Doctoral Thesis

Gelatin fibers: spinning processes, fiber modification and application

Author(s):
Stoessel, Philipp R.

Publication Date:
2015

Permanent Link:
https://doi.org/10.3929/ethz-a-010564948

Rights / License:
In Copyright - Non-Commercial Use Permitted
GELATIN FIBERS:
SPINNING PROCESSES, FIBER MODIFICATION AND APPLICATION

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH Zurich
(Dr. sc. ETH Zurich)

presented by

PHILIPP RENÉ STOESSEL

Master of Science ETH in Food Science

born on 28.04.1987
citizen of Wäldi (TG)
   Switzerland

accepted on the recommendation of

Prof. Dr. Wendelin J. Stark, examiner
Prof. Dr. André R. Studart, co-examiner

2015
Not everything that can be counted counts,
and not everything that counts
can be counted.

_Albert Einstein_
Acknowledgements

During my PhD studies I had the pleasure to receive support, advice and encouragement from numerous people. I would like to take the opportunity to thank them.

First and foremost I would like to express my profound gratitude to Prof. Dr. Wendelin J. Stark. By admitting me to his research group as a Master student, I got the possibility to immerse into a completely new scientific world. From the first day on I was inspired by his motivation to conduct research with a real impact. Wendelin has taught me to grow with the challenges, to think out of the box while never losing sight of the overall picture. Moreover, I appreciated the constant approachability and his open manner a lot. For all this I am greatly indebted to him. Wendelin, thank you for the confidence you have placed in me.

I am also very grateful to Prof. Dr. André R. Studart for investing his precious time to co-examine this thesis and for his interest in my work.

I especially would like to thank Dr. Robert N. Grass. In addition to being a great colleague, he is a brilliant scientist, receptive to any questions at any time. I greatly profited from his versatility, helpfulness, enthusiasm and humor. Thank you, Robert, for the endless discussions about science and everything else. The FML would not be the same without you.

This brings me to the pillar of the FML: a splendid conglomerate of people from different scientific disciplines! I thank all my colleagues for the exciting time: Stephanie Bubelhofer, Nicholas Cohrs, Roland Fuhrer, Lukas Gerber, Samuel Halim, Jonas Halter, Antoine Herzog, Samuel Hess, Corinne Hofer, Christoph Kellenberger, Fabian Köhler, Lukas Langenegger, Michael Loepfe, Norman Lüchinger, Gediminas Mikutis, Dirk Mohn, Carlos Mora, Daniela Paunescu-Bluhm, Michela Puddu, Renzo Raso, Michaël Rossier, Aline Rotzetter, Elia Schneider, Christoph Schumacher, Oleksander Stepuk, Robert Stettler, Mario Stucki, Martin Zeltner, Vladimir Zlateski and all undergraduate students and civilian service personnel. It was a privilege to work with you and I am happy that numerous friendships evolved from the prosperous working environment. I count myself lucky to having shared office space in E114 and E115 with Daniela, Martin, both Christops, Samuel and Gediminas. Thanks to all for the relaxing atmosphere and for brightening up the daily routine with jokes. I am thankful to the students who have contributed to my research work: Tim Kaufmann and Andri Mani. I especially would like to thank Mirjam Giacomin, not only for being an excellent secretary, but also for constantly spoiling us with chocolate.
A huge thank goes to the ICB workshop staff, namely Urs Krebs, Joël Jenni, Jean-Pierre Mächler, Max Wohlwend and Fredy Mettler. By realizing our crazy ideas, they provided the basis for my research.

While working on my PhD, I had the possibility to seek valuable advice from several experts at ETH, EMPA and ITV: Dr. Kirill Feldman and Dr. Thomas Schweizer (Department of Materials, ETH), Prof. Dr. Raffaele Mezzenga, Dr. Antoni Sánchez-Ferrer and Dr. Sreenath Bolisetty (Department of Health Sciences and Technology, ETH); Dr. Rudolf Hufenus and Marcel Halbeisen (Advanced Fibers Laboratory, EMPA St. Gallen); Dr. Martin Dauner (Institute of Textile Technology and Process Engineering, Denkendorf). They helped me to see beyond my nose and it was an enrichment to exchange views with them.

Although not shown in this thesis, I am glad to have been given the possibility to work in various projects and collaborate with the following people: Dr. Dirk Sewing, Hans-Jakob Schärer, Mathias Ludwig, Dr. Bodo Hattendorf, Marcel Burger and Franziska Grüneberger.

I hardly find the words to thank my parents and brothers. It is the absolute support of my parents which allowed me to pursue my studies. Without their aid I would not be where I am today. Thank you.

Finally, I would like to thank Fabienne. Her love, her interest in what I am doing and her encouraging words during harder times were the best source of motivation. Being married to you is the best thing in my life. Thank you so much.
# Table of contents

**Zusammenfassung**  
Summary  

1. **Towards the Utilization of Agricultural Waste Products as Biopolymer Fibers**  
   1.1. Biopolymers as substitutes for synthetic polymers  
   1.2. Biopolymers – an overview  
      1.2.1. Definition  
      1.2.2. Biopolymer classification  
      1.2.3. Biopolymer market today  
   1.3. Proteins from agricultural waste as building blocks for biopolymers  
      1.3.1. Proteins  
      1.3.2. Proteins as a resource for the biopolymer industry – a renaissance?  
      1.3.3. Waste proteins from agriculture  
      1.3.4. Collagen structure  
      1.3.5. Collagen and its transition to gelatin  
      1.3.6. Gel formation in aqueous gelatin solutions  
   1.4. Fiber spinning  
      1.4.1. History of fiber use  
      1.4.2. Fiber classification  
      1.4.3. Filament formation  
      1.4.4. Polymer chain orientation in man-made fibers  
   1.5. Conclusions  

2. **Spinning Angora Rabbit Wool-Like Porous Fibers from a Non-Equilibrated Gelatin/Water/2-Propanol Mixture**  
   2.1. Introduction  
   2.2. Experimental section  
      2.2.1 Preparation of gelatin/water/2-propanol mixture and spinning process  
      2.2.2. Crosslinking treatment and swelling experiments
2.2.3. Characterization of gelatin/water/2-propanol mixture 36
2.2.4. Fiber characterization 36
2.3. Results and discussion 37
  2.3.1. Characterization of the gelatin/water/2-propanol mixture 37
  2.3.2. Characterization of non-crosslinked gelatin fibers 39
  2.3.3. Crosslinking treatment and swelling experiments 45
2.4. Conclusions 47

3. Fibers Mechanically Similar to Sheep Wool Obtained by Wet Spinning of Gelatin and Optional Plasticizers 49
  3.1. Introduction 50
  3.2. Experimental section 51
    3.2.1. Fiber preparation 51
    3.2.2. Fiber characterization 52
  3.3. Results and discussion 53
    3.3.1. Characterization of non-plasticized fibers 53
    3.3.2. Characterization of plasticized fibers 57
  3.4. Conclusions 60

4. Porous, Water-Resistant Multifilament Yarn Spun from Gelatin 63
  4.1. Introduction 64
  4.2. Experimental section 65
    4.2.1. Materials 65
    4.2.2. Gelatin yarn production 66
    4.2.3. Yarn characterization 68
  4.3. Results and discussion 71
  4.4. Conclusions 83

5. General Conclusions and Outlook 85
  5.1. Conclusions 86
  5.2. Future research activities 87
Appendix

A.1. Supplementary data for chapter 2
A.2. Supplementary data for chapter 4

References

Curriculum Vitae
Zusammenfassung


In Kapitel 2 wurde eine robuste Trockenspinnmethode für die Herstellung von Gelatinefasern aus einer ternären Spinnmischung – bestehend aus Protein, entionisiertem Wasser und 2-Propanol – entwickelt. Beim Temperieren (50 °C) und Mischen durchlief die Spinnmischung


Die Praxistauglichkeit der Gelatinefasern konnte letztlich anhand einer Textilstruktur mit guter Wärmedämmung aufgezeigt werden.

Summary

Gelatin is the water-soluble degradation product of collagen. The latter is the most abundant structural protein in vertebrates and invertebrates. Based on the facts that gelatin has interesting properties and is available in megaton quantities from animal by-products, the prospects of gelatin as a substitute for petroleum-derived polymers are high. The thesis at hand demonstrates ways and means to fabricate biopolymer fibers from gelatin. Furthermore, strategies for tailoring the fibers’ mechanical and thermal properties as well as water-resistance are proposed.

Chapter 1 provides a general introduction into the topics addressed in this work. As a result of the outstanding properties and low-cost, most aspects of life involve synthetic plastics. The high disposability of synthetic polymers coupled with their persistence lead to adverse effects in the environment. In the last decades, a “green” rethinking has started and interest in biopolymer is increasing. Despite massive growth, the biopolymer market still is insignificant; only a handful of biopolymers is of commercial importance. Vegetable and animal proteins – intensively studied and applied as technical polymers before the end of the Second World War – felt into oblivion. Nonetheless, so called agro-proteins (e.g. soybean protein, zein, casein, keratin, collagen, gelatin) are promising building blocks for biopolymer-based products: they can be easily isolated and breaking down the macromolecule into monomers is not necessary; thus, workflow and energy consumption are minimized. Moreover, relying on agricultural waste protein does not interfere with the food chain.

As the focus of this work is the production of gelatin fibers, background on the triple-helical structure of collagen and its transition to gelatin is presented. Chapter 1 is complemented with general information on fibers. Besides recapitulating the historical evolution of fibers and textiles, well-established techniques for man-made fiber production are presented.

In Chapter 2, a robust dry spinning method for the fabrication of gelatin fibers from a ternary system consisting of protein, deionized water and 2-propanol is reported. This particular spinning mixture stands out because it underwent phase separation into a lower gelatin-rich opaque phase and an upper solvent-rich supernatant when being tempered (50 °C) and shaken. The precipitated gelatin (spinning dope) was extruded as a monofilament into a vertical spinning tube, where fiber solidification occurred due to evaporating solvent. Through continuous drawing of the gelatin fiber, the single and triple helical protein domains were aligned along the fiber axis. The anisotropy manifested itself in improved mechanical properties. A unique feature of the gelatin fibers is their high porosity (~ 30 %) as a result of
enclosed solvent droplets in the spinning dope, serving as templates and evaporated during the spinning process. This porous structure is in close morphological similarity with natural fibers from angora rabbits.

**Chapter 3** pursues the idea of using a phase-separated spinning dope from gelatin, solvent and non-solvent. Instead of dry spinning, a wet spinning process was explored, *i.e.* the fiber solidification was accelerated by continuously immersing the fibers in ethanol. Subsequent fiber drawing yielded good tensile properties: engineering tensile strength (180 MPa) and elastic modulus (3800 MPa) were comparable to sheep wool. However, fully drawn fibers were no longer porous. To address potential issues regarding fiber brittleness, the addition of plasticizers into the spinning dope was studied. Investigated plasticizers included ethylene glycol and triethylene glycol. The latter had a higher plasticizing capacity, reducing the gelatin fibers’ glass transition temperature from 82 °C to 57 °C.

In **Chapter 4**, the combination of the spinning processes from chapters 2 and 3 are presented (*i.e.* dry-wet spinning). Higher spinning speeds and parallel spinning of six filaments permitted a scale-up with a filament production rate of 200 m min⁻¹. About 1000 filaments, each ~ 25 µm diameter, were twisted into 2-ply yarns with good tenacity (4.7 cN tex⁻¹). Gelatin is per se susceptible to water: when in contact with water, untreated gelatin swells and dissolves. In order to obtain water-resistance, gelatin yarns were crosslinked by different polyfunctional epoxides. Examination of free lysyl amino groups and swelling degree in water revealed that ethylene glycol diglycidyl ether exhibited the highest crosslinking efficiency. Further post-treatment with gaseous formaldehyde and lanolin (wool grease) rendered the gelatin yarns water-resistant, allowing multiple swelling cycles in water or detergent solution without suffering damage. The yarns’ wet strength still was insufficient. In order to highlight the applicability of gelatin fibers in a consumer good, a textile structure with good thermal insulation was fabricated.

In **Chapter 5**, the developed spinning processes and methods for fiber tailoring are summarized and ideas for future research activities are specified. Overall, the results clearly show that gelatin fibers, compared to other biopolymer fibers, are an eligible and versatile alternative.
1. Towards the Utilization of Agricultural Waste Products as Biopolymer Fibers
1.1. Biopolymers as substitutes for synthetic polymers

Polymers are either made or natural macromolecules, which are composed of relatively simple organic monomers.\textsuperscript{1-3} Synthetic polymers have continuously gained in importance over the past half century as a result of their durability, low cost, outstanding mechanical properties, lightness, ease of processing etc.\textsuperscript{4,5} It is therefore not astonishing that synthetic polymers quickly found their way into various applications such as packaging (often single-use), agriculture (glass substitute), home applications and consumer goods (injection molded housings), paints and surface coatings, building and civil engineering (adhesives, window frames, pipes, insulation) or medicine (prostheses, hydrogels, scaffolds).\textsuperscript{1} Processing polymers into fibers did even expand the scope of polymer applications: synthetic fibers are nowadays ubiquitous, e.g. in clothing, furnishing, filters, sound absorber, fiber-reinforced materials and many more.\textsuperscript{6} Consequently, almost all aspects of everyday life involve plastics.\textsuperscript{7} In the last decades the production of plastics has impressively grown. From 1950 (~ 1.5 million tons global annual production) onwards, the production yearly increased by ~ 9 %.\textsuperscript{8} The global annual polymer production in 2008 amounted to 245 million tons and the usage of synthetic polymers was estimated at more than 90 kg per person and year in the USA.\textsuperscript{3,8}

However, the heavy consumption and omnipresent disposability of synthetic polymers has two major drawbacks: (1) the dependency on crude oil – a precursor with limited resources in the long term and bad general image in public;\textsuperscript{9-10} (2) the high resistance to biological degradation that leads to an accumulation in nature and landfills.\textsuperscript{4-5,11-12} It is well known that plastic debris have various adverse effects in nature, e.g. starving wildlife, accumulation as micro-plastics in organisms or absorbance of toxic chemicals.\textsuperscript{13} According to Scott, “the very technical advantages which made polymers so useful [durability, biological inertness] were disadvantages when polymer-based products were discarded at the end of their useful life and in particularly when they appeared as litter in the environment”.\textsuperscript{14} Household litter is the most prominent source of plastic waste (> 60 % of the total plastic waste), mostly because of the widespread application of single-use packaging.\textsuperscript{1} The latter makes up 40 % of all plastic consumption in Europe.\textsuperscript{15}

It would be unjust to demonize the plastic industry and to make it responsible for the misuse of plastics (e.g. littering). In our daily life we constantly profit from the advantages offered by synthetic polymers. Furthermore, it must be kept in mind that the production energy for synthetic plastics is low compared to e.g. aluminum, steel, glass or even paper.\textsuperscript{1} Nevertheless,
it is indispensable to consider alternative, more sustainable ways for production and usage of polymers. The growing awareness of the discussed disadvantages (limited availability of fossil fuel-based polymers, health, environmental and aesthetic problems) is the main driving force for a “green” rethinking and provokes a lot of interest in environmental benign polymers.\textsuperscript{16}

\textbf{Figure 1-1}: Life cycle of polymers from renewable and non-renewable (fossil fuel-derived) resources (adapted from Swift (2003)).\textsuperscript{17}

In Figure 1-1 the polymer processing routes and ways of disposal are illustrated. Apart from the non-renewability of the raw materials, petrol-based polymers also lead to problems at disposal. There is a shortage of landfill sites and plastics are persistent in the environment.\textsuperscript{18} Incineration is criticized for the CO\textsubscript{2} and toxic gas emission, while at least allowing recovery of some of the polymer’s energy.\textsuperscript{7, 17-18} Plastic recycling seems to be the best option. However, recycling is often hindered or made unfeasible due to contamination of polymers or blending of polymers with fillers. Recycling such material often is more expensive than the cost of virgin material.\textsuperscript{19} Primary recycling (re-extrusion) frequently yields materials with insufficient quality; secondary recycling (mechanical recycling) suffers from the degradation
and the heterogeneity of plastic waste; tertiary recycling (chemical recycling, i.e. the depolymerization into small molecules used for the production of new petrochemicals) is considered the most sustainable solution.

The benefit from using renewable resources is obvious: at the end of life, the material may be biodegraded by enzymes or microorganisms and the building blocks re-used – they thus stay in the life cycle. Despite their inferior properties, there is a broad consensus that bio-based and biodegradable polymeric materials are promising alternatives to man-made polymers.

The price of environmentally friendly polymers is not yet competitive (e.g. up to 10 times higher). However, according to market research, consumers are willing to pay more for bio-based polymers; as a consequence, the market in question shows massive growth. In 2013, the global biopolymer market already reached 3.5 million tons per year. Compared to the synthetic polymer market, this volume still is insignificant (< 1.5 % of the total polymer market), though.

1.2. Biopolymers – an overview

1.2.1. Definition

Biopolymers are generally defined as macromolecules fully or partially produced in a natural way by living organisms. Quite often the term “biopolymer” is used synonymously with “biodegradable polymer”, even though it is possible to produce fossil fuel-derived biodegradable polymers. Likewise, it is questionable whether non-biodegradable polymers from renewable resources should be called “biopolymers” (see chapter 1.2.2.).

This illustrates that the expression “biopolymer” is rather vague. As a consequence, a multitude of names are used to refer to environmentally friendly polymers (e.g. “green polymers”, “environmentally degradable polymers” or “bio-based polymers”). Yet the expression biopolymer nowadays is used the most and will be applied in the work at hand.

1.2.2. Biopolymer classification

The most straightforward differentiation among biopolymers is based on the raw material: biopolymers from (i) annually renewable resources, (ii) petroleum-based resources or (iii) mixed sources (combinations of bio-based and petroleum-derived monomers). The production method is another approach for classification: (i) chemical synthesis, (ii) direct biosynthesis in microorganisms or (iii) modification of natural macromolecular resources (so
Biopolymers can also be classified according to their chemical composition as proposed by Clarinval and Halleux (2005): polysaccharides, proteins, lipids, natural rubbers, polyesters (either produced from microorganisms, chemically synthesized from bio-based or petroleum-based monomers, respectively), polyvinylalcohols, polyolefins. Figure 1-2 attempts to combine different classification systems – production, resource, biodegradability – in order to give an overview of the common biopolymers available today.

Figure 1-2: Classification of biopolymers taking in account raw materials, production pathways, as well as degradability.

