Development of Polystyrene-based Organogels Swollen in *D*-limonene for Transdermal Drug Delivery

CHAROENSUMRAN Preeyarad

A thesis submitted in fulfillment of the requirements for

the degree of

Doctor of Philosophy in Materials Science

September 2019

Nara Institute of Science and Technology

Contents

Chapter 2

The Electrostatic Advantages on Cross-linked Polystyrene Organogels Swollen with Limonene for Selective Adsorbent and Hydrophobic Drug Storage.

Chapter 3

Cationic Moieties in Polystyrene Gels Swollen with *D*-limonene Improved Transdermal Delivery System

Chapter 4

Controlled Release of Testosterone by Polymer-polymer Interaction Enriched Poly(Vinyl benzyl-*N*-methyl-*D*-glucamine) Gel in Propylene Glycol As a novel Transdermal Drug Delivery System

Chapter 1

General introduction

1.1 Sustainability and Material Development

For the past few decades, the need for introducing environmental requirements into design and development of materials and products became a vital issue. The sustainable development is now significant for the public and industrial sectors and it is one of the fundamental objectives.[1] It has become an increasing demand for the application of natural products to address problems in the environment, in waste disposal, and in the depletion of non-renewable resources. Vegetable or plant oils represent a renewable resource that can be used as reliable starting material to access new products with a wide array of structural and functional variations. The abundant availability and the relatively low cost make plant oils an industrially attractive raw material for the plastics industry. Naturally occurring plant oils and fatty acids derived thereof, are considered to be the most important renewable feedstock processed in the chemical industry, as well as in the preparation of bio-based functional polymers and polymeric materials.[2]

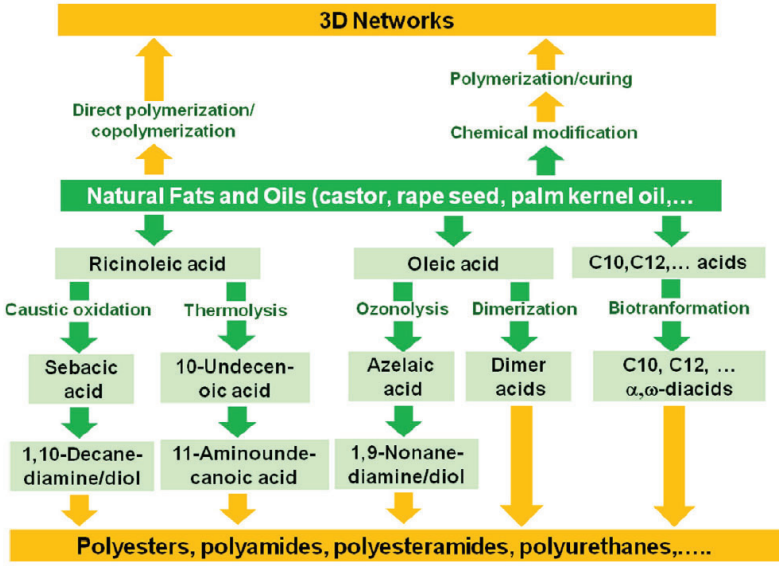


Figure 1-1. Main approaches to obtain thermoplastic and thermosetting materials from natural fats and oil-derived building blocks.[2]

Most of the scientific literature available today has been centered on modifying plant oils to prepare well-defined linear structures, the 3D networks, the matrices for biocomposites and hybrid materials as shown the example in **Figure 1-1**. From those reason, it is importance to develop the new materials based on the sustainability by using plant based oil.

1.2 Plant based oil and their modifications

So far, the plant oils and their derivatives have been used by polymer chemists due to their renewable nature, world wide availability, relatively low price, and their rich application possibilities. Although many different synthetic approaches have been used, more recent examples are pointing in the direction of the catalytic transformations and

the other efficient reactions to achieve the more sustainable productions of polymers from these renewable resources.[3]

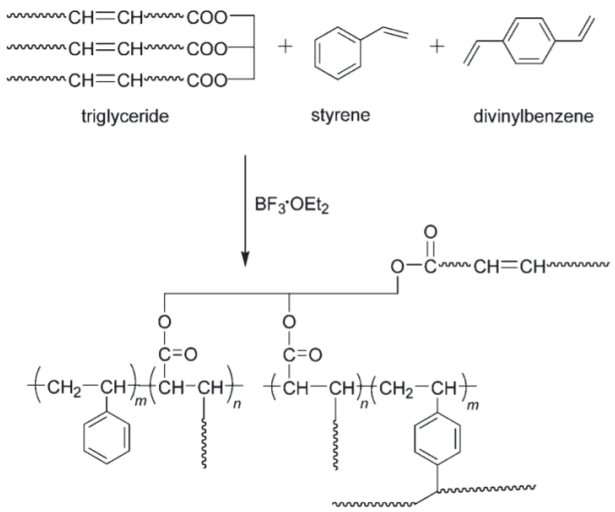


Figure 1-2. Cationic or thermal copolymerization of triglyceride oils with styrene (ST) and divinylbenzene (DVB).[4]

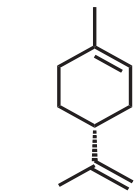
For example, the non-uniform cross-linking which performs in the soybean oil-divinylbenzene (DVB) systems has been optimized by using styrene (ST) and DVB as a cross-linker shown in **Figure 1-2**.[4,5] The mechanical properties of soybean oil-ST-DVB polymers are comparable to those of commercially available rubbery materials and conventional plastics. Thus they may serve as replacement in many applications. Some other works have also been reported on the nanocomposites obtained from the cationically polymerized vegetable oil resins reinforced with layered silicates.[6,7] Montmorillonite clay modified with triethyl (4-vinylbenzyl)ammonium chloride has been used to reinforce corn[6] and soybean oil resins.[7] Thus, there are abundance of plant based oil which are

useful for the material development.

D-limonene is one of them bearing the six-membered ring which similar structure to aromatic compound, specially a well-known industrial polymer; polystyrene (PS). Because of their miscible property between PS and limonene, thus there are many researches have been performed by the advantages.

1.3 *D*-limonene and its property

D-limonene, (4-isopropenyl-1-methylcyclohexene), monoterpene with a lemon-like favor, is the main constituent of all citrus-derived essential oils, such as lemon, lime, orange, and grapefruit. It is an environmental friendly compound, biodegradable solvent and natural-derived terpene compound. Limonene is used as a substitute for chlorinated hydrocarbons, chlorofluorocarbons, and other solvents. It is used for degreasing metals (30% limonene) prior to industrial painting, for cleaning in the electronic industry (50–100% limonene), for cleaning in the printing industry (30–100% limonene), and in paint as a solvent.[8] Limonene is also used as a solvent in histological laboratories and as a flavor and fragrance additive in food, household cleaning products, and perfumes. Limonene has been used as a gallstone solubilizer in humans.[9] As describe in aforementioned, limonene



***d*-Limonene**

is used in wide range of products. However it is noteworthy that limonene has the special selective solubilizing ability for PS, due to the similar structure of PS.[10,11] Because if its special property to dissolve PS, it has been applied for many researches.

1.3.1 Waste management of expanded polystyrene by *d*-limonene

Dissolution with suitable solvents is one of the cheapest and more efficient processes for PS waste management. A prototype production system for recycling expanded polystyrene (EPS), which uses an orange oil, *d*-limonene, as the EPS shrinking agent, has been developed. Tsutomu Noguchi *et al*. from Sony Corporation Research Center (**1998**) has reported a development of new recycling techniques which uses a natural derived solvent, *d*-limonene, to solve EPS (**Figure 1-3**).[12]

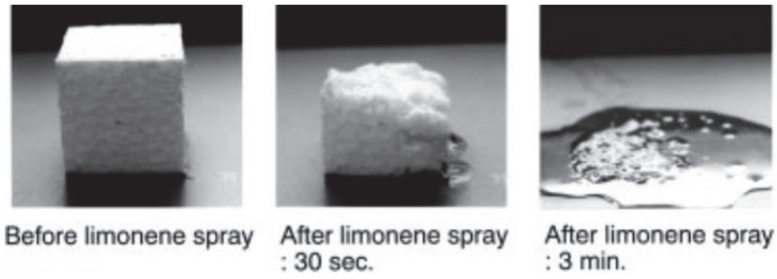


Figure 1-3. The shrinking process of EPS by limonene spray.[12]

The conventional methods of recycling EPS, such as crushing and shrinking by heated air or frictional heat have the disadvantages of molecular degradation caused by

oxidation and contamination by other materials, resulting in a reduction of the quality of the recycled PS. Therefore, such methods cannot be used to recycle EPS. In order to avoid the heated degradation from the recycling process, a method of shrinking using a solvent was promising method for improving the quality of recycled PS. An aromatic hydrocarbon, toluene, and a cyclic hydrocarbon, methyl cyclohexane, are well-known EPS solvents.

Table 1-1. The flash point and boiling point of solvent

Sample	Flash point (°C)	Boiling point (°C)
Limonene	47	175
Methyl cyclohexane	-3	101
Toluene	4	110
Acetone	-18	56

However, these solvents have the disadvantages of toxicity and a low flash point for use as EPS shrinking agents as shown in **Table 1-1**. Because of their molecular structure and the solubility parameters of solvents, monoterpene derivatives, such as *d*-limonene, isoamyl acetate, benzyl propionate and ethyl butyrate, are good solvent for EPS. Among these solvents *d*-limonene, which is a natural vegetable oil extracted from the rinds of citrus fruits, is the best shrinking agent because of its high solubility, safety, stable supply and fragrance. So the selective solubility of limonene against PS has been actually

employed for the recycling system,

1.3.2 Recycling system and renewable materials by *d*-limonene

D-limonene is considered as the best shrinking agent for EPS. Moreover, *d*-limonene dissolves only EPS, so contamination by other materials, such as expanded polyolefin, can easily be removed by filtering the limonene solution.[12,13]

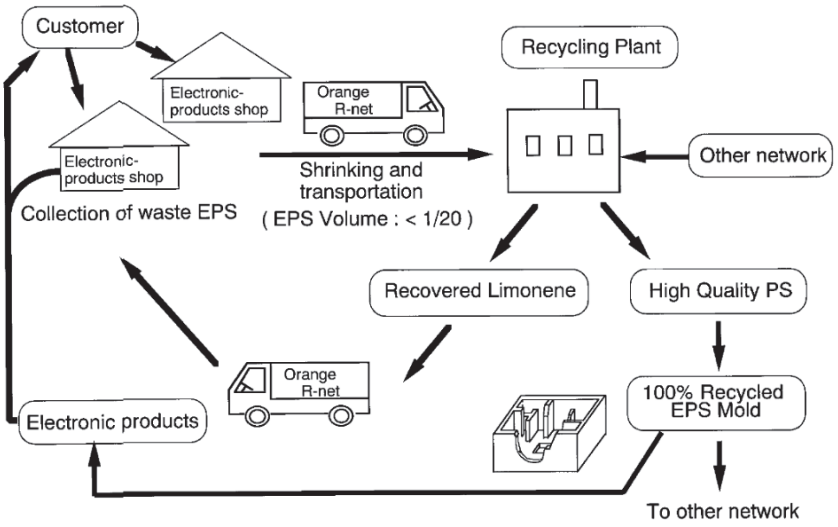
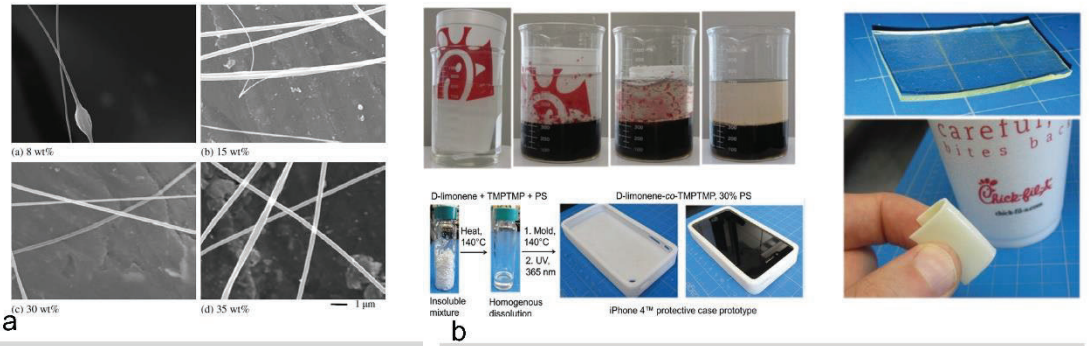


Figure 1-4. The recycling system of EPS from electronic product by *d*-limonene.

A schematic diagram of the recycling system is shown in **Figure 1-4**.[13] The limonene solution containing about 30 wt% PS is transported to the recycling plant by the truck and separated into PS and limonene. The recycled PS is used as the raw material for new EPS. The recovered limonene can be reused to shrink more EPS by distillation once every 10 cycles. This system consists of an apparatus to dissolve EPS and a recycling

plant to separate the limonene solution. The recycling plant can mass reproduce PS with the same mechanical properties as new PS and has lower greenhouse effect (-30%) acidification (-58%) and energy consumption (-20%).[14]

1.3.3 The fabrication of new materials by recycling PS with limonene



PS nanofibers electrospun from recycled EPS solution dissolved in *d*-limonene.

The recycling solution form EPS by synthesis of renewable poly(thioether) network derived from *d*-limonene

Figure 1-5. Example of recycling EPS from *d*-limonene solution.

Changyun Shin *et al.* (2005) describe the use of PS nanofibers electrospun from recycled EPS solution dissolved in *d*-limonene.[15] The electrospun PS nanofiber diameters vary from 300 to 900 nm, with an average diameter of about 700 nm as shown in **Figure 1-5a**.

Keith Hearon *et al.* (2014) has reported the recycling solution form EPS by synthesis of renewable poly(thioether) network derived from *d*-limonene.[16] They synthesized blended polymers containing recycled PS additives dispersed in *d*-limonene-co -polythiol network matrices to create a series of polymer blends with greater toughness

than either homopolymer exhibits individually as shown in **Figure 1-5b**. These demonstrated that environmental appropriation in new polymers can be achieved without sacrificing functional utility.

Satish Chandra Hari Mangalara *et al.* (2016) explored a completely green process for the recycling of EPS waste into value added fine particles. EPS waste is dissolved in *d*-limonene. Micro/nanosized PS particles were recovered through an emulsification−diffusion process.[17] The process is carried out under mild conditions and atmospheric pressure, which requires minimum energy. It also involves the recovery of *d*-limonene, so that the complete process loop can be closed.

As mentioned before, there are many researches either recycling or renewable system by capitalization of dissolving of PS in natural-based oil, limonene. Thus, this issue inspire me to develop new materials, such an organogels because limonene is oil product and it is still negligible.

1.4 Organogels

A gel is generally defined as a semisolid material, which exhibit appropriate solvent, is organic liquid or water. The liquid component is immobilized within the spaces available of a three-dimensional network structure. There are the self-assemble via

physical or chemical interactions into an extensive mesh network preventing solvent flow as a result of surface tension.[18,19] Gels are unique materials that are rigid and elastic in nature[20] and have a broad range of applications in cosmetics, medicine, biomaterials and food technologies[18,21-22].

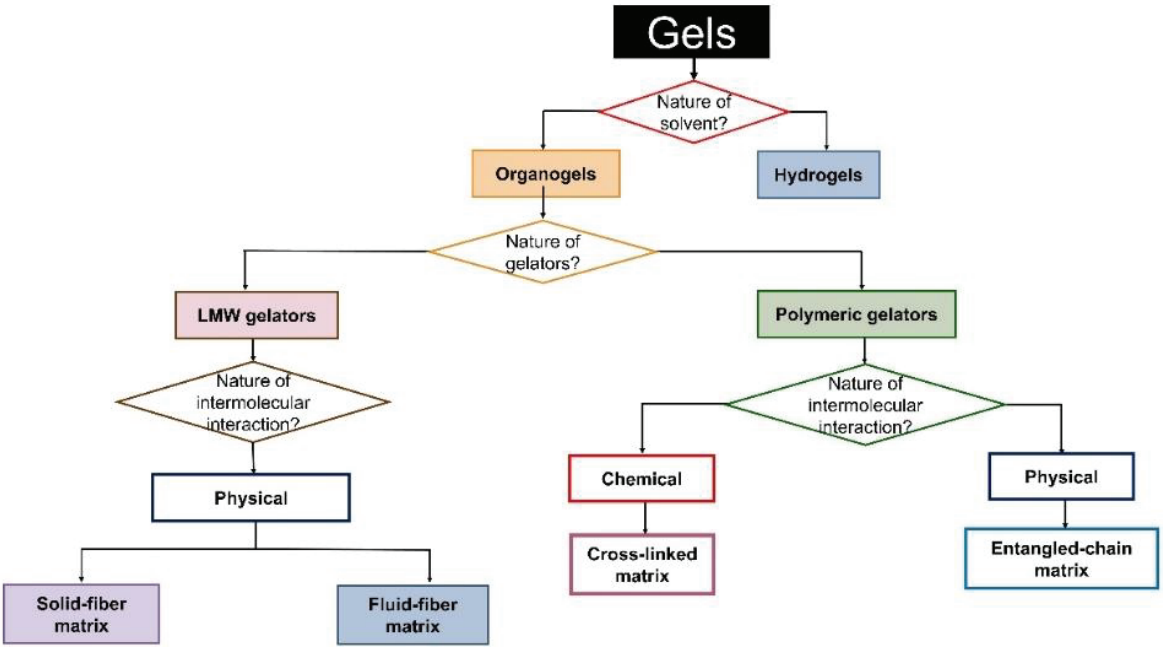


Figure 1-6. Gel classification.[19]

Organogels using limonene has been focused in this research. Typically, gels can be distinguished according to the nature of the liquid phase (**Figure 1-6**), organogels contain organic solvent, and hydrogels contain water. Organogels can then be further subdivided based on the nature of the gelling molecule: polymeric or low molecular weight organogelators. Polymers immobilize the organic solvent by forming a network

of either chemically cross-linked or physically cross-linked for chemical and physical gels, respectively. It have been extensively studied in various applications due to their unique behavior such as vehicles for drug delivery,[23-25] pollutant recovery materials,[26] and selective sensors.[27] Among those of application, I paid attention the drug delivery system with the property of oil gel.

1.4.1 Organogels for transdermal drug delivery system (TDDS)

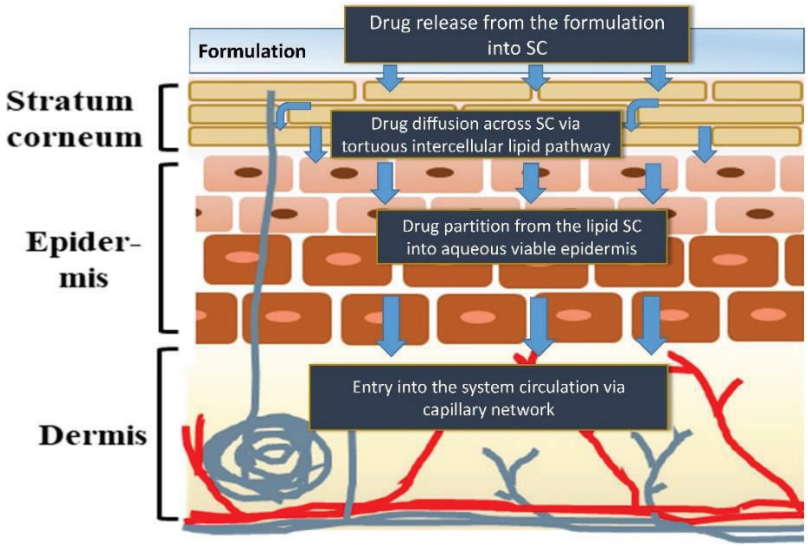


Figure 1-7. Pathway of transdermal absorption (SC stand for *stratum corneum*).

The gels can prove to be a beneficial vehicle for topical drug delivery or for the localized drug action on skin such as, the sprains or the acute musculoskeletal disorders. Although, there are huge researches focused on hydrogel, they still lack permeability and controllable property on skin due to their hydrophilic property. But organogels can

enhance drug penetration well through the skin barrier such a *stratum corneum* (SC) because skin itself can associate well with hydrophobic organogel. Recently, TDDS are a constant source of interest because of the benefits that they afford in overcoming over other drug delivery route (i.e. oral, intravenous). The TDDS is to delivery drugs as a controlled rate through the skin and into the blood stream as shown the transition pathway in **Figure 1-7**. The TDDS avoids first-pass metabolism by liver and gastro-intestinal tract, which is a significant hindrance for oral administration.[28,29] Nevertheless, there are the limitation of drug penetration because of the impermeable nature of the skin, especially SC. For this reason, a lot of research would approach to enhance the permeability through the skin[30,31]

For example, many researches have been reported on pluronic lecithin organogels (PLO) with various kind of drugs. Vikas Jhawat, *et al.* (2016) reported PLO gel formulation easily incorporated both hydrophilic and lipophilic types of drugs.[32] Various studies show that PLO gel formulations are much better than oral tablets in the treatment of inflammation. So, the present study was undertaken to develop PLO gel formulation of mefenamic acid for anti-inflammatory activity using in vivo method in rat model.

One of the most efficient and simplest way to improve the permeation of drug

for oil gel is chemical penetration enhancers (CPEs). The CPEs have been widely used to increase the skin permeability of many therapeutic molecules and anesthetics[33] by interacting with the SC, lipid bilayer or keratin, or by increasing the solubility of drug into SC lipid.[34,35] Extensive research during the past two decades has led to the formulation of several different classes of penetration enhancer such as terpene compound. Many reports have already provided substantial evidence that terpene[36,37] are capable of enhancing percutaneous transportation.

1.4.2 D-limonene as chemical penetration enhancer for transdermal delivery

Among of terpene compound, *d*-limonene is indicated as high efficiency penetration enhancer property. It can be used safely and effectively in a wide range of products which is well known as high efficiency permeation enhancer in TDDs.[38,39] D-limonene is also listed in the Code of Federal Regulations as generally recognized as a safe (GRAS).[40] Moreover, *d*-limonene has low toxicity, The oral LD_{50} for *d*-limonene in male and female mice is reported to be 5.6 and 6.6 g/kg body weight, respectively. So it is appropriate to boost up the transdermal delivery system (TDDS), as well as the efficient use if plant-based oil.

Sui Yung Chan *et al.* (2006) reported the preparation of limonene

dibutyllauroylglutamide (PG1)/propylene glycol (PG) organogels as physical gels.[41] Terpenes, namely limonene, linalool and cineole, in PG were first investigated *in vitro* for their capacity to enhance the percutaneous release of haloperidol (drug). Relative to oxygenated linalool and cineole, hydrocarbon limonene was more effective as a skin enhancer. It increased human skin permeability and decreased lag time. Limonene was thus incorporated in an organogels comprised of gelator GP1 in PG.

Zhen Yang *et al.* (2013) has reported that *d*-limonene was the most effective CPEs to improve the skin permeation of bufalin among other terpene compound and different synthetic CPEs. [42]

1.5 Scope of dissertation

"Cross-linked polystyrene swollen in *d*-limonene organogels for TDDS"

To develop the organogels for high mechanical property and controllable transdermal materials, some reports have studied the organogels as the physical gel which still lack of the stability against to temperature,[43,44] weak and have no specification interaction in delivery system. Additional, the organogels for TDDS, such as, emulsion, lipid bilayer and polymer solution are still unapproachable to desired application.

Thus, I aim to fabricate the sustainable materials as organogels with *d*-limonene

for not only reduce the limitation from the toxicity of organic solvent but also develop the organogels system both chemical and physical gel depends on the desired application in this dissertation. In order to enhance the interaction properties, various interaction moieties were introduced for example, cationic, anionic and hydrophilic group to enhance the reactive selectivity of their function. The research topic are processed and developed by 3 chapters illustrated in **Figure 1-8**.

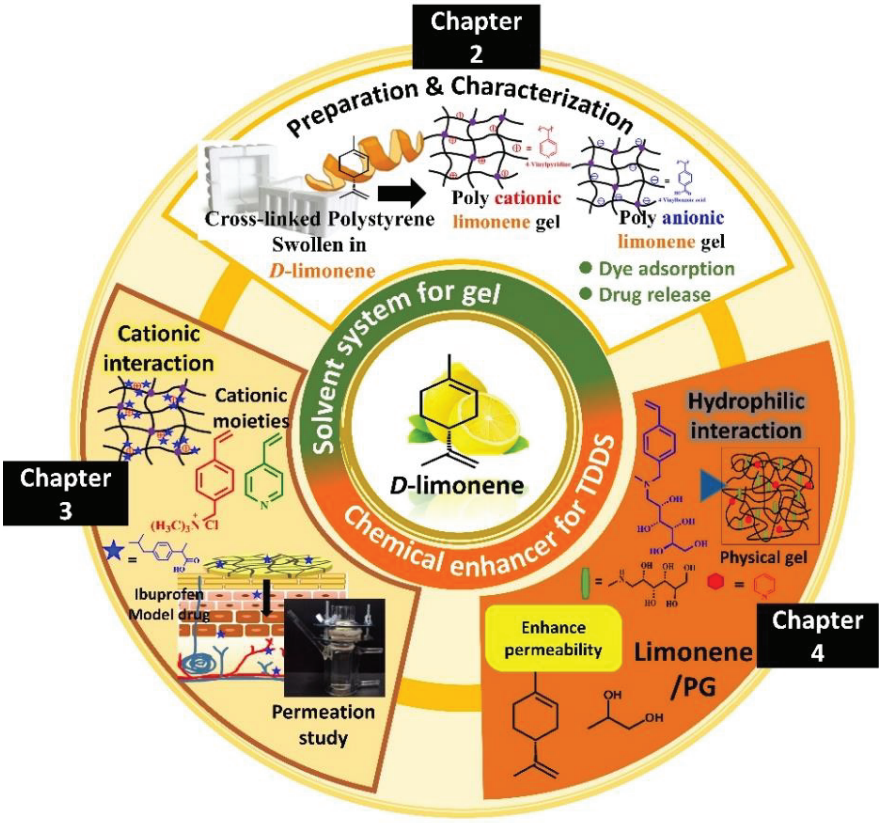


Figure 1-8. Illustrated scheme of this dissertation.

