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Abstract

Purpose: The pH discrepancy between healthy and atopic dermatitis skin was identified as a site specific trigger for delivering hydrocortisone from microcapsules.

Methods: Using Eudragit L100, a pH-responsive polymer which dissolves at pH 6, hydrocortisone-loaded microparticles were produced by oil-in-oil microencapsulation or spray drying. Release and permeation of hydrocortisone from microparticles alone or in gels was assessed and preliminary stability data was determined.

Results: Drug release from microparticles was pH-dependent though the particles produced by spray drying also gave significant non-pH dependent burst release, resulting from their porous nature or from drug enrichment on the surface of these particles. This pH-responsive release was maintained upon incorporation of the oil-in-oil microparticles into Carbopol- and HPMC-based gel formulations. *In-vitro* studies showed 4 to 5-fold higher drug permeation through porcine skin from the gels at pH 7 compared to pH 5.

Conclusions: Permeation studies showed that the oil-in-oil generated particles deliver essentially no drug at normal (intact) skin pH (5.0 – 5.5) but that delivery can be triggered and targeted to atopic dermatitis skin where the pH is elevated. The incorporation of these microparticles into Carbopol- and HPMC-based aqueous gel formulations demonstrated good stability and pH-responsive permeation into porcine skin.

Keywords

pH-responsive microparticles, Eudragit L100, hydrocortisone, Carbopol, hydroxypropyl methyl cellulose.

1 Introduction

The introduction of anti-inflammatory topical steroids into medicine about 50 years ago represents a significant landmark in dermatologic therapy and remains to date the mainstay of atopic dermatitis management (1). Although hydrocortisone is regarded as a safe alternative to the more potent corticosteroids, its "over-the-counter" availability makes it particularly liable for abuse. The use of more potent steroids, especially when treating paediatric patients, can cause serious systemic side effects such as hypothalamic-pituitary-adrenal axis suppression and it has been reported that as little as 2 g per day of clobetasol propionate, 0.05% w/w cream, can decrease morning cortisol levels after only a few days (2).

There is a clear need to optimise these therapies using drug delivery strategies. Ideally, new glucocorticoids with a better therapeutic index need to be developed. However, this is difficult to achieve as the receptors responsible for drug activity are also responsible for the occurrence of side effects. Novel selective glucocorticoid receptor ligands with better therapeutic profiles are being developed (3), but site-specific delivery strategies, such as drug encapsulation, offers an alternative approach for minimising adverse reactions.

The pH discrepancy between normal and atopic dermatitis skin provides a potential trigger for a controlled release formulation. Normal pH on the surface of adult skin is in the range of 5.0 to 5.9, depending on age, gender and body area (4-6). However, in the case of atopic dermatitis, there is a trend of increased stratum corneum surface alkalinity with reported pH values approximately 0.5 unit higher in patients with AD than in controls with healthy skin (pH of ~ 6 or over in diseased skin) (4, 7). A correlation between disease severity and surface skin pH was also observed. For instance, surface skin pH increases up to 7.3-7.4 in acute eczema with erosion (8). A trend towards more elevated pH values was also observed in non-involved skin of seborrhetic dermatitis, atopic dermatitis and xeroderma patients (9).

This study explores the use of pH-responsive polymeric microparticulates for site-specific corticosteroid delivery to treat atopic dermatitis. Eudragit L100, a polymer with a dissolution threshold of pH 6, was used to produce hydrocortisone-loaded microparticles by spray drying and oil-in-oil solvent evaporation methods. Drug release from both batches of microparticles was first evaluated to determine the degree of their pH-responsiveness and the mechanisms of drug release. The microparticles with the best pH-controlled release profiles were formulated into several aqueous gel preparations. *In-vitro* hydrocortisone permeation from the microparticles and formulations was assessed and a preliminary stability study was performed to examine drug leakage from the microparticles which would result in a loss of pH-responsiveness.

2 Materials and methods

2.1 Materials

Hydrocortisone was purchased from Sigma-Aldrich (UK). Eudragit L100 was kindly provided by Röhm (Germany). Ethanol, hexane (laboratory grades) and sorbitan sesquioleate were obtained from Sigma-Aldrich (UK). Sodium dodecyl sulfate and Liquid Paraffin BP were purchased from Fisher Scientific (UK). Sodium phosphate dibasic heptahydrate and sodium phosphate monobasic dehydrate (Sigma-Aldrich, UK) were used to prepare the dissolution media. Carbopol 940 (Acros Organics, Belgium) and Metolose 90SH-SR (hydroxypropyl methyl cellulose, HPMC) (Shin Etsu, Japan) were used for aqueous gel preparation. Nitrocellulose membranes (MF membrane, pore size 0.22 µm, 25 mm diameter) used in diffusion testing were purchased from Sigma Aldrich, UK.

2.2 Preparation of pH-responsive microparticles

Hydrocortisone-loaded Eudragit L100 microparticles were produced using the spray drying and oil-in-oil solvent emulsification methods as detailed in our previous articles (10, 11). Briefly, a 2.5% w/v polymeric feed solution was spray dried from a 50:50 v/v ethanol/water co-solvent system using the following parameters; a 1.5 ml/min feed rate, a 28 m^3/h flow of heated nitrogen and an inlet temperature of 70^oC. To obtain the oil-in-oil microparticles, a 10% w/v polymer ethanolic solution was emulsified in liquid paraffin and stirred overnight at 1200 rpm to allow for complete solvent evaporation and particle solidification (12). The drug was incorporated into the polymeric microparticles at 2.5, 10 and 25% w/w loading with respect to polymer weight.

