UNIVERSITY OF NAIROBI

DEPARTMENT OF CHEMISTRY

Extraction and Characterization of Gelatin from *Lates niloticus* Scales and Potential Industrial Applications

BY

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A thesis submitted to the Board of Post Graduate Studies in partial fulfillment for the requirement of the degree of Master of Science in Industrial Chemistry of the University of Nairobi.

31st JULY, 2016

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DECLARATION

This thesis is my original work developed as a requirement for the partial fulfillment of Master of Science in Chemistry and has not been presented before for a Degree in any other University.

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DEDICATION

This work is dedicated to my family.

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God for the unending grace he bestowed upon me during the duration of this work. Without his guidance and protection, I would not have been able to complete my work.

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ABSTRACT

This study sought to extract and characterize gelatin from *Lates niloticus* (Nile perch) scales, then blend it with polyvinyl alcohol (PVA). Hydrolysis of the scales was done using a crude alkaline protease harvested from a bacterium, *Bacillus cereus strain wwcp 1,* obtained from Lake Bogoria and the resulting solution lyophilized to obtain gelatin powder. The yield was calculated based on the dry weight of the scales and was shown to be 16.3%.

The sample was characterized using infrared spectroscopy and showed peaks at 3442 cm^{-1}, 1653 cm^{-1} and ~ 1590 cm^{-1} corresponding to Amide A, Amide I and Amide II bands respectively. Amino acid analysis of the sample was done using an Agilent 1260 HPLC. Glycine was the most abundant amino acid (21.7%), followed by proline (14.6%) and alanine (11.8%). Isoleucine, Histidine and Tyrosine were the least abundant (1.8, 1.4 and 0.9% respectively).

Polyvinyl alcohol-gelatin blend films of various compositions ranging from 10% to 90% PVA were prepared by solution casting method, Differential Scanning Calorimetry (DSC) and Thermo-gravimetric Analysis (TGA) tests showed the films had glass transition, melting and thermal decomposition onset temperatures intermediate between those of the respective individual polymers (PVA and gelatin). The thermal stability of the films reduced with the increase in the amount of the less thermally stable constituent.

Lastly, potential applications of the prepared blend films were investigated. Batch experiments to assess the potential of the polymer blend films as adsorbent material were done using Methylene Blue dye. The films were found to adsorb up to 64% of the dye. The percent dye removal varied with initial concentration of the dye and contact time.

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LIST OF ABBREVIATIONS

DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared spectroscopy
TGA	Thermo Gravimetric Analysis
PVA	Polyvinyl alcohol
PCL	Polycaprolactone
T_g	Glass transition temperature
T_m	Melting temperature
ACE	Angiotensin-I-Converting Enzyme
BSG	Bovine Skin Gelatin
PSG	Porcine Skin Gelatin
SD	Standard Deviation

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Gelatin is the main derivative of collagen, the most copious structural protein in animals. Collagen is found in the skin, bones, cartilage etc. In humans for example, it makes up a third of the total protein and overally comprises 75% of skin's dry weight (Shoulders and Raines, 2009). When collagen undergoes partial hydrolysis, gelatin, which is a water soluble polypeptide, is produced. Gelatin is one of the most used colloids in a number of industries including food and pharmaceuticals among others. It is produced in four grades; edible, pharmaceutical, photographic and industrial depending on its intended final use (Tavakolipour, 2011).Within the food industry for instance, gelatin is employed for texturization, gelling, stabilization and water binding properties. It is also widely applied in pharmaceuticals to make capsules, tablets, suppositories and blood plasma substitutes (Nik Aisyah *et al*., 2014).

Conventionally, gelatin is produced from mammalian sources. Bovine and porcine skins contribute 46% of total global output while bones and hooves contribute 23% and 29% respectively. Only 1% is from marine sources (Karim and Bhat, 2009; Wang *et al.,* 2010). Commercial gelatin production involves processes that generally lead to the destruction of the primary, secondary and tertiary structure of the parent collagen. These processes usually occur in two stages that involve pretreatment of the raw materials and extraction of gelatin. The pretreatment can be categorized into two steps; acid pretreatment and alkali pretreatment. The type of pretreatment to be used is determined by the raw material's source and the intended use.

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To produce gelatin from highly interconnected cattle connective tissue, alkaline pretreatment is preferred and is applied for up to twenty weeks (Karayannakidis and Zotos, 2014). Acid pretreatment is preferred for materials such as pig skin, and is usually done for shorter periods due to a lower degree of collagen cross linkage. The next stage in the extraction process involves neutralization of the pretreated material to neutral pH before gelatin is extracted using hot water.

The fish processing industry currently generates wastes up to 75% of the total catch weight. The wastes include scales, guts, heads, skins and bones, and are usually either used to produce low value goods such as fishmeal or are dumped in landfills or water bodies posing potential environmental harm. The dry mass of fish is largely composed of protein and therefore the fish processing by-products are candidates for alternative sources of high value protein ingredients such as food grade gelatin (Karayannakidis and Zotos, 2014).

Fish collagen has several intrinsic bioactive properties. It is able to stimulate skin collagen production and has anti-inflammatory, anti-wrinkle as well as UV damage repair activity. It has also been widely employed in the pharmaceuticals industry for the manufacture of wound dressings, drug delivery vehicles and vitreous implants. Fish collagen however has low thermal stability when compared to the mammalian counterpart and this poses a major problem for biomedical applications. The variation in thermal stability between mammalian and fish collagen is attributed to their imino acid content and distribution of molecular weights (Johnston-Banks, 1990).

Imino acids have a key role in stabilizing the collagen helix structure. The amount of imino acids in mammalian collagen is higher than that in fish collagen. Collagen sourced from cold water fish has fewer imino acids than warm water fish sourced collagen, and hence gelatin from

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mammalian sources and warm water fish have a higher melting point than cold water fish sourced gelatin (Singh *et al.,* 2011). In order to produce stable scaffolds for biomedical applications, it is essential to stabilize fish collagen either physically or microbiologically.

There are many fish processing plants in the country where fish filleting (both marine and freshwater fish) is done, both for domestic use and export to European Union and other markets. Fish scales, a byproduct of that processing, is largely not commercially utilized in Kenya. Furthermore, the establishment of an economic stimulus package by the government involving the establishment of fish ponds in all Counties in Kenya has increased production and consumption of fish that is expected to lead to more scales produced. This project therefore targeted utilization of fish scales waste obtained during fish processing to extract gelatin that was subsequently characterized and potential applications investigated.

The scales utilized were from *Lates niloticus*, commonly known as the Nile perch. This is a large fresh water fish species found extensively in Lake Victoria, East Africa that can grow up to 200 kg and two meters in length and thus a large amount of scales and other byproducts can be obtained from its processing.

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1.2 PROBLEM STATEMENT

Gelatin is widely applied in food processing. A large portion of the gelatin produced and used in the world is from porcine and bovine sources. With the outbreak of mad cow disease (BSE) and bird flu (avian influenza), concerns have developed over the possibility of transmission of prions via bovine products and bird flu in poultry products to humans. Additionally, in some communities, the consumption of porcine products is prohibited, for instance the Muslim and the Jewish communities due to halal and kosher restrictions respectively. In the Hindu community, cows are regarded as sacred animals and the consumption of bovine products is prohibited. All these factors in combination create a need for alternative sources of gelatin (Karim and Bhat, 2009; Kittiphattanabawon *et al.,* 2012).

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1.3 OBJECTIVES

1.3.1 Main objective

The overall objective in this study was to extract gelatin from *Lates niloticus* scales, prepare and characterize polymer blends containing gelatin, and investigate a potential industrial application

1.3.2 Specific Objectives:

(i) To extract gelatin from *Lates niloticus* scales using a crude alkaline protease from *Bacillus cereus strain wwcp 1*.

(ii) To determine the amino acid composition of gelatin.

(iii) To prepare polymer blends containing gelatin and characterize them using FTIR, DSC and TGA.

(iv) To investigate potential industrial applications.

1.4 JUSTIFICATION.

Fish production and consumption is at an all time high worldwide. The byproducts of fish processing industry including heads, skins and scales form a huge proportion of the total fish catch. These byproducts are often discarded causing environmental pollution. Fish is composed mainly of protein, and hence its byproducts contain huge amount of proteins meaning they can be used to produce high value protein goods such as gelatin. Consequently, over the last few years, there has been a rise in interest in these by-products as alternative sources of gelatin. Most studies however have focused on the skin as a gelatin source. Very few studies on scales as a

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source have been reported and none has been reported on *Lates niloticus* scales. This research seeks to investigate *Lates niloticus* scales as an alternative source of gelatin.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.0.1 Collagen and Gelatin

Gelatin is not found naturally but is obtained from collagen, which is the main protein in the skins, bones and connective tissues of animals (Karim and Bhat, 2009). Up to date, twenty nine collagen varieties have been identified. The varieties are collagen I-XXIX (Gelse, 2003), distributed widely in bones, tendons, skins, vascular system and intramuscular connective tissue, contributing to stability and structural integrity. Each variety is considerably different in terms of sequence, structure and function from the rest.

Collagen type I, a heterotrimeric molecule made up of two α1(I) chains and one α2(I) chain represented as $[\alpha1(I)]_2\alpha2(I)$, is the most copious in connective tissue. The α chains are intertwined to form what is widely known as the collagen triple helix (Gelse, 2003; Gomez-Guillen *et al.,* 2002; Liu et *al.,* 2008). Collagen II is largely found in cartilage tissue. A more detailed classification of collagens is shown in Table 1 below (Gelse, 2003).

Although individual collagen types vary widely in structure, they are all composed of a right handed triple helix made up of three α chains. The chains in the helices could be identical resulting in homotrimers such as collagen II, III, VII and X, or they could completely different hence heterotrimers such as collagens I,IV,VI and XI (Gelse, 2003).

The three chains, of similar size and each formed into a left-handed helix, are super coiled about a middle axis in a right handed way forming the triple helix. The triple helix results from having

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a glycine residue in all third positions on the chains producing a repetitive $(Gly\text{-}X\text{-}Y)_n$ structure. Proline and Hydroxyproline inhabit the X and Y positions in that order.

Table 1. Classification and tissue distribution of some collagens.

Type	**Molecular composition**	**Tissue distribution**
I	[α1(I)]2 α2(I)	Dermis, , ligaments, tendon, cornea, bone
II	[α 1(II)]3	Cartilage, nucleus pulposus, vitreous body,
III	[α1(III)]3	Skin, reticular fibres of most tissues ,vessel wall
IV	$[\alpha1(IV)]_2\alpha2(IV)$;α1–α6	basement membranes
V	α1(V), α2(V), α3(V)	Lung, cornea, fetal membranes; together with type I collagen, bone.
VI	α1(VI), α2(VI), α3(VI)	cartilage, placenta, lungs, vessel wall, intervertebral dermis, disc,
VII	[α 1(VII)]3	Skin, dermal – epidermal junctions
VIII	$[\alpha\ 1(VIII)]_2\ \alpha\ 2(VIII)$	Endothelial cells, Descemet's membrane
IX	α1(IX) α2(IX) α3(IX)	vitreous humor, cornea, cartilage,
X	$[\alpha\ 3(X)]_3$	Hypertrophic cartilage
XI	α1(XI) α2(XI) α3(XI)	Cartilage, vitreous body
XII	[α1(XII)]3	Perichondrium, ligaments, tendon

Source: Gelse et al (2003)

Hydrogen bonds between Glycine and proline in neighbouring chains stabilize the triple helix. There are short terminal regions that do not produce the triple helix structure. In these regions,

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referred to as telopeptides, inter-and-intramolecular covalent cross-links primarily concerning lysine and hydroxylysine residues are located. Four to eight collagen molecules are reinforced and stabilized by covalent bonds to form collagen fibrils (Gelse, 2003; Gomez-Guillen *et al.,* 2005; Karim and Bhat, 2009; Liu *et al.,* 2008).

In its native form, collagen is a water insoluble protein composed of high strength fibrils partly as a result of the triple helices adopting a 3D structure that favours interchain hydrogen bonding which in turn, stabilizes the triple helices.

2.0.2 Conversion to gelatin

Gelatin is obtained via partial hydrolysis of parent collagen. The hydrolysis can be catalyzed by enzymes, or more conventionally a chemical pretreatment which cleaves non-covalent bonds disorganizing the protein configuration resulting in collagen swelling and solubilization. A heat treatment at about 40°C destroys the hydrogen bonding that helps stabilize the helix and the collagen fibrils dissociate into tropocollagen units. Consequently, the intra-molecular bonds connecting the three strands of the helix are broken resulting in a helix to coil transformation (Gomez-Guillen *et al.,* 2002; Karim and Bhat, 2009; Mariod and Adam, 2013).

The chemical pretreatment of collagen can be carried out in two ways, an acid pretreatment or an alkali pretreatment. Gelatin from the acid pretreatment is referred to as gelatin type A and that from basic pretreatment gelatin type B. The type of pretreatment to be employed is determined by the type of raw material. Acidic pretreatment is preferred for lightly covalently cross-linked collagens such as those in porcine skins while alkaline pretreatment is preferred for the more cross linked bovine hide collagens (Karim and Bhat, 2009).

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As shown in figure 1 below, collagen conversion can take three paths; the first resulting in the formation of three separate randomly coiled α chains, the second path β chains (two covalently cross-linked, and one independent α chains) and finally a third, which results in γ chain (three covalently linked α chains). Gelatin therefore exists as a mixture of α, β and γ peptide chains in different proportions.

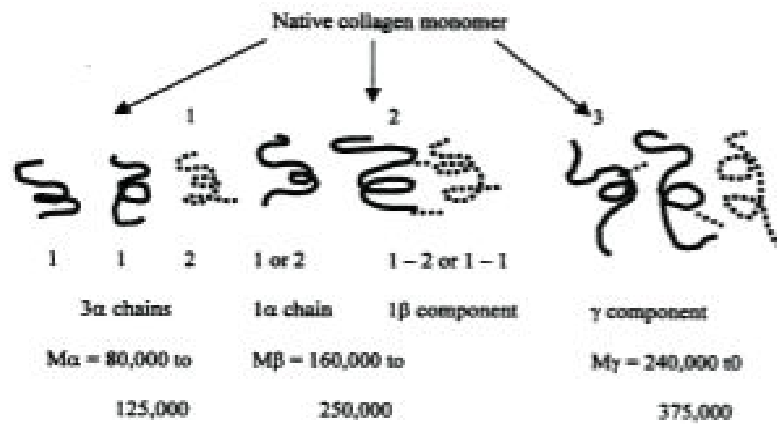

Figure 1: Collagen conversion paths.

The properties of gelatin resemble that of the parent collagen. Cleavage of collagen inter-chain cross linkages during extraction results in molecules of different masses and therefore, gelatin, composed of α-chains, α-chain dimers (β-components), high molecular polymers such as γ-components, and some low molecular weight fragments has a lower molecular weight as compared to collagen. This variation in molecular weights ultimately has an effect on the functional properties of gelatin (Karim and Bhat, 2009; Zhou *et al.,* 2006).

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2.0.3 Amino acid composition of gelatin

Consistent with its protein nature, gelatin comprises a long chain of amino acids each linked to its neighbor via a covalent bond known as the peptide bond, hence the name polypeptides for proteins (Alberts, 2002). There are 21 amino acids that make up proteins. The sequence and number of them in each protein varies, thus conferring uniqueness. The amino acid composition of collagen has been widely studied and a summary of the composition of collagen from bovine and porcine skins is presented in Table 2 below compiled by Nhari *et al.*, (2011).

Table 2: Amino acid profile of collagens from bovine and porcine skins.

Amino acid	BSG (residues/1000 total residues)	PSG (residues/1000 total residues)
Non polar		
Alanine	33	80
Valine	10	26
Leucine	12	29
Isoleucine	7	12
Phenylalanine	10	27
Methionine	4	10
Proline	63	151
Total	139	335
Polar uncharged		
Glycine	108	239
Serine	15	35
Threonine	10	26
Tyrosine	2	7
Total	135	307
Polar acidic		
Aspartic acid	17	41
Glutamic acid	34	83
Total	51	124
Polar basic		
Lysine	11	27
Arginine	47	111
Histidine	not detected	not detected

Source: Nhari et al (2011)

Collagen has a somewhat unusual amino acid composition relative to other proteins. It contains more glycine than any other vertebrate protein, with glycine forming a third of all residues. In

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fact, collagen can be thought of as a polymer of glycine led tri-peptides (Gly-X-Y)n (Talwar, 2003). The overall imino acid content is also uniquely high. Proline generally occupies the X position while the Y position is occupied by either hydroxyproline or hydroxyleucine. Collagen is the only mammalian protein containing hydroxyproline and hydroxyleucine. Alanine is also present in abundant quantities while methionine is the only sulphur containing amino acid present. Cysteine, and tryptophan are all absent (Podczeck and Jones, 2004).

Studies show that the amino acid composition of gelatin resembles that of the parent collagen. (Karayannakidis and Zotos, 2014) reported that gelatin contains all the amino acids found in proteins excluding tryptophan and cystine. Methionine and tyrosine are low and glycine is the most copious, making up 33% of the total amino acids. Table 3 below displays the amino acid content of gelatin from various sources as reported different studies (Nik Aisyah *et al.,* 2014).

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Table 3; Amino acid composition of gelatins from different sources

Amino acid (%)		[a]Chicken skin	[b]Porcine skin	[c]Bovine skin	[d]Fish skin
Alanine	Ala	101	112	113	123
Arginine	Arg	56	49	47	47
Aspartic acid	Asp	21	46	46	48
Cystine	Cys	1.6	0	0	0
Glycine	Gly	337	330	342	347
Glutamic acid	Glu	58	72	74	69
Histidine	His	30	4	4	6
Hydroxyproline	Hyp	121	91	83	79
Isoleucine	Ile	12	10	11	8
Leucine	Leu	26	24	24	23
Lysine	Lys	47	27	25	25
Methionine	Met	7	4	4	9
Phenylalanine	Phe	18	14	12	13
Proline	Pro	134	132	127	119
Serine	Ser	22	35	39	35
Threonine	Thr	10	18	33	24
Tyrosine	Tyr	12	3	4	2
Valine	Val	19	26	19	15
Imino acid	(Hyp+Pro)	255	223	215	198

Source: Nik Aisyah et al (2014)

As can be determined from Table 3, the amino acid composition of gelatin is variable, especially in the minor constituents, depending on the raw material and process used. The average percentage of individual amino acids by weight have been reported as follows, "Gly 21 %, Pro 12 %, Hyp 12 %, Glu 10 %, Alanine (Ala) 9 %, Arg 8 %, Asp 6 %, Lys 4 %, Serine (Ser) 4 %, Leucine (Leu) 3 %, Valine (Val) 2 %, Phenylalanine (Phe) 2 %, Threonine (Thr) 2 %, Isoleucine (Ile) 1 %, Hydroxylysine (Hyl)1 %, Methionine (Met), His < 1 % and Tyr < 0.5 %" (Gorgieva and Kokol, 2011). Figure 2 displays the structure of some of gelatin's amino acids.

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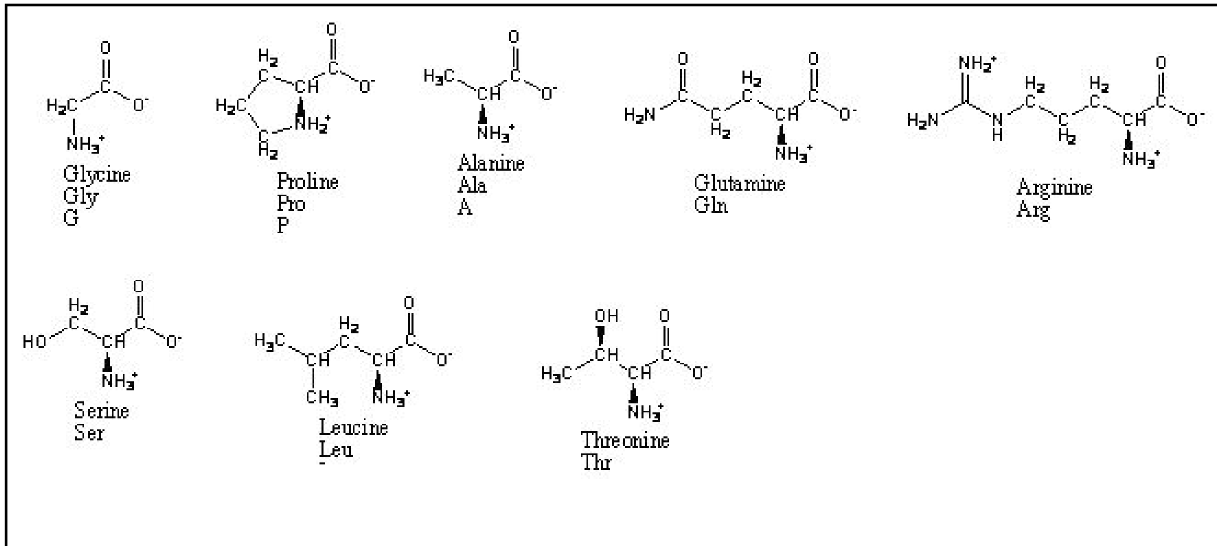

Figure 2: Structure of some amino acids found in gelatin.

The variation in the amino acid composition of gelatin with source species is a consequence of the different temperatures found in animals' various habitats (Jongjareonrak *et al.,* 2006). The imino acid content as well as proline hydroxylation degree of collagen from warm blooded animals and warm water fish is higher than that of cold water fish collagen. Mammalian gelatin has the highest imino acid concentration, about 30%, followed by warm water fish gelatin and then cold water fish gelatin at 22-25% and 17% respectively (Hoque, 2011).

The most outstanding features of gelatin are its capability to produce thermo-reversible gels and its remarkable solubility in water (Karim and Bhat, 2009). As established earlier, gelatin chains exist in coil conformation. When the temperature of a gelatin solution is lowered below a certain critical value known as the gelling temperature, fractional renaturation of the collagen molecules occurs and the coils undergo a reverse coil-helix transformation forming the gels (Djabourov *et al.,* 1988). Hydrogen bonds existing amongst the imino acids stabilize the gels and therefore, the more the imino acids; the stronger the bonds and the more the stability. The gels are said to be

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thermo-reversible, since they melt when the temperature is raised beyond a certain value – the gel melting point. This temperature is lower than the body temperature of humans, giving gelatin its melt property in the mouth, highly exploited in the food industry as well as pharmaceuticals. The gelling and melting points of the gels are also directly proportional to the amount of imino acids. (Gilsenan and Ross-Murphy, 2000; Karim and Bhat, 2009).

2.0.4 Commercial sources of gelatin

Mammalian gelatin is the most popular commercially. This is evidenced by the fact that out of the approximately 326,000 tons produced annually, pig skin derived gelatin accounts for 44%, bovine hide gelatin 28% and bovine bone gelatin 27% (Shyni *et al.,* 2014). In total, mammalian gelatin accounts for 99% of total world production, with other sources contributing a measly 1%.

The popularity of mammalian gelatins could be attributed to the fact that gels based on them have superior rheological properties such as viscosity and gel strength providing a wide variety of applications (Gomez-Guillen *et al.,* 2002). Gelatin from alternative sources, including marine, was 1.5% in the year 2002 but was almost double that in the year 2007 (Shyni *et al.,* 2014) indicating a rise in the importance of alternative, non mammalian gelatin sources. They attributed this to shortage of and competition amongst producers for bovine and porcine raw materials.

2.0.5 Alternative sources of gelatin

Various researchers (Jongjareonrak *et al.,* 2006; Karim and Bhat, 2009; Muyonga *et al.,* 2004; Wangtueai and Noomhorm, 2009) point to the outbreak of Bovine Spongiform Encelopathy and the concern that any kind of bovine product transmits it as another a reason for increased interest

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in alternative sources of gelatin. It is also well documented that consumption of pig related products is prohibited in Muslim and Jewish communities while in the Hindu community, cows are regarded as sacred and thus consumption of cow related products is forbidden.

There are various candidates for alternative sources of gelatin. Mariod and Adam (2013), reported gelatin extraction from insect sources, particularly the melon bug (*Aspongopus viduatus*) and the sorghum bug (*Agonoscelis pubescens*). Three different extraction methods were employed. The highest yield was 3% from hot water extraction followed by 1.5% from mild acid extraction and lastly 1.0% from distilled water extraction. They further reported that the FTIR spectra of the insect gelatin seemed similar to that of commercial gelatin and the amide II bands of the gelatins appeared at around 1542 to 1537 cm^{-1}. The low yields could limit insects as alternative sources of gelatin.

Another potential source of gelatin is poultry and its byproducts (Azzainurfina and Jamalulail, 2012; Nik Aisyah *et al.,* 2014; Widyasari and Rawdkuen, 2014). Azzainurfina and Jamalulail, (2012) extracted type B gelatin from chicken feet and compared its properties with commercial bovine gelatin. They reported the yield as 18% w/w and that the gelatins thus extracted were found to have lower bloom values and viscosity as compared to commercial bovine gelatin. In conclusion, they observed that the quality chicken feet gelatin is better than that of fish gelatin but poorer than that of commercial gelatin.

Chicken feet have also been studied as sources of gelatin (Widyasari and Rawdkuen, 2014). Acid and ultrasound assisted extraction was employed and the gelatin produced was reported to have properties that were not significantly different from those of commercial bovine gelatin.

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Even though gelatin from poultry seems to be the best alternative to mammalian gelatin, at least based on its superior qualities, its use is limited. This is mainly due the fact that obtaining certification for poultry products is difficult. This certification is essential for traceability, required for food ingredients especially from animal sources (Karim and Bhat, 2009). Furthemore, the production of poultry products is low when compared to mammalian products and also, poultry products are more expensive. Health concerns mainly due to avian influenza virus is also another factor (Nik Aisyah *et al.,* 2014).

Fish and its by-products are composed mainly of protein and can therefore be considered to be raw materials for the production of proteinaceous ingredients. Fish skin, heads, scales and fins contain a lot of collagen and thus can be utilized in the preparation of food grade gelatin (Karayannakidis and Zotos, 2014).

The global fish catch exceeds 100 million tons annually, with most of it processed into fish fillet and other products. This is on the rise, as evidenced by the data presented in Table 4 below.

Table 4: Annual fish production (million tons) from 2000-2011.

