REPUBLIC OF TURKEY
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOENGINEERING

RECOGNITION OF ANGIOTENSIN (II) VIA MOLECULARLY IMPRINTED POLYMERS

MEHTAP YILDIRIM

MASTER OF SCIENCE

ADANA 2020

REPUBLIC OF TURKEY
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOENGINEERING

RECOGNITION OF ANGIOTENSIN (II) VIA MOLECULARLY IMPRINTED POLYMERS

Mehtap YILDIRIM

MASTER OF SCIENCE

SUPERVISOR
Assoc. Prof. Gözde BAYDEMİR PEŞİNT

ADANA 2020

I hereby declare that all information in this thesis has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all information that is not original to this work.

Mehtap YILDIRIM

ABSTRACT

RECOGNITION OF ANGIOTENSIN (II) VIA MOLECULARLY IMPRINTED POLYMERS

Mehtap YILDIRIM
Department of Bioengineering
Supervisor: Assoc. Prof. Dr. Gözde BAYDEMİR PEŞİNT
June 2020, 50 pages

Angiotensin-II (ANGII) molecule is an important biomarker for the diagnosis and follow-up of some infections, tumors and especially cardiovascular diseases. Although the analysis of ANGII can be carried out routinely in health applications, the determination of this protein molecule from the blood is quite laborious and expensive. There is no study for the recognition of ANGII directly from blood using molecular imprinting technique which is a reliable technique for the preparation of affinity systems. In this study, ANGII-imprinted polymeric cryogels (ANGII-MIP) were designed and produced using cross-linker, functional monomer and template molecule (ANGII) at different ratios for the determination of ANGII, as an alternative to the present methods. Synthesized ANGII-MIP was characterized by surface area measurements (BET), swelling tests, and scanning electron microscopy (SEM). Surface area of ANGII-MIP was found to be 52 m^2/g, and the swelling ratio of the MIP columns was calculated as 94.3%. In order to obtain template-specific cavities in ANGII-MIP column, ANGII molecules was removed from the column with success over 80%, using 0.5 M NaCl solution. The AngII–non-imprinted (ANGII-NIP) cryogels synthesized without ANGII molecules and all studies were performed for ANGII-NIP, as well. Selectivity of ANGII-MIP was investigated using Arg8 Vasopressin, Angiotensin-I molecules. The reusability of the synthesized ANGII column was also studied in this study.

Keywords: Angiotensin II, Polymeric Cryogels, Molecular Imprinting

ÖZET

MOLEKÜLER BASKILANMIŞ POLİMERLERLE ANJİYOTENSİN II TAYİNİ

Mehtap YILDIRIM
Biyomühendislik Anabilim Dalı
Danışman: Doç. Dr. Gözde BAYDEMİR PEŞİNT
Haziran 2020, 50 sayfa

Anjiyotensin-II (ANGII) molekülü, bazı enfeksiyonların, tümörlerin ve özellikle kardiyovasküler hastalıkların tanı ve takibi için önemli bir biyobelirteçtir. Anjiyotensin analizinin sağlık uygulamalarında rutin olarak yapılabiliyor olmasına rağmen, bu proteinin kandan tayini oldukça zahmetli ve pahalıdır. ANGII'nin, afinite sistemlerinin hazırlanması için güvenilir bir teknik olan moleküler baskılama tekniği ile doğrudan kandan saptanması için gerçekleştirilmiş bir çalışma bulunmamaktadır. Yapılan tez çalışmasında, ANGII baskılanmış polimerik süngerler (ANGII-MIP), ANGII'nin saptanması için mevcut yöntemlere alternatif olarak farklı oranlarda çapraz bağlayıcı, fonksiyonel monomer ve kalıp molekülü (ANGII) kullanılarak tasarlanmış ve üretilmiştir. Sentezlenen ANGII-MIP kolonları, yüzey alanı ölçümleri (BET), şişme testleri, taramalı elektron mikroskopisi (SEM) ile karakterize edilmiştir. Karakterizasyon çalışmalarında, ANGII-MIP kolonlarının yüzey alanı 52 m^2/g, kolonların şişme oranı %94.3 olarak bulunmuştur. ANGII-MIP kolonunda kalıp moleküle özgü boşluklar elde etmek için ANGII molekülleri, 0,5 M NaCl çözeltisi kullanılarak %80'in üzerinde başarı ile kolondan söküldü. ANGII molekülleri olmadan sentezlenen AngII baskılanmamış polimerler (ANGII-NIP) için de karakterizasyon çalışmaları gerçekleştirildi. ANGII-MIP kolonunun ANGII molekülü için seçiciliği, Arg8 Vasopressin ve Angiotensin-I (AngI) yarışmacı moleküllerine karşı incelendi. Son olarak, sentezlenen ANGII-MIP kolonunun tekrar kullanılabilirliği, tekrar eden adsorpsiyon-desorpsiyon çalışmalarında incelenmiştir.

Anahtar Kelimeler: Anjiyotensin II, Polimerik Kriyojeller, Moleküler Baskılama

ACKNOWLEDGEMENTS

Firstly, I would like to express my gratitude to my supervisor Assoc. Prof. Dr. Gözde BAYDEMİR PEŞİNT, who was a perfect guide to me with numerous helps and advices. No matter how much I thank her, it will not be enough.

I sincerely thank to all members of our laboratory: to Okan ZENGER and Burcu EREN for contributing to this study; to Bürge ATAÖZÜ, Mustafa NERGİZ and Pırıl ARISOY for their help and kindness during the critical reading process of this master thesis, I am deeply grateful to them.

I would like to thank my dear lecturers, Asst. Prof. Dr. Hatice İmge OKTAY BAŞEĞMEZ and Asst. Prof. Dr. Sibel ÖZDAŞ, for all their positive attitudes and kindness during my master study.

I would like to thank my parents for their endless love and support and patience.

Finally, I will also include to my acknowledgements to my dear friend Gizem AKÇAY who was with me by support and patience in every time besides my family. I am grateful to her.

This thesis was supported by Adana Alparslan Turkes Science and Technology University Scientific Research Coordination Unit with project number of 19332002.

TABLE OF CONTENTS

ABSTRACT iv
ÖZET v
ACKNOWLEDGEMENTS vi
TABLE OF CONTENTS vii
LIST OF FIGURES ix
LIST OF TABLES x
NOMENCLATURE xi
1. INTRODUCTION 1
2. LITERATURE REVIEW 4
2.1. RENIN-ANGIOTENSIN-ALDESTERONE SYSTEM (RAAS) 4
2.2. ANGIOTENSIN II (ANGII) 5
2.3. HYPERTENSION 7
2.3.1. Hypertension and Cardiovascular Diseases 8
2.4. MOLECULAR IMPRINTING TECHNOLOGY 9
2.4.1. Main Components Of Molecular Imprinting 9
2.4.1.1. Template (Target) Molecule 10
2.4.1.2. Functional Monomers 11
2.4.1.3. Crosslinkers 11
2.4.1.4. Solvents 12
2.4.2. MOLECULARLY IMPRINTED POLYMERS 13
2.4.2.1. Covalent Imprinting 14
2.4.2.2. Non-Covalent Imprinting 15
2.4.3. CRYOGELS 16
3. MATERIAL AND METHODS 19
3.1. Materials 19
3.2. Device List 19
3.3. EXPERIMENTAL METHODS 20
3.3.1. Preparation of ANGII-MIP and NIPs 20
3.3.2. Removal of the Template ANGII from ANGII-MIP 21

3.4. CHARACTERIZATION STUDIES 21
3.4.1. Swelling Test 21
3.4.2. Surface Morphology 22
3.5. ADSORPTION STUDIES 22
3.5.1. Effect of Equilibrium ANGII concentration on Adsorption 22
3.5.2. Effect of Flow Rate on Adsorption 22
3.5.3. Effect of Interaction Time on Adsorption 23
3.6. Selectivity Studies 23
3.7. Desorption and Reusability Studies 24
3.8. Selective ANGII Rebinding From Human Serum 25
4. RESULTS AND DISCUSSION 26
4.1. Characterization of ANGII Imprinted Cryogels 26
4.1.1. Swelling test 26
4.1.2. Surface Morphology 27
4.2. Adsorption Studies 28
4.2.1. Effect of Equilibrium ANGII Concentration on ANGII Adsorption 29
4.2.1.1. Adsorption Isotherms 30
4.2.2. Effect of Interaction Time on ANGII Adsorption 33
4.2.2.1. Adsorption Kinetics 34
4.2.3. Effect of Flow Rate on ANGII Adsorption 35
4.3. Selectivity Studies 36
4.4. Reusability Studies 37
5. CONCLUSION 39
REFERENCES 40
6. CURRICULUM VITAE 47

LIST OF FIGURES

Figure 2.1. General demonstration of Renin–Angiotensin-Aldesterone System (RAAS) within tissue 4
Figure 2.2. Between cellular and extracellular compartments, the view of angiotensin II within tissues. 5
Figure 2.3. Chemical Structure of Angiotensin II 6
Figure 2.4. General principle of molecular imprinting 10
Figure 2.5. Functional monomers commonly used molecular imprinted polymers 11
Figure 2.6. The most commonly used crosslinkers in molecularly imprinted polymers 12
Figure 2.7. Schematic Representation of the Covalent and Non-covalent Molecular Imprinting Procedures 16
Figure 2.8. Demonstration of the Cryogelization Process 17
Figure. 3.1. ANGI (left) and VASP (right). 23
Figure 4.1. Structure of PHEMA 27
Figure 4.2. A, B) ANGII-MIP cryogel columns; C, D) SEM photos of NIP columns. 28
Figure 4.3. Effect of equilibrium initial ANGII concentration on ANGII adsorption: 29
Figure 4.4. Effect of equilibrium initial ANGII concentration and Langmuir and Freundlich Adsorption models. M_{dry}: 0.1027 g, V: 3 mL, time: 120 min, pH 7.4 Flow Rate: 0.5 mL/min, T: 25°C 32
Figure 4.5. Effects of interaction time on ANGII adsorption, ANGII conc.: 0.05 mg / mL, dry: 0.1027 g, V: 3 mL, pH 7.4, Flow Rate: 0.5 mL/min, T: 25°C 33
Figure 4.6. Effects of flow rate on ANGII adsorption: ANGII conc.: 0.05 mg/mL, m_{dry}: 0.1027 g, V: 3 mL, pH 7.4, Time: 120 min, T: 25°C 35
Figure 4.7. Molecular structures and properties of ANGII, which is a template molecule, and ANGI and VASP, which are competitor molecules 36
Figure 4.8. Selectivity Studies, ANGII, ANGI, VASP conc.: 0,05 mg/mL, m_{dry}:0,1027 g, Flow Rate: 0,5 mL/min, V: 3 mL, pH 7,4, Time: 120 min, T: 25°C 36
Figure 4.9. Reusability of ANGII-MIP columns. ANGII conc : 0.05 mg / mL, m_{dry}: 0.1027 g, V: 3 mL, pH 7.4, Time: 120 min, T: 25°C 38

LIST OF TABLES

Table 4.1. Swelling ratio, macroporosity, surface area and polymerization yield of ANGII-MIP and NIP 27
Table 4.2. Langmuir and Freundlich adsorption isotherm constants for ANGII 32
Table 4.3. Adsorption kinetic model constants and adsorption amounts 35
Table 4.4. Selectivity Studies 37

NOMENCLATURE

µL	: Microliter
µm	: Micrometre
mL	: Milliliter
mg	: Microgram
cm	: Centimetre
fM	: Femtomolar
fg	: Femtogram
fmg	: Femtomiligram
pM	: Picomolar
pg	: Picogram
ng	: Nanogram
L	: Liter
Min	: Minute
M	: Molarity
°C	: Celsius
HP	: Hypertension
CVD	: Cardiovascular Disease
BP	: Blood Pressure
Eq.	: Equation
ANGII	: Angiotensin II
ANGI	: Angiotensin I
VASP	: Arg8 Vasopressin
MIP	: Molecular Imprinted Polymer
NaCl	: Sodium Chloride
PBS	: Phosphate - buffered saline
TEMED	: N, N, N, N - Tetramethylethylenediamine
APS	: Ammonium persulfate
VIM	: 4-Vinylimidazole
HEMA	: 2-Hydroxyethylmethacrylate
P(HEMA)	: Poly(2-Hydroxyethylmethacrylate)
MBAAm	: N, N'-Methylenebisacrylamide

1. INTRODUCTION

ANGII is an active hormone of the renin-angiotensin-aldesterone system (RAAS) (Brunner et al., 1993; Unger, 2000; Yılmaz & Erdem, 2006) and has an important role within the complex network of other endocrine (Abalı & Kabakçı, 2004). The synthesis of ANGII begins with the production of angiotensinogen, an alpha 2 globulin, in the liver (Brunner et al., 1993; Unger, 2000; Yılmaz & Erdem, 2006).

RAAS is the system for regulating arterial pressure in hypertension physiopathology (Navar et al., 2001; Mıcılı et al., 2012). Therefore, it plays a very important role in the cardiovascular system (Rosenbaugh et al., 2013; Montezano et al., 2014; Pullareddy et al., 2009). Renin enzyme, Angiotensin (1-7) and Angiotensin converting enzyme (ACE) are essential parts of this system (Mehta & Griendling, 2006; Navar et al., 2001; Patel et al., 2016; Derad et al., 2013).

ANGII has central effects in vasoconstriction, aldosterone release, antidiuretic hormone synthesis, sympathetic activation, and salt reabsorption from kidney tubules, and all these effects lead to the development of hypertension. The most important effect in the hypertension is the direct contraction effect of ANGII on arterial smooth muscles. It has also a positive inotropic effect on the heart and elevating blood pressure. Additionally, ANGII causes irregularity in endothelial cells, medial hypertrophy and increase in connective tissue, causing atherosclerosis, growth of myositis in the heart muscle, development of left ventricular hypertrophy and development of heart failure (Brunner et al., 1993; Unger, 2000; Yılmaz & Erdem, 2006). For this reasons, ANGII can be considered as an important biomarker for certain diseases, including some infectious diseases such as COVID19 (Zheng et al., 2020) and influenza (Huang et al., 2014; Yan et al., 2015), as well as cardiovascular diseases (Úri et al., 2014; Zheng et al., 2020) and tumors (Munro et al., 2017; Xie et al., 2018), which are associated with hypertension and hypotension (Brunner et al., 1993). However, ANGII presents in blood in very low concentrations and it is not stable due to its reactivity, therefore spontaneous detection of ANGII is a big challenge (Navar et al., 2001).

Cardiovascular diseases (CVDs) appear to affect more human life than all types of cancer and chronic respiratory diseases with annual follow-up (Brown et al., 2005).

Cardiovascular diseases are stroke, congenital heart disease, rhythm disorders, subclinical atherosclerosis, coronary heart disease, heart failure, valvular disease, venous disease, peripheral

artery disease, and hypertension, hyperglycemia, dyslipidemia, and hyperhomocysteinemia (Lan et al., 2013), blood pressure, cholesterol and glucose control are risk factors as well as smoking, physical activity, diet and weight. Cardiovascular diseases are responsible most of deaths in the all over the world (Levenson et al., 2020; Bhatnagar et al., 2015; Swanoski et al., 2012). In this regard, early diagnosis and treatment of cardiovascular diseases are important for mortality and morbidity (Swanoski et al., 2012).

ANGII, as a significant biomarker in several diseases, is mostly detected in biological fluids using HPLC-radioimmunoassay (RIA) (Polyakov, 1931), enzyme-linked immunosorbent assay (ELISA) and LC-MS/MS methods. Although these methods are able to detect ANGII for diagnostic purposes, they have important drawbacks ie., that they are time consuming and laborious, they include hazardous radioactive materials (in case of RIA), and they have limited storage stability (in case of ELISA). Therefore, there is still a vital need to develop alternative methods for direct detection of ANGII in body fluids (Brosnihan & Chappell, 2017).

Molecular imprinting is a technique, which is used for recognizing a target molecule (eg, ion, protein, peptides, etc.) from a complex media, in single step with high selectivity. Basically, molecularly imprinted polymers (MIPs) are designed in the following steps: an interaction occurs between the functional monomers and the template molecule; the functional monomer-template complex is polymerized in presence of crosslinkers and monomers; and the target molecule is removed from the polymer to obtain target specific cavities with the shape and chemical structure memory for the target molecule (Polyakov, 1931; Mosbach & Ramström, 1996; Çiçek, 2014).

Molecularly imprinted polymers are resistant to mechanical stress, heat, acid, base, water and organic solvents and have high selectivity for the molecule of interest. Besides the fact that they can be stored for several years without any remarkable change in their performance, they are inexpensive and reusable. These advantages enables them to be used as synthetic recognition elements in many application fields, ie. in health, food and environmental sciences for the purpose of diagnosis, separation, purification, removal, etc (Piletska et al., 2004).