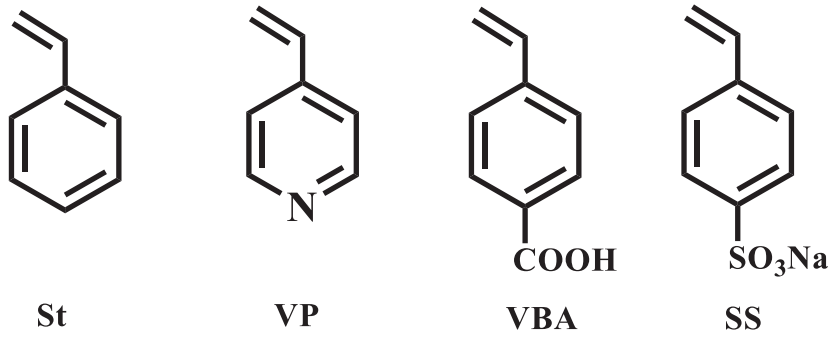


Figure 1-9. The chemical structure of monomer; styrene ((St), 4-vinyl pyridine (VP), 4-vinylbenzoic acid and 4-styrene sulfonate (SS).

In chapter 2, *d*-limonene was used as immobilized solvent in cross-linked polystyrene (CLPS) based chemical gel.[45] The limonene organogels has been increasing the recognition ability by introducing of styrene derivatives as electrostatic interaction moieties. That was 4-vinylpyridine (VP), 4-vinylbenzoic acid (VBA) and 4-styrenesulfonate (SS) as comonomers of PS (**Figure 1-9**). Poly(styrene-*co*-divinylbenzene) (PS gel), poly(styrene-*co*-divinylbenzene-*co*-vinylpyridine) (PS-VP gel), poly(styrene-*co*-divinylbenzene-*co*-vinylbenzoic acid) (PS-VBA gel) and poly(styrene-*co*-divinylbenzene-*co*-styrenesulfonate) (PS-SS gel) were prepared and swollen in limonene. Furthermore, we studied the controlled release from the organogels with hydrophobic drug; Testosterone and Ibuprofen as ionic drug. It was shown that the organogels could control release hydrophobic drug by network density and electrostatic interaction for ionic drug, respectively. The mechanical strength of these gels were investigated by swelling ratio, fracture stress and rheological behavior with increasing the

amount of crosslinking units from 5 to 10 mol%. Finally, dye adsorption test was also achieved to confirm the electrostatic interaction on surface in aqueous solution, by varying the amount and different co-monomers. This chapter showed the successful preparation of PS gel in limonene with interaction units as the first time.

In chapter 3, According to the limonene is predominant chemical permeation enhancer. Thus the CLPS based *d*-limonene organogels has been utilized as TDDS. [46] The CLPS bearing the cationic moieties of styrene derivatives which is 4-vinyl pyridine and (4-vinylbenzyl) trimethyl ammonium chloride were employed. The permeation through the rat skin were controlled by interaction between cationic moiety and drug molecule. In chapter 3, I aimed to study the model of controlled release of limonene gel to be develop as the appropriate TDDS Materials.

In chapter 4, in order to improve the pharmaceutics application of organogels in TDDS, the solvent system need to be compatible and reversible interacting with the skin. Thus propylene glycol (PG) is the most commonly use glycol in dermal and transdermal system.[47] Moreover, it was usually used as co-solvent with *d*-limonene due to their synergistic activity in permeability pathway. In this chapter, the novel sugar amine-derived gel, that was poly(vinyl benzyl-*N*-methyl-*D*-glucamine) gel (p(VbNMDG) gel), was obtained by the subsequently polymerization of neutral form;

VbNMDG and cationic form; VbNMDG-H included organic base as a promotor. The polymer-polymer interaction was determined by *in situ* gelation with rheological measurement. Plus the responsive behavior was shown shrink condition against to basic solution and urea solution. So as to enhance the permeability of testosterone, I studied the synergistic effect of limonene/PG as the solvent system and carbon chain length effect by benzalkonium chloride type as the co-monomer in polymer structure.

In short, this dissertation aims to prepare a new sustainable material as the organogels with *d*-limonene which is a plant-based oil. I used limonene as swollen solvent in CLPS as chemical gel because of their dissolution behavior. The CLPS with *d*-limonene was corporate with various interaction moieties in order to enhance the specification of organogels to hydrophobic molecule. It was shown the selective adsorption and desorption that could be the supportive for controlled release of delivery system. It was prepared, studied the mechanical property and applied for transdermal drug delivery that would be discussed in this thesis.

1.6 References

1) P. Koltun, *Pro. Nat. Sci-Mater.* **2010**, *20* 16.

2) G. Lligadas, J.C. Ronda, M. Galia, V. Ca´diz, *Mater. Today* **2013**, *16*, 337.

3) L.M. de Espinosa, M.A.R. Meier, *Eur. Polym. J.* **2011**, *47*, 837.

4) Y. Lu, R.C. Larock, *Chem.Sus.Chem.* **2009**, *2*, 136.

5) F. Li, R.C. Larock, *J. Polym. Sci. B: Polym. Phys.* **2001**, *39*, 60.

6) Y. Lu, R. C. Larock, *Biomacromolecules* **2006**, *7,* 2692.

7) Y. Lu, R. C. Larock, *Macromol. Mater. Eng.* **2007**, *292*, 863.

8) World Health Organization Geneva, Concise International Chemical Assessment Document (CICADS) 5 Limonene (**1998**)

9) H. Igimi, R. Tamura, K. Toraishi, F. Yamamoto, A. Kataoka, Y. Ikejiri, T. Hisatsugu, H. Shimura, *Dig. Dis. Sci.* **1991**, *36*, 200.

10) C.W. MONTE, D. LANDAU-WEST, *J. Food Sci.* **1982**, *47*, 1832

11) M.T. García, I. Gracia, G. Duque, A. de Lucas, J.F. Rodríguez, *Waste Manag.* **2009**, *29*, 1814.

12) T. Noguchi, M. Miyashita, Y. Inagaki, H. Watanabe, *Packag. Technol. Sci.* **1998**, *11*, 19.

13) T. Noguchi, M. Miyashita, Y. Inagaki, H. Watanabe, *Packag. Technol. Sci.* **1998**, *11*,

29.

14) T. Noguchi, M. Miyashita, Y. Inagaki, H. Watanabe, *Packag. Technol. Sci*. **1998**, *11*, 39.

15) C. Shin, G.G. Chase, *Polym. Bull.* **2005**, *55*, 209.

16) K. Hearon , L.D. Nash , J.N. Rodriguez, A.T. Lonnecker, J.E. Raymond, T.S. Wilson, K.L. Wooley, D.J. Maitland, *Adv. Mater.* **2014**, *26*, 1552.

17) S.C. H.Mangalara, S. Varughese, *ACS Sustainable Chem. Eng.* **2016**, *4*, 6095.

18) K. Rehman, M. H. Zulfakar, *Drug Dev. Ind. Pharm.* **2014** *40(4)*, 433.

19) A. Vintiloiu, J.-C. Leroux, *J. Control. Release* **2008**,*125*, 179.

20) D. J. Abdallah, R. G. Weiss, *Adv Mater* **2000**, *12*, 1237.

21) J. Kopeček, *Biomaterials* **2007**, *28(34)*, 5185.

22) S. H. Allan, *Adv. Drug Deliv.* Rev. **2012**, *64*, 18

23) S. Murdan, *Expert Opin. Drug Deli.* **2005**, *2*, 489.

24) Z. Li, J. Cao, H. Li, F. Han, Z. Lui, C. Tong, S. Li, *Drug Deliv.* **2016**, *23*, 3168.

25) B. Martin, F. Brouillet, S. Franceschi, E. Perez, *AAPS Pharm. Sci. Tech.* **2017**, *18*, 1261.

26) M. Durgun, G. O. Aydin, H. B. Sonmez, *React. Funct. Polym.* **2017**, *15,* 63.

27) X. Cao, N. Zhao, A. Gao, H. Lv, Y. Jia, R. Wu, Y. Wu, *Mater. Sci. Eng. C.* **2017**, *70*,

216.

28) D. Bhowmik, C. Bhattacharjee, M. C. Chandira, B. Jayakar, K.P. Sampath, *Int. J Pharm Tech Res.* **2010**, *2*, 68.

29) A. Kumar, N. Pullankandam, S. L. Prabhu, V. Gopal, *Int. J Pharm Sci. Review Res.* **2010**, *3*, 49.

30) Y. Yang, S. Sunoqrot, C. Stowell, J. Ji, C. W. Lee, J. W. Kim, S. A. Khan, S. Hong, *Biomacromolecules* **2012**, *13*, 2154.

31) H. Lee, C. Songa, S. Baika, D. Kima, T. Hyeona, D. H. Kima, *Adv. Drug Deli. Rev.* **2017**, *127*, 1.

32) V. Jhawat, S. Gupta, V. Saini, *Drug Des. Devel. Ther.* **2016**, *10*, 1101.

33) P. Karande, A. Jain, K. Ergun, V. Kispersky, S. Mitragotri, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102,* 4688.

34) S.V. Raut, L.S. Nemade, M.T. Desai, S.D. Bonde, S.U. Dongare, *IJPRR.* **2014**, *4*, 33.

35) A. C.Williams, B. W. Barry, *Pharm. Res.* **1991**, *8*, 17.

36) J. Chen, Q. D. Jiang, Y. P. Chai, H. Zhang, P. Peng, X. X. Yang, *Molecule* **2016**, *12*, 1709.

37) D. Monti, P. Chetoni, S. Burgalassi, M. Najarro, M. F. Saettone, E. Boldrini, *Int. J.*

Pharm. 2002, *237*, 209.

38) K. Inoue, J. Okada, K. Sugibayashi, *J Drug Deliv. Sci. Technol.* 2008, *18*, 335.

39) M. Almirall, J. Montaña, E. Escribano, R. Obach, J. D. Berrozpe, *Drug Res.* **1996**, *46*, 676.

40) J. Sun, *Altern. Med. Rev.* **2007**, *12*, 259.

41) P.F. Lim, X.Y. Liu, L. Kang, P.C. Ho, Y.W. Chan, S.Y. Chan, *Int. J. Pharm.* **2006**, *311*, 157.

42) Z. Yang, Y. Teng, H. Wang, H. Hou, *Int. J. Pharm*. **2013**, *447*, 231.

43) S. Saha, R. Shivarajakumar, V.V.S.R. Karri, *IJPSR.* **2018**, *9(11)*, 4540.

44) R. Kumar, O.P. Katare, *AAPS Pharma. Sci. Tech.* **2005**, *6*, 298

45) P. Charoensumran, H. *Polym. J.* **2018**, *50*, 1021.

46) P. Charoensumran, H. Ajiro, *Polymers* **2018**, *10(11)*, 1200.

47) L. Trottet, C. Merly, M. Mirza, J. Hadgraft, A.F. Davis, *Int. J. App. Pharm.* **2004**, *274*, 213.

Chapter 2

The Electrostatic Advantages on Cross-linked Polystyrene Organogels Swollen with Limonene for Selective Adsorbent and Hydrophobic Drug Storage.

2.1 Introduction

Organogels, which are semi-solid systems of three-dimensional network polymers, are immobilized in the organic liquid phase. Due to their hydrophobic structure, such gels have been widely used in various applications, such as vehicles for drug delivery,[1-3] pollutant recovery materials,[4] and sensor materials.[5] Their properties can be controlled by varying the components and their interactions in the gel network. In general, such gels can be classified into two categories, physical and chemical gels, depending on the type of interaction in the network structure. The framework of chemical gels consists of crosslinked subunits formed by covalent bonds, while physical gels are held together by noncovalent bonds, such as hydrogen bonds, π–π interactions, and van der Waals interactions.[6,7] Numerous physical gels made from oils have been extensively studied, probably due to the effective hydrogen bonding network in oils, but most of the resulting

gels lack the mechanical properties that would enable them revert to a sol solution.[8,9] Thus, chemical gels made from oil are sought to improve the mechanical properties and thermal stability. For this, we examined natural oils to avoid the toxicity of organic solvents, which is still a limitation in the application of oil gels.

D-Limonene (4-isopropenyl-1-methylcyclohexene) is an environmentally friendly, biodegradable solvent.[10] It can be used safely and effectively in a wide range of products. D-Limonene is listed in the Code of Federal Regulations as generally recognized as a safe.[11] It has possible applications for environmental and medical materials because of its pharmacological and biological properties.[12-15] Due to its properties, *D*-limonene is a good candidate solvent for environmentally friendly materials. In particular, network structures consisting of PS are well solubilized in *d*-limonene.[16-18] Since PS is a hydrophobic material as well as a biocompatible material,[19] it can be widely used in a variety of applications, such as the adsorbent material in pollutant recovery[20-22] and drug storage to control the release of lipophilic compounds.[23] A large number of different styrene copolymers have been produced,[24,25] but most are in the solid phase, such as the porous polymeric adsorbents of poly(styrene-*co*-divinylbenzene).[26] If PS derivatives could be used as an organogels, drug diffusion into the gel would make it possible to utilize the volume effectively.

In the previous work, we prepared chemical oil gels using a cross-linked copolymer composed of a trimethylene carbonate derivative and *L*-lactide for drug storage of testosterone.[27] This gel can be used as a delivery vehicle for androgen in bone fractures instead of costly bone morphogenetic proteins. The gel used dimethyl sulfoxide (DMSO) and dimethylcarbonate (DMC) as immobilized solvents, but the time for release was quite fast, occurring in the first 3 h, because of the good miscibility of the organic solvent in water.[28,29] Thus, I directed the attention to limonene gel, which is insoluble in water and could prolong release from the dense polymer network.

In this study, the preparation of chemically cross-linked polystyrene (CLPS) organogels including electrostatic interaction moieties with limonene as the solvent is presented for the first time, with the aim to control release and adsorption on the surface of aqueous media. The gel was designed to incorporate with poorly water-soluble compounds by using a hydrophobic core. Furthermore, we increased the recognition of organogels by introducing styrene derivatives as electrostatic interaction moieties, that is, by introducing 4-vinylpyridine (VP), 4-vinylbenzoic acid (VBA), and 4-styrenesulfonate (SS) as comonomers with styrene. Poly(styrene-*co*-divinylbenzene); PS gel (**1**), poly(styrene-*co*-divinylbenzene-*co*-vinylpyridine); PS-VP gel **(2)**, poly(styrene-*co*-divinylbenzene-*co*-vinylbenzoic acid); PS-VBA gel (**3**), and poly(styrene-*co*-

divinylbenzene-*co*-styrenesulfonate); PS-SS gel (**4**), were prepared and swollen in *d*-limonene shown the structure in **Figure 2-3**. The mechanical strength of these gels was investigated by the swelling ratio, fracture stress, and rheological behavior upon increasing the amount of cross-linking units from 5 to 10 mol%. Finally, dye adsorption tests were also performed to confirm the electrostatic interactions within these designed oil gels by varying the amount and comonomer.

2.2 Experimental section

2.2.1 Materials

Styrene (99.0%), azobisisobutyronitrile (AIBN) (98.0%), PBS (x10) were purchased from Wako Pure Chemical Industry Ltd. Japan. Super dehydrated toluene (99.5%), divinylbenzene (50.0%), 4-vinylbenzoic acid (97.0%), 4-vinylpyridine (95.0%), Sodium 4-styrenesulfonate (93.0%) and testosterone (98.0%) were all purchased from Tokyo Chemical Industry Co., Ltd. Japan (TCI). D-Limonene (90.0%), tetrahydrofuran (98.0%) dimethyl sulfoxide (99.0%) and bromocresol purple were supplied from Nacalai Tesque Inc. Japan. Rhodamine B was purchased from Sigma Aldrich.

2.2.2 Preparation of organogels

The CLPS were prepared via radical polymerization using AIBN as initiator. All monomers were purified by distillation or recrystallization to remove inhibitor before polymerization. To prepare the organogels, styrene, divinylbenzene as cross-linker and styrene derivative SD (VP, VBA and SS for PS-VP gel (**2**), PS-VBA gel **(3**) and PS-SS gel (**4**), respectively) as interaction unit were dissolve in toluene (4M), then 2.5 mol% of AIBN was added and sonicated. The mixture then was deoxygenated by nitrogen bubbling. The mixture was heated at 60 °C for 24 h. For PS-SS gel, the polymerization was done in DMSO due to SS is insoluble in toluene. The polymer network was polymerized randomly, providing PS_n-DVB_m-SD_p while n, m and p referred to feeding ratio of styrene, divinylbenzene and styrene derivative, respectively. The unreacted monomer, oligomer and linear polymer chains that are not connected into cross-linked network known as soluble fraction. To purify the gels, the completed organogels samples were soaked in THF to wash the soluble fraction for 72 h. The solvent was renew every 24 h. for 3 times. The THF gels were immersed in limonene (50mL-24h. 3times) lastly to replace THF with limonene in gel network providing limonene gel.

2.2.3 Characterization

The FT-IR of organogels were measured to investigate the additional functional group (that is pyridine, carboxylic and sulfonic group). The swelling ratio (Q) were evaluated in limonene solution at room temperature, followed by:

Swelling ratio $(Q) = \frac{(w_s - w_d)}{w_d}$

Where W_s stands for weight of swollen gel in limonene and W_d for weight of dry gel. The THF gel was dried under vacuum overnight and weight (W_d). The dried gels were immersed in limonene for 48h. Before weight the gel which is swollen in limonene (W_s). The fracture stress were measure by compressive mode of EZ test (EZ-SX, Shimadzu, Japan). The organogels were cut as square 8x8x2 mm^3 and compressed with 1 mm/min till break point. The rheological properties of the polystyrene organogels were measured using a Rheolometer (KNS2100, Kinexus, Japan). The organogels samples were cut as circle with diameter 20 mm. and thickness 4 mm. and placed between two plates while the lower plate is fixed and the upper circle plate (diameter 20 mm.) is connect with measuring system. The storage modulus (G') and loss modulus (G'') of swollen limonene organogels were measured at 25 °C.

2.2.4 Dye adsorption

The amount of dye adsorption were followed by UV-Visible spectroscopy. All limonene gels (8x8x4 mm^3, 180-200 mg) were immersed in 0.2 mM aqueous dye solution (bromocresol purple as anionic dye and rhodanmine B as cationic dye) 3.5 mL in cuvette for UV-Visible measurement. Absorption capability of organogels were followed by measuring the decreasing of dye concentration with UV-Vis spectrophotometer (UV-2600, Shimadzu, Japan). The concentration was curve fitted from calibration curve with 0.2-0.025 mM concentration range.

2.2.5 Drug release experiment

The drug were dissolved saturated in limonene. Then the limonene gel (30 mg) were soaked in the solution for 24 h. the loaded gel were wiped with tissue paper to remove the limonene on gel surface. Then loaded gels were released in PBS solution (pH 6.8, 10 mL, 37 °C). The releasing amount were determined by withdrawn 1 mL of solution and add 1 mL of fresh PBS solution to remain the same condition. Then it was detected by HPLC. The cumulative release of drug (%) was determined based on the following equation:

$$\text{Cumulative release} = \frac{M_n}{M_\infty} \times 100\%$$

Where M_n stands for the drug release amount (μg) at each sampling time and M_∞ for the drug release amount at highest releasing amount at 60h (for testosterone) and 72 h (for ibuprofen). While the drug releasing amount was calculated followed by:

Cumulative amount (M_t) $= C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i$

Where C_n stands for the drug concentration at each sampling time, C_i for the drug concentration at i^{th} sample, V_0 and V_i for volume of the receiver solution and the samples, respectively. The drug concentration (C_n) was derived from calibration curve of HPLC measurement with similar controlled condition shown in the next section.

2.2.6 Drug assay

Testosterone concentration was determined with a reverse phase high performance liquid chromatography (HPLC) from Shimadzu, Japan. HPLC system using Cosmosil Packed Column 5C18-MSII column (4.6 mm X 150 mm, 5 μm). The detection condition was 60 % MeOH in H_2O with flow rate 0.3 mL/min and 244 nm UV detector at 40 °C. 100 μL of sample was loaded. The testosterone characteristic peak appeared at 40.4 min. The concentration was curve fitted from calibration curve in range of 5-25 μg/mL (R^2 = 0.998) of testosterone in PBS solution in **Figure 2-1**.

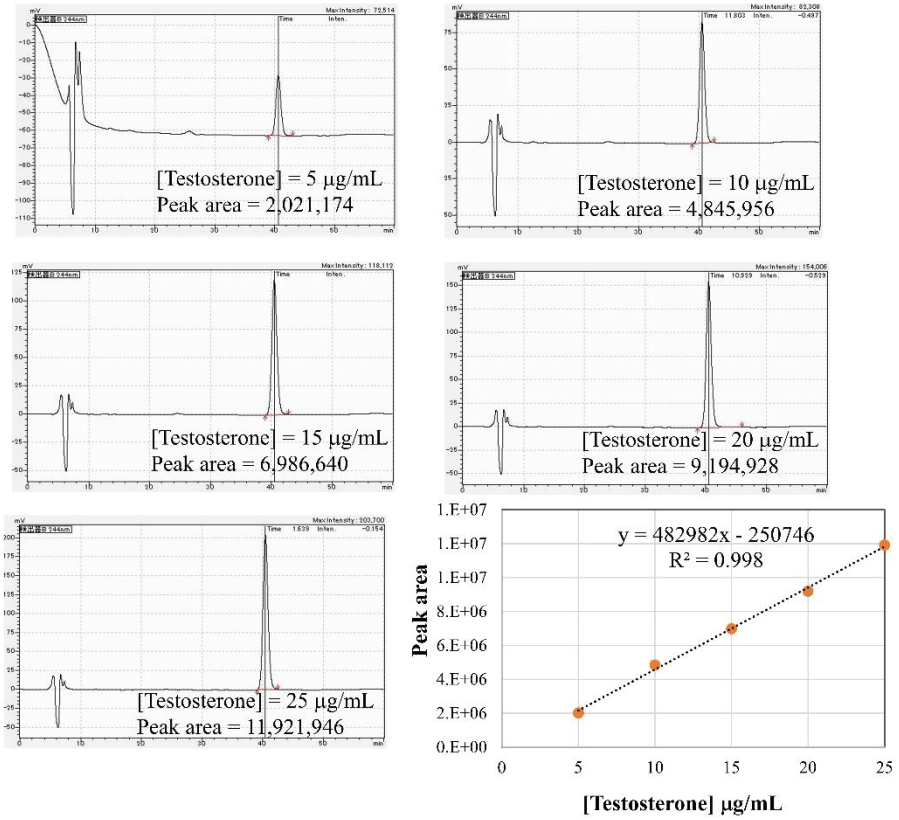


Figure 2-1. HPLC chromatogram of testosterone in PBS solution at 5 µg/mL to 25 µg/mL and standard calibration curve of peak area and [testosterone].

Ibuprofen (isobutylphenyl propionic acid) was detected by 60% PBS in acetonitrile as mobile phase with UV detector at 223 nm at 40 °C. The flow rate was set as 0.6 mL/min and 100 µL of sample was loaded. Under these condition the resolution time of ibuprofen was 5.01 min. A calibration curve was constructed by using ibuprofen standard solution in PBS solution from 6.5 – 46 µg/mL ($R^2 = 1$) as shown in Figure 2-2.

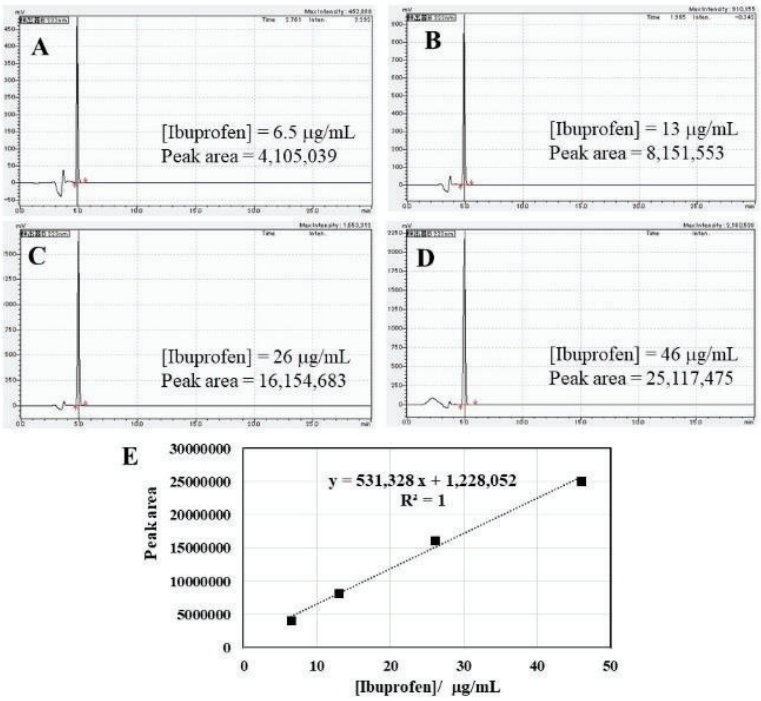


Figure 2-2. HPLC chromatogram of ibuprofen in PBS solution with 6.5 µg/mL, 13 µg/mL, 26 µg/mL, 46 µg/mL and standard calibration curve of peak area and concentration.

