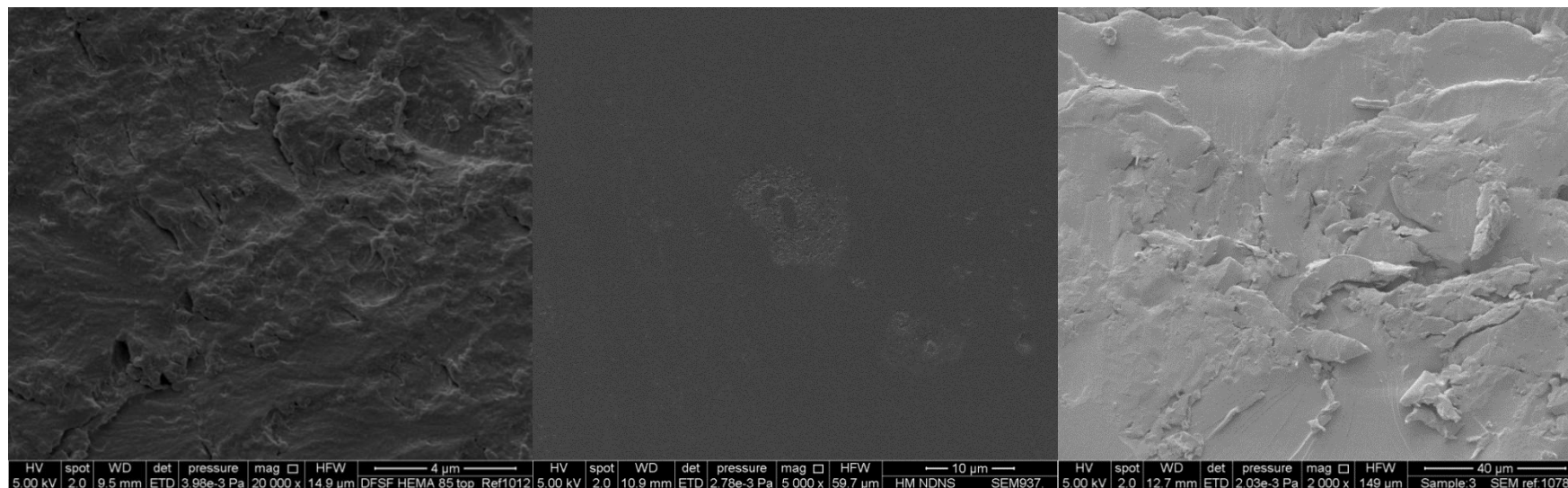**Ethyl methacrylate, MW 114, Viscosity 0.62 mPas**
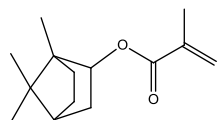
Isobornyl methacrylate, MW 222, Viscosity 7.40 mPas
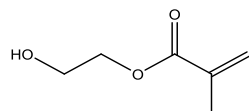
Hydroxyethyl methacrylate, MW 130, Viscosity 6.79 mPas

Figure 2: Chemical structures and viscosities of the different methacrylate-based monomers

| Top surface | Under surface | Cross-section |

Figure 3: Scanning electron micrographs of the top surface (i.e. exposed to UV light), under surface (i.e. in contact with the support), and cross-sectional surface of a drug-free, ethanol-free UV-cured film (DUDMA:HEMA 85:15). Films with the other methacrylate monomers, and ± ethanol and ± drug (amorolfine HCl or terbinafine HCl) had similar micrographs.
