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ARTICLE

Rational Design of Functionalized Polyacrylate-Based High Internal Phase Emulsion Materials for Analytical and Biomedical Uses

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This paper deals with the preparation of new poly(High Internal Phase Emulsion) [polyHIPE] materials, templated by varying different preparation parameters. The obtained systems are based on acrylic monomers, polymerized by free radical polymerization initiated by potassium persulfate and the redox initiator N,N,N',N'-Tetramethylethylenediamine (TEMED) to gain a crosslinked system at room temperature. To get a functional material, glycidyl methacrylate (GMA) has been introduced in the polymeric structure. The HIPE system was effectively stabilized by a co-polymer surfactant, while a polysorbate surfactant did not stabilize the emulsion. A water content as the internal phase ranging from 80 to 90 % was explored. A deep characterization of the materials, including SEM, DSC, TGA, BET, swelling and weight loss was carried out. The developed systems are promising and can find successful applications for analytical and biomedical purposes.

Introduction

Poly(High Internal Phase Emulsion) (polyHIPE) materials are a versatile class of polymers potentially useful in chemical analysis, biomedical applications and protein purification.¹⁻³ They can be formed by W/O emulsions (or even O/W) with water content (as internal phase) up to 90 % (v/v or w/w) and external phase composed by the liquid (at r.t.) hydrophobic monomer(s). The monomers can be polymerized (and crosslinked) applying classical polymerization techniques such as free radical polymerization or even by conjugate additions such as thiol-ene reactions as well as through the aldehyde-amine (urethane) reaction.⁴⁻⁷ Inversed phase polyHIPEs have also been explored in which O/W emulsion are used.⁸ Interestingly, not only the external phase could be polymerized but also the internal one. In this case, highly porous micro-nanoparticles could be obtained.⁹ A polyHIPE could not be obtained if the main polymer backbone would not have been crosslinked. The crosslinking could be achieved including a crosslinker in the monomer(s) mixture or by crosslinking a pre-formed polymer solubilized in a solvent

phase not miscible with the other phase of the emulsion. In literature, different monomers have been used even if the most explored polymer classes are polyacrylates and polystyrene. Among the pre-formed polymers, biodegradable polyesters,¹⁰⁻¹² polyurethane¹³ or polysaccharides have been used.^{7,14} In this field, several complete and exhaustive reviews have been published.¹⁵⁻¹⁹

In this work, new polyHIPEs based on butyl acrylate as the backbone monomer have been developed by a rational-based selection of the different components. The work is aimed to build-up a biocompatible material with functional groups able to covalently immobilize biomolecules to be used under continuous flow conditions (e.g., to catalyse specific reactions) or, if administered, able to promote the production of missing substances in pathologic processes.²⁰⁻²³ The surface area provided by the HIPE technique should lead to material with a high catalytic activity as a consequence of the facilitated mass transfer due to the peculiar interconnected porous structure. The monomers chosen for this study are hydrophobic, such as butyl acrylate (constitutional monomer), glycidyl methacrylate (functional monomer) and trimethylolpropanetriacrylate (crosslinker). Glycidyl methacrylate, after hydrolysis of its epoxy group with consequent formation of hydroxyl groups, adds a certain affinity of the material to water. The hydrophobic nature of the main components was taken into specific account to reduce the swelling of the material in water. This is important if enzymatic reactions are performed in aqueous medium. On the other side, the formation of hydroxyl groups from GMA should assure the correct conditions to yield the entrapped biomolecules with an appropriate reaction environment.²⁴

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Two different surfactants, both with low HLB values were considered, namely polysorbate 80 (Tween 80[®]) and Synperonic PE/L 121[™]. The former is a polyethylene sorbitan esterified with oleic acid which provides the hydrophobic portion, while the latter is a block-co-polymer of poly(ethylene glycol)-*block*-poly(polypropyleneglycol)-*block*-poly(ethylene glycol) with a Mw of 4400 Da.

Experimental

Materials

Trimethylolpropanetriacrylate (TMPT), Butyl acrylate (BA), Glycidyl methacrylate (GMA), Span[®] 80, N,N,N',N'-Tetramethylethylenediamine (TEMED), Potassium persulfate (KPS), tetrahydrofuran (THF), toluene, absolute ethanol and methanol were from Sigma-Aldrich (Milan, Italy). Synperonic PE/L 121[™] was kindly provided by Croda Italiana Spa. The water used in this study was obtained with a Milli-q system from Millipore.

Apparatus

Scanning electron microscopy (SEM) analyses were performed in a Zeiss EVO MA10 instrument (Carl Zeiss, Oberkochen, Germany) on gold sputtered samples. Images were acquired from upper, lower and lateral (cut) sides of the samples.

SEM pictures allowed also the evaluation of throat and void diameters by sampling 25 throats or voids. The results are expressed as \pm SD.

Specific surface area measurements were performed by a Sorptomatic 1990 instrument by Thermo Fisher Scientific.

ATR-FTIR spectra were recorded on powder in the range 4000–400 cm^{-1} using a Spectrum One Perkin-Elmer FTIR spectrophotometer (resolution 4 cm^{-1}) (Monza, Italy) equipped with a MIRacle[™] ATR device.

The thermal properties were examined with a Mettler STARE system (Mettler Toledo, Milano, Italy) equipped with a DSC821e Module and an Intracooler device for subambient temperature analysis to measure temperature and enthalpy values. Mass losses were recorded with a Mettler STARE system (Mettler Toledo, Milano, Italy) TGA with simultaneous DSC (TGA/DSC1).

The instruments were previously calibrated with Indium as standard reference.

