



NOVEL BIPHASIC GELS CONTAINING MIXTURE OF SILICONE OIL AND
SESAME OIL FOR TOPICAL DRUG DELIVERY



By
MISS Mya Thet Htar SWE

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy PHARMACEUTICAL ENGINEERING
(INTERNATIONAL PROGRAM)
Graduate School, Silpakorn University
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Novel biphasic gels containing mixture of silicone oil and sesame oil for topical drug delivery



โดย
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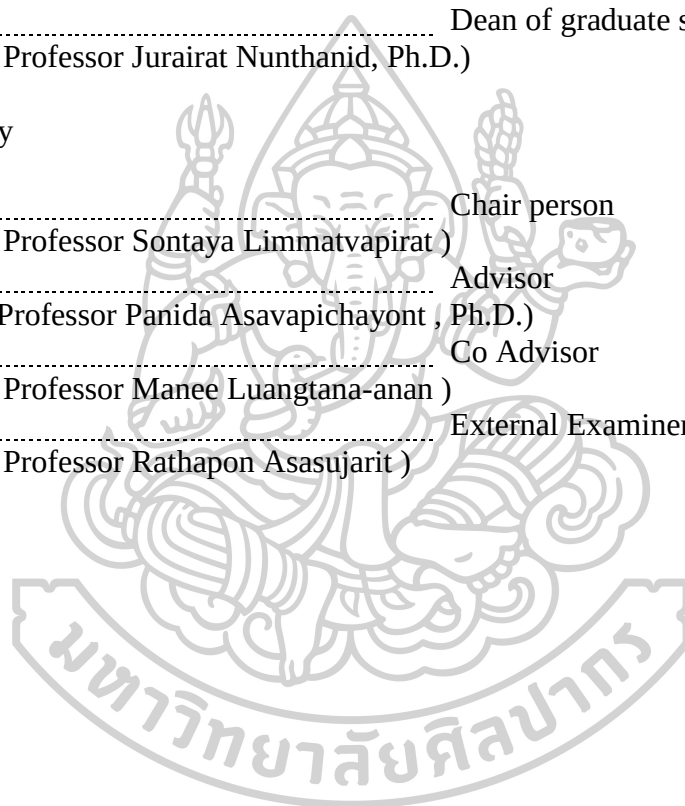
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MISS MYA THET HTAR SWE : NOVEL BIPHASIC GELS CONTAINING MIXTURE OF SILICONE OIL AND SESAME OIL FOR TOPICAL DRUG DELIVERY THESIS ADVISOR : ASSISTANT PROFESSOR PANIDA ASAVAPICHAYONT, Ph.D.

Bigels or biphasic gels are semisolid formulations offering the combined advantages of both hydrogels and oleogels. They are gaining interest in food, cosmetic and pharmaceutical applications. The aim of this study was to develop a new topical dosage form for both hydrophobic and hydrophilic drugs. A novel bigel was developed with the aid of cyclopentasiloxane (silicone oil). Bacitracin zinc (B) and neomycin sulfate were selected as hydrophobic and hydrophilic model drugs, respectively. Bigels were prepared by simply mixing hydrogels and sorbitan monostearate (SMS)-sesame oil oleogels. The hydrophobic drug was loaded in oleogel while the hydrophilic drug was loaded in hydrogel. Two types of hydrogel (xanthan gum (XG) and guar gum (GG)) were investigated for bacitracin zinc, whereas only guar gum was studied for neomycin sulfate. The physicochemical properties of drug loaded bigels (BXG and BGG) (microscopy, FTIR spectroscopy, rheology, and mechanical properties), drug content, *in vitro* drug release and antimicrobial activity were evaluated and comparisons were made with bigels containing a mixture of silicone oil and sesame oil (BXGS and BGGS). The findings showed that the properties of bacitracin loaded bigels were dependent on the type of hydrogel and composition of oleogel. Superior properties of those with silicone were reported due to strong gel formation and high drug solubility in the mixture of silicone oil and sesame oil. The superior properties were aesthetic and sensory profiles, rheological and mechanical properties, drug content, drug release, antimicrobial activity and stability. The order of drug content, drug release and antimicrobial activity were BGGS > BXGS > BGG > BXG. Although BXGS showed slightly inferior properties than BGGS, the stability of BXGS was far better and stable for 6-month storage. Similar results were demonstrated for neomycin sulfate bigels. The higher drug content, drug release, antimicrobial activity and stability were obtained with the incorporation of silicone oil. The silicone oil improved the properties of bigels, therefore, this novel bigel could be a good carrier for topical delivery of both hydrophobic and hydrophilic drugs.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	D
ACKNOWLEDGEMENTS.....	E
TABLE OF CONTENTS.....	F
LIST OF TABLES	K
LIST OF FIGURES	L
LIST OF ABBREVIATIONS.....	P
CHAPTER 1	1
INTRODUCTION	1
1.1 Statement and significance of the research problem	1
1.2 Objectives	4
1.3 The research hypothesis.....	4
CHAPTER 2	5
LITERATURE REVIEW	5
2.1 Topical drug delivery systems	5
2.2 Gels for topical drug delivery	6
2.3 Oleogels	8
2.3.1 Components of oleogels	9
2.3.1.1 Oils	9
2.3.1.2 Oleogelators.....	14
2.3.1.3 Mechanism of gelation	16
2.3.2 Silicone surfactant (cyclomethicone and PEG/PPG -20/15 dimethicone).....	17
2.4 Hydrogels.....	18
2.4.1 Components of hydrogels.....	19
2.4.1.1 Polymers used in the preparation of hydrogels	19
2.4.1.2 Xanthan Gum	20

2.4.1.3 Guar gum	21
2.4.1.4 Carbopol or carbomer polymers	22
2.5 Bigels	23
2.5.1 Types of bigels	25
2.5.2 Characterization techniques of bigels	27
2.5.3 Pharmaceutical applications of bigels	32
2.6 Model drugs loaded in bigels	33
2.6.1 Bacitracin zinc	33
2.6.2 Neomycin sulfate	35
CHAPTER 3	38
MATERIALS AND METHODS	38
3.1 Materials	38
3.2 Equipment	39
3.3 Methods	40
3.3.1 Development of oleogels with silicone oil	40
3.3.1.1 Preliminary study to choose the best miscible oil pair	40
3.3.1.2 Viscosity of the oil components	40
3.3.1.3 Determination of CGC for oleogels	41
3.3.1.4 Preparation of oleogels	41
3.3.1.5 Physicochemical characterization of the developed oleogels	42
3.3.1.5.1 FTIR analysis	42
3.3.1.5.2 Microstructure studies	42
3.3.1.5.3 Rheological characterization	42
3.3.1.5.4 X-ray diffraction studies	43
3.3.1.5.5 Differential scanning calorimetry (DSC)	43
3.3.1.6 Statistical analysis	43
3.3.2 Development of bigels containing mixture of silicone oil and sesame oil for topical drug delivery	44

3.3.2.1 Preliminary study to choose the polymers for hydrogel preparations and the best ratio of oleogel and hydrogel for the development of bigels.....	44
3.3.2.2 Development of hydrophobic drug loaded bigels	44
3.3.2.2.1 Determination of drug solubility	44
3.3.2.2.2 Preparation of hydrogels.....	45
3.3.2.2.3 Preparation of drug loaded oleogels	45
3.3.2.2.4 Preparation of bigels	45
3.3.2.3 Development of hydrophilic drug loaded bigels	46
3.3.2.3.1 Preparation of drug loaded hydrogels.....	46
3.3.2.3.2 Preparation of oleogels	47
3.3.2.3.3 Preparation of bigels.....	47
3.3.2.4 Physicochemical characterization of bigels	48
3.3.2.4.1 pH measurement	48
3.3.2.4.2 Microscopic studies	48
3.3.2.4.3 FTIR analysis.....	48
3.3.2.4.4 Rheological characterization	48
3.3.2.4.5 Mechanical properties.....	49
3.3.2.4.6 X-ray diffraction studies	49
3.3.2.4.7 Drug content determination	49
3.3.2.4.7.1 Drug content determination of bacitracin zinc loaded bigels	49
3.3.2.4.7.2 Drug content determination of neomycin sulfate loaded bigels	50
3.3.2.4.8 <i>In vitro</i> drug release study	50
3.3.2.4.9 Antimicrobial assay	51
3.3.2.4.10 Stability studies.....	52
3.3.2.5 Statistical analysis	52
CHAPTER 4	53
RESULTS AND DISCUSSION	53

4.1 Development of oleogels with silicone oil	53
4.1.1 Preliminary study to choose the best miscible oil pair	53
4.1.2. Viscosity of the oil components	56
4.1.3. Determination of CGC for oleogels	57
4.1.4. Preparation of oleogels	59
4.1.5 Physicochemical characterization of the developed oleogels	59
4.1.5.1 FTIR analysis	59
4.1.5.2 Microstructure studies	60
4.1.5.3 Rheological characterization	62
4.1.5.4 X-ray diffraction studies.....	67
4.1.5.5 Differential scanning calorimetry (DSC)	69
4.2 Development of bigels containing mixture of silicone oil and sesame oil for topical drug delivery.....	72
4.2.1 Preliminary study to choose the polymers for hydrogel preparations and the best ratio of oleogel and hydrogel for development of bigels	72
4.2.2 Development of hydrophobic drug loaded bigels	73
4.2.2.1 Determination of drug solubility	73
4.2.2.2 Preparation of hydrophobic drug loaded bigels.....	74
4.2.2.3 Physicochemical characterization of bigels	75
4.2.2.3.1 pH measurement.....	75
4.2.2.3.2 Microscopic studies	75
4.2.2.3.3 FTIR analysis.....	76
4.2.2.3.4 Rheological characterization	78
4.2.2.3.5 Mechanical properties.....	81
4.2.2.3.6 X-ray diffraction studies	82
4.2.2.3.7 Drug content determination	85
4.2.2.3.8 <i>In vitro</i> drug release study	85
4.2.2.3.9 Antimicrobial assay	88
4.2.2.3.10 Stability studies.....	90
4.2.3 Development of hydrophilic drug loaded bigels	94

4.2.3.1 Preparation of hydrophilic drug loaded bigels	94
4.2.3.2 Physicochemical characterization of bigels	94
4.2.3.2.1 pH measurement	94
4.2.3.2.2 Microscopic studies	94
4.2.3.2.3 FTIR analysis.....	96
4.2.3.2.4 Rheological characterization	97
4.2.3.2.5 Mechanical properties.....	100
4.2.3.2.6 X-ray diffraction studies	101
4.2.3.2.7 Drug content determination	102
4.2.3.2.8 <i>In vitro</i> drug release study	104
4.2.3.2.9 Antimicrobial assay	106
4.2.3.2.10 Stability studies.....	108
CHAPTER 5	112
CONCLUSION.....	112
REFERENCES	115
APPENDIX.....	124
VITA.....	135



LIST OF TABLES

	Page
Table 1 Composition of oleogels.....	41
Table 2 Composition of hydrogels and oleogels in bigels.	46
Table 3 Composition of hydrogels and oleogels in bigels	47
Table 4 Viscosity of oils (n=3).....	57
Table 5 FWHM, AUC and d-spacing values of oleogels and their components for XRD analysis.	67
Table 6 DSC studies of OG1, OG2 and OG3 (n=3, mean $\pm$ SD).....	70
Table 7 Determination of drug solubility (n=3).	74
Table 8 Flow properties of bigels determined by rheometer.....	79
Table 9 Mechanical properties of bigels determined by backward extrusion study using texture analyzer	82
Table 10 Release kinetics of bacitracin zinc from bigels	87
Table 11 Color, flow when inversion and pH of the bigels before and after passing the temp cycling for 6 cycles and intermediate stability test for 1,3 and 6 months.....	91
Table 12 Flow properties of bigels determined by rheometer.....	99
Table 13 Mechanical properties of bigels determined by texture analyzer.....	101
Table 14 Release kinetics of neomycin sulfate from bigels	106
Table 15 Color, flow when inversion and pH of the bigels before and after passing the temp cycling for 6 cycles and intermediate stability test for 1,3 and 6 months...	109

LIST OF FIGURES

	Page
Figure 1 The skin structure and its layers. Three main layers of the skin are epidermis, dermis and subcutaneous layer. The stratum corneum is known as the outermost layer of the epidermis (49).	6
Figure 2 General classification and types of gels (53).	8
Figure 3 Diverse application of oleogels in various fields (57).	9
Figure 4 Chemical structure of volatile silicone oil (cyclopentasiloxane).	13
Figure 5 Chemical structure of sorbitan monostearate.	16
Figure 6 Mechanism of gelation of oleogels (53).	17
Figure 7 Chemical structure of PEG/PPG -20/15 dimethicone.	18
Figure 8 Chemical structure of xanthan gum (76).	21
Figure 9 Chemical structure of guar gum.	22
Figure 10 Chemical structure of Carbopol polymers.	23
Figure 11 The microscopic arrangements of different types of bigels. (A) Oleogel dispersed in hydrogel, (B) hydrogel dispersed in oleogel, (C) both phases are dispersed, (D) emulgel, and (E) complex bigel (79).	29
Figure 12 Chemical structure of bacitracin zinc.	34
Figure 13 Structural components of Bacitracin. Asn-asparagine, Asp-aspartic acid, Glu-glutaminic acid, His-histidine, Ile-isoleucine, Leu-leucine, Lys-lysine, Orn-ornithine, Phe-phenylalanine; Val-valine. R: the end group of the side chain; (A): L-cysteine and L-isoleucine; (B): L-cysteine and L-valine; (C) and (D) are oxidative forms of their corresponding end groups (82).	35
Figure 14 Chemical structure of neomycin sulfate.	37
Figure 15 (A) Miscible and immiscible oil pairs of silicone oil and (1) olive oil, (2) safflower oil, (3) sesame oil, (4) castor oil, (5) sweet almond oil, and (6) sunflower oil and (B) miscibility study of sesame oil with silicone oil at various ratios (1) 1:4, (2) 2:3, (3) 1:1, (4) 3:2, and (5) 4:1 after keeping at 25°C for 24 h.	54
Figure 16 Determination of CGC of SMS for five different ratios of sesame oil and silicone oil oleogels (A) 1:4, (B) 2:3, (C) 1:1, (D) 3:2, and (E) 4:1. The concentration of SMS for each ratio was varied from 5-22% w/w. The CGC is the minimum gelator	

concentration which failed to flow when the tubes were inverted after keeping at 25°C for 24 h. 55

Figure 17 Polarized light micrographs of five different ratios of sesame oil and silicone oil oleogels at CGC of SMS. (A) 1:4, (B) 2:3, (C) 1:1, (D) 3:2, and (E) 4:1. The gel network formed by association of tubules at contact points and appeared at all ratios as demonstrated by arrows. 56

Figure 18 Determination of CGC of SMS for three oleogels (A) OG1, (B) OG2 and (C) OG3. The concentration of SMS for each oleogel was varied from 5-22% w/w. The CGC is the minimum gelator concentration which failed to flow when the tubes were inverted after keeping at 25°C for 24 h. 58

Figure 19 FTIR spectra of oleogels and their ingredients. 60

Figure 20 Polarized light micrographs of three oleogels at CGC of SMS (A) OG1, (B) OG2 and (C) OG3. 62

Figure 21 (A) Amplitude sweep result of oleogels: Storage modulus (G' , open symbols) and loss modulus (G'' , filled symbols) as function of shear strain for OG1 ($\circ/\bullet$), OG2 ($\diamond/\blacklozenge$) and OG3 ($\square/\blacksquare$). The strain range was 0.001 to 10 Pa at a fixed frequency of 1 Hz. (B) Frequency sweep result of oleogels: Storage modulus (G' , open symbols) and loss modulus (G'' , filled symbols) as function of frequency for OG1 ($\circ/\bullet$), OG2 ($\diamond/\blacklozenge$) and OG3 ($\square/\blacksquare$). The frequency range was 0.1-10 Hz at the strain value of 0.05 Pa. 64

Figure 22 Viscosity profiles of OG1 ($\circ$), OG2 ($\diamond$) and OG3 ($\square$) at the shear rate range of 0.1-100 s^{-1} 65

Figure 23 Thixotropic behavior of OG1 ($\circ$), OG2 ($\diamond$) and OG3 ($\square$) using three step shear rate sweeps. 66

Figure 24 XRD patterns of oleogels and their ingredients. 69

Figure 25 Thermal analysis of (A) heating cycle and (B) cooling cycle of oleogels and their ingredients. 71

Figure 26 Light micrographs of bigels (A) BXG, (B) BXGS, (C) BGG, and (D) BGGS. Green droplets represented the oleogel phase and the pale pink background was hydrogel phase. 76

Figure 27 FTIR spectra of drug loaded bigels and their ingredients 77

Figure 28 Viscosity profiles of drug loaded bigels BXG ($\bullet$), BXGS ($\circ$), BGG ($\blacksquare$) and BGGS ($\square$) at the shear rate range of 0.1-100 s^{-1} 78

Figure 29 Amplitude sweep test of bigels: Storage modulus (G' , solid line) and loss modulus (G'' , dashed line) as function of shear strain for BXG (●), BXGS (○), BGG (■) and BGGS (□). The shear stress range was 0.01 to 100 Pa at a fixed frequency of 1Hz. 81

Figure 30 XRD patterns of bacitracin zinc, SMS, drug loaded oleogels, hydrogels and drug loaded bigels. BOG3 is bacitracin zinc loaded oleogel containing mixture of silicone oil and sesame oil. BOG1 is bacitracin zinc loaded oleogel containing sesame oil. 84

Figure 31 Drug content determination of bacitracin zinc loaded bigels. 85

Figure 32 In vitro drug release study of bacitracin zinc loaded bigels 87

Figure 33 (A) Antimicrobial activity and (B) inhibition zone of bacitracin zinc loaded bigels against *Staphylococcus aureus*. 0.77 % w/w of bacitracin zinc in sodium acetate buffer (pH 5.5) and blank bigels were used as positive control and negative control. 89

Figure 34 Drug content determination of bacitracin zinc loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months. 92

Figure 35 Antimicrobial activity of the bacitracin zinc loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months. 93

Figure 36 Light micrographs of bigels (A) NGG and (B) NGGS. Biphasic nature of bigels with the oleogel phase (green droplets) and the hydrogel phase (pale pink background). 95

Figure 37 FTIR spectra of drug loaded bigels and their ingredients 97

Figure 38 Viscosity profiles of drug loaded bigels NGG (■) and NGGS (□) at the shear rate range of 0.1-100 s⁻¹. 99

Figure 39 Amplitude sweep test of bigels: Storage modulus (G' , solid line) and loss modulus (G'' , dashed line) as function of shear strain for NGG (■) and NGGS (□). The shear stress range was 0.01 to 100 Pa at a fixed frequency of 1Hz. 100

Figure 4.40 XRD patterns of neomycin sulfate, SMS, oleogels, drug loaded hydrogel and drug loaded bigels. OG3 is oleogels with a mixture of silicone oil and sesame oil and OG1 is oleogels containing sesame oil. 102

Figure 41 Drug content determination of neomycin sulfate loaded bigels. 104

Figure 42 In vitro drug release profile of neomycin sulfate loaded bigels 106

Figure 43 Antimicrobial activity of the neomycin sulfate loaded bigels against *Staphylococcus aureus*. The positive control and negative control were 0.5% w/w neomycin sulfate in water and blank bigels. 108

Figure 44 Drug content determination of neomycin sulfate loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months. 110

Figure 45 Antimicrobial activity of neomycin sulfate loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months. 111



LIST OF ABBREVIATIONS

®	registered trademark
±	plus-minus sign
2θ	two theta
%	percentage
% w/w	percent weight by weight
ΔH_m	melting enthalpy
ΔS_m	melting entropy
Å	angstrom
ATR	Attenuated Total Reflectance
AUC	area under the curve
BXG	Bacitracin zinc loaded xanthan gum bigel containing sesame oil
BXGS	Bacitracin zinc loaded xanthan gum bigel containing mixture of sesame oil and silicone oil
BGG	Bacitracin zinc loaded guar gum bigel containing sesame oil
BGGS	Bacitracin zinc loaded guar gum bigel containing mixture of sesame oil and silicone oil
BOG1	Bacitracin zinc loaded oleogel containing sesame oil
BOG 3	Bacitracin zinc loaded oleogel containing mixture of sesame oil and silicone oil
°C	degree Celsius
cal/g	calorie (s) per gram
cSt	centistoke (s)
cm	centimeter (s)
cm ⁻¹	wavenumber
C18	18 carbon atoms
CGC	critical gelling concentration
CFU	colony-forming unit
CPDR	cumulative percent drug release
DAD	diode array detector
DSC	Differential Scanning Calorimetry

EDTA	ethylenediaminetetraacetic acid
et al.	and others
etc.	et cetera (latin); and other things/ and so forth
FTIR	Fourier Transform Infrared Spectrometer
FWHM	full width of the peak at half maximum
g	gram (s)
gs ⁻¹	gram (s) per second
G'	storage or elastic modulus
G''	loss or viscous modulus
h	hour(s)
Hz	hertz
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
i.e.	id est (Latin); that is
Jg ⁻¹	joule per gram
Jg ⁻¹ K ⁻¹	joule per gram per kelvin
kg	kilogram (s)
kV	kilovolt (s)
K	consistency index
KBr	potassium bromide
LVER	linear viscoelastic region
M	molar
mM	millimolar
mA	milliampere
min	minute (s)
mg	milligram (s)
mL	milliliter (s)
mm	millimeter (s)
µm	micrometer (s)
µL	microliter (s)
mN/m	millinewton per meter
mPas	millipascal-second

n	flow behavior index
nm	nanometer (s)
NGG	Neomycin sulfate loaded guar gum bigel containing sesame oil
NGGS	Neomycin sulfate loaded guar gum bigel containing mixture of sesame oil and silicone oil
OG1	Oleogel containing sesame oil
OG2	Oleogel containing silicone oil
OG3	Oleogel containing mixture of sesame oil and silicone oil
Pa	pascal (s)
pH	potential hydrogenii (latin); power of hydrogen; hydrogen ion concentration
PEG	polyethylene glycol
PPG	polypropylene glycol
rpm	revolution (s) per minute
s	second (s)
SD	standard deviation
SMS	sorbitan monostearate
TSA	tryptic soy agar
T _{onset,m}	onset temperature
T _m	peak maximum temperature
USP	United States Pharmacopoeia
UV-visible	ultraviolet-visible
XRD	X-Ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the research problem

A topical delivery system is a desirable system for local drug delivery and offers significant advantages over other routes of administration. It has the ability to deliver drug substances more selectively to a specific site, improving patient compliance, avoiding fluctuations in drug levels, inter- and intra-patient variations, and an enhanced suitability for self-medication (1, 2). Topical products are categorized into three groups: liquid, semi-solid, and solid. The most commonly used topical products are semi-solid dosage forms (3, 4). Lotions, solutions and suspensions are liquid topical products; creams, foams, gels, ointments and pastes are semi-solid topical products and powders, patches, tapes and sticks are solid topical products (5). Among the topical semisolid preparations, gels are becoming more popular both in cosmetic and pharmaceutical applications due to their advantages such as ease of application and better percutaneous absorption (6). Gels are semisolid formulations in which a liquid phase is entrapped within a three-dimensional network. Unlike ointments or creams, gels contain a higher aqueous component that permits greater dissolution and facilitates migration of drugs through the vehicle. In general, there are two main types of gels based on the polarity of the liquid phase: hydrogels (polar) and organogels (non-polar) (7). Hydrogels are the most common and well-known type of topical drug delivery system due to increasing hydration of stratum corneum, easy spreading onto the skin, and cooling effect. They consist of a hydrophilic polymer network in which an aqueous phase has been trapped (7, 8). Natural and synthetic polymers have been used for hydrogel preparations, (9-16). Although hydrogels provide great benefits, they have several limitations such as difficulty in the loading of hydrophobic drugs, microbial contamination in polysaccharide-based hydrogels and the reduction of skin permeability across the stratum corneum (7). Due to the extensive amount of hydrophobic bioactive molecules in pharmaceutical, cosmetic and food applications, the interest in the potential use of organogels has grown rapidly (17). Organogels contain organic liquid which is physically immobilized within the network of self-assembled aggregates of gelator molecules (7). Animal and vegetable oils are used as organic solvents for organogel

preparations due to their non-toxic and biodegradable properties. These oil-based preparations are called oleogels rather than organogels. Similar to organogels, oleogels are a mixture of gelator and liquid oil (17). Sorbitan monostearate (SMS), an FDA approved non-ionic surfactant, is a commonly used gelator for oleogel preparations. It can form gel with a variety of solvents such as cyclohexane, octane, isopropyl myristate, ethyl oleate, sesame oil and olive oil (18-20). Sesame oil, a vegetable oil, has been extensively used in pharmaceutical and cosmetic applications as a result of its antioxidant, antiseptic, anti-inflammatory and antibacterial properties (21, 22). The oleogel systems offer a great benefit in delivering hydrophobic compounds since the components of oleogels such as surfactants and essential oils can serve as penetration enhancers and improve the penetration of drug (7). However, for cosmetic and pharmaceutical applications, oleogels have the major drawback of being greasy and unpleasant nature due to the oily nature of natural oils. To reduce the greasy and unpleasant nature of SMS-sesame oil oleogel, the combination of synthetic and vegetable oil for oleogel preparations was explored for the first time in this study. Silicone oil offers complementary benefits in topical preparations. It is widely used for topical products due to its compatibility with different types of lipophilic agents. The combination of vegetable oil and silicone oil can provide better moisturizing and occlusive properties, improving spreadability and aesthetic properties, and reducing stickiness of the formulations (23). It has been reported that the addition of 10% silicone oil in borage oil greatly reduced the surface tension and viscosity, and improved sensory attributes (24). There were several reports on the instability of SMS based oleogels upon long term storage. The problem of syneresis was observed and still remains unsolved (25-27). To tackle the stability problem with the application of silicone oil has never been investigated. The strong siloxane bond contributed to the high degree of thermal and oxidative stability of silicone oil enabling more stable formulations (28). The stability of vegetable oils can also be improved by reducing the unsaturation with less unsaturated oils. Boskou showed that the addition of 1mg/kg of methyl silicone in sunflower oil showed significant effect on preventing oxidation by lowering the degree of foaming and causing a reduction in oxidation products (29). In addition to the application of silicone oil in solving the weakness of oleogels, the search for advanced dosage form has so far been encouraging and warrants further investigation. The dosage

form which currently gains a wide benefit is bigel. An approach to solve this drawback of oleogel with the application of bigel together with silicone oil was then introduced.

Bigels are novel semisolid formulations offering the combined advantages of both hydrogels and oleogels. They have been of great interest in the food and cosmetics fields but have not been widely studied in pharmaceutical fields. A few studies showed bigels providing a good performance for controlled drug delivery system (12, 30-32). Due to its biphasic nature, there is a value in extending investigations into its pharmaceutical applications. They have the ability of loading both hydrophilic and hydrophobic drugs and can improve the hydration of the stratum corneum, ease of spreadability and water washability (9, 31, 33-35). Despite the fact that bigels are biphasic, the problems of thermodynamic instability and leaching of internal phase have never been reported. The stable bigels are a result of the semisolid nature of both phases, unlike emulsions and emulgels (9, 34). As it possesses superior properties over other topical dosage forms, bigel can be a promising candidate for topical drug delivery system.

In this study, bacitracin zinc is selected as the hydrophobic model drug and neomycin sulfate as the hydrophilic model drug. Bacitracin zinc, a polypeptide antibiotic, is mainly used in the treatment of ophthalmic and dermatological infections. Conventional formulations of bacitracin zinc such as ointments and creams are available in the commercial market. However, absorption of bacitracin zinc is limited by poor penetration through the stratum corneum due to its high molecular weight and size (36-38). The problems related to this kind of drug can be reduced by loading it into bigels. Silicone oil could improve the skin coverage and increase the bioavailability of the active ingredients due to its lower surface tension properties (39-41). Hence, the incorporation of bacitracin zinc into silicone-sesame oil oleogel and its formulation into bigel could improve the spreadability of drug over the skin and provide sufficient drug release. Neomycin sulfate, hydrophilic model drug was loaded into hydrogel phase. It is a hydrophilic aminoglycoside antibiotic with gram-positive and gram-negative bactericidal activity. It is widely used in many topical medications for the prevention or treatment of skin infections (42).

Therefore, the aim of this work is to develop a new topical dosage form for both hydrophobic and hydrophilic drugs. The novel bigel was then initiated with the addition

of silicone oil. The developed bigel was then characterized for their physicochemical properties such as microscopy, FTIR, rheological and mechanical properties, drug content, *in vitro* drug release and antimicrobial activity.

1.2 Objectives

The objectives of this research were:

- (1) To design a novel bigel with the application of silicone oil as a topical drug delivery carrier
- (2) To prove the potential of the developed bigel as a carrier for both hydrophobic and hydrophilic drugs using bacitracin zinc and neomycin sulfate as model drugs
- (3) To gain insight into oleogel and bigel as carriers for topical dosage form
- (4) To evaluate the effect of silicone oil on the properties of the developed bigel (i.e. drug solubility, drug release, antimicrobial activity and stability)

1.3 The research hypothesis

- (1) A novel bigel consisting of hydrogel and oleogel phases can be designed with the application of silicone oil.
- (2) The developed bigel can potentially be used as a carrier for topical delivery of both hydrophobic and hydrophilic drugs.
- (3) The addition of silicone oil can improve the properties of the developed bigels (i.e. drug content, drug release, antimicrobial activity and stability).

CHAPTER 2

LITERATURE REVIEW

2.1 Topical drug delivery systems

A topical drug delivery is the direct application of the pharmaceutical dosage form onto the skin for the treatment of the dermatological disorders or the cutaneous manifestations of systemic diseases (43). Nowadays, it has attracted the researchers' interest and various drugs have been delivered successfully by topical route for both local and systemic effects. Various types of drugs; antivirals, antibiotics, corticosteroids, antiseptics, local anesthetics, anti-fungal, and antineoplastic, can be delivered topically (44). The topical drug delivery system is more advantageous over conventional methods (injectable or oral delivery), in terms of many aspects (45). These potential advantages include ease of application, better patient compliance, and avoidance of first-pass metabolism. The main advantage is the ability to deliver drugs more selectively to a specific site (46). Topical products can be classified into three major groups: liquid, semi-solid, and solid (5). Among the topical preparations, semisolid dosage forms are the most commonly used and exist in multi-forms; ointments, lotions, gels, and creams (4). Due to their peculiar rheological characteristics, semisolid dosage forms can spread easily and adhere to the application surface for sufficiently long period before they are washed off. As discussed above, the major pluses of these semisolid dosage forms are ease of application, fast formulation, and ability to deliver various types of drug molecules (47). Besides this, there exist certain challenges and barriers for topical drug delivery as well. For example, skin's barrier property is the major challenge for topical delivery of many large and small molecules (43). This barrier property of the skin is usually associated with the thin outer layer of the epidermis; known as stratum corneum (48). The stratum corneum that comprises of keratinized cells ranges from 10 to several hundred micrometers in thickness and the macromolecules cannot easily pass through this dermal layer. Concludingly, it is regarded as the first line permeability barrier (7). Unlike the other body's tissues, the stratum corneum consists of the layers of dead corneocytes and these are neighbored by an extracellular lipid matrix. That extracellular lipid matrix resembles a "brick and mortar system" (Figure 1). These structured lipids prevent the

excessive loss of water from the body. In the same way, it blocks the entry of most commonly used topical drugs; except the ones that have a low molecular weight and are soluble in lipids. This fact put-forwarded a serious challenge to administering medications through the skin either as systemic therapy following their entry into the superficial dermal capillaries or for the local cutaneous effects (48). Although the recent advances in nanotechnology improved the drug pharmacokinetics and enhanced the drug penetration through the skin, a more innovative form of carrier system still need to develop for the topical drug delivery. However, in designing the effective topical delivery system, the gel can prove to be a beneficial vehicle for both local and systemic effects (7).

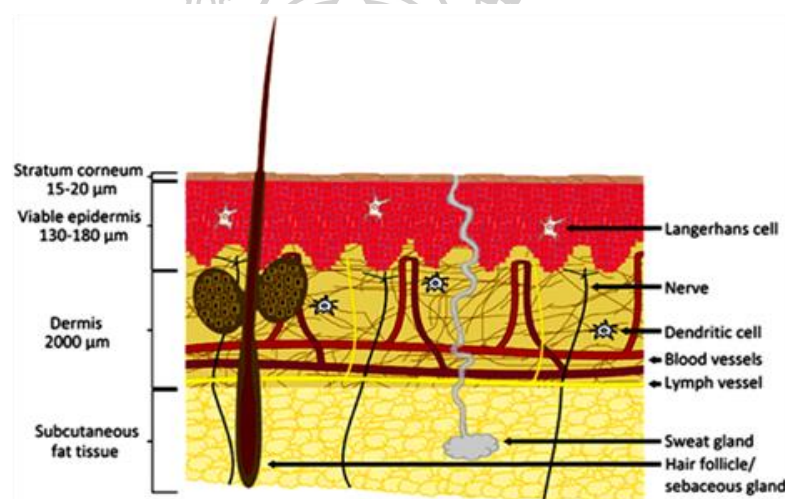


Figure 1 The skin structure and its layers. Three main layers of the skin are epidermis, dermis and subcutaneous layer. The stratum corneum is known as the outermost layer of the epidermis (49).

2.2 Gels for topical drug delivery

Gels are semisolid preparations consisting of a hydrophilic or hydrophobic liquid phase which is immobilized within a three-dimensional polymeric matrix. As compared to creams and ointments, gels contain a higher water content and thus offer greater dissolution and easy migration of the drugs through a vehicle. Moreover, the gels can provide skin hydration property since they can retain a significant amount of transepidermal water and in this way, they facilitate the drug transport. Based on the

nature of the liquid phase, gels may be differentiated into hydrogels and organogels (oleogels). The liquid phase is 'aqueous' in a hydrogel, whereas, the liquid phase is 'lipophilic' in an oleogel. Besides hydrogels and oleogels, the additional types of gels (Figure 2), such as emulgels, bigels, and aerogels have also been reported for dermal application of drugs (7). Emulgels resemble emulsions; are biphasic systems. However, unlike the emulsions, emulgels' external phase is semisolid in nature that assists in improving the emulgels' thermodynamic stability. This yields semisolid formulations with combined merits of both emulsions and gels. However, the major weakness of the emulgels is the leaching of the internal oil phase during long term storage. This stability problem therefore urged the researchers to explore more stable formulations for long term storage. This leaching problem was mainly due to mismatching of mechanical properties between the internal and the external phases of emulgels. Coping this, the bigel dosage form has been introduced in recent years. The bigels resemble emulsions and emulgels; are biphasic systems. However, in bigels, both the external and internal phases are semisolid in nature, unlike the emulsions and emulgels (50). The aerogels are formed when the liquid phase of the gels is replaced by the air with a moderate shrinkage in the solid gel network (51). Since aerogels and xerogels compose of silica, they are also considered as 'inorganic gels'. Nevertheless, the aerogels have been considered rather more effective than the xerogels as of the fact that their bio-availability and drug solubility can be adjusted by the addition of different functional groups which control their release kinetics (52).

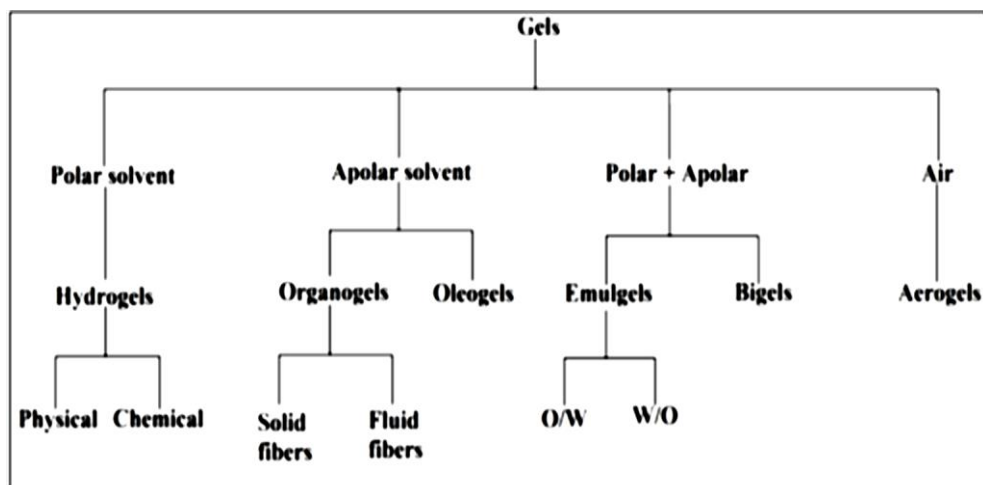


Figure 2 General classification and types of gels (53).

2.3 Oleogels

Organogels are gels containing non-polar liquids (alcohols, aliphatic and aromatic hydrocarbons, and dimethyl sulfoxide) (54, 55) or oils (silicone oil, olive oil, and castor oil) (54, 56) as the liquid phase. The gelator molecules entrapped the liquid phase within a three-dimensional solid network structure. Although many studies have done for the various organogel systems, only a few organogels can be applied for pharmaceutical fields due to the toxicity of the organic solvents. Thus, the vegetable oils are considered as an alternative to the conventional organic solvents to be used as the continuous phase of the organogels. It can provide the formation of non-cytotoxic organogels; that is suitable for application *in vivo*. These vegetable oil gels are called oleogels and they have attained the subsequent attention as its easy conversion from liquid oil into the gel-like structure, regardless of any change in the chemical properties of vegetable oil through oleogelation (57). As far as the gel-based products are considered, the usage of oleogels is being increased. That can be perhaps attributed to the easier preparation methods and long-term inherent stability of such products (58). Recently, many researchers have paid immense interests towards the oleogels in many aspects; for instance, cosmetics, pharmaceuticals, food, and petrochemistry as shown in Figure 3 (57).

