SENSOPAD

SENSING ENDOMETRIOSIS ON PORTABLE AUXILIARY DEVICES

D1.1

Preliminary SAPs

NTUA

31/05/2025

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Project Information

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TITLE	SENSING ENDOMETRIOSIS ON PORTABLE AUXILIARY DEVICES
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WEBSITE	www.sensopad.eu
COORDINATOR	National Technical University of Athens (NTUA)
PROJECT OVERVIEW	To significantly reduce the diagnosis time of endometriosis (ED) through innovative, non-invasive detection technologies, thereby empowering women with early, accurate, and accessible diagnostics that improve health outcomes and reduce healthcare costs.

Document Information

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Abbreviations

SAPs	Super absorbent polymers
HPMC	Hydroxy propyl methyl cellulose
CHIT	Chitosan
PMAA	Polymethacrylic acid
ACN	Acetonitrile
AIBN	Azobis-isobutyronitrile
MBA	Methylene bis-acrylamide
MW	Molecular weight

Table 1: Abbreviations

Executive Summary

Deliverable D1.1 aims to identify the types of SAPs that will be used and the experimental parameters for their synthesis and characterization as a filling material for the core in the novel PAD that will be fabricated within the scope of the SENSOPAD project. The strategy focuses on natural, synthetic, and hybrid (natural and synthetic) polymers, with the final selection of materials and processes assessed based on the water absorbance capacity of each formulation. Additionally, the parameters studied in this deliverable included the chemical composition of the SAPs and their morphology, specifically their shape and size. In addition, the parameters studied in this deliverable were the chemical composition of the SAPs and their morphology (shape and size).

Introduction

Super absorbent polymer (SAP) materials are crosslinked polymer networks that consist of water-soluble molecular structures. These molecular structures are often acids or bases that become charged under specific physicochemical conditions(crosslinking, morphology, pH) and subsequently attain the capacity to absorb large amounts of water several times their weight[1-3]. According to the intended application, the criteria for selecting the correct SAP material can vary. However, most SAPs must have certain basic characteristics, such as the ability to absorb several times their weight in water, to have a minor crosslinking degree to be flexible, stability during swelling, and excellent durability.[4]. Although there is an existing plethora of SAP materials, three categories stand out based on their composition: the natural polysaccharide-based, the polyacrylate-based, the semi-synthetic, which are past natural part synthetic parts, and the smart SAPs, which exhibit one or more additional properties according to application.[5]

Natural SAPs emerged as an alternative because they were considered biodegradable, abundant in nature, biocompatible, and non-toxic. Furthermore, their synthetic counterparts require raw materials based on crude oil, which are constantly increasing in price, whereas the raw materials for natural SAPs can be found in nature and can be chemically processed. Such a process is the acetylation of polysaccharides like cellulose to produce methylcellulose, hydroxypropyl methylcellulose, and carboxymethylcellulose. Other polysaccharides used for the synthesis of SAPs can be chitosan, dextrin, starch, and gellan gum.[6-8].

On the other hand, most SAPs nowadays utilize synthetic materials, mainly crosslinked polyacrylates.[9]. The main reason for this is that synthetic materials can be synthesized with precise control over their physicochemical properties, thus developing tailored properties. Parameters such as the molecular weight, the functional groups, the morphology and consequently the surface area of each polymer has an immediate effect on the water absorbent properties of the final product[10]. Nevertheless, when the application demands both biocompatibility and tailored properties, then hybrid natural synthetic SAPs can be utilized to answer the challenge. These hybrid structures are synthesized when acrylic monomers are polymerized in aqueous solution in the presence of water-soluble polysaccharide and a crosslinker usually methylene bis-acrylamide(MBA)[11].

During the preliminary phase of the SENSOPAD project, deionized water was chosen as the testing medium to evaluate the water absorption performance of natural, synthetic, and hybrid superabsorbent polymers (SAPs). This choice was based on the need to establish a baseline understanding of the material properties under highly controlled and reproducible conditions. Water provides a standardized medium that complies with ASTM and ISO testing protocols for hydrogels and SAPs, allowing for reliable comparative assessments across various formulations and manufacturing methods. Notably, using water reduces variability associated with complex biological fluids, such as viscosity, salt concentration, protein content, and pH—all of which can introduce confounding effects during the early stages of material optimization. Several studies in SAP development validate the common practice of using water in initial assessments before transitioning to more physiologically relevant media.

