Winter 2002

Improved swellable polymer microspheres for chemical sensing

Necati Kaval

University of New Hampshire, Durham

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IMPROVED SWELLABLE POLYMER MICROSPHERES
FOR CHEMICAL SENSING

By

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B.S., Hacettepe University, 1993
M.S., University of New Hampshire, 1998

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy

in

Chemistry

December, 2002
ACKNOWLEDGMENTS

First, I would like to express my sincere gratitude to Professor W. Rudolf Seitz for his invaluable mentoring, guidance, support and encouragements during my graduate study. I also wish to thank the other committee members: Professor Dennis Chasteen, Professor Sterling A. Tomellini, Professor Todd Gross and Professor Steven Levery for their time and assistance.

I want to thank other faculty members, especially Dr. Howard Mayne and Dr. Edward Wong, for their help and support. Also, I want to thank to Dr. Barry Lavine of the University of Clarkson for his support and guidance during his sabbatical at the University of New Hampshire.

I am grateful to my fellow group members for their friendship, collaboration and help.

Many thanks to Cindi Rohwer, Peggy Torch, Susan Higgins, Amy Lindsay, Kathy Gallagher, Sabrina Kirwan, Bob Constantine for their help and friendship.

I would like to thank Nancy Cherim and Art Anderson for technical and instrumental help.

Finally, I would like to acknowledge the Department of Chemistry and the University of New Hampshire for financial support.
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ABRAVIATIONS

AIBN 2,2-Azobisisobutyronitrile
BPO Benzoyl peroxide
DMAEMA 2-Dimethylaminoethyl methacrylate
DMPA 2,2-Dimethoxy-2-phenyl acetophenone
DVP Divinylbenzene
EGDMA Ethylene glycol dimethacrylate
HEMA 2-Hydroxyethyl methacrylate
LOD Limit of Detection
MMA Methyl methacrylate
PVA Poly(vinyl alcohol)
RI Refractive index
S Styrene
SDS Sodium dodecyl sulfate
SEM Scanning Electron Microscope
SPG Shirasu Porous Glass
TCPA 2,4,5-Trichloro phenylacrylate
VBC Vinylbenzyl chloride
4VP 4-Vinylpyridine
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ABSTRACT

IMPROVED SWELLABLE POLYMER MICROSPHERES
FOR CHEMICAL SENSING

By

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University of New Hampshire, December 2002

Derivatized lightly crosslinked polymer microspheres that swell and shrink as a function of pH have been investigated to develop polymeric transduction elements for fiber optic sensors and other sensors. The microspheres were immobilized in hydrogels forming a membrane. The membranes look turbid because of the difference between the refractive index of hydrogel and the refractive index of microspheres. As the microspheres swell as a function of pH, their refractive index decreases approaching the refractive index of the hydrogel. As a result, the turbidity of the membrane decreases. The change in the turbidity of the membrane was monitored by a UV-Vis-NIR spectrophotometer.

Optical measurements in our research involve turbidity, light reflection and light scattering. Therefore the conditions for turbidity and light scattering have been investigated. The turbidity of monodisperse polystyrene microspheres with diameters from 0.5 to 1.5 microns, suspended in water or embedded in hydrogels, has been studied. The turbidity increased with increasing particle size at longer wavelengths. At shorter wavelengths (i.e. shorter than 600 nm) the turbidity of smaller particle increased sharply.
while the turbidity of large particles decreased. The turbidity of microparticles increased with increasing particle concentration at all wavelengths. Scattered light intensity increases with decreasing particle size.

Microspheres of 2-(dimethylamine)ethyl methacrylate (DMAEMA) and 4-vinyl pyridine (4VP) were synthesized with various comonomers including 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA) and styrene. Microparticles made of DMAEMA and HEMA did not generate a measurable turbidity with pH changes. When MMA was added as a third monomer in the copolymer, swelling properties improved. Particles of DMAEMA, HEMA and MMA responded pH between 5.5 and 8. The apparent pKa of the membrane was 7.10 for decreasing pH and 7.24 for increasing pH. Membrane response times were less than 20 seconds.

Microspheres of 4VP-HEMA and 4VP-HEMA-Styrene were synthesized with 4VP contents from 10% to 50%. All formulations responded to pH between 3.5 and 5.5. The response time of 4VP-HEMA microparticles increased with increasing 4VP content but was less than 60 seconds for all formulations. Addition of styrene to 4VP-HEMA copolymer improved the performance of the microparticles. An approximately 50% decrease was observed in turbidity for all formulations.
CHAPTER 1

INTRODUCTION

1.1 Introduction to Sensors

In today's world the number of analyses conducted to acquire information about particular samples is increasing dramatically because there is a tremendous increase in the demand for analytical chemical information. It is thought that worldwide more than $10^9$ analyses are now performed every day - with a double-digit growth rate. Many complex systems are governed by time-dependent parameters, which makes it essential that as much information as possible be acquired within a particular period of time so that the various reactions taking place within the system can be followed, and the dynamics of the processes can be understood. Devices designed to permit continuous measurement of a quantity are called sensors. Various types of sensors have been developed for monitoring many important physical parameters, including temperature, pressure, speed, flow, etc. The number of potential interferences is rather limited in these cases, so it is not surprising that the corresponding sensors offer a level of reliability considerably higher than that for sensors that measure chemical quantities (i.e. the concentration of a certain species, the activity of an ion, or the partial pressure of a gaseous material).
1.2 Application Areas of Sensors

There are several major fields of application for chemical sensors. These include:

1. Environmental studies,
2. Biomedical analysis (especially for medical diagnostics),

In the field of *environmental analysis* there is a great demand for the type of continuous monitoring that only a sensor can provide, since the relevant parameter for toxicological risk assessment is always the *dose* (i.e., a concentration multiplied by an exposure time). The study of environmental chemistry also depends upon a continuous data output that provides not only baseline levels but also a reliable record of concentration bursts. *Data networks* can be used to make multiple determinations of a particular analyte regardless of whether these are acquired simultaneously or at different times within a three-dimensional sampling space (which may have dimensions on the order of miles), thereby providing information about concentration gradients and other factors essential for pinpointing the location of an emission source. The inevitable exponential increase in the number of chemical analyses associated with this need for maximum information threatens to create serious financial problems as well as ecological problems, since most classical analytical chemical techniques themselves produce a certain amount of chemical waste.

Modern societies are characterized by a population with a continuously increasing average age. This has stimulated the widespread *biomedical application of chemical sensors* for monitoring the health of the elderly without confining them to hospitals. Sensors for determining the concentrations of such key body substances as glucose,
creatinine, cholesterol, or blood gases could prove vitally important in controlling and maintaining personal health, at the same time enhancing the quality of the patient's life.

The same principle underlies sensor applications in intensive care wards. Sensors also offer the potential for significant cost reductions relative to conventional laboratory tests. They can even improve the quality of health because continuous monitoring can lower the risk of complications. For example, the monitoring of blood potassium levels can give early warning of the steady increase that often precedes an embolism, providing sufficient time for clinical countermeasures.

Another important field of application for chemical sensors is process control. Here the sensor is expected to deliver some crucial signal to the actuators (valves, pumps, etc.) that control the actual process. Fully automated process control is feasible with certain types of feedback circuits. Since key chemical parameters in many chemical or biochemical processes are not subject to sufficiently accurate direct determination by the human senses, sensor technology becomes the key to automatic process and quality control. Often the performance of an entire industrial process depends on the quality and reliability of the sensors employed. Successful adaptation of a batch process to flow-through reactor production technology is nearly impossible without chemical sensors for monitoring the input, the product quality, and also — an increasingly important consideration — the waste. Municipal incinerators would certainly be more acceptable if they offered sensor-based control of $\text{SO}_2$, $\text{NO}_x$, and, even more important, the most hazardous organic compounds, such as dioxins. Compared with the high construction costs for a plant complete with effective exhaust management, an extra investment in sensor research can certainly be justified.
These few examples illustrate the increasing importance of chemical sensor development as a key technology in various closed-looped sensor/actuator-based process control applications. Rapid, comprehensive, and reliable information regarding the chemical state of a system is indispensable in many high technology fields; for this reason there is also an increasing need for intelligent or "smart" sensors capable of controlling their own performance automatically.

1.3 Chemical Sensors

Until the introduction of the lambda (λ) probe in the 1980s for oxygen measurement in the exhaust systems of cars employing catalytic converters, the prototype of a successful chemical sensor was the electrochemical pH electrode, introduced over 50 years ago. This device can be used to illustrate essential features characterizing sensor performance, including reversibility, selectivity, sensitivity, linearity, dynamic working range, response time, and fouling conditions.

Potentiometric electrodes for chemical analysis have been known for over 110 years (the Nernst equation was published as early as 1889). They can, therefore, be considered as the forerunners of modern chemical sensors. In general, a chemical sensor is a small device placed directly on or in a sample and designed to produce an electrical signal that can be correlated in a specific way with the concentration of the analyte, the substance to be determined. If the analyte concentration varies in the sample under investigation, the sensor should faithfully follow the variation in both directions; i.e., it should function in a reversible way, and it should deliver analytical data at a rate greater than the rate of change of the system (quasi-reversible case). From the standpoint of
practical applications, complete and fast reversibility is relatively unimportant so long as measurements are acquired with sufficient rapidity compared with changes in the analyte concentration that are to be followed. Another restriction arises from the demand for a reagent-free measurement: an extremely selective or specific interaction between the analyte atom, ion, or molecule and the surface of a receptor-like sensor element must then be the sole basis for gaining information about the analyte concentration.

There are many different ways of classifying sensors. These include the mode of transduction, the application (clinical sensors, environmental sensors) or the size (micro, mini, macro, bench-type etc.). Sensor types classified by mode of transduction are potentiometric, amperometric, piezoelectric, thermal, mass, and optical.

1.3.1 Major Components of Chemical Sensors

The general construction principle of a chemical sensor is illustrated in Figure 1. The most important part is the sensing element (receptor) at which the molecular or ionic recognition process takes place, since this defines the overall selectivity of the entire sensor. The sensing-element surface is sometimes covered by an additional surface layer acting as a protective device to improve the lifetime and/or dynamic range of the sensor, or to prevent interfering substances from reaching the sensing-element surface. The analyte recognition process takes place either at the surface of the sensing element or in the bulk of the material, leading to a concentration-dependent change in some physical property that can be transformed into an electrical signal by the appropriate transducer.
Figure 1.1 Generalized scheme of the main elements of a sensor.
The transducer cannot itself improve the selectivity but it is responsible for the sensitivity of the sensor, and it functions together with the sensing element to establish a dynamic concentration range. The electrical signal of the transducer is usually amplified by a device positioned close to the transducer or even integrated into it.

If a biochemical mechanism (mostly enzymatic catalysis, immuno-chemical reaction or complementary DNA hybridization) is used in the molecular recognition step, the sensor is called a biosensor.¹

1.3.2 Key Features of Chemical Sensors

In order of decreasing importance, key features of chemical sensors are:

1. Selectivity
2. Limit of detection (LOD) for the analyte
3. Accuracy (more important than reproducibility)
4. Sensitivity (slope of the calibration curve)
5. Dynamic response range
6. Stability
7. Response time
8. Reliability (maintenance-free working time)
9. Lifetime

1.3.2.1 Selectivity

The selectivity of a chemical sensor toward the analyte is often expressed in terms of a dimension that compares the concentration of the corresponding interfering
substance with an analyte concentration that produces the same sensor signal. This factor is obtained by dividing the sensitivity (= slope of the calibration curve) of the sensing device toward the corresponding interfering substance by the sensitivity for the analyte. Typical selectivities range from $10^{-3}$ to $<10^{-12}$.

The selectivity is the most important parameter associated with a chemical sensor since it largely determines the trueness of the analytical method. Trueness (or accuracy) is more than simply precision, repeatability, or reproducibility: it represents agreement with the true content of the sample. This is the concentration found independent of the method, the time, the analyst, and the laboratory. All the other parameters mentioned are subject to systematic error, which can only be recognized if an analyte is determined by more than one method. Since selectivity is always limited, all chemical sensors are prone to report higher concentrations than a sample actually contains. In the case of environmental analysis this positive error can be regarded as a safety margin if relevant interferents are also present.

1.3.2.2 Limit of Detection (LOD) for the Analyte

In analytical chemistry the *limit of detection* (LOD) is exceeded when the signal of the analyte reaches at least three times the general noise level for the reading. No quantitative measurement is possible at this low concentration; only the presence of the analyte can be assumed with a high probability (> 99 %) in this concentration range. The lower range of quantification ends at ten times the LOD. Therefore, the LOD of an analytical instrument must always be ten times smaller than the lowest concentration to be quantified. In some cases with extremely sensitive sensors a zero-point calibration
might be difficult to perform, because traces of the analyte would always be present or might easily be carried into the calibration process by solvents or reagents. The lowest measurable level is then called the blank value. The LOD is then defined as three times the standard deviation of the blank value, expressed in concentration units (not in the units of the signal, as is sometimes improperly suggested). Normally the LOD becomes worse as a sensor ages.

1.3.2.3 Sensitivity

As mentioned above, the sensitivity of a sensor is defined by the signal it generates, expressed in the concentration units of the substance measured. This corresponds to the slope of the corresponding calibration curve when the substance is the analyte, or to the so-called response curve for interferents. With some sensors the sensitivity rises to a maximum during the device's lifetime. A check of the sensitivity is therefore a valuable quality-assessment step. Intelligent sensors are expected to carry out such checks automatically in the course of routine performance tests. Since in most cases the sensitivity depends on such other parameters as the sample matrix, temperature, pressure, and humidity, certain precautionary measurements are necessary to ensure that all these parameters remain constant both during calibration and in the analysis of real samples.

1.3.2.4 Dynamic Response Range

The dynamic response range is the concentration range over which a calibration curve can be described by a single mathematical equation. A potentiometric sensing
device follows a logarithmic relationship, while amperometric and most other
electrochemical sensors display linear relationships. Both types of signal-to-
concentration relationship are possible with optical sensors; in absorption measurements
it is the absorbance with its logarithmic base that is the determining factor, whereas a
fluorescence measurement can be described by a linearized function. The broader the
concentration range subject to measurement with a given sensing system, the less
important are dilution or enrichment steps during sample preparation. The measurement
range is limited by the LOD at the low concentration end, and by saturation effects at the
highest levels. A good chemical sensor should function over at least one or two
concentration decades. Sensors with excellent performance characteristics include the
lambda oxygen probe and the glass pH electrode, both of which cover analyte
concentration ranges exceeding twelve decades.

### 1.3.2.5 Stability

Several types of signal variation are associated with sensors. If the signal is found
to vary slowly in two directions it is unlikely to be regarded as having acceptable stability
and reproducibility, especially since it would probably not be subject to electronic
correction.

Output variation in a single direction is called drift. A steadily drifting signal can
be caused by a drifting zero point (if no analyte is present) and/or by changes in sensor
sensitivity (i.e., changes in the slope of the calibration curve). Drift in the sensor zero-
concentration signal (zero-point drift) can be corrected by comparison with a signal
produced by a sensor from the same production batch that has been immersed in an analyte-free solution.

The stability of a chemical sensor is usually subject to a significant aging process. In the course of aging most sensors lose some of their selectivity, sensitivity, and stability. Some sensors, like the glass pH electrode, can be rejuvenated, while others must be replaced if certain specifications are no longer fulfilled.

1.3.2.6 Response Time

The *response time* is not defined in an exact way. Some manufacturers prefer to specify the time interval over which a signal reaches 90% of its final value after a ten-fold concentration increase, while others sometimes prefer the 95% or even 99% level. The particular percentage value chosen represents a pragmatic decision, since most signal-time curves follow an exponential increase of the form:

\[
\text{signal} \times (1 - e^{-kt})
\]

where the true final value is unknown and/or will never be reached in a mathematical sense. Therefore, specification in terms of the time constant \( k \) is clearer. This corresponds to the time required for a signal to reach about 67% of its final value, which can be ascertained without waiting for a final reading: as the slope of the curve \( \ln(\text{signal}) \) vs. time. If the response is constant and independent of the sample matrix, this equation can be used to calculate a final reading immediately after the sensor has been introduced into the sample.

Typical response times for chemical sensors are in the range of seconds, but some biosensors require several minutes to reach a final reading. The response time for a
sensor is generally greater for a decreasing analyte concentration than for an increasing concentration. This effect is more pronounced in liquids than in gaseous samples. Both the surface roughness of the sensor and/or the dead volume of the measuring cell have some influence on the response time. Small cracks in the walls of a measuring cell can function as analyte reservoirs and diminish the rate of analyte dilution.

With certain sensors the response time can also depend on the sample matrix. In the presence of strongly interfering substances the response time for a chemical sensor might increase as a result of an increase in the time required to reach final equilibrium.

1.3.2.7 Reliability

There are various kinds of reliabilities. One involves the degree of trust that can be placed in an analytical result delivered by a particular chemical sensor. Another is a function of the real time during which the sensor actually performs satisfactorily without a breakdown and/or need for repair.

There is no way to judge fairly the analytical reliability of a chemical sensor, since this depends strongly upon the expert ability of the analyst to choose a suitable sensor and a suitable sample preparation routine in order to circumvent predictable problems. With respect to the first generation of sensors, the ion-selective electrodes, the experience of many users has led to the following "reliability hit list", with the most reliable devices cited first: glass pH electrodes, followed by fluoride, sodium glass, valinomycin-based potassium, sulfide, and iodide electrodes, and culminating in the electrodes for divalent ions. Reliability can be improved considerably if analytical conclusions are based on measurements obtained by different methods. For example, it is
possible to determine sodium either with an appropriate glass-membrane electrode or with a neutral carrier-based membrane electrode. If both give the same analytical results, the probability of a systematic error is very low.

The length of time over which a chemical sensor can be expected to function reliably can be remarkably great (in the range of years), as in the case of glass-membrane or solid-state membrane electrodes, the lambda probe, and the Taguchi gas sensor. On the other hand, a biosensor that depends upon a cascade of enzymes to produce an analyte signal will usually have a short span of proper functioning (a few days only).

Potentiometric ion-selective membrane electrodes and optical chemosensors have lifetimes of several months. In the case of biosensors it should be noted that anything that changes the quaternary space-orientation of the recognition biomolecules will destroy the proper functioning of the sensor. Enzymes can be influenced by such factors as pH, certain heavy-metal ions, certain inhibitors, and high temperature (resulting in denaturation).

1.3.2.8 Lifetime

Ion-selective electrodes and optical sensors based on membrane-bound recognition molecules often lose their ability to function by a leaching-out effect. In optical sensors the photobleaching effect may also reduce the lifetime to less than a year. On the other hand, amperometric cells work well for many years, albeit with restricted selectivities. Problems associated with inadequate lifetimes are best overcome with mass-produced miniaturized replacement sensors based on inexpensive materials. Minimizing replacement costs may well represent the future of biosensors.
1.4 Instrumentation

The instrumentation associated with chemical sensors is rather simple and inexpensive, especially in the case of electrochemical transducers. All that is normally required is a high-ohmic voltmeter (pH meter), a conductometer, and/or polarographic equipment. Fiber-optic devices, on the other hand, can be quite expensive depending on quality requirements with respect to the monochromatic light source. However, developments in optical fiber communication technology have dramatically reduced the cost. Spectrophotometers with optical fiber inputs and outputs are already commercially available.

The highest costs are usually related to development of a selective analyte recognition layer, perhaps the most important part of a complete chemical sensor. Transducer technology is much more highly developed; indeed, there seems to be little need here for further improvement without equivalent improvements in the selectivity of sensor elements. It should be noted that the development costs associated with chemical sensors are typically many orders of magnitude higher than those for a sensor measuring a physical quantity. A satisfactory return on investment therefore presupposes either mass production in the case of low-cost sensors or special applications that would justify an expensive sensor.

If extensive electrical wiring is required between the sensor and the readout – control unit, the electrical signal might also be digitized as one way of minimizing noise pick-up during signal transmission. Several chemical signals produced by multiple sensing elements, each connected to its own specific transducer, can be transmitted via
modern multiplexing techniques, which require only a two-wire electrical connection to
the control unit also delivering the electrical power.

1.5 Polymers for Sensor Applications

Polymeric materials are gaining increasing interest as sensitive, selective, and
stable layers of chemical sensors for the detection of ions, neutral molecules and, in
particular, inorganic and organic gases. Generally, these materials are based on a
variety of functional polymers, preferentially containing a backbone with a high
solubilizing power. The main advantage of these materials is the high flexibility for
tailoring recognition structures by controlled chemical synthesis and by thin layer
formation.

Sensors such as surface acoustic wave devices or electrochemical systems achieve
practical sensitivity by coating these materials with polymers at which adsorption or
absorption occur. These processes increase selectivity by discriminating against
interferents. In addition, polymer films can be used to prevent fouling and to immobilize
enzymes or chelators.

