

Evaluation of cellulose derivates for wound healing dressing

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ABSTRACT

The main of our work is to evaluate biomaterials, cellulose derivates and conclude if they would be suitable for wound healing dressing devices. Thence it follows the aim of this master thesis. It is necessary to determine properties of sorted cellulose derivates (OK CEL, HEC, CMC).

The evaluation is focused on temperature and concentration of cellulose solution influences on density, surface tension, contact angle and light absorbance. Work also determines influence of ionic concentration and pH of solution on particle size and zeta potential of cellulose solutions.

From our measurement we can conclude that density and surface tension of solutions depend on solution temperature and solution concentration. And the dependence for contact angle is fractional. The UV VIS spectroscopy determined that absorbance depends on solution concentration. Ions and pH of solution markedly influence only the light absorbance for OK CEL. The particle size varies by influence of varying pH of solutions or ionic concentration. The zeta potential is almost negative for determined pH range for our samples and that adding of ions does not influence this behaviour.

ABSTRAKT

Hlavním cílem naší práce je zhodnotit biomateriály, celulókové deriváty a zjistit zda tyto jsou vhodné pro aplikace používané k léčbě ran. Z toho vyplývá cíl naší práce, ten spočívá v zhodnocení vlastností vybraných celulókových derivátů (OK CEL, HEC, CMC).

Práce sleduje závislost hustoty, povrchového napětí, kontaktního úhlu smáčení a absorpce světla v UV oblasti na teplotě a koncentraci roztoků celulókových vzorků. Tato práce také sleduje vliv pH celulókových roztoků a koncentraci iontů na velikost koloidních částic a zeta potenciál.

Z měření vyplývá, že hustota a povrchové napětí roztoků závisí na teplotě i koncentraci roztoků derivátů. Teplota a koncentrace těchto roztoků nemá téměř žádný vliv na kontaktní úhly smáčení. Absorpce světla roste s rostoucí koncentrací roztoků. Koncentrace iontů a změna pH výrazně ovlivňují absorpci světla pouze v případě OK CEL. Velikost částic se mění v důsledku působení iontů i změnou pH roztoku. Zeta potenciál je negativní v celém zkoumaném rozsahu pH a přidavek iontů jej téměř neovlivní.

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INTRODUCTION

At the present time the importance of lowering the total costs per capita on post traumatic recovery is a driving force for intensive materials research oriented on surface coatings and different tissue regeneration vehicles. Both synthetic as well as the natural biopolymer systems are used. Our aim is the study of the polysaccharide based tissue recovery enhancement devices due to their both healing as well as anti-inflammatory effects. One of the possible applications seems to be cosmetics and wound healing dressings.

Skin is the human body's largest organ. Skin has many functions, which are essential for life. It helps to regulate body temperature, forms the natural barrier against the action of physical, chemical and bacterial agents from surrounding or it is the part of the system respiratory. The nerves in skin form the special end organs for the various sensations commonly grouped as the sense of touch. These noticed functions are only the best known. How is sketched out, the skin is very important for our living. On this account it must be insured against the reactive agents from surrounding environment and also against insult by serious emergency.

Unfortunately the skin is often serious injured during car accidents, burnt up by fire or electricity, etc. Many times these are very heavy injuries with far-reaching consequences. These are effected by problems during healing. The physical, chemical and bacterial agents influence process of healing, they make it slower and not so effective. The results are tedious treatment, cicatrix, burn scars, etc.

On that account an interest to make the healing process more effective is such enormous. A special tools and methods of the treatment are developed. Synthetic as well as natural polysaccharides are biocompatible with human skin. Both have positive effect on wound healing. These materials work on as a cover. They protect the wound against bacteria, physical and chemical processes from surrounding environment and help to speed up the healing process.

Our aim is to produced special plasters from biomaterials, polysaccharides. These plasters will be applied on the injury in a liquid form. By the medium of a cross-linking agent, liquid will form a very thin film. This film will protect the wound against aggravating factors from surrounding atmosphere. It will speed up the healing process and conserve the wound.

In a point of time the film will degrade. The wound will be clean and it will not be hurt again by painful whipping off the plasters as in the case of using the ordinary plasters. Then the wound will be prepared for other treatment or other film application.

Before the thin films will be produced, the evaluation of suitable biomaterial is required. The based material must be determined as well as its reaction on any influences.

This master thesis is focused on this material determination.

I. THEORETICAL PART

1 THE SKIN

1.1 Anatomy of human skin

Human skin is a uniquely engineered and the biggest organ of the human body.[1] It measures all around between 1,8 and 2 square meters and forms nearly 20% of human body weight. Thickness of the skin tissue is about one millimetre on most of the human body. But the skin thickness is much more higher on places, where is more wearing. These places are hands and feet. On feet the skin is thin four millimetres and the thinnest and the most sensitive skin is on the lips and the eyelids. [2]

It is the single place, where on the are one square centimetre 6 million cells, 100 sweat glands, 15 oil glands, 15 hairs, 100 centimetres of blood vessels and 5,000 sensory cells can be found.[3]

1.1.1 Types of skin cells

The skin is the collection of many cell types.

Keratinocytes

The most abundant cell type of the epidermis is the keratinocyte. Keratinocytes are stratified, squamous, epithelial cells, which comprise skin and mucosa, including oral, esophageal, and corneal, conjunctive and genital epithelia. Keratinocytes provide a barrier between the host and the environment. They prevent the entry of toxic substances from the environment and the loss of important constituents from the host. Keratinocytes differentiate as they progress from the basal layer to the skin surface. These cells produce keratin proteins that provide some of the rigidity of the outer layers of the skin. Keratinocytes also form the bulk of the material in hair follicles. Dandruff and hair are dead keratinocytes. Keratinocyte stem cells reside in the basal layer. These cells have a low rate of mitosis and give rise to a population of transient amplifying cells. Transient amplifying cells go through a limited number of divisions, differentiate and move up in the epidermis. The cells above the basal layer are known as the spinous layer. Under routine microscopy small bridges, resembling spines, can be seen between the keratinocytes, which represent intercellular adhesion complexes known as desmosomes. As the cells further differentiate, they synthesize keratohyaline granules, a prominent feature of cells in the granular layer. Proteins synthesized in the granular layer are important in the final

stages of epidermal differentiation and include profilagrin, loricrin, involucrin and cornifin. These molecules are important in the formation of the stratum corneum, the outer layer of the epidermis.