Synthesis of Trimethylolpropanetriacrylate Crosslinked poly(butyl acrylate-*stat*-glycidyl methacrylate) polyHIPEs

General procedure: to form the polyHIPE, an emulsion 80/20 v/v W/O was formed (also 85/15 and 90/10 v/v were tested for a selected sample). The oil phase was obtained with a total volume of 4 mL, while water phase had a total volume of 16 mL (total volume of HIPE 20 mL). Established amounts of BA were added to a three-neck round bottom flask purged with nitrogen, then GMA, TMPT and the surfactant (polysorbate or synperonic) were added (see Table 1 for the reagents amount). The rate BA to GMA was always kept constant as 2.5:1 v/v which means \approx 30 % mol/mol of GMA with respect to BA. In a typical experiment, e.g. HIPE 2.0 (see table 1), 1 mL of the oil

phase was composed by 0.5 mL of BA (4.383 mmol), 0.2 mL of GMA (1.315 mmol) [\approx 30 % mol/mol GMA to BA] for a total of 0.70 mL of BAplusGMA, 0.15 mL of TMPT (0.478 mmol) [\approx 8 % mol/mol TMPT to BAplusGMA] and 0.15 mL of surfactant. The mixture was stirred by an overhead stirrer provided with a glass rod fitted with a D-shaped PTFE paddle, at 300 rpm for 5 min, under nitrogen. One neck of the flask was provided with a 50 mL dropping funnel with a PTFE stopcock and filled with 16 mL of deionized (Milli-Q grade) and nitrogen/vacuum degassed water containing 0.8 g of KPS (2.9 mmol). The system was maintained under stirring and under nitrogen, thus the whole aqueous phase was added in 20 min, by manually regulating the dropping funnel PTFE stopcock and then left under stirring and nitrogen for additional 60 min. Finally, 0.13 mL of TEMED (1.45 mmol) were added and the mixture stirred for further 5 min at 400 rpm. After this period, the obtained emulsion (liquid or cream-like depending from the used surfactant) was quickly poured or transferred in a closed Petri dish and allowed to polymerize for 24 h at 25 °C in an oven purged with nitrogen. After 24 h reaction, the white solid was transferred in a 300 mL crystallizer and washed in the following conditions: two times with water (2x 200 mL), ethanol (2x 100mL), methanol (2x 100 mL) and, eventually, THF (2x 100 mL) [THF washing was performed or not, please refer to the discussion part]. Finally, the washed polyHIPEs were dried in an oven at 40 °C for 24 h. To verify the crosslinking of the materials, a dry sample of the obtained polyHIPEs were immersed in THF or toluene, both being good solvents for poly(butyl acrylate) and poly(glycidyl methacrylate) dissolution.

By following an experimental design, the above parameters were varied by maintaining constant the oil to water ratio in order to evaluate the effect of crosslinker and surfactant variation on the final output (Table 1 and 2).

Weight Loss and Swelling Studies in Water and Organic Solvents

This study aimed to verify the extractable material from the polyHIPEs in water or organic solvents after the polymerization.

Small monoliths of dried polyHIPE (\approx 10 mg each) were cut, the starting weight registered (Ws), and placed in a 50 mL beaker. 20 mL of water, THF or toluene were then added to each sample. The solvents (including water) were replaced with fresh ones at least 2 times in 24 h. After this time, the monolith sample was quickly dried on the surface with a filter paper, weighted (Wfw) and dried in an oven at 40 °C to constant weight (24 h is a sufficient time for all the tested materials). The dry weight was then registered (Wfd) and used for the following determination.

All the experiments were performed in triplicate and the results expressed as \pm SD.

Weight loss %, WL, was calculated as follow:

$$WL = \left(\frac{W_s - W_{fd}}{W_s} \right) * 100 \quad (\text{Eq 1})$$

where W_s is the starting weight of the sample before immersion in the solvent and W_{fd} is the final (dry) weight of the sample.

The swelling, SW , was calculated as follow:

$$SW = \frac{W_{fw} - W_{fd}}{W_{fd}} \quad (\text{Eq 2})$$

where W_{fw} is the weight of the wet sample at the end of the swelling experiment.

Apparent Density and (Calculated) Porosity of Obtained polyHIPEs

The weight/surface area ratio and the apparent density of the obtained polyHIPEs (weight/volume ratio) were evaluated after accurately cutting 1.00 cm x 1.00 cm samples and determination of the scaffold thickness by a certified micrometer. Furthermore, the porosity % (ϵ) of the scaffolds was calculated by applying equation (3):^{25, 26}

$$\epsilon = \left[1 - \left(\frac{\delta}{\delta_0} \right) \right] * 100 \quad (\text{Eq 3})$$

where δ is the apparent density of the scaffolds and δ_0 is the bulk density of poly(butyl acrylate) (1.00 g/cm³).¹⁰

All the experiments were performed in triplicate and the results expressed as mean value \pm SD.

SEM Analysis

Images were acquired from upper, lower and lateral (cut) sides of the samples.

SEM pictures allowed also the evaluation of throat and void diameters by sampling 25 throats or voids. The results are expressed as mean value \pm SD.

Specific Surface Area Measurements by BET

About 150 mg of sample was charged in the glass sample holder and degassed at 250 °C for 12 h. Subsequently, samples were cooled down at -196 °C and an adsorption run was performed (BET method, analyzing gas N₂, 20 points for run, blank done in He).

DSC and TGA Studies

Analyses were performed in sealed aluminium pans with a pierced lid. DSC curves were registered on 2–4 mg samples, over a temperature range of 10–400 °C ($\beta = 10 \text{ min}^{-1}$) in a nitrogen air atmosphere flux (50 mL min⁻¹).

Mass losses were recorded on 4–6 mg samples in alumina crucibles closed with a pierced lid [$\beta = 10 \text{ min}^{-1}$, nitrogen air atmosphere (flux 50 mL min⁻¹)], 30–400 °C range.

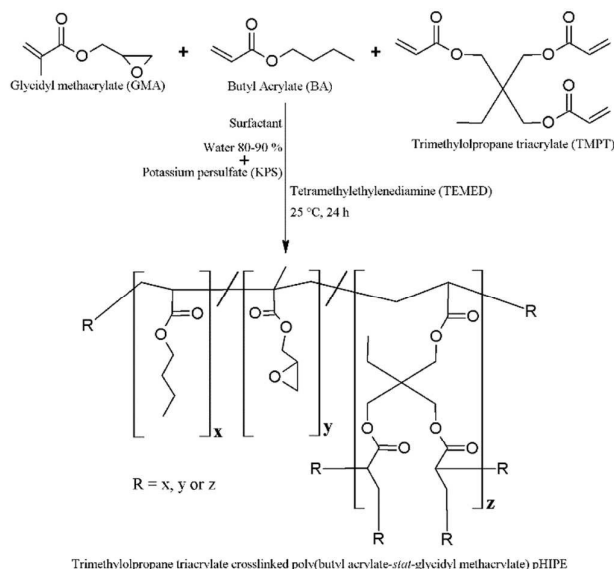
Measurements were carried out at least in triplicate and the result were expressed as mean value \pm SD.

