<Supplementary Information>

Ionic Liquid-Assisted Transdermal Delivery of Sparingly Soluble Drugs

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Materials and Supplies.

Tween-80 (polyoxyethylene sorbitan monooleate), Span-20 (sorbitan laurate) and Fluorescein sodium were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and were of the highest purity commercially available. They were used without further purification. All ionic liquids (ILs) except 1-ethyl-3-methylimodazolium acetate (from Sigma-Aldrich) were purchased from Solvent Innovation GmbH (Germany) and were used as received. Frozen (– 80°C) porcine skin samples, excised from a 5 month old female Yucatan Micropig (YMP), were obtained from Pierce (Rockford, IL USA). Acyclovir was obtained as a generous gift from ASPION pharmaceuticals, (Kobe, Japan) whereas acyclovir herpecia topical cream was received from Taisho pharmaceutical Co. Ltd., Tokyo, Japan. All other reagents used in the experiments were analytical grade.

Drug Solubility Determination. ACV was added in excess to water, IPM and ILs. All samples were stirred for 24 hrs at 25ºC. The precipitated drug was removed from microemulsions by filtration through a 0.8µm Millipore Millex-LG filter. The amount of ACV in the resulting clear filtrate was determined using a UV spectrophotometer at 252 nm after appropriate dilution with methanol keeping the respective blank. The same procedure was used to find the solubility efficiency of drug in each microemulsion.

The photographs appended in Fig. S1 show solubility of acyclovir in various systems. For this purpose, we added powder ACV in every system (5 mg mL^{-1}) and stirred for 24 hrs at 25ºC. The results showed that ionic liquid (IL) and IL-in-oil microemulsions can solubilize drugs, whereas the aqueous solution can not dissolve the ACV. In fact, all the ACV in water was precipated at the bottom as shown in the Fig. S1A.

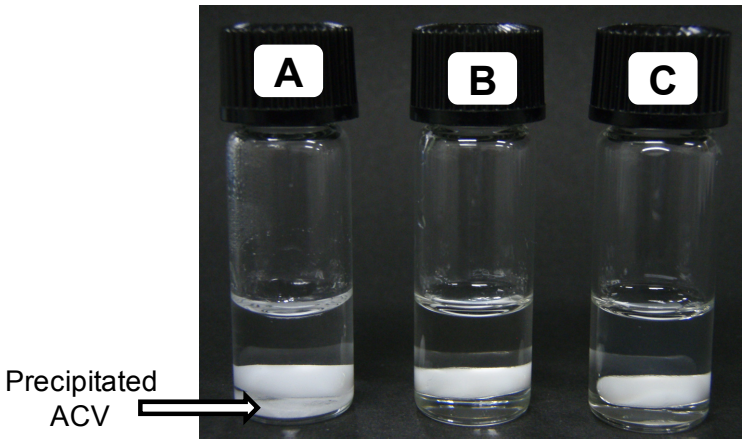

Fig.S1 Solubility of acyclovir (5mg mL^{-1}) in various systems. (A) water, (B) IL $[C_1mim][(MeO)_2PO_2]$ and (C) Tween-80/Span-20/IL/IPM microemulsions where the weight fraction of surfactants was 0.2 with R =0.7.

Phase Behavior Study: To study the phase behavior, the mixtures of Tween-80 and Span-20 wit different weight ratios were prepared. The mixtures dissolved in IPM to produce surfactants/IPM systems. The total surfactant concentrations varying from 10 to 40 (wt %) were studied. Then maximum solubility of IL was determined by adding IL successively until the solution started to become turbid. It was necessary to stir the preparation well after any addition of one of the components. Temperature was kept constant at 25 °C.

