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In Vitro Release Testing of a Peptide Gel

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The authors developed an in vitro release test for a gel formulation containing a topical microbicidal peptide. Polysulfone membranes and normal saline were used as the support membrane and receiving medium, respectively. Membranes with various pore sizes were tested to ensure no back-diffusion occurred and to identify a support medium that provided the least resistance to drug diffusion. The effects of peptide concentration, polymer concentration, and the addition of a cosolvent on the peptide release were determined. The degree of peptide binding to the support membranes was evaluated. The release profile of a solution containing the same peptide concentration was evaluated to show that the support membrane was not a rate-limiting step. Results show that the test can be modified to ensure product sameness after a scale-up and postapproval change.

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In May 1997, FDA issued a guidance document to reduce the regulatory burden when a company wishes to make limited changes and still retain the approved status of existing semisolid products. The document discusses scale-up and postapproval changes for semisolids (SUPAC-SS) and defines the actions a sponsor must take to maintain product certification after quantitative changes have been made. One of the tests recommended in SUPAC-SS for certain levels of changes is in vitro release testing of the semisolid dosage form. In vitro release testing is routinely used during product development to fine-tune a formulation. However, the suggested uses of the test to establish batch-to-batch uniformity, product certification, and possible bioequivalence are fairly new and have been the subject of much debate (1–4). The in vitro release test (IVRT) represents the diffusion of the drug out of the formulation and involves a synthetic membrane that should not be a rate-limiting barrier. It is important to properly validate a release test before using it for product qualification. Abundant literature exists about in vitro release of a drug present in a suspension semisolid formulation. However, there exists limited information about in vitro release of a drug in a solution formulation.

This article describes an IVRT for a peptide gel formulation in which the peptide is highly soluble. The method was validated by determining the effect of changing drug concentration, polymer (gelling agent) concentration, gel loading, and cosolvent addition on the release rate. Drug release from water was evaluated to establish that the membrane was not the rate-limiting step during the release process. Several polysulfone membranes of various pore sizes were tested as support media, and any potential binding of the drug to the membrane was established. A microbicidal peptide (D2A21, Demegen, Inc., Pittsburgh, PA) was used as the model drug.

Topical microbicides are chemicals that one can use intravaginally (or intrarectally) before sexual intercourse to block the transmission of HIV and other agents that cause sexually transmitted diseases (STDs) (5). The development of topical microbicides is a global priority and a central focus of the STD research effort of the National Institute of Allergy and Infectious Diseases (Bethesda, MD). The D2A21 peptide has been shown to prevent *Trichomonas vaginalis* infection in a mouse model and is being tested extensively in various laboratories for its activity against other pathogens and for its spermicidal effect (6).

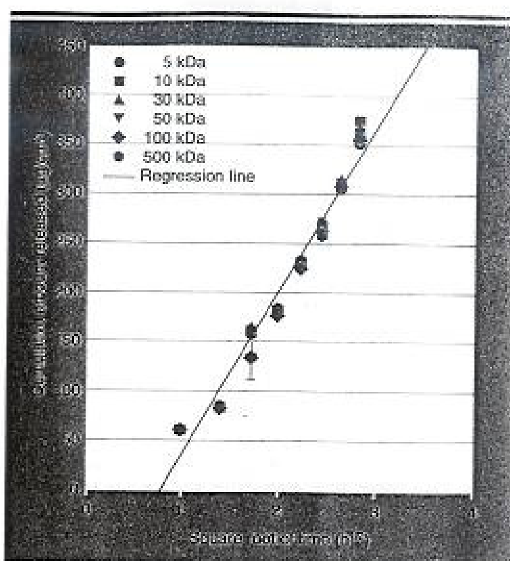

Figure 1: Effect of the molecular weight cutoff of the membrane on the release profile of D2A21 peptide (0.1%) from the gel ($r^2 = 0.96$).