Production	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
CAPTURE												
Inland	8.8	8.9	8.7	9.0	8.6	9.4	9.8	10.0	10.2	10.1	11.2	11.5
Marine	86.8	84.2	84.5	81.5	83.8	82.7	80.2	80.4	79.5	79.2	77.4	78.9
TOTAL	95.6	93.1	93.2	90.5	92.4	92.1	90.0	90.3	89.7	89.6	88.6	90.4
AQUACULTURE												
Inland	21.2	22.5	24.0	25.5	25.2	26.8	31.3	33.4	36.0	38.1	41.7	44.3
Marine	14.3	15.4	16.4	17.2	16.7	17.5	16.0	16.6	16.9	17.6	18.1	19.3
Total	35.5	37.9	40.4	42.7	41.9	44.3	47.3	49.9	52.9	55.7	59.9	63.6
TOTAL WORLD FISHERIES	131.1	131.0	133.6	133.2	134.3	136.4	137.3	140.2	142.6	145.3	148.5	154.0

Source: Brooks, (2013)

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The byproducts of fish processing account for about 70-85% of the total weight catch, a large amount that can be converted to value added products e.g. gelatin benefiting the food industry economically in terms of waste management (See *et al.,* 2010).

Fish gelatin has been studied from as early as the 1950's. However it is only recently that more intensive studies on fish gelatin have been carried out and started appearing in the literature (Karim and Bhat, 2009). A huge number of species have been studied as potential sources of gelatin. Researchers all over the world studying fish processing byproducts in their locality for gelatin extraction. Different byproducts-skin, heads, scales and even fins have been used.

Jongjareonrak *et al.,* (2006) extracted skin gelatin from two tropical fish species, the brownstripe red snapper and the bigeye snapper. They reported that the gelatin from the two species had slight variations in their amino acid compositions. However, the imino acid content of both gelatins was greater than that recorded for sole, hake, cod and megrim in a study by (Gomez-Guillen *et al.,* 2002).

Nikoo *et al.,* (2011) obtained relatively high gelatin yields from the skin of farmed Amur sturgeon (*Acipenser schrenckii*) at 19.6%. This was calculated from the weight of freeze dried gelatin vis-à-vis that of wet fresh skin. They investigated the amino acid profile of the gelatin and found its glycine and imino acid composition to be 336 and 138/1000 residues respectively. The gel strength was 316 g.

The quality of gelatin extracted varies depending on the source species, the species' habitat and the conditions under which the gelatin was extracted. There have been studies aimed at optimizing the conditions for gelatin extraction from fish skins. Mahmoodani *et al.,* (2014)

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studied and optimized the extraction of gelatin from the skin of Pangasius Catfish (*Pangasius sutchi*). They established that the optimal extraction occurred with a pretreatment of 0.2N sodium hydroxide, 0.1N acetic acid together and a hot water extraction at 63.7°C for 2.41 hours. Under these optimal conditions, the yield was impressively high, 23.61%. The gel strength was reported as 438 g which is high probably due to a high imino acid content at 18.01%.

Amiza *et al.* (2015) determined the effect of acid (CH_3COOH) concentration, the ratio of skin to water, heat and time on the yield of gelatin from Cobia (*Rachycentron canadum*) skin. They reported that the yield is linearly proportional to all the four parameters with optimal conditions being a CH_3COOH concentration of 0.15 ml/L, extraction temperature of 83.2°C, 6 hours and a skin to water ratio of 1:6 skin. These conditions produced gelatin with 20.1% yield and gel strength of 205.6 g. Further, they compared the properties of Cobia gelatin with those of commercial bovine gelatin and found that the foaming and emulsification properties of the two were similar at concentrations of 1% but at 2% and 3% bovine gelatin performed better. Cobia gelatin's fat binding capacity was higher than that of bovine gelatin but its water holding capacity was lower. They concluded that Cobia gelatin showed great potential to be used as an alternative to bovine gelatin.

Shyni *et al.*, (2014) isolated gelatin from skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*) skins and determined the physico-chemical properties. The yields were 19.7%, 17.2% and 11.3% for shark, tuna and rohu respectively. Amino acid analysis showed hydroxyproline content to be highest in dog shark skin gelatin at 9.85%, rohu had 6.78% and tuna 7.86% and therefore the gel strength of dog shark skin gelatin was highest at 206 g followed by tuna at 177 g and finally rohu at 124 g. Due to considerable

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differences in the yield and functional properties of dog shark, skipjack tuna and rohu skin gelatins, they concluded that the properties of a gelatin are dependent on the source species.

See *et al.,* (2010) extracted gelatin from the skins of four fish species that live in fresh water and compared the gelatin thus obtained to commercial gelatin. The species were the snakehead (*Channa striatus*), catfish (*Clarias batrachus*), pangasius catfish (*Pangasius sutchi*) and red tilapia (*Oreochromis niloticus*). They reported a higher pH, turbidity, lipid, moisture and ash content for each of the extracted gelatins when compared to the commercial gelatins but lower protein content. They further reported that the four extracted gelatins had gel strengths closer to those of mammalian gelatin than those of cold water fish.

Abbey *et al.,* (2008) also extracted and characterized gelatin from flying gurnard (*Dactylopterus volitans L)*, a fish found in warm to temperate waters on both sides of the Atlantic, skins. They then investigated the interactions of the gelatin thus extracted with starch. There have been many more studies on fish skins of various species as sources of gelatin including those by, Jaswir *et al.,* (2009) who isolated gelatin from the bigeye snapper and brown stripe red snapper, and studied its chemical composition as well as the effect of microbial trans-glutaminase on gel properties, and Muyonga *et al.,* (2004a) who did physicochemical characterization of gelatin extracted from Nile perch skin.

Other by-products of fish processing such as heads, scales and bones also contain huge quantities collagen and can be exploited as gelatin sources. In addition to the skin, Muyonga *et al.,* (2004a) also extracted gelatin from the bones of the Nile perch (*Lates niloticus*). They compared the properties of gelatins from the two sources and found out that they have the same amino acid composition, with about 21.5% imino acid composition. They however reported that the bone

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gelatin had lower yields, gel strength and viscosity as compared to skin gelatins. Skin gelatins had lower setting time and superior film tensile strength but poorer percent film elongation as contrasted with bone gelatin. They attributed the difference skin and bone gelatin properties to variations in molecular weight distribution of the gelatins.

Wang *et al.,* (2010) showed that collagens isolated from fish bones and scales have amino acid profiles similar to those of gelatins from fish skin. Bones and scales therefore can be employed as sources of gelatin. However, limited research on scales as a gelatin source has been carried out. Zhang *et al.,* (2011) extracted gelatin from the scales of grass carp (*Ctenopharyngodon idella*) then compared its properties to those of commercial porcine gelatin. They found that the gel strength and gel visco-elastic properties of grass carp gelatin were higher superior to those of commercial porcine skin gelatin. However, the imino acid content (16%) and gelling and melting points (20.8 and 26.9°C respectively) were shown to be lower.

Akagündüz et al. (2014) investigated the potential of sea bream bones and scales to produce gelatin, and managed to obtain gelatin with decent gelling properties. Gelatin obtained from scales had superior gel strength and visco-elastic properties. In contrast, bone gelatins gave low yields and displayed poor rheological properties. This could be as a consequence of, calling for more exhaustive measures employed to ensure sufficient pretreatment. Further, they tested the scale gelatin as a potential angiotensin-I-converting enzyme (ACE) inhibiting peptide. ACE is vital in the regulation of hypertension and blood pressure as it catalyzes the transformation of angiotensin-I into angiotensin-II and inhibits bradykinin (Akagunduz *et al.,* 2014).They concluded that sea brass scales have promising potential as a source of peptides with good ACE inhibitory activity.

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Another study isolated gelatin from the scales of farmed sea bass (*Dicentrarchus labrax*) (DinçEr *et al.,* 2015). The gelatin obtained had a high bloom value, 305 g, associated with the reported high imino acid content, 15751 mg/100 g and 10,223 mg/100 g for proline and hydroxyproline respectively. Amino acid analysis of the gelatin displayed high alanine content and a glycine content of 24,406.5 mg. These results are consistent with the theoretical expectation that glycine, proline and alanine are the most abundant amino acids in gelatin.

This study aimed to extract gelatin from Nile perch (*Lates niloticas*) scales. Nile perch is a large fresh water fish found extensively in Lake Victoria, East Africa, that can grow up to 200 kg and two meters in length and thus a large amount of scales and other byproducts can be obtained from its processing.

2.0.6 Natural polymers as alternatives for petroleum based polymers

Natural polymers have been used and studied since ancient times. However, the availability of petroleum and the low cost of petroleum based synthetic polymers basically drove natural polymers out of the picture. In the last few decades; natural polymers have experienced a revival due to environmental concerns associated with fossil fuels and fossil fuel based products especially their non biodegradability and carbon dioxide emissions (Babu *et al.,* 2013; Yu *et al.,* 2006).

Increasing efforts to find alternative non-fossil fuels has been the main reason for the accelerated research on bio-based polymers in recent decades. Despite this growing interest, bio-based polymers still occupied a small proportion of the universal plastics market. As of 2013, they accounted for less than 1% of the global plastics market, though the figure was expected to double by 2015. Incentives like the lead market initiative in the European Union and Bio-

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prefered in the USA have also created a demand to substitute petroleum-derived polymers with renewable resource/bio-based polymers (Babu *et al.,* 2013).

Natural polymers are also renewable resource based polymers, and are generally made up of two major categories, polysaccharides such as starch, lipids and proteins like gelatin. These natural polymers perform diverse functions in their indigenous surroundings for instance proteins act as structural components and catalysts, polysaccharides act as membranes and lipids as energy stores (Yu *et al.,* 2006).

Despite their important contribution in reducing dependence on fossil fuels and related environmental benefits due to their biodegradability, natural polymers have a largely hydrophilic disposition and a rapid degradation rate which disadvantages them by limiting their application particularly in wet environments. Furthermore, these polymers in some cases have inferior mechanical properties (Babu *et al.,* 2013; Yu *et al.,* 2006).

2.0.7 Blending natural and synthetic polymers

Developing and synthesizing new polymeric materials is usually an expensive affair that requires a lot of effort. Blending of polymers is an easier and more cost effective way to obtain a variety of chemical and physical properties from constituent polymers (Abdelrazek *et al.,* 2013). A polymer blend is a mixture of two different homopolymers, copolymers or terpolymers. They can be homogeneous (miscible) or heterogeneous (immiscible) depending on the thermodynamics of mixing. A polymer blend combines the characteristics of the individual polymers it is comprised of, resulting in a material that has qualities unique from those of the individual polymers and capable of satisfying performance demands (Kolbuk *et al.,* 2013; Sadiku-Agboola *et al.,* 2011).

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In the field of polymer blending, miscibility is of particular importance since the increase in properties is determined by the level of miscibility. The high molecular weight of polymers usually prevents polymers from mixing, and immiscible polymer blends result in heterogeneous microstructures that influence the final characteristics of the products. Poorly miscible blends can have worse characteristics than those of pure constituents (Viratyaporn *et al.,* 2007).

Differential scanning calorimetry (DSC) is used to study the thermal behavior of polymers especially to determine their glass transition temperature, (T_g). T_g trends of polymer blends can be used as a indicator for miscibility. A single T_g whose value is intermediate between the individual T_g of the pure polymers hints at molecular homogeneity in the blend, while many T_g's show phase separation of the blend's components (Ibrahim and Kadum, 2012).

Polymeric blends can be prepared in a number of ways. Viratyaporn *et al*. (2007) prepared immiscible blends of two systems, Polystyrene (PS)/High density polyethylene (HDPE), and Polystyrene (PS) /polypropylene (PP), by melt extrusion using a single screw extruder. Another study (Zhu *et al.,* 2013), used solution casting to prepare poly (vinyl alcohol/polyacrylonitrile blend films with dimethylsulfoxide as the solvent. Melt extrusion is usually employed in situations where a common solvent for the polymers to be blended cannot be found. The polymers are individually heated beyond their melting point and then they are mixed in the molten state.

In the packaging industry, a majority of the materials used to make the films have been synthetic. These synthetic materials have adverse environmental impacts and therefore focus on biodegradable materials are on the rise (Gómez-Guillén *et al.,* 2007). Also, biodegradable packaging is preferred to recyclable packaging since the recyclable packaging requires more

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energy to bring about the recycling process. Biopolymeric materials that can be used to make films can be categorized as; hydrocolloids (proteins and polysaccharides), lipids, resins and composites (Hoque, 2011).

The abundance, uniqueness in film-forming ability and conformational denaturation and amphipillic nature of proteins make them the highly favorable for the development of films (Jongjareonrak *et al.,* 2006). Biopolymers however have poor mechanical properties and due to their hydrophilicity, their application in wet environments is limited.

In an effort to improve their properties, biopolymers have been interblended or blended with synthetic polymers as reported by Warth *et al*., (1997), who developed starch/cellulose acetate blends by melt processing and reactive extrusion. The high cellulose contents improved the tensile strength and reduced the water vapor permeability of the films. Dermigoz *et al.,* (2000), devised a technique to manipulate the moisture sensitivity of starch/cellulose acetate blends by chemical modification.

Incorporation of proteins such as collagen or gelatin into synthetic polymers such as polycaprolactone (PCL) has been recently reported (Kolbuk *et al.,* 2013). PCL is a well-known route for improving cell adhesion in tissue engineering through introduction of amino-acid RGD sequence (Arginine-Glycine-Aspartic acid).

Gelatin/PVA blends have also been studied as a tool for antibiotic delivery in the eye (Jain *et al.,* 2011). Ocular inserts which proved able to deliver the antibiotic in the eye over a period of 24 hours were developed. Kim *et al.* (2008) made nano-fibrous matrices for improvement of cellular

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matrices using Polylactic acid (PLA)/gelatin blends. They reported that cell growth on the PLA/gelatin substrate improved significantly in comparison with that on pure gelatin substrate.

Gelatin is used extensively in the food and pharmaceutical industries. Its gel and film forming properties are of particular importance. Studies on gelatin films have been reported widely. Diop, (2009) fabricated and characterized gelatin films with the aim of establishing the method of preparation that would result in optimal improvement of mechanical properties. The methods used were spin coating and solution casting. Solution casting was found to be the better method.

Films have also been prepared from Horse Mackerelel (*Trachurus Japonicus*) scale gelatin (Le *et al.,* 2015). Effects of the extraction temperature and time on film properties were determined. It was demonstrated that films from gelatin extracted at 70°C for one hour had the highest tensile strength and elongation at break. Gómez-Guillén *et al.,* (2007) incorporated antioxidant extracts of two separate Murta (*Ugni molinae Turcz)* ecotype leaves into films prepared from tuna skin gelatin. They reported that the incorporation of the extracts lead to transparent films with amplified protection against UV radiation and antioxidant activity as well.

Jongjareonrak *et al.,* (2006) made and studied transparent and relatively strong films from brownskin snapper and bigeye snapper skin gelatins using glycerol as a plasticizer. The tensile strength of the films improved with protein content but reduced with glycerol content. Elongation at break increased with protein content and as well as glycerol content. Films from the brownstripe red sniper gelatin, however showed greater mechanical properties as contrasted with those from bigeye snapper gelatin.

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Avena-Bustillos *et al.,* (2006) compared films made from bovine gelatin to those made from fish gelatin (both warm and cold water fish). The water transmission of cold water fish gelatins was shown to be appreciably lower than that of warm water fish and bovine skin gelatins. This was associated with lower hydrophobicity owing to a lower imino acid content.

As evidenced by the aforementioned studies, gelatin has been used by itself in many studies. Gelatin is known to have low mechanical resistance and a high sensitivity to moisture due to its hydrophilic property (Sobral *et al.,* 2011). In an attempt to improve properties and/or instill new ones, blends and composites of gelatin with other biopolymers or synthetic polymers have been prepared for various applications.

Soliman and Furuta, (2014) reported starch-gelatin biodegradable, thermoplastic blend films with good miscibility between the two biopolymers. Good functional properties in the blends, with an improvement in tensile properties as well as water permeability depending on the starch/gelatin ratio were also indicated.

Films have been fabricated from starch-chitosan-gelatin blends (Nadiah and Aini, 2010) . The blend with a composition of 3% starch, 2% chitosan and 2% gelatin was found to be the most suited for food packaging application as it had the highest density, the least moisture and water uptake and exhibited a smooth surface with less agglomerates and no visible pore.

Phromsopha and Baimark, (2014) investigated starch-gelatin blend microparticles prepared using the water-in-oil emulsion technique for drug delivery application. Nearly spherical microparticles that exhibited promise as carriers of the water –soluble drug model, methylene blue were formed. Drug loaded microparticles showed continuous discharge of the drug model.

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Blends of gelatin with synthetic polymers have also been reported. Poly (vinyl pyrollidone)-gelatin blend films containing magnesium chloride ($MgCl_2$) have been investigated (Abdelrazek *et al.,* 2013). Results displayed an improvement in thermal stability of the blends as well as a reduction in the electrical resistivity, suggesting a possibility of the thin films being used in the preparation of electronic devices.

2.0.8 Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a nontoxic synthetic polymer with biodegradable, non-carcinogenic, water-soluble properties along with exceptional chemical stability and good film-forming capacity (Dou *et al.,* 2015). Suffice it to say, its desirable physical properties for example, elasticity and a extensive swelling in aqueous solutions, plus biocompatibility places it in a very popular position in biomaterials science. It has been used in many studies to produce biodegradable and biocompatible blends with polymers from renewable resources.

El Bahy, *et al.* (2012) prepared and characterized PVA/gelatin blend films. Despite the interaction between the two polymers in the blends being purely physical, there was a marked improvement in mechanical properties. According to the study, hydrogen bonding between the two polymers rearranges the chains in gelatin, reducing randomness inside the polymer thereby increasing the tensile strength together with elongation at break. The absence of chemical interaction was attributed to the fact that they did not heat the mixture of the two polymers prior to casting essentially rendering available energy insufficient for the formation of an ester bond between the hydroxyl group in the alcohol and the carboxylic groups in gelatin.

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In another study, Pawde *et al.,* (2008) investigated PVA/gelatin blend films using FTIR, DSC, XRD and dielectric measurements. FTIR analysis showed strong chemical interactions between PVA and gelatin amid creation of new peaks due to existence of gelatin in the blend films. DSC results indicated a change in thermal behavior, meaning the polymers were miscible with each other. This is also evidence of a change in the crystallite parameters and the degree of crystalinity as well as a change of electrical properties of the PVA/gelatin blends.

Said (2013), fabricated films based on PVA/gelatin blends and then exposed them to electron beam irradiation. The IR spectra showed that cross-linking occurred after the irradiation principally in the gelatin. Further, noticeable enhancement of the thermal properties of the films was realized probably due to the crystalinity of PVA.

Other studies on PVA/gelatin blends (Silva *et al.,* 2008; Sobral *et al.,* 2011; Wang *et al.,* 2010), have also been reported. This present study seeks to prepare films based on Nile perch scale gelatin and polyvinyl alcohol polymer blends.

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CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Raw Materials:

Fresh Nile Perch (*Lates niloticus*) skins, which had not been de-scaled, were purchased from the city market in Nairobi, packed in plastic bags and transported to the laboratory. Only large skins with a weight greater than one kilogram were chosen. On arrival in the laboratory, the skins were immediately stored in refrigerator at -20°C until further processing. Several skins were then chosen, thawed under ambient conditions for two hours, washed and rinsed thoroughly with warm water to degrease them.

When the skins were relatively dry, they were spread on the laboratory bench and scales removed using a knife. The scales were thoroughly washed in running tap water to rid them of any residual meat or lipids. The scales were then left to dry under ambient conditions. When completely dry, they were weighed and the dry weight recorded before being placed in airtight plastic bag awaiting further use.

3.2 Enzyme Preparation

The crude alkaline protease used in this study was prepared as outlined by Wanyonyi *et al.,* (2014). A culture medium containing 0.5 % casein and 0.25% glucose was prepared in 200 ml portions, in 500 mL conical flasks to allow for aeration. The flasks were then plugged with cotton wool and covered with aluminum foil before sterilization in an autoclave at 121ºC (15 lb) for 15 min. The flasks were removed and allowed to cool to room temperature before being

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moved into a laminar flow where they were inoculated with 5% over-night grown seed bacterial (*Bacillus cereus strain wwcp1*) culture and then transferred to a rotary shaker (140 rpm) incubator 45°C for 72 hours . The culture medium was then centrifuged at 5000 rpm for 15 min. The pellets were discarded and the supernatant, containing the crude enzyme stored in covered flasks at 4°C awaiting further use.

3.3 Gelatin extraction

One kilogram of completely dry scales was weighed into a conical flask. The solution carrying the crude enzyme prepared in procedure 3.2 above was transferred into a beaker. Using Sodium hydroxide and a pH meter, the pH was adjusted to a value of 11 which was the optimum value as established by Wanyonyi *et al.,* (2014). The solution was then poured over the scales until they were completely covered. The set up was transferred to an oven at 50°C. Every twelve hours, the pH of the solution was checked and restored to the optimum value. In order to ensure homogeneity, the system was stirred regularly.

Once the scales were completely hydrolyzed, the set up was removed from the oven and allowed to cool. The liquid part (gelatin solution) was decanted off and lyophilized to obtain gelatin powder. To determine the yield, the total weight of the dry gelatin powder was expressed as a percentage of total weight of the dry scales hydrolyzed.

3.4 Infrared spectroscopy

Infra-red spectroscopic analysis of the sample was performed as outlined by Muyonga *et al.,* (2004) with minor alterations. A 200 mg KBr disc containing 2-6 mg of the sample disc was

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prepared and used to obtain the spectrum. The analysis was performed on a Thermo Electron Corporation Nicolet 380 FTIR spectrometer.

3.5 Determination of Amino Acid Composition:

Amino acid analysis of Nile perch scale gelatin was performed using a narrow bore, (2.1 x 200 mm), (Hypersil AA-ODS), 5 µm reverse phase column purchased from Thermo Electron (part # 30105-202130). Samples were weighed and placed in a 13 x 100 mm Pyrex tube along with 1ml N HCl and 11 µmoles of Internal Standards (Norvaline and Sarcosine). After adding Internal Standards, the samples along with controls and blanks were exposed to liquid-phase 6N HCl for 22 hours at 100°C. Amino acids were separated on an Agilent 1260 with column heater, automatic injection programming UV and Fluorescence detection.

Five µl of the hydrolysate was dried down and re-suspended in 250 µl of 0.4 M Borate buffer. One µl was injected. The G1367E autosampler was used to perform pre-column derivatization and multiple sample handling. The derivatized amino acids were then eluted from the reverse phase column.

Primary amino acids (tagged with OPA, Agilent #5061-3335) were detected at 338/390 nm by the Variable Wavelength (UV) detector (G1365D) and secondary amino acids (tagged with FMOC, Agilent 5061-3337) at 266/324 nm. The flourometric detector (G1321B) was used to monitor the primaries at excitation/emission 340/450 and the secondaries at 266/305. The assay was calibrated by a standard (Agilent 5061-3331) which was subjected to the same treatment as the samples and control, including hydrolysis. The assay was controlled by a known protein, Human Serum Albumin. An aliquot from the same batch of HSA was run with every assay.

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3.6 Preparation of Polymer blends and films

The blends were prepared in 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80 and 10/90 PVA/gelatin proportions. The appropriate weights of PVA purchased from Merck (MW 60,000 and a degree of hydrolysis of 98%) were weighed out into separate 100 ml conical flasks. 30 ml distilled water at 95°C were added into each flask and swirled in a water bath at 90°C until complete dissolution occurred. The resulting solutions were labeled A.

Corresponding weights of gelatin were weighed into flasks and 10 ml distilled water added into each flask. The set up was transferred to a water bath at 50°C and vigorously stirred for 15 minutes to ensure complete dissolution. The resulting gelatin solutions were then labeled solution B. Solutions B were poured into the appropriate solutions A to obtain film-forming solutions (FFS) with the predetermined PVA/gelatin ratios. The film-forming solutions were homogenized with magnetic stirring at room temperature for 30 minutes to ensure complete mixing.

Identical volumes of the FFS, to guarantee same thickness, were then cast separately onto glass petri dishes which were subsequently placed in a chamber with air renewal circulation and left to dry at room temperature. When completely dry, thin films were peeled off the petri dishes and sent for analysis.

3.7 Differential scanning calorimetry

Differential scanning calorimetry data was recorded for pure gelatin, PVA/gelatin blends and pure PVA. The tests were done on a DSC Q100 V9.9 Build 303 (Universal V4.5A TA Instruments), with 1 to 9 mg samples sealed in aluminum pans. Thermal data (I and II heating scans) were recorded over the range of temperature from -50 to 250°C.

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3.8 Thermogravimetric analysis

Thermogravimetric analysis of pure gelatin, pure PVA and the PVA/gelatin blends was done on a TGA Q500 V20.13 Build 39 (Universal V4.5A TA Instruments) machine in a temperature range from 0 to 200°C with sample sizes ranging from 9 to 27 mg. The onset decomposition temperature and weight loss profiles were obtained.

3.9 Dye adsorption experiments

Methylene blue dye (MB, $C_{16}H_{18}N_3ClS$) with a molecular weight of 319.85 and λ_{max} at 664 nm procured from RANBAXY Fine Chemicals in Nairobi, Kenya was used without any refinement. Batch experiments were performed in 100 ml conical flasks, at 25°C. Films prepared from 60/40 PVA/gelatin (w/w) blend were chosen to be investigated for adsorption properties.

3.9.1 Calibration Curve

Five dilutions of 2.5×10^{-5}, 2.0×10^{-5}, 1.25×10^{-5}, 1.0×10^{-5} and 0.75×10^{-5} M were prepared from 4.0×10^{-5} M stock methylene blue solution and 30ml portions measured out into separate 100 ml conical flasks. Samples were the picked from each flask and measurements for absorbance on a UV-Vis spectrophotometer (Shimadzu UV mini 1240) at 664 nm determined. A graph of absorbance against concentration was plotted.

3.9.2 Effect of adsorbent concentration

The variation of equilibrium uptake of methylene blue with adsorbent concentration was deter in mined in 100 ml conical flasks on orbital shaker at room temperature. Varying weights (0.25, 0.50, 0.75 and 1 g) of the film were measured and placed in four separate flasks. Thirty ml of 0.000025 M methylene blue was then added to each flask. Samples were then collected from

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each flask at regular intervals and residual MB concentration determined using a UV-Vis spectrophotometer (Shimadzu UV mini 1240) at a wavelength of 664 nm.

