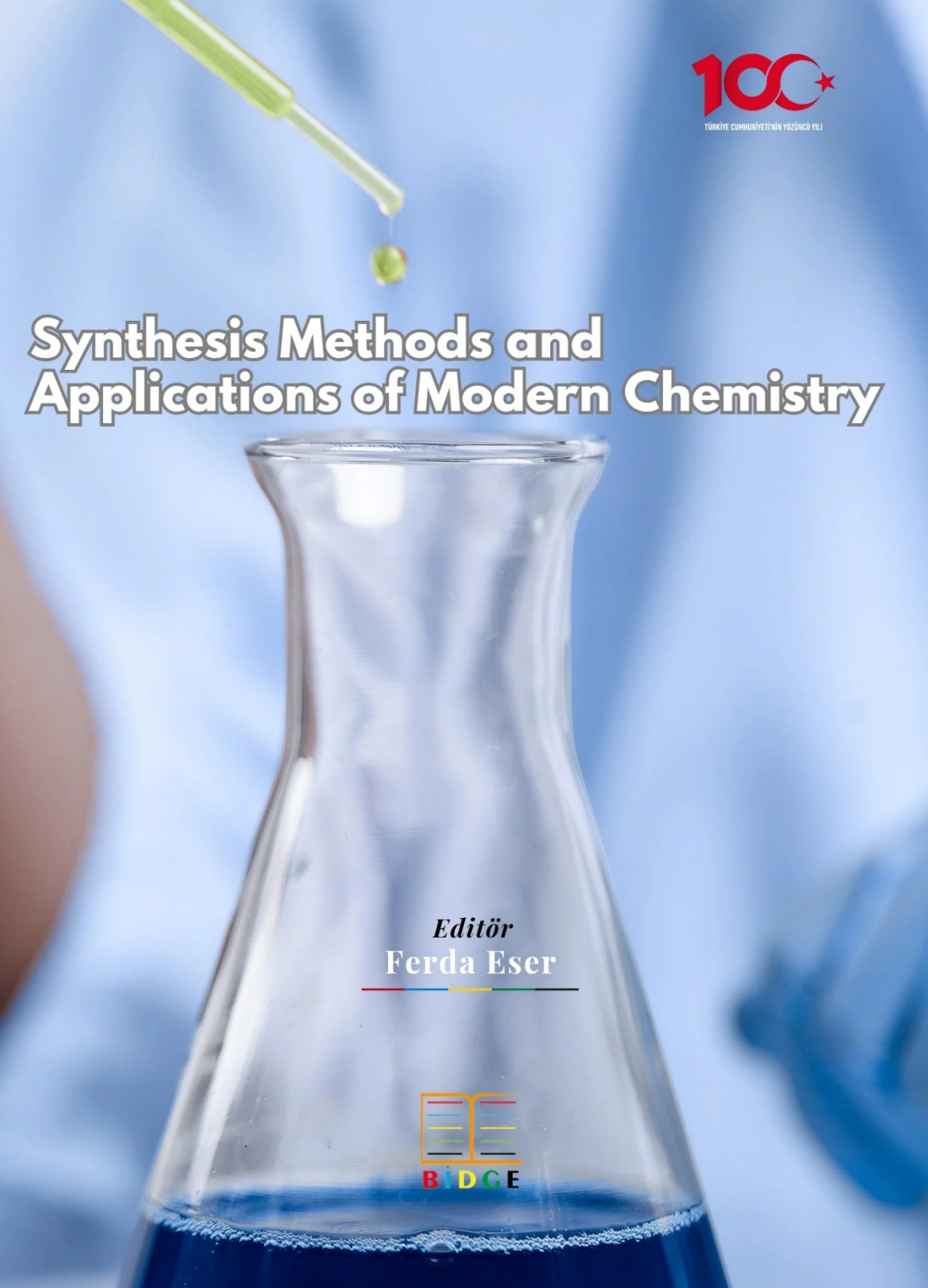
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Content

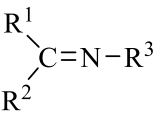
CHAPTER I

Synthesis of New Schiff Base-Palladium Complex

Emine Özge KARACA[1]

Introduction

Schiff bases (Figure 1), first synthesized in 1864 by Nobel Prize-winning German chemist Hugo Schiff, are compounds obtained by condensation of primary amines with carbonyl compounds [Schiff, 1869]. Schiff bases (imines) have become compounds of interest due to their stability and easy synthesis. This interest in imines can be explained by their usability in many biological systems, chemical catalysis, medicine and pharmacy, chemical analysis and new technologies [Upadhyay, 2008].



R^1,R^2 ,R^3= aryl or alkyl

Figure 1. *General display of Schiff bases*

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The importance of coordination compounds in both biological systems and industry is increasing day by day. Schiff bases are also among the ligands widely used in coordination chemistry. The metal complexes of Schiff bases, whose use as ligands was first reported by Pfeiffer in 1933, are being studied with interest [Pfeiffer, 1933; Seçkin, 2003]. Metal-imine complexes have been widely investigated due to their use as antitumor and herbicidal [Ashraf, 2011]. It has been reported that they have a protective effect on the hematopoietic system [Ozaslan, 2011]. In addition to their use as antivirals, they are considered antibacterial and antifungal agents. They are also used in the treatment of diabetes and AIDS [Golcu, 2005; Silva, 2011; Rehman, 2004]. In addition, Schiff bases are used in many areas such as corrosion inhibitors [Emregül, 2006], cation carriers, ion-selective electrode production [Aydınlı, 2006], and the paint industry [Serin, 1988].

In this study, new Schiff base compound suitable for green chemistry were synthesized, and it was aimed to contribute to the literature by elucidating their structures with spectrochemical methods.

METHOD

Since some synthesized compounds are sensitive to air humidity and oxygen, all experiments were carried out in an inert atmosphere and the Schlenk technique was used in the reactions. Before using the glass materials used in the reactions, vacuum was applied and heated to remove moisture and oxygen, and then they were filled with argon gas. Reagents used in the reactions were purchased commercially from Sigma Aldrich and Merck companies. NMR spectra were taken at İnönü University Catalysis Research and

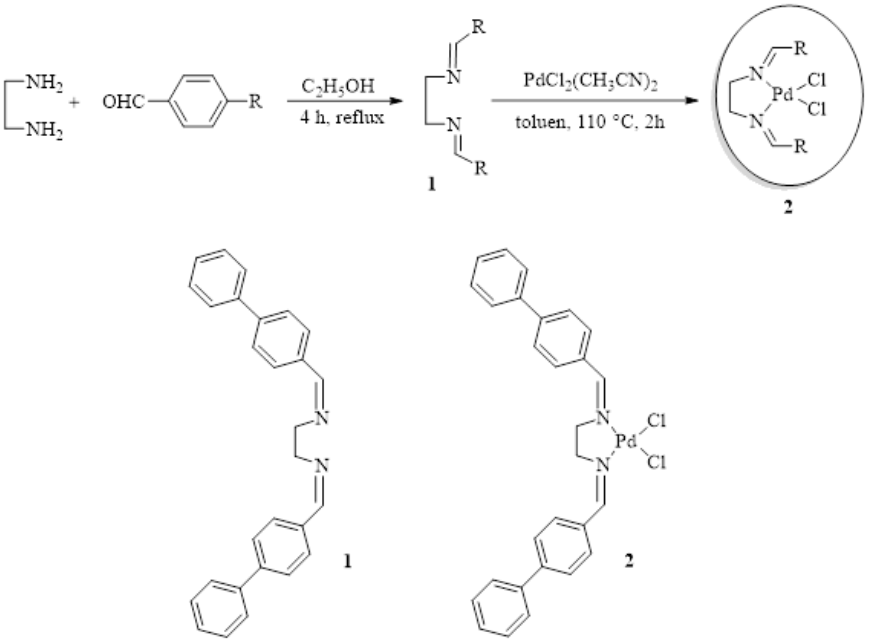
Application Center at Bruker Ascend 400 Avence III HD NMR spectrometer. DMSO was used as the solvent and TMS was used as the internal standard. FT-IR spectra were taken on the Perkin Elmer Spectrum 100 spectrometer in the range of 400-4000 cm-1. Melting points were determined with the electrothermal melting point determination device Stuart SMP 40 automatic melting point determination device.

Synthesis of Schiff Base:

Aldehyde (2 mmol) was completely dissolved in ethyl alcohol (25 ml) and ethylene diamine (1 mmol) was added. After boiling in an inert environment for 4 hours, half of the alcohol was removed in vacuum and crystallized by adding ether (Scheme 1.). The crystals obtained were filtered, washed with ether and dried in vacuum.

Synthesis of Schiff Bases-Palladium Complexe:

The synthesized Schiff base (1 mmol) and $PdCl_2(CH_3CN)_2$ (1 mmol) were stirred under argon in the presence of toluene (20 ml) at 110 ºC for 2 hours. After boiling in an inert environment for 2 hours, half of the alcohol was removed in vacuum and crystallized by adding ether (Scheme 1.). The crystals obtained were filtered, washed with ether and dried in vacuum.



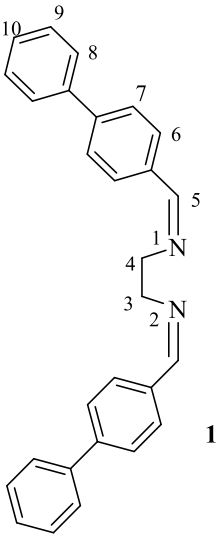
Scheme 1. *Synthesis of Schiff Bases-Palladium Complexes*

Findings and Discussion

Schiff base was obtained in high yield (90%) by reacting ethylenediamine and aldehydes in ethanol. Melting point, yield, FT-IR, elemental analysis, ^{1}H and ^{13}C NMR analysis results of Schiff base are given in the Table. It has been reported that C=N stretching vibrations in Schiff bases are generally observed as a sharp peak at 1610-1640 cm^{-1}, FT-IR specific ν(CN) vibrations of compounds carrying methylene groups attached to azomethine groups are around 1625-1640, and peaks are observed at 1600-1637 cm^{-1} in those not carrying methylene groups, i.e. those directly attached to aromatic rings [Karahan, 2013]. It was observed that the FT-IR specific ν(CN) vibrations of the synthesized Schiff base compounds gave sharp peaks at 1600-1603 cm^{-1}. These data support the completion of the

formation reaction of the compounds and the results are in agreement with the values given in the literature [Amer, 1988].

1,2-bis[phenylbenzylideneamino] ethane, 1:



1

Melting Point: 100-101 ºC
$\nu_{(CN)}$: 1602 cm^{-1}
Elemental analysis % $C_{28}H_{24}N_2$:
Calculated: C, 86.56; H, 6.23; N, 7.21.
Found: C, 86.49; H, 6.26; N, 7.17.

Table 1. *^{1}H ve ^{13}C-NMR data of Compound* ***1a***

Location	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	*J* (Hz)
3,4	3.94 (s, 4H)	47.7	-
5	8.41 (s, 2H)	146.0	-
6 and 7	7.82 (d, 4H)	130.6, 135.5	4.0
8 and 9	7.73 and 7.49 (t, 4H)	127.8, 129.0, 129.5	8.0
10	7.40 (t, 1H)	127.6	4.0

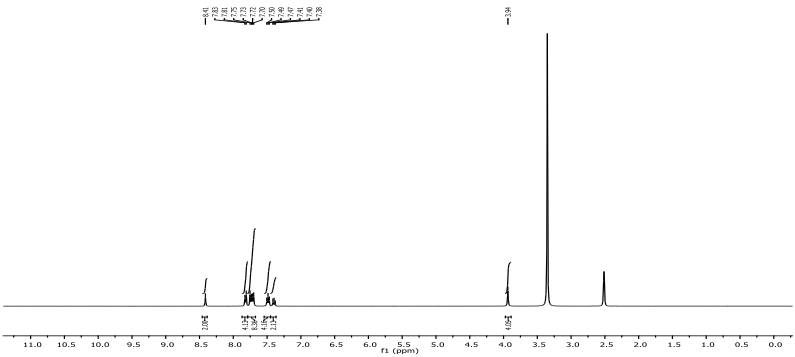


Figure 2. *^{1}H NMR spectrum of compound **1***

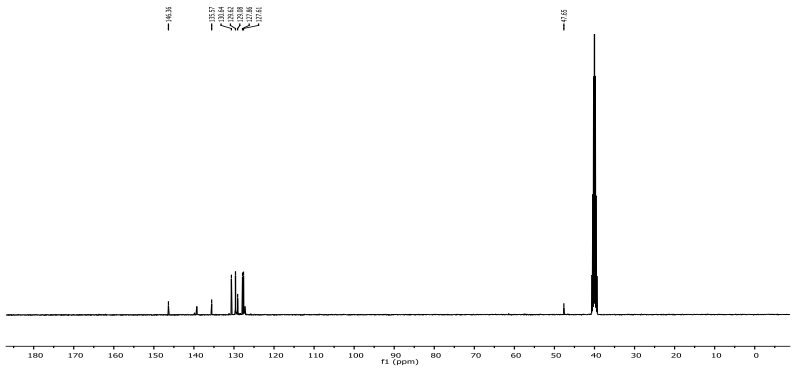
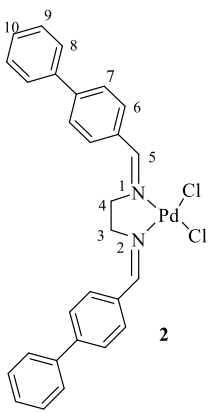


Figure 3. *^{13}C NMR spectrum of compound **1***

1,2-bis[fenilbenzilidenamino] etan dikloropalladyum(II), 2:



2

Melting Point: 118-1191 ºC
$\nu_{(CN)}$: 1604 cm^{-1}
Elemental analysis % $C_{28}H_{24}N_2$:
Calculated: C, 59.44; H, 4.28; N, 4.95.
Found: C, 59.47; H, 4.21; N, 4.92.

Table 2. *^{1}H ve ^{13}C-NMR data of Compound* ***1a***

Location	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	*J* (Hz)
3,4	3.35 (s, 4H)	46.7	-
5	10.1 (s, 2H)	193.2	-
6 and 7	7.78 (d, 4H)	135.6, 146.4	4.0
8 and 9	7.92 and 8.01 (t, 4H)	129.0, 129.6, 130.5	8.0
10	7.47 (t, 1H)	127.6, 127.8,	4.0

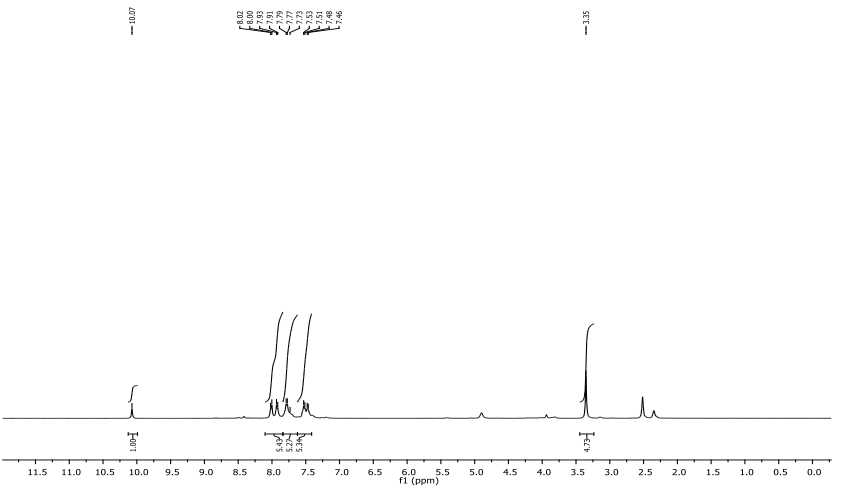


Figure 4. *^{1}H NMR spectrum of compound* ***2***

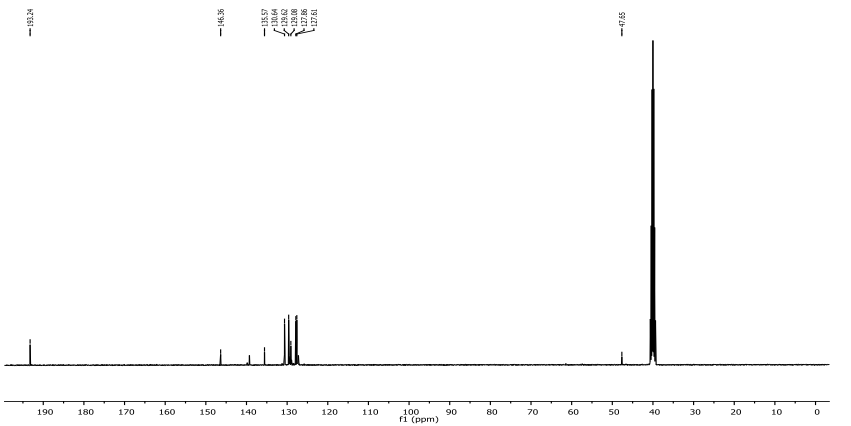


Figure 5. *^{13}C NMR spectrum of compound* ***2***

Conclusions and Recommendations

In this study, a new Schiff base compound and its palladium complex were synthesized by reacting aromatic aldehyde with ethylenediamine. The prepared compounds were obtained in high yields. When the analysis results of the synthesized compounds were compared with the literature, it was determined that the FT-IR, ^{1}H-^{13}C NMR data were in agreement with the literature. Schiff base compounds and metal complexes have widespread application areas. The synthesized compounds are important compounds with catalytic and biological properties. It is possible to use them in studies suitable for green chemistry by taking advantage of these properties. It is planned to examine the catalytic properties and antimicrobial activities of the obtained compounds in subsequent studies.

acknowledgment

I would like to thank my instructors, Prof. Dr. Nevin Gürbüz and Prof. Dr. İsmail Özdemir, for their help in my studies.

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CHAPTER II

Catalytic Activity of In-situ Pd-NHC Catalysts in Suzuki-Miyaura Reactions: A Study on Benzimidazolium Salts

Emine Özge KARACA[1]

Introduction

N-Heterocyclic carbenes (NHCs) have gained significant importance in organic and organometallic chemistry in recent years. NHC ligands are widely employed in transition metal catalysts due to their strong σ-donor properties and remarkable stability. In carbon-carbon bond-forming reactions such as the Suzuki-Miyaura reaction (Miyaura, 1979), NHCs exhibit high activity and selectivity, particularly when complexed with palladium (Pd). Pd-NHC complexes enable high yields and broad substrate compatibility in the coupling of boronic acids with aryl or vinyl halides. These complexes are especially preferred for synthesizing structurally

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complex compounds used in biologically active molecules and materials science. Moreover, Pd-NHC catalysts offer advantages over conventional phosphine-based catalysts, including greater stability and eco-friendliness, making them valuable in sustainable chemistry. The utilization of NHCs and Pd-NHC complexes continues to open new horizons in catalyst design and the development of chemical processes, enhancing interest in NHCs across both academic and industrial domains.

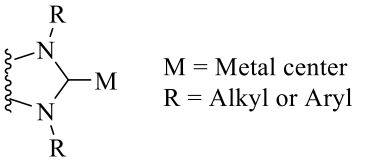


Figure 1. *Metal NHC Complexes*

N-Heterocyclic carbenes (NHCs) are often employed in-situ, meaning they are generated directly within the reaction medium. This method provides a practical and cost-effective approach, especially for preparing Pd-NHC complexes (Yaşar, 2015; Özdemir, 2015; Weskamp, 2001). In-situ synthesis typically involves using a precursor (e.g., benzimidazolium salts) and a base to generate the carbene ligand directly during the reaction. This approach not only saves time and resources but also minimizes issues arising from the air and moisture sensitivity of carbenes. In-situ Pd-NHC catalysts exhibit high efficiency and broad substrate compatibility in Suzuki-Miyaura reactions. Furthermore, this method reduces the steps required for catalyst preparation, making processes more environmentally friendly and playing a critical role in sustainable chemistry (Lee, 2000; Knochel, 2004; Hadei, 2005). In-situ synthesis facilitates the laboratory-scale application of NHCs and

accelerates their integration into industrial applications. In this study, benzimidazolium salts were utilized as in-situ Pd-NHC catalysts, and their catalytic activity in Suzuki-Miyaura reactions was investigated.

METHOD

Since some synthesized compounds are sensitive to air humidity and oxygen, all experiments were carried out in an inert atmosphere and the Schlenk technique was used in the reactions. Before using the glass materials used in the reactions, vacuum was applied and heated to remove moisture and oxygen, and then they were filled with argon gas. Reagents used in the reactions were purchased commercially from Sigma Aldrich and Merck companies. NMR spectra were taken at İnönü University Catalysis Research and Application Center at Bruker Ascend 400 Avence III HD NMR spectrometer. DMSO was used as the solvent and TMS was used as the internal standard. FT-IR spectra were taken on the Perkin Elmer Spectrum 100 spectrometer in the range of 400-4000 cm^{-1}. Melting points were determined with the electrothermal melting point determination device Stuart SMP 40 automatic melting point determination device.

General Method for the Preparation of Benzimidazolium Salt:

The 1-(4-vinylbenzyl)5,6-dimethylbenzimidazole (1 mmol) and alkyl or aryl halide (1 mmol) were stirred in DMF (5 mL) for 48 h at 80 °C. Ethyl ether (10 mL) was added to obtain a white crystalline solid, which was filtered off. The solid was washed with diethyl ether (3-10 mL) and dried under vacuum, and the crude product was recrystallized from DCM/diethyl ether (Hahn, 2008; Karaca, 2021).

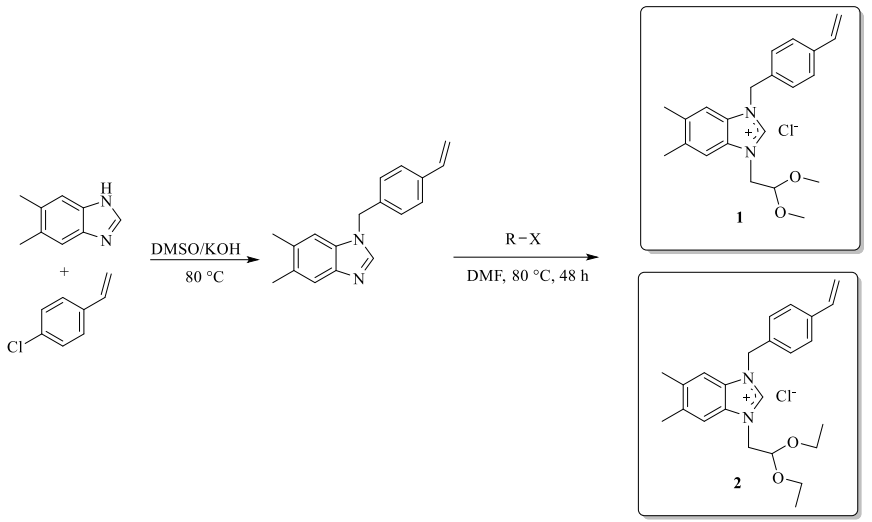
General Procedure for Suzuki Cross-Coupling Reaction

In air, **1-2** (1 mol%, aryl chloride (1.0 mmol), phenylboronic acid (1.5 mmol), K_2CO_3 (2 mmol) and 3 mL of a mixture of water and DMF (1:1) were added to a small round-bottom flask and the mixture was heated at 80 ^oC for an appropriate period of time. The reaction mixture was cooled to room temperature and 10 mL of water was added to the reaction mixture and extracted with Et_2O. The organic phase was dried with $MgSO_4$ and filtrated by short chromatography on silica gel column. Then volatiles were removed under reduced pressure and yield distribution was determined by GC using undecane as internal standard. The yields are based on corresponding aryl chlorides. All catalytic reactions were duplicated. All coupling products obtained via Suzuki-Miyaura coupling reaction are previously reported compounds, and were identified by comparison of our data with that available in the literature.

