Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(3): 189-192

Research Article ISSN 0975-248X

Evaluation of Track Etch Membrane as a Surrogate for *Ex-vivo* Drug Permeation Studies

Ravish Garg[1*], Priti Girotra[2], Shailendra Kumar Singh[2], Dinesh Kumar[3], S. K. Chakarvarti[4]

[1]*Department of Biomedical Engineering, G. J. University of Sci. & Tech., Hisar, Haryana, India*
[2]*Department of Pharmaceutical Sciences, G. J. University of Sci. & Tech., Hisar, Haryana, India*
[3]*Department of Electronic Science, Kurukshetra University, Kurukshetra, Haryana, India*
[4]*Manav Rachna International University, Faridabad, Haryana, India*

ABSTRACT
The track etch membranes has attracted the attention of researchers, as a kind of novel material, for their various applications such as precise separation of biological cells, detection of biomolecules and controlled drug release, especially for pharmaceutical and biomedical applications. In the present study, the efficacy of track etch membrane over goat skin for the *Ex-vivo* permeation of silver sulfadiazine gel has been investigated with the objective to evaluate track etch membrane as a surrogate for biological membrane owing to ethical issues and scarce availability of biological skins. The percent cumulative drug release from silver sulfadiazine gel using track etch membrane and goat skin were obtained as 18.18 % and 20.99 % respectively. The results obtained from track etch membrane and goat skin was similar with only a slight difference in percent drug diffusion which might be because of the lipids present in animal skin. Track etch membrane has the potential to mimic the animal skin for such *ex-vivo* drug diffusion studies with certain additional advantages such as sufficient mechanical strength and ruggedness. It can be efficiently reused and also surpasses the ethical issues involved with the use of biological membrane.

Keywords: Carbopol, Diffusion, Silver Sulfadiazine, Track-etch membrane.

INTRODUCTION

In recent years, dermatological formulations have been developed to diffuse active substances into and across the skin for their therapeutic effect. Topical semisolid formulations such as creams, ointments and gels are usually employed for local delivery, while transdermal patches have been successfully developed as time controlled drug release device for systematic action. Since years, skin from animal sources has been used for the evaluation of diffusion of drug from topical preparations, a number of devices and methods have also been utilized to evaluate the drug release and set specifications. [1]

Drugs and polymers of different nature, receptor media, and sink conditions are some of the key issues to decide the suitability of different devices for *in-vitro* release tests. [2-3] Several *ex-vivo* methods using excised animal skin have been investigated and developed for the evaluation of drug permeation through skin. Assessment of drug permeability through skin, using Franz diffusion cell, also known as "vertical diffusion cell" (VDC), has evolved as a major research tool for such investigations [4-5] intended to provide key insights into the relationships among skin, drug and formulation, which are very useful for evaluation and quality control of such novel drug delivery systems. [6-11] This method is widely accepted due to its simplicity, low cost and the easy controllable experimental conditions during investigations. Apart from excised human and animal skin, synthetic membranes are also being employed for the diffusion studies using Franz diffusion cell, mainly to achieve two functions- simulation of skin and quality control [12-15] owing to ethical issues and scarce availability of biological skins as well as their storage and preparation for such studies (removal of fatty tissues, treatment with trypsin etc.).

In the past few decades, membrane technology involving the application of porous synthetic membranes has rapidly evolved for the assessment of topical drug diffusion. A membrane is an intervening phase between two adjacent phases acting as an active or passive selective barrier, regulating transport of matter between the two compartments. [16] A man-made porous synthetic membrane, also called artificial membrane, is generally a continuous medium containing pores in which its active part (pores) allows

***Corresponding author: Mr. Ravish Garg,**
Department of Biomedical Engineering, G. J. University of Sci. & Tech., Hisar-125001, Haryana, India; **Tel.:** +91-9355250101; **E-mail:** ravishgarg2003@yahoo.com

selective transport of material, and auxiliary part meant for mechanical support of drainage. The Food and Drug Administration (FDA) has recommended that simple, porous synthetic membranes are suitable for assessing topical formulation performance as they act as a support but not as a rate limiting barrier. [17] However, recent studies has revealed that various types of porous synthetic membranes produce different drug flux values, which indicate that these membranes do offer resistance and simultaneously may also act as rate limiting barrier. [15, 18-19] Moreover, synthetic membranes, unlike skin, are almost inert and do not introduce biological and other variations.

