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Research Paper

Colorimetric Polymer Films for Predicting Lipid Interactions and Percutaneous Adsorption of Pharmaceutical Formulations

Izek Ben-Shlush,[1] Roman Volinsky,[1] Marina Katz,[1] Yogesh Scindia,[1] Racheli Itzhak,[1] Hila Tsahor Ohayon,[2] Ido Yosha,[2] and Raz Jelinek[1,3]

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Purpose. To develop and demonstrate a rapid and simple colorimetric film assay for evaluating lipid interactions of pharmaceutical compounds and gel formulations.
Methods. The colorimetric assay comprises glass-supported films of phospholipids and polydiacetylene, which undergo visible and quantifiable blue–red transformations induced by interactions with amphiphilic molecules applied in very small volumes on the film surface. The color transitions are recorded by scanning of the films, and quantified through a simple image analysis algorithm.
Results. We show that pharmaceutical molecules and gel formulations induce blue–red transformations after short incubation with the lipid/polydiacetylene (PDA) films. Colorimetric dose–response curves exhibit dependence upon the lipid affinity and extent of membrane binding of the pharmaceutical compounds examined. The colorimetric lipid/PDA film assay was employed for distinguishing the contributions of individual molecular components within gel formulations.
Conclusions. The colorimetric data yield insight into the degree of lipid binding of the molecules tested. The film assay is particularly advantageous for analysis of semi-solid (gel or lotion) formulations, elucidating the lipid interaction characteristics of specific molecular components within the mixtures. The new colorimetric film assay constitutes a generic, rapid, and easily applicable platform for predicting and screening interactions of pharmaceutical compounds and complex formulations with lipid barriers.

KEY WORDS: gel formulations; lipid barriers; lipid interactions; passive diffusion; percutaneous absorption.

INTRODUCTION

Evaluating the adsorption and interactions of pharmaceutical compounds and formulations with physiological lipid barriers, such as the blood–brain barrier and the stratum corneum (SC), is a critical parameter in pharmaceutical research and development. Lipid interactions play a particularly important role in situations of *passive diffusion,* in which the hydrophobic environment intimately affects the transport properties (1,2). Prediction of molecular diffusion through the skin is especially challenging due to its laminated and complex structure. The need to facilitate transfer of pharmaceutical substances through the hydrophobic SC, and in case of a systemic product through the hydrophilic epidermis and dermis, is a complicated task to achieve. The need for a fast and high throughput prediction tool is thus evident.

Delivery of drugs through the SC (or other physiological lipid barriers) is often facilitated through the creation of formulations comprising varied functional ingredients beside the active pharmaceutical ingredient (API), such as surfactants, penetration enhancers, and molecular stabilizers (3,4). *Gel-type* formulations have become an attractive configuration for topical administration of pharmaceutical substances and percutaneous drug delivery due to environmental stability, permeation effectiveness, and ease of practical use (5). When designing formulations, a critical need thus arises for evaluating the impact of individual constituents upon the interactions with the lipid barrier and upon the actual input (rate) of drug transport through human skin.

Existing assays for assessment of lipid barrier interactions and permeation are still limited. Prediction of molecular adsorption onto lipid barriers generally rely upon simplistic parameters such the molecular weights and partition coefficients between hydrophilic/hydrophobic phases (logP/logD values), determined by either the shake flask method (6), or by high performance liquid chromatography (HPLC) (7). Cell culture assays are experimentally demanding and suffer from poor reproducibility (8). Determination of molecular adsorption onto the air–water interface (9) and into lipid monolayers (10–12) have been utilized for analysis of lipophilic properties of pharmaceutical substances, however these techniques are experimentally demanding and time consuming.

We have recently introduced a new rapid colorimetric platform for detection and analysis of interactions and relative penetration of pharmaceutical compounds through

Izek Ben-Shlush and Roman Volinsky contributed equally.

[1] Department of Chemistry and Ilse Katz Institute for Nanotechnology, Ben Gurion University of the Negev, Beersheba 84105, Israel.
[2] Perrigo Israel Pharmaceutical Ltd., Yeruham Israel.
[3] To whom correspondence should be addressed. (e-mail: razj@bgu.ac.il)

lipid layers (13). The colorimetric assay consists of vesicles comprising phospholipids and polydiacetylene (PDA)—a cross-linked polymer which undergoes dramatic blue–red transformations corresponding to structural transitions within its conjugated framework (14). Previous studies demonstrated that PDA constitutes a useful biosensing element for membrane interactions since the colorimetric transitions can be directly induced by binding of lipophilic substances to PDA-based assemblies (15). In particular, vesicles comprising phospholipids and PDA were shown to exhibit distinct colorimetric dose–response profiles, which depend upon the degree of lipid binding and bilayer insertion, providing an effective platform for rapid analysis of membrane interactions of pharmaceutical compounds (16).

Application of vesicle-based assays is restricted, however, to evaluation of lipid interactions of *soluble* molecules. Here we demonstrate the utilization of glass-supported lipid/PDA *thin films* (17) for rapid colorimetric screening of the interactions of pharmaceutical compounds and semi-solid (gel) formulations with hydrophobic barriers. We show that PDA-based film systems offer significant advantages for practical pharmaceutical screening applications. The presented data demonstrate quantitative colorimetric analysis using conventional desktop scanning instrumentation and the possibility for testing samples in their actual physical states, such as gel formulations.

