UNIVERSITY OF SOUTHAMPTON

FACILITY OF SCIENCE

School of Chemistry

The Synthesis and Screening of Polymer Libraries using a High Throughput Approach

By

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ABSTRACT

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This thesis describes the development of blood compatible polymer libraries and a variety of novel high throughput screening techniques.

6 polymer libraries, comprising of 446 polymers prepared by free radical polymerisation and 90 polymers made by functionalisation of polymer side chains, were prepared by a variety of combinatorial approaches. High throughput polymer synthesis, purification, and analysis were established using a 96-well plate format and microarray techniques.

Novel high throughput methods, for evaluating the wettability of polymer and their thermal properties were developed. Protein adsorption onto polymers and leucocyte depletion abilities were investigated to determine the relationship between the binding of blood components and polymers in order to improve the biocompatibilities of polymeric materials.

CONTENTS

PREFACE

The research described in this thesis was carried out under the supervision of Prof. Mark Bradley at the University of Southampton between February 2002 and April 2004. No part of this thesis has been previously submitted at this or any other University.

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Firstly, I would like to express to my supervisor, Professor Mark Bradley, my deepest gratitude for all the advice and encouragements he has given during my research. I would like to thank my adviser, Professor George Attard, and all the staff of the School of Chemistry for their help.

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Finally I would like to thank Ai for her love and everlasting support.

ABBREVIATIONS

AAG-H	2-acrylamidoglycolic acid
ACHN	1,1'-azo-*bis*-1-cyclohexane carbonitrile
ACVA	4,4'-azo-*bis*-4-cyanovaleric acid
ADVN	2,2'-azo-*bis*-2,4- dimenthylvaleronitrile
AES-H	*mono*-2-(acryloyoxy)ethyl succinate
AFM	atomic force microscopy
A-H	acrylic acid
AIBN	2,2'-azo-*bis*-isobutyronitrile
Alb	albumin
Alexa555	Alexa Fluor 555
Alexa647	Alexa Fluor 647
AMBN	2,2'-azo-*bis*-2-menthylbutyronitrile
BACOEA	2-[[(butylamino)carbonyl]oxy]ethyl acrylate
BAEMA	2-(tert-Butylamino)ethyl methacrylate
1,4-BD	1,4-butanediol
BMA	*n*-butyl methacrylate
BnMA	*N*-benzylmethylaimne
t-BuOH	*tert*-butanol
calc.	calculated
CCD	charge coupled device
CD	circular dichroism
cHMA	cyclohexanemethylamine
CyOH	cyclohexanol
Da	dalton
DAAAm	diacetone acrylamide (*N*-(1,1-dimethyl-3-oxobutyl)-acrylamide)
DBnA	dibenzylamine
DcHA	dicyclohexylamine
DEAAm	*N,N*-diethyl acrylamide
DEAEA	2-(diethylamino)ethyl acrylate
DEAEMA	2-(diethylamino)ethyl methacrylate
DEGDMA	diethyleneglycol dimethacylate
DEGMEMA	di(ethylene glycol) methyl ether methacrylate
DEMEDA	*N,N*-dimethyl-*N'*-methylethylenediamine
DiPA	diisopropylamine
DMA	dimethylacetoamide
DMAEA	2-(dimethylamino)ethyl acrylate
DMAEMA	2-(dimethylamino)ethyl methacrylate
DMAAm	*N,N*-dimethyl acrylamide
DMAPMAAm	*N*-[3-(dimethylamino)propyl] methacrylamide
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMVBA	*N,N*-dimethylvinylbenzylamine
DNA	deoxyribonucleic acid
DnBA	di-*n*-butylamine
DnHA	di-*n*-hexylamine
DP	polymer length
DSC	differential scanning calorimetry

ELISA	enzyme linked immunosorbent assay
EG	ethylene glycol
EGMP-H	ethylene glycol methacrylate phosphate
EMA	ethyl methacrylate
EtOH	ethanol
eq.	equivalent
f	efficiency factor
FACS	fluorescence activated cell sorter
FCM	flow cytometry
FDA	Food and Drug Administration
Fib	fibrinogen
FITC	Fluorescein isothiocyanate
FT-IR	Fourier transform infrared
GMA	glycidyl methacrylate
GPC	gel permeation chromatography
HBMA	hydroxybutyl methacrylate
HDL	high density lipoprotein
HDPE	high density polyethylene
HEA	2-hydroxyethyl acrylate
HEMA	2-hydroxyethyl methacrylate
HPMA	hydroxypropyl methacrylate
hr	hour
HT-GPC	high throughput gel permeation chromatography
HTS	high throughput screening
[I]	initiator concentration
IgG	immunoglobulin G
IPA	isopropanol
k_d	rate constant of initiator decomposition
k_p	rate constant of propagation
k_t	rate constant of termination
LDL	low density lipoprotein
LDPE	low density polyethylene
[M]	monomer concentration
MAEPy	2-(2-methylaminoethyl)pyridine
MA-H	methacrylic acid
MAIB	dimethyl-2,2'-azo-*bis*-isobutyrate
MAn	*N*-methylaniline
MEA	2-methoxyethyl acrylate
MEMA	2-methoxyethyl methacrylate
MeOH	methanol
MMA	methyl methacrylate
Mn	number average molar mass
MnHA	*N*-methylhexylamine
MNPMA	2-methyl-2-nitropropyl methacrylate
MPC	2-methacryloyloxyethyl phosphocholine
Mpi	1-methylpiperazine
MTEMA	2-(methylthio)ethyl methacrylate
Mw	weight average molar mass
MWD	polydispersity index
NIPAAm	*N*-isopropyl acrylamide
NMP	1-methyl-2-pyrrolidinone

PA	polyamide
PBS	phosphate-buffered saline
PC	polycarbonate
PEO	poly(ethylene oxide)
PET	poly(ethylene terephthalate)
PDMAAm	poly(*N,N*-dimethyl acrylamide)
PF3	phospholipids and platelet factor 3
PHEMA	poly(2-hydroxyethyl methacrylate)
PMEA	poly(2-methoxyethyl acrylate)
P(MEA-*co*-DEAAm)	2-methoxyethyl acrylate – *N,N*-diethyl acrylamide copolymer
PMEMA	poly(2-methoxyethyl methacrylate)
P(MEMA-co-DEAEMA)	2-methoxyethyl methacrylate - 2-(dimethylamino)ethyl methacrylate copolymer
P(MEMA-*co*-GMA)	2-methoxyethyl methacrylate - glycidyl methacrylate copolymer
PMMA	poly(methyl methacrylate)
P(MMA-*co*-GMA)	methyl methacrylate - glycidyl methacrylate copolymer
PNIPAAm	poly(*N*-isopropyl acrylamide)
PP	polypropylene
ppm	parts per million
PSt	polystyrene
PTFE	poly(tetrafluoroethylene)
PU	polyurethane
PVA	poly(vinyl alcohol)
PVC	poly(vinyl chloride)
PVP	poly(*N*-vinyl-2-pyrrolidone)
quant.	quantitative
r	radii
RNA	ribonucleic acid
S	area (superficial measure)
SEM	scanning electron microscopy
St	styrene
TAG	triacylglycerol
TEDETA	*N,N,N',N'*-tetraethyldiethylenetriamine
TG	thermal gravimetric analysis
Tg	glass transition temperature
THF	tetrahydrofuran
THFFA	tetrahydrofurfuryl acrylate
THFFMA	tetrahydrofurfuryl methacrylate
Tm	melting point
TMEDA	*N,N,N'*-trimethylethylenediamine
TMPDA	*N,N,N'*-Trimethyl-1,3-propanediamine
TOF-SIMS	Time of flight secondary ion mass spectrometry
V	volume
VAA	*N*-vinylacetamide
VHDL	very high density lipoprotein
VI	1-vinylimidazol
VLDL	very low density lipoprotein
VP-2	2-vinylpyridine
VP-4	4-vinylpyridine
VPNO	1-vinyl-2-prrolidinone

XPS	X-ray photoelectron spectroscopy

Chapter 1

INTRODUCTION

1.1 Biocompatible polymers

1.1.1 General

The field of biomaterials has seen rapid development over the past few years.[1-3] A huge amount of financial resource and research effort has been invested to discover materials with improved biocompatibility.[4,5] A vast variety of materials are currently in routine use, especially for medical applications, including metals, ceramics, and polymers. However, many metals suffer from corrosion, brittleness, and high density, while the release of metal ions may cause allergic tissue reactions. The problems with ceramics include brittleness, low fracture strength, difficulty to fabricate, low mechanical reliability, lack of resilience, and high density. Therefore, polymers have made a very significant contribution in this area since the early 1950s with major improvements in medical devices.[5-12] Some of the benefits of polymers as medical products are as follows;

- Flexible, ductile, and tough
- Light weight
- Reduced cost
- Transparent (vital to monitor flows visually)

Furthermore, polymers can be prepared in a wide variety of compositions, properties, and forms (solids, fibers, fabrics, films, and gels), and be fabricated readily into various shapes and structures. Polymers have, importantly, excellent potential in producing highly biocompatible surfaces.[4,13]

1.1.2 Biocompatible polymers

The medical applications of polymers can be categorised into two groups. Firstly devices in medical fields where the polymers are not in direct contact with bodily fluids, and secondly where polymers are in direct contact with human tissues and fluids. Many biocompatible polymers are used to produce blood contacting devices in a range of applications including blood bags, artificial organs, sutures, syringes, surgical drapes, and so forth.[4-6]

Polymers that are in use generally for medical devices are based on a variety of

polymers including poly(vinyl chloride) (PVC), high density polyethylene (HDPE), low density polyethylene (LDPE), polypropylene (PP), silicone rubbers, poly(ethylene terephthalate) (PET), poly(tetrafluoroethylene) (PTFE), polystyrene (PSt), poly(methyl methacrylate) (PMMA), polycarbonate (PC), polyurethane (PU), polyacrylonitirile, polyacetals, polyamide, polyglycolate, and some natural polymers (Figure 1.1.1).[5,9]

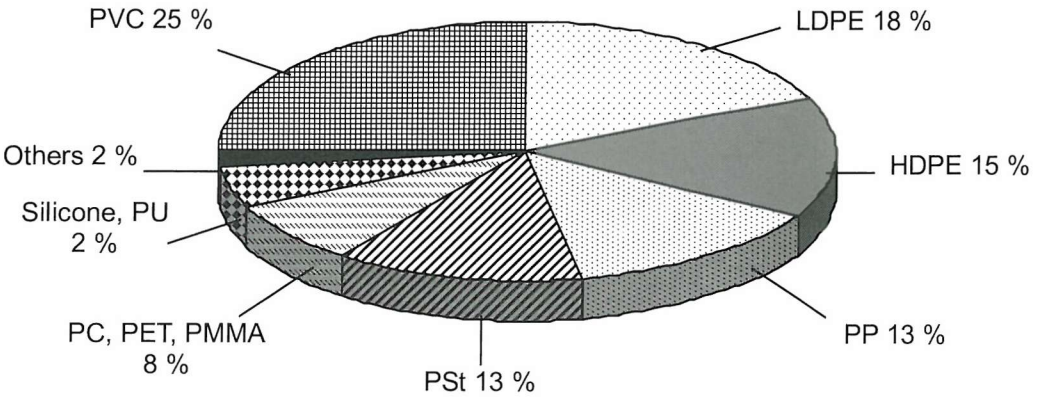


Figure 1.1.1. Weight distribution for blood contacting polymers.[5]

PVC is the most widely used polymer for short-term devices such as extracorporal blood-circulating devices, catheters, and blood bags. Silicone rubber and polyethylene are alternative materials for these applications. Commercially available vascular grafts and cardiac valves are made from polyesters and fluorinated polymers, mainly PET and PTFE. PUs and related polymers such as segmented polyurethanes have also been developed as biomaterials. The types of polymers currently used for the specific devices are shown in Table 1.1.1.

Table 1.1.1. Blood contacting polymers.[5]

Polymer	Device
Cellulose and derivatives	Membranes for dialysis
Cross-linked collagen	Heart valves
Dextran	Plasma expanders
Polyacetal	Heart valves
Polyacrylonitrile	Membranes for dialysis
Polyamides	Catheters, heart valves, membranes for dialysis
Polycarbonates	Syringes, catheters,
Poly(ethylene terephthalate)	Vascular prostheses
Polypropylene	Syringes, catheters,
Polysulfones	Membranes for dialysis
Poly(tetrafluoroethylene)	Vascular prostheses, artificial hearts, catheters
Polyurethanes	Vascular prostheses, artificial hearts, catheters
Poly(viniy chloride)	Blood bags, catheters
Silicones	Catheters, artificial hearts

Despite the large variety of polymers available, many of them are really unsatisfactory. For blood compatible polymers, any adverse reactions occurring on the material interface must be minimal, and require a polymer to interact as a natural material would in the presence of blood. In general, blood compatible polymers should NOT:

- Cause thrombus-formation.
- Destroy or sensitise cellular elements of the blood.
- Alter plasma proteins so as to trigger undesirable reactions.
- Cause adverse immune responses.
- Produce toxic and allergic responses.

So far, there are no ideal blood compatible polymers which totally satisfy these demands for long-term use *in vivo* due to their lack of blood compatibility.[5]

1.1.3 Blood constituents and their functions

Blood represents about 8 % of total body weight and the average volume is 5 litres in women and 5.5 litres in men. Blood has three main functions:

(i) Transport of oxygen, carbon dioxide, foods (glucose, lipids, amino acids, *etc.*), ions (Na^+, Ca^{2+}, HCO_3^-, *etc.*) and wastes (urea, *etc.*).

(ii) Defence against infections and other foreign materials.

(iii) Regulation of temperature.

Blood is a complex liquid plasma in which cellular elements such as erythrocytes, leucocytes, and platelets are suspended, and each component has an important role in the human body.

1.1.3.1 Red blood cells (Erythrocytes)[14,15]

Blood contains about 5 million red blood cells per cubic millimeter (mm^3). Red blood cells are flat, disc-shaped cells indented in the middle on both sides (like a doughnut with a flattened center instead of a hole), they are biconcave discs 8 μm in diameter, 2 μm thick at the outer edges, and 1 μm thick in the center.

Red blood cells are specialised for their primary function of O_2 transport in the blood, and do not contain a nucleus, organelles, or ribosomes but instead they are packed with hemoglobin, an iron–containing molecule that can bind reversibly with O_2, since O_2 is poorly soluble in blood, hemoglobin is indispensable for O_2 transport. Hemoglobin also contributes to CO_2 transport and buffering of blood by reversibly binding with CO_2 and H^+.

1.1.3.2 White blood cells (Leucocytes)[14,15]

White blood cells are much less numerous than red blood cells (the ratio between the two is around 1:700), have a nucleus, and participate in protecting the body from infection. There are five types of leucocytes, each with a different task: (1) Neutrophils are important in engulfing bacteria and debris. (2) Eosinophils specialise in attacking parasitic worms and play a key role in allergic responses. (3) Basophils release two chemicals: histamine, which is important in allergic responses, and heparin, which helps clear fat beads from the blood. (4) Monocytes, upon leaving the blood, set up residence in the tissues, and greatly enlarge to become the large tissue phagocytes known as macrophages. (5) Lymphocytes provide immune defence against bacteria, viruses, and other targets for which they are specifically programmed. Their defence tools include the production of antibodies and cell-mediated immune responses.

All leucocytes have a limited life span and must be replenished by ongoing differentiation and proliferation of precursor cells. The total number and percentage of each of the different types of leucocytes produced vary depending on the momentary defence needs of the body (Table 1.1.2).

Table 1.1.2. Percentage of the different types of leucocytes.

Type of leucocyte	Percentage distribution (%)
Neutrophiles	60 - 70
Eosinophiles	1 - 4
Basophiles	0.25 - 0.5
Monocytes	2 - 6
Lymphocytes	25 - 33

1.1.3.3 Platelets[14,15]

Blood contains about 0.25 million platelets per cubic millimetres (mm^3). Platelets are small cell fragments (about 2 - 4 μm in diameter) with no nucleus. Platelets play an important role in hemostasis, the arrest of bleeding from an injured vessel. The main steps in hemostasis are (1) vascular spasm, (2) platelet plugging, and (3) clot formation. Vascular spasm reduces blood flow thorough an injured vessel, whereas aggregation of platelets at the site of vessel injury quickly plugs the defect. Platelets start to aggregate upon contact with exposed collagen in the damaged vessel wall (Chapter 1.1.4).

1.1.3.4 Plasma[14,15]

Blood plasma is a pale yellow fluid, and the total bodily volume is around 2.5 to 3 litres. Plasma is composed of 90 % water, and serves as a medium for materials being carried in the blood (Table 1.1.3).

Table 1.1.3. Composition of blood plasma.

Constituent	Percentage distribution (%)
Water	~ 92
Protein	6 - 8
Salts	0.8
Lipids	0.6
Glucose (blood suger)	0.1

1.1.3.5 Plasma proteins[14,15]

Plasma proteins are one group of plasma constituents, and these important components normally remain in the plasma, where they perform many valuable functions. There are

three groups of plasma proteins; (i) albumins, (ii) globulins, and (iii) fibrinogen which are classified according to their various physical and chemical properties. Each type of plasma protein performs important specific tasks,

(i) Albumins, the most abundant of the plasma proteins bind many substances (bilirubin, bile salts, and penicillin) for transport though the plasma and contribute most extensively to the colloid osmotic pressure by virtue of their high concentration.

(ii) There are three subclasses of globulins: alpha (α), beta (β), and gamma (γ).

a. Specific α and β-globulins bind and transport a number of substances in the plasma, such as thyroid hormone, cholesterol, and iron.

b. Many of the factors involved in the process of blood clotting are α and β-globulins.

c. Inactive precursor protein molecules, which are activated as needed by specific regulatory inputs, belong to the α-globulin group.

d. The γ-globulins are the immunoglobulins (antibodies), which are crucial to the body defence mechanism.

(iii) Fibrinogen is a key factor in the blood clotting process (Chapter 1.1.4).

1.1.3.6 Lipids[14,15]

Serum lipids in plasma have become important parameters for cardiovascular diseases, because of their relationship to thrombus formation. Lipids are not able to move in body fluids due to their hydrophobic nature so to enable transport in the body they are combined with proteins to form globular micellelike beads, lipoproteins. The lipoproteins consist of a core of hydrophobic lipids surrounded by a shell of polar lipids, which is surrounded by a shell of protein. The proteins that are used in lipid transport are synthesised in the liver, and are called apolipoproteins (Apo A-1, Apo A-2, Apo B-48, Apo C-3, *etc.*).

Five lipoproteins have been classified according to increasing density: chylomicrons, very low density lipoproteins (VLDLs), low density lipoproteins (LDLs), high density lipoproteins (HDLs), and very high density lipoproteins (VHDLs). Since lipids are much less dense than proteins, there is an inverse relationship between the lipid content and density.

Chylomicrons and VLDLs transport both dietary and endogenous triacylglycerols (TAGs) around the body. LDLs and HDLs transport both dietary and endogenous

cholesterol around the body. HDLs and VHDLs transport both dietary and endogenous phospholipids around the body.

Together the lipoproteins maintain a blood lipid concentration of about 500 mg total lipid per 100 mL of blood. Of this 500, 120 mg will be TAG, 220 mg is cholesterol and 160 mg is phospholipid.

1.1.4 Clot formation[5,14]

Occasionally, when polymers come into contact with blood, clot formation can occur. The majority of factors necessary for clotting are always present in the plasma. The blood clotting system is a proteolytic cascade, but one of the most important blood clotting enzymes is thrombin. Thrombin acts on fibrinogen, converting it into the protein fibrin, which is insoluble and forms fibres across the wound, following which blood cells and platelets get caught up in the fibres, forming a clot. The generation of thrombin can be divided into two phases, the intrinsic and extrinsic pathways that provide alternative routes for the generation of factor X (Figure 1.1.2).

The intrinsic pathway is concerned with the contact of a foreign material. The intrinsic pathway requires the clotting factors VIII, IX, X, XI, and XII (also required are the proteins, prekallikrein/kallikrein (Fletcher factor), and high-molecular-weight kininogen) as well as calcium ions, phospholipids, and platelet factor 3 (PF3) secreted from platelets. The intrinsic pathway, which involves seven separate steps, is set off when factor XII (Hageman factor) is activated by coming into contact with exposed collagen when in contact with a foreign surface. This pathway brings about clotting of blood samples on a foreign surface.

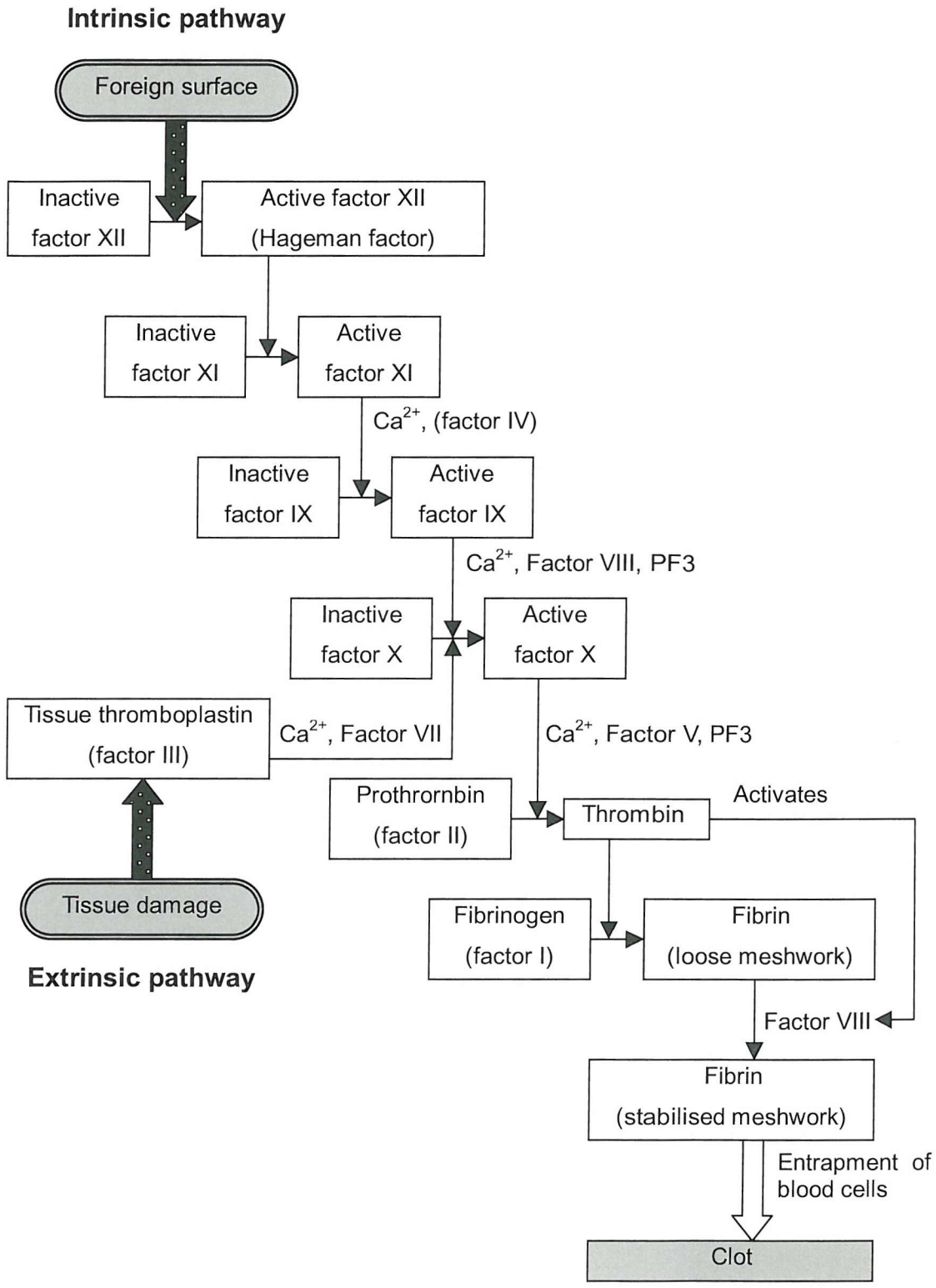


Figure 1.1.2. Clot pathways.

1.1.5 Blood coagulation on polymeric materials

In recent years, it has been shown that adhesion of platelets to materials pre-adsorbed with plasma or fibrinogen or coated with collagen can lead to activation of the procoagulant state of platelets.[5,16,17] Interaction of specific adhesion receptors on the platelet membrane with fibrinogen, collagen, or other adsorbed proteins stimulates various signal transduction pathways that lead to activation of the procoagulant state in adherent platelets. The amount of protein adsorbed and the composition of this protein layer depends on the property of the polymer surface. It is therefore important to understand the correlation between the polymer properties and protein adsorption in order to control blood coagulation.

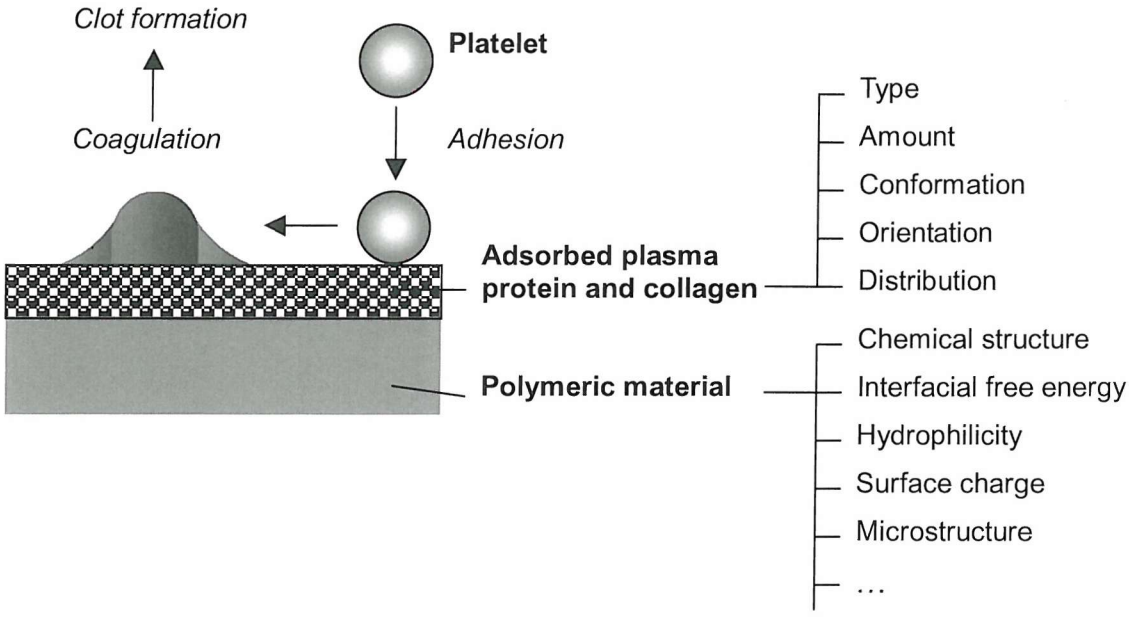


Figure 1.1.3. Mechanism and parameters of protein adsorption onto polymeric materials.

1.1.6 Polymer designs for blood compatible materials

The following section presents an overview of the concepts to control protein adsorption onto synthetic polymeric materials, showing there are several essential factors that can improve blood compatibility.

1.1.6.1 Interfacial free energy

Highly hydrophilic polymers show excellent blood compatibility. One possible

explanation of highly hydrophilic polymer's passivity may involve its minimum interfacial free energy with water. The basic concept of the minimal interfacial free-energy hypothesis is that as the interfacial free energy approaches zero, the driving force for protein adsorption decreases. Therefore, non-specific protein adsorption should not occur.

Hydrated cross-linked polymers, so-called hydrogels are well known examples of one of the most widely used hydrophilic polymers for medical use.[18-20] Hydrogels in tissue engineering must meet a number of design criteria to function appropriately and promote tissue formation. The interaction of cells with hydrogels will significantly affect their adhesion as well as migration and differentiation. The adhesion may be cell-type specific and is dependent on the interaction of specific cell receptors with ligands, that are a component of or adsorbed onto the materials. One of the most studied synthetic hydrogels is cross-linked poly(2-hydroxyethyl methacrylate) (PHEMA).[21-23] PHEMA gel has a high water content at equilibrium and exhibits rubbery type behaviour and good biocompatibility, thus resembling natural tissues more closely than other synthetic materials. The permeability and hydrophilicity of these gels are dependent on the cross-linking agents[24] and amounts[25]. Other synthetic hydrogels such as poly(*N*-isopropyl acrylamide) (PNIPAAm),[18,23,26] poly(*N,N*-dimethyl acrylamide) (PDMAAm),[23,26,27] poly(vinyl alcohol) (PVA),[28-30] poly(*N*-vinyl-2-pyrrolidone) (PVP),[31] and their derivatives have also been used for blood contact applications.

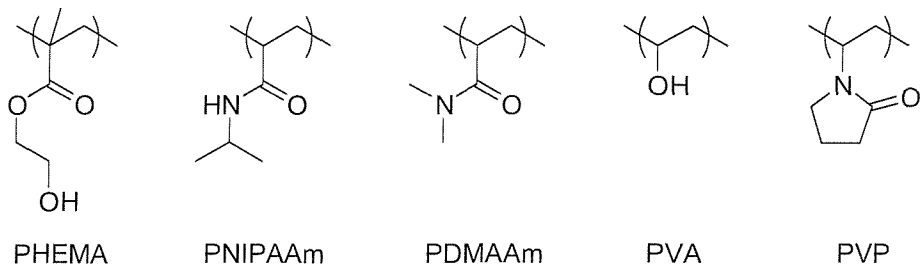


Figure 1.1.4. Several types of hydrogels.

1.1.6.2 Flexibility in water

Poly(ethylene oxide) (PEO) has been approved by the Food and Drug Administration (FDA) for several medical applications due to its biocompatibility and low toxicity.[18,32-40] PEO is also a highly hydrophilic polymer and shows complete water solubility,[33] because PEO does not have bulky side groups in its structure and its

segments nicely fit into the water structure. The hydrophilicity of PEO produces specific surfaces that are in a liquid-like state with the polymer chains exhibiting considerable flexibility and mobility.

Plasma protein adsorption and platelet adhesion on PEO gradient surfaces are gradually reduced by increasing PEO chain length and PEO surface density.[32,33,35] Rapidly moving hydrated PEO chains on a surface will effectively prevent stagnation of the proteins on the surface, probably because the contact time is shortened (Figure 1.1.5 (A)). The mobility of the hydrated PEO chains increases with chain length, and also the long PEO chains suppress the adsorption of proteins more effectively than shorter chains. It appears that PEO chains have a large excluded volume in water and tend to repel protein molecules which approach the surface (Figure 1.1.5 (B)).[33,41] The "repulsive forces" of the adsorbed PEO chains are generated by the loss of possible chain conformation (loss of entropy), as the volume available to the adsorbed chains is reduced between approaching surfaces.

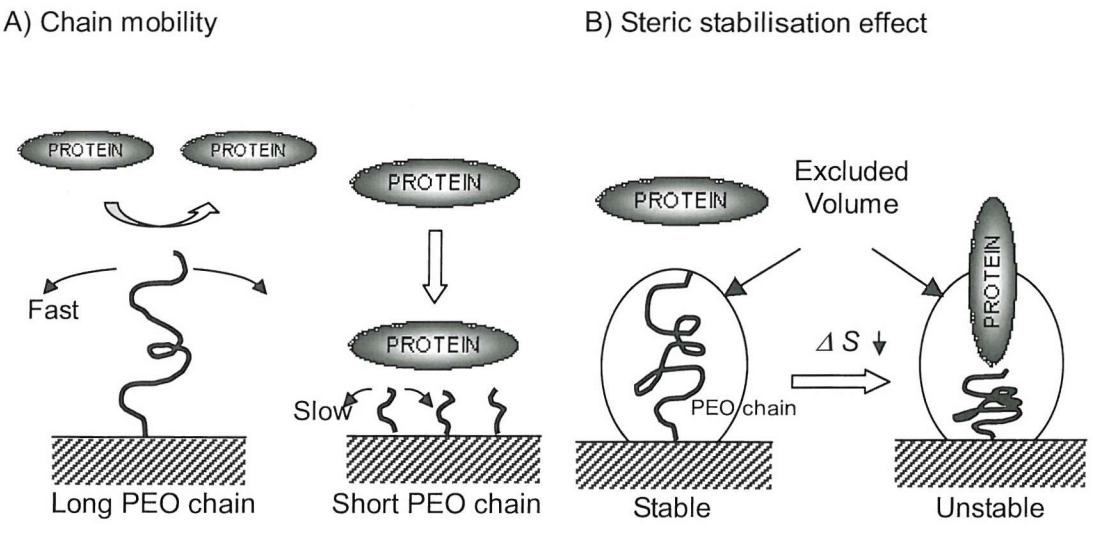


Figure 1.1.5. Basic mechanisms involved in protein accessibility of PEO surfaces.

1.1.6.3 Water in polymers

It has been reported recently by Tanaka and his co-workers that poly(2-methoxyethyl acrylate) (PMEA) shows blood compatibility,[42-47] by suppressing platelet adhesion and spreading. The amount of protein adsorbed onto PMEA was very low and was similar to that adsorbed onto PHEMA, which was used as a reference.

Circular dichroism (CD) was applied to investigate changes in the structure of the

proteins after adsorption onto the polymer surface, and it was found that the conformation of the proteins adsorbed onto PHEMA varied considerably, but that the proteins adsorbed onto PMEA differed little compared to the native state. These results suggest that low platelet adhesion and spreading are closely related to the low degree of denaturation of the protein adsorbed onto PMEA.

The reason why PMEA shows excellent blood compatibility was investigated by looking at the structure of water in the hydrated polymer.[43,46-49] Water in the polymer can be classified into three types: (i) non-freezing water, (ii) freezing bound water, and (iii) free water, which can be characterised by differential scanning calorimetry (DSC). PMEA had a significant amount of freezing bound water, suggesting the amount of the "freezing bound water" related to the platelet compatibility of the polymer.

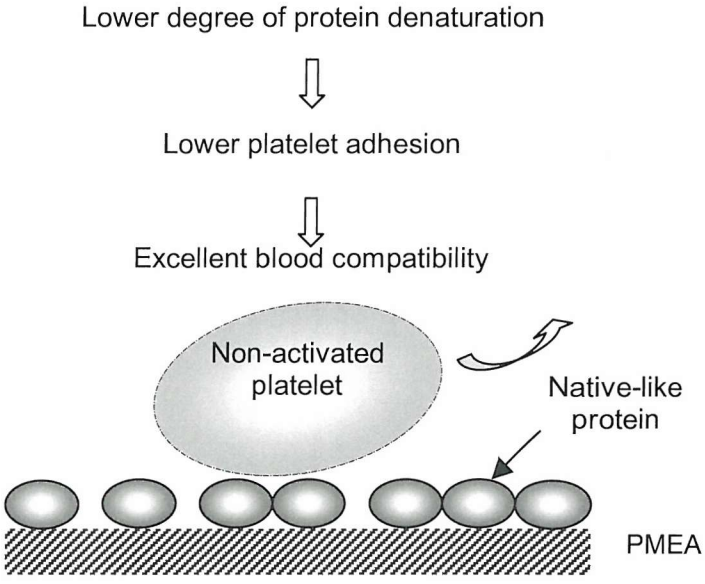


Figure 1.1.6. Schematic representation of the assumed adsorption state of Fibrinogen and platelet adhesion onto PMEA.

1.1.6.4 Micro-domain structure

One of the rational beliefs of blood compatible materials is the generation of a balanced distribution of hydrophilic and hydrophobic moieties of defined dimensions on the polymer surface,[50-52] due to the fact that cells and proteins have a number of surface domains of hydrophobic,[50,53] charged and polar character.[54] The control of the micro-domains, particularly hydrophilic/hydrophobic properties on a polymer surface is a useful approach for the development of blood compatible materials.

Two approaches to obtain micro-domain structures on the polymer surface are well known, co-polymerisation and polymer blending. In the case of co-polymerisation, polyurethanes (PUs) are widely used for the manufacture of conventional blood contacting devices.[51,55-57] PUs are multi-block copolymers usually consisting of hard segments and polyether or polyester soft segments to form the micro-domain structures. Much research[51,55-57] has been reported concerning the effects of the surface composition of PUs, their protein adsorption and platelet adhesion.

Recently it was reported by Okano that HEMA-styrene (St) block copolymers showed good blood compatibility owing to inhibition of platelet activation on the micro-domain surfaces.[58,59] Contacting platelets could be observed moving including rolling, detachment, oscillatory vibration, and change of direction on the HEMA-St block copolymer surface without activation. When a polymer surface is in contact with blood, the surface is rapidly covered with plasma proteins before interaction with platelets. On the HEMA-St block polymer-cast surface with hydrophilic-hydrophobic micro-domains, serum albumin was selectively adsorbed onto the hydrophilic micro-domains, whereas γ-globulin and fibrinogen were selectively adsorbed onto the hydrophobic micro-domains. The adsorbed blood proteins formed an organized structure regulated by the hydrophilic-hydrophobic micro-domain surface structure. Once platelets were in contact with the HEMA-St block polymer-cast surfaces, they passively adhered onto the surface but immediately unstuck from the surface. Platelet detachment from the surface was suggested to be the underlying mechanism for the non-thrombogenicity of HEMA-St block polymer-cast surfaces.[59]

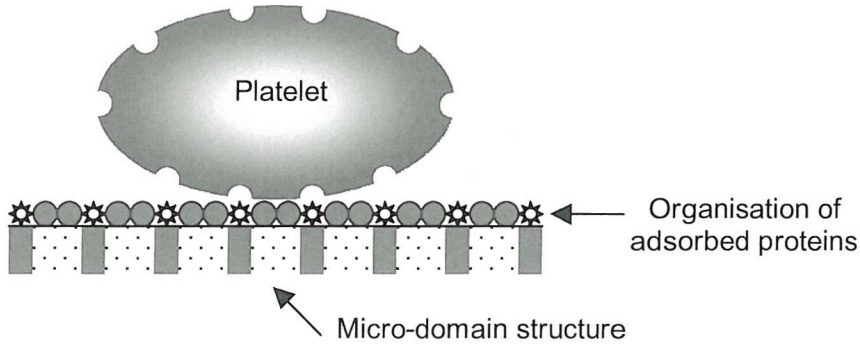


Figure 1.1.7. Schematic representation of platelet adhesion onto micro-domain surface.

Polymer blending for medical devices can be achieved to combine the properties of different polymers.[50,60] Hydrophilic polyamide membranes can be obtained by mixing

an aliphatic/aromatic polyamide (PA) with PVP, which is a hydrophilic polymer. (Hydrogen bonding between PVP and PA lead to good miscibility between the two polymers). Small hydrophilic patches in the 20 – 200 nm range are formed in the hydrophilic matrix, and this micro-domain structure is associated with blood compatibility.[50] The hydrophilic micro-domains significantly suppress the formation of thrombin, which is concerned with clot formation, compared with the hydrophobic polyamides without this micro-heterogeneity.

1.1.6.5 Negative charge

The deposition of the cells to a surface depends on the presence and the height of the energy barrier induced by several interactions such as ionic (electrostatic) interaction and *van der Waals* force between the cells and the surfaces (Chapter 1.1.7).[61,62] Most cells are negatively charged because of anionic groups, particularly phosphate and carboxylic acid groups. Therefore, materials that include negatively charged groups tend to be antithrombogenic, whereas positively charged surfaces are thrombogenic. Several investigations have introduced sulfonate and carboxylate groups into polymers for the improvement of blood compatibility.[61,63-72]

1.1.6.6 Biomembrane mimic systems

Biomembranes play an important role in a range of biological processes such as ion-transport, and lipids and protein are principal components of biomembranes.[15] There is a wide variation in the types of lipids and proteins as well as in their ratios. Phospholipids, the major lipid components of most biomembranes, are composed of glycerol bonded to two fatty acids and a phosphate group. The resulting phosphatidic acid contains the fatty acid components that are fat-soluble along with the charged phosphate groups that are water-soluble. Many phospholipids also have additional functional groups, such as choline, bound to the phosphate, and their bipolar character of phospholipids is essential for their biological function in cell membranes. The phospholipids of the cell membrane form into sheets two molecules thick with the fat-soluble portions inside shielded on both sides by the water-soluble portions.

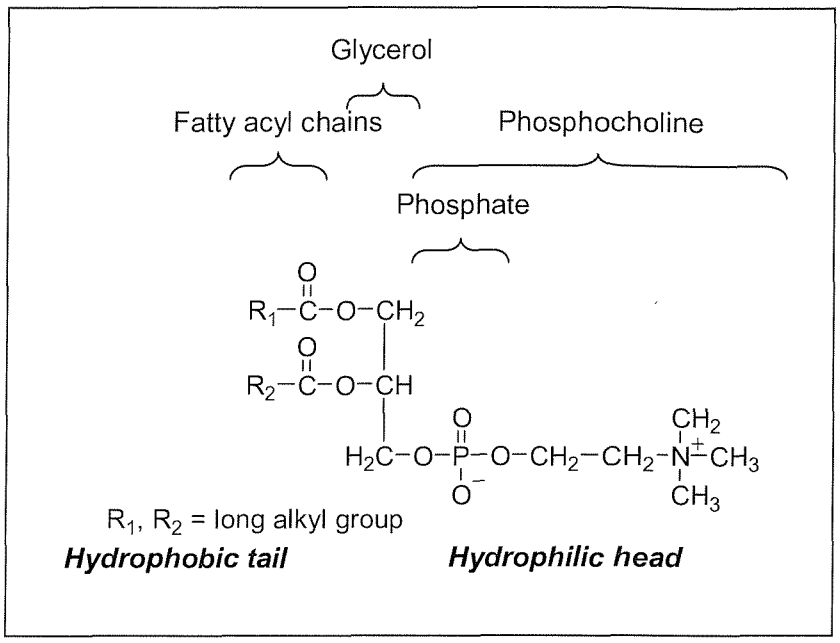


Figure 1.1.8. Structure of phospholipid.

Recently polymers containing phospholipid groups have been shown to have excellent blood compatibility.[9,73-83] (Ishihara synthesised a polymer with a phospholipid polar group). 2-methacryloyloxyethyl phosphocholine (MPC) and hydrophobic methacrylate monomers were polymerised by free radical polymerisation and applied in a number of intravascular applications[9,73,74,77,84-88] (Figure 1.1.9).

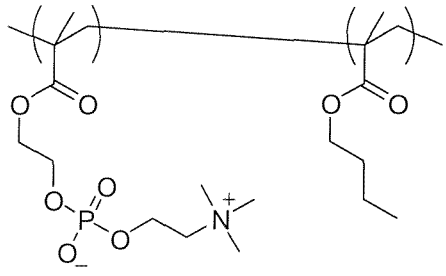


Figure 1.1.9. Chemical structure of poly(MPC-*co*-*n*-butyl methacrylate (BMA)).

The MPC copolymer generated a "biomembrane-like surface" when in contact with plasma and showed strong affinity to natural phospholipids. The surface could suppress protein adsorption and subsequent platelet adhesion and activation.[76,77,81]

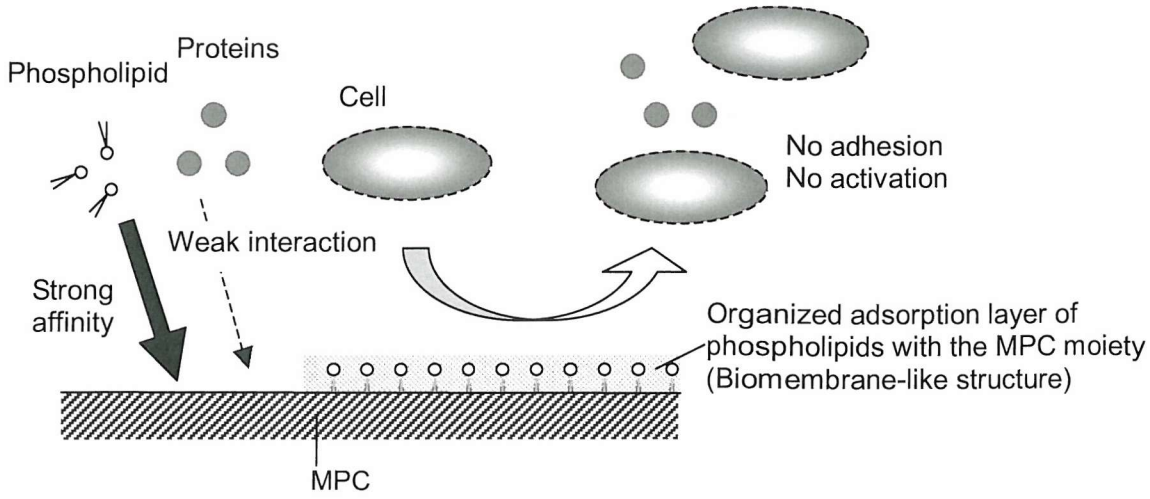


Figure 1.1.10. Schematic of interaction between blood components and MPC polymer surface.[76,77]

1.1.7 Interaction forces

When looking at the interaction between polymers and blood components, it is essential to understand the phenomena of intermolecular forces, because protein and cell adhesions onto polymer are caused by various forces (Table 1.1.4),[5,62,89] with several forces combining to produce significant interactions between the polymer and the blood components. The following forces are fundamental intermolecular interactions, all potentially important in biocompatibility.

Table 1.1.4. Interaction forces and the potential energies.[62]

Interaction type	Distance dependence of potential energy	Typical energy (kJ/mol)	Comment
Ion-ion	$1/r$	250	Only between ions
Ion-dipole	$1/r^2$	15	-
Dipole-dipole	$1/r^3$	2	Between polar molecules
Dipole-induced dipole	$1/r^6$	0.6	-
Induced dipole-induced dipole	$1/r^6$	2	Between all types of molecules

The energy of a hydrogen bond A-H…B is typically 20 kJ/mol and occurs on contact for A, B = N, O, or F.

1.1.7.1 Ionic (electrostatic) interactions[5,62]

Ionic bonds are formed by interaction between two oppositely charged species. However, ions are extensively solvated in solution, therefore, ionic interactions are relatively weak in an aqueous environment. The proteins consist of individual amino acids, and at physiological pH (pH 7.4) the protein the amino end carries a positive charge ($-NH_3^+$) and the carboxyl end carries a negative charge ($-CO_2^-$), which are solvated and neutralized by counterions of the salts present in the solution. There is potential to form the ionic interactions between proteins and polymeric materials, however this is complicated by desolvation and entropic terms.

1.1.7.2 Interactions between dipoles and hydrogen bonds[5,62]

Polar molecules with the bonds involving carbon linked to polar atoms such as O, N, S, Cl, and F can interact with other polar or ionic molecules. Even for non-polar molecules, the electron density can be polarised by neighbouring charged groups, and the induced dipole moment can interact with other molecules. These interactions are very dependent upon the orientation and strength of the dipole moment.

Hydrogen bonds are considered as the specific dipole-dipole interactions between the hydrogen attached to an electronegative atom of one molecule and an electronegative atom of a different molecule (Hydrogen bond, A-H...B, A, B = O, N, S, Cl, or F).

Dipole-dipole interactions are very important in protein research for a number of reasons. The chemical structure of proteins contain multiple peptide bonds, -CO-NH-, which tend to form hydrogen bonds, and the properties of proteins in water are directly related to its ability to form strong three dimensional hydrogen bonds with water or other proteins.

1.1.7.3 Hydrophobic interactions[62]

Proteins contain many hydrophobic groups that tend to spontaneously associate away from the aqueous environment, because strong interactions between water and hydrophobic groups of proteins are not possible. Therefore, each individual protein is surrounded by water molecules, and the water molecules disorder as they to attempt to associate with each other to maintain a maximal number of energetically favourable hydrogen bonds. Especially around hydrophobic patches, this increase in disorder is an increase in entropy and leads to a negative Gibbs free energy. As a result, the association of hydrophobic groups occurs the so-called hydrophobic effect.

1.1.7.4 Charge transfer complexes[62]

When a good electron donor comes into contact with a good electron acceptor, the donor might transfer some of its charge to the acceptor. The potential energy of this complex is proportional to the difference between the ionisation potential of the donor and the electron affinity of the acceptor. Electron donors contain π-electron such as alkenes, alkynes, and aromatic compounds, or groups with a pair of non-bonded electrons such as O, N, and S atoms. The acceptor groups contain available electron deficient π*-orbital such as alkenes, alkynes, and aromatic compounds with electron-withdrawing substituents.

1.1.8 From biocompatibility to biofunctionality

The term "biomaterial" is generally applied to an active material, used for a medical device intended to interact with biological systems. Therefore, the main objective in a biomaterial investigation is to understand the relationship between the biomaterial and the response it may cause with the components of the biological system, with the final aim of promoting the desirable and eliminating untoward effects during the development of new materials. Furthermore, nowadays biomaterials with specific responses under biological conditions are desired for various applications.

1.1.9 Protein purification

The development of techniques and methods for purification have been essential for much progress in biotechnology.[90,91] Protein purification varies from simple one step precipitation procedures to large scale validated production processes.[78,92-95] Most purification methods involve some forms of chromatography, which has become an essential tool in every laboratory where protein purification is needed. Different chromatography techniques with different selectivities can form powerful combinations for the purification biomolecules.

Table 1.1.5. Purification technique.

Type	Essential factor
Ion exchange chromatography	Charge
Hydrophobic interation chromatography	Hydrophobicity
Affinity chromatography	Reversible interaction between a protein and a specific ligands attached to a chromatography matrix
Gel filtration	Molecular size

However, there are still major problems such as selectivity and stability of the target protein and speed though the chromatography and so on. Therefore, materials which have selective interactions with proteins are desirable to improve protein purification.

1.1.10 Leucocyte depletion

In recent years it has become apparent that leucocytes in transfused blood are unnecessary in most of cases.[96-98] Leucocytes in the blood have also been found to cause non-hemolytic febrile reactions and alloimmunisation as well as to harbor viruses. The use of leuco-depleted blood minimises some of these adverse side effects.

Leucocyte removal from the blood has conventionally been conducted by centrifugation.[99] However this process has disadvantages such as incomplete leucocyte removal, damage of cells and proteins, expensive apparatus, and low throughput.

Filtration process which remove leucocytes by leucocyte adhesion onto fibers[98-100] are commercial available and consist of filters of cellulose acetate, cotton wool, polyester fibers, and polyurethane micro-porous materials. The filtration process has advantages in that leucocyte removal efficiency is higher than other methods, and the operation required is simple and can generally be performed at low cost. However, improvements are required in the selectivity and efficiency of blood filtration, and new polymeric materials were investigated in this PhD for blood filtration. It was thus important to establish the relationship between cell binding and the polymeric surface.

1.2 Combinatorial chemistry in polymer research

1.2.1 General

The combinatorial approach has been hugely successful in speeding up the process of drug discovery. In recent years, this concept has spread into a wide range of fields, such as material science, biotechnology and catalyst design.[101-103]

Interest in combinatorial strategies has been due to the following reasons; (i) fast and efficient improvements of existing processes and procedures, (ii) economics of industrial research, and (iii) introduction of numerous new technologies for high throughput screening (HTS). Combinatorial and high throughput methodologies are recognised as breakthrough technologies in various fields, and the development of suitable high throughput technologies will lead to new discoveries.

1.2.2 Material discovery using combinatorial methods

Research for the development of new materials is progressed by a cyclic process,[104,105] and the process flow of a material-discovery cycle is illustrated in Figure 1.2.1. Combinatorial efforts tend to be based on the parallel synthesis and HTS of large number of components with both technologies conducted on small scale and by automated processes. Nowadays a variety of technologies have been developed for the synthesis of compound libraries, and this progress has also had an influence on instrumentation development. Specially designed parallel reactors combined auto-samplers and liquid handling robotic systems are commercially available (Chapter 1.2.4).

HTS is the process for the rapid automated assessment of single or multiple properties of a large number of samples within a combinatorial library. A list of such material properties can include chemical, biological, thermal, optical, mechanical, and many others, and a variety of high throughput analytical tools are required for material analysis. The performance-testing steps in material-discovery cycles are also specific screenings in that they must simulate the end-use application. These data obtained in testing steps and by general analysis methods lead to the interpretation and identify the tendency and multidimensional relationships of the data.

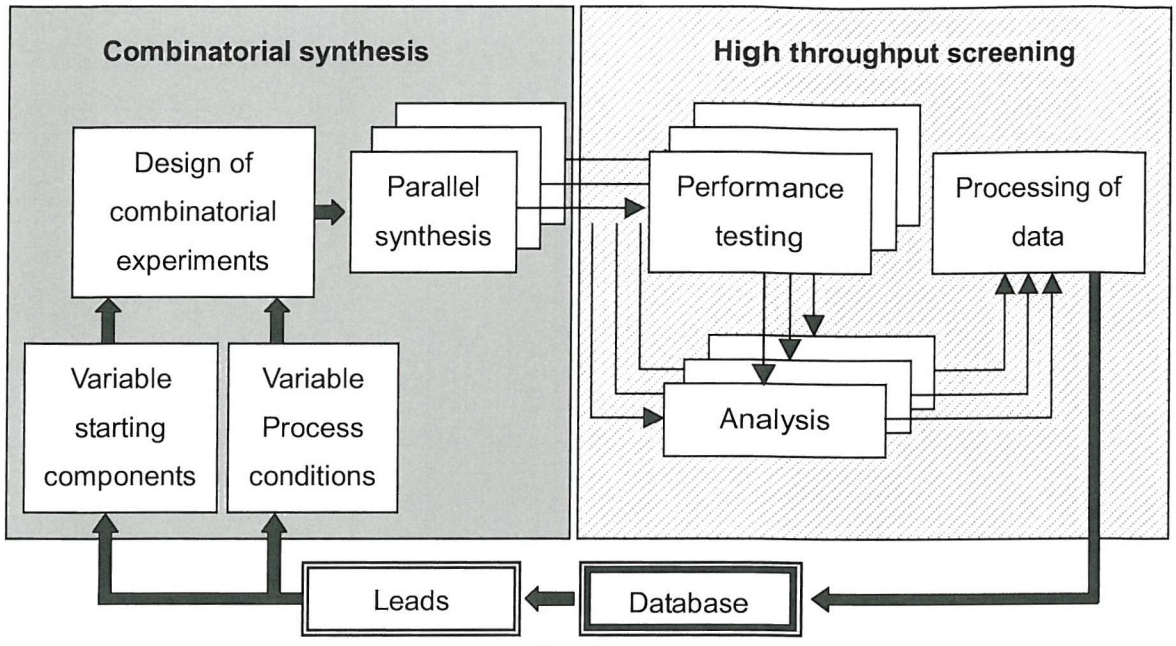


Figure 1.2.1. Combinatorial synthesis and HTS discovery cycle.[105]

In order to determine the property of materials, mathematical and statistical chemometric techniques are used in HTS, because it is not easy to interpret the large volumes of data generated by HTS directly. In the field of combinatorial chemistry, chemometrics has been successfully applied in a number of ways, for example via pattern-recognition and visualisation which with statistical design process can lead to identification and optimisation.[106] These techniques find similarities and differences between samples in libraries and serve as an efficient visualisation tool to identify the occurrence of trends or irregularities in the data sets.

The effective design of combinatorial experiments can lead to a more productive exploration of the variable compositions, processes, and performance space to generate new designs for building predictive models of materials performance.

1.2.3 Combinatorial approach for bio-polymers

Generally polymers are generated from monomers by polymerisation reactions where all the bond forming reactions are conducted in a single pot. This leads to high molecular weight and structural variation, and it is generally not possible to characterise the structure of each specific polymer molecule. Nevertheless, polymers must meet strict regulatory requirements for each application, especially bio-polymers. Many critical issues relating to toxicology, metabolic fate and immune response, need to be

applied to biomedical polymer research,[107] and traditionally, it has taken a long time to develop a new polymeric material using conventional methods. Recently several combinatorial approaches have been applied to the development of biocompatible polymers.[107-109]

1.2.4 Parallel synthesis of polymers

Theoretically, automated synthesisers that are utilized for organic synthesis can be considered as suitable for polymerisation reactions. However, most of the equipment has been developed for the synthesis of peptides, the reaction vessels of the workstations are based on a well-plate format made of polystyrene, polypropylene, or Teflon with limited reaction volume, and the systems are furthermore often not suitable for performing reactions under reflux conditions.

Most polymers are solid or rubbery, and the polymer solutions are usually highly viscous. Some polymer synthesis techniques such as free radical and ionic polymerisations must be carried out under inert atmospheres. It is therefore very hard to handle these materials, and only very recently has equipment become commercially available for this purpose.[108,110,111]

Some multi-reactors developed by *Radleys* are suitable for polymer synthesis. The *Radleys Carousel Reaction Stations*™ are capable of performing twelve parallel reactions in tubes varying from 3 to 25 mL with temperatures up to 160 °C. Refluxing can be performed with the aid of standard water-cooling, and it is possible to work under an inert atmosphere and under vacuum. *Mets Syn*[10] *Reaction Stations*™ are further developed versions, which can control the reaction temperature and individual stirring within each reaction vessel under an inert atmosphere, but are a simple advance of established systems.

There are some automated synthesisers suitable for polymerisation. The workstation *Argonaut Quest 205/210* designed by *Argonaut Technologies* integrates multi-step solution phase synthesis, product work-up/purification, and product collection. The instrument can maintain an inert environment, control the temperature, and agitate polymer solution. The reaction station is suitable for performing the polymerisation in a parallel way.

The *Chemspeed ASW1000/2000* workstation is one automated system available commercially. A maximum of up to 112 parallel reactions can be performed with controlled temperature and automated liquid/liquid extraction. An inert atmosphere and

the availability of a glove-box unit enable to handle highly sensitive reagents. Particularly for polymer synthesis, high-viscosity stirrers with spiral blades are available as an option. However, with all type systems, polymer work-up needs to be performed in a serial manner due to the uniqueness of each polymer.

1.2.5 High throughput screening (HTS) in bio-polymer research

Polymer structure-property correlations need to be optimised in material research. Polymer analysis needs specific techniques for the determination of chemical composition, molecular weight, polydispersity, and thermal properties such as glass transition temperature (Tg), melting point (Tm), and crystallisation point (Tc). For biocompatible polymers, it is important to investigate wettability[112,113] and cellular response,[109,114,115] *etc.* Many HTS techniques have been developed for polymer investigations.[108,110]

1.2.6 Thermal analysis of polymers

In polymer research, it is important to determine the melting point (Tm) and the glass transition temperature (Tg). Tm is a transition which happens to crystalline polymers, and melting is observed when the polymer chains fall out of their crystal structures, and become disordered liquids. Tg is a transition which occurs in amorphous polymers whose chains are not arranged in ordered crystals. As the temperature of a polymer drops below Tg, it behaves in an increasingly brittle manner. As the temperature rises above the Tg, the conformation of individual C-C bond can be changed and the polymer becomes more rubber-like.

The investigation of these thermal properties leads to control of the mechanical strength and mobility. Specially for biopolymers, flexibility in water might have an effect on protein and cell adsorption.[33,41,116]

Thermal properties can be analysed by various techniques. The most common techniques are differential scanning calorimetry (DSC), thermal gravimetric analysis (TG), dynamic mechanical analysis, and also application-oriented specialized tests such as heat deflection and melt index.[117,118]

DSC is essentially a technique which compares the difference between the energy input into a substance and a reference (or blank) as function of temperature while both the reference and the sample are subject to a controlled temperature rise (Figure 1.2.3), and the difference in the heat flow between the sample and reference is measured.

Recently, a high performance DSC which can measure at a very high, controlled temperature including constant cooling and heating rates of hundreds of degrees per minute, was reported by Mathot.[119,120] The high performance DSC could reduce the measurement time dramatically.