Fibroblasts

The dermis is produced largely by fibroblasts, which during embryonic development are part of the mesenchyme. The fibroblasts produce the collagens and elastins that make skin very durable, from within.

Melanocytes

Melanocytes are cells in low abundance in the basal layer in the epidermis that produces the pigment melanin. The pigment made in melanocytes is transferred to the cells of the hair or epidermis. In the inner layers of the epidermis the melanin granules are injected into the keratinocyte cells. There, the melanin granules form a protective cap over the outer part of keratinocyte nuclei.

In the stratum corneum, melanin granules are uniformly distributed to form a UV-absorbing blanket. Melanin absorbs harmful ultraviolet (UV) light before the UV radiation can reach the nucleus and protects the DNA in the nucleus from UV radiation damage. Then dividing cells are protected from mutations that might otherwise be caused by harmful UV light.

Differences in skin colour are due mostly to differences in the types and amount of pigment in our keratinocytes. Skin darkening (tanning) from sun exposure is caused by the movement of existing melanin into keratinocytes, and by increased production of melanin by the melanocyte. During embryonic development these cells migrate from the neural crest into the skin.

Langerhans Cells

These are star-shaped resident immune cells, macrophages. A macrophage is a cell that protects your body from injury or illness. Macrophages break up or destroy (phagocytise) the invading organisms. These macrophages process the invading organisms and present antigens to the T-lymphocytes. The T-lymphocytes are immune-system cells, which ultimately identify a substance as foreign or dangerous to the body.

Merkel's Cells

Only a few of these cells are present in skin; they are more numerous in the palms and soles (feet). These cells are probably sensory mechanical receptors that respond to stimulus, such as pressure or touch.[4]

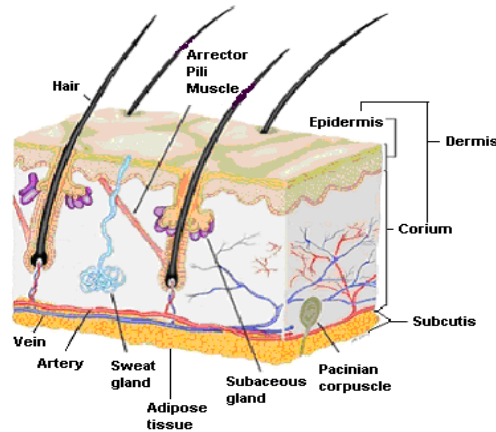


Fig. 1. The structure of human skin [3]

1.1.2 The skin layers

From the biological point of view, skin can be described as a layered structure. The two main layers are Subcutaneous fatty tissue and Dermis. Dermis consists of Epidermis and Coria. A boundary between these two basic layers forms membrane Lamina basalis.

The Epidermis

The epidermis is the topmost layer of the skin. It is the first barrier between the human body and the outside world. The total thickness of the epidermis is usually about 0.5 - 1 mm. It consists of three types of cells: keratinocytes, melanocytes, and langerhans cells.

Between the cells lie the epidermal lipids; the horny skin cells are thought of as bricks, and then lipids fill the spaces between the cells like mortar or cement. The lipid composition and moisture content of the epidermis change with increasing differentiation of the skin cells. Lipids are formed in the Golgi apparatus of the keratinocytes. Stored in these membrane-coated vacuoles, the Odland bodies, are the precursors of the skin-specific lipid barrier in the form of lamellar bilayer-lipid membranes. Through exocytosis, the contents of the Odland bodies are released into the extra cellular space. Only then are the epidermal lipids formed: as a horny cell cement these bilayer-lipid membranes lend the horny layer stability.

At the same time these intercellular lipid membranes are the decisive permeability barriers of the horny layer: Regulation of the water and fluid content is its most important function, as elasticity and firmness of the horny layer depend on moisture content.

Ceramides form the largest fraction with 40%. Also found are free fatty acids (25%) and cholesterol (25%) as well as cholesteryl sulphate. The ceramides are primarily responsible for the barrier forming and moisture-binding functions of the complex lipid mixture. Chemically, the ceramides are a group of sphingolipids. These are compounds formed from high-molecular weight alcohols, primarily sphingosine, and various fatty acids such as linoleic acid.

The epidermal lipids comprise 10 to 30 percent of the total volume of the horny layer (stratum corneum). That means they make up a 100 to 200 times more of the total volume of intercellular substance than that of other tissues. Accordingly, the horny layer makes an effective permeability barrier, which fulfils two important functions. It prevents invasion by certain substances such as micro-organisms, chemical substances and allergens. It minimizes trans epidermal water loss and thus protects the body from dehydration. The acid pH of the skin plays an essential role in the creation of the permeability barrier.

Trans epidermal water is the water diffusing to the skin surface. There it is removed from the body by means of evaporation. The less intact the horny skin layer is, the higher the loss rate.

The epidermis consists of many layers. Horny layer (stratum corneum), Clear layer (stratum lucidum), Granular layer (stratum granulosum), Prickle-cell layer (stratum spinosum) and Basal layer (stratum basale).

Cells in **basal layer** lie directly on the basal membrane that forms a definite border between the coria and epidermis. The basal cells acting as mother cells, by cell division, provide for the continuous regeneration of the skin. The daughter cells are slowly driven, by the active cell division, into the outer lying layers where they undergo various development stages. In the basal layer are also found the melanocytes, which are the pigment producing cells.

The basal cell layer is comprised mostly of keratinocytes which are either dividing or non-dividing. The cells contain keratin tonofibrils and are secured by hemidesmosomes to the basement membrane.

The ***stratum spinosum*** (spino = thorn, prickle/lat.), the prickle-cell layer, is above the basal layer. In it are, visible for the first time, the keratinosomes, membrane-bounded vacuoles, (Odland bodies). They contain the precursors of the epidermal lipids in the form of disk-like (lamellar) lipid bilayer membranes.