From the findings in chapter 1.2.1, it becomes clear that the raw material (renewable vs. fossil fuel) and degradability (biodegradable vs. persistent) have to be kept apart. For example, poly(thioesters) are biotechnologically produced in *Ralstonia eutropha*, the same bacterium that is able to synthesize polyhydroxy(alkanoates) under excess sugar substrate. Nonetheless, poly(thioesters) – such as poly(3-mercaptopropionate) – are persistent to microbial degradation or only very poorly biodegradable. From a chemical point of view, natural rubber (caoutchouc) is similar to the synthetic poly(isoprene) and both are biodegradable; as soon as they are industrially processed to commodity products (tires), the biodegradability is lost due to vulcanization and addition of antioxidants. The opposite is found in e.g. poly(caprolactone): despite being produced from fossil fuel-derived monomers, it is fully biodegradable. Furthermore, there are also polymers, such as polylactides, which
combine the biotechnological monomer production (lactic acid from polysaccharides) with a subsequent ring opening polymerization step.

The above examples demonstrate that “man-made” does not automatically exclude biodegradability and “bio-based” may include persistent materials. Instead, the chemical bonds (carbon backbone vs. hydrolysable backbones) and functional groups define whether a polymer can be decomposed by enzymes and microorganisms.\textsuperscript{28,37}

1.2.3. Biopolymer market today

Despite an attractive market with a massive growth potential, only few biopolymers are produced at a commercial scale, yet.\textsuperscript{23} These are mainly polylactic acid (PLA), polyhydroxyalkanoates (PHA), starch blends and cellulose (\textit{e.g.} Cellophane).\textsuperscript{23,32,38-40} The reason for this is twofold: (1) polymers such as PLA or PHA can be processed with various well established and widespread procedures used in the synthetic polymer industry (\textit{e.g.} extrusion, injection molding); (2) these materials are well explored and have a long tradition. PLA was discovered already in 1845 and PHA was characterized in 1920.\textsuperscript{21} The first cellulose thermoplastic dates back to 1870 and research on cellulose or its derivatives generated relevant progress in production technology – \textit{e.g.} the development of the lyocell process for cellulose fiber production as an environmentally friendly substitute to the viscose process.\textsuperscript{39} Cellulose and its disintegration products (cellulose micro- and nanofibrils) are increasingly considered as biodegradable additive in polymer reinforcement.\textsuperscript{41-43}

1.3. Proteins from agricultural waste as building blocks for biopolymers

1.3.1. Proteins

Proteins, or polypeptides, are a fundamental class of biomacromolecules, which are characteristic for all living systems and make up over 50\% of the dry weight of cells.\textsuperscript{44} All organisms apply the same range of 20 amino acids to synthesize protein chains, in which the amino acids are linked by peptide bonds.\textsuperscript{45} This linear sequence is known as primary structure; the secondary structure refers to the spatial arrangements of the backbone; the tertiary structure is the structure of an entire protein; and the quaternary structure refers to the structure of proteins containing more than one polypeptide chain.\textsuperscript{44,46}

The structural diversity likewise creates an immense range of protein functions. Figure 1-3 summarizes some of the most important functional roles.
1.3.2. Proteins as a resource for the biopolymer industry – a renaissance?

While materials such as PLA cause a veritable hype and are strongly promoted, other biopolymers fell into oblivion in the last few decades. For instance, proteins nowadays are of virtually no importance as raw material in the industrial polymer production. This was different in the late nineteenth and early twentieth century: proteins from soybean, corn or milk were commercially processed to plastics.\textsuperscript{47-49} Prime examples of this era are a Ford car with molded plastic panels on the basis of soybean protein in 1939 or various products from formaldehyde-hardened casein, such as buttons or jewelry.\textsuperscript{16} The World Wars I and II intensified the demand for new substances as conventional materials were lacking.\textsuperscript{48} It is well documented that the scarcity of wool was the driving force for the development of various substitute protein fibers.\textsuperscript{48, 50} Due to the launch of synthetic polymers after World War II, protein plastics suddenly disappeared; the petroleum-based polymers were cheaper, easier to produce and better performing.\textsuperscript{16, 48}

In the last decade, however, as a result of the interest in “green” polymers, research on proteinaceous material has impressively increased. As summarized by Cuq et al. (1998), numerous vegetable and animal proteins, so called agro-proteins, are investigated as polymer resource: zein (corn), gluten (wheat), soybean proteins, peanut proteins, milk proteins, collagen and gelatin, keratin, egg albumin or myofibrillar proteins.\textsuperscript{51}

What justifies research in protein-based polymers today?

Proteins are highly complex macromolecules built by nature (see chapter 1.3.1.). The processing of protein into technical products involves its isolation from the raw material, purification and modification (if needed), followed by the manufacturing procedure. In
comparison, the manufacturing of a polymer such as PLA requires a more complex workflow: sugars are fermented to obtain lactic acid, the fermentation broth is then down-stream processed (filtration, evaporation, recrystallization, acidification) prior to lactic acid polymerization. As indicated in Figure 1-1, the direct isolation of natural polymers from renewable resources makes the breaking down of the polymer into monomers dispensable and thus minimizes energy consumption in the industrial process.

1.3.3. Waste proteins from agriculture

In the biosynthesis of PLA or PHA, valuable supplies are diverted from the intended use, i.e. the food chain and livestock feed. At the same time, the bioethanol industry requires the same raw materials, typically some form of crop with high carbohydrate content. The high demand for energy crops coupled with financial speculation can raise the prize for staple food; this was monitored in the “Mexican Tortilla Crisis” in 2007. From this point of view, it would be beneficial to rely on renewable resources, which do not interfere with the food chain.

The agricultural and food industries generate large quantities of waste materials and byproducts rich in proteins, carbohydrates or oils. Along the food chain, valuable biomass is wasted from agricultural production to the final consumption. With estimated 1.3 billion tons per year, the global loss of food is tremendous. These wastes find limited industrial application and are not in competition with raw materials of major industries. However, a lot of waste biomass is used as inexpensive cattle feed, crop fertilizer or as source for biofuel. Interest in revalorizing waste biomass sources as additive or proper polymer, respectively, is increasing, and specific applications in the food packaging sector are proposed. According to Xu and Yang (2012), “turning these waste materials into profitable industrial products, such as bioplastics, will reduce cost for pollution-free treatments, save resources, energy and land, and increase value-added to the related industries”.

In terms of waste proteins, there are several types which accumulate in large quantities and comprise interesting properties: e.g. keratin from poultry feathers; casein from skimmed milk, remaining after removal of the butterfat; zein as a byproduct from corn starch manufacture; peanut and soybean protein, remaining unused after oil extraction. Another protein of particular interest is collagen, because it is largely available from meat industry byproducts. The latter consist of animal tissues such as bones, skin, hair or hides. Annually, approximately
25 million tons of animal byproducts are generated worldwide.\textsuperscript{59} In the European Union alone, waste from meat industry is estimated at 10 million tons.\textsuperscript{65}

### 1.3.4. Collagen structure

Collagen is the essential structural protein in all connective tissues of vertebrates and invertebrates.\textsuperscript{66-69} In mammals it amounts to about 25 - 35\% of total protein.\textsuperscript{45} As a structural protein, collagen is strong, stiff and water insoluble.\textsuperscript{46} The fibrous protein has a highly organized structure with an exceptional primary structure of repeating triplets (Gly - X - Y), in which X is often represented by proline, and Y frequently contains the proline-derivative hydroxyproline (\textbf{Figure 1-4a}).\textsuperscript{45} As a consequence, collagen has a high content of glycine (~ 33\%) and about 25\% imino acids (proline and hydroxyproline).\textsuperscript{68}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{collagen_structure.png}
\caption{Collagen at different length scales and levels of order. (a) The primary structure stands out due to the periodic triplet Gly - X - Y. (b) A collagen chain is folded into a left-handed helix (secondary structure). (c) Three helices form a right-handed super-helix, the so-called tropocollagen (tertiary structure). (d) Multiple tropocollagens build up a collagen microfibril, as e.g. found in the skin or tendons (quaternary structure).}
\end{figure}

Pioneering work on the structure analysis was conducted by G. N. Ramachandran and Kartha as well as Rich and Crick in the 1950s.\textsuperscript{70-73} But for the most part, the uncovering of the secondary and tertiary structure must be accredited to Ramachandran, because it was his work that gave rise to the famous coiled coil structure.\textsuperscript{74} The coiled coil structure is built up from...
three left-handed helices (secondary structure), which together are twisted into a right-handed triple helix (tertiary structure), the so called tropocollagen (Figures 1-4b and 1-4c).\textsuperscript{75-76} Hydrogen bonds are responsible for the stabilization of the tertiary structure.\textsuperscript{45, 69, 76} Finally, tropocollagens assemble into staggered arrays and build microfibrils (quaternary structure) stabilized by hydrophobic interactions (Figure 1-4d).\textsuperscript{46} The biosynthesis of collagen also involves covalent crosslinking of the protein chains. Lysyl oxidases convert lysine or hydroxylysine to an aldehyde that condenses with other aldehydes or unreacted lysines (Schiff bases).\textsuperscript{46, 77}

There is not one collagen, though. More than 30 genetically distinct protein chains are known, which assemble into > 20 different collagen varieties; the most abundant, Type 1, has a molecular weight of nearly 300 kDa and is composed from two so called α\textsubscript{1}(I) chains and one α\textsubscript{2}(I) chain.\textsuperscript{44, 46, 75}

1.3.5. Collagen and its transition to gelatin

Converting the water-insoluble collagen to water-soluble gelatin is relatively straightforward. In the easiest case, tropocollagen is heated in water, causing the collapse of the triple-helical structure into random coils.\textsuperscript{76} Gelatin can thus be considered to be partially denatured, hydrolyzed collagen, including any semicrystalline or amorphous state of collagen.\textsuperscript{76, 78-79} On an industrial scale, gelatin production involves (1) the removal of non-collagenous components from the raw material, (2) the conversion of the collagen to gelatin and (3) the recovery and drying of the gelatin.\textsuperscript{80} The process is described in detail by Veis (1964) or Schrieber and Gareis (2007).\textsuperscript{80-81} Briefly, the industrial extraction is either conducted under acid or alkaline conditions: pig skins – one of the most often used raw materials for gelatin production – are normally processed under acid conditions, yielding gelatin type A. After soaking the skins in inorganic acid solutions for about a day, they are conditioned at pH 4 and gelatin is extracted by means of heating. For type B gelatin production, the raw material (often bovine bones or hides) are conditioned in an alkaline bath for several weeks to months. After pH lowering, the gelatin can also be extracted by heating steps.

1.3.6. Gel formation in aqueous gelatin solutions

Gelatin readily dissolves in water at elevated temperatures (\textit{e.g.} 40 - 50 °C) and protein chains thereby switch from the helix to a random coil conformation.\textsuperscript{82} Gelatin solutions are remarkable since they undergo a thermoreversible coil to helix transition upon cooling below ~ 30 °C.\textsuperscript{82-84} That is to say, the protein chains partially intertwine and revert to ordered
structures with the same conformation as the tropocollagen. Yannas (1972) specifies that the quaternary structure is not restored and that in hot-cast gelation solutions \( (i.e. \) above 35 °C) no partial “renaturation” to the collagen-like structure occurs as the protein chains retain the random coil conformation (amorphous). Furthermore, Guo et al. (2003) and Djabourov (1988) describe the formation of triple helices by intramolecular hydrogen bonds at low gelatin concentrations. At higher protein concentrations (> 1 wt%), additional intermolecular hydrogen bonds stabilize the chains at junction zones, leading to network formation. This process is called gelation.

The viscoelastic properties and the ability to gel make gelatin solutions very versatile. For this reason, gelatin is broadly applied in industry and household as \( e.g. \) foaming agent, stabilizer, texturizing agent, emulsifier, encapsulation agent, fining agent, glue, plasticizer, corrosion inhibitor, molded item, (edible) film, coating, analytical tool in ballistics, and many more.

1.4. Fiber spinning

1.4.1. History of fiber use

The history of fibers is closely linked with the people’s need for clothing. The first clothes in the Stone Age were based on animal skin, fur or leaves. When people sought smoother textiles, most probably in the Neolithic (~ 4000 BC), they started to employ natural fibers: flax (linen), ramie, jute, hemp, cotton, wool or silk in China. These long and thin fibers were twisted together by hand to produce yarn, which was subsequently woven to flexible cloths on simple looms. Until around 1900, the raw materials remained the same, but the spinning and weaving processes were considerably improved and mechanized. At the same time, textile production, so far a home handicraft, was industrialized. In 1903 the first man-made fiber (cellulosic rayon) was invented, revolutionizing the fiber industry.

Research on fibers from polyamide, polyester, polyacrylnitrile \( etc. \) followed soon after, and the first synthetic fiber from polyamide (nylon) was commercialized in 1939. Man-made fibers have remarkable chemical and mechanical properties while at the same time being cheap. This led to a vast boom: while in 1950 roughly 70’000 tons of synthetic fibers (mainly nylon) were produced, the production increased to 2 megatons in 1970. Nowadays, the annual global production of synthetic fibers (~ 40 megatons) exceeds the production of natural fibers (~ 30 megatons; mainly cotton, wool, man-made cellulosic fibers).
1.4.2. Fiber classification

A well-founded classification of common fibers is proposed by Cook (1984). The corresponding scheme is illustrated in Figure 1-5 and explained below.

![Classification of natural and man-made fibers](image)

**Figure 1-5: Classification of natural and man-made fibers (adapted from Cook (1984)).**

Fibers are generally divided into two main categories: natural fibers and man-made fibers. With the exception of silk, all natural fibers are staple fiber, *i.e.* they have a limited length (millimeters to centimeters). Man-made fibers are continuous filaments, which can either be used as such or cut into staple fibers. On this account, the term “spinning” may be ambiguous, describing (1) the production of a filament (a fiber of continuous length) from synthetic or natural polymers and (2) the conversion of natural or man-made fibers into yarns (*e.g.* by twisting).

The next level of classification is based on the fiber’s raw material. Natural fibers are subdivided into cellulosic vegetable fibers (cotton, jute, hemp, flax *etc.*), proteinaceous animal fibers (wool, other hair fibers or silk) and mineral fibers (asbestos). Man-made filaments can be produced from natural or fossil fuel-based material. From a commercial point of view, cellulosic fibers are the only noteworthy man-made natural fiber to date.
1.4.3. Filament formation

The focus of this thesis is the production of man-made gelatin fibers. The principal techniques for filament production are specified below.

The processes for man-made fiber production are generally divided into either solution spinning or melt spinning.\(^6\) In the early days of filament spinning, it was attempted to spin natural polymers (proteins, cellulose). Those polymers decompose at high temperature, so they had to be processed in solution.\(^{98}\) That was the beginning of solution spinning. With the emergence of thermoplastic polymers, another spinning process was developed: melt spinning.\(^{98}\) The processes are compared in Figure 1-6.

![Figure 1-6: Illustration of the well-established spinning principles: melt spinning and solution spinning (dry or wet). Wet spinning is either performed with or without an air gap between spinning nozzle (spinneret) and solidification bath.](image)

At present, the most common synthetic fibers such as polyethylene terephthalate or nylon are produced by melt spinning.\(^{99-100}\) By heating the polymer in \textit{e.g.} an extruder, the polymer melt can be metered through a machined gear pump. It is then pressed through small orifices of a spinneret.\(^{100}\) The so formed filaments are quenched in a cool air stream.\(^6\) Advantages of this process are the very high production rate (\textit{> several thousand meters per minute}) and the relatively low cost, as no solvents are applied.\(^{100-101}\)
In solution spinning, the polymer is first dissolved in a suitable solvent. The viscous polymer solution, also called “dope”, is then extruded through a spinneret and fixed by the removal of the solvent.\textsuperscript{98, 100} Solution spun fibers typically include cellulosic fibers, polyvinyl chloride, acrylic fibers, spandex (an elastic polyurethane-based fiber).\textsuperscript{99–100} Two solution spinning methods are distinguished: dry and wet spinning, respectively.

In dry spinning, the highly volatile solvent is evaporated.\textsuperscript{99, 102} After leaving the spinneret, the filament increases in polymer concentration.\textsuperscript{102} A heated gas stream may accelerate the solidification.\textsuperscript{100} The production rate of industrial dry spinning is between 100 - 800 meters per minute.\textsuperscript{101}

In wet spinning, the polymer solution is extruded into a solidification bath containing a non-solvent. Thereby, solvent from the polymer solution is extracted and the filaments precipitate.\textsuperscript{100–101} Many different designs for wet spinning exist. As illustrated in Figure 1-6, the spinneret can be directly immersed in the coagulation bath or placed above an air gap. The latter is virtually a combination of dry and wet spinning and is therefore often referred to as dry-wet spinning.\textsuperscript{103} Occasionally, the term “gel spinning” is used as well.\textsuperscript{101} Because of the drag forces in the coagulation bath, wet spinning is generally the process with the lowest production rate (50 - 300 meters per minute).\textsuperscript{101} Nevertheless, wet spinning, and especially the dry-wet (or gel) spinning, is getting ever more important in the production of high-performance fibers.\textsuperscript{98, 104}

The major drawback of solution spinning methods is the use of organic solvents: volatile organic compounds (VOC) are emitted, the solvents are expensive and need to be recycled.\textsuperscript{100}

1.4.4. Polymer chain orientation in man-made fibers

The spinning methods presented in chapter 1.4.3. have a common flaw: after solidification of the fibers, they exhibit poor tensile properties.\textsuperscript{98} This is explained by the random orientation of polymer chains. Obviously, the macromolecules are not sufficiently oriented in the extrusion step. Instead, the die swell behavior (the polymer thread expands after exiting the orifice due to polymer chain relaxing) induces disorder.\textsuperscript{98, 105}

To improve the fibers’ mechanical properties in comparison to the bulk material, they must be subjected to a drawing step. On an industrial scale, continuous fiber drawing is mostly performed directly after spinning with the help of rolls (see Figure 1-6). The transfer of the fiber from the feed roll to drawing rolls with higher surface speeds results in longitudinal
Drawing is performed in ambient or hot air, in contact with hot rolls or in liquid baths.\textsuperscript{101} The effect of fiber drawing is illustrated in Figure 1-7a. The long polymer molecules are aligned along the longitudinal axis of the fibers.\textsuperscript{62} If the molecules are closely packed due to the applied strain, regions of crystallinity can arise. Between crystalline regions there are amorphous areas with only partially aligned molecules.

\textbf{Figure 1-7:} Orientation of polymer chains. (a) Filaments are subjected to drawing in order to align the polymer chains. (b) Enhanced alignment (as e.g. observed by X-ray scattering) goes in line with (c) the improvement of mechanical properties (e.g. strength, stiffness, strain at break, toughness).