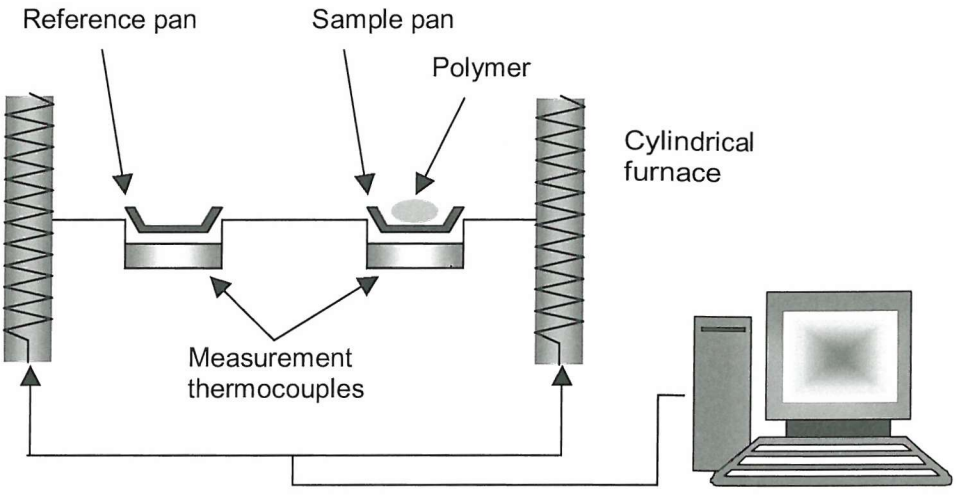


Figure 1.2.3. Differential scanning calorimetry (DSC).

1.2.7 Analysis of protein adsorption onto polymeric materials

1.2.7.1 General

In order to completely characterize and predict protein adsorption, information about adsorption isotherms, adsorption kinetics, conformation of adsorbed proteins, number and character of surface bound protein segments, and the physical parameters describing the adsorbed protein layer are examined. This information can be obtained using a number of different experimental techniques. When combined, such information can answer questions about the mechanism of protein adsorption and desorption from surfaces.

Conventionally, the amount of protein adsorbed onto a surface can be determined by a relative high throughput method, an enzyme linked immunosorbent assay (ELISA),[121] which is an immunologic assay to determine solid phase bound proteins based on the interactions between antigen (protein) and specific antibodies.

Newer methods of surface analysis have been developed in the field of physics and microelectronics. X-ray photoelectron spectroscopy (XPS),[122,123] time of flight secondary ion mass spectrometry (TOF-SIMS),[123] infrared (IR) spectroscopy,[47] ellipsometry,[124,125] scanning force microscopy (SFM),[126,127] and atomic force microscopy (AFM)[128,129] are suited to the analysis of surface structures and biological

interactions. Scanning electron microscopy (SEM) studies have also been carried out on the samples in order to investigate activation of platelets, fibrin clots, *etc.*[42,44,65,81]

1.2.7.2 Flow cytometry (FCM)

Flow cytometry (FCM) is a powerful HTS tool used in many applications including the isolation of bioactive molecules from synthetic combinatorial libraries.[130-133] In principle, when fluorescently labelled beads are allowed to flow rapidly through the instrument they are excited by a focused laser beam.[132] Up to 100,000 beads per second can be discriminated in modern sorters, although initially this technique was developed for the separation and identification of specific types of cells from heterogeneous populations on the basis of the size and the colour of fluorescent antibodies.

Nowadays, flow cytometry using fluorescence activated cell sorters are widely used in many branches of research, although most applications are within the medical sciences.[130,134-136] Only during the last decade has flow cytometry been applied in the areas of protein engineering, and the expanded use of flow cytometry as a HTS tool has stimulated the development of fluorescent probes and the growing applications of combinatorial libraries.

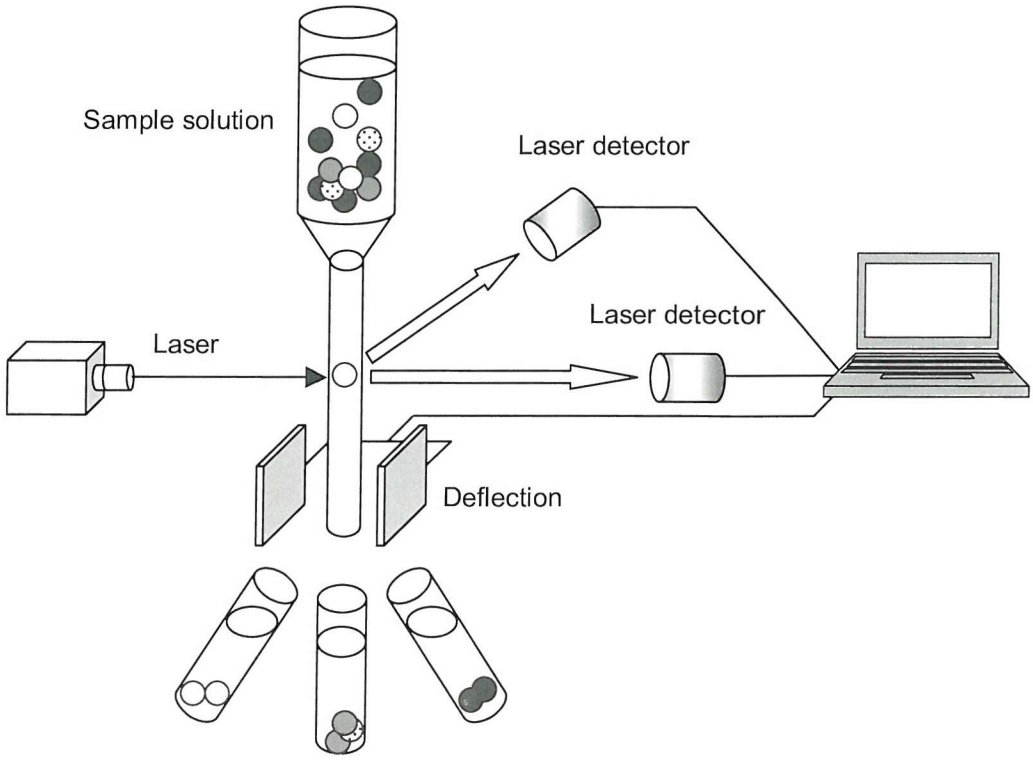


Figure 1.2.4. Principle of flow cytometry with fluorescence activated cell sorter (FACS).

1.2.7.3 Microarrays

Recently microarray technology has been developed as a screening method in biology.[137-139] Initially microarrays were developed for gene expression studies, for example to compare gene expression profiles between different tissue types, different treatments, in different disease models, and in human samples. With advances in microdissection techniques and the development of mRNA amplification protocols, it has become possible to identify gene expression patterns in neighbouring cells.[140,141]

Using the same basic principles, microarray applications are being designed for re-sequencing and mutational analysis for finding DNA copy number variations[142,143] and using protein microarrays to detect protein-protein interactions.[144-146]

As an example the general production of a protein and antibody microarray is shown in Figure 1.2.5. Microarrays were generated and processed in several steps.[146] First, either chemical groups or gels are attached to the microscope slide (commonly a glass slide or nylon membrane) to accommodate the reagents, and than the reagents are applied to the coated microscope slide by robotic spotting. Microarray formats provide a method for simultaneously probing thousands of bimolecular interactions using extremely small sample volumes (at a density of ~ 1,000 spots per square centimetre).[145,147] After processing the microarray is incubated with the fluorescently labelled sample, and the binding events are detected by confocal scanning in a laser scanner. The resulting signals are quantified and analysis performed.[148]

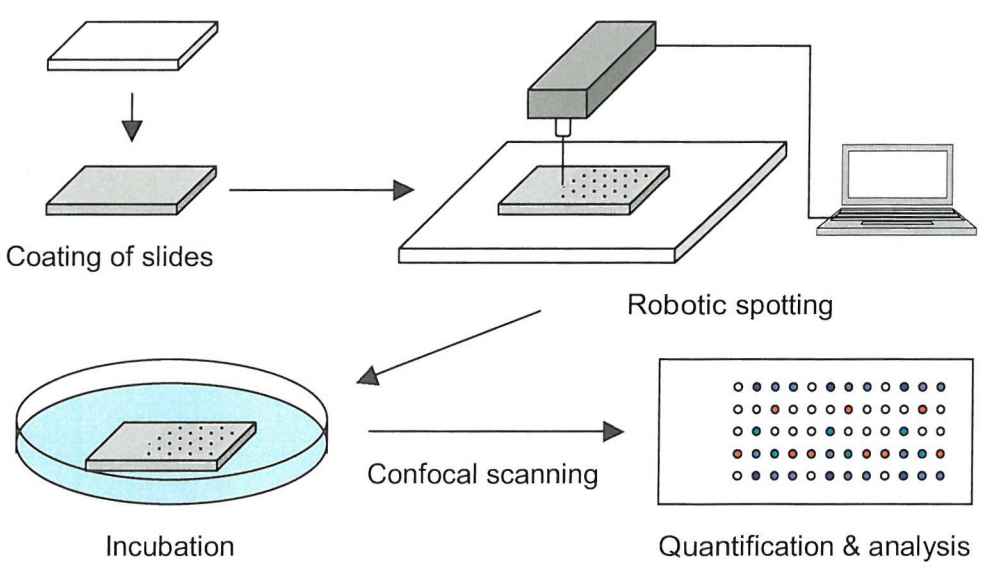


Figure 1.2.5. General production of protein and antibody microarray.

1.3 Aims for this thesis

A number of polymers are widely used in medical applications. Although many have excellent physical and mechanical properties, none is ideal for long-term use *in vivo* due to their lack of biocompatibility. When polymers come into contact with blood, cellular components are rapidly adsorbed, and it is therefore important to investigate the mechanism of binding between biocompatible polymers and cellular components, as such information is essential to aid the design of new blood compatible biomaterials. However blood is a complicated system, and numerous parameters are concerned with the cellular response to polymeric materials. In order to achieve the development of polymers with high biocompatibility, a large number of polymers have to be synthesised and screened.

The aims for this thesis were as follows;

(1) Synthesis of a "blood compatible" polymer library.

(2) Development of high throughput polymer synthesis.

(3) Establishment of high throughput screening methods to investigate blood compatibility.

(4) Investigation of the interactions between polymeric materials and cellular components.

(5) Development of novel biofunctional polymers with high leuco-depletion abilities.

Chapter 2

Synthesis of polymer libraries

2.1 Introduction

There are several techniques for polymer synthesis (Table 2.1.1),[117,118] but above all, free radical polymerisation is the most useful for the synthesis of polymer libraries, because a great number of monomers are applicable and available for radical polymerisation than other polymerisation methods. For example, ionic polymerisations are incompatible with many of the commercially available monomers that have versatile functional groups which might play an important role in biocompatibility. Therefore, a much greater diversity of polymers may be obtained through free radical polymerisation, and polymers based on a multitude of monomers can be readily achieved.

Table 2.1.1. Polymerisation techniques.[117,118]

Polymerisation type	
Chain polymerisation	Radical polymerisation
	Cationic polymerisation
	Anionic polymerisation
	Coordination polymerization
Step-growth polymerisaiton	Polycondensation
	Polyaddition
Ring opening polymerisation	

In this research project, using free radical polymerisation, polymer libraries with various functional monomers were synthesised for investigation of biocompatibility and leuco-depletion.

2.2 Optimisation of radical polymerisation

2.2.1 The theory of radical polymerisation[117,118]

In order to study the effects of various polymer structures on biocompatibility, polymers should be synthesised in high purity and yield, while molecular weight should be controlled. Polymerisation conditions have to be optimised to accomplish these objectives. Theoretically polymer length, *DP* is calculated from Equation 2-1.

$$DP = k_p[M]/2(fk_dk_t)^{1/2}[I]^{1/2}. \quad (2\text{-}1)$$

(Where k_d, k_p, and k_t define the rate constants of initiator decomposition, propagation, and termination, and f defines the efficiency factor, and [I] and [M] define initiator and monomer concentrations respectively). The variation with temperature, T of each of the rate constants, k_d, k_p, and k_t, is expected to be expressed by an *Arrhenius* type equation involving apparent activation energies, E_d, E_p, and E_t, respectively (*e.g.* $k_d = A_d \exp(-E_d/RT)$, where A defines frequency factor, and R defines the molar gas constant). Since the temperature term is represented in Equation 2-2, the value $(E_p - E_t/2) - E_d/2$ will normally be negative so that the polymerisation rate will increase as the temperature is raised.

$$d \ln DP/dT = [(E_p - E_t/2) - E_d/2]/RT^2 \quad (2\text{-}2)$$

Polymer length is associated with temperature, concentration of initiator, and monomer, and inevitably, because of the random nature of the growth process, the product is a mixture of chains of different length, and the polymer is characterised by a molar mass distribution and molar mass average, rather than by a single molar mass. A number average molar mass $<M>_n$ is defined by

$$<M>_n = \Sigma N_i M_i/\Sigma N_i \quad (2\text{-}3)$$

where N_i is the number of molecules of species i of molar mass M_i. The bracket < > indicates that it is average value, but by convention these are normally omitted. A weight average molar mass $<M>_w$ is defined as

$$<M>_w = \Sigma N_i M_i^2/\Sigma N_i M_i \quad (2\text{-}4)$$

The ratio $<M>_w/<M>_n$ is termed the polydispersity index, MWD. The value of MWD increases as the distribution of relative mass of molecules in the samples becomes broader.

2.2.2 Methods for the synthesis and characterisation of polymers

Polymerisations in these studies were carried out with up to twelve parallel reactions, in a *Radleys Carousel Reaction Station*^{TM} which can work with water-cooling and under nitrogen (Chapter 1.2.4 and Figure 2.2.1).

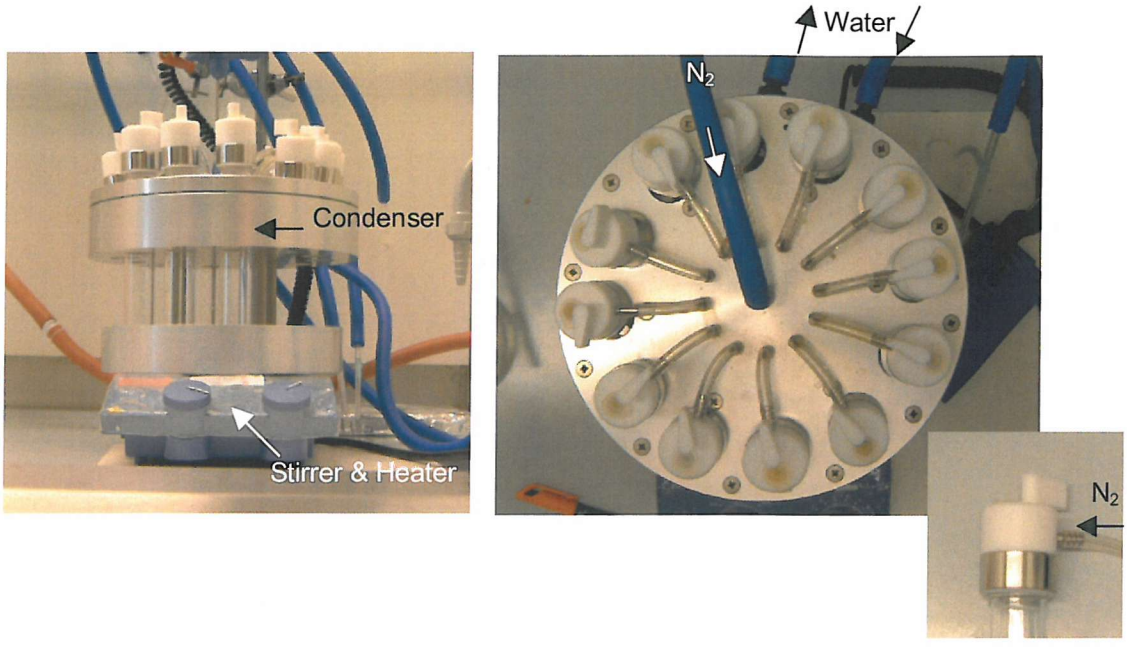


Figure 2.2.1. Multi-reactor for polymerisation, *Radleys Carousel Reaction Station*^{TM}.

In order to determine the molecular weight of the polymer high throughput gel permeation chromatography (HT-GPC) was performed with a column, PLgel HTS-D 150 x 7.5 mm or PLgel 5 μm MIXED-C 300 x 7.5 mm, purchased from Polymer Laboratories Co., Ltd., with a flow rate of 1.0 mL/min.

The relationship between the elution time and the molecular weight of standard polymers, polystyrene (PSt) and poly(methyl methacrylate) (PMMA) which were purchased from Polymer Laboratories Co., Ltd. is shown in Figure 2.2.2, and using HT-GPC, a polymer sample could be measured in about 6 min (10 samples/hr).

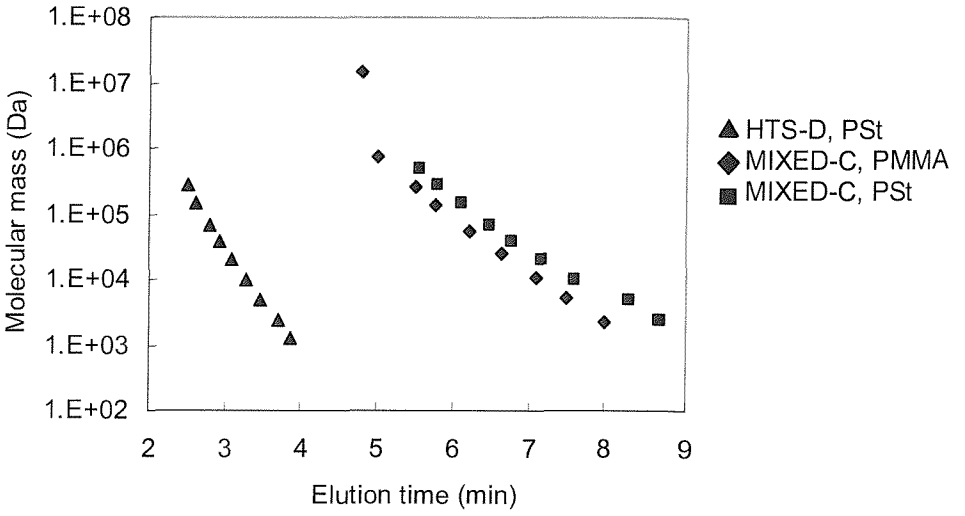


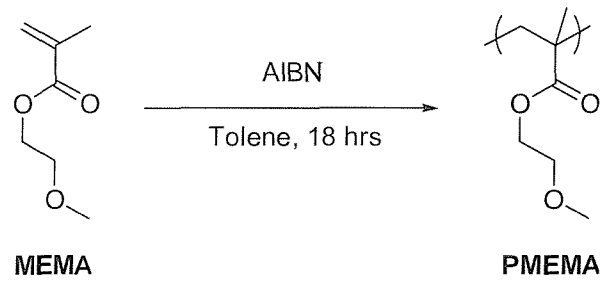
Figure 2.2.2. Performance of HT-GPC.

2.2.3 Effects of initiator concentrations

Optimisation of reaction conditions was studied by performing the polymerisation of 2-methoxyethyl methacrylate (MEMA). There are various types of azo-initiators commercially available (Table 2.2.1), the decomposition of which is normally first order with the rates unaffected by the solvent environment.[117,118] 2,2'-Azo-*bis*-isobutyronitrile (AIBN) was used here as the radical initiator (Scheme 2.2.1) and is commonly used for free radical polymerisation.[117,118]

Table 2.2.1. Azo-radical initiator.

Name	Formula	MW	M.p (°C)	Dec. (°C)	Temp. for 10hr half-life (°C)	Property
2,2'-Azo-*bis*-isobutyronitrile (AIBN)	$C_8H_{12}N_4$	164.2	100 - 103	100 - 103	65	Usable at moderate temperature
2,2'-Azo-*bis*-2-menthylbutyronitrile (AMBN)	$C_{10}H_{16}N_4$	192.3	48 - 52	105 - 107	67	Usable at moderate temperature
2,2'-Azo-*bis*-2,4-dimenthylvaleronitrile (ADVN)	$C_{14}H_{24}N_4$	248.4	45 - 70	97 - 99	52	Usable at low temperature
1,1'-Azo-*bis*-1-cyclohexane carbonitrile (ACHN)	$C_{14}H_{20}N_4$	244.3	110 - 115	138 - 143	87	Usable at high temperature
Dimethyl-2,2'-azo-*bis*-isobutyrate (MAIB)	$C_{10}H_{18}N_2O_4$	230.3	28 - 33	117 - 121	67	Non cyano type azo-catalyst
4,4'-Azo-*bis*-4-cyanovaleric acid (ACVA)	$C_{12}H_{16}N_4O_4$	280.3	110 - 120	110 - 120	68	Water soluble azo-catalyst



Scheme 2.2.1. Polymerisation of MEMA.

The effect of varying the amount of initiator on the molecular weight of the polymer was investigated. Polymerisations were carried out at 60 °C under nitrogen using the multi-reactor, and Figure 2.2.3 shows the correlation between the molecular weight of the MEMA polymer obtained and initiator ratios. The molecular weight of the polymer increased with decreasing initiator concentration, although the polydispersity did not change. Thus, the molecular weight could be clearly controlled by the amount of initiator used in the polymerisation.

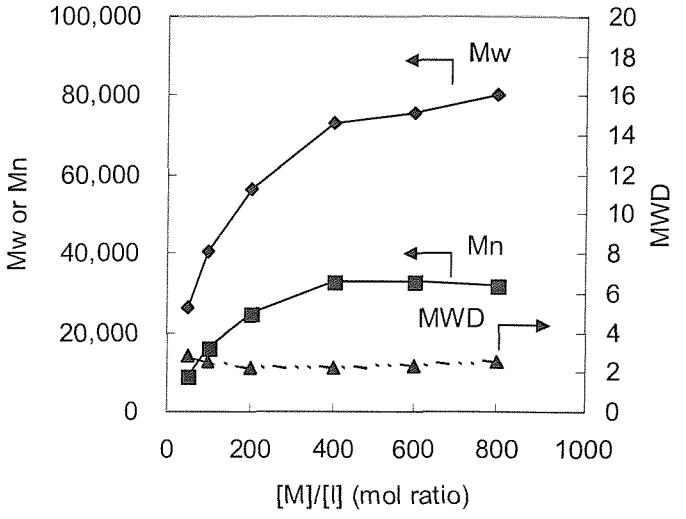


Figure 2.2.3. Effect of initiator concentration on molecular weight of PMEMA.

2.2.4 Effects of temperature on polymerisation

Temperature control during the reaction plays an important role of free radical polymerisation, and polymerisation of MEMA was carried out at several temperatures. No reaction was observed at 40 °C and with low initiator concentrations at 50 °C. At all temperatures studied, as the reaction temperature was increased, the molecular weight of polymer decreased as expected (Figure 2.2.4).

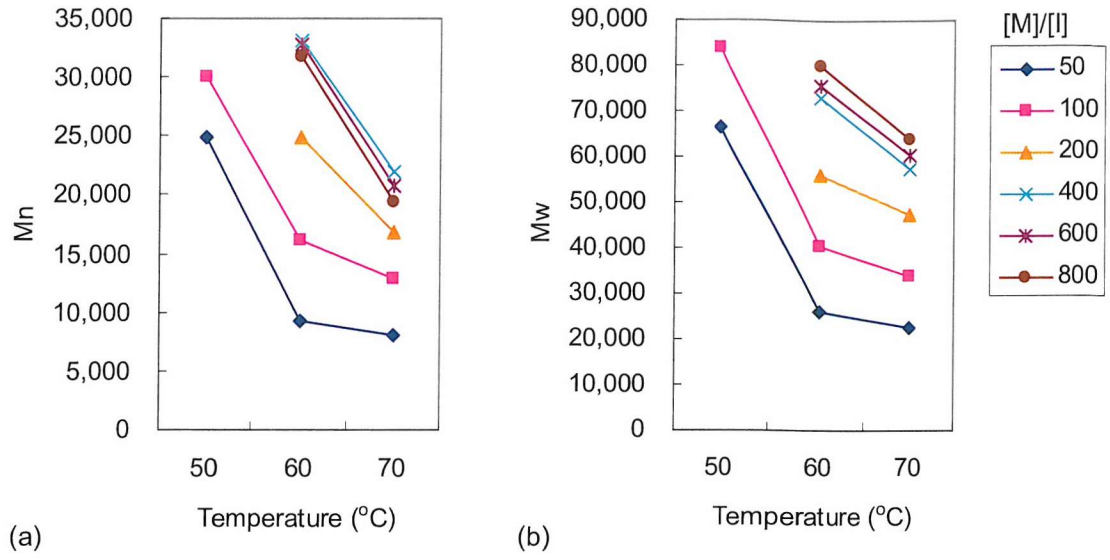


Figure 2.2.4. Effect of temperature during polymerisation on molecular weight. (a) Mn and (b) Mw.

2.2.5 Effects of monomer concentrations

The effect of monomer concentration was investigated to control the molecular weight of the polymer with polymerisation of MEMA carried out at 60 °C under nitrogen. Figure 2.2.5 shows the correlation between molecular weight and monomer concentration. When the monomer concentration was less than 30 vol.%, the molecular weight could be effectively controlled, and a monomodal distribution was obtained by GPC. However at higher monomer concentrations (more than 40 vol.%), the distribution became bimodal (Figure 2.2.6). Since free radical polymerisation is an exothermic process, the temperature of the polymerisation could not be controlled adequately at higher monomer concentrations, and it is hypothesized that 'thermal polymerisation' took place at higher temperature, giving rise to the bimodal peak in the GPC.

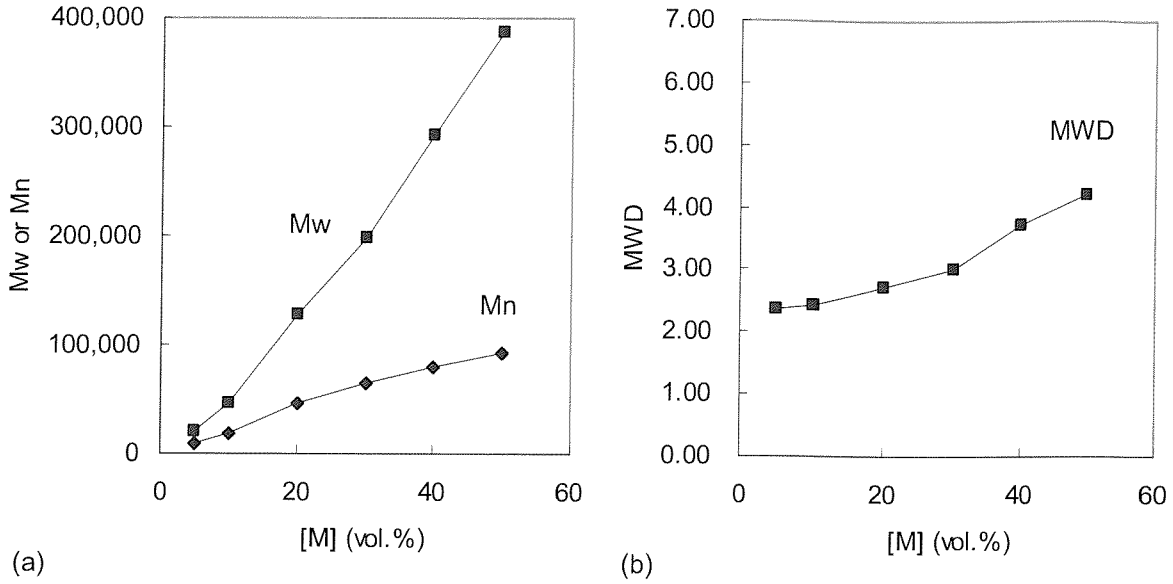


Figure 2.2.5. Effect of monomer concentration on (a) molecular weight and (b) polydispersity.

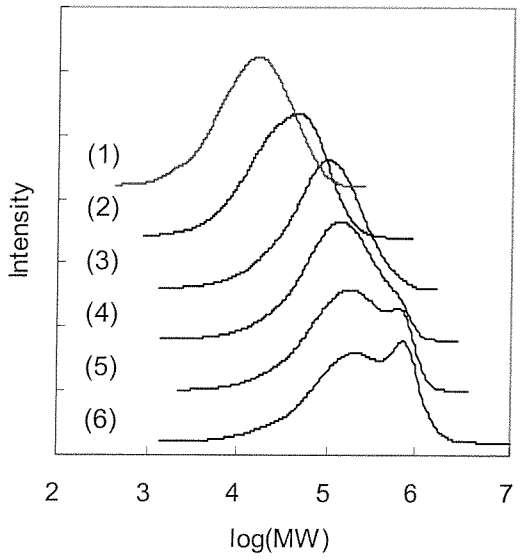


Figure 2.2.6. Gel permeation chromatographic analysis of PMEMA. Monomer concentration: (1) 5, (2) 10, (3) 20, (4) 30, (5) 40, and (6) 50 vol.%.

2.3 Design of polymer libraries

Research was aimed at the development of blood compatible polymers and determination of the interactions between blood components and polymeric surfaces. It is generally recognized that adhesion and proliferation of different types of cells on polymeric materials depend on the surface characteristics of the polymer such as wettability, hydrophilicity/hydrophobicity, surface charge, flexibility, *etc.*, and it is essential to optimise these parameters by controlling chemical structure.

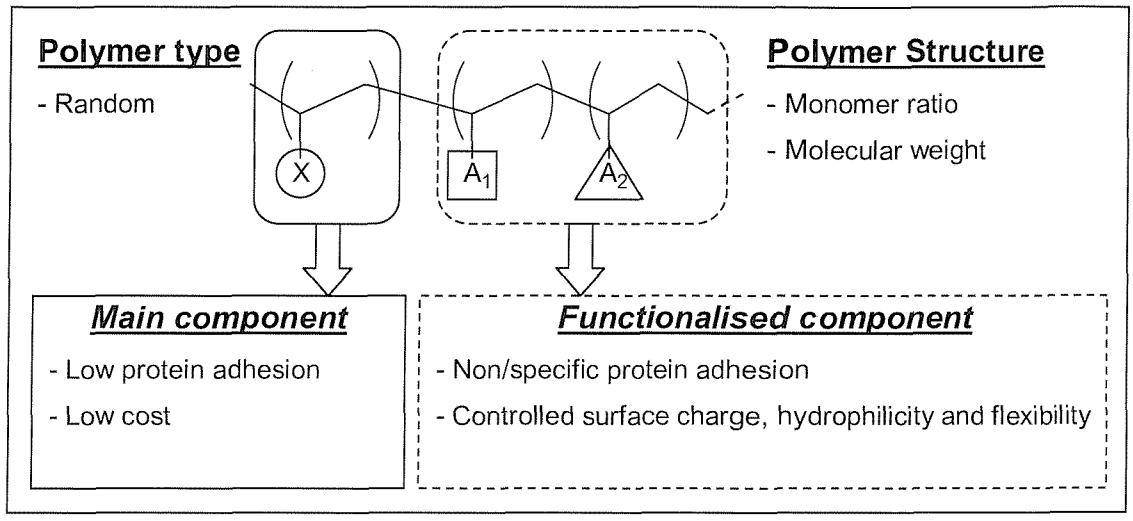


Figure 2.3.1. Polymer design for biocompatible polymer.

2.4 Syntheses of polymer libraries

2.4.1 Polymer library-1

Polymer library-1 was prepared by co-polymerisation with all possible combinations of 7 main components, (Styrene (St), methyl methacrylate (MMA), 2-methoxyethyl methacrylate (MEMA), 2-methoxyethyl acrylate (MEA), 2-hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA), and hydroxybutyl methacrylate (HBMA)) and 3 acrylamides (*N,N*-diethyl acrylamide (DEAAm), *N,N*-dimethyl acrylamide (DMAAm), and *N*-isopropyl acrylamide (NIPAAm)) with 3 different monomer compositions (90/10, 70/30, and 50/50 mol.%), resulting in 7 x 3 x 3 = 63 distinct polymers (Figure 2.4.1). Polymers containing MEA,[42-46] HEMA,[22,29] DMAAm,[23,25,27] and NIPAAm[18,23] monomers are generally thought to enhance biocompatibility (Chapter 1.1.6), and hence dictated their inclusion.

The reactions were carried out under nitrogen with AIBN as an initiator, toluene was used as reaction solvent for St, MMA, MEMA, and MEA-based polymer, and dimethylformamide (DMF) or 1-methyl-2-pyrrolidinone (NMP) was used for hydroxyalkyl methacrylate polymer due to the poor solubility of polymer in toluene. The reaction temperature was set to 60 °C, but in the case of hydroxyalkyl methacrylate copolymers the reaction temperature was changed to 80 °C to reduce the aggregation by hydrogen bonding between each monomer. After the reaction, the product was precipitated by dropwise addition into a poor solvent (hexane, cyclohexane, diethyl ether, or their mixture) to obtain a solid or rubber. The polymer was washed with a poor solvent or reprecipitated, and dried under vacuum at 40 °C for 4 hrs.

St-based copolymers were obtained as white solids in moderate yields, and the polydispersities (MWD) of these polymers were almost ideal for free radical polymerisation. All of the (meth)acrylate polymers were obtained in good yields. Most polymers were obtained as solids, though MEA-based polymers were obtained as highly viscous rubbers, because MEA is an acrylate type monomer, this gives a polymer with a low glass transition temperature (Chapter 4.3.3). Some polymers of NIPAAm gave multi-modal peaks and wide polydispersities by GPC analysis, which might be due to cross-linking during polymerisation. Cross-linking was observed despite the fact that reactions were carried out under low monomer concentrations. In the case of hydroxyalkyl methacrylate copolymers, the peaks obtained from GPC were bimodal in most cases and polydispersities became higher, and low molecular weights were observed.

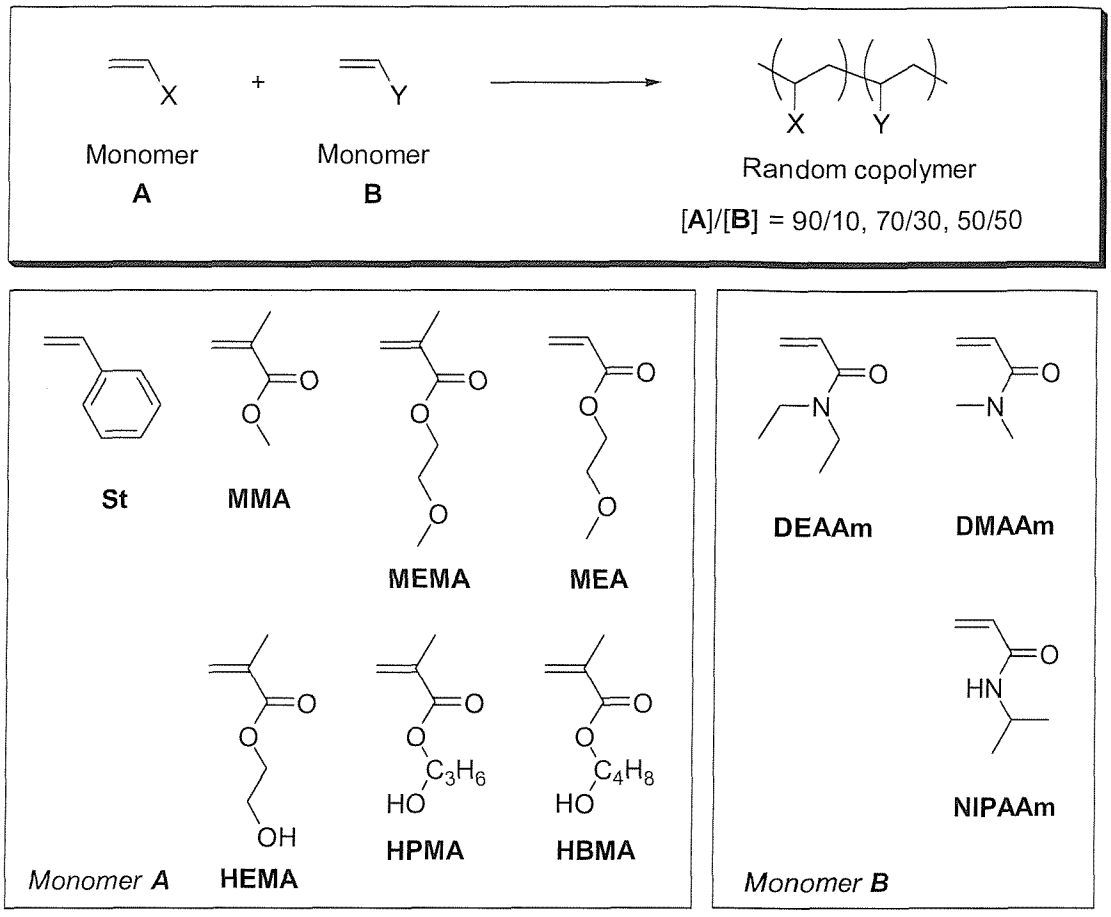


Figure 2.4.1. Monomers used in polymer library-1.

Table 2.4.1. Data for polymer library-1.

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL1-1	St	DEAAm	90/10	Powder	42%	63,600	2.2
PL1-2	St	DEAAm	70/30	Powder	50%	76,700	1.8
PL1-3	St	DEAAm	50/50	Powder	48%	56,000	2.0
PL1-4	St	DMAAm	90/10	Powder	31%	89,100	1.6
PL1-5	St	DMAAm	70/30	Powder	51%	62,500	2.0
PL1-6	St	DMAAm	50/50	Powder	60%	65,500	2.1
PL1-7	St	NIPAAm	90/10	Powder	32%	81,700	1.8
PL1-8	St	NIPAAm	70/30	Powder	55%	67,500	2.0
PL1-9	St	NIPAAm	50/50	Powder	60%	79,200	3.0

(*cont'd*)

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL1-10	MMA	DEAAm	90/10	Powder	78%	98,900	2.7
PL1-11	MMA	DEAAm	70/30	Powder	80%	135,000	2.2
PL1-12	MMA	DEAAm	50/50	Powder	Quant.	116,000	2.4
PL1-13	MMA	DMAAm	90/10	Powder	90%	114,000	2.4
PL1-14	MMA	DMAAm	70/30	Powder	89%	117,000	2.6
PL1-15	MMA	DMAAm	50/50	Powder	Quant.	129,000	2.6
PL1-16	MMA	NIPAAm	90/10	Powder	77%	106,000	2.3
PL1-17	MMA	NIPAAm	70/30	Powder	76%	172,000	11.2
PL1-18	MMA	NIPAAm	50/50	Powder	87%	108,000	9.3
PL1-19	MEMA	DEAAm	90/10	Solid	83%	73,200	3.0
PL1-20	MEMA	DEAAm	70/30	Solid	87%	62,200	3.5
PL1-21	MEMA	DEAAm	50/50	Solid	Quant.	62,400	3.1
PL1-22	MEMA	DMAAm	90/10	Solid	75%	67,100	3.0
PL1-23	MEMA	DMAAm	70/30	Solid	83%	70,300	3.0
PL1-24	MEMA	DMAAm	50/50	Solid	Quant.	63,600	3.6
PL1-25	MEMA	NIPAAm	90/10	Solid	79%	70,500	3.1
PL1-26	MEMA	NIPAAm	70/30	Solid	93%	82,500	7.7
PL1-27	MEMA	NIPAAm	50/50	Solid	Quant.	70,600	5.9
PL1-28	MEA	DEAAm	90/10	Rubber	98%	39,700	4.4
PL1-29	MEA	DEAAm	70/30	Rubber	96%	47,400	3.4
PL1-30	MEA	DEAAm	50/50	Rubber	93%	58,600	3.2
PL1-31	MEA	DMAAm	90/10	Rubber	97%	56,800	3.8
PL1-32	MEA	DMAAm	70/30	Rubber	93%	51,600	3.5
PL1-33	MEA	DMAAm	50/50	Rubber	Quant.	56,800	4.5
PL1-34	MEA	NIPAAm	90/10	Rubber	94%	46,800	11.7
PL1-35	MEA	NIPAAm	70/30	Rubber	91%	54,100	32.1
PL1-36	MEA	NIPAAm	50/50	Rubber	89%	82,800	41.1

(*cont'd*)

No.	Monomer A	Monomer B	Ratio (mol.%)	Appearance	Yield (mol.%)	Mn (Da)	MWD
PL1-37	HEMA	DEAAm	90/10	Solid	73%	59,300	7.2
PL1-38	HEMA	DEAAm	70/30	Solid	67%	29,000	6.6
PL1-39	HEMA	DEAAm	50/50	Solid	61%	15,100	8.9
PL1-40	HEMA	DMAAm	90/10	Solid	70%	68,300	7.5
PL1-41	HEMA	DMAAm	70/30	Solid	69%	41,500	6.2
PL1-42	HEMA	DMAAm	50/50	Solid	79%	21,900	5.2
PL1-43	HEMA	NIPAAm	90/10	Solid	82%	39,500	6.7
PL1-44	HEMA	NIPAAm	70/30	Solid	80%	20,600	7.3
PL1-45	HEMA	NIPAAm	50/50	Solid	80%	12,700	8.0
PL1-46	HPMA	DEAAm	90/10	Solid	75%	32,500	4.8
PL1-47	HPMA	DEAAm	70/30	Solid	63%	22,900	5.7
PL1-48	HPMA	DEAAm	50/50	Solid	51%	15,300	6.6
PL1-49	HPMA	DMAAm	90/10	Solid	66%	50,500	6.6
PL1-50	HPMA	DMAAm	70/30	Solid	75%	40,500	5.0
PL1-51	HPMA	DMAAm	50/50	Solid	77%	28,200	5.3
PL1-52	HPMA	NIPAAm	90/10	Solid	81%	26,800	3.0
PL1-53	HPMA	NIPAAm	70/30	Solid	74%	16,200	4.1
PL1-54	HPMA	NIPAAm	50/50	Solid	74%	13,700	7.8
PL1-55	HBMA	DEAAm	90/10	Solid	72%	30,500	5.7
PL1-56	HBMA	DEAAm	70/30	Solid	68%	19,900	6.4
PL1-57	HBMA	DEAAm	50/50	Solid	87%	11,600	5.3
PL1-58	HBMA	DMAAm	90/10	Solid	64%	52,500	8.2
PL1-59	HBMA	DMAAm	70/30	Solid	68%	46,200	6.7
PL1-60	HBMA	DMAAm	50/50	Solid	70%	27,000	5.0
PL1-61	HBMA	NIPAAm	90/10	Solid	77%	26,200	4.2
PL1-62	HBMA	NIPAAm	70/30	Solid	72%	17,000	4.2
PL1-63	HBMA	NIPAAm	50/50	Solid	70%	12,300	4.2

2.4.2 Polymer library-2

Polymer library-2 was prepared by co-polymerisation, using 2 main components (MEMA and HEMA) and 16 other monomers (in all combinations) with various functional groups (amine, amide, thioether, nitro, and heteroaromatic groups), at 3 different monomer compositions (90/10, 70/30, and 50/50 mol.%), resulting in 2 x 16 x 3 = 96 distinct polymers (Figure 2.4.2).

In the case of MEMA co-polymerisation, toluene was used as the reaction solvent except for the polymerisation of VAA, VI, DAAAm, and MNPMA as a co-monomer where DMF was used due to solubility issues. In all cases of HEMA co-polymerisation, DMF was used.

After precipitation, most of the MEMA type copolymers were obtained as solids, but acrylates (DEAEA and DMAEA) and long side chain containing co-monomers (BACOEA) gave rubbery solids as the product. Most of the HEMA type copolymers were obtained as powders in good yield. All polymers gave relatively high molecular weights.

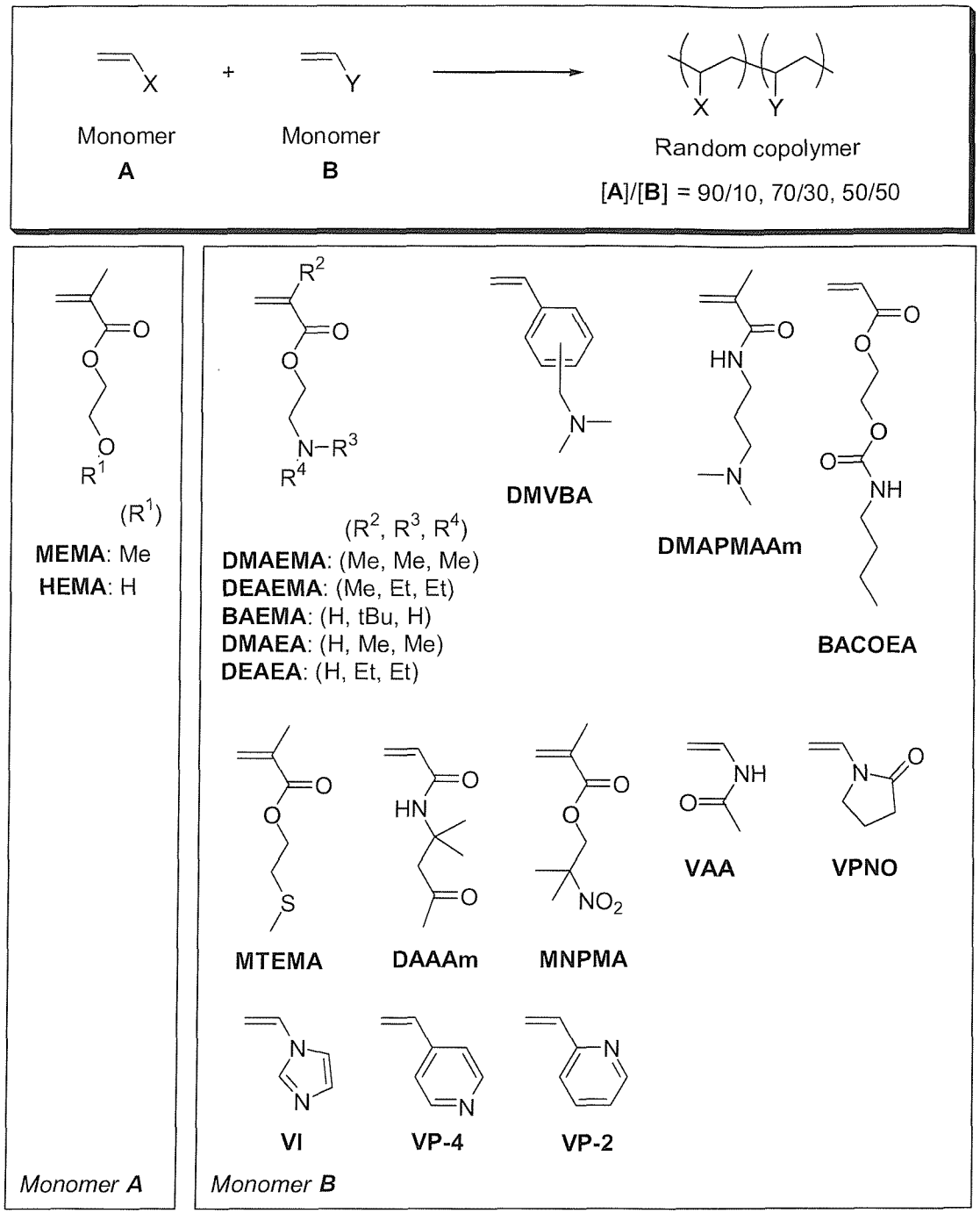


Figure 2.4.2. Monomers used in polymer library-2.

Table 2.4.2. Data for polymer library-2.

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL2-1	MEMA	DEAEMA	90/10	Solid	80%	102,000	4.1
PL2-2	MEMA	DEAEMA	70/30	Solid	77%	71,500	4.4
PL2-3	MEMA	DEAEMA	50/50	Solid	64%	99,200	3.0
PL2-4	MEMA	DMAEMA	90/10	Solid	64%	79,100	5.8
PL2-5	MEMA	DMAEMA	70/30	Solid	80%	87,600	4.2
PL2-6	MEMA	DMAEMA	50/50	Solid	56%	50,800	4.7
PL2-7	MEMA	DEAEA	90/10	Solid	89%	54,300	4.5
PL2-8	MEMA	DEAEA	70/30	Rubbery solid	65%	61,100	4.5
PL2-9	MEMA	DEAEA	50/50	Rubbery solid	44%	58,600	3.8
PL2-10	MEMA	DMAEA	90/10	Solid	82%	69,500	4.1
PL2-11	MEMA	DMAEA	70/30	Rubbery solid	74%	67,100	5.8
PL2-12	MEMA	DMAEA	50/50	Rubbery solid	67%	34,700	6.0
PL2-13	MEMA	MTEMA	90/10	Solid	86%	49,100	3.6
PL2-14	MEMA	MTEMA	70/30	Solid	90%	57,600	4.1
PL2-15	MEMA	MTEMA	50/50	Solid	62%	46,800	3.1
PL2-16	MEMA	BAEMA	90/10	Solid	82%	61,800	4.4
PL2-17	MEMA	BAEMA	70/30	Solid	77%	64,100	4.9
PL2-18	MEMA	BAEMA	50/50	Solid	76%	75,200	3.9
PL2-19	MEMA	DMAPMAAm	90/10	Solid	86%	68,300	4.0
PL2-20	MEMA	DMAPMAAm	70/30	Solid	81%	40,400	4.6
PL2-21	MEMA	DMAPMAAm	50/50	Solid	60%	35,400	4.4
PL2-22	MEMA	BACOEA	90/10	Solid	77%	58,700	4.1
PL2-23	MEMA	BACOEA	70/30	Rubbery solid	74%	66,800	4.5
PL2-24	MEMA	BACOEA	50/50	Rubbery solid	73%	49,000	6.9
PL2-25	MEMA	DMVBA	90/10	Solid	73%	67,800	3.5
PL2-26	MEMA	DMVBA	70/30	Solid	66%	40,700	3.1
PL2-27	MEMA	DMVBA	50/50	Solid	63%	35,900	2.9
PL2-28	MEMA	VAA	90/10	Solid	83%	79,900	4.7
PL2-29	MEMA	VAA	70/30	Rubbery solid	85%	94,700	3.7
PL2-30	MEMA	VAA	50/50	Rubbery solid	78%	68,600	3.1
PL2-31	MEMA	VI	90/10	Solid	82%	92,200	4.0
PL2-32	MEMA	VI	70/30	Solid	76%	72,200	3.7
PL2-33	MEMA	VI	50/50	Solid	70%	73,500	4.1

(cont'd)

No.	Monomer A	Monomer B	Ratio (mol.%)	Appearance	Yield (mol.%)	Mn (Da)	MWD
PL2-34	MEMA	VPNO	90/10	Solid	79%	58,900	3.7
PL2-35	MEMA	VPNO	70/30	Rubbery solid	81%	56,300	4.5
PL2-36	MEMA	VPNO	50/50	Rubbery solid	88%	37,900	6.7
PL2-37	MEMA	VP-4	90/10	Solid	76%	75,600	3.0
PL2-38	MEMA	VP-4	70/30	Solid	63%	103,000	3.0
PL2-39	MEMA	VP-4	50/50	Solid	63%	70,800	2.9
PL2-40	MEMA	VP-2	90/10	Solid	76%	82,400	2.9
PL2-41	MEMA	VP-2	70/30	Solid	65%	65,800	2.6
PL2-42	MEMA	VP-2	50/50	Solid	87%	164,000	2.3
PL2-43	MEMA	DAAAm	90/10	Solid	70%	56,900	2.7
PL2-44	MEMA	DAAAm	70/30	Solid	69%	74,200	3.1
PL2-45	MEMA	DAAAm	50/50	Solid	89%	51,400	4.1
PL2-46	MEMA	MNPMA	90/10	Solid	60%	50,600	2.5
PL2-47	MEMA	MNPMA	70/30	Solid	66%	98,800	2.9
PL2-48	MEMA	MNPMA	50/50	Solid	65%	58,500	3.0
PL2-49	HEMA	DEAEMA	90/10	Powder (Solid)	53%	35,500	4.9
PL2-50	HEMA	DEAEMA	70/30	Powder (Solid)	89%	32,000	4.9
PL2-51	HEMA	DEAEMA	50/50	Powder (Solid)	58%	48,800	4.1
PL2-52	HEMA	DMAEMA	90/10	Powder (Solid)	78%	40,500	4.4
PL2-53	HEMA	DMAEMA	70/30	Powder (Solid)	56%	47,600	4.2
PL2-54	HEMA	DMAEMA	50/50	Powder (Solid)	58%	46,100	3.6
PL2-55	HEMA	DEAEA	90/10	Powder (Solid)	81%	43,300	4.5
PL2-56	HEMA	DEAEA	70/30	Powder (Solid)	52%	37,000	5.0
PL2-57	HEMA	DEAEA	50/50	Powder (Solid)	33%	21,900	4.6
PL2-58	HEMA	DMAEA	90/10	Powder (Solid)	75%	46,700	4.5
PL2-59	HEMA	DMAEA	70/30	Powder (Solid)	63%	44,600	4.4
PL2-60	HEMA	DMAEA	50/50	Powder (Solid)	46%	31,500	5.1
PL2-61	HEMA	MTEMA	90/10	Powder (Solid)	83%	58,400	3.7
PL2-62	HEMA	MTEMA	70/30	Powder (Solid)	64%	42,000	3.8
PL2-63	HEMA	MTEMA	50/50	Powder (Solid)	67%	47,900	3.4
PL2-64	HEMA	BAEMA	90/10	Powder (Solid)	83%	55,300	4.3
PL2-65	HEMA	BAEMA	70/30	Powder (Solid)	60%	51,900	4.9
PL2-66	HEMA	BAEMA	50/50	Powder (Solid)	62%	54,900	3.9

(*cont'd*)

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL2-67	HEMA	DMAPMAAm	90/10	Powder (Solid)	83%	74,800	4.0
PL2-68	HEMA	DMAPMAAm	70/30	Powder (Solid)	64%	69,000	3.7
PL2-69	HEMA	DMAPMAAm	50/50	Powder (Solid)	52%	57,300	4.2
PL2-70	HEMA	BACOEA	90/10	Powder (Solid)	89%	60,600	4.9
PL2-71	HEMA	BACOEA	70/30	Powder (Solid)	72%	52,000	5.8
PL2-72	HEMA	BACOEA	50/50	Powder (Solid)	74%	20,700	9.5
PL2-73	HEMA	DMVBA	90/10	Rubbery solid	61%	63,600	3.8
PL2-74	HEMA	DMVBA	70/30	Powder (Solid)	42%	50,600	3.2
PL2-75	HEMA	DMVBA	50/50	Powder (Solid)	43%	43,800	2.5
PL2-76	HEMA	VAA	90/10	Powder (Solid)	78%	62,600	4.2
PL2-77	HEMA	VAA	70/30	Powder (Solid)	85%	40,900	5.2
PL2-78	HEMA	VAA	50/50	Powder (Solid)	Quant.	42,900	4.7
PL2-79	HEMA	VI	90/10	Powder (Solid)	81%	83,100	5.1
PL2-80	HEMA	VI	70/30	Powder (Solid)	72%	60,300	5.1
PL2-81	HEMA	VI	50/50	Powder (Solid)	66%	52,500	5.4
PL2-82	HEMA	VPNO	90/10	Powder (Solid)	83%	87,000	4.3
PL2-83	HEMA	VPNO	70/30	Powder (Solid)	70%	73,800	4.4
PL2-84	HEMA	VPNO	50/50	Powder (Solid)	Quant.	63,000	3.9
PL2-85	HEMA	VP-4	90/10	Powder (Solid)	Quant.	72,300	4.3
PL2-86	HEMA	VP-4	70/30	Powder (Solid)	49%	42,300	3.9
PL2-87	HEMA	VP-4	50/50	Powder (Solid)	90%	48,000	2.9
PL2-88	HEMA	VP-2	90/10	Powder (Solid)	44%	59,800	4.8
PL2-89	HEMA	VP-2	70/30	Powder (Solid)	42%	44,800	3.4
PL2-90	HEMA	VP-2	50/50	Powder (Solid)	51%	58,000	2.6
PL2-91	HEMA	DAAAm	90/10	Powder (Solid)	90%	88,300	6.1
PL2-92	HEMA	DAAAm	70/30	Powder (Solid)	75%	77,000	5.2
PL2-93	HEMA	DAAAm	50/50	Powder (Solid)	71%	47,600	5.0
PL2-94	HEMA	MNPMA	90/10	Powder (Solid)	Quant.	110,000	5.8
PL2-95	HEMA	MNPMA	70/30	Powder (Solid)	94%	99,500	5.6
PL2-96	HEMA	MNPMA	50/50	Powder (Solid)	82%	89,800	5.3

2.4.3 Polymer library-3

An amine group is a very attractive functionality for biofunctionality, because the amine group is protonated under biological conditions (pH 7.4), and the positive charge might have an effect on protein or cell binding. Therefore, it was interesting to diversify the polymers containing amine groups.

There were two ways for diversification of polymers containing amine groups, varying (1) the main monomer and (2) variation of the amine structure. The polymer library-3 was prepared with varying type of main monomers, and the diversification of an amine structure in polymers will be discussed in Chapter 2.5.

MMA, EMA, BMA, HPMA, and HBMA were used as the main monomers and DEAEMA, DMAEMA, DEAEMA, and DMAEA as amine containing monomers for polymer library-3 (Figure 2.4.3). Polymerisation was carried out under nitrogen using AIBN as an initiator.

After precipitation, the polymers were obtained as solids or rubbery solids with high molecular weights. Some BMA or HBMA-based polymers gave low yields, because the solubility of the product in organic solvents was very high, and the polymers were partially soluble in precipitation solvents (hexane, methanol, and diethyl ether).

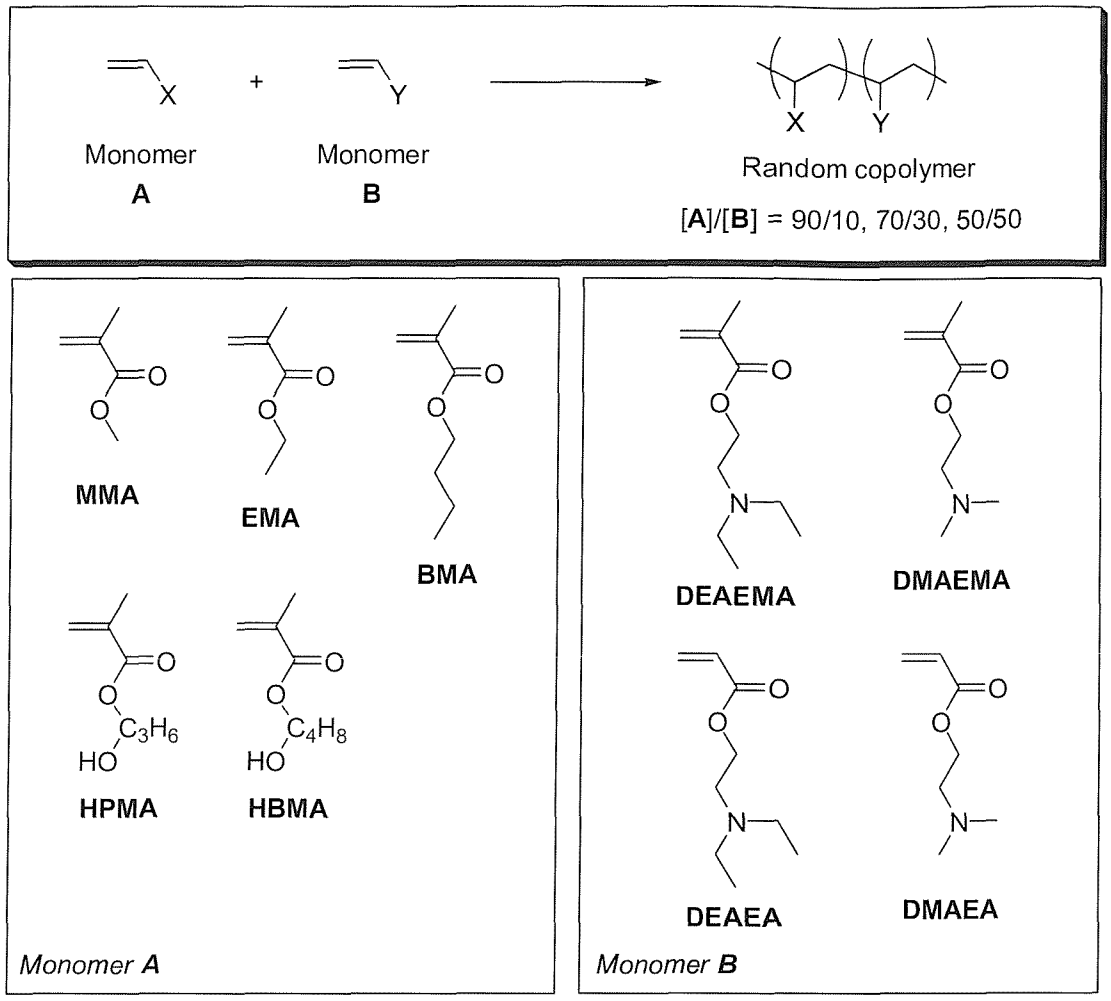


Figure 2.4.3. Monomers used in polymer library-3.