MATERIALS AND METHODS

Materials. 10,12-Tricosadiynoic acid was purchased from Alfa Aesar (Ward Hill , MA) and purified by dissolving the powder in chloroform, filtrating the resulting solution through a 0.45 μm Nylon filter, and evaporation of the solvent. Dimyristoylphosphatidylcholine (DMPC) [Avanti Polar Lipids, Alabaster, AL] was used as received. The diacetylene and phospholipids were dissolved in chloroform to give final concentration of 2 mM. Amitriptyline hydrochloride, promethazine hydrochloride, metoprolol tartrate salt, acebutolol hydrochloride, procaine hydrochloride, and procainamide hydrochloride were purchased from Sigma and were dissolved in water. All solvents were HPLC-grade pure.

Diclofenac gel formulations were supplied by Perrigo Israel Pharmaceutical Ltd, and contain (beside the API) polyethylene glycol monomethyl ether (MPEG 350), hydroxyethyl cellulose, diethylene glycol monoethyl ether (transcutol), and glycerin (18).

Film Preparation. All diacetylene/DMPC films were prepared at 20°C in a computerized Langmuir trough manufactured by NIMA (model Nima 312D (7×50 cm^2), Nima Technology Ltd, Coventry, U.K.) (19,20). The surface pressure was monitored using a 1-cm-wide filter paper as a Wilhelmy plate. For each experiment, 35 μL of the lipid/diacetylene chloroform solution was spread on the water subphase (pH 6.3). Compression started after solvent evaporation (15 min) and was carried out at a constant barrier speed of 8 cm^2 min^{-1}. The films were compressed up to 15 mN/m and then allowed to equilibrate at constant surface pressure for 10 min, subsequently transferred onto modified glass slides by the horizontal touch method (Langmuir–Schaefer method) (21,22). The glass-supported films were irradiated at 254 nm to polymerize the diacetylene.

Color scanning and image analysis. Aliquots of the tested compounds (6 μl) were placed on the surfaces of the polymerized films and incubated at 37°C for 45 min. The films were sealed in a Petri dish to minimize evaporation. Following incubation, the drops were removed with a filter paper and the dry films were scanned on an Epson 4990 Photo scanner to produce high resolution RGB images. The films were placed in a special film adaptor and scanned in transmitted mode at 2,400 dpi optical resolution and 24 bit color depth. Quantification of the blue–red color transformations was carried out using digital colorimetric analysis (DCA) (18). DCA was carried out by cropping the spots within the film images which the sample volume covered, and application of MATLAB® mathematical software for calculating the total intensity and abundance of red pixels on the surface (17).

Molecular adsorption at the air/water interface. The water subphase used in the Langmuir trough was doubly purified (18.3 mΩ resistivity) by using a Barnstead D7382 water purification system (Barnstead Thermolyne Corporation, Dubuque, IA-USA). Adsorption experiments were carried out at 20°C using the Nima 312D Teflon trough. Aliquots from the samples were injected into the gently-stirred water subphase (total volume of 50 cm^3) through a short vertical tube, yielding different initial concentrations used in the experiments. The adsorption isotherms ($\Delta\pi$-time) of the pharmaceutical compounds at a constant air/water interface area (12 cm^2) were monitored using the Wilhelmy plate. In the experiments examining compound penetration into lipid monolayers, the chloroform lipid solution was spread over the clean air/water interface prior to compound injection and allowed to equilibrate for 15 min reaching the desired initial surface pressure (π_i). The error in all measurements was ±0.2 mNm^{-1}.

RESULTS

Colorimetric Screening of Pharmaceutical Compounds Using Lipid/PDA Films

Figure 1 depicts the principles of the colorimetric pharmaceutical screening concept. The blue polymerized DMPC/PDA films constitute the adsorption platform and signal generator (Fig. 1A). Previous studies have determined that the phospholipids and polydiacetylene modules adopt microscopic domains within the film (20). We have further observed that mixing phospholipids with the diacetylene contributes to film stability, aids the efficient transfer from the air/water interface onto the glass surface, overall significantly improving the uniformity and long-term stability of the films. The thrust of the colorimetric screening methodology is the induction of visible blue–red transformations upon the film surface following incubation with membrane-active and lipophilic compounds which interact and bind to the film interface, disrupting the conjugated network of the polymer (15) (Fig. 1A).

Figure 1B depicts a typical colorimetric image of a lipid/PDA film incubated around 30 min with increasing concentrations of acebutolol, a β-adrenoceptor blocking agent which exhibits a wide range of physiological effects associated with cell membrane interactions (23,24). The scanned image in