The molecular alignment is manifested in e.g. an anisotropic X-ray scattering pattern (Figure 1-7b).\textsuperscript{98} This change in macromolecular structure goes along with altered properties. Tensile strength and stiffness of the fiber increase with better alignment and closer packaging of the molecules; at the same time, strain at break decreases (Figure 1-7c).\textsuperscript{62} The visual appearance of fibers is also modified: unoriented fibers are mostly opaque, while oriented fibers exhibit an attractive luster.\textsuperscript{62}
1.5. Conclusions

In the thesis at hand, the emphasis is on a niche product, namely man-made fibers from a waste protein (gelatin). Such fibers were produced at the beginning of the 19th century and soon after replaced by synthetics. Nowadays, interest in reusing waste streams is rising, and there is a clear need for sustainable, bio-based fibers.

It is concluded that transforming proteins of animal or vegetable origin into biomaterials is a desirable strategy. (1) Protein resources from agricultural waste are huge. (2) Proteins can directly be isolated from the raw material and do not need to be converted to monomers. (3) The use of waste proteins does not interfere with the food chain. (4) Furthermore, protein fibers are said to have a pleasant appearance, good dyeability and a favorable feeling.

To date, due to property constraints and cost, gelatin is not widely used as technical polymer. As mentioned by Schrieber and Gareis (2007), it is only a question of time and scarcity of petroleum, though, that technical polymers from gelatin will be implemented as an eligible alternative to synthetic polymers.
2. Spinning Angora Rabbit Wool-Like Porous Fibers from a Non-Equilibrated Gelatin/Water/2-Propanol Mixture

Published in parts as:

Philip R. Stoessel, Robert N. Grass, Antoni Sánchez-Ferrer, Roland Fuhrer, Thomas Schweizer, Raffaele Mezzenga, Wendelin J. Stark

2.1. Introduction

Along with other specialty animal fibers such as cashmere, mohair or alpaca, angora rabbit fibers make up for some of the highest quality animal fibers of textile relevance.\cite{107-110} Angora rabbit fibers stand out because of their shininess, small fiber diameter and the presence of a lattice-type medulla.\cite{107, 109-111} The latter is a hollow or partially filled central canal running lengthwise the fibers, yielding a porous fiber structure and decreased fiber density.\cite{109, 112} In most animal fibers, medullated fibers are contaminants and have adverse effects on its value and end-use potential.\cite{112-113} In contrast, angora rabbit fiber profits from the medulla structure in terms of superior insulation properties.\cite{111} Despite their extraordinary properties, angora rabbit fibers are not suited for applications on a technical scale due to the limited natural availability (global production of about 10,000 tons per year) and high price (approximately 20 $ kg$^{-1}).\cite{114} There is a clear need for fibers which combine high porosity and good mechanical stability at the same time. Ideally, the fibers should be built up from biopolymers as there is a permanent demand for environmentally friendly products.\cite{18, 115} A lot of biopolymers \textit{(e.g.} polylactic acid\textit{)} are synthetically produced from natural monomers and rely on starch sources such as corn or sugar cane.\cite{18} Thus, they may compete with the food chain and endanger food security. To our understanding, it is beneficial to produce biopolymers from waste materials, \textit{e.g.} proteins which are normally discarded. Proteins and peptides are extremely versatile building blocks for the fabrication of biomaterials.\cite{116} A fibrous protein of high relevance is collagen, the most abundant structural protein in the extracellular matrix of multicellular animals and in vertebrates where it accounts for about 30 - 60 wt\% of the total body protein.\cite{80, 117-118} Thus, collagen is a major component in animal slaughterhouse waste (including hides, bones, and tendon) of which large proportions are discarded.\cite{50, 119-121} With more than 10 million tons of slaughterhouse waste per year in the European Union alone, animal by-products are an immense source of collagen.\cite{65} If the water insoluble, quasi-crystalline collagen is partially denatured by thermal and chemical degradation, gelatin is prepared.\cite{67, 80, 119} Conversion of collagen to water-soluble gelatin involves breaking covalent interchain and hydrogen bondings. The network of linked tropocollagen units is then transformed to a water-soluble system consisting of random chains with a much lower degree of internal order.\cite{68, 80, 122} A recent study has put emphasis on the importance of applying materials science principles to understand biological materials: for collagen, the hierarchical structure consists of polypeptides, tropocollagen, fibrils and collagen fibers which allows predictions on the deformation behavior of collagen.\cite{118} The sol-gel transition of gelatin involves protein chains to switch from the random coil conformation to the native, collagen-
like triple-helical structure. If the triple helices are precisely aligned (e.g. by inducing stress), a renatured, collagen-like structure may be obtained. It is expected that the induced anisotropy improves the mechanical performance of gelatin-based materials, which in their isotropic status is mechanically weak.

In order to meet the introductory needs – fiber porosity and good mechanical properties as found in angora rabbit wool – a new fiber spinning process was developed. In a first step, a ternary mixture from gelatin, water and 2-propanol was prepared. This mixture is remarkable as it separates into two phases due to protein precipitation. The precipitate stands out because of its sponge-like structure and very good spinnability, which enabled simple spinning into a porous monofilament. Figure 2-1 highlights the novelty of this process, namely the porous nature of the gelatin fibers as a result of the sponge-like structure of the protein precipitate. Gelatin or collagen fiber spinning has mostly been carried out by means of wet spinning, gel spinning or electrospinning; these technological approaches produce dense protein fibers. In contrast, we opted for a dry spinning process, where the solidification of the polymer solution was achieved by solvent evaporation. Owing to the high protein content and limited mobility of protein chains in the precipitate, one might argue that the presented process also has analogies to gel spinning. By continuously drawing the fiber in process, anisotropy was induced, which led to improved mechanical properties.

![Figure 2-1](image-url)  
*Figure 2-1. Dry spinning of a gelatin precipitate from a ternary mixture (gelatin/water/2-propanol) allowed continuous spinning of a porous protein fiber.*
In this study, we demonstrate a reliable and continuous spinning process which allows producing porous biopolymer fibers in the kilometer scale. Despite their high porosity, the here obtained gelatin fibers attain mechanical performance similar to other bio- (e.g. wool, tendon collagen) and synthetic polymers (e.g. Polytetrafluoroethylene). Moreover, the fibers’ degradability can be tuned by different crosslinking treatments. These promising results may pave the way to the large-scale production of artificial angora rabbit wool-like fibers.

2.2. Experimental section

2.2.1 Preparation of gelatin/water/2-propanol mixture and spinning process

Type A gelatin from porcine skin (G2500, ~ 300 g Bloom strength) and 2-propanol (HPLC-grade) were used as received from Sigma-Aldrich. Deionized water (40 wt%) and 2-propanol (50 wt%) were added to gelatin powder (10 wt%). The mixture was tempered in a water bath at 50 °C for 30 min and shaken by hand every 5 min. This yielded a 2-phase system with an opaque, gelatin-rich lower phase (spinning phase) and an upper solvent-rich phase (supernatant). Subsequent to sedimentation (3 min), the supernatant was decanted and the lower gelatin-rich phase was transferred to a plastic syringe (25 mL), tempered at 45 °C. As illustrated in Figure 2-2, the spinning phase was extruded as a monofilament through a tempered syringe nozzle (35 °C) into a vertical spinning tube (50 ± 5 % relative humidity, 23 ± 2 °C) where evaporation and unspecific drawing by gravitational force took place. Active fiber drawing and evaporation of residual solvent was achieved by transferring the fiber onto a drawing device with motorized rollers. The speed of roller 1 was adjusted to match the speed of the falling fiber (~ 16 rpm). The successive rollers were each accelerated by a factor of 1.25. The ratio of different roller speeds was defined as draw ratio λ, ranging from \( \lambda = 1.25 \) (roller 2), \( \lambda = 1.56 \) (roller 3), \( \lambda = 1.95 \) (roller 4) to \( \lambda = 2.4 \) (roller 5). Depending on the chosen roller for fiber uptake, gelatin fibers at different draw ratios were prepared. For the purpose of reference, fiber samples were also taken directly after the syringe nozzle and denoted with \( \lambda = “0” \).
2.2.2. Crosslinking treatment and swelling experiments

Gas-phase crosslinking of gelatin fibers ($\lambda = 2$) was achieved by exposing them to formaldehyde (10 mL, Sigma-Aldrich, > 34.5 wt%) in a desiccator. The treatment was conducted for either 1 h or 15 h at room temperature ($23 \pm 2 ^\circ C$). An aliquot of FA crosslinked fibers (15 h) was further subjected to dehydrothermal treatment (DHT). The fibers were placed in a vacuum oven ($120 ^\circ C$, 50 mbar) for 10 h. After crosslinking, the fibers were equilibrated at room temperature and $45 \pm 5 \%$ relative humidity. Fibers with no, 1 h FA(g), 15 h FA(g) and 15 h FA(g)&DHT treatment were swelled in deionized water for 24 h. The mechanical properties (elastic modulus, true tensile strength) were measured before, directly after swelling (wet state) and after swelling with subsequent drying at $45 \pm 5 \%$ relative humidity (dry state).
2.2.3. Characterization of gelatin/water/2-propanol mixture

The gelatin/water/2-propanol mixture was kept in a water bath at 50 °C and sampled after different storage times of 30 min, 4 days and 30 days. The supernatant was centrifuged (15’000 g, 20 sec) and a precise amount of 1-octanol (Fluka, puriss.) was added as internal standard. The spinning phase was transferred into a round bottom flask and distilled into a second flask containing a defined amount of 1-octanol as internal standard. The solvent samples were analyzed using a Hewlett Packard 6890 Series gas chromatograph equipped with a phenyl methyl siloxane column (HP-5MS) and a flame ionization detector (GC-FID). Helium was used as carrier gas (1 mL min\(^{-1}\)) and the oven temperature was increased from 50 to 250 °C at a rate of 20 °C min\(^{-1}\). An appropriate GC-FID response factor was determined to quantify 2-propanol and 1-octanol. The dry weight of gelatin was gravimetrically determined. The water content was calculated as the residual weight percentage.

Rheological measurements were performed on a Paar Physica MCR300 rheometer equipped with a cone-plate geometry (diameter 30 mm, cone-angle 4°) and a Peltier element (set at 50 °C). The lower phase of a fresh gelatin/water/2-propanol mixture (spinning phase) and a 27 wt% aqueous gelatin solution (reference) were sheared from 0.1 to 22 s\(^{-1}\) and the flow curves were recorded.

2.2.4. Fiber characterization

Gelatin fibers were stored at room temperature (23 ± 2 °C) and constant humidity (45 ± 5 % relative humidity) for 2 days. Tensile tests (n = 6) were conducted according to ASTM D3822-07 on a Shimadzu Universal Testing Instrument AGS-X equipped with a 100 N load cell and pneumatic clamps. Fiber specimens (40 ± 1 mm) were tested at constant speed (24 mm min\(^{-1}\)). The cross-sectional area of every specimen was optically measured by light microscopy (Zeiss Axio Imager.M2m) and the appropriate dimension, representing the diameter of a corresponding circle, was calculated. In selected fiber samples, the cross-sectional area was cross checked by scanning electron microscopy (SEM, FEI, NovaNanoSEM 450) and imaging software (ImageJ, version 1.46). The porosity was not accounted for, though. Instead, the bulk apparent mechanical properties (elastic modulus (between 0.1 and 0.5 % strain), true tensile strength, true strain at break, toughness (area under the curve)) were determined from the stress vs. strain curves.

Wide-angle X-ray scattering (WAXS) experiments were performed using a Rigaku MicroMax-002+ microfocused beam (4 kW, 45 kV, 0.88 mA). The Cu Kα radiation (\(\lambda_{\text{Cu Kα}}\))
= 1.5418 Å) was collimated by three pinhole collimators and a bundle of parallel-aligned fibers was vertically placed to the detector. The scattered X-ray intensity was detected by a Fuji Film BAS-MS 2025 imaging plate system. An effective scattering-vector range of \(0.5 \text{ nm}^{-1} < q < 25 \text{ nm}^{-1}\) was obtained, where \(q\) is the scattering wave vector defined as \(q = 4\pi \sin\theta / \lambda_{\text{Cu K}_\alpha}\), with a scattering angle of 20. From the scattering intensities, the order parameter \(S\) was calculated. The order parameter \(S\) was determined according to Lovell and Mitchell and is given in the Appendix A.1., equation (2-1).

The fibers’ morphology was investigated by means of scanning electron microscopy (FEI Nova NanoSEM 450, 5 kV). The samples were sputter-coated with a 3 - 4 nm platinum layer (Leica EM SCD005). Cross-sections of gelatin fibers were obtained by freezing a fiber sample in liquid nitrogen for 30 s followed by cutting with a scalpel. Imaging software (ImageJ, version 1.46) was used to determine the cross-sectional area, the average pore diameter and the pore area for porosity calculation. The porosity was calculated by dividing the pore area by the total cross-sectional area.

2.3. Results and discussion

2.3.1. Characterization of the gelatin/water/2-propanol mixture

**Appearance and composition:** Upon addition of 2-propanol (50 wt%) to deionized water (40 wt%) and gelatin powder (10 wt%) at 50 °C, the ternary system underwent a phase separation (Figure 2-3). This is in line with previous reports on the addition of a water-miscible organic solvent to aqueous gelatin solutions leading to precipitation of the protein. This effect is often made use of in the context of nanoprecipitation for the production of polymeric nanoparticles.\(^{102, 131-132}\) In a study on poly(L-lactic acid) fibers, Qi *et al.* (2009) similarly characterized a ternary system of poly(L-lactic acid)/solvent/non-solvent, which underwent phase separation while being electrospun into nanofibers.\(^{133}\) In the present work, a two-phase system with a lower gelatin-rich opaque phase (spinning phase) and an upper solvent-rich phase (supernatant) was obtained. Observing the gelatin/water/2-propanol mixture at 50 °C over a prolonged storage time without shaking indicated that the system was not in an equilibrium state (Figure 2-3a): after several days, the spinning phase compacted, became transparent (similar to an aqueous gelatin solution) and could no longer be spun. In order to again reach the non-equilibrium state, the stored mixture could simply be shaken up and dry spinning of the opaque spinning phase was again possible and resulted in the same porous fibers. Gas chromatography (GC) and gravimetric analysis were used to determine the
composition of the two-phase system at 50 °C over the storage time. The respective results are summarized in Table 2-1. From these numbers it follows that 2-propanol enriched in the supernatant over the storage time. Due to compacting of the spinning phase, gelatin and water percentage increased therein.

**Figure 2-3.** (a) Preparation of a two-phase, non-equilibrium gelatin/water/2-propanol mixture (top left). The opaque, lower phase (spinning phase) was fed to a spinning process, while the supernatant was discarded. Stability of the spinning raw material solution: after 30 days of storage at 50 °C the mixture became homogeneous (equilibration) and could no longer be spun. (b) 300 meters of gelatin fiber spun at a draw ratio \( \lambda = 1.6 \). (c) Yarn of 10 monofilaments (\( \lambda = 2 \)) easily holding a weight of 140 g (17.8 MPa).

**Table 2-1.** Non-equilibrium, two-phase gelatin/water/2-propanol mixture; composition after different storage times. In the spinning process, the spinning phase after 30 minutes was used.

<table>
<thead>
<tr>
<th>Sample after 30 min \ (3 min sedimentation)</th>
<th>Sample after 4 days</th>
<th>Sample after 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinning phase</td>
<td>Supernatant</td>
<td>Spinning phase</td>
</tr>
<tr>
<td>Gelatin (wt%)</td>
<td>27.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Water (wt%)</td>
<td>41.2</td>
<td>46.8</td>
</tr>
<tr>
<td>2-propanol (wt%)</td>
<td>31.8</td>
<td>53.1</td>
</tr>
</tbody>
</table>

**Rheology:** Flow curves of the spinning phase and of an aqueous gelatin solution were recorded (see Appendix A.1., Figure A1-1). The protein content was equal in both samples in order to permit comparison (27 wt% gelatin). The ternary spinning phase (gelatin/water/2-propanol) showed a higher zero-shear viscosity \( \eta_0 \) \ (9.1 Pa·s) than the reference gelatin solution \ (1.3 Pa·s)\. Additionally, a more prominent shear-thinning behavior was observed in the spinning phase. This is another indication that the organic solvent induced severe changes.
in the protein structure, which in turn resulted in exceptional spinnability and properties of the spinning phase. While destabilizing the native protein structure, organic solvents can stabilize secondary structures; it was e.g. found that denatured myoglobin at 70 % ethanol concentration possesses as many helical structures as the native protein.\textsuperscript{134-135} This could explain the rheological behavior of our samples: while the aqueous gelatin solution was dominated by random peptide chains, the precipitated gelatin/water/2-propanol mixture contained structures of higher order, e.g. (triple-)helical structures and/or protein aggregates. This resulted in a higher $\eta_0$ and distinctive shear-thinning.

2.3.2. Characterization of non-crosslinked gelatin fibers

Gelatin fibers were obtained by extruding the spinning phase through a nozzle. Unspecific drawing was achieved by gravitational force and active drawing (denoted by $\lambda$) was achieved with the help of motorized rollers. In Figure 2-3b, a long gelatin monofilament (draw ratio $\lambda = 1.6$, diameter $\sim 110 \text{ \mu m}$) is depicted. Figure 2-3c shows a manually fabricated yarn (10 filaments), holding a balance weight.

*Mechanical properties:* Exemplary true stress vs. true strain curves for gelatin fibers are indicated in Figure 2-4.