Table 2.4.3. Data for polymer library-3.

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL3-1	MMA	DEAEMA	90/10	Solid	77%	66,800	3.1
PL3-2	MMA	DEAEMA	70/30	Solid	75%	53,400	3.4
PL3-3	MMA	DEAEMA	50/50	Rubbery solid	76%	63,200	3.3
PL3-4	MMA	DMAEMA	90/10	Solid	74%	75,300	2.7
PL3-5	MMA	DMAEMA	70/30	Solid	79%	64,100	3.1
PL3-6	MMA	DMAEMA	50/50	Rubbery solid	79%	65,100	3.1
PL3-7	MMA	DEAEA	90/10	Solid	80%	46,800	3.2
PL3-8	MMA	DEAEA	70/30	Solid	58%	33,600	3.6
PL3-9	MMA	DEAEA	50/50	Rubbery solid	46%	20,000	2.2
PL3-10	MMA	DMAEA	90/10	Solid	87%	58,500	3.2
PL3-11	MMA	DMAEA	70/30	Solid	82%	35,600	4.6
PL3-12	MMA	DMAEA	50/50	Rubbery solid	77%	26,100	5.5
PL3-13	HPMA	DEAEMA	90/10	Solid	80%	75,800	2.0
PL3-14	HPMA	DEAEMA	70/30	Solid	70%	57,000	2.0
PL3-15	HPMA	DEAEMA	50/50	Solid	60%	54,000	2.1
PL3-16	HPMA	DMAEMA	90/10	Solid	82%	83,200	2.0
PL3-17	HPMA	DMAEMA	70/30	Solid	75%	72,700	2.0
PL3-18	HPMA	DMAEMA	50/50	Solid	67%	67,400	2.0
PL3-19	HPMA	DEAEA	90/10	Rubbery solid	69%	70,700	2.1
PL3-20	HPMA	DEAEA	70/30	Rubbery solid	40%	46,800	2.0
PL3-21	HPMA	DEAEA	50/50	Rubbery solid	32%	29,600	1.9
PL3-22	HPMA	DMAEA	90/10	Rubbery solid	89%	60,400	2.0
PL3-23	HPMA	DMAEA	70/30	Rubbery solid	53%	23,800	2.3
PL3-24	HPMA	DMAEA	50/50	Rubbery solid	39%	16,300	2.0
PL3-25	HBMA	DEAEMA	90/10	Solid	77%	78,000	2.1
PL3-26	HBMA	DEAEMA	70/30	Solid	63%	58,900	2.1
PL3-27	HBMA	DEAEMA	50/50	Solid	63%	56,200	2.0
PL3-28	HBMA	DMAEMA	90/10	Solid	69%	89,000	2.2
PL3-29	HBMA	DMAEMA	70/30	Solid	67%	70,700	2.1
PL3-30	HBMA	DMAEMA	50/50	Solid	63%	63,900	2.2

(cont'd)

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL3-31	HBMA	DEAEA	90/10	Rubbery solid	55%	51,400	2.0
PL3-32	HBMA	DEAEA	70/30	Rubbery solid	45%	21,200	2.3
PL3-33	HBMA	DEAEA	50/50	Rubbery solid	31%	14,000	2.0
PL3-34	HBMA	DMAEA	90/10	Rubbery solid	66%	66,000	2.0
PL3-35	HBMA	DMAEA	70/30	Rubbery solid	71%	28,900	2.8
PL3-36	HBMA	DMAEA	50/50	Rubbery solid	59%	21,400	2.4
PL3-37	EMA	DEAEMA	90/10	Solid	80%	25,500	4.5
PL3-38	EMA	DEAEMA	70/30	Solid	76%	42,100	3.1
PL3-39	EMA	DEAEMA	50/50	Rubbery solid	72%	35,700	3.5
PL3-40	EMA	DMAEMA	90/10	Solid	83%	43,200	2.6
PL3-41	EMA	DMAEMA	70/30	Solid	79%	41,900	2.9
PL3-42	EMA	DMAEMA	50/50	Rubbery solid	80%	38,200	3.2
PL3-43	EMA	DEAEA	90/10	Rubbery solid	61%	45,000	2.7
PL3-44	EMA	DEAEA	70/30	Rubbery solid	56%	35,800	2.5
PL3-45	EMA	DEAEA	50/50	Rubbery solid	45%	17,800	2.5
PL3-46	EMA	DMAEA	90/10	Rubbery solid	63%	56,100	4.3
PL3-47	EMA	DMAEA	70/30	Rubbery solid	61%	42,500	8.7
PL3-48	EMA	DMAEA	50/50	Rubbery solid	47%	30,700	9.5
PL3-49	BMA	DEAEMA	90/10	Rubbery solid	62%	78,400	2.7
PL3-50	BMA	DEAEMA	70/30	Rubbery solid	28%	87,100	2.8
PL3-51	BMA	DEAEMA	50/50	Rubbery solid	21%	77,700	3.3
PL3-52	BMA	DMAEMA	90/10	Rubbery solid	72%	105,000	2.6
PL3-53	BMA	DMAEMA	70/30	Rubbery solid	74%	89,200	2.9
PL3-54	BMA	DMAEMA	50/50	Rubbery solid	77%	78,300	3.3
PL3-55	BMA	DEAEA	90/10	Rubbery solid	55%	74,100	2.7
PL3-56	BMA	DEAEA	70/30	Rubbery solid	50%	37,900	2.7
PL3-57	BMA	DEAEA	50/50	Rubbery solid	76%	22,300	2.2
PL3-58	BMA	DMAEA	90/10	Rubbery solid	63%	76,700	2.5
PL3-59	BMA	DMAEA	70/30	Rubbery solid	54%	51,100	4.2
PL3-60	BMA	DMAEA	50/50	Rubber	60%	30,600	6.7

2.4.4 Polymer library-4

In general, proteins are adsorbed more onto positively charged surfaces than on negatively charged ones, although some researchers have reported that negatively charged polymer surfaces show excellent blood compatibility.[63,71] Therefore, polymer library-4 was generated by selecting five different acidic monomers containing carboxylic acid and phosphoric acid groups, A-H, AES-H, MA-H, AAG-H, and EGMP-H. Since hydroxyalkyl (meth)acrylates such as HEMA might react with the acid group to form cross-linkages in the polymer chain, MMA and MEMA were selected as the main monomer (Figure 2.4.4).

Polymerisation was carried out at 60 °C under nitrogen, and DMF was used as a reaction solvent, however in the case of A-H and MA-H co-monomer, isopropyl alcohol (IPA) was used to aid solubility.

All polymers were obtained in high yield. However relatively low molecular weights were observed, because the polymerisation was carried out at low monomer concentrations to avoid aggregation by hydrogen bonding. The peaks corresponding to the polymer with a high content of AES-H and EGMP-H were not observed by the refractive index detector of the GPC.

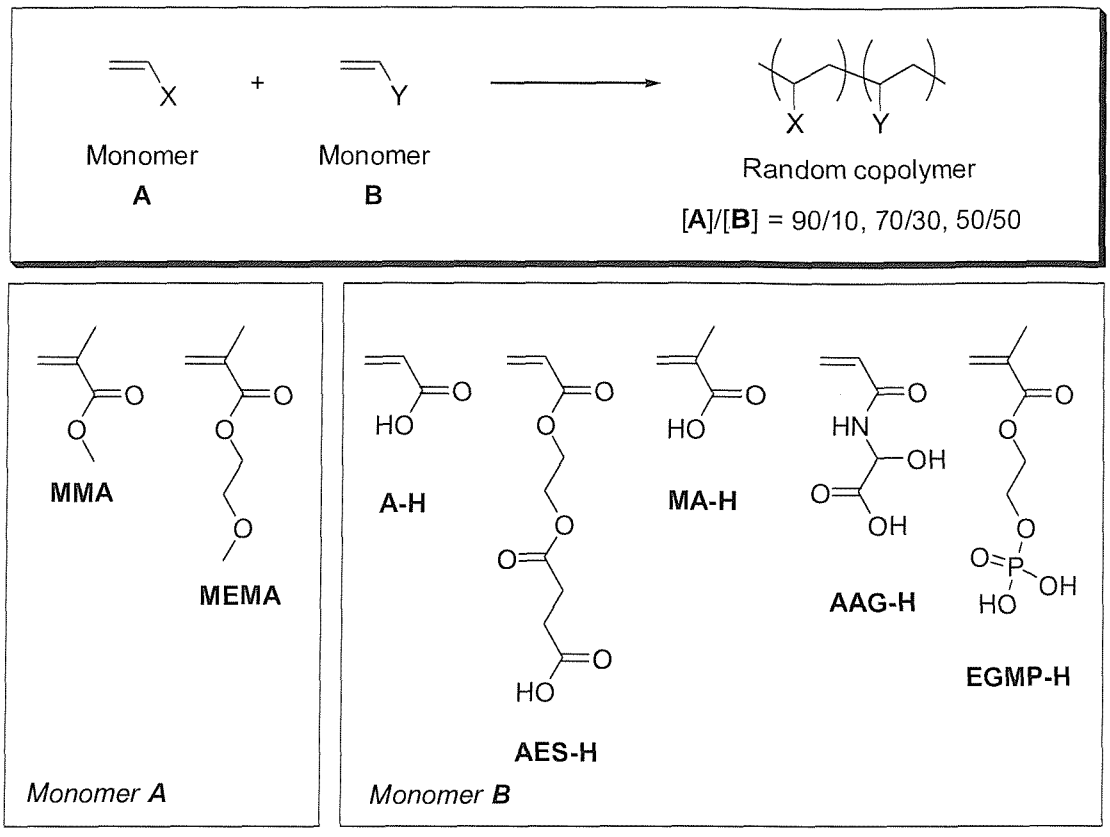


Figure 2.4.4. Monomers used in polymer library-4.

Table 2.4.4. Data for polymer library-4.

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
PL4-1	MMA	A-H	90/10	Powder	73%	12,400	2.2
PL4-2	MMA	A-H	70/30	Solid	81%	12,500	3.1
PL4-3	MMA	A-H	50/50	Solid	77%	11,000	2.5
PL4-4	MMA	AES-H	90/10	Rubbery solid	94%	16,900	2.3
PL4-5	MMA	AES-H	70/30	Rubbery solid	93%	Not detect	
PL4-6	MMA	AES-H	50/50	Rubbery solid	65%	Not detect	
PL4-7	MMA	MA-H	90/10	Powder	71%	11,000	2.2
PL4-8	MMA	MA-H	70/30	Powder	79%	13,100	2.3
PL4-9	MMA	MA-H	50/50	Powder	64%	10,100	2.1
PL4-10	MMA	AAG-H	90/10	Solid	92%	11,600	2.2
PL4-11	MMA	AAG-H	70/30	Rubbery solid	63%	13,500	2.1
PL4-12	MMA	AAG-H	50/50	Rubbery solid	64%	12,000	2.2
PL4-13	MMA	EGMP-H	90/10	Solid	81%	16,800	1.9
PL4-14	MMA	EGMP-H	70/30	Solid	96%	Not detect	
PL4-15	MMA	EGMP-H	50/50	Rubbery solid	95%	Not detect	
PL4-16	MEMA	A-H	90/10	Solid	78%	12,700	2.2
PL4-17	MEMA	A-H	70/30	Solid	86%	15,300	2.8
PL4-18	MEMA	A-H	50/50	Solid	89%	11,900	2.3
PL4-19	MEMA	AES-H	90/10	Rubbery solid	90%	20,300	2.9
PL4-20	MEMA	AES-H	70/30	Rubbery solid	83%	Not detect	
PL4-21	MEMA	AES-H	50/50	Rubbery solid	95%	Not detect	
PL4-22	MEMA	MA-H	90/10	Solid	89%	12,700	2.4
PL4-23	MEMA	MA-H	70/30	Powder	87%	15,200	2.6
PL4-24	MEMA	MA-H	50/50	Powder	88%	17,500	2.1
PL4-25	MEMA	AAG-H	90/10	Rubbery solid	78%	18,500	2.7
PL4-26	MEMA	AAG-H	70/30	Rubbery solid	84%	15,700	2.6
PL4-27	MEMA	AAG-H	50/50	Rubbery solid	96%	13,100	2.4
PL4-28	MEMA	EGMP-H	90/10	Solid	93%	17,700	3.0
PL4-29	MEMA	EGMP-H	70/30	Rubbery solid	76%	Not detect	
PL4-30	MEMA	EGMP-H	50/50	Rubbery solid	93%	Not detect	

2.4.5 Polymer library-5

It was important to investigate the effect of various functional groups on biocompatibility. Furthermore, the combined effects of mixtures of functional groups would have the potential to control protein adsorption. Terpolymers, which consist of three components, were designed to study polymer properties and biocompatibility. Terpolymers might control the physical properties of polymers such as charge, glass transition temperature, and hydrophilicity.[65,149]

MEMA was used as the main monomer, DEAE(M)A as the monomer containing an amino group which could be protonated, and 14 other monomers also used for the synthesis of the polymer library-5 (Figure 2.4.5). The third monomers were selected by functionality, hydrophilicity, hydrophobicity, glass transition temperature, and total charge. Figure 2.4.6 and Table 2.4.5 show the structures and the properties of the third components used. Hydrophilicity can be controlled by the number of hydroxy groups and ethylene glycol (EG) groups, and the length of the side chain and the difference between acrylate and methacrylate will have an effect on the glass transition temperature and hydrophilicity, while carboxylic acids and amine groups will control the charge within the polymer.[150,151]

The library was prepared by free radical polymerisation, and the feed compositions of the monomers were MEMA/DEAE(M)A/Third monomer = 40/30/30, 60/10/30, 60/30/10, and 80/10/10 mol.%. In total, 112 terpolymers were synthesised.

Polymers were obtained with molecular weights, Mn 13,200 – 60,000 Da in good yield. Most polymers with a high content of DEAEA were obtained as rubbery solids due to their low glass transition temperatures and as where most polymers with acrylate or long side chain containing monomers.

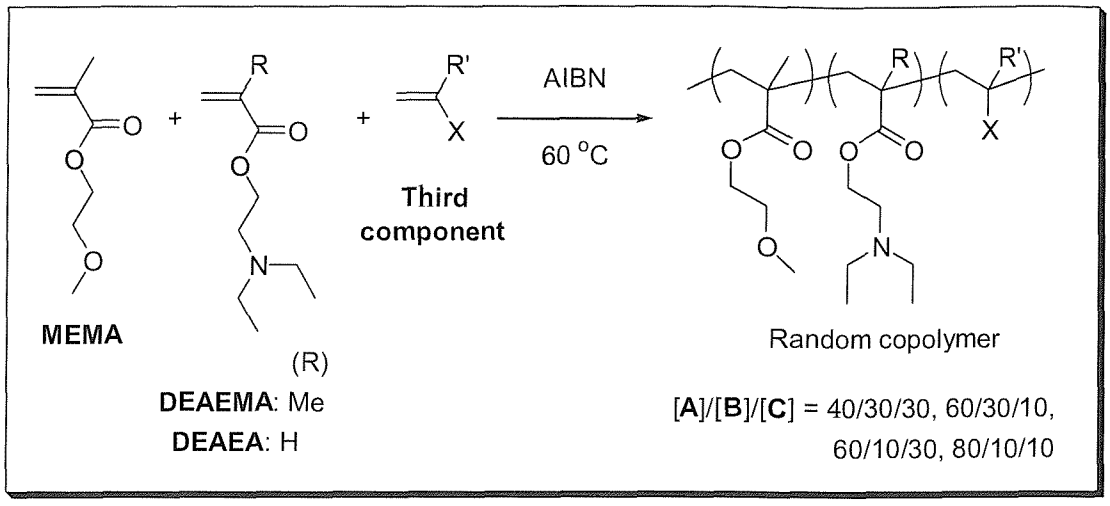


Figure 2.4.5. Polymer library-5.

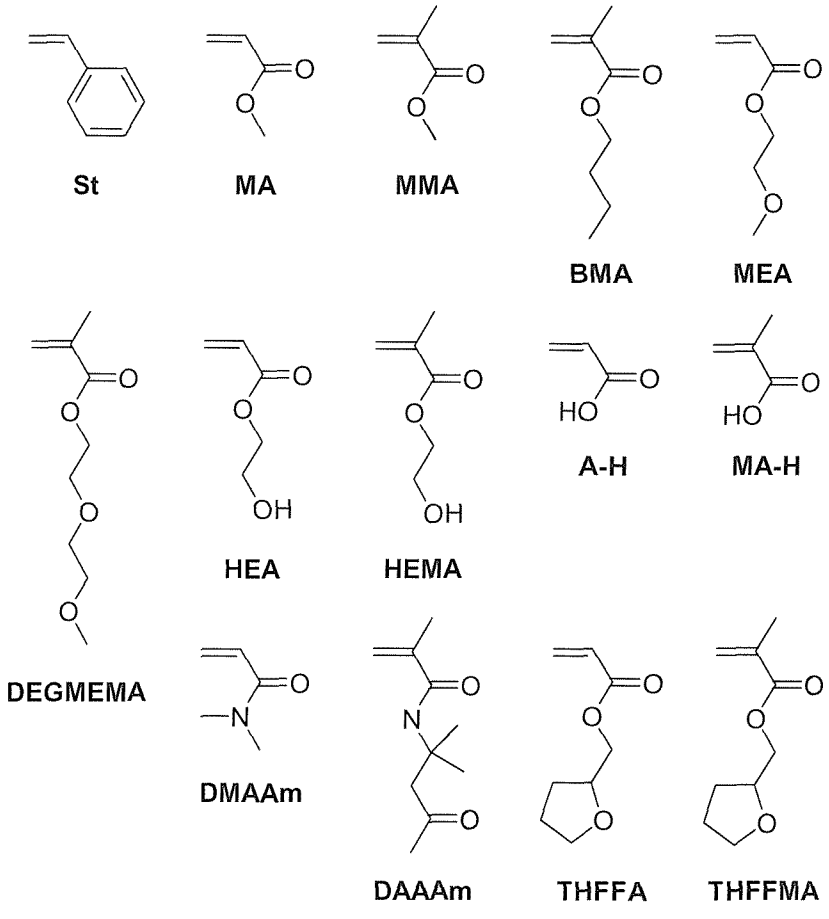


Figure 2.4.6. Structures of the third components selected for polymerisation.

Table 2.4.5. Properties of the third components.

Monomer		Functionality			Tg of Homopolymer* (K)
St	Vinyl	-	-	Aromatic ring	373
MA	Acrylate	-	-	-	283
MMA	Methacrylate	-	-	-	378
BMA	Methacrylate	-	-	-	293
MEA	Acrylate	-	EG unit	-	223
DEGMEMA	Methacrylate	-	EG unit	-	-
HEA	Acrylate	-OH	EG unit	-	-
HEMA	Methacrylate	-OH	EG unit	-	358
DMAAm	Acrylamide	-	-	Week cationic	362
DAAAm	Acrylamide	-	-	Week cationic	-
A-H	Acrylate	-COOH	-	Anionic	379
MA-H	Methacrylate	-COOH	-	Anionic	501
THFFA	Acrylate	-	(EG unit)	-	321
THFFMA	Methacrylate	-	(EG unit)	-	-

* Tg: glass transition temperature of homopolymer: reference[152]

Table 2.4.6. Data for polymer library-5.

No.	Monomer		Ratio (mol.%)	Appearance	Yield	Mn	MWD
	B	C	MEMA/B/C		(mol.%)	(Da)	
PL5-1	DEAEMA	MA	40/30/30	Solid	90%	39,000	4.2
PL5-2	DEAEMA	MA	60/10/30	Solid	78%	39,800	3.3
PL5-3	DEAEMA	MA	60/30/10	Solid	70%	35,200	3.0
PL5-4	DEAEMA	MA	80/10/10	Solid	81%	50,900	3.4
PL5-5	DEAEA	MA	40/30/30	Rubbery solid	78%	34,300	3.5
PL5-6	DEAEA	MA	60/10/30	Rubbery solid	74%	38,800	3.1
PL5-7	DEAEA	MA	60/30/10	Rubber	76%	23,500	3.7
PL5-8	DEAEA	MA	80/10/10	Rubbery solid	81%	45,700	3.3
PL5-9	DEAEMA	BMA	40/30/30	Solid	89%	37,700	4.0
PL5-10	DEAEMA	BMA	60/10/30	Solid	77%	37,700	3.0
PL5-11	DEAEMA	BMA	60/30/10	Solid	78%	36,700	3.0
PL5-12	DEAEMA	BMA	80/10/10	Solid	73%	38,200	3.0
PL5-13	DEAEA	BMA	40/30/30	Rubbery	71%	24,600	3.3
PL5-14	DEAEA	BMA	60/10/30	Rubbery solid	77%	37,400	3.0
PL5-15	DEAEA	BMA	60/30/10	Rubber	77%	28,100	3.6
PL5-16	DEAEA	BMA	80/10/10	Solid	80%	40,800	3.1
PL5-17	DEAEMA	MEA	40/30/30	Rubbery solid	84%	22,700	4.0
PL5-18	DEAEMA	MEA	60/10/30	Rubbery solid	88%	32,400	6.0
PL5-19	DEAEMA	MEA	60/30/10	Rubbery solid	88%	32,100	4.3
PL5-20	DEAEMA	MEA	80/10/10	Solid	90%	38,500	5.0
PL5-21	DEAEA	MEA	40/30/30	Rubbery solid	Quant.	18,700	4.0
PL5-22	DEAEA	MEA	60/10/30	Rubbery solid	84%	30,800	6.0
PL5-23	DEAEA	MEA	60/30/10	Rubbery solid	67%	23,500	3.3
PL5-24	DEAEA	MEA	80/10/10	Solid	88%	38,300	6.0
PL5-25	DEAEMA	DEGMEMA	40/30/30	Rubbery solid	85%	41,200	4.0
PL5-26	DEAEMA	DEGMEMA	60/10/30	Solid	86%	46,700	4.9
PL5-27	DEAEMA	DEGMEMA	60/30/10	Solid	81%	36,100	3.1
PL5-28	DEAEMA	DEGMEMA	80/10/10	Solid	79%	42,200	3.3
PL5-29	DEAEA	DEGMEMA	40/30/30	Rubbery	67%	30,700	3.0
PL5-30	DEAEA	DEGMEMA	60/10/30	Rubbery solid	68%	47,300	4.4
PL5-31	DEAEA	DEGMEMA	60/30/10	Rubber	81%	31,000	4.7
PL5-32	DEAEA	DEGMEMA	80/10/10	Solid	84%	40,600	3.7

(cont'd)

No.	Monomer		Ratio (mol.%)	Appearance	Yield	Mn	MWD
	B	C	MEMA/B/C		(mol.%)	(Da)	
PL5-33	DEAEMA	THFFA	40/30/30	Rubbery solid	67%	35,400	7.5
PL5-34	DEAEMA	THFFA	60/10/30	Rubbery solid	74%	46,400	4.4
PL5-35	DEAEMA	THFFA	60/30/10	Rubbery solid	71%	33,200	3.1
PL5-36	DEAEMA	THFFA	80/10/10	Solid	77%	40,900	3.8
PL5-37	DEAEA	THFFA	40/30/30	Rubber	66%	24,500	3.6
PL5-38	DEAEA	THFFA	60/10/30	Rubbery solid	66%	43,000	4.3
PL5-39	DEAEA	THFFA	60/30/10	Rubber	77%	30,500	5.1
PL5-40	DEAEA	THFFA	80/10/10	Solid	82%	54,100	4.6
PL5-41	DEAEMA	THFFMA	40/30/30	Solid	86%	42,200	3.6
PL5-42	DEAEMA	THFFMA	60/10/30	Solid	82%	50,200	3.6
PL5-43	DEAEMA	THFFMA	60/30/10	Solid	79%	59,500	2.6
PL5-44	DEAEMA	THFFMA	80/10/10	Solid	83%	57,400	2.7
PL5-45	DEAEA	THFFMA	40/30/30	Rubbery solid	84%	33,800	3.7
PL5-46	DEAEA	THFFMA	60/10/30	Solid	80%	47,600	3.1
PL5-47	DEAEA	THFFMA	60/30/10	Rubbery solid	81%	31,100	3.7
PL5-48	DEAEA	THFFMA	80/10/10	Solid	86%	50,600	3.0
PL5-49	DEAEMA	HEA	40/30/30	Rubbery solid	87%	23,000	3.2
PL5-50	DEAEMA	HEA	60/10/30	Rubbery solid	98%	31,400	4.7
PL5-51	DEAEMA	HEA	60/30/10	Rubbery solid	89%	30,100	3.5
PL5-52	DEAEMA	HEA	80/10/10	Rubbery solid	94%	41,100	4.5
PL5-53	DEAEA	HEA	40/30/30	Rubber	87%	21,300	3.5
PL5-54	DEAEA	HEA	60/10/30	Rubbery solid	94%	29,100	4.8
PL5-55	DEAEA	HEA	60/30/10	Rubber	87%	18,800	4.1
PL5-56	DEAEA	HEA	80/10/10	Rubbery solid	95%	32,600	4.4
PL5-57	DEAEMA	HEMA	40/30/30	Solid	95%	42,900	4.7
PL5-58	DEAEMA	HEMA	60/10/30	Solid	Quant.	57,300	6.3
PL5-59	DEAEMA	HEMA	60/30/10	Solid	93%	37,100	3.9
PL5-60	DEAEMA	HEMA	80/10/10	Solid	94%	46,400	4.3
PL5-61	DEAEA	HEMA	40/30/30	Rubbery solid	89%	22,300	3.7
PL5-62	DEAEA	HEMA	60/10/30	Solid	96%	48,500	5.6
PL5-63	DEAEA	HEMA	60/30/10	Rubbery solid	82%	30,900	3.1
PL5-64	DEAEA	HEMA	80/10/10	Solid	94%	37,800	4.7

(*cont'd*)

No.	Monomer		Ratio (mol.%)	Appearance	Yield	Mn	MWD
	B	C	MEMA/B/C		(mol.%)	(Da)	
PL5-65	DEAEMA	A-H	40/30/30	Solid	Quant.	37,800	2.2
PL5-66	DEAEMA	A-H	60/10/30	Solid	Quant.	52,600	2.2
PL5-67	DEAEMA	A-H	60/30/10	Solid	96%	40,800	3.3
PL5-68	DEAEMA	A-H	80/10/10	Solid	99%	53,400	3.4
PL5-69	DEAEA	A-H	40/30/30	Rubber	94%	13,200	2.8
PL5-70	DEAEA	A-H	60/10/30	Rubbery solid	Quant.	49,400	2.1
PL5-71	DEAEA	A-H	60/30/10	Rubber	88%	19,500	3.2
PL5-72	DEAEA	A-H	80/10/10	Rubbery solid	99%	54,600	2.9
PL5-73	DEAEMA	MA-H	40/30/30	Solid	Quant.	58,400	3.5
PL5-74	DEAEMA	MA-H	60/10/30	Solid	Quant.	46,500	2.8
PL5-75	DEAEMA	MA-H	60/30/10	Solid	Quant.	38,900	2.6
PL5-76	DEAEMA	MA-H	80/10/10	Solid	91%	60,000	3.0
PL5-77	DEAEA	MA-H	40/30/30	Solid	Quant.	43,900	2.8
PL5-78	DEAEA	MA-H	60/10/30	Rubbery solid	Quant.	36,500	3.0
PL5-79	DEAEA	MA-H	60/30/10	Solid	87%	35,300	3.5
PL5-80	DEAEA	MA-H	80/10/10	Rubbery solid	Quant.	54,300	3.0
PL5-81	DEAEMA	DMAAm	40/30/30	Solid	85%	28,500	3.3
PL5-82	DEAEMA	DMAAm	60/10/30	Solid	82%	34,800	3.4
PL5-83	DEAEMA	DMAAm	60/30/10	Solid	81%	32,800	3.1
PL5-84	DEAEMA	DMAAm	80/10/10	Solid	81%	40,400	3.3
PL5-85	DEAEA	DMAAm	40/30/30	Rubbery solid	76%	26,100	2.8
PL5-86	DEAEA	DMAAm	60/10/30	Solid	80%	32,100	3.3
PL5-87	DEAEA	DMAAm	60/30/10	Rubbery solid	73%	26,400	3.0
PL5-88	DEAEA	DMAAm	80/10/10	Solid	79%	35,400	3.2
PL5-89	DEAEMA	DAAAm	40/30/30	Rubbery solid	83%	33,400	3.9
PL5-90	DEAEMA	DAAAm	60/10/30	Rubbery solid	87%	44,400	4.4
PL5-91	DEAEMA	DAAAm	60/30/10	Rubbery solid	92%	38,700	4.4
PL5-92	DEAEMA	DAAAm	80/10/10	Rubbery solid	90%	46,600	5.2
PL5-93	DEAEA	DAAAm	40/30/30	Rubber	88%	29,400	3.4
PL5-94	DEAEA	DAAAm	60/10/30	Rubbery solid	89%	36,300	5.0
PL5-95	DEAEA	DAAAm	60/30/10	Rubber	78%	24,100	2.9
PL5-96	DEAEA	DAAAm	80/10/10	Rubbery solid	90%	43,400	4.9

(cont'd)

No.	Monomer		Ratio (mol.%)	Appearance	Yield	Mn	MWD
	B	C	MEMA/B/C		(mol.%)	(Da)	
PL5-97	DEAEMA	MMA	40/30/30	Solid	93%	46,500	3.6
PL5-98	DEAEMA	MMA	60/10/30	Solid	91%	54,100	3.8
PL5-99	DEAEMA	MMA	60/30/10	Solid	93%	51,700	4.2
PL5-100	DEAEMA	MMA	80/10/10	Solid	86%	58,300	4.2
PL5-101	DEAEA	MMA	40/30/30	Rubbery solid	77%	31,400	3.2
PL5-102	DEAEA	MMA	60/10/30	Solid	90%	51,147	3.5
PL5-103	DEAEA	MMA	60/30/10	Rubbery solid	79%	33,300	3.2
PL5-104	DEAEA	MMA	80/10/10	Solid	91%	49,100	4.2
PL5-105	DEAEMA	St	40/30/30	Solid	64%	35,700	2.0
PL5-106	DEAEMA	St	60/10/30	Solid	65%	42,400	2.0
PL5-107	DEAEMA	St	60/30/10	Solid	85%	51,300	2.8
PL5-108	DEAEMA	St	80/10/10	Solid	87%	59,100	2.9
PL5-109	DEAEA	St	40/30/30	Rubbery solid	Quant.	33,400	2.0
PL5-110	DEAEA	St	60/10/30	Solid	63%	37,500	2.1
PL5-111	DEAEA	St	60/30/10	Rubbery solid	68%	35,400	2.4
PL5-112	DEAEA	St	80/10/10	Solid	85%	55,400	2.8

2.4.6 Polymer library-6

Most biopolymers such as proteins and nucleotides contain ionic functionality. Ionic polymers may be divided into two categories, polyelectrolytes and polyzwitterions. Polyelectrolytes contain anionic or cationic groups, while polyzwitterions contain both anionic and cationic groups. Several researchers have focused on electrostatic interactions of polymers with cells and found that a small amount of positive charge induces cell adhesion, though too much causes cells lysis. It is thus important to control the surface charge of the polymer, and polyzwitterions are thus remarkable for bio-compatible materials.[150,151]

Though some polyzwitterions were prepared in polymer library-5, in this polymer library-6, the monomer type and composition of polyzwitterions were extended using MEMA or MMA as the main monomer, A-H or MA-H as the acidic monomer, and DEAEMA, DEAEA, DMAEMA, DMAEA, and DMAPMAAm as a monomer containing an amino group with varying monomer compositions (Figure 2.4.7).

Polymerisation was carried out at 60 °C in DMF using AIBN as an initiator. The results of polymerisation were shown in Table 2.4.7, and polymers were obtained with molecular weights, Mn 17,100 – 88,800 Da in good yields.

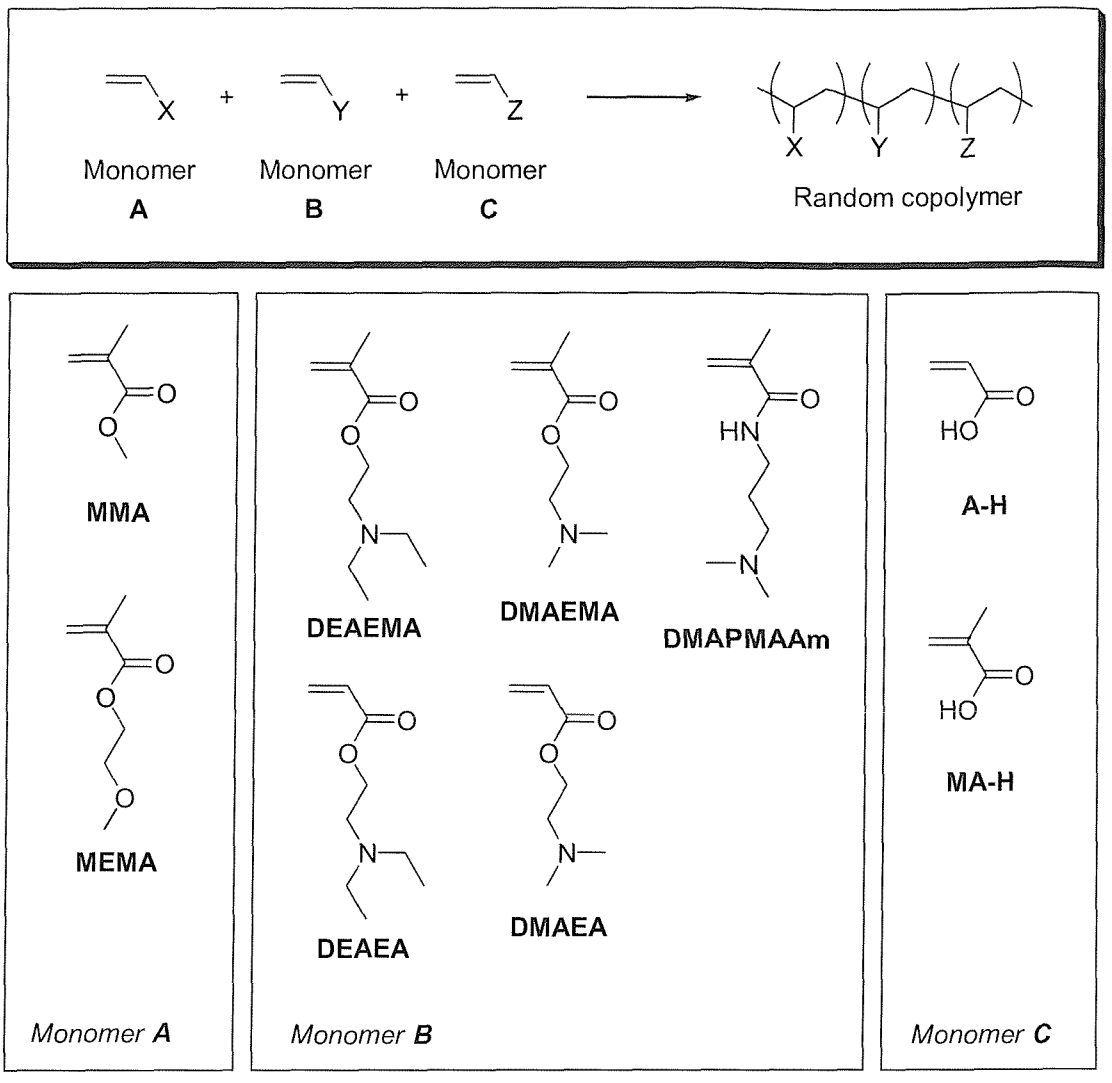


Figure 2.4.7. Monomers used in polymer library-6.

Table 2.4.7. Data for polymer library-6.

No.	Monomer*			Ratio (mol.%)	Appearance	Yield	Mn	MWD
	A	B	C	A/B/C		(mol.%)	(Da)	
PL6-1	A1	B1	C1	70/10/20	Solid	80%	36,900	2.0
PL6-2	A1	B1	C1	70/15/15	Solid	79%	42,200	2.1
PL6-3	A1	B1	C1	70/20/10	Solid	76%	44,700	2.4
PL6-4	A1	B2	C1	70/10/20	Solid	74%	27,500	2.0
PL6-5	A1	B2	C1	70/15/15	Solid	79%	31,600	2.0
PL6-6	A1	B2	C1	70/20/10	Solid	76%	28,300	2.2
PL6-7	A1	B1	C2	70/10/20	Solid	99%	34,400	1.7
PL6-8	A1	B1	C2	70/15/15	Solid	95%	31,700	1.9
PL6-9	A1	B1	C2	70/20/10	Solid	90%	42,300	2.0
PL6-10	A1	B2	C2	70/10/20	Solid	95%	33,200	1.7
PL6-11	A1	B2	C2	70/15/15	Solid	85%	25,500	2.1
PL6-12	A1	B2	C2	70/20/10	Solid	81%	26,100	2.2
PL6-13	A2	B1	C1	70/10/20	Solid	92%	60,600	2.6
PL6-14	A2	B1	C1	70/15/15	Solid	79%	53,000	3.0
PL6-15	A2	B1	C1	70/20/10	Solid	78%	58,100	2.9
PL6-16	A2	B2	C1	70/10/20	Solid	89%	61,600	2.4
PL6-17	A2	B2	C1	70/15/15	Solid	74%	50,300	2.5
PL6-18	A2	B2	C1	70/20/10	Solid	83%	35,700	3.1
PL6-19	A2	B1	C2	70/10/20	Solid	84%	50,200	2.2
PL6-20	A2	B1	C2	70/15/15	Solid	85%	48,300	2.6
PL6-21	A2	B1	C2	70/20/10	Solid	84%	54,200	2.6
PL6-22	A2	B2	C2	70/10/20	Solid	84%	46,000	2.3
PL6-23	A2	B2	C2	70/15/15	Solid	81%	37,000	2.9
PL6-24	A2	B2	C2	70/20/10	Solid	75%	38,900	2.7
PL6-25	A2	B1	C1	85/10/5	Solid	88%	48,400	4.2
PL6-26	A2	B1	C1	80/15/5	Solid	89%	52,300	4.0
PL6-27	A2	B1	C1	75/20/5	Solid	90%	41,300	4.0
PL6-28	A2	B1	C1	70/25/5	Solid	86%	42,000	3.9
PL6-29	A2	B1	C1	65/30/5	Solid	83%	43,100	3.7
PL6-30	A2	B1	C1	60/35/5	Solid	84%	42,700	3.7
PL6-31	A2	B1	C1	55/40/5	Solid	85%	37,100	3.3
PL6-32	A2	B1	C1	50/45/5	Solid	84%	35,600	3.6
PL6-33	A2	B1	C1	75/15/10	Solid	87%	50,500	3.4

(cont'd)

No.	Monomer*			Ratio (mol.%)	Appearance	Yield	Mn	MWD
	A	B	C	A/B/C		(mol.%)	(Da)	
PL6-34	A2	B1	C1	65/25/10	Solid	88%	33,100	3.1
PL6-35	A2	B1	C1	55/35/10	Solid	85%	36,400	2.9
PL6-36	A2	B1	C1	50/40/10	Solid	86%	34,500	2.9
PL6-37	A2	B1	C1	65/20/15	Solid	89%	48,800	2.6
PL6-38	A2	B1	C1	60/25/15	Solid	86%	43,100	2.9
PL6-39	A2	B1	C1	55/30/15	Solid	88%	34,200	3.0
PL6-40	A2	B1	C1	50/35/15	Solid	87%	33,900	3.0
PL6-41	A2	B1	C1	55/25/20	Solid	90%	42,000	2.4
PL6-42	A2	B1	C1	50/30/20	Solid	88%	38,300	2.2
PL6-43	A2	B1	C1	90/5/5	Solid	89%	52,300	3.7
PL6-44	A2	B1	C1	80/5/15	Solid	92%	49,300	3.0
PL6-45	A2	B1	C1	70/5/25	Solid	93%	49,300	2.4
PL6-46	A2	B1	C1	60/5/35	Solid	95%	50,500	2.2
PL6-47	A2	B1	C1	50/5/45	Solid	97%	40,500	1.9
PL6-48	A2	B1	C1	50/10/40	Solid	Quant.	52,800	1.8
PL6-49	A2	B1	C1	60/15/25	Solid	90%	53,500	2.4
PL6-50	A2	B1	C1	50/15/35	Solid	97%	51,200	2.2
PL6-51	A2	B1	C1	60/20/20	Solid	90%	44,800	2.8
PL6-52	A2	B1	C1	50/20/30	Solid	94%	45,900	2.3
PL6-53	A2	B1	C1	50/25/25	Solid	93%	45,800	2.3
PL6-54	A2	B1	C1	75/22.5/2.5	Solid	92%	48,500	4.6
PL6-55	A2	B1	C1	70/27.5/2.5	Solid	92%	60,800	3.7
PL6-56	A2	B1	C1	70/22.5/7.5	Solid	92%	77,000	3.2
PL6-57	A2	B1	C1	65/27.5/7.5	Solid	92%	47,400	4.6
PL6-58	A2	B1	C1	60/32.5/7.5	Solid	91%	57,200	3.5
PL6-59	A2	B1	C1	65/22.5/12.5	Solid	92%	51,300	3.9
PL6-60	A2	B1	C1	60/27.5/12.5	Solid	93%	51,600	3.8
PL6-61	A2	B1	C1	55/32.5/12.5	Solid	93%	55,600	3.5
PL6-62	A2	B2	C1	85/10/5	Solid	90%	47,800	3.2
PL6-63	A2	B2	C1	80/15/5	Solid	86%	45,000	3.2
PL6-64	A2	B2	C1	75/20/5	Solid	85%	44,400	3.0
PL6-65	A2	B2	C1	70/25/5	Solid	82%	26,500	4.2
PL6-66	A2	B2	C1	65/30/5	Rubbery solid	77%	23,700	3.8

(*cont'd*)

No.	Monomer*			Ratio (mol.%)	Appearance	Yield	Mn	MWD
	A	B	C	A/B/C		(mol.%)	(Da)	
PL6-67	A2	B2	C1	60/35/5	Rubbery solid	71%	25,300	2.8
PL6-68	A2	B2	C1	55/40/5	Rubbery solid	67%	23,300	2.8
PL6-69	A2	B2	C1	50/45/5	Rubbery solid	61%	20,700	3.5
PL6-70	A2	B2	C1	75/15/10	Solid	87%	59,400	2.4
PL6-71	A2	B2	C1	65/25/10	Solid	82%	34,100	3.0
PL6-72	A2	B2	C1	55/35/10	Solid	75%	22,500	3.0
PL6-73	A2	B2	C1	50/40/10	Rubbery solid	67%	17,100	3.1
PL6-74	A2	B2	C1	65/20/15	Rubbery solid	85%	41,700	3.0
PL6-75	A2	B2	C1	60/25/15	Solid	78%	36,600	2.8
PL6-76	A2	B2	C1	55/30/15	Solid	77%	36,600	2.3
PL6-77	A2	B2	C1	50/35/15	Rubbery solid	72%	21,200	3.0
PL6-78	A2	B2	C1	55/25/20	Solid	81%	27,000	2.6
PL6-79	A2	B2	C1	50/30/20	Solid	79%	46,400	2.2
PL6-80	A2	B3	C1	85/10/5	Solid	86%	44,500	3.7
PL6-81	A2	B3	C1	80/15/5	Solid	85%	83,500	2.8
PL6-82	A2	B4	C1	85/10/5	Solid	89%	67,000	3.0
PL6-83	A2	B4	C1	80/15/5	Solid	89%	73,900	2.7
PL6-84	A2	B5	C1	85/10/5	Solid	90%	65,400	3.0
PL6-85	A2	B5	C1	80/15/5	Solid	88%	88,800	2.1

*A1: MMA, A2: MEMA, B1: DEAEMA, B2: DEAEA, B3: DMAEMA, B4: DMAEA, B5: DMAPMAAm, C1: A-H, C2: MA-H

2.5 Diversification of polymers containing amino groups

Amines of various structures should have remarkable effects on protein adhesion (Chapter 4.4). However, most of the commercially available monomers with amine functionality were already investigated (Chapter 2.4). Therefore, an alternative approach was envisaged. Monomers with reactive functional groups such as epoxides, can react with secondary amines to form tertiary amines, and could be useful in introducing new amine environments into the polymers,[153-157] and importantly there are few limitations on the range of available amines. Glycidyl methacrylate (GMA) was chosen as a reactive functional monomer, and MEMA-GMA and MMA-GMA copolymers were synthesised with three different monomer compositions (90/10, 70/30, and 50/50 mol.%) by free radical polymerisation (Figure 2.5.1). All copolymers were obtained in good yields with high molecular weights (Mn 93,900 – 154,000 Da). The products were analysed by Infrared (IR), and no hydroxyl peaks (3650 - 3584 cm^{-1}) were observed confirming the stability of the epoxides to the free radical polymerisation conditions (Figure 2.5.2).[158]

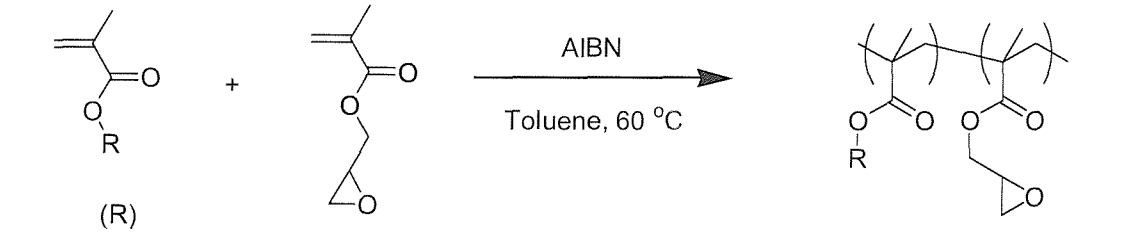


Figure 2.5.1. Syntheses of P(MEMA-*co*-GMA) and P(MMA-*co*-GMA).

Table 2.5.1. Data for GMA copolymers.

No.	Monomer		Ratio	Appearance	Yield	Mn	MWD
	A	B	(mol.%)		(mol.%)	(Da)	
G1	MEMA	GMA	90/10	Solid	85%	134,000	2.9
G2	MEMA	GMA	70/30	Solid	71%	139,000	3.6
G3	MEMA	GMA	50/50	Solid	97%	93,900	3.1
G4	MMA	GMA	90/10	Solid	60%	128,000	2.1
G5	MMA	GMA	70/30	Solid	81%	154,000	2.1
G6	MMA	GMA	50/50	Solid	70%	112,000	2.2

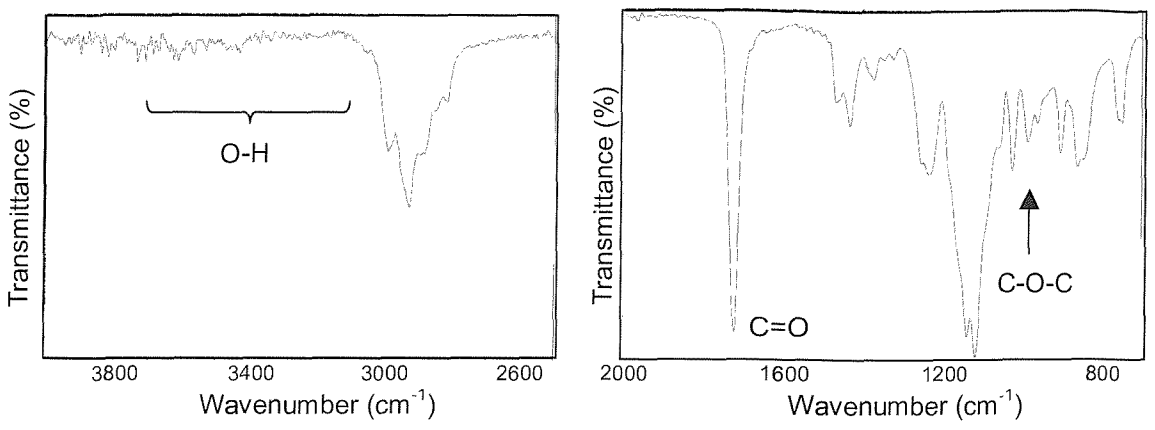


Figure 2.5.2. IR spectra of P(MEMA-*co*-GMA) (50/50 mol.%).

Functionalisation of the GMA copolymers with an excess of diisopropylamine (DiPA, b.p. 84 °C) was attempted in dioxane and/or *n*-butanol (*n*-BuOH) at 80 °C with silica gel as a catalyst, as it has been reported that the conversion is increased, and etherification as a side-reaction is not observed in the presence of the silica gel.[159] However no significant changes in the polymer structure could be observed under these conditions by IR analysis and NMR (Table 2.5.2). Therefore, the reaction solvent and the amine were changed to a mixture of *m*-xylene and cyclohexanol (CyOH) and di-*n*-butylamine (DnBA, b.p. 159 °C). Following reaction at 130 °C overnight, the epoxy peaks had disappeared, and resonances corresponding to the amine protons were observed in the NMR spectrum, the methoxy peak remained without change (Figure 2.5.3).

Table 2.5.2. Results of reaction between GMA copolymer and amine.

No.	Amine	Equiv.	Solvent	Cat.	Temp. (°C)	Results	
						Colour of solution	Conversion (%)*
1	DiPA	5	Dioxane	-	80	Colourless	0
2	DiPA	5	Dioxane	Silicagel 60	80	Colourless	0
3	DiPA	5	Dioxane/*n*-BuOH	-	80	Colourless	-
4	DiPA	5	Dioxane/*n*-BuOH	Silicagel 60	80	Colourless	-
5	DnBA	5	*m*-Xylene/CyOH	-	130	Brown	~ 100%
6	DnBA	5	*m*-Xylene/CyOH	Silicagel 60	130	Yellow	~ 100%
7	DnBA	1.1	*m*-Xylene/CyOH	-	130	Brown	-
8	DnBA	1.1	*m*-Xylene/CyOH	Silicagel 60	130	Colourless	~ 100%

* Conversion of epoxide was determined by NMR

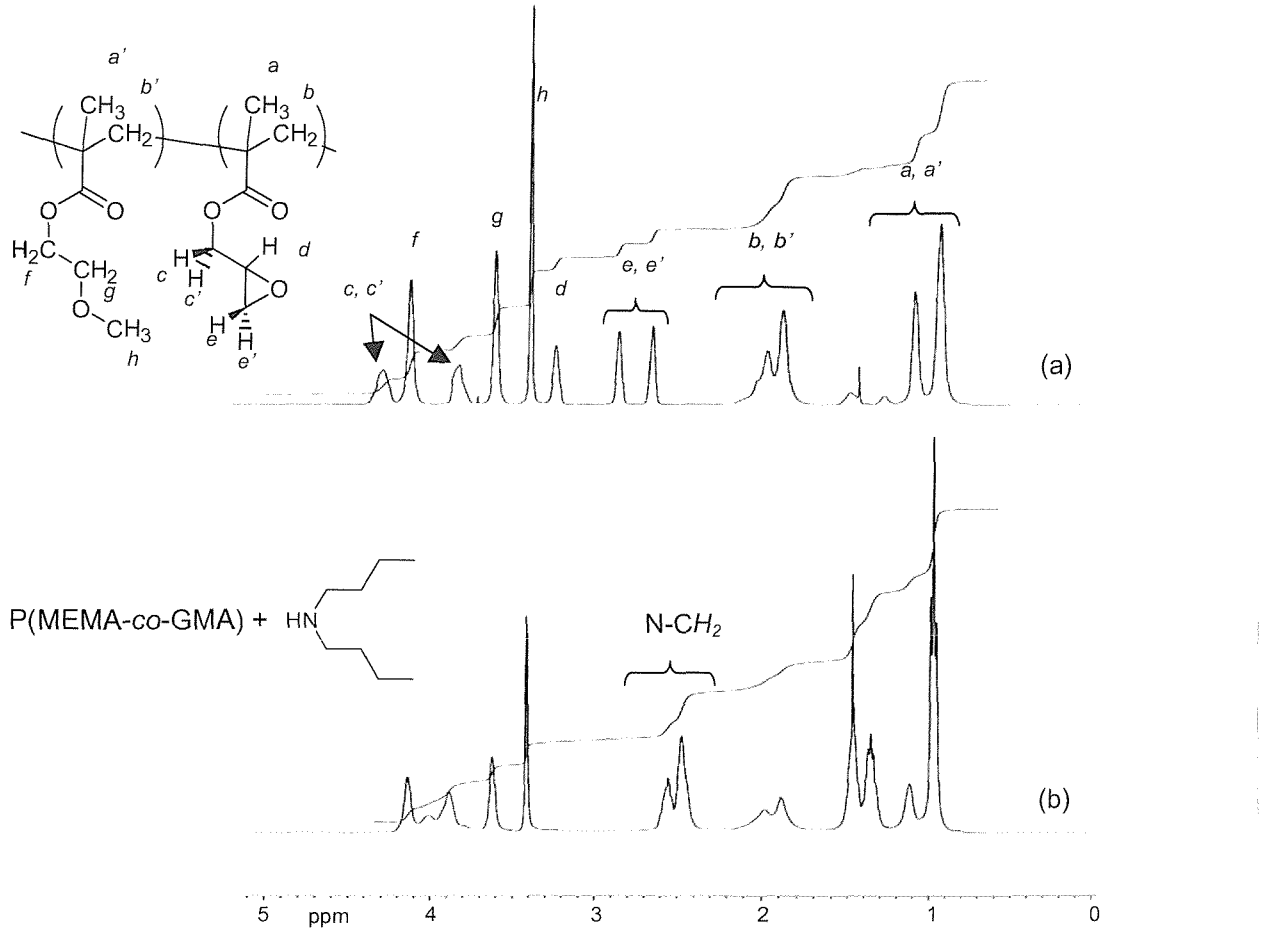


Figure 2.5.3. NMR spectra of (a) P(MEMA-*co*-GMA) and (b) the product after reaction of P(MEMA-*co*-GMA) with DnBA.

Different amines were used for the functionalisation of the GMA copolymer (Figure 2.5.4), although in some cases, a gel was formed during the reaction. Since the gel could not be purified and analysed, a number of reaction conditions were examined by varying the equivalents of amine and the polymer concentration used to give non gel like polymers.

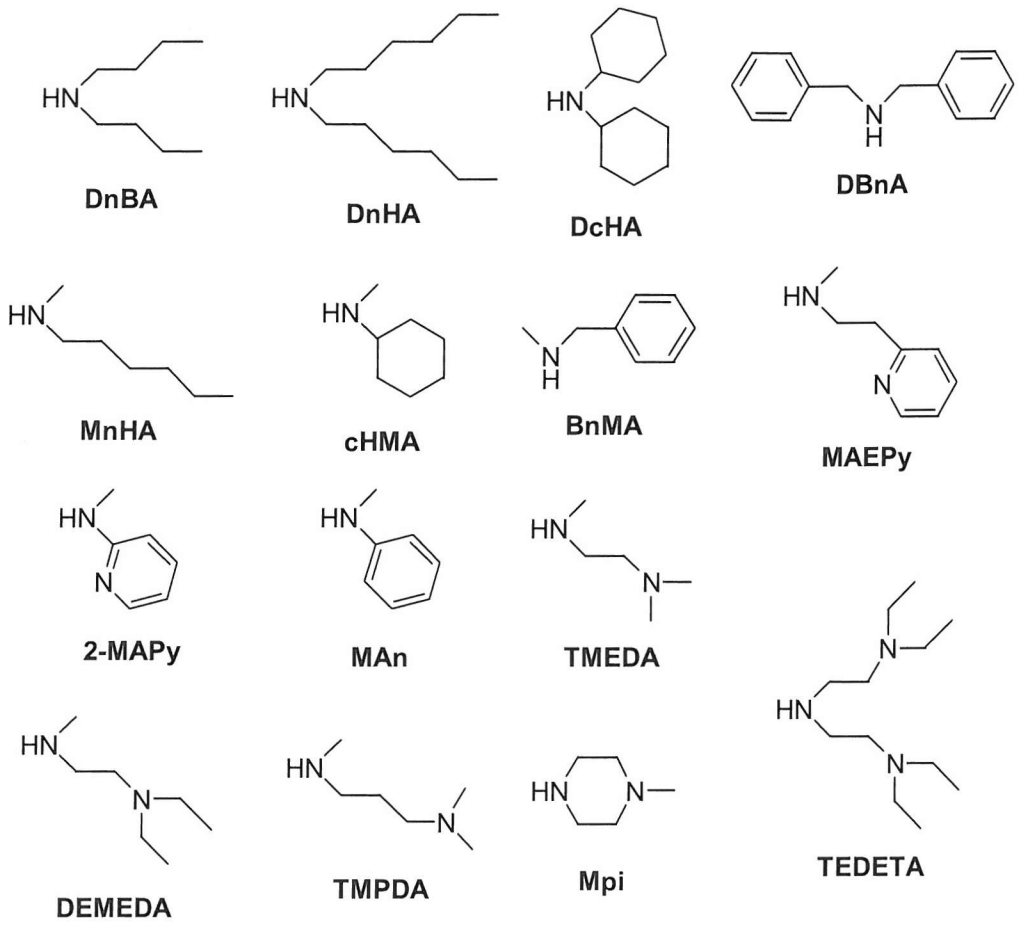


Figure 2.5.4. Different amines for functionalisation of GMA copolymer.

In the case of cyclohexanemethylamine (cHMA), a polymer gel was formed when 1.0 equivalents or less of amine was used with respect to the epoxy group. Even at 1.5 – 2.0 equivalents, partial gelation was observed. At higher concentrations of amine, the polymer gel was not observed (Figure 2.5.5).

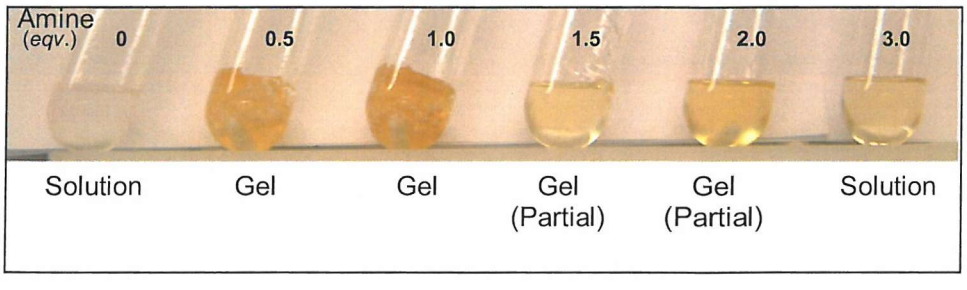


Figure 2.5.5. Products obtained from the reaction between P(MEMA-*co*-GMA) with cyclohexanemethylamine at a polymer concentration of 50 mg/mL.