Findings and Discussion

The benzimidazolium salts 1-(4-vinylbenzyl)-3-(2,2-dimethoxyethyl)-5,6-dimethylbenzimidazolium chloride and 1-(4-vinylbenzyl)-3-(2,2-diethoxyethyl)-5,6-dimethylbenzimidazolium chloride were isolated as white solids in very good yields and fully characterized by ^1H and ^{13}C NMR spectroscopy, and elemental analyses. The ^1H NMR spectra of the benzimidazolium salt further supported the assigned structures; the resonances for acidic C(2)-H were observed as a sharp singlet in the 11.08 and 9.95 ppm respectively for . ^{13}C NMR chemical shifts were consistent with the proposed structure; the imino carbon appeared as a typical singlet in

the ^{1}H-decoupled mode in the 142.7 and 143.7 ppm respectively for benzimidazolium salts (**1-2**). The NMR values are similar to those found for other benzimidazolium salts (Boztepe, 2011). This known compound was synthesized and characterized by m.p, ^{1}H and ^{13}C NMR and micro analyses. Results, which we found, are consistent with the literature.

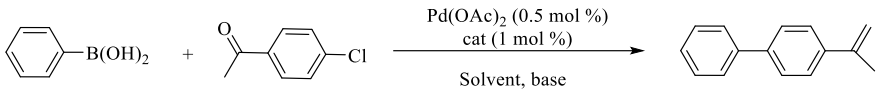


Scheme 1: *Synthesis of compound **1** and **2***

In the first instance, to find the optimum conditions for the Suzuki coupling reaction, an extensive screening of the reaction conditions was carried out using common mineral bases with different solvent variations under standard conditions. To assess the influence of the solvent, we used 4-chloroacetophenone as the substrate and K_2CO_3 as the base (**1**:1 mol%, 4-chloroacetophenone (1 mmol), $PhB(OH)_2$ (1.5 mmol), 80 ºC, 3h). In all cases, the reactions were heated for 3h at 80 ºC. After several reactions, the results showed that this catalytic system is effective with all solvents and bases, but the best one is K_2CO_3 -DMF/H_2O. The optimum yield

was obtained with the most polar solvents in an equal ratio of DMF/H_2O. The results are summarized in Table 1, entries 1-12 (Karaca, 2018).

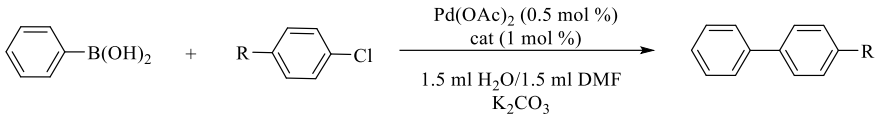
Table 1. *The effect of solvent and base on yield in the Suzuki coupling reaction.*



Entry	Solvent	Base(eq)	Yield [%]
1	Dioxane(6 mL)	Na_2CO_3 (2)	60
2	Dioxane (6 mL)	K_2CO_3 (2)	75
3	Dioxane(6 mL)	Cs_2CO_3 (2)	30
4	*i*-PrOH	Na_2CO_3 (2)	50
5	*i*-PrOH	K_2CO_3 (2)	52
6	*i*-PrOH	Cs_2CO_3 (2)	33
7	DMF (6 mL)	K_2CO_3 (2)	69
8	DMF/H_2O (4/2 mL)	K_2CO_3 (2)	74
9	DMF/H_2O (3/3 mL)	K_2CO_3 (2)	98
10	DMF/H_2O (2/4 mL)	K_2CO_3 (2)	88
11	H_2O (6 mL)	K_2CO_3 (2)	10
12	DMF/H_2O (3/3 mL)	Na_2CO_3 (2)	70
13	DMF/H_2O (3/3 mL)	Cs_2CO_3 (2)	45

[a] Reaction conditions: **1** (1 mol%), $Pd(OAc)_2$, 4-chloroacetophenone (1 mmol), $Ph(OH)_2$ (1.5 mmol), 80 °C, 3h.

Table 2. *The Suzuki coupling reaction of aryl chlorides.*



Entry	R	LHX	Yield (%)
1	$COCH_3$	1	96
2	$COCH_3$	2	94
3	CH_3	1	83
4	CH_3	2	75
5	CHO	1	78
6	CHO	2	74
7	OCH_3	1	90
8	OCH_3	2	86
9	H	1	66
10	H	2	64

ReactionConditions:1,0 mmol of R-C_6H_4Cl-*p*, 1,5 mmol of phenylboronicacid, 1,5 mmol K_2CO_3 % 1,0 mmol cat**,** % 0.5mmol $Pd(OAc)_2$, H_2O (1.5mL)/DMF (1.5 mL), 80^oC, 1h, yields are based on arylchloride; all reactions were monitored by GC.

Conclusions and Recommendations

In this study, 1-(4-Vinylbenzyl)-3-(2,2-diethoxyethyl)-5,6-dimethylbenzimidazolium chloride and 1-(4-Vinylbenzyl)-3-(2,2-diethoxyethyl)-5,6-dimethylbenzimidazolium chloride compounds were used for highly active, easy to produce and environmentally friendly new in-situ Pd-NHC complexes. Due to the structure of the Pd-NHC complexes, the carbene remains electron-rich upon coordination to palladium, which makes the palladium-carbon bond strong and stable. This outstanding property of Pd-NHC complexes

creates advantages over other complexes in catalytic cross-coupling reactions.

acknowledgment

I would like to thank my instructors, Prof. Dr. Nevin Gürbüz and Prof. Dr. İsmail Özdemir, for their help in my studies.

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CHAPTER III

Synthesis and Characterization of Vitamin B_1-Linked Waugh-Type Clusters

Hülya AVCI ÖZBEK[1]

Introduction

Polyoxometalates (POMs) are anionic metal-oxo nanoclusters of transition metals (V, Nb, Ta, Mo and W) with high oxidation step. They have attracted the attention of scientists in recent years as versatile inorganic building blocks that can be incorporated into organic compounds. They have the advantages of having more than one metal centre in their composition and increased reactivity due to allowing multiple electron transitions (Kastner & et al., 2017). These properties make POMs attractive for the design and synthesis of a wide variety of (bio)organic-inorganic hybrid functional materials with many areas (Song, 2018). POMs are

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known to have applications as drugs against infections, Alzheimer's disease, cancer, diabetes, etc. (Avcı Özbek, 2024; Avcı Özbek, 2023a, 2023b; Bijelic, Aureliano & Rompel, 2018; Bijelic, Aureliano & Rompel, 2019; Mahvash & et al., 2023; Ramezani-Aliakbari & et al., 2021; Ramezani-Aliakbari & et al., 2022; Van Rompuy & Parac-Vogt, 2019; Yamase, 2005; Yang & et al., 2013; Zhao & et al., 2020; Zhao & et al., 2020). The non-toxicity, cheapness and easy accessibility of the synthesised drug are important factors in the preference of POMs in these studies.

Vitamin B_1, also known as thiamine, is involved in the energy production mechanism in the body. Since it provides the energy needed by cells, its deficiency can lead to significant health problems. Vitamin B_1 is not stored like other water-soluble vitamins and therefore needs to be taken regularly on a daily basis. Therefore, the synthesis and characterisation of POM compounds containing vitamin B_1 is reported in this study. The limited number of studies on vitamin B_1-containing species in POMs and the lack of studies on Waugh species in POMs increase the value of this study. In this work, two novel Waugh-type POMS based on $[MMo_9O_{32}]^{6-}$ (M=Mn, Ni) anion with vitamin B_1 have been synthesized, characterized

Materials and Methods

All chemicals used were obtained from Aldrich and used without any purification. $K_3(NH_4)_3[MnMo_9O_{32}]\cdot 9H_2O$ and $(NH_4)_6[NiMo_9O_{32}]\cdot 9H_2O$ were synhthesized as previous report (Dunne, 1992a, 1992b). C, H, N and S elemental analyses were obtained on a LECO-932 CHNS elemental analyser. Mn, Mo and Ni were determined by Inductively coupled plasma mass spectrometry (ICP-MS) Agilent Technology 7700. The Fourier-

transform infrared spectroscopy (FT-IR) spectra were measured by a Perkin Elmer LR 64912 C spectrometer in the range 400-4000 cm^{-1} with KBr pellet. Thermal gravimetric analysis (TGA) was recorded on a Hitachi Exstar TG/DTA 7300 in flowing N_2 with a heating rate of 50.0 mL min 10 ºC/min at 25 and 800 ºC.

Synthesis of Compounds

$(C_{12}H_{17}N_4OS)_6[MnMo_9O_{32}]\cdot 9H_2O$ (1)

The solutions required for the reaction were prepared separately. Solution 1: $K_3(NH_4)_3[MnMo_9O_{32}]\cdot 9H_2O$ was (176 mg, 0.1 mmol) was dissolved in 5 mL hot water under stirring. Solution 2: Thiamine hydrochloride (excess) dissolved in 5 mL H_2O. Afterwards two solutions mixed stirred. The resulting mixture was kept at RT for 24 hours and filtered. The brown product washed with water and dried at 50 ºC. Yield: 569 mg, 18%. FT-IR data (cm^{-1}): 428 (m), 491 (m), 538 (m), 592 (m), 674 (m), 766 (m), 796 (m), 899 (m), 930 (m), 1046 (m), 1167 (m), 1224 (m), 1289 (m), 1362 (m), 1392 (m), 1436 (m), 1478 (m), 1502 (m), 1539 (m), 1607 (m), 1659 (m), 2883 (m), 2927 (m), 3068 (m), 3346 (w). Anal. Calcd. (%) for $C_{72}H_{120}N_{24}S_6MnMo_9O_{47}$ (3184.64 g/mol): C, 27.15; H, 3.80; N, 10.56; S, 6.04; Mo, 27.11; Mn, 1.73. Found (%): C, 27.05; H, 3.43; N, 9.54; S, 5.77; Mo, 26.82; Mn, 1.52. TGA (loss of 9 H_2O): calcd. 5.09%. found 4.53%; (loss of 6 $(C_{12}H_{17}N_4OS)^+$): calcd. 49.99%, found 48.97%.

$(C_{12}H_{17}N_4OS)_6[NiMo_9O_{32}]\cdot 9H_2O$ (2)

The solutions required for the reaction were prepared separately. Solution 1: $(NH_4)_6[NiMo_9O_{32}]\cdot 9H_2O$ was (170 mg, 0.1 mmol) was dissolved in 5 mL hot water under stirring. Solution 2: Thiamine hydrochloride (excess) dissolved in 5 mL H_2O.

Afterwards two solutions mixed stirred. The resulting mixture was kept at RT for 24 hours and filtered. The brown product washed with water and dried at 50 ºC. Yield: 728 mg, 23%. FT-IR data (cm^{-1}): 521(m), 569 (m), 644 (m), 678 (m), 728 (m), 805 (m), 866 (m), 912 (m), 990 (m), 1046 (m), 1218 (m), 1384 (m), 1437 (m), 1473 (m), 1538 (m), 1616 (m), 1659 (m), 2283 (m), 2918 (m), 3091 (m), 3215 (m), 3326 (w). Anal. Calcd. (%) for $C_{72}H_{120}N_{24}S_6NiMo_9O_{47}$ (3188.39 g/mol): C, 27.12; H, 3.79; N, 10.54; S, 6.03; Mo, 27.08; Ni, 1.84. Found (%): C, 26.56; H, 2.91; N, 9.01; S, 4.98; Mo, 26.87; Ni, 1.75. TGA (loss of 9 H_2O): calcd. 5.08%. found 5.17%; (loss of 6 $(C_{12}H_{17}N_4OS)^+$): calcd. 49.93%, found 50.55%.

Results and Discussion

1 and **2** were synthesized by reaction of $K_3(NH_4)_3[MnMo_9O_{32}]\cdot 9H_2O$ and $(NH_4)_6[NiMo_9O_{32}]\cdot 9H_2O$ with thiamine hydrochloride (vitamin B_1) in an aqueous solution at RT (Figure 1). Experimentally obtained spectroscopic data (ICP-MS, TGA and FT-IR) and elemental analyses results support **1** and **2** formulated as $(C_{12}H_{17}N_4OS)_6[MMo_9O_{32}]\cdot 9H_2O$ (M=Mn (**1**), Ni (**2**)). Elemental analysis data are given in Table 1 and ICP-MS data are given in Table 2.The colour of the compounds are salmon-coloured (**1**), pale yellow (**2**). Even though these compounds are stable in air, their low solubility in organic solvents and insolubility in water prevented the use of other spectroscopic methods (NMR, ESI) to characterise their structure and to obtain crystals suitable for X-ray analysis. The cluster structures of compounds **1** and **2** are reported to be similar to those in the literature (Gavrilova & et al., 2005; Kaziev & et al., 2007; Lin & et al., 2000; Quinones & et al., 2007; Tan & et al., 2009a, 2009b; Tan & et al., 2011).

Table 1: Results for the elemenal analysis

	C		H		N		S	
Compound	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**
1	27.15	27.05	3.80	3.43	10.56	9.54	6.04	5.72
2	27.12	26.56	3.79	2.91	10.54	9.01	6.03	4.98

Table 2: Results for the ICP-MS

	Mo		Mn		Ni	
Compound	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**
1	27.11	26.82	1.73	1.52	-	-
2	27.08	26.87	-	-	1.84	1.75

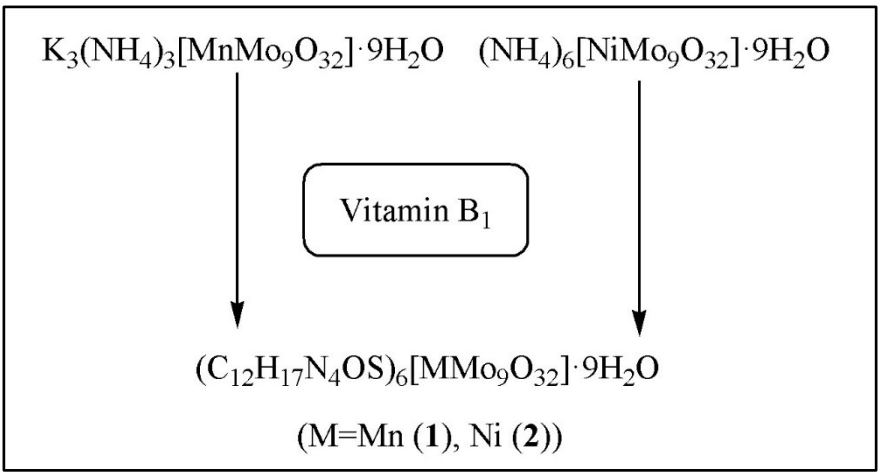

*Figure 1. Synthesis of **1** and **2***

In the FT-IR spectrum of POMs, the region between 1000 and 400 cm^{-1} is characterised as the fingerprint region. As shown in Figure 2-3, the FT-IR spectra of **1** and **2** show similar adsorption peaks, indicating the similar chemical bonds. Compounds **1** and **2** show characteristic FT-IR bands with the POMs in the $[MnMo_9O_{32}]^{6-}$ structure in the literature. At 899, 930 (**1**) 866, 912 (**2**) cm^{-1}, vibrations of terminal Mo=O bonds appear as a doublet. The

bands in the region of 428-592 cm^{-1} can be attributed to vibrations of Mo-O-Mo bridges. The adsorption band at 538(**1**), 521 (**2**) cm^{-1} is assigned to Mn-O respectively. The selected bands of vitamin B_1 are at 3346 (OH); 3068 (N–H in NH_2); 2927 (C–H in CH_3); 1659 (NH in NH_2); 1607, 1539, 1478; 1436 (CH in CH_2–CH_2); 1047 (C–O) cm^{-1} for **1**, 3400 (OH); 3326 (N–H in NH_2); 3215 (C–H in CH_3); 1659 (NH in NH_2); 1616, 1538, 1478 (CH in CH_2–CH_2); 1046 (C–O) cm^{-1} for **2**. The FT-IR spectra also show bands at 3346-3400 cm^{-1} associated with the vibrational modes of water molecules and hydroxyl groups (Ostroushko & et al., 2018). The data obtained from FT-IR analyses are in agreement with previously reported Waugh-type structures (Gavrilova & et al., 2005; Kaziev & et al., 2007; Lin & et al., 2000; Quinones & et al., 2007).

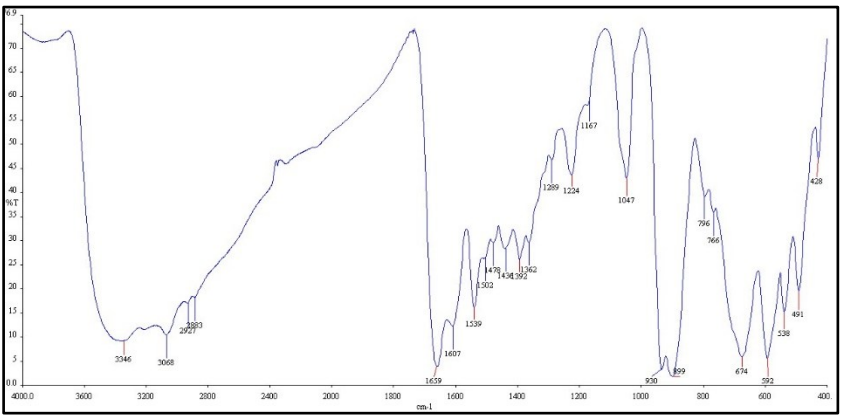

*Figure 2. FT-IR spectrum of **1***

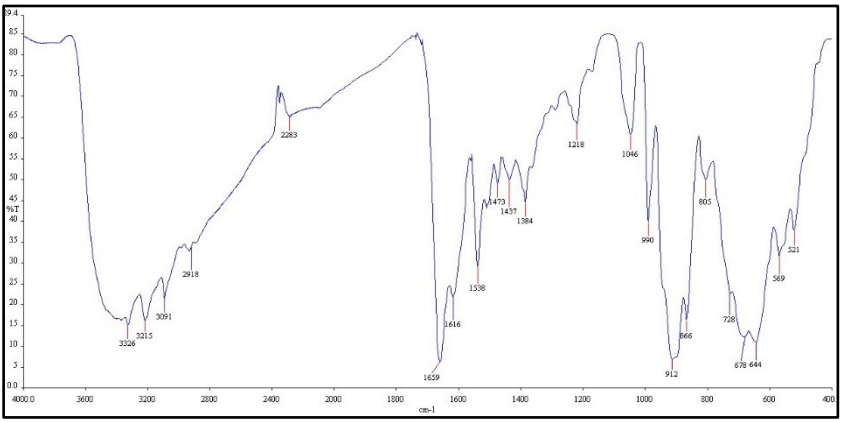

*Figure 3. FT-IR spectrum of **2***

TGA results of **1** (Figure 4) exhibits that 4.53% weight loss between 30-148 ºC can be assigned to approximately nine water molecules in the crystal lattice. The 48.97% weight loss between between 149-775 °C, is assigned to the removal of six thiamine cations. The TGA data for **2** (Figure 5) shows the two weight-loss steps between 25 °C and 800 °C too. The 5.17% weight loss of **2** is due to the loss of lattice water in the temperature range of 36°C-218°C. The 50.55% weight loss between 219-676 ºC is assigned to the removal of six thiamine cations (Table 3). The cluster is stable up to 800 ºC.

Table 3: Results for the TGA analysis

Compound	**Losses** Part	**Calc. (%)**	Exp. (%)	**Losses** Part	**Calc. (%)**	**Exp.** (%)
1	9 H_2O	5.09	4.53	6 Vitamin B_1	49.99	48.97
2	9 H_2O	5.08	5.17	6 Vitamin B_1	49.93	50.55

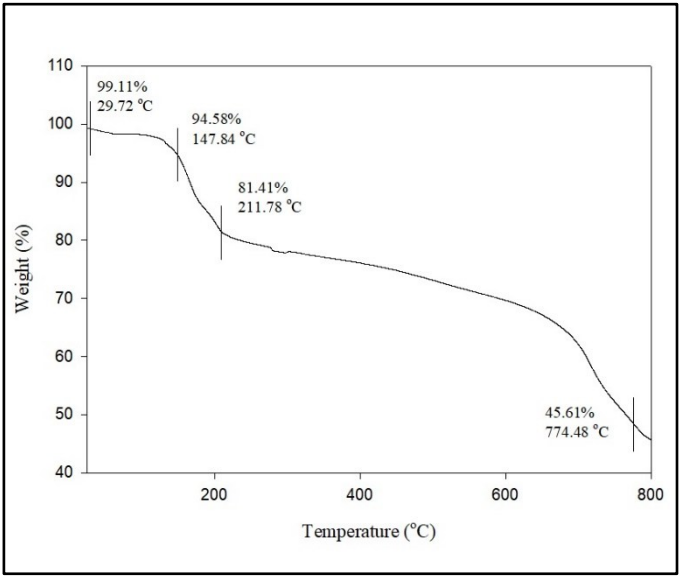

*Figure 4. TGA spectrum of **1***

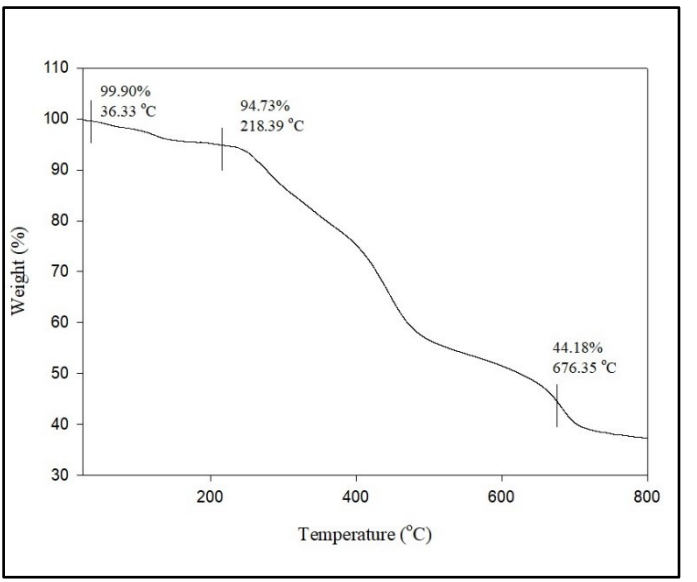

*Figure 5. TGA spectrum of **2***

Conclusion

In conclusion, two Waugh-type compounds containing vitamin B_1 have been synthesised from this study. The presence of $[MMo_9O_{32}]^{6-}$ (M=Mn, Ni) clusters is the main geometrical feature of this structure. The structure of these compounds was confirmed by elemental analysis, FT-IR, ICP-MS and TGA.