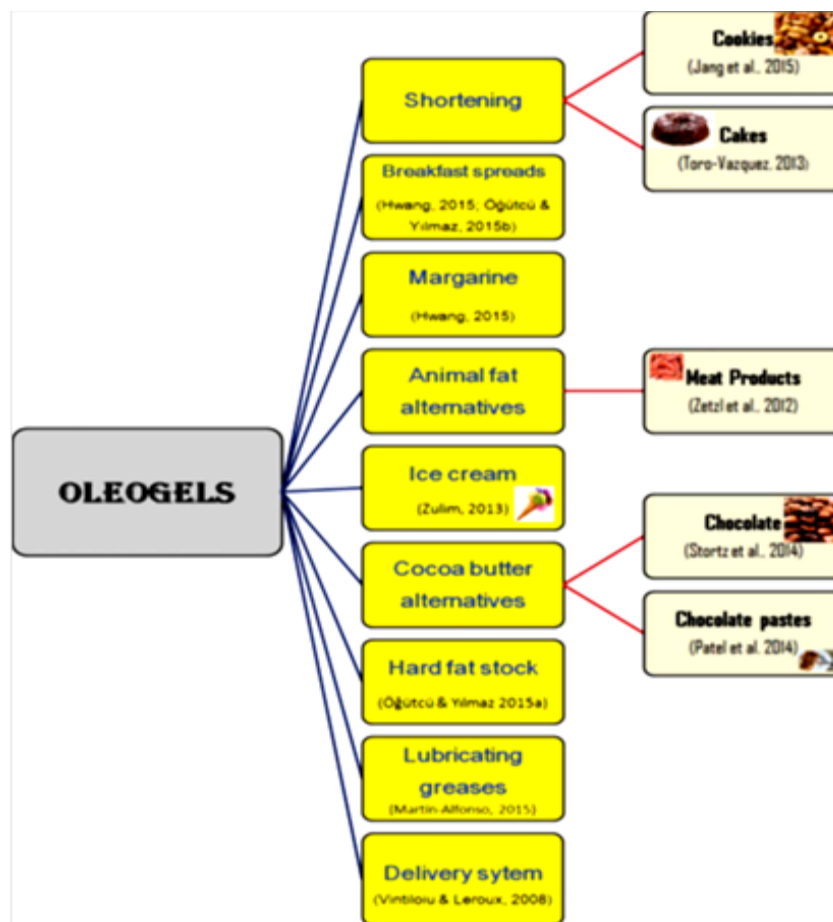


Figure 3 Diverse application of oleogels in various fields (57).

2.3.1 Components of oleogels

The formation of oleogels generally requires two materials; the solvent (oil) and the oleogelator that is used to entrap the liquid oil into a three-dimensional gel network.

2.3.1.1 Oils

The most commonly used organic solvent for oleogels are the oils as they have certain features such as their availability, emollient property, economical price, compatibility, and high spreading ability. Different types of oils that can be used for oleogel preparations include vegetable oils (sweet almond, soybean, and castor), mineral oils (vaseline oil), and synthetic oils (caprylic/capric triglycerides, 2-ethylhexyl palmitate, isopropyl myristate, and volatile or nonvolatile silicone oils) (59). The type of oils is an important factor that affects the textural, rheological and

thermal properties of the oleogels in addition to the concentration and the type of the oleogelator (57).

Types of oils used for oleogel preparations

(1) Vegetable oils

Vegetable oils are widely used in the preparation of various foods, pharmaceuticals, and cosmetic products mainly due to the functional and beneficial properties regardless of their inherent physical stability. The major ingredients of vegetable oils are glycerol esters, fatty acids with partially glyceridic material (lecithin), and tocopherol (60). Previous studies developed the oleogels with different vegetable oils such as mustard oil, sunflower oil, sesame oil, olive oil, and hazelnut oil (19, 20, 27, 61, 62). Edible oils' gelation is related to the gelation of other organic solvents. Nevertheless, the edible oils consist of a mixture of complex molecules and can be considered even more polar than the other organic solvents. Moreover, they influences the gelator molecules' solubility (63). These details can be outlined as the fact that gelators' gelling ability differ on the basis of the fatty acid composition, acyl chain length, and molecular weight of the edible oils. Lower concentration of gelators can be used for oleogels prepared with vegetable oils that contains high oleic acid content; for instance, olive oil, sunflower oil, and rice bran oil. The usage of lower amount of gelators can also result in economic benefits. In addition to textural properties and low cost, the healthy aspect due to presence of the high content of polyunsaturated fatty acids and wide availability are also the great benefits (57).

Sesame oil

Sesame oil is extracted from the *Sesamum indicum*'s ripe seeds. It is a pale yellowish color, clear oil that has nutty odor and taste. It is considered as the queen of the oils due to its highest antioxidant content among the primary edible oils (60). Sesamin, sesamol, sesamolin, and phytosterol are major active components and they provide remarkable stability against oxidative deterioration. The other components include stearic acid (7%), palmitic acid (11%), linoleic acid (35%), and oleic acid (43%) (22, 60). Due to its high nutritional and therapeutic values, it is markedly different from other vegetable oils and has wide applications in the food, cosmetic, and pharmaceutical

fields (21, 22). In the pharmaceutical fields, sesame oil is commonly used as a solvent for intramuscular injections and has demulcent, emollient, and mild laxative properties. It has antibacterial activity against *Staphylococcus* and *Streptococcus* and antifungal activity against common skin fungi, for example; athlete's foot fungus. In addition, sesame oil contains low-density lipoprotein (LDL) and high-density lipoprotein (HDL) and they provide lipid lowering property. Due to the presence of high antioxidant components like lignans, the refined sesame oil permits greater shelf-life of foods and also improve their flavor and taste (21). Sesame oil is regarded as one of the commonly used solvent for oleogel preparations and sesame oil based oleogels have investigated intensively with different types of gelators during recent years. Moghtadaei et al. (2018) investigated the potential of sesame oil-beeswax oleogel for food application. They reported that the good sensory attributes and the significant reduction of fat absorption were the good aspects of the oleogel which could improve the nutritional value and textural properties of food products (64). According to previous findings, it was demonstrated that sesame oil is the good solvent for oleogel preparation since it can use individually or by combination with other types of oils (20, 65). The critical gelling concentration of gelator (sorbitan monostearate) was dependent on the polarity and viscosity of the solvent and the CGC of SMS can reduce from 20% to 10% w/w by using a combination of sesame oil and silicone oil (65).

(2) Mineral oil

Mineral oil is produced from petroleum by hydrogenation or by intensive treatment with sulphuric and oleum, or a combination. It is a colorless, odorless, tasteless oily liquid and consists predominantly of saturated C15 – C50 hydrocarbons. According to the chemical composition, the mineral oil can be classified into different viscosity grades. The major difference between the heavy mineral oil (high viscosity) and the light mineral oil (low viscosity) is that the heavy mineral oil contains straight chain alkyls with 25 to 50 carbon atoms whereas the light mineral oil contains more saturated cyclic molecule. Mineral oil increases skin hydration by reducing the transepidermal water loss and provides occlusive and emollient effects. It has wide range of application in different areas like cosmetic, food, industrial, mechanical, and medicinal fields. It is very safe ingredient for cosmetic use due to the

limited penetration and its effects is mainly on the epidermal layers. The major difference between mineral oil and vegetable oil is their chemical composition. Mineral oil contains straight chain hydrocarbons whereas vegetable oil consists of various chemical constituents (unsaturated aromatic groups). It also enhances the skin softness better than fatty acids, triglycerides, and wax esters. In cosmetic field, mineral oil is used in higher concentration for its occlusive and emollient effects, however, only small amount of vegetable oils is used to obtain a specific effect of a specific ingredient (66). Recent works on mineral oil (liquid paraffin) based oleogels has focused on how the oil type affected their mechanical properties. Almedia and Bahia reported that the liquid paraffin oleogel was a weak gel with lower mechanical properties as compared to sweet almond oil oleogel (25). Gravelle et al. also investigated the mechanical properties of ethyl cellulose oleogels by partial replacement of vegetable oil with mineral oil and castor oil. They reported that mineral oil had no ability to form hydrogen bonds as it is composed exclusively of alkanes. Hence, the partial substitution of vegetable oil with mineral oil reduced the number of hydroxyl groups in the glycerol head and resulted in lower gel rigidity and firmness (67).

(3) Synthetic oils

Volatile silicone oil (Cyclomethicone or Cyclopentasiloxane)

Silicone products are well known and have been increasing attention around the world as a result of chemical stability and biological compatibility. They are synthetic polymers and consist of siloxane bonds (Si–O). Among the silicone products, polydimethylsiloxanes (PDMS) are the abundant silicone polymers. The PDMS are differentiated from other polymers (organic or inorganic). The distinct properties of PDMS such as highly flexible siloxane backbone, partial ionic nature and high bond energy of the siloxane bond, compact size of methyl groups, and the low intermolecular forces between pendant methyl groups are the main reasons for their applications in various fields. In addition, they had adopted in various healthcare applications due to their physiochemical properties and availability of different physical forms (68).

The silicone materials have been reported to use in a wide range of topical products. In pharmaceutical applications, dimethicone and cyclomethicone are

the most commonly used topically applied silicones as a result of their unique characteristics like low surface tension, emollient, and skin protection. Various types of silicones have been successfully applied in water-based products like emulsions even though they are very hydrophobic in nature. After the cosmetic industries had gained the great benefits from silicone-based products, the application of silicones had significantly extended to pharmaceutical fields (68). The silicone materials have generally been considered as safe and pleasant aesthetics preferred by consumers. The improvements of the physical properties such as silkier elegant, easy spreadability, less-tackiness, and more lubricious feel have been observed in silicone-containing formulations when compared with formulations without silicones (69).

Cyclopentasiloxane, a volatile silicone oil is commonly used for topical pharmaceutical products due to its compatibility with a wide range of lipophilic products. Its chemical structure is depicted in Figure 4. When the volatile siloxanes evaporate, they remove very little heat from the skin thus can avoid potentially undesirable, chilling or cooling effect and leave the skin feeling warm and soothed. Lotions and creams containing volatile silicone oil can impart the smooth spreading, ease of application and provide a smooth transition in spreadability without leaving an undesirable greasy or fatty feel. Due to the lower surface tension property, volatile silicone oil can improve the skin coverage thus may lead to increase the bioavailability of active ingredients.

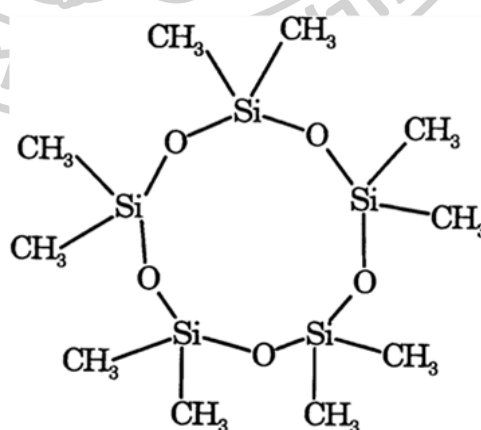


Figure 4 Chemical structure of volatile silicone oil (cyclopentasiloxane).

2.3.1.2 Oleogelators

The oleogelators also known as the structuring agents are the substances which immobilize the liquid oils during the conversion of liquid phase into solid gel network. Vegetable waxes, alcohols or esters of fatty acids, phospholipids, monodiglycerides, and phytosterols are commonly used oleogelators (57). Generally, most of the oleogelators can form gels at very low concentration. The concentration of oleogelators (0.5-2%) is adequate for gelation of edible oils whereas less than 0.5% is adequate for gelation of organic solvent (70). Typically, the higher concentration of gelator is used for food applications, however, the gelator concentration depends on the desired quality of the oleogel products.

The ideal properties of the oleogelators are:

- (i) presence of lipophilic and interactive groups
- (ii) surface activity
- (iii) thermoreversible in nature
- (iv) natural origin, and
- (v) generally recognized as safe (GRAS) grade (71, 72).

There are two types of oleogelators; one component and two component oleogelators. One component gelators can gel the organic solvent alone. In contrast, the two-component gelators require one or more other components to gel the organic liquid. According to the molecular weight of the gelators, the oleogelators can be categorized into two types. They are

- (i) Low molecular weight oleogelators (LMWOGs)
- (ii) Polymeric oleogelators (POGs)

(i) Low molecular weight oleogelators (LMWOGs)

Low molecular weight gelators have good characteristics like biocompatibility, low toxicity and greater flexibility. The great advantage is their good solubility in organic solvents when heating and smooth gelation after cooling. They can form stable and efficient gels with different types of organic solvents even at low concentrations ($\leq 2\%$ w/w). Their gelling capacity is (< 50 g/ L) and forms gelation by physical molecular interactions such as hydrogen bonding, electrostatic attraction, and London dispersion or van der Waals forces. This type of organogels shows thermoreversible and

thixotropic nature. Examples of low molecular weight organogelators are fatty acid and fatty acid esters (sorbitan fatty acid esters and 12-hydroxy stearic acid), sterols, waxes and wax esters (58).

Sorbitan Monostearate

Sorbitan monostearate (SMS), also called Span 60 is a hydrophobic non-ionic surfactant. Due to its non-toxic and biocompatible properties, SMS is commonly used as an emulsifier for food, cosmetic and pharmaceutical applications. Hydrophilic and lipophilic balance (HLB) of SMS is approximately 4.7 which favors water-in-oil emulsions. Recently, many research groups have tried to use SMS as a gelator (structuring agent) to develop the oleogels and it can form gels with various types of organic solvents (hexane, octane, squalene) and vegetable oils (canola oil, olive oil, sesame oil). The gel formation depends on the solvent-gelator interaction. The gelation occurs when the gelator molecules were dissolved or dispersed in the hot solvent to produce solution or dispersion. Upon cooling, solvent-gelator affinities decrease and the gelator molecules self-assemble into toroidal inverse vesicles. Further cooling, these vesicles convert into the rod-shaped tubules which associate with each other and form a three-dimensional gel network that immobilizes the liquid solvent. SMS based oleogels are opaque semisolids, thermoreversible in nature and stable for weeks at room temperature.

The gelation of SMS depends on the gelator concentration and types of the organic solvents. SMS has both polar and non-polar groups and the polarity of the solvent is the major factor which influences its solubility. The critical gelling concentration of the minimum gelator concentration which can form gels. In the study of Murdan et al, it was found that the CGC of SMS was significantly different due to the different solubility of SMS in both polar and non-polar solvents. Murdan also reported that the solvent should have optimum solubility and insolubility balance. It means that if the gelator is very soluble in solvent, the solution will produce instead of gel. Similarly, if the gelator is insoluble in the solvent, only precipitation and the gel cannot form. The presence of additives can also affect the properties of oleogels. The addition of hydrophilic surfactant (Tween 20) improved the stability of oleogels and altering the microstructure of oleogels into star-shaped clusters. This may be due to

greater solubility of SMS in hexadecane and also better stability induced by stronger gel network. Figure 5 shows the chemical structure of SMS (18, 20).

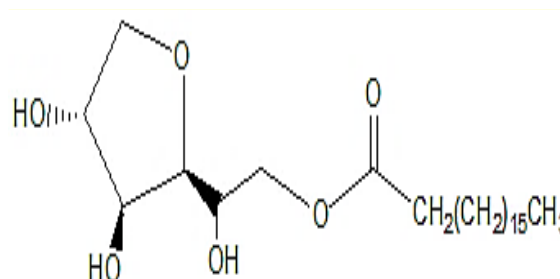


Figure 5 Chemical structure of sorbitan monostearate.

(ii) Polymeric organogelator

Polymeric organogelator acts in similar way like LMW organogelators. The gel network is formed by physical interactions between polymer molecules and this entraps the organic solvents. They produce higher gel strength when compared with LMW organogelators. Polyethylene is an example of polymeric organogelator it is used as gelator for mineral oil (Plastibase®) (58).

2.3.1.3 Mechanism of gelation

Hydrogen bonding, van der Waals (hydrophobic) and π -stacking interactions are the interactive forces that govern the formation of the three-dimensional network in the oleogels. Based on the mechanism of three-dimensional network formation, the oleogels are further differentiated into the solid fiber-based oleogels and fluid-filled structure (Figure 6). In the first method, the gelator is heated in an apolar solvent until it is completely solubilized and then the temperature is decreased below the crystallization temperature of the gelator. At that time, the gelator molecules start to precipitate and self-assemble to form solid aggregates by physical interaction. These resulting aggregates form a 3D gelation network which immobilizes the solvent. In the second mechanism, the oleogels are formed by the addition of polar solvents to the surfactant solution in apolar solvents enabling the reverse micelles formation. The reverse micelles form structures of different shapes which undergo aggregation to form 3D networks which entrap the solvent. Both gelation mechanisms cause the formation

of 3D networks by non-covalent interactions, however, the major difference is the stability between these types of the oleogels. Due to the presence of large junction points, solid fibers based oleogels is usually strong as compared to fluid-filled structure oleogels (54).

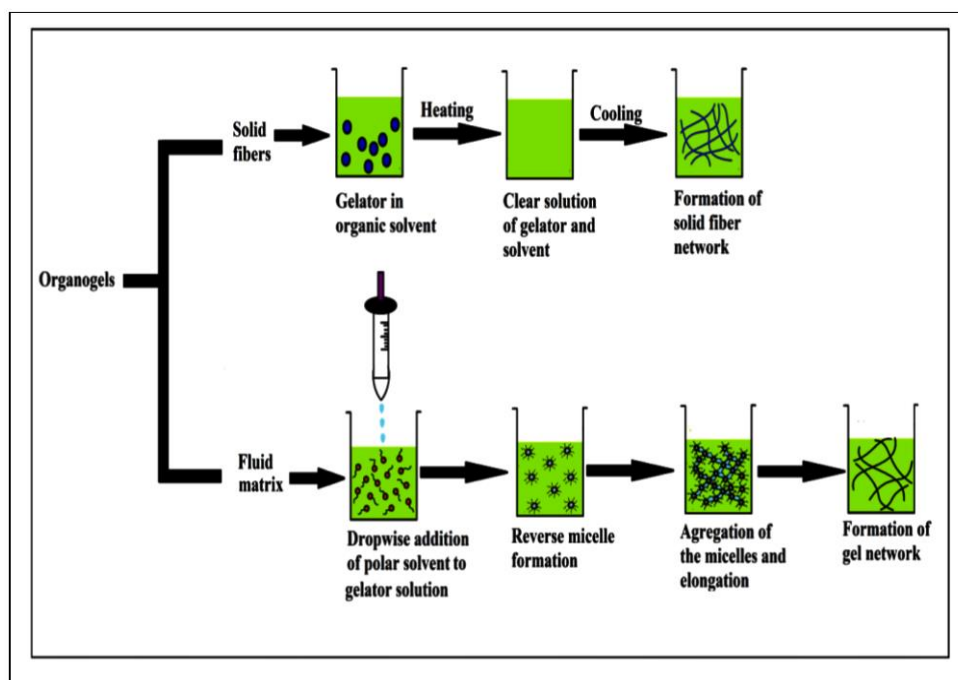


Figure 6 Mechanism of gelation of oleogels (53).

2.3.2 Silicone surfactant (cyclomethicone and PEG/PPG -20/15 dimethicone)

Silicone surfactant consists of both water and silicone-soluble groups within the same molecule. Dimethicone copolyol, amphiphilic compound is one of the commonly used silicone surfactants. Various types of dimethicone copolyol are available in the market and they offer different HLB values depend on the ratio of grafted groups onto a polydimethylsiloxane backbone. Due to the physical properties and usefulness, dimethicone has been extensively applied in various fields. However, the low solubility in both polar and apolar solvents is the major limitation of dimethicone. The grafting of either polyethylene glycol (PEG), polypropylene glycol (PPG) or both increases the solubility of dimethicone as a result of water-soluble

properties of polyoxyalkylene components. The siloxane backbone provides silicone soluble (siliphilic) property with low HLB value whereas polyoxyalkylene components offer water-soluble (hydrophilic) property with high HLB value. The ratio of PEG and PPG influences the hydrophilic and lipophilic properties of the dimethicone copolyol. The higher proportion of PEG provides more hydrophilic properties while the higher ratio of PPG offers better compatibility with oils. Lowering surface tension is the major property of PEG/PPG dimethicone and it is used as an emulsifier for silicone-in-water or water-in-oil emulsions. It should be added into the silicone phase or oil phase in the preparation of these emulsions. It can easily structure itself along the Si-O-Si bonds enabling the lowest free energy state and thus enabling the enhanced stability of emulsions. Figure 7 depicts the chemical structure of PEG/PPG -20/15 dimethicone(73, 74).

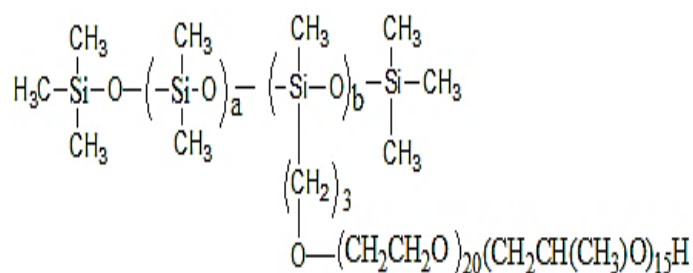


Figure 7 Chemical structure of PEG/PPG -20/15 dimethicone.

2.4 Hydrogels

An aqueous dispersion medium is gelled with a suitable hydrophilic gelling agent to form the hydrogel. Hydrogels are the gels with three-dimensional hydrophilic polymer networks, which can absorb large quantities of water. Hydrogels can be classified into chemical or physical gels based on the nature of chemical interactions. The physical hydrogels are reversible and the major interactions for the gel formation includes entanglements, Van der Waals interactions or hydrogen bonding. The physical hydrogel is formed mainly from physical crosslinks, in contrast, the chemical hydrogel is permanent and formed via covalently bonded cross-linked networks. The drug release from hydrogels via different mechanisms like diffusion and chemical

stimulation. Drug release by diffusion is regulated by bulk erosion of the hydrogel or movement of the polymer matrix. The chemical stimulation mechanism uses external stimuli like pH or temperature or enzymatic action to induce swelling and effective opening of the pores of hydrogels enabling the release of entrapped drugs. The drug release by diffusion mechanism is more common for nonspecific and localized drug release whereas drug release by chemical stimulation is more effective for selective treatment or oral drug delivery (7).

2.4.1 Components of hydrogels

Various types of polymers such as synthetic, semisynthetic, and/or natural polymers can be used for hydrogel preparations. Synthetic polymers commonly used in the preparation of hydrogels are carbomer or carbopol polymers, poloxamers, polyvinyl alcohol, poly N-vinyl pyrrolidone, polyethylene oxide, polyacrylic acid, polymethacrylic acid, polystyrene, polyethylene glycol diacrylate/methacrylate, and polyethylene glycol acrylate/methacrylate, etc. Methylcellulose, carboxymethyl cellulose, hydroxyethyl cellulose and hydroxypropyl methylcellulose are the extensively used semisynthetic polymers for hydrogels. Various types of natural polymers are widely used in the preparation of hydrogels due to their beneficial properties like biocompatibility, controlled swelling capability, water absorption ability, and economic reason. Chitosan, alginates, pectin, gellan, dextran, xanthan, guar, psyllium polysaccharide, tamarind polysaccharide, locust bean gum, sterculia gum, and tragacanth gum are the commonly used natural polymers.

2.4.1.1 Polymers used in the preparation of hydrogels

The polymers used in the preparation of hydrogels may be synthetic, semisynthetic, and/or natural polymers. Some of the synthetic polymers applied in the preparation of hydrogels are carbomer (Carbopol polymers), poloxamers, polyvinyl alcohol, poly N-vinyl pyrrolidone, polyethylene oxide, polyacrylic acid, polymethacrylic acid, polystyrene, polyethylene glycol diacrylate/methacrylate, polyethylene glycol acrylate/methacrylate, etc. The extensively used semisynthetic polymers for hydrogels are methylcellulose, carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, etc. Numerous natural polymers are being

employed in the preparation of hydrogels due to the improved biocompatibility, water absorption ability, controlled swelling capability, and economic reason. The commonly used natural polymers are chitosan, alginates, hyaluronic acid, pectins, gellan gum, dextran, xanthan gum, guar gum, psyllium polysaccharide, tamarind polysaccharide, locust bean gum, sterculia gum, gum tragacanth, etc.

2.4.1.2 Xanthan Gum

Figure 8 depicts the chemical structure of xanthan gum. Xanthan gum is secreted by the plant pathogenic bacteria *Xanthomonas campestris*. It is widely used natural polymer due to its water soluble, biodegradable, non-toxic properties and also it is less expensive. The chemical composition of xanthan gum includes pentasaccharide units consisting of two D-glucopyranosyl units, two D-mannopyranosyl units and one D-glucopyranosyluronic unit. It is a free-flowing powder and give viscous solutions at low concentrations. It gains increase interest in industrial use due to its solubility in both hot and cold water and ability to control the rheological properties of water-based products. It is a very useful thickener and stabilizer because it can give highly viscous solutions even at low concentrations as compared to other polysaccharide solutions. Xanthan gum solutions exhibit pseudoplastic behavior i.e. viscosity is regained immediately even at high shear rates, which enhances mouth feel effect and flavor release. These solutions have very good stability and are least affected by changes in pH, and the solution properties of xanthan are not affected in a pH range of 1–13. In addition, it is compatible with various types of commercially available thickeners such as sodium alginate, carboxymethyl cellulose and starch.

Xanthan gum is widely used in cosmetics and in toothpaste since the shear thinning flow behavior of xanthan gum facilitates the extrusion of the sample from the tube and also improve the dispersion and rinsing from the teeth. It is mainly used as a thickener and stabilizer in personal care products like creams, eye gels, etc. Xanthan gum also prevents the separation of insoluble ingredients in pharmaceutical emulsions or suspensions. It is often used in combination with other hydrocolloids to provide the desired flow behavior (75).

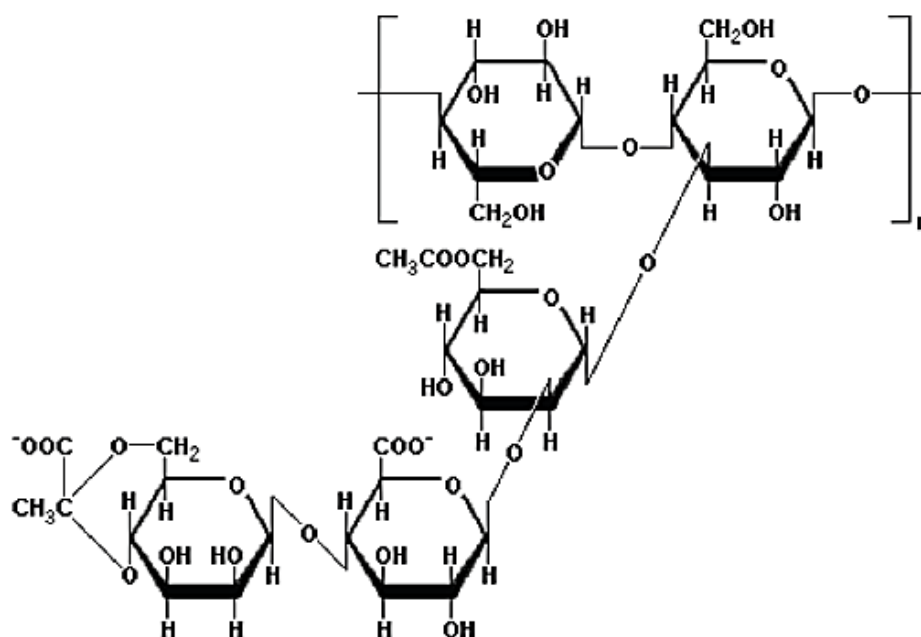


Figure 8 Chemical structure of xanthan gum (76).

2.4.1.3 Guar gum

Guar gum, natural polymer is extracted from *Cyamopsis tetragonoloba*. As shown in Figure 9, guar gum consists of high-molecular-weight polysaccharides composed of galactomannans which are linear chains of (1,4)-linked- β -D-mannopyranose backbone with branch points at their 6-positions linked to α -D-galactose residues as side chain. It is an FDA approved polymer to be used as emulsifier, stabilizer, and thickener for pharmaceuticals, cosmetics, and food. Recently, many research groups studied the swelling activity of guar gum as a rate controlling agent for controlled delivery of bioactive molecules. It is also used as a binder, disintegrant in tablet formulations and suspending agent in liquid formulations. Since it is easily degraded by colonic bacteria, guar gum has been widely used for colon specific drug delivery applications.

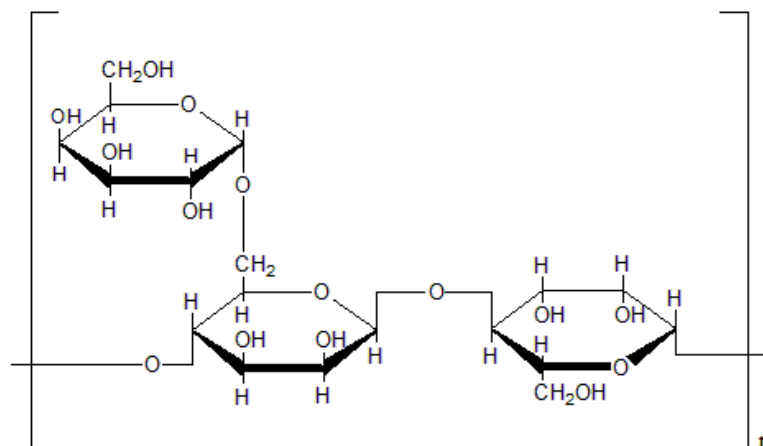


Figure 9 Chemical structure of guar gum.

2.4.1.4 Carbopol or carbomer polymers

Carbopol polymers are commonly used in the pharmaceutical and dermocosmetic fields. Benefits of using aqueous carbopol gels are of high viscosity at low concentration, wide viscosity interval and characteristic flow behavior, compatibility with many active ingredients, bioadhesive properties, good thermal stability, excellent organoleptic characteristics, and good patient acceptance. They are acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol and anionic polymers that need naturalization to become gellified. Organic amines like triethylamine can be used to neutralize these polymers in liquids (77). They have been used in the personal care industry in producing gels, creams, lotions and suntan products since many years ago. Carbopol gels have been used as drug vehicles for various routes of administration. They show a pseudoplastic behavior at high shear rate. The pseudoplastic (non-Newtonian) features of gels can be explained by the solvent-solvent and solvent-polymer interaction. The viscosity of Carbopol gels increases with pH as well as by the addition of glycerol (75, 77).

Carbopol ultrez 21 polymer is a hydrophobically modified crosslinked polyacrylate polymer. It is one of the newest carbopol polymers with very good ability of thickening, suspending and stabilizing properties. It wets quickly and gels could be prepared without extensive mixing. The major advantages of carbopol ultrez 21 are

high viscosity even at low concentrations, characteristic flow behavior, good bioadhesive properties, compatibility with many active ingredients, good aesthetics and good patient acceptance.

Carbopol 934 is a very common and well-suited polymer for the preparation of topical hydrogels. It has been widely used for preparations of many commercial topical products available today. The beneficial properties of carbopol 934 are safe and effective, biocompatible, and superior thickening, suspending and emulsification properties. In addition, due to its high molecular weight, it cannot penetrate the skin and affect the efficacy of the drug. The chemical structure of Carbopol polymer is depicted in Figure 10.

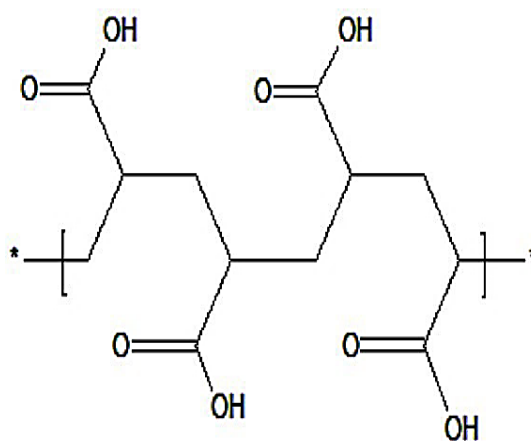


Figure 10 Chemical structure of Carbopol polymers.

2.5 Bigels

Bigel formulations possess characteristics of both hydrogel and oleogel such as enhancement of hydration of the stratum corneum, easily spreadable, emollients and water washability when applied onto the skin. The two gel systems in bigels can produce a synergetic effect such as improvement of skin hydration and drug penetration due to the presence of both water and oil phases. Unlike emulgels and creams, there is no surfactant or emulsifier in the preparation of bigels. Bigel are prepared by simply mixing aqueous or hydrophilic and lipophilic systems at a high shear rate or rpm. The mechanism of drug penetration of bigels is same nature as of hydrogels and oleogels. In bigels, the drug release mechanism is the combination of the conventional diffusion

of hydrogels and lipophilic nature of oils present in oleogels serve as penetration enhancers. Hence, the drug can easily pass through stratum corneum and provide the topical and transdermal effects on the skin. Since bigels possess the combined features and benefits of both organogels and hydrogels, they may potentially be used as a topical delivery vehicle for pharmaceutical and cosmetic applications. In addition, they do not require the addition of emulsifiers or surfactant to achieve physical stability, but still it remains unclear that how bigels can be stable under longer duration and different storage conditions. The occurrence of syneresis is an indicator of the life time of the bigel products. The major cause of syneresis is the contraction of a solid gel network which results the squeezing of the fluid and separation into two phases of the gel system. Bigels containing larger aggregates can occur syneresis easily. Although preparation methods of bigels are very simple and easy, only few studies have investigated these carrier systems for pharmaceutical or cosmetic purposes. The main reason may be due to the difficulty in obtaining a stable hydrogel and oleogel mixture without the addition of an emulsifier or a surfactant (7).

Advantages of bigels over organogels and hydrogels

Better stability of bigel over the emulsions (water-in-oil and oil-in-water), creams, emulgels, hydrogels, and oleogels is the main benefit of bigel. Bigel is a beneficial carrier for pharmaceutical or cosmetic applications. The enhanced physicochemical stability of bigels gives colloidal dispersions formulation and produces the immobilization of the mobile phases in a three-dimensional gel network (33). Unlike emulsions, the two components of the bigel interconnected with one another and hence they could not be easily separated during storage at the room temperature.

Hydrogels hold high patient compliance because they are easily washable with water and have cooling nature. However, they could deliver only hydrophilic drugs and thus limit the skin-penetration. On the other hand, oleogels are suitable carriers for the lipophilic drugs, but peculiar nature of oleagenous gives greasy feeling, and low consumer compliance (34). The conversion of oleogels, emulgels, and hydrogels into bigels produces easy washability and good patient compliance except compromising the beneficial effects of the oil. They are easy to be prepared and do not contain

surfactants which can cause skin irritation or toxic (7, 34). The combination of oleogel and hydrogel could be able to accommodate both hydrophilic and lipophilic drugs and have synergistic-effects of enhanced drug permeation due to the presence of both oil and water phases (34). Moreover, better skin penetration through the skin could be a choice for topical or transdermal drug deliveries (13, 31). The water phases in the bigels give raises electrical conductivity, and therefore bigels are suitable carriers for iontophoretic drug delivery (33). Bigels have a few obstacles such as phase separation due to the absence of emulsifiers. They are not thermo-reversible and they could be destabilized at higher temperature (32).

2.5.1 Types of bigels

Four types of bigels are:

- (1) oleogel dispersed in hydrogel system (O/W),
- (2) hydrogel dispersed in the oleogel system (W/O),
- (3) bicontinuous bigel, and
- (4) complex bigel based on the structural organization and the disposition of organogels and hydrogels.

(1) Oleogel dispersed in hydrogel system

This type of bigel is developed by dispersion of oleogel inside the hydrogel system as shown in Figure 2.11. Behera et al. had developed the bigels by the incorporation of Span 40 sunflower oil oleogel through aqueous gels (plural/singular) of water-soluble synthetic polymers (e.g., polyvinyl alcohol and polyvinyl pyrrolidone) (9). The micrographs showed that the oleogel as the internal phase dispersed in the external (continuous) hydrogel phase (10). Singh et al. developed an oil-in-water type of emulsion bigels by mixing carbopol 934 based hydrogel and Span 60 sesame oil based organogel (31).

(2) Hydrogel dispersed in the oleogel system

They are prepared by dispersing the hydrogel in the continuous oleogel phase (Lupi et al., 2016)(34). Patel et al. (2015) prepared by a combination of hydrophilic-fumed silica and sunflower oil based organogel with polysaccharides (72).

(3) Bicontinuous bigels

In bicontinuous bigels, the higher proportions of hydrogel/oleogel were dispersed in the lower proportions of oleogel/hydrogel phase. Singh et al. studied the bicontinuous bigels which consisted of guar gum-based hydrogel together with Span 60 sesame oil based organogel (31). Kodala et al. also developed the bicontinuous bigel with higher proportions of oleogel while preparing oleogel-in-hydrogel type bigels (78).

(4) Complex bigels

They are produced by the addition of organogel/hydrogel to an oil-in-water/water in-oil structured emulsion. Lupi et al. (2016) had studied the formation of a complex matrix-in-matrix system and the structural organization had been confirmed by microscopy (34).

Methods of preparation of bigels

The aqueous hydrogel phase and the oleogel phase were prepared separately by mixing the components at a defined speed and temperature.

Preparation of aqueous phase (hydrogel)

Hydrogels are prepared by mixing the required amount of hydrophilic gelling agent in water. Depend on the gelling behavior of the system, the process-parameters such as speed and temperature have been optimized. Hydrogels are formed by physical or chemical cross-linking (7).

Preparation of oleaginous phase (oleogel)

Oleogel is prepared by dissolving the accurately weighed-quantity of organogelator in an oil phase at a defined homogenization condition and a temperature higher than the melting point of the organogelator. When the temperature is reduced to room temperature (25°C), the gel formation occurs (31).

Preparation of bigels

Bigels are formed by mixing hydrogel and oleogel at high shear-rate. The homogenous mixture develops a smooth-gel or shows phase separation on cooling to room temperature and the formation of a stable bigel is checked by using tube inversion test (31).

2.5.2 Characterization techniques of bigels

Organoleptic evaluation

After the formation of bigels, they are cooled down to room temperature and the appearance, color, homogeneity, consistency, pH, and phase separation are analyzed. The color of bigel is white to off-white. They have viscous nature and should appear homogenous formulations on visualization. The composition and proportion of the oleogels strongly affected the color of the bigels (13, 31). An increased proportion of oleogel gives the bigel formulations with increased intensity of white color (78). The bigels with higher proportion of oleogel induced a higher degree of scattering as compared to aqueous gel as a result of the occurrence of interfaces between the two immiscible phases. Nonetheless, the increased proportion of oleogel provides bigels with increased brittleness and reduced firmness (31).

Stability studies

Accelerated stability test (freeze thaw thermocycling method) and long-term stability test are commonly tested to evaluate the stability of the bigels. A combination of two phases with semisolid in nature increases the thermodynamic stability of bigel to several folds. After the stability testing, the bigel formulations are evaluated for pH, color, homogeneity, consistency, phase separation, and overall appearance. Destabilization after each cycle of freeze thaw thermocycling and at

predefined time intervals (0, 3, 6, 12, 18, and 24 months or up to 36 months) during long-term stability study are also evaluated based on the active substance of the bigel (31).

Accelerated stability study

Typically, five cycles of freeze thaw thermocycling i.e. the formulations are freezing at -20°C for 15 min followed by thawing at 70°C for 15 min, are performed for accelerated stability testing which predicts the long-term stability of the bigels. After each cycle, the bigels are evaluated for different parameters like pH, color, homogeneity, consistency, phase separation, and overall appearance or sign of destabilization (31).

Long-term stability study

As per the ICH guidelines, long-term stability study or the real-time stability study of the bigels is performed. The duration for long-term stability study is up to 2 years or more (0, 3, 6, 12, 18, and 24 months or up to 36 months). After incubating the samples at different storage temperatures, the storage temperature of the formulations is decided by evaluating the drug-containing products. During the stability study period, the pH, color, homogeneity, consistency, phase separation, and overall appearance or sign of destabilization of the bigels are evaluated at regular intervals (31).