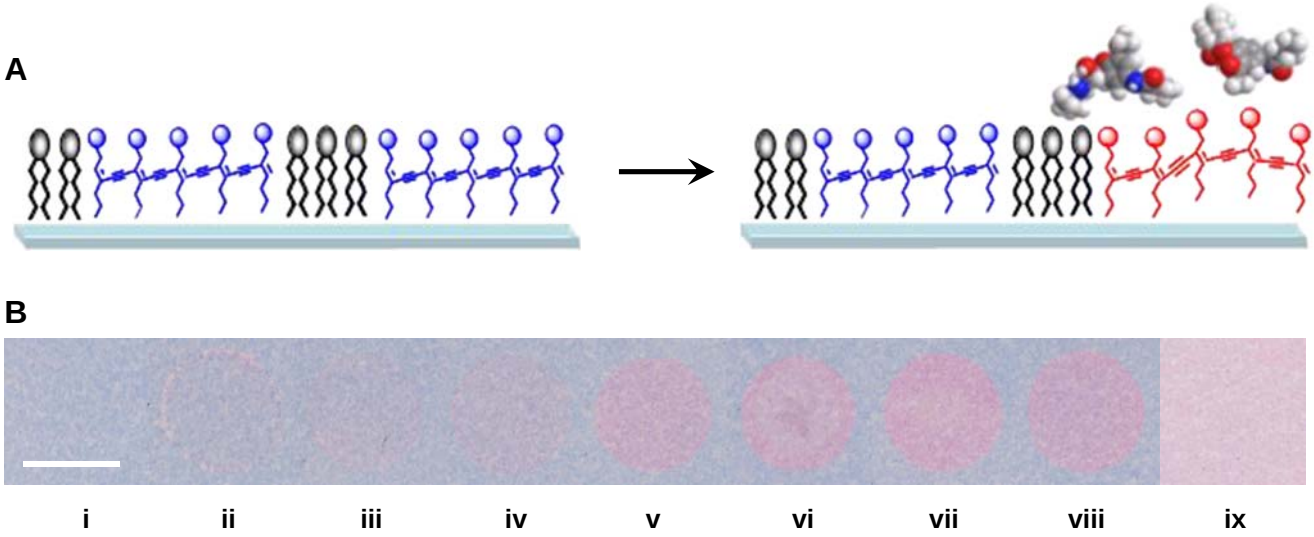

Fig. 1. Colorimetric pharmaceutical screening using lipid/PDA films. **A** Schematic figure showing the blue–red transformations induced in glass-supported lipid/PDA film by adsorbed compounds. Phospholipids are depicted in *black*, polydiacetylene is in *blue* (initially) and *red* (after structural transformation induced by adsorbed species); **B** Scanned film after incubation with acebutolol: (*i*) control (no addition), (*ii*) water, (*iii*) incubated with 0.0005 M acebutolol, (*iv*) 0.001 M, (*v*) 0.005 M, (*vi*) 0.01 M, (*vii*) 0.05 M, (*viii*) 0.1 M, (*ix*) most intense red color, induced upon film heating. *Scale bar* corresponds to 3 mm.

Fig. 1B highlights several important aspects of the colorimetric analysis. First, the intensity of red spots on the film surface is directly related to compound concentrations. Moreover, Fig. 1B demonstrates that blue–red transformations can be distinguished by the naked eye even at very low concentrations (sub-millimolar concentrations in case of acebutolol). The scanned image also shows that very low sample volumes can be analyzed using the chromatic film technology—the diameter of each spot in Fig. 1B is only around 3 mm.

The blue–red transformations occurring upon the film surface can be easily quantified through DCA, a simple image analysis algorithm designed to measure the abundance and intensity of the red pixels in a desired area within the scanned image (17). Figure 2 depicts the DCA results obtained for several pharmaceutical compounds (Table I) incubated on the DMPC/PDA film, revealing significant differences among the molecules tested. Figure 2 depicts the relationship between compound concentrations and the percentage of the red chromaticity shift (%RCS), which is a quantitative parameter measuring the extent of the blue-red transformation in the film surface (17). Amitriptyline and promethazine induced pronounced blue–red transitions at very low concentrations ($<10^{-4}$ M) (Fig. 2). Acebutolol and metoprolol, in comparison, gave rise to less intense red spots when placed upon the film surface (lower %RCS values), which were moreover induced at much higher concentrations (note the logarithmic concentration scale). Procaine and procaineamide did not give rise to blue–red transformations when placed on the film surface (Fig. 2).

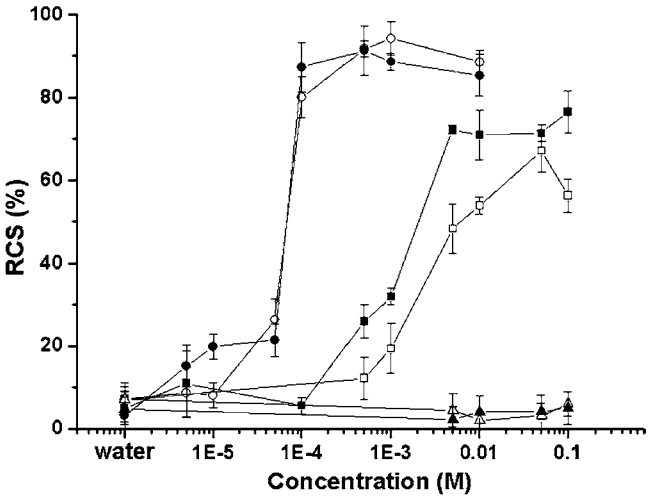

Fig. 2. Colorimetric dose response curves. Degree of blue–red transformations (%RCS) obtained following incubation of pharmaceutical compounds upon the surface of DMPC/PDA film: (*open circles*) Amitriptyline, (*closed circles*) Promethazine, (*open squares*) Acebutolol, (*closed squares*) Metoprolol, (*open triangles*) Procaine, (*closed triangles*) Procainamide.

