Chapter

Value Addition to Leather Industry Wastes and By-Products: Hydrolyzed Collagen and Collagen Peptides

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Abstract

Environmental consciousness and constraints in developed societies over the past 20 years have brought about a dramatic impact on tannery operations worldwide. Leather industry has been categorized as one of the most polluting industries, and it spoils the continuity of environmental rhythm because of the generation of liquid, solid and gaseous wastes and also by-products. Solid organic wastes involving untanned (trimmings, fleshings and splits) and tanned (trimmings, splits and shavings) wastes and by-products depending on their proteinic character have an advantage of recovery and reuse potentials instead of disposal to landfills in terms of environmental sustainability. These solid wastes and by-products are not properly treated and disposed of; hence, they can cause environmental damages to soil and groundwater as well as release emissions and poisonous greenhouse gases into the atmosphere. Valorization of these tannery solid wastes and by-products with different methods and processes is highly important for the perspective of eco-benignity and with respect to converting into new value-added products. This chapter focuses on the evaluation of the tannery solid wastes and by-products by partial and total denaturation and hydrolyzation. This paper also examines in general the specifications, production techniques and applications of collagen peptides in several industries.

Keywords: leather, solid wastes and by-products, technical gelatin, collagen peptides, collagen hydrolysates

1. Introduction

Leather manufacturing, which is an allied industry and subsector for textile, is the first making practice in primitive period of humankind. Different types of animal skin products were used throughout the first ages as parchment and vellum or by making the raw material resistant to putrefaction, heat, chemicals and environmental effects with smoke, potash alum and natural tannin extracts from different plant parts. Traditionally, these products obtained by modification of by-products of meat industry have all been classified as leather, which is a serviceable product. In this respect, the leather industry could have been distinguished as an environmental industry, since it processes waste products from meat production [1].

These natural products generally consist of long thick collagen fibers, fiber bundles and thin elastin fibers of interweaving in three-dimensional ways. Other features such as hairs and hair roots and also fat cells are present in three-dimensional woven structure that predominates and gives skin-based materials providing many of their unique physical and mechanical qualities [2].

The leather-making operation assists in converting the raw hide or skin, a highly putrescible material, into leather, a stable material, which can be used in manufacturing a wide range of products. These include shoes, clothing, leather goods, furniture, upholstery for car seats and interiors, boats and aircraft, and many other goods in daily use. The whole process involves a sequence of complex chemical reactions and mechanical processes [3].

The processing of leather involves four main stages: beamhouse, tanning, post-tanning and finishing. The first phase of the hide processing is called beamhouse operations and involves multiple mechanical, chemical and biological unit operations. Its objective is to remove dirt, hair, epidermis, noncollagenous proteins and grease from raw skin, and open up the collagen fibers to favor the subsequent tanning process [4]. The process is performed in a drum by mixing the raw hides with an alkaline solution containing lime and reducing agents, usually sulfide salts, the hair being chemically removed from the surface of the hide [5]. The beamhouse operations are the most water consuming and the effluents generated present very high organic load [6].

The tanning process is one of the oldest procedures in the world, and currently, these industrial activities are based on chemical processes involving several organic and inorganic compounds [7]. This step gives the leather stabilization against the wet and dry heat, bacterial growth, mechanical stress and enzymatic attack, among others, and forms the basis of leather production. This stabilization is attributed to the formation of new chemical cross-links in the matrix proteins [8]. The tanning stages are classified as mineral, vegetable and synthetic. When the skin stabilization is achieved by a suitable inorganic salt, the process is known as mineral tanning, and the most commonly used mineral tanning salt is the basic chromium sulfate ($Cr(OH)SO_4$). If the leather is tanned with chromium salt, it is called as wet-blue leather. Chromium (III) salts are the most extensively used compounds due to the quality and high stabilization ability they impart to leather [9].

The third part in leather production is post-tanning process. The tanned leather is considered a commodity, that is, it may be used to produce several articles. Each post-tanning operation is directed to the article that will be produced, such as garment, shoe upper and upholstery [10]. The aim of the post-tanning processes is to enhance the aesthetic properties of leather by coloring it and changing some physical and mechanical properties of the material by retanning, dyeing and fatliquoring stages [10].

The finishing step complements the previous stage, tanning, and provides the leather with the required physical and mechanical properties, such as color, tensile strength, impermeability, softness, flexibility and elasticity with different kinds of binder, pigment, wax and oils [11]. This operation consists of coating and changing the surface of leather. It is related to the fashion appearance, but also to conferring properties such as abrasion resistance, gloss, handle, flex, adhesion and rub fastness as well as other properties as required for the end use including extensibility, light and perspiration fastness, water vapor permeability and water resistance [10].

Leather industry has been categorized as one of the highly polluting industries because large quantities of water and different chemicals have been used during tanning process and different solid, gaseous and liquid wastes are generated that have an adverse effect on the environment [12]. These wastes have different characteristics because different chemicals are applied to the raw hides in different

ratios. Solid wastes generated in tanneries mainly include salts, raw trimmings, hair wastes, fleshings, splitting wastes, chrome shavings, buffing dusts, crust trimmings and finished trimmings. These solid wastes and by-products are not properly treated and disposed of, and they can cause environmental damages to soil and groundwater as well as release emissions of odor and poisonous greenhouse gases into the atmosphere by direct landfill or incineration, which is an unsustainable way [13].

Salt, which is used to preserve hides or skin thrown into open dumping areas or accumulated in piles outside the tanneries, is likely to create groundwater pollution when rain washes it away. Hair wastes and lime sludge discharged into the effluent can produce choking of treatment pipelines. Trimmings, fleshings and splitting wastes putrefy easily producing noxious odors [14]. Moreover, disposal of chromium-containing solid wastes into soil and water has potential effects on public health due to the possibility of oxidation of chromium (III) into hazardous chromium (VI) [15]. These tannery solid wastes have different characteristics that mainly constitute protein (collagen) as the main component [16].

Provisions for pollution control, waste minimization and disposal, the correct use of chemicals and accident prevention are essential for minimizing potential impact on air, water and soil from the processing of hides and skin.

Collagen derivatives are value-added products extracted from solid organic wastes and by-products, and they are utilized for several industrial applications such as preparation of technical-grade gelatin, protein hydrolysates, collagen peptides and subunits [17]. The processing of hides and skin also generates by-products, which find outlets in several industrial sectors such as pet and animal food production. They can be used in cosmetics, printing inks and photography, while the latter one is an ideal candidate for fertilizer or feeding additives due to their high nitrogen content [18].

The present chapter describes the leather solid wastes, general features of collagen peptides, and their preparation methods and applications in different industries.

2. Leather solid wastes

The tanning industry worldwide produces a significant amount of solid wastes and effluents, environmental concerns about discharge and escalating landfill costs are becoming increasingly serious problems for the industry, and their management alternatives regarding overall consideration have been based on multispot [19]. Huge amounts of solid wastes are generated at different stages of leather processing and there is no actual adopted utilization method available for solid wastes; hence, handling is more difficult for tanners. Leather solid wastes generated in fleshing, trimming, splitting and shaving processes and also sludges discharged from the wastewater treatment plant both contribute to increase the volume of the wastes [20].

Generally, out of 1000 kg of rawhide, nearly 800 kg of solid wastes are generated in leather-manufacturing industries, and only 200 kg of the raw material is converted into a usable product. About 600,000 tons of solid wastes annually are generated worldwide by leather industries [21]. An example of the types and quantities of solid wastes generated in leather processing based on one ton of raw hides/skin is given in **Table 1**.

The ways to disposals and valorizations for these wastes are defined by the chemical characteristics depending on the fact that the wastes are generated in either beamhouse or tanning and after tanning. This differentiation might be, namely, untanned wastes and tanned wastes accordingly.

Solid wastes generated from processing of raw hides/skin (1000 kg)	Quantity (kg)
Conservation salts	80
Hair	100
Raw trimmings	40
Lime sludges	60
Fleshings	120
Wet-blue trimmings	30
Chrome splittings	65
Chrome shavings	95
Buffing dusts	65
Crust trimmings	35
Dry sludge from common effluent treatment plants (CETPs)	125

Table 1.
Solid wastes from tannery [22].

2.1 Untanned solid wastes

Most of the solid wastes are generated in beamhouse, especially in fleshing operation. Fleshings are solid wastes generated during a mechanical process aiming at removing the flesh deposits or fats from the inner part of the skin [23]. Fleshings contain subcutaneous tissue, fat and flesh, which are composed of protein (5–7%), fat (4–18%), lime (2–6%), sulfide (2–4%), etc. [23].

Trimming is to cut out unwanted parts of processed hides/skin just after fleshing operation is completed. Trimmings are cut-outs from the operation and may be collected and shipped to glue manufactures or other by-product manufacturers or sent for disposal in a landfill [24].

Hides are generally subjected to mechanical operation called splitting to divide the hide into two or three layers horizontally. Splitting operation can also be applied at chromium tanning stage (wet-blue stage), which is called wet-blue splitting. Whether split is untanned and obtained after liming or tanned and obtained after tannage, it is a valuable part of a hide, which is a fibrous sheet, and hence it is in fact not a waste and more precisely it is a by-product.

The untanned solid wastes, mainly including leftovers from trimming of rawhide and surplus parts after liming and fleshing, are composed of large amount of collagen and grease. The chemical composition of these solid wastes varies depending on types and quality of the raw hides/skin and also process conditions. Fats and proteins are the main components of these wastes (10.5%). Moisture amounts might be up to 60%, meaning a high water content. The aforementioned solid wastes do not contain chromium compounds [25]. For sufficient usage of these protein-rich wastes, various kinds of methods and technologies have been proposed, focusing on the extraction of collagen/gelatin by using acid, alkali and enzyme hydrolysis and subsequent purification processes. Moreover, grease residue can also be used to extract oils and fats, which can be raw material for biofuel and leather fatliquor [26].

2.2 Chromium-tanned solid wastes

The chromium tanning is based on the cross-linkage of chromium ions with free carboxyl groups in the collagen. Chrome-tanned leather also called wet-blue leather

are characterized by top handling quality, high hydrothermal stability, user-specific properties and versatility [27]. At the end of the chrome-tanning process, 60–75% of the chrome offer (Cr_2O_3) remains in the collagen structure. Additionally, small amounts of other chemicals and auxiliaries such as tensides, acids and bases (in the form of soluble "reaction salts") remain in the wet-blue leathers. The main environmental impact of tannery solid wastes is the oxidation of trivalent chromium into the hexavalent form, which is highly toxic and has carcinogenic and mutagenic effect. Leakages from chromium-containing wastes when they come to the agricultural lands cause ground water pollution and soil contamination. Water pollution affects aquatic animals, which are common sources of food, and soil contamination poses health effects through food chain and also poses a health hazard through inhalation of toxic dust, which can be inhaled by both people and livestock [28].

The solid wastes containing chromium namely tanned wastes are wet-blue shavings, wet-blue trimmings, buffing dusts, finished leather trimmings and wastewater treatment sludge [29]. Their chemical composition consists of fats and oils (3–6%) and mineral matters (15%). As chromium has been already used worldwide, they normally contain 3.5–4.5% of chromium as Cr_2O_3. Sludge from effluent treatment plants contains mainly water (up to 65%), organic substances (30%) and chromium (III) (around 2.5%) [25, 30].

Chrome shaving wastes are generated during the machine process of thickness adjustment of wet-blue leathers based on the required thickness. Shavings are mainly the scraps from the flesh side of leather, which are carried out by cutting unusable parts of leather and rags created during shaving operation [31]. Utilization or safe disposal of shavings continues to pose a serious challenge in many countries and is more critical because of their compositions. While processing one ton of raw hide, approximately 95–100 kg of wet-blue shavings are produced [32, 33]. Currently, a part of the chrome shavings is used in the manufacture of different types of areas such as leather board, collagen peptides, gelatin, animal feed and fertilizers. Unused portion of shavings is dumped in open areas around tanneries posing a serious environmental hazard [34].

2.3 Environmental and health impacts of leather solid wastes

The tannery solid wastes can cause severe problems associated with its organic load, inorganic matter, chromium, suspended solids, total organic and ammoniacal nitrogen, sulfide, and chloride, among others, depending on the chemical and mechanical processes applied to the raw hides/skin. Accumulation of these wastes leads to sludge problem and choking of treatment pipes and finally results in the reduction in efficiency of the treatment plant [35].

Leather industry is facing a lot of solid waste problem and many tanneries are closed for not meeting biological oxygen demand (BOD) and total dissolved solids (TDS) norms [4]. It is very important to analyze the nature of these wastes in order to assure a safe disposal or application of them. Salt, which is used to preserve hides or skin, discharges huge amount of pollution load in terms of total dissolved solids and chlorides and creates groundwater pollution [36]. Hair waste and lime sludge if discharged along with the effluents are likely to choke the drains. Trimmings, raw fleshings, limed fleshings and splitting waste can putrefy easily by producing noxious smells. Some of the biodegradable tannery solid wastes cause volatile organic compound emissions and, moreover, are sources of pathogenic bacteria [37].

Shaving dust contains environmentally unfriendly chemical called chromium, and when it is dumped in the environment, it can easily enter into the surface and ground, and this heavy metal pollutes the surface water by erosion and the underground water by leaching and erosion, leading to serious health problems to aquatic

life in nearby rivers. As a result of this, pollution of surface and ground water results in shortage of drinking water for human beings and animals living at the downstream of the rivers [38].

According to Mu et al. [39], about 25% of tannery solid waste ends up as chromium-containing solid waste, which is more dangerous than other tannery solid waste. The waste generated from chrome-tanned leather is not biodegradable and toxic due to the chromium content [40]. Chromium-containing leather waste has been classified as one of the dangerous and hazardous waste if discharged into the environment without any pretreatment. Increased risks for a number of cancers such as lung cancer, testicular cancer, soft tissue sarcoma, pancreatic cancer and bladder cancer have been reported [41]. Chromium waste can also cause respiratory problems, a lower ability to fight disease, birth defects, infertility and tumor formation [42]. Chromium-containing solid waste percolates to the ground and causes ground water pollution and soil contamination. Water pollution affects aquatic animals that are common sources of food, and contamination of soil poses health effects through food chain and also poses a health hazard through inhalation of toxic dust, which can be inhaled by both people and livestock. It can damage the gills of fish; it can alter genetic materials and cause cancer [43]. Moreover, thermal incineration of these wastes is associated with serious air pollution problems due to emission of toxic hexavalent chromium (Cr+6), halogenated organic compounds, aromatic hydrocarbons, etc. into the environment [42].

3. Collagen peptides

The word collagen is derived from the Greek word "kola," which means gum, and "gen," which means producing. It is a fibrous structural protein present in extracellular matrix and connective tissue of animals [44]. Collagen is the most prevalent protein comprising approximately 30% of the total protein of animal and human bodies and is found primarily in connective tissues including animal hairs, bones, cartilages, tendons and blood vessels [45, 46].

There are many types of collagens, which are from collagen I to collagen XIX. Animal skin or hide contains collagen type I, mostly with approximately 90% of its dry weight [47]. Collagen precursors are synthesized in the endoplasmic reticulum of cells and transported to the Golgi apparatus in order to secrete into the extracellular spaces, and maturation of collagen can occur [47, 48].

Collagen polypeptide chains and cross-linkages can be broken down by hydrolytic processes and decomposition yielding in different subunits and fragments [49]. In other words, gelatin is produced by partial denaturation of collagen in triple helical structure. Gelatin and collagen peptides are new forms yielded by hydrolysis of native collagen with lower molecular weight fragments than original structure and including a wide range of subcategories having differentiated functionalities [50]. Native collagen exhibits superior and distinct properties from collagen peptides such as higher enthalpy, greater network structure of fibrils, basic isoelectric point and high resistance to protease hydrolysis [51]. The native triple helices and fibril networks in the native collagen are more rigid and firmer than gelatin and collagen peptides [52].

3.1 Structural and chemical characteristics of native collagen

All proteins are composed of linear chains of amino acids attached together by peptide bonds, thereby making oligomers, which are the primary structure of the protein. Being arranged into sequences of different amino acids gives way to fold

up the chains into a functional protein. Intermolecular and intramolecular bonds in the structure can enhance the folding of the peptide chains. This folding induces the weak forces such as hydrogen bonds and electrostatic, hydrophobic and van der Waals interactions [53]. Considering the reactivity of collagen molecule, the peptide bond itself is prioritized, capable of participating in hydrogen bonds with both hydrogen-bond donor and acceptor groups. For example, a carbonyl group in any protein has two lone pairs of electrons having the capability of accepting hydrogen bonds. In addition, the electronegative nitrogen induces a partial positive charge on its attached hydrogen, allowing the hydrogen to function as a hydrogen-bond donor [54]. Hydrogen bonding between peptide bonds is the basis of protein secondary structural formation, namely, helices, yielding in pleats and turns in the structure [55]. The side chains of amino acids are capable of a variety of interactions including hydrogen bonds, ionic bonding, hydrophobic interactions, van der Waals interactions and disulfide bonds. These interactions and secondary structural elements are responsible for taking a shape of the tertiary structure of proteins, their actual three-dimensional shape [56]. Due to the interactions between side chains of various amino acids, the protein molecule will bend and twist so as to gain individual stability or lower energy state.

Proteins included in the number and arrangements of subunits to give functionality are referred to as quaternary structure. The proteins comprising individual subunits may be identical, or they may be different. Like the secondary and tertiary structures, the quaternary structure of a protein is determined by its primary structure [57].

Collagens are trimeric molecules made up of three polypeptide chains, which contain the sequence repeat of (Gly-X-Y)n, X being frequently proline and Y hydroxyproline. These repeats allow the formation of a triple helix based on three polypeptide chains bound to each other by hydrogen bonding, which is the characteristic feature of the collagen [58]. The side chains of each X and Y residue are at the surface of triple helix, giving the collagen molecule a significant capacity for lateral interactions with other molecules of extracellular matrix and resulting in the formation of various supramolecular assemblies [59, 60]. An interchain hydrogen bonding between glycine and amide group in an adjacent chain is a key factor in stabilizing the collagen triple helix [61].

Collagen protein is more hydrophilic than lyophilic moieties due to the chemical nature of numerous amino acids present in its structure [62]. It has a highly complex structure and interacts with each other at the molecular level to form broader systems with distinctive properties [63]. The chemical structure of collagen type I is shown in **Figure 1**.

Basic properties create characteristic structure of collagen fibril helicoidal structure is; fibril diameters ranging from 10 to 500 nm [64], average molecular weight of 285,000 Da [65] and glycine ratio of 1/3 in the polypeptide chain consisting of 1400 amino acids [66].

Having been readily recognized in tissues with commonly white and opaque colors, collagen fibers area considered as viscoelastic materials having high tensile strength and low extensibility. The tensile strength of collagen depends on the formation of covalent intermolecular cross-links between the individual protein subunits [67].

The collagen family is highly complex and shows a remarkable diversity in molecular and supramolecular organization, tissue distribution and function. Collagen types are classified in several subfamilies according to sequence homologies, similarities in their structural organization and supramolecular assembly. The availability of 27 collagen types was reported and they are classified by their size, function and amino acid distribution that differ considerably in their

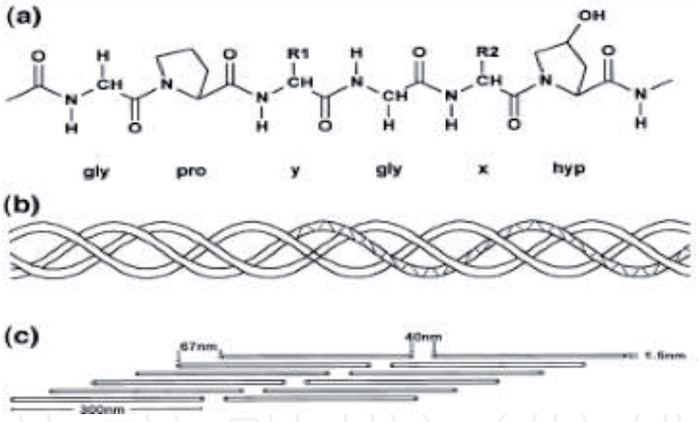

Figure 1.
Chemical structure of collagen type I. (a) Primary amino acid sequence, (b) secondary left-handed helix and tertiary right-handed triple-helix structure, and (c) staggered quaternary structure [63].

molecular structure [68]. The individual members are numbered with roman numerals. The family is subdivided into different classes: the fibrillar collagens (types I, II, III, V, XI, XXIV and XXVII), basement membrane collagens (type IV), fibril-associated collagens with interrupted triple helices (FACIT collagens, types IX, XII, XIV, XVI, XIX, XX and XXI), short chain collagens (types VIII and X), anchoring fibril collagen (type VII), multiplexins (types XV and XVIII), membrane-associated collagens with interrupted triple helices (MACIT collagens, types XIII, XVII, XXIII and XXV) and collagen type VI. The types indicated by an asterisk are heterotrimers, consisting of two or three different polypeptide chains. Type IV collagens contain six different polypeptide chains that form at least three distinct molecules and type V collagens contain three polypeptide chains in probably three molecules [68].

