

INVESTIGATING THE INTERACTION OF 3D CONDUCTIVE POLYMER COMPOSITES WITH BACTERIAL CELLS

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**Submitted in fulfillment of the requirements for the degree of Master
of Science in Biomedical Engineering**



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April 28, 2023

DECLARATION

I hereby declare that this manuscript, entitled “*Investigating the interaction of 3D conductive polymer composites with bacterial cells*”, is the result of my own work except for quotations and citations which have been duly acknowledged. I also declare that, to the best of my knowledge and belief, it has not been previously or concurrently submitted, in whole or in part, for any other degree or diploma at Nazarbayev University or any other national or international institution.



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Abstract

Flexible wearable sensors have attracted huge attention in a variety of fields, including sports, healthcare, and robotics, to overcome the limits of present sensor materials. Electrically conducting hydrogels are considered viable materials for wearable sensors, highlighting their unique features such as high-water content, biocompatibility, and tunable mechanical and electrical properties. Typical hydrogels comprise polysaccharides, synthetic hydrophilic polymers, and relevant crosslinkers. In this work, novel conductive polymer-based hydrogel systems with tunable electrical and mechanical properties were developed. More specifically, the individual contributions of Polyacrylamide (PAAm), Chitosan (CS), Phytic acid (PA), and Polyaniline (PAni) to the enhancement of the hydrogel's electrical sensitivity and stability under strain were investigated. PAAm, and chitosan with superior mechanical characteristics and biocompatibility, are widely utilized hydrogel materials, however, their strain and electrical sensitivity are limited. Hence, PA and PAni are two compounds that can increase the electrical sensitivity and mechanical stability of hydrogels. PA is known to create strong hydrogen bonds with polymer matrices, enhancing their mechanical strength. In contrast, PAni has exceptional electrical conductivity and may transmit electrical impulses in response to mechanical strain. Accordingly, the synthesis of conductive polymer-based hydrogel and its characterization, including mechanical and electrical properties sensitivity were investigated in detail. The study of the antibacterial properties of conductive polymer-based hydrogels was also provided.

Acknowledgments

I want to express my sincere thanks to my supervisors, Drs. Nurxat Nuraje, Tri Pham, and Salimgerey Adilov, for their countless discussions and advises. Throughout the research process, my thesis advisor, Dr. Nurxat Nuraje, provided valuable suggestions, support, and encouragement. Without the knowledge and support of Dr. Nurxat Nuraje, this thesis wouldn't have been achievable.

I would also like to thank my colleagues, PhD student Dana Kanzhigitova and Dr. Munziya Abutalip, and other researchers who provided insightful feedback and ideas on my work. Their insightful feedback and suggestions have assisted me in improving the quality of this work.

In addition, I would like to acknowledge the laboratory employees for their support and help during the experiments. Their technical knowledge and commitment were crucial to the accomplishment of this research.

Eventually, I would like to express my gratitude to my mother as well as to my family for their emotional support throughout my research.

Table of Contents

Abstract.....	3
Acknowledgments.....	4
Table of Contents.....	5
List of Abbreviations & Symbols.....	6
List of Figures.....	7
Chapter 1 – Introduction.....	8
1.1 Aim of research.....	9
1.2 Hypothesis and goals.....	10
Chapter 2 – Literature review.....	11
2.1 Conductive polymers: polyaniline.....	11
2.2 Polysaccharides: Acrylamide, Alginate, and Chitosan.....	12
2.3 Phytic acid.....	14
2.4 Conductive Polymer Hydrogels.....	15
Chapter 3 – Materials and Methods.....	18
3.1 Materials.....	18
3.2 Preparation of Alginate hydrogel with and without conductive polymer.....	18
3.3 Preparation of Alg-PA hydrogel with and without conductive polymer.....	19
3.4 Preparation of dual-crosslinked Alg-PA hydrogel with and without conductive polymer.....	19
3.5 Preparation of PAAm/PA-CS hydrogel with and without conductive polymer.....	20
3.6 Testing mechanical properties of hydrogels.....	21
3.7 Fourier Transform Infrared (FTIR) analysis.....	21

3.8 Scanning Electron Microscope (SEM).....	22
3.9 Swelling property test.....	22
3.10 Conductivity test.....	22
3.11 Antibacterial test.....	23
Chapter 4 – Results and Discussion.....	23
4.1 Alginate hydrogel sample preparation with CaCl ₂ and Phytic acid.....	23
4.2 PAAm/PA-CS hydrogel preparation with conductive polymer (PAni).....	27
4.3 Testing mechanical properties and electrical sensitivity of hydrogel.....	29
4.4 Conductivity test.....	33
4.5 Swelling properties.....	35
4.6 FTIR analysis.....	35
4.7 Morphology analysis by Scanning Electron Microscopy.....	37
4.8 Antibacterial test.....	40
Chapter 5 - Conclusion.....	42
Bibliography/References.....	43

List of Abbreviations & Symbols

Alg	Alginate
Ani-HCl	Aniline Hydrochloride
APS	Ammonium persulfate
CS	Chitosan
CP	Conductive polymer
DI	Deionized water
FTIR	Fourier Transform Infrared
KPS	Potassium persulfate
kPa	Kilo Pascals
k Ω	Kilo Ohms
MBAA	Methylene-bis-acrylamide
PAAm	Polyacrylamide
PAni	Polyaniline
PA	Phytic acid
SEM	Scanning Electron Microscope
SA	Sodium Alginate
SDE	Swelling Degree
S cm ⁻¹	Siemens per centimeter
UV	Ultraviolet
3D	Three-dimensional

List of Figures

Figure 2.1. Chemical structure of most used conjugated polymers [1]	13
Figure 2.2. Synthesis of polyaniline	14
Figure 2.3. Schematic synthesis of chitosan (Chandrasekaran et al.)	15
Figure 2.4. Chemical structure of Phytic acid	16
Figure 3.1. TA.XTplus, Stable Micro Systems, SIMAS™	20
Figure 3.2. Nicolet iS10 FT-IR Spectrometer	20
Figure 3.3. Lyovapor L-200 and SEM ZEISS Crossbeam 540. Gold coated samples	21
Figure 3.4. Resistance measurement	22
Figure 3.5. Antibacterial test	22
Figure 4.1.1. Schematic diagram of Alginate hydrogel synthesis with different percentages (% w/v) and the addition of monomer with polymerization scheme when it is soaked into oxidant.	23
Figure 4.1.2. SEM images of freeze-dried hydrogels, the general image of hydrogel and its antibacterial test on Escherichia coli.	24
Figure 4.1.3. Schematic representation of the formation of hydrogen bondings with the addition of phytic acid to alginate solution. Polymerization of monomers in a gel is also shown.	25
Figure 4.1.4. Images of obtaining conductive polymer hydrogels before and after polymerization. SEM images of freeze-dried hydrogels.	26
Figure 4.1.5. Schematic difference between hydrogels crosslinked with PA and dual-crosslinked with PA and CaCl ₂ .	27
Figure 4.1.6. Hydrogel was cut into cubical pieces and soaked in different pH solutions for 2 and 5 hours.	29
Figure 4.2.1. A) The general scheme of PAAm/PA-PAni hydrogel synthesis and B) its adhesive properties to a dry surface. C) Different volume ratios between Phytic acid and DI water.	32
Figure 4.2.2. Step by step conductive polymer hydrogel synthesis: a) obtained hydrogels; b) soaking into Ani-HCl solution; c) after one day in Ani-HCl; d) after one day in APS solution; e and f) final materials	33
Figure 4.3.1. Samples were cut into rectangular pieces s for the tensile test, as well as for the electrical sensitivity test (a). b) sample on a tensile tester; c) electrical sensitivity test with stretch; d) Stress-strain graphs of all samples; e) sensitivity results of all samples	33

Figure 4.3.2. Graphical results of tensile and sensitivity tests: a-c) stress-strain graphs; d-f) strain graphs until elastic deformation; g-i) electrical sensitivity graphs.	34
Figure 4.3.3. Graphical results of electrical sensitivity test by open chronoamperometry technique by stretching hydrogels for the 1-minute duration.	34
Figure 4.4.1. Resistance results in kilo Ohm for hydrogels without and with a conductive polymer.	35
Figure 4.4.2. Conductivity results in S/cm for hydrogels without and with a conductive polymer.	36
Figure 4.6. Fourier-transform IR spectra of PANi, Phytic acid, PAAm/PA hydrogel with and without PANi	37
Figure 4.7.1. a) freeze-drying of samples in a lyophilization machine; b) SEM images of PAAm/PA hydrogels with a ratio of 5:5 (PA:DI) without and c) with PANi at a magnification of 500 and 5000.	38
Figure 4.7.2. SEM images of PAAm/PA hydrogels with a ratio of 7,5:2,5 (PA:DI) without and b) with PANi at a magnification of 500 and 5000.	38
Figure 4.7.3. SEM images of PAAm/PA hydrogels with a ratio of 10:0 (PA:DI) without and b) with PANi and c) 0:10 without PA at a magnification of 500 and 5000.	39
Figure 4.8.1. Antibacterial test of Alg-PA dual cross-linked hydrogel on E. coli and S. aureus.	39
Figure 4.8.2. Antibacterial test of PAAm/PA and PAAm/PA-PANi hydrogel on E. coli (a) and B.subtilis (b). 1) 5:5; 2) 5:5 PANi; 3) 7,5:2,5; 4) 7,5:2,5 PANi	40
Table 1. Number of the weight of samples before and after swelling Ani-HCl solution	35

Chapter 1 - Introduction

In the past few decades, conductive polymers have been the research subject due to their unique electrical conductivity that can be tuned and processing flexibility. A class of organic materials known as conductive polymers (CPs) has unique electrical and optical properties that are comparable to those of inorganic semiconductors and metals [1]. The conductivity of conductive polymers is influenced by the component materials' molecular structures, the amount of doping, and the molecular packing arrangement [2]. CPs can be synthesized using simple, versatile, and cost-effective approaches. They can be readily assembled into supramolecular structures with multifunctional capabilities by using simple electropolymerization processes [3]. Conductive polymers have shown promise in inducing a variety of biological mechanisms, expanding the range of biomedical uses for these materials. Their intelligent reaction to electrical fields from diverse types of tissues, such as muscle, epithelial, and nerve tissue, makes them appealing for various biomedical applications [1]. Due to their wide range of physical and electro-conductive properties, inherent Conductive polymers like polyaniline (PAni), polythiophene (PTh), polypyrrole (PPy), and poly(acetylene) (PAc) have drawn a lot of attention during the past 10 years [4]. Because of its high electrical conductivity, environmental stability, and advantageous physicochemical features, PPy and PAni have drawn the most attention among them [5].

As a result of the more complicated microenvironmental settings that are inherent in three dimensions, numerous publications have demonstrated stark variations between the cellular activity found in two-dimensional (2D) [6] and three-dimensional (3D) systems [8]. Conductive polymers are one of these classes of materials, and they show promise because of their flexible mechanical characteristics, excellent compatibility with biological systems, mixed electrical and ionic conductivity, and capacity to form three-dimensional porous structures [7]. These large solid-state surface areas combined with electro-physical characteristics that are pertinent to both organic electronics and biology result in 3D porous scaffolds. The electrical conductivity of such a scaffold is a crucial characteristic for the local delivery of applied electrical stimuli as well as for the subsequent regulation of cell behavior, including adhesion, migration, and secretion. This property is in addition to the 3D structural

support that it offers [8]. Hydrogels are polymeric networks in which 3-dimensional microstructures resemble natural tissues, and also they have a high level of hydration. Possible electrical and optical characteristics, which are similar to metals or semiconductors, can be shown in conductive polymer-based hydrogels. These structural properties could facilitate the transport of charges and molecules [9].

Chitosan (CS) is a well-known biopolymer consisting of a polysaccharide backbone with numerous OH and NH₂ functional groups that can make hydrogels [10]. Recent studies suggest that natural materials, such as chitosan, have several advantages due to their biocompatibility, biodegradability, and hemostatic properties, but they lack flexibility, causing them to be easily split into tiny pieces [11]. Alginate has several favorable properties as a biomaterial, including biocompatibility and non-immunogenicity, which are probably connected to its hydrophilicity [12].

Phytic acid (PA), a biomaterial derived from grains, has 28.2% phosphorus in its molecule and has been exploited as a P-doping source for carbon compounds. Significantly, the PA molecule has six phosphoric groups that prefer to form ionic bonds with amino groups and metal ions [13].

1.1. Aim of research

This research aims to produce a novel CP based hydrogels for wearable strain sensors through rational design polyacrylamide, chitosan, phytic acid, and polyaniline (PAni), a conductive polymer, to generate material with higher strain sensitivity and stability. The particular aims of the study are as follows:

- Optimize the hydrogel synthesis by introducing PAni into the PAAm-chitosan-phytic acid matrix to attain the necessary mechanical and electrical properties.
- Evaluate the mechanical and electrical properties of the hydrogel as well as its sensitivity to strain utilizing various characterization techniques including scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and mechanical testing.
- To test the performance of the hydrogel as a wearable strain sensor in real-world circumstances and assess its possible applications in domains such as healthcare, sports, and robotics.

Due to their great electrical conductivity and ability to undergo reversible changes in conductivity when subjected to mechanical strain or pressure, conductive polymers, such as PANi, are vital for the creation of hydrogels for wearable strain sensors. By integrating PANi into the hydrogel matrix, it is possible to generate a material that is both biocompatible and conductive, making it perfect for usage in a variety of applications, especially in the medical field. The incorporation of PANi into the hydrogel matrix can improve its efficacy as a wearable strain sensor by increasing the hydrogel's sensitivity to strain.