Monolayer Adsorption Analysis

To examine whether the distinct colorimetric dose–response curves in Fig. 2 reflect different interactions of the pharmaceutical compounds with the lipid/PDA films, we carried out phospholipid monolayer adsorption analysis (Fig. 3). Isothermal adsorption experiments have been employed for characterization of lipid affinity and binding of pharmaceutical compounds (9,25). In such experiments, the surface-pressures of phospholipid monolayers at the air/water interface was shown to increase following injection of lipophilic molecules into the water subphase. Furthermore, the surface pressures increase mirrored the relative lipid affinity of the tested molecules (10–12). In the context of the colorimetric assay examined here, transfer of water-soluble molecules onto lipid monolayers at the air/water interface closely resembles the situation encountered in the lipid/PDA film experiments shown in Figs. 1 and 2, in which the blue–red transitions are induced through interactions between molecules dissolved in the aqueous solution and the lipophilic films.

Table I. Pharmaceutical Compounds Examined in this Work

Pharmaceutical compounds	LogP	Mode of action
Amitriptyline	5.04	Analgestic, antidepressant, adrenergic uptake inhibitor
Promethazine	4.946	Antiallergic, H_1-antagonist
Acebutolol	2.356	anti-arrhythmic, β_1-adrenoceptor blocking agent, local anesthetic
Metoprolol	2.477	Antihypertensive, β_1-adrenoceptor blocking agent
Procaine	1.845	Local anesthetic
Procainamide	1.282	Anti-arrhythmic

The adsorption isotherms in Fig. 3A point to significantly different lipid-monolayer affinities by the examined molecules, which echo the colorimetric dose–response curves in Fig. 2. Specifically, amitriptyline and promethazine induced the highest increase in surface pressures, followed by metoprolol and acebutolol (Fig. 3A, curves i–iv). Importantly, the injected concentrations of amitrptyline and promethazine used in the adsorption isotherms in Fig. 3A were an *order of magnitude lower* than the respective concentrations of metoprolol and acebutolol (50 μM for amitriptyline and promethazine compared to 500 μM in case of metoprolol and acebutolol)—indicating significantly stronger adsorption of amitrptyline and promethazine to the phospholipid monolayer. Procaine and procainamide gave rise to minimal increase in surface pressures, at higher injected concentrations of 1 mM (Fig. 3A, curves v, vi), mirroring their minimal interactions with the DMPC/PDA films (Fig. 2).

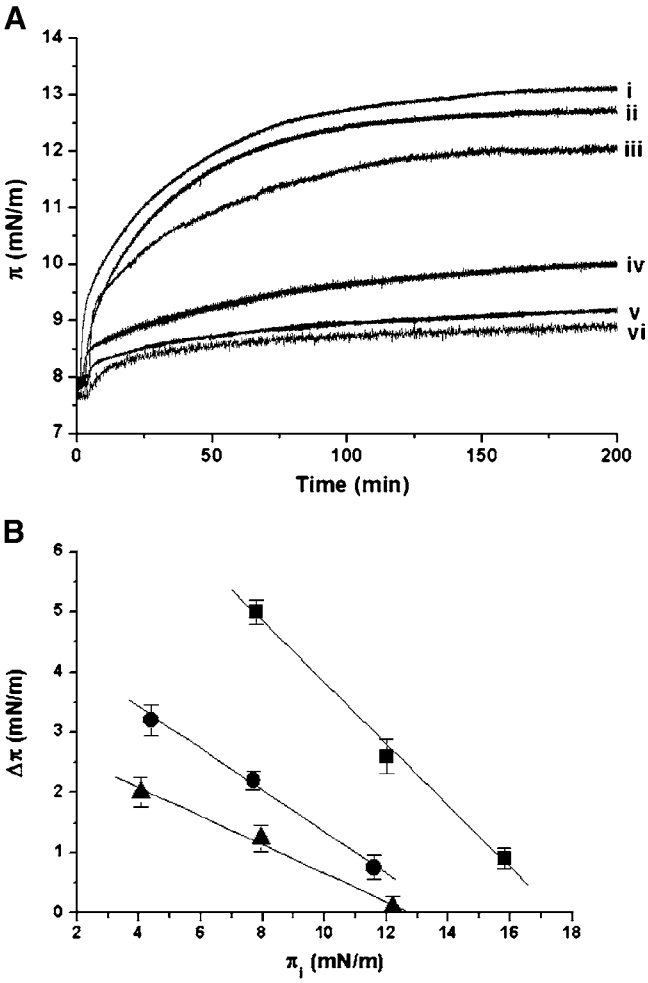

Fig. 3. Adsorption isotherms. **A** Adsorption isotherms onto DMPC monolayers at initial pressures of 8 mN/m: (*i*) amitriptyline at 50 μM concentration, (*ii*) promethazine 50 μM, (*iii*) metoprolol 500 μM, (*iv*) acebutolol 500 μM, (*v*) procaine 1 mM, (*vi*) procainamide 1 mM. **B** Correlations between Δπ (the difference between the final and initial surface pressures of the DMPC monolayer) and initial pressure π_i, after injection into the water subphase of (*squares*) promethazine 50 μM, (*circles*) acebutolol 500 μM, (*triangles*) procaine 1 mM.

