

High Throughput Screening of Transdermal Formulations

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Purpose. Applications of transdermal drug delivery are limited by low skin permeability. Many chemicals have been used to enhance skin permeability, however, only a handful are actually used in practice. Combinations of chemicals are likely to be more efficient in enhancing skin permeability compared to individual enhancers. However, identification of efficient enhancer combinations is quite challenging because many chemical enhancers interact with each other and with the skin in a complex manner. In the absence of a fundamental knowledge of such interactions, we need to rely on rapid methods to screen various enhancer combinations for their effectiveness. In this paper, we report a novel high throughput (HTP) method that is at least 50-fold more efficient in terms of skin utilization and up to 30-fold more efficient in terms of holdup times than the current methods for formulation screening (Franz diffusion cells).

Methods. A high throughput method was developed based on skin conductivity and mannitol penetration into the skin. This method was used to perform at least 100 simultaneous tests per day. Detailed studies were performed using two model enhancers, sodium lauryl sulfate (SLS) and dodecyl pyridinium chloride (DPC). The predictions of the high throughput method were validated using Franz diffusion cells.

Results. High throughput screening revealed that mixtures of SLS and DPC are significantly more effective in enhancing transdermal transport compared to each of them alone. Maximum efficiency was observed with near-equimolar mixtures of SLS: DPC. The predictions of the HTP method compared well against those made using Franz diffusion cells. Specifically, the effect of surfactant mixtures on skin conductivity and mannitol permeability measured using Franz cells also showed a maximum at near-equimolar mixtures of SLS: DPC.

Conclusions. The novel HTP method allows rapid screening of enhancer formulations for transdermal applications. This method can be used to discover new and effective enhancer mixtures. At the same time, these data may also broaden our understanding of the effect of enhancers on skin permeability.

KEY WORDS: combinatorial; discovery; chemical enhancers; mixtures; synergistic.

INTRODUCTION

Transdermal drug delivery offers several advantages over injections. However, applications of transdermal drug delivery are limited by low skin permeability (1). More than 200 chemical enhancers including surfactants, fatty acids, fatty alcohols, and organic solvents have been used to increase transdermal drug transport, however, only a handful of them are actually used in practice (2). This discrepancy results from the fact that among all the enhancers that have been used, only a few induce a significant enhancement of transdermal

drug transport (3–5). Furthermore, skin irritation and safety issues limit the applications of several enhancers. We hypothesize that this limitation may be overcome by using a combination of two or more enhancers (6). Especially, if individual enhancers increase transdermal transport via different mechanisms, their combination may be more effective than either of them alone. In addition, by combining two or more enhancers, concentration of each enhancer required to achieve the desired enhancement should be lower than that required if the enhancers were used individually. This may increase the safety of the formulation (6). Limited literature data available on combination of chemical enhancers indeed show that a combination of two or more enhancers is more effective in increasing transdermal transport compared to each of them alone (7). However, combinations of enhancers lead to myriad new formulations that need to be screened for their efficacy. Identification of appropriate combinations of enhancers *a priori* is quite challenging as the enhancers interact with each other and with the skin in a complex manner. In the absence of a fundamental understanding of such interactions, we are forced to search for efficient combinations through experiments.

We hypothesized that identification of efficient combinations of chemical enhancers may be greatly facilitated by using a high throughput (HTP) screening method. Screening of enhancers is currently performed using Franz diffusion cells, which can typically perform about one test per square inch of skin per day thus making them unsuitable for high throughput screening. Additional systems including Bronaugh's Flow Through Diffusion cell and Moody's AIVDA system, which operate on the same general principle of steady-state flux measurements, are also being used for transdermal testing. Although these methods offer several advantages over Franz diffusion cells including reduction in manual labor, their efficiency in screening enhancers is of the same order as Franz diffusion cells. Hence, we will use Franz Diffusion cells as a reference method for comparison. Here, we report a novel HTP method that is several fold more efficient compared to Franz diffusion cells. This should greatly improve the efficiency of formulation testing compared to most of the existing methods.