Above the prickle-cell layer is the ***stratum granulosum*** (granula = grain/Lat.), where the cornification (keratinisation) of the keratinocytes begins. It gets its name from its appearance, which is due to the presence of what are known as keratohyaline granules, a mixture of several smaller protein units.

The ***stratum lucidum*** is also called the clear layer or the translucent or transitional layer, as it is highly refractive. This layer is sometimes visible in thick skin; however, nuclei and other organelles are not visible. The cytoplasm (the amorphous area between the nucleus and the outer membrane of the cell) is mostly made of keratin filaments. The cells have been extremely flattened and are closely packed. The cell boundaries are no longer recognizable.

The ***horny layer*** also called stratum corneum (cornea = horny skin/Lat.). It is the uppermost layer of the epidermis. Between the cornified cells (corneocytes) lie the epidermal lipids. The horny layer - especially the bottom third - forms the permeability barrier, which is the skin's true barrier against exogeneous factors. This layer is made of flattened epithelial cells in multiple layers. These layers are called keratinised layers because of the build-up of the protein keratin in those cells. This layer of skin is, for the most part, dead. It is composed of cells that are almost pure protein. Dead cells from stratum corneum continuously slough off and are replaced by new ones coming from below. The skin completely renews itself every 3 - 5 weeks. Most mild peels work by partly removing the stratum corneum and thus speeding up skin renewal.

If horny skin layers are removed and with them the epidermal lipids, the skin becomes more permeable to water and other substances, including toxins and allergens.[4]

The Coria

The coria is the middle layer of the skin located between the epidermis and subcutaneous tissue. It is the thickest of the skin layers and comprises a tight, sturdy mesh of collagen and elastic fibres. Both collagen and elastin are critically important skin proteins: collagen is responsible for the structural support and elastin for the resilience of the skin. The key

type of cells in the coria is fibroblasts, which synthesize collagen, elastin and other structural molecules. The proper function of fibroblasts is highly important for overall skin health.

The coria also contains capillaries (tiny blood vessels) and lymph nodes (depots of immune cells). The former are important for oxygenating and nourishing the skin and for protecting it from invading micro-organisms.

Finally, the coria contains sebaceous glands, sweat glands, hair follicles as well as a relatively small number of nerve and muscle cells. Sebaceous glands, located around hair follicles, are of particular importance for skin health as they produce sebum, an oily protective substance that lubricates and waterproofs the skin and hair. When sebaceous gland produce too little sebum, as is common in older people, the skin becomes excessively dry and more prone to wrinkling. Conversely, overproduction or improper composition of sebum, as is common in adolescents, often leads to acne.

The coria is the layer responsible for the skin's structural integrity, elasticity and resilience. Wrinkles arise and develop in the coria. Therefore, an anti-wrinkle treatment has a chance to succeed only if it can reach as deep as the dermis. Typical collagen and elastin creams, for example, never reach the dermis because collagen and elastin molecules are too large to penetrate the epidermis.

The ***stratum reticulare*** (reticular = net-like/Lat.) makes up the lower part of the coria and shows a continuous transition to subcutis. The ***stratum papillare*** (papillae = protuberance/Lat.) is the upper layer, which is clearly demarcated from the epidermis by an undulated border. The wave-like structure increases the contact area with the epidermis, thus ensuring optimal nourishment of the deepest layer of the epidermis, the basal cells, by way of the blood vessels running through the papillae.

The main constituent of the dermis is the proteinous ***connective tissue*** made up of arc-shaped, elastic fibres and undulated, nearly inelastic collagen fibres. These are responsible for the high elasticity and tensile strength of the dermis.

Young collagen fibre, glycosaminoglycan, can bind large amounts of water and so determine the high intrinsic tension of young skin. As the skin ages, the interweaving of the collagen fibres increases and the water-binding capacity diminishes. The skin tends to wrinkle.

The space within the dermal meshwork contains a sort of "filling" made of long chains of sugar molecules (polysaccharides; poly = many, sacchar = sugar/Gk.). These are known as **glycos-aminoglycans** (also mucopolysaccharides). With the help of fibronectins a type of "glue", they bind to the proteinous connective tissue matrix to form proteoglycans, which can bind water molecules. This gel-like mass functions like a sponge. Under pressure it can expel the bound water and in a reverse process take it up again. This process is the probable route of nourishment for the dermis. Hyaluronic acid (hyalo = glass/Gk.) belongs to the group of glycosaminoglycans and so contributes to the water binding whole. Glycosaminoglycans are subject to a continuous waxing and waning. In contrast, the collagen fibres are only renewed when necessary, such as when injury is sustained.

Other constituents of the dermis are various types of cells such as **fibroblasts, mast cells** and other tissue cells, as well as a multitude of blood and lymph vessels, nerve endings, hot and cold receptors as well as tactile sensory organs.

Like the epidermis, the hair follicle manufactures a keratin structure, hair. These follicles are found everywhere on the body except for the palms and soles, though most of the hairs produced are fine, light hairs that, quite unlike the hair of the scalp, are scarcely visible to the naked eye. The sebaceous glands are attached to the hair follicles and through the follicles excrete an oily substance called sebum, which both lubricates and protects the skin. On most of the skin surface sebum appears constantly and imperceptibly, but in areas with a higher concentration of sebaceous glands, such as the face and back, there are wide variations in the amount of sebum produced.

There are two distinctive sweat-producing glands, the apocrine and the eccrine. The **apocrine gland** is best known for producing body odour but otherwise has no known physiological function and is apparently a holdover from times past. In the ear it forms a portion of what we see as earwax. It is also present under the arms, around the nipples and navel, and in the anal-genital area.

The **eccrine glands** are an advanced and extensive system of temperature control. Several million of these glands are distributed over the entire body, with the highest concentration in the palms, soles, forehead, and underarms.

Sweat, a dilute salt solution, evaporates from the skin's surface to cool the body. Excessive sweating without replacement of lost water can cause heat stroke. Eccrine glands sweat in

response to physical activity and hot environments, but emotional stress and eating spicy foods can also cause perspiring.

The dermis also regulates heat through a network of tiny blood vessels. In hot weather these vessels dilate to give off heat, causing the skin to flush. In cold weather, they constrict, conserving heat, causing pallor. The blood in these vessels nourishes the skin and provides protection for the cellular and fluid systems. Like the eccrine glands, blood vessels in the dermis are responsive to emotional stress, causing the colour changes mentioned previously.