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CHAPTER IV

Supercritical Fluids

Mehtap EANES[1]

Giriş

Imagine a substance that defies traditional states of matter, existing as neither a typical gas nor a liquid. Supercritical fluids (SCFs) exist in this unique realm—above a specific temperature and pressure where distinct liquid and gas phases cease to exist. In this "supercritical" state, a fluid combines a gas's penetration ability with a liquid's solvency power, creating a medium with exceptional versatility for various applications (Clifford & Williams, 2000). Supercritical fluids have garnered significant attention in various scientific and industrial fields due to their unique properties and versatile applications. We delve into the physical and chemical properties that make supercritical fluids distinctive and explore their

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significance in chemistry, materials science, pharmaceuticals, and environmental science. This chapter explores the science behind supercritical fluids, their properties, and their solid-state synthesis. Supercritical fluids exist at temperatures and pressures above their critical points, where the distinction between liquid and gas phases disappears. Carbon dioxide (CO_2) is one of the most commonly used supercritical fluids, with a critical point at 31.1 °C and 73.8 atm. Other examples include water, methane, and ethane. The transition from a gas to a supercritical fluid involves a continuous change in density, making it a unique phase with remarkable properties.

A supercritical fluid is a substance that exists above its critical temperature and pressure, exhibiting properties of both liquids and gases. In this state:

Critical Temperature (Tc) is the temperature above which a substance cannot be liquefied, no matter the applied pressure.

Critical Pressure (Pc) is the pressure required to liquefy a substance at its critical temperature.

Together, Tc and Pc define a point known as the critical point. Above this point, the fluid becomes "supercritical," with density and viscosity intermediate between the liquid and gaseous phases. Key Characteristics of Supercritical Fluids:

Density**:** Similar to liquids, which enables them to dissolve materials well.

Viscosity**:** Closer to gases, allowing better penetration into substances.

Diffusivity**:** Intermediate between liquids and gases, enabling efficient mass transport.

The basic principles of phase transitions and thermodynamics have to be known to be able to understand supercritical fluids. Figure 1 shows the critical point at the end of liquid-vapor existence at the critical pressure, P_c, and temperature, T_c, in a phase diagram of a pure homogenous substance. Supercritical fluids (SCF) is the state of a compound that is above its critical temperature and pressure (Jessop & Walter, 1999).

When the temperature increases, thermal expansion occurs, causing the liquid to become less dense, at the same time, the gas becomes denser as well. At the critical point, the densities of both phases become the same. Therefore, above the critical point, the compound is neither liquid nor gas anymore; it becomes a supercritical fluid. The properties of SCF will be between gas and liquid because there is no distinction between gas and liquid above the critical point.

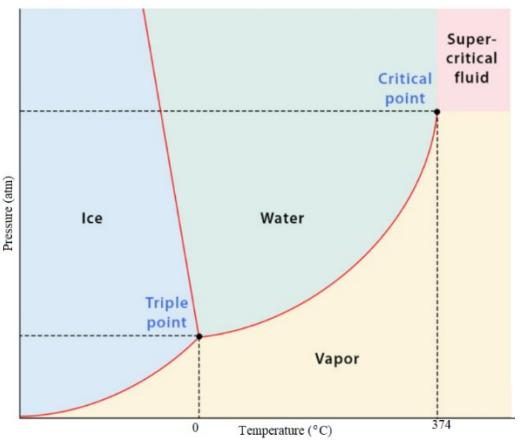

Figure 1. Phase diagram of water (Jessop & Walter, 1999).

In everyday conditions, matter can exist in three main phases: solid, liquid, and gas. These phases are defined by the arrangement and motion of particles (atoms or molecules).

Phase transitions occur when you change temperature or pressure. For example, water turns from a solid (ice) to a liquid (water) when heated, and further heating converts it into a gas (steam). Each substance has a unique set of conditions (temperature and pressure) at which its liquid and gas phases become indistinguishable. This point is called the critical point. Above the critical point, the substance is in the supercritical fluid state. It retains the properties of both a liquid and a gas. Supercritical fluids form when substances are subjected to pressures and temperatures above their critical points. Carbon dioxide (CO_2) and water (H_2O) are the most commonly used supercritical fluids, as they achieve supercritical states relatively easily. Here's an outline of their formation:

Compressing the Gas: The gas is pressurized at ambient conditions starting with a gaseous state.

Heating Beyond the Critical Temperature: The substance is heated to a temperature above its critical point.

Adjusting Pressure for Optimal Density: By adjusting the pressure, the supercritical fluid can be fine-tuned for various applications.

Properties of Supercritical Fluids

The properties of supercritical fluids, such as viscosity, density, and diffusion coefficient, change significantly with slight adjustments in pressure and temperature. This sensitivity enables

engineers to "tune" the properties of a supercritical fluid for specific applications.

Density and Solubility: A supercritical fluid has a density closer to a liquid, allowing it to dissolve a wide range of substances. This property is exploited in supercritical fluid extraction (SFE) processes to efficiently extract compounds from solid matrices. The solubility can be adjusted by varying pressure, making it suitable for extracting specific compounds without traditional solvents. In the supercritical region, density changes sharply but continuously with pressure. A considerable advantage of supercritical fluid is that a change in density affects solvating power. A decrease in the density causes a significant change in solvating ability. The dielectric constant also increases sharply with pressure in the compressible region, which is also related to a change in density (Figure 2) (Youssef & etal. 2020). Supercritical fluids are suitable for continuous flow due to high diffusivity, low viscosity, and intermediate density. These features allow for an increase in the reaction rate because of higher diffusivity and a weaker cage effect. Supercritical fluids are miscible with other gases, this feature leads to high rates of reactions. Supercritical fluids can dissolve a wide range of compounds, similar to liquids. Their solvation power can be tuned by adjusting temperature and pressure

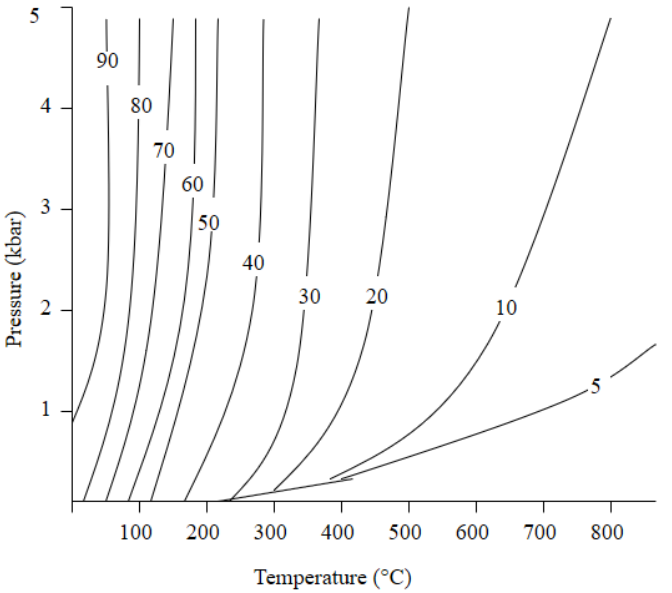

Figure 2. Variation of Dielectric constant of water with temperature and pressure (Youssef & etal. 2020).

Compressibility: Supercritical fluids are highly compressible, like gases. Small changes in pressure can lead to significant changes in density.

Viscosity and Diffusion: Supercritical fluids exhibit lower viscosity than liquids, which means they flow more easily through materials, facilitating deeper penetration. They also exhibit enhanced diffusion properties, meaning they can readily mix with other substances. Supercritical fluids have high diffusivity, promoting rapid mass transfer. This property is beneficial in applications such as supercritical fluid chromatography (SFC) for fast and efficient separations. In SCF, diffusivity increases and viscosity decreases in comparison with liquid solvent. Therefore, species will diffuse faster in SCF than in liquid solvents. Diffusivity

varies with temperature and pressure and has an inverse relationship with density and viscosity. The diffusion coefficient decreases with pressure. However, density and viscosity increase. At constant pressure, diffusivity increases with temperature.

Thermal Conductivity: The thermal conductivity of supercritical fluids is generally lower than that of liquids but higher than that of gases. This property is particularly useful in applications requiring controlled heat transfer, like supercritical water oxidation.

Tunable Properties: Supercritical fluids' density and solvating power can be tuned by adjusting temperature and pressure, offering control over their behavior. This tunability is advantageous in various industrial processes.

SCFs have several other advantages over liquid solvents. Most of the SCFs commonly used have low molecular weight, which causes them to have low critical temperatures. Thus, when the reactions are finished, the product is practically free of residual solvent. Water, CO_2, ethane, and ethane are the most commonly used SCFs. The last three solvents have a critical temperature below 35ºC. Because of its nontoxic, nonflammable, and availability in high purity properties of CO_2, it is the most widely used SCF. In comparison with organic solvents, SC water (SCW) has a very high critical pressure and temperature, but it is the most intensively investigated for the oxidation of organic wastes. Properties of SC water change drastically in comparison with liquid water.

For example, it becomes a nonpolar organic liquid in the SC region. The dielectric constant decreases to a point where organic compounds dissolve easily. This low polarity makes SCW ideal for

the oxidation of organic compounds to water and CO_2. Widely used SCFs are given in Table 1 (Jessop & Walter, 1999).

Table 1. Selected supercritical fluids.

SCF	T_c (°C)	P_c (bar)	d_c (g/mL)	m (Debye)
CO_2	31.1	73.8	0.466	0
HCl	51.5	82.6	0.42	1.08
HBr	90.0	85.5	n.a	0.82
H_2O	374	220.6	0.322	1.85
NH_3	132.4	113.2	0.235	1.47
SF_6	45.5	37.6	0.737	0
CH_4	-82.6	46.0	0.163	0
C_2H_4	9.2	50.4	0.214	0
C_2H_6	32.2	48.7	0.207	0
$C_2H_8N_2$	320	62.8	0.29	1.99
C_6H_6	289.5	49.2	0.300	0

Tc: Critical temperature, Pc: critical pressure, dc: Critical density, m: Dipol moment

The environmental benefit is one of the biggest advantages of using SCFs, especially when working with SC H_2O and SC CO_2 (Table 2) (Jessop & Walter, 1999). Replacing these solvents with harmful solvents would benefit environmental safety. These solvents, CO_2 and H_2O, are nontoxic, nonflammable, noncarcinogenic, nonmutagenic, and thermodynamically stable. This is very useful in chemical extraction and purification due to the volatility of SC fluids, so they can be easily removed from the product.

This is very valuable in preparing cosmetics, pharmaceuticals, and food additives. The most well-known application of supercritical fluids is using carbon dioxide to extract caffeine from green beans. A second application is supercritical fluid chromatography with carbon dioxide or nitrous oxide as a solvent. In supercritical fluid chromatography, the solute has a

higher diffusion rate than regular chromatography. Therefore, narrow peaks and better separation can be observed.

Table 2. Advantages of using SCFs over conventional solvents

Category	Advantage	Which SCFs
Environment	not contribute to smog	Most
	no damage the ozone layer	Most
	no acute ecotoxicity	CO_2, H_2O
	no liquid wastes	CO_2 and other volatile SCFs
Health and safety	Noncarcinogenic	most (but not C_6H_6)
	Nontoxic	most (but not HCl, en, NH_3)
	nonflammable	CO_2, H_2O, Xe, Kr, CHF_3
Process	no solvent residues	CO_2 and other volatile SCFs
	facile separation of products	CO_2 and other volatile SCFs
	high diffusion rates	All
	low viscosity	All
	adjustable solvent power	All
	adjustable density	All
	inexpensive	CO_2, H_2O, NH_3, CnHn
Chemical	high miscibility with gases	All
	variable dielectric constant	the polar SCFs
	high compressibility	all
	local density augmentation	all
	high diffusion rates	all
	altered cage strength	all

Applications of Supercritical Fluids

The unique properties of supercritical fluids lend themselves to a wide range of applications, from pharmaceuticals and food processing to materials science and environmental technologies.

Supercritical Fluid Extraction (SFE): SFE is widely employed for extracting valuable compounds from natural sources, such as essential oils, flavors, and pharmaceuticals. The process is

considered environmentally friendly due to the use of non-toxic supercritical fluids, especially CO_2. Supercritical CO_2 extraction is prized for producing high-purity extracts without toxic organic solvents, making it ideal for food, cosmetics, and natural health products.

Supercritical Fluid Chromatography (SFC): SFC is a powerful analytical technique combining gas and liquid chromatography principles. It is particularly effective for separating and analyzing complex mixtures with improved speed and resolution. SFC also uses supercritical CO_2 as a mobile phase to separate compounds in analytical and preparative chromatography. This technique is widely used in pharmaceutical research and quality control due to its ability to resolve complex mixtures rapidly and with high sensitivity.

Supercritical Fluids in Materials Science and Nanotechnology: Supercritical fluids are used to create nanoparticles and other advanced materials in materials science. Supercritical fluids' tunable density and diffusion properties facilitate the controlled formation of nano-sized particles and coatings, which are essential in electronics, medical devices, and pharmaceuticals. Supercritical fluids are utilized to fabricate nanomaterials, polymers, and aerogels. The precise control over density and solvating power enables the production of materials with unique structures and properties.

Pharmaceuticals: Supercritical fluid technology is employed in pharmaceutical processes, including particle size reduction, drug encapsulation, and purification. The use of supercritical fluids

minimizes the need for organic solvents, reducing environmental impact.

Supercritical Fluids in Solid State Synthesis: Next section will provide details on this part.

Supercritical Fluids in Solid State Synthesis

High temperatures, often between 600º C and 1500º C, and solid materials are used to prepare crystalline solids. All bonds in starting materials must be broken, and atoms must migrate to form the new structures, especially if there is a big difference in the structures of starting materials and the product; therefore, high temperatures must be used. (Jessop & Walter, 1999). Migration and the diffusion of the atoms will be impossibly slow unless very high temperatures are used. At high temperatures, very thermodynamically stable known compounds are usually formed; therefore, synthesizing new materials becomes difficult at high because thermodynamically stable known phases cannot be avoided (Kanatzidis, 1990). However, new metastable or kinetically stable compounds can be synthesized if relatively lower temperatures are used. The preparation of new kinetically stable solid-state chalcogenide compounds synthesized at temperatures lower than 500º C is the interest of researchers. There are many ways to prepare solid-state chalcogenides at low temperatures. Chemical vapor transport (CVT) or molten salts (or flux growth) are the most common techniques to dissolve reactants at a relatively low temperature (West, 1996).

The reactants are placed in a silica tube and kept under the atmosphere of a gaseous transporting agent while using the CVT

method. Reactants are put at a sealed end of the tube and inside a furnace so that a temperature gradient exists. The reactants combine with gases, to form products that decompose at the other end of the tube, yielding crystalline product. This method is very convenient for the growth of a single crystal or for the purification of a binary compound but is inefficient for more complex compounds. There are also flux growth techniques for preparing more complicated chalcogenide systems. The preparation of metal halides, chalcogenides, and alkali polychalcogenides includes three molten salt fluxes (Kanatzidis, 1989). These salt fluxes are high-temperature Melts (liquids); therefore, solubilization of starting materials occurs, and then during cooling of the melts, recrystallization occurs (Sunshine, Kang & Ibers, 1987; Kang & Ibers, 1988; Dhingra & Kanatzidis, 1989). Of these, the system that is of greatest current interest is alkali-metal polychalcogenides. It is known that low-dimensional polychalcogenide solids can be synthesized at room temperature with nonaqueous solvents, but it is difficult to grow single crystals under these conditions. Low to intermediate temperatures (T< 500º C) are generally needed to synthesize polychalcogenide systems because the polychalcogenide complexes will convert to denser chalcogenide fluxes at high temperatures. An alkali-metal poly chalcogenide melt system (Kanatzidis, 1990) is obtained as follows (A = alkali-metal, Q = chalcogenides):

$$A_2Q + (x-1)\,Q \longrightarrow A_2Q_x$$

Polychalcogenides stay liquid without significant evaporation or chalcogen loss over a wide temperature range (200-800ºC). Reactions are usually done in silica or Pyrex tubes. The

cooling procedure is typically very slow (2ºC/h). Since many alkali-metal poly chalcogenides melt at intermediate temperatures, they can act as reactants and solvent fluxes. Kanatzidis has used this very efficient route to new chalcogenides to synthesize a number of new metal polychalcogenide phases, such as $KCuS_4$, $NaAuSe_2$, and $KAuS_5$ (Kanatzidis, 1990). However, there are potential disadvantages to molten alkali-metal poly chalcogenides fluxes. For example, the alkali-metal invariably gets incorporated into the lattice. Also, the medium is chalcogen rich so metal poly chalcogenides are usually formed rather than metal chalcogenides. Finally, the flux must be removed after the completion of the reaction.

Supercritical Amines

Ammonothermal synthesis was first studied by Jacobs and Schmidt (Jacobs & Schmidt, 1982). In their work, the temperature and pressure far exceeded ($T \geq 500$ºC, $P \geq 6$ kbar) which is necessary to obtain supercritical ammonia. In this laboratory, we have demonstrated that many new phases can be prepared under milder conditions. For example, many new quaternary compounds (Schimek, 1996) have been recently synthesized in supercritical ammonia at 160º C. These new phases have the general formula $A_wM_xE_yQ_z$ (A=K, Rb, Cs; M=Cu, Ag; E=As, Sb; Q=S, Se) such as A_2AgSbS_4, $A_2Ag_3Sb_3S_8$ (Wood, Pennington & Kolis, 1992; Wood, Pennington & Kolis, 1994; Wood, Pennington & Kolis, 1996). These compounds have very complicated low dimensional structures. Anionic frameworks are made of clusters or polymers of 15/16 group anions and held together by Ag^{1+} or Cu^{1+}, while the alkali metals act as isolated cations. All the metals in these compounds are

monovalent. If divalent transition metal ions are used, then ammine complexes are isolated like $[Fe(NH_3)_6]Cu_3Sb_8S_{15}$ and $[Mn(NH_3)_6]Sb_4S_7$ (Schimek & et.al, 1996). Also, if lanthanide ions are substituted for alkali-metal ions, new lanthanide amine-transition metal sulfide phases can be synthesized, such as $[Yb(NH_3)_8][Ag(S_4)_2].2NH_3$ and $[La(NH_3)_9][Cu(S_4)_2]$ (Young, Schimek & Kolis, 1996).

It has been demonstrated that ethylenediamine (en) is also an excellent solution to generate novel solid-state phases at intermediate temperatures. Many new quaternary compounds, structural relatives of the sulfosalts, (Berry &Mason, 1959) have been successfully synthesized using superheated en. For example, $Cs_2Ag_3Sb_3S_7$ and $CsAgSb_4S_7$ (Jerome & etal., 1996) have been prepared in supercritical en, and they have very unusual and complex low-dimensional structures.

Research has proven that alkaline earth and alkali metals, as well as europium and ytterbium, rare earth, can be dissolved in liquid ammonia under normal conditions (Jolly, 1972). However, the solubility of most other inorganic compounds increases in water than in ammonia due to water's lower dielectric constant. Therefore, products obtained from ammonia are often microcrystalline or even amorphous. Using supercritical ammonia can solve this problem. The solubility of mainly ionic solid compounds increases with a high dielectric constant increases if the energy of solvation is higher than the lattice energy. The change in the dielectric constant of H_2O and NH_3 is shown in Figure 3 (Jacobs & Schimidt, 1982).

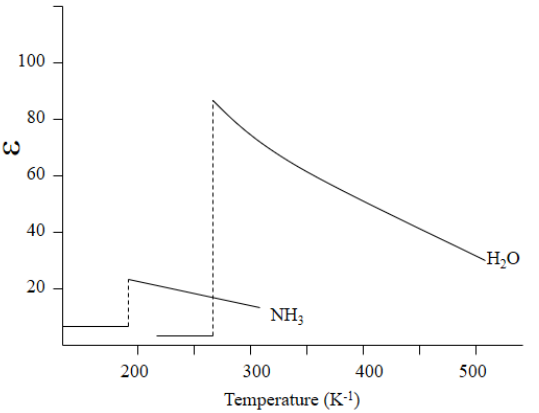

Figure 3. Dielectric constant vs Temperature diagram for water and Ammonia (Jacobs & Schimidt, 1982).

Dielectric constant depends on temperature and density so it increases up to the critical temperature then at the critical temperature, the dielectric constant starts to decrease due to the density decrease of the solvent in SCF. Because ammonia has a lower dielectric constant than water, it is less suitable for inorganic compounds however this problem can be solved by high ammonia pressure using high temperature that causes extreme decrease in the density of ammonia. Further data for NH_3, H_2O, and ethylenediamine are given in Table 3 (Jacobs & Schimidt, 1982).

Table 3. Physical data of ammonia, water and Ethylenediamine (en)

Physical constant	NH_3	H_2O	en
Melting point (K)	195.4	273.1	281.6
Boiling point (K)	239.7	373.1	389.6
Critical Temperature(K)	405.5	647.2	593.1
Critical Pressure (bar)	113	221	62.8
Conductivity ($S.m^{-1}$)	~$5x10^{-9}$ at 239.7 K	~$7x10^{-6}$ at 298.1	n.a
Dipole moment ($Cm.10^{30}$) (Debye)	 4.91 1.47	 6.16 1.85	 6.35 1.9
Proton affinity ($kj.mol^{-1}$)	875	761	n.a
Ionic Product	~10^{-29}	10^{-14}	n.a
Viscosity ($Pa.s.10^4$)	2.65 at 239.6 K	8.90 at 298.1 K	n.a

Supercritical Water

Water is one of the most important solvents in nature and has remarkable properties in its supercritical state. Numerous minerals have formed under hydrothermal conditions at temperatures higher than 100º C and pressures over one atmosphere (Barnes, 1979).

Researchers, especially mineralogists and geologists, have determined the conditions for forming minerals through simulations in laboratories (Shaw & et al. 1991). After World War II, the hydrothermal method that mimics the mineral forming conditions was introduced for the industrial growth of extremely pure crystals with interesting physical properties. (Laudise, 1985; Laudise & Ballman, 1969; Laudise, 1973; Laudise, 1962; Buchler & Walker, 1950). Single crystals of new materials were requested for improvements in the electronic industry. For that reason, Supercritical conditions are often adapted to obtain these materials as single crystals. Especially in producing large single-crystal

materials such as quartz crystals for electronic uses, hydrothermal conditions have been used in the research labs. The hydrothermal method was of interest because of the success in growing large crystals, and much research has been done on the physical chemistry of hydrothermal solvents (Roy & Tuttle, 1956). The hydrothermal method can be considered a case of chemical transport reactions. Highly soluble substances called mineralizers can be used as transport substances to increase the solubility of the reactants. The formation of ferromagnetic chromium (IV) oxides, given in equation 1, is an example of a compound of elements with unusual oxidation states. This reaction is an example of the advantages of the hydrothermal method over the conventional solid-state method (Rabenau, 1985).

$$Cr_2O_3 + CrO_3 \xrightarrow[H2O]{350^{o}\,C,\ 440\ bar} 3CrO_2. \qquad (1)$$

The hydrothermal method can also synthesize low-temperature phases and metastable compounds by simply using quartz ampoules.

The pVT data for water up to 1000ºC and 10 kbar are known accurately. The ionization of water increases sharply with temperature and pressure. Under supercritical conditions, water behaves like molten salt because it completely dissociated into H_3O^+ and OH^- with a 1.7-1.9 g/cm^3. Even nonpolar compounds dissolve in the water if the density is high enough because it behaves like a non-aqueous fluid. Mobility of species is higher in supercritical water than under normal conditions because viscosity decreases with temperature.

The viscosity of water above critical temperature is between $3x10^{-4}$ and $14x10^{-14}$ poise, depending on pressure. This value is much smaller than the room temperature viscosity of water, $1x10^{-2}$ poise. Due to the lower viscosity, the diffusion process is a lot faster. When diffusion is slow, solutions become supersaturated, and small fluctuations in concentration can cause imperfections in the crystal, such as dendritic growth.