2.3 Results and Discussion

2.3.1 Preparation of Organogels

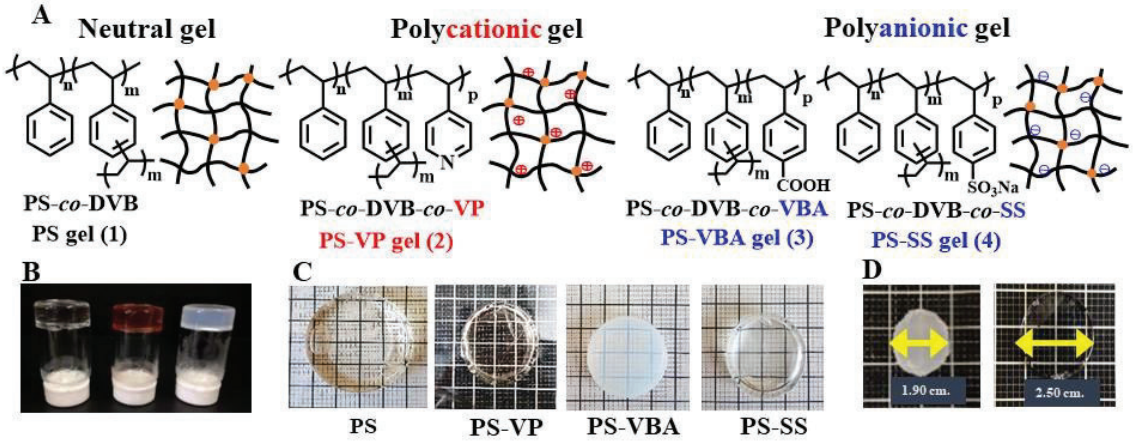


Figure 2-3. (A) Chemical structure and polymer model of PS gel **1** (neutral gel), PS-VP gel **2** (cationic gel), PS-VBA gel **3** and PS-SS gel **4** (anionic gel), (B) Inversion of vial of gel **1**, **2** and **3**, (C) Photograph of gel **1**, **2**, **3** and **4** (5% cross-linker) and (D) photograph of gel **1** between dry and swollen in limonene.

As shown in **Figure 2-3**, neutral gel (PS gel), polycationic gel (PS-VP gel) and polyanionic gel (PS-VBA and PS-SS gel) were prepared with polystyrene-co-divinylbenzene-*co*-styrene derivative (**Table 2-1**). Because the gel formation shows the quantitative yields, the formulas of polymer network could define by the feeding ratio of styrene to cross-linker (DVB) to styrene derivatives. The PS gel acted as neutral gel were prepared 5 and 10 mol% of divinylbenzene (cross-linker) for the variation of gel networks providing PS_{95}-*co*-DVB_5 and PS_{90}-*co*-DVB_{10} in order to observe the effect on the mechanical strength. We also selected VP and VBA as electrostatic interaction moieties providing 5 and 10% cross-linked PS-VP gel (**Table 2-1**, Entries 4 and 6) and PS-VBA gel (**Table 2-1**, Entries 8 and 9). Gelation was clearly confirmed when a homogeneous substance was obtained, which exhibited no gravitational flow upon inversion of the vial (**Figure 2-3B**). The reaction time was selected as 24 h in order to use the gel with quantitative reaction condition. All the gels with VP (**2**) yielded transparent gels, whereas over 5 mol% introduction of VBA gel (**3**) resulted in an opaque gel, probably due to low solubility of VP acid in toluene. The example of prepared gels were shown in **Figure 2-3C**. After the preparation of the gels in toluene and wash in THF, the gels were dipped into the excess amount of limonene to obtain the transparent gels swollen in limonene (**Figure 2-3D**).

Table 2-1. The sample parameter, percent yield and swelling ratio in limonene of synthesized organogels

Entry	Gel[a]	Formulas (PS-DVB-SD)[b]	Percent yield	Swelling ratio
1	5CL **1**	PS_{95}-DVB_5	96	3.39
2	10CL **1**	PS_{95}-DVB_5	95	2.10
3	5CL-2VP **2**	PS_{95}-DVB_5-VP_2	86	4.24
4	5CL-5VP **2**	PS_{95}-DVB_5-VP_5	94	2.58
5	5CL-8VP **2**	PS_{95}-DVB_5-VP_8	89	1.12
6	10CL-5VP **2**	PS_{90}-DVB_{10}-VP_5	95	1.40
7	5CL-2VBA **3**	PS_{95}-DVB_5-VBA_2	86	4.11
8	5CL-5VBA **3**	PS_{95}-DVB_5-VBA_5	95	1.52
9	10CL-5VBA **3**	PS_{95}-DVB_{10}-VBA_5	94	0.70
10	5CL-2SS **4**	PS_{95}-DVB_5-SS_2	93	1.05
11	5CL-5SS **4**	PS_{95}-DVB_5-SS_5	98	0.54
12	5CL-8SS **4**	PS_{95}-DVB_5-SS_{10}	98	0.31

(a) **1**= PS gel, **2** = PS-VP gel, **3** = PS-VBA gel and **4** = PS-SS gel, Numbers are represented feeding ratio (mol%).

(b) The subscripted number are represented to feeding ratio of each monomer.

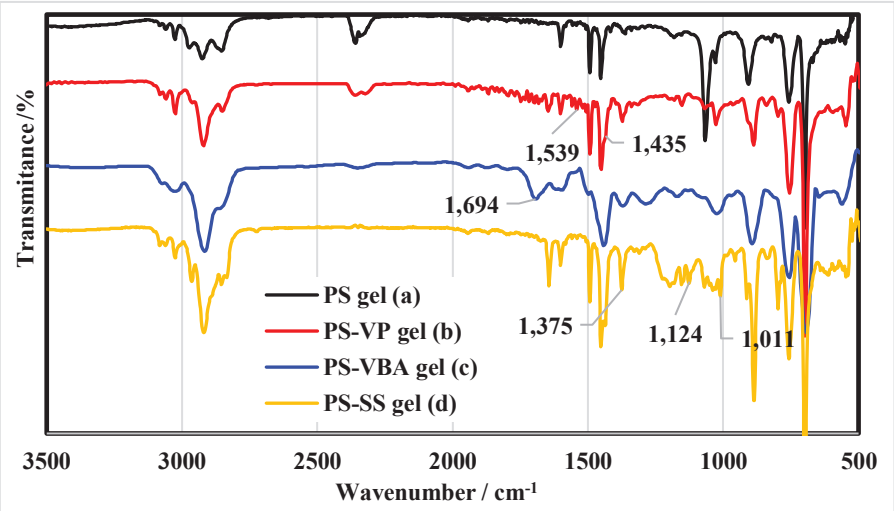


Figure 2-4. FT-IR of 5 %CL PS gel (a), PS-VP gel (b), PS-VBA gel (c) and PS-SS gel (d).

Incorporating of interaction unit as an electrostatic interaction was confirmed by FT-IR (**Figure 2-4**). The peaks at 1,434 cm^{-1} and 1,538 cm^{-1} were observed for PS-VP

gel, which was attributed to C-N and C=N stretching of aromatic amine. The significance of the C=O stretching peak at 1,694 cm^{-1} was also confirmed for PS-VBA gel, indicating the carbonyl group of carboxylic acid. While gel contains sulfonic acid, it appears peak at 1,011, 1,124 and 1,375 cm^{-1} for S=O and S-O bonds stretching.

2.3.2 Swelling Ratio and Mechanical Property

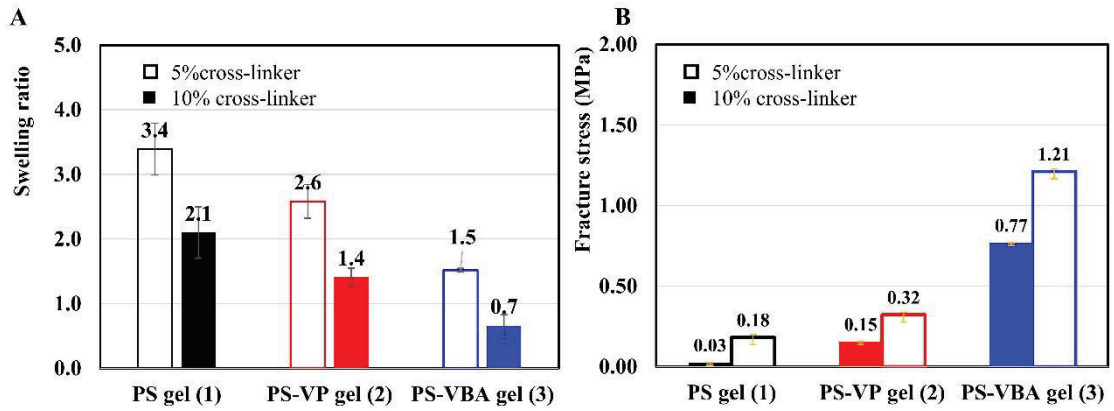


Figure 2-5. The mechanical measurement of PS gel **1**, PS-VP gel **2** and PS-VBA gel **3** between 5% and 10% cross-linker (A) Swelling ratio that the empty bar is represented 5% cross-linker and full bar is 10% cross-linker. (B) Fracture stress (n=3).

To study the mechanical strength, 5 mol% of a styrene derivative copolymer (VP and VBA) limonene gels were used to evaluate the effect of interaction unit and dense of polymer network between 5 and 10 mol% cross-linker (**Figure 2-5**). It was investigated by swelling ratio in limonene and fracture stress. The swelling property of an organogels is essential in biomedical and pharmaceutical applications because the degree of swelling influences the diffusion of solute, surface mobility and both the optical and mechanical

properties.

When organogels are to be used as a drug delivery system, the swelling ratios are directly related with drug absorption and release behaviors. It was generally shown that low swelling ratios were observed when the cross-linker increased. For example, swelling ratio of PS gel, PS-VP gel and PS-VBA gel, were 3.4, 2.6 and 1.5 in case of using 5 %CL while they reduced to 2.1, 1.4, and 0.7 in the cases of 10%CL (**Figure 2-5A**).

Furthermore, the fracture stress (**Figure 2-5B**) confirm stronger network structure of 10% CL gel. The fracture stress of 10% CL gels are higher about 2 times for gel **2** and **3**, and 6 times for gel **1**. The mechanical strength of limonene gel was thus influenced from both cross-linked degree and solubility of interaction unit. The result would make the gel strong, but the interaction units of carboxylic acid moiety might be low activity. Thus, the PS-SS gel, stronger anionic moiety, was studied the effect of electrostatic interaction by swelling property and dye adsorption instead of PS-VBA gel.

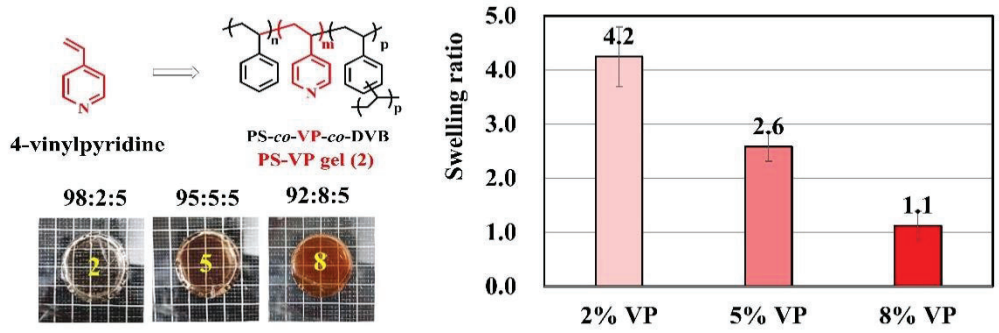


Figure 2-6. Chemical structure, photograph and swelling ratio of PS-VP gel **2** by varying feeding ratio of VP (2, 5 and 8 mol%).

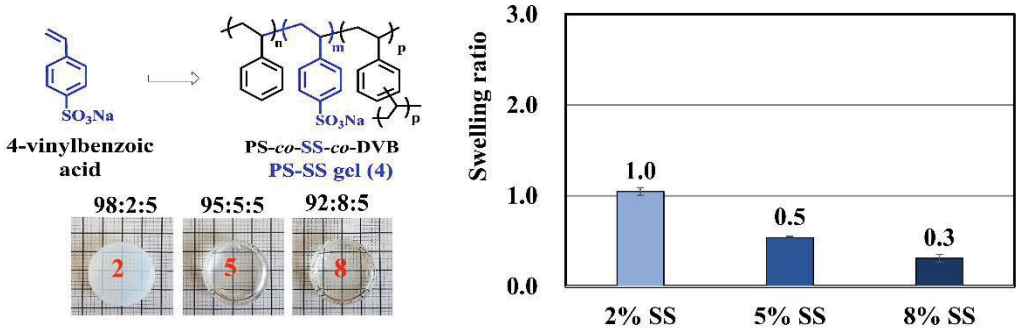


Figure 2-7. Chemical structure, photograph and swelling ratio of PS-SS gel **4** by varying feeding ratio of SS (2, 5 and 8 mol%).

The increase of comonomer ratio with VP as an interaction unit resulted in low swelling ratios: 4.24, 2.58, and 1.12 for gel **2** with 2, 5, 8 mol% of VP, respectively (**Figure 2-6**). Moreover, gel **4** was shown the similar trend the swelling degree in limonene were decreased from 1.05, 0.54 and 0.31 for 2, 5 and 8 mol% of SS, respectively (**Figure 2-7**), the higher anionic ratio (SS) the lower swelling ratio they show. However, gel **4** showed the lowest swelling ratios in limonene because of the lack of solubility of comonomer in limonene, including the heterogeneous domains.

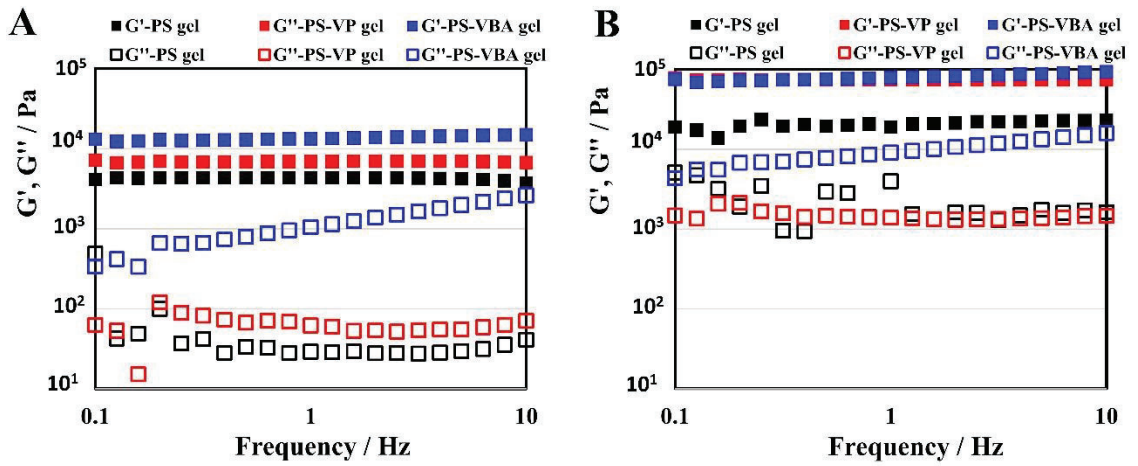


Figure 2-8. Rheological measurement; Storage modulus (G′=■) and loss modulus (G″=□) of (A) 5 mol% cross linker, and (B) 10 mol% cross linker (n=3).

In order to clarify the viscoelastic and rheological properties, the organogels in limonene were characterized between 5 (**Figure 2-8A**) and 10 mol% CL (**Figure 2-8B**) of the organogels in limonene. The oscillatory test was performed in the range of 0.1–10 Hz. In principle, the storage (elastic) modulus (G') represents the solid-like character and energy stored while the loss (viscous) modulus (G'') reflects the liquid-like behavior and energy lost. For all of them, the G' was higher than the G'' at all frequencies, showing gel-like behavior. The G' value of the 5 %CL was about six times lower than the value for the 10 %CL. For example, PS gel changed from 3,700 Pa with 5 %CL to 23,000 Pa with 10 %CL, as well as PS-VP gel and PS-VBA gel changed from 6,700 Pa and 15,000 Pa to 74,000 Pa and 93,000 Pa, respectively. This indicated strong mechanical strength from stable covalent network, especially when compared with the reported physical gel by PS main chain. [32] It has been reported that G' is only about 300 Pa to 1,400Pa as the

highest G' which the network was formed by charge-driven assembly. Furthermore, the value of G" for the viscosity also remained constant independent of the frequency, indicating the formation of a stable network via a strong covalent bond.[30-32] The G" value of 5 %CL are 30, 60 and 1,000 Pa for gel **1**, **2** and **3**, respectively which much more lower than the 10 %CL about 1,600, 1,400 and 9,000 Pa for gel **1**, **2** and **3**, respectively.

2.3.3 Adsorption Property by Electrostatic Behavior

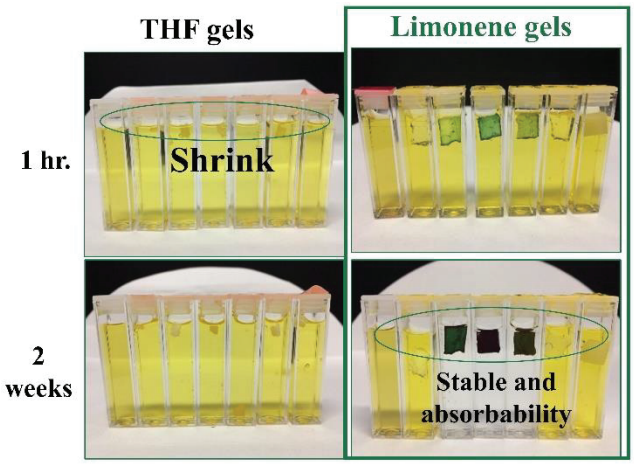


Figure 2-9. Photograph of THF gel (left) and limonene gel (right) in bromocresol purple aqueous solution between 1 h. and 2 weeks.

To reach the adsorption as well as drug-controlled release application, the organogels function need to perform via aqueous media. It thus is favorable for steady state of oil gel in aqueous solution. The limonene organogels were immersed in aqueous solution for about 2 weeks but the gels still retained their swollen size and adsorption

property. Because the limonene is insoluble in water, so the solvent is slowly released from the gels. This property is suitable for the prolonged release of a drug carrier in an aqueous solution (**Figure 2-9**). This was supported by the results of immersion of gels swollen in THF into the aqueous solution. THF is more easily dissolved in water and the gels quickly shrink twice and turn to opaque gels after immersed in water within 1 h.

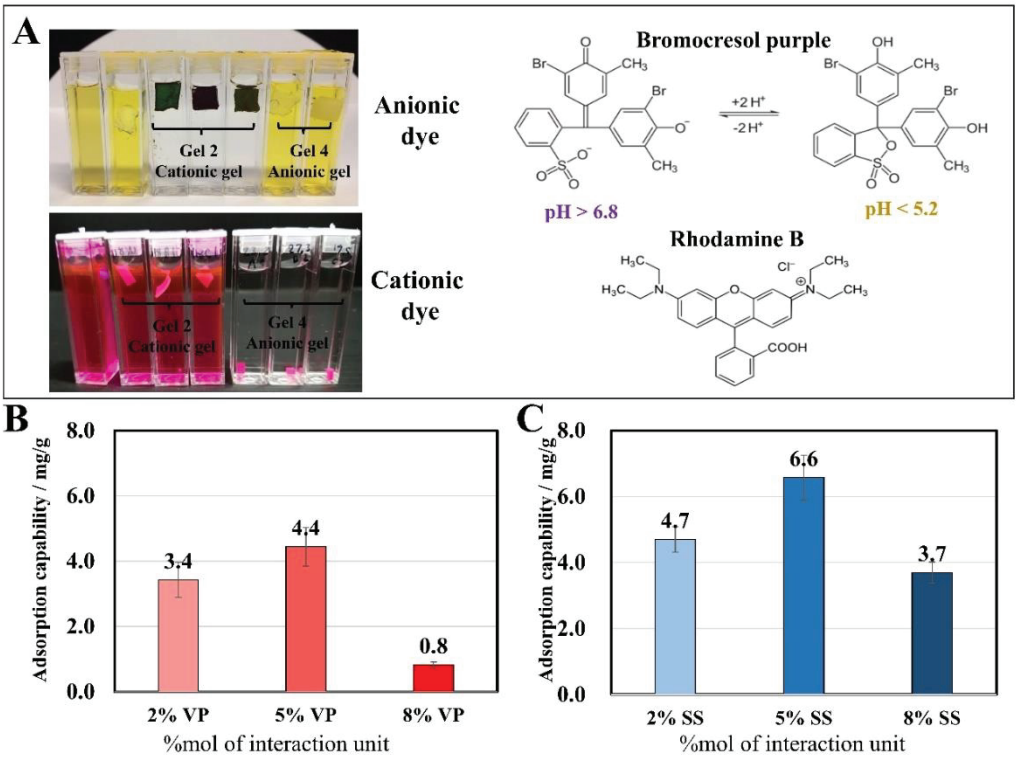


Figure 2-10. Dye adsorption experiment. (A) Photograph of dye adsorption test; anionic dye: bromocresol purpl and cationic dye: rhodamine B in aqueous solution (DI water, pH 5.9), (B) Adsorption capability to dye compound (mg/g) of PS-VP gel by variation of VP with 2, 5 and 8 mol% and (C) Adsorption capability to dye compound (mg/g) of PS-SS gel by variation of SS with 2, 5 and 8 mol% (n=3).

In order to study the adsorption capability by increasing interaction unit, the polyionic gels were fixed with 5% cross-linked ratio and varied the ratio of styrene

derivatives (that is VP and SS) 2, 5 and 8 mol% providing gel **2** (**Table 2-1**, Entries 3-5) and gel **4** (**Table 2-1**, Entries 10-12). The dye color were slowly turned to transparent (**Figure 2-10**), indicating the selectively adsorption by electrostatic interaction. Furthermore, we studied the adsorption ability of organic compound onto the organogels, using bromocresol purple as anionic hydrophobic drug and rhodamine B as a cationic hydrophobic drug in aqueous solution shown the structure in **Figure 2-10A**. In case of bromocresol purple, is pH indicator in range of weak acid condition (pH5.2-6.8), was equilibrium of neutral and anionic-form in pH 5.9 of aqueous solution. Whereas rhodamine B is usually in cationic-form of aqueous solution. The VP and SS were introduced in organogels showing electrostatic interaction as cationic and anionic, respectively. The different concentrations of interaction units were evaluated between 2, 5 and 8 mol%.

Only PS-VP gels show adsorption ability to bromocresol purple as an anion molecule in an aqueous solution while gel **4** and gel **1** have not appeared this property (**Figure 2-10A**). This evidence illustrated that the pyridine interaction unit shows electrostatic properties as a cation on a pyridine ring through surface in aqueous media. Also, the color of gels **2** were gradually changed to green while the color of the dye solution turned to transparent as observed by the naked eye while the gel **4** have not

changed. The gel **2** with 5 mol% VP moiety showed a higher adsorption capacity than the 2 mol% pyridine moiety gel as 4.4 mg/g and 3.4 mg/g, respectively because of the large amount of cationic parts (**Figure 2-10B**). However, gel **2** with 8 mol% VP moiety showed the lowest of about 0.8 mg/g, probably owing to the low swelling ratio (**Figure 2-6**) due to the low solubility of VP. The results indicate that the introduction amount of comonomer into polystyrene gel affected the limonene gel very much due to both solubility and electrostatic interaction with the target drug.

On the other hand, only gel **4** as the anionic gel are able to adsorb cationic dye efficiently shown which the adsorbed gel was changed to pink color. While cationic **2** gels cannot adsorb the cationic rhodamine B recognized by unchanging color because of the electrostatic repulsion activity (**Figure 2-10A**). The adsorption capability likewise depended on both electrostatic moiety and solubility of interaction unit in limonene. The 5 mol% of SS gel thus show the highest capability for 6.6 mg/g in **Figure 2-10C**.

2.3.4 Controlled Release of Drug Delivery System

To study the controlled release of limonene gel for testosterone, 5 mol% cross-linked limonene gels were used as drug storage for testosterone (**Figure 2-11**). The release profile was investigated in PBS at 37 °C. After 3 h., the 5 % cross-linked PS gel (**1**), PS-

VP gel (**2**), and PS-VBA gel (**3**) released about 36%, 25%, and 29%, respectively.

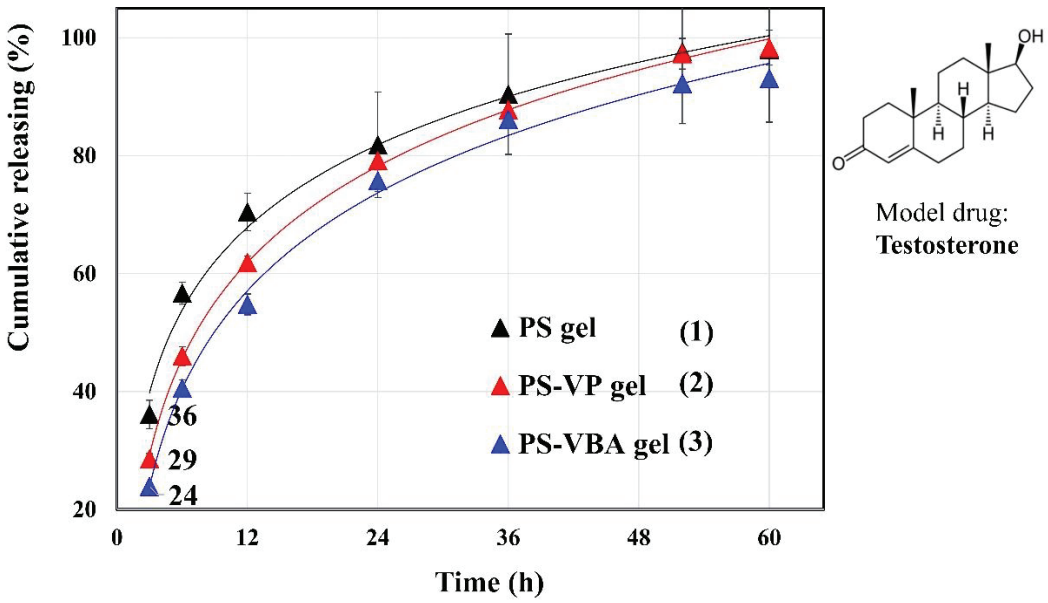


Figure 2-11. Cumulative release of testosterone from 5% cross-linker PS gel **1**, PS-VP gel **2** and PS-VBA gel **3** in PBS solution at 37° C (n=3) and chemical structure of testosterone.