FUNDAMENTAL BASIS OF THE HIGH-THROUGHPUT METHOD

A traditional method of formulation testing (Franz diffusion cells) relies on steady-state measurements of drug transport across the skin (8). This method, though useful for quantifying the drug dose delivered across the skin, is not suitable for HTP screening due to: (i) large area of skin required, (ii) time associated with sample collection and handling, and (iii) time required to reach the steady state. The new HTP method addresses these challenges. There are two end points that we use to determine the effect of chemical enhancers on skin permeability, namely solute penetration into the skin and skin conductivity. Although the former method directly measures the increase in solute delivery, the latter offers an indirect but rapid assay to determine the effect of the enhancers on skin permeability, especially for polar solutes.

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Solute Penetration into the Skin

This method follows the transport of a radiolabeled molecule, mannitol, into the skin. Solute diffusion in the stratum corneum (SC) can be described by Fick's law as follows (9):

$$\frac{\partial C_s}{\partial t} = D \frac{\partial^2 C_s}{\partial x^2} \quad (1)$$

where, D is the average solute diffusion coefficient in the SC, C_s is the solute concentration in the SC, and x is the distance from the SC surface. Equation (1) can be solved with the following boundary conditions:

$$C_s(x=0) = KC_0 \quad (2)$$

$$C_s(x=L) = 0 \quad (3)$$

where, $x=0$ corresponds to the SC surface and $x=L$ corresponds to the end of the SC, K is the average solute partition coefficient in the SC, and C_0 is the donor concentration of the solute. The resulting equation for solute concentration in the SC, C_s is given as follows (9):

$$\frac{C_s(t)}{C_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\{-D(2n+1)^2\pi^2 t/L^2\} \quad (4)$$

where, C_∞ is solute concentration in the SC at steady state ($C_\infty = KC_0/2$). For low values of Dt/L^2 (that is, for short times), Eq. (4) can be simplified as follows:

$$\frac{C_s(t)}{C_0} \approx \frac{4DKt}{L^2} \quad (5)$$

Mathematically, Eq. (5) is valid when $Dt/L^2 \ll 1$. Since, the lag time for diffusion, τ , is given by $\tau = L^2/6D$, Eq. (5) is valid when $t \ll 6\tau$. At long times, that is, at steady-state, Eq. (1) can also be solved to obtain solute permeability, P , with the same boundary conditions, as follows:

$$P = \frac{DK}{L} \quad (6)$$

Equations (5) and (6) can be combined to obtain the following equation.

$$C_s(t) = \left(\frac{4C_0 t}{L}\right)P \quad (7)$$

Equation (7) shows that the solute concentration in the SC measured at short times is proportional to its steady-state permeability. Accordingly we measured the amount of mannitol delivered into the skin at short times to screen the efficacy of the enhancers.

Skin Conductivity

The electrical conductivity of the skin is generally a good measure of its permeability to polar solutes. Transepidermal current is mediated by the movement of charge carrying ions and is thus related to the permeability of these ions. The ion flux across the skin can then be treated in the same way as the flux of solute molecules across the skin. Formal relationships relating ionic conductivity to permeability can be developed using Nernst-Planck flux equations and the Nernst-Einstein relations for ideal solutions (10,11). Such relations become

significant if one were to precisely estimate skin permeability based on its conductivity. However for screening purposes it is sufficient to know that skin possessing higher electrical conductivity exhibits higher permeability to polar solutes. Accordingly we monitor electrical conductivity of skin exposed to various formulations to identify the ones most efficient in increasing skin permeability.

MATERIALS AND METHODS

High Throughput Screening of Enhancers

A special cell was designed for HTP screening of formulations. The cell consisted of 2 polycarbonate or Teflon plates each having a thickness of 12.7 mm (half inch). One hundred holes (each a diameter of 3 mm) were drilled in the top and bottom plate. The top plate is shown in Fig. 1. The holes (wells) in the top plate act as the donors, and the holes (wells) in the bottom plate act as the receivers. The donor and the receiver plates were clamped using 4 screws.

Screening of formulations was performed using pigskin. Skin was obtained using methods described in Ref. 12. This skin was stored in a freezer at -70°C for later use. Skin was removed from the freezer prior to the experiment and was allowed to thaw at room temperature. All the wells in the receiver plate were filled with PBS. The skin was then placed between the two plates with the stratum corneum facing the donor plate. The plates were then clamped together. Two model enhancers, sodium lauryl sulfate (SLS, Sigma Chemicals, St. Louis, Missouri) and dodecyl pyridinium chloride (DPC, Sigma Chemicals, St. Louis, Missouri) were used in this study. To assess the efficacy of the HTP method in screening the formulations, various compositions of SLS and DPC were prepared. The total surfactant concentration (SLS+DPC) in these solutions was adjusted at various levels