Nerve endings in the dermis are the source of the body's sense of touch. They sense heat, cold, and pressure, providing both pain and pleasure.[4]

The Subcutaneous tissue

The subcutis (sub = under; cutis = skin/Lat.) refers to the fat tissue below the skin. It consists of spongy connective tissue interspersed with energy-storing adipocytes (fat cells). So the predominant type of cells in the subcutaneous tissue is fat cells. Subcutaneous fat acts as a shock absorber and heat insulator, protecting underlying tissues from cold and mechanical trauma. Interestingly, most mammals lack subcutaneous tissue because their fur serves as a shock absorber and heat insulator. Sweat glands and minute muscles attached to hair follicles originate in subcutaneous tissue.

The loss of subcutaneous tissue often occurs with age, leads to facial sag and accentuates wrinkles. A common procedure performed by dermatologists to counteract this process is to inject fat (collected elsewhere in the body) under the wrinkles on the face.

The subcutis is heavily interlaced with blood vessels, ensuring a quick delivery of stored nutrients as needed. The functions carried out by the subcutaneous fatty tissue, beside the storage of nutrients in the form of liquid fats, include the insulation of the body from cold and shock absorption. On the palms of the hand, the soles of the feet and the buttocks, fat padding serves almost exclusively for shock absorption.

The fat content of the subcutis is not the same in all body regions. Also men and women differ in the distribution of subcutaneous fat.[4]

The Epidermal Appendages

The epidermal appendages include the nails, hair and glands (glandulae cutis). They arise from invaginations of the epidermis into the dermis.

The **nails** are horny plates firmly attached to the nail bed. They are about 0.5 mm thick and consist of the front free edge - the body of the nail - and the nail matrix, which is embedded in the proximal nail fold.

The **hair** is divided into the protruding hair shaft and the hair root. The latter thickens at the end to become a bulb (bulbus), which together with the underlying dermal hair papillae are responsible for the nourishment, development and growth of hair. A dermal sheath of connective tissue surrounds the whole hair root and together these form a hair follicle. The sebaceous glands open into the infundibular part of the hair follicles.

The **glands** of the skin (glandulae cutis) include the sweat, scent, sebaceous and milk glands. The sebaceous glands are nearly always connected to hair follicles, which deliver the lipid-containing secretion to the surface through their funnel shaped openings. The size of the sebaceous gland and therefore the amount of sebum itself differ according to body region. An important influencing factor in sebaceous gland activity is the androgens.

Sebaceous and sweat glands are exocrine glands (exo = outer, external/Gk.), which means they deliver their secretion directly to a surface such as the skin. In the case of the sebaceous glands this occurs with complete disintegration of lipid-rich cells. They are continually replaced through division of the basal cells (holocrine glands). In the case of apocrine glands, like the mammary glands or the sweat glands of the axilla (underarm), only the outer parts of the cell body are lost with the secretion. The cells of the eccrine glands like the small sweat glands of the skin show no loss of cytoplasm after the secretion process.

Together with the sweat glands, the sebaceous glands deliver vital substances, which - along with the epidermal lipids - form the hydro lipid film.[4]

1.2 Function of human skin

The skin performs a complex role in human physiology. It serves as a barrier to the environment and some glands (sebaceous) may have weak anti-infective properties. This organ acts as a channel for communication to the outside world.

The most important property of the skin is that it contains our sense of touch. The sense of touch is difficult to investigate. All other senses have a definite key organ, which can be studied, but the skin is spread over the entire body and cannot easily be delimited.

Receptors (from the Latin word *receptor*, meaning “recorder”) located at the ends of nerve fibres are used to detect stimuli and convert them into neural impulses to be sent to the brain through the peripheral and central nervous systems. Receptors also are located in the internal organs, muscles and skeletal joints and can detect information such as the temperature of a cup of coffee or the roughness of sand paper. Although we “touch” with our epidermis, the sense of touch actually is recorded in the dermis and passed on to the central nervous system.

Other function is protection from water loss, friction wounds and impact wounds. The ability of the skin to store water depends in large part on the make-up of the barrier lipids in the horny layer. The protein structure of the horny cells, including the presence of the amino acid arginine, also influences the water-binding capacity of the skin. These substances that occur physiologically in the body and that retain water in the horny layer, are called natural moisturizing factors.

The skin contains specialized pigment cells to protect us from ultraviolet rays of the sun and produces vitamin D in the epidermal layer, when it is exposed to the sun's rays. It helps to regulate metabolism. [3], [4]

Another important function of the skin is that it helps the body keep a constant temperature. A person's average body temperature is 37 degrees of Celsius, but if it increases by 7 or 8 degrees and remains there for any of length of time, a person will almost certainly die. The body keep a generally constant temperature via a method of cooling known as perspiration. The main sources of body heat are the internal organs that work all the time, such as the heart and kidneys. The heat created by these organs is carried off by the blood and distributed evenly throughout the body. This is an efficient way to diffuse the heat at a slow pace.

Whole body equipped with tiny glands distributed all over our skin, about two million of them all together, the purpose of which is to bring water to the surface of the skin. On the surface this water is vaporized and heat is in this manner withdrawn from the body. The glands are *sweat glands* and the liquid produced is *sweat* or *perspiration*.

A sweat gland consists of a tiny-coiled tube, the main body of which is situated deep in the dermis. The tube straightens out finally and extends up through the epidermis. The tiny opening on the surface is a pore and is just barely visible to the naked eye. When man is working or playing hard and heat production is increased, the sweat glands accelerate their production of perspiration. This is also true when the temperature is unusually high. The rate of production may then outstrip the rate of evaporation, particularly if humidity is high, since the rate of evaporation declines with the rise in humidity. Perspiration will then collect on the body in visible drops and the body is conscious of sweating. The temperature determines how many sweat glands a person has.

The skin also has sometimes “very important” aesthetic and beauty qualities. [1], [4]

1.3 The wound healing process

Healing has many meanings. In its broadest context it can be used to describe the restoration of normality to any fractured situation. In its context as applied to wounds it should be taken to mean any process which acts, or is induced to act, to restore the integrity of the damaged tissues with replacement of lost tissues and repair of damaged tissues.