![Figure 2-4](image.png)

*Figure 2-4.* Illustrative true stress vs. true strain curves for selected gelatin fibers prepared at different draw ratios $\lambda$ (the ratio of different roller speeds, during active drawing). Reference fiber samples denoted with $\lambda = “0”$ were taken directly after the syringe. The inset displays an enlargement of the tensile test curves.
All fibers ($\lambda \geq 1.25$) showed a yield point followed by strain hardening. It is common for biopolymers to show an inflection point in the stress vs. strain curve.\textsuperscript{118} In their measurements of \textit{A. pernyi} silk fiber, Fu \textit{et al.} (2011) also determined a distinct yield point and strain hardening.\textsuperscript{136} After leaving the spinning nozzle, the gelatin fiber underwent a sol-gel transformation and the so-called renaturation process took place, leading to network formation of triple-helical, pseudo-crystalline structures or single protein chains.\textsuperscript{79-80, 82, 123} The stretching/drawing of the film or fiber can either be done during or after gelation. In the latter case, the fibers are \textit{e.g.} swollen in a hydrophilic solvent to make them stretchable. In our spinning process, the fibers are directly subjected to drawing (combination of unspecific and active drawing) in order to make the process attractive for a continuous large-scale production. Several reports have demonstrated that the mechanical properties of gelatin fibers or films can be improved by drawing because (1) segmental orientation of the fibrous protein is induced, (2) the development of triple-helical structures is facilitated and because (3) the overall material structure is improved (reduction in size and quantity of defects).\textsuperscript{120, 125-126, 128, 137-139}

These findings could be confirmed in the present study as the mechanical properties were clearly enhanced by drawing the fibers. The average values of the apparent mechanical parameters are summarized in Figure 2-5; toughness values are given in the Appendix A.1., Figure A1-2. As a consequence of mass conservation, the fiber diameter (corresponding to a circular cross-section) decreased from 240 ± 22 µm ($\lambda =$ “0”) to 96 ± 16 µm ($\lambda = 2.4$). By increasing the draw ratio, the elastic modulus increased from 1650 ± 356 MPa ($\lambda =$ “0”) to 2604 ± 282 MPa ($\lambda = 2.4$), the true tensile strength increased more than threefold from 51 ± 12 MPa ($\lambda =$ “0”) to 161 ± 10 MPa ($\lambda = 2.0$), the true strain at break from 4 ± 1 % ($\lambda =$ “0”) to 68 ± 15 % ($\lambda = 1.25$) and the toughness from 3 ± 5 MPa ($\lambda =$ “0”) to 62 ± 6 MPa ($\lambda = 2.0$). It must be noted that the above values have not been corrected for fiber porosity and that the effective mechanical properties would be significantly higher. While the elastic modulus reached a maximum at the highest draw ratio ($\lambda = 2.4$), maximal strength, strain and toughness were obtained at slightly lower drawing. Compared to other draw ratios, the fibers with $\lambda = 2.4$ frequently broke during the spinning process. It is assumed that they were overstrained, leading to structural defects, brittleness and lower strength. Altogether, drawing the fibers at $\lambda = 2$ delivered the best results with simultaneous high modulus, strength, strain at break and toughness. This is of particular interest because most materials show a clear strength-toughness trade-off and only some biological composite materials have high strength and toughness at the same time.\textsuperscript{139}
The above mechanical properties of gelatin fibers are on par with other biopolymers, biomaterials or some synthetic polymers.\textsuperscript{140-142} Fukae and Midorikawa (2008) have reported a method for gel spinning non-porous gelatin fibers with the help of dimethyl sulfoxide, ethylene glycol or glycerol as solvent and methanol as coagulation agent, resulting in non-crosslinked fibers with elastic moduli of up to 3100 MPa and tensile strength of up to 157 MPa.\textsuperscript{127} With an adapted method, Midorikawa \textit{et al.} (2012) achieved even higher mechanical properties (up to 400 MPa tensile strength).\textsuperscript{126} Compared to high-performance fibers such as fibers from carbon nanotubes (CNT)\textsuperscript{143} or polymer composites filled with CNTs or carbon black,\textsuperscript{144} the here presented gelatin fibers may appear weak. It must be noted, though, that high-performance fibers have drawbacks (\textit{e.g.} brittleness, costs, environmental concerns) and for many applications, the gelatin fibers’ properties are sufficient. Particularly, if targeting textile applications (\textit{e.g.} artificial angora rabbit wool), the prepared fibers are similar to natural fibers or hair.\textsuperscript{142, 145}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2-5.png}
\caption{Apparent mechanical properties of non-crosslinked gelatin fibers spun at different draw ratios $\lambda$. (a) Fiber diameter (corresponding to a circular cross-section), (b) elastic modulus, (c) true strength at break and (d) true strain at break. With increasing drawing, the fiber diameter decreased and the mechanical properties increased. Dotted lines indicate reference values for different polymer fibers produced and/or measured by other authors.\textsuperscript{140-142}}
\end{figure}
Wide-angle X-ray scattering (WAXS): Referring to published reports, the X-ray diffraction pattern of native collagen shows three prominent reflections: a strong inner equatorial spacing at $d_1 \approx 10 - 16$ Å ($2\theta \approx 8^\circ$), a diffuse equatorial halo at $d_2 \approx 4.6$ Å ($2\theta \approx 20^\circ$) and a strong meridional spacing at $d_3 \approx 2.9$ Å ($2\theta \approx 31^\circ$). The spacing at $d_3 \approx 2.9$ Å represents the interplanar distance across the fiber axis, corresponding to the mean distance between amino acid residues. The spacing at $d_2 \approx 4.6$ Å is practically the same for all proteins and corresponds to the mean distance between neighboring peptide chains (backbone spacing). The reflection at $d_1 \approx 11$ Å is assigned to the intermolecular lateral packaging of the triple helices. The WAXS patterns of gelatin fibers are depicted in Figure 2-6a. The reflection at 2.9 Å is not visible on the scattering patterns by eye but can be observed in the 1D scattering intensity distribution (Figure 2-6b). The least drawn fibers ($\lambda = 0$ and $\lambda = 1$) showed an isotropic distribution pattern with a diffused ring at $d_2$, indicating that the protein chains were randomly distributed. It is concluded that the unspecific drawing in the spinning tube had a minor effect on the fiber structure. Active drawing on motorized rollers was more effective in inducing orientation: at draw ratios $\lambda \geq 1.25$, the two collagen-characteristic equatorial reflections at $d_1$ and two maxima at $d_2$ appeared. For scattering wave vector $q_1$, this is also illustrated by the azimuthal intensity distributions for different gelatin fiber samples (Figure 2-6c). This anisotropy indicates that the drawn gelatin fibers were partially renatured (coil-to-helix transition) and that the gelatin fibers comprised highly ordered domains. Parts of the protein chains recover their native conformation in the sol-gel transformation; other chains stay in the random coil conformation. However, the single chains can also be oriented. A plot of the spacing distances vs. the draw ratio (Figure 2-6d) revealed, that the spacing distances $d_2$ and $d_3$ stayed constant, while $d_1$ was reduced with increasing fiber drawing from 13.5 to 11.0 Å due to alignment of the triple-helical structures. In order to quantify the anisotropy of the gelatin fibers, the order parameter $S$ corresponding to the $d_1$ spacing (scattering wave vector $q_1 = 5.5$ nm$^{-1}$) and to the $d_2$ spacing (scattering wave vector $q_2 = 14.0$ nm$^{-1}$) was calculated. The results are summarized in Figure 2-6e. For both characteristic spacings, the order parameter increased with increased fiber drawing. Isotropic systems are characterized by $S = 0$, and uniaxially oriented crystalline systems have $S = 1$. For $d_1$ (distance between triple helices), $S_{\text{max}}$ was 0.78 and for $d_2$ (distance between single peptide chains) $S_{\text{max}}$ was 0.41. Accordingly, the gelatin fibers correspond to a highly oriented structure with (triple and single) helical domains aligned along the fiber axis.
Figure 2-6. (a) 2D wide-angle X-ray scattering (WAXS) of gelatin fibers prepared at different draw ratios $\lambda$. The undrawn gelatin fiber ($\lambda = 0$) only showed diffused rings for the reflections corresponding to the spacings $d_1 = 11$ Å and $d_2 = 4.6$ Å, indicating isotropic orientation at these length scales. As soon as the fibers are drawn, the reflections at $d_1$ and $d_2$ get clearer and two-equatorial maxima start to appear. This demonstrates that the developed spinning process induces orientation in the fibrous protein. (b) 1D wide-angle X-ray scattering intensity distribution for a gelatin fiber ($\lambda = 2$). (c) Azimuthal intensity distributions for scattering wave vector $q_1$ of gelatin fibers prepared at different draw ratios. (d) Distances of the three prominent X-ray reflections of gelatin fibers prepared at different draw ratios. While distance $d_1$ decreases with drawing due to alignment of the gelatin’s triple helices, $d_2$ and $d_3$ are constant over the spectrum of differently spun fibers. (e) Order parameter $S$ at the scattering wave vector $q_1$ (corresponding to the distance between triple helices, $d_1 = 1.1$ nm) and $q_2$ (distance between single helices, $d_2 = 4.6$ Å) of the gelatin fibers prepared at different draw ratios.

**Scanning electron microscopy (SEM):** SEM micrographs of a gelatin fiber exhibited elliptical cross-sections and a furrow in the longitudinal direction which is attributed to the nozzle design and the flattening on the rollers (see **Figure 2-7a**). This morphology is in good agreement with other results. Furthermore, the fibers exhibited high porosity (~ 30 %) due to solvent droplets which were enclosed in the spinning phase and evaporated in the spinning process. The average pore size of a gelatin fiber ($\lambda = 2$) was ~ 1 µm. This immediately highlights that the bulk apparent mechanical properties evaluated above, would need to be rescaled by effective volume fractions, pointing at higher values. Featuring good mechanical
properties and high porosity at the same time is highly interesting. To allow comparison of the gelatin fibers to angora rabbit fibers, SEM micrographs of the animal fibers were taken (Figure 2-7b). The presence of the lattice-type medulla, which causes the porous fiber structure, was verified and the similarity to the here produced porous gelatin fibers is evident. As in most animal keratin fibers, the angora rabbit fibers’ surface is characterized by overlapping cuticle cells, whereas the artificial gelatin fibers have a smooth surface. The SEM micrographs in Figure 2-8 show fracture sites of gelatin fibers which were subjected to a tensile test. The breaking site of an unoriented fiber (λ = “0”) was straight and sharp (Figure 2-8a), while for an oriented fiber (λ = 2) it was fringy and fibrous (Figure 2-8b). This result again confirms that anisotropy was introduced by drawing the fiber in the spinning process.

![Figure 2-7. (a) Scanning electron microscopy (SEM) images of a gelatin fiber cross-section (λ = 2). The pore area was measured at different spots and the porosity was determined to be 30 %. (b) SEM micrographs of an angora rabbit fiber clearly show the morphological similarity to the produced porous gelatin fibers.](image-url)
2.3.3. Crosslinking treatment and swelling experiments

Gelatin, as many biopolymers, is water soluble and its properties are highly dependent on the water in the structure.\textsuperscript{118,149} While being advantageous in degradation, the moisture sensitivity limits the application of gelatin products.\textsuperscript{149-150} For this reason, protein structures are often crosslinked to reduce the swelling behavior and the rate of biodegradation. In this study, gaseous formaldehyde, FA(g), was used for chemical crosslinking of gelatin fibers ($\lambda = 2$) during 1 h or 15 h. FA was chosen for its small molecular size (good diffusion), low cost (only a fraction of other crosslinkers) and widespread use in the tanning or pharmaceutical industry.\textsuperscript{151-152} Crosslinking in the gas phase is advantageous over solution-based crosslinking because the anisotropic protein structure is not affected by solvents/swelling. Formaldehyde-treated protein fibers were also physically crosslinked by dehydrothermal treatment (DHT). DHT involves both the removal of residual moisture and the formation of covalent peptide bonds, especially ester and amide bonds.\textsuperscript{153-156}

The appearance and the obtained mechanical properties of (crosslinked) gelatin fibers, which were subjected to swelling in deionized water for 24 hours, are illustrated in Figure 2-9. The non-crosslinked fibers swelled in a matter of seconds, lost shape and became transparent. The swelling of crosslinked fibers (1 h FA(g)) was less pronounced and slower, while the crosslinked fibers (15 h FA(g)) did only minimally swell. As revealed by Figure 2-9a,
non-crosslinked fibers and the 1 h crosslinked fibers evidently sunk in the water because of water up-take. The 15 h crosslinked fibers still floated, indicating that the low density of the porous fiber was maintained and that less water was incorporated into the fiber matrix.

Figure 2-9. Appearance and mechanical properties of gelatin fibers \((\lambda = 2)\) as a function of crosslinking and swelling. (a) Fiber integrity after 24 hours of swelling in deionized water. Non-crosslinked fibers swelled in a matter of minutes and became transparent. Fibers crosslinked for 1 h swelled to a lesser degree while crosslinking for 15 h strongly increased the water-resistance. (b) Fiber diameter (corresponding to a circular cross-section), (c) elastic modulus (*: no elastic regime in the stress vs. strain curve) and (d) true tensile strength of gelatin fibers before swelling, in swelled state (24 hours in deionized water) and after swelling with subsequent drying. By combining formaldehyde crosslinking with a dehydrothermal treatment, the stiffness (elastic modulus) of the swelled fibers could clearly be increased. Interestingly, the mechanical properties after swelling/drying slightly increased compared to the initial results.
The reduction of swelling-degree was additionally monitored by fiber diameter measurements (Figure 2-9b): a fourfold reduction in swelling was observed in well crosslinked fibers (15 h FA(g) & DHT). These findings are in accordance with Fakirov et al. (1996), who stated that crosslinked gelatin is insoluble in water but it is still swollen by it. Drying the swollen samples caused the fibers to shrink, resulting in equal cross-sectional areas as before swelling. The elastic moduli are indicated in Figure 2-9c. When being tested in the wet state, fibers which were not crosslinked or only treated by FA did not show an elastic regime (see inset with schematic stress vs. strain curve in Figure 2-9c). However, combining FA crosslinking and DHT had a distinct effect on wet state stiffness and yielded an elastic modulus of 430 MPa. As shown by Bhushan (2010), human hair also swells in water and the elastic modulus follows a similar pattern as observed in this study: soaking the hair in water decreases the elastic modulus from about 3000 MPa to 500-900 MPa, while subsequent drying reestablishes the original elastic modulus. It is known that formaldehyde exhibits a fast diffusion rate, while having very slow endpoint fixation rates. Working with fish gelatin films and formaldehyde, Fraga and Williams (1985) concluded that the crosslinking reaction has a threshold temperature at 100 °C. Galembeck et al. (1977) concluded that crosslinking proteins with formaldehyde at room temperature and neutrality is slow. This would explain why crosslinking with gaseous formaldehyde at room temperature took several hours, while still not yielding satisfying wet state behavior of the fibers. Obviously, the DHT treatment did also have a positive side effect in increasing the reaction rate of FA.

The true strength at break (Figure 2-9d) strongly decreased due to swelling: for non-crosslinked fibers it was as low as 0.3 MPa, while 15 h FA(g) or 15 h FA(g) & DHT resulted in about 20 MPa strength. Compared to the initial results before swelling, the mechanical properties after swelling and drying increased to a small extent. Presumably, the swelling helped to diminish internal tension and defects in the fibers or the handling of gelatin fibers in swollen state led to further drawing of the fibrous structure.

2.4. Conclusions

The prominent advantages of gelatin are biodegradability, versatility and an immense availability from slaughterhouse waste. Because of its poor mechanical properties, untreated gelatin is hardly able to substitute synthetic polymers. However, it is generally acknowledged that the physical properties can be improved by promoting the renaturation of collagen, i.e. the aligning of triple-helical structures. In this work, a spinning process for the fabrication
of gelatin fibers is proposed, which only requires harmless solvents and minimal energy input. **Table 2-2** summarizes the major characteristics of the new process and relates it to the established methods for gelatin fiber spinning found in the literature.

**Table 2-2. Overview of different spinning processes for gelatin/collagen fiber production.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Process</th>
<th>Principle</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>This work</td>
<td>dry spinning</td>
<td>Spinning of non-equilibrium gelatin/water/2-propanol mixture; simultaneous drawing and solidification</td>
<td>porous fibers</td>
</tr>
<tr>
<td>Midorikawa et al. (2012)(^{126})</td>
<td>gel spinning</td>
<td>spinning gelatin/ethylene glycol gel; coagulation in cold methanol; extraction of ethylene glycol in methanol (10 days); drying</td>
<td>dense fibers</td>
</tr>
<tr>
<td>Fukae and Midorikawa (2008)(^{127})</td>
<td>gel spinning</td>
<td>spinning gelatin/DMSO gel; coagulation in cold methanol; batch-wise drawing of fibers; extraction of DMSO in methanol (10 days); drying</td>
<td>dense fibers</td>
</tr>
<tr>
<td>Meyer et al. (2010)(^{117})</td>
<td>wet spinning</td>
<td>spinning of collagen dispersion; coagulation in ethanol/acetone; drying</td>
<td>dense fibers</td>
</tr>
<tr>
<td>Ayres et al. (2007)(^{128})</td>
<td>electrospinning</td>
<td>gelatin in trifluoroethanol spun at 25 kV accelerating voltage</td>
<td>smooth scaffolds</td>
</tr>
</tbody>
</table>

Unlike the other methods, the here presented process is special owing to the fact that porous fibers were produced. Furthermore, it simultaneously allowed continuous spinning and drawing of the gelatin fibers in order to induce orientation in the protein structure. XRD measurements showed that the suggested orientation of single helices and triple helices was indeed achieved. Tensile tests revealed that drawing the fibers during the spinning improved the mechanical properties to the point where gelatin fibers can compete with other biopolymers and synthetic polymers. This is even more promising as the fibers are characterized by high porosity (up to 30 %), as observed by SEM. In a further treatment step, the fibers were crosslinked by gaseous formaldehyde and dehydrothermal treatment. This crosslinking combination significantly increased the water-resistance of the gelatin fibers to nearly the level of human hair. The resemblance of the produced gelatin fibers with highest quality natural fibers – such as angora rabbit fibers – is remarkable. These advantageous properties suggest the use of porous gelatin fibers in different forms (single fiber, yarn or woven structure) in textile applications.
3. Fibers Mechanically Similar to Sheep Wool Obtained by Wet Spinning of Gelatin and Optional Plasticizers

Published in parts as:

Philipp R. Stoessel, Renzo A. Raso, Tim Kaufmann, Robert N. Grass, Wendelin J. Stark

3.1. Introduction

Gelatin is a biopolymer derived from native collagen, the most abundant structural protein which is available from animal by-products in enormous quantities.\textsuperscript{67, 80} In the conversion of collagen to gelatin, the triple-helical structure of the water-insoluble collagen is cleaved into single protein chains, yielding gelatin as a water-soluble hydrolysis product.\textsuperscript{80, 120} In spite of being produced from animal waste (e.g. bones and hides), gelatin has interesting properties, such as high water solubility, biodegradability, biocompatibility, high micro-hardness and – most important – the ability to form thermally reversible gels.\textsuperscript{158} It is not surprising that gelatin is used in numerous commercial applications such as food, glue, cosmetics, medical products, pharmaceuticals (capsules), photography (emulsions), polymer industry (plasticizer) or printing (sizing).\textsuperscript{86} In the last five decades, a vast number of reports on gelatin films have been published, investigating mechanical and thermal properties, crosslinking reactions or effect of plasticizers. Many of these studies were motivated by the desire of finding biowaste-based substitutes for synthetic polymers, e.g. for packaging purposes.\textsuperscript{124, 159-160} Still, gelatin films have not found the way into our daily life as a suitable bioplastic, mainly because the films are too brittle and have a low water vapor resistance.\textsuperscript{124, 158-159} It is known that the mechanical properties of gelatin can significantly be improved by inducing anisotropy, \textit{i.e.} partial recovery of the collagen structure.\textsuperscript{125, 137-138, 158} For this reason, we decided to concentrate on gelatin fibers with the aim of producing a continuous filament which does not have the limitation of natural fibers, \textit{i.e.} the small staple fiber length.

