SYNTHETIC SCIENTIFIC REPORT

on the implementation of the project

ZWITTERIONIC POROUS MICROPARTICLES CONTAINING ZEIN AND BETAINE MOIETIES WITH ANTIMICROBIAL ACTIVITY AND DRUG DELIVERY CAPABILITIES

Project code: PN-III-P4-ID-PCE-2020-1541

PHASE 2021

POROUS CROSSLINKED MICROPARTICLES

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PHASE I. POROUS CROSSLINKED MICROPARTICLES

OBJECTIVE 1. SYNTHESIS OF POLYMERIC CROSSLINKED MICROPARTICLES. ADVANCED CHARACTERIZATION OF THE SYNTHESIZED MICROPARTICLES AND INTERMEDIATE DERIVATIVES

A1.1. Synthesis of porous microparticles based on glycidyl methacrylate, N-vinylimidazole and mono, di and triethylene glycol dimethacrylate

A1.2. Synthesis of porous microparticles based on glycidyl methacrylate, N-vinylimidazole and divinylbenzene

A1.3. Structural characterization of the porous crosslinked microparticles

A1.4. Morphological characterization of the porous crosslinked microparticles

A1.5. Evaluation of the effects of crosslinked monomers on their functionality

OBJECTIVE 2. RESULTS DISSEMINATION

The main goal of the research project is to obtain zwitterionic porous microparticles containing zein and betaine moieties that have antimicrobial properties and the capacity to immobilize and release different drugs. In this context, the **main objective** of the first phase consisted in the **synthesis of porous microparticles** based on glycidyl methacrylate (GMA), N-vinylimidazole (NVI) and various crosslinkers [monoethylene glycol dimethacrylate (EGDMA), diethylene glycol dimethacrylate (DEGDMA), triethylene glycol dimethacrylate (TEGDMA) and divinylbenzene (DVB)] that have different functional groups capable of participating in different chemical reactions, such as polymerization, ring opening and betainization reactions. Also, the influence of different reaction parameters (monomer ratio, types of crosslinking agents, types of diluents, crosslinking degree) on the reaction yield, the swelling capacity in different solvents and the morphology of the microparticles was studied to find the optimum conditions for the synthesis of microparticles with the desired properties for subsequent chemical transformations.

OBJECTIVE 1. SYNTHESIS OF POLYMERIC CROSSLINKED MICROPARTICLES. ADVANCED CHARACTERIZATION OF THE SYNTHESIZED MICROPARTICLES AND INTERMEDIATE DERIVATIVES

Due to their functional advantages, crosslinked polymeric materials can have various applications such as sorbent materials, inert catalysts, precursors for ion exchangers, support for the immobilization of biologically active principles or microorganism cells. Porous crosslinked materials have unique properties, like high specific surface, low density and a superior sorption capacity. The characteristics of a porous material depends on the size, arrangement and shape of the pores as well as the porosity and the composition of the material itself.

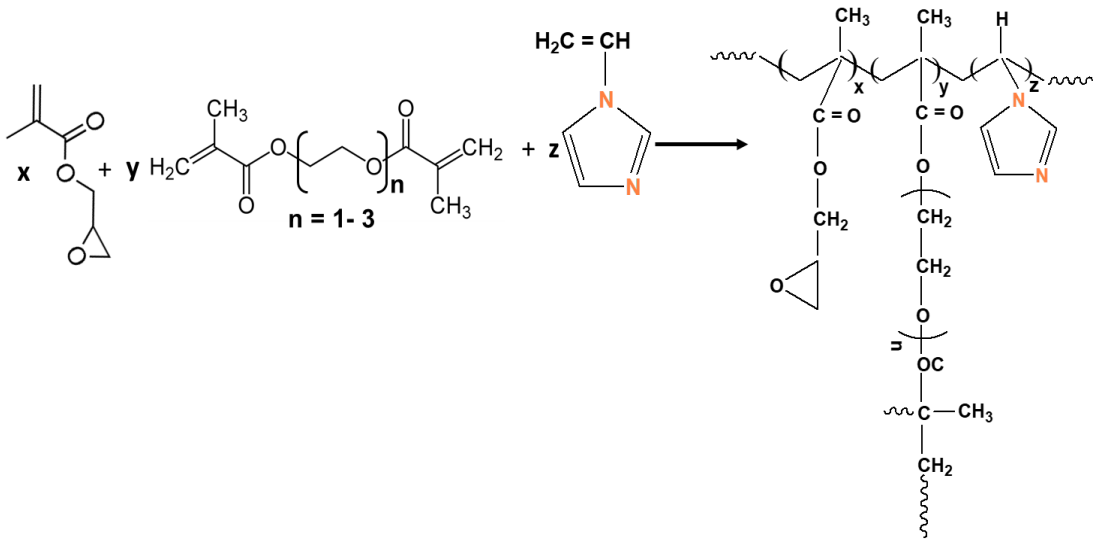
A 1.1. Synthesis of porous microparticles based on glycidyl methacrylate, N-vinylimidazole and mono, di and triethylene glycol dimethacrylate