In the case of trimethylethylenediamine (TMEDA), gel formation was observed even at 3.0 equivalents of amine. As the polymer concentration in the reaction mixture decreased, gel formation was found to be reduced (Table 2.5.3).

Table 2.5.3. Effect of polymer concentration on the polymer gel formation.

Polymer concentration (mg/mL)	50	20	13	10
Appearance	Gel	Gel (partially)	Solution	Solution

(TMEDA: 3.0 *eq.*)

The concentrations of both amine and polymer were thus found to affect gel formation. A mechanism of polymer gel formation is proposed in which the secondary amine reacts with the epoxy group on the polymer side chain to form a hydroxyl group and a tertiary amine. Though the reactivity of the hydroxyl group is much lower than an amino group, the hydroxyl group can react with the epoxy group, and the polymer will be cross-linked to form a gel (Figure 2.5.6). Although cyclohexanol has a hydroxyl group and was used as reaction solvent, it did not react with the epoxy group under the same conditions. Thus the amine acts as an internal base to promote cross-linking.

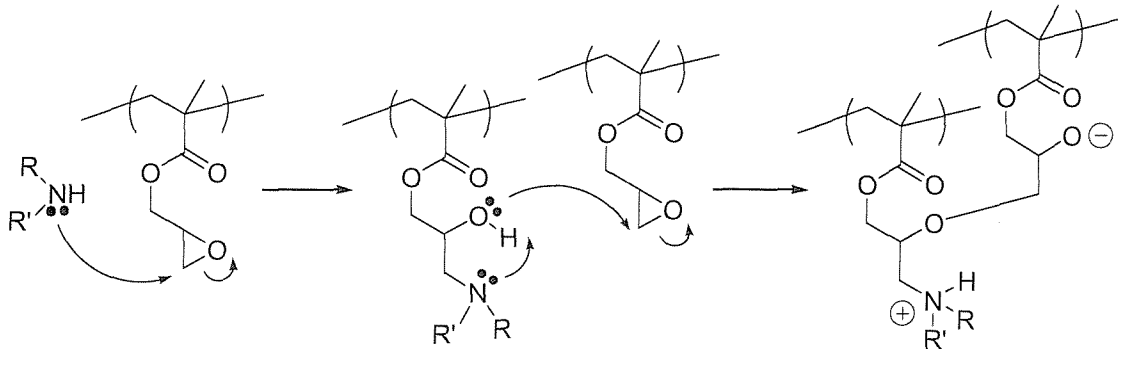


Figure 2.5.6. Proposed mechanism of polymer gel formation.

Functionalisations of the copolymers with 16 different amines were attempted. Figure 2.5.7 shows the NMR spectra of products after reaction of P(MEMA-*co*-GMA) with dibenzylamine (DBnA) and methylaniline (MAn). Though these amines are less nucleophilic and offer greater steric hindrance than dibutylamine, they reacted very well.

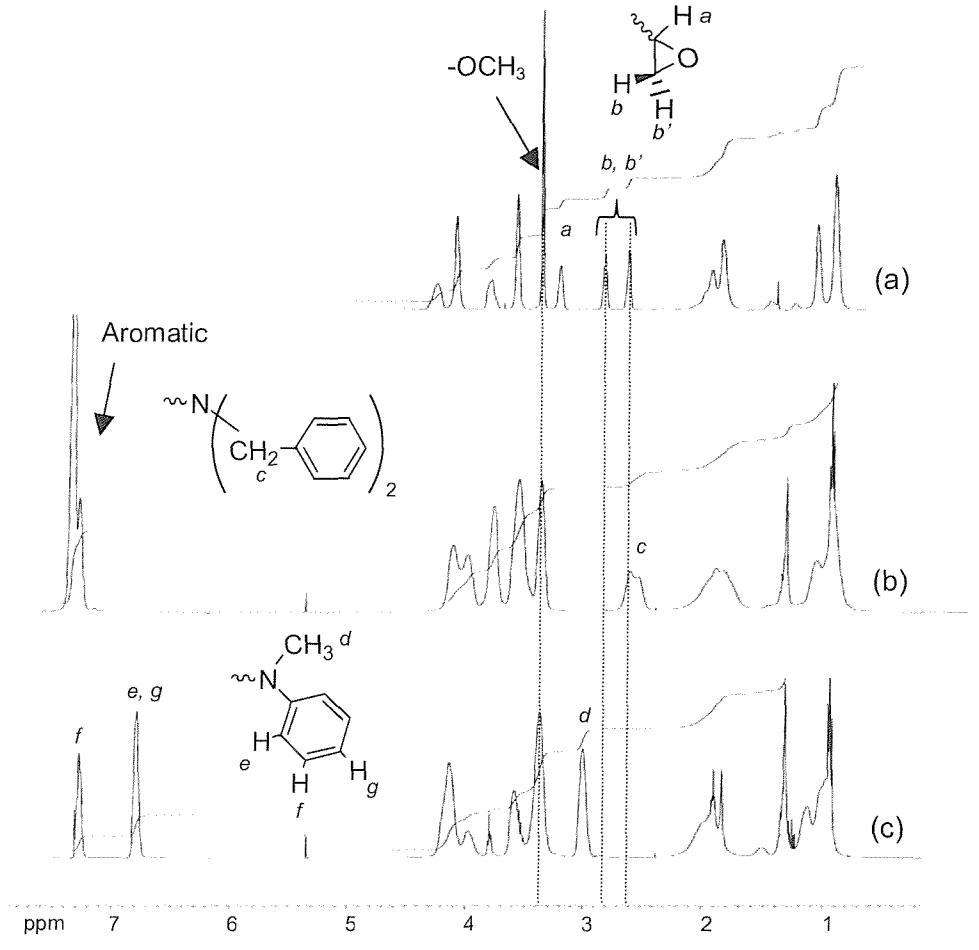


Figure 2.5.7. NMR spectra of (a) P(MEMA-*co*-GMA) and the product after reaction of P(MEMA-*co*-GMA) with (b) DBnA and (c) MAn.

All products were obtained in high yields, but unexpectedly, extremely high molecular weights were observed by GPC analysis, which might be caused by cross-linking. In some cases, the peaks corresponding to the polymer were not observed by the refractive index detector of the GPC. It was thought that the polymers were adsorbed on the column. The products of reaction between P(MMA-*co*-GMA) (50/50 mol.%) and 2-MEPy were not soluble in any solvent and could not be characterised.

Table 2.5.4. Results of functionalisation of GMA copolymer with amine.

No.	Prepolymer	Amine		Polymer conc.	Appearance	Yield	Mn	MWD
		Name	(eq.)	(mg/mL)		(mol%)	(Da)	
GA1	G1	DnBA	3	50	Solid	89%	149,000	5.1
GA2	G2	DnBA	3	50	Solid	94%	Mw > 2,000,000	
GA3	G3	DnBA	3	50	Rubber	78%	Mw > 2,000,000	
GA4	G1	DnHA	3	50	Solid	84%	134,000	7.0
GA5	G2	DnHA	3	50	Rubber	87%	Mw > 2,000,000	
GA6	G3	DnHA	3	50	Rubber	72%	Mw > 2,000,000	
GA7	G1	DcHA	3	50	Solid	83%	166,000	8.9
GA8	G2	DcHA	3	50	Solid	88%	Mw > 2,000,000	
GA9	G3	DcHA	3	50	Solid	79%	Mw > 2,000,000	
GA10	G1	DBnA	3	50	Solid	99%	131,000	5.2
GA11	G2	DBnA	3	50	Solid	Quant.	Mw > 2,000,000	
GA12	G3	DBnA	3	50	Solid	94%	Mw > 2,000,000	
GA13	G1	MnHA	3	50	Solid	90%	75,600	4.7
GA14	G2	MnHA	3	50	Solid	78%	Mw > 2,000,000	
GA15	G3	MnHA	3	50	Rubber	Quant.	Mw > 2,000,000	
GA16	G1	cHMA	10	50	Solid	98%	186,000	9.0
GA17	G2	cHMA	10	50	Solid	88%	Mw > 2,000,000	
GA18	G3	cHMA	10	50	Solid	92%	Mw > 2,000,000	
GA19	G1	BnMA	3	50	Solid	Quant.	235,000	7.7
GA20	G2	BnMA	3	50	Solid	Quant.	Mw > 2,000,000	
GA21	G3	BnMA	3	50	Solid	Quant.	Mw > 2,000,000	
GA22	G1	MAEPy	3	50	Solid	Quant.	Not soluble	
GA23	G2	MAEPy	3	50	Solid	Quant.	Not soluble	
GA24	G3	MAEPy	3	50	Solid	95%	Not soluble	
GA25	G1	2-MAPy	5	13	Solid	81%	Mw > 2,000,000	
GA26	G2	2-MAPy	5	13	Solid	71%	Mw > 2,000,000	
GA27	G3	2-MAPy	5	13	Solid	71%	Mw > 2,000,000	
GA28	G1	MAn	3	50	Solid	Quant.	292,000	2.5
GA29	G2	MAn	3	50	Solid	Quant.	Mw > 2,000,000	
GA30	G3	MAn	3	50	Solid	Quant.	Mw > 2,000,000	

(*cont'd*)

No.	Prepolymer	Amine		Polymer conc.	Appearance	Yield	Mn	MWD
		Name	(eq.)	(mg/mL)		(mol%)	(Da)	
GA31	G1	TMEDA	5	13	Solid	98%	Not detect	
GA32	G2	TMEDA	5	13	Solid	93%	Not detect	
GA33	G3	TMEDA	5	13	Solid	88%	Not detect	
GA34	G1	DEMEDA	3	50	Solid	99%	Not detect	
GA35	G2	DEMEDA	3	50	Rubber	99%	Not detect	
GA36	G3	DEMEDA	3	50	Rubber	Quant.	Not detect	
GA37	G1	TMPDA	5	13	Solid	82%	Not detect	
GA38	G2	TMPDA	5	13	Solid	81%	Not detect	
GA39	G3	TMPDA	5	13	Solid	78%	Not detect	
GA40	G1	Mpi	5	13	Solid	80%	Not detect	
GA41	G2	Mpi	5	13	Solid	Quant.	Not detect	
GA42	G3	Mpi	5	13	Solid	78%	Not detect	
GA43	G1	TEDETA	5	13	Solid	73%	Not detect	
GA44	G2	TEDETA	5	13	Solid	76%	Not detect	
GA45	G3	TEDETA	5	13	Solid	80%	Not detect	
GA46	G4	DnBA	3	50	Solid	83%	Mw > 2,000,000	
GA47	G5	DnBA	3	50	Solid	86%	Mw > 2,000,000	
GA48	G6	DnBA	3	50	Solid	Quant.	Mw > 2,000,000	
GA49	G4	DnHA	3	50	Solid	88%	529,000	3.6
GA50	G5	DnHA	3	50	Solid	77%	Mw > 2,000,000	
GA51	G6	DnHA	3	50	Solid	60%	Mw > 2,000,000	
GA52	G4	DcHA	3	50	Powder	75%	276,000	4.6
GA53	G5	DcHA	5	50	Powder	81%	Mw > 2,000,000	
GA54	G6	DcHA	5	50	Solid	78%	Mw > 2,000,000	
GA55	G4	DBnA	3	50	Powder	87%	170,000	3.7
GA56	G5	DBnA	3	50	Powder	80%	Mw > 2,000,000	
GA57	G6	DBnA	3	50	Solid	86%	Mw > 2,000,000	
GA58	G4	MnHA	3	50	Solid	77%	Mw > 2,000,000	
GA59	G5	MnHA	5	50	Solid	82%	Mw > 2,000,000	
GA60	G6	MnHA	5	50	Solid	87%	Mw > 2,000,000	

(*cont'd*)

No.	Prepolymer	Amine		Polymer conc.	Appearance	Yield	Mn	MWD
		Name	(eq.)	(mg/mL)		(mol%)	(Da)	
GA61	G4	cHMA	10	13	Powder	87%	Mw > 2,000,000	
GA62	G5	cHMA	10	13	Powder	84%	Mw > 2,000,000	
GA63	G6	cHMA	10	13	Powder	77%	Mw > 2,000,000	
GA64	G4	BnMA	3	50	Powder	86%	224,000	4.6
GA65	G5	BnMA	3	50	Solid	88%	Mw > 2,000,000	
GA66	G6	BnMA	3	50	Solid	91%	Mw > 2,000,000	
GA67	G4	MAEPy	3	50	Powder	81%	Mw > 2,000,000	
GA68	G5	MAEPy	5	13	Solid	Quant.	Mw > 2,000,000	
GA69	G6	MAEPy	5	50	Solid	68%	Mw > 2,000,000	
GA70	G4	2-MAPy	10	13	Powder	72%	Mw > 2,000,000	
GA71	G5	2-MAPy	10	13	Powder	71%	Mw > 2,000,000	
GA72	G6	2-MAPy	10	13		Gel		
GA73	G4	MAn	3	50	Powder	79%	Mw > 2,000,000	
GA74	G5	MAn	5	50	Powder	89%	Mw > 2,000,000	
GA75	G6	MAn	5	50	Solid	Quant.	Mw > 2,000,000	
GA76	G4	TMEDA	5	13	Solid	72%	Not detect	
GA77	G5	TMEDA	5	13	Solid	76%	Not detect	
GA78	G6	TMEDA	5	13	Solid	64%	Not detect	
GA79	G4	DEMEDA	3	50	Solid	79%	Not detect	
GA80	G5	DEMEDA	5	13	Solid	Quant.	Not detect	
GA81	G6	DEMEDA	5	13	Solid	87%	Not detect	
GA82	G4	TMPDA	5	13	Powder	79%	Not detect	
GA83	G5	TMPDA	5	13	Powder	76%	Not detect	
GA84	G6	TMPDA	5	13	Powder	62%	Not detect	
GA85	G4	Mpi	5	13	Solid	88%	Not detect	
GA86	G5	Mpi	5	13	Solid	82%	Not detect	
GA87	G6	Mpi	5	13	Solid	98%	Not detect	
GA88	G4	TEDETA	5	13	Solid	87%	Not detect	
GA89	G5	TEDETA	5	13	Solid	90%	Not detect	
GA90	G6	TEDETA	5	13	Solid	71%	Not detect	

2.6 Conclusions

In order to synthesise polymer libraries, free radical polymerisation techniques were utilised. The 12 parallel multi-reactor and HT-GPC were used to accelerate this process. Several parameters were examined for optimisation of free radical polymerisation, varying temperature, initiator, and monomer concentrations to allow control of molecular weight and polydispersity.

In totality, 6 different polymer libraries with 446 polymers in all, were prepared by varying the monomer type and composition. In order to expand the polymer library, diversification of a polymer containing an amine group was attempted by functionalisation of the epoxide with secondary amines, allowing 90 polymers to be synthesised. Most of these polymers were suitable for biological analysis/screening.

Chapter 3

High throughput polymer synthesis

3.1 Introduction

Conventionally polymerisation is usually carried out in a glass vessel under inert conditions with heating, and the polymer is purified by precipitation after reaction. Reaction conditions such as solvent, temperature, monomer, and initiator concentration depend on monomer type and often need to be optimised. Therefore, it takes a considerable amount of time to achieve the syntheses of the required polymers. The precipitation conditions also depend on the polymers properties such as its viscosity and solubility, and hence are unique for each polymer sample.

Nowadays various types of multi-reactors have been designed for combinatorial chemistry (Chapter 1.2). Some multi-reactors are useful for the polymer synthesis, and can perform several reactions at the same time under inert gas at high temperatures, but most of the robotic synthesisers, were designed for organic compound synthesis, and are not compatible with high throughput polymerisation chemistry.

3.1.1 Micro-plate formats

Micro-plate format arrays have been used in many biological fields.[105,110] There are many micro-plates commercially available with 24, 96, 384, and 1586 wells with various depths. The material used can be chosen from polystyrene, polypropylene, poly(tetrafluoroethylene), or glass depending on the required reaction, while filter plates are also available to combine with micro-plates for liquid/liquid or liquid/solid separation. Most micro-plate formats are compatible with automated robotic systems, and thus provide a great tool for organic synthesis.

3.1.2 Microarrays

Microarray technology has become a powerful tool for the miniaturisation of screening in various areas.[140] Compared to conventional assays, microarrays offer several advantages such as high density screening, good sensitivity, rapid detection, and the requirement for small quantities of material (in all arrays carried out under identical conditions). The key advantage of microarray-based methods is the parallel analysis of individual compounds at defined position on the array.

This technique is achieved with robotic instrumentation called a microarrayer which is used for sample spotting (Figure 3.1.1). Sample solutions are prepared in micro-plates (either 96 or 384 wells) and precisely spotted onto the surface of a substrate, commonly (functionalised) glass plates by equipment that can dispense tiny volumes (50 pL – 100 nL) of sample solution.[137] Usually the spot is brought by a pin or needle into contact with the surface with a positional accuracy of 1 μm. There are various types of pins which can furnish a variety of spots with various sizes and densities (Figure 3.1.2).[137] Thousands of individual compounds can be spotted on a single glass slide and can be analysed using numerous techniques developed for array screening.

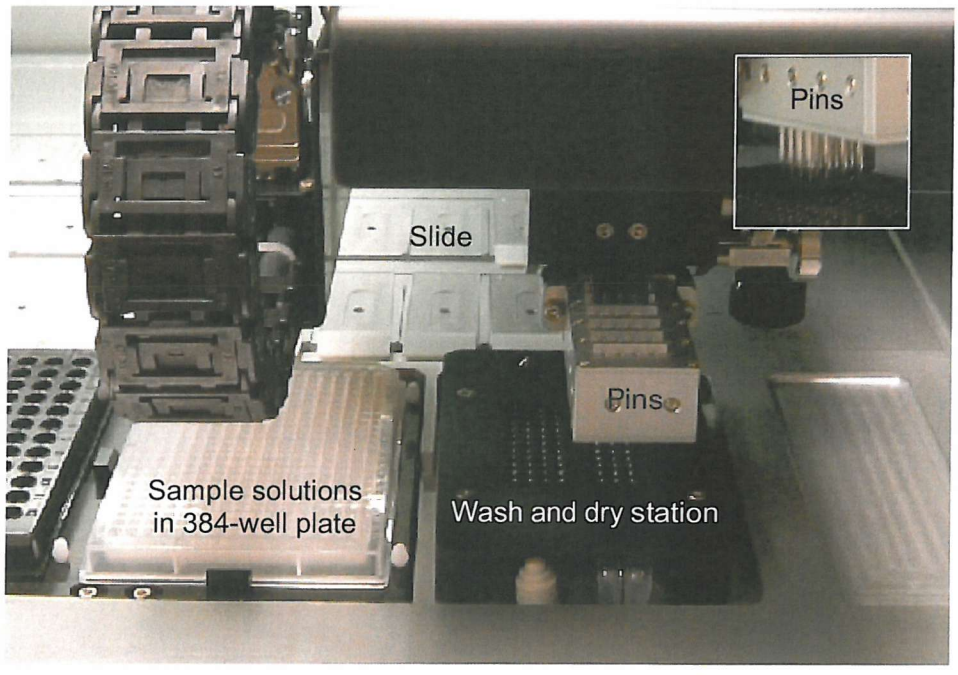


Figure 3.1.1. Instrument of microarray, Genetix Q Array mini arrayer (Genetix Ltd.).

Figure 3.1.2. Pin design for the delivery of pL volume.[137]

3.1.3 Direct synthesis of compounds on glass slides

Techniques in combinatorial chemistry encompass synthetic methods for the preparation of DNA chips *in situ*.[137] A major advantage of *in situ* synthesis is the preparation of many compounds with few reaction steps. A major strategy for *in situ* synthesis is masking, where a mask is used to isolate each spot from its surroundings. Photolithography is useful for the synthesis of oligonucleotide, where the oligonucleotide is synthesised in two stages, deprotection and coupling. Photocleavable protecting groups are coated onto a glass slide, and the pattern of irradiation deprotects specific areas, which are then coupled to monomer units that are exposed to the whole surface. The process is repeated to build up different sequences at different sites.

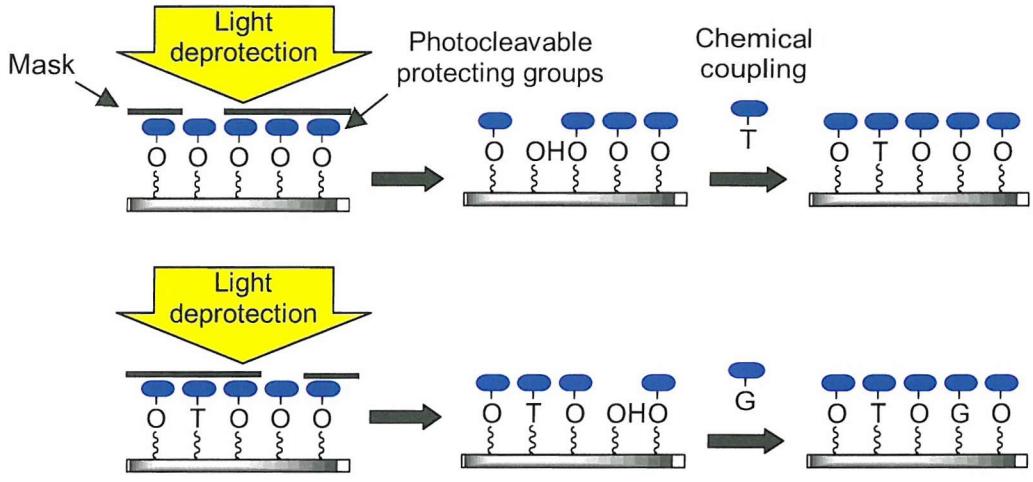


Figure 3.1.3. Photolithographic synthesis.

3.1.4 High throughput polymer synthesis

The aims here were directed towards microarray techniques and a micro-plate format for polymer synthesis, and the development of high throughput methods for the synthesis of a polymer library directly onto a glass slide.

3.2 Combinatorial polymer synthesis

3.2.1 Polymerisation using a high throughput approach

Two approaches for the synthesis of polymer libraries were attempted using microarray technology. The first approach was the spotting of monomer solutions onto a glass slide, followed by polymerisation, purification, and analysis on the glass slide directly. The second approach was polymerisation in a 96-well plate, and then spotting of the crude polymer solutions onto the glass plate for purification (washing) and analysis.

3.2.2 Polymerisation on a glass slide

36 different monomer solutions which consisted of 2-methoxyethyl methacrylate (MEMA), 2-(diethylamino)ethyl methacrylate (DEAEMA), 2,2'-azo-*bis*-isobutyronitrile (AIBN), diethyleneglycol dimethacylate (DEGDMA), and 1-methyl-2-pyrrolidinone (NMP) were prepared. The monomer concentrations were set to 50, 30, and 10 vol.%, and initiator amount was calculated as 1/100 molar of the total monomer. DEGDMA was used as cross-linking reagent with 3 different concentrations (0, 0.1, and 1.0 mol.%). NMP was chosen as solvent, because of its high boiling point, 202 °C and high solubility for monomers and polymers (Table 3.2.1).

The solutions were spotted onto a gold coated glass plate with a Genetix Q Array mini arrayer (Genetix Ltd.). The spotting was repeated 5 times on the same position, and four different spots were printed on the plate for each monomer solution. However, the spot sizes were not uniform and evaporated within 10 min of spotting.

The volatility of solvent is associated with several factors such as surface tension of solvent, humidity, temperature, and so on. One of most important factor is the boiling point of solvent. Even though the boiling point of NMP is quite high compared with other solvents (Table 3.2.1), it was evaporated on the gold coated glass plate due to high surface area. Therefore it was concluded that polymerization on the gold coated glass slide was very difficult.

Table 3.2.1. Solvents for polymerisation.

Solvent	Boiling point (°C)	Solubility parameter *1 ($MPa^{1/2}$)	Solubility of acrylate monomer *2	Solubility of Poly(acrylate) *2
NMP	202	23.1	+ +	+
DMF	153	24.8	+ +	+
DMA	164 - 166	22.1	+ +	+
DMSO	189	19.2	+ +	+
Toluene	110.6	18.2	+	+/-
EG	196 - 198	29.9	+/-	-
1,4-Butanediol	230	24.8	+/-	-
MMA	100	18	N/A	N/A
HEMA	189	N/A	N/A	N/A
St	145	19	N/A	N/A
PMMA	N/A	18.4 - 19.4	N/A	N/A

*1: Solubility parameter: reference[152]. *2: ++: Very good, +: Good, +/-: Poor, -: Not good.

3.2.3 Polymer synthesis in a 96-well plate and microarray purification

An array of polymerisations were carried out in a 96-well plate made of polypropylene, and various solvents such as NMP, toluene, dimethylformamide (DMF), and 1,4-butanediol (1,4-BD) were examined. The polymerisation of MEMA in a 96-well plate was carried out in a sealed glass vessel at 60 °C under nitrogen overnight. Polymers were obtained in all wells, but in certain cases, the solvents had evaporated (partially), and the polymers had precipitated during the reaction. NMP was found to be an ideal solvent with the least evaporation and precipitation.

An array of polymerisations was thus carried out in a 96-well plate made of glass. The plate was covered with a polypropylene sheet with a single small hole in each well to limit solvent evaporation. Polymers of 8 different MEMA/DEAEMA compositions were synthesised under 6 polymerisation conditions for each composition giving 48 kinds of polymers, but multiple 96 or 384-well plates could be used to make 1000's of polymers at a time. Figure 3.2.1 showed the polymerisation conditions (the monomer composition, the monomer concentration, [M], and the initiator concentration, [I]). NMP was used as the solvent and AIBN as the initiator. In order to examine the reproducibility, the same experiment was carried out in different positions on the same plate (experiment-2).

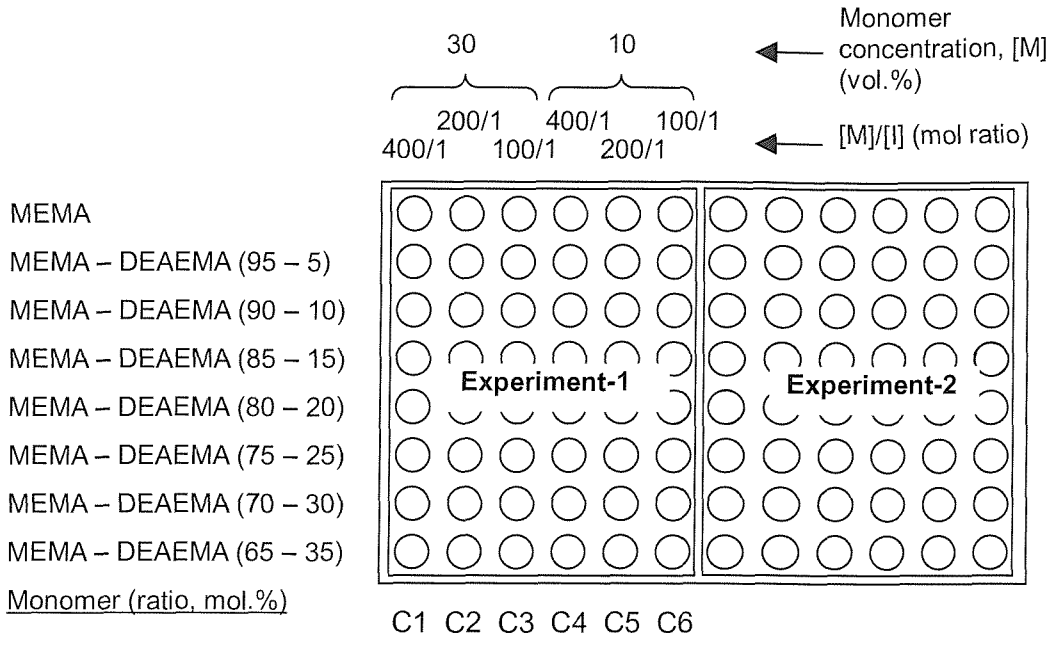


Figure 3.2.1. Polymerisation in a 96-well plate.

The polymerisation was carried out in a sealed glass vessel at 60 °C under nitrogen overnight. The polymers (in NMP) obtained in the wells were spotted onto the gold coated glass plate with a Genetix Q Array mini arrayer (Genetix Ltd.). Polymer solutions (30 vol.%), which could not be spotted uniformly due to high viscosity, were diluted to 20 vol.% with NMP. Four different spots were printed on the plate for each polymer, and polymers were repeatedly printed 5 times on each spot to give spot uniformly. After spotting, the slide was dried under vacuum at 45 °C for 2 hrs, washed with hexane to remove remaining solvents and monomers, and dried under vacuum at 45 °C overnight.

3.3 Analysis of polymer spots on a glass slide

In combinatorial chemistry, a wide range of analytical techniques are usually preformed to determine the chemical structure. Conventional nuclear magnetic resonance (NMR) spectroscopy is still useful to determine chemical structure, while several optical detection methods have a suite of attractive features that make them almost an ideal high throughput analysis tool for various applications. However, many of these techniques are not suitable for the tiny spots (~ 500 μm) of polymer on a glass slide, and therefore the development of analytical methods was required in order to characterise the polymers on the glass slide.

3.3.1 Microscope IR analysis

Fourier transform infrared (FT-IR) microscopy represents a useful tool for polymer analysis/characterisation, incorporating an integral CCD video camera, and a liquid nitrogen cooled mercury/cadmium-telluride (MCT) detector.

Polymer spots on the glass slide were analysed by an FT-IR microscope. Figure 3.3.1 shows the visible image of PMEMA (the polymerisation condition, [M]: 30 vol.%, [M]/[I]: 400/1 mol.%) spot on a gold coated glass slide. All polymer spots were circular in shape, and the sizes were 150 - 350 μm. The size might be dependent on the properties of polymer solution such as viscosity. An IR transmittance spectrum of PMEMA spotted onto the glass slide by FT-IR microscopy is shown in Figure 3.3.2. The resolution of the spectrum was high, and the carbonyl band at 1723 cm^{-1} was observed clearly. Bands of NMP which was used as reaction solvent were not observed, indicating that any solvent in the polymer had been removed by the washing protocol with hexane and drying under vacuum after spotting.

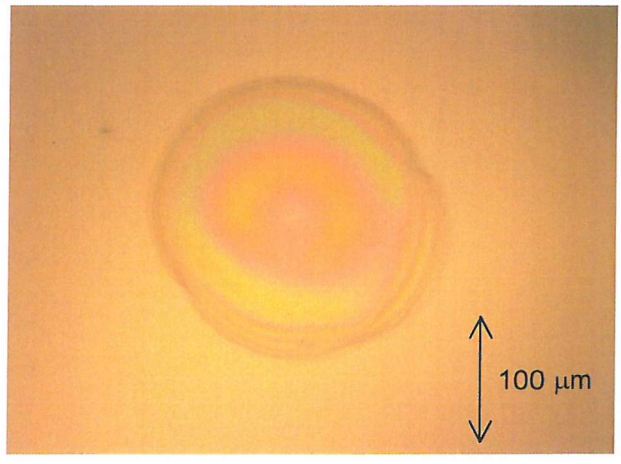


Figure 3.3.1. Visible image of a PMEMA spot on a glass slide.

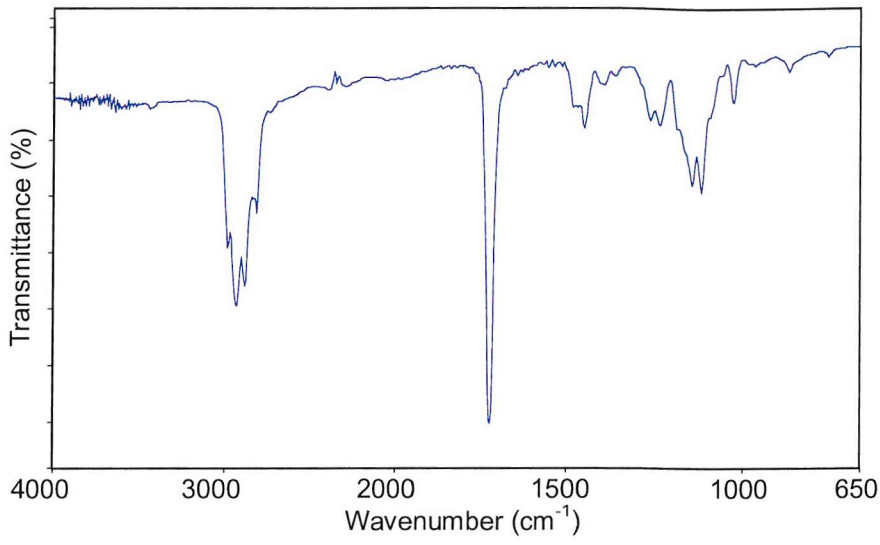


Figure 3.3.2. IR transmittance spectrum of PMEMA spot on a glass slide.

A series of copolymers with varying monomer compositions were measured on the array. The carbonyl bands at 1740 – 1720 cm^{-1} were normalised, and the spectra were overlaid as shown in Figure 3.3.3. The intensity of the alkyl band at 2700 – 3100 cm^{-1} increased with the DEAEMA content of the polymer.

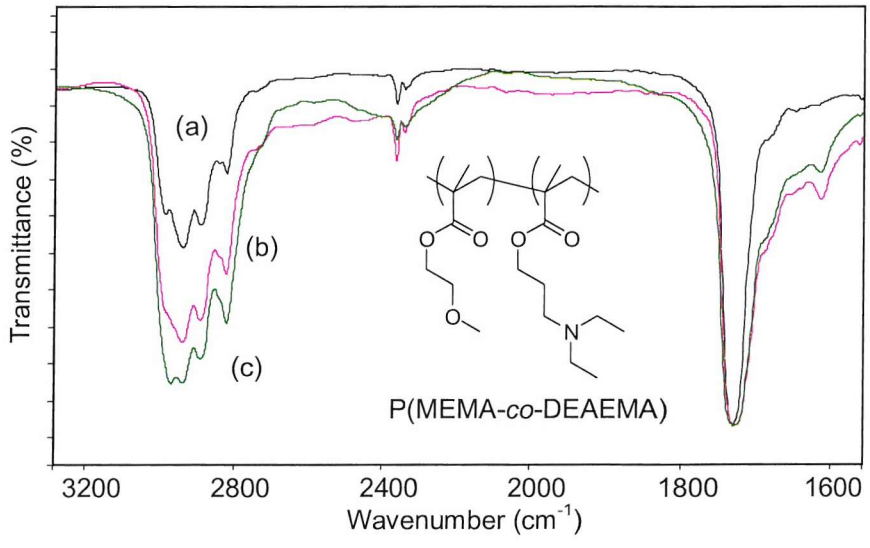


Figure 3.3.3. IR transmittance spectra of P(MEMA-*co*-DEAEMA) spotted on a glass slide. (a) 10, (b) 20, and (c) 30 mol.% content of DEAEMA.

Thus, the polymers spotted onto the glass plate could be analysed using FT-IR microscopy, and clear spectra observed. The functional groups in the polymer could be characterised, and the purity of polymer was estimated, furthermore, FT-IR microscopy provided quantitative information from the intensity of specific IR bands.

3.3.2 Analysis of polymer spot by time of flight secondary ion mass spectrometry (TOF-SIMS)

The polymers spotted onto the glass plates were analysed by TOF-SIMS. Positive ion TOF-SIMS spectra were obtained for each polymer, and three spots were measured for each polymer. All polymers showed a strong peak at m/z = 59, which was assigned to be the $^{+}C_2H_4$-O-CH_3 ion from MEMA. Polymers also showed a peak at m/z = 69, assigned to $^{+}C_4H_5O$. This peak stemmed from the acrylate main chain of the polymer. All polymers containing DEAEMA gave peaks at m/z = 72, 86, and 100, corresponding to $^{+}N(C_2H_5)_2$, $^{+}CH_2$-$N(C_2H_5)_2$, and $^{+}C_2H_4$-$N(C_2H_5)_2$, respectively. Using m/z ions of 59, 100, and 69 as representatives of MEMA, DEAEMA, and the main chain, the intensity ratios of these peaks in each polymer were compared (Figure 3.3.4 and 3.3.5).

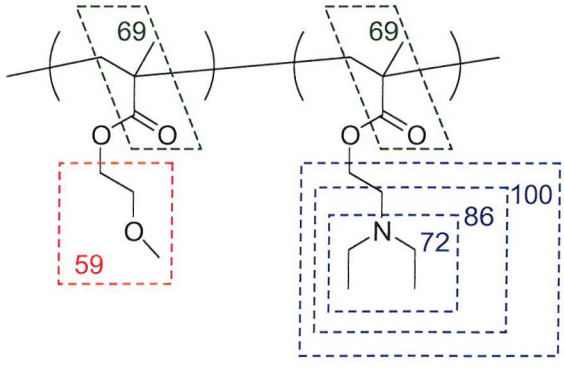


Figure 3.3.4. Ions assigned by TOF-SIMS.

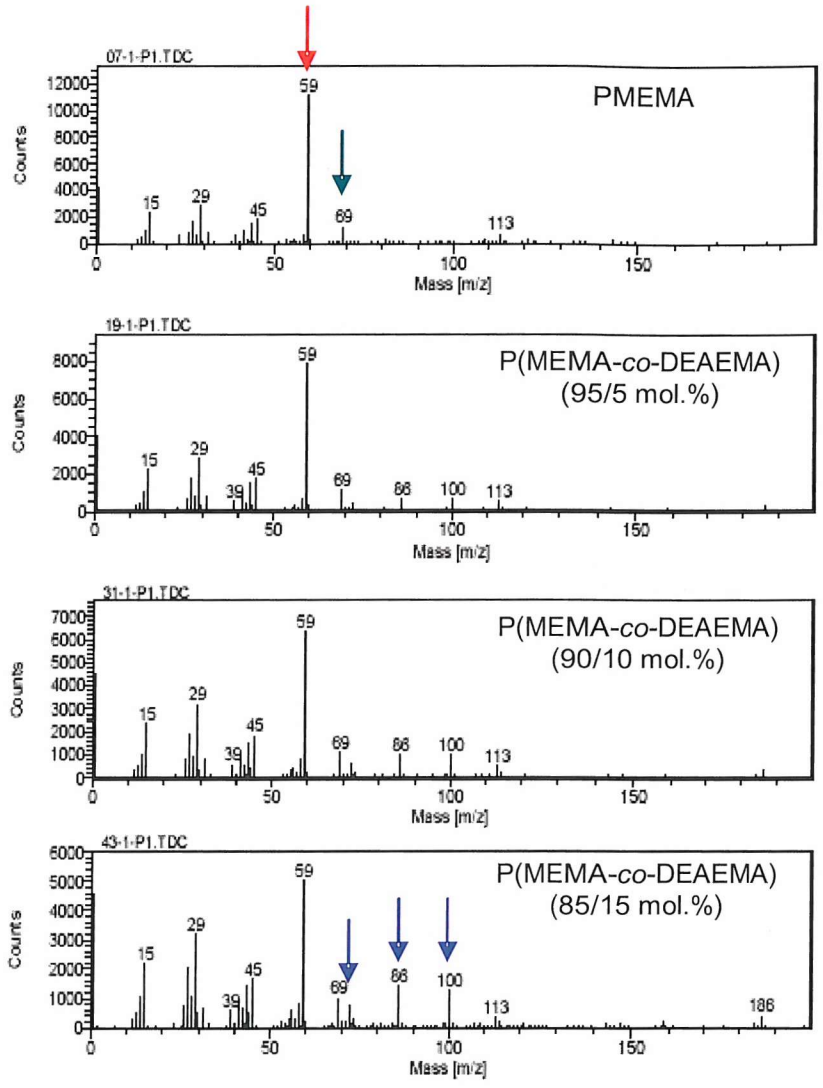
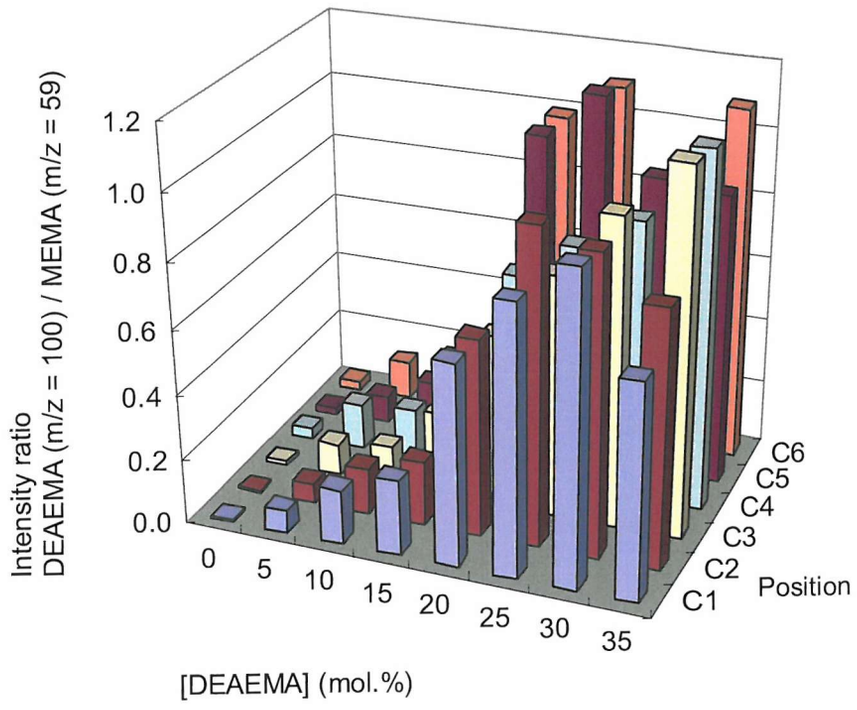


Figure 3.3.5. TOF-SIMS spectra.

Similar observations were observed in experiment 1 and 2, and the average intensities ratios of experiment 1 and 2 were determined. DEAEMA/MEMA ratios and DEAEMA/(main chain) ratios increased with increasing DEAEMA content in the reaction solution. However, in some cases the DEAEMA/MEMA intensity ratio appeared to decrease when the DEAEMA content was over 25 mol.% (Figure 3.3.6).



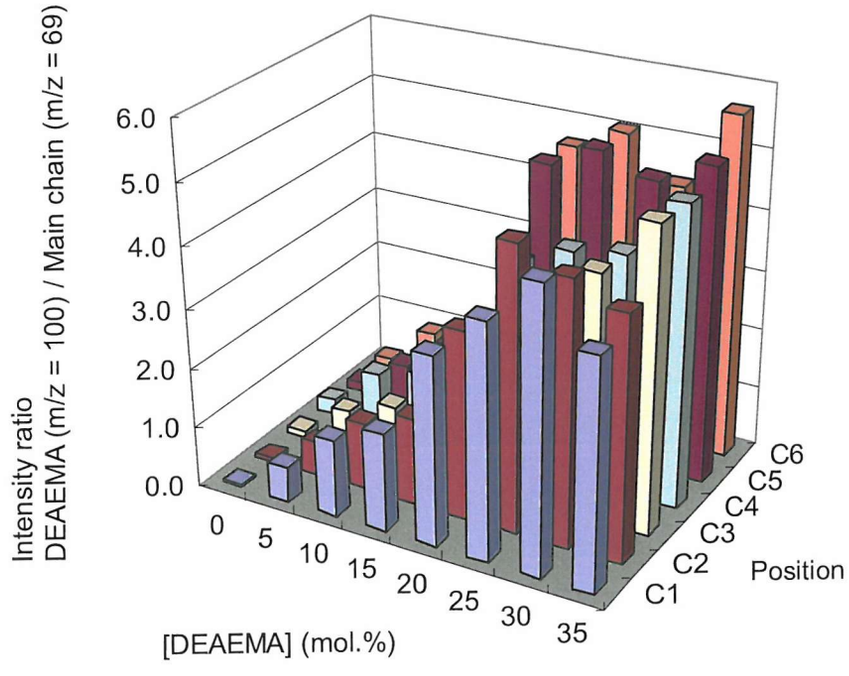


Figure 3.3.6. TOF-SIMS analysis of polymer spots on a gold coated glass plate.

3.4 Conclusions

Instead of conventional methods, a novel high throughput polymer synthesis was attempted using a 96-well plate format and a microarray technique.

Firstly, polymerisation on a glass plate was examined. Monomer solutions were spotted onto the glass plate by the microarray technique, however, solvents and monomers were evaporated.

Secondly, the various type of polymers were synthesised in a 96-well plate at the same time, and the polymers were spotted onto a glass plate by the microarray technique. These polymers on the glass plate could be purified by washing with hexane to remove the remaining solvents. Furthermore, FT-IR microscopy and TOF-SIMS techniques gave qualitative and quantitative information about functional groups of the polymers on the glass plate.

Thus, combinatorial polymer synthesis and analysis were established using a 96-well plate format and a microarray technique. This system provides a powerful tool for high throughput synthesis, purification, and screening in polymer research.

Chapter 4

Physical characterisation of polymers

4.1 General

Polymer characterisation is essential for development of new materials to understand the polymer function. For the development of biocompatible polymers, it is very important to characterise the physical and chemical properties, because protein and cell binding are highly dependent on the properties of the polymeric material.[107,109,114] In order to gain an understanding of the mechanism and more of cellular response on the polymeric material, it was therefore necessary to investigate the properties of the polymer library.

Polymer chemists need to investigate molecular weight, polydispersities, monomer compositions, end groups, thermal properties, morphologies, solution properties, viscosity, and so forth in addition to general characterisations.[110]

In this PhD, wettability and thermal analysis were selected, and high throughput screening (HTS) methods were developed for the investigation of these properties. Wettability is one key parameter for biocompatibility, because blood plasma is a solution contains 90 % water. When a polymer comes into contact with blood, the polymer rapidly adsorbs water molecules. Hence, the wettability of polymer might play a crucial role in biological processes. Thermal properties are also critical factors in polymer research. Especially glass transition temperature (Tg) and melting point (Tm) are essential to understand the polymer shape and stability.

Furthermore, it was attempted to find the correlation between protein binding and the polymer properties (Chapter 5).

4.2 Wettability

4.2.1 Wettability of polymer films

Wettability is one of the most essential properties for screening in the area of biopolymeric research.[112,113,122] Generally, contact angle measurements are commonly used to understand the hydrophilicity/hydrophobicity of the polymers.[160] However, this conventional method is relatively slow for screening a large number of polymers. In order to accelerate the screening of polymer wettability, a HTS method needed to be developed. Therefore, a novel HTS method was developed by which the wettability of

the polymeric surfaces was measured by an examination of the spreading area of water onto a polymer film exploiting the high throughput capability of image analysis (Figure 4.2.1).

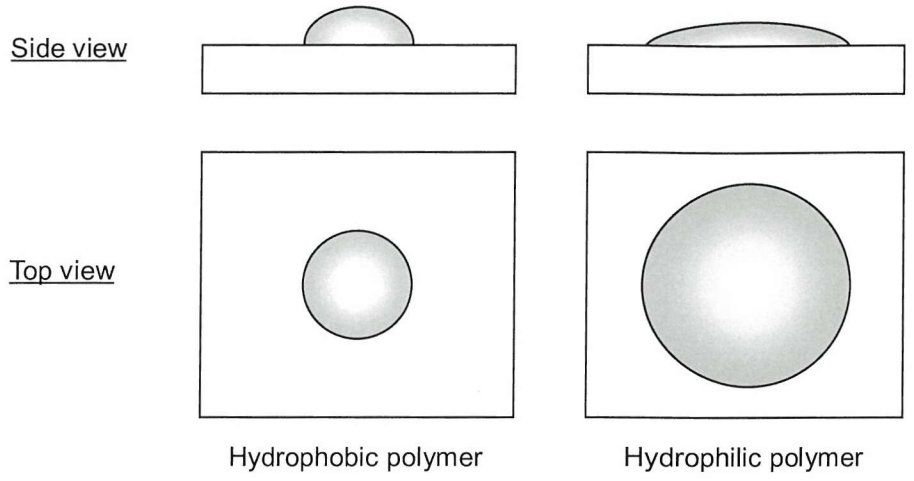


Figure 4.2.1. High throughput contact angle measurement.

A schematic representation of this method is shown in Figure 4.2.2. Polymer coated cover slips, were prepared by spin coating, and placed on the base of a liquid handler (Multiprobe IIx, Packard). A webcam (Sony, CMR PC4, 640 × 480 pixels) was fixed on the dispenser arm of the liquid handler, so images could be taken from above the droplet (Figure 4.2.3). The liquid handler was programmed to dispense one droplet of 9 μL of water onto each polymer coated glass, with a 20 sec interval between each polymer film. The dispensing volume of 9 μL was chosen arbitrarily in order to have a droplet of 3 – 4 mm in diameter. The spread of droplet was measured and quantified using image processing analysis software (Image Pro-Plus^{TM}, Media Cybernetics).

Thus, this system for evaluating the wettability was an automated method and could analyse by software easily.

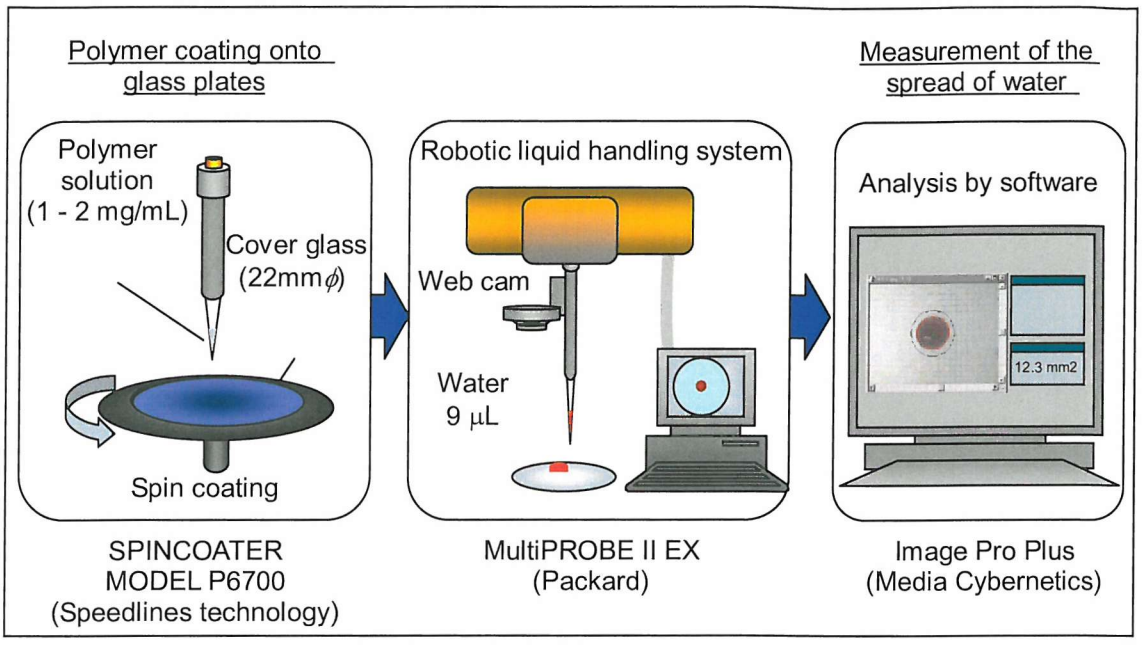


Figure 4.2.2. System for measurement of polymer wettability.

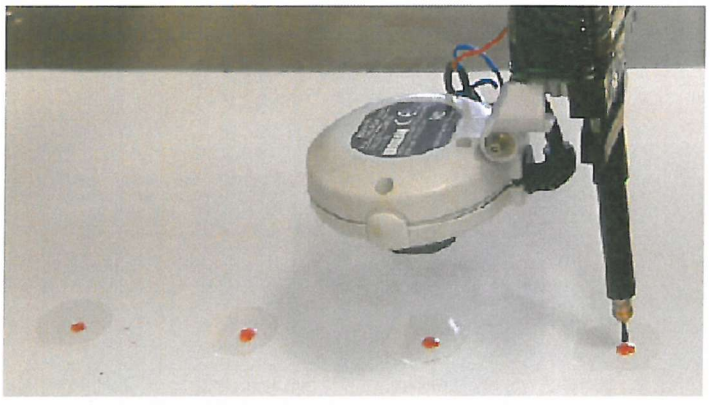


Figure 4.2.3. Webcam to take an image of solvent spread on the polymer coated cover slip.

The software used could analyse the area making use of the variations in pixel intensities. However, in order to analyse the spreading area of water-droplet accurately, it was necessary to obtain a clear contrast, hence, a water soluble dye, Congo Red was added to distinguish the water from the background. Experiments were performed varying the dye concentration, and at a concentration of 1.0 mg/mL, a clear distinction between the droplet and the background was achieved. (Figure 4.2.4) The addition of the dye did not significantly affect the spreading area of the droplet.

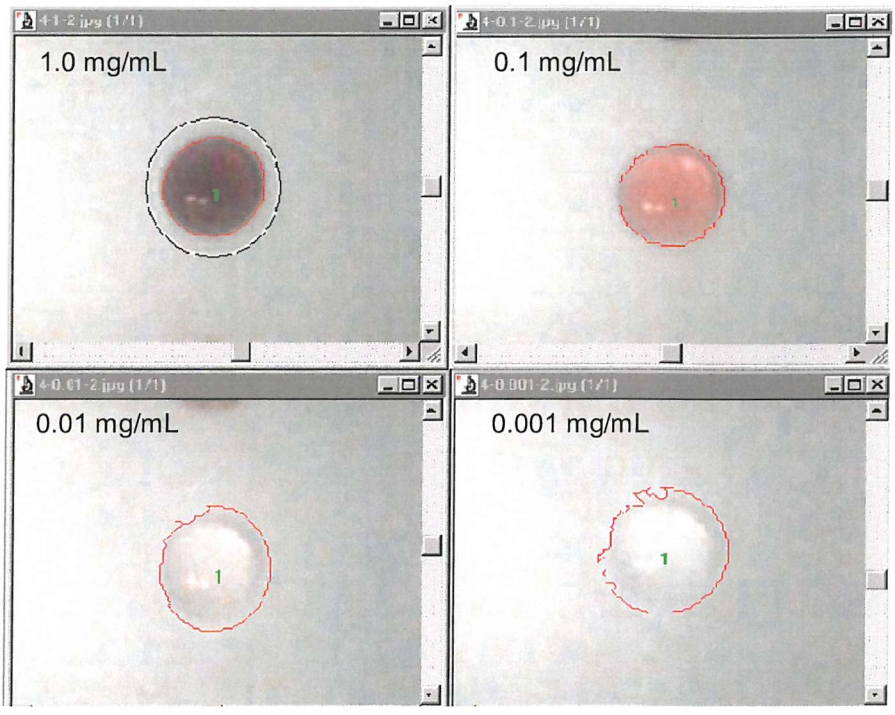


Figure 4.2.4. Effect of dye concentration on the contrast of the water-droplet intense.

4.2.2 Theoretical correlation between contact angle and spreading area

A correlation between the spreading area of water and contact angle is proposed. It is hypothesized that the water-droplets form part of a sphere, and the spreading area, S and water volume, V are represented with contact angle, θ and radii of sphere, r (Equation 4-1, 4-2, 4-3, and 4-4, Figure 4.2.5).[161]

In the case of contact angle, $0^\circ < \theta \leq 90^\circ$

$$S = \pi r^2 \sin^2\theta \qquad (4\text{-}1)$$

$$V = (1/3)\,\pi r^3\,(2 - 3\cos\theta + \sin^2\theta\cos\theta) \qquad (4\text{-}2)$$

In the case of contact angle, $90^\circ < \theta$

$$S = \pi r^2 \tag{4-3}$$

$$V = (2/3)\,\pi r^3\,(1 - \cos\theta) \tag{4-4}$$

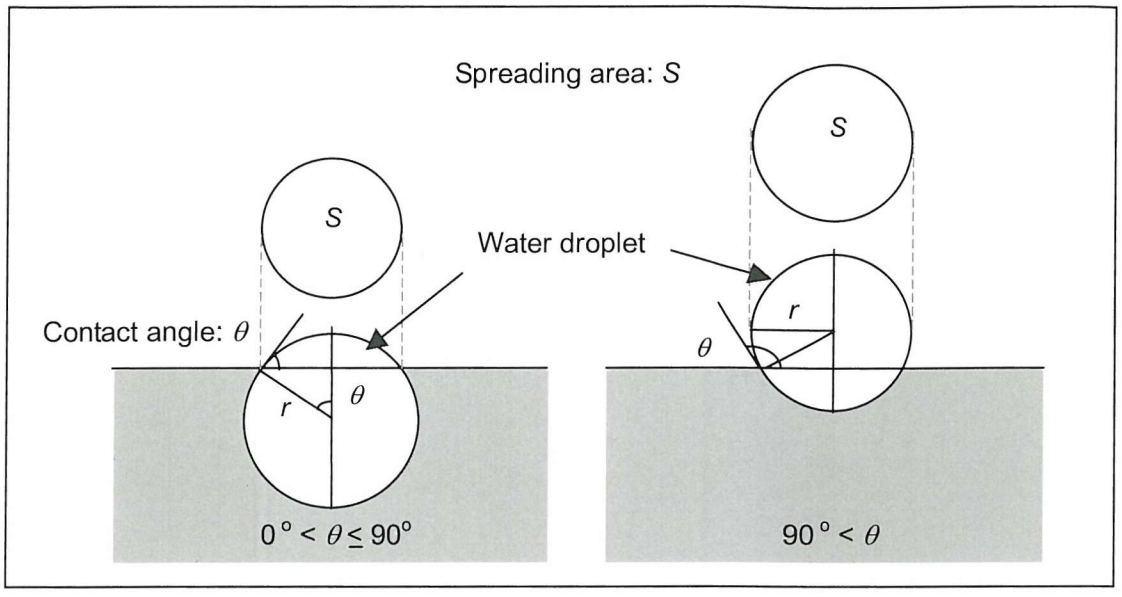


Figure 4.2.5. Contact angle and spreading area of water.

From these equations, spreading area, S can be represented with water volume, V (Equation 4-5 and 4-6).

$$S = [V^2\,\pi\sin^6\theta / (2 - 3\cos\theta + \sin^2\theta\cos\theta)^2]^{1/3} \qquad (0° < \theta \leq 90°) \tag{4-5}$$

$$S = [9V^2\,(1 - \cos\theta)^2]^{1/3} \qquad (90° < \theta) \tag{4-6}$$

When the water volume and contact angle are inserted into the equations, the spreading area can be calculated.

4.2.3 Experimental correlation between contact angle and spreading area

The contact angle and spreading area of water on the polymer films, PHEMA (Sigma-Aldrich Co., Ltd.), PSt, PMMA, PMEMA, their copolymers with acrylamides such as DEAAm, DMAAm, and NIPAAm, which were synthesised by free radical polymerisation (polymer library-1), were analysed after a contact of 20 sec. The contact angle was measured by an Automatic Solid Surface Free Energy Analyser (Kyowa Interface Science Co., Ltd.) with 1 μL of de-ionised water for each polymer film, and the spreading area was measured using the previously mentioned protocol (Chapter 4.2.1).

A clear correlation between the contact angle and surface area of water on polymer films was observed (Figure 4.2.6). The hydrophobic polymers showed a spreading area of less than 10 mm^2 which corresponds to a contact angle of more than 80°, and hydrophilic polymers showed a spreading area of more than 15 mm^2 which corresponds to a contact angle of less than 40°. The experimental data correlated with the theoretical value which was calculated from Equations 4-5 and 4-6 with water (9 μL) and contact angles varying from 40° to 110° in 1° increment. Though, highly hydrophilic polymers which had lower contact angle did not match the calculated values due to the declination from the hypothesis that the water-droplet forms part of a sphere, the experimental data was in good agreement with the calculated value in case of higher contact angles.