Cryogels, biomolecules and proteins separation-purification, immobilization of cells (Lozinsky et al., 2003), biosensors, enzymes and antibodies used as biomaterials, separation and purification of biomolecules such as plasmid DNA and enzyme; electrophoresis medium, isoforporesis and isoelectric focus in processes such as selectively identifying a target molecule among similar

molecules for biological and chemical processes; as the medium for immunodiffusion experiments, chromatographic supports (Baydemir, et al., 2009; Baydemir, et al., 2009), biomedical applications such as scaffolds in tissue engineering, and drug carriers are among the areas where it is used (Andaç & Denizli, 2014; Çavuş et al., 2013; Bereli et al., 2008; Lozinsky et al., 2003).

In this study, we offer an alternative method for direct ANGII detection from human serum in a single step with high selectivity. For this purpose, we prepared PHEMA-based ANGII imprinted cryogel polymers. The selectivity studies were performed against AngI and VASP, and ANGII detection were achieved from human serum (Brosnihan & Chappell, 2017).

2. LITERATURE REVIEW

2.1. RENIN-ANGIOTENSIN-ALDESTERONE SYSTEM (RAAS)

The RAAS activity, which was found centuries ago, is a peptidergic system (Patel et al., 2016), that is crucial for the endocrine system (Dzau, 2001), renal system (Patel et al., 2016), cardiovascular system (Mehta & Griendling, 2006), regulating vascular function (Montezano et al., 2014), blood pressure regulation and homeostasis (Pullareddy et al., 2009), sodium metabolism (Mitro et al., 2008).

In this system, renin enzyme which is an aspartyl protease is produced from juxtaglomerular cells in the afferent arterioles of the kidney, while the hepatic peptide angiotensinogen is produced by the liver cells. This angiotensinogen produced is converted to form of decapeptide angiotensin I by the effect of renin enzyme. Angiotensin I is also converted to an ANGII, an octapeptide, by the ACE released from the lung (Patel et al., 2016; Mehta & Griendling, 2006; Navar et al., 2001; Campbell, 1987; Baker et al., 1992). Among these components, angiotensin II (ANG II) shows the main effect of RAAS, angiotensin II is the main component of RAAS (Mıcılı et al., 2012; Pullareddy et al., 2009; Patel et al., 2016; Ferrario & Strawn, 2006; Sümbül, 2012; Bryson, et al., 2020).

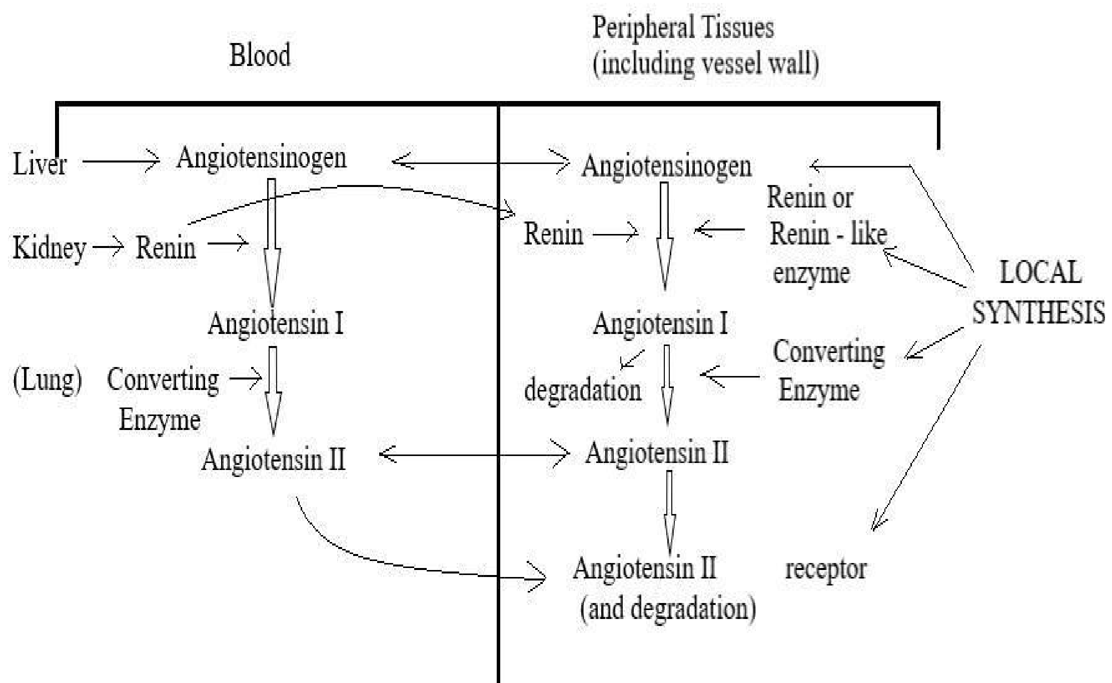


Figure 2.1. General demonstration of Renin–Angiotensin-Aldesterone System (RAAS) within tissue

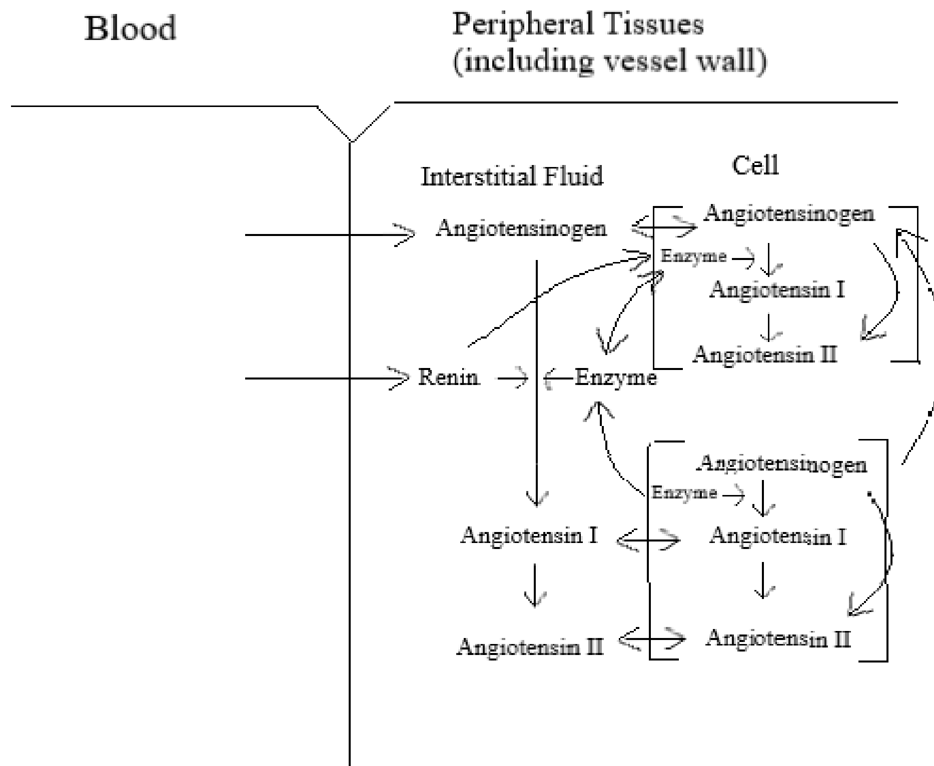


Figure 2.2. Between cellular and extracellular compartments, the view of angiotensin II within tissues.

2.2. ANGIOTENSIN II (ANGII)

ANGII is the main component of the RAAS activity and has two subtypes ANGII type 1 (AT_1) and ANGII type 2 (AT_2). While effects of AT_2 are not known as entirely, AT_1 is showed to effects, if not all (Pullareddy et al., 2009; Akbar et al., 2009; Baker et al., 1992).

Although ANGII is primarily effective in blood pressure, it has effect on plasma volume, neuronal function, and dispogenic responses (Griendling et al., 1996). Although it has proven to be more effective on AT_1, many studies have shown that AT_2 receptor has antagonistic effect on AT_1 receptor (Parlakpınar et al., 2004; Horiuchi et al., 1998).

In the studies conducted so far, AT_1 has been found to have the effects on growth promotion, vasoconstriction, inhibition of renin biosynthesis and release, pure appetite, thirst and sympathetic outflow; whereas AT_2 induces vasodilation and inhibits growth and cell proliferation (Akbar et al., 2009). While its molecular weight is 1046.2 g/mol, has 13 hydrogen bond donor and 15 hydrogen bond acceptor (National Center for Biotechnology Information, 2019).

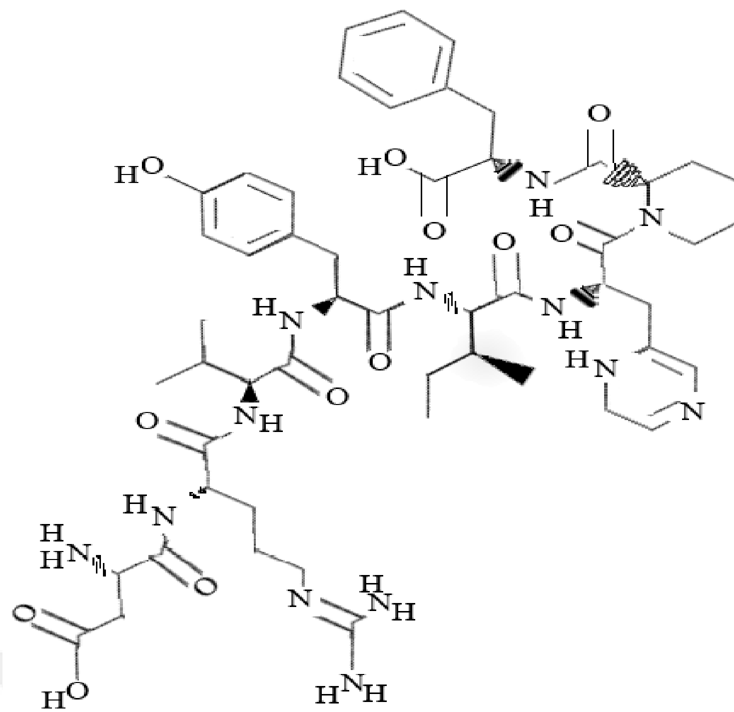


Figure 2.3. Chemical Structure of Angiotensin II

ANGII is important mediator of reduced NO activity and oxidative stress; induces the occuring of PAI-1 that is mediated special angiotensin receptors on the endothelial cells; activates to NADH/NADPH oxidase which powerful membrane oxidase and so, ANGII is effective to in endothelial dysfunction and vascular smooth muscle cells. At the same time, ANGII can be upset to coagulation systems (Dzau, 2001).

By causing vasoconstriction, especially hypertension-causing ANGII has a great importance in the cardiovascular system and is in the key point; myocardial infarction (MI), heart failure (HF), atherosclerosis, in the source of cardiovascular diseases (Navar et al., 2001; Rosenbaugh et al., 2013; Pullareddy et al., 2009).

These receptors are located on the different areas and they have different structures as genetic. So, gene of ANGII type 1 has five exons and four introns and the same time, length of it is > 55 kb and is existed on chromosome 3q21-q25 (Pullareddy et al., 2009; Akbar et al., 2009). According to some researches; ANGII type 1 has 4 or 5 exons in human and is divided into two as A and B in rats; A of type 1 has 4 exons and 3 introns, and B of type 1 has 3 exons and 2 introns. A gene in rats is found on chromosome 17 and B gene is on chromosome 2, while the human is located on the 3rd chromosome. Gene of ANGII type 2 is also examined from the mouse gene. And it has also, like the AT_1, 3 exons but in this receptor, all of coding sequence is

existed in the third exon. Despite of the fact that the gene of human has also been cloned, there is not enough to data. And, the gene of human is also intronless in the coding area, while it is existed on chromosome X (Griendling et al., 1996; Akbar et al., 2009). And, some studies are showed that these receptors are related to hypertensive diseases (Akbar et al., 2009).

ANGII is not produced only in one place/places. And ANGII is not only a endocrine hormone, in the same time it is produced in the different a lot of places like in the brain, in the heart, in the kidney and a lot of in the tissue of vascular walls. Thus, ANGII works as both paracrine or autocrine hormone (Parlakpınar et al., 2004; Wright et al., 1995; Mehta & Griendling, 2006; Patel et al., 2016).

While the changes in a small amount of ANGII cause a lot of effects, especially, in the endothelial structure, creates to vasoconstriction or other vascular injuries by changing structure of vascular wall. The endothelium structure is crucial for vascular function. In the between endothelial cells, a balance is achieved for vascular structure so, between vasodilators (e.g. NO) and vasoconstrictors (e.g. ANGII). Unless this balance can be achieved, structure of vascular is impaired and vascular damages occur (Dzau, 2001).

2.3. HYPERTENSION

Hypertension is a quite complicated a disease and affected by many factors such as genetics, environmental, homeostasis (Batkai & Thum, 2012). Hypertension is circumstance where the blood pressure is chronically higher than 140/90 mmHg. Normal values of blood pressure are systolic value < 120 mmHg and diastolic value < 80 mmHg (Aşık et al., 2018; Drummond et al., 2019).

Neurogenic hypertension, sympathetic overpressure defined as high pressure, parasympathetic mediated loss of cardiac diversity and high ANGII activity are common forms of hypertension (Fisher & Paton, 2012).

Hypertension causes vascular tissue remodeling, by triggering increase in its extracellular matrix (ECM), particularly to fibrillar collagen type I.

RAAS activation, imbalance between matrix metallopeptidases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), connective tissue growth factor (CTGF) upregulation, and abnormal

G protein-bound receptor signals are various pathophysiological factors at the molecular level of hypertension which cause vascular restructuring. Among all these factors RAAS activity, which shows its main effect with angiotensin II (ANG II), is the most important (Lan et al., 2013; Eskildsen et al., 2013; Bátkai & Thum, 2012; Lasocki, et al., 2002).

Hypertension can be controlled when diagnosed early, and causes microvascular and macrovascular diseases by affecting other organs such as the heart, cardiovascular diseases, brain and kidney when diagnosed late (Özdemir, 2010; Berker et al., 2014; Mıcılı et al., 2012).

Hypertension was the most common reason of death in the last century. Nowadays, hypertension is one of the risky factors with high mortality. According to the data of the World Health Organization, it causes 7.6 million deaths and 90 million disability in the world every year. One of every four deaths in our country is cardiovascular diseases and the most common underlying cause is hypertension. ("World Health Organization", 2020).

According to the American Heart Association 2019 statistics; hypertension was present in 46.0 % of Americans in 2013-2016; in 2016, it was observed that 82,735 deaths were due to hypertension, and in 2016, the age - adjusted death rate due to hypertension was 21.6 per 100,000. Moreover the data show that by 2035, the estimated total direct costs of High Blood Pressure (HBP) could rise to $220.9 billion (Association, 2019).

In Turkey, ratio of hypertension, in the data of the Turkish Hypertension Reconciliation Report, which was held for the first time in Turkey in 2015, has been shown that 30.2% of the population is hypertensive as of 2012 (Kılınç et al., 2016). 1 out of every 3 adults have hypertension. It is more common in women, and the frequency of hypertension seen under the age of 30 is 12%, which is a serious rate and not to be neglected. After the age of 60, this prevalence rises up to 60-80% (Arıcı, et al., 2019).

2.3.1. Hypertension and Cardiovascular Diseases

The vessels are subjected to pressure with numerous reasons and the functional, mechanical and structural changes occur in the vessel wall, that is, the vessel wall structure is disrupted. ANGII affects the development of cardiovascular diseases including hypertension, atherosclerosis, heart failure, myocardial infarction (MI). ANGII that has physiological role in maintaining blood pressure (BP) and electrolyte/fluid homeostasis, neurotransmitters and independent effects of

several hormones affect to organ damage by doing inflammation and fibrosis (Kvakan et al., 2009).

Hypertension can be defined as a permanent increase in systemic blood pressure (Batkai & Thum, 2012). It is qualified by an increase in extracellular matrix (ECM), vascular remodeling, which, among other alterations, particularly with fibrillar collagen type 1 (Lan, Huang, & Tan, 2013). Before the blood pressure increase, these pathologic features may be find and understand (Batkai & Thum, 2012).

2.4. MOLECULAR IMPRINTING TECHNOLOGY

Molecular imprinting is a technique used synthetic polymers for a specific target molecule. (Piletska et al., 2004; Haupt, 2001) Molecularly imprinted polymers (MIPs) therefore have specific molecular recognition, one of the most important properties of biological antibodies. It can be used for applications where immunoassays, affinity separation, biosensors and selective binding events such as directed synthesis and catalysis are important (Haupt, 2014; Lozinsky, 2018; Andaç & Denizli, 2014).