Each collagen type has its own specific amino acid composition and performs a distinctive role in tissues. Types I, II and III are of the most abundant collagens, which are responsible for tissue strength, elasticity and water retention capacity [69]. Type I collagen is the main structural component of extracellular matrix. It consists of one α2 chain and two α1 chains, which are encoded on chromosome 7 and 17 in humans [70]. Generally, type 1 collagen is the most commonly used in industrial scale especially in tissue repair and replacement, and they are intensive in skin, tendon, bone, cornea, dentin, fibrocartilage, large vessels, intestine, uterus, dermis, cornea and connective tissue [71]. It has outstanding mechanical properties and is present in virtually every extracellular tissue with mechanical function. In tendons and ligaments, collagen transmits the force from muscles to bones and stores elastic energy. Smooth walking would not be possible without these properties. Collagen also represents most of the organic matrix of bones and tooth dentin and confers them their fracture resistance. It is a major constituent of skin and blood vessels and is even present in muscles, which could not function without a collagen-rich matrix around the contractile cells. A slightly different type of collagen type II is a critical component of a tissue as soft as articular cartilage. The function of collagen is not only mechanical. In the cornea of the eye, for example, the ordering of collagen fibrils confers transparency in addition to mechanical stability [69]. Type II collagen is prevalent in hyaline cartilage, vitreous, nucleus pulposus, notochord and intervertebral disc. It provides biomarkers for osteoarthritis. Type III collagen is present in fetal dermis and epidermis, veins, uterus, synovium, connective tissue around muscles and also in small quantities in areas where type I collagen is present. Type III collagen is functional of fibrillogenesis of collagen I and for normal cardiovascular development [72].

3.2 Industrial sources of collagen peptides

The major sources of collagen for fabrication are bovine and porcine species, where collagen was extracted from the hides and skin and also bones of pigs and cows. Bovine hides, a by-product of meat production, are one of the major industrial sources of collagen [49]. The bovine hide is composed of approximately 30% protein, and the inner corium layer of the hide is rich in collagen. This collagen has a high denaturation temperature in comparison to collagen from other sources. Bovine hide is practiced upon in different development stages such as bovine dermis used for tendon regeneration, and skin and wound healing (in the form of collagen matrix); neonatal bovine dermis is used for hernia repair, plastic and reconstructive surgery [73].

Starting from the 1930s, the most significant raw material for large-scale industrial gelatin production is porcine skin [74]. The skin and bones of pigs are utilized as a collagen sources due to some advantages. Since porcine collagen is almost similar to human collagen, it does not cause much allergic response when used in health applications. But just like the bovine source, the zoonotic diseases poses a risk of contamination and pigs are proscribed due to religious reasons [60]. Halal certification of collagen derivatives is considered to be of main importance because of beliefs and it depends on the origin of raw materials used in its manufacture and traceability from the sources until product chain. Muslims and Jew people demand Halal-certified products for their needs, which is not prohibited and obtained by entirely traceable product chains. Nonspecific collagen is highly suspected of containing porcine elements and very strongly discouraged for use by the Muslims [75]. Nonetheless, adult porcine dermis and small intestinal mucosa are used for tendon regeneration, hernia repair, skin and wound healing, and plastic and reconstructive surgery [76].

There are some other sources of gelatin, somehow industrially applicable or not. Throughout the decade, huge numbers of fish species were investigated as alternatives to the source of collagen. Bones, skin, fins and scales of fresh or salt water fishes are mainly used for collagen procurement and gelatin extraction having different chemical composition. This in turn helps to reduce environmental pollution as considerable amount of wastes occurs during fish processing [77]. Collagen studies from marine origin are carried by on marine vertebrates and invertebrates [78, 79]. Marine sources are from some marine species such as fishes, starfish, jellyfish, sponges, sea urchin, octopus, squid, cuttlefish, sea anemone and prawn [80–82]. Some of the raw material sources of collagen peptides are given in **Figure 2**.

Collagen peptides can also be produced for research purposes in small quantities from other animal body parts such as eggshells, rat-tail tendons, frog skin, kangaroo tails, chicken and duck feet, sheepskin, poultry animal skin, feet, bones and many more [46, 83, 84].

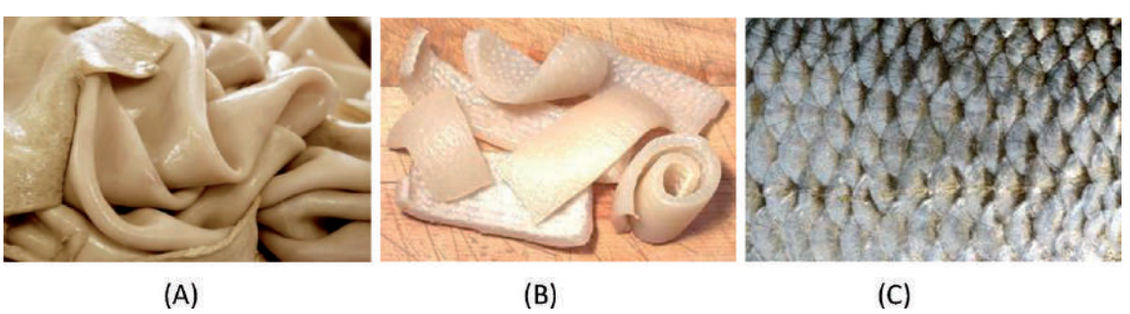

Figure 2.
Main raw material sources of collagen peptides. (A) Bovine split, (B) pig skin, and (C) fish skin.

4. Collagen peptide processing

The collagenic substances, which are involved in multiple collagen units (not subunits) in the quaternary structure and arguably misdefine the tertiary structure also, are normally processable structures. As known very well, leather manufacturing can convert low-value raw materials, which unless untanned and disposed of have detrimental effect to the environment, into valuable final products, and collagen is one of the most substantial structural protein, economically and biologically renewable material for processing. The wastes and by-products of leather processing are discarded parts and effluents from many steps, which are still valuable due to their composition. Lime splits and scraps, as ideal substances, go for gelatin and collagen peptide production.

In the production of industrial-scale collagen peptides, different animal's skin and bones that are easily available and contains collagen protein in high proportion are being used. Collagen peptide preparation steps are dependent on final products' properties. For the first step in general practice, acid and alkali extraction methods are used to remove noncollagenous components [85].

Enzymatic and chemical hydrolysis can be used in the extraction of collagen. Being affordable, chemical hydrolysis is the most commonly used method in industrial practices. Enzymatic hydrolysis is fast and produce waste in minimal amounts, but they are more expensive to carry out [86].

Collagen peptides can be produced by sensitive enzymatic reactions according to the desired molecular weights from collagen-rich raw materials by using protease enzymes. Depending on enzyme types and hydrolyzation conditions, final products can further differ with regard to molecular weight distribution [87]. The production processes could be optimized to obtain different peptides with different functionalities. In the structural level, the cleavage of triple helix is emerged and the collagen molecule is partially broken up. Long chains are hydrolyzed to form shorter chains and further hydrolysis leads to short peptides, some of which are bioactive with body-stimulating functions [88].

Chemical methods of collagen hydrolysis are carried out by means of strong acidic and highly alkaline conditions. Acid and alkaline hydrolysis methods are cost-effective and operation is simple. They have short hydrolysis time and are applicable to industrial processes [89]. However, the uses of strong acids or strong alkaline chemicals make the hydrolysis process environmentally unacceptable [90]. During the acidic treatment, the raw material is exposed to acid for a certain period of time. As this process occurs at a controlled temperature, the structure of the skin swells to twice or thrice more than its initial volume. Both organic acids such as acetic and citric acids and inorganic acids such as hydrochloric acid can be used during acid treatment; however, organic acids are more efficient for the purpose. Acidic treatment results unraveled the structural unity and the cleavage of the noncovalent inter- and intramolecular bonds. Materials with less intertwined collagen fibers such as fish and porcine skin are the preferred choice for the acidic process [85]. For the alkaline process, the raw materials are treated in basic solutions for a duration of a few days to weeks. The most commonly used process is through aqueous sodium hydroxide and calcium hydroxide solutions. However, other basic solutions can also be used in this alkaline process. This process entails the treatment of hard or thick substance that needs very aggressive penetration by the basic solutions [91].

To meet the technical needs of the different sectors, purification stage ensures the removal of ionic and nonionic impurities resulting from the processing of raw materials. Different filtration and purification systems can be used at this stage depending on the final product needs [92]. The purified and demineralized gelatin solution consists of over 95% water. This water has to be almost completely

removed. Only dried gelatin with its normal residual water content of 10–12% has an unlimited shelf life from the microbiological point of view. In addition, dilute gelatin solutions can neither be stored nor transported easily. In the next production step, the highly concentrated and filtered gelatin solutions are sterilized. For this step, both indirect sterilization via plate heat exchangers and direct steam sterilization are used. Both methods are microbiologically safe to a very high degree [93]. After sterilization, the prepared material needs to be dried to final form. There are different drying methods used in the production of collagen peptides. Spray drying is the most commonly used method and widely used in the production of small molecular weight peptides [94].

5. Industrial applications

5.1 Food

Collagen peptides have shown to be an important ingredient in the food and beverage industries worldwide [95]. It has been used for a long time in foods globally, such as in the United States, China, Japan and many countries in Europe. Approved as Generally Recognized As Safe (GRAS), the safety of collagen peptides has been affirmed by the Food and Drug Administration (FDA) and Center for Food Safety and Applied Nutrition (CFSAN) [96]. It has been applied as protein dietary supplements, carriers in the meat processing, edible film and coatings of products and food additive to improve product's functionality [97]. In addition, collagen may boost the health and nutritional value of the products relying on its inimitable properties on human bodies [75].

The source of the raw material and the degree of processing determine the properties of the collagen peptides like gelatin, which have several different applications in the food industry [98]. The major quality parameters are their higher gel strength and suitable melting and gelling temperatures for the food industry that uses them as an additive. Due to the fact that porcine and bovine gelatins are less preferred due to religious preferences, safety concerns and economic considerations, using fish skin or bones to obtain gelatin has become popular in recent years [99]. Thanks to its many unique properties, the numerous applications of gelatin include its usage as a thickener, stabilizer, setting agent, clarifying agent, water-retaining agent and adhesive in a wide range of foods, pharmaceuticals and household products. In the food industry, gelatin can be utilized in a wide range of confectioneries, beverages, snacks, desserts and meat products [100]. Gelatin is used as an additive to improve elasticity, consistency and stability of foods like desserts, candies, bakery products, jellied meats, ice cream and dairy products. Gelatin is also used as stabilizer to modify the structure of the food products. It is added to yogurt to reduce syneresis and increase firmness [100]. In addition, type A gelatin that is isolated with acid treatment with gel strength as 70–90 g, which is relatively low, is used to fine wines and juices. Type B gelatin is processed with an alkali treatment with gel strength as 125–250 g and is used in confectionery products [101]. Collagen peptides have also been reported to have antioxidant and antimicrobial activity [102]. However, the relationship between peptide characteristics and antimicrobial activity has not been clearly demonstrated.

5.2 Cosmetic

Collagen can be used in cosmetics due to its biodegradability, availability and biocompatibility properties for different purposes such as in dermal fillers, skin substitutes or scaffolding, wound repairs and facial products [103].

The formation of unwanted wrinkles in the body with aging is related to the damage of the fibers in the skin. In the researches about aging, it has been determined that collagen hydrolysates contribute greatly to the repair of these fibers [104]. The introduction of collagen hydrolysate into the body ensures the stimulation of collagen formation that enables the recovery and improved tissue appearance [105]. Hence, the cosmetic industry reclaims some functionalities of its products by incorporating this biomolecule.

Collagen peptide has been known to be used in cosmetic formulations for reasons such as protecting the structure and the function of the skin, enhancing its appearance and preventing premature aging [106].

Collagen peptide is prepared in the form of liquid ampoules, powder mixes or tablets in the food and cosmetic industries. It has a regenerative effect on skin wrinkles and other signs of skin aging: collagen helps the skin remain soft and pliant and improves the hydration of the epidermis [107]. Many studies have shown that collagen sleek thin lines and can prevent the development of deeper wrinkles and grooves. Collagen is not only effective for the skin on the face but also stimulates the fiber structure of the body to repair and reduce cellulite tissue [108].

Collagen hydrolysates have also shown bioactivities such as antioxidant properties, antihypertensive activity, lipid-lowering activity, as well as reparative properties in damaged skin [109]. Moreover, it has been also observed that collagen provides the building block for elastin and collagen formation and acts as ligands in fibroblast cells to stimulate hyaluronic acid [110].

5.3 Health

Collagen is the most abundant and ubiquitous protein in the body regarded as one of the most useful biomaterials. The excellent biocompatibility and safety due to its biological characteristics made collagen the primary resource in medical applications. It has various applications in some departments such as cardiology (heart valve), dermatology (for skin replacement, augmentation of soft tissue, skin tissue engineering and artificial skin dermis), surgery (as hemostatic agent, wound repair and dressing, nerve repair and blood vessel prostheses), orthopedy (tendon, bone and ligament repair and cartilage reconstruction), ophthalmology (corneal grafts and contact lenses), urology (hemodialysis and sphincter repair) and vascular surgery (vascular graft and vessel replacement) [111].

Collagen type I is considered to be the most valuable material for tissue engineering due to its high biocompatibility and immunogenicity. It is used as the basic matrix for cell culture [73, 112]. Biomaterials based on collagen are widely used in tissue engineering such as injectable matrices and scaffolds intended for bone regeneration [73, 113]. Moreover, collagen-based eye implants are preferred for the treatment of ophthalmic disorders. Such type of collagen-based implant preparation has shown considerable applicability because it provides stable and reasonable control over the postoperative complications such as intraocular pressure [114]. Collagen-based matrices find their use as corneal transplant and as temporary patches to repair perforations in case of emergencies [115].

Collagen is used in pharmaceutical industries for different functionalities as hard and soft dry capsules, microparticles, injectable dispersions, shields in ophthalmology sponges and drug delivery system. Its application in the pharmaceutical as well as biomedical field is due to its characteristics such as weak antigenicity, immunogenicity, biodegradability and biocompatibility [116].

As a collagen peptide, gelatin is the most important material for the production of hard and soft capsules as well as film-coated and effervescent tablets. Manufacturers take into account its adhesive, gelling and film-building properties.

Orally administered medicines and dietary supplements in particular are protected by gelatin-containing capsules or tablets from light, moisture and oxygen and given a long shelf life [107, 117, 118]. Gelatin is also used as a raw material in many field of health industry as is the case with the manufacture of blood substitute [119]. These products prevent hypovolemic shock by stopping bleeding in the wound-occurred area. As local hemostatic agents, collagen sponges and films have long been used in the surgical field (e.g., in oral cavity and ophthalmological surgery, urology or gynecology) and for the treatment of wounds in dental surgery. The structural composition of the collagen material enables the absorption of large amounts of blood and makes it possible for new tissue to grow into the sponges. Since it only takes a few days for the body to completely resorb the sponges or films, they can be left in the wound without any negative effects [107, 120, 121].

5.4 Sportive nutrition

Collagen peptides are ideal supply due to their numerous beneficial health effects for modern sportsperson nutrition as high-energy supplement to maximize muscle protein anabolism [122]. They are neutral in flavor, which means that they do not leave a bitter aftertaste that has to be masked in the final product, that is, through sugar or artificial sweeteners, as is often the case with soy, whey or other protein [107, 123]. Collagen peptides have been scientifically tested and have no undesirable side effects, and there is no evidence to elicit allergic reactions. It emulsifies foams and improves the shelf life of products [107, 124].

The more protein a body expends through physical exertion, the greater its needs for an external source, for example in the form of special dietary supplements such as protein shakes, energy bars, protein snacks or sports drinks. Several studies in the past few decades have reported that protein hydrolysates from various food sources, in addition to their nutritional properties, exhibited various biological functions including hypotensive activity, anticoagulant, cholesterol-lowering ability and hypoglycemic effect [125]. Consumption of hydrolyzed collagen increases collagen synthesis and decreases knee pain while standing and walking [126]. Shaw et al. [127] tested the role of gelatin consumption in collagen synthesis. In the study, double-blinded, placebo-controlled and crossover-designed research, subjected to whom consumed 15 g of gelatin showed double-fold collagen synthesis, measured through serum propeptide levels. From the results, it was observed that consuming hydrolyzed collagen might increase collagen synthesis and potentially decrease injury rate in athletes. Studies have also shown that products fortified with collagen peptide can promote joint health, bone synthesis and antisport fatigue ability [128].

5.5 Agriculture and animal feed

5.5.1 Fertilizer

Leather processing wastes like shavings that cause environmental pollution are opulent sources of novel and valuable biomolecule "collagen" [129]. Industry has been generally oriented on the recovery of collagen from leather waste, but the remaining waste also can be used for agricultural purposes. Collagen-based fertilizer products highly are demanded in agriculture industry because of being high amino acid and organic carbon source and nitrogen content [130].

The collagen hydrolysates obtained from leather wastes are being utilized as biofertilizer. Several plants can also take up and absorb amino acids as an example of biostimulants; these amino acids are sometimes better nitrogen sources than ammonia or nitrates [131]. Collagen peptides are recovered and channeled as an organic

nitrogenous fertilizer to increase the yield of the crop [132]. Both plants and animal organisms can more easily absorb microelements like iron, copper, zinc, calcium, magnesium and manganese chelated with hydrolyzed collagen. The use of collagen hydrolysates in combination with potassium polyphosphates increases agricultural production by increasing the absorption of phosphorus and potassium [133].

Collagen hydrolysates obtained by chemical and chemical-enzymatic processes under moderate reaction conditions were used in a study for preparation of foliar fertilizers [134]. Hydrolysates of chromium-tanned leather shavings were used in a study as nitrogen source for growth of common bean plants and banana cultivation [135]. De Oliveira et al. [136] have studied the use of leather wastes after extraction as a nitrogen source to elephant grass. The chrome shaving wastes can also be hydrolyzed in an autoclave (150°C). The obtained product contains moisture content (7–10%), total nitrogen (10–11%), organic carbon (40%) and chromium (III) (2.5–3%). By blending with other additive components, the product can be sold as a fertilizer [133].

Both gelatin and collagen hydrolysates have positive effect on the growth of plants when applied as fertilizer. The crop yield is comparable with those obtained by using inorganic fertilizers but with a significantly high value in view of the low nitrate content, which is 20 times less. Besides, organic fertilizer improves the soil quality unlike the inorganic ones [137].

5.5.2 Animal feed

Collagen peptide due to its organic compounds such as fats, proteins and minerals plays an important role in the preparation of highly valuable animal feed [84]. Fat, protein and mineral products are in especially high demand in the animal feed industry because pure fats are excellent sources of energy and collagen is of importance for the healthy growth of animals [138].

As a collagen peptide, which is recovered from leather solid wastes, gelatin is primarily added to animal feed based on its hydrophilic properties. Its jelly-like consistency holds feed together, making it transportable and extends its shelf life [107, 139]. When animal feed is enriched with vitamins, the gelatin coatings also protect these from light and oxygen. A positive side effect of adding gelatin to feed ensures that the fur of animal remains wonderfully glossy [107, 140].

There are also many fields for collagen peptides and gelatin usage. They can be used for photography and X-ray films and inkjet applications [141], industrial paper production [93], leather board [142], glue manufacture [143], feedstock for biodiesel production [144], leather tanning and retanning agent [145] and many more specific applications, etc.

6. Conclusion

The tanning industry is one of the oldest industries in the world and recently its pollution load onto environment has become seriously threatening for transferring the potential to next generations. It produces a significant amount of solid wastes and effluents. It is a well-known fact that removing undesired substances out of the structure in leather processing produces effluents; that is highlighted agenda which needs to be overcome and as per the composition those are able to handle for recover and reuse through the current technology.

Revaluation of leather solid wastes is one of the promising waste management strategies that provides raw materials to another industry such as food, agriculture, cosmetic, health, etc. This method may offer a solution for utilization of huge volume of leather solid wastes, which are often dumped in open landfills. Commercial

benefits of the system should be linked with both the value of the products and the disposal cost of solid wastes. In the economical point of view, feasibility should be based on converting them into value-added products instead of making a deposit for disposal.

Collagen peptides obtained from hides and their by-products have been practiced as healthful stuffs in many areas of our modern life. As the awareness of their technological value increases as time passes, this value-added material is considered to have higher interest with the usage in various fields. Analogous to collagen peptides, collagen hydrolysates and gelatins, emerged from this precious protein are involved in either partly or total denaturation. It is the process defined by disintegration of intra- and intermolecular bonds that keeps together the chains composed of amino acids in the conformation; thereby, a typical protein is formed. The discovery of benefits of collagen derivatives for health and their usage as additives has a long history and is dated back to some 8000 years ago. Today, its usage enlarges over many industries and applications includes in food, health, chemical, body care and agricultural etc. According to the molecular weight and properties, the usage of collagen derivatives increases as a gel or colloidal solution, their benefits are multiplied and this bio-based material supplies many valorization possibilities. As per the source and properties, they are bioavailable products that are digested and absorbed by human and animal body quickly and even by plants and are also easy to use in any industrial applications and processes.