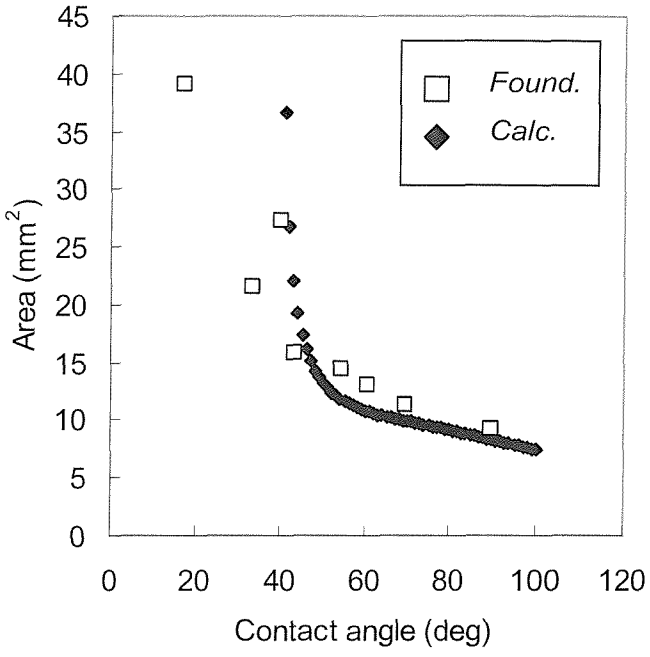


Figure 4.2.6. Correlation between contact angle and area of water droplet.

4.2.4 Characterisation of the polymer libraries

The spreading area of water on polymer in libraries 1 – 5 was investigated using this high throughput method. The polymers showed a very wide range of the spreading area (8.6 – 50.4 mm^2). In all cases, 3 or 2 cover slips were coated with the same polymer to confirm the reproducibility of the method (the average standard deviation was just 0.28 mm^2).

4.2.4.1 Effect of the main monomer on wettability

Polymer library-1 which consisted of 7 different monomers (St, MMA, MEMA, MEA, HEMA, HPMA, and HBMA) and 3 different acrylamides (DEAAm, DMAAm, and NIPAAm) with 3 different monomer compositions (90/10, 70/30, and 50/50 mol.%) was screened. (The structures are given in Chapter 2.4.1).

Monomer structure was found to have a strong effect on wettability (Figure 4.2.7). For example, DMAAm was the most hydrophilic monomer among the acrylamides. In particularly, copolymers with a high content of the DMAAm showed a relatively high hydrophilicity among the polymers (Figure 4.2.7, graph (b)). Thus, it was observed that *N*-substituents in the acrylamide had a large influence on wettability.

Also the structure of the major component reflected on the wettability of the polymer (Figure 4.2.7, graph (c)). The St-based copolymers showed spreading area of 10 mm^2 or less (corresponding to a contact angle of 80^o or more). Even the St copolymer with 50 mol.% of the DMAAm showed a spreading area of 9.6 mm^2, explained by the highly hydrophobic monomer, styrene.

Comparison of MMA and MEMA copolymers indicated that the ethylene glycol unit in the side chain had a profound effect on the wettability of the polymers. The copolymers with low content of acrylamide showed a smaller spreading area, which translates to hydrophobicity, in the both cases. However, as the percentage of DMAAm was increased, the MEMA copolymers showed a larger spreading area, while the spreading area of the MMA copolymer did not changed significantly. It can be concluded that the ethylene glycol unit in side chain of MEMA had a pronounced influence on polymer wettability.

From the differences between HEMA and MEMA, the effect of the hydroxy group on wettability is understandable. Thus hydroxy groups form strong hydrogen bonds with water, and a large spreading area on HEMA copolymers was observed (Figure 4.2.7, graph (c)).

Comparing HEMA, HPMA, and HBMA, the correlation between the number of carbon chains and the spreading area of water was observed. The size of the alkyl groups had a major effect on wettability (Figure 4.2.7, graph (c)).

The difference in the spreading area of acrylate and methacrylate polymers was explained by the effect of the methyl group in main polymer chain. In all cases, the MEA copolymers showed a higher spreading area of water compared with the MEMA copolymers.

Thus, by investigation of the spreading area on polymer films, it was found that various monomers containing hydroxy, ether, or alkyl groups in the side or main chain, significantly affected the wettability of the polymer.

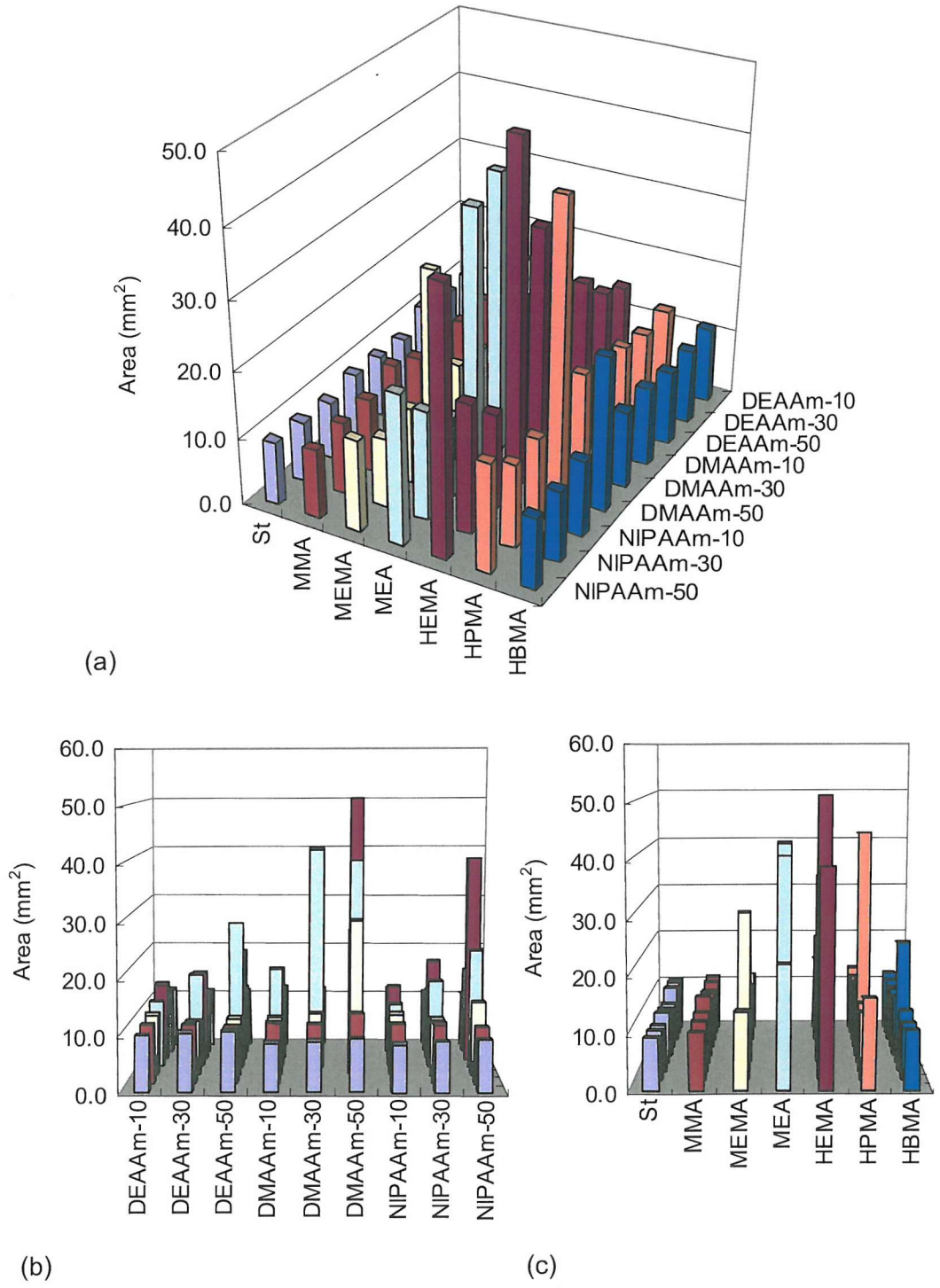


Figure 4.2.7. Effect of monomer type on wettability. Graphs (b) and (c) give the side and the front views of graph (a).

4.2.4.3 Effect of the specific functional group on wettability

The wettabilities of libraries 2 and 4 were determined. These polymer libraries consisted of copolymers with various types of functional groups, amines, amides, heteroaromatics, thioether, nitros, and acids. (Chapter 2.4.2 and 2.4.4).

Figure 4.2.8 shows the effect of functional groups of MEMA-based copolymers on wettability. The copolymer with substituted amine monomers DEAEMA, DMAEMA, and DMVBA showed smaller spreading areas, but the copolymers with a dimethylamino group containing acrylate, DMAEA, and acrylamide, DMAPMAAm showed larger spreading areas corresponding to low contact angle. Thus again the amine group, the main and side chain structure in the polymer had a significant effect on wettability.

The copolymers with heteroaromatic groups, VI, VP-2, and VP-4 showed smaller spreading areas, which showed the relatively hydrophobic properties of these polymers.

The less sterically hindered amide groups, VAA and VPNO showed larger spreading areas, due to the formation of hydrogen bonding between the amide groups of these polymers and water.

As for acid group containing copolymer, unexpectedly, MA-H and AES-H copolymers showed relatively high hydrophobicity despite of the presence of acidic functional groups. The AAG-H copolymers showed high hydrophilicity, because they contained both carboxylic acids and hydroxyl groups.

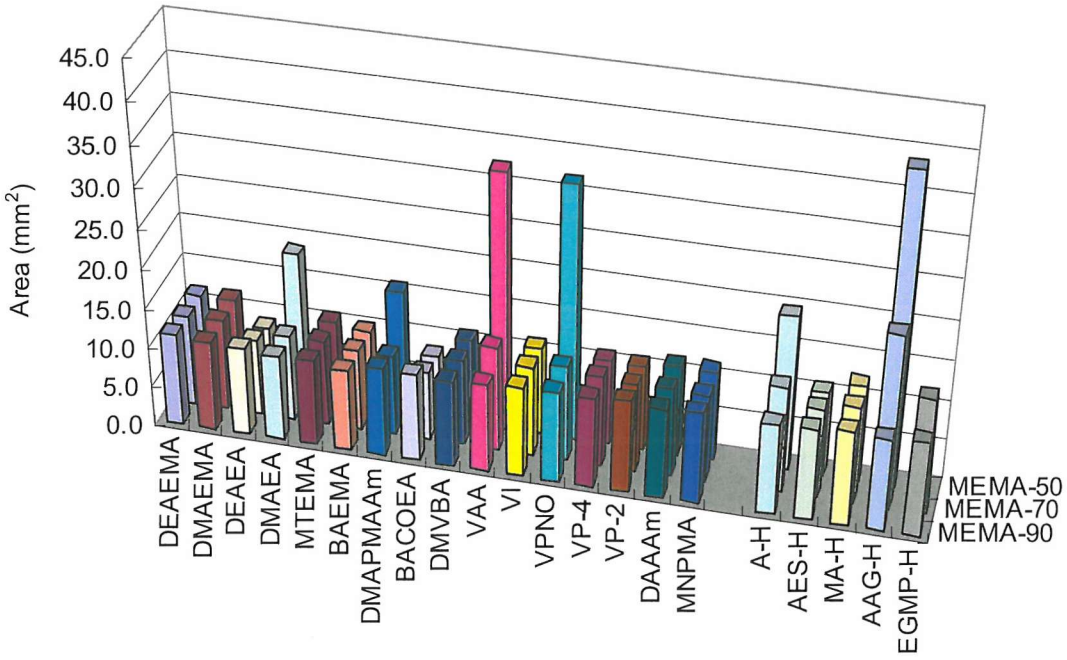


Figure 4.2.8. Effect of monomer type on wettability.

4.2.4.4 Effect of the third component

The terpolymers which consisted of MEMA, DEAE(M)A, and another functional monomer were screened to investigate the effect of third component (the structures are shown in Chapter 2.4.5).

Firstly, the second component, DEAEMA or DEAEA had an effect on wettability. Some polymers with DEAEA gave higher spreading areas compared with DEAEMA type polymers due to the differences of acrylate and methacrylate.

Although the introduction of MEA, DEAEMA, and HEMA as a third component was expected to increase the high hydrophilicity due to their characteristic functional groups (hydroxy groups and ethylene glycol units), the spreading areas of their terpolymer were effectively unchanged. Thus, in most cases, the addition of up to 30 mol.% of a third component did not have a significant effect on the wettability of the whole polymer. The exception was the spreading area on the terpolymers containing 30 (or more) mol.% of DMAAm was found be relatively higher than the other polymers. It was concluded that DMAAm was very effective for wettability enhancement.

Although the copolymers, P(MEMA-*co*-DEAE(M)A) and P(MEMA-*co*-(M)A-H) did not give large spreading areas, the terpolymers, composed of MEMA, DEAE(M)A, and (M)A-H, showed a very high spreading area. These polymers are termed polyzwitterionic, containing both anionic and cationic groups, hence, ionisation occurs in the polymer chain, and therefore high hydrophilicity of these polymers was observed (Figure 4.2.11).

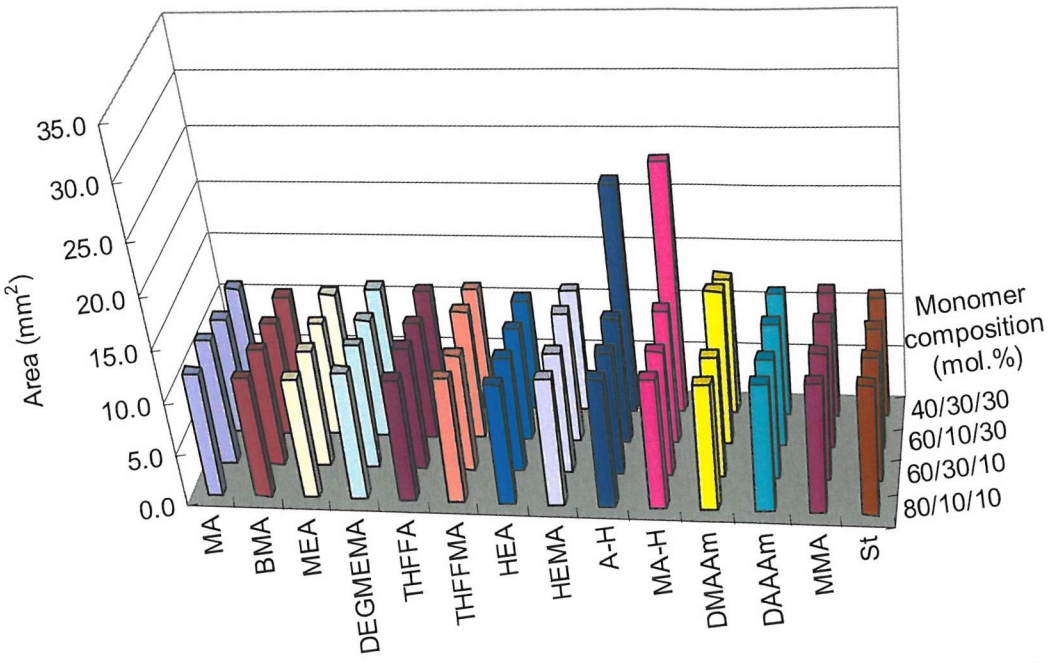


Figure 4.2.9. Wettability of terpolymer, MEMA/DEAEMA/Third component.

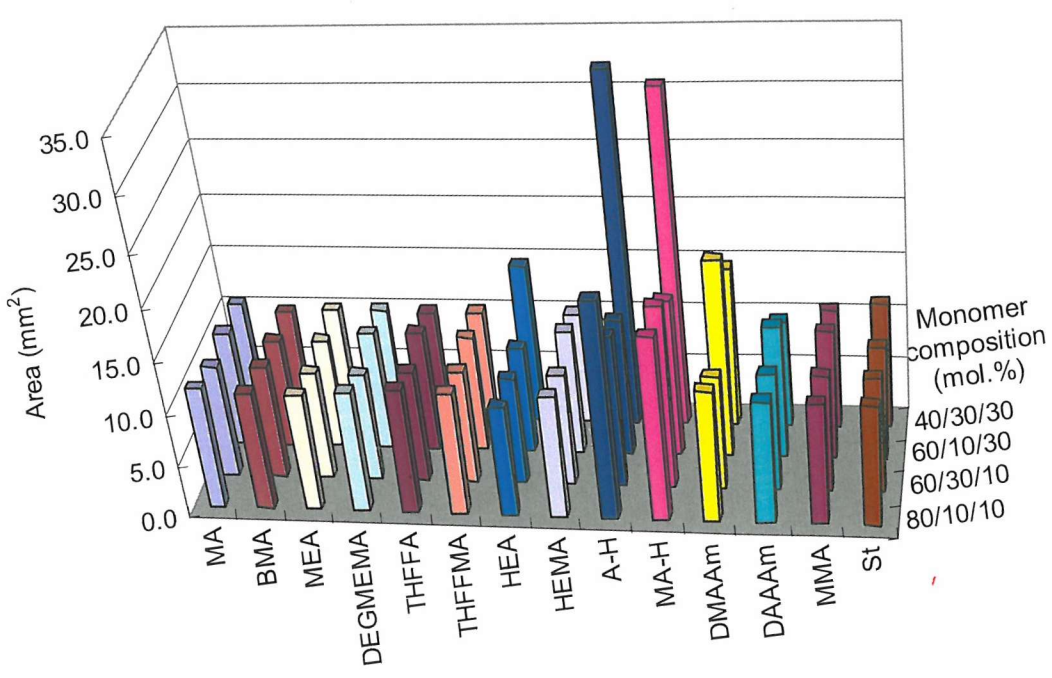


Figure 4.2.10. Wettability of terpolymer, MEMA/DEAEA/Third component.

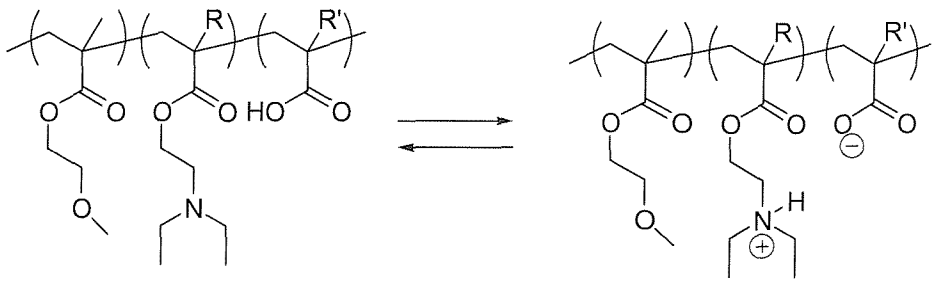


Figure 4.2.11. Ionisation in polymer side chains.

4.2.5 High throughput wettability measurements - Conclusions

A novel high throughput method for evaluating the wettability of polymer libraries, by measuring the spreading area of water droplets on the polymer films, was developed. This is a fast and automated method to determine the wettability on the polymer surface compared to those obtained by traditional methods, and the spreading areas can be directly related to contact angle.

The wettabilities of various polymers were investigated by using this technique, and it was clearly observed that the functional groups on the monomers and their combinations had a significant effect on wettability. Following conclusions can be described;

- The aromatic ring raised the hydrophobicity of the polymer.
- The hydroxy group and the ethylene glycol unit in the side chain improved the hydrophilicity.
- The methyl group in main polymer chain had a great effect on wettability of polymer and raised the hydrophobicity.
- The amide and amine groups gave hydrophilicity due to the hydrogen bonding between the functional groups and water.
- The polyzwitterions containing acid and amine groups gave high hydrophilicity due to the intermolecular and intramolecular ionisation.

Thus, the clear tendency was accomplished using high throughput method for wettability of polymer. This technique is quite useful to analyse the properties of the entire polymer library.

4.3 Analysis of thermal properties

4.3.1 High throughput (HT) - Differential scanning calorimetry (DSC)

Thermal properties are one of the most important characteristics of a polymer, as the hardness and rigidity of a polymer can be extrapolated from its melting point and its glass transition temperature data.

The most conventional thermal analysis technique for polymers is differential scanning calorimetry (DSC). Recently some excellent DSCs, which can operate at high heating and cooling rates, have become commercially available. One of the highest performance DSCs is the Diamond DSC™ (Perkin-Elmer), which provides a linear controlled scan rate at least 10 times higher than other standard DSC measurements (10 °C/min) up to 500 °C/min using a small furnace (Table 4.3.1). This technology is very useful for high throughput projects.

Table 4.3.1. Performance of Diamond DSC™ (Perkin-Elmer).

Diamond DSC™ (Perkin-Elmer)	
Temperatrue range	-170 to 730 degC
Controlled heating scanning rate	Up to 500 degC/min
Controlled cooling scanning rate	Up to 100 degC/min
Sensitivity	0.2 μW
Signal response: Indium (1mg)	
- Peak height	7.4 mW
- Width at half height	0.42 degC
- H/W ratio	17.6 mW/degC
Autosampler	44 samples

4.3.2 Effect of heating and cooling rates

In order to study the effect of varying scan rates on the thermal properties, PHEMA (approx. 30 mg) purchased from Sigma-Aldrich Co., Ltd. was used. Figure 4.3.1 shows the second heating curves at varying heating and cooling rates of 200, 100, 50, and 10 °C/min. It was observed that at higher heating rates, the peak corresponding to the glass transition temperature (Tg) broadened and also shifted to apparently higher temperatures ("thermal lag"). Thermal lag is in fact a general problem, which is always observed even at a moderate heating rate of 10 °C/min. Thermal lag depends on

numerous factors, such as the capability of the instrument to add energy during heating, the response time of the instrument, the heat conduction from heater to the pan used and subsequently within the sample, and the response from the sample to the sensor.[119,120] It is important to study to what extent the peaks are shifted with increasing scan rate. Figure 4.3.2 shows the correlation between the scan rate and Tg (onset temperature and temperature at half of the total heat capacity 1/2 ΔCp). When the scan rate was increased from 10 to 200 °C/min, apparent increases in Tg were observed, and the onset temperatures went up 14 °C. The 14 °C upward shift is large, and so the values measured with high scan rate may lead to misleading results. However, the relative values of Tg between the polymer samples can be understood. Furthermore, extremely high scan rates enable the measurement time to be drastically reduced.

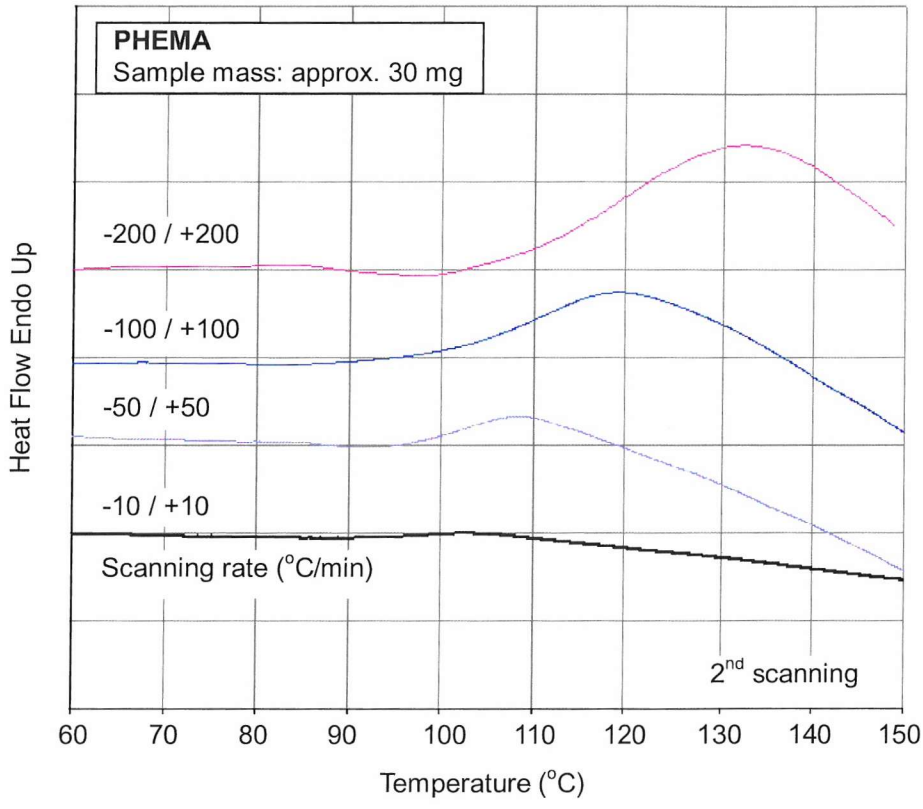


Figure 4.3.1. Second heating curve of PHEMA with various scan rates, 10, 50, 100, and 200 °C/min.

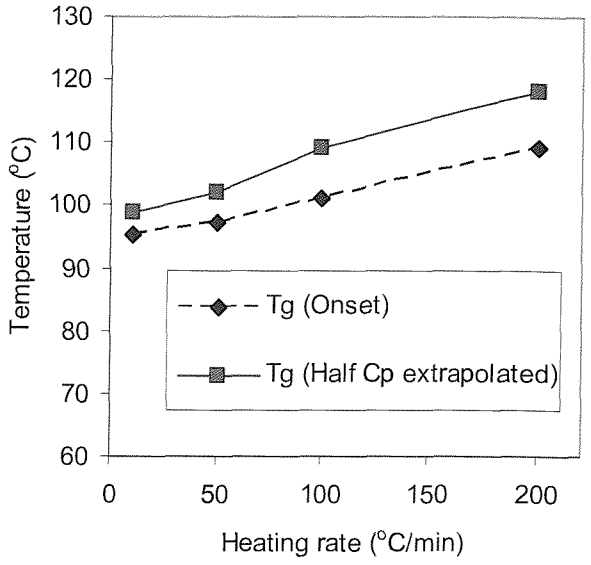


Figure 4.3.2. Glass transition temperatures of PHEMA as a function of scan rate.

4.3.3 Polymer structure and glass transition temperature

The polymers in libraries 1, 2, and 4 were analysed by the Diamond DSC™ (Perkin-Elmer). The heating and cooling rates were set to 100 °C/min which enables the measurement of a sample in less than 10 min. The onset temperature and temperature at which half of the total heat capacity, 1/2 ΔCp were calculated from a second scan, since the peak of residue solvent overlapped with polymer samples in the first scan. A clear glass transition temperature was observed in most polymers.

Figure 4.3.3 shows the glass transition temperatures of polymers in library-1 which consisted of 7 different monomers (St, MMA, MEMA, MEA, HEMA, HPMA, and HBMA) and 3 different acrylamides (DEAAm, DMAAm, and NIPAAm) with 3 different monomer compositions (90/10, 70/30, and 50/50 mol.%).

The St copolymers gave high glass transition temperature, because styrene has bulky pendant groups, benzene rings, which reduced the flexibility of the chain. The low flexibility raised the glass transition temperature. The MMA copolymer has no flexible long chain in polymer. As a result, The MMA copolymers gave high glass transition temperature.

The MEMA copolymers were found to give the lower glass transition temperature compared with MMA copolymers. This could be explained due to the fact that MEMA has an ethylene glycol unit in the side chain that can be categorised as a flexible group. This increases rotational motion and lowers glass transition temperatures. MEA

copolymers gave a lower glass transition than that of the MEMA copolymers, because the methyl group in the main chain decreased the flexibility of the polymer.

Monomer composition also had a significant effect on the glass transition temperature. In the case of MEA, clear tendency was observed. As the content of MEA was increased, the glass transition temperature decreased. The glass transition temperatures of some MEA copolymers were observed to be lower than room temperature. These results were associated with the appearances of the polymers, for example, the copolymers with a high content of MEA were rubbery.

Comparing HEMA, HPMA, and HBMA, the correlation between the size of alkyl chain and the glass transition temperature was observed. As the size of alkyl chain became larger, the range of glass transition temperatures became wider, and it was found the co-monomer contents had an effect on glass transition temperature.

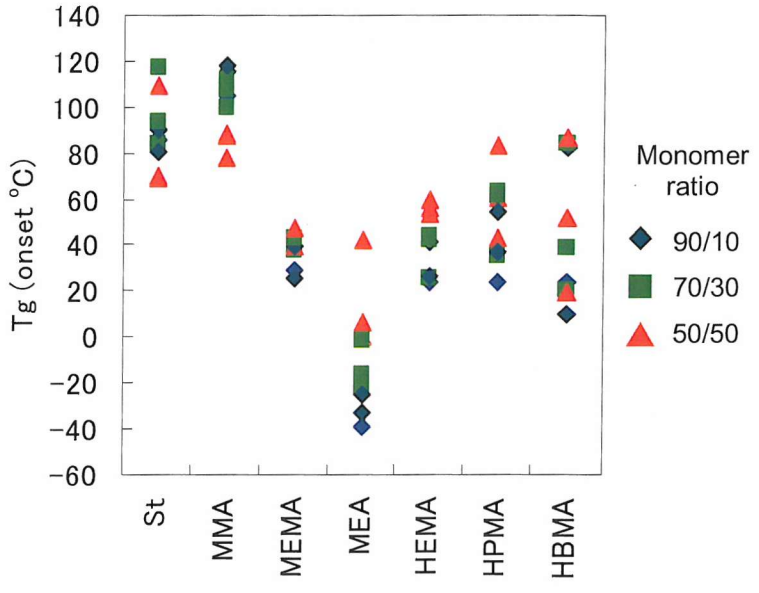


Figure 4.3.3. Effect of polymer structure on the glass transition temperature.

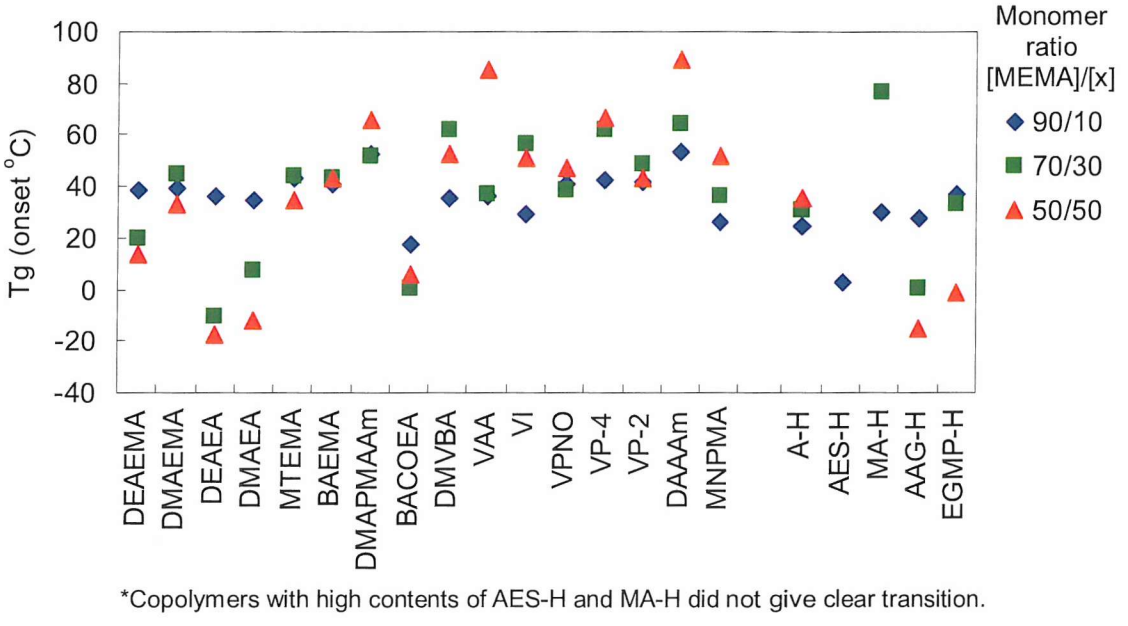
Figure 4.3.4 shows the effect of functional groups of MEMA-based copolymers in polymer libraries 2 and 4 on glass transition temperature. On the whole, the copolymers with 90 mol.% content of MEMA gave similar glass transition temperatures, meaning that the low content of co-monomer did not have a significant effect on the glass transition temperature.

The copolymers consisting of acrylate monomers with long side chains (DEAEA, DMAEA, and BACOEA) gave low glass transition temperature. Thus, the flexibility of

the polymer had a major effect on glass transition temperature. However, the acrylamide-containing polymers (DMAPMAAm and DAAAm) gave high glass transition temperature in spite of the long side chain. It was thought that the rigidity of polymer chain might be increased by the intermolecular and intramolecular hydrogen bonding between amide groups.

Aromatic ring containing polymers (DMVBA, VI, VP-4, and VP-2) did not give low glass temperature due to the bulkiness.

As for acidic group containing polymers, a similar tendency was observed. The polymers with long side chains (AAG and EGMP-H) gave low glass transition temperatures due to their flexibility. A-H is acrylate type monomer with short side chain, and has an acid group which can form hydrogen bonding, thus, the glass transition temperature was little changed.



*Copolymers with high contents of AES-H and MA-H did not give clear transition.

Figure 4.3.4. Effect of functional groups on the glass transition temperature.

4.3.4 Conclusions of thermal analysis by HT-DSC

High throughput synthetic methods allow the rapid generation of polymer libraries. In order to accelerate the research, high throughput analysis methods were developed. In the case of thermal analysis, the polymer sample could be analysed using HT-DSC which could measure at high heating and cooling rates (100 °C/min) and accordingly 10 times faster than other traditional DSCs.

Using HT-DSC, the libraries were analysed. The influence of polymer structure on the glass transition temperature was as follows;

- The polymers with long side chains gave low glass transition temperature.
- The methyl group raised the glass transition temperature.
- The aromatic ring raised the glass transition temperature.
- The acrylamide gave high glass transition temperature.

Thus, it was found that the value of glass transition temperature was concerned with the functional groups of the polymer chain, and this information will focus the direction of polymer design for targeted materials.

neutrophil activation and protein adhesion and enabling the measurement of protein adsorption on polymer beads in a high throughput manner.[130,134,135] However, for this process it was necessary to prepare polymer beads.

The design of functionalised polymer beads has attracted attention in various research areas, such as catalysis, biotechnology, medicine, and ecology. Generally, research has focused on the coating of materials onto spherical beads implying a core of defined size and the generation of a shell having the required stability on the outside. In our work it was a requirement to be able to coat different polymers onto spherical beads by an effective and simple coating method applicable to the entire polymer library.

Conventionally, polymers and the organic compound can be coated spray-drying.[162-166] In this process, the beads to be coated are suspended in a fluid bed and a coating solution of dissolved polymer or organic compound is sprayed onto the fluidised beads and then dried, and the polymer can be precipitated on the bead. However, in this case, specific equipment and large amounts of polymer are required.

A coating method in solution phase, the so-called Layer-by-Layer adsorption of oppositely charged polymers it based on the electrostatic interaction between polyanions and polycations. Various synthetic polyelectrolytes, biopolymers (proteins and nucleic acids), lipids, and inorganic beads have been used to produce multi-layer films on flat substrates allowing fabrication of nano-films with tailored physical and chemical properties.[167-174] Adsorption may occur by forces such as dipole-dipole interactions between the polymer and the beads, while the polymer may be coated onto beads via precipitation. Precipitation process can cause many problems, such as non-uniform coating of the polymer onto the beads and aggregation. Recently coating of beads has been achieved by precipitation with inorganic salts,[175] thus as the inorganic salts are added dropwise into the polymer solution, the solubility of the polymer decreases. Finally, the polymer precipitates onto the beads with interactions between the polymer and the bead. Using this technique, most polymers can be coated onto beads. However, the remaining inorganic salts can act as a contaminant affecting the bioassay. Therefore, for these studies a polymer coating technique was developed which would have no contaminants, yet provide a general method for all polymers.

It was decided to achieve this with the aid of phase separation from a polymer-solvent mixture onto the beads. Generally, the solubility of polymers in solvent decreases at with lower temperature, and it was considered that this phenomena might also be utilized for polymer coating onto beads.

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5.2.2 Polymer coating

Initially, silica and polystyrene beads (diameter 10 μm) were used as the core and PMEMA as the coating polymer. Polymer solutions (10 mg/mL) were prepared, and the beads were added to the solution, and the suspensions left for 2 hrs at room temperature. Following centrifugation, excess polymer solutions were removed carefully using a pipette, and the beads were freeze dried to obtain polymer coated beads. The surfaces on the beads were analysed by X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM).

5.2.3 Calculation of the coverage of polymers coated onto beads

In order to calculate the coverage of the polymer onto silica beads, the surface of the beads was investigated before and after coating by XPS. XPS detects all elements except hydrogen on the surface (depth: ~ 10 nm) and provided the composition.

Before polymer coating onto the silica surface, SiO_2, silicon and oxygen elements should be observed without any others. However, a peak corresponding to carbon was detected due to impurities (Table 5.2.1). Therefore, the percentage of silica surface, SiO_2 was estimated by the level of Si determined by XPS (Equation 5-1), and the coverage, C was represented with the percentage of silica surface, SiO_2, before and after polymer coating (Equations 5-2 and 5-3).

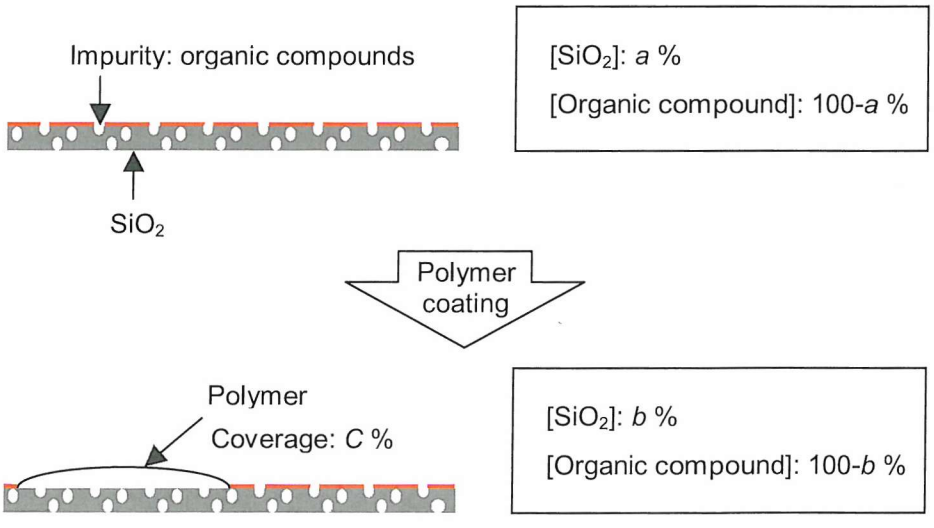


Figure 5.2.1. Calculation of the coverage of the polymer coated silica beads.

$$[SiO_2] = [Si] \times [O] \times 2 \div [Si] \times 3 \quad (5\text{-}1)$$

$$b = (100 - C)\, a / 100 \quad (5\text{-}2)$$

$$C = (1 - b/a) \times 100 \quad (5\text{-}3)$$

In the case of polystyrene (PSt) beads, the coverage, C' was calculated in same manner as the silica beads. Before polymer coating onto the PSt surface, only carbon elements should be observed without any others. However, a peak corresponding to oxygen was detected (Table 5.2.1).

The percentage of oxygen element in the polymer, d was represented with element compositions of coating polymer (Equation 5-4).

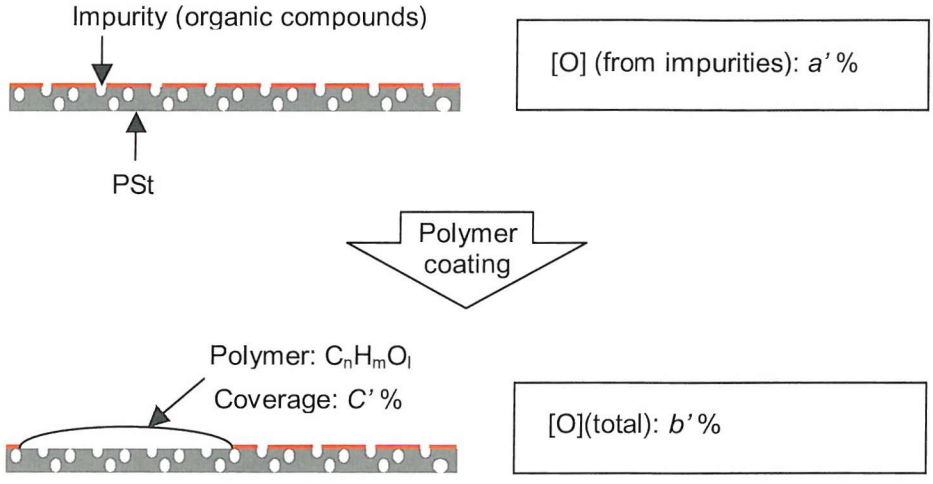


Figure 5.2.2. Calculation of the coverage of the polymer coated polystyrene beads.

$$d = l / (n + m + l) \times 100 \quad (5\text{-}4)$$

$$b' = a' (1 - C'/100) + d \times C'/100 \quad (5\text{-}5)$$

$$C' = (b' - a') \times 100 / (d - a) \quad (5\text{-}6)$$

Therefore, the coverage, C' was represented with the percentage of oxygen element, before and after polymer coating (Equations 5-5 and 5-6). Thus, the coverage of polymer coated beads was calculated.

5.2.4 Effects of beads type and solvent

Table 5.2.1 shows the elemental ratios on the surface of the beads and coverage determined by XPS. It was observed that when alcohol type solvents were used as the coating solvent, the coverage of polymers onto the silica beads was higher than with other solvents. The different beads had less influence on the coverage of the polymers. However, the state of the polymer coating were very different (Figure 5.2.3 and 5.2.4). Thus in one case, spheres of polymer were formed on the polystyrene beads, due to poor affinity between the polymer and beads, while in another, the polymer film was coated smoothly on the silica beads. Therefore, silica beads were used for subsequent experiments.

Table 5.2.1. Surface analysis of the coated beads by XPS.**

No.	Polymer	beads	Solvent (vol.%)	Element ratio (%)			Coverage*
				Si	C	O	(%)
1	-	PSt	-	0.9	94.2	4.9	-
2	-	Silica	-	28.8	8.2	63.0	-
3	PMEMA	PSt	IPA/H_2O (70/30)	0.0	83.4	16.6	47
4	PMEMA	Silica	IPA/H_2O (70/30)	18.2	35.6	46.2	37
5	PMEMA	Silica	THF/EtOH (50/50)	26.1	13.9	60.0	9
6	PMEMA	Silica	THF	26.3	14.4	59.3	9
7	PMEMA	Silica	$CHCl_3$	23.5	22.7	53.9	18
8	PMEMA (*calc.*)	-	-	0.0	70.0	30.0	-

PMEMA was coated with a polymer concentration of 10 mg/mL.

* Coverage: Chapter 5.2.3. ** XPS was performed using an ESCALAB250 (VG)

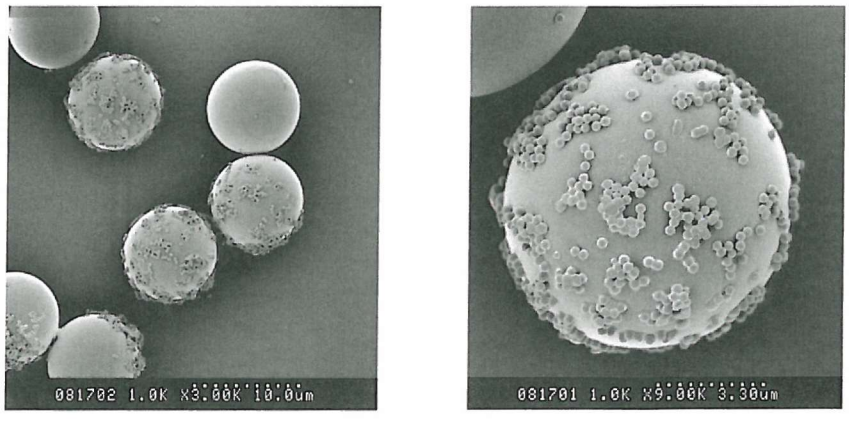


Figure 5.2.3. SEM images of PMEMA coated polystyrene beads (No.3 in Table 5.2.1).

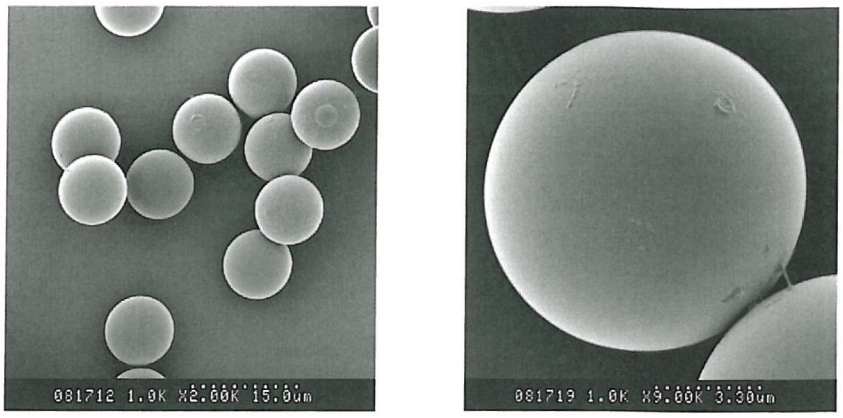


Figure 5.2.4. SEM images of PMEMA coated Silica beads (No.4 in Table 5.2.1).

Different types of alcohol, such as methanol (MeOH), ethanol (EtOH), isopropanol (IPA), and *tert*-butanol (*t*-BuOH), were studied as a coating solvent with a polymer concentration of 10 mg/mL. The highest coverage was achieved when a mixture of MeOH and water were used as the solvent (Figure 5.2.5). The polymer was found to be coated on the silica beads smoothly (Figure 5.2.6), but when the solvent was EtOH, IPA, or *t*-BuOH, coverage was lower.

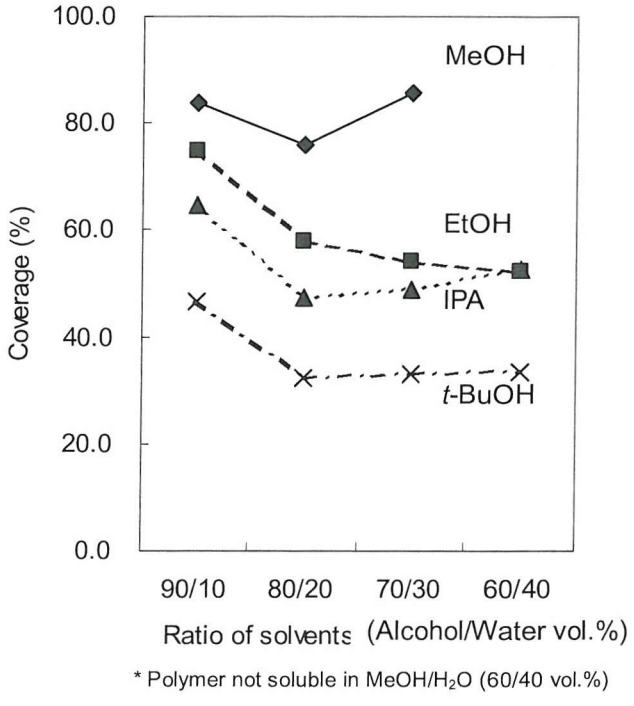


Figure 5.2.5. Effect of various solvents on polymer coverage on silica beads.

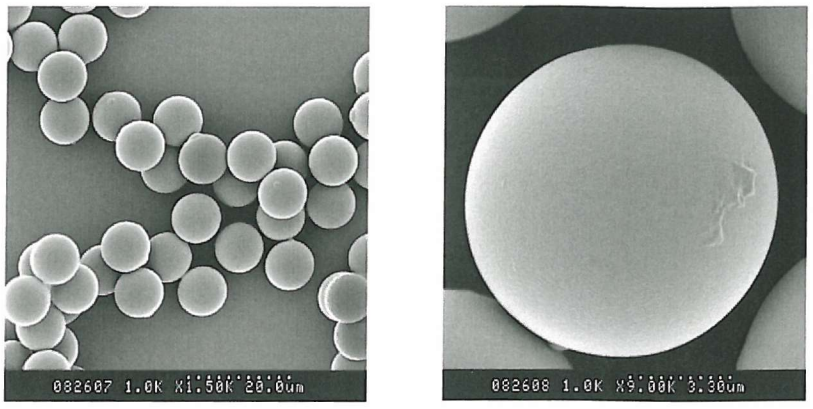


Figure 5.2.6. SEM images of PMEMA coated Silica beads prepared with a polymer concentration of 10 mg/mL. MeOH/H_2O (90/10 vol.%), coverage 84 %.

The solubility of the polymer was also believed to influence coating, thus as the polymer concentration increased, coverage was also found to increase (Figure 5.2.7), however polymer coating was uneven at higher polymer concentrations (*e.g.* 50 mg/mL, Figure 5.2.8).

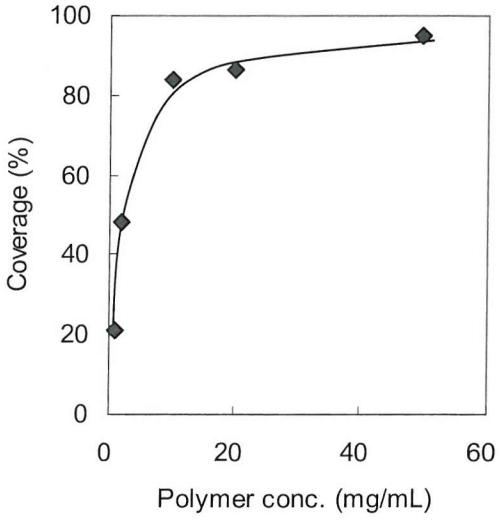


Figure 5.2.7. Correlation of the coverage and PMEMA concentration in mixed solvents of MeOH and water (90/10 vol.%).

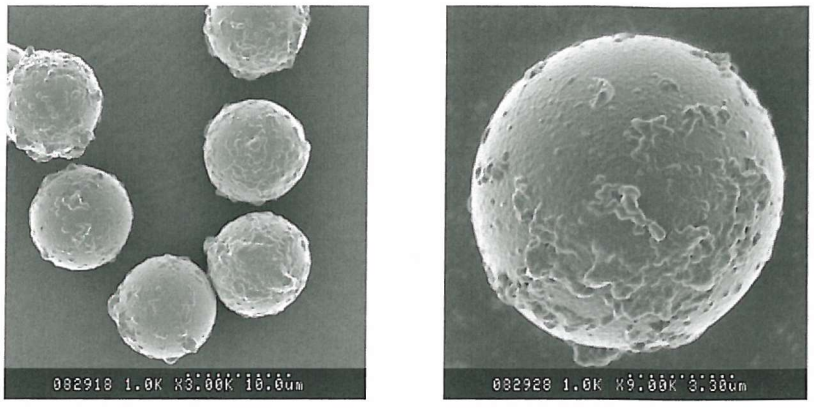


Figure 5.2.8. SEM images of PMEMA coated Silica beads prepared with a polymer concentration of 50 mg/mL. MeOH/H_2O (90/10 vol.%), coverage 95 %.

In conclusion, PMEMA could be coated onto silica beads with a smooth and high coverage layer using a suitable solvent, polymer concentration, and procedure.

5.2.5 Stability of PMEMA coated beads in water

The coated polymer was tested for its stability in water, since protein adsorption was to be measured in water by flow cytometry. The polymer coated beads were added to water for 2 or 24 hrs, and the bead surfaces analysed again by XPS. Table 5.2.2 shows the results of XPS, showing that the PMEMA coated beads were stable in water for at least 2 hrs.

Table 5.2.2. Stability of the coated polymer in water.

No.	Polymer	Time in water (hr)	Element ratio (%)			Coverage	Decrease in coverage.
			Si	C	O	(%)	(%)
1	PMEMA *	0	4.7	64.2	31.1	84	-
2	PMEMA *	2	5.0	66.3	28.7	83	1
3	PMEMA *	24	6.9	61.2	32.0	76	8

* PMEMA coated silica beads was prepared at the condition, MeOH/H_2O = 90/10 vol.%, a polymer concentration of 10 mg/mL.

5.2.6 Coverage and protein adhesion

Protein adsorption onto the polymer coated silica beads was measured by flow cytometry (FCM), using Fluorescein isothiocyanate (FITC)-conjugated human immunoglobulin G (IgG), Alexa Fluor 647 (Alexa647, Molecular Probes, Inc.)-conjugated human fibrinogen (Fib). (IgG-FITC, was excited by a 488 nm laser, had a fluorescence emission maximum around 520 nm and was detected in the fluorescence channel 1 (FL1, 530 nm filter). Fib-Alexa647, which was excited by a 633 nm laser, had fluorescence emission maximum around 650 nm and was detected in fluorescence channel 4 (FL4, 661 nm filter)).

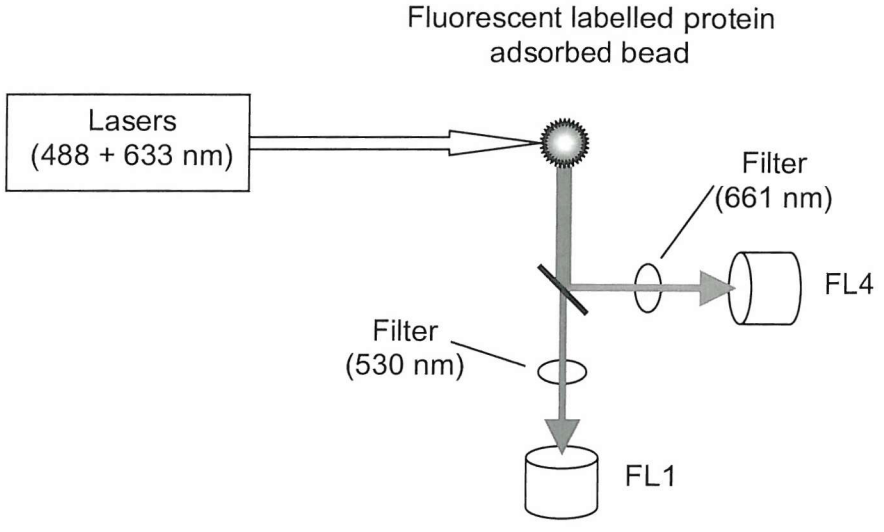


Figure 5.2.9. Fluorescent-activated cell sorting (FACS).

A mixture of the proteins (1 μg/mL of each protein) were prepared in 1 % goat serum / phosphate-buffered saline (PBS), and incubated with the polymer coated beads for 30 min (Figure 5.2.10). (Longer incubation times did not give significant changes in intensity). The protein and antibody associated fluorescence intensity on the bead was determined by FCM analysis using a FACSCalibur 4A (Becton Dickinson Co., Ltd.). Several thousands beads were measured, and the average value of fluorescence intensity was determined.

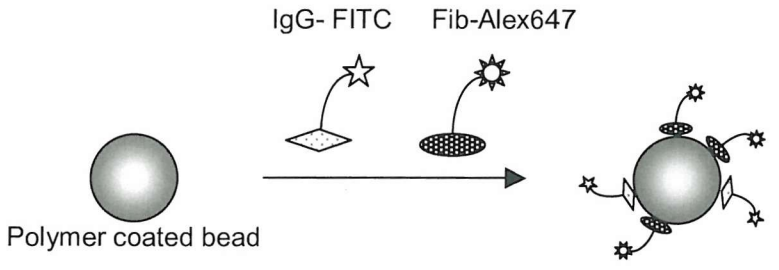


Figure 5.2.10. Protein adsorption onto polymer coated beads.

PMEMA coated silica beads with varying coverage were examined by FCM. Figure 5.2.11 shows the correlation between polymer coverage and the intensity of fluorescence corresponding to absorbed IgG and Fib. In the case of IgG adsorption, the fluorescence intensity was uniform from 0 to 80 % polymer coverage. While, in the case of Fib, high fluorescence intensity was observed with non-coated beads and the intensity decreased with higher coverage of polymer coated beads. The intensity of Fib-associated fluorescence was constant at more than 50 % coverage, but at 95 % coverage higher intensity was observed due to roughness on the surface of the polymer coated beads (Figure 5.2.8).

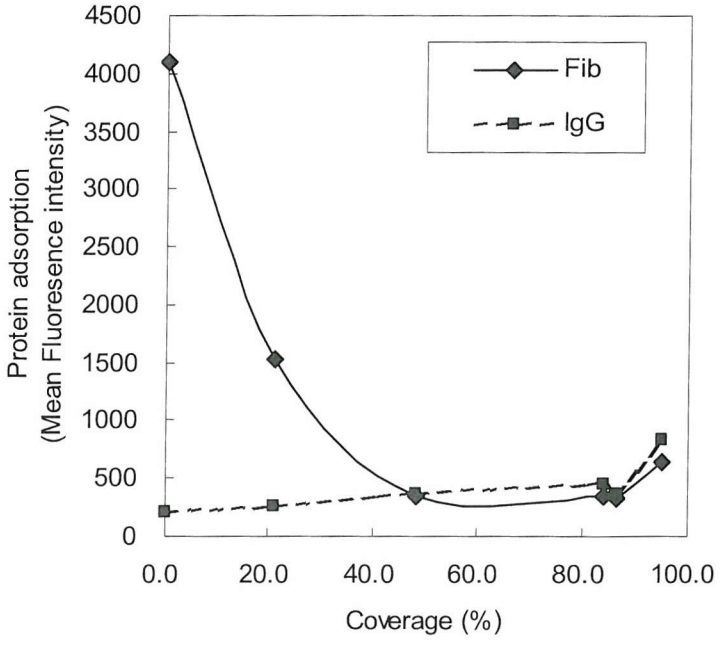


Figure 5.2.11. Correlation between coverage and protein absorption onto PMEMA coated Silica beads

5.2.7 Coating the polymer library

The polymers in libraries 1, 2, and 4 (189 polymers in total) were coated onto the silica beads. Since most PMEA copolymers were highly viscous liquids or rubbery, polymer coated beads aggregated after coating, and some polymers were not soluble in organic solvents with low boiling points which were appreciate for freeze-drying. However, most polymers could be used for this experiment. The coating method was thus found to be ideal for polymer coating onto silica beads, and all polymer coated beads were analysed by XPS.

Of the 189 polymers, 121 were coated onto the beads with a coverage exceeding 50 % coverage (Figure 5.2.12). Table 5.2.3 shows the effect of monomer types on the coverage of polymer coating, showing that some copolymers for example with hydroxy or acid group could not be coated with the required degree of coverage. The copolymers containing amine groups or styrene monomers were coated with high coverage.

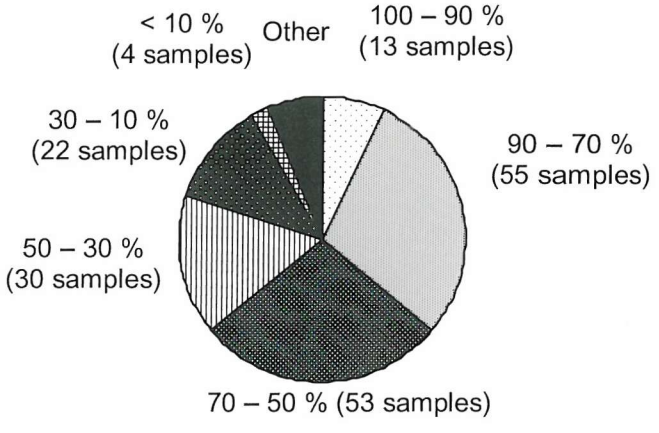


Figure 5.2.12. Sample distribution according to polymer coverage.

Table 5.2.3. Percentage and number of samples with more than 50 % coverage. Classified by type of (a) main monomer and (b) functional monomer.