Results and Discussion

PolyHIPE Synthesis

The novel polyHIPE systems were rationally though for the covalent binding of biomolecules able to catalyse specific reactions for analytical or biomedical purposes. The formation

of highly porous systems, such as the polyHIPEs are, should assure a high surface to weight ratio with subsequent high loading capacity of the selected biomolecule. The monomers used for the formation of the polyHIPEs were: i) butyl acrylate (BA) for its high glass transition temperature (T_g) which should assure a good mechanical stability under solvent flow; ii) glycidyl methacrylate (GMA) as a functional monomer; iii) trimethylolpropanetriacrylate as a tri-functional crosslinker to guarantee a high crosslinking density and subsequent structural stability. Moreover, the acrylate monomers were selected due to their proven biocompatibility in view of potential *in-vivo* applications.



Scheme 1: schematic representation of the reactions and processes involved in the preparation of Trimethylolpropanetriacrylate crosslinked poly(butyl acrylate-*stat*-glycidyl methacrylate) polyHIPEs.

Two different surfactants were evaluated: i) polysorbate 80 and ii) the block co-polymer Synperonic PE/L 121. As the radical initiator, a KPS/TEMED system was used to allow the polymerization at 25 °C instead of 60 °C as required to KPS for radical generation. In Scheme 1 the main chemical reactions involved together with the process parameters are reported.

In literature, several examples of polyHIPE materials based on polyacrylates are reported but, to the best of our knowledge, the composition selected in this study does not find similar references.^{2,10,27,28} Moreover, the selected composition provided a functional material rationally designed to obtain a polyHIPE with expected properties not feasible with different monomers (which do not provide functionalities) or processing solutions (not leading to interconnected materials); please see discussion part for the rationale of the work.

At the beginning of our studies, fixed amounts of oil phase composition (oil to water ratio and operating parameters),

only changing the kind of surfactant, were tested. In Tables 1 and 2, the amount of used monomers is reported fixing to 1 the whole composition of the oil phase. The rate is expressed as v/v.

Differently from other studies,^{10,29,30} polysorbate 80 was not able to stabilize the HIPE and thus to produce a polyHIPE. This negative result suggested the use of a polymeric surfactant such as the Synperonic. This surfactant led to a better steric and thermodynamic stabilization of the emulsion by forming a more stable interface between the continuous oil phase and the dispersed water phase. It could be hypothesized that the hydrophilic polyoxyethylene chains of the block-co-polymer would create an efficient environment for water droplet and, on the other side, the polypropylene oxide hydrophobic chains should “physically” separate and stabilize the oil phase against

the aqueous one. A schematic representation of the Synperonic stabilization is shown in Figure 1. To verify that the emulsion forming the HIPE was a W/O emulsion, a hydrophilic dye (methylene blue) was solubilized in the water phase and a drop of the emulsion was subjected to optical microscope visualization.

Table 1: Oil phase composition and macroscopic output of polyHIPEs obtained from the selected monomers by using two different surfactants. The amount of the used monomers is indicated by fixing to 1 the whole composition of the oil phase; the rate of each oil component is expressed as v/v. The rate BA to GMA was always kept at 2.5:1 v/v which means $\approx 30\%$ mol/mol of GMA with respect to BA. Oil to water amount was fixed to 20:80. For example, 10 mL of oil phase in HIPE 1.0 were composed by 7 mL of 2.5:1 v/v BA:GMA, 1.5 mL of synperonic and 1.5 mL of TMPT plus 40 mL of water.

Sample	BA+GMA	Surfactant	TMPT	Output
		kind/amount		
HIPE 1.0	0.70	Polysorbate/ 0.15	0.15	Flowing emulsion
HIPE 1.1	0.56	Polysorbate/ 0.30	0.15	Flowing emulsion
HIPE 2.0	0.70	Synperonic/ 0.15	0.15	Cream-like-emulsion
HIPE 2.0.1.2	NO GMA	Synperonic/ 0.15	0.15	Cream-like-emulsion
HIPE 2.1	0.56	Synperonic/ 0.30	0.15	Cream-like-emulsion

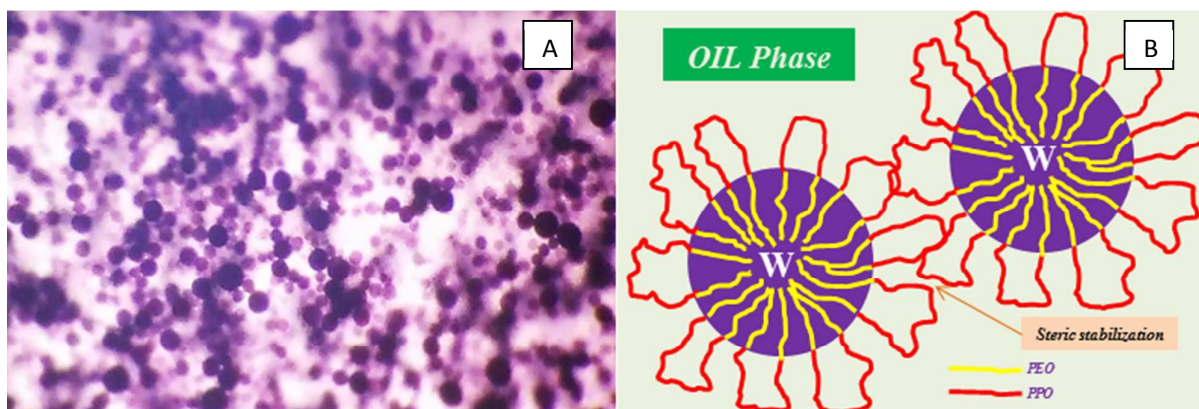


Figure 1: Optical image (A, scale bar not provided) of HIPE 2.0 emulsion acquired after a hydrophilic dye (methylene blue) was solubilized in the water phase. Water drops are clearly visualized as blue-violet spots. A pictorial drawing illustrating the Synperonic stabilization of the system is reported in B.

As from Figure 1, the water droplets are clearly found as internal phase while the continuous oil phase is not affected by the hydrophilic dye colour. This picture confirms that the obtained emulsions are W/O systems and that no macroscopic phase separation occurs in the tested time scale for optical visualization (almost 10 min). This time is arbitrary and mostly due to prevent water evaporation during the optical analysis (the analysis was performed on an open glass slide). All the materials obtained as crosslinked solid (as verified by insolubility in THF and Toluene) were subjected to SEM analysis. In Figure 2 the SEM pictures relative to the materials obtained from the trials indicated in Table 1 are shown.

