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Freeze-dried, mucoadhesive system for vaginal delivery of the HIV microbicide, dapivirine: Optimisation by an artificial neural network

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ABSTRACT

Dapivirine mucoadhesive gels and freeze-dried tablets were prepared using a $3 \times 3 \times 2$ factorial design. An artificial neural network (ANN) with multi-layer perception was used to investigate the effect of hydroxypropyl-methylcellulose (HPMC): polyvinylpyrrolidone (PVP) ratio (X1), mucoadhesive concentration (X2) and delivery system (gel or freeze-dried mucoadhesive tablet, X3) on response variables; cumulative release of dapivirine at 24 h (Q_{24}), mucoadhesive force (F_{max}) and zero-rate viscosity. Optimisation was performed by minimising the error between the experimental and predicted values of responses by ANN. The method was validated using check point analysis by preparing six formulations of gels and their corresponding freeze-dried tablets randomly selected from within the design space of contour plots. Experimental and predicted values of response variables were not significantly different ($p > 0.05$, two-sided paired t -test). For gels, Q_{24} values were higher than their corresponding freeze-dried tablets. F_{max} values for freeze-dried tablets were significantly different (2–4 times greater, $p > 0.05$, two-sided paired t -test) compared to equivalent gels. Freeze-dried tablets having lower values for X1 and higher values for X2 components offered the best compromise between effective dapivirine release, mucoadhesion and viscosity such that increased vaginal residence time was likely to be achieved.

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1. Introduction

Dapivirine (formerly TMC 120) is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) (Njai et al., 2005; Van Herwege et al., 2004) that is being developed as a vaginal microbicide to prevent the heterosexual transmission of the human immunodeficiency virus (HIV) (Nuttall et al., 2008; Jespers et al., 2007; Woolfson et al., 2006). Earlier formulation approaches to HIV microbicide candidates have focused largely on conventional aqueous gel systems, reflecting their predominant use in established vaginal products (Di Fabio et al., 2003; Malcolm et al., 2004). However, a major disadvantage with microbicide gels is that their relatively poor retention within the vaginal tract is likely to necessitate application immediately before each act of intercourse in order to elicit protection, thereby introducing issues around user acceptability and compliance. More recently, efforts have been aimed at developing sustained or controlled release, coitally independent vaginal dosage forms intended to provide protection over at least a 24 h period, irrespective of whether intercourse is imminent (Klasse et al., 2008). For example, vaginal ring devices, similar to those already used for contraception and estrogen replacement therapy (Woolfson et al., 1999; Malcolm, 2008) are being evalu-

ated for the controlled release of dapivirine over several weeks or months (Gupta et al., 2009). Reservoir-type silicone elastomer vaginal rings have been shown to provide in vitro release of dapivirine at a mean rate equivalent to 136 $\mu\text{g}/\text{day}$ over a period of 71 days (Malcolm et al., 2005). Matrix-type rings loaded with 25 mg micronised dapivirine are presently being evaluated in human clinical trials (Nel et al., 2009).

There is a strong consensus within the HIV microbicide field that a greater diversity of delivery technologies is needed to both effectively formulate the increasing number of microbicide candidates progressing through the development pipeline and to meet the needs and preferences of women in different cultural settings (Hardy et al., 2007; Romano et al., 2008). In particular, dosage forms that can overcome the inherent disadvantages associated with some gel-based delivery systems, such as poor user acceptability, leakage and short vaginal retention time, coital dependence and the inability to be used covertly without the knowledge of the male sexual partner, are high on the development agenda (Klasse et al., 2008; van de Wiggert and Shattock, 2008).

The concept of freeze-dried solid dosage forms is already well established in the pharmaceutical industry, in the context of rapid release oral drug delivery (Seager, 1998). This paper applies the concept of artificial neural networks (Takayama et al., 2003) to the optimisation of a novel microbicide delivery system, a freeze-dried vaginal tablet designed to reconstitute in vivo to a retentive mucoadhesive gel for the delivery of dapivirine. The potential

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advantages of this approach for HIV microbicides, compared to conventional semi-solid gels, include increased stability of water-labile actives, reduced transport costs (since gel transport effectively means the transport of water), ease of administration, potentially enhanced user acceptability, no necessity for the inclusion of antimicrobial preservatives and increased vaginal residence times through enhanced mucoadhesion and viscosity. Freeze-dried systems may also be particularly applicable to the formulation of macromolecular actives such as protein-based microbicides.

2. Materials and methods

2.1. Materials

Micronised dapivirine was supplied by the International Partnership for Microbicides (IPM, Silver Spring, MD, US). Carbopol (Noveon AA1, Surfachem Group Ltd., Leeds, UK), hydroxyethylcellulose (HEC, Natrosol® 250MR, Hercules–Aqualon, Wilmington, DE, USA), hydroxypropyl-methylcellulose (HPMC, mol. wt. ~120,000, viscosity 100,000 cps (2%, w/w in water) Sigma–Aldrich, St. Louis, MO, USA), polyvinylpyrrolidone (PVP, Plasdone® K-90, ISP Technology Inc., Wayne, NJ, USA) were all used as formulation excipients. Isopropanol (99.8%) and HPLC-grade methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultra-pure laboratory-grade water was obtained using an Elga Purelab Maxima system. Sodium hydroxide was purchased from VWR International Ltd., Poole, UK.