Materials and methods

Materials. The D2A21 peptide was a gift from Demegen, Inc. The polymer, hydroxyethylcellulose (HEC) (Natrosol HEC-250H), was a gift from Hercules, Inc. (Wilmington, DE). Polysulfone disk membranes were obtained from Millipore Corp. (Biomax 5, 10, 30, 50, 100, and ZM 500, Bedford, MA). Propylene glycol (PG) was from Fisher Scientific (Fair Lawn, NJ).

Methods. Preparation of gel. The gel was made by dissolving the peptide in normal saline and then adding HEC slowly during constant stirring. The dispersion then was refrigerated for a few minutes to allow it to gel and for any air bubbles or foam to subside. The gel was of the same consistency as K-Y Jelly (Advanced Care Products, Raritan, NJ). A typical formulation consisted of 0.1% peptide and 3.25% HEC unless otherwise specified.

The IVRT was carried out using Franz diffusion cells. The receptor compartment was filled with 5 mL of normal saline and was maintained at 37 °C. The polysulfone membranes used as support membranes for the release studies have a low binding capacity (Millipore Corp. Product Information Sheet). Most of the studies used a membrane with a molecular weight (MW) cutoff of 500 kDa although other membranes also were used. The membrane was soaked in the receptor phase before mounting on the Franz cell. The gel was carefully transferred to the donor compartment over the membrane. Gel loading was 500 mg unless otherwise specified. Samples were taken from the receptor every hour for 8 h unless otherwise specified. Factors investigated included peptide concentration (0.1, 0.5, and 1.0%), polymer concentration (2.0, 3.0, and 3.25%), and cosolvent addition (40% PG). All statistical comparisons were performed using single-factor analysis of variance (ANOVA) ($p<.01$). All experiments were done in triplicate.

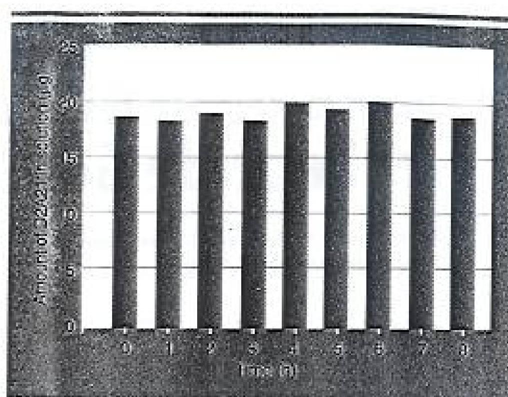

Figure 2: Binding of D2A21 peptide to the polysulfone support membrane.

Assay. Samples were analyzed for the peptide using a high-performance liquid chromatography (HPLC) assay with a C18 column (Varian, Inc., Walnut Creek, CA) and an HPLC device (Waters, Milford, MA). The mobile phase consisted of 46.5% acetonitrile, 52.6% distilled water, and 0.9% trifluoroacetic acid. The wavelength of detection was 215 nm and retention time was ~6.8 min.

Results and discussion

Peptide release profiles were expressed in terms of the amount released as a function of time and the square root of time. Higuchi's equation, which describes a square root of time relationship, was used to calculate the diffusion coefficient. The amount of drug released from the vehicle in which the drug is uniformly dissolved is

$$Q = 2C_0\left[\frac{Dt}{\pi}\right]^{1/2} \qquad [1]$$

where Q is the amount of drug released in time t, C_0 is the initial concentration of the drug in the vehicle, and D is the diffusion coefficient of the drug in the vehicle. The equation is valid when only the drug is able to diffuse out of the vehicle — that is, the components of the vehicle cannot diffuse out (7). To select an appropriate membrane for the test, the authors evaluated membranes with various MW cutoffs (5, 10, 30, 50, 100, and 500 kDa). Figure 1 shows the cumulative amount of peptide released as a function of the square root of time. A linear relationship was observed for Q versus $t^{1/2}$, but there was no statistical difference among peptide release rates from the various membranes. The D2A21 peptide has a MW of 2776 Da, which is smaller than the membrane with the lowest MW cutoff. The rationale for using membranes with various MW cutoffs was to determine whether there was any difference in potential back-diffusion of the receptor media into the gel, which may solubilize the gel and increase the release rate. Results suggest that back-diffusion was not a significant problem in this case. All subsequent studies were performed with the 500 kDa–cutoff membrane.