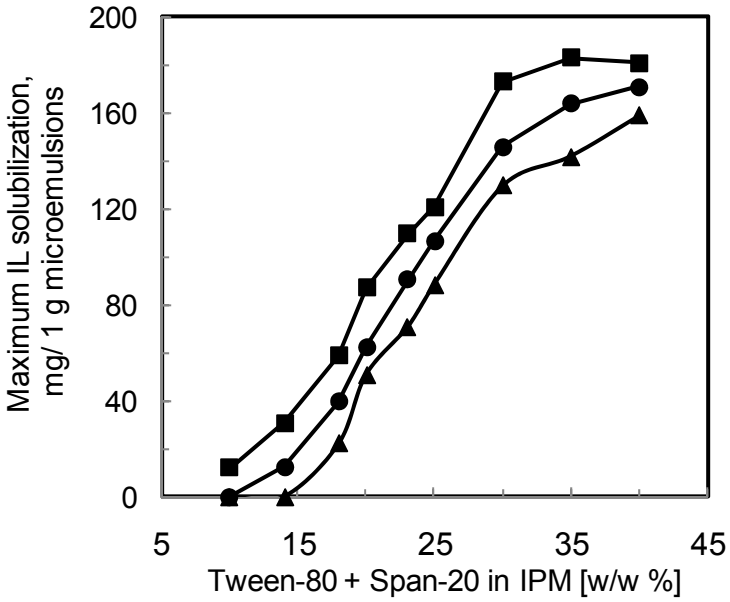

Fig. S2 Phase diagrams of IL $[C_1mim][(CH_3O)_2PO_2]$/Tween-80/Span-20/ IPM system at 25 °C with different proportion of surfactant mixtures. (▲) Tween-80: Span-20 = 3:1 (w/w) (●) 1:1 (■) 1:2

Schematic Representation of Ionic Liquid-in-Oil (IL/o) Microemulsions Containing Drug Molecules

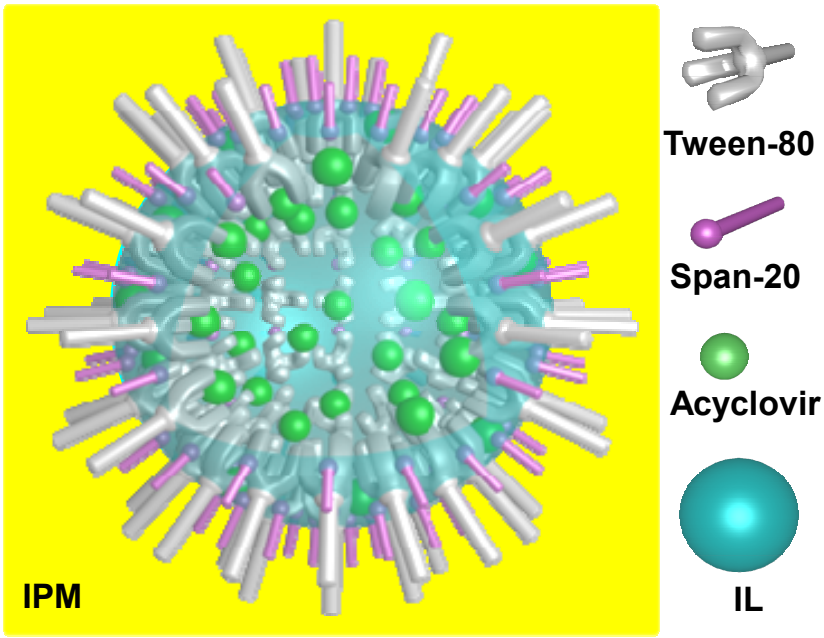

Fig. S3

Dynamic Light Scattering. Dynamic light scattering measurements were performed using a Malvern Zetasizer Nano ZS light scattering instrument (Malvern Instruments Ltd., Malvern, Worcester, U.K.) with a 532 nm laser. The scattering angle was set at 90º. The experiments were performed on a stable single phase, formed by the injection method. After microemulsion preparation as described in the text, all samples were equilibrated for 24 hrs, and no macroscopic heterogeneity was visible before measurments.. Samples were filtered through a 0.2μm Millipore Millex-LG filter to remove any traces of dust or contaminants prior to the measurement of particle size for each sample. Measurements were conducted at 25±0.1ºC in triplicate. Samples were equilibrated for 20 minutes before data collection. The droplet size and polydispersity of the dispersed ionic liquid phase were evaluated with the aid of Malvern DTS software.