2.3 Preparation of gel formulations

Eudragit L100 microparticles prepared from the oil-in-oil emulsification process at 10% w/w drug loading were incorporated into aqueous formulations gelled with either 1.5% w/v Carbopol 940 or 2% w/v Metolose 90SH-SR and containing 1% w/v phenoxyethanol (ethyleneglycol-monophenyl ether) as a preservative. The final concentration of hydrocortisone within all gel preparations was 1% w/w.

2.4 Release studies

Release testing of hydrocortisone-loaded Eudragit L100 microparticles used nitrocellulose membranes in Franz-type diffusion cells with receptor compartments containing 15 ml of 0.1 M phosphate buffer solutions with pH's ranging from 5 to 7. A finite amount of the microparticles was used in the donor compartment: 0.5 mg, 3.3 mg and 12 mg for 25, 10 and 2.5% w/w drug-loaded microparticles ($C<0.1\ C_s$ upon complete drug release), over a surface area of 3.14 cm^2. The Franz-cells were immersed in a water bath at 37 ± 1^0 C in order to maintain the membrane surface temperature at 32 ± 1^0 C. Samples (1ml) were taken

periodically from the receptor compartment, replaced with fresh pre-heated phosphate buffer and analysed using UV spectroscopy at 248 nm.

2.5 Permeation studies

Although human skin is the most relevant membrane for percutaneous drug absorption, due to its limited availability for experimental use, a wide range of animal models has been investigated as a replacement. Porcine skin was found to be a good surrogate for human skin in a number of in-vitro studies (e.g. 13, 14) and was used in this study. Pig ears were obtained from a local abattoir (within 6 hrs of animal sacrifice), cleaned under cold running water before full thickness skin was removed from the underlying cartilage (frozen skin was used within 2 month). Permeation experiments were performed using Franz-type diffusion cells (receptor volume 15 ml, surface area 3.14 cm^2). Full thickness skin (mean thickness 1.20±0.15 mm, n=20) was mounted between the donor and receptor compartments with the stratum corneum side up before equilibration with the receptor buffer pH for 2 hours prior to the experiment. 10% w/w Hydrocortisone-loaded microparticles or 1% w/w hydrocortisone Carbopol- and Metolose-based gel preparations were then applied to the skin at a finite dose (3.3 mg of microparticles and 30 mg of gel). The permeation studies were performed under occlusion with Parafilm to ensure hydration and equilibration of the stratum corneum with the phosphate buffer receptor at pH 5 or 7. Samples (1ml) of receptor solution were taken periodically, replaced with fresh pre-heated buffer and analysed with reverse-phase high performance liquid chromatography (Hypersil ODS, 5 µm particle size, 250 x 4 mm) using UV detection at 248 nm. The high performance liquid chromatography (HPLC) system consisted of a Spectra-Physics SP800 ternary HPLC pump, a Spectroflow 757 UV detector and an HP 3395 integrator. A mobile phase of 60:40 v/v methanol/water was used at 1.1 ml/min which gave a retention time (R_t) of 5.2 min. The calibration curve was linear over 0-

25 µg/ml (R^2> 0.99) with a limit of detection (LoD) and quantification (LoQ) of 0.87 µg/ml and 2.89 µg/ml respectively. The injection volume was set at 20 µl.

2.6 Stability testing

Stability testing of the 1% w/w hydrocortisone gel formulations (Carbopol- and Metolose-based) was carried out over twelve weeks at room temperature. Gel samples were diluted with water, centrifuged then filtered before the filtrate was analysed by reverse-phase HPLC to determine drug leakage from the particles into the gel, whereas the collected microparticles were dissolved in ethanol and analysed by UV to establish the percentage of encapsulated drug remaining.

2.7 Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical significance was assessed using the Student's t-test, for two batch drug release comparison, and one way analysis of variance (ANOVA), when the difference between the results of more than two microparticulate systems are evaluated (Genstat; version 12). The correlation coefficient (R^2) is reported in these cases. For permeation experiments, statistical analysis was carried out using the Mann-Whitney U test. In all cases, $p < 0.05$ denotes significance.

3 Results

The characteristics of our pH-responsive microparticles were described in our previous articles (10, 11). Briefly, the encapsulation efficiency of the drug was relatively high with over 70% of the drug encapsulated in all cases (10, 11). Differential scanning calorimetry and X-ray powder diffraction data showed that hydrocortisone was encapsulated as a solid solution, except for the oil-in-oil microparticles produced at 25% w/w drug-loading where about 50% of the drug was incorporated in its crystalline form (10). Scanning electron

microscopy confirmed these results with visible drug crystals only observed at the surface of 25% w/w drug-loaded oil-in-oil microparticles and not in images of particles with lower drug contents.

