SYNTHESIS OF ACRYLIC BASED HIGH INTERNAL PHASE EMULSION POLYMERS AND THEIR APPLICATION IN CHROMATOGRAPHY

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SYNTHESIS OF ACRYLIC BASED HIGH INTERNAL PHASE EMULSION POLYMERS AND THEIR APPLICATION IN CHROMATOGRAPHY

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ABSTRACT

SYNTHESIS OF ACRYLIC BASED HIGH INTERNAL PHASE EMULSION POLYMERS AND THEIR APPLICATION IN CHROMATOGRAPHY

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High internal phase emulsion polymers (PolyHIPEs) are new generation materials with their high porosity and interconnected open-cell structures and finds applications in areas such as supports for catalytic systems, separation media and tissue engineering scaffolds. Styrene based PolyHIPEs are currently the most popular choice, but solvent compatibility and poor mechanical properties of these materials prevent their applications. Therefore development of new polyHIPEs with desired mechanical and cellular properties is needed to extend the range of applications.

The objective of this thesis was to synthesize new polyHIPEs with different mechanical characteristics changing from ductile to elastomeric. For this purpose, acrylic based polyHIPEs with various cellular structure and mechanical

characteristics were developed by using stearyl acrylate (SA), isodecyl acrylate (IDA), isobornyl methacrylate (IBMA) and divinylbenzene (DVB). All materials were highly porous (90%) and had open cellular structure with uniform voids in the range of 5.2-12.9 μm. The PolyHIPEs produced from the monomers of SA and IDA demonstrated elastomeric property and had high ability of recovery when the applied stress is removed. IBMA based polyHIPEs were ductile and demonstrated higher Young's modulus and compression strength than that of conventional styrene based polyHIPEs. Therefore, by varying the composition, it became possible to alter the mechanical properties of polyHIPEs from ductile to elastomeric, without changing the interconnected cellular structures.

One of the prepared IDA based polyHIPE was evaluated as stationary phase for capillary electrochromatography for the first time in literature. The column was very efficient in the separation of alkylbenzenes namely thiourea, benzene, toluene, ethylbenzene, propylbenzene and butylbenzene with high column efficiency (up to 200.000 plates/m).

Keywords: High internal phase emulsion polymers, polyHIPE, Open-cell structure, Monolithic columns, Capillary electrochromatography

ÖZ

AKRİLİK BAZLI YÜKSEK İÇ FAZ EMÜLSİYON POLİMERLERİNİN SENTEZİ VE KROMATOGRAFİDE KULLANIMI

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Yüksek iç faz emülsiyon polimerleri (polyHIPEs) yüksek gözenekli ve birbirine bağlı açık hücre yapılarıyla yeni nesil malzemelerdir ve katalitik ile ayırım sistemleri ve doku mühendisliği gibi alanlarda destek malzemesi olarak uygulamaları mevcuttur. Günümüzde, stiren bazlı polyHIPE malzemeler en popüler tercihtir, ancak çözücü uyumluluğu ve zayıf mekanik özellikleri bu malzemelerin uygulamalarını kısıtlamaktadır. Dolayısıyla, uygulama alanının genişletilmesi için istenilen mekanik ve hücresel özellikli yeni polyHIPE malzemelerin üretilmesine ihtiyaç vardır.

Bu tez araştırmasının amacı sünekten elastomerik yapıya değişen, farklı mekanik özelliklere sahip yeni polyHIPE malzemeler sentezlemektir. Bu amaçla, akrilik bazlı çeşitli hücre yapısı ve mekanik özelliklere sahip polyHIPE yapılar, stearil akrilat (SA) izodesil akrilat (IDA) izobornil metakrilat (IBMA) ve

divinilbenzen (DVB) kullanılarak geliştirilmiştir. Bütün malzemeler yüksek gözenekli ve 5.2-12.9 µm aralığındaki düzenli küresel boşluklarıyla açık hücresel yapıya sahiptirler. SA ve IDA monomerlerinden üretilen elastomerik PolyHIPE malzemeler, uygulanan gerinim kaldırıldığında kendini toplamada yüksek kabiliyet ve elastomerik özellik sergilemişlerdir. IBMA bazlı polyHIPE malzemeler sünektirler ve yaygın stiren bazlı polyHIPE malzemelerden daha yüksek Young Modülü ve basma dayanımı göstermişlerdir. Buna bağlı olarak, polyHIPE malzemelerin mekanik özelliklerini, birbirine bağlı hücre yapısını bozmadan sünek yapıdan elastomerik yapıya değiştirmek sadece içerik değişikliği ile mümkündür.

IDA bazlı polyHIPE malzemelerden bir tanesi, literatürde ilk kez olarak kapiler elektrokromatografisi katı fazı olarak değerlendirilmiştir. Kolon, tiyoüre, benzen, toluen, etilbenzen, propilbenzen ve bütilbenzen isimli alkil benzenlerin ayırımında yüksek bir kolon etkinliği ile (200.000 tabaka/m) oldukça başarılı olmuştur.

Anahtar Kelimeler: Yüksek iç faz emülsiyon polimerleri, polyHIPE, açık hücre yapısı, monolitik kolonlar, kapiler elektrokromatografisi

To my family...

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ABBREVIATIONS

ACN	Acetonitrile
BET	Brunauer-Emmet-Teller
CEC	Capillary Electrochromatography
DVB	Divinylbenzene
E	Young's Modulus
EOF	Electroosmotic Flow
HIPE	High Internal Phase Emulsion
IBMA	Isobornyl Methacrylate
IDA	Isodecyl Acrylate
OT-CEC	Open-Tubular Capillary Electrochromatography
PolyHIPE	High Internal Phase Emulsion Polymer
SA	Stearyl Acrylate
SAB	Strain At Break
SEM	Scanning Electron Microscopy
ST	Styrene
TPN	Theoretical Plate Number
UCS	Ultimate Comprssion Strength

CHAPTER 1

1. INTRODUCTION

1.1 High Internal Phase Emulsion Polymers

1.1.1 High Internal Phase Emulsions

Emulsions are a class of two immiscible liquid system in which liquid droplets are dispersed in the other liquid medium [1]. They are described as either water-in-oil (w/o) or oil-in-water (o/w) systems. High internal phase emulsion (HIPE) systems are the class of emulsions which have more than 74 % internal phase in the most compact arrangement of liquid droplets separated by thin film of continuous phase [2]. These emulsions are also known as highly concentrated emulsions [3], gel emulsions [4] and biliquid foams [5]. In these concentrated emulsions, the internal phase droplets are deformed against the neighbour droplets and form foam like structures with polyhedron shapes [6]. These emulsions are widely used in different applications such as food, pharmacy and cosmetic industries, and as protective films [2, 7]. In recent years HIPE systems have attracted great attention for the production of solid macroporous foams called polyHIPE polymers.

1.1.2 High Internal Phase Emulsion Templated Polymers

Polymerization of an emulsion can be used to produce polymeric materials in colloid form and porous monolith form (Figure1).

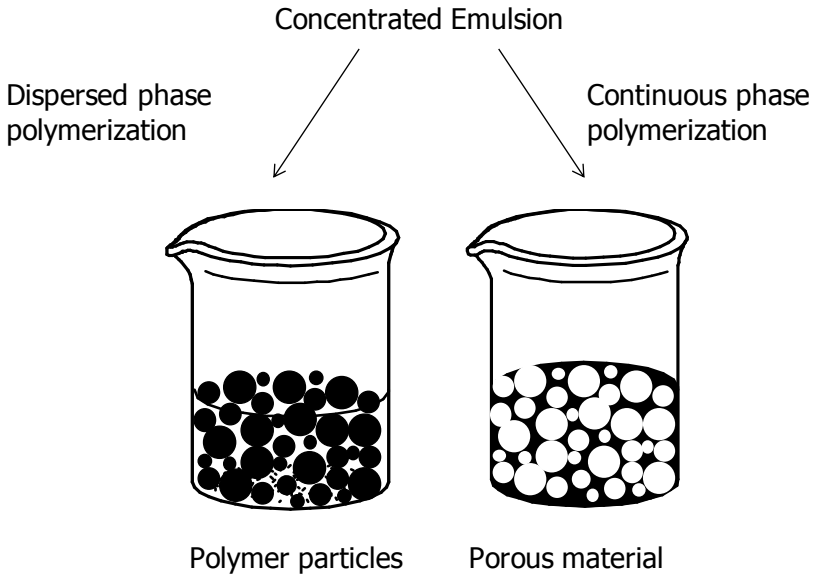


Figure 1 Polymerization of a concentrated emulsion in the dispersed phase and continous phase.

In an emulsion, colloids form if the polymerization takes place in the dispersed phase. By contrast, the polymerization of the continuous monomer phase produces emulsion templated porous polymer. This technique can produce open or closed cell structure materials depending on the internal phase ratio in the emulsion. The internal phase volume higher than 74% produce open pore

structure, while the internal phase volume lower than 60% produce closed pore structure materials.

In recent years, high internal phase emulsion (HIPE) systems have attracted attention for the production of solid macroporous foams called polyHIPE polymers. The use of HIPE as a template to produce macroporous foams was first described by the researchers from Unilever [8]. The conventional method for the production of these materials is the polymerization of the continuous phase of a HIPE (Figure 2). The continuous phase of the HIPE contains monomer/monomers, a crosslinker and a surfactant for the stabilization of the emulsion. The internal phase is an aqueous solution of an initiator and an electrolyte. Polymerization of continuous monomer phase of the HIPE around the aqueous emulsion droplets and following the removal of the dispersed phase forms a highly porous and low density polymeric material. During the polymerization process, the emulsion acts as a template for the resulting polymeric foam and interconnecting windows form in the contact points of emulsion droplets entrapped in the emulsion.

This emulsion templating technique can form foams that have density less than 0.15 g/cm^3 [9], porosities in the range of 75-99 %, pore sizes of 5-100 μm and open or closed type cellular nature by varying emulsion composition [10]. The classical polyHIPE morphology is characterized by highly porous structure with regular micron size voids that were all interconnected through the windows. In the polyHIPE structure, the large spherical cavities are termed as voids and the circular holes in between the voids are termed as interconnecting windows (Figure 3).

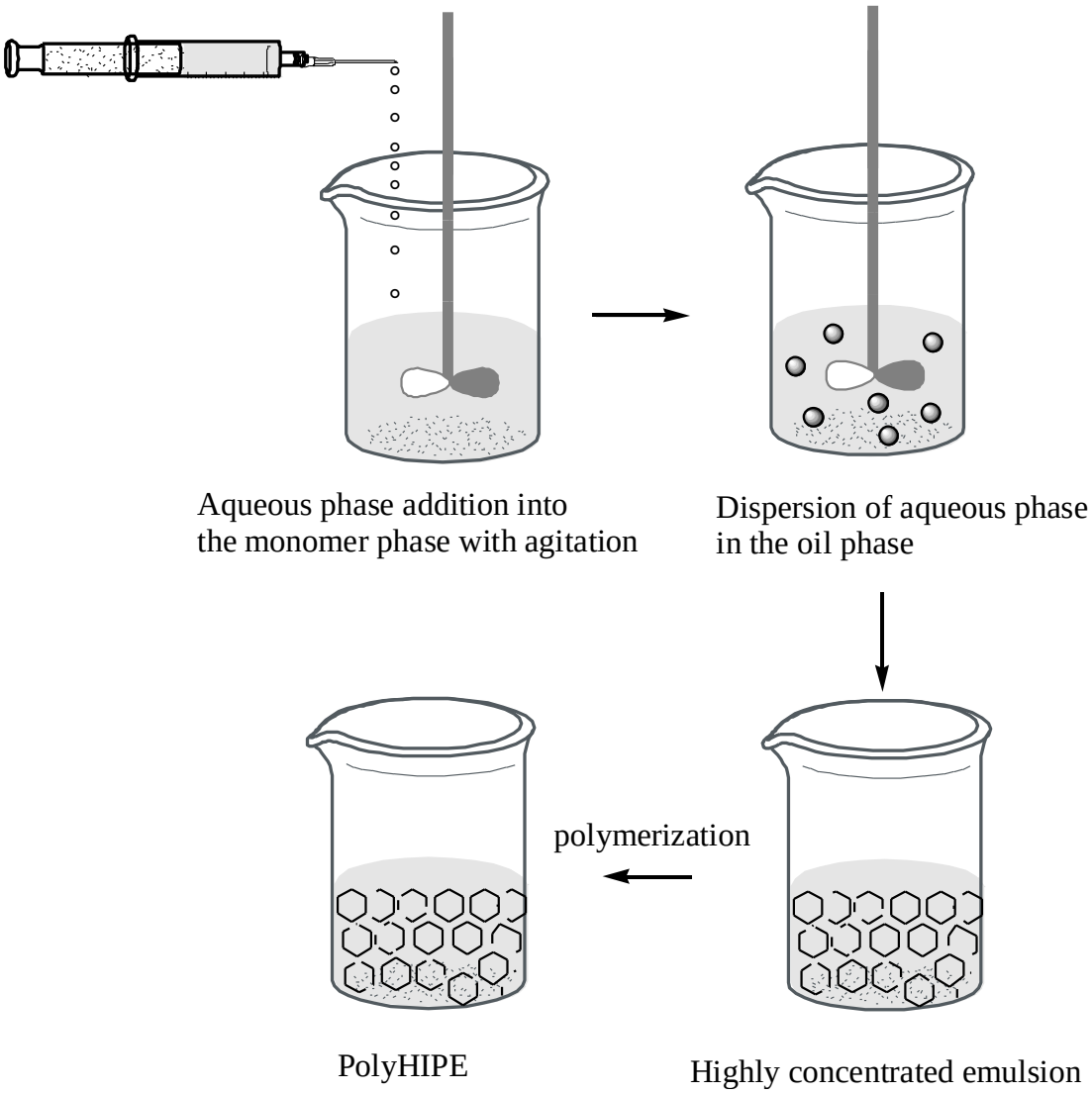


Figure 2 PolyHIPE production process.

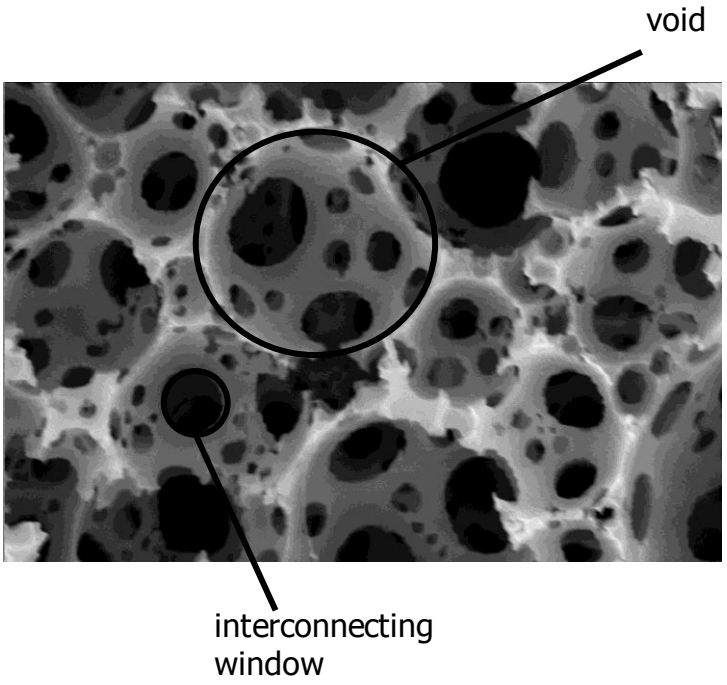


Figure 3 Definition of void and interconnecting window in polyHIPE structure.

They are defined to have a hierarchical pore structure with large voids which are imprints of dispersed phase; interconnecting windows between the voids and pores within the polymer walls [11]. PolyHIPEs have unique properties due to their special cellular structure. Therefore they have been useful as a support material in the applications requiring high porosity, permeability and low density.

1.1.3 Chemistry of High Internal Phase Emulsion Polymers

The structural architecture of polyHIPE materials depends on emulsion stability. Accordingly, it is determined by continuous and dispersed phase compositions, surfactant type and content and preparation conditions [12]. The formation of such a structure is a complex phenomenon and various studies have been performed to determine the parameters that affect the polyHIPE properties. It is known that monomer type and content in the continuous phase are the most important parameters since they affect the emulsion stability. The monomer phase is either hydrophilic or hydrophobic enough to form a stable emulsion. The first and the most common polyHIPE system is a water-in-oil emulsion containing styrene (ST) and divinylbenzene (DVB) in the continuous phase. Since, styrene and DVB are hydrophobic and water immiscible monomers, they are very suitable for the formation of a stable water-in-oil emulsion with water. Free radical polymerization of these hydrophobic monomers in the continuous phase produces polyHIPE materials. Some other hydrophobic monomers such as 4-vinylbenzyl chloride [13], 2-ethylhexyl acrylate [14] also have been used to prepare polyHIPEs.