In spite of the tremendous development in technology and sciences, there are still challenges ahead to better understand the collagen types and sources, structure and properties, gelatin processes and product characteristics. It seems that in the future the researches on bio-based materials as well as the efforts for their commercialization will continue intensively in a wider range of products.

Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter

Characterization of Grafted Acrylamide onto Pine Magnetite Composite for the Removal of Methylene Blue from Wastewater

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Abstract

Much attention has been focused on chemical modification of natural biomass through grafting. Modification of natural polymers by graft copolymerization has shown to be a promising technique as it functionalizes a biopolymer to its potential, imparting desirable properties onto them. The present study focuses on functional groups such as —CO—NH_2 which were grafted into cellulose from acrylamides. The characterization of the composite was done using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), Brunauer-Emmett-Teller (BET), thermal gravimetric analysis (TGA), and transmission electron microscopy (TEM). These techniques were used to further demonstrate the formation of the grafted acrylamide composite (GACA). SEM analyses showed existence of strong chemical interactions between pine cone magnetite and acrylamides.

Keywords: characterization, adsorption, acrylamide, methylene blue, grafting

1. Introduction

The rapid development of the textile industry has resulted to a large proportion of industrial wastewater pollution. Methylene blue (MB) dye is a most widely used dye by industries like textile, paper, rubber, plastics, leather, cosmetics, food industries and pharmaceuticals. The textile industry is classified into three main categories, namely; cellulose fibers (cotton, rayon, linen, ramie, hemp and lyocell), protein fibers (wool, angora, mohair, cashmere and silk) and synthetic fibers (polyester, nylon, spandex, acetate, acrylic, ingeo and polypropylene) [1]. The type of dyes and chemicals used in the textile industry are found to differ depending on the fabrics manufactured. Reactive dyes (remazol, procion MX and cibacron F), direct dyes (congo red, direct yellow 50 and direct brown 116), naphthol dyes (fast yellow GC, fast scarlet R and fast blue B) and indigo dyes (indigo white, tyrian purple and indigo carmine) are some of the dyes used to dye cellulose fibers [1]. The textile industry is known to be the main creator of wastewater effluents because it

consumes more water for its wet processes. Therefore, globally it is estimated that all wastewater discharge is highly populated. According to the world bank estimation, textile dyeing and finishing treatment given to a fabric generates at least 17–20% of world's industrial wastewater [2, 3].

Dyes often discharged in water effluents contain residues that are highly visible and undesirable even at low concentrations [4]. In addition, they are toxic due to their harmful effects on the human beings. Therefore, it is of vital importance that they are removed from water [5]. Wastewater containing dyes needs to be treated before being discharged into water bodies [6]. Various techniques including chemical oxidation, coagulation-flocculation, membrane processes and biological treatment have shown effectiveness in the removal of methylene blue from waste water [7]. The limitation most of these techniques possess is the incomplete dye removal, poor detection, requirement of expensive equipment and monitoring systems [6]. The performance of adsorption techniques have been applied due to their effectiveness since they remove the entire dye molecule, leaving no fragments in the effluent [8].

Extensive research in recent years has focused on utilizing waste materials from agricultural products (such as pine cones and others) since they are eco-friendly, cost-effective and renewable [9]. Pine cones are naturally occurring agricultural wastes widely found in a plantation in Vanderbijlpark, Gauteng, South Africa. They are of commercial importance and value which is extensively used in different industries [10]. One pine cone consists of 46.5% hemicellulose, 37.4% lignin, 18.8% cellulose and 15.4% extractives [11]. Pine cone powder has been studied extensively in the removal of heavy metal pollutants such as lead, caesium, copper nickel and arsenic from water systems. Activated carbon has been the most employed adsorbent for the removal of dyes due to its outstanding adsorption properties. However, it has limitations by being expensive and it cannot be used in large applications of wastewater treatment. The use of biomass and other microbial cultures in the removal of methylene blue has been extensively studied in recent years. Among others, carbonized organic materials, fly ashes, peat moss, recycled alum sludge, fishery residues and microorganisms such as fungus and algae [12].

The present study reports the development and characterization of grafted pine magnetite composite using grafted acrylamide (GACA) for the removal of methylene blue in wastewater. Grafting is a process of chemically or physically manipulating the surface properties of plant materials such as type and amount of functional groups, surface area and porosity by extraction of plant chemical components in order to improve its adsorptive ability. Grafting of synthetic monomers onto pure biological materials has been successfully performed, e.g., grafting of acrylonitrile onto starch [13] and methyl acrylonitrile onto cotton [14].

2. Materials and methods

2.1 Materials

Pine cones are naturally occurring agricultural wastes found in a plantation in Vanderbijlpark, Gauteng, South Africa. All the chemicals and reagents used throughout this study were of analytical grade reagents and used without any further purification. Acrylicamide, ceric ammonium nitrate (CAN), nitric acid (HNO_3), sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), ferric sulfate ($FeSO_4$) and methylene blue was supplied by Merck, South Africa. Deionized was used for the preparation of all solutions. The stock solution for methylene blue (1000 mg/L) was prepared by dissolving the required amount of dyes in a 1000 ml of deionized water and the stock solution was further diluted for batch experiments.

2.2 Methods

2.2.1 Synthesis of pine-magnetite composites

A mixture of $FeSO_4 \cdot 7H_2O$ (2.1 g) and of $Fe(SO_4)_3 \cdot XH_2O$ (3.1 g) were dissolved under inert atmosphere in 100 cm^3 of double-distilled water with vigorous stirring. Thereafter, 20 cm^3 of 28% ammonium hydroxide and the appropriate amount of pine powder was added. The reaction was left to run for 45 min at 80°C under constant stirring. The resulting particles, consisting of magnetite attached to the cellulose (hereafter referred to as bio-composite) were washed several times with deionized water and ethanol and dried in a vacuum oven at 60°C overnight. To determine the optimum conditions to achieve the desired products of the biocomposites, we experimented with the following variables: volume of NH_4OH 5, 10, 20, 30, and 40 cm^3; weight of pine powder 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 g; temperatures 40, 60, 80 and 100°C and reaction times 15, 30, 45 and 60 min.

2.2.2 Synthesis of grafted pine magnetite composite

1 g of pine magnetite composite (PMC), 20 ml of 1.5 M acrylicamide and 135 ml of deionised water were transferred into a three neck round bottom flask at a temperature of 42°C. The reaction was bubbled under nitrogen gas for 30 min to remove the dissolved oxygen under stirring. 10 ml of 0.5 M CAN, dissolved in 0.3 M HNO_3 was slowly added to the reaction to initiate graft co-polymerization and stirring was continued for 2 h. Reaction mass was neutralised by 50% NaOH and precipitated in methanol and thereafter washed with methanol/water (90:10) several times, so that the unreacted PMC and ceric salt were removed. The final residue was dried in a vacuum oven at 40°C.

2.3 Characterization

Qualitative and fundamental identification of the functional chemical groups of grafted pine magnetite composite (GPMC) were carried out with a FTIR (Perkin-Elmer) in the range 450–4000 cm^{-1}. An X'Pert PRO X-ray diffractometer (PAN analytical, PW3040/60 XRD; CuKα anode; λ = 0.154 nm) was used for particle size measurements. The size of the synthesized particles was observed using transmission electron microscope (TEM, FEI TECNAI G^2 SPIRIT) at an accelerating voltage of 150 kV. TGA (Perkin-Elmer (USA) Simultaneous Thermal Analyzer 6000 instrument) was used for determining the weight loss as a function of temperature. Changes in morphology were studied using scanning electron microscopy (SEM), HRSEM Instrument Specs Model: Jeol JSM 7800F field emission scanning electron microscope run operational voltage: 5kVEDS specs Model: Thermo Fischer UltraDry EDS Detector for the graft co-polymerization and incorporation of iron oxide magnetite (Fe_3O_4 PMC).

3. Results and discussions

3.1 FT-IR spectroscopy results

The FT-IR spectrum shown in **Figure 1(a)** represents the pine-Fe_3O_4 magnetite (PMC). The FT-IR spectrum showed some changes in band intensities, indicating the functional groups on the surface that had been modified. A compressed —OH peak at 3350 cm^{-1} with an increase in intensity was observed. This might have been

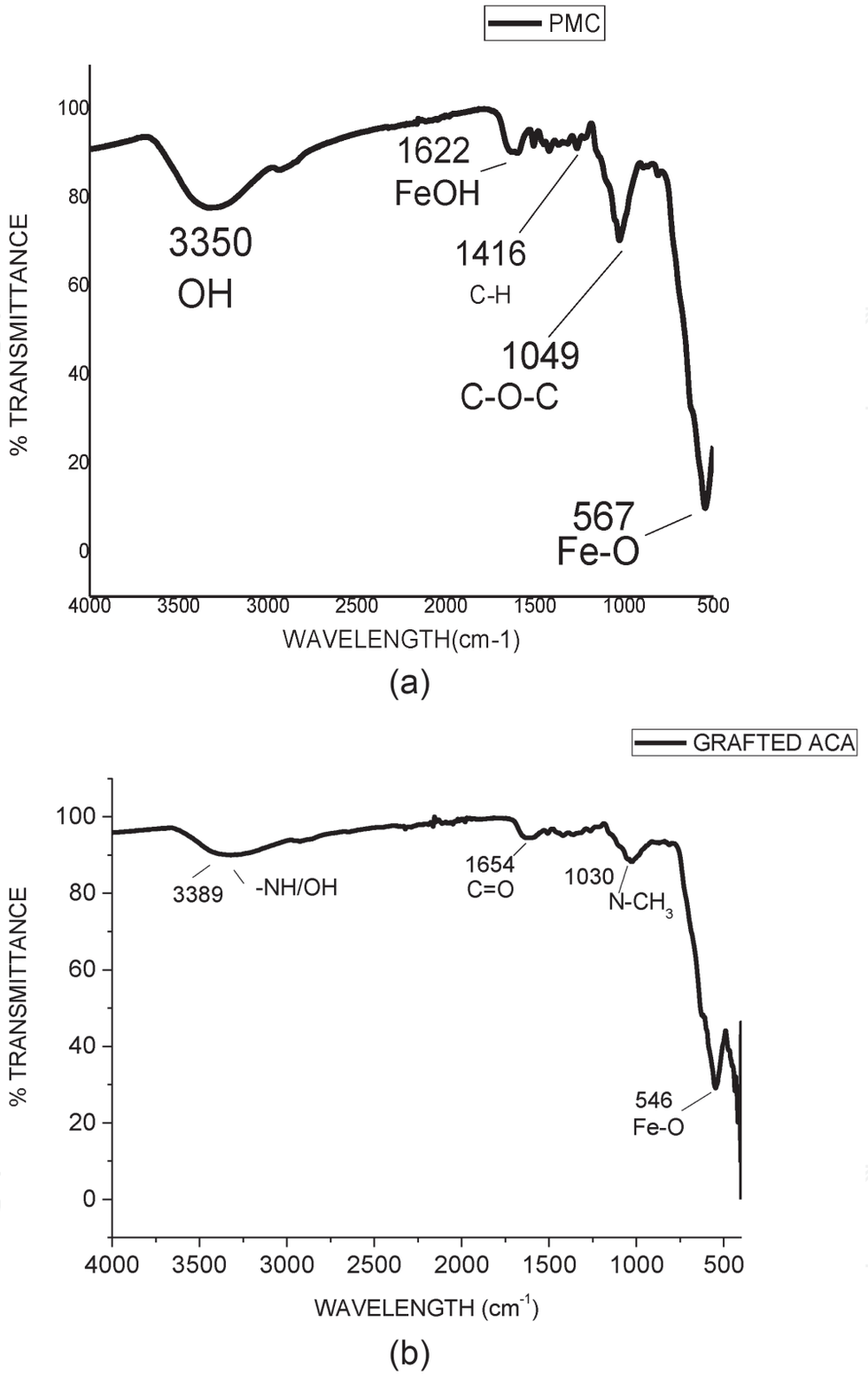


Figure 1.
(a) FT-IR spectrum for PMC and (b) FT-IR spectrum for GACA.

due to the presence of extracted lignin in pine cone. Clearly, the COO— peak was converted to esters at 1622 cm^{-1}, C—H aliphatic peaks were observed at 1416 cm^{-1} which represent the increase in the internal surface of the pine cone and a new peak was found at 567 cm^{-1} which was assigned to the vibration of Fe—O band of Fe_3O_4.

The FT-IR spectrum of GACA (**Figure 1(b)**) shows a slightly broad band observed at 3389 cm^{-1} ascribed to the existence of OH— and —NH groups [15]. The compressed peak at 1654 cm^{-1} corresponds to a carbonyl functional group of

acrylamide [16, 17], whereas the one at 1030 cm^{-1} reflects on the vibrations of N—CH_2 groups. The last peak at 546 cm^{-1} reflects the Fe—O functional groups. These functional groups might have participated in the interactions with MB which involved the mechanism of surface complex, hydrogen bonding, and electrostatic attractions.

3.2 XRD analyses

The XRD spectrum of grafted pine magnetite composite with acrylamide is shown in **Figure 2a**. The prominent peaks at 2θ values of 30.5°, 38.7°, 43.9.0°, 59.8° and 63.7° corresponding to (220), (311), (400), (422), (511), respectively, attributes to the cellulose peaks due to the presence of iron oxide magnetite composite and crystal planes of grafted pine magnetic composite respectively [18]. The composite has shown a cubic crystal structure. It is observed that diffraction intensity of the broad peak at 43.9° was weakened indicating that the crystallinity of the PMC decreased after grafting. This phenomenon might be due to the strong interaction of covalent bonds between the PMC and the acrylamide.

3.3 TGA analyses

The TGA and DTG curves shown in **Figure 3a** demonstrate the thermal stability of grafted pine magnetite composite. The incorporation of the Fe_3O_4 magnetite composite showed the changes in the thermal properties of the cellulose. The initial thermal decomposition of GACA occurred at 100–240°C temperature range which corresponds to loss of water molecules and volatile compounds. The second stage thermal decomposition in the temperature range 380–640°C may be due to the breakdown of the polymer matrix and cross-links between different polymeric chains. The last stage of decomposition at a temperature of 700°C corresponds to the lignin degradation [19]. Grafting with acrylamide presented a better thermal stability due to the different types of covalent bonds in the grafting of copolymer backbone [20]. Differential thermal analysis (DTA) showed endothermic peaks associated with degradation of various materials. The degradation behaviour exhibited two stage decomposition effects. Observation at different temperatures (380–620°C) was attributed to the cellulose decomposition at low temperature and grafted acrylamide composite at higher temperatures. This confirmed the stabilizing effect of the incorporation of Fe_3O_4 composite onto acrylamide.

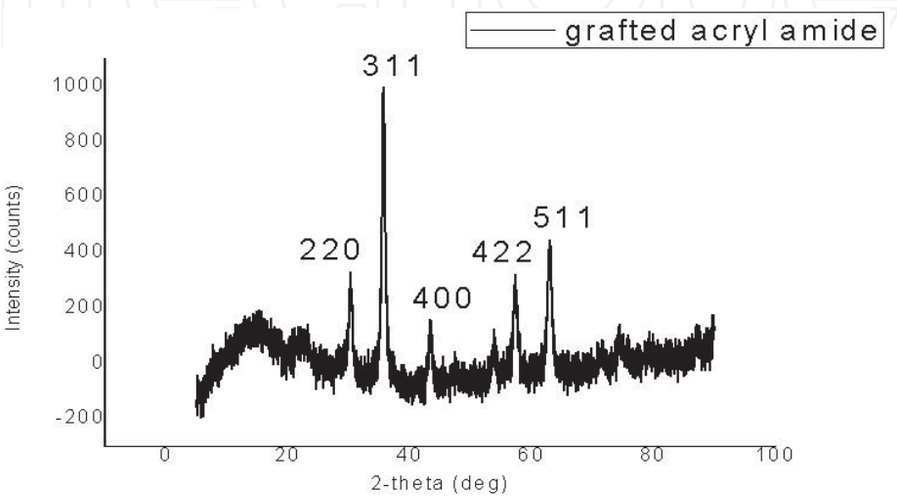


Figure 2.
XRD spectrum for GACA.

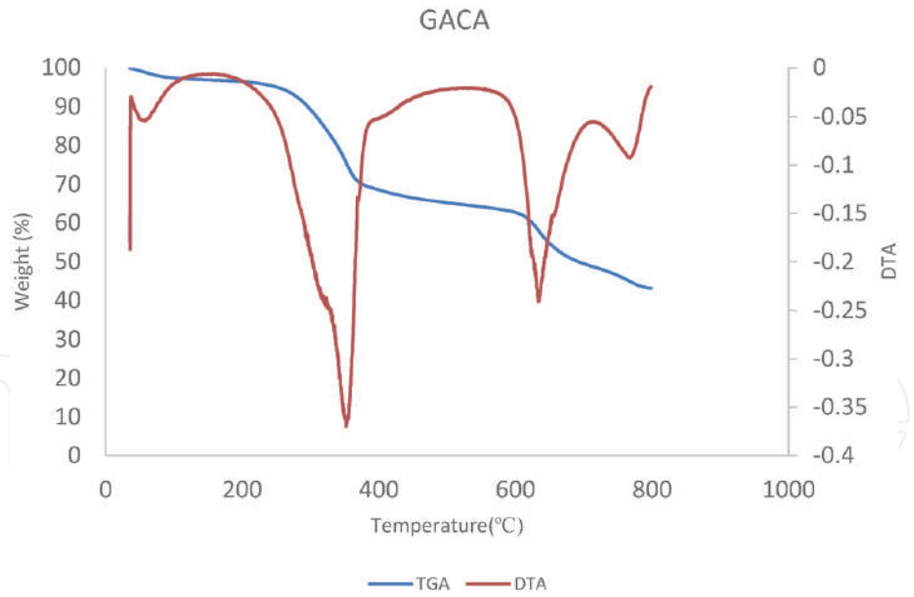


Figure 3.
TGA and DTA curves for GACA.

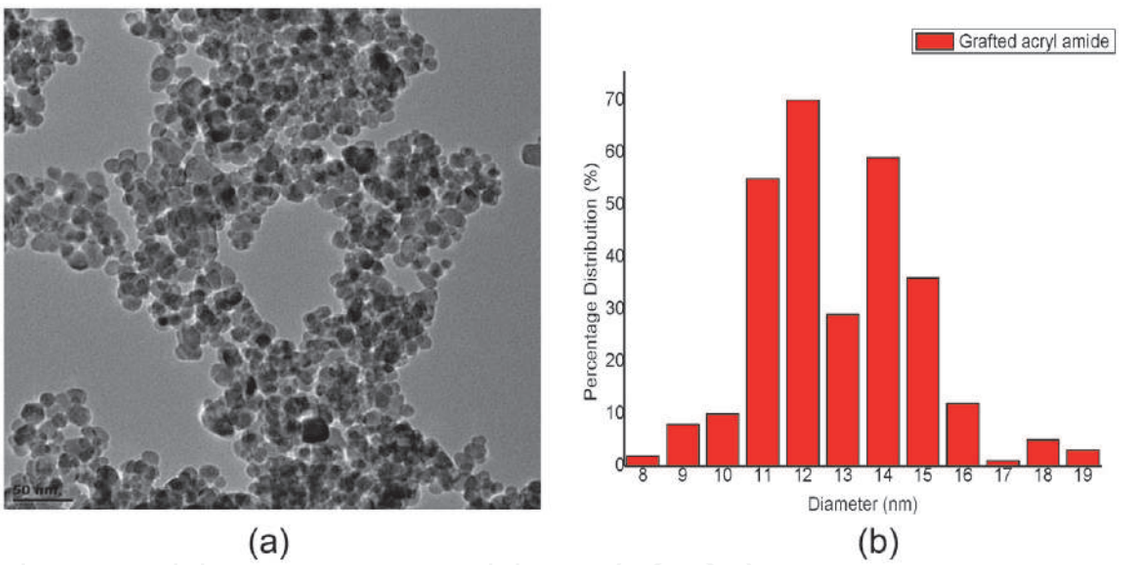


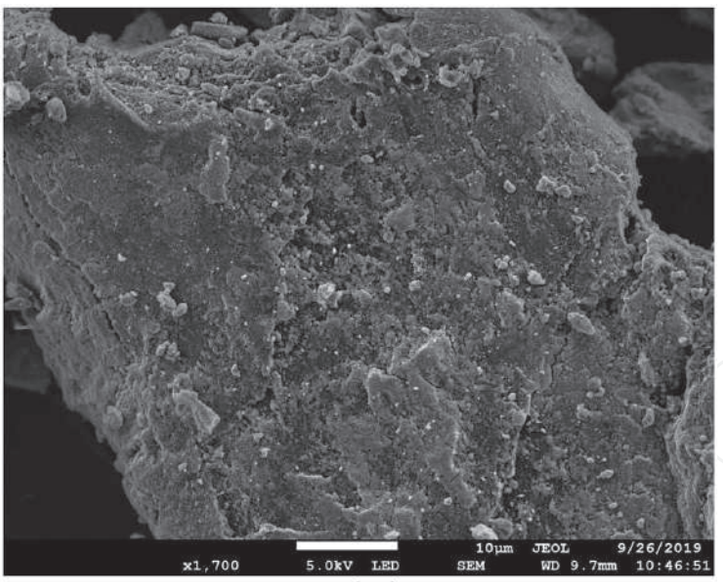
Figure 4.
(a) TEM image and (b) size distribution of GACA.

