Complex Polymer Particles via Microfluidics

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to Nadia
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شكراً كثيراً لكل شيء. أحبكما جداً

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talha
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Complex Polymer Particles via Microfluidics

List of Abbreviations

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<tbody>
<tr>
<td>AIBN</td>
<td>azobisisobutyronitrile</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>BDM</td>
<td>2-benzyl-2 (dimethylamino) -4’-morpholino- butyrophenone</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer, Emmett and Teller</td>
</tr>
<tr>
<td>BMA</td>
<td>n-butyl methacrylate</td>
</tr>
<tr>
<td>CH</td>
<td>cyclohexanol</td>
</tr>
<tr>
<td>Con A</td>
<td>concanavalin A</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Cu(I) catalyzed azide-alkyne cycloaddition;</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation defined as ( (\sigma/D_p) \times 100 ) where ( D_p ) is the number average diameter and ( \sigma ) is the standard deviation on the diameter</td>
</tr>
<tr>
<td>DBP</td>
<td>di-n-butyl phthalate</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloro methane</td>
</tr>
<tr>
<td>DD</td>
<td>1-dodecanol</td>
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<tr>
<td>DEE</td>
<td>diethyl ether</td>
</tr>
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<td>DEGDA</td>
<td>di(ethylene glycol) diacrylate</td>
</tr>
<tr>
<td>DEP</td>
<td>diethyl phthalate</td>
</tr>
<tr>
<td>di-alkyne</td>
<td>1,7-octadiyne</td>
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<td>DMAEMA</td>
<td>2-(dimethylamino)ethyl methacrylate</td>
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<tr>
<td>DMF</td>
<td>( N,N )-dimethylformamide</td>
</tr>
<tr>
<td>DMPA</td>
<td>2,2-dimethoxy-2-phenylacetophenone</td>
</tr>
<tr>
<td>DMPP</td>
<td>dimethylphenylphosphine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DOP</td>
<td>di-n-octyl phthalate</td>
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<tr>
<td>DVB</td>
<td>divinylbenzene</td>
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<td>EGDMA</td>
<td>ethylene glycol dimethacrylate</td>
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<tr>
<td>EHA</td>
<td>2-ethylhexyl acrylate</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>Fmoc</td>
<td>fluorenlymethyloxycarbonyl</td>
</tr>
<tr>
<td>FTIR</td>
<td>fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GMA</td>
<td>glycidyl methacrylate</td>
</tr>
<tr>
<td>HDA</td>
<td>hexanediol diacrylate</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>HFBMA</td>
<td>2,2,3,4,4,4-hexafluorobutyl methacrylate</td>
</tr>
<tr>
<td>HIPE</td>
<td>high internal phase emulsion</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>LCMS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LMA</td>
<td>lauryl methacrylate</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MMA</td>
<td>methyl methacrylate</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NaOAsc</td>
<td>sodium ascorbate</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OA</td>
<td>oleic acid</td>
</tr>
<tr>
<td>O/W</td>
<td>oil-in-water</td>
</tr>
<tr>
<td>PDMS</td>
<td>poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>PEGMA</td>
<td>poly(ethylene glycol) methyl ether methacrylate</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
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<tr>
<td>PMDETA</td>
<td>$N,N',N''-pentamethyldiethylenetriamine</td>
</tr>
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<td>PMMA</td>
<td>poly(methyl methacrylate)</td>
</tr>
<tr>
<td>PNA</td>
<td>peanut agglutinin</td>
</tr>
<tr>
<td>PPO</td>
<td>poly(propylene oxide)</td>
</tr>
<tr>
<td>PS</td>
<td>polystyrene</td>
</tr>
<tr>
<td>PtNPs</td>
<td>platinum nanoparticles</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>PVA</td>
<td>poly(vinylalcohol)</td>
</tr>
<tr>
<td>PVAc</td>
<td>poly(vinylacetate)</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SPOS</td>
<td>solid phase organic synthesis</td>
</tr>
<tr>
<td>SPPS</td>
<td>solid phase peptide synthesis</td>
</tr>
<tr>
<td>SPG</td>
<td>Shirazu porous glass</td>
</tr>
<tr>
<td>TBTA</td>
<td>tris-(benzyltriazolyl methyl) amine</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
</tr>
<tr>
<td>tetra-thiol</td>
<td>pentaerythritol tetrakis-(3-mercaptopropionate)</td>
</tr>
<tr>
<td>( T_g )</td>
<td>glass transition temperature</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
</tr>
<tr>
<td>W/O</td>
<td>water-in-oil</td>
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Chapter I. Aim and Outline

I. 1. Aim of the Work

Polymer particles are generally spherical materials, which have been subject of a wide range of applications over decades. Ion exchange, solid phase synthesis, catalyst/enzyme immobilization and chromatography all depend on particles. These polymer particles can be mainly classified into two groups: porous and nonporous. Nonporous particles are loosely crosslinked, which are being utilized more than their porous counterparts. Nonporous microgels swell when subjected to compatible solvents due to the low degree of crosslinking and this swelling is necessary in some applications such as solid phase synthesis. Porous polymer particles on the other hand, are generally highly crosslinked. Porous particles are not expected to swell with solvents since the pore network allows solvents, even non-compatible ones, to diffuse into inner zones of the particles. Porous particles are generally opaque and more brittle due to the pores and higher crosslinking. Both nonporous and porous particles can be either monodisperse or polydisperse in size. Monodisperse particles are the ones with a very low coefficient of variation in diameter. Monodisperse particles perform in a reproducible manner, which is very important from the application viewpoint, and monodispersity is even compulsory in some applications such as chromatography.

The importance of microgels became more prominent when solid phase synthesis was introduced by B. Merrifield, who eventually became a Nobel Prize laureate for this invention. The chloromethylated polystyrene resin, referred to as Merrifield resin since then and used by Merrifield as early as 1963, is still being widely used in solid phase synthesis, together with the later discovered Tentagel resin. In solid phase synthesis, the term resin is used as a synonym for polymer support. It is surprising that the type of resin remained the same over decades although much advance is realized in the field of polymer particle preparation. First of all, several novel particle preparation techniques have been discovered, which enabled one to prepare polymer particles with unprecedented control over size, shape, monodispersity, chemical functionality and anisotropy. Moreover, novel highly efficient coupling reactions have been discovered during the last decade since the introduction of the “click” chemistry concept by Sharpless et al. However, only few chemical strategies have been used for coupling onto resins. One of the aims of this research was to develop such uniform resins by using one of the newest methods, which should allow performing
click reactions. These resins will be highly interesting, not only for conducting solid phase synthesis but also for several other applications.

Another application field of microgels is utilizing them as templates for coatings with additional material such as polyelectrolytes\textsuperscript{11} or a crosslinked shell.\textsuperscript{12} Capsules of various nature are obtained by dissolving the template microgels after coating with the secondary shell. It is obvious that the final capsules are monodisperse only in case that the corresponding template microgels are monodisperse. Monodispersity is important for obtaining reproducible results as mentioned before. Although well established methods exist for preparing up to 10 µm sized monodisperse microgel templates, it is rather impossible to prepare larger (hundreds of micrometers) monodisperse microgels with the traditional methods. Such large capsules may find application areas such as microreactor technology. Preparing monodisperse, large, degradable microgels has been another task described in this thesis.

Improving properties of porous particles has also been among the aims of this thesis. Porous particles are classified into three categories according to the average pore size: microporous (< 2nm), mesoporous (50-2 nm) and macroporous (> 50 nm). Whereas microporous particles are more useful for gas sorption applications, macroporous ones are better for reagent/solvent diffusion. Macroporous resins are traditionally prepared by adding a porogenic solvent to the monomer mixtures, which is a nonsolvent for the polymer. Macropores are obtained via the phase separation between the forming polymer network and the porogen. These pores are in the submicron range. A general method for creating uniform polymer particles with pores well over a micrometer, does not exist. Those “megapores” should allow faster reagent diffusion and perform better in various applications such as catalysis. A totally new approach is necessary to obtain such “megaporous” particles, which is also aimed via this thesis.

Last but not least, we were also aiming to establish a straightforward strategy for anisotropical modification of polymer beads to obtain anisotropical particles, also referred to as “Janus” or “patchy” particles.\textsuperscript{13} Janus particles may combine incompatible chemical groups on their distinct regions, leading to totally unique properties for many different applications. Few well defined methods are recently established to create Janus particles via co-flow methods.\textsuperscript{14-16} The obtained Janus particles via these methodologies are composed of two distinct hemispheres. However, an easy method that selectively modifies only surface regions of polymer beads did not exist and was developed in the framework of this thesis.

In fact, this thesis has been part of a European Union funded Marie Curie Early Stage Training (EST) project named "Sensing Endocrine Disrupting Chemicals: Development of novel solid phase
extraction systems” (sEnDiChem). sEnDiChem is a multidisciplinary project devoted to develop novel custom made sorbents furnished with artificial receptors mimicking the endocrine receptor. The Department of Organic Chemistry of Ghent University is the host location of this project with 4 doctoral trainings offered.

The sEnDiChem project has the focal point of detecting endocrine disrupting chemicals (EDCs) in wastewater. Presence of EDCs in wastewater constitute significant risks on human and animal health. EDC is defined as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process” by the U.S. Environmental Protection Agency. The effect of EDCs in the human body happens via binding hormone receptors instead of the hormones, which causes several hormonal abnormalities. The list of EDCs is highly populated with chemicals with a broad range of chemical nature, which makes establishment of a specific detection system rather difficult.

Schematic depiction of an SPE cartridge built to adsorb EDCs via specifically designed peptidic receptors on the polymer support.

Since EDCs are generally hydrophobic, their concentrations in wastewater are very low and they are mixed with a huge number of other substances, which makes the analysis very difficult. There is a need for a system which can specifically enrich EDCs in wastewater for analysis. sEnDiChem project proposes a solid phase extraction (SPE) system that is packed with a sorbent that has artificial peptide based EDC receptors as schematically depicted in the figure. Once the wastewater is passed over, the SPE cartridge should specifically adsorb EDCs. EDCs will be recovered from the sorbent via
washing with small amounts of an organic solvent which will continue to the analysis part by chromatographic techniques. Consequently, the sEnDiChem project constitutes of 3 main activities:

- development of novel peptide based receptors,
- tailor made polymer supports for synthesizing/attaching receptor peptides,
- analyzing the efficiency of obtained system in terms of EDC affinity.

The development of suitable polymer supports for the sEnDiChem project was within the initial tasks of this thesis.

I. 2. The Outline

The thesis starts with an extensive literature survey about polymer particles, which constitutes Chapter II (accepted as a review article for Progress in Polymer Science). It describes and compares all of the available particle production methodologies. Established characterization and functionalization strategies are also discussed, together with information on applications.

Chapter III and IV are devoted to nonporous microgels. Chapter III starts with information about the developed tubing-needle microfluidic setup utilized for manufacturing all the particles in this thesis. This introduction also mentions the modifications made on the system by us for performance improvement. Later, Chapter III describes the synthesis of monodisperse large dextran based microgels and their usage as degradable “giant” templates for layer by layer coating with a polyelectrolyte and with Pt nanoparticles, which possessed opposite charges. Capsule formation via core dissolution is presented.

Chapter IV describes two novel reactive microgels. Monodisperse thiol and yne functionalized resins were prepared utilizing the same microfluidic setup via thiol-yn chemistry by using only two monomers. Functionalization of these resins by various reagents are also presented in a comparative manner. The thiol resin is treated with 9 reagents in parallel and the kinetics are compared by using IR spectroscopy. At least two highly efficient functionalizations are demonstrated for this thiol resin, which has a very high amount of thiol groups. In this sense, this thiol microgel can be a good candidate for a novel resin for attachment of various precious species for different applications, avoiding any loss during the attachment. It can also be a novel resin for solid phase peptide synthesis, when the sEnDiChem project is considered, where novel high loading resins were needed.
Chapter V and VI describe porous particles. The preparation of macroporous “clickable” beads via the porogen method is described in Chapter V. Several approaches were presented as solutions for the associated problems of the method. After the optimizations, the obtained beads were monodisperse, non-fluorescent, macroporous and carrying epoxy groups for functionalization. These beads underwent a microcontact printing methodology that covalently printed two different amine molecules on both faces of the beads, which is recognized as being a universal mild method for Janus particle production.

Finally Chapter VI describes the unique contribution of this thesis to the polymer particles science: polymerizing high internal phase emulsion (HIPE) droplets in microfluidics. Particles with pores reaching up to 20 µm, so-called “megapores”, were obtained. These poly(HIPE) beads were compared with the macroporous beads of the same composition (described in the Chapter V) in a “click”-“click” reaction sequence to see if these huge pores make a difference. The obtained poly(HIPE) particles were not only spherical: rods, hollow rods, capsules, doughnut structures and core-shell monoliths were obtained, all of which possessing huge pores. Moreover, the assembly of the hydrophobic poly(HIPE) beads around water droplets was successfully realized yielding highly porous liquid marbles. Thanks to these huge pores and various shapes, the poly(HIPE) particles are envisaged to solve some problems of aforementioned applications such as limited reagent diffusion, reactivity and grafting large compounds.
References

(2) http://www.rapp-polymere.com/
Abstract

This chapter surveys the literature published on polymer particles. The large synthesis chapter discusses and compares microfluidics, membrane/microchannel, suspension, emulsion, dispersion, precipitation, seeded suspension polymerizations and a few other less known methods. The comparison includes size, size monodispersity, porosity, pore characteristics and chemical functionality of the obtained beads, and the ability to yield nonspherical particles, easiness and scaling-up possibilities, which are summarized in a table. Functionalization and characterization techniques are also discussed, efficient reactions such as click chemistry being highlighted. Finally, applications of polymer particles are also briefly mentioned.

Chapter II. Literature Background

II. 1. Introduction

Polymer particles, especially the ones that are spherical in shape, have been utilized in numerous applications for decades. Classification of polymer particles can be based on the shape (spherical or nonspherical), synthesis method, application, dispersity, functional groups possessed, crosslinking degree and porosity. A huge majority of the synthesized particles are spherical due to the nature of synthetic methods, which will be discussed further in detail in this chapter. In terms of synthesis, nonporous particles can be regarded easier to manufacture compared to the porous ones. Crosslinking degree is important in terms of swelling behavior. Highly crosslinked resins do not swell in solvents as good as lightly crosslinked counterparts.

Together with these classifications, this literature survey will mainly use the porosity point of view while comparing polymer particles. Depending on the application, nonporous microgels are generally very lightly crosslinked and can even be totally uncrosslinked. Porous particles, on the other hand, need to be highly crosslinked. They have been further classified as macro-, meso- and microporous depending on the size of the pores, respectively > 50 nm, 50-2 nm and < 2nm. Porosity and higher crosslinking degree give rise to different characteristics such as high surface area, ability to uptake various solvents with different polarity and increased brittleness for porous particles. Size, size dispersity, chemical nature and functionality can be mentioned as the features that porous particles share with their nonporous counterparts. The variety of applications require different combinations of the mentioned features. For instance, while chromatography requires highly monodisperse (uniform in size, low coefficient of variation (CV)) sub 5 μm beads, solid phase peptide synthesis (SPPS) is usually conducted with 100-200 μm beads and monodispersity is not that crucial. On the other hand, functionality is a must for SPPS but can be undesired for chromatography.

In general, polymer particles are produced by heterogeneous polymerizations using the immiscibility of two or more liquids. Suspension, emulsion, miniemulsion, dispersion, precipitation, seeded suspension, membrane/microchannel emulsification and microfluidic polymerizations are the main techniques to prepare polymer particles. In all cases, the application should be kept in mind prior to choosing the method of production. While it is possible to easily produce nonporous particles with all the aforementioned techniques, it is virtually impossible to prepare porous particles via emulsion
and miniemulsion polymerization techniques. Other techniques also require additional ingredients such as a porogen when porosity is desired.

To the best of our knowledge, a review or book covering all these techniques does not exist. There are several reviews for polymer particles but the older ones\textsuperscript{1-3} merely cover the conventional methods (suspension, emulsion, dispersion, precipitation and seeded suspension) while the new ones\textsuperscript{4-6} focus on the new methods (membrane/microchannel emulsification and microfluidics). We will try to compare all these techniques in this introduction, porosity being examined in detail. Moreover, chemistry viewpoints will be focused on using basic phenomena, rather than highlighting the technical aspects of the mentioned methods. Together with the characterization and applications section, we will also discuss how one can effectively conduct chemical transformations on these particles. This so-called functionalization subchapter aimed to summarize what is flourishing in polymer science as click chemistry strategies. Last but not least, nonspherical particles are also discussed throughout the text since this is an immature field with lots of opportunities waiting to be exploited to our belief.

This chapter covers and compares all the synthetic methods for polymer particle production. For the most relevant discussion with regard to the experimental work in the following chapters, the reader is referred to the section on microfluidics (Chapter II. 7).

II. 2. Synthesis

II. 2. 1. Introduction to the Production of Polymer Particles

For decades chemists learnt how to use physical principles to design their reactors, rather than chemical principles. Temperature, pressure, viscosity, stirring and fluid dynamics are the most important principles to name at first. Liquid immiscibility is another ‘tool’ that chemists are familiar with and make it serve to their ambitions, for instance to prepare regular particulate material. From daily life, we all know that oil and water are immiscible and will phase separate when added to each other. When it is desired to form a dispersion of one of the two liquids in the other, which is called an emulsion, a sufficient amount of emulsion stabilizer should be added together with applied shear. The words emulsifier, surfactant, surface active molecules and many more are all used to describe emulsion stabilizers that are readily present in our daily life, such as soap and detergents. Emulsifiers are molecules that possess both hydrophilic and hydrophobic parts, recognized by water and oil, respectively. When oil and water are mixed in the presence of an emulsifier, emulsifier molecules
cover the surface of the dispersed phase droplets by reducing the interfacial tension. Milk is a well known example of a stable emulsion from nature where oil (butterfat) droplets are dispersed in water by the aid of phospholipids and proteins.

Already in 1912 chemists realized that an emulsion can be utilized to produce polymer particles. Keeping water as the continuous phase, the discrete phase could be droplets of hydrophobic monomeric species, which should be converted into polymer particles after polymerization. The emulsion stabilizer can be a soap, a polymeric stabilizer or a natural surface active material such as gum, starch and gelatin. A free radical polymerization initiator is used and can be added to either phases. These ingredients and their immiscibility are the basis of heterogeneous polymerizations (also called heterophase polymerizations), with the exception of dispersion and precipitation polymerizations where the initial mixture starts from a completely homogeneous solution, which will be discussed later in this chapter. It is also worth to mention that water soluble monomers can be polymerized as discrete phase droplets in an organic solvent (the oil phase). These type of W/O systems are generally denoted as inverse polymerizations.

The importance of controlling interfacial tension is already discussed in the introduction paragraph. However, it is also necessary to stress that the spherical shape of the monomer droplets is caused by this interfacial tension. Indeed, the sphere is the shape with the lowest surface to volume ratio, which is the reason why almost all of the particulate polymeric material is spherical in shape and why it is much more difficult to make regular nonspherical particles.

Emulsion polymerization, miniemulsion polymerization, suspension polymerization, dispersion polymerization, precipitation polymerization, membrane emulsification and microfluidics are the techniques utilized for polymer particle manufacture. All but emulsion and mini-emulsion polymerizations can yield porous particles, hence they will be discussed lesser in detail. Microfluidics will be covered in greater detail since this is the youngest among all heterogeneous polymerization techniques and allow researchers to achieve unprecedented structures such as regular nonspherical forms or core-shell structures. Moreover, suspension polymerization occupies a significant space by explaining all the pore formation mechanisms in this literature survey, which sheds light into other techniques including microfluidics.

II. 2. 2. Suspension Polymerization and General Pore Formation Techniques

In terms of physical categorization, whereas an emulsion denotes a liquid/liquid dispersion, a suspension denotes a solid/liquid dispersion. However, this does not apply for heterogeneous
polymerizations since both emulsion and suspension polymerizations start with liquid/liquid mixtures in the beginning and end up as solid/liquid dispersions. A suspension polymerization starts with monomer droplets dispersed in the continuous phase with the aid of surfactants such as sodium dodecyl sulphate (SDS) (see Fig. II. 1). A monomer soluble initiator is added, aiming to drive both initiation and chain growth inside the monomer droplets. This is the main difference with the emulsion polymerization where a continuous phase soluble initiator is used, hence the mechanism completely changes. Moreover, radical trapping species such as NO$_2^-$ salts, can also be added to suspension polymerization recipes$^8$ to prevent nucleation in the continuous phase$^9$. To obtain porous particles however, a porogen should be added to the monomer phase of the reaction mixture.

**Fig. II. 1.** Basic depiction of the suspension polymerization technique.

Suspension polymerization can be considered as the least complicated heterogeneous polymerization technique in terms of its mechanism. As seen in the Fig. II. 1, on one hand, there is the discrete phase droplets with monomeric species (monomers and crosslinkers) and initiator (+ porogen if desired) and on the other hand, there is the continuous phase with dissolved surfactant and/or polymeric stabilizers. The molecular transfer between the two phases needs to be minimized in suspension polymerization because initiation and propagation all take place in these monomer droplets. Two parameters are very important: solubility of monomers in the continuous phase and the role of surfactants and stabilizers. The monomer solubility in the continuous phase becomes important where a monomer to continuous phase ratio is low. If there are no phase transfer limitations, a significant amount of monomer would be present in the continuous phase in the beginning and will transfer back to discrete phase droplets by time since polymerization consumes
the monomers in these droplets. This process would result in retardation of polymerization. Also the porous nature would be affected since porosity depends very much on the concentration difference between monomers to porogen(s). Nevertheless, a porogen can also be used to increase the partition of a water soluble monomer in the discrete phase droplets where the continuous phase is water. For example, Fréchet et al. managed to polymerize a completely water soluble crosslinker in a "classical" suspension polymerization by using cyclohexanol as the porogen.\textsuperscript{8}

In suspension polymerization, continuous mechanical agitation with a constant speed is applied throughout the whole process to keep monomer droplets well dispersed (see Fig. II. 1). However, droplet collision and break-up cannot be prevented. Since the droplet formation is governed by the chaotic agitation and since droplet collision/break-up takes place continuously throughout the process, particles obtained via suspension polymerization are always polydisperse. Notwithstanding the fact that this polydispersity is the main drawback of suspension polymerization, this technique is much applied in industry because of its simplicity and upscaling possibilities. Obtained particles are sieved to certain size ranges when needed. Agitation speed and the shape of reactor and mechanical stirrer are the main factors influencing the size distribution and size of final particles, without forgetting the importance of surfactant concentration and interfacial tension between phases.

Here we will discuss the general pore formation mechanisms that can be directly applied to the other techniques as well, especially membrane/microchannel emulsification and microfluidics. Other techniques about pore formation, which are not applicable to suspension polymerization, are discussed in the section of the concerned technique.

II. 2. 2. 1. Nonporous Particles

Polymerization of styrene in water can be accepted as a text-book example of the suspension polymerization. As an example, the discrete phase may consist of styrene (monomer) and azobisisobutyronitrile (AIBN, initiator), whereas the continuous phase can be an aqueous poly(vinylalcohol) (PVA, emulsion stabilizer) solution. With the applied shear and heat, AIBN decomposes and polymerizes styrene that is dispersed as droplets in aqueous PVA solution. Each droplet of styrene can be considered as a micro polymerization reactor without forgetting the fact that collision and breakup occur throughout the process. Uncrosslinked PS microgels will be obtained. To obtain crosslinked microgels, divinylbenzene (DVB) is by far the mostly preferred crosslinker for styrene beads. DVB is included to the discrete phase in this case, i.e., 1% compared to the amount of styrene. Nonporous particles will be obtained in this way since no porogen is utilized.
Chapter II. Literature Background

II. 2. 2. 2. Using a Solvent as the Porogen (ν-Induced Syneresis)

When porous PS beads are needed, toluene can be added to the recipe as the porogen, which will be part of the discrete phase. Toluene is a thermodynamically good solvent for the polymer, which means that it can readily swell the final crosslinked beads. A good solvent is characterized by possessing a value of the Hildebrand solubility parameter close to that of the polymer. Inside every discrete phase monomer droplet, a continuous network grows by addition of monomer and after a certain time, the network becomes incapable of absorbing more toluene due to an increasing amount of crosslinking. A precipitation or deswelling (phase separation) occurs at this point, which is after the gelation point of the network, and this phase separation yields the porous nature of the particle. The amount of the crosslinker is of great importance as it determines the time of precipitation and the extent of porosity. Micro- and mesopores are predominant, resulting in beads with high surface area values but low pore volumes. This type of pore formation is called ν-induced syneresis and explained in detail elsewhere in the literature.

II. 2. 2. 3. Using a Nonsolvent as the Porogen (χ-Induced Syneresis)

On the other hand, if a nonsolvent for the final polymer is used as a porogen, such as n-heptane instead of toluene for the previous case, pore formation occurs via χ-induced syneresis. In this case, phase separation occurs before the gelation point since heptane cannot swell/dissolve the growing polymer chains. At the start of the initiation, separated smaller particles of polymer (nuclei) grow as a discontinuous phase (early phase separation due to the nonsolvent) inside every discrete monomer phase droplet. These nuclei agglomerate via inter-nuclei crosslinking and the final bead is formed. In contrast to the previous case macropores are predominant, resulting in significantly lower surface area values but larger pore volumes. Moreover, suspension polymerization of monomers like vinyl chloride and acrylonitrile yields intrinsically macroporous particles without the need of adding any external nonsolvent due to the fact that these monomers cannot dissolve/swell their corresponding polymers; this could be referred to as a ‘self-porogen’ effect.

II. 2. 2. 4. Using Linear Polymers as the Porogen

Various polymers and oligomers can also be used, generally together with a solvent, as the porogen. Also in this case pore formation occurs via χ-induced syneresis. Examples of polymers and oligomers used as porogens include poly(methyl methacrylate) (PMMA), poly(ethylene oxide) (PEO), poly(propylene oxide) and poly(dimethylsiloxane) (PDMS). It is important to note that Okubo et al. reported that the amount and the nature of polymeric porogen may either induce a porous or a nonporous hollow final structure. The pioneers of methacrylate based porous particles, Svec and
Horák, reported the differences between the use of a good solvent (toluene), nonsolvent (dodecanol) and a polymeric porogen (polystyrene in toluene, 15%) for the synthesis of a copolymer of glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EGDMA).\textsuperscript{2} In the order of good solvent, nonsolvent and polymeric porogen, the specific surface area decreases below 1 m\textsuperscript{2}/g whereas the size of microglobules and total pore volume increase (see Fig. II. 2). It should be noted that nonporous particles have totally smooth surfaces.

![Fig. II. 2. Scanning electron microscopy (SEM) images from surface of GMA\textsubscript{60}:EGDMA\textsubscript{40} beads prepared by using different porogens at 60 vol\% ratio: a) toluene (good solvent), b) dodecanol (nonsolvent), c) PS solution in toluene (polymeric porogen, 50000 g/mol, 15%). From left to right, pore size increases and total surface area decreases. Reproduced from literature.\textsuperscript{2}]

Sherrington et al.\textsuperscript{15} reported that a bimodal pore size distribution can be obtained in some cases by using a mixture of toluene (good solvent, inducing micropores) and PDMS (polymeric porogen, inducing macropores) for a bead composed of DVB alone. Although it should be against expectations that from a single porogen the combination of high surface area and high pore volume could be reached, Li et al.\textsuperscript{18} found out that polyDVB particles exhibit a surface area equal to 720 m\textsuperscript{2}/g, together with a very high pore volume of 68\%, when prepared in the presence of 1-chlorodecane alone, which is a nonsolvent for polyDVB. However, the authors were unable to explain this unexpected behavior.

An important problem of using a nonsolvent as the porogen is the possibility of the formation of a dense and often impermeable polymer layer on the surface of particles, although the internal structure is highly porous. In literature, this nonporous layer is referred to as either "skin"\textsuperscript{19-22} or
"shell"\textsuperscript{23-24}. As mentioned before, a nonsolvent should possess a solubility parameter value that significantly differs from that of the polymer. However, when the difference in the solubility parameter is too large, "skin" formation is promoted, as reported in detail by Kumacheva et al.\textsuperscript{25} In their case, the difference in the solubility parameter was increasing by a decreasing polarity of porogen. Since the continuous phase was water in their system, highly nonpolar porogens disliked to be present in the water/oil interface due to the high interfacial tension. Thus, the interface became rich in monomer and polymer, resulting in a "skin" layer, while the interior was porous. On the other hand, more polar solvents resulted in "skin"-free macroporous particles. Moreover, they provided an excellent solution to this problem by decreasing the interfacial tension without changing the highly nonpolar porogen. They lowered the interfacial tension by decreasing the polarity of the continuous aqueous phase or by adding a specific surfactant next to a polymeric stabilizer. However, this solution avoiding the skin formation may not be valid for every monomer/continuous phase system since the interfacial tension and solubility parameters may not follow the same trend. Although the technique utilized was microfluidics in this case,\textsuperscript{25} these results should also be applicable to suspension polymerization. The similarities between the two techniques will be discussed in the microfluidics section.

II. 2. 2. 5. Using Water as the Porogen

Unlike a nonsolvent, solvent or a polymer, a porogen that is even immiscible with the initial monomer mixture can also be utilized to obtain porous particles. The most common example of such strategy is using water as the porogen. A water-in-oil-in-water (W/O/W) double emulsion is formed by adding oil soluble surfactants to the discrete monomer (oil) phase. Water is absorbed from the continuous water phase by the monomer droplets as a result of the stabilizing effect of the oil soluble surfactants.\textsuperscript{26} Although porogen water droplets should have been separated initially inside the monomer phase, highly porous polymer beads with pore sizes around 80 nm and surface area values reaching up to 200 m\textsuperscript{2}/g (proving the interconnectivity of pores) are obtained after polymerization. The same authors also published that a combination of surfactants can produce hollow porous beads (see Fig. II. 3. B).\textsuperscript{27} Although produced by a uniquely facile template-free approach, such hollow porous particles were not further discussed in their paper.\textsuperscript{27} However, the same authors published later that hollow porous particles can also be obtained via addition of a W/O emulsion (oil is the monomer phase) into a second water phase,\textsuperscript{28} thanks to ripening.
Fig. II. 3. A) Porous and B) hollow porous particles prepared by aqueous suspension polymerization, utilizing water as the porogen with the aid of various monomer soluble surfactants. Monomer soluble surfactants captured water from the continuous phase, resulting in the hollow and/or porous structure. The hollow core only formed with specific surfactants. Scale bars indicate 10 µm. Reproduced from literature with modifications.27

II. 2. 2. 6. HIPE Technique

The highest amount of a liquid dispersed as monodisperse droplets in another liquid can be 74 vol%.29 However, by a careful choice of the surfactant and dropwise addition of the internal phase over a vigorously stirred continuous phase (including the surfactant), high internal phase emulsions (HIPE) can be obtained with internal phase volumes exceeding 99%.30 thanks to the nonspherical packing of internal phase droplets.29 When the continuous phase is polymerized, a poly(HIPE) is obtained, i.e. a very light, highly porous material with fully interconnected pores exceeding 10 µm in diameter.29-30 Particulate poly(HIPE) with regular shapes has been a challenge for scientists due to the difficulties faced during the formation of HIPE droplets in a second continuous phase, referred to as a double emulsion. Nevertheless there are few reports in patents31-32 and in open literature33-36 of polymerizing HIPE formulations in a suspension media yielding polydisperse beads with ultra large pore sizes (see Fig. II. 4. A). Deleuze et al. reported33 a surface area value of 124 m²/g when they added 20% petroleum ether (a volatile porogen) to the monomer phase of HIPE whereas the surface area was 20 m²/g in the absence of petroleum ether. This is another example where a combination of porogens is utilized to obtain different pore sizes. Based on the W/O/W double emulsion approach, Nelissen et al.37 prepared water absorbed PS beads. The obtained beads were heated above their transition temperature (Tg) in order to use entrapped water molecules as blowing agents, which resulted in pores reaching up to 100 µm (see Fig. II. 4. B). In this example water replaces the traditional blowing agent pentane,38 which results in avoiding the use of volatile organic compounds.
II. 2. 2. 7. Using Solids as the Porogen

Another type of immiscible porogen can be a solid instead of water, which results in the realization of solid-in-oil-in-water (S/O/W) dispersions. Pores are obtained after the removal of solid particles embedded on polymer beads via washing or etching. Washing is also needed to reveal the porous structure in the previous cases where a liquid porogen is used, with the exception of volatile solvents, which can be removed via evaporation. As an example for S/O/W dispersion, Wu et.al. dispersed \( \sim 0.8 \) µm \( \text{CaCO}_3 \) particles in a EGDMA-GMA monomer mixture and suspension polymerized this S/O dispersion in water.\(^{39}\) After removal of \( \text{CaCO}_3 \) via HCl etching, the beads exhibited pores as large as 10 µm and a surface area value of 79 m\(^2\)/g. In another report,\(^{40}\) a mixture of solid (\( \text{CaCO}_3 \)), nonsolvent (dodecanol) and good solvent (cyclohexanol) porogens are utilized all together for the suspension polymerization of the same EGDMA-GMA monomer mixture. Together with a total surface area of 91 m\(^2\)/g, the formation of a bimodal distribution of micropores (10-90 nm) and macropores (180-4000 nm) is observed. We would like to stress here that, in principle, also gas forming reactive porogens can be used to obtain larger pores but no example was reported to the best of our knowledge.