In the context of clinical practice in wound treatment ‘healing’ could be taken to have two main connotations. The first represents the innate properties of the systems of the body, which act to replace and repair damaged tissues. The second is the intervention of a third party who carries out an active process designed to accelerate or modify the natural process of healing for the benefit of the patient. This third party intervention is conventionally understood as the surgical intervention necessary to produce the conditions that will lead to the spontaneous healing of the wound or the application of biological or biocompatible materials, which act to close the wound and reach a stable acceptable end point to the healing process. [6]

The process by which tissue repair takes place is a continuous sequence of inflammation and repair, in which epithelial, endothelial, inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains, interact to restore a semblance of their usual discipline and having done so resume their normal function.

The process of wound repair differs little from one kind of tissue to another and is generally independent of the form of injury. Although the different elements of the wound

healing process occur in a continuous, integrated manner, it is convenient to divide the overall process into three overlapping phases and several natural components for descriptive purposes. [7]

1.3.1 Phases of wound repairing

Conditions for wound healing are more favourable when less tissue is damaged. The best prognosis for successful wound healing is found with smooth, closely abutting incision wounds without substantial tissue loss or presence of foreign bodies, in well-vascularised areas of the organism. In these cases, healing proceeds by *first intention* (per primam intentionem) as long as wound infections can be avoided.

Wound healing by *secondary intention* (per secundam intentionem) typically occurs in those situations when tissue defects have to be refilled or when pus formation interferes with the direct reassociation of the wound edges. Here, the wound surfaces do not lie closely adjacent to each other, but rather split, more or less away from each other. To close this wound, new tissue, the so-called granulation tissue, must be grown. The energy demand placed upon the organism on this occasion is incomparably greater than in the case of primary wound healing.

Wound healing has been arbitrarily divided into three phases: inflammation, proliferation and maturation. [7]

Inflammatory Phase (Day 0-5)

The healing response is initiated at the moment of injury. Surgical or traumatic wounds disrupt the tissue architecture and cause haemorrhage. Initially, blood fills the wound defect and exposure of this blood to collagen in the wound leads to platelet degranulation and activation of Hageman factor. This in turn sets into motion a number of biological amplification systems including the complement kinin and clotting cascades and plasmin generation. These serve to amplify the original injury signal and lead not only to clot formation, which unites the wound edges, but also to the accumulation of a number of mitogens and chemo attractants at the site of wounding.

Production of both kinins and prostaglandins leads to vasodilatation and increased small vessel permeability in the region of the wound. This results in oedema in the area of the injury and is responsible for the pain and swelling which occurs early after injury. Within 6h, circulating immune cells start to appear in the wound. Polymorphonuclear leucocytes

(PMN) are the first blood leucocytes to enter the wound site. They initially appear in the wound shortly after injury and subsequently their numbers increase steadily, peaking at 24-48h. Their main function appears to be phagocytosis of the bacteria, which have been introduced into the wound during injury. The presence of PMN in the wound following injury does not appear to be essential in order for normal wound healing to take place, with healing proceeding normally in their absence provided that bacterial contamination has not occurred. In the absence of infection, PMN have a relatively short life span in the wound and their numbers decrease rapidly after the third day.

The next cellular, immune element to enter the wound are macrophages. These cells are derived from circulating monocytes by a combination of migration and chemotaxis. They first appear within 48-96h post-injury and reach a peak around the third day post-injury. These macrophages have a much longer life span than the PMN and persist in the wound until healing is complete. Their appearance is followed somewhat later by T-lymphocytes, which appear in significant numbers around the fifth day post-injury, with peak numbers occurring about the seventh day after injury. In contrast to PMN, the presence and activation of both macrophages and lymphocytes in the wound is critical to the progress of the normal healing process. [7]

Proliferative Phase (Day 3-14)

In the absence of significant infection or contamination the inflammatory phase is short, and after the wound has been successfully cleared of devitalised and unwanted material it gives way to the proliferate phase of healing. The proliferate phase is characterized by the formation of granulation tissue in the wound. Granulation tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells, along with new capillaries embedded in a loose extra cellular matrix of collagen, fibronectin and hyaluronic acid. Fibroblasts first appear in significant numbers in the wound on the third day post-injury and achieve peak numbers around the seventh day. Fibroblasts are derived from local mesenchymal cells and attracted into the wound by a combination of cytokines produced initially by platelets and subsequently by macrophages and lymphocytes. Fibroblasts are the primary synthetic element in the repair process and are responsible for production of the majority of structural proteins used during tissue reconstruction. In particular, fibroblasts produce large quantities of collagen, a family of triple-chain

glycoproteins, which form the main constituent of the extra cellular wound matrix and which are ultimately responsible for imparting tensile strength to the scar. Collagen is first detected in the wound around the third day post-injury and thereafter the levels increase rapidly for approximately 3 weeks. It then continues to accumulate at a more gradual pace for up to 3 months post wounding. The collagen is initially deposited in a seemingly haphazard fashion and these individual collagen fibrils are subsequently reorganized, by cross-linking, into regularly aligned bundles oriented along the lines of stress in the healing wound. Fibroblasts are also responsible for the production of other matrix constituents including fibronectin, hyaluronic acid and the glycosaminoglycans. The process of fibroblast proliferation and synthetic activity is known as fibroplasia.

Revascularization of the wound proceeds in parallel with fibroplasia. Capillary buds sprout from blood vessels adjacent to the wound and extend into the wound space. On the second day post-injury, endothelial cells from the side of the venule closest to the wound begin to migrate in response to angiogenic stimuli. It appears that angiogenesis occurs by a combination of proliferation and migration. Putative mediators for endothelial cell growth and chemotaxis include cytokines produced by platelets, macrophages and lymphocytes in the wound, low oxygen tension, lactic acid and biogenic amines.