Several recent reviews have appeared that address the topic of polymer coatings
on amperometric devices. Sensors based on solid polymer electrolytes (SPEs) utilize
the polymer as a supporting electrolyte for use in nonconducting media or the gas phase.
Nafion is the most common in SPE. It is a perfluorinated polymer consisting of a
tetrafluoroethylene backbone with perfluorinated vinyl ether side chains that terminate
with sulfonic acid groups. The use of Nafion in amperometric sensors exploits its proton
conductivity, water diffusivity, gas permeability (CO, CO_{2} and O_{2}), and chemical and
electrochemical inertness. A sensor for NO in physiological media was reported by Malinski and Taha.\textsuperscript{21} Nafion was used to discriminate against anionic interferents such as NO\textsubscript{2}\textsuperscript{-}.

An amperometric sensor for glucose and lactose was constructed by Liu et al.\textsuperscript{22} using a β-cyclodextrin polymer. Holtz and Asher\textsuperscript{23} fabricated biosensors from crystalline colloidal arrays polymerized in a solid matrix (PCCA). The PCCAs were doped with molecular recognition groups that bind an analyte selectively (such as crown ethers for metal ions) or molecular-recognition agents that react specifically to a single analyte (enzymes). The detection of oxygen by photoluminescent quenching of platinum octoethylporphyrin was demonstrated by Lee and Okura.\textsuperscript{24} The porphyrin-polymer film was excited at 535 nm, and the luminescence was monitored at 646 nm. Polyvinyl chloride, polystyrene and silicone polymer (GP-197) were mixed with the porphyrin using a cosolvent. The sensor responded to concentrations of oxygen down to 3% by volume and was stable for one year.

Conjugated polymers such as poly (phenylene vinylene) (PPV) and its soluble derivatives are known to exhibit photoluminescence with high quantum efficiency.\textsuperscript{25} Very recently, it was reported that the luminescence of a semiconducting polymer in aqueous solution can be quenched by using extremely low concentration of a cationic electron acceptor.\textsuperscript{26}

\textbf{1.6 Chemical Sensors Based On Polymer Swelling}

One of the useful properties of lightly crosslinked polymers is that they can swell and shrink in response to the changes in the environment. This phenomenon has been
described by Tanaka. Although there is considerable amount of research on the use of swellable polymers for controlled drug delivery systems, sensors based on reversible swelling of polymers are a relatively new concept and have not matured enough for widespread commercial applications. One of the most successful applications is humidity sensors which involve swelling of hydrophilic polymers.

Our group and others have been investigating swellable polymers for their potential chemical and biological sensor applications. Our interest in this area first started with commercial ion exchange resins in early 1990s for detecting ionic strength of aqueous solutions. This earlier sensor (Figure 1.2) used the size change of a polymer to displace a reflective diaphragm, affecting the amount of light reflected into an optical fiber. The lifetime of this sensor was short because the beads were highly crosslinked and subject to cracking after repeated swelling and shrinking cycles. We decided to make our own beads so that we could control their physical and chemical properties to get better performance. Poly(vinyl benzyl chloride) (Poly-VBC) has been investigated because vinyl benzyl chloride monomer can be polymerized easily by various polymerization methods. Furthermore it can be easily derivatized before or after polymerization to introduce functionality for sensing. The derivatized poly-VBC
particles did not swell with enough force to move the diaphragm in the sensor. As an alternative to optical displacement, polymer membranes bound to a substrate were investigated. In these systems (Figure 1.3), the intensity of light reflected from the polymer-substrate interface is measured. As the polymer, in contact with the analyte, swells and shrinks the reflected light intensity changes because of the change in the refractive index of the polymer. The problem associated with these two systems was delamination of the polymer from the surface due to the shear forces at the polymer substrate interface. However, it was observed during these studies that derivatized poly-VBC beads become clearer as they swelled due to the dilution of the polymer with water uptake. This change was reversible. When the polymer shrunk, it became opaque again. This observation resulted in a new sensing scheme (Figure 1.4). Derivatized poly-VBC beads were suspended in a hydrogel, forming a cloudy membrane, and the intensity of the

Figure 1.3 Fiber optic sensor based on change in reflection accompanying polymer swelling.

Figure 1.4 Swellable microspheres embedded in a hydrogel forming membrane for chemical sensing
light scattered in the membrane was measured. As the beads in the hydrogel swell and
shrink their refractive index changed, causing a change in the opacity of the membrane.
This system has been investigated for several years. Different polymerization methods
and formulations have been tested to improve the sensing system.

A diagram of fiber optic sensor system built and developed by our group
members for microsphere immobilized membranes is shown in Figure 1.5. This
instrument was designed to monitor the change in reflectance at the distal end of two long
lengths of optical fibers. Some portion of the light pulses that reach the membrane at the
tip of the indicator fiber is reflected back by the fiber-membrane interface, and some
other portion is reflected back by the particles embedded in the membrane (Figure 1.5b).
The former is unwanted background noise and the latter is the analytical signal. A pulse
of light from the diode laser is split by the 2x2 coupler and is directed into two optical
fibers with different lengths. The light pulse travels to the tip of the each fiber and the
reflected light from each fiber reaches the detector at different times. The transit time is
approximately 3μs for the reference fiber and 5μs for the indicator fiber. Milde
discovered that by polishing the fiber tip at an angle (ca. 8 degree) before attaching the
membrane, the signal to background ratio can be greatly improved.

Besides optical sensors, Doherty and Liu investigated a new type of magnetic
sensor based on polymer swelling in our group. First developed by Grimes, this new
sensor has the advantage that no physical connection between the sensing element and
the detection electronics is required. Furthermore, the sensing element does not require
an external power source. This allows the sensor to be placed in enclosed or opaque
containers, which are completely isolated from the outside environment. The sensing
**Figure 1.5** Schematic diagram of fiber optic sensor based on polymer swelling. (Figure 1.5b: $I_s$ is light reflection from the particles embedded in hydrogel and $I_f$ is light reflection from the fiber-membrane interface.)
element consists of swellable microparticles coated on a magnetic strip. The strips mechanically vibrate at a characteristic frequency that varies with the mass and viscosity of the coating. As the microspheres swell, the characteristic frequency shifts.

1.7. Objectives

The goal of my research was to develop and improve swellable polymer microspheres for optical pH sensing. Since the optical measurements in our research involve turbidity measurements, my first project was focused on investigation of the conditions for turbidity measurements. This work has been performed using polystyrene microspheres suspended in water or embedded in various hydrogels. The research also involved measurements of light scattering by microspheres in hydrogel membranes.

The second project was to develop swellable copolymer microspheres by varying the functional monomer concentration. The functional monomers, 2-(dimethylamino)ethyl methacrylate and 4-vinyl pyridine, are sensitive to pH. Protonation introduces a charge on the polymer causing the microspheres to swell. The project involves dispersion copolymerization of the functional monomers with various comonomers. The goal was to improve the swelling properties of the copolymer by varying the type and the concentration of the functional monomer.

The third project was to investigate a new emulsification method, called Shirasu Porous Glass (SPG) emulsification, to synthesize monodisperse microspheres by suspension polymerization. The suspension polymerization method requires monomer/water emulsification prior to polymerization. Emulsification of monomers in water in the traditional way is carried out by vigorously stirring the monomer/water.
mixture. This process produces monomer droplets in a very wide size distribution varying from a few micrometers to a few millimeters. However, we want to make uniform microspheres a few micrometers in diameter. SPG membranes are made of porous glass with uniform pore sizes. Emulsification is carried out by forcing the monomer to permeate through the membrane into water. We expected to make monodisperse microparticles by this method.
CHAPTER 2

THEORY

Our current research involves synthesis of swellable polymer microspheres and evaluation of the microspheres for optical chemical sensing. This chapter will briefly discuss the background and the theory of these processes by covering free radical polymerization, dispersion and suspension polymerization methods, the theory of polymer swelling and finally optical properties of polymers.

2.1 Free Radical Polymerization

This section provides a basic introduction to free radical polymerization, because both the dispersion and suspension polymerization techniques employed in this research involve free radical polymerization.

Free radical polymerization is considered a chain polymerization or an addition polymerization, which involves the linking together of the small molecules of vinyl monomers to form a long chain polymer molecule. This process can be described as

\[ nM \rightarrow \{M\}_n \]

where \( M \) represents both a molecule of monomer and a monomer unit in the polymer chain. In a chain polymerization, the polymer grows by reaction of monomer molecule with the end group on the growing chain. Monomers for addition polymerization usually contain double bonds, which are called vinyl groups.
Free radicals are species that contain an unpaired electron and are denoted as $R^\bullet$. They are extremely reactive and will react with monomers containing a double bond to form an active center that is capable of reacting with further monomer molecules to give a macromolecular chain. In order for an initiator molecule to be effective, it must be capable of breaking down into radicals which are stable long enough to react with a monomer molecule and form an active center.

The whole free radical polymerization process is divided into three distinct stages: initiation, propagation and termination.

**Initiation**

Initiation of free radical polymerization requires the production of free radicals in the presence of unsaturated monomers. Although the free radicals can be initiated directly from the monomer (by irradiating with high-energy radiation, for example), the most common method is to add a small amount of initiator reagent. Such an initiator is usually a molecule that can be decomposed thermally or photolytically to yield a pair of initiating radicals. Commonly used initiators are 2,2'-azo-bis-isobutyronitrile (AIBN) and benzoyl peroxide (BPO), because of their convenient decomposition temperature range, (50-100 °C). Decomposition temperature varies with the type of monomer and solvent. The initiation mechanisms of AIBN (Figure 2.1a) and BPO (Figure 2.1b) are shown below.

Free radicals formed from the initiator system are known as primary free radicals and must react with monomer to initiate polymerization.
Propagation

Chain propagation involves the addition of a free radical to the double bond of a monomer molecule. The product must itself be a free radical, and the process can then be repeated. The average lifetimes of growing chains are extremely short, and several thousand additions can take place within a few seconds. This means that there may be a monomer addition every few milliseconds to each growing chain. The propagation step of styrene polymerization is shown in Figure 2.2.
The most likely monomer addition is *head-to-tail* (Figure 2.3a) reaction. Alternatively, the reaction may involve a *head-to-head* (Figure 2.3b) or a *tail-to-tail* (Figure 2.3c) reaction.

**Figure 2.2** Propagation in free radical polymerization of styrene.

**Figure 2.3** Types of monomer addition in free radical polymerization.

**Termination**

Free radical chains can be terminated by reaction of a growing polymer radical with some other free radical in the system. Combination (Figure 2.4a) and disproportionation (Figure 2.4b) are the two most common termination reactions.
Combination involves the coupling together of growing chains to form a single polymer molecule.

Termination by combination can bring about a considerable increase in the molecular weight of the final polymer. Termination by disproportionation occurs by transfer of an atom (usually hydrogen) from one polymer radical to another resulting in the formation of two polymer molecules, one with a saturated end-group and the other with an unsaturated end-group.

![Figure 2.4](image)

**Figure 2.4** Types of termination in free radical polymerization. (a) Combination and (b) disproportionation.

In addition to combination and disproportionation, there are a number of other reactions that contribute to termination. These reactions are collectively known as chain transfer reactions and require the addition of a chain transfer agent into the reaction medium. This process results in decreased chain length in accordance with the concentration of chain transfer agent.

### 2.2 Copolymerization

In our research, almost all the microparticles we synthesize are copolymers. Incorporating a second monomer in the system allows us to control the physical and
chemical properties of microspheres. Some of properties that we wish to control are porosity, hydrophilicity, and chemical functionality (e.g. acidity).

A copolymer contains more than one type of monomer. Besides affecting the chemical and physical properties, the inclusion of a second monomer greatly complicates the polymerization kinetics. This brings additional requirements, the most important being the need to understand how differences in monomer reactivity affect copolymer composition and the sequence distribution of the different repeat units in the copolymer molecules formed.

The reactivity of a monomer is strongly dependent upon the ability of its substituent group(s) to stabilize the corresponding polymeric radical. The more reactive monomers have substituent groups that stabilize the polymeric radical by delocalization of the unpaired electron (i.e. by resonance). Hence, a monomer of high reactivity yields a polymeric radical of low reactivity and visa versa. The order in which common substituent groups provide increasing resonance stabilization is

$$\text{Ph} > -\text{CH}=\text{CH}_2 > -\text{CO-R} > -\text{C}=\text{N} > -\text{CO-OR} > -\text{Cl} > -\text{R} > -\text{OCO-R} > -\text{OR}$$

where R is an alkyl group.

When two monomers ($M_1$ and $M_2$) react to form a copolymer, the relation between the composition of the initially formed copolymer and the initial monomer mixture is given by

$$\frac{dm_1}{dm_2} = \frac{M_1(r_1M_1 + M_2)}{M_2(r_2M_2 + M_1)}$$
Where, \( m_1 \) = the molecules of monomer 1 entering the copolymer
\( m_2 \) = the molecules of monomer 2 entering the copolymer
\( M_1 \) = the molecules of monomer 1 in the monomer mixture
\( M_2 \) = the molecules of monomer 2 in the monomer mixture

Monomer reactivity ratios \( r_1 \) and \( r_2 \) for any monomer pair are the ratios of the rate constants of the different propagation reactions.

\[
\begin{align*}
\text{-} \text{M}_1^\cdot + M_1 & \rightarrow \text{-} \text{M}_1^\cdot M_1^\cdot \quad \text{for } k_{11} \\
\text{-} \text{M}_1^\cdot + M_2 & \rightarrow \text{-} \text{M}_1^\cdot M_2^\cdot \quad \text{for } k_{12} \\
\text{-} \text{M}_2^\cdot + M_2 & \rightarrow \text{-} \text{M}_2^\cdot M_2^\cdot \quad \text{for } k_{22} \\
\text{-} \text{M}_2^\cdot + M_1 & \rightarrow \text{-} \text{M}_2^\cdot M_1^\cdot \quad \text{for } k_{21}
\end{align*}
\]

where \( \text{-} \text{M}^\cdot \) represents a polymer chain ending in a radical derived from a monomer.

The reactivity ratios, \( r \), are the ratios of the reactivity of each species with its own monomer to its reactivity with the other type of monomer. Reactivity ratios of the monomers \( M_1 \) and \( M_2 \) are expressed as

\[
\begin{align*}
  r_1 &= \frac{k_{11}}{k_{12}} \quad \text{and} \quad r_2 = \frac{k_{22}}{k_{21}}
\end{align*}
\]

The structure of the polymer produced can be controlled both by the composition of the feed and the reactivity ratios. In the case of \( r_1 \equiv r_2 \equiv 1 \), there is no preference for either type of monomer to add onto a particular type of active center. The copolymer produced will therefore have entirely random distribution of monomer units. When the reactivity ratios are of the order of unity, it means that \( k_{11} \equiv k_{21} \) and \( k_{22} \equiv k_{21} \).
Copolymers that have completely random sequences of monomer units are known as *ideal copolymers*.

In the case of $r_1 \equiv r_2 \equiv 0$, $k_{11}$ and $k_{22}$ are very small and it means there is no tendency for the monomer to homopolymerize, but they may form a copolymer. In the copolymer, the two types of monomer units must therefore alternate along the polymer chain leading to a completely alternating copolymer.

In the case of $r_1 >> 1$ and $r_2 << 1$, the monomer $M_1$ is incorporated in the copolymer at a much greater rate than $M_2$ and so the polymer produced has long sequences of $M_1$ interspersed with a few $M_2$ units. However, things change towards the end of the reaction since the concentration of $M_1$ decreases at a much greater rate than the concentration of $M_2$, and so longer sequences of $M_2$ are found in the latter stages of reaction.

Normally reactivity ratios lie between 0 and 1, so there is usually a tendency toward alternation in most copolymerization reactions.

### 2.3. Monodisperse Polymer Microspheres

Monodisperse microspheres of known size have many applications in research and industry, including instrument calibration standards, standards for the determination of pore size and the efficiency of filters, column packing materials for chromatography, support materials for biochemicals, and additives in the coating and paint industry.$^{32,33}$

Synthesis of highly monodisperse submicron (nano) particles is relatively easy by emulsion or dispersion polymerization techniques. The larger particles are usually prepared by the suspension or the seeded polymerization methods. Seeded
polymerizations are two-step procedures in which particles obtained by emulsion or dispersion polymerization are swollen with additional monomer and polymerized again. Suspension polymerization produces particles with a broad size distribution. Therefore, the method requires post-polymerization procedures to isolate particles with the desired size. Ugelstad developed a two step suspension polymerization method to prepare very uniform particles by using the particles obtained in the emulsion polymerization as the seed. Okuba et.al. proposed a dynamic swelling method to prepare large particles with diameters around 7 μm by using the particles obtained in the dispersion polymerization as the seed. Both of these techniques are called two-step seed polymerization. In the first step, the seeds are produced by either emulsion polymerization or dispersion polymerization. In the second step, the seeds are allowed to swell in their monomer to the desired size, and the swollen seeds are then polymerized by the suspension polymerization. The swelling techniques can provide very uniform particles. However, these techniques are labor-intensive multi step procedures that may fail to yield the desired product.

The ideal particle size in our current research is on the order of 1-2 micrometer. To synthesize microparticles in this range we employ suspension and dispersion polymerization methods. The latter is easy and provides satisfactory particles. However, as indicated before, the suspension polymerization method produces large and highly polydisperse particles. On the other hand, the two-step seed polymerizations are time consuming and labor intensive. Therefore, we evaluated an alternative method to prepare large monodisperse particles in one step, combining the SPG (Shirasu Porous Glass).
membrane emulsification technique with suspension polymerization. Both dispersion and the SPG techniques will be discussed in the following sections.

2.4. Suspension Polymerization

Suspension polymerization is similar to bulk polymerization. It could be considered "bulk polymerization within a droplet." The speed at which reactions take place at a given temperature is the same as for bulk polymerization. For suspension polymerization, there are two phases, water and organic, typically 10 parts water and 1 part monomer. There are two separate phases throughout the whole process. The water insoluble initiator is dissolved in the monomer, and the solution suspended as droplets in an aqueous medium using continuous vigorous agitation. The low viscosity and the high thermal conductivity of the aqueous dispersion medium, together with the high surface area to volume ratio of the droplets, facilitate good heat transfer. Suspending agents (usually called stabilizers or surfactants) such as polyvinyl alcohol, methylcellulose or other water-soluble polymers are used to keep the monomer droplets in a state of suspension. Continuous mechanical agitation is required to maintain the liquid in suspension. For polymers that are soluble in their monomer, an interruption in stirring during polymerization, or excessive or insufficient stirring, may lead to agglomeration of the droplets when they are in a partially polymerized, tacky state. An insufficiency of suspending agent will also permit agglomeration, even for polymers insoluble in their monomer.

Particle size in suspension polymerization is affected by the following four factors:
1. Stirring rate
2. Ratio of reactants
3. Suspending agent
4. Temperature

These four parameters must be controlled carefully to produce uniform particles with low size distribution. Diameters typically fall between 0.01 to 0.5 cm, but may be as small as 1 micron.

In conventional suspension preparations, high-speed rotor systems or high-pressure dispersing systems are used to break down the monomer into droplets. These techniques produce emulsions with considerable polydisperse. The formation of emulsions with a narrow droplet size distribution is highly desirable, but quite difficult to accomplish. The production of uniform emulsion droplets requires that large mechanical energy be introduced into the system. In our research, we employed a newly developed emulsification technique known as Shirasu Porous Glass (SPG) Membrane Emulsification. In the membrane emulsification, the applied shear stress is far smaller than for conventional technique, because the droplets are produced in a different way.

2.5. Principle of Membrane Emulsification

The membrane emulsification method uses the surface chemistry of a microporous membrane to disperse one of two immiscible liquids into another liquid by applying pressure to cause the dispersion phase to permeate through the membrane. This method makes it possible to produce continuously not only oil in water (O/W) or water in oil (W/O) emulsion of uniform particle size, but also O/W/O or W/O/W type double
emulsions.

The major components of the SPG membrane emulsification apparatus (Figure 2.5) include a pressure-tight glass (or stainless-steel) monomer tank connected to a nitrogen gas tank, a membrane module which is a stainless-steel pressure-tight cylinder containing a cylindrical porous glass membrane, and a pump equipped with tubing, valves and pressure gauges.

![Diagram of SPG Membrane Emulsification Apparatus]

**Figure 2.5 SPG Membrane emulsification apparatus.**

The dispersion phase (mixture of monomer, solvent and initiator) is stored in the monomer tank and allowed to permeate through the glass membrane under appropriate pressure into the circulating flow. The droplets are suspended in the continuous phase (aqueous solution of stabilizers and surfactants). The suspension that forms is stored in the emulsion tank with a gradual increase of droplet concentration.