(a)	Number of samples	Number of samples with > 50 % coverage	%
St	9	9	100
MMA	24	11	46
MEMA	72	58	81
MEA	9	-	-
HEMA	57	41	72
HPMA	9	2	22
HBMA	9	0	0
Total	189	121	64

*MEA: Aggregated

(b)	Number of samples	Number of samples with > 50 % coverage	%
Amide	87	53	61
Amine	36	30	83
Amine, Amide	6	5	83
thioether	6	6	100
Aromatic	18	17	94
Nitro	6	4	67
Acid	30	6	20
Total	189	121	64

5.2.8 Protein adsorption on polymer coated beads

Protein adsorption onto the polymer coated beads with more than 50 % coverage was investigated by FCM. The fluorescent-labelled protein, human albumin (Alb), IgG, and Fib, (which are thought to be concerned with clot formation in blood,) were used for the assay, and the setting of laser, filter, and fluorescence channel on flow cytometry are given in Table 5.2.4.

Table 5.2.4. Labelled fluorescents and instrument setting.

Protein associated fluorescent	Laser (nm)	Fluorescence channel	Filter (nm)
IgG-FITC	488	FL1	530
Alb-Alexa555	488	FL2	585
Fib-Alexa647	633	FL4	661

A mixture of proteins and antibody suspensions (1 μg/mL for each protein) were prepared in serum-free PBS, and incubated with the polymer coated beads at room temperature for 30 min. Fluorescence on the bead was determined by FCM analysis. The average value of fluorescence intensities of several thousands beads was calculated. The data obtained was analysed using Spotfire® which allowed correlation between a number of parameters to be determined. The results are shown in Figure 5.2.13. (The axes in the graphs indicate the intensities of fluorescence which were corresponding to Alb, IgG, and Fib, the colours of graph (a) and (b) were categorised by the main monomer A and the functional monomer type B.)

Most St copolymers with amide groups had higher Fib adsorption compared to acrylate type polymers. It was thought that the property of styrene, such as aromaticity, rigidness, and hydrophobicity, might have a significant effect on Fib adsorption.

Copolymers with MEMA or HEMA had a various range of protein adsorptions. As shown in graph (b), amide, heteroaromatic, and acidic groups in the MEMA copolymer did not have any great effect on protein bindings. However, IgG and Alb adhered onto the amine group containing copolymers. Accordingly, it was concluded that MEMA and HEMA are not important monomers for specific protein adsorption, and the amine group had an essential effect on IgG and Alb adsorption. The amine group might be protonated to form the positive charge at physiological pH (pH 7.4). It was considered that the positive charge caused the interaction between proteins (IgG and Alb) and polymeric materials.

The MEMA copolymers containing nitro group had relatively high adsorptions of Fib, though most of the other MEMA copolymers showed low adsorption.

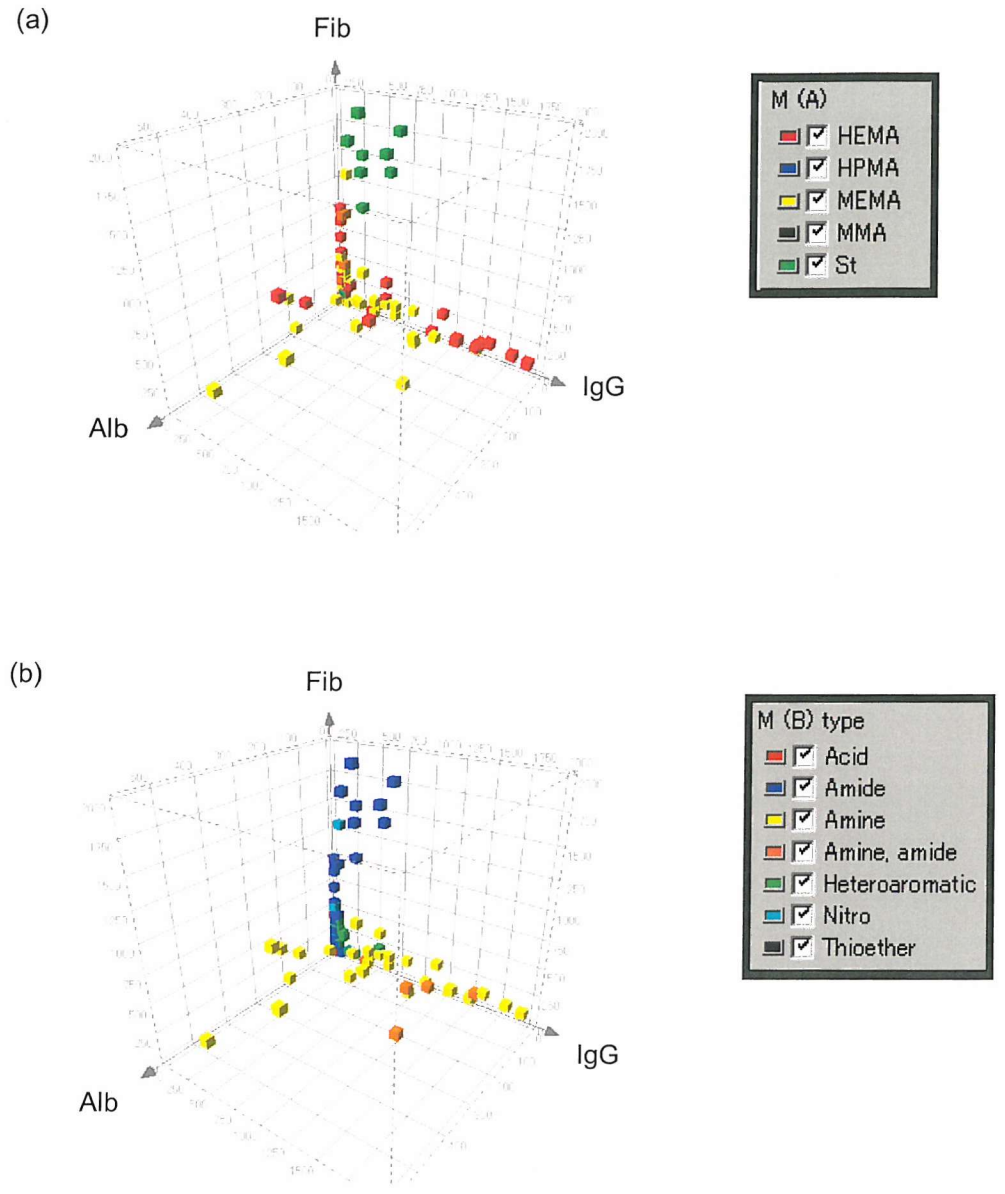


Figure 5.2.13. Effect of monomer type on protein adsorption. The colours correspond to specific monomer types (a) main monomer and (b) functional monomer.

5.2.9 Correlation between protein adsorption and polymer properties

The correlation between protein adsorption and polymer properties was evaluated by the data analysis software Spotfire®. The data of protein adsorption measured by FCM, the spreading area (wettability parameter) and glass transition temperature were used for this investigation. Figure 5.2.14 and 5.2.15 show the results of the correlation between each parameter, and the colours were categorised by the main monomer and functionalised monomer in the copolymer.

Wettability had a significant effect on protein adsorption. In particularly, Fib and Alb were less adsorbed onto hydrophilic polymers. At lower wettability, the protein adsorptions were observed in a wide range. This indicates the potential to obtain high protein adsorption on hydrophobic surfaces, and that protein adsorption is dependent upon many other factors which are not within the scope of this project. On the other hand, no correlation between glass transition temperature of the polymer and protein adsorption was observed. For Fib adsorption, it seemed that the polymers with a higher Tg gave higher protein adsorption, but most polymers with a high Tg were styrene based polymers. The aromatic ring might have positive interactions (such as π-π interactions) with Fib.

Importantly, the most essential factor for protein adsorption was the functional groups in the polymer, and the functional groups had a remarkable effect on protein adsorption (Chapter 5.2.8 and Figure 5.2.13).

In conclusion, it was found that there were correlations between polymer properties and protein adsorption, with wettability and functional groups on the polymer being key parameters for protein adsorption. Controlling the wettability suppresses Fib and Alb adsorption, and the suitable functional groups on polymer surface might control the specific protein adsorption. Accordingly, these results might lead the development of the next generation of biocompatible and functional polymers.

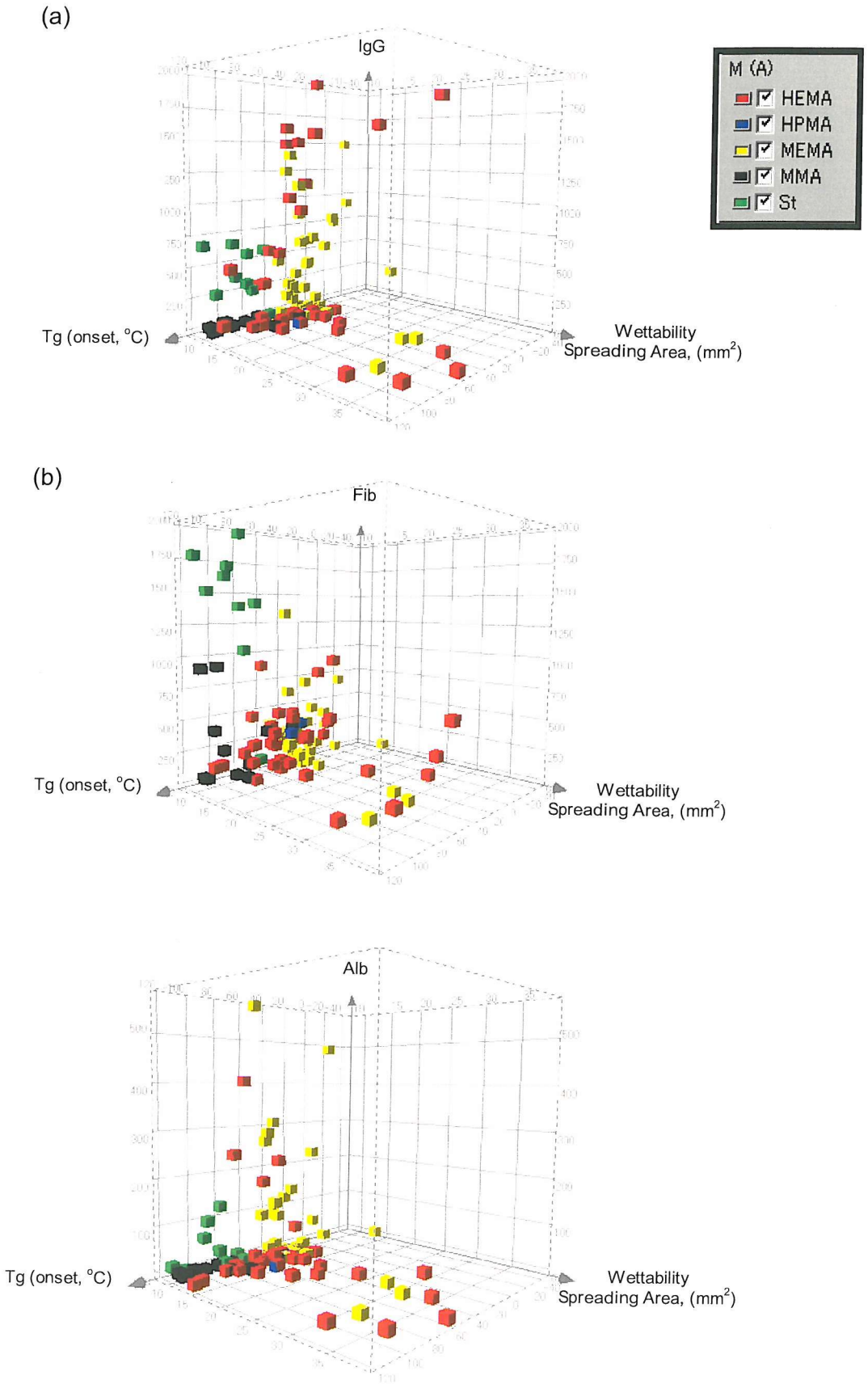


Figure 5.2.14. Correlation between protein adsorption, wettability, and glass transition temperature. (a) IgG, (b)Fib, and (c) Alb. The colour was categorized by main monomer.

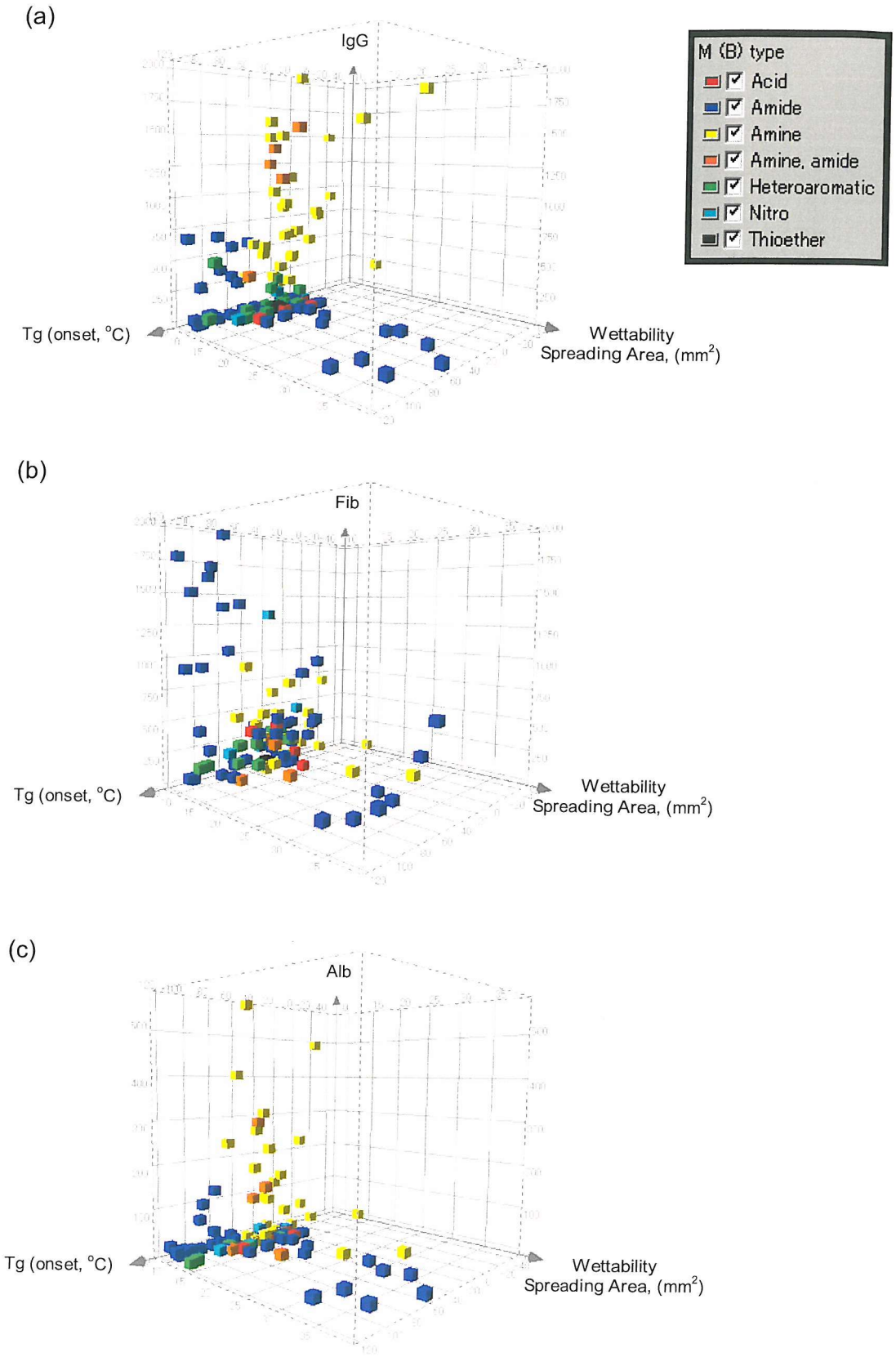


Figure 5.2.15. Correlation between protein adsorption, wettability, and glass transition temperature. (a) IgG, (b)Fib, and (c) Alb. The colour was categorized by functionalised monomer.

5.3 Leuco-depletion

5.3.1 Background

In processing whole blood for therapeutic administration to patients, it is desirable to separate the various cellular components. In particular, it is important to remove leucocytes in order to avoid adverse clinical events (Chapter 1.1.10). In recent years, a filtration process to remove leucocytes has been developed, however, improvement in selectivity and efficiency is still required.

5.3.2 Experimental for blood filtration

In order to develop new polymers for blood filtration, the following requirements are necessary,

(a) Improvement in leuco-depletion.

(b) Negligible hemolysis.

(c) High-efficiency platelet removal.

These issues were investigated in blood filtration experiments.

Experiments were carried out using a published method,[99,176] using polymer coated onto a filter base material, non-woven fabric (thickness: 0.20 mm, polyethylene terephthalate fibre with an average fibre diameter of 1.2 μm). Leuco-depletion ability and platelet recovery were calculated from Equations 5-7 and 5-8. The tendency to produce hemolysis was evaluated by removing blood cell components from the filtered blood by centrifugation (1500 rpm, 10 min), and then detecting haemoglobin by measuring absorbance at 576 nm.[177]

Leuco-depletion ability (-Log) = -Log (leucocyte concentration after filtration / leucocyte concentration before filtration) (5-7)

Platelet recovery (%) = (platelet concentration after filtration / platelet concentration before filtration) x 100 (5-8)

As a positive control, a known material[99] with high leuco-depletion ability was used, and the abilities of the positive control in this experiment are shown in Table 5.3.1. The target was the development of a polymer with higher leuco-depletion and similar or less hemolysis. In all experiments, the difference of leuco-depletion ability from the positive control was calculated to understand the improvement.

Table 5.3.1. Data for the positive control.

	Ave.	(Standard deviation)
Leuco-depletion ability (-log)	2.98	(0.38)
Platelet recovery (%)	1.6	(2.7)
Hemolysis (ABS at 576 nm)	0.25	(0.20)

5.3.3 Screening of copolymers

33 copolymers with various functional groups were investigated for blood filtration. The results are shown in Figure 5.3.1. As shown in graph (a) (Figure 5.3.1), some of MEMA and MMA polymers gave high leuco-depletion ability, but it was difficult to understand the polymer structure trends that gave good blood filtration. According to graph (b) (Figure 5.3.1), most of the polymers that gave higher leuco-depletion ability contained amine groups, but some polymers showed hemolysis. It was thus difficult to achieve the both high leuco-depletion and low hemolysis.

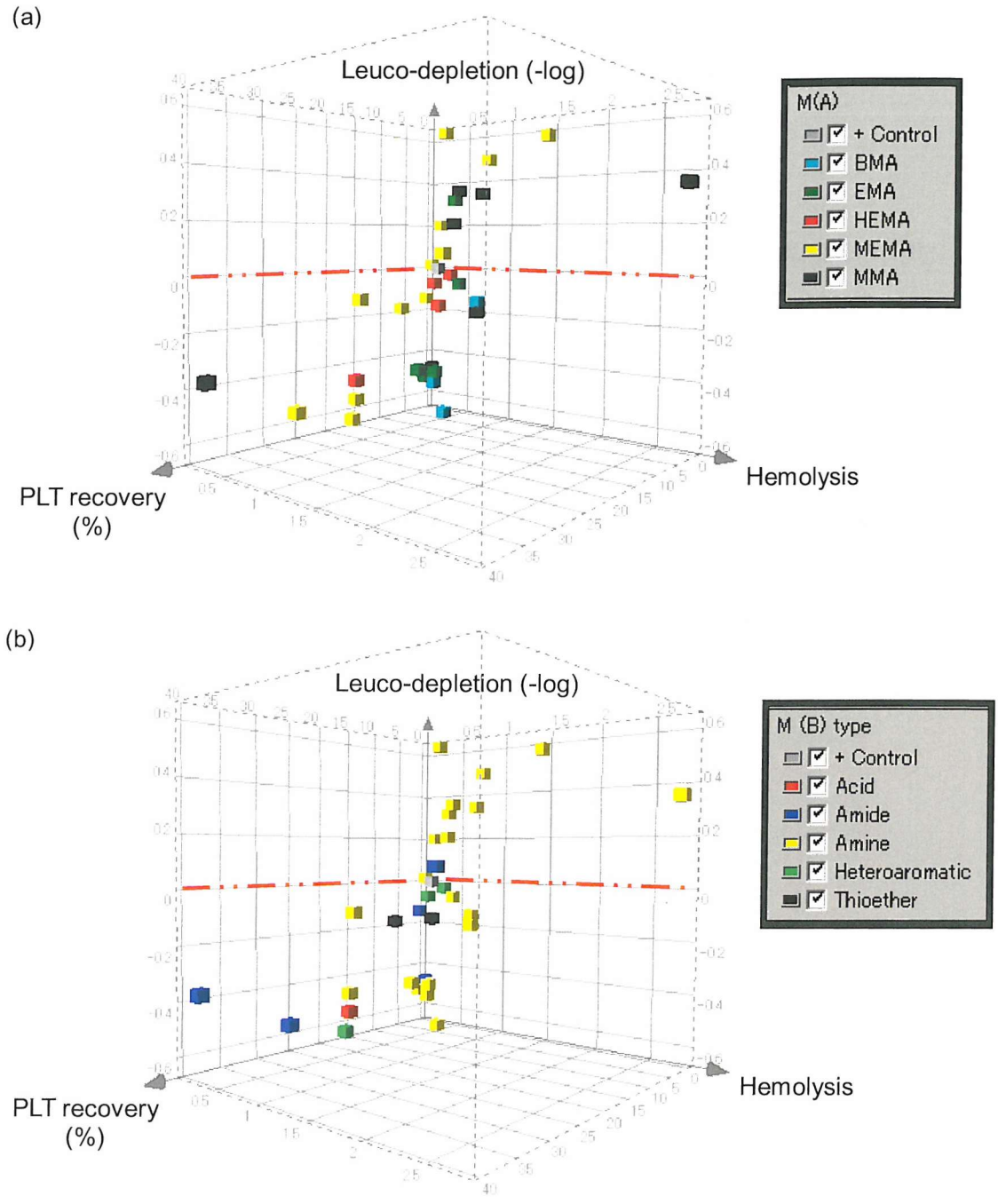


Figure 5.3.1. Effect of the polymer structure on the blood filtration. The colours correspond to specific monomer types (a) main monomer and (b) functional monomer.

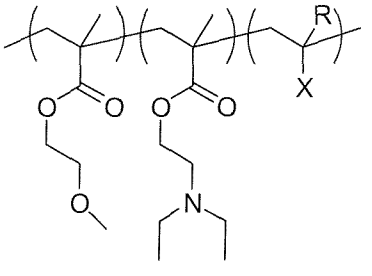
5.3.4 Effect of the third component on leuco-depletion

MEMA as a main component and DEAEMA as a monomer containing amine groups were selected, and the effect of the third component was investigated to achieve higher leuco-depletion and lower hemolysis. 14 different monomers were used (The structures are given in Chapter 2.4.5), and the monomer composition was set at 40/30/30 mol.% (MEMA/DEAEMA/Third component).

The third component had a remarkable effect on leuco-depletion ability (Figure 5.3.3). DEGMEMA, THFFMA, and DAAAm polymers gave higher leuco-depletion ability compared with the positive control and copolymer, P(MEMA-*co*-DEAEMA) (70/30 mol.%), however THFFMA and DAAAm containing polymers caused hemolysis.

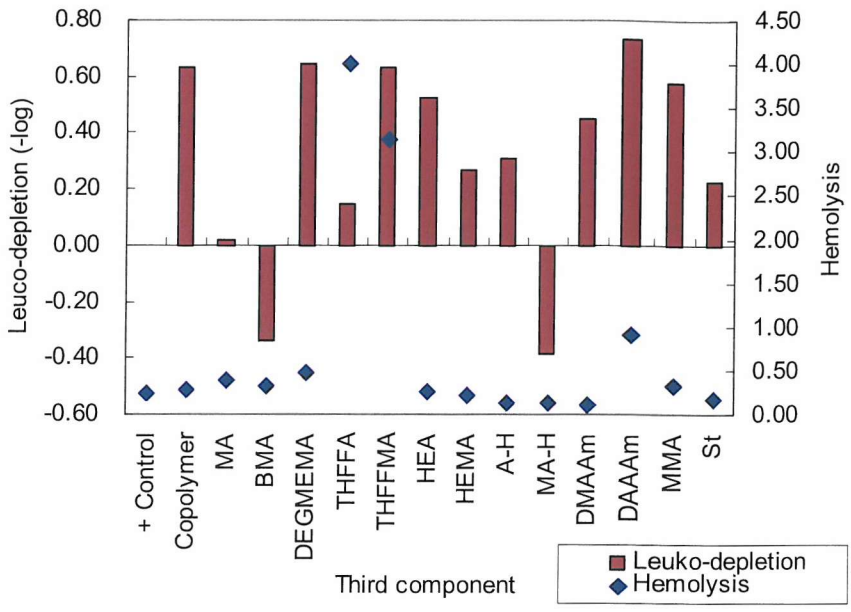
The terpolymer containing MMA did not give the change of the leuco-depletion ability and hemolysis of the copolymer. The polymers with the hydrophilic monomers, HEMA, HEA, and DMAAm had lower leuco-depletion ability than copolymer, but they had higher leuco-depletion ability than the positive control and kept low hemolysis. These polymers might be useful for application as new blood filtration devices.

Interestingly, the polymer with acidic monomers (A-H and MA-H) as a third component gave the great change of leuco-depletion ability compared with the copolymer, P(MEMA-*co*-DEAEMA) (70/30 mol.%). It was considered that the charge of the polymer was concerned with the leuco-depletion.



[MEMA]/[DEAEMA]/[Third component]
(40/30/30 mol.%)

Figure 5.3.2. Structure of terpolymers.



* Copolymer: P(MEMA-*co*-DEAEMA): 70/30 mol.%

Figure 5.3.3. Effect of the third component on leuco-depletion.

5.3.5 Polyzwitterions for blood filtration

In order to investigate the effect of the formal charge on the polymers, MEMA, DEAEMA, and A-H in varying monomer ratios were examined.

The results are shown in Figure 5.3.4. Polymers with more than 60 mol.% of DEAEMA and A-H were soluble in water. However with insoluble polymers, it was observed that as the content of A-H was increased, leuco-depletion ability decreased, and platelet recovery increased. On the other hand, as the content of DEAEMA increased, leuco-depletion ability increased, and platelet recovery decreased. Furthermore, polymers with a low content of A-H and a high content of DEAEMA caused hemolysis.

The effect of the difference between DEAEMA and A-H on leuco-depletion is shown in Figure 5.3.5. Unpredictably, some polyzwitterionic polymers which contained DEAEMA and A-H showed higher leuco-depletion abilities than the copolymers without A-H. In particularly, polymers which had a composition, MEMA/DEAEMA/A-H = 70/20/10 mol.%, showed high leuco-depletion ability with a low level of hemolysis. Thus, the balance of DEAEMA and A-H was important in improving the leuco-depletion ability without hemolysis.

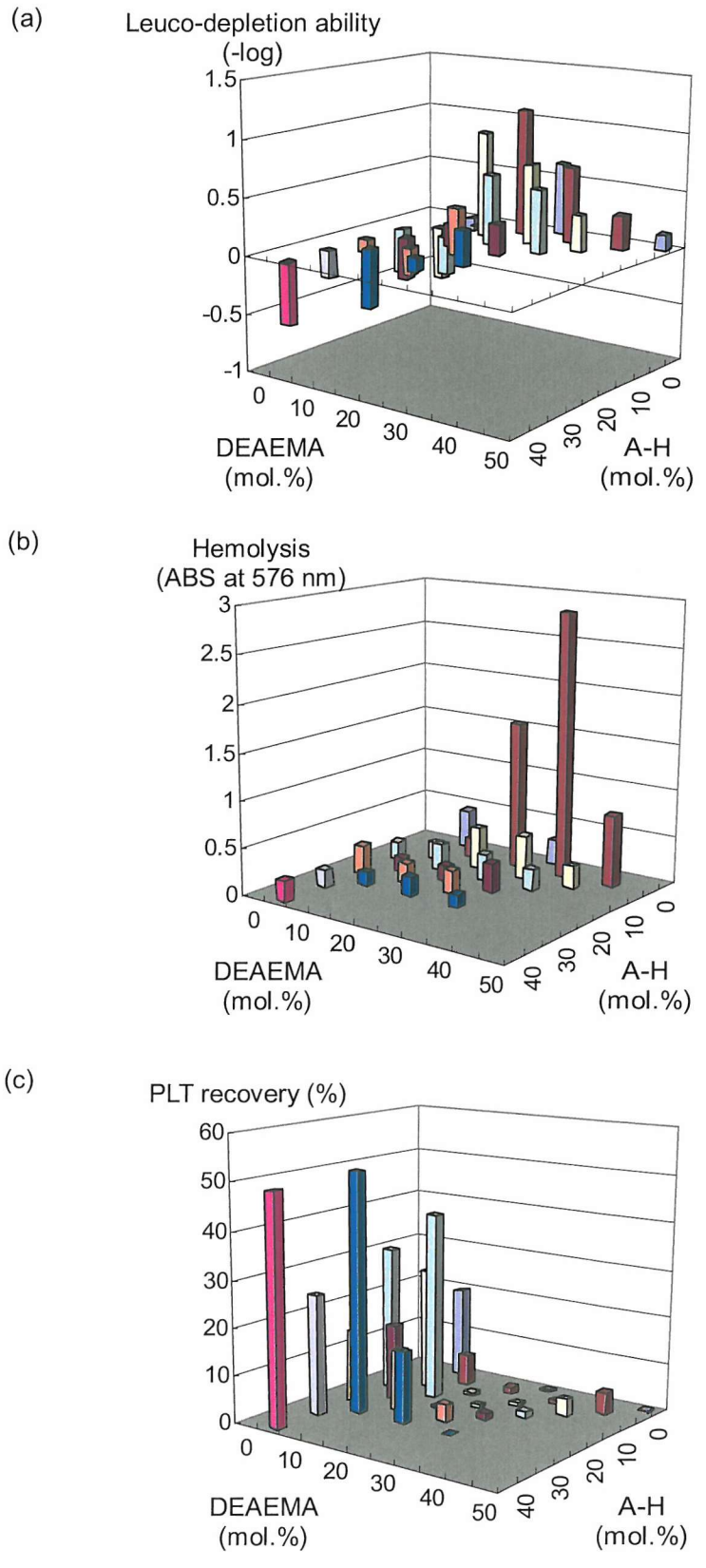
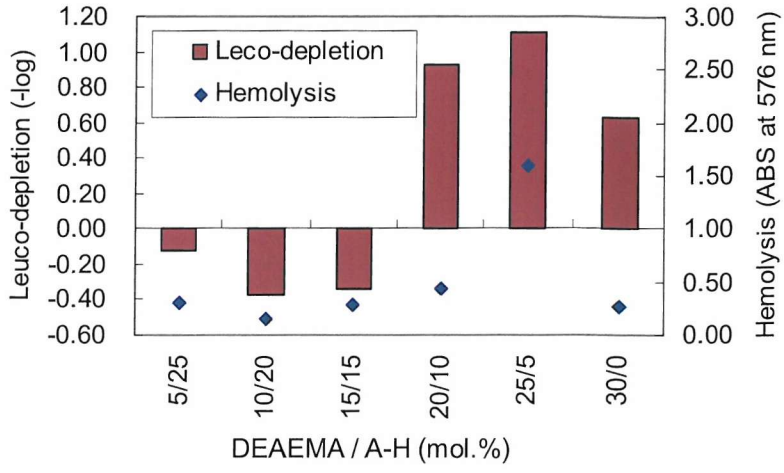
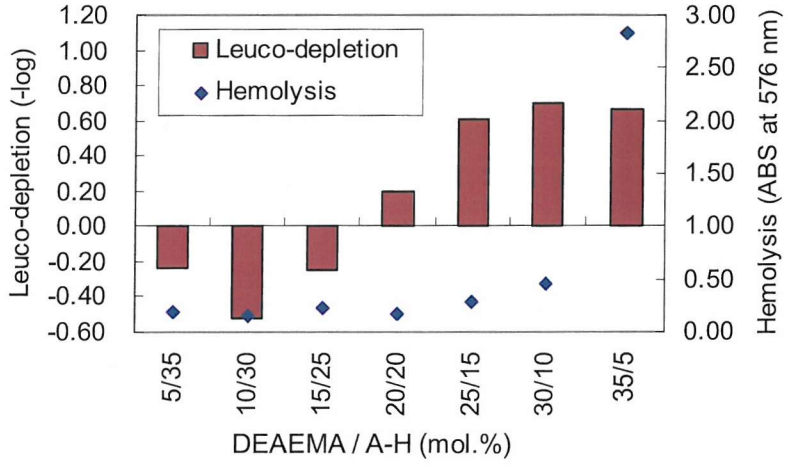


Figure 5.3.4. Effect of monomer composition on polyzwitterions on (a) leuco-depletion, (b) hemolysis, and (c) platelet recovery. The composition MEMA/DEAEMA/A-H = 70/20/10 mol.% gave high leuco-depletion ability, a low level of hemolysis, and low PLT recovery.

(a) MEMA 70 mol.%



(b) MEMA 60 mol.%



(c) MEMA 50 mol.%

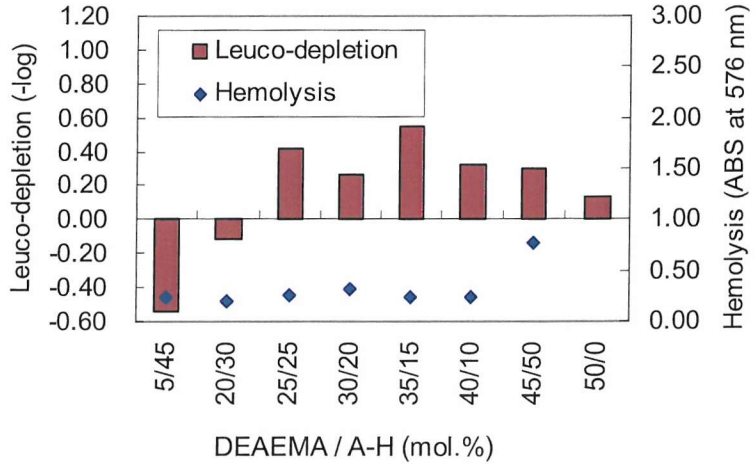


Figure 5.3.5. Correlation between monomer composition and leuco-depletion.

5.3.6 Effect of polymer structure on leuco-depletion abilities

The effect of polymer structure was examined by varying monomer type in order to improve the ability for leuco-depletion. Polymers with a 70/20/10 (Main/Amine/Acid) mol ratio of monomers were tested, because in the case of MEMA, DEAEMA, and A-H, the terpolymer with this monomer composition gave the highest leuco-depletion ability with low hemolysis.

Table 5.3.2 shows the results of the blood filtration tests. No polymers caused significant hemolysis. MEMA type polymers gave higher leuco-depletion ability compared to MMA polymers. DMAEMA and DMAEA copolymers gave relatively high leuco-depletion abilities, although the polymer containing DMAPMAAm had a low leuco-depletion ability. A-H containing copolymers gave higher leuco-depletion ability compared to MA-H polymers.

In conclusions, although the leuco-depletion ability of these polymers may be improved by optimisation of monomer composition in each case, polymers with high leuco-depletion abilities were not discovered compared to the polymer which consisted of MEMA, DEAEMA, and A-H.

Table 5.3.2. Results of blood filtration test.

Code	Polymer structure			Leuco-depletion	Homolysis
	Monomer (1)	Monomer (2)	Monomer (3)	(-log)	(ABS at 576 nm)
PL6-3	MEMA	DEAEMA	A-H	0.92	0.44
PL6-6	MEMA	DEAEA	A-H	0.67	0.31
PL6-9	MEMA	DEAEMA	MA-H	0.13	0.24
PL6-12	MEMA	DEAEA	MA-H	0.39	0.16
PL6-15	MMA	DEAEMA	A-H	0.00	0.13
PL6-18	MMA	DEAEA	A-H	0.49	0.15
PL6-21	MMA	DEAEMA	MA-H	-0.22	0.23
PL6-24	MMA	DEAEA	MA-H	0.39	0.13
PL6-80	MEMA	DMAEMA	A-H	0.60	0.19
PL6-82	MEMA	DMAEA	A-H	0.56	0.26
PL6-84	MEMA	DMAPMAAm	A-H	-0.04	0.14

5.3.7 Correlation between leuco-depletion and protein adsorption

As discussed in Chapter 5.2, the polymer structure had a significant effect on protein adsorption. In particularly, it was found that the (protonated) amine groups gave high IgG adsorption. As for cell adsorption, it was also found the polymer containing amine and acid groups gave high leuco-depletion ability. Therefore, the correlation between IgG adsorption and leuco-depletion ability was studied.

The polymers which consisted of MEMA, DEAEMA, and A-H with varying compositions were used for this study, and the data of IgG adsorption which were investigated by FCM analysis were used. (The procedure was described in Chapter 5.2.) Figure 5.3.6 – 5.3.8 show the correlation between IgG adsorption, leuco-depletion ability, and difference of monomer content ([DEAEMA] – [A-H]) which indicate the polymer charge. It was found that monomer composition correlated poorly with IgG adsorption (Figure 5.3.6), and the polymer with higher contents of DEAEMA than A-H adsorbed IgG.

The correlation between monomer composition and leuco-depletion was also observed. Polymers with lower values for the difference between DEAEMA and A-H gave low leuco-depletion abilities, while the leuco-depletion ability increased upon increasing the differences between DEAEMA and A-H. However at more than 30 mol.% differences between DEAEMA and A-H, leuco-depletion decreased. Accordingly, the mountainous looking curve was observed (Figure 5.3.7).

No correlation was observed between IgG adsorption and leuco-depletion, thus the monomer composition that gives high IgG adsorption was not same as that which gave high leuco-depletion ability (Figure 5.3.8).

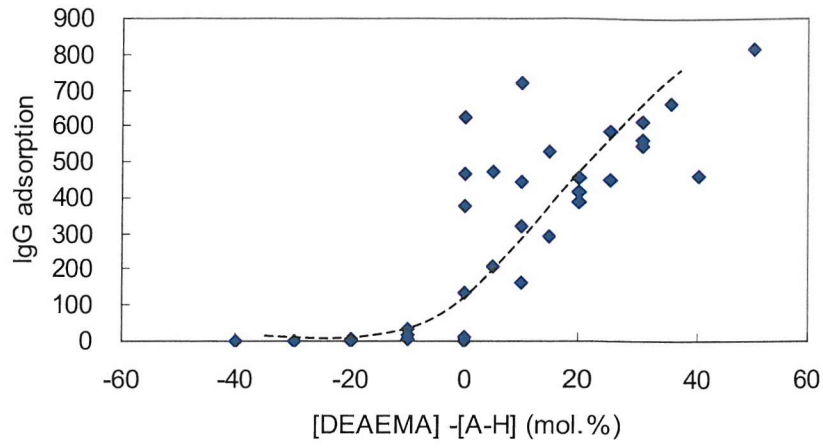


Figure 5.3.6. Correlation between IgG adsorption and monomer composition.

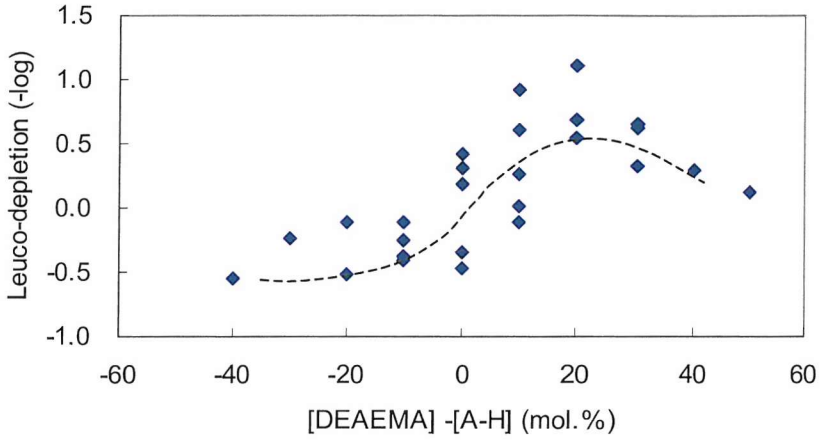


Figure 5.3.7. Correlation between leuco-depletion ability and monomer composition.

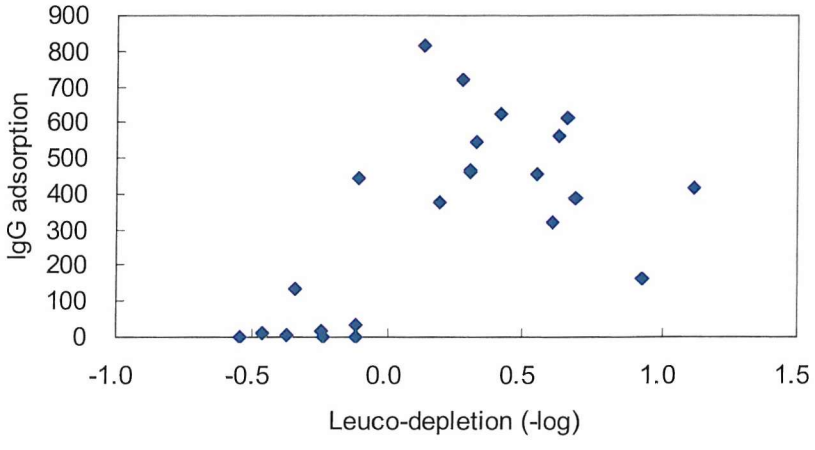


Figure 5.3.8. Correlation between IgG adsorption and leuco-depletion.

5.4 Conclusions

In order to investigate the protein adsorption on polymer with high throughput, flow cytometry (FCM) was utilised. The flow cytometric method can analyse submicron sized objects. Therefore, the silica beads (10 μm) were used as core substrates and a simple polymer coating method was established. Most polymers could be coated onto the silica beads with more than 50 % coverage. The polymer coated silica beads (121 types) were investigated for protein adsorption by FCM analysis.

It was observed that the functional groups of the polymer had a significant effect on protein binding. The St-based copolymers gave high Fib adsorption due to the hydrophobicity, aromaticity and so on. The amine group gave high IgG and Alb adsorptions, because of the positive charge which might be formed by protonation of amine group. Furthermore, the control of polymer wettability reduced the protein adsorption.

Leuco-depletion is one of the most desirable ability for the development of blood filtration. The polymer libraries were screened in order to discover new polymeric materials with leuco-depletion ability. It was found that polyzwitterions which consisted of amine and carboxylic acid groups gave high leuco-depletion abilities, while monomer composition had a remarkable effect on leuco-depletion ability, and some polymers showed quite higher leuco-depletion ability without hemolysis than positive control. These new polymers identical could be utilised for advanced blood filtration devices.

Thus, controlling the polymer properties such as the charge and wettability is important for both cell and protein adsorption. These results enabled the development of polymers with high biocompatibility and biofunctionality.

Chapter 6

EXPERIMENTAL

6.1 General information

6.1.1 Materials

All reagents and solvents were obtained from commercial sources and were used without further purification unless specified. Monomers, St, MEMA, MMA, DMAAm, NIPAAm, HEMA, HBMA, DMAEMA, DEAEMA, BAEMA, DMAEA, DEAEA, DMVBA, DMAPMAAm, BACOEA, MTEMA, DAAAm, MNPMA, VAA, VPNO, VI, VP-4, VP-2, DAAA, A-H, AES-H, MA-H, AAG-H, EGMP-H, GMA, MA, BMA, DEGMEMA, HEA, THFFMA, THFFA, and amine compounds, DnHA, DcHA, DBnA, MnHA, cHMA, BnMA, MAEPy, 2-MePy, MAn, TMEDA, DEMEDA, TMPDA, Mpi, TEDETA were purchased from Sigma-Aldrich Co., Ltd. MEA was purchased from Fisher Scientific UK. DEAAm was purchased from Lancaster Synthesis Ltd. HPMA, amine compound DnBA, and initiator AIBN were purchased from Acros Organics. Solvents, DMF, NMP, toluene, hexane, cyclohexane, and diethyl ether of analytical grade were used. Catalysts for reaction of epoxide with amines (silicagel 60) were purchased from Merck & Co., Inc. Polystyrene beads, Polybead® (10 μm) were purchased from Polyscience, Inc. Silica beads, UNK HIPRESICA (3N3, 10 μm) were purchased from UBE-NITTO KASEI Co., Ltd. Human fibrinogen (Fib) was purchased from American Diagnostica Inc. Human immunoglobulin G (IgG) and Human albumin (Alb) were purchased from SIGMA. Fib, IgG, and Alb were labelled with Alexa Fluor 647, fluorescein, and Alexa Fluor 555 labelling kits purchased from Molecular Probes.

6.1.2 Characterization

6.1.2.1 Gel permeation chromatography (GPC)

The average molecular weights of the polymers were measured using GPC, carried out using a HP1090 Liquid Chromatograph equipped with a refractive index detector (Hewlett Packard Co., Ltd.). The columns used were a PLgel HTS-D (Polymer Laboratories Co., Ltd.) and a PLgel 5 μm MIXED-C 300 x 7.5 mm (Polymer Laboratories Co., Ltd.). THF was used as mobile phase for the experiments of optimisation of polymer condition (Chapter 2.2) with a flow rate of 1.0 mL/min, and 10 mM LiCl/DMF solute or NMR was used for polymer libraries.

6.1.2.2 Infrared (IR) spectroscopy

IR spectra were obtained on a Satellite™ FTIR (Thermo Electron Corporation). Each spectrum from 4000 to 600 cm^{-1} was averaged over 32 scans at 4 cm^{-1} resolution.

6.1.2.3 Fourier transform infrared (FT-IR) microscopy

IR experiments of polymers on a glass slide were performed using a FT-IR Perkin-Elmer 2000 Spectrometer (Parkin-Elmer) combined with an AutoIMAGE FT-IR microscope, and spectral processing was carried out using AutoIMAGE™ and Spectrum 3.02v. The data was collected between 4000 - 650 cm^{-1} with a resolution of 8 cm^{-1}, with a summation of 16 scans.

6.1.2.4 Nuclear magnetic resonance (NMR) spectroscopy

^{1}H and ^{13}C NMR spectra were recorded in the solvent indicated at 298 K using a Bruker AC-300 or DPX400 spectrometer. All chemical shifts are quoted in ppm on the δ scale using residual protic solvent as the internal standard.

6.1.2.5 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed using an S-900 (Hitachi) with a 1 kV electron beam.

6.1.2.6 X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB250 (VG) at the following conditions,

X-ray source: monochromatic Al Kα

X-ray power: 15 kV x 10 mA

Analysis area: 600 μm x 300 μm (oval shape)

Pass energy: 20 eV

Peaks were characterised by the energy of the photoelectrons, and elemental ratios were calculated from the percentage of integrals of each peak in total.

6.1.2.7 Time of flight secondary ion mass spectrometry (TOF-SIMS)

Time of flight secondary ion mass spectrometry (TOF-SIMS) was performed using a TRIFT-III (PHI) at the following conditions,

Primary ion: Ga^+

Primary beam energy: 15 kV

Primary beam current: 600 pA

Secondary ion polarity: positive

Mass range: 0.5 – 2000 Da

Analysis area: 120*120 micron

Beam diameter: ca. 1 micron

Accumulation time: 1 min.

Charge neutralization: 28 eV electron beam

6.1.2.8 Flow cytometry (FCM)

Flow cytometry (FCM) was performed using an FACSCalibur 4A (Becton Dickinson Co., Ltd.) with analysis software Cell Quest (BD Bioscience, the U.S.). The gate was set to remove the aggregated polymer beads, and three band pass filters (530, 585, and 661 nm) were used for the investigation of fluorescence.

6.1.2.9 Differential scanning calorimetry (DSC)

A thermal analyser model Diamond DSC (Perkin-Elmer Inc.) calibrated with indium was used to determine polymer glass transition temperatures. Pyris Software Version 4.0 was used in order to operate the analyser and calculate the glass transition temperature.

6.1.3 Polymer spotting

Polymers were spotted onto a gold coated glass slide with Genetix Q Array mini arrayer (Genetix Ltd.) at following conditions,

Pin type: Solid pin

Number of stamps per spot: 5

Stamp time: 10 ms

Inking time: 200 ms

Washing condition of pin

Solvent: Ethanol

Washing time: 5000 ms

Dry time: 5000 ms

6.2 Experimental for Chapter-2

6.2.1 Free radical polymerisation

A mixture of AIBN, monomers and solvents were mixed under nitrogen. Polymerisation was carried out overnight. After the reaction, the product was precipitated by dropwise addition into a poor solvent (hexane, cyclohexane, diethyl ether, or their mixture) to give a solid or rubber. The polymer was washed with a poor solvent or reprecipitated, and dried under vacuum at 40 °C for 4 hrs.

Table 6.2.1 shows the reaction condition and results for the optimisation of the polymerisation conditions (Chapter 2.2). Table 6.2.2 - 6.2.7 show the reaction conditions for the synthesis of polymer libraries 1 - 6 (Chapter 2.4). The data for the polymers are given in Chapter 2.3.

6.2.2 Functionalisation of GMA copolymer

The glycidyl methacrylate (GMA) copolymer, synthesised by free radical polymerisation, was dissolved in a mixed solvent of *m*-xylene and cyclohexanol. Silicagel 60 was added to polymer solution as catalyst. Amine was added to the reaction mixture at 60 °C, and the reaction temperature was increased to 120 - 130 °C, and the reaction was carried out overnight. The product was precipitated by dropwise addition into a poor solvent (hexane, cyclohexane, diethyl ether, or their mixture). The polymer was dissolved in THF and reprecipitated, and the product was dried under vacuum at 40 °C.

The reaction conditions and results of functionalisation are described in Chapter 2.5, Table 2.5.4.

Table 6.2.1. Reaction conditions and results for polymerisation of MEMA.

No.	[M]/[I] (mol ratio)	Temp. (°C)	[M] (vol.%)	Appearance	Yield (mol.%)	Mn (Da)	Mw (Da)	MWD
1	800/1	50	20	No polymerisation				
2	600/1	50	20	No polymerisation				
3	400/1	50	20	No polymerisation				
4	200/1	50	20	Solid	27	20,500	57,600	2.8
5	100/1	50	20	Solid	76	30,100	83,700	2.8
6	50/1	50	20	Solid	94	24,900	66,300	2.7
7	800/1	60	20	Solid	76	31,800	80,000	2.5
8	600/1	60	20	Solid	77	32,800	75,600	2.3
9	400/1	60	20	Solid	74	33,100	73,000	2.2
10	200/1	60	20	Solid	Quant.	24,800	56,300	2.3
11	100/1	60	20	Solid	95	16,050	40,500	2.5
12	50/1	60	20	Solid	101	9,350	26,300	2.8
13	800/1	70	20	Solid	55	19,400	64,000	3.3
14	600/1	70	20	Solid	79	20,700	60,400	2.9
15	400/1	70	20	Solid	81	21,900	57,200	2.6
16	200/1	70	20	Solid	98	16,800	47,400	2.8
17	100/1	70	20	Solid	96	12,900	33,900	2.6
18	50/1	70	20	Solid	Quant.	8,050	22,600	2.8
19	400/1	60	5	Solid	26.5	8,520	20,100	2.4
20	400/1	60	10	Solid	53.6	19,100	46,700	2.4
21	400/1	60	20	Solid	96.2	47,200	128,000	2.7
22	400/1	60	30	Rubbery solid	99.8	65,700	198,000	3.0
23	400/1	60	40	Rubbery solid	Quant.	78,900	293,000	3.7
24	400/1	60	50	Rubbery solid	Quant.	92,000	387,000	4.2
25	400/1	60	60	Gel	N/A	N/A	N/A	N/A

Table 6.2.2. Reaction conditions for synthesis of polymer library-1.

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL1-1	St	DEAAm	90/10	800/1	60	Toluene	50
PL1-2	St	DEAAm	70/30	800/1	60	Toluene	50
PL1-3	St	DEAAm	50/50	800/1	60	Toluene	50
PL1-4	St	DMAAm	90/10	800/1	60	Toluene	50
PL1-5	St	DMAAm	70/30	800/1	60	Toluene	50
PL1-6	St	DMAAm	50/50	800/1	60	Toluene	50
PL1-7	St	NIPAAm	90/10	800/1	60	Toluene	50
PL1-8	St	NIPAAm	70/30	800/1	60	Toluene	50
PL1-9	St	NIPAAm	50/50	800/1	60	Toluene	50
PL1-10	MMA	DEAAm	90/10	800/1	60	Toluene	50
PL1-11	MMA	DEAAm	70/30	800/1	60	Toluene	50
PL1-12	MMA	DEAAm	50/50	800/1	60	Toluene	50
PL1-13	MMA	DMAAm	90/10	800/1	60	Toluene	50
PL1-14	MMA	DMAAm	70/30	800/1	60	Toluene	50
PL1-15	MMA	DMAAm	50/50	800/1	60	Toluene	50
PL1-16	MMA	NIPAAm	90/10	800/1	60	Toluene	50
PL1-17	MMA	NIPAAm	70/30	800/1	60	Toluene	50
PL1-18	MMA	NIPAAm	50/50	800/1	60	Toluene	30
PL1-19	MEMA	DEAAm	90/10	400/1	60	Toluene	30
PL1-20	MEMA	DEAAm	70/30	400/1	60	Toluene	30
PL1-21	MEMA	DEAAm	50/50	400/1	60	Toluene	30
PL1-22	MEMA	DMAAm	90/10	400/1	60	Toluene	30
PL1-23	MEMA	DMAAm	70/30	400/1	60	Toluene	30
PL1-24	MEMA	DMAAm	50/50	400/1	60	Toluene	30
PL1-25	MEMA	NIPAAm	90/10	400/1	60	Toluene	30
PL1-26	MEMA	NIPAAm	70/30	400/1	60	Toluene	30
PL1-27	MEMA	NIPAAm	50/50	100/1	60	Toluene	30
PL1-28	MEA	DEAAm	90/10	400/1	60	Toluene	20
PL1-29	MEA	DEAAm	70/30	400/1	60	Toluene	20
PL1-30	MEA	DEAAm	50/50	400/1	60	Toluene	25
PL1-31	MEA	DMAAm	90/10	400/1	60	Toluene	20
PL1-32	MEA	DMAAm	70/30	400/1	60	Toluene	20
PL1-33	MEA	DMAAm	50/50	400/1	60	Toluene	25

(*cont'd*)

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL1-34	MEA	NIPAAm	90/10	400/1	60	Toluene	20
PL1-35	MEA	NIPAAm	70/30	400/1	60	Toluene	20
PL1-36	MEA	NIPAAm	50/50	400/1	60	Toluene	10
PL1-37	HEMA	DEAAm	90/10	800/1	80	NMP	5
PL1-38	HEMA	DEAAm	70/30	800/1	80	NMP	5
PL1-39	HEMA	DEAAm	50/50	800/1	80	NMP	5
PL1-40	HEMA	DMAAm	90/10	800/1	80	DMF	5
PL1-41	HEMA	DMAAm	70/30	800/1	80	DMF	5
PL1-42	HEMA	DMAAm	50/50	800/1	80	DMF	5
PL1-43	HEMA	NIPAAm	90/10	800/1	80	NMP	5
PL1-44	HEMA	NIPAAm	70/30	800/1	80	NMP	5
PL1-45	HEMA	NIPAAm	50/50	800/1	80	NMP	5
PL1-46	HPMA	DEAAm	90/10	800/1	80	NMP	5
PL1-47	HPMA	DEAAm	70/30	800/1	80	NMP	5
PL1-48	HPMA	DEAAm	50/50	800/1	80	NMP	5
PL1-49	HPMA	DMAAm	90/10	800/1	80	DMF	5
PL1-50	HPMA	DMAAm	70/30	800/1	80	DMF	5
PL1-51	HPMA	DMAAm	50/50	800/1	80	DMF	5
PL1-52	HPMA	NIPAAm	90/10	800/1	80	NMP	5
PL1-53	HPMA	NIPAAm	70/30	800/1	80	NMP	5
PL1-54	HPMA	NIPAAm	50/50	800/1	80	NMP	5
PL1-55	HBMA	DEAAm	90/10	800/1	80	NMP	5
PL1-56	HBMA	DEAAm	70/30	800/1	80	NMP	5
PL1-57	HBMA	DEAAm	50/50	800/1	80	NMP	5
PL1-58	HBMA	DMAAm	90/10	800/1	80	DMF	5
PL1-59	HBMA	DMAAm	70/30	800/1	80	DMF	5
PL1-60	HBMA	DMAAm	50/50	800/1	80	DMF	5
PL1-61	HBMA	NIPAAm	90/10	800/1	80	NMP	5
PL1-62	HBMA	NIPAAm	70/30	800/1	80	NMP	5
PL1-63	HBMA	NIPAAm	50/50	800/1	80	NMP	5

Table 6.2.3. Reaction conditions for synthesis of polymer library-2.