3.9.3 Effect of initial dye concentration

The influence of the initial methylene blue concentration was investigated in 100 ml conical flasks on an orbital shaker at room temperature. 0.25g of the film was used. The concentrations of methylene blue used were 1.25 X 10^{-5}M, 2.0 X 10^{-5}M and 2.5 X 10^{-5}M. Samples were taken from each flask at consistent time intervals and absorbance recorded on a UV-Vis spectrophotometer at 664 nm (Shimadzu UV mini 1240).

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CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Gelatin yield

A yield of 16.3% of dry gelatin was obtained after freeze drying of the gelatin solution. This is comparable to gelatin yield of 18.49% reported by DinçEr *et al.,* (2015) from the scales of farmed sea brass (*Dicentrarchus labrax*) using acetic acid. Jiang, (2013) reported gelatin yield of 48.1% from the scales of cultured carp using an alkaline protease. Before the enzyme hydrolysis, the scales in the study by Jiang, (2013) were ground into a fine paste, increasing the surface area available for enzyme action hence the higher yield.

(a) (b)

Figure 3: (a) Nile perch scales (b) scales during hydrolysis

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The lower yield from Nile perch scales could possibly be due to the longer extraction process (approximately 13 days), which could have resulted in a greater loss of collagenous material. Since gelatin is a direct derivative of collagen, the collagen content of raw materials has determines the yields. The collagen content varies with species and this could also explain the variable yields amongst the three fish species.

The extractability of gelatin from different tissues could vary due to differences in the type and quantity of cross links in collagens from various tissues (Muyonga *et al.,* 2004). It is therefore expected that the yields of gelatin extracted from different tissues will vary. The gelatin yield of 16.3% obtained in present study, is higher than reported for isolation of gelatin from the heads of Mackerel (*Scomber scombrus*) heads using different organic acids with average yield of 3.5 % (Khiari *et al.,* 2011).

The yield in this study also differed from that reported for skin extraction from farmed Amur sturgeon at 19.6%, catfish (*Clarias batrachus*) 27.79%, snakehead (*Channa striatus*) 16.57%, red tilapia (*Oreochromis niloticus*) 11.75%, pangasius catfish (*Pangasius sutchi*) 10.78%, bigeye snapper (*Priacanthus macracanthus*) 6.5% and brownstripe red snapper (*Lutjanus vitta*) at 9.4% (Jongjareonrak *et al.,* 2006; Nikoo *et al.,* 2011; See *et al.,* 2010).

4.2 IR analysis

The IR spectra of the gelatin obtained from Nile Perch scales is presented in Figure 4 below. As shown, about nine characteristic IR absorption bands can be observed from the IR spectrum. These are the amide A, B, and I through VII bands. The amide I band is very sensitive and is the most frequently used to study secondary protein makeup. As shown in Figure 4, the amide I peak

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occurs at 1653 cm^{-1}. It corresponds to a combination of a C=O stretching vibration of the amide group and N-H bending and usually occurs at 1600 cm^{-1} to1700 cm^{-1} (Ahmad and Benjakul, 2011; Nikoo *et al.,* 2011). Amide II is largely due to in plane NH bending and CN stretching vibration and reveals less protein conformation than amide I. Other bands are rarely used in protein conformational studies (Nikoo *et al.,* 2011)

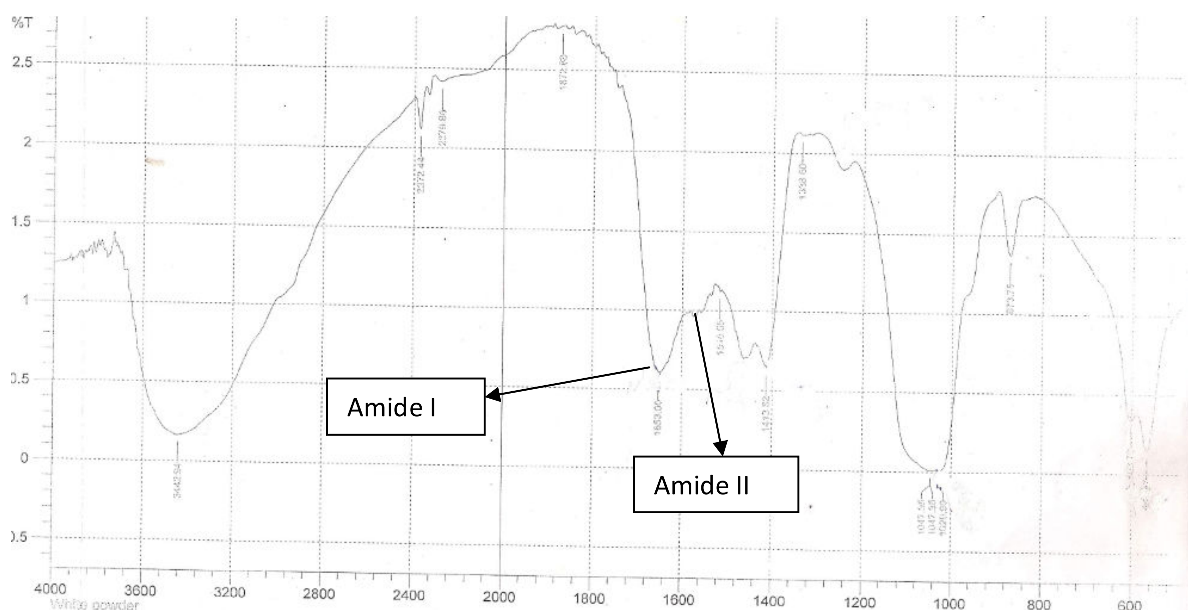

Figure 4: IR spectra of gelatin obtained from Nile Perch scales.

The amide I peak absorption of 1653 cm^{-1} is within the expected range of 1650 to 1660 cm^{-1}. This is slightly higher than values of 1630 to 1640 cm^{-1}, observed in other studies (Muyonga *et al.,* 2004a; Nikoo *et al.,* 2011). The higher frequency of the amide I band can be attributed to a higher degree of disruption of intermolecular bonding when gelatin extraction is done at elevated temperatures and for longer periods (Kittiphattanabawon *et al.,* 2012; Nikoo *et al.,* 2011) as was the case in this study. The amide A in this study is the wide peak occurring at 3442 cm^{-1}. This

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peak corresponds to NH stretching coupled with hydrogen bonding. Hydrogen bonding between OH groups of the amino acids in gelatin is responsible for the width (Muyonga *et al*, 2004b).

Referring to Figure 4, it is evident that the Amide II and Amide III bands occurred at 1590 cm^{-1} and 1230 cm^{-1} respectively, consistent with observations of Ahmad and Benjakul (2011). The low intensity of Amide III is attributable to low molecular interaction in gelatins extracted under high temperature conditions (Muyonga *et al.,* 2004).

4.3 Amino acid analysis

Table 5 displays the amino acid composition of gelatin obtained in this study from the Nile perch in this study. The sample was analyzed in duplicate and the results of each repetition are displayed in Appendix 1.

Table 5: Amino acid composition of Nile perch scale gelatin.

Amino acid	Composition (weight %)	SD
Asparigine/Aspartic acid	6.6	0
Glutamine/Glutamic acid	10.8	0.0707
Serine	2.4	0.0707
Histidine	1.4	0.1414
Glycine	21.7	0.7778
Threonine	2.1	0.0707
Alanine	11.8	0.2828
Arginine	8.9	0
Tyrosine	0.9	0.2121
Valine	3	0
Methionine	3.4	0.1414
Phenylalanine	3.1	0
Isoleucine	1.8	0
Leucine	3.3	0.0707
Lysine	4.3	0.1414
Proline	14.6	0.7778
Total %	100	

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The Glycine content was found to be the highest at 21.7% by weight followed by proline at 14.6%. Gelatin is composed of repeating Gly-X-Y motifs. Gly, X, and Y correspond to glycine, proline and hydroxyproline respectively. It therefore follows that these amino acids are the most abundant in any gelatin, irrespective of the source. Glycine and proline were also reported as the most abundant amino acids, at 33.7% and 13.42% respectively, in gelatin sourced from chicken skins (Sarbon *et al.,* 2013). Tavakolipour (2011), studied gelatin from silver carp fish waste (skins and fins) also reported glycine and proline as the most abundant amino acids. In fact, glycine made up a third of all amino acid residues at 31.7% while proline was reported at 12.4%. The proline content of Nile Perch scale gelatin in this study is lower than that of bovine gelatin and porcine gelatin. It is however, higher than that observed for Pollock (10.09%) and Salmon (10.79%) skin gelatins (Avena-Bustillos *et al.,* 2006). Pollock and Salmon are both cold water fish. Gelatins from animals that inhabit high temperature habitats have higher imino acid content than those from low temperature habitats. Consequently, fish gelatins generally have lower imino acid concentration than mammalian gelatin. Further warm water fish have a higher concentration of imino acids compared to cold water fish. Proline together with hydroxyproline makes up the imino acid complex, which is of particular importance in determining the gel properties of a particular gelatin. It is thought that proline and hydroxyproline stabilize the gel network by hydrogen bonding. The more the imino acid content, the better the properties (Gomez-Guillen *et al.,* 2002; Nikoo *et al.,* 2011).

Alanine was another major amino acid being the third highest in terms of composition at 11.8%. It ma occupies the non-polar regions in which gly-pro-y sequences are predominant, with the third positions inhabited by either hydroxyproline or alanine. Methionine and Threonine were substantially low. The lowest amino acids by composition were tyrosine (0.9%), histidine (1.4%)

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and isoleucine (1.8%) while tryptophan and cystein were completely absent. All these are inherent properties of gelatin (Mahmoodani *et al.,* 2014). The amino acid analysis results are further confirmed in the chromatograms in Figure 5_a and 5_b. The height of the peak corresponds to the amount of amino acid.

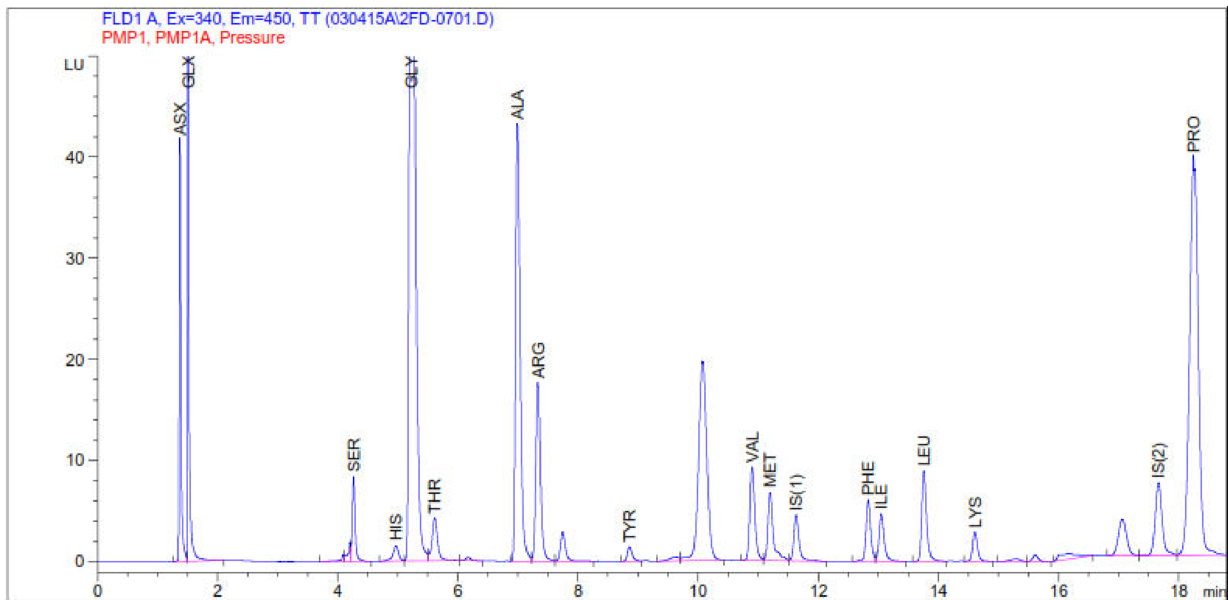

Figure 5a: HPLC chromatogram of the amino acid analysis of Nile perch scale gelatin, first repetition

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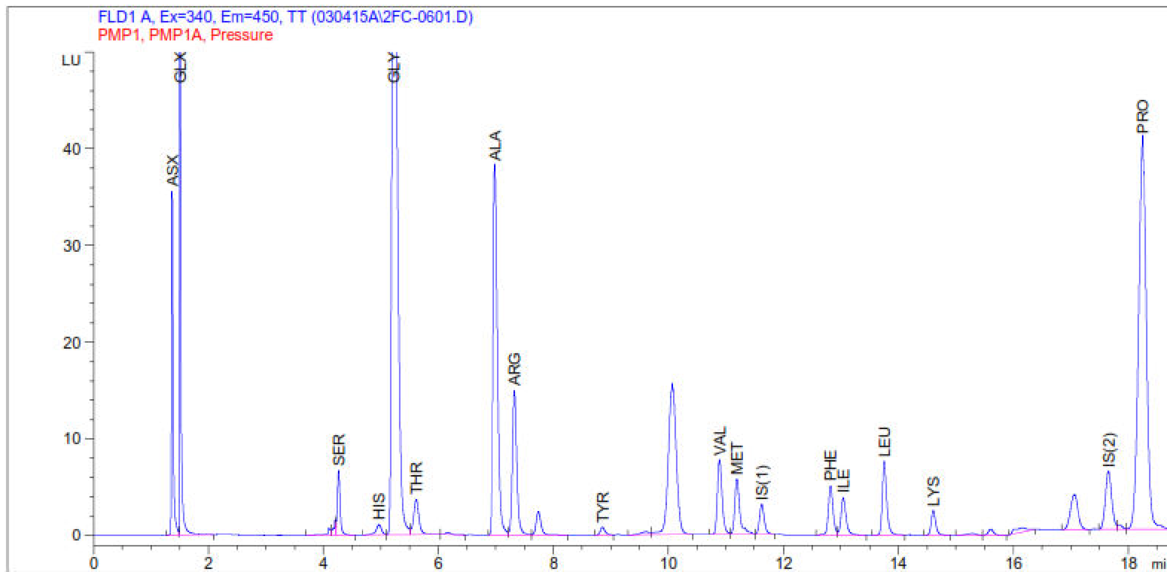

Figure 5b: HPLC chromatogram of the amino acid analysis of Nile perch scale gelatin second repetition.

These findings are similar with those of other studies on the amino acid composition of gelatin (Karim and Bhat, 2009) that reported the absence of the tryptophan and cysteine in fish skin gelatins. Jongjareonrak *et al.,* (2006) found that gelatin isolated from the bigeye snapper skins had glycine and proline as the most abundant amino acids at 19.3% and 13.4% respectively.

The amino acid profile of Nile perch scale gelatin is similar to that reported by Wangtueai and Noomhorm, (2009) for lizardfish scale that yielded gelatin content of 18.3% glycine, 16.5% proline and 12.4% alanine. Threonine and histidine were the least abundant amino acids at 0.9% and 1.52% respectively. This similarity can possibly be explained by similar habitats (warm water) for both the Nile perch and lizard fish.

Analysis of Gelatin from sea bream bones and scales also revealed that gelatin from the two tissues had a similar amino acid profiles. Akagündüz *et al.,* (2014) suggested that this was due to conservation of the chemical composition of type I collagen across tissues. This finding could be

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used to explain the similarity in the amino acid profiles of Nile Perch bone gelatin (Muyonga *et al.,* 2004a) and that of Nile Perch scales reported in this study. The slight variations could be attributable to the different extraction methods (Amiza *et al.,* 2015). Suffice to say, Muyonga *et al.,* (2004a) employed an acid pretreatment before extraction while in the present study, an enzymatic extraction was used.

4.4 Blend films preparation

Figure 6 shows PVA/gelatin blend solutions in the process of drying, done for four days. Once completely dry, PVA/gelatin films of various compositions were peeled off the glass petri dishes on which they were drying. It was possible to peel

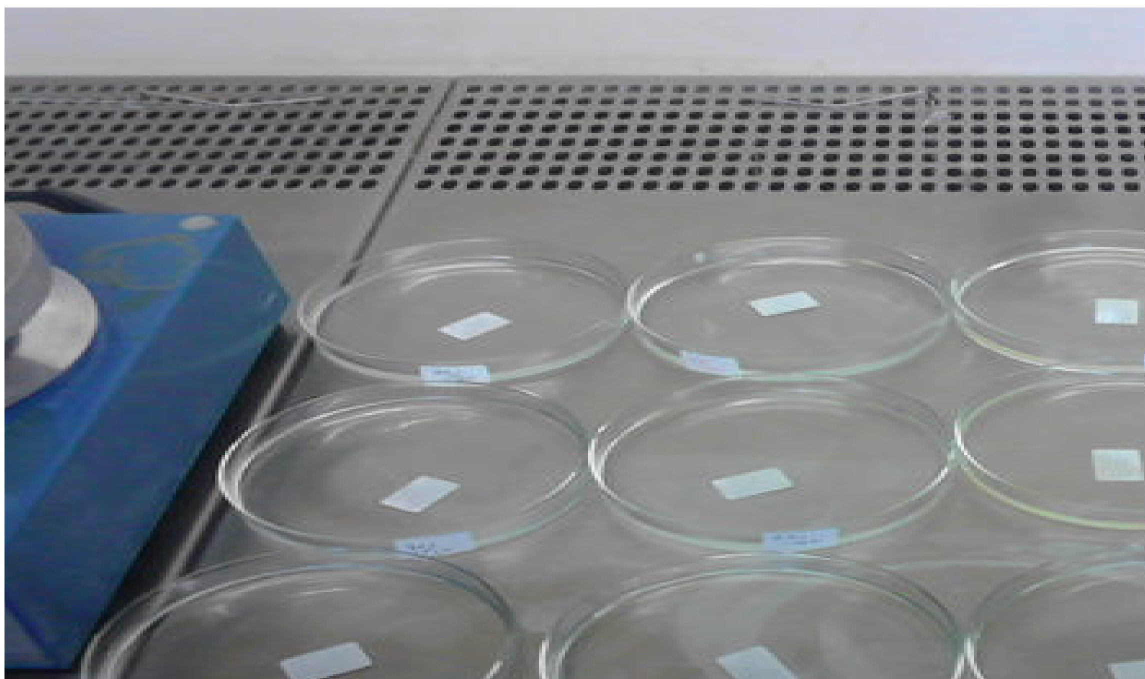

Figure 6: PVA/gelatin solutions of various compositions drying in petridishes.

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off films for all PVA/gelatin ratios except for the 10/90 composition. Figure 7 below shows the 60/40 PVA/gelatin films being peeled off. Generally, films made from proteins are delicate and therefore plasticizers are often added, so as to lower the protein-protein chain interactions stabilizing the films network, increasing the mobility of protein molecule (Peesan *et al.,* 2005; Vanin *et al.,* 2005).

Figure 7: PVA/gelatin films of 60/40 PVA/gelatin composition.

In this study, no plasticizer was used and after complete drying of the films, it was observed that blend films with the most gelatin content were brittle and difficult to peel off. Gelatin has a dominant hydrophilic character and is highly water soluble. Therefore, it has poor mechanical properties such as tensile strength in damp environments (Yu *et al.,* 2006). The low tensile strength of gelatin explains the ineffectiveness of moisturizing, to allow peeling off of the 10/90 blends, unlike other blends containing lower gelatin content.

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4.5 Thermal analysis of the polymer blends

4.5.1 DSC Analysis

4.5.1.1 DSC analysis of pure gelatin and pure PVA

Figure 8 below shows the DSC thermogram obtained from the analysis of pure gelatin from Nile Perch scales.

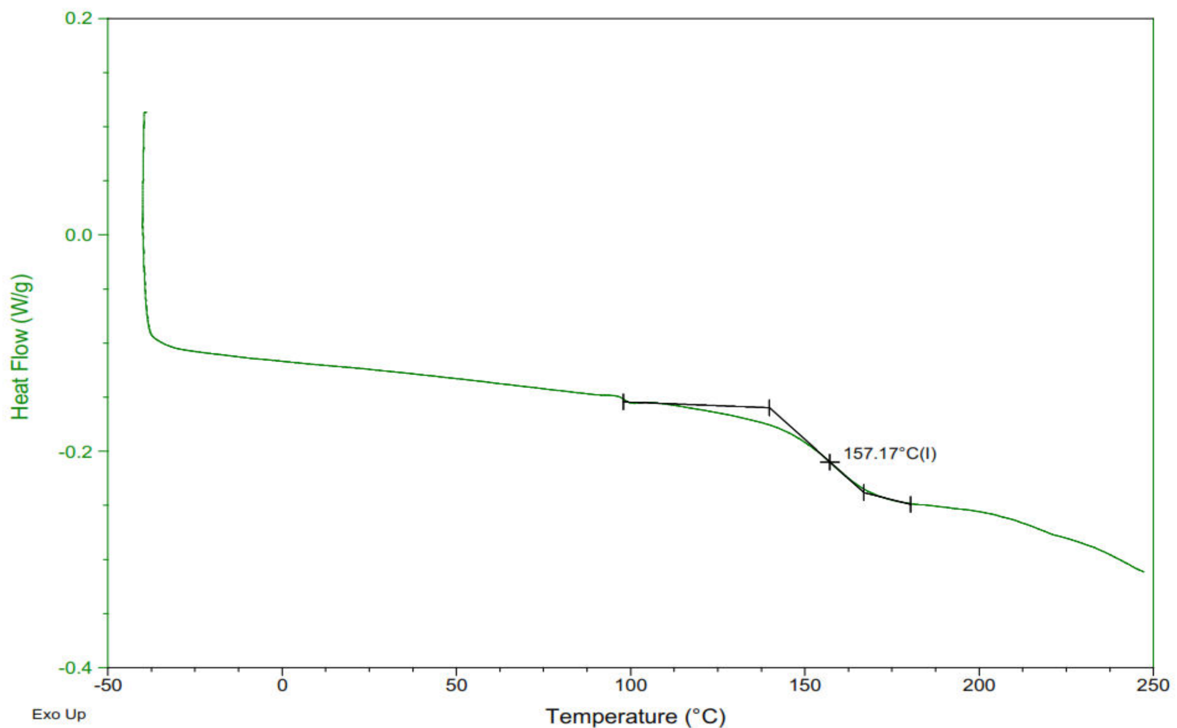

Figure 8: DSC thermogram of gelatin obtained from Nile Perch scales.

The thermogram shows no endothermic peak except for an inflection which corresponds to the glass transition temperature (T_g) at approximately 157°C. The high glass transition temperature suggests a low degree of crystalinity. As a polymer gets more amorphous, its chains become more entangled and the amount of energy required to disentangle them during glass transition

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increases, raising the glass transition temperature (Elsergany, 2014). The T_g of gelatin in this study has a higher value than that observed by Gao *et al.,* (2014) and Silva *et al.,* (2008) who reported values of 121.9°C and 57.5°C respectively. Pawde *et al.,* (2008) reported a much higher value of 220°C to 230°C. T_g is a kinetic event, thus the same substance may have different T_g's as reported in the literature depending on the manufacturing conditions (Elsergany, 2014). As shown in Figure 9, T_g and T_m values of 84.9°C and 190.8°C were obtained for pure PVA

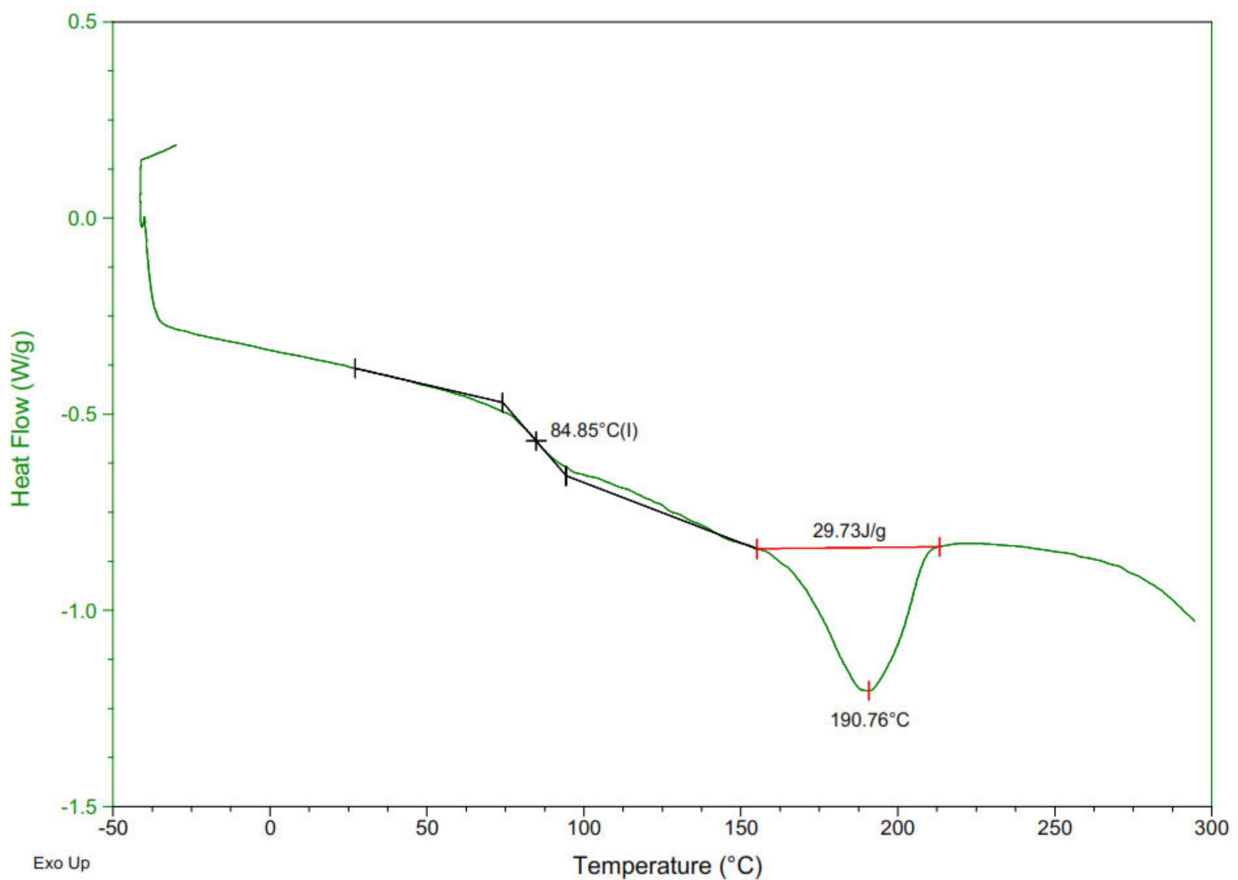

Figure 9: DSC thermogram of pure PVA.