A wide range of porous synthetic membranes, ranging from semi-synthetic to synthetic polymers, is commercially available in market that motivates researchers for selection of membranes according to their requirements for evaluation of such topical formulation. Most porous synthetic membranes used in Franz diffusion cells are borrowed from separation and filtration applications in membrane technology. It is evident from the literature that many investigators have employed synthetic membranes consisting of a diverse range of materials, pore sizes and thickness to evaluate different drugs and formulation preparations. [20-28] The properties of synthetic membranes can be tailored to a great extent so that their separation capability could comply with the specific separation task. The common membranes for such biological applications are silicone, cellulose and polysulfone membranes.

The most important type of artificial porous polymeric membrane, formed by one of the phase separation techniques, is track etch membrane, which has generated significant interest in disposable biomedical devices and in applications where a large number of identical samples are required to study many biological events and processes. Track etch membranes are porous systems consisting of a thin polymer foil with pores constituting channels across the surface of membrane. A variety of polymeric materials are available with good biocompatibility, tensile strength and inertness for myriad applications. Track etch membrane production is well-known established technique involving [29-33] two steps. Firstly, the irradiation of heavy charged ions creates damages in a material along their trajectory, called latent tracks, causing structural and/or chemical changes and deposit energy in the material resulting in active sites which are subsequently etched by hydrofluoric acid to form pores. The size and shape of pores in track etch membrane can suitably controlled, which depends on the nature of incident ions, detector material and etching conditions. The shape of the pores can be tailored cylindrical, conical, double conical and funnel-like under controlled etching conditions, whereas density and orientation of pores depend on the collimation of ion beam during irradiation process. [31, 34, 35-37] In the last few decades special attention has been paid to the study of track etch membrane with respect to their ability to mimic biological ion channels. [38] It can serve as potential candidate for drug release/permeation studies because of its distinct properties, which are controllable such as optical transparency, smooth surface, pore size, pore density, shape, inertness, thinness, high mechanical strength, toughness, low diffusion rate that gives the membrane a unique overall performance. [33]

The present investigation was aimed to evaluate the efficacy of the prepared track etch membrane for the permeation of drug particles in order to replace the use of animal skin in drug evaluation due to ethical and procurement issues. In this work, comparison of ex-vivo permeation of silver sulfadiazine in carbopol gel (2%) through goat skin with *in-vitro* diffusion through track etch membrane have been carried out using Franz diffusion cell.

MATERIALS AND METHODS

Materials: Silver sulfadiazine, generously gifted by Galentic Pharma (India) Pvt. Ltd., was used as model drug. Track etch membrane of average pore size 0.2µm was prepared in the laboratory and goat skin was procured from the slaughter house at Hisar (Haryana), India. The Franz diffusion cell was used for drug release study. All the chemicals used in the study were of suitable analytical grade and were used as and when required.

Methods

Formulation of Silver sulfadiazine gel

In the present study, 2% silver sulfadiazine (SSD) gel was formulated using carbopol 934 as the gel forming agent.

Synthesis of Track Etch Membrane

Samples of polycarbonate (monomer composition $C_{16}H_{14}O_3$, trade name Makrofol, Bayer, clear film of thickness 20µm), irradiated with 13.02 MeV/n Xe (fluence 10^6 ions/cm^2) using the heavy ion accelerator (UNILAC facility at GSI, Darmstadt, Germany) were taken for preparation of track etch membrane. The charged-particle irradiated on polycarbonate foil resulted into the formation of latent tracks in the material along the trajectory of the ions which were subsequently chemically etched by dipping the irradiated sample in 6N NaOH solution at 50°C for 10 minutes resulting into the formation of discrete cylindrical pores [39-41] with an average pore size of 0.2µm. This prepared track etch membrane was used for the dug release study and compared with the goat skin to act as a surrogate for *ex-vivo* drug permeation studies.