Nonetheless, we fully acknowledge that water does not replicate the complexity of menstrual fluid, which is known to have higher viscosity, non-Newtonian flow characteristics, a variable pH, and a higher ionic and organic content. As such, the next steps of the project (Task 2.1) will explicitly focus on the use of artificial menstrual fluid (AMF) formulations that simulate the rheological and biochemical properties of real menses. Our proposed staged approach enables robust benchmarking: by first assessing SAP performance in water, we can isolate and fine-tune intrinsic material parameters (e.g. porosity, crosslinking degree, polymer ratio) without interference from biological factors. Subsequently, testing under AMF conditions will provide clinically and commercially relevant performance metrics. Our strategy aligns with recent literature advocating for a two-step SAP evaluation process, beginning with standardized water-based tests and progressing to simulated or real biological fluids. Our two-step design minimizes risk, ensures scientific rigor, and allows us to adapt SAP compositions with greater confidence when moving into application-specific testing environments. The absorption of menstrual fluids commercially is currently governed by synthetic SAP materials. However, efforts are being made to incorporate natural components to render the final product more biocompatible[12]. The main issue in the absorption of menstrual fluids is the fundamental differences with water in terms of viscosity, salt concentration, pH, and temperature. This phenomenon is attributed to lower water absorbance that SAPs exhibit in salt water compared to distilled water, due to the differences in osmosis. These challenges are the focus in contemporary SAP technology, whereas addressing those challenges is the aim of Tasks 1.1 and 2.1 of the SENSOPAD project.[13].

1. Materials and Methods

1.1 Materials

Chitosan (MW 100000-300000), Hydroxy propyl methyl cellulose (HPMC, MN: 86000, cP: 4000) acetic acid, where purchased from thermo scientific and were used to synthesize natural polymer hydrogels via the physical crosslinking method. Acetonitrile (ACN), methacrylic- acid (MAA), methylene bis-acrylamide (MBA), azobis-isobutyronitrile were purchased from Acros organics and were used to synthesize the synthetic SAP microparticles via the distillation precipitation. The hybrid SAP was synthesized by polymerizing methacrylic acid in the presence of HPMC (natural polymer) and the crosslinker MBA and the initiator ammonium persulfate (APS) in aqueous medium.

For the investigation of candidate materials for electrospinning (by BIOG3D), desired properties were first clearly defined to facilitate the selection of suitable materials. These properties include high liquid absorption capacity, mechanical stability, biodegradability, sustainability, biocompatibility, and electrospinnability. Based on these requirements, biopolymers such as cellulose acetate, chitosan, and silk fibroin were chosen for the formation of a multilayered membrane intended to serve as the absorbent core of the pad. Cellulose acetate (30kDa), low molecular weight chitosan and methacrylated silk fibroin were purchased from Sigma Aldrich. Acetic Acid (99.8%) and Acetone (99.8%) were purchased from Honeywell, Dimethyl-Formamide (99.8%) was purchased from Sigma Aldrich and Formic acid (99.8%) was purchased from Carlo Erba Reagents to serve as solvents for the polymers. Genipin (99.8%) and Glycerol were purchased from Sigma Aldrich to potentially serve as additives (crosslinker for chitosan, plasticizer).

1.2 Methods

Scanning electron microscopy (SEM) was employed to determine the size and shape of the synthesized SAPs, utilizing an FEI Inspect microscope with a Tungsten filament operating at 25 KeV. SEM images were analyzed in the ImageJ software to measure the average diameter of microspheres in the case of the synthetic SAPs. Infrared spectra (FT-IR) were recorded using a Cary 630 Agilent FTIR spectrometer equipped with a diamond ATR crystal, whereas Yingtai freeze drier was utilized to dry the SAPs prior to the water absorbance tests. Water absorbance was measured according to the following protocol:

- 100 mg of freeze-dried SAPs were weighed (Wd) in a glass petri dish
- 10 ml of water was added to fully submerge the material
- The sample was left to rest for 30 minutes
- The swollen SAP is separated via filtration (cellulose filter) and is weighted (Ws)

Subsequently, the water absorption (A%) is calculated according to Equation 1

Equation 1

$$A = \frac{\mathrm{Ws} - \mathrm{Wd}}{\mathrm{Wd}}$$

2. Experimental

The synthesis of the natural polymer hydrogel was accomplished via hydrogen bonding formation. In short, two separate 2% solutions of chitosan and HPMC were prepared in phosphoric acid (0.1M) and water, respectively. The HPMC was slowly added to the water solution after it had reached a temperature of 85 °C. After the solution cooled down, the hydrogel solutions were mixed and stirred magnetically until a homogenous solution was evident. Subsequently, an appropriate amount of acetic acid (0.1M) was added to the solution to induce hydrolysis and promote the hydrogen bonding between the 2 polymers. The solution was left to stir for 30 minutes. Two different formulations were prepared concerning the ratio of chitosan to HPMC (80:20, 70:30).

The synthetic PMAA microparticles were synthesized according to the distillation precipitation method. In short, 350 mL of ACN were added to 5 mL of methacrylic acid, 3% (relevant to the monomer) of the crosslinker MBA. The solution was kept under N2 flow and constant stirring until the temperature reached 75 °C, at which point the initiator AIBN was inserted to generate radicals and initiate the polymerization. After approximately 10 minutes, the color of the solution transitioned from transparent to milky white, indicating particle formation. At this point, the temperature increased to 95 °C to start the distillation. after 40 minutes, 80 ml of ACN were distilled. The size of the particles varied by adjusting the monomer concentration (nucleation) and the amount of distilled ACN (growth).