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The endothermic peak, with a heat of fusion of 29.7 J/kg indicates semicrystalline properties. Semicrystalline systems typically display a peak after T_g transition (Silva *et al.,* 2008) indicative of the melting of the crystalline domains of the polymer and is absent in case of amorphus polymers.

4.5.1.2 DSC analysis of PVA/gelatin blends

Table 6 summarizes the DSC data obtained for the various compositions of the prepared blends.

Table 6: DSC data (T_g and T_m) for PVA/Gelatin polymer blends.

PVA/Gelatin	**T_g (°C)**	**T_m (°C)**
20/80	97.14	-
30/70	96.78	-
40/60	97.47	187.6
50/50	96.81	188.77
60/40	96.95	197.88
70/30	96.79	207.54
80/20	96.75	207.0
90/10	87.36	173.55
Gelatin 100%	157.2	-
PVA 100%	84.85	190.76

Glass transition temperature is an important tool in polymer blend technology used to determine the miscibility of two polymers in the amorphous phase. The presence of a solitary, composition dependent glass transition temperature intermediate between those of individual constituents (Peesan *et al.,* 2005) usually indicate miscibility of polymer blends. Table 6 above shows the DSC data obtained for all the blend films studied.

For all the films investigated, the T_g falls in between those observed for gelatin and PVA and therefore miscibility between PVA and gelatin can be deduced. A single T_g intermediate between the T_g's of the homopolymers implies a greater degree of miscibility in the amorphous phase

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(Onyari and Huang, 2009). It can therefore be deduced (Table 6) that miscibility is highest in the 90/10 PVA/gelatin blend.

The outcome of this study demonstrates that the T_g of the PVA/Gelatin blends studied, lie between 96°C to 98°C for all compositions except 90/10 PVA/gelatin blend film. This constancy of T_g values could be due to restricted mobility of PVA molecules in the amorphous phase due to the presence of gelatin molecules as observed by Peesan *et al.,* (2005).

The T_g inflections in the DSC curves for all films in this study were followed by large endothermic peaks as seen in Figure 10, the DSC thermogram of the 40/60 PVA/gelatin blend.

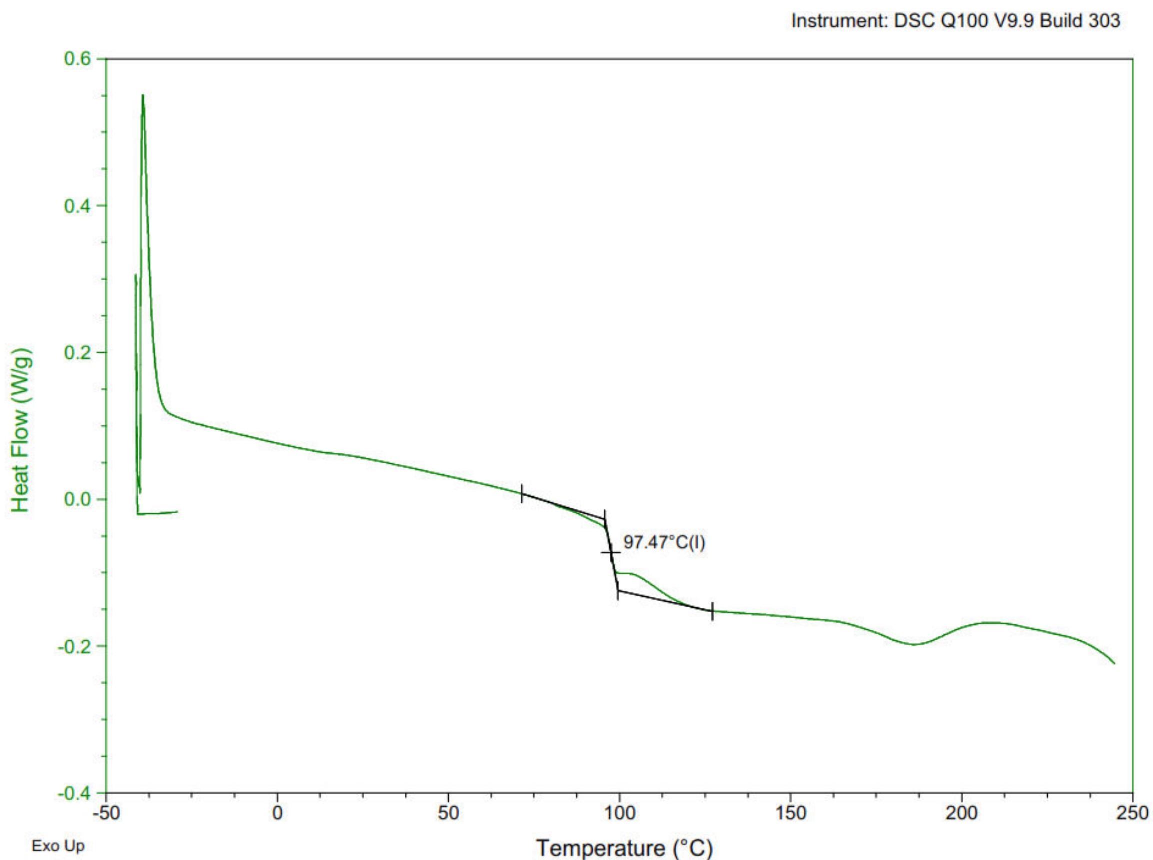

Figure 10: DSC thermogram of PVA/gelatin blend film in 40/60 composition.

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Melting is a first order transition unlike glass transition which is second order. A first order transition is one which occurs with changes in both heat capacity and enthalpy while a second order is occurs with a change in the heat capacity only (Chiellini *et al.,* 2001; Zhang and Li, 2009). This explains the presence and association of the peaks to melting process (melting peak). The apex of the melting peak gives the melting temperature point (T_m) of a polymer, which is the temperature at which the polymer's crystallites lose the highly ordered arrangement in their structure.

Defined melting points are characteristic of crystalline polymers and glass transition is a feature of amorphous polymers. The DSC curves of the PVA/gelatin films in this study showed both T_g and T_m values suggesting semi-crystalline properties, concurring with the findings of other studies (Mendieta-Taboada *et al.,* 2008; Silva *et al.,* 2008). The T_m of the films occur between 174°C to 207°C for different compositions. In the films with 40, 50 and 90% PVA composition there is a T_m depression (187.6, 188.77 and 173.56°C respectively) from the observed T_m value for pure PVA (190.76°C). This, as observed by Pawde et al., (2008) is indicative of strong intermolecular interactions between PVA and gelatin molecules.

The area under the endothermic peak corresponds to the value of the enthalpy of fusion of the crystalline portion and is qualitatively related to the amount of crystallites/degree of crystallinity in a film sample. The larger the area, the bigger the amount of crystallites and the more crystalline a substance is.

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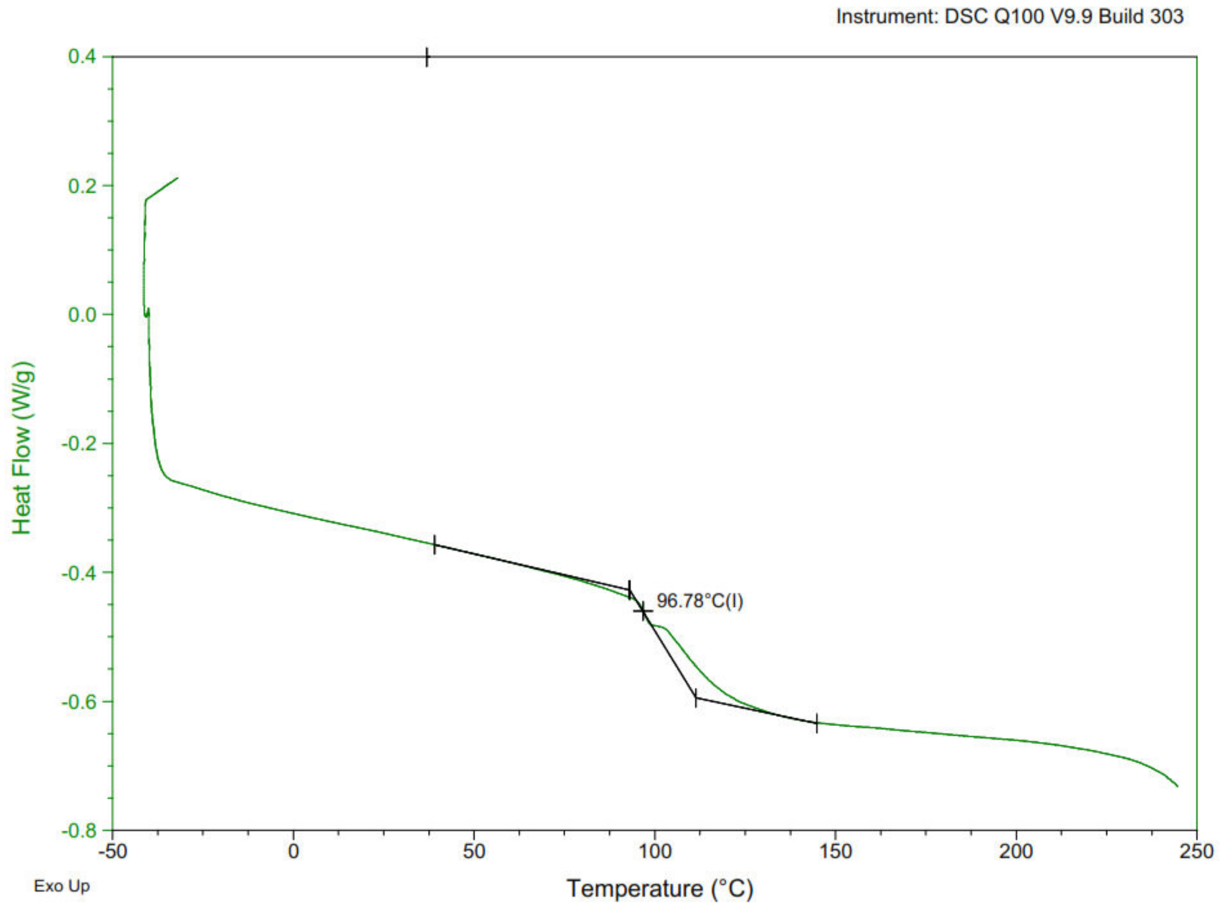

Figure 11: DSC thermogram of the 30/70 PVA/gelatin blend.

Figures 11 to 13 display the DSC curves of the films of various compositions. It is observed that as the gelatin content increases and the PVA reduces, the size of the area under the endothermic peak reduces indicating a decline in the level of crystallinity associated with PVA. From figure 11, barely any endothermic peak is observed. This is the thermogram of the film with a composition of 30% PVA and 70% gelatin. The more the gelatin content in a sample, the closer the properties of that sample to those of gelatin.

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Gelatin is amorphous while PVA is more crystalline. An increase in gelatin or a decrease in the PVA content in the sample films evidently results in a reduction of the amount of crystallites in the blend samples as s also observed by Pawde *et al*, (2008).

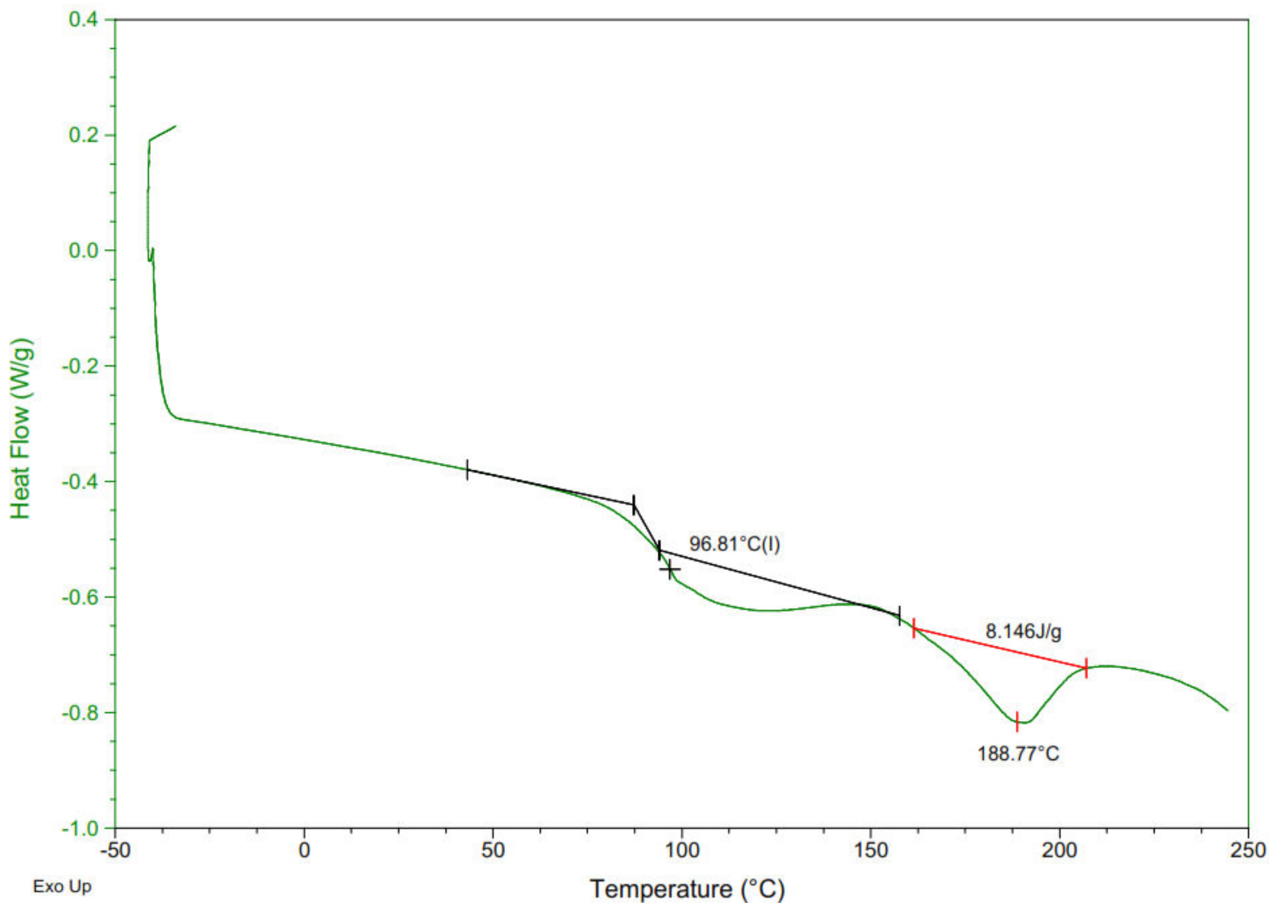

Figure 12: DSC thermogram of 50/50 PVA/gelatin blend film.

Figure 12 above shows the DSC thermogram of the film made of PVA/ gelatin (50/50%) blend. As shown, increasing the amount of PVA also increased the area under the endothermic peak. An increase in the quantity of PVA results in an increase in the amount of crystallites and therefore the degree of crystalinity. This is further illustrated in the thermogram of film made from the blend with 70% PVA composition in Figure 13.

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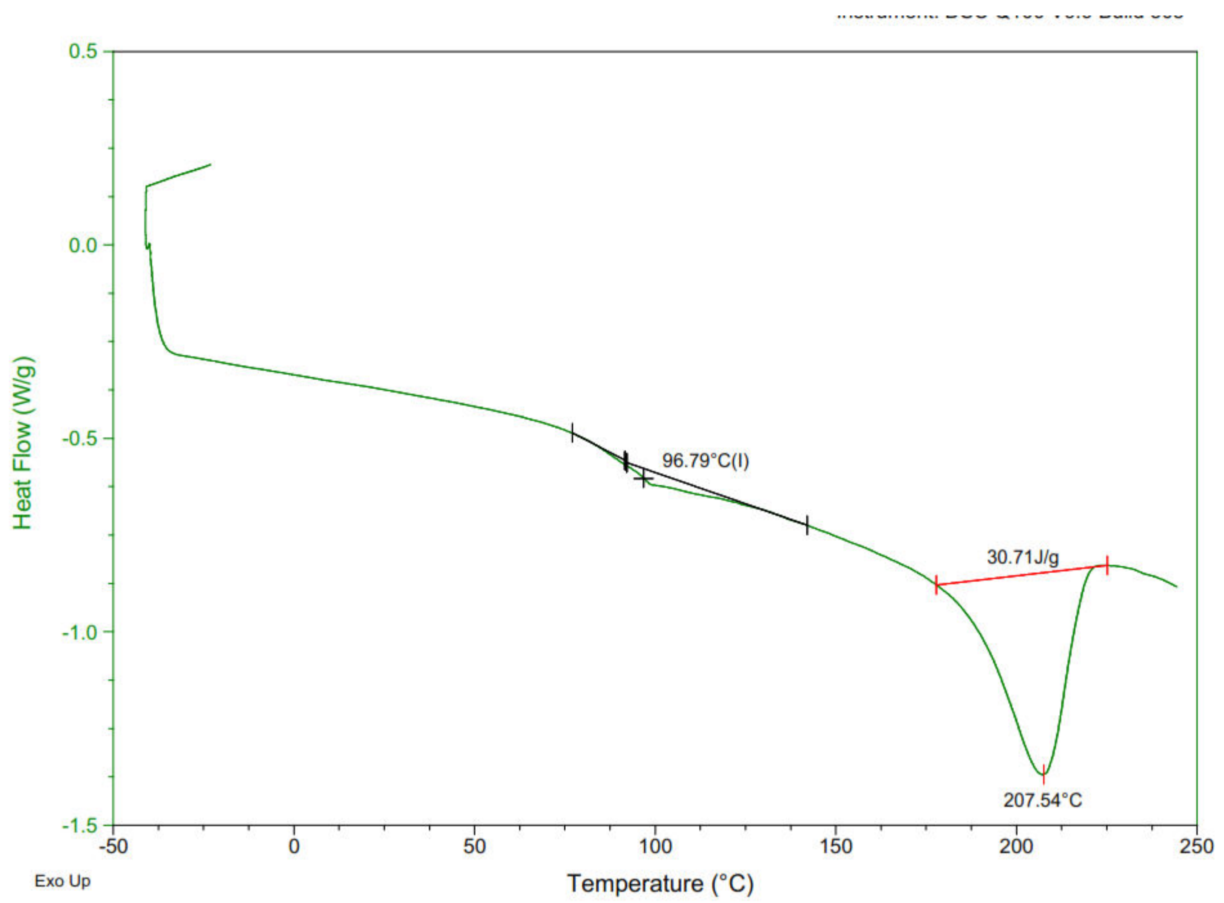

Figure 13: DSC thermogram of 70/30 PVA/gelatin blend film.

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4.5.2 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) is used to evaluate the thermal stability and decomposition behaviour of polymer samples. A sample is heated to high temperatures in a controlled environment and its weight loss profile is plotted against the temperature in the TGA curves. The TGA thermograms of pure PVA and pure gelatin are displayed in figures 14 and 15 respectively.

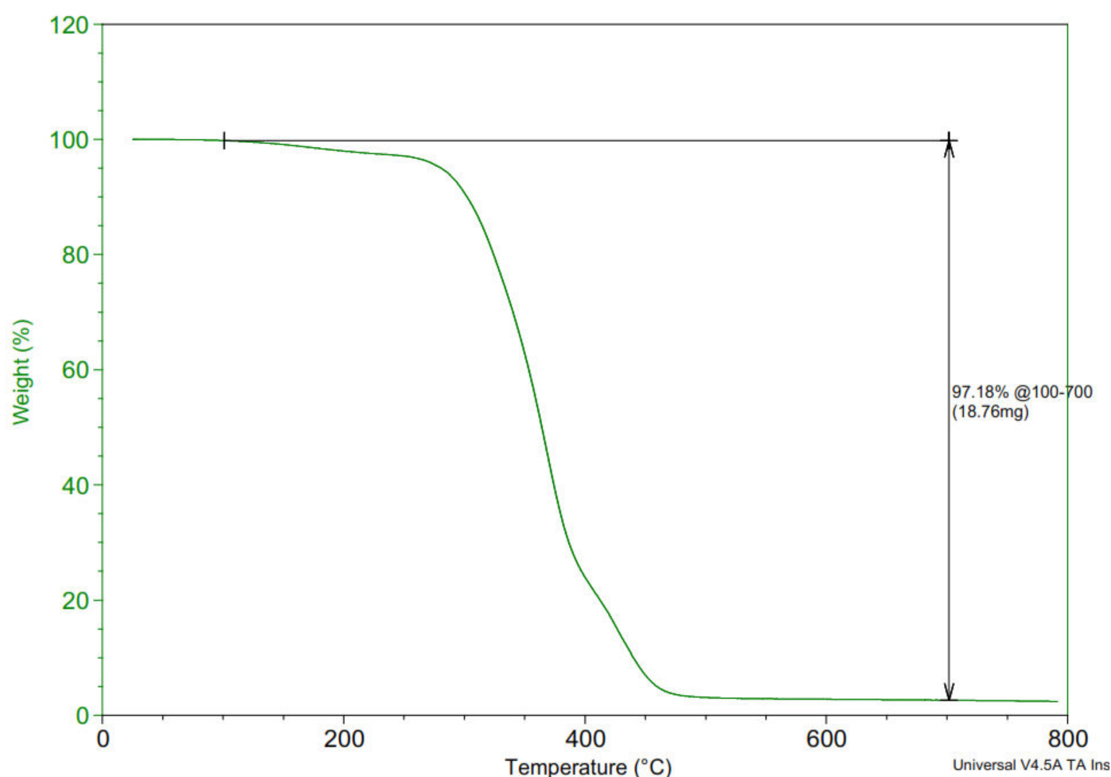

Figure 14 : TGA thermogram of pure PVA.

Both curves show two zones of weight loss. The first regions are due to moisture loss and/or the evaporation of volatile components. The second region is due to pyrolytic reactions/thermal decomposition. The onset temperature for the decomposition of PVA is approximately 260°C. After the onset decomposition temperature, the slope of the curve is very steep indicating a fast decomposition rate. Between 250°C and 400°C, PVA looses most of its weight, remaining with

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just 20% at around 400°C. The rate slows down and completely levels out about 475°C. As shown in Figure 14, PVA looses 97.2% of its weight and a char yield of 2.82% between the temperature range 100°C - 700°C.

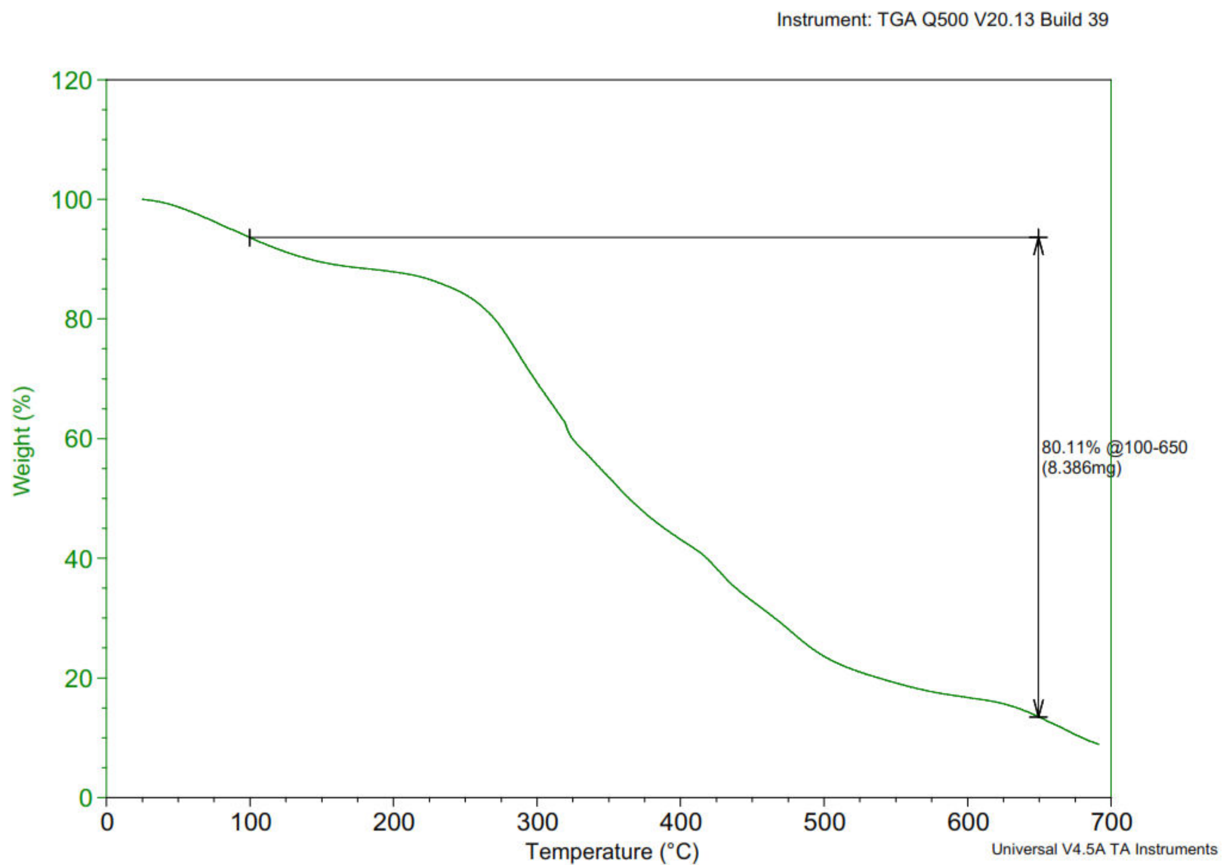

Figure 15: TGA themogram of pure gelatin obtained from Nile Perch fish scales.