1.2. Hypothesis and goals

We hypothesize that with the addition of the conductive polymer polyaniline (PANi) into a polyacrylamide-chitosan-phytic acid hydrogel matrix, we can build a wearable strain sensor with increased electrical sensitivity and mechanical stability under strain. Further, we expect that the hydrogel will exhibit reversible conductivity changes when subjected to mechanical strain or pressure, making it an attractive candidate for use as a wearable strain sensor. Additionally, material will have good antibacterial properties to avoid allergic reaction to human skin. However, since PANi has low antimicrobial activity, it can possibly decrease this property of hydrogel.

Investigation of the effect of PANi on mechanical and electrical properties by measuring strain and conductivity can be a main goal as well as the testing materials to observe changes in the antibacterial properties of hydrogels.

Chapter 2 - Literature Review

2.1 Conductive polymers: polyaniline

Hodgson et al. [3] claim that the class of organic materials known as conductive polymers (CPs) has distinctive electrical and optical characteristics that are comparable to those of inorganic semiconductors and metals. CPs may be created utilizing straightforward, flexible, and economical methods. Simple electropolymerization techniques may be used to easily assemble them into supramolecular structures with several functionalities.

This class of organic materials is called conjugated polymers with electrons carrying in their unsaturated backbones, where the pathway for moving charges could be created by the free motion of delocalized π -electrons [14].

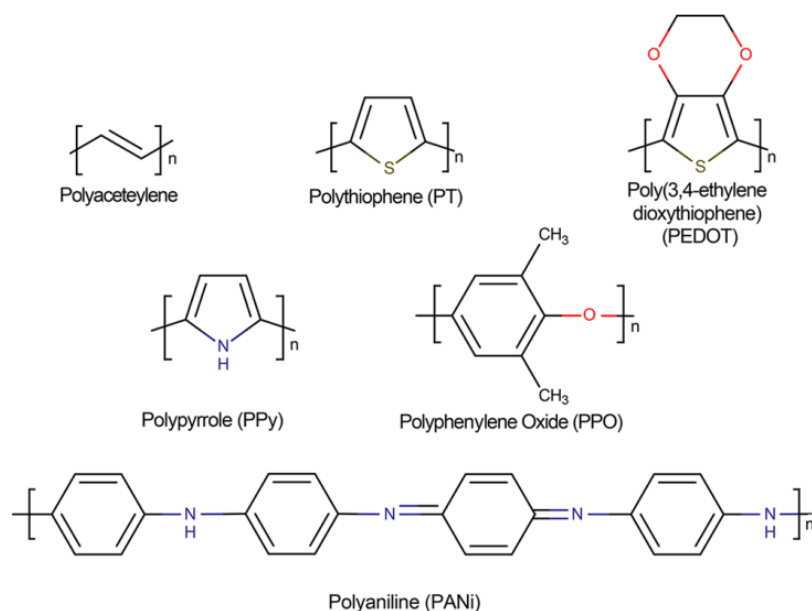


Figure 2.1. Chemical structure of most used conjugated polymers. Reprinted with permission from [1].

Due to its simple synthesis, high electrical conductivity, and excellent environmental stability, PANi is one of the most promising conjugated CPs [15]. Chemical composition of

PAni It is based on the aniline monomer for polymerization, and it can exist in one of three idealized oxidation states that influence its conductivity. Emeraldine, a partly oxidized structure of interest, is doped with acid to form a CP that is very stable at ambient temperature [16].

During the obtaining process of PAni, the polymer can be in three states according to its components: leucoemeraldine $[(C_6H_4NH)_n]$, emeraldine $[(C_6H_4NH)_2(C_6H_4N)_2]_n$, and per-nigraniline $[(C_6H_4N)_n]$, where the most conductive one is Emeraldine. Doping with acid helps to get conductive and stable structured PAni, for which ammonium persulfate is required as an oxidant and polymer is soluble in an aqueous hydrochloric acid solution (Figure 2).

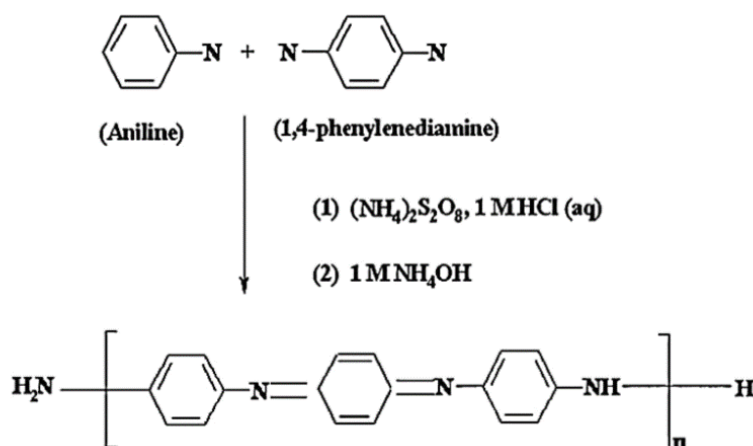


Figure 2.2. Synthesis of polyaniline.

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2.2 Polysaccharides: Acrylamide, Alginate, and Chitosan

Polymers are generally categorized in a variety of ways, but the basic categorization utilized in the pharmaceutical industry divides them into natural and synthetic polymers. The development of desired release formulations and controlled-release dosage forms frequently uses polysaccharides as natural polymers. Two commonly synthesized biopolymers that have been used for many years in biomedical and pharmaceutical applications are chitosan (CS) and sodium alginate (SA) [17].

Chitosan (CS) is a superior cationic biopolymer with great ability to interact with negatively charged macromolecules, polymers, and poly ions. In-depth research is being done on CS-based matrices for oral, transdermal, rectal, and ocular drug delivery systems [18]. According to Figure 2.3, the linear structure of chitosan, which is obtained from chitin by deacetylation, consists of amino and hydroxyl groups for the reaction with other polymers in the solution. These bindings can construct network structures, which is a beneficial feature for hydrogel synthesis. Moreover, chitosan has biocompatibility and antimicrobial properties, which makes it a great candidate for the production of materials and devices with biomedical applications.

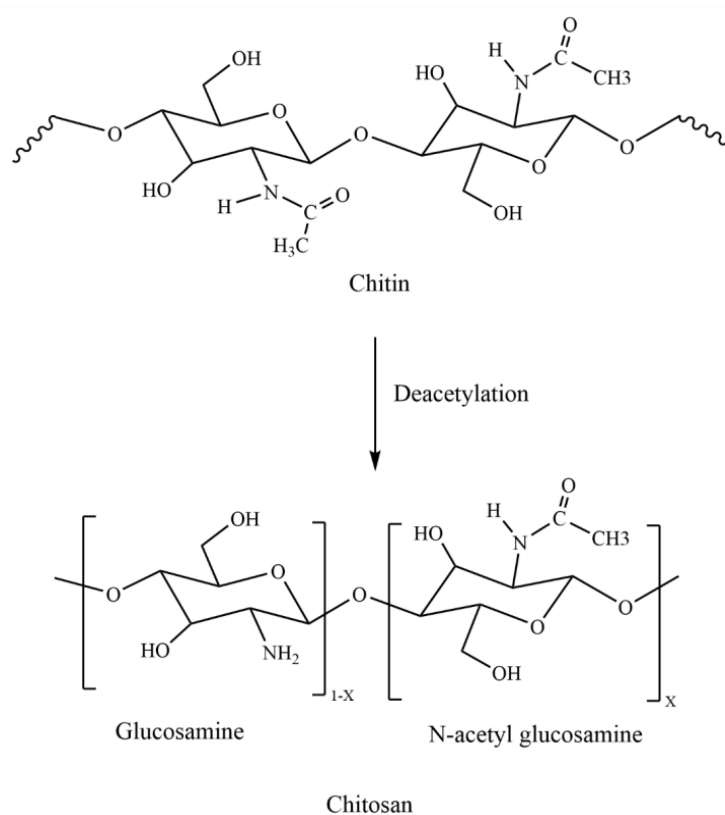


Figure 2.3. Schematic synthesis of chitosan.

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A well-known natural polymer of plant origin called sodium alginate (SA) is mostly made up of (1-4) connected -D-mannuronic acid and -L-guluronic acid units [20]. It has exceptional gel-forming capabilities, is biocompatible, and is biodegradable, making it a good choice for biomedical, controlled release applications, and matrices for enzyme immobilization, among other uses [21]. Furthermore, SA may form insoluble calcium alginate when crosslinked with multivalent cations like calcium ions. SA exhibits less swelling in

various solvents as a result of its special crosslinking properties, which minimizes the absorption of various solutes. This makes it possible to employ drug-embedded alginate matrices as sustained-release formulations for applications that require controlled drug delivery [22]. SA may function as a polyelectrolyte to provide the hydrogel with strong conductivity in addition to its good biocompatibility. An ionic hydrogel sensor, for instance, was described in several recent investigations employing SA as the conducting substance (as a polyelectrolyte). The hydrogel sensor that was obtained was biocompatible and could be used to track different human movements. When SA is spread in a hydrogel system, it can also increase the mechanical strength of hydrogels. Recently, scientists created a PVA-based hydrogel that quickly gels to gather dangerous substances. The hydrogel produced by adding SA showed greater tensile stress than a hydrogel produced without adding SA and subsequently, it is a suitable choice for creating wearable hydrogel sensors as a result [23].

Moreover, hydrogels produced from synthetic polymers have drawn a lot of interest over the years due to their positive characteristics to those of hydrogels produced from natural polymers. Since the structure may be adjusted to its effectiveness, hydrogels composed of synthetic polymers have enhanced mechanical features, a long life before degradation, and a significant capacity to absorb water [24].

2.3 Phytic acid

Due to its strong antioxidant activity and capacity to chelate Fe^{3+} ions, phytic acid (PA), which is extracted from oilseeds, legumes, cereals, nuts, and pollen by acid solutions while being heated and/or stirred and then purified, has demonstrated positive health and physiological benefits. The number of publications on PA has risen, particularly those that discuss how it affects disease prevention and treatment. Moreover, recent research has indicated that PA is an effective inhibitor of foodborne bacteria. As a result, phytic acid has received more attention because of its many documented benefits than owing to its traditional designation as only an antinutrient. An overview of PA structure, sources, biosynthesis, extraction, purification, and uses has been provided by reviewing recent and previous works [25].

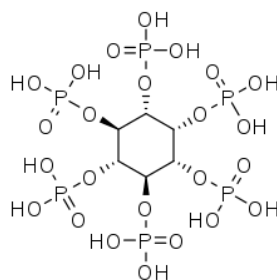


Figure 2.4. Chemical structure of Phytic acid.

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2.4 Conductive Polymer Hydrogels

The polymer networks known as hydrogels can hold and absorb large amounts of water, because they contain hydrophilic groups that hydrate in aqueous media to create a hydrogel structure. Cross-links are necessary to avoid the breakdown of the polymer chains prior to usage since the word "network" is implied. Rheological investigation of hydrogels is also possible. Water soluble polymer solutions often display "Newtonian" behavior when they are present at low or intermediate concentrations and there is little to no chain tangling. Moreover, networks formed demonstrate viscoelastic and occasionally completely elastic behavior as cross-links between polymeric chains are added. Due to their capacity for water absorption, hydrogels are being studied to better understand the underlying principles of swollen polymer networks. They are also widely used in a variety of technical fields, including the production of contact lenses, materials for protein separation, and devices for the controlled release of drugs and proteins [26].

Doping is a key technique in the contemporary semiconductor industry because it enables adjusting the electronic characteristics of semiconductors. Yet, conductive polymers' doping differs significantly from that of their inorganic analogs [17]. Acid-base chemistry or charge-transfer redox chemistry is mostly used to dope conjugated polymers (protonation). Examples include the frequent use of protonation via acid-base chemistry in reversible chemical doping of Polyaniline. The polaronic or bipolaronic structure along the polymer chain, or equilibrium between polarons and bipolarons, is caused by the acidic dopant protonating the imine groups in PANi. The polyaniline that has been doped with proton acid shows a transformation from the emerald base (EB) to the emerald salt (ES) form, as well as a conductivity enhancement [17]. Acidic medium from Phytic acid can easily dope polymerized aniline in the hydrogel.

Chapter 3 - Materials and Methods

3.1 Materials

Aniline (Ani, 99%), Sodium Alginate (SA), Calcium chloride dihydrate (CaCl_2 , $\geq 99\%$), and Ammonium persulfate (APS, $\geq 98.0\%$) were purchased from Sigma Aldrich for preparation of Alginate hydrogels with and without PAni. Phytic acid solution (PA, 50% w/w in H_2O), Hydrochloric acid (HCl, 37%), Acrylamide (AAM, $\geq 99\%$), N, N'-Methylenebis(acrylamide) (MBA, 99%), Potassium persulfate (KPS, $\geq 99\%$) and Chitosan (CS, medium molecular weight) were also purchased from Sigma Aldrich in the aim of preparation of PAAm/PA-CS hydrogels with and without PAni. Nutrient Broth (NutriSelect[®] Plus), Nutrient Agar (NutriSelect[®] Plus), and Mueller-Hinton Broth (NutriSelect[®] Plus) were purchased from Millipore, Sigma Aldrich for the preparation of nutrient media for the antibacterial test on Gram (+) and Gram (-) bacterial species.