The three requirements given below must be met absolutely for the successful preparation of such emulsions by membrane emulsification.
1. The microporous membrane must provide uniform pore size distribution as well as reasonable mechanical strength.

2. The membrane must be wetted with the continuous phase prior to contact with the dispersion phase. Membrane wetting with the dispersion phase must be avoided.

3. To stabilize the emulsion, a surfactant must be added to both the dispersion phase and the continuous phase or just to the latter.

The formation of droplets by membrane emulsification (Figure 2.6.) can be explained as follows. When the dispersion phase is pressurized and penetrates into the micropores, the following equation is derived from the pressure critical, $P_c$, and the micropore size $D_m$.

$$P_c = 4 \gamma_{ow} \cos \theta / D_m$$

![Diagram showing dispersion phase intrusion into the micropore and formation of droplets.](image)

- $P_a < P_c$
- $P_a = P_c$
- $P_a > P_c$

$P_a$ : Applied pressure  
$P_c$ : Critical pressure

**Figure 2.6** Dispersion phase intrusion into the micropore and formation of droplets.
\( \gamma_{ow} \) represents the interfacial tension of the O/W interface and \( \theta \) represents the oil contact angle with the water surface.\(^5^9\)

As shown in the above figure, the dispersion phase does not permeate through the membrane when the pressure \( P \) is lower than \( P_c \). However, at the moment when \( P \) has exceeded \( P_c \), it is able to pass through the micropores for the first time, then creating a dispersion of droplets into the continuous phase. Thus, \( P_c \) is the minimum pressure where one can observe the flux of the dispersion phase and is called the critical pressure. Equation (1) also reveals that higher pressure is required as the micropore size of the membrane becomes smaller, and that the critical pressure can be reduced by reducing the interfacial tension.

The experimental procedure used to prepare emulsions by the SPG technique is covered in Chapter 3. More detailed information on the membrane emulsification technique can be found in the literature.\(^5^9\)

**2.6 Dispersion Polymerization**

Dispersion polymerization is a technique to produce polymer particles in a narrow size distribution with diameters in the range of 0.1-15 micrometer.\(^6^5-6^7\) The method, a modified precipitation polymerization, was invented by Osmond and coworkers, and has reviewed by Barret up to 1975.\(^6^8\) The process is basically the polymerization of a monomer in the presence of a dispersing agent called a stabilizer or surfactant. The main components are monomer, solvent, initiator and steric stabilizer.\(^5^5-6^8\)

The monomer must be soluble in the reaction mixture while its resulting polymer is insoluble. There is a wide range of monomers, both water-soluble and oil-soluble, that
satisfy this requirement. Styrene and methyl methacrylate are the monomers that have been most commonly investigated by researchers studying the mechanism of the method. Dispersion copolymerization is also possible; however, there are fewer examples of this in the literature. Most of our research has involved in copolymerization to control the hydrophilicity, porosity and the reagent density of the beads. *The stabilizer plays an important role in dispersion polymerization*. Steric stabilizers are preferred because the usual electrostatic stabilization (as in emulsion and suspension polymerizations) does not apply in dispersion polymerization, due to the low dielectric constant of the solvents used. Steric stabilizers contain two segments; one segment has an affinity for the polymer while the other has affinity for the solvent. Stabilizers can be classified into three groups: homopolymers, block copolymers, and macromonomers. The first two groups of polymers are the most often used because they have two chemically distinct segments. On the other hand, macromonomers can rapidly react with the principal monomer to generate graft copolymer species, which grow to form bead particles. In early work, petroleum distillates were used as the *solvent* in dispersion polymerizations. However, recent trends are to use polar solvents such as ethanol and methanol. Some less polar or non-polar solvents may be added as a cosolvent to control the particle size. *The initiator* must be completely soluble in the medium, like all other ingredients. The most common initiators are azobis(isobutyronitrile) (AIBN) and benzoyl peroxide (BPO). Dispersion polymerizations are usually carried out with free radical polymerization; however, anionic and cationic dispersion polymerizations are also possible.
2.7 Mechanism of Particle Formation in Dispersion Polymerization \textsuperscript{68,69}

A schematic representation of the mechanism is given in Figure 2.7. At the beginning, there is only a homogeneous solution of monomers, initiator and stabilizer (a). In the second step, the reaction starts with a free radical formation by thermal decomposition of the initiator. Oligomers appear and grow until reaching a critical length (b). At the same time, grafting with the stabilizer begins and particles start to precipitate from solution to form particle nuclei (c). Until this point, the solution remains clear and homogeneous. Once nucleation occurs, the solution becomes cloudy. Then, particles grow with diffusional capture of more oligomer and monomer (d), and this process concludes with the termination of radicals in the particles. Stabilizer stabilizes the particles by surrounding them at the interfacial region (e).
a) All reagents are dissolved in the solvent. Polymerization is initiated by increasing the temperature.

b) Oligomers have formed and the chains are growing. The solution is still clear. There is no phase separation.

c) Nucleation stage. Phase separation begins and the solution becomes milky.

d) Particles grow by diffusional capture of other oligomers and monomers.

e) Particles reach their final size, and stay suspended with the help of the stabilizer.

Figure 2.7 Schematic depiction of particle growth in dispersion polymerization
2.8 Polymer Swelling

When placed in a suitable solvent, a lightly crosslinked polymer will absorb a portion of the solvent and swell, rather than dissolving completely. The swollen gel can be characterized as a solution, although it is an elastic rather than a viscous solution. The extent of swelling represents a competition between two forces: (1) the free energy of mixing will cause the solvent to penetrate and try to dilute the polymer solution, (2) as the polymer chains in the crosslinked polymer network begin to elongate under the swelling action of the solvent, they generate an elastic retractive force in opposition to this deformation. The degree of swelling also increases significantly when there are fixed charges on the polymer network. The volumetric swelling reaches steady state when the two forces balance each other. This is called the swelling equilibrium. Equilibrium is determined by interaction between the gel and the solvent, and influenced by various factors such as temperature, solvent composition, pH, hydrostatic pressure, light and external electric or magnetic field. Furthermore, depending on the network and solvent, a volumetric phase transition may occur. The volume can change as much as 10-100 times at the phase transition point, responding to even a slight change of external conditions. There are no solids other than gels that show such phenomena.

The swelling behavior of crosslinked polymers has led to their widespread application in drug delivery, molecular separation systems, biomedical devices including implants and contact lenses, and in emerging technologies such as gel-based valves and actuators, sensors, artificial muscles and display devices. 

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2.8.1 Thermodynamics of Polymer Swelling

The first molecular description of gel swelling was that of Flory and Rehner, who suggested that the change in Helmholtz free energy of a polymer gel upon swelling could be expressed as the sum of a polymer-solvent mixing free energy term and an elastic free energy term,

\[ \Delta G_{\text{swell}} = \Delta G_m + \Delta G_e \]  

where \( \Delta G_m \) is the free energy change on mixing of the equivalent uncrosslinked polymer and solvent, and \( \Delta G_e \) is the elastic free energy change due to the conformational rearranging and stretching of the crosslinked network chains during the swelling process.

Free energy of mixing

If the entropy of mixing is written as \( \Delta S_m \) and the enthalpy of mixing as \( \Delta H_m \), then the free energy of mixing can be expressed as,

\[ \Delta G_m = \Delta H_m + T \Delta S_m \]  

where \( \Delta S_m \) is the entropy of mixing when \( n_1 \) solvent molecules and \( n_2 \) polymer chains mix and form a homogeneous solution. \( \Delta S_m \) can be expressed in the following form:

\[ \Delta S_m = -k_B [n_1 \ln(1 - \phi) + n_2 \ln \phi] \]  

where \( k_B \) is Boltzmann constant and \( \phi \) is the volume fraction of the solute and ideal behavior is assumed.

Next, we will consider change of enthalpy upon mixing. The change of internal energy of mixing is generated by replacement of the contact of the same species, such as solvent-solvent and segment-segment, with contact between dissimilar species such as
solvent-segment. Therefore, the parameter $\varepsilon$, which expresses the change of contact energy, will be introduced as,

$$\varepsilon = u_{12} - \frac{(u_{11} + u_{22})}{2}$$  \hspace{1cm} (4)

where $u_{12}$ is the energy of 1 (solvent) and 2 (solute) pair formation. By multiplying the number of contacts, $p$, between solvent and solute by $\varepsilon$, the change of contact energy, the enthalpy of mixing for the entire system can be obtained. The quantity $p$ can be obtained by multiplying of valence number $z$, the number of solvent molecules $n_1$, and the volume fraction of polymer $\phi$. Thus the enthalpy of mixing is

$$\Delta H_m = p\varepsilon = zn_1\phi\varepsilon = k_B T n_t \phi X$$  \hspace{1cm} (5)

where $X$ is defined as

$$X = \frac{z\varepsilon}{k_B T}$$  \hspace{1cm} (6)

and is called the polymer-solvent interaction parameter or the $X$ parameter. By combining the equations 2, 3 and 5, the free energy of mixing is expressed as

$$\Delta G_m = k_B T [n_1 \ln(1-\phi) + n_2 \ln \phi + X n_1 \phi]$$  \hspace{1cm} (7)

**Free energy of elasticity**

According to Flory’s work, the deformation of a polymer gel network may be modeled as a two step processes; first, expansion due to free swelling and second, stretching at constant volume without changing the enthalpy of the gel system. Because the elasticity of polymer networks involves a change in entropy, and the enthalpy change due to the deformation is assumed to be negligible, the elasticity term can be written as

$$\Delta G_e = -T \Delta S_e$$  \hspace{1cm} (8)
where $\Delta S_e$ is the change in the entropy due to deformation including the swelling of networks. The deformation ratio ($\alpha$) is introduced as the ratio of the length before and after elongation as $\alpha = L/L_o$.

To calculate the change in entropy of network swelling, it is necessary to know the change of entropy in deforming the network expressed as the deformation parameter $\alpha$, from the $N_c$ polymer chains at the reference state (before deformation). This can be expressed by the sum of the change of entropy $\Delta S_1$, which is generated by placing the $N_c$ uncrosslinked and undeformed chains into the position of deformed networks, and the change of entropy $\Delta S_2$, which is generated by crosslink formation within a pair of such chains. Flory expressed $\Delta S_1$ and $\Delta S_2$ as

$$\Delta S_1 = -\left(\frac{3N_c}{2}\right)(\alpha^2 - 1 - 2 \ln \alpha) \quad (9)$$

$$\Delta S_2 = -\left(\frac{3}{2}\right)N_c \ln \alpha \quad (10)$$

From equation 9 and equation 10 the entropy change for the swelling of the gel is given as

$$\Delta S_e = -\left(\frac{3k_b}{2}\right)N_c(\alpha^2 - 1 - \ln \alpha) \quad (11)$$

Accordingly, the change of elastic free energy for the swelling of the gel is

$$\Delta G_e = \left(\frac{3}{2}\right)k_b N_c T(\alpha^2 - 1 - \ln \alpha) \quad (12)$$

When equation 7 and equation 12 are substituted into equation 1, the free energy of swelling can be obtained as
\[ \Delta G_{\text{swelling}} = n_i k_B T [\ln(1 - \phi) + X_i \phi] + \left( \frac{3}{2} \right) k_B N_c T (\alpha^2 - 1 - \ln \alpha) \] (13)

Because \( n_2 = 1 \) for the network, the term containing \( n_2 \) in equation 13 is omitted.

2.8.2 Swelling of Neutral Polymers

Swelling equilibrium is achieved when the chemical potentials of the solvent inside and outside of the gel are equal. The swelling equilibrium of neutral polymer gels has been expressed by Flory as:

\[ q_m^{5/3} \equiv \frac{\left( \bar{V} M_c \left( \frac{1}{2} - X_i \right) \right)}{\left( 1 - \frac{2M_c}{M} \right) V_i} \] (15)

where: \( q_m \) = equilibrium swelling ratio

\( \bar{V} \) = specific volume of the polymer

\( M_c \) = molecular weight per cross-linked unit

\( M \) = molecular weight of the polymer network

\( X_i \) = polymer-solvent interaction parameter

\( V_i \) = molar volume of the solvent

The swelling ratio, \( q \), is equal to the ratio of the volume of the swollen \( (V) \) and unswollen \( (V_0) \) structures. The subscript \( m \) indicates maximum, or equilibrium swelling.

Equation 15 describes the dependence of the equilibrium swelling ratio \( (q) \) on a quality of the solvent, expressed as \( X_i \), and on the extent of crosslinking, expressed as \( M_c \). The polymer-solute interaction parameter, \( X_i \), is the energy change that occurs when a mole of solvent molecule is removed from the pure solvent and immersed in an infinite amount of
pure polymer. $X_I$ values for solvent polymer pairs are determined experimentally, and can be found in the Polymer Handbook.\(^5\)

### 2.8.3 Swelling of Ionic Polymers

The degree of swelling can be increased significantly by fixed charges on the polymer network due to the electrostatic forces between charges. Fixed charges on the polymer backbone also cause osmotic pressure because of the charge density difference inside and outside the polymer. To equalize this difference, solvent enters the polymer causing further swelling.

![Swelling of an ionic polymer](image_url)

**Figure 2.8** Swelling of an ionic polymer.

Flory\(^5\) expressed the swelling of ionic polymers as:

\[
q_m^{5/3} \equiv \frac{\left( \frac{i}{2V_o \sqrt{S}} \right)^2 + \left( \frac{1/2 - X_I}{V_i} \right)}{\frac{V_o}{V_o}}
\]  

\((16)\)

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where

\[ i \] is the fraction of the charged functional groups on the polymer,

\[ V_u \] is the molar volume of a monomer unit.

\[ S \] is the ionic strength in the external solution

\[ X_i \] is the polymer-solvent interaction parameter

\[ v_e \] is the effective number of chains in the network

\[ V_o \] is the volume of unswollen polymer

The first term, \( (i/2V_uS^{1/2})^2 \), in the equation deals with electrostatic swelling. Here, \( i/V_u \) is the concentration of fixed charge in an unswollen state. The swelling ratio increases with the square of the fixed ion concentration, but decreases with the increase in ionic strength of the external solution. The second term, \( (1/2-X_i)/V_e \), involves swelling due to solvent compatibility. The denominator reflects the effect of crosslinking on swelling. Increased crosslinking density will decrease the degree of swelling.

The effect of the first term on swelling can be explained by both electrostatic and osmotic arguments. If the polymer has charged sites along the chains, the chains will try to maximize the distance between the charged sites to minimize the charge repulsion energy. This results in an increase in the polymer volume. It is obvious that the degree of swelling will increase with the number of charged sites. Conversely, increasing ionic strength in the first term will decrease the swelling due to the shielding effect of the mobile ions. The osmotic pressure effect on the swelling is the result of the charge density difference between the inside and outside of the polymer. In this case, the solvent will enter the polymer to equalize the charge difference. The increase of the solvent
content in the polymer will create an osmotic pressure resulting in an increase in the polymer volume.

Flory's equation does not include an important factor, porosity, which greatly affects the degree of polymer swelling. Porous polymers will allow better access of the analyte in the polymer, which promotes faster and more efficient interaction of the analyte and the functional groups on the polymer backbone. Furthermore, the solvent will have faster and better access to the interior of a porous polymer. As a result, the degree and the rate of swelling can be increased significantly.

2.9 Optical Properties of Polymers

Polymers have many important optical properties, such as the refractive index, reflection, scattering, transparency, gloss, haze, birefringence, and stress-optic coefficient. Among these optical properties the first four are the most important in our research and therefore this section will focus on them.

Refractive index.

Many of the optical properties of a polymer are related to the refractive index \( n \), which is a measure of the ability of the polymer to refract or bend light as it passes through the polymer. If the first medium is vacuum or air, \( n_1 \) is assumed to be 1, in this case refractive index of the polymer, \( n_2 \), is equal to the ratio of the sine of the angles of incidence, \( i \), and refraction, \( r \), of light passing through the polymer:

\[
\frac{n_2}{n_1} = \frac{\sin i}{\sin r}
\]
The magnitude of $n$ is related to the density of the substance and varies from 1.000 and 1.333 for air and water, respectively, to about 1.5 for many polymers. The value of $n$ is high for crystals and is dependent on the wavelength of the incident light and the temperature; it is usually reported for the wavelength of the transparent sodium D line at 298 K. For example, the $n_D$ value of polystyrene (PS) is 1.592.

The velocity of light passing through a polymer is affected by the polarity of the bonds in the molecule. Polarizability, $P$, is related to the molecular weight per unit volume, $M$, and density, $\rho$, as follows (the Lorentz-Lorentz relationship):

$$P = \left( \frac{n^2 - 1}{n^2 + 2} \right) \frac{M}{\rho}$$

The polarizability of a polymer is related to the number of molecules present per unit volume, and the polarizability of each molecule is related to the number and mobility of the electrons present in the molecule.

Reflection

The reflectance at an interface between two non-absorbing media (e.g. polymer and air) is a function of the refractive indices of the two media and the angle of incidence. The fraction of light reflected at the interface for normal incidence is given by Fresnel’s relation

$$r = \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2$$

where $n_2$ is the refractive index of the denser medium (polymer) and $n_1$ is the refractive index of the other medium. Total reflection is observed at an angle called Brewster’s angle ($\theta_B$), which depends on the refractive indices of the two media.
\[ \tan \theta_s = \frac{n_2}{n_1} \]

Scattering

Scattering is the basis of turbidimetry and nephelometry. In the usual type of scattering, electromagnetic radiation interacts with small particles in its path by inducing oscillations in the electric charge of the matter. The dipoles that are induced radiate secondary waves in all directions. In the process, energy is removed from the beam of incident light and emitted without a change in its wavelength.

Particles smaller than the wavelength of the incident radiation can scatter the radiation elastically without a change in its energy. This type of scattering is called Rayleigh scattering, and it typically occurs with atoms or molecules. Scattering from larger particles with dimensions on the order of the wavelength of the incident radiation is often called Debye scattering. When the dimensions of particles are greater than 10 percent of wavelength, Mie scattering occurs. Large particle scattering (Debye and Mie) can be used to determine particle sizes and is important in turbidimetry and nephelometry where suspended particles are the scatterers.

Transparency of polymers

Polymers can be described as highly transparent, translucent (semi transparent), or opaque. Opacity occurs when refractive index fluctuations in the polymer cause light to be scattered. Translucence is just weak opacity.
Turbidity

When light passes through a suspension of particles, some is absorbed, some scattered and a portion is unaffected, the relative proportions depend on the particle size, the wavelength of the light and the refractive indices of media. The reduction in transmitted light intensity due to scattering is called the sample’s turbidity. Extinction includes the effects of both absorption and scattering. The Beer-Lambert law describes the effects of both absorption and turbidity on the transmitted intensity. For suspended polymer particles this law can be expressed as

\[ I = I_0 e^{-(\varepsilon + \tau) b} \]

Where \( I \) is intensity of the light transmitted through the sample, \( I_0 \) is the intensity of the incident light, \( \varepsilon \) is absorption coefficient per unit length, \( \tau \) is the turbidity coefficient (or simply the turbidity), with the units of \( \text{cm}^{-1} \), \( b \) is the length of the light path in the sample. The equation can be reorganized for turbidity as,

\[ \tau = \frac{1}{b} \ln \frac{I}{I_0} - \varepsilon \]

Nephelometry is an alternative way of measuring the scattered light intensity by the suspended particles. In this method, light scattered at 90° to the incident beam by the suspended particles is measured.
CHAPTER 3

EXPERIMENTAL

3.1 Reagents

Aldrich Chemical Company, Inc., Milwaukee, WI 53233

Acryloyl chloride, F.W. 90.51, b.p. 72-76°C

2,2'-Azobisisobutyronitrile (AIBN), 98%, F.W. 164.21, m.p. 103-105°C

Benzoyl peroxide (BPO), 97%, F.W. 242.23

Divinylbenzene, 55% or 80%, mixture of isomers, F.W. 130.19, b.p. 195°C

2,2-Dimethoxy-2-phenyl-acetophenone (DMPA), 99%, F.W. 256.30, m.p. 67-70°C

2-(Dimethylamino)ethyl methacrylate, 98%, F.W. 157.22, b.p. 182-192

Dimethylamine, 40% w/w of soln. in water

Diethylamine, 98%, F.W. 73.14, b.p. 55°C

Diethanolamine, 99%, F.W. 61.08, b.p. 170°C

Diisopropylamine, 99%, F.W. 101.19, b.p. 105-110°C

Dipropylamine, 99%, F.W. 101.19, b.p. 105-110°C

Dibutylamine, 99%, F.W. 129.25, b.p. 159°C

Ethylene glycol dimethacrylate (EGDMA), 98%, F.W. 198.22, b.p. 98-100°C/5mm

2-Hydroxyethyl methacrylate (HEMA), 97%, F.W. 130.14, b.p. 67°C/3.5 mm

Methyl methacrylate (MMA), 99%, FW 100.12, bp 100
Poly (Vinyl alcohol), 100% hydrolyzed, Aver. Mw: 14,000

Poly (vinyl alcohol), 87-89% hydrolyzed, Aver. Mw: 85,000-146,000

Poly (Vinyl alcohol), 98% hydrolyzed, Aver. Mw: 11,000-31,000

Potassium Iodide, 99%, FW: 166.00

Sodium thiocyanate, 98%, FW: 81.07

Styrene, 99%, F.W. 104.15, b.p. 145-146°C

4-Vinylpyridine, 95%, FW 105.14, bp 62-65/15 mm

Sigma Chemical Company, P.O. BOX 14508, St. Louis, MO 63178

Polyvinylpyrrolidone (PVP), Av. Mol. Wt. 40.000, K value: 29-32

TCI America

2,4,5-Trichlorophenol, F.W. 197.45, m.p. 67-69°C

The Dow-Chemical Company, Midland, MI 48674

Vinylbenzyl chloride (VBC), Mixture of 3- and 4- isomers, F.W. 152.62, b.p. 229°C


Glutaraldehyde, 50 wt. % soln. in water, F.W. 100.12.