For the hybrid natural-synthetic SAPs a combination of the previous procedures was utilized. In short, the HPMC water solution was prepared according to the previous procedure and then MAA and MBA were added and left to stir together to homogenize. Subsequently, the initiator was added when the temperature reached 75 °C to initiate polymerization. Three different HPMC to MAA ratio were prepared (1:1, 1:2, and 1:4).

All the samples, upon completion of each procedure, were freeze dried at 0.1mbar/-70°C for three hours. The crosslinking degree for the synthetic and the hybrid formulation was kept constant at 3% in relation to the monomer.

Each sample was ionized by dissolving (1mg/ml) of SAPs in 0.1M NaOH solution and was left to stir overnight. Upon completion the hydrogel formed was freeze-dried as previously.

3. Results and Discussion

3.1 Natural polymers HPMC-Chitosan

Figure 1 exhibits the FTIR spectra of HPMC and chitosan (Fugure1a) and the FTIR spectra of the corresponding hydrogels with different HPMC to Chitosan ratios (Figure1b). From Figure 1 a) it is evident that both HPMC and Chitosan exhibit similar vibrations since both polymers are in the polysaccharide class. Nevertheless, from the HPMC spectra, the band at 3444.60 cm^{-1} is assigned to the stretching vibration frequency of the hydroxyl (OH) group, whereas the band at 1373.63 cm^{-1} is due to the bending vibration of -OH. Other stretching vibration bands related to C-H and C-O were observed at 2929 cm^{-1} and 1055.52 cm^{-1}, respectively. However, the bands that separate chitosan from HPMC are those corresponding to the amide bonds and in Figure 1b are located at 1650 and 1595 cm^{-1}, whereas the bands located at 3500 and 3000 cm^{-1} are attributed to the OH and NH stretch vibrations, respectively. Assessing figure 1b, both hydrogels exhibit the same bands observed in the individual polymers before the physical crosslinking, however, the 80:20 sample presents increased intensities in the bands between 3200 and 3000 cm^{-1} and the band 1400 cm^{-1}. The difference in intensities was attributed to the residual water content of each sample after the freeze-drying procedure. From their spectra, it is evident that the hydrogel with increased chitosan content (70:30) contained more water than the 80:20, which was drier. This observation agrees with the corresponding water absorption results for the natural polymers (Figure 2). The bands observed for HPMC and Chitosan aligned with the results found in the literature[14, 15].

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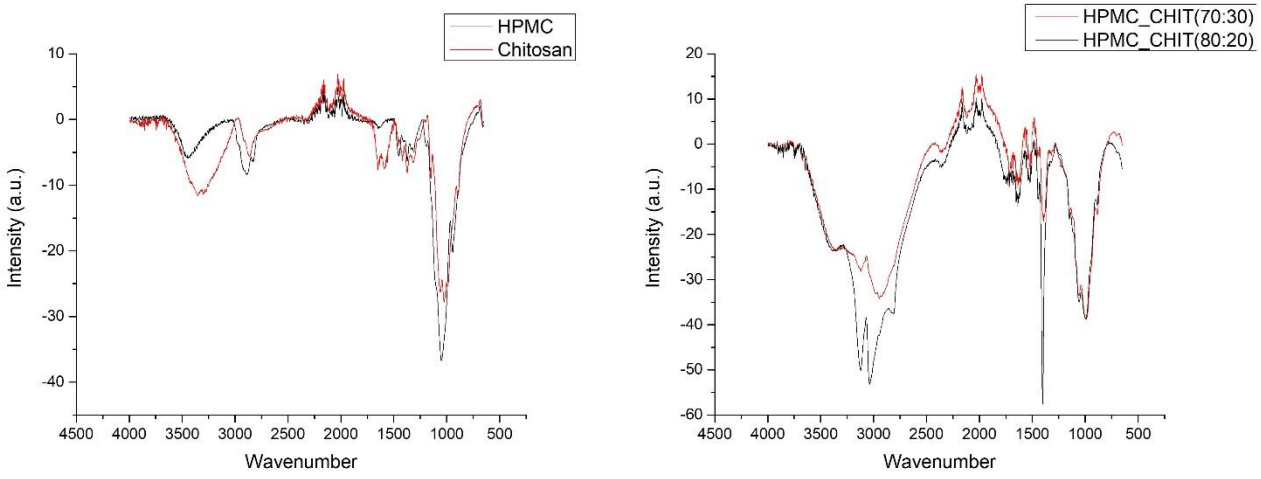

a) b)

Figure 1 a) FTIR spectra of HPMC and Chitosan, b) FTIR spectra of hydrogels with 2 different HPMC to Chitosan ratios (70:30, 80:20)

Figure 2 shows the water absorption results for the two hydrogels. It is evident that the sample with increased chitosan content (70:30) absorbed more water, however, both samples presented low water absorption capacity, below 10g/g.