Optical microscopy

The microscopic arrangement of different types of bigels was showed in Figure 11. Various microscopy techniques such as confocal microscopy, scanning electron microscope (cryo-SEM), transmission electron microscopy, and phase contrast microscopy are used to study the biphasic nature as well as the mutual disposition of organic and aqueous phases within the bigels. Lupi et al. used the confocal microscopy to identify the oil and/or aqueous phases by incorporating the fluorescent dyes in the two phases (34). Furthermore, Patel et al. used cryo-SEM to study the distribution pattern of aqueous and organic phases by converting the bigels into freeze-fractured samples (72). Ibrahim et al. and Rehman et al. also investigated the microarchitecture of different bigel systems using the compound optical microscope and polarized light microscope (11, 12). The bigels containing higher proportions of

the oleogels produced bigger droplet sizes. Further increment of the oleogel concentration resulted in irregularly shaped globular structures. When the oleogel proportion beyond a critical oleogel concentration, bicontinuous bigels is formed (78).

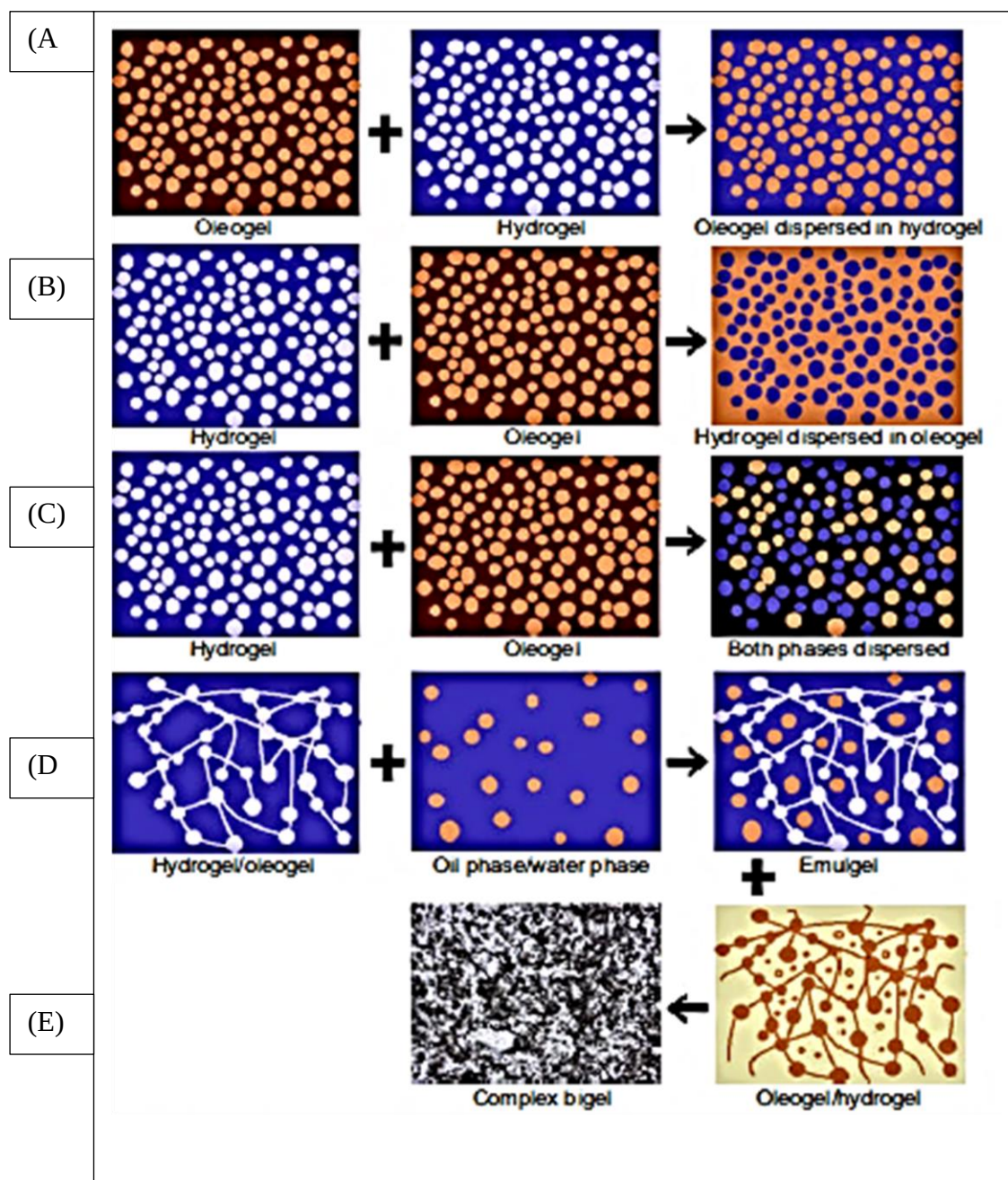


Figure 11 The microscopic arrangements of different types of bigels. (A) Oleogel dispersed in hydrogel, (B) hydrogel dispersed in oleogel, (C) both phases are dispersed, (D) emulgel, and (E) complex bigel (79).

Droplet size distribution

The droplet size distribution is a critical factor in bigel formulations for drug absorption and colloidal stability. The smaller droplet size provides large interfacial surface area, which in turn increases the drug absorption and provides excellent colloidal stability and vice versa (80). ImageJ software is used as analytical tool for analysis of the droplet size distribution of the dispersed phase in bigels. A dimensionless number also known as SPAN number calculated by width of the droplet size is used to determine the droplet size distribution (31). Singh et al. reported that the average droplet size of guar gum-sesame oil bigels became smaller when an increase in proportion of organogel which was related to closer packing of dispersed droplets (31). In contrast, an increase in average droplet size and number of droplets was found in carbopol-sesame oil based bigels with increase in organogel fraction (14).

Fourier transform infrared spectroscopy analysis

The functional groups present in the bigel formulations are evaluated by mean of fourier transform infrared (FTIR) spectroscopy (15). Most of the functional groups of molecules absorb infrared rays in a wavelength range between 4000 and 1500 cm^{-1} thus the spectrum is recorded in this range to determine the lipophilic and the hydrophilic nature of the colloidal mixtures (30). The “fingerprint region” of the spectrum is the absorption bands below 1500 cm^{-1} which indicates deformation, bending- and ring-vibration. To identify the chemical composition directly in the undisturbed state without any requirement of sample preparation, attenuated total reflection is a helpful analytical tool. The intra- or intermolecular hydrogen bonds within the hydrogel is attributed by a broad hump in the range of 3300-3200 cm^{-1} (31). The formation of the bigel is the incorporation of the oleogel within the agar hydrogel, which causes a considerable decreased in the intra- and intermolecular hydrogen bonding of the agar gels (78). Rehman and Zulfakar also reported as the oleogel ratio inside the colloidal mixture is increased from 10% to 50%, transmittance in the region of 3330 cm^{-1} reduced (30).

Mechanical properties

Various instruments such as rheometer, viscometer, and mechanical tester are mainly used to analyze the mechanical properties of the bigels such as viscosity and stress relaxation. A cone- and plate viscometer is used to measure the viscosity of the bigels with shear rate ranging between 20 and 100 s⁻¹ and the measurement is generally operated at room temperature (25°C). The measured data are fitted using the Ostwald-de Waele Power Law, which is used to represent the viscosity profiles of the non-Newtonian fluids. When organic and aqueous phases were mixed together, the rheology of the bigels increased synergistically, which is due to the interpenetrating network of the organic and the aqueous phases (72).

The bigel did not form when the aqueous gel concentration is higher than 50% of its proportion with organogel. At higher ratios of an aqueous gel, only viscous sols were formed instead of bigel formation (72). To measure the mechanical properties of bigels, different parameters like hardness or firmness, adhesiveness, cohesiveness, stickiness, gel strength and spreadability were mainly extracted from the results of various tests such as stress relaxation, cyclic creep and recovery. The effect of organogel and hydrogel contents on the mechanical properties of bigels was reported by many researchers. The higher cohesiveness, adhesiveness, firmness and stickiness values were found in the bigels with higher organogel proportion (31). A decrease in hardness was observed with a corresponding decrease in hydrogel content. This result linked to the important role of hydrogel in imparting firmness and gel strength because of hydrogen bonding present in hydrogel (12).

Thermal properties

Differential scanning calorimetry (DSC) is used to study the thermal properties of the bigels and the experiment is performed under an inert (nitrogen) gas atmosphere using aluminum crucibles with pierced aluminum lid. Approximately 15 mg of the bigels formulations are hermetically sealed in aluminum pans, with pierced lids. The melting and crystallization events are recorded in a defined temperature range under an inert (nitrogen) atmosphere (31).

***In vitro* release study**

In vitro release study is done using Franz's diffusion cell. The volume required for dissolution, pH of dissolution media, temperature, and stirring speed is selected and the test is done for a predefined time period. The drug aliquot is collected at different time points, filtered through a 0.45 mm millipore filter, and is assayed using UV-Vis spectrophotometer or HPLC. Various mathematical models like zero-order, first-order, Higuchi and Korsmeyer-Peppas model are used to fit the drug release kinetics. There are many factors which influence the amount of drug release such as initial water content, drug loading, electrostatic interaction between drugs, lipid bilayers, and swelling capacity. Zero-order kinetics represents release of drug from the delivery vehicle at constant rate, e.g., reservoir type delivery systems whereas, first-order release kinetics suggests a constant rate of release of the drug over the period of time. Higuchi proposed a model to explain the release of the drug from the matrix-type delivery vehicle which is based on the uniform dispersion of drug particles within the insoluble homogenous polymer matrix. The model suggests that the solutes diffuse from the polymer matrix through Fickian diffusion. The n value characterizes different types of release and the n value of 0.45 corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n \approx 0.89$ to case II (relaxational) transport, and $n > 0.89$ to super case II transport (31).

2.5.3 Pharmaceutical applications of bigels

The bigels are recently getting popularity and widespread usage among the all other topicals as well as transdermal delivery. Numerous bigel systems are recently being investigated for wide range of applications. They are mostly used as controlled release vehicles for topical and transdermal applications. Their great benefits over single gel systems have been reported by Ibrahim et al., who found that the release rate of diltiazem hydrochloride bigel was higher than that of hydrogel and organogel (11). In addition, the developed bigels exhibited the higher permeation rate as compared to organogels. The beneficial properties of bigels for cosmetic applications have been investigated by Lupi and co-workers and Andonova and groups. According to their findings, the moisturizing effects and rheological behavior of the bigels confirmed that they are promising candidates for topical application (Lupi et al., 2016, Andonova et

al., 2017)(34, 81). In recent years, there are increasing interest for usage for bigels for food applications and edible bigels have been prepared from gelatin and maltodextrin for the very first time. To form stable bigel formulations, various ratios of hydrogel and organogel (80/20, 70/30, and 60/40 w/w) were thoroughly investigated. Among the tested ratios, the formulation prepared with the hydrogel: organogel ratio of 60/40 w/w was found to be unstable during the early stage of storage and syneresis was observed. However, the hydrogel and organogel ratio of 80/20 and 70/30 were stable for longer period and displayed very similar mechanical properties. Bigels of testosterone are commercially available as anti-inflammatory and antimicrobial formulation.

2.6 Model drugs loaded in bigels

2.6.1 Bacitracin zinc

Bacitracin is cyclic polypeptide antibiotic and produced from *Bacillus licheniformis* and *Bacillus subtilis*. Its mechanism of action is the interruption of cell wall synthesis. It is active against gram-positive organisms such as staphylococci, streptococci, clostridia and has limited eschar-penetrating ability. It is mainly used in the treatment of ophthalmic and dermatological infections. A white petroleum-based formulation of bacitracin zinc can produce soothing and comfortable application. Adverse effects of bacitracin zinc are very rare; but hypersensitivity reaction can occur. The chemical structure of bacitracin consists of seven amino acids membered ring and five amino acids in the side chain (Figure 12). The terminal of the side chain ends with thiazole ring which is composed of L-cysteine and L-isoleucine or L-valine. Bacitracin has 15 major and minor components such as A, B1, B2, B3, C1, C2, C3, E, F, H1, H2, H3, I1, I2, and I3 (Figure 13). Among these components, the microbiologically active components are A, B1, B2 and B3 and they represent about 96% of total microbiological activity. Bacitracin F is degradation product and it is microbiologically inactive and also nephrotoxic (82, 83). Bacitracin zinc is a more stable complex form of bacitracin. It has very low water solubility, high molecular weight and size which may lead to poor skin penetration (37).

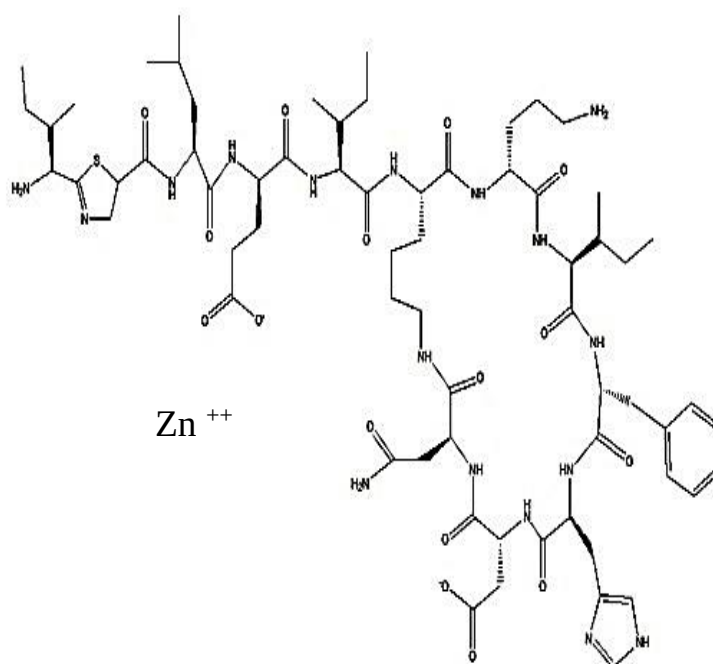


Figure 12 Chemical structure of bacitracin zinc.

Physicochemical properties

- (1) Melting point : 221-225°C
- (2) Molecular formula: C₆₆H₁₀₃N₁₇O₁₆S (Bacitracin A)
- (3) Molecular weight : 1422.7 (Bacitracin A)
- (4) Solubility

Slightly soluble in water and in alcohol.

- (5) Stability

Bacitracin zinc is more stable than bacitracin but aqueous solutions can degrade rapidly at room temperature. Bacitracin zinc is relatively stable in acidic solutions and unstable above pH 9. Loss in activity is probably due to transformation of bacitracin A into bacitracin F, which has low antimicrobial activity.

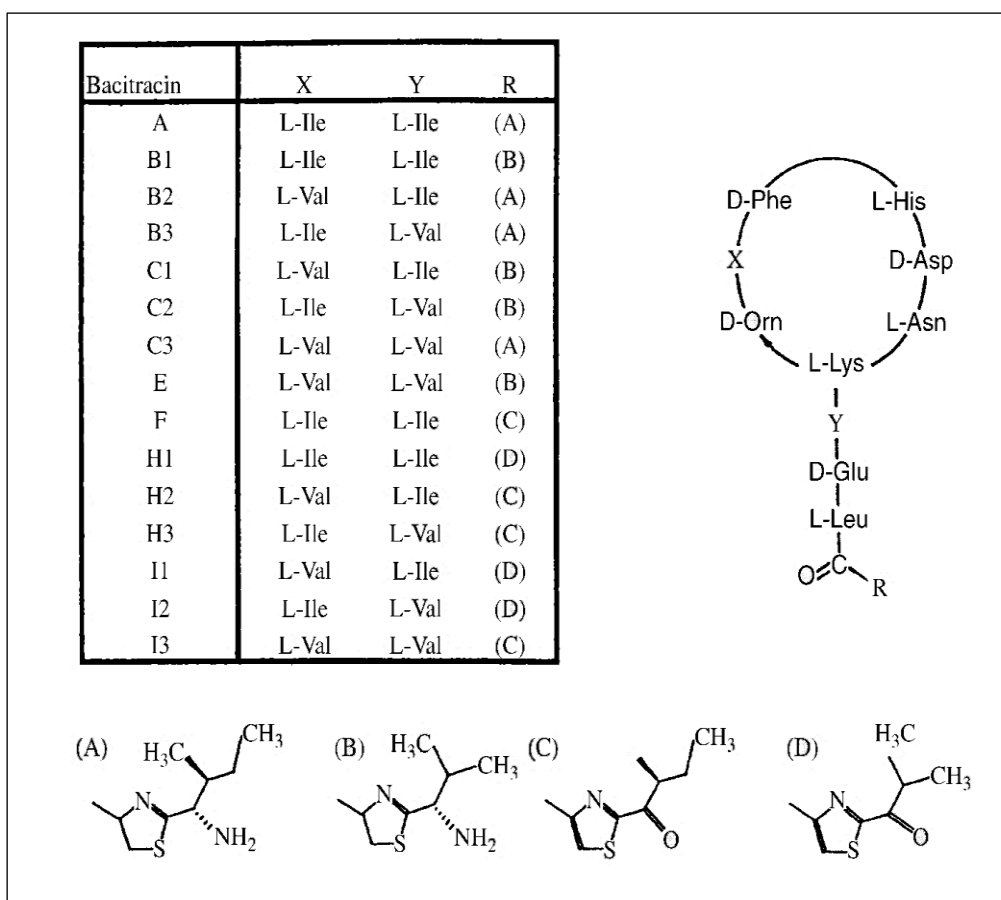


Figure 13 Structural components of Bacitracin. Asn-asparagine, Asp-aspartic acid, Glu-glutaminic acid, His-histidine, Ile-isoleucine, Leu-leucine, Lys-lysine, Orn-ornithine, Phe-phenylalanine; Val-valine. R: the end group of the side chain; (A): L-cysteine and L-isoleucine; (B): L-cysteine and L-valine; (C) and (D) are oxidative forms of their corresponding end groups (82).

2.6.2 Neomycin sulfate

Neomycin sulfate, an aminoglycoside antibiotic is one of the commonly used topical antibiotics in treatment of skin infection. It is sulfate salt of neomycin B and C and categorized as BCS class-III drug which has high solubility and poor permeability. It has bactericidal activity and mechanism of action is to inhibit bacterial protein synthesis by binding with ribosomal RNA and causing misreading of the bacterial genetic code. They are effective against both gram-negative and gram-positive aerobic organisms including *S aureus* and some strains of *S epidermidis*. They have a

low incidence of adverse reactions when applied to the skin, but hypersensitivity reactions have been reported. Neomycin sulfate is available in the commercial market as ointment or cream and is frequently combined with other topical antibiotics to improve its coverage against gram-positive bacteria. It is mainly used for treatment of superficial infections, prophylaxis against infection in minor wounds and postoperative wounds, adjunctive treatment of burns, and management of superinfection in chronic dermatoses (84). Due to its physicochemical properties (high molecular weight (908.87D), poor skin permeability (<3%), and highly polar nature), neomycin sulfate has poor skin penetration (85). Figure 14 shows the structural components of neomycin.

Physicochemical properties

(1) Molecular formula : $C_{23}H_{48}N_6O_{17}S$

(2) Molecular weight : 712.72

(3) Melting point : $>187^{\circ}\text{C}$ (dec.)

(4) Main components

Neomycin B and C and small amount of neamine (inactive)

(5) Solubility

Freely soluble in water; very slightly soluble in ethanol

(6) Stability

It is susceptible to atmospheric oxidation. It can be heated at 110°C for 10 hrs without loss of activity, however, change the color of the drug. It is stable at a pH range of 2-9 and shows optimal activity at pH 7.

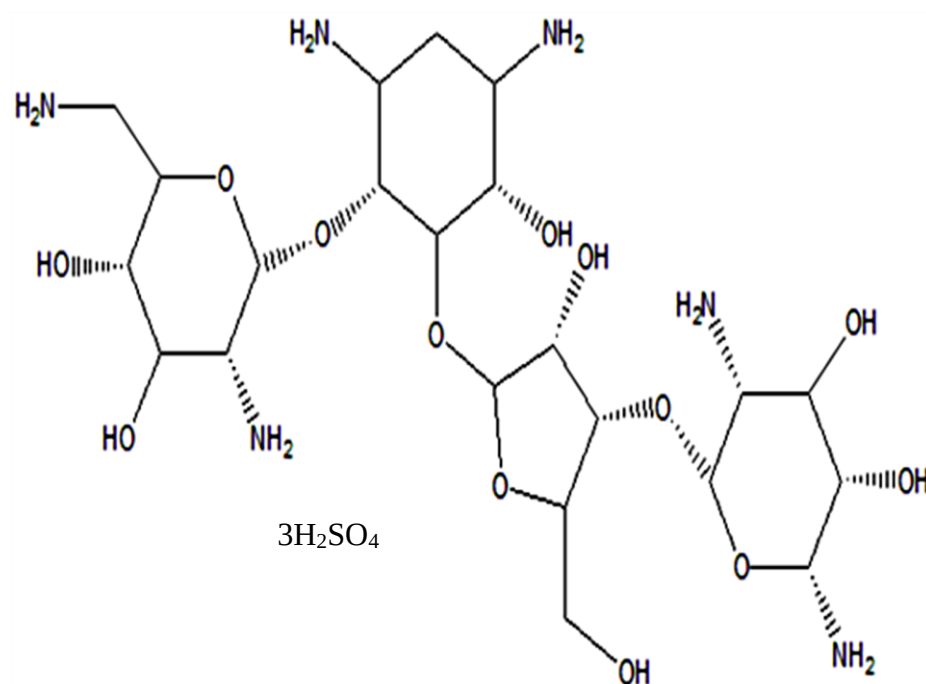
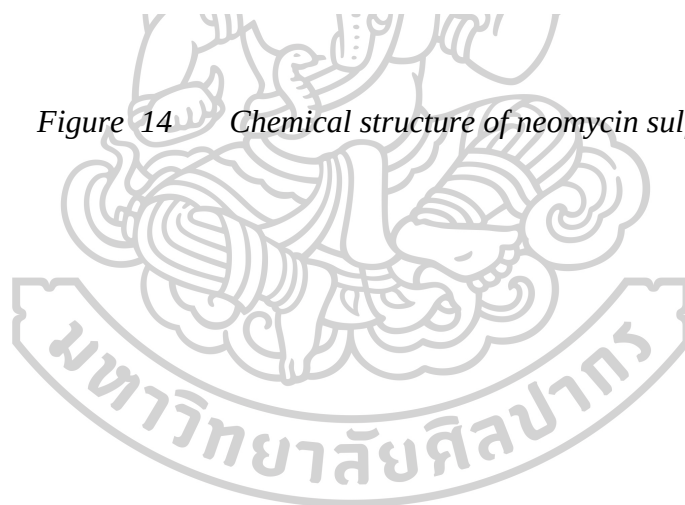


Figure 14 Chemical structure of neomycin sulfate.



CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

1. Bacitracin zinc (Lot No. SPMAR00063, Greater Pharma Co., Ltd., Thailand)
2. Neomycin sulfate (Lot No. 14080076, Greater Pharma Co., Ltd., Thailand)
3. Volatile silicone oil (silicone oil, TSF 405[®]) (Lot No. 10E116, Momentive Performance Materials, Thailand)
4. Silicone surfactant (cyclomethicone (and) PEG/PPG-20/15 dimethicone, SF 1328[®]) Lot No. 15AWFA010, Momentive Performance Materials, Thailand
5. Sorbitan monostearate (span 60, Nonion SPA 60[®]) (Lot No. 70213B, NOF Corporation, Japan)
6. Sesame oil, refined (Lot No. 590200000, Henry Lamotte Oils, GMBH)
7. Sweet almond oil (P.C. Drug Center Co., Ltd., Thailand)
8. Castor oil (P.C. Drug Center Co., Ltd., Thailand)
9. Safflower oil (Lot No. ESO 112-28, P.C. Drug Center Co., Ltd., Thailand)
10. Olive oil (Lot No. 562252, P.C. Drug Center Co., Ltd., Thailand)
11. Sunflower oil (P.C. Drug Center Co., Ltd., Thailand)
12. Guar gum (Lot No. E/091/01, P.C. Drug Center Co., Ltd., Thailand)
13. Xanthan gum (Lot No. OC7891, P.C. Drug Center Co., Ltd., Thailand)
14. Carbopol 934 (P.C. Drug Center Co., Ltd., Thailand)
15. Carbopol ultrez 21 (Lot No. 0101279359, P.C. Drug Center Co., Ltd., Thailand)
16. Methanol (HPLC grade) (Lot No. 15070220, Lab-Scan, Thailand)
17. Acetonitrile (HPLC grade) (Lot No. 16110020, Lab-Scan, Thailand)
18. Sodium acetate (Analytical grade) (Lot No. AF604190, Ajax Finechem Co., Ltd., Australia)
19. Potassium dihydrogen orthophosphate (Analytical grade) (Lot No. 1102246, Ajax Finechem Co., Ltd., Australia)
20. di- Potassium hydrogen orthophosphate (Analytical grade) (Lot No. 0711005, Ajax Finechem Co., Ltd., Australia)

21. Ethylene diamine tetra acetic acid disodium (Lot No. J069H11, RFCL, New Delhi)
22. *o*-Phthaladehyde (Analytical grade) (Lot No. EOFBD-1L, Tokyo Chemical Industry Co., Ltd., Japan)

3.2 Equipment

1. Analytical balance (CP224S, Sartorius, Germany)
2. Digital eyepiece (Dinolite, AM423X, Taiwan)
3. Digital pH meter (Mettler Toledo SevenEasy, Switzerland)
4. Differential Scanning Calorimeter (Pyris Sapphire DSC, Perkin-Elmer, USA)
5. Franz diffusion cells (Logan DHC 6T, Logan Instruments Corp, New Jersey)
6. FTIR spectrometer (Nicolet 4700, Thermo Electron Corporation, USA)
7. High Performance Liquid Chromatography (Agilent 1200 series, Agilent Technologies, USA)
8. Laboratory Centrifuges (Hettich Zentrifugen, Germany)
9. Light microscope (CX31, CX41RF, Olympus, Japan)
10. Magnetic stirrer (Schott, CAT)
11. Overhead stirrer (SS10, Stuart, UK)
12. Rheometer (Kinexus Pro, Malvern Instruments, UK)
13. Texture analyzer (TA. XT plus, Stable Micro Systems, UK)
14. UV-visible spectrophotometer (Agilent G1103A, Agilent Technologies, USA)
15. Vortex mixer (Gibthai VX-100, Thailand)
16. Water bath (AH-30-110, Scientific Promotion, Thailand)
17. X-ray diffractometer (MiniFlex II, Rigaku, Tokyo, Japan)

3.3 Methods

3.3.1 Development of oleogels with silicone oil

3.3.1.1 Preliminary study to choose the best miscible oil pair

Castor oil, olive oil, safflower oil, sesame oil, sweet almond oil and sunflower oil were studied for their miscibility with silicone oil. An equal volume (2.5 mL) of silicone oil and each vegetable oil were mixed in a screw-cap glass vial. The mixture was stirred vigorously for 5 min using a magnetic stirrer (Schott, CAT) at 1500 rpm and 25°C. Phase separation of the oil pairs was observed visually after 24 h. The pair of sesame oil and silicone oil was chosen for further determination of miscibility and CGC using 5 different ratios of sesame oil and silicone oil; 1:4, 2:3, 1:1, 3:2, and 4:1. The CGC is the minimum gelator concentration required to form gel and started failing to flow when the tubes were inverted after keeping at 25°C for 24 h. In CGC testing, the range of concentration of SMS (5%-22% w/w) was used to investigate with five different ratios of sesame oil and silicone oil. The microscopical characteristics of oleogels formed by CGC of SMS with five different ratios of sesame oil and silicone oil were examined. The best ratio of sesame oil and silicone oil which produced oleogels with the strongest network formation was selected for the preparation of oleogel. The physicochemical properties of the developed oleogel were compared with that of the sesame oil oleogel and silicone oil oleogel.

3.3.1.2 Viscosity of the oil components

Viscosity of sesame oil, silicone oil and their combination were measured using a rheometer (Kinexus Pro, Malvern Instruments, UK). Cup and bob (C25SW 1130 ss) geometry was selected and 25 mL of samples were sheared at the shear rate range from 0.1 to 100 s⁻¹ at temperature of 25 ± 0.1°C; controlled by a Peltier cylinder cartridge. All measurements were done in triplicate. Mean and standard deviation (SD) were used to analyze the data.

3.3.1.3 Determination of CGC for oleogels

CGC of SMS was determined by heating the mixture of oil phase (sesame oil, silicone oil or a mixture of silicone oil and sesame oil) and SMS gelator (5% - 22% w/w) at 55°C in glass tubes with screw caps. The formulations were considered as oleogels if they failed to flow when the tubes were inverted. The CGC of SMS that induces gel formation were used for the preparation of oleogels.

3.3.1.4 Preparation of oleogels

Table 1 shows the composition of oleogels. The formulations consisted of SMS, sesame oil and silicone oil. Oleogels containing sesame oil was designated as OG1, silicone oil was coded as OG2 and sesame oil and silicone oil combination was designated as OG3. The concentrations of SMS gelator for OG1, OG2 and OG3 were selected based on the CGC results. Oleogels were prepared by dissolving CGC of SMS in three different oil phases (sesame oil, silicone oil or a mixture of silicone oil and sesame oil). The mixture of gelator and oil was heated in a temperature-controlled water bath at 55°C and stirred at 300 rpm until a clear homogenous solution was obtained. Afterwards, the solution was allowed to cool to room temperature to induce gel formation. The samples were stored at $25 \pm 1^\circ\text{C}$ for further characterization. The physical appearances of the obtaining oleogels were observed visually and their physicochemical properties (FTIR, microstructure, rheology, X-ray diffraction and DSC) were investigated.

Table 1 Composition of oleogels.

Sample	Sorbitan monostearate (%w/w)	Sesame oil (%w/w)	Silicone oil (%w/w)
OG 1	20	80	—
OG 2	18	—	82
OG 3	10	36	54

3.3.1.5 Physicochemical characterization of the developed oleogels

3.3.1.5.1 FTIR analysis

FTIR spectra of the ingredients and oleogels were analysed using a Fourier transform infrared spectrometer (Nicolet 4700, Thermo Electron Corporation, USA). Solid samples were recorded as an intimate mixture with powdered KBr. Oil and gel samples were operated in attenuated total reflectance (ATR) mode. The scanning range was 4000-600 cm^{-1} and spectral manipulations of the samples were performed using Omnic software (version 8, Thermo Electron Corporation, USA).

3.3.1.5.2 Microstructure studies

The microstructure of the oleogels was observed under polarized light using a CX-31 optical microscope (Olympus Optical Co., Ltd., Japan) equipped with a Canon EOS (Rebel T5i) 700D camera. Thin smears of oleogels were prepared on glass slides and visualized under 40x magnification.

3.3.1.5.3 Rheological characterization

Rheological properties of oleogels were characterized using a rheometer (Kinexus Pro, Malvern Instruments, UK) with Peltier plate and active hood cartridges to control the temperature. All rheological measurements were performed at 25°C using a 40 mm diameter parallel plate geometry with a gap value of 1 mm. The viscoelastic properties of the oleogel samples were investigated with oscillation tests. First, the amplitude sweep was run by increasing stress from 0.001 to 10 Pa at a fixed frequency of 1Hz to determine the linear viscoelastic region (LVER) of the samples. Then a strain of 0.05% (within the LVER) was selected to run a frequency sweep from 0.1 to 10 Hz. The flow properties were characterized using the shear rate range of 0.1-100 s^{-1} . Three step shear rate sweep (up-down-up) was used to evaluate the thixotropic behaviors of the samples during application on the skin. All measurements were performed in duplicate.

3.3.1.5.4 X-ray diffraction studies

The crystalline behaviors of oleogels and their ingredients were evaluated using an X-ray diffractometer (MiniFlex II; Rigaku, Tokyo, Japan) at room temperature using monochromatic $\text{CuK}\alpha$ radiation at 15 mA and 30 kV over the range of 2θ angles from 5° to 40° with the scanning speed of $2^\circ/\text{min}$. Data analysis was carried out using a free trial version of Origin Pro 8 software.

3.3.1.5.5 Differential scanning calorimetry (DSC)

Thermal properties of the ingredients and oleogels were examined using a DSC (Pyris Sapphire DSC, Perkin-Elmer, USA). About 15 mg of samples were weighed and hermetically sealed in aluminum pans. The melting and crystallization events of samples were recorded in the temperature range of $25\text{--}80^\circ\text{C}$ with a thermal scanning rate of $5^\circ\text{C}/\text{min}$. Thermal characteristics such as onset and peak maximum temperature, heat of melting (ΔH_m) and heat of crystallization (ΔH_c) were determined using Pyris manager software. The measurements were performed in triplicate and mean and standard deviation (SD) were used to analyze the data.

3.3.1.6 Statistical analysis

The resulting data from viscosity of oils and thermal analysis of oleogels were compared by one-way ANOVA, Tukey's test using SPSS software version 16.0. The level of significance was considered as $p < 0.05$.

3.3.2 Development of bigels containing mixture of silicone oil and sesame oil for topical drug delivery

3.3.2.1 Preliminary study to choose the polymers for hydrogel preparations and the best ratio of oleogel and hydrogel for the development of bigels

Four types of hydrogels; two types of natural polymers (guar gum and xanthan gum) and two types of synthetic polymers (carbopol 934 and carbopol ultrez 21) were prepared. Xanthan gum (2% w/w) or guar gum (1.5% w/w) or two carbopol polymers at the concentration of 0.5% and 0.2% w/w respectively were dispersed in water containing the paraben preservatives (0.1% w/w methyl paraben and 0.02% w/w propyl paraben). These solutions were heated at 55°C and stirred using an overhead stirrer at 1000 rpm for 5 min until a smooth transparent mixture was obtained. The polymers which showed no interactions with drugs were selected for the preparation of hydrogels. The ratio of oleogel and hydrogel for the preparation of bigels was chosen using 5 different ratios; 10:90, 20:80, 30:70, 40:60, and 50:50. The 20:80 ratio was the best ratio which produced bigels with good consistency, homogeneity without lump formation and the absence of flow when inverted. This ratio was selected for the preparation of bigels for further studies.

3.3.2.2 Development of hydrophobic drug loaded bigels

3.3.2.2.1 Determination of drug solubility

Bacitracin zinc was selected as a hydrophobic model drug and the solubility of bacitracin zinc in various solvents; silicone oil, sesame oil, and a mixture of silicone oil and sesame oil at the ratio of 2:3, was determined. This ratio was chosen from the preliminary study due to producing the strongest oleogel network with thick and long tubules (65). An excess amount of bacitracin zinc was added in glass tubes filled with 10 mL of solvents. The tubes were equilibrated at 25°C in a thermostatically controlled water bath for 72 h. The equilibrated samples were then centrifuged at 8,000 rpm for 15 min, followed by filtration through a 0.45µm nylon syringe filter. The filtrates were suitably diluted with methanol and the concentration of bacitracin zinc in each vehicle was determined by an Agilent G1103A model UV-visible spectrophotometer (Agilent Technologies, USA). The solubility study was

performed in triplicate and the data was presented using mean and standard deviation (SD).

3.3.2.2.2 Preparation of hydrogels

Two types of hydrogels, guar gum and xanthan gum were prepared. Xanthan gum and guar gum at the concentration of 1.5 and 2% w/w respectively was dispersed in water containing the mixture of 0.1% w/w methyl paraben and 0.02% w/w propyl paraben (55°C). These solutions were stirred using an overhead stirrer at 1000 rpm for 5 min until a smooth transparent mixture was obtained. The hot mixtures were then allowed to cool to room temperature.

3.3.2.2.3 Preparation of drug loaded oleogels

According to preliminary study, oleogels prepared with the two oil phases (sesame oil phase and a mixture of silicone oil and sesame oil) showed a 3-dimensional gel network whereas silicone oil oleogel showed only tubules aggregation without forming a network (65). Hence, these two phases were selected for further preparation of oleogels. Oleogels were prepared using SMS as the gelator with two different oil phases. Bacitracin zinc was dissolved in either sesame oil or a mixture of silicone oil and sesame oil and heated at 55°C in a temperature-controlled water bath. SMS (20% w/w) was added to each oil phase with continuous stirring (300 rpm) until a clear homogenous solution was obtained. The clear solution was then mixed with 5% w/w silicone surfactant and allowed to cool to room temperature to induce gel formation. The final concentration of bacitracin zinc was 3.85% w/w in oleogel (0.77% w/w in bigel).

3.3.2.2.4 Preparation of bigels

The prepared hydrogels and oleogels were stored at 25°C for 24 h. The bigels were prepared by simply mixing the heated hydrogel (55°C) and the molten oleogel (55°C) using 20:80 ratio of the oleogel and hydrogel. The mixtures were continuously stirred at 1000 rpm for 5 min using an overhead stirrer and cooled to room temperature. The physical appearance of the prepared bigels was observed visually and

their physicochemical properties (pH, microscopy, FTIR, rheology, mechanical properties), *in vitro* drug release and antimicrobial activities were investigated. The composition of the hydrogel and oleogel in bigel was presented in Table 2. Xanthan gum and guar gum bigels with the loaded bacitracin zinc were designated as BXG, BGG and bigels with the combination of silicone oil were coded as BXGS and BGS.

Table 2 Composition of hydrogels and oleogels in bigels.

Sample	Hydrogels 100g (% w/w)					Oleogels 100g (% w/w)				
	Guar gum	Xanthan gum	Methyl paraben	Propyl paraben	Water	Silicone oil	Sesame oil	SMS	Silicone surfactant	Bacitracin zinc
BXG	–	2	0.1	0.02	q.s	–	71.15	20	5	3.85
BXGS	–	2	0.1	0.02	q.s	41.15	30.00	20	5	3.85
BGG	1.5	–	0.1	0.02	q.s	–	71.15	20	5	3.85
BGS	1.5	–	0.1	0.02	q.s	41.15	30.00	20	5	3.85

* The ratio of oleogel and hydrogel for all bigels was 20:80.

3.3.2.3 Development of hydrophilic drug loaded bigels

3.3.2.3.1 Preparation of drug loaded hydrogels

The hydrophilic drug selected for this study was neomycin sulfate. Hydrogel was prepared by dissolving the guar gum (1.5% w/w) in water containing 0.1% w/w methyl paraben and 0.02% w/w propyl paraben maintained at 55°C. The mixture was stirred at 1000 rpm for 5 min using an overhead stirrer and neomycin sulfate solution (0.5% w/w) was added with continuous stirring. The hot mixture was cooled down to room temperature.

3.3.2.3.2 Preparation of oleogels

Oleogels were prepared by dissolving 20% w/w SMS in either sesame oil or a mixture of silicone oil and sesame oil (55°C) and stirred at 300 rpm until a clear homogenous solution was obtained. The clear solution was then mixed with 5% w/w silicone surfactant and allowed to cool to room temperature to induce gel formation.