Hydrocortisone release profiles from the microparticles at different pH values are presented in Figure 1. Eudragit L100 microparticles prepared from the oil-in-oil emulsification method showed better pH-controlled release than the spray dried microparticles, with negligible hydrocortisone release at pH 5 at 2.5% and 10% w/w drug-loading (Figure 1, A and B) and in agreement with results obtained from dissolution testing (10). In contrast, significant drug release was observed at both pH 5.0 and 5.5 for 2.5% w/w drug-loaded Eudragit L100 microparticles produced from the spray drying method (Figure 1, D). In both cases, the drug was incorporated at a level well below its solubility within the polymer matrix (11) suggesting that differences in drug release profiles are related to the method of microencapsulation rather than the level of drug loading. In fact, solvent evaporation during the spray drying process occurs rapidly (due to the high temperatures used) leading to the formation of porous microparticles. The porosity of these microparticles might be in part implicated in the burst release effect observed at low pH values (5.0 and 5.5), where the polymers are not soluble. Another explanation for this phenomenon is the possible deposition of the drug on or near the surface of the microparticles leading to non-pH-controlled drug leakage. Loading the microparticles with 25% hydrocortisone exceeds the solubility of the drug in the polymer and results in uncontrolled burst release of the excess drug from both the spray dried and oil-in-oil generated materials (Figures 1C and F)

INSERT FIGURE 1

Despite differences in drug release profiles, further analysis of hydrocortisone release from the 2.5% w/w drug-containing Eudragit L100 microparticles at different time points (Table 1) showed no statistical difference in terms of pH-responsiveness ($p>0.05$). The pH-

responsiveness of Eudragit L100 is reported in Table 1 as percentage hydrocortisone release per pH unit. These values were obtained by plotting the percentage drug release against pH and calculated from the gradient of the line of best fit. These data confirm that the preparation method did not alter the pH-responsiveness of the polymer and is supported by DSC, X-ray and Raman analysis reported in our previous study (10) as no differences were observed between unprocessed polymer and the microparticles obtained from the different preparation methods at the various drug loadings.

INSERT TABLE 1

Maximum release rates (J_{max}) for the different microparticles batches are reported in Table 2 At pH 5, maximum drug release rates were significantly higher for the spray dried microparticles than the oil-in-oil produced material ($p<0.05$). At pH7, where the polymer dissolves, the maximum release rate and time to achieve this were not significantly different ($P>0.05$) between the two production methods. These findings support the view that apart from the burst release effect observed with the spray dried microparticles (about 30% after 3 hrs, Figure 1, D), which is responsible for the relatively high flux values at pH 5, the two preparation methods produce equally pH-responsive particles.

INSERT TABLE 2

Release testing showed that incorporating 10% w/w hydrocortisone-loaded microparticles into the Carbopol- and HPMC-based gel formulations maintained pH-responsiveness (Figure 2, A and C), similar to that observed for the microparticles alone (Figure 1, B). However, drug release from the neutralised Carbopol gel showed significantly lower drug release at pH 7 (Figure 2, B), which is probably due to increased viscosity of the formulation. Because the pKa of Carbopol is 6.0±0.5, the carboxylate moiety on the polymer backbone ionizes with increased pH, resulting in repulsion between the negative charges, which adds to the swelling

of the polymer thus increases viscosity (15). This also demonstrates that adjusting formulation viscosity can confer further slow-release properties to the pH-responsive formulation.

INSERT FIGURE 2

Permeation across full thickness porcine skin was measured using receptor compartments containing phosphate buffer at pH 5 or 7. The skin was equilibrated with the buffer for 2 hrs prior to the experiment to develop the appropriate surface pH. A comparison of the cumulative amount of hydrocortisone permeating the skin after 24 hours is given in Figure 3. Permeation from Carbopol hydrogels before and after neutralisation showed similar pH-responsiveness to the free powder, with about 4-fold greater permeation of the drug after 24 hours at pH 7 compared to pH 5 ($p<0.05$). Similarly, the HPMC-based gel formulation showed significantly higher drug permeation at pH 7 (about 5-fold greater than the amount permeated after 24 hours at pH 5). In contrast to drug permeation from the particles, the difference in hydrocortisone permeation from a saturated aqueous solution at pH 5 and 7 is statistically non-significant. This is expected as the solubility of the drug at pH 5 and 7 was found to be essentially the same (0.252±0.01 mg/ml) (11).

INSERT FIGURE 3

Increasing the pH of the Carbopol formulation to pH 5, in order to prevent skin pH alteration, did not affect the amount of drug permeating the skin at pH 5 and 7 (Figure 3). Nonetheless, increasing the pH of the formulation from 3 to 5 could potentially negatively influence the efficient encapsulation of the drug within Eudragit L100 microparticles. Eudragit L100 has a reported pKa value of 6.45±0.03, this means that a greater degree of carboxyl group ionisation will be present at pH 5 compared to pH 3. Increased polymer ionisation might lead to greater swelling and potentially a greater degree of drug loss into the external hydrogel phase through diffusion; drug leakage from the incorporated microparticles can result in the

loss of formulation pH-responsive properties. In this study, the possible effects of Carbopol hydrogel neutralisation on formulation stability was monitored before and after the addition of the base (Figure 4, A), and compared to drug leakage from microparticles in the HPMC gel (Figure 4, B).

INSERT FIGURE 4

4 Discussion

In recent years, polymeric nano- and micro-particulate systems have emerged as promising systems for site-specific drug delivery and they have been extensively studied for oral, parenteral and pulmonary drug administration (e.g. 12, 16). The application of such polymeric systems in topical drug delivery is thought to offer a number of advantages, including control of drug release and protection of unstable compounds (17).

Microencapsulation, using polymers such as poly(lactide-co-glycolide acid) (PLGA), for controlled-release topical drug delivery has previously been reported, for example to prolong the residence time of sunscreen agents in the stratum corneum (18) or to target vitamin A to the upper layers of the skin (19). However, reports concerning percutaneous uptake of nano- and micro-particles are contradictory (20, 21), though deposition into the follicles is well documented (22, 23).