First, the molecular imprinting system was used by MV Polyakov in 1930 to selectively capture various additives in a silica matrix. In the 1940s, Linus Pauling argued that a process similar to molecular pressure could be used because of the selectivity of antibodies to their antigens. By the 1970s, Günter Wulff stated that highly crosslinked organic polymers could be used to make molecular imprints with high specificity, and in later years, imprinted polymers were used to capture everything from steroids to TNT (Haupt, 2003; Haupt, 2014).

2.4.1. Main Components Of Molecular Imprinting

The target molecule, functional monomers, crosslinkers, initiators and solvent are required for the preparation of molecularly imprinted polymers, and the amount of each determines the structure of the polymer, such as the pore size (Narula et al., 2014; Jian et al., 2015; Wulff, 1995; Yan & Row, 2006; Çorman, 2014; Çetin, 2013; Ersoy, 2018; Biçen, 2009; Öncel, 2013).

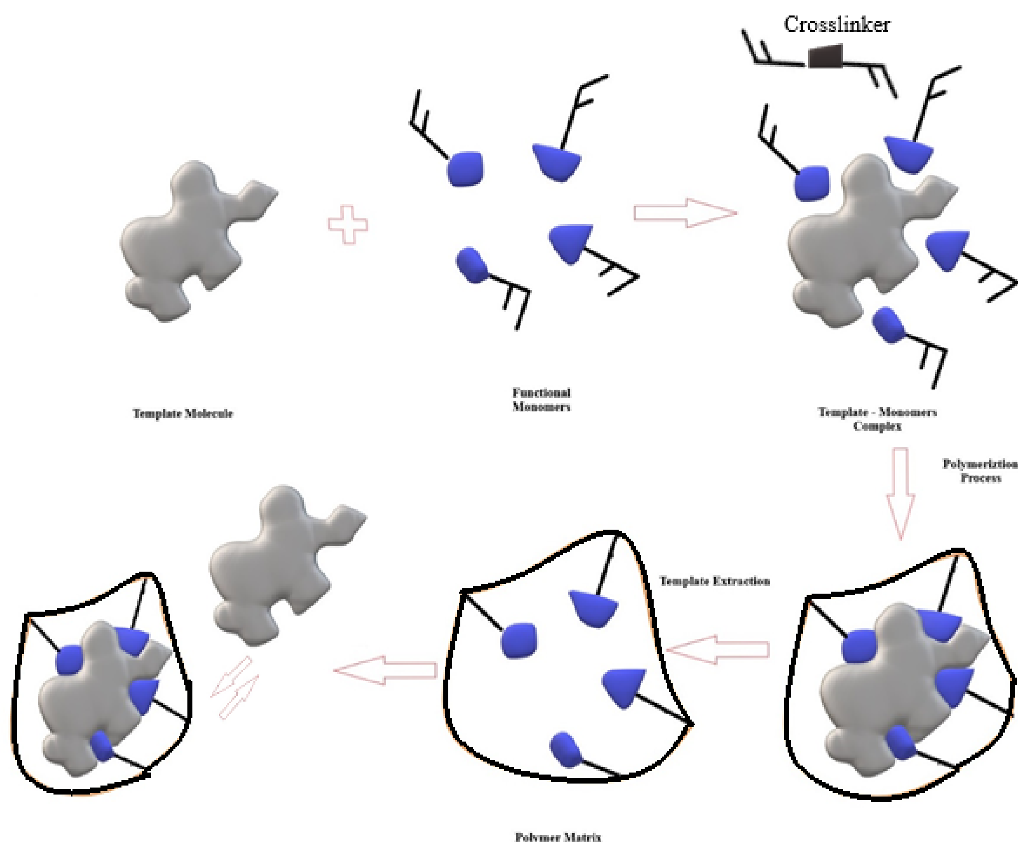


Figure 2.4. General principle of molecular imprinting.

2.4.1.1. Template (Target) Molecule

Any inorganic or organic molecules or biomacromolecules can be used as template molecules (target molecule) in molecular imprinting. Since the template molecule will interact with functional monomers, it should be noted whether there are properties that may adversely affect polymerization (Vasopollo et al., 2011).

Whether the template has groups that can be polymerized, whether it has a feature that delays the formation of free radical polymerization, whether it will be stabilized under high temperatures or UV rays are the main factors to be considered (Yan & Row, 2006; Jian et al., 2015; Vasopollo et al., 2011; Biçen, 2009; Çetin, 2013; Ersoy, 2018; Çorman, 2014; Öncel, 2013; Haupt & Mosbach, 2000).

2.4.1.2. Functional Monomers

The template must interact with functional monomers for the synthesis of MIPs, hence functional monomers such as template are also very important and constitute the key point (Yan & Row, 2006; Vasopollo et al., 2011; Çetin, 2013).

The amount of functional monomers is an important factor as it affects the binding sites; while this amount varies according to the template in covalent molecular imprinting, the template/functional monomer ratio should be 1: 4 according to Le Chatelier principle in non-covalent molecular imprinting (Yan & Row, 2006; Vasopollo et al., 2011; Biçen, 2009; Öncel, 2013).

If the functional monomer is less than necessary, no binding sites occur, whereas more than necessary, nonspecific adsorption regions are formed (Vasopollo et al., 2011; Öncel, 2013; Çetin, 2013; Biçen, 2009).

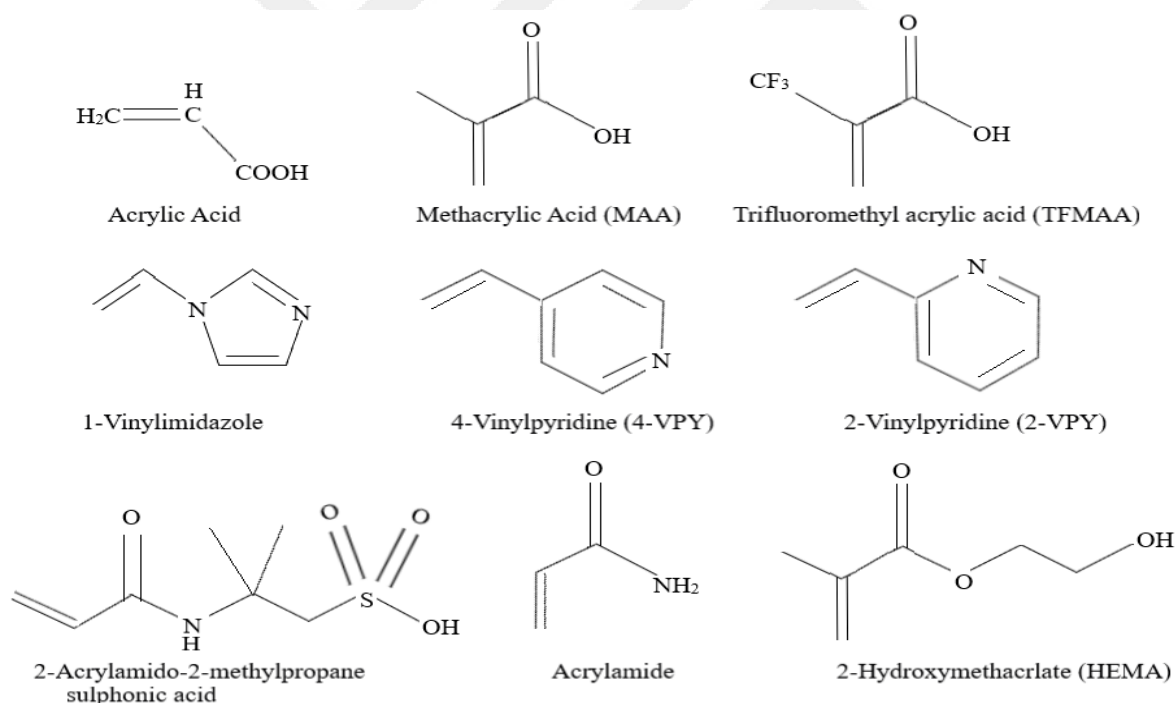


Figure 2.5. Functional monomers commonly used molecular imprinted polymers

2.4.1.3. Crosslinkers

Crosslinkers essentially integrate with 3 steps:

- The first is to control the morphology of the cryogel regardless of whether it is gel type, macroporous or microgel structure.
- Secondly, it provides stabilization of the imprinted area.
- Finally provides stabilization of the polymer (Vasopollo et al., 2011; Yan & Row, 2006; Çetin, 2013; Öncel, 2013; Biçen, 2009).

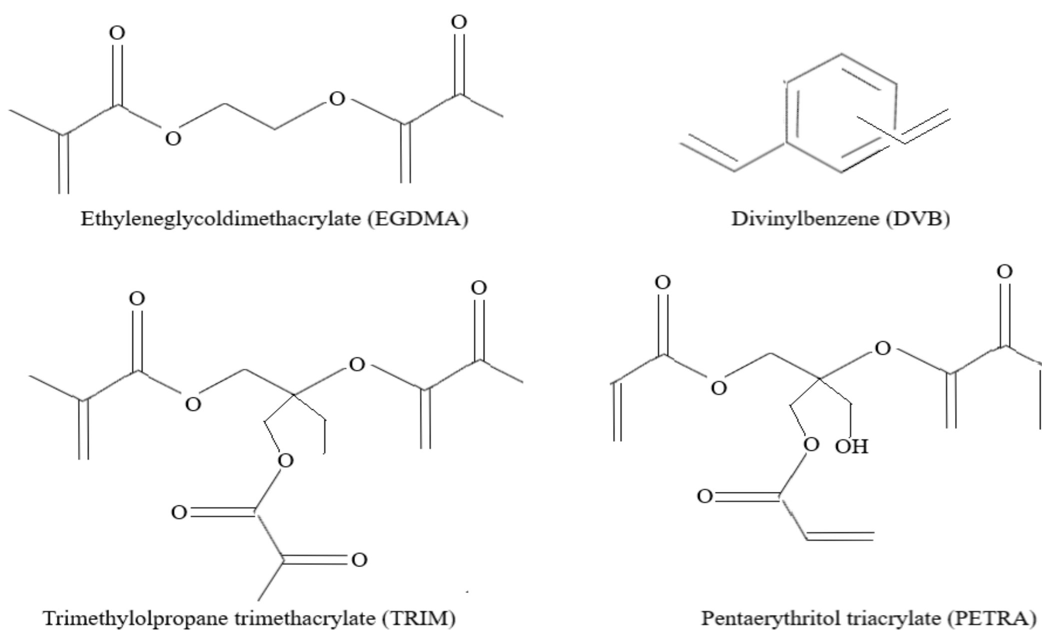


Figure 2.6. The most commonly used crosslinkers in molecularly imprinted polymers

If the amount of crosslinker is less than necessary, polymers cannot stabilize in the gap due to insufficient crosslinking; if it is more than necessary, non-covalent interactions occur, homogeneous copolymerization does not occur, the number of recognition zones decreases (Öncel, 2013; Çetin, 2013; Biçen, 2009; Vasopollo et al., 2011; Yan & Row, 2006).

2.4.1.4. Solvents

Solvents increase to the complex structure between the functional monomer and the template, determines the pore volume and morphology, so it is called "porogen". It also has a role to adjust the reaction temperature (Biçen, 2009; Yan & Row, 2006; Vasopollo et al., 2011).

MIPs show different swelling features in different solvents (Biçen, 2009), therefore, like other components, solvent also plays an important role in molecular imprinting. The most commonly used solvents toluene, chloroform, dichlorometane or acetonitrile (Vasopollo et al., 2011) are cause that the polymers are porous structure (Yan & Row, 2006; Öncel, 2013; Çetin, 2013).

The choice of solvent is significant according to the type of imprinting. While the choice of solvent is not very important in covalent imprinting, in non-covalent imprinting, it is important to increase the formation and imprinting effect of the interaction, and it should be less polar and aprotic (Öncel, 2013) in order to polar interactions to occur (Yan & Row, 2006; Biçen, 2009; Öncel, 2013; Çetin, 2013; Vasopollo et al., 2011).

In polymers with less than the desired amount of solvent, it causes the structure of the polymer to be very hard and intense and the template is hardly attached (Vasopollo et al., 2011; Çetin, 2013).

2.4.2. MOLECULARLY IMPRINTED POLYMERS

Molecular imprinting is a technique used synthetic polymers for a specific target molecule. Molecularly imprinted polymers (MIPs) therefore have specific molecular recognition, one of the most important properties of biological antibodies (Haupt, 2014).

At the present time, the majority of reports on MIPs describe organic polymers synthesized by radical polymerization of functional and cross-linking monomers having vinyl groups, and using non-covalent interactions with the template (Haupt, 2014).
MIPs are created by a mixture of targeted template molecules, functional monomers and crosslinkers (Haupt, 2001; Piletska et al., 2004).

Polymers such as acrylic and vinyl polymers, silica, polyphenols, poly(aminophenyl boronate), poly(phenylenediamine), poly(phenylenediamine-co-aniline), polyurethanes, overoxidized polypyrrole are among the matrices used as imprinting matrix. For specific studies, it continues to be developed to synthesize the polymer in the desired form (Haupt, 2001).

Although imprinting of many small molecules is successfully applied in MIPs, there are still difficulties in the printing of large molecules such as protein. So far, polymerization methods such as bulk (3D), surface (2D) or partial (epitope) printing has been used for proteins and bulk polymerization is generally used due to its water compatibility (Haupt, 2001; Rabieizadeh et al. 2014).

Several application areas of MIPs are given below:

- Separation techniques (such as chromatographic techniques and HPLC, SPE and CEC (Rabieizadeh et al. 2014)),
- Binding assays (such as radioimmunoassays (RIA) and enzyme immunoassays (ELISA)),
- Sensor applications (Haupt, 2001; Piletska et al., 2004),
- Food analysis,
- Medical diagnostics,
- Environmental analysis
- Drug delivery systems (Kartal & Denizli, 2020; Lozinsky, 2018).

The molecular imprinting method, which is a selective-recognition technique, was discovered by imprinting the desired template molecule by various bonding methods. In this context, there are two different approaches that are crucial to molecular imprinting: covalent imprinting and non-covalent imprinting (Haupt, 2014).

2.4.2.1. Covalent Imprinting

Covalent imprinting, pioneered by Wulff et al. (Haupt, 2001; Ye & Mosbach, 2008; Haupt & Mosbach, 2000) and Shea in California (Mosbach, 1994), is a reversible kinetic bonding created by polymerization (Ye & Mosbach, 2008), with greater stability between the template and functional monomers, thus requiring some chemical synthesis prior to the synthesis of the MIP itself (Haupt, 2001; Haupt & Mosbach, 2000).

After polymerization, the imprint molecule is removed by chemical cleavage. Binding of the analyte occurs via the same covalent interactions. The obvious advantage of this technique is that the monomer/template complex is stoichiometric and hence results in a more homogeneous population of binding sites within the polymer (Wulff & Sarhan, 1972).

There are some disadvantages:

- The synthesis process is not very economical and the target molecule binds to the polymer in a limited number of reversible directions;
- Their binding kinetics are slow due to covalent bond formation; and
- It is difficult to remove the target molecule after polymerization (Shea & Thompson, 1978; Whitcombe et al, 1985).

2.4.2.2. Non-Covalent Imprinting

Non-covalent imprinting, discovered by Mosbach et al., is a type of binding where the process of separation with a simple solvent (Haupt, 2001) is easy and convenient after forming the pattern between the target molecule and functional molecules (Haupt & Mosbach, 2000; Mosbach, 1994).

Types of bonding such as hydrophobic, hydrogen bonding, ionic bonding, wan der waals bonding (Ye & Mosbach, 2008), electrostatic interaction (Ikegami et al., 2004) are non-covalent bonding types. This so-called non-covalent or self-assembly protocol is today the most commonly employed, since it is relatively easy to put into practice, and rather flexible since a large number of functional monomers able to interact with almost any kind of target molecule are available. In this approach, the complex between the template and the functional monomer is formed by interactions such as hydrogen bonds, ionic bonds, van der Waals forces, and the hydrophobic effect (Mosbach & Arshady, 1981).

The removal of the target molecule takes place directly by a simple solvent extraction and the target molecule is easily removed from the polymer after polymerization because the non-covalent interactions are weaker. The target molecule has a fast re-binding kinetics. For this reason, this method is easier and forms binding sites with higher affinity than covalent imprinting method (Haupt, 2014).

Besides, it has some disadvantages: polymerization conditions that can be applied to increase interactions are limited and functional monomers are used excessively to increase the bond formation balance and this can lead to the formation of non-specific binding sites (Haupt, 2014).