2.2. Determination of dapivirine solubility in isopropanol/water mixtures

The solubility of dapivirine in a range of isopropanol (IPA)/water mixtures (30%, 40%, 50%, 60% and 70%, v/v IPA) was measured at both 20 °C and 37 °C. Dapivirine (25–30 mg) was added to 20.0 mL of each solvent mixture. After 24 h, a sample of the supernatant was removed from the excess solid dapivirine, appropriately diluted and the dapivirine concentration measured by reverse-phase high performance liquid chromatography (HPLC) with ultra-violet (UV) detections, as described in Section 2.5. Each solubility measurement was performed in triplicate.

2.3. Preparation of mucoadhesive dapivirine gels and their corresponding freeze-dried systems

Table 3 describes the compositions of dapivirine mucoadhesive gels (18 series) and their corresponding freeze-fried systems, prepared as follows. Carbopol was added to pH-adjusted water, entrapped air removed under vacuum and dapivirine added to form a suspension (0.5% (w/w), pH 7.0). HEC, HPMC and PVP were added sequentially, with the application of vacuum following the addition of each component. The gels were centrifuged (3000 rpm, 10 min), before dispensing into aluminium tablet blister packs and freeze-drying in an AdVantage Freeze Dryer (VirTis, Gardiner, NY, USA) according to the protocol in Table 2. The freeze-dried vaginal solid dosage forms were removed from blisters and kept in airtight vials at ambient temperature until further use.

2.4. Factorial design

Based on data from preliminary experiments, HPMC: PVP ratio (%) (X1), Carbopol (% w/w) (X2) and formulation type (gel or freeze-dried vaginal tablet) (X3) were found as the contributing factors to determine response variables (the cumulative amount of dapivirine released at 24 h (Q_{24}), maximum detachment force (F_{max}) and zero-rate viscosity). In this study, a $3 \times 3 \times 2$ factorial design (18 series)

Table 1

Correspondence between real and transformed values for dapivirine formulations.

| Formulation variables | Transformed values | | |
|-----------------------|--------------------|-----|---------------------|
| | –1 | 0 | +1 |
| HPMC:PVP ratio (%) | 1:3 | 2:2 | 3:1 |
| Carbopol % | 1 | 2 | 3 |
| Type of formulations | Gel | – | Freeze-dried tablet |

was used to study the effect of X1, X2 and X3 on response variables. The correspondence between real and transformed values is presented in Table 1.

2.5. In vitro drug release studies

Gels or freeze-dried tablets, each comprising 2.25 mg dapivirine, were placed in sealed borosilicate glass containers with 100 mL of release medium (60:40, v/v IPA/water), placed in a shaking incubator (60 rpm, 25 mm throw; Unitron, Infors HT, Switzerland) and maintained at 37 ± 0.5 °C. Release medium (20.0 mL aliquot) was withdrawn and replaced by fresh medium at regular time intervals. The samples were analyzed for dapivirine content by reverse-phase HPLC/UV, as follows: Waters Breeze HPLC system; Phenomenex Luna 5 μ C18(2) column 4.6 mm i.d., 150 mm length; temperature 25 °C; isocratic mode; methanol/water mobile phase (85:15, v/v); flow rate 1.0 mL/min; detection wavelength 287 nm; 10 μ L injection volume; dapivirine retention time 2.9 min. A linear calibration plot for dapivirine was obtained over the range 0.05–100 μ g/mL in methanol ($r^2 = 0.999$). The Q_{24} values were calculated and reported in Table 3.

2.6. In vitro mucoadhesion measurements of gels and freeze-dried dapivirine formulations

Mucoadhesion measurements were performed as previously described (Curran et al., 2009) using a TA-TX2 Texture analyzer (Stable Micro Systems, Surrey, UK). Briefly, mucin discs (13 mm diameter, 250 mg) were prepared by compressing mucin powder in a single punch tablet press (Riva Minipress, Buenos Aires, Argentina). Each formulation was fixed with double-sided adhesive tape to the sample holder. One side of the mucin disc was attached to a polycarbonate probe (attached to the moveable carriage of the analyzer) using double-sided adhesive tape. The other side of the disc was then wetted for 30 s by bringing its surface into contact with water in a beaker. Excess water was removed by gently blotting with absorbent paper before bringing the mucin disc and the formulation (gel or tablet) into contact with a preload of

Table 2

Operational parameters for the production of freeze-dried vaginal tablets.

| | Temperature (°C) | Time (min) | Vacuum (mTorr) | Condition |
|----------|------------------|------------|----------------|-----------|
| Freezing | –50 | 60 | – | Ramp |
| | –50 | 90 | – | Hold |
| Drying | –40 | 30 | 600 | Ramp |
| | –40 | 60 | 600 | Hold |
| | –30 | 30 | 600 | Ramp |
| | –30 | 60 | 600 | Hold |
| | –20 | 30 | 600 | Ramp |
| | –20 | 60 | 600 | Hold |
| | –10 | 30 | 600 | Ramp |
| | –10 | 600 | 600 | Hold |
| | 0 | 30 | 600 | Ramp |
| | 0 | 60 | 600 | Hold |
| | 10 | 30 | 600 | Ramp |
| | 10 | 60 | 600 | Hold |
| | 25 | 60 | 600 | Ramp |
| 25 | 300 | 400 | Hold | |

Table 3
Series matrix, experimental and ANN-predicted responses.