Unlike previous studies where polymeric particulate systems have been designed to cross the intact skin layer or to accumulate in follicular openings, this study relies on a targeted drug delivery approach whereby microparticle dissolution occurs on the surface of the skin according to pH and results in drug release at the diseased site. Thus, the discrepancy in skin surface pH observed in normal and diseased skin is used to trigger drug release at the target site and so minimises delivery to non-diseased sites.

4.1 Hydrocortisone release from Eudragit L100 microparticles

The usefulness of drug release data in formulation design has been discussed by Flynn and Poulsen (24, 25). Because the efficiency of creams, ointments and gel products is dependent on drug release, *in-vitro* release testing constitutes an important and valuable tool for quality control. This is particularly important for controlled release drug formulations. In fact, the use of the diffusion model for polyvinyl alcohol (PVA) microparticles was shown to better differentiate between samples in terms of controlled-drug release kinetics compared to other methods of release testing such as dissolution studies (26).

The data in Figure 1 and Tables 1 and 2 clearly illustrate pH control over drug release from the Eudragit microparticles. Comparing Figures 1A and 1D, the spray dried materials give significantly greater drug release at pH 5 than from the oil-in-oil particles. However, with increasing pH, release from particles produced by either process were similarly pH responsive (Table 1) which indicates that the initial non-pH controlled release from the spray dried materials is due to poor drug encapsulation, perhaps from surface enrichment or the production of porous materials in which the polymer does not control release. Well-controlled drug release from oil-in-oil manufactured particles was achieved; for example, at a drug loading of 10%, only 1.02 μg cm^{-2} hr^{-1} hydrocortisone was released at pH 5 whereas the same particles liberated 26.6 μg cm^{-2} hr^{-1} at pH 7.

4.2 Formulation development

There are numerous examples on the market for powdered topical formulations such as, talc, zinc oxide and kaolin (27). However, such formulations are applied as drying, protective or lubricant agents. Difficulties associated with the application and dosing of powders can be overcome by incorporating them into a vehicle such as a cream, ointment, lotion or a gel.

The main criteria for incorporating powders intended for topical drug delivery into conventional formulations is that the individual powder particles should be impalpable, i.e. incapable of being perceived as individual particles by our sense of touch (28). In general, particles less than 50 μm in their longest dimension are invisible to the senses (17). A coarser powder can make the preparation gritty and cosmetically unacceptable. Microparticles prepared from the oil-in-oil emulsification process at 2.5% and 10% w/w drug-content fulfil this criterion with a mean equivalent circular area particle diameter of 41.5±0.6 and 39.3±11.2 μm respectively. The mean size of these microparticles can be further reduced using a higher homogenisation speed during the manufacturing process.

In this study, 10% w/w hydrocortisone-loaded Eudragit L100 microparticles were incorporated into a gel formulation to obtain a final drug content of 1% w/w, which is within the 0.5 to 2.5% w/w topical concentration commonly used for hydrocortisone. The use of microparticles with a lower drug-loading, e.g. 2.5% w/w, will require the incorporation of a larger amount of powder into the gel formulation, which would result in a preparation with a paste-like consistency that is difficult to spread (29).

For formulation development, two commonly used gelling materials were investigated; Carbopol 940 and Metolose 90SH-SR (HPMC). The carbomer (Carbopol 940) used in this study is an acrylic acid polymer cross-linked with allyl ethers of pentaerythritol (30, 31). The individual Carbopol cross-linked polymer particles swell when dispersed in an aqueous solution forming a colloidal, mucilage-like dispersion. Dermatological applications of Carbopol polymers are numerous; examples include formulations of local anaesthetics and cosmetics (e.g. 32-34). Carbopol polymers are also reported to have good suspending properties (35). For example, a permanent sand (average particle size of 0.06 cm) suspension was achieved using as little as 0.25% Carbopol 934 (36). The same physical stability was

reported for other insoluble compounds such as, pentobarbital sodium, chloramphenicol and sulphadimidine (34, 37).

Metolose 90SH-SR, a high-viscosity grade of hydroxypropyl methyl cellulose (HPMC), was also used. This is an example of a fully soluble polymer, which dissolves in aqueous media to form a highly viscous gel due to its relatively high molecular weight. The use of HPMC for dermatological gels has also been reported in the literature (34, 38). However, unlike Carbopol, this is a neutral polymer. In fact, the pH of the two prepared gels using 1.5% w/w Carbopol 940 or 2% w/w Metolose 90SH-SR were found to be 3.23±0.01 and 7.40±0.09 respectively. The addition of Eudragit L100 microparticles did not change the pH of the Carbopol gel, but for the HPMC-containing gel the pH was reduced to about 5.2 due to the anionic nature of Eudragit L100.

Carbopol 940 is also an anionic polymer whose use in a topical drug formulation can also modulate skin surface pH. This effect can be detrimental for the controlled pH-responsive release of hydrocortisone from the microparticles, as a reduced pH can result in reduced or no drug release at the affected areas of the skin. To circumvent this problem, the Carbopol hydrogel formulation was neutralised to pH 5.0 using 0.5 M NaOH. The use of other bases for Carbopol neutralisation, such as monoethaloamine and triethylamine, has been reported in the literature (28, 32, 33). However, these stronger bases are usually used for gel formulations prepared from a hydro-alcoholic co-solvent system, as the inclusion of an alcohol can affect polymer solubility leading to the precipitation of the neutralised Carbopol salt (39). It is therefore essential to select the appropriate neutraliser depending on the alcohol content of the formulation. Since, in this study an aqueous Carbopol formulation was employed, sodium hydroxide was selected to neutralise the polymer.