The SEM images obtained for HIPE 1.0 and 1.1 materials suggest that a phase inversion occurred. The globular structures point out on a crosslinking of the droplets (internal phase) instead of the continuous phase has taken place. HIPE 1.1 was obtained under the same experimental conditions used for HIPE 1.0 doubling the amount of the surfactant. As evident in Figure 2b, a product similar to that of HIPE 1.0 was obtained. Moreover, the samples in Figure 2a and b were further subjected to toluene extraction (polyacrylates are soluble in toluene) which revealed a porous structure mostly formed by globular interconnected structure as seen by SEM, further confirming that the polymerization does not happen in the continuous phase. We can also speculate that under these conditions the crosslinking was not complete, leading to great amounts of “soluble” polymer (SI1). It should be also noted that by using the polysorbate, only liquid emulsions were obtained, while cream-like semi-solids were always obtained with Synperonic as surfactant. In fact, Synperonic, used as surfactant in place of polysorbate, allowed obtaining an organized polyHIPE structure (Figure 1c), named HIPE 2.0. However, by increasing the amount of Synperonic led to a collapsed structure (see Figure 2d, HIPE 2.1) without evident voids or connections which formation is attributed to volume contraction during the conversion of monomers to polymer.¹⁶ It should be also noted that HIPE 2.1 (doubled amount of Synperonic with respect to HIPE 2.0) was obtained with lower yields after washing (final weight/theoretical weight $100 \approx 45$

% w/w) with respect to HIPE 2.0. The effect of the surfactant on the formation of polyHIPEs was deeply studied in the past, and, what we found experimentally, is supported by different studies in literature. For example, it has been shown that by increasing the surfactant concentration, thinner monomer films separating adjacent emulsion droplets, could be produced. If a critical (lower) film thickness is reached, it could be hypothesized a massive coalescence of the emulsion with consequent phase separation.^{16,30} In 1988, Williams and Wroblewski found that by increasing the amount of surfactant above an upper limit, the emulsion did not maintain a coherent structure upon drying, leading to unstructured materials.³¹ Based on their observations the authors stated: “As the surfactant level is raised even higher, the thin oil layer retracts even more until it disappears. The foam generated under these conditions has no walls, only struts.”, so, “...the structure of the emulsion is not clearly indicated by the resulting foam, since the polymeric material produced is no longer able to maintain continuity when dried.”

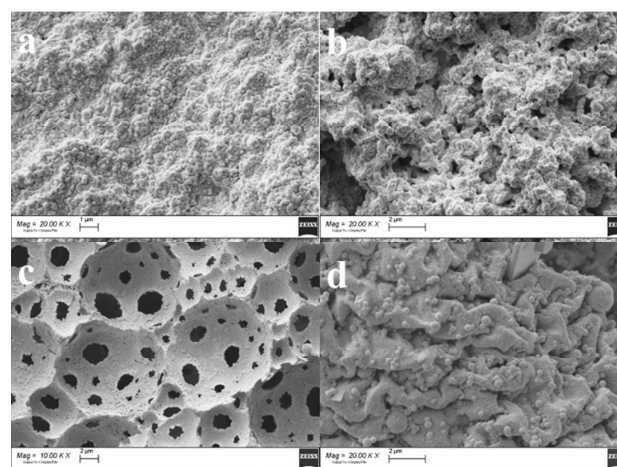


Figure 2: SEM analysis on HIPE 1.0 (a) and HIPE 1.1 (b) obtained by using polysorbate 80 as the surfactant and SEM inspection on HIPE 2.0 (c) and HIPE 2.1 (d) obtained by using Synperonic PE/L 121 as the surfactant.

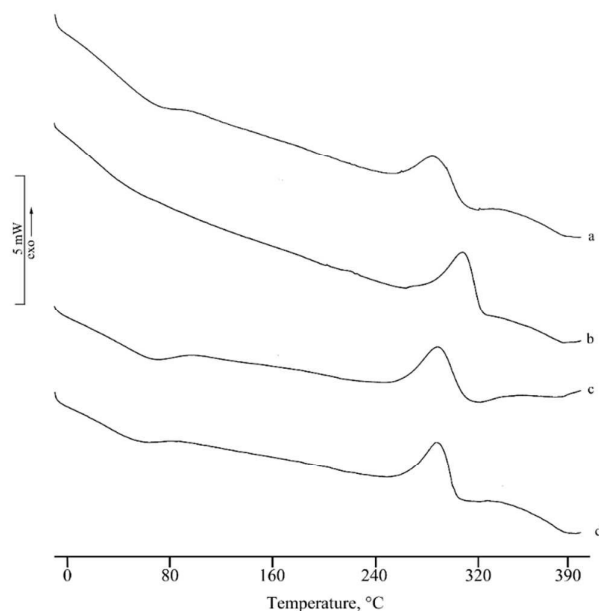


Figure 3. DSC curves for a) HIPE 2.0 before THF washing; b) HIPE 2.0 after THF washing; c) HIPE 2.2.1 (THF washed); and d) HIPE 2.3.1 (THF washed).

PolyHIPEs Characterization

The purification of the obtained HIPE was performed by subsequent washing of the materials with water, ethanol and methanol. A final washing step in THF was carried out to remove not-crosslinked portion of the polymer since the formation of soluble portions cannot be excluded. To verify whether the THF washing can influence the thermal and morphological behaviours of the studied polyHIPEs, DSC, TGA (TGA spectra are reported in SI) and SEM analyses were performed. DSC and TGA studies were conducted due to the possibility that different thermal behaviours might be evidenced on the materials if a soluble (not crosslinked) polymer is found within the structure of the network (Figure 3 and SI2). SEM analysis before and after THF washing aimed to

evaluate morphological changes in the material structure (Figure 4).