The reaction system and the synthesis method were chosen depending on the final goal of this project, namely obtaining zwitterionic porous materials containing zein and betaine moieties. Thus:

- glycidyl methacrylate is a methacrylic monomer with a low toxicity as compared to other acrylic and methacrylic monomers and has two functional groups: a methacrylic group that can participate in radical polymerization reactions and an epoxy group which can subsequently participate in open ring reactions, thus making it possible to graft the zein in basic medium;

- N-vinylimidazole is a vinylic monomer capable of participating in radical polymerization reactions and forming biocompatible and biodegradable materials with antimicrobial activity. Also, its chemical structure contains the imidazole ring with a tertiary N atom unhindered sterically that, through polymer analogous reactions in the presence of appropriate betainization agents, can lead to polymers with betaine moieties;
- dimethacrylic monomers with two methacrylic functional groups are capable of forming crosslinked polymeric networks.

The synthesis of porous microparticles based on GMA, NVI and mono, di and triethylene glycol dimethacrylate was done by suspension polymerization following the reaction presented in Scheme 1:



Scheme 1. *The synthesis reaction of crosslinked polymeric networks based on GMA, NVI and mono, di and triethylene glycol dimethacrylate*

The suspension polymerization was chosen from the classical and modern methods of porous microparticle preparation due to a series of technical and economic advantages: low costs as compared to the diversity of porous particle properties; obtaining of pre-established internal structure; obtaining of porous structures; ease of use and separation of the resulted particles; low number of components used in the polymerization system compared to the emulsion technique; the final products purification is made through simple processes (washing, steam distillation). The suspension polymerization process consists of two phases:

- the organic phase, formed by GMA, NVI, crosslinking agent, radical polymerization initiator and the porogen agent;
- the aqueous phase, formed by distilled water as the dispersion medium and the suspension stabilizer.

The aqueous phase is introduced in the reactor, stirring the mixture for 30 minutes at 50-55°C, after which the organic phase is added dropwise.

To achieve the crosslinking radical copolymerization reaction, the temperature is raised to 78-80 °C for 8 hours, and for the ripening of the copolymer spherical particles the temperature needs to be kept at 90°C for 1 hour. After the copolymerization reaction is finished, the crosslinked porous microparticles are separated through filtration and washed with warm water and subsequently introduced in a Soxhlet apparatus for the solvent extraction of the porogen agent and possible homopolymers formed during the copolymerization reaction. For the structural and morphological characterization, the crosslinked porous microparticles are dried in vacuum, at 50°C, for 48h.

The formation of crosslinked copolymeric structures depends on the quantity and nature of both the monomers and the porogen agents. The formation mechanism of the porous microparticles through suspension polymerization is extremely complex and is presented in Figure 1.