The degradation onset temperature (figure 15) for gelatin in this study was approximately 210°C, approximately 50°C lower than that of PVA corroborating with the findings of Chiellini *et al.,* (2001) who reported a difference of approximately 40°C. A more detailed analysis of the TGA curve of gelatin reveals a degradation rate slower than that of PVA. At 400°C, gelatin had lost about 65% of its weight unlike PVA which had lost 80%. The slope of the curve is generally less

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steep as compared to that of PVA and ultimately, gelatin has a char yield of 19.9%. It can therefore be concluded that PVA thermally decomposes faster and more fully than gelatin.

Figure 16 shows the DSC thermograms for the film of 30/70 PVA/gelatin blend composition.

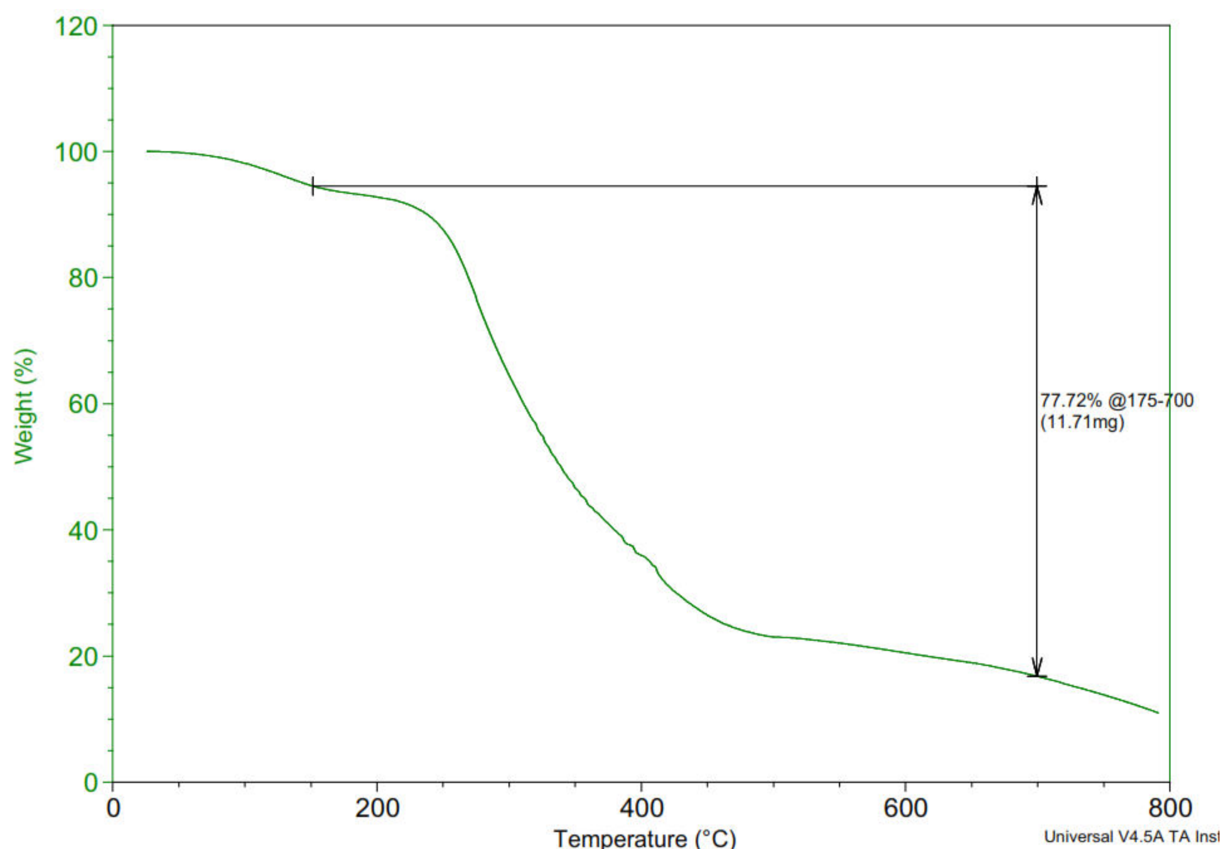

Figure 16: TGA thermogram of 30/70 PVA/gelatin blend film.

The film also shows two regions of weight loss. The first region can be attributed to evaporation of moisture and the second region pyrolytic reactions. The onset temperature for the film is 230°C, an intermediate between the onset temperatures of the individual pure components. The curve is not as steep as that of PVA, but it is steeper than that of gelatin suggesting an intermediate degradation rate. At 400°C, the film had lost 60% of its weight. These observations

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all display an alteration of individual polymer properties by the introduction of the other polymer component during blending. The properties of the 30/70 PVA/gelatin blend film are more similar to those of gelatin than those of PVA probably due to the higher gelatin content.

As the percentage of PVA in a film is increased, the thermal properties of the films become more like those of PVA. The rate of degradation increases and the residue left at 700°C decreases. This can be observed clearly in the TGA thermograms of the 50/50 and 70/30 PVA/Gelatin blends in Figures 17 and 18 respectively. This indicates that the thermal stability of the films significantly varies from that of the individual polymers and depends on the ratio of each component in the blend. Chiellini *et al*. (2001) also reported the similar findings, noting that thermal stability reduced with an increase in the less stable component.

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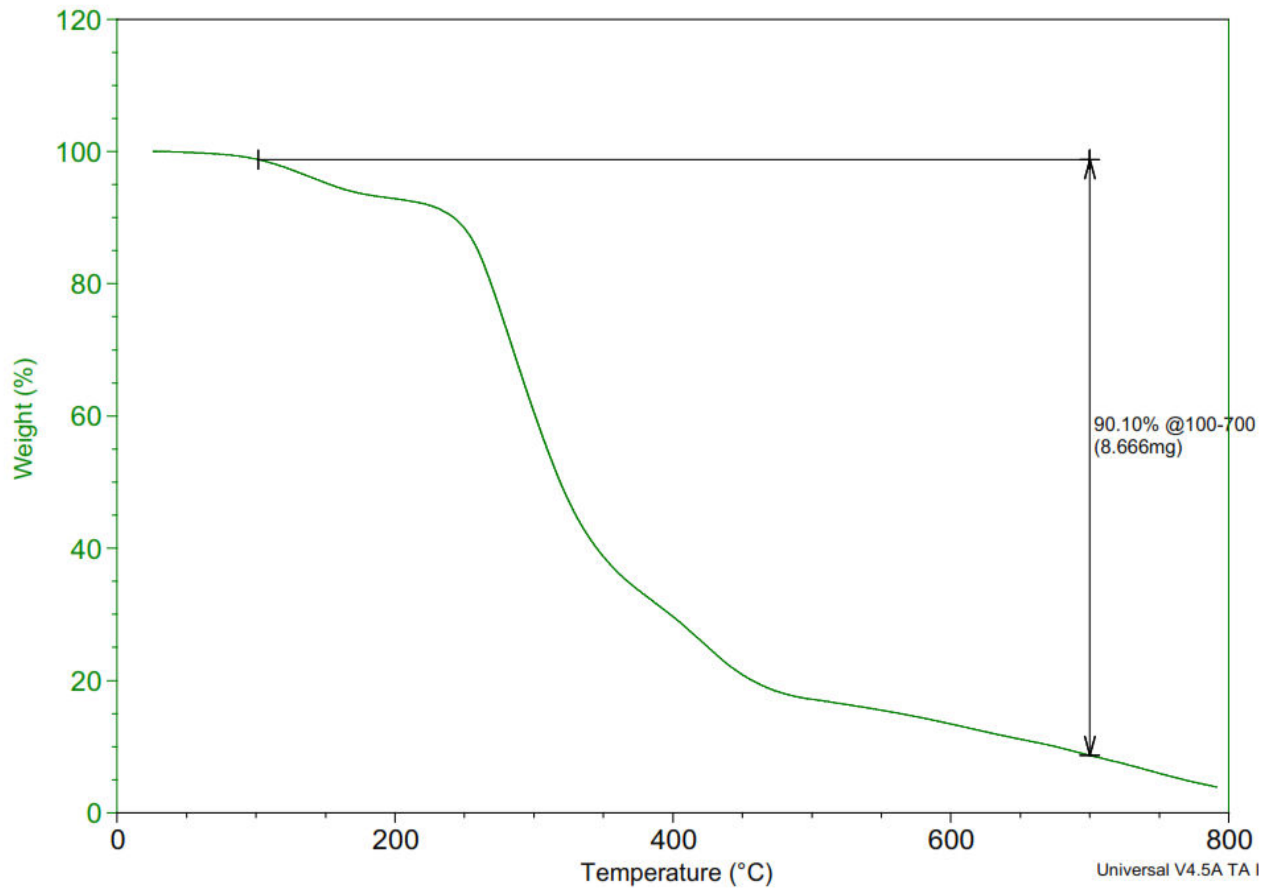

Figure 17: TGA thermogram of the 50/50 PVA/gelatin blend film.

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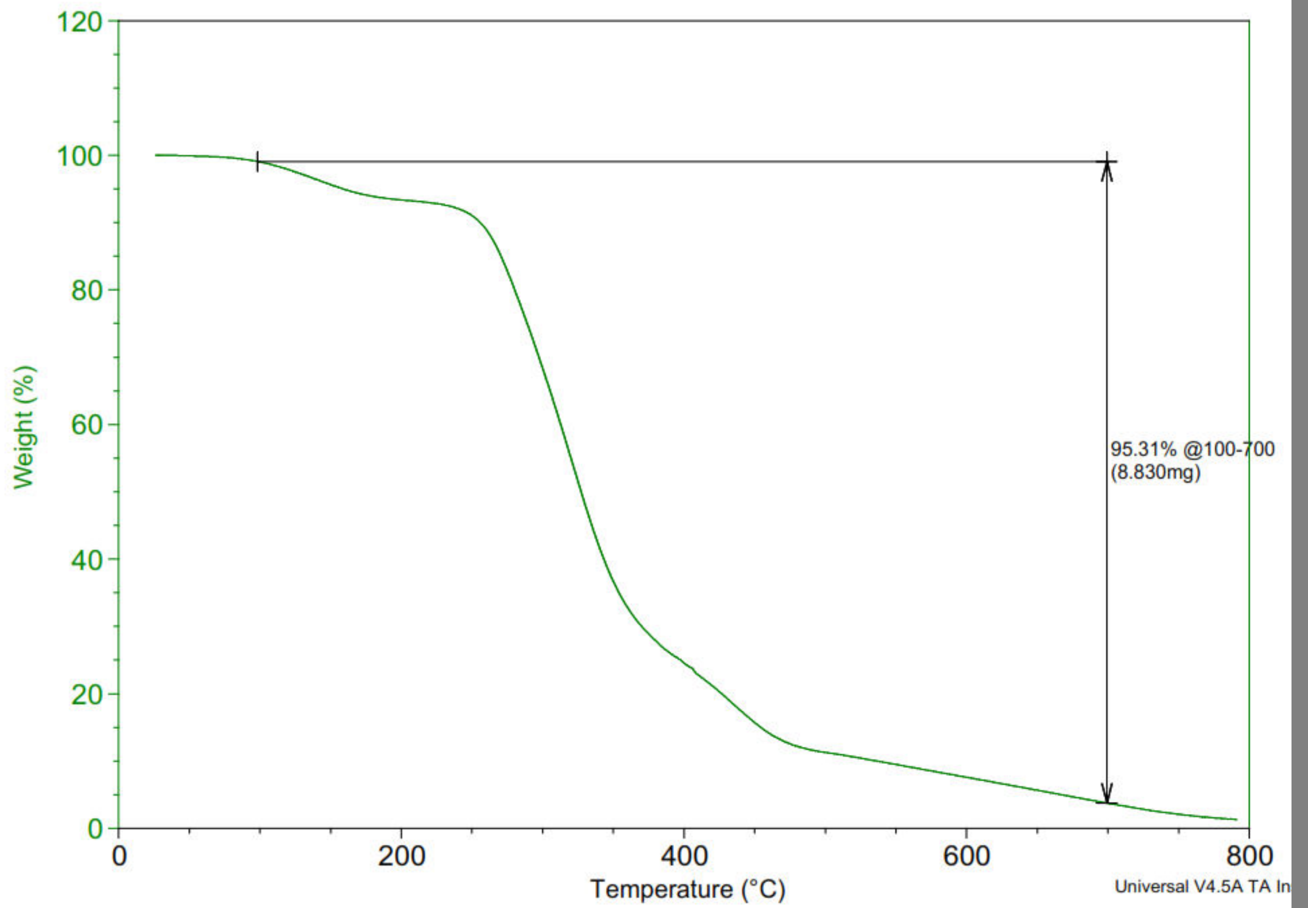

Figure 18: TGA thermogram of the 70/30 PVA/gelatin blend film.

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4.6 Dye Adsorption

This study also sought to determine potential industrial applications. The potential of the films to be employed in the adsorption of dyes from aqueous solutions was studied using Methylene blue dye. The influence of different parameters such as contact time, adsorbent amount and initial concentration of the dye in adsorption process was determined and the results are presented below.

4.6.1 Calibration curve

The calibration graph for the dilute solutions of methylene blue prepared from a stock solution of 4.0 X 10^{-5} is displayed in figure 19 below.

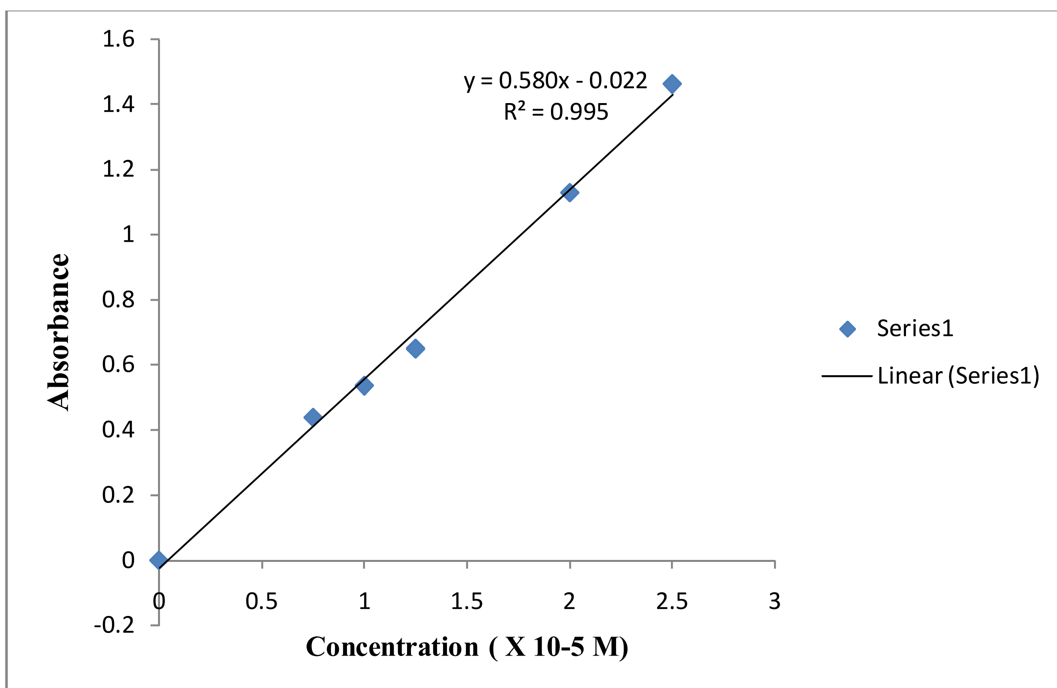

Figure 19: Calibration curve adsorption of methylene blue solutions onto 60/40 PVA/gelatin blend film.

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4.6.2 Effect of contact time on adsorption of methylene blue

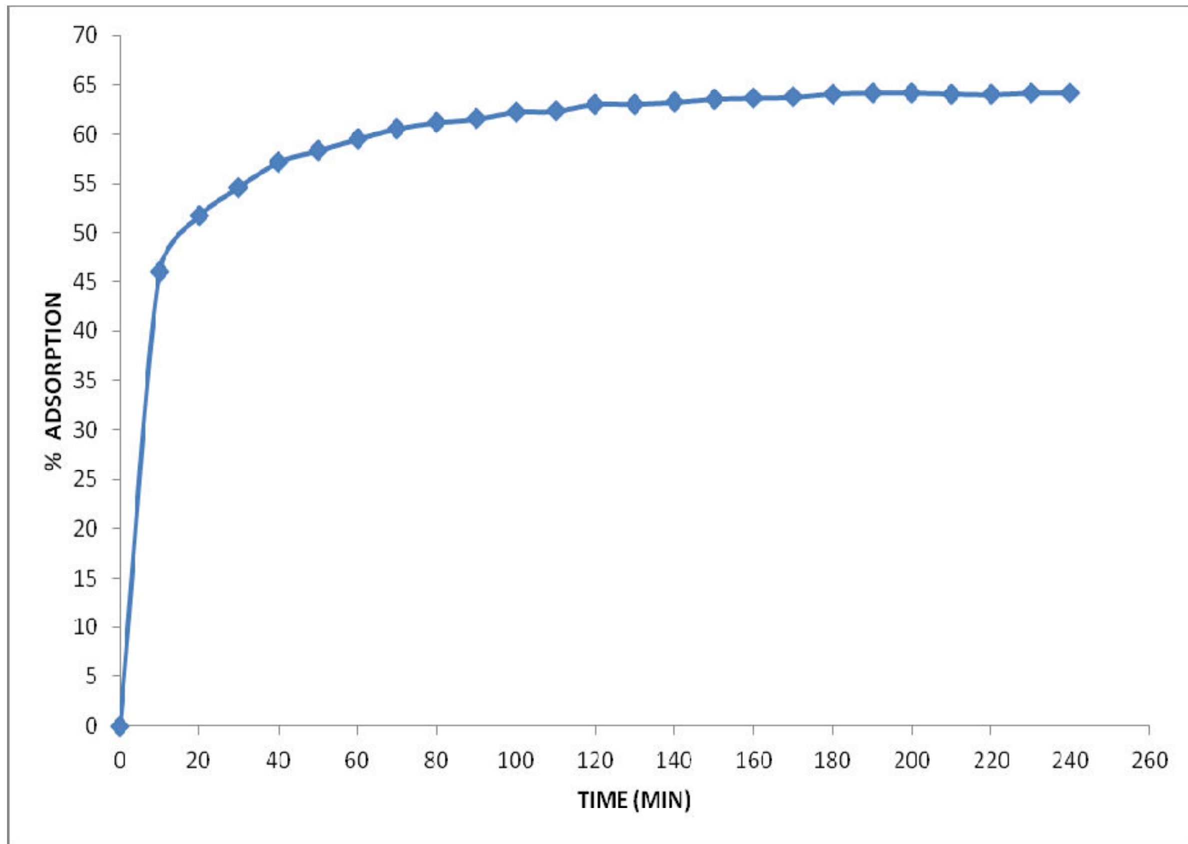

Figure 20: Effect of contact time on the adsorption of methylene blue dye solution, 2.0 X 10^{-5} M concentration onto 60/40 PVA/gelatin blend film.

The contact time between the adsorbent and dye molecules is used in the determination of the efficiency, kinetics and equilibrium time of an adsorption process (Shee, 2011). From figure 20, it is evident that initially the amount of dye adsorbed increased rapidly but slowed on approaching equilibrium. At equilibrium,the graph flattens completely indicating little or no adsorption. It can be seen that the adsorption reaches equilibrium after 110 minutes when the percent dye adsorbed is 64%.

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There are three regimes of adsorption. In the first region,the dye uptake proceeds very fast. In the second, the adsorption slows down and eventually flattens out in the third. This behaviour can be explained in that in the first region, there is an abundant amount of vacant surface sites available for adsorption. However, as time passes, more molecules occupy them and thus the number of available sites reduces,bringing down the rate of absorption. Additionally, repulsive forces between adsorbate molecules on the adsorbent surface and those in the bulk solution increase (Wanyonyi, 2011).

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4.6.3 Effect of amount of adsorbent on adsorption

The variation of the percent adsorption of 0.000025M methylene blue dye solution with the amount of PVA-gelatin film(60/40 composition) is illustrated in figure 21.

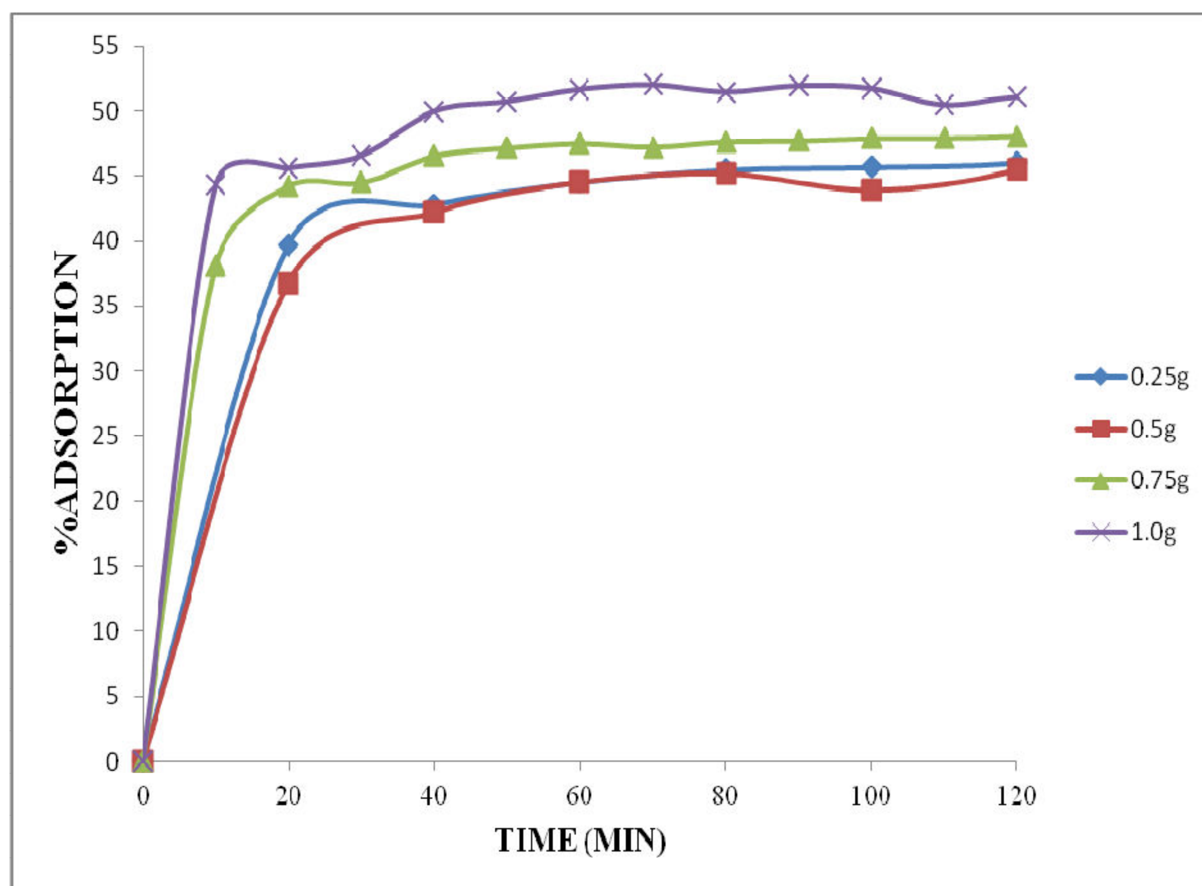

Figure 21: Effect of adsorbent weight on adsorption of 2.5 X 10^{-5}M by PVA-gelatin film.

Evidently, as the weight of the PVA-gelatin film used in the experiment increases, so does the percent dye removal/efficiency. At equilibrium, for 0.25g of PVA-gelatin film, the percent dye removal is 43% while for 1.0 g it is 50%. When more of the adsorbent is used, then possibly more binding/active sites are availed, increasing the efficiency. Wanyonyi, (2011) and Shee, (2011) reported similar findings.

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4.6.4 Effect of initial dye concentration

Initial concentration of the dye being adsorbed has a huge effect on the adsorption kinetics. It creates the driving force essential for overcoming mass transfer resistances that exist between molecules in both the aqueous and the solid phases. Adsorption of methylene blue onto 0.25g PVA-gelatin film was studied at three different concentrations and the results are displayed in figure 22.

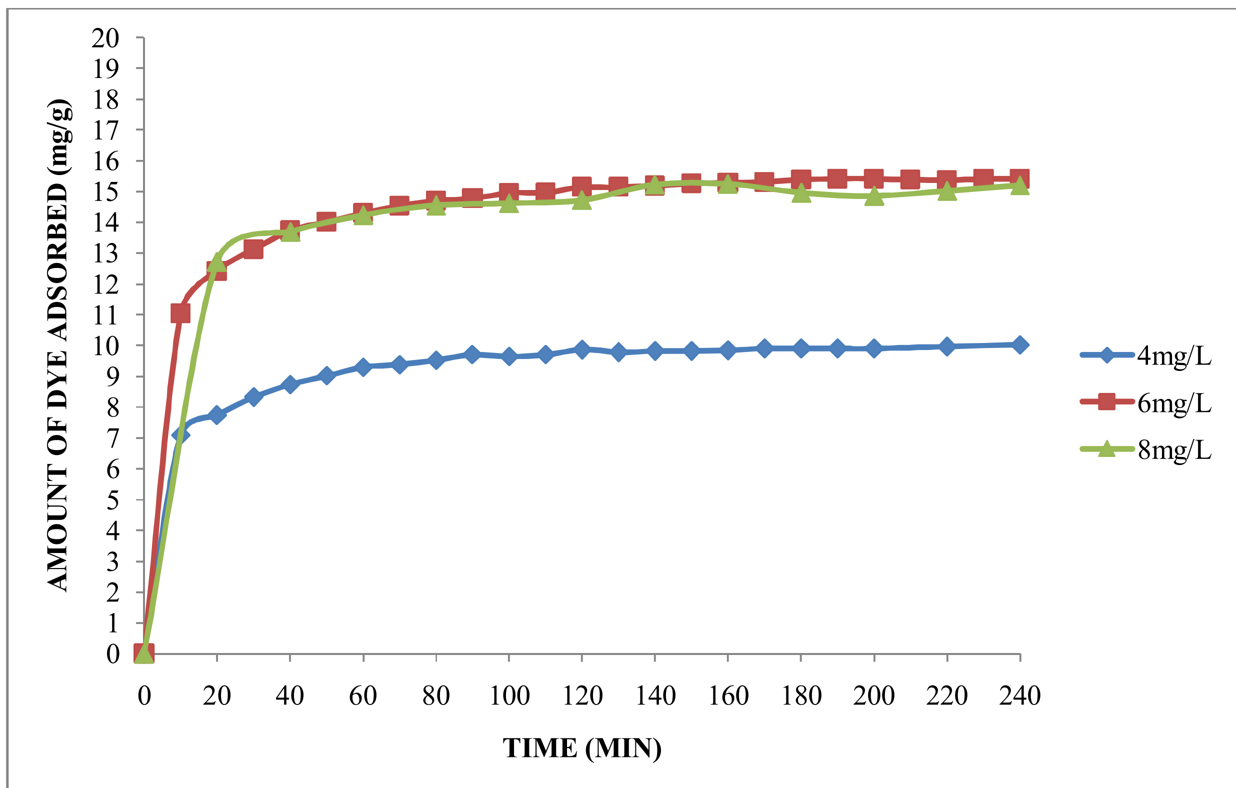

Figure 22: Effect of initial methylene blue dye concentration on equilibrium dye uptake using PVA/Gelatin blend film (60/40 composition).

