T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT of NUTRITION AND DIETETICS

ENCAPSULATION OF VITAMIN B12 WITH pH-SENSITIVE BIOPOLYMERS AND INVESTIGATION OF ITS RELEASE THROUGH THE GASTROINTESTINAL TRACT

MASTER OF SCIENCE THESIS

ALI ONAL

ISTANBUL, 2022

T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT of NUTRITION AND DIETETICS

ENCAPSULATION OF VITAMIN B12 WITH pH-SENSITIVE BIOPOLYMERS AND INVESTIGATION OF ITS RELEASE THROUGH THE GASTROINTESTINAL TRACT

MASTER OF SCIENCE THESIS

Ali Onal

ADVISOR
Assoc. Prof. Dr. Hülya Demir

CO - ADVISOR
Prof. Dr. Olcay Kaplan Ince

İSTANBUL-2022

THESIS APPROVAL

Institute : Yeditepe Üniversity Institute of Health Sciences

Programme : Nutrition and Dietetics

Title of the Thesis : Encapsulation of Vitamin B12 With pH-Sensitive Biopolymers and Investigation of Its Release Through the Gastrointestinal Tract

Owner of the Thesis : Ali ONAL

Examination Date : 07.07.2022

This study have approved as a Master Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)
Chair of the Jury	Prof. Dr. Muharrem INCE Munzur University
Advısor	Assoc. Prof. Dr. Hülya DEMIR Yeditepe University
Co- Advısor	Prof. Dr. Olcay KAPLAN INCE Munzur University
Member:	Asst. Prof. Dr. Ozge TASTAN Yeditepe University
Member:	Prof. Dr. Muharrem INCE Munzur University

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..…/…../……. and numbered ……………..

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

That this thesis is my own work, that I did not have any unethical behavior at all stages from planning, conducting the experiments to writing, I obtained all the information in the thesis within academic and ethical rules, I cited all the information and comments that were not obtained through the thesis study and included these sources in the list of sources, and I declare that I have not acted in violation of patents and copyrights during my writing.

07.07.2022

Ali ONAL

ACKNOWLEDGEMENT

Endless Thanks,

I would like to thank my esteemed thesis advisors, Assoc. Prof. Dr. Hülya Demir and Prof. Dr. Olcay KAPLAN INCE,

Not withholding his contributions during the preparation of the thesis, Prof. Dr. Muharrem INCE and Burcu AYDOGDU to my teachers,

To my father Mehmet ONAL and Berrin EKMEKCİOGLU, who have been with me in every period of my life and have not hesitated to.

TABLE OF CONTENTS

THESIS APPROVAL FORM……………………………..…………………….………….ii
DECLARATION…………………………………………………………..………...iii
ACKNOWLEDGEMENT……………………………………………………..….............iv
TABLE OF CONTENTS v
LISTOFTABLES……………………………………………………………….....vii
LIST OF FIGURES viii
ABSTRACT x
ÖZET..xi
1. INTRODUCTION...1
2. LITERATURE REVIEW...4
2.1. VITAMIN B12 4
2.1.1. Definition 4
2.1.2. Molecule Structure and General Properties 4
2.1.3. Synthesis of Vitamin B12 5
2.1.4. B12 Resources and Need...8
2.2. Encapsulation Technology and General Features 14
2.2.1. Encapsulated Active Ingredients..16
2.2.2. Coating Materials Used in Encapsulation 17
2.2.2.1. Carbohydrates...18
2.2.2.2. Gums 20
2.2.2.3. Proteins...23
2.2.3. Encapsulation Methods 24
2.2.3.1. Spray Drying 24
2.2.3.2. Coacervation...26
2.2.3.3. Emulsification 27
2.2.3.4. Freeze Drying...27
2.3. Polymers..34
2.3.1. Intelligent Biopolymers...35
2.3.1.1. pH-Sensitive Biopolymers 36
2.3.1.2. Usage Areas of Biopolymers..40

2.4. Drug Release Systems 41
3. MATERIALS AND METHODS 50
3.1. Chemicals 50
3.2 Devices 50
3.3. Use of Central Composite Design in Experimental Studies 50
3.4. Synthesis of Biopolymer Material and Loading of Vitamin B12 53
4. RESULTS AND DISCUSSION 54
4.1. UV-Vis Spectrophotometer Analyzes 54
4.2. FT-IR Analyzes 54
4.3. TGA-DTA Analyzes 56
4.4. SEM-EDS Analyzes 58
5. CONCLUSION AND RECOMMENDATIONS 76
6. REFERENCES 77

LIST OF TABLES

Table 2. 1. Nutritional sources with more than 3 µg of vitamin B12 per 100 grams............8
Table 2. 2. Coating materials used in the encapsulation of food ingredients.......................18
Table 3. 1. Used chemicals, their usage purpose and brands..50
Table 3. 2. Used devices, their usage purpose and brands..50
Table 3. 3. Independent variables and their levels..52
Table 4. 1. Ideal model selection for the vitamin B12 release behavior of chitosan-g-polyacrylic acid...61
Table 4. 2. ANOVA results of response surface quadratic model......................................61
Table 4. 3. The lower-upper limits and severity of the quadratic model of vitamin B12 release from biopolymer...72
Table 4. 4. Possible solutions...73
Table 4. 5. Confirmation report of the quadratic model at 95% confidence interval 74
Table 4. 6. Coefficients obtained from the quadratic model..75

LIST OF FIGURES

Figure 2. 1. Structure of vitamin B12 5
Figure 2. 2. Structure of vitamin B12 7
Figure 2. 3. The role of methionine Synthase enzyme in vitamin B12 metabolism 9
Figure 2. 4. Role of methylmalonyl CoA mutase enzyme in vitamin B12 metabolism 10
Figure 2. 5. Vitamin B12'nin emilimi 14
Figure 2. 6. Structure of sodium alginate 21
Figure 2. 7. Spray drying system 25
Figure 2. 8. Calcium-alginate cross-linking mechanism 29
Figure 2. 9. Dropping system 30
Figure 2. 10. Schematic representation of stimulus sensitive biopolymers 36
Figure 2. 11. Swelling rate change depending on pH 37
Figure 2. 12. Chemical structures of some polymers that react to pH in drug release applications 38
Figure 2. 13. Schematic view of swelling behavior of pH sensitive polymers containing acidic side groups 39
Figure 2. 14. Schematic view of swelling behavior of pH sensitive polymers containing basic side groups 40
Figure 2. 15. Comparison of time-dependent change of blood drug level between classical drug applications and controlled release systems application. 43
Figure 2. 16. Schematic view of diffusion controlled membrane systems 44
Figure 2. 17. Schematic view of diffusion controlled matrix systems 45
Figure 2. 18. Schematic representation of systems worn in the body 45
Figure 2. 19. Schematic view of chain attached systems 46
Figure 2. 20. Schematic view of swelling controlled systems 47
Figure 2. 21. Swelling-shrinking behavior of environmentally sensitive biopolymers 48
Figure 2. 22. Schematic view of osmotic controlled systems 49
Figure 4. 1. UV-Vis spectrum of vitamin B12 54
Figure 4. 2. FTIR spectrum of chitosan, chitosan–grafted-poly (acrylic acid), vitamin B12 loaded chitosan–grafted-poly (acrylic acid) 55

Figure 4. 3. TGA thermograms of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid 57
Figure 4. 4. DTA thermograms of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid 58
Figure 4. 5. EDS image of chitosan-g-polyacrylic acid 59
Figure 4. 6. EDS image of vitamin B12 is loaded chitosan-g-polyacrylic acid 59
Figure 4. 7. The overlap ratio of the estimated and actual values obtained 63
Figure 4. 8. Interaction between pH and initial concentration a) 3D graph b) 2D graph 64
Figure 4. 9. Interaction between pH and release time a) 3D graph b) 2D graph 65
Figure 4. 10. Effect of interaction between pH and solution volume a) 3D graph b) 2D graph 66
Figure 4. 11. Effect of interaction between release time and initial concentration a) 3D graph b) 2D graph 67
Figure 4. 12. Effect of interaction between solution volume and initial concentration a) 3D graph b) 2D graph 68
Figure 4. 13. Effect of interaction between solution volume and release time a) 3D graph b) 2D graph 69
Figure 4. 14. Diagnostic plots: Power conversion and disorder plot for vitamin B12 release from biopolymer at optimum conditions 70
Figure 4. 15. Desirability plot for vitamin B12 release from biopolymer at optimum conditions 71
Figure 4. 16. Ramp chart for statistically optimized factors for vitamin B12 release from biopolymer 74

ABSTRACT

Onal, A., Encapsulation of vitamin B12 with pH-sensitive biopolymers and investigation of its release through the gastrointestinal tract. Yeditepe University, Institute of Health Science, Nutrition and Dietetics Department, Master of Science Thesis,Istanbul,2022.

In this study, a biofilm was produced using chitosan, a natural polymer, and the release potential in the gastrointestinal tract was investigated by loading vitamin B12 on this biofilm. Characterization analyzes of the synthesized biofilm were performed using FT-IR, TGA-DTA, XRD, SEM-EDS methods. In addition, the analysis results of the film before loading were compared with the analyzes made after adding vitamin B12 to the film. While examining the release potential, parameters such as pH, initial concentration, release time and solution volume were optimized using RSM. The interactive effects of these parameters in RSM were also examined. The loading and release potential of vitamin B12 to the synthesized and characterized biopolymer were investigated as a function of solution pH, initial concentration of vitamin B12, release time and solution volume. The release conditions of vitamin B12 from the biofilm were determined as pH 4.2, the initial concentration of vitamin B12 as 54.0 mg L^{-1}, the release time as 117 min, and the solution volume as 2.1 mL. The maximum vitamin B12 release amount of the synthesized biofilm was calculated as 100%. Optimization of the experimental parameters was carried out with the MKT approach and a mathematical model for vitamin B12 release was obtained from the proposed quadratic model. Analysis of variance results showed that the determination coefficients for the use of the synthesized biofilm in vitamin B12 release were high and the R^2 value was 0.9704.

Keywords: Vitamin B12, biopolymer, characterization, response surface methodology, analysis of variance

ÖZET

Onal, A., B12 vitamininin pH'a duyarlı biyopolimerlerle kapsüllenmesi ve gastrointestinal sistemden salımının araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Anabilim Dalı, Yüksek Lisans Tezi, İstanbul, 2022.

Bu çalışmada doğal bir polimer olan kitosan kullanılarak biyofilm üretildi ve bu biyofilme B12 vitamini yüklenerek gastrointestinal sistemde salım potansiyeli incelendi. Sentezlenen biyofilmin karakterizasyon analizleri FT-IR, TGA-DTA, XRD, SEM-EDS yöntemleri kullanılarak gerçekleştirildi. Ayrıca filme vitamin B12 eklendikten sonra da yapılan analizler ile yüklemeden önceki filmin analiz sonuçları karşılaştırıldı. Salım potansiyeli incelenirken salıma etki eden faktörlerden pH, başlangıç konsantrasyonu, salım süresi ve çözelti hacmi gibi parametreler RSM kullanılarak optimize edildi. RSM'de ayrıca bu parametrelerin etkileşimli etkileri de incelendi. Sentezlenen ve karakterize edilen söz konusu biyopolimere, B12 vitaminin yüklenmesi ve salım potansiyeli çözelti pH'ı, B12 vitamini başlangıç konsantrasyonu, salım süresi ve çözelti hacminin bir fonksiyonu olarak incelendi. B12 vitamininin biyofilmden salım koşulları pH 4.2, B12 vitamini başlangıç konsantrasyonu 54.0 mg L^{-1}, salım süresi 117 dk ve çözelti hacmi 2.1 mL olarak belirlendi. Sentezlenen biyofilmin maksimum B12 vitamini salım miktarı % 100 olarak hesaplandı. Deneysel parametrelerin optimizasyonu MKT yaklaşımı ile gerçekleştirildi ve önerilen kuadratik modelden B12 vitamini salımı için bir matematiksel model elde edilerek önerildi. Varyans analizi sonuçları sentezlenen biyofilmin B12 vitamini salımında kullanılmasındaki belirleme katsayılarının yüksek olduğunu ve R^2 değerinin 0.9704 olduğu tespit edildi.

Anahtar Kelimeler: Vitamin B12, biyopolimer, karakterizasyon, yanıt yüzey metodolojisi, varyans analizi

1. INTRODUCTION

Hydrogels are three-dimensional, hydrophilic, polymeric network structures that can absorb large amounts of water or biological fluids (1). They are prepared by the polymerization reaction of one or more monomers and are insoluble due to the presence of chemical or physical crosslinks between the main chains. Hydrogels have a wide usage especially in medicine and pharmacy (2). They resemble living tissues more than other synthetic biomaterials. This is due to the high-water content and soft structure of the hydrogels. Due to the high biocompatibility of hydrogels; they are used in contact lenses, membranes for biosensors, artificial skin material and drug delivery systems. Hydrogels can be classified according to their ionic charge as neutral (uncharged), anionic (negatively charged), cationic (positively charged) or ampholytic (both charged). Hydrogels can exhibit swelling behavior that changes depending on the external environment conditions. These polymers are termed "physiologically-responsive hydrogels" (3). pH, temperature, ionic strength and electromagnetic radiation are the main factors affecting the swelling behavior of physiologically sensitive or environmentally sensitive hydrogels. All pH-sensitive polymers contain acidic groups (e.g., carboxylic and sulfonic acids) and basic groups (e.g., sulphonic acids) that are capable of either accepting or donating protons in response to environmental changes in pH (4). Contains ammonium salts). Changes in environmental pH lead to structural changes as ionizable groups are attached to the polymer structure, and there is a change in the dissolution of polymers and swelling behavior of hydrogels. The most studied ionic polymers for pH sensitive behavior are Poly(acrylamide) (PAAm), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), Poly(diethylaminoethyl methacrylate) (PDEAEMA) and poly(dimethylaminoethyl methacrylate) (PDMAEMA). However, polymers containing phosphoric acid derivatives have also been investigated (5). The degree of swelling of pH-sensitive hydrogels varies depending on the degree of ionization, protonation, or deprotonation. Upon exposure to the aqueous medium of appropriate pH and ionic strength, some groups ionize and develop fixed charges in the polymer network, and pH-dependent swelling or shrinkage of the hydrogel causes electrostatic repulsive forces, resulting in drug release (5). The aim of this study is to synthesize and characterize various pH-sensitive polymers, to examine the release of vitamin B12 by loading into the synthesized polymers and to develop hydrogels suitable for vitamin B12 release.

For any drug to be administered orally, some physiological aspects of the body and more specifically the gastrointestinal tract must be known. For example, pH change occurs throughout the gastrointestinal (2); however, more delicate changes occur in various body tissues. Various approaches have been used for colon-targeted drug delivery, including pH-dependent swelling-controlled systems (Bajpai et al., 2004), delayed-release delivery systems (3), intestinal pressure-controlled colon delivery capsules (4), and enzymatically degradable systems using a variety of enzymes (5). A comprehensive review of the literature available so far on drug release studies reveals that most in vitro release studies involve measuring release rates from the drug-loaded polymer matrix at different pH environments. That is, HCl solution was used to simulate gastric juice, and pH 6.8 phosphate buffer was used to simulate intestinal fluid. Oral administration of therapeutic compounds is most convenient, although it is not used for many drugs that cannot overcome their many natural barriers (1). The presence of pathological conditions affecting the gastrointestinal tract also inhibits the absorption of active ingredients after oral administration. Vitamin B12 is an example of such drugs. Vitamin B12 deficiency can cause various diseases. Controlled release of vitamin B12 increases its concentration in the liver and blood serum level, which helps prevent or treat cobalt deficiency in the body. Vitamin B12 has been preferred as a model drug because of its neutral behavior and high solubility in water. Since patients with pernicious anemia and other intestinal disorders cannot absorb the small amount of vitamin B12 found in food, vitamin B12 deficiency occurs and therefore lifelong intramuscular injections are needed (2). Adequate oral absorption of vitamin B12 can only be achieved by using high daily doses (3).

To date, no serious effort has been made to increase the oral bioavailability of vitamin B12 by improving its penetration into the intestinal mucosa. Poly (N-vinyl-2-pyrrolidone) (PVP) is known as a good biocompatible material. The non-toxic nature and higher hydrophilicity of PVP encourage its use for drug delivery devices. Another important point is that the synthesis of a gel does not require a high temperature or the use of some organic solvents, so the probability of loss of activity during the incorporation of the drug is minimal. In summary, the aim of the proposed study is to close this gap in the literature by regulating the swelling behavior with the help of a crosslinker, by designing drug delivery systems that can deliver the desired amount of vitamin B12 for the desired time and with the experimental data to be obtained.

Polyacrylic acid (PAA) and its copolymers have been used as carriers in drug delivery systems due to their multifunctional structure, unique properties and good biocompatibility (4). Inter-polymer complexes between poly(vinyl pyrrolidinone) (PVP) and poly(acrylic acid) have been investigated to develop new mucoadhesive drug carriers by taking advantage of hydrogen bonding between the carboxyl groups of PAA and the carbonyl groups of PVP. The adhesion strength and drug release rate of PVP/PAA inter-polymer complexes can be controlled by varying the pH values as well as the mole ratios of PVP and PAA. The complex appears to be an adequate carrier for the mucoadhesive drug delivery system (4). The copolymer can form both intrapolymer and interpolymer complexes between PVP and PAA components. Poly(acrylic acid)-cysteine conjugate (PAA-cys) has been shown to provide a relatively higher absorption of substrates in the rat intestinal mucosa in vitro and in vivo (4,5). In addition, PAA-cys solution and microparticles have recently been highlighted in in vitro studies as having a promising potential as intestinal permeability enhancer for vitamin B12 (5). Therefore, with the designed study, while investigating the potential of vitamin B12 to act as an efflux pump substrate (thereby explaining the release behavior in line with previous studies), on the other hand, examining the experimental variables to increase the release capacity of PAA-cys-based mini-tablets is the main unique value of this study. forms. At the same time, in this study, the potential of using chemically crosslinked PAA-based hydrogels to delay the release of vitamin B12 will be investigated. For this purpose, cross-linked copolymers of acrylic acid (AA) will be synthesized and their interactions with B12 during diffusion will also be examined.

2. LITERATURE REVIEW

2.1. Vitamin B12

2.1.1. Definition

B12, also known as cobalamin, is a water-soluble vitamin. It is involved in the immune system, protein metabolism, nervous system, and the formation of blood cells in the bone marrow in organisms (6).

Rickes et al. (7) isolated a substance that has a therapeutic effect on anemia from the liver. They named the red-colored crystalline substance antianemic vitamin or B12. In the same year, Hodgkin defined the molecular structure of vitamin B12 and received the Nobel Prize in Medicine and Physiology for this study.

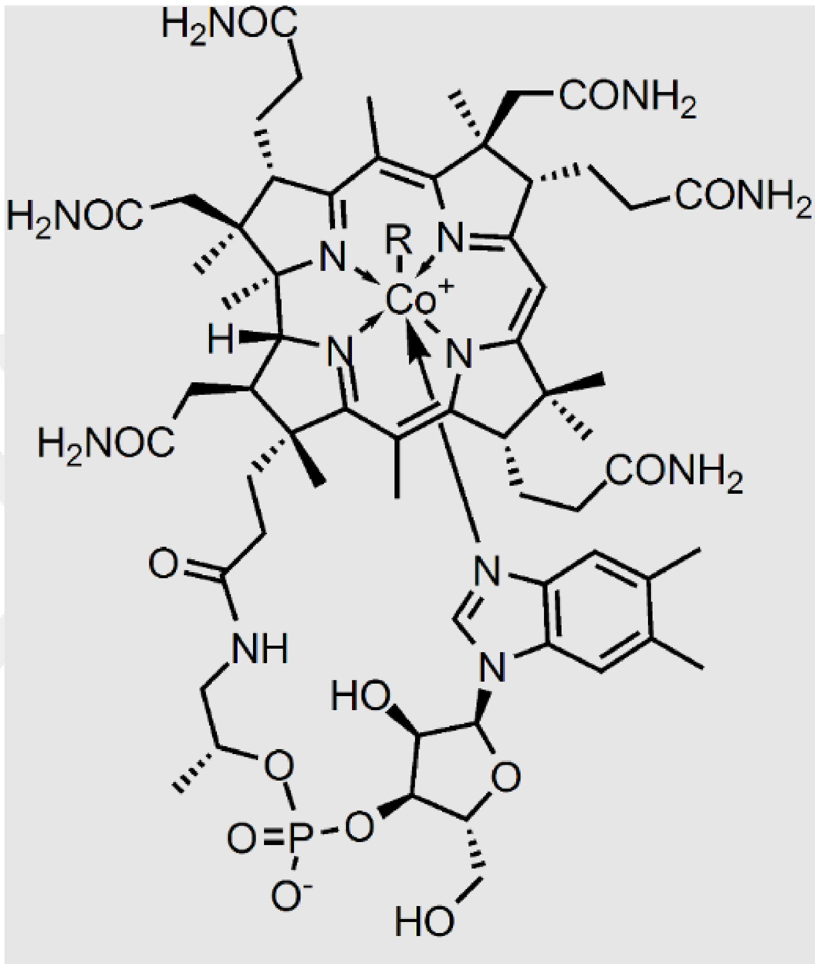
2.1.2. Molecule Structure and General Properties

Vitamin B12 has a corrin ring system and is the only vitamin with metallprotein properties for the cobalt ion content in its structure 8). Although this structure of vitamin B12 is quite similar to the porphyrin ring, it differs from the porphyrin structure by the fact that the four pyrrole rings in the corrin structure are directly connected to each other (9).

Vitamin B12 has different forms according to the R groups in its structure. Methylcobalamin is formed when the R group is a methyl group (CH3), and deoxyadenosylcobalamin is formed with a 5'-deoxyadenosine group. These two forms give vitamin B12 a coenzyme function. Although the term vitamin B12 is commonly preferred in the literature, it is more specific to name it this way, since the term cobalamin refers to all corrins with coenzyme function in humans (10).

The circulating form of vitamin B12 is methylcobalamin, and the intracellular form is 5'-deoxyadenosylcobalamin (11).

If the R group of vitamin B12 is hydroxyl group (OH-), it is called hydroxycobalamin, if the glutathionyl (GS) group is glutathionylcobalamin, and if the cyanide (CN) group is, it is called cyanocobalamin. These formed structures are also inactive forms of vitamin B12 (Figure 2.1.) (11,12). These forms act as precursors for active forms of vitamin B12. Since it is the most durable form among the compounds in the cyanocobalamin group, it generally forms the structure of commercial B12 preparations (11).



R= 5- Deoxyadenosyl CN, OH, CH3

Figure 2.1. Structure of Vitamin B12 (12)

2.1.3. Synthesis of Vitamin B12

Synthesis of vitamin B12 takes place by aerobic or anaerobic biosynthetic pathways. While *Pseudomonas denitrificans* synthesizes Vitamin B12 in the aerobic pathway, it is synthesized by *Bacillus magaterium*, *P.shermanii* and *Salmonella typhimurium* in the anaerobic pathway (13,14).

Vitamin B12 synthesis begins from the C-5 skeleton of glutamate. In the first step, glutamate bound to tRNA is reduced to glutamate-1-semialdehyde by the enzyme glutamyltRNA reductase. When the aldehyde in the structure of glutamate-1-semialdehyde passes to C-1 in the molecule, it forms 5-aminolevulinic acid, the first precursor of succinyl CoA and glycine and tetrapyrrole. Then, porphobilinogen is synthesized by the condensation of two molecules of 5-aminolevulinic acid (13). The first cyclic intermediate, uroporphyrinogen III, is formed by polymerization of the pyrrole rings of four porphobilinogens. Uroporphyrinogen III undergoes methylation at C-2 and C-7 to form precorrin-2, the final common product of coenzyme synthesis (13).

After the precorrin-2 formation step, vitamin B12 synthesis is divided into anaerobic and aerobic pathways. While cobalt chelation occurs without the need for ATP in the anaerobic pathway, there are nine preliminary steps and ATP requirement for this chelation in the aerobic pathway. While the 20th carbon atom of Precorrin-2 is removed from the ring as acetaldehyde in the anaerobic pathway, it is removed as acetic acid in the aerobic pathway and cobiric acid synthesis is carried out. The synthesized cobiric acid turns into cobinamide by attaching an aminopropanol arm to the propionic acid side chain of the D ring in its structure (13,14).

In the last step of vitamin B12 synthesis, dimethyl benzimidazole (DMB) binds to cobinamide and synthesizes vitamin B12 (Figure 2.2.) (13).

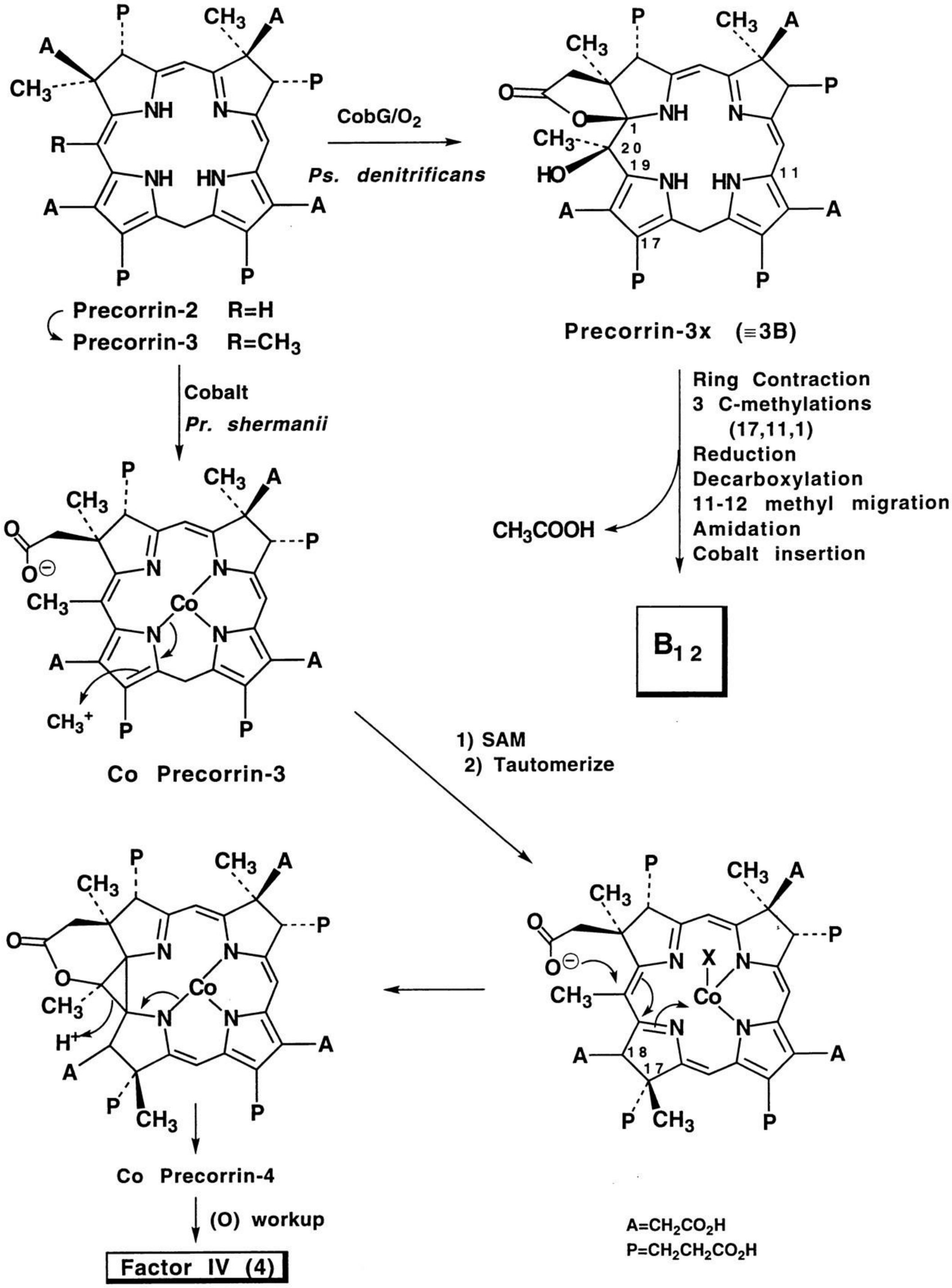


Figure 2.2. Structure of Vitamin B12 (12)

B12 synthesis cannot be performed in humans because the relevant genes are not available (15,16). However, although colon bacteria synthesize B12, the amount needed by the organism cannot be met due to the fact that this synthesis takes place in the distal of the absorption region and is at insufficient levels (11). For this reason, vitamin B12, which has important functions in the organism, must be taken from outside with food, that is, vitamin B12 is an essential vitamin.