The fabrication of polymer fibers is a diversified field and numerous spinning methods exist. Electrospinning\textsuperscript{161-162}, centrifugal\textsuperscript{162-163} and solution/melt blow spinning\textsuperscript{162, 164-165} are often used for the fabrication of nanofibers. Fibers of larger diameter are generally produced by melt spinning\textsuperscript{166-168}, wet spinning\textsuperscript{169-171}, gel spinning\textsuperscript{125-126, 171} or dry spinning\textsuperscript{171-172}. In a recent study, we successfully explored a dry spinning process for gelatin fibers with promising properties (see chapter 2).\textsuperscript{173} Dry spinning is highly interesting as the fiber solidification is achieved by solvent evaporation only. However, this step is relatively slow as it has to be performed at low temperatures in order not to melt the gelatin. Furthermore, the device must have a considerable height to achieve good spinning velocities and large amounts of volatile organic compounds (VOC) are produced. In the present study, we expanded the research on gelatin fibers by introducing a wet spinning process. Wet spinning implies rapid fiber coagulation and dehydration in a solvent bath which is beneficial for large-scale processes.\textsuperscript{102} Instead of working with an aqueous gelatin solution, a ternary mixture consisting
of gelatin, 2-propanol and water was produced. The presence of 2-propanol led to a separation of the mixture into a lower phase (spinning phase) and a supernatant (see Figure 3-1a). Only the spinning phase, essentially a protein precipitate, was further processed. Robust wet spinning by the air-gap method with continuous fiber drawing was achieved. In comparison to other processes, e.g. the gel spinning of a gelatin solution into cold methanol with subsequent solvent extraction, no fiber post-treatment was necessary and fibers could be spun at 11 m min⁻¹ in a lab set-up (Figure 3-1b). As such, the proposed method qualifies for up-scaling. To address potential issues regarding fiber brittleness, we systematically investigated the application of plasticizers, namely ethylene glycol (EG) and triethylene glycol (TEG).

Figure 3-1. (a) Phase separation of the spinning mixture yielded an opaque spinning phase and a supernatant. (b) Wet spinning equipment for the continuous spinning and drawing of gelatin fibers. The spinning phase was filled into a syringe (25 mL) and held at a temperature of 60-65 °C. The syringe nozzle (0.8 mm opening diameter) was tempered at 35 °C and a syringe pump (flow rate Q = 0.1 ml min⁻¹) was used to extrude a gelatin fiber into an ethanol bath (length of 1.8 m). The air gap was 5 cm and the residence time in the coagulation bath was 35 seconds. The fiber was conveyed in air (23 ± 2 °C, 30 ± 10 % r.h.) over a distance of 3 m before being drawn on a motorized roller system. (c) Final product: approx. 400 m of gelatin fiber on a bobbin.

3.2. Experimental section

3.2.1. Fiber preparation

Type A gelatin from porcine skin (G2500, ~ 300 g Bloom strength), 2-propanol (HPLC-grade), ethylene glycol (EG) (≥ 99 %) and triethylene glycol (TEG) (99 %) were used as received from Sigma Aldrich. The spinning mixture, consisting of gelatin powder, plasticizer, deionized water and 2-propanol, was tempered in a water bath at 50 °C for 1 h and repeatedly shaken by hand. The plasticizer percentage was varied between 10 wt% and 200 wt% based
on the gelatin weight. The exact spinning mixture compositions are disclosed in Table 3-1. After phase separation, the supernatant was decanted and the opaque spinning phase was wet spun into continuous gelatin filaments (Figure 3-1b). Ethanol (technical grade) was used as a cheap and relatively green coagulation agent. Drawing of the fibers was achieved on a motorized roller system. The speed of the first roller was adjusted to match the speed of the conveyed fiber (draw ratio $\lambda = 1$) and the successive rollers were accelerated in order to achieve draw ratios (i.e. ratios of speeds of first and last roller) of $\lambda = 2, 3$ and 4.

**Table 3-1. Composition of different spinning mixtures.** Mixing of gelatin, plasticizer, deionized water and 2-propanol and subsequent heating at 50 °C yielded two-phase systems. The lower, opaque spinning phase was fed to a wet spinning process.

<table>
<thead>
<tr>
<th>Plasticizer content (wt% based on gelatin)</th>
<th>Gelatin (wt%)</th>
<th>Plasticizer (wt%)</th>
<th>Deion. water (wt%)</th>
<th>2-propanol (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plasticizer</td>
<td>10.0</td>
<td>-</td>
<td>40.0</td>
<td>50.0</td>
</tr>
<tr>
<td>TEG 10</td>
<td>10.0</td>
<td>1.0</td>
<td>40.0</td>
<td>49.0</td>
</tr>
<tr>
<td>TEG 25</td>
<td>10.0</td>
<td>2.5</td>
<td>40.0</td>
<td>47.5</td>
</tr>
<tr>
<td>TEG 50</td>
<td>10.0</td>
<td>5.0</td>
<td>40.0</td>
<td>45.0</td>
</tr>
<tr>
<td>TEG 100</td>
<td>10.0</td>
<td>10.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>TEG 200</td>
<td>9.1</td>
<td>18.2</td>
<td>36.4</td>
<td>36.4</td>
</tr>
<tr>
<td>EG 10</td>
<td>10.0</td>
<td>1.0</td>
<td>40.0</td>
<td>49.0</td>
</tr>
<tr>
<td>EG 25</td>
<td>10.0</td>
<td>2.5</td>
<td>40.0</td>
<td>47.5</td>
</tr>
<tr>
<td>EG 50</td>
<td>10.0</td>
<td>5.0</td>
<td>40.0</td>
<td>45.0</td>
</tr>
<tr>
<td>EG 100</td>
<td>9.1</td>
<td>9.1</td>
<td>36.4</td>
<td>45.5</td>
</tr>
<tr>
<td>EG 200</td>
<td>7.7</td>
<td>15.4</td>
<td>30.8</td>
<td>46.1</td>
</tr>
</tbody>
</table>

### 3.2.2. Fiber characterization

Prior to all measurements, gelatin fibers were equilibrated at room temperature (23 ± 2 °C) and constant relative humidity (45 ± 5 % r.h.) for at least 48 h. Tensile tests ($n = 10$) were conducted according to ASTM D3822-07 on a Shimadzu AGS-X equipped with a 100 N load cell. Fiber cross-sections were prepared by freezing the sample in N$_2$(l) followed by cutting with a scalpel. The diameter of every fiber specimen was determined by light microscopy (Zeiss Axio Imager.M2m) prior to tensile testing. The morphology of gelatin fibers was studied with the help of scanning electron microscopy (SEM) (FEI, NovaNanoSEM 450, operating at 5 kV). High-performance liquid chromatography (HPLC, Agilent 1100) coupled with a mass sensitive detector (MSD) was used to quantify the plasticizer concentration in the
supernatant of the spinning mixtures. A flow injection method with methanol (LC/MS grade, Fisher Chemicals) and 0.1 vol% formic acid (≥ 98 %, Merck) as mobile phase was used. EG or TEG in Millipore water (18.2 MΩ cm) at different concentrations were used as calibration solutions. The plasticizer content in the spinning phase was calculated from the difference between the total used plasticizer and the plasticizer discarded in the supernatant. In order to quantify the plasticizer content in the gelatin fibers, microanalysis was performed (Elementar vario MICRO cube). The results were standardized to the nitrogen content of a non-plasticized gelatin fiber and the effective plasticizer concentration was calculated via the increase of carbon. Differential scanning calorimetry (DSC) was carried out using a DSC Q1000 (TAinstruments). Samples of 8 - 11 mg were hermetically sealed in steel pans and analyzed from 0 - 150 °C at 10 °C min⁻¹. Two heating runs were performed and the glass transition temperature T_g was extracted from the second heating run.

### 3.3. Results and discussion

#### 3.3.1. Characterization of non-plasticized fibers

A spinning mixture, consisting of gelatin, 2-propanol and deionized water was prepared. The spinning mixture is unique owing to the fact that it underwent phase separation into a lower spinning phase and a supernatant (see Figure 3-1a). It was only after the separation that the spinning phase was wet spun into an ethanol bath. After the coagulation bath, different degrees of fiber drawing were applied in order to study the orientation of the fibrous protein. Figure 3-2 summarizes the average mechanical properties of non-plasticized gelatin fibers. In accordance with our previous work (chapter 2) and other studies on gelatin/collagen fibers\(^{117, 125-126}\) or oriented films\(^{120, 137-138}\), an overall increase in mechanical properties was observed with increasing draw ratio. The mechanical improvements are explained by the alignment of single and triple-helical protein domains during or after the coil-helix transition of gelatin.\(^{80, 83, 125}\) The stiffness of fully drawn fibers (\(\lambda = 4\)) was 3.8 GPa, and the strength at break could be raised from 47 MPa (\(\lambda = 1\)) to 178 MPa (\(\lambda = 4\)). The strain at break was highest for fibers at \(\lambda = 3\) (65 %). Interestingly, the difference between samples at \(\lambda = 1\) and \(\lambda = 2\) was negligible although drawing occurred (\textit{i.e.} decrease in fiber diameter). This may indicate that the fiber was mainly tapered at low draw ratios, while draw ratios of \(\lambda > 2\) were required for orientation of the coagulated protein. Due to its mechanical qualities, fully oriented gelatin fibers (\(\lambda = 4\)) as produced in the present work are an eligible alternative to other protein fibers, such as sheep wool: single fibers of commercial sheep wool (measured with the same...
set-up and test parameters) had an elastic modulus of 2.7 GPa and a tensile strength of 116 MPa. According to the literature, merino wool has higher stiffness (3.9 GPa) and tensile strength (212 MPa).\textsuperscript{176}

\textbf{Figure 3-2.} (a) Fiber diameter, (b) elastic modulus, (c) engineering tensile strength at break and (d) engineering strain at break of non-plasticized gelatin fibers. Samples were taken directly from the coagulation bath and from the different rollers (draw ratios $\lambda = 1 - 4$). Increased drawing led to decreased fiber diameter and considerably increased mechanical properties.

In \textbf{Figure 3-3} the mechanical properties of the produced gelatin fibers are compared with major commodity fibers; the relevant properties were measured in-house or taken from literature.\textsuperscript{177-178} The Ashby plot emphasizes the mechanical similarity between gelatin fibers and sheep wool. Furthermore, it illustrates that many vegetable fibers, man-made synthetic polymer and metallic fibers or even some specialty protein fibers such as silk have considerably superior mechanical properties.
Figure 3.3. Ashby plot comparing the elastic modulus and tensile strength of gelatin fibers and major commodity fibers.

Figure 3.4. (a) Cross-sections of non-plasticized gelatin fibers produced at different draw ratios $\lambda = 1 - 4$. Drawing led to smaller pore size and diminished pore volume, extensive drawing ($\lambda = 4$) to a denser fiber structure. Plasticized gelatin fibers with (b) triethylene glycol (9 wt% TEG in final fiber) and (c) ethylene glycol (3 wt% in final fiber) show the same fiber morphology.
Up to present, gelatin fibers have mostly been spun from a gel with subsequent coagulation in cold methanol. Fibers produced from a gelatin/dimethyl sulfoxide gel yielded similar tensile strengths (180 MPa) and elastic moduli (3.4 GPa). Gel spinning of gelatin in ethylene glycol yielded higher mechanical properties (up to 400 MPa tensile strength) but only after extraction of ethylene glycol during several days which is impractical for a large-scale application. In comparison to dry spun gelatin fibers prepared in chapter 2 (elastic modulus 2.6 GPa; engineering tensile strength 85 MPa; true tensile strength 160 MPa), the here presented wet spun gelatin fibers have superior mechanical properties.

Cross-sections of non-plasticized fibers were visualized by SEM and are disclosed in Figure 3-4a. Undrawn fibers from the first roller exhibited large, circular pores and a major pore volume (porosity of ~ 30 %). The porosity was a result of the sponge-like structure of the spinning phase: enclosed solvent droplets served as pore template. Drawing the fibers to $\lambda = 2$ induced folding of the pore walls, yielding shrunk pores and decreased porosity (~ 14 %). This is in line with the above hypothesis of fiber tapering at low draw ratios: between $\lambda = 1$ and $\lambda = 2$, the fiber lost pore volume but did not mechanically improve. At draw ratios $\lambda \geq 3$, the pores disappeared, yielding (nearly) dense fibers. In Figure 3-5, the fiber porosity is compared to the fiber strength at different draw ratios. Even if the tensile strength is corrected for the fibers’ effective volume fraction, it is apparent that the increase in strength is not a function of decreasing porosity but a function of the draw ratio – this means that the anisotropy is indeed responsible for the improved fiber properties.

**Figure 3-5.** Fiber porosity (squares) vs. tensile strength at break (dots) of gelatin fibers prepared at different draw ratios. With increasing drawing of the fibers, the pores shrunk and fiber porosity decreased from 31 % ($\lambda = 1$) to 0 % ($\lambda = 4$). The tensile strength was also corrected for the fibers’ effective volume fraction. This yielded slightly higher tensile strength values for fibers with $\lambda = 1-3$ (open dots).
3.3.2. Characterization of plasticized fibers

In its dry state, gelatin exhibits a high brittleness, which may be disadvantageous. In order to lower the glass transition temperature and to improve protein chain mobility, plasticizers are often applied. Repeatedly investigated plasticizers include oligosaccharides, sugar alcohols, (poly)ethylene glycol species or citrate species. Here, we added ethylene glycol (EG) and triethylene glycol (TEG) to the spinning mixtures. As a consequence of phase separation during spinning mixture preparation, a fraction of the plasticizer was lost in the supernatant. Plasticizer was also lost in the wet spinning process by diffusion into the coagulation bath. It must be noted, though, that plasticizer and solvents could easily be recycled.

![Figure 3-6](image)

**Figure 3-6.** (a) TEG percentage in the spinning phase (black dots) and in the final equilibrated gelatin fiber (blue squares), respectively, expressed as percentage of total TEG used. TEG was lost due to phase separation (supernatant discarded) and transfer into the coagulation bath. (b) Engineering stress vs. engineering strain curves of gelatin fibers ($\lambda = 4$) showing the effect of EG and TEG addition (plasticizer percentage given as wt% of gelatin fiber). Mechanical properties of fibers plasticized by (c) TEG and (d) EG. The draw ratio was held constant at $\lambda = 4$. Addition of plasticizer led to a higher strain at break, accompanied by reduced stiffness and strength. Compared to TEG the plasticizing effect of EG was less pronounced.
The loss of EG and TEG is in line with the fact that both compounds are highly soluble in the supernatant of the spinning mixture (water, 2-propanol) and in the coagulation bath (ethanol). The distribution of TEG in the spinning phase and in the final gelatin fibers is presented in Figure 3-6a. About 60% of the initially used TEG was detected in the spinning phase. About 50% - 90% of the remaining TEG subsequently leached from the fiber into the coagulation bath. Finished and equilibrated TEG plasticized fibers showed a TEG percentage between 2.5 wt% and 12.0 wt% (based on fiber weight). Loss of EG in the supernatant was smaller (~ 30% of the initially used EG), but EG diffusion into the coagulation bath was more prominent (> 90%), yielding final fibers with an EG percentage between 0.1 wt% and 5.5 wt% (see Figure 3-7).

**Figure 3-7.** EG content in the spinning phase (black dots) and in the final fibers (blue squares), respectively, expressed as a function of total plasticizer used. Losses of EG in the supernatant of the spinning mixture and in the coagulation bath were higher than for TEG.

Plasticized gelatin fibers with TEG and EG had the same fiber morphology as non-plasticized fibers: undrawn fibers were highly porous, while fully drawn fibers ($\lambda = 4$) exhibited a dense fiber structure (see Figures 3-4b and 3-4c). Representative tensile test curves (Figure 3-6b) highlight major mechanically relevant differences between reference and plasticized fibers. In non-plasticized fibers, a gradual transition from the elastic to the plastic regime was followed by uniform extension and strain hardening. This curve shape is similar to that of other biopolymer fibers such as keratin (sheep wool$^{186,187}$, human hair$^{188}$) or silk$^{189}$. Both EG and TEG plasticized fibers showed an intrinsic yield point, *i.e.* a stress maximum after the elastic regime. The yielding was followed by cold drawing, and strain hardening set in after the propagation of the neck at ~ 20% elongation. This behavior is characteristic for amorphous and partially crystalline polymers.$^{190}$ The distinctive yielding is related to the neck
Necking is an indication for sample weaknesses, such as structural defects, impurities, interphase cracks, crazes, or shape defects. It is therefore concluded that the plasticized fibers are structurally inferior to non-plasticized ones. It is known that plasticizer addition comes with a trade-off: while ductility increases and glass transition temperature decreases, the plasticizer adversely affects stiffness and strength of a material. The tensile test results of TEG plasticized fibers are summarized in Figure 3-6c. The findings are in agreement with Cao et al. (2009) presenting results from gelatin films produced with 20 wt% TEG, yielding a 50% decrease of elastic modulus and strength and a 30% increase in strain at break. The same authors stated that TEG is among the most effective plasticizers. In contrast, the plasticizing effect of EG was less pronounced in the gelatin fibers (see Figure 3-6d). This is not only because of higher EG losses into the coagulation bath but also for its smaller plasticizing capacity as shown previously.

As shown by several studies on protein films, the changes in mechanical characteristics due to plasticizer addition are directly reflected in a change of the glass transition temperature $T_g$. Water also acts as a plasticizer and has a strong effect on transition temperatures. At low water content (8 - 10 wt% in the gelatin fibers) the glass transition and melting temperatures are very close to each other. This may explain why the helix-coil transition was sometimes hard to detect in the first heating scan. $T_g$ values are indicated in Figure 3-6b and Table 3-2. An illustrative DSC thermogram is given in Figure 3-8.

**Table 3-2. Glass transition temperatures for pure gelatin and wet spun gelatin fibers drawn to $\lambda = 4$.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plasticizer</th>
<th>Plasticizer (wt% of gelatin)</th>
<th>Glass transition temperature $T_g$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-plasticized gelatin powder</td>
<td>TEG</td>
<td>2.5</td>
<td>79.9</td>
</tr>
<tr>
<td>Non-plasticized fiber</td>
<td>TEG</td>
<td>6.1</td>
<td>71.2</td>
</tr>
<tr>
<td>Plasticized fiber</td>
<td>TEG</td>
<td>12.0</td>
<td>57.0</td>
</tr>
<tr>
<td>Plasticized fiber</td>
<td>EG</td>
<td>0.2</td>
<td>81.9</td>
</tr>
<tr>
<td>Plasticized fiber</td>
<td>EG</td>
<td>5.5</td>
<td>78.9</td>
</tr>
</tbody>
</table>
The glass transition temperature ($T_g$) of an non-plasticized fiber (81.8 °C) was nearly identical to $T_g$ of pure gelatin powder (82.5 °C). The addition of EG had almost no effect, which is in accordance with the above mechanical results and those in other studies. In contrast, with increasing TEG, $T_g$ linearly decreased to 57.0 °C (at 12 wt% TEG), thus strongly resembling keratin fibers (e.g. sheep wool, human hair) with similar $T_g$ at the same moisture content.