From figure 22 above, it can be seen that adsorption is directly proportional to the initial methylene blue concentration. Equilibrium uptake for 0.25g PVA-gelatin film when the dye

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concentration is 4mg/L is 9.5 mg/g while that when the dye concentration is 6 mg/L is 14 mg/g. This increase is explained as follows. Increasing the dye concentration, increases the amount of driving force available to overcome resistances to mass transfer of the dye onto the film (Wanyonyi, 2011). It also means an increase in the amount of dye molecules in solution, thereby increasing the interaction between the dye and the adsorbent, which effectively increases adsorption. Shee (2011), also reported similar findings in the adsorption of methylene blue dye onto mangrove bark, mangrove leaves and coconut husks.

Additionally, it can be observed that there is very little change in the percent equilibrium adsorption when the initial methylene blue concentration is increased from 6 mg/L to 8 mg/L. Since the weight of the adsorbent remains the same, the amount of binding sites also remain the same. An increase in concentration means an increase in the number of dye molecules, but the number of sites to bind to remains the same and will at some point, beyond which an increase in concentration will have no significant effect on adsorption, that is, the sites will be fully occupied.

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CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In this study the potential of fish scales to substitute bovine and porcine sources of gelatin was clearly demonstrated. Nile perch scales were treated with an enzyme hydrolysis using crude alkaline protease from *Bacillus cereus strain wwcp 1,* and the resulting hydrolysate freeze dried to obtain pale yellow gelatin powder, with a yield of 16.3%. The use of an enzymatic method during hydrolysis poses extra environmental gains since it is considered more green (less chemicals are used) than the conventional acid or base hydrolysis employed in industry.

An infrared analysis of the sample showed peaks at 3442 cm^{-1}, 1653 cm^{-1} and ~1590cm^{-1} corresponding to Amide A, Amide I and Amide II bands respectively. Amino acid analysis revealed that glycine was the most abundant amino acid at 21.7% (w/w) followed by proline at 14.6%. Alanine was the third most abundant at 14.6%. The high proline content suggests that the gelatin obtained from Nile Perch fish scales could have interesting gel properties, demonstrating its commercial potential. It can therefore be concluded that Nile Perch fish scales are a viable source of gelatin and can be exploited as an alternative to bovine and porcine gelatin.

Blends of polyvinyl alcohol and the extracted gelatin were then prepared and thermal analysis of the blends via DSC and TGA was performed on the films obtained by solution casting method. The DSC thermograms for all the films displayed a single glass transition temperature that was intermediate between that of pure PVA and pure gelatin, suggesting that the two polymers are miscible in the amorphous phase. The DSC thermograms for the PVA/Gelatin blends also displayed endothermic peaks corresponding to the melting temperatures for certain blend

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compositions, i.e T_m of the films. The area under the peaks corresponds to the degree of crystallinity and it increased as the PVA content increased. TGA analysis of the films displayed decomposition onset temperatures intermediate between those of the pure components.

The potential of the PVA/gelatin films in 60/40 composition to be applied industrially as adsorbents was also studied. Methylene blue dye was used as the adsorbate and the film showed up to 64% dye adsorption at equilibrium, showing good potential for application. The dye adsorption capacity of the film increased with contact time, adsorbent dosage and initial dye concentration.

5.2 RECOMMENDATIONS

1. It is recommended that scales from other readily available fish such as Tilapia sp, *(Oreochromis spp)* be studied as an alternative source of gelatin. The viability of bones from the same, for gelatin extraction should also be investigated.
2. The visco-elastic and gelling properties such as viscosity and gel strength of Nile perch gelatin should also be investigated. It is further recommended that the effect of the age of the fish from which scales are sourced, storage conditions and also extraction conditions on those properties be studied.

3. The kinetics of the adsorption of methylene blue dye onto Polyvinyl alcohol-gelatin blend films should be investigated. The effect of parameters such as pH, temperature and salinity on the adsorption should also be established.

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APPENDICES

Appendix 1: Amino acid composition

Appendix 1a: amino acid composition of gelatin, first repetition

FIRST REPETITION					
amino acid	M.W.	raw data umoles rep. 1	mgms AA found by analysis	molar % Composition of total protein	by weight % Compositon
ASX	115.1	52.40	6.03	5.1%	6.6%
GLX	129.1	76.48	9.87	7.5%	10.8%
SER	87.1	24.71	2.15	2.4%	2.4%
HIS	137.2	8.65	1.19	0.8%	1.3%
GLY	57.1	356.94	20.38	34.9%	22.3%
THR	101.1	18.85	1.91	1.8%	2.1%
ALA	71.1	153.90	10.94	15.1%	12.0%
ARG	156.2	52.33	8.17	5.1%	8.9%
TYR	163.2	3.97	0.65	0.4%	0.7%
VAL	99.1	27.59	2.73	2.7%	3.0%
MET	131.2	23.21	3.05	2.3%	3.3%
IS(1)		11.00			
PHE	147.2	19.28	2.84	1.9%	3.1%
ILE	113.2	14.44	1.63	1.4%	1.8%
LEU	113.2	26.39	2.99	2.6%	3.3%
LYS	128.2	31.51	4.04	3.1%	4.4%
IS(2)		11.00			
PRO	97.1	131.88	12.81	12.9%	14.0%
	Sum:	**1022.53**	**91.38**	**100%**	**100%**

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Appendix 1b : Amino acid composition of gelatin second repetition

SECOND REPETITION					
Amino Acid	M.W.	raw data umoles rep. 2	mgms AA found by analysis	molar % Composition of total protein	by weight % Compositon
ASX	115.1	37.85	4.36	5.1%	6.6%
GLX	129.1	55.231	7.13	7.5%	10.7%
SER	87.1	19.11	1.66	2.6%	2.5%
HIS	137.2	7.2932	1.00	1.0%	1.5%
GLY	57.1	245.98	14.05	33.4%	21.2%
THR	101.1	13.408	1.36	1.8%	2.0%
ALA	71.1	108.2	7.69	14.7%	11.6%
ARG	156.2	37.839	5.91	5.1%	8.9%
TYR	163.2	4.0971	0.67	0.6%	1.0%
VAL	99.1	20.391	2.02	2.8%	3.0%
MET	131.2	17.527	2.30	2.4%	3.5%
IS(1)		11			
PHE	147.2	13.95	2.05	1.9%	3.1%
ILE	113.2	10.552	1.19	1.4%	1.8%
LEU	113.2	19.001	2.15	2.6%	3.2%
LYS	128.2	22.009	2.82	3.0%	4.2%
IS(2)		11			
PRO	97.1	103.35	10.04	14.0%	15.1%
	Sum:	**735.79**	**66.40**	**100%**	**100%**

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Appendix 1c: Amino acid composition of gelatin, Average.

AVERAGE		
AA	molar % Composition of total protein	by weight % Compositon
ASX	5.1%	6.6%
GLX	7.5%	10.8%
SER	2.5%	2.4%
HIS	0.9%	1.4%
GLY	34.2%	21.7%
THR	1.8%	2.1%
ALA	14.9%	11.8%
ARG	5.1%	8.9%
TYR	0.5%	0.9%
VAL	2.7%	3.0%
MET	2.3%	3.4%
IS(1)		
PHE	1.9%	3.1%
ILE	1.4%	1.8%
LEU	2.6%	3.3%
LYS	3.0%	4.3%
IS(2)		
PRO	13.5%	14.6%
TOTAL	**100%**	**100%**

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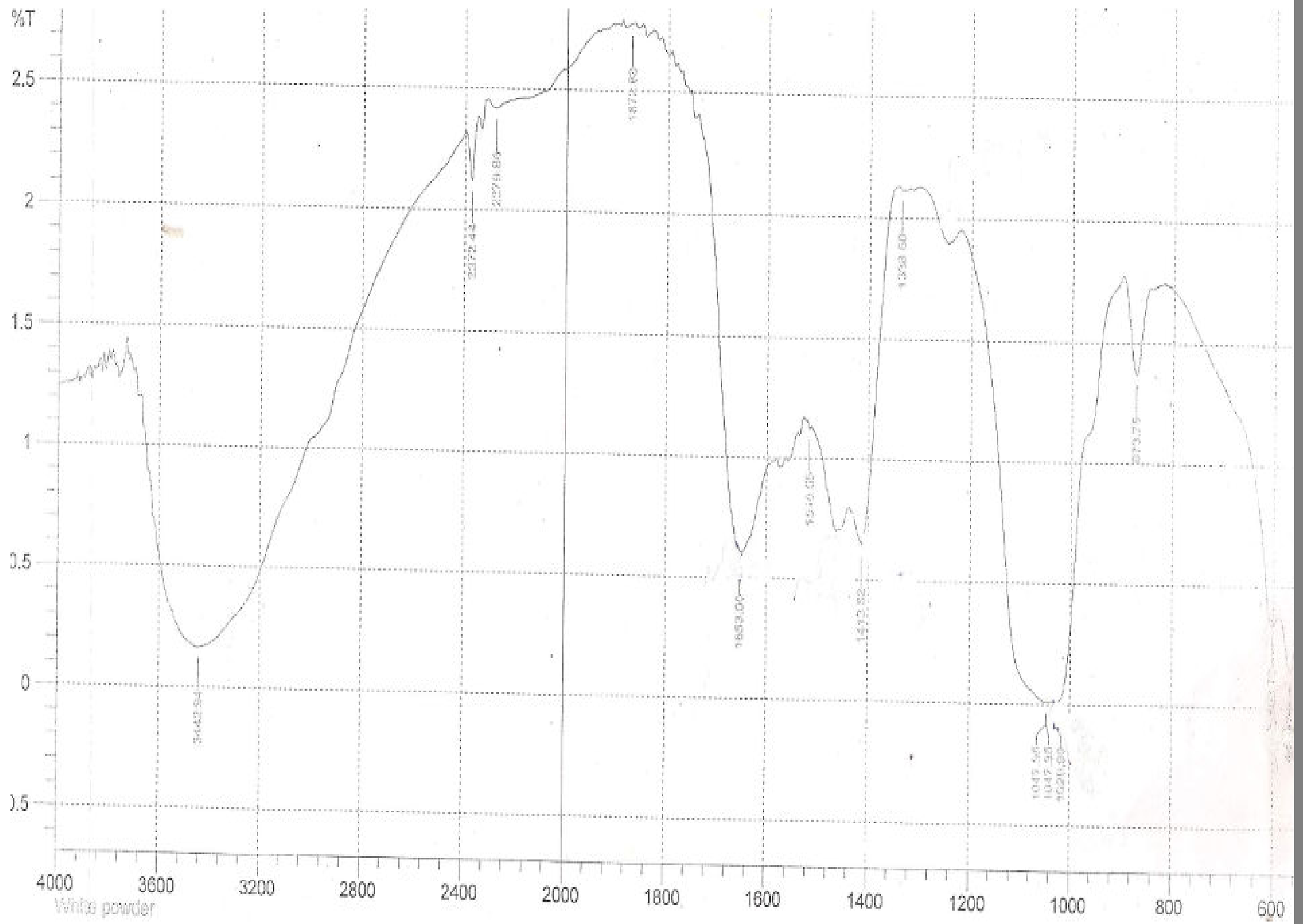
Appendix 2: IR spectra of gelatin.



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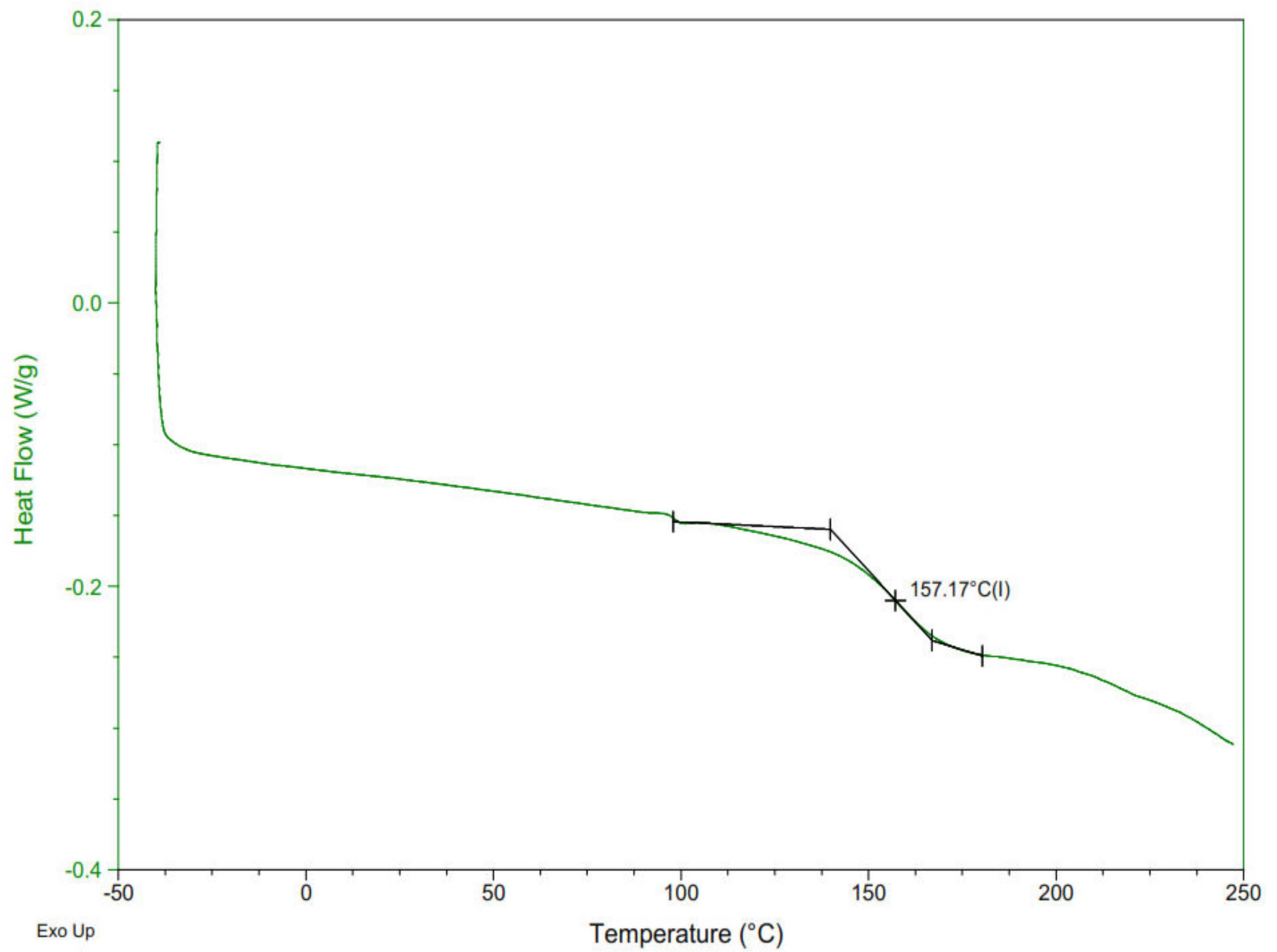
Appendix 3: DSC thermograms

Appendix 3a: DSC of pure gelatin



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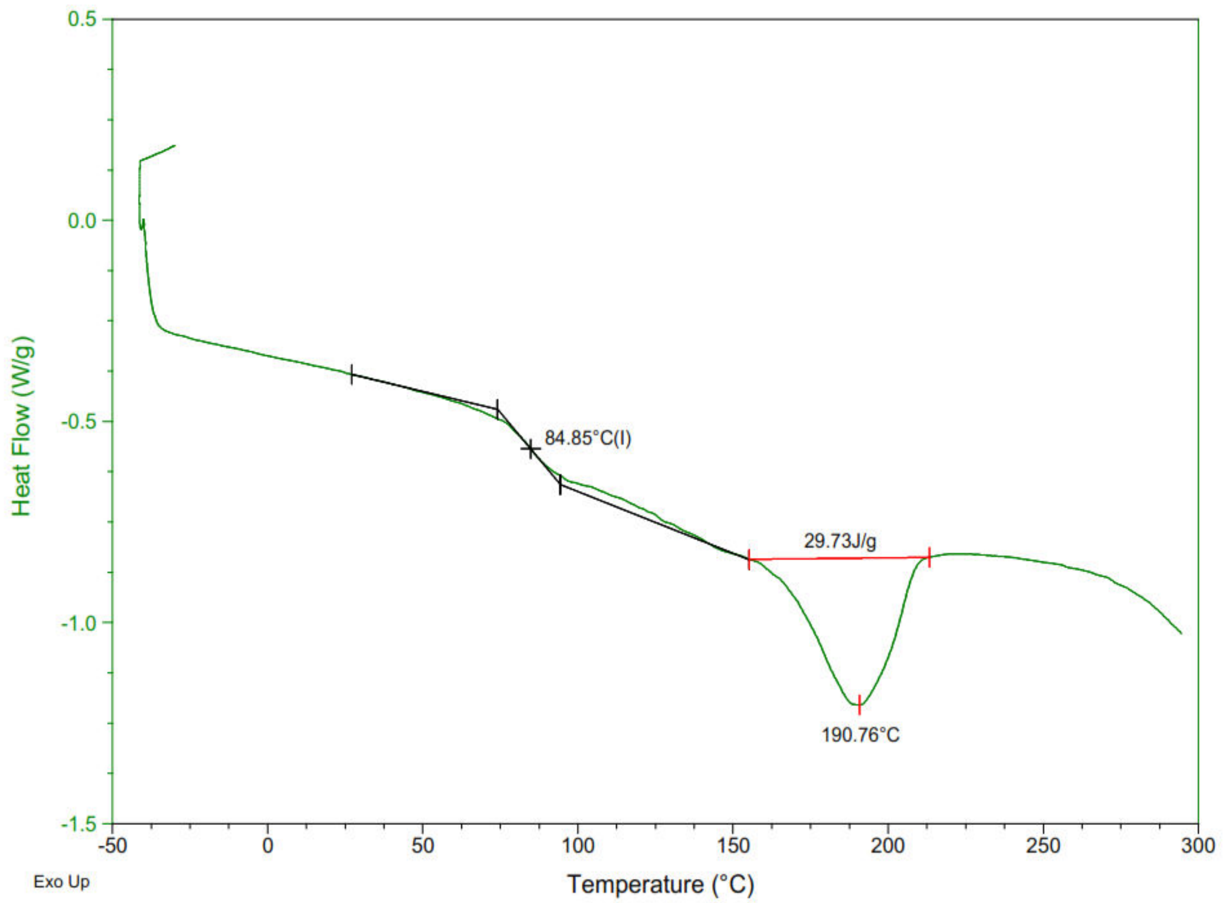
Appendix 3 b: DSC thermogram of pure PVA



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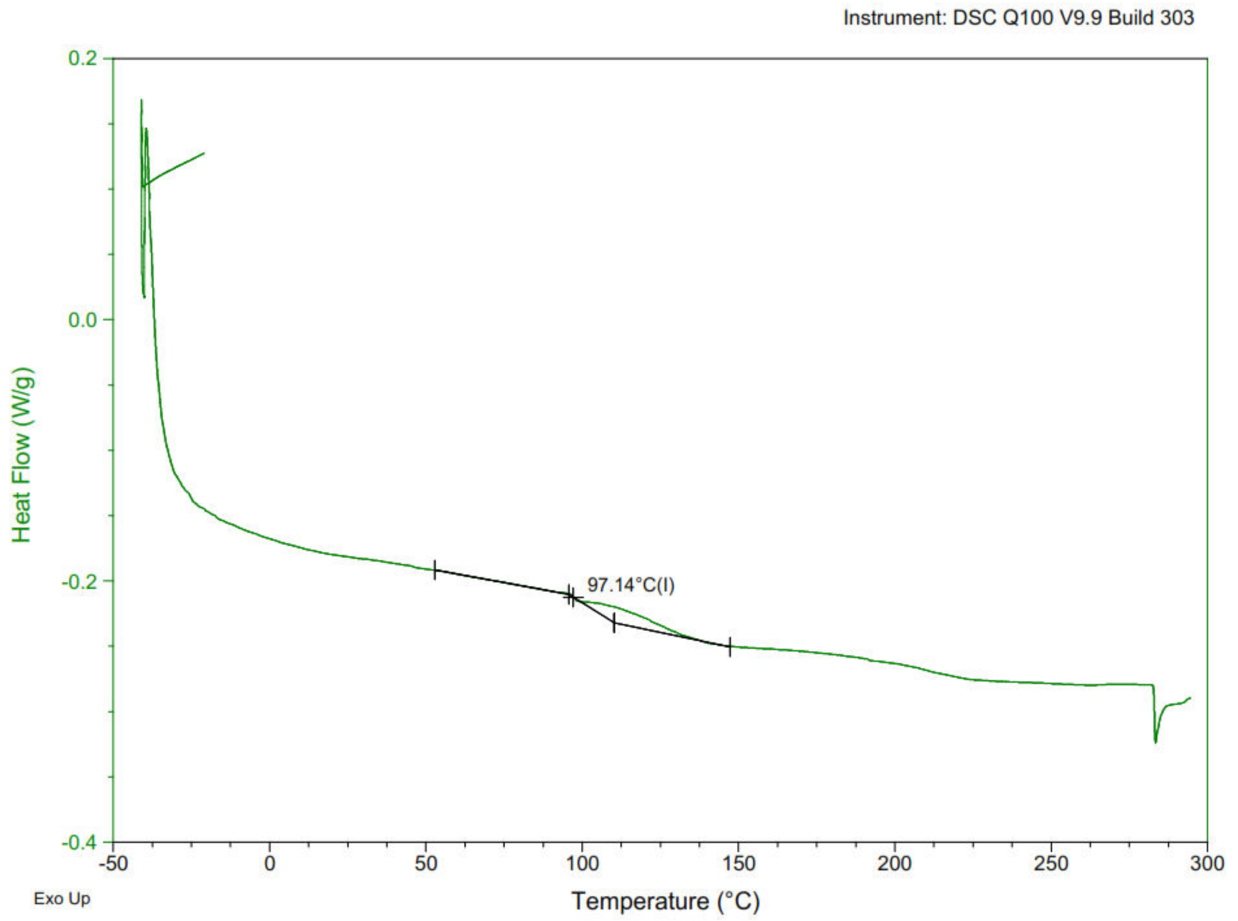
Appendix 3 c: DSC thermogram of 20/80 PVA/gelatin film.