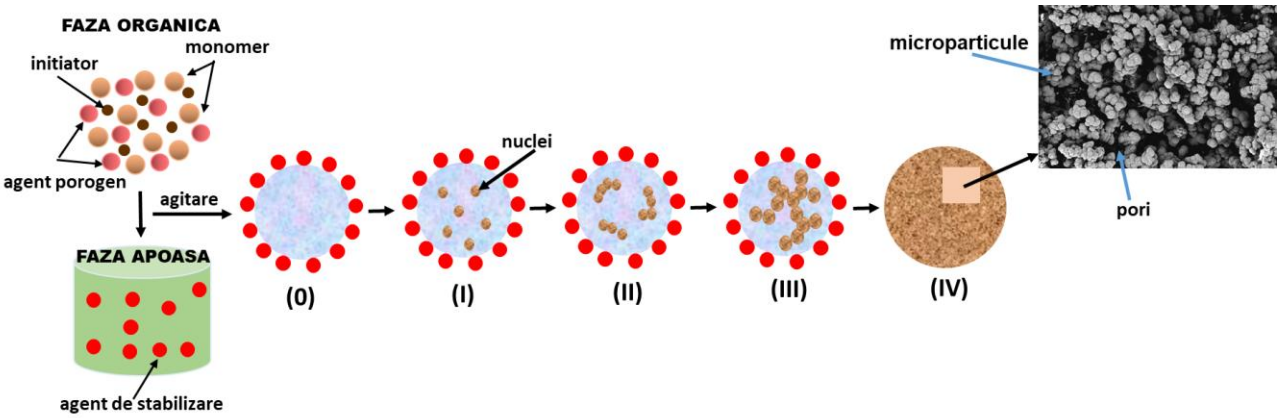


Figure 1. *The formation mechanism of the porous microparticles obtained through the suspension polymerization*

The crosslinked porous microparticles codes were established as follows:

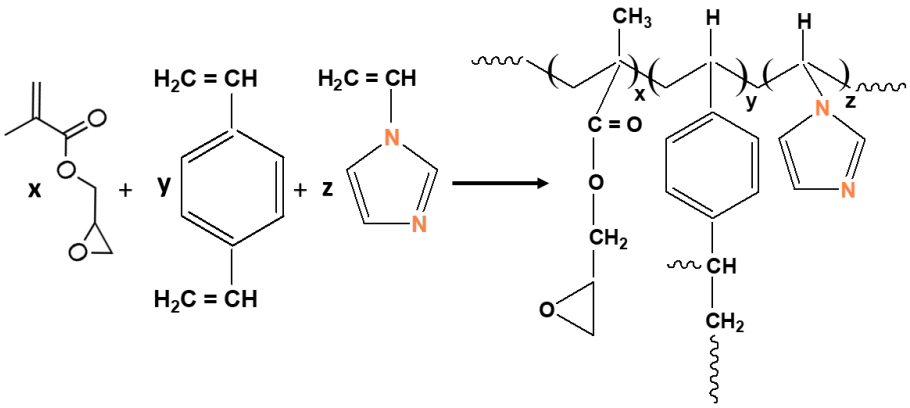
$G_xN_yE_z$ – crosslinked porous microparticles based on GMA, NVI and EGDMA;
$G_xN_yD_z$ - crosslinked porous microparticles based on GMA, NVI and DEGDMA;
$G_xN_yT_z$ - crosslinked porous microparticles based on GMA, NVI and TEGDMA.

In all cases, x, y and z are the number of moles of monomers used in the suspension polymerization reaction.

The crosslinked porous microparticles were obtained by modifying the following parameters: the molar ratio between monomers, the molecular mass and hydrolysis degree of poly(vinyl alcohol), the nature and quantity of porogen agent (toluene and n-butyl acetate).

A1.2. Synthesis of porous microparticles based on glycidyl methacrylate, N-vinylimidazole and divinylbenzene

The synthesis of porous microparticles based on GMA, NVI and DVB was also achieved through the suspension polymerization technique and can be highlighted by the chemical reaction in Scheme 2:



Scheme 2. *The synthesis reaction of the crosslinked polymeric networks based on GMA, NVI and DVB*

The suspension polymerization of GMA and NVI in the presence of DVB follows the same reaction mechanism specific to the radical polymerization and the formation of porous structures is identical to the one presented in the case of crosslinked porous microparticles obtained in the presence of dimethacrylic monomers used as crosslinking agents.