3.2 Preparation of Alginate hydrogel with and without conductive polymer

Firstly, 1% w/v (0,25 g) of sodium alginate was dissolved in 25 ml of DI water with continuous magnetic stirring, until it became a viscous solution. Then, a thick solution was poured into the CaCl_2 solution, which was prepared separately by dissolving 2,5g of calcium chloride in water, in order to obtain a transparent hydrogel. After this trial, 3 hydrogel samples were prepared with different percentages (w/v%) of sodium alginate in water: 1%, 1,5%, and 2%. Considering the swelling properties of these gels, which were gelated in 5 minutes, were soaked in a liquid aniline monomer for 1 day. As a next step, after 1 day, hydrogels were

replaced and left for another 1 day in 0.5M of APS solution, where there is enough time for total oxidation of the swelled monomer. Consequently, the obtained hydrogels, with the black color, were washed with acetone in order to clean from undesired unreacted molecules.

3.3 Preparation of Alg-PA hydrogel with and without conductive polymer

The process of Alg-PA hydrogel preparation is similar to the previous method, however, the phytic acid played the role of cross-linker and gelator instead of calcium chloride. As mentioned previously, 3 different volumes of PA (160 μ l, 200 μ l, and 250 μ l) were dropwise added to 2% w/v 20 ml viscous solutions of alginate under magnetic stirring and left for 24 hours for the maturation of hydrogel. As a following step, obtained hydrogels were also soaked in aniline for one day, and in APS oxidant solution the next day for 24 hours.

3.4 Preparation of dual-crosslinked Alg-PA hydrogel with and without conductive polymer

In this case, hydrogels also were prepared by the addition of a PA cross-linker to Alg solution and obtained after soaking and oxidizing them as in the above-mentioned method. Next, these gels were soaked in CaCl_2 solution, which has to cross-link with unbonded alginate molecules in order to get stronger hydrogel.

3.5 Preparation of PAAm/PA-CS hydrogel with and without conductive polymer

This method includes several steps of hydrogel synthesis, where the first is to get 10 ml of the viscous solution by mixing phytic acid with water in different ratios by decreasing the volume of phytic acid (10:0, 7.5:2.5, 5:5, 2.5:7.5, 0:10). As a next step, 2.5 g of AAm, 0.125g of CS and 0.001 g of MBA was added to this solution under magnetic stirring until chitosan was fully dispersed. The obtained viscous solution was cooled down to 0^o C, in order to add 0.005 g of KPS initiator. After 2 minutes of magnetic stirring, solutions were poured into vials and put in an oil bath for 5 hours at 50^oC until the solution was gelled.

Synthesis of PAAm/PA-CS hydrogel with CP includes the whole process above and after obtaining strong and stretchable gel, it was soaked into 0.4 M of Ani-HCl solution, which

was prepared separately. After 1 day the gel was transferred into 0.5 M APS solution for another 24 hours in order to get an oxidized monomer in a hydrogel.

3.6 Testing mechanical properties of hydrogels

A tensile test was done (by TA.XTplus, Stable Micro Systems, SIMAS™) for checking the tensile properties of hydrogels and measuring strain. For these measurements, hydrogels were cut into rectangular shapes (30 mm x 10 mm x 4 mm) and stretched with a 1 mm per s running speed.



Figure 3.1. TA.XTplus, Stable Micro Systems, SIMAS™

3.7 Fourier Transform Infrared (FTIR) analysis

Analysis of chemical properties of materials was done by using Nicolet iS10 FT-IR Spectrometer



Figure 3.2. Nicolet iS10 FT-IR Spectrometer

3.8 Scanning Electron Microscope (SEM)

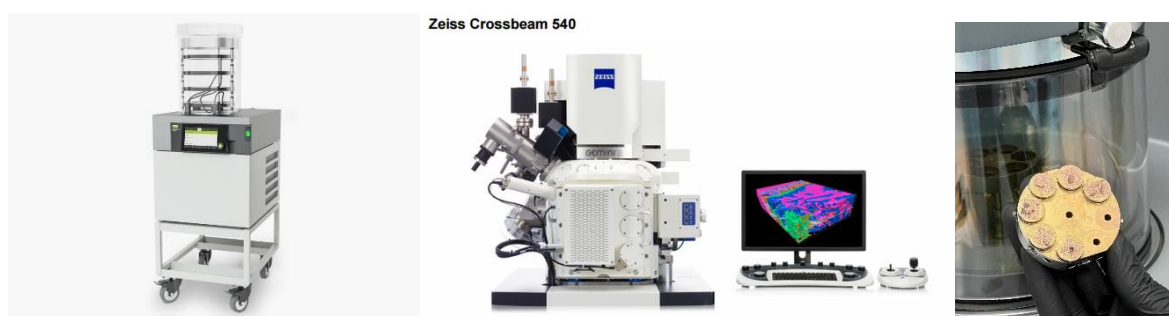


Figure 3.3. Lyovapor L-200 and SEM ZEISS Crossbeam 540. Gold coated samples

Lyovapor™ L-200, BUCHI was used for the preparation of materials by freeze-drying technique for 24 hours and Scanning Electron Microscope ZEISS Crossbeam 540 was used for the analysis of surface morphology of cross-sectional parts, which were gold-coated at 10 nm.

3.9 Swelling property test

The swelling ability of hydrogels was tested and measured during the synthesis of PAAm/PA-PAni hydrogel, where materials were soaked into a 0,4 M Ani-HCl aqueous solution with a weakly acidic medium. The degree of swelling was calculated by the following formula:

$$SDE = \left(\frac{W_t - W_d}{W_d} \right) \times 100$$

where W_t and W_d are wet and dried phase weights of samples.

3.10 Conductivity test

The conductivity test was done by measuring the resistance of rectangular-shaped samples with 20 mm of length, 10 mm of width, and 0,5 cm² of cross-sectional area by using a digital multimeter (Vici VC97A).



Figure 3.4. Resistance measurement

Consequently, electrical conductivity (σ) was measured by the formula:

$$\sigma = \frac{1}{R \frac{A}{L}}$$

where R is electrical resistance (Ω), A is the cross-sectional area of the sample (cm²) and L is the length of the hydrogels (cm).

3.11 Antibacterial test

The antimicrobial properties of obtained hydrogels were tested on Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*). All hydrogel samples were cut into rectangular shapes (10 mm x 5 mm x 5 mm) and sterilized with UV for 15 minutes. As a next step, 200 μ l of bacterial suspension were inoculated on nutrient agar plates and hydrogel samples were placed on them. To observe the inhibition of samples, plates were incubated in an oven at 37°C for 24 hours until the total growth of bacteria.



Figure 3.5. Antibacterial test

Chapter 4 - Results and Discussion

4.1 Alginate hydrogel sample preparation with CaCl_2 and Phytic acid

After several failed trials with the addition of monomer during hydrogel synthesis, where no gel was obtained, it was decided to get hydrogel separately and soak it into monomer, because of its swelling properties. The first experiment, which is shown in Figure 4, was done with sodium alginate and calcium chloride giving the easiest way of obtaining hydrogel, as in the food industry. Three different alginate percentages were chosen for dissolving in a determined volume of water (1 %, 1.5 %, and 2 % (w/v)), which were gelled after a few minutes in the CaCl_2 solution. Because of ionic crosslinking, the poor mechanical properties of these gels showed very soft and not stable shaped hydrogels. Obtained materials were frozen at -30°C for 5 hours and freeze-dried for 24 hours to do a morphological analysis. Figure 5, shows images, where with the increasing alginate percentage, the density of material increased respectively, which is seen as a thick film. Additionally, polyaniline in a gel can be shown as particles, without binding to any gel network. As an alginate gel is a mostly biocompatible and edible hydrogel and PANi also has this property, there is no antibacterial effect observed after testing on *E. coli*.

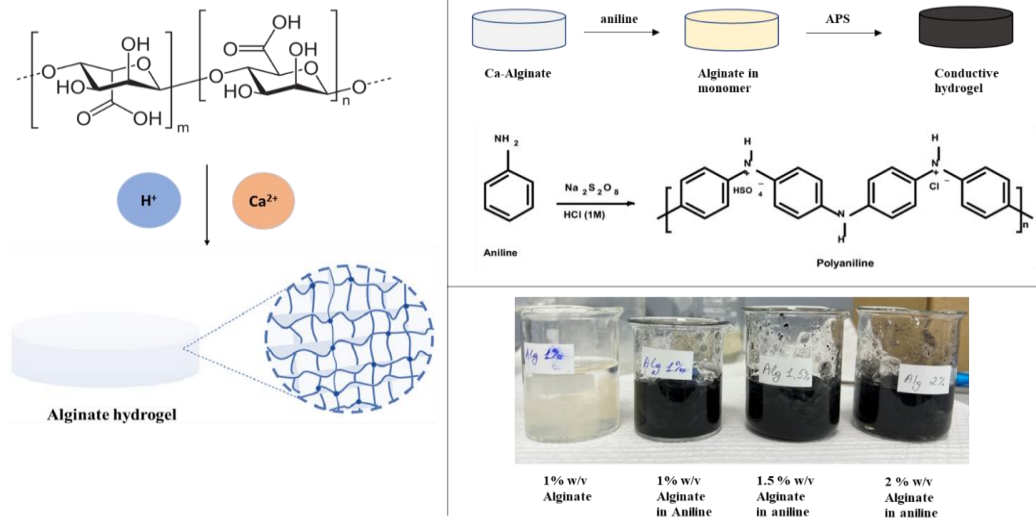


Figure 4.1.1. Schematic diagram of Alginate hydrogel synthesis with different percentages (% w/v) and the addition of monomer with polymerization scheme when it is soaked into oxidant.

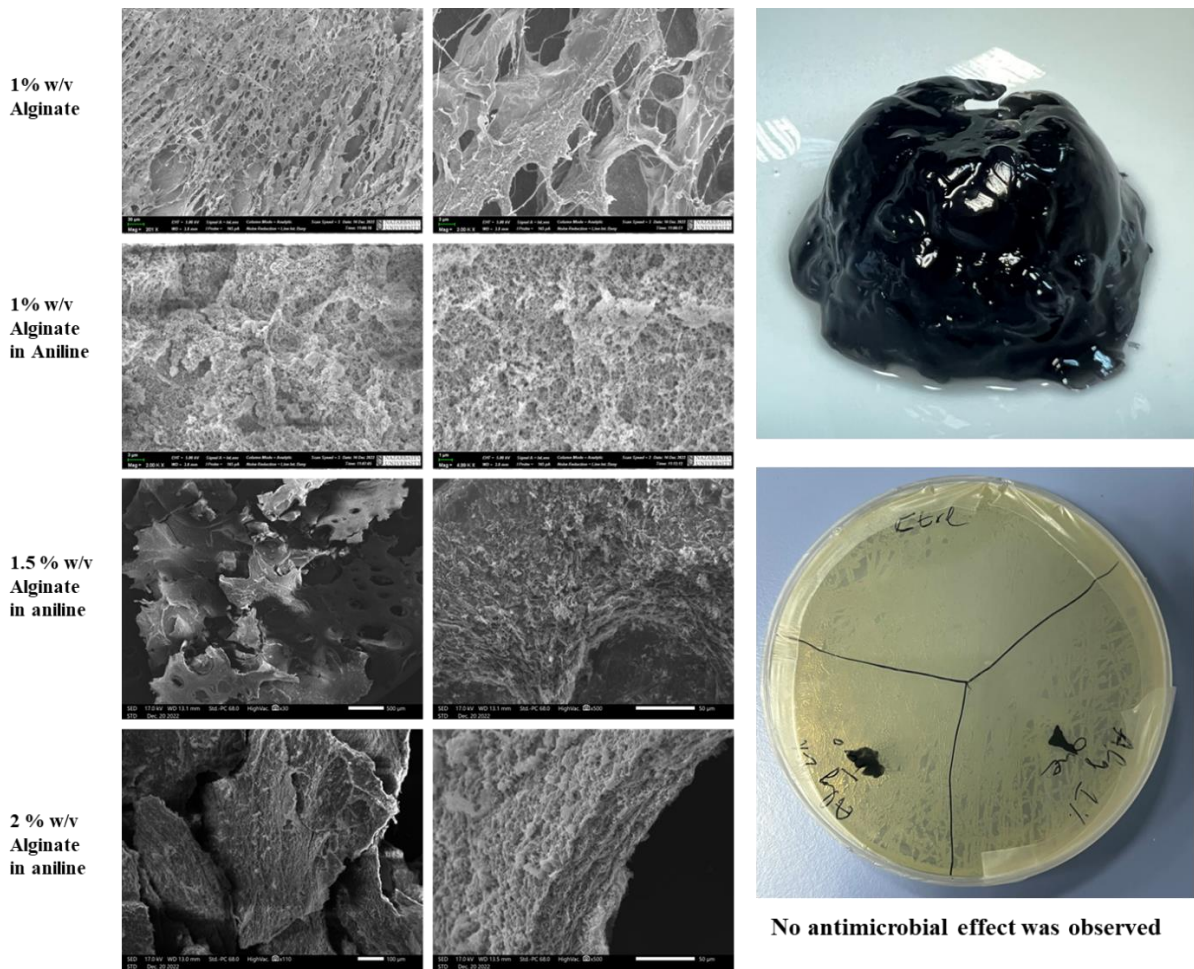


Figure 4.1.2. SEM images of freeze-dried hydrogels, the general image of hydrogel and its antibacterial test on Escherichia coli.