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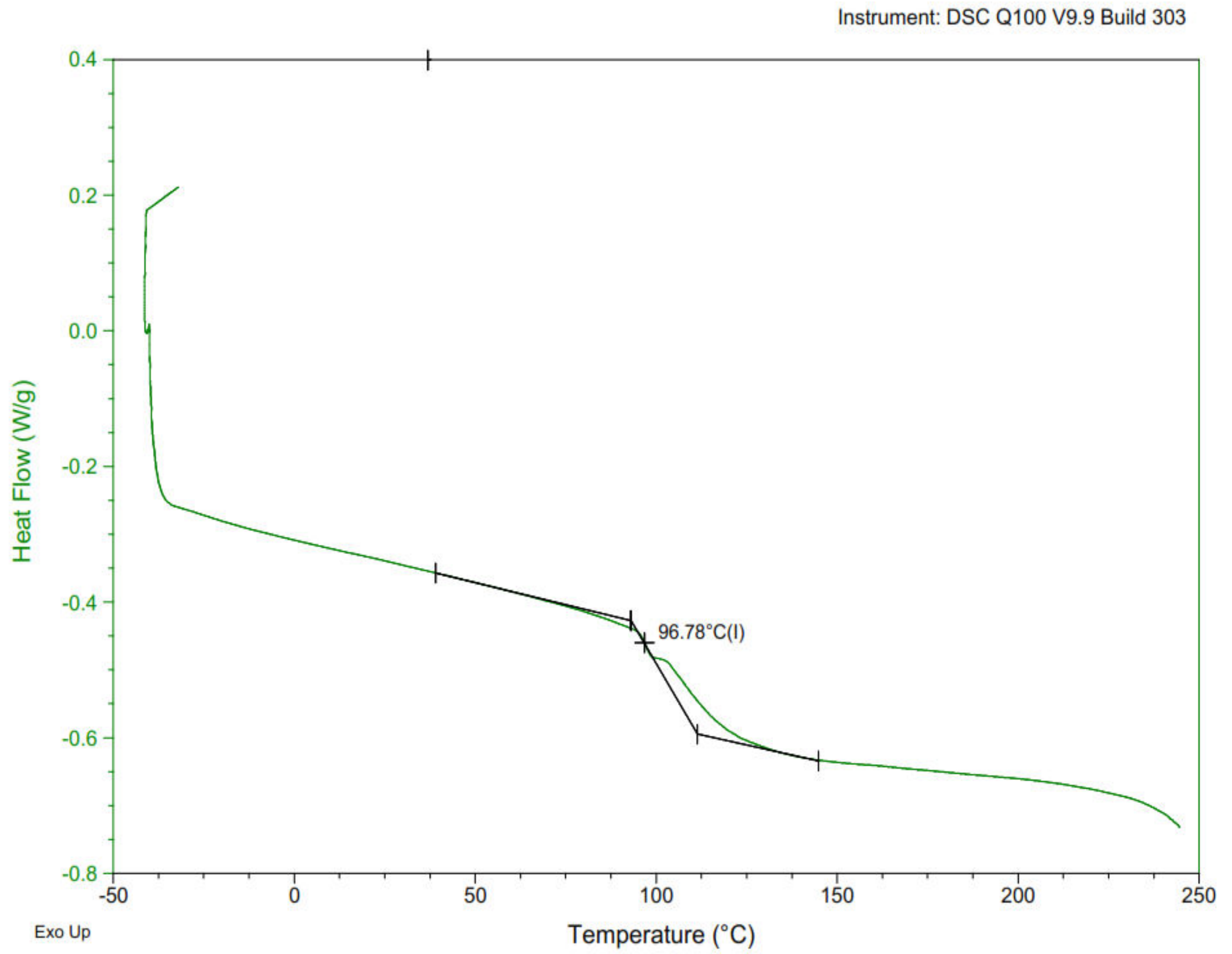
Appendix 3 d: DSC thermogram of 30/70 PVA/gelatin blend film



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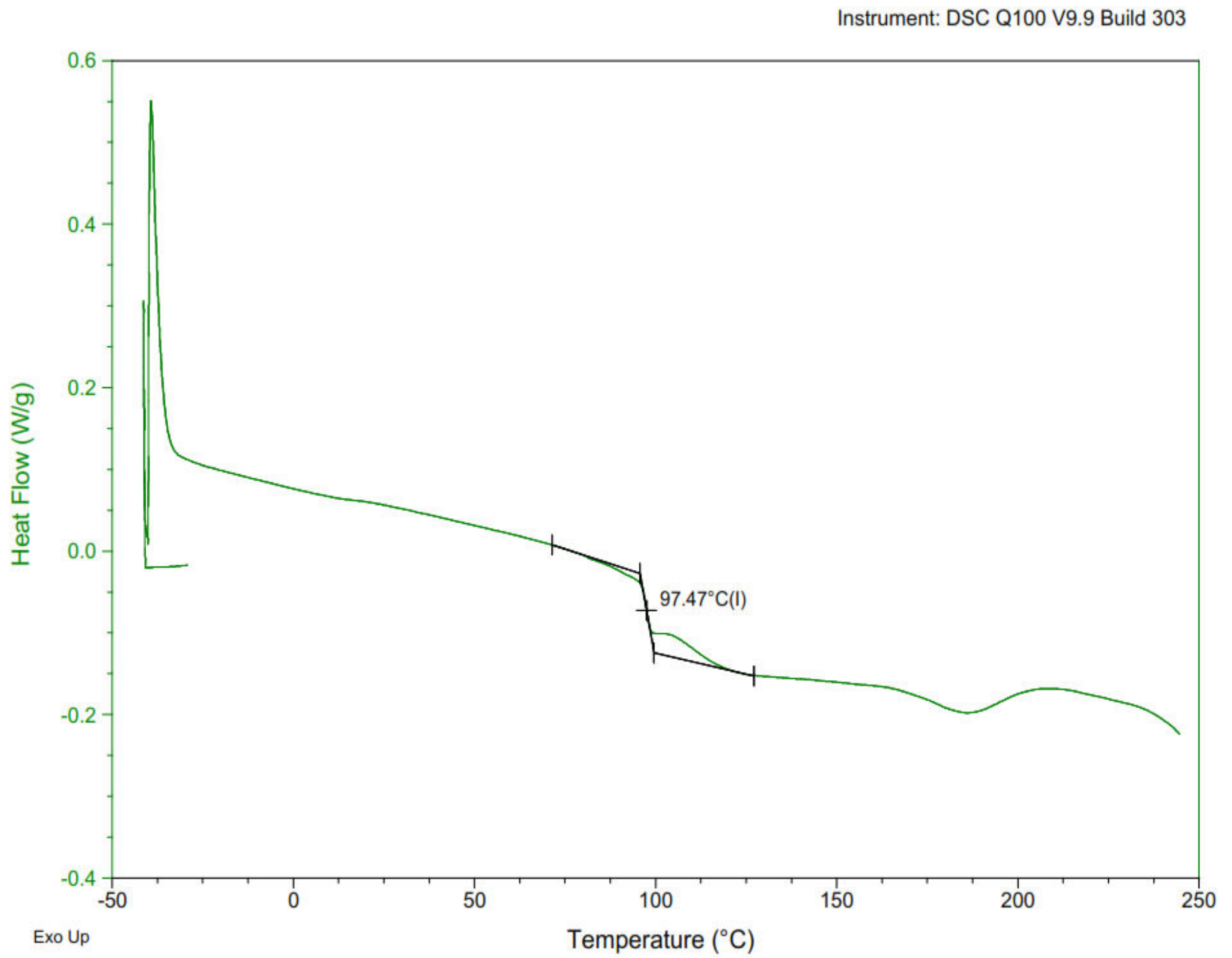
Appendix 3e: DSC themogram of 40/60 PVA/gelatin blend film:



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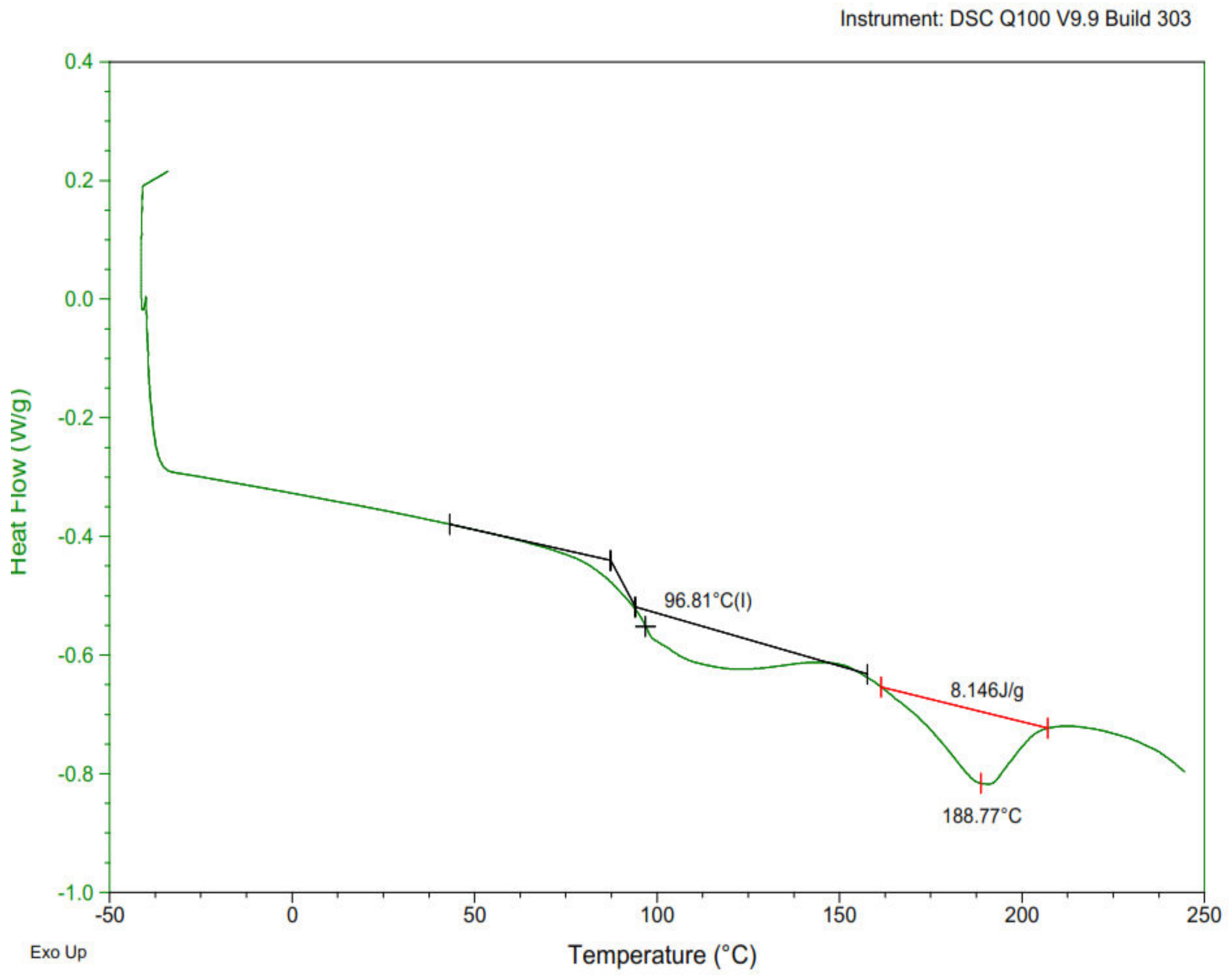
Appendix 3f : DSC themogram of 50/50 PVA/gelatin blend film



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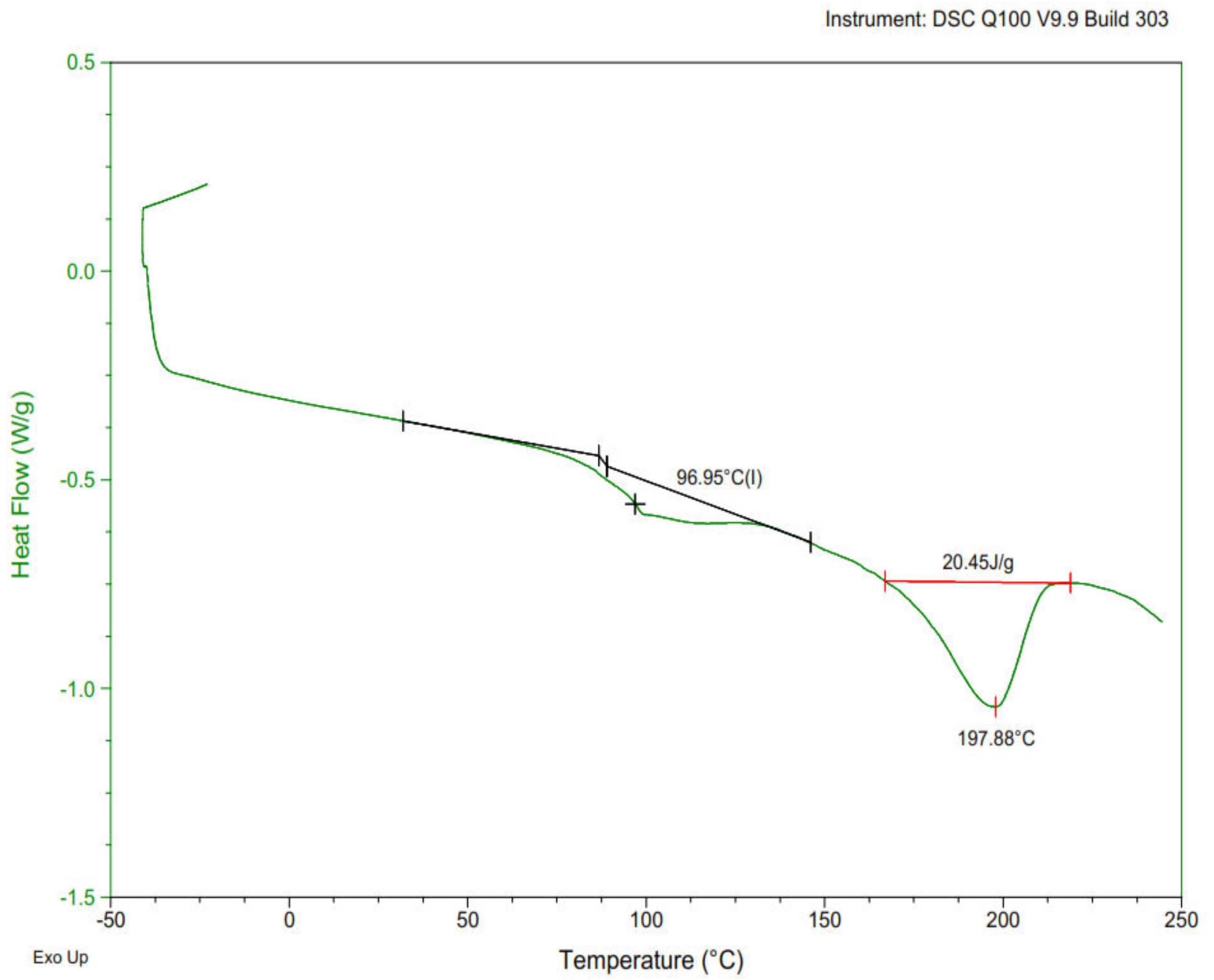
Appendix 3g DSC themogram of 60/40 PVA/gelatin blend film:



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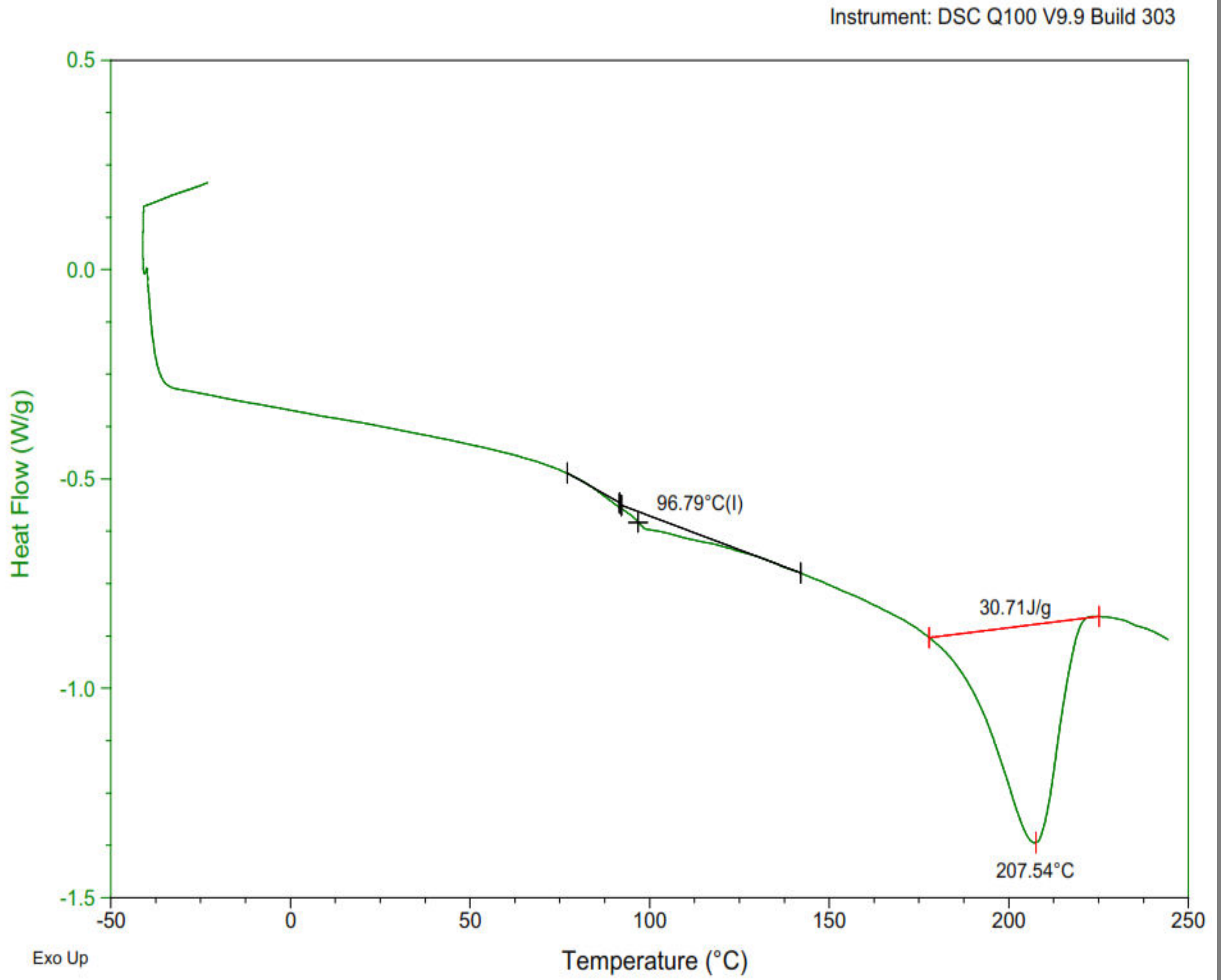
Appendix 3h DSC themogram of 70/30 PVA/gelatin blend film



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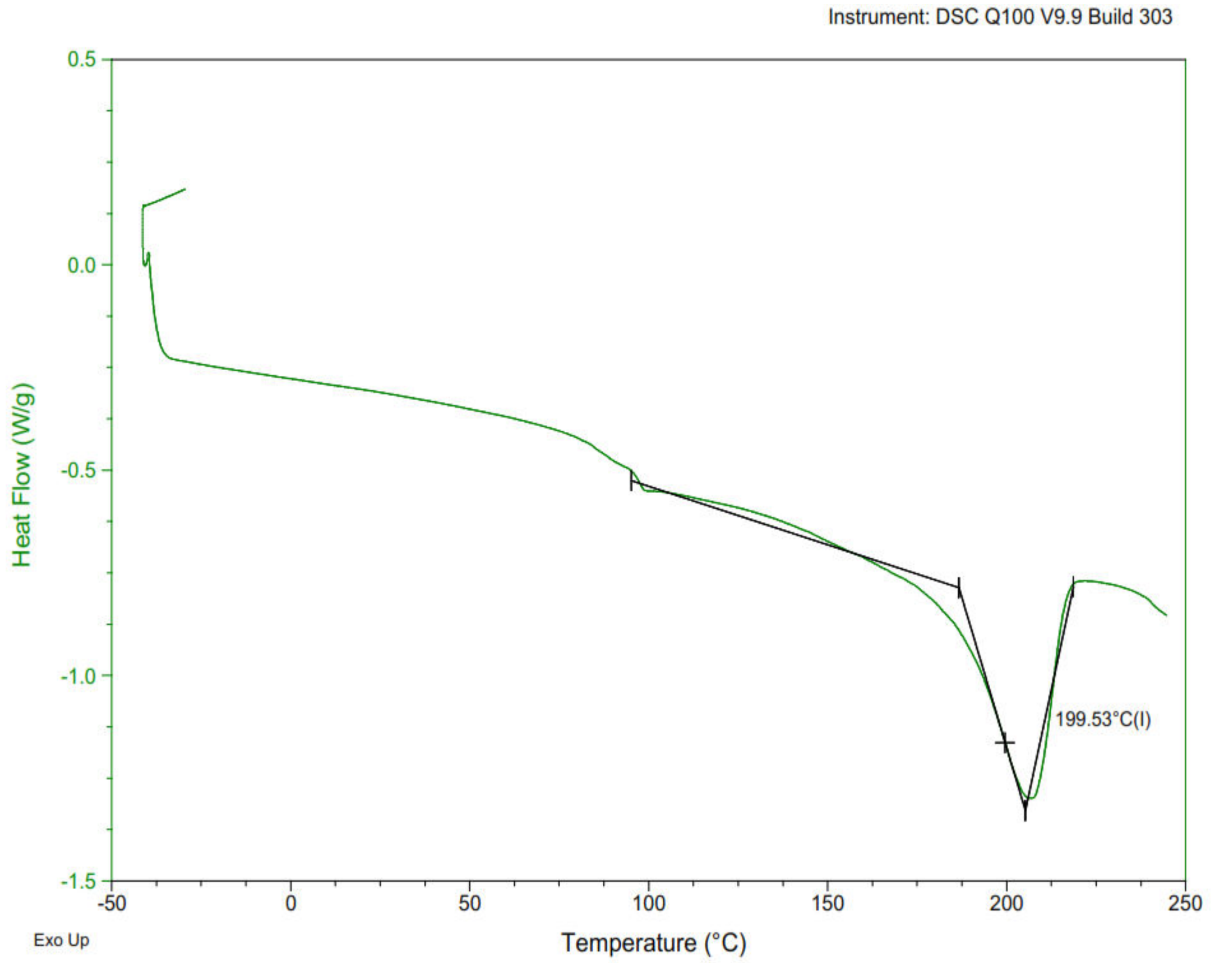
Appendix 3 i: DSC themogram of 80/20 PVA/gelatin blend film.



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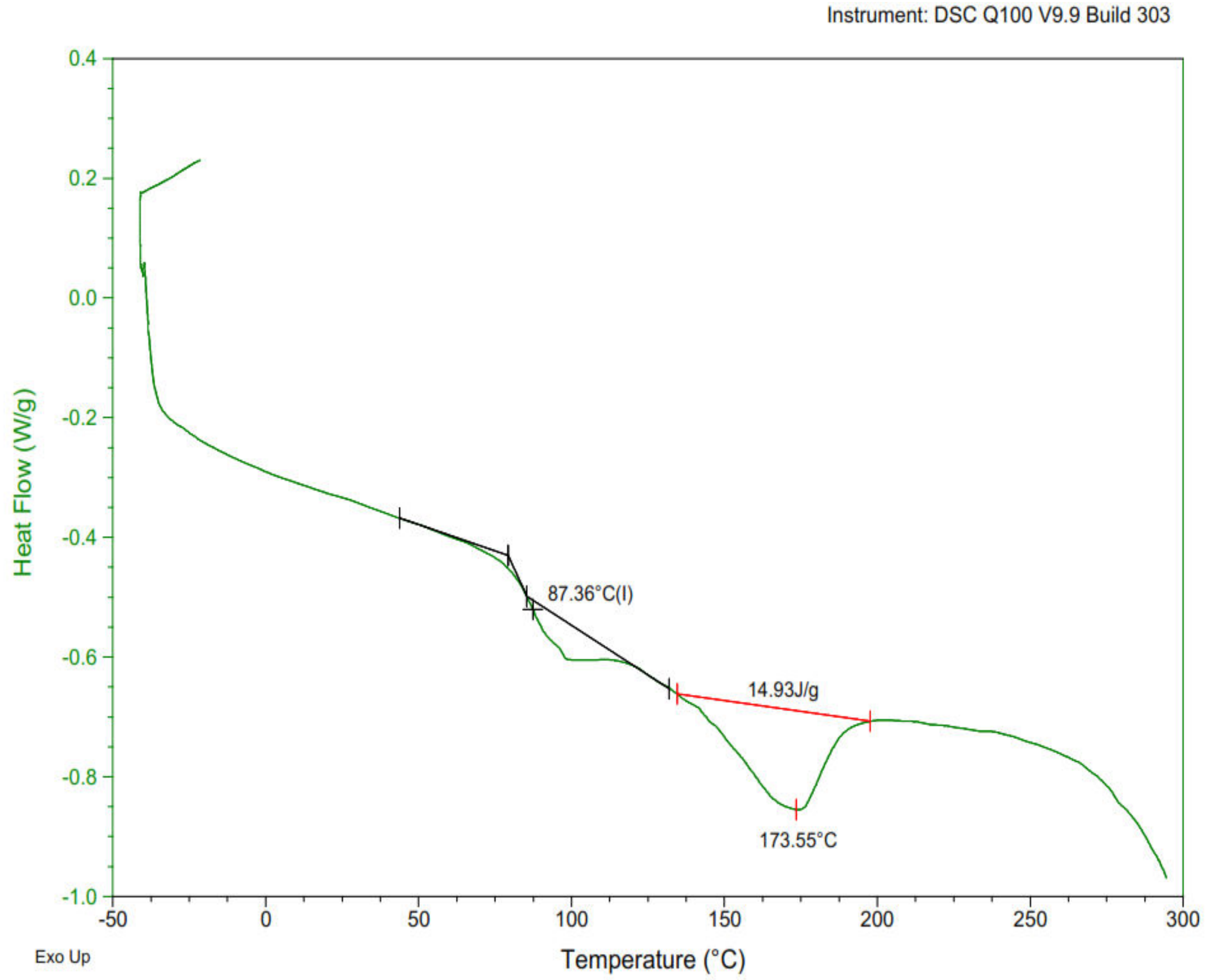
Appendix 3 j: DSC themogram of 90/10 PVA/gelatin blend film



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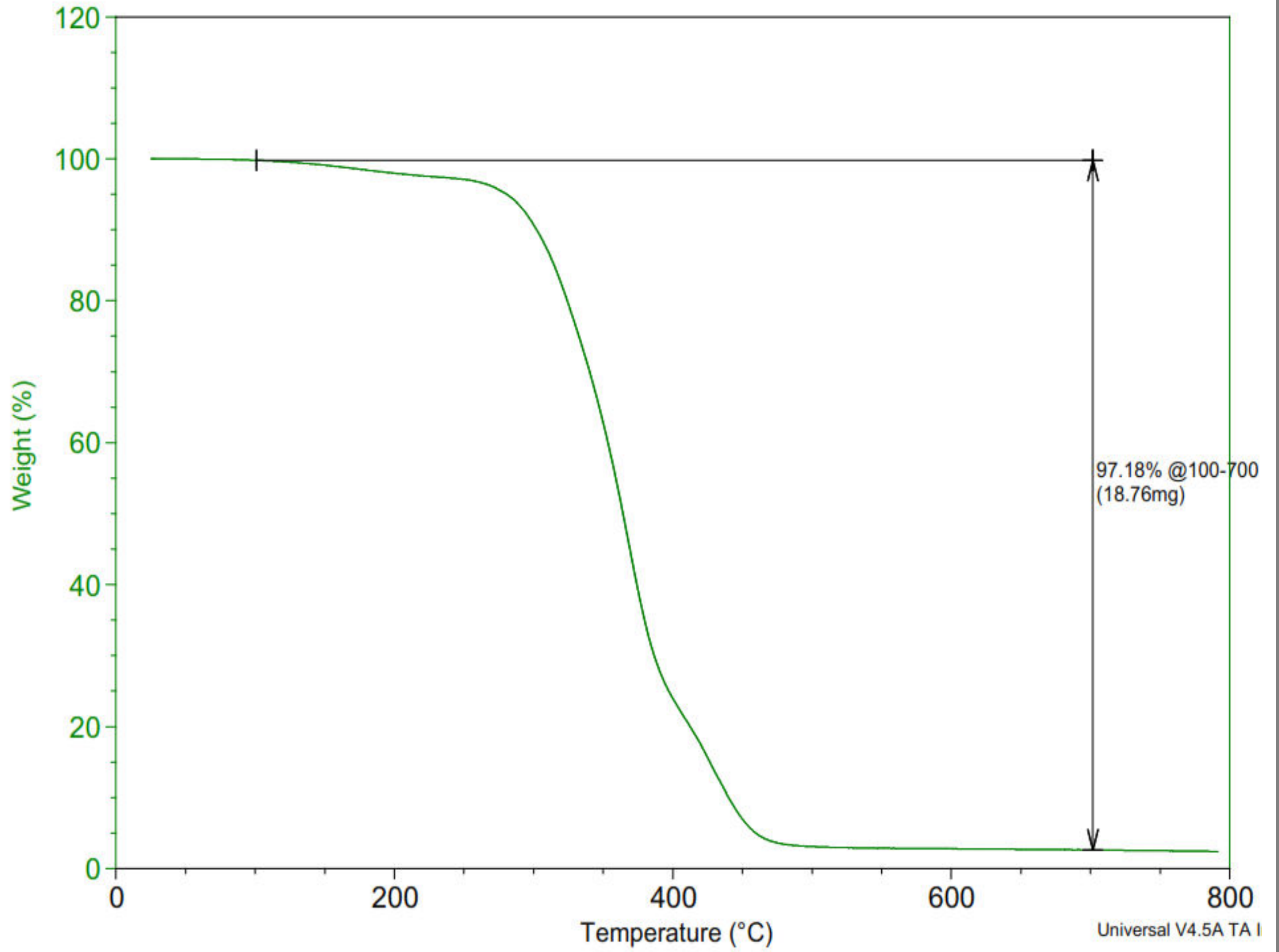
Appendix 4: TGA thermograms