The DSC studies showed that a first decomposition peak can be detected at around 285 °C while, after washing the HIPE in THF, the same decomposition can be found at higher temperature ($T_{\text{onset,dec}} = 308$ °C). It should be noted that these are not the main decomposition peaks which, in turn are found at temperatures above 380 °C, as also confirmed by TGA studies. TGA studies also evidenced a mass loss corresponding to the first decomposition peaks of ≈ 8 % (see TGA curves in SI2). This result indicates that the decomposition at 285 °C for HIPE 2.0 before THF washing and at 308 °C for HIPE 2.0 after THF washing, led to the same mass loss of about 8 %, demonstrating that what decomposed upon heating is not a portion of network soluble in THF. The same results were obtained washing the polyHIPEs in toluene, confirming that, as expected, both solvents (THF and toluene) are good solvents for polyacrylates. To better understand this phenomenon, the obtained polyHIPEs were subjected to mass loss experiments (see section 2.4) and the results are shown in Table 3. Mass loss of HIPE 2.0 after washing in THF was 8.6 % w/w indicating that a certain amount of soluble polymer is found as extractable material into the network. Moreover, a DSC study performed on pure Synperonic, showed only a decomposition peak at around 380 °C, demonstrating that the decomposition at around 300 °C, found in the polyHIPEs samples, could not be attributed to unwashed Synperonic. Although in literature DSC studies performed on inverse-HIPE based on polyacrylates showed crystallization exothermic peak or glass transition effect (T_g), in our samples these thermic effects have not been detected.⁸

Indeed, this is not a surprising result because, as known, in crosslinked systems the chain mobility could be so much impeded that small thermal transitions such as T_g is, could not be detected.

On the other side, no crystalline clusters can be detected by DSC indicating that the obtained HIPEs are amorphous. Last but not least, an extremely high thermal stability (up to 380 °C) is noticeable.

HIPE 2.0 was further analysed by SEM, before and after washing with THF, Figure 4.

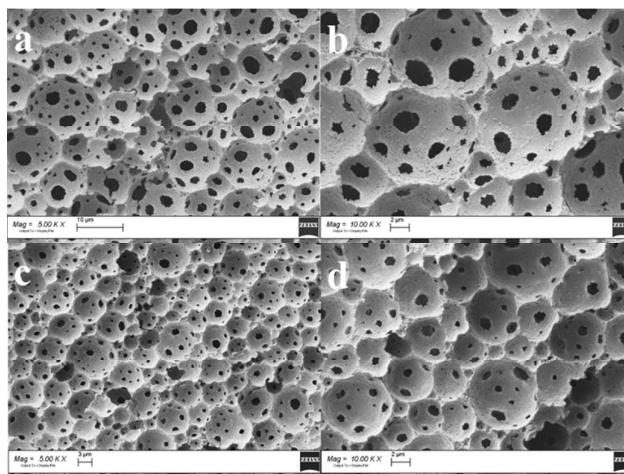


Figure 4: SEM study on HIPE 2.0 obtained by using Synperonic PE/L 121 as the surfactant; a) and b) show two magnifications, 5000 and 10000 X of the raw material before washing in THF; c) and d) show two magnifications, 5000 and 10000 X after washing the monolith in THF.

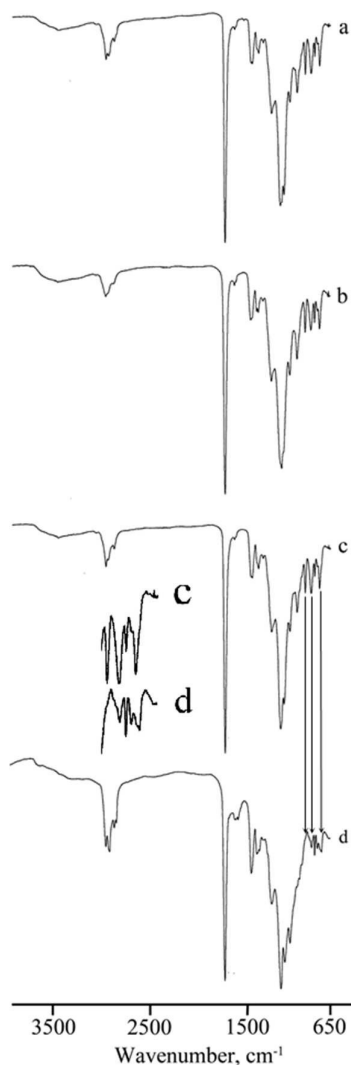


Figure 5. ATR-FTIR spectra of a) HIPE 2.0, b) 2.2.1 (W/O 85:15), c) 2.3.1 (W/O 90:10) and d) 2.0.1.2 (without GMA), the spectra were acquired in absorbance mode. In the inset, a magnification of the most significant peaks.

As from Figure 4, the macroscopic structure of the HIPE 2.0 is not affected by the THF washing while some difference could be noted in terms of voids and throats size, Table 3. The mean diameter of the voids before THF washing was $9.1 \pm 2.7 \mu\text{m}$ ($n = 25$) while the throats mean diameter was $1.6 \pm 0.6 \mu\text{m}$ ($n = 25$). After THF washing the voids were $8.3 \pm 2.5 \mu\text{m}$ and the throats $1.0 \pm 0.3 \mu\text{m}$ ($n = 25$) for each measurement. The slight difference in voids and throats mean diameters could be attributed to shrinkage of the polymer after THF washing (see Table 3 for mass loss results). For these reasons, all the obtained materials, were routinely washed with THF before further characterizations.

ATR-FTIR analysis was performed to verify the main chemical structure of the polymeric materials and to reveal the presence of the epoxy groups in the polyHIPEs, that is fundamental to accomplish our aim in obtaining a functionalized material with reactive epoxy groups. To better attribute ATR-FTIR peaks to the epoxy group, a HIPE 2.0 sample was prepared under the conditions previously reported but without GMA (HIPE 2.0.1.2). ATR-FTIR spectra are reported in Figure 5.

The ATR-FTIR spectra evidence the presence of the main peaks of polyacrylates. In particular, the peaks at 3300 cm^{-1} are relative to hydrogen-bonded hydroxyl groups from epoxy group hydrolysis, the peaks at $3000\text{--}2850 \text{ cm}^{-1}$ are attributed to the C-H stretching of the polymer chain, the peak at 1724 cm^{-1} corresponds to the stretching of carbonyl group, the signal at 1159 cm^{-1} is due to the stretching of the C-O bond and the signals at 911 and 839 cm^{-1} are vibrational bands attributed to the epoxy group ring.³² From ATR-FTIR studies, at least two fundamental evidences can be deduced: i) due to the low intensity of the band at 3300 cm^{-1} , it can be inferred that only a few amount of hydrolysed epoxy groups are present in the materials; ii) the strong bands at 911 and 839 cm^{-1} , relative to the epoxy group ring vibration, was present in samples HIPE 2.0. When GMA was excluded from the HIPE preparation, HIPE 2.0.1.2 (Figure 5d), no peaks at 959 , 911 , 839 and 759 cm^{-1} were detected confirming that those seen in the BA/GMA HIPEs were from GMA. In literature it is reported that the hydrolysis of epoxy groups increases by increasing the amount of GMA in the HIPE composition, this is the reason why we maintained constant the BA to GMA ratio at 2.5:1.¹⁷ Even if a quantitative analysis has not been performed, it could be supposed, from the ATR-FTR spectra, that the amount of hydrolysed epoxy group is relatively low.