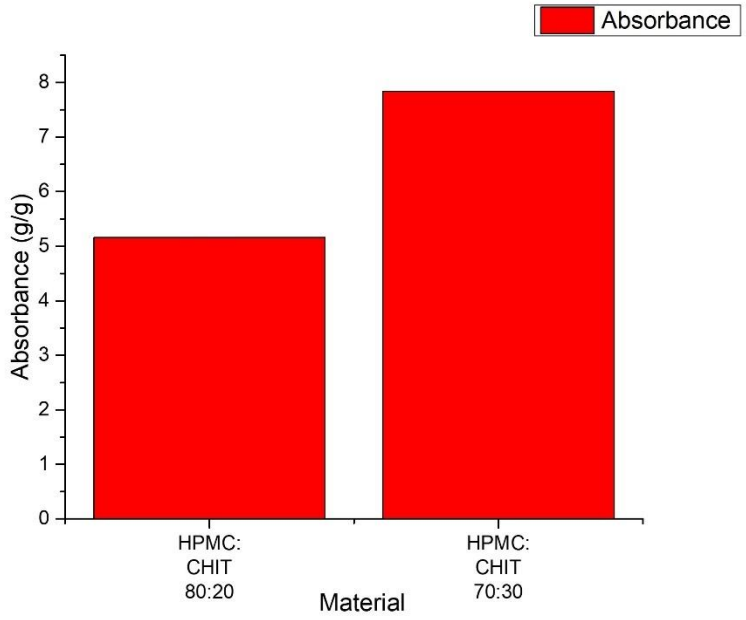

Figure 2 Water absorption results for the two natural hydrogels.

3.2 Synthetic SAPs, PMAA microspheres.

Crosslinking of the polymer microsphere is vital for the spherical shape to be retained in aqueous media; Infrared spectroscopy was utilized to qualitatively identify the crosslinking of the P(MAA-co-MBA) microspheres. Therefore, an additional sample of PMAA microspheres was prepared following identical concentrations and conditions, but in the absence of a crosslinker; Figure 2 illustrates the results. It can be observed that both spectra exhibit a strong vibration at approximately 1699 $cm^{-1,}$ which is attributed to the carbonyl vibration, yet the P(MAA-co-MBA) IR spectrum exhibits an additional peak at 1531 cm^{-1} corresponding to the amide vibration crosslinker molecule.

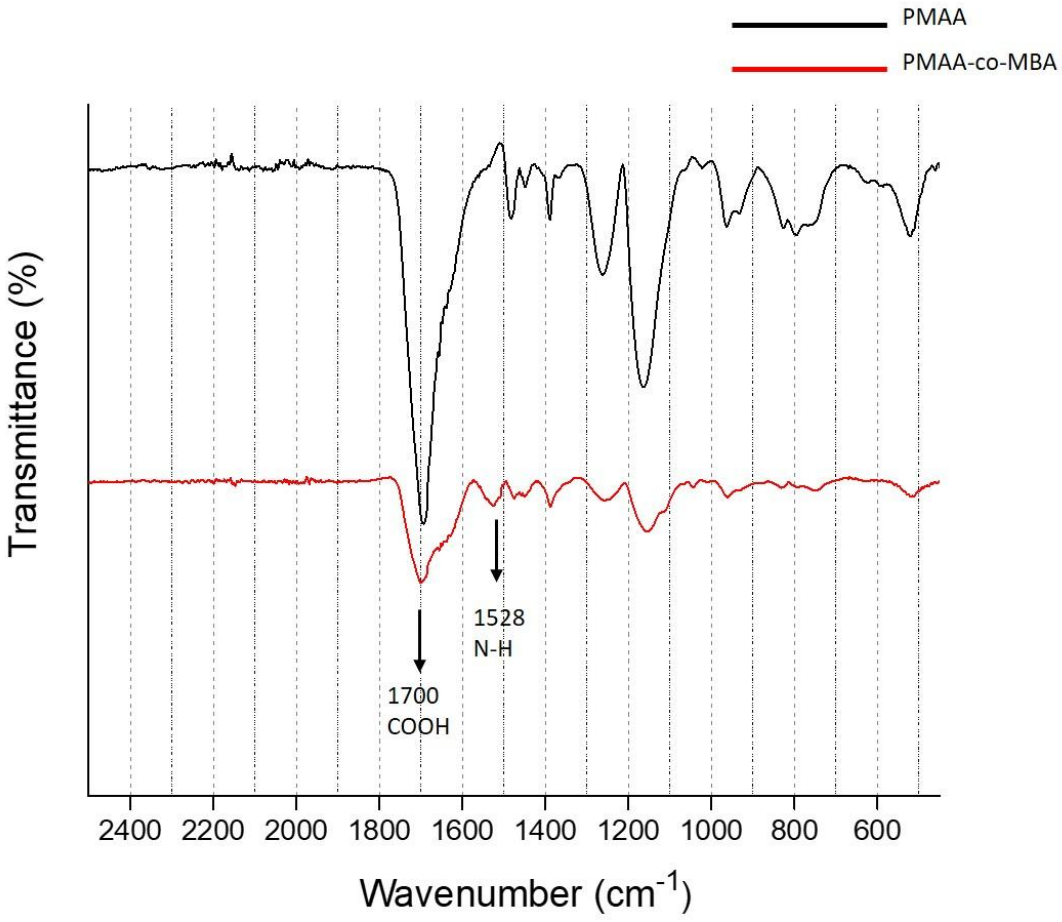

Figure 3 FTIR spectra of PMAA and PMAA-co-MBA crosslinked microspheres

The initial experiments regarding the PMAA microspheres focused on assessing the effect of the size of the microspheres on the water absorption, therefore, four different samples were synthesized. The results of the SEM microscopy are presented in Figure 4.