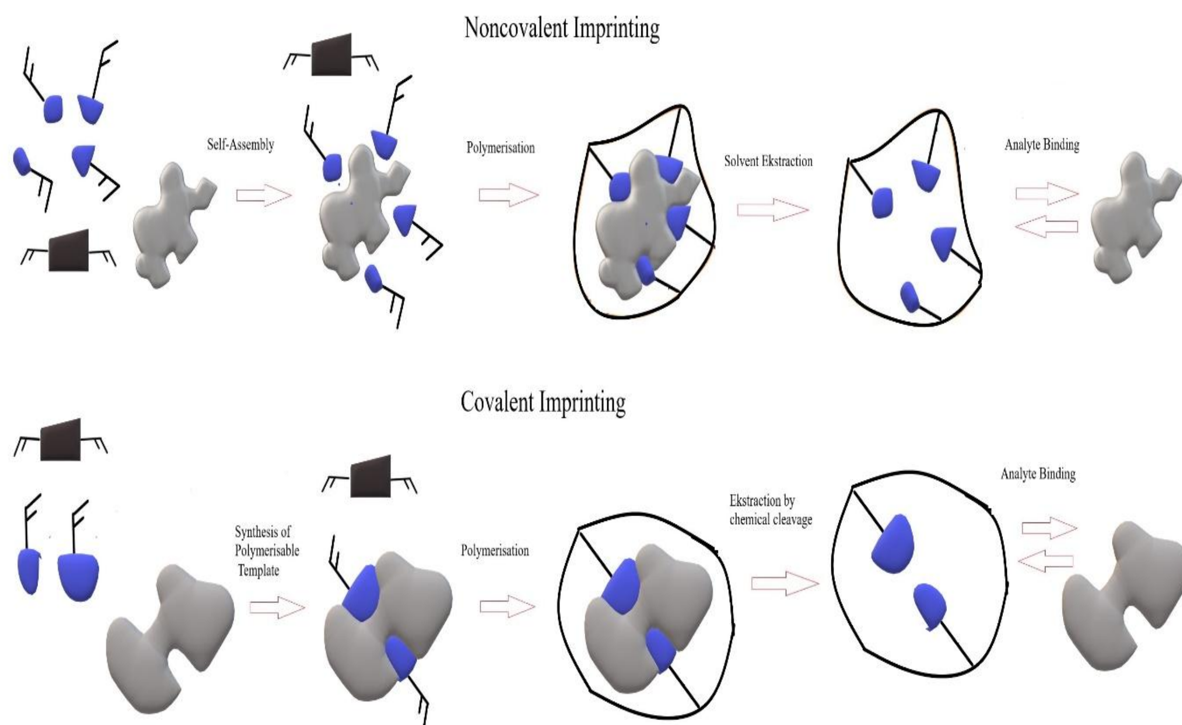


Figure 2.7. Schematic Representation of the Covalent and Non-covalent Molecular Imprinting Procedures

2.4.3. CRYOGELS

Scientists who have been affected by natural recognition models such as antibody-antigen, receptor-effector, enzyme-inhibitor have been working on the key-lock model structure based on biomimetics for many years (Rachkov, et al., 1998; Rachkov, Cheong, El'skaya, Yano, & Karube, 1998). These studies, firstly in the 1950s, have evolved starting with Linus Pauling step by step until today (Ye & Mosbach, 2008; Mosbach, 1994; Flam, Molecular Imprints Make a Mark, 4 March 1994).

In line with the purpose of diagnosis or treatment in health and industrial fields, natural models, which has been established for many years, have been taken as examples and important steps have been taken by producing products with artificial or even more economical or durable products. (Reichelt, 2015).

The term "cryogel" was first used for polymeric materials prepared by polymeric chemical crosslinking in 1984 (Reichelt, 2015). Cryogels are soft materials which are synthesized at subzero temperatures (between 0 and -20) (Rabieizadeh et al., 2014), with three-dimensional sponge-like elastic morphology and pore sizes from a few microns to several hundred microns.

Because of their unique properties such as high porosity, mechanical stability and durability, and hydrophilic characteristics that reduce non-specific adsorption of biomolecules, cryogels are ideally seen in separation and purification studies. (Kartal & Denizli, 2020; Lozinsky, 2018; Andaç & Denizli, 2014).

In general, these gels are prepared using physical interactions or by covalent chemical reactions. Gel formation can be observed through hydrogen bonds and ionic bonds. Cryogels containing polyvinyl alcohol can be given as an example for gels created by hydrogen bond interactions (Reichelt, 2015).

Cryogels are formed via cryogelization process. The cryogelization process is ideally carried out in 4 stages: First of the these, ice crystal formation; second stage, crosslinking and polymerization; other stage is phase separation and final stage is thawing of ice crystals and forming a porous cryo-network interconnect (Reichelt, 2015; Kahveci et al., 2010; Lozinsky, 2018).

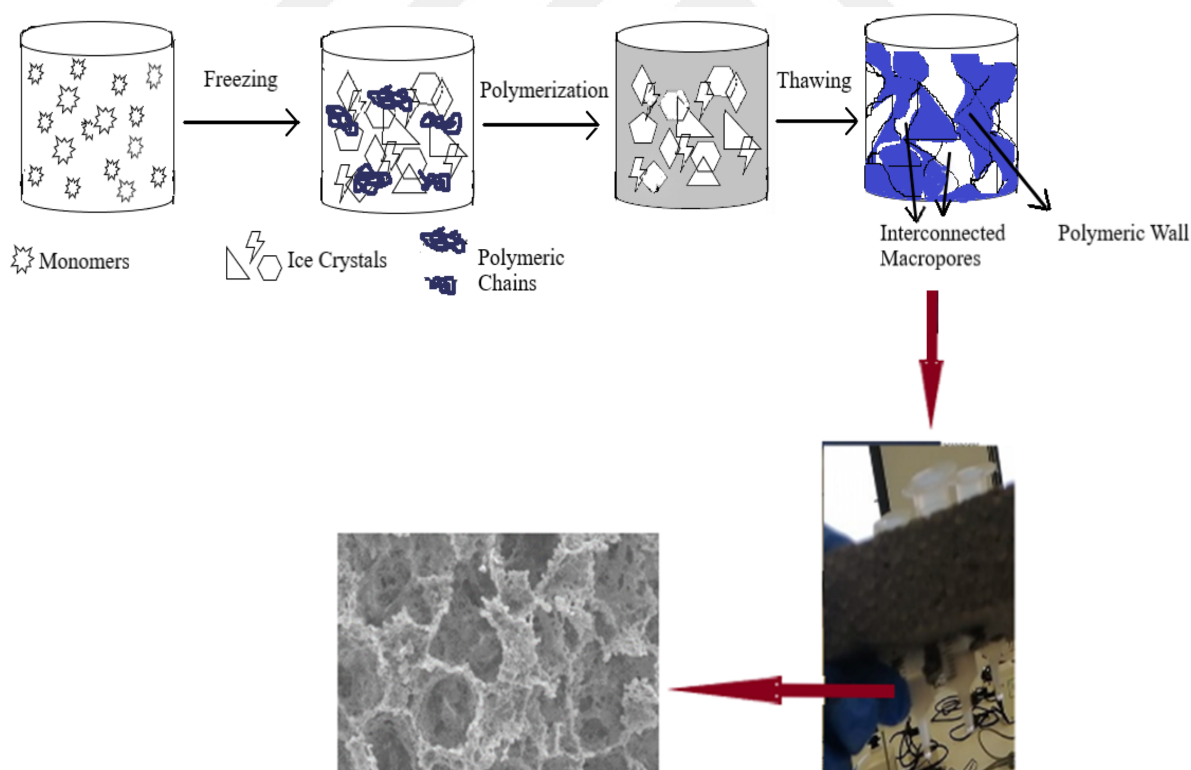


Figure 2.8. Demonstration of the Cryogelization Process

Preferred cryogels for MIPs have specific recognition sites and are formed by preparing a mixture containing the target molecule and other components (Andaç & Denizli, 2014).

Advantages of cryogels such as large pore sizes (up to 100 nm), short diffusion path, good biocompatibility, flexibility and high mechanical strength have made them indispensable in biodegradation and purification processes (Lozinsky, 2018; Andaç & Denizli, 2014). Cryogels are easy to prepare and are also cost-effective adsorbents. Therefore, in order to prevent cross-contamination during the sequential studies of a cryogel sample, it can be discarded and re-prepared after use (Hajizadeh et al., 2012; Andaç & Denizli, 2014; Kirsebom et al., 2010).

Macroporosity, which is one of their characteristic features, depends on many factors in size and shape (Lozinsky, 2018; Andaç & Denizli, 2014), some of these factors are:

- The precursors' nature and concentration,
- The solvent used and its cryoscopic properties,
- the presence and amount of foreign solutes or disperse fillers,
- As well as the thermal regimes of cryogenic processing, namely, the cooling rate during freezing,
- The freezing temperature itself, frozen storage duration,
- The rate of the frozen samples heating for their thawing,
- The number of the freeze-thaw cycles (the latter two parameters are of especial significance (Lozinsky, 2018; Andaç & Denizli, 2014)

Because of the fact that cryogels are three-dimensional cross-linked polymers, they have some disadvantages such as a very high cross-linking, low efficiency in template removal. In addition, the type of functional monomer that will complex with the target molecule can affect reconnection efficiency and selectivity (Kirsebom et al., 2010).

3. MATERIAL AND METHODS

3.1. Materials

- Angiotensin II used as template, Angiotensin I and Arg8-Vasopressin Acetate used as competitor and 4-Vinylimidazole (VIM) used as functional monomer were provided from Sigma - Aldrich (St. Louis, MO, USA).

- 2-hydroxyethylmethacrylate (HEMA), methylenebisacrylamide (MBAAm) and N, N, N, N - tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) were provided from BioRad and used for polymerization reactions.

- Sodium phosphate dibasic, sodium phosphate monobasic, potassium chloride, acetate buffer, carbonate buffer, PBS were also provided from Sigma-Aldrich (St. Louis, MO, USA).

- All chemicals used in the experiments are of reagent grade.

- The resistance of the water used during the experiments was 18 MΩ/cm.

- The materials used in the experiments had been used after washed with pure water and by drying in a dust-free environment before use.

3.2. Device List

- Shimadzu Type ATX224 No. D307039788 Electronic Balance was used for weighing of chemicals and samples.

- Scientific Vortex Mixer (Daihan, Korea) was used to mix solutions.

- Model of Millipore DIRECT-Q®3 ultrapure (type 1) water with BioPak®ultrafiltration cartridge with Millipak®Express 20 (0.22 µm) membrane filter was used for water system.

- Nanodrop 2000c Spectrophotometer were purchased from Thermo Scientific (USA) and used for quantification and assessment of proteins of interest, in order to examine adsorption capacity, selectivity and other characterisics of obtained cryogel columns.

- Synthesized ANGII-MIP and NIP morphologies were characterized by Scanning Electron Microscope SEM (JEOL JSM 5600, Jeol Co.,Tokyo, Japan).

- Specific surface area of the ANGII-MIP and NIP was identified as using Brunauer-Emmett-Teller (BET) analysis (Flowsorb II 2300, Micrometrics Instrument Corporation, Norcross, GA) with multipoint method.

3.3. EXPERIMENTAL METHODS

For the preparation of ANGII imprinted polymers, batch polymerization method was applied. 2 different polymers were prepared within the extent of our study:

(i) non-imprinted polymer is PHEMA (polymer code: NIP) and

(ii) molecularly imprinted polymers containing functional monomer-ANGII complex, that is poly (HEMA–VIM) (polymer code: ANGII-MIP).

3.3.1. Preparation of ANGII-MIP and NIPs

The method followed for the preparation of ANGII-MIP cryogel columns (ANGII-MIP):

ANGII-VIM complex was prepared by dissolving ANGII and VIM functional monomer in 1/10 n/n mol ratios in 1 mL of deionized water and stored at +4°C for 12 hours to complete complexation. Here, this 1:10 ratio was chosen as the maximum rate of attachment as a result of analyzes and calculations.

HEMA and MBAA was dissolved in 5 mL and 10 mL deionized water, respectively, and these two solutions were mixed to achieve monomer concentration of 10%. Then, pre-prepared pre-complex was added into the the polymer solution.

Finally, APS solution (10% w/w) and TEMED was added to polymerization mixture as a initiator/catalyst system and mixed in the ice bath for 5 minutes. Then, prepared mixture was equally divided into 3 mL syringes and frozen in a cryostat at -16°C for 10 h to complete the polymerization process.

Polymers taken from the cryostat and left to room temperature for melting ice crystals in frozen polymeric column and creation of interconnected macropores were allowed for obtain cryogel columns. In order to remove the unreacted monomers from the cryogels, washing was performed by passing pure water through the cryogel.

Non Imprinted crogel columns (NIP) were also prepared in the same way without using ANGII, these columns functioned as a control group.

3.3.2. Removal of the Template ANGII from ANGII-MIP

0.5 M NaCl solution was used to break the interaction caused by secondary forces between ANGII and HEMA monomer and to remove template molecule ANGII and also this process was repeated 3 times. After the template molecule was removed, washing was carried out again with pure water for half an hour.

3.4. CHARACTERIZATION STUDIES

3.4.1. Swelling Test

Swelling test were performed to determine the water uptake properties of cryogels. ANGII-MIP and NIP were dried and weighed with an accuracy of ± 0.0001 and placed in a beaker containing 20 mL of distilled water for 5 hours at 25 ± 0.5ºC at constant temperature. Then it was removed from beaker and weighed by removing excess water from the surface. Dry and wet weights are averaged of three repeated operations, the swelling rate of the material is determined with the following equation:

$$\text{Swelling rate (\%)} = [(W_s - W_0) / W_0] \times 100 \quad (1)$$

W_0 and Ws are the weights of the cryogel before and after swelling in gram, respectively.

In order to determine the macropore volume of cryogels, the cryogels were swelled and swelled samples weighed (W_1) in gram. Then, swollen cryogel sample was squeezed precisely to get rid of the water in the macro pores of the cryogels and weighed (W_2) in gram. The macropore volume of ANGII-MIP and NIP were calculated using the equation below:

$$\text{Macropore amount (\%)} = [(W_1 - W_2) / W_1] \times 100\% \quad (2)$$

The polymerization efficiency of the prepared cryogels is calculated as using the dry weight of produced crogels (W_p) and the weight of total reactant monomers (W_m) used for polymerization as follows:

$$\text{Yield (\%)} = (W_p) / (W_m) \times 100\% \quad (3)$$

3.4.2. Surface Morphology

The Ang-MIP and NIP samples were swollen in DI and then dried in the lyophilizer. Then they were covered with gold under vacuum to taken scanning electron microscopy (SEM) photographs. The surface and bulk structure of ANGII-MIP and NIP cryogels were examined by SEM FEI Quanta 650 Field Emission SEM (USA).

3.5. ADSORPTION STUDIES

ANGII adsorption onto ANGII-MIP and NIP from aqueous solutions was performed by continuous system using a peristaltic pump to determine the maximum amount of ANGII adsorption of columns.

3.5.1. Effect of Equilibrium ANGII concentration on Adsorption

The effects of the equilibrium ANGII concentration onto ANGII rebinding were investigated in the range of 0.01-0.2 mg/mL ANGII concentrations.

All parameters were evaluated using UV-spectrophotometer at 283 nm and the adsorption capacities were calculated using;

$$Q = (C_o - C_f) * V/m \quad (4)$$

Here, Q is adsorption capacity (mg/g), C_o and C_f are ANGII concentrations of the ANGII solutions before and after the interaction with cryogel columns (mg/mL), V is volume of the solution (mL), m is the dried mass of the columns (g).

3.5.2. Effect of Flow Rate on Adsorption

Effect of flow rate on ANGII rebinding capacity was studied in the range of 0.1-2 mL/min. 3 mL of 0.05 M ANGII solution was passed through the ANGII-MIP and NIP columns using peristaltic

pump. Flow rates were adjusted to 0.1, 0.5, 1 and 2 mL/min, respectively. At each cycle, adsorption capacity was calculated using Eq. 4.

3.5.3. Effect of Interaction Time on Adsorption

Effect of interaction time on ANGII rebinding capacity was investigated at different interaction times. For this purpose, 3 mL of 0.05 M ANGII solution was passed through the ANGII-MIP and NIP columns using peristaltic pump. 50 µl of samples were taken at 0, 5, 10, 15, 20, 30, 60, 90 and 120th minutes, and they were analyzed using nanodrop.

ANGII amounts were calculated by taking samples at specific range. Using the obtained adsorption data, the suitability of adsorption to kinetic models (pseudo-first degree kinetic model, pseudo-second degree kinetic model) was investigated.

The parameters of the pseudo-first degree kinetic model and pseudo-second-degree kinetic models were calculated by using linear and nonlinear equations, described in Results section.