3.3.2.3.3 Preparation of bigels

Bigels were prepared by following the similar procedure of bacitracin zinc loaded bigels using the same ratio of oleogel and hydrogel. Briefly, the heated oleogel at 55°C was slowly incorporated into the hydrogel maintained at 55°C and mixed by mechanical stirring (1000 rpm for 5 min) using an overhead stirrer. Afterwards, the mixture was allowed to cool to room temperature. The physicochemical properties (pH, microscopy, FTIR, rheology, mechanical properties), drug content, *in vitro* drug release and antimicrobial activity of the prepared bigels were studied. The composition of bigel has been tabulated in Table 3. Neomycin sulfate loaded bigel containing sesame oil was designated as NGG and bigel with combination of silicone oil and sesame oil was coded as NGGS.

Table 3 Composition of hydrogels and oleogels in bigels

Sample	Hydrogels 100g (% w/w)					Oleogels 100g (% w/w)			
	Neomycin sulfate	Guar gum	Methyl paraben	Propyl paraben	Water	Silicone oil	Sesame oil	SMS	Silicone surfactant
NGG	0.625	1.5	0.1	0.02	q.s	—	75	20	5
NGGS	0.625	1.5	0.1	0.02	q.s	45	30	20	5

* The ratio of oleogel and hydrogel for all bigels was 20:80.

3.3.2.4 Physicochemical characterization of bigels

3.3.2.4.1 pH measurement

The pH of the prepared bigels was determined using a digital pH meter (Mettler Toledo SevenEasy, Switzerland) at 25°C. The measurement was performed in triplicate.

3.3.2.4.2 Microscopic studies

The CX41RF light microscope (Olympus, Japan) equipped with the digital eyepiece (Dinolite, AM423X, Taiwan) was used to observe the biphasic nature and homogeneity of the bigel formulations. The samples were stained with 0.1% w/w water soluble dye (amaranth) and 0.1% w/w oil soluble green dye and visualized under 10x magnification to differentiate the oleogel region from the aqueous hydrogel region and to determine the homogeneity of the formulations. The free version of Image J software 1.43v (NIH) was used to analyze the droplet sizes of the bigels. The droplet size was calculated in triplicate using the average of 500 droplets.

3.3.2.4.3 FTIR analysis

The interaction between the components of the bigels was analyzed using a FTIR spectrometer (Nicolet 4700, Thermo Electron Corporation, USA). Solid samples were recorded as an intimate mixture with dry potassium bromide (KBr). Attenuated total reflectance (ATR) mode was used to operate oil and gel samples. The scanning was processed in the wavelength range of 4000-600 cm^{-1} at 4 cm^{-1} resolution. The FTIR spectrum of each sample was analyzed using Omnic software (version 8, Thermo Electron Corporation, USA).

3.3.2.4.4 Rheological characterization

A rheometer (Kinexus Pro, Malvern Instruments, UK) with Peltier temperature control system was used for rheological characterization of the bigels. A cone and plate geometry of 40 mm diameter and 4° cone angle (CP4/40) was used for rheological characterization and all measurements were performed at 25°C. To characterize the flow properties of the bigels, the shear rate range of 0.1-100 s^{-1} was used and Power law model was used to fit the measured data. Dynamic oscillatory tests

were performed to investigate the viscoelastic properties. The amplitude sweep with a range of 0.01 to 100 Pa at a fixed frequency of 1Hz was run to determine the linear viscoelastic region (LVER) of the bigels. All experiments were performed in triplicate.

3.3.2.4.5 Mechanical properties

The mechanical properties of the bigels were characterized using a texture analyzer (TA. XT plus, Stable Micro Systems, UK) with a 5-kg load cell. A backward extrusion rig (A/BE) with cylindrical probe was used and the compression test was conducted to study the mechanical properties (hardness, cohesiveness and consistency) of the bigels. A 35 mm diameter disc was attached to the cylindrical probe. Twenty grams of sample was filled into a standard container and the probe with the disc attached was compressed until it penetrated from the gel surface for 5mm at a speed of 2mm/s, and the probe was subsequently withdrawn at the same speed. The resulting force-time plots were used to analyze the mechanical properties of the samples.

3.3.2.4.6 X-ray diffraction studies

The relative changes in the crystallinity of the bigels were evaluated using an X-ray diffractometer (MiniFlex II; Rigaku, Tokyo, Japan) at room temperature using monochromatic $\text{CuK}\alpha$ radiation at 15 mA and 30 kV over the range of 2θ angles from 5° to 40° with the scanning speed of $2^\circ/\text{min}$. The free trial version of Origin Pro 8 software was used for data analysis.

3.3.2.4.7 Drug content determination

3.3.2.4.7.1 Drug content determination of bacitracin zinc loaded bigels

One gram of all bigel formulations, equivalent to 0.77% w/w of bacitracin zinc, was accurately weighed into a 100 mL volumetric flask and mixed with 50 mL of sodium acetate buffer (0.05M, pH 5.5). The solution was then shaken in an ultrasonic bath for 30 min, adjusted to 100 mL with buffer solution and filtered through a $0.45\ \mu\text{m}$ nylon syringe filter. The drug content determination was carried out using a HPLC-DAD (Agilent 1200 series, Agilent Technologies, USA) and Hypersil

gold C-18 column (250 x 4.6 mm, 5 μ m). The official analytical method (USP 39) for bacitracin zinc was used for drug content determination (86). The mobile phase was a mixture of organic solvent and aqueous system at the ratio of 63:37. The organic phase consisted of methanol and acetonitrile at the ratio of 27:2, while the aqueous phase was a mixture of 0.2 M phosphate buffer aqueous solution and 0.1mM EDTA adjusted to pH 6. Samples were injected at a flow rate of 1mL/min with an injection volume of 100 μ L. Bacitracin zinc was detected at 254 nm and the drug content was calculated against the calibration curve.

3.3.2.4.7.2 Drug content determination of neomycin sulfate loaded bigels

The concentration of neomycin sulfate was determined by derivatization method using o-phthaldialdehyde reagent (87). The method was validated according to USP guidelines in terms of specificity, linearity, accuracy and precision (88). One gram of all bigel formulations, equivalent to 0.5% w/w of neomycin sulfate, was accurately weighed into a 100 mL volumetric flask and mixed with 50 mL of phosphate buffer (0.05M, pH 5.5). The solution was then shaken in an ultrasonic bath for 30 min, adjusted to 100 mL with buffer solution and filtered through a 0.45 μ m nylon syringe filter. An equal volume (1 mL) of sample, o-phthaldialdehyde reagent, and isopropanol were mixed using a vortex mixer and stored for 40 min at room temperature. The drug content determination was carried out using an Agilent G1103A model UV-visible spectrophotometer (Agilent Technologies, USA). The absorbance was measured at 335 nm and the drug content was calculated against the calibration curve.

3.3.2.4.8 *In vitro* drug release study

The *in vitro* drug release study was performed using Franz diffusion cells (Logan DHC 6T, Logan Instruments Corp, New Jersey). Sample (0.5 g) were accurately weighed and spread over a cellulose acetate membrane (0.45 μ m pore size and 25 mm diameter) which was mounted between the donor and receptor chambers of the diffusion cell. The volume of the receptor chamber was 12 mL and the

receptor mediums were sodium acetate buffer (pH 5.5) for bacitracin zinc bigels and phosphate buffer (pH 5.5) for neomycin sulfate bigels. The receptor medium was kept at $32 \pm 0.5^\circ\text{C}$ and continuously stirred at 600 rpm. At definite time intervals (0.5, 1, 1.5, 2, 4, 6, 8 and 10h), 1.5 mL samples (bacitracin zinc bigels) or 3 mL samples (neomycin sulfate bigels) were removed and replenished with an equal volume of fresh sodium acetate or phosphate buffer. For bacitracin zinc loaded bigels, the amount of drug released was analyzed using a HPLC-DAD (Agilent 1200 series, Agilent Technologies, USA) at 254 nm under the identical HPLC conditions utilized for the drug content assay. An Agilent G1103A model UV-visible spectrophotometer was used to analyze the amount of drug release of neomycin sulfate bigels and measured at 335 nm using the same method of the drug content assay. The *in vitro* drug release experiments were carried out in triplicate and the cumulative percentage of drug release (CPDR) was calculated. To investigate the mechanism of drug release from the bigel formulations, *in vitro* drug release data was fitted with various kinetic models such as zero order, first order, Higuchi and Korsmeyer-Peppas models.

3.3.2.4.9 Antimicrobial assay

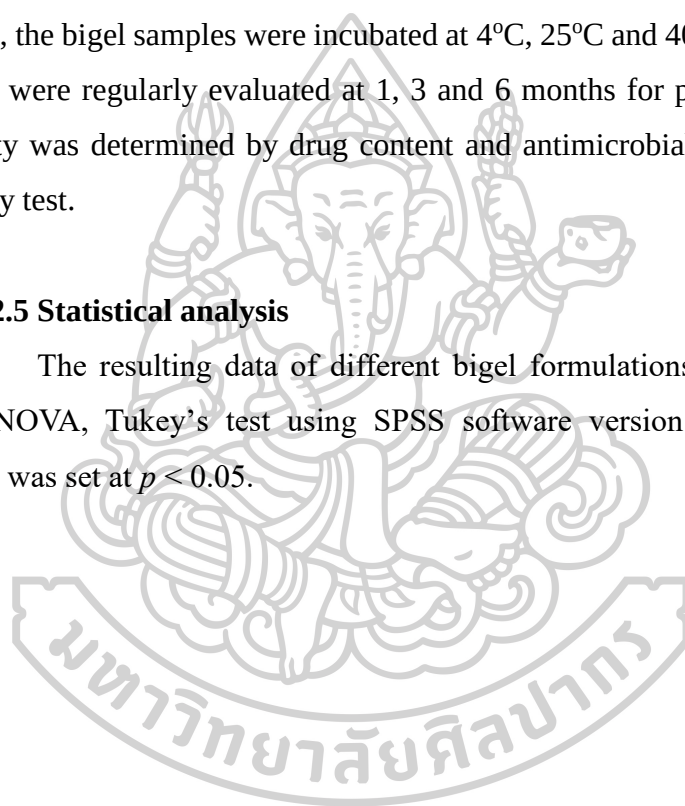
Drug loaded bigels were investigated for their antimicrobial activity against *Staphylococcus aureus* using agar well diffusion technique. The culture medium for antimicrobial assay was tryptic soy agar (TSA). The actively growing broth culture of *Staphylococcus aureus* (1×10^8 CFU/mL) was spread onto the TSA agar plate, the wells (diameter = 6mm) were filled with 100 μL of the test samples and incubated at 37°C for 24 h. For bacitracin zinc loaded bigels, the standard solution of bacitracin zinc (0.77% w/w) in sodium acetate buffer (pH 5.5) was used as a positive control while neomycin sulfate standard solution (0.5% w/w in phosphate buffer, pH 5.5) was used as a positive control for neomycin sulfate loaded bigels. The blank bigel was used as a negative control for both types of bigels. The diameter (mm) of the clear inhibition zone indicated the antimicrobial activities was detected. The tests were performed in triplicate and the mean inhibition zones $\pm$ SD were calculated.

3.3.2.4.10 Stability studies

The accelerated stability test and intermediate stability test (ICH guidelines) were used to determine the stability of the bigels (89). For the accelerated stability test, the temperature cycling method was carried out by alternately storing the bigels samples at 4°C for 24 h and at 45°C for 24 h for 6 cycles. At the end of 6 cycles, the bigels were evaluated for physical or organoleptic changes (color, flow at inversion, pH) and the stability of drug was evaluated by the determination of drug content and antimicrobial activity at initial and after stability test. For intermediate stability test, the bigel samples were incubated at 4°C, 25°C and 40°C for 6 months and the samples were regularly evaluated at 1, 3 and 6 months for physical changes and drug stability was determined by drug content and antimicrobial assay at initial and after stability test.

3.3.2.5 Statistical analysis

The resulting data of different bigel formulations were compared by one-way ANOVA, Tukey's test using SPSS software version 16.0. The level of significance was set at $p < 0.05$.



CHAPTER 4

RESULTS AND DISCUSSION

4.1 Development of oleogels with silicone oil

To reduce the greasy and unpleasant feature of vegetable oil oleogels and to develop the topical oleogel formulation with improved aesthetic and sensory profiles, oleogels were prepared by combination of silicone oil and vegetable oil. Firstly, the mutual miscibility of the oil pair was evaluated by mixing silicone oil with 6 types of vegetable oils. The best miscible oil pair (sesame oil and silicone oil) with the best ratio was used to prepare oleogel with SMS gelator and compared with that of SMS-sesame oil oleogel and SMS-silicone oil oleogel.

4.1.1 Preliminary study to choose the best miscible oil pair

The result of miscible and immiscible oil pairs was shown in Figure 15A. Among the 6 types of oils; castor, sunflower, and sweet almond oils were completely immiscible with silicone oil and showed phase separation. However, olive, safflower, and sesame oils were fully miscible with silicone oil. From these 3 miscible pairs, sesame oil was selected to combine with silicone oil in oleogel preparations because it has the highest antioxidant content among the 3 vegetable oils. This leads to better stability of the final oleogels. In addition, sesame oil has a wide range of applications in pharmaceutical and cosmetic fields which are the intended applications (20, 22). Afterwards, the miscibility of sesame oil and silicone oil at different ratios was investigated. The miscibility of the two oils was observed in all 5 ratios and the result was shown in Figure 15B. Figure 16 displayed the CGC determination results of these 5 ratios. The CGC values were 10, 10, 14, 16, and 20% w/w at the ratios of 1:4, 2:3, 1:1, 3:2, and 4:1, respectively. The lower CGC value of 10% w/w for SMS was obtained at the lower ratios of sesame oil to silicone oil (1:4 and 2:3). This might be due to the lower solubility of SMS at the lower amount of sesame oil. The ratio of 2:3 was chosen for the preparation of oleogels because the microstructure shown in Figure 17 revealed the formation of stronger gel network at this ratio.

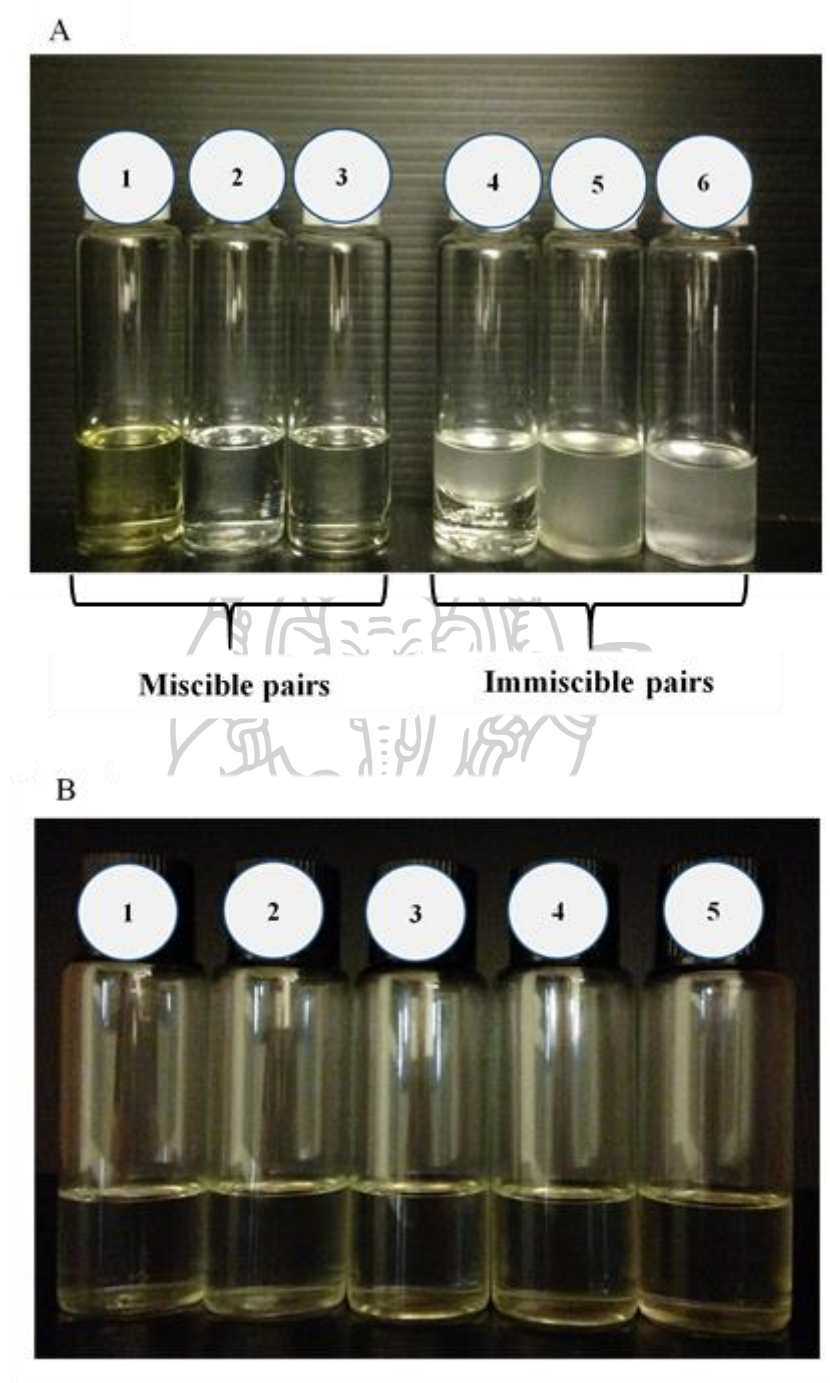


Figure 15 (A) Miscible and immiscible oil pairs of silicone oil and (1) olive oil, (2) safflower oil, (3) sesame oil, (4) castor oil, (5) sweet almond oil, and (6) sunflower oil and (B) miscibility study of sesame oil with silicone oil at various ratios (1) 1:4, (2) 2:3, (3) 1:1, (4) 3:2, and (5) 4:1 after keeping at 25°C for 24 h.

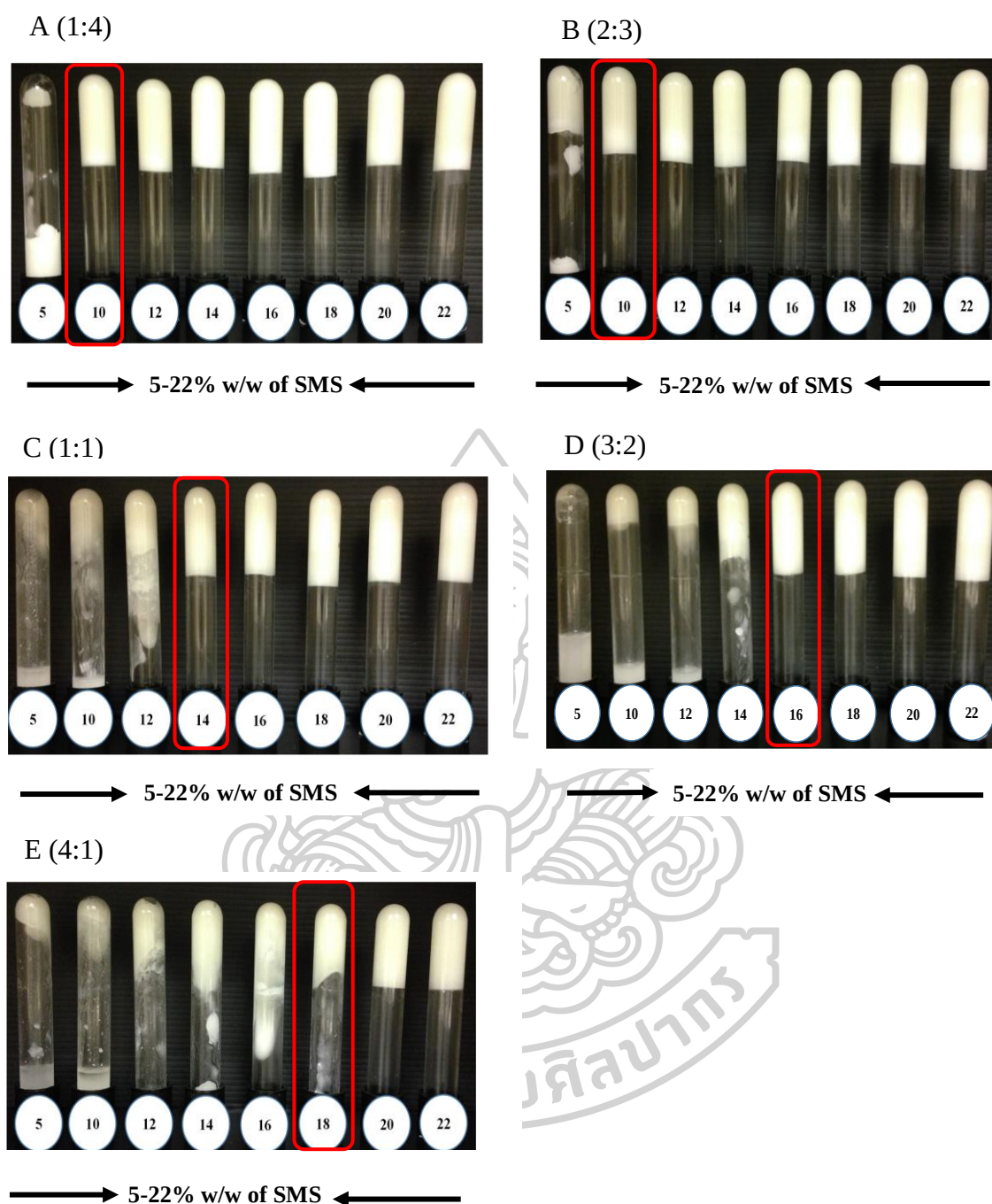


Figure 16 Determination of CGC of SMS for five different ratios of sesame oil and silicone oil oleogels (A) 1:4, (B) 2:3, (C) 1:1, (D) 3:2, and (E) 4:1. The concentration of SMS for each ratio was varied from 5-22% w/w. The CGC is the minimum gelator concentration which failed to flow when the tubes were inverted after keeping at 25°C for 24 h.

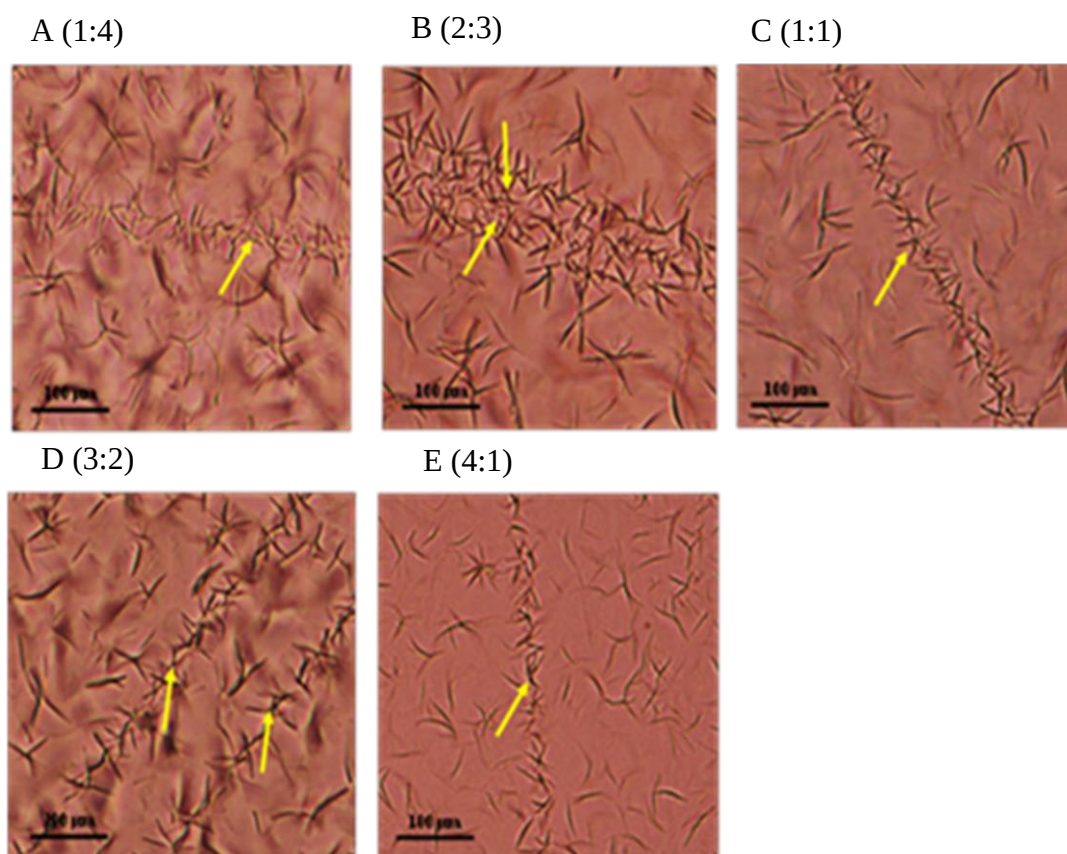


Figure 17 Polarized light micrographs of five different ratios of sesame oil and silicone oil oleogels at CGC of SMS. (A) 1:4, (B) 2:3, (C) 1:1, (D) 3:2, and (E) 4:1. The gel network formed by association of tubules at contact points and appeared at all ratios as demonstrated by arrows.

4.1.2. Viscosity of the oil components

The viscosity of the oil components was determined using a rheometer with viscosity-shear rate ramp. All the oil samples in this study showed Newtonian flow characteristics as the viscosity was constant, independent of shear rate. The absolute viscosity of the samples was obtained from either the linear regression of the slope from shear stress (Y-axis) against shear rate (X-axis) plot or from the average readings of viscosity at different shear rates. The results were shown in Table 4. The absolute viscosity of both sesame oil and silicone oil were comparable to those published values (90-92). Sesame oil had the significant highest viscosity value ($p < 0.05$) as it consisted mainly of long-chain fatty acids (linolenic acid 46.9%, oleic acid 37.4%, and palmitic acid 9.1%) (20, 22). Silicone oil, a short-chain cyclic polydimethylsiloxane, has low

heat of vaporization of 31 cal/g and low viscosity of 4.2 cSt (90). Due to very light and excellent spreading properties, silicone oil was used to blend with high viscosity silicone oils to improve spreadability (23). In this study, silicone oil showed low viscosity value of 4.0 mPas which is equal to 4.2 cSt. Addition of silicone oil significantly reduced the viscosity of sesame oil which could improve the spreading property of the oleogel preparations. This is in accordance with the findings from previous study (24).

Table 4 Viscosity of oils (n=3).

Oil	Viscosity at 25°C (mPas) (mean $\pm$ SD)	Reference viscosity at 25/26°C
Sesame oil	57.80 $\pm$ 0.13 ^a	54.37, 59.3 mPas (91, 92)
Silicone oil	4.00 $\pm$ 0.12 ^c	4.2 cSt (90)
Mixture of sesame oil and silicone oil (2:3)	18.60 $\pm$ 0.45 ^b	

a, b, c mean values are significantly different ($p < 0.05$).

4.1.3. Determination of CGC for oleogels

Oleogels with 5-22% w/w of SMS were prepared and the CGC of each oleogel preparation was determined. The results were shown in Figure 18. The CGCs of OG1 and OG2 were 20% and 18% w/w respectively which were not very different. However, the CGC of OG3 which contained combination of sesame and silicone oils decreased by half to only 10% w/w of SMS. To confirm the CGC of OG3, the concentration of SMS (5, 7, 9 and 10% w/w) was tested. At the lower concentration of SMS (<10% w/w), all samples flowed when the tubes were inverted. Gel formation and CGC values of SMS oleogel were mainly influenced by the nature of solvent. According to previous studies, CGC of SMS was dependent on solvents. The vegetable oil tends to show high CGC. The CGC values of SMS for hexadecane, sesame oil, olive oil and sunflower oil were 1% w/v, 15% w/w, 20% w/w and 18% w/w (18, 20, 27, 93).

Different type of vegetable oils had different composition of fatty acids which could influence the solubility of the gelator molecules (94). In this study, the CGC value of SMS in sesame oil oleogel was found to be different from previous studies. It might be due to the different sources of the oil and SMS. Since SMS is a non-ionic surfactant which comprised both polar and non-polar segments, the higher CGC of SMS for sesame oil and silicone oil might be due to the higher solubility of SMS in both polar sesame oil and non-polar silicone oil. Interestingly, the combination of silicone oil and sesame oil reduced the CGC of SMS because the combination of polar and non-polar solvents could provide an optimum solubility and insolubility balance of the solvent. Solvent modification could affect the CGC of oleogels due to the influence of solvent on the gelator solubility; hence altering the crystallization behavior and microstructure of the gel network (95).

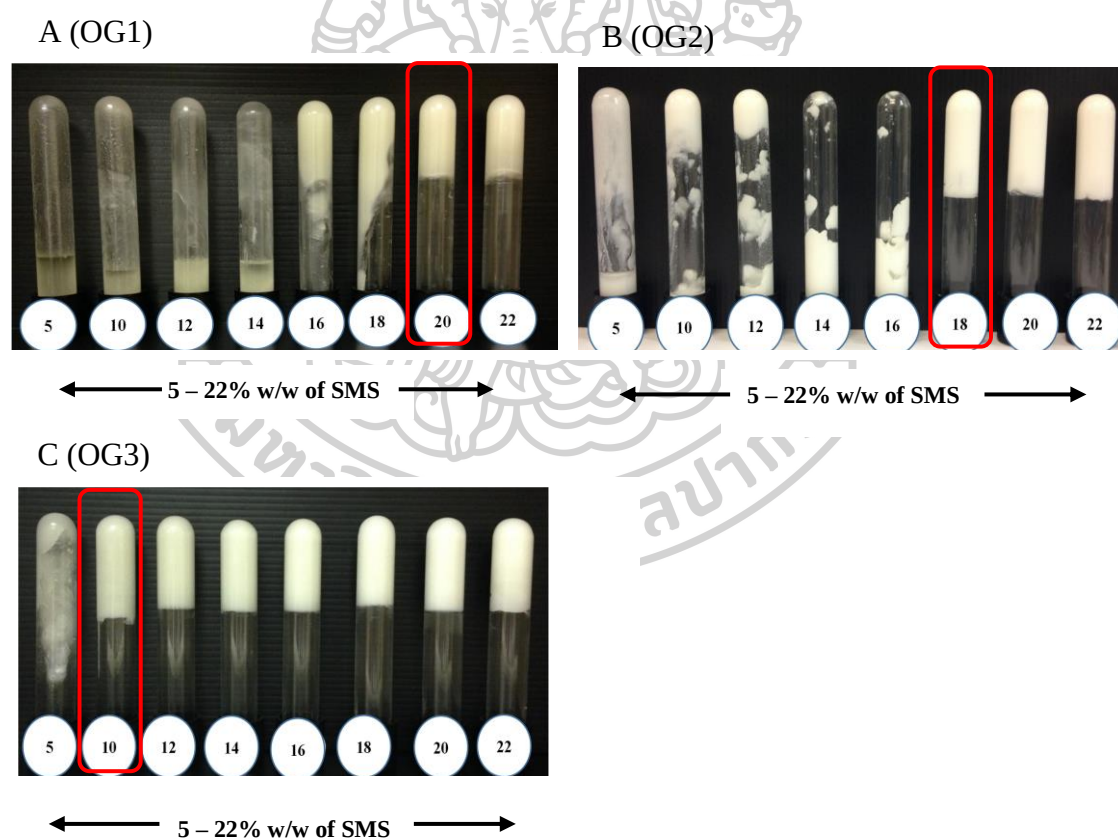


Figure 18 Determination of CGC of SMS for three oleogels (A) OG1, (B) OG2 and (C) OG3. The concentration of SMS for each oleogel was varied from 5-22% w/w. The CGC is the minimum gelator concentration which failed to flow when the tubes were inverted after keeping at 25°C for 24 h.

4.1.4. Preparation of oleogels

Oleogels were formed when the hot mixture of gelator and solvent was cooled down to room temperature. The hot solution of gelator was transparent and slowly turned turbid and finally converted into opaque oleogels. This might be accounted to the change in the solubility of the gelator molecules. The color of the oleogels in this study was significantly different due to the different nature of solvents. OG1 was yellowish in color and very oily, which is the major drawback of vegetable oil oleogels. OG2 was white in color but did not spread evenly when applied on the skin. However, OG3 was white in color, less oily when applied and better in spreadability compared to OG1. This might be due to the lower surface tension (17.38 ± 0.10 mN/m), and the light and dry feel property of silicone oil added to sesame oil.

4.1.5 Physicochemical characterization of the developed oleogels

4.1.5.1 FTIR analysis

The FTIR spectra of SMS gelator, oils (sesame oil and silicone oil) and oleogels are shown in Figure 19. FTIR spectra indicated the presence of molecular interactions amongst the components presented in the oleogels. A shallow broad peak at $3700\text{--}3100\text{ cm}^{-1}$ in OG1 and OG3 revealed the presence of stretched hydrogen-bonded O-H groups. This indicated the presence of intermolecular hydrogen bonding between the hydroxyl groups present in SMS and sesame oil, resulting in the formation of the gel network (19). As this bond was absent in OG2, it might also explain the different gel network formation of OG2. The spectra of OG3 and OG2 showed additional peaks at about 800 cm^{-1} , 1200 cm^{-1} , and a wide band around $1270\text{--}850\text{ cm}^{-1}$. This indicated the presence of Si-C stretching and CH_3 deformation in Si- CH_3 and Si-O-Si stretching (96). The C-H asymmetric and symmetric stretching vibrations of SMS, sesame oil and silicone oil produced absorption bands at about 2920 and 2850 cm^{-1} . The absorption band at around 1750 cm^{-1} was attributed to C=O stretching vibration due to the ester group present in SMS (19, 20).

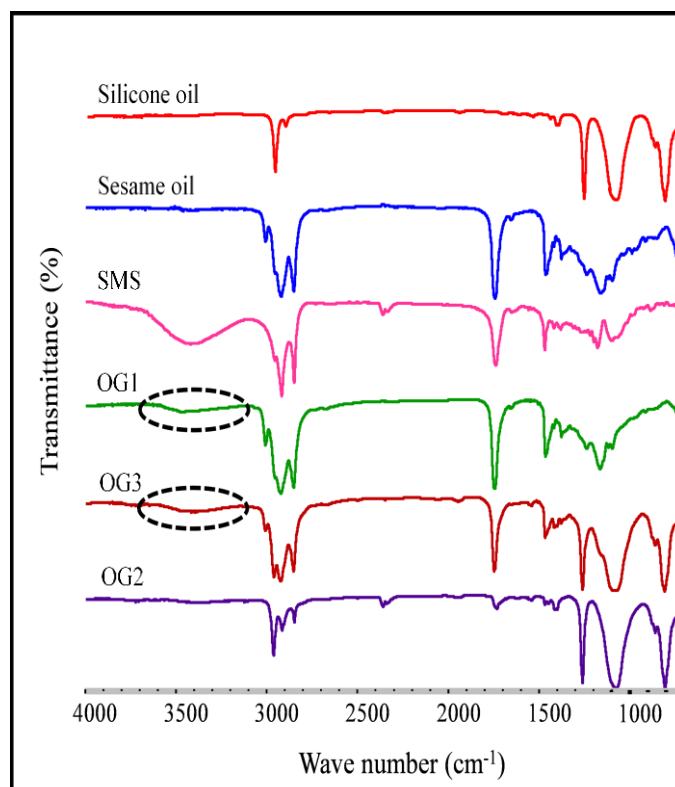


Figure 19 FTIR spectra of oleogels and their ingredients.

4.1.5.2 Microstructure studies

The microstructure of the oleogels was observed under polarized light microscope and the results were depicted in Figure 20. The oleogels were prepared by melting/dissolving the gelator in the solvent and allowing the system to cool down gradually. Upon cooling down, the solubility of the gelator in the solvent reduced according to the decreasing affinity between the gelator and the solvent. Consequently, gelator molecules self-assembled and toroidal vesicular tubules were formed, followed by the association of the tubules at contact points and the 3-dimensional network was developed (18). From Figure 20A, only small and thin 3-dimensional network with distinct contact points was seen in OG1 despite its high gelator concentration of 20% w/w. Furthermore, with 18% w/w SMS concentration, OG2 showed only toroidal vesicular tubules aggregation of the gelator molecules without establishing contact points, as shown in Figure 20B. In contrast, the long and thick 3-dimensional network with distinct contact points was observed in OG3 with only 10% w/w of the gelator

concentration (Figure 20C). The nature and functional groups of the solvent significantly affect the microstructure of the gelator molecules aggregation (18, 97). In this study, the network formation of the oleogels was mainly driven by hydrogen bonding between the hydroxyl groups present in SMS and sesame oil. Silicone oil was non-polar and contained no functional groups to form hydrogen bond with SMS, confirmed by the absence of hydrogen bonding in the FTIR analysis of OG2. Although there might be hydrophobic interactions between silicone oil and the hydrophobic part of SMS (which is a surfactant), this interaction is weak and at CGC level, it might not be sufficient to produce a stable gel, leading to syneresis on storage (27). The high concentration of SMS in OG2 could only increase the viscosity of the bulk sample in the liquid phase, thus favored stronger gelator aggregate-aggregate interaction rather than solvent-gelator interaction. Consequently, only solvent entrapment occurred during the cooling stage, and gave rise to a gel with weaker strength. From the oil viscosity results, the addition of silicone oil reduced the viscosity and also decreased the polarity of the sesame oil. The viscosity reduction as well as the polarity alteration of the solvent could influence the self-assembly of SMS and favored the formation of the long and thick 3-dimensional network with distinct contact points, as observed in OG3. The polarity and viscosity of the oil component controlled the self-assembly of the gelator molecules which in turn affected the crystallization, gelation behavior, and gel strength (18, 54, 98). Therefore, it could be concluded that the hydrogen bonding (between the gelator-gelator and the gelator-solvent), the solvent polarity and the viscosity of the oil phase were the major factors influencing the formation of the gel network. In addition, combination of the 2 oils at a suitable ratio in this study also improved the solvent entrapment property of the gelator hence reduced the possibility of syneresis.