Another approach to vary the polyHIPE chemistry is functionalization. There have been many studies on the functionalization of a polyHIPEs [15, 16] by mainly two different approaches: (1) post-functionalization of a preformed polyHIPE; and (2) grafting a functionalized chain on the surface of polyHIPE [17]. For example, 4-vinylbenzoyl chloride containing polyHIPEs can be easily functionalized by nucleophilic amines because of the reactive benzyl chloride groups [18]. Benicewicz et. al. [19] grafted poly(4-vinylpyridine) onto 4-vinylbenzoyl chloride based polyHIPEs and studied removal of heavy metals

from aqueous media. It was observed that the resultant material had faster kinetics than 4-vinylpyridine based material to remove the metals. Active ester acrylate based polyHIPEs were also used to produce materials bearing amino or hydroxyl functionality [20]. It was also reported that functionalization was applied to unreacted vinyl groups of a polyHIPEs containing high ratio of DVB [16, 17].

1.1.4 Morphology Control of HIPEs

Emulsion templating is an easy to control technique to produce macroporous materials whose morphologies are optimized for a specific application. The cellular structure, porosity and mechanical characteristics of polyHIPEs can be varied by changing the emulsion composition and process parameters.

1.1.4.1 Cellular Structure

The cellular structure of the material can be varied and produced as open or closed cell by controlling mainly the internal phase volume and surfactant concentration. Here, the surfactant concentration was found to be a very effective parameter by Williams and Wrobleski [21]. It was reported that a surfactant level less than 5% relative to organic phase volume caused closed cell type materials even if the emulsion contains an internal phase which is 97% of the total emulsion. On the other hand, open-cell structures could be produced by using an emulsion containing 60% dispersed phase volume with 20% surfactant with respect to organic phase volume. This behaviour was explained by the increasing surface area of droplet phase and hence the

thinning of the continous phase film in between the droplets with increasing surfactant concentration. Thickness of the continous phase film is very important for the formation of interconnecting windows between the droplets. Cameron et. al. [22] concluded that window formation is a result of volume contraction on conversion of monomer to polymer at the thinnest point between the adjacent droplets. Another approach to explain the formation of windows came from Menner and Bismarck [23]. They reported that aqueous droplets were enclosed by the continuous phase during the polymerization reaction and the windows formed during removal of the internal phase from the resultant material.

1.1.4.2 Void Size

In emulsion templating method, the void size is determined by the size of the dispersed droplets in the emulsion before the polymerization of the continuous phase. Therefore it is affected by any parameter controlling emulsion stability. In general, the void size of a polyHIPE material can be varied by monomer phase composition, surfactant and electrolyte concentration. Williams et. al. [21, 24] studied the effects of these parameters (monomer composition and surfactant and salt concentration) on styrene/DVB system. They found that increasing DVB ratio in the continuous phase decreased the average void diameter. This was explained by the increased emulsion stability. DVB is more hydrophobic than styrene and this result was explained by the increased emulsion stability which causes lower interfacial tension and smaller droplet size. Increasing surfactant concentration has a similar effect. The void diameter decreased with increasing surfactant concentration as a result of the increased emulsion stability. Another parameter, electrolyte concentration is

important to suppress the Ostwald ripening. Ostwald ripening is the tendency of small dispersed droplets to form larger droplets to decrease polydispersity of the system. In a water-in-oil emulsion, the rate of Ostwald ripening can be reduced by the addition of electrolytes into the dispersed phase. As expected, increasing electrolyte concentration was found to decrease the droplet size and hence the void size of the material.

Different applications require different void diameters. In some applications such as tissue engineering, it can be advantageous to prepare large voids in the structure. To increase the void size, Ostwald ripening effect can be induced by addition of a water miscible solvent to the aqueous phase. Hayman et. al. [25] produced styrene/DVB based polyHIPEs with the voids ~50-100 μm in diameter by the addition of 1% (v/v) tetrahydrofuran to the aqueous phase.

Another effect on the void size of polyHIPEs is the type of the functional monomer. Barbetta et al. [26] reported that when a different hydrophobic monomer, for example 4-vinylbenzyl chloride was used instead of styrene, the resultant polyHIPE composed of 4-vinylbenzyl chloride and DVB had smaller void diameters than the polyHIPE composed of styrene and DVB. This effect of 4-vinylbenzyl chloride on void size was explained by its co-surfactant bahaviour in the system.

1.1.4.3 Surface Area

As a result of the large voids in the structure, polyHIPE materials have low surface area. The surface area can be increased by increasing the crosslinker ratio and by addition of a porogen to the monomer phase, thus porosity can be generated within the polyHIPE walls. Although polyHIPE materials are highly porous, they have low surface area values in the range of 3-20 m^2g^{-1} due to large void size in their structure [27]. Cameron et.al. [28] reported that surface area of polyHIPEs could be increased up to 554 m^2g^{-1} by replacing some of the monomer with a porogenic solvent in polyDVB foam.

1.1.5 Mechanical Characteristic of High Internal Phase Emulsion Polymers

PolyHIPEs can be used in many applications in which high porosity and open-cell structure are required, but poor mechanical characteristic of these materials prevent their use in industry [29]. Various approaches have been applied to improve the mechanical properties of these materials. Some of these approaches propose to use nanosilica particles for reinforcement [30], to increase the continuous phase of emulsions [29] and to prepare hybrid polyHIPEs with interpenetrating polymer-inorganic networks [31]. On the other hand, it is also important to preserve the unique cellular structure of these materials while improving their mechanical properties. In conclusion, development of new polyHIPEs structures with desired mechanical and cellular properties is necessary for different applications.

1.1.6 Applications of High Internal Phase Emulsion Polymers

The most important application areas of polyHIPEs are tissue engineering and cell culture, separation of chemicals and biosensor productions. A short summary about these applications are given below.

1.1.6.1 Tissue Engineering and Cell Culture

Tissue engineering has the potential to create new tissue and organs from cultured cells for transplantation. For this purpose, three dimensional tissues are formed by growing the cells within a porous scaffold [32]. Therefore it is very important to develop a material which is suitable for cell seeding and growth of tissues. An ideal scaffold should have interconnecting pore structure for migration of cells and growth of new tissue. Interconnected macroporous structure of PolyHIPEs makes them very desirable candidates for tissue engineering applications. Furthermore, their easy fabrication technique is an advantage for the production in various sizes and shapes. Akay et. al. [33] investigated styrene/DVB based polyHIPE materials as supports for the growth of osteoblasts. Their material demonstrated in-vitro cell-polymer compatibility and maturation of osteoblast-like cells for 35 days. In addition, Poly(ε-caprolactone) [34] and poly(lactic acid) [35] containing polyHIPEs were prepared and investigated as supports for tissue growth. Cell and tissue growth studies of the materials containing 20% poly(ε-caprolactone) or poly(lactic acid) showed good biocompatibility with whole chicken embryo explants, rat skin explants and individual human fibroblasts. Comparison of these two materials showed that poly(ε-caprolactone) containing polyHIPE indicated more rapid attachment of cells. Fully biodegradable polyHIPE

scaffolds with different void sizes (10-300 μm) and morphologies were also prepared by using macromer poly(propylene fumarate) and the crosslinker propylene fumarate diacrylate [36]. In this study, fully biodegradable scaffolds were prepared by the emulsion templating technique for the first time in literature.

1.1.6.2 Separation Materials

Macroporous polymeric materials have attracted an increasing interest as monolithic materials in separation technologies and they are commonly used in chromatographic applications. An ideal monolith should have high permeability and low flow resistance. In this sense, polyHIPEs are potential materials as chromatographic separation media. Because of interconnections in the polyHIPE structures, diffusion limitations are minimized and mass transfer properties are suitable especially for large molecules. Krajnc et. al. [37] prepared glycidyl methacrylate/ethylene glycol dimethacrylate based polyHIPE monolith materials for the separation of proteins. Chromatographic studies showed that the monolith demonstrated acceptable separation performance but low dispersion. PolyHIPEs are promising materials as a monolith providing fast chromatographic separations, low back pressure at high flow rates and hence high efficiency.

1.1.6.3 Catalyst Materials

A catalyst or reagent attached to a PolyHIPE has high accessibility to the reactants because of the large voids and the interconnections. Therefore they are used as support for heterogeneous reagents and catalysts. The reaction mixture is forced through the interconnected macroporous structure of the polyHIPE functionalized for a specific aim. For example sulfonated styrene/DVB based polyHIPE was used as catalyst in liquid-liquid hydration of cyclohexene to cyclohexanol [38]. Sulphonated polyHIPEs were also used to produce ion exchange resins by incorporating Na^+ onto their surface and showed better column utilization than the commercial resins in single-pass dynamic adsorption tests [39].

In a more sophisticated application, enzymes were immobilized covalently onto a polyHIPE support to prepare a material for biocatalysis [40]. The results of the study showed the polyHIPE structures are very suitable for immobilizing enzymes. Besides, the enzyme immobilized polyHIPEs demonstrated high biocatalytic activity compared to the enzyme immobilized beads or particles.

1.1.6.4 Sensors

Macroporous structure of polyHIPEs makes them excellent supports providing separation with sensing in real liquid media [11]. The high porosity can enhance the capabilities of sensors. To produce a sensor for detecting and measurement of a specific chemical substance in vapour form, Silverstain et. al. [14] produced intrinsically conductive polymer coated polyHIPEs for

acetone vapour sensing. The conductivity was provided by the oxidative polymerization of pyrrole and aniline on the polyHIPEs surface. The resulting material showed a reversible change in conductivity on exposure to acetone vapour, exhibiting its potential use as sensor material.

1.2 Capillary Electrochromatography

1.2.1 What is Capillary Electrochromatography?

Capillary electrochromatography (CEC) is a microscale separation technique that combines capillary electrophoresis and capillary chromatography [41, 42]. It uses the separation mechanism of both techniques by utilizing chromatographic retention and electrophoretic migration [43]. In CEC, a capillary column (50-100 μm in diameter) is used and the mobile phase is driven through this capillary column by electroosmosis generated by an electric field. The source of the electroosmotic flow (EOF) is the electrical double layer formed at the charged solid-liquid interface of the stationary phase which is in contact with the electrolyte solution. When a voltage is applied across the column, the counterions at the electrical double layer move to the corresponding electrode and carry the mobile phase [44, 45]. Electroosmotic flow has various advantageous over conventional pressure driven flow. Firstly, unlike the parabolic flow profile of pressure driven flow, EOF has a uniform plug-like velocity profile, because the dragging effect of friction on the wall is low [46]. Therefore it leads higher column efficiency than pressure driven flow. Another result of using EOF is that the double layer thickness is assumed to be much smaller than the column diameter and so the

column diameter does not affect the EOF velocity. Thus, longer columns can be used to increase efficiency. These advantages of EOF lead to development of alternative column technologies for CEC.

Although the use of EOF was first reported by Strain [47] in the separation of dyes in 1939, CEC was introduced by Pretorius et. al. [48] who pointed out the advantages of EOF in chromatography in 1974. Then Jorgenson and Lukacs [49] used electrochromatography by filling the capillaries with octadecylsilica particles. Since that time, CEC has been a significant alternative technique to the conventional liquid chromatographic techniques.

1.2.2 Capillary Electrochromatography Instrumentation

A schematic diagram of a basic CEC instrument is represented in Figure 4. CEC is composed of a capillary column, two buffer solution reservoirs, a high voltage source and a detector.

Capillaries usually have the inner diameter less than 100 μm and the length in between 30-100 cm. A high voltage up to 30 kV is applied across the column on which the ends are immersed into the vials filled with electrolyte solution. The mobile phase and the analytes are driven through the column by EOF and separated due to the chromatographic retention and electrophoretic migration of ionic analytes. The separated analytes are detected at the detection window on the capillary by detector.

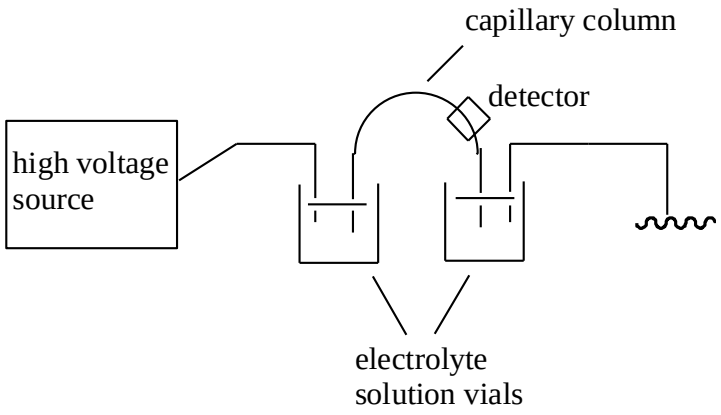


Figure 4 Schematic representation for capillary electrochromatography.

1.2.3 Electroosmosis and Flow in Capillary Electrochromatography

Electroosmosis is the movement of a liquid containing electrolyte relative to charged stationary phase under an applied electric field [50]. In CEC systems, the electroosmotic flow is a result of surface charge of ionized stationary surface that affect the ion distribution in the solution. A double layer of electric charge is formed by the counter ions attracted by the surface and the repelled ions of like charge [45]. This double layer formation can be explained by Gouy–Chapman theory. Figure 5 shows the Gouy-Chapman model illustrating the distribution of ions in the presence of a charged surface.

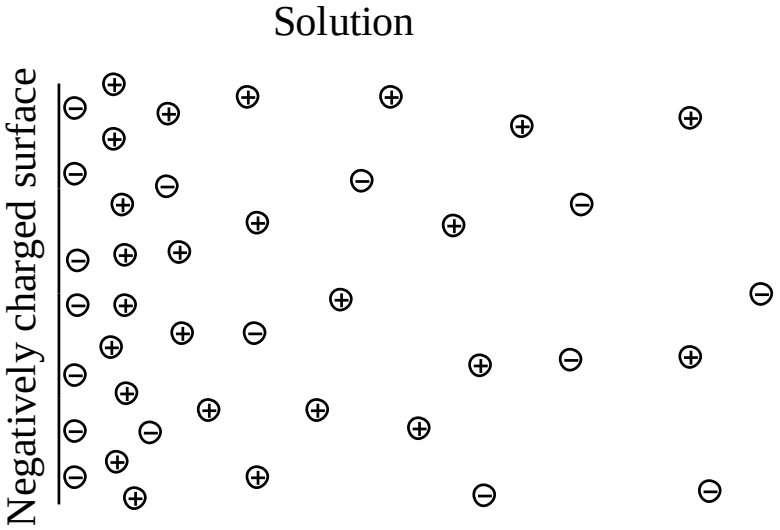


Figure 5 Gouy-Chapman model illustrating the distribution of ions in the presence of a charged surface.

According to this theory, counterions next to a charged surface does not form a homoionic layer but exist as a diffuse cloud in which counterions are concentrated at the surface and decrease exponentially as the distance increases [51]. The counterions exist in two layers fixed and diffuse which form shear plane next to their interface. The potential between this shear plane and the wall is known as zeta potential (ζ). Zeta potential depends on surface charge density and the ionic strength of the electrolyte and is expressed by the Equation 1;

$$\zeta = \frac{\sigma}{\varepsilon_0 \varepsilon \kappa} \qquad (1)$$

where σ is the surface charge density, ε_0 is the permittivity of the vacuum, ε is permittivity of the mobile phase and κ is the Debye-Huckel parameter.

When a potential difference is applied across the column, the counterions at the electrical double layer move to the corresponding electrode. The linear velocity of the resulting EOF (u_{eo}) depends on the zeta potential, permittivity and viscosity of the mobile phase and also electric field strength. Equation 2 defines the linear velocity of EOF [45].

$$u_{eo} = \frac{\varepsilon_0 \varepsilon \zeta}{\eta} E \qquad (2)$$

where ζ is the zeta potential, ε_0 is the permittivity of the vacuum, ε and η are permittivity and viscosity of the mobile phase, respectively and E is the electric field strength.

In CEC, the neutral species move in the same direction with the mobile phase and separated according to chromatographic retention factor. On the other hand, the charged species have electrophoretic migration in addition to EOF. The direction of the electrophoretic migration is determined by the charge of the species. Therefore, the electrophoretic migration can be in the same or the opposite direction with the EOF and lead to co- or counter-directional separation process [44].

1.2.4 Stationary Phases for Capillary Electrochromatography

CEC is hybrid technique combining liquid chromatography and capillary electrophoresis. The role of stationary phase is very important because it generates electroosmotic flow of the mobile phase in addition to its separation role. Therefore the most rapidly developing research area in CEC is the column technology. The stationary phase types for CEC includes open tubular columns, packed columns and monolithic columns.

1.2.4.1 Open Tubular Columns

An open tubular column is a small diameter column in which the inner wall or a liquid or active solid on the tube wall has the stationary phase function [52]. The simplest column type in CEC is capillary open tubular columns that have charged groups and separation groups on the capillary wall as stationary phase. Open tubular capillary electrochromatography (OT-CEC) columns have small internal diameter (10-25 μm) hence higher mass sensitivity compared to other column types [53]. Generally, OT-CEC fused silica columns are prepared by the derivatization of inner wall of the silica capillaries with silane reagent [54]. However, the surface area, hence the phase ratio of such a column is so low that it has low sample capacity. The phase ratio of OT-CEC columns have been increased by two major approaches including etching the inner surface [55, 56] and coating the column with a porous layer [57-59]. Although many techniques have been studied to increase the surface area and sample capacity, these columns still need higher phase ratio.