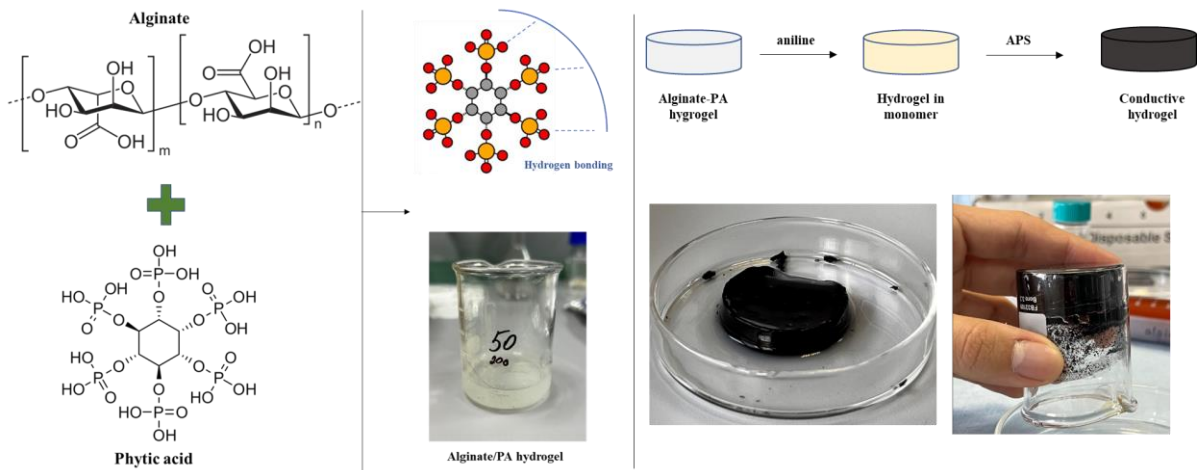


Figure 4.1.3. Schematic representation of the formation of hydrogen bondings with the addition of phytic acid to alginate solution. Polymerization of monomers in a gel is also shown.

Phytic acid, which is a gelator, natural cross-linker, and dopant, is added for hydrogel synthesis as a next step, according to the research work done by Nita et al [27]. PA has several anions and hydroxyl groups that can create a network by forming many ionic and hydrogen bonds.

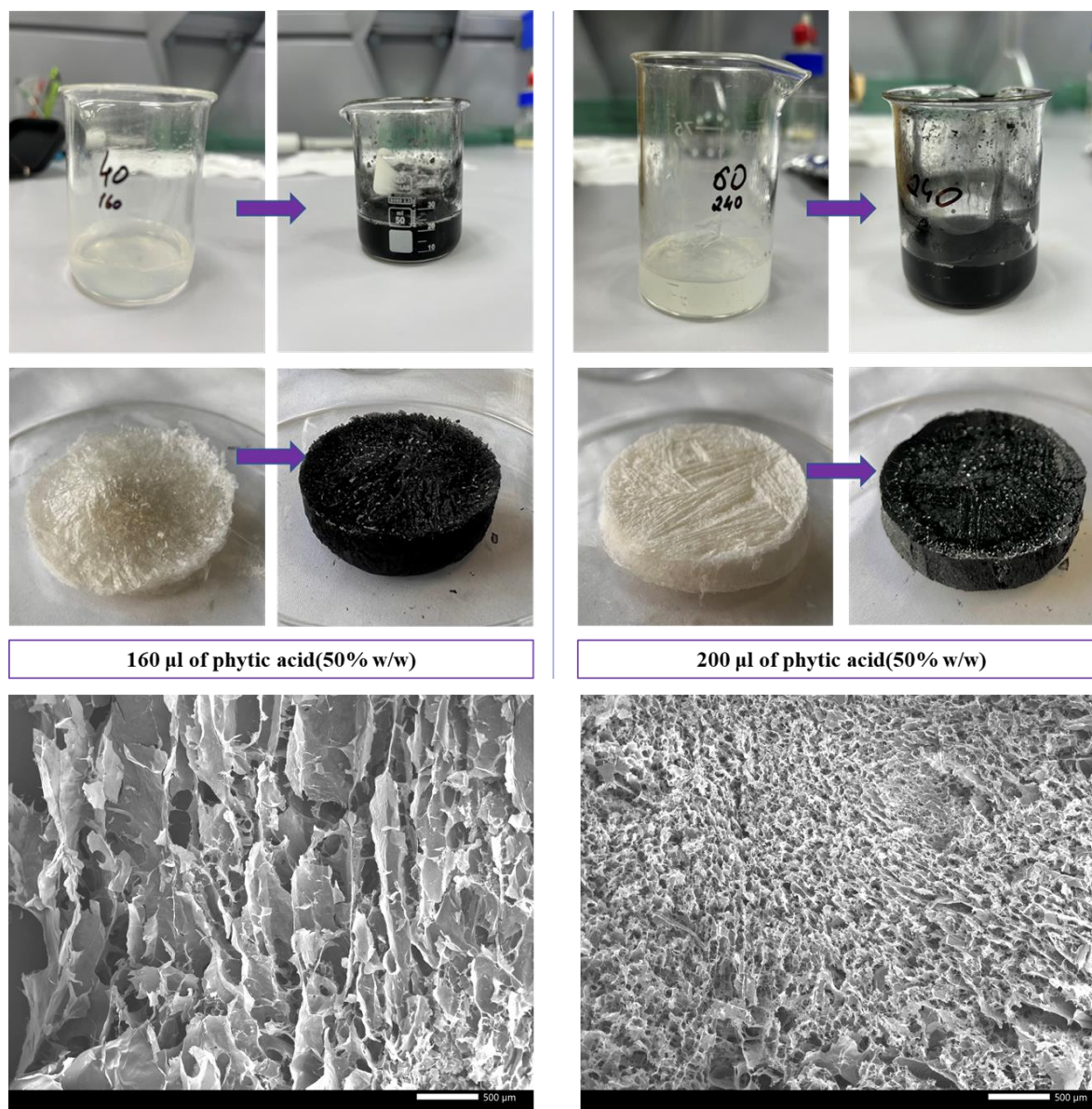


Figure 4.1.4. Images of obtaining conductive polymer hydrogels before and after polymerization. SEM images of freeze-dried hydrogels.

In Figure 6, the binding sites of PA molecules are illustrated, as well as the process of addition and obtaining of the hydrogel. Mixing a small amount of PA with alginate solution and leaving it for maturation for 24 hours at room temperature, gave us a physically crosslinked hydrogel with poor mechanical properties also. According to a phase diagram [27], where the gelation depends on the volume of added PA, it is important to use a determined number of volumes to obtain desired hydrogels. According to Figure 4.1.4, where SEM images of gels are given, the porosity and density rise with the increasing PA, however excess PA cannot form a hydrogel.

However, the addition of only phytic acid was not enough for the cross-linking of hydrogel, because the addition of it gave a porous and sponge-like structure, which was weak and unstable. Additionally, in the previous experiments, it was noticed that soaking alginate solution into calcium chloride gives strong shelled hydrogel with thick films inside, showing highly porous hydrogel. Considering this, it was decided to use both crosslinkers, as a dual-crosslinking method, in order to improve the net structure and mechanical properties with ionic and hydrogen bonding from hydroxyl groups of PA, and dynamic ionic bonding between Ca^{2+} and alginate. In Figure 4.1.5, it is shown that the addition of a PA crosslinker leads to the synthesis of weak hydrogel, which started to destroy when rinsed with DI water. Whereas the dual-crosslinked hydrogel showed smooth and dense structure on the surface as well as in the cross-sectional part.

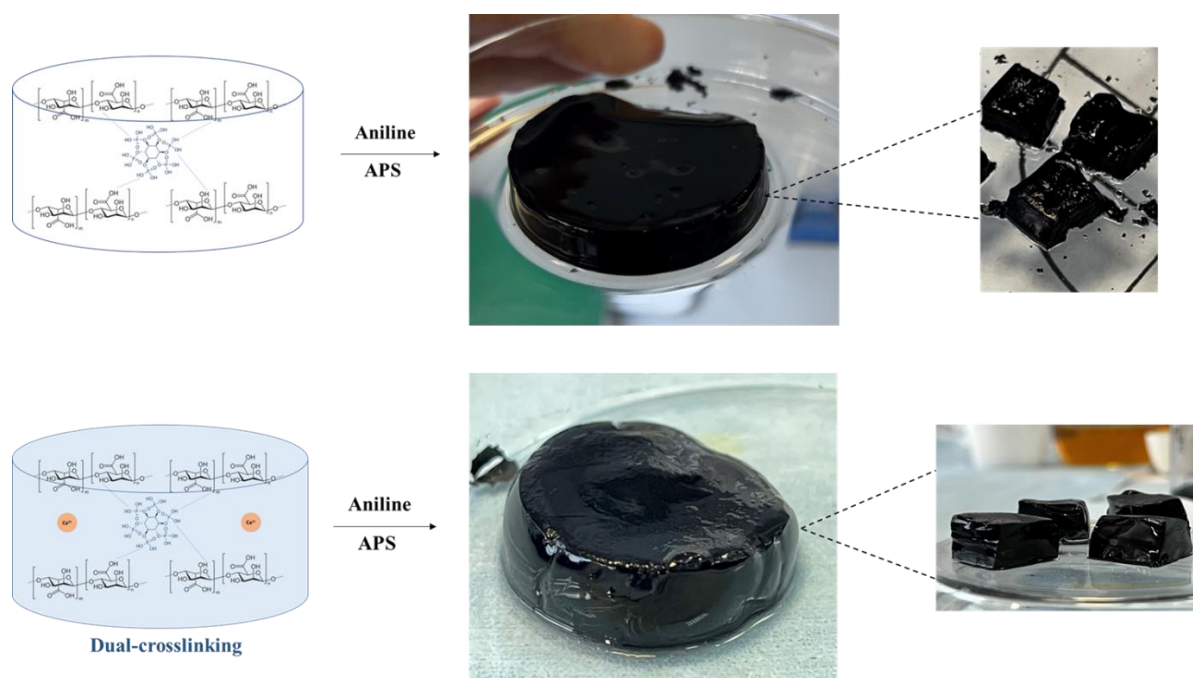


Figure 4.1.5. Schematic difference between hydrogels crosslinked with PA and dual-crosslinked with PA and CaCl_2 .

After washing with DI water, the dual-crosslinked Alg-PA hydrogel was tested for stability in different pH medium solutions: 3, 7, and 12. Hydrogel was cut into cubes with 5 mm side lengths and was soaked into separately prepared solutions with different pH levels for 2 and 5 hours. Before soaking, the mass of cubical hydrogels was measured to determine the swelling ability of a material, which is calculated after 2 hours in solutions. In the table of Figure 4.1.6, it is shown that in acidic and base solutions, the mass of hydrogel was increased approximately 1,4 times, whereas, in a neutral solution, it shows a 2,5 times mass increase.

After 3 hours more, the full degradation was observed at pH=12 and destroying of the gel structure at pH=7, while in an acidic solution, it showed a more stable form.

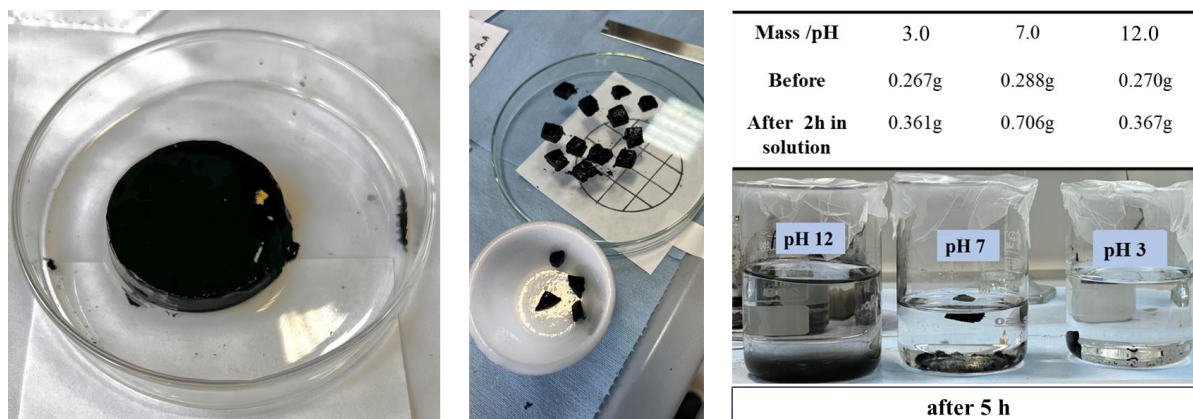


Figure 4.1.6. Hydrogel was cut into cubical pieces and soaked in different pH solutions for 2 and 5 hours.

4.2 PAAm/PA-CS hydrogel preparation with conductive polymer (PAni)

Zhang et al. in their research work synthesized a stretchable and conductive hydrogel using the double network, binding PAAm, MBAA initiated with KPS, and dissolving Chitosan in Phytic acid solution. After 5 hours of hydrogel synthesis, obtained material was soaked into an aniline hydrochloride solution, polymerizing after in APS solution. The hydrophilic property of phytic acid allows it to dilute in water, consequently creating an acidic medium for dissolving chitosan, where these molecules cross-linked physically. Stretchable and other mechanical properties come with the covalently bound PAAm hydrogel network and PA-CS linkages. Figure 10A gives a schematic illustration of the general hydrogel synthesis process with conductive polymer, where also the adhesiveness of the hydrogels is shown by sticking of material to a dry surface of a medical glove (Figure 4.2.1B), as well as it can easily attach to human skin, without leaving wastes after removal. . It is explained that hydrogen bonds from phytic acid and electrostatic interactions between molecules possibly contribute to the adhesive property of hydrogels [28]. For the first step of the synthesis, different volume ratios between phytic acid and DI water were chosen, as shown in Figure 4.2.1C, where the total volume of solution for the hydrogel synthesis is 10 ml. The acetic acid solution was used for the dissolving of CS by making an acidic medium in the ratio PA:DI (0:10).