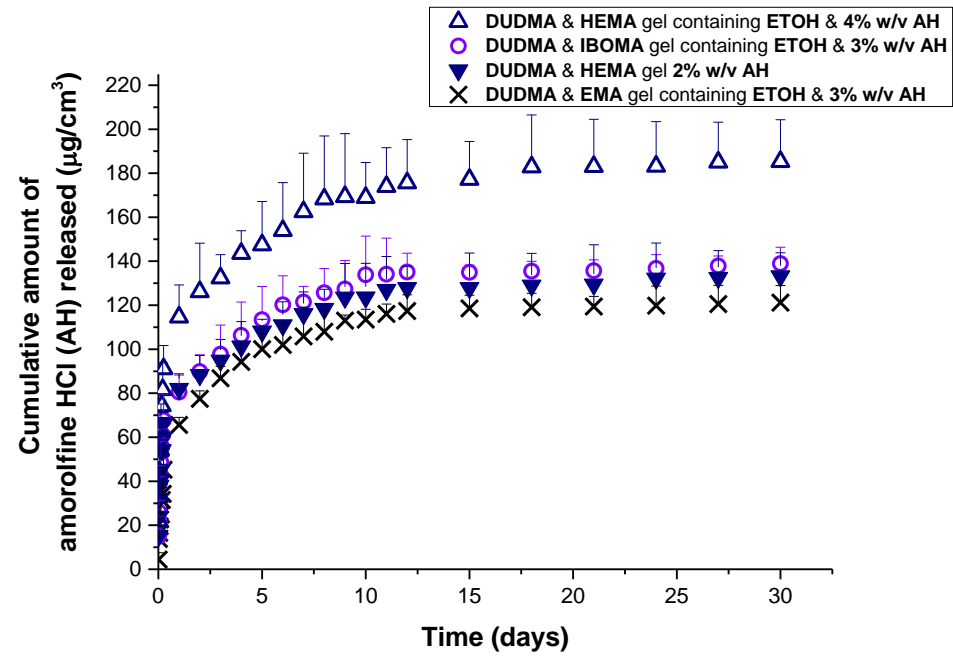
Figure 4: Cumulative amount of drug released from the amorolfine HCl loaded UV-cured films. Means and standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl.
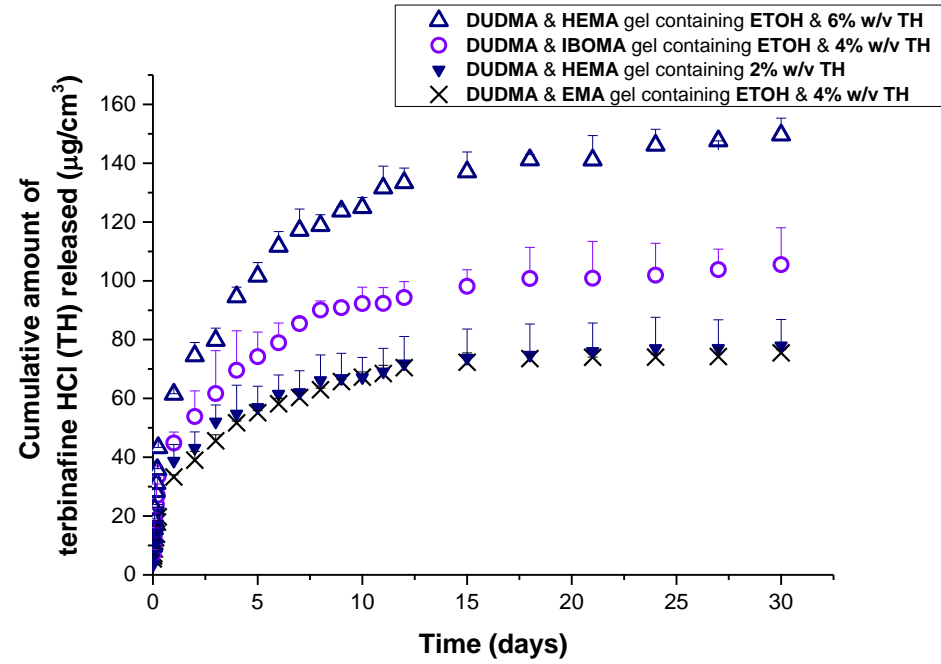
Figure 5: Cumulative amount of drug released from the terbinafine HCl loaded UV-cured films. Means and standard deviations are shown, n=3. Abbreviations: TH, terbinafine HCl.