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL2-1	MEMA	DEAEMA	90/10	400/1	60	Toluene	30
PL2-2	MEMA	DEAEMA	70/30	400/1	60	Toluene	30
PL2-3	MEMA	DEAEMA	50/50	400/1	60	Toluene	30
PL2-4	MEMA	DMAEMA	90/10	400/1	60	Toluene	30
PL2-5	MEMA	DMAEMA	70/30	400/1	60	Toluene	30
PL2-6	MEMA	DMAEMA	50/50	400/1	60	Toluene	30
PL2-7	MEMA	DEAEA	90/10	400/1	60	Toluene	30
PL2-8	MEMA	DEAEA	70/30	400/1	60	Toluene	50
PL2-9	MEMA	DEAEA	50/50	400/1	60	Toluene	70
PL2-10	MEMA	DMAEA	90/10	400/1	60	Toluene	30
PL2-11	MEMA	DMAEA	70/30	400/1	60	Toluene	30
PL2-12	MEMA	DMAEA	50/50	400/1	60	Toluene	30
PL2-13	MEMA	MTEMA	90/10	400/1	60	Toluene	30
PL2-14	MEMA	MTEMA	70/30	400/1	60	Toluene	40
PL2-15	MEMA	MTEMA	50/50	400/1	60	Toluene	50
PL2-16	MEMA	BAEMA	90/10	400/1	60	Toluene	30
PL2-17	MEMA	BAEMA	70/30	400/1	60	Toluene	30
PL2-18	MEMA	BAEMA	50/50	400/1	60	Toluene	30
PL2-19	MEMA	DMAPMAAm	90/10	400/1	60	Toluene	30
PL2-20	MEMA	DMAPMAAm	70/30	400/1	60	Toluene	30
PL2-21	MEMA	DMAPMAAm	50/50	400/1	60	Toluene	30
PL2-22	MEMA	BACOEA	90/10	400/1	60	Toluene	30
PL2-23	MEMA	BACOEA	70/30	400/1	60	Toluene	30
PL2-24	MEMA	BACOEA	50/50	400/1	60	Toluene	30
PL2-25	MEMA	DMVBA	90/10	400/1	60	Toluene	40
PL2-26	MEMA	DMVBA	70/30	400/1	60	Toluene	50
PL2-27	MEMA	DMVBA	50/50	400/1	60	Toluene	60
PL2-28	MEMA	VAA	90/10	400/1	60	DMF	30
PL2-29	MEMA	VAA	70/30	400/1	60	DMF	20
PL2-30	MEMA	VAA	50/50	400/1	60	DMF	10
PL2-31	MEMA	VI	90/10	400/1	60	DMF	30
PL2-32	MEMA	VI	70/30	400/1	60	DMF	15
PL2-33	MEMA	VI	50/50	400/1	60	DMF	15

(*cont'd*)

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL2-34	MEMA	VPNO	90/10	400/1	60	Toluene	30
PL2-35	MEMA	VPNO	70/30	400/1	60	Toluene	30
PL2-36	MEMA	VPNO	50/50	400/1	60	Toluene	30
PL2-37	MEMA	VP-4	90/10	400/1	60	Toluene	30
PL2-38	MEMA	VP-4	70/30	400/1	60	Toluene	40
PL2-39	MEMA	VP-4	50/50	400/1	60	Toluene	40
PL2-40	MEMA	VP-2	90/10	400/1	60	Toluene	30
PL2-41	MEMA	VP-2	70/30	400/1	60	Toluene	30
PL2-42	MEMA	VP-2	50/50	400/1	60	Toluene	60
PL2-43	MEMA	DAAAm	90/10	400/1	60	DMF	10
PL2-44	MEMA	DAAAm	70/30	400/1	60	DMF	15
PL2-45	MEMA	DAAAm	50/50	400/1	60	DMF	20
PL2-46	MEMA	MNPMA	90/10	400/1	60	DMF	10
PL2-47	MEMA	MNPMA	70/30	400/1	60	DMF	10
PL2-48	MEMA	MNPMA	50/50	400/1	60	DMF	10
PL2-49	HEMA	DEAEMA	90/10	800/1	80	DMF	5
PL2-50	HEMA	DEAEMA	70/30	800/1	80	DMF	5
PL2-51	HEMA	DEAEMA	50/50	800/1	80	DMF	10
PL2-52	HEMA	DMAEMA	90/10	800/1	80	DMF	5
PL2-53	HEMA	DMAEMA	70/30	800/1	80	DMF	5
PL2-54	HEMA	DMAEMA	50/50	800/1	80	DMF	5
PL2-55	HEMA	DEAEA	90/10	800/1	80	DMF	5
PL2-56	HEMA	DEAEA	70/30	800/1	80	DMF	5
PL2-57	HEMA	DEAEA	50/50	800/1	80	DMF	10
PL2-58	HEMA	DMAEA	90/10	800/1	80	DMF	5
PL2-59	HEMA	DMAEA	70/30	800/1	80	DMF	5
PL2-60	HEMA	DMAEA	50/50	800/1	80	DMF	5
PL2-61	HEMA	MTEMA	90/10	800/1	80	DMF	5
PL2-62	HEMA	MTEMA	70/30	800/1	80	DMF	5
PL2-63	HEMA	MTEMA	50/50	800/1	80	DMF	10
PL2-64	HEMA	BAEMA	90/10	800/1	80	DMF	5
PL2-65	HEMA	BAEMA	70/30	800/1	80	DMF	5
PL2-66	HEMA	BAEMA	50/50	800/1	80	DMF	5

(*cont'd*)

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL2-67	HEMA	DMAPMAAm	90/10	800/1	80	DMF	5
PL2-68	HEMA	DMAPMAAm	70/30	800/1	80	DMF	5
PL2-69	HEMA	DMAPMAAm	50/50	800/1	80	DMF	5
PL2-70	HEMA	BACOEA	90/10	800/1	80	DMF	5
PL2-71	HEMA	BACOEA	70/30	800/1	80	DMF	5
PL2-72	HEMA	BACOEA	50/50	800/1	80	DMF	5
PL2-73	HEMA	DMVBA	90/10	800/1	80	DMF	5
PL2-74	HEMA	DMVBA	70/30	800/1	80	DMF	15
PL2-75	HEMA	DMVBA	50/50	800/1	80	DMF	20
PL2-76	HEMA	VAA	90/10	800/1	80	DMF	5
PL2-77	HEMA	VAA	70/30	800/1	80	DMF	5
PL2-78	HEMA	VAA	50/50	800/1	80	DMF	5
PL2-79	HEMA	VI	90/10	800/1	80	DMF	5
PL2-80	HEMA	VI	70/30	800/1	80	DMF	5
PL2-81	HEMA	VI	50/50	800/1	80	DMF	5
PL2-82	HEMA	VPNO	90/10	800/1	80	DMF	5
PL2-83	HEMA	VPNO	70/30	800/1	80	DMF	5
PL2-84	HEMA	VPNO	50/50	800/1	80	DMF	5
PL2-85	HEMA	VP-4	90/10	800/1	80	DMF	5
PL2-86	HEMA	VP-4	70/30	800/1	80	DMF	5
PL2-87	HEMA	VP-4	50/50	800/1	80	DMF	10
PL2-88	HEMA	VP-2	90/10	800/1	80	DMF	5
PL2-89	HEMA	VP-2	70/30	800/1	80	DMF	10
PL2-90	HEMA	VP-2	50/50	800/1	80	DMF	20
PL2-91	HEMA	DAAAm	90/10	800/1	80	DMF	5
PL2-92	HEMA	DAAAm	70/30	800/1	80	DMF	5
PL2-93	HEMA	DAAAm	50/50	800/1	80	DMF	5
PL2-94	HEMA	MNPMA	90/10	800/1	80	DMF	5
PL2-95	HEMA	MNPMA	70/30	800/1	80	DMF	5
PL2-96	HEMA	MNPMA	50/50	800/1	80	DMF	5

Table 6.2.4. Reaction conditions for synthesis of polymer library-3.

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL3-1	MMA	DEAEMA	90/10	800/1	60	Toluene	50
PL3-2	MMA	DEAEMA	70/30	800/1	60	Toluene	45
PL3-3	MMA	DEAEMA	50/50	800/1	60	Toluene	40
PL3-4	MMA	DMAEMA	90/10	800/1	60	Toluene	50
PL3-5	MMA	DMAEMA	70/30	800/1	60	Toluene	45
PL3-6	MMA	DMAEMA	50/50	800/1	60	Toluene	40
PL3-7	MMA	DEAEA	90/10	800/1	60	Toluene	50
PL3-8	MMA	DEAEA	70/30	800/1	60	Toluene	50
PL3-9	MMA	DEAEA	50/50	800/1	60	Toluene	50
PL3-10	MMA	DMAEA	90/10	800/1	60	Toluene	50
PL3-11	MMA	DMAEA	70/30	800/1	60	Toluene	45
PL3-12	MMA	DMAEA	50/50	800/1	60	Toluene	40
PL3-13	HPMA	DEAEMA	90/10	800/1	80	DMF	5
PL3-14	HPMA	DEAEMA	70/30	800/1	80	DMF	7
PL3-15	HPMA	DEAEMA	50/50	800/1	80	DMF	9
PL3-16	HPMA	DMAEMA	90/10	800/1	80	DMF	5
PL3-17	HPMA	DMAEMA	70/30	800/1	80	DMF	7
PL3-18	HPMA	DMAEMA	50/50	800/1	80	DMF	9
PL3-19	HPMA	DEAEA	90/10	800/1	80	DMF	5
PL3-20	HPMA	DEAEA	70/30	800/1	80	DMF	7
PL3-21	HPMA	DEAEA	50/50	800/1	80	DMF	9
PL3-22	HPMA	DMAEA	90/10	800/1	80	DMF	5
PL3-23	HPMA	DMAEA	70/30	800/1	80	DMF	7
PL3-24	HPMA	DMAEA	50/50	800/1	80	DMF	9
PL3-25	HBMA	DEAEMA	90/10	800/1	80	DMF	5
PL3-26	HBMA	DEAEMA	70/30	800/1	80	DMF	7
PL3-27	HBMA	DEAEMA	50/50	800/1	80	DMF	9
PL3-28	HBMA	DMAEMA	90/10	800/1	80	DMF	5
PL3-29	HBMA	DMAEMA	70/30	800/1	80	DMF	7
PL3-30	HBMA	DMAEMA	50/50	800/1	80	DMF	9
PL3-31	HBMA	DEAEA	90/10	800/1	80	DMF	5
PL3-32	HBMA	DEAEA	70/30	800/1	80	DMF	7
PL3-33	HBMA	DEAEA	50/50	800/1	80	DMF	9

(*cont'd*)

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL3-34	HBMA	DMAEA	90/10	800/1	80	DMF	5
PL3-35	HBMA	DMAEA	70/30	800/1	80	DMF	7
PL3-36	HBMA	DMAEA	50/50	800/1	80	DMF	9
PL3-37	EMA	DEAEMA	90/10	800/1	60	Toluene	30
PL3-38	EMA	DEAEMA	70/30	800/1	60	Toluene	30
PL3-39	EMA	DEAEMA	50/50	800/1	60	Toluene	30
PL3-40	EMA	DMAEMA	90/10	800/1	60	Toluene	30
PL3-41	EMA	DMAEMA	70/30	800/1	60	Toluene	30
PL3-42	EMA	DMAEMA	50/50	800/1	60	Toluene	30
PL3-43	EMA	DEAEA	90/10	800/1	60	Toluene	50
PL3-44	EMA	DEAEA	70/30	800/1	60	Toluene	50
PL3-45	EMA	DEAEA	50/50	800/1	60	Toluene	50
PL3-46	EMA	DMAEA	90/10	800/1	60	Toluene	50
PL3-47	EMA	DMAEA	70/30	800/1	60	Toluene	50
PL3-48	EMA	DMAEA	50/50	800/1	60	Toluene	50
PL3-49	BMA	DEAEMA	90/10	800/1	60	Toluene	40
PL3-50	BMA	DEAEMA	70/30	800/1	60	Toluene	40
PL3-51	BMA	DEAEMA	50/50	800/1	60	Toluene	40
PL3-52	BMA	DMAEMA	90/10	800/1	60	Toluene	40
PL3-53	BMA	DMAEMA	70/30	800/1	60	Toluene	40
PL3-54	BMA	DMAEMA	50/50	800/1	60	Toluene	40
PL3-55	BMA	DEAEA	90/10	800/1	60	Toluene	50
PL3-56	BMA	DEAEA	70/30	800/1	60	Toluene	50
PL3-57	BMA	DEAEA	50/50	800/1	60	Toluene	60
PL3-58	BMA	DMAEA	90/10	800/1	60	Toluene	40
PL3-59	BMA	DMAEA	70/30	800/1	60	Toluene	40
PL3-60	BMA	DMAEA	50/50	800/1	60	Toluene	30

Table 6.2.5. Reaction conditions for synthesis of polymer library-4.

No.	Monomer		Monomer ratio	[M]/[I]	Temp	Solvent	[M]
	A	B	(mol.%)	mol ratio	(°C)		(vol.%)
PL4-1	MMA	A-H	90/10	100/1	60	IPA	10
PL4-2	MMA	A-H	70/30	100/1	60	IPA	10
PL4-3	MMA	A-H	50/50	100/1	60	IPA	5
PL4-4	MMA	AES-H	90/10	100/1	60	DMF	10
PL4-5	MMA	AES-H	70/30	100/1	60	DMF	10
PL4-6	MMA	AES-H	50/50	100/1	60	DMF	5
PL4-7	MMA	MA-H	90/10	100/1	60	IPA	10
PL4-8	MMA	MA-H	70/30	100/1	60	IPA	10
PL4-9	MMA	MA-H	50/50	100/1	60	IPA	5
PL4-10	MMA	AAG-H	90/10	100/1	60	DMF	10
PL4-11	MMA	AAG-H	70/30	100/1	60	DMF	5
PL4-12	MMA	AAG-H	50/50	100/1	60	DMF	5
PL4-13	MMA	EGMP-H	90/10	100/1	60	DMF	5
PL4-14	MMA	EGMP-H	70/30	100/1	60	DMF	5
PL4-15	MMA	EGMP-H	50/50	100/1	60	DMF	5
PL4-16	MEMA	A-H	90/10	100/1	60	IPA	5
PL4-17	MEMA	A-H	70/30	100/1	60	IPA	10
PL4-18	MEMA	A-H	50/50	100/1	60	IPA	10
PL4-19	MEMA	AES-H	90/10	100/1	60	DMF	10
PL4-20	MEMA	AES-H	70/30	100/1	60	DMF	10
PL4-21	MEMA	AES-H	50/50	100/1	60	DMF	10
PL4-22	MEMA	MA-H	90/10	100/1	60	IPA	5
PL4-23	MEMA	MA-H	70/30	100/1	60	IPA	10
PL4-24	MEMA	MA-H	50/50	100/1	60	IPA	10
PL4-25	MEMA	AAG-H	90/10	100/1	60	DMF	5
PL4-26	MEMA	AAG-H	70/30	100/1	60	DMF	5
PL4-27	MEMA	AAG-H	50/50	100/1	60	DMF	5
PL4-28	MEMA	EGMP-H	90/10	100/1	60	DMF	5
PL4-29	MEMA	EGMP-H	70/30	100/1	60	DMF	5
PL4-30	MEMA	EGMP-H	50/50	100/1	60	DMF	5

Table 6.2.6. Reaction conditions for synthesis of polymer library-5.

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL5-1	MEMA	DEAEMA	MA	40/30/30	Toluene	40
PL5-2	MEMA	DEAEMA	MA	60/10/30	Toluene	25
PL5-3	MEMA	DEAEMA	MA	60/30/10	Toluene	25
PL5-4	MEMA	DEAEMA	MA	80/10/10	Toluene	25
PL5-5	MEMA	DEAEA	MA	40/30/30	Toluene	25
PL5-6	MEMA	DEAEA	MA	60/10/30	Toluene	25
PL5-7	MEMA	DEAEA	MA	60/30/10	Toluene	40
PL5-8	MEMA	DEAEA	MA	80/10/10	Toluene	25
PL5-9	MEMA	DEAEMA	BMA	40/30/30	Toluene	40
PL5-10	MEMA	DEAEMA	BMA	60/10/30	Toluene	25
PL5-11	MEMA	DEAEMA	BMA	60/30/10	Toluene	25
PL5-12	MEMA	DEAEMA	BMA	80/10/10	Toluene	25
PL5-13	MEMA	DEAEA	BMA	40/30/30	Toluene	40
PL5-14	MEMA	DEAEA	BMA	60/10/30	Toluene	25
PL5-15	MEMA	DEAEA	BMA	60/30/10	Toluene	40
PL5-16	MEMA	DEAEA	BMA	80/10/10	Toluene	25
PL5-17	MEMA	DEAEMA	MEA	40/30/30	Toluene	40
PL5-18	MEMA	DEAEMA	MEA	60/10/30	Toluene	40
PL5-19	MEMA	DEAEMA	MEA	60/30/10	Toluene	40
PL5-20	MEMA	DEAEMA	MEA	80/10/10	Toluene	40
PL5-21	MEMA	DEAEA	MEA	40/30/30	Toluene	50
PL5-22	MEMA	DEAEA	MEA	60/10/30	Toluene	40
PL5-23	MEMA	DEAEA	MEA	60/30/10	Toluene	40
PL5-24	MEMA	DEAEA	MEA	80/10/10	Toluene	40
PL5-25	MEMA	DEAEMA	DEGMEMA	40/30/30	Toluene	25
PL5-26	MEMA	DEAEMA	DEGMEMA	60/10/30	Toluene	25
PL5-27	MEMA	DEAEMA	DEGMEMA	60/30/10	Toluene	25
PL5-28	MEMA	DEAEMA	DEGMEMA	80/10/10	Toluene	25
PL5-29	MEMA	DEAEA	DEGMEMA	40/30/30	Toluene	25
PL5-30	MEMA	DEAEA	DEGMEMA	60/10/30	Toluene	25
PL5-31	MEMA	DEAEA	DEGMEMA	60/30/10	Toluene	40
PL5-32	MEMA	DEAEA	DEGMEMA	80/10/10	Toluene	25
PL5-33	MEMA	DEAEMA	THFFA	40/30/30	Toluene	40

(*cont'd*)

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL5-34	MEMA	DEAEMA	THFFA	60/10/30	Toluene	25
PL5-35	MEMA	DEAEMA	THFFA	60/30/10	Toluene	25
PL5-36	MEMA	DEAEMA	THFFA	80/10/10	Toluene	25
PL5-37	MEMA	DEAEA	THFFA	40/30/30	Toluene	40
PL5-38	MEMA	DEAEA	THFFA	60/10/30	Toluene	25
PL5-39	MEMA	DEAEA	THFFA	60/30/10	Toluene	40
PL5-40	MEMA	DEAEA	THFFA	80/10/10	Toluene	25
PL5-41	MEMA	DEAEMA	THFFMA	40/30/30	Toluene	25
PL5-42	MEMA	DEAEMA	THFFMA	60/10/30	Toluene	25
PL5-43	MEMA	DEAEMA	THFFMA	60/30/10	Toluene	25
PL5-44	MEMA	DEAEMA	THFFMA	80/10/10	Toluene	25
PL5-45	MEMA	DEAEA	THFFMA	40/30/30	Toluene	40
PL5-46	MEMA	DEAEA	THFFMA	60/10/30	Toluene	25
PL5-47	MEMA	DEAEA	THFFMA	60/30/10	Toluene	40
PL5-48	MEMA	DEAEA	THFFMA	80/10/10	Toluene	25
PL5-49	MEMA	DEAEMA	HEA	40/30/30	DMF	25
PL5-50	MEMA	DEAEMA	HEA	60/10/30	DMF	25
PL5-51	MEMA	DEAEMA	HEA	60/30/10	DMF	25
PL5-52	MEMA	DEAEMA	HEA	80/10/10	DMF	25
PL5-53	MEMA	DEAEA	HEA	40/30/30	DMF	50
PL5-54	MEMA	DEAEA	HEA	60/10/30	DMF	25
PL5-55	MEMA	DEAEA	HEA	60/30/10	DMF	40
PL5-56	MEMA	DEAEA	HEA	80/10/10	DMF	25
PL5-57	MEMA	DEAEMA	HEMA	40/30/30	DMF	25
PL5-58	MEMA	DEAEMA	HEMA	60/10/30	DMF	25
PL5-59	MEMA	DEAEMA	HEMA	60/30/10	DMF	25
PL5-60	MEMA	DEAEMA	HEMA	80/10/10	DMF	25
PL5-61	MEMA	DEAEA	HEMA	40/30/30	DMF	25
PL5-62	MEMA	DEAEA	HEMA	60/10/30	DMF	25
PL5-63	MEMA	DEAEA	HEMA	60/30/10	DMF	40
PL5-64	MEMA	DEAEA	HEMA	80/10/10	DMF	25
PL5-65	MEMA	DEAEMA	A-H	40/30/30	DMF	25
PL5-66	MEMA	DEAEMA	A-H	60/10/30	DMF	25

(*cont'd*)

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL5-67	MEMA	DEAEMA	A-H	60/30/10	DMF	25
PL5-68	MEMA	DEAEMA	A-H	80/10/10	DMF	25
PL5-69	MEMA	DEAEA	A-H	40/30/30	DMF	25
PL5-70	MEMA	DEAEA	A-H	60/10/30	DMF	25
PL5-71	MEMA	DEAEA	A-H	60/30/10	DMF	25
PL5-72	MEMA	DEAEA	A-H	80/10/10	DMF	25
PL5-73	MEMA	DEAEMA	MA-H	40/30/30	DMF	25
PL5-74	MEMA	DEAEMA	MA-H	60/10/30	DMF	25
PL5-75	MEMA	DEAEMA	MA-H	60/30/10	DMF	25
PL5-76	MEMA	DEAEMA	MA-H	80/10/10	DMF	25
PL5-77	MEMA	DEAEA	MA-H	40/30/30	DMF	25
PL5-78	MEMA	DEAEA	MA-H	60/10/30	DMF	25
PL5-79	MEMA	DEAEA	MA-H	60/30/10	DMF	25
PL5-80	MEMA	DEAEA	MA-H	80/10/10	DMF	25
PL5-81	MEMA	DEAEMA	DMAAm	40/30/30	Toluene	25
PL5-82	MEMA	DEAEMA	DMAAm	60/10/30	Toluene	25
PL5-83	MEMA	DEAEMA	DMAAm	60/30/10	Toluene	25
PL5-84	MEMA	DEAEMA	DMAAm	80/10/10	Toluene	25
PL5-85	MEMA	DEAEA	DMAAm	40/30/30	Toluene	40
PL5-86	MEMA	DEAEA	DMAAm	60/10/30	Toluene	25
PL5-87	MEMA	DEAEA	DMAAm	60/30/10	Toluene	40
PL5-88	MEMA	DEAEA	DMAAm	80/10/10	Toluene	25
PL5-89	MEMA	DEAEMA	DAAAm	40/30/30	DMF	40
PL5-90	MEMA	DEAEMA	DAAAm	60/10/30	DMF	40
PL5-91	MEMA	DEAEMA	DAAAm	60/30/10	DMF	40
PL5-92	MEMA	DEAEMA	DAAAm	80/10/10	DMF	40
PL5-93	MEMA	DEAEA	DAAAm	40/30/30	DMF	50
PL5-94	MEMA	DEAEA	DAAAm	60/10/30	DMF	40
PL5-95	MEMA	DEAEA	DAAAm	60/30/10	DMF	40
PL5-96	MEMA	DEAEA	DAAAm	80/10/10	DMF	40
PL5-97	MEMA	DEAEMA	MMA	40/30/30	Toluene	40
PL5-98	MEMA	DEAEMA	MMA	60/10/30	Toluene	40
PL5-99	MEMA	DEAEMA	MMA	60/30/10	Toluene	40

(*cont'd*)

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL5-100	MEMA	DEAEMA	MMA	80/10/10	Toluene	40
PL5-101	MEMA	DEAEA	MMA	40/30/30	Toluene	40
PL5-102	MEMA	DEAEA	MMA	60/10/30	Toluene	40
PL5-103	MEMA	DEAEA	MMA	60/30/10	Toluene	40
PL5-104	MEMA	DEAEA	MMA	80/10/10	Toluene	40
PL5-105	MEMA	DEAEMA	St	40/30/30	Toluene	40
PL5-106	MEMA	DEAEMA	St	60/10/30	Toluene	40
PL5-107	MEMA	DEAEMA	St	60/30/10	Toluene	40
PL5-108	MEMA	DEAEMA	St	80/10/10	Toluene	40
PL5-109	MEMA	DEAEA	St	40/30/30	Toluene	40
PL5-110	MEMA	DEAEA	St	60/10/30	Toluene	40
PL5-111	MEMA	DEAEA	St	60/30/10	Toluene	40
PL5-112	MEMA	DEAEA	St	80/10/10	Toluene	40

[M]/[I] = 400/1 mol ratio, reaction temperature 60 °C.

Table 6.2.7. Reaction conditions for synthesis of polymer library-6.

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL6-1	MMA	DEAEMA	A-H	70/10/20	DMF	20
PL6-2	MMA	DEAEMA	A-H	70/15/15	DMF	20
PL6-3	MMA	DEAEMA	A-H	70/20/10	DMF	20
PL6-4	MMA	DEAEA	A-H	70/10/20	DMF	20
PL6-5	MMA	DEAEA	A-H	70/15/15	DMF	20
PL6-6	MMA	DEAEA	A-H	70/20/10	DMF	20
PL6-7	MMA	DEAEMA	MA-H	70/10/20	DMF	20
PL6-8	MMA	DEAEMA	MA-H	70/15/15	DMF	20
PL6-9	MMA	DEAEMA	MA-H	70/20/10	DMF	20
PL6-10	MMA	DEAEA	MA-H	70/10/20	DMF	20
PL6-11	MMA	DEAEA	MA-H	70/15/15	DMF	20
PL6-12	MMA	DEAEA	MA-H	70/20/10	DMF	20
PL6-13	MEMA	DEAEMA	A-H	70/10/20	DMF	20
PL6-14	MEMA	DEAEMA	A-H	70/15/15	DMF	20
PL6-15	MEMA	DEAEMA	A-H	70/20/10	DMF	20
PL6-16	MEMA	DEAEA	A-H	70/10/20	DMF	20
PL6-17	MEMA	DEAEA	A-H	70/15/15	DMF	20
PL6-18	MEMA	DEAEA	A-H	70/20/10	DMF	20
PL6-19	MEMA	DEAEMA	MA-H	70/10/20	DMF	20
PL6-20	MEMA	DEAEMA	MA-H	70/15/15	DMF	20
PL6-21	MEMA	DEAEMA	MA-H	70/20/10	DMF	20
PL6-22	MEMA	DEAEA	MA-H	70/10/20	DMF	20
PL6-23	MEMA	DEAEA	MA-H	70/15/15	DMF	20
PL6-24	MEMA	DEAEA	MA-H	70/20/10	DMF	20
PL6-25	MEMA	DEAEMA	A-H	85/10/5	DMF	25
PL6-26	MEMA	DEAEMA	A-H	80/15/5	DMF	25
PL6-27	MEMA	DEAEMA	A-H	75/20/5	DMF	25
PL6-28	MEMA	DEAEMA	A-H	70/25/5	DMF	25
PL6-29	MEMA	DEAEMA	A-H	65/30/5	DMF	25
PL6-30	MEMA	DEAEMA	A-H	60/35/5	DMF	25
PL6-31	MEMA	DEAEMA	A-H	55/40/5	DMF	25
PL6-32	MEMA	DEAEMA	A-H	50/45/5	DMF	25
PL6-33	MEMA	DEAEMA	A-H	75/15/10	DMF	25

(*cont'd*)

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL6-34	MEMA	DEAEMA	A-H	65/25/10	DMF	25
PL6-35	MEMA	DEAEMA	A-H	55/35/10	DMF	25
PL6-36	MEMA	DEAEMA	A-H	50/40/10	DMF	25
PL6-37	MEMA	DEAEMA	A-H	65/20/15	DMF	25
PL6-38	MEMA	DEAEMA	A-H	60/25/15	DMF	25
PL6-39	MEMA	DEAEMA	A-H	55/30/15	DMF	25
PL6-40	MEMA	DEAEMA	A-H	50/35/15	DMF	25
PL6-41	MEMA	DEAEMA	A-H	55/25/20	DMF	25
PL6-42	MEMA	DEAEMA	A-H	50/30/20	DMF	25
PL6-43	MEMA	DEAEMA	A-H	90/5/5	DMF	25
PL6-44	MEMA	DEAEMA	A-H	80/5/15	DMF	25
PL6-45	MEMA	DEAEMA	A-H	70/5/25	DMF	25
PL6-46	MEMA	DEAEMA	A-H	60/5/35	DMF	25
PL6-47	MEMA	DEAEMA	A-H	50/5/45	DMF	25
PL6-48	MEMA	DEAEMA	A-H	50/10/40	DMF	25
PL6-49	MEMA	DEAEMA	A-H	60/15/25	DMF	25
PL6-50	MEMA	DEAEMA	A-H	50/15/35	DMF	25
PL6-51	MEMA	DEAEMA	A-H	60/20/20	DMF	25
PL6-52	MEMA	DEAEMA	A-H	50/20/30	DMF	25
PL6-53	MEMA	DEAEMA	A-H	50/25/25	DMF	25
PL6-54	MEMA	DEAEMA	A-H	75/22.5/2.5	DMF	25
PL6-55	MEMA	DEAEMA	A-H	70/27.5/2.5	DMF	25
PL6-56	MEMA	DEAEMA	A-H	70/22.5/7.5	DMF	25
PL6-57	MEMA	DEAEMA	A-H	65/27.5/7.5	DMF	25
PL6-58	MEMA	DEAEMA	A-H	60/32.5/7.5	DMF	25
PL6-59	MEMA	DEAEMA	A-H	65/22.5/12.5	DMF	25
PL6-60	MEMA	DEAEMA	A-H	60/27.5/12.5	DMF	25
PL6-61	MEMA	DEAEMA	A-H	55/32.5/12.5	DMF	25
PL6-62	MEMA	DEAEA	A-H	85/10/5	DMF	25
PL6-63	MEMA	DEAEA	A-H	80/15/5	DMF	25
PL6-64	MEMA	DEAEA	A-H	75/20/5	DMF	30
PL6-65	MEMA	DEAEA	A-H	70/25/5	DMF	30
PL6-66	MEMA	DEAEA	A-H	65/30/5	DMF	30

(cont'd)

No.	Monomer			Monomer ratio	Solvent	[M]
	A	B	C	(mol.%)		(vol.%)
PL6-67	MEMA	DEAEA	A-H	60/35/5	DMF	30
PL6-68	MEMA	DEAEA	A-H	55/40/5	DMF	30
PL6-69	MEMA	DEAEA	A-H	50/45/5	DMF	30
PL6-70	MEMA	DEAEA	A-H	75/15/10	DMF	25
PL6-71	MEMA	DEAEA	A-H	65/25/10	DMF	30
PL6-72	MEMA	DEAEA	A-H	55/35/10	DMF	30
PL6-73	MEMA	DEAEA	A-H	50/40/10	DMF	30
PL6-74	MEMA	DEAEA	A-H	65/20/15	DMF	30
PL6-75	MEMA	DEAEA	A-H	60/25/15	DMF	30
PL6-76	MEMA	DEAEA	A-H	55/30/15	DMF	30
PL6-77	MEMA	DEAEA	A-H	50/35/15	DMF	30
PL6-78	MEMA	DEAEA	A-H	55/25/20	DMF	30
PL6-79	MEMA	DEAEA	A-H	50/30/20	DMF	30
PL6-80	MEMA	DMAEMA	A-H	85/10/5	DMF	25
PL6-81	MEMA	DMAEMA	A-H	80/15/5	DMF	25
PL6-82	MEMA	DMAEA	A-H	85/10/5	DMF	25
PL6-83	MEMA	DMAEA	A-H	80/15/5	DMF	25
PL6-84	MEMA	DMAPMAAm	A-H	85/10/5	DMF	25
PL6-85	MEMA	DMAPMAAm	A-H	80/15/5	DMF	25

[M]/[I] = 400/1 mol ratio, reaction temperature 60 °C.

6.3 Experimental for Chapter-3

6.3.1 Polymer synthesis in a 96-well plate and microarray purification

A mixture of AIBN, monomers and solvents was prepared. Table 6.3.1 shows the amounts of the reagents. Solutions (200 μL) were dispensed into each wells of the glass 96-well plate, and same experiment was carried out in different positions in the same plate (Figure 3.2.3). The plate was covered by a polypropylene sheet, and holes were made by needle into each well. The plate was put into a sealed glass vessel, and nitrogen gas purged for 30 min. Polymerisation was carried out at 60 °C overnight.

After the reaction, polymer solutions (30 vol.%) were diluted to 20 vol.% with NMP. The polymers were spotted on the gold coated glass plate with Genetix Q Array mini arrayer (Genetix Ltd.)

After spotting, the polymer spots were dried under vacuum at 45 °C for 2 hrs. The plate was washed with hexane to remove the remaining solvents and monomers, and dried under vacuum at 45 °C overnight.

Table 6.3.1. Reaction conditions of polymer synthesis in a 96-well plate.

No.	MEMA (M1)		DEAEMA (M2)		AIBN (Ini)		Mol ratio			NMP	[M]
	(mmol)	(mL)	(mmol)	(mL)	(μmol)	(mg)	M1	M2	Ini	(mL)	(vol.%)
1	1.0	150	0	0	2.6	0.42	400	0	1	0.35	30
2	1.0	150	0	0	5.2	0.85	200	0	1	0.35	30
3	1.0	150	0	0	10	1.70	100	0	1	0.35	30
4	0.48	70	0	0	1.2	0.20	400	0	1	0.63	10
5	0.48	70	0	0	2.4	0.40	200	0	1	0.63	10
6	0.48	70	0	0	4.8	0.79	100	0	1	0.63	10
7	1.0	150	0.054	11	2.7	0.45	380	20	1	0.38	30
8	1.0	150	0.054	11	5.4	0.89	190	10	1	0.37	30
9	1.0	150	0.054	11	11	1.79	95	5	1	0.37	30
10	0.48	70	0.025	5	1.3	0.21	380	20	1	0.68	10
11	0.48	70	0.025	5	2.5	0.42	190	10	1	0.68	10
12	0.48	70	0.025	5	5.1	0.83	95	5	1	0.68	10
13	1.0	150	0.11	23	2.9	0.47	360	40	1	0.40	30
14	1.0	150	0.11	23	5.7	0.94	180	20	1	0.40	30
15	1.0	150	0.11	23	11	1.89	90	10	1	0.40	30
16	0.48	70	0.054	11	1.3	0.22	360	40	1	0.73	10
17	0.48	70	0.054	11	2.7	0.44	180	20	1	0.73	10
18	0.48	70	0.054	11	5.4	0.88	90	10	1	0.73	10
19	1.0	150	0.18	37	3.0	0.50	340	60	1	0.43	30
20	1.0	150	0.18	37	6.1	1.00	170	30	1	0.43	30
21	1.0	150	0.18	37	12	2.00	85	15	1	0.43	30
22	0.48	70	0.085	17	1.4	0.23	340	60	1	0.78	10
23	0.48	70	0.085	17	2.8	0.47	170	30	1	0.78	10
24	0.48	70	0.085	17	5.7	0.93	85	15	1	0.78	10
25	1.0	150	0.26	52	3.2	0.53	320	80	1	0.47	30
26	1.0	150	0.26	52	6.5	1.06	160	40	1	0.47	30
27	1.0	150	0.26	52	13	2.12	80	20	1	0.47	30
28	0.48	70	0.12	24	1.5	0.25	320	80	1	0.85	10
29	0.48	70	0.12	24	3.0	0.49	160	40	1	0.85	10
30	0.48	70	0.12	24	6.0	0.99	80	20	1	0.85	10
31	1.0	150	0.34	69	3.4	0.57	300	100	1	0.51	30
32	1.0	150	0.34	69	6.9	1.13	150	50	1	0.51	30
33	1.0	150	0.34	69	14	2.26	75	25	1	0.51	30

(cont'd)

No.	MEMA (M1)		DEAEMA (M2)		AIBN (Ini)		Mol ratio			NMP	[M]
	(mmol)	(mL)	(mmol)	(mL)	(μmol)	(mg)	M1	M2	Ini	(mL)	(vol.%)
34	0.48	70	0.16	32	1.6	0.26	300	100	1	0.92	10
35	0.48	70	0.16	32	3.2	0.53	150	50	1	0.92	10
36	0.48	70	0.16	32	6.4	1.06	75	25	1	0.92	10
37	1.0	150	0.44	89	3.7	0.61	280	120	1	0.56	30
38	1.0	150	0.44	89	7.4	1.21	140	60	1	0.56	30
39	1.0	150	0.44	89	15	2.42	70	30	1	0.56	30
40	0.48	70	0.21	42	1.7	0.28	280	120	1	1.00	10
41	0.48	70	0.21	42	3.4	0.57	140	60	1	1.00	10
42	0.48	70	0.21	42	6.9	1.13	70	30	1	1.00	10
43	1.0	150	0.56	112	4.0	0.65	260	140	1	0.61	30
44	1.0	150	0.56	112	7.9	1.31	130	70	1	0.61	30
45	1.0	150	0.56	112	16	2.61	65	35	1	0.61	30
46	0.48	70	0.26	52	1.9	0.30	260	140	1	1.10	10
47	0.48	70	0.26	52	3.7	0.61	130	70	1	1.10	10
48	0.48	70	0.26	52	7.4	1.22	65	35	1	1.10	10

6.3.2 Analysis of polymers by time of flight secondary ion mass spectrometry (TOF-SIMS)

Polymers spotted on a glass slide was analysed by time of flight secondary ion mass spectrometry (TOF-SIMS), which was performed using a TRIFT-III (PHI).

A peak at m/z = 59 was assigned to be $^{+}C_2H_4$-O-CH_3 ion from MEMA, and a peak at m/z = 69, assigned to $^{+}C_4H_5O$, which came from acrylate main chain of the polymers. Peaks at m/z = 72, 86, and 100, which correspond to $^{+}N(C_2H_5)_2$, $^{+}CH_2$-$N(C_2H_5)_2$, and $^{+}C_2H_4$-$N(C_2H_5)_2$ ions from DEAEMA. Using m/z = 59, 100, and 69 as representatives of MEMA, DEAEMA, and the main chain, the intensity ratios of these peaks were calculated in each polymer (Figure 3.2.5 and Table 6.3.2).

Table 6.3.2. Results of polymer spotted on the glass plate by TOF-SIMS.

No.	Condition				Intensity ratio					
	DEAEMA	Position	[M]	[M]/[I]	DEAEMA/MEMA Peak: 100/59			DEAEMA/main Peak: 100/69		
	(vol.%)		(vol.%)	(mol)	Ex.1	Ex.2	Ave.	Ex.1	Ex.2	Ave.
1	0	C1	30	400/1	0.00	0.01	0.01	0.02	0.10	0.06
2	0	C2	30	200/1	0.01	0.01	0.01	0.04	0.07	0.05
3	0	C3	30	100/1	0.02	0.00	0.01	0.21	0.03	0.12
4	0	C4	10	400/1	0.05	0.01	0.03	0.42	0.07	0.25
5	0	C5	10	200/1	0.03	0.01	0.02	0.21	0.08	0.15
6	0	C6	10	100/1	0.02	0.03	0.03	0.13	0.28	0.20
7	5	C1	30	400/1	0.07	0.07	0.07	0.57	0.61	0.59
8	5	C2	30	200/1	0.07	0.06	0.06	0.65	0.46	0.56
9	5	C3	30	100/1	N/A	0.10	0.10	N/A	0.69	0.69
10	5	C4	10	400/1	N/A	0.14	0.14	N/A	0.92	0.92
11	5	C5	10	200/1	0.10	0.08	0.09	0.75	0.61	0.68
12	5	C6	10	100/1	0.13	0.11	0.12	0.95	0.81	0.88
13	10	C1	30	400/1	0.21	0.12	0.17	1.68	0.90	1.29
14	10	C2	30	200/1	0.16	0.13	0.15	1.31	0.93	1.12
15	10	C3	30	100/1	0.15	0.10	0.12	1.03	0.95	0.99
16	10	C4	10	400/1	0.17	0.14	0.16	1.27	1.02	1.14
17	10	C5	10	200/1	0.18	0.15	0.16	1.48	1.03	1.26
18	10	C6	10	100/1	0.17	0.16	0.17	1.26	1.10	1.18
19	15	C1	30	400/1	0.22	0.24	0.23	1.73	1.53	1.63
20	15	C2	30	200/1	0.17	0.23	0.20	1.37	1.50	1.43
21	15	C3	30	100/1	0.25	0.28	0.26	1.61	2.04	1.82
22	15	C4	10	400/1	0.27	0.19	0.23	1.76	1.49	1.62
23	15	C5	10	200/1	0.21	0.23	0.22	1.69	1.58	1.64
24	15	C6	10	100/1	0.17	0.25	0.21	1.39	1.59	1.49

(cont'd)

No.	Condition				Intensity ratio					
	DEAEMA	Position	[M]	[M]/[I]	DEAEMA/MEMA Peak: 100/59			DEAEMA/main Peak: 100/69		
	(vol.%)		(vol.%)	(mol)	Ex.1	Ex.2	Ave.	Ex.1	Ex.2	Ave.
25	20	C1	30	400/1	0.52	0.72	0.62	2.76	3.47	3.11
26	20	C2	30	200/1	0.60	0.62	0.61	3.01	3.19	3.10
27	20	C3	30	100/1	0.32	0.75	0.53	1.92	3.82	2.87
28	20	C4	10	400/1	0.31	0.98	0.64	1.94	4.99	3.47
29	20	C5	10	200/1	1.05	0.95	1.00	5.18	4.26	4.72
30	20	C6	10	100/1	1.06	0.93	0.99	5.20	4.15	4.67
31	25	C1	30	400/1	0.68	0.97	0.82	3.39	4.34	3.86
32	25	C2	30	200/1	1.06	0.86	0.96	5.48	3.85	4.66
33	25	C3	30	100/1	0.45	1.04	0.75	2.42	4.65	3.53
34	25	C4	10	400/1	0.48	1.04	0.76	2.61	5.07	3.84
35	25	C5	10	200/1	1.30	0.97	1.14	5.74	4.43	5.09
36	25	C6	10	100/1	1.22	0.98	1.10	5.47	4.67	5.07
37	30	C1	30	400/1	0.95	0.94	0.95	4.09	5.16	4.63
38	30	C2	30	200/1	1.01	0.81	0.91	4.40	4.25	4.33
39	30	C3	30	100/1	0.59	1.30	0.95	2.72	5.37	4.04
40	30	C4	10	400/1	0.46	1.26	0.86	2.48	5.45	3.96
41	30	C5	10	200/1	0.88	0.95	0.92	4.85	4.73	4.79
42	30	C6	10	100/1	0.63	0.94	0.78	4.00	4.74	4.37
43	35	C1	30	400/1	0.62	0.70	0.66	3.39	4.14	3.76
44	35	C2	30	200/1	0.73	0.85	0.79	3.77	4.22	3.99
45	35	C3	30	100/1	0.73	1.51	1.12	3.37	6.59	4.98
46	35	C4	10	400/1	0.76	1.43	1.09	3.71	6.16	4.94
47	35	C5	10	200/1	0.78	1.01	0.90	5.01	5.36	5.19
48	35	C6	10	100/1	1.04	1.14	1.09	5.68	5.67	5.68

6.4 Experimental for Chapter-4

6.4.1 Wettability

6.4.1.1 Preparation of polymer films

Polymer films were prepared as follows. The polymer was spin-coated onto a glass cover-slip (diameter: 22 mm) by a SPINCOATER MODEL P6700 (Speedlines technology) with a polymer concentration of 20 mg/mL. The polymer film was dried under vacuum for 2 hrs before using.

6.4.1.2 Measurement of spreading areas

A droplet of aqueous solution (9 μL), which was mixed with dye (Congo red, Sigma-Aldrich Co., 1.0 mg/mL), was placed on the polymer film using an automated liquid handling system, MultiPROBE II EX (Packard). The image of the water-droplet was taken by a webcam from the top after a contact time of 20 sec. The spreading area of the droplet was calculated by software, Image Pro Plus^{TM} (Media Cybernetics). In all cases, 2 or 3 cover slips, were coated with the same polymer and evaluated, and the average value and the standard deviation calculated.

6.4.1.3 Contact angle measurements

Contact angle measurement was performed with an Automatic Solid Surface Free Energy Analyser (Kyowa Interface Science Co., Ltd.). A droplet of de-ionised water (1 μL) was placed on the polymer film, and the contact angle was calculated after a contact of 20 sec. In all cases, 2 or 3 cover slips with the same polymer were evaluated, and the average value and the standard deviation were calculated.

Table 6.4.1 shows the comparison between the measurement of contact angle and spreading area. The data of all polymers were shown in Table 6.4.2 – 6.4.7.

Table 6.4.1. Comparison between the measurement of contact angle and spreading area.

Polymer sample	Contact angle		Spreading area	
	(deg)	STDEV	(mm^2)	STDEV
St	90.2	0.9	9.1	0.01
MMA	69.7	2.2	11.3	0.06
HEMA	54.2	2.0	14.5	0.21
PL1-18	60.6	0.3	13.6	0.07
PL1-33	17.0	0.4	39.1	0.04
PL1-36	22.3	0.1	22.2	0.00
PL1-39	33.3	18.6	21.1	0.65
PL1-40	43.2	0.6	15.9	0.45

Table 6.4.2. Spreading area on polymer film (Polymer library-1).

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL1-1	St	DEAAm	90	10	10.1	0.08
PL1-2	St	DEAAm	70	30	10.6	0.03
PL1-3	St	DEAAm	50	50	10.8	0.13
PL1-4	St	DMAAm	90	10	8.7	0.11
PL1-5	St	DMAAm	70	30	9.1	0.08
PL1-6	St	DMAAm	50	50	9.6	0.26
PL1-7	St	NIPAAm	90	10	8.6	0.18
PL1-8	St	NIPAAm	70	30	9.0	0.11
PL1-9	St	NIPAAm	50	50	9.4	0.01
PL1-10	MMA	DEAAm	90	10	10.6	0.30
PL1-11	MMA	DEAAm	70	30	10.7	0.16
PL1-12	MMA	DEAAm	50	50	10.2	0.06
PL1-13	MMA	DMAAm	90	10	10.9	0.22
PL1-14	MMA	DMAAm	70	30	10.7	0.22
PL1-15	MMA	DMAAm	50	50	12.7	0.92
PL1-16	MMA	NIPAAm	90	10	10.9	0.11
PL1-17	MMA	NIPAAm	70	30	10.7	0.77
PL1-18	MMA	NIPAAm	50	50	10.1	0.42
PL1-19	MEMA	DEAAm	90	10	11.2	0.16
PL1-20	MEMA	DEAAm	70	30	10.0	0.05
PL1-21	MEMA	DEAAm	50	50	10.5	0.14
PL1-22	MEMA	DMAAm	90	10	10.8	0.27
PL1-23	MEMA	DMAAm	70	30	11.5	0.24
PL1-24	MEMA	DMAAm	50	50	28.6	0.62
PL1-25	MEMA	NIPAAm	90	10	11.3	0.21
PL1-26	MEMA	NIPAAm	70	30	10.3	0.11
PL1-27	MEMA	NIPAAm	50	50	13.6	0.07
PL1-28	MEA	DEAAm	90	10	12.5	0.02
PL1-29	MEA	DEAAm	70	30	17.5	0.54
PL1-30	MEA	DEAAm	50	50	27.3	1.25
PL1-31	MEA	DMAAm	90	10	18.7	0.40
PL1-32	MEA	DMAAm	70	30	41.2	0.01
PL1-33	MEA	DMAAm	50	50	39.1	0.04

(*cont'd*)

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL1-34	MEA	NIPAAm	90	10	12.0	0.33
PL1-35	MEA	NIPAAm	70	30	16.2	0.00
PL1-36	MEA	NIPAAm	50	50	22.2	0.00
PL1-37	HEMA	DEAAm	90	10	14.5	0.45
PL1-38	HEMA	DEAAm	70	30	16.7	0.13
PL1-39	HEMA	DEAAm	50	50	21.1	0.65
PL1-40	HEMA	DMAAm	90	10	15.9	0.45
PL1-41	HEMA	DMAAm	70	30	34.3	0.88
PL1-42	HEMA	DMAAm	50	50	51.1	0.88
PL1-43	HEMA	NIPAAm	90	10	14.2	0.01
PL1-44	HEMA	NIPAAm	70	30	19.1	0.11
PL1-45	HEMA	NIPAAm	50	50	39.0	1.48
PL1-46	HPMA	DEAAm	90	10	12.5	0.52
PL1-47	HPMA	DEAAm	70	30	12.0	0.74
PL1-48	HPMA	DEAAm	50	50	12.9	0.03
PL1-49	HPMA	DMAAm	90	10	12.9	0.13
PL1-50	HPMA	DMAAm	70	30	15.4	0.21
PL1-51	HPMA	DMAAm	50	50	43.7	0.35
PL1-52	HPMA	NIPAAm	90	10	12.6	0.01
PL1-53	HPMA	NIPAAm	70	30	12.4	0.44
PL1-54	HPMA	NIPAAm	50	50	16.2	0.93
PL1-55	HBMA	DEAAm	90	10	11.4	0.12
PL1-56	HBMA	DEAAm	70	30	11.1	0.02
PL1-57	HBMA	DEAAm	50	50	10.9	0.32
PL1-58	HBMA	DMAAm	90	10	11.8	0.04
PL1-59	HBMA	DMAAm	70	30	11.5	0.13
PL1-60	HBMA	DMAAm	50	50	23.0	1.15
PL1-61	HBMA	NIPAAm	90	10	11.3	0.08
PL1-62	HBMA	NIPAAm	70	30	10.5	0.03
PL1-63	HBMA	NIPAAm	50	50	10.4	0.21

Table 6.4.3. Spreading area on polymer film (Polymer library-2).

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL2-1	MEMA	DEAEMA	90	10	11.6	0.11
PL2-2	MEMA	DEAEMA	70	30	11.4	0.22
PL2-3	MEMA	DEAEMA	50	50	11.7	0.12
PL2-4	MEMA	DMAEMA	90	10	11.3	0.06
PL2-5	MEMA	DMAEMA	70	30	11.4	0.17
PL2-6	MEMA	DMAEMA	50	50	11.6	0.10
PL2-7	MEMA	DEAEA	90	10	11.0	0.24
PL2-8	MEMA	DEAEA	70	30	9.5	0.02
PL2-9	MEMA	DEAEA	50	50	8.6	0.00
PL2-10	MEMA	DMAEA	90	10	10.7	0.06
PL2-11	MEMA	DMAEA	70	30	10.7	0.18
PL2-12	MEMA	DMAEA	50	50	18.7	0.00
PL2-13	MEMA	MTEMA	90	10	10.9	0.05
PL2-14	MEMA	MTEMA	70	30	10.7	0.00
PL2-15	MEMA	MTEMA	50	50	10.6	0.12
PL2-16	MEMA	BAEMA	90	10	10.3	0.13
PL2-17	MEMA	BAEMA	70	30	10.1	0.04
PL2-18	MEMA	BAEMA	50	50	10.1	0.04
PL2-19	MEMA	DMAPMAAm	90	10	11.1	0.37
PL2-20	MEMA	DMAPMAAm	70	30	10.1	0.04
PL2-21	MEMA	DMAPMAAm	50	50	15.7	0.40
PL2-22	MEMA	BACOEA	90	10	11.0	0.05
PL2-23	MEMA	BACOEA	70	30	8.6	0.09
PL2-24	MEMA	BACOEA	50	50	8.1	0.02
PL2-25	MEMA	DMVBA	90	10	10.8	0.11
PL2-26	MEMA	DMVBA	70	30	10.8	0.08
PL2-27	MEMA	DMVBA	50	50	10.9	0.03
PL2-28	MEMA	VAA	90	10	11.1	0.18
PL2-29	MEMA	VAA	70	30	13.2	0.13
PL2-30	MEMA	VAA	50	50	32.3	1.41
PL2-31	MEMA	VI	90	10	11.4	0.04
PL2-32	MEMA	VI	70	30	11.3	0.13
PL2-33	MEMA	VI	50	50	11.1	0.18

(cont'd)

Code	Polymer structure		Ratio (mol.%)		Wettability Spreading area (mm^2)	
	M(1)	M(2)	M (1)	M (2)	Ave.	STDEV
PL2-34	MEMA	VPNO	90	10	11.4	0.22
PL2-35	MEMA	VPNO	70	30	12.1	0.01
PL2-36	MEMA	VPNO	50	50	31.9	0.62
PL2-37	MEMA	VP-4	90	10	11.4	0.03
PL2-38	MEMA	VP-4	70	30	11.4	0.04
PL2-39	MEMA	VP-4	50	50	10.8	0.04
PL2-40	MEMA	VP-2	90	10	11.7	0.11
PL2-41	MEMA	VP-2	70	30	11.1	0.03
PL2-42	MEMA	VP-2	50	50	10.7	0.13
PL2-43	MEMA	DAAAm	90	10	11.3	0.08
PL2-44	MEMA	DAAAm	70	30	11.2	0.03
PL2-45	MEMA	DAAAm	50	50	11.4	0.36
PL2-46	MEMA	MNPMA	90	10	11.5	0.08
PL2-47	MEMA	MNPMA	70	30	11.0	0.19
PL2-48	MEMA	MNPMA	50	50	11.4	0.02
PL2-49	HEMA	DEAEMA	90	10	11.5	0.02
PL2-50	HEMA	DEAEMA	70	30	12.2	0.23
PL2-51	HEMA	DEAEMA	50	50	11.3	0.14
PL2-52	HEMA	DMAEMA	90	10	12.8	0.16
PL2-53	HEMA	DMAEMA	70	30	13.0	0.14
PL2-54	HEMA	DMAEMA	50	50	14.7	0.11
PL2-55	HEMA	DEAEA	90	10	12.6	0.06
PL2-56	HEMA	DEAEA	70	30	14.0	0.13
PL2-57	HEMA	DEAEA	50	50	25.1	0.99
PL2-58	HEMA	DMAEA	90	10	13.8	0.55
PL2-59	HEMA	DMAEA	70	30	26.6	0.04
PL2-60	HEMA	DMAEA	50	50	32.4	0.33
PL2-61	HEMA	MTEMA	90	10	12.9	0.06
PL2-62	HEMA	MTEMA	70	30	11.9	0.07
PL2-63	HEMA	MTEMA	50	50	11.7	0.08
PL2-64	HEMA	BAEMA	90	10	12.0	0.08
PL2-65	HEMA	BAEMA	70	30	11.3	0.21
PL2-66	HEMA	BAEMA	50	50	11.0	0.04

(*cont'd*)

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL2-67	HEMA	DMAPMAAm	90	10	13.5	0.37
PL2-68	HEMA	DMAPMAAm	70	30	21.1	0.91
PL2-69	HEMA	DMAPMAAm	50	50	26.4	0.88
PL2-70	HEMA	BACOEA	90	10	12.6	0.03
PL2-71	HEMA	BACOEA	70	30	10.3	0.14
PL2-72	HEMA	BACOEA	50	50	9.5	0.33
PL2-73	HEMA	DMVBA	90	10	11.5	0.16
PL2-74	HEMA	DMVBA	70	30	11.1	0.16
PL2-75	HEMA	DMVBA	50	50	11.1	0.07
PL2-76	HEMA	VAA	90	10	15.5	0.50
PL2-77	HEMA	VAA	70	30	31.8	1.05
PL2-78	HEMA	VAA	50	50	36.5	0.69
PL2-79	HEMA	VI	90	10	13.7	0.44
PL2-80	HEMA	VI	70	30	13.9	0.17
PL2-81	HEMA	VI	50	50	-	-
PL2-82	HEMA	VPNO	90	10	14.8	0.64
PL2-83	HEMA	VPNO	70	30	16.1	0.33
PL2-84	HEMA	VPNO	50	50	31.4	0.33
PL2-85	HEMA	VP-4	90	10	13.6	0.21
PL2-86	HEMA	VP-4	70	30	14.4	0.02
PL2-87	HEMA	VP-4	50	50	13.7	0.18
PL2-88	HEMA	VP-2	90	10	12.9	0.12
PL2-89	HEMA	VP-2	70	30	12.2	0.02
PL2-90	HEMA	VP-2	50	50	11.9	0.06
PL2-91	HEMA	DAAAm	90	10	13.7	0.25
PL2-92	HEMA	DAAAm	70	30	12.8	0.47
PL2-93	HEMA	DAAAm	50	50	12.1	0.38
PL2-94	HEMA	MNPMA	90	10	13.4	0.11
PL2-95	HEMA	MNPMA	70	30	12.7	0.16
PL2-96	HEMA	MNPMA	50	50	12.2	0.12

Table 6.4.4. Spreading area on polymer film (Polymer library-3).

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL3-1	MMA	DEAEMA	90	10	12.8	0.13
PL3-2	MMA	DEAEMA	70	30	12.7	0.30
PL3-3	MMA	DEAEMA	50	50	12.6	0.25
PL3-4	MMA	DMAEMA	90	10	12.0	0.33
PL3-5	MMA	DMAEMA	70	30	12.4	0.43
PL3-6	MMA	DMAEMA	50	50	12.3	0.05
PL3-7	MMA	DEAEA	90	10	12.1	0.66
PL3-8	MMA	DEAEA	70	30	12.5	0.65
PL3-9	MMA	DEAEA	50	50	12.6	0.60
PL3-10	MMA	DMAEA	90	10	13.8	0.11
PL3-11	MMA	DMAEA	70	30	13.5	0.07
PL3-12	MMA	DMAEA	50	50	11.7	0.42
PL3-13	HPMA	DEAEMA	90	10	13.2	0.18
PL3-14	HPMA	DEAEMA	70	30	12.5	0.06
PL3-15	HPMA	DEAEMA	50	50	11.9	0.01
PL3-16	HPMA	DMAEMA	90	10	13.4	0.01
PL3-17	HPMA	DMAEMA	70	30	12.9	0.02
PL3-18	HPMA	DMAEMA	50	50	13.0	0.04
PL3-19	HPMA	DEAEA	90	10	13.2	0.22
PL3-20	HPMA	DEAEA	70	30	13.8	0.41
PL3-21	HPMA	DEAEA	50	50	17.9	0.14
PL3-22	HPMA	DMAEA	90	10	13.7	0.22
PL3-23	HPMA	DMAEA	70	30	15.0	0.37
PL3-24	HPMA	DMAEA	50	50	30.9	2.19
PL3-25	HBMA	DEAEMA	90	10	12.8	1.08
PL3-26	HBMA	DEAEMA	70	30	11.9	0.05
PL3-27	HBMA	DEAEMA	50	50	11.7	0.21
PL3-28	HBMA	DMAEMA	90	10	12.3	0.31
PL3-29	HBMA	DMAEMA	70	30	12.2	0.51
PL3-30	HBMA	DMAEMA	50	50	12.3	0.36
PL3-31	HBMA	DEAEA	90	10	11.4	0.48
PL3-32	HBMA	DEAEA	70	30	11.5	0.28
PL3-33	HBMA	DEAEA	50	50	11.3	1.05

(*cont'd*)

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL3-34	HBMA	DMAEA	90	10	11.8	0.42
PL3-35	HBMA	DMAEA	70	30	11.4	0.45
PL3-36	HBMA	DMAEA	50	50	9.5	0.40
PL3-37	EMA	DEAEMA	90	10	11.9	0.43
PL3-38	EMA	DEAEMA	70	30	11.9	0.16
PL3-39	EMA	DEAEMA	50	50	11.9	0.06
PL3-40	EMA	DMAEMA	90	10	11.6	0.30
PL3-41	EMA	DMAEMA	70	30	12.0	0.66
PL3-42	EMA	DMAEMA	50	50	12.3	0.04
PL3-43	EMA	DEAEA	90	10	11.7	0.50
PL3-44	EMA	DEAEA	70	30	12.2	0.05
PL3-45	EMA	DEAEA	50	50	10.8	0.21
PL3-46	EMA	DMAEA	90	10	12.1	0.10
PL3-47	EMA	DMAEA	70	30	11.4	0.42
PL3-48	EMA	DMAEA	50	50	10.1	0.11
PL3-49	BMA	DEAEMA	90	10	9.6	0.25
PL3-50	BMA	DEAEMA	70	30	10.2	0.14
PL3-51	BMA	DEAEMA	50	50	10.4	0.05
PL3-52	BMA	DMAEMA	90	10	10.2	0.04
PL3-53	BMA	DMAEMA	70	30	10.9	0.34
PL3-54	BMA	DMAEMA	50	50	10.9	0.62
PL3-55	BMA	DEAEA	90	10	9.7	0.42
PL3-56	BMA	DEAEA	70	30	9.7	0.25
PL3-57	BMA	DEAEA	50	50	9.4	0.12
PL3-58	BMA	DMAEA	90	10	9.6	0.23
PL3-59	BMA	DMAEA	70	30	9.3	0.02
PL3-60	BMA	DMAEA	50	50	9.6	0.04

Table 6.4.5. Spreading area on polymer film (Polymer library-4).

Code	Polymer structure				Wettability	
	M(1)	M(2)	Ratio (mol.%)		Spreading area (mm^2)	
			M (1)	M (2)	Ave.	STDEV
PL4-1	MMA	A-H	90	10	11.7	0.49
PL4-2	MMA	A-H	70	30	11.7	0.08
PL4-3	MMA	A-H	50	50	14.0	0.21
PL4-4	MMA	AES-H	90	10	11.7	0.52
PL4-5	MMA	AES-H	70	30	11.5	0.15
PL4-6	MMA	AES-H	50	50	12.0	0.18
PL4-7	MMA	MA-H	90	10	11.7	0.02
PL4-8	MMA	MA-H	70	30	11.8	0.11
PL4-9	MMA	MA-H	50	50	12.0	0.33
PL4-10	MMA	AAG-H	90	10	11.4	0.17
PL4-11	MMA	AAG-H	70	30	25.1	0.28
PL4-12	MMA	AAG-H	50	50	26.4	1.03
PL4-13	MMA	EGMP-H	90	10	14.1	0.08
PL4-14	MMA	EGMP-H	70	30	13.4	0.21
PL4-15	MMA	EGMP-H	50	50	-	-
PL4-16	MEMA	A-H	90	10	11.5	0.01
PL4-17	MEMA	A-H	70	30	13.4	0.77
PL4-18	MEMA	A-H	50	50	19.6	0.54
PL4-19	MEMA	AES-H	90	10	11.5	0.04
PL4-20	MEMA	AES-H	70	30	11.3	0.09
PL4-21	MEMA	AES-H	50	50	10.7	0.27
PL4-22	MEMA	MA-H	90	10	11.9	0.04
PL4-23	MEMA	MA-H	70	30	12.2	0.19
PL4-24	MEMA	MA-H	50	50	12.2	0.01
PL4-25	MEMA	AAG-H	90	10	14.2	0.37
PL4-26	MEMA	AAG-H	70	30	21.8	0.99
PL4-27	MEMA	AAG-H	50	50	38.7	0.21
PL4-28	MEMA	EGMP-H	90	10	12.0	0.08
PL4-29	MEMA	EGMP-H	70	30	14.6	1.05
PL4-30	MEMA	EGMP-H	50	50	-	-

Table 6.4.6. Spreading area on polymer film (Polymer library-5).