For the above described strategies about pore formation in suspension polymerization, the continuous phase was water in every single case. Water soluble monomers are also suspension polymerized but in that case the continuous phase is an organic solvent. Thus the overall medium
should be a W/O emulsion, which is also referred to as an inverse suspension polymerization. The aforementioned porogen types are applicable (at least theoretically) to inverse suspension polymerization under the condition that the porogen is chosen accordingly.\textsuperscript{41-43}

We would like to note that in suspension polymerization, every single monomer droplet behaves like a microreactor of a bulk polymerization if a porogen is absent. These droplets will become microreactors of a solution polymerization where a good solvent is added as porogen. Addition of a poor solvent will make the droplets microreactors for precipitation polymerization. Finally, droplets can be regarded as microreactors of monolith polymerization in the case of HIPE.

\section*{II. 2. 3. Emulsion and Miniemulsion Polymerizations}

Emulsion polymerization is much used in industry to obtain so-called latexes, i.e., for paints. Compared to the suspension polymerization, the only difference in the ingredients is the initiator which is not discrete phase soluble but continuous phase soluble. In the case of styrene in water, a water soluble initiator is used such as ammonium persulfate instead of AIBN. This small change however, completely alters the outcome. Obtained particles are in the sub-micron range and may be monodisperse unlike the suspension polymerized particles.\textsuperscript{44-45} The reason is the completely different mechanism of emulsion polymerization.\textsuperscript{3,46} The monomer and the initiator are on different locations from the start. With the applied thermal energy, initiator decomposes in continuous water phase and form radicals whereas the monomers are residing mostly in discrete phase droplets. Thanks to the slight water solubility (which is necessary) there is a little portion of the monomer (i.e., styrene) in the water phase. Initiation starts with these monomers. When the growing chains in the aqueous phase reach a certain length, micelles form with the help of surfactants or emulsion stabilizers. It is also possible and common to conduct emulsion polymerization without surfactants; emulsifier-free or soap-free emulsion polymerization.\textsuperscript{47-49} The monomer concentration in water is kept constant during the emulsion polymerization since new monomer is supplied to the water phase from large monomer droplets as soon as they are consumed by growing radicals. This is why discrete phase droplets are also often called monomer reservoirs.

On the miniemulsion polymerization however, a super-hydrophobe such as hexadecane is added to the system.\textsuperscript{44,50} Large discrete phase droplets are broken down to much smaller droplets by applying high shear force prior to the polymerization. Initiation again starts in the aqueous phase. Since the super-hydrophobe greatly reduces monomer transfer to the water phase, formed radicals enter discrete phase monomer droplets to start the polymerization instead of encountering free monomer
molecules in the water phase. It should be also noted that the probability of growing radicals to hit
discrete phase droplets is greatly increased since the total surface area of discrete phase droplets is
greatly increased by being divided into smaller droplets with the effect of shear and the super-
hydrophobe. As a matter of fact, the mechanism of miniemulsion polymerization is rather similar to
suspension polymerization than emulsion polymerization.

II. 2. 4. Dispersion and Precipitation Polymerizations: Homogeneous at Start

In contrast to all other techniques described in this literature survey, dispersion and precipitation
polymerizations start as completely homogeneous solutions. However, they are still classified as
members of heterogeneous polymerizations since phase separation takes place in an early stage as a
result of the polymerization and the system becomes heterogeneous after some time. Although they
have similar mechanisms, there are two main differences: 1) a stabilizer is used in dispersion
polymerization but not in precipitation polymerization, 2) whereas a crosslinker is most of the cases
omitted in dispersion polymerization recipes, in precipitation polymerization a crosslinker is
necessary and only small amounts of monomer are used.

II. 2. 4. 1. Dispersion Polymerization

Dispersion polymerization is generally used to obtain non-crosslinked and nonporous particles. As
seen from Fig. II. 5, a monomer, initiator and a polymeric stabilizer are generally dissolved in an
alcohol with the applied mechanical stirring, such is in the case of the suspension polymerization
setup (see Fig. II. 1). After application of heat, the initiator decomposes to give radicals and
oligomers start to form, which are still soluble in the media (see Fig. II. 5. B). This homogeneous
mixture becomes cloudy as the oligomers grow and precipitate out, forming the nuclei of the final
particles (see Fig. II. 5. C). The nuclei are stabilized by the polymeric stabilizer added in the beginning
of the reaction. If no external intervention is made at this stage, such as addition of other species,
nuclei grow by capturing new monomers and oligomers/polymers from the medium. Most of the
dispersion polymerization reactions are conducted this way, hence the obtained particles are non-
crosslinked.\textsuperscript{51-54} A porogen may be added from the beginning to the medium to obtain porous but
still non-crosslinked particles.

If crosslinked particles are needed, a crosslinker should be added after completion of nuclei growth
(Fig. II. 5. C), as explained by Winnik et al.\textsuperscript{55} They reported that crosslinkers and polar monomers
significantly influence the particle growth and the monodispersity may be lost in such cases. The
most important stage for the monodispersity of final particles was found to be the nucleation step.
After the nucleation by styrene only (the seeds), crosslinker and polar monomers can be added and perfectly monodisperse crosslinked particles are obtained (see Fig. II. 5. D).

![Fig. II. 5. Schematic description of the stages of dispersion polymerization.](image)

In a report on the preparation of porous poly (methacrylic acid) particles via this route, 11 wt% of methacrylic acid was polymerized in a medium of a chloroform/EtOH mixture (∼5/1). The obtained porous particles were then crosslinked. The monomer concentration is much higher in the medium compared to the precipitation polymerization procedures (see below) thanks to the high amount (6.5 wt%) of polymeric stabilizer used.

II. 2. 4. 2. Precipitation Polymerization

Precipitation polymerization seems similar to dispersion polymerization. One of the main differences is that polymeric stabilizers are not used in precipitation polymerization. There are even reports defining precipitation polymerization as ‘stabilizer-free dispersion polymerization’, in analogy with soap-free (or emulsifier-free) emulsion polymerization. As a consequence, particles prepared via precipitation are always free of surfactant/stabilizer.

As depicted in Fig. II. 6. A, precipitation polymerization also starts as a homogeneous solution. Opposite to the dispersion polymerization where the usage of a crosslinker is avoided (at least in the beginning), precipitation polymerization requires a high amount of crosslinker. DVB is polymerized alone in many cases. Note that commercial DVB is technical, composed of either 55% or 80% DVB with the rest being mostly p-ethyl styrene monomer. Methacrylate crosslinkers are also polymerized via precipitation polymerization with a low percentage of added monomer. As a result,
precipitation polymerized particles are rich in remaining double bonds, which have then be used for efficient post-functionalization\textsuperscript{63} (see also Chapter II. 4).

![Schematic description of the stages of precipitation polymerization.](image)

**Fig. II. 6.** Schematic description of the stages of precipitation polymerization.

As the polymerization starts, oligomers and nuclei are formed (see **Fig. II. 6. B**). Whereas the oligomers are still soluble in the medium, the nuclei precipitate and the mixture becomes heterogeneous. The medium should be a near $\Theta$-solvent for the crosslinker. Although no stabilizer is used, the nuclei are stabilized by a layer of oligomers that are swollen by the medium and because of the fact that the system is very diluted. The polymerization continues at the particle-continuous phase interface.\textsuperscript{64-65} The nuclei do not overlap but only grow by adding fresh monomer and oligomers from the medium (see **Fig. II. 6. C**). As a result of this fact, highly monodisperse particles, generally in the size range of 1-5 µm, are obtained.\textsuperscript{66} Recently, monodisperse nanoparticles are also reported.\textsuperscript{61}

A cosolvent can be added to the initial medium, which acts as the porogen resulting in porous particles. For precipitation polymerization, it has been reported earlier that, whereas a good solvent as the porogen should give only small pores (below 10nm) and thus very high surface area values (800 m$^2$/g), a poor solvent should result in larger pores and thus lower surface area values.\textsuperscript{59} This theory seems to overlap with the $\nu$-induced and $\chi$-induced synereses, as explained in the previous section. However, a latest report on precipitation polymerization of DVB do not coincide with the initial results. In this paper, 1-decanol resulted in a surface area as high as 419 m$^2$/g and lower pore sizes (2.7 nm), which were 29.8 m$^2$/g and 5.9 nm respectively when toluene was used.\textsuperscript{67} The effect of
porogens on the structure and porous character of the final beads prepared from DVB/vinylbenzyl chloride mixture can be observed from Fig. II. 7.

**Fig. II. 7.** Effect of the media on porosity of DVB/vinylbenzyl chloride (56/44) particles prepared via precipitation polymerization. a) Acetonitrile/toluene 80/20, b) acetonitrile/toluene/cyclohexanol 70/15/15, c) acetonitrile/toluene/dodecanol 70/15/15. Total surface area values are 89.8 m$^2$/g for (c), 2.8 m$^2$/g for (b) and not available for (a). Reproduced from literature.

As mentioned earlier, precipitation polymerization needs highly diluted monomer concentrations (2-5 %) hence a high amount of continuous phase, which seems to be a drawback of the method. However, Stöver et al. reported the repeated usage of the continuous phase for subsequent precipitation polymerizations while still obtaining monodisperse particles. The polymerization is rather slow due to the high monomer dilution in comparison to suspension polymerization where high local monomer concentrations are achieved. The particles grow constantly and the polymerization can be stopped when the desired size, which falls in the range of 1 to 10 microns, is obtained. This capability to allow size control seems to be very advantageous for precipitation and dispersion polymerizations over suspension polymerization. Another advantage is that monodisperse particles are readily prepared via precipitation and dispersion polymerizations.

Polymerization of DVB (2%) in an acetonitrile/toluene mixture together with AIBN could be considered as the basic procedure for precipitation polymerization. Acetonitrile is the mostly used continuous phase in precipitation polymerization, however other solvents are also in use. Toluene is the porogen, which can be added up to 40% compared to the continuous phase. Depending on the crosslinker and monomer, this porogen can be a good solvent, a non solvent or even a polymer. Whereas the thermal initiation is the preferred route, there is a recent report about ultraviolet (UV) initiated precipitation polymerization to obtain porous particles.
II. 2. 5. Seeded Suspension Polymerization: Extensive Swelling

Ugelstad et al. discovered that polymer particles can absorb slightly hydrophilic molecules up to 100 times more of their own volume and form stable emulsions. An important observation was that the final droplet size and size distribution were completely determined by the initial polymer particles, the so called "seeds". A polymerization in the second stage yields much larger monodisperse latexes provided that the seeds are monodisperse. This is the basis of so called seeded (also called templated) suspension polymerization today.

Seeds need to be not only monodisperse but also non-crosslinked to allow swelling in the second stage. (Soap free) emulsion and dispersion polymerizations are readily utilized to obtain seeds. Since emulsion prepared seeds are generally in the submicron range, they are suitable for obtaining particles up to 10 µm in diameter after the suspension polymerization stage. On the other hand, dispersion polymerized seeds can be over 10 µm, so that particles with sizes over 100 µm can be obtained in the suspension polymerization stage. Note that, a volume enlargement of $10^6$ times would be needed for a 1 µm seed to be swollen by the new monomer(s) to 100 µm.

![Fig. II. 8. Schematic description of seeded suspension polymerization.](image)

The approach of Fréchet et al. is a good example of seeded suspension polymerization as depicted in Fig. II. 8. Polystyrene seeds with a diameter of 560 nm were first prepared by emulsifier-free emulsion polymerization. In the second stage, these seeds are first swollen by dibutyl phthalate in an aqueous emulsion, which is necessary to "activate" the seeds prior to swelling with the monomers. The amount of activator was 6-7 times higher in volume compared to the seeds. Finally these activated seeds were added to a new aqueous emulsion where the dispersed phase consisted of
propargyl acrylate and EGDMA as monomers, a mixture of cyclohexanol/dodecanol (9/1) as solvent and nonsolvent porogens respectively and AIBN as the thermal initiator. The aqueous phase contained PVA as the stabilizer, SDS as the surfactant and NaNO₂ as the radical trapping species. At the end of this successful second suspension polymerization stage, 5 µm monodisperse functional (alkyne groups) beads were obtained with surface area values reaching up to 243 m²/g and 10 nm pore size. In this example, a volume enlargement of 800 times is achieved without sacrificing the monodispersity. In addition, it is also reported by Margel et al.86-87 that porous particles can be prepared by just dissolving the PS seeds after the second stage. In this case the swelling medium included DVB but excluded the use of any porogen. A surface area of 630 m²/g is obtained.

The power of seeded suspension polymerization is that advantages of two techniques can readily be merged such as monodispersity of emulsion/dispersion polymerizations with porosity-functionality-larger size of suspension polymerization. On the other hand, this is ultimately a multi-step approach and thus needs knowledge and experience about two different polymerization techniques.

**II. 2. 6. Membrane/Microchannel Emulsification: Controlling the Droplet Formation**

It was discussed in the previous section that seeded suspension polymerization leads to monodisperse particles provided that the seeds are monodisperse. Thus, it is clear that the control of the final size dispersity in a suspension polymerization is merely connected to controlling the initial droplet size distribution. As a matter of fact, the invention of the Shirasu Porous Glass (SPG) with uniform pore sizes and leading to uniform emulsions thereby paved the way for controlled suspension polymerizations.88-89

The name membrane emulsification is the appropriate one for such technique and low CV (around 10%) beads with diameters ranging from 1 to 100 µm can be easily prepared in a single stage avoiding seed preparation and swelling steps. However, particles prepared via SPG are generally not monodisperse since the CV of SPG pores varies between 10-17% (see Fig. II. 9. B).90 Later, other ceramic membranes have also been invented next to SPG.91-92 Moreover, researchers developed microchannel emulsification for which every hole (channel) for discrete phase droplet formation is custom made. Silicon,93-96 metal97-98 and polymer99-100 based highly uniform microchannels have been used for monodisperse (CV <5%) particle manufacture.101 The difference between membrane and microchannel emulsification is the fabrication of the emulsification material, which in turn affects the pore size distribution. Microchannels (see Fig. II. 9. C) are manmade uniform holes on a suitable material while a membrane (see Fig. II. 9. B) is a material where the production method is controlled
in a way to reduce the polydispersity of the pores. In this overview, the two techniques have been combined in the same section since they are basically the same. However, we kept the given names to indicate the difference, especially with regard to monodispersity and cost. Microchannels offer highly monodisperse particles but need to be custom-made, which can be expensive and may require a lot of experience.

**Fig. II. 9.** A) Representation of membrane/microchannel emulsification process.\(^{102}\) The monomer phase (discontinuous phase) is pumped from bottom through a microchannel network or a membrane towards the continuous phase. An agitator helps the droplets to pinch off. These monodisperse droplets are then polymerized to obtain particles. B) SEM image of a SPG membrane with a mean pore size of 15 µm,\(^{90}\) C) circular pore microchannel network.\(^{102}\)

Reproduced from literature after modifications.\(^{90,102}\)

Particle production using membrane/microchannel emulsification can be simply depicted by **Fig. II. 9.** A. A discrete monomer phase is pumped through the membrane towards the continuous phase to form uniform droplets to be polymerized. In a representative case, by using a silicon microplate with multiple microchannels, Seki et.al.\(^{103}\) prepared nonporous polyDVB particles of two sizes: 9.2 µm diameter beads with a CV of 5.7% and 3.4 µm beads with a CV of 7.4%. Numerous other articles exist on nonporous particle production via membrane/microchannel emulsification\(^{96,104-105}\) which even include submicron particles.\(^{91}\) On porous particle production on the other hand, Su et. al.\(^{106}\) reported the following recipe: GMA, DVB, benzoyl peroxide and a mixture of solvating and non-solvating porogens for the discrete phase and an aqueous solution of NaNO\(_2\) and emulsion stabilizers for the continuous phase. This recipe can be transferred exactly to a basic suspension polymerization reactor.
to obtain porous particles. A propeller helps the monomer droplets to pinch off from the membrane surface. However, many reactor designs are proposed that do not necessitate the usage of a propeller or a stirring bar. Although thermal initiation is utilized in this report, photopolymerization is mostly applied in continuous flow membrane/microchannel emulsification reactors.

II. 2. 7. Microfluidics: The Ultimate Control

An ultimate control of droplet formation is achieved by the youngest particle production technique, called microfluidics. This technique can be considered as the miniaturized version of microchannel emulsification where flow plays a crucial role. Spherical particles with CV below 2% can readily be produced by various microfluidic setups. It is the elaborate chip design that allowed researchers not only to miniaturize microchannel emulsification reactors and prepare narrowly monodisperse spherical beads but also to achieve unprecedented control over structure and shape of particles. This unique capability of control resulted in the realization of perfectly controlled multiple emulsions, Janus particles, regular nonspherical shapes, and even gas bubbles, almost all of which were impossible to achieve before.

II. 2. 7. 1. Types of Microfluidic Devices

It was the introduction of a soft lithography technique for the design of PDMS devices by Whitesides in the late 90’s that popularized studying behavior of fluids at laminar flow in fine channel dimensions, which is referred to as microfluidics today. Since then, PDMS based chips became the most popular devices, also for microfluidic particle production. This technique is basically based on consecutive steps of molding, casting and curing steps. Soft lithography allows an easy way to manufacture an unlimited number of 2D device designs including T-junction, co-flow and flow-focusing geometries (see Fig. II.10). Multiple emulsification points to obtain multiple emulsions can also be easily fabricated. However, PDMS is not compatible with several organic solvents, mainly due to swelling. The most important alternative to PDMS based microfluidic devices is assembled glass capillaries introduced by Weitz et al. In this approach, chemically robust and solvent resistant glass capillary tubes are fitted in each other to form truly 3D microfluidic geometries including co-flow and flow-focusing (see Fig. II.10). Droplets, hence particles, smaller than the orifice can be fabricated in a flow-focusing glass capillary device compared to a flow-focusing PDMS device. However, Weitz-type glass capillary device preparation can be tedious and requires expertise. Recently, Weitz et al. proposed a route to coat inner walls of PDMS devices with
glass,\textsuperscript{154-155} thereby cleverly merging the easiness of soft lithography with the inertness of glass. Other studies exist in which pure glass\textsuperscript{156} or organic polymers\textsuperscript{157-159} are used instead of PDMS for the chip manufacture.

<table>
<thead>
<tr>
<th>PDMS device</th>
<th>geometry</th>
<th>Capillary device</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="PDMS device" /></td>
<td>T-junction</td>
<td><img src="image2.png" alt="Capillary device" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="PDMS device" /></td>
<td>Co-flow</td>
<td><img src="image4.png" alt="Capillary device" /></td>
</tr>
<tr>
<td><img src="image5.png" alt="PDMS device" /></td>
<td>Flow focusing</td>
<td><img src="image6.png" alt="Capillary device" /></td>
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**Fig. II. 10.** T-junction, co-flow and flow-focusing geometries for PDMS and glass capillary microfluidic devices for comparison. The lighter liquid is the monomer phase and the darker is the continuous (cont.) phase. The largest arrows point the direction of total flow and droplets. Capillary device pictures are taken from literature.\textsuperscript{151}

In connection to these two mainstream microfluidic devices, a few other setups are also drawing attention. The first one is the so called ‘simple’ microfluidic device which was originally published by Suh et.al.\textsuperscript{160} but later popularized by McQuade et. al.\textsuperscript{161} Here the microchannel is as simple as a commercial transparent polymer tubing and the discrete phase orifice is a blunt needle punched into this tubing (see **Fig. II. 11. A**). Syringes, syringe pumps and a UV source are also needed like in the case of PDMS and glass capillary setups. In this device, chip preparation is avoided and even highly monodisperse double emulsions\textsuperscript{162} are prepared together with particles.\textsuperscript{163} For this simplified setup, three main drawbacks are present: first of all, commercially available tubing is generally hydrophobic, which can be problematic in terms of wettability in case that mainstream hydrophobic monomers are used. Second, the smallest needle available has an internal diameter of 110 µm (32G), which gives an idea about the possible smallest particles prepared with such device. Finally, only a T-junction geometry is reported so far with this setup. Nevertheless, such a setup is very attractive
from the viewpoint of ease of use. Another co-flow device, similar to this simple setup, was reported by Serra et al.\textsuperscript{164-165} utilizing a steel tee to fix the discrete phase needle (see Fig. II. 11. B). This design was important in the sense that it later allowed the same research group to use capillaries\textsuperscript{132,135,166-167} instead of the discrete phase needle. Theoretically, monodisperse particles with few µmeters in diameter should be possible to achieve since down to 2 µm internal diameter capillaries are available.

Fig. II. 11. A) Tubing-needle based microfluidic T-junction device.\textsuperscript{160} The needle pumps the monomer phase into the flowing continuous phase that is a PVA solution in this case. Droplets are pinched off from the tip of the needle and polymerized thermally in the reactor. B) The latter developed tubing-needle/capillary device. The needle or capillary are fixed by using a commercially available tee.\textsuperscript{165} Smaller particles are obtained in this way due to the usage of capillaries. Adapted from references.

**II. 2. 7. 2. Droplet Formation in Microfluidic Channels**

The core of microfluidics is the droplet formation. To this extent, the dripping-jetting transition is of great importance for low CV particle production and satellite formation. The droplet formation mechanism in microfluidic emulsification will be discussed on a co-flow device, which is the most utilized geometry in microfluidics. In this section, parameters effecting droplet formation, which are familiar to chemists, such as flow rates, polarity differences, viscosity, wettability and channel dimensions will be discussed. A representative scheme of a co-flow microfluidic device is shown in Fig. II. 10, where the orifice of the dispersed phase is located in the middle of the surrounding continuous phase and the flow directions of either liquids are the same. The outer liquid (continuous
phase) flows around the inner liquid (dispersed phase) and provides the droplet breakup from the inner liquid orifice. Droplet breakup can take place either in the dripping regime (Fig. II. 12 upper image) or jetting regime (Fig. II. 12 bottom image) where the inner liquid forms a long thread before breaking up into droplets. The dripping regime is desired for the formation of low CV spherical particles. However, the production of low CV and smaller particles (compared to particles prepared in the dripping regime) is reported in the jetting regime and once the jet is stabilized uniform fibers and tubes can be obtained.

![Real time images of dripping (above) and jetting regimes (below) in a co-flow device.](image)

*Fig. II. 12. Real time images of dripping (above) and jetting regimes (below) in a co-flow device. The long thread of the inner phase is called the jet. Droplet break-up is often irregular in the jetting regime. Adapted from literature.*

In the co-flow device (Fig. II. 12) both the inner and outer liquids are pressurized with constant flow rates, generally by the aid of syringe pumps. It is the immiscibility between two liquids, hence the interfacial tension, that allows the discrete phase droplet to grow at the tip of the orifice. More inner liquid fills the droplet in the first stage, resulting in the growth of the droplet. Thus the growing droplet occupies more and more space from the available microchannel, hence the pressure of the surrounding outer liquid increases. By the time that a critical size for the droplet is reached, the pressure of the outer liquid overcomes the interfacial tension and forces the droplet to pinch off from the orifice, which is the second and the last stage of droplet formation in microfluidics.

Flow rates are important in terms of the dripping-jetting transition. Indeed, when the flow rate of the outer liquid is too high (which means high pressure), it suppresses the proper droplet growth, so that the first stage is blocked. On the other hand, for too high flow rates of the inner liquid, this liquid adds more and more discrete phase into the forming jet and thus does not let the outer liquid to narrow the thread, resulting in the blockage of the second stage of the droplet formation. Consequently, there is a safe zone, the dripping regime, where both flow rates are low. Fischer et al. showed this trend by plotting a graph (Fig. II. 13) showing the relationship between two flow
rates and discussed about a ‘critical jetting velocity’ for the continuous phase where the jetting regime is reached above this velocity. It is important to note that the authors mentioned that this critical jetting velocity may slightly vary depending on the starting regime.

![Graph](image)

**Fig. II. 13.** Critical jetting velocity of the continuous phase as a function of the rate of discrete phase for an O/W emulsion in a co-flow microfluidic device. The plot is divided into 3 imaginary sections for this overview. Optimum conditions are reached when both of the flow rates are low. Reprinted from literature after modifications.\(^{169}\)

As mentioned above, it is crucial to work in the dripping regime to form droplets with CV below 2%. Consequently, ‘low flow rates’ should be the main principle of a researcher wishing to obtain particles in a microfluidic device. However, low flow rates have certain issues that cannot be neglected. First of all, a low flow rate for the discrete phase logically means a lower droplet production rate, hence a lower particle production rate, which is the main drawback of microfluidics. On the other hand, an increase in the discrete phase flow rate will generally increase the size of the final particles, which may not be desired for the application. The continuous phase flow rate can be increased (without exceeding the critical jetting velocity) to keep the particle size lower, without decreasing the discrete phase flow rate. However, an increase in the continuous phase would result in a higher consumption of continuous phase liquid. More importantly, a higher continuous phase rate would result in a higher ratio of continuous phase over discrete phase droplets. This dilution is certainly problematic when more hydrophilic monomers are emulsified in water. Taking these facts into consideration, the dripping-jetting transition figure from reference\(^ {169}\) was divided into 3 imaginary parts for this chapter: Large droplets, optimum conditions and a large amount of the continuous phase (see **Fig. II. 13**).
Next to the flow rates, another important factor effecting dripping-jetting transition is the polarity of both phases. For an O/W emulsion, the effect of polarity can be very prominent for porous particles. Since it is the interfacial tension, hence the polarity difference between two phases, that allows droplet growth at the tip of the inner liquid orifice, an increase in polarity of the monomer phase will lead to smaller droplets and a smaller value of critical jetting velocity (undesired). In other words, polar porogens narrow the polarity gap between the two phases, resulting in a drop of the critical jetting velocity. On the other hand, addition of a hydrophobic porogen such as hexadecane should increase the droplet size if desired. Finally, addition of salts to the continuous phase increases its ionic strength and thereby increases the polarity difference between the two phases, which then increases the droplet size and critical jetting velocity, i.e. salt-out effect.\(^{170}\)

The last ‘internal’ factor affecting dripping-jetting transition discussed here will be the viscosity before switching to ‘external’ factors arising from the device itself. A highly viscous inner liquid would prefer jetting instead of dripping\(^{168-169}\) due to the viscous attraction of inner liquid molecules, thus suppressing the breakup. This behavior can also be explained by the stabilization of the interface between two phases. Moreover, whereas some reports emphasized the effect of continuous phase viscosity on droplet formation,\(^{171}\) some others figured out that it does not have a significant effect.\(^{172}\) Viscosity has also found to be playing a role\(^{173}\) in undesired satellite formation, which can be defined as the formation of much smaller droplets accompanying the larger monodisperse droplets.\(^{127,129,174-177}\) Kumacheva et al. reported for O/W emulsions that there are larger and narrower safe ranges (no satellite formation) of flow rate ratios for different viscosity values of oils emulsified.\(^{173}\) It is also important to mention that the jetting regime is also one of the main reasons of satellite formation.\(^{178}\) However, a high viscosity of the jet can suppress satellite formation,\(^{179}\) again due to the viscous attraction.

When considering the effect of the microfluidic device on droplet formation, two main factors will be encountered: channel dimensions and wettability. As in the case of membrane/microchannel emulsification, the continuous phase should preferably wet the channel walls for a proper droplet breakup. In the case of opposite wettability, the monomer phase may form a flowing thin layer on the channel walls and let the continuous phase flow in the middle. Since most of the monomers of interest are hydrophobic, hydrophilic channels should be preferred.

Two mainstream microfluidic devices differ on such issue. While Weitz-type glass capillary based devices are inherently hydrophilic, Whitesides-type PDMS devices are hydrophobic and generally treated prior to use,\(^{180}\) with a plasma\(^{181}\) for instance, to change its wettability. In the case of W/O emulsions, the glass capillary device needs to be adapted, which can be easily done via chemical
Complex Polymer Particles via Microfluidics

treatment by silanes. In terms of channel dimensions, the rule of thumb is ‘the smaller the better’ provided that the wettability is adjusted. Less amount of continuous phase will be needed for the same flow rate if the channel is narrower, which is important for monomer transfer and cost issues as discussed earlier. Wettability will be more prominent when miniaturizing the channel since the inner liquid droplets will become closer to the channel walls.

A last issue to be discussed in this sub-chapter is the effect of the initiation on porosity. Although few exceptions exist, microfluidic particle synthesis is almost completely based on fast UV curing, whereas the other manufacturing techniques mostly employ thermal initiation. Thermal initiation is much slower in terms of monomer conversion. Temperature also has an effect on the porosity due to the number of radicals generated and more importantly due to the change in solvating power of the porogen. Polymerization in UV initiated droplets is so fast that the phase separation process should be different compared to a thermally initiated polymerization. Moreover, although the temperature locally increases in a UV initiated droplet due to the exothermic polymerization, this temperature should not reach 60-70 °C, which is typically the temperature used in suspension polymerization. This should theoretically influence the porous nature of the final particles since all the theory of porosity is mainly based on phase separation and solvating power of the porogen.

II. 2. 7. 3. Examples of Microfluidic Particle Production

To start with the examples of particle production in microfluidics, we should state that the discussion in Chapter II. 2. 2 about suspension polymerization is the starting point to understand the bead and pore formation in microfluidics. The reader will find out that most of the pore formation techniques mentioned in that section can be easily adopted to microfluidics since the latter can be considered as an advanced version of suspension polymerization. Moreover, microfluidics enables one to produce not only monodisperse particles but also regular nonspherical particles, which is virtually impossible to achieve by suspension polymerization.

To the best of our knowledge, the first crosslinked vinyl polymer particles synthesized via microfluidics appeared in literature in 2004. Nisisako et.al. prepared nonporous acrylate beads (see Fig. II. 14. A) in the size range of 30-120 µm diameter with CV values below 2% by using a quartz chip. Same paper was also reporting black-white bicolored Janus beads (see Fig. II. 14. B) from isobornyl acrylate by using carbon black and titanium white dopings. Later, Suh et. al. published nonporous polyDVB beads (see Fig. II. 14. C-D) via tubing-needle setup (see Fig. II. 11. A) with a size range of 50-210 µm with CV values below 5% in few cases.
Fig. II. 14. A) First crosslinked beads prepared by microfluidics. B) Janus droplet formation in the quartz chip. Adapted from literature. C-D) Nonporous polyDVB particles prepared via the tubing-needle device. Adapted from literature.