Another criterion for membrane selection is to ensure that the drug does not bind to it. To test this, the 500 kDA–cutoff

membrane was soaked in a peptide solution (1% D2A21) for 8 h, and the amount of peptide remaining in the solution was analyzed each hour. Figure 2 shows that no significant peptide–membrane binding occurred. To demonstrate that the membrane was not a rate-limiting barrier to diffusion of the D2A21 peptide, results of IVRTs for solution and gel formulations were compared at three concentrations (0.1, 0.5, and 1.0%). Figures 3a–c show that the amount of drug released from the solution formulations was greater in all cases, approximately three times the amount released from the gel formulations. This suggests that peptide diffusion across the membrane is not a rate-limiting step for these membranes. The fact that release from the solution formulation occurred during 8 h and not instantaneously (as a result of gel dissolution by back-diffusion of receptor media) further supports the observation that the membrane was suitable for the IVRT.

The relationship between the amount of gel loaded on the membrane and the release rate of the peptide also was investigated. Peptide release was observed for three loading levels (150, 300, and 500 mg). Figure 4 shows the cumulative amount transported versus the square root of time. The difference in slopes for each loading level was statistically insignificant ($p<.01$). This suggests that infinite dosing probably was achieved at the lowest loading dose (150 mg) and thus Higuchi kinetics applies.

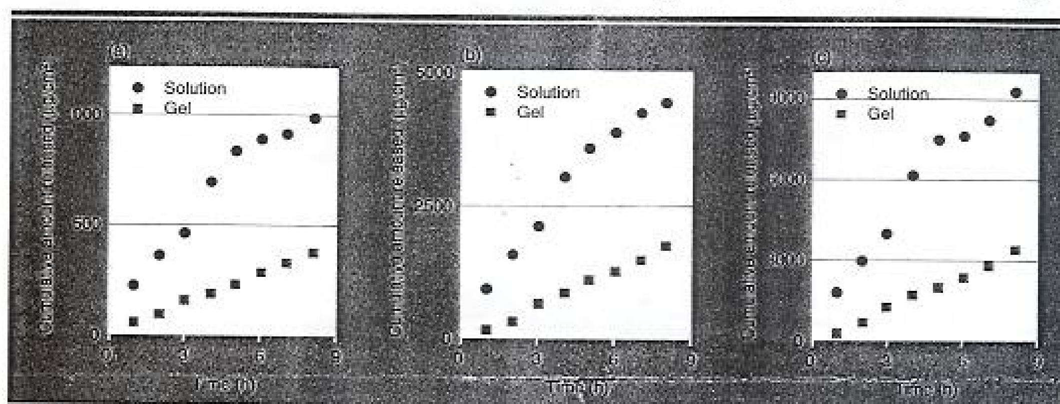

Figure 3: Effect of D2A21 peptide concentration on in vitro release: (a) 0.1% D2A21, (b) 0.5% D2A21, and (c) 1.0% D2A21.

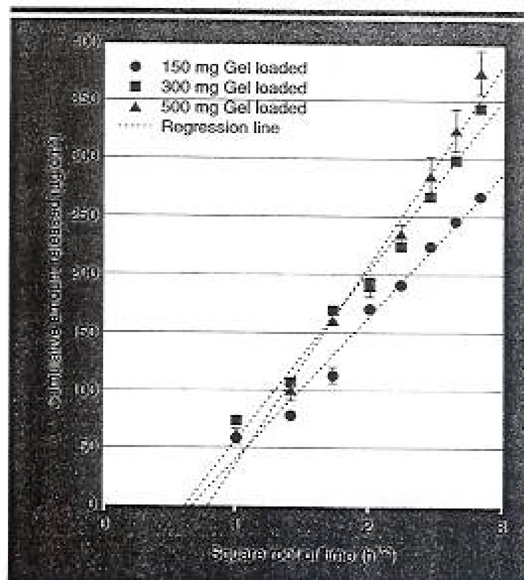

Figure 4: The effect of the amount of gel loaded on the membrane on the release profile of D2A21 peptide (r^2 = 0.97–0.98).