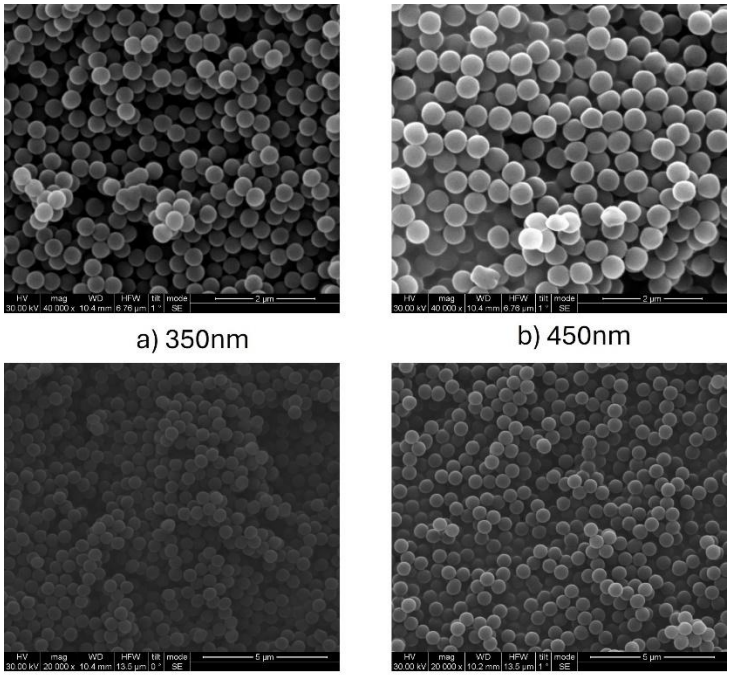

Figure 4 SEM microscopy images for the PMAA samples with different sizes

From Figure 4, it is evident that with increasing monomer concentration as well as distillation volume, the size of the microspheres increases, as it has been previously reported for these nanostructures[16]. Upon polymerization, the four samples were centrifuged and completely dried under vacuum overnight. The next day, all samples were ionized with NaOH and then freeze-dried to be tested for water absorbance. according to the protocol described in the experimental section. Figure 5 a,b) illustrates the SEM images of the PMAA samples with diameters 350 and 570 nm before and after water absorption, whereas Figure 5 c) exhibits the water absorption results for all 4 samples.

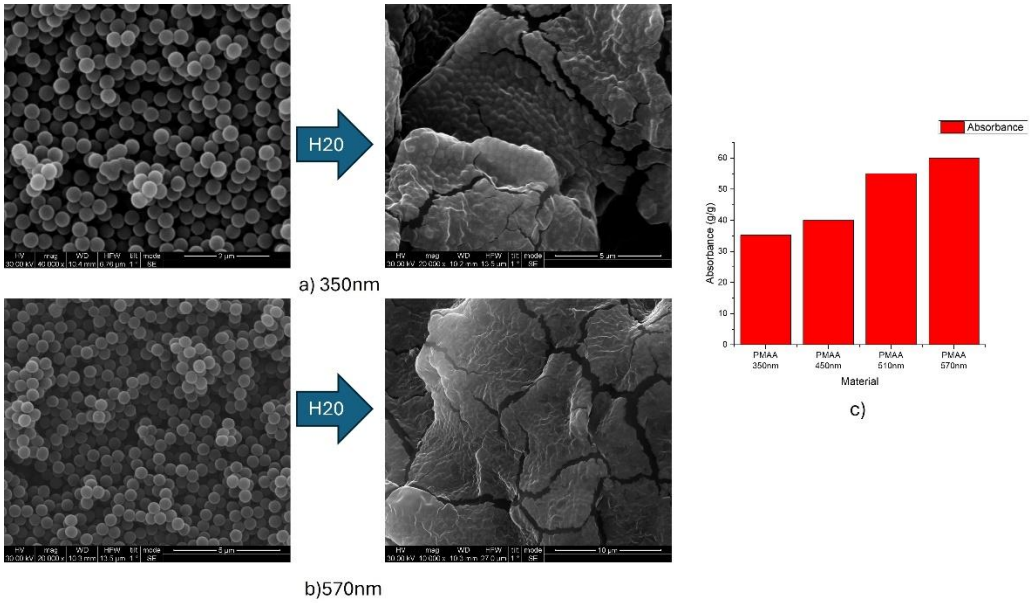

Figure 5 SEM images of PMAA microspheres (a) 350nm, b) 570 nm) before and after water absorption and c) water absorption results for all PMAA samples

From figure 5a, b it is evident that the microspheres with 350nm diameter aggregate but still retain their spherical shape after their water immersion, whereas the larger microspheres (570nm diameter), after their water immersion lose their spherical shape and aggregate with each other to form a homogenous hydrogel. On the other hand, from Figure 5 c, it is observed that water absorption increases linearly with increasing microsphere diameter reaching a maximum value of 65% for the larger microspheres (570nm). Comparing the water absorption results with the SEM morphology images, before and after water absorption, it can be hypothesized that the larger microspheres, when immersed in water, increase in size enough to form a hydrogel that traps water, whereas smaller microspheres aggregate but do not form a homogenous hydrogel after water absorption. Nevertheless, these results are preliminary, and further research is needed, particularly considering the degree of crosslinking.