2.1.4. B12 Resources and Need

Vitamin B12 is synthesized by anaerobic bacteria and is only found in foods of animal origin as storage B12 (17,18).

The best sources of B12 can be listed as red meat, fish, poultry, dairy products and eggs (18). When the foods containing more than 3 µg of vitamin B12 in 100 grams of B12 sources listed in the national food composition database TURKOMP are examined, it is seen that all of them are animal meat and eggs, and offal meats are in the first place (Table 2.1).

Table 2.1. Nutritional sources with more than 3 µg of vitamin B12 per 100 grams

ood	Average amount of B12 in 100 grams of food
dible offal, veal liver	133.52 µg
dible offal, veal kidney	25.19 µg
gg, ostrich	10.95 µg
dible offal, veal tongue	7.61 µg
abbit meat, skinless	7.60 µg
ussels, Mediterranean, black mussels	7.52 µg
orse mackerel	7.39 µg
dible offal, veal heart	7.35 µg
irsi	6.89 µg
idney beans (red bean)	5.51 µg
trich meat, thigh	5.48 µg
strich meat, breast	4.97 µg
nchovy	4.83 µg
onito	4.21 µg
out, rainbow, rearing	4.01 µg
ullet, pacific (Russian mullet)	3.92 µg
rch, rearing	3.74 µg
dible offal, veal brain	3.65 µg
dible offal, sheep intestines	3.54 µg

The recommended daily intake of B12 is as follows:

≈0.05 µg/kg for 0-1 year old

0.9 µg/day for 1–3 years old

1.2 µg/day for 4–8 years old

1.5 µg/day for 9-13 years old

For persons over 14 years of age, it is 2 µg/day.

The need for B12 may increase in special circumstances. It is 2.2 µg/day during pregnancy and 2.4 µg/day during breastfeeding (19). It is estimated that only 50% of dietary vitamin B12 is absorbed by healthy adults (18).

2.1.5. Physiological Importance and Function of Vitamin B12

Vitamin B12 is the basic coenzyme for two important enzyme reactions in the human body (20). The first of these reactions is the remethylation of homocysteine, and the second is the isomerization of methylmalonyl CoA (21).

2.1.5.1. Remethylation of Homocysteine

The methionine synthase enzyme converts homocysteine to methionine, which is of great importance in maintaining the integrity of the nervous system (22). In this reaction, the form of vitamin B12 found in the cytoplasm acts as a methylcobalamin coenzyme (Figure 2.3) (21,23).

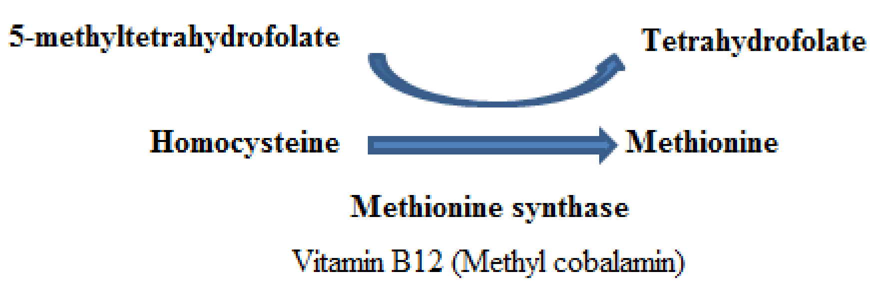


Figure 2.3. The Role of Methionine Synthase Enzyme in Vitamin B12 Metabolism (21)

After being synthesized, methionine is adenosylated to S-adenosylmethionine (SAM). SAM, on the other hand, is involved in transmethylation reactions (DNA, RNA, lipid and neurotransmitter methylation), myelin synthesis and neurotransmitter regulation (24,25).

This reaction is also the intersection point of vitamin B12 and folate (26), and tetrahydrofolate (THF) is synthesized from 5-methyltetrahydrofolate (5-MTHF), which is a methyl donor in the reaction (27). THF takes part in DNA synthesis by methyl transfer (20).

2.1.5.2. Isomerization of Methylmalonyl CoA

The isomerization of methylmalonyl CoA and its conversion to succinyl CoA occur with the methylmalonyl CoA mutase enzyme in the mitochondria (Figure 2.4) (10), and 5'-deoxyadenosyl cobalamin, the form of vitamin B12 found in the mitochondria, takes part in this reaction (26). The succinyl CoA formed participates in the Krebs cycle and takes part in energy production and meeting the need for fat and protein (22).

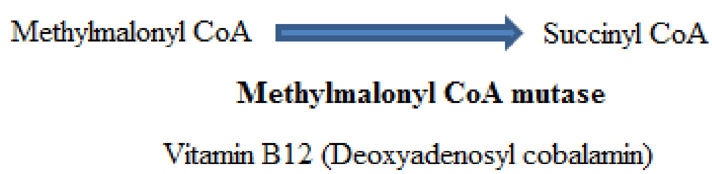


Figure 2.4. Role of Methylmalonyl CoA Mutase Enzyme in Vitamin B12 Metabolism (10)

2.1.6. Absorption, Storage and Elimination of Vitamin B12

Cobalamin trafficking, transport, and access to cells are dependent on three cobalamin-binding proteins: intrinsic factor (IF), transcobalamin (TC), and haptocorrin. Only IF and transcobalamin interact with specific cell receptors and allow efficient intracellular uptake of low concentrations of cobalamin, but these systems have a relatively low capacity (28).

Intrinsic factor

IF is a 45 kDa glycoprotein synthesized by gastric parietal cells in humans; Under certain conditions, ectopic IF synthesis can also occur in gastric chief cells, which are the normal synthesis site in many other mammals (29). It effectively mediates cobalamin uptake in the gut. It binds cobalamins less tightly than TC or haptocorrin, but has a much greater binding affinity for cobalamins than non-functional corrin analogs, helping to limit intestinal absorption of the analogs (30). IF is encoded by the GIF (gastric intrinsic factor) gene on chromosome 11q13 (31).

Transcobalamin (also called transcobalamin II (TC II)

It is synthesized by endothelial and other cells and mediates the entry of cobalamin into most peripheral cell types (9). The TCN2 gene is on the 22q12-13 chromosome (32).

Haptocorrin (also called transcobalamin I (TC I)

Distinct from IF and TC in that it has a high affinity for non-functional corrinoid analogues and cobalamin and lacks a specific cell receptor for intracellular uptake. The protein encoded by the TCN1 gene on chromosome 11q12.1 is approximately 46 kDa. Due to its secretion, some glycosylation variability has led to artificial distinctions for obsolete haptocorrin nomenclature (eg, Transcobalamin III and R binder) (9). The name haptocorrin had been used in the early 1980s to include cobalamin-binding proteins with the same protein backbone, which until then had a number of different names, including TCI and TCIII, cobalophilins, R-linker, and saliva-binding (33).

Haptocorrin is synthesized in myeloid precursors in the early stages, when its specific granules are formed; this plasma appears to be the major source of haptochorria. Epithelial or ductal cells of exocrine glands also synthesize haptocorrin (34).

More than 70% of cobalamin in plasma is bound to haptocorrin at any one time, but this apparent superiority is misleading because haptocorrin is gradually cleared from plasma (half-life; 9.5-12 days). However, TC (half-life of only 90 minutes) carries 40 times more cobalamin daily than haptocorrin (35).

Almost all of the cobalamin in foods is in a protein-bound form. After food intake, cobalamin, which is separated from proteins with the help of acid and proteases in the stomach, first binds to haptocorrin in saliva and secretions in the upper gastrointestinal tract, and then haptocorrin is broken down by gastrointestinal enzymes in the duodenum, and then cobalamin binds to IF secreted from the gastric wall (36).

The IF-cobalamin complex is taken up by receptor-mediated endocytosis by binding to receptors at the apical brush border of enterocytes in the presence of calcium and at neutral pH; these receptors are most commonly found in the distal ileum. The ileal receptor consists of two proteins: cubilin (the product of the CUBN gene on chromosome 10p12.1) and amnionless (the product of the AMN gene on chromosome 14q32) and is called “cubam” (37). Cubilin receptors can also bind to ligands other than IF, such as transferrin and vitamin D-binding proteins (38).

Intrinsic factor is not resistant to lysosomal enzymes, so it has now completed its mission in the terminal ileum. Free cobalamin is then bound by TC inside or outside the cell (39). The transcobalamin-cobalamin complex is then recognized in the liver and other organs by an as yet unknown receptor structure, which is predicted to weigh 58-kDa (40).

Transcobalamin is the main carrier of cobalamin in plasma, and the level of transcobalamin-cobalamin (holotranscobalamin (holo-TC)) is an indicator of cobalamin status (41). A significant amount of cobalamin is also bound to haptocorrin in plasma. Haptocorrin-bound cobalamin is a much less dynamic cobalamin pool, and the biological significance of haptocorrin in the cellular supply of cobalamin is unclear (38).

High cobalamin intake with food or non-food supplements (eg 5-10 μg) exceeds the capacity of IF receptors, and excess free cobalamin is taken up into the cell with an efficiency of about 1% by nonspecific diffusion alone (9).

About two-thirds of the cobalamin secreted in the bile is recovered during this process by the enterohepatic circulation. Approximately 1.5 μg of cobalamin bound to haptocorrin and varying amounts of cobalamin analogues are excreted daily via bile (42). Pancreatic proteases degrade biliary haptochorrin. While the released cobalamin is reabsorbed in the ileum by binding to IF, other free analogues that cannot bind to IF are excreted via feces (43).

The amount of cobalamin filtered daily by the renal route is approximately 1.5 μg (44). Under normal physiological conditions, small amounts of cobalamin are detected in the urine because more than 99.8% of it is reabsorbed in the proximal tubules (45).

Cubilin and megalin receptors are expressed in the proximal renal tubule, filtered holo-TC is reabsorbed by megalin (46). Unbound cobalamin is not normally found in plasma, but free filtered cobalamin cannot be reabsorbed in the proximal tubule if ingested by parenteral injection (9). Particularly when the overall cobalamin load in the body is high, significant amounts of cobalamin seem to be recirculated through this renal pathway, and the kidneys thus act as a short-term storage organ for cobalamin (47).

Cobalamin is also efficiently transported across the placenta during pregnancy, and the concentration of cobalamin is higher than in the maternal circulation, even in cases of maternal cobalamin deficiency (48).

Maternal-fetal cobalamin transfer favors the fetus even if maternal stores are limited, and although cord blood cobalamin levels exceed maternal serum levels, maternal serum cobalamin levels are found to be falsely low during pregnancy (9). While the mean liver cobalamin content of term newborns was 27.3 μg; total liver content is only 10.7 μg in premature neonates and 4.1 μg in fetus (49).

Cobalamin storage in adults is 2-5 mg and is mostly stored in the liver. Total body cobalamin stores in newborns are approximately 50 μg, and the ratio of stores to daily need is 1000/1 in adults and decreases to 100/1 in newborns (9).

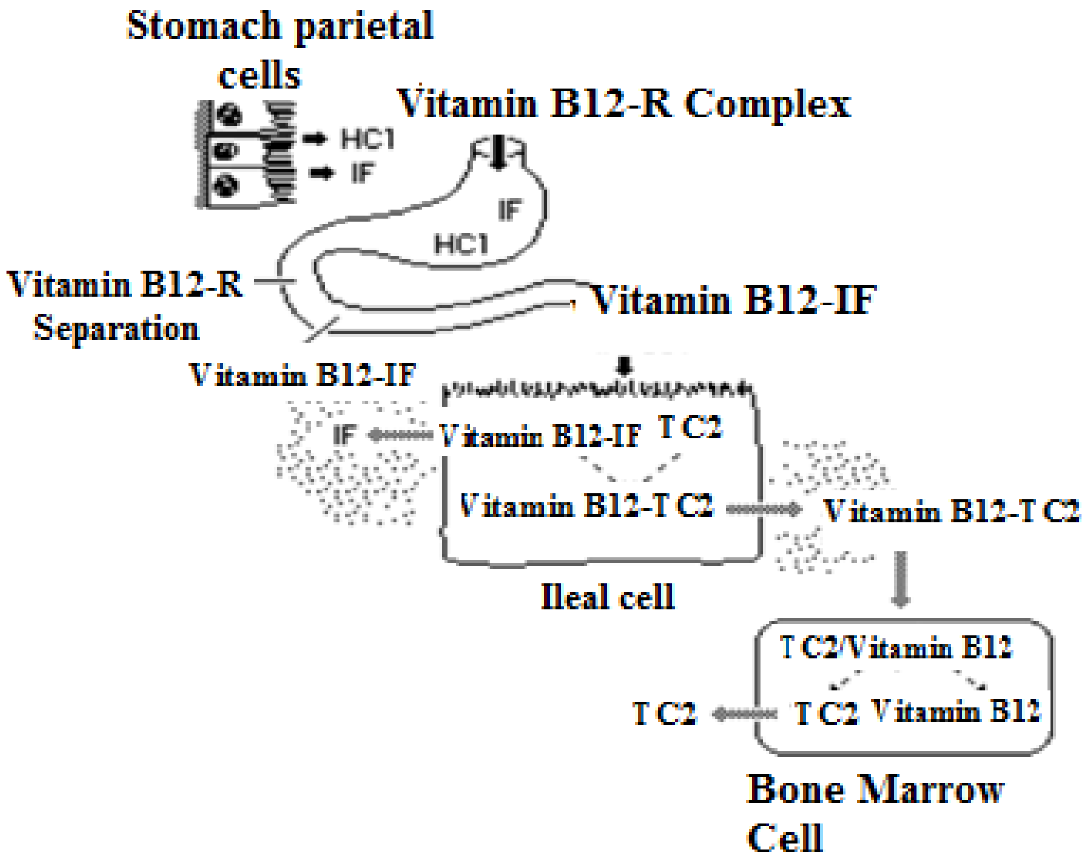


Figure 2.5. Vitamin B12’nin Emilimi (50)

2.2. Encapsulation Technology and General Features

Encapsulation is an important technology that enables the materials used as solid, liquid and gas to be released in the desired environment at a certain speed under the control of the capsule in a certain period of time, by covering them with various materials and encapsulating them in a capsule, preserving very small substances or the entire composition. The substance or mixture that is coated by being placed in a microcapsule is expressed as "fill", "core" or "inner phase". The outer wall is called “membrane”, “shell”, “covering” or “wall” (51). Particles are classified as “nano particles” when their size is below 1 µm, “microparticle” when they are between 3-800 µm, and “macro particles” when they are larger than 1000 µm (52,53).

The encapsulation process is a technology that can be applied in many different fields such as food, biotechnology, veterinary, agriculture, feed, medicine and pharmacy and is developing rapidly day by day (54). The use of microencapsulation technique in the food industry has a long history. Especially in recent years, with the rapid increase in the importance of functional foods, microencapsulation technique has started to gain importance for the food industry (55). This technique is mostly evaluated in the food industry for coating liquid droplets, gaseous components or solid particles with coating materials close to a food grade (56).

Substances that are successfully microencapsulated include enzymes, flavor compounds, fats and oils, color components, minerals and vitamins. The purpose of microencapsulation technology in the food industry is to protect the material to be coated against external factors (heat, humidity, light and air, etc.), prevent it from being lost by evaporation, protect its physical properties in the best way, facilitate the transfer of the material from one place to another by coating, where it is desired. It can be summarized as enabling it to be active at any time, preventing the taste and odor of the material to be coated, preventing it from interacting with different substances, keeping it homogeneous by diluting when it is desired to be used in small quantities (57).

Encapsulation technology works in principle on two pillars. By mixing in an active ingredient polymer solution and dispersing it in small droplets, the formed drops are mixed with techniques such as drying, cooling, gelation, coacervation. Solid powder particles are mixed in the fluidized bed, the coating solution formed is coated by spraying method or mixed by cooling or drying method (58). Coating of active components during encapsulation is done by applying different techniques. These different techniques are examined under 3 headings:

1. Physical processes: Spray drying, spray cooling, fluid bed coating and extrusion,

2. Physicochemical processes: Liposome entrapment and simple or complex coacervation,

3. Chemical processes: Molecular inclusion (entrapment within a structure) and interface polymerization (58).

Moisture control, protection against oxidation, sequential distribution of multiple active ingredients are provided, while loss of functionality, incompatibilities between the peel and active ingredient, unpleasant aroma, odor formation, deterioration of structure, loss of enzyme activity can be reduced or prevented by encapsulation or during storage. and the bioavailability of the components is increased (58).

2.2.1. Encapsulated Active Ingredients

Thanks to the encapsulation technology, many active ingredients can be encapsulated in the direction of the determined target, making them sheltered. The most preferred components in these process applications can be listed as follows (59):

- Antioxidants (polyphenols, tocopherol, flavonoids)
- Sweeteners, flavors, colorants, preservatives
- Organic acids
- Fatty acids (omega-3, conjugated linoleic acid)
- Essential oils
- Minerals and vitamins
- Proteins and enzymes
- Carotenoids (β-carotene, lycopene)
- Probiotics and prebiotics

Selection of the most suitable encapsulation method for the material to be encapsulated should be selected by making preliminary tests in accordance with the content structure and intended use. Desired properties should be based on consumer demands, nutritional value, or unknown new ideas for improvement in food processing and storage stability. Another important issue is to determine whether the encapsulation process is an ergonomic method that can provide the desired feature. Encapsulation technology is a suitable method for the preservation of functional properties as a result of the portability of the active material with a suitable food matrix structure (59).

- When the encapsulation method is preferred in obtaining the desired properties, the method design should be done first. According to this;
- Physicochemical properties of the active material,
- Which process conditions will be used during the production and processing of the applied foodstuff,
- How long the material to be encapsulated will be stored before use,
- What will be the storage conditions of food products containing the encapsulated substance before consumer use,
- What particle size and density will be in the food products,
- Calculation of the total process cost,
- Selection of coating material,
- Selection of the coating method,
- Considering its applicability to legal regulations, the design should be completed in terms of obtaining a capsule product (59).

2.2.2. Coating Materials Used in Encapsulation

The composition of the carrier system is the main factor determining the functional properties of the final product. The properties that an ideal coating material should have can be listed as follows (60):

- It should be able to perform the encapsulation process in such a way that it affects the functional agent in the desired ratios and it should be able to trap it well.
- Its rheological properties should be very useful in high concentrations and it should be easily processed during the encapsulation process.
- It should be compatible with the solid or liquid food matrix around it, and should not adversely affect the appearance, taste, shelf life and rheology of the obtained material.
- In order for the encapsulated material to remain active, it must be protected from chemical degradation. It should be able to control the release of the encapsulated material.
- It should not adversely react to the bioavailability of the encapsulated material.

- In addition to its low cost, it should be able to be dissolved in the desired solvent.
- It should be resistant to environmental impacts that may occur during production, storage, transportation and processing.

It is difficult for a coating material to fulfill the mentioned properties alone. For this reason, it is recommended to apply different coating materials together. There are also modified coating materials (for example, modified cellulose) with better physical and mechanical qualities (61). In the encapsulation method, mostly carbohydrates such as maltodextrin, sucrose, starch and maltose; Proteins such as whey proteins, gelatin, caseinates and casein, gums such as carrageenan and gum arabic are preferred as coating materials. These materials used as coating agents are described in groups in Table 2.2 (62).

Table 2.2. Coating materials used in the encapsulation of food ingredients

arbohydrates	işasta, maltodekstrinler, mısır şurubu, dekstran, sukroz, siklodekstinler
ums	arob gum, carrageenan, alginate
ats/lipids	eeswax, parafilm, beeswax, tristearic acid, diglyceride, monoglyceride, fats ıd oils
oteins	luten, casein, gelatin, albumin, hemoglobin, peptides
elluloses	arboxy methylcellulose, methylcellulose, ethylcellulose, acetylcellulose, llulose acetate-phthalate

2.2.2.1. Carbohydrates

It is generally used as a coating material or carrier (carrier) in encapsulation processes made from carbohydrates by spray drying method. Carbohydrates not only cause spherical and smooth microcapsule formation, but also increase the bond strength between the coating material and the core material (63). During the microencapsulation process, macromolecule carbohydrates such as corn syrup powder, starch and maltodextrin are mostly preferred. These materials are suitable coating materials due to their low viscosity and good solubility even at high concentrations. In addition, they are preferred as coating materials due to their affordability and widespread use in the food industry. However, due to the lack or low emulsifying properties, they are preferred to be used together with proteins rather than being used alone in the microencapsulation process (63).

Starch is a polysaccharide that can carry many biologically active substances and is formed by the glycosidic bonding of many glucose molecules. It has a wide range of applications because of its low cost, easy to use, biodegradable and biocompatible materials. It consists of starch, amylose and amylopectin. Amylose is a linear molecule composed of D-glucopyranose linked by α-(1-4) bonds.

The pancreas is resistant to amylase, but can be degraded by enzymes in the colon (64). Amylopectin is a branched glucose molecule composed of an α- (1-4) glycosidic bond and an α- (1-6) glycosidic bond. Modified starch can be used for this purpose, although native starch is not a suitable carrier for controlled release as it causes rapid release of biologically active components (51).

Starch can be modified by processes such as etherification, esterification or acidification to make it resistant (64). Resistant starch is starch that cannot be broken down and therefore digested by pancreatic enzymes in the small intestine. Resistant starch reaches the colon and is fermented there. The use of probiotics in a delivery system targeting the colon has been reported (64). With appropriate modification, it can effectively prevent colon cancer, lower cholesterol, and reduce cardiovascular disease (51). If starch derivatives are used for the controlled release of biologically active ingredients, they should be designed so that they are not digested in the upper digestive tract (antacid and enzymatic degradation), but fermented in the colon (511).

Pullulan reduces oxygen permeability by forming a strong membrane. It can be used as a coating for oxidation sensitive microencapsulation compounds. In some studies, pullulan was used as a dye. In the study of the degradation kinetics of encapsulated saffron carotenoids, pullulan was chosen as one of the coating materials (65). In addition, in the study of water/oil/water type microcapsules prepared by single-droplet drying method and changes in encapsulation efficiency of hydrophilic materials during storage, pullulan was chosen as one of the coatings. In this study, it was stated that the microencapsulation efficiency provided by amylopectin was approximately 95% (66).

It is used for coating sucrose, maltose, glucose, lactose, volatile compounds. The permeability of the surface film they form at low moisture content is very low. Therefore, they are highly resistant to diffusion. Various studies have observed that lactose is preferred as the coating material. In a study using a whey protein-based dye and lactose mixture, ethyl butyrate and ethyl caprylate volatile compounds were microencapsulated by spray drying (67).

Maltodextrin is a substance obtained by partial hydrolysis of maize starch by acid or enzymatic methods, and its glucose equivalent: DE is 20 or less than 20 (68). Under high temperature (100 °C) and pH 4.0-5.0 conditions, starch is decomposed into its monomers by α-amylase, thus yielding maltodextrin and corn syrup. Maltodextrin, with its low DE value, is used as a fat replacer in some foods (such as ice cream). Maltodextrins are surfactants and their solutions have low viscosity. However, it is not recommended to be used as an emulsifier. Maltodextrin reduces the rate of the Maillard reaction during microencapsulation of oils, vitamins, minerals and colorants. In the microencapsulation process, maltodextrins are often used as auxiliary coating materials due to their low emulsification properties. Maltodextrin has good oxidation stability in microencapsulated oil (51,69).

2.2.2.2. Gums

Hydrocolloids, generally known as water-soluble gums, are polymeric carbohydrates obtained from various biological sources and subjected to various purification processes and mainly composed of soluble fibers (70). Most of the hydrocolloids form a group of anionic or neutral complex and branched heteropolysaccharides containing calcium, potassium, magnesium and sometimes other metallic cations combined in the molecule (71).

Alginate is one of the most used coating materials in encapsulation applications and is obtained from brown algae such as Laminaria hyperborean, Ascophyllum nodosum and Macrocystis pyrifera. Commercial alginates occur mainly as salts of Mg^{2+}, Sr^{2+}, Ba^{2+} and Na^{+} cations (72). Sodium alginate is a water-soluble salt of alginic acid, a linear natural polysaccharide containing α-Lguronic acid and β-D-mannuronic acid (73). It is known that in an aqueous environment, sodium alginate will form gels and gel spheres in the presence of divalent ionic cations (such as Ca, Zn, Sr^{2+} and Ba^{2+}) (51,72). Figure 2.7 shows the chemical structure of the sodium alginate molecule (74).

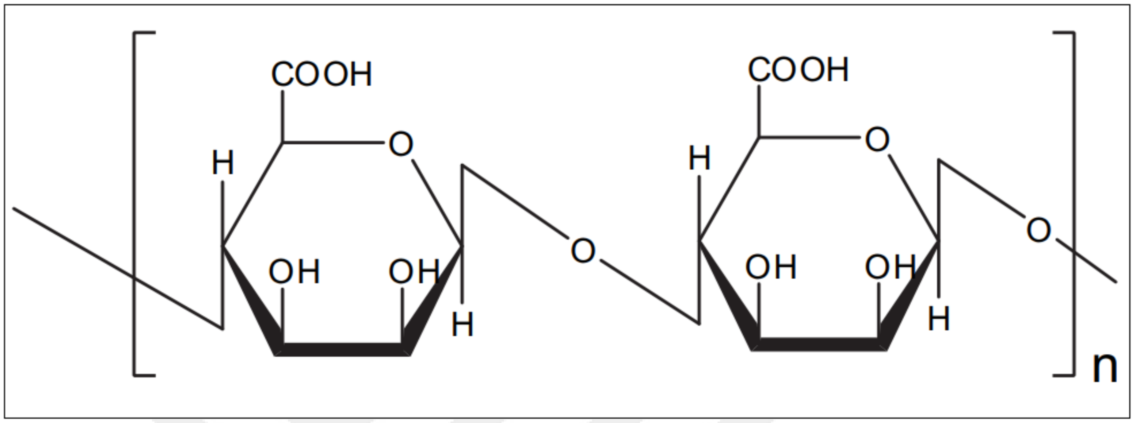


Figure 2.6. Structure of sodium alginate (74)

The physical properties of alginate depend on its composition, molecular weight and chain length. Alginate is also used as a thickener, emulsifier and stabilizer in the food industry as well as its gel forming feature. The ratio of guluronic acid to mannuronic acid (G/M) varies from species to species, providing different mechanical stability (64). The G block is wrinkled and the M block has a wide stripe shape. When two G-block sites are arranged side by side, they form a crystalline cavity of size and structure to which calcium ions can bind (72).