Fig. II. 15. A) A representation of the flow-focusing Whitesides’ PDMS device for droplet generation. B) Representation of photochemical solidification of particles. C–E) Representations for formation of various drop shapes in the microfluidic channel. When the volume of the droplet is large enough, it deforms into disks (D) or rods(E). SEM pictures of F) porous bead, G) rods, H) disks and I) elipsoids. A-I are adapted from literature. J) Doyle’s PDMS device for making plugs (K) and disks (L). J-L are adapted from literature.
Complex Polymer Particles via Microfluidics

After these two reports, Whitesides and Kumacheva et al. reported ∼250 µm porous particles (see Fig. II. 15. F) with a mean pore size of 0.90 µm by photopolymerizing tripropyleneglycol diacrylate mixed with 20% dioctyl phthalate (non-solvating porogen). A PDMS based flow-focusing device (see Fig. II. 15. A-C) was used and the continuous carrier phase was 2% SDS in water. There were no further data about the surface area of the particles. The same paper also reported nonporous rods, disks and ellipsoids (see Fig. II. 15. G-I). Furthermore, Doyle et al. prepared plugs (see Fig. II. 15. K) and disks (see Fig. II. 15. L) by keeping the polymerization channel smaller than the spherical volume of monomer droplets so that they adopted the channel dimensions and were polymerized on-flight (see Fig. II. 15. J). Later, numerous other groups reported spherical nonporous particles in microfluidics, similar to the earlier approaches.

A detailed study about porous particle production in microfluidics was published by Kumacheva et al. reporting the effect of 4 different phthalates as porogens for an EGDMA-GMA monomer mixture. In the order from a solvating to a non-solvating phthalate, the pore size increased and the specific surface area decreased for the final particles (see Table II.1). CV values as low as 0.83 and particle diameters as low as 60 µm were reported. The authors also conducted suspension polymerization for the same mixtures and concluded that the particles prepared by microfluidics have a finer porous structure. In a following work, Kumacheva et al. reported that after scaling up, they observed a skin layer on a portion of beads when dioctyl phthalate and diisodecyl phthalate were used. The solution proposed was to change the continuous phase instead of the discrete phase, which has already been discussed in the suspension polymerization section.

Table II.1. Comparison of the effect of porogenic phthalates on EGDMA-GMA particles. The solubility parameter of the polymer was calculated to be 24 (MPa)$^{1/2}$ by the authors. Adapted from literature.

<table>
<thead>
<tr>
<th>Porogen</th>
<th>Diethyl phthalate</th>
<th>Diisobutyl phthalate</th>
<th>Dioctyl phthalate</th>
<th>Diisodecyl phthalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility parameter</td>
<td>20.5</td>
<td>19.0</td>
<td>16.2</td>
<td>14.7</td>
</tr>
<tr>
<td>(MPa)$^{1/2}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area m$^2$/g</td>
<td>28.7</td>
<td>13.9</td>
<td>6.6</td>
<td>3.4</td>
</tr>
</tbody>
</table>
In another study, Kumacheva et al.\textsuperscript{184} reported the fabrication of beads with an acrylate-urethane interpenetrating network structure. The heat generated from the photopolymerization of the acrylic crosslinker triggered the formation of a urethane network. It has been shown that porous beads can be obtained when a lower amount of urethane precursors is used, suggesting that the urethane chains act as a polymeric porogen for the acrylate. Zourob et al.\textsuperscript{196} made use of a solvating porogen to obtain particles with the highest surface area prepared in a microfluidic reactor. A specific surface area of 201 m$^2$/g with a mean pore size of 8.1 nm was realized upon addition of acetonitrile added to the monomer mixture. A polycarbonate based chip was fabricated and different batches of beads in the size range from 10 to 120 µm with CV values below 2% were achieved. In another work\textsuperscript{197} the effect of initiator on morphology of the beads is studied in a capillary device. While the continuous phase was water, the discrete phase was a mixture of HEMA, MMA and 1-octanol. A macroporous morphology was only obtained when an oil soluble initiator is used. On the other hand, a water soluble UV initiator resulted in nonporous but hollow particles. The waterborne radicals started the polymerization from the periphery towards the core and 1-octanol stayed inside, forming the hollow core for the final beads.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig16.png}
\caption{Gas foamed particles prepared by microfluidics; A) Hydrophilic particles, scale bar represents 200 µm,\textsuperscript{198} B) Hydrophobic particles made by gas forming H$_2$O$_2$. Note that the number of internal cavities can be controlled. Scale bars represent 50 µm.\textsuperscript{199} Adapted from literature.\textsuperscript{198,199}}
\end{figure}

An approach realized exclusively by microfluidics is using gas bubbles instead of any liquid or solid porogen. Stone et al.\textsuperscript{198} were able to capture a controlled number of gas bubbles in an aqueous
monomer phase, which was then emulsified in the carrier oil phase, thus forming G/W/O double emulsions. Upon solidification of the monomer phase, ~20 µm sized beads with uniform spherical cavities were formed. It is worth to mention that the interior of the obtained beads were in closed-cell foam structure (see Fig. II. 16. A), which designates the absence of interconnectivity between the cavities.

Another unique approach made use of a gas forming reactive porogen. Small droplets containing H₂O₂ molecules were captured in bigger oil phase monomer droplets flowing in a carrier aqueous phase. UV exposure not only solidified the oil phase but also decomposed H₂O₂ molecules exhausting gas molecules, similar to the blowing agent strategy discussed in Chapter II. 2. 2. 6. A controlled number of voids is reported, however interconnectivity within the porous structure is poor (see Fig. II. 16. B). These two reports may be the inspiration point to exploit the usage of bubble capture or gas forming porogens to prepare very light polymer particles with well interconnected pore structure. For instance, combination of a liquid porogen with bubble capture/formation should lead to porous particles possessing ultra large voids connected to each other through smaller pores. Selective functionalization of those pores depending on their size may pave the way to novel particles with unique properties..

As previously mentioned, the fabrication of nonspherical particles is one of the distinctive capabilities of microfluidics devices. Porous Janus fibers are obtained from a photocurable polyurethane resin in a co-flow PDMS chip by making use of a stabilized jet. The inner jet, which was composed of the polyurethane resin, reacted with the continuous aqueous phase releasing CO₂, thus forming pores only on one side of the fiber. The effect of the water was proven by replacing it with glycerol, which led to the formation of nonporous fibers. Another approach was based on gas bubble capture in a stable aqueous monomer jet to form hydrophilic polymer threads with ordered, embedded uniform gas bubbles.

Microfluidics has also been utilized to form monodisperse ‘supraballs’, consisting of an assembly of smaller particles to form larger aggregates. As early as 2002, Pine et al. reported the assembly of nanoscale spherical polymer beads into monodisperse (~5 µm) supraballs (see Fig. II. 17) thanks to a co-flow PDMS chip and a tubing/pipette tip device. Similar results were also reported by Gu et al. Again nano-sized seed particles in aqueous suspension droplets were emulsified in oil and the assembly was realized via the removal of water.
II. 2. 8. Other Techniques

In addition to all the aforementioned mainstream production methods, there are few other techniques. The first one is called aerosol polymerization, which utilizes a gas, for instance air, as the continuous phase instead of a liquid. The interfacial tension between monomer droplets and the surrounding gas also renders the former spherical, such as in the case of rain droplets. Although this seems to be a very efficient method, reports are scarce in the open literature. A recent paper by Ray et al. describes the photopolymerization of a commercial multiacrylate resin via aerosol polymerization. The resin was dissolved in EtOH and atomized, also referred as nebulized, by an aerosol generator. EtOH was quickly removed thanks to the \( \text{N}_2 \) current and droplets were rapidly cured by UV. The particle size varied from 14 to 22 µm with CV values below 1%. All particles were twofold smaller compared to the orifice diameter due to the removal of EtOH. This kind of atomization is a very well known technique in industry, called spray drying, which is used for drying detergent for instance. Another paper described the usage of a simple airbrush for atomization.

A similar technique is the electrospray method, where a high voltage is applied between the aerosol generator and the collection substrate. Although being extensively used for non-crosslinked particle synthesis via precipitation of polymers from their solutions, reports describing monomer polymerization is not many. Loscertales et al. electrified a coaxial jet of two immiscible liquids, the outer being a commercial UV resin. Jet breakup resulted in monodisperse compound droplets and UV curing gave uniform submicron capsules with an encapsulate. Like in the previous case, addition of a porogen to make porous particles needs to be exploited.
Another technique waiting to be used for porous particle production is called selective withdrawal.\textsuperscript{222-226} Reported initially by Nagel et al.\textsuperscript{222} the bottom liquid, which is going to be the dispersed phase, is withdrawn just from the interface by a tube where the continuous phase liquid is on top. The formed liquid cone breaks up into regular droplets in the microchannel. The setup looks very similar to microfluidics, however it does not necessitate any device preparation. Nevertheless, few parameters such as viscosity and tube distance to the lower phase are of importance.

Finally, flow lithography techniques pioneered by Doyle\textsuperscript{227-230} have drawn attention as a potential technique for porous particle production. Although generally being considered as a microfluidic technique, there are distinct differences. First of all, there is no immiscible carrier phase. The monomer mixture flows as a single homogenous phase in a PDMS channel and polymerization is done in seconds via UV light masked with a template. Polymerization near the PDMS channel is inhibited thanks to the high O\textsubscript{2} permeability of PDMS,\textsuperscript{231} which avoids clogging of the channel. The non-polymerized monomer flow basically acts as the carrier phase for the polymerized particles. The shapes and resolution of particles achieved with flow lithography techniques\textsuperscript{230-237} are certainly unmatched by any other technique. There are also other techniques based on PDMS and wettability\textsuperscript{238-239} such as PRINT.\textsuperscript{240-244}

II. 2. 9. Final Comparison of Heterogeneous Polymerizations

This introduction chapter aims to give an overview of all the techniques that can be used for particle production. Basics for each individual technique is explained by giving recipes. Finally, we compare all these techniques in Table II. 2. Monodispersity, average particle diameter, functionality, extent of porosity, shape and certainly the cost should be considered all together. It would be wise to select the production method according to the final application. For instance, if the final application does not require monodisperse particles, there may not be a need for membrane/microchannel emulsification or microfluidics. If a bimodal pore size distribution is aimed, using dispersion and precipitation polymerizations may be quite challenging for that purpose. From economic viewpoint, suspension polymerization is probably the most attractive one but is limited for certain applications with regard to size and size dispersity. Moreover, if nonspherical porous particles are targeted, one will probably be directed to microfluidic approaches. However, it does not mean that it is impossible to produce nonspherical porous particles with other techniques, just because it is not realized so far. For sure, scientists will continue to challenge the limits of techniques in the near future.
### Table II. 2. Comparison of several heterogeneous polymerizations

<table>
<thead>
<tr>
<th></th>
<th>Suspension polymerization</th>
<th>Emulsion polymerization</th>
<th>Dispersion polymerization</th>
<th>Precipitation polymerization</th>
<th>Seeded susp. polymerization</th>
<th>Membrane/µchannel emulsification</th>
<th>Microfluidics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discovery</strong></td>
<td>20’s</td>
<td>Early 20(^{th}) century</td>
<td>70’s</td>
<td>Early 90’s</td>
<td>Early 80’s</td>
<td>90’s</td>
<td>2000’s</td>
</tr>
<tr>
<td><strong>Particle size (µm)</strong></td>
<td>5-2000</td>
<td>0.1-1</td>
<td>0.1-20</td>
<td>0.1-8</td>
<td>0.5-1000</td>
<td>10-1000</td>
<td>10-1000</td>
</tr>
<tr>
<td><strong>Minimum CV of beads produced</strong></td>
<td>Generally very high</td>
<td>2-3%</td>
<td>2-3%</td>
<td>2-3%</td>
<td>2-3%</td>
<td>10 % (memb.) / 2-3 % (µchan.)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td><strong>Ability to scale up</strong></td>
<td>✓</td>
<td>✓</td>
<td>~</td>
<td>~</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Porosity</strong></td>
<td>✓</td>
<td>X</td>
<td>~</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Chemical functionality</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Shape variation</strong></td>
<td>X</td>
<td>~</td>
<td>X</td>
<td>X</td>
<td>~</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Multiple emulsions/core-shell structures</strong></td>
<td>~</td>
<td>X</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>~</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Ease/ cost</strong></td>
<td>Easy and cheap</td>
<td>Easy and cheap</td>
<td>Easy and cheap</td>
<td>Easy but can be costly</td>
<td>Cheap but can be time consuming</td>
<td>Membrane/µchannel can be costly</td>
<td>Tedious device preparation</td>
</tr>
<tr>
<td><strong>Reviews published</strong></td>
<td>1,3,248,252-254</td>
<td>3,7,46,251</td>
<td>13,54,250</td>
<td>164,249</td>
<td>1-2,248</td>
<td>4-5,101,247</td>
<td>4-6,151,245-246</td>
</tr>
</tbody>
</table>

✓: Facile  ~: Possible but not easy  X: Not achieved so far
II. 3. Characterization

II. 3. 1. Size and Size Dispersity

Perhaps the first 'label' to define a batch of spherical particles will be the size and size dispersity. Size can be easily deduced from microscopy images, especially in the case of monodisperse particles. Sieving is an old method that is still used in industry to fractionate and also to determine roughly the size range of micron sized particles. There are several ways to determine a CV value for both monodisperse and polydisperse particles. One way is analyzing microscopy images via various available software, which will also calculate the average size. Moreover, light scattering is another method to determine size and size distribution, especially for sub-micron particles. Porous particles are almost always micron sized, so that microscopy and image analysis combination is considered as the most easiest method.

II. 3. 2. Porosity: Surface area, Total Pore Volume and Pore Size Distribution

Surface area, total pore volume and pore size distribution data would define the porous nature. These characteristics can be measured by N\textsubscript{2} sorption and Hg intrusion techniques, which both depend on penetration of the mentioned fluids into the pores. N\textsubscript{2} sorption is more suitable for determining micro- and mesopores and gives less data about macropores. On the other hand, Hg intrusion is only able to provide data about macropores and mesopores but not about micropores. This is attributed to the higher ability of N\textsubscript{2} gas to penetrate into smaller pores compared to Hg. Consequently, these methods are complimentary to each other and the proper one should be chosen depending on the type of the particle. The surface area is generally calculated from N\textsubscript{2} sorption isotherms by using the BET method. Commercial instruments measuring N\textsubscript{2} sorption isotherms include the necessary software. The total pore volume in the dry state is measured by using both methods but again the size of the pores should be taken into consideration.

Besides N\textsubscript{2} sorption and Hg intrusion, other techniques exist for quantifying the pore size distribution such as inverse size exclusion chromatography and analysis of microscopy imaging. It is important to note that inverse size exclusion chromatography is performed intrinsically in a solvent, so that the data can be considered as swollen state porosity. N\textsubscript{2} sorption analysis seems as the most facile method since it gives quite reliable data for surface area, total pore volume and pore size distribution unless the pores are extremely large. There are also reports with equations to calculate several aspects of porosity by using density measurements as the only variable.
The effect of the washing solvent, used prior to drying, on dry state porosity is also important. Indeed, a fraction of the pores can collapse if the particles are dried from a good solvent. However, these pores ‘open’ again after drying from a poor solvent.

II. 3. 3. Swelling/Solvent Uptake

Swelling is one of the important differences between porous and nonporous resins. Although swelling is crucial for nonporous resins, it may not be expected from porous (especially macroporous) resins. Reagents can only reach the inner reactive sites of nonporous resins if they swell reasonably in the medium. For that reason, nonporous resins are fabricated by using very low amount of crosslinkers, for instance 1% for the Merrifield resin. On the other hand, a high amount of crosslinker is certainly needed for producing a macroporous resin to facilitate the phase separation between the polymer and porogen during the synthesis, as discussed before. This high amount of crosslinking limits the swelling of porous resins. Although the porous resins cannot swell as much as their lowly crosslinked nonporous counter parts, the pores can ‘accommodate’ some solvent, a process that is better referred to as ‘solvent uptake’. It should not be considered as a disadvantage that porous resins do not swell to high extents. Pores certainly facilitate the diffusion of reagents and solvents to the inside, even if the liquids are not a good solvent for the particle.

The swelling degree or solvent uptake can be expressed either in volume or weight expansion. In the case of weight expansion, a weighed sample of dried resin is swollen by a solvent and the excess of the solvent is removed after the equilibrium swelling is reached. The swelling degree is the ratio of the swollen over dry weight. In the case of volume expansion on the other hand, dry beads are packed into a volumetric syringe fitted with a frit at the tip, then swollen by a solvent and the volume difference is recorded as the swelling degree.

II. 3. 4. Mechanical Strength

Perhaps the weakest point of porous, especially macroporous resins is the lower mechanical strength compared to nonporous ones. For this reason, it is certainly not advised to use a magnetic stir bar when a resin (even nonporous) is treated with reagents. Several shakers are being used and rotary evaporation is useful when heating is also needed. Although few methods are proposed to measure the mechanical strength, the best way seems to utilize the resin for the desired application and observe if any change in morphology takes place due to the stress generated for this specific application.
II. 3. 5. Chemical Analysis

The chemical nature of the particles is of utmost importance for some of the applications where functionalization is needed. Although analysis techniques of other solid phase chemistries (bulk polymer materials, inorganic particles, surfaces etc) are to some extent applicable to polymer particles, a particle scientist cannot use certain tools such as liquid phase NMR and LC-MS. Most suitable techniques for the analysis of particles are elemental analysis, IR and color based essays, which are briefly described herein.

Elemental analysis can provide information about functional groups that carry atoms different from the backbone. For instance, halogen, azide and thiol groups will be nicely detected for a C, H, O based particle but not C-C triple bonds. If the function to be monitored possesses elements that are also present in the backbone, derivatization can be a solution. –NH₂ groups on a N based resin is such an example. Elemental analysis will give the total amount of N present in the resin. If free –NH₂ groups are completely capped (for instance) with a -Cl containing isocyanate, the amount of Cl atoms in the final resin will give the desired information about the accessible –NH₂ groups of the initial batch. It is worth to be mentioned that the sample should obviously be totally free of any residual reagents or solvents for elemental analysis.

Infrared analysis is probably the most facile instrument based method to detect functional groups (such as –OH, –NH₂, C=O, C≡C, C≡C, –SH and –N₃) and monitor the evolution of reactions on particles. IR spectrometers are abundant and analysis time is short. In addition, the decrease of a reagent due to the reaction with the present particles can also be followed by real-time IR measurement. As a complementary method to IR, Raman spectroscopy can reveal other functional groups (such as C-Cl and C≡N) that can be difficult to detect by IR. Moreover, solid state, gel phase and high-resolution magic angle spinning (HRMAS) NMR techniques can be quite successfully applied to detect the functional groups and monitor reactions on particles. Availability of probes, operator experience and need of a suitable solvent for the analysis can be the parameters to tackle.

Real time monitoring of reactions taking place on particles can be realized by several spectroscopy methods. NMR, IR, UV-Vis and fluorescence spectroscopy techniques will give qualitative data on reaction kinetics in this way. Once the flask is well isolated, decrease of a reagent due to the reaction with the present particles will enable online monitoring. However, one should be careful not to conclude that all the functional groups on the polymer are consumed when the consumption of the followed reagent is stopped. Mostly there are inaccessible functionalities on the polymer, which will
give a positive signal when analyzed. This is generally troublesome since it is very difficult to quantify remaining functional groups.

The fact that reliable quantification of remaining functional groups on particles is rather difficult, stimulated solid phase peptide synthesizers to develop highly efficient coupling strategies. These strategies will be further discussed in the next section. In solid phase synthesis, color tests are the equivalence of thin layer chromatography (TLC) in solution phase organic synthesis. Once a resin undergoes a chemical transformation with a reagent, a small portion of the resin is treated with a dye that is highly reactive for the chemical function that has to be consumed in the actual reaction. Lack of coloring of the resin judged by the naked eye designates the completeness of the main reaction. In the classical fluorenylmethyloxycarbonyl (Fmoc) based solid phase peptide synthesis (see Scheme II. 1), a resin possessing –NH$_2$ or –OH groups is treated with an Fmoc protected amino acid in the presence of some well known organic catalysts. Here the completeness of the reaction is checked with a color test, i.e. ninhydrin. If the amidation/esterification is complete, the Fmoc group can be released by piperidine and quantitatively detected by a well established UV measurement.
This in turn will give reliable data for initial –NH₂–OH loading (amount of a functional group on a resin generally expressed as mmol/g) of the resin. Finally, back titration²⁸⁸-²⁸⁹ can also be a reliable method for quantitative analysis.

II. 4. Functionalization

This subchapter will briefly discuss the strategies employed for particle functionalization. As in the case of characterization, strategies used for monoliths, surfaces, inorganic materials and so on can be applied to polymer particles in many cases. It should be known beforehand that reactions are much slower and yields are generally lower on solid phase compared to homogeneous reactions. In addition, quantitative detection of unreacted remaining groups may not be straightforward as already mentioned in the previous section. Even more troublesome is that one may not be able to simply separate unreacted groups on the particles, like organic chemists extract or distill their unreacted starting compounds from the final product. These constraints necessitate the use of high yielding reactions on solid phase. "Click chemistry"²⁹⁰ is the term coined almost a decade ago to describe reactions basically running with high yields in mild conditions and without any offensive by-products. Thus, click type reactions should be well appreciated for functionalization of polymer particles. For that reason, this section will mainly discuss click type reactions. It is important to mention that by-products or excess of the reagents generally do not constitute a problem for solid phase functionalization since purification is done by some washing steps.

Scheme II. 2. CuAAC and thiol-ene/yne click chemistries

From the several proposed click type reactions in the literature,²⁹¹-²⁹² two of them received much attention within the polymer society: Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC)²⁹³-²⁹⁷ and the addition of a thyl radical to olefins (thiol-ene and thio-yne)²⁹⁸-³⁰⁰ (see Scheme. II. 2). There is a huge number of publications utilizing CuAAC since in the first report in 2002 and thio-click reactions
are recently becoming very popular. Obviously, one of the reactive groups should be present on the solid support and the complementary one(s) in solution for these click reactions to take place. The presence of azide and thiol on the support and not in the solution should be considered for practical reasons. Low MW azide compounds can be seriously explosive while low MW thiol compounds generally have a deterring smell. Moreover, there are also color tests to detect remaining amounts of both azide and thiol groups. As mentioned in the previous section, azide, alkyne, alkene and thiol groups can be followed by IR spectroscopy which makes these reactions further attractive on solid phase.

![Scheme II. 3. Monitoring the agarose functionalization by the released \( p \)-nitrophenol group. Reproduced from literature.](image)

Although the number of reported studies CuAAC functionalization of porous beads, silica and metal particles is limited, there are numerous publications on CuAAC functionalization of nonporous polymers. Actually, the first report on using Cu(I) as a catalyst for azide-alkyne cycloaddition was on a PEG based resin by Meldal et. al. Many other following publications used the strategy of converting \(-\text{Cl}\) groups Merrifield resin into \(-\text{N}_3\) groups by treatment with NaN\(_3\). Via an interesting approach, Finn et al. described the click functionalization of a commercial porous agarose resin for affinity chromatography. In two parallel experiments, amino agarose beads were treated with azide and alkyne carrying activated esters respectively (see Scheme II. 3). The interesting point of this approach was that the azide/alkyne carrying ester released the UV active \( p \)-nitrophenol group upon amidation, thus the azide/alkyne loading could be determined by online spectroscopic techniques in a similar way to the Fmoc test. Several compounds of interest were subsequently clicked on these agarose beads in the next step and the coupling efficiency was shown by clicking a fluorophore. More studies about clicking onto commercial agarose beads are reported but information about the nature (porous or not) of the beads is missing.
The preparation of custom made porous azide and alkyne beads for chromatographic applications was later published by Fréchet et al.\textsuperscript{9} utilizing the seeded suspension approach. Alkyne bearing beads were prepared in a straightforward fashion by using an alkyne monomer for the second swelling stage. For the azide bearing beads however, an epoxy monomer was used for the second swelling stage and azide introduction was realized in another step. Although the authors do not mention the reason for the need of another step instead of utilizing an azide monomer for swelling, we believe that it is due to the loss of azide groups during the polymerization of double bonds as recently reported by Perrier et al.\textsuperscript{329} Azide groups are not only sensitive to temperature but also to UV; the UV triggered self crosslinking ability of azide groups is even used as a strategy to obtain networks.\textsuperscript{330} Nevertheless, Chan et al.\textsuperscript{310} recently reported a one-pot preparation of azide carrying nano-beads by a delayed addition of azide monomer into their inverse microemulsion polymerization batch.

Another strategy to introduce azide groups on a (porous) resin was published by Oyelere et al.,\textsuperscript{331} i.e. NH$_2$ groups on commercial Argopore resin have been converted to azides via diazo-transfer reaction by using triflyl azide and further clicked with nucleosides. Despite the handling difficulties of triflyl azide (explosive, needs to be freshly prepared each time), this method should be widely applicable since there are numerous amino resins available on the market.

A combination of thiol-ene and CuAAC click reactions on nonporous polyDVB particles was published by Müller et al.\textsuperscript{63} Remaining double bonds of precipitation polymerized polyDVB particles first underwent thiol-ene click by treatment with 1-azidoundecan-11-thiol. In a second step, the azide functions have been treated with an alkyne terminated linear polymer. The same strategy was applied to metal doped nanoparticles by Hawker et al.\textsuperscript{332} The efficiency of the thiol-click reaction was shown by the change in dispersing ability of the particles in THF after grafting thiol terminated PEG chains. Addition of thiol groups onto (meth)acrylate\textsuperscript{333-334} or epoxy groups\textsuperscript{335} of particles has also been published. Finally, catalyst and heat free grafting of PS chains onto precipitation polymerized porous DVB particles via hetero-Diels-Alder chemistry developed by Barner-Kowollik et al. also needs to be mentioned here as a novel highly efficient functionalization reaction.\textsuperscript{336} The microspheres were functionalized with cyclopentadiene and PS chains were furnished with thiocarbonyl moiety as dienophile. Very high PS couplings were reported for time scales as short as 2h without heat treatment.

Strategies developed over decades for solid phase peptide synthesis\textsuperscript{337} and solid phase organic synthesis\textsuperscript{338} are generally very efficient. Coupling of an amino acid on a resin carrying –NH$_2$ groups (Scheme. II. 1) can be completed in less than 1h at room temperature\textsuperscript{339} thanks to various efficient catalysts\textsuperscript{340} developed over decades. This chemistry is certainly as efficient as any well accepted click
reaction. In addition to amidation, several highly efficient esterification strategies are also well established.$^{341}$ Various other peptide ligation strategies such as native chemical ligation$^{342}$ and Staudinger ligation$^{343}$ are well described in literature.$^{344}$

Finally, we would like to conclude this functionalization section by mentioning further possibilities offered with epoxy carrier particles. The potential of spring loaded epoxy ring for effective transformations constitutes an important part in the review of Sharpless et al.$^{290}$ where ‘click chemistry’ was first defined. Opening the three-membered ring with an azide anion (acid catalyzed) or a thiol (base catalyzed) is already mentioned in this overview. Amines (preferably primary) can also open the ring (Scheme. II. 4) without the need of any catalyst or heat. Consequently epoxy groups are good starting points for several modifications. Moreover, the most commonly used epoxy carrying vinyl monomer GMA is stable in (neutral) water based emulsions. It should be noted however that opening of the epoxy ring with a nucleophile results in secondary $–OH$ groups, which may interfere with some chemistries.

![Scheme. II. 4](image)

Scheme. II. 4. Some of the effective modifications of epoxy particles. Only the attack to the less hindered carbon atom is considered.
II. 5. Applications

Applications of polymer particles greatly depends on their size and nature. As for nonporous particles, submicron emulsion polymerized particles, so called latex, is used in paint industry. Uncrosslinked nonporous particles are also good for templating studies where the particle as the template is dissolved after being coated with a secondary material. Using these sacrificial bead templates, hollow particles can be easily obtained such as via layer by layer polyelectrolyte deposition or via secondary shell crosslinking. Size monodispersity of the obtained secondary hollow particles obviously depend on the sacrificial templates used. Advanced applications such as drug delivery necessitates monodisperse capsules for uniform loading, hence monodisperse templates are needed. Nano/microgels themselves are also used for drug delivery purposes.

In terms of porous resins, ion exchange has been the first area where there was successful commercialization such as Dowex resins. These resins possess ionic groups such as $\text{SO}_3^-$, $\text{CO}_2^-$ and $\text{NH}_3^+$, together with a complementary anion or cation such as $\text{H}^+$, $\text{Na}^+$, $\text{Cl}^-$ or $\text{OH}^-$. In the classical example, a PS-sulphonic acid based ion exchange column can soften water by exchanging $\text{Ca}^{++}$ and $\text{Mg}^{++}$ with $\text{Na}^+$. Toxic heavy metals can also be removed from water thanks to their high affinity to polar groups such as carboxylates. Later, it has been discovered that this metal complexing ability of ion exchange resins can be used in heterogeneous catalysis. Once the resin is loaded with the desired metal, the organic transformation can be realized either in batch or in a continuous process. Moreover, $\text{H}^+$ carrying cation exchange resins can be used for acid catalyzed organic reactions. On the other hand, non-ionic porous resins are also used in catalysis. The catalyst is either a covalently attached organic molecule or a metal that is chelated to the resin thanks to the electron donating ligands. Simple precipitation of the metal to the pores is also reported.

Physical absorption, electrochemical absorption and covalent absorption abilities of porous resins lead to several applications. As discussed in the seeded suspension polymerization section (Chapter II. 5), particles can swell to a great extent by absorbing hydrophobic species. This can be used for removing undesired species either from water or from organic media. Moreover, gaseous species can also be absorbed by particles. Scavenging is another application area of polymer particles. Scavenger resins ideally possess chemical groups that selectively react and therefore remove undesired compounds from a mixture.

Another absorption based application area is solid phase extraction (SPE). Small particles packed in a cartridge absorb (generally hydrophobic) solutes from an analyte. Solutes are removed from the sorbent by washing with an organic liquid in the second stage. In this way, solutes are enriched and
ready for analysis. Since the interaction time is relatively short, a high performance of absorption is requested from particles (=sorbent). In that respect, hypercrosslinked particles are suitable due to their extremely high surface area. Seed preparation accompanied by hypercrosslinking in the second stage seems to be a suitable approach to produce such sorbents.

Chromatography\textsuperscript{2,248} is perhaps the most delicate of all the mainstream applications of porous polymer particles. In the range of 2-5 µm, highly spherical and narrowly monodisperse beads are necessary to obtain reproducible results from a packed chromatographic column. Whereas, silica packed columns are preferred over polymer packed columns in HPLC, polymer particles are mainly used due to their ‘configurable’ pore size and pore size distribution, in size exclusion chromatography (SEC). In SEC,\textsuperscript{378} smaller polymer chains spend more time in pores of packed beads compared to the larger chains, which is the basis for the separation. For a batch of higher MW polymer to be analyzed, beads with a higher pore size are necessary for better separation. On the other hand, a lower average pore size is needed for the separation of a lower MW polymer. Seeded polymerizations seem to be suitable for production of SEC beads\textsuperscript{379} since the pore size and pore size distribution can be easily controlled in the second swelling step. For HPLC columns, aerosol and precipitation\textsuperscript{62,380} polymerizations are also available, together with seeded polymerizations\textsuperscript{9,74-75,381-382}.