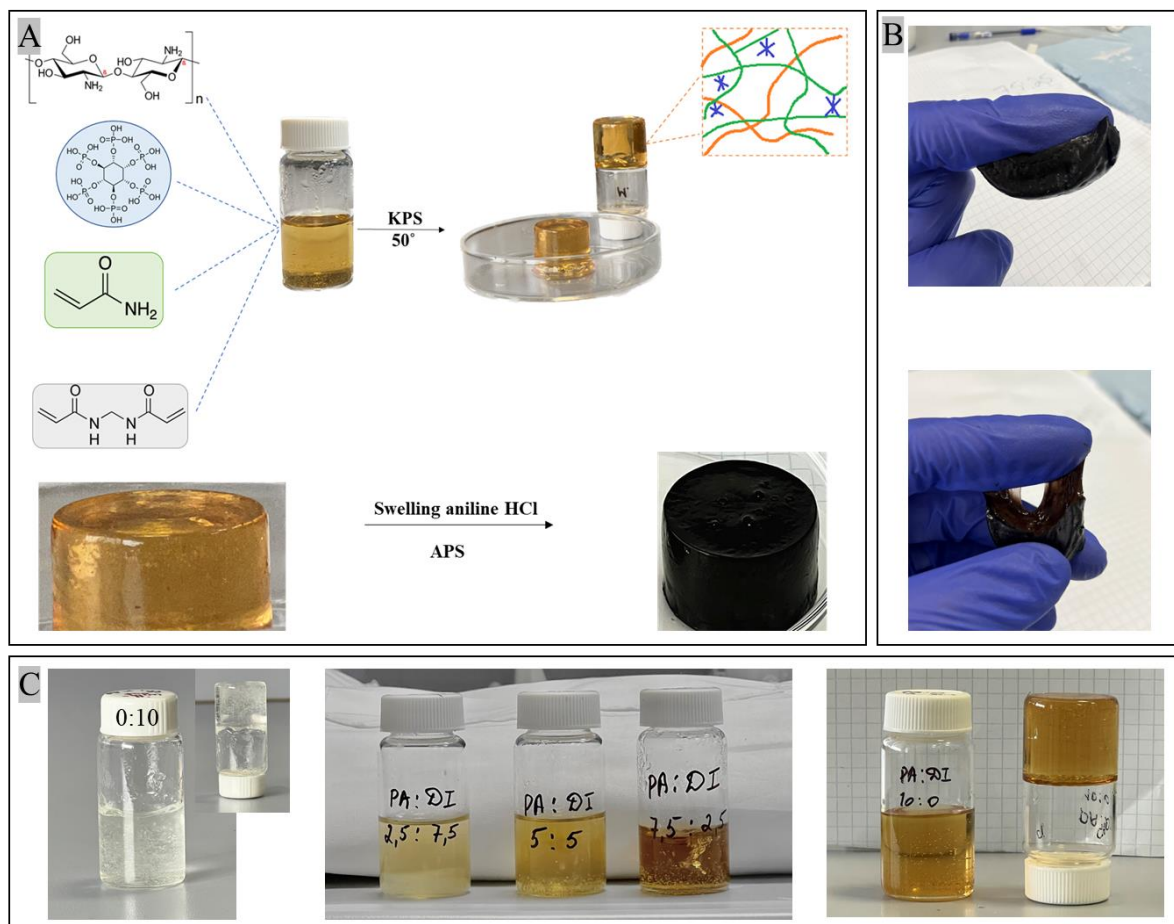


Figure 4.2.1. A) The general scheme of PAAm/PA-PANI hydrogel synthesis and B) its adhesive properties to a dry surface. C) Different volume ratios between Phytic acid and DI water.

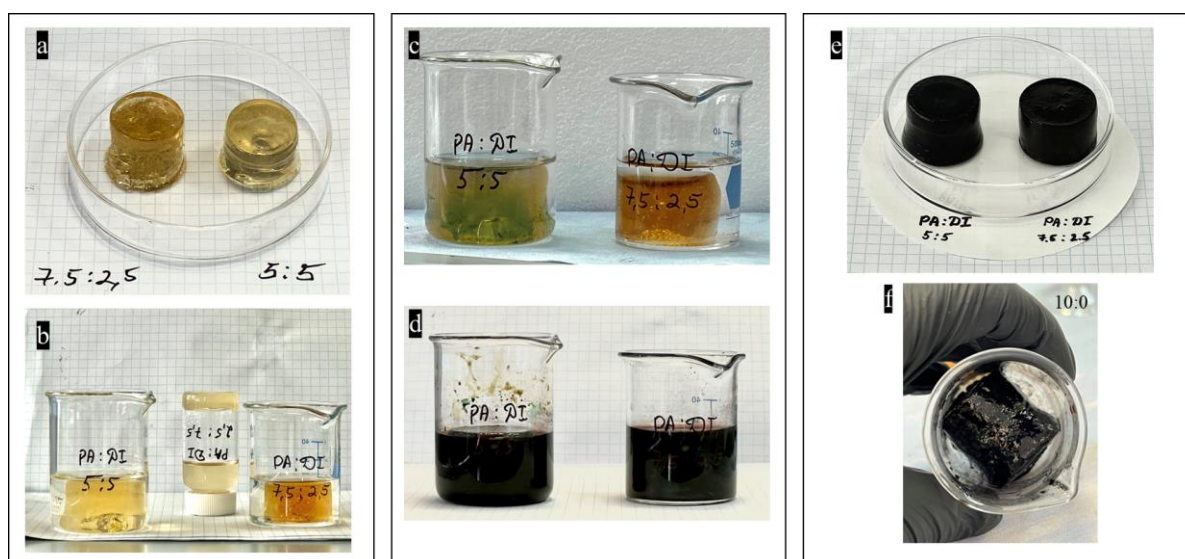


Figure 4.2.2. Step by step conductive polymer hydrogel synthesis: a) obtained hydrogels; b) soaking into Anis-HCl solution; c) after one day in Anis-HCl; d) after one day in APS solution; e and f) final materials

Additionally, no gel was formed in the PA:DI (2,5:7,5) ratio because of phytic acid deficiency, which can be clearly seen in Figure 4.2.2b. The increase of PA in hydrogel also leads to a raise of density and shape stability (Figure 4.2.2e), showing a vice versa effect on swelling property, which can be obviously seen in Figure 4.2.2c by early-started polymerization in a hydrogel with a 5:5 ratio of PA and DI.

4.3 Testing Mechanical Properties and electrical sensitivity of Hydrogel

Hydrogels were cut into rectangular shapes with a size of 30 mm x 10 mm x 4 mm and a cross-sectional area of 40 mm², as shown in Figure 4.3.1a. The TA.XTplus model of Stable Micro Systems was applied for measuring the stress and strain of hydrogels, where samples were attached for 5 mm on both sides equally, leaving 20 mm for the test. The test was done at 1 mm per second speed until the breaking of samples, and Figure 4.3.1d shows at which level of strain samples stopped to stretch. Also, it is clearly seen the growth of elasticity level with the increase of phytic acid volume, shows a maximum of 1100% of stain, while the addition of PANi shows the difference in a strength level showing higher numbers, at 90 kPa as a maximum point.

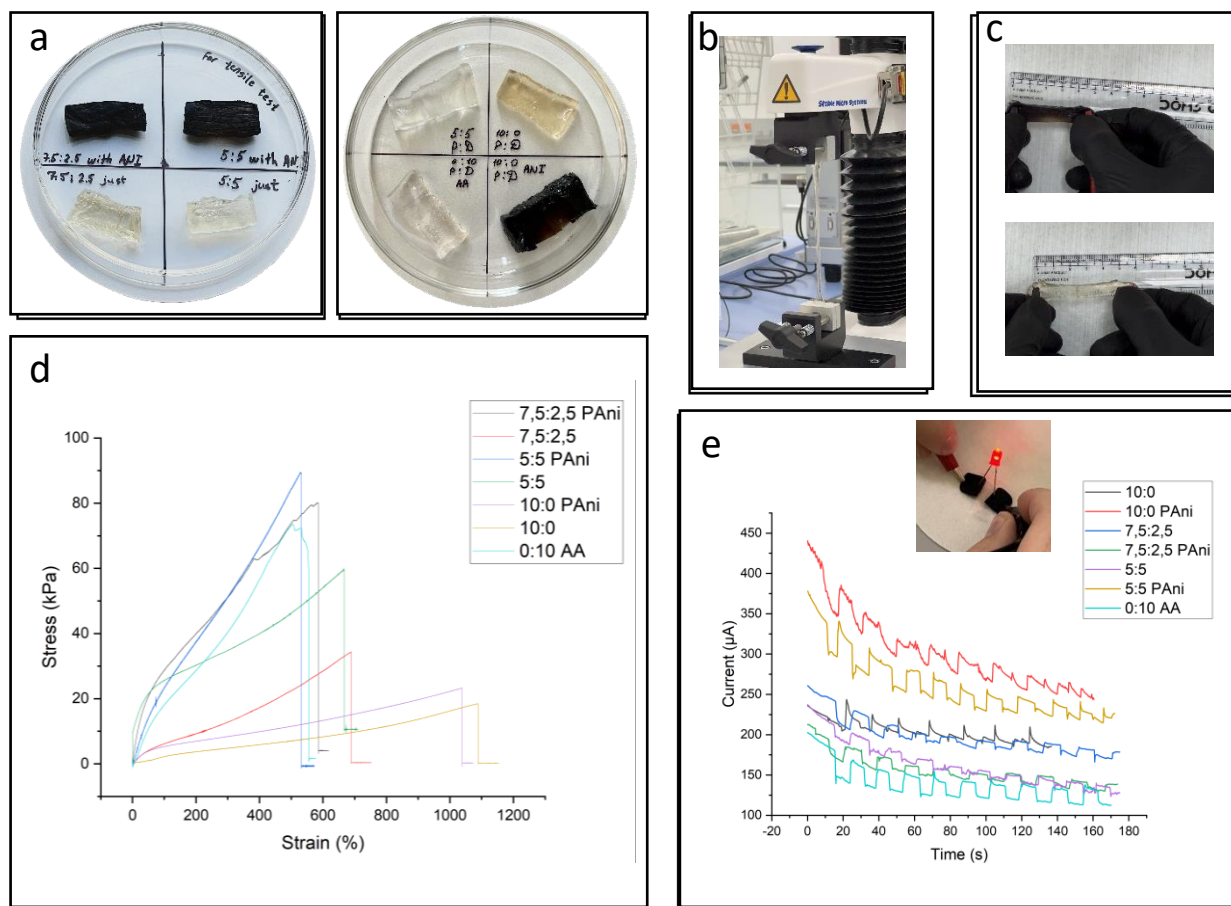


Figure 4.3.1. Samples were cut into rectangular pieces (a) for the tensile test, as well as for the electrical sensitivity test. b) sample on a tensile tester; c) electrical sensitivity test with stretch; d) Stress-strain graphs of all samples; e) sensitivity results of all samples

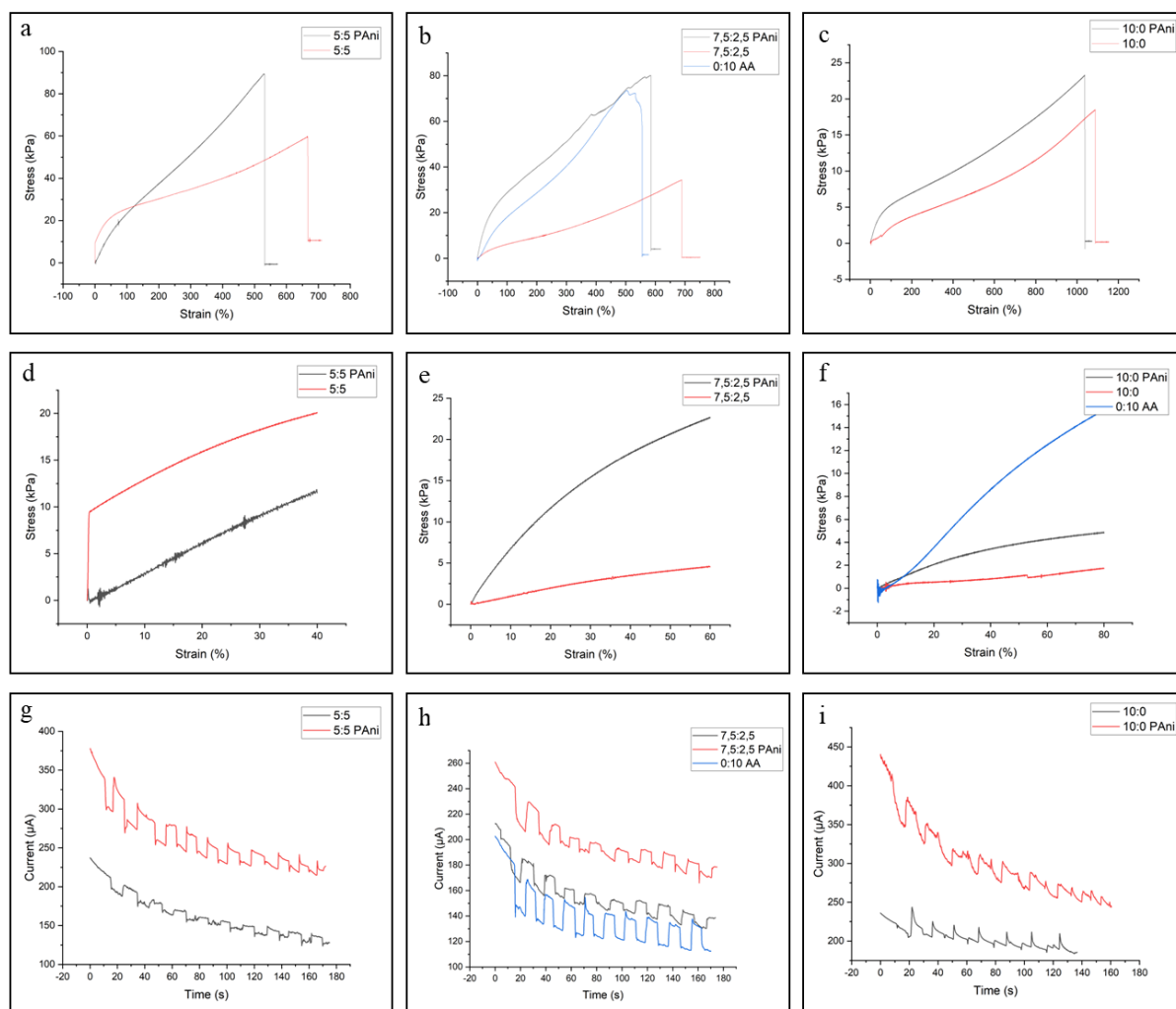


Figure 4.3.2. Graphical results of tensile and sensitivity tests: a-c) stress-strain graphs of samples divided by ratios with and without CP; d-f) strain graphs until elastic deformation; g-i) electrical sensitivity graphs.