![Illustrative differential scanning calorimetry (DSC) thermogram for a gelatin fiber plasticized by 12.0 wt% TEG ($\lambda = 4$). The endothermic peak in the first heating scan corresponds to melting, i.e. the helix-coil transition of gelatin. The glass transition temperature ($T_g = 57$ °C) was extracted from the second heating scan. While $T_g$ could be clearly detected in second heating scans, melting – normally a prominent endothermic peak in the first heating scan – was sometimes vague. This may be caused by overlapping of glass transition and melting. A further explanation is the relatively low sensitivity of the method applied: heavy hermetically sealed crucibles in combination with relatively small sample weights were used in order to avoid a big water evaporation overlapping with glass transition and melting peak.]

### Figure 3-8.

3.4. **Conclusions**

We developed a wet spinning process for the continuous fabrication of gelatin fibers. The proposed method is beneficial as gelatin, a relatively cheap polymer derived from waste, is continuously spun into a biofiber with the help of environmentally favourable and low-toxicity solvents. The latter can easily be recycled. The presented process stands out against other methods for gelatin spinning because a previously phase-separated spinning
mixture is used. Likewise, this sponge-like protein precipitate allows spinning of porous fibers. Due to rapid fiber coagulation in ethanol, the fiber can directly be subjected to drawing or further treatment steps, which makes the process interesting for up-scaling. With elastic moduli of up to 3.8 GPa and tensile strength of 180 MPa, fully oriented fibers showed mechanical properties comparable or even superior to sheep wool. The addition of plasticizers and variations in the degree of fiber drawing enabled to tailor the fiber characteristics such as porosity (0 to 30 %), glass transition temperature (57 to 82 °C) or mechanical properties.
4. Porous, Water-Resistant Multifilament Yarn Spun from Gelatin

Published in parts as:

Philipp R. Stoessel, Urs Krebs, Rudolf Hufenus, Marcel Halbeisen, Martin Zeltner, Robert N. Grass, Wendelin J. Stark

4.1. Introduction

Annually, over 70 million tons of fibers are traded globally. Man-made fibers from synthetic polymers dominate the market (nearly 60% share nowadays), while cotton and wool (<40%) – the only two noteworthy natural fibers – constantly lose importance. This is a massive change considering that 150 years back the dominant fibers were either cellulosic (cotton and linen) or proteinaceous (wool and silk). From 1890 to 1950 much effort has been put into so called regenerated protein fibers made from either animal or vegetable proteins, e.g. to find a substitute for the scarce wool fiber at war time. Relatively poor fiber performance and the development of low-cost synthetic fibers led to a quick economic decline of regenerated protein fibers and commercial products such as Fibrolane (casein), Ardl (peanut protein) or Vicara (zein) disappeared from the market.

During the last decades, sustainability, eco-friendliness, renewability and biodegradability have become important trends in the polymer industry. Interest in “green” biomolecular materials mainly originates from the limited abundance of petrochemical products, regulations regarding synthetic waste and the consumers’ concern for the environment. It is hence not astonishing that the idea of using agriculturally derived waste as polymeric building blocks has been undergoing a remarkable revival. Fiber production from polysaccharide waste streams mainly concentrates on cellulose and chitin. While chitin fibers are a niche product from the biomedical field (sutures), regenerated cellulosic fibers are omnipresent in the textile industry as viscose, rayon, cupro or lyocell. In contrast, the field of regenerated proteins is much more diverse and numerous waste proteins were and still are considered for fiber production: keratin from feathers or wool, zein from maize (marketed as Vicara in the 1950s; nowadays electro-spun to nanofibrous mats), wheat gluten, casein from milk (marketed as Aralac or Fibrolane in the 1930s/1940s; recently revived as Qmilk), soy bean protein, regenerated silk proteins, egg white lysozyme and others. One of the most abundant agricultural waste proteins is gelatin. It is derived from collagen, the main structural protein in the skin, bones and tendons of vertebrates. Collagen exhibits an unusual amino acid composition, i.e. high glycine, proline and hydroxyproline content, and a striking primary structure consisting of Gly - X - Y repeats (see Figure 4-1a). When collagen is subjected to chemical and heat treatment, the fibrous protein is partially hydrolyzed, interchain covalent and hydrogen bondings are broken, yielding gelatin as a water-soluble biopolymer. Aqueous gelatin solutions have the remarkable ability to reverse into a collagen-like structure and thus to form gels upon cooling. The motivation
for gelatin fiber production originates from its enormous availability: the raw material, namely slaughterhouse waste, accumulates at about 10 million tons per year in the European Union and the global gelatin market is expected to reach 450’000 tons in 2018. In the early days of man-made fiber production (1890 and later), several patents on gelatin fibers were filed and for a short time a fiber called Vanduara was commercially produced in Scotland. However, as with all regenerated protein fibers, production was stopped as a result of the booming synthetics. Nowadays, gelatin and collagen based materials are exploited for medical applications (bone repair, tissue engineering) and quite recently research on macroscopic gelatin fibers has gained in importance. While acceptable mechanical properties are achieved, the manufactured quantities are still very small and the fibers’ stability in water is insufficient for most applications.

In the present study, we demonstrate the production of gelatin filaments from a precipitated spinning dope by dry-wet spinning. The continuous lab-scale spinning setup was able to manufacture 200 m min⁻¹ of filaments, which were subsequently twisted into yarns by hand. Furthermore, we systematically investigated different crosslinking methods based on epoxide and aldehyde reactive groups (see Figure 4-b-e) to render the yarns water-resistant. As proof of concept we fabricated a knitted glove from gelatin multifilament yarn, which showed water-resistance as well as high thermal insulation capacity.

4.2. Experimental section

4.2.1. Materials

Type A gelatin from porcine skin (G2500, ~ 300 g Bloom strength), 2-propanol (HPLC-grade), poly(propylene glycol) diglycidyl ether (PPGDE, MW = 640 g mol⁻¹), formaldehyde solution (37 wt% in H₂O), lanolin (sheep wool wax) and picrylsulfonic acid solution (5 % in water) were used as received from Sigma-Aldrich. Ethylene glycol diglycidyl ether (EGDE, 174 g mol⁻¹) from TCI and poly(ethylene glycol) diglycidyl ether (PEGDE, 330 g mol⁻¹ and 530 g mol⁻¹) from Polysciences Inc. were utilized. The trifunctional epoxide Grilonit® V51-31 (320 g mol⁻¹, EMS-GRILTECH, Switzerland) and tetrafunctional epoxide Epotec RD129 (469 g mol⁻¹ as determined by mass spectroscopy (see Appendix A.2., Figure A2-1), Aditya Birla Chemicals, Thailand) were kindly provided by the manufacturers. Technical grade ethanol was used to wet the rolls. For filament dying, red acid dye (Bezanyl red E-3G 200, Bezema AG) was mixed into the spinning dope. Merino sheep wool (LANG yarns, Thema Nuova, Merino fine superwash) was used as reference material.
Figure 4-1. Chemical structures of gelatin and the applied crosslinking agents. (a) Typical sequence of the gelatin polypeptide. (b) Ethylene glycol diglycidyl ether, poly(ethylene glycol) diglycidyl ethers with $n = 4-5$ and $n = 9$, respectively. (c) Poly(propylene glycol) diglycidyl ether with $n = 8-9$. (d) Trifunctional EMS-GRILTECH Grilonit® V51-31. (e) Formaldehyde.

4.2.2. Gelatin yarn production

A process chart is given in Figure 4-2a. The spinning and twisting process is illustrated in Figure 4-2b. Briefly, a mixture of porcine gelatin (Type A), deionized water and 2-propanol was tempered in a water bath at 50 °C and repeatedly shaken by hand. This resulted in a 2-phase system with a lower, opaque spinning phase (dope) and a supernatant. After 2 - 3 hours at 50 °C, epoxides were added. The compositions of the spinning mixtures are summarized in Table 4-1. The epoxide contents are indicated with respect to the gelatin weight. The applied epoxide content was 50 wt%; in case of EGDE the content was varied (25 wt%, 50 wt%, 100 wt%). After thorough shaking, the spinning mixture was degassed in a vacuum oven set at 50 °C. The supernatant was decanted and the lower phase (the spinning dope) was transferred into six plastic syringes (25 mL). An incubator set at 50 °C was used to temper the syringes and the multi-channel syringe pump (adapted VIT-FIT pump, Lambda Instruments). The dope was extruded at 0.05 mL min$^{-1}$ through tempered polytetrafluoroethylene (PTFE) hosing; except for the PTFE tubes with 1.3 mm inner diameter, no special nozzle was needed.
The six parallel monofilaments were then directed via two PTFE-coated rolls (0.15 m in diameter) to a conveyor belt (5 m length), where they were continuously taken up around the whole belt (10 m circumference). In order to coagulate the fibers and to prevent the sticking of the fibers, the rolls were continuously wetted in an ethanol bath (technical grade). The air gap (distance from nozzle to first roll) was 1 m; the distance between the two rolls was 0.5 m; the distance from the second roll to the end of the conveyor belt was 5.5 m. The first roll was running at 35 rpm (16.5 m min\(^{-1}\)), the second at 70 rpm (33 m min\(^{-1}\)) and the conveyor belt at 33 m min\(^{-1}\) (= take-up speed). The spinning velocity (spinning dope flow rate divided by the cross-sectional area of the PTFE hosing) was 0.038 m min\(^{-1}\). The overall fiber draw-down ratio, \(i.e.\) the ratio of the take-up speed and the extrusion speed, was \(\sim 900\).

**Figure 4-2.** (a) Process chart of gelatin yarn and cloth production. (b) Spinning process and yarn twisting. (1) Spinning dope in 6 syringes was pumped through (2) PTFE-hosings (1.3 mm inner diameter) with a multi-channel syringe pump (flow rate 0.05 mL min\(^{-1}\)). (3) An incubator set at 50 °C was used to temper the dope. (4) PTFE-coated rolls (0.15 m in diameter), constantly wetted in an ethanol bath. The second roll (70 rpm) was rotating twice as fast as the first one (35 rpm). (5) The gelatin filaments were continuously taken up around a conveyor belt (10 m circumference), running at the same speed as the second roll (33 m min\(^{-1}\)). (6) After 30 - 40 min of spinning, a gelatin roving could be removed from the conveyor belt. (7) The roving was first twisted into a yarn with Z-twists (clockwise twisting). (8) Two of these yarns were then plied together (counterclockwise twisting, S-twists) and wound up on a spool (9).
**Table 4-1.** Compositions of different spinning mixtures. Mixing of gelatin, deionized water and 2-propanol and subsequent heating yielded two-phase systems. The epoxide was added directly before degassing and spinning of the lower phase.

<table>
<thead>
<tr>
<th>Epoxide content (wt% based on gelatin)</th>
<th>Gelatin (wt%)</th>
<th>Deion. water (wt%)</th>
<th>2-propanol (wt%)</th>
<th>Epoxide (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>40</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>40</td>
<td>47.5</td>
<td>2.5</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>40</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>40</td>
<td>40</td>
<td>10</td>
</tr>
</tbody>
</table>

Yarn twisting. After continuous uptake of gelatin filaments on the conveyor belt for 30 - 40 min, the 10 m long fiber roving (~ 500 filaments) was removed and twisted into a yarn with a wooden drop spindle (clockwise, Z-twists). Two Z-twist yarns were afterwards plied together with another spindle to form a 2-ply yarn (counterclockwise, S-twists, ~ 1’000 filaments). The loose yarns were conditioned overnight at room climate (23 ± 2 °C, 20 - 30 % r.h.) and heat treated (120 °C, 4 h) the next day.

Knitting. As a presentation object and proof of concept, a glove from gelatin yarn was knitted by hand. The yarn was prepared with 50 wt% EGDE in the spinning mixture (effective epoxide content in final product = 6 wt%). Heat-treatment (120 °C, 4 h) and post-treatments (FA(g) crosslinking, lanolin impregnation) were conducted after knitting.

Post-treatments. A second crosslinking step was conducted by gas-phase treatment with formaldehyde at room temperature (23 ± 2 °C). Gelatin yarns were placed in a desiccator (volume 2.5 L), containing 5 mL of formaldehyde solution (37 wt% in H₂O) and vacuum was applied once. To study the kinetics, crosslinking duration was varied between 30 min and 8 h. After crosslinking, the yarns were conditioned overnight at room climate (23 ± 2 °C, 20 - 30 % r.h.) and heat treated (120 °C, 4 h) the next day. In a last step, the crosslinked yarns were impregnated with lanolin (sheep wool wax). 1 wt% lanolin was dissolved in EtOH at 45 °C and yarn samples were immersed in the solution for 5 min and afterwards dried.

4.2.3. Yarn characterization

The amount of free ε-amino groups in gelatin fibers – a direct measure for the extent of crosslinking – was determined by an established UV-assay using picrylsulfonic acid.²¹⁹-²²⁰ Briefly, 11 mg of gelatin yarn were mixed with 1 mL of 4 % NaHCO₃ solution and 1 mL of
0.5 % picrylsulfonic acid solution (TNBS) and heated at 40 °C for 4 h. After addition of 3 mL HCl (6 M), the samples were autoclaved at 120 °C for 1 h. The hydrolysate was diluted with 5 mL deionized water and extracted 3 times with ethyl ether. 3 mL of the aqueous phase were then heated in a water bath (60 °C) for 15 min. After dilution with 9 mL deionized water, the UV-absorbance at 346 nm was measured against blank samples with a Nanodrop 2000c (Thermo Scientific). The blank samples were analogically prepared except that the HCl was added before TNBS addition. The moles ε-amino groups per g gelatin were calculated from equation (4-1). $1.46 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ is the absorptivity of TNP-lys, $b$ is the cuvette path length (1 cm) and $x$ is the sample weight (in g).

$$\frac{\text{moles } \varepsilon-\text{amino groups}}{g \text{ gelatin}} = \frac{2x(\text{absorbance}) \times (0.020 \text{ L})}{(1.46 \times 10^4 \text{ L mole}^{-1} \text{ cm}) (b)(x)} \quad (4-1)$$

The swelling behavior of gelatin yarns in deionized water was evaluated at room temperature (23 ± 2 °C): yarn pieces of 5 - 10 cm (with a knot on each end to prevent unravelling of the yarn) were swelled for 1 hour and subsequently blotted with filter paper to remove excess water. The specimen’s length (knot-knot distance) and its weight were measured before, directly after swelling and after drying. Conditioning and drying of the specimens were conducted at room climate (23 ± 2 °C, 20 - 30 % r.h.). The degree of swelling was calculated from equation (4-2). The standard deviation achieved with this method was 6 - 10 % (measurement of 4 samples with 4 specimens each).

$$SD = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \quad (4-2)$$

For simulated washing of yarns, detergent powder (Persil Universal Megaperls, Henkel) was dissolved in tap water (manufacturer instruction: 87.4 mg detergent powder in 50 mL water). The gelatin yarns were then shaken in the detergent solution for 30 minutes on a laboratory shaker (500 rpm) at 30 °C, rinsed three times with tap water and dried at room climate.

Microanalysis (Elementar vario MICRO cube) was performed in order to measure the epoxide content in the yarns. The results were standardized to the nitrogen content of a yarn without epoxide (control sample) and the effective epoxide concentration was calculated via the increase in carbon.

Prior to the tensile tests, the yarn samples were conditioned at room temperature (23 ± 2 °C) and constant relative humidity (65 ± 5 % r.h.) according to ISO 139:2005. Tensile tests ($n = 5$) were conducted following ISO method 2062:2009 on a Shimadzu AGS-X equipped
with a 100 N load cell and pneumatic clamps. The gauge length was 250 mm and the test speed 250 mm min\(^{-1}\) (strain rate 1 min\(^{-1}\)). A preload of 0.01 cN tex\(^{-1}\) was chosen. The linear density was determined by weighing a yarn specimen of a defined length. The wet state tensile properties of gelatin yarns were determined after immersing the sample in deionized water for 1 h. The linear density of the dry yarn was applied for tenacity calculations. Single filaments were removed from the yarns and tested according to ASTM D3822-07 \((n = 5)\). The average diameter of the filaments was determined from SEM pictures of cross-sections.

The morphology of gelatin yarns was studied by means of scanning electron microscopy (SEM) (FEI, NovaNano SEM 450, operating at 3 - 5 kV). For this purpose, the samples were sputter-coated with a platinum layer (3 - 4 nm). Cross-sections of yarns were obtained by freezing the samples in liquid nitrogen followed by cutting with a cooled scalpel. Imaging software (ImageJ, version 1.46) was used to manually determine the porosity of single filaments (total pore area divided by the cross-sectional area). Three filaments were considered for every sample. In heterogeneous samples, unequal filaments, which represent the sample as a whole, were analyzed. From the area of single pores, the pore diameters – representing the diameter of a corresponding circle – were calculated. The yarn morphology was also investigated by light microscopy (Zeiss Axio Imager.M2m, 25× magnification, circular differential interference contrast mode).

The thermal conductivity of different samples was measured with the help of a heat flux sensor and two temperature sensors (greenTEG, gSKIN® KIT-2615C) under free convection. An aluminum block, placed in a water bath and heated to 40 °C, was used as heat reservoir. The heat flux sensor and a temperature sensor were mounted on the aluminum block; a second temperature sensor measured the ambient temperature 10 cm above the flow sensor. The sample was then laid directly above the heat flow sensor. The setup is depicted in the Appendix A.2., Figure A2-2. Recording and averaging of the parameters during 30 min was done after the heat flux reached equilibrium. The specimen thickness was determined with the help of the tensile testing machine: instead of clamps, a stamp (diameter 12.75 mm) was fixed to the cross head. The sample was laid flat beneath and the stamp was slowly moved in the direction of the specimen surface until a force of -0.05 N was reached. The gauge length was taken as the sample thickness. Apart from the two gloves (gelatin yarn and merino sheep wool), a polyester fleece and an expanded polystyrene plate were tested. The thermal conductivity \((\lambda_{fc})\) and the heat transfer coefficient \((\alpha_{fc})\) under free convection (fc) were calculated with the help of equation (4-3), of which \(\dot{Q}/A\) (heat flux per area), \(d\) (sample
thickness) and $\Delta T$ (temperature difference between the two sensors) were experimentally
determined.

$$\frac{Q}{A} = \frac{1}{\alpha_{fe} \cdot \varphi_{fe}} \Delta T$$ (4-3)

For all samples, at least two different sample thicknesses were measured (e.g. gelatin glove as such $[d_1 = 2$ layers of cloth$]$ and folded glove $[d_2 = 4$ layers of cloth$]$). With the justified assumption that the two unknowns $\lambda_{fe}$ and $\alpha_{fe}$ are independent from the sample thickness, a system of two equations was solved.