Efficacy evaluation and comparison of release profile

The synthesized track etch membrane was evaluated for its efficacy of permeating the gel by studying the *in-vitro* diffusion rate of the prepared gel through the membrane. The membrane for *in-vitro* study and goat skin for *ex- vivo* study were tied onto two similar Franz diffusion cell assemblies. Distilled water was taken as the diffusion medium. At regular intervals of 15 minutes, 1 mL sample was withdrawn from each assembly and was regularly replenished with fresh diffusion medium. The samples were analyzed after diluting them with 9 mL of 0.05% ammonia solution, essential for measuring their UV absorbance at λ_{max} equal to 254 nm. The study was carried out for time duration of 3 hours. A marketed depilatory cream (Veet® Hair Removal Cream) was used to depilate the animal few minutes before performing the experiment, so that a completely hairless and smooth skin could be obtained. The experiments were carried out in triplicate and the results were recorded as mean ±standard deviation.

The model independent approach, computation of difference factor (f_1) and similarity factor (f_2), was used to compare the drug release profiles from Track-etch membrane and goat skin [42]. The difference factor (f_1) is used to determine the percent difference between each time point interval of the release profiles for both the reference and test batch and thus is a measure for the relative error between the two respective curves. Similarity factor (f_2), on the other hand, is a measure

for obtaining the similarity in the percent dissolution of the two curves (reference and test batch). The factors (f_1) and (f_2) can be represented by the following expressions:

$$f_1 = \left\{\frac{\sum_{t=1}^{n}(R_t - T_t)}{\sum_{t=1}^{n} R_t}\right\} \times 100$$

$$f_2 = 50 \log\left[\left\{1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right\}^{-0.5} \times 100\right]$$

Where, n is number of time points; R_t is cumulative percentage release of the reference batch (goat skin) at time t; T_t is cumulative percentage release of the test batch (track etch membrane) at time t.

Table 1: *In-vitro* diffusion study of model drug (SSD) in 2% Carbopol gel through track etch membrane and goat skin

Time (min)	% Cumulative Drug release (Track-etch membrane)	% Cumulative Drug release (Goat skin)
30	0.74 ± 0.003	1.54 ± 0.004
45	1.64 ± 0.005	2.39 ± 0.006
60	2.68 ± 0.004	5.26 ± 0.008
75	4.02 ± 0.004	6.40 ± 0.007
90	7.52 ± 0.006	9.64 ± 0.007
105	9.14 ± 0.005	12.96 ± 0.008
120	10.84 ± 0.008	13.32 ± 0.009
135	12.72 ± 0.010	15.03 ± 0.005
150	13.88 ± 0.012	16.90 ± 0.010
175	17.33 ± 0.011	18.85 ± 0.012
190	18.18 ± 0.018	20.99 ± 0.016

RESULTS

The comparison of diffusion rate of silver sulfadiazine gel through goat skin and track etch membrane using two similar sets of Franz diffusion cell assemblies have been shown in Table 1. The results have been graphically represented in Fig. 1, which illustrates that drug release profile through track etch membrane is similar and slightly less than goat skin. These results were also compared using model independent approach by calculating the difference factor (f_1) and similarity factor (f_2), which was found to be 19.95% and 79% respectively. The similarity and difference factors depict that the release profile from the two different membranes was significantly similar and analogous.