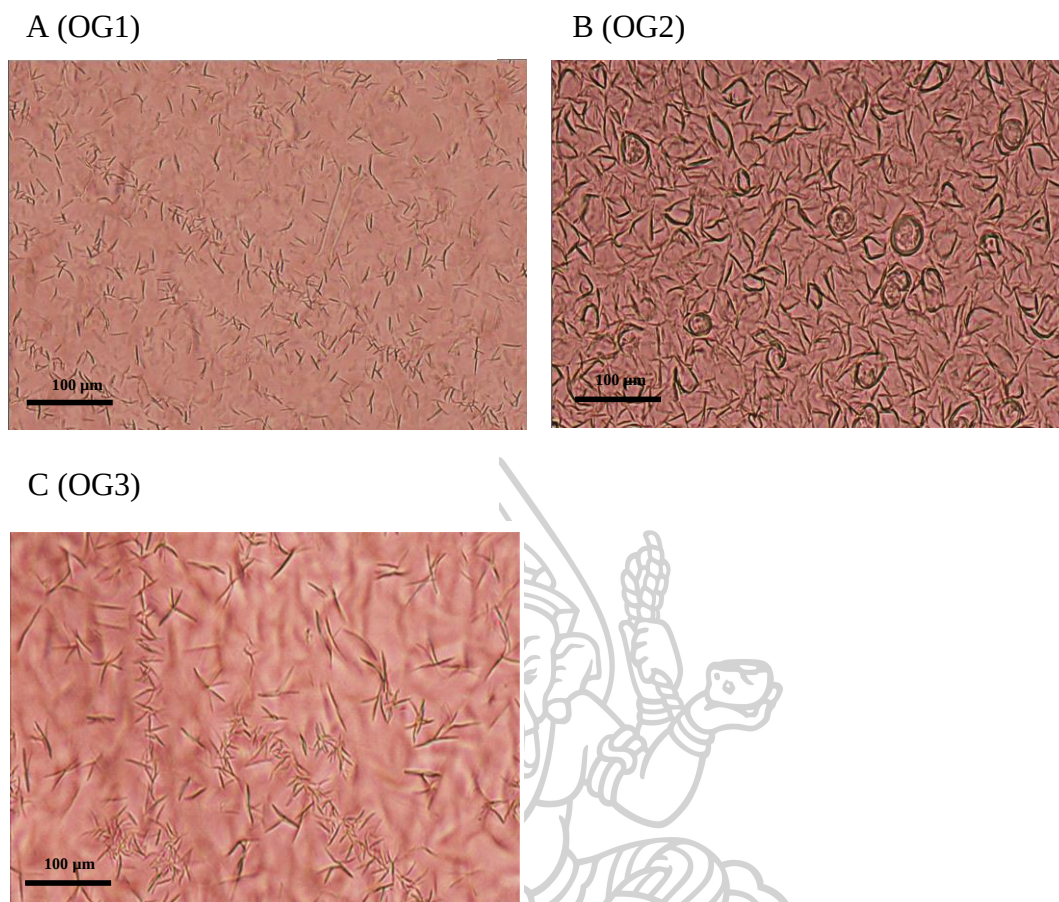


Figure 20 Polarized light micrographs of three oleogels at CGC of SMS (A) OG1, (B) OG2 and (C) OG3.

4.1.5.3 Rheological characterization

Non-destructive oscillation tests provide the rheological parameters such as the storage or elastic modulus (G'), the loss or viscous modulus (G''), and the LVER. The viscoelastic behavior involves the structural changes within the gel formulation. It combines 2 components; G' and G'' which refer to solid-like and liquid-like properties, respectively. During an amplitude sweep, the amplitude of the shear stress is varied while the frequency is kept constant. The G' and G'' are plotted against the strain. At low strain, G' and G'' are constant, meaning that the sample structure is undisturbed. This region is called LVER. Once the moduli start to decrease, the structure is disturbed, and the end of the LVER is reached. The plateau value of G' in the LVER-region describes the rigidity of the sample at rest while the plateau G'' value

implies the viscosity of the unsheared sample. Therefore, LVER provides the information involving resistance of the material to deformation (99). The long LVER presented in a product have higher stability as compared to products with a weak and sensitive structure (100). From the amplitude sweep result in Figure 21A, all oleogels had 1 decade higher G' than G'' , indicating that the system was more elastic than viscous at the studied frequency range, hence they had strong gel property. The plateau G' value of OG3 was the highest among all preparations, and showed 1 decade higher than the other two, implying the highest gel strength. OG3 also possessed the longest LVER suggesting the highest stability of the gel formulation and resistance to syneresis. As mentioned in the microstructure studies, the gel formation of OG2 was mainly from self-aggregation of the high gelator concentration and also no contact points were established. Therefore, the gel strength of OG2 was the lowest as seen from the plateau G' values in Figure 21A and a shorter LVER than that of OG3. The plateau G' and G'' values of OG1 were in between those of OG3 and OG2, meaning weaker gel strength than OG3 but better than OG2. This was supported by the polarized light micrographs in Figure 20 as small and thin fibers gel network was observed for OG1 while the long and thick 3-dimensional network with distinct contact points was obvious in OG3.

The frequency sweep test measures the response of a system as a function of frequency at a constant amplitude within the LVER. It provides the specific information of G' and G'' of a viscoelastic system without disrupting its structure (101). Figure 21B displayed the frequency sweep result of the 3 oleogels. All preparations were regarded as true gels because G' values were higher than G'' in all cases and G' was not dependent upon the applied frequency.

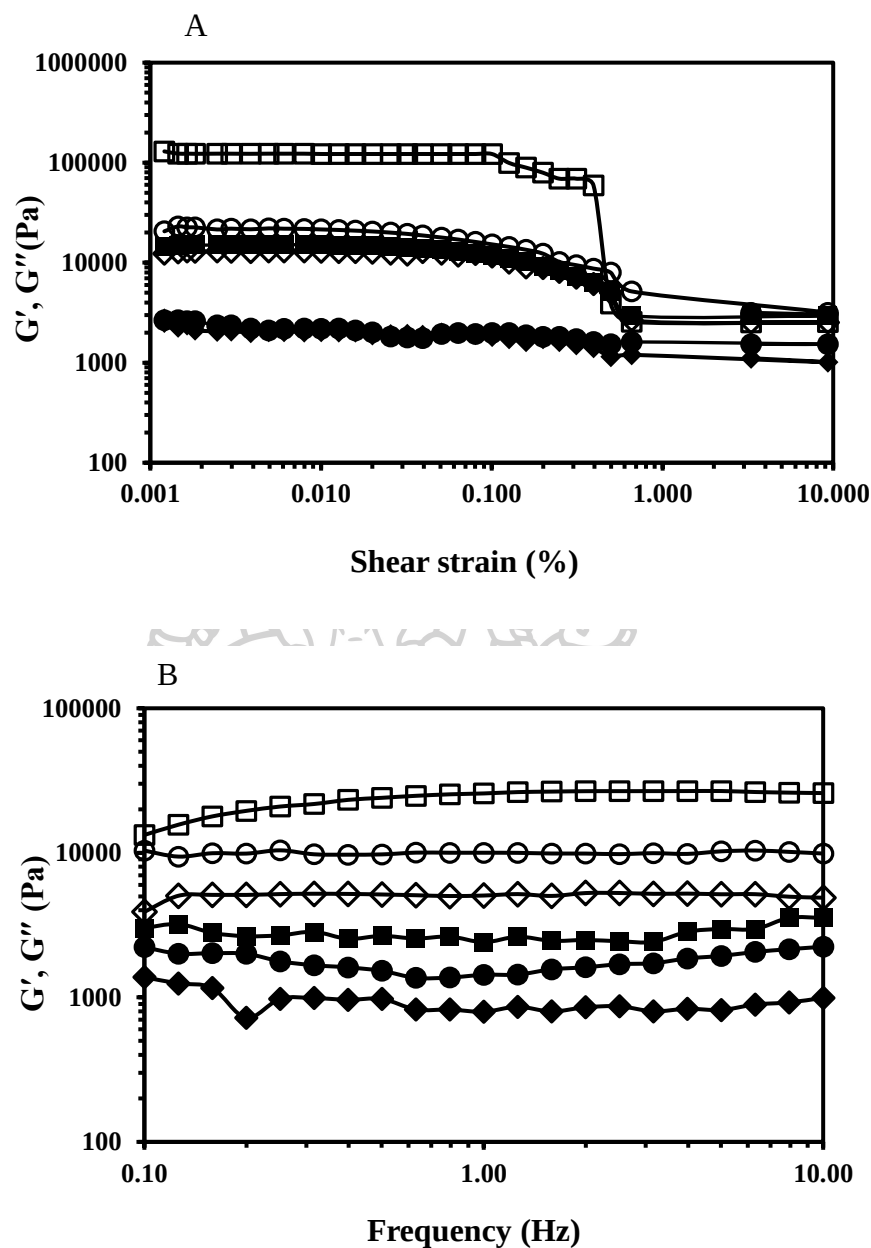


Figure 21 (A) Amplitude sweep result of oleogels: Storage modulus (G' , open symbols) and loss modulus (G'' , filled symbols) as function of shear strain for OG1 ($\circ/\bullet$), OG2 ($\diamond/\blacklozenge$) and OG3 ($\square/\blacksquare$). The strain range was 0.001 to 10 Pa at a fixed frequency of 1 Hz. (B) Frequency sweep result of oleogels: Storage modulus (G' , open symbols) and loss modulus (G'' , filled symbols) as function of frequency for OG1 ($\circ/\bullet$), OG2 ($\diamond/\blacklozenge$) and OG3 ($\square/\blacksquare$). The frequency range was 0.1-10 Hz at the strain value of 0.05 Pa.

The viscosity profiles of the oleogels were demonstrated in Figure 22. All showed pseudoplastic flow characteristic. OG3 had the highest viscosity at rest but the viscosity reduced quickly when sheared thus suggested easiest spreadability which was suited for topical applications.

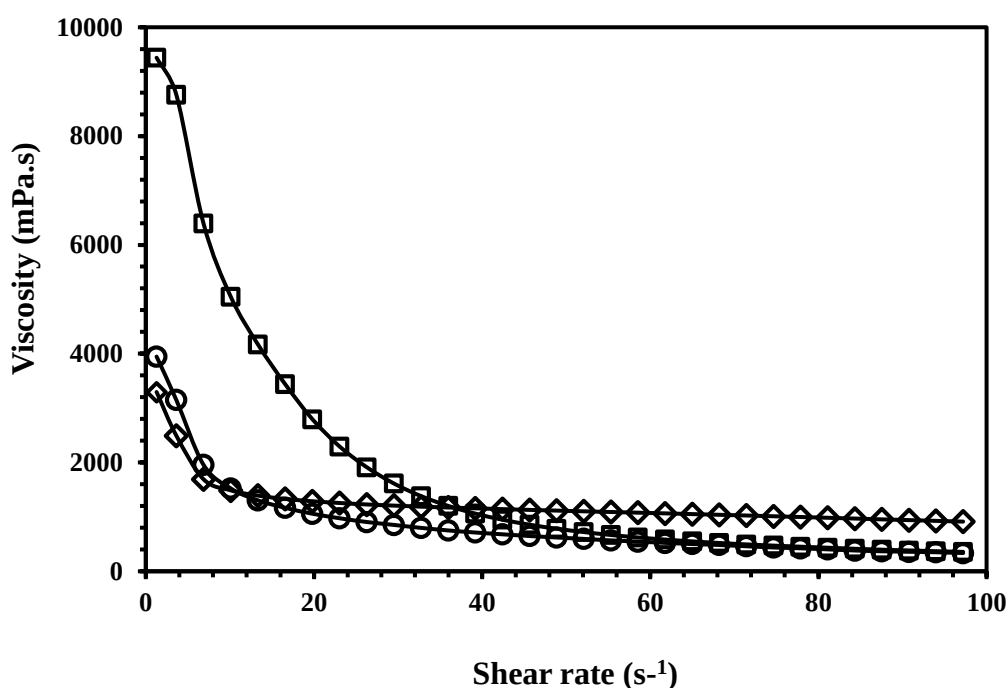


Figure 22 Viscosity profiles of OG1 (o), OG2 ($\diamond$) and OG3 ($\square$) at the shear rate range of 0.1 - 100 s^{-1} .

Thixotropy refers to time-dependent decrease in viscosity at a constant shear rate with reversible structure recovery after removal of the shear stress or shear rate. The recovery can be a complete recovery or a partial recovery and can be rapid or gradual. Therefore, the thixotropic behavior is best studied by changing the shear rate stepwise and tracking the response viscosity over time (102). The thixotropic behavior of the three oleogels was measured using a specific rheology test called “three-step shear rate sweep” which consists of three consecutive phases in the control rate mode with alternate low and high shear rates. The results were presented in Figure 23. In the first phase (at the shear rate of 0.1 s^{-1}) which represented the viscosity at rest, all three oleogels had high viscosity. In the second phase, the shear rate was rapidly shifted

from 0.1 s^{-1} to 100 s^{-1} which related to the shear rate during application of semisolid product on the skin, the viscosity of all oleogels immediately dropped as the network structure was completely broken down by the high shear. In the third phase, the shear rate was quickly shifted back to 0.1 s^{-1} , representing the end of application process on the skin. The oleogel viscosity increased to different values as their structure showed some recovery depending on the gel strength. OG2 had the weakest gel strength, hence easily broke down and flowed with the lowest viscosity. Its recovery was very rapid (2 seconds) in the third phase. This might be because no strong connection points between the tubules were established in the gel network. This result was in agreement with previous study of Doan et al. (2015), which stated that weak gels broke easier, flowed at lower shear values and had higher regeneration percentage than stronger gels (71). In contrast, the gel network structures of OG1 and OG3 were stronger than that of OG2 due to the presence of hydrogen bonding, therefore the structure breakdown in the second phase was less and the viscosity decrease was in the order of $\text{OG3} < \text{OG1} < \text{OG2}$. All 3 oleogels showed very quick partial structure recovery in the third phase. The recovery time was within a few seconds.

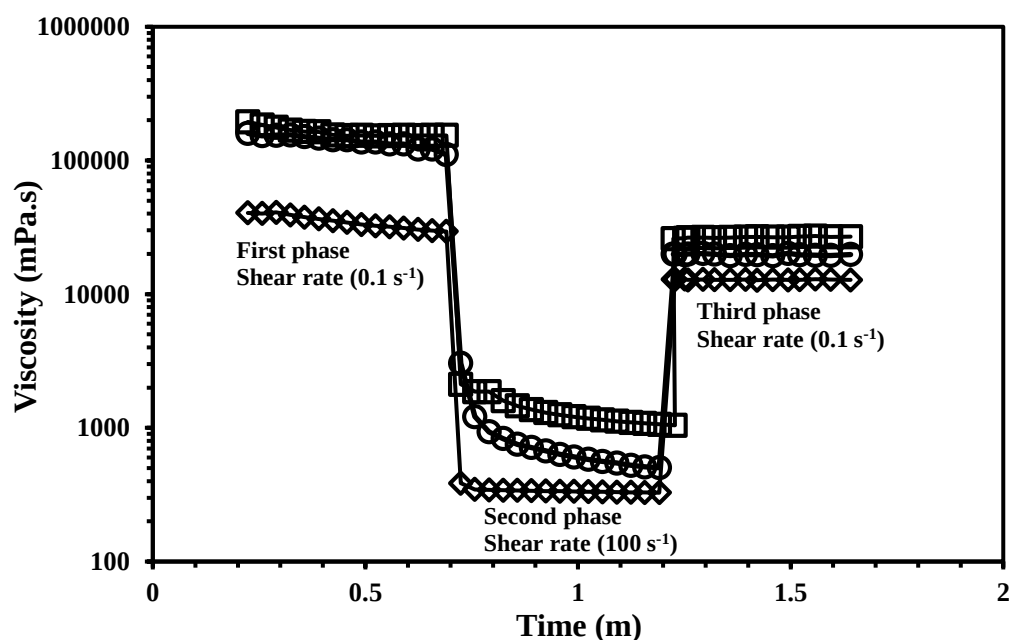


Figure 23 Thixotropic behavior of OG1 (o), OG2 ($\diamond$) and OG3 ($\square$) using three step shear rate sweeps.

4.1.5.4 X-ray diffraction studies

The oleogels and their components were analyzed using XRD to study the influence of solvent on crystalline behaviors of oleogels. The underlying peaks of the profiles were determined using the peak-fitting tool of Origin Pro 8 and Gaussian model was used for peak-fitting. The full width of the peak at half maximum (FWHM), area under the curve (AUC), and the Bragg distance (d -spacing) values were calculated using the Origin Pro 8 software and the results were tabulated in Table 5.

Table 5 FWHM, AUC and d-spacing values of oleogels and their components for XRD analysis.

Sample	FWHM			AUC			d-spacing (Å)
	Halo 1 ($11^\circ 2\theta$)	Halo 2 ($19^\circ 2\theta$)	Peak 3 ($21^\circ 2\theta$)	Halo 1 ($11^\circ 2\theta$)	Halo 2 ($19^\circ 2\theta$)	Peak 3 ($21^\circ 2\theta$)	Peak 3 ($21^\circ 2\theta$)
SMS	—	—	1.90	—	—	6027.68	4.1120
Silicone oil	2.63	—	—	1153.38	—	—	—
Sesame oil	—	7.33	—	—	8673.62	—	—
OG 1	—	6.76	0.56	—	6512.62	181.65	4.1146
OG 2	2.39	—	6.67	1199.28	15.71	2512.72	4.1414
OG 3	3.66	14.26	3.72	826.91	2565.69	349.97	4.1241

Figure 24 showed the presence of broad halos, silicone oil at $11^\circ 2\theta$ (halo 1), and sesame oil at $19^\circ 2\theta$ (halo 2), and single sharp peak of SMS at $21^\circ 2\theta$ (peak 3). SMS showed a strong sharp signal with an associated Bragg distance (d -spacing) of 4.11 Å. When compared to the XRD patterns of the neat SMS, all three oleogels showed a dominant peak at 4.11-4.14 Å which was attributed to the crystalline nature of SMS. The same d -spacing values were noted in the XRD patterns of SMS-mustard oil oleogels as reported by Sagiri and coworkers (2016) (19). Although the

peak position of SMS and the *d*-values of all oleogels did not significantly changed, the FWHM and AUC values of oleogels had altered.

The FWHM is dependent on the crystallinity of the sample, which in turn is dependent on the interactions amongst the gelator molecules responsible for the formation of an ordered structure. For FWHM determination, the XRD profile data was smoothened by Savitzky-Golay filter using 50 points of window and polynomial order of 5. Then the width of the smoothened curve was calculated resulting the FWHM value (103). The FWHM values of the broad halos (halos 1 and 2) were higher than that of peak 3, indicating the comparatively liquid nature as compared to single sharp peak of peak 3. Therefore, only peak 3 (SMS) was chosen to compare the crystalline behavior of different oleogels. The FWHM values of SMS peak for OG1, OG2 and OG3 were found to be 0.56, 6.67, and 3.72, respectively. The lower FWHM value relates to the higher crystallinity and vice versa (103). The low FWHM value of OG1 indicated an increase in the crystallinity from the high concentration of SMS (20% w/w). In contrast, the highest FWHM value of OG2 showed the low crystalline nature although it contained the high gelator concentration of 18% w/w. This might come from decrease in the ordered structure of OG2. This finding was in agreement with the microscopic character of OG2. The AUC values of OG1, OG2, and OG 3 were 181.65, 2512.72, and 349.97, respectively. The AUC results were similar to those of FWHM results. The higher AUC favored the lower crystalline nature. Therefore, it could be concluded that the combination of silicone oil and sesame oil in OG3 exhibited crystalline behavior even with 10% w/w of SMS. The crystallinity of OG3 might be attributed to the presence of network structure made up of toroidal vesicular tubules formed in the solvent.

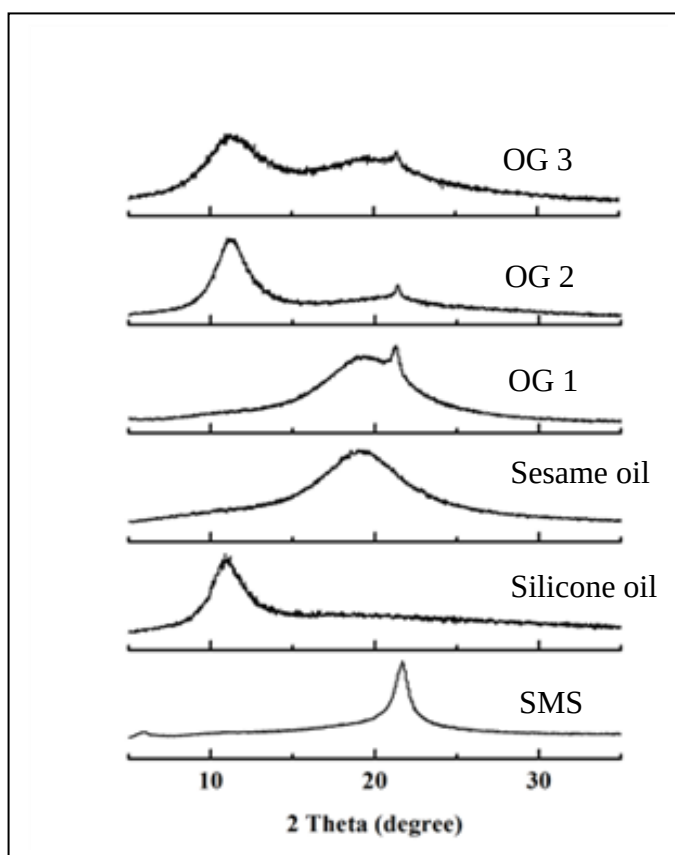


Figure 24 XRD patterns of oleogels and their ingredients.

4.1.5.5 Differential scanning calorimetry (DSC)

The thermal profiles of SMS, sesame oil, silicone oil and oleogels were characterized with DSC and the results were displayed in Figure 25. In this study, the endothermic and exothermic peaks of SMS were found to be at 52 °C and 46 °C during the heating and cooling cycles, respectively. This result showed some variation from previous studies; 51 °C for endothermic (18, 20) and 45°C for exothermic (Singh et al., 2015) which might be from different sources of SMS. All oleogels showed thermoreversible nature. The endothermic and exothermic peaks assigned to the melting and crystallization of SMS were found at 43 – 46 °C and at 44– 48 °C, respectively in all three oleogels (18). The slight shifting of the corresponding peaks during the heating and cooling cycle might be due to the structural changes of SMS molecules. Due to thermal hysteresis property, the position of the melting and crystallization peaks might be shifted. In addition, different microstructure of oleogels affected the gel strength, which in turn affected the melting and crystallization behavior

of oleogels. The melting and crystallization enthalpies were determined using the area under the respective peaks and the values were tabulated in Table 6.

Table 6 DSC studies of OG1, OG2 and OG3 (n=3, mean $\pm$ SD).

Sample	Melting endotherm				Crystallization exotherm			
	T _{onset,m} (°C)	T _m (°C)	ΔH_m (Jg ⁻¹)	ΔS_m (Jg ⁻¹ K ⁻¹)	T _{onset,m} (°C)	T _m (°C)	ΔH_m (Jg ⁻¹)	ΔS_m (Jg ⁻¹ K ⁻¹)
OG1	44.55 $\pm$ 0.12 ^b	50.26 $\pm$ 0.24 ^a	5.62 $\pm$ 0.37 ^b	0.085 $\pm$ 0.45 ^b	47.36 $\pm$ 0.14 ^a	44.61 $\pm$ 0.15 ^b	2.45 $\pm$ 0.23 ^b	0.038 $\pm$ 0.14 ^b
OG2	46.45 $\pm$ 0.28 ^a	49.28 $\pm$ 0.12 ^b	2.58 $\pm$ 0.19 ^c	0.043 $\pm$ 0.38 ^c	46.84 $\pm$ 0.36 ^b	45.56 $\pm$ 0.27 ^a	2.40 $\pm$ 0.16 ^c	0.037 $\pm$ 0.35 ^c
OG3	43.15 $\pm$ 0.35 ^c	46.54 $\pm$ 0.46 ^c	5.68 $\pm$ 0.23 ^a	0.091 $\pm$ 0.15 ^a	44.96 $\pm$ 0.25 ^c	43.24 $\pm$ 0.43 ^c	3.69 $\pm$ 0.34 ^a	0.059 $\pm$ 0.22 ^a

a, b, c mean values are significantly different ($p < 0.05$).

In general, melting enthalpy (ΔH_m) and entropy (ΔS_m) values increase with higher gelator concentration confirmed by the work of Singh et al. (2015) (20). In this study, the SMS concentration was not varied but fixed at the CGC of each oleogel formulation. In contrast to previous study, ΔH_m and ΔS_m of our study were not depended on the concentration of SMS. ΔH_m and ΔS_m values of OG3 were the highest among all three preparations despite the lower SMS concentration of OG3 than that of OG1 and OG2. Combination of the 2 oils in OG3 improved its thermodynamic stability even with low concentration of gelator. This might be due to the long and thick gel network formation in OG3 which lead to stronger gel strength.

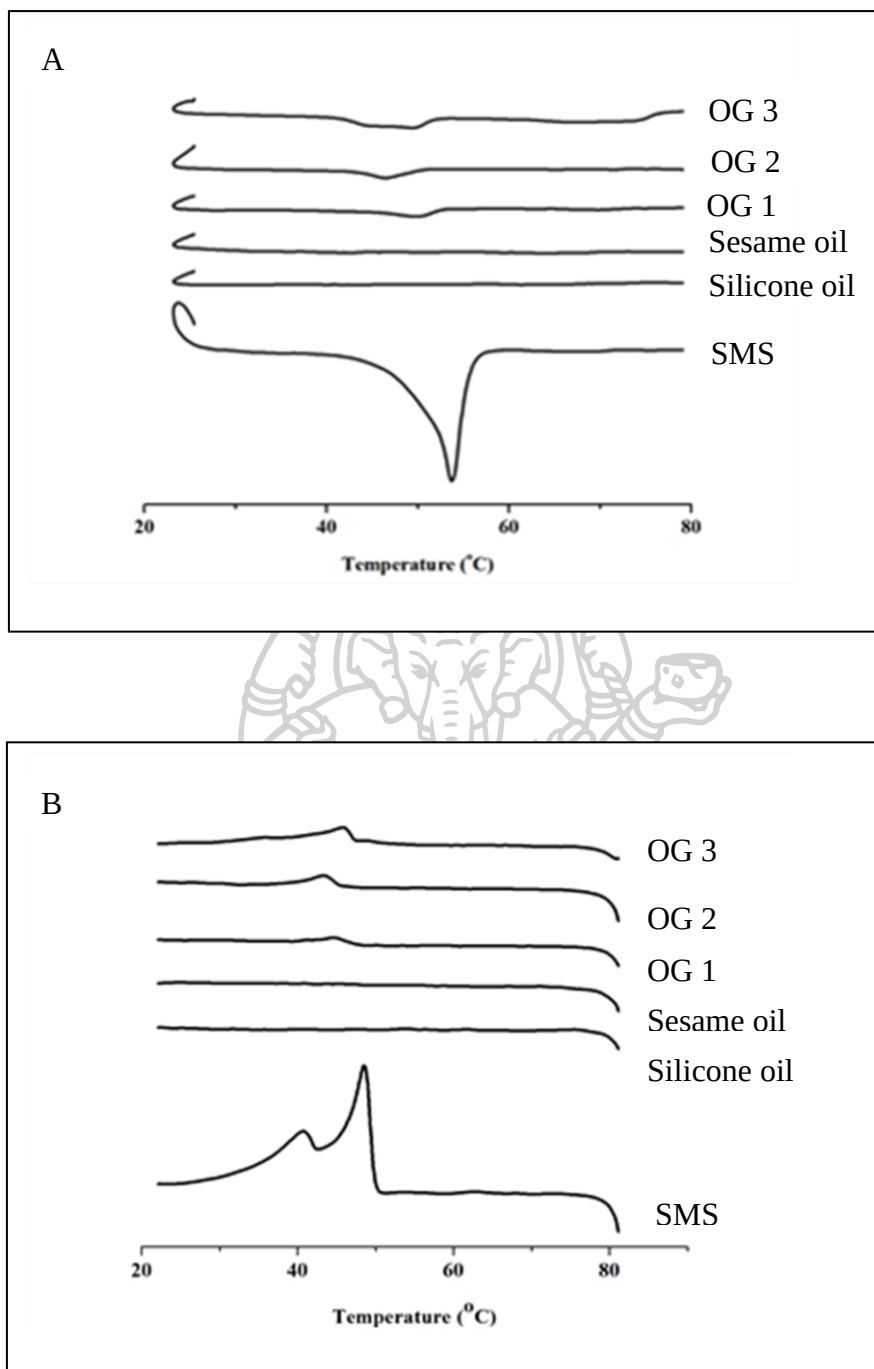


Figure 25 Thermal analysis of (A) heating cycle and (B) cooling cycle of oleogels and their ingredients.

4.2 Development of bigels containing mixture of silicone oil and sesame oil for topical drug delivery

4.2.1 Preliminary study to choose the polymers for hydrogel preparations and the best ratio of oleogel and hydrogel for development of bigels

Before the development of drug loaded bigels, the preliminary study was done to screen the suitable polymers used for hydrogel preparations and the best ratio of oleogel and hydrogel for preparation of bigels. Among the 4 types of polymers, carbopol 934, carbopol ultrez 21 and xanthan gum were incompatible with the model drug, neomycin sulfate and precipitation or coagulation was occurred. Guar gum was the only polymer which did not show polymer-drug interactions. This could be the result of cation-anion interactions between drug and polymers. The two carbopols and xanthan gum are anionic polymers and guar gum is a non-ionic neutral polysaccharide (104). Neomycin sulfate is polycationic aminoglycoside antibiotic and provide broad spectrum antimicrobial activity related to the presence of numerous amino and hydroxyl groups (42). Due to its polycationic nature, neomycin sulfate can cause interactions with anionic polyelectrolytes. The ionic nature of polymers played an important role in developing the neomycin sulfate hydrogels. Allen also reported the immediate precipitation or coagulation of neomycin sulfate with other anionic gelling agents (Allen, 1971)(105). The cation-anion drug interaction was also found when neomycin sulfate was incorporated into aqueous cream containing anionic surfactant, sodium lauryl sulfate (106). The neutral character of guar gum did not favor ionic interactions and hence it was selected as the polymer of choice for further preparation of hydrogels. Similarly, 2 synthetic polymers, carbopol 934 and carbopol ultrez 21 were incompatible with bacitracin zinc and showed unstable bigel formulations with soft texture and easy to flow when inversion. Carbopols, crosslinked polymers of acrylic acid are anionic in nature and neutralization to pH range 6.5 to 7.5 is required to occur gel formation by electrostatic repulsion (107). Bacitracin is cyclic polypeptide antibiotic and the major components for antimicrobial activity are bacitracin A, B1, B2 and B3 (82). Bacitracin can be decomposed by oxidation, hydrolysis and deamidation and its decomposition depend on pH. Pavli and Kmetec stated that bacitracin A rapidly degrades into multiple unstable products through oxidation and hydrolysis at near neutral pH (83). Hence, the neutralized carbopol hydrogel in this study may favor the decomposition of bacitracin

and resulting in soft and unstable bigels. However, 2 natural polymers (guar gum and xanthan gum) did not show interactions with drug and these two gums were selected as polymers used for hydrogel preparations of bacitracin zinc bigels. The ratio of oleogel and hydrogel for bigel preparations was selected using 5 different ratios. Among the 5 ratios, 20:80 was the best ratio for both bacitracin zinc bigels and neomycin sulfate bigels and showed good consistency, homogeneity and absence of flow. Hence, this ratio was used for further preparation of bigels.

4.2.2 Development of hydrophobic drug loaded bigels

4.2.2.1 Determination of drug solubility

The solubility of bacitracin zinc in various solvents was shown in Table 7. The order of solubility was sesame oil < silicone oil < mixture of both solvents. Bacitracin, a highly polar compound, is stable in zinc compound form. The compound is a metallo-antibiotic polypeptide and its antimicrobial activity is dependent on the presence of the metals (108, 109). Tay et al. also reported that the hydrophobic site of bacitracin zinc played an important role for its antimicrobial activity (110). The predominance of hydrophobic amino acids (valine, leucine, and isoleucine) in the structure of bacitracin zinc was confirmed by IR analysis (111). The solubility of bacitracin zinc was dependent on the degree of hydrophobic and hydrophilic characteristic of the solute and the solvent. Sesame oil having hydroxyl groups (112) shows hydrophilic property contributing to the lowest solubility (0.88 ± 0.42 mg/mL) while silicone oil which is hydrophobic in nature having dielectric constant of 2.5 (113) had higher solubility (1.05 ± 0.38 mg/mL). The mixture of sesame oil and silicone oil displaying between both properties showed the highest solubility (1.27 ± 0.45 mg/mL). This was the result of the distribution of the polar and non-polar side chains around the surface of bacitracin-zinc (108), contributing to the hydrophobic and hydrophilic nature of bacitracin zinc. The mixture of both solvents may behave like co-solvent system and favored maximum solubility of the drug. Therefore, mixture of sesame oil and silicone oil was selected as the solvent for further preparation of oleogels.

Table 7 Determination of drug solubility (n=3).

Solvent	Solubility of bacitracin zinc (mg/mL)
Silicone oil	1.05 ± 0.38
Sesame oil	0.88 ± 0.42
Mixture of silicone oil and sesame oil (2:3)	1.27 ± 0.45

4.2.2.2 Preparation of hydrophobic drug loaded bigels

Bigel formulations consisted of two phases; the hydrogels (guar gum or xanthan gum) and bacitracin zinc loaded oleogels (SMS–sesame oil either with or without silicone oil). After mixing both phases at a high shear rate and let the mixture cool down, the gelation occurred during temperature decreasing. The mixture failed to flow when tested by inverted tube method. Silicone surfactant (cyclomethicone (and) PEG/PPG 20/15 dimethicone) was required to form bigel as silicone oil was hardly miscible with the hydrogel phase due to its hydrophobic nature. The amphiphilic nature of silicone surfactant caused a reduction in interfacial tension (108, 114, 115) leading to a stabilized bigel. In addition, silicone surfactant could eliminate the foaming problem during preparation of sesame oil oleogel at a high stirring speed. As a result of multi hydrophilic groups (PEG and PPG) of silicone surfactant, it contributed to higher surface tension causing the more liquid drainage between the two surfaces of the foam, hence the foam destruction (114). The results showed that all prepared bigels were smooth, homogenous and did not show phase separation or oil leakage. The physical properties of the prepared bigels were dependent on the composition of both hydrogel and oleogel (11, 16). It was observed that both types of hydrogel were able to form bigels and the color of the hydrogel phase affected the color of the bigel. BXG and BXGS were slightly yellowish in color due to the color of xanthan gum while BGG and BGGS were milky white as guar gum hydrogel was white in color. Silicone oil improved product aesthetics of BXGS and BGGS as a result of its light, dry feel and lower surface tension properties (68, 116). The result was in agreement with our

previous study which reported better aesthetic oleogel with the application of silicone oil (65).

4.2.2.3 Physicochemical characterization of bigels

4.2.2.3.1 pH measurement

The pH of all bigels were in the range of 6 to 7, these values were within the acceptable range to avoid skin irritation for topical formulations.

4.2.2.3.2 Microscopic studies

The light micrographs of the bigels (Figure 26) showed the existence of both hydrogels and oleogels. 0.1% w/w amaranth was used as water soluble dye and 0.1% w/w green colorant as oil soluble dye. All bigels showed green droplets dispersed in pale pink background indicating that the oleogel was the disperse phase and the hydrogel was the continuous phase. The viscosity of the hydrogel played a role in controlling the droplet size. The average droplet size of BXG and BGG were 22 and 38 μm , respectively. Xanthan gum had a high viscosity attributing to a high firmness and preventing the coalescence of oil droplets. This finding was in agreement with Behera et al. (117) who showed that xanthan gum hydrogels exhibited the higher viscosity than guar gum hydrogels at the same concentration, resulting in smaller droplet sizes. Addition of silicone oil had a significant effect on droplet size ($p < 0.05$). The average droplet size of BXGS and BGGS were 11 and 20 μm , respectively which were about half the size of BXG and BGG. Oleogels containing silicone oil had stronger gel strength (65) enabling closer packing of dispersed phase hence smaller droplet size. Therefore, the distinct variations in droplet sizes of all types of bigel resulted from the viscosity of hydrogel (10, 117) and the composition of oleogel (11).

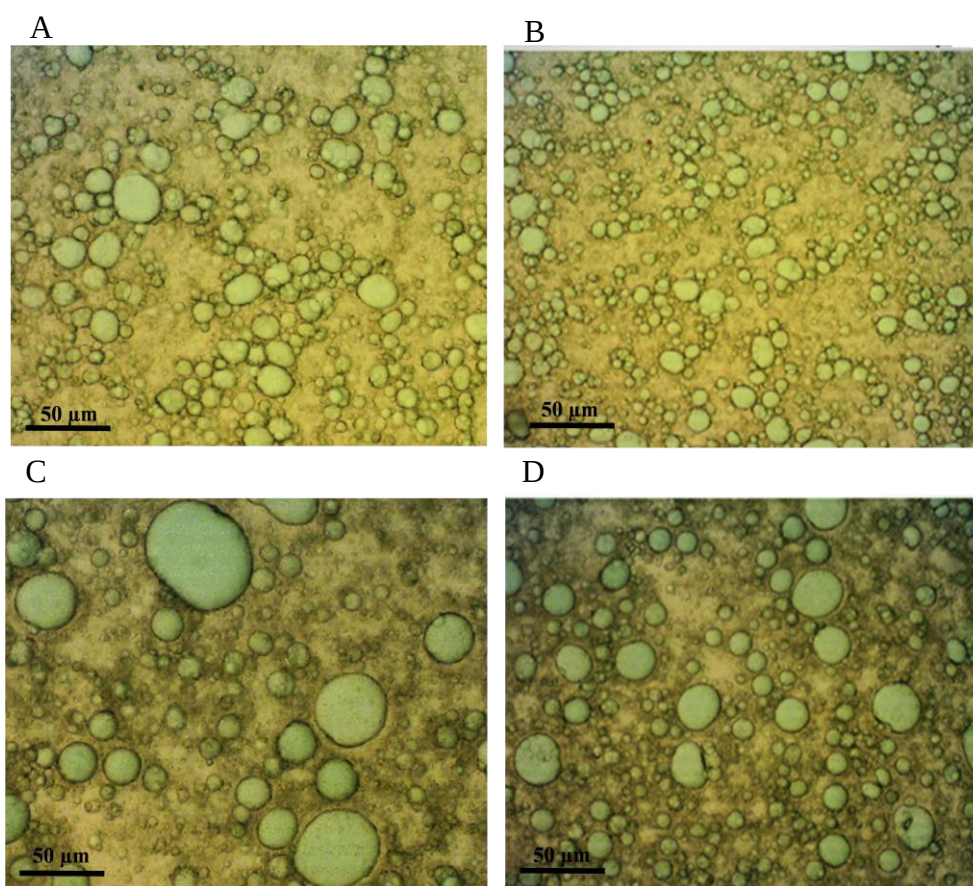


Figure 26 Light micrographs of bigels (A) BXG, (B) BXGS, (C) BGG, and (D) BGGS. Green droplets represented the oleogel phase and the pale pink background was hydrogel phase.