1.2.4.2 Packed Columns

Capillary electrochromatography columns are typically packed by using 3-5 μm silica based packing materials. In these columns the electrical double layer forms on the particle and the wall of the column. Silanol groups on the surface of silica particles ionize and generate EOF. Since there is no back pressure, the particles of packed CEC columns can be smaller than that of liquid chromatography columns. On the other hand, double layer overlap which can decrease the EOF limits the minimum size of the particles. The minimum particle size is in between 0.08-0.8 μm [53].

Packing of the capillary columns can be performed by several techniques. These techniques include solvent slurry packing, supercritical CO_2 slurry packing, electrokinetic packing and packing by centripetal forces. A study to compare these techniques were performed by Maloney and Colon [60] and showed that the columns packed by using these four techniques displayed similar performances and efficiencies of 270.000-300.000 plates/m under the same CEC conditions.

The packed columns require end fittings to keep the packing material within the column. Therefore the preparation of these columns includes fabrication of end frits and filling of silica based material into the capillary as illustrated in Figure 6. In the fabrication process, first, the end fitting is prepared by sintering a small amount of silica within the column. The silica is packed into the capillary and a second end frit is formed by sintering. Then the excess packing material is flushed out.

Due to the packing materials, these columns provide high surface area and sample capacity compared to OT-CEC columns. Since the packed columns with right material and packing technique can provide high performance, they have been widely used [61]. On the other hand, especially the fabrication of the end fittings in the preparation process may cause technical problems. The heat application for the formation of fitting changes the properties of the packing material. Also the surface chemistry difference at the frits can lead to bubble formation in the column. Due to these disadvantages of the end fittings, different column technologies have been developed to eliminate the use of end fittings.

1.2.4.3 Monolithic Columns

Monolithic columns are continuous macroporous solids bound to capillary wall which are prepared by in-situ polymerization [62]. In the recent years there has been significant progress in the development of macroporous monolithic materials for capillary electrochromatography (CEC). Since the problems of preparation of packed capillary columns for these systems such as inhomogeneous packing or bubble formation can be reduced by monolithic columns [63], they are very good alternatives to the conventional packed columns. Monolithic columns offer various advantages over the packed columns. The flow property of a liquid in monolithic column is totally different than that of the packed columns. The interparticular voids in the packed columns cause slower mass transfer because of diffusion between these voids and the pores within the particles [64]. On the other hand, the liquid in the monolithic column flows through the continuous phase of the macroporous material.

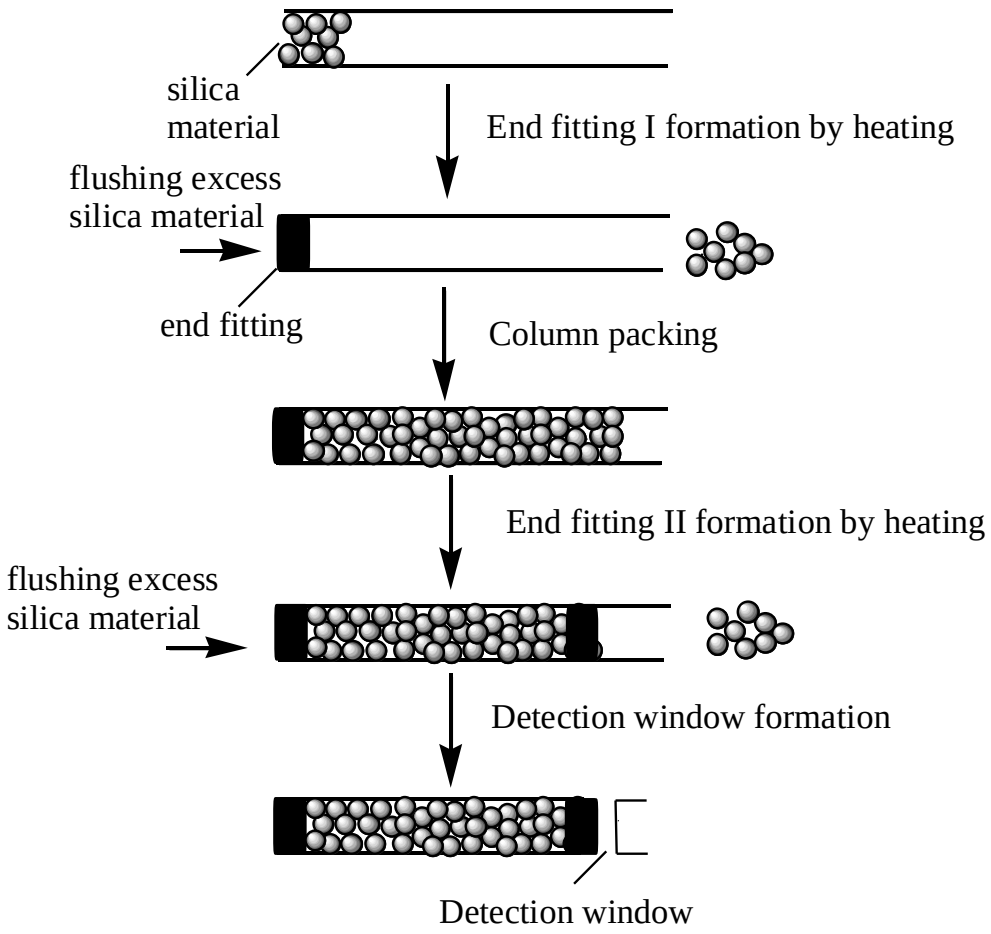


Figure 6 CEC column packing process.

The monoliths allow high flow velocities and superior mass transport at low back pressure due to their large pores and more open pore structure in comparison to packed columns [65, 66].As a consequence, they provide high permeability and fast separation. Moreover, since they bind to the wall of capillaries during in situ polymerization, they do not require any retaining frits that lead to inhomogenity in the separation media [67]. Another advantage of monolithic columns is the easy of the morphology control to optimize the

chromatographic performance. The morphology of monolithic CEC columns is a very important parameter in order to control the flow velocity, efficiency and selectivity of the monolith [46]. Many studies on the effect of pore structure of the monolits on chromatographic efficiency have been performed [68-70]. Ideally, a monolith should be a highly porous material that have interconnected large pores to facilitate flow [64]. In the conventional macroporous polymers, the porous structure is formed by the porogen which is a solvating or non-solvating inert solvent for the polymer. In addition to the type of porogen, type and percentage of monomer, crosslinker and initiator and polymerization temperature control the network structure and the porosity of these polymers.

Polymeric monolith columns are widely used to prepare for CEC applications. As shown in Figure 7, the preparation process of monolithic columns includes (1) functionalization of the capillary wall by the reaction of silanol groups with 3-(trimethoxysilyl)propyl methacrylate whose methacrylate parts then inserted into the monolith; (2) filling the capillary with the mixture of the monomer, crosslinker, initiator and a progen; (3) initiation of the in-situ polymerization reaction within the capillary by thermally or radiation; (4) removal of the unreacted species from the resultant monolithic polymer.

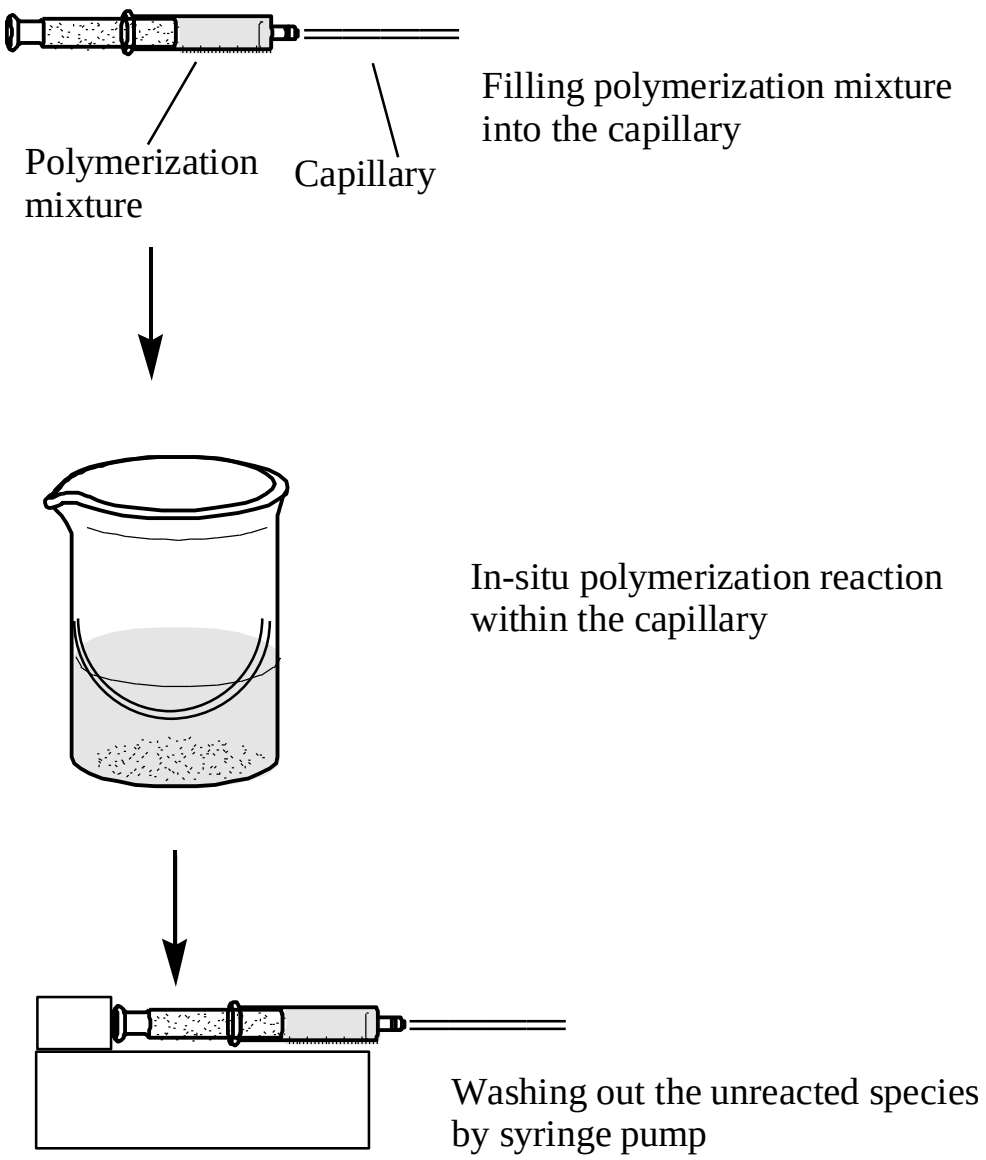


Figure 7 Monolithic column preparation process.

1.3 The Aim of This Study

The aim of the first part of this study was to develop new highly porous materials that have open-cellular structures with various mechanical characteristics by the emulsion templating technique by using hydrophobic acrylic monomers of stearyl acrylate, isodecyl acrylate, isobornyl methacrylate and divinylbenzene. The resultant materials which are called as high internal phase emulsion polymers (polyHIPEs) are promising for a wide range of applications that require highly porous structures with different mechanical properties changing from ductile to elastomeric. The morphology, density, porosity and the mechanical properties of the developed materials were investigated and compared with the styrene based conventional ones.

The aim of the second part of this study was to evaluate the most suitable HIPE structure as a stationary phase for capillary electrochromatography for the first time in literature. For this purpose, a novel stationary phase was prepared by in-situ polymerization of the continous phase of a HIPE containing isodecylacrylate and divinylbenzene. The resultant polyHIPE capillary monolith was studied with respect to influence of organic modifier concentration, pH and voltage in the electrochromatographic separation of neutral molecules.

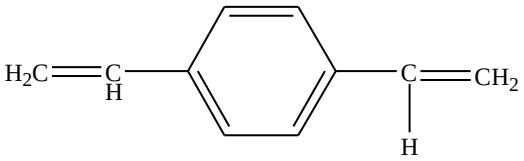
CHAPTER 2

2. EXPERIMENTAL

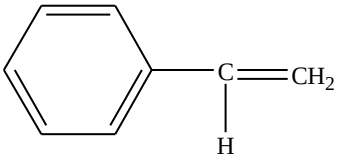
2.1 High Internal Phase Emulsion Polymer Preparation

2.1.1 Materials for High Internal Phase Emulsion Polymer Preparation

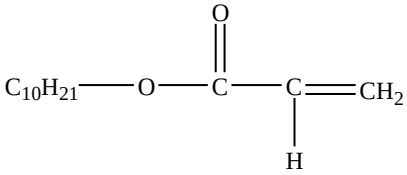
The monomers used for polyHIPE synthesis were styrene (ST) (99%, Merck), divinylbenzene (DVB) (80% Fluka), isobornyl methacrylate (IBMA) (Aldrich), isodecyl acrylate (IDA) (Aldrich) and stearyl acrylate (SA) (Aldrich). DVB and styrene were purified by distillation under vacuum and IBMA, IDA and SA were purified by using inhibitor remover replacement packing for hydroquinone and hydroquinone monomethyl ether (Aldrich) prior to use to remove inhibitors.The surfactant sorbitan monooleate (Span 80, Fluka), potassium peroxodisulfate (98%, Merck) and calcium chloride dihydrate (99%, Riedel) were used as supplied. Chemical structures of the monomers used are listed in Figure 8.



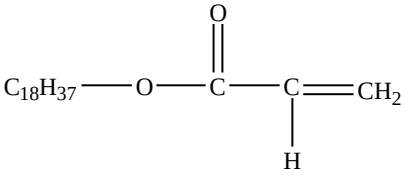
Divinylbenzene (DVB)



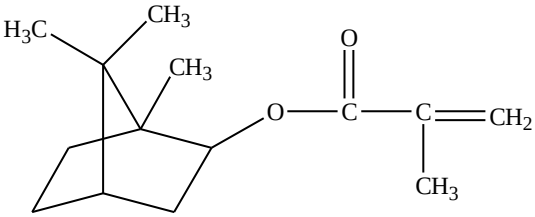
Styrene (ST)



Isodecyl acrylate (IDA)



Stearyl acrylate (SA)



Isobornyl methacrylate (IBMA)

Figure 8 Structures of the monomers used.

2.1.2 Method for High Internal Phase Emulsion Polymer Preparation

The polyHIPE copolymer compositions prepared in this study are listed in Table 1. The materials produced were 90 % porous based on aqueous phase content. The organic phase comprised monomer I, the crosslinker DVB and the surfactant Span 80 (20 vol % relative to total monomer volume). The aqueous phase involved potasium persulfate (0.2 wt %) and $CaCl_2.2H_2O$ (1 wt %). The high internal phase emulsion (HIPE) was prepared by mixing organic phase at 320 rpm while the liquid phase was added slowly. After all aqueous phase has been added, the emulsion was stirred for an additional 15 minutes. The resulting HIPE was then placed in a water bath at 60^0C for 48 hour. After the polymerization was complete, the product was removed from the beaker and washed in a Soxleth apparatus with ethanol for 16 hours. Then the polymer was dried in a vacuum oven to constant mass.

All styrene and acrylic based PolyHIPEs were prepared at 20, 40 or 80% crosslinker DVB into the reaction medium.

Table 1 The formulations of the polyHIPEs prepared in this study.

Poly(HIPE)	Monomer I	Monomer II
Poly(DVB)	-	DVB (100%)
Poly(ST-co-DVB)[1]	ST (20%)	DVB (80%)
Poly(ST-co-DVB)[2]	ST (60%)	DVB (40%)
Poly(ST-co-DVB)[3]	ST (80%)	DVB (20%)
Poly(SA-co-DVB)[1]	SA (20%)	DVB (80%)
Poly(SA-co-DVB)[2]	SA (60%)	DVB (40%)
Poly(SA-co-DVB)[3]	SA (80%)	DVB (20%)
Poly(IDA-co-DVB)[1]	IDA (20%)	DVB (80%)
Poly(IDA-co-DVB)[2]	IDA (60%)	DVB (40%)
Poly(IDA-co-DVB)[3]	IDA (80%)	DVB (20%)
Poly(IBMA-co-DVB)[1]	IBMA (20%)	DVB (80%)
Poly(IBMA-co-DVB)[2]	IBMA (60%)	DVB (40%)
Poly(IBMA-co-DVB)[3]	IBMA (80%)	DVB (20%)

2.2 Polymer Characterization Methods

2.2.1 SEM Analysis

Scanning electron microscopy (SEM) analysis was performed to characterize the resulting polyHIPE materials and to see the effect of monomer composition on morphology. The polymer samples were examined by SEM JEOL 6400 Model scanning electron microscope. For this purpose, the cryogenic fracture surfaces were coated with gold and their microphotographs were obtained at magnifications of 750 x and 1500 x. Average void and interconnecting window diameters were detected from these micrographs. For each sample at least 20 voides and 40 windows were measured to calculate the average values.