Impurities can lead to imperfections that produce dendritic growth while growing crystals from melt. During the growth process, impurities will be trapped, resulting in defective crystals in case of solution growth, and the solvent is often entrapped, called liquid inclusion. Synthetic growth is to prepare a perfect bulk single crystal without any impurities.

The hydrothermal method is one of the crystal growth techniques from a liquid solution with a lower viscosity than viscosity at ambient temperature; therefore, diffusion is not a problem. Supersaturation and dendritic growth are less likely due to fast diffusion under hydrothermal conditions. The diffusion is fast even in materials with low solubility at ambient temperatures, such as quartz, which can't be grown at low temperatures. However, quartz can be grown at high rates under hydrothermal conditions. Unfortunately, there is a downside to the hydrothermal method because it requires high pressure, and it is difficult to gather data.

For hydrothermal experiments, the behavior of water under various conditions of pressure, volume, and temperature is important. A detailed study on the pressure-temperature behavior of water was reported by Laudise (Laudise, 1973). With a 32% fill, the liquid remains unchanged until the critical temperature. At the

critical point of water, 374º C and 220 bar, gas and liquid density are 1.32 g/cm^3 (Rofer, 1991). Over 32% fill and the liquid level will fill the autoclave before reaching the critical temperature. The higher the percentage fill, the lower the temperature at which the autoclave is filled with liquid (Figure 4). When the autoclave is filled less than 32%, the level of liquid will drop as the temperature rises, and the autoclave will be filled with gas before the critical temperature.

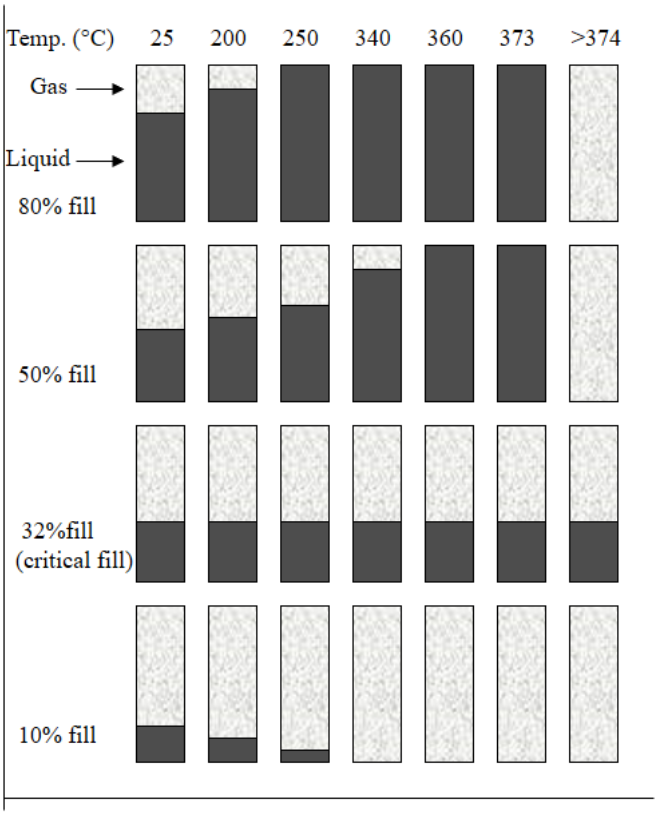

Figure 4. Presentation of the P-T behavior of the water at various degrees of fill (Rofer, 1991).

Mineralizers have a very important role in inorganic synthesis under hydrothermal conditions. The solubility of many compounds can be increased by adding mineralizers to the reaction medium (Franck, 1968; Demianets & Lobachev 1979). Mineralizers are complexing agents which promote the solubilization and recrystallization of materials. Quartz has a very low solubility in water (eq. 1) however, the solubility of quartz can be increased up to several percentages by adding sodium hydroxide to the solution (eq.2)

$$4SiO_2 + 2OH^- \xrightarrow{1M\ NaOH} Si_2O_7^{2-} + H_2O \qquad (2)$$

Common inorganic mineralizers are OH^-, Cl^-, F^-, NH_4^+, H^+, and S^{-2}. The complexes they form should not be very stable, or they precipitate.

Industrial Crystal Growth Using the Hydrothermal Method

The biggest industrial use of the hydrothermal method is in producing quartz, which has wide use in electronic materials (Laudise, 1985). Quartz is piezoelectric, so it can produce an electric dipole in a crystal when deformed. Piezoelectricity is optimum in α-quartz, meaning the synthesis temperature must be below 570ºC. Above this temperature, the transition from α-quartz to β-quartz happens, and piezoelectricity will decrease. Melt growth can be ruled out due to the melting point of silicon dioxide (above 1700ºC). For solution growth, an effective solvent should be found because of the high viscosity of the silica solution. Hydrothermal synthesis is the logical choice to grow α-quartz crystals.

Another industrial method is the production of $AlPO_4$. This compound has piezoelectricity as well (Kolb & Laudise, 1982). $AlPO_4$ was of interest for electronic devices, especially surface acoustic wave devices. $AlPO_4$ is isostructural and isoelectronic with quartz. The hydrothermal method is appropriate for $AlPO_4$ growth also because the α phase is only stable below 584ºC.

Potassium titanyl phosphate, $KTiOPO_4$ (KTP), is another important compound synthesized by hydrothermal technique (Rabenau & Rau, 1969). KTP is an optical material with a large nonlinear optical (NLO) coefficient (Zaldo, Rico & Diaz, 1999). NLO is a measure of efficiency to double the frequency of light or convert the light of one wavelength to the light of another.

There are a number of economically very important minerals deposited hydrothermally (Barnes, 1979). Tin, Tungsten, zinc sulfides, gold sulfide, quartz, mica, tourmaline, topaz and emerald are all grow hydrothermally (Bloembergen, 1999; Labochev, 1971). Hydrothermal occurrence of the minerals led scientists to explore their synthesis in the laboratory.

Our goal is to prepare new compounds by using supercritical fluids. The first part of our work includes synthesizing chalcogenides using supercritical amines. Synthesis of chalcogenides, particularly sulfides, sometimes requires a closed system because of the reagents' high vapor pressure and volatility. The versatility of the reaction medium led us to synthesize quite a number of chalcogenides temperatures with unique structures at relatively low.

Our group used supercritical water to prepare new phosphates under acidic conditions. A number of different structure types were obtained by making small temperature changes. Based on results and experiences from this early research, we have explored the hydrothermal synthesis of oxides under basic conditions. Alkali metal hydroxides are used as a solvent mineralizer and increase the solubility of metal oxides, particularly rare earth oxides. In basic conditions, GeO_2 and metal oxides react to form new compounds, and alkali metals can interact, forming quaternary compounds of good yield and large size.

The development of supercritical ethylenediamine and water as unique reaction media will be studied in detail to synthesize novel chalcogenides and germanates, respectively. The crystallographic and physical characterization data are used to support the discovery of these compounds.

Conclusion

Supercritical fluids represent a unique state of matter with distinctive properties that make them valuable in numerous scientific and industrial applications. Their tunable properties, environmentally friendly nature, and diverse applications make them an exciting and promising area of research. As technology advances and our understanding of supercritical fluids deepens, we can expect further innovations and breakthroughs in chemistry, materials science, and beyond.

Supercritical fluids have revolutionized various industrial processes by offering an eco-friendly and versatile alternative to traditional solvents. From extraction and chromatography to

advanced material synthesis and environmental cleanup, the unique properties of SCFs enable applications that were once considered unachievable. As research in this field continues, supercritical fluids will likely play an increasingly important role in science, industry, and environmental stewardship.

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CHAPTER V

Glycemıc Index And Its Role In Managıng Inflammatory Dısorders

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Gökay VARDAR[3]

1.Introduction

Carbohydrates are essential nutrients in our daily lives. Carbohydrates function as a source of energy in the body and play an important role in metabolism. It is recommended that healthy individuals consume about 200 to 300 grams of carbohydrates per day. Carbohydrates are our body's major source of energy and, when

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consumed, they are processed into glucose and released into the bloodstream. The glycemic index (GI) was created to determine the amount of carbohydrates in foods and observe its effect on blood sugar. In simple terms, glycemic index measurement measures how fast and how much the carbohydrates in a food raise blood glucose. This measurement is usually defined as the area under the area of increase (iAUC) of a food consumed under standard conditions within 2 hours. Test and reference foods should contain 50 g of available carbohydrate. The formula shows the effect of the food on blood glucose after consumption. To calculate the glycemic index of foods, it is necessary to determine the amount of available carbohydrates. Available carbohydrates, also known as glycated carbohydrates, refer to carbohydrates that are digested, absorbed and metabolized in the small intestine. This is usually taken as the sum of starches and sugars and does not take into account resistant starches and fibers (Venn & Green,2007; Vega-López, Venn, & Slavin, 2018).

Foods are categorized into groups after careful calculation of their glycemic index. Depending on their measured GI, foods are classified as low (<55), medium (55-69) or high (>70) GI foods. Carbohydrate-rich foods that are rapidly broken down and rapidly absorbed in the body are referred to as high GI foods. Conversely, carbohydrate-rich foods that are slower to digest and absorb are called low GI foods. Low GI foods have a slower effect on postprandial blood glucose levels and insulin response. There are many determinants of the amount of glycemic index in foods, including the type of carbohydrate, protein, fat, amount and type of fiber (soluble fiber foods have a lower glycemic index, whereas

insoluble fiber foods have a higher glycemic index), pH (low pH foods and beverages have a higher glycemic index), and combinations and processing of foods. High glycemic index foods in particular affect insulin, glucagon and lipid metabolism, as well as inflammation, leading to many diseases. Frequent consumption of high glycemic index foods in diets is known to cause type 2 diabetes, obesity, cardiovascular diseases, stroke, cancer, rheumatoid arthritis, neurological diseases such as epilepsy (Hardy, Garvin & Xu 2020; Jenkins & et al., 2021).

Biochemical Basis of Glycemic Index

Carbohydrates are divided into three groups: disaccharides and monosaccharides (sugars) and long-chain polysaccharides. Monosaccharides, the basic building blocks of carbohydrates, are compounds with a general chemical structure of $C_6H_{12}O_6$. Examples of monosaccharides include glucose, galactose and fructose. Disaccharides are compound sugars formed by the combination of two monosaccharides in such a way that a water molecule is released. Examples of disaccharides with a general chemical structure of $C_{12}H_{22}O_{11}$ include sucrose and lactose. Polysaccharides are polymers containing long monosaccharide chains linked together by glycoside bonds. Examples include amylose and cellulose. Carbohydrates can only be absorbed as monosaccharides, so other carbohydrate compounds with longer chains must first be digested enzymatically. The digestion of carbohydrates starts in the mouth with amylase in saliva and continues through enzymes in the stomach and small intestine. Carbohydrates enter the circulation via specialized transporters such as GLUT1 and GLUT2 (Holesh &et al., 2023; McQuilken, 2021).

Once monosaccharides enter circulation, glucose causes complex metabolic responses in the body. Pancreatic beta cells increase secretion of the enzyme insulin, producing hyperinsulinemia, which brings plasma glucose levels within normal values. Insulin also inhibits the secretion of the enzyme glucagon, released from pancreatic alpha cells, which acts as an antagonist and inhibits glucose production in the liver. Muscles and other tissues take up glucose and use it for energy production or storage. This hormone also promotes glucose uptake in adipose tissues (adipocytes) and reduces the concentration of free fatty acids (Norton & et al., 2021).

The types of carbohydrates that produce a high glycemic index are usually monosaccharides such as glucose and fructose. It is known that this is because they pass directly into circulation without being digested. Recent studies have focused on the fact that GI diets with high monosaccharide content may cause long-term hyperinsulinemia and glucotoxicity, leading to destruction of pancreatic beta cells. At the same time, chronic consumption of fructose decreases peripheral insulin sensitivity. Other studies have shown that a high glycemic index diet reduces the level of the enzyme lipoprotein lipase, leading to higher postprandial triglyceride and triacylglycerol formation. Lipoprotein lipase is an enzyme that prevents excess fat accumulation by breaking down excess triglycerides in the blood into fatty acids and glycerol. Suppression of this enzyme leads to hyperlipidemia and high cholesterol. (Yuan & et al.,2020; de Farias Lelis et al.,2020; Teymoori et al.,2021).

There are many factors that affect the formation of the glycemic index of foods. Among the most important internal factors is the content of the food. Carbohydrate foods contain starch, a polysaccharide, and the structure of starch is a major factor in the glycemic response of food. Starch is composed of two different substances, amylase and amylopectin. Amylase is more resistant to enzymatic hydrolysis and thus produces a jelly-like consistency with a lower glycemic index, whereas amylopectin forms a better jelly and is less resistant to enzymatic hydrolysis (Lal & et al., 2021). Another factor among the internal factors is resistant starch. The chemical composition of resistant starch is the same as starch and consists of amylose and amylopectin. However, the configuration of amylose changes in resistant starch. Studies have shown that resistant starch reduces the glycemic index (Bayar & Yurttagül, 2023).

When we look at the external factors, the effect of cooking methods is seen. When we look at foods with high starch content such as oats, it is known that cooking swells starch granules and makes them more suitable for enzymatic hydrolysis. This increases the GI compared to raw products. In particular, cooking methods that require less water should be used because higher moisture facilitates starch digestion (Zhang & et al., 2021). There are studies suggesting that cooling processes increase the formation of resistant starch and lower the glycemic index (Lal & et al., 2021).

2.Role of Glycemic Index in Inflammation and Diseases

As we mentioned at the beginning of this article, a high-glycemic diet plays a major role in the development of many

diseases. Research has suggested that a high GI diet increases inflammation in the body. Inflammation is a protective biological response through the immune system, tissues and organs to various harmful conditions in the body. Markers of inflammation in the body are usually determined by blood tests. Meta-analyses and studies have shown that generally low glycemic index (LGI) diets are associated with lower levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), leptin, interleukin-6 (IL-6), (IL-1β) and interleukin-10 (IL-10), whereas high glycemic index (HGI) diets show an increase or lesser decrease in these markers. In this chapter, we examined the association of different diseases with the glycemic index and its potential implications for inflammatory expression (Milajerdi & et al., 2018; Kim & et al., 2018).

2.1.Glycemic Index and Diabetes Mellitus

Diabetes mellitus (DM), or colloquially diabetes, is a macromolecular disorder of metabolism characterized by an individual's impaired ability to respond to the body's hormonal system, resulting in blood glucose levels that are higher than they should be. This disorder occurs in a situation in which the pancreas is unable to produce enough of the hormone insulin, or the insulin it produces is unable to sense the body's use of it. The main symptoms of diabetes include high blood glucose levels over a long period of time, frequent urination, increased thirst (polydipsia), increased hunger (polyphagia) and fatigue. Some biochemical tests are routinely performed to diagnose prediabetes or diabetes. Examples of these tests include Hemoglobin A1c (HbA1c) and oral glucose tolerance tests (OGTT) (Alam & et al.,; Kumar & et al.,2020; 2021).

According to WHO, diabetes is a major disease that causes blindness, kidney failure, heart attack, stroke and amputation. In 2019, diabetes and diabetes-related kidney disease caused an estimated 2 million deaths. At the same time, 422 million individuals worldwide have been diagnosed with diabetes and the disease has become a global problem in recent years (WHO, 2022). Diabetes is diagnosed by dividing into three subgroups.

Type 1 Diabetes (T1D): T1D is a type of diabetes, commonly diagnosed in childhood and adolescence, in which the pancreas produces insufficient or no insulin. This type of diabetes appears when the immune system wrongly targets the body's own pancreatic beta cells. This is why T1D is classified as an autoimmune disease. According to current models, it is thought to occur in three stages with genetic predisposition (Syed, 2022; Popoviciu & et al., 2023).

In the first stage, the individual is normoglycemic (normal blood sugar) but has islet-directed autoantibodies in the blood. Autoantibodies indicate that the autoimmune process has started. In the second stage, while the presence of autoantibodies is still detected, insulin secretion begins to be inadequate after food consumption, which causes dysglycemia (abnormal blood sugar). In the next stage, the general symptoms of diabetes appear, T1D is clinically diagnosed and insulin treatment is initiated. Research has shown that individuals progress through these stages at different rates, which is because T1D is a heterogeneous disease. Treatment of T1D involves lifelong insulin therapy. In addition, nutrition, exercise and blood glucose monitoring are important (Janež & et al., 2020; Powers, 2021).

Type 2 Diabetes (T2D): This type of diabetes is characterized by insulin resistance caused by the inability of pancreatic beta cells to use insulin. Although T2D is usually seen in older adults, recent studies suggest that its prevalence is increasing in children and adolescents. Unlike T1D, insulin intake is ineffective in the control of this type of diabetes. It is controlled by lifestyle, diet and some antibiotics. More than 90% of diagnosed diabetes belongs to this group (Galicia-Garcia & et al., 2020; Ahmad & et al., 2022). From a biochemical perspective, β-cell dysfunction is associated not only with β-cell death but also with a complex network of interactions between environmental interactions and molecular pathways.Especially in conditions such as obesity, which is a significant risk factor for the progression of T2D, hyperglycemia and hyperlipidemia increase insulin resistance and damage β-cells. Excess free fatty acids and hyperglycemia cause endoplasmic reticulum (ER) stress by activating apoptotic unfolded protein response (UPR) pathways. These conditions increase metabolic and oxidative stress, leading to β-cell damage (Serbis & et al., 2021).

Gestational Diabetes (GDM): GDM is a type of diabetes seen in pregnant women. In pregnant women, more glucose than normal is required for fetal growth and development, and hormones such as placental lactogen, estrogen and progesterone secreted during pregnancy can cause maternal insulin resistance, which can lead to hyperglycemia. This is thought to contribute to the development of type 2 diabetes in both mother and fetus(Sweeting & et al., 2022). As a result of recent studies, it is known that GDM is diagnosed in 14% or more of pregnant women. Especially as a result of the researches, advanced age (over 30) pregnancy, having a

macrosomic baby, number of previous births depending on age, family history of individuals with GDM and PCOS (polycystic ovary syndrome) treatment can significantly increase the risk of GDM in the maternal (Modzelewski & et al., 2022). As a result, diabetes is a disease typified by higher than normal blood sugar levels. In line with the researches, there are studies showing that foods with a high glycemic index can increase blood sugar. In this section, we will give examples of these studies to help the reader understand this relationship based on the results.

In another study conducted in Japan, researchers examined how participants' diets were distributed by quartiles in terms of glycemic index (GI) and glycemic load (GL) and the relationship of these factors with diabetes risk. According to the findings of the study, it was observed that high glycemic load and high glycemic index diet were more significantly associated with diabetes risk in female participants than in male participants. The reasons for this situation are that female individuals are fed a diet containing higher carbohydrate and fat, have a lower body mass index (BMI), and have beta cell dysfunctions (Oba & et al., 2013). A meta-analysis investigated the association of the glycemic index (GI) and glycemic load (GL) of foods with metabolic syndrome (MetS) in generally healthy adult participants. The studies were conducted between 2004 and 2018 in various geographical regions such as the USA, Korea, Iran, Malaysia and Russia. Dietary assessment methods included food frequency questionnaires (FFQ), 24-hour food recall (24DR) and 3-day food records (3FR). Different criteria were used to measure GI and GL using a variety of reference foods, and various criteria were used to diagnose MetS across studies. Results showed

a significant positive link between diet and GI and increased risk of MetS (Zhang et al., 2020) As a result, consuming people with diabetes foods with a high glycemic index can make blood glucose control difficult and may significantly increase the risk of diabetes in the long term. Therefore, for diabetes management, it is important to choose foods that have a low glycemic index and regulate blood sugar more consistently. Choosing a balanced diet of low glycemic index foods can play an important role in preventing complications of diabetes and improving overall health. The types of diets will be presented in detail at the end of the chapter.

2.2. Glycemic Index and Cardiovascular Diseases

Cardiovascular diseases (CVDs) are known to cause approximately 17.9 million deaths each year. Examples of cardiovascular diseases include coronary artery disease, stroke, hypertension, heart failure, arrhythmia, atherosclerosis and congenital heart disease. Factors such as hypertension, high cholesterol levels, diabetes, smoking and alcohol use, sedentary lifestyle, unhealthy diet, diabetes, obesity, genetics and stress can be given as examples of the causes of cardiovascular disease. Although CVD is known to occur more frequently in men, there are also important studies showing that early menopause increases this risk in women. More than four-fifths of CVD deaths are due to heart attack and stroke, and one-third of these deaths occur at an early age in people under 70 years of age. In recent years, CVD deaths have been known to outnumber cancer deaths (WHO n.d.; Eckel, Bornfeldt & Goldberg, 2021; Yoshida et al.,2021; Townsend et al.,2022). Before discussing the relationship between the glycemic index and CVDs, the most common cardiovascular diseases in

Turkey and the world will be explained to familiarize the reader with these diseases.

Coronary Artery Disease: This situation arises when plaque buildup obstructs the coronary arteries, leading to an insufficient supply of blood and oxygen to the myocardium. Plaques are formed when cholesterol, fat and other substances build up on the artery walls. These plaques narrow or block the lining of the arteries, so that blood flow to the heart muscle is reduced or stopped. This can lead to serious events such as a heart attack. Types of coronary artery disease include stable angina (angina pectoris), acute coronary syndromes (acute myocardial infarction, unstable angina), coronary artery spasm and sudden coronary death (Shao & et al., 2021; Komilovich, 2023).

Hypertension: Hypertension (commonly known as high blood pressure) is one of the most prevalent illnesses in society today. It is characterized by a narrowing or compression of the arterioles, which causes the blood to exert a higher pressure on the vessel walls. In this case, the heart works harder and relies on a higher pressure to pump the blood. According to the WHO, an estimated 1.28 billion adults aged 30-79 years worldwide have hypertension. Hypertension plays a very important role in the development of a lot of diseases. For the prevention and early detection of hypertension, it is recommended to measure the blood pressure of an individual at regular intervals (Zhou & et al., 2021; WHO, 2022; Sekhon, Kaur & Airi, 2024)

Normal blood pressure values are considered to be 120/80 mmHg. In this measurement, "120" is systolic blood pressure and

represents the maximum pressure exerted on the arterial walls during contraction of the heart muscle. "80" is diastolic blood pressure and represents the minimum pressure exerted on the arterial walls by the heart muscle at rest. SBP greater than 140 mm Hg and DBP greater than 90 mm Hg indicate an abnormal blood pressure (Lee & et al., 2020).

In addition to the fact that the formation of cardiovascular diseases depends on many reasons, especially the glycemic index has a great effect on the formation of these diseases. In a study conducted in Europe, the diet of individuals of different weights with coronary heart disease and the increase in the disease were monitored. The researchers found that the course of the disease increased in individuals who ate high GI foods. In addition, it has been suggested that the effect of a diet with a high glycemic index may be greater, especially in individuals with a higher BMI (Sieri & et al., 2020)

Further studies have observed the correlation between glycemic index and risk of CVD diseases in women participating in weight loss programs in Japan. The results show significant associations between HDL-cholesterol, triglycerides (TG) and insulin resistance (IRI). In the study, individuals with low dietary GI consumed less carbohydrates (Anjom-Shoae & et al.,2023).