*Molecular mass determination.* The structure of the tetrafunctional epoxide Epotec RD129 (Aditya Birla Chemicals, Thailand) was not known. In order to determine its molar mass, a mass spectrum was recorded on a Bruker Daltonics ESI-QTOF instrument.

### 4.3. Results and discussion

In previous studies we investigated the use of a spinning dope consisting of gelatin, 2-propanol and water for lab-scale solution spinning (see chapters 2 and 3).\textsuperscript{173,221} We found that upon heating, the water-miscible organic solvent (2-propanol) induced phase-separation of the ternary mixture into a transparent supernatant and a lower phase (spinning dope). This dope was continuously processed into gelatin fibers by both dry spinning (fiber solidification by solvent evaporation) and wet spinning (fiber coagulation and dehydration in a solvent bath). While most research on protein fibers relies on coagulation after spinning, \textit{i.e.} by organic solvents or salt solutions,\textsuperscript{125,213,218} we experienced very good spinnability by pre-coagulating the gelatin with 2-propanol in the actual spinning dope. In the present study we therefore opted to pursue the spinning of precipitated gelatin and we combined the proven techniques of dry spinning (long air gap) and wet spinning (secondary coagulation in ethanol). Instead of a traditional coagulation bath, the two rolls were constantly wetted with ethanol, thus combining fiber drawing and coagulation as well as preventing gelatin filaments from sticking to the rolls. The filaments were then hand-twisted to 2-ply yarns of 400 - 600 tex (1 tex = 1 g per 1’000 m yarn).

The morphology of gelatin yarns was visualized by scanning electron microscopy (SEM) and displayed in Figure 4-3. The smooth surface of single filaments is characteristic for man-made fibers. In comparison, animal fibers have scaled surfaces, \textit{e.g.} due to overlapping cuticle
cells in sheep wool (see Appendix A.2., Figure A2-3). Sections of the yarns revealed slightly lobed, bean-shaped cross-sectional areas. This is common for wet and dry spun filaments due to fast solvent removal from the outer part and subsequent collapsing. An eye-catching attribute is the filaments’ porosity. The fibers held a multitude of longitudinal pores (average pore diameter 0.6 ± 0.4 µm), yielding an overall filament porosity of about 32%. This was a result of the applied phase-separated gelatin/solvent/non-solvent system, where 2-propanol droplets were most likely enclosed in the spinning dope and served as pore template. A diagonally fractured gelatin fiber (see Appendix A.2., Figure A2-4a) revealed pores with a high length to diameter ratio (> 25) which were not interconnected. Such a porous structure is believed to be favorable for thermal insulation.

**Figure 4-3.** Scanning electron microscopy (SEM) images of a gelatin yarn produced without the addition of crosslinkers (control sample). (a) Top view of a 500 tex yarn (= linear density of 500 g per 1’000 m). The 2-ply yarns consisted of ~ 1’000 monofilaments. (b, c, d) Cross-section of the same yarn at different magnifications. The filaments exhibited longitudinal pores and a porosity of ~ 32%.

Gelatinous material degrades in water. The water-sensitivity is a major drawback and submitting gelatin to crosslinking is typically performed to induce water-resistance. For this purpose heat treatment, UV irradiation, enzymes and various chemicals such as aldehydes (formaldehyde, glutaraldehyde, glyoxal), carbodiimides or (poly)epoxide compounds have been proposed. Due to their availability and diversity, we investigated the effect of different glycidyl ethers for linkage of the amino groups in lysine residues (see Figure 4-1).
Under neutral pH condition the reaction of epoxides with amine groups is highly favored over the reaction with hydroxyl groups or carboxylic acids.\(^{227, 229-230}\) In order to test the crosslinking effect, each epoxide was added in excess to the spinning mixture (50 wt% of gelatin weight). As a consequence of phase separation during the spinning mixture preparation, the majority of the crosslinker stayed in the supernatant, from where it can easily be recycled. Furthermore, epoxide was presumably diffused into the ethanol used for fiber coagulation. As evaluated by microanalysis, the effective epoxide content was similar in all yarn types samples (5 - 7 wt%). This is congruent with our previous work on plasticized gelatin fibers where a similar loss of triethylene glycol was observed in a wet spinning process (see chapter 3). Despite the loss of crosslinker, the molar excess of epoxide in relation to the free amino groups of lysine was huge: 230-fold for the smallest difunctional epoxide (EGDE) and 50-fold for the heaviest crosslinker (PPGDE). The crosslinking efficiency was measured by the decrease in free ε-amino groups by a UV-assay using picrylsulfonic acid and compared to a control sample without crosslinker (see Figure 4-4a).However, this method was not able to discriminate between true crosslinks (protein chains linked) and incomplete linkages (only one epoxide group reacted with one lysyl amino group). The actual effect of the crosslinking procedure was monitored by swelling tests in deionized water. Heat treating the control samples had a marginal effect, which is in line with the data of heat treated gelatin films.\(^{154}\) However, the application of epoxides with subsequent heat treatment (i.e. 120 °C, 4 h) clearly altered the yarns’ properties. The application of tri- and tetrafunctional epoxides caused a relatively small decrease in free amino groups while still reducing the swelling degree. The multiple reactive sites per molecule obviously helped in establishing crosslinks between lysyl amino groups. In case of the difunctional glycidyl ethers, an explicit correlation between molecular weight and crosslinking efficiency was observed: epoxides of lower molecular weight yielded a higher decrease in amino group content and less swelling in water. It is assumed that the long diglycidyl ethers were sterically hindered and therefore only reacted with one lysyl amino group, not forming true crosslinks.

Being the most efficient epoxide, EGDE was selected for further investigations. As shown in Figure 4-4b, heat treatment of EGDE containing fibers significantly decreased the free ε-amino groups. It is hypothesized that the curing reaction was very slow at room temperature and that elevated temperatures were necessary for good reactivity between linker and amino groups. If EGDE containing yarns were not heat treated, they were completely unstable in water and had a swelling degree SD > 10. Variation of the EGDE content proved the correlation of the ε-amino group content and the swelling degree. Working at > 50 wt%
(effective 6 wt% EGDE in yarn) did only minimally decrease the free amino groups, while not improving the swelling behavior. In a study on the EGDE-crosslinking of gelatin films, Vargas et al. (2008) also showed that the wet-state properties improved with increasing EGDE content up to 7 - 10 wt%; further EGDE addition had no effect.\textsuperscript{227} This is in accordance with our work. On these grounds it was concluded that 50 wt% EGDE (= 6 wt% in yarn) in the spinning mixture was optimally chosen for gelatin yarn crosslinking.

![Graph showing the crosslinking of gelatin yarns with different epoxides and heat treatment.](image)

**Figure 4-4.** (a) Di-, tri- and tetrafunctional epoxides were considered for crosslinking gelatin yarns. The smallest linker, ethylene glycol diglycidyl ether (EGDE), was the most efficient one, allowing the highest reduction of free ε-amino groups. (b) Addition of ethylene glycol diglycidyl ether (EGDE) at different concentrations combined with heat treatment. The amount of free ε-amino groups correlated well with the swelling degree (weight increase of yarns when subjected to deionized water). Working at high epoxide concentrations (> 50 wt% EGDE) did not improve the swelling behavior.
The above discussed EGDE crosslinking improved the swelling behavior. Meanwhile, the yarn still considerably expanded in water and the dried samples tended to be too stiff for practical use. For this reason a subsequent crosslinking procedure with formaldehyde was conducted, exposing the yarns to gaseous formaldehyde (FA(g)) and heat treating them \(i.e.\) 120 °C, 4 h. Due to its small molecular size, FA only crosslinks closely associated proteins.\(^{151}\) This makes it an ideal supplement to the longer diglycidyl ether. However, formaldehyde is a hazardous volatile organic compound (VOC) which has been found to have adverse health effects \(e.g.\) allergic reactions and is nowadays classified as a carcinogenic substance.\(^{231-232}\) Formaldehyde is often applied in textile finishing products \(e.g.\) as \(N\)-methylol compounds such as dimethylol ethylene urea), which release formaldehyde over time.\(^{233-234}\) In contrast, the suggested formaldehyde treatment for gelatin crosslinking is unproblematic: (1) It is known that free formaldehyde is removed in washing.\(^{233}\) (2) Pure formaldehyde boils at -19 °C, while its aqueous solution (37 %) boils at 96 °C (in comparison to > 300 °C for formaldehyde-based finishing products). It is thus concluded that free formaldehyde was removed from the gelatin yarn during heat treatment at 120 °C. As the reaction of FA with proteins at room temperature is rather slow,\(^{152}\) heat treatment was likewise beneficial.

The effect of FA(g) as a function of crosslinking duration is illustrated in Figure 4-5. For both EGDE & FA as well as FA-only samples a prominent consumption of lysyl amino groups was measured. For instance, in a yarn previously crosslinked with EGDE, the free \(\varepsilon\)-amino groups were reduced from \(12 \times 10^{-5}\) to \(2 \times 10^{-5}\) moles g\(^{-1}\). The pure, unprocessed gelatin held \(31 \times 10^{-5}\) moles g\(^{-1}\). This is in accordance with Ofner and Bubnis (1996), who reduced the amino group content in glutaraldehyde treated gelatin from \(33 \times 10^{-5}\) to \(2 \times 10^{-5}\) moles g\(^{-1}\). Analogous to EGDE crosslinking (see Figure 4-4b), the swelling degree closely correlated with the amino group content. EGDE crosslinking prior to FA(g) treatment had two advantages: (1) the smallest swelling degree was achieved (SD = 1.9), and (2) longitudinal shrinking in consequence of the FA(g) treatment was limited (10 % compared to 16 % for FA(g)-only samples).
Figure 4-5. Gelatin yarns (uncrosslinked samples or diglycidyl ether crosslinked) were subjected to an additional crosslinking step using gaseous formaldehyde, FA(g), with successive heat treatment. This led to a massive decrease in free amino groups which went along with improved water-resistance (= decreasing swelling degree).

Figure 4-6. Different gelatin yarns swelled in deionized water for a prolonged time span: (a) one day and (b) one week. The yarn samples did not visually differ from the ones which were swelled for 1 hour only (see Appendix A.2., Figure A2-5).

Generally, the appearance of swelled yarn samples did not change over prolonged swelling in water (i.e. 1 hour, 1 day or even 1 week, see Figure 4-6 and Appendix A.2., Figure A2-5), indicating that swelling cycles of 1 hour were reasonable to investigate the yarns’ wet-state properties. The effect of swelling in deionized water was further surveyed by macroscopic
observation and light microscopy (see Figure 4-7a). After immersion in deionized water, non-crosslinked control samples immediately became transparent and lost the yarn structure. Upon drying, the samples compacted to a block-like, translucent gelatin structure. EGDE crosslinking clearly improved the swelling behavior, but the dried yarns also compacted to a degree where they could not be bent anymore. Inserting additional crosslinks with formaldehyde helped to fully stabilize the gelatin yarn: after 5 swelling cycles, the yarn was still bendable, however its integrity (i.e. the filament cohesion) was deteriorated.

Natural sheep wool contains internal and external lipids. Furthermore, the cuticle cells of sheep wool contain covalently bound C18 fatty acids, making the fibers naturally water- and soil-repellent. A common fraction of wool grease is known as lanolin, a mixture of various alcohol esters and fatty acids. In an attempt to mimic the hydrophobic nature of sheep wool, fully EGDE and FA(g) crosslinked gelatin yarns were impregnated with lanolin (see experimental section for details). In our experiments, we were able to incorporate a lanolin loading in yarns of up to 6.5 wt%. This approach improved the yarn integrity and reduced the hairiness (i.e. filaments protruding out from the yarn) of dried samples (see Figure 4-7a). Likewise, the swelling degree was further reduced to SD = 1.75. Gelatin yarns were subjected to multiple swelling cycles (see Figure 4-7b). Each cycle consisted of swelling the sample for 1 h in deionized water followed by drying. Over the five cycles, the yarns’ dry weight decreased by ~ 5 %. This was mainly due to residual, loose filaments falling off. Upon measuring the length change (see Appendix A.2., Figure A2-6) it was observed that the length in the dry state was unaltered and that lanolin-impregnated samples showed less longitudinal swelling.

Cross-sections of repeatedly swelled yarns (containing EGDE and post-treated with FA(g) and lanolin) were analyzed by SEM (see Figure 4-7c). Swelling had a distinctive effect: yarn porosity decreased and the average pore diameter increased due to merging of single pores. It is hypothesized that the water plasticized and softened the yarns despite the thorough crosslinking and – when taken out of the water – some of the pores collapsed. Judging by the heterogeneous filament cross-sections (nearly no pores vs. unaltered porosity), the pore collapsing was random. The porosity – initially at ~ 25 % – reached a plateau at 8 - 9 % after 3 swelling cycles. In contrast, the porosity was completely lost in samples without formaldehyde crosslinking after only one swelling cycle (see Appendix A.2., Figures A2-7 and A2-8).
Figure 4-7. (a) Macroscopic appearance and light microscopy images of gelatin yarns. After swelling in water, non-crosslinked control samples compacted to a block-like structure. EGDE-crosslinked yarns were also compacted and stiffened. Additional crosslinking with FA(g) conserved the filaments during multiple swelling cycles. Finally, lanolin impregnation further improved the filament cohesion and the yarn’s swelling behavior. Scale bars are 1 mm. (b) Weight change of gelatin yarns during several swelling cycles. (c) SEM images of EGDE and FA(g) crosslinked, lanolin-impregnated yarns which were swelled for up to 5 times. Scale bars are 40 µm.

To test the practicality in e.g. textile applications, gelatin yarns were also subjected to a simulated washing cycle (30 min shaking in detergent solution at 30 °C and rinsing with tap water). Similar to the above findings, the yarn structure was lost in EGDE-crosslinked samples (see Figure 4-8). Fully crosslinked samples with FA(g), however, were not harmed and optional lanolin impregnation did further improve the resistance to the detergent solution. As with swelling in water, the washing led to a decrease in filament porosity (see Appendix A.2., Figure A2-9).
In order to simulate a washing cycle, gelatin yarns were shaken in detergent solution at 30 °C for 30 min, rinsed with tap water and dried. In samples with EGDE only, the yarn structure was lost. Samples with FA(g) and optional lanolin-impregnation were not harmed. Scale bars are 1 mm.

The average mechanical properties are summarized in Table 4-1 and representative stress-strain curves for gelatin yarns and merino sheep wool are indicated in Figure 4-9. Out of the gelatin yarns, the control samples held the best mechanical properties with a tensile strength of 4.7 cN tex\(^{-1}\) (= breaking force in cN divided by the linear density in g per 1’000 m), thus approaching the value of merino wool (6.2 cN tex\(^{-1}\)). In comparison to sheep wool, the control gelatin yarn was significantly stiffer and evidently had a lower strain at break. Yet, the comparison of the strain at break is difficult as the tensile tests were conducted at small preloads (0.01 cN tex\(^{-1}\)) and as the two yarn types were twisted in different ways. Furthermore, a significant amount of strain in wool is attributed to its characteristic crimp: in the pre-yield region, only a small stress is required to straighten the crimp in the fiber, leading to an initial large strain.\(^{238}\) It is apparent that the sheep wool exhibited a small plastic regime, while the gelatin yarns broke at the yield point, comparable to stiffer natural fibers such as cotton.\(^{239}\) Gelatin materials tend to be brittle and stiff.\(^{76}\) As shown in a previous study (chapter 3), this characteristic could be altered by plasticizers. The addition of EGDE and heat treatment did minimally influence the yarns’ mechanical properties. The FA(g) crosslinking and lanolin impregnation had a more severe effect, though: both the tenacity and stiffness decreased to about 60 % of the control sample. In gas-phase crosslinking, the yarns were partially wetted as the formaldehyde was dissolved in water. It is known that crystalline structures and hydrogen bonds are lost in wetted protein, rendering the fibers more rubbery.\(^{200}\) Likewise, the incorporated lanolin did harm and weaken the filament structure in a similar way as plasticizers do, \textit{i.e.} by increasing the free space and mobility between polymer chains. This also explains the higher strain at break for post-treated yarns compared to EGDE yarns. Despite this trade-off (water-resistance vs. decrease in mechanical strength), the gelatin yarns’
mechanical properties are within reach of sheep wool. The strength of gelatin yarn (control sample) is also comparable to e.g. fibers from regenerated wool or commercialized protein fibers from the 1940-1950’s (casein, peanut, soybean, zein).

Table 4-1. Tensile test results of different yarns and commercial merino wool as a reference.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tenacity (cN tex⁻¹)</th>
<th>Elastic modulus (N tex⁻¹)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control no epoxide</td>
<td>4.73 ± 0.52</td>
<td>0.89 ± 0.04</td>
<td>8.91 ± 1.67</td>
</tr>
<tr>
<td>&quot; 120 °C, 4 h</td>
<td>4.66 ± 0.38</td>
<td>0.88 ± 0.11</td>
<td>8.44 ± 1.80</td>
</tr>
<tr>
<td>EGDE 50 wt%</td>
<td>4.19 ± 0.48</td>
<td>0.96 ± 0.08</td>
<td>6.64 ± 0.62</td>
</tr>
<tr>
<td>&quot; 50 wt% 120 °C, 4 h</td>
<td>4.33 ± 0.62</td>
<td>1.06 ± 0.11</td>
<td>5.98 ± 0.42</td>
</tr>
<tr>
<td>&quot; 100 wt%</td>
<td>4.32 ± 0.50</td>
<td>1.19 ± 0.16</td>
<td>5.79 ± 1.13</td>
</tr>
<tr>
<td>&quot; 50 wt% FA(g) 8 h 120 °C, 4 h</td>
<td>3.49 ± 0.48</td>
<td>0.63 ± 0.07</td>
<td>7.66 ± 1.26</td>
</tr>
<tr>
<td>&quot; 50 wt% &quot; lanolin</td>
<td>2.76 ± 0.23</td>
<td>0.52 ± 0.06</td>
<td>7.43 ± 1.02</td>
</tr>
<tr>
<td>&quot; 50 wt% &quot; swelled</td>
<td>2.64 ± 0.34</td>
<td>0.63 ± 0.09</td>
<td>7.11 ± 0.86</td>
</tr>
<tr>
<td>Merino wool</td>
<td>6.16 ± 0.10</td>
<td>0.65 ± 0.03</td>
<td>26.91 ± 2.71</td>
</tr>
</tbody>
</table>

Figure 4-9. (a) Illustrative tensile test curves of differently produced gelatin yarns. The crosslinking with ethylene glycol diglycidyl and formaldehyde as well as the impregnation with lanolin weakened the yarns. Interestingly, the lanolin impregnated and washed sample (gray) exhibited a different breaking behavior. It is hypothesized that the single filaments were sticking together more strongly due to the lanolin impregnation and washing, leading to a prolonged rupture. (b) Tensile test curve of commercial merino sheep wool.
A yarn with EGDE, FA(g) and lanolin as well as the reference merino yarn were disassembled in order to measure the mechanical properties of single filaments (see Appendix A.2., Table A2-1 and Figure A2-10). The high error of measurement was a result of (1) the delicate determination of the filament cross-sectional area and (2) possible damage of the filaments during disassembly of the yarns. In accordance with the yarn strength, the filament strength of the fully treated gelatin filament was ~ 45 \% of the merino wool. Both filaments showed a small elastic regime followed by a plastic regime and some strain hardening. This is in line with the findings of our previous studies (chapters 2 and 3). While the gelatin filaments exhibited high stiffness, the strength at break was smaller than in wet-spun fibers (up to 175 MPa).\textsuperscript{221} It is beyond question that the gelatin yarns or single fibers cannot compete with high-performance protein fibers from (regenerated) silk\textsuperscript{213}, \textsuperscript{240-241}, amyloid fibrils\textsuperscript{214} or hagfish slime thread.\textsuperscript{203} Still, these specialty fibers are not relevant on an industrial scale as the raw materials are scarce.