3.4 TEM studies

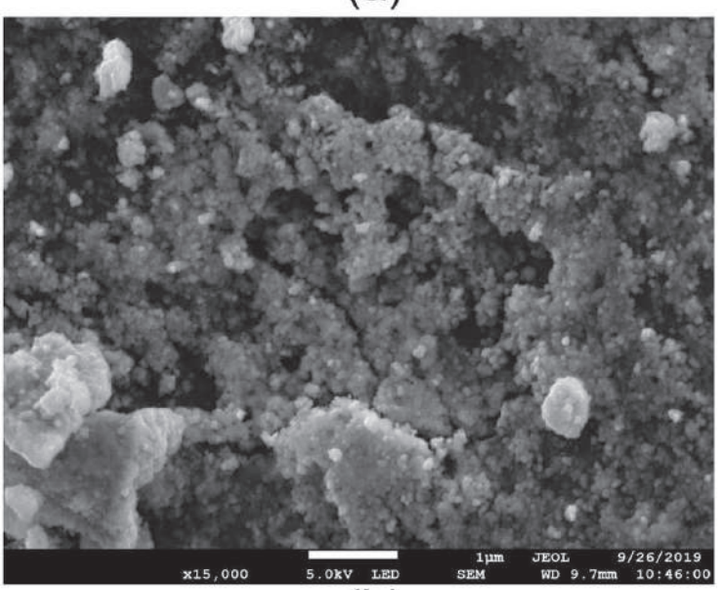
The TEM image in **Figure 4(a)** shows the appearance of the typical images of grafted pine magnetic composite with acrylamide. The supporting information showed spherical nano-particles as attributed to the shape and the incorporation of the magnetic nanoparticles in the polymer matrix. **Figure 4(b)** shows the size distribution of the pine magnetite particles with a peak at 12 nm.

3.5 SEM studies

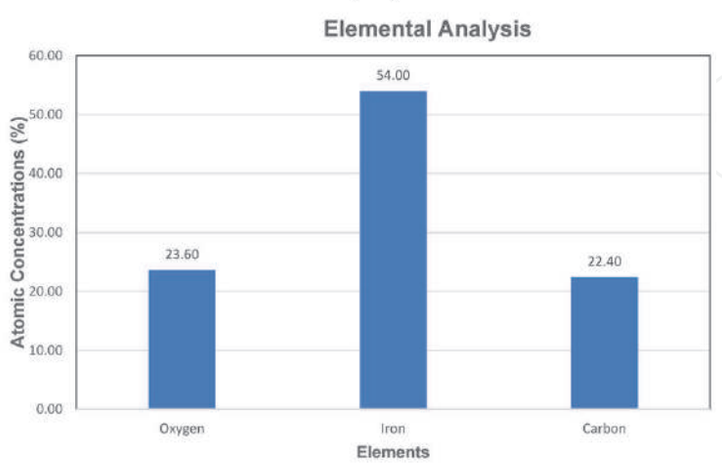
SEM images of grafted pine magnetite composite with acrylamide are shown in **Figure 5(a)** and **(b)**. The observation showed changes in morphology of the GACA



(a)



(b)



(c)

Figure 5.
(a) SEM image of the GACA, (b) SEM image of the GACA and (c) elemental analysis from SEM-EDX.

because of the graft copolymerization process and incorporation iron oxide magnetite. Supporting information showed the granular smooth surface. Roughness of the surface increased after modification, better matrix coherence was achieved after incorporation of the iron oxide magnetite nanoparticles. All the observations confirmed that grafting pine magnetite composite with acrylamide allows better compatibility. The presence of the Fe peak in the EDX of the nanocomposite showed successful incorporation of iron oxide composite in the polymer matrix **Figure 5(c)**.

3.6 BET (surface area) analyses

A surface property of an adsorbent describes the effect of modification on the surface area of the adsorbent. **Table 1** shows comparison of the effect of modification on the surface area of the materials. The pure pine magnetite nanoparticles showed a surface area of 113.60 m^2/g, pore volume of 0.6321 cm^3/g and pore size of 25.86 nm. On the other hand, the NaOH treated pine had a surface area of 2.25 m^2/g, pore volume of 0.0177 cm^3/g and pore size of 10.17 nm. Pine magnetite composite exhibited surface area of 54.80 m^2/g, pore volume of 0.1522 cm^3/g and pore size of 23.10 nm. Grafted acrylamide reflected the surface area of 57.77 m^2/g, pore volume of 0.1591 cm^3/g and pore size of 17.33 nm. The higher surface area was due to the pine cone structure which was found to be important for the improvement of mass diffusion and adsorptive capacity. An increase in surface area, pore volume and pore size confirmed that GACA can adsorb MB more efficiently than the PMC. The distinct pore structure size enables fast transportation of particles.

3.7 Point zero charge (pH_{pzc})

To further investigate the effects of modifications on the suitability of the synthesized materials for adsorption, the isoelectric point or point of zero charge (pH_{pzc}) was determined. The solution pH is an important parameter for dye adsorption because it does not only change the surface charge of the adsorbent but also it affects the molecular structure of the dye. As MB is a cationic dye, it can easily form positively charged species over a wide pH range. The solid addition method was used to determine the pH_{pzc} of the pine cone composite. To a series of 100 cm^3 volumetric flasks, 45 cm^3 of 0.01 mol/dm^3 KNO_3 solution were transferred. The pH_i values of the solutions were roughly adjusted between pH 2 and 12 by the addition of either 0.1 mol/dm^3 HCl or NaOH on a pH meter with constant stirring. The total volume of the solution in each flask was made up to 50 cm^3 by the addition of KNO_3 solution of the same strength. The pH_i of the solutions was accurately noted, and 0.1 g of pine cone composite were added to each volumetric flask, which was then immediately closed. The suspensions were allowed to equilibrate for 48 h on a shaker operating at 200 rpm. The pH_f values of the supernatant were accurately noted and the difference between the initial and final pH values ($\Delta pH = pH_f - pH_i$) were plotted against the pH_i. The solution pH is an important parameter for

Properties	Pure magnetite composite	NaOH treated pine	Pine magnetite composite (PMC)	Grafted acrylamide
Surface area (m^2/g)	113.60	2.25	54.80	57.77
Pore volume (cm^3/g)	0.6321	0.0177	0.1522	0.1591
Ave. pore size (nm)	25.86	10.17	23.10	17.33

Table 1.
BET surface area and pore characteristics for synthesized materials.

dye adsorption because it does not only change the surface charge of an adsorbent, but it also reflects the molecular structure of the dye.

Changes in the point of zero charge values within the sample can be attributed by the difference in types and amounts of surface functional groups present on the surface of the adsorbent. pH_{pzc} is observed when modification on the suitability of the synthesized materials is determined. It is known to be the pH at which the amount of positive charges on a biosorbent surface equals the amount of the negative charge, i.e., the pH at which the biosorbent surface has net electrical neutrality [21, 22]. Methylene blue is a cationic dye and can easily form positively charged species over a wide pH range. The pH_{pzc} of pine magnetite composite was found to be 8.56 and grafted pine magnetite with acrylamide was found to be 6.2. The decrease in the pH_{pzc} is attributed to the modification of the surface area.

3.8 Adsorption studies

3.8.1 Effect of solution pH

The adsorption experiments were carried out using batch equilibration techniques. Various methylene blue (MB) solutions with different pH range, initial concentrations and mass dosage were prepared by diluting 1000 mg/dm^3. Equilibrium experiments, to determine the adsorption capacity of pine magnetite composite were conducted using 250 cm^3 bottles. 0.1 g of PMC and 100 cm^3 of the MB solution were added and shaken for 2 h at 26°C. Thereafter, absorbance was determined using UV-VIS spectrophotometer at the wavelength corresponding to the maximum absorbance (λ_{max} = 665 nm) as determined from the plot. This wavelength was used for measuring the absorbance of residual concentration of MB. pH of the solution was adjusted using 0.1 M HCl and 0.1 M NaOH. **Figure 6** showed the effect of pH on the adsorption of MB. An increase in pH showed an increase in percentage removal. When the pH was 2.0 and 4.0, the removal rate of MB was 99.4 and 99.5%, respectively. This indicated that the lower adsorption of MB at acidic pH was due to the presence of excess H^+ ions. The influence of low pH to MB adsorption was that H^+ ions could occupy the binding sites; this was not favorable for the adsorption of MB. Furthermore, MB possessed positive surface charges and could be repulsed by H^+ ions to prevent MB adsorption onto grafted pine magnetic composite.

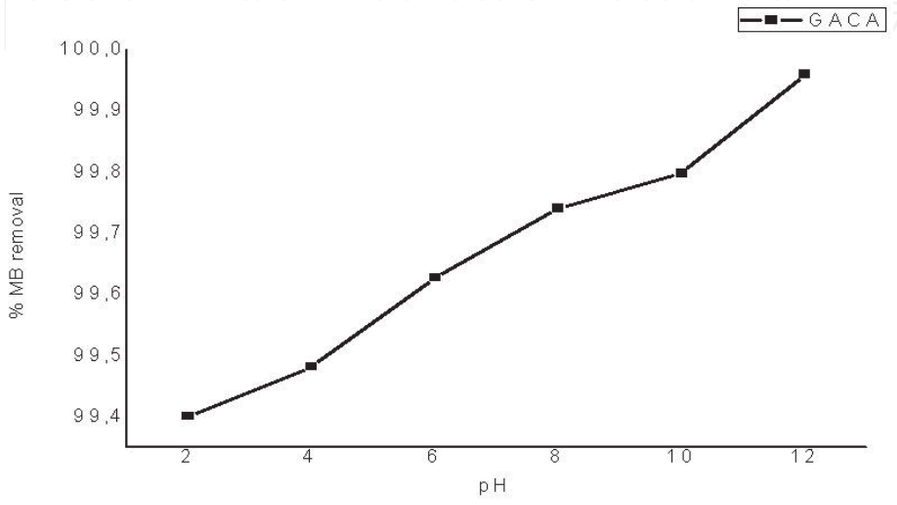


Figure 6.
Effect of pH on the adsorption of MB.

With increasing pH, the number of hydrogen ions i solution was reduced and the competitive effect, repulsive interaction weakened, lead to an increase in the removal rate. The MB removal rate became stable when the pH reached 12, where the higher percentage removal for MB was observed in comparison to other pH values.

3.8.2 Effect of adsorbent dose

Figure 7 shows the effect of adsorbent dose on the percentage removal and amount of dye that was adsorbed. This effect was necessary in order to observe how the novel adsorbent used impacted on the adsorption stoichiometry. It also gave an idea of the propensity of dye molecules to be adsorbed with the smallest amount of adsorbent. When the mass of the adsorbent was 0.5 g, the percentage adsorption removal increased rapidly, which contributed to the increased surface area of the adsorbent which in turn increased the number of binding sites [23]. The adsorption capacity decreased as the amount of adsorbent GACA increased because more active sites were available for the adsorption of dye, which resulted in more interactions between dye and adsorbent thus increasing the MB percentage removal. At mass 0.5 g the highest percentage removal of 99.8% was achieved.

3.8.3 Effect of contact time

The effect of contact time on the grafted pine magnetite composite with acrylamide for the adsorption of methylene blue is shown in **Figure 8**. The adsorption experiment was done at 100 mg/L concentration. The adsorption rate of the grafted composite on the removal of MB is faster from the beginning which might be influenced by the grafted composite with higher specific gravity which makes them better in dispersity and more efficient contact with MB. The adsorption capacity of the grafted composite is higher due to its high surface area.

3.9 Adsorption isotherms

The adsorption isotherm explains the relationship between an adsorbate in the liquid phase and the adsorbate adsorbed on the surface of the adsorbent at

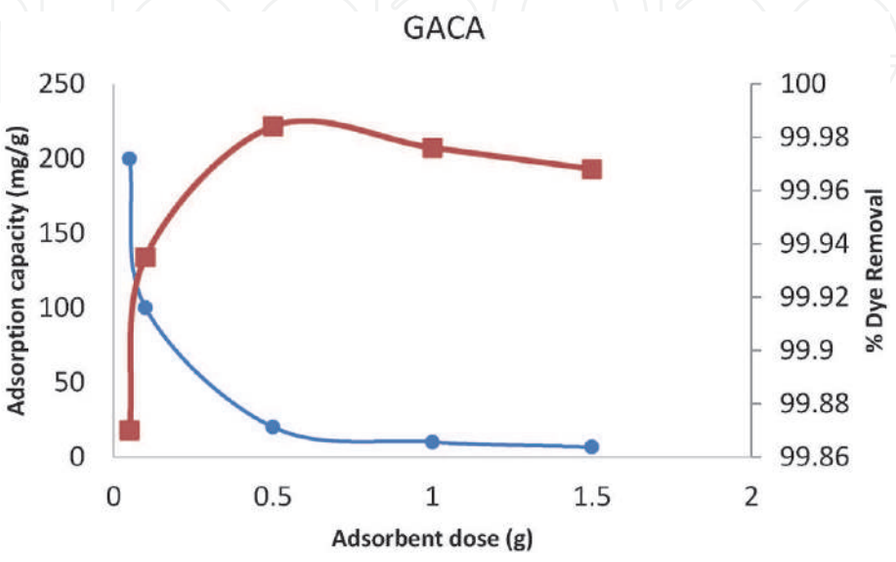


Figure 7.
Effect of adsorbent dose on the adsorption of MB.

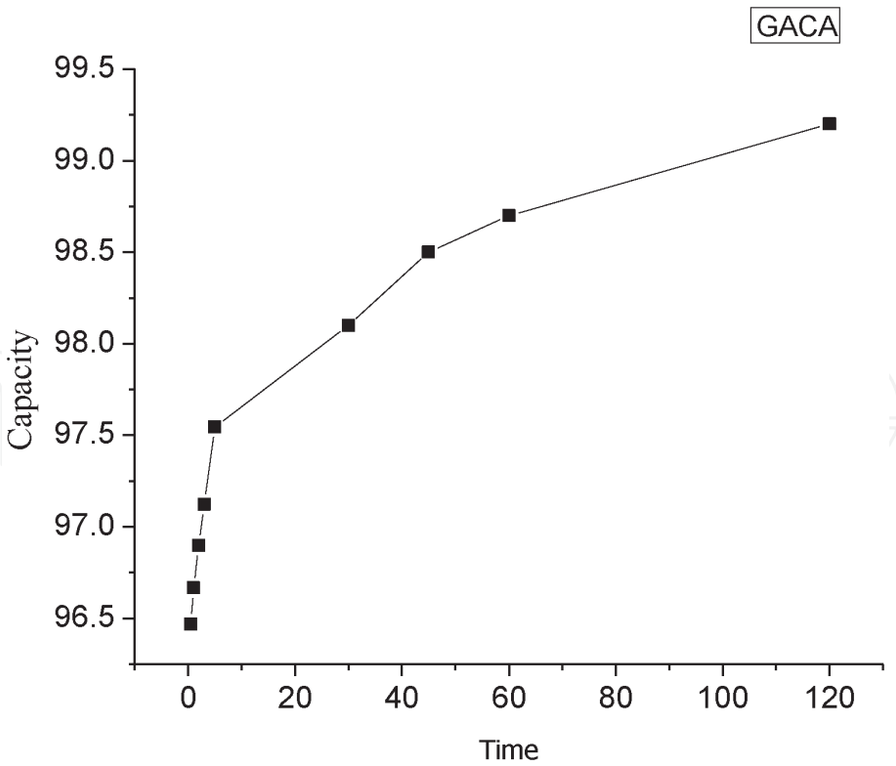


Figure 8.
Effect of contact time at 100 mg/L on the MB adsorption of the GACA.

equilibrium at constant temperature [24, 25]. To successfully obtain the adsorptive behaviour of any substance from the liquid to the solid phase, it is important to have a satisfactory description of the equilibrium state between two phases composing the adsorption system. Langmuir and Freundlich isotherms are the well-known isotherms which have been used to describe the equilibrium of adsorption systems. Typically, the Langmuir model describes the monolayer sorption on a surface containing a limited number of sites and predicting a homogeneous distribution of sorption energies [25]. Freundlich describe the heterogeneity distribution. The results of the MB concentration dependence study were subjected to analyses by using Langmuir and Freundlich isotherm models.

The theoretical Langmuir isotherm is represented by the following equation:

$$C_e/q_e = 1/q_m K_L + C_e/q_m \tag{1}$$

where q_e is the amount of dye adsorbed at the equilibrium time (mg/g), C_e is the equilibrium dye concentration (dm^3/mg), q_m is the maximum adsorption capacity (mg/g) and K_L is the Langmuir adsorption equilibrium constant (dm^3/mg). Freundlich linear expression was represented by:

$$\text{Log}\, q_e = \log\, K_F + 1/n\, \log\, C_e \tag{2}$$

where K_F is the equilibrium adsorption coefficient (dm^3/mg) and $1/n$ is an empirical constant. The parameters of the isotherm models are calculated from the experimental data and the values of correlation coefficient (R^2) are demonstrated in **Table 2**. The results show R^2 values for the Langmuir are higher than those of Freundlich isotherm model. This implies that the equilibrium adsorption data comply with the Langmuir isotherm, suggesting that the adsorption process occurs in a homogeneous surface. Also, it can be stated that the results demonstrate no interaction and transmigration of dyes in the plane of the neighboring surface [26].

	Langmuir isotherm model			Freundlich isotherm model		
Temperature (K)	Q_m (mg/m)	K_L (dm^3/mg)	R^2	K_f (mg/g) (dm^3/mg)	N	R^2
299	57.47	0.2107	0.9957	17.7174	2.862	0.9819
304	82.64	0.0588	0.9745	7.66655	1.725	0.9635
309	78.74	0.0968	0.9825	10.7226	1.873	0.9691
314	68.03	0.1786	0.9851	15.01067	2.293	0.9097
319	67.11	0.2569	0.9925	18.02603	2.455	0.8879

Table 2.
Isotherm parameters for methylene blue dye adsorption on GACA.

Higher K_f value for GACA indicates a higher adsorption capacity for methylene blue and a value of $n > 1$ indicates favorable adsorption conditions [27, 28].

3.10 Desorption and regeneration

The main goal of desorption studies is the competitiveness of adsorbents reusability in the multiple adsorption or desorption cycles and their beneficial potential in practical and economical applications. Desorption studies were performed with 0.01 M, 0.05 M and 0.1 M HCl. Typically, 1 g of PMC saturated with 100 mg/L of MB was placed in different desorption solutions and constantly stirred in a water bath at 200 rpm for 2 h. The adsorbent solutions were centrifuged and analysed using UV-VIS spectrophotometer. **Figure 9(a)** demonstrates the effect of eluent concentrations on MB dye desorption efficiency. It was observed that desorption efficiency increased with increase in the eluent concentration even though the shift is small in percentage. The maximum desorption percentage was found at 0.1 M HCl (99.8%) whereby 0.01 M HCl showed the minimum desorption efficiency (98.8%). An increase in HCl concentration resulted in an increase in H^+ ions concentration which led to a subsequent increase in dye desorption efficiency.