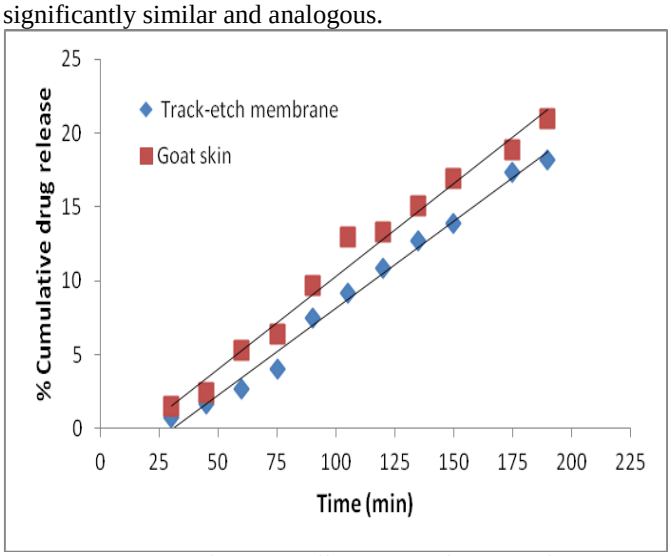

Fig. 1: Comparison of *in-vitro* diffusion rate of silver sulfadiazine gel through goat skin and track etch membrane

DISCUSSION

It is observed from the results that there is no significant differences between the cumulative percent drug releases from track etch membrane and the goat skin. The slight difference obtained in the drug release may be because of presence of lipids in the animal skin which facilitate the passive diffusion of drug through the skin membrane. The results of computation of similarity factor and difference factor also strongly indicate that the release profile from both track etch membrane and goat skin is analogous. The release profiles are considered to be similar when the value of similarity factor (f_2) ranges from 50% to 100%. Higher value of the (f_2) factor implies more closeness of the two release profiles. The graph (figure 1) representing the percentage cumulative drug release with time suggests that the drug release from the gel follows zero order kinetics which infers that the drug is released by diffusion through the polymeric gel network. The advantages of track etch membrane over animal skin are that it is rugged in nature and has sufficient mechanical strength to withstand the contact with diffusion medium for a longer time. It is also environmentally stable and can easily be reused after washing. Also, it surpasses the ethical issues involved in the use of animal skin for the evaluation of drug permeation or diffusion rate as well as variations introduced by source of sample (animal), anatomical region and subject variability.

Hence, the present investigation illustrates that the drug diffused through track etch membrane is comparable with that through goat skin. Track etch membrane has the capability to mimic the animal skin for drug diffusion studies with certain added advantages such as its ruggedness, mechanical strength, reusability and evasion of the ethical issues involved with the biological membrane. Controllability of the shape and size of track etch membrane during its manufacturing process also provide researchers an ample scope to mimic the various biological membranes. This study provides a platform to the researchers for further investigations to explore the applicability of track etch membrane for the diffusion studies of various drug formulation preparations. Track etch membranes holds a lot of potential to become an effective surrogate for *ex-vivo* studies but investigators should also be careful while employing them for such drugs whose transport across biological membrane is carrier mediated. Functionalization of pores can also impart the property of selective permeation which may be explored in future for mimicking other biological membranes like the blood brain barrier.

ACKNOWLEDGEMENTS

The authors express gratitude to Galentic Pharma (India) Pvt. Ltd. for providing gift sample of silver sulfadiazine. The first author is thankful to Guru Jambheshwar University of Science & Technology, Hisar to provide financial aid in the form of minor project.

REFERENCES

1. Shiow-Fern NG, Jennifer JR, Francis DS, Gillian ME. The Relevance of Polymeric Synthetic Membranes in Topical Formulation Assessment and Drug Diffusion Study. Arch. Pharm. Res. 2012; 35(4): 579-593.
2. Martinez M, Rathbone M, Burgess D, Huynh M. *In vitro* and *In vivo* Considerations Associated with Parenteral Sustained Release Products: A Review Based upon Information Presented and Points Expressed At the 2007 Controlled Release Society Annual Meeting. J. Control. Release 2008; 129 (2): 79-87.