According to the detailed data from Figure 4.3.2, hydrogels show superior mechanical properties. The effect of increasing phytic acid content can be determined by respectively increased strain levels of samples in graphs a-c. For the ratio of 5:5 between PA and water, where hydrogel showing 670% of strain at 60 kPa of strength is changed after the addition of conducting PANi, which decreased the strain to 540% while increasing the strength level up to 90 kPa. The PAAm/PA-PANi hydrogel (7,5:2,5) with the same level of stress showed 580% of strain, however, a significant difference can be seen for samples without PANi, where the strain is 700% but the stress rate is below 40 kPa. The stress level of 75 kPa and 550% of strain was shown by the hydrogel sample without phytic acid (0:10). Lowest stress level was demonstrated by a ratio of 10:0 with and without PANi, showing 24 kPa and 17 kPa respectively. On the other hand, great elasticity was shown by the last samples, where the strain

percentage is higher than others with 1040% for samples with PANi and 1100% for PAAm/PA hydrogel.

Generally, stress is calculated from the force (N) and the cross-sectional area (mm^2) of the hydrogel at the initial point, which gives numbers after turning results to a kPa. Strain graphs in Figure 4.3.2 (d-f) illustrate the level of strength at the elastic deformation point, where strain shows 40% for a 5:5 ratio, about 60% for 7,5:2,5 and 80% for 10:0 and 0:10. These numbers were used for an electrical sensitivity test by stretching it until given above percentages and released back after every 10 seconds for about 3 minutes of measurement. Furthermore, the addition of a conductive polymer shows a higher level of current flow through the hydrogel than the samples without PANi, where 0.5 V electrical voltage was applied during the whole process. Additionally, those figures demonstrate the strain until the elastic deformation happens, where Young's (E) modulus can be calculated according to this formula:

$$E = \frac{\sigma}{\varepsilon} = \frac{F * L_0}{A(L - L_0)}$$

where, σ is stress and ε is strain, which is determined already. In Figure 4.3.2d, the maximum strength level is $20 \pm 0,8$ kPa and the modulus is 50 ± 2 kPa for the ratio of 5:5 without PANi, whereas the strength for the sample with CP is $12 \pm 1,3$ kPa with a modulus of $30 \pm 3,3$ kPa. Increasing phytic acid content could be the main reason for the decrease in strength, which can be seen in the graph for the ratio of 7,5:2,5 (without PANi), where the strength rate significantly lowered to a number of $4 \pm 0,7$ kPa and $6 \pm 1,8$ kPa of modulus. On the other hand, the sample with CP is increased in comparison with the previous sample, showing $22 \pm 0,6$ kPa of stress level and $36 \pm 1,1$ kPa modulus. The lowest stress rate is demonstrated by a ratio of 10:0 with and without PANi, where strengths are $5 \pm 0,2$ kPa and $2 \pm 0,4$ kPa respectively as well as with the modulus of $6,2 \pm 0,3$ kPa and $2,6 \pm 0,6$ kPa.

Another test was done by using the chronoamperometry technique, by applying 0.5V electricity into a sample and keeping it stretched 2, 3, and 4 times more than the initial length for one minute, as shown in Figure 4.3.3.

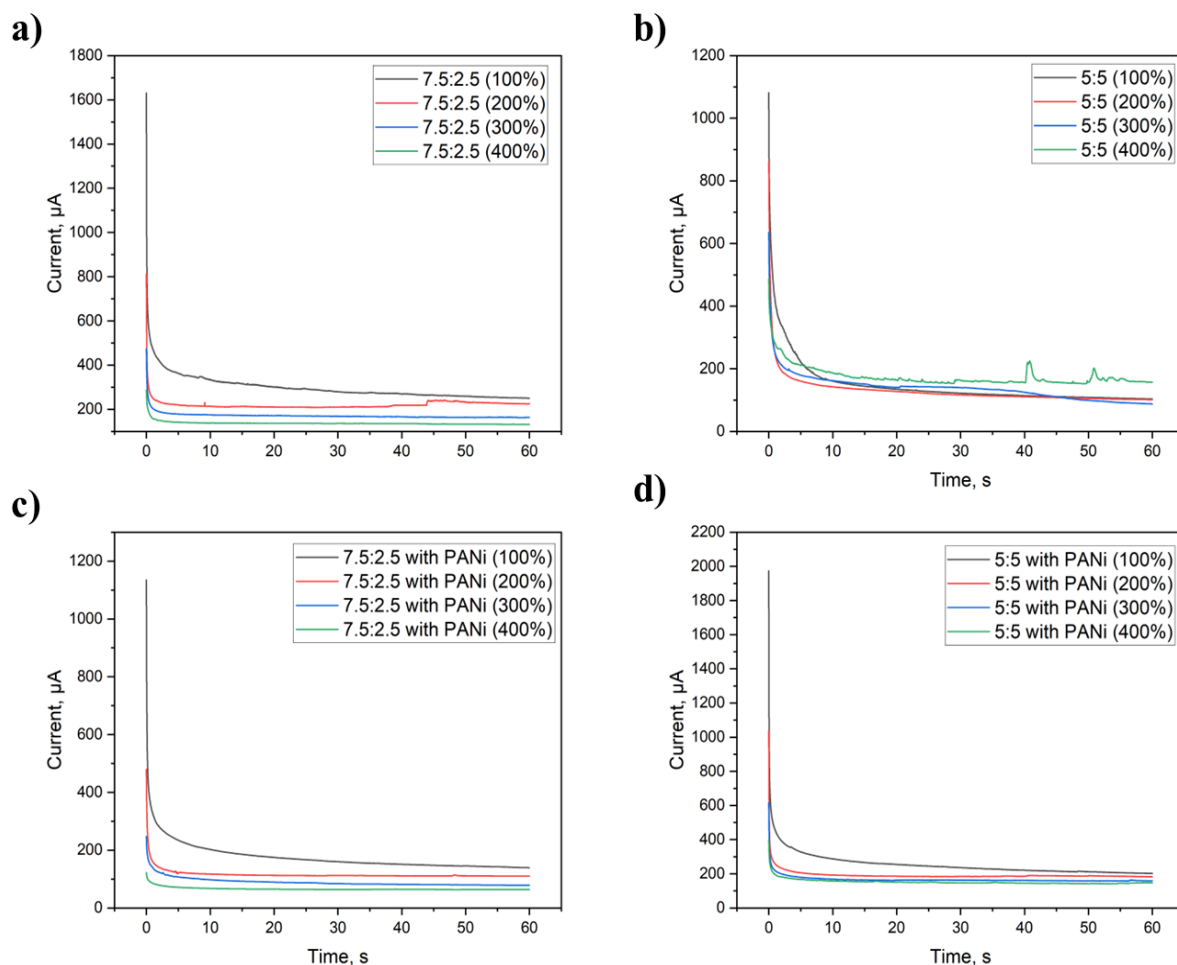


Figure 4.3.3. Graphical results of electrical sensitivity test by open chronoamperometry technique by stretching hydrogels for the 1-minute duration.

4.4 Conductivity test

Conductivity measurement of hydrogels was done by simple two-probe methods for which samples were cut in a rectangular shape with a size of 20 mm x 10 mm x 5 mm and a cross-sectional area is 50 mm².

The result of the resistance measurement is demonstrated in Figure 4.4.1, where significant differences can be observed between samples with and without PANi. The resistance measurement of hydrogels without CP, with two probes, gives 10,88 k Ω for 5:5, 8,43 k Ω for 7,5:2,5, and 16,63 k Ω for 10:0. Whereas, for gels with PANi, there are 1,782 k Ω , 1,362 k Ω , and 2,245 k Ω respectively. According to the electrical conductivity formula, calculated numbers are shown in Figure 4.4.2.

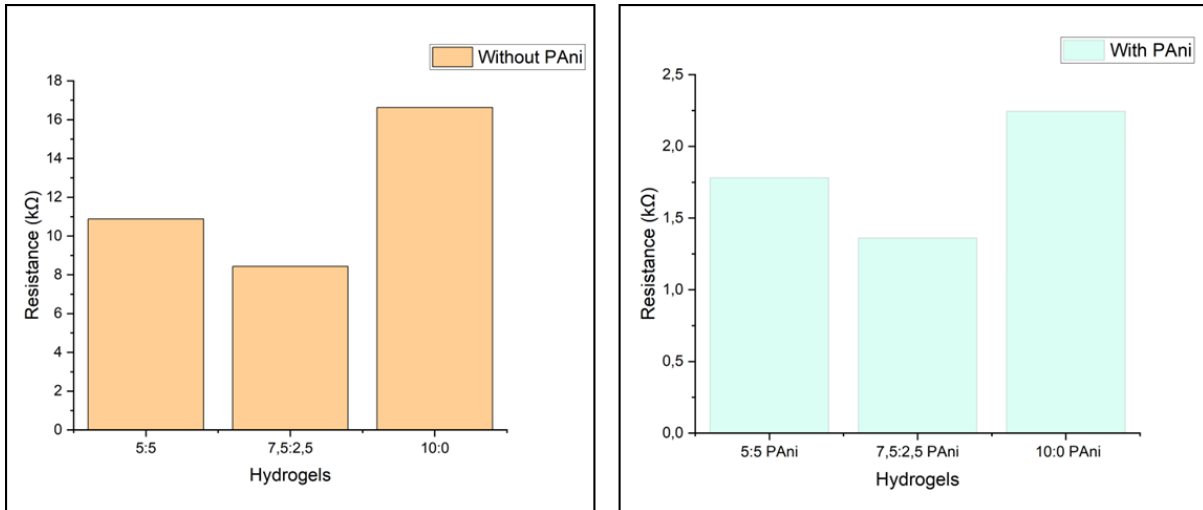


Figure 4.4.1. Resistance results in kilo Ohm for hydrogels without and with conductive polymer.

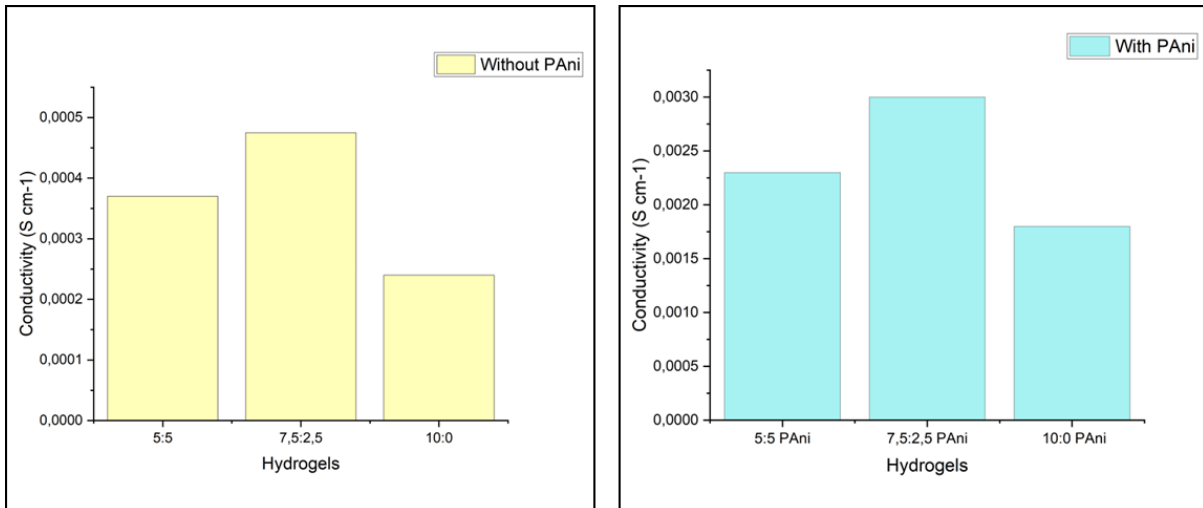


Figure 4.4.2. Conductivity results in S/cm for hydrogels without and with conductive polymer.