2.2.2 Density Measurements

The densities of the samples were determined from the weight and the volume measurements. Five samples were analysed for each composition to obtain a statistically acceptable data.

2.2.3 Surface Area Measurements

The surface area of the polyHIPE samples was determined from nitrogen adsorption isotherms applying the Brunauer-Emmet-Teller (BET) model. The measurements were performed using a Quantachrome Autosorb 1 Automated

Gas Sorption System. Surface area measurements utilized seven points adsorption isotherm collected over 0.05 to 0.3 P/Po.

2.2.4 Mercury Porosimetry Analysis

Mercury porosimetry analysis was performed by using Quantachrome Corporation Poremaster 60. Samples were autogassed under vacuum before analysis. The intrusion mercury contact angle was 140^{o}. The pressure range was 0.2 PSI to 55000 PSI. The measurements were performed according to scan mode.

2.2.5 Mechanical Characterization

The mechanical properties of polyHIPEs were characterized by compression tests using a Lloyd Instuments (LRX5K) equiped with 100 N and 5 kN load cell. For this purpose, cubic samples (10x10x10 mm^3) were loaded at a rate of 0.5 mm/min. The elastic modulus values were determined from the initial linear slopes of the stres-strain plots.

2.3 High Internal Phase Emulsion Monolith Column Preparation

2.3.1 Materials for High Internal Phase Emulsion Monolith Column

The polyimide coated fused silica capillary with 100-μm I.D. and 360-μm O.D. was purchased from Polymicro Technologies (Phoenix, AZ, USA). The derivatization agent 3-(trimethoxysilyl)propyl methacrylate was obtained from Aldrich. The monomers used for polyHIPE synthesis were divinylbenzene (DVB) (80 % Fluka) and isodecyl acrylate (IDA) (Aldrich). DVB was purified by distillation under vacuum and IDA was purified by using inhibitor remover replacement packing for hydroquinone and hydroquinone monomethyl ether (Aldrich). The surfactant sorbitan monooleate (Span 80, Fluka), potassium peroxodisulfate (98 %, Merck) and calcium chloride dihydrate (99 %, Riedel), acetonitrile (99.9 %, Sigma-Aldrich), ethanol (99.8 % Riedel) were used as supplied.

2.3.2 Preparation of The Column

Before the preparation of monolithic column, the pretreatment and vinylization of the inner wall of a fused silica capillary tubing was carried out by following procedure: First, to increase the surface density of silanol groups on the inner wall, the capillary tubing 40 cm in length was washed with 0.2 M NaOH for 1.5 h, followed with distilled water for 30 min and methanol for 30 min at room temperature. Thereafter, the capillary was purged with nitrogen for 30 min.

Finally 3-(trimethoxysilyl)propyl methacrylate – methanol solution (50/50, v/v) was flushed through the capillary for 20 min. This tubing filled with this solution was sealed by rubber septum and then kept in a water bath at 35 ^{o}C for 20 h. Then it was washed with methanol for 10 min. and dried by nitrogen for 30 min. So that vinyl bonds were created on the surface of the inner wall of the capillary. These vinyl bonds provided covalent attachment of the monolith to the capillary wall during in-suti polymerization reaction.

The HIPE produced for the column preparation was 90 % porous based on aqueous phase content. The organic phase of the emulsion comprised isodecyl acrylate (IDA) (80 vol %), the crosslinker DVB (20 vol %), the surfactant Span 80 (20 vol % relative to total monomer volume) and the aqueous phase involved potasium persulfate (0.2 wt %) and $CaCl_2.2H_2O$ (1 wt %). Both phases were purged with nitrogen for 15 min. The HIPE was prepared by mixing organic phase at 320 rpm while the liquid phase was added slowly. After all aqueous phase has been added, the emulsion was stirred for 45 min. Then the HIPE was filled into the capillary of 40 cm length by applying an external pressure of 2.5 bar in the CEC system. This emulsion within the capillary was kept in a water bath at 60^{o}C for 48 h. Poly(IDA-co-DVB)[3] monolith had a 28 cm length in the capillary. The monolithic column was connected to a liquid chromatography pump to wash with absolute ethanol (2 h) then with distilled water (1 h) and acetonitrile (1 h) at the flow rate of 2 µL/min. The detection window was opened by burning off the 2-3 mm of coating at the end of polymer bed during ethanol wash. The column was flushed with ethanol before and after opening the detection window. Prior to the connection of the capillary to the CEC system, the column was equilibrated by acetonitrile/5 mM phosphate buffer (60/40, v/v) at the flow rate of 3 µL/min overnight.

2.4 Monolith Column Characterization Methods

2.4.1 SEM Analysis

Scanning electron microscopy (SEM) analysis was performed to characterize the resulting monolith structure by SEM JEOL 6400 Model. For this purpose, surfaces were coated with gold and their microphotographs were obtained. The internal structure micrograph at a magnification of 750 x was used to calculate average void and interconnecting window diameters. At least 20 voides and 40 windows were measured to calculate the average value.

2.5 Electrochromatography

Electrochromatographic experiments were performed by using Prince CEC 760 model capillary electrochromatograpy system equipped with photodiode array detector. To investigate the ability of the column as stationary phase, the column was connected to CEC system and equilibrated with ACN/5mM phosphate buffer solution (60/40, v/v) at pH 7 by applying a voltage of 10 kV and an external pressure of 2 bar at the inlet of the column for 30 min. Then it was equilibrated further by applying a voltage of 20 kV without any external pressure for 15 min. A mixture of alkylbenzene derivatives including thiourea, benzene, toluene, ethylbenzene, propylbenzene and butylbenzene was used as sample for separation studies. The sample mixture was prepared at a concentration of 1% (v/v) in ACN/buffer solution (80/20, v/v). The detection wavelength was 200 nm. The electrochromatographic separations were carried

out by using various volume fractions of ACN in mobile phase and different voltages from 10 to 30 kV.

The electrophoretic mobility, μ_{EOF}, was obtained by the following equation 3 where L_t was the total length of the column, L_e the distance from inlet to the detection point, V the applied voltage and t_R retention time of the unretained analyte, thiourea.

$$\mu_{EOF} = L_e L_t / V t_R \quad (3)$$

The theoretical plate number (N) and the plate height (h) were calculated by the Equation 4 and Equation 5 respectively. In Equation 4, t_r and t_w are the retention time and the peak width at half-height, respectively.

$$N = 5.54(t_r/t_w)^2 \quad (4)$$

$$h = L / N \quad (5)$$

The retention factor (k) was calculated by the following Equation 6 where t was the retention time of analyte and t_0 was the retention time of thiourea.

$$k = (t-t_0) / t_0 \quad (6)$$

CHAPTER 3

3. RESULTS AND DISCUSSION

3.1 Morphology

The foam constructions of polyHIPEs structures were confirmed by SEM analysis (Figures 9-12). SEM microphotographs showed that all the foams have the cellular structures similar to classic styrene and DVB based polyHIPEs. Samples were highly porous with regular micron size voids that were all interconnected to each other through the windows.

As seen from the figures, the cellular structure of the polyHIPEs changed depending on the chemical composition. The differences between the structures are the results of the nature of the acrylic monomers and the crosslinking ratio. The quantitative characterization of the polyHIPEs by average void diameter (D), window diameter (d) and degree of interconnection (d/D) was performed by using SEM micrographs and the obtained results are given in Table 2.

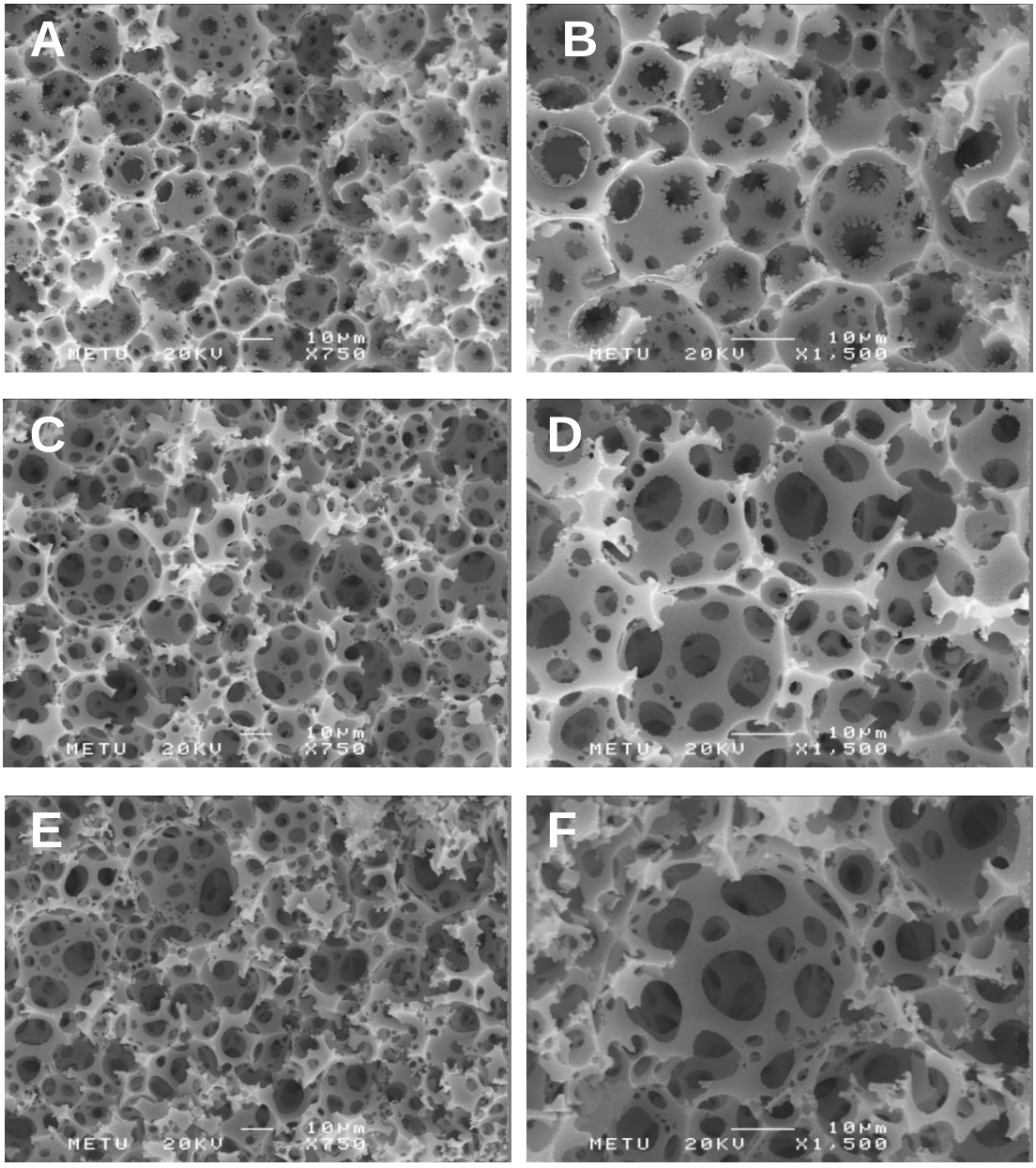


Figure 9 SEM of (A,B) poly(ST-co-DVB)^{1}, (C,D) poly(ST-co-DVB)^{2}, (E,F) poly(ST-co-DVB)^{3}.

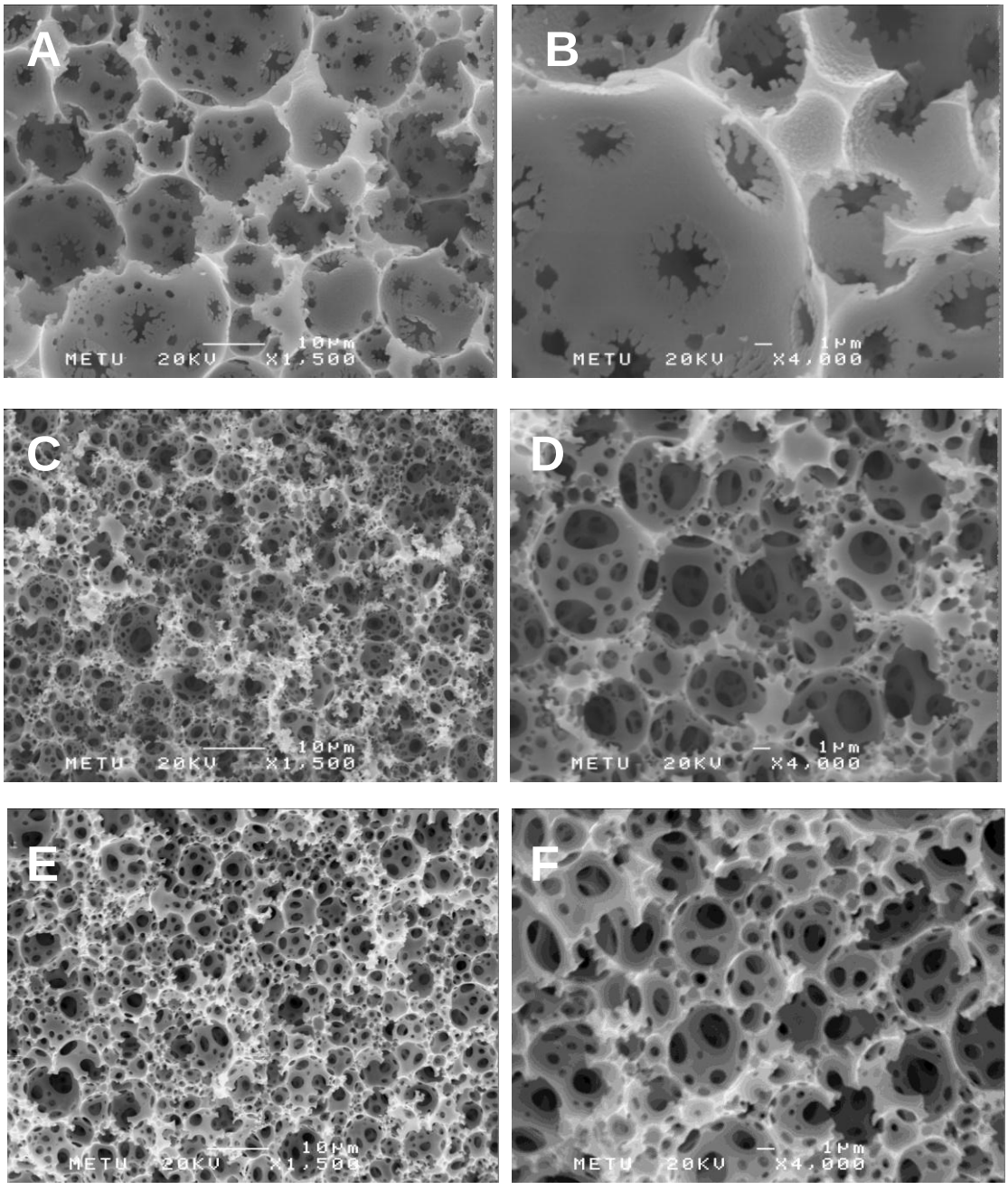


Figure 10 SEM of (A, B) poly(SA-co-DVB)[1], (C, D) poly(SA-co-DVB)[2], (E, F) poly(SA-co-DVB)[3].