| Series code | X1 | X2 | X3 | Q ₂₄ (mg) | | F _{max} (N) | | Viscosity (Pa S) | |
|-------------|----|----|----|----------------------|--------|----------------------|--------|---------------------|-----------|
| | | | | Experimental | By ANN | Experimental | By ANN | Experimental | By ANN |
| 11– | –1 | –1 | –1 | 1.970 ± 0.063 | 1.945 | 0.130 ± 0.017 | 0.128 | 661.50 ± 26.31 | 706.80 |
| 21– | 0 | –1 | –1 | 1.955 ± 0.110 | 1.912 | 0.140 ± 0.015 | 0.135 | 1086.00 ± 67.55 | 1007.30 |
| 31– | 1 | –1 | –1 | 1.759 ± 0.016 | 1.765 | 0.142 ± 0.012 | 0.138 | 1217.00 ± 7.94 | 1330.90 |
| 12– | –1 | 0 | –1 | 1.826 ± 0.058 | 1.860 | 0.123 ± 0.021 | 0.124 | 6637.33 ± 407.50 | 6567.70 |
| 22– | 0 | 0 | –1 | 1.706 ± 0.024 | 1.728 | 0.127 ± 0.023 | 0.127 | 8057.00 ± 751.42 | 8007.30 |
| 32– | 1 | 0 | –1 | 1.619 ± 0.032 | 1.608 | 0.123 ± 0.022 | 0.124 | 9228.00 ± 556.31 | 9271.90 |
| 13– | –1 | 1 | –1 | 1.736 ± 0.078 | 1.726 | 0.128 ± 0.015 | 0.124 | 10,332.00 ± 742.40 | 10,398.50 |
| 23– | 0 | 1 | –1 | 1.587 ± 0.086 | 1.592 | 0.131 ± 0.010 | 0.126 | 14,343.33 ± 1913.28 | 14,267.60 |
| 33– | 1 | 1 | –1 | 1.470 ± 0.115 | 1.475 | 0.127 ± 0.012 | 0.125 | 24,943.33 ± 3015.86 | 24,989.20 |
| 11+ | –1 | –1 | 1 | 1.860 ± 0.111 | 1.860 | 0.276 ± 0.023 | 0.285 | 3837.67 ± 159.16 | 3888.80 |
| 21+ | 0 | –1 | 1 | 1.743 ± 0.041 | 1.750 | 0.256 ± 0.030 | 0.242 | 7411.33 ± 903.04 | 7482.10 |
| 31+ | 1 | –1 | 1 | 1.722 ± 0.051 | 1.707 | 0.178 ± 0.020 | 0.182 | 9640.33 ± 714.34 | 9757.30 |
| 12+ | –1 | 0 | 1 | 1.717 ± 0.057 | 1.711 | 0.337 ± 0.035 | 0.333 | 13,450.00 ± 420.00 | 13,544.70 |
| 22+ | 0 | 0 | 1 | 1.615 ± 0.068 | 1.638 | 0.314 ± 0.022 | 0.322 | 21,850.00 ± 269.63 | 21,776.00 |
| 32+ | 1 | 0 | 1 | 1.556 ± 0.030 | 1.560 | 0.295 ± 0.042 | 0.290 | 27,293.33 ± 875.58 | 27,347.90 |
| 13+ | –1 | 1 | 1 | 1.570 ± 0.048 | 1.575 | 0.588 ± 0.038 | 0.585 | 28,036.67 ± 452.81 | 27,921.70 |
| 23+ | 0 | 1 | 1 | 1.489 ± 0.045 | 1.466 | 0.511 ± 0.071 | 0.514 | 44,400.00 ± 2765.45 | 44,602.2 |
| 33+ | 1 | 1 | 1 | 1.343 ± 0.079 | 1.369 | 0.403 ± 0.013 | 0.409 | 51,460.00 ± 1076.85 | 51,878.30 |

0.1 N applied for 30 s. The probe carriage was then moved away at a constant speed of 1 mm/s to complete separation of the surfaces. Both displacement and force of detachment were recorded. From the force versus displacement curves, the maximum force of detachment F_{max} was measured for each formulation (Table 3).

2.7. Dynamic (oscillatory) rheological analysis

Dapivirine gel samples were analysed directly. Freeze-dried dapivirine tablets were reconstituted with SVF (simulated vaginal fluid) to a total weight of 450 mg per tablet (equivalent to dapivirine gel weight) prior to use. Dynamic (oscillatory) rheological analyses of candidate formulations were performed using a rheometer (AR 1500, TA Instruments, Surrey, England) at 37.0 ± 0.1 °C with a 4 cm parallel plate geometry (dependent on sample consistency) and a sample gap of 1 mm. Samples of each formulation were applied to the lower plate of the rheometer and allowed to equilibrate for at least 2 min prior to analysis. Initially, the linear viscoelastic region for each system was identified following a stress sweep from 0.1 Pa to 250 Pa at frequencies of 10 Hz as the region where stress was directly proportional to strain, and the storage modulus (G') remained constant. All frequency sweep analysis was investigated over the frequency range of 0.1–10 Hz following application of a constant stress (5 Pa). Data were converted to flow rheometric experiments using onboard software (TA Instruments). Using the Cross model (Cross, 1965) the zero-rate viscosities of all formulations were determined as previously described (Jones et al.,

1997). In each case, the dynamic rheological properties of five replicates were determined. Mean zero-rate viscosities are reported in Table 3.