Bioactive drugs, enzymes, proteins, etc. Calcium alginate particles are often preferred for their controlled release. Alginate calcium spheres in "eggbox" form are obtained by cross-linking, by replacing the sodium ions of gulonic acid contained in alginate with diionic cations such as calcium. Although this alginate matrix is stable at low pH and partially resistant to gastric acidity, it swells and disperses in a high pH environment (such as the small intestine) (75). In the study of enrichment of probiotic yogurt with encapsulated iron, ferrous sulfate (Fe^{+2}) was selected using sodium alginate emulsification technology. It was made using the sodium alginate:starch (2:0.05) extrusion method to encapsulate Lactobacillus acidophilus bacteria. Iron supplements are used in two types of products, yoghurt with and without probiotic capsules. When the effect of iron supplementation on unencapsulated probiotic-containing yoghurt was examined, it was seen that there was no effect on the number of probiotics during storage. It was found that the thiobutyric acid (TBA) numbers of the samples containing unencapsulated iron were higher than the others (76).

Acacia gum, also known as gum arabic gum, is widely used due to its excellent emulsifying properties. Gum arabic contains D-glucuronic acid, L-rhamnose, D-galactose and L-arabinose and approximately 2% protein. The emulsifying properties of gum arabic are related to the protein in its structure. Due to its high solubility, low viscosity, emulsifier and good retention of volatile components, it is widely used as a coating in the microencapsulation process. In research on the encapsulation of cardamom essential oleoresin, gum arabic is said to be a better coating material than maltodextrin and modified starch (77). In another study, gum arabic was found to be a good coating material in the spray drying encapsulation process of fennel oleorin (78). The use of gum arabic in the encapsulation of oils is very common. This is because gum arabic can form a stable emulsion with many different oils over a wide pH range. In the microencapsulation process of soybean oil in which glucose was used instead of 50% of the gum arabic used, the microencapsulation efficiency increased from 74% to 92% (79). However, another study found that gum arabic encapsulated in 5 different monoterpenoids (citral, linalool, monene, and pineene) failed to perform effectively (80). The semi-permeability of the membrane formed with gum arabic only limits its oxidation resistance to a certain extent. For this reason, oxygen transfer occurs and directly affects the shelf life of the core material negatively. In addition, its use in the food industry is limited due to the high price of gum arabic. Another study found that a blend of 60% gum arabic and 40% legume encapsulated 93.5% of the orange peel oil. A recent study showed that a mixture of 40% cardamom gum and 60% maltodextrin can encapsulate 84.6% of cardamom oil (81).

Chitosan is a natural linear cationic polysaccharide obtained by deacetylation of chitin, which is abundant in the shells of shellfish, insect epidermis and cell walls of some fungi (82). Along with alginate, chitosan is one of the best coating materials for food applications and drug delivery systems due to its biocompatibility, low toxicity/non-toxicity and biodegradability (83). The ratio of N-acetyl-D-glucosamine to D-glucosamine is used to determine the properties, biodegradability and biological effects of chitosan. Due to its cationic nature, chitosan can be used as a fat binder to lower cholesterol and weaken it. Chitosan is soluble at acidic pH but stable at high pH. The pH-sensitive swelling/growth mechanism involves the protonation of amine groups in chitosan under low pH conditions. This protonation can lead to chain repulsion, proton diffusion, penetration of opposite charges and water, and destruction of the secondary structure (72).

K-carrageenans consist of sulfated and unsulfated α- (1, 3) and β- (1, 4) polysaccharides. Due to its gelling feature, it can be converted into spiral form according to temperature, ionic strength and biopolymer concentration. In addition, it has the function of interacting with certain milk proteins, such as forming complexes with casein micelles (84). To encapsulate probiotic cells, cells can be added to the polymer solution at 40-50°C and gelled by cooling the mixture to room temperature (85).

Pectin is a linear polymer of plant origin and consists of β-(1-4) linked D-galacturonic acid and 1,2-linked L-rhamnose units. The percentage of carboxyl groups indicates the degree of esterification of pectin. The degree of esterification of low methoxylated pectin is less than 50% and can form a gel with calcium. It is suitable for the target transport system as it does not cause deterioration in the stomach and small intestine. Due to its high water solubility, unmodified applications are limited. Therefore, it is often combined with cations or polymers (such as chitosan) for slower degradation (64).

2.2.2.3. Proteins

Protein is a good coating material for the microencapsulation process due to its functional properties. Protein is especially good at combining flavor components. In general, gelatin, whey protein, casein and caseinate are preferred as coating materials in the microencapsulation process.

Gelatin is a good coating material microencapsulated by spray drying due to its good emulsifying properties and film-forming effect, high water solubility and edibility. The mentioned properties and morphology of gelatin particles can be further improved by adding the sugar alcohol mannitol (63).

In microencapsulation studies of some *Lactobacillus plantarum* species (*Lactobacillus plantarum* 299v, *Lactobacillus plantarum* 800 and *Lactobacillus plantarum* CIP A159), whey protein is used as a coating material together with alginate to increase coating effectiveness. In the inactivation test of artificial gastric juice and intestinal juice, the release of bacteria in the alginate matrix was investigated by opening the crosslinks in the lyophilized pellet. The results of this study reveal that the researchers' whey protein is a suitable, inexpensive and effective coating material for coating alginate beads containing bacteria (86).

Casein and caseinate are the most important parts of milk protein found only in natural milk and make up about 80%. There are many studies in which sodium caseinate is used as a coating material in the packaging process. Casein and caseinate are more heat sensitive than whey protein, but due to their high surface activity, they are used as emulsifiers in many applications, including spray drying (86).

2.2.3. Encapsulation Methods

2.2.3.1. Spray Drying

Spray drying is the most widely used and oldest method in encapsulation technology. This method is frequently used in the food industry due to its low cost, convenience and durability. With this method, high quality particles can be obtained. Starch, maltodextrin and gum are used as carrier materials of the method (87). Alginate, guar gum, chitosan and other polysaccharides, sodium caseinate, whey protein, soy protein and other proteins can also be used as coating materials for spray drying. Their disadvantages are low water solubility and high cost (88). After the material to be coated with the carrier system is homogenized, the water in contact with the hot air of the atomized rotating head is evaporated, and it is sent to a spray dryer to dry the capsules. Capsules in the range of 10-100 µm are obtained. The disadvantages of this method are that the water-soluble coating materials used are limited and heat-sensitive bioactive materials can easily degrade at high temperatures (87). The basis of spray drying is to dry the droplets in contact with the hot air stream through the atomization process of the solution or dispersion containing the active ingredient to be coated. The resulting particles are generally spherical and their topology depends on the polymer used. The release profiles and retention of molecules can be optimized by varying the type and length of the polymer (89).

Although studies show that spray drying is not suitable for heat sensitive biologically active materials (oil, etc.), shorter contact time in the dryer can minimize this effect (90). The size of the atomized droplets depends on the viscosity and surface tension of the liquid, the spray rate, and the pressure drop in the ejector. The material used as a carrier should be able to protect the active substance with its high water solubility, molecular weight, glass transition, crystallinity, diffusion ability, good film forming ability, good emulsification performance and low cost (59).

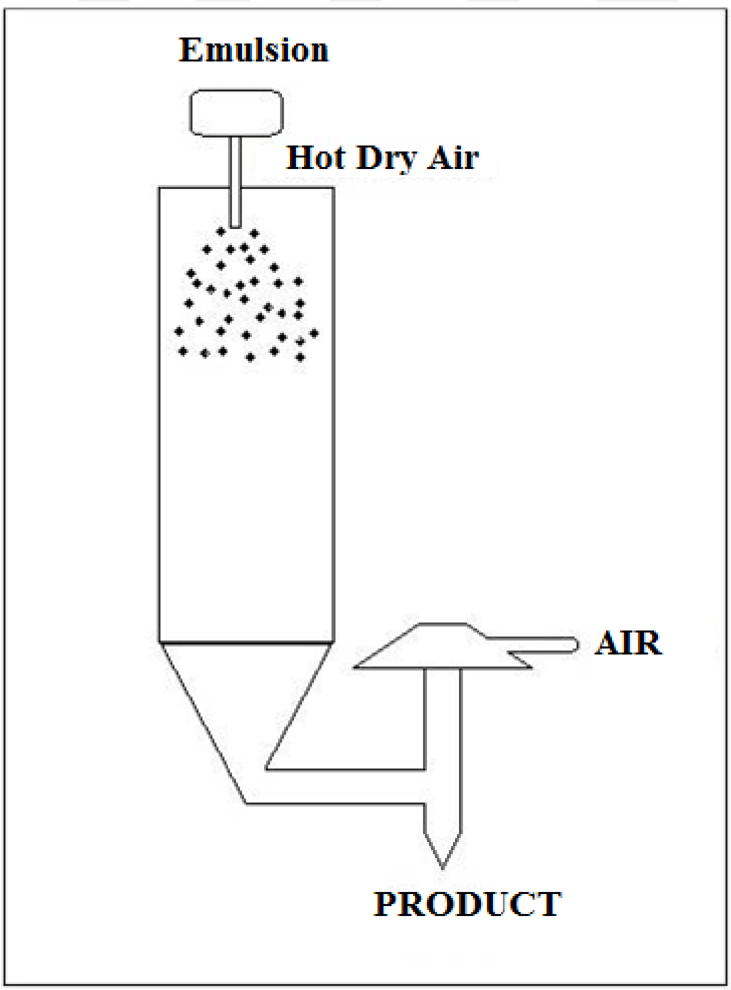


Figure 2.7. Spray Drying System (59)

2.2.3.2. Coacervation

Coacervation is the phase separation of one or more hydrocolloids from the initial solution from the newly formed coacervate phase suspended or emulsified in the same reaction medium surrounding the active ingredient. Only colloids (eg gelatin) can be used in this method, or a combination of gelatin and gum arabic can be used in more complex processes (87). Milk protein can be used instead of gelatin, negatively charged molecules such as pectin, carrageenan, carboxymethyl cellulose, pectin, carrageenan, alginate or polyphosphate can be used instead of gum arabic (59). Although coacervation is an expensive encapsulation method, it can be used to encapsulate highly perishable biologically active substances such as polyphenols (87). Although coacervation is widely used for the encapsulation of essential oils, it is also a useful method for fish oils, vitamins, preservatives and enzymes. Although this method has advantages over other encapsulation methods, it has some issues that are restricted by laws and regulations in many countries, such as expensive commercialization, complex processes, and the need to use glutaraldehyde as a secondary containment material (88).

While only one type of polymer is used for simple coacervation, two or more oppositely charged polymers are used for complex coacervation. coacervation; It is triggered by environmental factors such as heating, adding salt, which affect the solubility of polymers, or the use of oppositely charged polymers (89). Complex coacervation is achieved by adjusting the pH to 7 to 4 in a mixing bowl at 35°C and dissolving it in the water phase with an oil/water emulsion containing varying proportions of gelatin, acacia and 2-4% of each polymer. In this way, an immiscible three-phase system (oil phase, polymer-rich phase and polymer-poor phase) is obtained. As an alternative to complex coacervation pH adjustment, the oil emulsified in gelatin solution can be diluted with water to emulsify and gum arabic can be added to the mixture. When the temperature is lowered, the gelatin solidifies and forms a crust. Polymer concentration, pH, ionic strength, emulsion size, system turbulence and temperature are the factors affecting the coacervation preparation process (590). The encapsulation efficiency of this method is 60% and the size of the coated capsule is in the range of 30-100 nm (90).

2.2.3.3. Emulsification

The emulsion is obtained by dispersing two immiscible and kinetically stable phases (oil and water) into each other in small droplets. High speed mixers and homogenizers are devices used to produce emulsions. Water-soluble components may be encapsulated in a water/oil emulsion or dual emulsion (eg, water/oil/water). An oil/water emulsion changes the volume of the water phase, thereby affecting the flavor by changing the concentration of flavor molecules in the water. Beta-carotene, lycopene, sterols and fat-soluble vitamins can be added to foods in powder form by forming an oil/water emulsion or drying by spray drying and freeze drying methods. In addition, it is part of other encapsulation methods such as emulsification, extrusion and coacervation (89).

Droplets with a diameter in the range of 0.1-100 μm are usually obtained. The system in which oil droplets are dispersed in the water phase is called oil in water (oil/water) emulsion, while the system in which water droplets are dispersed in the oil phase is called water in oil (water/oil) emulsion. It is also possible to obtain a multi-layered emulsion form. In addition, some emulsifiers can be used to increase the stability of the emulsion (87). The initial droplet concentration and size distribution depend on the emulsifier used to stabilize the droplets. Emulsifier selection is important to control environmental responses (pH, temperature, enzyme activity and ionic strength), charge and thickness interface properties. The nanoemulsion is in the range of 10 nm to 100 nm, while the droplet diameter of the conventional emulsion is in the range of 100 nm to 100 μm. It has been stated that the bioavailability of nanoemulsions is high. Because nanoemulsions are more stable than conventional emulsions, they have a clearer appearance, a higher active ingredient release rate, a larger surface area, and a high rate of chemical reaction (60).

2.2.3.4. Freeze Drying

Freeze drying, also known as lyophilization, is a method used to dry heat-sensitive ingredients and minimize flavor loss. For this purpose, an aqueous solution or emulsion of the carrier and active substance is prepared. This mixture is then frozen. Water is then removed from the frozen structure by sublimation under reduced pressure (usually <2 Pa).

In this way, the active ingredient and the carrier material are water-soluble and a freeze-dried product with a porous structure is obtained. The disadvantages of freeze-drying are poor barrier properties due to the open-hole structure of the capsules, high energy consumption, long processing time, and a relatively expensive method (89). However, water-soluble components are preferred because it is a simple technique for encapsulating drugs, despite the need for general natural flavors, and heat-sensitive biologically active substances are well preserved (87).

2.2.3.5. Dropping (Ionic Gelation) Method

This method, which is an economical and practical method, is generally preferred in laboratory applications and is not suitable for the production of large quantities of products. In this method, the polymer solution and the active material are homogenized and released into the dispersed phase (eg $CaCl_2$) to obtain spherical gel particles via a syringe. The drip method is a method for obtaining calcium alginate particles. Crosslinking; The cross-linking mechanism between calcium and sodium ions is based on the substitution of calcium in the CaCl2 molecule and sodium in the sodium alginate molecule as shown in Figure 2.8. The calcium-alginate cross-linking mechanism is shown in Figure 2.8 (91). Gel formation is observed by dropping the biopolymer solution into another solution. Particles can be obtained by dripping the medium (hot gelling) or the gelatin solution into a cold liquid (cold gelling) (92). The appearance of the drip system is shown in Figure 2.9 (93).

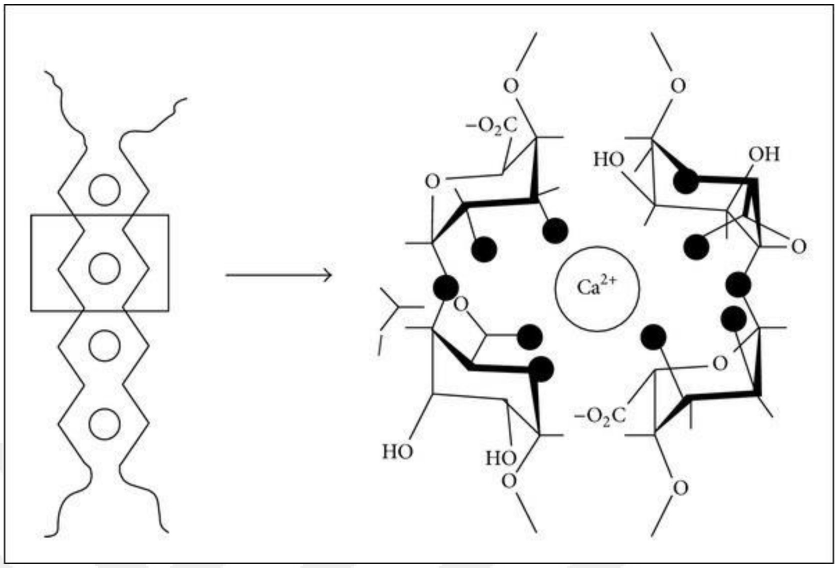


Figure 2.8. Calcium-alginate cross-linking mechanism (91).

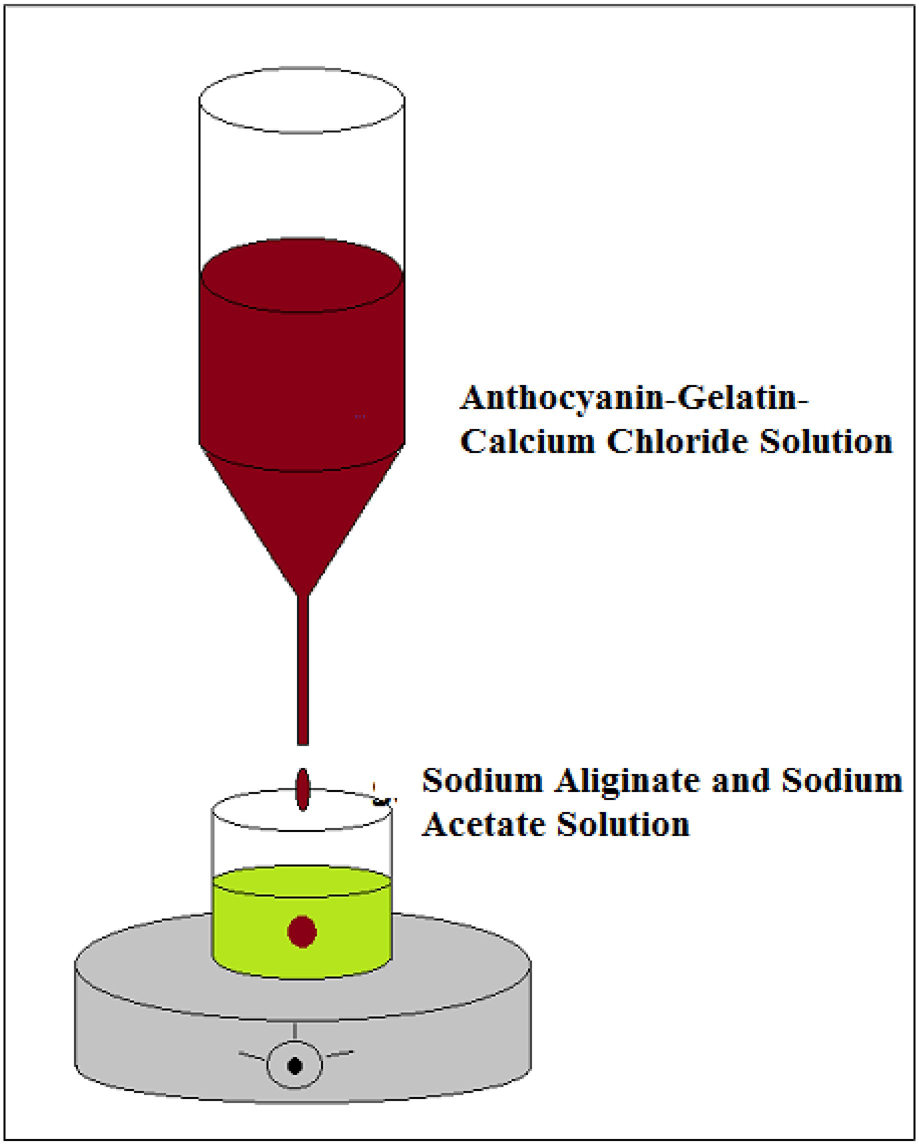


Figure 2.9. Dropping system (93).

It is generally preferred because of the positive properties of this method, such as very easy to manufacture in laboratory scale, hydrophilic or hydrophobic, heat sensitive, fluid or viscous, solid or liquid. Almost all components can be encapsulated by this method. Also, while it is easy to obtain capsules at low scale, it can be difficult and expensive at large scales. The resulting capsules are very porous and well suited for diffusing water or other liquids through the inlet and outlet of the alginate matrix (89). The oppositely charged chitosan of the negatively charged alginate forms a semipermeable membrane, making the surface of the capsule smoother and having lower permeability to water-soluble molecules (94).

Non-spherical and multi-directional scattered spheres are formed, the size of which cannot be controlled by simple dripping techniques. However, by applying the drop coating technique, monodisperse and spherical microspheres can be obtained. Despite this shortcoming, simple dripping techniques are generally preferred due to the ease of application. In the production of calcium alginate particles, the preferred concentration of alginate is in the range of 0.6-3% and the concentration of calcium chloride solution is 0.05-1.5 M. The size and shape of the capsule produced depend on the diameter of the syringe, the vibration system, the viscosity of the alginate solution and the distance between the syringe and the $CaCl_2$ solution. Whether the relationship between chains (bonds) is permanent or temporary depends on the calcium content in the system. When the calcium content is low, the bonding becomes transient while a high viscosity thixotropic (viscosity increases rapidly) solution is obtained. Precipitation or gel formation is observed at higher calcium levels due to the persistence of the web. Chemical structure is highly influential on functional properties such as molecular size, gel formation kinetics, cationic porosity, swelling behavior, biodegradability, stability, and gel forming ability (72).

The use of alginate alone in ion gelling results in porous, open mesh particles that result in a low encapsulation capacity for low molecular weight and water-soluble drugs (73). Coating these particles with other polymers (such as chitosan) plays a role in improving shelf-life stability and half-lives in biological fluids. The alginate-chitosan complex can be formed by ion gelation, in which the amine group in the cationic chitosan molecule interacts with the carboxyl group on the anionic alginate molecule (95). This complex preserves the active ingredient in the capsule and limits its release, making it more effective than a system using alginate or chitosan alone. Alginate-chitosan coated microbeads are used to encapsulate drugs, oligonucleotides, proteins and cells (96).

2.2.3.6. Cocrystallization

A new coating method, which is a combination of sucrose matrix and core material, is cocrystallization. The sucrose syrup is adjusted by concentrating to supersaturation and using temperature to prevent crystallization. A predetermined amount of core material is added to the vigorously mixed concentrated syrup. With the help of the mixing process, the encapsulation process takes place by intertwining the sucrose and the core material (97).

2.2.3.7. Liposome Arrest

Due to the dispersion of phospholipids in an aqueous medium, a layer of lipid biomolecules called liposomes or vesicles is formed. Liposomes can selectively penetrate ions like natural membranes (98). There are three types of liposomes and they are defined as multiple vesicles, single vesicles and macro vesicles, respectively. While liposome capture methods and microencapsulation processes were used more widely in the pharmaceutical and cosmetic industries compared to the food industry, they have become suitable for the food industry with the development of technology. Libosomes have been successfully used to encapsulate enzyme systems to increase the ripening rate of cheese. In one study, an encapsulation process was performed to extend the shelf life of vitamin C. The half-life of pure ascorbic acid stored at 4°C is 18 days, but this time has been increased to 100 days by liposome encapsulation (99). Liposomes are also used to increase the nutritional value of dairy products. According to reports, liposome treatment preserved the encapsulated vitamin D added to cheese and cream (95). In addition, it has been reported that the addition of ferrous sulfate liposomes to milk prolongs the bitterness and metallic taste formation time during storage. In a study, soluble and stable negatively charged nanocomposites were obtained by adding negatively charged pectin to a positively charged medium with a lower isoelectric pH value than lactoglobulin, and these composites were used. These compounds were also used in the encapsulation of docosahexaenoic acid, and it was determined that the β-lactoglobulin-pectin electrostatic nanocomposite encapsulating DHA molecules showed very good colloidal stability and had a particle size of about 100 nm (100).

In a study, soluble and stable negatively charged nanocomposites were obtained by adding negatively charged pectin to a positively charged medium with a lower isoelectric pH value than lactoglobulin, and these composites were used. These compounds were also used in the encapsulation of docosahexaenoic acid, and it was determined that the β-lactoglobulin-pectin electrostatic nanocomposite encapsulating DHA molecules showed very good colloidal stability and had a particle size of about 100 nm (100). In another study, the effects of microemulsion composition, polymer concentration and complex aggregation conditions on the performance of nanocapsules were investigated. By using oil-in-water microemulsions, agglomeration of gum arabic and gelatin complexes, nanocapsules containing cypermethrin pesticides insoluble in water and smaller than 100 nm in size were obtained. The encapsulation efficiency determined in this study was approximately 60% (101).

2.2.3.8. Encapsulation Studies with Ionic Gelation Method

In a study, purified lycopene was microencapsulated by ionic gelation method using syringe pump-extrusion system. Different ratios of sodium alginate were used as coating material and different flow rates were tested. Shapes and size distributions of the capsules were determined by optical microscope and scanning electron microscope images. The obtained microcapsules were added to the cake mix selected as model food and it was determined that they were stable in the product (102).

In another study, anthocyanin extract obtained from mulberry was mixed with alginate solution in the ionic gelation method and homogenized, and then this mixture was dripped into calcium chloride solution with chitosan with the help of a syringe and capsules were obtained. Accordingly, while the calcium molecules in calcium chloride and sodium molecules in sodium alginate form crosslinks, an ionic bonding was detected between the oppositely charged chitosan and alginate (58).

2.3. Polymers

Long chain structures formed by small molecule structures called monomers coming together are called polymers, and this phenomenon is called polymerization (103).

For a substance to be considered a polymer, hundreds of monomers must combine to form a large molecule. A polymer (macromolecule) is a large molecule consisting of simple and small repeating units. These simple and small chemical units that are repeated in polymer molecules are called “repeated units (mers)”, and substances with small molecules and organic structures that are applied in the first place to obtain polymers are called “monomers”.

If the units of the macromolecule are the same, this type of polymer is called "homopolymer". If the polymer consists of a combination of two or more monomers, then it is expressed as "copolymer" (103).

The main group that makes up the polymers is in different numbers depending on the polymer type. Apart from this, these groups form a linear, branched or cross-linked structure. The number of groups in molecules is called the degree of polymerization. The molecular weight of the plastic material is calculated by multiplying the group molecular mass and the degree of polymerization. The molecular weight of the majority of polymers is generally between 10,000-100,000. All polymers have starting monomers. The monomers that make up the polymers undergo various structural changes during the transition to the main group. The materials obtained as a result of polymerization are in their natural color. Apart from this, they are put on the market by forming films, sheets, blocks, profiles or various shapes for their ease of use and purposes, which are specially colored (104)..

$$nCH_2 = CH_2 \xrightarrow{\text{Polymerization}} (\text{-}CH2\text{-}CH2\text{-})^n \quad (1)$$

In Equation 1 written above, it is seen that polyethylene containing (n) monomers is obtained by polymerization of ethylene monomers. Polymers are divided into two groups as natural and synthetic polymers. Polymers used in industrial applications are generally synthetic polymers.

When we look at the structure of polymers, there are three different structures: homopolymer, which is formed by the repetition of a single monomer, copolymer formed by the polymerization of two different monomers, and thermopolymer, which is formed by the repetition of three different monomers. When we look at the areas where polymers are used, fibers are divided into three as coatings and adhesives, while physically they are also divided into three as amorphous, crystalline and partially crystalline structures. The most used one during the classification of the polymer is the classification made according to the processing method. Accordingly, polymers are divided into two as thermoplastic and thermoset (105).