Optimization of PolyHIPEs Formation

Additional studies were aimed to evaluate if polyHIPEs up to 90 % v/v in water could be obtained and stabilized under the same experimental conditions used for the HIPE 2.0. For this reason, the formulation used for the production of HIPE 2.0 was used, and water amounts of 85% and 90% (v/v) were considered to obtain, respectively, HIPE 2.2.1 and HIPE 2.3.1 polymers. HIPE 2.2.1 and HIPE 2.3.1 polymers were characterized by FTIR (Figure 5) and SEM (Figure 6). The other parameters are reported in Table 3.

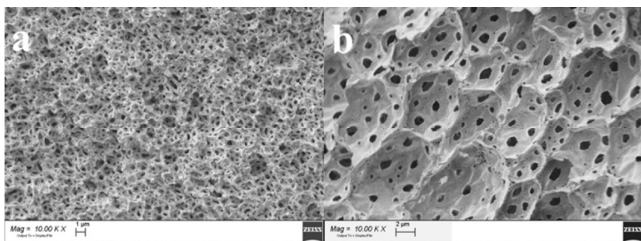


Figure 6. SEM study of a) HIPE 2.2.1 (85 % water) and b) HIPE 2.3.1(90 % water).

As from Figure 6, the established formulation resulted able to stabilize the system up to 90 % v/v of water content. This result is in agreement with other studies where it is reported that the amount of water is less important than other parameters such as surfactant concentration, in determining structural changes in the polyHIPE.^{16,31}

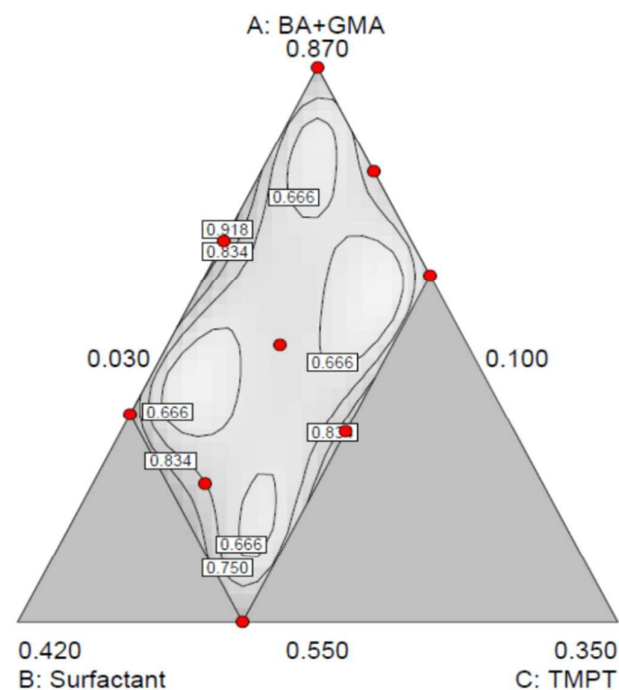


Figure 7: Contour map of the leverage function in the experimental domain and points selected for the study. See main text for components specifications.

After the first experiments, aimed to establish the main reaction and process conditions, further studies were performed by setting up an experimental design. Synperonic PE/L 121 was selected as the surfactant system. In particular, since high amounts of surfactant did not produce a typical polyHIPE structure, we were interested in understanding the upper surfactant limit and the effect of the TMTP crosslinker on the HIPE formation and structure. The lower limit in surfactant was not studied because, as known, low surfactant concentration leads to a close-cell (no throats) structure which is out of our aim to obtain a highly porous interconnected material.³⁰ The BA to GMA ratio was maintained constant, while varying the amount of surfactant (Synperonic) and crosslinker. On the other side, the ratio oil phase (BA plus GMA plus TMTP plus surfactant) to water was maintained 20:80 v/v for all the experiments.

The experiments planned for studying the polyHIPE formation, involved several components and a number of process variables. However, most process variables were fixed, and out the many ingredients involved, only three were considered for the study of the polyHIPE formation by means of a mixture design. The oil to water ratio selected was 20:80 (v/v). The BA to GMA ratio was kept fixed at 2.5:1 v/v (which correspond to 30 % mol/mol of GMA with respect to BA) and the amount of this binary mixture was varied in the rate 0.55-0.87 v/v with respect to the whole oil phase. The Surfactant in the oil phase was varied from 0.10-0.30 v/v with respect to the whole oil phase. The TMPT amount was varied from 0.03-0.15 v/v with respect to the whole oil phase. In a typical experiment, e.g. HIPE 2.8.1 (please see table 2), 1 mL of the oil phase was composed by 0.536 mL of BA (4.699 mmol), 0.214 mL of GMA (1.407 mmol) [≈ 30 % mol/mol GMA to BA] for a total of 0.75 mL of BAplusGMA, 0.15 mL of TMPT (0.478 mmol) [≈ 8 % mol/mol TMPT to BAplusGMA] and 0.10 mL of surfactant. It is known that “the percentage of epoxy groups that underwent hydrolysis during polyHIPE synthesis and purification increased from 25 to 77% with increasing GMA content”¹⁷, this is the reason why we did not use higher amounts of GMA in our study nor we used a lower amount to avoid a substantial reduction of the epoxy functional groups to be used for subsequent chemical conjugations. Since the three components of the mixture (BA+GMA, Surfactant, and TMPT) had to be used in amounts constrained with upper and lower limits, a D-optimal design was studied.³³ The experiments were planned thus under the hypothesis that a suitable model for studying the properties of the polymers obtained could be the special cubic model. Under this hypothesis the model postulated was

$$Y = b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X_3 + b_{12} \cdot X_1 \cdot X_2 + b_{13} \cdot X_1 \cdot X_3 + b_{23} \cdot X_2 \cdot X_3 + b_{123} \cdot X_1 \cdot X_2 \cdot X_3$$

where Y is the experimental value of a given measurable response provided by the polymer.

All computations were carried out using Microsoft Excel and R version 3.1.0 (2014-04-10) Copyright (C) 2014 The R Foundation for Statistical Computing. R-based chemometric software routines were used for DOE calculations. The R-based

software has been developed by the Group of Chemometrics of the Italian Chemical Society [http://gruppochemiometria.it/gruppo-lavoro-r-in-chemiometria.html].