Figure 3B depicts the net pressure increase (Δπ—difference between the initial surface pressure and final equilibrium pressure) recorded in DMPC monolayers compressed to different initial pressures (π_i) following injection of representative compounds into the water subphase. The linear dependences apparent in Fig. 3B reflect the extent of binding and insertion of the tested molecule into the lipid monolayer (26). Specifically, for all π_i examined, the net pressures (Δπ) decreased in the order promethazine > acebutolol > procaine.

The extent of lipid interactions is also manifested in the *intersections* of the straight lines in Fig. 3B with the x axis (π_i). The intersection values are essentially the "exclusion pressures" (π_{ex}) at which the molecules cannot transfer from the aqueous phase into the phospholipid monolayer (26). The π_{ex} values, extracted from Fig. 3B, of approximately 17 mN/m (promethazine), 14 mN/m (acebutolol), and 12.7 mN/m (procaine), clearly distinguish between the capacities of the molecules to adsorb and penetrate into the lipid monolayers, and again point to a relative monolayer affinity of promethazine > acebutolol > procaine.

Colorimetric Screening of Gel Formulations

Prediction of lipid interactions and membrane permeation of semi-solid *gel formulations* poses particular challenges since analysis cannot be carried out in conventional liquid environments (i.e. the *gel* configuration of the sample needs to be maintained). Figure 4 depicts an experimental setup for analysis of lipid interactions of gel formulations using glass-supported lipid/PDA films. The gel samples are placed upon circular glass-supported DMPC/PDA films, and subsequently covered with a thin glass slip to maintain stability and prevent environmental contamination (Fig. 4A). Visible colorimetric transformations induced by gel formulations of different compositions are shown in Fig. 4B.

The color transitions induced by the formulations can be also monitored *in situ* through color scanning (Fig. 4C). Figure 4C depicts the kinetic colorimetric profiles of several diclofenac formulations designed for enhanced skin permeation (18). The tested formulations comprised diclofenac (the

API), transcutol (penetration enhancer) and other molecular components (18). Importantly, neither diclofenac nor transcutol gave rise to color transitions when placed *individually* on DMPC/PDA films (data not shown). This result indicates that the colorimetric transformations in Fig. 4B and C correspond to membrane interactions affected by the formulations as a whole. Furthermore, the distinct kinetic behavior of formulations having different compositions, as shown in Fig. 4C, points to contributions of specific constituents within the formulation to lipid interactions.

In particular, increasing the concentration of diclofenac in the gel formulation from 3% (Fig. 4C, curve ii) to 5% (Fig. 4C, curve iv) significantly enhanced film interaction of the gel mixture—apparent as the higher %RCS values in Fig. 4C. This result is consistent with previously-reported observations suggesting that enhanced skin permeation goes hand in hand with an increase in diclofenac concentration (27). In contrast, *decreasing* the weight-percentage of transcutol from 5% (Fig. 4C, curve ii) to 1% (Fig. 4C, curve iii) seems to actually *enhance* lipid binding (i.e. more pronounced blue–red transformation). Such an inverse relationship between transcutol concentration and lipid permeation has indeed been reported in high transcutol concentration ranges (28).

Figure 5 depicts an application of the colorimetric film assay for multicomponent analysis of gel formulations. Specifically, the 3-dimensional bar diagram in Fig. 5A

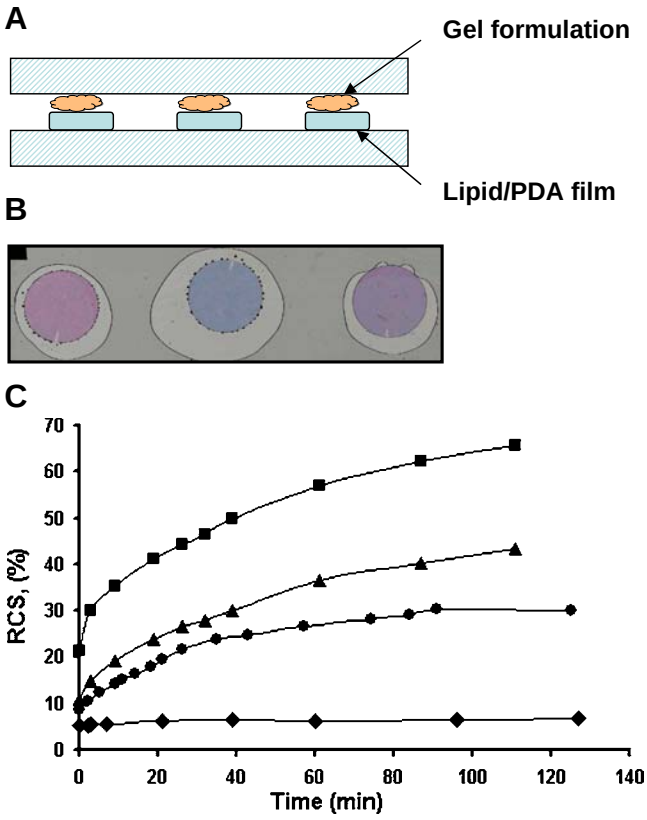

Fig. 4. Colorimetric analysis of gel formulations: **A** schematic description; **B** scanned image recorded after 1 h incubation of diclofenac gel formulations: 5% diclofenac, 5% transcutol (*left spot*), 0%, 0% (*middle spot*), 3%, 5% (*right spot*). **C** Kinetic curves recorded *in situ* for formulations containing: *squares*—5% diclofenac, 5% transcutol, *triangles*—3%, 1%, *circles*—3%, 5%, *diamonds*—0%, 0%.