3. Rathbone MJ, Martinez MN, Huynh M, Burgess DJ. CPS/AAPS Joint Workshop on Critical Variables in the *In vitro* and *In vivo* Performance of Parenteral Sustained-Release Products. Dissolution Technol. 2009; 16 (2): 55-56.
4. Franz TJ. Percutaneous Absorption. On The Relevance of *In vitro* Data. J. Invest. Dermatol. 1975; 64: 190-195.
5. Franz TJ. The Finite Dose Technique as a Valid *In vitro* Model for the Study of Percutaneous Absorption. Curr. Probl. Dermatol. 1978; 7: 58-68.
6. Zorin S, Kuylenstierna F, Thulin H. *In vitro* Test of Nicotine's Permeability through Human Skin-Risk Evaluation and Safety Aspects. Am. Occup. Hyg. 1999; 43: 405-413.
7. Siewert M, Dressman J, Brown CK, Shah VP. FIP/AAPS Guidelines to Dissolution/*in vitro* Release Testing of Novel/Special Dosage Forms. AAPS Pharm Sci. Tech. 2003; 4: Article 7.
8. Shah V, Elkins J, Williams R. Evaluation of the Test System Used For *In vitro* Release of Drugs For Topical Dermatological Drug Products. Pharm. Dev. Technol. 1999; 4: 377-385.
9. Ueda CT, Shah VP, Derdzinski K, Ewing G, Flynn G, Maibach H, Marques M, Rytting H, Shaw S, Thakker K, Yacobi A. Topical and Transdermal Drug Products. Pharmacopeial Forum 2009; 35(3): 750-764.
10. Farinha A, Toscano C, Campos R, Bica A, Hadgraft J. Permeation of Naproxen from Saturated Solutions and Commercial Formulations through Synthetic Membranes. Drug Dev Ind Pharm. 2003; 29: 489-94.
11. Shah V, Elkins J, Lam S, Skelly J. Determination of *In Vitro* Drug Release from Hydrocortisone Creams. Int J Pharm. 1989; 53: 53-59.
12. Twist JN, Zatz JL. Influence of Solvents on Paraben Permeation through Idealized Skin Model Membranes. J. Soc. Cosmet. Chem. 1986; 37: 429-444.
13. Twist J, Zatz J. Membrane - Solvent - Solute Interaction in a Model Permeation System. J. Pharm. Sci. 1988; 77: 538-540.
14. Corbo M, Schultz TW, Wong GK, Van Buskirk GA. Development and Validation of *In Vitro* Release Testing Methods for Semisolid Formulations. Pharm. Tech. 1993; 9: 112-128.
15. Shiow-Fern NG, Jennifer R, Sanderson D, Eccleston, A Comparative Study of Transmembrane Diffusion and Permeation of Ibuprofen across Synthetic Membranes Using Franz Diffusion Cells. Pharmaceutics 2010; 2: 209-223.
16. Ulbricht M. Advanced Functional Polymer Membranes. Polymer 2006; 47: 2217-2262.
17. FDA-SUPAC-SS. Guidance for Industry. SUPAC-SS Non-sterile Semisolid Dosage Forms. Scale-up and Post approval Changes: Chemistry, Manufacturing and Controls. *In vitro* Release Testing and *In Vivo* Bioequivalence Documentation 1997; 19-24.
18. Wu ST, Shiu GK, Simmons JE, Bronaugh RL, Skelly JP. *In Vitro* Release of Nitroglycerin from Topical Products by Use of Artificial Membranes. J. Pharm. Sci. 1992; 81: 1153-1156.
19. Gallagher SJ, Trottet L, Carter TP, Heard CM. Effects of Membrane Type and Liquid/Liquid Phase Boundary on *In Vitro* Release of Ketoprofen from Gel Formulations. J. Drug target. 2003; 11: 373-379.
20. Clement P, Laugel C, Marty JP. Influence of Three Synthetic Membranes on the Release of Caffeine from Concentrated W/O Emulsions. J. Control Release 2000; 66: 243-254.
21. Dias M, Raghavan SL, Hadgraft J. ATR-FTIR Spectroscopic Investigation on The Effect of Solvents on The Permeation of Benzoic Acid and Salicylic Acid Through Silicone Membranes. Int. J. Pharm. 2001; 216: 51-59.
22. Friend D, Catz P, Heller J, Okagaki M. Transdermal Delivery of Levonorgestrel IV: Evaluation of Membranes. J. Pharm. Sci. 1989; 78: 477-481.