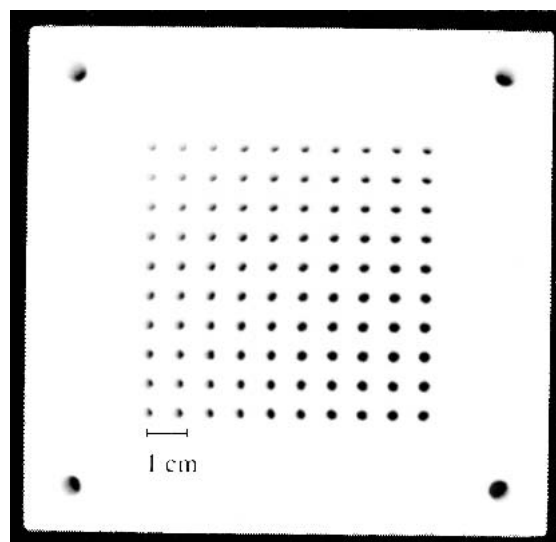


Fig. 1. The Figure shows the top view of the cell used for high throughput screening. The cell contains an array of 100 holes (10×10) each having a diameter of 3 mm. The top plate of the cell was prepared from Teflon and the bottom plate was prepared from polycarbonate. To reduce the glare from the white Teflon plate the picture was taken at an angle. This causes a part of the wells in the top left corner to look smaller than the rest. The scale bar corresponds to 1 cm.

including 0%, 0.5%, 1.0%, 1.5%, and 2.0% (all w/v) prepared in 1:1 Ethanol: PBS. Ethanol was used to avoid precipitation of surfactants. At each concentration, the relative weight fraction of SLS: DPC was varied between 0 and 1 in increments of 0.1. Thus, a relatively large number of SLS: DPC mixtures (formulations) were screened for their efficacy in enhancing transdermal transport.

Donor chambers were filled with 85 μL formulation. Each formulation was filled in 4 wells. Current was measured across the skin in each well with the help of 2 metal electrodes. One electrode was inserted into the dermis and used as a common electrode while the second electrode was placed sequentially into each donor compartment. Conductivity measurements were performed using a multimeter (Fluke 189, Everett, Washington), which has a resolution of 0.01 microamperes. A waveform generator (Agilent 33120A, Palo Alto, California) was used to generate an AC signal 143 mV pp at 100 Hz. The current readings were taken periodically over a span of 25 h (0 h, 4 h, 8 h, 18 h, and 25 h). The trend in conductivity enhancement is clear even at relatively low times. The advantage of using HTP method precisely comes from the ability to identify efficient formulations at very low times. The enhancement in the conductivity at a time 't' was then calculated as

$$E_C = \frac{I_t}{I_0},$$

where I_t is the current measured at time 't' and I_0 is the current measured at time 0.

Radiolabeled mannitol was added to all formulations at a concentration of 10 $\mu\text{Ci/mL}$. The donor compartments were filled with these formulations. Each formulation was filled in 4 different wells. The skin was then incubated for 90 minutes. Solutions from the donor compartments were then removed. The skin was rinsed gently to remove free mannitol that could be sticking to the surface of the skin. The skin was then cut into pieces and dissolved in 0.5 M Solvable, a tissue and gel solubilizer (Perkin Elmer, Wellesley, Massachusetts), at 60°C overnight. Concentration of radiolabeled mannitol in solubilized tissue was measured using a scintillation counter (Packard Tri-Carb 2100 TR, Meriden, Connecticut).

Transdermal Transport Experiments

Transdermal transport experiments were performed using pigskin to validate the data obtained from the HTP method. Transdermal experiments were carried out using a vertical Franz diffusion cell (receiver volume = 12 ml, area = 1.7 cm^2), which consists of a donor and a receiver compartment. 1 ml of test formulation was added to the donor chamber. A small stir bar and an Ag/AgCl disk electrode (E242 In Vivo Metric, Ukiah, California) were added to the receiver chamber. The conductivity measurement assembly used was the same as that used in the case of HTP system except that an Ag/AgCl electrode was used in the receiver compartment instead of inside the skin. The electric resistance of the electrodes used in both the systems was the same. The receiver chamber was filled with PBS. Pigskin was thawed and was mounted on the diffusion cell with the stratum corneum side facing up. The donor and the receiver compartments were clamped making sure there were no bubbles in the receiver chamber. Before each experiment, structural integrity of the skin was confirmed by measuring its conductivity using meth-