A1.3. Structural characterization of the porous crosslinked microparticles

Structurally, the crosslinked porous microparticles were characterized through FTIR spectroscopy (Figure 2), thermogravimetry (Figure 3) and elemental analysis by determining the content of nitrogen and epoxy groups (Table 1).

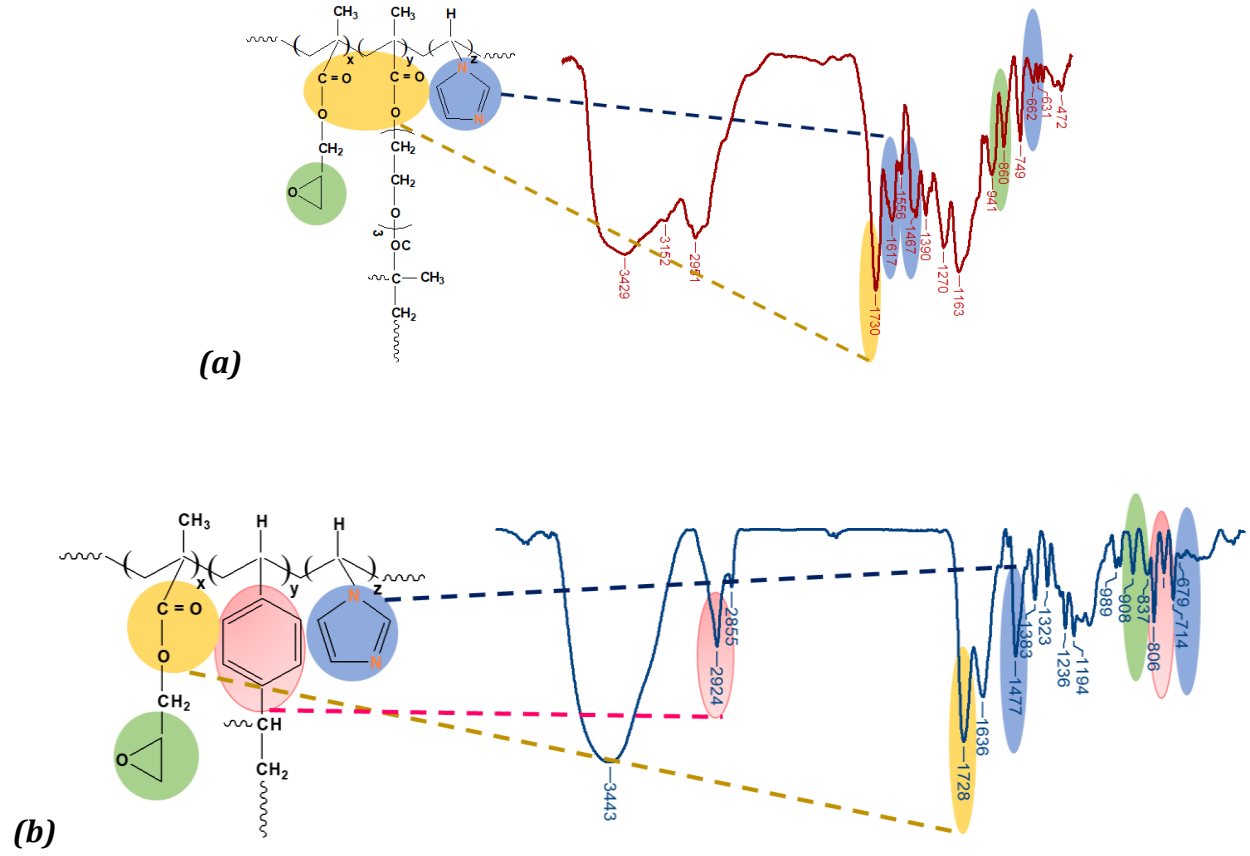


Figure 2. *The structural characterization through FTIR spectroscopy of the crosslinked porous microparticles (a)* ***G40N30T30*** *and (b)* ***G40N30DVB30***