2.3.1. Intelligent Biopolymers

One of the most important properties of biopolymers is that they swell in the presence of water and shrink in the absence of water. However, the swelling and shrinkage capabilities of biopolymers should not be considered as the only parameter in conducting studies on biopolymers. Recently, new biopolymers that can respond to a signal (swelling, shrinking, deteriorating, etc.) by attaching some functional groups to biopolymers have been prepared by many researchers. The hydrophilicity of the polymeric chain and the crosslink density are the factors controlling swelling. The presence of hydrophilic groups such as hydroxyl, carboxyl, carbonyl, amine and amide, which have the ability to make hydrogen bonds in the main chain or side branches, is necessary for a polymer to show biopolymer properties. By adding stimulant-reactive comonomers to the network structure, biopolymers sensitive to the desired property can be prepared. Superabsorbent biopolymers are smart polymers made for this purpose. Superabsorbent biopolymers can be prepared by forming pores in the biopolymer structure, or by attaching suspended groups to the biopolymer or by reducing the biopolymer dimensions. The ability of a polymer to respond to external stimuli, similar to real systems, enables this polymer to be used in various applications. Such biopolymers are capable of swelling, shrinking, bending, and even fragmentation by undergoing structural changes. These biopolymers are called smart biopolymers or stimulus-response polymers. Examples of environmental factors that change the volume of biopolymers are pH, temperature, electric field, ionic strength and salts (106).

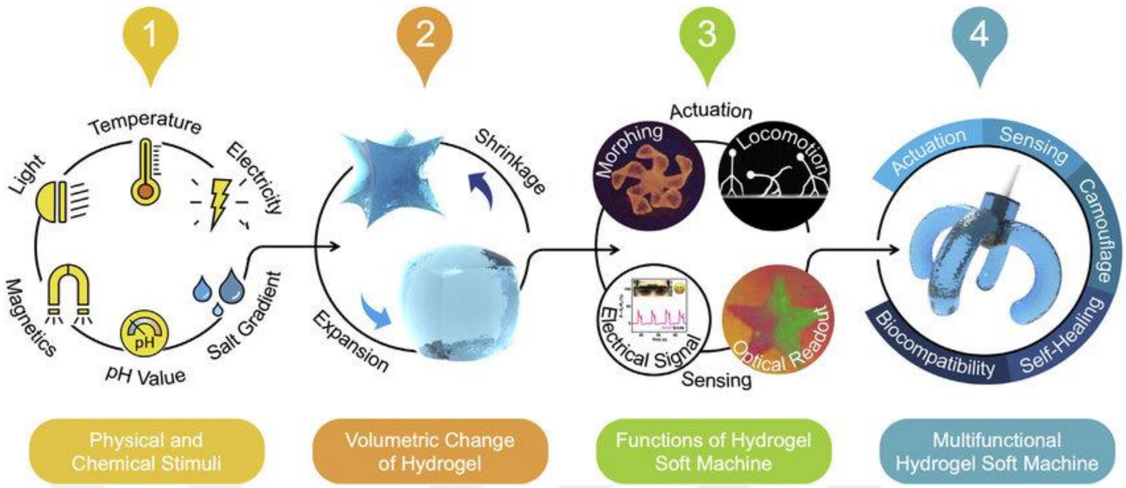


Figure 2.10. Schematic representation of stimulus sensitive biopolymers

2.3.1.1. pH-Sensitive Biopolymers

pH-responsive biopolymers contain side groups and ionizable groups in the cross-link that accept or donate protons in response to environmental pH (Figure 2.11). In this case, changing the pH across the pKa threshold of the polymer causes a rapid change in the net charge and hydrodynamic volume of the polymer chains. In other words, the presence of a high-loaded structure causes the mesh to swell as a result of high-load repellency and the biopolymer to change from collapsed to swollen state. The osmotic pressure created by the presence of mobile counterions also helps to explain the increase in the hydrodynamic volume of the biopolymer network chains. It is particularly interesting to exploit pH differences in the human body (for example, throughout the gastrointestinal tract) to achieve delivery of a drug to the targeted site using polymers that respond to pH changes. In addition, in anti-cancer drug delivery, the release of drugs can be triggered by the acidic extracellular pH of tumors; this contributes to higher efficacy and lower toxicity to peripheral tissues. Poly(acrylic acid) (Figure 2.12) is generally used in drug delivery systems targeting the intestine. Due to the presence of ionizable -COOH groups, this polyelectrolyte structure is protonated so that it becomes uncharged at low pH and negatively charged at high pH. The presence of a protonated structure at low pH (as in gastric media) eliminates electrostatic repulsive forces and contributes to the formation of a tighter structure (106).

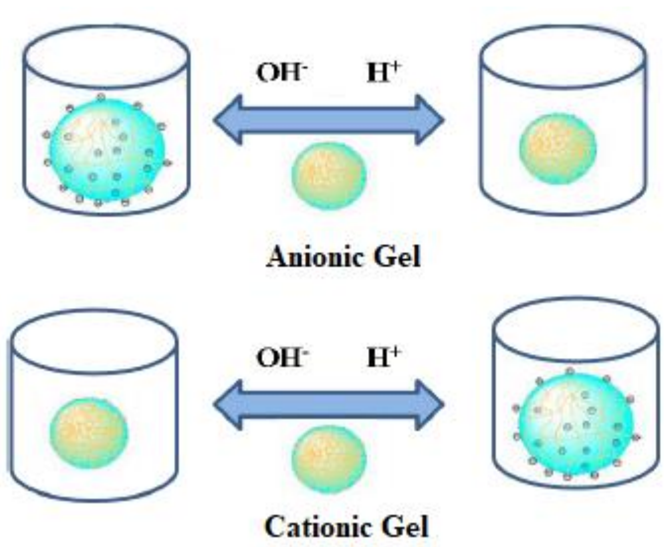


Figure 2.11. Swelling rate change depending on pH

Formation of a low elasticity network is also promoted by the formation of hydrogen bonds. When the pH rises above the pKa of the biopolymer, the dominant negative charges cause swelling of the network with high charge repellency and release of the drug. Accordingly, as a result of higher pH (7.4) in the intestinal environment, the drug may be released from the poly(acrylic acid) polymer network (106).

For other applications, it can also specifically trigger drug release in response to acidic environments. Polybases with amino groups, such as poly(N,N'-dimethyl aminoethyl methacrylate) (pDMAEMA) (Fig. 2.12) and poly(amino esters), are deprotonated at neutral pH but become positively charged by acquiring protons at acidic pH. This is particularly effective in the delivery of cancer drugs. Since the extracellular environment of solid tumors is weakly acidic (pH<6.5), the pH in endosomes and lysosomes of cancer cells is lower (pH 4.0-6.0). In this context, the particles formed by polybases are stable at physiological pH. However, in tumor tissues or their intracellular compartments, the particles become positively charged and dissolve rapidly in response to lower pH, thereby releasing the cytotoxic drug. In normal tissue, the slightly acidic environment of endosomes can also be used to trigger cargo release in gene delivery applications. Polyethyleneimine (pEI) and poly(L-lysine) (pLL) (Fig. 2.12) are two well-known transporters in non-viral gene delivery (106).

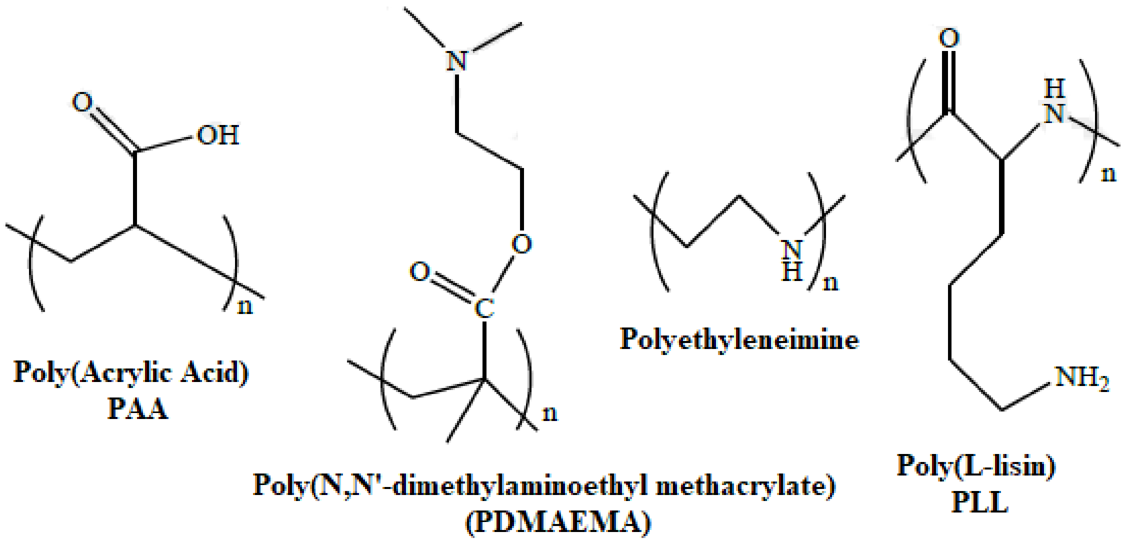


Figure 2.12. Chemical structures of some polymers that react to pH in drug release applications

pH sensitive polymers are used to release harmful drugs for the stomach in the intestine. The gels that shrink at gastric pH (pH<2.0) swell in the intestinal environment (pH>7.0) and release the drug. In another application made in the opposite direction with this process, bad-tasting drugs are released with the help of polymers that can swell at low pH. Since the polymer has a low swelling degree at the neutral pH of the mouth (pH=7.0), drug release due to the structure does not occur. In the acidic environment of the stomach, where the pH decreases, drug release is ensured (106,107).

pH sensitive polymers are smart polymers that can respond to pH change as a variable condition. There are acidic or basic ionizable groups in the structures of these polymers. These ionizable groups in the structure of the pH-sensitive polymer take or donate protons depending on the pH change. This causes the conformation of the polymer chain to change, resulting in swelling or shrinkage of the polymer (108).

pH-sensitive polymers are classified according to the presence of acidic or basic functional groups in their structures. One of the pH sensitive polymers are those containing acidic functional groups such as carboxylic acid or sulfonic acid. pH sensitive polymers with acidic side groups are found in a shrunken state when the pH of the environment is low because they are not in the ionic state.

Acidic side groups of pH-sensitive polymers are ionized in neutral or alkaline solutions. The ionization of acidic side groups depends on the logarithmic value of the acid dissociation constant of the polymer, known as pKa. In cases where the pH value of the medium is greater than the pKa value, the acidic side groups ionize. Due to the electrostatic repulsion forces between the ionized groups, the molecular conformation of the polymer changes and the polymer swells (Figure 2.13). These polymers are widely used for drug release (109,110).

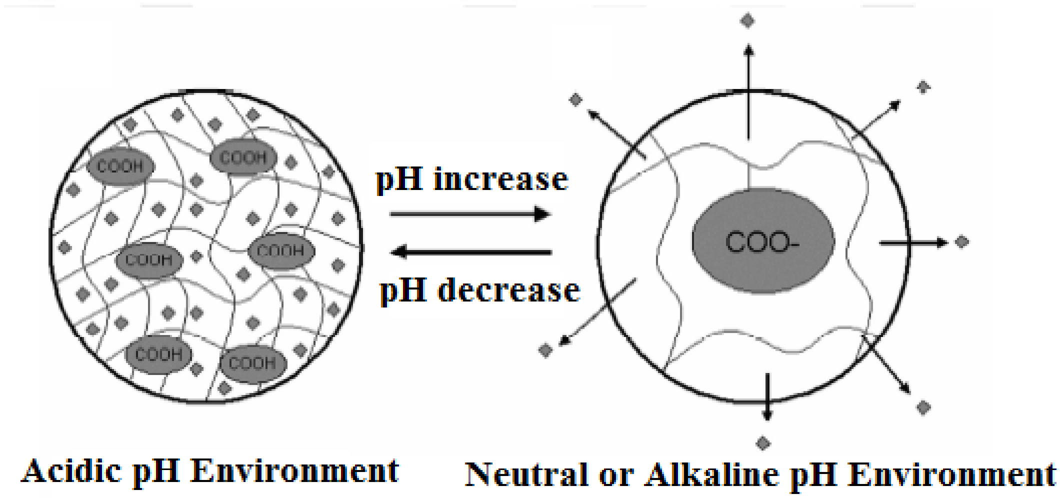


Figure 2.13. Schematic view of swelling behavior of pH sensitive polymers containing acidic side groups (109).

The second class of pH-sensitive polymers are polymers containing basic functional groups, that is, cationic groups. Poly(2-(dimethylamino)ethyl methacrylate) and poly(vinyl amine) are widely used pH sensitive cationic polymers. Unlike polymers containing acidic groups, these polymers show a structure change at pH 8 or higher. A schematic view of the swelling behavior of pH sensitive polymers containing basic functional groups is given in Figure 2.14. As can be seen from the schematic view, pH sensitive polymers containing basic functional groups are not ionized in neutral and alkaline environments, and the polymer is in a shrunken state in this environment. However, as soon as the pH value drops below the pKb (basic ionization constant) value of the cationic group-containing polymer, the cationic groups take up protons and the polymer swells because the positively charged groups repel each other with electrostatic interaction (109).

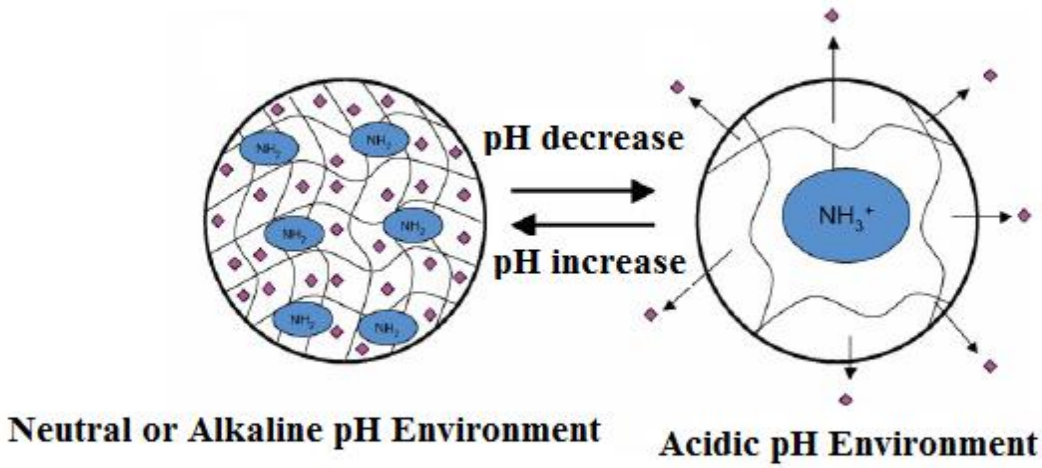


Figure 2.14. Schematic view of swelling behavior of pH sensitive polymers containing basic side groups (109)

2.3.1.2. Usage Areas of Biopolymers

Biopolymers are used extensively in fields such as biotechnology, bioengineering, biomedical sciences, pharmaceuticals, agriculture, veterinary medicine, food industry, wastewater treatment technology due to their ability to hold water very well and swell in an aqueous environment (111).

Most biopolymers respond in a controlled manner to changes in external environmental conditions such as pH, ionic strength, solvent composition, electromagnetic radiation, and electric field. This unique property creates a wide range of applications where a biopolymer can effectively serve. These include tissue engineering materials, sensors, metal particle preparation, agriculture (fertilizer and pesticide controlled release), purification and removal of water from oil and oil-containing aqueous wastes in industry, inspection of industrial wastewater containing paint and heavy metals, prevention of corrosion in telecommunications, and hospital wastes. There are filters, catalysis and optically transparent materials in the absorption of some physiological (blood and urea, etc.) fluids that are water-soluble or can be absorbed in harmony with the biopolymer (111,112,113).

While biopolymers with biomaterial properties are used in fields such as biomedicine, pharmaceuticals, veterinary medicine, controlled release systems, artificial organ construction, contact lens production, enzyme immobilization systems, drug delivery systems, artificial cornea, materials for the treatment of bone diseases, in-ear applications, synthetic cartilage, gall bladder and esophagus, wound dressing/membrane-surgery thread and many similar applications (114-118). Depending on the application, these biopolymers can be prepared in different ways such as cubes, hollow tubes, rods, sheets and films (119).

2.4. Drug Release Systems

In recent years, there has been an increase in studies on the development of systems that can direct the drug to the target areas of the body and control the drug release rate in the long term. These developed systems have been widely used in various fields such as medicine, medicine, biology, biomedicine, and gene engineering. R&D studies on a new drug take a long time. For this reason, since drugs given by traditional methods require frequent dosing and high doses of drug are required to stay in the therapeutic range, systems that will provide longer and controlled release of drug active ingredient are being researched, and controlled drug release systems, in which polymers are used, are the leading ones. These systems are the systems that best respond to expectations such as reducing the dose of drugs, extending the dosing interval, reducing side and harmful effects, targeting drugs to certain cell types or tissues.

When the drug is bound to a polymer or a lipid (oil) or encapsulated, drug safety and the drug's ability to provide the desired efficacy can be greatly enhanced. The development of controlled drug release systems is very important in terms of increasing the efficiency of drug molecules. In some treatments, it is necessary to keep the drugs at constant concentration in the aqueous phase and to release them periodically. Drug delivery systems developed for this purpose can be designed to improve the solubility of drug molecules as well as being targeted. Increasing the solubility of drugs in water makes it easier to overcome physiological barriers. Controlled release systems can be designed to be activated by spontaneous diffusion, fragmentation and dissolution mechanisms, or they can be designed to be affected by different factors such as pH, temperature, ionic forces, light, electricity and enzymes. Biopolymers formed by physical and chemical cross-links are frequently preferred especially in controlled drug release systems because they can change their polymer structures by reacting to physical conditions such as pH, temperature, ionic composition and can be easily prepared in different geometric forms.

With such systems, success is achieved in increasing the protection of vaccines, as well as prolonging the life of the drug in the body circulation, accelerating its absorption and targeting to the place of action. Drug active molecules can be easily loaded into a biopolymer prepared in cylindrical form for use in controlled drug release studies, and binders, colorants, etc., which are inevitable to be used in conventional drug formulations such as tablets and capsules. types are not required. In this way, the parameters that negatively affect the release of drug active molecules from the biopolymer structure used as the support material are eliminated. In addition, mechanical strength, which is an important parameter for controlled drug release formulations to be recommended in the gastrointestinal tract, will also be increased (120).

2.4.1. Controlled Drug Release Systems

Controlled release systems are systems that allow the active substance to be released to the targeted area at a predetermined rate and at specific time intervals. The main purpose of these systems is to keep the amount of active substance in the blood plasma concentration constant. Polymers are one of the most used materials as a controlled drug system. The release of the active substance is closely related to the properties of the polymer used, as well as physiological conditions such as pH, ionic strength, temperature and enzyme. In classical applications, there is a possibility that the drug concentration in the plasma may decrease below the effective level or rise above the safe level (toxic limit). Areas below the mentioned effective level and above the safe level represent the amount of wasted active substance (Figure 2.15). The main advantages of controlled drug release systems over conventional drug applications are:

- The ability to keep the drug level constant at the therapeutic rate
- Elimination of possible side effects against the body due to the fact that the drug can be sent directly to the desired area.

- There is no need for the use of drugs above the amount required for treatment, they are economical in this regard.

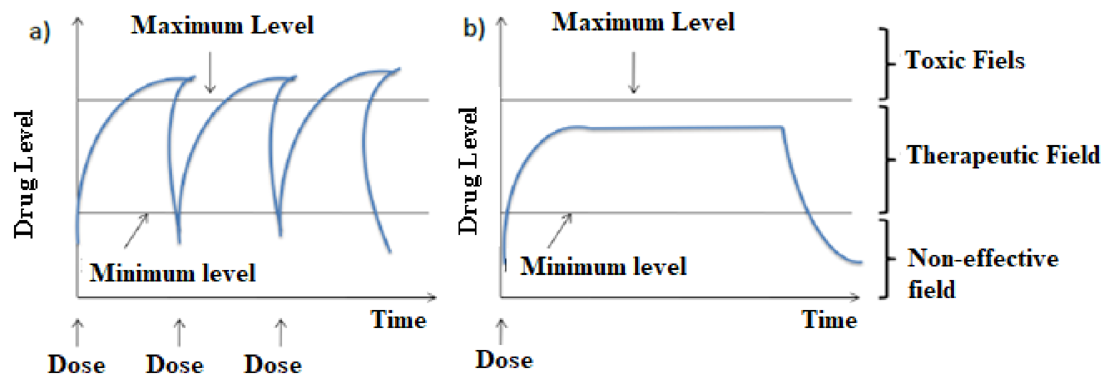


Figure 2.15. Comparison of Time-Dependent Change of Blood Drug Level between Classical Drug Applications and Controlled Release Systems Application (121).

In addition to these advantages of controlled drug release systems, attention should be paid to the following points:

- The polymer carrying the drug or the degradation products of this polymer should not have toxicities.
- It should release the drug quickly and not pose a danger to the body.
- There should be no discomfort from the system itself or the way it is applied to the body.
- The polymeric material forming the system itself and its preparation should not be costly.
- There should be no cracks in the structure of the system that could cause sudden release of the drug, and its mechanical properties should be good.

Drug delivery systems can be broadly divided into three groups in terms of drug delivery:

- Delayed release systems: Systems that release in the targeted area of the body due to the elimination of the side effects of the drug
- Sustained release systems: Systems that ensure that the drug is released continuously and remains at the same level in the blood.

- Controlled release systems: Systems where release occurs in response to a variable in the external environment (121).

2.4.1.1. Classification of controlled drug release systems

Release of drugs from controlled release systems occurs primarily through one or a combination of the following mechanisms:

2.4.1.1.1. Difüzyon kontrollü sistemler

a) Membrane systems: In these systems, polymers prepared in the form of sheets, films, capsules or microcapsules are used. The drug to be released is placed in these membranes as dissolved or dispersed (122) (Figure 2.16). During the release, the drug diffuses through the membrane and rises to the outer surface of the membrane. Diffusion of the drug across the membrane is the rate-determining step.

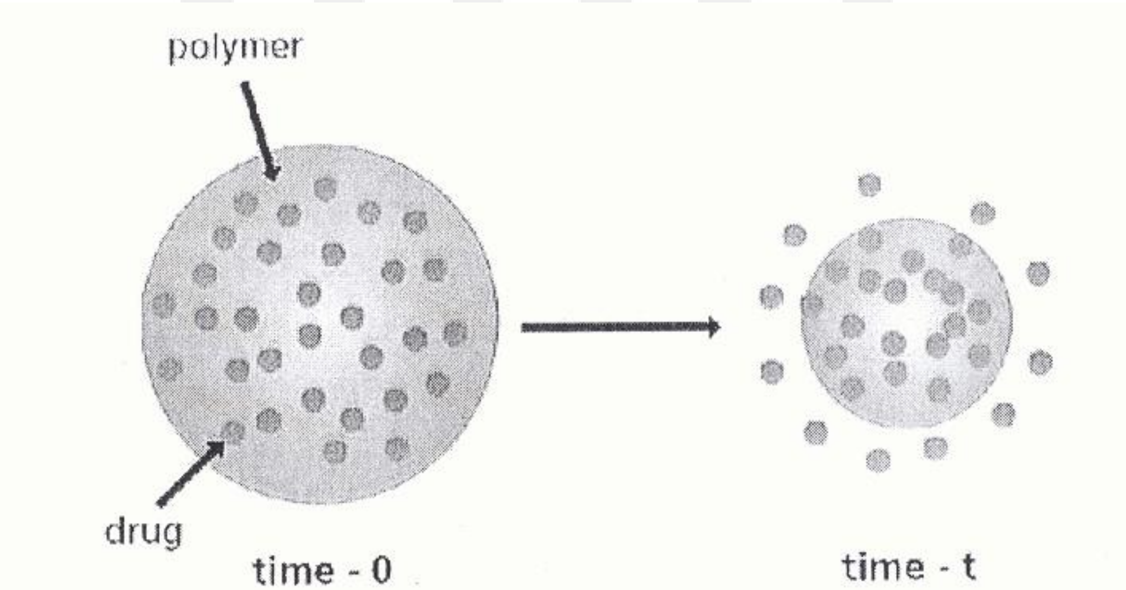


Figure 2.16. Schematic View of Diffusion Controlled Membrane Systems (122).

b) Matrix systems: These systems are prepared by dissolving the drug in a solid polymer or dispersing it homogeneously. It is easy and cheap to produce, but since there is first-degree release, the rate of release and thus the amount of drug given to the outside environment is constantly decreasing. This is a disadvantage of the system (123) (Fig.2.17).

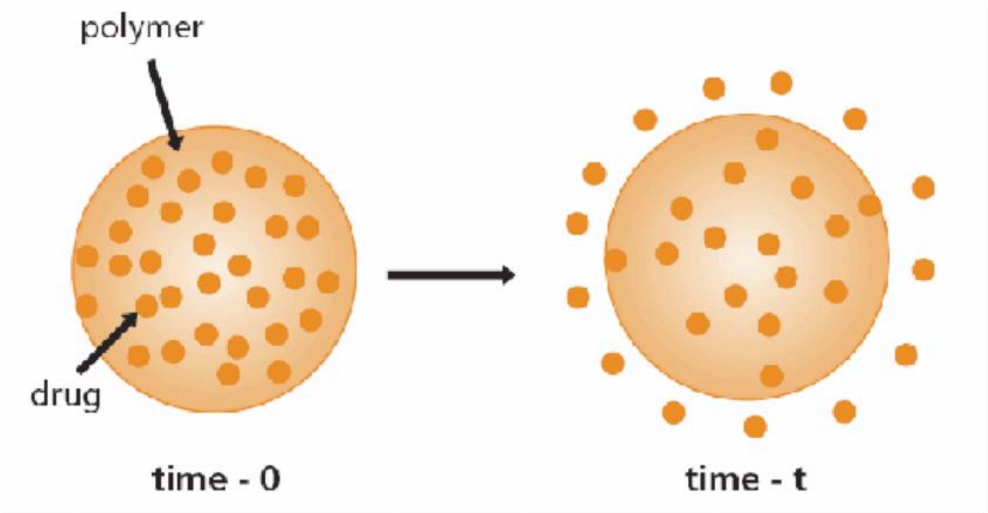


Figure 2.17. Schematic View of Diffusion Controlled Matrix Systems (123).

2.4.1.1.2. Chemical controlled systems

a) Worn systems in the body: In these systems, there are hydrolytically or enzymatically degradable bonds within the structure. For this reason, these systems are also called "biodegradable systems". As a result of the biodegradation of the said bonds, the polymer is eroded and the drug is released. The biggest advantage of these systems is that they can be eliminated from the body naturally without the need for surgical intervention. However, here, the material itself and its decomposition products should not have any health problems (124) (Figure 2.18).

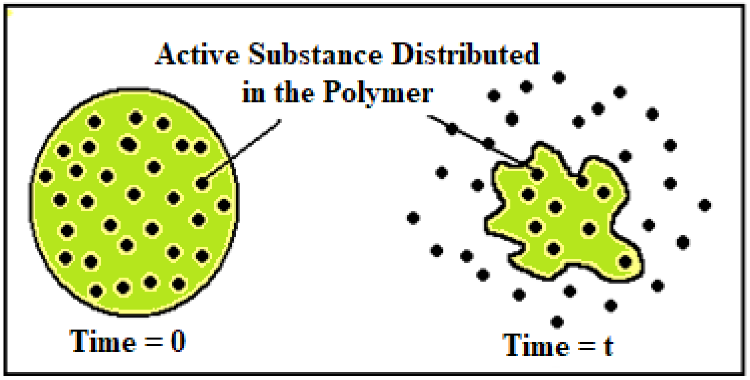


Figure 2.18. Schematic representation of systems worn in the body

b) Chain-mounted systems: In these systems, the drug molecule planned to be sent to the body is bound to the polymer chain by covalent bonds that can be broken down by enzymatic or hydrolytic ways, and by these means, the drug is released into the body. With the appropriate design of the polymer material, the breakdown of bonds, thus the release rate of the drug, can be controlled. These systems are generally used for short-term drug release applications. The biggest advantage of the system compared to other systems is that it can contain about 80% drug by weight (125) (Figure 2.19).