4.3 Release and permeation testing of microparticle-containing formulations

The results in Figure 2 illustrate that pH responsive release of hydrocortisone from the oil-in-oil generated particles was maintained when incorporated into formulations gelled with Carbopol or HPMC, though neutralizing the Carbopol gel did negatively impact on drug release. As described above, release from topical preparations provides a good indication as to permeation across skin membranes and the release data did indeed mirror the percutaneous absorption values in Figure 3. Thus the particles alone or in gelled formulations all delivered significantly greater amounts of hydrocortisone through porcine skin at pH 7 than at pH 5, in contrast to a standard saturated aqueous solution from which drug permeation was the same at both pH values. It is notable that the 10% particles showed a 26-fold increase in drug release at pH 7 compared to pH 5 (Table 2) but only a 4-fold increase in permeation at the higher pH value. This discrepancy reflects the presence of the stratum corneum as the rate limiting barrier to permeation of the liberated drug whereas the release studies provide no such barrier.

Our approach demonstrates that it is feasible to deliver the active ingredient to diseased skin sites with minimal delivery to surrounding (non-involved) tissue. Current formulations, such as typical creams, contain various ingredients which can act as permeation enhancers, including surfactants and fragrance agents such as terpenes. Such enhancers could promote the formation of a drug depot within the non-involved skin and hence increase potential adverse effects. Whilst we have not explored the potential for depot formation on long term use at diseased skin sites, our *in-vitro* proof-of-principle studies demonstrate that an *in-vivo* evaluation of our pH-triggered and targeted drug delivery formulation is merited.

4.4 Stability testing

The Eudragit L100 microparticles were predominantly stable within the Carbopol hydrogel with about 5% drug leakage into the bulk of the formulation before neutralisation (Figure 4). This is probably due to the high glass transition temperature of Eudragit L100 (150^0 C, (40)) which limits drug movement and diffusion into the external environment. In fact, the prevention of drug molecule aggregation, which can lead to crystal growth, through encapsulation into polymers with high transition temperatures, has been extensively studied (17, 41, 42). The limited diffusion of hydrocortisone from the Eudragit L100 microparticles coupled with the poor solubility of the drug in aqueous media explains the minimal drug leakage from the microparticles.

After Carbopol hydrogel neutralisation at week 6, drug leakage from the microparticles increased to about 15%. This is probably due to increased Eudragit L100 ionisation and swelling as discussed earlier. However, this value remained constant over the next 6 weeks, with 85% of the drug originally encapsulated remaining within the microparticles. With the HPMC-based gel formulation, hydrocortisone leakage from Eudragit L100 microparticles was minimal over the 12 weeks testing period, as demonstrated in Figure 4, B. A longer-term stability study is nonetheless necessary to fully evaluate the commercial viability of these novel pH-responsive formulations.

5 Conclusion

Eudragit L100 microparticles loaded with hydrocortisone intended for targeted delivery to atopic dermatitis areas of the skin were produced by two different methods, oil-in-oil microencapsulation and spray drying. Release from both batches of microparticles was equally pH-dependent though the particles produced by spray drying also gave significant

non-polymer controlled burst release, probably due to their porous nature or as a result of drug enrichment on the surface of these particles.

The release studies showed that the oil-in-oil generated particles deliver essentially no drug at normal (intact) skin pH (5.0 – 5.5) but that delivery can be targeted and controlled to atopic dermatitis skin where the pH is elevated to around pH 6.0-6.5. This pH-responsive release was maintained upon the incorporation of these microparticles into Carbopol- and HPMC-based gel formulations. *In-vitro* hydrocortisone permeation studies from these novel pH-responsive formulations showed a 4 to 5-fold increase in drug permeation after 24 hours at pH 7 compared to pH 5. A 12-week preliminary stability study monitored drug leakage from the incorporated microparticles which would result in a loss of pH-responsiveness. The study demonstrated relatively good stability of both formulations with better stability of the microparticles incorporated in the HPMC gel. Nonetheless, a long-term stability study and a blanching assay are necessary to fully evaluate the commercial viability and clinical efficacy of such formulations.

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Legend to Figures

Figure 1. Hydrocortisone release profiles from: A, B and C) 2.5%, 10% and 25% w/w hydrocortisone-containing microparticles produced from the oil/oil method and D, E and F) 2.5%, 10% and 25% w/w drug-loaded microparticles obtained from the spray drying technique (mean ± SD, n=3).

Figure 2. Release of hydrocortisone from: A) Carbopol hydrogel, B) neutralised Carbopol hydrogel and C) HPMC gel. Results are presented as mean ± SD, n=3-5.

Figure 3. Cumulative hydrocortisone (HC) permeation at pH 5 and 7 after 24 hrs from a saturated solution of hydrocortisone, 10% w/w hydrocortisone-loaded Eudragit L100 microparticles and various microparticle-containing gel formulations. Data are shown as mean ± SD, n=5.

Figure 4. Stability testing of 10% w/w hydrocortisone-loaded Eudragit L100 microparticles incorporated into; Top) Carbopol hydrogel and Bottom) HPMC gel. The percentage of drug leaking into the bulk of the formulation and that remaining within the microparticles was determined before and after Carbopol neutralisation at 6 weeks. Data is presented as mean ± SD, n= 3.