4.2.2.3.3 FTIR analysis

The FTIR spectra of bigels and their ingredients were shown in Figure 27. A shallow broad peak at $3700\text{--}3100\text{ cm}^{-1}$ revealed the presence of extensive hydrogen bonding between the hydroxyl groups present in the components of the bigels (10, 14, 117). In this study, BXG and BXGS showed less hydrogen bonding as compared to BGG and BGGS due to the presence of large number of free hydroxyl groups. It was reported that the larger the number of hydrogen bonded hydroxyl groups, the stronger the hydrogen bonding (Behera et al., 2014)(117). Tipvarakarnkoon and Senge also stated that guar gum formed the network by strong hydrogen bonding whereas xanthan gum provided the complex network of weakly bound molecules by intermolecular associations (118). BXGS and BGGS showed the strong peaks at about 800 cm^{-1} , 1200 cm^{-1} , and a wide band around $1270\text{--}850\text{ cm}^{-1}$. This was associated with

the presence of Si-C stretching and CH₃ deformation in Si-CH₃ and Si-O-Si stretching (96). The absorption bands at about 2920 and 2850 cm⁻¹ were related to C-H asymmetric and symmetric stretching vibrations of silicone oil, sesame oil and SMS. The C=O stretching vibration due to the ester group present in SMS produced absorption band at around 1750 cm⁻¹ (19, 31). For drug loaded bigels, there was no corresponding peak for bacitracin zinc. This could be explained by the presence of very low concentration of the drug in the formulation hence completely suppressed by the strong peaks of other excipients (119).

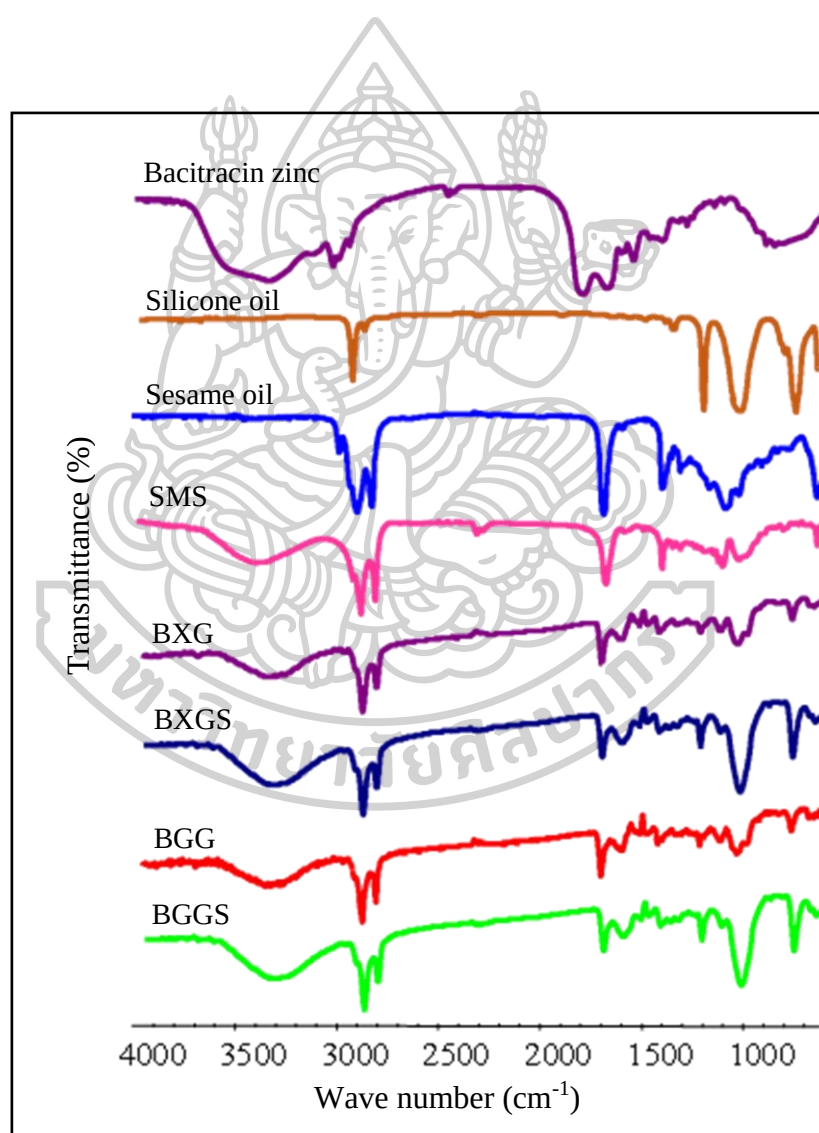


Figure 27 FTIR spectra of drug loaded bigels and their ingredients

4.2.2.3.4 Rheological characterization

The viscosity profiles of the bigels were presented in Figure 28. All bigels exhibited pseudoplastic (shear-thinning) flow, where viscosity reduced significantly with increasing shear rate. Power law model was used to characterize the flow behavior and the parameters of rheological measurements are shown in Table 8. The n value is the flow behavior index which indicates the structural behavior of the sample, shear thinning ($n < 1$) or shear thickening ($n > 1$). The polymer conformation of the hydrogel strongly affected the rheological properties of the bigels (120). The n value of BXG was found to be lower than BGG. The lower the n value, the higher the pseudoplasticity (121). Xanthan gum provide the lower strength as a result of electrostatic repulsion between the anionic charged groups on the side chains causing the disordered or partly ordered helix enabling to the weaker network (117, 118, 121). This can be assumed that the weaker structure and lower gel strength of xanthan gum displayed pronounced shear thinning behavior of BXG (117, 121). The addition of silicone oil in oleogel imparted a great benefit in spreadability of the resulting bigels. BXGS and BGGS had lower n value as compared to BXG and BGG. This might be resulted from the lower surface tension properties of silicone oil (Girboux and Starch, 2009) enhancing the spreadability of the bigels(23).

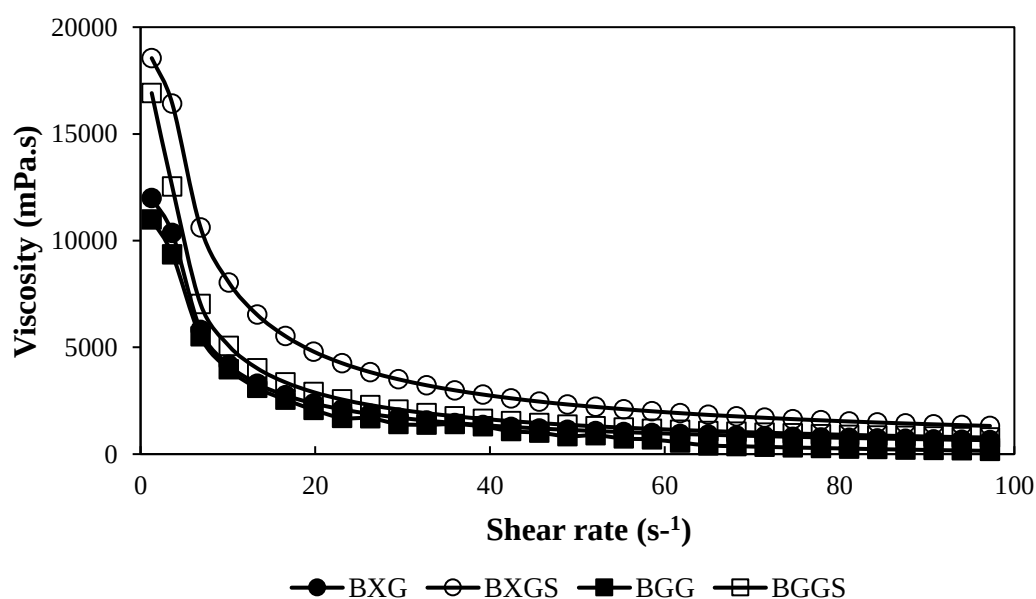


Figure 28 Viscosity profiles of drug loaded bigels BXG (●), BXGS (○), BGG (■) and BGGS (□) at the shear rate range of 0.1-100 s⁻¹.

Table 8 Flow properties of bigels determined by rheometer

Samples	Power law		
	n- value	K-value	R-square
BXG	0.30 ± 0.33	48.29 ± 0.27	0.996
BXGS	0.16 ± 0.28	62.66 ± 0.15	0.997
BGG	0.38 ± 0.43	44.36 ± 0.29	0.997
BGGS	0.27 ± 0.19	52.68 ± 0.38	0.998

All are significantly different ($p < 0.05$).

The consistency index (K) is used to measure the viscosity of the system. The viscosity of the bigels was influenced by the concentration and molecular weight of the gum and viscosity of the hydrogel (9, 117). The viscosity of BXG was higher than BGG which might be due to higher concentration of xanthan gum used in this study (10). The optimum concentration of xanthan gum and guar gum for preparation of hydrogels were 2% w/w and 1.5% w/w, respectively. The complex molecular structure and high molecular weight of xanthan gum also contributed to the higher viscosity of hydrogel which in turn increased the viscosity of bigels (117, 122). Moreover, IR analysis of Behera et al. stated that the network formation of xanthan gum involved both hydrogen bonding and polymer entanglement which resulted in higher viscosity of the bigels (117). Both the viscosity and composition of the oleogel had a significant effect on the viscosity of the bigels ($p < 0.05$). The viscosity of BXGS and BGGS were higher than that of BXG and BGG. This result might be attributed to the higher viscosity of the oleogels. From our previous study, it was found that the oleogel with a mixture of silicone oil and sesame oil exhibited the higher viscosity as compared to sesame oil oleogel (65). Furthermore, incorporation of silicone oil provided the strong oleogel (65), which could increase the viscosity of BXGS and BGGS. The light micrographs in Figure 4.12 also supported this result, as higher

viscosity induced smaller droplet size and closer packing were observed in BXGS and BGGS.

The rheological parameters such as the storage or elastic modulus (G'), the loss or viscous modulus (G''), and the LVER can be obtained from non-destructive oscillation tests. The viscoelastic behavior relates to the structural changes within the gel formulation and can be separated into 2 components; G' (solid-like) and G'' (liquid-like) properties. To observe viscoelastic behavior, the amplitude sweep test was run by varying the amplitude of the shear stress while the frequency is kept constant. The amplitude sweep consists of two regions. The first region is the linear viscoelastic region (LVER) where G' and G'' are constant at low strain, indicating that the sample structure is undisturbed. The second region is the non-linear response of material to larger strain value. Therefore, resistance of the material to deformation can be estimated from the LVER. It means that the longer the LVER, the higher the stability of the products. The LVER of the bigels was controlled by the viscoelastic behavior of the hydrogel. From the amplitude sweep result in Figure 4.15, BXG showed higher LVER as compared to BGG which was resulted from the high elastic behavior of xanthan gum. Similarly, Moraes et al. reported that xanthan gum showed a weak gel behavior with more elastic character whereas, guar gum did not present yield stress and displayed a concentrated solution behavior (121). On the other hand, the viscoelastic properties of the oleogel also influenced the LVER of the bigels. BXGS and BGGS had higher G' than G'' indicating that the system was more elastic than viscous at the studied frequency range, hence they had strong gel property. In addition, the plateau G' value of BXGS and BGGS were also higher than that of the BXG and BGG, implying the higher gel strength which is accordance with Behera et al (117). This was supported by our previous study result, the oleogel with combination of silicone oil and sesame oil showed higher gel strength with longer LVER as compared to sesame oil oleogel.

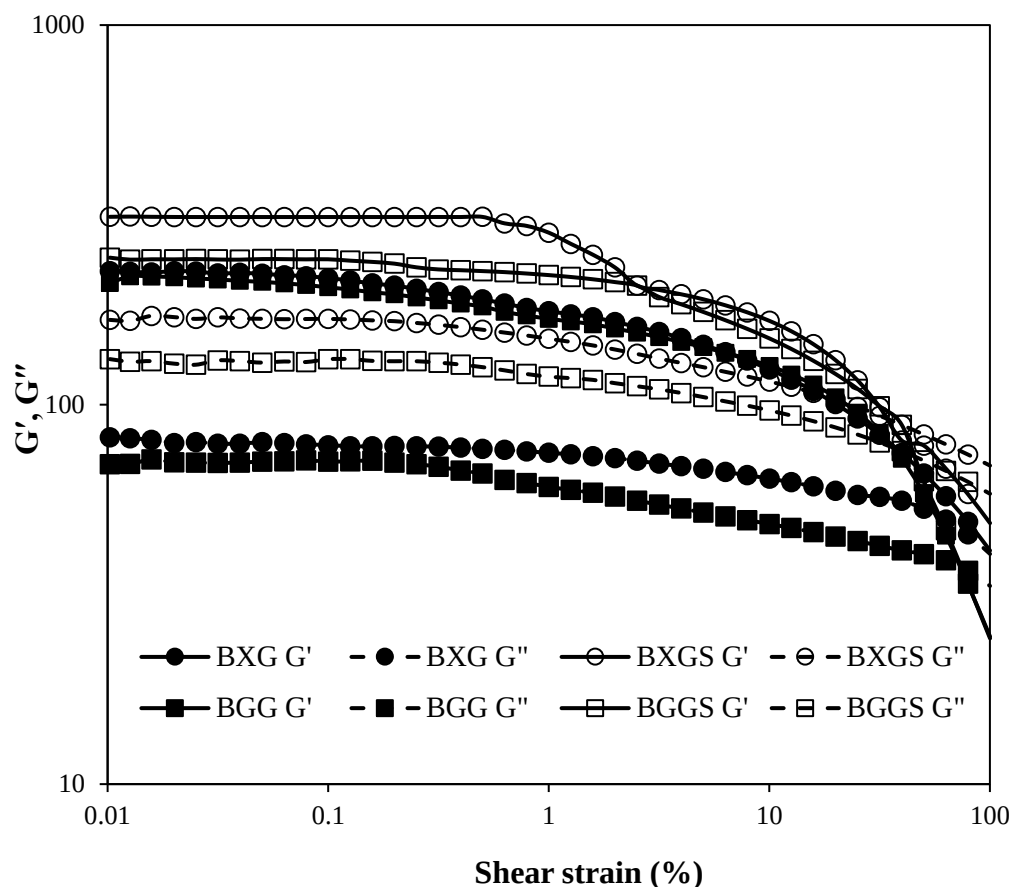


Figure 29 Amplitude sweep test of bigels: Storage modulus (G' , solid line) and loss modulus (G'' , dashed line) as function of shear strain for BXG (●), BXGS (○), BGG (■) and BGGS (□). The shear stress range was 0.01 to 100 Pa at a fixed frequency of 1Hz.

4.2.2.3.5 Mechanical properties

The parameters such as hardness, cohesiveness, index of viscosity and spreadability were quantified from the force versus time plot and the results were presented in Table 9. Positive peak force is defined as the firmness of the formulations. Hardness or firmness displays the substantial resistance to deformation of a product and is measured in terms of maximum force (g). Positive and negative area indicate the consistency and index of viscosity of the formulations. Index of viscosity provides information about the bulk viscosity of the bigels. Negative peak force indicates the cohesiveness of the formulations. The hydrogel played an important role in controlling the mechanical properties of the bigels. The firmness of BXG (308 g) was significantly

greater ($p<0.05$) than BGG (238 g) which might be associated with the molecular conformation and high viscoelastic properties of xanthan gum as discussed in the rheological characterization. The high firmness values were found in BXGS (524 g) and BGGS (433g). In addition, the index of viscosity and cohesiveness values were significantly higher as compared to BXG and BGG ($p<0.05$). This was in accordance with the rheological results which suggested the stiffer and stronger gel strength of silicone oil oleogel provided the better mechanical properties of BXGS and BGGS. An important characteristic of topical formulations is spreadability which is responsible for ease of application, extrudability from the container, correct dosage transfer to the target site, and consumer preference. Spreadability can be determined from cohesiveness which is inversely proportionate. The formulation with strong cohesive force can retard its flowability and thereby its spreadability on the substrate. The higher viscosity of BXG offered the higher cohesiveness resulting the lower spreadability. Even though BXGS and BGGS had high cohesiveness value, the lower surface tension properties of silicone oil could increase the spreadability of the bigels.

Table 9 Mechanical properties of bigels determined by backward extrusion study using texture analyzer

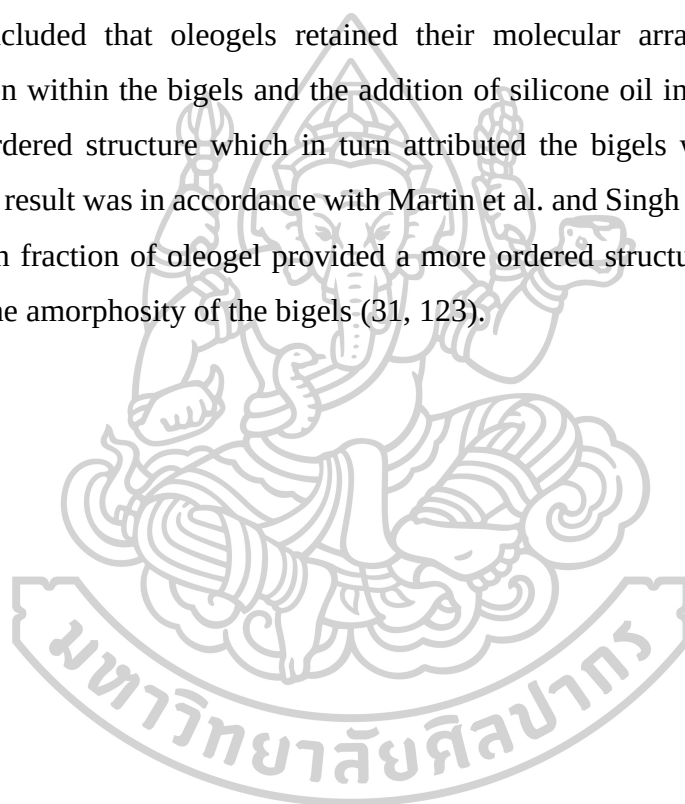
Sample	Firmness (g)	Cohesiveness (g)	Consistency (gs^{-1})	Index of viscosity (gs^{-1})
BXG	308.77 ± 0.13	135.93 ± 0.39	220.05 ± 0.45	370 ± 0.48
BXGS	524.64 ± 0.21	300.31 ± 0.44	236.06 ± 0.37	452 ± 0.36
BGG	238.72 ± 0.49	116.45 ± 0.20	148.27 ± 0.19	295 ± 0.25
BGGS	433.07 ± 0.33	186.81 ± 0.14	226.38 ± 0.44	423 ± 0.10

All are significantly different ($p<0.05$).

4.2.2.3.6 X-ray diffraction studies

The diffraction patterns of bigels and their components were shown in Figure 30. Oleogels exhibited the presence of broad peaks ($11^\circ 2\theta$ and $19^\circ 2\theta$) related to the non-crystalline nature of solvents and a single sharp peak ($21^\circ 2\theta$) associated with

the crystalline nature of SMS as reported in our previous study (65). Guar and xanthan hydrogels displayed broad humps around $25^\circ 2\theta$ due to the presence of amorphous region in the polymers which is in agreement with previous study of Martins et al. (123). All patterns of bigels with broad humps at $25^\circ 2\theta$ and the crystalline peak at $21^\circ 2\theta$ indicated the presence of both oleogel and hydrogel in the bigels. Both oleogels and bigels retained the peaks of SMS, however, a little variation in diffraction was visible between different formulations. This alteration might be associated with the strong gel properties of silicone oil oleogel which attributed to more ordered structure. Thus, it can be concluded that oleogels retained their molecular arrangement even after incorporation within the bigels and the addition of silicone oil into oleogels provided the more ordered structure which in turn attributed the bigels with less amorphous nature. This result was in accordance with Martin et al. and Singh et al., who suggested that the high fraction of oleogel provided a more ordered structural arrangement and decreased the amorphosity of the bigels (31, 123).



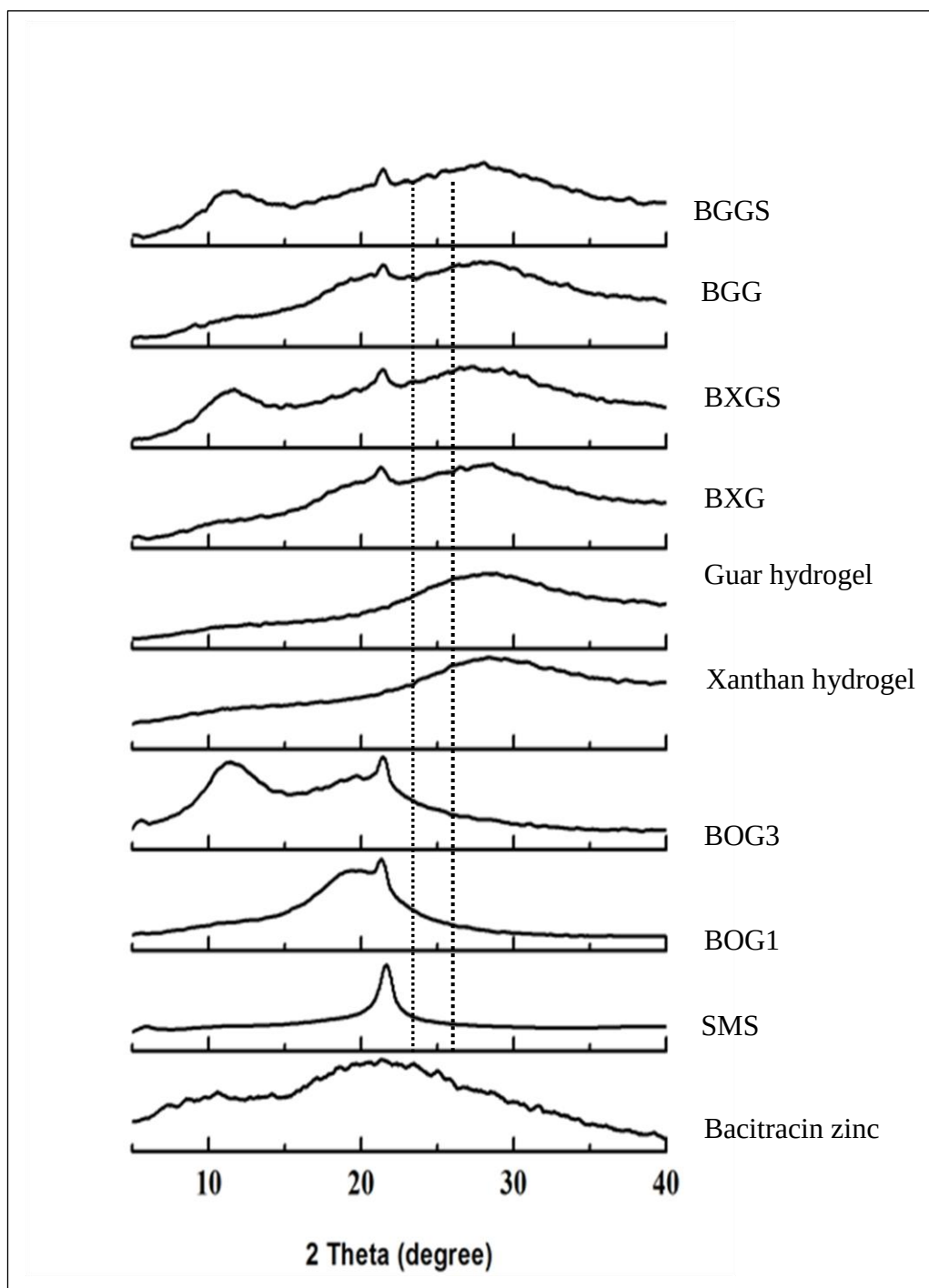


Figure 30 XRD patterns of bacitracin zinc, SMS, drug loaded oleogels, hydrogels and drug loaded bigels. BOG3 is bacitracin zinc loaded oleogel containing mixture of silicone oil and sesame oil. BOG1 is bacitracin zinc loaded oleogel containing sesame oil.

4.2.2.3.7 Drug content determination

Bacitracin zinc content of all formulations was determined using HPLC. The label amount claimed according to the United States Pharmacopoeia is 90-140% (86). It was found that the average drug content of bacitracin zinc in bigel formulations was in the range of 90.93-110.82% (Figure 31), which was within the acceptable limit. This result indicated that the preparation process of bigel did not cause the drug degradation and the loaded drug into the formulations could be well dispersed. The average drug content of BXG and BGG were 91.93% and 101.21% respectively. The addition of silicone oil significantly improved the drug content ($p < 0.05$). BXGS and BGGS showed higher drug content of 106.36% and 110.82%, respectively. This resulted from the higher solubility of the drug in the mixture of silicone oil and sesame oil leading to higher drug content in BXGS and BGGS.

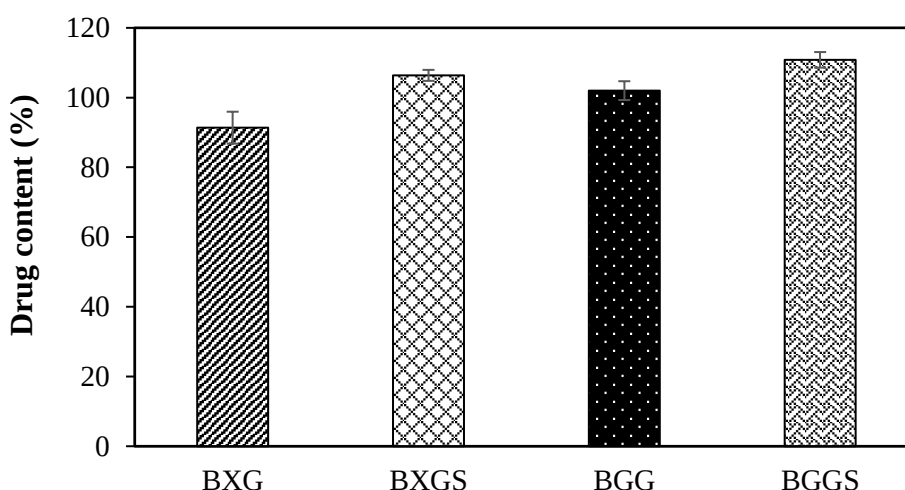


Figure 31 Drug content determination of bacitracin zinc loaded bigels.

4.2.2.3.8 *In vitro* drug release study

The *in vitro* drug release profile of bacitracin zinc from bigel formulations was shown in Figure 32. The release was dependent on the viscosity and firmness of the bigels, the amount of drug content, and the release mechanism. The results showed that the CPDR of bigel formulations was significantly different ($p < 0.05$). The CPDR of bacitracin zinc from BXG and BGG was 40.5% and 42.8%, respectively. The lower release from BXG was due to the viscosity and firmness rendering to the difficulty in diffusion of drug hence lower drug release. The high

viscosity and firmness of BXG was proved by rheological characterization and mechanical properties. The result was in agreement with the study of Mughal et al. They reported the lower release of xanthan gum at higher concentration as a result of high viscosity enabling to the difficulty in penetration of medium (124). In addition, Behera et al stated that the elastic retraction of acetyl side chains caused the lower swelling of xanthan gum enabling to the slow drug release from xanthan gum bigels (120). The CPDR of BXGS and BGGS was 55.3% and 58.6% respectively. The solubility of drug in the internal phase is the major driving force for the enhancement of drug release. The higher release of drug with the addition of silicone oil compared with those without silicone oil was due to the factors of drug content and release mechanism. The BXGS and BGGS had the higher drug content due to the high solubility in the mixture of sesame oil and silicone oil. The release mechanism was investigated as shown in Table 10. The mechanism might be associated with the partition of drug from oleogel to hydrogel phase. The drug must diffuse from the internal phase to external phase where the drug molecules are released freely. Hence, the higher drug solubility in the oleogel phase favors the higher drug partition consequently increased the drug release. The release kinetics of the drugs were estimated by fitting various models (zero-order, first-order and Higuchi models). The correlation coefficient values suggested that the drug release pattern followed the Higuchi model and the release was diffusion. To identify Fickian or non-Fickian diffusion, the n value (release exponent) was calculated from Korsmeyer-Peppas model. BXG and BGG showed Fickian diffusion as the n value of these two bigels were less than 0.5. It was reported that xanthan gum showed the lower release and the higher concentration caused the lower water permeation and erosion resulting in lower drug release (124-126). Generally, natural gums followed the Fickian diffusion, however, due to the presence of other additives, the mechanism might be mixture of diffusion and chain relaxation (124). Hence, the addition of silicone oil could alter the release mechanism and the n value of BXGS and BGGS were found to be greater than 0.5 indicating non-Fickian diffusion. The higher release of BXGS and BGGS could be the combination of mechanism of drug release such as swelling and subsequent diffusion. Therefore, the amount of release could be modified by types of hydrogel and the enhancement of release by the addition of silicone oil. This result was in accordance

with the work of Eimen et.al., which reported the enhancement of drug penetration across the skin with the addition of silicone products (127).

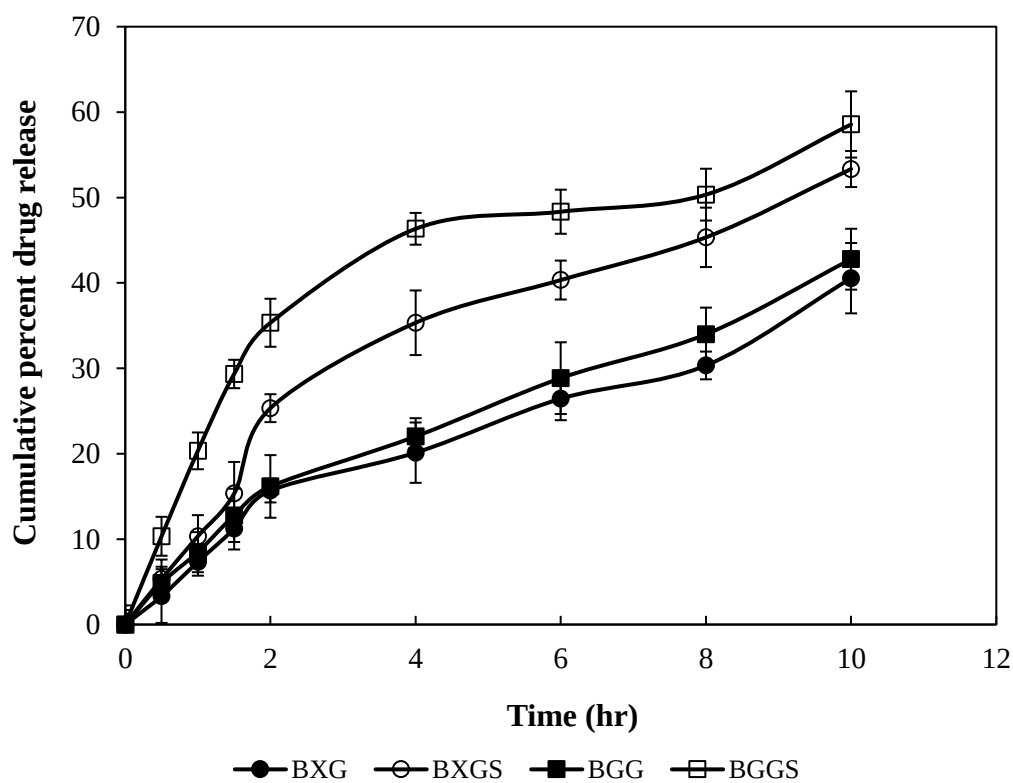


Figure 32 In vitro drug release study of bacitracin zinc loaded bigels

Table 10 Release kinetics of bacitracin zinc from bigels

Sample	Zero order (r^2)	Higuchi (r^2)	Korsmeyer-Peppas (r^2)	n	Release mechanism
BXG	0.81	0.98	0.97	0.43	Fickian
BXGS	0.93	0.99	0.99	0.56	Non-Fickian
BGG	0.85	0.99	0.98	0.45	Fickian
BGGS	0.88	0.99	0.98	0.50	Non-Fickian

4.2.2.3.9 Antimicrobial assay

Antimicrobial efficiency of bacitracin zinc loaded bigels were evaluated against *Staphylococcus aureus*. Bacitracin zinc is only available as ointment in commercial markets. Due to the very hydrophobic nature of ointment base, the commercial ointment could not easily diffuse and contributed to the smaller zone diameter after 72 h. Similar result was obtained and bacitracin showed smaller inhibition zones (3-8 mm) from different types of bases after 1-week study (128). Hence, a standard solution of bacitracin zinc (0.77% w/w) was used as a positive control instead of the market sample and a negative control was a blank bigel. The antimicrobial activity of the bigels was found to be influenced by several factors such as the viscosity of the bigels, the drug content, and the rate of drug release as well as the ability of drug diffusion. All drug loaded bigels showed good antimicrobial activity against *Staphylococcus aureus*. As shown in Figure 33, the diameter of inhibition zone was BGG>BXG (23, 21mm). The difference was associated with the limited diffusion of drug through the higher viscosity of BXG bigel, hence resulting the lower antimicrobial activity. The increase in inhibition zones were shown for bigels with silicone oil. The inhibition zones of BGGS and BXGS were 27 and 25 mm, respectively. The improvement in activity with the addition of silicone oil could be the result of the higher drug content and higher diffusion. The finding was also correlated with the result of *in vitro* drug release. The inhibition zones proved that the release of bacitracin zinc from all bigels were in an active form. The tremendous higher zone diameter of all developed bigels proved that they were far better than bacitracin ointment base investigated by Plaxco et al. (128). Thus, our fabricated bigels could be a suitable carrier for delivery of antibiotics.

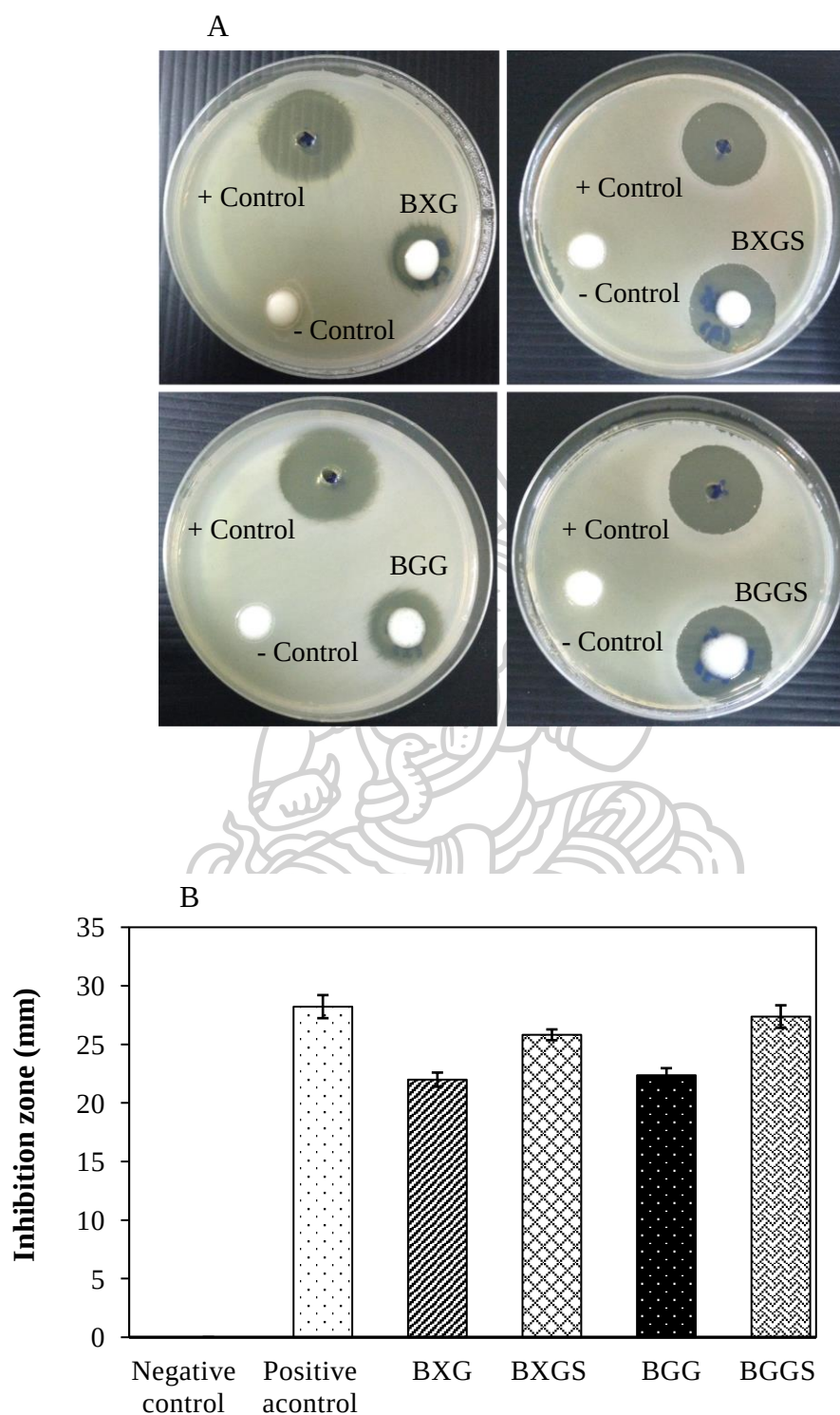


Figure 33 (A) Antimicrobial activity and (B) inhibition zone of bacitracin zinc loaded bigels against *Staphylococcus aureus*. 0.77 % w/w of bacitracin zinc in sodium acetate buffer (pH 5.5) and blank bigels were used as positive control and negative control.

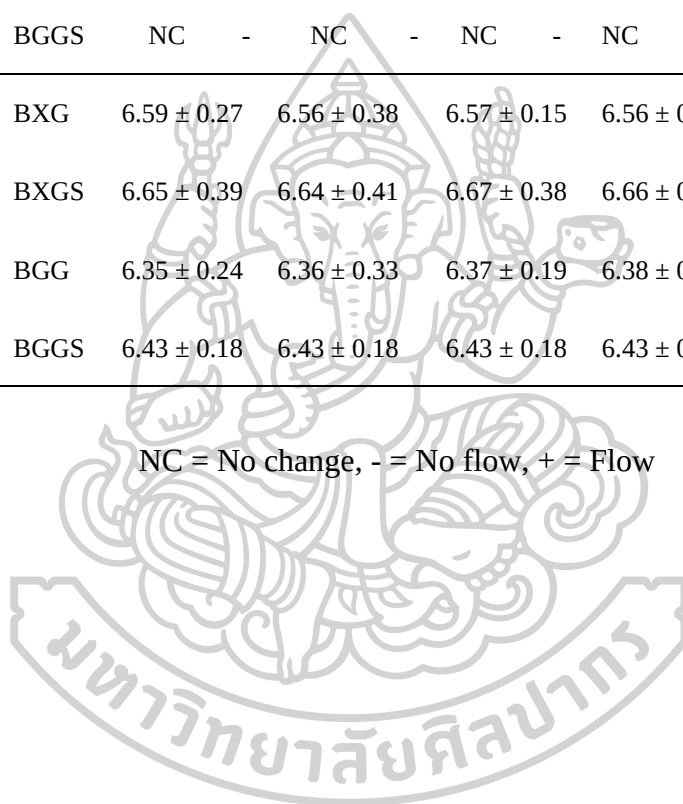
4.2.2.3.10 Stability studies

The physical appearance, pH and drug content of all bigel formulations were investigated for the physical or chemical changes. The stability of all bigels was performed for temperature cycling and intermediate stability tests. There were no changes in color and pH and the drug content was between 90-110% which was within the acceptable range as shown in Table 11 and Figure 34. Figure 35 shows antimicrobial activity of all bigels after stability tests. The results exhibited that all bigels were stable and their antimicrobial activities did not change after the stability tests. One of the indicators to detect the physical appearance was the stable texture and absence of flow when the inversion test was performed. All bigels remained stable after six cycles of temperature cycling, however, the intermediate stability test displayed differently dependent on composition of bigel. The findings showed that the viscosity played a significant effect on the stability ($p < 0.05$). After 3-months stability test, BGG was the only formulation starting to flow. The lowest stability of BGG was due to the dispersion of larger droplet size within the lower viscosity of hydrogel contributing to the tendency of coalescence. Unlike BGG, BXG exhibited a longer period of stability due to higher viscosity preventing from coalescence. The influence of addition of silicone oil improved the stability significantly ($p < 0.05$). The improvement of stability of BXGS and BGGS compared with those without silicone oil resulted from a formation of stronger gel strength proved by our previous study (65) which may help in maintaining the structural integrity of bigels. However, the longer the time of stability test, the flow on inversion of BGGS was detected, as a result of lower firmness and viscosity of the bigels. After 6 months of stability test, BXGS remained firm texture and no flow or phase separation occurred. Hence, it can be concluded that the highest stability of bigel was BXGS due to synergistic effect of both stronger oleogel induced by silicone oil and the higher viscosity hydrogel.