2.8. Artificial neural network (ANN), contour plots and check point analysis

A multi-layer perception back propagation model with a single hidden layer (NeuroSolutions v5.07, NeuroDimension Inc., FL, USA) was used to predict three different responses for candidate dapivirine formulations. The ANN consisted of an input layer of 3 neurons (formulation variables), an output layer of 3 neurons (response variables) and one hidden layer. Input and output variables were used to train the network. During training, the step size, the momentum of the hidden layer and number of epochs were optimised until a minimum standard error observed between actual and predicted values of responses. Two-dimensional contour plots for ANN-predicted responses using two variables, X1 (HPMC/PVP ratio) and X2 (Carbopol concentration), at –1 (gel) and +1 (freeze-dried tablet) levels of X3 (as indicated in Table 1) were established using NCSS software 2007 (Kaysville, Utah, USA). A checkpoint analysis was performed to validate the prediction from the ANN and the utility of the contour plots (Fig. 1) in defining the design space. Values of X1 and X2 were randomly selected from each contour plot (Fig. 1), representing data for the three chosen variables for X3 = –1 (Fig. 1a–c) and X3 = +1 (Fig. 1d–f). The values of Q₂₄, F_{max} and viscosity corresponding to the randomly selected val-

Table 4
Cross-validation set for ANN-predicted and experimental responses.

| Series no. | X1 | | X2 | | X3 | | Responses estimated by ANN | | | Experimental values (mean ± SD) | | |
|--------------------------------------|----------|------------|----------|------------|----------|------------|----------------------------|------------------|------------------|---------------------------------|------------------|------------------|
| | TF value | Real value | TF value | Real value | TF value | Real value | Q ₂₄ | F _{max} | Viscosity (Pa S) | Q ₂₄ | F _{max} | Viscosity (Pa S) |
| 1 | –0.6 | 1.4:2.6 | –1 | 1% | –1 | Gel | 1.961 | 0.131 | 919.8 | 1.904 ± 0.05 | 0.135 ± 0.01 | 1003.6 ± 60.8 |
| 2 | –0.6 | 1.4:2.6 | –1 | 1% | +1 | FDT | 1.835 | 0.278 | 4814.8 | 1.820 ± 0.08 | 0.286 ± 0.04 | 4782.2 ± 107.3 |
| 3 | 0.4 | 2.4:1.6 | 0.6 | 2.6% | –1 | Gel | 1.594 | 0.127 | 14,536.2 | 1.614 ± 0.03 | 0.129 ± 0.01 | 14,551.4 ± 410.3 |
| 4 | 0.4 | 2.4:1.6 | 0.6 | 2.6% | +1 | FDT | 1.494 | 0.430 | 39,288.2 | 1.477 ± 0.06 | 0.447 ± 0.04 | 39,050.6 ± 642.8 |
| 5 | 0.8 | 2.8:1.2 | –0.4 | 1.6% | –1 | Gel | 1.658 | 0.124 | 7609.9 | 1.667 ± 0.05 | 0.122 ± 0.00 | 7570.8 ± 186.3 |
| 6 | 0.8 | 2.8:1.2 | –0.4 | 1.6% | +1 | FDT | 1.595 | 0.278 | 23,489.0 | 1.609 ± 0.05 | 0.295 ± 0.03 | 23,332.6 ± 435.2 |
| ^a t _{calculated} | | | | | | 0.627 | 2.372 | 1.278 | | | | |
| t _{tabulated} | | | | | | 2.570 | 2.570 | 2.570 | | | | |
| ^b p-Value | | | | | | 0.558 | 0.063 | 0.257 | | | | |
| ^c Normalised error | | | | | | 0.036 | 0.083 | 0.085 | | | | |

^a In all cases t_{calculated} < t_{tabulated} (statistically insignificant different).

^b In all cases p > 0.05 (statistically insignificant different).

^c Normalised error = [Σ{(Er – Pr)/Pr}²]¹/², where Pr = practical response and Er = estimated response.