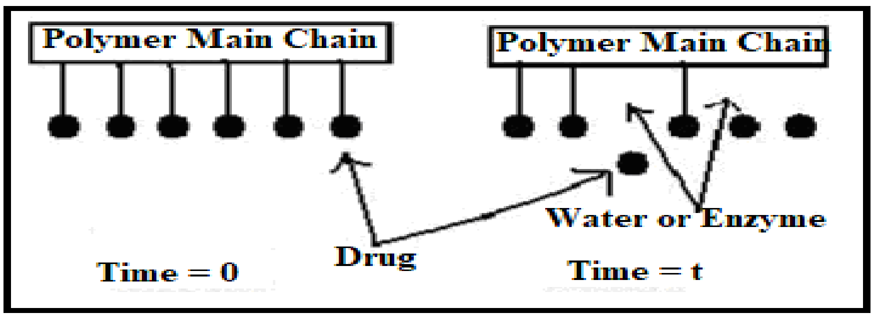


Figure 2.19. Schematic View of Chain Attached Systems (125).

2.4.1.1.3. Solvent activated systems

a) Swelling controlled systems: Cross-linked biopolymers form the basis of these systems. The drug molecules in the biopolymer diffuse into the external environment by swelling of the biopolymer in the release medium (water or biological fluid). Here, the chemical structure of the biopolymer, the cross-link ratio, in other words the pore size, play an important role and determine the rate and amount of drug release (125) (Figure 2.20).

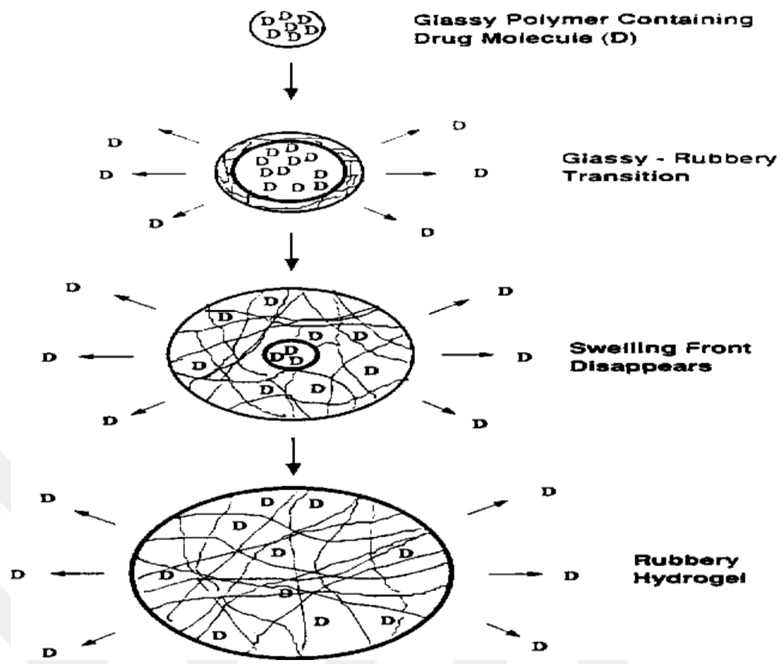


Figure 2.20. Schematic View of Swelling Controlled Systems (125).

On the other hand, controlled drug release systems can be prepared by taking advantage of the environmental (pH, temperature, light, magnetic, electric field, etc.) intumescent biopolymers properties, which can release drugs sensitive to pH, temperature, light, magnetic and electric fields. Here again, there is drug release via the swelling mechanism (125) (Figure 2.21).

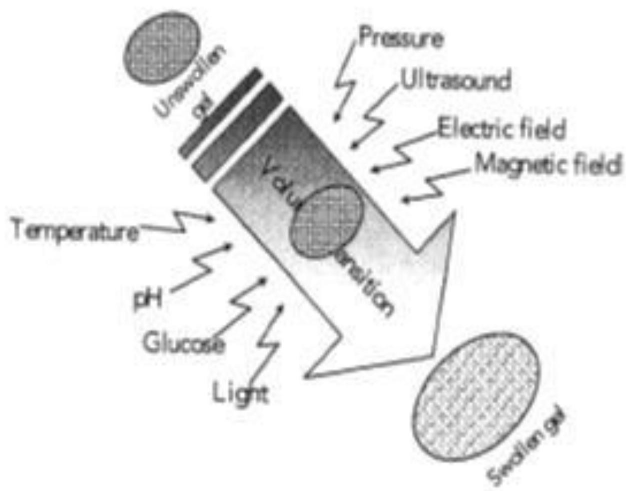


Figure 2.21. Swelling-Shrinking Behavior of Environmentally Sensitive Biopolymers (125).

b) Osmotic controlled systems: These systems consist of a semi-permeable membrane and the drug placed in this membrane. When they are placed in water or biological fluid, the molecules in the environment penetrate through the pores of the semipermeable membrane and dissolve the drug molecules inside. However, in order for these drug molecules to diffuse through the membrane, a hole must be drilled from a suitable part of the system. For this, the laser method is used. Because the drug concentration in these systems is very high (above the saturation limit), the release is usually zero-order and osmosis-controlled (125) (Fig. 2.22).

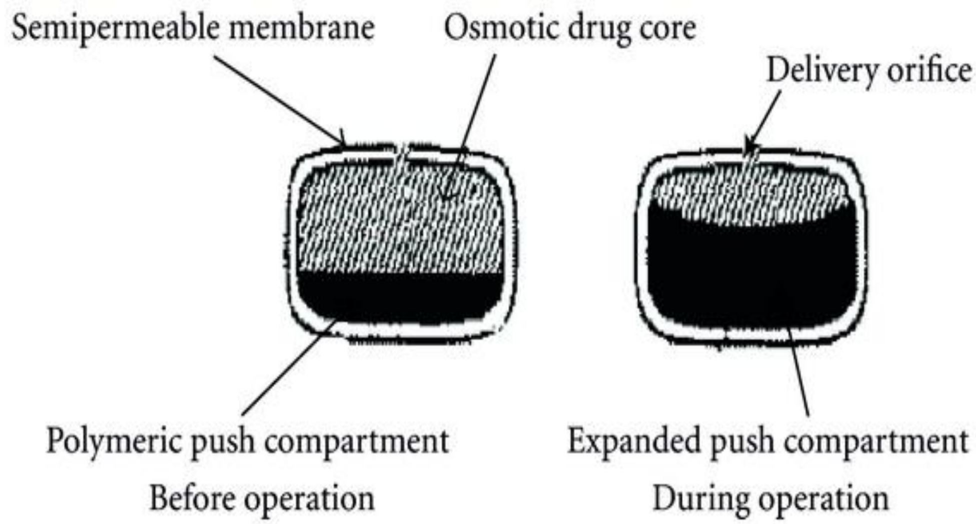


Figure 2.22. Schematic View of Osmotic Controlled Systems (125).

3. MATERIALS AND METHODS

3.1. Chemicals

All chemicals used in experimental studies are of analytical purity. Used chemicals, their usage purpose and brands are presented in Table 3.1.

Table 3.1. Used chemicals, their usage purpose and brands

hemical Name	sage purpose	rand and origin
crylic acid	ır acid-base balance	bcr GmbH, Germany
hitosan	ır encapsulation	ıgma-Aldrich, ABD
, N`-methylene sacrylamide	ır encapsulation	ıgma-Aldrich, ABD
cetic acid (CH_3COOH)	ır solubilization of chitosan	ıgma-Aldrich, ABD
mmonium persulfate	; a free radical generator	ıgma-Aldrich, ABD
itamin B12	; drug	ıgma-Aldrich, ABD

3.2 Devices

The devices, their usage purpose and brands used for analysis and/or characterization in experimental studies are presented in Table 3.2.

Table 3.2. Used devices, their usage purpose and brands

evice Name	sage purpose	rand-Origin
ɔurier transform frared spectrometer TIR)	was used to elucidate the functional oups of the synthesized biopolymer aterial.	ʌSCO, 67000, Japan
:anning electron icroscope -energy spersive X-ray ectrometer (SEM-ɔS)	was used to examine the surface orphologies of the synthesized biopolymer aterial.	ıtachi SU3500, Japan
ltraviolet-visible ectrophotometer (UV-is)	was used for vitamin B12 release.	ıimadzu UV-1800, Japan
agnetic stirrer	was used for mixing the solutions.	TOPS, South Korea
I meter	is used to determine the pH values of lutions.	ZDO, PL-700PV, Taiwan)
ɔrtex	is used for homogenization of solutions	:agon Lab, China
ecision scales	is used for weighing solid sample ıantities	ıdwag AS220/R/2, Poland

3.3. Use of Central Composite Design in Experimental Studies

The central composite design (CCD) combined with the response surface method (RSM) is a suitable system for optimizing the levels of parameters that affect drug release in the experimental study. This method is a useful method for analyzing the effects of some independent variable on the response along with a set of statistical and mathematical data. In addition, various estimations and tests such as estimation and testing of the regression equation with the CCD technique, factor effect testing, model determination coefficient calculation, optimal level combination estimation and response surface properties around it are provided. CCD is known as regression analysis and is used to estimate parameters and establish relationships between test indices and periodic variables. The experimental design approach is particularly suitable for optimizing complex synergies or antagonistic effects between variable states (126). Since it has wide application areas, RSM is used in many areas.

In this study, a CCD containing four and five levels of each of these factors was chosen to examine and optimize the effect of the selected variables. A CCD experiment design (Table 3.3) was created to measure the effect of each variable, namely pH (X_1), initial concentration (X_2), release (X_3) and solution volume (X_4), on drug release. Variable levels were coded as -1, 0, and +1, respectively, and were defined as low, medium, and high. Also, +2 and -2 star points were defined for each experimental group, corresponding to $+\alpha$ and $-\alpha$, respectively. The effects of main, interaction and quadratic variables were modeled using CCD. This experimental design was carried out to minimize the effects of uncontrollable parameters and based on controllable factors. Also, for optimizing the process parameters and their interactions were performed using a CCD with a minimum number of experiments. The CCD design in question includes the ranges of the independent variables that are preferred in the experimental study and that have an effect on adsorption, and the responses corresponding to these variables (Table 3.3).

Table 3.3. Independent variables and their levels

	Factor 1	Factor 2	Factor 3	Factor 4	Response
Run	X_1: pH	X_2: Initial concentration (mg L^{-1})	X_3: Release time (min)	X_4: Solution volume (mL)	Release (%) of vitamin B12 (mg L^{-1})
1	3	50	60	1	49.8
2	3	80	120	1	80.75
3	7	50	120	1	78.4
4	7	80	120	1	92.2
5	7	50	60	2.5	78.4
6	3	50	120	1	73.1
7	5	65	30	1.75	78.2
8	3	80	60	1	66
9	7	80	120	2.5	99
10	5	65	90	1.75	100
11	7	80	60	2.5	93.8
12	7	50	60	1	58.8
13	5	65	90	1.75	95
14	5	65	90	1.75	98
15	9	65	90	1.75	74.2
16	3	50	120	2.5	95.1
17	5	95	90	1.75	95
18	5	65	90	1.75	99
19	3	80	60	2.5	85.7
20	5	65	90	1.75	95
21	5	65	90	0.25	44.4
22	7	80	60	1	67.2
23	5	65	90	1.75	96.7
24	3	50	60	2.5	84.2
25	3	80	120	2.5	93.3
26	7	50	120	2.5	95
27	1	65	90	1.75	73.1
28	5	65	150	1.75	100
29	5	65	90	3.25	90.1
30	5	35	90	1.75	90.7

3.4. Synthesis of Biopolymer Material and Loading of Vitamin B12

3.4.1. Biopolymer production

10 mL of acrylic acid was taken, the final volume was filled up to 50 mL with ultrapure water and mixed in a magnetic heater stirrer until homogeneous. Then 0.5 g of chitosan was added and mixing was continued for 15 minutes. Mixing was continued by adding 0.6 g of N, N`-methylene bisacrylamide. After adding N, N`-methylene bisacrylamide 0.3 g ammonium persulfate (APS) added and mixed.

3.4.2. Loading the biopolymer with vitamin B12

The films were loaded with vitamin B12 according to the concentrations and volumes according the Table 3.3. Then the vitamin B12 release was determined by using UV-Vis spectrometer.

4. RESULTS AND DISCUSSION

4.1. UV-Vis Spectrophotometer Analyzes

Measurement of the attenuation of a beam of light after it has passed through the sample or is reflected from a sample surface is performed using visible region UV-Vis absorption spectrophotometer. For example, the decrease in the intensity of light passing through the solution is an indication that absorption has occurred. This spectroscopic technique is generally preferred for the measurement of molecules or complexes in solution. In this study, UV-Vis spectrophotometer was used to examine the B12 release of biopolymer. By scanning the wavelength in the range of 300-600 nm the spectra (Figure 4.1) was obtained and the maximum absorption wavelength was determined as 360 nm. Then vitamin B12 release was measured at this wavelength.

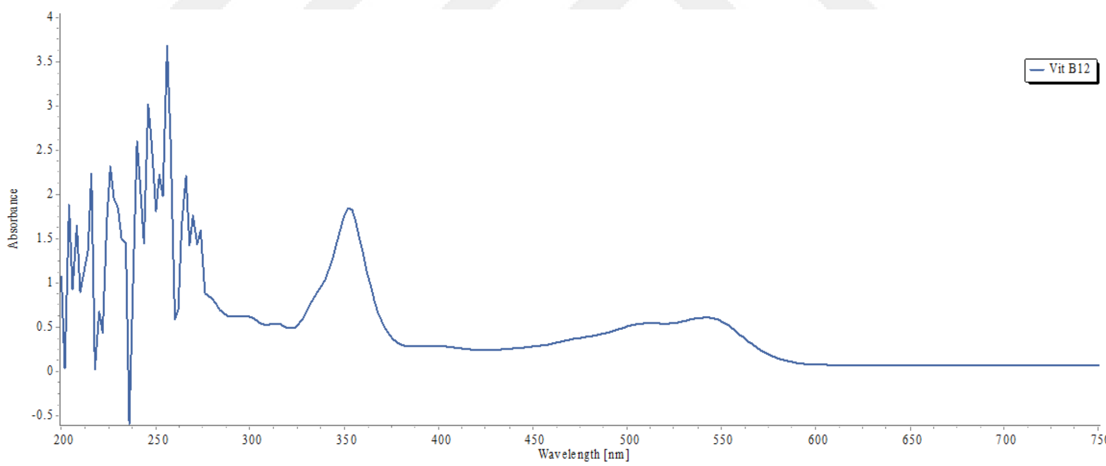


Figure 4.1. UV-Vis spectrum of vitamin B12

4.2. FT-IR Analyzes

For determination the the functional groups that help determine the surface chemistry, chemical properties of the synthesized biopolymer and vitamin B12-loaded biopolymer FT-IR spectrometer was used. The FT-IR spectrum of chitosan, chitosan –

grafted-poly (acrylic acid), vitamin B12 loaded chitosan –grafted-poly (acrylic acid) were shown in Figure 4.2.

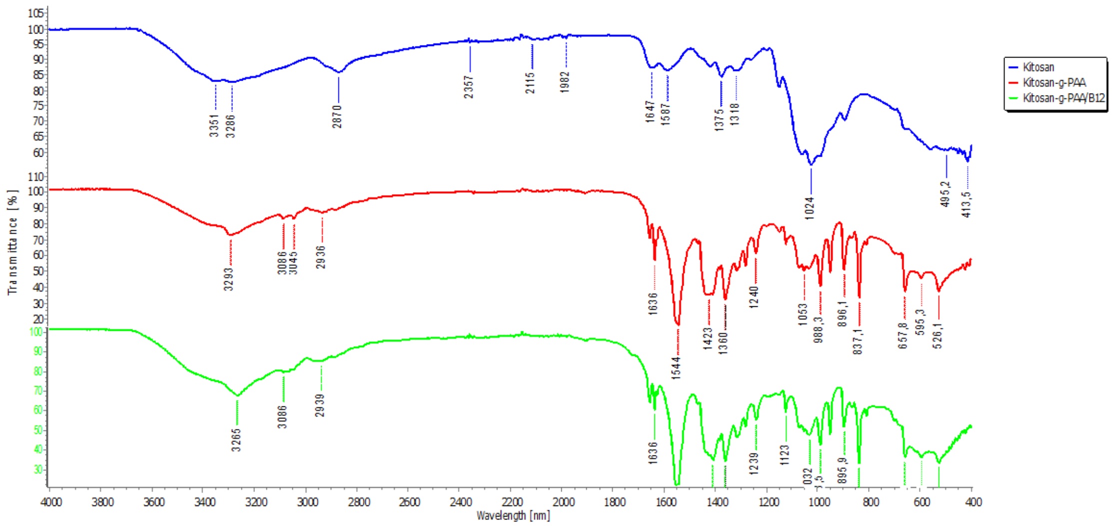


Figure 4.2. FTIR spectrum of chitosan, chitosan–grafted-poly (acrylic acid), vitamin B12 loaded chitosan–grafted-poly (acrylic acid)

Chitosan peaks at 3287 cm^{-1} can be assigned to O-H stretching and at 2864 cm^{-1} C-H stretching. Bands at 1647 cm^{-1}, 1590 cm^{-1} and 1322 cm^{-1} are indicated amide I, amide II and amide II, respectively. Bridge O stretching, C-O stretching and pyranoid ring stretching are revealed at 1151 cm^{-1}, 1061 cm^{-1}, and 890 cm^{-1}. The band at 1026 cm^{-1} is derived from C-O stretching of the primary –OH. Asymmetric stretching of C–O–C is seen at 1154 cm^{-1}. After the reaction, these characteristic absorption bands are weakened or masked by other absorption bands, which means that it is involved in the copolymerization reaction. In the spectrum of chitosan-g-PAA, in addition to the characteristic peaks of chitosan, some new absorption peaks appear. The peaks at 1658, 1551 and 1435 cm^{-1} are characteristic peaks of PAA and are stretching of C-H vibration, asymmetric –COO− stretching vibration and C–H bending vibration, respectively. The peak at 1658 cm^{-1} corresponds to carboxy absorption by grafted poly (acrylic acid), and the peaks at 809 and 620 cm^{-1} are also characteristic for poly (acrylic acid). Also, the bands at 1551 and 1409 cm^{-1} correspond to the sodium carboxyl group. These show that the acrylic acid in the polymer is grafted onto chitosan and can also be assigned to asymmetric and symmetric stretching vibrations of the COO− anion groups. This indicated that the carboxylic groups of PAA dissociate into COO- groups, which form complexes with protonated chitosan amino groups via electrostatic interaction to form polyelectrolyte complex during the polymerization procedure. The main bands of vitamin B12 are in the range of 2800-3500 cm^{-1} and 1000-1750 cm^{-1}. The band widening and intensity reductions in the FT-IR spectra between the unloaded flim and the B12 loaded flim prove the chemical bond formation between the vitamin B12 molecules and the biopolymer film. 2940 ve 2920 cm^{-1} (127). The peak of the CONH2 group is observed at 1655 cm^{-1} (128). The peaks at 1555 cm^{-1} are the peaks of the amide N-H bands (129). There are peaks of CH2 swing bands at 1410 cm^{-1} (130). Peaks of N-C tensile vibration are observed at 1295 cm^{-1} (130). The peak of C-O stretching vibration in C-O-C bonds is observed at 1160 cm^{-1} (131). A peak of skeletal stress is observed at 1075 cm^{-1} (130). The peak observed at 1025-1005 cm^{-1} of the saccharide structure of chitosan is also observed here (132).

4.3. TGA-DTA Analyzes

The thermal stability of biopolymers and vitamin B12 loaded biopolymers was investigated using TGA from room temperature to 600 °C at a heating rate of N_2 was 4 °C min^{-1}. The percentage of mass loss of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid is given in Figure 4.3. Major mass loss up to 160 °C is related to the evaporation of water molecules. In the second step between the 160 °C and 275 °C it can be seen the decay of –COOH groups and in the third step (275 °C- 490 °C), where mass loss is evident, it was assigned to deconstruction. Polymers are the basic inputs used in the production of materials such as plastic, rubber, fiber, paint, adhesive that we use frequently in every aspect of our daily life. Polymers that can occur spontaneously in nature are called natural polymers and most of the natural polymers are found in living structures (133). Examples of these are polysaccharides such as lignin, cellulose, hemicellulose, alginate, chitin, chitosan, heparin, and proteins such as collagen, fibrin, keratin, eggshell membrane (134,135). Thermogravimetric analysis (TGA) is widely used to study the fundamental stability and properties of thermal decomposition of polymers. TGA and DSC studies of other chitosan-based polymers reported a decomposition temperature of 310 °C (136-138). These results are similar to our study.

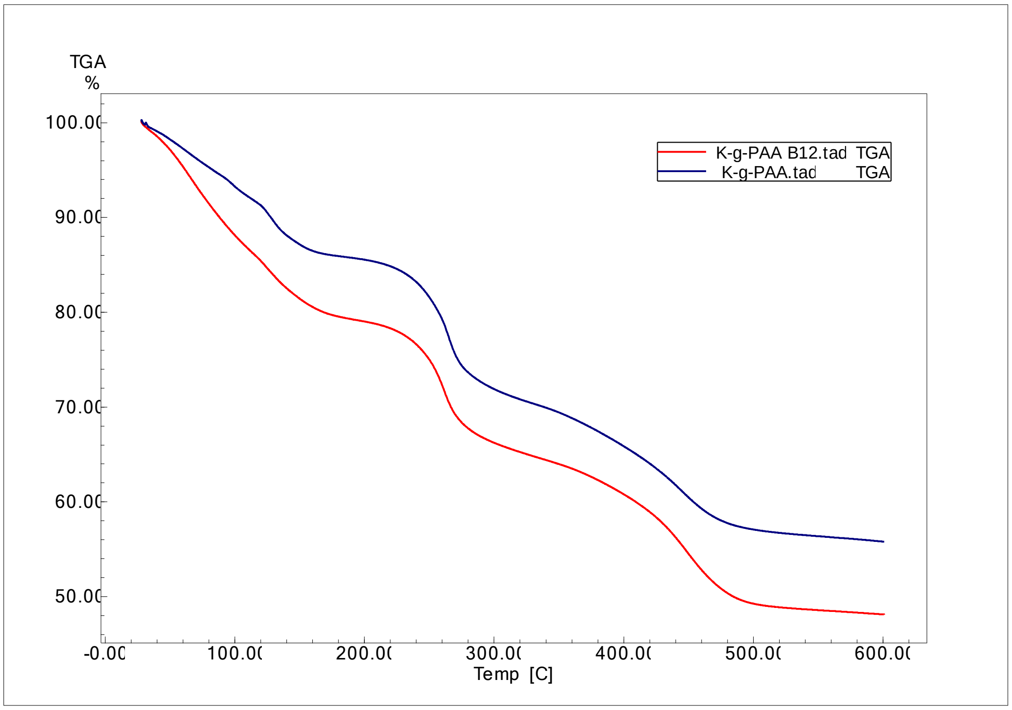


Figure 4.3. TGA thermograms of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid

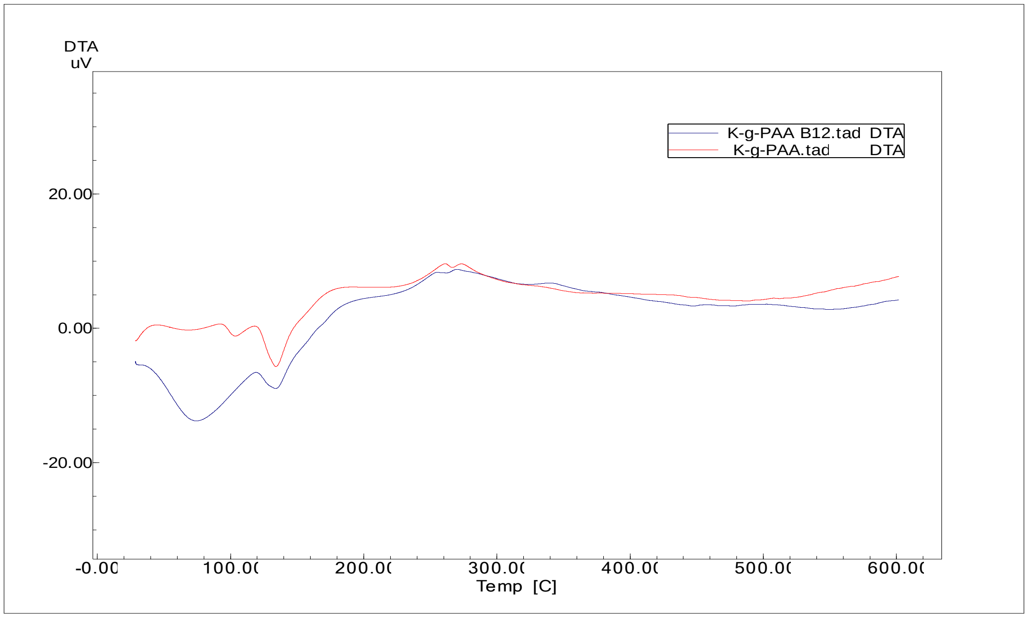


Figure 4.4. DTA thermograms of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid

Differential thermal analysis (DTA) of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid was given in Figure 4.4. In Figure 4.4 it is seen an endothermic peak at 75°C and at TGA thermogram (Figure 4.3) the decomposition is propotionally higher. These are supported that the vitamin B12 is loaded on chitosan-g-polyacrylic acid.

4.4. SEM-EDS Analyzes

SEM-EDS images of chitosan-g-polyacrylic acid and vitamin B12 loaded chitosan-g-polyacrylic acid was depict in Figure 4.5 and Figure 4.6, respectively. From the EDS images in Figure 4.6 it is understood that vitamin B12 is bound on the biopolymer. While the peak of Co element is not seen in the EDS image of chitosan-g-polyacrylic acid (Figure 4.5), the peaks of Co element are seen after vitamin B12 is loaded on chitosan-g-polyacrylic acid (Figure 4.6).

SEM is used to examine cracks or scratches on the surfaces, phase boundaries in the material, agglomeration of the support and additive materials, the attraction between the materials and the surface roughness of the material in all studies and applications involving polymers. Roughnesses such as indentations and protrusions on the surface are very important as they affect the wetting feature intensely. This property, which is known as the wettability feature in solids and similar to the tendency of fluids to stick to a surface, is directly related to the structure of the surface.