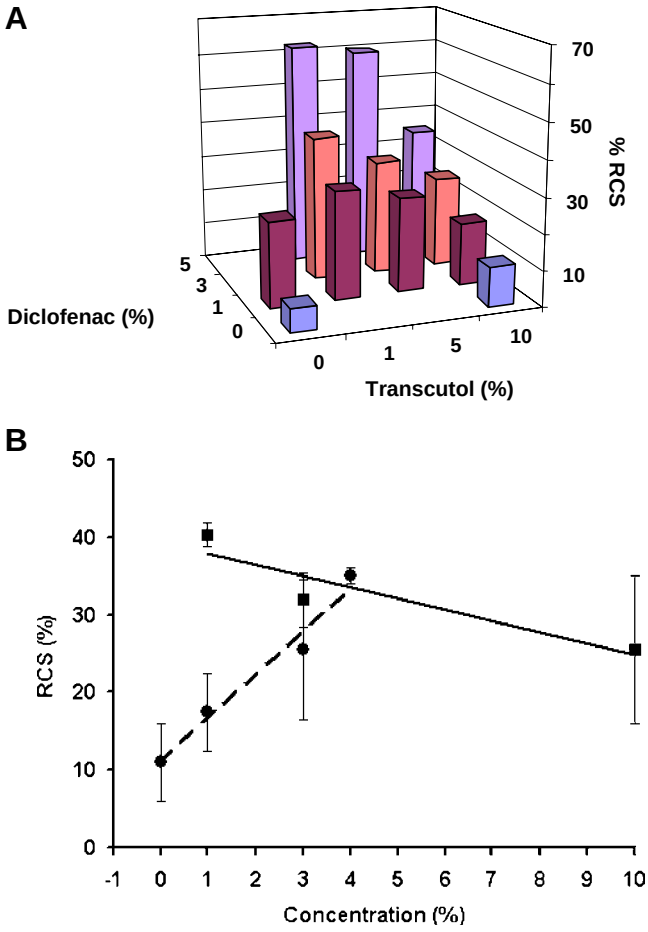

Fig. 5. Multi-component analysis of gel formulations: **A** three-dimensional matrix depicting %RCS values recorded for formulations having different contents of diclofenac and transcutol; **B** Dependence of %RCS upon the concentrations of diclofenac (*circles–broken line*, at a constant transcutol concentration of 1%), and transcutol (*squares–solid line*, at a constant diclofenac concentration of 3%).

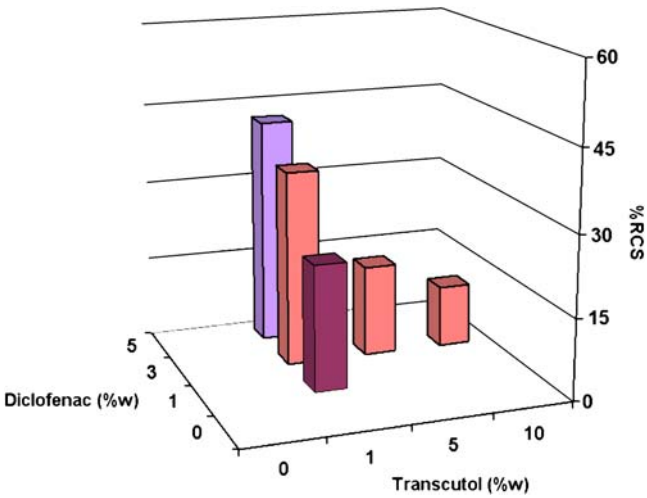

Fig. 6. Multi-component analysis of ceramide/PDA film: Three-dimensional matrix depicting %RCS values recorded for formulations shown in Fig. 5 (having different contents of diclofenac and transcutol).

summarizes the quantitative determination of the blue–red transformations (e.g. DCA analysis) induced by formulations having different compositions. The colorimetric data in Fig. 5A, recorded approximately 60 min after deposition of the formulations upon the film surface, highlight the different effects of diclofenac and transcutol in the mixtures upon lipid interactions.

Indeed, the linear relationships in Fig. 5B, extracted from the bar diagram in Fig. 5A, clearly point to the opposing effects of diclofenac and transcutol upon lipid interactions. While increasing diclofenac mole percentage seems to linearly amplify the color response of the film (broken line in Fig. 5B), the impact of transcutol upon the color change was opposite (solid line in Fig. 5B). In particular, increasing transcutol concentration (at a constant diclofenac concentration) actually reduced lipid binding. This inverse relationship essentially replicates the kinetic curves depicted in Fig. 4C.

It should be emphasized that the choice of DMPC as the lipid molecule incorporated within the PDA films was due to the fact that this phospholipid is inexpensive, readily available, and widely-used in diverse model systems of membrane and physiological barriers. However, different lipid compositions can be readily incorporated within the PDA framework. Ceramide, for example, which comprises a major component of the SC, has been included in the chromatic films and yielded colorimetric data that were similar to the results reported above for the DMPC/PDA film (Fig. 6).