3.6. Selectivity Studies

In order to determine the selectivity of ANGII-MIP columns against the ANGII molecule, ANGI and VASP molecules, which are similar to ANGII as their molecular structure and coexist with ANGII in the blood, were selected as competitors. The chemical structure of the competitor molecules used in selectivity studies is as follows:

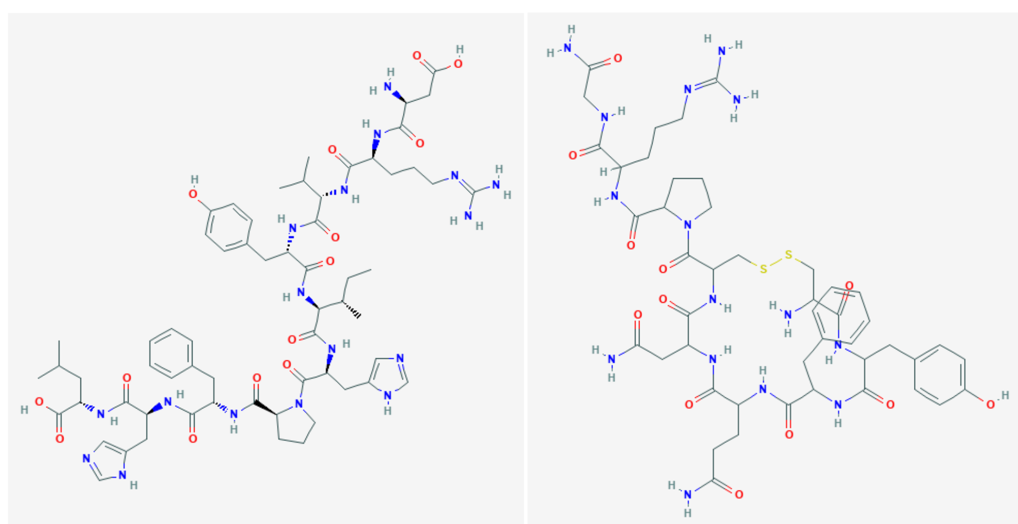


Figure. 3.1. ANGI (left) and VASP (right).

The distribution and selectivity constants of ANGII with respect to other competitor molecules were calculated. The distribution coefficient calculated according to following equation;

$$Q = [(C_i - C_f) / C_f] \times V / m \quad (5)$$

Here C_i: Initial solution concentration (mg/mL); C_f: Concentration of the solution after interaction with the column (mg/mL); V: volume of solution (mL); m: dry column weight (g)

The recognition characteristic of ANGII-MIP columns is determined by the imprinting factor (IF). IF value allowed us to compare the selective adsorption behavior of imprinted and non-imprinted columns.

$$IF: Q_{MIP} / Q_{NIP} \quad (6)$$

In this equation, Q_{MIP} is the maximum adsorption amounts of MIP columns and Q_{NIP} is the maximum adsorption amounts of NIP columns.

The selectivity coefficient (k) is an indicator of the selectivity of the column for the template molecule. Selectivity coefficients were calculated by comparing the selective adsorption behavior of ANGII-MIP towards ANGII versus AngI and VASP. k is calculated with the equation given below:

$$k: Q_{MIP\ template} / Q_{MIP\ competitor} \quad (7)$$

$Q_{MIP\ template}$ is maximum ANGII adsorption onto ANGII-MIP and Q_{MIP} competitors is maximum adsorption capacity of competitors onto ANGII-MIP.

3.7. Desorption and Reusability Studies

Depending on the change in binding efficiency, the reuse numbers of cryogel columns were determined.

Reusability of ANGII-MIP and NIP were tested by applying 0,5 mg/mL of ANGII to the same column 10 times. After each adsorption step, ANGII molecules desorbed from ANGII-MIP/NIP,

by circulating 0.5 M NaCl in 10 mM of pH 7.4 phosphate through ANGII-MIP/NIPs for 2 hours at room temperature.

ANGII concentration in desorption medium was measured at 283 nm.

The ANGII desorption ratio was calculated using;

$$Desorption\ ratio\ (\%) = \frac{Amount\ of\ the\ desorbed\ ANGII\ (mg)}{Amount\ of\ the\ adsorbed\ ANGII\ (mg)}\ X\ 100 \quad (8)$$

3.8. Selective ANGII Rebinding From Human Serum

The selective rebinding ANGII from human serum were achieved using 1:10, 1:20 diluted human serum which have 0,005 and 0,0025 mg/mL ANGII concentrations, respectively.

The ELISA results were evaluated and the ANGII rebinding amounts were found as 0.054 mg/g and 0.028 mg/g as 7% and 92% recovery. And when 5pg/mL serum applied to column; selective rebinding capacity was calculated as 60.5 pg/g, it is important to note that 96% of the ANGII can successfully rebinded from crude human serum.

4. RESULTS AND DISCUSSION

4.1. Characterization of ANGII Imprinted Cryogels

4.1.1. Swelling test

Morphological structures of polymers are directly related to their swelling behavior. Swelling behavior is one of the key parameters in polymeric materials. This behavior is a function of the crosslinker density in the polymeric network structure.

In molecular imprinting technology, due to the stability of the pore structures, a high amount of crosslinker is used and a rigid structure is achieved, in this case a low swelling rate is inevitable (Fareghi et al., 2017).

As cryogels are cross-linked polymers capable of swelling in the aqueous medium, these polymeric gels swell with water molecules entering the chains according to the hydrophilicity and molecular weight of the matrix. Thanks to the large macropores in the cryogels, the template molecule can also diffuse easily (Çorman, 2014).

Swelling tests were performed for analyzing water uptake capacity, and macropore amount. It was summarized the swelling tests results both for ANGII-MIP and NIP on Table 4.1. Swelling ratios were calculated as 94.3% and 92% and the macroporosity ratio was calculated as 82% and 80% for ANGII-MIP and NIP respectively.

Polymerization yields were found as 88% and 84% for ANGII-MIP and NIP respectively. The columns were produced several times and the nearly the same results obtained so it can be say that the columns can be synthesized in high production yield.

The BET (Brunauer, Emmett and Teller) analysis method is the main technique used to determine specific surface area (m^2/g), specific pore volume (cm^3/g), pore size distribution and average pore diameter, described in Baydemir et. al. (Baydemir, 2009). Briefly, in the technique of Brunauer, Emmett and Teller (BET), the most common method for determining the surface area of powders and porous materials, the sample is exposed to a series of N_2 gases at specific pressures. As the pressure increases, the gas condenses and fills the pores. The amount of gas is measured as a function of pressure. The amount of gas required to coat the sample surface with a single molecular layer is determined and the surface area is calculated using the Brunauer Emmett and Teller theory (Baydemir, 2009).

Table 4.1. also shows the BET analysis results and the specific surface areas of ANGII-MIP and NIP were found to be 52 and 47 m^2/g, which leads sufficient interaction areas for selective recognition studies.

Table 4.1. Swelling ratio, macroporosity, surface area and polymerization yield of ANGII-MIP and NIP

	Swelling Ratio %	Macroporosity %	Surface Area m^2/g	Polymerization Yield %
ANGII-MIP	94.3	82	52	88
NIP	92	80	47	84

4.1.2. Surface Morphology

The supermacroporous network structure formed by cryogelization creates low flow resistance, thus, diffusion flow and mass transfer are provided easily. However, due to the presence of large pores in the cryogel, the surface area is small and high adsorption capacity cannot be achieved in the adsorption of various substances (Baydemir et al., 2009; Hajizadeh et al., 2012).

2-Hydroxyethyl methacrylate) (HEMA), used in this thesis, is a hydrophilic polymer (Figure 4.1).

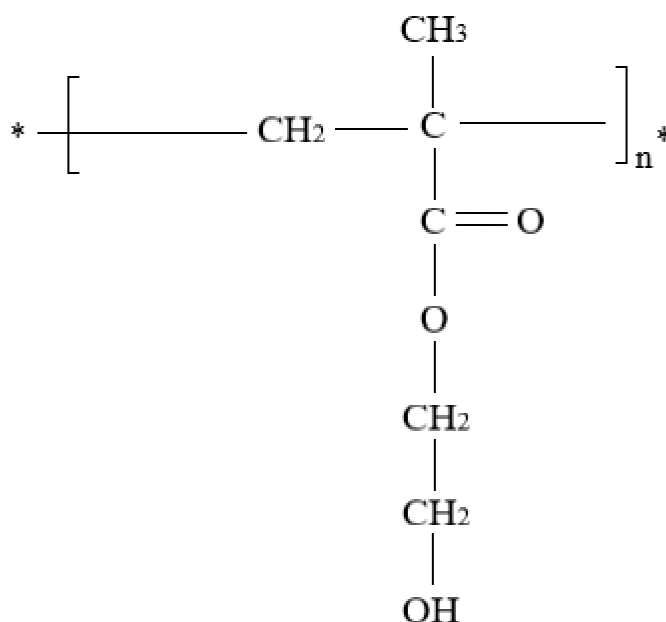


Figure 4.1. Structure of PHEMA

In this study, the main reasons of choosing HEMA monomers are their physiological compatibility, mechanical endurance, chemical and biological stability, minimal non-specific protein interactions, and compatibility with blood (Uzunoğlu, 2013; Denizli, 2002). PHEMA-based matrices can be used to purify proteins from the blood, and thanks to this compatibility, it is possible to purify proteins in their natural conformation (Uzunoğlu, 2013).

SEM images showing the pore structure of the supermacroporous cryogel columns are given in Figure 4.2.

Figure 4.2A and 4.2B Show the interior structures of ANGII-MIP and NIP respectively. As can be clearly seen that the cryogel columns obtained have interconnected pores and the pores have around 100 μM in diameter which allowed the easy fluid flow through the column. SEM photos also showed rough walls, which leads high surface area for effective interaction with ANGII and ANGII specific cavities on ANGII-MIP.

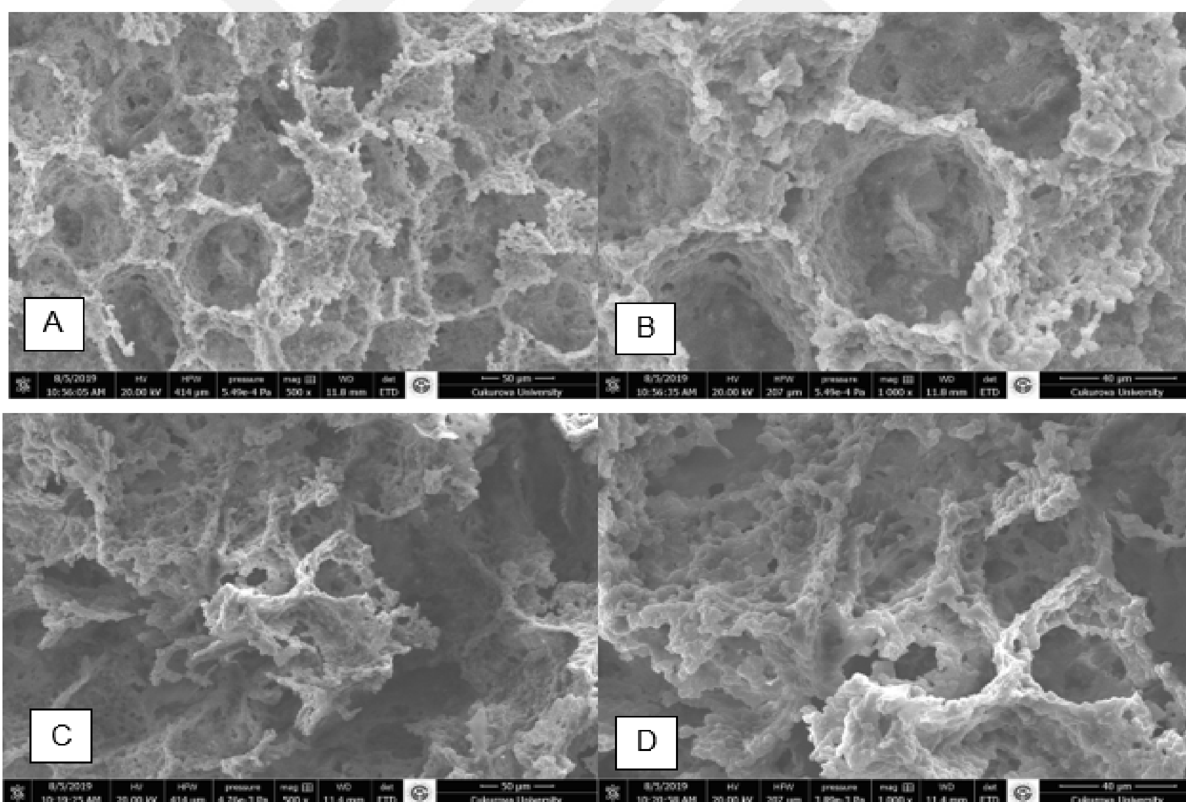


Figure 4.2. A, B) ANGII-MIP cryogel columns; C, D) SEM photos of NIP columns.

4.2. Adsorption Studies

ANGII adsorption onto ANGII-MIP and NIP from aqueous solutions was performed and the effects of the equilibrium ANGII concentration, adsorption time and flow rate were evaluated

within the scope of optimization studies. All parameters were evaluated using UV-spectrophotometer at 283 nm.

4.2.1. Effect of Equilibrium ANGII Concentration on ANGII Adsorption

The effect of equilibrium ANGII solution concentration on maximum ANGII adsorption was determined by applying aqueous solutions of ANGII molecules in concentrations ranging from 0.01-0.2 mg/mL to the column under the same conditions.

As expected, ANGII adsorption increase at beginning, then the maximum adsorption capacity of the column remained unchanged after the concentration of 0.05 mg/mL. This behavior can be explained by the ANGII concentration difference (ΔC_{ANGII}). ΔC_{ANGII} is driving force for ANGII adsorption on ANGII-MIP and it increases with increasing ANGII concentration so an increase in adsorption capacity is also observed with increasing driver force. Saturation of the recognition zones then prohibits the adsorption.

Maximum ANGII adsorption capacity was calculated as 0.667 mg/g ANGII-MIP and 0.222 mg ANGII/g NIP (Figure 4.3).

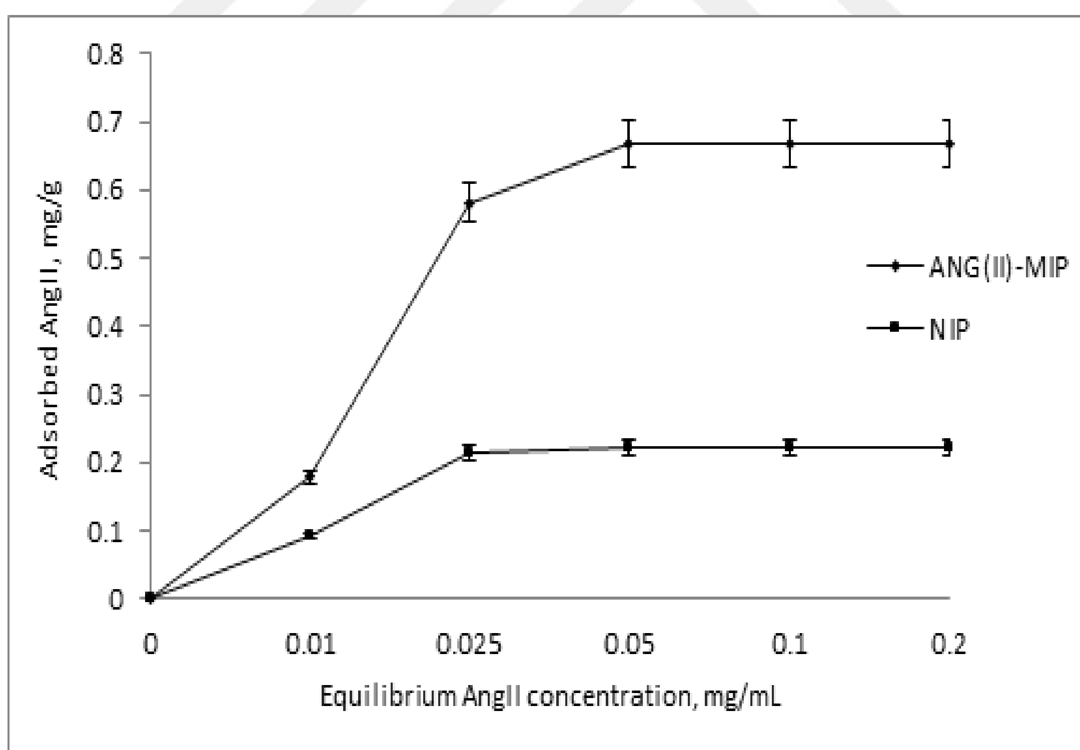


Figure 4.3. Effect of equilibrium initial ANGII concentration on ANGII adsorption: m_{dry}: 0.1027 g, V: 3 mL, time: 120 min, pH 7.4 Flow Rate: 0.5 mL/min, T: 25°C, Max ANGII adsorption capacity: 0.667 mg/g ANGII-MIP and 0.2221 mg ANGII/g NIP polymers.