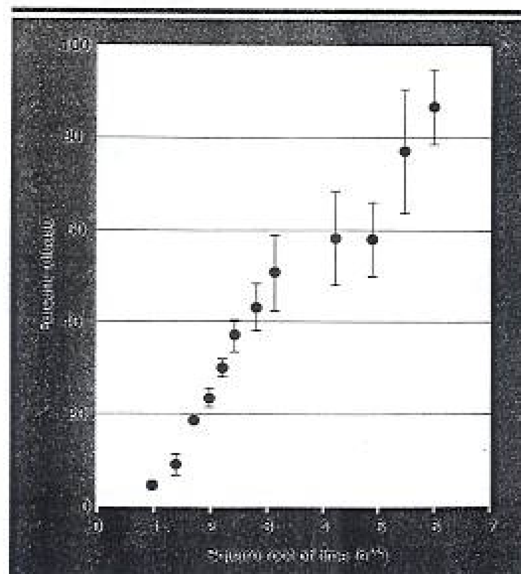

Figure 5: Total percentage of D2A21 peptide released over 36 h.

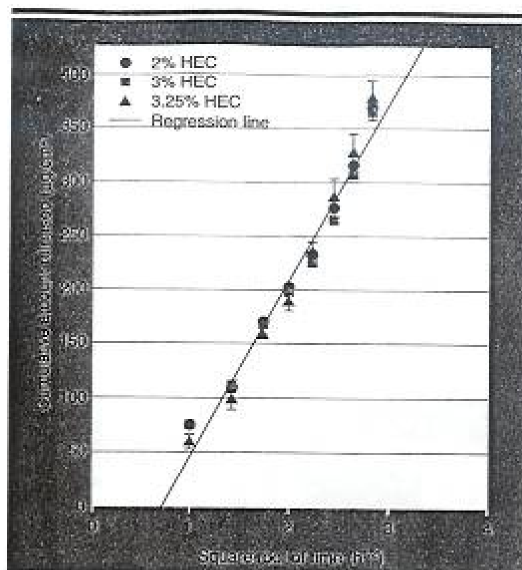

Figure 6: Effect of change of HEC concentration on the release rate of D2A21 peptide from gel ($r^2 = 0.97$).

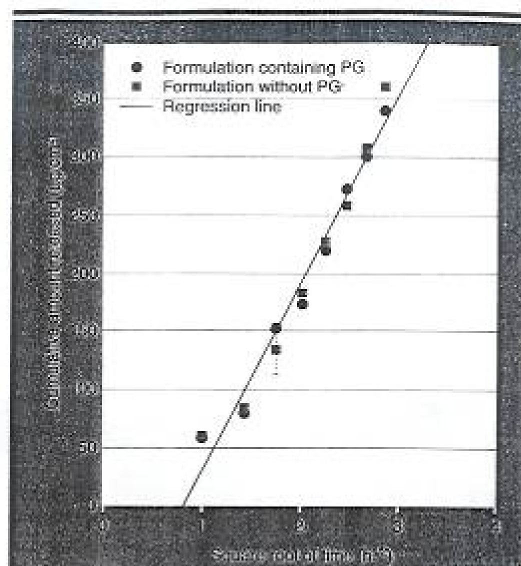

Figure 7: Effect of PG addition on the release of D2A21 peptide from gel ($r^2 = 0.97$).