A diet high in GI increases the likelihood of CVD, especially in individuals with other diseases (obesity, polycystic ovary syndrome (PCOS), diabetes, etc.). A study in 2020 examined the diets of individuals of different weights with PCOS. It shows that women fed diets with a low glycemic index improved cardiometabolic and reproductive profiles. This diet can improve

glucoregulation and lipid profiles, while reducing waist circumference and preventing hyperandrogenism (Kazemi & et al., 2021).

Another study in patients with type 2 diabetes showed that patients on a high-GI diet had lower Flow-Mediated Dilation (FMD) and more severe coronary artery disease than those on a low-GI diet. FMD assesses changes in the inner diameter of vessels by measuring the contraction and relaxation of an artery. A low FMD value is considered an indicator of endothelial dysfunction. Decreased FMD is a marker of coronary artery disease and this association is more pronounced in patients with type 2 diabetes than in patients with high GI control. These findings also emphasize the importance of glycemic control in patients with T2DM (Chen & et al.2021)

2.3.Glycemic Index and Autoimmune Diseases

Autoimmune diseases are diseases that occur when an individual's immune system attacks its own tissues as a result of certain factors. Autoantibodies that cause autoimmune diseases are antibodies produced against the body's own tissues or components. Normally, the immune system produces antibodies to fight against foreign pathogens, while autoantibodies mistakenly attack the body's own cells or tissues. Autoantibodies can also occur in conditions other than autoimmune diseases. These include infections, vaccines, medication or injuries. Examples of autoimmune diseases include type 1 diabetes, rheumatoid arthritis, Hashimoto's thyroid, Lupus Erythematosus, Sjögren's Syndrome (Negrini & et al.,2022; Harroud & Hafler,2023)

Rheumatoid Arthritis (RA): Rheumatoid arthritis (RA) is a systemic autoimmune disease and a chronic inflammatory condition that can affect the joints as well as organs such as the heart, kidneys and lungs. The course of the disease is variable and in the absence of appropriate treatment, conditions such as joint damage are seen. It is often genetically inherited. As RA progresses, joint damage and deformities can develop, especially affecting the wrist and fingers, including subluxation and ulnar deviation of the MCP joints (Dedmon, 2020; Radu & Bungau, 2021).

Vitiligo: Vitiligo is a skin disorder that features the loss of melanocytes, leading to reduced pigmentation in affected areas. This lack of pigmentation usually occurs in areas such as the face, back of the hands, nipples, armpits, belly, sacral and groin. Vitiligo is divided into two types: segmental (SV) and nonsegmental (NSV). SV is characterized by depigmented patches located in a single segment of the body according to the distribution of a particular nerve, while NSV is characterized by white patches that are common in different parts of the body. There are cases where a person with SV also has NSV, and this is classified as "mixed vitiligo" (Bergqvist & Ezzedine, 2020).

The disease is the most common depigmenting skin disorder worldwide. Vitiligo patients and their first-degree relatives are more likely to have other autoimmune compared to the general population. Treatment options for this autoimmune disease include phototherapy, medical therapies and surgical treatments (Bertolani & et al., 2021).

This increased likelihood of comorbid autoimmune diseases extends to metabolic conditions as well. In a study, metabolic syndrome (MetS) in vitiligo patients was investigated. Looking more closely at the diagnostic components of MetS, a significantly higher fasting glycemic index was observed in vitiligo patients compared to non-patients. This leads to early insulin resistance changes, higher LDL cholesterol levels and higher BMI levels in vitiligo patients (Xia & et al., 2021).

Further research indicates the association of the nutritional indices DII and DIL with the risk of Rheumatoid Arthritis (RA). The findings show that those with higher DII and DIL scores have an increased risk of RA. Individuals with a high glycemic diet were found to have a higher risk of developing RA. Higher DII and DIL scores are generally associated with consuming more red meat, processed foods, sweets and refined grains, while lower scores generally indicate a diet with lower fat and energy intake (Damaneh& et al., 2023).

At the same time, the glycemic index increases the risk of complications of the diseases that will occur. Another study in women with SLE suggests that a pro-inflammatory diet may be associated with a lipid profile that increases CVD risk. The underlying mechanisms are not entirely clear, but previous researchs suggests that an unhealthy diet can increase the risk of complications by increasing pro-inflammatory cytokines and altering the gut microbiota (Pocovi-Gerardino & et al., 2020).

2.4.Glycemic Index and Neurological Diseases

Neurological diseases generally occur when parts of the nervous system such as the brain, spinal cord and nerves are affected. The function of the nervous system depends on many internal and external factors. Internal factors are called neurotrophic elements and control the survival, growth and functioning of nerve tissue. The functioning of the brain also depends on various external factors. Stress, an unhealthy lifestyle and an unbalanced work-rest schedule, environmental pollution lead to mental and neurological diseases that occur in patients of all ages (Kurowska & et al., 2023).

An important new study published by The Lancet Neurology shows that more than 3 billion people worldwide will face a neurological problem in 2021. The amount of disability, morbidity and premature death caused by neurological conditions has increased by 18% since 1990. While the risk of death is higher in low-income countries because access to treatment is more difficult, access to treatment is higher in higher income countries (WHO, 2024). Migraine, Alzheimer's disease, multiple sclerosis (MS) and epilepsy are examples of neurological diseases whose prevalence has increased in recent years.

Alzheimer's Disease (AD): Alzheimer's is the most common type of dementia. The disease is characterized by cognitive, functional and behavioral impairments, usually beginning with memory loss. The hallmark pathological features in the brain tissues of these patients are elevated levels of amyloid-β (Aβ), which forms extracellular senile plaques, and neurofibrillary clusters formed by intracellularly deposited hyperphosphorylated tau (p-tau) (Zhang &

et al., 2021). AD follows a progressive disease process, starting from a symptom-free phase (a priori AD), progressing to mild cognitive impairment and/or neurobehavioral (mild behavioral impairment) changes, and finally to AD dementia. Various stage systems exist to categorize these stages of AD. There is no treatment that can completely cure Alzheimer's disease. The medications available on the market only provide symptomatic relief. In recent years, the incidence of Alzheimer's disease has reached high levels in the population and for this reason, there is a lot of research on this disease (Srivastava, Ahmad & Khare 2021; Porsteinsson & et al., 2021).

Parkinson's Disease (PD): The clinical syndrome known as Parkinson's disease (PD) is a motor disorder characterized by tremor, bradykinesia, rigidity and postural instability. Parkinson's disease is caused by the loss of cells in the dopamine-producing region of the brain stem, resulting in an under-release of this substance. PD subtypes were created by dividing patients into different clinical clusters, such as tremor-dominant and postural instability-gait disorder (PIGD) subtypes. PIGD is usually the most severe type. Environmental and genetic factors are effective in its formation. It is controlled with medication. Apart from motor disorders, constipation, rapid eye movement sleep behavior disorder (RBD) and depression are findings that occur before the onset of motor symptoms (Jankovic & Tan,2020; Hirayama & Ohno,2021).

Migraine: Migraine is defined as “an episodic headache associated with specific features such as sensitivity to light, sound or movement” or “a recurrent headache syndrome associated with a specific mixture of other neurological dysfunctions”. Several risk

factors are known as causes of migraine, including advanced age, head trauma, low socioeconomic status, drug use, stress, sleep problems (e.g. snoring), obesity, pain syndrome and pro-inflammatory or pro-thrombotic conditions (Amiri & et al., 2022).

Migraine is classified into three main types. Migraine without aura involves recurrent headaches lasting 4-72 hours, characterized by unilateral, pulsating pain that ranges from moderate to severe and worsens with physical activity. Migraine with aura affects about one-third of individuals and includes transient neurological symptoms, often visual, that occur before or during the headache. Chronic migraine is defined by experiencing headaches on 15 or more days per month (Eigenbrodt & et al., 2021).

For years, the relationship between neurological diseases and nutrition has been investigated and its importance in the formation of diseases is known. Especially in recent years, many studies have focused on the relationship between the glycemic index and the occurrence of specific neurological diseases in certain groups. A study by Taylor et al. confirms that a high glycemic diet is associated with increased amyloid burden in cognitively normal older adults. In particular, the amount of disease-causing amyloid was found to accumulate in the precuneus of the brain. According to this research, AD is increasingly associated with metabolic disease, including impaired brain and peripheral glucose metabolism, which in turn is associated with diet. In particular, high glucose and high fat levels are associated with brain amyloid deposition. These metabolic disturbances in the energy flow required for protein homeostasis can alter the processing of amyloid precursor protein and increase amyloid deposition (Taylor & et al., 2021).

A recent literature review has suggested that a low-glycemic diet may be beneficial for migraine. It has been suggested that this diet may reduce inflammation. In particular, a balance between the intake of omega-6 and omega-3 fatty acids has been suggested to reduce inflammatory responses, increase platelet function and regulate vascular tone. Therefore, it has been shown that a nutritional strategy that reduces omega-6 intake and increases omega-3 fatty acid intake may be beneficial for migraine (Gazerani, 2020).

Parkinson's disease (PD), another neurological disease in which the cells that produce dopamine in the brain are damaged, is also closely related to nutrition. PD is a disease for which there is no complete solution, and therefore a therapeutic dietary approach is of extra importance in terms of the development of the disease. The results of the studies suggest that high GI diets increase alpha-synuclein oligomerization and increased levels of DJ-1 protein are expected to regulate this. Alpha-synuclein (a-sin) is a protein found in the brain and plays an valuable part in the development of neurodegenerative diseases such as Parkinson's disease. Oligomerization is the abnormal assembly of this protein into small molecular structures called oligomers. These oligomers activate various biochemical and cellular pathways that can cause toxic effects in nerve cells and neuronal death. This is known to increase the likelihood of Parkinson's disease (Jafarzadehgharehziaaddin & et al., 2021).

4. Management of Inflammation-Based Diseases through Glycemic Index and Nutrition

As we described in the previous part of our study, the control of the glycemic index influences the occurrence of many diseases. The general view is that low glycemic index foods reduce inflammation. As a result, the risk of disease development is reduced and the progression of diseases is slowed down. For this, individuals' diets should be controlled. It is known that the fatty diet, which has increased in recent years, increases inflammation significantly. This dietary model is called the Western diet. The Western diet usually consists of ultra-processed supermarket products, ready-to-drink beverages, fast-food meals and foods with high fat content. Research proves that this dietary pattern activates pro-inflammatory cytokines, causes dysbiosis by disrupting the microbiota, and impairs intestinal permeability and bile acids. It is known that this diet plays a role in the development of many diseases, especially obesity and diabetes, and neurodegenerative diseases such as Alzheimer's disease (Azzam, 2021; Więckowska-Gacek & et al., 2021; Malesza & et al., 2021).

Low GI (LGI) diets, unlikely to the Western diet, are associated with increased body fat consumption, reduced total fat mass, rapid recovery after exercise and less hunger between meals. Furthermore, low GI diets have been shown to have positive effects on glucose and lipid metabolism and insulin resistance. In a study, the relationship between low glycemic index and diabetes was examined. It was observed that glycated hemoglobin (HbA1c), fasting glucose, blood lipids, adiposity values, systolic blood pressure and inflammation in the blood values of individuals fed

with LGI diet decreased (Chiavaroli & et al., 2021). In other studies, it has been suggested that the LGI diet reduces symptoms in female individuals with polycystic ovary syndrome with high BMI. In these individuals, testosterone glycoregulatory status decreased, abdominal adiposity decreased with a decrease in waist circumference (WC) with weight loss, and hyperandrogenism improved with a decrease in total testosterone (TT) (Kazemi et al.,2021). In a study conducted on epilepsy patients, there are findings that low glycemic index diet therapy (LGIT) reduces the number of seizures (Lakshminarayanan & et al., 2021).

Based on these studies, it can be argued that low-GI nutrition should be integrated into individuals' meals in order to maintain their health and reduce the risk of disease occurrence. Recent studies have argued that not only the GI content of the food should not be focused on, but also the time of consumption of the food is a very important factor. Given this, a meta-analysis showed that consuming low-GI foods, especially at breakfast, may be beneficial in reducing the risk of type 2 diabetes and other cardiometabolic diseases in individuals with metabolic disorders (Toh, Koh, & Kim 2020). Other studies have shown that consuming low-GI breakfasts improves cognitive performance, especially in young individuals (Peña-Jorquera & et al., 2021).

Another way to control GI is to enrich foods in this direction. Many countries, especially Asian countries with high carbohydrate consumption, are working in this direction. The first example of these efforts is rice. White rice, being refined and having its nutritious outer layer (bran and kernel) removed, can cause a sudden rise in blood sugar. Some types of rice, such as glutinous rice, which

has a particularly high amylopectin content, have a higher GI. However, whole grain and wholemeal rice types, such as brown rice, are recommended as low-GI alternatives. Similarly, rice and wheat flours are often used in cooking. These flours are classified as high glycemic index. As an alternative to reduce the GI, the use of flours derived from seeds, grains, nuts, fruits and root crops is recommended (Wee & Henry, 2020). The amount of antioxidant compounds in foods also affects GI. Phytochemicals containing important antioxidant groups contribute to more stable blood glucose levels. Phytochemicals include many different compound groups such as saponins, tannins, steroids, cardiac glycosides, alkaloids and flavonoids. Flavonoids (hesperidin, narengin, etc.), a particularly important group of antioxidants, are known to be associated with a lower GI by increasing insulin sensitivity and slowing glucose absorption. In addition to these factors, the amount of fiber, amount of fat and protein, and retrogradation status are other factors affecting GI. The table below provides examples of studies conducted worldwide in recent years to lower the GI of foods (Mohammed & Mhya, 2021; Ademosun, Oboh & Ajeigbe, 2021)

Table 1: Recent Studies on Reducing the Glycemic Index (GI) of Foods

Samples	Methods/Devices	Result of study	Cited
Wheat bran breads	Colorectometric Analysis	Wheat bran reduced GI	(Jimenez-Pulido et al.,2022)
Ice cream enriched with orange and shaddock	GI calculation formula	Phenolic compounds and antioxidants reduced GI level	(Ademosun, Oboh & Ajeigbe, 2021)
Chocolate energy bars prepared with %1,5 of guar gum	GI calculation formula	Soluble fiber property reduces sugar absorption, potentially leading to lower blood sugar levels.	(Saupi&et al.,2023)
Chips prepared with red lentil flour	GI calculation formula	The fiber and other components in red lentil flour slow blood sugar rise by reducing digestibility	(Babacan Cevik, Kahraman,& Ekici, et al.,2022)
Watermelon seeds and broken brown rice extruded snacks	Hexokinase enzymatic spectrophotometer	Extrusion partially gelatinized the starch, resulting in the intermediate GI of the snacks	(Sanusi&et al.,2023)
Cookies enriched with Dragon Fruit peels (DFP)	GI calculation formula	Dietary fibers and phytochemicals in DFP led to a slight decrease in the GI level	(Chumroenvidhayakul& et al.,2023)
Resistant starch-rich purple sweet potato noodles	GI calculation formula	Partially gelatinized and retrogradated flour reduces the GI level	(Nurdjanah&et al.,2022)
Mushroom(Termitomyces le-testui) bread	Glucometer	Mushroom flour reduced carb release, decreasing GI level	(Ashi& et al.,2022)
Gluten free sweet potato (Ipomoea batatas L.)	GI calculation formula	Protein content (WPC) of spaghetti reduced GI	(Giri& Sakhale,2022)
Fresh Pasta with Maqui Berry Powder(Aristotelia chilensis)	GI calculation formula	The fiber content of Maqui Berry Powder has reduced GI levels	(Bianchi& et al.,2022)

In recent years, with advances in nutrition science and technology, different studies have been conducted. One of these studies is to provide a comprehensive guide to the GI values of foods and a technological tool such as a website to increase the level of knowledge and self-efficacy in adopting low-GI dietary practices by supporting participants who prefer the materials. Thus, individuals' awareness can be increased (Avedzi & et al., 2019). Another study on this subject is a training program that lowers the glycemic index (GI). As a result of GI trainings given to individuals in Canada, participants were satisfied with the training, increased their knowledge about GI and reduced GI in their diets (Grant & et al.,2020). Another application is glycemic index labeling. It is emphasized that food labeling should take into account not only the total amount of carbohydrates, but also factors such as GI and glycemic load (GL). In particular, labeling the GI of the relevant food provides postprandial glucose control in diabetes management. In some countries (e.g. Australia, India, New Zealand, Singapore, South Africa), this labeling method is subject to specific regulations for GI-related nutritional content or endorsements, while in other countries (Canada, European Union) such claims are not allowed (Barclay & et al., 2021).

5.Conclusion

In conclusion, GI is known to be a very serious factor in the development of many diseases, especially obesity and diabetes. High GI dietary patterns that include high-fat and ultra-processed foods, such as the Western diet, which has increased in recent years, have been shown to increase inflammation, disrupt microbiota balance and lead to serious diseases. In contrast, low-GI foods have been

shown to reduce inflammation, lower disease risk and slow the rate of progression of existing diseases. In this context, it has been revealed that the GI value does not only depend on the amount of carbohydrates in the diet, but is also affected by many factors such as the type of carbohydrate, the way the food is processed and the meal consumed.

In this study, we have focused on the factors that reduce the GI of foods, how to eat and what to choose. Research for this purpose focuses on the use of alternatives to foods with high GI and enriching foods with antioxidant compounds. In addition, food labeling practices that enable GI control play an important role in protecting individuals' health by changing their eating habits. In this context, ensuring the adoption of low GI diets by the society is very important for maintaining public and individual health and reducing the risk of disease occurrence. In conclusion, understanding the effects of GI on nutrition and adopting low GI diets is one of the most important steps to lead a healthier life and the widespread adoption of this understanding will make a great contribution to public health.

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CHAPTER VI

Tragacanth Gum-based Films in Food Packaging

F. Fulya TAKTAK[1]

Introduction

Food packaging plays a vital role in preserving food quality, extending shelf life, and ensuring safety (Robertson, 2009). However, traditional synthetic packaging materials like plastics have raised serious environmental concerns due to their non-recyclability, long degradation times, and dependence on fossil fuels. This has created a strong demand for sustainable and eco-friendly alternatives in the packaging industry (El Alami El Hassani, 2024). Among the most promising solutions are natural polymers, which are derived from renewable resources and are biodegradable, making them ideal for reducing environmental impact while meeting the functional

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requirements of food packaging (Mangaraj, 2019; Gupta, 2022; Ponnusamy, 2022).

Natural polymers are categorized based on their origin and properties (Iravani, 2019; Aaliya, 2021). They include:

i. **Plant-Based Polymers**: Polysaccharides such as starch, cellulose, pectin, and alginates, as well as plant-derived proteins like soy protein and wheat gluten.

ii. **Animal-Based Polymers**: Gelatin, chitosan, and collagen, which offer excellent film-forming and antimicrobial properties.

iii. **Microbial Polymers**: Examples include xanthan gum and polyhydroxyalkanoates (PHAs), which are produced by microorganisms.

iv. **Lipid-Based Polymers**: Materials like beeswax and carnauba wax provide moisture barriers for coatings.

v. **Synthetic Bio-Derived Polymers**: Polymers like poly(lactic acid) (PLA), made from renewable monomers, offer strong biodegradability and mechanical properties (Iravani, 2019; Aaliya, 2021).

Natural polymers are widely used in food packaging due to their biodegradable and functional properties. They can be crafted into:

i. **Biodegradable Films**: These films can directly contact food, allowing the controlled release of active compounds like

antioxidants and antimicrobials. This helps extend shelf life, reduce spoilage, and maintain nutritional and sensory quality (Deshmukh, 2022; Pradhan, 2024).

ii. **Edible Coatings**: Materials like tragacanth gum or chitosan are used to coat fruits, vegetables, and meat products. These coatings act as protective layers, reducing moisture loss, delaying spoilage, and maintaining food freshness (Raghav, 2016).

iii. **Active and Intelligent Packaging**: Active packaging integrates features like oxygen absorbers or moisture regulators to improve preservation, while intelligent packaging uses freshness indicators to monitor food quality (Realini & Marcos, 2014). These innovations not only improve food safety but also reduce waste by encouraging timely consumption.

Polysaccharides and gums stand out for their unique properties and diverse applications. Polysaccharides such as cellulose, starch, alginates, and pectin are valued for their film-forming abilities, water retention, and compatibility with other biopolymers. Gums like tragacanth, xanthan, and guar are particularly important due to their functionality. They improve the mechanical strength and barrier properties of films, stabilize emulsions, and enhance the structural integrity of coatings (Gajbhiye, 2024; Choudhary, 2024). These features make them suitable for a wide range of food packaging solutions, from edible films to composite bioplastics.

Life-cycle assessments reveal that natural polymer-based materials significantly reduce environmental impact compared to traditional plastics. These materials require less energy to produce, generate minimal waste, and naturally degrade in the environment, reducing pollution and promoting sustainability (Ahmed, 2023; Saha, 2017).

The biodegradability of these polymers ensures they decompose naturally into non-toxic components, aligning perfectly with circular economic principles and providing a promising solution to the escalating plastic waste problem. Embracing natural polymers in food packaging signifies a transformative step toward sustainability. These materials seamlessly combine eco-friendliness with functional performance, meeting the growing demand for environmentally conscious alternatives while actively contributing to the protection of our planet. Polysaccharides and gums show significant potential in advancing the packaging industry by providing practical and sustainable alternatives to conventional materials.

2. Tragacanth Gum: Properties and Applications

2.1. Composition and structure

Tragacanth gum is a natural polysaccharide with a unique composition and versatile applications. Its chemical makeup includes moisture (8.79–12.94% w/w), minerals (1.8–3.2% w/w), protein (0.31–3.82% w/w), and carbohydrates (83.81–86.52% w/w). Tragacanth gum contains high levels of calcium and potassium, which are its main minerals. These minerals are consistently present across different species and growing conditions, making Tragacanth

gum a reliable source of these nutrients. Among the species, Astragalus rahensis has the highest protein content, making it particularly useful for applications needing protein-rich materials. On the other hand, Astragalus gossypinus has much lower protein content. Interestingly, the protein levels in A. gossypinus are like other natural gums like gum Arabic, agar, Persian gum, and gum Karaya. This indicates that although TG species differ, they all possess valuable properties that make them suitable for various applications (Nejatian, 2020).

Tragacanth gum is made up of two main components that are key to its unique properties. The first component, the soluble fraction of gum tragacanth (SFGT), is water soluble and accounts for approximately 20-40% of the weight of Tragacanth gum. This fraction plays an important role in the gum's ability to dissolve in water and enhance its functionality. The second component, the insoluble fraction of gum tragacanth (IFGT), does not dissolve in water but swells when hydrated to form a gel-like structure. This fraction accounts for 60-80% of the weight of tragacanth gum and contributes to its thickening and gelling properties (Bahraseman, 2022). Together, these two fractions give Tragacanth gum its high viscosity, ability to form gels, and overall versatility in various applications. The soluble fraction, tragacanthin, is further divided into sub-fractions, including arabinogalactan (which dissolves in ethanol) and tragacanthic acid (which does not). These sub-fractions add to the functional properties of tragacanth gum, but too much separation can reduce its overall performance. The balance of these fractions makes Tragacanth gum a highly effective and valuable natural polymer for many uses (Nazemi, 2023).

Tragacanth Gum also contains monosaccharides such as fucose, galacturonic acid and arabinose. Fucose, a hydrophobic monosaccharide, enhances the efficacy of tragacanth gum in gel-based systems, while the water-insoluble bassorin provides structural integrity. Together, these components make Tragacanth Gum suitable for a wide range of applications (Fattahi, 2013).