A final mainstream application area of beads is solid phase peptide (SPPS) and organic (SPOS) syntheses.\textsuperscript{383-384} Porous particles are used to some extent for SPPS and SPOS,\textsuperscript{385-386} however gel-type nonporous particles are mainly preferred. Thanks to their very lightly crosslinked nature (1% in general) nonporous gels can swell to a great extent when immersed in a good solvent, such as toluene for styrene-DVB based resins. However, if a nonsolvent is necessary for transformation, especially in the case of SPOS, permanently porous resins can perform better. It is also worth to mention that by the growth of the desired molecule (such as peptide), space restrictions become more prominent. Porous (especially macroporous) resins can offer room to accommodate such large molecules and prevent ‘saturation of resin’. Too small resins are not easy to handle as supports for solid phase synthesis due to clogging of the filters and loss of visibility by naked eye. Therefore beads with sizes ranging from 100 to 500 µm are used for this purpose. In addition, shape and monodispersity are not the highest priorities. Suspension polymerization\textsuperscript{387} accompanied by sieving, seeded suspension polymerization, microfluidics and also membrane/microchannel emulsification methods are all appropriate.
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The strategy followed in this chapter for making capsules.

Abstract

This chapter describes the synthesis of degradable microgels that are used as sacrificial templates for the fabrication of ‘giant’ hollow polyelectrolyte capsules with rigid walls consisting of covalently cross-linked polyelectrolytes and metal nanoparticles. The size monodisperse, degradable ‘giant’ microgels consisted of dextrane chains linked via carbonate esters. These monodisperse microgels are subjected to a layer-by-layer coating of negatively charged platinum nanoparticles (PtNP’s) and a positively charged diazo resin (DAR). Three alternate layers of PtNP’s and DAR are used to obtain a stable membrane on the microgels. Finally, the sacrificial dextrane based microgel cores are smoothly hydrolyzed and removed without rupturing the polyelectrolyte membrane. The ability of encapsulating materials of interest is also shown by adding fluorescent polystyrene particles to the monomer mixture and subsequent visualization of embedded polystyrene particles in coated microgels after microfluidic polymerization and layer-by-layer coating. The obtained ‘giant’ microcapsules are envisaged to be used as microreactors or drug delivery systems.

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Chapter III. Degradable Microgels

III. 1. General Introduction to Microgels

It can be stated that nonporous microgels are easier to prepare compared to porous ones since no porogen is required. Any heterophase polymerization of any monomer-crosslinker system will yield nonporous microgels as soon as the resulting polymer swells in its monomer and the amount of crosslinker is kept low. The loosely crosslinked nature of microgels allow them to swell to a great extent, which can be calculated from the Flory-Huggins-Rehner theory equation. A low crosslinking density also results in a less brittle character, which is perhaps the most important advantage of these particles during their production and use. These two features, high swelling capacity and mechanical strength, made nonporous microgels preferred in several applications, especially in SPPS.

For the manufacture of microgels, microfluidics was the method of choice in this thesis due to advantages discussed in the previous chapter. Our system was based on a previously published\textsuperscript{1-2} tubing-needle based “simplified” microfluidic setup: two syringe pumps, one large syringe for continuous carrier phase and another smaller syringe for monomer phase, a flexible and transparent tubing, needles and UV lamps (see Fig. III. 1). The large syringe mounted on the first syringe pump injects the continuous carrier phase with a relatively high rate from the beginning of the tubing. The needle of the second syringe is punched a little further on the tubing, which injects the monomer phase with a much lower pumping rate. As discussed earlier, this rate difference provides the droplet breakup. Monomer droplets travel downstream thanks to the stream of continuous carrier phase and get polymerized by UV exposure. Although on-flight exposure solidifies the droplets, extra off-tubing UV curing is applied for approximately 30 min to achieve the highest possible monomer conversion. Finally, the obtained particles are washed with different solvents.

The most important modification of the published system was done via simply bending the monomer needle. By this modification, the system was no longer a T-junction but was transferred to a co-flow device. Significant improvements in monodispersity and reproducibility together with suppression of satellite droplet formation were observed. We also used flexible tubing (PVC or Tygon) instead of rigid Teflon as originally reported.\textsuperscript{1-2} This helped to minimize the leakage in the system. Finally, using smaller diameter tubing was important in terms of a lower amount of continuous phase liquid compared to the monomer phase pumped into the system. This reduction of diameter not only
This chapter explains the first of the 2 different classes of nonporous microgels prepared in our research: degradable dextran based hydrophilic microgels that were used as templates for crosslinked layer-by-layer (LbL) capsules. Their synthesis, particle loading, polyelectrolyte deposition and obtained capsules are described. This work was performed in collaboration with Dr. ir. Bruno De Geest from the Faculty of Pharmaceutical Sciences.

Fig. III. 1. Scheme of the tubing-needle based, in-house built microfluidic setup utilized throughout this thesis.

### III. 2. Degradable Monodisperse Dextran Microgels as Templates for Giant Layer-by-Layer Capsules

Polyelectrolyte microcapsules have attracted increased attention for different purposes such as for delivery, bio-sensors and microreactors. These microcapsules are usually fabricated by alternate layer-by-layer\(^3\) deposition of charged species onto an oppositely charged template followed by the decomposition of this template.\(^7\)\(^-\)\(^14\) Both hydrogels\(^15\)\(^-\)\(^16\) and emulsions\(^17\)\(^-\)\(^19\) have recently gathered increased interest to serve as such sacrificial templates in which the species of interest are being
Complex Polymer Particles via Microfluidics

encapsulated within the template during their synthesis. This method benefits from the easy encapsulation procedure and mild conditions required to remove the template after polyelectrolyte coating. An inherent feature of emulsions is the polydispersity of their size distribution. However, production of monodisperse emulsions would offer considerable benefits such as equal distribution of encapsulated material over different droplets and a better prediction and control of the behavior of the entire population.

Polyelectrolyte microcapsules are traditionally fabricated on microparticles such as polystyrene, melamine formaldehyde, silica or inorganic carbonate with dimensions varying from 1 to 10 µm. In this research however, we aimed to fabricate stable “giant” hollow polyelectrolyte capsules since such microcapsules could find application as e.g. microreactor because their size would allow higher production capacities while still having the benefits of a confined geometry. Microfluidics is utilized to obtain monodisperse dex-HEMA microgels as sacrificial templates for LbL ‘giant’ capsules with sizes of several hundreds of microns. Dex-HEMA designates 2-hydroxyethyl methacrylate (HEMA) modified dextran chains with degradable carbonate ester linkages (see Fig. III. 2. A).

Usually research on polyelectrolyte coated microgels has the purpose to develop self-exploding microcapsules. Such microcapsules consist of a degradable dex-HEMA microgel core surrounded by a polyelectrolyte membrane. Upon microgel core degradation, the swelling pressure increases and at a certain moment, when the swelling pressure exceeds the membrane’s tensile strength, the capsule explodes and releases its content. In this concept two parameters play a prominent role: tensile strength of the membrane and swelling pressure of the microgels. However, as stable polyelectrolyte capsules were aimed in this project, i.e. avoiding the self-exploding property, some fine-tuning has been required on two levels. First the swelling pressure of the degrading microgels were lowered by decreasing the solid content of the microgels from 30 wt % to 17 wt %. Secondly, we attempted to strengthen the capsules’ membrane by employing a so-called ‘hybrid’ polyelectrolyte/nanoparticle coating comprising alternating layers of negatively charged platinum nanoparticles (PtNP’s, see Fig. III. 2. C) and a diazoresin (DAR, see Fig. III. 2. D), a cationic polyelectrolyte. Several groups have already reported on the enhanced mechanical properties of multilayer films containing nanoparticles. Moreover, the diazoresin is able to form a covalent linkage with the carboxylic acid moieties present on the surface of the PtNP’s, which was expected to further increase the membrane’s strength.
Chapter III. Degradable Microgels

III. 3. Results and Discussion

An aqueous solution of dex-HEMA, (dimethylamino)ethyl methacrylate (DMAEMA, see Fig. III. 2. B), and the photo-initiator Irgacure 2959 was pressurized by a syringe pump and fed in an in-line fashion through a blunt needle into a continuous oil stream containing a non-ionic surfactant; ABIL EM90 (see Fig. III. 3). DMAEMA is a cationic methacrylate at physiological conditions,\(^\text{30}\) providing the microgels with a positive surface charge.\(^\text{31}\) After formation of monodisperse aqueous droplets in the oil-stream, the tubing was directed into a UV chamber where radical polymerisation of the methacrylate groups was initiated, resulting in the formation of solid monodisperse microgels. The oil stream was collected and the microgels were transferred to water by several washing steps with acetone to remove the oil, finally allowing the microgels to be suspended in water. Fig. III. 3. B shows a stereomicroscopy image of the obtained microgels. The dense packing of the microgels in a hexagonal conformation illustrates their monodispersity. Image analysis was performed to determine
the size distribution of the microgels and the corresponding histogram is shown in Fig. III. 3. C, indicating a mean diameter of 290 µm and a narrow size distribution.

Fig. III. 3. A) Schematic representation of the tubing-based microfluidic setup; B) Optical microscopy image of the obtained monodisperse microgels; C) Size distribution histogram of the microgels with the red curve representing a Gaussian fit.

In a next step, the monodisperse microgels have been coated with 3 alternate layers of PtNP’s and DAR. A schematic representation of the process is shown in Fig. III. 4. The PtNP’s were synthesized according to Chen et al. by reduction of hydrogen hexachloroplatinate with sodium borohydride. Capping with mercaptosuccinic acid functionalizes the PtNP’s surface with carboxylic acid moieties, providing them with a negative surface charge as confirmed by measuring the electrophoretic mobility.

Fig. III. 4. Schematic representation of the fabrication of hollow capsules. Dex-HEMA microgels (A) are coated with a multilayer membrane consisting of anionic PtNP’s and cationic DAR (B). Hollow capsules (C) are obtained after dissolution of the dex-HEMA microgel template.
Fig. III. 2. C shows a transmission electron microscopy image of the Pt\textsubscript{NP}'s exhibiting size values roughly between 0.5 and 2 nm. The molecular structure of the cationic diazoresin and its light induced cross-linking (which occurs even under ambient light conditions)\textsuperscript{25} with the Pt\textsubscript{NP}'s carboxylic acid moieties are shown in Fig. III. 2. D. As the monodisperse microgels are positively charged as a result of the incorporation of the cationic DMAEMA, the multilayer build-up was initiated with the anionic Pt\textsubscript{NP}'s followed by the DAR. We have chosen for a deposition of three alternate layers as a number of alternate layers between 2 and 4 is usually well suited for the fabrication of stable multilayer capsules.\textsuperscript{33-35}

![Image of microgels](image1.png)

**Fig. III. 5.** Confocal microscopy (transmission channel) of (A) uncoated and (B) (Pt\textsubscript{NP}/DAR\textsubscript{3}) coated monodisperse dex-HEMA microgels. C and D show the scanning electron microscopy images corresponding to A and B.

The (Pt\textsubscript{NP}/DAR\textsubscript{3}) coated microgels were visualized with confocal microscopy (see **Fig. III. 5. B**) and scanning electron microscopy (see **Fig. III. 5. D**). Whereas uncoated microgels are observed as being optically very transparent (see **Fig. III. 5. A**), the (Pt\textsubscript{NP}/DAR\textsubscript{3}) coated microgels appear black and do not transmit light. This is due to the high density of the Pt\textsubscript{NP}'s, which are kept together by the DAR. Similar observations were reported using anionic gold nanoparticles in conjunction with the cationic...
poly(allylamine hydrochloride) for the synthesis of 3 µm sized hollow capsules templated on CaCO$_3$ microparticles.$^{33-34}$ Scanning electron microscopy also reveals the presence of a coating surrounding the microgels. Whereas the surface of the uncoated microgels is smooth, the (Pt$_{NP}$/DAR)$_3$ coated microgels exhibit a ‘ball-in-a-bag’ appearance due to the difference in drying kinetics between the microgels and the (Pt$_{NP}$/DAR)$_3$ coating. When comparing the size of the microgels measured by optical and electron microscopy a twofold decrease in diameter is observed by electron microscopy. This dramatic shrinkage is ascribed to the dehydratation step under vacuum prior to sputtering and SEM imaging.

![Fig. III. 6. Confocal microscopy images of microgels encapsulating green fluorescent polystyrene beads (A) before and (B) after coating with (Pt$_{NP}$/DAR)$_3$. The upper row represents encapsulation of 100 nm sized green fluorescent polystyrene beads while the lower row represents encapsulation of 1 µm sized polystyrene beads.](image)

To illustrate the possibility of encapsulation within the microgels, hydrophilic (carboxylated) green fluorescent polystyrene beads with diameters of 100 nm and 1 µm were added to the aqueous dex-HEMA feed stream prior to microfluidic emulsification in two different experiments. The rationale behind the encapsulation of these particles was to use them in future research for on bead detection assays within the confined volume of a capsule, which could exhibit selective permeability for solutes of different size and would also allow easy transportation of the beads from one medium to another. **Fig. III. 6** shows the corresponding confocal microscopy images of the microgels obtained after UV polymerisation. The fluorescent polystyrene particles are distributed throughout the volume of the microgels and can still be detected after coating with (Pt$_{NP}$/DAR)$_3$. Note that for transferring the
polystyrene loaded microgels to the aqueous phase, ethanol was used as the intermediate solvent instead of acetone as this would have lead to dissolution of the polystyrene particles.

Fig. III. 7. (A) Confocal microscopy (transmission channel) snapshots taken during the (NaOH accelerated) degradation of (PtNP/DAR)$_3$ coated monodisperse dex-HEMA microgels resulting in hollow (PtNP/DAR)$_3$ capsules. (B) Evolution of the microgel diameter during the degradation process as measured by the confocal software. (C) Scanning electron microscopy image of a hollow (PtNP/DAR)$_3$ capsule.

Finally, in order to obtain hollow capsules the microgel templates have to be dissolved. Dex-HEMA hydrogels are degradable through hydrolysis of carbonate esters, which connect the dextran backbone with the polymerized HEMA. Therefore the (PtNP/DAR)$_3$ coated microgels were suspended in a 1 M sodium hydroxide (NaOH) solution for 5 min and allowed to sediment, followed by removal of the supernatant and resuspension in distilled water. This process was repeated three times to ensure sufficient removal of residual NaOH. Note that centrifugation should be avoided to avoid capsule break-up. Fig. III. 7 shows in detail the influence of the microgel degradation on the capsule properties. Confocal microscopy (see Fig. III. 7. A) shows that upon addition of NaOH, the microcapsules start to swell until equilibrium is reached after approximately 100 s of swelling as illustrated by Fig. III. 7. B that shows the gradual increase in capsule diameter as a function of degradation time until a plateau is reached. The physico-chemical reason for this swelling is the swelling pressure that increases upon degradation of the hydrogel network. As the number of cross-
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links decreases, the dextran chains have more flexibility within the network, allowing it to expand. Note that no relaxation of the capsule membrane was observed and the capsules retained their final diameter over prolonged times. The proof that hollow capsules are generated in this way is given by scanning electron microscopy as a collapsed folded structure is observed (see Fig. III. 7. C), which is typical for hollow polyelectrolyte capsules in the dried state.

III. 4. Conclusions

In this work, giant hollow polyelectrolyte capsules, with dimensions much larger than common (1-10 µm sized) polyelectrolyte capsules, have been fabricated using degradable microgels as sacrificial templates. The microgels were synthesized by microfluidic emulsification in a co-flow geometry, allowing large monodisperse microgels to be produced. In order to help the capsule membrane withstand the swelling pressure of the degrading microgel, the membrane was reinforced using covalent linkages between a cationic polyelectrolyte and metal nanoparticles. Microgels loaded with nano- or micro-sized fluorescent polystyrene particles have also been prepared for encapsulation studies. Both types of polystyrene particles have been visualized, even after multilayer build-up. These microcapsules can find applications as microreactor or delivery system after remote opening of the microcapsules’ shell by an external physical source such as laser light or ultrasound.

III. 5. Experimental

Materials and Methods:

For the microfluidic setup, syringe pumps were KDS Scientific and New Era NE-300 model. Tygon tubing (0.8 mm internal diameter, Saint-Gobain Performance Plastics) were purchased from VWR International. 30G disposable needles were purchased from Becton Dickinson and blunted. A Metalight UV box operating with 12 (320-400 nm) lamps was used for solidification.

Light mineral oil, DMAEMA and suspensions of fluorescent polystyrene beads with diameters of both 100 nm and 1 µm were purchased from Sigma-Aldrich. Irgacure 2959 was obtained from Ciba. ABIL EM-90 was kindly donated by Evonik Degussa.

Dex-HEMA was synthesized by Dr. ir. B. G. De Geest according to the literature. Briefly, HEMA was first coupled with carbonyldiimidazole and this HEMA-carbonylimidazole was then used to derivate dextran chains. PtNP’s were prepared by adding an aqueous solution of H₂PtCl₆·6H₂O to a solution of mercaptosuccinic acid in methanol and stirred for 30 min. Nanoparticles were formed by dropwise
addition of aqueous NaBH₄ over the H₂PtCl₆/mercaptosuccinic acid suspension under vigorous stirring. Nanoparticles were isolated by repeated centrifugation and washing steps and finally dried under vacuum. The diazoresin (diazo-10, 4-diazo-diphenylamine/formaldehyde condensate hydrogen sulfate/zinc chloride salt) was purchased from Livingston Associates, USA.

Confocal microscopy images were recorded with the transmission channel on a Nikon EZ-C1. Scanning electron microscopy (SEM) images were recorded with a Quanta 200 FEG FEI scanning electron microscope operated at an acceleration voltage of 5 kV. A drop of the particle or capsule suspension was deposited onto a silicon wafer and dried under a nitrogen stream followed by sputtering with gold. Transmission electron microscopy (TEM) images were recorded with a CM-200 FEG Philips transmission electron microscope operated at an acceleration voltage of 120 kV. A drop of PtNP’s suspension was deposited and dried onto a copper grid modified with amorphous carbon.

**Microfluidic emulsification**: Pumping rates of continuous and discrete phases were 60 and 1 mL/h respectively. A 2 m piece of Tygon tubing was used, which allowed the formed droplets to be exposed to UV light for approximately 45 seconds. The tubing makes few helices inside the chamber in which the UV lamps are distributed on the outer circle. These 45 seconds were enough for fixing the structure of the beads but complete conversion was ensured with 15 min of extra off-tubing UV exposure by simply leaving the collected beads in the UV chamber. The continuous phase was prepared by dissolving 4.5 vol% of ABIL EM-90 in mineral oil. The discrete phase was prepared by first dissolving 20 mg Irgacure 2959 in 1 mL distilled water, which was warmed up to 70-80 °C in a water bath. After cooling down to room temperature, 200 mg dex-HEMA and 5 μL DMAEMA were added, the mixture was stirred and sonicated, resulting in a 17 % (w/w) dex-HEMA solution in water. For polystyrene encapsulation studies, 20 μL of corresponding polystyrene suspension was also added to the monomer phase and the mixture was vortexed vigorously.

**Microcapsule fabrication**: Monodisperse microgels were coated with alternating layers of PtNP’s and diazoresin. In total three alternate layers were deposited. The PtNP’s solution consisted of 0.5 mg/ml PtNP’s in the presence of 0.5 M NaCl in deionized water and the diazoresin consisted of 1 mg/ml resin in the presence of 0.5 M NaCl in deionized water. Adsorption was carried out for 10 minutes under continuous shaking and three washing/centrifugation steps were carried out to remove non-adsorbed species.
References


Thiol functionalized bead undergoes various click transformations with different rates.

Abstract

This chapter describes microgels prepared via thiol-ynne chemistry in microfluidics. By changing the ratio of the tetra-thiol and di-alkyne monomers used for the construction of these microgels, thiol and yne functionalized resins were obtained. The yne functionalized bead was then subjected to the two well known click reactions in a comparative manner: thiol-ynne and azide-alkyne additions. More interestingly, the thiol bead allowed the comparison of 9 click reactions: thiol-ene, thiol-norbornene, thiol-acrylate, thiol-maleimide, thiol-α bromide, thiol-epoxy, thiol-aziridine, thiol-isocyanate and thiol-isothiocyanate. The kinetics of these reactions were followed and compared by FTIR spectroscopy. In brief, this chapter tries to fulfill the need for novel highly efficient resin functionalization strategies.

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Chapter IV. Thiol-Yne Microgels

IV. 1. Introduction

The introduction of the “click” chemistry concept by Sharpless et al., focusing on constructing carbon-heteroatom linkages in a modular and highly efficient manner instead of C-C bond formation, revolutionized the way how research is being conducted in numerous fields and even ‘lead to a paradigm shift’ in certain research fields. For the area of click chemistry, this impact has recently been highlighted by our and other groups in Angewandte Chemie. Long time enduring synthetic challenges such as rotaxanes and catenanes are being overcome, supramolecules met novel building blocks, two huge homopolymers or polymers with peptides can be easily coupled, macromolecular rings can be closed, novel materials with improved performances are being designed, cells and DNA molecules are getting accustomed to ground-breaking functionalization approaches, just to mention some of the breakthroughs. Cu catalyzed azide-alkyne cycloaddition (CuAAC) had been dominating the click world exploration of the click characteristics of radical mediated addition of thiols to olefins (thiol-ene). Moreover, the click potential of several other thiol-based conjugations (thio-click) has been recently highlighted, thiol-yne being the main example. The click repertoire is broader than ever and an efficiency comparison of these reactions is of prime interest within this strongly evolving field, which has been the starting point of this chapter.

Solid phase synthesis (SPS) was defined by Sharpless as a process where “ordinary” reactions are employed under click conditions, due to the large excess of reagents used in the mobile phase and to the possibility of using washing as a simple work-up procedure. Since working with 5 to 10 equivalents is a common practice in SPS, making it very expensive by wasting a large amount of reagents, we believe that it is reasonable to compare a series of click reactions on the solid state by utilizing a small excess of reagents in the mobile phase. We therefore decided to start with a thiol-functionalized resin and screen the click efficiency of a large variety of reaction partners. With this approach, we aim for the introduction of novel ligation reactions for several applications such as solid phase peptide synthesis (SPPS), which is currently “mind-locked” on a few reactions.

This chapter covers an easy approach to construct not only thiol but also yne functionalized monodisperse beads, which underwent several click reactions in a comparative manner. By using
only a tetra-thiol (1) and a di-alkyne (2), fabrication of either a thiol or an yne resin is possible by changing the ratios of these starting reagents. Whereas CuAAC and thiol-yne were compared on yne bead, 9 different (click) reactions on thiol beads were compared.

**IV. 2. Results and Discussion**

Fig. IV. 1. A shows the production of thiol and yne beads. Desired functionalities were proven by FTIR spectra (see Fig. IV. 2); S–H stretch at 2570 cm\(^{-1}\) for the thiol bead, \(\equiv\text{C–H}\) stretch at 3280 cm\(^{-1}\) and C≡C stretch at 2115 cm\(^{-1}\) for the yne bead. It is important to note that the IR spectrum of thiol beads does not possess any of the alkyne peaks and vice versa. The tubing-needle based microfluidic setup (see Fig. III. 1) is used to obtain monodisperse particles (see Fig. IV. 1. A). Monodispersity is important for reproducible functionalization, hence comparison. Nonporous beads are preferred in this study to porous ones because of the fact that the latter are generally opaque and can retard UV based click reactions by forbidding light penetration.

Fig. IV. 1. A) Making thiol and yne beads via thiol-yne chemistry and fluorogenic click functionalizations. B) Light microscopy image showing the nonporous and monodisperse nature of thiol beads. C) Fluorescent thiol bead after clicking with 3-azido-7-hydroxycoumarin (4).

Initially, the reactivity of the beads was attempted to be visualized by making use of fluorogenic reactions. Fluorogenic molecules are indeed effective tools for reaction monitoring since they only
become fluorescent after the desired reaction occurs, i.e. triazole formation. Whereas the yne beads reacted with 3-azido-7-hydroxycoumarin (4 in Fig. IV. 1. A) to give fluorescent beads (see Fig. IV. 1. C), thiol beads did not react with another fluorogenic thiol probe (3), which is also based on coumarin. Addition of NEt₃ as a catalyst did not make any change for this failing reaction.

**Fig. IV. 2.** FTIR spectra of intact yne (thin spectrum) and thiol (bold spectrum) beads.

After proving the reactivity towards the fluorogenic azide, the yne-functionalized beads were exposed to another CuAAC reaction as well as to a thiol-yne click reaction with similar compounds (see Table IV. 1). An intrinsic difficulty of this comparison was the double addition of thiols to the alkynes while the azide reacts only once (see Table IV. 1). The double addition of thiols can be considered advantageous if high loadings are desired. The long C12-chain of the chosen reagents avoided the explosive character and unpleasant smell of small azides and thiols, respectively. Commonly practiced literature conditions have been employed for both reactions (see Table IV. 1), including continuous UV-irradiation for the thiol-yne setup. Dimethyl formamide (DMF) was chosen as the solvent for both reactions because of its high solvating capability, which is crucial for solid phase reactions. For each reaction, several batches of the yne beads have been prepared and the reactions have been stopped by washing the beads after the predetermined time intervals (30 min, 1h, 2h and 4h).
Table IV. 1. Click reactions performed in this study on the yne bead

<table>
<thead>
<tr>
<th>BEAD (1eq)</th>
<th>REACTANT</th>
<th>Catalyst/Initiator</th>
<th>ANTICIPATED PRODUCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSC₁₂H₂₅ (4eq)</td>
<td>0.05 eq DMPA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N₃C₁₂H₂₅ (2eq)</td>
<td>0.1 eq CuBr 0.2 eq PMDETA</td>
<td></td>
</tr>
</tbody>
</table>

The kinetics of both reactions were followed by FTIR.⁵¹,⁵³ Both C≡C and ≡C–H peaks were clearly degrading as a function of time as a result of both CuAAC and thiol-yne reactions (see Fig. IV. 3). The larger ≡C–H (3280 cm⁻¹) signal was used for reaction monitoring after normalization by using the carbonyl peak at 1740 cm⁻¹. While the area ratio is generally the most appropriate choice for kinetic studies, the height ratio has been used in this case to have a comparable set of data with the results on thiol beads (see Table IV. 2).

Fig. IV. 3. Evolution of FTIR spectra of yne bead by the addition of 1-dodecanethiol. Spectra was normalized using the carbonyl peak at 1740 cm⁻¹. Addition of 1-dodecaneazide has a similar but slower depletion of ≡C–H peak.
**Fig. IV. 4** compares the addition of 1-dodecanethiol (5) and 1-dodeceneazide (6) to yne beads. While thiol-yne reaches to near completion in about 4 hours, CuAAC consumes 60% of the yne peak height in 2 hours without further improvement for longer reaction times. The thiol-yne reaction appears to be superior to CuAAC in these conditions, without neglecting the fact that the thiol concentration was two times higher and UV exposure was necessary. Of course, other ligands can be utilized to improve CuAAC.\textsuperscript{54-55} It should be noted that a further proof of the CuAAC was the appearance of a C=C peak\textsuperscript{56} at 1660 cm\textsuperscript{-1} originating from the triazole ring (not shown). A larger C=C peak appears for the thiol-yne reaction (see **Fig. IV. 3**), suggesting that the second thiol addition is not as fast as the first one, although reported otherwise.\textsuperscript{51} Furthermore, alkyne-nitrile oxide\textsuperscript{57} and alkyne-tetrazine\textsuperscript{58} conjugations were skipped in this study due to the unstability of the nitrile oxides and the necessity of extensive heating for the latter.

![Graph](image.jpg)

**Fig. IV. 4.** Comparison of thiol-yne and CuAAC click reactions kinetics on yne beads. Red squares for 1-dodecanethiol and blue diamonds for 1-dodeceneazide. Curves were drawn arbitrarily.

More interestingly, the thiol beads allowed the comparison of 9 different thiol-X reactions, which are listed in **Table IV. 2** including reagents and anticipated products. Together with a non-activated ene (7), norbornene (8) has been used since it receives thiol radicals the fastest among enes.\textsuperscript{59-61} Two Michael additions were also selected: phosphine catalyzed thiol-acrylate\textsuperscript{62-64} and amine catalyzed thiol-maleimide reactions.\textsuperscript{65-68} Thiol-bromide exchange,\textsuperscript{69-70} which is also accepted as a click reaction despite the evolving HBr, has also been performed. Furthermore, we focused on two types of three-membered heterocyclic rings. It is surprising that the thiol-epoxy conjugation\textsuperscript{71-73} is described as being a click reaction instead of the thiol-aziridine reaction,\textsuperscript{74-78} although the latter was highlighted in the Sharpless’ review. Finally, amine catalyzed thiol-isocyanate\textsuperscript{38,63,79} and isothiocyanate\textsuperscript{80}
conjugations were added to our comparison. Thiol-yne was deliberately avoided due to the double addition.

Table IV. 2. Overview of thiol-X reactions performed on thiol beads

<table>
<thead>
<tr>
<th>BEAD (1eq) Class</th>
<th>REACTANT (2eq)</th>
<th>Catalyst/Initiator</th>
<th>ANTICIPATED PRODUCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S$_{N}$2 Reaction</td>
<td>CH$_3$COOH</td>
<td>0.05 eq DMPA</td>
<td>S$_2$CH$_3$COOH</td>
</tr>
<tr>
<td>Michael addition</td>
<td>CH$_3$COOH</td>
<td>0.1 eq DMPP</td>
<td>S$_2$CH$_3$COOH</td>
</tr>
<tr>
<td>S$_{N}$2 Reaction</td>
<td>CH$_3$COOH</td>
<td>0.1 eq NEt$_3$</td>
<td>S$_2$CH$_3$COOH</td>
</tr>
<tr>
<td>S$_{N}$2 Reaction</td>
<td>CH$_3$COOH</td>
<td>0.1 eq NEt$_3$</td>
<td>S$_2$CH$_3$COOH</td>
</tr>
<tr>
<td>iso(thio)cyonate</td>
<td>CH$_3$COOH</td>
<td>0.1 eq NEt$_3$</td>
<td>S$_2$CH$_3$COOH</td>
</tr>
</tbody>
</table>

Two equivalents of click reagents, 0.05 eq. initiator (for radical mediated click reactions) and 0.1 eq. of the base catalysts were always used, except for the thiol-bromo reaction where 1 eq. NEt$_3$ was...
necessary for the evolving HBr. The thiol beads had a quite high theoretical loading of 2.07 mmol/g compared to the common resins used in SPPS. With such a high thiol loading, the use of only 2 eq. of the click reagent and 0.1 eq of the respective catalyst, the click status of these 9 reactions should be revealed, at least for the solid phase reactions. No heat, shaking or deoxygenation were applied, however UV light treatment was necessary for the radical-mediated thiol-ene reactions.

![Graph](image)

**Fig. IV. 5**. Kinetic comparison of 9 thiol-X reactions on the high loading thiol functionalized bead. Red triangles for n-hexyl isocyanate, open circles for 2-norbornene, open triangles for n-butyl acrylate (solid line), blue diamonds for n-hexyl isothiocyanate (dashed-dotted curve), open squares for maleimide (dotted line, shared with undecylenic acid), green circles for undecylenic acid (dotted line, shared with maleimide) and black squares for methyl bromopropionate. No data points or lines for N-tosyl aziridine and 1,2-epoxybutane. Curves were drawn arbitrarily to guide the eye.