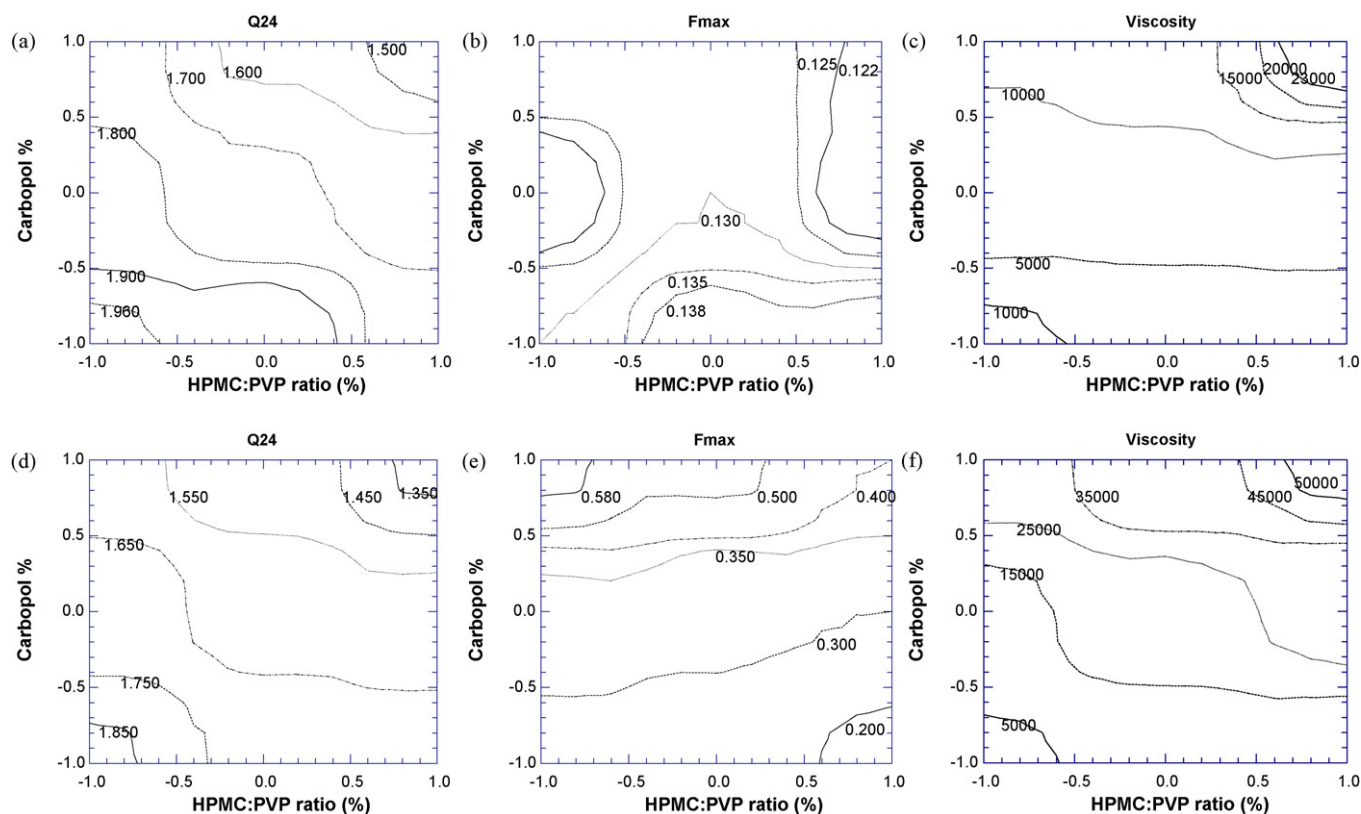


Fig. 1. Contour plots for Q_{24} , F_{max} and viscosity at -1 (a–c) and $+1$ (d–f) levels of X_3 .

ues of X_1 and X_2 were thus predicted. Gels and freeze-dried tablets were subsequently prepared on the same checkpoints, responses were measured and compared with those predicted by the ANN (Table 4) using a paired t -test and normalised error estimation.

3. Results and discussion

3.1. Dapivirine solubility

The highly hydrophobic character of dapivirine (mean predicted $\log P = 4.73 \pm 0.64$, mean predicted water solubility $S = 8.8 \mu\text{g/mL}$) (Tetko et al., 2005) is appropriate to its development as a vaginal microbicide. Dapivirine can effectively permeate cell membranes and the HIV lipid envelope, and is capable of irreversible tight-binding to the HIV reverse transcriptase enzyme (Liang and Chen, 2007). However, from a drug delivery perspective, the poor aqueous solubility of dapivirine is problematic, particularly in relation to performing in vitro release testing where sink conditions are usually employed.

One of the common criticisms of in vitro release testing of vaginal products is that the large volumes of release medium typically employed and the non-aqueous nature of the media used for hydrophobic drugs do not mimic the typical volume (approx. 2 mL) of aqueous fluid present in the human vagina (Woolfson et al., 2000). However, in addition to the fluid component, the surrounding mucosal tissue also serves as a sink for vaginally administered drugs, particularly hydrophobic molecules that can readily partition into the cell lipid membranes. Use of solvent/water mixtures for in vitro release testing of vaginal products may therefore be considered reasonable as they combine both fluid and tissue components into a single compartment release model.

The measured solubilities of dapivirine in various water/isopropanol mixtures increased with isopropanol con-

centration in the solvent mixture and with temperature, ranging from 0.06 to 1.24 mg/mL and were of the same order as predicted values calculated from the SMILES notation for dapivirine using ALOGPS 2.1 software (VCCLAB, 2005). Based on the use of a 100 mL volume and 37 °C for in vitro release testing, and the maintenance of sink conditions at no more than 10% of saturation solubility, a 60:40 isopropanol/water mixture (dapivirine solubility 0.73 ± 0.08 and 1.09 ± 0.02 at 20 °C and 37 °C, respectively) was deemed appropriate for use as a sink condition release medium.