Figure 1

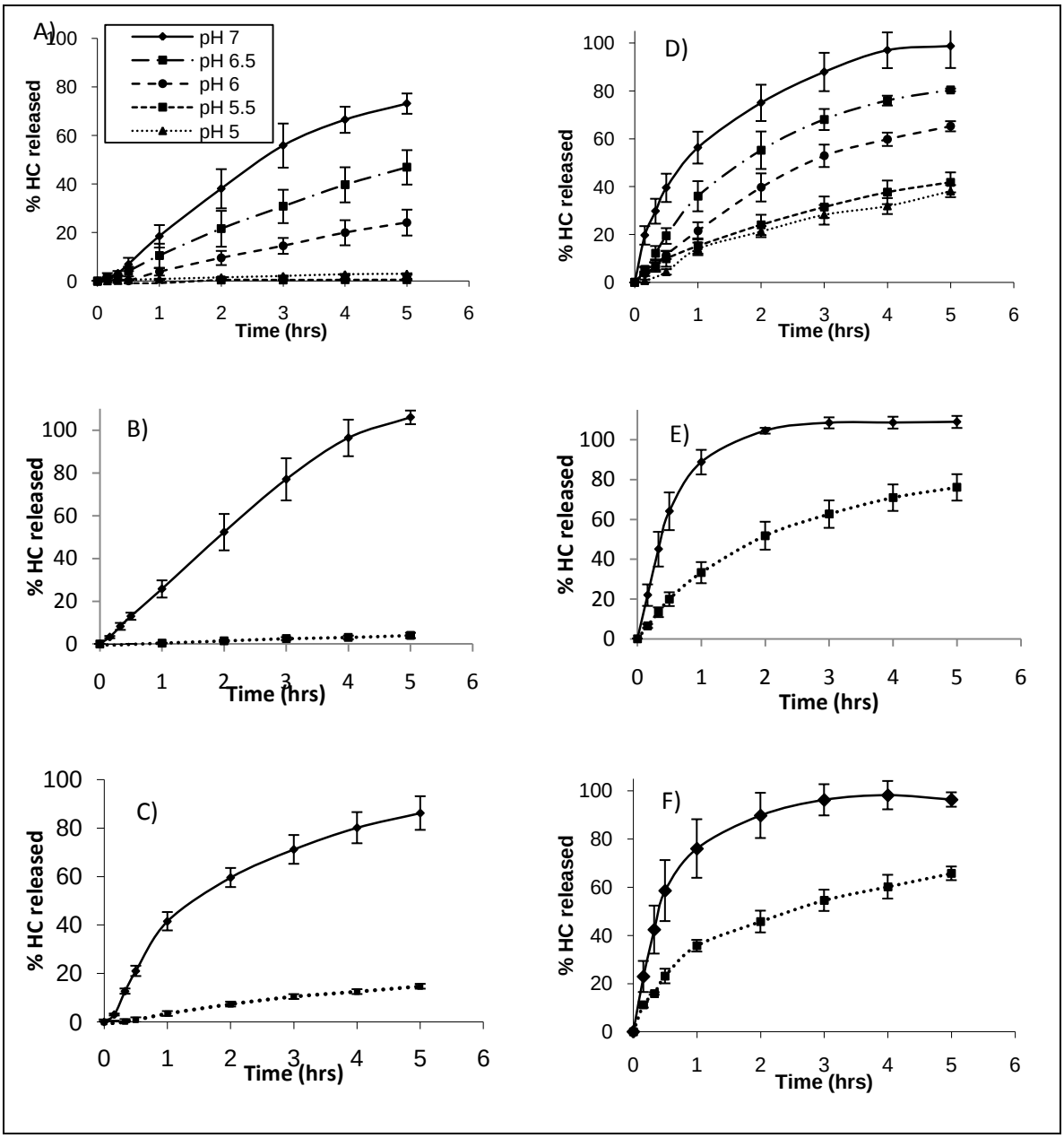


Figure 2

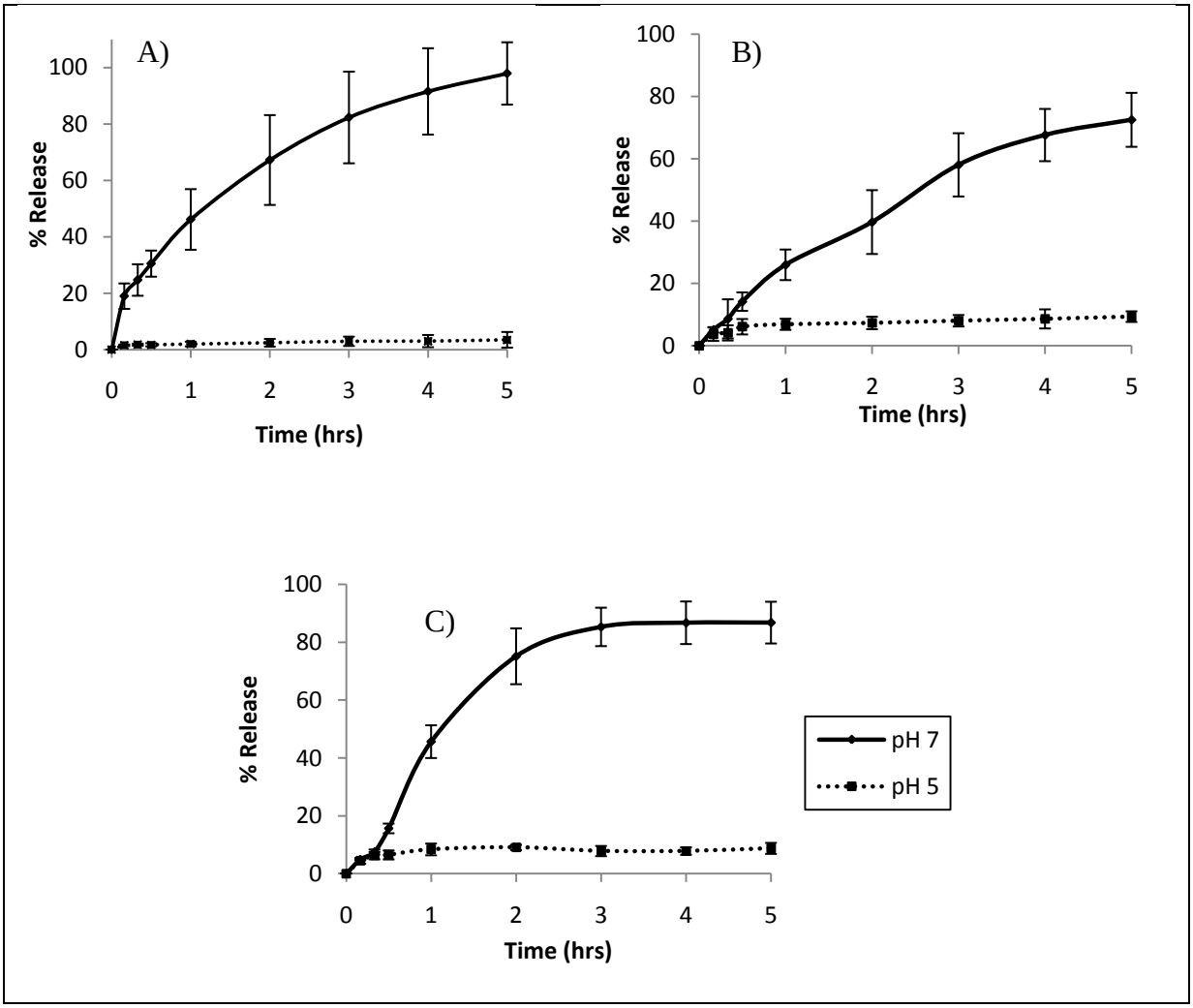

A)
% Release
Time (hrs)
B)
% Release