Table S1. Mean particle diameter (mean ± standard error of the mean) with various ionic liquid (IL) content of Tween-80 (15 wt %)/span-20 (5wt %) reversed micelles in IPM, as measured by dynamic light scattering

IL content as molar ratio of IL to surfactants (*R*)	Mean diameter (nm)	Polydispersity index
0.0	7.79 ± 1.23	0.146 ± 0.041
0.15	9.97 ± 1.0	0.124 ± 0.025
0.35	13.85 ± 2.35	0.101 ± 0.041
0.50	17.76 ± 1.60	0.100 ± 0.030
0.70	20.52 ± 2.10	0.114 ± 0.029
0.85	26.90 ± 2.00	0.097 ± 0.030
1.0	31.34 ± 2.80	0.085 ± 0.020

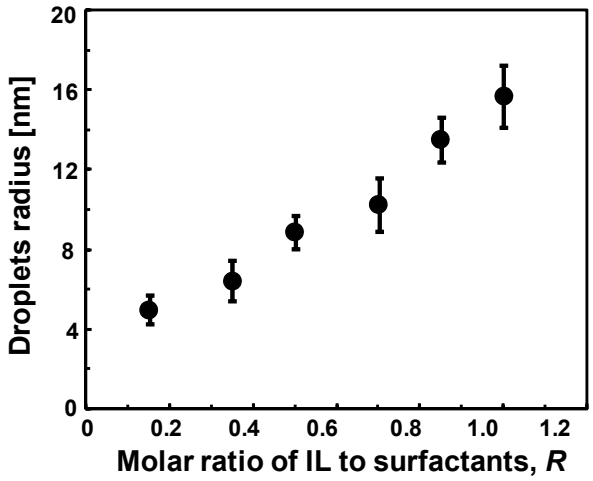

Fig.S4 Dependence of droplet radius on *R* for Tween-80/Span-20/ IL/ isopropyl myristate microemulsions with Tween-20 (15 wt%) and Span-20 (5 wt%) at 25 ºC