**Fig. IV. 5** compares the kinetics of the 9 thiol-X reactions, many of which being already accepted as click reactions. As mentioned above, we utilized the height depletion of the thiol peak (IR), normalized to the carbonyl peak of the resin, since area normalization was not possible anymore where a new carbonyl peak was being formed or carried by the clicking reagent. From this figure, it becomes clear that the thiol-isocyanate reaction is the fastest conjugation in our particular conditions, reaching up to 72% thiol peak height consumption in only an hour, which is extraordinary for such a highly loaded resin. The UV-stimulated thiol-norbornene reaction also reached high
conversions in about 4 hours, although it started slower compared to the reaction with isocyanate. Following these two reactions, thiol-acrylate (phosphine catalyzed) and thiol-isothiocyanate ($\text{NET}_3$ catalyzed) reactions exhibited quite comparable performances, reaching 75% thiol peak height depletion in 8 hours. Thus, $n$-hexyl isothiocyanate (15) had a lower but still acceptable reactivity towards thiols in comparison to $n$-hexyl isocyanate (14). Moreover, it should be noted that the actual conversions, which are proportional to area rather than to height ratios, should be even higher in all cases as the decrease in peak area is higher than the decrease of the height of the same peak. However, we still do not agree on advocating amine catalyzed thiol-iso(thio)cyanate conjugations as a member of the click family instead of amine-iso(thio)cyanate conjugations, which do not need any catalyst. The same applies for catalyst demanding thiol-acrylate Michael addition; amines add to acrylates without any catalyst. Evolution of FTIR spectra for the addition of isocyanate to our thiol bead is given in Fig. IV. 6 as an example.

**Fig. IV. 6.** Evolution of FTIR spectra of thiol bead by the addition of $n$-hexyl isocyanate (14). Spectra was normalized using the carbonyl peak at 1740 cm$^{-1}$.
Chapter IV. Thiol-Yne Microgels

Further interpretation of the Fig. IV. 5 shows comparable performances for N-methyl maleimide (10) and isolated ene (7) although they work via anionic and radical mechanisms, respectively (dotted line represents both). Their maximum reaches ~60% thiol peak height depletion after 8 hours. We believe that these yields can further be improved with more efficient catalysts such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and stronger UV sources, respectively. Furthermore, the thiol-bromide exchange reaction was found to be the slowest working conjugation. It has a poor maximum conversion of only 50% at 8 hours. Finally, neither the epoxide nor the aziridine resulted in any conversion when NEt₃ was used as the catalyst. The effect of the catalyst was further analyzed and it has been found out that while DBU¹ and ZnCl₂² can catalyze the addition of N-tosyl aziridine to our thiol beads to a maximum of approximately 30% in 8h, BF₃ and tri-butylphosphine¹ totaly failed such as NEt₃. Similarly, 62% thiol peak depletion was observed when DBU was used for 1,2-epoxybutane (12). Other conjugations can also be further improved by extended reaction times, other catalysts and by shaking the reaction mixture. For instance thiol-acrylate and thiol-maleimide reactions reached to full conversion with NEt₃ in 15 and 24 hours, respectively.

IV. 3. Conclusions

This study demonstrated that novel, high loading monodisperse alkyne or thiol functionalized beads can be easily manufactured by a tubing-needle based microfluidic setup via thiol-yne chemistry. Their ability to serve as click platforms has been demonstrated in a comparative manner. The yne beads react either with an azide or with two thiols. Thiol addition is found to be faster than the triazole formation with the continuous UV irradiation for the former being the penalty to pay. On the other hand, 9 different thiol-X reactions have been compared on the thiol beads. The thiol-isocyanate reaction is the fastest, being closely followed by the thiol-norbornene reaction. The full comparison is as follows: isocyanate > norbornene > acrylate ≈ isothiocyanate > maleimide ≈ isolated ene > α bromo ester > epoxide ≈ aziridine. The kinetics of the reaction is found to be more dependent on the reactivity of a compound than to the class it belongs to. This comparison may be the first step to guide researchers, especially in the field of SPPS, to choose the most suitable click reaction (or ligation) according to their needs since a vast number of conjugations are recently being claimed to be part of the click family.
IV. 4. Experimental

These experiments have been conducted together with Jérémy Brassinne (exchange student, UCL).

Materials and Methods: See also Chapter III. 5 for information about some materials and methods utilized also for the work of this chapter but not mentioned here. Pentaerythritol tetrakis-(3-mercaptopropionate) (tetra-thiol), 1,7-octadiyne (di-alkyne), 2,2-dimethoxy-2-phenylacetophenone (DMPA), dodecane-1-thiol, 10-undecylenic acid, n-hexyl isocyanate, n-hexyl isothiocyanate, methyl-2-bromopropionate, N-methylmaleimide, 1,2-epoxybutane, triethylamine (NEt₃), dimethylformamide (DMF), azidotrimethyl-silane, tetrabutylammonium fluoride (1M in THF), 2-norbornene, n-butyl acrylate, dimethylphenyl phosphine (DMPP), N-tosylaziridine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), ZnCl₂, BF₃ and tri-butylphosphine were purchased from Aldrich and used as received. CuBr was washed with glacier acetic acid overnight to remove oxidized species, further washed with ethanol and DEE, and dried under vacuum. Pentamethyldiethylenetriamine (PMDETA) was distilled prior to use. Washing solvents were technical grade and used as received. The fluorogenic maleate (3) was synthesized according to the literature.⁵⁰

A Nikon SMZ800 microscope was used for the bead image. Fourier transform infrared (FTIR) spectra of polymer beads, crushed and dispersed in KBr, were recorded by a Perkin-Elmer Spectrum 1000, in order to provide relevant qualitative information about IR active groups. Elemental analysis measurements were done in CNRS Paris (Centre National de la Recherche Scientifique). GC-MS analysis was done with a Hewlett-Packard GCD-Plus equipped with an electron ionization detector and a HP-SMS (Agilent Technologies, 30m x 0.25 mm x 0.25 µm) column. The carrier gas was He and temperature gradient was 3’ at 70°C, 17.5°C/min upto 315°C, 3’ at 315°C. Tygon tubing (0.8 mm internal diameter) was purchased from VWR international and 32G blunt needles were purchased from Ellsworth Adhesives.

Synthesis of 1-dodecanedezide:⁸⁴ 1-dodecanedezide was synthesized by reaction of 1-iodododecane (6 ml) with azidotrimethyl-silane (5 ml, 1.5 eq), in the presence of tetrabutylammonium fluoride (36 ml, 1M in THF, 1.5 eq). This mixture is stirred at room temperature for 24h. 25 ml of THF and water were added to the crude and the aqueous phase was extracted with pentane (3x25 ml). After washing with 3x25 ml water and drying with Na₂SO₄, pentane was removed under vacuum. GC-MS spectra (negative mode) of the product has a single peak with a retention time of 11.12 min whereas the retention time of the iodide was 11.81 min. MS spectra of the product (peak at 11.12 min) showed a minor peak at 210 m/z (211.20 calculated) but there was a larger peak at 182 m/z, which is attributed to product with loss of N₂. MS of iodododecane was completely different and showed the
mother peak of 296 m/z (calculated 296.24). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 3.18 (t, 2H), 1.53 (m, 2H), 1.19 (bs, 18H), 0.81 (t, 3H).

**Synthesis of 3-azido-7-hydroxycoumarin (4):** According to literature, \(^{49}\) 2.76g (20 mmol) 2,4-dihydroxy benzaldehyde, 2.34g (20 mmol) N-acetylglycine and 4.92g sodium acetate (60 mmol) was refluxed (140-150 °C) in 100 mL acetic anhydride at for 4h. There was some solid on the bottom at the end. The reaction mixture was poured onto ice (300-400 mL) to give a yellow precipitate. Precipitation was sluggish. After filtration, the yellow solid was washed by ice-water before it was refluxed in a mixture of concentrated HCl and ethanol (2:1, 30 mL) for 1 hour (mixture turns into dark red when the acid is added), then ice-water (40 mL) was added to dilute the solution. The solution was then cooled in an ice bath and NaNO\(_2\) (40 mmol, 2.76g) was added. The mixture was stirred for 5-10 minutes and NaN\(_3\) (60 mmol, 3.90g) was added in portions. After stirring for another 15 minutes, the resulting precipitate was filtered off, washed with water, and dried under reduced pressure to afford a brown solid; 215 mg (5% overall yield). NMR (DMSO) peaks fit with the original report. \(^{49}\) The product was also analyzed with LCMS. In positive mode, we obtained 176 and 148 m/z peaks, the latter being the larger peak. 176 peak should correspond to the product with -N\(_2\) loss. This kind of N\(_2\) loss is very usual for azides. Later, we clicked it with phenyl acetylene and obtained single large peak with a value of 304 m/z in negative mode. MW of the clicked product should be 305 g/mol.

**Bead manufacture:** Beads were synthesized by using the tubing-needle microfluidic setup. Continuous phase was 3 wt% SDS. Flow rates were 120 ml/h and 1 ml/h for continuous phase and monomer phase, respectively. Beads were exposed to UV for one more hour after collection and then washed with 3 x 5 ml of water, methanol, dichloromethane, acetone, and diethyl ether, and dried under vacuum at 90 °C overnight.

The monomer phase for the thiol beads was prepared as follows: 0.375 g (5 mol% compared to the total thiol functions) photoinitiator DMPA is dissolved in a mixture of 3.666 g tetra-thiol (7.502 mmol) and 0.545 g 1,7-octadiyne (5.133 mmol).

For the yne beads, 0.133 g (5 mol% compared to the total thiol functions) photoinitiator DMPA is dissolved in a mixture of 1.297 g tetra-thiol (2.654 mmol) and 0.424 g 1,7-octadiyne (3.994 mmol).

**Theoretical Loading Calculations**

**Thiol bead:**

\[
\text{Thiol loading} = \frac{n_{\text{remaining thiol groups after bead formation}}}{\text{total mass of ingredients}} \\
= \frac{n_{\text{initial thiol groups}} - n_{\text{initial }} n\text{-bonds}}{\text{total mass of ingredients}}
\]
\[ \frac{(4x \text{ n}_{\text{tetraphiol}} - 4x \text{ n}_{\text{dialkyne}})}{m_{\text{tetraphiol}} + m_{\text{dialkyne}} + m_{\text{DMPA}}} \]

\[ = \frac{(4x 7.502 \text{ mmol} - 4x 5.133 \text{ mmol})}{(3.666g + 0.545g + 0.375g)} \]

\[ = 2.067 \text{ mmol/g} \]

**Yne bead:**

Yne loading \[ = \frac{\text{n}_{\text{remaining yne groups after bead formation}}}{\text{total mass of ingredients}} \]

\[ = [0.5x (\text{n}_{\text{remaining π-bonds after bead formation}})] / (m_{\text{tetraphiol}} + m_{\text{dialkyne}} + m_{\text{DMPA}}) \]

\[ = [0.5x (\text{n}_{\text{initial π-bonds}} - \text{n}_{\text{initial thiol groups}})] / (m_{\text{tetraphiol}} + m_{\text{dialkyne}} + m_{\text{DMPA}}) \]

\[ = [0.5x (4x \text{n}_{\text{dialkyne}} - 4x \text{n}_{\text{tetraphiol}})] / (1.297g + 0.424g + 0.133 g) \]

\[ = [0.5x (4x 3.994 \text{ mmol} - 4x 2.654 \text{ mmol})] / (1.854g) \]

\[ = 1.446 \text{ mmol/g} \]

**Post-modifications of beads**

Note: Samples were prepared individually for each time scale of every reaction, instead of preparing one batch per reaction and taking samples from the same batch. Namely, for the thiol-norbornene reaction for instance, 5 vials were prepared containing exactly the same ingredients. The first one is stopped via washing after 30 min, second after 1h and so on. DMF was selected as the solvent because of its high solvating capability and its ability to swell the beads.

**CuAAC on yne beads:** 20 mg yne beads (28.8 µmol theoretical yne groups) were weighed into a 5 ml vial, followed by addition of a solution of 1-dodecaneazide (12.2 mg, 57.6 µmol), copper bromide (0.8 mg, 5.58 µmol) and PMDETA (2.5 µL, 11.97 µmol) in DMF (0.5 mL). Reaction with 3-azido-7-hydroxycoumarin was performed in a similar way using TBTA as the ligand instead of PMDETA.

**Thiol-yne on yne beads:** 20 mg beads were weighed into a 5 ml vial, followed by addition of a solution of dodecanethiol (23.3 mg, 115.1 µmol) and DMPA (1.5 mg, 5.85 µmol) in DMF (0.5 mL). The reaction setup was then exposed to the same UV lamps as used to perform beads synthesis.

**Representative base mediated thiol-X reaction on thiol beads:** A solution of n-hexyl isocyanate (12 µL, 82 µmol) and NEt₃ (0.56 µL, 4 µmol) in DMF (0.5 mL) was added on 20 mg of the thiol-containing bead (theoretically 41 µmol) in a glass vial and capped. Instead of 0.56 µL NEt₃, 5.6 µL NEt₃ was used
for the methyl-2-bromopropionate reaction and 0.57 µL DMPP (4 µmol) was used for the thiol-acrylate reaction. Reaction with fluorogenic maleate was performed in a similar manner by using NEt₃.

**Representative radical mediated thiol-ene on thiol beads:** 20 mg thiol beads (41 µmol theoretical thiol groups) were weighed into a 5 ml vial, followed by addition of a solution of 2-norbornene (0.5 ml, 82 µmol) and DMPA (0.53 mg, 2 µmol) in DMF. The reaction setup was then exposed to the same UV lamps as used to perform beads synthesis. Reaction with 10-undecylenic acid is performed in a similar manner.

After the necessary time passed, all the beads were washed with 3 x 5 ml of methanol, dichloromethane, acetone, and diethyl ether, and dried under vacuum at 90 °C overnight. The beads are mixed with some KBr, crushed together and IR pellets were prepared.

**Elemental analysis calculations for the loading**

2.05% = Amount of nitrogen found by elemental analysis after fully treating the thiol bead with N-methyl maleimide (further denoted here as maleimide)

0.0205g nitrogen in 1 g final maleimide capped bead

\[ n_{\text{nitrogen}} = \frac{m_{\text{nitrogen}}}{14 \text{ g mol}^{-1}} = \frac{0.0205 \text{ g}}{14 \text{ g mol}^{-1}} = 0.00146 \text{ mol} \]

\[ n_{\text{nitrogen}} = n_{\text{maleimide}} = n_{\text{SH groups}} = 0.001464 \text{ mol} \]

\[ m_{\text{maleimide}} = n_{\text{maleimide}} \times MW_{\text{maleimide}} = 0.001464 \text{ mol} \times 111.10 \text{ g mol}^{-1} = 0.163 \text{ g} \]

\[ m_{\text{untreated bead}} = m_{\text{final bead}} - m_{\text{maleimide}} = 1 \text{ g} - 0.163 \text{ g} = 0.837 \text{ g} \]

Initial SH loading = \[ \frac{n_{\text{SH groups}}}{m_{\text{untreated bead}}} = \frac{1.464 \text{ mmol}}{0.837 \text{ g}} = 1.75 \text{ mmol g}^{-1} \]

Theoretical loading = 2.067 mmol/g  
Found = 84.7%
References

References:


Abstract

This chapter describes the preparation of macroporous beads prepared by the tubing-needle microfluidic setup and their subsequent modification via “sandwich” microcontact printing that is a soft lithography technique. Microcontact printing necessitated the beads to be monodisperse and reactive. Porosity was important for easier handling and visualization during the microcontact printing. Moreover, porosity offers additional possibilities in terms of applications. Porogen trials cover a large part of the chapter together with encountered problems such as skin formation, low yields and inherent fluorescence. Several solutions were proposed for these problems. In addition, nonporous epoxy-functionalised, nonporous unreactive and porous unreactive beads were also prepared as reference materials.

Chapter V. Porous Janus Beads

V. 1. Introduction: Microfluidics and Microcontact Printing Married

Anisotropy is much sought recently when making particles to achieve performances which are virtually impossible by homogeneous particles.\(^1\)\(^-\)\(^2\) Anisotropy can either be realized as shape complexity\(^3\)\(^-\)\(^9\) or heterogeneity of chemical nature\(^10\)\(^-\)\(^12\) or even a combination of both.\(^13\)\(^-\)\(^16\) Chemically heterogeneous particles can be further classified into two main sub-classes: core to shell anisotropic particles\(^17\)\(^-\)\(^18\) and opposite face anisotropic particles.\(^19\) The latter is better known as Janus particles, the name of which is given after the ancient Roman god Janus believed to have two opposing faces.\(^20\)

Much attention is drawn onto Janus particles since they may possess two chemically incompatible parts, which give rise to improved and even unprecedented performances.\(^21\) Janus particles can better stabilize emulsions (Pickering emulsions)\(^22\)\(^-\)\(^24\) by locating themselves at the liquid-liquid interface, move/rotate due to external stimuli\(^25\)\(^-\)\(^27\) and self assemble in nano/macro scale.\(^28\)\(^-\)\(^34\) These unique features of Janus particles led to novel applications such as liquid marbles,\(^35\) particle based displays,\(^36\)\(^-\)\(^37\) barcoded detection,\(^14\) directionally controlled association with human cells\(^38\) and catalysis of two distinctive reactions in both phases while stabilizing a water-oil emulsion at the interface.\(^39\)

Microfluidics,\(^36\)\(^-\)\(^37\),\(^40\)\(^-\)\(^42\) flow lithography\(^15\),\(^43\) and electrohydrodynamic co-jetting\(^38\),\(^44\)\(^-\)\(^46\) techniques recently enabled researchers to manufacture as such Janus particles via solidifying (i.e. polymerization) co-flowing solutions. Although very efficient and lacking any post-modification steps, these methods are not devoid of drawbacks. Several biologically active molecules may not be compatible either with the solidification technique or the solution which is the precursor of the particle. Even if the compounds of interest are compatible with the medium, burying majority of these precious compounds to inner unreachable zones is not desired. On the other hand, several post-modification techniques have been developed over the last decade to convert homogeneous particles into Janus structures. These techniques are all based on multi-step interface chemistry; protecting one face of the particles in one phase (often reversible gelled), manipulating the other face first, removing the gelled phase and finally manipulating the opposite face of particles.\(^30\),\(^47\)\(^-\)\(^49\)

Another approach is based on fixing particles as a monolayer and treating upper faces with metal vapor,\(^27\),\(^33\),\(^50\)-\(^51\) etching,\(^35\) UV\(^52\)\(^-\)\(^53\) or laser.\(^54\) Although very efficient, these techniques are limited to
their special substrates (metals, monomers) and yet a mild and straightforward technique for surface engineering of particles with an unlimited number of (bio-)organic molecules is not available.

Since late 90’s, different lithography techniques are being utilized to engineer surfaces. Although an ever increasing work has been done on flat surfaces with ever increasing resolution of patterns, manipulating curved surfaces such as colloids is rather unknown. Microcontact printing (µcp) is one of the most efficient lithography techniques used to transfer a chemical (the ink), which is templated on a soft elastomer, to a flat substrate. There are only a handful of reports about µcp on colloids. While the earlier reports55-58 utilized electrostatic forces to transfer an ink onto single face of particles without covalent bonding, Granick et al. recently reported59 covalent µcp on silica colloids via silane chemistry. However, the latter is not only restricted to the number of available silanes but also to silica particles. Furthermore, silica particles need to be small enough in size so that they can be lifted up with the PDMS stamp and the PDMS stickiness also needs to adjusted. Full potential of the µcp method to covalently transfer a wide range of ink molecules onto tailor-made particles and not only to a single face but to both faces is still waiting to be exploited.

In this chapter, creating totally custom made Janus beads with two compounds of interest covalently attached on opposing faces via a µcp route is explored. Epoxy-amine chemistry is chosen as an efficient platform for covalent µcp since it does not require any catalyst, works at room temperature and both components are rather stable at ambient atmosphere. Monodispersity of the beads is of crucial importance to easily prepare a hexagonally packed monolayer of beads. Monodispersity also provided a uniform height of the bead monolayer, which was very important to avoid any smaller bead to be excluded (since they are out of contact of the stamp) during the µcp process. Once a monolayer of epoxy-functionalized beads is prepared on a stamp inked with an amino compound, the second stamp inked with another amine can be brought into contact with the upper side of the bead monolayer. This marriage of microfluidics and µcp is unique in the sense that we can first produce highly sophisticated polymer particles with any shape (core-shell, hollow, non-spherical), porosity and functionality and later convert them into Janus versions with an unlimited number of (bio-active) amine molecules.

V. 2. Preparation of Monodisperse Porous Beads

As discussed in detail in Chapter II, porous beads possess almost completely different characteristics when compared to nonporous microgels. Porous particles are generally opaque (especially macroporous), highly crosslinked, lighter and more brittle due to pores and higher degree of crosslinking. As advantages, porous beads possess surface area values as large as 2000 m²/g, which
allows intensive interaction of polymer with the surrounding liquid. Another distinctive feature is the fact that even non-solvents can be utilized with porous particles since interaction with the surrounding liquid is not based on swelling but on liquid penetration into pores. Nonporous microgels are only useful if they swell in the solvent utilized.

Most of the research on porous beads has been done on PS. Nevertheless, there is also extensive information about acrylates and methacrylates in the literature, especially from the Institute of Macromolecular Chemistry, Prague (Kalal, Horak, Bleha, Svec).\(^60\)-\(^62\) Styrenics photopolymerize rather slowly hence they are not suitable for our microfluidic system that requires polymerization times as short as a few minutes. This necessity turned our attention onto acrylates and methacrylates. Although slower than acrylates, methacrylates polymerize fast enough under UV exposure. Due to fast enough kinetics and extensive literature\(^63\)-\(^64\) that enables comparison and knowledge transfer, methacrylates were monomers of choice for this thesis. A longer introduction is believed not to be necessary since Chapter II discusses most of the available literature.

The study of Kumacheva et al. reporting macroporous beads composed of ethyleneglycol dimethacrylate (EGDMA) and glycidyl methacrylate (GMA) made by microfluidics had been the starting point of this research. They polymerized GMA and EGDMA in a 60:40 ratio with phthalate porogens in a PDMS chip. Diethyl, diisobutyl, dioctyl and diisodecyl phthalates are the four porogens that were used. As expected, longer alkyl chains of the porogen increased the pore size but decreased the total surface area. Diethyl phthalate (DEP) yielded a maximum surface area of 28.7 m\(^2\)/g when used 60 vol%.

**Fig. V. 7.** Light microscopy image of monodisperse GMA\(_{60}\):EGDMA\(_{40}\)/\((\text{CH}_{80}:\text{DD}_{20})_{60}\) beads.

We initially started with repeating phthalate experiments and also tried the cyclohexanol\(_{80}\):dodecanol\(_{20}\) (\(\text{CH}_{80}:\text{DD}_{20}\)) porogen mixture inspired by monoliths of Fréchet and Svec.\(^65\) First of all, our simplified microfluidic system was able to produce highly monodisperse porous beads.
(see Fig. V. 7) in most of the cases. Fig. V. 8 shows SEM images of GMA$_{60}$:EGDMA$_{40}$ beads prepared by using three different porogens; GMA$_{60}$:EGDMA$_{40}$//DEP$_{60}$ (see Fig. V. 8. A-B), GMA$_{60}$:EGDMA$_{40}$//DOP$_{33}$ (see Fig. V. 8. C-D) and GMA$_{60}$:EGDMA$_{40}$//(CH$_{80}$:DD$_{20}$)$_{60}$ (see Fig. V. 8. E-F) beads where DEP stands for diethyl phthalate, DOP for dioctyl phthalate, CH for cyclohexanol and DD for dodecanol.

![SEM images of GMA$_{60}$:EGDMA$_{40}$ beads](https://example.com/image1.png)

Fig. V. 8. SEM images of GMA$_{60}$:EGDMA$_{40}$ beads. Complete beads are shown in the upper row and their corresponding close-up images are shown in the bottom row. A) Bead prepared with 60 vol% DEP (GMA$_{60}$:EGDMA$_{40}$//DEP$_{60}$ bead); B) Surface close-up of the GMA$_{60}$:EGDMA$_{40}$//DEP$_{60}$ bead; C) Bead prepared with 33 vol% DOP (GMA$_{60}$:EGDMA$_{40}$//DOP$_{33}$ bead); D) Surface close-up of the GMA$_{60}$:EGDMA$_{40}$//DOP$_{33}$ bead; E) Bead prepared with 60 vol% CH$_{80}$:DD$_{20}$ (GMA$_{60}$:EGDMA$_{40}$//(CH$_{80}$:DD$_{20}$)$_{60}$ bead); F) Surface close-up of the GMA$_{60}$:EGDMA$_{40}$//(CH$_{80}$:DD$_{20}$)$_{60}$ bead.

The systematic nomenclature of beads used throughout this thesis is as follows: monomer’$_{XX}$:monomer”$_{YY}$:crosslinker$_{ZZ}$//(porogen’$_{AA}$:porogen”$_{BB}$)$_{CC}$. Here, the final composition of the beads comes before the // sign, with the corresponding volume ratios; XX, YY and ZZ. XX + YY + ZZ is always equal to 100 since porogens are, in theory, completely washed out after polymerization. The porogens come after the // sign, which are used for the preparation of the corresponding discrete phase mixture for this bead with their corresponding volume ratios subscripted. AA + BB is also equal to 100. In addition, CC designates the initial volume ratio of the sum of these porogens compared to
the sum of monomers and crosslinker. As an example, \( \text{GMA}_{60} : \text{EGDMA}_{40} / (\text{CH}_{80} : \text{DD}_{20})_{60} \) describes a bead that is composed of GMA and EGDMA with 60 and 40 vol%, respectively. For the preparation of this bead, a mixture of CH:DD is used in 60 vol% (where the ratio between these porogens was 80:20). Hence the monomers constituted 40 vol% in the initial discrete phase mixture. Initiator was always dissolved in the monomer mixture with a ratio of 4 w% and will never be mentioned in this nomenclature.

The \( \text{GMA}_{60} : \text{EGDMA}_{40} / \text{DEP}_{60} \) bead has a much smoother surface compared to the same one published by Kumacheva group. On the other hand, both the \( \text{GMA}_{60} : \text{EGDMA}_{40} / \text{DOP}_{33} \) and the \( \text{GMA}_{60} : \text{EGDMA}_{40} / (\text{CH}_{80} : \text{DD}_{20})_{60} \) beads are clearly macroporous; even if the ratio of DOP was only 33 vol%. The \( \text{GMA}_{60} : \text{EGDMA}_{40} / \text{DOP}_{33} \) beads also seem to be perfectly spherical compared to the \( \text{GMA}_{60} : \text{EGDMA}_{40} / (\text{CH}_{80} : \text{DD}_{20})_{60} \) beads, which is attributed to a lower amount of porogen utilized in the DOP case. Beads from 50% dibutyl phthalate (DBP) were also prepared; however the obtained surfaces of those beads were rather smooth (not shown) such as in the case of DEP. Surface area measurements on the other hand were interesting. Although the \( \text{GMA}_{60} : \text{EGDMA}_{40} / (\text{CH}_{80} : \text{DD}_{20})_{60} \) bead possessed a surface area of 49.4 m\(^2\)/g (rather high for a macroporous bead), \( \text{GMA}_{60} : \text{EGDMA}_{40} / \text{DEP}_{60} \) bead had no surface area value. However, a value of 28.7 m\(^2\)/g was published by Kumacheva for the \( \text{GMA}_{60} : \text{EGDMA}_{40} / \text{DEP}_{60} \) bead and the only difference with Kumacheva’s bead prepared by using DEP in our system was the initiator utilized. This fact suggests that the polymerization kinetics (emerging from initiator difference) may have a significant effect on pore formation. In addition, while the \( \text{GMA}_{60} : \text{EGDMA}_{40} / (\text{CH}_{80} : \text{DD}_{20})_{60} \) and \( \text{GMA}_{60} : \text{EGDMA}_{40} / \text{DOP}_{33} \) beads were opaque to the naked eye, our DEP beads were transparent. Being opaque is a good measure of macroporosity.\(^{66}\) It should also be noticed that the \( \text{GMA}_{60} : \text{EGDMA}_{40} / (\text{CH}_{80} : \text{DD}_{20})_{60} \) bead is the smallest among the three although pumping rates were the same. This is ascribed to the polarity difference between the porogens: the highly polar \( \text{CH}_{80} : \text{DD}_{20} \) mixture decreases the surface tension between the droplets and the continuous phase, so that smaller droplets are formed.

V. 3. Low Yields in Bead Preparation

It is important that one can obtain high yields in bead manufacture, especially in microfluidics that is generally suffering from low production rates due to miniaturization. During the sample preparation for surface area measurements, it was observed that yields for our porous beads are rather low. \( \text{N}_2 \) absorption-desorption analysis provide more reproducible data if the amount of the sample is higher. As an example, when 1 mL of discrete phase composed of EGDMA, GMA and \( \text{CH}_{80} : \text{DD}_{20} \) porogen (60 vol%) is emulsified and polymerized in the microfluidic system, approximately 0.4 g of
beads are expected to be obtained. However, less than half of this amount was being obtained in each case. Several facts could be the reason of the low yields: monomer transfer to the continuous phase, the effect of porogens on monomer transfer and incomplete polymerization due to photopolymerization (since bead diameters are rather large and porosity makes beads opaque).

First, the effect of porogen amount and the type of photoinitiator is analyzed. Results in previous chapter was obtained by using 2-benzyl-2 (dimethylamino)-4’-morpholino-butyrophene (BDM) initiator. 2,2-dimethoxy-2-phénylacétophene (DMPA) initiator was included in this study together with BDM. Results are summarized in Table V. 3.

**Table V. 3.** Effect of initiator and amount of porogen on yields. Porogen used is CH:DD (80:20) mixture.

<table>
<thead>
<tr>
<th>Initial amount of porogen (vol%)</th>
<th>BDM</th>
<th>DMPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Yield (%)</td>
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First of all, Table V. 3 confirms the theory about porosity in two ways. There is a minimum limit for the amount of porogen needed to be added to achieve phase separation hence the pores. This limit seems to be around 40 vol% for the system studied. On the other hand, the porogen can greatly reduce the diameter of the beads. There is approximately a 2-fold decrease in diameter when 0 and 60 vol% porogen beads are compared for both initiators. This observation is ascribed to the decreased interfacial tension between the discrete and continuous phases since porogens are polar alcohol molecules.

Several other conclusions can be drawn from Table V. 3, especially regarding the yield. First of all, yield (mass conversion) greatly depends on the amount of porogen used. In the case of BDM initiator, the yield gradually drops from 69.5% to 37.5% by increasing the amount of porogen from 0 to 60%. Higher amount of the porogen means higher dilution of monomers, hence slower
polymerization. Since polymerization is slower when more porogen is used, monomers may find more time to diffuse to the continuous water phase. EGDMA has a negligible water solubility but GMA has a water solubility of 3 w%. This low amount becomes significantly high when the continuous phase is pumped ~100 times faster compared to the monomer phase. Indeed, mostly GMA but also EGDMA and the porogens are detected by NMR when the continuous phase of a collected batch of beads is extracted with DCM. The amount of monomers found in the continuous phase was matching the missing portion of the yield. That clearly dictates that monomers, especially GMA, are diffusing out to the continuous water phase before the polymerization is complete.

Generally porogens are expected to help keeping monomers in the discrete phase by increasing the partitioning of monomers between discrete and continuous phases. In our system however, porogens were also diffusing out to the continuous phase and carrying the monomers along. Even in the both cases where no porogen is added, the loss of the monomers to the aqueous phase was above 30%. On the other hand, when the combined organic washing steps of the beads is concentrated and analyzed by NMR, only the porogens were detected and not the monomers (GMA or EGDMA). This fact proves that 5-10 min polymerization times are sufficient, and also no monomer remains in the beads. This was also proven by following the kinetics of polymerization via IR. GMA_{60}:EGDMA_{40} mixtures were polymerized in little glass vials with 3 different initiators while the evolution of the acrylic double bond at 1638 cm\(^{-1}\) (see Fig. V. 9) is monitored. The peak at 813 cm\(^{-1}\) was not useful for following kinetics in our hands although reported by Kumacheva group.