3.2. Artificial neural network optimisation and check point analysis

Various models are available for the optimisation of formulation and processing variables during the development of novel drug delivery systems, including multiple regression analysis (Umrethia and Murthy, 2004), response surface methodology (McCarron et al., 1999), genetic algorithms and the artificial neural networks (ANN) concept (Turkoglu et al., 1999). ANNs are a type of mathematical model that simulates the biological nervous system, drawing on analogues of adaptive biological neurons. A major advantage of ANNs compared to other statistical models is that they do not require rigidly structured experimental designs and can map functions using historical or incomplete data. A significant difference between the ANN concept and a statistical model is that the ANN can generalize the relationship between independent and dependent variables without a specific mathematical function. Thus, ANNs work well for solving non-linear problems of multivariate and multi-response systems. Multi-layer perception with a back propagation network is the most widely and successfully applied architecture, consisting of an input layer, one or more hidden layers and one output layer. Use of at least one hidden layer enables the ANN to describe non-linear systems and provide adequate prediction. Each layer has some neuronal units that are fully inter-

connected to synapses, with the strength of the connection known as 'weights'. Using varying weight values, a non-linear relationship (a search process for the optimised set of weight values) is generated by training the ANN, which can minimise the squared error between the estimated and experimental data of units in the output layer. The ANN concept has been successfully applied to various areas of pharmaceutical development in recent years (Mansa et al., 2008; Leonardi et al., 2009). Essentially, the use of ANN allows a design space to be defined through the use of relatively few experiments such that the ANN model, once validated, can be used to predict the performance of any formulation combination within that defined space.

Three different levels of HPMC: PVP ratio (%) and Carbopol (%) concentration, and two levels of formulation type, were used as each unit of input layer. Multi-layer perception (MLP) with a back propagation algorithm was used as the artificial neural network to predict three different response variables such as Q_{24} , F_{max} and zero-rate viscosity, which were the units of the output layer. In order to train the network various trials were carried out with different numbers of neurons in hidden layers (from 1 to 35), epoch values (from 100 to 2000), step size (from 0.1 to 1) and momentum for hidden and output layer (from 0.1 to 1). The optimised values for neurons in the hidden layer, epoch value, step size and momentum for the hidden and output layers were 17, 1000, 0.1 and 0.7 and 0.01 and 0.7, respectively, which predicted minimum error. Further increases or decreases in the optimised values produced higher errors when the network was validated. The trained ANN was then used to obtain predicted response values. The practical and ANN-predicted values were found not to be significantly different (0.048, 0.676 and 1.613 t_{cal} for Q_{24} , F_{max} and viscosity, respectively ($p > 0.05$, paired two-sided t -test)). These values were used to produce contour plots using NCSS statistical software (Fig. 1). Six different randomly selected series were prepared (Table 4) and the ANN-predicted responses for Q_{24} , F_{max} and viscosity then compared with the corresponding experimentally determined values. Differences were not statistically significant ($p > 0.05$, paired two-tailed t -test).

3.3. Relationship between formulation and response variables: formulation optimisation

Fig. 1a–c shows the contour plots drawn at –1 level (gel) of X3 for prefixed values of Q_{24} , F_{max} and viscosity, respectively. There was an inverse and directly proportional relationship between Q_{24} and viscosity with X1 and X2. As the transformed values of X1 and X2 increased from –1 to +1, Q_{24} decreased from 1.960 mg to 1.500 mg (Fig. 1a) and the viscosity increased from 1000 Pa S to 23,000 Pa S (Fig. 1c). There was no linear relationship between F_{max} and X1 and X2 (Fig. 1b). Fig. 1d–f represents the contour plots drawn at the +1 level (freeze-dried tablets) of X3 for prefixed values of Q_{24} , F_{max} and viscosity, respectively. It was found that as X1 and X2 values increased from –1 to +1, Q_{24} decreased from 1.850 mg to 1.350 mg (Fig. 1d) and the viscosity increased from 5000 Pa S to 50,000 Pa S (Fig. 1f). F_{max} was directly proportional to X1 and inversely proportional to X2. It was concluded from the contour plots (Fig. 1d–f) that a lower HPMC:PVP ratio and higher Carbopol concentration in freeze-dried tablets was desirable in order to achieve a balance between good mucoadhesion, prolonged release of dapivirine and a predicted prolonged vaginal retention time, aided by increased viscosity as the system reconstitutes in vivo.

3.4. Mucoadhesion, viscosity and dapivirine release

Formulations described in Table 1 comprised the following components: a structural polymer (HPMC), a mucoadhesive poly(acrylic acid) (Carbopol) and a high water affinity polymer (PVP) intended