Time (hrs)
C)
% Release
Time (hrs)
pH 7
pH 5

Figure 3.

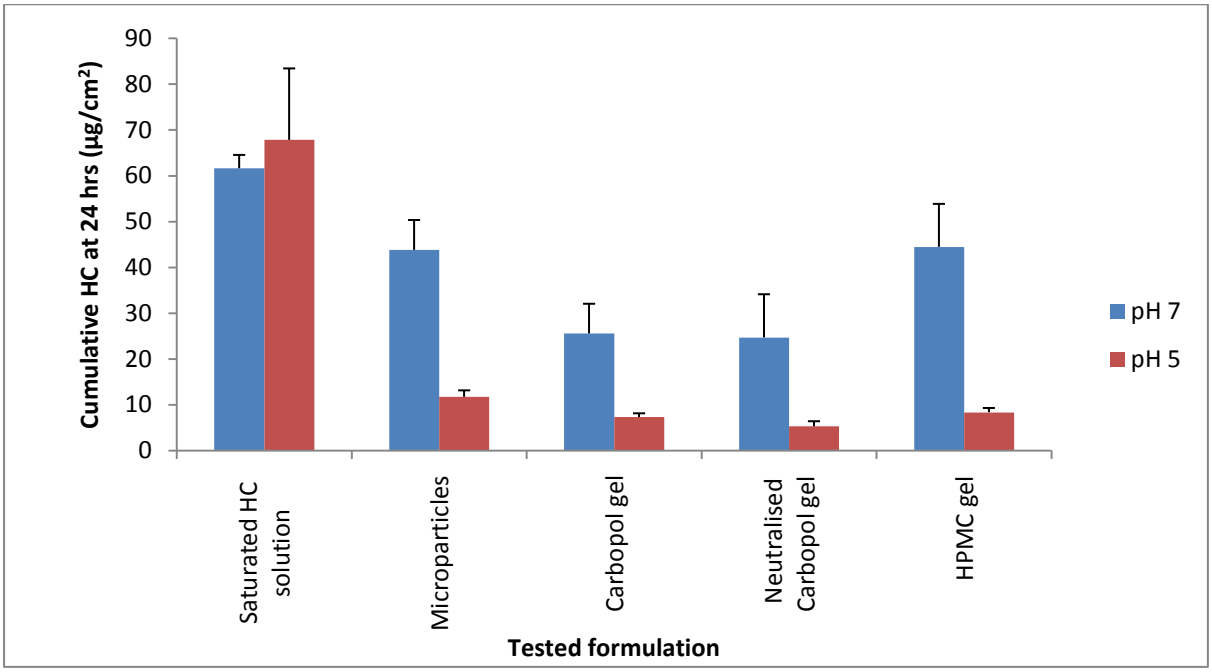
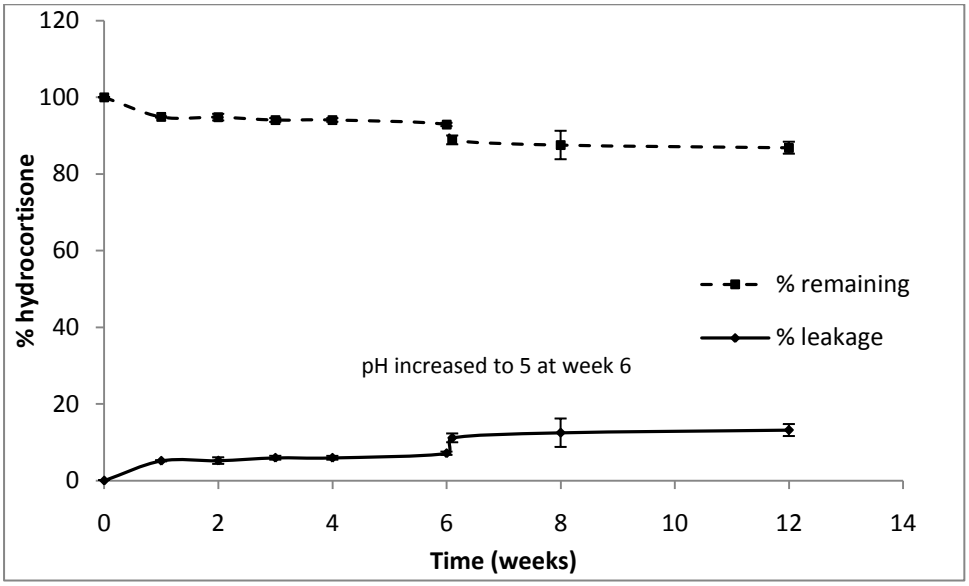