Regeneration shows the competitiveness of the adsorbent where it expresses the good reusability and recycling abilities. **Figure 9(b)** demonstrates the possibility of regeneration and reusability of the grafted pine magnetite composite with acrylamide. Adsorption-desorption reaction cycles were repeated 4 times using 0.1 M HCl

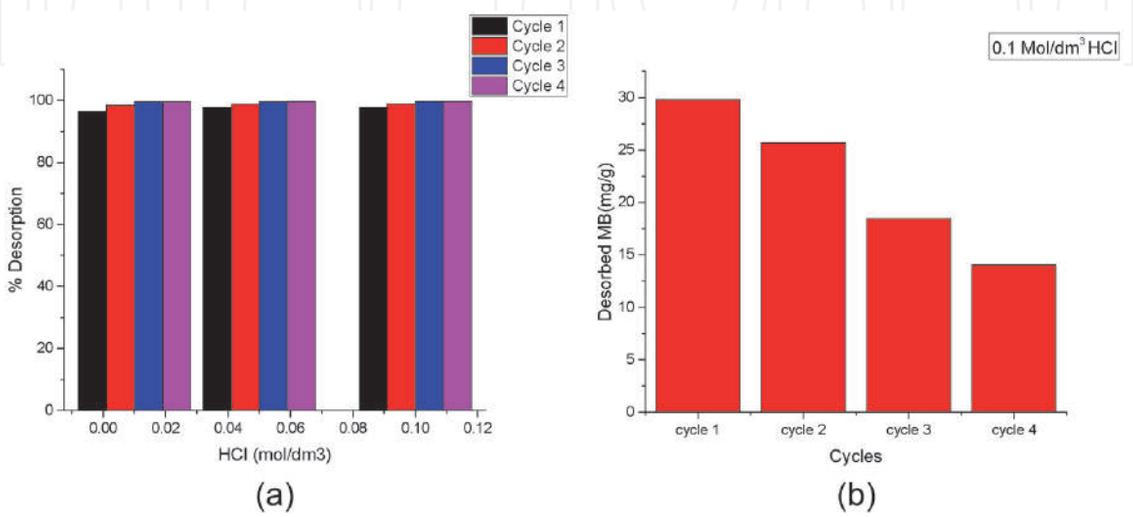


Figure 9.
(a) Desorption characteristics of MB adsorbed and (b) regeneration characteristics of MB adsorbed.

as the desorbing agent. Observation showed that there was a gradual reduction from 29.8% to 14.03% after cycle 4. The results explain that the higher adsorption capacity proves the adsorbent to be a good adsorbent for the removal of MB.

3.11 Conclusions

The study showed that acrylamide was successfully grafted onto pine magnetite composites. FT-IR, BET, SEM, TEM and XRD characterization provided sufficient evidence to demonstrate the incorporation and distribution of the iron oxide nanoparticles within the polymer matrix. GACA nanocomposites were shown to be effective in the adsorption of methylene blue at a pH of 12. The role of adsorbent dose and contact time demonstrated excellent results in the adsorption of methylene blue due to the increased surface area and high rate of the adsorption were achieved. The adsorption data was adequately interpreted by Langmuir and Freundlich isotherm models respectively. It was found that Langmuir isotherm model gave the best equilibrium fit.

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Chapter

Wastewater Treatment Using Imprinted Polymeric Adsorbents

Burcu Okutucu

Abstract

In twenty-first century, numbers of synthetic dyes are used in many industries, for example paper, textile, cosmetic, leather for coloring, vs. The dyeing industries wastes is the most found contaminant to be recognized in wastewater. There are various treatment methods including oxidation processes, biological degradation, membrane filtration and coagulation/flocculation have been studied to treat dyeing wastewater. Unfortunately, these methods are high operational costs, complicated operations and possibility of producing more toxic products. Molecularly imprinted polymers (MIPs) are interesting and alternative polymeric adsorbents that can be applied in wastewater treatment for sample preparation and for the quantification of dyes present in wastewater. Molecular imprinting is a process in which functional and crosslinking monomers are co-polymerized in the presence of the target analyte, the imprint molecule. Initially, the functional monomer forms a complex and, after polymerization, their functional groups are held by the highly crosslinking polymeric structure. Upon leaching of the imprint molecule from the polymer matrix, a polymer with binding sites complementary in size and shape to the imprint molecule is created. MIPs can function under extreme conditions of pH, temperature and complex environment. Also, MIPs present wide recognition due to their stability, ease of production and low-cost potential.

Keywords: molecularly imprinted polymer, dye, solid phase extraction, magnetic molecularly imprinted polymers, adsorbent

1. Introduction

With the growth of human; society, science, technology the world is reaching to new high horizons but the cost which will pay in near future is going to be too high. The bad result of this rapid growth is big environmental pollution problem. One of the biggest risks is the water pollution. The major environmental concern worldwide is water pollution associated with release of many different pollutants [1]. Pollutants released into the environment in wastewater effluent, contaminate freshwater resources and are harmful to humans and the environment. These pollutants were released from many different chemical industries such as textile, dyestuffs, metal plating, paper, mining, fertilizer, battery manufacturing metallurgical, pesticides, fossil fuel, tannery, mining, various plastics. Micropollutants, which are mostly found in wastewater, can be pharmaceuticals, industrial chemicals, pesticides and so on. The textile industries micropollutants released in wastewater and sludge can be dyes, phosphorus, sulfamethazine and heavy metals [2]. The release of these contaminants has been increased because of recent rapid industrialization.

Many industries such as textile, dyestuffs, paper and plastics which are used dyes, use substantial volumes of water in process and also, they generate a considerable amount of colored wastewater [3]. Dye is found a lot amount of pollutant in wastewater. More than 10,000 commercially available dyes are existed and over 7×10^5 of dyes are produced annually throughout the world [4].

Wastewater which is rich by dyestuffs must be treated by many different techniques to eliminate dyes before they are discharged to the ecosystem because of large content of organic pollutants (polycyclic aromatic hydrocarbons, nonphenols, phatales, so on.). The results of many researches showed that about 2% of dyes produced form textile industries are directly discharged in wastewater. Many of these dyes are hazardous and a big threat to aquatic life due to their toxicity and carcinogenicity [3].

Dyes are typically classified in two ways: chemical composition and application class or end use. Based on the application method, dyes can be divided into acid, azoic, basic, direct, disperse, mordant, reactive, sulfur, and vat dyes. According to chemical compositions, dyes can be divided into azo, nitro, nitroso, diarylmethane, triarylmethane, xanthene, anthraquinoid, acridine, cyanine, quinone-imine, pthalocyanine, and thiazole dyes. The azo and anthraquinone dyes are two major classes of reactive dyes, which is released to wastewater as 90% of all organic pollutants. Due to their interaction with hydroxyl ions in the solution, these dyes are always wasted in the dyeing process, remained in the effluents and are hardly eliminated under aerobic conditions. Also, the azo dyes have stability against microbial attack. Reduction of azo bond frequently releases aromatic amines, which resist further degradation under anaerobic condition. The acute toxicity of azo dyes to humans are rather low according to the European Union criteria. The toxicity is generally not because of the dye itself, but because of its degradation products. Azo dyes can be caused contact dermatitis in the local lymph node assays. However azo dyes can be very toxic to several aquatic organisms including algae, fish. Because of presence of azo dyes in water; viability, reproduction rate, filtration feeding and O_2 consumption of the fishes reduced in the freshwater. Azo dyes also inhibit several biological processes, such as they can inhibit algal photosynthesis by reducing the penetration of light, they inhibit chemical oxygen demand (COD) reduction and respiratory activities of microbial populations. Which are ecologically very important. This may result in inhibition of microbial processes like wastewater treatment systems and natural biogeochemical cycles [2, 5, 6].

Over the last years, different physical, chemical and biological techniques have been developed to remove toxic dyes from wastewater and water reservoirs. All kinds of wastewater containing dyes cannot be treated with one technology. The methods for clean up wastewater are adsorption, electrolysis, ozonation, coagulation/flocculation, physical membrane separation, advanced oxidation processes and biological degradation process. Each technology has some merits and demerits. The demerits of many of these techniques are high cost and the formation of hazardous by products. The problems of some techniques can be listed; the quantification of different dyes related to complicated sample preparation procedures, long analysis times, and the use of large quantities of solvents [7]. Coagulation/flocculation usually generates large amounts of toxic sludge difficult to deal with. Physical membrane separation employing nanofiltration (NF) membrane is easy to bring pollution and lead to decreasing removal efficiency Advanced oxidation processes including chemical oxidation, catalytic degradation and electro chemical treatment consume high energy and are still quite costly for practical application [8]. Biological degradation with activated sludge cannot clean up wastewater due to the biodegradable difficulty of many synthetic dyes. The problem of anionic dyes is highly water soluble and difficult to remove by conventional methods. The nonionic

dyes (disperse dyes) do not ionize in an aqueous solution and their fused aromatic ring structure makes them highly resistant to degradation. However, a few cationic dyes like methyl blue can be easily removed by adsorption and advanced oxidation processes [4]. With the realization that a single technique cannot give universal solution, the recent trend in dye wastewater treatment is focused around integration of multiple techniques [7, 9].

Adsorption is a low-cost and effective method for expurgate of dye wastewater. The adsorption process is the best choice for the decolorization of dyes and gives the best results for removal of various types of dissolved coloring materials and also removes the entire dye molecule, leaving no fragments in the effluent. The adsorption has a high treatment efficiency and adsorbents can be regenerated for multiple reuses. The initial dye concentration, solution pH, temperature, contact time and adsorbent dosage are usually the main factors that govern the performance. Generally, removal of dyes from water and wastewater is carried out by adsorption using activated carbon. The disadvantage of activated carbon are expensive regeneration process and the decrease of adsorption capacity after regeneration. The other adsorption material can be nanoparticle adsorbents, low cost waste-based adsorbents and polymeric adsorbents. Polymeric adsorbents have the advantages of high flexibility in design, physical stability, porosity, uniform pore size distribution, high surface area, and chemical stability towards acids and bases, feasible regeneration and thermal durability but they are generally expensive materials [4]. The interesting and urgent research needs for a high-efficiency, low cost, attractive and reusable adsorbent for clean up wastewater [10].

2. Molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers (MIPs) are interesting materials that can be applied in environmental research for sample preparation, to clean up and for the quantification of contaminants present in wastewater. The advantages of MIPs are related to their small size, high surface area, and specific selectivity towards target molecules. Molecular imprinting can be prepared with nanoscale surface recognition sites for target molecules, providing high binding capacities and fast mass transfer rates. MIPs have resistance under extreme conditions of pH, temperature, and complex environment. The preparation route of molecular imprinting is in which functional and crosslinking monomers are co-polymerized in the presence of the target analyte (the imprint molecule). Initially, the functional monomer forms a complex (monomer-target analyte) via covalent or noncovalent interactions and, after polymerization, their functional groups are held by the highly crosslinking polymeric structure. Upon leaching of the imprint molecule from the polymer matrix, a polymer with binding sites complementary in size and shape to the imprint molecule is created. This polymer is now capable of selectively rebinding the imprint molecule [6, 9–11]. In covalent approach; the imprinted molecule is covalently coupled to a polymerizable molecule. The binding of template with monomers are relies on reversible covalent bonds. After copolymerization with crosslinker, the imprint molecule is chemically cleaved from the highly crosslinked polymer with harsh conditions. In non-covalent approach which is usually used for preparing MIP is composed of self-assembly between the template and monomer, followed by a crosslinked co-polymerization. The template molecules interact with monomers (during imprinting procedure and the rebinding) via non-covalent interactions, e.g. ionic, hydrophobic and hydrogen bonding [12].

MIPs have been successfully used as an alternative adsorbent in several analytical fields such as separation of enantiomers in liquid chromatography (LC) or capillary

electrochromatography (CEC), binding assays, sensors and solid-phase extraction (SPE). SPE is the most advanced application area of the MIP. In recent years, the number of papers which the MIP is used for SPE sorbents (MISPE) increased MISPE is used in the areas of environmental, food and pharmaceutical analysis as selective sorbents for the extraction or for the clean-up of different classes of compounds from various complex matrices [13]. Concerning more specifically the determination of dyes in wastewater, there were too many examples that mentioned above with MISPE. The application of MIP particles in chromatography, can be packed in a column between two frits and be used off-line or they can also pack in a small column to be coupled on-line with LC. The principle of the extraction on a MIP is the same classical SPE sorbents. The desorption of the analytes is achieved by percolating a solvent in order to disrupt the interactions between the analytes and the MIP. Nevertheless, there are more and more applications of MIPs directly to real wastewater samples without a preliminary treatment [14]. The importance of the washing step and the difficulty to optimize this step was the most important problem of MIP usage.

The successful preparation of MIPs depends on the choice of monomers, the crosslinkers, and the appropriate polymerization conditions. The structure and the functionalities of the template molecule-monomer interaction define the subsequent properties of the binding sites. The criteria to consider when selecting a candidate template molecule are its cost, its availability and its chemical functionalities defining which is the ability to strongly interact with monomers. In non-covalent imprinting, the interactions involved are weak; and the excess amount of template should be used. The main factor which is important for MIP process is choosing the best monomer. The role of the monomer is to provide functional groups which can form a complex with the template by covalent or non-covalent interactions. The strength of the interactions between template and monomer affects the affinity of MIPs and determines the accuracy and selectivity of recognition sites [13]. Many techniques are used to select best suitable monomer. The rational design (computer simulation) and analytical techniques includes nuclear magnetic resonance, UV-vis, Fourier-transform infrared spectroscopy and isothermal titration calorimetry have been studied [15].

The common monomers, which are used for molecular imprinting, are methacrylic acid (MAA), acrylic acid (AA), 2- or 4-vinylpyridine (2- or 4-VP), acrylamide, trifluoromethacrylic acid, 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate, acrylic acid, 2-acrylamido-2-methyl-1-propanesulfonic acid, 4-vinylbenzene boronic acid, 1-vinyl imidazole, allylamine, itaconic acid, urocanic ethyl ester, methacrylamide, acrylonitrile and styrene. MAA named as a "universal" functional monomer due to its unique characteristics, being capable to act as a hydrogen-bond donor and acceptor and showing good suitability for ionic interactions. The monomer is chosen according to functional groups of template molecule. The attempts for finding new functional monomers for synthesis of MIPs continued. Because of their special structures, β-cyclodextrins (β-CDs) can be interesting monomers. The β-CDs are composed of cyclic oligosaccharides with a hydrophilic exterior and a hydrophobic cavity. The molar ratio between template and monomer are important because this ratio affect the affinity and imprinting efficiency of MIPs. The lower molar ratios mean less binding sites in polymers. The over-high ratios mean higher non-specific binding capacity and decrease the binding selectivity. For best imprinting efficiency, the molar ratio of templates to monomers have to be optimized [14, 15].

The role of the cross-linker in imprinted polymer is to organize the functional groups of functional monomers around imprinted molecules, and to form highly rigid polymer The common cross-linkers which is used ethylene glycol

dimethacrylate (EGDMA), trimethylolpropane trimethacrylate (TRIM), N,N-methylene bisacrylamide (MBAA) and divinylbenzene (DVB), pentaerythritol triacrylate, 1,4-diacryloyl piperazine [15]. Types and amounts of cross-linkers effects the selectivity and binding capacity of MIPs. If the amount of cross-linkers is too low, MIPs cannot maintain stable cavity configuration. The over-high amounts of cross-linkers will reduce the number of recognition sites.

The solvent is one of the other most important factors determining effective molecular recognition. Because the accuracy of the assembly between the template and the monomer is related to the physical and chemical characteristics of the solvent. The solvent, which is used while preparing MIPs, named as porogen. The porogen acts as not only a solvent but also cavity maker. Aprotic/low polar organic solvents (toluene, acetonitrile, chloroform) are often used in non-covalent polymerization processes in order to obtain good imprinting efficiency. Nevertheless, the MIPs prepared in organic solvent cannot be compatible with aqueous media usage. A few studies reported the use of polar/protic media (methanol, ethanol, water) for the synthesis of MIPs. The development of water-compatible MIPs is an interesting area are going to study [16].

Actually, MIPs are synthesized by free radical polymerization, generated by the thermal or photo homolysis of a chemical bond on an initiator. The most widely used initiators for MIPs synthesis are benzoyl peroxide (BPO), 2,2-dimethoxy-2-phenylacetophenone (DMPA) and 2-azobis(2-methylpropionitrile) (AIBN) and 2,20-azo-bis(2,4-dimethyl) valeronitrile (ABDV) [17].

3. Synthesis methods of MIPs

The synthesis method of MIPs can be a different type. Bulk, suspension, emulsion, precipitation, multi-step swelling, and surface imprinting were the methods which were summarized. Bulk polymerization is the common method to prepare MIPs. The bulk polymerization is rapid, simple preparation, needs no sophisticated or expensive instrumentation. But after polymerization of the bulk polymer, it has to be crushed, ground and sieved to an appropriate size. After grinding, the polymer was in irregular particles in shape and size, and also some high affinity binding sites are destroyed. Bulk polymers have a heterogeneous binding site distribution which is limited the use of MIPs in chromatography. The other techniques of preparing imprinting polymers are suspension polymerization, emulsion polymerization, seed polymerization, precipitation polymerization and surface imprinting. The more homogeneous binding site distribution can be taken by these techniques. The MIPs which is prepared by conventional suspension polymerization, where water is used as a continuous phase to form a droplet of pre-polymerization mixtures in the presence of a stabilizer or surfactant. However, the MIPs prepared by suspension polymerization is polydisperse in size (a few to a few hundred micrometers) and displayed poor recognition. The liquid perfluorocarbon or mineral oil (liquid paraffin) can be used while preparing MIPs by suspension polymerization technique to prevent poor recognition. But, liquid perfluorocarbons immiscible with almost all organic solvents (chloroform, dichloromethane, toluene), monomers and cross-linkers. The emulsion polymerization is as an effective method to produce monodispersed polymeric particles. The disadvantage of emulsion polymerization was the presence of remnants of surfactant in polymerization media. Precipitation polymerization have some advantages in synthesizing spherical particles such as free of surfactant, in one single preparative step and with excellent control over the particle size. The seed polymerization, a typical multi-step swelling and polymerization, produced monodispersed

MIPs. In this technique, the use of water was weakened recognition. In addition, the multistep procedure is very time-consuming. Surface imprinting is another technique nowadays using MIPs mostly synthesized. The biggest problem of MIP was bleaching of template molecule due to the high cross-linking nature of MIPs, which will result in incomplete template removal, and slow mass transfer. Fortunately, this problem can be resolved by surface imprinting, in which the imprinted templates are situated at the surface of the material's surface. Compared to traditional MIPs, surface imprinted polymers possess not only higher binding capacity but also faster mass transfer and binding kinetics. Many particles have been used for the surface imprinting process, such as activated silica, Fe_3O_4 (magnetic) nanoparticles, chitosan, activated polystyrene beads, quantum dots (QDs) and alumina membranes. The magnetic separation is an effective technique for separation of complicated samples. The magnetic nanoparticles have an advantage of its fast recovery, high efficiency, low cost, and direct purification from a mixture without any pretreatment. In recent years, magnetic MIPs have become a hotspot based on the significant advantages of magnetic separation over conventional methods. Generally, preparation of MIPs-coated magnetic nanoparticles (MNPs) involves three steps: (1) preparation of Fe_3O_4 MNPs; (2) surface modification of Fe_3O_4 MNP with TEOS, oleic acid, ethylene glycol or poly (vinyl alcohol) and (3) synthesis of surface imprinted MNPs using a sol–gel process or free radical polymerization [16–18].

4. Examples of MIPs applied for wastewater treatment

Till 1980s, according to MIP database number of MIP paper was published. Eighty percent of these papers were the usage of MIPs as SPE adsorbent. By the usage of nanoparticles for MIPs, the new papers were about the surface imprinting and also to use them in SPE. The some examples were summarized above. The most attractive and useful examples were chosen.

First example was the paper of Deng et al. [8]. Deng et al. was studied Ti(IV) functionalized chitosan molecularly imprinted polymer (Ti-CSMIP). While preparing chitosan imprinted polymer he used Ti^{4+} as Lewis acidic for producing metal hydroxyl group and protonated surface of MIP. He used Ti(IV) functionalized chitosan molecularly imprinted polymer to recognize reactive brilliant red (X-3B) in aqueous solution. The dye behaved as a Lewis base. The MIP was characterized by FTIR, SEM, XRD, BET, elemental and zeta potential analysis. Batch adsorption experiments (sorption isotherm, kinetics) and reusability were performed to evaluate adsorption condition. Regeneration experiments indicated that Ti-CSMIP was an effective sorbent for the selective removal of azo anionic dye in aqueous solutions. The difference of the study was; the Ti-CSMIP used as adsorption and also recognition. By using Ti^{4+} as a Lewis acid, the recognition problem of dye, which is limited due to its macromolecular structure, was overcoming. The Ti(IV) functionalized chitosan MIP was recognized dye macromolecules as template, because X-3B was behaved as a Lewis base. In this study, the first step is preparing Tİ-CSMIP. First of all, chitosan was dissolved and mixed with $Ti(SO_4)_2$ Then $Ti(SO_4)_2$/CS mixture was cross-linked with KH-560. After gelation, the product was treated by microwave for 20 min and the obtained product was washed with distilled water and dried. The product was added into X-3B solution after stirring the product was ready. The X-3B was removed using NaOH and HCl, respectively. Non-imprinted polymer (Ti-CSNIP) was prepared with the same procedure in the absence of the template molecule. Experiment of pH effect indicated X-3B sorption on Ti-CSMIP

was dependent on solution. The Temkin and Sips models was used as equilibrium model recycling experiments demonstrated that Ti-CSMIP had a greater potential even after several cycles of regeneration.