3.1 Hybrid SAPs, HPMC-PMAA hydrogels.

As was previously described in the experimental section, three formulations of HPMC-PMAA were synthesized with different HPMC to PMAA ratios, 1:1, 1:2, and 1:4. Figure 6 summarizes the FTIR results.

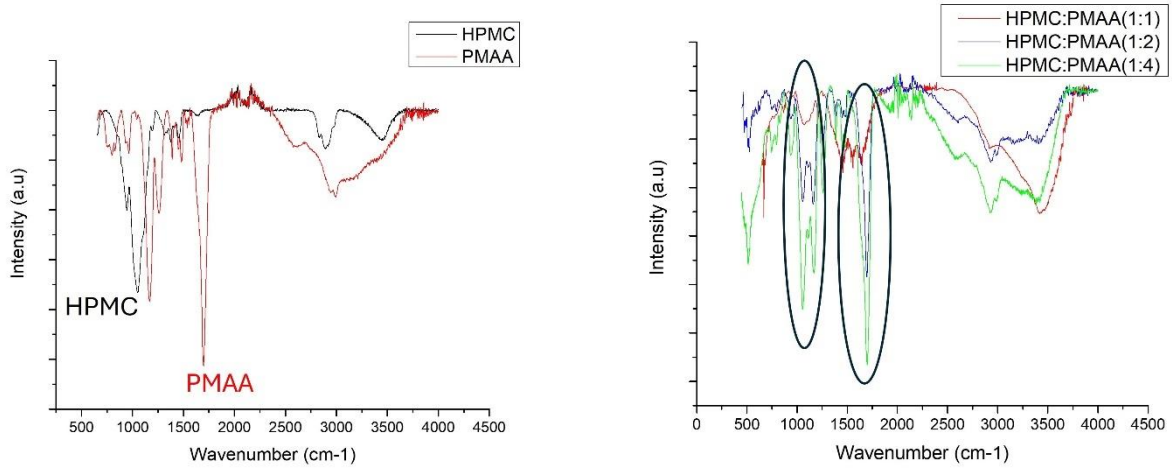

Figure 6 FTIR spectra of a) HMPC and PMAA polymers, b) hybrid HPMC-PMAA polymers with different ratios.

The most characteristic peaks identified for each polymer in Figure 6a) are the C-O stretch vibration at 1055 cm^{-1} and the C=O at 1650 $cm^{-1,}$ which are attributed to the HPMC and PMAA molecules respectively. Furthermore, these bands are also identified in the FTIR hybrid hydrogel structures in figure 6b), however, they present different intensities. The intensity of the C-O stretch vibration, related to HPMC, decreases, whereas the C=O stretch vibration increases its intensity with increases PMAA content. These results agree with similar efforts reported in the literature regarding the fabrication of hybrid natural-synthetic hydrogels[11, 17]. The morphology of the formulations with different ratios was further characterized with SEM analysis, and the results are presented in Figure 7a-d.