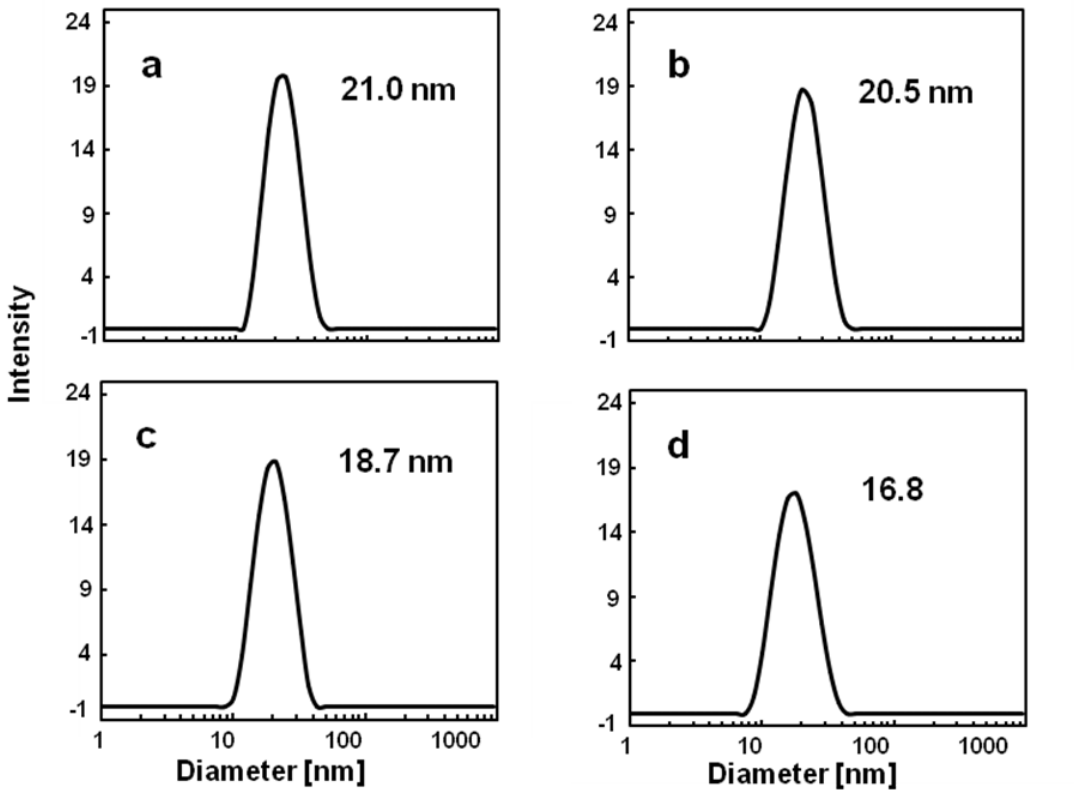

Fig. S5 Sizes and size distributions of the domains of the IL-in-IPM microemulsions with various drug concentrations at 25 ºC. The weight fraction of surfactants was 20 wt% (Tween-80: Span-20 = 3:1 (w/w)) and R =0.70. (a) ACV = 0, (b) 1 mg/mL, (c) 3 mg/mL and (d) 5 mg/ mL.

Storage Physical Stability of Microemulsions. The physical stability of microemulsions loaded with acyclovir was evaluated by measuring particle size changes at designated time intervals as well as direct visual observations. For the size measurement, microemulsions with ACV were prepared as described in the text and kept at 25 ºC. Samples were withdrawn periodically and the size was measured.

Skin Permeation Studies. Drug release was evaluated *in vitro*. The penetration of ACV through full thickness skin pieces of Yucatan hairless micropigs (YMPs) were performed using a hand-made Franz diffusion cells with a diameter of 10 mm. The porcine skin was prepared as we reported previously. [S1] The skin piece was clamped between the donor and receptor compartments of the diffusion cell. The stratum corneum faced the donor side of the cell whereas, the dermal side of the skin was allowed to be in contact with PBS solution (pH 7.4). The buffer solution was maintained at 32.5 ± 0.1ºC using a thermostatically controlled water bath and was magnetically stirred at 600 rpm throughout the experiments.Skin pieces were hydrated with PBS for 1 hr before permeation experiments and then 0.5 mL of formulations containing ACV were placed over the skin. After 24 hr, the receptor chambers were emptied and solutions were analyzed for ACV content. Donor solutions were removed and the skin was washed twice with 0.1M HCl acid before unclamping the diffusion cells. Finally, tested skin pieces were processed to determine the ACV content inside the skin.

Acyclovir contents in the receptor solutions and the skin were detected using a modern HPLC system. Column: Shiseido CAPCELL PAK C18 MG (4.6 x 250); mobile phase: 0.1 wt% trifluoroacetic acid / acetonitrile = 1: 99; flow rate = 0.5 ml/min; detection: UV at 252 nm

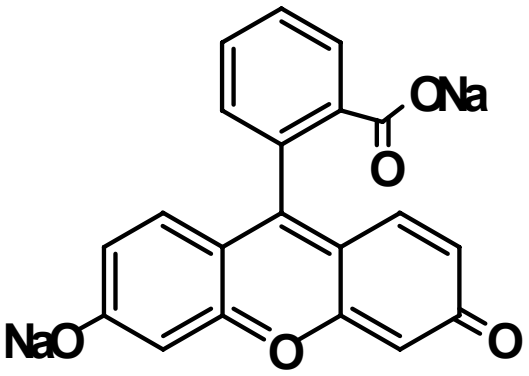

Fig. S6 Fluorescein sodium (M.W. 376.28)

Skin Morphology. Skin histology was studied as reported in our previous articles. [S-1] Skin penetration experiments with a fluorescent probe, fluorescein sodium (see the Fig. S4), were carried out *in vitro,* as described in the above section. After the post-application of fluorescence probe for 24 hr, the diffusion area of the skin samples was embedded in Histo Prep™ compound (Fisher Scientific, NJ, USA), frozen at -80 ºC in a deep-freezer and sectioned using a cryostat microtome (CM1510; Leica, Wetzlar, Germany). Each skin section (20μm) was mounted on the glass slides. The slides of fluorescein sodium were visualized, without any additional staining or treatment, through a 10X objective using a microscope (Olympus, Tokyo, Japan) equipped with a filter for fluorescein. The shutter exposure time of the camera was adjusted until the green fluorescence from the aqueous solution samples became invisible in the dark.