Sodium bicarbonate, ACS, F.W. 84.01.

Acetone, F.W. 58.08, b.p. 56.01°C.

HYMEDIX International, Inc.

HYPAN Polymers: HYPAN HN50, HYPAN HN68, and HYPAN HN80
Polysicence, Inc., Warrington, PA

Polystyrene microparticles

A) Diameter: 0.535 μm (Standard Deviation: 0.01 μm)
B) Diameter: 1.072 μm (Standard Deviation: 0.019 μm)
C) Diameter: 1.414 μm (Standard Deviation: 0.044 μm)

The structures of the major reagents used for preparation of microbeads and hydrogels are shown in Figure 3.1.
Figure 3.1 Structures of monomers, crosslinkers, initiators and polymers used for preparation of microbeads and hydrogels.
3.2 Apparatus

An Orion 901 digital Analyzer with an Orion 91/55 combination pH electrode were used for buffer preparation. A Fisher laboratory centrifuge (3400 rpm) was used for separation of polymer microparticles. A VWR Model 1217 reciprocal shaker bath with temperature controller was used for derivatization of the microparticles. A Branson model 1210 sonicator was used to redisperse the particles. Scanning Electron Micrographs (SEM) and the CHN analysis of the functionalized polymer microparticles were obtained at the University of New Hampshire Instrumentation Center by Nancy Cherim using an Amray Model 3300FE Scanning Electron Microscope and a Perkin Elmer Model 2400 CHN analyzer, respectively. A Zeiss model light microscope equipped with a CCD camera was used for examination and characterization of the microparticles. A Bausch and Lomb Abbe refractometer was used for refractive index measurements. Photo-polymerization of HEMA was carried out with a 400 Watt UV lamp. Membrane turbidity and absorbance spectra were recorded with a Cary 500 UV-Vis spectrophotometer. A Perkin-Elmer 204 fluorescence spectrophotometer and a SLM model fluorescence spectrophotometer were used to obtain nephelometry and scattering data. A Nicolet Model 520 FT-IR spectrophotometer was used for monomer and polymer characterizations.

3.3 Procedures

3.3.1 Dispersion Polymerization

Polymerizations were carried out on 5 or 10 g monomer scales in a 500ml 3-neck round bottom flask immersed in a water bath maintained at 70 °C. In a typical procedure,
toluene was used as solvent, sometimes with a cosolvent. A typical recipe is given below in Table 3.1.

<table>
<thead>
<tr>
<th>Monomer(s)</th>
<th>5 or 10 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilizer (Kraton G1650)</td>
<td>15 weight % of monomer</td>
</tr>
<tr>
<td>Initiator (BPO or AIBN)</td>
<td>1 weight % of monomer</td>
</tr>
<tr>
<td>Crosslinker (EGDMA)</td>
<td>2 mol % of monomer</td>
</tr>
</tbody>
</table>

Table 3.1 Formulation for dispersion polymerization of HEMA and its copolymers

After the complete dissolution of all the reagents in the solvent, the solution was degassed by ultra-sonication for 1 to 2 minutes and then purged with nitrogen gas for 15 to 20 minutes. Due to the slow dissolution of Kraton in toluene, the appropriate amount of Kraton was dissolved in 30 ml toluene in advance. This solution was mixed with the monomer solution prior to purging with nitrogen for removing the oxygen. After purging, the flask was immersed in the water bath and a water condenser was attached to the flask. The solution was stirred with a magnetic stirrer. Usually polymerization is complete in about 4 hours, but in all batches the reaction was allowed to continue for 6 to 10 hours to ensure maximum conversion. The product was cleaned as described in section 3.3.4.

3.3.2 Suspension Polymerization

Suspension polymerization is carried out in two steps: emulsification and polymerization. In conventional suspension polymerization, the monomer (organic
phase) is emulsified in water by vigorous agitation. This method produces highly polydisperse monomer droplets with a diameter range of 0.1 to 2 mm. In our research, we need highly monodisperse particles in the range of a few micrometers. Therefore, the traditional emulsification method is not appropriate for our purpose. For this reason, we have employed a relatively new emulsification method known as Shirasu Porous Glass (SPG) membrane emulsification. A typical recipe for the suspension polymerization of vinylbenzyl chloride (VBC) is given in Table 3.2. This recipe is for a 300 ml total suspension volume.

<table>
<thead>
<tr>
<th>Monomer(s)</th>
<th>30 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porogenic solvents</td>
<td>Up to 30 % v/v of monomer</td>
</tr>
<tr>
<td>Initiator (BPO or AIBN)</td>
<td>2 weight % of monomer</td>
</tr>
<tr>
<td>Crosslinker (DVP)</td>
<td>2 mol % of monomer</td>
</tr>
<tr>
<td>Stabilizer (0.5% PVA solution)</td>
<td>270 ml</td>
</tr>
</tbody>
</table>

**Table 3.2** Formulation for suspension polymerization of VBC

**Emulsification**

A schematic diagram of the emulsification apparatus is shown in Figure 2.5. A cylindrical SPG membrane (10 mm OD x 125 mm length) was installed in a pressure tight stainless-steel module. SPG membranes were immersed in aqueous phase solution (PVA and SDS in water) and treated with ultrasonic energy so that the surface and the pores are thoroughly wetted with the aqueous phase. The dispersion phase (a mixture of
monomer, inert solvents and initiator) was stored in a pressure tight storage tank and allowed to permeate through the membrane under an appropriate pressure into circulating aqueous phase. The droplets were suspended in the continuous phase and stabilized by the surfactants. During emulsification, samples from the aqueous phase were taken, and monomer droplets were examined using an optical microscope to determine droplet size and the size distribution. When emulsification was complete, the emulsion was removed from SPG module for polymerization.

**Polymerization**

The emulsion was transferred to a 3-neck round bottom flask equipped with a semicircular anchor-type stirring blade and a nitrogen inlet nozzle. After purging with nitrogen gas for 10 to 20 minutes, the flask was immersed in a water bath maintained at 70 °C. The polymerization was carried out for 24 hours. The beads were cleaned as described in section 3.3.4 before and after the derivatization process.

**3.3.3. Derivatization of Polymer Beads**

If the polymer is not functional by itself, it needs to be derivatized so that it can swell and shrink when it contacted with the analyte solution. In dispersion polymerization, we used 4-vinyl pyridine and 2-(dimethylamino)ethyl methacrylate as functional co-monomers. However, the monomer, vinylbenzyl chloride, was derivatized after polymerization. The beads were pre-swollen in 1,4-dioxane and then derivatized in excess diethyl amine and triethylamine. The derivatization reaction was carried out at room temperature for 3-4 days in a shaking water bath. In this reaction, diethylamine
displaces the chloride from the chloromethyl group producing a tertiary amine. Triethylamine in the system catalyses the reaction by absorbing protons and forming the hydrochloride. Derivatized particles were cleaned with a similar procedure described below, and then stored suspended in water.

3.3.4 Cleaning the Polymer Beads

Microspheres are made by dispersion and suspension polymerization using surfactants and steric stabilizers. Before the particles are derivatized with a functional group, surfactant and other solutes need to be removed. In suspension polymerization, sodium dodecyl sulfate (SDS) and polyvinyl alcohol (PVA) were used to stabilize the emulsions and the final polymer beads. SDS molecules adsorbed on the particle surface; there they give the particles a negative charge, which increases colloidal stability. On the other side, PVA provides steric stability. Dispersion polymerizations were carried out in toluene, which requires the use of an organic soluble stabilizer. Our choice of stabilizer was an ethylene-butylene-styrene triblock copolymer, commercially known as Kraton G1650. Removal of this stabilizer from the bead suspension after polymerization is completed is vitally important, because it is hydrophobic and insoluble in water. In an aqueous medium Kraton polymers plug the surface of the beads resulting in a decrease of swelling ability of the beads.

Although it is probably a good idea to use thoroughly cleaned microspheres, it is often not necessary to remove all surfactant prior to derivatization or after derivatization. This decision depends on the type and concentration of the surfactant, and the type of membrane. Each system must be evaluated independently.
We employed a repetitive washing and centrifuging process for cleaning the particles. This method is described below. Standard filtration seems to be an easier method but it is generally unacceptable because the small microspheres can easily plug any filter designed to catch them.

Washing

Repetitive centrifuging, decanting, and resuspending is the cleaning method we employ. The solvent chosen should not affect the particles but should effectively remove the surfactant and other solutes. The microspheres must be spun down to form a tight "button" to permit the clean separation (decantation) of liquid from the solids. If the brake is used to stop the centrifuge, the particles may be partially resuspended and some of them lost on decanting. After decanting, fresh washing liquid (water, alcohol, or organic solvents) is added, and the microspheres are resuspended by means of vortex stirring and ultrasonication. Effective washing requires that the microspheres be completely redispersed. Resuspension can be monitored by some reliable method, such as microscopic examination to verify that the particles are dispersed efficiently. The more the surfactant is removed, the more tightly the microspheres adhere to one another. Larger particles (>0.8 \( \mu m \)) are more easily spun down, less likely to stick firmly together, and more easily resuspended. Hydrophilic microspheres are much less likely to stick together after centrifuging than hydrophobic microspheres.

Calculations of microsphere settling velocity and gravitational (G) forces generated by a centrifuge can be performed to determine the amount of time necessary to
spin down microspheres of a specific size. Microspheres <50 nm (<0.050 μm) may require >300,000 G to sediment them efficiently (i.e., a 10-cm/hr settling rate).

As the underivatized hydrophobic microspheres go through successive cleaning steps, however, they become more hydrophobic and, therefore, more difficult to resuspend and separate. An ultrasonic bath can greatly assist resuspension.

### 3.3.5 Characterization of Polymer Beads

It is advisable to test microspheres at various stages in their processing. Attributes to monitor include the microspheres' monodispersity, colloidal stability, surface charge, functional group density, and percent solids. The characterization techniques we used are described below.

**Scanning Electron Microscopy (SEM) analysis** provides information about the surface morphology and the size distribution the particles. Samples were prepared by placing a drop of suspended (in alcohol or water) particles onto a SEM sample stub and allowing the drop to air dry. The sample was coated with a gold/palladium alloy under vacuum to a depth of about 300Å.

**Light Microscopy Observations:** Prior to polymerization, the size distribution of monomer droplets in an emulsion obtained by SPG emulsification was monitored by a Zeiss model microscope equipped with a computer controlled CCD camera system. The size of the final polymer particles was also determined by the same microscope system. A drop of emulsion or bead suspension was placed on a microscope slide and picture of
particles was taken. This picture, was then, analyzed using the microscope's particle analysis software.

**CHN Analysis:** CHN data is needed to determine the functional group concentration of the amine containing microparticles. These data also helps us to get an approximate idea of the proportion of the monomer units in a copolymer. For CHN analysis, microparticles were washed a few times with 0.1M HCl solution and a few times with deionized water in order to remove any excess unreacted amine. Cleaned beads were first air dried to remove the excess water and then vacuum dried to remove the trace water. Samples were sent for CHN analysis in air-tight glass vials.

**FTIR Analysis:** A portion of the sample prepared for CHN analysis was used to obtain the FTIR spectra of the polymer microparticles. This analysis was needed to confirm the derivatization reaction. Disappearance of C-Cl peaks and formation of amine peaks (or amide peaks if TCPA used as comonomer) were monitored.

### 3.3.6 Determination of Water Content in Microparticles

Since the copolymers of 2(dimethylamino)ethyl methacrylate (DMAEMA) and 4-vinylpyridine (4VP) are hydrophilic, they swell by absorbing substantial amounts of water. Water content changes their optical properties, especially their refractive index, which is an important parameter in our sensor technology. Determining the water content provides information on the degree of hydrophilicity of the polymer. In addition, we can also calculate the hydrated refractive index of the polymer.
The water content of the microparticles was determined by gravimetry. Surface water of triplicate samples (approximately 1 g each) was removed by using Kimwipes and the samples were quickly weighed in an aluminum weighing boat. The samples were dried overnight in an oven at 80 °C. The mass of dried samples was recorded until it became constant and the percentage of water lost calculated.

The data collected by this technique cannot be considered accurate enough, due to the small size (a few micrometers) and high hydrophilicity of microparticles. Despite care and effort, it is not possible to remove all the surface water. Water content data given in Chapter 5 are averages of triplicate measurements and presented to give approximate values.

3.3.7 Preparation of Hydrogel Membranes

We employed various hydrogel materials for preparing membranes to immobilize the swellable microspheres for turbidity measurements. These included poly-vinyl alcohol (PVA), poly-2-hydroxyethyl methacrylate (poly-HEMA), HYPAN™ and polyurethane (PU). All these materials have different characteristics (e.g. refractive index, water content, hydrophilicity, etc.) and different methods of preparation (e.g. physical, chemical, radiation, etc.). The general characteristics of all hydrogels we used are (1) low crosslinking, (2) inertness to analyte (e.g. pH), (3) optical transparency, and (4) sufficient mechanical strength.
**Preparation of PVA membranes:**

Poly-vinyl alcohol (PVA) is a hydrophilic, water-soluble polymer. PVA is commercially available with varying degrees of hydration and molecular weight. The best PVA polymers for hydrogel preparation are those with high hydration (e.g. over 90%) and low molecular weight (ca.10,000-30,000). Membranes are prepared from 10% (w/w) stock PVA solutions. Higher concentrations of PVA solutions are difficult to prepare and handle due to their high viscosity. PVA membranes prepared from solutions with lower concentrations (lower than 10%) do not provide sufficient mechanical strength.

PVA hydrogel membranes are prepared by crosslinking a 10% PVA solution with glutaraldehyde (crosslinker) by acidic initiation. A typical recipe and preparation procedure is given below.

**Reagents:**
- 10% PVA solution (w/w)
- 10% Glutaraldehyde solution (w/w)
- 4M HCl solution

- Weigh appropriate amount of beads (e.g. 1% w/w) in a small glass vial.
- Add 10% PVA solution to bring the mass to 1g.
- Mix the beads and PVA using sonication to obtain a homogeneous suspension.
- Add 25 μl of 10% glutaraldehyde solution and continue sonication for about 5 to 10 minutes to ensure the homogeneous distribution of crosslinker.
- Add 50 μl of 4M HCl solution and sonicate for 1 minute.

* See App. A for dependence of hydrogel formation on crosslinker and initiator levels.
• Transfer a portion of the mixture onto a microscope slide with a Teflon spacer and place a second microscope slide on top as a cover. Tighten the two slides with clamps.

• Allow approximately one hour for gelation. Remove the membrane from the slides, wash, and store in DI water.

**Preparation of poly-HEMA membranes:**

Poly-HEMA membranes are prepared by crosslinking 2-hydroxyethyl methacrylate (HEMA). Crosslinking is a photochemical process, which requires use of a mercury lamp as an UV source. A typical procedure for preparing poly-HEMA membranes is described below.

Poly-HEMA membrane recipe for a 3 g total hydrogel solution:

**Reagents:**

- **Monomer:** 2-Hydroxyethyl methacrylate (HEMA)
- **Crosslinker:** Ethylene glycol dimethacrylate (EGDMA)
- **Initiator:** 2,2-Dimethoxy-2-phenyl-acetophenone (DMPA)

• Preparation of a mold for the membrane: Cover one surface of a glass slide with Teflon tape. Cut thin Teflon tape strips (3-4 mm thick) and place the strips at the edges of the Teflon covered surface of the glass slide creating a rectangular well at the center. Cover one surface of a second glass slide with Teflon tape. This slide will be used as a cover.

• Weigh appropriate amount of beads (e.g. 1% w/w of monomer) in a small glass vial.
• Add 0.046 g EGDMA (1 mol % of HEMA) and 0.060 g DMPA (2 w/w % of HEMA).
• Add more HEMA to bring the mass to 3 g.
• (OPTIONAL) Add approximately 5% w/w of water. Water will plasticize the membrane and make it less fragile.
• Sonicate the mixture for 5 minutes to obtain a homogeneous suspension.
• Transfer a portion (2-3 drops) of the solution onto the glass slide with a Teflon spacer, place the second slide, and tighten them with clamps.
• Expose the slides to a UV light source for 10 minutes.
• Remove the membrane from the slides and store in DI water.
• The vial containing the unused portion of the Bead-HEMA mixture should be covered with aluminum foil and stored in a refrigerator for future use.

**Preparation of HYPAN membranes:**

HYPAN™ polymers are hydrophilic acrylate derivatives with a unique multiblock copolymer structure. Each polymer chain of any HYPAN™ polymer is composed of sequences of units with pendant hydrophilic groups (soft block) and sequences of units with nitrile groups (hard block). HYPAN polymers are commercially available in dry granule form with various water uptake capacities. In our research, we used polymers with trade name: HYPAN HN30, HYPAN HN50, HYPAN HN68 and HYPAN HN80. The HN# indicates the maximum water uptake capacity (% w/w) of the fully hydrated membrane. Physical properties of the membranes, such as mechanical strength and refractive index, vary with their water content. Unlike other hydrogels we used, HYPAN does not require crosslinker and initiator to form a gel. Crosslinking occurs due to
interactions between nitrile blocks in the polymer chains when the polymer is placed in aqueous medium.

HYPAN membranes are usually prepared from solutions of 10% HYPAN (w/w) in DMSO. The appropriate amount of beads is added into 2-3 grams of HYPAN solution and the mixture is stirred with a magnetic stirrer at an elevated temperature (~ 40-50 °C) to form a homogeneous suspension. Stirring may produce air bubbles, which can be removed by ultrasonification. A small portion of the suspension (2-3 drops) is then applied on a glass slide with a Teflon spacer. A glass cover slip is used like a squeegee to pull the viscous suspension down across the slide to fill the well forming a layer. Excess mixture is removed and the slide is placed in a petri dish with a small amount of water. A cover is placed on top of the petri dish to create high humidity inside, and the bead suspension in HYPAN solution is allowed to hydrate. Once the gel forms, the slide is inserted into water to extract DMSO. Then, the membrane is removed from the slide surface and washed with excess water to remove the remaining DMSO. The cleaned membrane is stored in DI water.

Aqueous HYPAN solutions can also be prepared for membrane fabrication. For this, HYPAN polymer is dissolved in 55% aqueous NaSCN solution. Membranes are prepared as described above.

Preparation of polyurethane membranes: 84

Polyurethane membranes can be prepared from ethanol solutions of the HydroMed D series of polymers from Cardiotech International, Inc. For membrane preparation, a 10% stock ethanol solution of polyurethane is prepared. The rest of the
procedure is similar to HYPAN membrane preparation. In order to get a good suspension of beads in the polyurethane solution, the excess water should be remove from the beads. If possible, the beads should be washed with ethanol prior to mixing with the polyurethane solution.

3.3.8 Coupling PVA and HYPAN Membranes on Glass Surfaces

Membranes used for turbidity measurements with the flow-cell described in section 3.3.8 need to be bonded to glass slides. Most of the hydrogel membranes do not stick on glass surface. Some (e.g. poly-HEMA) stick on the glass surface during gelation, but easily come off the surface after hydration. The method used to form the membranes on the glass surfaces is described for PVA and HYPAN membranes below. Despite the slight differences, this method is basically the same for all membranes. The basic idea is to form a very thin film of hydrogel polymer on a glass surface prior to forming the membrane on the glass.