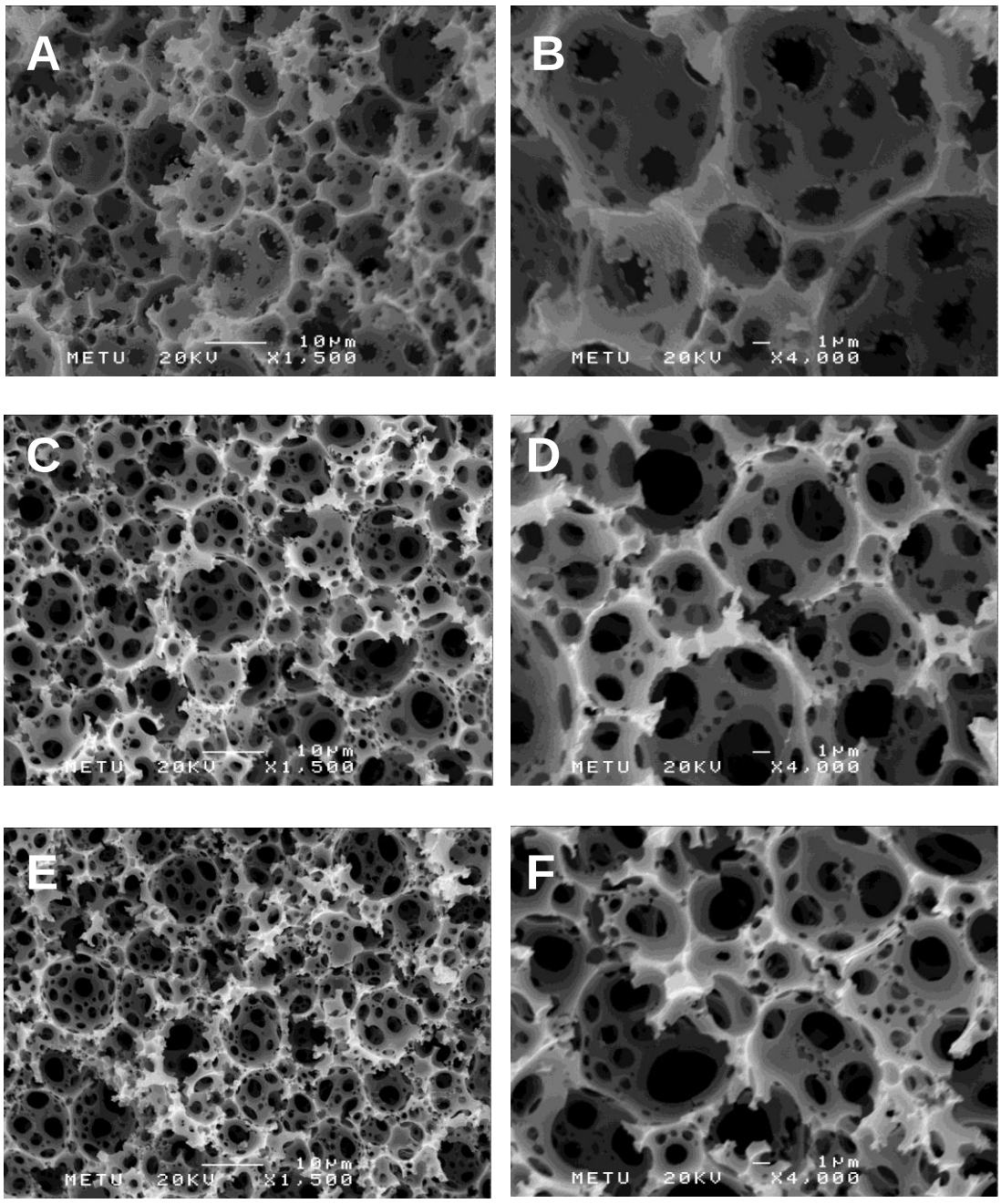


Figure 11 SEM of a) poly(IDA-co-DVB)[1], b) poly(IDA-co-DVB)[2], c) poly(IDA-co-DVB)[3].

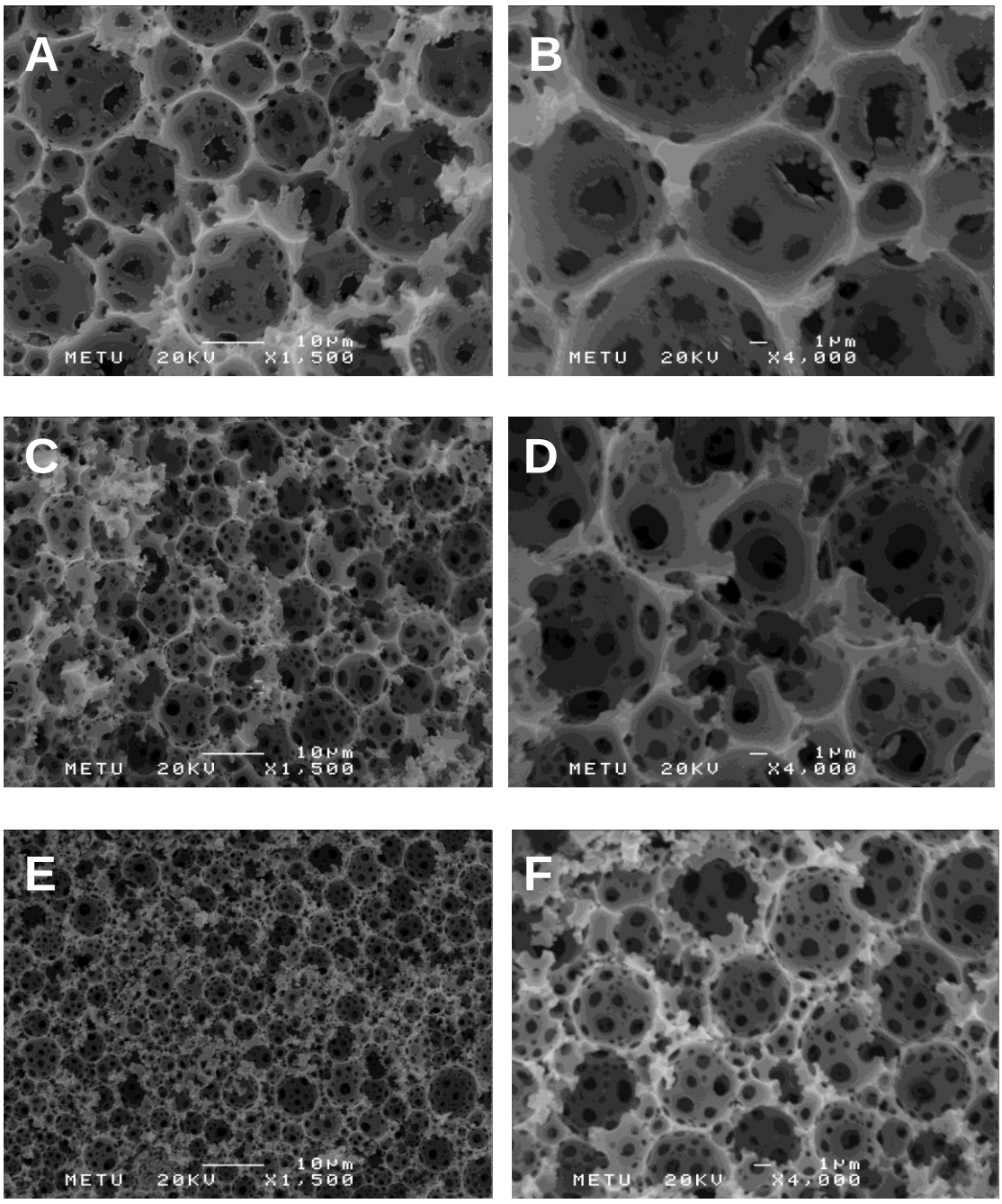


Figure 12 SEM of (A, B) poly(IBMA-co-DVB)[1], (C, D) poly(IBMA-co-DVB)[2], (E, F) poly(IBMA-co-DVB)[3].

When the hydrophobic acrylic monomers were used as comonomer, the resultant polyHIPEs had smaller void diameters than those prepared from styrene and DVB. The styrene based foams have spherical voids in the range of 15.0-20.5 μm with circular windows which have diameters of 3.7-5.5 μm. On the other hand, the spherical void diameter of SA, IDA and IBMA based foams were obtained in the ranges of 5.9-13.8 μm, 8.7-8.9 μm and 5.2-12.9 μm, respectively. Besides the interconnecting window diameters of acrylic based polyHIPEs were smaller than that of styrene based ones.

In this emulsion templating technique, hydrophobicity of the organic phase is very important in terms of the emulsion stability which affect the void diameter in polyHIPE structure. Increased emulsion stability results in a smaller average droplet size, because of lower interfacial tension. Increasing emulsion stability also reduces the tendency of the emulsion for Ostwald repining, coalescence of small droplets into larger ones. A suggestion for this behavior also came from a study by Barbetta et.al. [71]. They reported that when another hydrophobic monomer, 4-vinylbenzyl chloride was used instead of styrene, the resultant polyHIPE composed of 4-vinylbenzyl chloride and DVB had smaller void diameters than the polyHIPE composed of styrene and DVB. This effect of 4-vinylbenzyl chloride on void size was explained by its co-surfactant behaviour in the system. It was also observed from the SEM micrographs that, the void diameters become more uniform with increasing acrylic monomer in acrylic based foams because of increasing emulsion stability.

Table 2 Void diameters (D), interconnecting window diameters (d), and degree of interconnection (d/D) of polyHIPEs.

Poly(HIPE)	**D (μm)**	**d (μm)**	**d/D**
Poly(ST-co-DVB)[1]	15.0 ± 4.8	3.7 ± 1.8	0.25
Poly(ST-co-DVB)[2]	19.2 ± 5.6	6.1 ± 2.1	0.32
Poly(ST-co-DVB)[3]	20.5 ± 8.3	5.5 ± 2.7	0.27
Poly(SA-co-DVB)[1]	13.8 ± 6.4	1.7 ± 0.7	0.12
Poly(SA-co-DVB)[2]	6.4 ± 1.6	1.6 ± 0.8	0.25
Poly(SA-co-DVB)[3]	5.9 ± 1.8	1.7 ± 0.7	0.29
Poly(IDA-co-DVB)[1]	8.7 ± 3.8	1.9 ± 0.8	0.22
Poly(IDA-co-DVB)[2]	8.9 ± 3.3	2.6 ± 1.1	0.29
Poly(IDA-co-DVB)[3]	8.9 ± 2.7	2.0 ± 0.9	0.22
Poly(IBMA-co-DVB)[1]	12.9 ± 4.7	1.8 ± 0.6	0.14
Poly(IBMA-co-DVB)[2]	8.3 ± 1.9	1.4 ± 0.6	0.17
Poly(IBMA-co-DVB)[3]	5.2 ± 1.1	0.9 ± 0.4	0.17

A similar result was reported in a previous study by Tai et al.[72]. They prepared HIPE copolymer from 2-ethylhexyl acrylate, styrene and divinylbenzene and observed that increase in styrene content made the cell diameters of the HIPE polymer more uniform because of increasing monomer hydrophobicity. The cellular structure of different compositions of the poly(ST-co-DVB) foams (Figure 9) changed in a similar way. DVB is more hydrophobic than styrene, and therefore the HIPE with higher DVB content produced foams with smaller voids than that of the foams with lower DVB content.

Degree of interconnection (d/D) gives information about interfacial tension of the emulsion prior to polymerization of the continuous phase [73]. A high ratio of d/D means large interconnecting windows relative to voids in the structure. Therefore degree of interconnection gives important information about the mechanical properties of such materials. The degree of interconnection was in the range of 0.25-0.32 for ST based foams, 0.12-0.29 for SA based foams, 0.22-0.29 for IDA based and 0.14-0.17 for IBMA based foams. It was clearly seen that IBMA based polyHIPEs had lower interconnection compared to the other polyHIPEs probably due to the higher emulsion stability of the IBMA based emulsions.

3.2 Density, Surface Area and Porosity

The densities and surface area values of the polyHIPEs are listed in Table 3. Since the void volume ratio of the polyHIPEs studied in this study was 90%, they had the densities of about 0.1 g/cm^3. The lowest value was obtained for poly(ST-co-DVB)^3 as 0.084 while the highest value was obtained for poly(IBMA-co-DVB)^3 as 0.160 g/cm^3. The higher density values of the IBMA

based polyHIPEs was explained by the higher density of IBMA compared to the other acrylic monomers used in this study.

Although the polyHIPE materials are highly porous, they have low surface area values in the range of 3-20 m^2g^{-1} due to large void size in their structure [27]. Cameron et.al. reported that surface area of polyHIPEs could be increased by replacing some of the monomers with a porogenic solvent. With this technique, it became possible to increase the surface area of poly(DVB) foam up to 554 m^2g^{-1} [28].

The surface area values of the polyHIPE samples prepared in this study were determined from nitrogen adsorption isotherms applying the Brunauer-Emmet-Teller (BET) model and the results are given in Table 3. The lowest surface area was obtained for poly(SA-co-DVB)^3 as 4.29 m^2/g while the highest surface area was obtained for poly(SA-co-DVB)^1 as 61.00 m^2/g. Differences in the surface area data of different compositions of polyHIPEs confirm the idea that the surface area depends on the nature and composition of the continuous phase of the emulsion, as expected. The data showed that when the crosslinker ratio was decreased, the surface area also decreased. The reason of this trend is explained by the decrease in the porosity generated within the walls of the polyHIPEs. In vinyl/divinyl copolymers, the pores (< 2nm) arise from spaces between nuclei which are highly crosslinked particles forming spheres which are about 100 nm in diameter. Mesopores in the range of 2-50 nm appear between these spheres whose aggregates form in mesopores and macropores (>50 nm) [74]. Increasing crosslinker content

increases stiffness and decreases the size of nuclei and thus increases the surface area which is determined mainly by the surface of nuclei.

On the other hand, the differences between the surface areas of the polyHIPEs prepared from different monomers could not be explained in a simple fashion, because the solubility parameters of the acrylic monomers used in this study are very close to each other.

Pore sizes in the range of 0.0036–200 μm were analyzed by mercury porosimetry and plots of logarithm of differential intrusion (dV/dlog(d)) where V was the volume of mercury intruded into the pores vs diameter are given in Figures 13-16. The results showed the presence of two groups of size distributions. One group was in the range of 0.1–0.005 μm and the other group was in the range of 2–20 μm. The pore size distribution of poly(IDA-co-DVB)[3] (Figure 15) had an unexpectedly significant peak for pore size at 0.1–5 μm. This peak was thought to be a consequence of some collapse in the structure because of high pressure of mercury during analysis. The pore sizes derived from mercury intrusion porosimetry and SEM analysis were different probably due to the measuring drawbacks in mercury intrusion porosimetry method.

Table 3 Surface area and density data of the polyHIPEs.

PolyHIPEs	**Density (g/cm^3)**	**Surface Area (m^2/g)**
Poly(DVB)	0.104 ± 0.039	33.51
Poly(ST-co-DVB)[1]	0.103 ± 0.010	43.03
Poly(ST-co-DVB)[2]	0.088 ± 0.010	26.52
Poly(ST-co-DVB)[3]	0.084 ± 0.004	19.99
Poly(SA-co-DVB)[1]	0.102 ± 0.005	61.00
Poly(SA-co-DVB)[2]	0.110 ± 0.0015	51.88
Poly(SA-co-DVB)[3]	0.123 ± 0.002	4.29
Poly(IDA-co-DVB)[1]	0.107 ± 0.008	33.20
Poly(IDA-co-DVB)[2]	0.114 ± 0.001	10.78
Poly(IDA-co-DVB)[3]	0.120 ± 0.012	5.44
Poly(IBMA-co-DVB)[1]	0.112 ± 0.009	26.59
Poly(IBMA-co-DVB)[2]	0.117 ± 0.005	18.09
Poly(IBMA-co-DVB)[3]	0.160 ± 0.003	9.46

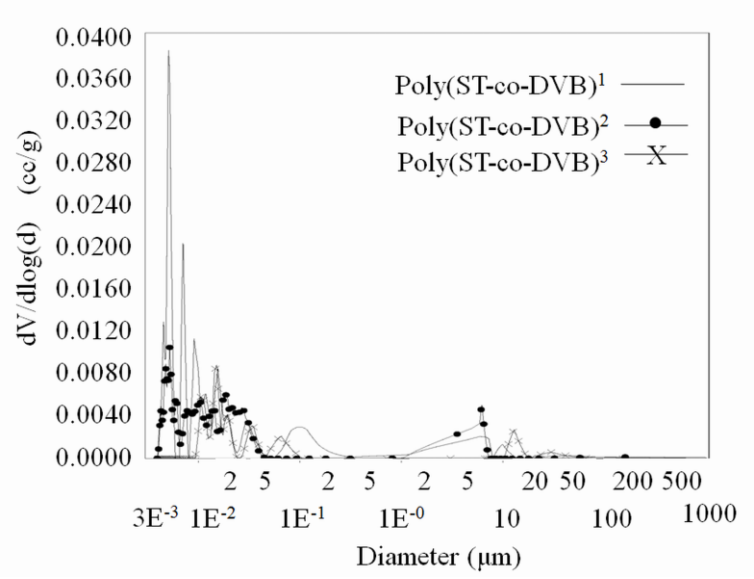


Figure 13 Pore size distribution of poly(ST-co-DVB)[1], poly(ST-co-DVB)[2] and poly(ST-co-DVB)[3].

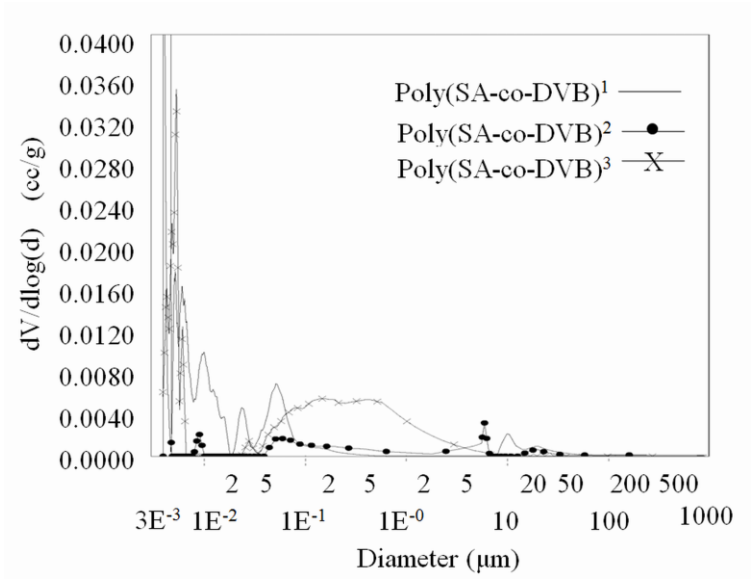


Figure 14 Pore size distribution of poly(SA-co-DVB)[1], poly(SA-co-DVB)[2] and poly(SA-co-DVB)[3].