Cumulative HC at 24 hrs (µg/cm²)
90
80
70
60
50
40
30
20
10
0
Saturated HC solution
Microparticles
Carbopol gel
Neutralised Carbopol gel
HPMC gel
Tested formulation
pH 7
pH 5

Figure 4.


120
100
80
60
40
20
0
% hydrocortisone
% remaining
% leakage
pH increased to 5 at week 6
0
2
4
6
8
10
12
14
Time (weeks)

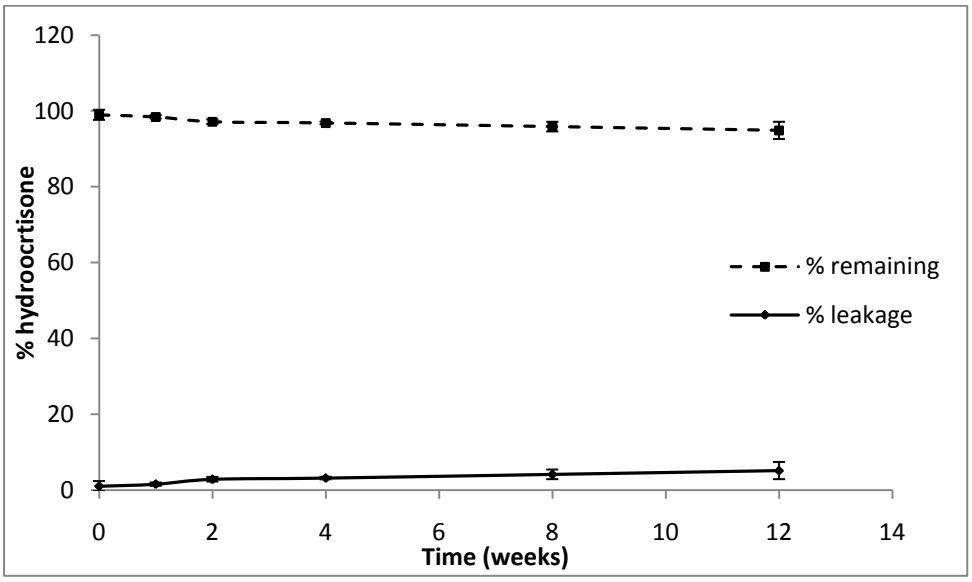

120
100
80
60
40
20
0
% hydroocrtisone
% remaining
% leakage
0
2
4
6
8
10
12
14
Time (weeks)

Table 1. pH-responsiveness of 2.5% w/w hydrocortisone-loaded Eudragit L100 microparticles reported as percentage hydrocortisone released per unit pH, calculated at 3, 4 and 5 hours.

Time	**Spray drying**	**Oil/oil method**
3 hours	31.2±3.66	37.2±8.62
4 hours	33.7±3.14	44.3±6.69
5 hours	31.9±4.19	48.8±5.33

Table 2. Maximum release rates (J_{max}) (μg cm^{-2} hr^{-1}) and time to maximum release rates (T_{max}) calculated from the release profile of hydrocortisone for the different microparticles prepared from the spray drying and oil-in-oil emulsification methods.

Method	**Drug loading (% w/w)**	**pH 5**		**pH 7**	
		J_{max}	**T_{max}**	**J_{max}**	**T_{max}**
Spray drying	2.5%	7.23±2.11	1.5 hr	17.3±2.94	1.5 hr
	10%	18.5±1.81	1.5 hr	24.6±4.48	45 min
	25%	12.6±0.71	45 min	16.6±5.98	45 min
Oil/oil method	2.5%	1.26±0.16	1.5 hr	19.6±3.54	1.5 hr
	10%	1.02±0.24	1.5 hr	26.6±4.50	1.5 hr
	25%	5.20±2.26	1.5 hr	18.8±1.30	1.5 hr