These results showed the improvement of the long-term release, compared to previous study with the organogels in dimethyl sulfoxide and dimenthyl carbonate as solvents.[27] The gel **3** can control the most prolong release compared to gel **2** and **1**, respectively. Thus, the release profiles (**Figure 2-11**) were controlled by dense of polymer network of limonene gel because the gel **3** show the highest mechanical strength followed by gel **2** and **1**, respectively. The testosterone that is neutral molecule have weak interaction to polymer chain (interaction unit moiety and polystyrene). The controlled release thus were not significantly difference by various electrostatic moiety. Therefore, the electrostatic activity in limonene gel would be effective to ionic drug molecule.

Because of strong interaction, it could be controlled release of drug.

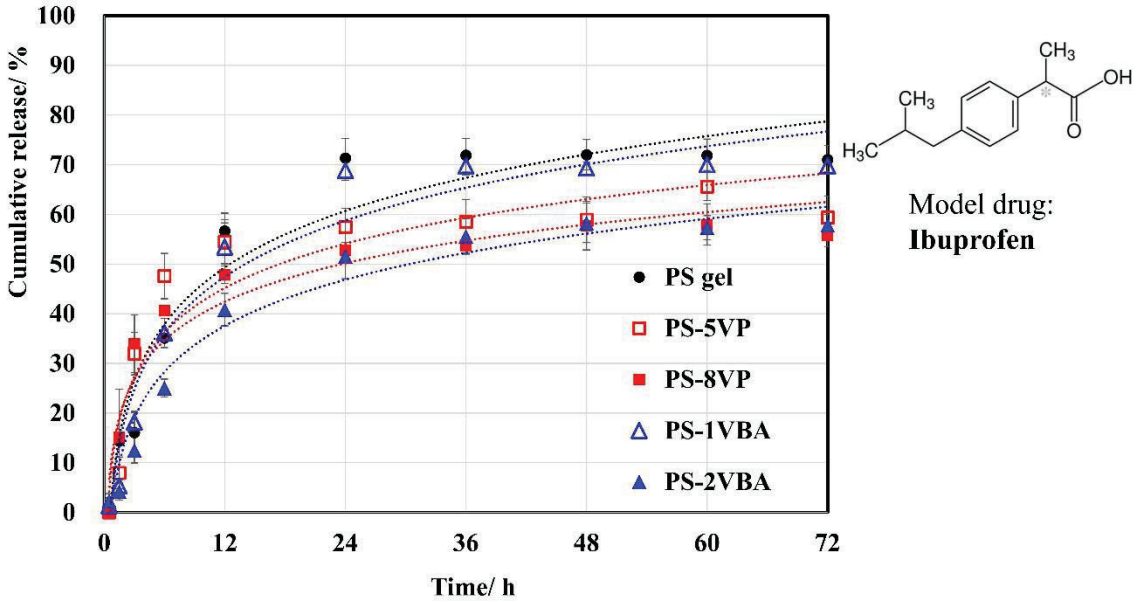


Figure 2-12. Cumulative release of ibuprofen in PBS at 37°C, using the PS gel **1**, the PS-VP gels **2**with 5 and 8 mol% VP, and the PS-VBA gels **3** with 1 and 2 mol% VBA (n=3) and chemical structure of ibuprofen.

In order to clarify the controlled release by electrostatic interaction, the ibuprofen release experiments were performed as model in **Figure 2-12**. The drug contains carboxylic group, which supposed to bind with pyridine by dipole-dipole interaction or electrostatic interaction. The cumulative release were plotted against to the releasing time and trend line were shown in logarithmic-plot theory. The gels **2** with 5 and 8 mol% VP and the gels **3** with 1 and 2 mol% VBA were employed for the observation on the release of ibuprofen. Interestingly, the trend releasing profiles of the drug from the gels with 8 mol% VP and 2 mol % VBA were prolonged more than those including the 5mol% VP and 1 mol % VBA as well as the neutral gel **1**. Thereby, the pyridine and carboxylic

moieties in limonene gels which are aprotic solvent were claimed to controlled release of ionic drug by interaction between gels network-drug.

2.4 Conclusions

In conclusion, the novel cross-linked polystyrene with electrostatic moieties based organogels in limonene were firstly prepared. The chemical organogels improved the mechanical strength, showing a G' of about 90,000 Pa and fracture stress is 1.21 MPa for PS-VBA gel (**3**), compared to the physical organogels. The dense of polymer network can be controlled by varying the concentration of the cross-linker (DVB) and the co-monomers, such as VP, VBA and SS. The selectivity by electrostatic interaction was also used for the drug adsorption. Only PS-VP gel (**2**) shows the adsorption ability to anionic dye compound with slowly adsorption through surface. The 5 mol% of VP shows the highest capability about 4.4 mg/g to anionic dye (bromocresol purple). On the other hand the PS-SS gel (**4**) could adsorb the cationic dye (rhodamine B) about 6.6 mg/g because of the introduction of electrostatic interaction units. Moreover the limonene gel served as hydrophobic drug storage and controlled release. The gel **3** could control release longer than gel **2** and **1**, respectively influenced by mechanical strength. Furthermore, the introducing of Cat/Anionic moiety could be controlled release of charged drug by

interaction with drug molecule. The novel chemically limonene gels with interaction unit are promising environmental friendly materials to serve as selective adsorbent and controlled release for hydrophobic drug reservoirs.

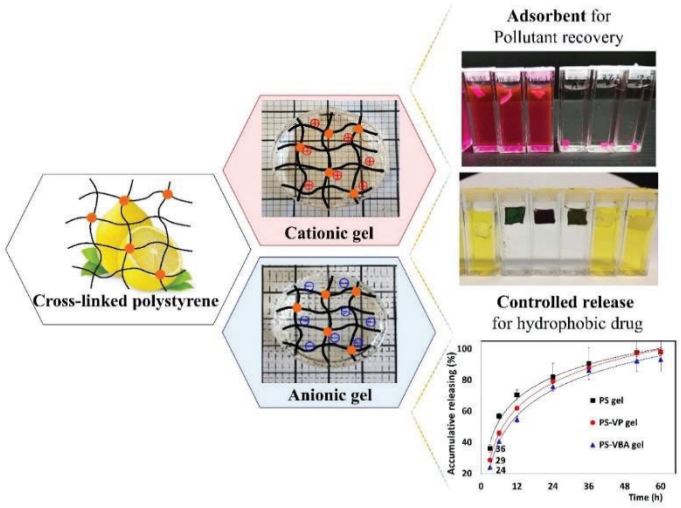


Figure 2-13. Schematic of oil gels bearing with electrostatic moiety for selective adsorption and controlled release of drug.

2.5 References

1) S. Murdan, *Expert Opin. Drug Deli.* **2005**, *2*, 489.

2) Z. Li, J. Cao, H. Li, F. Han, Z. Lui, C. Tong, S. Li, *Drug Deliv.* **2016**, *23*, 3168.

3) B. Martin, F. Brouillet, S. Franceschi, E. Perez, *AAPS Pharm. Sci. Tech.* **2017**, *18*, 1261.

4) M. Durgun, G. O. Aydin, H. B. Sonmez, *React. Funct. Polym.* **2017**, *15*, 63.

5) X. Cao, N. Zhao, A. Gao, H. Lv, Y. Jia, R. Wu, Y. Wu, *Mater. Sci. Eng. C.* **2017**, *70,* 216.

6) A. Vinloiu, J. C. Leroux, *J. Control. Release* **2008**, *125*, 179.

7) M. A. Cerqueira, L. H. Fasolin, C. S. F. Picone, L. M. Pastrana, R. L. Cunha, A. A. Vicente, *Food Res. Int.* **2017**, *96*, 161.

8) X. Cao, A. Gao, H. Lv, Y. Wu, X. Wang, Y. Fan, *Org. Biomol. Chem.* **2013**, *11*, 7931.

9) D. M. Pinhas, S. Barbut, A. G. Marangoni, *Annu. Rev. Food Sci. Technol.* **2016**, *7*, 41.

10) World Health Organization Geneva, Concise International Chemical Assessment Document (CICADS) 5 Limonene (**1998**).

11) J. Sun, *Altern. Med. Rev.* **2007**, *12*, 259.

12) M. R, Zahi, P. Wan, H. Liang, Q. Yuan, *J. Agric. Food Chem.* **2014**, *62*, 12563.

13) D. Roberto, P. Micucci, T. Sebastian, F. Graciela, C. Anesini, *Basic Clin. Pharmacol.* **2009**, *106*, 38.

14) S. F. Vuuren, A. M. Viljoen, *Flavour Fragr. J.* **2007**, *22*, 540.

15) M. N. Gould, *Environ. Health. Persp.* **1997**, *105*, 977.

16) W. C. Monte, D. Landau-West, *J. Food Sci.* **1982**, *47*, 1832.

17) M. T. García, I. Gracia, G. Duque, A. Lucas, J. F. Rodríguez, *Waste Manage*. **2009**, *29*, 1814.

18) C. H. M. Satish, V. Susy, *ACS sustainable Chem. Eng.* **2016**, *4*, 6095.

19) D. M. Monti, D. Guarnieri, G. Napolitano, R. Piccoli, P. Nettic, S. Fuscoc, A. Arciello, *J. Biotechnol.* **2015**, *193*, 3.

20) M. P. Tsyurupa, M. M. Ilyin, A. I.Andreeva, V. A. Davankov, *J. Anal. Chem.* **1995**, *352*, 672.

21) K. P. O. Mahesh, Y. Tsujita, H. Yoshimizu, S. Okamoto, D. Mohan, *J. Polym. Sci. Pol. Phys.* **2006**, *43*, 2380.

22) H. Zhu, S. Qiu, W. Jiang, D. Wu, C. Zhang, *Environ. Sci. Technol.* **2011**, *45*, 4527.

23) X. Wang, H. Yan, *Mater. Sci. Eng. C.* **2017**, *78*, 596.

24) E. Lizundia, M. Sáenz-Pérez, D. Patrocinio, I. Aurrekoetxea, Md. M. Vivanco, J. L. Vilas, *Mater. Sci. Eng. C.* **2017**, *75*, 229.

25) T. Koriyama, T. A. Asoha, A. Kikuchia, *Colloid Surface B.* **2016**, *147*, 408.

26) W. C. Lai, P. H. Lai, *Macromol. Chem. Phys.* **2010**, *211*, 685.

27) B. H. Cheng, T. M. Chu, C. Chang, H. Y. Kang, K. E. Huang, *PLOS ONE 8*, e702342013. DOI:10.1371/journal.pone.0070234 (**2013**).

28) K. Takemura, H. Ajiro, T. Fujiwara, M. Akashi, *RSC Adv.* **2014**, *4*, 33462.

29) Takemura K, Ajiro H, Fujiwara T, Akashi M. *Polym. J.* **2015**, *47*, 460.

30) B. J. Kong, A. Kim, S. N. Park, *Carbohyd. Polym.* **2016**, *147*, 473.

31) D. Khondkara, R. F. Testera, N. Hudsonb, J. Karkalasa, J. Morrow, *Food Hydrocolloids* **2007**, *21*, 1296.

32) T. Zhang, Q. Guo, *Chem. Commun.* **2013**, *49*, 5076.

Chapter 3

Cationic Moieties in Polystyrene Gels Swollen with *D*-limonene Improved Transdermal Delivery System.

3.1 Introduction

Transdermal drug delivery (TDD), the transportation of drugs across the skin, has been an attractive research area because of its obvious advantages over other routes of delivery.[1,2] Transdermal delivery systems provide convenient, pain-free, and self-administrated use for the patient. It avoids the gastrointestinal side effects, usually entailed by many oral preparations. TDD also avoids fluctuations in plasma drug concentration, which helps minimizing adverse effects and therapeutic failure.

The main challenge in TDD, however, is to overcome the inherent barrier of the skin. It has been reported that the rate limiting step in transdermal delivery is the ~30 μm thick *stratum corneum* (SC) which acts as a protective barrier against exogenous molecules including drugs.[3] For this reason, a variety of molecules[4] and materials[5] have been investigated as candidates to enable or facilitate skin permeation.

Chemical penetration enhancers (CPEs) have been widely used to increase the

skin permeability of many therapeutic molecules and anesthetics[6] by interacting with the SC lipid or keratin,[7] or by increasing the solubility of drug into SC lipid.[8,9] Extensive research during the past two decades has led to the formulation of several different classes of penetration enhancer such as terpene compound. Many reports have already provided substantial evidence that terpene[10,11] are capable of enhancing percutaneous transportation, especially *d*-limonene. Zhen Yang and co-workers[12] has been reported the *d*-limonene was the most effective permeation enhancer (PE) to enhance skin permeation of bufalin among other terpene compound and different synthetic PEs. D-limonene is a neutral-derived terpene compound which is well known as permeation enhancer in transdermal delivery system.[13,14] Moreover, it has low toxicity, so it is appropriate to boost up the transdermal delivery system

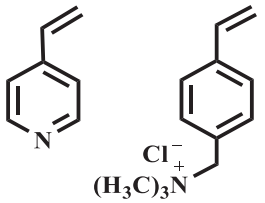
Regarding the material side, organogels are promising candidates for drug delivery system including dermal and transdermal application,[15,16] because of their intrinsic properties. They are lipophilic, non-irritating easy-to-use, and moisture insensitive. In an earlier work, Chan and co-workers reported the preparation of limonene PG1/propylene glycol (PG) organogels as a physical gel.[17] However, it has a limitation on increasing the amount of limonene because it affects the stability of organogels. Yang and co-workers reported transdermal delivery of ibuprofen using microemulsion as

vehicle.[18] Microemulsions are spreadable materials requiring a specific ratio of the oil-surfactant-water system. In order to open the door to facile preparation, we suggest that the convenient use and stability against various conditions, organogels as a chemical gel swollen with limonene[19] are an auspicious approach to solve the limitation of TDD in both of applications and permeability.

Actually, it is known that the *d*-limonene is the excellent solvent for PS which is well known aromatic polymer.[20] In addition, it can use as eco-friendly solvent for dissolving wasted expanded PS.[21] According to this advantage, the network structure consists of cross-linked PS gel is possible to swollen in *d*-limonene. Since PS has hydrophobic and biocompatible properties,[22] it can be widely used in a variety of applications such as adsorbent materials in pollutant recovery,[23,24] drug storage to control release for lipophilic compounds[25] and transdermal delivery systems.[26,27]

In this work, the cross-linked polystyrene swollen in *d*-limonene as reservoir-type transdermal system was studied. We developed transdermal materials by using *d*-limonene as solvent for chemical organogels because of its chemical enhancer property and I investigated the permeation behavior of ibuprofen via the limonene gel through skin in this study in order to clarify the controlled release by electrostatic interaction of cationic moiety on the surface, although the limonene gel as drug reservoir will be studied

with adhesive membrane to abate effect of limonene contact to skin in the future. Herein, effect of network density of PS gels was studied by rheological measurements, as well as its influence on permeability and controlled release behaviors. The cationic moieties, 4-vinylpyridine (VP) and vinylbenzyl trimethylammonium chloride (VBAC), were selected as interaction units with drug molecules for the prolong release. The interaction between drug and cationic moieties were observed by FT-IR.



VP VBAC

The relationship betwee n elastic moduli and permeability were also discussed.

3.2 Experimental section

3.2.1 Materials

Styrene (St) (99.0%), azobisisobutyronitrile (AIBN) (98.0%), and PBS buffer solution (x10) were purchased from Wako Pure Chemical Industry Ltd. Japan. Super dehydrated toluene (99.5%), divinylbenzene (DVB) (50.0%), 4-vinylpyridine (VP) (95.0%), sodium 4-styrenesulfonate (93.0%), acetonitrile (99.0%) and isobutylphenyl propionic acid (Ibuprofen, > 98.0%) were all purchased from Tokyo Chemical Industry Co., Ltd. Japan (TCI). D-limonene (90.0%), tetrahydrofuran (98.0%), dimethyl sulfoxide (99.0%) and bromocresol purple were purchased from Nacalai Tesque Inc. Japan.

Rhodamine B was purchased from Sigma Aldrich. Vinylbenzyl trimethylammonium chloride (VBAC) was purchased from Santa Cruz Biotechnology.

3.2.2 Preparation of Organogels

The transdermal patch gel was prepared by cross-linked polymers swollen with *d*-limonene as the solvent. All monomers were purified by distillation to remove inhibitor before the polymerization. Firstly, as model drug, Ibuprofen solution in *d*-limonene (33.3 mg/mL) was prepared. Then, St, DVB as cross-linker, and styrene derivative (SD), that is VP or VBAC, as a cationic moieties were added. After that, 2.5 mol% of AIBN was added and the solution was sonicated for 5 minutes. In the following step, mixture was deoxygenated by nitrogen bubbling and heated at 60 °C for 24 h. The polymer network was radical polymerized, providing PSn-DVBm-SDp while n, m and p are referring to the feeding ratio of St, DVB, and SD, respectively. The gels in this study are different from those reported in previous study,[19] because PS gels were prepared in *d*-limonene while previously reported PS gels were prepared in toluene.[19] The gels were removed from the container and cut into 13 mm-diameter, 2 mm-thick discs (V = 0.265 cm^3) for permeation test with a Franz diffusion cell (**Figure 3-8**). The drug concentration per disc was calculated from the total prepared gel containing 31 mg/cm^3 of Ibuprofen, thus, each

discs was found to contain 8.23 mg.

3.2.3 Swelling property

The PS gels swollen in limonene were freeze-dried after limonene had been washed out by benzene. Swelling properties of the synthesized gels were determined after 24 h. re-swelling in limonene by the following equation:

$$\text{Swelling ratio} \qquad (Q) = \frac{(W_s - W_d)}{W_d}$$

Where W_s stands for weight of swollen gel in limonene and W_d is weight of dry gel.

3.2.4 Rheological study

The rheological properties of the PS gels swollen in limonene[19] were measured using a Rheometer (KNS2100, Kinexus, Japan). The organogels were cut in discs with 20 mm diameter and ~ 4 mm thickness and placed between two plates while the lower plate is fixed and the upper circle plate (20 mm diameter) is connect with the measuring system. The elastic modulus (G') and viscous modulus (G") of the organogels swollen with limonene were measured at controlled frequency from 0.1 to 10 Hz at 25 °C in triplicate.

3.2.5 Preparation of Rat Skin

The rat skin was received from System Neurobiology and Medicine Laboratory, NAIST, Japan. All relevant aspect of experiment was approved by the Institutional Animal Care and Use Committee of Nara Institute of Science and Technology (reference No. 1802). The abdominal skins of female rat (adult pregnant wistar rat, weighing 250-300 g) were excised after sacrifice by cervical dislocation of rat. Adhering fat and other visceral debris were carefully removed. The processed skin was cut into pieces of appropriate size and used freshly without storing.

3.2.6 *In vitro* Skin Permeation Study

In vitro skin permeation study[19] was carried out by using Franz-cell diffusion with receptor volume of 10 mL and an exposed area of 1.33 cm^2. The 10 mL pH7.4 phosphate buffer saline (PBS) was considered as receptor medium for the maintenance of physiological environment. The prepared skin sample was then mounted between acceptor and donor compartment of the cell and clamped with its dermal side in contact with the receptor medium. The prepared 13 mm. diameter PS gel swollen in limonene was placed into the donor chamber. The diffusion cells were keep in 37 °C incubator. At designed time interval, 1 ml receptor medium was withdrawn from the receptor chamber

and immediately replaced with the same amount of fresh PBS solution.

3.2.7 HPLC Measurements for Drug Assay

Ibuprofen (isobutylphenyl propionic acid) concentration was determined with a HPLC from Shimadzu, Japan. HPLC system using Cosmosil Packed Column 5C18-MSII column (4.6 mm × 150 mm, 5 μm). The detection condition was 60% PBS in acetonitrile as mobile phase with UV detector at 223 nm, 40 °C. The flow rate was set as 0.6 mL/min and 100 μL of sample was loaded. Under these condition the resolution time of ibuprofen was 4.97-5.02 min. A calibration curve was constructed by using ibuprofen standard solution in PBS solution from 6.5 – 46 μg/mL ($R^2 = 1$) shown in **Figure 2-2**.

3.2.8 Calculation of permeation parameter

The cumulative permeated amount of drug (Q) permeating through the skin from the donor chamber at constant concentration (C_0) to the receptor phase at the sink condition can be described by Fick's 2nd law of diffusion, Eq. (1)[28] where A is the surface area, L is the thickness of the skin and K is the diffusion coefficient of the skin. Permeation parameters are interpreted from cumulative permeated drug per unit skin area Q/A versus time t plot. The steady-state flux (Jss) and lag time t_L were obtained from slope

and x-interception value of the linear portion as shown the illustration in **Figure 3-1**. The flux Jss over drug concentration C_0 in the donor solution gives permeability coefficient KD/L.[29] Diffusion parameter D/L^2 reflects the mobility of the drug solute in the skin.[30]

$$Q = AKLC_0\left[\frac{D}{L^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\alpha}\frac{(-1)^n}{n^2}e^{-(\frac{D}{L^2})n^2\pi^2 t}\right] \quad (1)$$

$$\frac{KD}{L} = \frac{J_{ss}}{C_0} \quad (2)$$

$$\frac{D}{L^2} = \frac{1}{t_L 6} \quad (3)$$

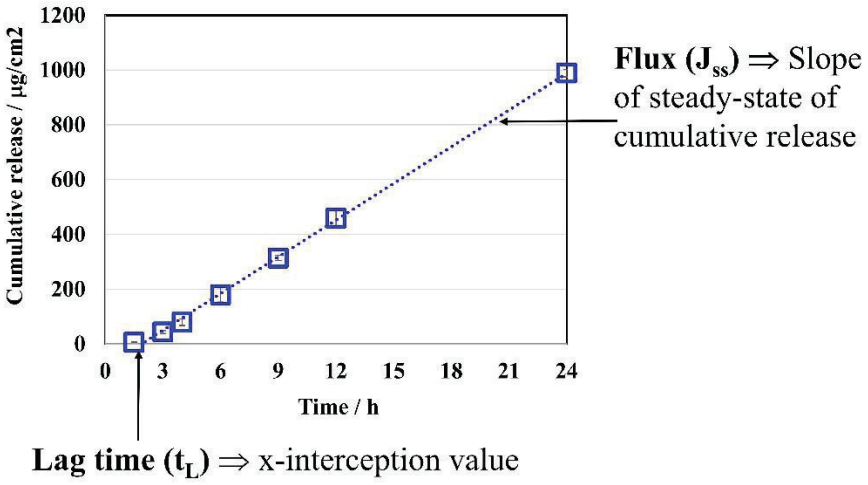


Figure 3-1. The illustration of parameter (flux: J_{ss} and lag time: t_L) from steady-state (linear portion) release.

3.3 Results and Discussion

3.3.1 Synthesis and characterization of limonene organogels

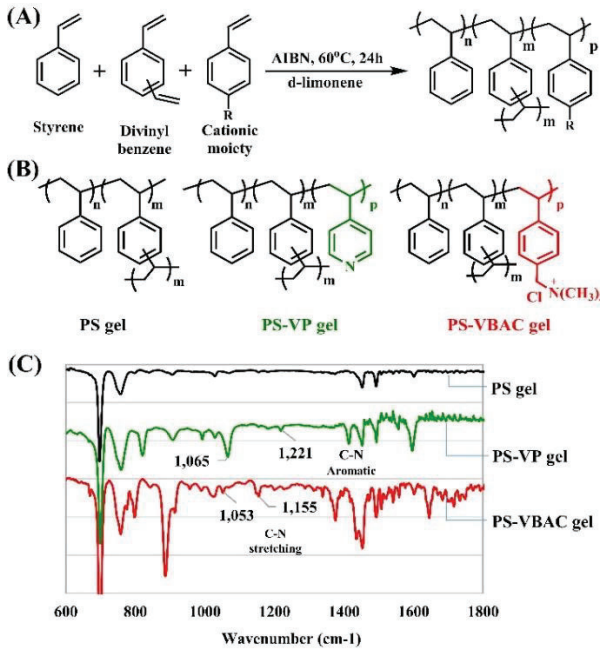


Figure 3-2. (a) Preparation of cross-linked PS gel in *d*-limonene. (b) Chemical structure of cross-linked PS gels. (c) FT-IR spectra of dry PS gel, PS-VP gel, and PS-VBAC.

The limonene gels, poly(styrene-*co*-divinylbenzene) (PS gel), poly(styrene-*co*-divinylbenzene-*co*-4-vinylpyridine) (PS-VP gel) and poly(styrene-*co*-divinylbenzene-*co*-(vinylbenzyl) trimethylammonium chloride) (PS-VBAC gel) were prepared in *d*-limonene via radical polymerization (**Figure 3-1a and 3-2b**). The prepared gels in this study were listed in Table 2-1. The PS gels were prepared by varying the cross-linker ratio between 5 and 10 mol% and the concentration of *d*-limonene between 4 and 8M resulting in gel A-D (**Table 3-1**, entries 1-4). These gels were used to study the effect of the network

density. As a comparison, the PS gel was prepared in toluene as solvent for gel E (**Table 3-1**, entry 5). Moreover, the VP which acts as cationic moiety was introduced at 2-5 mol% being PS-VP gel in gel F-J (**Table 3-1**, entries 6-10). As the other cationic moiety, the VBAC was introduced 0.5 and 1 mol% being PS-VBAC gel in gel K and L (**Table 3-1**, entries 11 and 12). Their elastic modulus (G') were also listed in **Table 3-1**.