4.2.1.1. Adsorption Isotherms

By conducting a series of adsorption experiments, the adsorption mechanism can be determined under optimum conditions (solution pH, ionic intensity, amount of adsorbent and particle size, liquid/solid ratio, etc.). When the adsorption process reaches equilibrium, appropriate adsorption isotherm is determined with the help of experimental data obtained to characterize the interaction between them and to define the relationship between adsorbate and adsorbent in the solution.
Adsorption isotherms describe the relationship between the amount of adsorbate (q_e) adsorbed by the adsorbent at a constant temperature and the adsorbate concentration (C_e) remaining in the solution after equilibrium.

The parameters obtained from the adsorption isotherms provide useful information about the surface properties, the adsorption mechanism and the interaction between the adsorbent and the adsorbate.

The Langmuir isotherm model was proposed by Irwing Langmuir in 1918 (Langmuir 1918). It is the first adsorption model and is widely used to study the thermodynamics of the molecular imprinting technique. This isotherm model assumes that the adsorption is monolayer and the surface is homogeneous. (Biçen, 2009; Çorman, 2014)

According to the Langmuir isotherm, all binding sites in the adsorbent are equally energized and suitable for binding of at most one adsorbate molecule. The layer formed by bonding is a molecule thick. The adsorbed molecules fill the homogeneous surface until equilibrium (Yeşilova, 2019).

Eq. 9 explains Langmuir adsorption isotherm which assumes that the molecules bind to a particular number of binding sites, each with the ability of binding only one molecule and these binding sites are equal in terms of energy.

$$Q=Q_{max}*b*C_e/(1 + b*C_e) \quad (9)$$

Q is the capacity of molecules that bind to the material (mg/ml), Ce is the concentration of the given molecule in the solution (mg/L), b is constant of Langmuir (ml/mg) and Q_{max} is the highest adsorption capacity (mg/g). Linearized equation is given below:

$$1/Q_e=1/(Q_{max}*b) * (1/C_e) + 1/Q_{max} \quad (10)$$

1/Qmax is calculated by the point where 1/Q graph crosses the y-axis against 1/Ce and the slope gives the value of 1/Qmax * b.

The Freundlich isotherm model was proposed by the German Physicochemist Herbert Max Finlay Freundlich to define the balance data and adsorption properties of clean and non-homogeneous, that is, heterogeneous surfaces (Freundlich, 1906; Ersoy, 2014; Biçen, 2009). The Freundlich isotherm assumes that adsorption occurs physically and reversibly on heterogeneous surfaces. According to this isotherm model, which can be used for multi-layer adsorption, the heat and affinity (affinity) of the adsorption on the heterogeneous surface are not evenly distributed (Yeşilova, 2019).

The Freundlich isotherm model is an exponential equation and assumes that as the adsorbent concentration increases, the adsorbent concentration on the adsorbent surface increases. Moreover, according to this model, adsorption occurs through multiple layers instead of a single layer. Consequently, the Freundlich isotherm model has wide application in heterogeneous systems. (Yeşilova, 2019)

Freundlich Eq. 11 assumes that the binding of the adsorbent to the adsorbent varies depending on if the adjacent binding regions are full.

$$Q_{eq}=K_f*C_{eq}^{1/n} \quad (11)$$

In this equation, Q_{eq} is the amount of adsorption (mg/g) and Ce is the equilibrium concentration in the solution (mg/L). K_f and 1/n are Freundlich constants which are indicating adsorption capacity and adsorption intensity. When this equation is taken as the logarithm of both sides, Eq. 12 is obtained.

$$\ln Q_{eq}=\ln K_f + 1/n \ln C_{eq} \quad (12)$$

Experimental data were adapted to Freundlich model and $\ln C_{eq}$ was plotted against $\ln Q_{eq}$. Adsorption constants were calculated from the cut-off point and slope.

In this study, the suitability of the adsorption data obtained to illuminate the interaction between PHEMA-MIP and ANGII was investigated for the Langmuir and Freundlich isotherm models.

Linear equation of the isotherm models was used to investigate the suitability of the Langmuir and Freundlich isotherm models.

Langmuir and Freundlich isotherm equations (linear) in which the compatibility of ANGII adsorption data are investigated are summarized in Table 4.2.

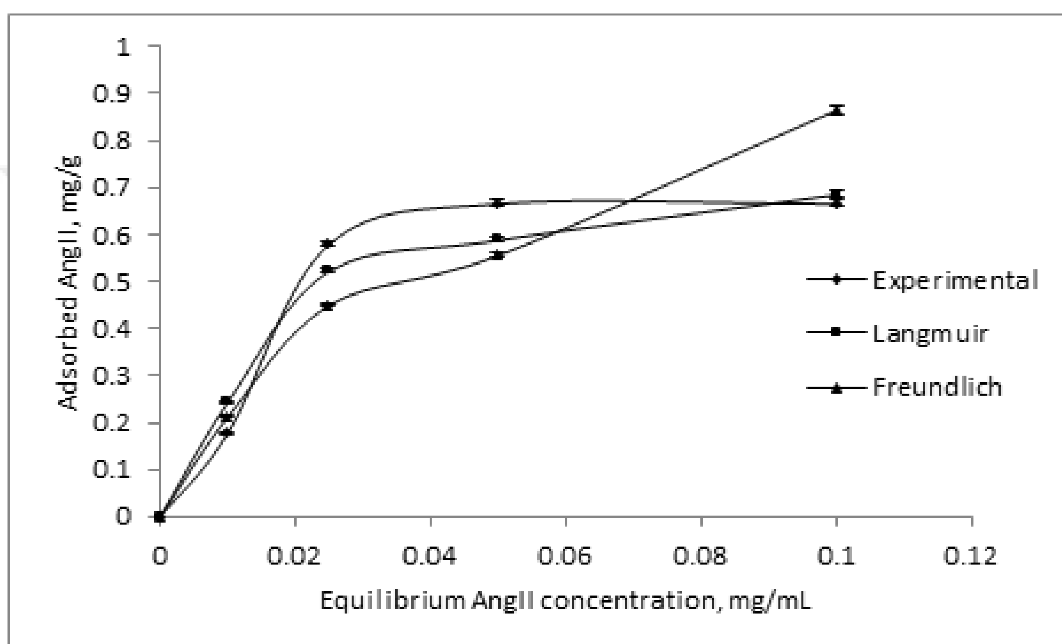


Figure 4.4. Effect of equilibrium initial ANGII concentration and Langmuir and Freundlich Adsorption models. M_{dry}: 0.1027 g, V: 3 mL, time: 120 min, pH 7.4 Flow Rate: 0.5 mL/min, T: 25°C

Table 4.2. Langmuir and Freundlich adsorption isotherm constants for ANGII

Experimental	Langmuir Constants			Freundlich Constants		
Q_{ex} (mg/g)	Q_L (mg/g)	b (ml/mg)	R^2	Q_F (mg/g)	n	R^2
0,66	0,77	57,24	0,9766	2,37	2,00	0,8406

n; number of experiments and b; number of parameters in the model, Q_{ex} (mg/g); the amount of experimentally determined adsorption, Q_L and Q_F (mg/g) are the adsorption amounts calculated by the model.

The parameters Q_L and Q_F obtained, and the R^2 values showed that the adsorption data is more compatible with Langmuir isotherm, and it can be concluded that the monolayer adsorption is favorable.

4.2.2. Effect of Interaction Time on ANGII Adsorption

In order to determine the effect of interaction time on the ANGII adsorption amount, 0.05 mg/mL ANGII solution was interacted with the column with a flow rate of 0.5 mL/min at pH 7.4. Samples were taken for 2 hours with certain time intervals and the maximum adsorption time interval was determined. It was observed that the maximum adsorption was reached after 60 minutes, so, 60 minutes of adsorption time was taken as a basis in all subsequent experiments (Figure 4.5).

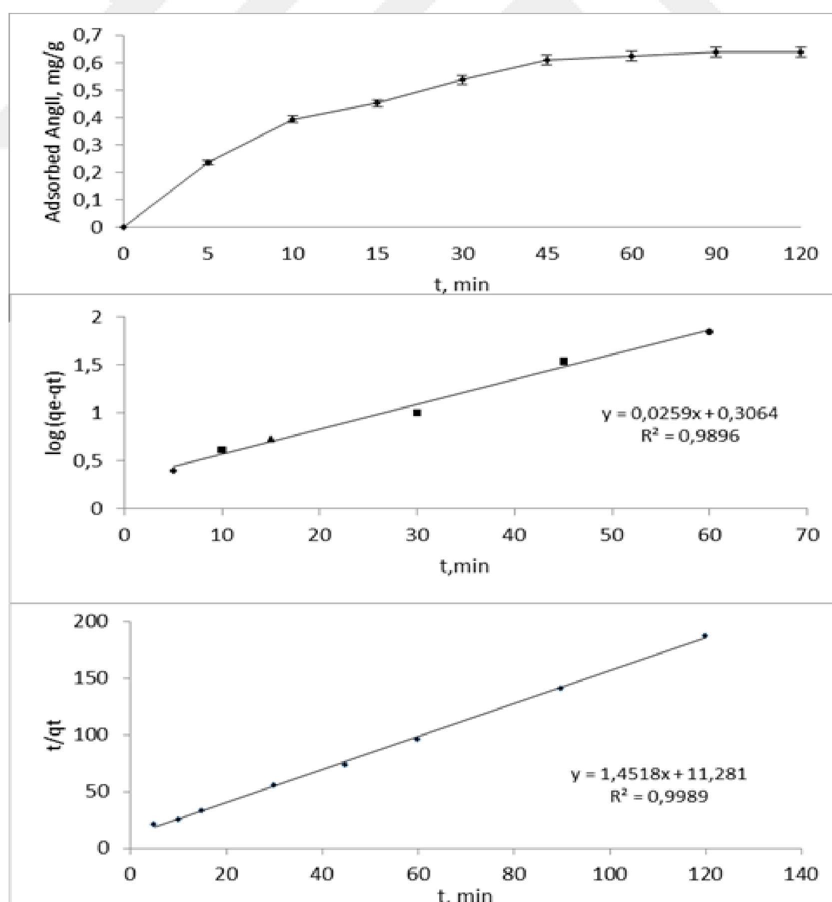


Figure 4.5. Effects of interaction time on ANGII adsorption, ANGII conc.: 0.05 mg / mL, dry: 0.1027 g, V: 3 mL, pH 7.4, Flow Rate: 0.5 mL/min, T: 25°C

4.2.2.1. Adsorption Kinetics

Pseudo first-order and Pseudo second-order reactions

Adsorption kinetic studies are important for determining the adsorption mechanism. Time-adsorption relationship was used in kinetic model calculations to understand adsorption controlling mechanism. In here Pseudo-first order kinetic and Pseudo second-order kinetic equations were used for the adsorption of an analyte from its aqueous solution.

The pseudo first-order kinetic model calculated using Lagergren's equation;

$$\Delta q_t/dt = k_1 (q_{eq}-q_t) \quad (13)$$

and linearized as:

$$\log(q_{eq}-q_t) = \log(q_{eq}) - (k_1 t)/2.303 \quad (14)$$

In here; k_1 is first order adsorption rate constant (min^{-1}); q_{eq} and q_t are ANGII adsorption amount at equilibrium and at time t (mg/g), respectively.

The pseudo-second order equation based on adsorption equilibrium capacity expressed as;

$$\Delta q_t /dt = k_2(q_{eq}-q_t)^2 \quad (15)$$

and can be linearized as:

$$(t/q_t) = (1/k_{2qeq}{}^2) + (1/q_{eq})t \quad (16)$$

Here k_2 is pseudo-second order adsorption rate constant (g mg^{-1} min^{-1}).

The rate constants k_1, k_2 and equilibrium adsorption amounts q_{eq} obtained intercepts and slopes of $\log(q_{eq}-q_t)$ vs t plot for first order and (t/q_t) vs t plots for second order, respectively and summarized in Table 4.3.

Table 4.3. Adsorption kinetic model constants and adsorption amounts

Initial Conc.	Exp.	Pseudo-first-order-kinetic			Pseudo-second-order-kinetic		
(mg/mL)	q_{eq} (mg/g)	k_1 (1/min)	q_{eq} (mg/g)	R^2	k_2 (1/min)	q_{eq} (mg/g)	R^2
0,05	0,6381	0,059	2,024	0,9896	23,832	0,688	0,9989

According to results shown in Table 4.3 the adsorption process can be expressed via second order mechanism. It is obvious that the R^2 of pseudo-second order is 0,9989 greater than pseudo-first order that means pseudo-second order mechanisms control the adsorption process via chemisorption rather than diffusion.

4.2.3. Effect of Flow Rate on ANGII Adsorption

To investigate the effects of flow rate on ANGII adsorption, 0.05 mg /mL ANGII solution was applied to the column at different flow rates of 0.1 to 2 mL/min. also decreased. Considering the effective experiment time, the appropriate flow rate for the column synthesized was determined as 0.5 mL/min.

The decrease of the flow rate means that the interaction decreases depending on the speed of attachment ANGII molecules to specific cavities in the PHEMA-MIP cryogel.

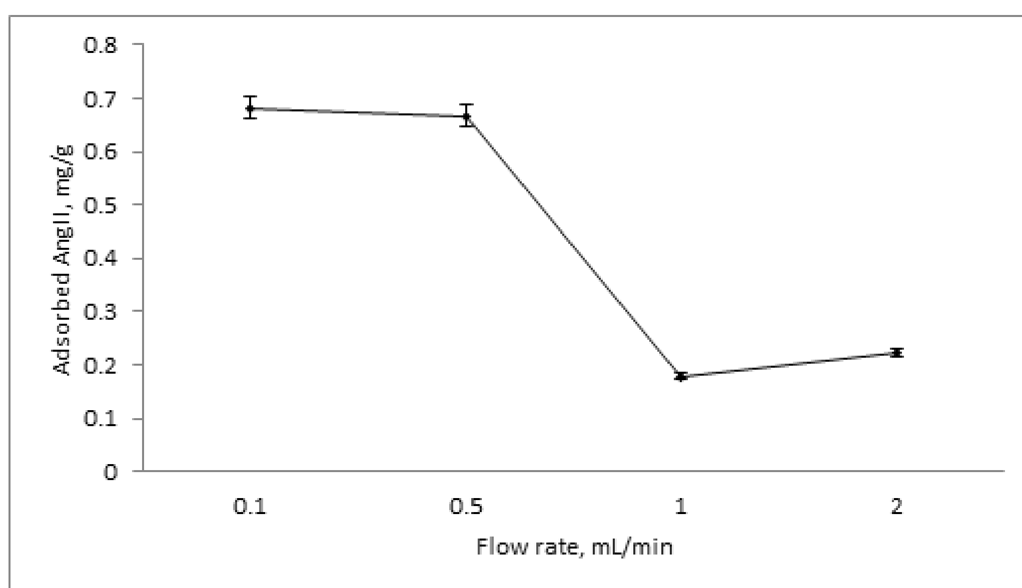
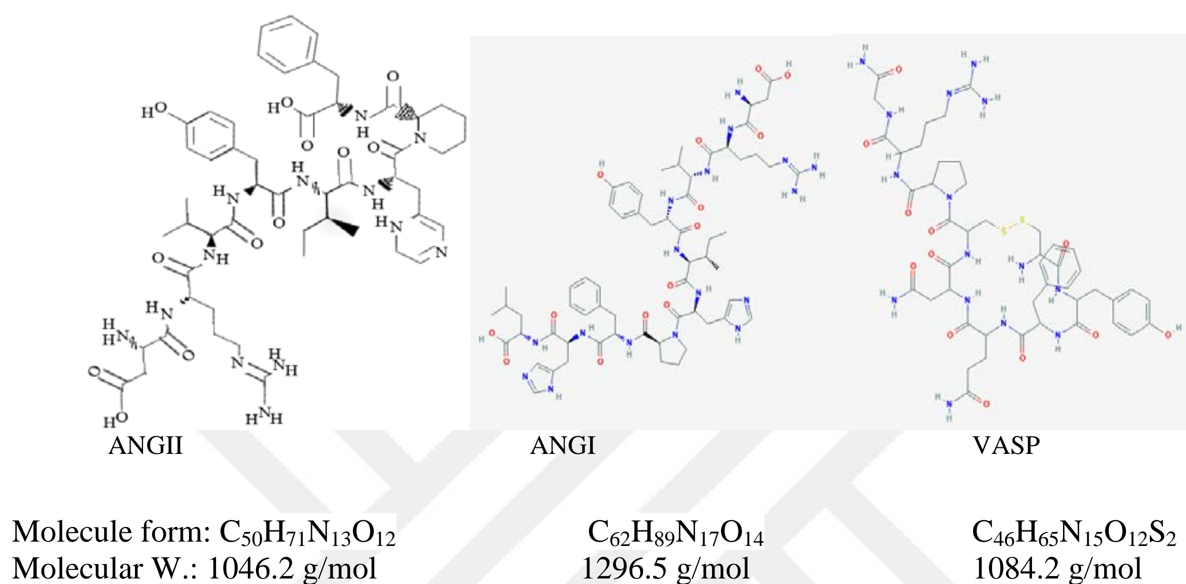


Figure 4.6. Effects of flow rate on ANGII adsorption: ANGII conc.: 0.05 mg/mL, m_{dry}: 0.1027 g, V: 3 mL, pH 7.4, Time: 120 min, T: 25°C

4.3. Selectivity Studies

ANGII and competitor molecules adsorption were performed to demonstrate the selectivity of ANGII-MIP and NIP. ANGII, AngI Human Acetate and Arg8-Vasopressin Acetate aqueous solutions were prepared separately and interacted with both ANGII-MIP and NIP one by one.