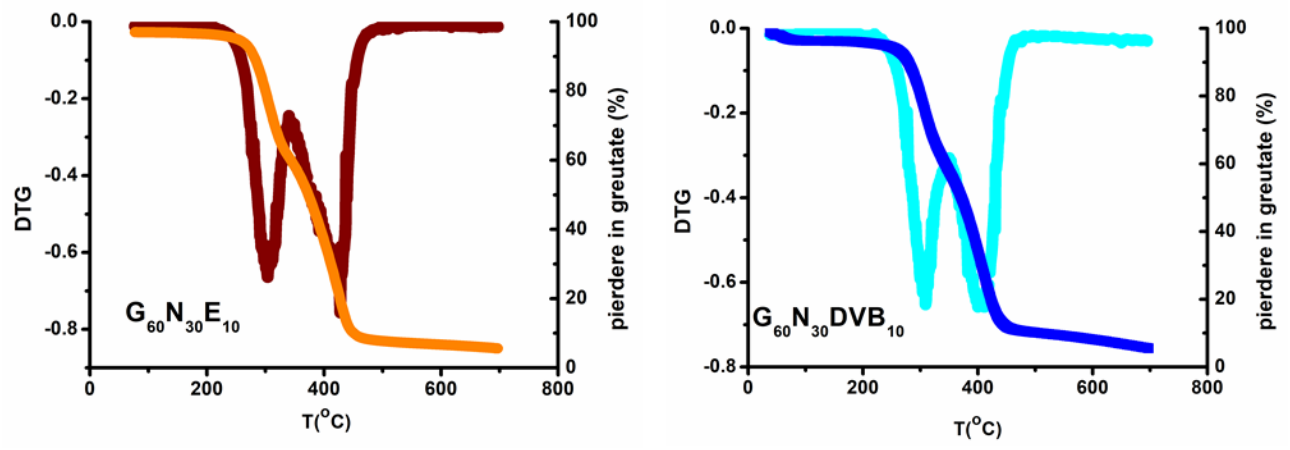


Figure 3. *Thermogravimetric analysis of the crosslinked porous microparticles*

In the FTIR spectra corresponding to $G_{40}N_{30}T_{30}$ and $G_{40}N_{30}DVB_{30}$ microparticles, the specific adsorption peaks for GMA as well as for NVI and TEGDMA or DVB can be seen, confirming the obtaining of crosslinked microparticles based on GMA, NVI and TEGDMA or DVB. Also, the thermogravimetric behaviour of the $G_{60}N_{30}E_{10}$ and $G_{60}N_{30}DVB_{10}$ porous microparticles shows that these samples are thermically stabile up to 270°C and have two degradation stages.

Regarding the elemental analysis, the determination of the epoxy groups content was achieved through the titration method that consists in opening the epoxy ring by HCl in a dioxan solution, at room temperature, and the nitrogen percent was determined through the Kjeldhal method (Table 1).

Table 1. *The porous microparticles characterization through elemental analysis (% N and % epoxy groups)*

Sample code	N (%)		Epoxy groups (%)	
	theoretical	experimental	theoretical	experimental
$G_{60}N_{30}E_{10}$	6,30	2,65	19,35	7,23
$G_{60}N_{30}DVB_{10}$	6,47	2,11	19,88	6,23

The obtained results suggest that the titration method can be useful to determine the number of epoxy groups on the surface and in the superficial layers, but not in the volume.

A1.4. Morphological characterization of the porous crosslinked microparticles

The microparticles were morphologically characterized using the scanning electron microscopy (Figure 4), atomic force microscopy (Figure 5) and particle size and shape image analyzer (Figure 6).