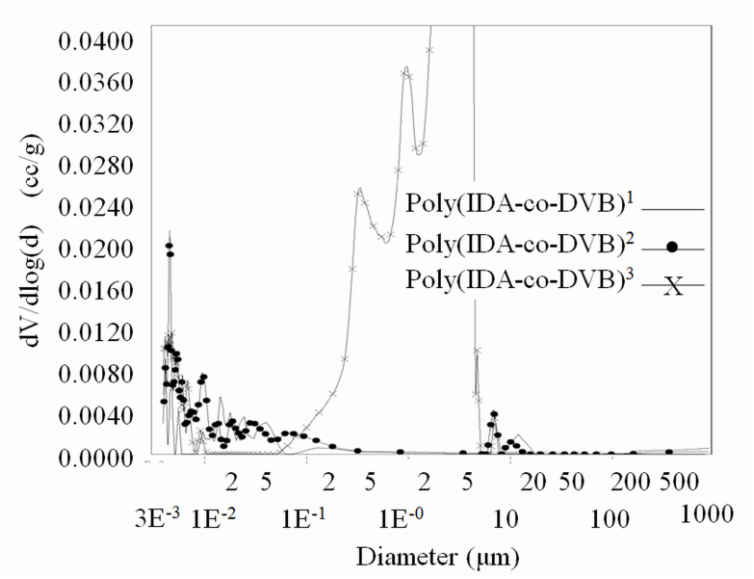


Figure 15 Pore size distribution of poly(IDA-co-DVB)[1], poly(IDA-co-DVB)[2] and poly(IDA-co-DVB)[3].

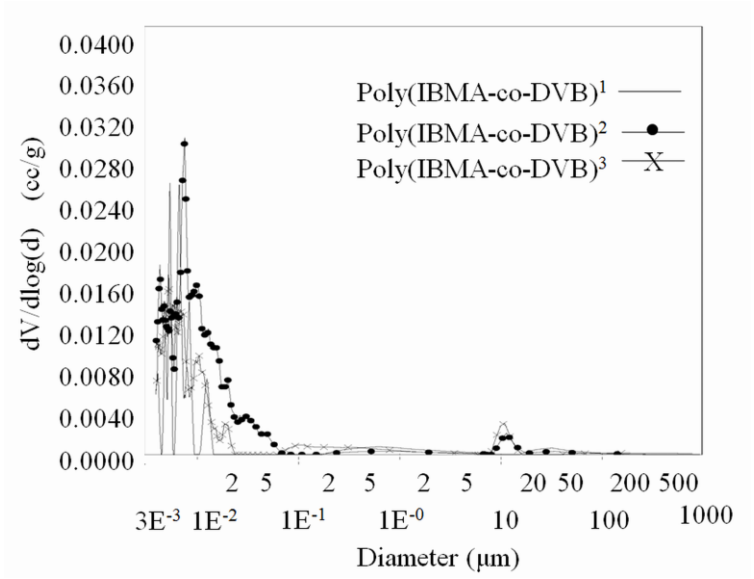


Figure 16 Pore size distribution of poly(IBMA-co-DVB)[1], poly(IBMA-co-DVB)[2] and poly(IBMA-co-DVB)[3].

3.3 Mechanical Properties

The poor mechanical property of the polyHIPEs is a major problem in many applications. Our aim was to synthesize new polyHIPEs with desired mechanical properties having high pore volume and interconnectivity. Mechanical properties of styrene and acrylic based polyHIPEs prepared in this study are given in Table 4 by ultimate compression strength (UCS), strain at break (SAB) and Young's modulus (E) data. Besides, Figures 17-20 show the representative stress versus strain plots of the polyHIPEs for compression tests.

Compression test results are given as stress versus strain plots for poly(ST-co-DVB) and poly(DVB) materials (Figure 17) and the graphs demonstrated an elastic and linear zone, with increasing stress level and then densification and larger strain caused the cell walls to break, finally the cells crushed. This behavior is the characteristic of ductile foams [75]. The deformation at high stress is unrecoverable for the ductile materials. For these samples, it was seen that increasing styrene content decreased the UCS and modulus values. While the UCS of poly(DVB) and its modulus were 0.805 MPa and 11.79 MPa, respectively, increasing the styrene percent to 80% at the constant organic phase volume, the UCS and modulus of the samples decreased to 0.476 MPa and 5.16 MPa, respectively. This tendency is probably due to the decreasing trend in density and increasing void interconnectivity in the structure with increasing styrene content (Figure 9). Relative low modulus and crush strength of polyHIPEs can be associated with high degree of interconnectivity affecting the structural integrity [29].

Table 4 Mechanical properties: ultimate compression strength (UCS), Young's modulus (E) and strain at break (SAB) of polyHIPEs.

Poly(HIPE)	**UCS (MPa)**	**E (MPa)**	**SAB (%)**
Poly(DVB)	0.805 ± 0.053	11.79 ± 1.62	10.64 ± 0.93
Poly(ST-co-DVB)[1]	0,778 ± 0.051	10.42 ± 0.21	10.66 ± 2.03
Poly(ST-co-DVB)[2]	0.611 ± 0.164	8.26 ± 1.45	11.67 ± 2.28
Poly(ST-co-DVB)[3]	0.476 ± 0.045	5.16 ± 0.21	15.81 ± 2.11
Poly(SA-co-DVB)[1]	0.517 ± 0.119	12.54 ± 1.05	7.78 ± 1.48
Poly(SA-co-DVB)[2]	-	2.45 ± 0.11	-
Poly(SA-co-DVB)[3]	-	0.33 ± 0.002	-
Poly(IDA-co-DVB)$_1$	0.769 ± 0.057	9.55 ± 1.14	11.34 ± 0.65
Poly(IDA-co-DVB)[2]	0.381 ± 0.014	3.98 ± 0.52	16.55 ± 1.69
Poly(IDA-co-DVB)[3]	-	0,13 ± 0.05	-
Poly(IBMA-co-DVB)[1]	0.854 ± 0.013	10.74 ± 1.51	10.87 ± 1.96
Poly(IBMA-co-DVB)[2]	0.971 ± 0.017	11.47 ± 0.72	13.89 ± 1.19
Poly(IBMA-co-DVB)[3]	1.048 ± 0.021	16.43 ± 1.44	8.60 ± 0.71

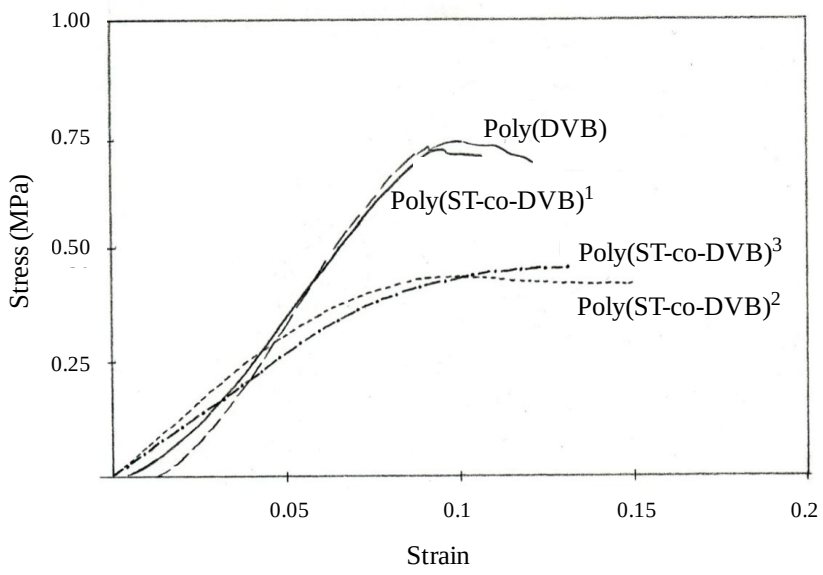


Figure 17 Stress vs strain plot of poly(ST-co-DVB)[1], poly(ST-co-DVB)[2] and poly(ST-co-DVB)[3].

The use of SA as comonomer with DVB at 20 % caused a decrease of the Young's modulus and UCS of poly(DVB). However, the poly(SA-co-DVB) samples including 60 and 80% SA in organic phase showed elastomeric behavior differently from the styrene based samples. The elastomeric materials can recover themselves with little or no hysteresis when the stress is removed [76]. In the stress vs. strain plots of these samples (Figure 18), there are a very small elastic linear zone and then a plateau region in which strain increases at nearly constant and after this plateau, there is a densification region where the slope increased significantly with strain. This behavior can be explained as follows. Bending and elastic buckling of the cell ribs give linear elasticity and the plateau and the contact between these ribs results in

densification. In elastomeric foams rib buckling is fully reversible, while in ductile foams, it is associated with permanent micro damage [75].

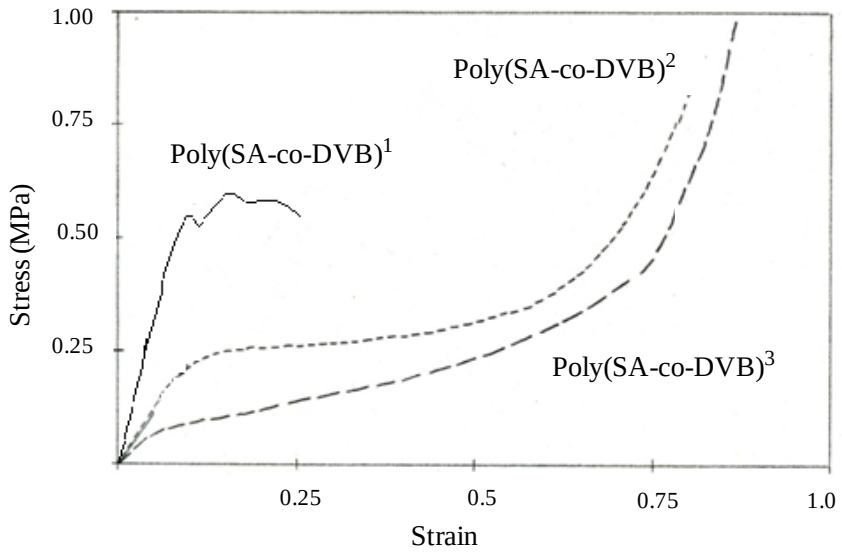


Figure 18 Stress vs strain plot of poly(SA-co-DVB)^1, poly(SA-co-DVB)^2 and poly(SA-co-DVB)^3.

A similar elastomeric behavior is also observed in poly(IDA-co-DVB)^3 sample including 80% IDA (Figure 19). However, lower content of IDA in the poly(IDA-co-DVB) samples showed a ductile behavior. They had lower Young's modulus and UCS when compared with the poly(ST-co-DVB) samples. Use of IDA at 60% at the constant organic phase volume decreased the UCS and modulus of the samples to 0.381 MPa and 3.98 MPa, respectively.

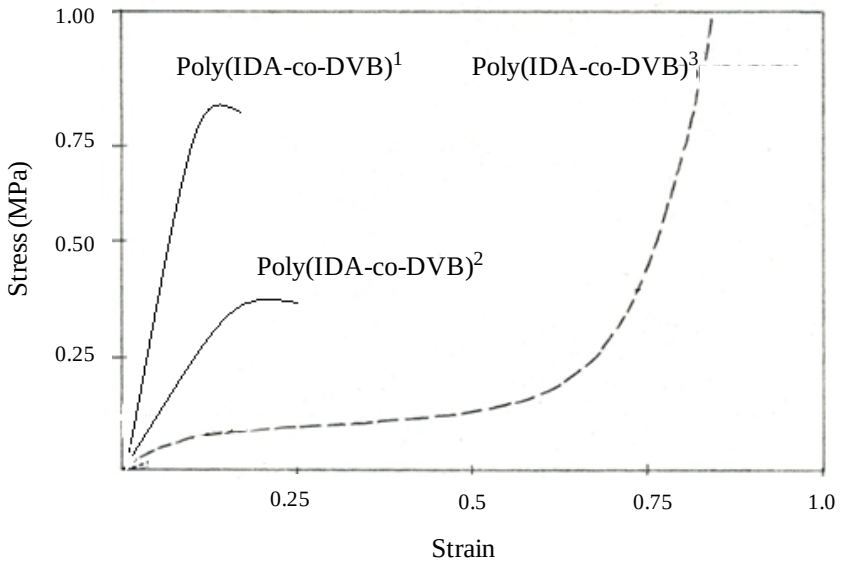


Figure 19 Stress vs strain plot of poly(IDA-co-DVB)[1], poly(IDA-co-DVB)[2] and poly(IDA-co-DVB)[3].

Ductile IBMA based polyHIPEs (Figure 20) had the highest Young's modulus which had the increase of 69% compared to styrene based polyHIPEs at 20% DVB content. This result can be associated with smaller void and window size, higher density and also narrower void size distribution compared to the other polyHIPEs. Besides there is a significant decrease in the interconnection degree (d/D) data of IBMA based polyHIPEs compared to the other polyHIPEs (Table 2). Low degree of interconnection means small interconnecting windows relative to the voids. Such a low interconnection makes the material tougher and stronger.

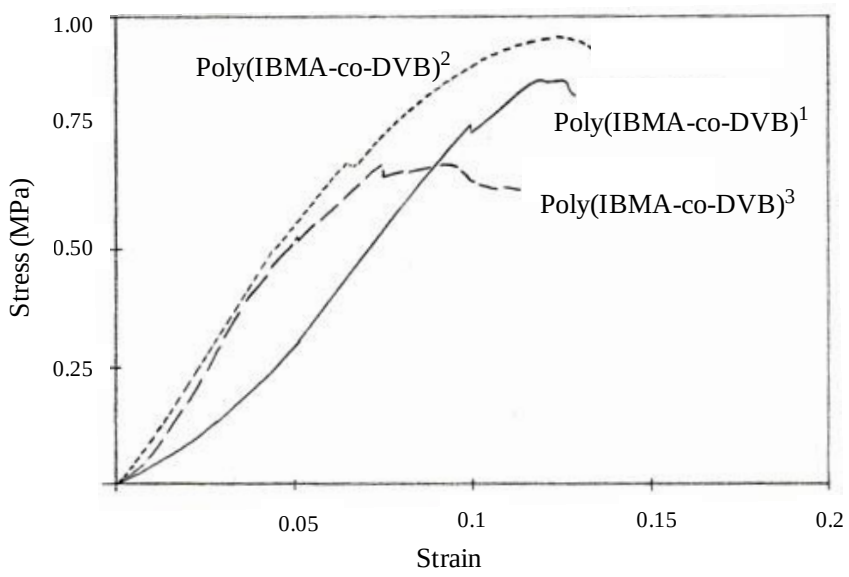


Figure 20 Stress vs strain plot of poly(IBMA-co-DVB)[1], poly(IBMA-co-DVB)[2] and poly(IBMA-co-DVB)[3].

Figure 21 shows that Young's modulus increases with increasing crosslinker content for SA and IDA based samples. Crosslinking degree determines the mechanical properties of polymers. It affects creep resistance, dimensional stability and mechanical strength linearly. Consequently, increasing Young's modulus with crosslinker ratio is an expected result. This is also a result of increase in window diameters and so interconnectivity of voids with decreasing DVB content. This trend can be seen clearly in the SEM micrographs of SA and IDA based polyHIPEs in Figure 10 and 11. Increasing interconnectivity with decreasing DVB in these structures decreases structural integrity and so Young's modulus values. Interestingly IBMA based samples displayed an opposite trend; the modulus of these samples decreased with increasing DVB content. This trend probably results from the dramatic decrease in void and

window size, increase in density and also narrow void size distribution in the structure of these polyHIPEs. Another important parameter that influence the mechanical bahaviour of the IBMA based polyHIPEs is the chemical structure of the IBMA monomer. As shown in Figure 8, it has a rigid cyclic side chain attached to the methacrylate group. This cyclic side chain close to the polymer backbone in the structure is expected to improve the mechanical property, particularly modulus, of these materials.