DISCUSSION

This study depicts the design and application of a simple colorimetric film platform for prediction and analysis of lipid interactions of pharmaceutical compounds and formulations. The new colorimetric approach relies upon color scanning and quantification of the blue–red transformations induced by pharmaceutical substances in glass-supported phospholipid/PDA films; the color changes are directly related to the extent of lipid binding by the molecules tested. The technology is generic, easily applicable, and capable of distinguishing among different lipid interaction and binding properties of the tested molecules.

The experiments revealed significantly different colorimetric responses induced by representative pharmaceutical compounds. Specifically, the diverging colorimetric dose response curves depicted in Fig. 2 most likely reflect the different affinities of the examined compounds to lipid barriers, and the lipid interaction/penetration profiles of the compounds. As summarized in Table I, amitriptyline and promethazine are highly lipophilic, exhibiting logP values that are greater than 4, which most likely contribute to the considerable affinity to the lipid/PDA film as manifested in Fig. 2. The logP values of acebutolol and metoprolol are lower – around 2.5 – which might explain their lesser association with the lipid/PDA film compared to amitriptyline and promethazine (i.e lower %RCS values, further induced at a higher concentration range, as depicted in Fig. 2). The colorimetric dose response analysis in Fig. 2 also suggests that procaine and procainamide *do not* adsorb onto lipid barriers. This observation might be related to the relatively low logP values, at around 2.0, of these two compounds (Table I).

Monolayer adsorption experiments (Fig. 3) corroborated the proposed lipid interactions corresponding to the colorimetric phenomena. The isothermal adsorption profiles in Fig. 3 feature differences among the pharmaceutical molecules which exactly mirror the colorimetric data, and confirmed the relationships between the color changes and lipid interactions. In particular, the data highlighted the pronounced affinity of amitriptyline and promethazine to lipid monolayers compared to the other molecules examined, the lesser lipid binding of acebutolol and metoprolol, and the minimal lipid interactions of procaine and procainamide.

An important advantage of the lipid/PDA film platform as a practical pharmaceutical screening tool is the feasibility for analysis of semi-solid, gel formulations. Figures 4 and 5 demonstrate that lipid interactions of physically-intact gel formulations can be easily monitored through incubation upon the film surface. In particular, the color response of different formulations facilitates elucidation of the contributions of specific components. The colorimetric analysis depicted in Fig. 5 revealed, on the one hand, the increased lipid adsorption induced by higher concentrations of dicloefnac (the API) and, and, on the other hand, the *inverse* relationship between lipid binding and transcutol concentration in the gel formulation. It should be emphasized that the formulations we have examined here were thoroughly analyzed for their skin penetration capabilities using conventional techniques such as Franz cell apparatus (18). Such methods, which are overall more cumbersome and time-consuming, yielded similar information concerning lipid interactions as the simple model presented here.

The colorimetric lipid/PDA film platform constitutes a generic, easy-to-apply technology for evaluation of binding, interactions, and penetration into lipid barriers by pharmaceutical substances. The colorimetric approach is a fast and simple technique for evaluating membrane/lipid affinities, particularly in comparison with common methods such as logP/logD measurements, both because of the ease of performing the experiments and data interpretation, and also because the lipid/PDA films mimic two-dimensional lipid barriers. The lipid/PDA film platform can be employed for high throughput screening utilizing extremely low sample volumes, making the technique a practical R&D tool. The technology is particularly amenable for investigating lipid interactions and percutaneous adsorption of gel-type formulations and pharmaceutical lotions as the analysis examines the actual semi-solid configuration of the tested materials.

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REFERENCES

1. G. Grubauer, K. R. Feingold, R. M. Harris, and P. M. Elias. Lipid content and lipid type as determinants of the epidermal permeability barrier. *J. Lipid. Res*. **30**:89–96 (1989).
2. T. Marjukka Suhonen, J. A. Bouwstra, and A. Urtti. Chemical enhancement of percutaneous absorption in relation to stratum