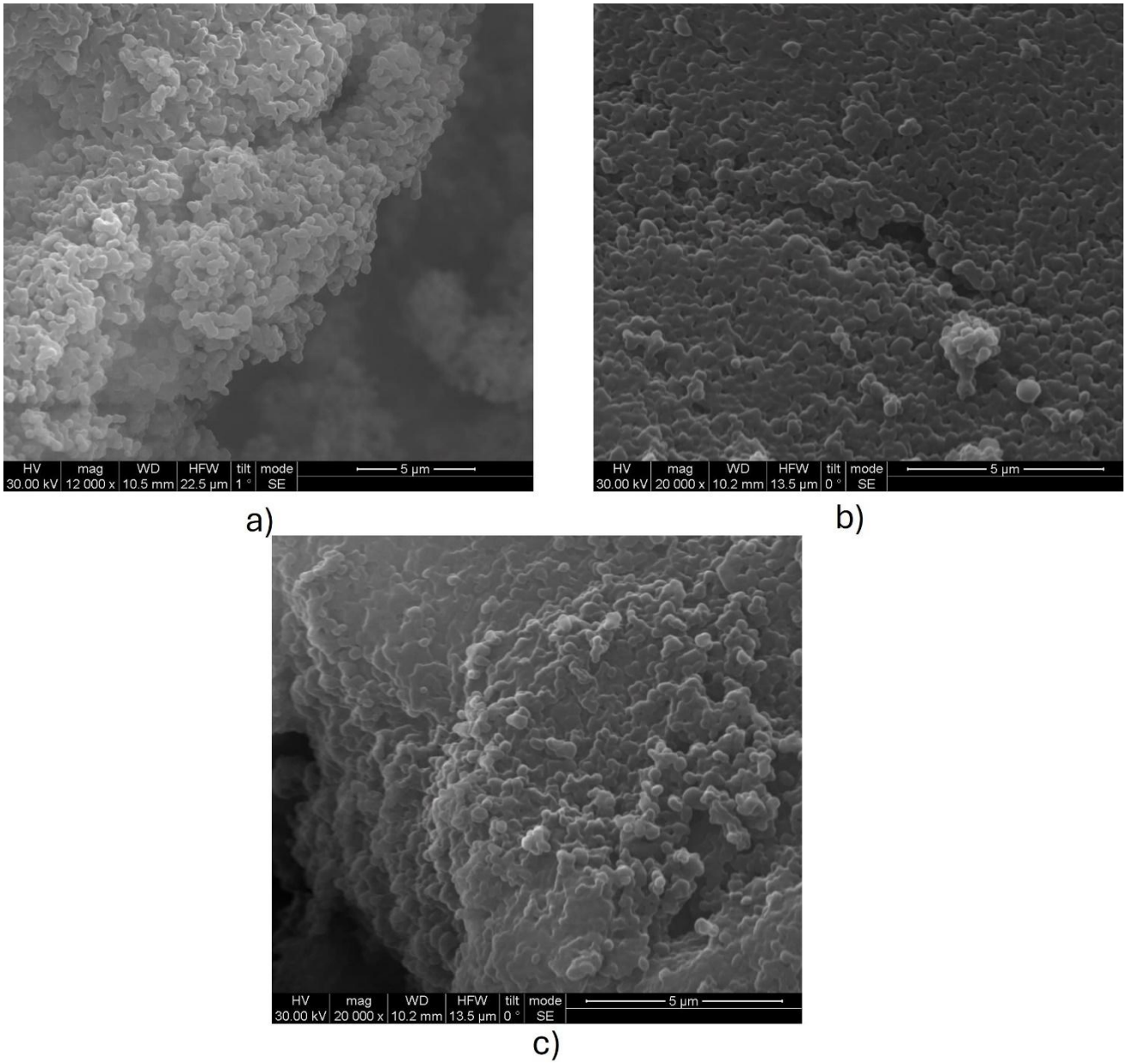

Figure 7. SEM pictures of a) HPMC-PMAA 1:1, b) HPMC-PMAA 1:2, c) HPMC-PMAA 1:4

It is evident from Figure 7 that all formulations exhibit the morphology of agglomerated microparticles with diameters ranging from 250 to 500nm. Nevertheless, upon closer inspection of the images, the particle aggregation intensifies with increasing PMAA content. According to the literature, the particle formation takes place due to the HPMC's low critical solution temperature (LCST), which is at 50 °C, whereas the polymerization occurs at 65- 70 °C. In temperatures above the LCST, the HPMC polymer chains become hydrophobic, thus favouring polymer-polymer interactions and therefore the formation of spherical structures. In addition, the subsequent polymerization and crosslinking of MAA, which is physically bonded (H bonding) to the HPMC molecule, secures the overall structure in this formation even after the temperature is decreased below 50 °C[18]. The microsphere aggregation observed is hypothesized to originate from the hydrogen bonding between the hydroxyl groups of PMAA.