2.2. Applications of Tragacanth Gum

Food Industry

Tragacanth gum is highly valued in the food industry for its role as a stabiliser, emulsifier and thickener. It helps maintain the consistency of products such as sauces, dressings and dairy products by stabilising emulsions and preventing separation (Azarikia & Abbasi 2010; Mallakpour, 2023). The excellent film-forming properties of Tragacanth gum are used to produce edible films and food packaging materials that act as a barrier to moisture and oxygen, thereby extending the shelf life of food products (Tabassum, 2024; Amani, 2022; Zare-Bavani, 2024). In addition, Tragacanth gum is added to food formulations as a dietary fibre to improve texture, nutritional value and overall product quality (Hojjatoleslami, 2015).

Biomedical Applications

Tragacanth gum has important applications in healthcare, particularly in tissue engineering. It serves as a scaffold material that supports cell adhesion, growth and regeneration in both bone and skin tissues (Abdi, 2024; Zarekhalili, 2017; Najafian, 2023). Tragacanth gum-based hydrogels and nanofibres are widely used in controlled drug delivery systems, effectively releasing antibacterial,

anti-inflammatory and anticancer agents (Soleiman, 2021; Singh, 2023). Their hydrogels are also ideal for wound dressings, providing a protective barrier that promotes faster healing while delivering therapeutic agents directly to the wound site (Singh, 2027; Mohamadi-Sodkouieh, 2024).

Environmental Applications

The chemical structure of tragacanth gum contains hydroxyl and carboxyl groups, making it highly effective in environmental applications such as wastewater treatment (Fakhri, 2023; Rahimdokht, 2019; Ahmad & Imran, 2024). It acts as a bio adsorbent and efficiently removes heavy metals, nitrates and dyes from polluted water, making Tragacanth gum an environmentally friendly solution to water treatment, contributing to sustainability and pollution control.

Green Chemistry

Tragacanth gum plays a role in green chemistry as a natural reducing and stabilizing agent for the environmentally friendly synthesis of nanoparticles (Rao, 2017; Darroudi, 2013; Indana, 2016). This process replaces harmful chemicals typically used in nanoparticle production, making it a safer and more sustainable alternative. Due to its ability to form stable nanoparticle systems, tragacanth gum has applications in medicine, electronics and materials science.

Pharmaceutical Industry

Tragacanth gum is an important excipient in pharmaceutical formulation due to its non-toxicity, chemical stability, and

biodegradability. It is used to enhance the stability of drugs and improve their delivery. tragacanth gum also plays a critical role in encapsulating drugs, allowing for targeted and controlled release, particularly in pH-sensitive environments. These features make it a valuable component in modern drug development and delivery systems.

Cosmetic Industry

Tragacanth gum is widely used in personal care products like lotions, creams, and shampoos for its emulsifying and stabilizing properties (Bhasha, 2013). It helps create smooth textures and ensures the uniform mixing of ingredients, improving product performance and shelf life. Its natural origin and biodegradability make it an appealing choice for eco-conscious consumers and brands in the cosmetic industry.

3. Film Forming and Applications in Food Preservation

Tragacanth gum is well-known for its excellent film-forming abilities, making it a valuable ingredient in creating edible, biodegradable, and functional food packaging films. As a hydrophilic material, Tragacanth gum forms cohesive and flexible films that are highly effective for food preservation (Abbasi, 2019; Tokasi, 2024). These films act as strong barriers to moisture and oxygen, ensuring durability, flexibility, and biodegradability, which are essential for advanced food packaging solutions.

Recent studies have highlighted the potential of tragacanth gum to create smart, biodegradable food packaging materials that not only preserve food but also provide visual indicators of spoilage. For example, tragacanth gum (GT) has been combined with Kodo millet

starch (KMS), zinc oxide nanoparticles (ZNP) and beetroot peel extract (BPE) rich in betalain to produce films with advanced functionality. These films exhibit pH-dependent color changes and offer improved mechanical strength, UV protection and antimicrobial properties. These features make them effective against harmful bacteria such as E. coli and Bacillus cereus, while offering innovative ways to monitor food freshness. An important application of these films is the tracking of shrimp freshness during storage. Using KMS/GT/ZNP/BPE200% films, researchers monitored changes in total volatile basic nitrogen (TVB-N), an indicator of protein degradation. As the shrimp deteriorated over 48 hours, the films visibly changed color. After 12 hours, the TVB-N level increased to 10.70 mg/100 g and the redness of the film decreased while the yellowness increased slightly. At 36 hours, TVB-N reached 27.46 mg/100 g and the film turned completely yellow, indicating that the shrimp were no longer fresh (TVB-N > 20 mg/100 g, according to Chinese standards). After 48 hours, TVB-N increased further to 36.77 mg/100 g and the film turned pale brown, indicating advanced spoilage (Thakur, 2024).

Tragacanth gum is widely used as an edible coating for fruits, offering a natural way to extend their shelf life and maintain quality during storage. For example, a study on mangoes demonstrated that Tragacanth gum coatings in concentrations of 0.5%, 1%, and 1.5% effectively delayed ripening, reduced weight loss, and preserved the firmness of the fruit. Among these, the 1.5% Tragacanth gum coating showed the most significant results. It lowered ethylene production and respiration rates, which are key factors in the ripening process. Additionally, Tragacanth gum coating suppressed

the activity of enzymes responsible for fruit softening, such as polygalacturonase , cellulase, pectin methylesterase, β-glucosidase (β-Glu), and β-galactosidase. Tragacanth gum coating boosted antioxidative enzyme activity, reducing oxidative stress by minimizing reactive oxygen species like hydrogen peroxide (H_2O_2) and superoxide anions ($O_2^{\bullet-}$). This protection preserved cell wall components, such as protopectins, pectins, cellulose, and hemicellulose, delaying their degradation and reducing water-soluble pectin production, which slows fruit softening. Furthermore, Tragacanth gum coatings inhibited chlorophyll breakdown and slowed the accumulation of carotenoids, delaying changes in peel color. They also maintained higher levels of titratable acidity while slowing the increase in soluble solids content and the ripening index, ensuring better taste and nutritional quality. Overall, Tragacanth gum coatings offer a natural, effective solution for protecting perishable fruits like mangoes, preserving their freshness and extending their marketability under normal storage conditions (Ali, 2022).

Research on sweet peppers showed that a 1% tragacanth gum coating helped improve their quality after harvest by reducing weight loss, keeping them firm and slowing ripening. The coating created a thin barrier that reduced water and gas exchange, lowering respiration rates and delaying ripening. It also reduced the activity of enzymes such as pectin esterase and polygalacturonase, which are responsible for softening the fruit, helping it to retain its firmness and structure. To prepare the Tragacanth coating, Tragacanth gum powder (1.% w/w) was dissolved in water at 40°C for 10 minutes, together with glycerol (1%) to soften it and Tween 80 (0.05%) as an

emulsifier. After stirring for 30 minutes, the solution was refrigerated for 24 hours and mixed again before use. Peppers were disinfected with a 0.05% sodium hypochlorite solution, dried and immersed in the tragacanth gum solution for 5 minutes. The coated peppers were then dried, packed in boxes and stored at 8°C with high humidity. Their quality was checked weekly for 28 days. Tragacanth gum coating increased the activity of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase, which protect the fruit by reducing harmful reactive oxygen species. This helped the peppers stay fresher for longer, retaining more phenolic compounds and antioxidant activity than uncoated peppers. The coating also reduced lipid oxidation, further protecting the quality of the peppers. In addition, the Tragacanth gum-coated peppers showed a slower increase in sugar and a smaller decrease in acidity, i.e. ripening was delayed. These results demonstrate that a 1% TG coating is a simple and effective way to extend the shelf life and quality of sweet peppers during storage (Zare-Bavani, 2024).

Tragacanth gum is not only useful in coatings, it also significantly improves the strength and flexibility of composite films, making them ideal for food packaging. For example, adding 12% Tragacanth gum to alginate/aloe vera films increased their strength from 20.92 MPa to 67.49 MPa and their flexibility from 0.7% to 5.50%. This is due to the strong bonds formed between tragacanth gum and alginate, making the films tougher. Tragacanth gum also acts as a plasticizer, helping the films to remain flexible and durable for various applications. (Hadi, 2022).

4. Preparation of Tragacanth-Based Films

4.1. Solution Casting Method

Solvent casting is a widely used and highly reliable technique due to its simplicity, cost effectiveness and flexibility. The process involves dissolving polymers and optional plasticizers in a volatile solvent such as ethanol, acetone or water, or a combination of solvents. The polymer solution is then applied evenly to a substrate or mold, where the solvent is allowed to evaporate, resulting in the formation of a thin, uniform film. This evaporation process results in the alignment of the polymer chains and the integration of plasticizer molecules, if present, to form a cohesive film.

Solvent casting offers a significant advantage in terms of adaptability, as it can accommodate the incorporation of active ingredients such as essential oils, plant extracts or pharmaceutical compounds. These agents can be dissolved or suspended in the polymer blend prior to casting, allowing the production of functional films with tailored properties for specific applications. The process also allows film properties such as thickness, mechanical strength and optical clarity to be tuned by adjusting processing parameters such as solvent composition, drying time and temperature.

Solvent casting is recognized as a robust technique in various industrial and biomedical contexts. It is particularly valued for its ability to produce films with desirable properties such as uniform thickness, transparency and cost effectiveness, making it suitable for applications such as food packaging, wound dressings and advanced materials requiring functional enhancements through incorporation of active ingredients (Borbolla-Jiménez, 2023).

The solution casting method was effectively used by Goudar et al. (2020) to prepare various films, including control PVA films, poly(vinyl alcohol)/tragacanth gum (PT) blend films, and GA-doped PT films (PTG). In this study, PVA and tragacanth gum were dissolved in Millipore water with continuous stirring at 80-90°C for one hour to obtain a clear and uniform solution. After cooling to room temperature (approximately 26 ± 3 °C), the viscous solution was poured into clean Petri dishes. The films were formed by solvent evaporation over 96 hours at room temperature. After drying, the films were carefully peeled off and stored in desiccators for further testing. Composite films with improved mechanical and thermal properties were prepared by incorporating different weight percentages of gallic acid (GA) into the PVA and tragacanth gum blend using the same procedure. This approach demonstrated the versatility and effectiveness of the solution casting method for developing Tragacanth gum -based composite films for advanced applications (Goudar, 2020).

Amani et al. (2022) used the solution casting method to produce active packaging films using complex coacervates of gelatin and the soluble fraction of tragacanth gum (SFTG). SFTG was prepared by hydrating tragacanth gum, followed by centrifugation to separate the soluble fraction. Optimised ratios of gelatin and SFTG were mixed and the pH adjusted to promote coacervate formation, which was then freeze-dried. The resulting coacervates were dissolved in acetate buffer using glycerol as a plasticiser and curcumin as an active ingredient. The prepared solution was poured onto plates and dried under controlled conditions to produce films

with improved antioxidant properties, demonstrating their potential for use in food packaging applications (Amani, 2022).

4.2. Layer-by-layer (LbL) asssembly with Tragacanth gum

The LbL method involves the sequential deposition of materials to create multilayer thin films. This technique utilizes various materials such as polyelectrolytes, proteins, nucleic acids, and nanoparticles, forming layers through electrostatic interactions, hydrogen bonding, hydrophobic interactions, and covalent bonding. LbL assembly can be performed using techniques like dipping, spraying, spin coating, and microfluidic systems. Its versatility, low cost, and ability to precisely tune structural properties make it widely applicable in fields such as biomedical engineering, food packaging, and environmental sciences (Zhang, 2022).

The LbL method offers a powerful approach to fabricating multilayer thin films with tailored properties for diverse applications. Utilizing natural and synthetic materials, this method enables precise control over film composition and structure. Recent advancements have incorporated materials like Tragacanth gum and konjac glucomannan (KGM) into LbL systems, significantly enhancing mechanical, thermal, and functional properties. A noteworthy application of this method is the creation of the ε-poly-L-lysine (ε-PLL)/chitosan (CS)/tannic acid (TA)–KGM/TG–ε-PLL/CS/TA multilayer film, termed "ECTKT." This film combines the complementary properties of its layers to achieve superior physical and antibacterial performance. The ECT layers, composed of ε-PLL, CS, and TA, contribute robust antibacterial activity and strong mechanical strength, while the central KGM/ Tragacanth gum

layer enhances film flexibility and barrier properties. Cationic chitosan (CS) and ε-PLL interacted electrostatically with negatively charged Tragacanth gum. This interaction fostered strong adhesion between the layers, resulting in an integrated film structure (Mu, 2023). Although not a classic LbL assembly, the stepwise solvent casting approach used in this study mimics the sequential layering process of LbL. Each layer was cast and partially dried before adding the next, ensuring seamless adhesion and integration. This modified approach enabled precise control over the film's architecture while maintaining simplicity and scalability. The synergistic integration of these materials creates a multilayer structure optimized for food packaging applications, providing both functional versatility and environmental sustainability.

4.3. Electrospinning

Electrospinning is an advanced technology used to produce nanofibrous films by applying a high-voltage electric field to a polymer solution. The process involves stretching and solidifying the polymer solution into fine fibers, which are deposited onto a receiving surface to form a continuous film. The key components of an electrospinning device include a high-voltage power supply, injection pump, spinneret (needle), and a collector plate. When the electric field is applied, the polymer solution forms a "Taylor cone," and fine fibers are ejected as the electric field overcomes the surface tension of the solution. As the solvent evaporates, solid fibers are deposited onto the collector, creating a film with high porosity and a large surface area.

One of the main advantages of electrospinning is its ability to produce films at ambient temperatures, making it suitable for incorporating thermally sensitive bioactive compounds, such as antioxidants, antimicrobial agents, or vitamins. Electrospun films are highly versatile, offering controlled release properties, improved barrier performance, and enhanced bioactive compound stability compared to traditional methods like casting or spin coating. These properties make electrospinning particularly useful for applications in active food packaging and biomedical materials (Cui, 2023).

Electrospinning technology was used to develop PVA/ konjac glucomannan (KGM) nanofiber films enhanced with tea polyphenols (TPs) for food preservation. The films demonstrated excellent mechanical strength, hydrophilicity, oxygen barrier properties, and strong antioxidants and antibacterial activities, making them highly effective for extending the shelf life of bananas during storage. The preparation involved blending PVA and KGM solutions with TPs, followed by electrospinning at optimized parameters (9.5 kV spinning voltage, 1 mL/h feeding speed, and 15 cm receiving distance), resulting in nanofiber films with tailored properties. These films significantly reduced weight loss, maintained banana firmness, and slowed ripening by minimizing moisture loss, gas exchange, and organic acid consumption. Additionally, the films showed controlled TPs release, further enhancing antioxidant effects. Physical and chemical characterizations, including DPPH radical scavenging assays, confirmed their excellent performance in preserving food quality, offering a sustainable and effective solution for fresh produce packaging (Huang, 2024).

5. Environmental Benefits

5.1. Biodegradability and Sustainability

Tragacanth gum-based films are gaining significant attention for their environmental benefits, particularly due to their biodegradability and sustainability. Tragacanth gum, a natural polysaccharide derived from plant exudates, decomposes into non-toxic organic matter under natural conditions, posing minimal risk to the environment. This positions Tragacanth gum as an eco-friendly alternative to conventional petroleum-based synthetic polymers, contributing to global efforts to mitigate pollution and ecological degradation (Ma, 2024; Zare, 2019). The natural origin and compatibility of Tragacanth gum with other biopolymers further enhances its appeal for applications in sustainable material development.

Biodegradability is a key advantage of incorporating Tragacanth gum into packaging films, as demonstrated in bioactive nanocomposite films made with Tragacanth gum, polyvinyl alcohol (PVA), zinc oxide nanoparticles (ZnO NPs), and ascorbic acid (AA). These films exhibit exceptional mechanical strength, antioxidant activity, and antibacterial properties, making them ideal candidates for sustainable food packaging. In addition to their functional advantages, Tragacanth gum -based nanocomposite films offer remarkable environmental benefits. The incorporation of Tragacanth gum significantly enhances the biodegradability of the films, addressing a critical issue in reducing long-term waste accumulation. Soil burial studies demonstrate that Tragacanth gum-based films degrade completely within 60 days, a stark contrast to the minimal

5% weight loss observed in pure PVA films over the same period. This rapid biodegradation is attributed to Tragacanth gum 's natural polysaccharide structure, which supports microbial activity by providing an organic substrate for decomposition (Janani, 2020). Moreover, Tragacanth gum-based nanocomposite films align with the principles of a circular economy by offering a renewable and biodegradable alternative to synthetic plastics. These films provide effective oxygen and water vapor barriers, crucial for preserving food quality and extending shelf life, while simultaneously minimizing their environmental footprint. The inclusion of active agents like ascorbic acid enhances antioxidant properties, making the films suitable for packaging perishable food items, reducing spoilage, and contributing to waste reduction.

By combining biodegradability, sustainability, and functional performance, Tragacanth gum-based nanocomposite films exemplify the potential of natural materials in addressing pressing environmental challenges. Their development represents a significant step toward reducing the reliance on petroleum-based materials in packaging and promoting sustainable practices across industries.

5. Conclusion: Future Perspectives in Food Packaging

The development of sustainable and functional food packaging is a critical focus for addressing global environmental and food preservation challenges. Emerging materials like Tragacanth gum-based bioactive nanocomposite films present exciting opportunities for advancing this field. These materials combine biodegradability, antimicrobial activity, and enhanced mechanical

and barrier properties, making them ideal for eco-friendly packaging solutions.

Future research should explore the integration of other natural biopolymers and active agents to further enhance the multifunctional properties of Tragacanth gum -based films. Innovations in nanotechnology and fabrication methods, such as electrospinning and LbL assembly, offer potential to create even more advanced materials tailored for specific packaging needs. Additionally, large-scale production methods need to be optimized to reduce costs and facilitate commercial applications.

Advancements in intelligent packaging, including films capable of indicating food freshness or releasing active compounds in response to environmental changes, represent another promising direction. By aligning with circular economy principles, future food packaging solutions can contribute to reducing waste, extending shelf life, and promoting global sustainability. Tragacanth gum-based films, with their remarkable properties and environmental benefits, are poised to play a pivotal role in shaping the future of food packaging.

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CHAPTER VII

Fatty Acid Composition of Three Different Grape Pekmez in Konya, Turkey

Özcan Barış ÇİTİL[1]

1. Introduction

Pekmez is the one of the most common and known mulberry and grape products in Turkey. (Tekeli, 1965). It can be assumed as a natural food containing natural sugars such as glucose, galactose and minerals. Since pekmez contains high amounts of sugar, mineral and organic acid, it is a very important food product in human nutrition (Demirozu et al., 2002). This content refers to about 250.000 tones/year pekmez (Koylu, 1997).

Grape pekmez is produced with classical and vacuum (modern) methods in Turkey. Content of production by classical

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method is higher than vacuum method. Specially, in rural regions pekmez is produced by classical method (Batu et al., 1993)

Pekmez is a good source of energy and carbohydrate content source due to its high sugar content ranges betwen 50%-80%. Average energy value is 280 kcal/100g (Guven, 1982). Pekmez are rich in point of zinc, copper, manganese and iron minerals (Kayisoglu and Demirci, 2006). On the other hand, grape pekmez is a best nutritious dietary supplement for all ages owing to its daily nutrition needs, caloric value and aroma compounds; iron and calcium, vitamins, minerals, proteins, balanced natural sugars of fructose and glucose and antioxidant agents (Batu, 1993).

40% of the grapes produced in our country are consumed fresh, 35% are dried, 23% are used in the making of various products such as molasses, dried fruit sheets and sherbet while 2% are processed to be made wine Baskan and Pala, 2008. Approximately 37% of grapes produced in Turkey is used in pekmez production every year (Koylu, 1997).

The n-3 and n-6 PUFAs are also considered essential for the growth and development of children and they are precursors of composite hormones known as eicosanoids, involved in several metabolic processes of great importance for the human body, mainly related to cardiovascular activity (Eder, 1995; Inhamuns and Franco, 2008). Among the polyunsaturated fatty acids, linolenic acid (C18:3 n-3), EPA (eicosapentaenoic acid, C20:5 n-3) and DHA (docosahexaenoic acid, C22:6 n-3) are the dominant n-3 fatty acids in sunflower oil (Citil et al., 2010). These fatty acids are of great importance to humans for the prevention of coronary heart disease

(Kinsella, 1987). ω-3 PUFA have been shown to have positive effects on cardiovascular diseases (Connor, 1997).

There are a number of researches about grape pekmez (Kaya and Belibagli, 2002; Kayisoglu and Demirci, 2006; Sengul et al., 2005). However, there is little information about other properties of pekmez. There is no work on the fatty acid composition of grape pekmez species. Therefore, the aim of this study was to determine the total fatty acid composition, PUFA, MUFA and SFA of some pekmez samples in eating.

2. Materials and Methods

2.1. Collection of Samples

The pekmez samples were done from three different Turkish grape cultivars, Mix Pekmez, Müşküle, Siyah Pekmezlik, in 2010. Seven samples were taken from different pekmez species. It was stored at room temperature until analysis.

2.2. Fatty Acid Analysis

Fat extraction was carried out according to the Tarım ve Köy İşleri Bakanlığı (2004). Fat (crude) was determined gravimetrically on 2 or 4 g portions of each pekmez species by AOAC (1990) method 922.06. Samples were transesterified with BF3-methanol (Moss et al., 1974). The resultant fatty acid methyl esters were separated and stored at -20 ^{o}C. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature and analysed by gas liquid chromatography (Shimadzu 15-A), equipped with dual flame ionisation detector and a 1.8 m × 3 mm internal diameter packed glass column containing GP 10 % SP–2330 on

100/120 Chromosorb WAW, cat no: 11851. Column temperature was 190 °C for 31 min., and then rose progressively at 30 ºC/min up to 220 °C where it was maintained for 10 min at 220 °C. Carrier gas was nitrogen (2 ml/min). The injector and detector temperatures were 235 and 245 °C respectively. Conditions were chosen to separate fatty acids of carbon chain length from 8 to 24. The fatty acids were identified by comparison of retention times with known external standard mixtures (Alltech), quantified by a Shimadzu Class-Vp software were expressed as percentage distribution of fatty acid methyl esters. Each of the experiments was repeated three times.

Identification of fatty acids was carried out comparing sample FAME peak relative retantion times with those obtained for Sigma standarts. Results were expressed as relative percentages. All solvents and reagents were analytical grade.