The second example was magnetic MIPs which was studied by Luo et al. [19]. He was studied magnetic and hydrophilic molecularly imprinted polymers (mag-MIPs) which were prepared by an inverse emulsion–suspension polymerization technique to remove water-soluble acid dyes from contaminated water. The attractive point of study was using 1-(1-methyl acrylate)-3-methylimidazolium bromide (1-MA-3-MI-Br) as a new functional monomer. The thermal stability, chemical structure and magnetic property of the 1-MA-3-MI-Br-mag-MIPs were characterized by the thermal-gravimetric analyzer (TGA), Fourier transform infrared spectrometer (FT-IR) and vibrating sample magnetometer (VSM), respectively. The first step was preparing novel magnetic nanoparticles and modifying surface by PEG. The final brown suspension was placed for 24 h quiescence, and the supernatant was discarded. The second step was preparing inverse emulsion. According to the method; 2 mL toluene with 0.1 g AIBN was mixed in 50 mL beaker. When AIBN was dissolved, 10 mL TRIM and a drop of Span 80 were added in the mixture and stirred to be uniform. Then 10 mL Fe_3O_4 magnetic fluid was added in the mixture. The mixture was stirred for 5 min, and then the mixture was submerged in the ultrasonic bath for 5-min. Finally, the inverse emulsion can be obtained. The third step was preparing mag-MIPs and magnetic non-imprinted polymers (mag-NIPs) were prepared via inverse emulsion–suspension polymerization. The procedure was summarized, 1 mmol tartrazine and 4 mmol 1-MA-3MI-Br were dissolved and prepolymerized for 30 min and mixed with inverse emulsion. Thirdly, the reaction mixture was heated and purged by N_2 to remove the oxygen. The reaction temperature was at 70°C for 12 h. After the reaction completed, the resulting mag-MIPs with uneluted molecule were filtered by 120 mesh sieve and washed by methanol and 60°C water. Finally, the products were washed by methanol:ammonia solution (9:1, v/v) for 24 h. The mag-NIPs were prepared by the same manner in the absence of template molecules. The several advantages of the water-compatible 1-MA-3-MI-Br-mag-MIPs to remove and recycle the water-soluble acid dyes in water media could be existed. Firstly, the removal efficiency towards water-soluble acid dyes is very high with all above 95% in wastewater. Secondly, the 1-MA-3-MI-Br-mag-MIPs can be reused at least five times without obvious decrease in the removal efficiency. Thirdly, due to the encapsulated Fe_3O_4, the 1-MA-3-MI-Br-mag-MIPs can be easily separated by external magnetic field.

The third example was the combination of MIP with biosensor [20]. Khan et al. was studied a biomimetic sensor which was prepared on carbon paste with magnetic molecularly imprinted polymer (mag-MIP) for sensitive and selective detection of methyl green dye. The mag-MIP was synthesized using a functional monomer that was selected before by computational simulation. The findings showed that imprinted biosensor can be suitable for real samples. The mag-MIP was synthesized by the process of polymerization in the existence of methyl green and the functional monomer acrylamide were dissolved in ethanol The reaction mixture was agitated in a water bath at 25°C for 12 h, followed by addition of Fe_3O_4@ SiO_2—C═C and shaking for a further 3 h. In the next step, EGDMA and AIBN were put to the system to the mixture that was further sonicated in a water bath for 5 min. The reaction mixture was thermal polymerized at 60°C for 24 h. The analyte molecule was then removed by Soxhlet extraction apparatus via methanol:acetic acid (9:1, v/v) washing solution. The product obtained (mag-MIP) was dried out at 60°C in vacuum. The magnetic non-molecularly imprinted polymer (mag-MIP) was prepared under the same conditions, but without the presence of the analyte.

This polymer showed higher binding capacity for methyl green, compared to the corresponding non-imprinted polymer. Electrochemical sensors using the mag-MIP produced by this method have some advantages that it was offering mechanical stability in solution and providing satisfactory performance of sensor in terms of sensitivity and selectivity.

The fourth example was different example from the others [7]. In this study, Yu et al. is studied selective, sensitive and reliable magnetic molecularly imprinted material to enrich and separate aromatic amines from azo dyes. The 4,4′-methylenebis(2-chloroaniline) (MOCA) was used as template molecule. The synthesis of magnetic molecularly imprinted polymer was performed on the surfaces of the magnetic silica gel spheres via thermal polymerization as mentioned paper, detailly. The steps can be summarized, $Fe_3O_4@SiO_2$ was prepared and washed sequentially with both ethanol and toluene. The second step was the surface of $Fe_3O_4@SiO_2$ was grafted with vinyl by using VETS and triethylamine. The final product of $Fe_3O_4@SiO_2@-CH=CH_2$ was separated and then dispersed in toluene. The synthesis of magnetic molecularly imprinted nanoparticles was done with EGDMA and 2-VP were used as the cross-linker and functional monomer, respectively. A template molecule of MOCA and 2-VP were dispersed in of toluene. Then, particles $Fe_3O_4@SiO_2@-CH=CH_2$, EDGMA and AIBN mixed with the MOCA+2-VP solution and purged with nitrogen. The thermal polymerization was performed with changing temperature. The non-imprinted particles were synthesized with the same procedures in the absence of MOCA. A solution of methanol and acetic acid (9:1, v/v) was used in a Soxhlet to remove the template molecule. The physical properties of mag-MIPs and NIPs were characterized, and the adsorption isotherms were studied. The adsorption process was described by a pseudo-second order model and the equilibrium data fitted well to a Freundlich equation. The other advantages of these magnetic molecularly imprinted polymers were dye removing from wastewaters effectively, and also discriminating carcinogenic aromatic amines which were the main structure of textile dyes.

The fifth example was one of real sample application [21]. Foguel et al. is studied MIPs for the Acid Green 16 (AG16) textile dye and the used this MIP for rebinding, selectivity and application of in wastewater samples. MIP synthesis was performed using AG16 dye (template), 1-vinylimidazole (functional monomer), ethyleneglycol-dimethacrylate (cross-linker), 2,2′-azobis(2-methylpropionitrile) (initiator) and methanol (solvent) by bulk polymer synthesis. The imprinted polymer presented excellent rebinding of 83%, an imprinted factor of 6.91 and great selectivity in comparison with other textile dyes. Additionally, the MIP showed high efficiency in the extraction of this dye in wastewater and have a better performance when compared to commercial SPE cartridges. In this study the selectivity of the MIP for AG16 was evaluated using four dyes, commonly used in the textile industry, with different structures and chromophore groups: Direct Yellow 50 (DY50), Acid Red 1 (AR1), Basic Red 9 (BR9) and Methyl Green (MG). MIP for AG16 is quite selective compared to the dyes DY50, AR1, MG and BR9, since approximately 86% of AG16 was bound to the MIP, while the binding percentage for these other four dyes was between 4% and 11%. The MIP proposed in this work showed great efficiency in the determination of the AG16 dye, since the synthesized MIP presented good rebinding of the analyte to the selective cavities of the MIP, high selectivity compared to other textile dyes and efficiency in the extraction of the compound of interest, when applied in a sample of textile wastewater.

The sixth example was about removal and also adsorption of dye on MIPs [22]. Okutucu et al. is studied a molecularly imprinted polymer (MIP) which textile dye (Direct Red 23) was used as template for decolorization of textile wastewater and also used for leaching of this dye from the wastewater by adsorption of onto

polymer. Acrylamide was used as a monomer and dimethyl sulfoxide was used as a porogen. The ethylene-glycol-dimethacrylate (cross-link), 2,2′-azobis(2-methylpropionitrile) (initiator) was used while preparing bulk polymer by thermal synthesis. The template was removed by methanol:conc. HCl washings. The selective recognition ability of the MIP was studied by an equilibrium-adsorption batch method. The effective adsorption properties of the polymer were tested in synthetic dye wastewater. The high adsorption rate and the amount of imprinted dye that was removed from the polymer was nearly 65%. Eighty percent of the dye was adsorbed by imprinted polymer in synthetic wastewater. The goal of this study is to prepare dye-imprinted polymer to research the molecular recognition characteristics, so that to get valuable results for contamination of dyes in wastewater. It is also important and valuable to recognize dye molecules and their removal from wastewater for the protection of the environment. The dye-imprinted polymer can also be used as a solid-phase adsorbent for Direct Red 23 dye to detect whether it was present in wastewater. Adsorption of dye molecules onto a sorbent can be an effective, low-cost method of decolorization of textile wastewater. Most of the techniques used for this aim were the high cost of production and the regeneration also makes them uneconomical. Molecular imprinting polymers are a new kind of materials which can be economical and effective adsorbents. The removing effect of MIP was seen at **Figure 1**.

The seventh example was the example of using β-cyclodextrin as a monomer for MIP synthesizing [23]. In this study, Hu et al. were synthesized a magnetic β-cyclodextrin polymer (MNP-CM-CDP) which could be used in aqueous media. Kinetic isotherms and a dye adsorption method provided Langmuir. By using MNP-CM-CDP model pollutants (BPA, MB, BO_2, RhB, Cr(III), Pb(II), Zn(II), and Cu(II)) were rapidly and efficiently removed from the aqueous solution. Because of magnetic character, the polymer could be easily separated from the solution under an external magnetic field. The synthesis of the magnetic β-cyclodextrin polymer was done at three steps, with TFTPN used as rigid crosslinker, EPI used as flexible crosslinker, chloroacetic used as carboxymethyl agent, Fe_3O_4 used as magnetic matrix and deionized water used as solvent. First step was, β-TFTPN was dissolved in EPI and then added dropwise to cyclodextrin in NaOH solution. After magnetic stirring for 3 h at 100°C, the solution was filtered. The precipitate was washed with water and THF, and then dried. The second step was synthesis of the CME-CDP. The T-E-CDP was dispersed into NaOH and chloroacetic acid was added. After magnetic stirring, the solution was cooled and neutralized with hydrochloric acid. The CME-CDP was obtained. Third step was synthesis of the MNP-CM-CDP. The magnetic β-cyclodextrin polymer (MNP-CM-CDP) was synthesized by one-step coprecipitation. Fe solutions and the CME-CDP were mixed,

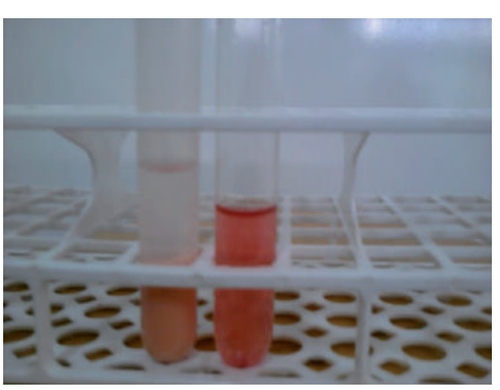

Figure 1.
The removal of Direct Red 23 with MIP [12].

then $NH_3 \cdot H_2O$ (25%, 5 mL) was added dropwise under nitrogen. The precipitate was washed with deionized water five times and then dried. The adsorption of dyes and metal ions is mainly dependent on the pH and this condition was also studied and optimized. The polymerization was performed by green synthesis route, due to use β-cyclodextrin good adsorption performance, easy regeneration, and simple operation. The results indicated that the MNP-CM-CDP is a promising adsorbent in commerce for purifying dyeing wastewater and can be produced on a large scale in industry.

5. Conclusion

Saving water to save the planet and to make the future of human was the most important aim. There are many materials existed that can be used. These are activated carbon, clay minerals, zeolites and biomaterials. The main purpose of all these materials was adsorption of wastes in water. Such as zeolites are crystalline substances with pores which was permitted only the passage of certain size. Zeolites can occur naturally or synthesized in laboratory. They are mainly constituted by Al, O and some metals. They are mostly negatively charged, and their surface can be modified. So, the surface can be treated with multiple classes of contaminants. There are many studies about dye adsorption with different zeolites in wastewater [24, 25]. The zeolites only can remove dyes by adsorption on their surface, but MIPs can ability of recognition, quantification and adsorption. All of these excellent characteristics make MIPs good alternative adsorbents for wastewater treatment. The ease of preparation, resistance to hard conditions (pH, temperature, storage stability, vs.), easily to study with real complex examples (directly wastewater) make MIPs more suitable for different samples. In this chapter, the characteristic components of MIP, synthesizing methods and some examples of treatment of wastewater by MIPs were summarized.

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Chapter

Considerations Regarding the Research for the Conservation of Heritage Textiles in Romania

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Abstract

Textiles are valuable elements that make up Romania's cultural heritage, being unique through the production techniques, materials used and their significance for the Romanian population. Heritage textiles represent bridges between past and present, kept in collections from different types of buildings. Many of them are preserved and exposed in heritage buildings that are open for public viewing and do not benefit from internal microclimate monitoring systems. These things can have serious repercussions on the integrity and conservation status of these fragile materials. The chapter proposes to analyze the approaches used in different studies to evaluate the risks to which the historical textile collections from Romania are exposed, depending on the place and the way in which they are kept. All these approaches aim to determine the degree of conservation of the materials and their implications on the health of the people with whom they come into contact. Based on the methodology applied in the studies already published, examined in the first part of the chapter, in the second part, a case study was performed on a different sample of historical textiles from Romania. This comes to complete the sphere of knowledge in the field.

Keywords: textiles, heritage buildings, historical textiles, methodological approaches, microclimate, protection, conservation

1. Introduction

The textile materials represented a major coordinated on the evolution of human society both temporally and spatially. The objects made of textile materials have, over time, experienced a great typological, functional and structural diversity, in close relation with the needs of the human society at a certain stage regarding the technological progress achieved and means of processing the textile materials. Therefore, textile objects are part of the cultural heritage, with roles and functions in asserting the specific identity of the place [1–4]. The specificity and identity of the objects made out of textile are derived from the genetic, evolutionary and qualitative features that they incorporate (occupations, customs, technological level achieved, beliefs, superstitions, etc.) [5].

The mutations that have occurred lately against the backdrop of globalization call for sustained efforts to conserve these elements of cultural heritage and local identity. The necessity of their conservation has required the carrying out of numerous specialized studies that have highlighted the anthropogenic and environmental degradation factors [1, 6–8] and their effects on textile, yellowing, decreased elasticity and tear resistance, microflora development [9–15], as well as the necessary measures to prevent their degradation and to conserve them [16–19].

Considering that one of the most objective and important factors with direct effects on the degradation of textile is "time itself", a solution may represent digitization, constituting an informational bridge between past, present and future [20–23]. During the past decades, digitization has become one of the main concerns of cultural institutions and governments all over the world [22] as there is a constant need for preserving cultural heritage in a digital form as well. From a process that transforms a physical object into a digital one [21], digitization became an art itself involving various specialists from different domains having the same aim—preservation. But, still the final goal of digitization is not only to turn a material object into bytes, but making it accessible for everyone interested in it, in an online environment [24] as culture is considered a basic human right [25]. Thus, through digitization the past is connected to the future as the digital world may seem a better option for preserving and conservation of the cultural heritage resources. We are living in the "Digitization Age" and our society understood that cultural heritage belongs to the mass and plays an essential role not only for the social and economic development of the present society but for the future one as well.

From all the cultural resources, maybe the most sensitive in preserving are textile [3] as requires certain storage conditions. Temperature, humidity, light exposure and the mycological content in the composition of the materials are few parameters to be analyzed in order to assure proper storage conditions. In this context, digitization seems a better solution for preservation and promotion, their life span will be undeniable higher although it will be in the cyberspace not in the real world.

Among tests, an analysis that can be performed on textile materials for their digitization, the SEM analysis and digital radiography are highlighted practices whose results have a high degree of plausibility.

The scanning electron microscope (SEM) allows three-dimensional views of external morphology of the fibers, in order to determine their level of conservation [26]. SEM is used to identify fibers and to assess the level and type of fiber wear, degradation or structural alteration [27–29]. The environmental scanning electron microscope (ESEM) can be used to "characterise the surface, interface and dynamic properties of textile materials" [30].

X-radiography and digital radiography uses non-destructive techniques very useful in the study of textile materials, documentation and interpretation in order to conserve valuable objects from cultural heritage [31–33]. The limitation of this method is inaccessibility on a large scale due to the high costs. Another technique for studying old textile materials is the one proposed by Ahmed et al. [34]—laser induced breakdown spectroscopy (LIBS), which also provides chemical information; also for the determination of the chemical composition of the colors used, we mention the use of X-ray fluorescent spectrometry (Spectroscan Max G, Spectron), with the type of spectrometer being wavelength dispersive [35].

Mannes et al. [36] propose a non-invasive approach for the analysis of fragile materials of cultural heritage, neutron and X-ray imaging. Valuable information about the old manufacturing techniques used in the creation of textile but also about the possible treatments for cleaning and preserving them for a longer period of time can be obtained using all the techniques and X-ray spectroscopy

methods: scanning electron microscopy with energy dispersive X-ray spectroscopy (SEMEDX), X-ray fluorescence spectroscopy (XRF), particle induced X-ray spectroscopy (PIXE) and also Rutherford backscattering spectroscopy (RBS) [37].

A simple technique that could be encountered for analyzing the colors of the cultural heritage objects is colorimetry (CIELab color system). This technique might be useful when a change in color is desired to be quantified when varying the parameters of the microclimate [38]. Moreover, colorimetry could be a suitable method to determine the change in color when a preservation treatment is tested on a heritage object [39], as the modification of its main features should be avoided.

The color of a cultural heritage object is given by pigments or dyes. These substances can be evaluated from the compositional point of view by using infrared spectroscopy (Fourier Transform infrared spectroscopy). Usually, this technique requires the use of a potassium bromide pellet, which is a time-consuming and destructive operation [40]. Fortunately, a new version of this technique, called attenuated total reflectance FT-IR, was developed, having the main advantage of its non-destructive fashion, as it allows the measurements to be performed without any previous preparation [41]. ATR-FTIR is a valuable technique when analyzing dyes and pigments because it can offer information about the molecular fragments of this substances [42, 43]. In this way, it is possible to determine what type of dye or pigment has been used in the original process of painting. This information could be very helpful when choosing the restoration materials.

But without a doubt, the best way to conserve historical textiles is to keep them in an interior environment that is less harmful for them. The great diversity of the buildings in which they are exposed (museums, traditional constructions, private collections, etc.) determines a great variety of indoor microclimate conditions, which could have repercussions on the degree of conservation of textile collections. Thus, of a major importance is both the determination of the state of conservation of the textiles, and the establishment of the influence that the microclimate from the different storage spaces exerts on them.

In this context, the present study aims to highlight some methodological aspects and the results obtained following their implementation in a series of researches carried out on three structural elements defining for the Romanian society, in which different collections of textiles are kept: wooden church "Saint Martyrs Constantin Brancoveanu and his sons", The Museum House from Sălacea and National Archives of Romania—Bihor County Service from Oradea Municipality (**Table 1**). About the three locations where the research was carried out, we can say that they are representative regarding the Romanian rural communities, offering different environment conditions, including the anthropic impact with implications in manifesting in a form or another the negative effects of the textile objects hosted. Nevertheless, in order to diversify and deepening the obtained results by the research team from University of Oradea and collaborators, in the last part of this chapter a new case study regarding a traditional women's shirt over 100 years old from a private collection, has been conducted.

2. Case studies

This paper aims to analyze from the point of view of approach, methodology and research object, three case studies already carries out regarding the current state and the conservation conditions of some textile collections from three buildings in Bihor County, Romania (**Figure 1**) serving different purposes. These three collections have been extensively examined in numerous specialized papers, some being already published and some being in print.

No.	Location of study	*Title of the studies*—Status (journal, volume, issue, page)—Year
1	Wooden church *"Saint Martyrs Constantin Brancoveanu and his sons"*	*Investigations on air quality in the historic wooden church in Oradea city, Romania*—Published (Environmental Engineering and Management Journal, 17, 11, 2731–2739)—2018
2		*Study on microbial and fungal contamination of air and wooden surfaces inside of a historical Church from Romania*—Published (Journal of Environmental Biology, 39, 6, 980–984)—2018
3		*Indoor air quality assessment and its perception. Case study—historic wooden church, Romania*—In print (Romanian Biotechnological Letters)—2020
4		*Exploring the Indoor Environment of Heritage Buildings and its Role in the Conservation of Valuable Objects*—Published (Environmental Engineering and Management Journal, 18, 12, 2579–2586)—2019
5		*Preserving textile objects in Romanian Wooden Churches. Case study of the heritage wooden church from Oradea*—In print (Industria Textila Journal, 2)—2020
6		*Spectrometry Study of Heritage Objects for the Digitisation of Cultural Heritage—In print (Environmental Engineering and Management Journal)—2020*
7	Sălacea Museum House	*Indoor air quality of museums and conservation of textiles art works. Case study: Salacea Museum House, Romania*—Published (Industria Textila Journal, 70, 1, 88–93)—2019
8		*Analyzing indoor museum air quality implications: Case study of Salacea Museum House in Romania*—Conference paper (Global and Regional in Environmental Protection GLOREP, Ed. Politehnica, 89–91)—2018
9		*Microbial and fungal contamination of air and wooden surfaces inside of Museum House Salacea, Romania*—Under evaluation—2020
10	National Archives of Romania—Bihor County Service	*Microclimatic characteristics and air quality inside of the National Archives of the Bihor County (Romania) for the long-term preservation of the documents and the health of the employees*—Under evaluation—2020
11		*SEM investigations on old maps with canvas support*—Conference paper (International Conference TexTeh IX. Advances textiles for a better world, Proceedings, 9, 153–157)—2019

Table 1.
Details of the studies already carried out by the research team from the University of Oradea and collaborators, on the three buildings in which textile collections are kept.