PVA Membranes:

A thin layer of PVA film is formed on a glass slide using 2-3% w/w solution of PVA by spin coating. This slide is heated at 80 °C for about 1-2 hours in an oven. A gluteraldehyde layer is formed on top of the PVA film by spin coating, and the film is dried at room temperature for about 5 minutes. The PVA membrane is then prepared on the modified glass surface as described in section 3.3.7.
HYPAN Membranes:

A thin layer of HYPAN film is coated on a glass slide using a 2-3% solution of HYPAN by spin coating. This slide is heated at 80 °C for about 1-2 hours in an oven. After this, the procedure for membrane preparation described in section 3.3.7 is followed.

3.3.9 Collection of Data

Turbidity measurements

Samples for turbidity measurements were prepared in two different forms. Microparticles were (1) suspended in water and (2) immobilized in hydrogel membranes. The turbidity of aqueous suspensions of standard polystyrene microparticles was measured in standard 1 cm × 1 cm cells. Custom-built membrane holders were used for the measurements of membrane turbidity. All the measurements were performed using a UV-Vis-NIR spectrophotometer. How the membrane is held in the light path of the spectrophotometer is crucial for reliable measurements. The membrane must be positioned perpendicular to the light beam, and it shouldn’t curl. We developed two different membrane holders for this purpose. The holder shown in Figure 3.2 was developed to use with standard 1 cm spectrophotometer cells. This holder has a circular window with a diameter of ¼ inches, and holds the membrane perpendicular to the light beam. The small window area ensures that the membrane stays straight and does not curl during measurements. Once the holder with a membrane is placed in the spectrophotometer cell, the holder is not disturbed during the measurements, so that its orientation does not change. Buffer solutions are removed and replaced with a syringe or a Pasteur pipette.
A more advanced membrane holder with a flow cell is shown in Figure 3.3. The membrane is prepared on a glass slide, and the slide is used as a window of the flow cell. The volume of flow cell is usually approximately 0.5 ml. It can be varied by changing the dimensions, especially the thickness, of the rubber spacer. The advantages of this holder are: (1) it holds the membrane more stably, (2) there is no need to remove the membrane from the spectrophotometer when changing reagents, (3) reagents can be circulated continuously, (4) more accurate response times can be measured, (5) it allows use of membranes thinner than 76 μm, and (6) the membranes are more easily handled.

B) Nephelometry measurements

Only particles suspended in water were used for nephelometry measurements. Highly monodisperse polystyrene microparticles with three different diameters, 0.535 μm, 1.072 μm, 1.414 μm, were used as samples. From now on, diameters of these particles will be given as 0.5, 1.0 and 1.5 for simplicity. Samples were prepared by diluting the stock suspensions (approximately 2.6% w/w) with DI water. Each sample was treated in an ultrasonic bath for about 5 to 10 minutes to break up all particle aggregates. Standard 1cm x 1cm cells were used for these measurements.
Figure 3.2 Membrane holders for turbidity measurements.

Figure 3.3 Membrane holders with flow cell for turbidity measurements.
C) Light scattering measurements

Samples for light scattering measurements were approximately 50-70 µm thick hydrogel membranes. Microparticles were immobilized in the hydrogel membrane and the membrane was formed on the surface of a glass slide as described in section 3.3.7. The slide was placed in the specially designed membrane holder shown in Figure 3.4. The holder was equipped with a flow cell that allows circulation of a liquid over the membrane. The holder can also be rotated to vary the angle of incident light hitting the membrane surface. The holder can be moved up and down or back and forth, which allows focusing of the incident light on the membrane surface. The SLM fluorescence spectrophotometer was used for scattering measurements because this instrument had a large sample compartment, which can hold various cell and membrane holders.

D) Measuring the size of the microparticles as a function of pH

We used a custom-made flow cell designed for use with a microscope, to monitor the size of the swollen and shrunken microspheres (Figure 3.5). Beads were immobilized on the inner wall of the flow cell window. The window is a standard 1-inch microscope cover slide. Using a syringe or a mini pump, buffers of varying pH were circulated in the flow cell. Particles were equilibrated by circulating the buffer for a few minutes before measuring their diameters.
Rubber spacers Incident Light

Liquid In

Liquid Out

Glass slide with immobilized microparticles at its center

Incident Light

Scattered Light

Reflected Light

Figure 3.4 Membrane holder for light scattering measurements at variable angles.
Figure 3.5 Flow cell for microparticle size measurements using a light microscope.
CHAPTER 4

TURBIDITY AND LIGHT SCATTERING MEASUREMENTS OF POLYMER MICROSPHERES SUSPENDED IN WATER AND HYDROGELS

4.1 Introduction

Our chemically sensitive membranes consist of swellable microparticles embedded in a hydrogel. The membrane appears turbid due to the light scattering caused by the difference between the refractive index of the microparticles and the refractive index of the hydrogel. Optical measurements in our research mainly involve turbidity, light reflection and light scattering. We employ an UV-Vis-NIR spectrophotometer for turbidity measurements. However, our eventual goal is to incorporate the membranes into a fiber optic sensor device described in chapter 1. In this instrument, the membrane is placed at the distal end of an optical fiber, and the scattered and reflected light from the membrane is measured (Figure 4.1a).

![Diagram of fiber optic sensor and membrane sensor arrays](image)

**Figure 4.1:** (a) Fiber optic sensor, (b) Membrane sensor arrays
Another direction we will pursue is to make tiny membrane arrays on a flat substrate for multi sensing applications (Figure 4.1b). In this system, the light scattered and reflected from the arrays will be monitored using a CCD camera.

The purpose of this chapter is to investigate the conditions for turbidity and light scattering measurements. Experiments were performed under controlled conditions by using monodisperse polystyrene microparticles. Diameters of the particles were chosen as 0.5 μm, 1.0 μm and 1.5 μm because the diameters of the swellable microparticles we synthesize for our sensor applications in this size range.

4.2 Turbidity and Light Scattering

Light scattering is an extremely important analytical tool in polymer science and in other disciplines. One of the most common uses of light scattering is in the determination of particle size distribution due to the dependence of scattered light intensity on particle size. Other factors, which affect the scattered light intensity, are the refractive index of particles relative to the refractive index of the medium, wavelength of the light and the particle concentration.

When a beam of light strikes an assembly of particles, some of it is transmitted, some absorbed and some scattered. In the usual type of scattering, electromagnetic radiation interacts with small particles in its path by inducing oscillations in the electric charges of the matter. The dipoles that are induced radiate secondary waves in all directions. In the process energy is removed from the beam of incident light and emitted without a change in wavelength. The necessary criteria for this type scattering are that the particles have dimensions of about the same order of magnitude or smaller than the...
incident wavelength of light and that the particles be distributed in a medium of refractive index different from their own. For particles larger than approximately $2\lambda$, refraction and reflection occur.

Turbidimetry and nephelometry are techniques of analysis based on the scattering of light by particles suspended in a solution. The two techniques differ only in the manner of measuring the scattered radiation. In turbidimetry (Figure 4.2a) the source radiation is passed directly through the sample solution and the decrease in intensity is measured. In nephelometry (Figure 4.2b) the beam of radiation is measured at an angle (usually 90°) to the incident beam. Because of the difference in the angle of these measurements, turbidimetry is best suited for determining relatively high concentrations of suspended particles, whereas nephelometry is best suited for determining very low concentrations. Nephelometry is much more sensitive and precise at low concentrations, since the small amount of scattered light would be measured against a black background. On the other hand, for denser suspensions turbidimetry is the method of choice since it can accurately measure small changes in transmitted intensity when the absolute intensity is relatively low.

Reflection and scattering play an important role in turbidimetry and nephelometry. The criterion for deciding whether reflection or scattering is responsible for the deviation of the light from its original path is based on the size of the suspended particles compared with the wavelength of the light used. Specifically, if the suspended particles have dimensions about the same order of magnitude or smaller than the incident wavelength, the light will be scattered, whereas if the particles are larger than the wavelength of light, reflection will occur. The distinction is important because it affects
Figure 4.2 (a) Turbidimetry, (b) Nephelometry, (c) Light Scattering.
the sensitivity of the measurement as well as how the measurement is made.

4.3 Results and Discussion

The purpose of this section is to investigate how the turbidity is affected by the particle size, particle concentration, wavelength of the incident light and the refractive index of the medium. Turbidity of both aqueous suspensions and the membranes were investigated and compared.

4.3.1 Turbidity of Microparticles Suspended in Water

Turbidity spectra of aqueous suspensions of microparticles in three different diameters are shown in Figure 4.3. Turbidity increases with increasing particle size at long wavelengths. This is surprising when we consider the number of particles in the suspensions. The concentration of each suspension was prepared as 0.1 % w/w. Therefore, in terms of the number of particle per unit volume, the concentration of each suspension is different because of the differences in their sizes. The relative number of particles in the unit volume of each suspension is illustrated in Figure 4.4. There will be fewer particles per unit volume as the particle size increases. Fewer particles mean less scattering centers and, therefore, turbidity could be expected to be lower as the particle size increases. Despite the fewer number of particles, turbidity is higher with larger particles at longer wavelengths.

If the particles are larger than the incident wavelength, the scattering phenomenon terminates, and reflection dominates scattering. In the Figure 4.3, the turbidity of 0.5-micron particles is the lowest, at longer wavelengths. However, as the wavelength
decreases reflection dominates over scattering and turbidity increases gradually. When the wavelength became shorter than the particle size at around 500 nm, turbidity increases much more steeply.

High turbidity with large particles at longer wavelengths is advantageous for our fiber optic sensor system because we want to use a light source in the near IR region that is used for fiber optic telecommunications. Furthermore, large turbidity means more back-reflected light. In our sensor system, there will be more light reflected back into the fiber if we use large particles. This means increased sensitivity. On the other hand, swelling and shrinking time of the large particles will be longer than that of smaller particles. This may increase the response time of the sensor. Therefore, we need to find the optimum conditions for large signal and fast response.

Dependence of turbidity spectra on particle concentration in three different particle sizes is shown in Figure 4.5. When we look at each individual spectrum in Figure 4.8, we see that turbidity increases as the wavelength of incident light decreases. This increase is much higher and steeper with smaller particles. The spectrum of 0.5-micron particles indicates that there is a dramatic increase in turbidity as the wavelength of incident light approaches the particle’s diameter. After a gradual increase, the turbidity of 1.0 and 1.5 micron particles again decreases in the region of shorter wavelengths.

There is a linear relationship between the turbidity and the particle concentration provided that the wavelength is larger than the particle size. But, once the wavelength becomes equal to or smaller than the particle size the relationship is no longer linear. This is shown in Figure 4.6 for 0.5 μm particles.
Figure 4.3 Dependence of turbidity on microparticle size.

Figure 4.4 Relative number of 0.5 μm, 1.0 μm and 1.5 μm particles. Suspensions of these particles have the same concentration by weight percent. Number of scattering sites increase as the particles size decreases.
Figure 4.5 Dependence of turbidity on microparticle concentration. Concentrations are given as weight percent.
Figure 4.6 Dependence of turbidity on particle concentration. Diameter of the particles is 0.5 μm.
In this study particles were suspended in water, as mentioned before. In order to see how the spectra of particles with different sizes look like when they are on a flat 2D surface, an experiment was performed by immobilizing the microparticles on glass slides by spin-coating. In this case we don't have good control of particle concentrations on the glass surface. We just made tightly and well-organized monolayers of beads. Turbidity spectra of a monolayer of microparticles (Figure 4.7) appear to be similar to what we saw in Figure 4.3. Turbidity increased as the particle size increased at longer wavelengths. However, at wavelengths shorter than 800 nm the spectra are more complex, possibly due to interference phenomenon.

![Graph showing turbidity spectra for different particle sizes.](image)

**Figure 4.7** Dependence of the turbidity on the size of the microparticles coated as a monolayer on a glass surface.
4.3.2 Turbidity of Microparticles Embedded in Hydrogels

In our research, polymer particles that are sensitive to an analyte are immobilized in a hydrogel to form a membrane. The membrane becomes turbid, because the particles in the hydrogel act as scattering centers due to the refractive index differences between the particles and the hydrogel. We used a few different hydrogels, including poly-vinyl alcohol (PVA), poly(HEMA) and HYPAN. These hydrogels differ in physical and optical properties such as refractive index, hydrophilicity, degree of swelling, etc. Therefore, it is possible that turbidity spectra and the turbidity intensity can differ from one hydrogel to another.

Turbidity spectra of microparticles in PVA and poly-HEMA hydrogels are given in Figure 4.8a and Figure 4.8b, respectively. The general trend is the same as before. Turbidity increases with increasing particle size at longer wavelengths. As observed in aqueous suspensions, the turbidity of particles in hydrogels increases with decreasing wavelength. In both cases, turbidity of 0.5-micron particles increases sharply at shorter wavelengths.

Even though the microsphere concentration in a PVA membrane is much lower than in a poly-HEMA membrane, the turbidity of PVA membrane is quite high. This is because the refractive index of a PVA hydrogel (n=1.34) is lower than that of a poly-HEMA hydrogel (n=1.42). The refractive index of polystyrene particles is 1.59. The turbidity increases as the refractive index difference between the hydrogel and the microparticles increases.
Figure 4.8 Turbidity of polystyrene microparticles in a (a) PVA hydrogel (b) poly-HEMA hydrogel. The thickness of the hydrogels is 76 μm. The concentrations of microparticles in the membranes are 0.25% in PVA and 1% in poly-HEMA.
Figure 4.9a, 4.9b and 4.9c show the turbidity spectra of microparticles embedded in HYPAN hydrogels. We used three different types of HYPAN hydrogels: HN50, HN68 and HN80. The HN# indicates the percent water content of the hydrogel in fully hydrated form in weight percent. The variation in turbidity for all HYPANs with increasing particle size looks similar to that of PVA and poly-HEMA membranes. When HYPAN membranes are compared among themselves (Figure 4.10), it can be seen that turbidity increases with decreasing water content. This is more significant at shorter wavelengths. However, we did not expect to see this, because the turbidity is proportional to the refractive index difference between the hydrogel and the microparticles (Table 4.1), and the turbidity difference decreases with increasing HN#. The refractive index of polystyrene microparticles is 1.59. We know that the larger the difference between the refractive indexes of the microspheres and the hydrogel, the more turbid the membrane. According to this, the turbidity of the HN80 membrane is expected to be the highest and the turbidity of HN50 membrane to be the lowest. One the other

<table>
<thead>
<tr>
<th>Re refractive index (n)</th>
<th>HYPAN HN50</th>
<th>HYPAN HN68</th>
<th>HYPAN HN80</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.40</td>
<td>1.38</td>
<td>1.36</td>
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<tr>
<td>0.19</td>
<td>0.21</td>
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Table 4.1 Difference between the refractive index of polystyrene and the refractive index of HYPAN hydrogels. 
(Δn = Refractive index of polystyrene – Refractive index of hydrogels)
Figure 4.9 Turbidity of polystyrene microparticles in (a) HYPAN HN50 hydrogel, (b) HYPAN HN68 hydrogel and (c) HYPAN HN80 hydrogel. Sizes of the microparticles are A: 0.5μm, B: 1.0μm, C: 1.5μm.
Figure 10 Turbidity spectra of 0.5 μm polystyrene microparticles embedded in HYPAN hydrogels.

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hand, one of the factors that determines the turbidity is the particle concentration. However, all the HYPAN membranes were prepared with equal particle concentration, 0.5 % w/w. There might be something that affects particle concentrations. We noticed this effect when we measured the size of the each type of HYPAN membrane. HYPAN hydrogels were prepared by casting the HYPAN polymer solution in a mold on a microscope slide. After the formation of HYPAN membranes, we noticed that the membranes shrink, and the amount of shrinking depends on the type of the HYPAN hydrogel (Table 4.2). It is seen that the order of shrinking from the highest to the lowest is; HN50> HN68> HN80. This means, shrinking changes the particle concentration in the membranes. The more the membrane shrinks the higher the particle concentration in a unit area of the membrane. On the other hand, shrinking decreases the thickness of the

![Image](image.png)

**Table 4.2.** Percent shrinking of HYPAN hydrogels after gel formation.
membranes. As a result, the pathlength also decreases. Since the turbidity is proportional to pathlength, shrinking is expected to cause lower turbidity. It is not clear to us why we observed the opposite.

4.3.3 Light Scattering Measurements

In the scattering measurement setup, we needed to position the membrane at an angle to the incident light so that we can prevent specularly reflected light reflection from reaching the detector. Initially we estimated that the angle should be about 65°.

A preliminary experiment was performed, to determine the appropriate angle. One surface of a glass slide was covered with a membrane carrying polystyrene particles and the membrane was placed in the holder as shown in the Figure 3.3. The holder was rotated step by step from θ = 0° to θ = 90° and the detector signal was recorded at every step. The plot on Figure 4.11 shows that the highest scattered light intensity was detected between θ = 70° and θ = 75°. Another less intense peak appeared around 20°. The large signal between 40° and 65° is due to the incident light reflected from the glass surface. From the plot, the angle for light scattering measurements was determined as 73°.

4.3.3.1 Effect of Particle Size on Scattering Light Intensity

In order to see the effect of size of the microparticles and the wavelength of the incident light on scattered light intensity, some slides were coated with 1.0 μm and 1.5 μm particles. Spectra of the slides were obtained by scanning the incident wavelength between 200 nm and 600 nm. We were limited to these wavelengths because we were using a fluorescence spectrophotometer for scattering measurements. Figure 4.12 shows
Figure 4.11 Intensity of the light reflected and scattered from the surface of a glass slide coated with polystyrene microparticles. The size of the particles was 1.5 μm. The wavelength of the incident light was 600 nm.
Figure 4.12 Effect of Particle Size on Scattering Light Intensity. Particles were coated on a glass slide surface as a thin layer. Data were collected at 75°. Small particles scatter light more than large particles.
that scattered intensity was highest at 400 nm. There is a clear difference between the scattered light intensity of 1.0 μm particles and that of 1.5 μm particles. Here 1.0 μm particles scatter the light more than 1.5 μm particles. Since the small particles scatter the incident light more than large particles, scattered light intensity generated by 1.0 μm particles is larger than that of 1.5 μm particles. This means, using smaller particles in sensor arrays is more advantageous than using larger particles, in terms of high sensitivity and fast response time. One other reason that the magnitude of scattered light intensity generated by smaller particles is higher than that of larger particles is because the concentration of 1.0 μm particles in a unit area is higher than that of 1.5 μm particles due to the smaller volume of 1.0 μm particles. More particles mean more scattering centers.

4.3.3.2 Effect of Particle Swelling on Scattering Light Intensity

A slide was covered with a membrane immobilized with pH sensitive microparticles (≈ 0.5μm diameter), and the membrane was treated with pH4 and pH10 buffer solutions. The spectra of this membrane under these two conditions are shown in Figure 4.16.

Amine derivatized poly (vinyl benzyl chloride) microparticles swell in pH 4 buffer and shrinks in pH 10 buffer. Shrunken microparticles have a higher refractive index than that of the swollen particles. This means, there is larger refractive index difference between the hydrogel and the microparticles in pH 10 buffer than in pH 4 buffer. As a result, the membrane is expected to be more turbid and scatter more light.
Figure 4.13 Effect of particle swelling on scattered light intensity. Aminated poly-VBC microparticles with 0.5 μm diameter were coated on a glass slide surface. Change in the scattered light intensity was measured in acidic (pH 4) and basic (pH 10) buffer solutions at 75° angle.
4.3.4 Effects of refractive index of medium on turbidity

The purpose of this experiment is to see how the refractive index of the medium surrounding the particles affects the turbidity. The refractive index of water was varied by adding potassium iodide (KI). A set of KI solutions with varying concentrations was prepared and the refractive index of each solution was measured using an Abbe refractometer. Equal amounts of 0.5 μm polystyrene particles were suspended in KI solutions. As the KI concentration increases, it becomes more difficult to suspend polystyrene particles because the density of KI solutions increases with increasing KI concentration. (The density of solid KI is 3.13 g/ml) Once the particles are suspended, the suspension needs to be sonicated and the measurements should be done quickly.

The spectra of microparticles in varying refractive index media are given in Figure 4.14. It is seen that the turbidity decreases gradually with increasing refractive index. It can be noticed that there is a decrease in turbidity of some of the spectra as the wavelength gets shorter. This is actually not an effect of wavelength. The reason is that the particles start rising in the solution due to their lower density and some of them accumulate at the top. This process causes dilution of the suspension resulting in a slight decrease in turbidity in the area of shorter wavelengths.