2.3. Statistical Analysis

Table 1. *Fatty acid profiles of grape pekmez (%, w/w).*

Fatty acids	Mix pekmez	Müşküle	Siyah Pekmezlik
C 8:0	1,06±0,03a	0,94±0,03b	0,95±0,02b
C 10:0*	0,65±0,02a	0,03±0,00c	0,32±0,01b
C 12:0*	0,54±0,02a	0,01±0,00b	0,26±0,02c
C 14:0*	1,66±0,02c	2,56±0,04a	2,01±0,01b
C 15:0	1,13±0,03a	1,15±0,03a	1,07±0,03a
C 16:0*	21,46±0,02b	21,07±0,04c	22,66±0,03a
C 17:0	1,66±0,02a	1,39±0,06b	1,45±0,02b
C 18:0*	0,08±0,00a	0,27±0,01b	0,17±0,02c
C 20:0	2,53±0,00a	2,28±0,05b	2,29±0,03b
C 21:0	1,14±0,02a	0,87±0,04b	0,82±0,02b

C 22:0*	0,59±0,01a	0,41±0,01b	0,48±0,02c
C 24:0	0,17±0,02a	0,14±0,01b	0,14±0,00ab
∑SFA	32,68	31,13	32,62
C 14:1 ω5	0,32±0,15b	0,41±0,02a	0,35±0,02b
C 16:1 ω7*	3,25±0,03c	4,62±0,04a	3,75±0,04c
C 18:1 ω9*	36,09±0,03b	33,58±0,07c	36,33±0,04a
C 20:1 ω9	0,63±0,02a	0,48±0,02b	0,53±0,02b
∑MUFA	40,28	39,09	40,97
C 18:2 ω6*	15,91±0,04b	18,24±0,04a	18,36±0,09a
C 18:3 ω3*	9,51±0,05b	10,46±0,02a	6,74±0,02c
C 20:3 ω3	0,27±0,03a	0,21±0,01a	0,24±0,01a
C 20:4 ω6*	0,17±0,01a	0,11±0,00b	0,13±0,00c
C 20:5 ω3*	0,52±0,01a	0,27±0,03c	0,39±0,01b
C 22:3 ω3	0,36±0,02a	0,27±0,02b	0,30±0,01b
C 22:4 ω6*	0,30±0,00a	0,21±0,00c	0,25±0,00b
∑PUFA	27,03	29,78	26,41

* a – c Mean values within the same row sharing a common superscripts are not significantly different at $P<0.05$.

The proximate compositions and GLC analyses were repeated by three times. In fatty acid analysis, seven data (n=7) were obtained for each variety. The results are reported as means ± SD. Statistical comparisons were made using SPSS Software (version 15.0). Analysis of variance (ANOVA) was used to compare three different pekmez with each other. P-value <0.05 was considered to be significant. The mean values were compared by Tukey's test.

3. Result and Discussion

The pekmez included in this study were contained fatty acids of 8-24 carbon chain lengths. Total lipid contents of the pekmez investigated in Konya province in Turkey. The lipid of pekmez was found to be 0,1%, 0,12% and 0,09%, Mix Pekmez, Müşküle and Siyah Pekmezlik, respectively. Similarly, Temiz and Yesilsu (2010) found that contents of pekmez was around 0.1.

In the present study, the most abundant fatty acids in siyah pekmezlik were oleic (C18:1), palmitic (C16:0), linoleic (C18:2), linolenic acid (C18:3), palmitoleic acid (C16:1), eikosanoic acid (C20:0) and myristic acid (C14:0) at 36.33%, 22.66%, 18.36%, 6.74%, 3.75, 2.29% and 2.01%, respectively. These seven fatty acids represented about the 92.14% of total fatty acids. Similar results were observed by Boschin et al. (2008) for C16:0, C16:1 ω7, C18:0, C18:1 ω9, C18:2 ω6, C18:3 ω3, C20:1 ω9 and C22:0 in *Lupinus albus*.

Fatty acid composition of total 3 pekmez in eating is shown in Table 1. In the present study, palmitic acid was the major SFA (21.07 - 22.66% of total fatty acids) in all samples. Similar results were obtained by Akin (2011) with 18.91, 15.84 and 24.23% for Ekşi Kara, Gök Üzüm and Karadimrit, respectively.

Oleic acid was identified as the major MUFA 36.09%, 33.58% and 36.33% at Mix Pekmez, Müşküle, Siyah Pekmezlik, respectively (Table 1). Citil and Tekinsen (2011) found similar results for all salep species Palustris (28.65%), for Anatolica (23.77%), for Coriophora (14.44%), for Tridentata (56.89%). The main interest in pekmez oil is the high content of the

monounsaturated fatty acids such as oleic acid (33.58-36.33%). Similarly, Ertan et al. (2001) found that oleic acid was the predominant fatty acid (25.4%) in Sideritis athoa and Sideritis niveotomentosa (24.8%).

Linoleic acid (LA) was the most abundant PUFA 15.91%, 18.24% and 18,36% at Mix Pekmez, Müşküle, Siyah Pekmezlik, respectively (Table 1). Similar results were obtained by Tangolar et al. (2009) and Pardo et al. (2009) in all varieties.

Palmitic acid was the major SFA and the amount of fatty acids in pekmez species was found to be between 21.07% and 22.66%. In accordance with our results, in the fatty acid composition of vegetable oil, palmitic acid was shown to have the highest proportion in SFAs (Citil et al., 2010). However, it has been determined that the palmitic acid in the seeds of five Nepeta species is lower than that found in our study, but palmitic acid is the major fatty acid identified in SFAs (Akpinar et al., 2008).

The highest value of the saturated (SFA) to polyunsaturated (PUFA) fatty acids ratio was in siyah pekmezlik. The SFA/PUFA value in siyah pekmezlik was around 1.24. Similar results obtained by Tangolar et al. (2009) for Horoz karası, Narince; 0.23, 0.19, respectively. In the present trial, the values for ω-6/ω-3 ratio were higher than 4. This ratio was 0.65, 0.60 and 0.40, in Mix Pekmez, Müşküle and Siyah Pekmezlik, respectively.

4. Conclusion

In conclusion, it was determined that fatty acid compositions varied between pekmez samples. Unsaturated fat content was determined the highest level (68.87%) in all pekmez species.

According to these results, the oil of pekmez species may be a good source of essential fatty acids. As PUFAs are accepted to be beneficial to health, the oils obtained in pekmez species having high content of PUFAs could improve human health.

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CHAPTER VII

Significance Of Kinetic Studies On Myeloperoxidase Enzyme

Hande Usanmaz[1]

1. Introduction

Enzymes are proteinaceous biological catalysts that expedite chemical reactions. A substantial segment of the human genome encodes for enzymes (Lehninger, Nelson & Cox, 2005). The principal roles of enzymes are their catalytic efficacy and selectivity. The location where catalysis transpires in enzymes is referred to as the active site. The active site consists of elements that can bind the enzyme's substrate and, if available, its cofactor, facilitating bond formation and cleavage. Enzymes lower the activation energy of a process, hence enhancing the reaction rate (Berg, Tymoczko & Stryer, 2014).

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The enzymatic process occurs millions of times more rapidly than the uncatalyzed reaction. Enzymes exhibit remarkable specificity for the processes they catalyze and the substrates they utilize in those reactions. These protein molecules remain unchanged, are not used, and do not affect the equilibrium of the process they facilitate. Enzymes employ all molecular forces to effectively align substrates, facilitating the creation and cleavage of chemical bonds (Keha & Küfrevioğlu, 2000).[2]

The function of several enzymes can be obstructed by the attachment of certain molecules and ions. Enzyme inhibition serves as a regulatory mechanism inside biological systems. Moreover, several pharmaceuticals and hazardous compounds can impede enzyme activity. Enzyme inhibition is categorized into reversible and irreversible kinds. Irreversible inhibitors bind firmly to enzymes and are challenging to separate. Certain pharmaceuticals function as irreversible enzyme inhibitors. Reversible inhibition allows the inhibitor to swiftly dissolve from the enzyme and is categorized into competitive and non-competitive kinds (Berg, Tymoczko & Stryer, 2014). In competitive inhibition, the enzyme interacts with the substrate to create an enzyme-substrate (ES) complex or an enzyme-inhibitor (EI) complex, but an enzyme-substrate-inhibitor (ESI) complex is not formed. In non-competitive inhibition, the inhibitor exclusively binds to the enzyme-substrate complex, therefore

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increasing substrate concentration does not mitigate inhibition (Gilbert, 1992).

Metabolic activities in live cells need protection from free radicals and the deleterious consequences of oxygen consumption. Antioxidant enzymes, including superoxide dismutase, catalase, glutathione peroxidase, and peroxidases, are integral to this protective mechanism. Antioxidant enzymes and molecules safeguard cells in aerobic organisms from oxidative stress by blocking lipid peroxidation and other free radical-mediated processes, therefore averting the advancement of several chronic ailments, including lipid peroxidation (Gülçin et al., 2010a).

Free radicals possess one or more unpaired electrons in their outer shells, enabling them to oxidize, assault biomolecules, and impair biological membrane structures. Molecular oxygen, with a high electron affinity, serves as the thermodynamic impetus for the adoption of the terminal electron in the electron transport chain. Harmful chemicals, including superoxide and peroxide, may be generated during this reduction process. These two chemicals may harm cells. Superoxide (O2•-), hydrogen peroxide (H^2O^2), and hydroxyl radical (OH•-) generated from these are together termed reactive oxygen species (ROS). Reactive oxygen species (ROS) can be generated metabolically through the electron transport chain, specific enzymatic activities, and oxidation processes, or may result from external influences such as ultraviolet radiation, ionizing radiation, pharmaceutical side effects, tobacco use, dietary variables, and carcinogenic agents (Aksoy 2002; Gülçin 2012).

Despite the role of cytochrome C oxidase and other proteins in the electron transport chain in neutralizing reactive intermediates, a minimal quantity of superoxide and peroxide is always generated. Superoxide dismutase acts as a superoxide scavenger, converting superoxide into hydrogen peroxide and molecular oxygen. In eukaryotic cells, it is located in the mitochondria and cytoplasm, with the mitochondrial enzyme associated with copper and zinc. The catalase enzyme captures superoxide dismutase and hydrogen peroxide generated through several activities, converting them into molecular oxygen and water. Both enzymes exhibit great efficacy. Glutathione peroxidase is crucial for the sequestration of hydrogen peroxide (Doumonted & Rousset, 1983).

Peroxidases (POD: H^2O^2 -Oxidoreductase E.C.1.11.1.7): Enzymes transform reactive oxygen species produced during metabolism into innocuous compounds (Champe, Harvey & Ferrier, 2007). Peroxidases demonstrate antioxidant capabilities by facilitating the oxidation of organic and inorganic substrates with hydrogen peroxide serving as the electron acceptor (Hussain, Slikker & Ali, 1995). Peroxidases are prevalent in prokaryotic, eukaryotic, and photosynthetic cells. They are found in several species of radishes, legumes, elevated plants like as tobacco, yeasts, fungi, and bacteria. Numerous isoenzymes of peroxidase have been identified from various plant species.

In mammals, peroxidase enzymes are found in milk, salivary glands, and tears as lactoperoxidase (LPO) (Kumar & Bhatla, 1995), in leukocytes and platelets as myeloperoxidase, in the liver, spleen, uterus, lung walls, cytoplasm, and mitochondria as glutathione

peroxidase, and in microsomes and lysosomes as peroxidases (Pütter & Becker, 1987).

The protein component of peroxidase is produced within the organism, rendering the enzyme inactive. The holoenzyme acquires functioning by the association of the heme group with the apoprotein (Fric, 1976; Van Huystee, 1987).

The first prosthetic group of peroxidases is protoheme, which is loosely associated with the apoprotein, in contrast to other hemoproteins. In peroxidase-catalyzed processes, H2O2 is reduced utilizing several compounds, including ascorbate, quinones, and cytochrome C, which serve as electron acceptors (Doumonted & Rousset, 1983). The oxidative nature of H2O2 requires its prompt elimination from the medium. This function is performed by crucial antioxidant enzymes within cells, including catalase and peroxidase (Halliwell & Gutteridge, 1989; Robert et al., 1993). Antioxidant enzymes also neutralize free oxygen radicals, so averting cellular oxidative damage (Harris, 1992).

The secondary prosthetic group on the peptide chain consists of glycosidic side chains. Most peroxidase isoenzymes have carbohydrates that constitute 15-17% of their molecular weight (Van Huystee, 1987). Alongside the heme and glycosidic groups, the calcium (Ca2+) ion is essential for the release and structural integrity of peroxidase (Fric, 1976; Van Huystee, 1987). Various investigations have shown that horseradish peroxidase had a molecular weight of 31-33.8 kD and includes a glycosidic side chain beside the heme group (Van Huystee, 1987).

Peroxidases are extensively utilized in the research of metabolic processes, enzyme functionalities, and protein architectures because their activities can be readily quantified by chromogenic techniques and their thermal stability (Van Huystee, 1987). POD is frequently preferred as an important enzyme in clinical diagnosis, micro-analytical applications, pharmaceuticals, and the food industry (Kwak Ark., 1996).

2.Myeloperoxidase Enzyme (MPO) (EC: 1.11.1.7)

The myeloperoxidase enzyme (EC: 1.11.1.7) was first purified by Agner in 1940. According to some researchers, the MPO enzyme constitutes 1-2% of the cell's dry weight, while others report it constitutes 5% (Olsson, Bulow, Hansson, 2004). The MPO enzyme belongs to the mammalian peroxidase family, which comprises eosinophil peroxidase, lactoperoxidase, and thyroid peroxidase, and it possesses three isoenzymes: MPO I, II, and III (Wright & Ark. 1990, Miyasaki & Ark. 1987). MPO is a prominent protein in azurophilic granular leukocytes, located in the cytoplasm of azurophilic granules with digestive enzymes and several antimicrobial proteins (Olsson, Bulow, Hansson, 2004).

MPO is a cationic protein containing glucose, with a molecular weight of 144 kD. It consists of two similar dimers connected by a disulfide bridge. Selective cleavage of the disulfide bond connecting the two subunits yields a hemi-enzyme (half-enzyme) that is not significantly different from the full enzyme in terms of spectral and catalytic properties (Fiedler, Davey & Fenna, 2000). Each dimeric structure of MPO contains a light and a heavy subunit (composed of 108 and 466 amino acids respectively) with a

protoporphyrin IX group containing a central iron atom. The heme groups of MPO are functionally identical and are attached to the apoprotein by two ester bonds and one sulfonium ion bond. This unique triple bonding of heme, compared to other heme proteins, causes a slight distortion of the porphyrin ring and a “red shift” of the Soret band to 428 nm for MPO (Arnhold, 2004). This shift is responsible for the strong absorption band in the UV region, which gives the enzyme its characteristic green color (Fiedler, Davey & Fenna, 2000).

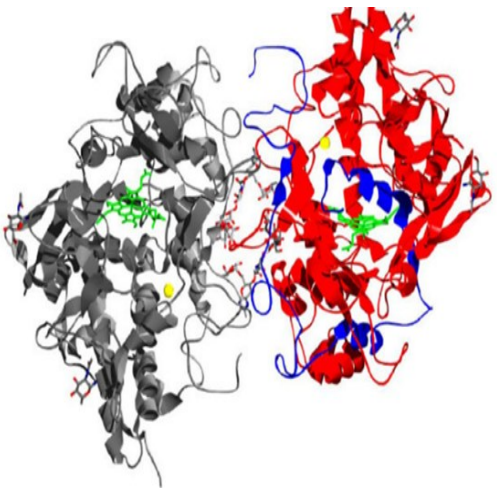

Figure 2.1. The Comprehensive Structure of Natural Homodimeric Human Myeloperoxidase. *Each subunit comprises a light chain (blue), a heavy chain (red), and a modified heme group (green). Furthermore, calcium ions (depicted as yellow spheres) and glycosylated areas are shown (Furtmüller et al., 2006).*

Leukocytes execute their antimicrobial functions through the process of phagocytosis, wherein the microorganism is engulfed and digested. During this process, respiratory burst, which involves a rapid increase in oxygen consumption and the formation of

superoxide radicals, occurs via NADPH oxidase localized on the phagosome membrane. As a result of excessive oxygen usage during phagocytosis, superoxide anions and hydrogen peroxide are produced. Superoxide is a free radical resulting from the loss of an electron from an oxygen molecule (Hampton Kettle & Winterbourn, 1998). The myeloperoxidase enzyme catalyzes the formation of hypohalites by converting H^2O^2 and halogens (Cl-, F-, Br-, and I-). For instance, myeloperoxidase catalyzes the formation of the bactericidal agent hypochlorite from H^2O^2 and Cl-, as well as hypobromite from H^2O^2 and Br-, among others. These substances are highly toxic to bacteria, fungi, and mammalian cells (Klebanoff, 1968).

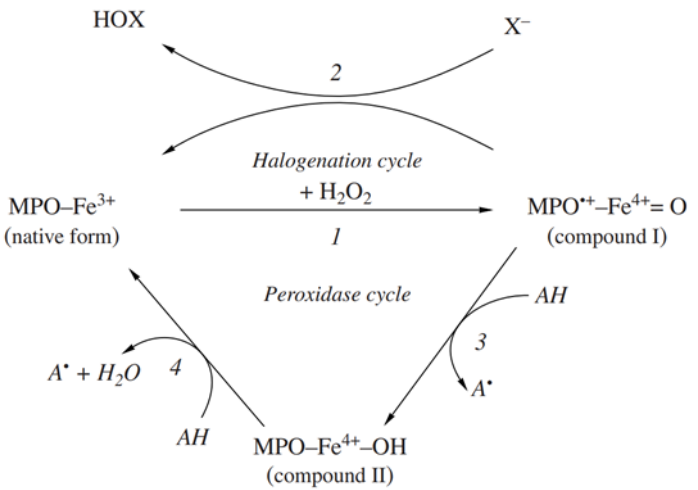

Figure 2.2. Principal reactions of the halogenation and peroxidase cycles mediated by MPO. *X− denotes halogenide or pseudohalogenide (SCN−); AH represents the peroxidase substrate (Gorudko & Ark. 2009).*

Figure 2.2. illustrates a schematic representation of the MPO mechanism. Hydrogen peroxide is generated in vivo during the respiratory burst. It transforms MPO produced by active neutrophils into compound I. This MPO form has a significant redox potential. In the first segment, halides (Cl-, Br-, and I-) and pseudohalides (SCN-) experience two-electron oxidation. During this step, extremely reactive hypohalous acids are generated, the halogenation cycle is completed, and the enzyme reverts to its natural MPO-Fe3+ state. In the subsequent phase, compound I is first transformed into compound II and then reverted to the original enzyme by a series of one-electron oxidations involving several peroxidase substrates. The enzyme exhibits both halogenation and peroxidase activity (Gorudko et al., 2009).

The MPO enzyme is found only in neutrophils and monocytes (Arnhold, 2004). Many studies have indicated that the unique antimicrobial property of MPO is due to the production of hypochlorous acid and other toxic agents, creating an environment within neutrophil phagolysosomes that inhibits or kills microbes. The presence of MPO-catalyzed modifications, such as tyrosyl radical formation, chlorination, tyrosine peroxide production, and serum lipoprotein oxidation, is known to support toxicity in the body. The role of MPO in inflammatory diseases, along with structural studies and the accumulated knowledge about the function of the peroxidase family, provides insights into the development of many diseases and plays a role in their prevention, diagnosis, and treatment before they occur (Fiedler, Davey & Fenna, 2000).

2.1.Myeloperoxidase Activity Assay

Each monomer of dimeric MPO contains an iron and a calcium bound to the heme group. The calcium ion's binding site has a pentagonal bipyramidal structure. This structure includes a similar sequence well-preserved in all mammalian peroxidases. It consists of Ser174 and Phe170 at the two ends and Asp96, Thr168, and Asp172 in the central plane. Of these amino acids, Asp96, adjacent to the distal His95, is located on a small polypeptide, indicating that the calcium ion not only correctly positions the distal histidine but also plays a role in holding the two chains together. Removal of calcium results in protein precipitation (Booth et al., 1989), demonstrating that the calcium ion organizes the tertiary structure. Native MPO contains Fe3+ with five bonds in the heme group. Four of these bonds are attached to the nitrogen atoms of the pyrrole rings of porphyrin, and the fifth is connected to the ε-nitrogen of the proximal histidine (His336). The δ-nitrogen of this histidine is hydrogen-bonded to the NH2 group of Asn421. This structure also binds via hydrogen bonds to water molecules, forming a complex that provides peroxidase activity of MPO (Fiedler, Davey & Fenna, 2000; Martina, 2007).

To determine the KM and Vmax values, activity measurements are conducted at the optimum pH and temperature using five different substrate concentrations with O-dianisidine. For this purpose, aliquots of the stock solution of O-dianisidine ranging from 0.25 to 1.5 ml are used. For each experiment with a different O-dianisidine concentration, the total volume is brought to 2.8 ml with the buffer solution in which enzyme activity is measured. Then, 0.1 ml enzyme solution and finally 0.1 ml H^2O^2 are added to each

tube, and the absorbance values are measured at 460 nm for O-dianisidine in a spectrophotometer. Each measurement is repeated three times. By finding 1/V and 1/[S] values, Lineweaver-Burk plots are drawn. The least squares regression line equation is used for plotting. KM and Vmax values are calculated using the graphs and line equations.

The MPO enzyme is found only in neutrophils and monocytes (Arnhold, 2004). Many studies have indicated that the unique antimicrobial property of MPO is due to the production of hypochlorous acid and other toxic agents, creating an environment in neutrophil phagolysosomes that inhibits or kills microbes. MPO-catalyzed modifications, such as tyrosyl radical formation, chlorination, tyrosine peroxide production, and serum lipoprotein oxidation, are known to contribute to the body's toxicity. The role of MPO in inflammatory diseases, along with structural studies and the accumulated knowledge about the function of the peroxidase family, provides insights into the development of many diseases and plays a role in their prevention, diagnosis, and treatment before they occur (Fiedler, Davey & Fenna, 2000).

3.Conclusion

The MPO enzyme is found only in neutrophils and monocytes (Arnhold, 2004). Numerous studies have indicated that MPO's unique antimicrobial property is due to the production of hypochlorous acid and other toxic agents, creating an environment within neutrophil phagolysosomes that inhibits or kills microbes. The presence of MPO-catalyzed modifications, such as tyrosyl radical formation, chlorination, tyrosine peroxide production, and

serum lipoprotein oxidation, is known to support the body's toxicity. The role of MPO in inflammatory diseases, along with structural studies and the accumulated knowledge about the function of the peroxidase family, provides insights into the development of many diseases and plays a role in their prevention, diagnosis, and treatment before they occur (Fiedler, Davey & Fenna, 2000).

The unique structural properties of MPO, including its heme group and glycosylated regions, emphasize the enzyme's stability and catalytic efficiency. The enzyme's high specificity and potent oxidative activity make it a critical subject of research in the context of oxidative stress-related diseases, such as cardiovascular disorders, chronic inflammation, and cancer (Edwards, 1994; Sengeloev, 1993; Butterfield et al., 1998; Babior, Kipnes & Curnutte, 1976). Additionally, kinetic studies, including the determination of KM and Vmax parameters, enhance our understanding of the enzyme's functionality under various conditions and its interactions with inhibitors.

The therapeutic implications of MPO inhibition in mitigating inflammation and oxidative damage are promising (Gülçin & Ark., 2010a). However, the dual role of MPO, as both a protector against pathogens and a contributor to oxidative damage, necessitates a balanced approach to its modulation. Future studies should focus on designing selective inhibitors and exploring the enzyme's role in non-immune cells, which could unlock new avenues for disease prevention and treatment.

Further studies are needed to understand the role of MPO in non-immune cells and tissues, as this could reveal new functions and

therapeutic targets beyond its known role in the immune system. High-resolution structural studies could help us better understand the unique properties of MPO and contribute to the development of treatments that target its specific enzymatic mechanisms.

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BÖLÜM VIII

Efficient Adsorptive Removal of Penicillin G by PVA/Tragacanth Gum Nanocomposite Films

Süleyman GÖKCE[1]
F. Fulya TAKTAK[2]

1. INTRODUCTION

The contamination of water bodies with pharmaceuticals has become a growing problem due to the widespread use and improper disposal of antibiotics (Monika Hejna, 2022: 1) Among the various antibiotics, penicillin G is widely used in both human and veterinary medicine, resulting in its recurring presence in wastewater (Viviana Carmen Ciucă, 2023: 1). Penicillin G, a widely used β-lactam antibiotic, poses a significant environmental risk when discharged into water bodies, as it can contribute to the development of antibiotic-resistant bacteria and disrupt aquatic ecosystems.