According to the figure above, for hydrogel without conductive polymer with a PA and water ratio 5:5 is $3,7 \times 10^{-4} \text{ S cm}^{-1}$, which increased to $2,3 \times 10^{-3} \text{ S cm}^{-1}$ after the addition of PANi. This phenomenon can be explained by the role of phytic acid as a dopant for aniline monomer, showing electrical conductivity in addition to an ionic conductivity of PA. The same dramatic change can be observed in samples with a ratio of 7,5:2,5, where conductivity is increased from $4,75 \times 10^{-4} \text{ S cm}^{-1}$ to $3 \times 10^{-3} \text{ S cm}^{-1}$ after PANi was added. The last samples with a ratio of 10:0 (PA:DI) indicate approximately the same level of difference, changing from $2,4 \times 10^{-4} \text{ S cm}^{-1}$ to $1,8 \times 10^{-3} \text{ S cm}^{-1}$. However, this ratio shows lower conductivity in

comparison with others, because of possible depression of ionization of phytic acid at a high concentration of this chemical, leading to a fall of free ions.

4.5 Swelling properties

Table 1. Number of the weight of samples before and after swelling Ani-HCl solution

Mass	5:5	7,5:2,5	10:0
Before	15,54 g	14,78 g	15,36 g
After 24 h in Ani-HCl	43,41 g	39,97 g	34,63 g

The swelling rate of hydrogels was about in the same range, where samples with a ratio of 5:5 and 7,5:2,5 showed 179,3 % and 170,4% respectively, while 10:0 showed only 125,4%. An increase of phytic acid content in hydrogels can decrease the swelling property because of higher density and low porosity. This can lead to a lower content of PANi in the case of swelling Ani-HCl, polymerizing inside of the hydrogel, consequently decreasing conductivity.

4.6 FTIR analysis

According to Figure 4.6, the peaks at 1563 and 1471 cm^{-1} in the FTIR spectrum of polyaniline (black line) correspond to the C=N and C=C stretching modes of the quinoid and benzenoid rings, respectively. The 1297 cm^{-1} band corresponds to the C-N stretching of a secondary aromatic amine. The band at 1234 cm^{-1} can be read as a C-N-C stretching vibration in the polaron structure, whereas the band at 1101 cm^{-1} corresponds to a structure-forming -NH⁺ = vibration mode. The broad band at 3223 cm^{-1} is attributable to the N-H stretching vibration caused by the protonation of nitrogen, whereas the broadband at 3050 cm^{-1} is attributable to the stretching vibration of the aromatic C-H bond [30]. Polyaniline is a polymer with numerous intrinsically intriguing redox states.

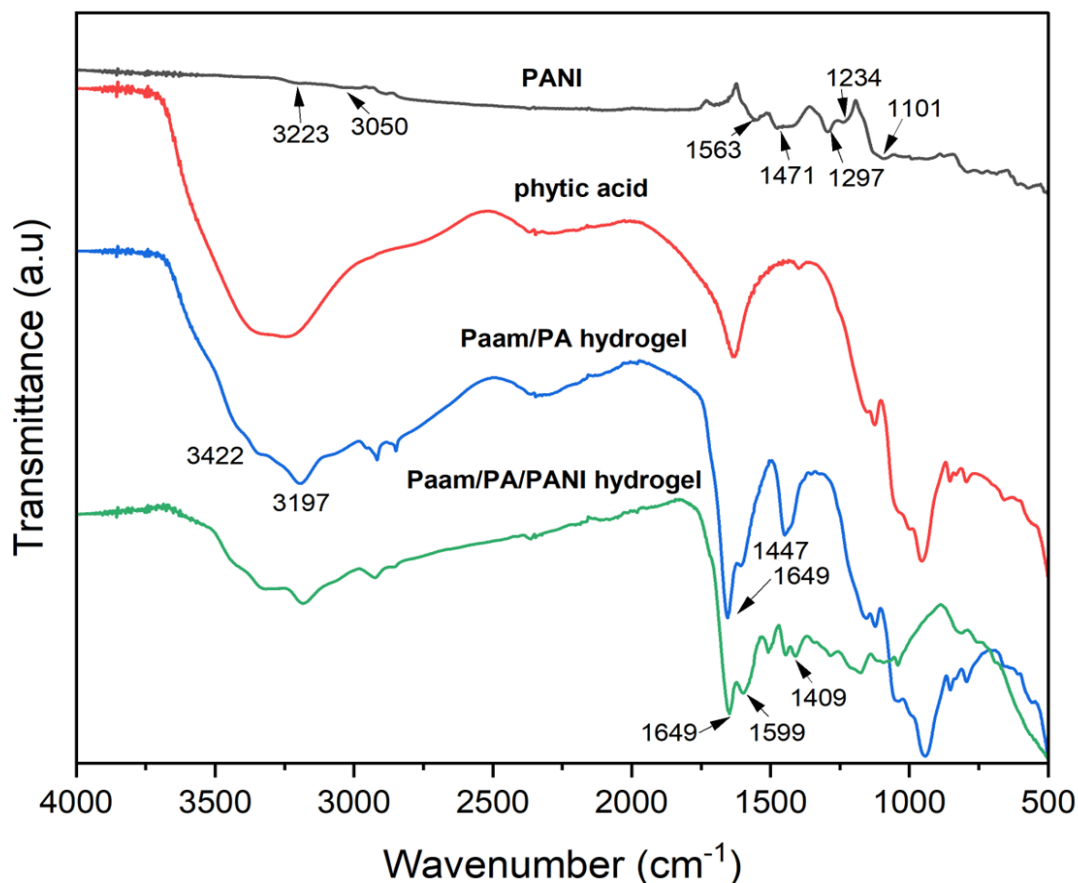


Figure 4.6. Fourier-transform IR spectra of PANi, Phytic acid, PAAM/PA hydrogel with and without PANi

The absorption peak at 3197 cm^{-1} in the PANi /PAM composite corresponds to the N-H bending, 1649 cm^{-1} to the C=O bending in PAM, 1599 cm^{-1} to the N-H in-plane bending in the $-\text{CONH}_2$ group, and 1409 cm^{-1} to the C-N stretching in the composite hydrogel system.

The hydroxyl groups of chitosan are responsible for the peak at 3422 cm^{-1} . Moreover, the peaks at 1447 cm^{-1} are attributable to CO groups in the hydrogel structure. The absorption band at 3197 cm^{-1} in hydrogel FTIR spectra, attributable to the OH and NH_2 groups of chitosan and acrylamide [30].

4.7 Morphology analysis by Scanning Electron Microscopy

After 24 hours of freeze-drying of samples (Figure 4.7.1a), SEM images were obtained from the cross-section part of the samples. Figures 4.7.1b and 4.7.1c show the 3D porous sponge-like structure inside hydrogels with a 5:5 ratio of phytic acid and water, without and with PANi.

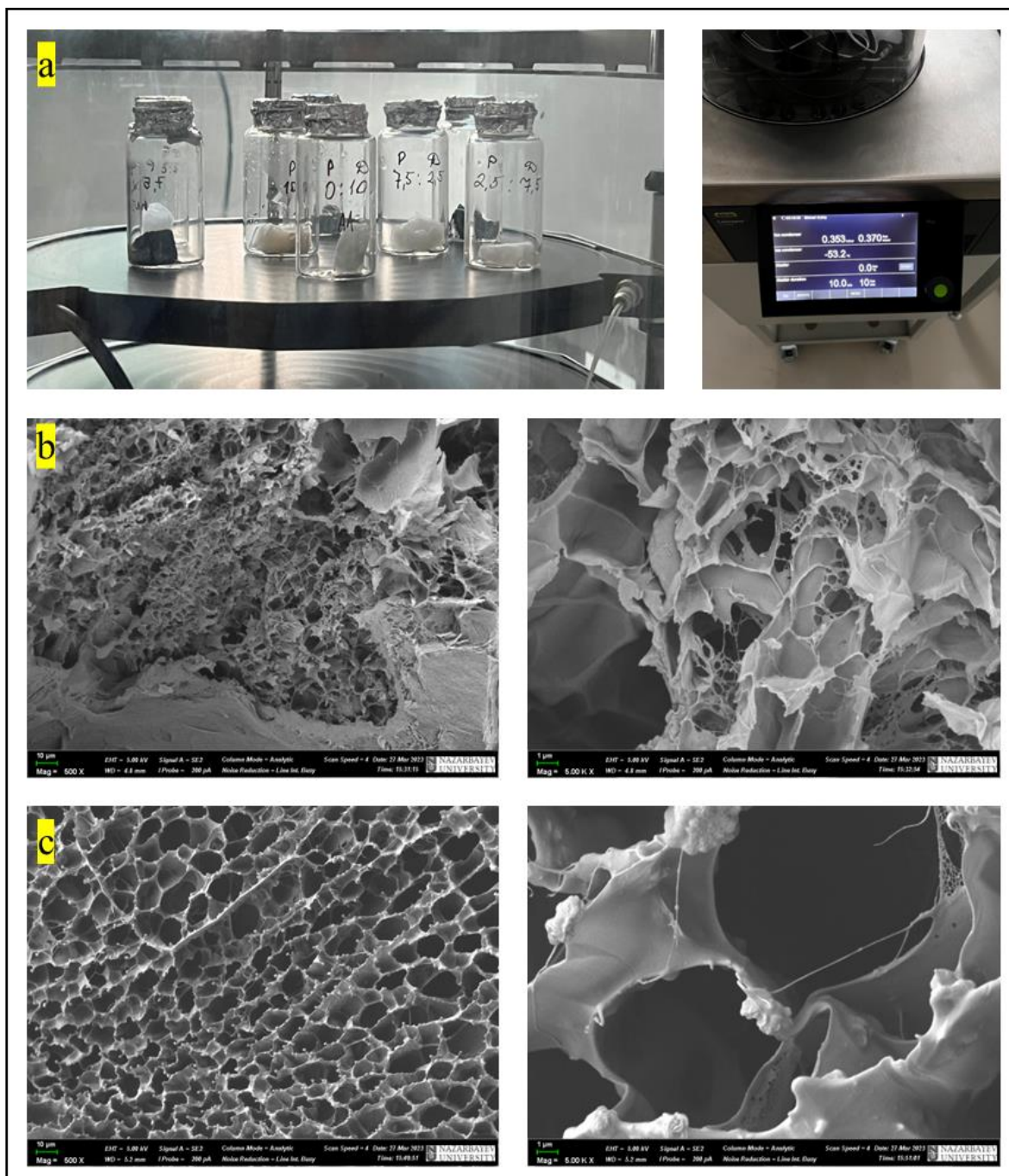


Figure 4.7.1. a) freeze-drying of samples in a lyophilization machine; b) SEM images of PAAm/PA hydrogels with a ratio of 5:5 (PA:DI) without and c) with PANi at a magnification of 500 and 5000.

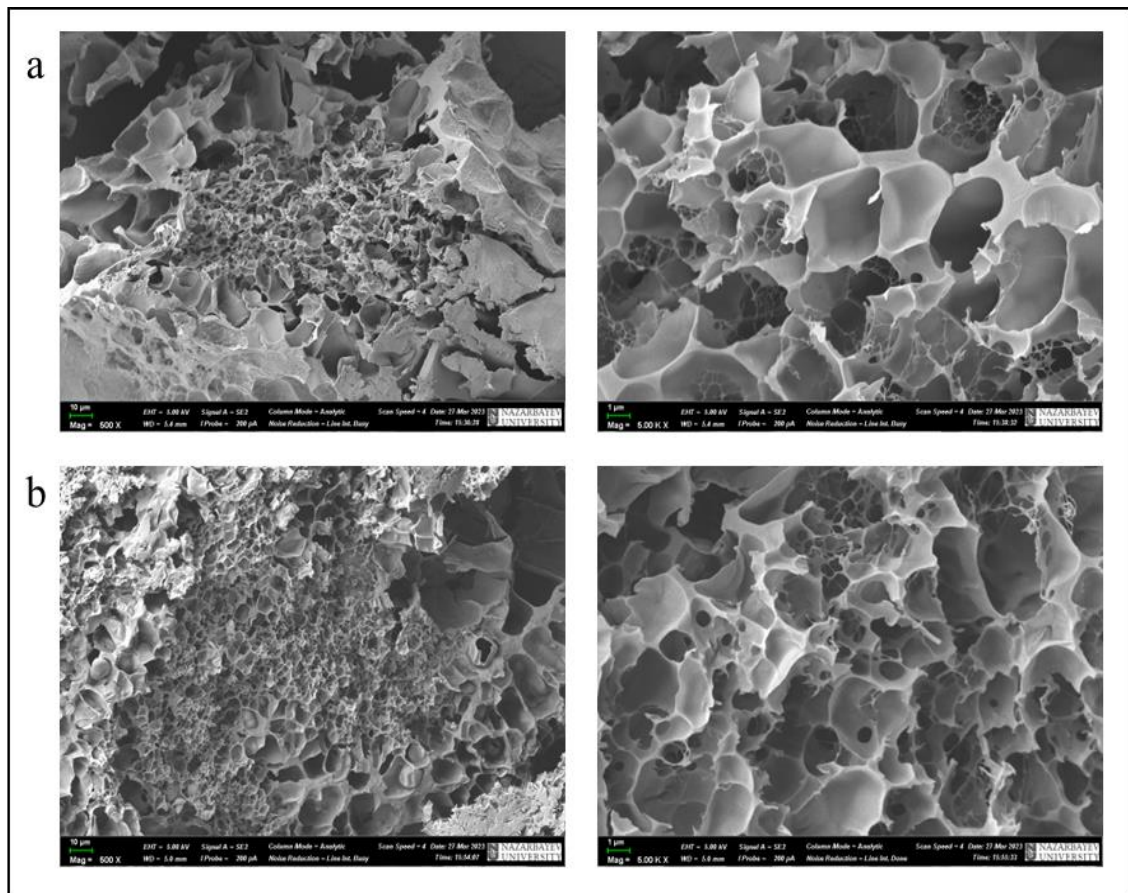


Figure 4.7.2. SEM images of PAAm/PA hydrogels with a ratio of 7,5:2,5 (PA:DI) without and b) with PANi at a magnification of 500 and 5000.