Figure 4.15 shows that there is a linear relation between the refractive index of the medium and the scattered light intensity. The only factor that affects the scattered light intensity is the change in the refractive index of medium. The refractive index of polystyrene particles is approximately 1.59. As the refractive index of the medium increases, the difference between the refractive index of the medium and that of the polystyrene particles decreases, resulting in less light scattering. It can be predicted from
the curve that when the scattered light intensity becomes zero, refractive index of the medium (KI solution) becomes equal to the refractive index of polystyrene particles. However, when the curve was extrapolated to x-axis, the refractive index at zero turbidity was obtained as 1.51, which is far from the actual refractive index of polystyrene. Perhaps this is because the polystyrene particles are porous. The solution absorbed by the particles may decrease their refractive index.

![Figure 4.14 Effect of refractive index of the suspension medium on the turbidity of polystyrene microspheres.](image)

**Figure 4.14** Effect of refractive index of the suspension medium on the turbidity of polystyrene microspheres.
4.4 Conclusions

The turbidity of microparticles suspended in water increases with increasing particle size. The turbidity varies with the wavelength of the incident light. This variation increases with decreasing particle size.

The turbidity spectra of particles in different physical environments (such as water, air and hydrgel) are similar with some minor differences. In all cases, turbidity increases with increasing particle size at wavelengths longer than about 800 nm. At shorter wavelengths turbidity spectra become complicated.

The turbidity decreases as the difference between the refractive index of the particles and the refractive index of the suspension medium decreases.
Unlike turbidity, scattered light intensity increases with decreasing particle size. Maximum light scattering from a monolayer of microparticles on a flat surface occurs when the angle between the surface and the incident light beam is between 70 and 75 degree. This angle may depend on particle size, but was not tested in this research.

According to the results discussed in this chapter:

1. In our fiber optic sensor system, using microparticles larger than the wavelength of the incident light is more advantageous. If the particles are larger than the wavelength of the incident light, the light is reflected at the interface rather than scattered. This means, more light is reflected back into the fiber after interacting with the particles, resulting in increased sensitivity.

2. In our sensor array approach, using microparticles, which are smaller than the wavelength of the incident light, is more advantageous. In this case, light will be scattered rather than being reflected when interacting with the particles. In order to direct the maximum amount of scattered light into the detector, the sensor array surface should be positioned at an appropriate angle.
CHAPTER 5

PREPARATION OF AMINE CONTAINING SWELLABLE POLYMER MICROSPHERES BY DISPERSION POLYMERIZATION AND THE EVALUATION OF MICROSPHERES FOR pH SENSING

5.1 Introduction

This chapter explores the preparation and evaluation of copolymers of 4-vinyl pyridine (4VP) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) microspheres for optical pH sensing. The advantage of using these polymers is that no further derivatization is required since both polymers have basic amine groups.

\[
\begin{align*}
\text{2-(dimethylamino) ethyl methacrylate} & \quad \text{4-vinyl pyridine}
\end{align*}
\]

Amine based polymers are called poly-bases or cationic polyelectrolytes. The main interest in such polymers is related to their partial or complete solubility in water and their swelling ability when they are lightly crosslinked. In aqueous solution, their...
conformation results mainly from equilibrium between the electrostatic repulsions, hydrophobic or dipolar (hydrogen bonds) attractions and also depends on various intrinsic and extrinsic parameters. The intrinsic parameters are the nature of the basic group (primary, secondary, or tertiary amine) and its basic strength, which varies with its position inside the main chain or in lateral groups and, the nature of the hydrophobic substituents, and the tacticity and the molecular weight of the polymer. The extrinsic parameters usually considered are the pH, the average ionic strength of the medium, and the temperature.

Polymers synthesized from monomers like DMAEMA and 4VP are also called polyelectrolytes. Cationic polyelectrolytes have industrial importance. Quarternized poly (dimethylaminoethyl acrylate) is widely used in the treatment of urban or industrial wastewaters. Many different cationic polyelectrolytes have been used in hair care.

In our research, we want to make copolymers of 4VP and DMAEMA in a lightly crosslinked form so that they swell in water instead of dissolving. Our group has investigated 4VP in the past for making pH sensitive swellable microspheres. Firman and Hassen attempted to synthesize poly-4VP particles by suspension polymerization. This technique produced large and highly polydisperse particles with low mechanical strength. In our current research, the desired particles are small (ca. 1-2 μm) and monodisperse. Firman and Milde investigated the synthesis of 4VP particles by dispersion polymerization. They found that dispersion polymerization of 4VP in toluene with Kraton G1650 as a stabilizer produces particles with satisfactory size and dispersity.

Milde studied the pH dependent swelling of 4VP particles synthesized by dispersion polymerization in toluene. Swelling and shrinking times of the particles was
found to be approximately 1 minute. The magnitude of the turbidity change between the membranes of swollen and shrunken particles was low.

Poly-DMAEMA is a weak polybase, which is water-soluble (if not crosslinked) both at neutral pH and in acidic media due to protonation of the tertiary amine groups. Recently several research groups have described the synthesis of well-defined, near-monodisperse copolymers based on DMAEMA via living polymerization techniques. Doherty prepared bulk copolymer membranes of poly-DMAEMA-co-HEMA by photopolymerization to investigate its swelling as a function of pH. He prepared membranes a few centimeters in length, and measured the change in the size of the membranes as a function of pH. He did not report the swelling rate of the membranes, but bulk polymers generally require a long time to reach swelling equilibrium. In general, the response of most of the hydrogels to changes in environmental factors is too slow for most applications. Fast response time is the key for successful applications of smart hydrogels in drug delivery, artificial muscles, microswitches and sensors. However, we have achieved response times as short as a few second by making the polymers in the form of tiny microspheres.

In my work, lightly crosslinked microspheres of poly-DMAEMA-co-HEMA and poly-4VP-co-HEMA were prepared by dispersion polymerization. These particles were embedded in a hydrogel forming a membrane, and the turbidity of the membrane was measured as a function of pH.

There are two main advantages of incorporating the functional polymers (poly-DMAEMA and poly-4VP) into poly-HEMA.
1) The degree of swelling can be controlled by varying the functional polymer concentration: Doherty reported that as the poly-DMAEMA concentration in poly-HEMA increased, the mechanical stability of the membrane decreased. At higher than a 50% (w/w) poly-DMAEMA concentration, the membranes started to break apart due to the excessive swelling force.

2) Response time can be improved: Lower functional group concentrations mean fewer ionizeable sites. As a result particles can reach the swelling equilibrium faster.

One more advantage of adding HEMA as a comonomer is that the dispersion polymerization of poly-DMAEMA in toluene was not achieved, despite several attempts. However, DMAEMA can copolymerize with HEMA in toluene at low DMAEMA levels. High DMAEMA levels (around 70%) did not yield microparticles, either.

We expect that using HEMA as a comonomer will improve the swelling and shrinking rate of the microparticles. Fast response is essential for sensors performing real-time detection and monitoring. We focused on two things to make microparticles that can swell and shrink fast: particle size and porosity. Making the particles small enough (ca. a few microns) and porous helps water and the analyte penetrate into the particle faster. Porous microparticles can be synthesized by adding inert solvents, or monomers with large leaving groups, into the polymer formulation. After polymerization is complete, inert solvents or the large leaving group are extracted out. With this process, it is possible to create macro and/or micro pores in the particles.
Polymers like poly-HEMA are classified as hydrogels because they are hydrophilic and can hold water in varying amounts, up to hundreds of times of their own weight. Therefore, they have pores and channels when they are hydrated, and we don’t need an extra procedure to make them porous. Because the hydrogels have some water, ions and molecules can easily diffuse in and out of them. The ideal amount of water in the hydrogel is about 20-30%. If the water content is too high, it will decrease the initial refractive index of the polymer resulting in decreased sensitivity. If the polymer has too little water, it may not be porous enough for fast access.

The magnitude of the change in the refractive index of the polymer as a result of swelling is one of the crucial factors for our sensing system. This also determines the sensitivity of our sensor. The ideal case is the maximum refractive index change with the minimum swelling. Refractive index of the microspheres is determined by the amount of water they absorb as they swell. Water causes the refractive index of the polymer to decrease by diluting it. If the initial refractive index of the polymer is known, the refractive index of the swollen polymer can be calculated by using the equation below.

\[
RI_{sp} = \frac{(RI_{up}V_{up} + RI_wV_w)}{V_T}
\]

Where, \(RI_{sp}\) is the refractive index of swollen polymer, 
\(RI_{up}\) is the refractive index of unswollen polymer (dry polymer),
\(RI_w\) is the refractive index of water,
\(V_{up}\) is the volume of unswollen polymer,
\(V_w\) is the volume of water absorbed by the polymer, and
\(V_T\) is the total volume of polymer and water.
Figure 5.1 shows how the refractive index of poly-HEMA changes with increasing swelling index. The water content increases as the swelling index increases. Lightly crosslinked poly-HEMA can absorb about 35% water by weight, but we assumed that the polymer here could absorb more water. The figure gives us some important

Figure 5.1 Theoretical relation between the swelling index and the refractive index of poly-HEMA. (V_s: volume of swollen polymer, V_us: volume of unswollen polymer)

* See the table in Appendix B for water content corresponding to the swelling index.
information about the refractive index change. The largest refractive index change occurs at the beginning of swelling, and the change decreases as the swelling proceeds. The figure also tells us that the larger the initial refractive index, the larger the change due to swelling. For example, if the initial (unswollen) refractive index of the polymer is 1.51, it drops to 1.45 when the polymer volume increased 50% by absorbing water. The difference is 0.06. If the initial refractive index is 1.45, the refractive index will decrease to 1.41 when the polymer increases its volume by 50%. This is a change of 0.04 refractive index units. This can be seen in Figure 5.2.

What these figures tell us is that high initial water content is disadvantageous for our purpose, because initial water decreases the refractive index of the polymer. If the polymer is highly hydrophilic, its initial water content will be high. DMAEMA is this type of polymer. To decrease its water content, a hydrophobic monomer, MMA, was added to the formulation. MMA also increases the mechanical strength of the polymer. 4VP is less hydrophilic than DMAEMA, but for the same reasons, a hydrophobic monomer, styrene, was added in its formulation.

Three different types of monomers were used to make pH sensitive microparticles. Some of their functions and properties are summarized in the Table 5.1.

In order to make the copolymer microspheres described above, the conditions to make poly-HEMA particles were first investigated, because it is the main component of the polymer. We want to make monodisperse poly-HEMA particles with diameter of 1 μm or larger in size for best performance. Then, microparticles with varying percentages functional monomers were synthesized.
Figure 5.2 Theoretical relation between the swelling index and the refractive index of swellable polymers with varying initial refractive indices. (\( V_s \): volume of swollen polymer, \( V_{us} \): volume of unswollen polymer)
### Table 5.1 Monomers used for making pH sensitive swellable microspheres

<table>
<thead>
<tr>
<th>FORMULATION-1</th>
<th>FORMULATION-2</th>
<th>TYPES AND FUNCTIONS OF THE MONOMERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMA</td>
<td>HEMA</td>
<td>Substrate (base) monomer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Hydrophilic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Provides flexibility, strength and porosity</td>
</tr>
<tr>
<td>4VP</td>
<td>DMAEMA</td>
<td>Functional (sensitive) monomer</td>
</tr>
<tr>
<td>Styrene</td>
<td>MMA</td>
<td>Auxiliary monomer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Hydrophobic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduces the hydrophilicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Increases the mechanical strength</td>
</tr>
</tbody>
</table>
In the next sections of this chapter, experimental procedures are first described. Then, synthesis of poly-HEMA microparticles is discussed. Copolymers of HEMA with 4VP and DMAEMA are evaluated for their response to pH. The chapter ends with conclusions.

5.2 Preparation of poly-HEMA Microparticles by Dispersion Polymerization

Poly-HEMA is usually prepared by free radical solution polymerization. The free radicals for the polymerization of HEMA are generated by chemical initiators, ionizing radiation, or photochemical initiation. Volume contraction occurs when the initial soft matrix is formed. As the network forms completely, it becomes more rigid with no further contractions.

\[
\begin{align*}
\text{HEMA} & \xrightarrow{\text{BPO or AIBN}} \text{Poly-HEMA} \\
& \quad \text{75 °C}
\end{align*}
\]

The critical properties of poly-HEMA, such as sorption and desorption, mechanical behavior, swelling properties, etc., can be controlled by network characteristics, that is the degree of crosslinking and the density, length of crosslinks, and the type and concentration of functional groups.

Ethylene glycol dimethacrylate (EGDMA) is the most commonly used crosslinking agent. Other crosslinking agents are 3-oxapentamethylene dimethacrylate,
2,3-dihydroxytetramethylene dimethacrylate, and trimethylopropane trimethacrylate. The crosslinking agents also play an important role in controlling hydrophilicity and mechanical properties. For fast swelling and shrinking, we prefer the crosslinking density to be very low (ca. 2 mole % of monomers). High crosslinking density makes the polymer hard and rigid, which causes little or no swelling.

A lot of comonomers can be copolymerized with HEMA to change its resultant properties. To increase the tensile strength of HEMA, it can be copolymerized with various hydrophobic monomers. Similarly, more hydrophilic comonomers can be copolymerized with HEMA to improve its hydrophilicity. In my research, two hydrophobic monomers, methyl methacrylate and styrene, were to increase tensile strength and control the water content of the resultant copolymer.

Any factor changing the water content will cause a change in hydrogel dimensions. Poly-HEMA is a very stable hydrogel and its water content is not easily influenced by pH or temperature. By making copolymers of HEMA with acidic or basic monomers, poly-HEMA can be designed to respond to the pH of the environment. In my research, 2-(dimethylamino)ethyl methacrylate and 4-vinyl pyridine were added to the polymer formulation to make copolymers that respond to pH.

Even though HEMA can be polymerized in both polar (including water) and nonpolar solvents, toluene was chosen as the reaction solvent in our research. The reason for this is that polymerization of both 4VP and DMAEMA in polar solvents is not successful due to their high solubility in this type of solvent. As mentioned before, polymerization of 4VP in toluene has been achieved by our group and others.
5.2.1 Stabilizers for Poly-HEMA Synthesis

Determining a suitable stabilizer is very important in dispersion polymerization. Without a stabilizer, particles can easily stick to each other during the soft and sticky phase of polymerization, yielding bulk polymer or large granules. Stabilization in dispersion polymerization occurs sterically. For a material to be a good stabilizer, it must be amphipatic—i.e. contain both an “anchor” segment, with an affinity for the final polymer particle surface, and a solvent soluble segment. The most commonly used amphipatic stabilizers are (1) homopolymers, (2) block and graft copolymers and (3) macromonomers. One can predict what material to use as a stabilizer, depending on the characteristics of the monomer, such as polarity, hydrophobicity and aromacity. However, most often researchers prefer to perform experiments to find the right stabilizer, because there are many factors that affect a polymerization reaction. For this reason, several stabilizers available in our lab were tested. Results are summarized in Table 5.2.

Beads produced in the presence of polystyrene (280 K) or Kraton G1650 were highly monodisperse and non-coagulated (Figures 5.3A and 5.3B). Kraton G1650 was chosen as the most appropriate stabilizer because it dissolves in toluene much faster than polystyrene. Furthermore it is easier to remove Kraton from the suspension of particles during cleaning. Formulation 4-19B was done to confirm the formation of monodisperse poly-HEMA beads obtained in the formulation 4-18B.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Stabilizer</th>
<th>Solvent</th>
<th>Initiator</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-16A</td>
<td>S/E-B(89K)</td>
<td>Toluene</td>
<td>BPO</td>
<td>Large, polydisperse and coagulated particles.</td>
</tr>
<tr>
<td>4-16B</td>
<td>PVP</td>
<td>Toluene</td>
<td>BPO</td>
<td>Large, coagulated particles</td>
</tr>
<tr>
<td>4-17A</td>
<td>PVP (Increased)</td>
<td>Toluene</td>
<td>BPO</td>
<td>Large, coagulated particles</td>
</tr>
<tr>
<td>4-17B</td>
<td>PS (MW: 5K)</td>
<td>Toluene</td>
<td>BPO</td>
<td>Polydisperse particles</td>
</tr>
<tr>
<td>4-18A</td>
<td>PS (MW: 280K)</td>
<td>Toluene</td>
<td>BPO</td>
<td>Highly monodisperse particles formed</td>
</tr>
<tr>
<td>4-18B</td>
<td>Kraton G1650</td>
<td>Toluene</td>
<td>BPO</td>
<td>Highly monodisperse particles formed</td>
</tr>
<tr>
<td>4-19A</td>
<td>ABA (89K)</td>
<td>Toluene</td>
<td>BPO</td>
<td>Some coagulated rubbery particles</td>
</tr>
<tr>
<td>4-19B</td>
<td>Kraton G1650</td>
<td>Toluene</td>
<td>BPO</td>
<td>Highly monodisperse particles formed</td>
</tr>
</tbody>
</table>

**Table 5.2** List of dispersion polymerizations of HEMA for determining a suitable stabilizer.

CAP: Cellulose Acetate Butyrate  

PVP: Poly(vinylpyrrolidone)  

PS: Polystyrene  

S/E-B (89K): Styrene/Ethylene-Buthylene, an ABA block copolymer with MW: 89K  

Kraton G1650: Ethylene, butylene-styrene triblock copolymer
Figure 5.3 a) Poly-HEMA microparticles stabilized with polystyrene (280K), b) poly-HEMA microparticles stabilized with Kraton G1650.
Kraton is the trade name of a series of styrene/ethylene-butylene block copolymers, and the type of the Kraton is indicated with G#. Even though ABA is also a styrene/ethylene-butylene block copolymer, it did not produce the desired poly-HEMA particles. Our past experience with the synthesis of poly (4-vinyl pyridine) showed that, different Kraton types produce particles with different size and morphologies.

5.2.2 Effect of Solvent on Dispersion Polymerization of HEMA

After determining the right stabilizer, the next step was to investigate the conditions to control the particle size. In general, we want the size of the microparticles to be about 1 μm or larger, because turbidity of the large particles changes more than that of smaller particles at longer wavelengths. The wavelength of the light source to be used in our fiber optic sensor system will be 1310 nm, a telecommunications wavelength where optical fiber attenuation is very low. Furthermore, the wavelength dependence of the turbidity change is less with larger particles.

Miele reported in his systematic work on dispersion polymerization that the two major factors that affect microparticle size and formation are solvent composition and monomer concentration. Others factors are initiator concentration, reaction temperature and stabilizer concentration. Controlling initiator concentration and reaction temperature to vary the particle size is difficult and unreliable. In general, all the parameters in dispersion polymerization interact with each other. Therefore, searching for optimum conditions to control particle size is a difficult and laborious task. For example, as you try to optimize reaction temperature your optimum initiator concentration changes, because the decomposition rate of the initiator increases with
increasing temperature. Similarly, as you increase the monomer concentration, properties of the reaction solvent (e.g. polarity of solvent) change because the monomer itself also acts as a solvent for its polymer.

Increasing the solubility of the resulting polymer in the solvent, usually leads to an increase in the diameter of the microparticles. Poly-HEMA is more soluble in polar solvents. In order to vary the polarity of the reaction solvent (toluene) a set of alcohols with varying polarities were added in the reaction mixture. The alcohols used were ethanol, 1-propanol, 1-butanol and 1-dodecanol. The amount of each alcohol added in the reaction mixture was 10% of total volume. Table 5.3 summarizes the result of this work. The SEM micrographs of the microparticles are shown in Figure 5.4.

Table 5.3 shows that small changes in the solubility parameter of the reaction solvent have a larger impact on particle formation. The solubility parameter of the poly-HEMA is given in the literature as 13.4. As the solubility parameter of the reaction solvent increases approaching to 13.4, the particle size increases but the particles become soft and squishy. In the presence of 1-propanol and ethanol only coagulated bulk polymer forms. The change in the particle size is not as large as observed in Miele's study. In his work, an increased solubility parameter significantly increased the particle size. His formulations involve hydrophobic monomers (e.g. styrene and chloromethyl styrene) in polar solvents (e.g. methanol and ethanol). However, the reaction components in my case were opposite. I worked with a hydrophobic solvent (toluene) and a hydrophilic monomer (HEMA). I should consider that some other factors (e.g. type of co-solvents) might affect polymerization in my system. Therefore, some other co-solvents (e.g. DMSO) may need to be tested.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility parameter of pure solvent, $\delta_i$ (cal/cm$^3$)$^{1/2}$</th>
<th>Average solubility parameter of alcohol-toluene mixtures, $\delta_i$ (cal/cm$^3$)$^{1/2}$</th>
<th>Result (Size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>12.7</td>
<td>9.33</td>
<td>Coagulated product</td>
</tr>
<tr>
<td>1-propanol</td>
<td>11.9</td>
<td>9.24</td>
<td>Coagulated product</td>
</tr>
<tr>
<td>1-buthanol</td>
<td>11.4</td>
<td>9.19</td>
<td>Particles with slightly irregular shape $\left(1.09 \pm 0.04 , \mu m\right)$</td>
</tr>
<tr>
<td>1-dodecanol</td>
<td>9.80</td>
<td>9.00</td>
<td>Particles with increased size $\left(0.93 \pm 0.02 , \mu m\right)$</td>
</tr>
<tr>
<td>Toluene (No co-solvent)</td>
<td>8.91</td>
<td>8.91</td>
<td>Highly monodisperse particles $\left(0.50 \pm 0.01 , \mu m\right)$</td>
</tr>
</tbody>
</table>

*Table 5.3 Dependence of dispersion polymerization of HEMA on solvent polarity.*
Figure 5.4 SEM micrographs of poly-HEMA microparticles synthesized by dispersion polymerization in toluene with various cosolvents: A) ethanol, B) 1-propanol, C) 1-buthanol, D) 1-dodecanol.
5.2.3 Effect of Monomer Concentration on Dispersion Polymerization of HEMA

Monomer concentration is the most convenient parameter for controlling particle size. One should get larger particles with increasing monomer concentration under well-controlled conditions. Table 5.4 shows the results of polymerizations for three different monomer concentrations. The monomer concentrations varied from 5% to 15%. SEM micrographs of the resulting products are shown in Figure 5.5.