An omnipresent issue of most protein fibers is their limited wet strength.\textsuperscript{199-200, 212, 223} Wool stands out in this matter: due to high cysteine content, the keratinous material is stabilized by disulfide bridges.\textsuperscript{235} They play an important role in stabilizing the keratinous material and enable relatively high wet state strength (~ 70 \% of the dry strength) and moderate swelling.\textsuperscript{235, 242-243} This was reflected by the small swelling degree of merino sheep wool (SD = 1, own measurement). In comparison, more water protruded into gelatin yarns: although thoroughly crosslinked by EGDE and FA(g), these yarns absorbed 80 \% more water than wool (SD = 1.8). As water acts as plasticizer,\textsuperscript{179} the filament structure was accordingly weakened: in gelatin yarns (EGDE, FA(g) crosslinked and lanolin impregnated), the tensile strength decreased to a mere 0.4 cN tex\textsuperscript{-1} (15 \% of the dry strength) when being immersed in water for 1 h. Consequently, the strain at break increased from 7 \% to 30 \%. To circumvent the problem of insufficient wet state strength, blending of the protein with cellulose or synthetic polymers is proposed.\textsuperscript{201}

In order to demonstrate the processability of gelatin yarn into a textile, a glove was knitted (see Figure 4-10a-c). All yarns contained EGDE and in one spinning run, a red acid dye was added to the spinning dope to obtain red yarn. After knitting, the gelatin glove was heat treated and post-treated with formaldehyde and lanolin. The glove did not only persist swelling in water; after drying, it still had the same shape and haptics (see Appendix A.2., Figure A2-11). Besides, the gelatin glove resembled its counterpart from commercial merino sheep wool (Figure 4-10d).
Figure 4-10. (a, b) Spools with gelatin yarn (each ~ 10 m) of which a glove was knitted. (c) Crosslinking with ethylene glycol diglycidyl ether and gaseous formaldehyde (8 h) and impregnation with lanolin stabilized the gelatin glove to such an extent that it could easily withstand swelling in water for 1 h. (d) The analogy with a glove knitted from commercial merino sheep wool is remarkable.

In addition, the knitted gelatin glove and reference materials were examined in terms of their thermal insulation under free convection (see Table 4-2). The thermal conductivity from a heated plate through the textile (in contact with air) was measured with a heat flux sensor, allowing more sensitive measurements compared to the popular hot-plate methods, relying on the temperature drop only. The method was verified by measuring the conductivity of expanded polystyrene (0.039 W m\(^{-1}\) K\(^{-1}\)) which has literature values in the range of 0.03 - 0.04 W m\(^{-1}\) K\(^{-1}\).\(^{244}\) To study the effect of gelatin filament porosity, the gelatin cloth was measured in pristine condition (high filament porosity, ~ 27 %), after post-treatment (formaldehyde treatment, lanolin impregnation) as well as after swelling in water (low filament porosity, ~ 15 %). Contrary to the expectations, the thermal conductivity decreased with decreasing porosity. Consequently, it is hypothesized that the filament porosity did not significantly improve the thermal resistance. Instead, the cloth structure and density were more crucial for conductivity: the post-treatments (formaldehyde crosslinking, lanolin
impregnation) and swelling led to a shrinking in yarn length and thus to a denser cloth structure. Understandably, the denser gelatin cloth structure yielded a reduction in thermal conductivity. Altogether, the gelatin cloth showed very similar – or even slightly superior – thermal resistance to the merino sheep wool. On the other hand it goes without saying, that the high-performance materials (polyester fleece, expanded polystyrene) were superior thermal insulators compared to the gelatin and wool textiles.

**Table 4-2.** Thermal conductivity for knitted gelatin yarn cloth and reference materials (knitted merino wool, polyester fleece, expanded polystyrene).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thermal conductivity $\lambda$ (W m$^{-1}$ K$^{-1}$) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin cloth with EGDE pristine</td>
<td>0.060</td>
</tr>
<tr>
<td>&quot; post-treated</td>
<td>0.059</td>
</tr>
<tr>
<td>&quot; post-treated &amp; swelled</td>
<td>0.054</td>
</tr>
<tr>
<td>Merino wool cloth</td>
<td>0.060</td>
</tr>
<tr>
<td>Polyester fleece</td>
<td>0.046</td>
</tr>
<tr>
<td>Polystyrene (expanded)</td>
<td>0.039</td>
</tr>
</tbody>
</table>

$^a$ standard deviation determined from 3 independent measurements: ± 0.003 W m$^{-1}$ K$^{-1}$

### 4.4. Conclusions

Gelatin multifilament yarns of 400 - 600 tex (= g per 1’000 m) were fabricated by a dry-wet spinning process. A phase-separated spinning dope consisting of gelatin, deionized water and 2-propanol was used. Ethanol was applied as coagulation agent. When in contact with water, untreated gelatin readily swells and dissolves. In order to render the yarns water-resistant, we systematically investigated different crosslinking agents. The combination of ethylene glycol diglycidyl ether and formaldehyde yielded high crosslinking degrees, that is, a reduction of free amino groups from $28 \times 10^{-5}$ moles g$^{-1}$ (control sample) to $2 \times 10^{-5}$ moles g$^{-1}$. In an attempt to mimic the hydrophobic nature of sheep wool and further improving their stability and integrity, the gelatin yarns were impregnated with natural wool grease (lanolin). Gelatin yarns treated in this manner could repeatedly be swelled in water (SD = 1.8) or even washed in detergent solution. The only observed change was a decrease in filament porosity, which ceased after 3 swelling cycles at just below 10%. The non-crosslinked control samples showed good mechanical properties with a tensile strength (4.7 cN tex$^{-1}$) comparable to
merino wool yarn. However, the epoxide addition and post-treatments weakened the filaments. To demonstrate a possible use of gelatin textiles, a glove was knitted. The glove exhibited good thermal insulation capacity and haptics similar to its sheep wool analogue. As with most protein fibers, though, the poor wet state performance limit their applicability. Justifiably, Butler and McGrath state that protein polymers will never meet all industrial requirements and that petrochemical polymers cannot be fully substituted. Nevertheless, protein fibers are increasingly attractive for numerous applications where *e.g.* high-performance mechanical properties are dispensable. The applications may range from the biomedical field, where proteins such as gelatin or collagen are desired because of the similarity to tissue constituents, to textiles.
5. General Conclusions and Outlook
5.1. Conclusions

This work demonstrates how gelatin can be used as building block for the fabrication of biopolymer fibers. Three different spinning processes were investigated: dry spinning, wet spinning and a combination thereof. All methods have in common that a ternary mixture (gelatin/water/2-propanol) was used. The particular spinning dope stands out because the protein was previously precipitated, allowing robust spinning of porous gelatin filaments. Compared to other gelatin fiber spinning methods, the described processes presumably are more economic and have less ecological implications. Continuous fiber drawing promoted anisotropy and consequently improved mechanical properties.

The engineering of spinning processes was accompanied by investigations on the tailoring of fiber and yarn properties. The addition of plasticizers affected the fibers’ thermal and mechanical properties; they can, thus, be easily controlled and adjusted to specific requirements. To render the gelatin fibers water-resistant, different crosslinking methods based on epoxide and aldehyde reactive groups as well as impregnation with lanolin were explored. This yielded high crosslinking degrees and a significant reduction in swelling.

The combination of high disposability of the raw material, facile processability and good material properties (porosity, promising mechanical properties, nice luster, etc.) motivate for further investigations on the large-scale production and use of gelatin filaments or related products. Admittedly, a scale-up often bears difficulties. Potential challenges in this regard are listed below:

- It is yet unclear if gelatin from a phase-separated dope is processable on conventional industry-scale spinning machinery. Ideally, pilot experiments on an adapted wet spinning line are performed.
- A system for solvent recovery from the supernatant of the spinning mixture and from 2-propanol containing fumes has to be established.
- The applicability of gelatin filaments for mechanized processing steps (yarn twisting, cloth production) has to be assessed.
- Post-processing steps such as heat treatment, formaldehyde crosslinking and lanolin impregnation were conducted batch-wise. Continuous processing would be required for industrial production.
- Cost constraints: Synthetic polymers are popular not only because of their extraordinary properties but also due to the low price (e.g. 1 - 2 $ per kilogram polyethylene). In comparison, pig skin gelatin with high gel strength and no chromium contamination from leather industry is traded for at least 5 - 10 $ per kilogram. Furthermore, solution spinning of gelatin requires organic solvents, in Switzerland subjected to VOC-taxation. However, none of the commercialized biopolymers are price competitive and consumers generally are willing to pay more for bio-based polymers.

5.2. Future research activities

To date, man-made fibers from proteinaceous agro-waste streams have little importance due to the omnipresence of synthetic and cellulosic fibers. The main reason is the protein fibers’ insufficient wet strength. In order to generate a successful product, gelatin fibers have to be improved in this respect. Several approaches need to be investigated, e.g.:

- Chemical functionalization of the filament surface by means of atom-transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer (RAFT) polymerization or reaction with hydrophobic acyl chlorides.
- Chemical functionalization or crosslinking of carboxylic acid containing amino acids.
- Inclusion of fillers into the fiber (e.g. layered silicates such as montmorillonite).
- Blending with other (bio-)polymers suited for solution spinning.

Present understanding of the pore formation mechanism is insufficient. It is hypothesized that solvent droplets in the phase-separated spinning dope act as pore template. Future work should identify whether porosity and pore size can be controlled.

In this work, gelatin fibers were particularly studied in view of textile applications. It suggests itself that gelatin fibers could be applied in numerous other applications. Especially the biomedical field, where water-solubility may be an advantage, is of high interest.
Appendix

A.1. Supplementary data for chapter 2

**Figure A1-1.** Flow curves of the spinning phase and a 27 wt% aqueous gelatin solution at 50 °C. The spinning phase showed a higher zero-shear viscosity (9.1 Pa·s) and more prominent shear-thinning behavior than the reference gelatin solution (1.3 Pa·s).

**Figure A1-2.** Toughness (area under the stress vs. strain curve) of gelatin fibers spun at different draw ratios. A simultaneous increase in true strain at break, elastic modulus and strain at break led to a sharp increase in toughness for fibers with $\lambda > 1$. Analogous to the true strain at break, the toughness of gelatin fibers at maximal draw ratio ($\lambda = 2.4$) decreased.
Equation (2-1), defining the order parameter $S$ for XRD measurements according to Lovell, Mitchell and Windle.\textsuperscript{129-130}

$$S = \frac{1}{2} (3 \cos^2(\theta) - 1) = \frac{1}{2} \frac{\int_0^\pi I(\theta) (3 \cos^2(\theta) - 1) \sin(\theta) d\theta}{\int_0^\pi I(\theta) \sin(\theta) d\theta}$$

A.2. Supplementary data for chapter 4

Figure A2-1. ESI-MS spectrum of Epotec RD129, a tetrafunctional epoxide with an average molecular weight of approximately 469 g mol\textsuperscript{-1} (peak at m/z = 470 g mol\textsuperscript{-1} [M+H\textsuperscript{+}]).

Figure A2-2. Setup for measuring the thermal properties. (a) An aluminum block with mounted heat flux sensor and two temperature sensors. The sample was fixed directly above the heat flux sensor. (b) Wind shield screen (for free convection conditions) and data logger.
Figure A2-3. Scanning electron microscopy (SEM) images of commercial merino wool at different magnifications.

Figure A2-4. Scanning electron microscopy (SEM) images of gelatin filaments produced without the addition of crosslinkers (control sample). (a) Diagonally fractured fiber illustrating the high ratio of pore length to diameter. (b) Surface of the same sample.

Figure A2-5. Different gelatin yarns swelled in deionized water for 1 hour.
Figure A2-6. Length change of gelatin yarns during several swelling cycles. Each cycle consisted of swelling the yarns for 1 h in deionized water followed by drying. The yarns’ dry length was not affected by the multiple swelling (final length 98 % and 100 %, respectively) but the wet length did decrease in both samples.

Figure A2-7. Fiber porosity as a function of the swelling cycles. Gelatin yarns, which were only crosslinked by EGDE, lost all porosity after 1 cycle already. FA(g) crosslinked samples with optional lanolin impregnation kept their porous nature. However, a significant decrease in porosity was observed until a plateau at 8 - 9 % was reached after 3 swelling cycles.
Figure A2-8. Scanning electron microscopy (SEM) images of different yarns before, after one, three and five times of swelling in deionized water (each swelling was conducted for 1 h). In EGDE crosslinked yarns, the pores collapsed after one swelling cycle only. FA(g) treatment clearly increased the water-resistance. In terms of porosity and pore size, the lanolin impregnation did not have a positive effect. The scale bar corresponds to 50 µm.

Figure A2-9. Scanning electron microscopy (SEM) image of a gelatin yarn which was subjected to a simulated washing cycle. Prior to washing, the gelatin yarn was crosslinked with EGDE and FA(g) as well as impregnated with lanolin. Simulated washing led to slightly lower filament porosity and larger pore diameters. The effect was similar to swelling the yarn in deionized water.
**Table A2-1.** Tensile test results of single filaments. A gelatin yarn was prepared from a spinning mixture with 50 wt% EGDE, heat treated (120 °C, 4 h), FA(g) crosslinked (with subsequent heat treatment) and impregnated with lanolin. The yarn was disassembled into single filaments and mechanically tested. Single filaments from commercial merino wool were taken as reference.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Engineering tensile strength at break (MPa)</th>
<th>Elastic modulus (MPa)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin filament with EGDE, post-treated with FA(g) and lanolin</td>
<td>70.9 ± 23.6</td>
<td>3226 ± 551</td>
<td>35.4 ± 40.8</td>
</tr>
<tr>
<td>Merino wool filament</td>
<td>162.3 ± 101.3</td>
<td>4768 ± 2104</td>
<td>25.4 ± 16.5</td>
</tr>
</tbody>
</table>

**Figure A2-10.** Illustrative tensile test curves of single filaments.

**Figure A2-11.** (a) A glove made from gelatin yarn, containing ethylene glycol diglycidyl ether. The glove was post-treated with gaseous formaldehyde and impregnated with lanolin. (b) The same glove after immersion in deionized water (1 h) and drying.
References


154. Vaz, C. M.; De Graaf, L. A.; Reis, R. L.; Cunha, A. M., Effect of crosslinking, thermal treatment and UV irradiation on the mechanical properties and in vitro degradation


Curriculum Vitae

Philipp René Stoessel

Functional Materials Laboratory
Department of Chemistry and Applied Biosciences
ETH Zurich, Vladimir-Prelog-Weg 1, HCI E115
8093 Zurich
Switzerland
Phone: +41 44 633 75 87
Email: philipp.stoessel@chem.ethz.ch
Homepage: www.fml.ethz.ch

Private Address:
Salstrasse 21
8400 Winterthur
Switzerland

Born: April 28, 1987 in Vevey (VD), Switzerland
Citizen of Switzerland
Education

11/2012 - 09/2015  PhD studies at the Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, Functional Materials Laboratory, ETH Zurich, Switzerland
Advisor: Prof. Dr. Wendelin J. Stark
Title: Gelatin fibers: Spinning processes, fiber modification and application

02/2011 - 08/2012  MSc studies in Food Science, Department of Health Sciences and Technology (former D-AGRL), ETH Zurich
Major in Food Process Engineering, Minor in Food Chemistry
Master thesis at the Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, Functional Materials Laboratory
Advisor: Prof. Dr. Wendelin J. Stark
Title: Dry spinning process for the fabrication of oriented gelatin fibers

08/2007 - 06/2010  BSc studies in Food Science, Department of Health Sciences and Technology (former D-AGRL), ETH Zurich
Bachelor thesis at the Institute of Food Science and Nutrition, Laboratory of Food Process Engineering
Advisor: Prof. Dr. Erich J. Windhab
Title: Dispersing droplets in turbulent flow field using static mixers with different surface characteristics

08/2002 - 06/2006  High School, Aarau (Alte Kantonsschule Aarau)
Core subjects: music, mathematics, chemistry
Refereed Journal Articles


Patent

Presentations


Undergraduate student supervision

09/2013 - 02/2014 Madeleine Bloch (Food Science, ETH Zurich)
Master thesis: Labeling food products with silica/DNA nanoparticles

03/2015 - 04/2015 Andri Mani (Chemistry, ETH Zurich)
Research project: Fabrication of non-woven gelatin fibers by multiple centrifugal jet spinning
Teaching experience

Lecture “Chemical Engineering”, D-CHAB, ETHZ, 529-0625-00L
Teaching assistant, spring semester 2013 & 2014

Chemistry Laboratory Course, D-HEST & D-UWIS, ETHZ, 529-0030-00L
Course assistant, autumn semester 2013 & 2014

Professional Experience

09/2010 - 01/2011 Internship at Buhler India Pvt. Ltd. Bangalore
Establishing a new analytical laboratory for rice R&D activities, studying rice processing and collecting detailed data on rice properties.

08/2010 - 09/2010 Internship at Buhler AG Uzwil
Training in grain analytics

12/2006 - 09/2007 Internship at the Cantonal Laboratory Aargau (food safety & control)
Dairy inspections and studies on yeast-contamination in yogurt production, validation of methods, development of a PCR-method for fast germ detection

Activities & Varia

2010 - 2011 ETH Zurich, D-HEST (former D-AGRL)
Teaching assistant in Mathematics and Chemistry
Research assistant in Food Processing

12/2007 - present Member of the Swiss Student Foundation

04/2006 National contest “Schweizer Jugend forscht”

Languages

German (native), English (fluent), French (intermediate), Spanish (basic)