In the first case, it is a wooden church located in the University of Oradea Campus, built in the second half of the eighteenth century and currently considered a historical monument, being registered in the list of historical monuments with the code BH-II-m-B-20958. It houses numerous paintings, some of which are made on textile material (**Figures 2** and **3**). These are very valuable in terms of the techniques used for painting (al secco), the colors used (some with gold composition), the dimensions and materials on which they are made. The studies envisaged for the methodological and result analysis are focused on determining the interior microclimate of the monument [7, 44, 45] as well as the preservation of the valuable objects inside [35, 46, 47].

The second case study is represented by a former nineteenth century peasant household from the village of Sălacea, which was later refurbished and is currently functioning as a village museum. Numerous ethnographic items are exposed inside such as: furniture, traditional fabrics and other household items (**Figure 4**);

precious due to the prominent place formerly occupied in the life of the Romanian village, especially in the villages from Bihor County. The museum house has been the object of study for three scientific works, one focused on the determination and analysis of the internal microclimate [48], and the second on its influence on the conservation of the exposed textile materials [2].

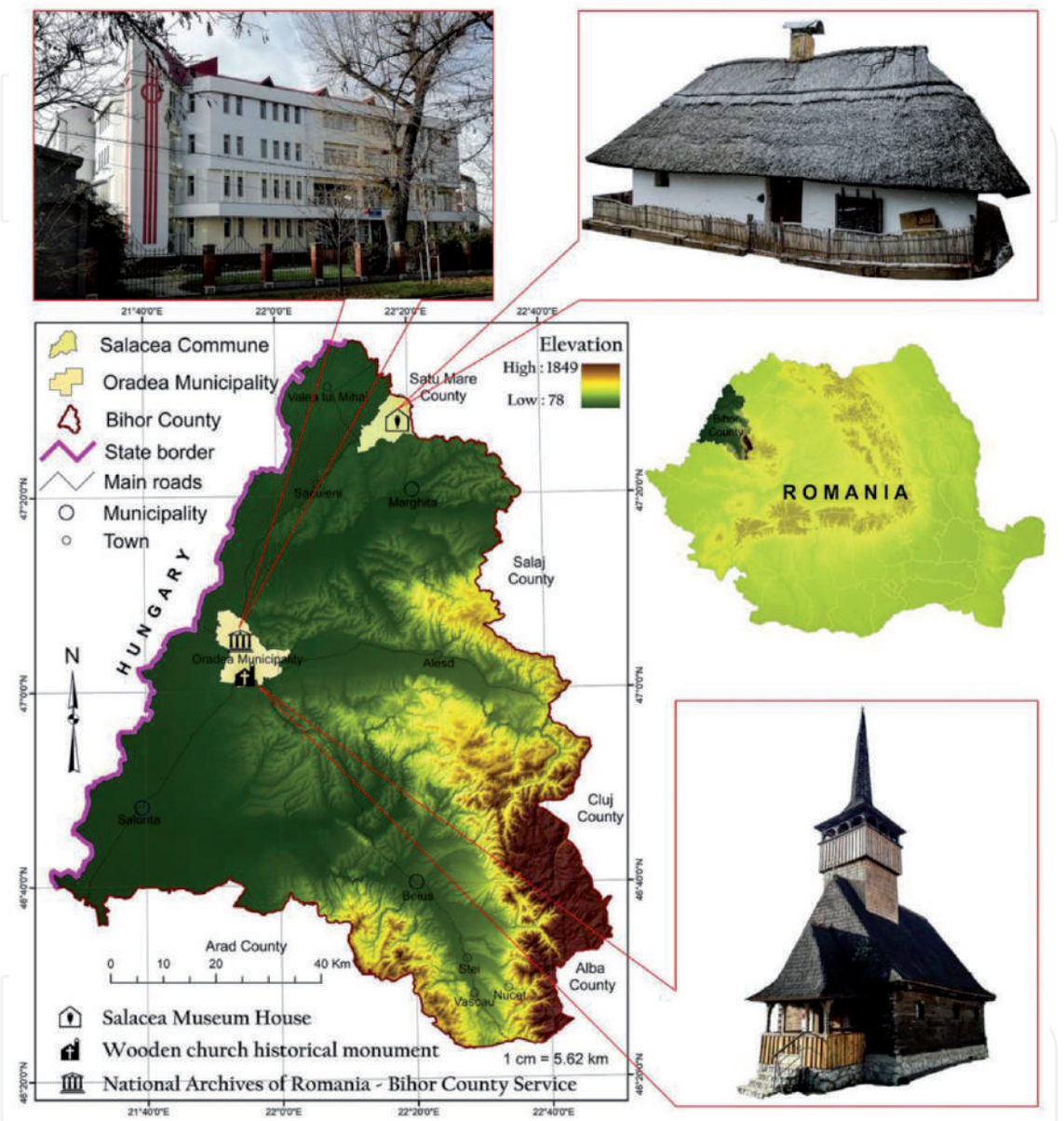

Figure 1.
The location of the three case studies at the Bihor County level.

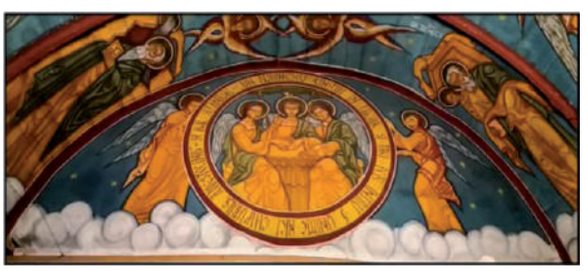

Figure 2.
Painting on canvas inside the wooden church historical monument depicting The Holy Trinity.

Figure 3.
Painting on canvas from the ceiling of pronaos inside the wooden church depicting the Virgin Mary with Baby Jesus.

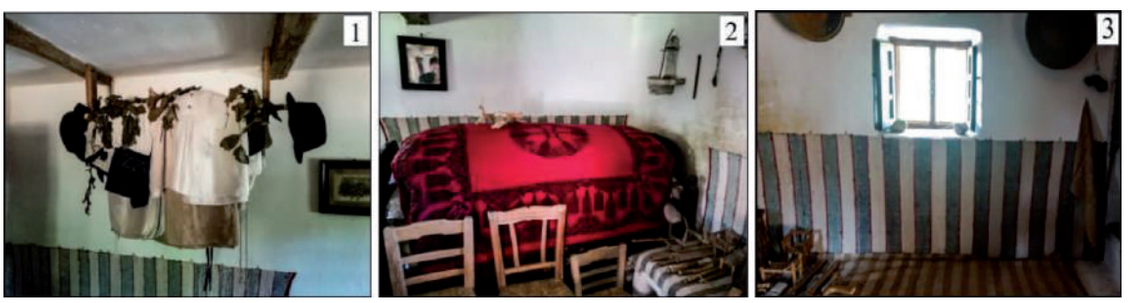

Figure 4.
Traditional port and household items from Sălacea Museum House made of textile materials: (1) elements of the traditional port from the Ier Valley; (2) and (3) items for daily use made from different textile materials.

In the last case study, the conditions and the degree of conservation of the documents and textile materials deposited in the National Archives of Romania—Bihor County Service, based in Oradea Municipality—were examined. The researches focused on a thorough analysis of both the main parameters of the internal microclimate from several deposits [49] and of old maps dated between 1895 and 1910 with the support of textile materials (especially cloth) [50].

Due to the different age of the three buildings, the different construction materials and the purpose they serve, the collections of textiles inside them are subjected to various anthropogenic and environmental pressures. If the building of the National Archives of Romania—Bihor County Service is recently built, using modern building materials and access is restricted, so the textiles inside are subject to minimal external influences in order to conserve them for as long as possible; at the opposite pole is the wooden church from the University of Oradea Campus. It is about 260 years old, built mostly of organic material (wood) and is a "living" monument (it still serves the purpose for which it was built) that houses an average of 60 parishioners at each religious service [7], the paintings on the canvas inside are exposed to much amplified pressures. As for the Museum House in Sălacea, it was built in the last century, made of beaten clay and covered with reed; functioning as a village museum, it is occasionally visited by tourist groups, the textiles not being exposed to a very high stress (at least anthropic).

2.1 The methodology used and its relevance

The three case studies already conducted are based on a common principle regarding methodology; namely the monitoring of the internal microclimate of the spaces where the textiles are stored in order to determine the characteristics of

its main elements (temperature, humidity, luminosity, CO_2, contamination with bacteriological microflora and fungi). This has been achieved because it is known that historical textiles are highly susceptible to being damaged by the action of these environmental factors [51]. Theoretically, for textiles to be stored in an environment that is most suitable for storage, the indoor temperature should not exceed 22°C, with a relative humidity between 50 and 65% and a brightness between 50 and 80 luces [52]. To determine the temperature, humidity, brightness and CO_2 concentration, a large number of electronic devices were used in the research, most of them being data loggers for the simultaneous monitoring and storage of the values. The values of the temperature and the relative humidity were determined using Klimalogg Pro, Thermal Imaging Camera FLIR I7 and Delta Ohm HD 32.3, brightness with the help of Digi-Sense Data Logging Luxmeter and Luxmeter data logger Extexh SDL400, and the amount of carbon dioxide with Nova 5000.

Regardless of whether the textiles are made from fibers of plant or animal origin, too high temperature can determine tissue weakening and discoloration. The increased relative humidity of the environment can cause its absorption to the textile fibers and as a result of the humidothermal treatments contractions can occur. Mechanical technological processes can result in a loss of elasticity, flexibility and tensile strength [53]. Furthermore, excess natural light can cause the oxidation of polymers from the composition of natural textile fibers, leading, in time, to the breaking of the intermolecular bonds, yellowing of the material, facilitating the breakdown and penetration of microbial enzymes [13], especially in the case of wool.

The pressure that the characteristics of the main parameters of the microclimate exert on the textile materials in general and the historical ones in particular, should not be considered as an intrinsic relation. These factors are interconditioning and acting simultaneously in the process of material degradation. Thus, there is a directly proportional relationship between the amount of natural light and the interior temperature, as well as inversely proportional between the temperature and relative humidity variations. Further, all these environmental factors contribute to the emergence and spread of microorganisms (such as bacteria and fungi), which present an increased risk of deterioration for materials of organic origin, such as textiles. The most favorable conditions for the colonies of fungi and bacteria are high temperatures (between 24 and 30°C) and very high relative humidity [51]. As a consequence of the activity of certain groups of microorganisms on textiles, we can mention: fiber degradation, discoloration, loss of structural resistance, shading of the affected areas in red, brown, orange or black, cracking and fragmentation areas [1]. For the determination of microbial contamination, for all three case studies the method of Koch sedimentation of the conventional techniques of open plates was used, the samples being collected both from the air and from the surfaces of the textiles. The sampling of the types of fungi and bacteria was done by microscope analysis, and the calculation of the average value of the number of colonies of fungi was materialized by applying Omeliansky's method [54].

If all these methods presented above were of a general nature, being used to establish the influence that the internal microclimate exerts on the textile collections of each of the three analyzed buildings, further on an individual investigation of the fabric samples was carried out. In the wooden church, it was analyzed and represented in cartographic form, with the help of GIS techniques [55, 56], the areas of paintings on canvas anthropic degraded, as well as the distribution of temperatures and brightness within the frescoes. Finally, the colors used for painting inside were examined from the point of view of the internal composition, by digital techniques (X-ray fluorescent spectrometry) [35]. A digital technique was also used to examine two samples collected from old maps with textile support deposited in the

National Archives of Romania—Bihor County Service. Namely scanning electron microscope (SEM); a technique often used in studies targeting textiles, but with a high efficiency in determining their degree of conservation. The use of SEM aimed to identify morphological aspects and irregularities of the fibers that make up the fabrics, as well as the presence of microorganisms and dust [47].

For centuries, the purpose of textiles has been to serve man. Offering protection against the glazes of nature through clothing and material or building houses (tents of nomadic populations), means of purchasing food (fishing nets and various traps), mobility in the territory (sails of ships), and many other practical uses. This has not changed until today, fabrics still occupying a central place in the everyday life of modern society. The relationship of interdependence created along the time between man and textile makes it impossible to analyze the latter separately from the creative element. Therefore, in the accomplishment of the three case studies, the influence of the internal microclimate on the conservation of textiles, as well as their integrated action (internal microclimate + textiles) on human health, was considered.

Furthermore, it is worth mentioning that all the interventions for determining and monitoring the degree of conservation of textiles have been chosen so that they are non-invasive for materials; ensuring that they are kept in the best conditions.

2.2 Results obtained

2.2.1 Wooden church "Saint Martyrs Constantin Brancoveanu and his sons"

Following the microclimate measurements carried out in two periods, March-April (2016) and October-December (2018) respectively, a fluctuating evolution of the main parameters was found. Between March and April 2016, the average temperature inside was 25.8°C, with an average relative humidity of 38% [7]; none of these indicators are complying with the rules in force. The situation is improving, and the values approach the ideal parameters (according to the GD no. 1546/18.12.2003, see [52]), at the level of 2018, when between October and December, the temperature has an average value of 21.1°C, and the relative humidity of 44.3% [46]. Oscillating quantities have also been recorded in terms of carbon dioxide. During the religious services it exceeds in multiple times the value of 2000 ppm, so that during the periods without human activity it is constantly maintained between 400 and 500 ppm.

Following analyses with X-ray fluorescent spectrometry, it was determined that these oscillations are responsible for modifying several properties of the pigments that make up the paintings, eventually leading to their degradation [35].

In order to establish the damaged areas of the paintings on the canvas, the mapping of the areas in which they were degraded by temperature, humidity and rainwater infiltrated in the painted canvas was performed. Also in this sense, the distribution and influence of heat and light on the painted canvas were analyzed (**Figure 5**), at different times of the day and in different seasons [47].

Regarding the contamination with bacteriological microflora and fungi, following the laboratory analyses and sampling of results, 47 colonies of bacteria and 31 of fungi colonies were identified in the air [45]. Fungi belong to a number of 18 species, the most common being *Aspergillus sp., Alternaria sp., Absidia sp., Penicillium sp.*, as well as *Rhodotorula* and *Candida*; and four types of bacteria were identified (*Staphylococcus, Micrococcus, Bacillus,* and *Actinomyces*) [44]. The large number of microorganisms identified in the air inside the wooden church can represent a potential danger to the health of the parishioners, but at the same time they can colonize the textile materials [47]. The organic components that characterize

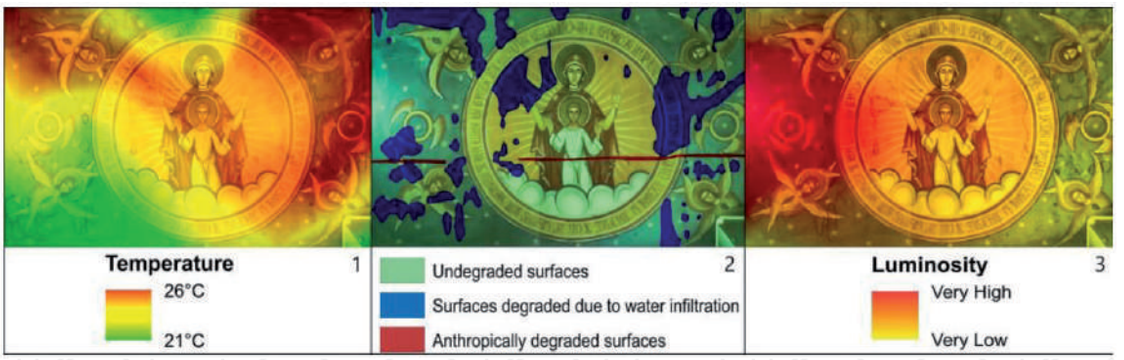

Figure 5.
Distribution of temperature, luminosity and degraded areas on the painting on canvas "Virgin Mary with the Baby Jesus" (after Oana et al., see [42]): (1). Temperature distribution at the canvas level; (2). Distribution of degraded areas at the canvas level; (3). Distribution of natural light at the canvas level.

the paintings on canvas [35] represent a nutritional source for a wide range of microorganisms [45], proving the large number of identified colonies. The samples collected from the paintings on canvas revealed the presence of no less than seven fungi and one type of bacteria [45, 47]. The integrated action of these microbes, both from the air and from the surfaces, can, in time, lead to the decomposition of textile materials, damage that is practically irreversible.

2.2.2 Sălacea Museum House

The measurements made between 03.06.2018–2102.07.2018 in the Museum House in Sălacea Village indicated that the values of temperature, humidity and brightness comply, with small exceptions, the norms set in GD no. 1546/18.12.2003 [2]. Only the temperature recorded an average value higher by 1.3°C than the optimal one (22°C); while humidity, with an average of 65%, is at the upper limit of the ideal. The brightness, having values between 10 and 20 luces, does not influence textiles in any way.

However, these environmental conditions cause the development of microorganisms, which attack the tissues and endanger the health of the visitors. A number of 73 colonies were identified in the center of the main room, 63 colonies in the corners and 39 in the ceiling [2]. Due to the fact that the museum is visited only periodically by tourists, the number of fungal species was obviously smaller than in the wooden church, only three being observed (*Alternaria, Geotrichum* and *Cladosporium*) [48]. However, the action of the bacteriological microflora present inside can cause degradation of textile fibers, as well as health problems (allergies) in humans [6].

2.2.3 National Archives of Romania: Bihor County Service

According to the order No. 235/05.07.1996, supplemented by the Daily Provision of the General Director of the National Archives No. 92/14.05.2009 [57], for the optimum preservation of the documents (including those of textile materials) the microclimate of the interior should be kept between 15 and 25°C regarding the average air temperature, between 40 and 65% the average relative humidity and below 0.3 m/s the speed of air currents [49]. Excluding the relative humidity, which registered a value with 2% (38%) lower than the limit, the other elements are included in these norms (average air temperature of 23.3°C; air currents speed of 0 m/s). The amount of CO_2 recorded an average of 570 ppm, a value considered to be within normal parameters [58].

Aeromicroflora was determined to be composed of fungi species: *Alternaria, Botrytis, Cladosporium, Penicillium, Scopulariopsis* and various subspecies of

Aspergillus [49]. These microorganisms affect human health and integrity of textile fibers; the second case being detected after the SEM analysis of the samples of old maps with textile support. These revealed that the fibers are strongly damaged due to the presence of fungi and dust. Among the microorganisms identified on the maps, there are different subspecies of *Penicillium* and *Fusarium* [50].

3. A new case study

In order to diversify the study objects, a new research on a textile material with historical implications was conducted. It is about a traditional women's shirt called "ie" (**Figure 6**), specific to Alba County, Romania. The value of this fabric lies in its very old age (over 100 years), due to the fact that it is handmade from natural cotton fibers, but also due to the special significance it has in the life of local communities.

Like the other collections of textiles presented above, which are stored in buildings with different environmental conditions, the present study comes to complete the way historical textiles are preserved in Romania. The traditional shirt, being part of a private collection, to which only the owner has access, its preservation conditions are different from all the other samples. It is stored in household microclimate, which commonly consists of an average temperature in the range of 20–24°C (during wintertime) and 23–26°C (during summertime). Also, the humidity level range between 30 and 60% [59]. Moreover, due to the fact that the piece still serves the purpose for which it was achieved, being occasionally worn, it is necessary to determine the bilateral implications of this action; both on the degree of conservation of the shirt and on the health of the wearer.