corneum structural alterations. *J. Control. Release*. **59**:149–161 (1999), doi:10.1016/S0168-3659(98)00187-4.
3. B. W. Barry, and A. C. Williams. *Encyclopedia of Pharmaceutical Technology*. Informa Healthcare, London, 1995.
4. R. H. Guy, and J. Hadgraft. Physicochemical aspects of percutaneous penetration and its enhancement. *Pharm. Res*. **5**:753–758 (1988), doi:10.1023/A:1015980516564.
5. C. Surber, and E. W. Smith. The mystical effects of dermatological vehicles. *Dermatology*. **210**:157–168 (2005), doi:10.1159/000082572.
6. A. Leo, C. Hansch, and D. Elkins. Partition coefficients and their uses. *Chem. Rev*. **71**:525–616 (1971), doi:10.1021/cr60274a001.
7. L. Escuder-Gilabert, M. Molero-Monfort, R. M. Villanueva-Camanas, S. Sagrado, and M. J. Medina-Hernandez. Potential of biopartitioning micellar chromatography as an *in vitro* technique for predicting drug penetration across the blood brain barrier. *J. Chromatogr*. **807**:193–201 (2004).
8. P. Artursson, K. Palm, and K. Luthman. Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv. Drug Deliv. Rev*. **46**:27–43 (2001), doi:10.1016/S0169-409X(00)00128-9.
9. A. Seelig, R. Gottsclichi, and R. M. Devant. A method to determine the ability of drugs to diffuse through the blood–brain barrier. *Proc. Nat. Acad. Sci. U. S. A*. **91**:68–72 (1994), doi:10.1073/pnas.91.1.68.
10. S. L. Krill, K. Y. Lau, W. Z. Plachy, and S. J. Rehfeld. Penetration of dimyristoylphosphatidylcholine monolayers and bilayers by model â-blocker agents of varying lipophilicity. *J. Pharm. Sci*. **87**:751–756 (1998), doi:10.1021/js970374z.
11. M. Makino, M. Kamiya, N. Nakajo, and K. Yoshikawa. Effects of local anesthetics on the dynamic behavior of phospholipid thin film. *Langmuir*. **12**:4211–4217 (1996), doi:10.1021/la950917p.
12. A. Seelig. Local anesthetics and pressure: a comparison of dibucaine binding to lipid monolayers and bilayers. *Biochim. Biophys. Acta*. **899**:196–204 (1987), doi:10.1016/0005-2736(87)90400-7.
13. D. Evrard, E. Touitou, S. Kolusheva, Y. Fishov, and R. Jelinek. A new colorimetric assay for studying and rapid screening of membrane penetration enhancers. *Pharm. Res*. **18**:943–949 (2001), doi:10.1023/A:1010980009823.
14. H. Ringsdorf, B. Schlarb, and J. Venzmer. Molecular architecture and function of polymeric oriented systems—models for the study of organization, surface recognition, and dynamics of biomembranes. *Angew Chem, Int. Ed. Engl*. **27**:113–162 (1988), doi:10.1002/anie.198801131.
15. Q. Cheng, and R. C. Stevens. Coupling of an induced fit enzyme to polydiacetylene thin films colorimetric detection of glucose. *Adv. Mater*. **9**:481–483 (1997), doi:10.1002/adma.19970090605.
16. M. Katz, I. Ben-Shlush, S. Kolusheva, and R. Jelinek. Rapid colorimetric screening of drug interaction and penetration through lipid barriers. *Pharm. Res*. **23**:580–588 (2006), doi:10.1007/s11095-006-9569-1.
17. R. Volinsky, S. Kolusheva, T. Sheynis, M. Kliger, and R. Jelinek. Glass-supported lipid/polydiacetylene films for colour sensing of membrane-active compounds. *Biosens. Bioelectron*. **22**:3247–3251 (2007), doi:10.1016/j.bios.2006.12.024.
18. M. Arkin, *et al*. Diclofenac compositions for the treatment of skin disorders. U.S.A. patent US 20050137164 A1. 2005.
19. F. Gaboriaud, R. Golan, R. Volinsky, A. Berman, and R. Jelinek. Organization and structural properties of langmuir films composed of conjugated polydiacetylene and phospholipids. *Langmuir*. **17**:3651–3657 (2001), doi:10.1021/la0012790.
20. R. Volinsky, F. Gaboriaud, A. Berman, and R. Jelinek. Morphology and Organization of Phospholipid/Diacetylene Langmuir Films Studied by Brewster Angle Microscopy and Fluorescence Microscopy. *J. Phys. Chem. B*. **106**:9231–9236 (2002), doi:10.1021/jp020393j.
21. V. Tscharner, and H. M. McConell. Physical properties of lipid monolayers on alkylated planar glass surfaces. *Biophys. J*. **36**:421–427 (1981).
22. G. L. Gaines Jr. Insoluble Monolayers at Liquid–Gas Interfaces. Wiley-Interscience, New York, 1966.
23. D. Attwood, and S. P. Agarwal. The surface activity and self-association of some beta-adrenoceptor blocking agents in aqueous solution. *J. Pharm. Pharmacol*. **31**:392–395 (1979).
24. B. Basil, R. Jordan, A. H. Loveless, and D. R. Maxwell. A comparison of the experimental anti-arrhythmic properties of acebutolol (M & B 17,803), propranolol and practolol. *Br. J. Pharmacol*. **50**:323–333 (1974).
25. P. Suomalainen, C. Johans, T. Soderlund, and P. K. J. Kinnunen. Surface activity profiling of drugs applied to the prediction of blood–brain barrier permeability. *J. Med. Chem*. **47**:1783–1788 (2004), doi:10.1021/jm0309001.
26. R. Maget-Dana. The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes. *Biochim. Biophys. Acta*. **1462**:109–140 (1999), doi:10.1016/S0005-2736(99)00203-5.
27. E. Touitou, B. Godin, M. Shumilov, E. Roitman, A. Zeevi, E. Asculai, I. Yosha, and H. Tsahor-Ohayon. A new approach for *in vitro* testing bioequivalence of dermal products, *2nd EUFEPS/APGI Conference on Optimizing Drug Delivery and Formulation "Evaluation of Drug Delivery Systems—Issues and Perspectives"*. Versailles, France, 2005.
28. D. A. Godwin, N. H. Kim, and L. A. Felton. Influence of transcutol CG on the skin accumulation and transdermal permeation of ultraviolet absorbers. *Eur. J. Pharm. Biopharm*. **53**:23–27 (2002), doi:10.1016/S0939-6411(01)00215-6.