While these events are proceeding deep in the wound, restoration of epithelial integrity is taking place at the wound surface. Re-epithelialization of the wound begins within a couple of hours of the injury. Epithelial cells, arising from either the wound margins or residual dermal epithelial appendages within the wound bed, begin to migrate under the scab and over the underlying viable connective tissue. The epidermis immediately adjacent to the wound edge begins thickening within 24 h after injury. Marginal basal cells at the edge of the wound loose their firm attachment to the underlying dermis, enlarge and begin to migrate across the surface of the provisional matrix filling the wound. Fixed basal cells in a zone near the cut edge undergo a series of rapid mitotic divisions, and these cells appear to migrate by moving over one another in a leapfrog fashion until the defect is covered. Once the defect is bridged, the migrating epithelial cells loose their flattened appearance, become more columnar in shape and increase in mitotic activity. Layering of the epithelium is re-established and the surface layer eventually keratinized. Reepithelialization is complete in less than 48 h in the case of approximated incised wounds, but may take substantially longer in the case of larger wounds where there is a

significant tissue defect. If only the epithelium is damaged, such as occurs in split thickness skin graft donor sites, then repair consists primarily of re-epithelization with minimal or absent fibroplasia and granulation tissue formation. The stimuli for re-epithelization remain incompletely determined, but it appears that the process is mediated by a combination of loss of contact inhibition, exposure of constituents of the extra cellular matrix, particularly fibronectin and by cytokines produced by immune mononuclear cells. [7]

Maturation Phase (Day 7 to 1 Year)

Almost as soon as the extra cellular matrix is laid down, its reorganization begins. Initially, the extra cellular matrix is rich in fibronectin, which forms a provisional fibre network. This serves not only as a substratum for migration and ingrowths of cells, but also as a template for collagen deposition by fibroblasts. There are also significant quantities of hyaluronic acid and large molecular weight proteoglycans present, which contribute to the gel-like consistency of the extra cellular matrix and aid cellular infiltration. Collagen rapidly becomes the predominant constituent of the matrix. The initially randomly distributed collagen fibres become cross-linked and aggregated into fibrillar bundles, which gradually provide the healing tissue with increasing stiffness and tensile strength. After a 5-day lag period, which corresponds to early granulation tissue formation and a matrix largely composed of fibronectin and hyaluronic acid, there is a rapid increase in wound breaking strength due to collagen fibrogenesis. The subsequent rate of gain in wound tensile strength is slow, with the wound having gained only 20% of its final strength after 3 weeks. The final strength of the wound remains less than that of uninjured skin, with the maximum breaking strength of the scar reaching only 70% of that of the intact skin.

This gradual gain in tensile strength is due not only to continuing collagen deposition, but also to collagen remodelling, with formation of larger collagen bundles and alteration of intermolecular cross-linking. Collagen remodelling during scar formation is dependent on both continued collagen synthesis and collagen catabolism. The degradation of wound collagen is controlled by a variety of collagenase enzymes, and the balance of these opposing mechanisms determines the net increasing in wound collagen. The high rate of collagen synthesis within the wound returns to normal tissue levels by 6-12 months, while

active remodelling of the scar continues for up to 1 year after injury and indeed appears to continue at a very slow rate for life.

As remodelling progresses, there is a gradual reduction in the cellularity and vascularity of the reparative tissue, which results in the formation of a relatively avascular and acellular collagen scar. Grossly this can be observed as a reduction in erythema associated with the earlier scar and some reduction in the scar volume, resulting in a pale thin scar. [7]

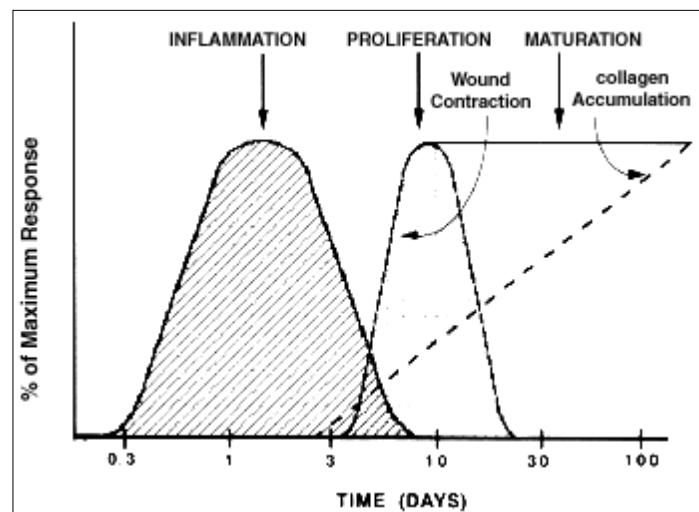


Fig. 2. Phases of wound repair. [7]

1.3.2 Scar Formation

As mentioned previously, the process of wound healing is essentially similar in all tissues and is relatively independent of the mode of injury; however, slight variation in the relative contribution of the different elements to the overall result may occur. The final product of the healing process is a scar. This relatively avascular and acellular mass of collagen serves to restore tissue continuity, strength and function. Delays in the healing process cause the prolonged presence of wounds, while abnormalities of the healing process may lead to abnormal scar formation.

Wound contraction is a further important element in the healing process and should be distinguished from contracture. Sharply incised wounds without significant tissue loss, approximated early after injury, heal rapidly without the need for significant reduction in the wound volume. Such wounds are described as having healed by primary intention. Large wounds, however, particularly those associated with significant tissue loss, heal by secondary intention, with granulation tissue gradually filling the defect and epithelization

proceeding slowly from the wound edges. Contraction of the wound edges can lead to a significant reduction in the quantity of granulation tissue required to fill the wound defect and a reduction in the area requiring reepithelization, with a consequent reduction in scar volume. Contraction is only undesirable where it leads to unacceptable tissue distortion and an unsatisfactory cosmetic result. Initially following injury, where the wound edges are not approximated, there is a slight retraction of the wound edges due to the release of normal elastic tension in the skin, with a resultant increase in wound volume. The wound area starts to decrease rapidly from the third day onwards. While this is due in part to reepithelization, the main reason is an inward movement of the uninjured skin edges. Wound contraction usually begins around the fifth day post wounding and is complete by 12-15 days after wounding. Fibroblasts within the wound appear to be responsible for providing the force for this contractile activity. The wound contraction occurs as a result of an interaction between fibroblast locomotion and collagen reorganization. The contraction is thought to be mediated via the attachment of collagen fibrils to cell surface receptors, with the resulting tractional forces generated by cell motility bringing the attached collagen fibrils closer together and eventually compacting them. [7]

So successful completion of wound healing may not always yield the desired clinical result, particularly where the final cosmetic appearance of the scar is of primary importance.