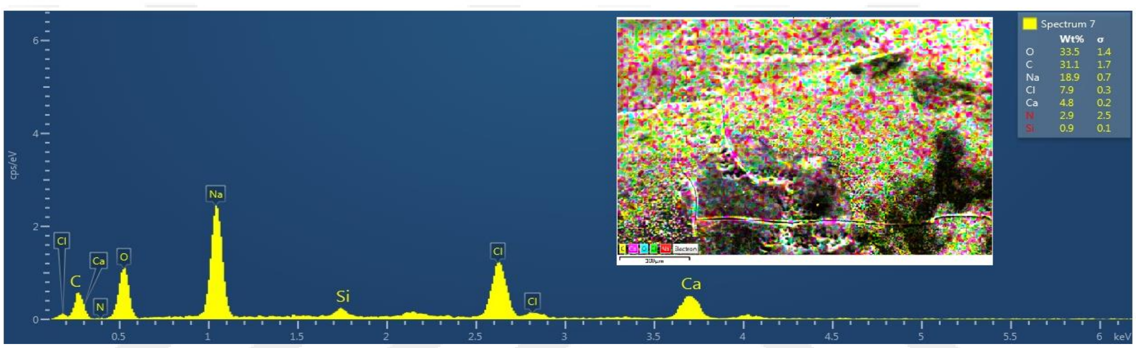


Figure 4.5. EDS image of chitosan-g-polyacrylic acid

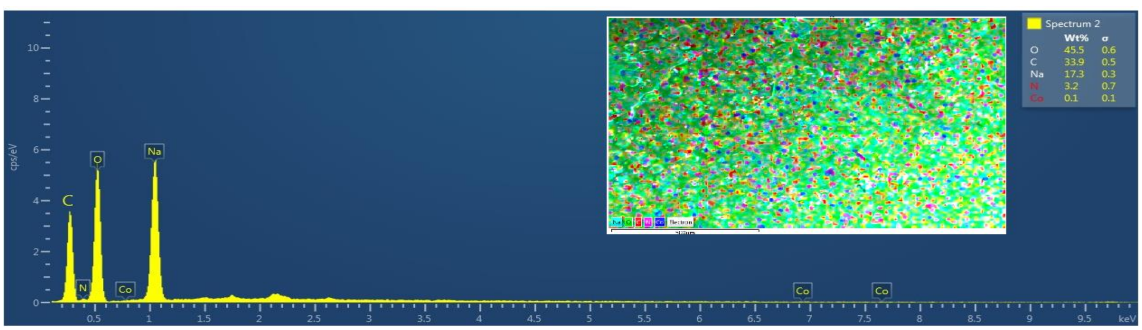


Figure 4.6. EDS image of vitamin B12 is loaded chitosan-g-polyacrylic acid

4.5. Ideal Model Choice for Vitamin B12 Release

In order to optimize the range and levels of variables that affect the vitamin B12 release process of biopolymers and to elucidate the nature of the response surface in the experimental design, the CCD coupled with the RSM was selected and developed considering all the important interactions. In the study, it is understood from the obtained results in Table 4.2 that the CCD model is quadratic and it is understood that there is no need for any power transform.

Among the various applications, the use of biopolymers as drug carriers is one of the most promising applications. High loading capacity, encapsulation efficiency, simultaneous application of various treatments, ease of operation, low cost are the features that make the use of nanofibers produced by electrospinning method attractive in this field (139).

Controlled drug release is a method in which the active substance is designed to be delivered in the desired amount at a certain speed in the system for the desired time (140). In classical drug release, the active substance is released suddenly and the toxic drug concentration in the plasma rises above its value, and this may cause undesirable side effects in the patient. In drug release with nanofibers, the drug substance can be added to the electrospinning solution or it can be produced by encapsulation. Thus, nanofibers can be used both as a drug carrier and as a drug delivery system (141).

Among the various applications, the use of biopolymers as drug carriers is one of the most promising applications. High loading capacity, encapsulation efficiency, simultaneous application of various treatments, ease of operation, low cost are the features that make the use of nanofibers produced by electrospinning method attractive in this field (139).

Controlled drug release is a method in which the active substance is designed to be delivered in the desired amount at a certain speed in the system for the desired time (140). In classical drug release, the active substance is released suddenly and the toxic drug concentration in the plasma rises above its value, and this may cause undesirable side effects in the patient. In drug release with nanofibers, the drug substance can be added to the electrospinning solution or it can be produced by encapsulation. Thus, biopolymers can be used both as a drug carrier and as a drug delivery system (141).

When the literature studies for the release of vitamin B12 are examined, it is seen that the release of vitamin B12 from nanofiber systems developed on different polymers has been examined. Today, there are many studies for the controlled release of vitamin B12 (142,143).

Table 4.1. Ideal model selection for the vitamin B12 release behavior of chitosan-g-polyacrylic acid

ırce	Sequential p-value	Lack of Fit p-value	$R^2_{Adjusted}$	$R^2_{Predicted}$	
ear	< 0.0001	0.0006	0.5408	0.4611	
	0.9310	0.0004	0.4476	0.3456	
adratic	< 0.0001	0.0670	0.9427	0.8432	Suggested
pic	0.1267	0.1113	0.9678	0.3402	Aliased

Table 4.2. ANOVA results of response surface quadratic model

ırce		**n of ıares**	**an ıare**	**ue**	**alue ob > F**	
del		32.93	3.07	08	.0001	ıificant
pH		20	20	3	549	
Initial concentration		5.63	5.63	17	009	
Release time		55.79	55.79	55	.0001	
Solution volume		96.88	96.88	5.70	.0001	
X_2		36	36	4	333	
X_3		6	6	6	083	
X_4		68	68	2	097	
X_3		91	91	1	383	
X_4		39	39	4	835	
X_4		2.10	2.10	9	107	
		9.68	9.68	69	.0001	
		11	11	8	288	
		5.56	5.56	5	098	
		19.08	19.08	7.34	.0001	
sidual		3.03	20			
ck of Fit		5.42	64	8	670	significant
e Error	61	2				
Total	30.96					
	704					
j R^2	427					
d R^2	432					
eq Precision	004					

The ANOVA results and regression coefficients presented in Table 4.2 and the results showed that the contribution of the quadratic model ($p<0.0001$) is significant for Vitamin B12 release.

The "Fisher test" shows that larger "F values" and smaller "p values" are more significant than the suggested model terms. In this study; A p value less than 0.05 indicates significans at the 95% confidence level, and an F value of 35.08 indicates that the model is significant. The "probe>F" values are a measure of whether the model terms are statistically significant. If the value of "probe>F" is less than 0.05, it means that the term is statistically significant for the model and a value greater than 0.05 means that term is statistically in significant for the model. In this case; Model terms X_1, X_2, X_3, and X_4 are statistically significant model terms for vitamin B12 release from biopolymer. Also, the variation of the data around the model that fits the experimental data is expressed as the model's lack of fit (LOF). The LOF p values measure the fit of the model and indicate that the LOF is not significant with respect to pure error. This value is a sufficient test for the adequacy of model fit without the effects of additional higher order terms. The LOF value in the ANOVA table was obtained as 0.0670. These LOF p values of the vitamin B12 release method from biopolymer confirm the applicability for good reaction settlement. It also shows that the number of experiments performed is sufficient to determine the effects of independent variables on B12 loading for drug release of biopolymer. Since the R^2 and adjusted R^2 values describe the percentage of variation in response, the validity of the polynomial models was evaluated with these values in mind. Since the R^2 value of the quadratic model used was obtained as 0.9704, it can be said that 97.04% of the values estimated by the model matched the experimental values of the release behavior of vitamin B12 loaded biopolymers (Figure 4.7).

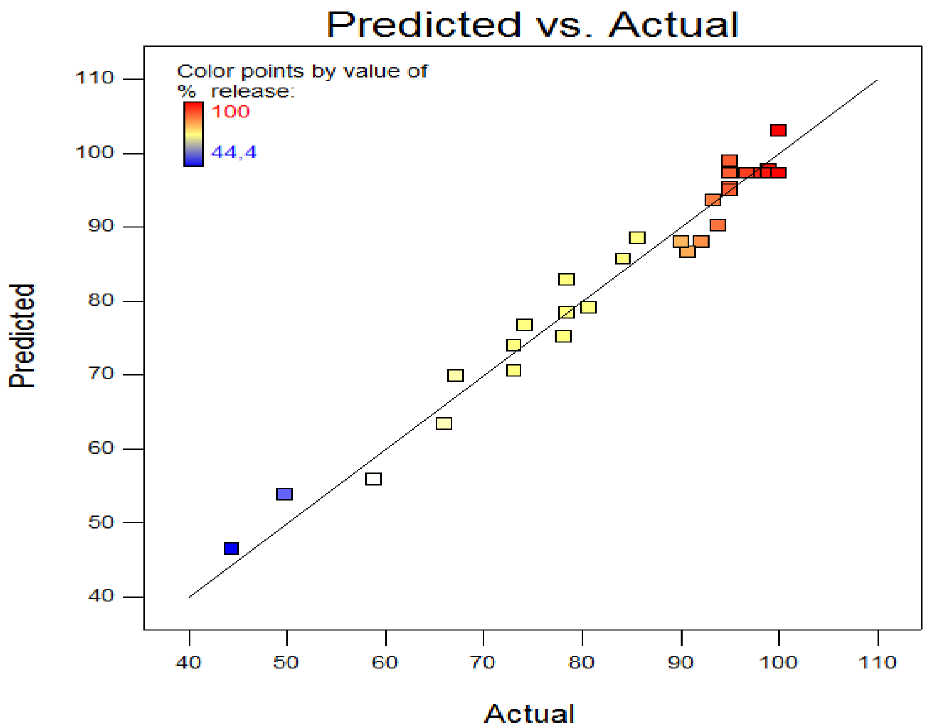


Figure 4.7. The overlap ratio of the estimated and actual values obtained

The sensitivity term (AP) measures the signal-to-noise ratio (S/N) and it is desirable that this ratio be greater than 4. This value was obtained as 22.004, which is a clear indication that the model is giving sufficient signal. In the light of preliminary experimental studies, four critical parameters affecting B12 release from biopolymers were selected as independent variables, and drug release was considered as the dependent variable. As can be seen from Table 4.4, the quadratic model with the highest R2 (0.9704) value was preferred according to the statistical test results of the model used. To express the relationship between the independent variables and responses, the experimental data were expressed with a quadratic polynomial mathematical equation. A quadratic mathematical equation derived from the model in question was derived using coded factors and presented in Equation 2.

$$Release\ \% = -122,44 + 13,58X_1 + 1,14X_2 + 0,95X_3 + 84,91X_4 + 0,037X_1X_2 + 0,01X_1X_3 - 0,79X_1X_4 - 2,47917\mathrm{E} - 003X_2X_3 - 0,14X_2X_4 - 0,11X_3X_4 - 1,47X_1^2 - 4,95X_2^2 - 2,28X_3^2 - 13,36X_4^2$$

Eq. 2

The best method to determine the optimum point where the parameters selected as independent variables have the maximum effect and to obtain the highest removal performance is the arrangement of two-dimensional (2D) and three-dimensional (3D) surface graphs of vitamin B12 release from biopolymer.

The effects and interactions of each parameter on the vitamin B12 release of biopolymers are presented in Figure 4.8. 3D response surface plots derived from CCD are used to define the response maximum, mid, and minimum points.

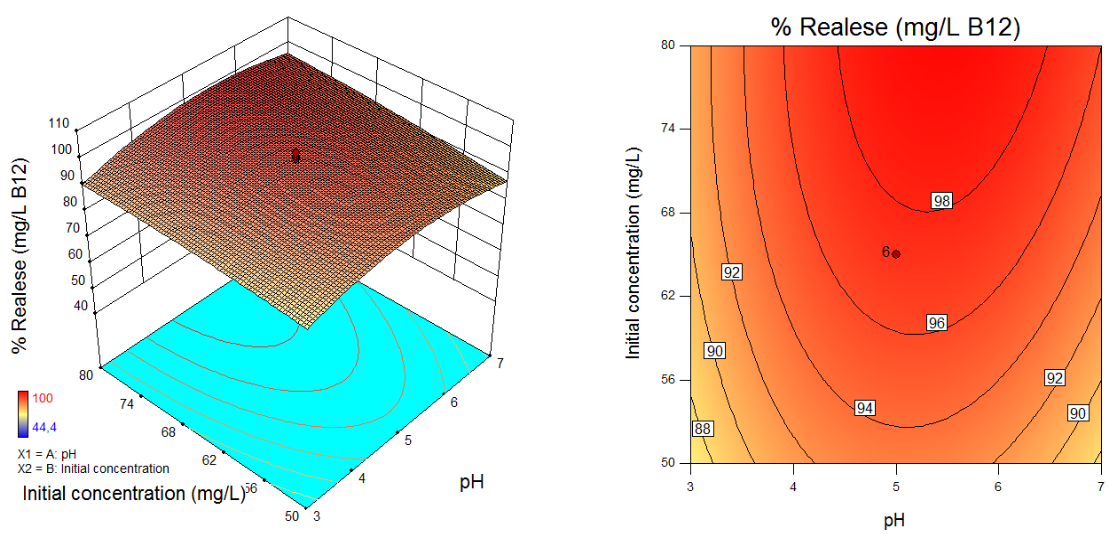


Figure 4.8. Interaction between pH and initial concentration a) 3D graph b) 2D graph

Figures 4.8 (a) and (b) represent 3D and 2D response surface plots of the effect of initial concentration and pH on vitamin B12 release efficiency from biopolymers. When pH increased from 3 to 7, vitamin B12 release from the biopolymer increased up to pH 5, followed by a partial decrease in vitamin B12 release as pH increased to 7. While the initial concentration had a significant effect on vitamin B12 release from biopolymers, it was observed that the effect of the interaction between the initial concentration and pH was not statistically important ($p>0.005$). In the study by Nath et al (2020), they investigated an acrylic acid grafted gelatin/LDH based biocompatible hydrogel with pH-controlled B12 release. As a result of the study, it was reported that there was a certain increase in the release of vitamin B12 from the biopolymer as the pH value increased, in parallel with our study, and a partial decrease in the release of vitamin B12 from the biopolymer when the pH value increased to 7.

Similarly, in the study conducted by Maiti et al. (144), it was reported that the release of vitamin B12 increased until the pH value was 7, and that there was a decrease in the release of B12 with the increase of the pH value to 7.

In the study performed by Bajpai and Dubey (145), it was determined that vitamin B12 released approximately 8.6% ± 2.1% and 83.2% ± 4.8% in environments with pH 1.2 and 6.8, respectively. However, a new test model called diffusion cell flow was also used to examine the drug release behavior of the device to include in vivo GI conditions such as acidic pH in the stomach and high water content, low water content, and the presence of semisolid mass in the large intestine.

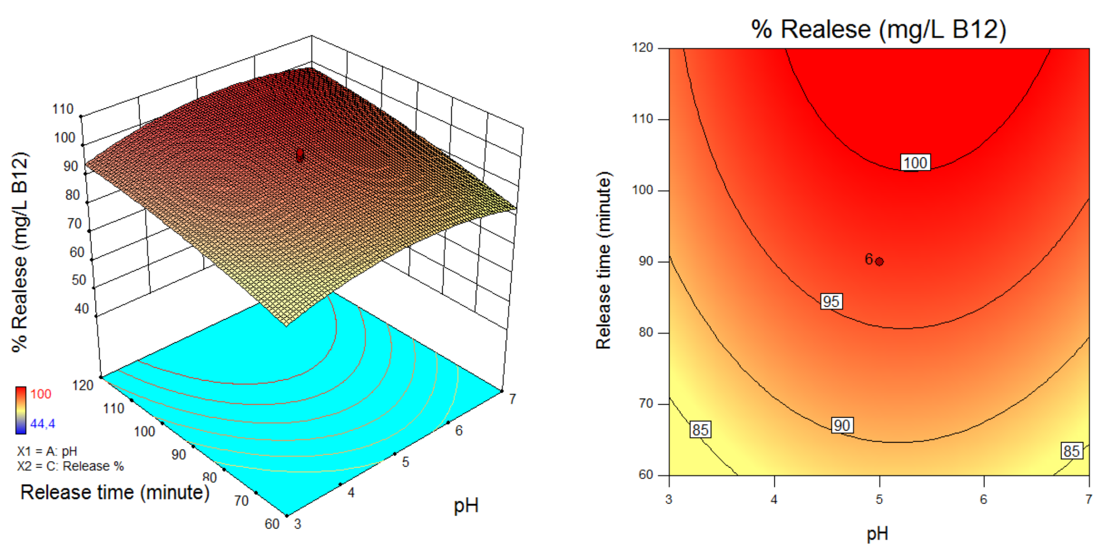


Figure 4.9. Interaction between pH and release time a) 3D graph b) 2D graph

The 3D and 2D response surface plots of the effect of release time and pH vitamin B12 release efficiency are presented in Figure 4.9 (a) and 4.9 (b). While the increase in the release time caused a significant increase in the vitamin B12 release from the biopolymer, it was observed that the vitamin B12 release of the biopolymer increased as the pH increased from 3 to 5, but there was a partial decrease after pH 5. It was observed that the effect of the interaction between pH and release time was not statistically significant ($p>0.05$). Maiti et al. (144) reported that the release of vitamin B12 increased until the pH value was 7, and that there was a decrease in the release of B12 with the increase of the pH value to 7.

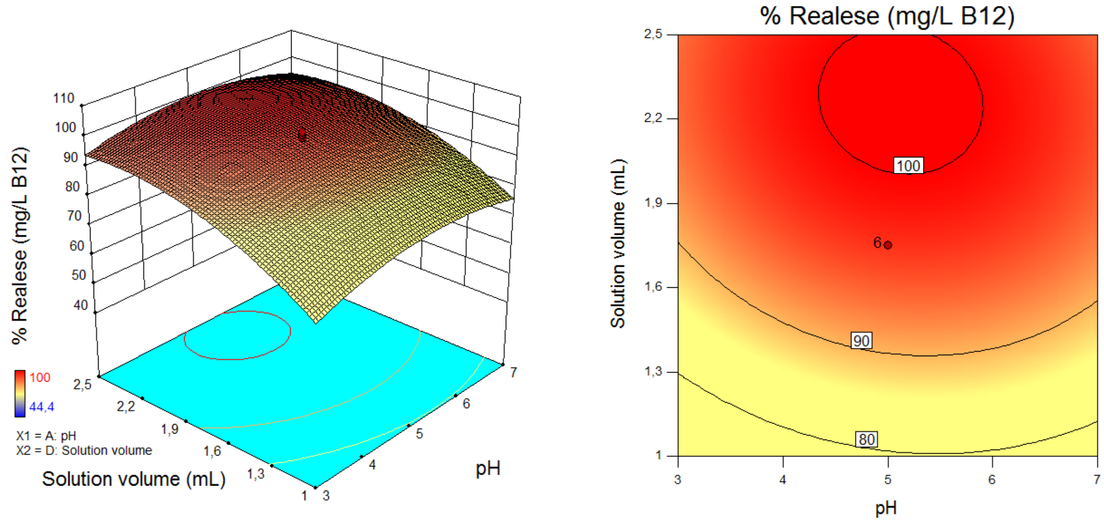


Figure 4.10. Effect of interaction between pH and solution volume a) 3D graph b) 2D graph

The effect of solution volume and pH on vitamin B12 release efficiency is presented in Figure 4.10 (a) and 4.10 (b). While the increase in the solution volume caused a significant increase in the vitamin B12 release from the biopolymer, it was observed that the vitamin B12 release of the biopolymer increased as the pH increased from 3 to 5, but then there was a partial decrease as the pH increased. It was observed that the effect of the interaction between pH and solution volume was not statistically significant ($p>0.05$). In the study conducted by Moradi et al (146), it was reported that the initial volume is effective on vitamin B12 release. In the same study, the optimal initial concentration for vitamin B12 was determined as 1000 mol/m^3.

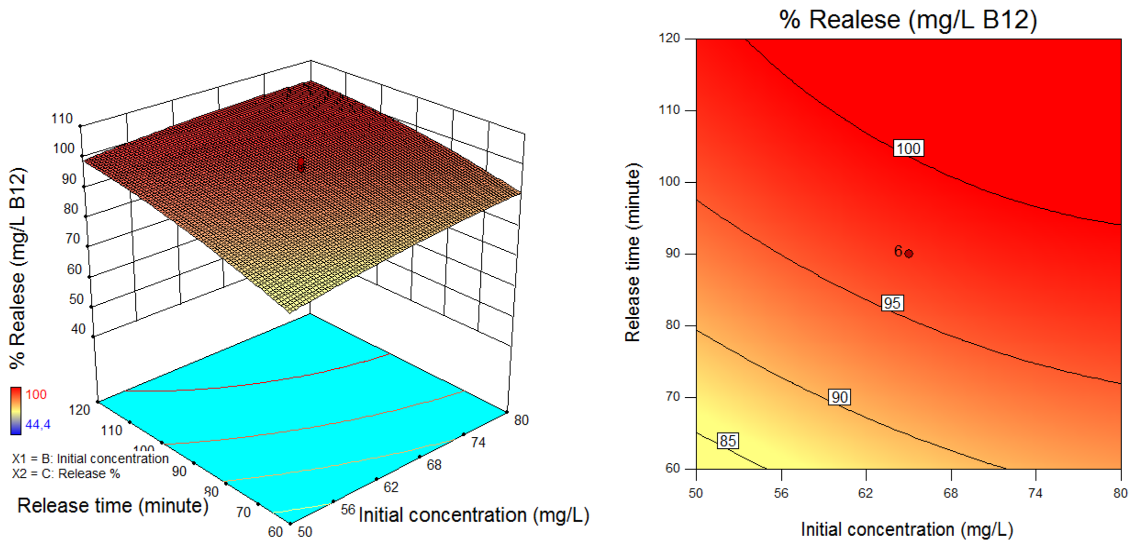


Figure 4.11. Effect of interaction between release time and initial concentration a) 3D graph b) 2D graph

The 3D and 2D response surface plots of the effect of release time and initial concentration on vitamin B12 release efficiency are presented in Figure 4.11 (a) and Figure 4.11 (b). It has been determined that with the increase of the release time and the initial concentration, it contributes positively the release of vitamin B12. However, it was found that the interactive effect of both variables was not statistically significant by using the ANOVA table ($p>0.05$). Moradi et al. (146) investigated the effect of hydroxyethyl methacrylate polymer on the hydrogel carrier on the production and release of vitamin B12 in their study. The system boundary velocity, the initial concentration of the drug loaded on the carrier, and the hydrogel membrane thickness were investigated in stimulation. As a result of the study, it was determined that the drug concentration on the carrier increased more than the desired amount for a certain time in order to control the optimum drug release from the carrier to the body, and at the same time, the speed of the boundary layer was proportional to the rate of water infusion into the hydrogel. Optimal simulation results were obtained with an initial concentration of 1000 mol/m3 for vitamin B12, a thickness of 0.0004 mm for the hydrogel membrane, and a constant velocity of $2x10^{-11}$ m/s. In this study, the time required to reach the equilibrium concentration for the stationary boundary of the system is 120000 s, while this figure is 210000 s for a moving boundary with a constant velocity of 2×10^{-11} m/s.

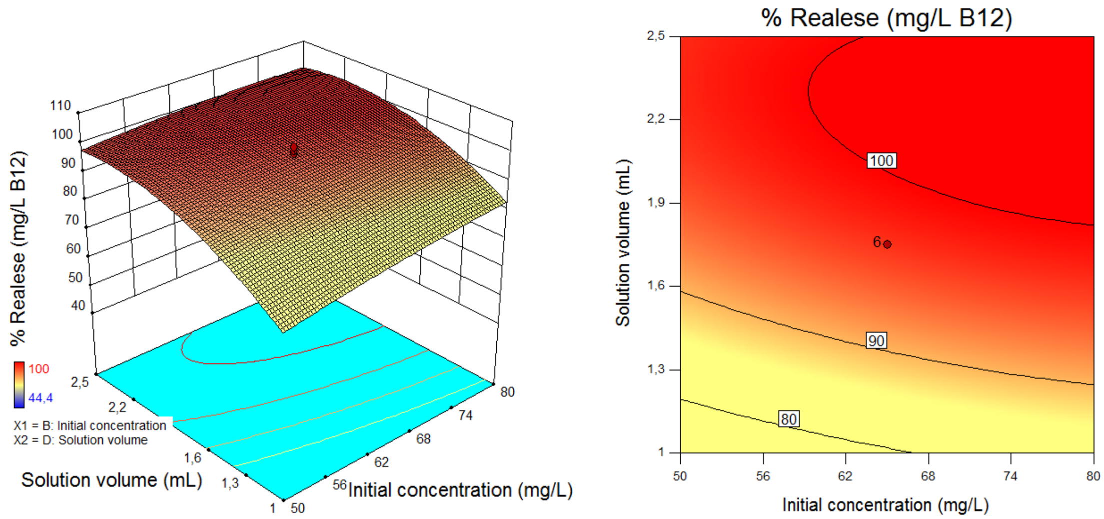


Figure 4.12. Effect of interaction between solution volume and initial concentration a) 3D graph b) 2D graph

The effect of solution volume and initial concentration on vitamin B12 release efficiency are presented in Figure 4.12(a) and 4.12(b). It contributes positively to vitamin B12 release by increasing the solution volume and initial concentration. However, using the ANOVA table, it was found that the interactive effect of both variables was not statistically significant (p>0.05). In the study conducted by Moradi et al (146), it was reported that the initial concentration is effective on vitamin B12 release. In the same study, the optimal initial concentration for vitamin B12 was determined as 1000 mol/m^3.

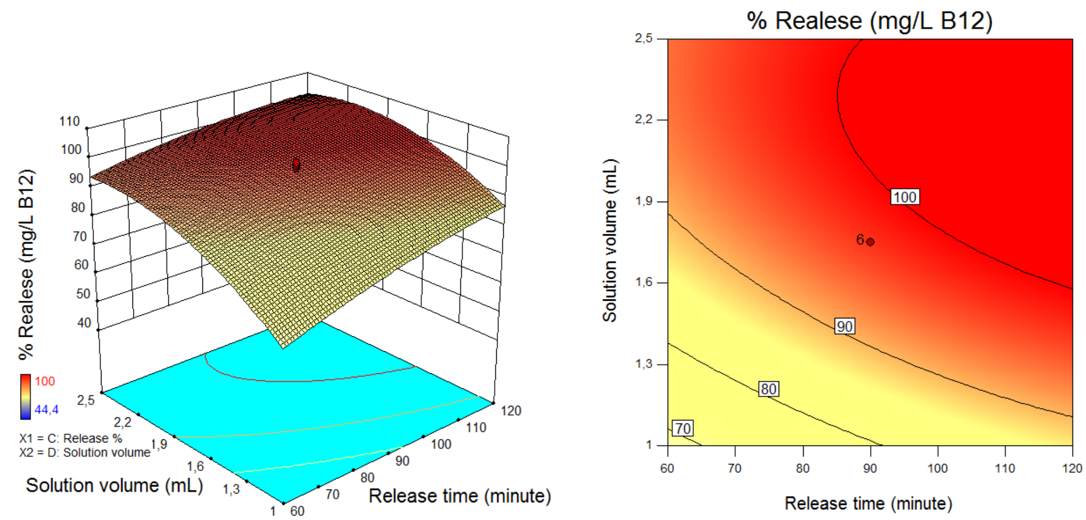


Figure 4.13. Effect of interaction between solution volume and release time a) 3D graph b) 2D graph

The 3D and 2D response surface plots of the effect of solution volume and release time on B12 release efficiency are presented in Figures 4.13(a) and Figures 4.13(b). The increase in solution volume and release time contributed positively to vitamin B12 release from biopolymer. Also, based on the ANOVA table, it was observed that the interactive effect of solution volume and release time was statistically significant ($p<0.01$). In the study conducted by de Abreu Figueiredo et al. (147), it was reported that solution volume and release time are associated with drug release from biopolymers. Nath et al. (148) investigated the effect of acrylic acid grafted gelatin/LDH-based biocompatible hydrogel with pH-controlled vitamin B12 release in their study. As a result of the study, they reported that the volume of the solution and the release time were related to the release of B12 from the biopolymer.