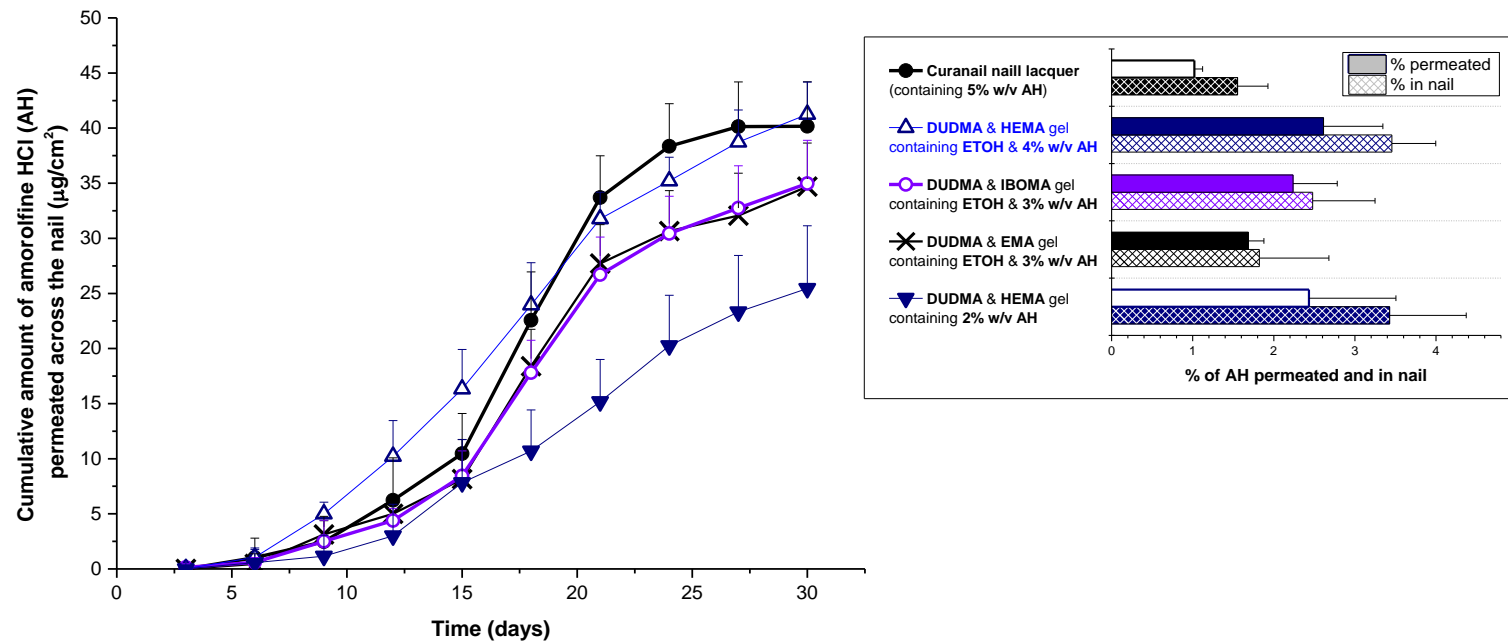
Figure 6 Cumulative amount of amorolfine HCl permeated across the nail with time from the UV-cured and Curanail® films, and the % of amorolfine HCl permeated across the nail and remaining in the nail at day 30. Means and standard deviations are shown, n=6. Abbreviations: AH, amorolfine HCl; ETOH, ethanol.

The permeation profiles for the gel containing the highest drug load (4% amorolfine HCl) was significantly different ($p<0.05$) to those containing 3% w/v amorolfine HCl which were similar ($p>0.05$), and which were in turn significantly higher ($p<0.05$) than the gel containing the lowest drug load (2% w/v amorolfine HCl). The steady-state flux was lowest ($p<0.05$) from the film with the lowest drug load.