1.3.3 Types of wound healing treatments

Major injuries resulting in extensive damage to the skin necessitate immediate coverage to aid repair and regeneration to restore normal skin function. However skin injuries are traumatic events, which are seldom accompanied by complete structural and functional restoration of the original tissue. There is a substantial expense by way of health care and loss of personal freedom, quality of life and productivity.

Research efforts to deal with skin loss, have focused on three principal directions. Firstly, towards improvement of wound healing by factors which speed up the process and reduce scarring. Secondly, the design and development of skin substitutes as functional equivalents of auto-graft skin. Thirdly, to identify cues that induce the skin to heal by regeneration rather than repair (scarring).

Management of wounds due to injury, burns, surgery, other types of trauma and non-healing ulcers poses a great challenge to the surgeons, plastic surgeons and all others

associated in this mission. The type of wounds (acute or chronic), extent and depth of lesion and factors like nutritional status, age, systemic disease and medication that is specific to the individual, complicate the healing process. [9]

If the wound is by secondary intention, it means that the wound surface do not lie closely, there is not any possibility how can the organisms renew the skin itself. In this case it is necessary to use third part. It could be by a specific surgical invention which helps to make the conditions that will lead to the spontaneous healing of the wound or the application of biological or biocompatible material which acts to close the wound and helps to speed up healing process or makes cover to protect the wound against influences from surrounding environment.

Recently, there are various types of inventions, which try to speed up or make the healing process more effective.

The treatment of large or deep burn injuries due to thermal, chemical or electrical means is very tedious and it brings many complications. One possibility of the treatment, which is widely used, is the **transplantation** of the skin from different healthy body parts, especially from the hips and the backside of the legs. But it is also production of new scars and it brings a new endurance for the human body, the skin and whole organisms. So it gives questions and big interest to find new ways of treatment.

Another possibility is to apply the **artificial skin**. This process is called grafting by an ideal. This type of treatment consists of the several steps. At the beginning the wound is debrided followed by chemotherapy and subsequent preparation of the wound bed for grafting by providing an optimal environment for healing with an ideal wound dressing, which can act as a bacterial barrier and provide adequate gaseous exchange and remain non-antigenic. After excision of injury and stabilization of the patient followed grafting. It is application of skin substitutes: split thickness auto-grafts, allo-grafts (homografts), xenografts (heterografts) or their refined versions.

Research and development in the field of wound dressing has resulted in the fabrication and production of a wide variety of synthetic and biological dressings.

In the case of the **biological dressing** the major technological achievement leads to the production of cultured keratinocytes, which were suitable for grafting in burns and acute and chronic wounds. The whole invention embody in culturing epithelium from an allogenic donor. This technique is found to be beneficial in burns, leg ulcers and split

thickness skin graft donor sites. Keratinocytes isolated from older donors exhibited slow growth in culture and also had a reduced culture life span and colony forming ability, but keratinocytes derived from neonatal foreskin provided a potent stimulus to healing in a variety of wounds. Due to accelerated proliferation of newborn epidermal cells, the preparation of allo-graft is faster and easier. The allo-graft made in culture, however, provides a temporary wound covering, but release of multiple cytokines, promotes permanent re-epithelialization by quiescent host keratinocytes. [9]

The cultured epidermal grafts alone did not yield satisfactory results, because of the mechanical instability of these extremely delicate entities. They were fragile and blistering of the epidermis took place. So the development of auto or allo grafts were improved by the delivery system, which also serves as a substrate for cell growth. For example biological delivery system is based on Laser skin. It is a thin transparent membrane, which is a semi-synthetic derivative of hyaluronic acid, a naturally occurring molecule and a major component of human skin. The hyaluronic acid is completely esterified to give a benzyl derivative. Micro holes are drilled with a laser across the membrane. The seeded keratinocytes are found to populate the pores and grow out as colonies both above and beneath the membrane. [9]

When dermis as well as epidermis is lost in full thickness burns or wounds are too large, composite replacement of cultured epidermis and dermis is a logical objective of creation of a dermal wound bed onto which keratinocytes may be applied .

Dermal tissue is proven to contribute to durability, elasticity and cosmetic appearance of normal skin. In deep partial thickness and full thickness burns, dermis is repaired by formation of granulation tissue, which later gives rise to scar tissue. There is also overwhelming evidence that dermal substitutes should approximate live human dermis as close as possible.

The dermal equivalent must be stable i.e. contraction and reorganization of the lattice by dermal fibroblasts must be complete and the matrix must be resistant to collagenase hydrolysis present in the wound site. On the basis of this conclusions acellular dermal allo-graft with the basement membrane is produced. The allo-derm has been proved to maintain typical dermal structure and biochemical features, which are distinct from granulation tissue. [9]

The biological dressing includes allo-graft, heterograft from pigs and amniotic membrane, reconstituted collagen films and sponges from bovine and other sources. Allo-grafts from cadavers carry the risk of viral contaminants and are potentially antigenic, hence they will be rejected if immunosuppressants are not administered.

Understanding of the disadvantages of allo-grafts helps to develop collagen dressing. The major applications of collagen based dressings are collagen films, collagen gels and collagen sponges. Though the biological dressings reduce healing time and improve the quality of healing among other properties, they serve only as temporary covers and for ultimate healing the patient's own skin has to be grafted.

The ***synthetic dressings*** based on polymeric sheets, these are suitable for superficial wounds and not for larger and deeper wounds.

Further improvements gave rise to composite synthetic dressings composed of two or more layers of polymers. The outer layer is designed for durability and the inner layer for maximum adherence and elasticity. These composites are temporary and do not help in extensive and deep burn injuries.