<table>
<thead>
<tr>
<th>Monomer Concentration (% V/V)</th>
<th>% Recovery</th>
<th>Average Particle Size (μm)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>&lt;50</td>
<td>0.50 ±0.01</td>
<td>5</td>
</tr>
<tr>
<td>10%</td>
<td>&gt;70</td>
<td>0.80 ±0.04</td>
<td>5</td>
</tr>
<tr>
<td>15%</td>
<td>&gt;90</td>
<td>1.03 ±0.11</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 5.4 Effect of monomer concentration on particle size and distribution in dispersion polymerization of HEMA.

As the monomer concentration increased, the particle diameter increased from 0.50 μm to 0.80 μm at the 10% monomer level and 1.03 μm at the 15% monomer level. However, particles started to become less uniform at high monomer concentrations, the polydispersity increased from 5% to 11%. At monomer levels lower than 5% the yield decreased dramatically. For some batches, no product was obtained. At monomer levels higher than 15%, a very large amounts of coagulated product were obtained.
Figure 5.5 SEM micrographs of poly-HEMA microparticles synthesized by dispersion polymerization at various monomer concentrations: A) 5%, B) 10% C) 15%
Recoveries given in the table are approximate, because it was difficult to recover the product due to the losses during the cleaning procedures. The numbers presented here are intended to give an idea that the % yields increase with increasing monomer concentration.

The reason why the particles coagulated at high monomer levels might be similar to the reasons for the solvent effect (Section 5.2.2). The monomer itself also acts as a solvent for its polymer. This effect increases with increasing monomer concentration. Furthermore, it was indicated before that particles pass through a soft and sticky phase during their formation in dispersion polymerization. If insufficiently stabilized, which is often the case at high monomer concentrations, particles can easily coagulate with each other forming a bulk polymer. In addition, it can be expected that the probability of particle collisions, which may cause coagulation, will increase with increasing monomer concentration.

In an attempt to improve stabilization at high monomer concentration (15% v/v), the amount of stabilizer (Kraton G1650) was increased. The result was smaller and more coagulated particles. Kraton is a rubbery block co-polymer. Increasing its amount in the reaction medium causes an increase in the viscosity. Maybe this is the reason for getting more coagulated product.

5.3 Synthesis of Copolymers of HEMA with DMAEMA and 4VP by Dispersion Polymerization

Copolymers of HEMA with functional monomers, DMAEMA and 4VP, were prepared by varying the amount of DMAEMA and 4VP. The conditions that yielded the
best poly-HEMA particles were employed for copolymer preparations. Ethyleneglycol dimethacrylate (EGDMA) was used as a crosslinker at 2 mole % of the monomers level. 1.0-1.5% (w/w) Kraton G1650 solution in toluene was used as stabilizer. Unlike the poly-HEMA synthesis, only AIBN was used as an initiator in the copolymer synthesis. Compounds with amine groups decrease the decomposition temperature of BPO. If the amine level is high, BPO may react vigorously at room temperature or at lower temperatures. Toluene was used as the reaction solvent and the reactions were carried out at 75-80 °C for 12 to 24 hours.

HEMA-DMAEMA microspheres were prepared at DMAEMA concentrations ranging from 10% to 30%. Microparticles with a higher DMAEMA percentage were too soft and often deformed during cleaning. Attempts to make HEMA-DMAEMA microparticles with 70% or higher DMAEMA levels were not successful.

HEMA-4VP microparticles were relatively easier to synthesize. Particles with 10% to 50% 4VP were prepared successfully. Particles with 4VP concentrations greater than 50% were partially coagulated. Therefore, particles used for swelling and turbidity measurements studies contained 50% or less 4VP.

Size characterization of the microparticles has been performed using a light microscope. Figure 5.6 A and B show pictures of HEMA-DMAEMA (10%) and HEMA-4VP(10%) microparticles, respectively. HEMA-DMAEMA microparticles were usually larger than HEMA-4VP microparticles with diameters ranging from 2 to 4 microns. These particles were also more polydisperse compared to HEMA-4VP particles. HEMA-4VP particles were smaller, (ca. 1.0-1.5 microns in diameter) but more uniform.

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Figure 5.6 Light microscopy images of A) copolymer of HEMA-DMAEMA(10%) and B) copolymer of HEMA-4VP(10%).
5.4 Evaluation of Copolymers of DMAEMA for Swelling and pH Sensing

Lightly crosslinked poly-HEMA has approximately 35% water when it is fully hydrated. Hydration lowers its refractive index from 1.51 to approximately 1.44. When co-polymerized with DMAEMA, the water content of poly-HEMA increases because DMAEMA is more hydrophilic than HEMA.

Doherty investigated the water content and swelling properties of poly-HEMA-DMAEMA membranes. He found that the water content of poly-HEMA with 10% DMAEMA was 32% in pH 10 buffer solution and 41% in pH 4 buffer solutions. Poly-HEMA with 50% DMAEMA contains 35% water in pH 10 buffer solution and 62% water in pH 4 buffer solution. These numbers also show that HEMA-DMAEMA copolymers contain large amounts of water in the shrunken state. As mentioned in Chapter 3, it was difficult to determine the water content of HEMA-DMAEMA microparticles because they were too soft and swollen.

High initial water content is not good for our purpose, because it decreases the refractive index of the polymer. The magnitude of the change in the refractive index of the polymer as a result of swelling is a crucial factor for our sensing system. The ideal case is the maximum refractive index change with minimum swelling. The membranes with poly-HEMA-DMAEMA particles having 10% and 30% DMAEMA show very little turbidity change when exposed to pH 4 and pH 10 buffers. Particles with 50% DMAEMA showed almost no turbidity change. Synthesis of microparticles with 70% or higher DMAEMA content was unsuccessful by dispersion polymerization in toluene.

In order to reduce the initial water content, a hydrophobic monomer, methyl methacrylate (MMA), was added to the formulation. Table 5.5 shows the initial water content.
content of the poly-HEMA-DMAEMA-MMA copolymers. It was expected that the presence of MMA in the copolymer would decrease the water content. The presence of hydrophobic monomers increases the mechanical strength of hydrophilic polymers. MMA makes the copolymer more brittle in the dry form, but when hydrated it becomes flexible due to the plasticizing effect of water. For long-term applications, hydrogels must have structural resilience to maintain their property of repeated expansion and collapse.55

Since we don’t know the water content of HEMA-DMAEMA particles, we can not compare HEMA-DMAEMA and HEMA-DMAEMA-MMA particles in terms of the change in their water content. However, Table 5.5 helps us to calculate the refractive indices of hydrated HEMA-DMAEMA-MMA microparticles.

<table>
<thead>
<tr>
<th>% DMAEMA</th>
<th>% HEMA</th>
<th>% MMA</th>
<th>% WATER</th>
<th>RI&lt;sub&gt;DP&lt;/sub&gt;</th>
<th>RI&lt;sub&gt;HP&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>70</td>
<td>20</td>
<td>37</td>
<td>1.516</td>
<td>1.444</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>20</td>
<td>49</td>
<td>1.513</td>
<td>1.421</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>20</td>
<td>54</td>
<td>1.511</td>
<td>1.412</td>
</tr>
</tbody>
</table>

Table 5.5 Water content of DMAEMA-HEMA-MMA copolymers at varying DMAEMA concentrations. RI<sub>DP</sub> is the refractive index of dry polymer and RI<sub>HP</sub> is the refractive index of hydrated polymer.
Microparticles of poly-HEMA-DMAEMA-MMA were embedded in HYPAN HN68 hydrogel forming 76 µm thick membranes. The turbidity of the membranes was measured by exposing them to acidic (pH 4) and basic (pH 10) buffer solutions. Figure 5.7 shows the turbidity spectra of the membranes. The magnitude of the turbidity change of the particles with 30% DMAEMA was surprisingly high, 0.472. As expected, the turbidity change decreases with decreasing DMAEMA concentration.

Response times of the particles were measured by monitoring the turbidity change as a function of time. As seen in the Figure 5.8, swelling and shrinking response times of the particles become faster as the DMAEMA concentration decreases. This is an expected result because there will be fewer sites to be protonate as the DMAEMA concentration decreases. As a result, the particles will reach their equilibrium swollen or shrunken state more rapidly. Particles with 20% DMAEMA swell and shrink in about 2 seconds, while the particles with 30% DMAEMA swell in 7 seconds and shrink in 17 seconds. The swelling and shrinking rates of 10% DMAEMA could not be determined because of the low turbidity change. We often observe a difference between the rates of swelling and shrinking. This will be discussed at the end of the chapter.

There are many factors that control the swelling and shrinking of microparticles, such as particle size, hydrophilicity, functional group density, particle morphology, etc. All these factors cumulatively affect water and ion diffusion in and out of the particles, and the mechanical force generated by swelling and shrinking.
Figure 5.7 Effect of DMAEMA concentration on the turbidity of DMAEMA-HEMA-MMA copolymer microspheres embedded in a hydrogel.
Figure 5.8 Effect of DMAEMA concentration on the response time of DMAEMA-HEMA-MMA copolymer microspheres embedded in a hydrogel.
The size of the channels in the polymer decrease as shrinking proceeds. This naturally slows the diffusion of the materials in and out of the polymer resulting in a slower shrinking. On the other hand, as the polymer swells the channels open gradually, allowing the water to enter the polymer more easily.

Mechanical force applied to the microparticles may affect the swelling and shrinking rate, too. As the particles expand during swelling, they may need to overcome an opposing force applied by the surrounding hydrogel network. This force will increase with increasing stiffness and crosslinking of the hydrogel network.

**pH Dependence of Microparticles**

The pH sensitivity of hydrogels results from the presence of weak acid or base functionalities on the polymer backbone. The window of pH at which hydrogels undergo a substantial volume changes depends on the type of weak acid or base used. If the hydrogels contain a weak acid functionality, they will swell more as the pH of the medium increases. The reverse is true with hydrogels containing weak bases.

Since the membrane with 30% DMAEMA beads showed the largest turbidity change between basic and acidic conditions, this membrane was used to determine the response of the poly-HEMA-DMAEMA-MMA copolymer system to varying pH. The turbidity of the membrane was recorded for both increasing and decreasing pH. Figure 5.8 shows that the sensitive pH range is approximately 6 to 8. The sinusoidal curves were linearized to obtain the dynamic range and the apparent pKa of the membrane. Figure 5.9A and 5.9B show that apparent pKa of the membrane is 7.10 for decreasing pH and 7.24 for increasing pH. The pKa of the DMAEMA monomer is given as 7.94 in the
Figure 5.9 Turbidity of DMAEMA-HEMA microspheres as a function of pH.
Figure 5.10 Turbidity of HEMA-DMAEMA copolymer microspheres as a function of pH. A) Increasing pH, B) Decreasing pH.
We often observe a change in the apparent pKa of the acidic or basic compounds after they were bound on a polymer chain. For example, pKa of diethyl amine decreased from 10.9 to 7.4-7.5 when it was bound to a polystyrene backbone.

5.5 Evaluation of Copolymers of 4VP for Swelling and pH Sensing

Unlike DMAEMA, 4VP is a convenient monomer to work with. Synthesis of poly-4VP microparticles by dispersion polymerization is easier than synthesis of poly-DMAEMA microparticles. 4VP has a higher refractive index and higher mechanical strength than DMAEMA. Refractive indices of poly-4VP and poly-DMAEMA are 1.626 and 1.517, respectively. For these reasons, 4VP has been more intensively studied by our group.

In my research microparticles of HEMA-4VP and HEMA-4VP-Styrene copolymers were synthesized by dispersion polymerization using toluene as the solvent. A typical recipe is shown in Table 5.6. Polymerizations were carried out in 500-ml round bottom flasks in a hot water bath maintained at 80 °C. The total volume of each batch was 100 ml.

<table>
<thead>
<tr>
<th>Monomer(s)</th>
<th>Crosslinker</th>
<th>Initiator</th>
<th>Stabilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 g</td>
<td>2 mol % of mon</td>
<td>1% w/w</td>
<td>1.5 g</td>
</tr>
</tbody>
</table>

Table 5.6 A typical recipe for dispersion polymerization of HEMA-4VP and HEMA-4VP-Styrene copolymers.
Table 5.7 shows the average diameters of the microparticles with varying monomer contents. Figure 5.11 and Figure 5.12 show the microscope images of HEMA-4VP and HEMA-4VP-Styrene microaparticles respectively. The dispersion polymerization method produced fairly monodisperse particles.

<table>
<thead>
<tr>
<th>EXP#</th>
<th>%4VP</th>
<th>HEMA</th>
<th>Styrene</th>
<th>Particle Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-62</td>
<td>10</td>
<td>90</td>
<td>0</td>
<td>1.84 ± 0.17</td>
</tr>
<tr>
<td>4-25B</td>
<td>30</td>
<td>70</td>
<td>0</td>
<td>1.64 ± 0.13</td>
</tr>
<tr>
<td>4-54</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>1.59 ± 0.10</td>
</tr>
<tr>
<td>4-61</td>
<td>10</td>
<td>70</td>
<td>20</td>
<td>1.73 ± 0.20</td>
</tr>
<tr>
<td>4-60</td>
<td>30</td>
<td>50</td>
<td>20</td>
<td>1.97 ± 0.24</td>
</tr>
<tr>
<td>4-59</td>
<td>50</td>
<td>30</td>
<td>20</td>
<td>2.13 ± 0.12</td>
</tr>
</tbody>
</table>

**Table 5.7** Diameters of HEMA-4VP and HEMA-4VP-Styrene microparticles produced by dispersion polymerization

Milde reports that the membranes with poly-4VP particles have very low turbidity changes with response times about 1 minute or longer. The apparent pKa reported by Milde was 4.6. Because of the low apparent pKa, poly-4VP particles are shrunken at neutral pH. However, they can still absorb a large amount of water due to their hydrophilicity. This causes a decrease in their initial refractive index. Since the sensitivity of our sensors depends on the refractive index change, a low initial refractive index means a low turbidity change, and low sensitivity.
Figure 5.11 Microscope pictures of HEMA-4VP microparticles with varying monomer contents.
Figure 5.12 Microscope pictures of HEMA-4VP-Styrene microparticles with varying monomer contents.

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Table 5.8 shows the water content of copolymers of 4VP and HEMA microparticles and their calculated refractive indices in the dry and hydrated forms. Refractive indices of the dry copolymers were calculated using the ChemSketch Molecular Modeling Program (ACD LAB. Inc.). The refractive index of the hydrated beads for each formulation is calculated by considering their experimental water contents, which were determined gravimetrically as described in Chapter 3.

<table>
<thead>
<tr>
<th>% 4VP</th>
<th>% HEMA</th>
<th>% WATER</th>
<th>RI&lt;sub&gt;DP&lt;/sub&gt;</th>
<th>RI&lt;sub&gt;HP&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>90</td>
<td>27</td>
<td>1.528</td>
<td>1.475</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>40</td>
<td>1.547</td>
<td>1.462</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>56</td>
<td>1.568</td>
<td>1.465</td>
</tr>
</tbody>
</table>

**Table 5.8** Water content and calculated refractive index of 4VP-HEMA copolymers at varying 4VP concentrations. RI<sub>DP</sub> is the refractive index of dry polymer and RI<sub>HP</sub> is the refractive index of hydrated polymer.

In order to decrease the initial water content of the copolymers, 20 % styrene was included in the formulation. Table 5.9 shows that styrene has a large effect on the water content of 4VP-HEMA microparticles. As a result, the calculated refractive indices of hydrated microparticles increase significantly.
Three different formulations have been studied to determine the effect of varying the percentage of functional monomer and the effect of decreasing the water content by adding a hydrophobic monomer.

The change in the turbidity of membranes membranes containing 1% w/w HEMA-4VP microspheres was determined for each system for comparison. Figure 5.13 shows that as the 4VP concentration in the copolymer increases the turbidity difference increases. Turbidities of the membranes in pH 3 and pH 6 buffer solutions (Buffer concentration: 0.1 M and IS: 0.1M) at 1000 nm are given in Table 5.10 for comparison.

Swelling and shrinking times of the particles embedded in a HYPAN HN68 membrane were measured at pH 3 and pH 6. Time measurements for swelling and shrinking cycles were repeated for a few times for each particle type. The particles reach their maximum swollen state at pH 3 and maximum shrunken state at pH 6. Figure 5.14 shows that the swelling of the particles is very fast compared to shrinking. We often observe this asymmetric behavior for swellable polymers. In the case described here the swelling is extremely fast compared to shrinking. Possible reasons will be discussed at
the end of this chapter. The shrinking response time of each formulation is listed in Table 5.10 for comparison.

<table>
<thead>
<tr>
<th>% 4VP</th>
<th>% HEMA</th>
<th>Turbidity of at 1000nm</th>
<th>Response Time/Seconds (Shrinking)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH 3</td>
<td>pH 6</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>0.058</td>
<td>0.126</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>0.052</td>
<td>0.162</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>0.176</td>
<td>0.337</td>
</tr>
</tbody>
</table>

**Table 5.10** Turbidities and response times of 4VP-HEMA particles.

**Turbidity –pH relation**

The turbidity of particles with 50% 4VP content was measured in buffer solutions between pH 3 and pH 6. Measurements were performed for both increasing and decreasing pH. Figure 5.15 shows that the membrane is sensitive to pH between 3.5 and 5.5. Linearized curves of sinusoidal turbidity-pH relationship are given in Figure 5.15 a and b. The apparent pKa of the membrane is obtained from both curves as 4.60. The slope of both curves is greater than 1.00 indicating that response does not follow the Henderson-Hasselbach equation.
Figure 5.13 The effect of 4VP concentration on the turbidity of HEMA-4VP microspheres embedded in HYPAN HN68 hydrogels.
Figure 5.14 The effect of 4VP concentration on swelling (decreasing turbidity) and shrinking (increasing turbidity) response of HEMA-4VP particles embedded in 76 μm thick HYPAN HN68 membranes. Data were collected at 600 nm.
Figure 5.15 Turbidity of 4VP-HEMA microspheres as a function of pH. Microspheres were embedded in 76 μm thick HYPAN HN68 hydrogels and the data were collected at 600 nm.
Figure 5.16 Response times of 4VP-HEMA copolymer microspheres as a function of pH. a) Decreasing pH, b) Increasing pH.
5.6 Evaluations of copolymer microspheres of 4VP, HEMA and Styrene

The turbidity spectra of microparticles containing 4VP, HEMA and styrene are shown in Figure 5.17. The turbidities of each formulation in the swollen and shrunken forms are given in Table 5.11. The general trend is that the turbidity difference increases with increasing 4VP concentration.

Swelling and shrinking times of the particles embedded in a HYPAN membrane were measured at pH 3 and pH 6. Time measurements for swelling and shrinking cycles were repeated a few times for each particle type. The particles reach their maximum swollen state at pH 3 and maximum shrunken state at pH 6. Figure 5.18 shows that the shrinking of the particles is very fast comparing to swelling. The striking result is that this is opposite to 4VP-HEMA, which swells very rapidly but shrinks more slowly. 4VP-HEMA-S particles shrink fast but swell slowly.

Turbidity differences and swelling rates of 4VP-HEMA-S microparticles are given in Table 5.11.