Since monomers are lost to the continuous phase, pre-saturation of the continuous phase with monomers should, in theory, stop the monomer transfer. To check this possibility, the continuous aqueous phase (3 w% SDS) was first washed with a mixture of GMA_{60}:EGDMA_{40} and this one was used as the continuous phase. The discrete phase in this experiment consisted of monomers without any porogen. There was only a ~8% improvement in yield after pre-saturation: 77.3 % (average of two experiments) in the case of pre-saturation and 69.5 % without any pre-saturation of the continuous phase.

The effect of photo-initiation was questioned since the pre-saturation did not make a significant improvement in yields. An experiment was needed to be designed where other factors are eliminated such as the continuous phase. It was therefore decided to prepare monoliths, one of which via photo-initiation and the other with combination of thermal and photo initiation. A plastic syringe with a diameter of 0.47 mm was used as the mold and GMA_{60}:EGDMA_{40}/(CH_{80}:DD_{20})_{60} mixtures were polymerized by UV and UV+heat, respectively. The improvement was satisfactory: 43.6% for only UV cured monolith and 85.5% for doubly cured monolith. This result suggested that thermal initiation may improve the yield of bead manufacture, although the diameter of the mold
was larger than the diameter of the droplets generally obtained in microfluidics. To see this, an addition was made to our microfluidic setup. Indeed a thermal bath was added to the end of the tubing in order to expose the droplets first to UV and then to heat. While UV curing will fix the structure, it was believed that the thermal curing would go deeper to cure inner zones. Unfortunately, again no improvement was obtained in terms of the yield.

Fig. V. 9. Comparison of effect of initiators on polymerization kinetics for GMA$_{60}$:EGDMA$_{40}$//DEP$_{60}$. IR spectra are focused on 1638 and 813 cm$^{-1}$. 40 mg/mL photoinitiator was used compared to the sum of EGDMA and GMA. The blue curve belongs to the initial mixture without irradiation, black one belongs to BDM, green to benzoin ethyl ether and red to DMPA. All mixtures were irradiated for 90 sec.

In conclusion, a significant improvement in yield could not be made for the bead preparation although several trials were performed. The reason is believed to be the huge excess of the continuous phase used in this setup. The ratio of the discrete phase to the continuous phase should come closer to 1, which would reduce the monomer transfer to an insignificant value. That could, in principle, be realized first by using tubing with smaller diameters. However, rather large tubing is needed, so that the discrete phase needle does not make contact with the inner walls of tubing. Larger tubing exponentially increases the amount of aqueous phase needed. Another idea could be the use of hydrophilic tubing. Since the tubing in our research was hydrophobic, the contact of droplets with inner walls of the tubes were clogging the system. That is why larger tubing and a huge
excess of continuous phase were used. Finally, better emulsion stabilizers will also help reducing the ratio between the discrete and continuous phases. Polymeric stabilizers could be used instead of or together with SDS.

V. 4. Inherent Fluorescence Problem

Our initial tests with confocal microscopy showed that previously prepared beads were already highly fluorescent without any treatment with a fluorophore. The reason is found to be the initiator, which is needed to be used in high amounts to have very fast photopolymerization. Among the 9 different commercial photoinitiators tried (Irgacure 2022, Irgacure 819, Irgacure 184, Irgacure 2959, Darocure 4265, benzoin ethyl ether, Michler’s ketone, BDM and DMPA), the most suitable photoinitiator is found to be DMPA, due to negligible fluorescence and the formation of spherical particles. It was also interesting to found out that initiators significantly change the jetting rate of the discrete phase although being added only 1.6 w% in total monomer mixture including the porogen.

V. 5. Skin Formation

Since DMPA was found to be the most appropriate initiator, we decided to scale up previously found GMA\textsubscript{60}:EGDMA\textsubscript{40}/((\text{CH\textsubscript{80}}:DD\textsubscript{20})\textsubscript{60}} beads (see Fig. V. 8. E-F and Fig. V. 10. A) with DMPA instead of BDM. However, new batches prepared with DMPA possessed a skin layer (see Fig. V. 10. D). This skin formation was initially attributed to the DMPA but later it has been discovered that new batches of beads prepared with BDM (see Fig. V. 10. B), Irgacure 2022, Irgacure 819, Darocure 4265 and benzoin ethyl ether also possess skin layers. The reason was certainly the CH\textsubscript{80}:DD\textsubscript{20} porogen and the polarity difference between the discrete phase and the continuous water phase. There was no skin formation when the same DMPA formulation is polymerized in a petri dish as a thin film open to atmosphere (see Fig. V. 10. E) and inner the sides of the beads prepared by using BDM and DMPA initiators were both porous (see Fig. V. 10. C, F). This kind of irreproducible results in terms of skin formation was also reported by the Kumacheva group.\textsuperscript{68}

It was obvious that the CH\textsubscript{80}:DD\textsubscript{20} mixture is not suitable for our purposes. A proof of this statement was realized when click type azide addition (by using NaN\textsubscript{3}) to epoxy groups\textsuperscript{69} of the beads failed. Although the initial GMA\textsubscript{60}:EGDMA\textsubscript{40}/(\text{CH\textsubscript{80}}:DD\textsubscript{20})\textsubscript{60} bead (prepared by using BDM) and GMA\textsubscript{60}:EGDMA\textsubscript{40}/(\text{CH\textsubscript{80}}:DD\textsubscript{20})\textsubscript{60} film (prepared by using DMPA) were capable of undergoing azide addition, later prepared beads (exhibiting skin layer) were not responding to the NaN\textsubscript{3} treatment, which was revealed by IR (not shown). Obviously the skin layer was not permitting the solvents and reagents to penetrate inside of the beads.
Several newer porogens were tried to find reproducibly skin-free particles since CH\textsubscript{80}:DD\textsubscript{20} was found to be the reason of skin formation. CH:DD in other ratios,\textsuperscript{70} n-butyl acetate,\textsuperscript{71} xylene,\textsuperscript{71} toluene, phenetyl alcohol, oleic acid, n-butanol, n-octanol,\textsuperscript{72} iso-octane:toluene mixtures\textsuperscript{73} were tried as 60 vol% additive to GMA\textsubscript{60}:EGDMA\textsubscript{40} monomer mixture. CaCO\textsubscript{3} was also tried to be dispersed in the monomer mixture (with or without oleic acid) as a solid porogen\textsuperscript{74} without success. Initial check of the behavior of porogens was done via monolith preparation in glass vials (see Fig. V. 11). Since opaqueness is a measure of macroporosity,\textsuperscript{66} only the porogens that make the polymer fully opaque have been chosen for further microfluidic emulsification (see Table V. 4).

As revealed from the study summarized in Table V. 4, n-octanol is found to be the proper porogen. Different batches showed that beads are skin-free in all cases. BET measurement revealed a total surface area of 6 m\textsuperscript{2}/g, which is not unusually low for a macroporous particle. Moreover, these beads were also totally non-fluorescent when prepared in combination with DMPA initiator. The GMA\textsubscript{60}:EGDMA\textsubscript{40}/DOP\textsubscript{60} bead on the other hand, was also suitable. Although the reported surface area\textsuperscript{64} for this bead was comparable to that of GMA\textsubscript{60}:EGDMA\textsubscript{40}/n-octanol\textsubscript{60} bead, the latter was
stronger in mechanical properties, which was needed for µcp. This is valid for beads where 60 vol% DOP was used. Beads with less amount of DOP could be checked since 33 vol% DOP was enough for macroporosity (see Fig. V. 8. C-D).

![Image](image.jpg)

**Fig. V. 11.** Opaqueness test to see the suitability of porogen for macroporosity.

<table>
<thead>
<tr>
<th>Table V. 4. Porogen trials for macroporous skin-free EGDMA-GMA beads</th>
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<tr>
<td><strong>Porogen 60 vol%</strong></td>
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<td>n-Butyl acetate</td>
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In the final study, the minimum amount of \( n \)-octanol needed for macroporosity is checked since beads with 60 vol\% \( n \)-octanol were too brittle for the \( \mu \)cp process. The limit is found to be between 45 and 50 vol\% since the former was nonporous (at least on the surface, see Fig. V. 12. A-B) and the latter is macroporous (see Fig. V. 12. C-D). Furthermore, 3 other beads were prepared for the sake of comparison in \( \mu \)cp studies: a nonporous epoxy-functionalized bead, a porous unreactive (no GMA) bead and a nonporous unreactive bead. The nonporous epoxy-functionalized bead was easily prepared from GMA\(_{60}:\)EGDMA\(_{40}\) mixture by omitting any porogen. For the nonporous and unreactive bead, GMA is replaced by butyl methacrylate (BMA) using the same ratio: BMA\(_{60}:\)EGDMA\(_{40}\). BMA is chosen since the butyl group has a similar size compared to the glycidyl group but lacking any functionality. However, we were not able to prepare porous beads from BMA\(_{60}:\)EGDMA\(_{40}\) formulation using following porogens: \( n \)-octanol, DOP, DBP, CH, CH\(_{80}:\)DD\(_{20}\), \( n \)-octanol\(_{50}:\)DBP\(_{50}\), \( n \)-butyl acetate and di-isopropyl ether. This results once again showed us that finding a proper porogen for a specific monomer mixture needs trial and error type work. Solubility parameters, as reported by Kumacheva group,\(^{64,68}\) were helpless for us since we tried a whole range of solvents and solvent mixtures with a varying range of solubility parameters. Finally the MMA\(_{60}:\)EGDMA\(_{40}:\)DOP\(_{50}\) formulation resulted in porous unreactive beads.

![Fig. V. 12. SEM images of GMA\(_{60}:\)EGDMA\(_{40}\) beads prepared with two different amounts of \( n \)-octanol. A) A whole GMA\(_{60}:\)EGDMA\(_{40}:\)\( n \)-octanol\(_{45}\) bead; B) Surface close-up of GMA\(_{60}:\)EGDMA\(_{40}:\)\( n \)-octanol\(_{45}\) bead; C) A whole GMA\(_{60}:\)EGDMA\(_{40}:\)\( n \)-octanol\(_{50}\) bead; B) Surface close-up of GMA\(_{60}:\)EGDMA\(_{40}:\)\( n \)-octanol\(_{50}\) bead.](image)
V. 6. Microcontact Printing (μcp) Experiments

Microcontact printing experiments have been conducted in collaboration with the group of Prof Ravoo from Münster University. Fig. V. 13 schematically depicts the reactive “sandwich” μcp approach developed via the collaboration of both laboratories. A monolayer of beads is prepared on the first PDMS step which is inked with the ‘red’ amine. A second stamp inked with ‘yellow’ amine is brought into contact of the top of the bead monolayer to complete the “sandwich”. 2-10h of μcp produces high resolution “patches” on Janus beads shown on the right. Primary amine groups of inks attack to the epoxy groups of beads forming irreversible covalent bonds. This approach has been exploited in developing 3 different types of Janus beads.

To prove the concept, a red and a blue dye (rhodamine ethylenediamine and dansylcadaverine, respectively) were printed on porous beads for which the development stage was explained in previous parts of this chapter. A monolayer of beads was sandwiched between two PDMS stamps, one of which is inked with the red and the other with the blue dye. After heating this pressurized stamp-bead-stamp system in an oven at 120 °C for 4h, stamps were removed and beads were washed extensively with hot solvents. Results are shown in Fig. V. 14. It is clearly visible, especially from Fig. V. 14. C, that this approach successfully yielded Janus beads with blue and red faces.

Based on this successful result another class of Janus beads were prepared by printing selective biological receptors in a subsequent experiment. Amino-β-galactoside and amino-α-mannoside were first printed on beads in the same manner. It is well known that peanut agglutinin (PNA) binds to β-galactosides and concanavalin A (Con A) to α-mannosides via carbohydrate-lectin affinity.75 To exploit this possibility of selective binding the carbohydrate-Janus particles were incubated in a dilute solution of PNA and Con A, containing both lectins in equimolar ratio. It is obvious from Fig. V. 14. D-F that rhodamine labeled PNA (red) and fluorescein labeled Con A (green) adsorbed selectively to the opposing poles of the carbohydrate-Janus beads. These observations demonstrate that the carbohydrates were immobilized exclusively in the contact areas between the particles and each stamp and that both carbohydrates retain their characteristic affinity for each lectin, also when they are immobilized on the polymer particles by μcp.

A third and final type of investigated Janus particles were magnet responsive ones. This is achieved via printing magnetite nanoparticles on one face and rhodamine ethylenediamine on the other face. The utilized magnetite nanoparticles were dopamine modified, hence carrying amine groups for establishing covalent linkage with epoxy-functionalized beads. As for inking, a dilute suspension of these nanoparticles were applied to one of the stamps and dried. After the μcp process, Janus beads with nanoparticle-dye faces are obtained (see Fig. V. 15. A-B). The responsive behavior of these
Janus beads to a magnet is shown in Fig. V. 15 C-D. Beads are aligned by facing towards the same direction under a magnetic field, whereas the same beads are disoriented (see Fig. V. 15. A-B) when the magnetic field is absent.

**Fig. V. 13.** Schematic description of reactive µcp on monodisperse epoxy-functionalized beads.

**Fig. V. 14.** Upper row: Janus polymer beads obtained by printing two distinctive amine dyes; A) red rhodamine ethylenediamine (green filter), (B) blue dansylcadaverine (UV filter), (C) overlay of A and B, insert: bright field image. Bottom row: Janus polymer beads obtained by printing carbohydrates; (D) rhodamine labeled PNA bound to β-galactoside modified faces (green filter), (E) FITC labeled ConA bound to β-mannoside faces (blue filter), (F) overlay of D and E.
Control experiments proved the covalent character of this μcp process. Both nonporous BMA$_{60}$:EGDMA$_{40}$ and porous MMA$_{50}$:EGDMA$_{50}$/DOP$_{50}$ beads (no epoxy groups, see Chapter V. 5) were irresponsive to μcp experiments, meaning that no dye attachment was realized under the same conditions. Moreover, nonporous GMA$_{60}$:EGDMA$_{40}$ bead also underwent a Janus transformation μcp process, proving the necessity and power of amine-epoxy addition.

![Fig. V. 15. Bright field images (A, C) and fluorescence microscopy images (B, D) of Janus particles possessing magnetite nanoparticles on one face and rhodamine ethylenediamine on the opposing face. A magnetic field is present for C and D.](image)

**V. 7. Conclusions**

In this chapter, preparation of macroporous beads and their subsequent conversion into various Janus particles via a μcp process is described. Beads were dictated by the μcp process to possess several crucial features. Monodispersity was the first feature, which was rather easy due to the microfluidic setup we have been working with. Epoxy functions were provided by GMA monomer incorporated in the initial mixture. It was also necessary that beads were non-fluorescent as such prior to any stamping. By screening various initiators, DMPA was found to be the most suitable initiator. There was an important problem of low yields that we were unable to solve despite several attempts. Finally, porosity was not compulsory for the μcp process but porous beads offered several advantages. First of all, the application area of porous beads are broader than that of nonporous...
ones. Showing that µcp is possible both on nonporous and porous beads is a proof of versatility of this µcp approach. There were also two technical reasons: 1) due to the added polar porogen (n-octanol), porous beads become significantly smaller in microfluidics as a result of lowering the interfacial tension, 2) porous beads certainly offer a higher contact area with stamps due to increased surface area emerging from macropores. Skin formation was problematic in this sense but it has been overcome thanks to various trials with different porogens. We also demonstrated that, for a given monomer mixture, finding a suitable porogen and avoiding the skin formation needs extensive trials.

The final part of this study shows isotropic to anisotropic conversion of particles in a very efficient manner. Via reactive sandwich µcp, three different Janus particles (anistropic) were prepared from isotropic epoxy-functionalized beads: 1) Beads with red and blue dyes on opposing faces; 2) Carbohydrate Janus beads that selectively bind different lectins on opposing faces; 3) Magnetic Janus beads that respond to external magnetic field via aligning. This unique marriage of microfluidics and microcontact printing allowed us to create such anisotropic particles.

V. 8. Experimental

Materials and Methods: See also Chapter III. 5 and IV. 4 for information about some materials and methods utilized also for the work of this chapter but not mentioned here. The chemicals were obtained from Sigma-Aldrich or Acros Organics and used as received except the monomers. MMA was distilled, GMA, BMA and EGDMA were passed over basic Al₂O₃ to remove the inhibitors. Irgacure and Darocure initiators were kindly donated by Ciba Specialty Chemicals.

N₂ sorption isotherms were measured on a Belsorp-Mini II apparatus at 77K. The surface area was calculated using the BET method.

General bead manufacture: Continuous phase was 3 wt% aqueous SDS solution. Tubing utilized was Tygon (0.8 mm internal diameter, 2 m long). 30G or 32G blunt bent needles were used for the monomer phase. A generic monomer phase was as follows: 16 mg photoinitiator was dissolved in a solution of EGDMA (0.16 mL), GMA (0.24mL) and porogen (0.60 mL) and the mixture is kept in dark. GMA:EGDMA volume ratio was always 60:40 even if the ratio of the porogen was different than 60 vol%. The amount of initiator was also adjusted in these cases, by taking 4 w% compared to the total amount of EGDMA and GMA. MMA and BMA beads were prepared in a very similar manner, replacing GMA in calculations. Pumping rates were 1.20 ml/min and 0.6-0.8 ml/h for continuous and monomer phases, respectively. The droplets were polymerized on-flight by UV irradiation and
exposed for another 5 min after collection. The beads were then washed with 3 x 5 ml of water,
DCM, MeOH, DCM and DEE, and dried under vacuum at RT overnight. Samples sent for surface area
measurements were soxhleted with MeOH and acetone for 6-8h each and dried at 90 °C overnight
prior to the analysis.

**Yield experiments:** In addition to the above mentioned procedure, the discrete phase was purged
with N₂ stream for 5 min for yield experiments. For the calculations, volumetric amount of monomer
phase fed into the tubing for droplet formation is recorded, the amount of porogen was subtracted
from this volume, \( \delta_{\text{mix}} = 0.4 \times \delta_{\text{EGDMA}} + 0.6 \times \delta_{\text{GMA}} \) approximation was used for the density of
GMA:EGDMA mixture and theoretically expected bead amount was calculated in this manner. Yield
was calculated finally by comparing the theoretical amount and obtained beads after whole washing
and drying steps. Details are given in Table V.3.

\[
\delta_{\text{mix}} = 0.4 \times \delta_{\text{EGDMA}} + 0.6 \times \delta_{\text{GMA}} = 0.4 \times 1.051 \text{ g/mL} + 0.6 \times 1.06 \text{ g/mL} = 1.056 \text{ g/mL}
\]

These calculations are approximations and were certainly able to demonstrate the effect of different
parameters on the bead preparation yield.

For the monomer transfer experiments, after separating the beads from their continuous phase, the
latter was extracted 3 times with DCM, DCM phases were dried, concentrated in the rotavapor. The
amount of transferred species was then measured via weighing and NMR samples were prepared by
adding CDCl₃.

**Comparison of 3 different initiators for kinetics + other monoliths:** Monomer mixtures were
prepared in a similar manner as explained in ‘General bead manufacture’. Porogen was DEP. 0.1 mL
of these monomer mixtures with different initiators were added to 2mL transparent glass vials. The
amount was kept low to reduce the thickness of the film. Polymerized in UV box by placing the vials
close to the lamps. After 90 sec UV curing, vials were broken and films were analyzed as such by ATR-
IR. Other monoliths were prepared in the same way changing the ingredients.

**Pre-saturation of continuous phase:** 100 mL water was shaked with ~10 mL GMA₆₀:EGDMA₄₀
mixture in a separation funnel. After phase separation, water phase on top is taken, but there were
still some visible oil droplets in. 3 g of SDS is added and mixed. The final mixture was clear without
any droplets.

**Photo and thermal initiation:** 10 mg AIBN and 20 mg BDM were dissolved together in a mixture of
0.4 mL EGDMA, 0.6 mL GMA and 1.5 mL CH₈₀:DD₂₀. Beads were being exposed to UV for ~40 sec and
immediately arriving into a flask equipped with a condenser maintained at 70 °C. Thermal treatment took place overnight.

**Film preparation (Fig. V. 10. E):** 16 mg DMPA was dissolved in a solution of EGDMA (0.16 mL), GMA (0.24 mL) and CH$_8$:DD$_{20}$ (0.60 mL) and polymerized as a thin layer in a plastic weighing cup (as a mold) under UV treatment for 30 min. The films were removed from the mold, washed with DCM, MeOH, DCM and DEE, and dried.

**Epoxy-azide conversion:** Beads or pieces of film (50 mg) were preswollen in DMF (7 mL) in a flask, NaN$_3$ (0.25 g) and NH$_4$Cl (0.21 g) were added, and the reaction took place for 24 h at 50 °C while the flask is being rotated with a rotary evaporator motor. Beads were washed on a glass filter with warm H$_2$O, DMF, MeOH, DCM, and DEE and dried under vacuum at room temperature overnight. Introduction of azide groups was confirmed by IR spectroscopy with the characteristic azide band at 2100 cm$^{-1}$.

**Microcontact printing (mostly conducted by Tobias Kaufmann):** Prepolymer Sylgard 184 and curing agent (10:1) was mixed thoroughly in a plastic cup. The mixture was degassed at reduced pressure until no more bubbles raised from the polymer. The mixture was poured onto a silicon master (flat, fluorosilanized wafers) embedded into a bowl of aluminum foil and degassed again at reduced pressure. The prepolymer mixture was cured at 65 °C overnight. Finally, small (ca. 1 x 1 cm) sections of the polymer stamp were cut out and oxidized in a UV decontamination system (creating ozone with air oxygen) for 50 min. The hydrophilized stamps were stored under water to preserve their polar surface until use.

For printing, dried stamps were loaded with a few drops of the respective ink solution (1-10 mM in ethanol, added NEt$_3$ as a base) for 1 min. Excess ink solution was removed under a stream of argon for approximately 1 min. A layer of polymer particles was loaded onto the stamp and a second stamp loaded with the second ink (and dried like the first one) was put on top using a press. The samples were then left to react at a given temperature (typically: 120°C) for 4 h. When the reaction was complete, particles were collected in a filter and washed extensively with water, ethanol, acetone and diethylether. In some case, samples were additionally cleaned in hot ethanol for 10-12 h.
References

(16) Bong, K. W.; Pregibon, D. C.; Doyle, P. S. Lab Chip 2009, 9, 863.
Complex Polymer Particles via Microfluidics


Abstract

This chapter describes unique particles that are formed via polymerizing various HIPE formulations in a microfluidic channel. The obtained poly(HIPE) particles vary in shape, core-shell morphology and chemical nature. Moreover, all the particles are monodisperse and possess huge interconnected pores thanks to the applied emulsion templating strategy. The effect of these huge pores is also investigated by comparing poly(HIPE) beads with macroporous beads in a click-click modification scheme.

A part of this chapter is published as Fabrication of Porous “Clickable” Polymer Beads and Rods through Generation of High Internal Phase Emulsion (HIPE) Droplets in a Simple Microfluidic Device, Gokmen M.T., Van Camp W., Colver P.J., Bon S.A.F., Du Prez F.E. Macromolecules 2009, 42, 9289-9294.
Chapter VI. HIPE in Microfluidics

VI. 1. Introduction: Uniform Double Emulsions in Microfluidics

As discussed in Chapter II, poly(HIPE)\(^1\) is a unique class of polymeric materials due to pores that are several microns in size and huge pore volume values. These properties emerge from the emulsion templating procedure. More than 99% water (discrete phase, porogen here) can be dispersed in the continuous monomer phase if the surfactant is well chosen and this emulsion, the high internal phase emulsion (HIPE) or concentrated emulsion, will be stable for days. Polymerization, generally via thermal treatment, produces poly(HIPE). Mayonnaise is also an example of HIPE, where a large fraction of oil is dispersed in water by the lecithin of egg yolk.

Since its discovery by scientists of Unilever,\(^2\) several improvements and application areas are proposed for poly(HIPE)s. It is not surprising that applications proposed for poly(HIPE) are very similar to other monolithic supports; column adsorbent\(^3\), ion-exchange matrix\(^4\), scavenger\(^5-7\) and catalysis\(^8-13\) being the prime examples. As for the more sophisticated applications, an organic nanoparticle releasing, temperature responsive poly(HIPE) “particle pump” was designed by Cooper et al.\(^14\) by using thermal shrinking properties of \(N\)-isopropyl acrylamide (NIPAM) monomer. Carbonaceous monoliths\(^15\) were prepared from poly(HIPE) as electrodes.\(^16\) Poly(HIPE)s were also used in polymer\(^17\) and enzyme immobilization.\(^18\) Last but not least, Silverstein et al.\(^19\) managed to produce biodegradable poly(HIPE) scaffolds that can be used for cell growth\(^20-22\) and tissue engineering.\(^23\)

In terms of modifications, the most important recent development in poly(HIPE) preparation can be mentioned as improving the brittleness and chalkiness of poly(HIPE)s.\(^24\) Bismarck et al. achieved these results via using a flexible crosslinker based on PEG and via the Pickering emulsion route\(^25\) using silica particles.\(^26\) Poly(HIPE)s can also be prepared by using other emulsion stabilizers such as inorganic particles,\(^27-28\) clay,\(^29\) carbon nanotubes\(^30\) and micro-nano polymer particles.\(^31-34\) Another improvement was done in terms of total surface area. Due to huge pores, poly(HIPE) generally possess very low surface area values. Micropores were added on cell walls of poly(HIPE) by using monomer phase soluble porogens.\(^35-37\) This is another example of using porogen mixtures; water creating the huge pores and oil soluble porogen creating the micro- and mesopores. Moreover, Kaskel et al.\(^38\) utilized the Davankov approach (post hypercrosslinking) to create poly(HIPE) with a
final surface area of 1210 m$^2$/g. The obtained monoliths showed improved liquid and H$_2$ uptake properties. There has been also reports on functionalization of poly(HIPE). Heise et al.$^{39}$ polymerized GMA monomer from the poly(HIPE) surface, opened the epoxy rings with NaN$_3$ and clicked propargyl alcohol via CuAAC. Thio-click reactions were utilized on poly(HIPE) even well before the acceptance of these reactions in the click family. Mondain-Monval et al.$^{40-41}$ functionalized poly(HIPE) by thiols thanks to remaining double bonds of DVB on the matrix. More recently, Cameron et al.$^{18}$ utilized the remaining methacrylate bond of EGDMA also as a thiol receptor.

Chapter II described that there has been few attempts in the past to produce granulated$^{42}$ poly(HIPE), especially via suspension polymerization. The obtained particles however, were either polydisperse in size$^{43}$ or monodisperse but too large in diameter ($\sim$2 mm).$^{44}$ For sure there is a need for miniaturization of poly(HIPE) particles without sacrificing the monodispersity since poly(HIPE) is a unique material class due to huge interconnected pores. An obvious choice for this miniaturization step is microfluidics. Here in this chapter, we exploited the possibilities of second emulsification of HIPE formulations in our simplified microfluidic setup. The initial aim was to produce spherical particles but during the trials it has been found that there are other and more interesting possibilities that can be realized via the “HIPE-in-microfluidics” approach. These possibilities were exploited in an opportunistic manner and several unprecedented particles were manufactured in a very simple way. Photopolymerization is a way to solidify particles and it is generally preferred in microfluidics to fast cure the droplets on flight. However, studies in this chapter revealed that photopolymerization may play a very significant role in the structure of particles, producing characteristics that are not possible via thermal polymerization unless a template is used. Moreover, the obtained poly(HIPE) beads were compared by ‘classical’ porous beads, described in Chapter IV, in a double click reaction sequence to see whether the huge pores of poly(HIPE) is advantageous or not.

VI. 2. Poly(HIPE) Rods: Importance of Viscosity

HIPE preparation needs some experience and is not as easy as mixing two components. Since the critical 74% barrier is exceeded, addition of the discrete phase needs to be slow and mixing should be well controlled. For these reasons, an overhead stirrer with a fixed stirring rate is chosen. The stirrer paddle is made out of Teflon (obtained from Radleys Discovery Technologies, UK), which perfectly fits on the inner bottom of a 50 mL round bottom flask (see Fig. VI. 1). This provided homogeneous mixing during the addition of the water phase. First the photoinitiator is dissolved in the monomer mixture in a 50 mL round bottom flask that is covered with aluminum foil to prevent light penetration, surfactant is added and stirring is kept for few minutes to complete mixing. The
subsequent step is the addition of NaCl solution dropwise by the aid of a syringe pump over 30 min. When the addition is complete, overhead stirring is kept for 5 min more to ensure complete homogenization. The obtained HIPE resembles a shaving foam in terms of look and consistency. If kept in the dark, these concentrated emulsions were stable for several days. Photoinitiation was again preferred over its thermal equivalent, as heating is known to destabilize HIPE formulations, especially those with limited polarity differences between the two phases.

![Image of HIPE preparation setup]

**Fig. VI. 1.** HIPE preparation setup

Initially, HIPE formulations were prepared from highly hydrophobic acrylate monomers. Typically, the HIPE formulation consisted of 90% internal water phase (0.1M aqueous NaCl solution) and 10% continuous reactive oil phase, which contains a solution of BDM photoinitiator (2-benzyl-2-(dimethylamino)-4'-morpholino-butyrophenone, 4% wt) in 2-ethylhexyl acrylate (EHA, 65 vol%), di(ethylene glycol) diacrylate (DEGDA, 15 vol%) and the surfactant Span 80 (20 vol%) (see **Fig. VI. 2** for all the monomers used in this chapter for the preparation of HIPE formulations). An aqueous solution of sodium dodecyl sulfate (SDS, 3 w%) was used as the continuous carrier phase in the microfluidic device. Under these conditions, non-spherical poly(HIPE) segments were obtained (see **Fig. VI. 3**) that are named as (HIPE)EHA_{81}:DEGDA_{15}/(Span 80)_{20}:(NaCl solution)_{90} (see below for the explanation of the systematic nomenclature). The high viscosity of the HIPE led to a jetting rather than dripping regime, with the cylindrical jet of HIPE being broken up. The formed HIPE segments were of too high viscosity to adjust to a spherical lowest-surface-area droplet. Intriguing fibril-like side filaments were observed.
These preliminary results were exciting as there is a growing interest in non-spherical particles\(^{46-47}\), such as rods\(^{48-50}\) due to their unique packing, self-assembly and mechanical properties. Non-spherical particles or droplets can be prepared in microfluidic devices, but the key in all cases is the use of a confined space, determining the final shape of the droplets or particles. The present case is different in that the non-spherical shape is directly correlated to the viscous behavior of the HIPE phase. We
therefore took an interest in optimizing the process in order to improve control of the shape of the non-spherical segments.

Reducing the pumping rate of the HIPE phase resulted in a decreased average length of the obtained irregular segments. Attempts to improve the regularity of the obtained non-spherical structures, including a reduction of the internal water phase to 80% of the HIPE or changing EHA with lauryl acrylate did not result in any improvement. Further trials to increase the viscosity of the continuous phase by using 80% glycerol in water as the continuous phase or decreasing the viscosity of the HIPE phase, by changing the surfactant that was used for the HIPE preparation to ABIL EM 90, did not improve the process either.

However, a different HIPE formulation dramatically improved the regularity and reproducibility of the non-spherical segments, now showing resemblance with rods (also referred as worms). DMPA was dissolved in a mixture of 0.34 mL EGDMA, 0.51 mL GMA and a polymeric surfactant, i.e. poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO, MW 4400, 0.15 mL). Addition of 3.6 mL aqueous CaCl$_2\cdot$2H$_2$O solution (1.3 % wt) to this oil phase resulted in 78% internal aqueous phase in the final HIPE, which was viscous. In our co-flow device, porous uniform poly(HIPE) rods (see Fig. VI. 4) were fabricated. These rods were named as (HIPE)GMA$_{60}$:EGDMA$_{40}$/(PEO-PPO-PEO 4400)$_{3.3}$:(CaCl$_2$ solution)$_{78.3}$.