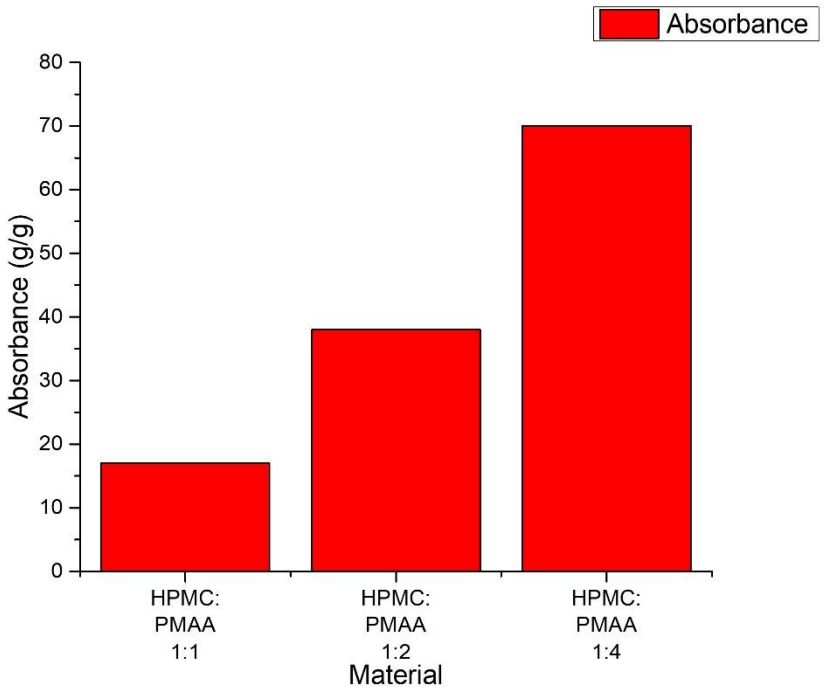

Figure 8. Water absorption results

Figure 8 exhibits the water absorption results for the three different HPMC to PMAA ratios. Water absorption increases with PMAA content, reaching a maximum of 74 g/g. This is occurring either due to the superiority of PMAA as a water absorbent material or due to the aggregation of the microspheres, as previously described in the synthetic samples. Nevertheless, it is possible that both mechanisms could take place simultaneously. Further experimental evidence will take place to reach an outcome.

Conclusion

Figure 9 exhibits the water absorption results of all the materials produced for comparison.

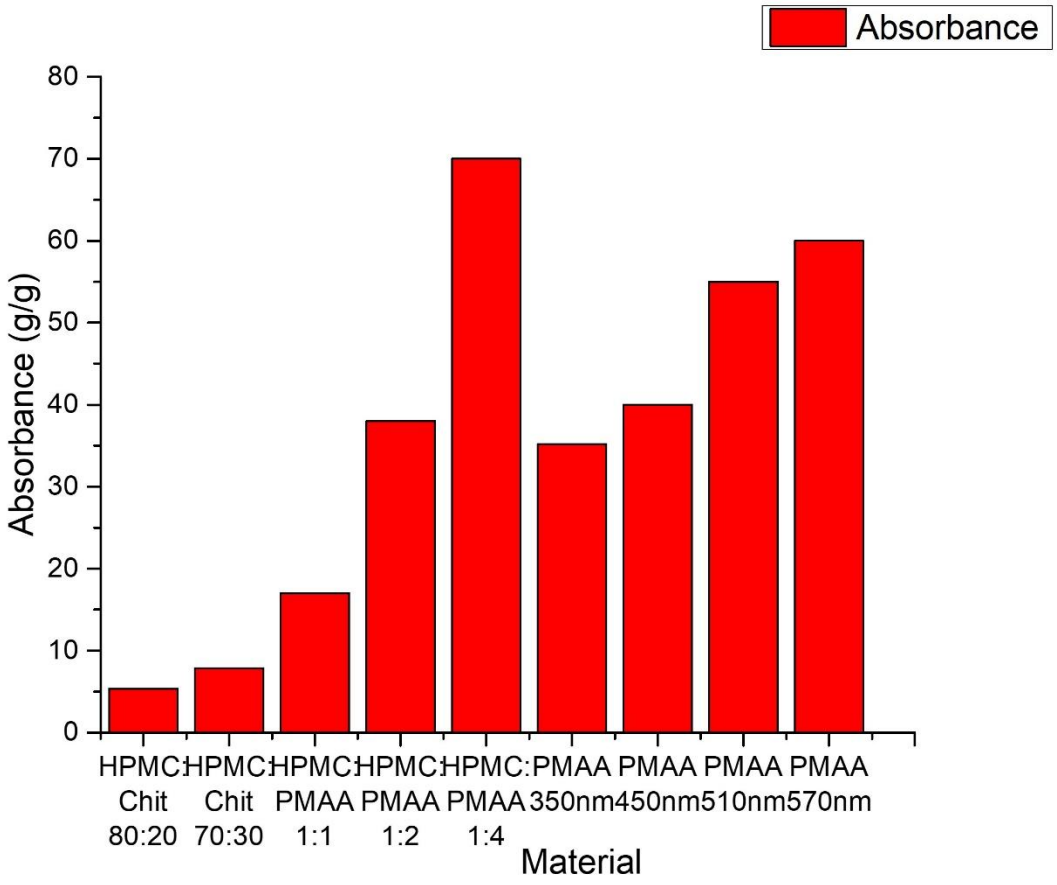

Figure 9 Summary of water absorption results

HPMC-PMAA 1:4 and PMAA microparticles with a diameter of 570 nm showed the most promising results. The common factor between these two materials was the aggregation potential of the polymer particles before and after water absorption. The natural polymers exhibited lower water absorption compared to the hybrid and synthetic materials, likely due to the nature of the physical crosslinking between the natural polymer HPMC and chitosan. Although many techniques exist to promote chemical crosslinking in natural polymers, the processes and materials used are not environmentally friendly (e.g., organic solvents, elevated temperatures, complex compounds), which undermines the goal of producing materials through green processes. Therefore, the next steps in the synthesis of SAPs will focus on further optimizing the hybrid and synthetic formulations.

4. Next Steps

Following the completion of D1.1, the next steps will focus on:

- Study of the effect of the crosslinking degree of selected SAP
- Study of the ionization effect on the water absorbent properties.
- Execution of the water absorption measurements according to standardized protocols (ISO, ASTM)
- Water retainability measurements, and
- Salt water and menstrual fluid absorption capacity measurements.

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