ods described previously (13). Skin samples with a resistivity less than 20 kohm-cm^2 were assumed to be defective and not used. Skin conductivity was measured throughout the experiment to assess the effect of the formulation on skin structure. Effect of different formulations (total surfactant [SLS+DPC] concentration of 1.0% w/v, SLS:DPC ratios in the range of 0 to 1) on skin conductivity was tested. Each formulation was prepared in a 1:1 mixture of ethanol: PBS. Skin conductivity was measured over a period of 25 h. The enhancement of skin conductivity was calculated as $E_C = K_t/K_0$, where K_t is skin conductivity at time t, K_0 is skin conductivity at time 0.

To assess the effect of formulations on skin permeability, radiolabeled mannitol (^3H labeled) was added to the formulation at a concentration of 10 $\mu\text{Ci/ml}$. Samples were taken from the receiver compartment periodically to measure the amount of mannitol transported transdermally over 48 h. Concentration of radiolabeled mannitol was measured using a scintillation counter (Packard Tri-Carb 2100 TR, Meriden, Connecticut). Skin permeability increased over 48 h without achieving a steady state. Hence, the enhancement of transdermal mannitol transport due to the formulations was calculated using the equation, $E_T = M_F / M_o$, where M_F is the amount of mannitol transported in 48 h in the presence of surfactants and M_o is the amount of mannitol transported in the same time under the same donor mannitol concentration in the absence of surfactants, (i.e., control).

RESULTS AND DISCUSSION

Fundamentals of the New HTP Screening Method

Transdermal drug transport is traditionally measured using Franz diffusion cells. This method, though suitable for measuring the actual dose of drug delivered transdermally, is not ideal for screening a large number of enhancers. Specifically, Franz diffusion cells can be used to perform permeability measurements by taking frequent samples from the receiver compartment. These measurements typically use about 4–5 sq. cm of skin (in a diffusion cell of diameter 16 mm) per test and are relatively slow due to the requirement of frequent sampling as well as due to the time required to achieve the steady state. The novel HTP method described in this paper is at least 50-fold more efficient than Franz diffusion cells in terms of skin utilization and up to 30-fold more efficient than Franz diffusion cells in terms of hold up times. Thus a large number of formulations can be screened per unit skin area per day. This improvement in efficiency comes from two factors. First, the new method can perform 50 tests per square inch of skin compared to about one test per square inch of skin as in the case of Franz diffusion cells (16 mm diameter). Second, the new method is based on unsteady-state measurements of solute permeation as opposed to steady-state measurements used by Franz diffusion cells. As shown by Eq. (7), unsteady state measurements at short times reveal the same trend as that revealed under steady state conditions. The objective of the new method is primarily to compare efficiency of various enhancers. Effective enhancers that are identified through the HTP method should be subsequently tested using Franz diffusion cells to measure the actual amount of drug delivered across the skin.

Use of the HTP Method

To assess the ability of the HTP method in determining the efficiency of formulations, various formulations contain-

ing SLS and DPC were generated. These two surfactants were chosen for this study because they are effective transdermal transport enhancers. In addition, because they possess opposite charges (SLS being anionic and DPC being cationic), their mixtures are likely to exhibit peculiar thermodynamic properties (14,15) that may offer unique opportunities for formulation discovery. Total surfactant concentration in these formulations was varied between 0–2% w/v. At each concentration, the relative ratio of SLS: DPC was varied between 0 and 1 in increments of 0.1. Thus, a large number of compositions were generated.