Appendix 4a: TGA thermogram of pure PVA:



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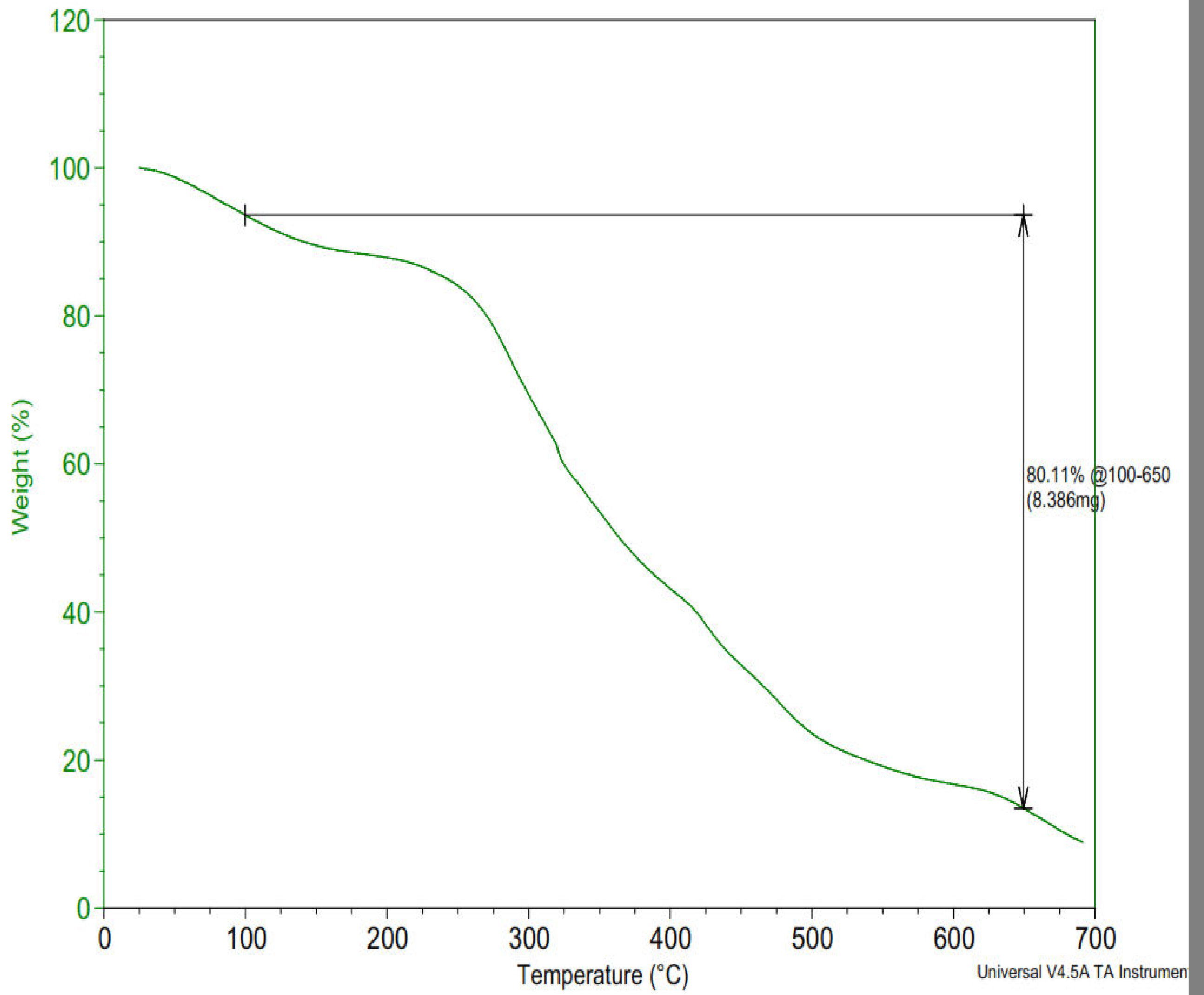
Appendix 4b: TGA thermogram of pure gelatin:



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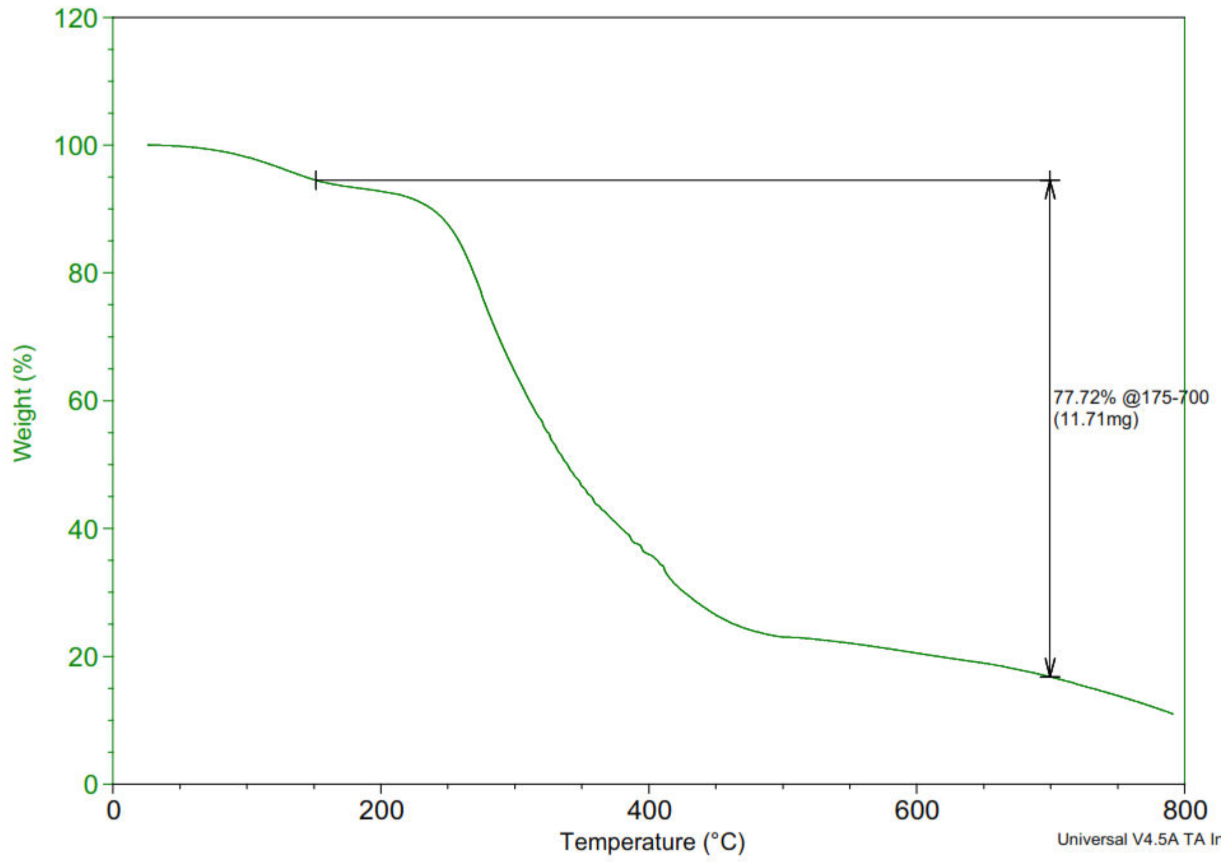
Appendix 4c: TGA thermogram of 30/70 PVA/gelatin blend film



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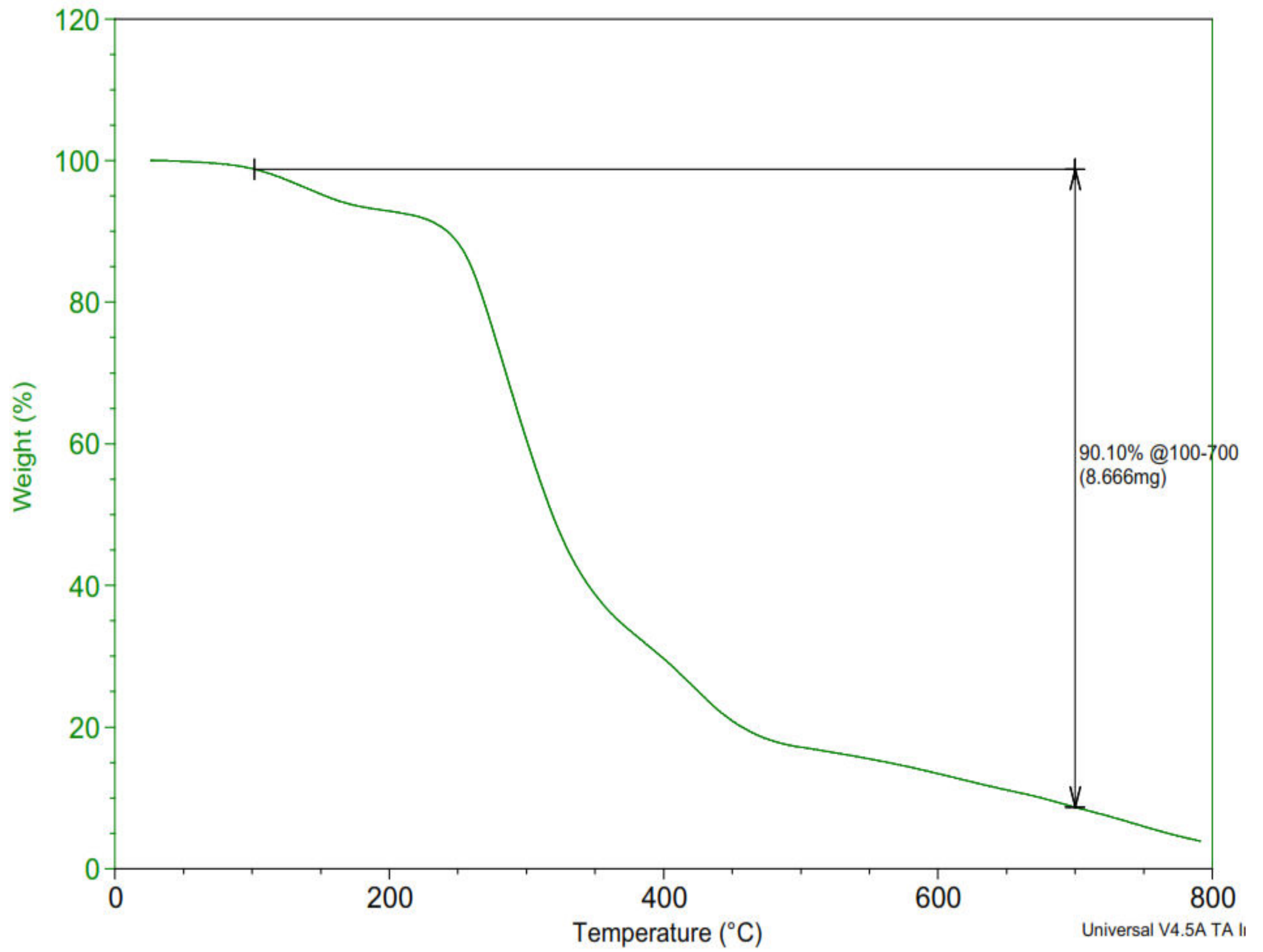
Appendix 4d: TGA thermogram of 50/50 PVA/gelatin blend film



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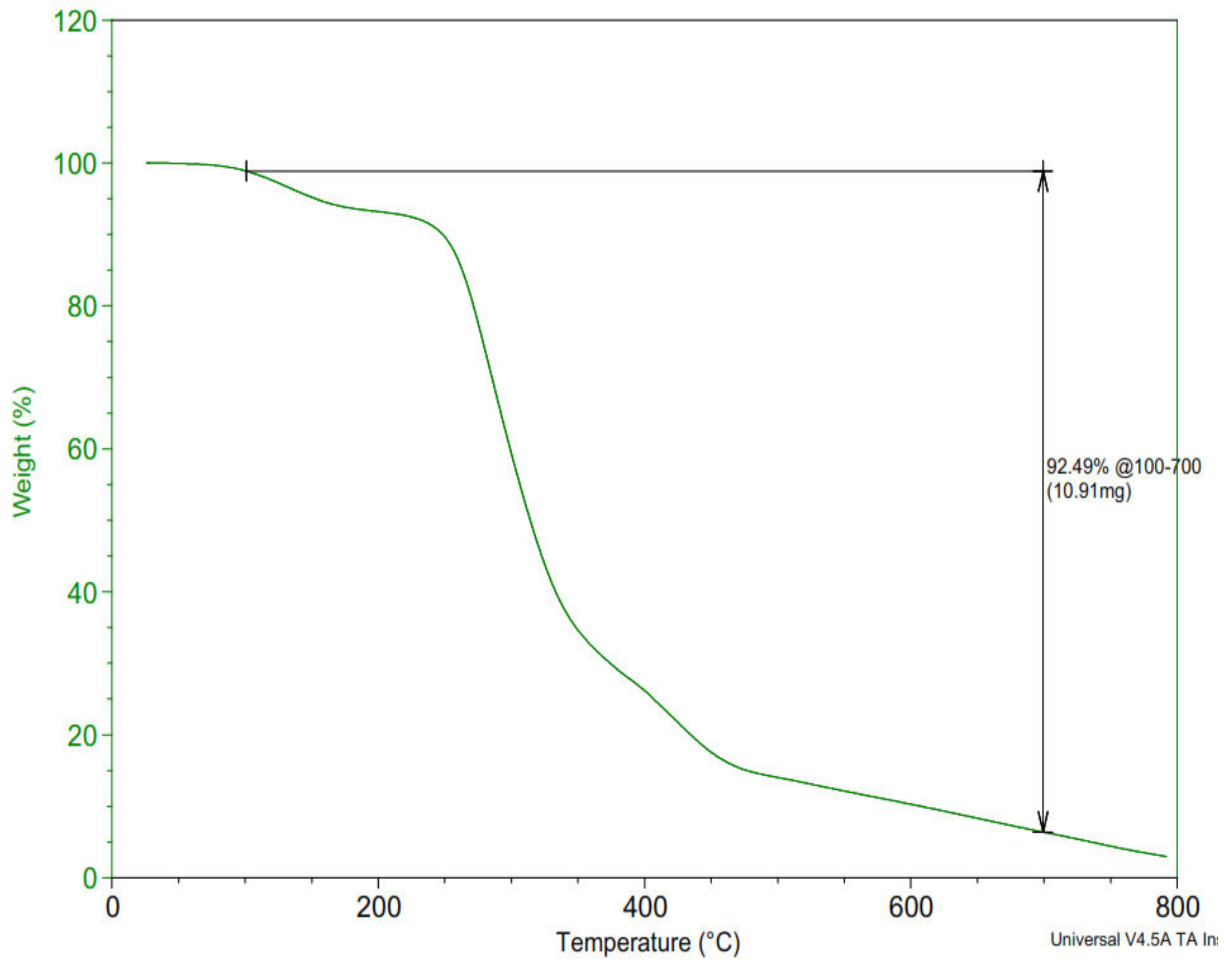
Appendix 4e: TGA thermogram of 60/40 PVA/gelatin blend film



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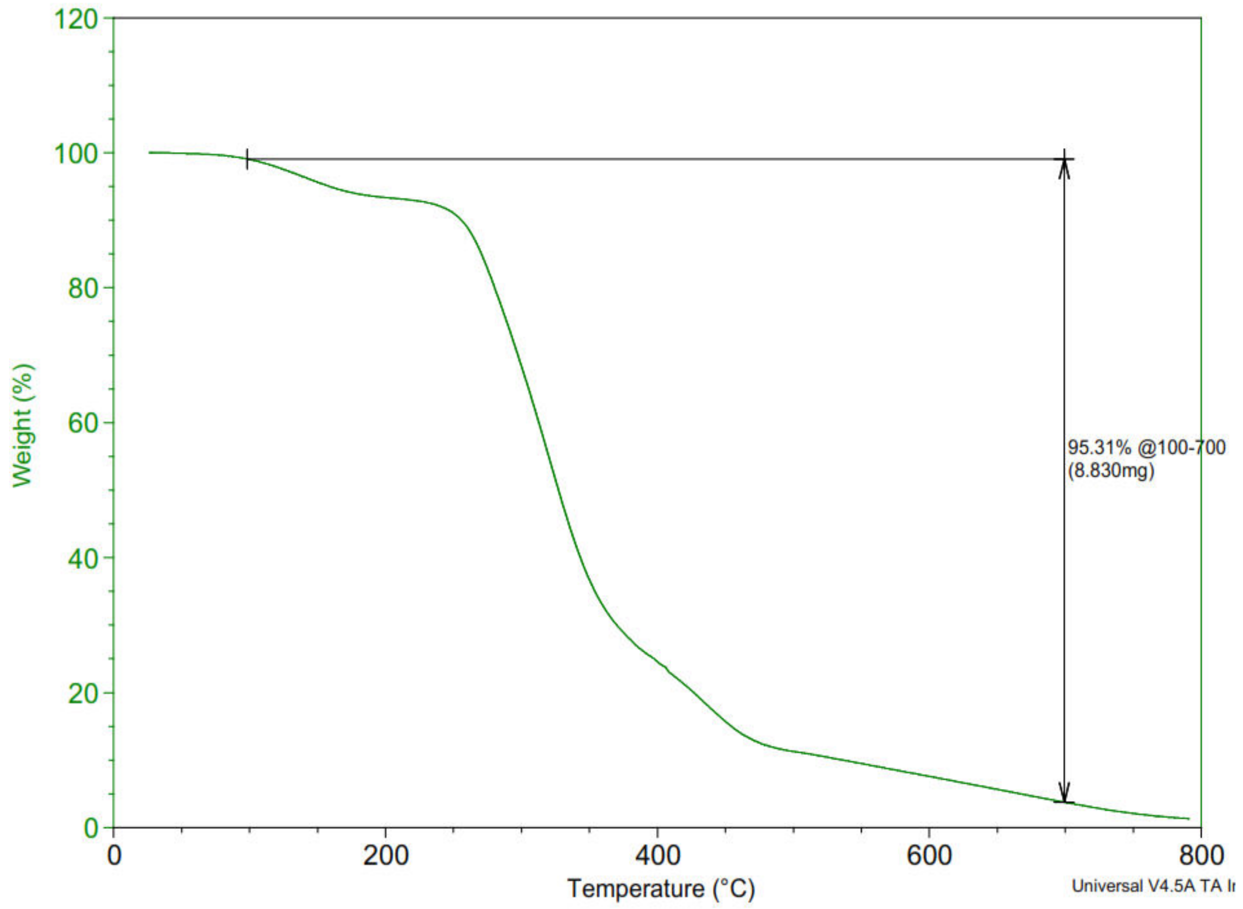
Appendix 4f: TGA thermogram of 70/30 PVA/gelatin blend film



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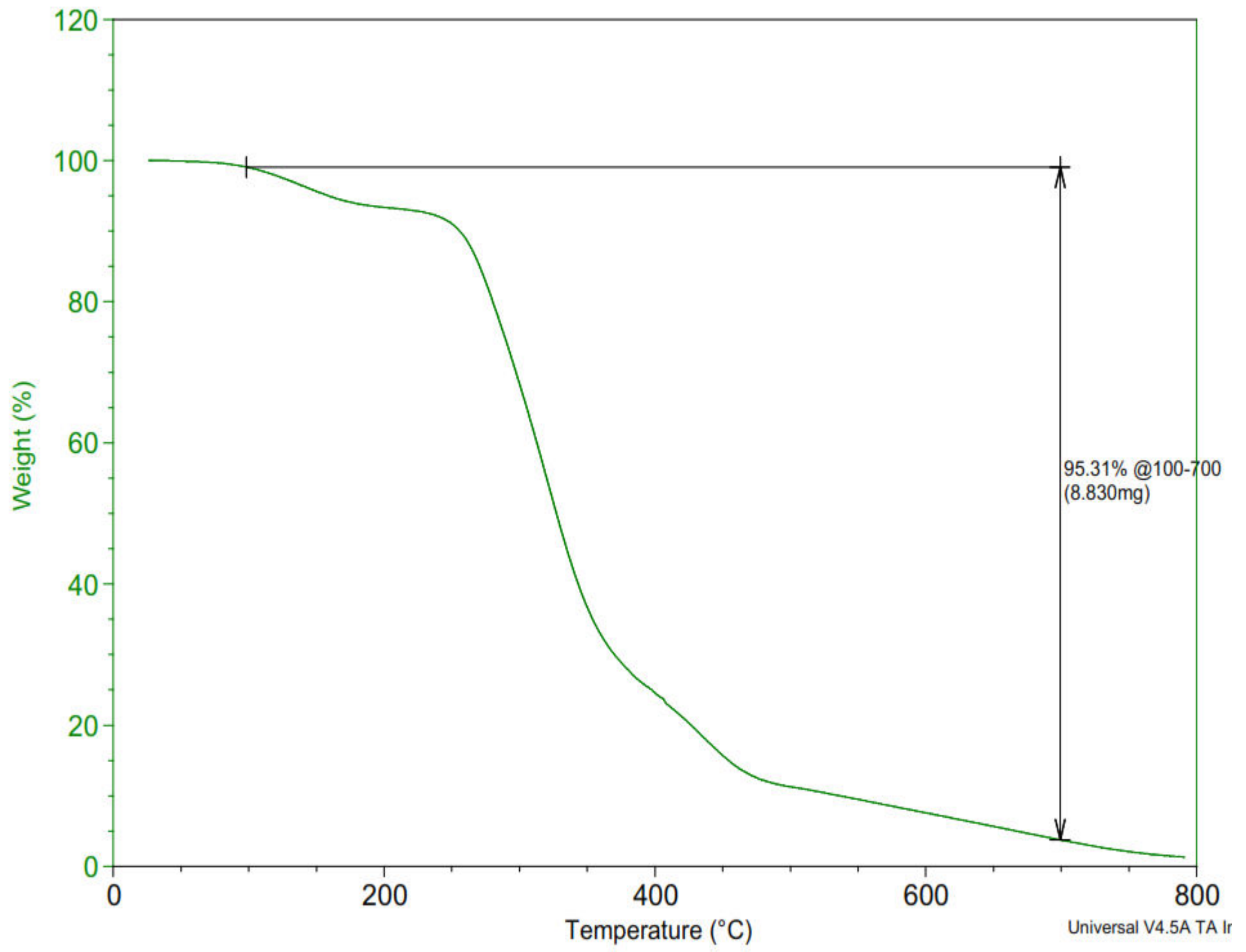
Appendix 4g: TGA thermogram for 90/10 PVA/gelatin blend films



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Appendix 5: Adsorption data

Appendix 5 a: Data for establishment of calibration curve for the adsorption of methylene blue by PVA/gelatin blend films

CONCENTRATION ($X10^{-5}M$)	ABSORBANCE
0	0
0.75	0.4389
1	0.536
1.25	0.65
2	1.129
2.5	1.463

Appendix 5 b: Determination of the effect of adsorbent dosage on the adsorption of methylene blue by PVA/gelatin blend film

Time (Min)	**Absorbance 0.25 g**	**Absorbance 0.5g**	**Absorbance 0.75g**	**Absorbance 1g**
0	1.463	1.463	1.463	1.463
20	0.882	0.925	0.816	0.796
40	0.837	0.846	0.782	0.732
60	0.812	0.812	0.768	0.707
80	0.798	0.802	0.766	0.71
100	0.795	0.821	0.762	0.706
120	0.79	0.798	0.76	0.716
140	0.767	0.738	0.75	0.712
160	0.766	0.72	0.758	0.733
180	0.779	0.705	0.737	0.73
200	0.784	0.698	0.74	0.75
220	0.776	0.685	0.74	0.753
240	0.768	0.702	0.771	0.756

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Appendix 5c: Determination of effect of initial dye concentration on adsorption by 0.25g of PVA/gelatin blend film.

Time	**Absorbance (conc 1.25 x 10^{-5})**	**Absorbance (conc 2.0 x 10^{-5})**
0	0.65	1.129
10	0.362	0.61
20	0.336	0.545
30	0.312	0.512
40	0.295	0.483
50	0.284	0.47
60	0.272	0.457
70	0.269	0.445
80	0.263	0.438
90	0.256	0.434
100	0.258	0.426
110	0.256	0.425
120	0.249	0.417
130	0.253	0.417
140	0.251	0.415
150	0.251	0.411
160	0.25	0.41
170	0.248	0.409
180	0.248	0.405
190	0.244	0.404
200	0.248	0.404
210		0.405
220	0.245	0.406
230		0.404
240	0.243	0.404

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Appendix 5 d: Determination of adsorption of 2.5 x 10^{-5}M methylene blue dye by 0.25g PVA/gelatin film

Time (Min)	Absorbance
0	1.463
20	0.882
40	0.837
60	0.812
80	0.798
100	0.795
120	0.79
140	0.767
160	0.766
180	0.779
200	0.784
220	0.776
240	0.768

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