Table 3-1 Gel preparation and elastic modulus (G′)

Entry	Sample	Gel type	Ratio of PS_n-DVB_m-SD_p (n: m: p)[a]	comonomer	comonomer (mol%)	Conc. (M)	G' (Pa)
1	Gel A	PS gel	95:5:0	-	-	4	680
2	Gel B		90:10:0	-	-	4	6,800
3	Gel C		95:5:0	-	-	8	44,200
4	Gel D		90:10:0	-	-	8	98,600
5	Gel E[b]		95:5:0	-	-	4	1,600
6	Gel F	PS-VP gel	95:5:2	VP	2	4	2,600
7	Gel G		95:5:2.5	VP	2.5	4	10,300
8	Gel H		95:5:3	VP	3	4	11,700
9	Gel I		95:5:4	VP	4	4	21,300
10	Gel J		95:5:5	VP	5	4	14,000
11	Gel K	PS-VBAC gel	95:5:0.5	VBAC	0.5	4	5,000
12	Gel L		95:5:1	VBAC	1	4	1,400

a) the n, m and p are referred to the feeding ratio of St, DVB and SD (VP and VBAC) , respectively from PSn-DVBm-SDp formulation.

b) Gel E was polymerized in toluene, while all the other gels were polymerized in limonene.

The existence of cationic moieties were verified by FT-IR spectra of dry gels in **Figure 3-1c**. The PS-VP gel, bearing pyridine group, was shown characteristic of C-N

aromatic stretching at 1,221 cm^{-1} and C-N stretching at 1065 cm^{-1}. The PS-VBAC gel contained trimethyl ammonium group illustrated C-N stretching at 1,053 and 1,155 cm^{-1}.

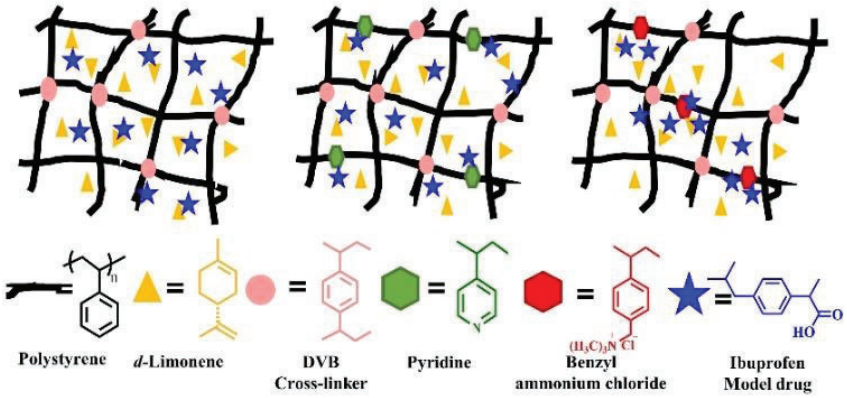


Figure 3-3. Schematic illustration of the interaction with drugs and scheme of polymer network with and without cationic moieties at the interface of aqueous media.

The VP and VBAC served as interaction unit in the copolymer for the controlled release by interacting with the drug. The positively charged VBAC would be expected to show stronger interaction with ibuprofen than VP (**Figure 3-3**). According to the interaction between drug and polymer chain, it could release ibuprofen through the skin in a controlled manner.

2.3.2 Swelling behavior and rheological property of PS gels swollen in limonene

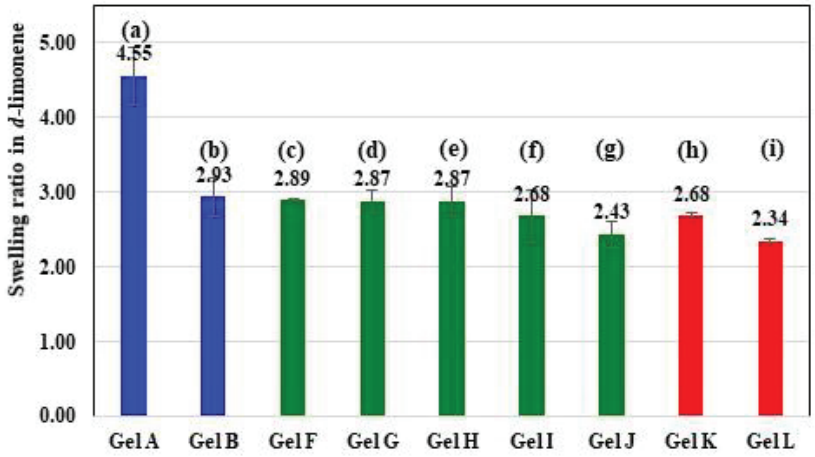


Figure 3-4. Swelling ratios of gel A (a), Gel B (b), Gel F (c), gel G (d), gel H (e), gel I (f), gel J (g), gel K (h), and gel L (i) (n=3, error bars present standard deviation).

All gels were prepared directly by polymerization in *d*-limonene as solvent due to the good solubility of St in limonene.[18,19] Swelling ratios of various compositions of gels were determined as shown in **Figure 3-4**. The cross-linker amount of PS gel was compared at DVB contents of 5 and 10 mol% to evaluate the effect of the cross-linking degree on the density of the network structure and swelling properties. The swelling degree was decreasing as expected upon increasing the amount of cross-linker from 4.5 and 2.9 for 5 and 10 mol%, respectively (**Figure 3-4a and 3-4b**). These results confirmed the formation of a denser network structure when the cross-linking degree of PS gel was increased. Furthermore, the swelling ratio of PS-VP gel (gel F-J in **Figure 3-4c—3-4g**) and PS-VBAC gel (gel K and L in **Figure 3-4h and 3-4i**) with 5 mol% cross-linker were determined. The swelling ratios of PS-VP gels were not significantly affected by

enhancing of VP ratio in a range of 2-3 mol% (**Table 3-1**, entries 6-8), illustrating a swelling ratio of about 2.9 (**Figure 3-4c-e**). However, the swelling ratio of 4 and 5 mol% (**Table 3-1**, entries 9 and 10) of VP (gel I and J) were slightly decreased to 2.68 and 2.43, respectively. The shrinkage of gel I and J was caused by the low solubility of VP in *d*-limonene. In the same way, the VBAC was introduced at only 0.5 and 1 mol% (**Table 3-1**, entries 11 and 12). Their swelling ratio was calculated as 2.68 and 2.34, respectively (**Figure 3-4h and 3-4i**). As a result, the swelling ratio of PS gel was about 1.5 time higher for the cationic gels as PS-VP gel and PS-VBAC gel which were referred to limitation of solubility in limonene.

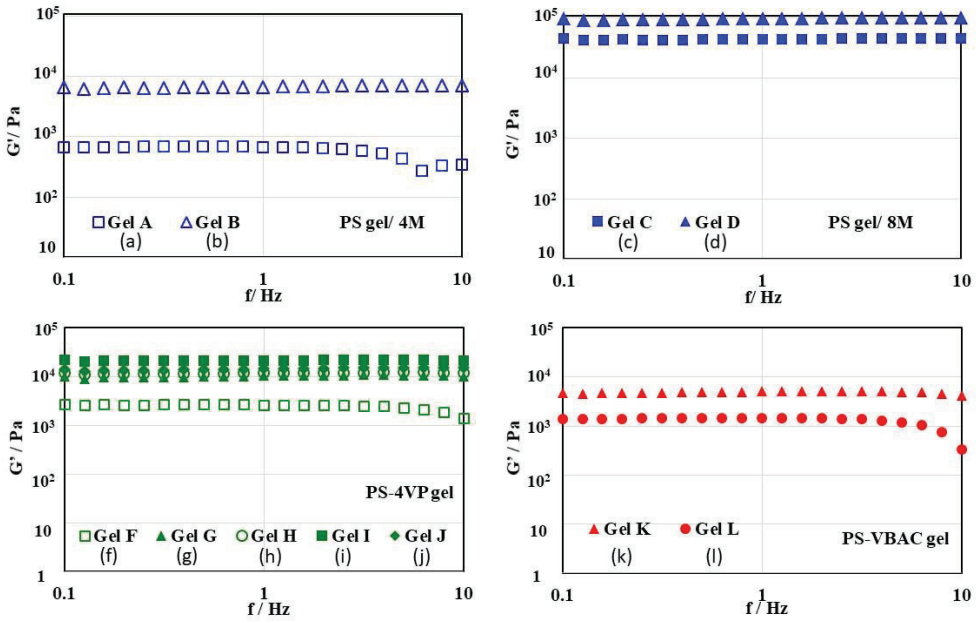


Figure 3-5. Elastic (storage) modulus (G') from rheological measurement at frequency 0.1-10 Hz of 5 mol% cross-linker PS gel; Gel A (a) and Gel B (b), 10 mol% cross-linker PS gel; Gel C (c) and Gel D (d), PS-VP gel; Gel F (f), Gel G (g), Gel H (h), Gel I (i) and Gel J (j) and PS-VBAC; Gel K (k) and Gel L (l) in d-limonene, (n=3).

Previously, the rheological analyses on PS gels has been reported.[19] In this study, we further investigated the rheological properties with the detailed crosslinking and comonomer's ratios regarding the PS gels obtained in *d*-limonene (**Figure 3-5**). The elastic modulus (G') of gel B (**Figure 3-5b**) showed higher than that of gel A (**Figure 3-5a**), based on the higher crosslinking degree under 4M condition. The same tendency was recognized between gel D (**Figure 3-5d**) and gel C (**Figure 3-5c**) under 8M condition. Gel D illustrated the most rigid network structure providing an elastic modulus of 98 kPa at a frequency of 1 Hz (**Figure 3-5d**). This is about 2 times higher than gel C of 44 kPa (**Figure 3-5c**) and about 6 times higher than gel B of 6.7kPa (**Figure 3-5b**). On the other hand, the concentration of the gel effects the strength of the network structure more than the cross-linking control as indicated by the elastic modulus.

It is shown that elastic moduli of the cationic gels, PS-VP gel as gel F-J from 2-5 mol%-VP (**Figure 3-5f-j**) and PS-VBAC gel as gel K and M between 0.5-1 mol%-VBAC (**Figure 3-5k** and **3-5l**). The addition of 2% VP improved the elastic modulus from 680 Pa to 2600 Pa, when gel A and gel F were compared (**Figure 3-4a** and **3-5f**). While the amount of VP in gel increased from 2 to 4 mol% (**Table 3-1**, entries 6-9), the G' value gradually increased from 2,600 Pa to 21 kPa (**Figure 3-5f—3-5i**). However increasing ratio of VP until 5 mol% (**Table 3-1**, entry 10), the elastic modulus dropped from gel I

which is 21 kPa to 14 kPa (**Figure 3-5j**). These results suggest that there is an optimized value for the strongest composition due to the complicated factors, such as electrostatic repulsion and the composition of monomers. Correspondingly, introducing a higher ratio of VBAC resulted in a lower elastic modulus in PS-VBAC gel (**Table 3-1**, entries 11 and 12), resulting from stronger repulsive forces of positively charged ammonium making the gel softer (**Figure 3-5k** and **3-5l**). After the evaluation of mechanical strength, the influence of the cationic moieties in the gel-drug interaction were evaluated.

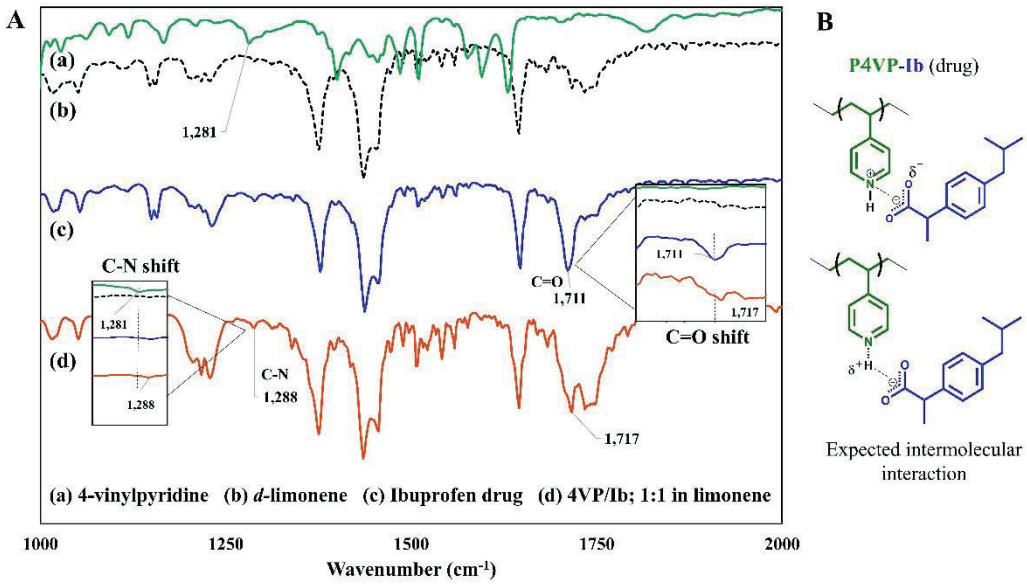


Figure 3-6. (A) FT-IR spectrum of 4-vinylpyridine (a-green), *d*-limonene (bdotted black), Ibuprofen (c-blue), and 4-vinylpyridine: ibuprofen, 1:1 in limonene (d-orange) and (B) the expected intermolecular interaction between 4-vinylpyridine and ibuprofen.

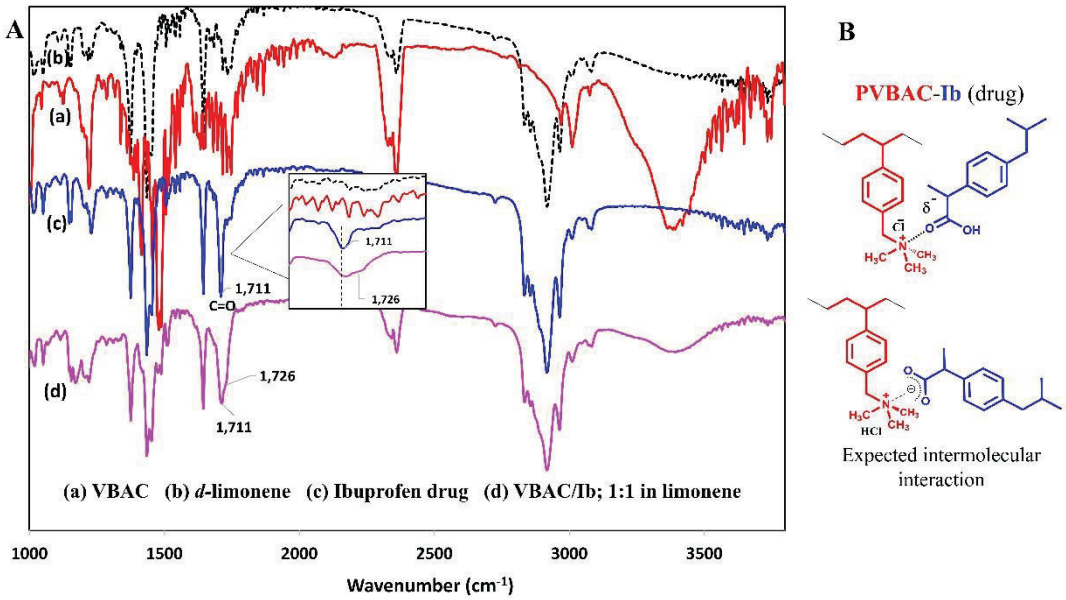


Figure 3-7. (A) FT-IR spectrum of (vinylbenzyl) trimethylammonium chloride; VBAC (a-red), *d*-limonene (b-dotted black), Ibuprofen (c-blue), and VBAC: ibuprofen, 1:1 in limonene (d-violet) and (B) the expected intermolecular interaction between VBAC and ibuprofen.

The FT-IR spectrum of cationic monomer and drug in *d*-limonene were evaluated to confirm the interaction between VP and ibuprofen (drug) in **Figure 3-6** and VBAC with drug in **Figure 3-7**. The interaction could perform by electrostatic interaction and H-bond. The VP contained C-N aromatic bond appeared at 1,281 cm^{-1} while ibuprofen has C=O from carboxylic group at 1,711 cm^{-1} in **Figure 3-6A**. The carbonyl peak shifted from 1,711 cm^{-1} to 1,717 cm^{-1} and C-N peak shifted 1,281 cm^{-1} to 1,288 cm^{-1} (in set **Figure 3-6A**). These could be confirm the interaction between VP and ibuprofen as expected in **Figure 3-6B**. However, the introducing moiety of interaction unit are trifling. It was difficult to find the peak shift from the gel formulation. Likewise, the FT-

IR of mixture VBAC and ibuprofen in *d*-limonene (**Figure 3-7**) shown the carbonyl significant shifted from 1,711 cm^{-1} to 1,726 cm^{-1} (in set **Figure 3-7A**).

3.3.3 Effect of D-limonene as Chemical Enhancer and Density of The Network Structure on Permeability

In order to investigate the drug permeation as a controlled release by the cross-linked PS gel swollen in limonene, ibuprofen was selected as a model drug (**Figure 3-8**). The drug was released through the rat skin into the receiver solution of pH 7.4 PBS at 37 °C using a Franz diffusion cell as shown in **Figure 3-8c**.

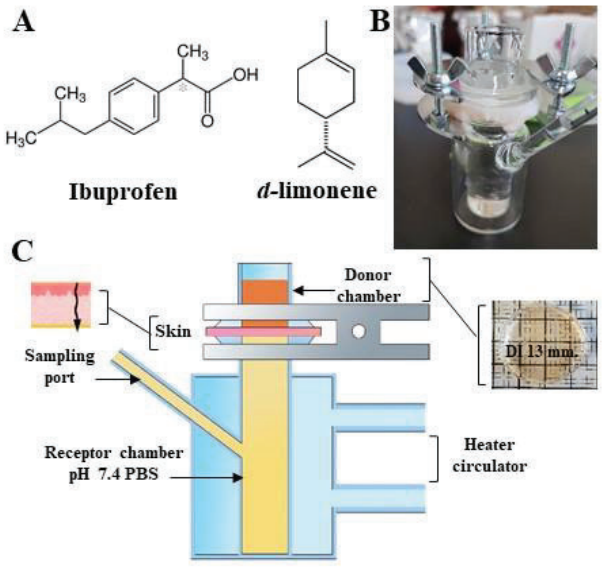


Figure 3-8. (a) Chemical structure of Ibuprofen as model drug and *d*-limonene, photograph of in vitro permeation experiment by using Franz diffusion cell (b) and illustration of permeation study (c).

All PS gels swollen in limonene formulations show high potential releasing properties through the skin because limonene can enhance the permeation of the drug through the epidermis by increasing the activity of drug with SC or decrease the tortuous pathway in SC or both.[14] Nevertheless lack of solvent in PS-*co*-DVB would not be able to permeate drug in transdermal system. These exhibit the advantage of chemical organogels swollen in *d*-limonene could perform prolong release with simple preparation of cross-linked polystyrene. Permeability of drug from the PS gels swollen in limonene (**Table 3-1**, entry 1) was compared to the gel swollen in toluene (**Table 3-1**, entry 5) with the similar crosslinking condition (**Figure 3-9a and 3-9e**).

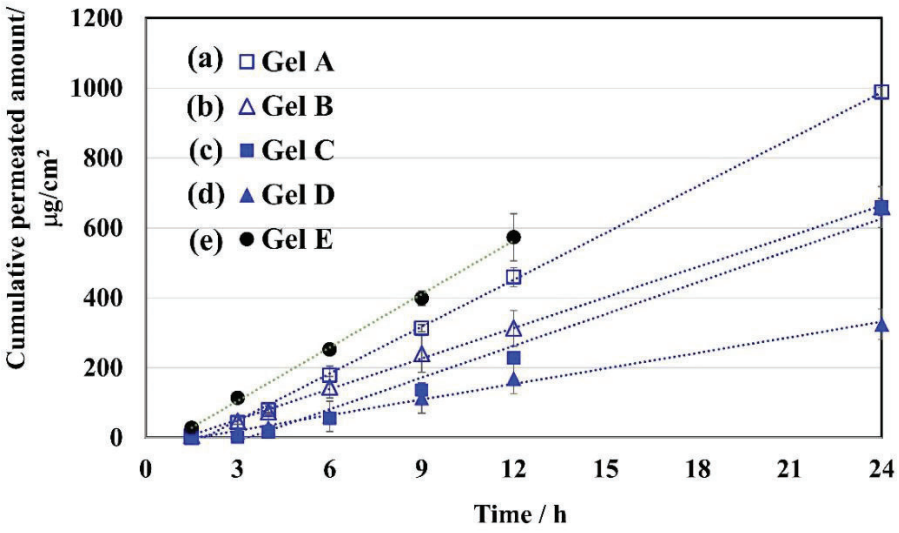


Figure 3-9. In vitro cumulative permeated amount of ibuprofen from PS gel; Gel A (a), Gel B (b), Gel C (c), Gel D (d) and Gel E (e) (n=3).

Table 3-2. The skin permeation parameters of PS gel (gel A-D) and toluene gel (gel E)

Entry	Gel	Flux, J_{ss} ($\mu g/cm^2 \cdot h$)	lag time (h)	D/L^2 (h^{-1})	KD/L ($*10^{-4}$ cm/h)
1	Gel A	43.7±0.3	1.72±0.16	0.10±1.03	53.1±0.03
2	Gel B	29.4±2.5	1.31±0.29	0.13±0.58	35.8±0.30
3	Gel C	29.9±1.0	3.15±0.16	0.05±1.06	36.4±0.12
4	Gel D	14.7±1.6	1.18±0.36	0.14±0.46	17.9±0.20
5	Gel E	50.9±5.5	0.91±0.16	0.18±1.06	61.9±0.67

Toluene can be also defined as chemical enhancer for skin penetration.[31] However, it is extremely toxic and irritating to the skin, showing the merit of the present gels in limonene with the similar enhancer effect. The PS gels swollen in limonene A-D could prolong controlled release depending on the gel preparation conditions (**Table 3-1**, entries 1-4). Additionally, limonene has a low skin irritancy and it allows a reversible change in the skin structure when administered with a pretreatment method.[17] The permeability coefficient (KD/L) indicates the effect of the enhancer on the diffusion coefficient in SC, whereas the lag time decreases as the diffusion path length decreases. They are determined from **Figure 3-9** and listed in **Table 3-2**. During the lag time period, SC would be conditioned for higher permeability and permeation reaches steady state after the lag time. Therefore, the decrease in lag time could also be due to fast SC conditioning times.[8] Gel A showed higher flux and the permeation coefficient as 53.1±0.03 $x10^{-4}$ cm/h (**Table 3-2**, entry 1) than that of gel B as 35.8±0.30 $x10^{-4}$ cm/h (**Table 3-2**, entry 2), influenced of the difference of the crosslinking ratios with 5%

(**Table 2-1**, entry 1) and 10 % (**Table 3-1**, entry 2), respectively. The same tendency was observed between gel C as 36.4±0.12 x10^{-4} cm/h (**Table 3-2**, entry 3) and gel D as 17.9±0.20 x10^{-4} cm/h (**Table 3-2**, entry 4) which were prepared both under 8 M with 5% (**Table 3-1**, entry 3) and 10% cross-linked (**Table 3-1**, entry 4), respectively. The concentration of gel preparation condition were also recognized compared with gel A as 53.1±0.03 x10^{-4} cm/h (**Table 3-2**, entry 1) and gel C as 36.4±0.12 x10^{-4} cm/h (**Table 3-2**, entry 3), which prepared with the same 5% crosslinking ratio (**Table 3-1**, entries 1 and 3). It is probably due to the cross-linked network structure of PS gel on activity through gel network. The mobility of drug solute in the skin is represented as D/L^2 diffusion parameter. It raised when increase the cross-linked degree, gel A as 0.10±1.03 h^{-1} (**Table 3-1**, entry 1) and gel B 0.13±0.58 h^{-1} (**Table 3-1**, entry 2) for 4M and gel C as 0.05±1.06 h^{-1} (**Table 3-1**, entry 3) and gel D 0.14±0.46 h^{-1} (**Table 3-1**, entry 4) for 8M.

Table 3-3. Storage modulus and permeation parameter of PS gel

Entry	Gel	Cross linker (mol%)	Conc.	Elastic modulus (G')	Flux, J_{ss} ($\mu g/cm^2 \cdot h$)
1	A	5	4	680	43.7±0.25
2	B	10	4	6,700	29.4±2.47
3	C	5	8	44,200	29.9±1.02
4	D	10	8	98,000	14.7±1.61

In order to clarify the relationship of permeability and gel network structure, the cross-linker ratio were plotted against to the steady flux of ibuprofen permeation

through the skin (J_{ss}) and elastic modulus (G') as shown in **Table 3-3**. The steady flux decreased when the cross-linking degree and moduli increased for both concentration (4 and 8 M).