3.1 Methodology

Based on the methodology applied in previous studies, for the traditional shirt it was decided to evaluate the state of the material, its physical characteristics, the

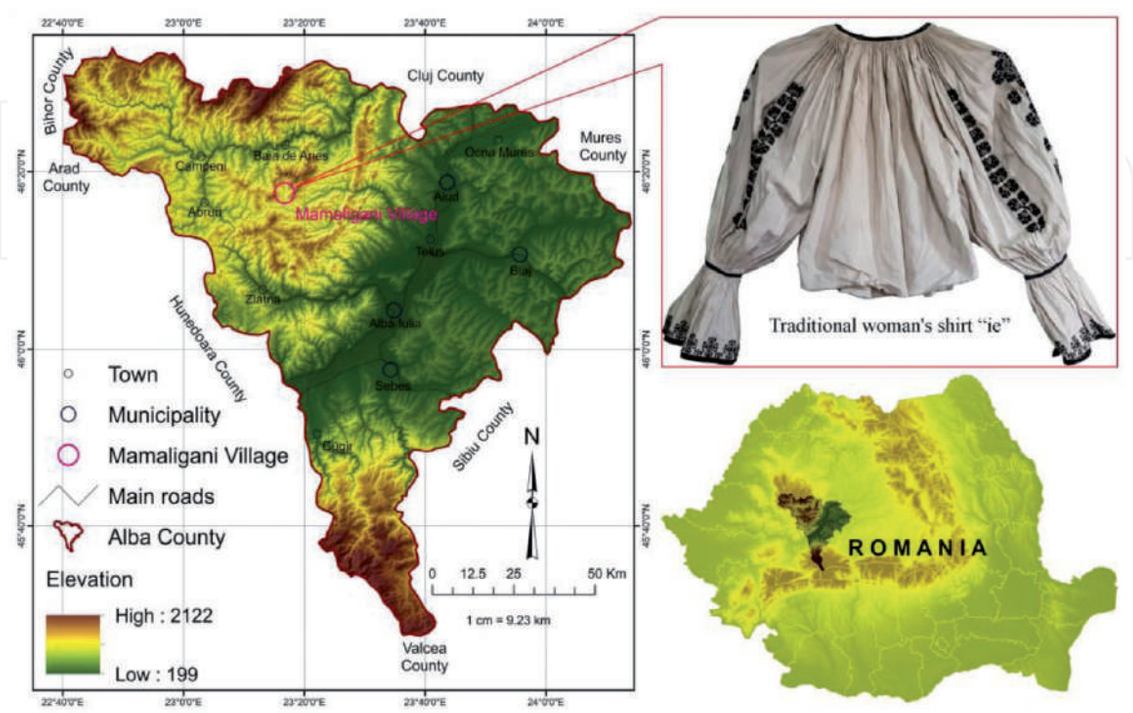

Figure 6.
The geographical location of the area of origin of the traditional women's shirt "ie".

degree of mechanical destruction and the possible influence of atmospheric factors. The samples collected from the textile material were examined using the scanning electron microscope (SEM), to determine the current state of the fibers. The morphology of the fiber system was determined in order to identify visible damage under the microscope and verify the occurrence of dust on the tested impurities. An attempt was made to determine the fungi and other microorganisms potentially occurring on the tested material [60, 61]. The test samples were presented in successively increased magnifications, from 178× to 2.31K× at EHT 20.00 kV. Using the FEI Quanta 200 microscope, a comparative examination was performed between a white and a black thread from the shirt's composition, in order to determine any differences in terms of material or degree of preservation. The whiteness index was determined with the Datacolor Spectrophotometer with a D65/10 lamp, by measuring the X, Y and Z chromatic components from three areas (**Figure 7**) with different bleaching levels.

To identify the microorganisms that are currently colonizing the material, Dichloran culture medium 18% glycerol agar with chloramphenicol (DG 18) was used; the diluent used being peptonated water. Further, petri plates were seeded, and the samples were incubated under aerobic conditions, at 25 ± 1°C, for 5–7 days. Macroscopic and microscopic examination of fungal colonies [62, 63] highlighted the following types of fungi: *Aspergillus niger, Penicillium spp., Cladosporium spp., Alternaria spp. and Candida spp*. After dishes incubation, fungal and bacterial colonies were counted. Colony forming units per cubic meter (CFU • m^{-3}) were determined, following the Omeliansky's equation [64, 65]:

$$N = 5a \times 10^{4} (bt)^{-1}, \quad (1)$$

where N = microbial CFU • m^{-3} of indoor air, a = number of colonies per Petri dish, b = dish surface (cm^2), t = exposure time (min). Determination of the number of fungi (NTF) per gram of product ufc/g showed NTG/ml or /g = greater than 300 at both 22°C and 37°C, NTF/ml or /g = 2.1×10^3.

3.2 Results and discussions

The first pair of images at 178× magnification (**Figure 8**) and 270× (**Figure 9**) allowed the determination of the tested fiber's condition, as good. The first image

Figure 7.
The three points in which the whiteness index was measured.

clearly shows the weave of threads constituting the basic fiber of the material of the costume under examination. Its good condition may indicate that the folk costume ("ie") is not used very often (within the scope of the test sample), as well as it is kept in good storage conditions. This is confirmed by another image (**Figure 9**), which shows only slight mechanical damage to the fibers. The entire fiber bundle tested is in good condition. However, even at this low magnification, enhanced by image enlargement using computer tools, we can see atmospheric dust covering the fibers.

Successive image magnifications, 818× (**Figure 10**) and 877× (**Figure 11**) confirm previous observation. At this magnification, dust and dirt are clearly visible on individual fibers. Also, it can be observed in **Figure 10**, the structure of a single fiber of natural origin. In the case of natural fibers, with a lose arrangement, the rough, lamellar surface structure means that they are able to transport significant amounts of material, including microbial origin [66]. Computer enlargement of the image of the same fragment of the sample, showing the tested fibers, allows to detect the presence of dust particles and microbiological contamination.

The images of the next sample at 1.31× magnification (**Figure 12**) with clear contamination and at 2.31× magnification with single fiber (**Figure 13**) confirm this observation. The first of them clearly shows mechanical impurities, although the individual fiber in the next image is free of impurities.

Using a datacolor instrument, the whiteness indices of the shirt was measured on three portions, from the portion where the textile is clean to the portions where yellow spots are observed (**Figure 7**). Berger and CIE whiteness indices are presented in **Table 2**. When comparing to literature, the indices for the traditional shirt are significantly lower [67, 68]. This is a proof of the shirt age, even if it has been kept in better conditions. Due to the variations of humidity and temperature, over time the cotton fibers have oxidative reactions, in fact an aging of the cellulose fibers of cotton occurs, thus causing the yellowing of the material.

The SEM images (**Figure 14**) resulting from the analyzes on the two different color yarns (black and white) collected from the shirt, confirm the similarities regarding the cellulose nature of the fibers, both being made of cotton [69]. At the same time, there are no noticeable differences in the state of conservation between the white and black fibers (from the manually embroidered model), both of which are in very good state of conservation.

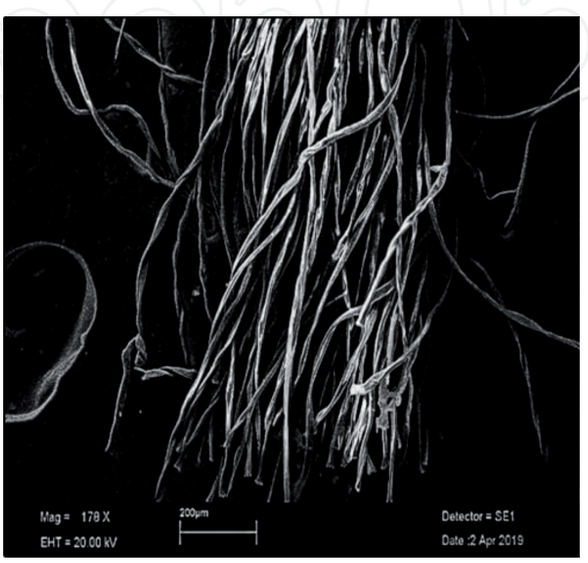

Figure 8.
SEM image of the fibers from traditional shirt at magnification 178×.

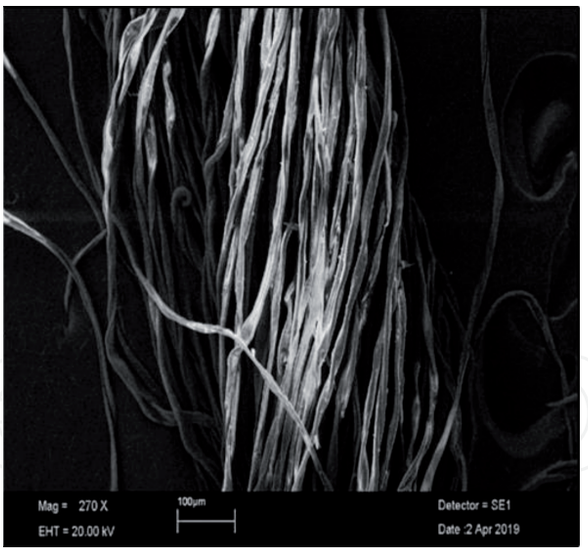

Figure 9.
SEM image of the fibers from traditional shirt at magnification 270×.

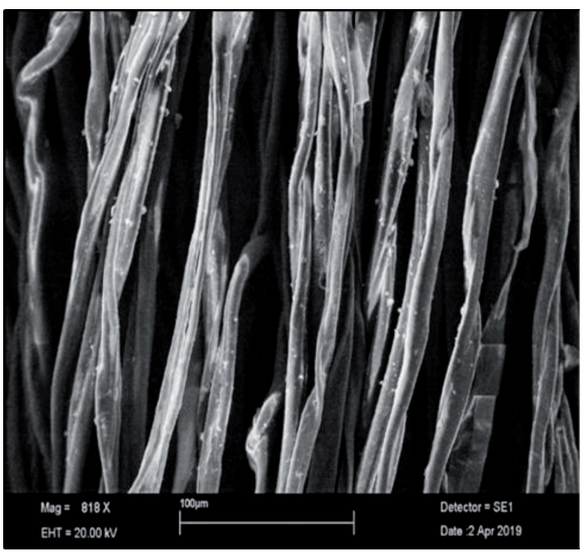

Figure 10.
SEM image of the fibers from traditional shirt at magnification 818×.

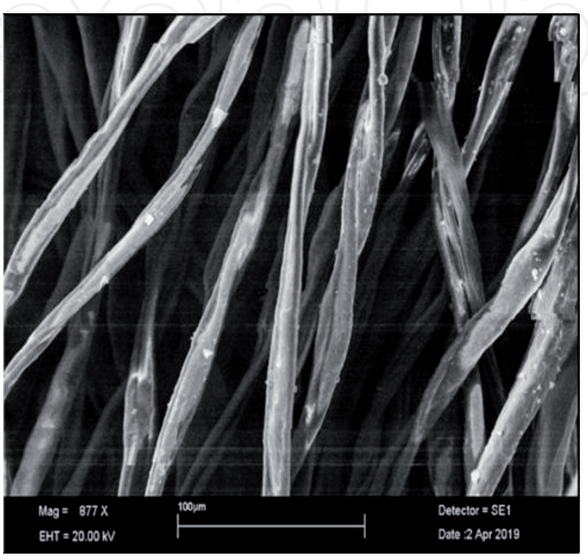

Figure 11.
SEM image of the fibers from traditional shirt at magnification 877×.

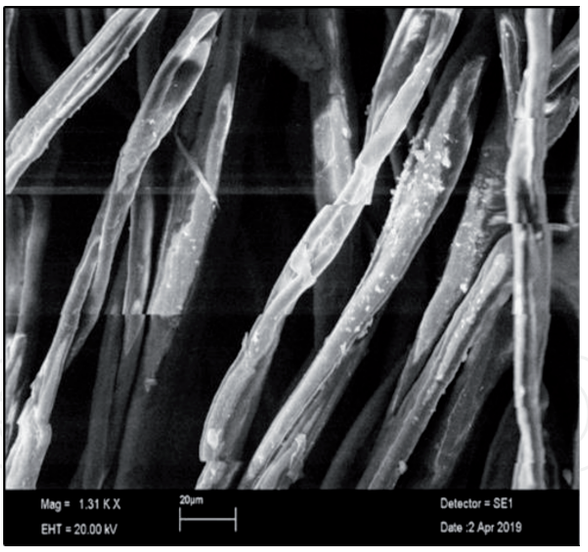

Figure 12.
SEM image of the fibers from traditional shirt at magnification 1.31×.

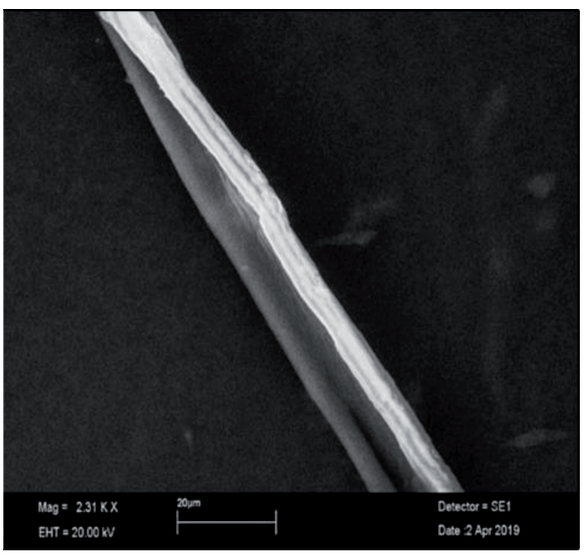

Figure 13.
SEM image of the fibers from traditional shirt at magnification 2.31×.

According to the literature on the subject [66, 70, 71] and the conducted research, it can be stated that the most common fungi found on the tested material (folk costume) and cellulose textiles (cotton, linen) include different species of *Aspergillus, Penicillium, Alternaria, Botrytis, Chaetomium*, etc. [70].

Portion	X	Y	Z	W (Berger)	W (CIE)	T	Obs.
1	65.64	68.92	67.68	46.07	43.86	−3.16	Ref.
2	63.15	66.73	62.74	36.24	30.44	−3.57	Darker More saturated More green
3	63.51	66.69	64.5	41.18	37.34	−3.48	Darker More saturated More green

Table 2.
The whiteness index of the traditional shirt measured in three different portions.

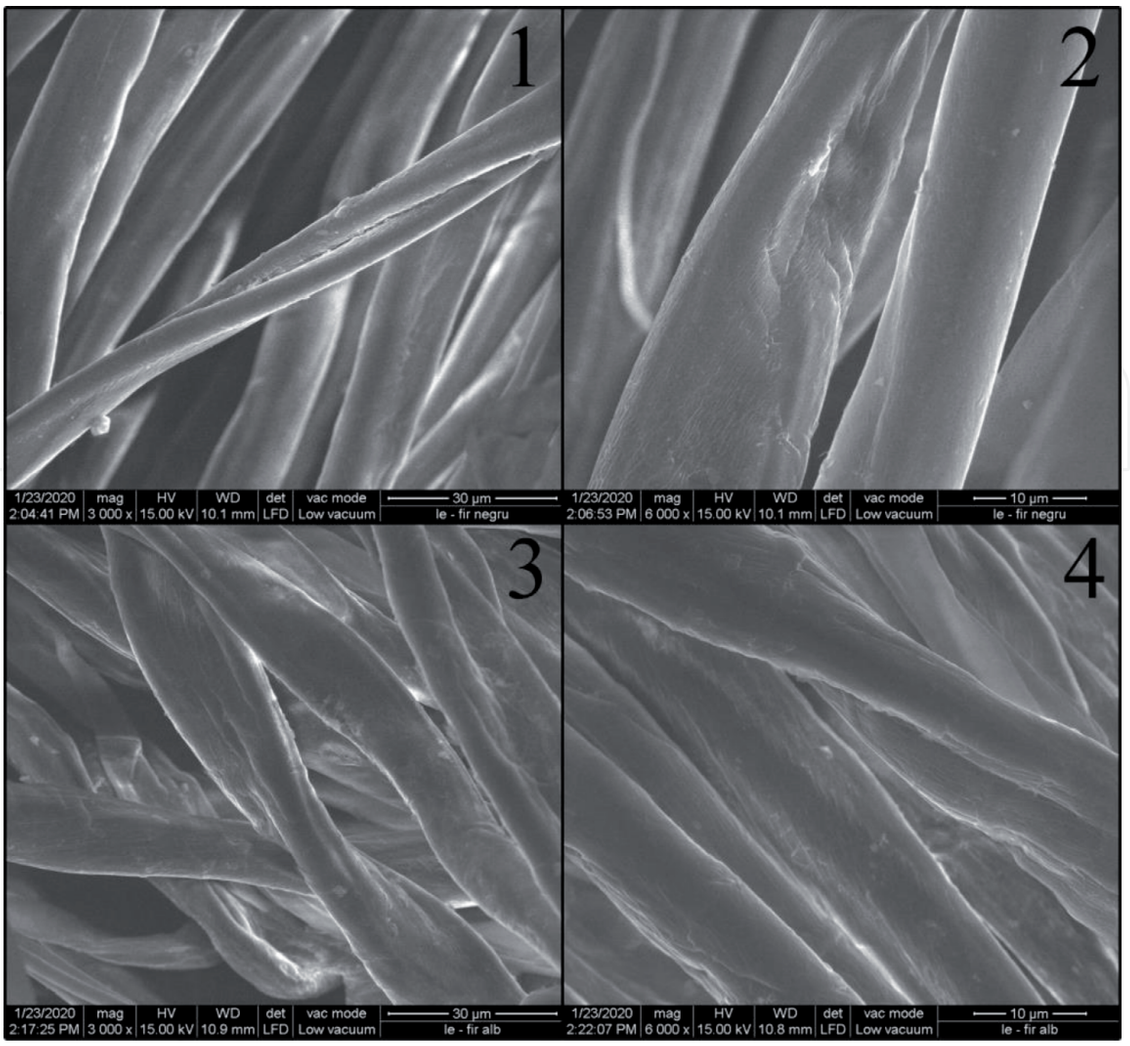

Figure 14.
SEM images of the two white and black wires from the traditional shirt: (1) SEM image of the black wire at 3000× magnification; (2) SEM image of the black wire at 6000× magnification; (3) SEM image of the white wire at 3000× magnification; (4) SEM image of the white wire at 6000× magnification.

The conducted tests of the described fiber samples confirmed the presence of *Aspergillus niger, Penicillium spp., Cladosporium spp. Alternaria spp.* and *Candida spp*. The penultimate enlargement of the tested fabric (**Figure 10**) clearly shows microbiological contamination and the process of fiber development and possible colonization by *Aspergillus* and *Penicillium.*

People are frequently exposed to spores and vegetative forms of *Aspergillus niger*, present on various textile objects. *Aspergillus niger* can cause allergic symptoms and produce certain mycotoxins that can especially affect people with a weak immune system, respiratory, renal, immune system or hearing aids; they can also cause local lesions in both the internal and external ears, postoperative cavities, etc. [72].

Aspergillus and *Candida* cause deaths due to invasive infections. The fungal species in the *Candida* family are the most common etiologic fungal agent of invasive infections that can endanger life in patients: immunocompromised; they have undergone invasive clinical procedures or major trauma and require long-term hospital care [73]. *Cladosporium spp.* can cause allergic reactions in humans, which sometimes results in asthma. Rarely, it can cause opportunistic infections, mainly located in the skin and subcutaneous cellular tissue [74]. Exposure to fungi of the *Alternaria* has been recognized as a risk factor for the development, persistence and severity of asthma and allergic respiratory diseases. They can cause rhinosinusitis, onychomycosis, and skin and subcutaneous infections, generally

in immunocompromised patients. Infections with *Penicillium* can be mainly by inhalation and sometimes by ingestion. Pathologies that are the result of infection with *Penicillium spp*. are generally referred to as penicilliosis. Prolonged exposure to fungi from the *Penicillium* family which typically produce mycotoxins ochratoxin A and citrinin, has been reported in combination with opportunistic infections such as keratitis, otomycosis and urinary tract infections [75, 76].

4. Conclusions

In all four case studies, it was proved that the values of the main elements of the microclimate play a decisive role both in the degradation of the textile materials and development in the air and on the surfaces of the bacteriological microflora. In time, its leads to the deterioration of the historical fabrics exposed inside, at the same time endangering human health. The degree of accessibility of the public and the age of the building, is directly proportional to the quality of the elements of the microclimate. Thus, in the wooden church (the oldest building among those analyzed, where human activity is the most intense), textiles are exposed to the highest risk of being degraded due to poor environmental conditions. At the opposite pole, in the Museum House and National Archives of Bihor County, due to the more recent dating of buildings, the nature of the materials and largely limited human activity, the fabrics find better conditions of conservation, and people are exposed to much reduced risks.

By far, in the best state of conservation is the traditional Romanian shirt ("ie"), which due to the fact that it is part of a private collection, is exposed to anthropogenic and lower environmental pressure. It is stored in good conditions, the fibers are very little affected, they do not show discoloration or breakage as a consequence of the action of the microflora.

The analyzes carried out in this study show that some of the historical textiles are in a poor state of conservation, or are threatened to be degraded by human action or environmental factors. In order to conserve the materials for a longer period of time, it is necessary first of all a careful and continuous monitoring of the internal microclimate. The values of its main parameters should be kept as far as possible within the allowed limits, and the impact of the bacteriological microflora and the anthropic factor must be limited. Furthermore, where appropriate, exhibits are preferably to be protected by their installation in special glass boxes to reduce mechanical and chemical damage. All these interventions have both the role of creating a favorable environment for the conservation of textile materials, as well as of minimizing the implications on human health.

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