<table>
<thead>
<tr>
<th>% 4VP</th>
<th>% HEMA</th>
<th>% S</th>
<th>Turbidity at 1000nm</th>
<th>Response Time/Seconds (Swelling)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pH 3</td>
<td>pH 6</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>20</td>
<td>0.569</td>
<td>0.488</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>20</td>
<td>0.326</td>
<td>0.534</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>20</td>
<td>0.270</td>
<td>0.399</td>
</tr>
</tbody>
</table>

Table 5.11 Turbidities and response times of 4VP-HEMA-S particles.
Figure 5.17 The effect of 4VP concentration on the turbidity of HEMA-4VP-S microspheres embedded in HYPAN HN68 hydrogels.

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Figure 5.18 The effect of 4VP concentration on the swelling and shrinking response of 4VP-HEMA-S copolymer microspheres embedded in a 76 μm thick HYPAN HN68 membrane. Data were collected at 1000 nm.
**Turbidity-pH Relation**

Since the formulation with 30% 4VP produced the largest turbidity change, particles of this formulation were used to determine how turbidity varies with pH. The turbidity of HYPAN HN68 membranes containing the particles with 30% 4VP were measured as a function of pH. Figure 4.18 shows that the membrane is sensitive to pH between approximately 3.5 and 5.5. Membrane turbidity was measured for both increasing and decreasing pH using buffer solutions with 0.1M ionic strength.

![Turbidity-pH Relation](image)

**Figure 5.19** Turbidity of 4VP-HEMA-S copolymer microspheres with 30% 4VP content as a function of pH. Data was collected at 1000 nm.

Figure 5.19 A and B show the linearized form of the turbidity vs. pH curves. These figures indicate that the apparent pKa of the membrane is about 4.4 for increasing pH and about 4.2 for decreasing pH. The difference in pKa is caused by the hysteresis, which is a problem we often deal with. As it was observed before (Figure 5.16), the slope is considerably larger than 1.00 indicating that this system does not follow the Henderson-Hasselbach equation.
Figure 5.20 Turbidity of 4VP-HEMA-S copolymer microspheres as a function of pH. A) Increasing pH, B) Decreasing pH.
5.7 Discussion of the Results

Addition of styrene to the 4VP-HEMA copolymer decreased the initial water content and, as a result, increased the turbidity change caused by swelling and shrinking.

When the three turbidity spectra of 4VP-HEMA microparticles (Figure 5.12) and 4VP-HEMA-S microparticles (Figure 5.16) are compared, it can be seen that addition of styrene increased the turbidities of the membranes in both the swollen and the shrunken state. This is an expected result of lowering the initial water content, which resulted in an increase in the initial refractive index of the microparticles. Larger refractive index means larger turbidity. If the turbidity of the membranes in the swollen state could be decreased, bringing it closer to zero, this would further improve membrane sensitivity. This can be achieved by a systematic study of copolymer formulation. In my work, only one styrene concentration (20%) was investigated to determine its effect on the initial water content and the turbidity of the microparticles. The concentration of styrene can be varied to determine the optimum conditions, or other hydrophobic monomers can be tested.

When the response times (Figure 5.14 and 5.18) are compared, it can be seen that the response time of the microspheres decreased after adding styrene to the formulation. The most interesting result is the shift in the response times for swelling and shrinking. While 4VP-HEMA particles swell fast and shrink slowly, 4VP-HEMA-S particles act oppositely. They swell slowly and shrink fast.

There are many factors that affect the swelling and shrinking rates of polymers, such as functional group concentration, particle morphology, particle size, porosity and
polymer memory. All these factors cumulatively affect the swelling and shrinking rates of a polymer.

It was mentioned before that the smaller the particles the faster they can swell and shrink, because there will be a shorter distance for water or the molecules to travel to reach the center of the particle. Porosity helps the water and the molecules to access the center easier. Microparticles used in this part of the research were about 2-3 micrometers in size, which are small enough for fast response. The particles also had 20% to 50% initial water content. Containing water means they were porous. Both small size and porosity provide fast response.

As the functional group concentration increases, the response time is expected to increase because the time needed to protonate or deprotonate the amine groups will increase.

4VP-HEMA particles swell rapidly and shrink slowly (Figure 5.14), but 4VP-HEMA-S particles swell slowly and shrink rapidly (Figure 5.18). This can be explained by the degree of swelling of the microparticles when they were embedded in the hydrogel. 50% 4VP-50%HEMA particles have higher initial water content (56%) than 50% 4VP-30%HEMA-20%S particles (28%). This means that 50% 4VP-50%HEMA particles are initially more swollen than 50% 4VP-30%HEMA-20%S particles. When they are embedded in a hydrogel, their initial volume will be their relaxation volume. They will generate an opposing force to any external or internal disturbance that changes their volume. They will prefer to go back to their relaxation state. Therefore, 4VP-HEMA particles will try to reach the swollen state faster. On the other hand, 4VP-HEMA-S particles will reach the shrunken state faster. Similarly, DMAEMA copolymer
particles behave like 50% (4VP-HEMA) particles. DMAEMA particles are also in a partial swollen state. Therefore, they swell faster than they shrink (Figure 5.8). One possible method to overcome the hysteresis is to condition the polymer by cycling it between the swollen and shrunken states repeatedly.

The apparent pKa of 4VP-HEMA particles was determined as 4.6, which is consistent with Milde’s results. However, the apparent pKa of 4VP-HEMA-S particles was determined as 4.4 when the pH increased and 4.2 when the pH decreased. It seems that addition of styrene causes hysteresis. To solve the problem, optimum conditions for particle formulation should be investigated systematically.

5.8 Conclusions

The refractive index of swellable polymers decreases with increasing swelling and water content. The rate of refractive index change is high at the beginning of swelling and decreases as swelling proceeds. In order to get a high turbidity change, the initial refractive index of the swellable polymer should be as high as possible and the initial water content should be low, ca. 20%-30% w/w.

Poly-HEMA microparticles up to 1μm diameter can be successfully produced by dispersion polymerization in toluene using Kraton G1650 as the stabilizer. Copolymers of both DMAEMA and 4VP with HEMA can be produced successfully using the same conditions. Addition of hydrophobic monomers MMA and styrene to the HEMA-DMAEMA copolymer and HEMA-4VP copolymer, respectively, improved the turbidity and response time of the microspheres.
HEMA-DMAEMA microspheres are not suitable for pH sensing because the membranes containing these particles do not have a turbidity changes large enough to measure. Adding MMA to the HEMA-DMAEMA copolymer significantly improved turbidity and response time. This copolymer swells in about 17 seconds and shrinks in 7 seconds. The response time of the copolymer decreases with decreasing DMAEMA percentage. The dynamic range of HEMA-DEMA-MMA microparticles is between pH 5.5 and 8.5. The apparent pKa of microparticles is 7.10 for decreasing pH and 7.24 for increasing pH.

Both HEMA-4VP and HEMA-4VP-S microparticles are sensitive to pH between 3.5 and 5.5. HEMA-4VP microparticles swell rapidly but shrink relatively slowly. On the other hand, HEMA-4VP-S microparticles swell relatively slowly but shrink rapidly. The response times of both copolymer formulations decrease with decreasing 4VP concentration. The apparent pKa of HEMA-4VP copolymer was 4.6, consistent with our earlier determination. The apparent pKa of HEMA-4VP-S depends on the direction of pH change. If the pH increased the apparent pKa is 4.4, but it is 4.2 if the pH is decreased. In general, the addition of styrene in 4VP-HEMA copolymers increases the turbidity and shortened the response time.
CHAPTER 6

SYNTHESIS OF POLYMER MICROSPHERES BY SUSPENSION POLYMERIZATION AND EVALUATION OF THE MICROPARTICLES FOR OPTICAL pH SENSING

6.1 Introduction

The key properties of the swellable polymer microspheres for our sensor applications are particle size, size distribution, porosity and polymer composition. The method of choice to make the particles affects these properties. We have employed two synthesis methods: dispersion polymerization and suspension polymerization. We can make highly monodisperse particles by dispersion polymerization, but we don't have good control on particle size, porosity and particle composition. An important limitation of dispersion polymerization is that it is not possible to control porosity using porogenic solvents. Suspension polymerization is more suited to controlling porosity and composition but this method produces particles with a very large size distribution. Our group has successfully employed a modified form of suspension polymerization method, two-step seeded emulsion polymerization, for making particles that have the features we need. But this method is labor intensive, and is difficult to carry out repeatably. Recently we have been testing a new method called Shirasu Porous Glass (SPG) emulsification technique, in an effort to make microparticles with ideal characteristics in
less time. In the traditional suspension polymerization, the monomer (oil or organic phase) is emulsified in water by vigorous stirring to form small droplets. This technique produces large polydisperse particles. In SPG emulsification, the monomer is forced through a glass membrane with very fine pores. This process produces fairly monodisperse droplets.

Polymer microparticles synthesized by this new method behaved very differently from particles prepared by other methods. Particles swell and shrink at a certain pH rapidly, like a switch. This chapter will discuss the synthesis of polymer microparticles using the SPG emulsification method and subsequent suspension polymerization, and the switch-like response of the particles to pH.

6.2 Synthesis of Poly-VBC Microspheres by Suspension Polymerization Employing SPG Emulsification Method

SPG emulsification and suspension polymerization methods were described in sections 2.4, 2.5 and 3.3. An emulsion of vinylbenzyl chloride monomer (VBC) is formed by SPG emulsification. Approximately 30 ml of monomer and inert porogenic solvents are dispersed in 0.5% aqueous PVA solution. Besides PVA, the aqueous solution (dispersion medium) contains 0.05% sodium dodecyl sulfate (SDS) and 0.025% sodium sulfate to help stabilize the microdroplets. The amount of porogenic solvent added in the monomer was 30% by volume. The purpose of using inert solvents (Xylene and Dodecane) is to create micro and macro pores in the polymer particles. Xylene is a good solvent for poly-VBC. Therefore it mixes with monomer homogenously and creates micro pores in the resulting polymer. On the other hand, dodecane is a poor
solvent for poly-VBC. It spreads in the monomer droplets less homogenously and creates macro pores in the polymer.

The pore size of the glass membrane used in the SPG apparatus was 0.5 μm. This membrane can form monomer droplets with diameters from 1 to 2 μm. The droplets formed during the emulsification were monitored using a light microscope. Figure 6.1 shows a microscope image of a VBC/Water emulsion. The emulsions prepared by SPG are highly stable and can be stored for hours before polymerization.

In the usual polymerization process, the emulsion was transferred into a 500-ml 3-neck round bottom flask, and the flask was immersed in a water bath maintained at 70 °C. The emulsion was stirred during polymerization to prevent coagulation of droplets and to provide homogeneous heat distribution. After polymerization was complete, microparticles were washed repeatedly to remove the stabilizer and unreacted reagents. Inert solvents are extracted during cleaning process. The final product is stored in DI water. The size and the surface morphology of the particles were analyzed by SEM. Figure 6.2 shows an SEM of poly-VBC particles. The average diameter of the particles in this picture is 1.46 μm.

After the particles have been synthesized, the next step is to derivatize them to introduce functionality so that they swell with an external stimulus, pH. For this, the particles were derivatized with diethyl amine. In the process, diethyl amine replaces a –Cl group forming a tertiary amine group bound on the polymer chain (Figure 6.3).
Figure 6.1  
a) Light micrograph of monomer droplets in VBC/Water emulsion  
b) SEM micrograph of poly-VBC particles
6.3 Swelling and Turbidity Measurements for Aminated Poly-VBC Microparticles

Derivatized particles microparticles were immobilized in a HYPAN HN68 hydrogel forming an approximately 50 μm thick membrane for collecting turbidity data. Figure 6.4 shows the relation between turbidity of the membrane and varying pH at 1000 nm. Turbidity increases abruptly between pH 7.0 and 7.2 for increasing pH, and decreases abruptly between pH 6.8 and 6.6 for decreasing pH. We have not observed this switch-like behavior before. The usual behavior is a gradual change in turbidity with pH over a range of 1 to 2 pH units. The synthesis was repeated several times but the result was always the same.
Figure 6.3 Turbidity of HYPAN HN68 membranes containing aminated poly-VBC particles vs. pH.

Response of Poly-VBC Particles to Varying pH

Turbidity

pH

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1

3 4 5 6 7 8 9 10 11

pH 4-10

pH 10-4

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In order to understand the switch-like behavior of the microparticles we decided to monitor the swelling and shrinking of particles by two other independent methods: measuring the size of the particles as a function of pH and titrating the particles to determine the percentage of protonated or unprotonated amine groups as a function of pH. Due to the technical difficulties, the second procedure produced unreliable data. But the first method was successful, and is discussed below.

6.4 Measuring the Change in the Size of the Microparticles as a Function Of pH

A highly polydisperse batch of poly-VBC particles was chosen for size measurements, because it was difficult and unreliable to measure the size particles with diameters less than few micrometers. Therefore, particles as large as 10 μm were chosen for this experiment. Figure 6.5 shows how the size of these particles changes when they were exposed to buffer solutions between pH 10 and 4. And, Figure 6.6 shows the relation between particle size and pH. The result is similar to the relationship between pH and membrane turbidity.

6.5 Possible Reasons for the Switch-Like Swelling and Shrinking Behavior of Poly-VBC Particles and Attempts to Eliminate It

The major difference between these particles and the particles we investigated before is the synthesis method. Previously we employed dispersion polymerization and two-step emulsion polymerization methods to make microparticles. The particles produced by these techniques acted normally.
Figure 6.4 Light micrograph of aminated poly-VBC microparticles, a) shrunken, b) swollen.
Change in the Diameter of Swellable Microparaticles as a Function of pH

Figure 6.5 Dependence of aminated poly-VBC particle size on pH.

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Dispersion and suspension polymerization are different procedures (see the related sections in chapters 2 and 3). By considering the differences, some possible reasons for switch-like behavior might be:

- Different distribution of crosslinking,
- Structural differences: e.g. polymer chains and groups oriented differently,
- Excessive porosity.

A series of experiments was performed to investigate this reason (Table 6.1).

The crosslinker level was increased from 2% to 5%, 10% and 15%. The magnitude of the turbidity change decreased when the crosslinking increased due to increased rigidity of the particles but switch-like behavior continued.

In order to vary the polymer structure, some co-monomers in varying amounts were added to the formulations. To investigate the effect of porosity, a series of microparticles was prepared by varying the amount and percentages of inert solvents. Particles without any inert solvent were also synthesized. After testing many different formulations, the switch-like behavior continued and remained a mystery.

Even though the microparticles prepared by SPG emulsification and subsequent suspension polymerization cannot be used for pH sensing, they can be used as pH indicator because they swell and shrink at a certain pH. Furthermore, these microparticles may find other applications, such as pH controlled micro valves and pumps to be used in lab-on-a-chip technology.
### Table 6.1 Formulations for suspension polymerization.

<table>
<thead>
<tr>
<th>A</th>
<th>VBC</th>
<th>Comonomer</th>
<th>BPO % w/w</th>
<th>Porogenic Solvents % v/v</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>S: 30%</td>
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<tr>
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<td>TCPA: 20%</td>
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<tr>
<td>70</td>
<td>TCPA: 30%</td>
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VBC: Vinyl benzyl chloride, S: Styrene, VA: Vinyl acetate, TCPA: 2,4,5-Trichlorophenyl acrylate, BPO: Benzoyl peroxide

<table>
<thead>
<tr>
<th>B</th>
<th>VBC (g)</th>
<th>DVB % mol/mol</th>
<th>BPO % w/w</th>
<th>Porogenic Solvents % v/v</th>
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DVB: Divinyl benzene (crosslinker)

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<thead>
<tr>
<th>C</th>
<th>VBC (g)</th>
<th>DVB % mol/mol</th>
<th>BPO % w/w</th>
<th>Porogenic Solvents % v/v</th>
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6.6 Conclusions

Fairly monodisperse poly-VBC microparticles with diameters of a few micrometers can be produced by SPG emulsification and subsequent suspension polymerization methods by using a glass membrane with 0.5 micron pore size.

Poly-VBC microparticles synthesized by suspension polymerization swell and shrink very rapidly with a switch-like action. These particles are not suitable for pH sensing, but they can be used as pH level indicators.
CHAPTER 7

CONCLUSIONS

The turbidity of microparticles suspended in water increases with increasing particle size. The magnitude of the turbidity is dependent on the wavelength of the incident light. This dependence increases with decreasing particle size.

Turbidity spectra of particles in different physical environments (such as water, air and hydgel) are similar with some minor differences. In all cases turbidity increases with increasing particle size at longer wavelengths (longer than about 800 nm). At shorter wavelengths turbidity spectra become more complicated.

The magnitude of turbidity decreases as the difference between the refractive index of the particles and the refractive index of the suspension medium decreases.

HYPAN hydrogels shrink during gelation. Shrinking affects the particle concentration in the final membrane, which eventually affects the turbidity. This should be considered in HYPAN membrane preparations.

Unlike turbidity, scattered light intensity increases with decreasing particle size. Maximum light scattering from a monolayer of microparticles on a flat surface occurs when the angle between the surface and the incident light beam is between 70 and 75 degree. This angle might be dependent on particle size, but this was not tested in this research. According to the results discussed in this chapter:
1. In our fiber optic sensor system, using microparticles larger than the wavelength of the incident light is advantageous. If the particles are larger than the wavelength of the incident light, the light is reflected at the interface rather than being scattered. This means, more light is reflected back into the fiber after interacting with particles, resulting in increased sensitivity.

2. In our sensor array approach, using microparticles smaller than the wavelength of the incident light is more advantageous. In this case, light will be scattered rather than being reflected when interacting with particles. In order to direct the maximum amount of scattered light into a detector, the sensor array should be positioned at an appropriate angle to the detection system.

The refractive index of swellable polymers decreases with increasing swelling and water content. The rate of refractive index change is high at the beginning of swelling and decreases as the swelling proceeds. In order to get a high turbidity change, the initial refractive index of the swellable polymer should be as high as possible and the initial water content should be low ca. 20%-30% w/w.

Poly-HEMA microparticles up to 1μm diameter can be successfully produced by dispersion polymerization in toluene using Kraton G1650 as stabilizer. Copolymers of both DMAEMA and 4VP with HEMA can be produced successfully with the same condition. Addition of hydrophobic monomers MMA and styrene to HEMA-DMAEMA and HEMA-4VP respectively, improved the turbidity and response time of microspheres.

HEMA-DMAEMA microspheres are not suitable for pH sensing because the membranes containing these particles do not have a turbidity change large enough to
measure. Adding MMA to a HEMA-DMAEMA copolymer significantly improved its turbidity and response time. This copolymer can swell in about 17 seconds and shrinks in 7 seconds. The response time of the copolymer decreases with decreasing DMAEMA percentage. The dynamic range of HEMA-DEMA-MMA microparticles is between pH 5.5 and 8.5. The apparent pKa of microparticles is 7.10 for decreasing pH and 7.24 for increasing pH.

Both HEMA-4VP and HEMA-4VP-MMA microparticles are sensitive to pH between 3.5 and 5.5. HEMA-4VP microparticles swell rapidly but shrink relatively slowly. On the other hand, HEMA-4VP-ST microparticles swell relatively slowly but shrink rapidly. The response times of both copolymer formulations decreases with decreasing 4VP content. The apparent pKa of HEMA-4VP copolymer was found to be 4.6, which is consistent with our earlier determination. The apparent pKa of HEMA-4VP-ST depends on the direction of the pH change. If the pH increased the apparent pKa is 4.4, but is 4.2 if the pH is decreased.

Fairly monodisperse poly-VBC microparticles with diameters of a few micrometers can be produced by SPG emulsification and subsequent suspension polymerization methods by using a glass membrane with 0.5 micron pore size.

Poly-VBC microparticles synthesized by suspension polymerization swell and shrink very rapidly with a switch-like action. These particles are not suitable for pH sensing, but they can be used as pH level indicators.
REFERENCES


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82. HYPAN Structural Hydrogels, Hymedix International, Inc.


84. Hydromed D Series Hydrophilic Polymers, Cardiotech International.


89. Polymer 2001, 42, 5993-6008. (2 and 3 are from ref of 1)


101. Polymer 1982, 23,748.


APPENDIX A

The tables below show how the physical properties of the resulting PVA hydrogel changes by varying the amount of the initiator (HCl) and the crosslinker (Gluteraldehyde).

### Change in the size of PVA membrane after gel formation.
(Diameter of the mold / Diameter of the final gel)

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<tr>
<th>10% Glutaraldehyde (µL)</th>
<th>25</th>
<th>50</th>
<th>75</th>
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<td>25</td>
<td>1.00</td>
<td>0.92</td>
<td>0.84</td>
<td>0.76</td>
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<td>0.86</td>
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- More opaque
- Harder
- Faster gelation

% Water content of PVA Membranes

<table>
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<tr>
<th>10% Glutaraldehyde (µL)</th>
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- More opaque
- Harder
- Faster gelation

---

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## APPENDIX B

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Vs : Volume of swollen polymer  
Vus : Volume of unswollen polymer