3.3.4 Effect of the Cationic Moiety on the Controlled Release of Ibuprofen for Permeation through the Skin

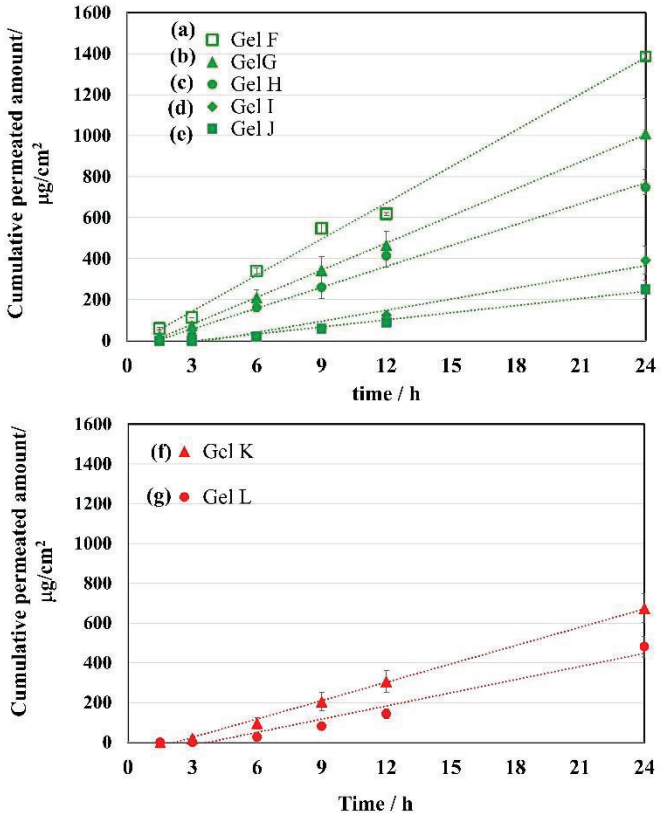


Figure 3-10. In vitro cumulative permeated amount of ibuprofen from PS-VP gel by various feeding ratio of VP from 2-5 mol%; Gel F (a), Gel G (b), Gel H (c), Gel I (d), Gel J (e), and PS-VBAC gel by various feeding ratio of VBAC 0.5 and 1 mol%; Gel K (f) and Gel L (g) (n=3).

Since the sustainable controlled release is one of the concerning factors in drug delivery systems, it is important to fabricate controllable materials for TDD by organogels. The VP and VBAC were incorporated in order to control the permeation of drug through the skin by interacting with drug molecules. **Figure 3-10** showed the plot of the released amount of the drugs against time, and then the permeability (KD/L), diffusion parameter (D/L^2) and lag time were calculated as shown in Table 3. Upon the gradual increased introduction of VP with 2 to 5 %, the permeability of PS-VP gels was reduced significantly from 67.1±0.10 to 14.1±0.25 cm/h (**Table 3-4**, entries 1-5). Moreover, the lag time tended to increase together with the cationic interaction moiety while diffusion parameter (D/L^2) trend to decrease from 0.32±1.14 h^{-1} to 0.04±0.93 (**Table 3-4**, entries 1-4). The increase in lagging time and the decrease in permeability and diffusion was attributed to the higher interaction of the cationic moiety

Table 3-4. The skin permeation parameters of ibuprofen from PS-VP gel (gel F-J) and PS-VBAC (gel K and L)

Entry	Gel	Cationic moiety	Mol%	Flux, J_{ss} (μg/cm^2·h)	lag time (h)	D/L^2 (h^{-1})	KD/L (*10^{-4} cm/h)
1	Gel F	VP	2	55.2±0.80	0.51±0.15	0.32±1.14	67.1±0.10
2	Gel G	VP	2.5	44.2±7.22	1.22±0.37	0.14±0.45	53.7±0.88
3	Gel H	VP	3	34.4±1.71	1.67±0.48	0.10±0.35	41.8±0.21
4	Gel I	VP	4	18.1±3.13	3.77±0.18	0.04±0.93	22.0±0.38
5	Gel J	VP	5	11.6±2.03	3.27±0.08	0.05±2.00	14.1±0.25
6	Gel K	VBAC	0.5	30.8±3.14	2.22±0.42	0.07±0.40	37.4±0.38
7	Gel L	VBAC	1	22.1±2.42	3.02±0.90	0.06±0.18	26.9±0.29

To emphasize the effect of interaction between drug and gel network structure, a stronger cationic moiety as ammonium salt was introduced as interaction unit in polymer chain in PS gel producing PS-VBAC gel. These interaction force of cationic moiety and ibuprofen (drug) was investigated by FT-IR in **Figure 3-6** and **3-7**. The VBAC was incorporated only 0.5 and 1 mol% due to poor solubility in *d*-limonene. However, it is not strong effect to lagging time and diffusion parameter, it was shown insignificantly difference of D/L^2; 0.07±0.40 h^{-1} and 0.06±0.18 h^{-1}, respectively (**Table 3-4**, entries 6 and 7). Resulting in the small amount of cationic moiety is not effect to the mechanism of permeation through the *stratum corneum*. The effect of cationic moiety was still stronger than in PS-VP gel. Gel K and L was shown the permeability with 37.4±0.38 and 26.9±0.29 cm/h, respectively (**Table 3-4**, entries 6 and 7), while Gel F, G and H showed 67.1±0.10, 53.7±0.88 and 41.8±0.21 cm/h (**Table 3-4**, entries 1-3). It was indicated that the PS-VBAC gels included smaller amount of cationic moiety (0.5-1 mol%-VBAC) could possess higher efficiency on their prolonged release of ibuprofen than that of PS-VP gels.

3.4 Conclusion

In conclusion, the limonene oil gels were successfully fabricated from cross-linked PS and its derivatives, which included VP andVBAC. They were prepared for TDDS by using the advantages of *d*-limonene as an effective chemical permeation enhancer and a low-toxic organic solvent. The stable chemical network of the PS gels swollen in limonene illustrated the highest elastic modulus at 98 kPa. Moreover, the efficiency of the permeability of ibuprofen was successfully enhanced by *d*-limonene and the controllable of the network density indicated by relative permeability coefficient from 53.1±0.03 to 17.9±0.20 x10 cm/h. Cationic moieties were introduced to control the drug releasing behavior of the gel with the slowest steady flux at 14.1±0.25 cm/h of 5 mol%-VP of PS-VP gel because of drug-polymer chain interaction. However, upon increasing the cationic moiety repulsive effects appeared. Overall, we achieved a steady controlled release of ibuprofen from modified PS gels swollen in limonene.

3.5 References

1) M. R. Prausnitz, S. Mitragotri, R. Langer, *Nat Rev Drug Discov.* **2004**, *3*, 115.

2) T. Tanner, R. Marks, *Skin Res Technol.* **2008**, *14*, 249.

3) G. Cevc, U. Vierl, *J Control Release* **2010**, *141*, 277.

4) Y. Yang, S. Sunoqrot, C. Stowell, J. Ji, C. W. Lee, J. W. Kim, S. A. Khan, S. Hong, *Biomacromolecules* **2012**, *13*, 2154.

5) H. Lee, C. Songa, S. Baika, D. Kima, T. Hyeona, D. H. Kima, *Adv. Drug Deli. Rev.* **2017**, *127*, 1.

6) P. Karande, A. Jain, K. Ergun, V. Kispersky, S. Mitragotri, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 4688.

7) T. Aoyagi, O. Terashima, N. Suzuki, K. Matsui Y. Nagase, *J Control Release* **1990**, *13*, 63.

8) H. K. Vaddi, P. C. Ho, Y. W. Chan, S. Y. Chan, *J Control Release* **2002**, *81*, 121.

9) A. C. Williams, B. W. Barry, *Pharm. Res.* **1991**, *8*, 17.

10) J. Chen, Q. D. Jiang, Y. P. Chai, H. Zhang, P. Peng, X. X. Yang, *Molecule* **2016**, *12*, 1709.

11) D. Monti, P. Chetoni, S. Burgalassi, M. Najarro, M. F. Saettone, E. Boldrini, *skin. Int. J. Pharm.* **2002**, *237*, 209.

12) Z. Yang, Y. Teng, H. Wang, H. Hou, *Int. J. Pharm.* **2013**, *447*, 231.

13) K. Inoue, J. Okada, K. Sugibayashi, *J Drug Deliv. Sci. Technol.* **2008**, *18*, 335.

14) M. Almirall, J. Montaña, E. Escribano, R. Obach, J. D. Berrozpe, *Drug Res.* **1996**, *46*, 676.

15) K. Saroha1, S. Singh, A. Aggarwal, S. Nanda, *IJPCBS* **2003**, *3*, 495.

16) C. L. Espositoa, P. Kirilovb, V. G. Roullina, *J Control Release* **2018**, *271*, 1.

17) P. F. Lim, X. Y. Liu, L. Kang, P. C. Ho, Y. W. Chan, S. Y. Chan, *Int. J Pharm.* **2006**, *311*, 157.

18) H. Liandong, H. Qiaofeng, Y. J. Yang, *Iran J Basic Med. Sci.* **2014**, *17*, 760.

19) P. Charoensumran, H. Ajiro, *Polym. J.* **2018**, *50*, 1021.

20) W. C. Monte, D. Landau-West, *J. Food Sci.* **1982**, *47*, 1832.

21) S. Chandra, H. Mangalara, S. Varughese, *ACS Sustainable Chem.* **2016**, *4*, 6095.

22) D. M. Monti, D. Guarnieri, G. Napolitano, R. Piccoli, P. Nettic, S. Fuscoc, A. Arciello, *J. Biotechnol.* **2015**, *193*, 3.

23) M. P. Tsyurupa, M. M. Ilyin, A. I. Andreeva, V. A. Davankov, *J. Anal. Chem.* **1995**, *352*, 672.

24) H. Zhu, S. Qiu, W. Jiang, D. Wu, C. Zhang, *Environ. Sci. Technol.* **2011**, *45*, 4527.

25) X. Wang, H. Yan, *Mater. Sci. Eng.* C **2017**, *78*, 598.

26) M. Rajabi, N. Roxhed, R. Z. Shafagh, T. Haraldson, A. C. Fischer, W. Wijngaart, G. Stemme, F. Niklaus, *PLOS ONE* **2016**.

27) S. A. Coulman, A. Anstey, C. Gateley, A. Morrissey, P. McLoughlin, C. Allendera, J. C. Birchall, *Int. J. Pharm.* **2009**, *366*, 190.

28) J. Crank, The Mathematics of diffusion. Oxford University Press, England **1975**.

29) H. K. Vaddi, L. Z. Wang, P. C. Ho, S. Y. Chan, *Int. J. Pharm.* **2001**, *212*, 247.

30) H. K. Vaddi, P. C. Ho, S. Y. Chan, *J. Pharm. Sci.* **2002**, *91*, 1639.

31) H. Tsuruta, *Industrial Health.* **1996**, *34*, 369.

Chapter 4

Controlled Release of Testosterone by Polymer-polymer Interaction Enriched Poly(Vinyl benzyl-*N*-methyl-*D*-glucamine) Gel in Propylene Glycol As a novel Transdermal Drug Delivery System.

4.1 Introduction

Transdermal drug delivery systems (TDDS) are designed to deliver drugs at a controlled rate through the skin and into the blood stream which has obvious advantages over other routes. The TDDS avoids first-pass metabolism by the liver and gastro-intestinal tract, which is a significant hindrance for oral administration.[1-3] The loaded drug can avoid degradation by enzymes and by pH-associated deactivation, thus achieving an efficient therapy.[4] TDDS is also painless in comparison to parenteral administration, hence improving patient acceptability and compliance.[5] Nevertheless, there are limitations of drug penetration because of the impermeable nature of the skin, especially the *stratum corneum*. For this reason, a number of technologies have been developed to enhance the ability of molecules to pass through the skin.[6-10] To advance an appropriate

vehicle for use as a non-invasive technique, it is necessary to design a new material by using polymer chemistry along with permitted CPEs.

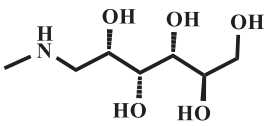
Generally, the gels are widely used as a beneficial vehicle for topical drug delivery likewise improving the drug permeability.[11-13] It is known that the organogels (oleogels) contain organic solvents, although hydrogels contain water, which can select the incorporating compounds. Gels are unique materials whose rigid and elastic properties can be controlled, and they have a broad range of application in cosmetics, food technologies, biomaterials and pharmaceutics.[14,15] Hydrogels consisting of an aqueous medium such as chitosan hydrogel[16,17] and some sugar-derived molecular gels[18] have been widely investigated for topical delivery systems.[19-21] However, they are limited and problematic for transdermal drug delivery, because of their hydrophilic nature. Organogels can effectively enhance drug penetration through the skin barrier such as the SC because of their hydrophobic nature and favorable adsorption.[22,23] They are also miscible with many CPEs compounds.[24,25] Thus, organogels are promising vehicles for cutaneous administration.[26,27] For the past decade, Lecithin and Pluronic Lecithin Organogel (PLO) gel have been intensively studied for the formulation of controlled release which shows low skin irritation and increases drug permeation.[28,29] It has been largely used as a biocompatible penetration enhancer instead of conventional ones.

However, it still lacks thermal stability and has limited permeability. So it would be worthwhile to study organogels for their potential in a range of applications.[30]

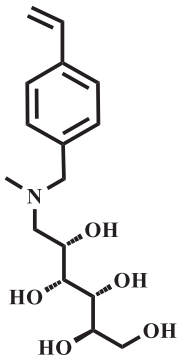
In the previous works, it has been reported an oilgel with polylactides as a chemical organogel for controlled release of testosterone.[31] Testosterone is a male hormone that is used to treat male hypogonadism — a condition in which the body does not produce enough testosterone. Transdermal testosterone gels are available and popular among both patients and clinicians.[32,33] It is necessary to further develop the controlled release by the transdermal testosterone system.

In chapter 3, I have reported on cross-linked polystyrene in *d*-limonene as a chemical gel in order to solve the conventional problems, in particular the low mechanical strengths of physical gel, as well as polylactide oilgel. Actually, the polystyrene swollen in limonene gel showed the high mechanical property and drug permeability. [34,35] Other problems such as the skin irritation caused by using the highly concentrated d-limonene still remained however and it lacked sufficient flexibility due to the chemical cross-linking. I therefore started to consider other approaches, focusing on the strong and various polymer-polymer interactions to form physical gels.

In this study, N-methyl-D-glucamine (NMDG) is used to modify a PS core structure to increase the hydrophilicity of an organogels system and fabricate the physical cross-links by chain entanglement and various interactions. The NMDG group is well known for arsenic sorption and selective boron removal from an aqueous solution.[36,37] For the organic solvent, we used propylene glycol (PG) since this is the most commonly used glycol in dermal and transdermal formulations[38] and is usually used to improve drug permeability. The limonene/PG was evaluated as a solvent system for the synergistic effect. Finally, in order to study the effect of carbon-chain length from cationic surfactant polymers,[39] benzalkonium chloride type,[40] was selected to improve permeability by varying carbon side chains (C1, C4, C8 and C12). Using these new components with various polymer-polymer interactions, we developed a self-standing gel network for a transdermal drug delivery system, which exhibits simple gel formation without chemical cross-linking, especially for the controlled release of testosterone.



NMDG



VbNMDG

4.2 Experimental section

4.2.1 Materials

4-vinylbenzylchloride (90.0%) was purchased from Sigma Aldrich. Azo-Bis(isobutyro)nitrile (AIBN) (98.0%), PBS (x10) were purchased from Wako Pure Chemical Industry Ltd. Japan. Testosterone (98.0%) and *N*-methyl- *D*-glucamine (>99.0%) *N,N*-dimethyl-1-butylamine (>98.0%), *N,N*-dimethyl-1-1ctylamine (>97.0%) and *N,N*-dimethyl-1-dodecylamine (>96.0%) were all purchased from Tokyo Chemical Industry Co., Ltd. Japan (TCI). *D*-Limonene (90.0%), tetrahydrofuran (98.0%) dimethyl sulfoxide (99.0%) and propylene glycol (>99.0%) were supplied from Nacalai Tesque Inc. Japan. Vinylbenzyl trimethylammonium chloride (VBAC) was purchased from Santa Cruz Biotechnology. All chemicals were used as supplied without further purification, except where noted otherwise.

4.2.2 Synthesis of 4-Vinylbenzyl-*N*-methyl-*D*-glucamine; VbNMDG

The *N*-methyl- *D*-glucamine (2 g, 10.2 mmol) was dissolved with pyridine (0.5 M) in round bottom flask. The 4-vinylbenzylchloride (1.74 mL, 12.29 mmol) then was added dropwise. The mixture solution was stirred at 30 °C to protect the polymerization for 24 h. The pyridine was removed by co-evaporate with water under vacuum. The

residue was washed with toluene and diethyl ether twice to remove remained starting compound. The solvent was removed by evaporate again under vacuum.

Then the monomer was recrystallized in water. ^{1}H NMR (DMSO-*d6*, 400 MHz) δ_H 7.442-7.28 (4H, dd, H_{Ar}), 6.75-6.67 (1H, dd, H_a; CH_a=CH_2), 5.82 (1H, d, H_c; CH=C(H_c)H), 524 (1H, d, H_b; CH=C(H_b)H), 4.61 (1H, d, OH), 4.52 (2H, dd, OH*2), 4.33 (2H, m, OH*2), 3.77 (1H, t, H_i), 3.67 (1H, t, H_j,), 3.59 (1H, m, H), 3.54-3.40 (5H, m, $H_{e,k,l}$), 2.56-2.37 (2H, m, H_g), 2.10 (3H, s, CH_3), HRMS (ESI) m/z: [M + H]+ calculation 312.1733 found [M+H]; 312.16917

4.2.3 Synthesis of *N*-alkyl-*N,N*-dimethyl-4-Vinylbezenaminium (C4, C8 and C12)

The *N,N*-dimethyl-1-alkylamine (*N,N*-dimethyl-1-butylamine; C4, *N,N*-dimethyl-1-1ctylamine; C8 and *N,N*-dimethyl-1-dodecylamine; C12) was mixed directly with 4-vinylbenzyl chloride. The mixture was stirred at 30 ˚C overnight. The product was recrystallized and yielded as shown in **Table 4-1**. Detail of ^{1}H NMR were shown in **Figure 4-1**.

Table 4-1. Synthesis of N-alkyl-N,N-dimethyl-4- Vinyl bezenaminium monomers

Entry	Tertiary amine	Solvent for recrystallization	Yield (%)
1	N,N-dimethyl-1-butylamine (C4)	Acetone	80%
2	N,N-dimethyl-1-octylamine (C8)	Hexane	98%
3	N,N-dimethyl-1-dodecylamine (C12)	Acetone	95%

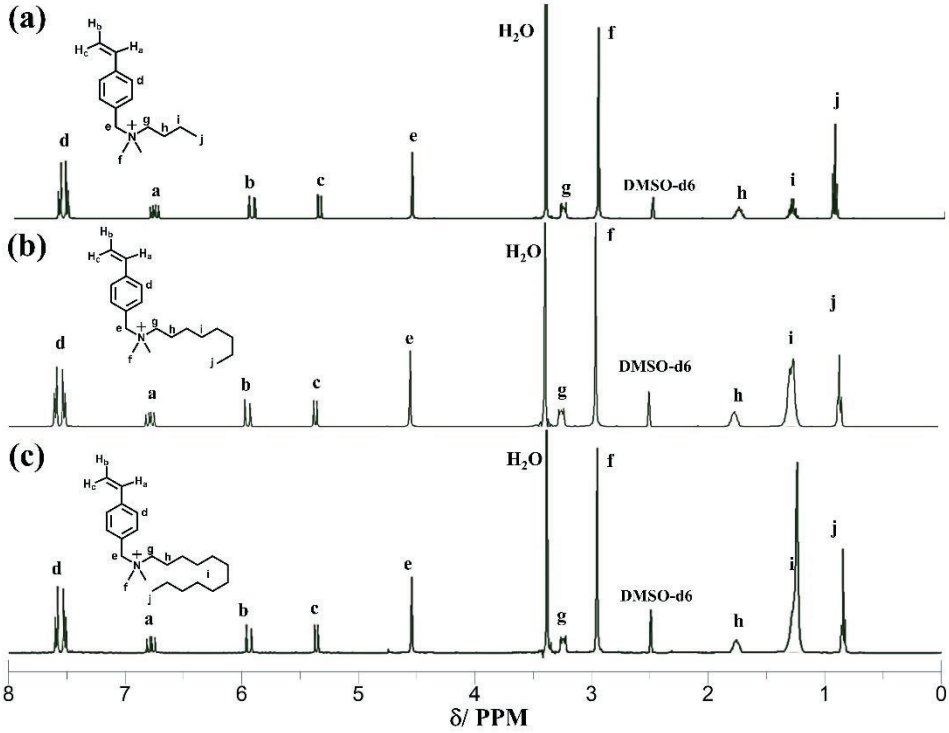


Figure 4-1. [1]HNMR (400 MHz, DMSO-*d6*) of (a) N-butyl-N,N-dimethyl-4-Vinylbezenaminium; C_4, (b) N-butyl-N,N-dimethyl-4-Vinylbezenaminium; C_8 and (c) N-butyl-N,N-dimethyl-4-Vinylbezenaminium; C_{12}

4.2.4 Gel preparation

The gels were prepared by dissolving the crude product of monomer (shown NMR in **Figure 4-3d**) in solvent (water or PG, 1M) and 2,2-azobis(2-methylpropionamidine) dihydrochloride: V-50 (1%) as initiator. After that, the solution

mixture was bubbled with N_2 gas for 5 min. and vacuum to remove the bubble. The gel was set in sandwich template, and polymerized at 60 °C in desiccator for 18 h shown the detail in **Table 4-2**. The drug (Testosterone) was added in monomer solution with 5 mg/mL for the transdermal permeation experiment.

Table 4-2. Synthesis of poly(VbNMDG) gel

Entry	Sample	Formulation	Solvent [a)]
1	poly(VbNMDG)-Aq	poly(VbNMDG)	H_2O
2	poly(VbNMDG)-PG	poly(VbNMDG)	PG
3	poly(VbNMDG)-2.5Lim-PG	poly(VbNMDG)	2.5%limonene/PG
4	poly(VbNMDG)-5Lim-PG	poly(VbNMDG)	5%limonene/PG
5	poly(VbNMDG)-10Lim-PG	poly(VbNMDG)	10%limonene/PG

a) the concentration of monomer are 1M

To study the effect of carbon side chain, the gels were prepared by co-polymerization with *N*-alkyl (C1, C4, C8 and C12)-*N,N*-dimethyl-4-vinylbenzenammi nium for 5 Mol% in PG solution. The gel was synthesized similarly to poly(VbNMDG) gel shown the detail in **Table 4-3**.

Table 4-3. Synthesis of p(VbNMDG-*co*-C_nDMVB)

Entry	Sample	Formulation [a)]	Co-monomer
1	poly(VbNMDG)-PG-C1	p(VbNMDG-C_1DMVB)	N,N,N-trimethyl-4-vinylbenzenamminium
2	poly(VbNMDG)-PG-C4	p(VbNMDG-C_4DMVB)	N-butyl-N,N-dimethyl-4-vinylbenzenamminium
3	poly(VbNMDG)-PG-C8	p(VbNMDG-C_8DMVB)	N-octyl-N,N-dimethyl-4-vinylbenzenamminium
4	poly(VbNMDG)-PG-C12	p(VbNMDG-C_{12}DMVB)	N-dodecyl-N,N-dimethyl-4-vinylbenzenamminium

a) The gel was all prepared in PG solution

The gels were removed from the sandwich template and cut into 13 mm-diameter, 2 mm-thick discs (V = 0.265 cm^3) for permeation test with a Franz diffusion cell. The drug concentration per disc was calculated from the total prepared gel containing 5 mg/cm^3 of testosterone, thus, each discs sample contains 1.325 mg of drug.

Responsive property: The swelling ratio was calculated for 3 different conditions; 1) normal aqueous solution, 2) Alkali solution of NaOH at pH 12, and 3) 0.5 M urea solution. The hydrogel was swollen in each condition for 24 h. After that they was dried by freeze dry under vacuum. The swelling ratio was calculated by the equation (1);

Swelling ratio $$(Q) = \frac{(W_s - W_d)}{W_d} \qquad (1)$$

Where W_s stands for the weight of the swollen gel in each condition and W_d for the weight of the dried gel after freeze-dried.

4.2.5 FTIR characterization

The monomer solution and prepared gel were studied the functional group by FTIR measurement. IR Affinity-1S (Shimadzu, Japan) was used to record FT-IR spectra in a wavelength range between 400 and 4000 cm^{-1}. All spectra were measured with a resolution of 4 cm^{-1} at room temperature.

4.2.6 Rheological study

The rheological properties of the hydrogels or PG gels were measured using a Rheometer (KNS2100, Kinexus, Japan). The gel formation was monitored by concentric cylinder rheological measurement. The monomer solution (1M) was added in cylinder and heat up to 70 °C for polymerization during the measurement at constant frequency 0.1 Hz and 1% stain shown in **Figure 4-5**. Furthermore, to study the effect of water content, the gel was varied the solvent from pure PG to 10, 50, 90% of water/PG and pure water (100%). Then they were polymerized *in situ* to evaluate the different of gel formation by solvent effect shown in **Figure 4-7**. Plus, in order to study the rheology of synthesized gel, the gels were cut in discs with 20 mm diameter and ~2 mm thickness and placed on plate type and the upper circle plate (20 mm diameter) is connect to the measuring system. The elastic modulus (G') and viscous modulus (G") of the organogels

swollen with limonene were measured at controlled frequency from 0.1 to 10 Hz at 25 ºC.

4.2.7 *In vitro* skin permeation study

The abdominal rat skin was used as membrane referred preparation by experimental section 3.2.7. *In vitro* skin permeation study was carried out by using Franz-cell diffusion followed by experimental part of 3.2.8. The testosterone then was determined by HPLC analysis.

4.2.8 HPLC measurements for drug assay

Testosterone concentration was determined with a reverse phase high performance liquid chromatography (HPLC) from Shimadzu, Japan. HPLC system using Cosmosil Packed Column $5C_{18}$-MSII column (4.6 mm × 150 mm, 5 μm). The detection condition was 60% Methanol in water as mobile phase with UV detector at 244 nm, 40 °C. The flow rate was set as 0.2 mL/min and 50 μL of sample was loaded. Under these condition the retention time of testosterone was 37.72 min. A calibration curve was constructed by using testosterone standard solution in PBS solution from 5-33.33 μg/mL (R^2 = 0.9988) shown in **Figure 4-2**.