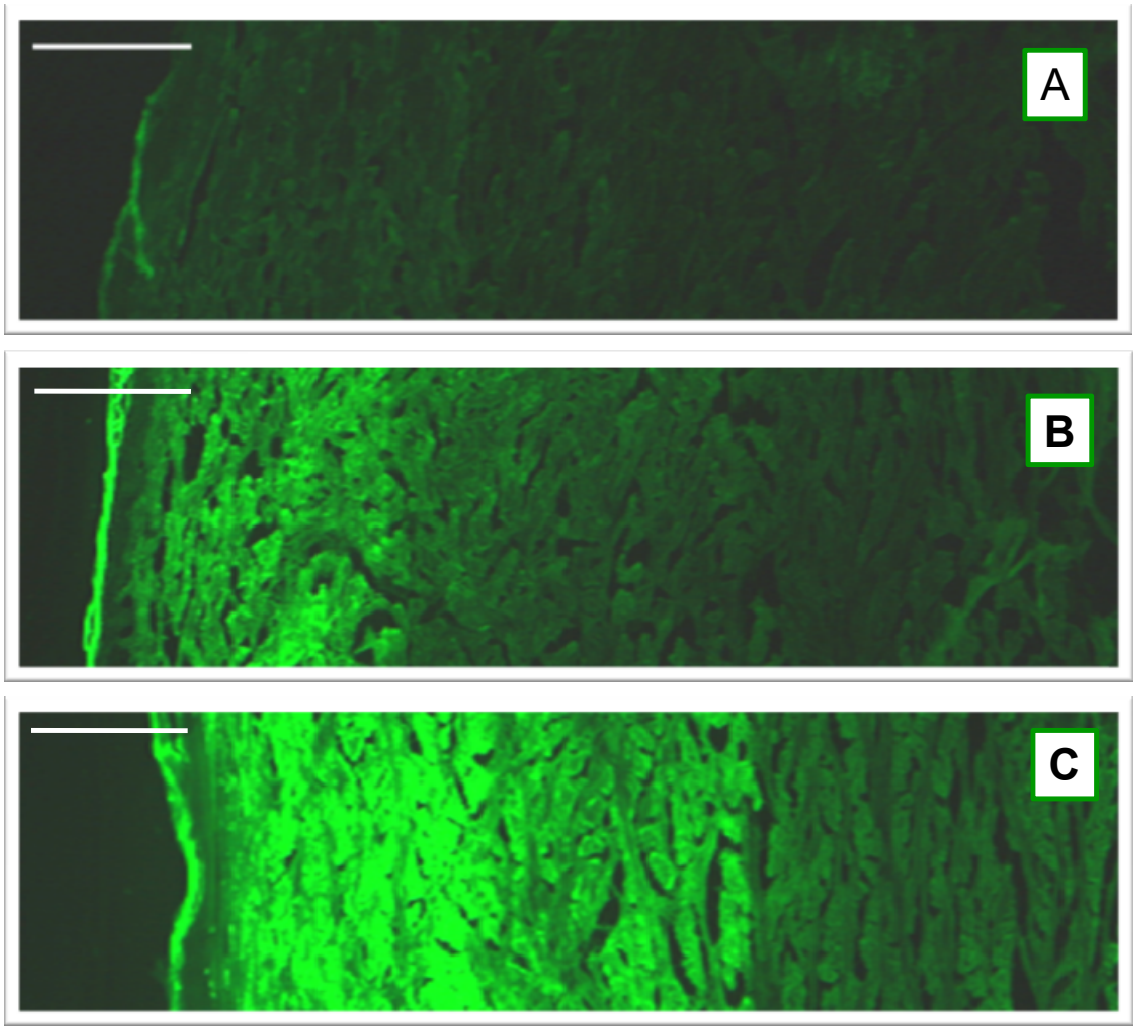

Fig. S7 Fluorescence microscopy of the skin sections treated with the samples containing fluorescein sodium salt visualized through a 10X objective (scale bar: 200 μm). (A) probe in IL, penetration time = 72 hrs (B) probe encapsulated in IL/o microemulsions with penetration time 48 hrs and (C) probe in IL/o microemulsions for 72 hrs. [pbobe] = 1mg mL^{-1}.

References

S1 Y. Tahara, S. Honda, N. Kamiya, H. Piao, A. Hirata, E. Hayakawa, T. Fujii, M. Goto, *J. Control. Release,* 2008, **131**, 14–18.