Nine experiments were carried out as reported in Table 2. Figure 7 shows the experimental domain and the leverage associated with the experimental points.

Table 2. Main experimental conditions gained from the experimental design. BA to GMA ratio was maintained constant to 2.5:1 v/v which correspond to a constant amount of $\approx 30\%$ mol/mol in GMA vs BA. The numbers indicate the relative amount of each component in the oil phase, the sum of any formulation is equal to 1. Oil to water rate is fixed to 20:80 v/v. Each experiment was performed in triplicate.

Sample	BA+GMA	Surfactant	TMPT	Output
HIPE 2.0*	0.70	0.15	0.15	Yes/SEM
HIPE 2.12	0.87	0.10	0.03	Powdered after washing
HIPE 2.5.1	0.77	0.20	0.03	No solid material gained
HIPE 2.13	0.67	0.30	0.03	No solid material gained
HIPE 2.11	0.81	0.10	0.09	Yes/SEM
HIPE 2.10	0.71	0.20	0.09	Yes/SEM
HIPE 2.6.1	0.63	0.28	0.09	No solid material gained
HIPE 2.8.1	0.75	0.10	0.15	Yes/SEM
HIPE 2.7.1	0.66	0.19	0.15	Yes/SEM
HIPE 2.9.1	0.55	0.30	0.15	No solid material gained

Powdered after washing = the obtained HIPE powdered upon washing

No solid material gained = an emulsion was obtained which did not solidify

Yes/SEM = a solid was obtained upon crosslinking which was analyzed by SEM

***HIPE 2.0** is included for completeness but is not part of the experimental design

From the experiments shown in Table 2, different experimental evidences could be gained: i) by fixing the crosslinker amount at 0.03 with respect to the oil phase, no structured materials have been obtained; ii) by increasing the amount of surfactant above 0.20 no structured materials have been obtained; iii) no solid materials were obtained when the BA+GMA composition was below 0.66. It could be hypothesized that the reduction of the crosslinker amount, limits the formation of the netpoints between the polymer chain and, consequently, even if the polymerization takes place, a stable tri-dimensional structure could not be formed. As far as the surfactant is considered, increasing its amount

could reduce the thickness of the monomer layer leading to coalescence or insufficient polymerization. Another explanation is that increasing the amount of surfactant which enters in the oil composition, the lower (absolute) amount in BA, GMA and TMPT does not guarantee a structural integrity of the system. These experimental evidences also point on the low stability of the emulsion and on the number of variables governing the formation of such systems. For this reason, we maintained unaltered the process conditions throughout the experiments, including: i) stirring speed, ii) time for emulsion formation, iii) temperature of emulsion formation and polymerization.

The SEM study in Figure 8 gives a clear evidence on how the different parameters of preparation affect the polyHIPE structure. For example, Figure 8a and 8c show that in polyHIPE at the same crosslinker concentration, obtained by increasing the surfactant amount (HIPE 2.7.1 with respect to HIPE 2.8.1) led to a less structured material which shows, macro/microscopically, more interconnections. Even reducing the amount of crosslinker, by maintaining the same concentration of surfactant, Figure 8b vs 8a (HIPE 2.8.1 with respect to HIPE 2.11) led to morphological modifications in the main structure.

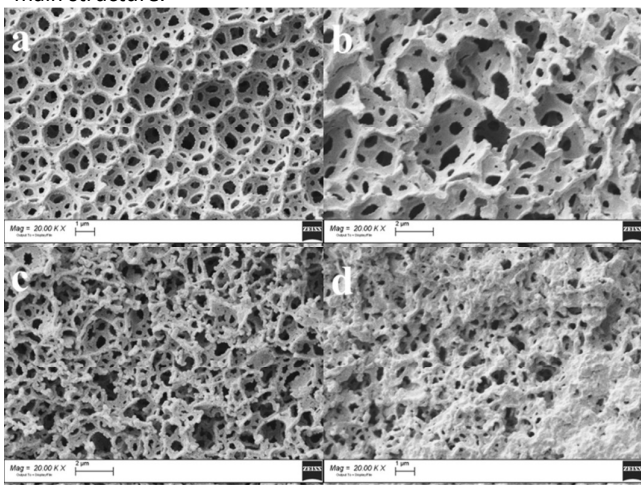


Figure 8: SEM analysis of a) HIPE 2.8.1, b) HIPE 2.11, c) HIPE 2.7.1 and d) HIPE 2.10.

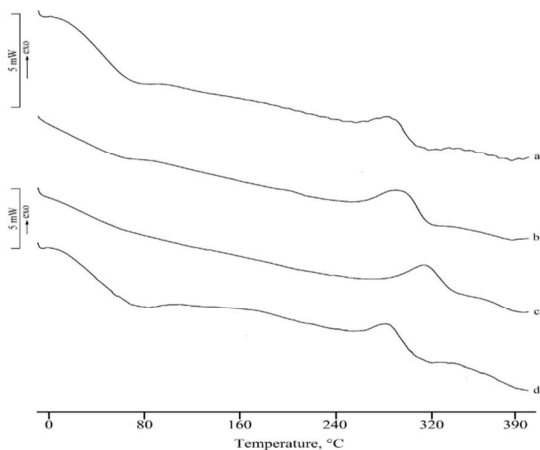


Figure 9. DSC curves for a) HIPE 2.7.1 b) HIPE 2.8.1 c) HIPE 2.10.1 and d) 2.11.1.

These structural differences were measured by evaluating the variation of specific parameters such as, calculated porosity (see section 2.5), porosity by BET, swelling studies in water, THF and Toluene, mass loss, voids and throats mean diameter

(when applicable), thermal data and the results are summarized in Table 3 and 4.

DSC studies were aimed to verify the thermal behaviours of the obtained polyHIPEs. Also for the four samples which gained a network from Table 2, before the main decomposition peak at around 380 °C a first decomposition can be detected in a range between 283-307 °C depending from the sample, Figure 9.

The data from Table 3 and 4 and the results from SEM (Figure 8 and S14-7) allowed us to select two polyHIPEs as the most promising for our aims. As shown, the reduction of the amount of surfactant from 0.15 to 0.10 (HIPE 2.0 vs HIPE 2.8.1) resulted in a marked reduction in both voids and throat diameters. Moreover, HIPE 2.0 and HIPE 2.8.1 showed a good affinity for water which is fundamental to assure a good contact with the solvent (i.e. to perform an enzymatic catalysis).