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Therefore, the removal of penicillin G from wastewater has gained increasing attention in recent years (Emmanuel Sunday Okeke, 2022: 2) (Mehdi Sadeghi, 2023: 3)

Various conventional methods such as biological treatments, chemical oxidation and membrane filtration have been used to remove antibiotics from water. However, these techniques often suffer from limitations such as high operating costs, incomplete removal and the generation of harmful by-products. Adsorption has emerged as a promising alternative for the efficient and cost-effective removal of contaminants from aqueous solutions due to its simplicity, high efficiency and reusability of adsorbents (B. Senthil Rathi, 2021: 2); (Omid Moradi, 2021: 2)

In recent years, the development of biopolymer-based nanocomposite adsorbents has attracted great interest due to their environmental friendliness and improved adsorption capacity. Polyvinyl alcohol (PVA) is a biodegradable polymer known for its excellent film-forming properties, high water solubility and chemical stability. It is used in numerous applications, including the production of adsorbents Tragacanth, a natural gum derived from the dried sap of certain Middle Eastern plants, is another biopolymer with remarkable swelling properties and biocompatibility, making it an attractive candidate for adsorbent applications (Mohammad Ehsan Taghavizadeh, 2021: 2) By combining PVA and tragacanth in nanocomposite films, an adsorbent with increased mechanical strength, swelling capacity and adsorption efficiency can be produced for the removal of contaminants such as penicillin G.

The aim of this study is to investigate the potential of PVA/TG nanocomposite films for the adsorption of penicillin G from aqueous solutions. Key parameters such as pH, initial antibiotic concentration and adsorbent dosage will be evaluated to determine their effects on the adsorption process. In addition, the adsorption mechanism will

be explored through kinetic and isothermal studies and the reusability of the adsorbent will be evaluated through multiple adsorption-desorption cycles. The results of this study will contribute to the development of sustainable and effective solutions for the removal of pharmaceutical impurities from wastewater.

2 EXPERIMENTAL METHODOLOGIES

2.1 Chemicals

The materials used in this study were of high analytical quality. Polyvinyl alcohol (PVA) was purchased from Thermo Scientific, while gum tragacanth was purchased from a local distributor. Hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), methanol, phosphoric acid and potassium dichromate were purchased from Sigma-Aldrich. All chemicals were used in their original form without additional purification. Deionized water was used throughout the study for both solution preparation and rinsing steps.,

2.2 Preparation of PVA/Tragacanth gum Films

Firstly, a 10% (w/v) PVA solution was prepared by dissolving 10 g of PVA in 100 mL of water using a reflux condenser for 2 hours. From the prepared PVA solution, 2.5 g was taken, and Tragacanth gum in varying amounts (20, 40, 60, and 80 mg) along with 1 g of glycerol was added. The mixture was stirred for 3 hours at 400 rpm using a magnetic stir bar, then 2.5 mL of the solution was poured into molds. The films were left to dry for 24 hours. In the adsorption experiment, a 15 mg film was placed in contact with a 25 ppm Penicillin G solution at pH 8 for 45 minutes. The Penicillin G concentration was measured using UV spectrophotometry at 288 nm.,

2.3 Determination of the Point of Zero Charge (pHpzc) of PVA/TG Composite Films

The point of zero charge (pHpzc) refers to the pH value at which the surface of the PVA/TG nanocomposite film is electrically neutral. The solid addition method was used to determine the pHpzc. A 0.1 mol/L NaCl solution was adjusted to pH values of 2, 4, 6, 8, 10 and 12 with 0.1 mol/L HCl or NaOH. Fifty milliliters of each solution was added to individual flasks and 0.5 g of PVA/TG film was added to each. Once equilibrium was reached, the final pH values were recorded. The pHpzc was calculated by plotting the change in pH (ΔpH = pHi - pHf) against the initial pH values and determining the intercept of the curve with the axis.

2.5 Optimization of Adsorption Parameters for Efficient Removal of Penicillin G

The optimal conditions for the adsorption of Penicillin G onto the PVA/TG nanocomposite film were determined by evaluating various factors, such as pH, initial Penicillin G concentration, and adsorbent dosage. In addition, the concentration of Penicillin G in the solution was monitored using UV-Vis spectrophotometry at a wavelength of 288 nm to measure the absorbance and determine the adsorption efficiency. By adjusting these parameters, the most effective adsorption conditions were identified, ensuring maximum removal of Penicillin G from aqueous solutions.

The adsorption capacity (qe) and the percentage of Penicillin G removal (R%) were calculated using equations (3) and (4).

$$q_e = \frac{(C_0 - C_e)}{m\,x\,V} \tag{3}$$

$$(\%) = \frac{(C_0 - C_e)}{C_0} x100$$

The reusability of the PVA/tragacanth composite film for Penicillin G adsorption was assessed through multiple adsorption-desorption cycles. To desorb Penicillin G, the Penicillin G-loaded PVA/TG film was treated with different desorption agents, including methanol, acetonitrile, and HCl, at concentrations of 0.1 M, 0.5 M, 1 M, and 2 M. Following desorption, the film was soaked in 0.1 M NaOH for 15 minutes. The composite was then thoroughly rinsed with deionized water, dried, and reused for up to four cycles to evaluate its reusability and structural stability.

3. RESULT AND DISCUSSION

3.1 FTIR

The FTIR spectra of TG, PVA, and PVA/TG nanocomposite films are presented in Figure 1, highlighting the characteristic functional groups for each material. The FTIR spectrum of TG (dotted line) showed a broad band at 3293.66 cm^{-1} (O–H stretching vibrations), a weak band at 2937 cm^{-1} (asymmetric C–H stretching), a band at 1728 cm^{-1} (C=O groups of gum polysaccharides), 1369 cm^{-1} (–CH_2 bending vibrations), and a band at 1016 cm^{-1} (C–O stretching vibrations of polysaccharides). In the PVA spectrum (solid line), a broad band at 3293.66 cm^{-1} (O–H stretching) was observed, along with peaks at 2937 cm^{-1} (C–H stretching), and 1016 cm^{-1} (C–O stretching), confirming the polymeric nature of PVA. The PVA/Tragacanth gum composite (dashed line) displayed a broad O–H stretching band at 3272 cm^{-1}, a peak at 2937 cm^{-1} (C–H stretching), a band at 1417 cm^{-1} (–CH_2 bending), and a peak at 1041 cm^{-1} (C–O stretching), indicating strong interactions between the components. The shifts in the FTIR bands suggest effective bonding between PVA and TG, enhancing the structural integrity and functional performance of the composite material.

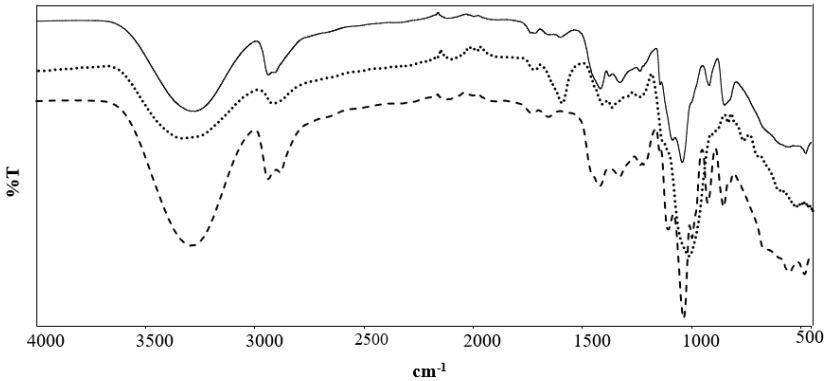

Figure 1 *FTIR spectra of TG, PVA and PVA/TG film.*

3.2 Optimal parameters for Penicillin G adsorption

3.2.1 pH effect and swelling behavior.

Adsorption processes are highly sensitive to the pH value of the solution, as the interactions between the adsorbent and the adsorbate are significantly influenced by pH. Determining the optimal pH is essential for achieving the most efficient adsorption. The pHpzc of the PVA/TG composite film, shown in Figure 2, was determined to be 2.8. Below this value, the surface of the film is positively charged, favoring the adsorption of negatively charged species, while above pH 2.8, the surface becomes negatively charged, which may lead to electrostatic repulsion with anionic species like Penicillin G (pKa = 2.7).

Despite this potential repulsion, the removal capacity increases as the pH rises, reaching its peak at pH 8, as illustrated in Figure 3. This can be attributed to the film's enhanced swelling behavior at higher pH levels, as shown in Figure 4, which increases the surface area and porosity of the film, allowing for more effective adsorption. At pH 8, the swelling ratio is the highest, leading to greater accessibility of

adsorption sites and better diffusion of Penicillin G molecules into the film.

At pH values higher than 8, the removal capacity decreases slightly. This is likely due to stronger electrostatic repulsion between the negatively charged Penicillin G molecules and the negatively charged surface of the film. However, the continued swelling of the film, combined with non-electrostatic interactions such as hydrogen bonding and Van der Waals forces, helps maintain relatively high adsorption efficiency.

In conclusion, the interplay between surface charge and swelling behavior is crucial for optimizing the adsorption process. pH 8 is the optimal condition, where the swelling is maximized, allowing for efficient Penicillin G removal despite electrostatic repulsion.

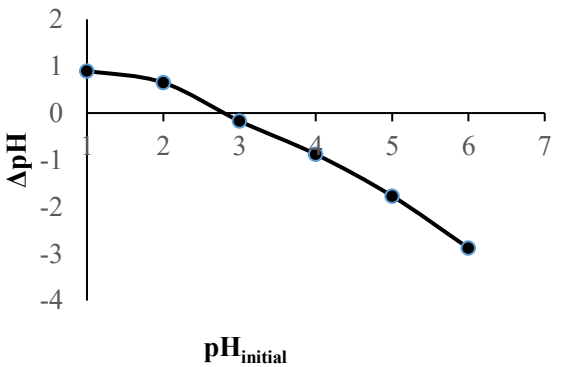

Figure 2 *The pHpzc determination curve for PVA/TG film.*

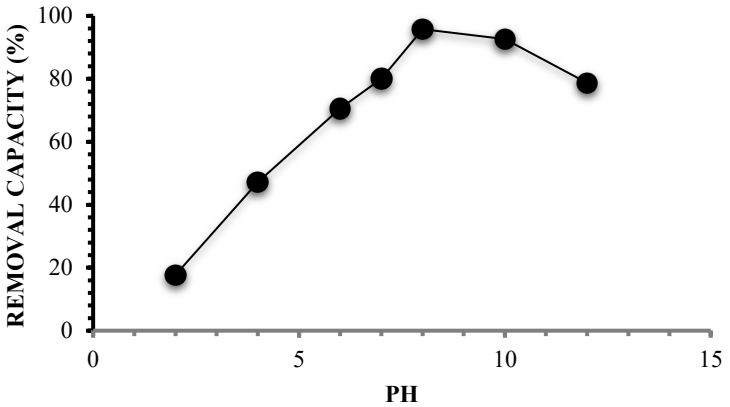

Figure 3 *Effect of pH on PVA/TG removal capacity.*

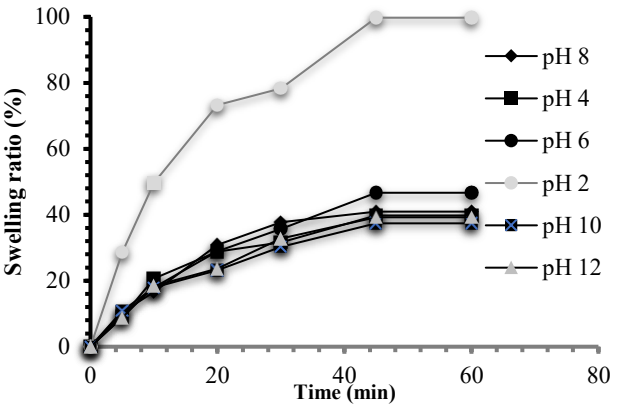

Figure 4. *Swelling behavior of PVA/TG films at different pH values over time.*

3.2.2 Effect of adsorbent dosage.

The effect of PVA/TG composite film dosage on the removal capacity of Penicillin G is depicted in Figure 5. The removal capacity shows a sharp increase as the film dosage increased from 2.5 mg to 7.5 mg, reaching around 89%. This rapid initial rise can be explained by the increased availability of active adsorption sites, which

enhances the interaction between the adsorbent and Penicillin G molecules in solutions. Beyond 10 mg, however, the removal capacity begins to plateau, with only a slight increase in removal efficiency as the dosage is raised to 15 mg and higher. This suggests that the adsorption sites become saturated after a certain dosage, and further increases in film dosage do not significantly improve removal efficiency. The marginal improvement seen at higher dosages (up to 20 mg) is likely due to the complete saturation of Penicillin G in the solution, where most molecules are already adsorbed at dosages around 15 mg. Therefore, the optimal dosage for the PVA/TG composite film is around 15 mg, where the removal capacity is maximized without excessive use of adsorbent. Increasing the dosage beyond this point does not provide any meaningful enhancement in performance and may be inefficient for practical applications.

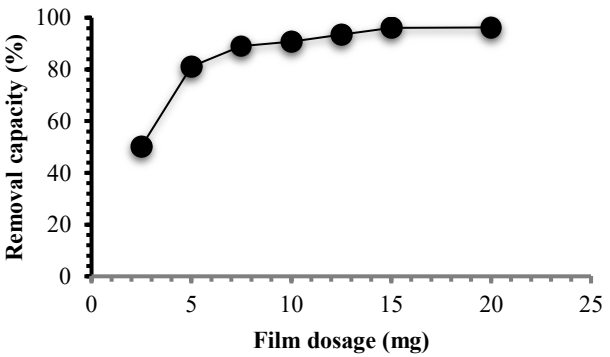

Figure 5 *Effect of dosage on PVA/TG removal capacity.*

3.2.3 Effect of TG content.

As shown in Figure 6, the removal capacity of Penicillin G increases with rising TG content in the PVA/TG composite film, reaching a maximum at around 60%. This increase is likely due to the additional functional groups (such as hydroxyl and carboxyl

groups) provided by the TG, which enhance the film's interaction with Penicillin G. However, increasing the TG content beyond 60% does not lead to noticeable improvements in removal efficiency, suggesting that the adsorption sites are fully utilized at this point. Adding more TG after this level does not provide additional benefits in terms of adsorption. Therefore, 60% TG content is optimal for maximizing removal efficiency without overloading the system with unnecessary material.

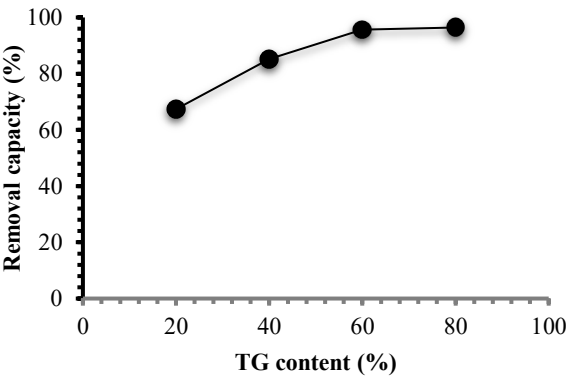

Figure 6 *Effect of TG content on PVA/TG removal capacity.*

3.3 Kinetic Analysis of Penicillin G Adsorption

The kinetics of Penicillin G adsorption onto the PVA/TG composite film were analyzed using both the pseudo-first-order and pseudo-second-order models. The results are presented in Figure 7(a) and Figure 7(b), respectively. In Figure 7(b), the pseudo-first-order model shows a non-linear fit with a low correlation between experimental data and the linearized model. This suggests that the adsorption of Penicillin G does not follow the pseudo-first-order kinetic model, indicating that the rate of adsorption is not solely dependent on the concentration of Penicillin G in the solution. On the other hand, Figure 7(b) presents the pseudo-second-order kinetic

model, where the plot of t/qt versus time (t) shows a highly linear relationship, with a correlation coefficient (R^2) of 0.9945. The linearity of this plot indicates that the adsorption process is better described by the pseudo-second-order model, suggesting that the adsorption rate is more dependent on the availability of active sites on the PVA/TG composite rather than just the concentration of Penicillin G in the solution. The strong fit of the pseudo-second-order model implies that chemisorption, involving valence forces or covalent bonding, is the predominant mechanism for Penicillin G adsorption. This finding is consistent with the presence of functional groups on the TG component of the composite, which can form strong interactions with the Penicillin G molecules. In conclusion, the adsorption kinetics of Penicillin G onto the PVA/TG composite film are best described by the pseudo-second-order model, suggesting that the process is controlled by chemisorption involving surface active sites.

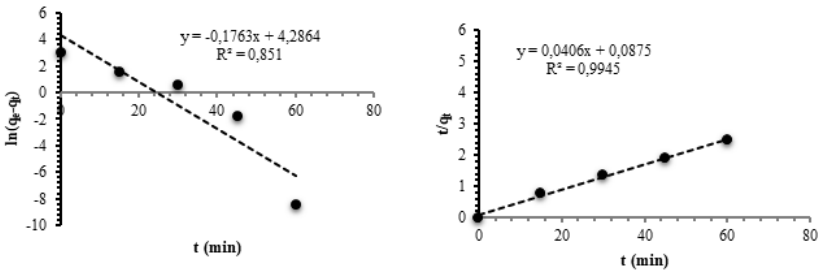

Figure 7 *(a) Pseudo-first-order kinetic, (b) pseudo-second-order kinetic and c intra-particular diffusion for adsorption of Penicillin G by PVA/TG film*

3.3 Adsorption Isotherms (Langmuir and Freundlich) and Initial Concentration Effect

As shown in Figure 8, the removal capacity remains high (around 95%) at Penicillin G concentrations below 25 mg/L.

However, beyond this point, efficiency gradually decreases, dropping to about 88% at 50 mg/L. This decline is likely due to the saturation of adsorption sites on the PVA/TG composite. Thus, the adsorbent performs optimally at lower Penicillin G concentrations, where more active sites are available for adsorption.

Figure 9presents the adsorption isotherms for both Langmuir and Freundlich models. The Langmuir isotherm (Figure 10a) shows the linear relationship between Ce/qe and Ce with an R2 value of 0.9858, indicating a strong fit and suggesting adsorption occurs on a homogeneous surface with a limited number of identical active sites. In contrast, the Freundlich isotherm (Figure 10b) displays the log-log relationship between log qe and log Ce , with an R2 value of 0.8317, representing a moderately good fit that aligns with heterogeneous surface adsorption. The higher R2 value for the Langmuir model suggests that the adsorption process is better explained by the assumptions of this model, implying that the adsorption occurs predominantly on a homogeneous surface.

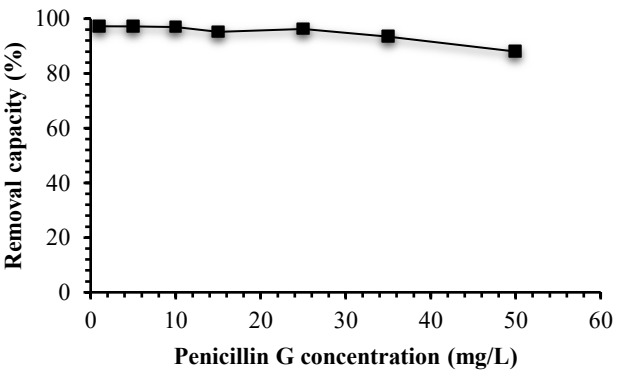

Figure 8 *Effect of initial Penicillin G concentration on PVA/TG removal capacity*

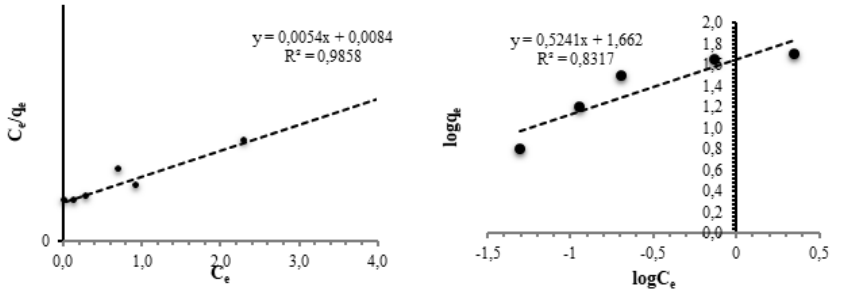

Figure 9 *(a) Langmuir isotherm plot for the adsorption of Penicillin G on PVA/TG film; (b) Freundlich isotherm plot for the adsorption of Penicillin G on PVA/TG film.*

3.4 Desorption Efficiency and Reusability of PVA/TG Nanocomposite Film

Methanol, acetonitrile, and HCl solutions at different molarities (0.1 M, 0.5 M, 1 M, and 2 M) were used for the desorption of Penicillin G. Methanol demonstrated the highest desorption efficiency at 95.54%,

The performance of the composite was evaluated over five consecutive adsorption-desorption cycles, and the results are presented in the figure. In the first cycle, the removal capacity was high, but there was a slight decrease in the second and third cycles, indicating some reduction in adsorption efficiency with repeated use. By the fourth and fifth cycles, the decline became more significant, with the removal capacity dropping to lower levels. This reduction in efficiency over cycles may be due to the partial loss of active adsorption sites on the composite material, possibly caused by incomplete regeneration after each cycle or structural deterioration of the adsorbent.

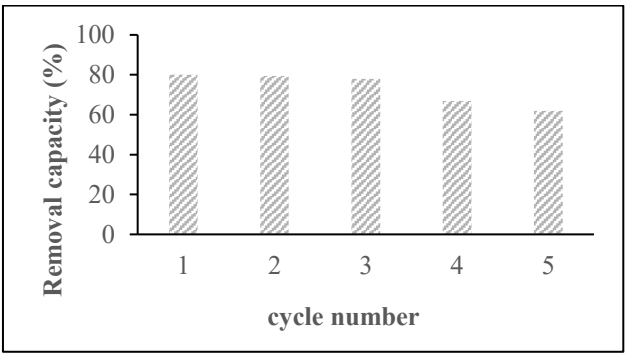

Figure 10 *Reusability performance of PVA/TG film over five successive adsorption-desorption cycle.*

CONCLUSION

This study demonstrated the effective removal of Penicillin G from aqueous solutions using PVA/Tragacanth gum nanocomposite films. The adsorption process was influenced by key parameters such as pH, initial Penicillin G concentration, tragacanth gum content, and adsorbent dosage. The nanocomposite's swelling behavior, particularly at pH 8, played a significant role in enhancing the adsorption capacity. The adsorption kinetics followed a pseudo-second-order model, suggesting that chemisorption is the dominant mechanism. Adsorption data fit well with the Langmuir isotherm, indicating monolayer adsorption on a homogeneous surface.

The desorption and reusability tests revealed that methanol was the most efficient desorption agent, with the composite maintaining structural integrity across five cycles, although there was a gradual decrease in removal efficiency. This decline may be due to the loss of active adsorption sites or incomplete regeneration. Overall, the PVA/Tragacanth gum nanocomposite films exhibit great potential as eco-friendly and efficient adsorbents for the removal of Penicillin G from pharmaceutical wastewater. Future studies should focus on enhancing the durability and reusability of

the composite to further improve its long-term performance in wastewater treatment applications.

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