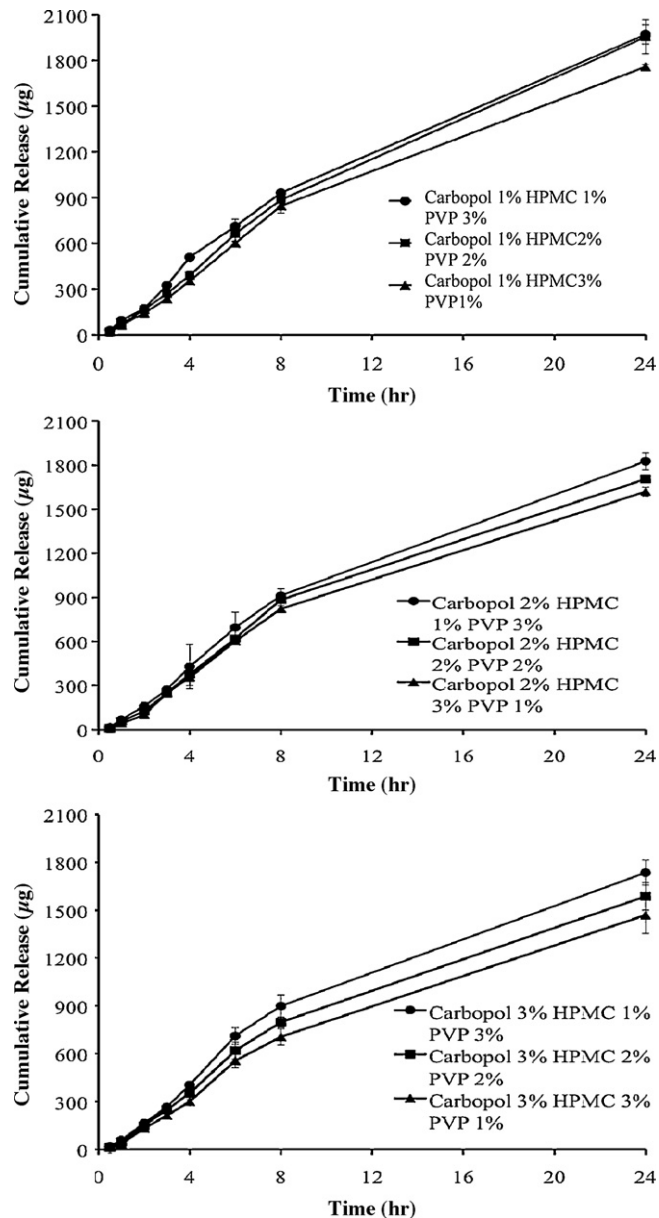


Fig. 2. Cumulative release of dapivirine from gels.

to absorb vaginal fluid in vivo, a concept described as a rheologically structured vehicle (Curran et al., 2009). Viscoelastic, highly structured gels of this type have a high water uptake capacity at a relatively slow rate, thus maintaining gel structure and drug release for prolonged periods, and have previously been extensively characterised in respect of rheological performance for use in the oral cavity (Jones et al., 1996, 1997). Their use for the delivery of vaginal microbicides is designed to overcome issues of poor vaginal retention associated with conventional gel delivery systems (Woolfson et al., 2000), and to provide a coitally independent vaginal delivery for once-daily administration.

Within the vagina, mucoadhesive drug delivery systems operate in an environment in which a mucus gel layer (derived from the cervix) acts as an interface between adherent and epithelial surfaces. Water, in particular, plays an important role in adhesion to mucin, the invading water molecules liberating polymer chains within the formulation from their twisted and entangled state and, thus, exposing reactive sites that can bond to tissue macromolecules. In semi-solid mucoadhesive gels the mucoad-