Table 11 Color, flow when inversion and pH of the bigels before and after passing the temp cycling for 6 cycles and intermediate stability test for 1,3 and 6 months.

Parameters	Sample	Initial		Temp-cycling	Intermediate stability test						
					1 month		3 months		6 months		
Color / Flow when inversion	BXG	NC	-	NC	-	NC	-	NC	-	NC	+
	BXGS	NC	-	NC	-	NC	-	NC	-	NC	-
	BGG	NC	-	NC	-	NC	-	NC	+	NC	+
	BGGS	NC	-	NC	-	NC	-	NC	-	NC	+
pH	BXG	6.59 ± 0.27		6.56 ± 0.38		6.57 ± 0.15		6.56 ± 0.25		6.58 ± 0.46	
	BXGS	6.65 ± 0.39		6.64 ± 0.41		6.67 ± 0.38		6.66 ± 0.18		6.64 ± 0.26	
	BGG	6.35 ± 0.24		6.36 ± 0.33		6.37 ± 0.19		6.38 ± 0.29		6.36 ± 0.45	
	BGGS	6.43 ± 0.18		6.43 ± 0.18		6.43 ± 0.18		6.43 ± 0.18		6.43 ± 0.18	

NC = No change, - = No flow, + = Flow



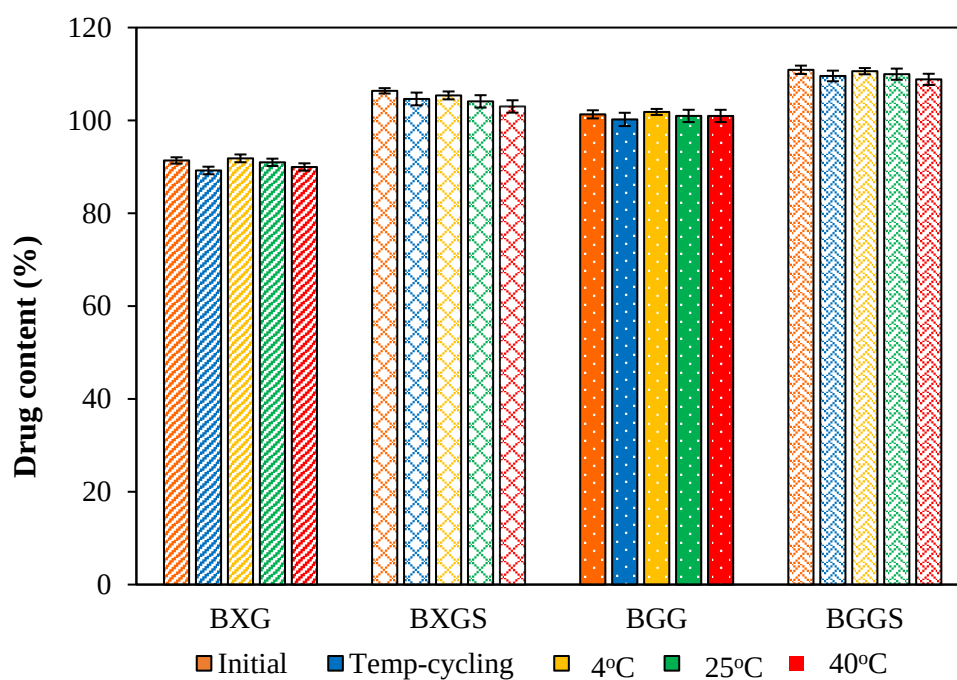


Figure 34 Drug content determination of bacitracin zinc loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months.



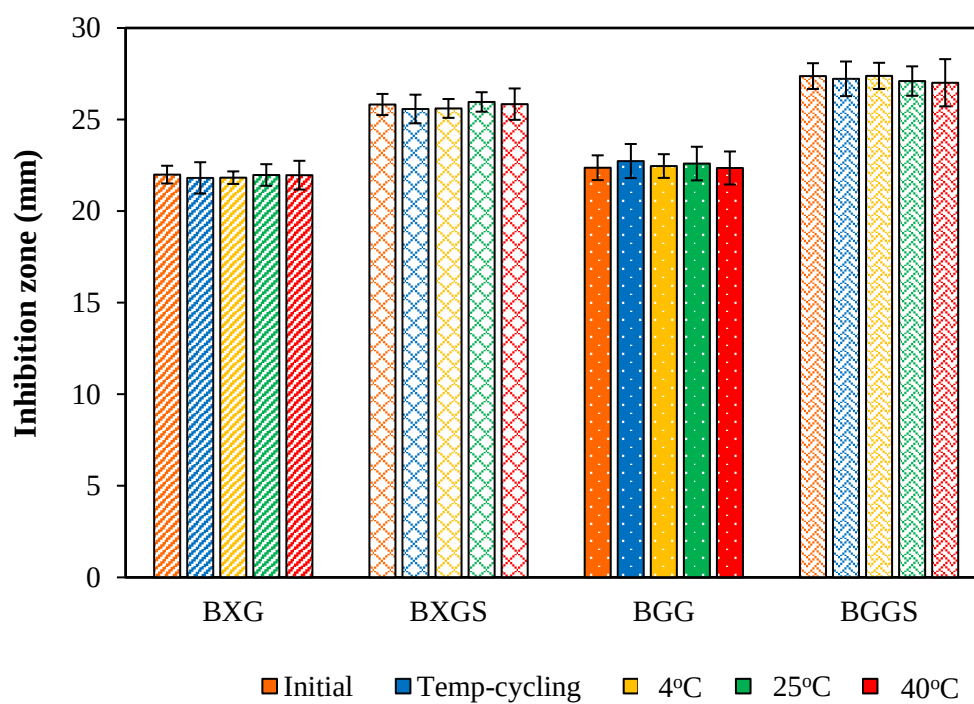


Figure 35 Antimicrobial activity of the bacitracin zinc loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months.



4.2.3 Development of hydrophilic drug loaded bigels

4.2.3.1 Preparation of hydrophilic drug loaded bigels

The hydrogel phase was guar gum hydrogel loaded with neomycin sulfate and the oleogel phase was SMS-sesame oil oleogel either with or without silicone oil. Bigels were formed when the two phases were heated and mixed at a high shear rate and the absence of flow in inverted tube method confirmed the gel formation. The bigels were white in color, smooth, homogeneous and did not show oil migration or phase separation. An improvement in product aesthetics was observed in bigels with mixture of silicone oil and sesame oil which might be due to unique properties of silicone oil like light, dry feel and lower surface tension. Sene also stated that volatile silicone oil enhanced the product aesthetics and sensory attributes as compared with petrolatum as a result of distinct physical properties of silicone oil (69). Similar result was found in our previous study which reported the addition of silicone oil improved the product aesthetics of oleogel (65).

4.2.3.2 Physicochemical characterization of bigels

4.2.3.2.1 pH measurement

It was found that the pH of NGG and NGGS were 5.5 and 5.8, respectively, which were within the acceptable limit for topical products to avoid skin irritation.

4.2.3.2.2 Microscopic studies

The light micrographs depicted in Figure 36 confirmed the biphasic nature of bigels due to the presence of both oleogel and hydrogel phases. All bigels showed the oleogel as the dispersed phase (green droplets) and the hydrogel as the continuous phase (pale pink background), hence the type of bigel was oleogel in hydrogel type. The addition of silicone oil into oleogel significantly controlled the droplet size of bigels ($p < 0.05$). The droplet sizes were found to be in the range of 20-40 μm and the average droplet size of NGG and NGGS were 35 and 25 μm , respectively. NGGS showed the smaller droplet size and closer packing of the dispersed phase compared with NGG. The result could be the effect of higher viscosity of silicone

containing oleogel which was proved by our previous study (65). Similar result was found in bacitracin zinc bigels, in which silicone and sesame oil based bigels showed smaller droplet size than that of sesame oil bigels. The effect of high viscosity played a part in controlling the particle size which was in agreement with the previous study of Singh et al (31). They found that the higher concentration of oleogel showed the higher viscosity attributing to closer packing of the dispersed phase and the smaller droplet size. In addition, the lower surface tension property of silicone oil may reduce the interfacial tension between the oleogel and hydrogel phases resulting in a smaller droplet size. Bae et al. stated that the interfacial tension of silicone oil emulsion was lower than mineral oil emulsion at the same concentration of emulsifier and enabling to the smaller droplet size (129). In this study, it was found that the incorporation of silicone oil, having low surface tension (17.38 ± 0.10 mN/m), caused the reduction in surface tension from 32.82 ± 0.35 mN/m to 20.85 ± 0.22 mN/m. This was in agreement with the study of O'Lenick and Thomas (130). They reported the reduction in surface tension of soybean oil from 30-35 dynes/cm to 20-25 dynes/cm with the addition of the silicone oil (alkyl dimethicone) (130). Hence, silicone oil not only plays as a solvent in oleogel part but also as a surfactant attributing to the reduction in droplet sizes of bigels.

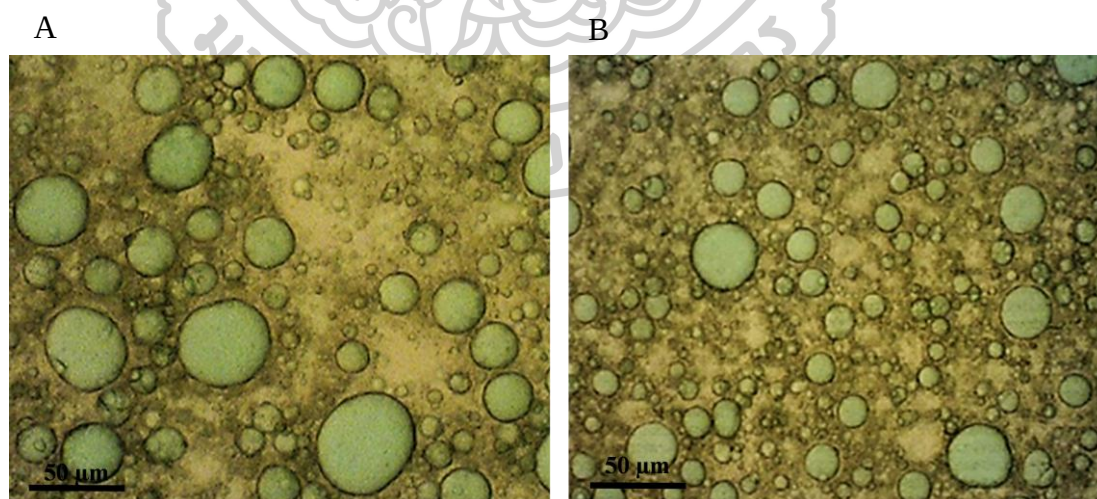
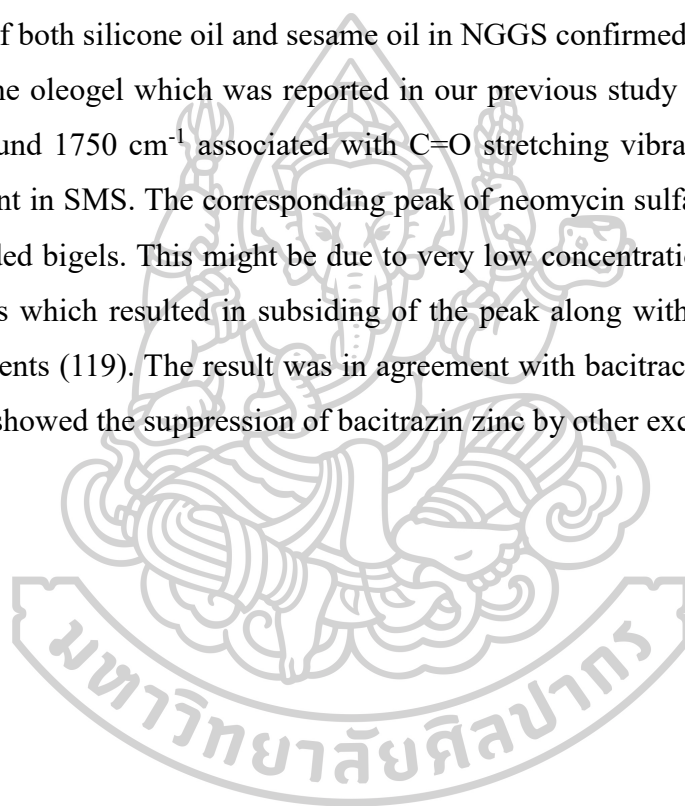


Figure 36 Light micrographs of bigels (A) NGG and (B) NGGS. Biphasic nature of bigels with the oleogel phase (green droplets) and the hydrogel phase (pale pink background).

4.2.3.2.3 FTIR analysis

The FTIR spectra of bigels (Figure 37) showed a shallow broad peak at 3700-3100 cm^{-1} which was due to hydrogen bonding between the hydroxyl groups present in the components of the bigels (10, 14, 117). Silicone oil, sesame oil and SMS showed the absorption bands at about 2920 and 2850 cm^{-1} related to C-H asymmetric and symmetric stretching vibrations. The strong peaks at about 800 cm^{-1} , 1200 cm^{-1} , and a wide band around 1270 -850 cm^{-1} were found in NGGS. This peaks revealed the presence of Si-C stretching and CH_3 deformation in Si- CH_3 and Si-O-Si stretching (96). The peaks of both silicone oil and sesame oil in NGGS confirmed the presence of both oil within the oleogel which was reported in our previous study (65). The absorption band at around 1750 cm^{-1} associated with C=O stretching vibration due to the ester group present in SMS. The corresponding peak of neomycin sulfate was not observed in drug loaded bigels. This might be due to very low concentration of the drug in the formulations which resulted in subsiding of the peak along with the strong peaks of other excipients (119). The result was in agreement with bacitracin zinc loaded bigels which also showed the suppression of bacitracin zinc by other excipients.



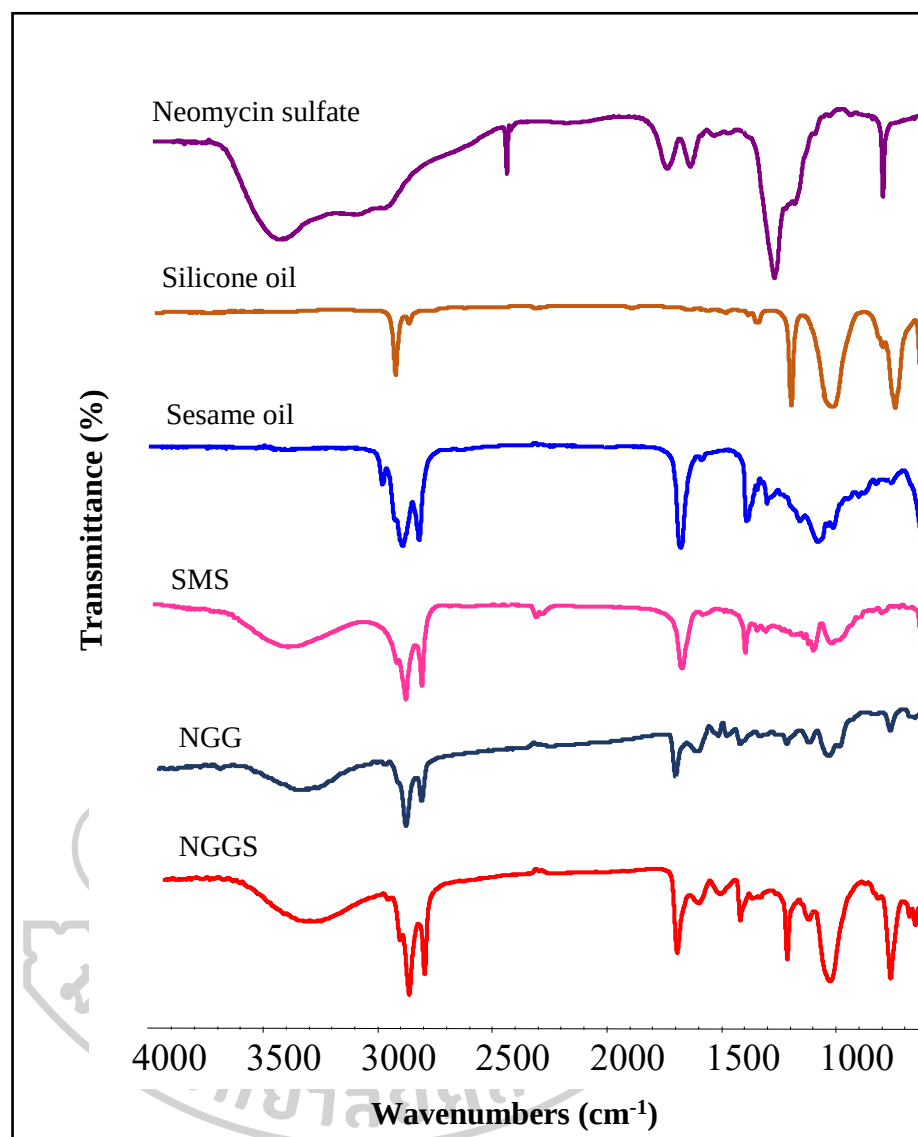


Figure 37 FTIR spectra of drug loaded bigels and their ingredients

4.2.3.2.4 Rheological characterization

The viscosity profiles (viscosity versus shear rate) of the bigels were plotted in Figure 38. This measurement provided the information relating to the structural changes of materials complied with the applied shear in different conditions like processing, storage and application. The applied shear during an application will cause the change in viscosity as a result of internal structure change of the sample (131). The relation between shear rate and shear stress was analyzed with Power law model

and the rheological parameters were presented in Table 12. The consistency index (K) and the flow behavior index (n) were used to analyse the viscosity and pseudoplastic characteristics of the bigels. Both bigels showed pseudoplastic (shear-thinning) flow which displayed the viscosity reduction while increasing the shear rates thus ensuring in easy spreadability and uniform distribution on the skin. The pseudoplastic behavior was also confirmed by the flow behavior indices of the power law model ($n = 0.21-0.42$). It was found that the n value of NGGS was lower than NGG which indicated the greater degree of pseudoplasticity. Bacitracin zinc bigels with silicone oil also showed the more pseudoplastic characteristics with smaller n value. This might be due to lower viscosity and surface tension properties of silicone oil caused the reduction in viscosity and surface tension of sesame oil thus improving the spreadability. Similarly, the previous studies of Litvin et al., and Girboux and Couborn showed that the addition of silicone oil decreased the viscosity and surface tension of vegetable oils and petrolatum and greatly enhanced the spreadability (23, 132). The viscosity of bigels was found to be dependent on the viscosity of the oleogel and the results were significantly different ($p < 0.05$). NGGS had higher viscosity than NGG which was due to the higher viscosity of silicone oil containing oleogel. Although the addition of silicone oil decreased the viscosity of sesame oil, it attributed to the oleogel with stronger gel network which could increase the viscosity of oleogel. An increase in viscosity of oleogel resulted in the increased viscosity of bigel enabling the closer packing and smaller droplet as shown in microscopic study.

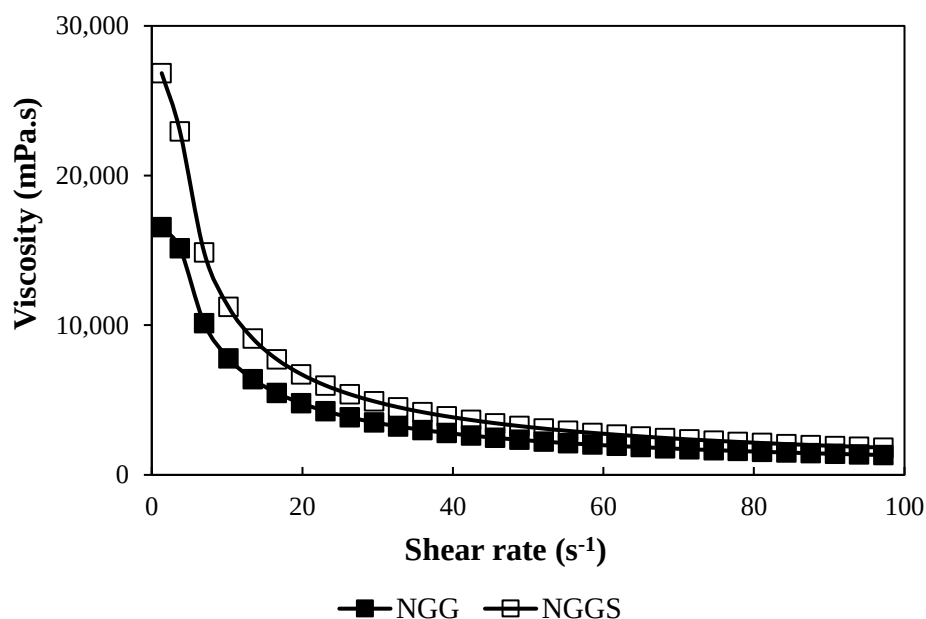


Figure 38 Viscosity profiles of drug loaded bigels NGG (■) and NGGS (□) at the shear rate range of 0.1-100 s⁻¹.

Table 12 Flow properties of bigels determined by rheometer

Sample	Power law		
	n- value	K-value	R-square
NGG	0.42 ± 0.35	52.42 ± 0.21	0.993
NGGS	0.21 ± 0.41	74.77 ± 0.16	0.999

The amplitude sweep analysis in Figure 39 demonstrated that NGGS were more elastic than viscous because G' had higher magnitude than G'' at the selected frequency. The higher and longer LVER value of NGGS confirmed the higher gel strength and this result might be associated with the strong gel property of oleogel. The same behavior was observed for silicone oil containing bacitracin zinc bigels, they behaved more solid like due to the higher gel strength of oleogel.

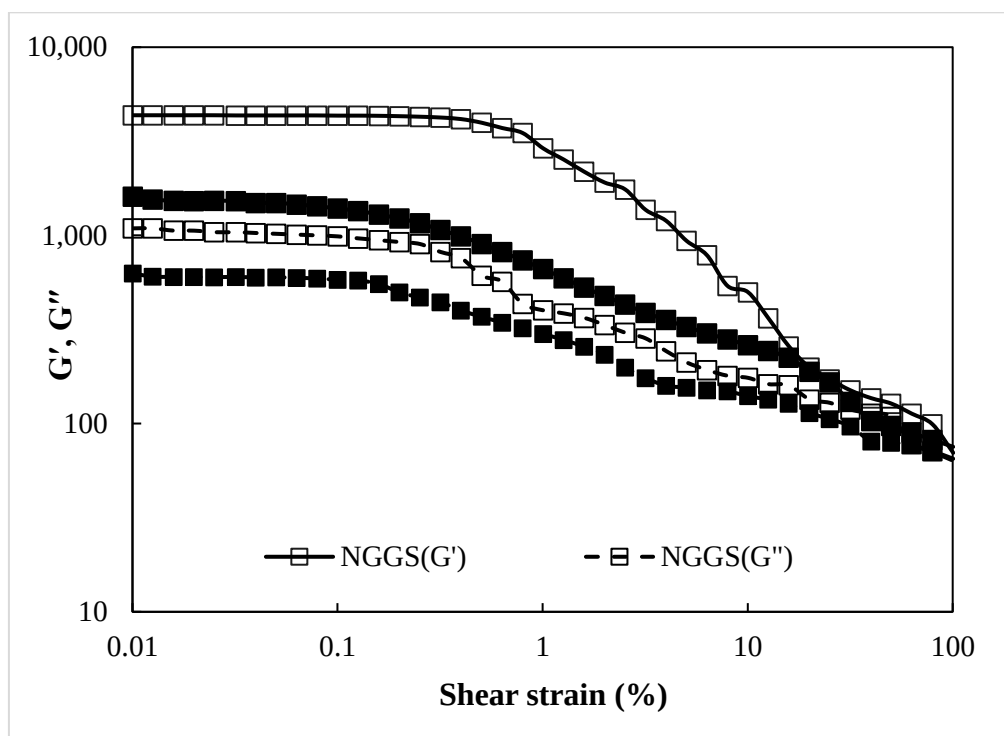


Figure 39 Amplitude sweep test of bigels: Storage modulus (G' , solid line) and loss modulus (G'' , dashed line) as function of shear strain for NGG (■) and NGGS (□). The shear stress range was 0.01 to 100 Pa at a fixed frequency of 1Hz.

4.2.3.2.5 Mechanical properties

The mechanical properties of bigels were analysed in terms of firmness, cohesiveness, consistency and index of viscosity as shown in Table 13. The findings demonstrated that the mechanical properties of bigels was totally dependent on the viscoelastic properties of the oleogel. The firmness, consistency and index of viscosity of NGGS were significantly higher than NGG ($p < 0.05$) which indicated an increase in mechanical properties of the bigels with a corresponding increase in gel strength of the oleogel. The results were correlated well with the rheology data which suggested NGGS had more elastic behavior as a result of strong gel properties of silicone oil containing oleogel. The higher cohesiveness of NGGS associated with the higher viscosity of bigels. Actually, spreadability is an inverse function of cohesiveness

and the higher cohesiveness means the lower spreadability. However, the lower surface tension properties of silicone oil could improve the spreadability of the bigels.

Table 13 Mechanical properties of bigels determined by texture analyzer

Sample	Firmness (g)	Cohesiveness (g)	Consistency (gs ⁻¹)	Index of viscosity (gs ⁻¹)
NGG	215.36 ± 0.32	124.50 ± 0.45	139.16 ± 0.28	289 ± 0.17
NGGS	489.12 ± 0.41	195.76 ± 0.23	259.42 ± 0.52	443 ± 0.34

All are significantly different ($p < 0.05$).

4.2.3.2.6 X-ray diffraction studies

Figure 40 displayed the X-ray diffraction patterns of drug, gelator, oleogels, hydrogel and bigels. All bigels showed the broad hump and a single sharp peak associated with the amorphous nature of guar gum and crystalline nature of SMS, which revealed the presence of both phases in bigels. Since there is variation in SMS diffraction peak of bigels, which suggested the oleogel phase was the dominant factor which controlled the crystalline behavior of the bigels. Similar to bacitracin bigels, the silicone oil containing oleogels increased the ordered structural arrangement of oleogels contributing to less amorphous behavior in bigels.

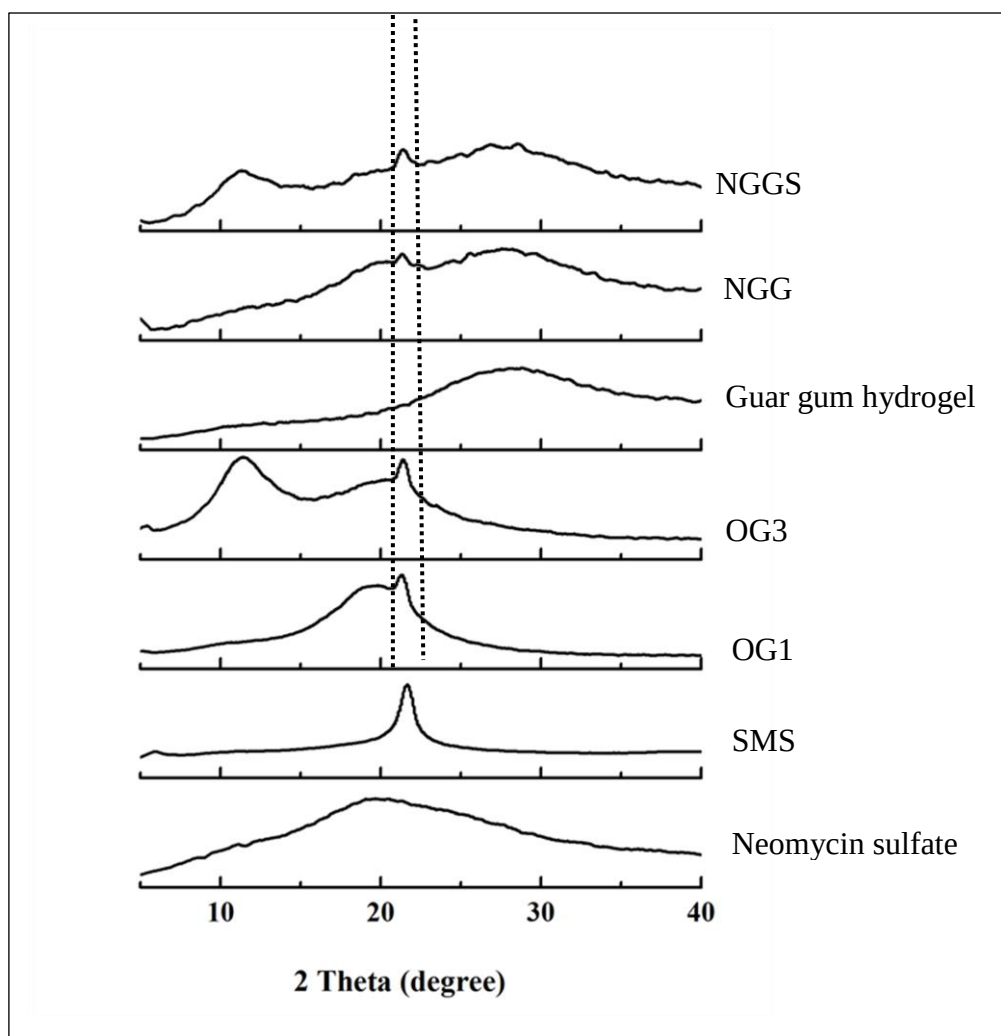


Figure 4.40 XRD patterns of neomycin sulfate, SMS, oleogels, drug loaded hydrogel and drug loaded bigels. OG3 is oleogels with a mixture of silicone oil and sesame oil and OG1 is oleogels containing sesame oil.

4.2.3.2.7 Drug content determination

The content of neomycin sulfate in bigel formulations was determined by derivatization method using UV-visible spectrophotometer. It could be seen in Figure 41 that the average drug content of all bigels was in the range of 95.84% to 105.72%. These results were within the acceptable limit of the claimed label amount of the United States Pharmacopoeia (90-120%) (86) which revealed that the drug was

homogenously dispersed within the formulations and did not degrade during the preparation process. The amount of drug content was depended on drug solubility in hydrogel and water content of hydrogel. Silicone oil has a low surface tension, high permeability to oxygen, nitrogen and water vapor and is capable of wetting most surfaces. The presence of methyl groups pointing outside provides the hydrophobic film with a good film forming property. As a result of hydrophobic film formation of silicone oil, it contributes to a protective film on the skin surface and prevent water evaporation (133). It was found that NGGS had the higher drug content (103.54%) compared with NGG (96.35%). This might result from the formation of silicone oil film on the water surface of hydrogel thus retarding the water evaporation and contributing to higher water content of hydrogel. Neomycin sulfate is hydrophilic drug and the higher water content of hydrogel could enhance the drug solubility. This result was in agreement with the study of Strachan et al., who reported that the addition of silicone oil (PDMS) assisted in retardation of water evaporation due to its permeability to water vapor and formation of surface film (134). Similarly, in the study of Zukas and Gupta, the incorporation of 50% silicone oil in alginate solution provided the water barrier properties and significantly decreased the water evaporation from fabricated capsules (135). Therefore, the higher drug content of NGGS could be the result of higher retaining of water owing to the property of water barrier of silicone enabling to the high solubility of neomycin in hydrogel.

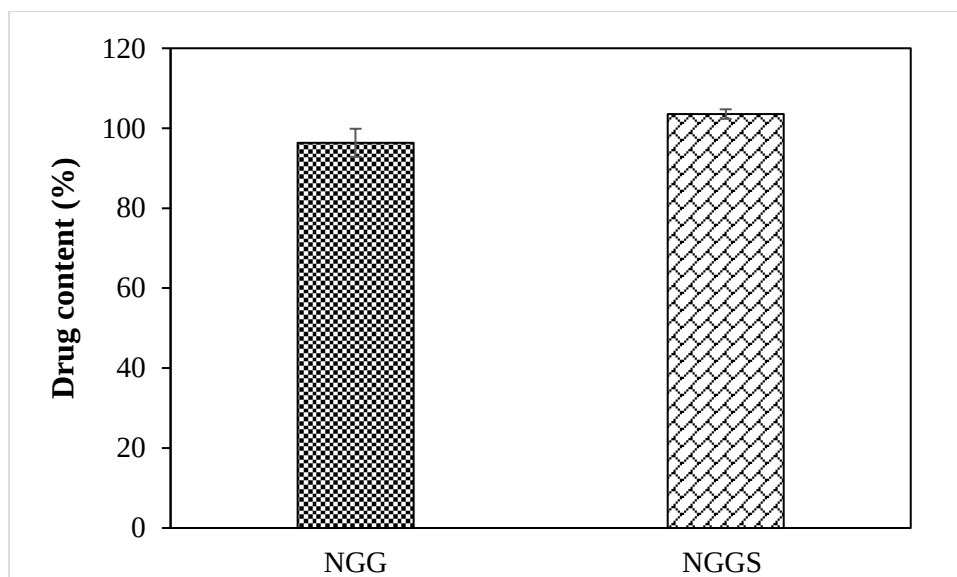


Figure 41 Drug content determination of neomycin sulfate loaded bigels.

4.2.3.2.8 *In vitro* drug release study

Figure 42 shows the *in vitro* drug release profile of neomycin sulfate from bigel formulations. The addition of silicone oil and the viscosity of oleogel strongly influenced the drug release from bigels. The CPDR of NGG and NGGS were 47.55% and 60.33%, respectively. It was found that the CPDR of NGGS was significantly higher than NGG ($p < 0.05$). For NGGS, the higher drug content resulted in higher drug release. Similar to bacitracin zinc bigels the silicone oil containing bigels had higher drug release due to high drug solubility and drug content. According to microscopic analysis, the type of bigel was oleogel in hydrogel type and the ratio used was 20:80. It could be expected the hydrophilic nature and higher water content of external phase of bigel facilitates the release of hydrophilic drug from external hydrogel phase. Ibrahim et al. reported that the release of hydrophilic drug (diltiazem hydrochloride) from bigels was higher than oleogels as a result of large amount of water in external phase provided the higher drug release of hydrophilic drug (11). Sieg et al. found that the addition of 5% of silicone oil (cyclopentasiloxane) to hydrogel enhanced the drug penetration of lidocaine due to strong influence of silicone oil on the diffusion and partition of drug from the formulation (136). Winckle et al. also stated that emulsion containing silicone fluid promoted the penetration of hydrophilic drugs by

dissolving the sebaceous lipids causing the reduction of skin barrier and enabling the delivery of hydrophilic drugs through sebaceous lipid barrier (137). The beneficial properties of silicone oil for delivery of hydrophilic nucleic acids has also reported by Vij et al. They indicated silicone oil was safe and effective chemical enhancer because it remained on the skin surface in spite of entering into deeper skin tissue. The main mechanism was that silicone oil induced alteration of skin hydration and mild disruption of microstructure of skin lipids leading to possible opening of many porous channels which can mediate efficient delivery of hydrophilic molecules (138). Hence, the high release rate of NGGS could be due to silicone oil induced higher water content of hydrogel and higher drug content. The results were fitted with various models (zero-order, first-order and Higuchi models) to estimate the release kinetics of the drugs. The drug release pattern followed the Higuchi model and the release mechanism was diffusion according to the correlation coefficient values. The n value (release exponent) was calculated from Korsmeyer-Peppas model to identify Fickian or non-Fickian diffusion. NGG had n value of less than 0.5 which indicated that Fickian diffusion was the major release mechanism of NGG. However, the greater n value of NGGS might be associated with the addition of silicone oil which could alter the release mechanism. NGGS showed non-Fickian diffusion and the higher drug release could be resulted from the combined mechanism of drug release such as swelling and subsequent diffusion. It was in agreement with bacitracin zinc bigels which was found that the addition of silicone oil altered the drug release mechanism of bigels from Fickian to non-Fickian diffusion.