All formulations were tested using the methods described earlier. Figure 2A shows the data for the conductivity enhancement for different SLS: DPC formulations (relative concentration in the range 0 to 1) at a total surfactant concentration of 1% w/v. These data were plotted at various times (diamonds = 0 h, circles = 8 h, squares = 18 h, triangles = 25 h). The maximum efficiency exists at equimolar concentration ($P < 0.02$). The position of the maximum on the enhancement curve remains consistent over all these time intervals. The error observed in the data (<30%) is comparable to a typical error observed in traditional transdermal experiments. We repeated the same set of experiments over varying total surfactant concentration of 0.5% w/v, 1.5% w/v, 1.5% w/v, and 2% w/v. The data were analyzed to generate two-dimensional contour maps of various compositions of SLS and DPC with respect to their efficiency in enhancing conductivity at varying total surfactant concentrations. These maps were generated at various time periods (i.e., 4 h, 8 h, 18 h, and 25 h). The maps at these different times are generally similar and show the same trend in dependence of conductivity enhancement on the composition. For example the map at 25 h is shown in Fig. 2B. In this figure, the y-axis shows the total surfactant concentration in the formulation (SLS+DPC). The x-axis shows the relative fraction of SLS in the formulation. Lines of constant enhancement (0–16) appear as closed contours on the map. The efficiency of the formulations varied significantly with their composition. Specifically, a near-equimolar mixture of SLS and DPC was more effective in enhancing transport compared to either of them alone.

The effectiveness of near equimolar mixtures of SLS: DPC in enhancing skin permeability to mannitol can be clearly seen from Fig. 3, which shows the dependence of conductivity enhancement (squares) and mannitol delivery (circles) on the composition of the formulation at a constant total surfactant composition of 0.5 w/v%. The conductivity data in Fig. 3 correspond to a section of the data from Fig. 2B. The maximum in the transport is observed at a composition of about 50% \pm 10% SLS: DPC (that is, in the range of 40–60% SLS). The occurrence as well as the location of this maximum would have been difficult to predict from the first principles. Specifically, several studies have been performed on the use of various surfactants on transdermal drug transport (16,17); however, relatively little is known about the effect of surfactant mixtures on skin permeability. Surfactant mixtures exhibit a complex phase behavior, thus leading to a plethora of microscopic structures (18,19). Because relatively little is known about the interactions of such complex phases with the skin, it is difficult to predict the effect of surfactant mixtures on skin permeability. The HTP method presented here may not only allow discovery of efficient enhancer composition in such situations, but also assist in creating a knowledge base