23. Proniuk S, Dixon SE, Blanchard J. Investigation of the Utility of an *In vitro* Release Test for Optimizing Semisolid Dosage Forms. Pharm. Dev. Technol. 2001; 6: 469-476.
24. Santoyo S, Arrelano A, Ygartua P, Martin C. *In vitro* Percutaneous Absorption of Piroxicam through Synthetic Membranes and Abdominal Rat Skin. Pharm. Acta. Helv. 1996; 71: 141-146.
25. Schwarb FP, Imanidis G, Smith EW, Haigh JM, Surber C. Effect of Concentration and Degree of Saturation of Topical Fluocinonide Formulations on *In vitro* Membrane Transport and *In Vivo* Availability on Human Skin. Pharm. Res. 1999; 16: 909-915.
26. Smith EW, Haigh JM. *In vitro* Diffusion Cell Design and Validation II: Temperature, Agitation and Membrane Effects on Betamethasone 17-Valerate Permeation. Acta. Pharm. Nord. 1992; 4: 171-178.
27. Thakker KD, Chern H. Development and Validation of In Vitro Release Tests for Semisolid Dosage Forms - Case Study. Disso. Tech. 2003; 10: 10-15.
28. Welin-Berger K, Neelissen JAM, Bergenstahl B. The Effect of Rheological Behaviour of A Topical Anaesthetic Formulation on The Release and Permeation Rates of The Active Compound. Eur. J. Pharm. Sci. 2001; 13: 309-318.
29. Fleischer R, Price P, Walker R. Nuclear Tracks in Solids: Principles and Applications: University of California Press, Berkeley, 1975.
30. Ferain E, Legras R. Heavy Ion Tracks in Polycarbonate. Radiation Effects and Defects in Solids 1993; 126(1-4): 243-246.
31. Apel P. Track Etching Technique in Membrane Technology. Radiation Measurements 2001; 34(1-6): 559-566.
32. Trautmann C, Brueckle W, Sophr R, Vetter J, Angert N. Pore Geometry of Etched Ion Tracks In Polyimide. Nucl. Instr. and Meth. Phys. Res. B. 1996a; 111: 70-74.
33. Chakarvarti SK. Track-Etch Membranes As Templates Enabled Nano/Micro Technology: A Review. Indian J. Phys. 2009; 83(6): 737-749.
34. Ferain E, Legras R. Pore Shape Control in Nanoporous Particle Track Etched Membrane. Nucl. Instr. and Meth. Phys. Res B. 2001; 174: 116-122.
35. Karim S, Ensinger W, Mujahid SA, Maaz K, Khan EU. Effect of Etching Conditions on Pore Shape in Etched Ion-Track Polycarbonate Membranes. Radiation Measurements 2009; 44(9-10): 779-782.
36. Garg R, Kumar V, Kumar D, Chakarvarti SK. Electrical Transport Through Micro Porous Track Etch Membranes of Same Porosity. Mod. Phys. Lett. B. 2012; 26(31): 12502091-12502097.
37. Quinn JA, Anderson JL, Ho WS, Petzny WJ. Model Pores of Molecular Dimension- The Preparation and Characterization of Track- Etched Membranes. Biophys J. 1972; 12(8): 990-1007.
38. Bashford CL. Membrane Pores- from Biology to Track-Etched Membranes. Bioscience Reports. 1995; 15(6): 553-565.
39. Kumar S, Zagorski DL, Kumar S, Chakarvarti SK. Chemical Synthesis of Polypyrrole Nano/Microstructures using Track Etch Membranes. J. Mater Sci. 2004; 39: 6137 – 6139.
40. Sanjeev K, Chakarvarti SK. Chemical Synthesis of Agcl Microstructures Using Etched Ion Track Polycarbonate Membranes. Synthesis and Reactivity in Inorganic, Metal-Organic, And Nano-Metal Chemistry 2012; 42: 1242-1245.
41. Kumar S, Chakarvarti SK. Electrodeposition of Copper Nanowires in Ion-Crafted Membranes as Templates. Dig. J. of Nanomater Bios. 2006; 1(4): 139-143.
42. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic Modeling on Drug Release from Controlled Drug Delivery Systems. Acta Poloniae Pharmaceutica- Drug Research 2010; 67: 217-223.