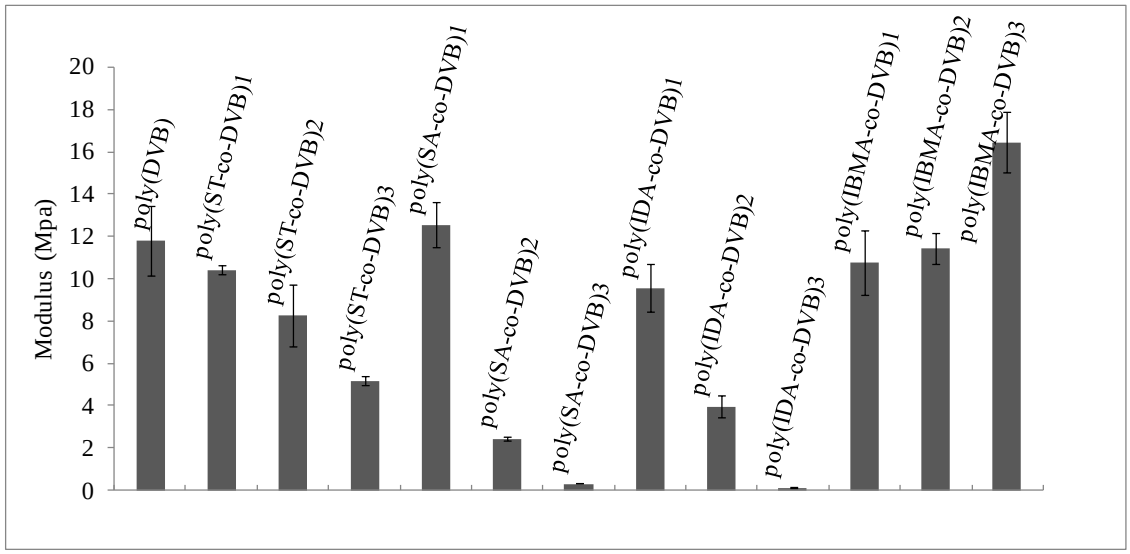


Figure 21 Young's modulus (E) of the polyHIPEs studied.

3.4 Development of a PolyHIPE Monolithic Column for Capillary Electrochromatography

HIPE polymers having high porosity, and interconnected open-cell structure, were developed as stationary phase for CEC for the first time. For this aim, a novel polyHIPE monolithic column was prepared by the in-situ polymerization of continous phase of a HIPE in a silica capillary. Poly(IDA-co-DVB)^3 was chosen due to its large voids and interconnecting windows and its elastomeric behavior. Poly(IDA-co-DVB)^3 monoliths were studied with respect to influence of organic modifier concentration, pH and voltage in the electrochromatographic separation of neutral molecules.

3.4.1 Characterization of Poly(IDA-co-DVB)^3 Monolithic Column

PolyHIPE structure of poly(IDA-co-DVB)^3 monolith in the capillary was confirmed by SEM analysis (Figure 22). SEM micrographs demonstrated that the monolith was well attached to the silica capillary. They also showed that the monolith had classic cellular structure of polyHIPEs. It was a highly porous material with regular micron size voids which were all interconnected through the windows. Due to the regular micron size voids and open pore structure of this 90 % porous monolith, it provides a significant advantage to provide a higher mass transfer in comparison to conventional macroporous monoliths. Another advantage of this monolith is its elastomeric behavior (Figure 19). An elastomeric polyHIPE has the advantage of that when a stress is applied to the monolith; it shows reversible elastic behavior without any permanent damage.

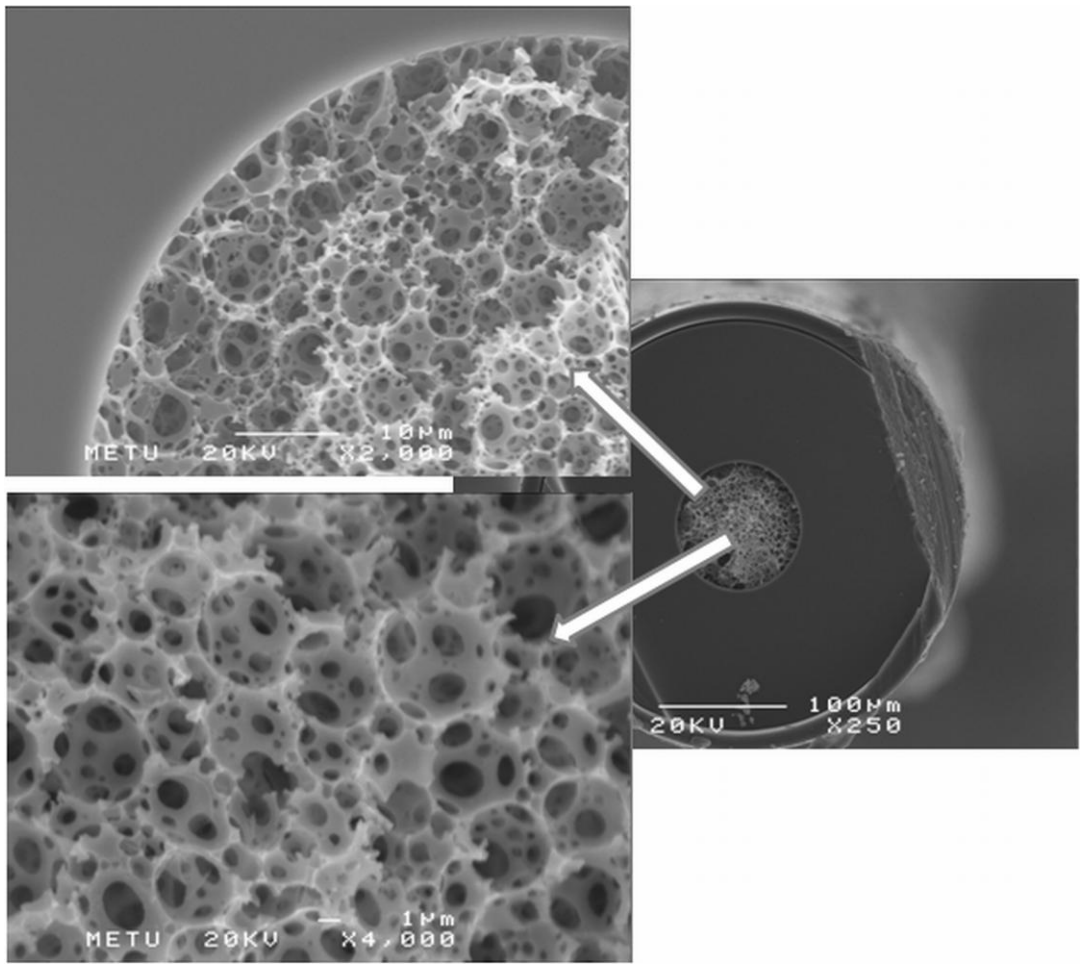


Figure 22 SEM microphotographs of poly(IDA-co-DVB) polyHIPE monolith.

The variation of back-pressure of the column with ethanol flow rate was determined in a μ-HPLC system and shown in Figure 23. As shown in the figure, the back pressure increased with increasing flow rate.

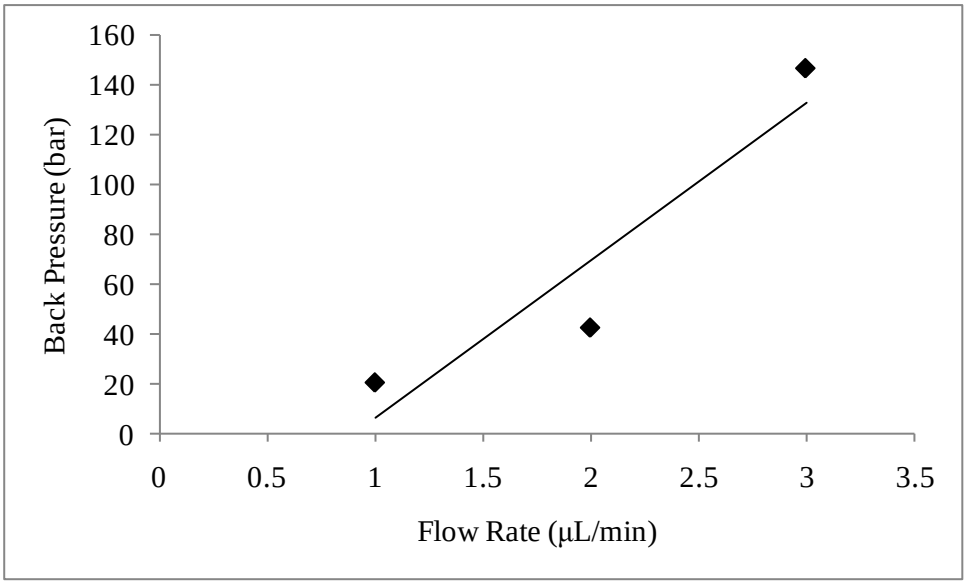


Figure 23 The effect of flow rate on back-pressure, mobile phase: ethanol, effective column length: 28cm, 100 μm id.

3.4.2 Capillary Electrochromatography

In CEC, the mobile phase and the analytes are driven by electroosmotic flow (EOF). The EOF velocity depends on zeta potential an so the charge density on the stationary phase surface [77]. Generally, an ionizable monomer such as 2-acrylamido-2-methylpropanesulfonic acid is used to generate EOF in the monolithic separation media used in CEC. It should be noted that the poly(IDA-co-DVB)^3 monolith did not require any EOF generating monomer which makes it superior than the other conventional monoliths prepared by using comonomers with strong ionizable groups. The reason is probably that the presence of ionizable sulphate groups generated by the decomposition of water-soluble initiator ($K_2S_2O_8$) tended to be on the surface of the material

which surrounded by the dispersed aqueous phase. These groups probably provide sufficient surface charge to generate the EOF.

3.4.2.1 Effect of pH Value on EOF

The influence of pH on electrophoretic mobility was studied and no appreciable change was observed in the pH range of 5-10 as seen in Figure 24. This result indicates that the monolith provides sufficient EOF over a wide pH range.

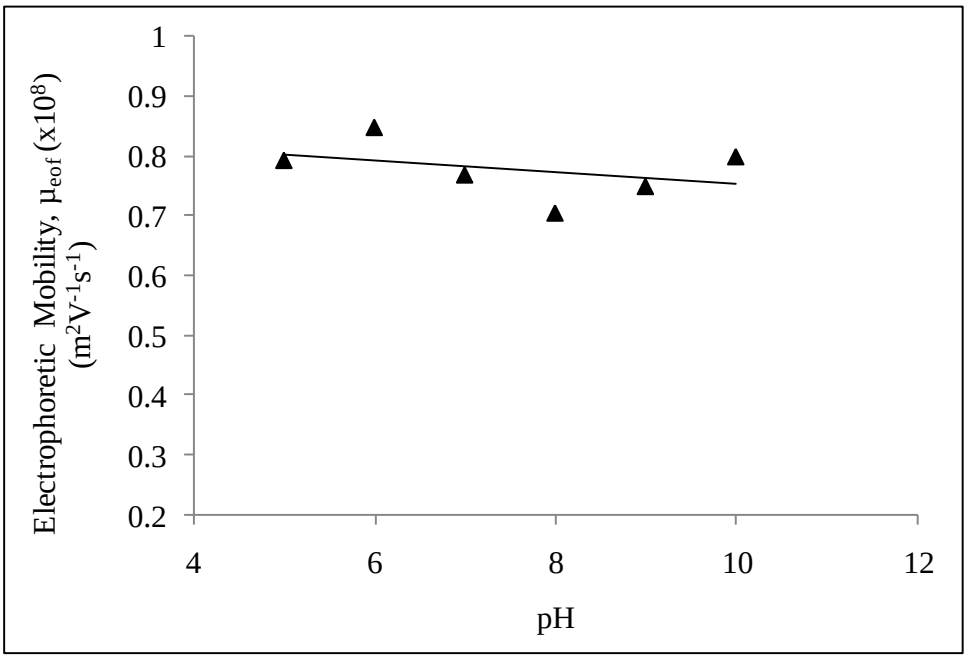


Figure 24 The variation of electrophoretic mobility with pH, ACN/5mM Phosphate Buffer:70/30.

3.4.2.2 Effect of Acetonitrile Composition on EOF

The effect of ACN content in the mobile phase on EOF is shown in Figure 25. In the range of 60-70 % (v/v), as the content of ACN increased, the EOF mobility decreased. Similar behavior was found in previous studies [78, 79]. This trend was explained by Schwer et al. [80] with the decrease in zeta potential on addition of organic solvent.

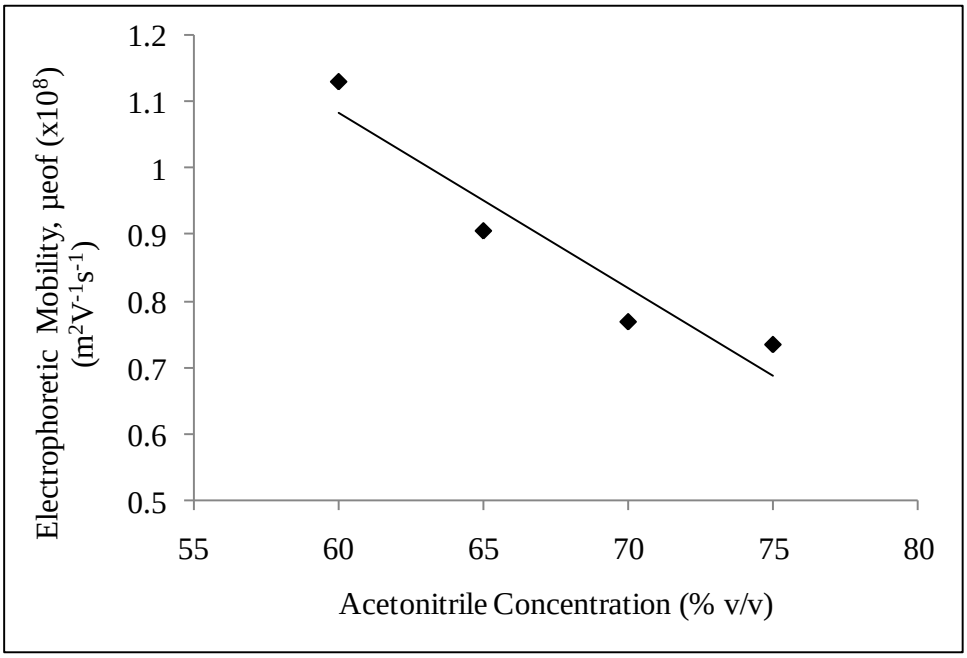


Figure 25 The variation of electrophoretic mobility with ACN concentration in the mobile phase (ACN/5 mM phosphate buffer, pH: 7). The unretained marker: thiourea.

3.4.2.3 Chromatographic Performance

The chromatographic performance of the poly(IDA-co-DVB)^3 monolith was investigated by the separation of selected alkylbenzenes in CEC. It is clearly known that separation behaviors of the analytes are highly dependent on the mobile phase composition [81, 82]. Therefore, first, the effect of ACN/buffer ratio on the electrochromatographic separation was evaluated. Figure 26 shows the representative electrochromatograms obtained by using ACN percentage in the range of 60-75 % (v/v). It was seen that as the ACN concentration in the mobile phase was increased, the retention of the analytes decreased. 60 % (v/v) ACN in the mobile phase provided a good separation, but it also caused a long analysis time. On the other hand, use of 70% ACN provided an optimum separation with shorter separation time. This trend can be seen also in Figure 27 which shows the linear dependence of retention factor with increasing ACN concentration. This behavior is typical for the separation of alkylbenzenes in the reverse phase systems [78, 83].

In CEC systems, the conductivity of mobile phase can be increased by increasing the applied voltage [84]. Thus, the retention of analytes can be adjusted by changing the applied voltage to improve separation performance. To investigate the effect of applied voltage on the electrochromatographic separation, the electrochromatograms of alkylbenzenes were obtained by using the voltages in the range of 10-30 kV at the ACN/buffer ratio of 70/30 (v/v).

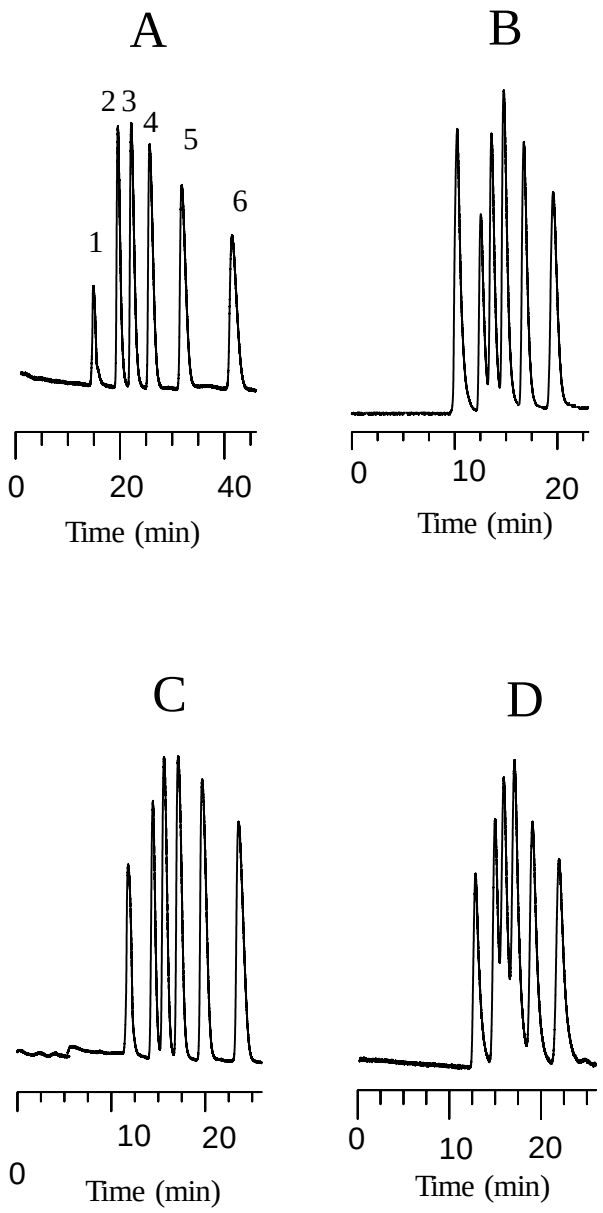


Figure 26 Effect of ACN/buffer on the electrochromatographic separation of alkylbenzenes by poly (IDA-co-DVB) monolithic column. ACN/buffer ratio (v/v): (A): 60/40, (B): 65/35 , (C): 70/30 , (D): 75/25, mobile phase : ACN/5 mM phosphate buffer at pH 7, 22 ^{o}C, id: 100 µm, effective column length: 28 cm, injection: 5 kV , 3 s; 200 nm. Order of peaks: (1): thiourea, (2) benzene, (3) toluene, (4) ethylbenzene, (5) propylbenzene, (6) butylbenzene.