Fig. VI. 4. Poly(HIPE) rods: (HIPE)GMA$_{60}$:EGDMA$_{40}$/(PEO-PPO-PEO 4400)$_{3.3}$:(CaCl$_2$ solution)$_{78.3}$. A) Optical microscopy image showing uniformity and SEM images of B) a partial rod and C) surface of a rod.

To our knowledge, the direct preparation of non-spherical poly(HIPE) particles has not been reported so far. Moreover, the preparation of non-spherical particles with a diameter (~0.2 mm) considerably smaller than the dimension of the continuous channel (0.8 mm inner diameter), and dictated by the viscosity of the dispersed phase is unprecedented.
For the systematic nomenclature of HIPE particles, the following method is used similar to the one for ‘classical’ beads explained in Chapter V. 2: (HIPE) monomer\textsuperscript{XX} : monomer\textsuperscript{YY} : crosslinker\textsuperscript{ZZ} // (oil phase surfactant)\textsubscript{AA} : (Internal aqueous phase)\textsubscript{BB}. High internal phase emulsion is designated in the beginning with (HIPE). Monomers (the term includes also the crosslinker) follow with corresponding ratios. XX + YY + ZZ again equals to 100, since surfactant and internal phase are, in theory, completely washed out after polymerization. The oil phase surfactant, added to the monomers prior to the dropwise internal phase addition, follows the // sign. AA is the initial volume ratio of the surfactant to the whole emulsion including monomers and the internal phase. Finally, the internal aqueous phase is mentioned with the initial volume ratio. As an example, (HIPE) GMA\textsubscript{60} : EGDMA\textsubscript{40} // (PEO-PPO-PEO 4400)\textsubscript{3.3} : (CaCl\textsubscript{2} solution)\textsubscript{78.3} designates that the initial ratio of GMA\textsubscript{60} : EGDMA\textsubscript{40} in HIPE was 18.4% since 100 – (3.3 + 78.3) = 18.4. 60:40 is the ratio in between these monomers and the theoretical composition of the final bead. 3.3% PEO-PPO-PEO 4400 is dissolved in 18.4% GMA\textsubscript{60} : EGDMA\textsubscript{40} mixture and 78.3% CaCl\textsubscript{2} solution was added to form the reactive HIPE. The initiator was always dissolved in the monomer mixture with a ratio of 4 w% and has not been mentioned in this nomenclature.

VI. 3. Poly(HIPE) Beads: Do Huge Pores Matter?

Based on the promising results about rods, further attempts were done to reduce the viscosity of HIPE to be polymerized. That has been obtained by reducing the molecular weight of the surfactant PEO-PPO-PEO to 2800 g/mol. This enabled us to produce nearly spherical droplets of HIPE, and the corresponding poly(HIPE) beads; (HIPE) GMA\textsubscript{60} : EGDMA\textsubscript{40} // (PEO-PPO-PEO 2800)\textsubscript{3.3} : (CaCl\textsubscript{2} solution)\textsubscript{78.3}. While the use of SDS solution as the carrier phase yielded polydisperse beads, monodisperse beads were obtained using an aqueous poly(vinyl alcohol) solution (PVA, 3%) (see Fig. VI. 5).

SEM images of the obtained beads show that the pores are predominantly micron-sized (as we aimed for) reaching up to 15 μm. In addition, it is important to stress that the beads show no ‘skin’ effect. In our case, the pores resulted from the 78% internal aqueous phase in the HIPE formulation, which can be increased up to approximately 87% if spherical bead formation is desired. Above this limit, the HIPE phase becomes too viscous, preventing proper droplet breakup. The surface area of the obtained beads was determined as low as 16 m\textsuperscript{2}/g, which is not surprising for poly(HIPE).\textsuperscript{12} The (HIPE) GMA\textsubscript{60} : EGDMA\textsubscript{40} // (PEO-PPO-PEO 2800)\textsubscript{3.3} : (CaCl\textsubscript{2} solution)\textsubscript{78.3} beads exhibited an approximate diameter of 400 μm, which is 5-fold smaller compared to the previously reported monodisperse poly(HIPE) beads.\textsuperscript{44} We want to emphasize the importance of the co-flow geometry (bent needle) for
obtaining monodisperse poly(HIPE) beads instead of a T-junction (straight needle) as originally reported.

Fig. VI. 5. Porous, size-monodisperse near-spherical poly(HIPE) beads; \((\text{HIPE})\text{GMA}_{60}:\text{EGDMA}_{40}/(\text{PEO-PPO-PEO} 2800)_{3.3}:(\text{CaCl}_2 \text{ solution})_{78.3}\). A) Optical microscopy image showing size monodispersity, SEM images of B) a whole bead, C) surface of a bead, D) inner part of a broken bead.

Monomer conversion for the poly(HIPE) beads exceeded 75%, which is roughly two-fold higher when compared to beads prepared with the conventional porogen method. This result indicates a good stability of the HIPE formulation during the second emulsification process in the microfluidic channel, to give a water-in-oil-in-water double emulsion.

In addition to the monomer conversion, poly(HIPE) \((\text{HIPE})\text{GMA}_{60}:\text{EGDMA}_{40}/(\text{PEO-PPO-PEO} 2800)_{3.3}:(\text{CaCl}_2 \text{ solution})_{78.3}\) beads have been compared, in terms of functionalization, with ‘classical’ macroporous beads \((\text{GMA}_{40}:\text{EGDMA}_{40}/(\text{CH}_{80}:\text{DD}_{20})_{60}\), see Fig. V. 2. E-F\) described in the previous chapter. Both beads are composed of the same monomer mixture: \(\text{GMA}_{60}:\text{EGDMA}_{40}\). However pores of the ‘classical’ beads are much smaller (see Fig. V. 2. F) compared to the pores of the poly(HIPE) beads (see Fig. VI. 5). It is worth to mention that an accompanying advantage of the HIPE pathway is the elimination of the use of organic porogens, thanks to the water-in-oil emulsion template. Comparison of the two beads was done by using the epoxy groups that originated from the glycidyl group.
methacrylate (GMA) monomer. The epoxy group allows a two-step ‘click’-‘click’ process\textsuperscript{53-55} (see Fig. VI. 6), which involves ring opening of the epoxy groups with NaN\textsubscript{3} to introduce azide groups, followed by Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC, see Chapter II. 4).

Using phenyl acetylene as a model compound, we compared the performance of poly(HIPE) beads and ‘classical’ macroporous beads. Although the surface area of these ‘classical’ beads was 49.4 m\textsuperscript{2}/g (3 times higher than that of poly(HIPE) beads), they were outperformed by poly(HIPE) beads in terms of reaction rates in CuAAC (see Fig. VI. 7), which was revealed by comparing the change in the azide signal located at 2100 cm\textsuperscript{-1} in ATR-IR spectra as a function of time. It should be noted that the spectra were normalized by using the carbonyl peak (see Fig. VI. 8).

The same trend was observed when the reaction was performed with CuBr catalyst at room temperature and at 50 °C (see Fig. VI. 7. C-D). In the latter case, more than 90% of the azide peak disappeared in only 5h, meaning near quantitative triazole formation, which is remarkable taking into account the rather high azide loading: 0.81 mmol/g (from elemental analysis). It is also important to mention that poly(HIPE) beads have more azide groups than the ‘classical’ beads, indicating that even the first ‘click’ reaction is more efficient for poly(HIPE) beads. Elemental analysis confirmed the difference in azide loading: 0.81 and 0.31 mmol/g for poly(HIPE) and ‘classical’ beads respectively. This performance is attributed to the large macroporous open cellular structure of the poly(HIPE) beads, with excellent accessibility of the functional groups. These results indicate that poly(HIPE) beads can serve as a universal platform for ‘clicking’ compounds of interest such as biomolecules, drugs and catalysts.
Fig. VI. 7. Monitoring -N₃ peaks during CuAAC reactions. A) Poly(HIPE) beads with CuSO₄; B) ‘Classical’ beads with CuSO₄; C) Poly(HIPE) beads with CuBr; D) Poly(HIPE) beads with CuBr at 50 °C. The IR spectra were normalized with respect to the carbonyl peaks (see Fig. VI. 8).

Fig. VI. 8. IR spectra, monitoring CuAAC on poly(HIPE) beads (left) and ‘classical’ beads (right) using CuSO₄. Spectra were normalized using C=O peaks.

VI. 4. Pickering Poly(HIPE)

Since the poly(HIPE) bead production is well understood, the next step was improving the mechanical properties of the obtained beads. Poor mechanical properties is a general problem of poly(HIPE) and (HIPE)GMA₆₀:EGDMA₄₀/(PEO-PPO-PEO 2800)ₐ.ₙ.(CaCl₂ solution)ₗₙ.ₘ beads were no exception. According to the literature, there are two common ways of improving toughness of poly(HIPE). First one is adding particulate emulsion stabilizers (Pickering emulsion), especially inorganic particles, and the second is enriching the monomer phase with long alkyl chain monomers (see next subchapter). The importance of using particles for stabilizing HIPE formulations and obtaining hybrid materials after polymerization is well appreciated by the poly(HIPE) community in recent years. Metals are not only useful for stabilizing the concentrated emulsion but also can be used as catalysts.
Chapter V

I. HIPE in Microfluidics

since they are immobilized on the walls of a highly porous polymer support. To this extent, we planned to make poly(HIPE) beads stabilized with Cu salts aiming to catalyze azide alkyne cycloaddition after polymerization. Cu stabilized poly(HIPE) catalyzing this famous type of click reaction has never been reported in literature to the best of our knowledge. Briefly, three different Cu salts were treated with oleic acid and these copper-oleic acid complexes were used in previously developed HIPE formulations, directly replacing PEO-PPO-PEO surfactants. The monomer formulation has also been altered. However, obtained formulations were not stable (see experimental section for details). This route is not continued since time consuming optimization was needed and new monomers were already giving better results, which will be explained in the following sections.

VI. 5. Toughened Poly(HIPE) Beads

Further research was concentrated on the effect of different monomers in terms of toughness and structure of the poly(HIPE) beads. As mentioned earlier, long alkyl chain monomers should decrease the brittle character by decreasing the $T_g$. The previously described successful monomer mixture for poly(HIPE) bead preparation was $(HIPE)_{GMA_{60}}:EGDMA_{40}://(PEO-PPO-PEO\ 2800)_{3.3}:\{(CaCl_2\ solution)_{78.3}}$. Four new monomers were used together with the previous bead forming formulation (see Fig. VI. 2). BMA, LMA and PEGMA300 monomers have been used for improving toughness of the beads, while 2,2,3,4,4,4-hexafluorobutyl methacrylate (HFBMA) has been used for preparing highly fluorinated, superhydrophobic particles.

From these four new monomers, LMA yielded a highly viscous HIPE even at low concentrations, and $(HIPE)_{LMA_{40}}:GMA_{20}:EGDMA_{40}://(PEO-PPO-PEO\ 2800)_{3.3}:\{(CaCl_2\ solution)_{78.3}}$ rods were obtained (see Fig. VI. 9. C) after microfluidic polymerization. The first interesting result were obtained with BMA. As seen from Fig. VI. 9. B, the $(HIPE)_{BMA_{40}}:GMA_{20}:EGDMA_{40}://(PEO-PPO-PEO\ 2800)_{3.3}:\{(CaCl_2\ solution)_{78.3}}$ bead has a smoother surface structure compared to the previous $(HIPE)_{GMA_{60}}:EGDMA_{40}://(PEO-PPO-PEO\ 2800)_{3.3}:\{(CaCl_2\ solution)_{78.3}}$ bead (Fig. VI. 5. B). These beads were also monodisperse (see Fig. VI. 9. A) and less brittle (observed during handling).

It should be noted that we were not able to prepare porous beads from the BMA$_{60}$:EGDMA$_{40}$ ratio via the classical porogen method although 8 different porogens were tried (see Chapter V. 5). On the other hand, highly porous beads can be obtained from a similar monomer composition via the HIPE approach: $(HIPE)_{BMA_{65}}:EGDMA_{35}://(PEO-PPO-PEO\ 2800)_{3.3}:\{(CaCl_2\ solution)_{78.3}}$ beads were prepared (not shown). This alone shows the versatility of HIPE approach: Pore formation is almost warranted by using immiscible water for any (reasonably hydrophobic) monomer formulation without
extensively trying different porogens. For hydrophilic monomers, even oil-based internal phases can be utilized. \(^44\)

**Fig. VI. 9.** SEM images of A) (HIPE)BMA\(_{40}\):GMA\(_{20}\):EGDMA\(_{40}\)//(PEO-PPO-PEO 2800)\(_{3.3}\):(CaCl\(_2\) solution)\(_{78.3}\) beads; B) Single (HIPE)BMA\(_{40}\):GMA\(_{20}\):EGDMA\(_{40}\)//(PEO-PPO-PEO 2800)\(_{3.3}\):(CaCl\(_2\) solution)\(_{78.3}\) bead; C) (HIPE)LMA\(_{40}\):GMA\(_{20}\):EGDMA\(_{40}\)//(PEO-PPO-PEO 2800)\(_{3.3}\):(CaCl\(_2\) solution)\(_{78.3}\) rods. Inset shows a broken rod.

**VI. 6. Porous Liquid Marbles**

Similar to the BMA formulations, we were able to prepare porous super-hydrophobic beads (see **Fig. VI. 10. A-B**) by using the HFBMA monomer (see **Fig. VI. 2**) via this method at the first trial. First of all, we prepared poly(HIPE) beads with 60% HFBMA: (HIPE)HFBMA\(_{60}\):GMA\(_{20}\):EGDMA\(_{20}\)//(PEO-PPO-PEO 2800)\(_{3.3}\):(CaCl\(_2\) solution)\(_{78.3}\) beads. The obtained beads were monodisperse, not too brittle and had a regular surface (see **Fig. VI. 10. A**). The interior of the HFBMA beads also appears to be highly porous with single pores reaching up to 50 µm (see **Fig. VI. 10. B**).

The most important feature of fluorinated particles is their super-hydrophobic nature, which gives rise to extremely high water (and even hydrophobic solvents) repelling characteristics. In other words, these particles cannot be wetted by water. By using this phenomenon, Aussillous and Quéré reported the stabilization of water droplets by a layer of highly hydrophobic particles that covers the whole droplet surface, referred to as liquid marbles. \(^58\) The water droplet was shielded by these particles so well that the whole assembly (the liquid marble) was stable in spherical shape when placed on glass (hydrophilic) and even floated on a water pool. Liquid marbles can be transferred easily although there is no physical or chemical link between the particles surrounding the droplet. Recently there has been a growing interest in liquid marbles recently\(^59\)-\(^63\). As an example, magnetic
liquid marbles\textsuperscript{64-65} were prepared and used as microreactors that can contain various solvents inside and can be manipulated externally by a magnet such as opening a window on the particle shell.

![Image of fluorinated poly(HIPE) beads and liquid marbles prepared thereof. A) SEM image of (HIPE)$\text{HFBMA}_{40}:\text{GMA}_{20}:\text{EGDMA}_{20}/(\text{PEO-PPO-PEO} \ 2800)_{3.3}:\text{(CaCl}_2 \ \text{solution)}_{78.3}$ bead; B) The inner structure of the same bead; C) Liquid marble not wetting the paper below. Prepared from 8 µL water and (HIPE)$\text{HFBMA}_{76}:\text{EGDMA}_{34}/(\text{PEO-PPO-PEO} \ 2800)_{3.8}:\text{(CaCl}_2 \ \text{solution)}_{75}$ beads; E) Liquid marble can be transported by tweezers.](image)

Preparation of liquid marbles is spontaneous: addition of a water droplet on a batch of fluorinated particles and simple shaking paves the way. We were able to prepare liquid marbles (see Fig. VI. 10. C-D) both by using (HIPE)$\text{HFBMA}_{40}:\text{GMA}_{20}:\text{EGDMA}_{20}/(\text{PEO-PPO-PEO} \ 2800)_{3.3}:\text{(CaCl}_2 \ \text{solution)}_{78.3}$ beads (see Fig. VI. 10. A) and (HIPE)$\text{HFBMA}_{76}:\text{EGDMA}_{34}/(\text{PEO-PPO-PEO} \ 2800)_{3.8}:\text{(CaCl}_2 \ \text{solution)}_{75}$ beads (increased HFBMA content, less internal aqueous phase). Further investigation revealed that fluorinated HFBMA monomer is not compulsory for liquid marble formation. Whereas (HIPE)$\text{BMA}_{50}:\text{GMA}_{20}:\text{EGDMA}_{40}/(\text{PEO-PPO-PEO} \ 2800)_{3.3}:\text{(CaCl}_2 \ \text{solution)}_{78.3}$ beads easily form liquid marbles, (HIPE)$\text{GMA}_{40}:\text{EGDMA}_{40}/(\text{PEO-PPO-PEO} \ 2800)_{3.3}:\text{(CaCl}_2 \ \text{solution)}_{78.3}$ beads are certainly not suitable for liquid marble formation. GMA monomer renders the latter highly hydrophilic and water droplets immediately wet these particles. Similarly, GMA$\text{CH}_{80}:\text{DD}_{20} \ 60$ beads (same composition, ‘classical’ porous beads) also do not form transportable liquid marbles. On the other hand, liquid marbles prepared by using (HIPE)$\text{BMA}_{50}:\text{GMA}_{10}:\text{EGDMA}_{40}/(\text{PEO-PPO-PEO} \ 2800)_{3.3}:\text{(CaCl}_2 \ \text{solution)}_{78.3}$ beads, are quite stable, easily transported and can be placed on a water pool. It is also observed that these liquid marbles buckle in less than 30 min by loosing their water core (drying) when placed on a flat surface. Interestingly, this buckled structure was also stable and could be transported. Moreover, the liquid marble floating on water was stable for hours. It seems that the
water lost by drying from the core of the liquid marble is replaced by water vapor coming from the water pool beneath.

There are only a few examples of liquid marbles in literature prepared from porous material. The huge pores of poly(HIPE) shelled liquid marbles are certainly unique and may lead to novel applications due to increased gas and liquid permeability. Moreover, their highly porous shell makes these liquid marbles very light, which probably makes them more stable against gravity when transported.

VI. 7. Poly(HIPE) Capsules and Hollow Rods

The most interesting results were obtained when the PEGMA300 monomer was used. Indeed, to our surprise, (HIPE)PEGMA40:GMA20:EGDMA40/(PEO-PPO-PEO 2800)3.3:(CaCl2 solution)78.3 particles were not only porous but also hollow (see Fig. VI. 11). In general, hollow particles can be made by using sacrificial templates (see also Chapter III), emulsion templating, via core formation by using hexadecane or via using a suitable oil soluble surfactant added to monomer phase. These methods however either need tedious device preparation or multiple steps including long optimization studies. In our case, there was no need of preparing special devices, using sacrificial templates or utilizing hexadecane/porogen mixtures. The hollow core was formed due to an unexpected effect coming from the combination of the hydrophilic PEGMA300 monomer, water (internal phase of the HIPE) and pluronic surfactant. Only PEGMA300 monomer yielded hollow particles out of the 5 monomers tried, which can be regarded as a drawback of the method. Moreover the monomer composition needed to be optimized since different particles in Fig. VI. 11 show different structures, especially in terms of wall thickness. Some particles have huge openings as in the case of Fig. VI. 11. B.

![SEM images of (HIPE)PEGMA40:GMA20:EGDMA40/(PEO-PPO-PEO 2800)3.3:(CaCl2 solution)78.3 particles.](image)
Ratios between GMA, EGDMA and PEGMA300 monomers were changed for optimization of the structure. Consistent homogenous results came with 30% PEGDMA as shown in Fig. VI. 12. The wall thickness is increased by decreasing the amount of PEGMA300 used (from 40% to 30%). These (HIPE)PEGMA₃₀:GMA₂₅:EGDMA₄₅/(PEO-PPO-PEO 2₈₀₀)₃·₃·₇(CaCl₂ solution)₇₈·₃ beads are monodisperse and physically stable enough to withstand the sample preparation process (see Fig. VI. 12. A). As a matter of fact, it was our intention to improve the mechanical properties by adding low Tg monomers and PEGMA300 provided some extra toughness. The hollow nature of the beads is exposed (see Fig. VI. 12. B) when some force is applied, for instance by a spatula. To further investigate the homogeneity of wall thickness, hollow (HIPE)PEGMA₃₀:GMA₂₅:EGDMA₄₅/(PEO-PPO-PEO 2₈₀₀)₃·₃·₇(CaCl₂ solution)₇₈·₃ beads were microtomed and results are shown in Fig. VI. 12. C. With a mean diameter of 370 µm, beads exhibit a shell thickness of 70 ± 5 µm. This <10% deviation should be regarded quite low for such an uncontrolled shell formation. To the best of our knowledge, these are the first monodisperse hollow porous poly(HIPE) particles and they are quite unique among previously fabricated hollow-porous particles due to their huge pores. It should be stressed that no template, organic porogen or special device is used. Moreover, epoxy groups are also available for functionalization (see Chapter VI. 9).

**Fig. VI. 12.** SEM images of (HIPE)PEGMA₃₀:GMA₂₅:EGDMA₄₅/(PEO-PPO-PEO 2₈₀₀)₃·₃·₇(CaCl₂ solution)₇₈·₃ particles. A) A general image showing monodispersity and physical stability of the particles; B) Broken particle; C) Microtomed particle.

Based on the previous experience, the next challenge was the preparation of hollow poly(HIPE) rods. As in the case of the initial GMA₆₀:EGDMA₄₀ version (see Chapter VI. 2-3), using 4400 MW Pluronic as the surfactant should increase the viscosity and lead to jetting. Indeed Pluronic 4400 enabled us to obtain a viscous HIPE from the 30% PEGMA300 formulation: (HIPE)PEGMA₃₀:GMA₂₅:EGDMA₄₅/(PEO-
PPO-PEO 4400)_{3.3}:(CaCl_2 \text{ solution})_{78.3}. Particles obtained after microfluidic emulsification of this viscous PEGMA300 HIPE are shown in Fig. VI. 13. First of all, rod formation was successful as intended (see Fig. VI. 13. A). However, these rods were not hollow in a consistent manner such as the beads prepared from the same 30% PEGMA300 formulation (only difference being the MW of the pluronic surfactant). Only a small percentage of the rods were spotted as being hollow (see Fig. VI. 13. B-C) and they seemed to be partially hollow. No further investigation could be done to improve this preparation procedure.

![Image](image_url)

**Fig. VI. 13.** Hollow (HIPE)PEGMA_{30}:GMA_{25}:EGDMA_{45}/(PEO-PPO-PEO 4400)_{3.3}:(CaCl_2 \text{ solution})_{78.3} rods. A) General overview; B) Single broken rod; C) Focus on the broken part of the rod in C.

### VI. 8. Factors Playing a Role in the Core Formation

Hollow core formation can be due to several effects including I) interaction between the ethylene glycol units of both the surfactant (PEO-PPO-PEO) and PEGMA300 monomer, II) photopolymerization, III) hydrophilicity of PEGMA300 and aqueous carrier phase. For a better understanding of the effect of photopolymerization and the aqueous carrier phase, HIPE formulations have been polymerized in glass vials that have an approximate diameter of 11 mm. These experiments enabled us to disable the effect of the continuous carrier phase and to use thermal initiators. The obtained poly(HIPE) monoliths can be seen in Fig. VI. 14.

Comparing the monoliths in Fig. VI. 14 reveals the importance of photopolymerization on core formation. Monoliths in Fig. VI. 14. A and Fig. VI. 14. C are composed of exactly the same monomer mixture ((HIPE)PEGMA_{30}:GMA_{25}:EGDMA_{45}/(PEO-PPO-PEO 2800)_{3.3}:(CaCl_2 \text{ solution})_{78.3}) but with photo and thermal initiators, respectively. Whereas thermal initiation yielded a non-hollow monolith,
photoinitiation provided a hollow core. The hollow core formation became more prominent and uniform (see Fig. VI. 14. B) when the percentage of PEGMA300 was increased to 70% in the monomer mixture. On the other hand, photopolymerization alone does not give hollow monoliths but core-shell structures (see Fig. VI. 14. D-E) if PEGMA300 is not present in the HIPE composition. Consequently, combination of PEGMA300 and photopolymerization is necessary for hollow core formation, and the aqueous carrier phase in the microfluidic channel does not seem to play a significant role on this since the monoliths prepared in glass vials are also hollow.

**Fig. VI. 14.** Poly(HIPE) monoliths with \( \sim 11 \text{ mm diameter} \). A) \((HIPE)\text{PEGMA}_{30} \text{GMA}_{25} \text{EGDMA}_{45} / (\text{PEO-PPO-PEO 2800})_{3.3} : (\text{CaCl}_2 \text{ solution})_{78.3} \text{ with photoinitiator; B) (HIPE)PEGMA}_{70} \text{GMA}_{10} \text{HDA}_{20} / (\text{PEO-PPO-PEO 2800})_{3.3} : (\text{CaCl}_2 \text{ solution})_{78.3} \text{ with photoinitiator; C) (HIPE)PEGMA}_{30} \text{GMA}_{25} \text{EGDMA}_{45} / (\text{PEO-PPO-PEO 2800})_{3.3} : (\text{CaCl}_2 \text{ solution})_{78.3} \text{ with thermal initiator; D) (HIPE)GMA}_{60} \text{EGDMA}_{40} / (\text{PEO-PPO-PEO 2800})_{3.3} : (\text{CaCl}_2 \text{ solution})_{78.3} \text{ with photoinitiator; E) (HIPE)HFBMA}_{76} \text{EGDMA}_{34} / (\text{PEO-PPO-PEO 2800})_{3.8} : (\text{CaCl}_2 \text{ solution})_{75} \text{ with photoinitiator.}

GMA, BMA and HFBMA HIPE formulations (excluding PEGMA300) resulted in \( \sim 0.4 \text{ mm complete beads (neither hollow nor core-shell) in the microfluidic channel, 11 mm core-shell monoliths were obtained in glass vials. This fact points out the importance of UV penetration, which could also play a
role in hollow core formation in PEGMA300 HIPEs. The thickness of the shell seems very similar in both hollow and core-shell monoliths, which is approximately 1.5 mm. This is probably the depth limit that UV light is highly effective. First the shell polymerizes with a thickness of 1.5 mm and the core polymerizes slower with the released radicals from the shell. It is also probable that the heat released from the photopolymerization of the shell helps the photoinitiator molecules in the core to decompose for radical production.

In the case of PEGMA300, monomer transfer from the unpolymerized core towards the polymerized monolithic shell should be faster than the polymerization of the core. Hence our hypothesis is that PEGMA300 monomer rapidly transfers to the solidified shell and polymerizes there once the UV curing starts. That could be facilitated by the hydrophilicity of PEGMA300 monomer and the similar structure of the surfactant (PEO-PPO-PEO). This process should be taking place in a smaller scale in microfluidic channel where droplets are as small as 400 µm. Ostwald ripening, which is defined as the depletion of small droplets in favor of larger droplets in an emulsion, can be another explanation of hollow core formation. Smaller (aqueous) internal phase droplets can be transferred via the monomer phase (the continuous phase of HIPE), forming continuously larger water droplets and eventually the water core, which becomes the hollow core after drying. It is believed that the highly hydrophilic PEGMA300 monomer should facilitate the water transfer via the monomer phase.

VI. 9. Reactivity of the Poly(HIPE) Capsules

Although lower in percentage, the GMA monomer was also used to construct poly(HIPE) capsules. Like in Chapter VI. 3, capsules were exposed to click chemistry conditions to see if these (HIPE)PEGMA$_{30}$:GMA$_{25}$:EGDMA$_{45}$/((PEO-PPO-PEO 2800)$_{3.3}$-](CaCl$_2$ solution)$_{78.3}$ capsules can also be regarded as reactive structures. The intact capsule was first treated with NaN$_3$ and then with 4-ethynyl-N-ethyl-1,8-naphthalimide (denoted further as the naphthalimide), a fluorogenic alkyne molecule (see Fig. VI. 15. A). The theoretical epoxy loading of the intact capsule was calculated (from 25% initial GMA) as 1.88 mmol/g. In two parallel experiments, the first batch of the azido capsules was clicked with 0.3 eq and the second batch with 3 eq of the naphthalimide. First of all, the azido capsule did not show any fluorescence, hence light microscopy image is shown in Fig. VI. 15. B instead of the completely dark confocal image. After the triazole formation however, there is a clear difference in fluorescence of the capsules that are treated with different amounts of the naphthalimide. Whereas capsules treated with 3 eq of the naphthalimide exhibited a very bright confocal image (see Fig. VI. 15. D), the 0.3 eq naphthalimide treated counterpart exhibited still
visible but much lower fluorescence (see Fig. VI. 15. C). It should be noted that settings of the confocal microscope, especially the “gain value” were unchanged between the analyses.

**Fig. VI. 15.** Click reactions of (HIPE)PEGMA\textsubscript{30}:GMA\textsubscript{25}:EGDMA\textsubscript{45}/(PEO-PPO-PEO 2800)\textsubscript{3.3}:(CaCl\textsubscript{2} solution)\textsubscript{78.3} poly(HIPE) capsules. A) Opening of the epoxy groups with NaN\textsubscript{3} and subsequent fluorogenic reaction with 4-ethynyl-N-ethyl-1,8-naphthalimide; B) Light microscopy image of microtomed azido capsule; C) Microtomed capsule after treatment with 0.3 eq 4-ethynyl-N-ethyl-1,8-naphthalimide; D) Microtomed capsule after treatment with 3 eq 4-ethynyl-N-ethyl-1,8-naphthalimide; E) Evolution of the 2100 cm\textsuperscript{-1} IR azide peak; large, medium and small peaks belong to the capsules in B, C and D, respectively.

The course of the reaction was again followed by FTIR (see Fig. VI. 15. E). The disappearance of the azide peak was \(\sim\)60% and \(\sim\)90% for the capsules treated with 0.3 eq (Fig. VI. 15. C) and 3 eq (Fig. VI. 15. D) naphthalimide, respectively. Fluorescence of the capsule in Fig. VI. 15. C can be considered to be uniform throughout the shell. The naphthalimide did not concentrate on the periphery although 0.3 eq was utilized compared to the azide groups. Similarly, the fully treated capsule in Fig. VI. 15. D also exhibits quite uniform fluorescence, except some additional fluorescence from the periphery. These facts lead to several conclusions:

- there is no problem of reactant diffusion for the capsules,
- pores are interconnected,
- azide groups are uniformly distributed on the capsules,

- a high fraction of the azide groups are accessible for click reaction,

- the epoxy groups are uniformly distributed and are also all accessible.

These features make the capsules very attractive. As discussed earlier, diffusion of reactants is one of the most important problems for solid supports. It is often difficult to reach all the reactive groups. However, poly(HIPE) capsules lack a core. These highly porous capsules may have quite unique applications.

**VI. 10. Embedding PS Beads into the Poly(HIPE) Particles**

Furthermore, we wanted to see if the hollow core and the highly porous shell of these poly(HIPE) particles (spherical or rod shaped) can be advantageous in some applications. Utilizing these particles as microreactors seems plausible in case that the hollow core can be used as the reaction location. The huge pores of the poly(HIPE) shell make these particles unique among other capsules since these pores allow a highly increased diffusivity. Permeability of common non-porous capsules may depend on the solvent-capsule compatibility.