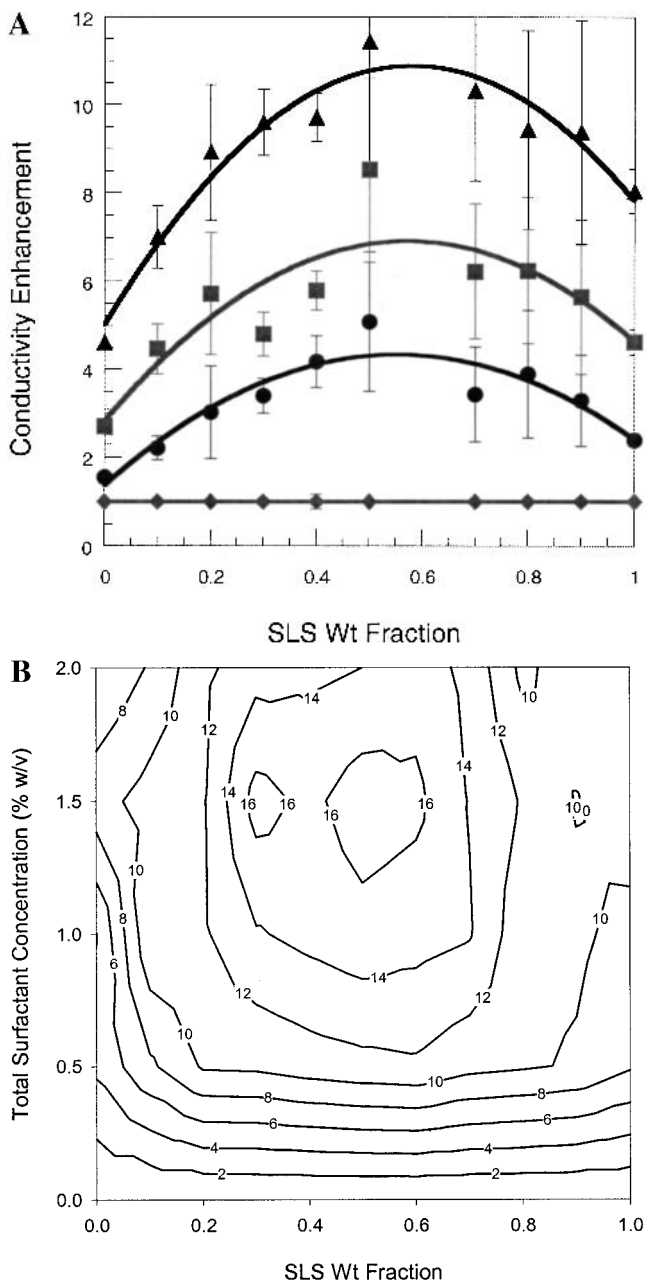


Fig. 2. (A) The dependence of skin conductivity enhancement on SLS: DPC composition at a fixed total surfactant concentration of 1.0% in the HTP array. The conductivity enhancement is plotted at different times (diamonds-0 h, circles-8 h, squares-18 h, triangles-25 h). The trend lines are used to guide the eye. Error bars correspond to $n = 8$. (B) Contour map showing the dependence of skin conductance enhancement on SLS: DPC composition at varying total surfactant concentrations at 25 h in the HTP array. At each total surfactant concentration the wt fraction of SLS in the mixture formulation is varied from 0 to 1 in increments of 0.1. The enhancement varies from 0 to 16 as indicated along the lines of constant enhancement.

that will broaden the understanding of the effect of enhancer mixtures on transdermal drug transport.

Validation of the HTP Method against Franz Diffusion Cells

The predictions of the HTP method were validated using Franz Diffusion Cells. Specifically, we assessed whether the

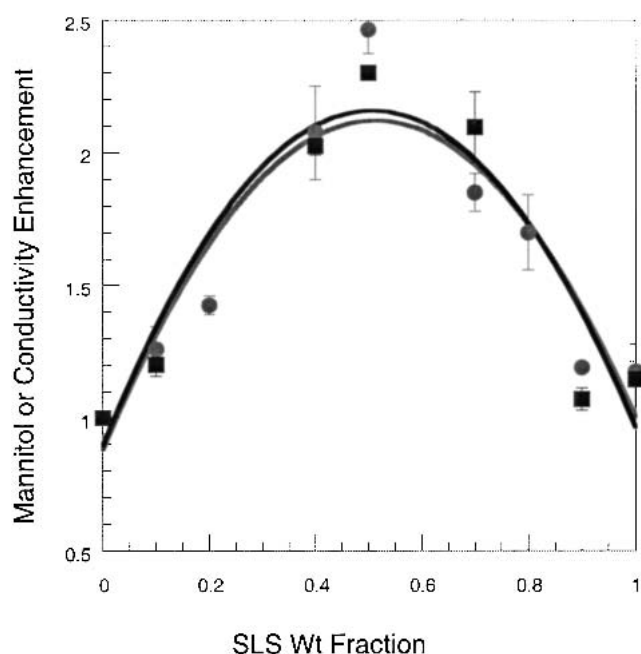


Fig. 3. The dependence of skin conductance enhancement and mannitol permeability enhancement on SLS:DPC composition at a fixed total surfactant concentration of 0.5% as obtained in the HTP array at the end of 90 min. Circles correspond to conductance enhancement ($n = 4$) and squares correspond to mannitol permeability enhancement ($n = 3$).

data shown in Fig. 3 can be confirmed using Franz diffusion cells. In these experiments, the effect of various mixtures of SLS and DPC on electrical conductivity and mannitol permeability of pigskin was measured using Franz diffusion cells. Various formulations containing SLS and DPC were prepared such that the total surfactant concentration was constant at 1.0%. Relative fraction of SLS and DPC was varied in the range of 0 to 1 in increments of 0.1.

Skin conductivity increased significantly on skin exposure to surfactant mixtures (total surfactant concentration of 1.0% w/v). Figure 4 shows the enhancement of skin conductivity on exposure to various compositions of SLS and DPC in Franz diffusion cells (circles) and HTP array (squares) at the end of 25 h. A maximum in conductivity enhancement was obtained for near equimolar mixture of SLS:DPC ($P < 0.05$). The location of the maximum is consistent with that observed during the HTP screening (Fig. 2A). Figure 5 shows the dependence of mannitol permeability on surfactant composition at a constant total surfactant concentration of 1.0% w/v at the end of 40 h in the Franz diffusion cells (circles) and at the end of 90 mins in the HTP array (squares). A maximum is observed in permeability enhancement for near equimolar concentrations of SLS and DPC (0.5 ± 0.1) in both cases ($P < 0.05$). Note that though the qualitative nature of both these data is the same it should not be inferred that they would be quantitatively comparable. HTP screening, as suggested earlier, is only a tool to identify efficient enhancers at low holdup times. The most important conclusion of these data is that the dependence of permeability enhancement on the formulation composition in Fig. 5 is similar to that discovered by the HTP method (Fig. 3). Yet, the new HTP method is much more efficient in screening the formulations (at least 50 fold in terms of area use and up to 30 fold in terms of holdup times).