Molecule form: $C_{50}H_{71}N_{13}O_{12}$	$C_{62}H_{89}N_{17}O_{14}$	$C_{46}H_{65}N_{15}O_{12}S_2$
Molecular W.: 1046.2 g/mol	1296.5 g/mol	1084.2 g/mol

Figure 4.7. Molecular structures and properties of ANGII, which is a template molecule, and ANGI and VASP, which are competitor molecules

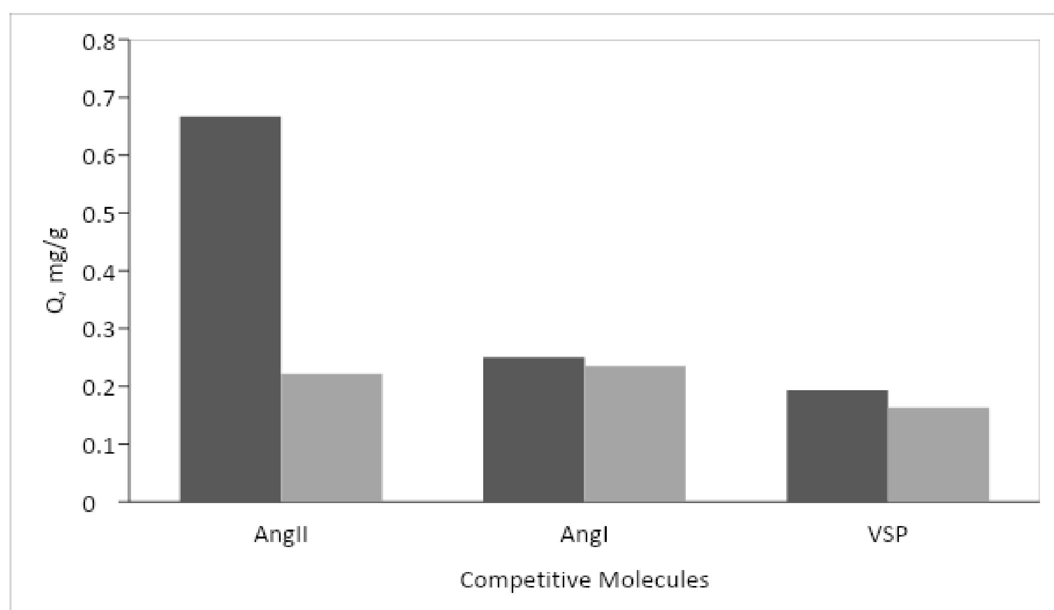


Figure 4.8. Selectivity Studies, ANGII, ANGI, VASP conc.: 0,05 mg/mL, m_{dry}:0,1027 g, Flow Rate: 0,5 mL/min, V: 3 mL, pH 7,4, Time: 120 min, T: 25°C

Figure 4.8. and Table 4.4. show the selective adsorption results. The selectivity of the column was investigated in the presence of competing agents with similar molecular structures, and it was calculated that the ANGII-MIP was 2.66 times more selective against the AngI molecule and 3.46 times more selective against VASP. It was concluded that the ANGII-MIP can recognize ANGII selectively.

Table 4.4. Selectivity Studies

	ANGII-MIP	NIP		
	Q, mg/g	Q, mg/g	IF	k
ANGII	0,667	0,221	3,01	
AngI	0,25	0,235	1,06	2,66
VASP	0,193	0,163	1,18	3,46

The molecular weight of ANGII, the template molecule, is 1046.2 g/mol, while the molecular weight of ANGI is 1296.5 g/mol and the molecular weight of VASP is 1084.2 g/mol.

4.4. Reusability Studies

Reusability is one of the most important criteria for the production of cost-effective materials. In order to examine the reusability of the produced columns, the ANGII solution in a certain concentration was applied to the same column 10 times and the results obtained after 10 adsorption desorption cycles are shown in Figure 4.9. According to the results obtained, the decrease in capacity of ANGII adsorption of the columns is negligible (3%).

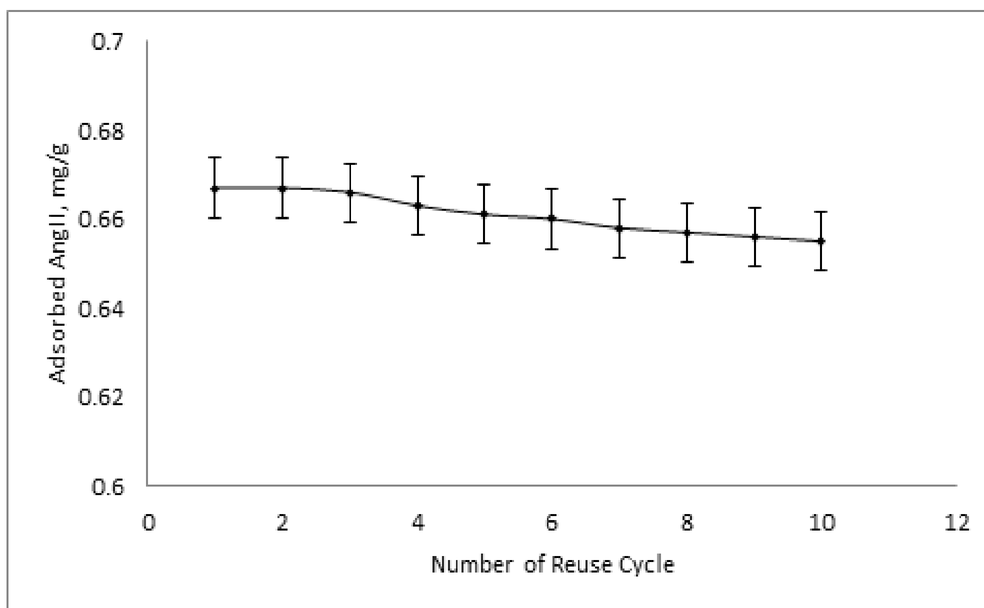


Figure 4.9. Reusability of ANGII-MIP columns. ANGII conc : 0.05 mg / mL, m_{dry}: 0.1027 g, V: 3 mL, pH 7.4, Time: 120 min, T: 25°C

5. CONCLUSION

ANGII, an important biomarker in the blood for cardiovascular diseases, and the synthesis of angiotensin-imprinted polymeric cryogels (ANGII-MIP) for the detection of ANGII from human blood for the purpose of early diagnosis, follow-up and treatment in cardiovascular diseases. In this study, polymeric cryogels specific to HEMA-based ANGII molecule recognition sites were synthesized and the synthesized polymeric structure was analyzed by BET, SEM, Swelling tests and techniques. It was determined that the structure obtained as a result of the characterizations consisted of macro pores interconnected at high swelling rates.

When the ANGII recognition capacity of cryogels synthesized from aqueous solutions by ANGII recognition studies was investigated, the column was calculated as 0.667 mg per dry gram of polymer. The selectivity of the column was performed in the presence of competing agents with similar molecular structures, and it was calculated that the column was 2.66 times more selective against AngI molecule and 3.46 more profitable against vasopressin. It was concluded that the polymeric structure obtained by the ANGII recognition studies from the aqueous medium in the presence of competitor molecules similar in shape and size to the ANGII structure of the column has ANGII recognition selectivity. It is seen that the column synthesized under the light of all these studies can be used as an alternative method for the diagnosis of ANGII from blood and has the potential to be commercialized as it has reusability.

REFERENCES

Abalı, G., Kabakcı, M. G. (2004). Anjiyotensin Reseptör Blokerleri ve Kardiyovasküler Hastalıklarda Kullanım Alanları, Türkiye Tıp Dergisi, 11(1), 18-24.

Akbar, S. A., Khawaja, N. P., Brown, P. R., Tayyeb, R., Bamfo, J., & Nicolaides, K. H. (2009). Angiotensin II Type 1 and 2 Receptors Gene Polymorphisms in Pre-eclampsia and Normal Pregnancy in three Different Populations. *Acta Obstetricia et Gynecologica*, 606-611.

Andaç, M., & Denizli, A. (2014). Affinity-recognition-based polymeric cryogels for protein depletion studies. *Royal Sociaty of Chemistry,* 31130-31141.

Angiotensin II. (n.d.). Retrieved June 11, 2020, from https://pubchem.ncbi.nlm.nih.gov/compound/172198

Arıcı, M., Altun, B., Erdem, Y., Derici, Ü., Nergizoğlu, G., Turgan, Ç., . . . Derneği, T. H. (2019). *TÜRK HİPERTANSİYON PREVALANS ÇALIŞMASI.* Ankara: http://www.turkhipertansiyon.org/.

Association, A. H. (2019). *Heart Disease and Stroke Statistics-2019 At-a-Glance.* America: American Heart Association.

Aşık, M., Aydoğdu, A., Bayram, F., Bilen, H., Can, S., Cesur, M., . . . Yılmaz, M. (2018, Mayıs). *Hipertansiyon Tanı ve Tedavi Kılavuzu.* Ankara: Türkiye Endokrinoloji ve Metabolizma Derneği.

Baker, K. M., Booz, G. W., & Dostal, D. E. (1992). Cardiac Actions of Angiotensin II: Role of an Intracardiac Renin-Angiotensin System. *Annual Review of Psychology*, 227-241.

Bátkai, S., & Thum, T. (2012). MicroRNAs in Hypertension: Mechanisms and Therapeutic Targets. *Current Hypertension Reports*, 79-87.

Baydemir, G., Bereli, N., Andaç, M., Say, R., Galaevc, I. Y., & Denizli, A. (2009). Bilirubin recognition via molecularly imprinted supermacroporous cryogels. *Colloids and Surfaces B: Biointerfaces*, 33-38.

Baydemir, G., Bereli, N., Andaç, M., Say, Galaev, I. Y., & Denizli, A. (2009). Supermacroporous poly(hydroxyethyl methacrylate) based cryogel with embedded bilirubin imprinted particles. *Reactive & Functional Polymers*, 36-42.

Bereli, N., Andaç, M., Baydemir, G., Say, R., Galaev, I. Y., & Denizli, A. (2008). Protein recognition via ion-coordinated molecularly imprinted supermacroporous cryogels. *Journal of Chromatography A,* 18-26.

Bereli, N., Ertürk, G., Tümer, M.A., Say, R., Denizli, A. (2013). Oriented immobilized anti-hIgG via Fc fragment-imprinted PHEMA cryogel for IgG purification. Biomed. Chromatogr., 27, 599–607.

Berker, B., Çakmak, T., Darka, İ., Dokumacıoğlu, İ., & Kuloğlu, U. (2014). Hipertansiyon Hastalarında Yaşam Tarzı Değişikliklerine Uyma ve Kan Basıncı Kontrolünün Değerlendirilmesi. *XVI. ÖĞRENCİ SEMPOZYUMU ÇALIŞMA GRUBU* (pp. 1-18). Ankara: Başkent Üniversitesi.

Bhatnagar, D., Kaurb, I., & Kumara, A. (2015). Ultrasensitive cardiac troponin I antibody based nanohybrid sensor for rapid detection of human heart attack . *International Journal of Biological Macromolecules*, 1-8.

Brosnihan, K. B., & Chappell, M. C. (2017). Measurement of Angiotensin Peptides: HPLC- RIA. In R. M. Touyz, & E. L. (eds.), Hypertension: Methods and Protocols, Methods in Molecular Biology (Vol. Chapter 7, pp. 81-99). Switzerland: Humana Press.

Brown, R. D., Ambler, S. K., Mitchell, M. D., & Long, C. S. (2005). The Cardiac Fibroblast: Therapeutic Target in Myocardial Remodeling and Failure. *Annual Review of Pharmacology and Toxicology*, 657-687.

Brunner H. R., Nussberger J., Waeber B., (1993). Inhibitors of the renin-angiotensin system, Arzneimittelforschung, 43, 274-8.

Bryson, T. D., Pandrangi, T. S., Khan, S. Z., Xu, J., Pavlov, T. S., Ortiz, P. A., . . . Harding, P. (2020). The Deleterious Role of the Prostaglandin E2 EP3 Receptor in Angiotensin II Hypertension. *American Journal of Physiology - Heart and Circulatory Physiology*, 867-882.

Campbell, D. J. (1987). Tissue Renin-Angiotensin System: Sites of Angiotensin Formation. *Journal of Cardiovascular Pharmacology*, 1-8.

Çavuş, A., Baysal, Z.,&Alkan, H. (2013). Preparation of poly(hydroxyethyl methacrylate) cryogels containing I-histidine for insulin recognition. *Colloids and Surfaces B: Biointerfaces*, 84-89.

Çetin, K. (2013). *Moleküler Baskılanmış Kriyojel Disklerden Florourasil Salınımı.* Ankara: Hacettepe Ünivesitesi.

Çiçek, Ç. (2014). Preparation Of Molecularly Imprinted Sensor For Bilirubin Detection, Yüksek Lisans Tezi.

Çorman, M. E. (2014). *Polisiklik Aromatik Hidrokarbonların Moleküler Baskılanmış Polimerler ile Ön-Deriştirilmesi ve Tayini.* Ankara: Hacettepe Üniversitesi.

Denizli A. , (2002). Preparation of immuno-affinity membranes for cholesterol removal from human plasma, *Journal of Chromatography B*.; 772, 357-367.

Derad, I., Sayk, F., Lhnert, H., Marshall, L., Born, J., & Nitschke, M. (2013). Intranasal Angiotensin II in Humans Reduces Blood Pressure When Angiotensin II Type 1 Receptors Are Blocked. *Hypertension*, 762-767.

Drummond, G. R., Vinh, A., Guzik, T. J., & Sobey, C. G. (2019). Immune Mechanisms of Hypertension. *Nature Reviews Immunology* , 517-532.

Dzau, V. J. (2001). Tissue Angiotensin and Pathobiology of Vascular Disease A Unifying Hypothesis. *75231 Hypertension* , 1047-1052.

Eskildsen, T. V., Jeppesen, P. L., Schneider, M., Nossent, A. Y., Sandberg, M. B., Hansen, P. B., . . . Sheikh, S. P. (2013). Angiotensin II Regulates microRNA-132/-212 in Hypertensive Rats and Humans . *International Journal of Molecular Sciences*, 11190-11207.

Ersoy, Ş. K. (2014). *Fenolik Bileşiklerin Seçimli Ayrımı ve Tayininde Yeni Malzemelerden Yararlanılması.* İstanbul: İstanbul Üniversitesi.

Fareghi, A. R., Moghadam, P. N., Khalafy, J., Bahram, M., & Moghtader, M. (2017). Preparation of a new molecularly imprinted polymer based on self-crosslinkable cellulose acrylate in aqueous solution: A drug delivery system for furosemide. *Journal of Applied Polymer Science*, 1-11.

Ferrario, C. M., & Strawn, W. B. (2006). Role of the Renin-Angiotensin-Aldesterone System and Proinflammatory Mediators in Cardiovascular Disease. *The American Journal of Cardiology*, 121-128.

Fisher, J., & Paton, J. (2012). The Sympathetic Nervous System and Blood Pressure in Humans: Implications for Hypertension. *Journal of Human Hypertension*, 463-475.

Griendling, K. K., Lassegue, B., & Alexander, R. W. (1996). Angiotensin Receptors And Their Therapeutic Implications. *Annual Review of Pharmacology and Toxicology*, 281-306.

Hajizadeh, S., Kirsebom, H., Leistner, A., Mattiasson, B. (2012). Composite cryogel with immobilized concanavalin A for affinity chromatography of glycoproteins. J. Sep. Sci., 35, 2978–2985.

Haupt, K. (2001). Molecularly Imprinted Polymers In Analytical Chemistry. *The Royal Society of Chemistry/The Analyst*, 747-756.