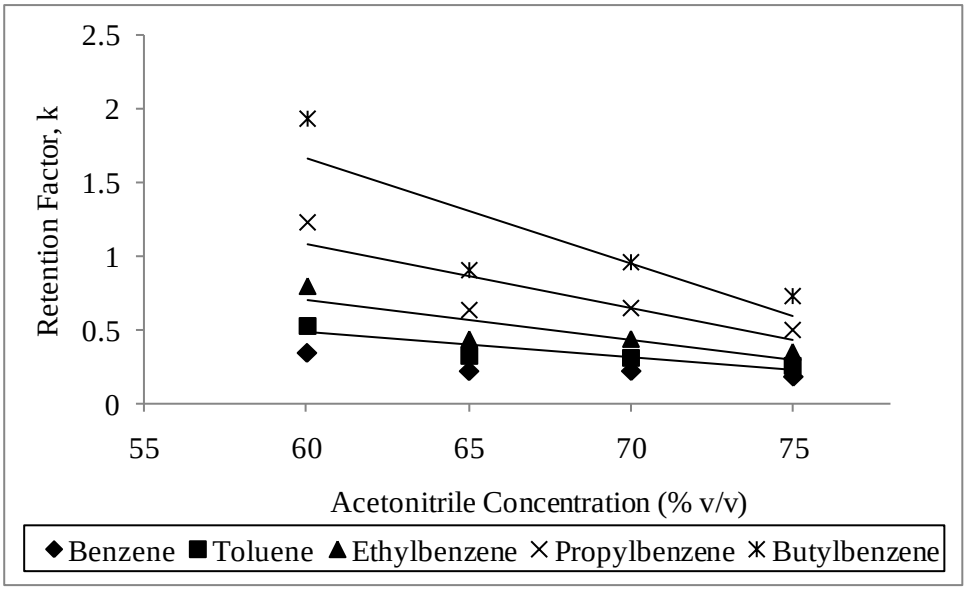


Figure 27 The variation of retention factor with ACN concentration in the mobile phase, mobile phase: ACN/5 mM phosphate buffer at pH: 7 , 22 ^{o}C, id: 100 µm, effective column length: 28 cm, injection: 5 kV , 3 s; 200 nm.

As it is seen from Figure 28, the column afforded the separation of the analytes within 15 min without any significant change in separation power at the voltage of 25 kv. However, when 30 kV was applied, a decrease in resolution was observed in the electrochromatogram (Figure 28E). On the other hand, no significant change was observed in retention factor with varying voltage (Figure 29). As we expected, the retention factor of the neutral analytes did not vary with the EOF.

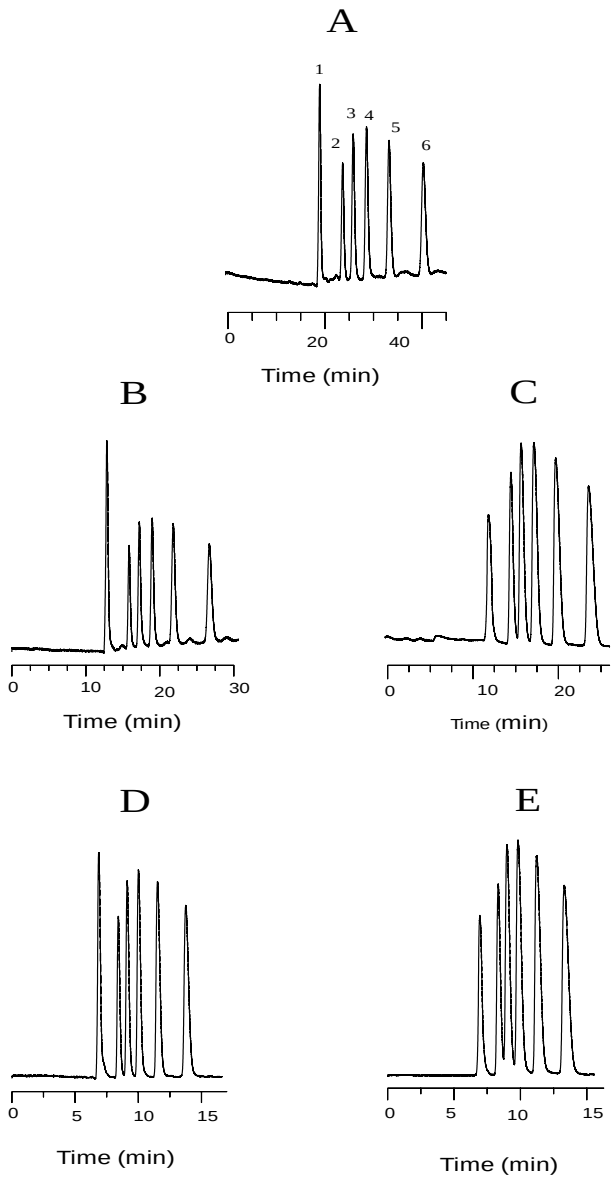


Figure 28 Effect of applied voltage on the electrochromatographic separation of alkylbenzenes by poly (IDA-co-DVB) monolithic column. Applied voltage (kv): (A): 10, (B): 15, (C): 20, (D): 25, (E): 30. ACN/phosphate buffer (pH 7, 5mM) ratio: 70/30 v/v , 22 oC, id: 100 µm, effective column length: 28 cm, injection: 5 kV , 3 s; 200 nm. Order of peaks: (1): thiourea, (2) benzene, (3) toluene, (4) ethylbenzene, (5) propylbenzene, (6) butylbenzene.

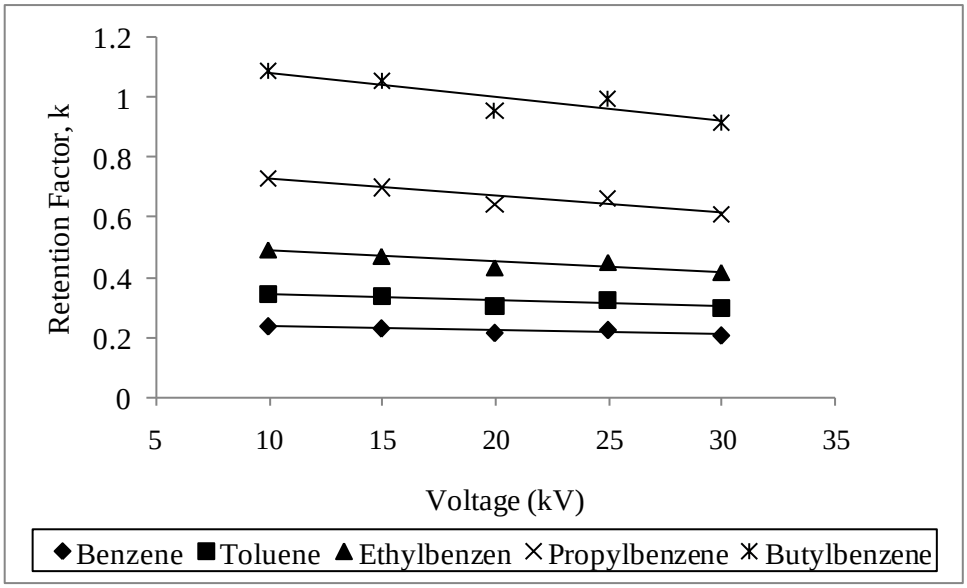


Figure 29 The variation of retention factor with voltage, mobile phase: ACN/5mM Buffer : 70/30, at pH: 7, 22^{o}C, id: 100 µm, effective column length: 28 cm, injection: 5 kV , 3 s; 200 nm.

The peak resolutions of electrochromatographic separation of the alkylbenzenes at different ACN/buffer ratios are indicated in Table 5. As shown here, the resolution of the analytes were higher than 1.5 when the ACN percentage in the mobile phase was 60 and 70%. However further increase in ACN percentage caused decrease in the resolution of some analytes, especially for benzene and toluene. Besides, Table 6 shows the effect of applied voltage on the peak resolutions at the ACN/buffer ratio of 70/30 (v/v). From these data, it is seen that the resolutions decrease with increasing voltage for all analytes.

Table 5 The effect of ACN/buffer ratio on peak resolution for the separation of benzene derivatives at different ACN/buffer ratios.

ACN/buffer	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
60/40	3.40	1.51	1.83	2.64	3.76
70/30	4.20	1.64	1.80	2.64	3.16
75/52	2.35	0.94	1.16	1.62	2.17

Table 6 The effect of applied voltage on peak resolution for the separation of benzene derivatives, mobile phase: ACN/5mM Buffer: 70/30.

Voltage (kV)	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
10	6.86	2.93	3.23	4.90	5.54
15	6.10	2.90	2.86	4.56	5.42
20	4,20	1.64	1.80	2.64	3.16
25	3.60	1.50	1.53	2.35	3.05
30	3.15	1.33	1.42	2.12	2.65

3.4.2.4 Efficiency of the Column

The efficiency of the column was evaluated by theoretical plate number (TPN) and plate height values. The effect of flow velocity on TPN and plate height is seen in Figure 30. The TPNs up to 200.000 and the plate heights with a minimum of 5 µm were obtained by using ACN / buffer ratio of 70/30 (v/v). As shown in Figure 31, the column had the highest TPN (60.000 m^{-1}) and the lowest plate height (15 µm) at 70 % ACN in the mobile phase.

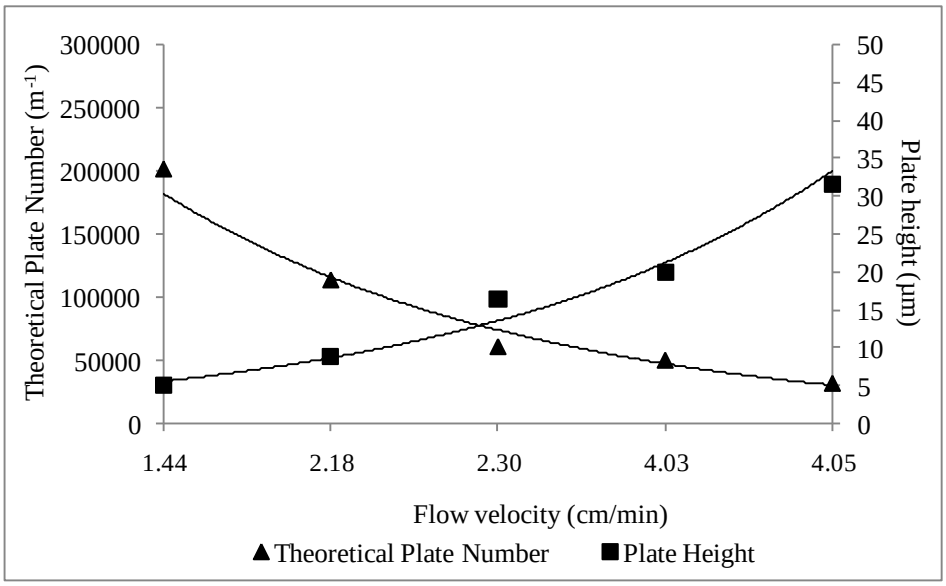


Figure 30 The effect of linear velocity on the theoretical plate number and plate height of poly (IDA-co-DVB)column , ACN / 5 mM Buffer : 70/30, analyte: thiourea, 22 ^{o}C , column id: 100 mm, effective column length: 28cm, injection: 5 kV , 3 s; 200nm.

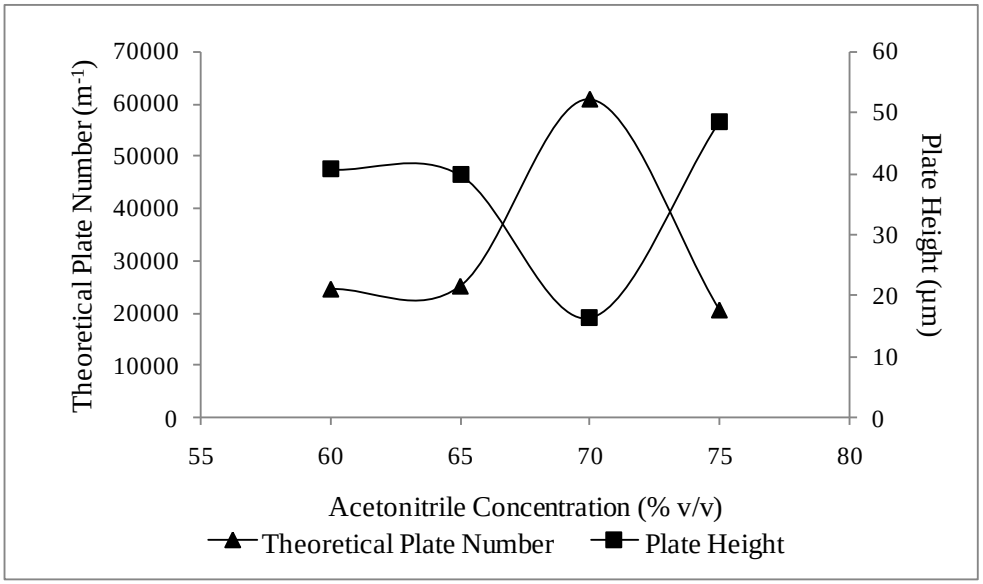


Figure 31 The effect of ACN concentration on the theoretical plate number and plate height of poly (IDA-co-DVB) column , Applied voltage: 20 kV , analyte: thiourea , 22 ^{o}C , column id: 100 mm, effective column length: 28cm, injection: 5 kV , 3 s; 200nm.

CHAPTER 4

4. CONCLUSIONS

High internal phase emulsion polymers (polyHIPEs) are a group of a new generation porous materials with their highly porous, light, and open-cell structures and they can be used in various applications. This study focused on the preparation of new acrylic based polyHIPEs that have various microstructure and mechanical properties and evaluation of these materials as stationary phase for capillary electrochromatography for the first time in literature. The results obtained in the current study are summarized as follows:

- Acrylic based PolyHIPE materials that have 90% porosity were successfully prepared by using high internal phase emulsion polymerization technique. All samples demonstrated very high pore volumes with properly interconnected cellular structures. For best of our knowledge, it was the first time that stearyl acrylate (SA), isodecyl acrylate (IDA), isobornyl methacrylate (IBMA) and divinylbenzene (DVB) were used to prepare HIPE polymers.

- The obtained results showed that monomer nature, composition and crosslinker content determine the physical and mechanical structure of the polyHIPEs. Therefore, by varying the initial parameters it becomes possible to control both microstructure and mechanical properties of the

resultant material changing from ductile to elastomeric. Besides, the mechanical performance which is usually quite low for these kind of materials can be improved without decreasing the void volume and interconnected cellular structure of the resultant polyHIPE.

- A polyHIPE monolithic column which had 90 % porosity and a well-defined structure with interconnected micron size spherical voids was successfully prepared within a capillary and it was evaluated as stationary phase for CEC for the first time.

- The column did not require any EOF generating monomer and indicated a strong electroosmotic flow (EOF) without any additional EOF generating monomer. This is explained by the presence of ionizable sulfate groups coming from the water soluble initiator used in the preparation of polyHIPE matrix. This property makes our column superior than the other conventional monoliths prepared by using comonomers with strong ionizable groups.

- Due to its well-defined polyHIPE structure with interconnected micron size spherical voids, the column showed successful chromatographic performance in the separation of alkylbenzenes.

As a result, it can be concluded that new polyHIPE materials that have various structure and mechanical characteristics were prepared. These materials are very promising to be used in various applications that require different mechanical characteristics. Furthermore, the evaluation of them as a stationary phase for CEC showed that these materials can be a good alternative to the commercial monolithic columns with their flexible and easy to control fabrication process.

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