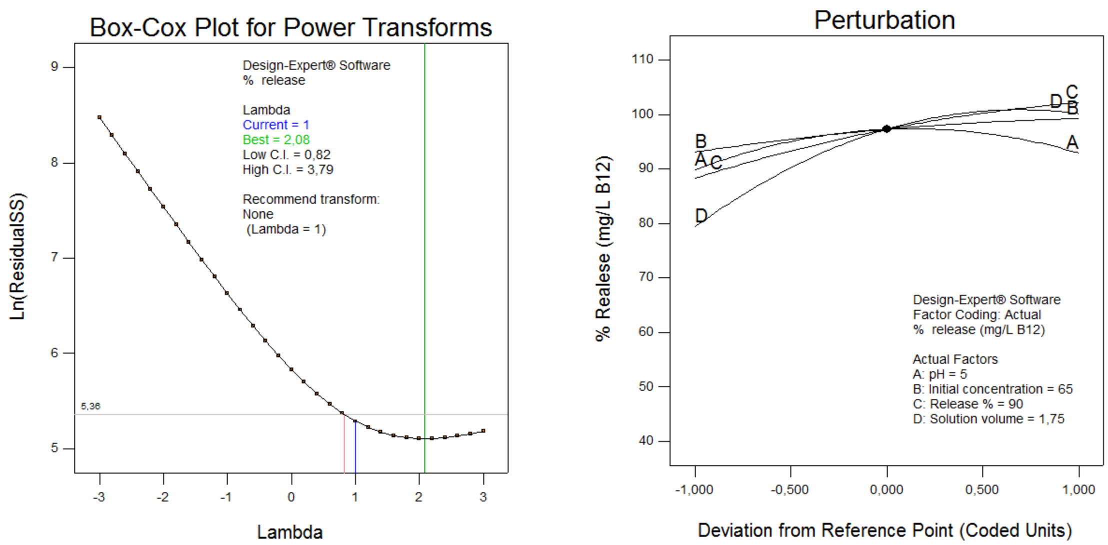


Figure 4.14. Diagnostic plots: Power conversion and disorder plot for vitamin B12 release from biopolymer at optimum conditions

In Figure 4.14 (a), a Box-Cox graph for vitamin B12 release was obtained to control the lambda (λ) value. The Box-Cox plot is often preferred diagnostic plot for estimating any necessary transformation of the experimental value to increase the significance of the model. As can be seen from the power conversion graph, it has been confirmed by the model that no conversion is needed according to the obtained λ value (λ: 1, the most ideal value is 0.78).

A perturbation graph (Figure 4.14 (b)) was performed to compare the influence of all factors on the optimum conditions for vitamin B12 release from biopolymer. It was also used to analyze the variation of factors as well as the combined effect of all factors on a process. In addition, a power conversion plot for vitamin B12 release was obtained to control the lambda (λ) value. Because the power conversion plot is often a preferred diagnostic plot to estimate any necessary conversion of the experimental value to increase the significance of the model. As can be seen from the power conversion graph, it has been confirmed by the model that there is no need for any conversion according to the obtained λ value (1).

To select the optimal model and the maximum desirability function obtained, the most important statistical metrics such as the lowest LOF and the highest p-value and the highest R^2 and F-value were searched. The desirability value of the optimization process was found as 1.0 and presented in Figure 4.15. The optimum values of the variables used for optimization and the highest desirability values obtained depending on these values were given in the same way.

The lower and upper limits of vitamin B12 release of the quadratic model, which were obtained at the end of the experimental study, are presented in Table 4.4 and the significance level was determined to be 3.

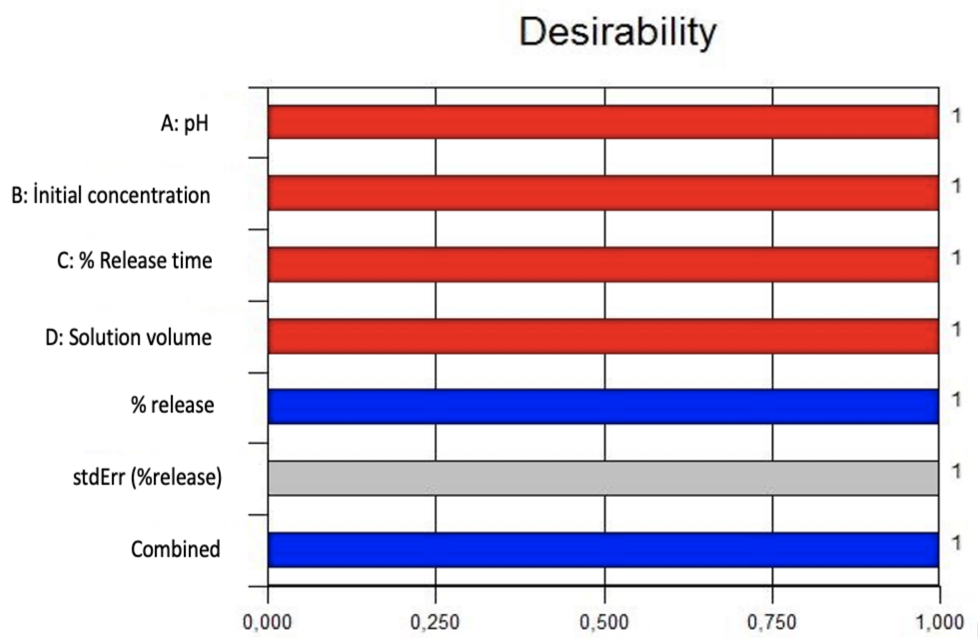


Figure 4.15. Desirability plot for vitamin B12 release from biopolymer at optimum conditions

Table 4.3. The lower-upper limits and severity of the quadratic model of vitamin B12 release from biopolymer

nstraints

me	**al**	**wer it**	**per it**	**wer ight**	**per ight**	**portance**
pH	n range					
Initial concentration	n range					
Release time	n range		)			
Solution volume	n range					
ease %	ximize	4	)			
Err (release %)	ıe	8335	751			

Based on the possible global solution (Table 4.4), validation experiments were performed to support the optimized data from numerical modeling under optimum conditions. The experimental results obtained were also compared with the possible vitamin B12 release results suggested by the quadratic model, and it was observed that the suggested model results and the validation values overlapped by 97.04%.

Table 4.4. Possible solutions

lutions								
mber	H	tial concentration	lease time	olution olume	lease %	lErr (Release %)	sirability	
1	:41	54.039	117.066	2.084	)1.258	1.627	1.000	lected
2	i60	74.436	111.860	l.938	)1.890	1.597	1.000	
3	:73	72.369	83.579	2.138	)0.370	1.429	1.000	
4	)33	54.903	108.754	2.240	)0.587	1.579	1.000	
5	i04	72.534	106.784	2.058	)2.901	1.436	1.000	
6	i36	68.508	113.608	l.892	)3.286	1.437	1.000	
7	:00	67.250	94.500	l.863	)0.094	1.468	1.000	
8	i62	77.469	117.750	2.373	)0.753	2.172	1.000	
9	)79	51.111	119.889	2.489	)0.050	2.227	1.000	
10	i79	71.394	94.881	2.355	)1.804	1.452	1.000	
11	l18	57.967	104.873	2.403	)1.113	1.518	1.000	
12	'52	79.317	116.573	l.899	)1.805	1.959	1.000	
13	l24	51.930	115.446	2.473	)1.011	1.935	1.000	
14	.53	69.467	90.213	2.148	)1.335	1.436	1.000	
15	l46	78.783	111.762	2.429	)1.497	1.852	1.000	
16	i32	74.078	94.113	l.820	)0.441	1.430	1.000	
17	:54	75.914	111.612	2.452	)2.348	1.712	1.000	
18	l29	71.344	117.734	l.871	)2.417	1.483	1.000	
19	:87	64.830	107.867	2.303	)1.983	1.464	1.000	
20	'09	68.688	93.893	2.289	)1.478	1.433	1.000	
21	)90	61.704	106.371	2.386	)0.861	1.510	1.000	
22	)59	68.664	107.579	l.894	)1.891	1.433	1.000	
23	:15	71.770	111.034	2.481	)2.220	1.615	1.000	
24	.71	79.770	91.874	2.372	)1.631	1.620	1.000	
25	i81	73.361	108.515	2.450	)2.446	1.613	1.000	

By loading the values obtained from the experimental design into the CCD system, the ramp chart of the optimum points of the independent variables in vitamin B12 release from biopolymer is given in Figure 4.16. Besides presenting the ranges of all variables in the figure, the desirability value was obtained as high as 1.000.

When the approval report (Table 4.5) of the quadratic model used was examined at the 95% confidence interval, it was determined that the standard deviation was obtained as 0.000 and the model was significant and valid. In addition, the coefficients obtained from the quadratic model and their significance are presented in Table 4.6.

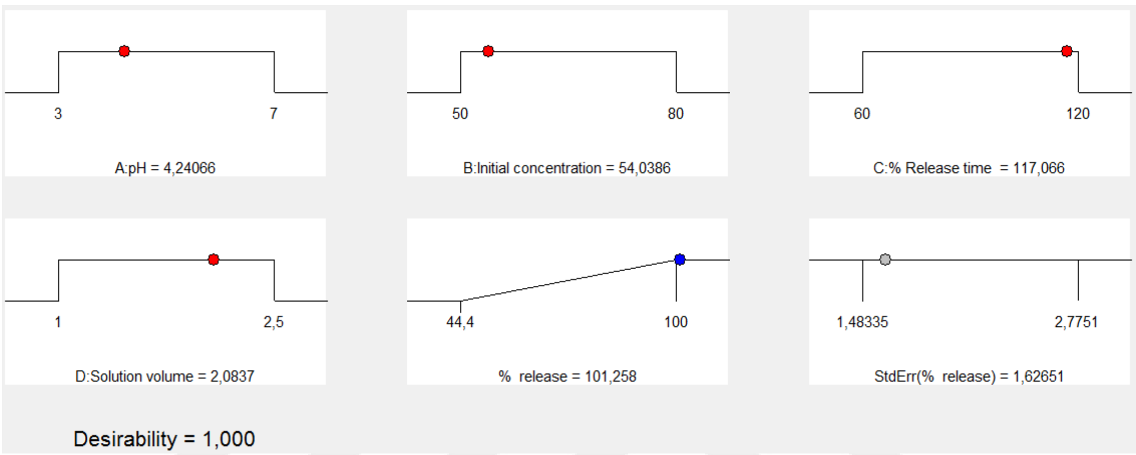


Figure 4.16. Ramp chart for statistically optimized factors for vitamin B12 release from biopolymer

Table 4.5. Confirmation report of the quadratic model at 95% confidence interval

ınfirmation Report

ʋo-sided **Confidence = %** **n =**

ctor	ıme	.evel	Low Level	High Level	Std. Dev.	Coding
	:	4.50	3.00	7.00	0.000	Actual
	tial concentration	0.36	50.00	80.00	0.000	Actual
	lease time	9.71	60.00	120.00	0.000	Actual
	lution volume	1.77	1.00	2.50	0.000	Actual

Table 4.6. Coefficients obtained from the quadratic model

nse	ept														
ease	33	75	92	58	21	13	625	062	562	437	587	51	51	26	51
		9	9	001	001	3	3	7	3	5	7	001	8	8	001
nd		1	p <.05	p <.10	10										

NOTE: Colored in red: Very important; colored green: Important; colored blue: Important; black color: unimportant

As can be seen from Table 4.7, it was observed that most of the coefficients derived from the quadratic model were very important or important, and the statistical effect of a few of them was insignificant.

5. CONCLUSION AND RECOMMENDATIONS

In recent studies, pH sensitive biopolymers have been developed and their use in colon-specific drug release has been investigated. Based on these studies, by loading vitamin B12 onto pH-sensitive biopolymers synthesized with chitosan, its release throughout the intestinal tract was studied. The synthesized biopolymers were characterized using FT-IR, TGA-DTA and SEM-EDS techniques. Biopolymers, whose characterization was completed and produced in film form, were used for release by loading B12. The following results were obtained by performing the experimental study design and multi-parameter optimization.

The proposed mathematical models (Equation 2) also provided critical analyzes for the interactive effects of selected independent variables on the vitamin B12 release process from pH-sensitive biopolymer.

It was concluded that the values of R^2 values (for chitosan-synthesized ebiopolymer material: R^2=0.9704, and R^2adjusted=0.9427) obtained from the analysis of variance (ANOVA) tables were a satisfactory level and the overlap ratios of the estimated and actual values were better than 97%. That is, it can be said that more than 97% of the values predicted by the model match the vitamin B12 release values experimentally on biopolymer material.

Based on the 3D and 2D plots, it was observed that the percentage of vitamin B12 release (%) of pH-sensitive biopolymer increased significantly as the pH increased from 3 to 5 and the initial concentration increased.

Data from the proposed three-factor CCD combined with RSM clearly confirmed that optimization is an effective approach to model the B12 release process of pH-sensitive biopolymer, understanding the relationships between independent and response variables and maximizing process efficiency.

In conclusion, it is undisputed that biopolymer materials synthesized, characterized and used are good candidates for the release of other drugs due to their release capacity.

6. REFERENCES

1. Ayhan, H., & Ayhan, F. Kontrollu ilaç salımı için fotoçapraz bağlı poli (etilen glikol) hidrojeller. *Turkish Journal of Biochemistry/Turk Biyokimya Dergisi*, 2014;*39*(4).

2. Bajpai, S. K., & Sharma, S. Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca2+ and Ba2+ ions. *Reactive and Functional Polymers*, 2004;*59*(2), 129-140.

3. Gupta, S., Sharma, A., & Verma, R. S. Polymers in biosensor devices for cardiovascular applications. *Current Opinion in Biomedical Engineering*, 2020;*13*, 69-75.

4. Jin, S. G., Yousaf, A. M., Kim, K. S., Kim, D. W., Kim, D. S., Kim, J. K., ... & Choi, H. G. Influence of hydrophilic polymers on functional properties and wound healing efficacy of hydrocolloid based wound dressings. *International journal of pharmaceutics*, 2016;*501*(1-2), 160-166.

5. Ghorbanloo, M., & Heydari, A. pH sensitive hydrogel based acrylic acid for controlled drug release. *Physical Chemistry Research*, 2017;*5*(1), 185-193.

6. Santos, J. A., Rempel, S., Mous, S. T., Pereira, C. T., ter Beek, J., de Gier, J.-W., Slotboom, D. J. Functional and structural characterization of an ECF-type ABC transporter for vitamin B12. eLife, 2018;7

7. Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., & Folkers, K. Crystalline vitamin B12. Science, 1948;107(2781), 396-397

8. Yılmaz, S. Vitamin B12 yetersizliğine yaklaşım. Konuralp Tıp Dergisi 2019;11(3): 482-488

9. Carmel R. Cobalamin-binding proteins in man. In Silber R, Gordon AS, LoBue J, et al, editors: Contemporary hematology-oncology, vol 2, New York, 1981, Plenum Publishing p 79–129

10. Ferrier, D.R, *Lippincott Illustrated Reviews: Biochemistry*, Seventh Edition, Wolters Kluwer 2017. p.375-77

11. Güngören, MS. *Mersin bölgesinde vitamin b12 ve folik asit düzeylerine ait referans aralıklarının belirlenmesi,* Uzmanlık Tezi, Mersin Üniversitesi Tıp Fakültesi Tıbbi Biyokimya Anabilim Dalı, Mersin, 2008.

12. Özkanay, H. *Vitamin d, vitamin b12, folat ve ferritin parametrelerinin biyolojik varyasyonu*, Uzmanlık Tezi, İzmir Kâtip Çelebi Üniversitesi Tıp Fakültesi Tıbbi Biyokimya Anabilim Dalı, İzmir, 2020

13. Goraya JS, Kaur S, Mehra B. *Neurology of Nutritional Vitamin B12 Deficiency in Infants:* Case Series From India and Literature Review. Journal of child neurology. 2015;30(13):1831 7.

14. Çuhadar, G. *Yetişkin bireylerde serum folat ve b12 vitamini düzeyleri ile duygudurum ve beslenme durumu arasındaki ilişkinin değerlendirilmesi,* Yüksek Lisans Tezi, Başkent Üniversitesi Sağlık Bilimleri Enstitüsü Beslenme Ve Diyetetik Anabilim Dalı Beslenme Ve Diyetetik Yüksek Lisans Programı, Ankara, 2021.

15. Murray, R., Bender, D., Botham, K. M., Kennelly, P. J., Rodwell, V., & Weill, P. A. Micronutrients, Vitamins & Minerals. *Harper's Illustrated Biochemistry. New York: McGraw Hill Medical* 2012;467-478

16. Antony AC. *Megaloblastic Anemias*. Twenty-Fir. Vol. 12, Hematology: Basic Principles and Practice. Elsevier Inc. 2018;514-545.e7 p.

17. Herbert V. Vitamin B-12: plant sources, requirements, and assay. The American Journal of Clinical Nutrition. 1988;48(3):852-8

18. Lachner, C., Steinle, N. I., & Regenold, W. T. The neuropsychiatry of vitamin B12 deficiency in elderly patients. The Journal of neuropsychiatry and clinical neurosciences, 2012;24(1), 5-15

19. Pitkin RM, Allen LH, Bailey LB, Bernfield M. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, Folate, vitamin B12, pantothenic acid, biotin and choline. Washington DC: National Academy Press, 2000.

20. Nielsen MJ, Rasmussen MR, Andersen CB, Nexo E, Moestrup SK. (2012). *Vitamin B12 transport from food to the body's cells--a sophisticated, multistep pathway.* Nat Rev Gastroenterol Hepatol. 9(6):345-54

21. Çağatay, Ü., Güvenç, B. (2004). *Megaloblastik Anemiler. Çeviri Editörü: Sağlıker Y. Harrison İç Hastalıkları Prensipleri*. 15. Baskıdan Çeviri. İstanbul: Nobel Tıp Kitabevleri, 2004: 674-680

22. Wong C.W. (2015). Vitamin B12 deficiency in the elderly: Is it worth screening? Hong Kong Med J. 21(2):155–64

23. Olteanu H, Banerjee R. (2001). *Cobalamin-dependent remethylatio*n. In: Carmel R, Jacobsen DW, editors. Homocysteine in health and disease. Cambridge UCUP 2001. p. 135–44

24. Clarke, S, Banfield K (2001). *S-adenosylmethionine-dependent methyltransferases*. In Carmel R, Jacobsen DW, editors: Homocysteine in health and disease, Cambridge, 2001, Cambridge University Press p. 63–78

25. Issac TG, Soundarya S, Christopher R, Chandra SR. (2015). *Vitamin B12 deficiency: an important reversible co-morbidity in neuropsychiatric manifestations*. Indian J Psychol Med. 37(1):26-9

26. Leal NA. (2004). B1*2 Metabolisms In Humans*. Dissertation for Doctora Of Philosophy. University of Florida. Florida

27. Rasmussen, S.A., Fernhoff, P.M., & Scanlon, K.S. (2001). *Vitamin B12 deficiency in children and adolescents, Medical Progress*, 2001; 10-7.

28. Reisner Jr, E. H., Weiner, L., Schittone, M. T., & Henck, E. A. (1955). Oral treatment of pernicious anemia with vitamin B12 without intrinsic factor. New England Journal of Medicine, 253(12), 502-506

29. Shao, J. S., Carmel, R., & Alpers, D. H. (2011). Production of ectopic gastric intrinsic factor in gastric mucosa of humans with chronic gastritis. *Digestive diseases and sciences*, *56*(11), 3209-3219

30. Sennett, C., Rosenberg, L. E., & Mellman, I. S. (1981). Transmembrane transport of cobalamin in prokaryotic and eukaryotic cells. Annual Review of Biochemistry, *50*(1), 1053-1086

31. Hewitt, J. E., Gordon, M. M., Taggart, R. T., Mohandas, T. K., & Alpers, D. H. (1991). Human gastric intrinsic factor: characterization of cDNA and genomic clones and localization to human chromosome 11. *Genomics*, *10*(2), 432-440

32. Li, N., Seetharam, S., & Seetharam, B. (1995). Genomic structure of human transcobalamin II: comparison to human intrinsic factor and transcobalamin I. *Biochemical and biophysical research communications*, *208*(2), 756-764

33. Nexø, E., & Olesen, H. (1982). Intrinsic factor, transcobalamin, and haptocorrin. *Dolphin D, editor. B*, *12*, 57-85

34. Morkbak, A. L., Poulsen, S. S., & Nexo, E. (2007). Haptocorrin in humans. Clin Chem Lab Med. 45(12):1751–9

35. Hardlei, T. F., & Nexo, E. (2009). A new principle for measurement of cobalamin and corrinoids, used for studies of cobalamin analogs on serum haptocorrin. *Clinical chemistry*, *55*(5), 1002-1010

36. Alpers, D. H., & Russell-Jones, G. J. (1999). Intrinsic factor, haptocorrin, and their receptors. *Chemistry and Biochemistry of B*, *12*, 411-440

37. Fyfe, J. C., Madsen, M., Højrup, P., Christensen, E. I., Tanner, S. M., de la Chapelle, A., ... & Moestrup, S. K. (2004). The functional cobalamin (vitamin B12)–intrinsic factor receptor is a novel complex of cubilin and amnionless. *Blood*, *103*(5), 1573-1579

38. Moestrup, S. K., Kozyraki, R., Kristiansen, M., Kaysen, J. H., Rasmussen, H. H., Brault, D., ... & Verroust, P. J. (1998). The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral membrane protein with homology to developmental proteins. *Journal of Biological Chemistry*, *273*(9), 5235-5242

39. Russell-Jones GJ, Alpers DH. (1999). Vitamin B12 transporters. Pharm Biotechnol. 12:493–520

40. Quadros, E. V., Nakayama, Y., & Sequeira, J. M. (2005). The binding properties of the human receptor for the cellular uptake of vitamin B12. *Biochemical and biophysical research communications*, *327*(4), 1006-1010

41. Miller, J. W., Garrod, M. G., Rockwood, A. L., Kushnir, M. M., Allen, L. H., Haan, M. N., & Green, R. (2006). Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. *Clinical chemistry*, *52*(2), 278-285

42. El Kholty, S., Gueant, J. L., Bressler, L., Djalali, M., Boissel, P., Gerard, P., & Nicolas, J. P. (1991). Portal and biliary phases of enterohepatic circulation of corrinoids in humans. *Gastroenterology*, *101*(5), 1399-1408

43. Allen, R. H., & Stabler, S. P. (2008). Identification and quantitation of cobalamin and cobalamin analogues in human feces. *The American journal of clinical nutrition*, *87*(5), 1324-1335

44. Lindemans, J., Kapel, J. V., & Abels, J. (1986). Uptake of transcobalamin II-bound cobalamin by isolated rat kidney tubule cells. *Scandinavian journal of clinical and laboratory investigation*, *46*(3), 223-232

45. Hazin, H., Neelakantappa, K., Ramasahayam, L., Abdel-Maksoud, A., Fogel, J., Moestrup, S. K., & Carmel, R. (2008). Vitamin B12 Handling by the Kidney, a Major Site of B12 Traffic: Normal and Abnormal Physiology. Blood, 16;112(11):1855

46. Christensen, E. I., Birn, H., Storm, T., Weyer, K., & Nielsen, R. (2012). Endocytic receptors in the renal proximal tubule. *Physiology*, *27*(4), 223-236

47. Birn, H., Willnow, T. E., Nielsen, R., Norden, A. G., Bönsch, C., Moestrup, S. K., ... & Christensen, E. I. (2002). Megalin is essential for renal proximal tubule reabsorption and accumulation of transcobalamin-B12. *American Journal of Physiology-Renal Physiology*, *282*(3), F408-F416

48. Acilmis, Y. G., Dikensoy, E., Kutlar, A. I., Balat, O., Cebesoy, F. B., Ozturk, E., ... & Pence, S. (2011). Homocysteine, folic acid and vitamin B12 levels in maternal and umbilical cord plasma and homocysteine levels in placenta in pregnant women with pre-eclampsia. *Journal of Obstetrics and Gynaecology Research*, *37*(1), 45-50

49. Vaz Pinto, A., Torras, V., Sandoval, J. F., Dillman, E., Mateos, C. R., & Cordova, M. S. (1975). Folic acid and vitamin B12 determination in fetal liver. The American Journal of Clinical Nutrition, 28(10), 1085-1086

50. Koç A. (2017), *Genetik Bozukluklarla İlişkili B12 Vitamini Eksiklikleri,* Uzmanlık Tezi, Marmara Üniversitesi Tıp Fakültesi Çocuk Hematolojisi ve Onkolojisi Bilim Dalı

51. Wang, X., Li, X., Chen, L., Xie, F., Yu, L., Li, B., (2011). Preparation and characterisation of octenyl succinate starch as a delivery carrier for bioactive food components. Food Chemistry, 126: 1218-1225

52. Jyothi, N. V. N., Prasanna, P. M., Sakarkar, S. N., Prabha, K. S., Ramaiah, P. S., Srawan, G. Y., (2010). Microencapsulation techniques, factors influencing encapsulation efficiency. Journal of Microencapsulation, 27(3): 187-197

53. Abbas, S., Azar, S., (2011). Efficiency of novel iron microencapsulation techniques: fortification of milk. International Journal of Food Science and Technology, 9(46): 1927-1933

54. Ersus, S., Yurdagel, U., (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota L.*) by spray drier. Journal of Food Engineering, 80: 805–812

55. Kunz, B., Krückeberg, S., Weissbrodt, J., (2003). Chancen und grenzen der mikroverkapselung in der modernen lebensmittelverarbeitung. Chemie Ingenieur Technik, 75: 1733-1740

56. Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., (2007), Application of spray-drying in microencapsulation of food ingredients: An overview. Food Research International, 40(5): 1107–1121

57. Desai, K.G.H., Park, H.J., (2005). Recent developments in microencapsulation of food ingredients. Drying Technology, 23(8): 1361–1394

58. Çoruhli T., (2013). Kara Dut Antosiyaninlerinin İyonik Jelasyon Yöntemi İle Enkapsülasyonu Ve Enkapsülasyon Parametrelerinin Tepki Yüzeyi Metodu İle Optimize Edilmesi, İstanbul Teknik Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İstanbul

59. Zuidam, N.J., Shimoni, E., (2010). Overview of microencapsulates for use in food products or processes and methods to make them. In: encapsulation technologies for active food ıngredients and food processing. Springer, London

60. McClements, D.J., (2009). Structural design principles for ımproved food performance: nanolaminated biopolymer structures in foods. In: micro/nanoencapsulation of active food ıngredients, American Chemical Society, 1: 4-7

61. Mazza, G., Miniati, E. (1993). Anthocyanins in fruits, vegetables and grains. CRC Press. 362, London, England

62. Shahidi, F., Han, X.Q., (1993). Encapsulation of food ingredients. Critical Review in Food Science and Nutrition, 33: 501–547

63. Bruschi, M.L., Cardoso, M.L.C., Lucchesi, M.B., Gremiao, M. P.D., (2003). Gelatin microparticles containing propolis obtained by spray-drying technique: preparation and characterization. International Journal of Pharmaceutics, 264(6): 45–55

64. Vos, P., Faas, M. M., Spasojevic, M., Sikkema, J., (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. International Dairy Journal, 20: 292-302

65. Adachi, S., Imaoka, H., Hasegawa, Y., Matsuno, R., (2003). Preperation of a water-in-oil-in-water (w/o/w) type microcapsules by a single-droplet-drying method and change in encapsulation efficiency of a hydrophilic substance during storage. Bioscience, Biotechnology and Biochemistry, 67: 1376- 1381

66. Bayram, O.A., Bayram, M., Tekin, A.R., (2005). Spray drying of sumac flavour using sodium chloride, sucrose, glucose and starch as carriers, Journal of Food Engineering, 69: 253–269

67. Kaushik, V., Roos, Y.H., (2005). Effects of different homogenisation processes on olive oil encapsulation in freeze dried sugar/gelatin matrices. 15th International Symposium on Microencapsulation, 2005, Parma, Italy