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL5-1	MEMA	DEAEMA	MA	40	30	30	11.3	0.16
PL5-2	MEMA	DEAEMA	MA	60	10	30	11.3	0.19
PL5-3	MEMA	DEAEMA	MA	60	30	10	12.1	0.67
PL5-4	MEMA	DEAEMA	MA	80	10	10	11.8	0.21
PL5-5	MEMA	DEAEA	MA	40	30	30	11.1	0.03
PL5-6	MEMA	DEAEA	MA	60	10	30	11.0	0.13
PL5-7	MEMA	DEAEA	MA	60	30	10	10.7	0.51
PL5-8	MEMA	DEAEA	MA	80	10	10	11.6	0.26
PL5-9	MEMA	DEAEMA	BMA	40	30	30	10.6	0.34
PL5-10	MEMA	DEAEMA	BMA	60	10	30	10.8	0.21
PL5-11	MEMA	DEAEMA	BMA	60	30	10	11.2	0.27
PL5-12	MEMA	DEAEMA	BMA	80	10	10	11.5	0.33
PL5-13	MEMA	DEAEA	BMA	40	30	30	10.3	0.11
PL5-14	MEMA	DEAEA	BMA	60	10	30	10.3	0.03
PL5-15	MEMA	DEAEA	BMA	60	30	10	10.8	0.03
PL5-16	MEMA	DEAEA	BMA	80	10	10	11.2	0.06
PL5-17	MEMA	DEAEMA	MEA	40	30	30	10.9	0.05
PL5-18	MEMA	DEAEMA	MEA	60	10	30	10.9	0.08
PL5-19	MEMA	DEAEMA	MEA	60	30	10	11.3	0.13
PL5-20	MEMA	DEAEMA	MEA	80	10	10	11.5	0.22
PL5-21	MEMA	DEAEA	MEA	40	30	30	10.6	0.29
PL5-22	MEMA	DEAEA	MEA	60	10	30	10.4	0.66
PL5-23	MEMA	DEAEA	MEA	60	30	10	10.2	0.25
PL5-24	MEMA	DEAEA	MEA	80	10	10	11.1	0.58
PL5-25	MEMA	DEAEMA	DEGMEMA	40	30	30	11.5	0.56
PL5-26	MEMA	DEAEMA	DEGMEMA	60	10	30	11.4	0.10
PL5-27	MEMA	DEAEMA	DEGMEMA	60	30	10	11.9	0.27
PL5-28	MEMA	DEAEMA	DEGMEMA	80	10	10	12.2	0.36
PL5-29	MEMA	DEAEA	DEGMEMA	40	30	30	10.6	0.00
PL5-30	MEMA	DEAEA	DEGMEMA	60	10	30	11.2	0.13
PL5-31	MEMA	DEAEA	DEGMEMA	60	30	10	10.2	0.18
PL5-32	MEMA	DEAEA	DEGMEMA	80	10	10	11.5	0.36
PL5-33	MEMA	DEAEMA	THFFA	40	30	30	11.3	0.03

(*cont'd*)

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL5-34	MEMA	DEAEMA	THFFA	60	10	30	11.1	0.15
PL5-35	MEMA	DEAEMA	THFFA	60	30	10	11.7	0.53
PL5-36	MEMA	DEAEMA	THFFA	80	10	10	11.7	0.37
PL5-37	MEMA	DEAEA	THFFA	40	30	30	10.5	0.33
PL5-38	MEMA	DEAEA	THFFA	60	10	30	11.4	0.59
PL5-39	MEMA	DEAEA	THFFA	60	30	10	10.5	0.22
PL5-40	MEMA	DEAEA	THFFA	80	10	10	11.8	0.17
PL5-41	MEMA	DEAEMA	THFFMA	40	30	30	11.6	0.09
PL5-42	MEMA	DEAEMA	THFFMA	60	10	30	12.3	0.92
PL5-43	MEMA	DEAEMA	THFFMA	60	30	10	11.1	0.00
PL5-44	MEMA	DEAEMA	THFFMA	80	10	10	11.9	0.24
PL5-45	MEMA	DEAEA	THFFMA	40	30	30	10.5	0.37
PL5-46	MEMA	DEAEA	THFFMA	60	10	30	11.0	0.09
PL5-47	MEMA	DEAEA	THFFMA	60	30	10	10.7	0.14
PL5-48	MEMA	DEAEA	THFFMA	80	10	10	11.6	0.39
PL5-49	MEMA	DEAEMA	HEA	40	30	30	10.6	0.49
PL5-50	MEMA	DEAEMA	HEA	60	10	30	10.8	0.50
PL5-51	MEMA	DEAEMA	HEA	60	30	10	11.0	0.16
PL5-52	MEMA	DEAEMA	HEA	80	10	10	11.5	0.41
PL5-53	MEMA	DEAEA	HEA	40	30	30	15.0	0.53
PL5-54	MEMA	DEAEA	HEA	60	10	30	10.1	0.24
PL5-55	MEMA	DEAEA	HEA	60	30	10	10.1	0.21
PL5-56	MEMA	DEAEA	HEA	80	10	10	10.4	0.05
PL5-57	MEMA	DEAEMA	HEMA	40	30	30	11.6	0.20
PL5-58	MEMA	DEAEMA	HEMA	60	10	30	12.3	0.33
PL5-59	MEMA	DEAEMA	HEMA	60	30	10	11.5	0.13
PL5-60	MEMA	DEAEMA	HEMA	80	10	10	12.2	0.07
PL5-61	MEMA	DEAEA	HEMA	40	30	30	10.6	0.06
PL5-62	MEMA	DEAEA	HEMA	60	10	30	11.8	0.27
PL5-63	MEMA	DEAEA	HEMA	60	30	10	10.6	0.14
PL5-64	MEMA	DEAEA	HEMA	80	10	10	11.6	0.35
PL5-65	MEMA	DEAEMA	A-H	40	30	30	21.6	0.28
PL5-66	MEMA	DEAEMA	A-H	60	10	30	11.9	0.01

(*cont'd*)

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL5-67	MEMA	DEAEMA	A-H	60	30	10	11.6	0.23
PL5-68	MEMA	DEAEMA	A-H	80	10	10	12.2	0.28
PL5-69	MEMA	DEAEA	A-H	40	30	30	32.6	0.44
PL5-70	MEMA	DEAEA	A-H	60	10	30	12.9	0.11
PL5-71	MEMA	DEAEA	A-H	60	30	10	14.3	0.21
PL5-72	MEMA	DEAEA	A-H	80	10	10	20.5	0.44
PL5-73	MEMA	DEAEMA	MA-H	40	30	30	23.5	0.25
PL5-74	MEMA	DEAEMA	MA-H	60	10	30	12.8	0.16
PL5-75	MEMA	DEAEMA	MA-H	60	30	10	11.9	0.35
PL5-76	MEMA	DEAEMA	MA-H	80	10	10	12.3	0.08
PL5-77	MEMA	DEAEA	MA-H	40	30	30	31.1	0.64
PL5-78	MEMA	DEAEA	MA-H	60	10	30	14.7	0.47
PL5-79	MEMA	DEAEA	MA-H	60	30	10	17.1	1.69
PL5-80	MEMA	DEAEA	MA-H	80	10	10	17.3	0.48
PL5-81	MEMA	DEAEMA	DMAAm	40	30	30	12.9	0.19
PL5-82	MEMA	DEAEMA	DMAAm	60	10	30	14.6	0.59
PL5-83	MEMA	DEAEMA	DMAAm	60	30	10	11.4	0.21
PL5-84	MEMA	DEAEMA	DMAAm	80	10	10	11.9	0.06
PL5-85	MEMA	DEAEA	DMAAm	40	30	30	15.0	0.18
PL5-86	MEMA	DEAEA	DMAAm	60	10	30	18.5	0.67
PL5-87	MEMA	DEAEA	DMAAm	60	30	10	10.7	0.02
PL5-88	MEMA	DEAEA	DMAAm	80	10	10	12.4	0.66
PL5-89	MEMA	DEAEMA	DAAAm	40	30	30	11.6	0.26
PL5-90	MEMA	DEAEMA	DAAAm	60	10	30	11.7	0.15
PL5-91	MEMA	DEAEMA	DAAAm	60	30	10	11.3	0.06
PL5-92	MEMA	DEAEMA	DAAAm	80	10	10	12.1	0.01
PL5-93	MEMA	DEAEA	DAAAm	40	30	30	10.1	0.04
PL5-94	MEMA	DEAEA	DAAAm	60	10	30	12.5	1.04
PL5-95	MEMA	DEAEA	DAAAm	60	30	10	11.0	0.21
PL5-96	MEMA	DEAEA	DAAAm	80	10	10	11.5	0.52
PL5-97	MEMA	DEAEMA	MMA	40	30	30	11.9	0.04
PL5-98	MEMA	DEAEMA	MMA	60	10	30	12.1	0.11
PL5-99	MEMA	DEAEMA	MMA	60	30	10	12.0	0.03

(*cont'd*)

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL5-100	MEMA	DEAEMA	MMA	80	10	10	12.2	0.14
PL5-101	MEMA	DEAEA	MMA	40	30	30	11.2	0.08
PL5-102	MEMA	DEAEA	MMA	60	10	30	12.1	0.13
PL5-103	MEMA	DEAEA	MMA	60	30	10	10.9	0.16
PL5-104	MEMA	DEAEA	MMA	80	10	10	11.4	0.03
PL5-105	MEMA	DEAEMA	St	40	30	30	11.4	0.04
PL5-106	MEMA	DEAEMA	St	60	10	30	11.5	0.06
PL5-107	MEMA	DEAEMA	St	60	30	10	11.7	0.37
PL5-108	MEMA	DEAEMA	St	80	10	10	12.2	0.42
PL5-109	MEMA	DEAEA	St	40	30	30	11.9	0.15
PL5-110	MEMA	DEAEA	St	60	10	30	10.7	0.00
PL5-111	MEMA	DEAEA	St	60	30	10	10.7	0.23
PL5-112	MEMA	DEAEA	St	80	10	10	11.3	0.07

Table 6.4.7. Spreading area on polymer film (Polymer library-6).

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL6-1	MMA	DEAEMA	A-H	70	10	20	11.2	0.29
PL6-2	MMA	DEAEMA	A-H	70	15	15	11.3	0.04
PL6-3	MMA	DEAEMA	A-H	70	20	10	11.1	0.01
PL6-4	MMA	DEAEA	A-H	70	10	20	15.1	0.51
PL6-5	MMA	DEAEA	A-H	70	15	15	11.2	0.14
PL6-6	MMA	DEAEA	A-H	70	20	10	11.2	0.16
PL6-7	MMA	DEAEMA	MA-H	70	10	20	12.0	0.13
PL6-8	MMA	DEAEMA	MA-H	70	15	15	12.1	0.08
PL6-9	MMA	DEAEMA	MA-H	70	20	10	12.8	0.14
PL6-10	MMA	DEAEA	MA-H	70	10	20	12.5	0.13
PL6-11	MMA	DEAEA	MA-H	70	15	15	10.9	0.05
PL6-12	MMA	DEAEA	MA-H	70	20	10	10.9	0.23
PL6-13	MEMA	DEAEMA	A-H	70	10	20	11.8	0.18
PL6-14	MEMA	DEAEMA	A-H	70	15	15	11.7	0.54
PL6-15	MEMA	DEAEMA	A-H	70	20	10	11.7	0.13
PL6-16	MEMA	DEAEA	A-H	70	10	20	11.8	0.30
PL6-17	MEMA	DEAEA	A-H	70	15	15	11.5	0.11
PL6-18	MEMA	DEAEA	A-H	70	20	10	11.4	0.45
PL6-19	MEMA	DEAEMA	MA-H	70	10	20	12.8	0.22
PL6-20	MEMA	DEAEMA	MA-H	70	15	15	12.9	0.13
PL6-21	MEMA	DEAEMA	MA-H	70	20	10	12.2	0.11
PL6-22	MEMA	DEAEA	MA-H	70	10	20	12.5	0.05
PL6-23	MEMA	DEAEA	MA-H	70	15	15	13.7	0.26
PL6-24	MEMA	DEAEA	MA-H	70	20	10	13.0	0.19
PL6-25	MEMA	DEAEMA	A-H	85	10	5	12.5	0.22
PL6-26	MEMA	DEAEMA	A-H	80	15	5	12.5	0.06
PL6-27	MEMA	DEAEMA	A-H	75	20	5	12.4	0.03
PL6-28	MEMA	DEAEMA	A-H	70	25	5	12.5	0.18
PL6-29	MEMA	DEAEMA	A-H	65	30	5	12.6	0.08
PL6-30	MEMA	DEAEMA	A-H	60	35	5	12.4	0.00
PL6-31	MEMA	DEAEMA	A-H	55	40	5	12.2	0.09
PL6-32	MEMA	DEAEMA	A-H	50	45	5	12.6	0.08
PL6-33	MEMA	DEAEMA	A-H	75	15	10	12.2	0.13

(*cont'd*)

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL6-34	MEMA	DEAEMA	A-H	65	25	10	12.0	0.16
PL6-35	MEMA	DEAEMA	A-H	55	35	10	11.9	0.01
PL6-36	MEMA	DEAEMA	A-H	50	40	10	11.7	0.23
PL6-37	MEMA	DEAEMA	A-H	65	20	15	12.0	0.01
PL6-38	MEMA	DEAEMA	A-H	60	25	15	11.9	0.15
PL6-39	MEMA	DEAEMA	A-H	55	30	15	11.5	0.27
PL6-40	MEMA	DEAEMA	A-H	50	35	15	11.7	0.11
PL6-41	MEMA	DEAEMA	A-H	55	25	20	11.6	0.05
PL6-42	MEMA	DEAEMA	A-H	50	30	20	11.4	0.10
PL6-43	MEMA	DEAEMA	A-H	90	5	5	12.2	0.38
PL6-44	MEMA	DEAEMA	A-H	80	5	15	12.1	0.18
PL6-45	MEMA	DEAEMA	A-H	70	5	25	11.8	0.12
PL6-46	MEMA	DEAEMA	A-H	60	5	35	11.8	0.17
PL6-47	MEMA	DEAEMA	A-H	50	5	45	11.0	0.40
PL6-48	MEMA	DEAEMA	A-H	50	10	40	14.2	1.09
PL6-49	MEMA	DEAEMA	A-H	60	15	25	18.3	0.27
PL6-50	MEMA	DEAEMA	A-H	50	15	35	22.3	0.33
PL6-51	MEMA	DEAEMA	A-H	60	20	20	12.7	0.71
PL6-52	MEMA	DEAEMA	A-H	50	20	30	23.1	0.10
PL6-53	MEMA	DEAEMA	A-H	50	25	25	16.6	0.93
PL6-54	MEMA	DEAEMA	A-H	75	22.5	2.5	12.3	0.28
PL6-55	MEMA	DEAEMA	A-H	70	27.5	2.5	11.9	0.18
PL6-56	MEMA	DEAEMA	A-H	70	22.5	7.5	12.0	0.02
PL6-57	MEMA	DEAEMA	A-H	65	27.5	7.5	12.1	0.04
PL6-58	MEMA	DEAEMA	A-H	60	32.5	7.5	12.4	0.01
PL6-59	MEMA	DEAEMA	A-H	65	22.5	12.5	11.8	0.25
PL6-60	MEMA	DEAEMA	A-H	60	27.5	12.5	12.3	0.64
PL6-61	MEMA	DEAEMA	A-H	55	32.5	12.5	12.2	0.28
PL6-62	MEMA	DEAEA	A-H	85	10	5	11.9	0.19
PL6-63	MEMA	DEAEA	A-H	80	15	5	11.1	0.18
PL6-64	MEMA	DEAEA	A-H	75	20	5	10.8	0.30
PL6-65	MEMA	DEAEA	A-H	70	25	5	11.2	0.58
PL6-66	MEMA	DEAEA	A-H	65	30	5	11.6	0.08

(*cont'd*)

Code	Polymer structure						Wettability	
	M(1)	M(2)	M(3)	Ratio (mol.%)			Spreading area (mm^2)	
				M (1)	M (2)	M (3)	Ave.	STDEV
PL6-67	MEMA	DEAEA	A-H	60	35	5	11.0	0.45
PL6-68	MEMA	DEAEA	A-H	55	40	5	11.5	0.32
PL6-69	MEMA	DEAEA	A-H	50	45	5	11.1	0.09
PL6-70	MEMA	DEAEA	A-H	75	15	10	12.3	0.49
PL6-71	MEMA	DEAEA	A-H	65	25	10	11.5	0.91
PL6-72	MEMA	DEAEA	A-H	55	35	10	11.7	0.21
PL6-73	MEMA	DEAEA	A-H	50	40	10	16.5	0.57
PL6-74	MEMA	DEAEA	A-H	65	20	15	10.9	0.06
PL6-75	MEMA	DEAEA	A-H	60	25	15	10.1	1.11
PL6-76	MEMA	DEAEA	A-H	55	30	15	13.4	1.79
PL6-77	MEMA	DEAEA	A-H	50	35	15	18.4	0.54
PL6-78	MEMA	DEAEA	A-H	55	25	20	20.3	1.32
PL6-79	MEMA	DEAEA	A-H	50	30	20	15.7	0.14
PL6-80	MEMA	DMAEMA	A-H	70	20	10	12.5	0.39
PL6-81	MEMA	DMAEMA	A-H	60	30	10	12.3	0.45
PL6-82	MEMA	DMAEA	A-H	70	20	10	10.9	0.08
PL6-83	MEMA	DMAEA	A-H	60	30	10	10.2	1.04
PL6-84	MEMA	DMAPMAAm	A-H	70	20	10	10.2	0.06
PL6-85	MEMA	DMAPMAAm	A-H	60	30	10	11.2	2.01

6.4.2 Thermal analysis by Differential scanning calorimetry (DSC)

6.4.2.1 Investigation of the effect of scanning rate on glass transition temperature (Tg)

Thermal properties were analysed by a thermal analyser model Diamond DSC (Perkin-Elmer Inc.). Polymer sample, PHEMA (Sigma-Aldrich Co., Ltd., 30 mg) was heated to 150 °C and cooled to -20 °C at a set scan rate. The second scan was run at same scan rate, and the glass transition temperatures (the onset temperature and the temperature at half of the total heat capacity 1/2 ΔCp) were calculated from the second scan.

Table 6.4.8. Effect of scan rate on glass transition temperature.

Scan rate (°C/min)	Tg (Onset) (°C)	Tg (Half Cp extrapolated) (°C)
10	95.2	98.7
50	97.2	102.1
100	101.2	109.1
200	109.0	118.2

6.4.2.2 Investigation of the thermal properties of various polymers

Polymers in libraries 1, 2, and 4 were analysed by DSC. The sample (5 - 30 mg) was heated to at least 30 °C above the glass transition temperature and cooled to at least 30 °C below the glass transition temperature at 100 °C/min. The second scan was run at the same scan rate, and the glass transition temperatures were calculated from the second scan.

Table 6.4.9. Glass transition temperature (Polymer library-1).

No.	Glass transition temperature	
	Onset (°C)	Half Cp extrapolated (°C)
PL1-1	86.2	92.2
PL1-2	83.8	91.7
PL1-3	70.0	75.8
PL1-4	80.8	91.6
PL1-5	116.9	121.7
PL1-6	109.1	115.6
PL1-7	90.0	97.3
PL1-8	93.7	101.3
PL1-9	69.5	76.7
PL1-10	104.9	115.3
PL1-11	107.3	111.7
PL1-12	77.8	88.1
PL1-13	115.6	122.8
PL1-14	112.1	119.0
PL1-15	88.2	98.2
PL1-16	118.0	124.1
PL1-17	99.6	103.4
PL1-18	88.0	93.2
PL1-19	28.7	35.4
PL1-20	39.5	46.2
PL1-21	39.7	46.9
PL1-22	25.8	33.5
PL1-23	37.7	45.4
PL1-24	39.5	45.7
PL1-25	39.5	48.1
PL1-26	43.2	50.7
PL1-27	47.2	54.9
PL1-28	-39.3	-32.4
PL1-29	-21.3	-13.2
PL1-30	0.1	6.2
PL1-31	-33.0	-28.0
PL1-32	-16.2	-6.1
PL1-33	6.5	13.2
PL1-34	-25.4	-20.8
PL1-35	-1.3	5.1
PL1-36	42.4	48.4
PL1-37	23.8	31.7
PL1-38	25.8	31.4
PL1-39	56.4	63.0
PL1-40	41.5	43.7
PL1-41	41.9	49.6
PL1-42	53.3	61.8
PL1-43	26.8	29.7
PL1-44	44.2	45.4
PL1-45	60.1	67.4
PL1-46	24.1	31.6
PL1-47	35.2	40.6
PL1-48	42.6	45.6
PL1-49	36.9	43.2
PL1-50	63.4	74.8
PL1-51	60.9	70.2
PL1-52	54.2	58.0
PL1-53	61.3	69.7
PL1-54	83.3	87.1
PL1-55	24.1	30.8
PL1-56	20.4	28.2
PL1-57	19.6	27.2
PL1-58	82.3	88.7
PL1-59	83.8	90.8
PL1-60	86.8	92.8
PL1-61	10.3	21.2
PL1-62	38.5	47.7
PL1-63	51.7	61.2

Table 6.4.10. Glass transition temperature (Polymer library-2).

No.	Glass transition temperature		No.	Glass transition temperature	
	Onset (°C)	Half Cp extrapolated (°C)		Onset (°C)	Half Cp extrapolated (°C)
PL2-1	38.2	44.7	PL2-34	41.1	45.5
PL2-2	20.2	30.2	PL2-35	38.2	44.9
PL2-3	14.0	21.3	PL2-36	47.4	55.4
PL2-4	39.7	47.2	PL2-37	42.6	47.3
PL2-5	44.4	46.8	PL2-38	61.7	70.6
PL2-6	33.1	41.9	PL2-39	66.8	73.1
PL2-7	36.6	41.9	PL2-40	41.7	48.5
PL2-8	-10.2	-0.5	PL2-41	48.5	54.2
PL2-9	-17.7	-13.6	PL2-42	43.2	50.0
PL2-10	34.9	40.6	PL2-43	53.2	66.8
PL2-11	7.7	15.5	PL2-44	64.2	72.2
PL2-12	-12.3	-6.6	PL2-45	89.1	96.7
PL2-13	43.4	50.7	PL2-46	25.7	36.7
PL2-14	43.9	53.2	PL2-47	36.1	43.2
PL2-15	34.7	43.9	PL2-48	51.5	59.0
PL2-16	40.7	49.1	PL2-49	59.0	66.9
PL2-17	42.9	50.4	PL2-50	43.9	53.4
PL2-18	43.3	49.8	PL2-51	46.5	54.1
PL2-19	52.3	59.3	PL2-52	60.6	69.0
PL2-20	52.1	59.1	PL2-53	63.8	70.0
PL2-21	65.7	73.1	PL2-54	59.0	69.7
PL2-22	17.9	27.9	PL2-55	62.1	76.1
PL2-23	0.1	8.3	PL2-56	56.5	62.6
PL2-24	5.7	8.8	PL2-57	27.6	36.9
PL2-25	35.8	44.1	PL2-58	57.6	69.1
PL2-26	61.8	72.4	PL2-59	51.8	60.4
PL2-27	52.8	59.1	PL2-60	30.4	38.2
PL2-28	35.9	45.5	PL2-61	51.8	59.1
PL2-29	36.6	41.2	PL2-62	56.8	66.4
PL2-30	85.2	89.2	PL2-63	51.9	60.3
PL2-31	29.2	40.8	PL2-64	66.0	75.9
PL2-32	56.1	62.7	PL2-65	84.6	93.0
PL2-33	50.7	59.4	PL2-66	78.3	85.6

(*cont'd*)

No.	Glass transition temperature	
	Onset (°C)	Half Cp extrapolated (°C)
PL2-67	85.0	95.1
PL2-68	80.4	85.1
PL2-69	76.1	85.8
PL2-70	52.0	66.1
PL2-71	39.1	47.0
PL2-72	8.2	11.5
PL2-73	28.9	53.4
PL2-74	42.3	47.4
PL2-75	71.2	76.3
PL2-76	62.6	68.7
PL2-77	88.2	96.2
PL2-78	91.7	100.8
PL2-79	43.2	58.3
PL2-80	72.3	78.5
PL2-81	86.0	94.8
PL2-82	45.4	62.6
PL2-83	79.9	89.9
PL2-84	104.8	110.3
PL2-85	72.7	83.0
PL2-86	119.6	126.0
PL2-87	112.3	118.2
PL2-88	84.8	92.6
PL2-89	80.4	89.0
PL2-90	81.2	95.0
PL2-91	72.5	79.7
PL2-92	77.2	85.5
PL2-93	79.8	86.7
PL2-94	68.7	76.1
PL2-95	91.3	98.0
PL2-96	80.9	92.8

Table 6.4.11. Glass transition temperature (Polymer library-4).

No.	Glass transition temperature	
	Onset (°C)	Half Cp extrapolated (°C)
PL4-1	-	-
PL4-2	65.2	72.1
PL4-3	66.9	73.3
PL4-4	61.4	72.4
PL4-5	8.1	15.6
PL4-6	-6.1	0.7
PL4-7	-	-
PL4-8	-	-
PL4-9	-	-
PL4-10	80.1	91.1
PL4-11	61.3	81.3
PL4-12	-	
PL4-13	78.4	82.8
PL4-14	53.5	59.9
PL4-15	-	-
PL4-16	24.7	30.4
PL4-17	30.6	35.8
PL4-18	35.5	43.4
PL4-19	2.7	10.4
PL4-20	-	-
PL4-21	-	-
PL4-22	29.9	39.7
PL4-23	76.5	84.7
PL4-24	-	-
PL4-25	27.6	36.5
PL4-26	0.4	10.7
PL4-27	-15.2	-11.0
PL4-28	36.8	42.1
PL4-29	33.4	38.4
PL4-30	-0.9	4.2

6.5 Experimental for Chapter-5

6.5.1 Polymer coating onto beads

Beads (Polybead® or UNK HIPRESICA, 10 μm, 40 mg) and polymer solution (1 mL) were added into a glass vial. After sonication, the samples were left mixing for 2 hrs at room temperature. The suspension was centrifuged, and excess polymer solution was carefully removed. The remaining suspension was freeze-dried. The polymer coated beads were analysed by scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS).

6.5.2 Analysis of polymer coated beads by X-ray photo-electron spectroscopy (XPS)

The energy of photoelectrons is characteristic of each element, hence XPS can provide elemental characteristics of the surface. XPS can identify all elements (except H and He) present in the outer 10 nm of a sample.

Peaks were characterised by the energy of the photoelectrons, and elemental ratios were calculated from the percentage of integrals of each peak in total. The coverage of polymer coated beads was calculated from Equations 5-2 and 5-6 in Chapter 5, and results were shown in Table 6.5.1 – 6.5.5.

Table 6.5.1. Conditions and results for PMEMA coating onto silica beads.

No.	Solvent (vol.%)	Polym.conc. (mg/mL)	Element ratio (%)			Coverage (%)
			Si	C	O	
1	MeOH/H_2O (90/10)	10	4.7	64.2	31.1	84
2	MeOH/H_2O (80/20)	10	6.9	59.4	33.8	76
3	MeOH/H_2O (70/30)	10	4.1	64.7	31.2	86
4	MeOH/H_2O (60/40)	PMEMA was not soluble in this solvent.				
5	EtOH/H_2O (90/10)	10	7.3	58.5	34.2	75
6	EtOH/H_2O (80/20)	10	12.2	48.5	39.3	58
7	EtOH/H_2O (70/30)	10	13.3	46.2	40.6	54
8	EtOH/H_2O (60/40)	10	13.8	45.0	41.2	52
9	IPA/H_2O (90/10)	10	10.2	52.6	37.3	65
10	IPA/H_2O (80/20)	10	15.2	42.7	42.1	47
11	IPA/H_2O (70/30)	10	14.8	42.3	42.9	49
12	IPA/H_2O (60/40)	10	13.7	44.8	41.5	53
13	*t*-BuOH/H_2O (90/10)	10	15.4	40.6	44.0	47
14	*t*-BuOH/H_2O (80/20)	10	19.5	31.0	49.5	32
15	*t*-BuOH/H_2O (70/30)	10	19.3	31.6	49.1	33
16	*t*-BuOH/H_2O (60/40)	10	19.1	31.6	49.3	34

6.5.3 Investigation of protein adsorption by flow cytometry (FCM)

Protein adsorption onto the polymer coated silica beads was measured by flow cytometry (FCM), using fluorescence-conjugated proteins. In all experiments, several thousands beads was measured, and the average value of fluorescence intensity was determined.

6.5.3.1 Investigation of the effect of polymer coverage onto beads

A mixture of the IgG-FITC and Fib-Alexa647 (1 μg/mL of each protein) were prepared in 1 % goat serum / phosphate-buffered saline (PBS), and incubated with the polymer coated beads for 30 min. The fluorescence intensity was determined by FCM analysis. The results were shown in Table 6.3.2 and Figure 5.2.11.

Table 6.5.2. Effect of coverage on protein adsorption.

No.	Coating condition		Polymer coated particle				Bio-analysis	
	Solvent (vol.%)	Polymer conc.	XPS (%)			Coverage	FACS (Intensity)	
		(mg/mL)	Si	C	O	(%)	IgG	Fib
1	-	-	28.8	8.2	63.0	0	211	4105
2	MeOH/H_2O (90/10)	1	22.8	24.0	53.2	21	258	1526
3	MeOH/H_2O (90/10)	2	14.9	43.1	41.9	48	371	356
4	MeOH/H_2O (90/10)	10	4.6	63.3	32.1	84	443	354
5	MeOH/H_2O (90/10)	20	3.9	63.9	32.2	86	364	328
6	MeOH/H_2O (90/10)	50	1.5	68.4	30.1	95	827	635

*No.1: Non-coated silica beads.

**No.2 – 6: PMEMA was coated onto silica beads with varying polymer concentration.

6.5.3.2 Investigation of polymer libraries 1, 2, and 4

A mixture of IgG-FITC, Fib-Alexa647 and Alb-Alexa555 (1 μg/mL for each protein) were prepared in serum-free PBS, and incubated with the polymer coated beads at room temperature for 30 min. The intensity of fluorescence on the bead was determined by FCM analysis. The results were shown in Table 6.5.3 – 6.5.6 and Figure 5.2.13.

Table 6.5.3. XPS and FACS results of polymer library-1.

No.	Monomer B type*	Polymer coated particle					Bio-analysis		
		XPS analysis (%)				Coverage	FACS (Intensity)		
		C	O	N	Si	(%)	IgG	Fib	Alb
PL1-1	P	68.3	18.5	3.1	10.1	65	327	1393	32
PL1-2	P	74.2	14.8	3.8	7.2	75	277	1041	7
PL1-3	P	60.8	22.8	5.9	10.6	63	38	112	4
PL1-4	P	69.1	18.7	1.9	10.2	65	558	1976	125
PL1-5	P	58.7	24.8	2.4	14.1	51	676	1774	15
PL1-6	P	83.0	9.0	5.0	3.0	90	277	1507	5
PL1-7	P	60.8	25.2	0.9	13.1	54	371	1715	95
PL1-8	P	64.5	22.4	1.4	11.8	59	662	1630	61
PL1-9	P	78.4	12.2	3.6	5.9	80	582	1419	11
PL1-10	P	51.5	35.5	1.3	11.7	59	56	924	1
PL1-11	P	49.2	36.0	3.0	11.8	59	61	423	8
PL1-12	P	62.5	25.2	5.7	6.6	77	28	80	1
PL1-13	P	55.3	33.1	3.2	8.4	71	8	73	2
PL1-14	P	63.4	27.6	4.0	5.0	83	7	57	2
PL1-15	P	64.4	25.6	6.2	3.8	87	3	32	2
PL1-16	P	42.1	42.5	1.2	14.2	51	51	922	9
PL1-17	P	51.5	33.9	4.4	10.2	65	13	252	4
PL1-18	P	58.3	27.5	6.5	7.7	73	11	18	2
PL1-19	P	43.7	41.7	0.8	13.8	52	10	368	2
PL1-20	P	45.1	38.1	2.6	14.2	51		N/A	
PL1-21	P	46.5	36.3	3.8	13.4	53	11	88	1
PL1-22	P	46.6	40.8	1.2	11.3	61	4	171	1
PL1-23	P	58.3	30.7	4.0	7.1	75	42	63	2
PL1-24	P	66.6	25.4	5.4	2.5	91	2	8	1
PL1-25	P	55.1	35.9	1.6	7.4	74	6	244	1
PL1-26	P	44.0	38.5	3.2	14.2	51	5	145	1
PL1-27	P	53.0	31.2	5.5	10.3	64	4	122	1
PL1-28	P	Rubber (highly viscous liquid)						N/A	
PL1-29	P	Rubber (highly viscous liquid)						N/A	
PL1-30	P	Rubber (highly viscous liquid)						N/A	
PL1-31	P	Rubber (highly viscous liquid)						N/A	
PL1-32	P	Rubber (highly viscous liquid)						N/A	
PL1-33	P	Rubber (highly viscous liquid)						N/A	

(*cont'd*)

No.	Monomer B type*	Polymer coated particle					Bio-analysis		
		XPS analysis (%)				Coverage	FACS (Intensity)		
		C	O	N	Si	(%)	IgG	Fib	Alb
PL1-34	P	Rubber (highly viscous liquid)						N/A	
PL1-35	P	Rubber (highly viscous liquid)						N/A	
PL1-36	P	Rubber (highly viscous liquid)						N/A	
PL1-37	P	42.4	44.5	0.8	12.4	57	4	383	1
PL1-38	P	37.0	45.5	1.5	16.0	45		N/A	
PL1-39	P	44.5	39.5	3.7	12.4	57	7	530	1
PL1-40	P	56.8	37.8	0.6	4.7	84	7	848	1
PL1-41	P	46.6	40.4	2.1	10.9	62	4	361	1
PL1-42	P	37.9	43.7	3.6	14.7	49		N/A	
PL1-43	P	41.9	45.8	0.4	11.9	59	2	275	1
PL1-44	P	43.7	42.0	1.4	13.0	55	7	966	1
PL1-45	P	44.8	39.6	3.3	12.4	57	5	685	1
PL1-46	P	28.6	50.4	1.7	19.4	33		N/A	
PL1-47	P	32.3	46.8	2.4	18.5	36		N/A	
PL1-48	P	55.2	32.6	4.1	8.1	72	6	391	1
PL1-49	P	34.7	47.4	0.6	17.2	40		N/A	
PL1-50	P	53.0	31.2	5.5	10.3	64	4	369	1
PL1-51	P	36.7	45.5	1.5	16.3	43		N/A	
PL1-52	P	25.1	54.0	0.5	20.5	29		N/A	
PL1-53	P	23.4	54.8	0.3	21.4	26		N/A	
PL1-54	P	36.4	45.8	1.2	16.5	43		N/A	
PL1-55	P	30.1	50.8	0.4	18.8	35		N/A	
PL1-56	P	31.1	48.3	1.5	19.1	34		N/A	
PL1-57	P	27.4	49.3	2.4	20.9	28		N/A	
PL1-58	P	31.9	45.6	3.3	19.2	33		N/A	
PL1-59	P	18.3	58.1	0.2	23.3	19		N/A	
PL1-60	P	29.2	48.8	2.6	19.4	33		N/A	
PL1-61	P	18.5	58.4	0.0	23.1	20		N/A	
PL1-62	P	20.8	56.3	0.8	22.1	23		N/A	
PL1-63	P	32.0	48.8	1.6	17.6	39		N/A	

* P: Amide

Table 6.5.4. XPS and FACS results of polymer library-2.

No.	Monomer B type*	Polymer coated particle						Bio-analysis		
		XPS analysis (%)					Coverage	FACS (Intensity)		
		C	O	N	Si	S	(%)	IgG	Fib	Alb
PL2-1	Q	60.1	33.3	0.9	5.7	-	80	442	102	1
PL2-2	Q	66.4	28.0	2.7	3.0	-	90	561	169	12
PL2-3	Q	71.7	22.5	4.5	1.3	-	96	815	433	234
PL2-4	Q	61.9	32.8	0.5	4.9	-	83	213	45	1
PL2-5	Q	58.0	32.6	2.3	7.2	-	75	628	361	93
PL2-6	Q	65.4	26.7	4.3	3.7	-	87	660	732	150
PL2-7	Q	56.2	34.9	0.5	8.4	-	71	635	67	2
PL2-8	Q	58.7	32.7	1.4	7.2	-	75	1489	46	6
PL2-9	Q	67.9	26.0	3.3	2.8	-	90	918	683	489
PL2-10	Q	57.3	33.6	1.2	8.0	-	72	412	23	4
PL2-11	Q	55.4	34.2	2.3	8.1	-	72	769	300	59
PL2-12	Q	58.7	30.9	3.7	6.8	-	77	331	156	42
PL2-13	R	42.4	43.3	0.0	14.0	0.3	51		N/A	
PL2-14	R	49.5	37.7	0.0	11.2	1.6	61		N/A	
PL2-15	R	51.3	35.4	0.0	10.1	3.2	65		N/A	
PL2-16	Q	42.4	42.4	0.9	14.3	-	50	869	222	20
PL2-17	Q	62.1	30.2	2.2	5.5	-	81	333	648	309
PL2-18	Q	65.7	26.5	4.0	3.8	-	87	194	230	93
PL2-19	P, Q	58.7	32.7	1.7	6.8	-	76	1389	247	287
PL2-20	P, Q	53.3	32.5	4.4	9.7	-	66	1246	235	94
PL2-21	P, Q	47.0	34.4	5.9	12.7	-	56	1149	285	145
PL2-22	P	59.8	35.1	0.6	4.5	-	85		N/A	
PL2-23	P	59.7	33.2	2.4	4.7	-	84		N/A	
PL2-24	P	62.9	30.0	4.4	2.8	-	90		N/A	
PL2-25	Q	59.0	32.3	1.0	7.8	-	73	1132	211	131
PL2-26	Q	66.9	25.3	2.5	5.2	-	82	486	400	571
PL2-27	Q	77.5	15.6	4.7	2.1	-	93	267	278	268
PL2-28	P	50.9	37.4	1.5	10.2	-	65		N/A	
PL2-29	P	64.7	29.1	3.1	3.1	-	89	5	51	1
PL2-30	P	60.5	29.6	4.8	5.1	-	82	2	14	1
PL2-31	S	60.6	32.7	1.5	5.2	-	82	103	234	1
PL2-32	S	56.9	31.5	4.1	7.5	-	74	177	63	1
PL2-33	S	59.8	28.8	5.9	5.4	-	81	248	55	1

(*cont'd*)

No.	Monomer B type*	Polymer coated particle						Bio-analysis		
		XPS analysis (%)					Coverage	FACS (Intensity)		
		C	O	N	Si	S	(%)	IgG	Fib	Alb
PL2-34	P	47.0	39.1	1.2	12.7	-	56		N/A	
PL2-35	P	63.0	28.6	3.3	5.1	-	82	5	25	1
PL2-36	P	60.4	25.4	7.1	7.0	-	76	77	16	1
PL2-37	S	58.3	33.5	1.3	6.9	-	76	25	322	1
PL2-38	S	65.2	27.1	3.1	4.6	-	84	40	339	1
PL2-39	S	58.6	28.2	4.1	9.0	-	69		N/A	
PL2-40	S	59.1	33.5	0.9	6.4	-	78	4	326	1
PL2-41	S	61.8	29.1	2.8	6.4	-	78	9	321	1
PL2-42	S	58.6	28.2	4.4	8.7	-	70	55	264	2
PL2-43	P	32.6	48.8	0.4	18.2	-	37		N/A	
PL2-44	P	58.9	33.1	1.7	6.3	-	78	4	393	1
PL2-45	P	38.5	41.7	3.2	16.5	-	43		N/A	
PL2-46	T	40.9	44.6	0.1	14.4	-	50	16	495	5
PL2-47	T	32.2	48.7	1.2	17.9	-	38		N/A	
PL2-48	T	48.7	39.6	1.9	9.9	-	66	136	1333	21
PL2-49	Q	40.1	42.7	1.7	15.5	-	46		N/A	
PL2-50	Q	50.0	39.2	1.0	9.8	-	66	1145	261	6
PL2-51	Q	38.4	42.4	2.2	17.0	-	41		N/A	
PL2-52	Q	59.8	35.0	1.0	4.2	-	85	1031	36	2
PL2-53	Q	47.7	38.6	2.2	11.5	-	60	1609	514	183
PL2-54	Q	46.9	37.3	3.5	12.4	-	57	938	523	232
PL2-55	Q	50.4	39.5	0.6	9.5	-	67	1480	60	4
PL2-56	Q	40.4	43.6	1.3	14.7	-	49		N/A	
PL2-57	Q	39.8	43.0	2.0	15.2	-	47		N/A	
PL2-58	Q	52.0	39.0	1.1	7.9	-	73	1495	84	18
PL2-59	Q	41.3	42.7	2.1	13.9	-	52	1620	173	20
PL2-60	Q	42.2	41.4	2.9	13.6	-	53	1836	164	29
PL2-61	R	57.2	37.1	0.0	4.9	0.8	83	6	296	1
PL2-62	R	63.0	31.5	0.0	2.5	3.0	91	6	89	1
PL2-63	R	64.1	28.7	0.0	2.2	5.0	92	14	62	2
PL2-64	Q	44.3	42.2	0.7	12.8	-	56	567	400	14
PL2-65	Q	35.9	45.1	1.2	17.8	-	38		N/A	
PL2-66	Q	47.6	36.8	3.0	12.6	-	56	313	490	244

(*cont'd*)

No.	Monomer B type*	Polymer coated particle						Bio-analysis		
		XPS analysis (%)					Coverage	FACS (Intensity)		
		C	O	N	Si	S	(%)	IgG	Fib	Alb
PL2-67	Q, P	52.1	38.3	1.0	8.5	-	71	361	14	1
PL2-68	Q, P	45.0	39.5	2.8	12.6	-	56	1547	149	20
PL2-69	Q, P	40.1	41.0	3.7	15.2	-	47		N/A	
PL2-70	P	35.3	47.8	0.6	16.3	-	43		N/A	
PL2-71	P	35.8	46.7	1.2	16.3	-	43		N/A	
PL2-72	P	30.8	49.0	1.9	18.2	-	37		N/A	
PL2-73	Q	50.8	37.5	1.0	10.7	-	63	2006	213	57
PL2-74	Q	44.9	38.4	1.4	15.3	-	47		N/A	
PL2-75	Q	51.1	32.8	2.9	13.2	-	54	585	905	404
PL2-76	P	45.6	42.1	1.0	11.3	-	61	7	495	4
PL2-77	P	24.9	54.4	1.3	19.5	-	32		N/A	
PL2-78	P	58.3	33.3	4.0	4.4	-	85	3	177	1
PL2-79	S	58.1	36.3	1.0	4.5	-	84	37	266	1
PL2-80	S	62.0	32.9	2.8	2.3	-	92	38	112	1
PL2-81	S				N/A				N/A	
PL2-82	P	55.7	37.9	0.7	5.7	-	80	13	313	1
PL2-83	P	59.6	34.5	1.8	4.1	-	86	7	405	1
PL2-84	P	64.4	30.7	2.8	2.1	-	93	6	56	1
PL2-85	S	62.8	33.1	1.3	2.8	-	90	3	287	1
PL2-86	S	61.0	29.8	3.6	5.6	-	80	116	209	4
PL2-87	S	52.4	31.4	4.4	11.8	-	59	526	200	1
PL2-88	S	62.7	32.5	1.6	3.2	-	89	10	284	1
PL2-89	S	63.4	28.6	3.6	4.4	-	85	6	275	1
PL2-90	S	59.2	28.0	4.7	8.1	-	72	52	123	2
PL2-91	P	43.3	44.1	0.5	12.1	-	58		N/A	
PL2-92	P	37.5	46.4	1.2	14.9	-	48		N/A	
PL2-93	P	40.9	42.7	2.1	14.4	-	50		N/A	
PL2-94	T	54.5	39.3	0.5	5.7	-	80	6	256	1
PL2-95	T	49.1	41.4	1.8	7.7	-	73	10	235	2
PL2-96	T	6.9	65.4	0.2	27.4	-	5		N/A	

* P: Amide, Q: Amine, R: Thioether, S: Heteroaromatic, T: Nitro

Table 6.5.5. XPS and FACS results of polymer library-4.

No.	Monomer B type*	Polymer coated particle						Bio-analysis		
		XPS analysis (%)					Coverage	FACS (Intensity)		
		C	O	N	Si	P	(%)	IgG	Fib	Alb
PL4-1	U	5.7	65.9	-	28.4	-	2		N/A	
PL4-2	U	10.8	62.8	-	26.4	-	8		N/A	
PL4-3	U	9.4	63.8	-	26.8	-	7		N/A	
PL4-4	U	11.7	62.3	-	26.0	-	10		N/A	
PL4-5	U	12.8	61.7	-	25.5	-	12		N/A	
PL4-6	U	14.6	60.9	-	24.5	-	15		N/A	
PL4-7	U	14.8	60.5	-	24.7	-	14		N/A	
PL4-8	U	12.0	62.1	-	25.9	-	10		N/A	
PL4-9	U	17.2	59.2	-	23.6	-	18		N/A	
PL4-10	U	36.9	46.7	1.4	15.0	-	48		N/A	
PL4-11	U	20.8	56.4	1.5	21.3	-	26		N/A	
PL4-12	U	17.9	57.6	1.7	22.8	-	21		N/A	
PL4-13	U	53.8	37.8	-	7.3	1.1	75	2	403	1
PL4-14	U	42.1	44.7	-	10.5	2.6	63	4	389	1
PL4-15	U				N/A				N/A	
PL4-16	U	26.0	53.3	-	20.8	-	28		N/A	
PL4-17	U	20.4	57.0	-	22.6	-	22		N/A	
PL4-18	U	15.9	59.7	-	24.4	-	15		N/A	
PL4-19	U	14.8	60.4	-	24.8	-	14		N/A	
PL4-20	U	25.7	53.7	-	20.6	-	29		N/A	
PL4-21	U	35.4	48.7	-	15.9	-	45		N/A	
PL4-22	U	19.2	57.4	-	23.4	-	19		N/A	
PL4-23	U	14.2	60.5	-	25.3	-	12		N/A	
PL4-24	U	12.4	62.0	-	25.6	-	11		N/A	
PL4-25	U	73.2	23.0	0.7	3.0	-	90	3	95	1
PL4-26	U	39.2	44.6	2.7	13.5	-	53		N/A	
PL4-27	U	23.3	55.0	1.6	20.1	-	30		N/A	
PL4-28	U	50.9	38.5	-	9.8	0.8	66	2	30	1
PL4-29	U	46.0	42.4	-	9.3	2.3	68	3	18	2
PL4-30	U				N/A				N/A	

*U: Acid

6.5.4 Analysis for leucocyte depletion on blood samples

Experiments were carried out using a published method.[99,176]

The polymer coated non-woven fabric (thickness: 0.20 mm, polyethylene terephthalate fibre with an average fibre diameter of 1.2 μm) was cut into the circular configuration with a diameter of 20 mm, and a filter holder was filled with 9 sheets of this material. The filtration of fresh human whole blood (4 mL) was carried out, and the filtrate was collected at a fixed rate-of-flow of 0.74 mL/min using a syringe pump. Leucocyte concentrations were measured by the LeucoCOUNT™ kit, flow cytometer FACSCalibur, and the analysis software CELL Quest (BD Bioscience, the U.S.). Platelet concentrations were measured by an automatic blood cell counter, MAX A/L-Retic (BECKMAN COULTER, U.S.). Leuco-depletion ability and platelet recovery were calculated from Equations 5-7 and 5-8 in Chapter 5. The tendency to produce hemolysis was evaluated by removing blood cell components from the filtered blood by centrifugation (1500 rpm, 10 min), and then detecting haemoglobin by measuring absorbance at 576 nm.[177]

As a positive control, a known material[99] with high leuco-depletion ability was used, and the difference of leuco-depletion ability from the positive control was calculated. The results were shown in Table 6.5.6 – 6.5.9.

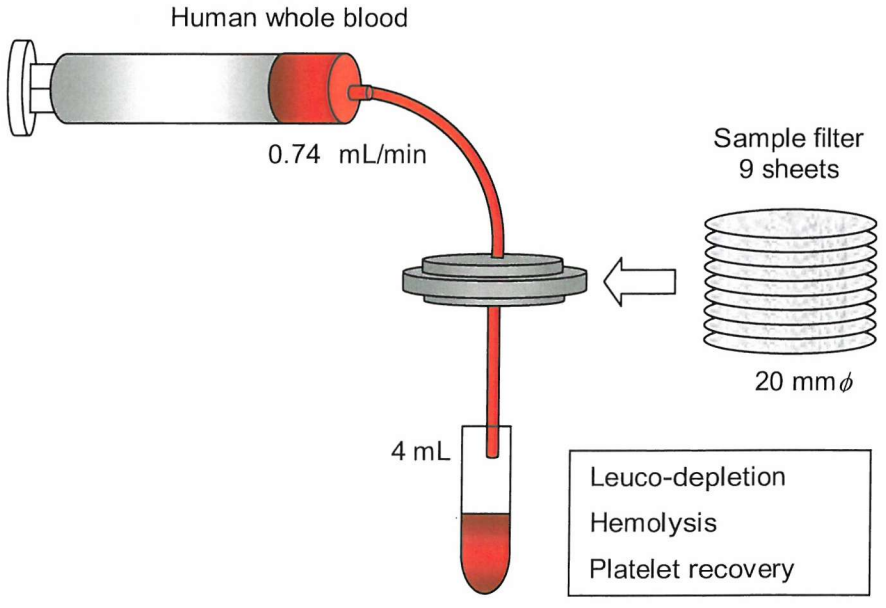


Figure 6.5.1. Experimental for blood filtration.

Table 6.5.6. Results of blood test of copolymers.

No.	Polymer structure		Leuco-depletion (-log)		PLT recovery (%)		Homolysis (ABS at 576 nm)	
	M (A)	M (B) Type*	Ave.	STDEV	Ave.	STDEV	Ave.	STDEV
Control	-		0	0	1.6	2.7	0.25	0.20
PL1-10	MMA	P	-0.46	0.20	2.1	2.3	0.21	0.06
PL1-18	MMA	P	-0.37	N/A	39.5	N/A	0.21	N/A
PL2-1	MEMA	Q	-0.11	0.10	19.3	8.3	0.41	0.29
PL2-2	MEMA	Q	0.63	0.17	0.4	0.3	0.27	0.11
PL2-3	MEMA	Q	0.13	0.06	0.3	0.3	N/A	N/A
PL2-8	MEMA	Q	0.57	0.11	0.4	0.4	1.46	0.52
PL2-10	MEMA	Q	0.20	N/A	0.0	N/A	0.19	N/A
PL2-11	MEMA	Q	0.49	0.23	0.2	0.3	0.81	0.24
PL2-13	MEMA	R	-0.18	N/A	6.9	N/A	0.10	N/A
PL2-16	MEMA	Q	0.01	N/A	0.4	N/A	0.10	N/A
PL2-23	MEMA	P	-0.14	N/A	2.6	N/A	0.16	N/A
PL2-25	MEMA	Q	-0.17	N/A	0.8	N/A	0.22	N/A
PL2-33	MEMA	S	-0.62	0.19	18.0	16.2	0.22	0.08
PL2-36	MEMA	P	-0.54	0.16	27.6	10.5	0.26	0.05
PL2-44	MEMA	P	0.08	0.19	11.1	3.0	0.86	0.89
PL2-58	HEMA	Q	-0.46	N/A	17.1	N/A	0.21	N/A
PL2-63	HEMA	R	-0.16	N/A	5.4	N/A	0.48	N/A
PL2-87	HEMA	S	-0.03	N/A	0.0	N/A	0.32	N/A
PL2-89	HEMA	S	-0.07	N/A	1.6	N/A	0.21	N/A
PL4-27	MEMA	U	-0.54	N/A	17.1	N/A	0.20	N/A
PL3-2	MMA	Q	-0.19	N/A	1.8	N/A	0.75	N/A
PL3-5	MMA	Q	0.21	N/A	0.4	N/A	0.38	N/A
PL3-6	MMA	Q	0.35	N/A	1.2	N/A	2.77	N/A
PL3-8	MMA	Q	0.36	N/A	0.4	N/A	0.44	N/A
PL3-11	MMA	Q	0.34	N/A	0.0	N/A	0.71	N/A
PL3-38	EMA	Q	-0.45	N/A	6.2	N/A	0.50	N/A

* P: Amide, Q: Amine, R: Thioether, S: Heteroaromatic, T: Nitro, U: Acid.

(*cont'd*)

No.	Polymer structure		Leuco-depletion (-log)		PLT recovery (%)		Homolysis (ABS at 576 nm)	
	M (A)	M (B) Type*	Ave.	STDEV	Ave.	STDEV	Ave.	STDEV
PL3-40	EMA	Q	-0.50	N/A	3.0	N/A	0.17	N/A
PL3-41	EMA	Q	-0.43	N/A	9.3	N/A	0.47	N/A
PL3-44	EMA	Q	-0.07	N/A	0.4	N/A	0.46	N/A
PL3-47	EMA	Q	0.32	N/A	0.4	N/A	0.40	N/A
PL3-52	BMA	Q	-0.53	N/A	2.6	N/A	0.27	N/A
PL3-53	BMA	Q	-0.64	N/A	4.9	N/A	0.52	N/A
PL3-59	BMA	Q	-0.15	N/A	0.4	N/A	0.68	N/A

* P: Amide, Q: Amine, R: Thioether, S: Heteroaromatic, T: Nitro, U: Acid.

Table 6.5.7. Effect of third component in terpolymer on blood filtration.

No.	Monomer (C)	Leuco-depletion (-log)		PLT recovery (%)		Homolysis (ABS at 576 nm)	
		Ave.	STDEV	Ave.	STDEV	Ave.	STDEV
Control	-	0	0	1.6	2.7	0.25	0.20
PL2-2	-	0.63	0.17	0.4	0.3	0.27	0.11
PL6-1	MA	0.02	N/A	0.8	N/A	0.38	N/A
PL6-9	BMA	-0.33	N/A	13.3	N/A	0.33	N/A
PL6-25	DEGMEMA	0.65	0.13	0.5	0.1	0.48	0.31
PL6-33	THFFA	0.15	N/A	6.5	N/A	4.00	N/A
PL6-41	THFFMA	0.63	N/A	0.8	N/A	3.14	N/A
PL6-49	HEA	0.52	0.32	0.6	0.3	0.26	0.15
PL6-57	HEMA	0.27	N/A	1.7	N/A	0.22	N/A
PL6-65	A-H	0.31	N/A	0.0	N/A	0.13	N/A
PL6-73	MA-H	-0.39	N/A	8.9	N/A	0.12	N/A
PL6-81	DMAAm	0.45	N/A	0.8	N/A	0.10	N/A
PL6-89	DAAAm	0.73	0.45	0.6	0.5	0.91	0.89
PL6-97	MMA	0.57	0.50	0.2	0.3	0.32	0.20
PL6-105	St	0.23	N/A	8.5	N/A	0.18	N/A

Table 6.5.8. Effect of monomer composition in terpolymer on blood filtration.

No.	[DEAEMA] - [A-H]	Protein adsorption			Blood test					
		FACS (Intensity)			Leuco-depletion (-log)		PLT recovery (%)		Homolysis (ABS at 576 nm)	
	(mol.%)	IgG	Fib	Alb	Ave.	STDEV	Ave.	STDEV	Ave.	STDEV
PL2-1	10	442	102	1	-0.11	0.10	19.3	8.3	0.41	0.29
PL2-2	30	561	169	12	0.63	0.17	0.4	0.3	0.27	0.11
PL2-3	50	815	433	234	0.13	0.06	0.3	0.3	N/A	N/A
PL5-65	0	465	21	2	0.31	N/A	0.0	N/A	0.13	N/A
PL5-66	-20		N/A		-0.52	N/A	51.3	N/A	0.15	N/A
PL5-67	20	390	46	9	0.69	0.12	0.5	0.5	0.46	0.26
PL5-68	0	13	11	1	-0.46	N/A	25.8	N/A	0.17	N/A
PL6-13	-10	3	6	1	-0.37	N/A	16.3	N/A	0.15	N/A
PL6-14	0	137	8	1	-0.34	N/A	40.1	N/A	0.28	N/A
PL6-15	10	164	9	1	0.92	0.14	0.6	0.4	0.44	0.11
PL6-26	10		N/A		0.01	N/A	6.5	N/A	0.20	N/A
PL6-28	20	414	29	2	1.11	N/A	1.4	N/A	1.59	N/A
PL6-30	30	616	109	13	0.66	N/A	0.5	N/A	2.83	N/A
PL6-32	40	464	236	43	0.30	N/A	4.6	N/A	0.77	N/A
PL6-36	30	548	88	22	0.32	N/A	3.7	N/A	0.24	N/A
PL6-38	10	319	12	2	0.61	N/A	0.5	N/A	0.28	N/A
PL6-40	20	458	77	15	0.55	N/A	1.4	N/A	0.23	N/A
PL6-42	10	722	62	10	0.27	N/A	1.4	N/A	0.31	N/A
PL6-44	-10		N/A		-0.40	N/A	31.0	N/A	0.21	N/A
PL6-45	-20	2	8	1	-0.12	N/A	15.3	N/A	0.31	N/A
PL6-46	-30	2	7	1	-0.24	N/A	25.5	N/A	0.19	N/A
PL6-47	-40	3	18	2	-0.55	N/A	49.1	N/A	0.23	N/A
PL6-49	-10	17	5	1	-0.24	N/A	13.0	N/A	0.22	N/A
PL6-51	0	377	15	2	0.19	N/A	0.5	N/A	0.17	N/A
PL6-52	-10	34	7	1	-0.12	N/A	15.3	N/A	0.21	N/A
PL6-53	0	624	23	2	0.41	N/A	3.7	N/A	0.26	N/A

* FACS measurement: Polymers was coated onto silica beads, and protein adsorption was investigated by FCM. The procedure was described in Chapter 6.5.1 – 6.5.5.

Table 6.5.9. Effect of polymer structure on blood filtration.

No.	Leuco-depletion (-log)		PLT recovery (%)		Homolysis (ABS at 576 nm)	
	Ave.	STDEV	Ave.	STDEV	Ave.	STDEV
PL6-3	0.92	0.14	0.61	0.43	0.44	0.11
PL6-6	0.67	0.08	0.84	0.61	0.31	0.04
PL6-9	0.13	N/A	0.41	N/A	0.24	N/A
PL6-12	0.39	N/A	1.27	N/A	0.16	N/A
PL6-15	0.00	N/A	0.58	N/A	0.13	N/A
PL6-18	0.49	0.23	0.21	0.30	0.15	0.03
PL6-21	-0.22	N/A	2.31	N/A	0.23	N/A
PL6-24	0.39	N/A	0.00	N/A	0.13	N/A
PL6-80	0.60	N/A	0.00	N/A	0.19	N/A
PL6-82	0.56	N/A	0.00	N/A	0.26	N/A
PL6-84	-0.04	N/A	2.45	N/A	0.14	N/A

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