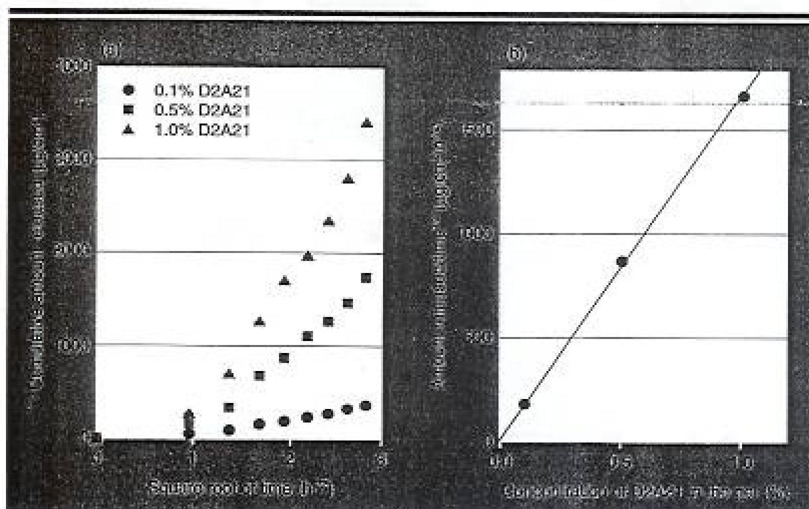

Figure 8: (a) Effect of change in D2A21 peptide concentration in gel on release rates. (b) The corresponding curve of flux versus concentration.

Similar observations have been reported in the literature (2). Approximately 86% of the peptide was released from the gel during 36 h. A typical study was carried out for ~8 h, at which time ~50% of the peptide was released. Figure 5 shows a linear relationship for the first ~8 h between the percentage of peptide released and the square root of time. The release percentage was rather high, most likely resulting from a very hydrophilic drug in a water-soluble gel.

The release rate was not significantly affected ($p<.01$) by changing the concentration of structure-forming agent (HEC) in the gel over the range investigated (2–3.25%) (see Figure 6). Similarly, the addition of 40% cosolvent (PG) had no effect on the in vitro release rate (see Figure 7). This result may indicate the structure of the gel was not affected to any significant extent by changes in HEC concentration or by the addition of PG, in which case the resistance barrier to drug diffusion remained more or less the same. This could be because there was no appreciable change in the number, dimensions, and the microscopic viscosity of the fluid channels in the gel matrix, which would have no effect on the solubility of the peptide in the matrix. Also, during diffusion through the gel, the solute travels primarily through the fluid phase. The microscopic viscosity and the resistance offered by these fluid channels determine the diffusivity (8).

The IVRT did respond to changes in the peptide concentration in the gel. The cumulative amount of peptide released increased as the peptide concentration in the gel increased (see Figure 8a). A linear relationship ($r^2 = 0.99$) was observed for the release flux and peptide concentration in the gel (see Figure 8b). The diffusion coefficient D was calculated for each of the

three initial concentrations (0.1, 0.5, and 1.0%) using Equation 1. The values, 0.012, 0.012, and 0.0113 cm^2/h, respectively, are statistically identical. Thus, D was observed to be independent of C_0. This is expected if the concentration is less than the solubility of the drug in the vehicle. In this case, even the highest initial concentration of the peptide (1%) is much lower than the solubility of the peptide in the vehicle. The authors were able to dissolve 10% peptide in the vehicle but did not check higher concentrations because of a limited supply of peptide. The actual solubility is expected to be much higher. The diffusion coefficient is related to frictional resistance experienced by the drug when moving through the medium. This resistance is constant for differing concentrations if the overall gel structure is the same, that is, if the microscopic viscosity of the fluid channels has not changed.

Conclusions

The authors developed a method to test the in vitro release of D2A21 peptide from HEC gel using polysulfone membranes. The peptide did not bind to the membrane, and there was no significant back-diffusion of receptor media into the formulation. The percentage of peptide release from solution formulations was higher than that from gel formulations, suggesting that the membrane was not a rate-limiting step. The test failed to detect any changes in release rate when the HEC concentration was changed or when a cosolvent was added. The test did respond to changes in peptide concentration in the gel. Overall, it seems the method could be refined into a differentiating test to ensure product sameness following changes in the formulation or manufacturing process or manufacturing site.

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