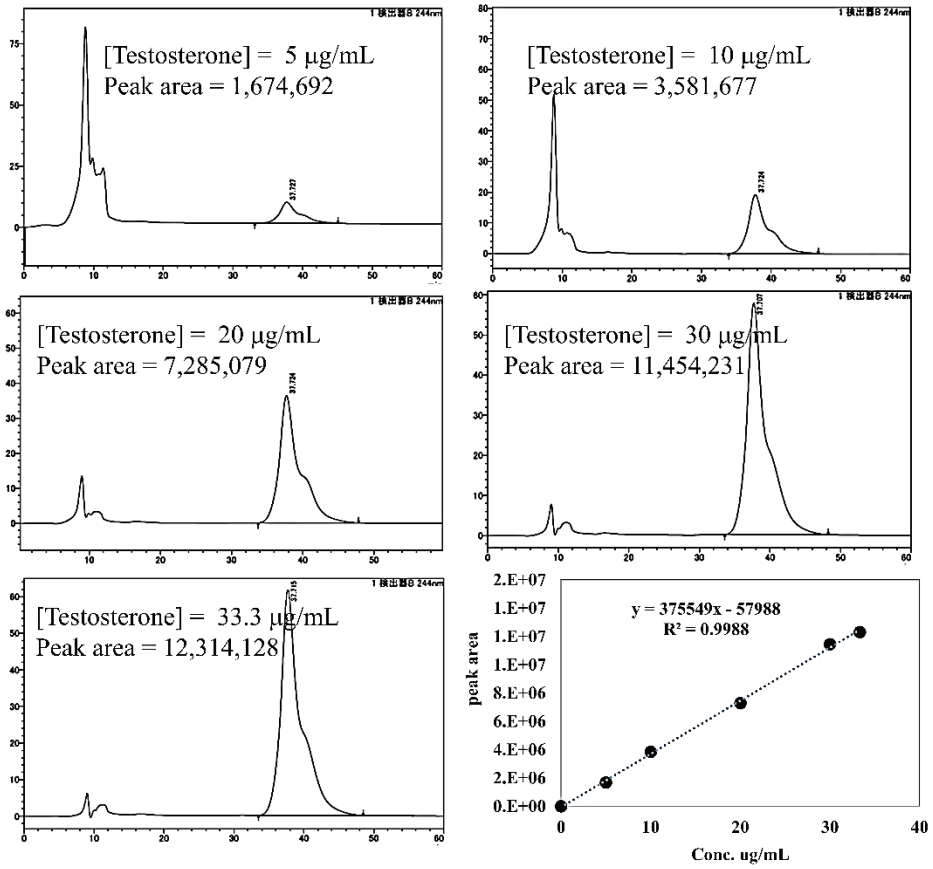


Figure 4-2. Standard calibration curve between peak area and concentration with inset HPLC chromatogram of testosterone in PBS solution from 5 µg/mL to 33.3 µg/mL.

4.2.9 Calculation of permeation parameter

The cumulative amount of drug (Q) permeating through the skin from the donor chamber at constant concentration (C_0) to the receptor phase at the sink condition can be described by Fick's 2nd law of diffusion, Eq. (1)[41] where A is the surface area, L is the thickness of the skin and K is the diffusion coefficient of the skin. Permeation parameters are interpreted from cumulative permeated drug per unit skin area Q/A versus time t plot. The steady-state flux (J_{ss}) and lag time t_L were obtained from slope and x-interception

value of the linear portion. The flux J_{ss} over drug concentration C_0 in the donor solution gives permeability coefficient KD/L.[42]

$$Q = AKLC_0\left[\frac{D}{L^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\alpha}\frac{(-1)^n}{n^2}e^{-(\frac{D}{L^2})n^2\pi^2 t}\right] \quad (2)$$

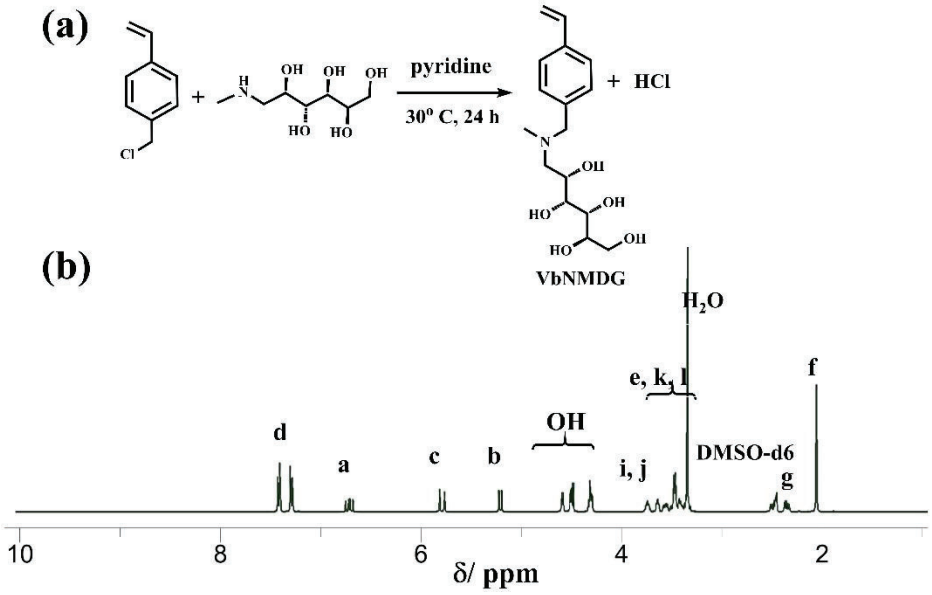
$$\frac{KD}{L} = \frac{J_{ss}}{C_0} \quad (3)$$

Flux enhancement ratio (E_r) was calculated by dividing the J_{ss} of the respective formulation (synergistic effect or carbon-chain length) by J_{ss} of the control formulation (PG as solvent) using the equation (4).

$$E_r = \frac{J_{ss}\ (variation\ formulation)}{J_{ss}\ (control\ formulation)} \quad (4)$$

4.3 Results and discussion

4.3.1 Preparation of poly(VbNMDG) gel



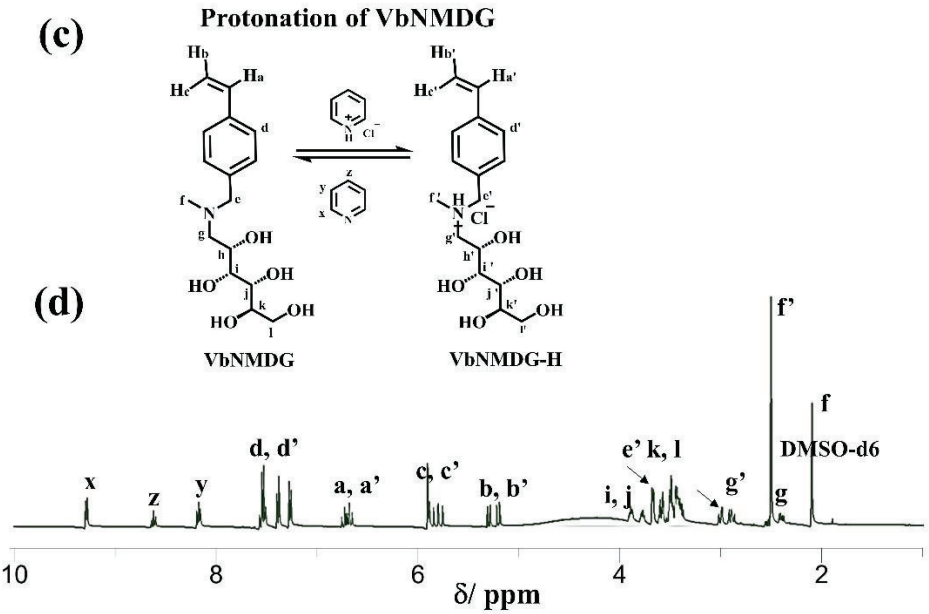


Figure 4-3. (a) Synthetic scheme of vinyl benzyl-*N*-methyl-*D*-glucamine (VbNMDG), (b) ^{1}H NMR (400 MHz, DMSO-*d6*) of recrystallized VbNMDG, (c) Protonation of VbNMDG, (d) ^{1}H NMR (400 MHz, DMSO-*d6*) of protonation of VbNMDG incorporated with pyridine indicated by VbNMDG and VbNMDG-H.

To develop the organogels for a TDDS, we fabricated the novel entangled chain matrix organogels which were composed of several polymer-polymer interactions. The network polymers are clearly formed by hydrogen bond and electrostatic interaction between linear polymer chain of poly(vinylbenzyl-*N*-methyl-D-glucamine); poly(VbNMDG) with organic base as a promotor. To prepare the gel, the NMDG based monomer was synthesized by the reaction of 4-vinyl benzyl chloride and NMDG with pyridine (organic base) as a catalyst and solvent to keep the temperature low thus protecting polymerization as shown in **Figure 4-3a**. The ^{1}H NMR of recrystallized VbNMDG monomer was clearly assigned (**Figure 4-3b)**. It was observed that the

VbMNDG-monomer was protonated by base (pyridine) to provide VbMNDG-H (protonated form) as shown the ^{1}H NMR in **Figure 4-3d**. The spectrum from H_a to H_d which was the styrene core structure was paternally splitting at 7.26-7.51 ppm. The adjacent methylene of quaternary amine (VbNMDG-H); H_g was obviously shifted to the low field from 2.42 to 2.98 ppm (arrow) and the H_e was shifted from 3.59 to about 3.66 ppm (arrow). Also, the methyl group (H_f) on *N*-atom clearly shifted with two singlet peaks at 2.10 and 2.50 ppm (overlapped with DMSO-d_6). It was confirmed that there are VbNMDG and VbNMDG-H in the mixture. From these results, we assumed that the organic base compound plays the role for proton exchange and interacting molecules. This system was then used as a polymeric gelator model, due to the mixture of the neutral form and cationic form properly establishing the network structure after subsequent polymerization. We therefore believe that the gel could be formed by polymer-polymer interaction of physical cross-links including the polymer entanglement.

.

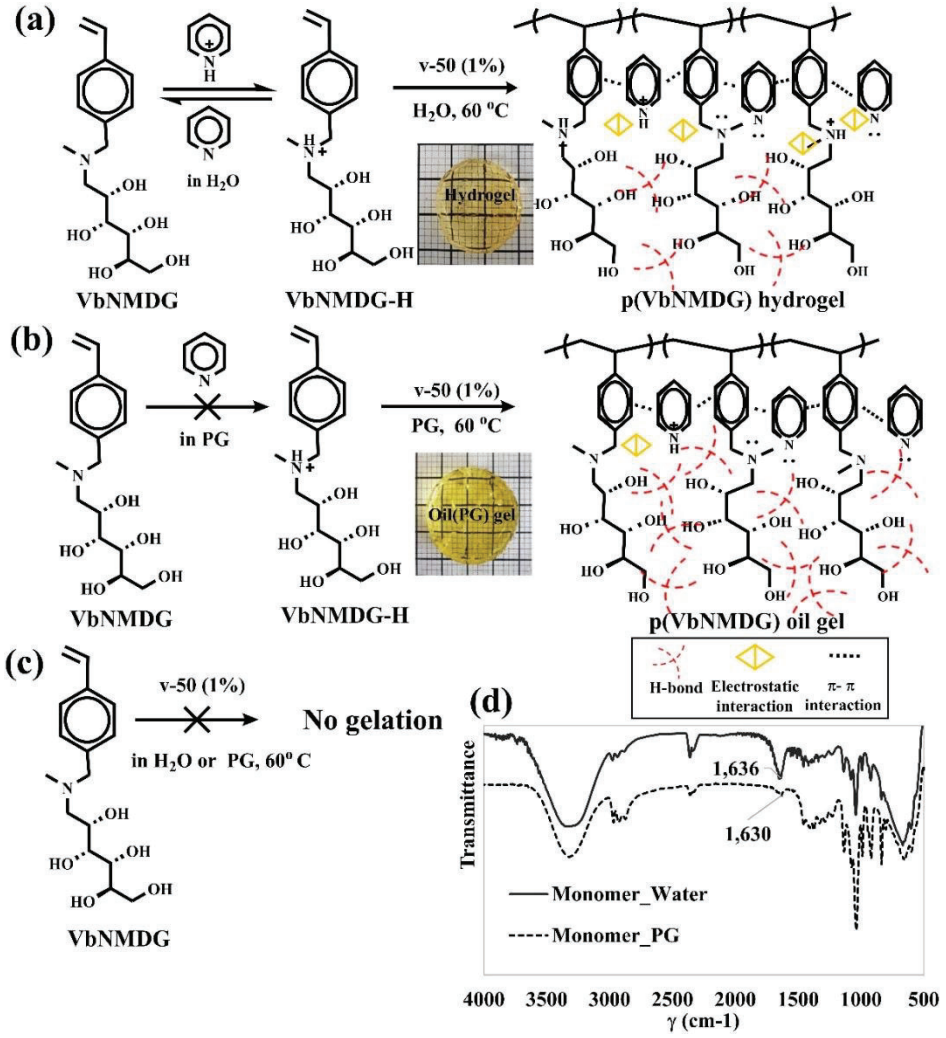


Figure 4-4. Schematic preparation of (a) poly(VbNMDG)-Aq gel, (b) poly(VbNMDG)-PG gel and proposed physical interaction, (c) polymerization of neutral form of VbNMDG and (d) FTIR of 4-vinylbenzyl-N-methyl- D-glucamine (monomer) in aqueous (solid line) and PG (dotted line) solution.

In order to clarify the interacting model, we compared the oil gel and hydrogel illustrated in **Figure 4-4**. The interactions would be the electrostatic interaction, π-π interaction, and hydrogen bonding between the linear polymer chains of vinyl benzyl-*N*-methyl-D-glucamine (VbNMDG) incorporated in the presence of an aromatic base compound (**Figure 4-4a**). Subsequently, the gel was derived after radical polymerization

of VbNMDG in both aqueous and oil (PG) solutions (**Figure 4-4a and 4-4b**). Interestingly, these gels could be formed without any chemical cross-linkers, so the network should be formed by non-covalent interaction, namely 1) electrostatic interaction, 2) hydrogen bond, and 3) π-π interaction. Electrostatic interaction would exist between the cationic form (VbNMDG-H) and its base pair (VbNMDG). Hydrogen bond would exist between hydroxyl groups of glucamine. The π-π interaction would exist between the benzene ring on the polymer chain and the pyridine ring in aqueous solution (**Figure 4-4a**). All of these interactions would be strong enough to provide the network structure between linear polymer chains.

I then selected PG to prepare organogels for the TDDS (**Figure 4-4b**), which is most favorable for both gel network formation and TDDS. Moreover hydrogel was prepared to clarify their interaction as mentioned earlier. Owing to the PG solution lacks of the ionization ability of amine group with pyridine which is supported by comparison of FT-IR as shown in **Figure 4-4d**. The FT-IR of the monomer in an aqueous solution showed an N-H peak at 1,636 cm^{-1} due to protonation of VbNMDG while the PG solution showed a very weak signal at 1,630 cm^{-1}. This indicated the low electrostatic behavior in PG gel. Thus, the oil gel could be derived from the stronger H-bond while increasing the hydrophobicity of solvent. Above all, the polymerization of the neutral form (VbNMDG)

could not form gel in any solutions (**Figure 4-4c**) due to the absence of both electrostatic and π-π interaction. In order to confirm this assumption, the hydrogel and PG gel formation were investigated by the rheological measurements under the various solvents.

4.3.2 Rheological property

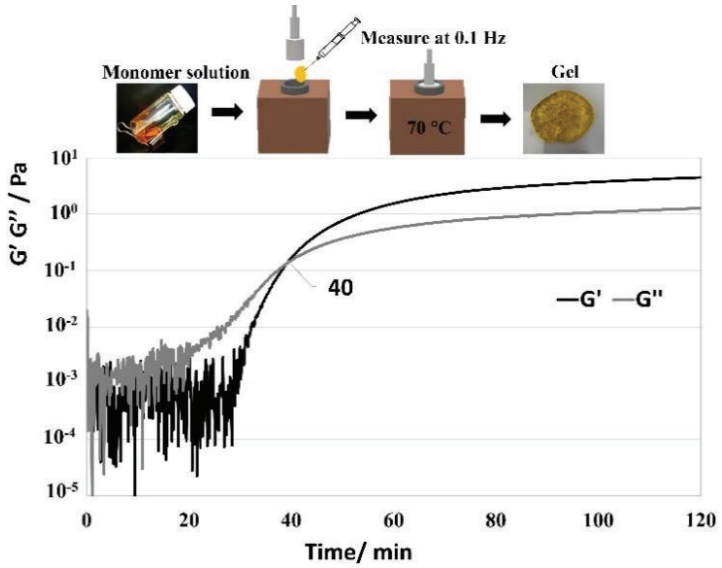


Figure 4-5. The frequency constant measurement at 0.1 Hz monitoring the *in situ* poly(VbNMDG)-PG gel with concentric cylinder rheological equipment at 70 °C for 120 min. (G′ is the black line, G″ is the gray line).

In order to monitor and understand the physical interaction between polymer chains, the *in situ* polymerization was performed during rheological measurement which supports the following discussion as illustrated in **Figure 4-5**. The frequency and strain constant measurement indicated elastic (G′) and viscous (G″) modulus to give the gel formation of poly(VbNMDG) in PG from the linear polymer chain after polymerization.

The network structure is formed gradually after heating up for 20 to 40 min. The beginning state shows the sol-like behavior (G″>G′) of the monomer solution. After polymerization, G′ gradually increases until it is greater than G″, illustrating gel-like behavior. These behaviors identify physical gel formation which thoroughly differs from the chemical cross-linked bond.

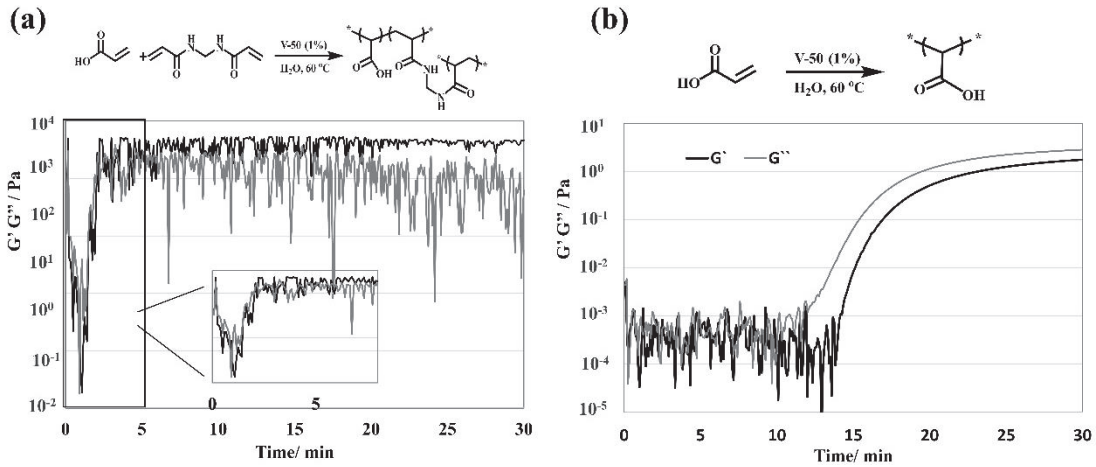


Figure 4-6. The frequency constant measurement at 0.1 Hz monitoring the in situ gelation of (a) PAA-MBA and (b) PAA at 2M with concentric cylinder rheological equipment at 40 °C for 30 min. (G′ is black line, G″ is grey line).

In order to clarify the gelation by this system, the PAA-MBA chemical hydrogel was also monitored during *in situ* polymerization shown in **Figure 4-6a.** It changes rapidly from sol to gel-like behavior within 1 min. after the cross-linked structure was bonded. Moreover, when it was compared the experiment without cross-linker of polyacrylic acid which is a linear polymer chain in **Figure 4-6b**, the sol-like behavior over measurement was clearly indicated. However the G′ and G″ values gradually

increased indicated the growing viscosity of the longer linear polymer chain after the polymerization.

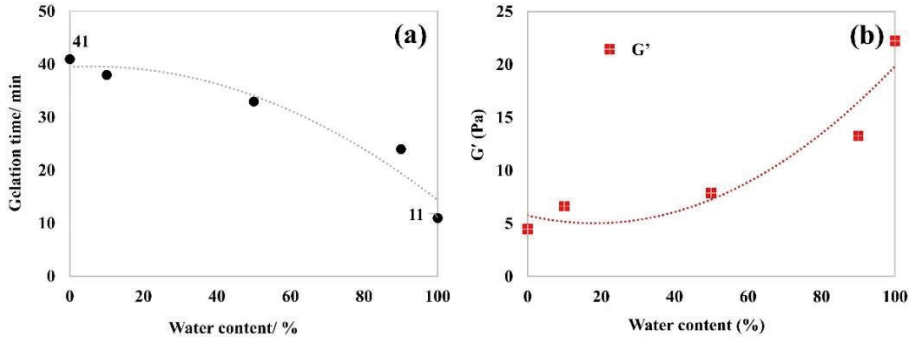


Figure 4-7. Study of water content in gel formation monitoring by rheological measurement (a) Plot of gelation time versus water content (gelation time is determined by changing point from sol-like to gel-like behavior as shown in Figure 4-5) and (b) Plot of elastic modulus versus water content.

Table 4-4. Gelation time and G′ value by varied the water content in PG solution

Water content (%)	Condition	Gelation time (min)	G' (Pa)	G'' (Pa)
0	PG	41	4.46	0.94
10	10% H_2O/PG	38	6.64	1.80
50	50% H_2O/PG	33	7.89	1.72
90	90% H_2O/PG	24	13.28	0.75
100	H_2O	11	22.25	1.12

Moreover, to emphasize the stronger physical interaction of hydrogel compared to PG gel, the *in situ* rheological experiment was conducted while varying the water content in the PG solution. The hydrogel could be formed about 4 times faster in an aqueous solution compared to organogels (PG) as shown in **Figure 4-7a**. Furthermore, the G′ value increased gradually as the water content was increased (**Figure 4-7b)**. Following on from

the previous discussion, the monomer in an aqueous solution could be protonated which generated the electrostatic behavior.

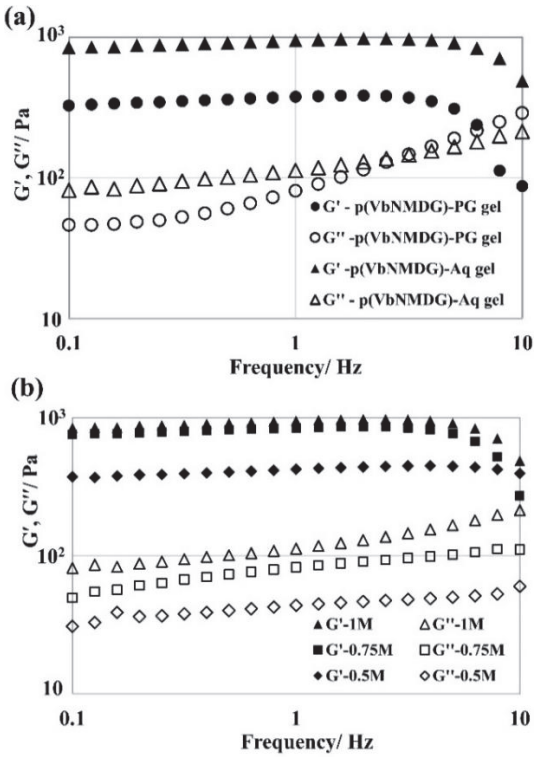


Figure 4-8. Frequency sweep measurement (0.1-10 Hz) and a constant strain (1%) for (a) poly(VbNMDG)-PG (● and ○) and poly(VbNMDG)-Aq gel (▲ and △). (b) poly(VbNMDG)-Aq at 1 M (▲ and △), 0.75 M (■ and □) and 0.5 M (◆ and ◇)concentration.

Furthermore, the hydrogel showed a higher elastic modulus (G′) at 950 Pa while the oil gel was only 370 Pa and the viscous modulus (G″) was less than the elastic modulus (G′) with no intersection, indicating the elastic nature and characteristic feature of the gel network. Whereas, the G″ value gradually increased, indicating the transformation from an elastic state to a viscous state of oil gel (f > 6.31 Hz) when the frequency was increased (**Figure 4-8a)**. Because the network structure of PG gel lacks an

electrostatic interaction as mentioned earlier, this results in the weaker network structure of oil gel. Considering the results for the flexible physical organogels, it could be useful for drug delivery application. It is noteworthy that the network density of hydrogel can be controlled by concentration. The G′ increased from 420, 840, and 950 Pa at higher concentrations for 0.5, 0.75, and 1 M, respectively (**Figure 4-8b**).

4.3.3 Responsive and swelling behavior

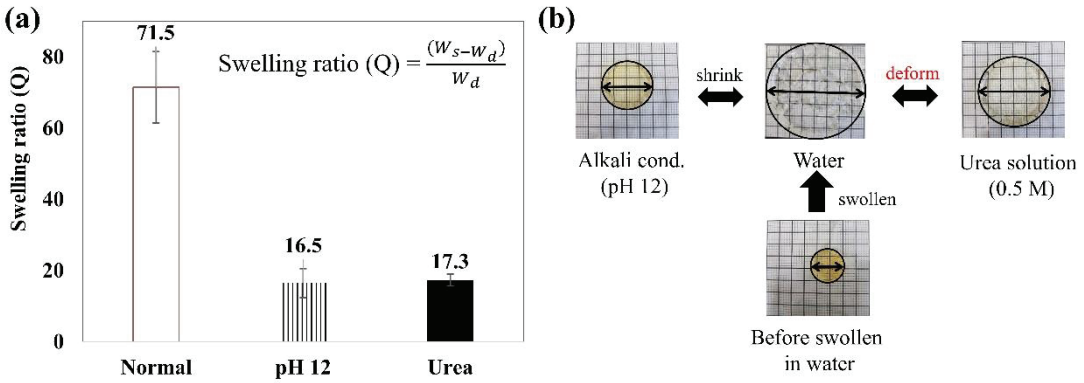
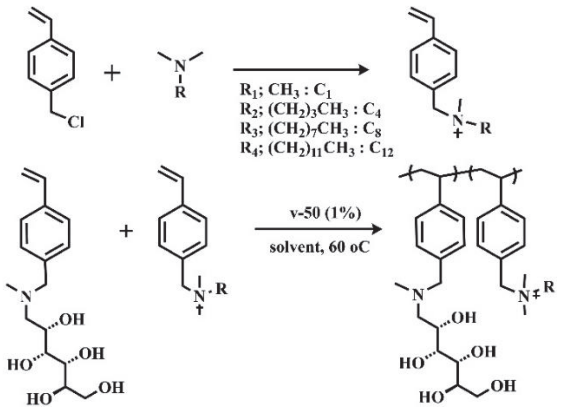


Figure 4-9. Swelling ratio of hydrogel in (a) normal aqueous solution (□), alkali condition pH 12 (||||), 5 M Urea solution (■) (n=3) and (b) photograph of gel in each condition.