As reported in literature, polyHIPEs prepared from more hydrophilic acrylic monomers showed higher swelling values in water. This is in agreement with our approach aimed to maintain low the swelling in water by using hydrophobic monomers.^{34,35,36}

The porosity shown from these two materials can be exploited to allow an optimal amount of conjugated molecules per gram of material. Moreover, from the gained data, the optimal crosslinker amount of our formulation, was 0.15 even if the explored range (0.15-0.09) allowed the polyHIPEs formation (see Table 2) while BA+GMA could be varied between 0.7 and 0.75 in the oil phase fraction. In addition, the optimal amount of surfactant could be varied between 0.10-0.20 of the oil phase fraction. Finally, it is interesting to note that by increasing the amount of water as internal phase from 80 to 90 % HIPE 2.0→HIPE 2.2.1→HIPE 2.3.1, the obtained polyHIPEs showed a reduced swelling in water by increasing the amount of water as internal phase. This could be explained by considering that higher amount in water during the preparation of the HIPE, brings to more stretched continuous phases which, upon polymerization, “freeze” in a mechanically stressed structure less prone to stretch further. Speaking about water, the swelling mechanism, due to the hydrophobicity of the material, could be mostly attributed to a capillary effect which allows water penetration with subsequent uptake and weight growth. This phenomenon could be inhibited by mechanical stress in the polymer structure. On the other side, THF swelling is mostly due to the affinity of the solvent with the polymer which could freely swell incorporating the solvent not being affected by possible mechanical stress. This would explain why, in THF, the swelling resulted almost the same for the three considered samples.

Table 3. Characterization of the obtained polyHIPE by, swelling in water (Sw_{water}) or THF (Sw_{THF}), mass loss by extraction (ML), void size (Vs), throat size (Ts). (n=3)

Sample	Sw_{water}	Sw_{THF}	ML%	Vs (μm)	Ts (μm)
HIPE 2.0	4.8 \pm 0.1	6.4 \pm 0.1	8.6 \pm 0.4	8.3 \pm 2.5	1.0 \pm 0.3
HIPE 2.2.1	2.3 \pm 0.1	6.5 \pm 0.1	14.4 \pm 1.1	NC*	NC*
HIPE 2.3.1	1.9 \pm 0.1	6.1 \pm 0.1	13.1 \pm 2.3	7.4 \pm 2.1	1.0 \pm 0.2
HIPE 2.7.1	1.8 \pm 0.2	5.3 \pm 0.2	8.3 \pm 1.2	NC*	NC*
HIPE 2.8.1	3.8 \pm 1.3	6.4 \pm 0.2	8.4 \pm 1.5	1.8 \pm 0.5	0.5 \pm 0.1
HIPE 2.10	0.6 \pm 0.1	1.1 \pm 0.1	16.1 \pm 2.8	NC*	NC*
HIPE 2.11	1.1 \pm 0.1	3.6 \pm 0.8	11.0 \pm 4.9	4.7 \pm 0.5	0.8 \pm 0.2

*NC = not calculated due to undefined morphology

Table 4. Characterization of the obtained polyHIPE by, apparent density (δ_a) and calculated porosity ($\epsilon_{c\%}$), total corrected area by BET (ϵ_b), first decomposition temperature by DSC ($D_{T^{\circ}}$) and the corresponding mass loss recorded by TGA (ML_{TGA}).

Sample	δ_a (g/cm^3)	$\epsilon_{c\%}$	ϵ_b (m^2)	$D_{T^{\circ}}$ ($^{\circ}\text{C}$) peak	ML_{TGA} (% w/w)
HIPE 2.0	0.17	83	4.38	308	8.2
HIPE 2.2.1	NC	NC	NC	289	11.6
HIPE 2.3.1	0.32	68	2.61	288	8.3
HIPE 2.7.1	0.28	72	2.12	283	12.4
HIPE 2.8.1	0.17	83	3.12	294	9.9
HIPE 2.10	NC	NC	NC	307	9.5
HIPE 2.11	0.43	57	1.24	283	10.0

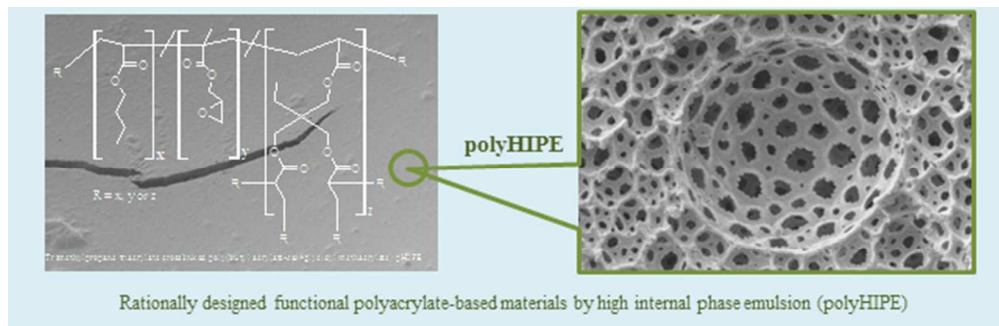
Conclusions

The synthesis and characterization of new functionalized polyacrylate-based poly High Internal Phase Emulsions developed by a rational design have been reported. The used monomers comprised butyl acrylate as the backbone, glycidyl methacrylate as the functional monomer and trimethylol propane triacrylate as the tri-functional crosslinker. Synperonic PE/L 121 resulted as the most promising surfactant for the polyHIPEs formation with respect to polysorbate 80. The polyHIPEs stabilized by the Synperonic had a W/O ratio equal to 80:20 but the same formulation resulted also able to stabilize water contents of 85:15 and 90:10 W/O. From the first data gained with the selected surfactant, an experimental

design was developed. This design, allowed to perform a series of experiments that led to establish upper and lower concentration limits for each component (monomers amount, crosslinker amount and surfactant amount) and to obtain structured materials with five different compositions. In particular, two samples reached our attention in terms of morphology, affinity for water, thermal stability and porosity. The selected polyHIPEs will be used as the base for the development of new biomedical materials to be used for the immobilization of active biomolecules to exploit their activity in different pharmaceutical fields. Analytical application, with particular regards to chromatographic techniques, will be further studied. A new experimental design will be developed based on the actual outputs.

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