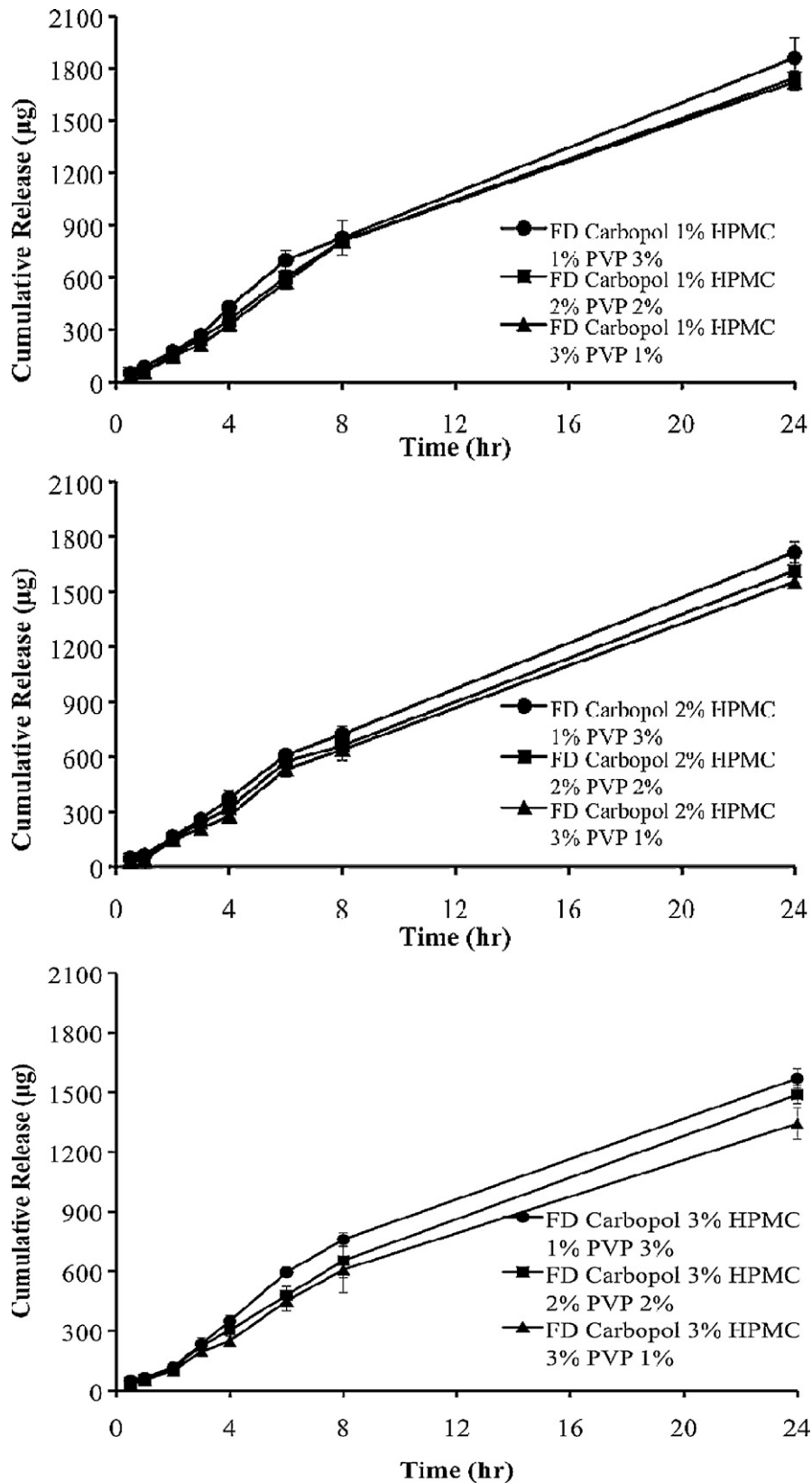


Fig. 3. Cumulative release of dapivirine from freeze-dried tablets.

hesive polymers are fully hydrated and display poorer structural and mucoadhesive properties compared to solid systems. Thus, vaginal retention by a mucoadhesive mechanism is progressively weakened as vaginal fluid is continually absorbed into an already

hydrated system. By contrast, solid systems with low water contents are known to optimise the mucoadhesion of a given polymer or polymeric combination compared to their semi-solid gel counterparts. Upon contact with mucus, water diffuses into the

formulation and controls swelling of the mucoadhesive polymer, which, in turn allows interpenetration of the fluidised polymer chains with mucus and hence ensures physical and chemical adhesion (Marshall et al., 2004). Thus, reconstitution of the semi-solid gel from a freeze-dried solid may be expected to reduce over-hydration and slow the onset of mucilage formation, thus aiding vaginal retention.

Table 3 allows comparison of mucoadhesion data determined in vitro for gel formulations and their corresponding freeze-dried systems. For example, series 11– is the gel product and series 11+ is the freeze-dried product prepared from the same formulation. In all cases, it can be seen that the '+1' (freeze-dried) products have superior F_{\max} (mucoadhesive force) values than the corresponding gel. This is mirrored by the viscosity data, where the freeze-dried products again have substantially higher values. Thus, the largest mucoadhesion and viscosity data were recorded for a freeze-dried product having a high HPMC/PVP ratio (less water-absorbing capacity) and high mucoadhesive polymer content, both values being accurately predicted by ANN and representing approximately 40 and 50% increases over the corresponding gel, respectively.

From Table 3, products with the lowest in vitro viscosity (and mucoadhesion) showed the highest dapivirine drug release over 24 h. Thus, dapivirine release from gel products (Fig. 2) was, in all cases, greater than for the corresponding freeze-dried product (Fig. 3), with excellent predicted values from ANN. These highly structured, viscoelastic gels comprise, predominantly, an aqueous dispersion of micronised solid dapivirine particles within a viscous gel matrix. Thus, the major steps in drug release are considered to be (i) influx of the release medium into the aqueous gel matrix, (ii) subsequent dissolution of the solid dapivirine within the modified gel matrix, (iii) diffusion of the solvated dapivirine molecules through the gel matrix into the bulk release medium and (iv) break up of the gel with accelerated drug release towards the end of the product life, typical of Super Case II behaviour. The higher viscosity of the gel formed from the reconstituted freeze-dried tablet, and the resultant higher tortuosity for diffusing drug, dictates the overall slower release rate of dapivirine, compared to the pre-hydrated gel, reflected in lower Q_{24} data in Table 3. Interestingly, within groups (Table 3), formulations with a higher PVP content also have a higher Q_{24} value, presumably reflecting increased water uptake into the formulation due to the higher water affinity of PVP compared to HPMC. However, the overall correspondence between Figs. 2 and 3, for drug release from the gels and freeze-dried tablets, respectively, demonstrates the key point that the freeze-dried systems retain the essential drug release properties of the gels, while having numerous practical advantages for the user. Of course, drug release data obtained under sink conditions does not necessarily translate easily to the in vivo situation, since vaginal fluid is aqueous in nature and dapivirine is highly hydrophobic. In vivo, it is likely that vaginal tissue uptake of dapivirine will significantly influence the release rate and, thus, vaginal fluid concentration of dapivirine (Romano et al., 2009). In addition, reconstitution of the tablet in vivo was dependent on the volume of vaginal fluid, with a suggested value of between 0.5 mL and 0.75 mL present at any one time (Owen and Katz, 1999). Hence, in this study, for example, viscosity measurements on reconstituted freeze-dried tablets were based on a total reconstituted weight of 450 mg, which is a realistic estimate of the in vivo situation.

4. Conclusion

The enhanced mucoadhesive performance of the novel freeze-dried mucoadhesive vaginal tablets is important in providing the possibility of increased vaginal retention of the dosage forms. In freeze-dried form, they provide enhanced mucoadhesion,

increased convenience for the patient, improved economy due to the reduced mass of product to be transported (in the absence of water) and, potentially, enhanced stability for moisture-sensitive actives. In order to maximise mucoadhesion and viscosity, and thus to promote prolonged vaginal retention, and to achieve sufficient dapivirine release for effective microbicide protection against HIV, a balance of formulation variables is necessary. As predicted by ANN, and confirmed by experimental data, freeze-dried systems with a lower HPMC:PVP ratio and higher Carbopol concentration in freeze-dried tablets represent the optimal candidates for further formulation development, as exemplified by series 13+ in Table 3.

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