To this extent, possibilities of anchoring catalysts or enzymes in the core of poly(HIPE) particles were examined. One model approach was embedding micron sized PS particles in the core or core/pore combination. There are few examples of similar particles with movable single cores in the literature that are often called rattle-type particles which are generally inorganic.\(^{80-81}\) Our aim was different however, namely to embed thousands of much smaller PS beads in one poly(HIPE) capsule or hollow rod. There is a large difference between the sizes of the poly(HIPE) particles and PS particles to be embedded. While poly(HIPE) particles have a diameter of 300-400 µm and a core diameter of 100-200 µm, PS particles are only in the 1-10 µm range. These type of micron-sized particles are very useful in bioassays such as immobilization of precious bioactive molecules.\(^{82}\) Once such micro PS beads are embedded in the poly(HIPE) particles, active biomolecules or catalysts can be immobilized on the PS beads. In that sense a single hollow poly(HIPE) bead or rod can be truly used as a microreactor since it is possible to handle single poly(HIPE) particles due to their relatively large size. The number of the PS beads embedded in a single poly(HIPE) particle can be very low, allowing one to downscale those type of particle based bioassays. Downscaling bioassays is often very important since it allows one to reduce the cost of an experiment, especially when highly expensive molecules are involved.
To achieve such PS-in-poly(HIPE) microreactors, we envisaged that adding PS microbeads to the internal phase of HIPE, that is being the CaCl$_2$ solution, prior to the dropwise addition could be the easiest way. There are many aqueous PS bead suspensions commercially available where the suspension is stabilized with surfactants. These new HIPE formulations should accommodate PS beads in the water droplets dispersed in the monomer-Pluronic continuous phase. It is possible that hollow core formation happens via merging of a large percentage of internal phase droplets at the core via some kind of Ostwald ripening process.\textsuperscript{36,83-84} If this hypothesis is true, a significant percentage of PS particles should agglomerate in the core, the remaining ones being mostly trapped in the pores. The argument that the huge pores should allow PS particles to escape out is debatable since there is also a huge amount of submicron pores throughout the shell. A PS bead should encounter such sub-micron pores on the way to escape out from its host poly(HIPE) particle. Moreover, the inner surface of the core has smaller pores as seen in Fig. VI. 12. In conclusion, most of the PS beads should remain inside if our hypotheses are correct.

Since the PS beads will be used for further chemical manipulation, it was necessary to prepare unreactive poly(HIPE) capsules, namely free from epoxide residues. Trials with methyl methacrylate (MMA) instead of GMA were successful; 25% of GMA is totally replaced with MMA and the obtained particles were hollow again (not shown). First of all, this is a further proof that hollow core formation depends mostly on the PEGMA$_{300}$ monomer. Secondly, we are able to prepare not only reactive but also “inert” poly(HIPE) capsules.

Another expected problem was the viscosity increase due to localization of PS particles at the water-monomer interface, which is known as the Pickering effect. The amount of the Pluronic surfactant used is reduced by 33% to counterbalance this effect and again non-viscous HIPE formulations were obtained containing MMA, PS particles (batches of 1 and 10 µm in size) and less surfactant. As discussed earlier, it is also possible to stabilize HIPEs by only using polymer beads instead of surfactants.\textsuperscript{31-34} However, PS particles alone failed to stabilize our HIPE formulations and a small amount of the Pluronic is found to be necessary. This failure of stabilization is attributed to the larger size of PS particles.

After extensive trials and optimization, we managed to obtain some promising results on embedding 10 micron PS particles into hollow beads and rods (see Fig. VI. 16.A). Keeping the amount of aqueous internal phase fixed at 75%, playing with the ratio of CaCl$_2$ solution to the PS suspension yields viscous and non-viscous HIPE formulations. As expected, these formulations result in hollow rods and capsules, respectively. For instance, while \textbf{(HIPE)PEGMA$_{30}$:MMA$_{25}$:EGDMA$_{45}$/(PEO-PPO-PEO 2800)$_{2.6}$:(CaCl$_2$ solution)$_{38.4}$:(PS suspension)$_{36.8}$} formulation resulted in beads,
resulted in hollow rods. As mentioned earlier, PS particles act as emulsion stabilizers at the water-oil interface; Pickering emulsion.

Fig. VI. 16. A) Confocal and B,C) SEM images of (HYPE)PEGMA₃₀:MMₐ₂₅:EGDMA₄₅/(PEO-PPO-PEO 2800)₂.₆:(CaCl₂ solution)₂₂.₄:(PS suspension)₅₂.₆ rods.

Fig. VI. 16. A shows the exterior of a rod with PS particles clearly visible. However, we could not see a cluster of PS microbeads located in the core of rods or capsules. Moreover, the capsules or hollow rods themselves are also extremely fluorescent, pointing out the fact that PS microbeads are dissolved by the methacrylate monomers prior to polymerization. That should also be the reason that there was no PS microbead cluster located in the core. These results are however quite promising and should be repeated with particles that will not be dissolved by the methacrylate monomers such as particles composed of silica or CaCO₃.

VI. 11. Conclusions

Possibilities of polymerizing different HIPE formulations in microfluidic channels are exploited in this chapter. Unique and even unprecedented particles have been realized thanks to the special properties of HIPEs such as high viscosity, stability and high content of internal phase. The obtained particles not only varied in physical shape but also in chemical nature. Different particles have been prepared from hydrophilic (PEGMA300), hydrophobic (LMA) and even super-hydrophobic monomers (HFBMA). Spheres, rods, capsules, doughnut structures and hollow rods can be prepared by only using three different monomers and two different surfactants. As default features, all the particles were monodisperse thanks to microfluidics and highly porous thanks to emulsion templating.
Chapter VI. HIPE in Microfluidics

The poly(HIPE) beads exhibited a superior performance compared to their classical macroporous counterparts in a double click reaction sequence. These click reactions included epoxy-azide addition and CuAAC. Since the compositions of both the poly(HIPE) and classical macroporous beads were theoretically the same, the performance difference was attributed to the pore size difference. The larger the pores, the higher the diffusion and the faster the reactions. Higher yields, higher functionality and higher reaction rates make poly(HIPE) beads very attractive for immobilization and catalysis applications. The huge pores of poly(HIPE) beads may also be advantageous for attaching macromolecules such as proteins.

It is also shown that highly hydrophobic poly(HIPE) beads can prevent water droplets from wetting their surroundings. These liquid marbles are unique due to their highly porous shell. If cleverly managed, this highly porous shell may lead to novel applications for liquid marbles. There are less than 50 manuscripts in literature reporting about liquid marbles since they are discovered in 2001 in spite of their unique properties, which means that there is a huge room for innovation.

The use of PEGMA300 monomer for HIPE preparation on the other hand, resulted in hollow beads and rods. First of all, nonspherical particle (rods) production in microfluidics without using restricted channel dimensions, has been achieved for the first time in literature. Moreover, the obtained rods were hollow although no sacrificial template or special reactor design is used. Physical parameters alone have been utilized to both externally and internally shape the hollow rods, such as viscosity, phase separation, and photopolymerization. The effect of PEGMA300 monomer and photopolymerization is proved by the experiments with monoliths. Further research is needed to be performed to better understand the hollow core formation.

Both reactive and unreactive versions of hollow particles have been prepared. Reactive capsules for instance, also exhibited a high click performance. Porous doughnut structures are revealed via microtomy of the poly(HIPE) capsules. We were also able to embed PS microbeads on the walls of the unreactive capsules and hollow rods. Such uniform, reactive, highly porous, various shaped particles by means of a simple setup is certainly unique and deserves further attention and research to our belief.

VI. 12. Experimental

*Materials and Methods:* See also Chapter III. 5 and IV. 4 for information about some materials and methods utilized also for the work of this chapter but not mentioned here. Solvents used for the bead wash procedure were technical grade and used as received. H₂O used was MilliRO grade.
DMSO, DMPA, polyvinyl alcohol (PVA, 95% hydrolyzed, 95000 MW), NaN\textsubscript{3}, DMF, sodium ascorbate (NaOAsc), CuSO\textsubscript{4} and EGDMA were purchased from Acros Organics. GMA, PEGMA300, HFBMA, LMA, DCM, tripropargylamine, Pluronic surfactants (PEO-PPO-PEO), basic Al\textsubscript{2}O\textsubscript{3}, EHA, benzyl bromide, phenyl acetylene, DEGDA (technical grade), BDM, cyclohexanol, dodecanol and CuBr were purchased from Sigma-Aldrich. Benzyl azide was synthesized as reported.\textsuperscript{85} ABIL EM 90 was kindly donated by Evonik Degussa. PVA solution was prepared by stirring the corresponding amount of PVA in water that was heated up to 70-80 °C. The elevated temperature was kept till all polymer is dissolved and then the solution was cooled down. No precipitation was observed. MMA was distilled prior to use. GMA, EGDMA, BMA and LMA were passed over basic Al\textsubscript{2}O\textsubscript{3} to remove the inhibitors.

For microtomy, samples were embedded within a TissueTec matrix and subsequently frozen at -20°C. Freeze-sections with a thickness of 10 µm thick were cut with a Microm Vacutome HM 500 OM cryomicrotome. The freeze-sections were collected onto a glass substrate and extensively washed with water to remove the TissueTec matrix.

**HIPE Preparation:** The name of the particle designates the composition. For instance \((\text{HIPE})\text{PEGMA}_{30}:\text{MMA}_{25}:\text{EGDMA}_{45}/(\text{PEO-PPO-PEO} \; 2800)_{3.3}:(\text{CaCl}_2 \; \text{solution})_{78.3}\) beads (capsules) were prepared by dissolving 33 mg DMPA photoinitiator (always 4 w% compared to the monomers+crosslinker) was dissolved in a mixture of 255 µL PEGMA300, 210 µL GMA, 385 µL EGDMA and 150 µL PEO-PPO-PEO 2800 in a 50 mL flask that was covered with a piece of aluminum foil to avoid light penetration. This is the continuous oil phase of the HIPE. Preparation of other particles was done in similar manner with different monomers. When less surfactant was designated such as in the case of \((\text{HIPE})\text{PEGMA}_{30}:\text{MMA}_{25}:\text{EGDMA}_{45}/(\text{PEO-PPO-PEO} \; 2800)_{2.6}:(\text{CaCl}_2 \; \text{solution})_{38.4}:(\text{PS suspension})_{16.8}\) beads, 100 µL PEO-PPO-PEO 2800 was used while keeping the total amount of monomers+crosslinker at 850 µL.

While this oil phase is being stirred at 350-400 rpm by an overhead stirrer equipped with a Teflon propeller that fits to the bottom of the flask (see Fig. VI. 1), an aqueous CaCl\textsubscript{2}.2H\textsubscript{2}O solution (3.6 mL, 1.3 w%) was added dropwise during 30-40 min. Adding 3.6 mL aqueous phase over 1 mL oil phase makes 78.3 % HIPE. For the particles prepared on the 75% HIPE ratio, 3 mL CaCl\textsubscript{2} solution was added dropwise on 1 mL oil phase. For the hollow poly(HIPE) particles embedding PS microbeads such as \((\text{HIPE})\text{PEGMA}_{30}:\text{MMA}_{25}:\text{EGDMA}_{45}/(\text{PEO-PPO-PEO} \; 2800)_{2.6}:(\text{CaCl}_2 \; \text{solution})_{16.8}:(\text{PS suspension})_{38.4}\) the oil phase was 950 µL in total. 1.4 mL PS suspension was mixed with 1.46 mL CaCl\textsubscript{2}.2H\textsubscript{2}O solution (with a concentration of 2.6 w% this time to have 1.3 w% after dilution with PS suspension) and this aqueous phase was added over the oil phase while mixing. 10 µm sized monodisperse PS particle suspensions were purchased from Invitrogen.\textsuperscript{86}
**Polymerization of HIPE via Microfluidics:** Either Tygon (2 m long, 0.8 mm internal diameter) or PVC (75 cm long, 1.6 mm internal diameter) tubing were used as the channel. The HIPE phase needles used were 27G for viscous formulations and 30G for nonviscous formulations. The HIPE phase was pumped with pumping rates in a range of 0.20-1 mL h\(^{-1}\), again depending on viscosity. Generally viscous HIPEs were pumped in higher rates. The continuous carrier phase was an aqueous PVA solution (3 w%, 96000 MW, 95% hydrolyzed) pumped with a rate of 1.2 mL h\(^{-1}\). Further off-tubing UV curing was performed for at least 15 min to complete polymerization.

The obtained beads were washed on a glass filter with warm H\(_2\)O, DCM, MeOH, DCM and DEE respectively and dried under vacuum at room temperature overnight. In the case of PS embedded poly(HIPE) particles, washing was performed only with H\(_2\)O and MeOH in order not to dissolve the PS microbeads.

**Monoliths (Chapter VI. 8):** HIPE formulations were prepared as explained above. Monoliths were prepared by polymerizing HIPE formulations in 11 mm diameter glass vials for a UV treatment of 1-2h. Polymerized monoliths were self dried in room conditions at least for a day and taken out from the glass mold by breaking the mold. Thermally cured PEGMA300 monoliths were prepared by using V-70 (Wako Chemicals) initiator instead of DMPA. These monoliths were cured either at room temperature or at 35 °C in an oven. V-70 initiator was useful since thermal treatment at 60 °C destabilized the HIPE formulations (prepared from AIBN) before the polymerization.

**Oleic acid treatment of copper salts:** 1g of a copper salt (CuSO\(_4\), Cu(OAc)\(_2\) or CuCl\(_2\)) was suspended in 7 ml of chloroform in an erlenmeyer. 8 ml of oleic acid was added to the suspension and then the mixture was stirred by a magnetic stirrer. 30 ml of methanol was added to this mixture after 3h for the precipitation. Particles were separated from the liquid phase and 10 ml of chloroform was added to re-suspend the particles and the suspension was sonicated for about 10 minutes. Afterwards, particles were precipitated via the addition of MeOH and centrifuged. The liquid phase is discarded after centrifugation. Purification process (MeOH-centrifuge) was repeated 5 times. OA-Cu particles were dried for 24 h at 120 °C. To prepare Pickering HIPE formulations, OA-Cu particles were added to the appropriate monomer mixture and stirred vigorously. Internal aqueous phase was then added to particle-monomer suspension. While precipitates were obtained in the case of CuSO\(_4\) and Cu(OAc)\(_2\), there was no precipitate for CuCl\(_2\). These copper-oleic acid complexes were used in previously developed HIPE formulations, directly replacing PEO-PPO-PEO surfactants. LMA, BMA and PEGMA300 are also used in monomer formulations in 40% ratio. DMPA was dissolved in chosen monomer mixture and the OA-Cu (either acetate or sulfate) complex were added. Three situations were observed after the addition of CaCl\(_2\).2H\(_2\)O solution: no emulsion, highly viscous emulsion and
non-viscous emulsion. The most promising example was the Cu(OAc)$_2$:EGDMA:GMA case, however this non-viscous emulsion got phase separated when it was subjected to UV.

**Liquid Marbles (Chapter VI. 6):** Hydrophobic beads were added on a small plastic lid. An 8 µL water droplet was added on the beads with a micropipette. Little movements of the lid was enough for liquid marble formation.

**Synthesis of tris-(benzyltriazolylmethyl)amine (TBTA) (Chapter VI. 3):** Based on a reported procedure$^{87}$ TBTA was synthesized as follows: tripropargylamine (1mL, 7.1 mmol), benzyl azide (3.14g, 23.6 mmol), CuSO$_4$:5H$_2$O (0.29g, 1.2 mmol) and NaOAsc (0.68g, 3.4 mmol) were added into a mixture of DCM (20 mL) and H$_2$O (20 mL) and reaction was performed by stirring for 24h at room temperature. H$_2$O phase was washed with DCM (3x 20 mL), DCM phases were all combined and dried over MgSO$_4$. Product was purified by column chromatography using silicagel and MeOH:EtOAc (1:9) mixture as the eluent. A white solid is obtained with 70% yield. H$^1$ NMR: (CDCl$_3$) δ (ppm) = 3.70 (s, 6H), 5.45 (s, 6H), 7.10-7.36 (15H), 7.60 (s, 3H) which fits with the original report.$^{88}$

**Epoxy-Azide Conversion for Beads and Capsules:** This conversion was performed in the same way as explained in Chapter V. 8. Azide loading for the (HIPE)GMA$_{60}$:EGDMA$_{40}$/((PEO-PPO-PEO 2800)$_{3.3}$):(CaCl$_2$ solution)$_{78.3}$ bead was calculated by elemental analysis. For the capsules on the other hand, azide loading was calculated by comparing IR azide peaks with that of the beads.

**CuAAC Reactions on Azide Containing Beads (Chapter VI. 3)**

**CuSO$_4$: (HIPE)GMA$_{60}$:EGDMA$_{40}$/((PEO-PPO-PEO 2800)$_{3.3}$):(CaCl$_2$ solution)$_{78.3}$** beads (10mg) were added into a syringe fitted with a frit and a solution of TBTA (4.8 mg), CuSO$_4$ (1.12 mg), NaOAsc (1.78 mg) and phenyl acetylene (25 μL) in DMSO:H$_2$O (0.5 mL, 4:1) was added. The syringe reactor was gently shaken by a vortex mixer. Samples were taken with time intervals and washed with acetone, H$_2$O, acetone and DEE respectively. Same was applied to the azidified GMA$_{60}$:EGDMA$_{40}$/((CH$_8$0:DD$_{20}$)$_{60}$) beads.

**CuBr:** (HIPE)GMA$_{60}$:EGDMA$_{40}$/((PEO-PPO-PEO 2800)$_{3.3}$):(CaCl$_2$ solution)$_{78.3}$ beads (10mg) were added into a syringe fitted with a frit and a solution of TBTA (4.8 mg), CuBr (6.5 mg) and phenyl acetylene (25 μL) in DMF (0.75 mL) was added. The syringe reactor was gently shaken by a vortex mixer. Samples were taken with time intervals and washed with acetone, H$_2$O, acetone and DEE respectively.

**CuBr + 50 °C:** (HIPE)GMA$_{60}$:EGDMA$_{40}$/((PEO-PPO-PEO 2800)$_{3.3}$):(CaCl$_2$ solution)$_{78.3}$ beads (10mg) were added into a 5 mL flask and a solution of TBTA (4.8 mg), CuBr (6.5 mg) and phenyl acetylene (25 μL)
in DMF (0.75 mL) was added. The flask was placed in an oil bath that is heated up to 50 °C and rotated by a rotary evaporator motor. Samples were taken with time intervals and washed with acetone, H₂O, acetone and DEE respectively.

**CuAAC Reactions on Azide Containing Capsules (Chapter VI. 9)**

15 mg azidified (HIPE)PEGMA₃₀:GMA₂₅:EGDMA₄₅/(PEO-PPO-PEO 2800)₃.₃:(CaCl₂ solution)₇₈.₃ capsules with a theoretical azide loading 0.2 mmol/g (calculated by comparing IR azide peaks) were treated with 0.2 mg CuBr (0.5 eq), 0.8 mg TBTA (0.5 eq), 0.3 ml DMF and either 2.5 mg (3 eq) or 0.25 mg (0.3 eq) naphthalimide (synthesized according to the literature⁷⁹). The reaction was rotated at 50 °C for 24h. At the end, capsules were washed with DMF, DCM, MeOH, DEE and dried. For IR analysis, KBr pellets were prepared. Capsules were microtomed for confocal microscopy.
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Chapter VII. Conclusions

This thesis aimed to occupy a unique place in the field of polymer particle synthesis, where the majority of the previously reported research is about polystyrene-based beads. Similarly, only a handful of chemistries have been used in the past for particle functionalization, a fact that is limiting the actual possibilities. Nonspherical and anisotropic particles have been scarcely reported although they may have unique properties. Moreover, porous particles have been produced the same way as decades ago, namely by making use of phase separating porogens. As a result, the obtained porous particles do not differ significantly when compared with the ones reported in previous decades. Novel approaches, and even combinations of novel approaches, are needed to expand the possibilities and opportunities in this field. For the sake of applicability, these approaches should also be easy.

The need for unique particles is also driven by the applications. Currently available resins fall short on satisfying the varying demands. For several applications such as solid phase synthesis or solid supported (enzymatic) catalysis, various molecules and even (bio)macromolecules need to be attached on polymer particles. Currently utilized chemistries for such kind of attachments suffer from low yields. Novel highly efficient resin functionalization strategies need to be developed, which in turn necessitates the development of novel resins. Modifying an existing resin with an intermediate molecule that possesses the final desired functionality for the efficient attachment of important molecules, is not ideal since it requires additional steps. Ideally, resins should be synthesized where the desired functional group is incorporated during the synthesis, which would allow direct functionalization with the molecules of importance.

Resin overloading is another problem in several applications. This problem becomes prominent when the molecule to be attached is too large such as synthetic polymers or enzymes. As one may expect, such large molecules hardly diffuse into inner zones of the support. As a result, the reactive groups located on the inner zones of resins remain largely intact after a functionalization step. This problem is also encountered in solid phase peptide synthesis after several amino acid coupling steps. Low loading resins are utilized as a solution to this problem but higher amounts of the resin are then required, which is material inefficient.

Started as a part of the Marie Curie sEnDiChem project (see Chapter I), the challenge of this thesis was to expand the research scope of particle synthesis. A previously reported tubing-needle based
microfluidic system is used for the whole thesis including some important modifications such as the use of a bent needle for the discrete phase, forming a co-flow device.

The tubing-needle device is easy to set up and to operate in contrast to the chip-based microfluidic systems that require experience and knowledge transfer from an expert. On the other hand, the tubing-needle device is certainly not free from drawbacks. To our experience, the most prominent problem of the system is the hydrophobicity of polymer tubing since most of the monomers are hydrophobic. Generally, the continuous to discrete phase ratio was about 100 to avoid the clogging, which resulted in other problems such as monomer transfer to the continuous aqueous phase and low production rates.

The exact location of the tip of the needle was also important to avoid the contact between droplets and tubing. However, it is not possible to manually place the needle always right in the middle of the tubing. It has also been experienced that droplet size slightly differs depending on the exact place of the needle in the tubing. Another aspect of the tubing-needle device is the fact that the smallest available commercial needle has 110 µm inner and 240 µm outer diameter, which limits the minimum bead size. Produced beads are often larger than 300 µm, while the smallest we could obtain was 150 µm in diameter. Since the needle is large (compared to the capillary and PDMS devices), the tubing also needs to be large: 0.5 to 1.6 mm inner diameter. The term “millifluidic” is therefore also utilized for such devices. However in this thesis, “microfluidics” is used to designate the method and thus not the dimensions of the channel.

Despite the shortcomings of the tubing-needle device, some significant achievements have been made in this thesis. First of all, monodisperse large microgels were prepared from dextran chains furnished with methacrylate groups, as explained in Chapter III. The dextran microgels were degradable thanks to the carbonate ester groups located between the dextran backbone and the attached methacrylate groups. These template dextran microgels were layer-by-layer coated by negatively charged Pt nanoparticles and a positively charged polymer and the three bi-layers were fixed by a light reaction. Covalent linking was necessary to keep the “giant” capsules stable after the dissolution of the dextran core. Much smaller PS particles were also embedded in these capsules in another experiment, by physically incorporating them to the dextran microgels during the microfluidic synthesis. By making use of microfluidics, monodisperse large beads were easily produced, which cannot be done by any of the classical methods such as suspension polymerization.

Chapter IV is also about nonporous beads but with a completely different composition. Almost all of the organic particles reported in literature are based on polymerization of vinyl monomers such as styrene. Instead, thiol-yne chemistry was utilized for the first time to construct resins, to the best of
our knowledge. Similarly, we also prepared porous and nonporous particles with different functional groups by using either thiol-yne or thiol-ene chemistries, which is not included in this thesis. The advantage of the thiol-yne approach was that the thiol and yne functionalized beads were readily obtained by changing the ratio between the two monomers, a tetra-thiol and a di-alkyne. Both thiol and yne groups are becoming popular, especially in the polymer society thanks to the many “click” reactions one may conduct via these functionalities. As a matter of fact, both azide-alkyne and thiol-yne click reactions were conducted on the yne functionalized resin in a comparative manner.

The thiol functionalized resin allowed more click reactions to be conducted: 9 different type of reactions, many of which are regarded as being part of click chemistry, were performed and followed by IR. The isocyanate reacted fastest with the thiol bead, followed by norbornene via anionic and radical mechanisms, respectively. In addition, quantitative couplings were obtained by many of these 9 reagents after 15-24h reaction times without heating or shaking, although this thiol bead had a high thiol loading. This thiol bead seems to be a novel, highly efficient polymer support, as a candidate that could overcome problems of traditional resins, as explained in the initial paragraphs of this chapter. Further research should be done to see how these thiol and yne resins perform in several applications such as solid phase peptide synthesis.

Chapter V reports a fruitful combination of two novel techniques: microfluidics and microcontact printing. The porous epoxy-functionalized beads synthesized in our group were used in the development of the “sandwich” microcontact printing method in the research group of Prof. B. J. Ravoo from the University of Münster. Three types of Janus particles were obtained after printing different combinations of functional amines on the epoxy beads. First, the methodology was proven by printing two different dyes. Second, functional Janus beads were obtained by printing two carbohydrate molecules, which bind different proteins, on each face of beads. Finally, a dye and Fe₃O₄ nanoparticles were printed on each face, resulting in Janus beads responding to external magnetic fields.

The printing was also done successfully on nonporous beads. However, porous particles were preferred because of several reasons. First of all, porous particles have additional applications. Pores can be covalently or physically filled with a third compound of interest. Moreover, porous beads have a larger surface area as a result of the pores, which increases the contact area between the stamp and the beads. Finally, porous beads were easier to be visualized after the microcontact printing, thanks to the opacity. The reproducible production of porous particles however, was not straightforward and further research can be done on this topic for optimization.
The complexity of the obtained particles by the tubing-needle setup reached its maximum in the Chapter VI. “Megaporous” hollow rods were obtained that are certainly unprecedented. Neither a confined channel, nor a template for the hollow core, nor any organic porogen was used for the construction of these unique particles. All the previous microfluidic synthesized rod-like particles necessitated confined channels.\textsuperscript{11-14} Hollow particles also often require a template for the empty core,\textsuperscript{15-17} such as the capsules of the Chapter III. Finally, instead of organic porogens, only water and some surfactant are used to form the huge pores of the shell.

Together with the hollow rods, non-hollow rods, capsules and beads were obtained in this Chapter VI. To obtain these particles, a combination of microfluidics and high internal phase emulsion (HIPE) approaches were combined. In other words, water-in-monomer HIPE droplets were polymerized in microfluidics. Unique poly(HIPE) structures with uniform size and “megapores” (up to 20 µm) were obtained. Bead to rod, and non-hollow to hollow transitions were provided by controlling the viscosity and the use of a PEG based monomer, respectively. In addition, the obtained poly(HIPE) particles were highly reactive; they outperformed the macroporous beads of the same composition in consecutive click reactions. Thanks to high diffusion rates of reagents and solvents, these poly(HIPE) beads can solve some of the long standing problems of resin functionalization.

In addition, so-called liquid marbles were also formed via the assembly of hydrophobic poly(HIPE) beads around water droplets. They are unique thanks to the “megapores”, which may allow triggered delivery of some compounds that can be loaded into the pores.

To sum up, various particles that differ in shape, morphology and composition have been obtained via a tubing-needle based microfluidic system. Different chemical post-modifications, especially click chemistry type, were also conducted on the obtained particles. Future research can be devoted on the actual use of these beads, for instance the thiol functionalized beads, in suitable applications. HIPE formulations from thiol-ene/yne monomers can be obtained and novel poly(HIPE) monoliths and particles can be prepared thereof. A proper understanding of the hollow core formation could even lead to more complex structures via HIPE in microfluidics approach. There is certainly more to discover in this field. In terms of microcontact printing on beads, the work was ongoing during the writing of this thesis. For example other particles, such as the thiol functionalized bead, are also being “sandwich” printed. Moreover, the same method could be applied on nonspherical particles to obtain Janus rods.
References

Addendum. Curriculum Vitae

Short Biography

Muhammed Talha Gökmen was born in Ankara on 13 October 1981. He obtained his high school degree from Tevfik İleri Anadolu İmam-Hatip Lisesi (Ankara) in 1999. The same year, he started studying chemistry in Gazi University (Ankara) in the Sciences and Letters Faculty and obtained his B.Sc. degree in 2003. In 2006, he obtained his M.Sc. degree in chemistry from Boğaziçi University (İstanbul), Instiute of Sciences with the thesis entitled “Synthesis of Segment-Block Dendrimers via Chemical Ligation”. In September 2006, he started his Ph.D. study in Ghent University. He is married to Nadia Faris since 2010. He enjoys travelling, reading about history and architecture and performing several sports. Besides Turkish and English, he speaks Arabic, Italian and Dutch to some extent.

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Oral Presentations

11/06/2010  Group of Prof Andrew Griffiths, Université de Strasbourg, France
Complex Polymer Architectures via a Tubing-Needle Based Simple Microfluidic Setup

25/05/2010  Belgian Polymer Group Annual Meeting 2010, Blankenberge, Belgium
Microfluidic Synthesis of Previously Unprecedented Porous Uniform Reactive Beads, Rods and Capsules

25/03/2010  ACS National Meeting, Spring 2010, San Francisco, USA
Fabrication of Porous “Clickable” Polymer Beads and Rods Through Generation of High Internal Phase Emulsion (HIPE) Droplets in a Simple Microfluidic Device

01/03/2010  10th Flemish Youth Conference of Chemistry, Blankenberge, Belgium
Highly Porous Monodisperse Polymer Particles via Microfluidics: Novel Shapes, Novel Structures, Novel Features

05/02/2010  3rd EST-GOA sEnDiChem Meeting, Ghent University, Ghent, Belgium
Highly Porous Monodisperse Polymer Particles via Microfluidics: Novel Shapes, Novel Structures, Novel Features

02/02/2009  2nd EST-GOA sEnDiChem Meeting, Ghent University, Ghent, Belgium
Size monodisperse, highly porous, functional polymer beads as solid supports for various applications

31/01/2008  1st EST-GOA sEnDiChem Meeting, Ghent University, Ghent, Belgium
Challenging problems of polymeric solid supports

Poster Presentations

01/07/2010  Marie Curie Workshop of Euroscience Open Forum (ESOF), Torino, Italy
Using Syringes, Needles And Simple Tubing To Make Unprecedented Little Plastic Particles

24/03/2010  ACS National Meeting, Spring 2010, San Francisco, USA
Novel Monodisperse Porous Polymer Particles with Various Shapes and Reactive Groups via a Simple Microfluidic Device

07-09/06/2009  Frontiers in Polymer Science Conference, Mainz, Germany
Fabrication of ‘Clickable’ Particles via a Simple Microfluidic System

14-15/05/2009  Belgian Polymer Group Annual Meeting 2009, Houffalize, Belgium
Uniform ‘Clickable’ Macroporous Beads and ‘Worms’ by a Simple Microfluidic Device

20-24/07/2008  48th Micro Symposium on Polymer Colloids: From Design to Biomedical and Industrial Applications, Prague, Czech Republic
Single and Multiple Emulsion Droplets in Flow; Fabrication of ‘Clickable’ Particles via a Simple Microfluidic System

22-23/05/2008 Belgian Polymer Group Annual Meeting 2008, De Haan, Belgium
Single and Multiple Emulsion Droplets in Flow; Fabrication of ‘Clickable’ Particles via a Simple Microfluidic System

16-18/09/2007 UKPCF 2007 Conference, University of Warwick, Coventry, UK
Clickable Particles Produced via Simple Microfluidic Device

Academic Visits

2009 Work in the lab of Prof. Stefan Bon for 3 weeks, University of Warwick, UK
2009 Work in the lab of Prof. Bart Ravoo for 2 days, Münster University, Germany
2007 Work in the lab of Prof. Stefan Bon for 6 weeks, University of Warwick, UK