68. Hogan, S.A., McNamee, B.F., O'Riordan, E.D., O'Sullivan, M., (2001). Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. International Dairy Journal, 11(9): 137– 144

69. George, M., Abraham, T.E., (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review. Journal of Controlled Release, 114: 1–14

70. Ward, F.M. and Andon, S.A. (1993). Water-soluble gums used in snack foods and cereal products. Cereal Foods World, 38: 748-752

71. Glicksman, M. (1980). Food Hydrocolloids. Vol.1-3. CRC. Press, Boca Raton.F.L

72. Gong, R., Li, C., Zhu, S., Zhang, Y., Du, Y., Jiang, J., (2011). A novel pHsensitive biopolymer based on dual crosslinked alginate/N-a-glutaric acid chitosan for oral delivery of protein. Carbohydrate Polymers, 85: 869- 874

73. Yoo, S-H., Song, Y-B., Chang, P-S., Lee, H., G., (2006). Microencapsulation of α-tocopherol using sodium alginate and its controlled release properties. International Journal of Biological Macromolecules, 38(1): 25–30

74. Cemeroğlu, B., Yemenicioğlu, A. and Özkan, M. (2001). Meyve ve sebzelerin bileşimi, Soğukta depolanmaları, Meyve ve Sebze İşleme Teknoloisi, Cilt I, s. 1–328, Gıda Teknolojisi Derneği Yayınları, Ankara

75. Madene, A., Jacquot, M., Scher, J., Desobry, S., (2006). Flavour encapsulation and controlled release – a review. International Journal of Food Science and Technology, 41: 1–21

76. Krishnan, S., Bhosale, R., Singhal, R.S. (2005). Microencapsulation of cardamom oleoresin: Evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials. Carbohydrate Polymers, 61: 95–102

77. McNamee, B.F., O'Riordan, E.D., O'Sullivan, M., (1998). Emulsification and Microencapsulation properties of gum Arabic. Journal of Agricultural Food Chemistry, 46: 4551–4555

78. Bertolini, A.C., Siani, A.C., Grosso, C.R.F., (2001). Stability of monoterpenes encapsulated in gum arabic by spray-drying. Journal of Agriculture and Food Chemistry, 49: 780–785

79. Beristain, C.I., Garcia, H.S., Vernon-Carter, E.J., (2001). Spray-dried encapsulation of cardamom (Elettaria cardamomum) essential oil with mesquite (*Prosopis juliflora*) gum. Lebensmittel-Wissenschaft undTechnologie, 34: 398–401

80. Luo, Y., Zhang, B., Whent, M., Yu, L. Wang, Q., (2011). Preparation and characterization of zein/chitosan complex for encapsulation of α-tocopherol, and its in vitro controlled release study. Colloids and Surfaces Biointerfaces, 85: 145–152

81. Abreu, F.O.M.S., Bianchini, C., Forte, M.M.C., Kist, T.B.L., (2008). Influence of the composition and preparation method on the morphology and swelling behavior of alginate–chitosan biopolymers. Carbohydrate Polymers, 74: 283–289

82. Lee, P.S., Yim, S.G., Choi, Y., Van Anh Ha, T., Ko, S., (2012). Physiochemical properties and prolonged release behaviours of chitosan-denatured β-lactoglobulin microcapsules for potential food applications. Food Chemistry, 2(34): 92-98

83. Perrechil, F.A., Sato, A.C.K., Cunha, R.L., (2011). κ-Carrageenan - sodium caseinate microgel production by atomization: Critical analysis of the experimental procedure. Journal of Food Engineering, 104: 123-133

84. Caiyuan Y., Wei W., Hui Y., Hongjing L., (2007). preparation of phospholipid microcapsule by spray drying. Drying Technology, 25: 695–702

85. Selim, K., Tsimidou, M., Biliaderis, C.G., (2000). Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. Food Chemistry, 71(3):199-206

86. Faldt, P., Bergenstahl, B., (1996). Spray-dried whey protein/lactose/soy-bean oil emulsions. 1. Surface composition and particle structure. Food Hydrocolloids, 10: 421–429

87. Gouin, S., (2004). Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science and Technology, 15: 330-347

88. Zuidam, N.J., Nedovic, V.A., (2010). Encapsulation Technologies for Active Food Ingredients and Food Processing. Springer, London

89. Gonnet, M., Lethuaut, L., Boury, F., (2010). New trends in encapsulation of liposoluble vitamins. Journal of Controlled Release, 146: 276-290

90. Lakshmi V., Sudha T., Dandona R., Teja V.D., Kumar G.A., Dandona L., (2009). Application of human immunodeficiency virus type 1 BED enzyme immunoassay on dried blood spots in India. Journal of Medical Microbiology, 58: 312–317

91. Çilek B., (2012). Microencapsulation of Phenolic Compounds Extracted From Sour Cherry (*Prunus cerasus L.*) Pomace, Orta Doğu TeknikÜniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Ankara

92. Shi, P., He, P., Teh, T.K.H., Morsi, Y.S., Goh, J.C.H., (2011). Parametric analysis of shape changes of alginate beads. Powder Technology, 210: 60- 66

93. Acosta, E., (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Current Opinion in Colloid & Interface Science, 14: 3– 15

94. Davarcı F., (2012). Effects of Some Physical Parameters on Penetration, Size and Shape in Alginate Gel Microencapsulation, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İstanbul

95. Banville, C., Vuillemard, J. C., Lacroix, C., (2000). Comparison of different methods for fortifying Cheddar cheese with vitamin D. International Dairy Journal, 56(10): 375-382

96. Gazori, T., Khoshayand, M.R., Azizi, E., Yazdizade, P., Nomani, A., Haririan, I., (2009). Evaluation of Alginate/Chitosan nanoparticles as antisense delivery vector: Formulation, optimization and in vitro characterization. Carbohydrate Polymers, 77: 599–606

97. Beristain, C.I., Vazquez, A., Garcia, H.S., Vernon-Carter, E.J., (1996). Encapsulation of orange peel oil by cocrystallization. Lebensmittel-Wissenschaft und Technologie, 29: 645–647

98. Jakson, L.S., Lee, K., (1991). Microencapsulation and food industry. Lebensmittel-Wissenschaft und Technologie, 24: 289-297

99. Schrooyen, P. M. M., Van der Meer, R., De Kruif, C. G., (2001). Microencapsulation: its application in nutrition. Proceedings of the Nutrition Society, 60(4): 475-479

100. Abbas, S., Da Wei, C., Hayat, K., Zhang, X. M., (2012). Ascorbic acid: microencapsulation techniques and trends-a review. Food Reviews International, 28(4): 343-374

101. Zimet, P., Livney, Y.D., (2009). Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for ω-3 polyunsaturated fatty acids. Food Hydrocolloids, 23:1120-1126

102. Özgen, S., (2014). Domatesten Likopen İzolasyonuna Yönelik Monodispers Kromatografik Kolon Dolgu Maddelerinin Sentezi, Likopenin Saflaştırılması ve Enkapsülasyonu. Erciyes Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, Kayseri

103. Silva, L. R., Davim, J. P., Festas, A., & Abrão, A. M. (2009). Machinability aspects concerning micro-turning of PA66-GF30-reinforced polyamide. The International Journal of Advanced Manufacturing Technology, 41(9), 839-845

104. Cheng, K., Teraguchi, M., Kaneko, T., & Aoki, T. (2021). Improved oxygen permeation of a multi-stranded network two-dimensional polymer synthesized by three-step polymerizations of a novel monomer bearing three different polymerizable groups followed by photoexfoliation. *Polymer*, *228*, 123857

105. Aliyeva, N., Sas, H. S., & Okan, B. S. (2021). Recent developments on the overmolding process for the fabrication of thermoset and thermoplastic composites by the integration of nano/micron-scale reinforcements. *Composites Part A: Applied Science and Manufacturing*, 106525

106. Calejo M. T. R. (2013). *Thermoresponsive polymers in controlled drug delivery and gene delivery.* (Thesis submitted for the degree of Philosophiae Doctor). University of Oslo, School of Pharmacy, Faculty of Mathematics and Natural Sciences, Oslo

107. Ilmain, F., Tanaka,T., Kokufuta, E., (1991). Volume Transition in a Gel Driven by Hydrogen Bonding. *Nature*, 349, 400-401

108. Reyes-Ortega, F., (2014). pH-responsive polymers: properties, synthesis and applications, Maria Rosa Aguilar (Ed.), Smart Polymers And Their Aplication (45-92), Woodhead Publishing, 650s, Spain

109. Grainger, S. J. and El-Sayed, M.E.H., (2010). Stimuli-Sensitive Particles for Drug Delivery. Esmaiel Jabbari and Ali Khademhosseini (Ed.) In Biologically- Responsive Hybrid Biomaterials (171-190), World Scientific, 432s, Singapore

110. Şimşek, C., (2016). İlaç İçeren NIPAMm Hidrojellerinin Salınım Kinetiğinin İncelenmesi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İstanbul

111. Camishra, A., Pande, P.P., (2017). Polymer Biopolymers and Their Applications. *International Journal of Materials Science*, 12 (1): 11-14

112. Calvert, P., (2009). Biopolymers for soft machines. *Advanced Materials*, 21: 743-756

113. Ottenbrite R.M., Park K., Okano T., (2010). *Biomedical Applications of Biopolymers Handbook.* Springer New York. 438

114. Ozay, H., & Ozay, O. (2014). Synthesis and characterization of drug microspheres containing phosphazene for biomedical applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *450*, 99-105

115. Prabhakar, M.N., Sudhakara, P., Subha, M.C.S., Rao, K.C., Song, J., (2015). Novel thermoresponsive biodegradable nanocomposite biopolymers for dual function in biomedical applications. *Polymer Plastic Technology and Engineering* 54: 1704-1714

116. Ozay, H., & Ozay, O. (2013). Rhodamine based reusable and colorimetric naked-eye biopolymer sensors for Fe3+ ion. *Chemical engineering journal*, *232*, 364-371

117. Fang, R., He, W., Xue, H., Chen, W., (2016). Synthesis and characterization of a high-capacity cationic biopolymer adsorbent and its application in the removal of Acid Black 1 from aqueous solution. *Reactive and Functional Polymers* 102: 1-10

118. Khan, M., Lo, I.M.C., (2017). Removal of ionizable aromatic pollutants from contaminated waterusing nano ɣ-Fe2O3 based magnetic cationic biopolymer: Sorptive performance, magnetic separation and reusability. *Journal of Hazardous Materials* 322: 195-204

119. Kabiri, M., Emami, S.H., Rafinia, M., Tahriri, M. (2011). Preparation and characterization of absorbable hemostat crosslinked gelatin sponges for surgical application. *Current Applied Physics*, 11: 457-461

120. Ekici, S., (2016). Kitosan temelli hidrojellerin hazırlanması ve 5-Florourasil salımı davranışlarının incelenmesi. *Balıkesir Üniversitesi - Fen Bilimleri Enstitüsü* 18 (2): 12-24

121. Baker, Ş.B., (2012). Kontrollü ilaç salım hidrojel sistemleri ve uygulama çalışmaları, Yüksek Lisans Tezi, Osmangazi Üniversitesi, Eskişehir

122. Blagoeva, Rumiana & Assen, Nedev. (2006). Monolithic Controlled Delivery Systems: Part I. Basic Characteristics and Mechanisms. International Journal Bioautomation. 4.

123. Reddy, A & Karthikeyan, Ramadoss & Vejandla, Ramya & Babu, Puttagunta. (2017). Controlled release matrix drug delivery system – a review. Int. J. of Allied Med. Sci. and Clin. Research. 5. 2017-384

124. Lee, J. H., & Yeo, Y. (2015). Controlled drug release from pharmaceutical nanocarriers. *Chemical engineering science*, *125*, 75-84

125. Kişmir Y. (2011), Bazı polimerik jellerin hazırlanması ve ilaç aktif madde salınımında kullanılması, Doktora Tezi, İstanbul Üniversitesi, İstanbul

126. Ince, M., & Ince, O. K. (Eds.). (2019). *Hydrocarbon Pollution and its Effect on the Environment*. BoD–Books on Demand.

127. Zhang, Z., Li, J., Zhao, X. Q., Wang, J., Wong, G. K. S., & Yu, J. (2006). KaKs_Calculator: calculating Ka and Ks through model selection and model averaging. *Genomics, proteomics & bioinformatics*, *4*(4), 259-263.

128. Qi, L., Xu, Z., Jiang, X., Hu, C. and Zou, X., (2004). Preparation and antibacterial activity of chitosan nanoparticles, Carbohydrate Research, 339, 2693–2700

129. Tangpasuthadol, W., Pongchaisirikul, N. and Hoven, V. P., (2003). Surface modification of chitosan films. effects of hydrophobicity on protein adsorption, Carbohydrate Research 338, 937–942

130. Osman, Z. and Aro, A.K., (2003). FTIR studies of chitosan acetate based polymer electrolytes, Electrochimica Acta, 48, 993-999

131. Yuan, Q., Hein, S. and Misra R.D.K., (2010). New generation of chitosan-encapsulated ZnO quantum dots loaded with drug: Synthesis, characterization and in vitro drug delivery response, Acta Biomaterialia, 6, 2732–2739

132. Boonsongrit, Y., Mueller, W. B. and Mitrevej, A., (2008). Characterization of drug-chitosan interaction by H NMR, FTIR and isothermal titration calorimetry, European Journal of Pharmaceutics and Biopharmaceutics, 69, 388-395

133. Saçak, M., (2005). Polimer Kimyası, Gazi Kitapevi, Ankara, 431

134. Sandak, A., Sandak, J., Babinski, L., Pauliny D., Riggio, M., (2014). Spectral analysis of changes to pine and oak wood natural polymers after short-term waterlogging, Polymer Degradation and Stability, 99, 68-79

135. Mogosanu, D. G., Grumezescu, A. M, (2013). Natural and synthetic polymers for wounds and burns dressing, International Journal of Pharmaceutics, 463, 127-136

136. Cárdenas, G,.Bernal L, Tagle L.H. (1992). Thermochim. Acta, 195, 33

137. Cárdenas, G., Retamal J, Tagle L. H (1993). Polymeric Mater. Intern. J. 21, 137

138. Kittur, F.S, Harish Prashanth, K.V, Udaya Sankar, K, Tharanathan, R.N (2002). Carbohydrate Polymers, 2002, 49, 185

139. Hu, X., Liu, S., Zhou, G., Huang, Y., Xie, Z., & Jing, X. (2014). Electrospinning of polymeric nanofibers for drug delivery applications. *Journal of controlled release*, *185*, 12-21

140. Öner, F. (2002). Biyomalzemeler, Biyoetkin Moleküller ve Kontrollü Salım. *Yeni Ufuklar: Biyomalzemeler, Bilim ve Teknik Dergisi*, *416*

141. Çelik, G. (2013). İlaç Yüklü Nanolif Sistemlerden Kontrollü İlaç Salınımı. *Yüksek Lisans Tezi Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Kimya Anabilim Dalı*

142. Mendes, A. C., Gorzelanny, C., Halter, N., Schneider, S. W., & Chronakis, I. S. (2016). Hybrid electrospun chitosan-phospholipids nanofibers for transdermal drug delivery. *International journal of pharmaceutics*, *510*(1), 48-56

143. Baskakova, A., Awwad, S., Jiménez, J. Q., Gill, H., Novikov, O., Khaw, P. T., Williams, G. R. (2016). Electrospun formulations of acyclovir, ciprofloxacin and cyanocobalamin for ocular drug delivery. *International Journal of Pharmaceutics*, *502*(1–2), 208–218

144. Maiti, M., Roy, A., & Roy, S. (2014). Effect of pH and amphiphile concentration on the gel-emulsion of sodium salt of 2-dodecylpyridine-5-boronic acid: Entrapment and release of vitamin B12. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *461*, 76-84

145. Bajpai, S. K., & Dubey, S. (2005). In vitro dissolution studies for release of vitamin B12 from poly (N-vinyl-2-pyrrolidone-co-acrylic acid) hydrogels. *Reactive and Functional Polymers*, *62*(1), 93-104

146. Moradi, H., Khadiv-Parsi, P., Karimi Sabet, J., & Esmaeili, M. (2020). Simulation of Release of Vitamin B12 on Hydrogel Drug Delivery Systems. *Iranian Chemical Engineering Journal*, *18*(107), 28-35

147. de Abreu Figueiredo, J., Teixeira, M. A., Campelo, P. H., Lago, A. M. T., de Souza, T. P., Yoshida, M. I., ... & Borges, S. V. (2020). Encapsulation of camu-camu extracts using prebiotic biopolymers: Controlled release of bioactive compounds and effect on their physicochemical and thermal properties. *Food Research International*, *137*, 109563.

148. Nath, J., Ahmed, A., Saikia, P., Chowdhury, A., & Dolui, S. K. (2020). Acrylic acid grafted gelatin/LDH based biocompatible hydrogel with pH-controllable release of vitamin B12. *Applied Clay Science*, *190*, 105569

CURRICULUM VITAE

Personal information

Name	Ali	**Surname**	Önal

Education status

Degree	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Master degree	Beslenme ve Diyetetik	**Yeditepe Üniversitesi**	2022
Graduation	Gıda Mühendisliği	**Munzur Üniversitesi**	2018

Scientific Studies

Articles published in journals entering SCI, SSCI, AHCI indexes

- Hevidar Alp, Muharrem Ince, Olcay Kaplan Ince, **Ali Onal** (2020) Adsorptive removal of Eriochrome Black-T using Agaricus campestris: Parameters optimization with response surface methodology. Desalination and Water Treatment, in press.
- H Alp, M Ince, OK Ince, **A Onal** (2020) Evaluation the Weekly Intake of Some Wild Edible Indigenous Mushrooms Collected in Different Regions in Tunceli, Turkey. Biological Trace Element Research, in press.
- O Serdar, A Pala, M Ince, **A Onal** (2019) Modelling cadmium bioaccumulation in *Gammarus pulex* by using experimental design approach, Chemistry and Ecology, 40(3), 98-103.
- Aysegul Pala, Osman Serdar, Muharrem Ince, and **Ali Onal**. (2019) Modeling Approach with Box-Behnken Design for Optimization of Pb Bioaccumulation Parameters in *Gammarus pulex* (L., 1758), Atomic Spectroscopy, Accepted in press.
- Kaplan Ince, O., Ince, M., **Onal, A.** 2018. Response surface modeling of Pb(II) removal from alcoholic beverage by clay: Process optimization using Box-Behnken experimental design, Atomic Spectroscopy, 39(6): 242-250.
- Ince, M., Kaplan Ince, O., Asam, E., **Onal, A**. 2017. Using Food Waste Biomass as Effective Adsorbents in Water and Wastewater Treatment for Cu (II) Removal. Atomic Spectroscopy, 38(5): 142-148.

Papers presented at international scientific meetings and published in the proceedings book

- 6. International Congress on Food Technology, Athens-Greece, 18-19 March 2017.
- International Conference on Agriculture, Forest, Food Sciences and Technologies (ICAFOF), Cappadocia-Turkey, 15-17 May 2017.
- 4th Edition of International Conference on Environmental Science and Technology, Vienna-Austria, 29-31 2018.
- 2nd International Conference on Agriculture, Forest, Food Sciences and Technologies (ICAFOF), Çeşme/İzmir-Turkey, 02-05 April 2018.
- 3. International Conference on Civil and Environmental Engineering (ICOCEE), Çeşme/İzmir-Turkey, 24-27 April 2018.
- International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2018), Ankara-Turkey, 26-27 April 2018.

Publications in the proceedings of refereed conferences/symposiums

1. Ince Muharrem, Alp Hevidar, Kaplan Ince Olcay, **Onal Ali** (2018). Bio-removal potential of *Pleurotus eryngii var. ferulae* for methylene blue: variables optimization using experimental design approach, International Health Sciences Conference (IHSC 2018), November. (Full Text)
2. Kaplan Ince Olcay, Ince Muharrem, Alp Hevidar, **Onal Ali** (2018). The investigation of element contents of wild growing edible mushrooms: comparison of different habitats in Tunceli region, International Health Sciences Conference (IHSC 2018), November.
3. Alp Hevidar, Kaplan Ince Olcay, Ince Muharrem, **Onal Ali** (2018). Multivariate analysis of methylene blue: proses optimization using experimental design approach, International Engineering and Natural Sciences Conference (IENSC 2018), November. (Full Text)
4. Kaplan Ince Olcay, Alp Hevidar, Ince Muharrem, **Onal Ali** (2018). Bio-decolorization of Eriochrome Black T using experimental design approach, International Engineering and Natural Sciences Conference (IENSC 2018), November. (Full Text)
5. Ince Muharrem, Alp Hevidar, Kaplan Ince Olcay, **Onal Ali** (2018). Comparison of Copper Level in Some Wild Edible Mushrooms Collected From Tunceli Province, Turkey. 4th Edition of International Conference on Environmental Science & Technology-Vienna/Austria.
6. Ince Muharrem, Kaplan Ince Olcay, **Onal Ali** (2018). Process optimization for Pb (II) removal from alcoholic beverage by clay: Using Box-Behnken experimental design. 4th Edition of International Conference on Environmental Science & Technology-Vienna/Austria.
7. **Onal Ali**, Ince Muharrem, Kaplan Ince Olcay, Benzer Fulya, Kandemir F.M. (2018). A Comparison of Trace Metal Levels in Doxorubicin Induced Rat Liver Using ETAAS. 4th Edition of International Conference on Environmental

Science & Technology-Vienna/Austria.

8. **Onal Ali**, Kaplan Ince Olcay, Ince Muharrem (2018). Application of Response Surface Methodology to Optimize the Process Variables for Cupper Ion Determination in Beer Using Clay. 4th Edition of International Conference on Environmental Science & Technology-Vienna/Austria.
9. Ince Muharrem, Alp Hevidar, Kaplan Ince Olcay**, Onal Ali** (2018). Decolorization of Methylene Blue from Aqueous Solution by *Agaricus campestris* (AC) using Response Surface Methodology. 3. International Conference on Civil and Environmental Engineering-Çeşme-İzmir/Turkey
10. Kaplan Ince Olcay, Alp Hevidar, Ince Muharrem**, Onal Ali** (2018). Determination of zinc contents in Tunceli species of wild growing edible mushrooms: Comparison of different habitats. 3. International Conference on Civil and Environmental Engineering-Çeşme-İzmir/Turkey
11. Alp Hevidar, Kaplan Ince Olcay, Ince Muharrem**, Onal Ali** (2018). Comparison of iron contents in different habitat of some wild edible mushrooms collected from Tunceli-Turkey. International Eurasian Conference on Biological and Chemical Sciences-Ankara/Turkey.
12. Alp Hevidar, Kaplan Ince Olcay, Ince Muharrem**, Onal Ali** (2018). Evaluation of copper contents of selected three wild edible mushrooms collected from Tunceli, Turkey: A comparative study. International Eurasian Conference on Biological and Chemical Sciences-Ankara/Turkey.
13. Ince Muharrem, Alp Hevidar, Kaplan Ince Olcay**, Onal Ali** (2018). Experimental design and response surface modeling for optimization of Eriochrome Black T removal from aqueous solution using *Langermannia gigantea.* International Eurasian Conference on Biological and Chemical Sciences-Ankara/Turkey.
14. Kaplan Ince Olcay, Alp Hevidar, Ince Muharrem**, Onal Ali** (2018). Response surface methodology and process optimization of methylene blue using central composite design. International Eurasian Conference on Biological and Chemical Sciences-Ankara/Turkey.
15. Ince Muharrem, Alp Hevidar, Kaplan Ince Olcay, **Onal Ali** (2018). Adsorption of Erichrome Black T from Aqueous Solution onto AC: Process optimization using designed experiments. International conference on Agriculture, Forests, Food Sciences and Technoogies-İzmir/Turkey.
16. Ince Muharrem, Alp Hevidar, Kaplan Ince Olcay, **Onal Ali** (2018). Response surface methodology for the modelling of methyl orange removal from aqueous solutions using *Langermannia gigantea.* International conference on Agriculture, Forests, Food Sciences and Technoogies-İzmir/Turkey.
17. Kaplan Ince Olcay, Ince Muharrem, Alp Hevidar, **Onal Ali** (2018). Effect of species and region on iron and zinc contents in wild edible mushrooms. International conference on Agriculture, Forests, Food Sciences and Technoogies-İzmir/Turkey.
18. Ince M., Kaplan Ince O., Asam E., **Onal A.** (2017). Monitoring Weekly Changes of Element Levels in the Milk Using Faas. 6th International Congress

on Food Technology. Greece-Athens.

19. Eryilmaz H., Kaplan Ince O., Yuksel E., Ince M., **Onal A**., Asam E. (2017). Some Chemical and Physical Parameters of Kefir. 6th International Congress on Food Technology. Greece-Athens.
20. Karaca Sanyurek N., Kaplan Ince O., Celik G., **Onal A.** (2017). Antioxidant activity of Mardin Assyrian Wines. 6th International Congress on Food Technology. Greece-Athens.
21. Kaplan Ince O., Ince M., **Onal A.** (2017). Application an Experimental Design Methodology to Copper Removal from Wastewater Using a Green and Economical Sorbent. International Conference on Agriculture, Forest, Food Science and Technologies. Capadocia-Turkey.
22. **Onal A.,** Ince M., Kaplan Ince O., Karaca Sanyurek N. (2017). Comparison elemental content of home-made and factory-produced Mardin Assyrian wines. International Conference on Agriculture, Forest, Food Science and Technologies. Capadocia-Turkey.
23. Ince M., Kaplan Ince O., **Onal A.** (2017). Copper Sorption on Clay: Application of Response Surface Methodology. International Conference on Agriculture, Forest, Food Science and Technologies. Capadocia-Turkey.

Other (Projects Involved/Certificates/Awards)

➢ Jasco FT-IR 6700 model cihaz kullanım eğitimi (Tetra Teknolojik Sistemleri-2018) ➢ ISO 27001 Bilgi Güvenliği Yönetim Sistemi (Information Security Management System) ➢ 14001-2015 Çevre Yönetim Sistemi (Environmental Mana Gement System) ➢ TKY-Toplam Kalite Yönetimİ (Total Quality Management) ➢ ISO 50001 Enerji Yönetim Sistemi (Energy Management System) ➢ 9001-2015 Kalite Yönetim Sistemİ (9001-2015 Quality Management System) ➢ 18001-2007 OHSAS İş Sağlığı ve Güvenliği (Occupational Healthy and Safety) ➢ Entegre Yönetim Sistemi (Integrated Management System) ➢ Mülakat Teknikleri (Interview Techniques) ➢ 19011-2011 İç Denetçi (Internal Auditor) ➢ 10002 Müşteri Memnuniyeti (Customer Happiness) ➢ Proje Geliştirme Eğitimi (Project Development Training) ➢ Kriz Yönetimi (Crisis Management) ➢ Stratejik Yönetim (Strategic Management) ➢ 22000-2005 HACCP Gıda Güvenliği (Food Safety System) ➢ Risk Analizi (Risk Analysis)
➢ Munzur Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2014-2015 öğretim yılı bahar dönemi Onur Belgesi. ➢ Munzur Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2015-2016 öğretim yılı güz dönemi Onur Belgesi. ➢ Munzur Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2015-2016 öğretim yılı bahar dönemi Onur Belgesi. ➢ Munzur Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2016-2017 öğretim yılı güz dönemi Onur Belgesi. ➢ Munzur Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2016-2017 öğretim yılı bahar dönemi Onur Belgesi. ➢ Munzur Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 2017-2018 öğretim yılı güz dönemi Yüksek Onur Belgesi.