Both the SEM and the AFM images show that by adding a porogen agent in the reaction system, microparticles with porous structure are obtained. The microparticles size distribution is influenced by a series of factors: geometrics (reactor diameter and shape, type of stirrer), functionalities (stirring speed and temperature), suspension mediums physical characteristics (density, interfacial tension and viscosity), but also other reaction parameters (monomer ratio, the nature of crosslinker and porogen agent). The crosslinkers nature influences the size distribution of the porous microparticles, by using dimethacrylic monomers as crosslinking agents microparticles with larger diameters being obtained, as compared to the ones obtained by using DVB.

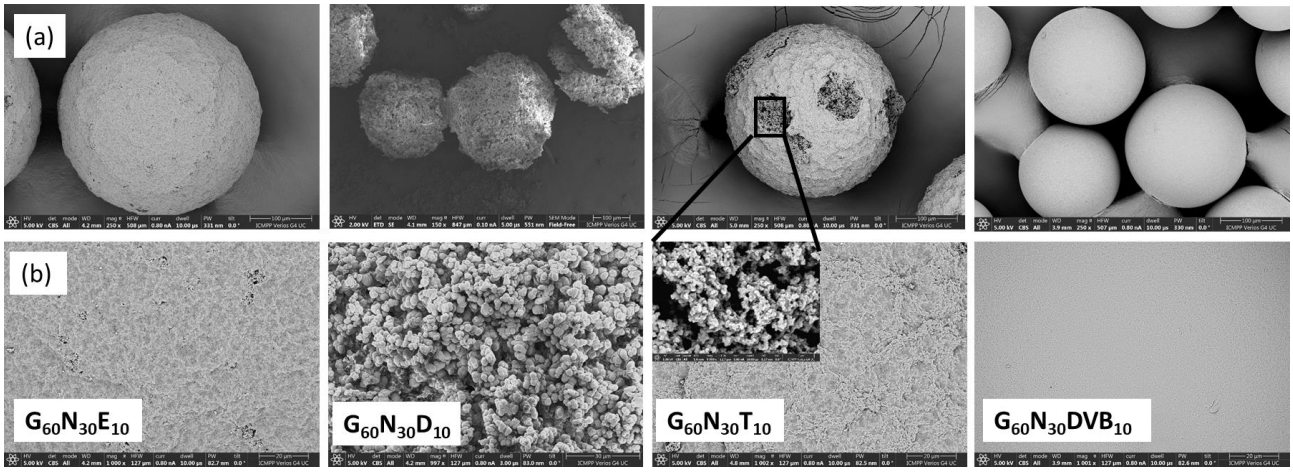


Figure 4. *SEM images of crosslinked porous microparticles (a) overview; (b) microparticles surface images*

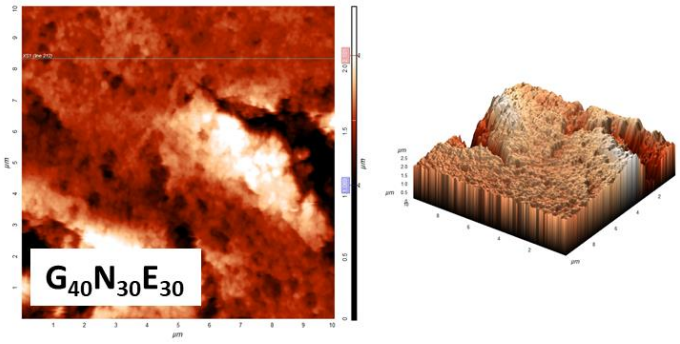


Figure 5. *AFM images (10 µm x 10 µm) of the $G_{40}N_{30}E_{30}$ porous microparticles*

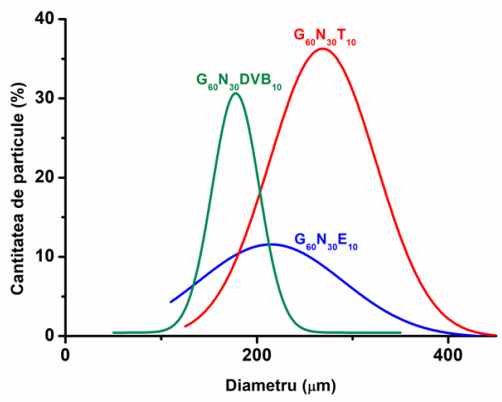


Figure 6. *The influence of the crosslinkers nature on the diameter of porous microparticles based on GMA, NVI, dimethacrylic monomers and DVB*