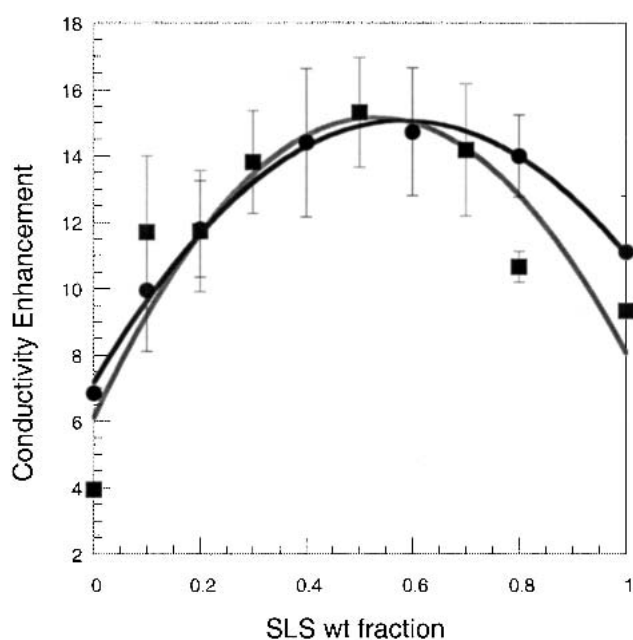


Fig. 4. The dependence of skin conductivity enhancement on SLS:DPC composition at a fixed total surfactant concentration of 1.0% in the Franz diffusion cell (circles, $n = 3$) and HTP array (squares, $n = 4$) at the end of 25 h.

The high-throughput method presented here offers a novel way of screening the enhancer formulations. This method is highly efficient in assessing the efficiency of enhancers. There is no physical, experimental, or fundamental limit on the size of wells used in the HTP array, thereby offering further scope to increase efficiency. The efficiency of the HTP method may also be improved by automated formulation preparation, analysis (especially for conductivity), and

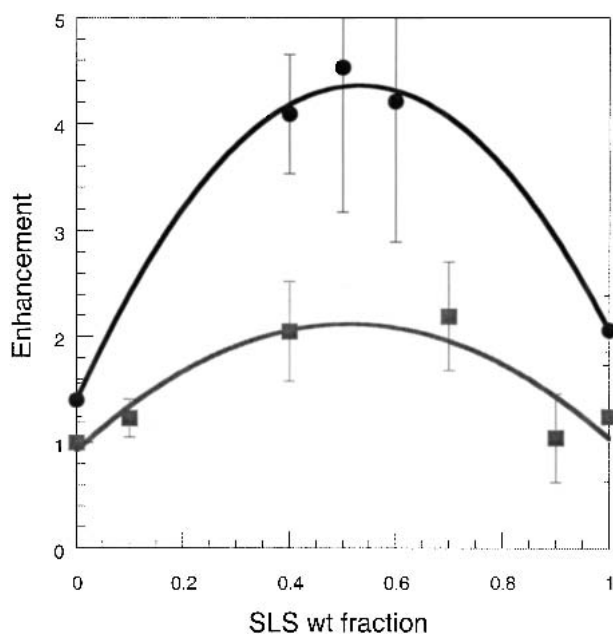


Fig. 5. The dependence of skin mannitol transport enhancement on SLS:DPC composition at a fixed total surfactant concentration of 1.0% in the Franz diffusion cell at the end of 40 h (circles, $n = 5$) and in HTP array at the end of 90 mins (squares, $n = 3$).

by increasing the detection limit of the method, thereby lowering the incubation time. Knowing the relative efficiency of one enhancer over another from HTP screening, it should be sufficient to perform transport experiments in Franz diffusion cells for only efficient enhancers.

The HTP method is particularly beneficial for testing mixtures of enhancers whose efficiency may be difficult to predict *a priori*. Since appropriate mixtures of enhancers are likely to be more efficient than their individual components, the HTP method may be used to discover novel enhancers comprising of enhancer mixtures. This method may also be used to explore synergies between various enhancers, which may lead to novel formulations for transdermal drug delivery as well as cosmetic agents (6).

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