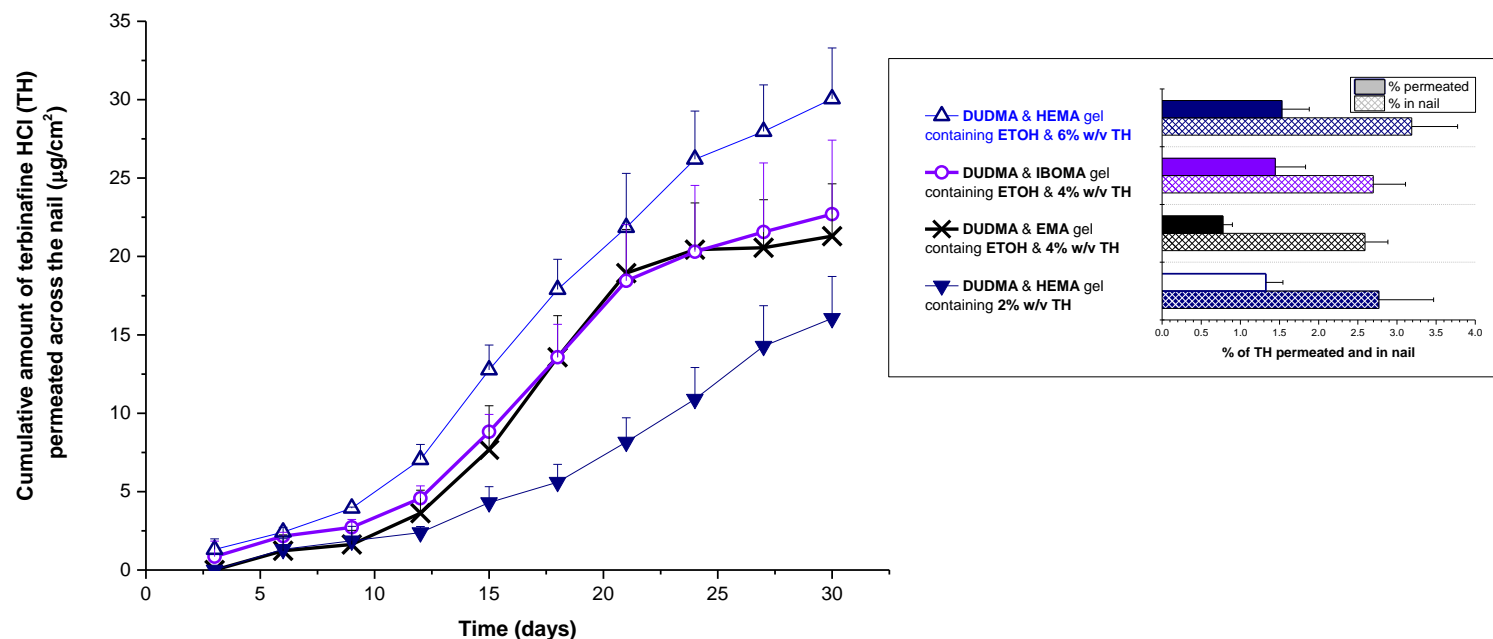
Figure 7 Cumulative amount of terbinafine HCl permeated across the nail with time from the UV-cured films, and the % of terbinafine HCl permeated across the nail and remaining in the nail at day 30. Means and standard deviations are shown, n=6. Abbreviations: TH, terbinafine HCl; ETOH, ethanol.

The permeation profiles for the gel containing the highest drug load (6% terbinafine HCl) was significantly different ($p<0.05$) to those containing 3% w/v terbinafine HCl, which were similar ($p>0.05$), and which were in turn significantly higher ($p<0.05$) than the gel containing the lowest drug load (2% w/v terbinafine HCl). The steady-state flux was lowest ($p<0.05$) from the film with the lowest drug load.
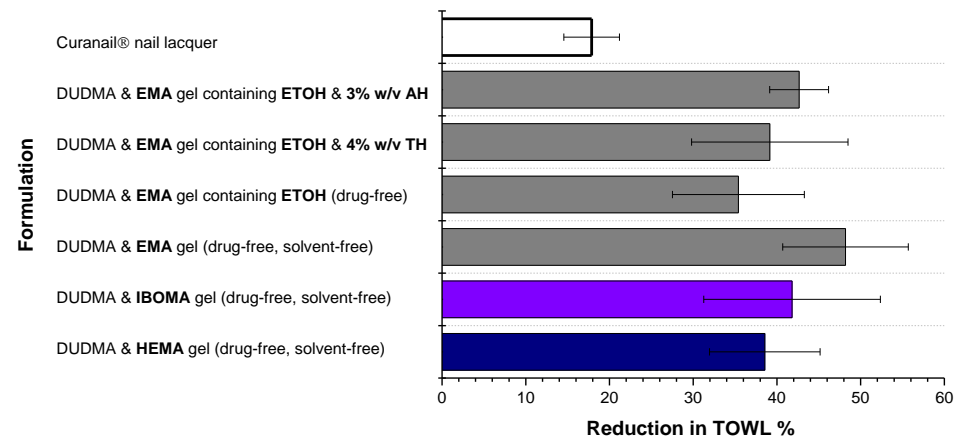
Figure 8: Reduction of TOWL by the different UV-cured films and the Curanail lacquer film control. Means and standard deviations are shown. n=5

Supplementary Material