To investigate the polymer-polymer interaction, the swelling ratio of hydrogel was observed (**Figure 4-9)**. The swelling ratio of hydrogel in an aqueous solution was around 70, which is a very high swelling ability due to repulsive behavior. However, the gel showed a response against the alkali condition (NaOH, pH 12) by shrinking twice (**Figure 4-9b**) and the swelling ratio was around 4 times lower at about 16.5 (**Figure 4-**

9a). As a result of decreasing of electrostatic repulsion the quaternary amine changed to neutral molecule in alkali condition. Similarly, the swelling ratio in the urea solution was decreased to 17, which indicated the deformation hydrogen bonding between side chains by hydrogen bond disruption of urea in the aqueous solution. Moreover, the urea solution is alkali where it could neutralize the cationic form. These findings confirmed the electrostatic attraction resulting in a diminishing the swollen activity response in those solution. These responsive behaviors can be applied across a wide range of gel applications.

4.3.4 Permeation study of Limonene/PG synergistic and carbon chain length effect



Scheme 4-1. Synthetic scheme of (a) *N*-alkyl-*N,N*-dimethyl-4-vinyl benzenamminium and (b) poly(VbNMDG-*co*-C_nDMVB); n =1, 4, 8 and 12.

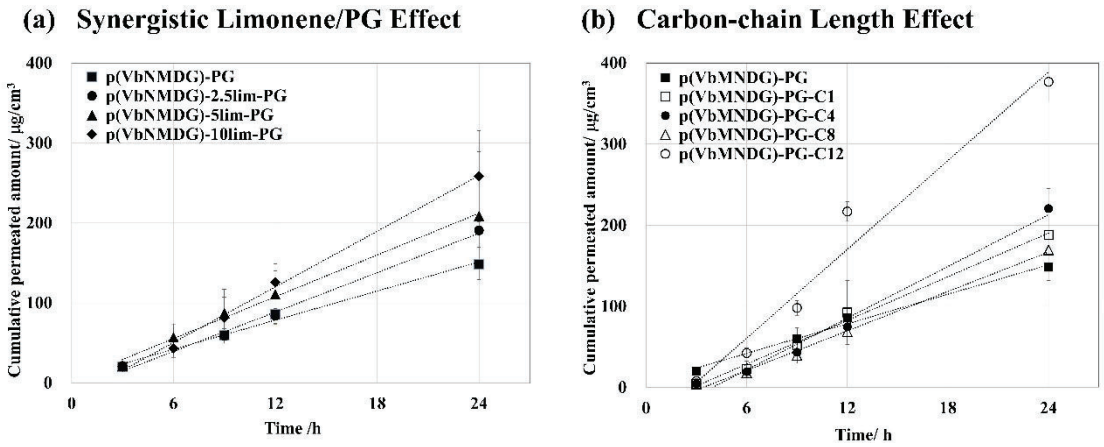


Figure 4-10. *In vitro* cumulative permeated amount of testosterone from (a) Limonene/PG effect and (b) Carbon chain length effect by Franz diffusion cell at 37 °C (n=3).

Summarizing the aforementioned results, the present gels were originally designed with a monomer structure in order to control release for TDDS. The structure of glucose derivatives was introduced because it plays the role of a polar enhancer to interact with the hydrophilic pathway. The alkyl groups were introduced because they immobilized in the PG solution for the interaction with skin barriers. Furthermore, the core structure is a hydrophobic benzene ring which reacts with the lipophilic pathway and improves solubility in the organic solvent. Methanol and ethanol might be used for the system, but it was found that the monomer reverted to a starting compound via deamination in alcohol. But the VbNMDG-monomer is selective to PG for the oil gel, as well as being an enhancer.

Table 4-5. Flux and permeation coefficients (KD/L)

Entry	Gel	Flux, J_{ss} ($\mu g/cm^2 \cdot h$)	KD/L ($*10^{-3}$ cm/h)
1a	poly(VbNMDG)-Aq	-	-
2a	poly(VbNMDG)-PG	6.07±1.00	4.58±0.75
3a	poly(VbNMDG)-2.5Lim-PG	8.21±1.13	6.20±0.85
4a	poly(VbNMDG)-5Lim-PG	8.71±3.71	6.57±2.79
5a	poly(VbNMDG)-10Lim-PG	11.6±2.60	8.80±1.95
1b	poly(VbNMDG)-PG-C1	8.98±1.13	6.78±0.85
2b	poly(VbNMDG)-PG-C4	10.62±383	8.02±2.88
3b	poly(VbNMDG)-PG-C8	8.10±3.27	6.11±2.46
4b	poly(VbNMDG)-PG-C12	18.23±7.25	13.76±5.45

Poly(VbNMDG) gel was then finally applied to the transdermal drug delivery system. The poly(VbNMDG)-PG gel showed quite a high permeation coefficient (KD/L) at 4.58±0.75 $x10^{-3}$ cm/h (**Table 4-5**, entry 2) which is close to the Androderm patch commercial product.[43] According to previous reports, PG is used synergistically with terpene for organogels in order to enhance their penetration by lipid disruption.[44,45] It has also been proposed that propylene glycol increases the partitioning of the terpene into the SC, where it can exert its enhancer effect.[46] To study this synergistic effect, *d*-limonene was used for 2.5, 5 and 10% with PG in gel preparation in **Table 4-2**. The results show that the higher the *d*-limonene content, the more permeation coefficient (KD/L) was obtained (**Table 4-5**, entries 3-5) as shown by the cumulative permeated profile in **Figure 4-10a**. Followed by these results, *d*-limonene could perform synergistic action by increased drug diffusivity and partitioning into the SC.[47] The flux enhancement ratio (E_r)

was 1.91 for 10 % limonene/PG. Moreover, to fabricate the polymeric enhancer and investigate the effect of carbon chain length on the cationic surfactant polymer, the benzalkonium chloride type was co-polymerized for 5 mol% in a PG solution as shown in **Scheme 4-1** (**Table 4-3**). In the previous report, the polymeric enhancer was studied in the alcohol solution.[40] Hence, it would be convenient, highly efficient and low irritation by co-polymerizing as a gel formulation. Here, the poly(VbNMDG)-PG-C_n gels were prepared by varying side chains of the alkyl group (C_1, C_4, C_8 and C_{12}) and the cumulative permeated was studied as indicated in **Figure 4-10b**. It was shown that poly(VbNMDG)-PG-C_{12} significantly raised the permeability as indicated by the highest KD/L of 13.76±5.45 x10^{-3} cm/h and highest flux enhancement ratio of 3.00 (**Table 4-5**, entry 4). While in the C_1, C_4, and C_8 series, there were no significant differences in penetration efficiency (KD/L) (**Figure 4-10b**, **Table 4-5**, entry entries 1-3). Referring to a previous report, the carbon chain length with homologous fatty acid series from C_6 to C_{18} chain length has been studied.[48] The lauric acid (C_{12}) hydrophobic group may have an optimal balance of partition coefficient and affinity to skin. This is suggested in a study by Florence *et al*.[49] which showed that increasing the carbon chain length within a homologous series increased the lipophilicity while the short-chain hydrocarbon had insufficient lipophilicity for skin penetration, findings which support our results. In

summary, the poly(VbNMDG)-PG gel indicated the appropriate efficiency in TDDS. It is compatible and enhanced by a CPEs solvent system, synergistic activity and co-polymerized with polymeric penetration.

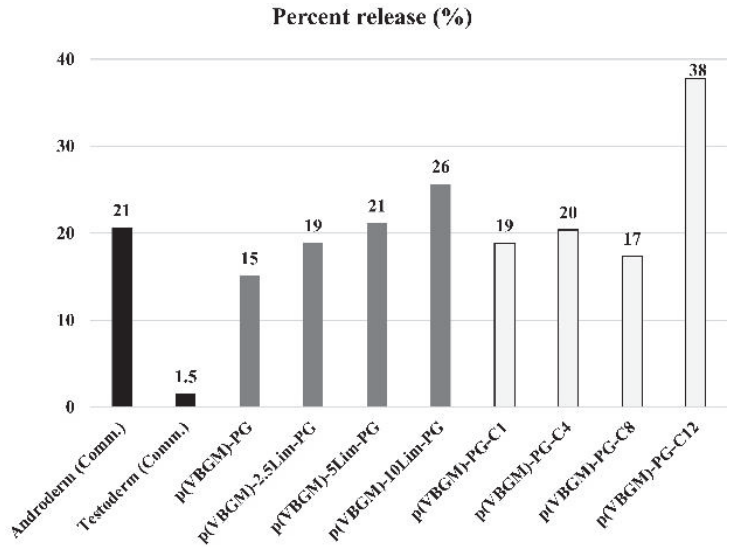


Figure 4-11. Drug releasing efficiency of testosterone.

Interestingly, the prepared gels were compared with testosterone patch commercial product such as Androderm and Testoderm shown in **Figure 4-11**. The permeability dose for 24 h approached the Androderm which could transport for 2mg dosage from 9.7 mg (21%, **Table 4-6**) and higher than Testoderm (1.5%). Especially, the percent release of p(VbNMDG)-10Lim-PG gel and p(VbNMDG)-PG-C12 are 26 and 38%, it is higher than the commercial product. These were confirmed that the novel p(VbNMDG) show very high permeability of testosterone via TDDs and it delivery the proper demand dosage for treatment.

Table 4-6. Drug releasing efficiency of commercial and synthesized transdermal formulas

Formular	Release Doase (mg/day)	Initial drug (mg)	Percent release (%)
Androderm (Comm.)	2	9.7	21
Testoderm(Comm.)	5	328	2
p(VBGM)-PG	0.2	1.325	15
p(VBGM)-2.5Lim-PG	0.25	1.325	19
p(VBGM)-5Lim-PG	0.28	1.325	21
p(VBGM)-10Lim-PG	0.34	1.325	26
p(VBGM)-PG-C1	0.25	1.325	19
p(VBGM)-PG-C4	0.27	1.325	20
p(VBGM)-PG-C8	0.23	1.325	17
p(VBGM)-PG-C12	0.5	1.325	38

4.4 Conclusion

A novel poly(VbNMDG) gel was fabricated by physical cross-linking of polymer-polymer interaction. Electrostatic interaction and hydrogen bond by using an organic base as the promotor was confirmed, suggesting the novel interacting model of the physical organogels. In addition, we confirmed the gel formation by *in situ* gelation at 0.1 Hz frequency by rheological measurement. The gel was formed by gradually changing from sol-like to gel-like behavior clearly in both aqueous and PG solution without chemical cross-links. The G′ value of the poly(VbNMDG)-Aq gel was 950 Pa and poly(VbNMDG)-PG gel was 370 Pa which is rigid enough for use as a drug reservoir

in transdermal application. For the permeation study, the synergistic action of limonene/PG as the solvent system was clearly indicated. The effect of the co-solvent with limonene could enhance the permeation coefficient (KD/L) from 4.58±0.75 $x10^{-3}$ cm/h (pure PG) to 8.80±1.95 $x10^{-3}$ cm/h (10% Lim/PG). Moreover, we studied the carbon chain length effect on permeability. The poly(VbNMDG)-PG-C12 which contains *N,N*-dimethyl-*N*-dodecyl benzenamminium showed the maximum permeation coefficient at 13.76±5.45 $x10^{-3}$ cm/h. As a result, the novel poly(VbNMDG) gel could control the release of testosterone which is needed for optimal dosage over 24 h depending on the conditions. Overall, the poly(VbNMDG)-PG gel illustrated the novel polymer-polymer interaction which can improve the permeability of transdermal testosterone and be compatible with a chemical penetration enhancer system.

4.5 References

1) D. Bhowmik, C. Bhattacharjee, M. C. Chandira, B. Jayakar, K.P. Sampath, *Int J Pharm Tech Res.* **2010**, *2*, 68.

2) A. Kumar, N. Pullankandam, S. L. Prabhu, V. Gopal, *Int. J Pharm Sci Review Res.* **2010**, *3*, 49.

3) M. N. Pastore, Y. N. Kalia, M. Horstmann, M. S. Roberts, *Br J Pharmacol.* **2015**, *172*, 2179.

4) M. R. Saboktakin, S. Akhyari, F.A. Nasirov. *Int J Biol Macromol*. **2014**, *69*, 442.

5) N. M. Saidin, N. K. Anuar, M. M. R. Meor Mohd Affandi, *J. Appl Pharm Sci.* **2018**, *8*, 141.

6) Y. Yang, S. Sunoqrot, C. Stowell, J. Ji, C. W. Lee, J. W. Kim, S. A. Khan, S. Hong, *Biomacromolecules* **2012**, *13*, 2154.

7) H. Lee, C. Songa, S. Baika, D. Kima, T. Hyeona, D. H. Kima, *Adv. Drug Deli. Rev.* **2017**, *127*, 1.

8) M. Lapteva, Y. N. *Expert Opin Drug Deliv.* **2013**, *10*, 1043.

9) A. C. Watkinson, M. C. Kearney, H. L. Quinn, A. J. Courtenay, R. F. Donnelly, *Expert Opin Drug Deliv.* **2016**, *13*, 523.

10) T. Gratieri, Y. N. Kalia, *Adv Drug Deliv Rev.* **2013**, *65*, 315.

11) K. Rehman, M. H. Zulfakar, *Drug Dev Ind Pharm*, **2014**, *40*, 443.

12) C. L. Espositoa, P. Kirilovb, V.G. Roullina, *J Control Release* **2018**, *271*, 1.

13) R. Hamed, A. AbuRezeq, O. Tarawneh, *Drug Dev Ind Pharm.* **2018**, *44*, 1488.

14) P. Kirilov, K. Le cong Anh, A. Denis, H. Rabehi, S. Rum, C. Villa, M. Haftek, F. Pirot, *Pers Care Today* **2015**, *10*, 15.

15) P. Matricardi, C. Di Meo, T. Coviello, W. E. Hennink, F. Alhaique, *Adv Drug Deliv Rev.* **2013**, *65*, 1172.

16) B. Li, L. Wang, F. Xu, X. Gang, U. Demirci, D. Wei, Y. Li, Y. Feng, D. Jia, Y. Zhou, *Acta Biomaterialia* **2015**, *22*, 59.

17) B. Naryan, g. Jonathan, Z. Miqin, *Adv. Drug Deliv. Rev.* **2010**, *62*, 83.

18) S. Datta, S. Bhattacharya, *Chem Soc. Rev.* **2015**, *44*, 5596.

19) S. H. Allan, *Adv. Drug Deliv. Rev.* **2012**, *64*, 18.

20) D. Chouhan, T. U. Lohe, P. K. Samudrala, *Adv Healthc Mater.* **2018**, *7*, 180.

21) A. M. Cardoso, E. G. de Oliveira, K. Coradini, F. A. Bruinsmann, T. Aguirre, R. Lorenzoni, R. C. S. Barcelos, K. Roversi, D. R. Rossato, A. R. Pohlmann, S. S. Guterres, M. E. Burger, R. C. R. Beck, *Mater Sci Eng C Mater Biol Appl.* **2019**, *96*, 205.

22) C. C. Chen, C. L. Fang, S. A. Al-Suwayeh, Y. L Leu, J. Y. Fang. *Int J Pharm.* **2011**, *415*, 119.

23) H. Richards, C. P. Thomas, J. L. Bowen, C. M. Heard, *J Pharm Pharmacol.* **2006**, *58*, 903.

24) P. Karande, A. Jain, K. Ergun, V. Kispersky, S. Mitragotri, *Proc. Natl. Acad. Sci.* **2005**, *102*, 4688.

25) H. Marwah, T. Garg, A. K. Goyal, G. Rath, *Drug Deliv.* **2016**, *23,* 564.

26) A. Vintiloiu, J.C. Leroux, *J Control Release* **2008**, *125*, 179.

27) C. L. Espositoa, P. Kirilovb, V. G. Roullin, *J Control Release* **2018**, *271*, 1.

28) V. Jhawat, S. Gupta, V. Saini, *Drug Deliv.* **2016**, *23*, 3573.

29) F.M. Mady, H. Essa, T. El-Ammawi, H. Abdelkader, A.K. Hussein, *Drug Des Dev Ther.* **2016**, *10*, 1101.

30) H. Alsaab, S. P. Bonam, D. Bahl, P. Chowdhury, K. Alexander, S.H. Boddu, *J Pharm Pharm. Sci.* **2016**, *19*, 252.

31) K. Takemura, H. Ajiro, T. Fujiwara, M. Akashi, *Polym J.* **2015**, *47,* 460.

32) P. Surampudi, R. S. Swerdloff, C. Wang, *Expert Opin. Pharmacother* **2014**, *15*, 1247.

33) A. Thirumalai, K. E. Berkseth, J. K. Amory, *F1000Research* **2017**, 6.

34) P. Charoensumran, H. *Polym J.* **2018**, *50*, 1021.

35) P. Charoensumran, H. Ajiro, *Polymers* **2018**, *10*, 1200.

36) N. Kabay, S. sarp, M. Yuksel, O. Arar, M. Bryjak, *React funct polym.* **2007**, *67*, 1643.

37) B. F. Urbano, B. L. Livas, F. Martinez, S.D. Alexandratos, *React funct polym.* **2012**, *72*, 642.

38) L. Trottet,C. Merly, M. Mirza, J. Hadgraft, A.F. Davis, *Int. J. App. Pharm.* **2004**, 274.

39) I. Som, K. Bhatia, M. Yasir, *J. Pharm. Bioallied Sci.* **2012**, *4*, 2.

40) T. Aoyagi, O. Terashima, N. SuZuki, K. Matsui Y. Nakase, *J of controlled release*, **1990**, *13*, 63.

41) J. Crank, The Mathematics of diffusion, Oxford University Press, England **1975**.

42) H. K. Vaddi, L. Z. Wang, P. C. Ho, S. Y. Chan, *Int. J. Pharm.* **2001**, *212*, 247.

43) A. Thirumalai, K.E. Berkseth, J. K. Amory, *F1000Res.* **2017**, *68*, 1.

44) P.F. Lim, X.Y. Liu, L. Kang, P.C. Ho, Y.W. Chan, S.Y. Chan, *Int. J Pharm.* **2006**, 311.

45) D. Monti, P. Chetoni, S. Burgalassi, M. Najarro, M.F. Saettone, E. Boldrini, *Int J Pharm.* **2002**, *237*, 209.

46) B.W. Barry, *Eur J Pharm Sci* **2001**, 2, 101.

47) N. Dragicevic, H.I. Maibach, *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement*, Springer, UCSF, CA **2015**.

48) T. Ogiso, M. J. Shintani, *Pharm. Sci.* **1990**, *79*, 1065.

49) A. T. Florence, I. G. Tucker, K. A.Walters, *In Structure Performance Relationships in Surfactants*, American Chemical Society, Washindon DC **1984**.

Chapter 5

Concluding remark

The aim of this research is to develop a new sustainable materials as the organogels from cross-linked polystyrene; CLPS with *d*-limonene which is non-toxic and plant-based oil. It was fabricated functionalized oil gel by introducing several interacting unit for example, cationic, anionic and hydrophilic moiety. They show selective absorbability by electrostatic behavior on surface of organogels. Also, I studied the controlled release of drug in transdermal delivery system. It has been developed to approach the application for TDDS by using PS as core structure.

In chapter 2, the electrostatic moieties were introduced into CLPS based *d*-limonene organogels in order to improve the functionality of oil gel. The chemical gel showed high stability and mechanical property by increase the network density. The selective adsorption to organic molecule (dye) by electrostatic interaction at aqueous surface was indicated which is supportive to drug storage system. I found, the release of hydrophobic drug (Testosterone and Ibuprofen) was controlled by network density and electrostatic behavior, respectively. However, to fabricate advance application of CLPS limonene, we applied the gels for TDDS in the next chapter.

In chapter3, in order to enhance the permeability and controllable of drug

through the skin, I used the CLPS copolymerized with cationic moiety. Owing to *d*-limonene as effective CPEs terpene, the CLPS in limonene is suitable for study model of TDDS. It was shown that, the efficiency of the permeability of ibuprofen was successfully enhanced by *d*-limonene and the controllable of the network density indicated by relative permeability coefficient from 53.1±0.03 to 17.9±0.20 x10 cm/h. Cationic moieties were introduced to control the drug releasing behavior of the gel with the slowest steady flux at 14.1±0.25 cm/h of 5 mol%-VP of PS-VP gel because of drug-polymer chain interaction. We achieved a steady controlled release of ibuprofen from modified PS gels swollen in limonene.

In the chapter 4, it focuses on TDDS application for testosterone which is extensively used for percutaneous administration. It is the first time to study the novel Poly(Vinyl benzyl-*N*-methyl-*D*-glucamine) gel in PG formed by physical cross-linked. The synergistic effect of limonene/PG were studied, increasing of limonene content till 10% V/V enhance the penetration efficiency and releasing rate. Moreover, we studied the carbon chain length effect on permeability. The C12 carbon chain length; poly(VbNMDG)-PG-C12 shows significantly the highest permeation coefficient.

In summary, we have explored the field of “organogels” and their application on the concept of environmental friendly organic solvent by using *d*-limonene. The PS was

use as polymer network due to their fine solubility that appeared many reports as sustainability system. I created the novel oil gel which functionalize by interaction unit and studied and developed the PS organogels both chemical and physical gel to improve the permeability and controlled release for TDDS. I expect this dissertation will be useful for the further study.

List of Publications

Chapter 2:

Preeyarad Charoensumran, Hiroharu Ajiro,

"The electrostatic advantages on cross-linked polystyrene organogels swollen with limonene for selective adsorbent and hydrophobic drug storage",

Polym. J. **2018**, 50, 1021-1028.

Chapter 3:

Preeyarad Charoensumran, Hiroharu Ajiro,

"Cationic Moieties in Polystyrene Gels Swollen with D-limonene Improved Transdermal Delivery System"

Polymers **2018**, 10(11), 1200-1211.

Chapter 4:

Preeyarad Charoensumran, Hiroharu Ajiro,

"Controlled Release of Testosterone by Polymer-polymer Interaction Enriched Organogel as a Novel Transdermal Drug Delivery System; Effect of Limonene/PG and Carbon-chain Length on Drug Permeability."

In preparation

Other Publications

Mohamed F. Mady, Preeyarad Charoensumran, Hiroharu Ajiro Malcolm A. Kelland

“Synthesis and Characterization of Modified Aliphatic Polycarbonates as

Environmentally Friendly Oilfield Scale Inhibitors” *Energy Fuels* **2018**, 32, 6746−6755.

Patent

P. Charoensumran, H. Ajiro (2018) Kyoto international Patent Japan (110001069)

「オイルゲル組成物及び経皮吸収 製剤」

Acknowledgement

First and foremost, I would like to express my deep and sincere gratitude to my supervisor, Professor Hiroharu AJIRO to give me the important opportunity to study the PhD and especially his recommendation for MEXT scholarship at NAIST. During my studying, He has taught me the methodology to carry out the research, go through and solve the problem. It was a great privilege and honor to work and study under his guidance. His dynamism, vision, sincerity and motivation have deeply inspired me. He always give me many knowledge and the comment to improve myself. I am extending my heartfelt thanks to his wife, family for their friendly and kindness during 3 years. I could not have imagined having a better advisor and mentor for my PhD studying.

Besides, I would like to deeply thank all co-supervisors; Prof. Hiroko YAMADA, Prof. Michiya FUJIKI, Assoc. Prof. Takashi MATSUO and Prof. Takahiro HONDA for their insightful advice and comment to improve my research throughout my PhD studying. I also thanks for their comment, patent and well-intentioned to help me for improve myself. I also really appreciate to Prof. Naoyuki INAGAKI (Laboratory of Systems Neurobiology and Medicine, Bioscience NAIST) for his generous to donate the rat skin for all experiments in my research. I am so thankful for Ria Fajarwati Kastian-san and Abe-san for their helps to excise the rat skin.

My sincere thanks also goes to Prof. Malcolm A. Kelland and Dr. Mohamed F. Mady for their great opportunity to let me join the project and co-author for a publication.

Moreover, I would like to thank Materials Science Department and NAIST for financial support of English Program at University of California, Davis, USA.

I would like to say sincerely thanks to my friend, Nalin san who introduce me to Ajiro sensei at first and her helps during the student life in Japan. Also I am thankful for Steffen for his helps, advice and kindness. Plus I would like to deeply thank to all fellow labmates in Ajiro's group especially, Ken san, Iwami chan and Nobu kung for all their helps and kindness. I have learn a lot from them, thanks for their warm and friendship to this Thai girl.

Moreover, I would like to thank all staffs in NASIT, MS division and international student affair for their support, helps and well management during this three years.

Last but not the least, I am extremely grateful to my family for their love, caring, believe and encouragement for my education. Also I am thankful to all Thai friends for their helps, friendship and sharing good time together.