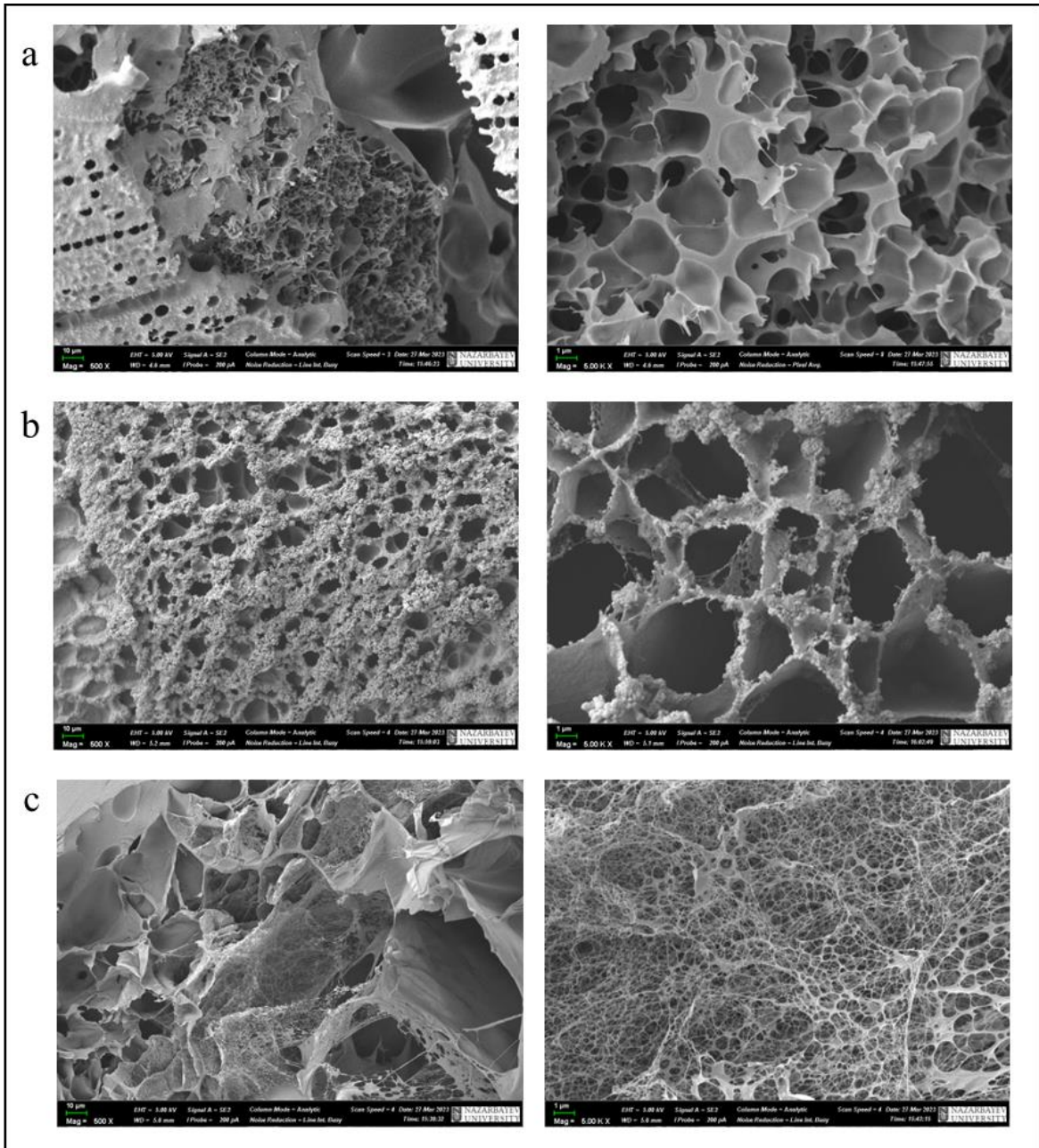


Figure 4.7.3. SEM images of PAAM/PA hydrogels with a ratio of 10:0 (PA:DI) without and b) with PANi and c) 0:10 without PA at a magnification of 500 and 5000.

4.8 Antibacterial test

Testing hydrogels for antimicrobial activity takes beginning with hydrogel, which was synthesized by dual cross-linking of alginate with phytic acid and calcium chloride.

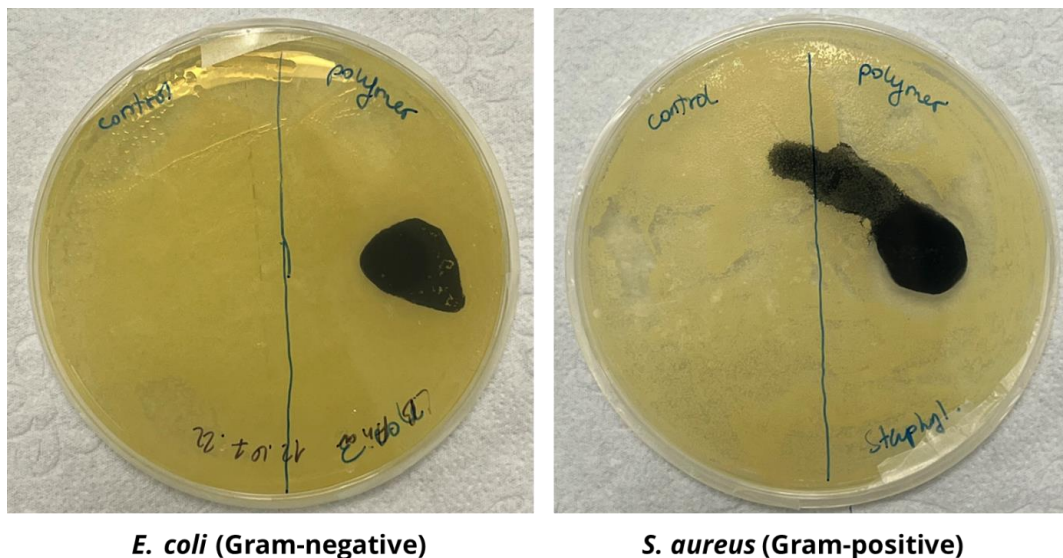


Figure 4.8.1. Antibacterial test of Alg-PA dual cross-linked hydrogel on *E. coli* and *S. aureus*.

Zero antibacterial activity was observed in testing on Gram-negative *Escherichia coli* and a small radius of activity has to be detected against Gram-positive *Staphylococcus aureus*, however, a sample was melted at 37°C showing weak temperature resistance (Figure 4.8.1). Additionally, it can be obvious that alginate and PANi are highly biocompatible polymers and the very low antimicrobial activity means that is coming from the little amount of phytic acid content. The reason for that is the molecule of phytic acid has highly reactive phosphate groups, which can bind to metal cations (like Ca or Mg) on the membrane of bacteria and lead to damage of it. Consequently, the microbial inhibition zone can expand with the increase of phytic acid content in a hydrogel, which is clearly demonstrated in Figure 4.8.2.

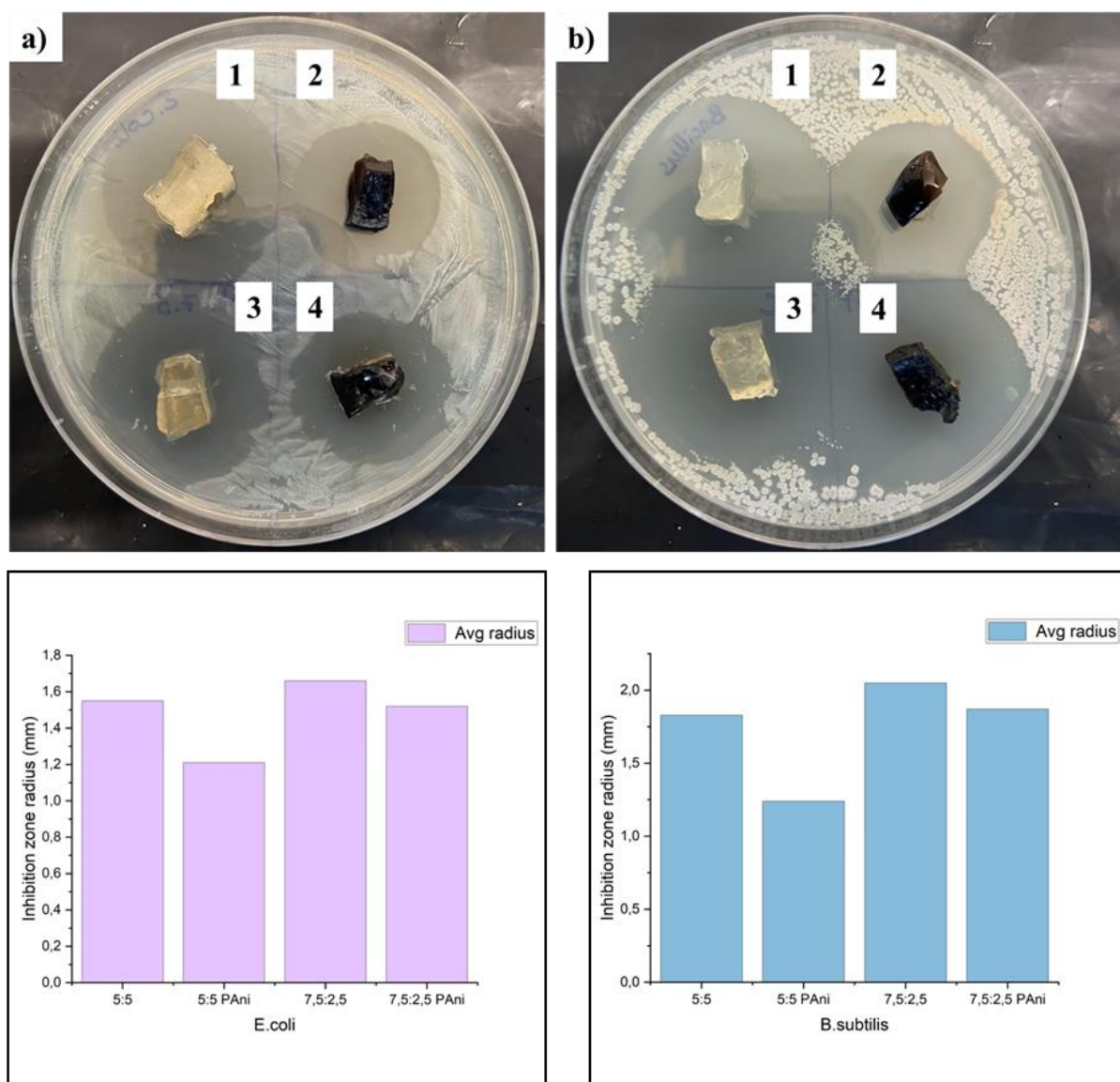


Figure 4.8.2. Antibacterial test of PAAm/PA and PAAm/PA-PAni hydrogel on *E. coli* (a) and *B. subtilis* (b). 1) 5:5; 2) 5:5 PAni; 3) 7,5:2,5; 4) 7,5:2,5 PAni

Excellent antimicrobial activity was clearly demonstrated in Figure 4.8.2, where hydrogels with a ratio of 5:5 and 7,5:2,5 showed the average radius of inhibition zone 1,55 mm and 1,67 mm against *E. coli* and 1,83 mm and 2,05 mm against *B. subtilis*, respectively. Because of the low antibacterial property and high biocompatibility of PAni, the radius of inhibition is lowered after the addition of conductive polymer to the hydrogel, but not significantly.

Chapter 5 – Conclusion

In conclusion, electrical sensitivity and mechanical properties were measured showing significant difference between added and non-added PANi. Strength of materials increased twice after addition of conductive polymer, whereas the elasticity of them decreased. Electrical sensitivity also rise after addition of PANi making these materials as a great candidate for biomedical application as a flexible and wearable sensor. Additionally, antibacterial property was not decreased and showed great bacterial killing activity on both Gram-positive and Gram-negative bacterial species. Chemical content of hydrogels were studied by FTIR and morphological analysis was done using SEM investigation. Conductivity of materials were measured and after addition of PANi, it is increased about 10 times.

However, swelling properties of materials changes depending on the phytic acid content, where ratio of 10:0 showed lower rate than other samples, because of high density. Furthermore, increase of phytic acid more than 80% affect to a conductivity of materials by lowering it. Nevertheless, the effect of the addition of conductive polymer was well studied and suitable ratio of phytic acid and water (7,5:2,5) was found for the production of conductive polymer-based hydrogel sensors. In the future, this method can be modified to obtain more electrically conductive hydrogels and will have a potential use in many branches of biomedicine and engineering.

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