The behaviour in aqueous medium study of the microparticles obtained in this project phase is important for their future functionalization studies, this being the subject of the current project future phases. For example, Figure 7 presents the water swelling behaviour for:

- $G_{70}N_{20}E_{10}$, $G_{60}N_{30}E_{10}$ and $G_{45}N_{45}E_{10}$ microparticles, when the ratio between GMA and NVI (Figure 7a) is varied, the crosslinker percentage remaining constant;
- $G_{60}N_{30}E_{10}$, $G_{50}N_{30}E_{20}$ and $G_{40}N_{30}E_{30}$ microparticles, when the crosslinking degree is varied and the NVI percentage is kept constant (Figure 7b).

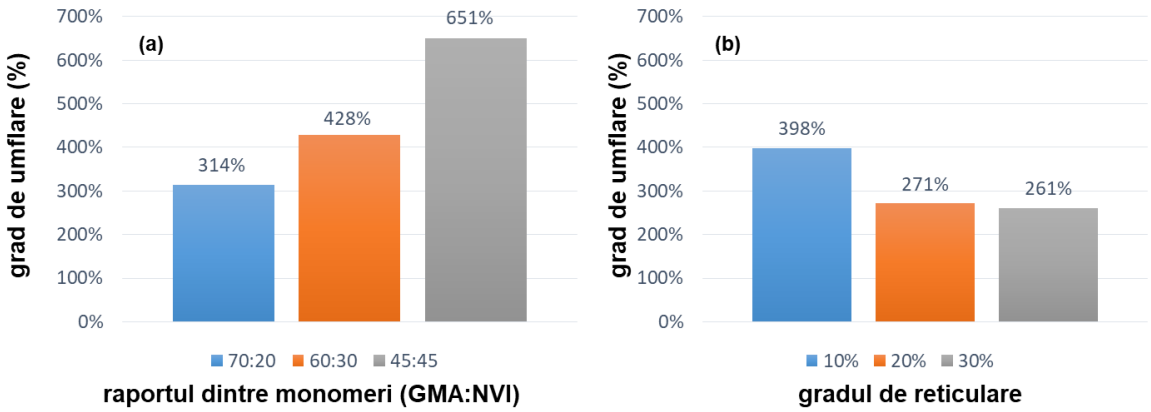


Figure 7. *The influence of the monomer ratio (a) and crosslinking degree (b) over the water swelling degree*

The water swelling degree increases with the increase of the hydrophile component (NVI) in the microparticles structure. As expected, a high crosslinking degree leads to more compact microparticles that swell less in water.

A1.5. Evaluation of the effects of crosslinked monomers on their functionality

Using a high concentration of dimethacrylic or divinylic monomer in the crosslinked network structures obtained through suspension polymerization leads to:

- the increase of the pendant double bonds number in the formed crosslinked structure;
- the increase of the reaction possibility of both double bonds;
- the decrease of the crosslinking efficiency with the conversion increase;
- the decrease of the formed structures mobility.

All of these behaviours can be interpreted based on the 3D structures evolution, the reactivity of the pendant double bonds being highly affected by the spatial correlations. This phenomenon results in the increase of the simple or multiple cyclization possibility and leads to a decrease of the double bonds reactivity due to the steric effects of the formed structures. As mentioned before, the nature and quantity of crosslinking agent influences the morphology and size of the porous microparticles, as well as the swelling degree.

The results obtained in this project phase demonstrate that using the aqueous suspension polymerization, depending on the reaction conditions, a wide range of crosslinked porous microparticles with different structures and properties can be obtained. After the optimization of the reaction conditions, the microparticles with the best properties (high specific surface, good swelling capacity and a reaction yield close to 100%) will be chosen for further adequate chemical transformations in order to obtain zwitterionic porous microparticles based on zein and betaine moieties.