Haupt, K., & Mosbach, K. (2000). Molecularly Imprinted Polymers and Their Use in Biomimetic Sensors. *American Chemical Society*, 2495-2504.

Horiuchi , M., Akishita, M., & Dzau, V. J. (1998). Molecular And Cellular Mechanism of Angiotensin II-Mediated Apoptosis. *Endocrine Research*, 307-314.

Huang, F., Guo, J., Zou, Z., Liu, W., Cao, B., Zhang, S., Li, H., Wang, W., Sheng, M., Liu, S., Pan, J., Bao, C., et al., (2014). Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients, Nature Communications.

Ikegami, T., Mukawa, T., Nariai, H., & Takeuchi, T. (2004). Bisphenol A-recognition polymers prepared by covalent molecular imprinting. Analytica Chimica Acta, 504(1), 131–135. doi:10.1016/j.aca.2003.08.032

Jian, W., Wang Quin - Mei, T. L.-L., Chen, Y., Hua, Y. S., & Chun, Y. (2015). Research Progress of the Molecularly Imprinted Cryogel. *Chinese Journal of Analytical Chemistry*, 1777-1784.

Kahveci, M.U., Beyazkılıç, Z., Yagci, Y. (2010). Polyacrylamide cryogels by photoinitiated free radical polymerization. J. Polym. Sci: Part A: Polym. Chem., 48, 4989–4994.

Kartal, F., & Denizli, A. (2020). Molecularly Imprinted Cryogel Beads for Cholesterol Removal from Milk Samples. *Journal Pre-proof*, 1-28.

Kılınç, K., Bedir, A., Arık, N., Alvur, M., & Bodur, A. F. (2016). Esansiyel Hipertansiyonlu Hastalarda M235T Anjiyotensinojen Gen Polimorfizmi. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi*, 56-63.

Kirsebom, H., Topgaard, D., Galaev, I.Y., Mattiasson, B. (2010). Modulating the porosity of cryogels by influencing the non-frozen liquid phase through the addition of inert solutes. Langmuir, 26, 16129–16133.

Kvakan, H., Kleinewietfeld, M., Qadri, F., Park, J.-K., Fischer, R., Shwarz, I., . . . Muller, D. N. (2009). Regulatory T Cells Ameliorate Angiotensin II - Induced Cardiac Damage. *Circulation Journal of the American Heart Association*, 1-17.

Lasocki, S., Igalrz, M., François, P., Barrot, S. V., Vicaut, E., Henrion, D., . . . Bénessiano, J. (2002). Involvement of Renin-Angiotensin System in Pressure-Flow Relationship. *Anesthesilogy*, 271-275.

Lan, T.-H., Huang, X.-Q., & Tan, H.-M. (2013). Vascular Fibrozis in Atherosclerosis. *Cardiovacular Pathology*, 1-7.

Levenson, B., Herrera, C., & Wilson, B. H. (2020). New ACC Global Heart Attack Treatment Initiative (Improving STEMI Care Worldwide). *Journal of the American College of Cardiology*, 1605-1608.

Lozinsky, V. I., Galaev, I. Y., Plieva, F. M., Savina, I. N., Jungvid, H., & Mattiasson, B. (2003). Polymeric cryogels as promising materials of biotechnological interest. *TRENDS in Biotechnology*, 445-451.

Lozinsky, V. I. (2018). Cryostructuring of Polymeric Systems. 50.† Cryogels and Cryotropic Gel-Formation: Terms and Definitions. *Gels*, 1-12.

Mehta, P. K., & Griendling, K. K. (2006). Angiotensin II Cell Signaling: Physiological And Pathological Effetcs In The Cardiovascular System. *The American Physiological Society*, C82-C97.

Mıcılı C., S., Ergür, B. U., Özoğul, C., Saroğlu, S., Bağrıyanık, A., Tuğyan, K., . . . Buldan, Z. (2012). Deneysel Hipertansiyon ve Diyabet Modeli Oluşturulan Sıçanlarda Böbreğin İmmünohistokimyasal Olarak İncelenmesi. *DEÜ Tıp Fakültesi Dergisi*, 91-101.

Mitro, P., Mudrakova, K., Mığkova, H., Dudas, J., Kırsch, P., & Valoğık, G. (2008). Hemodynamic Parameters and Heart Rate Variability during a Tilt Test in Relation to Gene Polymorphism of Renin-Angiotensin and Serotonin System . *Journal compilation* , 1571-1581.

Montezano, A. C., Cat, A. N., Rios, F. J., & Touyz, R. M. (2014). Angiotensin II and Vascular Injury. *Current Hypertension Reports*, 431.

Mosbach, K., Arshady, R. (1981). Synthesis of substrate-selective polymers by host–guest polymerization, *Makromolecular Chemistry and Physics*, 182, 687–692.

Mosbach, K., Ramström, O. (1996). The emerging technique of molecular imprinting and its future impact on biotechnology, Nature Biotechnology, 14, 163-170.

Munro, M., Wickremesekera, A., Davis, P., Marsh, R., Tan, S., Itinteang, T., (2017). Renin-angiotensin system and cancer: A review", Integrative Cancer Science and Therapeutics (ICST), 4, 1-6.

Navar, G. L., Mitchell, K. D., Harrison - Bernard, L. M., Kobori, H., & Nisbiyama, A. (2001). Intrarenal Angiotensin II Levels in Normal and Hypertensive States. *Journal of the Renin-Aldosterone System*, 176-184.

Narula, P., Singh, R., Kaur, V., & Malik, A. K. (2014). Recent Progress, Challenges and Prospects in Monitoring Plastic-Derived Xenoestrogens Using Molecularly Imprinted Sorbents. *Chromatographia*, 207-221.

Öncel, P. (2013). *İlaç Salımı için Moleküler Baskılanmış Kriyojel Membranlar.* Ankara: Hacettepe Üniversitesi.

Özdemir, Z. N. (2010). *Anjiyotensin II-Bağımlı Renovasküler Hipertansiyon Modelinde Egzersizin İyileştirici Etkisinin Araştırılması*. İstanbul: Marmara Üniversitesi.

Parlakpınar, H., Yanılmaz, M., Ağlamış, S., & Acet, A. (2004). Kardiyovasküler Sistem ve Anjiotensin II Tip 2 Reseptörü (AT2) . *İnönü Üniversitesi Tıp Fakültesi Dergisi* , 49-52.

Patel, V. B., Zhong, J.-C., Grant, M. B., & Oudit, G. Y. (2016). Role Of The ACE2/Angiotensin 1-7 Axis Of The Renin - Angiotensin System In Heart Failure. *Circulation Research*, 1313-1326.

Piletska, E., Piletsky, S., Karim, K., Terpetschnig, E., & Turner, A. (2004). Biotin-Specific Synthetic Receptors Prepared Using Molecular Imprinting. Analytica Chimica Acta, 179-183.

Polyakov, M. V. (1931). Adsorption properties and structure of silica gel adsorption properties on the character of its porosity, Zhurnal Fizieskoj Khimii, 2, 799-805**.**

Pullareddy, B. R., Babu, B. M., Karunakar, K. V., Yasovanthi, J., Kumar , P. S., Sbarath, A., &
Jyothy, A. (2009). Angiotensin II Type 1 Receptor Gene Polymorphism In Myocardial Infarction Patients. *Journal Of Renin Angiotensin Aldosterone System*, 174-178.

Rabieizadeh, M., Kashefimofrad, S.M., Naeimpoor, F. (2014). Monolithic molecularly imprinted cryogel for lysozyme recognition. J. Sep. Sci., 37, 2983–2990.

Reichelt, S. (2015). Affinity chromatography: Methods and protocols. New York: Humana Press.

Rosenbaugh, E. G., Savalia, K. K., Manickam, D. S., & Zimmerman, M. C. (2013). Antioxidant-Based Therapies for Angiotensin II - Associated Cardiovascular Diseases. *American Journal of Physiology Regulatory Integrative and Comparative Physiology*, R917-R928.

Swanoski, M. T., Lutfiyya, M. N., Amaro, M. L., Akers, M. F., & Huot, K. L. (2012). Knowledge of Heart Attack and Stroke Symptomology: a cross-sectional comparison of rural and non-rural US adults. *BMC Public Health*, 1-8.

Sümbül, A. T. (2012). *Metastatik Kolorektal Karsinomlu Hastalarda Bevacızumab Kullanımının Hipertansiyon Gelişmesi ve Serum Vasküler Endotelyal Büyüme Faktörü, Nitrik Oksit, Anjiyotensin II Düzeyleri Üzerindeki Etkisi ve Tedaviye Yanıt ile Olan İlişkisi.* Ankara: Başkent Üniversitesi Tıp Fakültesi.

Unger T. (2000). Neurohormonal modulation in cardiovascular disease, *American Heart Journal,* 139, 2-8.

Úri, K., Fagyas, M., Mányiné Siket, I., Kertész, A., Csanádi, Z., Sándorfi, G., Clemens, M., Fedor, R., Papp, Z., Édes, I., Tóth, A., Lizanecz, E., (2014). "New perspectives in the renin-angiotensin-aldosterone system (RAAS) IV: circulating ACE2 as a biomarker of systolic dysfunction in human hypertension and heart failure", PloS one, 9(4), e87845.

Uzunoğlu, G. (2013). *Düşük Molekül Ağırlıklı Heparin Takılı PHEMA Kriyojel ile Kandan Kolesterol Uzaklaştırılması.* Ankara: Hacettepe Üniversitesi.

Vasopollo, G., Sole, R. D., Mergola, L., Lazzoni, M. R., Scardino, A., Scorrano, S., & Mele, G. (2011). Molecularly Imprinted Polymers: Present and Future Prospective. *International Journal of Molecular Sciences*, 5908-5945.

Wright, J. W., Krebs, L. T., Stobb, J. W., & Harding, J. W. (1995). The Angiotensin IV System: Functional Implications. *Frontiers In Neuroendocrinology*, 23-52.

Wulf, G. (1995). Molecular Imprinting in Cross-Linked Materials with the Aid of Molecular Templates-A Way towards Artificial Antibodies. *Angewandte Chemie International Edition in English*, 1812-1832.

Wulff, G., Sarhan, A. (1972). Über die Anwendung von enzymanalog gebauten Polymeren zur Racemattrennung. *Angewandte Chemie*, 84(8), 364–364.

Xie, G., Cheng, T., Lin, J. et al., (2018). Local angiotensin II contributes to tumor resistance to checkpoint immunotherapy, J. Immunotherapy Cancer 6, 88.

Yan, H., & Row, K. H. (2006). Characteristic and Synthetic Approach of Molecularly Imprinted Polymer . *International Journal of Molecular Sciences* , 155-178.

Yan, Y., Liu, Q., Li, N., Du, J., Li, X., Li, C., Jin, N., Jiang, C. (2015). "Angiotensin II receptor blocker as a novel therapy in acute lung injury induced by avian influenza A H5N1 virus infection in mouse", Science China. Life sciences, 58, 208-11.

Yılmaz, R., & Erdem, Y. (2006). Anjiyotensin II Reseptör Blokerleri ve Yeni Bir Ajan: Olmesartan Medoksomil. *Dahili Tıp Bilimleri Dergisi*, 176-182.

Ye, L., & Mosbach, K. (2008). Molecular Imprinting: Synthetic Materials As Subtitutes for Biological Antibodies And Receptors. *American Chemical Society*, 859-868.

Yeşilova, E. (2019). *Tetrasiklin Baskılanmış Nanopartikül Gömülü Kriyojel Sentezi, Karakterizasyonu ve Adsorpsiyon Parametrelerinin İncelenmesi.* Bursa: Uludağ Üniversitesi.

Zheng, Y., Ma, Y., Zhang, J., Xie, X., (2020). COVID-19 and the cardiovascular system, Nat Rev Cardiol, 17, 259-260.

6. CURRICULUM VITAE

MEHTAP YILDIRIM

Date of Birth	: 31.12.1991
Place of Birth	: Adana, TURKEY
Gender	: Female
Nationality	: Turkish
Marital Status	: Single
Foreign Language	: English, German, Portuguese
Address	: ADANA / TURKEY
Mobile Phone	: +90 (0536) 9323445
Email	: mhtpyldrm01@hotmail.com

EDUCATION INFORMATION

2016 Gaziosmanpaşa University, Tokat/TURKEY, Department of Bioengineering

2009 Usak Anatolian High School, Usak/TURKEY, Field of Science

EXPERIENCES

Dokuz Eylul University Oncology Institute

Intern / July – September 2013 / 3 months / At Izmir, Turkey

- DNA - RNA isolation from neuroblastoma tumor tissues and lung tumor tissues with various kits
- Mononuclear cell isolation from pleural effusions, cisplatin ototoxicity samples and blood
- Preparing complex media for cells, changing, opening, passaging and closing (cell culture)
- Cell cultivation
- Painting of lung tumor and normal tissues
- Cell staining with Wescor aerospray hematology device
- Using of the Light Cycler Nano Instrument Real - Time PCR
- Using of Inverted Microscope
- Application of TUNEL method for in situ detection of Apoptesis
- Immunohistochemistry application, hematoxin staining
- Examination of DNA - RNA samples in spectrophotometer

- Flow cytometry device usage, DNA index study
- Following the studies carried out on experimental animals
- Gene amplification with CISH technique
- Electrophoresis application in agarose gel
- Interactive basic oncology training (Sequencing, biobanking, cancer etiology, cancer cell cultures, tumor tissue selection, RNA extraction and cDNA conversion, DNA isolation from paraffin block)
- Scientific thesis - patent preparation and writing

Çukurova University Medicine Faculty Department of Medical Biology

Volunteer Intern / July – August 2012 / 3 weeks / At Adana, Turkey

- Amniocentesis applications
- Examination of chromosome maps, learning of chromosome diseases

Çukurova University Medicine Faculty Department of Medical Biochemistry

Volunteer Intern / June 2012 – July 2012 / 2 weeks / Adana, Turkey

- DNA - RNA isolation from blood samples of hemoglobinopathy patients
- PCR applications
- Agarose gel electrophoresis applications

Çukurova University Medicine Faculty Department, Medical Sciences Experimental Research and Application Center

Volunteer Intern / June 2012 – July 2012 / 3 weeks / Adana, Turkey

- Care of experimental animals (mouse, rat, rabbit, pig, sheep), surgical operations and medical applications

Gaziosmanpasa University -- Bachelor's Degree – Bioengineering /

(My applications during my undergraduate education) 2010-2014

- Protein and enzyme purification applications
- Genetic studies on microorganisms
- Plant breeding and tissue culture studies
- Basic engineering experiments (thermodynamics, heat transfer, mass transfer, bioprocess applications)

- Bioinformatics applications

HOBBIES and AREAS of INTEREST

Folk Dances, Latin Dances, Mountaineering, Rock Climbing

MEETINGS AND CERTIFICATIONS:

1) 8th Graduate Student Symposium on Molecular Imprinting, 28-30 August 2019, (oral presentation), Bundesanstalt für Materialforschung und - prüfung (BAM), in Berlin, Germany

2) Affinity Based Sensors Applied Summer School / Gold Nanoparticles and Sensor Application, 22-24 May 2019, Hacettepe University, ANKARA

3) 14. Bioengineering Days, 2017, Ege University, IZMIR

4) BIOSUPPORT WORKSHOP ON TISSUE ENGINEERING: Polymeric Materials, Bioceramic Composites, Tissue Identities; 2016, Biomaterials and Tissue Engineering Association - Kocaeli University, KOCAELI

5) ISO Certificates; ISO 9001: 2008, Internal Auditor, Documentation, 2016, YDS Consultancy, ADANA

6) 11. Bioengineering Days, 2014, (poster presentation), Ege University, IZMIR

7) Bioengineering Student Congress, 2014, Ege University, IZMIR; Poster Presentation Certificate

8) International IUGEN Molecular Biology and Genetics Student Winter School, 2014, Istanbul University, ISTANBUL

9) Bioengineering Student Congress, 2014, Gaziosmanpaşa University, TOKAT

10) METU Synthetic Biology Day, 2013, Middle East Technical University (METU), ANKARA

11) Basic Oncology Summer Course, 12 August-12 September 2013 Dokuz Eylül University, IZMIR

12) Congress of the International Society of Molecular Biology, 2012, Bogazici University, ISTANBUL

13) BIOMED 2012: 18th International Biomedical Science and Technology Symposium, 2012, Gaziosmanpaşa University, TOKAT

14) Traditional 4th Stem Cell Symposium, Ege University, 2012, IZMIR

15) International 8. IUGEN Molecular Biology and Genetics Student Winter School, 2011, Istanbul University, ISTANBUL

I AM A MEMBER OF THE INSTITUTION AND TASKS

Board Member: Chamber of Chemical Engineers BIOMEDAK (Bioengineering Profession Main Commission)

Board Member: TMMOB Chamber of Chemical Engineers South Branch