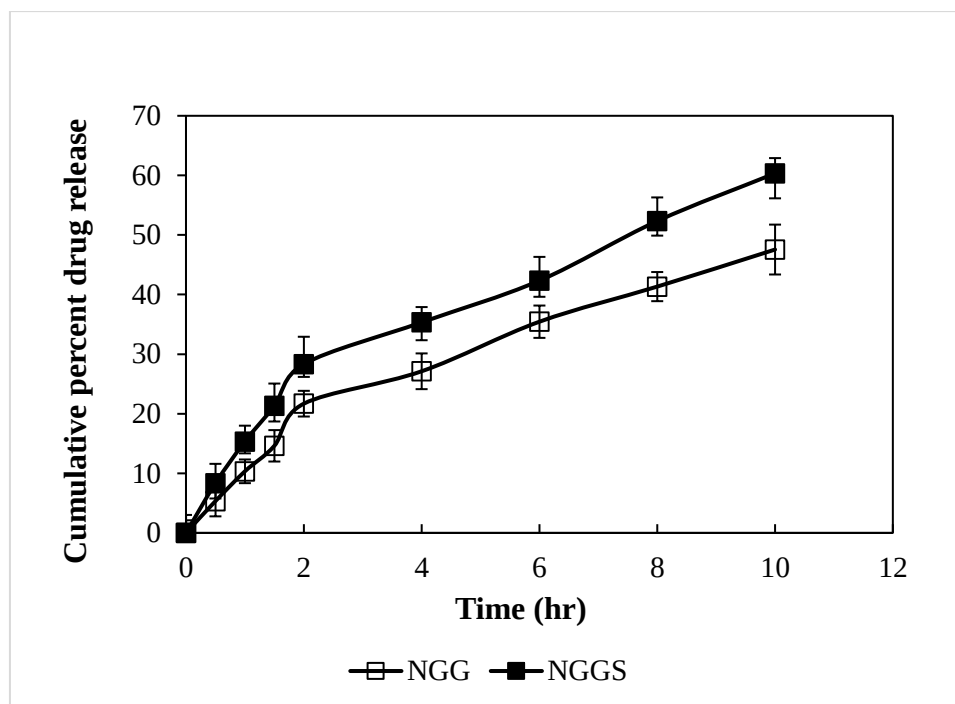


Figure 42 In vitro drug release profile of neomycin sulfate loaded bigels

Table 14 Release kinetics of neomycin sulfate from bigels

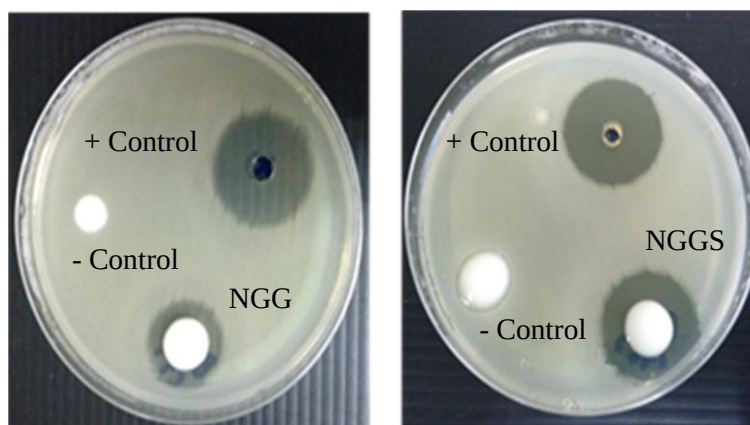
Samples	Zero order (r^2)	Higuchi (r^2)	Korsmeyer-Peppas (r^2)	n	Release mechanism
NGG	0.87	0.97	0.98	0.41	Fickian
NGGS	0.83	0.99	0.99	0.52	Non-Fickian

4.2.3.2.9 Antimicrobial assay

Neomycin sulfate loaded bigels were evaluated for antimicrobial activity against *Staphylococcus aureus*. In the commercial market, most of the topical products of neomycin sulfate are only available as the combination of neomycin sulfate, bacitracin zinc and polymyxin B. Thus, neomycin sulfate standard solution (0.5% w/w) and blank bigel were used as positive and negative controls, respectively. The drug content and diffusion of drug from bigels are the factors which controlled the antimicrobial activity of the bigels. As shown in Figure 43, the antimicrobial assay

revealed that bigels without drug had no antimicrobial activity against *Staphylococcus aureus*. All drug loaded bigels showed good antimicrobial activity and the diameter of inhibition zone of NGGS (28 mm) was found to be significantly larger ($p < 0.05$) than NGG (23 mm). The inhibition zones indicated that the released neomycin sulfate was in the active form. It was found that the zone diameter of positive control and NGGS were not significantly different. Hence, it can be concluded that bigel showed easy diffusion as compared to the drug solution even though it was semisolid in nature. Similar to bacitracin zinc bigels, the improvement in antimicrobial activity with the addition of silicone oil could also be due to the higher drug content and release of NGGS. The finding was also correlated with the result of *in vitro* drug release.

A



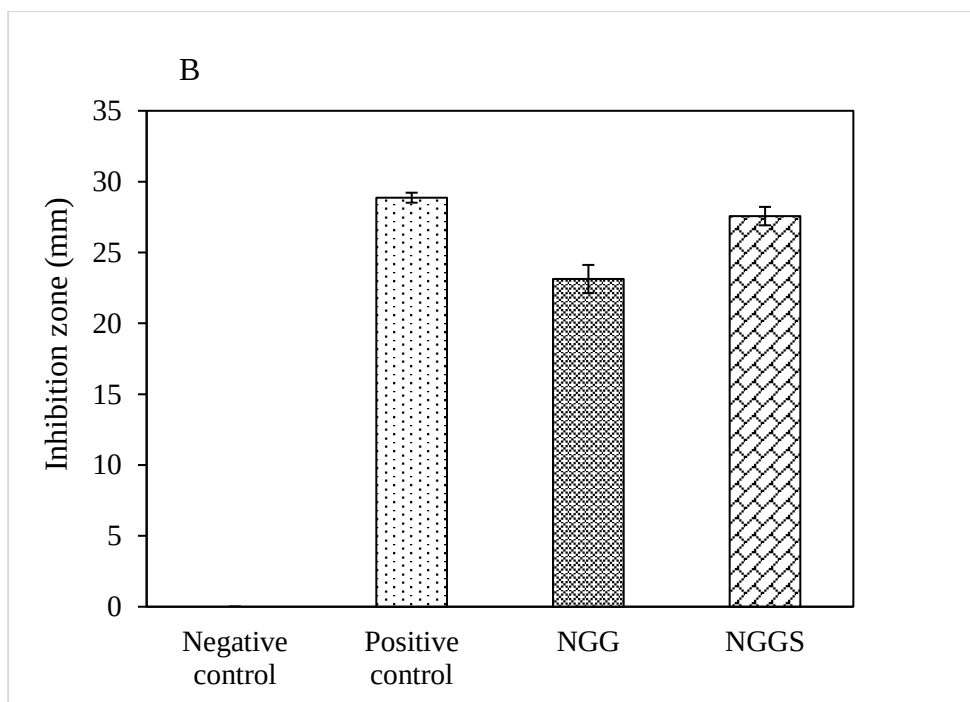


Figure 43 Antimicrobial activity of the neomycin sulfate loaded bigels against *Staphylococcus aureus*. The positive control and negative control were 0.5% w/w neomycin sulfate in water and blank bigels.

4.2.3.2.10 Stability studies

The stability of all bigels was evaluated by temperature cycling and intermediate stability tests and the parameters such as the physical appearance, pH and drug content of all bigel formulations were investigated to confirm the physicochemical changes. There were no changes in color and pH and the drug content (Figure 44) was also within the acceptable range (90-120%) which indicated that all bigels were stable and their antimicrobial activities (Figure 45) did not change after the stability tests. One of the indicators to detect the physical appearance was the stable texture and absence of flow when the inversion test was performed. All bigels remained stable after six cycles of temperature cycling, however, the intermediate stability test showed differently. NGGS remained firm texture and absence of flow or phase separation after 4 months stability test whereas NGG showed flow and separation into two phases after 2 months. The addition of silicone oil improved the stability

significantly ($p < 0.05$) and similar results were found in bacitracin zinc bigels. This could be due to stronger gel strength of silicone oil containing oleogel which may help in supporting the structural integrity of bigels. Similar improvement of stability was found in previous study of Eeman et al., who reported that the incorporation of ascorbic acid in a silicone matrix enhanced the chemical stability by reducing the oxidative decomposition (127). Hence, it can be concluded that the addition of silicone oil into oleogel not only increase the drug release and antimicrobial activity but also improve the stability.

Table 15 Color, flow when inversion and pH of the bigels before and after passing the temp cycling for 6 cycles and intermediate stability test for 1,3 and 6 months

Parameters	Sample	Initial		Temp-cycling		Intermediate stability test					
						1 month		3 months		6 months	
Color / Flow when inversion	NGG	NC	-	NC	-	NC	-	NC	+	NC	+
	NGGS	NC	-	NC	-	NC	-	NC	-	NC	+
pH	NGG	5.59 ± 0.31		5.57 ± 0.13		5.52 ± 0.26		5.54 ± 0.17		5.55 ± 0.39	
	NGGS	5.83 ± 0.21		5.85 ± 0.31		5.84 ± 0.32		5.87 ± 0.19		5.82 ± 0.29	

NC = No change, - = No flow, + = Flow

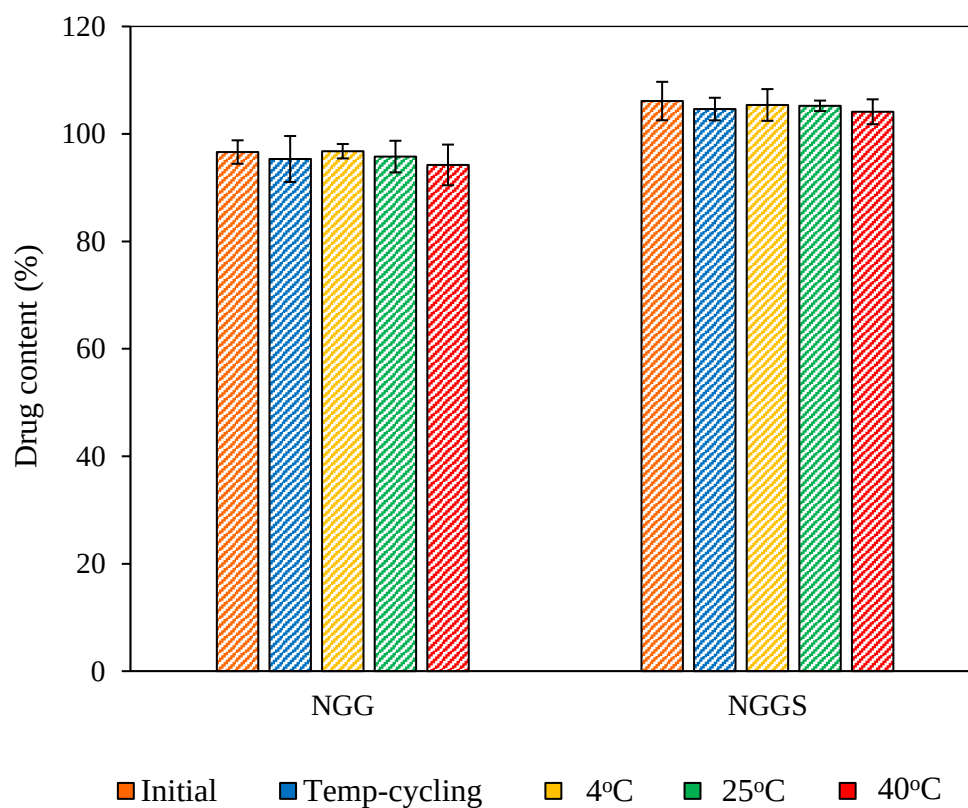


Figure 44 Drug content determination of neomycin sulfate loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months.

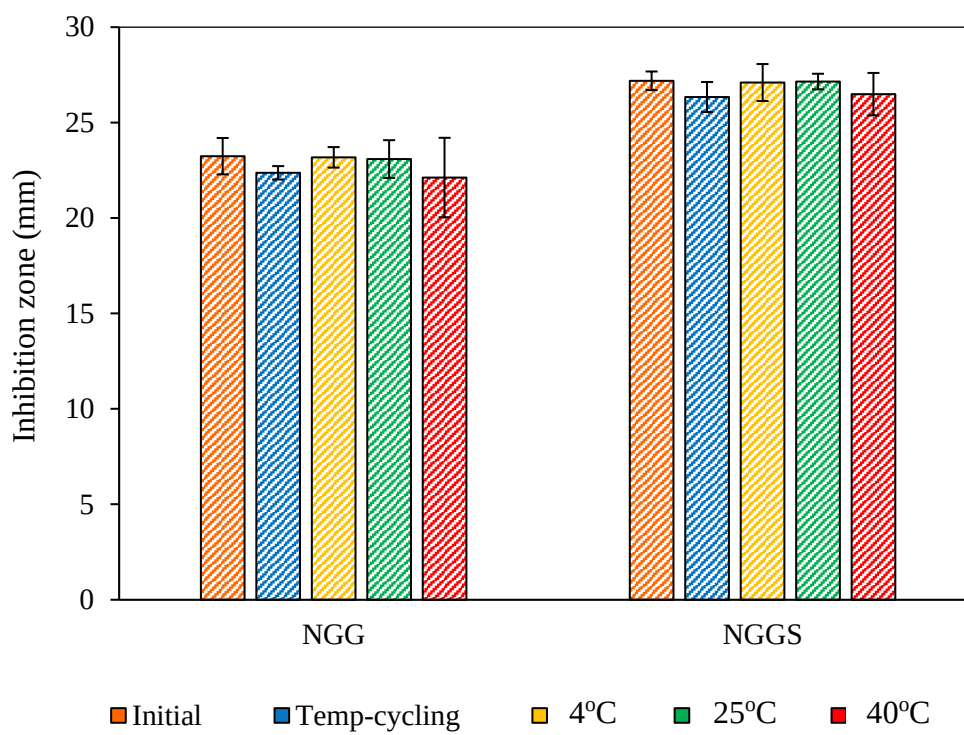


Figure 45 Antimicrobial activity of neomycin sulfate loaded bigels before and after passing the temp cycling for 6 cycles and intermediate stability test at 4°C, 25°C and 40°C for the period of 6 months.

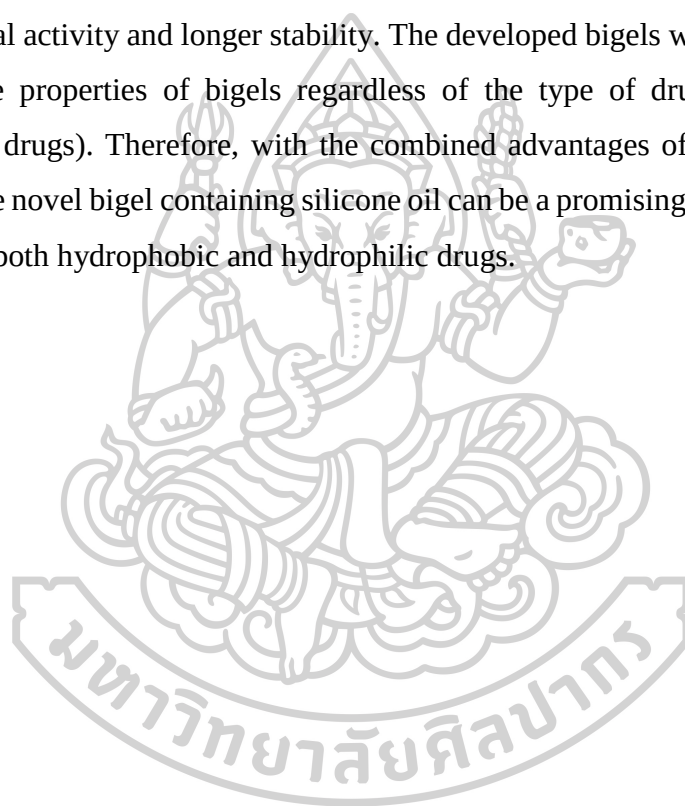
CHAPTER 5

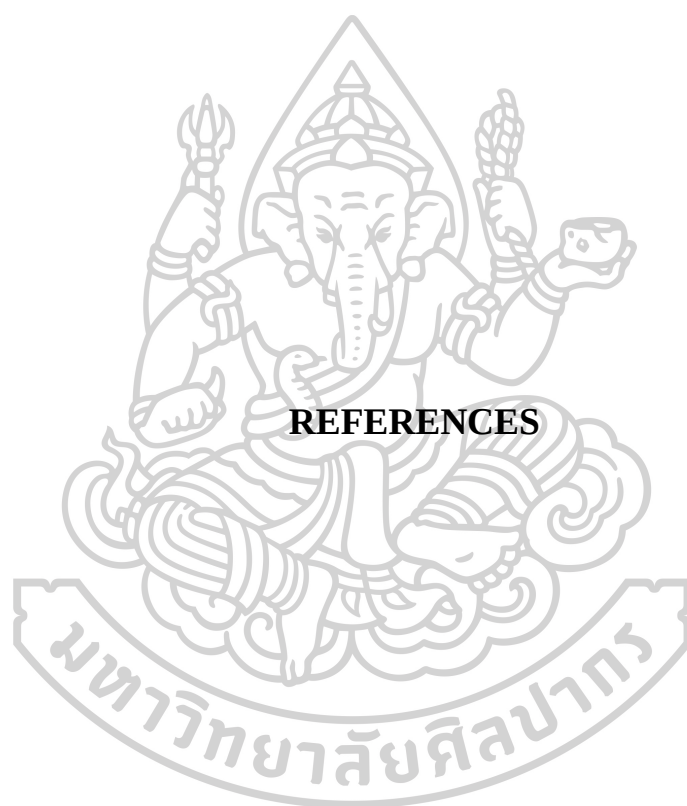
CONCLUSION

A novel sorbitan monostearate (SMS)-sesame oil oleogel could be formulated with the aid of silicone oil. Addition of silicone oil to sesame oil-SMS oleogel system resulted in a formation of good oleogel which was a true gel with longer LVER and better gel strength at only 10% w/w gelator concentration (as compared to 20% and 18% for the single oil systems of sesame oil and silicone oil). The polarized light micrographs revealed the long and thick three-dimensional network which can enhance solvent entrapment properties of the gelator and prevent syneresis. Moreover, the obtained oleogel was thermoreversible in nature. It had pseudoplastic flow with thixotropic behavior and high viscosity at rest thus suitable for topical application. The improved physical and thermal properties, greater sensory profile, better product aesthetics and stability of oleogel was achieved. The improvement of the oleogel with the addition of silicone oil could gain a high potential for the development of new carrier system called bigel.

The novel bigel was developed by mixing SMS-silicone and sesame oil oleogel with hydrogel. The aim of this study was to develop a novel bigel which has capability of carrying hydrophobic and hydrophilic drugs. The hydrophobic drug (bacitracin zinc) was loaded into the oleogel and hydrophilic drug (neomycin sulfate) was loaded into the hydrogel. Xanthan gum and guar gum hydrogels were used for bacitracin zinc bigels, however, only guar gum hydrogel was selected for neomycin sulfate bigels due to polymer-drug interactions. The oleogel to hydrogel at the ratio of 20:80 was the optimum ratio for the formation of bigels with good homogeneity, absence of flow, satisfactory rheological and mechanical properties and good stability. The microscopic study confirmed the biphasic nature, indicating by the oleogel phase dispersed in the continuous hydrogel phase. The physicochemical properties of bacitracin zinc loaded bigels could be modified by varying type of hydrogel and composition of oleogel. BXG displayed lower release and diffusion causing smaller inhibition zone as a result of higher viscosity compared with BGG. The modification of bigels based on SMS-sesame oil oleogel and hydrogel by silicone oil could significantly enhance drug content, release and diffusion and hence large inhibition zone was obtained. Although

all bigels could stand the temperature cycling test for 6 cycles, BXGS was the only bigel passed the intermediate stability test for 6-months study. The superior properties of BXGS resulted from higher viscosity and stronger formation of oleogel network due to the addition of silicone oil preventing the coalescence of oleogel and hence lower chance of phase separation. The antimicrobial activity of BXGS remained constant after 6-months stability test. Similar results were obtained for neomycin sulfate bigels, the drug was successfully loaded into hydrogel phase and the addition of silicone oil improved the overall aspects of bigels such as higher drug content and release, greater antimicrobial activity and longer stability. The developed bigels with silicone oil could enhance the properties of bigels regardless of the type of drugs (hydrophobic or hydrophilic drugs). Therefore, with the combined advantages of both hydrogels and oleogels, the novel bigel containing silicone oil can be a promising candidate for topical delivery of both hydrophobic and hydrophilic drugs.





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APPENDIX

Appendix I

Development of blank bigels

1. Screening of polymers for hydrogel preparations

Synthetic polymers (carbopol 934 and carbopol ultrez 21), semi-synthetic polymer (hydroxy propyl methyl cellulose), and natural polymers (xanthan, guar, chitosan, sodium alginate, and carrageenan) were studied for the development of hydrogels. After developing the hydrogels, they were combined with oleogel to develop the bigels. Among the tested polymers, gum gum, xanthan gum, carbopol 934 and carbopol ultrez 21 were the suitable polymers for hydrogel preparations since they provided the bigel formulations with smooth, homogeneous and good aesthetics. Hence, these four polymers were selected for further preparation of hydrogels. Thereafter, the optimum gelling concentration of the polymers for hydrogel preparations were determined and the results were shown in Figure 46-49. It was found that xanthan (2% w/w), guar (1.5% w/w), carbopol 934 (0.5% w/w), and carbopol ultrez 21 (0.2% w/w) were the optimum concentration since they produced bigels with good consistency, homogeneity and absence of flow.

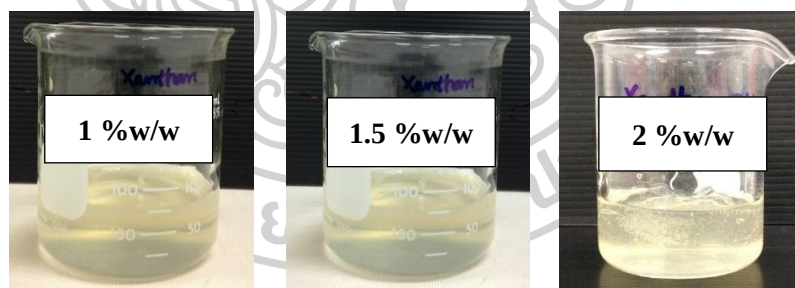


Figure 46 Determination of gelling concentration of xanthan gum hydrogel.

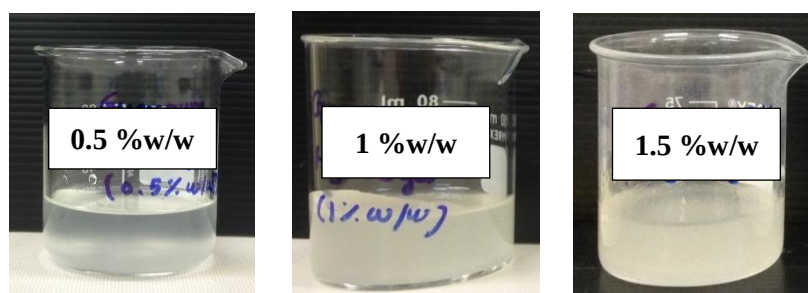


Figure 47 Determination of gelling concentration of guar gum hydrogel.

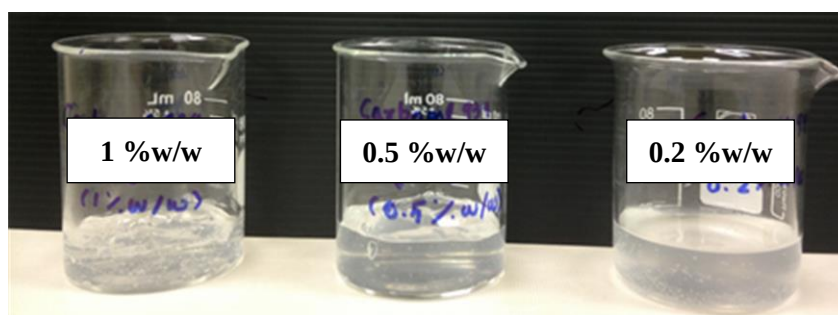


Figure 48 Determination of gelling concentration of carbopol 934 hydrogel.

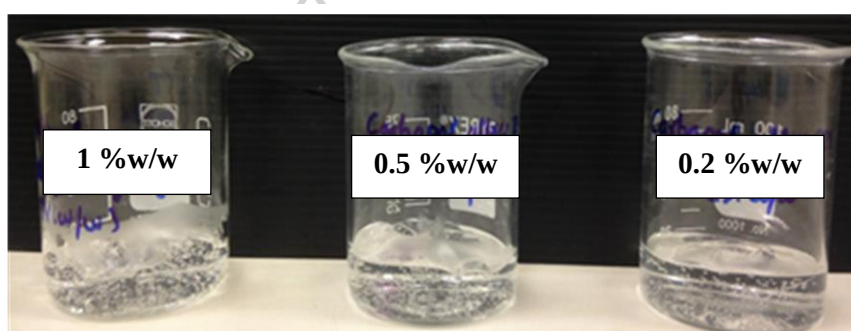


Figure 49 Determination of gelling concentration of carbopol ultrez 21 hydrogel.



2. Selection of oleogel and hydrogel ratio for the development of bigels

The ratio of oleogel and hydrogel for the preparation of bigels was chosen using 5 different ratios; 10:90, 20:80, 30:70, 40:60, and 50:50. The results were presented in Table 16. As shown in Figure 50, the 20:80 ratio was the best ratio which produced bigels with good consistency, homogeneity without lump formation and the absence of flow when inverted. This ratio was selected for the preparation of bigels for further studies.

Table 16 Selection of the best ratio of oleogel and hydrogel for the development of bigels.

Ratio of oleogel and hydrogel	Consistency	Flow when inverted	Lumps formation
50:50	Very thick	Not flow	Lumps
40:60	Thick	Not flow	Lumps
30:70	Thick	Not flow	Lumps
20:80	Good	Not flow	No lumps
10:90	Soft	Easily flow	No lumps



Figure 50 Selection of the best ratio of oleogel and hydrogel for the development of bigels.

Appendix II

Measurement of surface tension

Table 17 displays the surface tension of the tested samples. The surface tension of the samples was measured using Goniometer (FTA 1000, First Ten Angstroms, USA).

Table 17 Surface tension of sesame oil, silicone oil, silicone surfactant and their combination.

Sample	Surface tension (mN/m)			
	1	2	3	Average $\pm$ SD
Sesame oil	32.82	32.86	32.75	32.81 $\pm$ 0.06
Silicone oil	17.45	17.38	17.29	17.37 $\pm$ 0.08
Silicone surfactant	17.29	17.26	17.20	17.25 $\pm$ 0.05
Mixture of sesame oil and silicone oil	20.85	20.93	20.83	20.87 $\pm$ 0.05
Mixture of sesame oil, silicone oil and silicone surfactant	17.84	17.70	17.80	17.87 $\pm$ 0.07

Appendix III

Standard curve of bacitracin zinc

1. Determination of bacitracin zinc in the sample

The content of bacitracin zinc in the sample was determined by HPLC method using the following condition. The standard curve shown in Figure 51 was used for drug content determination and *in vitro* drug release study.

Standard	:	Bacitracin zinc
Method	:	HPLC analysis
Model of HPLC	:	HPLC-DAD, Agilent 1200 series, Agilent Technologies, USA
Analytical column	:	Thermo scientific® Hypersil GOLD™ C-18 column, USA (250 x 4.6 mm i.d.)
Mobile phase	:	63:37 v/v of organic phase (27:2 methanol and acetonitrile) and aqueous phase (0.2 M phosphate buffer aqueous solution and 0.1 mM EDTA adjusted to pH 6)
Flow rate	:	1 mL/min
Injection volume	:	100 µL
Detector	:	Diode array detector at 254 nm
Concentration (µg/mL)	:	10, 30, 50, 70, 90

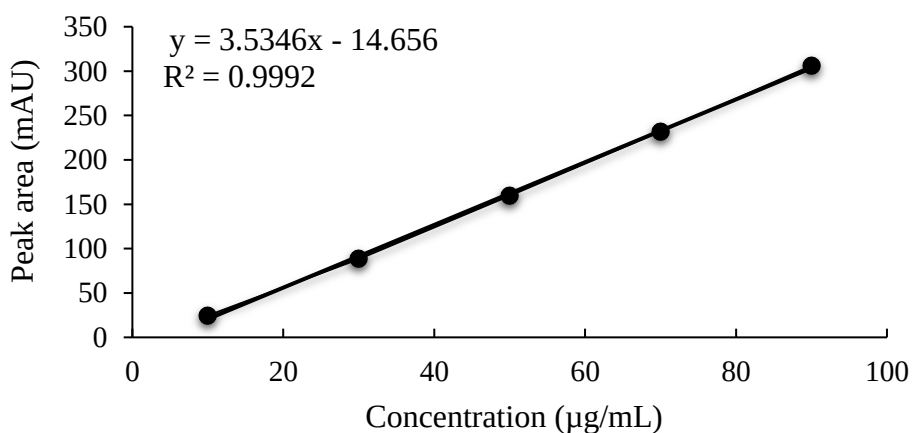


Figure 51 Standard curve of bacitracin zinc for drug content determination and *in vitro* drug release study.

Appendix IV

Standard curve of neomycin sulfate

2. Determination of neomycin sulfate in sample

The content of neomycin sulfate in the sample was determined by UV derivatization method using the following condition. The standard curve shown in Figure 52 was used for drug content determination and *in vitro* drug release study.

Standard	:	Neomycin sulfate
Method	:	UV-Visible spectrophotometry at 335 nm using derivatization method
Derivatization reagent	:	o-phthaldialdehyde reagent
Model of UV-Visible spectrophotometer	:	Agilent G1103A model UV-Visible spectrophotometer, Agilent Technologies, USA
Concentration (µg/mL)	:	10, 30, 50, 70, 90

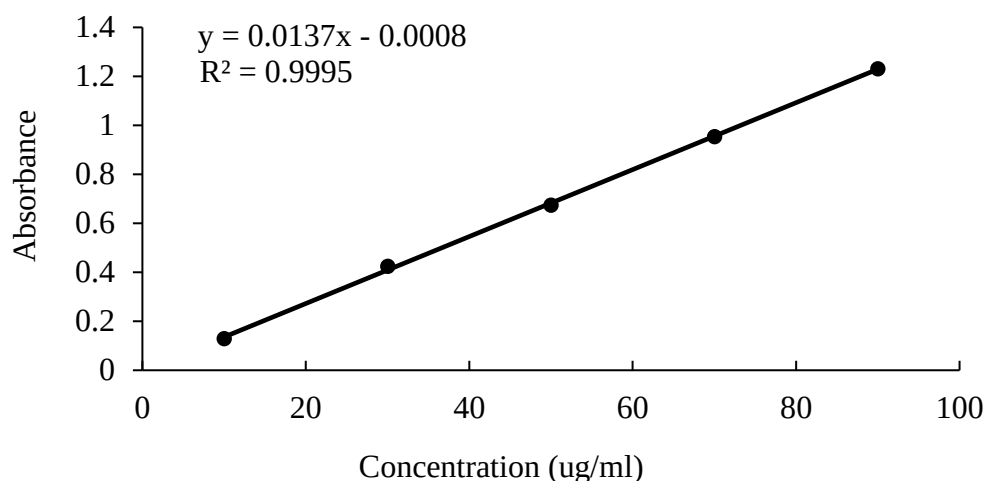


Figure 52 Standard curve of neomycin sulfate for drug content determination and *in vitro* drug release study.

Appendix V

Drug content determination of bacitracin zinc

Figure 53 shows the typical chromatogram of standard bacitracin zinc. The concentration of bacitracin zinc was determined over the concentration range of (10-90 $\mu\text{g/mL}$) and the peak area values were displayed in Table 18.

Table 18 Peak area of bacitracin zinc standard solution analyzed by HPLC method for the calibration curve (n=3).

Concentration ($\mu\text{g/mL}$)	Peak area			
	1	2	3	Average $\pm$ SD
10	24.914	23.5162	25.2046	24.5449 $\pm$ 0.9027
30	88.8126	88.7644	87.0138	88.1969 $\pm$ 1.0249
50	159.9234	159.5315	158.8557	159.4369 $\pm$ 0.5401
70	231.4014	231.9532	233.2083	232.1876 $\pm$ 0.9260
90	305.4844	307.141	305.4132	306.0129 $\pm$ 0.9776

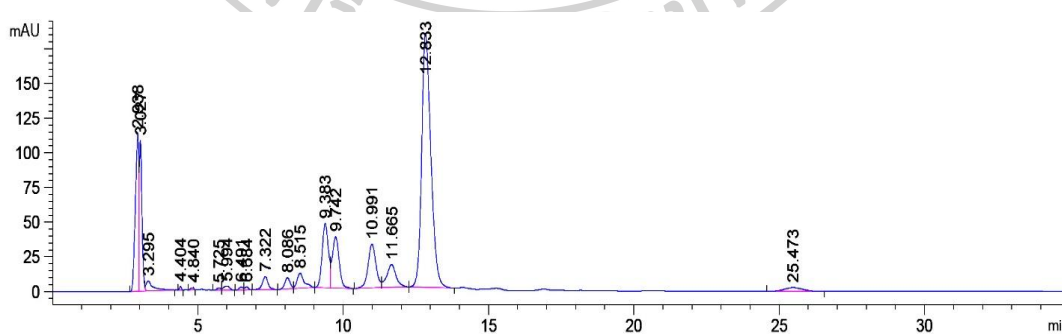


Figure 53 Typical chromatogram of standard bacitracin zinc.

Appendix VI

Drug content determination of neomycin sulfate

Ultraviolet-visible spectrophotometry and method validation

Typical validation characteristics which should be considered are accuracy, precision as repeatability and intermediate precision, linearity and range for assay (ICH harmonized tripartite guideline, Validation of analytical procedures: text and methodology Q2 (R1), 1994). The content of neomycin sulfate was analyzed using Ultraviolet-visible spectrophotometry.

1. Linearity

Table 19 shows the absorbance of standard neomycin sulfate. The UV-Visible spectrophotometric method for determination of neomycin sulfate obeyed the Beer's law at the concentration range of 10, 30, 50, 70 and 90 $\mu\text{g/mL}$. The calibration curve was found to be linear over the concentration range stated, with correlation coefficient (R^2) of 0.9998 and the equation for the line was $y = 0.0129x + 0.0017$ (Figure 54).

Table 19 Absorbance of neomycin sulfate standard solution in the range of 10-90 $\mu\text{g/mL}$ by UV-Visible spectrophotometric method at 335 nm ($n=3$.)

Concentration ($\mu\text{g/mL}$)	Absorbance			
	1	2	3	Average $\pm$ SD
10	0.1297	0.1292	0.1289	0.1293 $\pm$ 0.0004
30	0.3845	0.3783	0.4247	0.3958 $\pm$ 0.0251
50	0.6172	0.6236	0.6742	0.6383 $\pm$ 0.0312
70	0.8809	0.8892	0.8892	0.8864 $\pm$ 0.0048
90	1.1367	1.1239	1.2310	1.1639 $\pm$ 0.0585

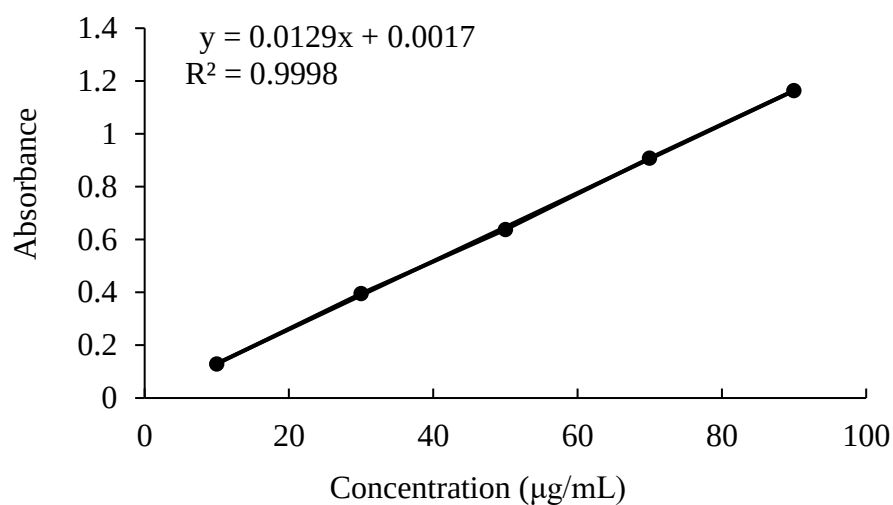


Figure 54 Calibration curve of neomycin sulfate (n=3).

2. Accuracy

Standard addition method was used to access accuracy. Placebo sample was prepared and then spiked with three concentration levels (30, 50 and 70 µg/mL) of neomycin sulfate and the resulting solutions were analyzed to calculate the % recovery. The %RSD was used to determine the accuracy of this method and results were described in Table 18.

Table 18 Accuracy test of UV-Visible spectrophotometric method for neomycin sulfate at concentration of 30, 50 and 70 µg/mL (n=3).

Actual concentration (µg/mL)	Calculated concentration (µg/mL)	Recovery (%)	Mean recovery	SD	RSD (%)
30	29.9399	96.667	96.445	0.2499	0.259
	28.9490	96.497			
	28.8526	96.175			
50	48.5635	97.127	97.541	0.9636	0.988
	49.3210	98.642			
	48.4265	96.853			
70	69.7269	99.61	100.018	0.8727	0.873
	70.7102	101.02			
	69.5965	99.424			

3. Precision

Repeatability (intra-day precision)

The repeatability was evaluated as relative standard deviation (%RSD). Six replicate measurements for the same concentration of neomycin sulfate (50 µg/mL) were performed on the same day and the results were shown in Table 19.

Table 19 Repeatability test of UV-Visible spectrophotometric method for neomycin sulfate at concentration of 50 µg/mL (n=6).

Actual concentration (µg/mL)	Calculated concentration (µg/mL)		
	Mean	SD	RSD (%)
50	49.092	0.0121	0.026

Intermediate precision (inter-day precision)

The reproducibility was assessed using the same concentration of neomycin sulfate (50 µg/mL) over a period of three days and the results were depicted in Table 20.

Table 20 Intermediate precision of UV-Visible spectrophotometric method for neomycin sulfate at concentration of 50 µg/mL (n=3).

Day	Actual concentration (µg/mL)	Calculated concentration (µg/mL)		
		Mean	SD	RSD (%)
1	50	49.195	0.0056	0.011
2	50	48.997	0.0231	0.047
3	50	48.268	0.0135	0.028

Appendix VII

Antimicrobial assay of drug loaded bigels

Tryptic soy agar (TSA)

Suspend 40.0 g of the powder in 1 L of purified water and mix completely. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Afterwards, autoclave at 121°C for 15 minutes.

Tryptic soy broth (TSB)

Suspend 30.0 g of the powder in 1 L of purified water and mix thoroughly. Warm slightly to completely dissolve the powder and autoclave at 121°C for 15 minutes.

Measurement of inoculum concentration of *Staphylococcus aureus*

For antimicrobial assay of drug loaded bigels, the inoculum concentration of *Staphylococcus aureus* was determined using the calibration curve (Figure 55).

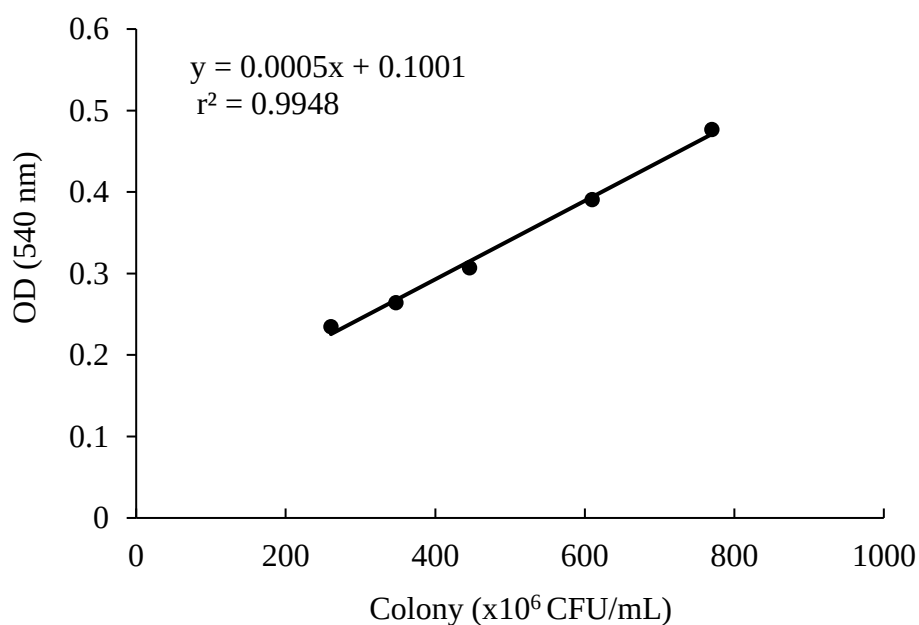


Figure 55 Calibration curve of *Staphylococcus aureus* DMST 8013.

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