In burns or injuries with limited skin loss, a split thickness auto-graft enables faster wound closure, minimizing other complications at the wound site is applied. [9]

Efficacy of a skin substitute depends on biocompatibility, uniformity in composition when prepared in vitro, control of biochemical and biological composition and standardized evaluation before specialized purposes. Plasticity of preparation procedure and of composition provides an opportunity to modify the cellular biopolymer and biomolecular components of skin substitutes to promote epithelialization and vascularization to minimize rejection and inflammatory response in wounds. The above information can readily be translated to other tissues and organ substitutes.⁹

These methods described formerly are demanding and sometimes very expensive. They are very ticklish on the culture environmental quality, the purification of synthesis of the special substances or the exactness of the donor skin cells lock and separation. This is reflected on the high costs of the surgical material and the whole healing treatment.

Here is big interest to find new options in the wound dressing materials which shall reduce the treatments costs. The attention starts to focus on biomaterials, which could be extracted from plants. Biomaterials extracted from plants do not carry the risk of viral contamination. The big interest is focused on biomaterial based on polysaccharides. The most discussed

polysaccharides is cellulose and its derivatives. Cellulose and some of its derivatives are biocompatible with human body and human body fluids. They could be biochemically controlled. Cellulose and cellulose derivatives are widely used in cosmetics and health care. So cellulose brings free field for discovery and evaluation for these applications.

2 CELLULOSE AND CELLULOSE DERIVATES

2.1 Cellulose structure

Cellulose is the primary constituent of wood, paper, and cotton. Cellulose is found in plants as micro fibrils (2-20 nm diameter and 100 - 40 000 nm long). These form the structurally strong framework in the cell walls. Cellulose is mostly prepared from wood pulp. [19]

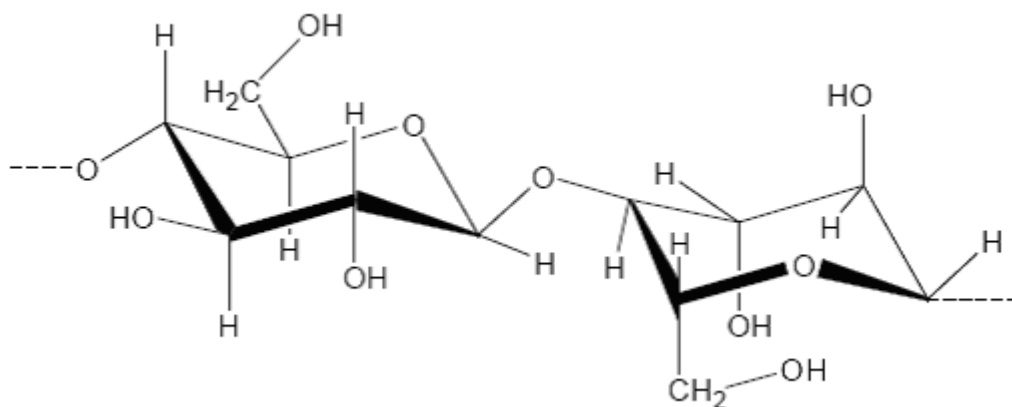


Fig. 3. The structure unit of cellulose. [24]

Cellulose is carbohydrate made up of glucose units. It is a linear polymer of β -(1 $\rightarrow$ 4)-D-glucopyranose units in 4C_1 conformation. Strong intermolecular and intramolecular hydrogen bonds link the chains. The fully equatorial conformation of β -linked glucopyranose residues stabilizes the chair structure, minimizing its flexibility. [22]

Cellulose is an insoluble molecule consisting of between 2000 - 14000 residues with some preparations being somewhat shorter. It forms crystals where intra-molecular and intra-strand hydrogen bonds holds the network flat allowing the more hydrophobic ribbon faces to stack. Each residue is oriented 180° to the next with the chain synthesized two residues at a time. Although individual strand of cellulose are intrinsically no less hydrophilic this tendency to form crystals utilizing extensive intra- and intermolecular hydrogen bonding makes it completely insoluble in normal aqueous solution. It is thought that water molecules catalyse the formation of the natural cellulose crystals by helping to align the chains through hydrogen-bonded bridging. [19]

Part of a cellulose preparation is amorphous between these crystalline sections. The overall structure is of aggregated particles with extensive pores capable of holding relatively large amounts of water by capillarity.

The natural crystal is made up from metastable Cellulose I with all the cellulose strands parallel and no inter-sheet hydrogen bonding. This cellulose I (*i.e.* natural cellulose) contains two coexisting phases cellulose I_α (triclinic) and cellulose I_β (monoclinic) in varying proportions dependent on its origin; I_α being found more in algae and bacteria whilst I_β is the major form in higher plants. Cellulose I_α and cellulose I_β have the same fibre repeat distance (1.043 nm for the repeat dimmer interior to the crystal, 1.029 nm on the surface) but differing displacements of the sheets relative to one another. The neighbouring sheets of cellulose I_α (consisting of identical chains with two alternating glucose conformers) are regularly displaced from each other in the same direction whereas sheets of cellulose I_β (consisting of two conformational distinct alternating sheets, each made up of crystallographically identical glucose conformers) are staggered. Cellulose I_α and cellulose I_β are interconverted by bending during microfibril formation and metastable cellulose I_α converts to cellulose I_β on annealing. If it can be recrystallised (*e.g.* from base or CS_2) cellulose I gives the thermodynamically more stable Cellulose II structure with an antiparallel arrangement of the strands and some inter-sheet hydrogen-bonding. Cellulose II contains two different types of anhydroglucose (A and B) with different backbone structures; the chains consisting of -A-A- or -B-B- repeat units. Cellulose III is formed from cellulose mercerised in ammonia and is similar cellulose II but with the chains parallel, as in cellulose I_α and cellulose I_β . [19]

2.1.1 Cellulose functionality

Cellulose has many uses as an anticake agent, emulsifier, stabilizer, dispersing agent, thickener, and gelling agent but these are generally subsidiary to its most important use of holding on to water. Water cannot penetrate crystalline cellulose but dry amorphous cellulose absorbs water becoming soft and flexible. Some of this water is non-freezing but most is simply trapped. Less water is bound by direct hydrogen bonding if the cellulose has high crystallinity but some fibrous cellulose products can hold on to considerable water in pores and its typically straw-like cavities; water holding ability correlating well with the amorphous (surface area effect) and void fraction (the porosity). [19]

2.1.2 Cellulose derivates

About a third of the world's production of purified cellulose is used as the base material for a number of water-soluble derivatives with pre-designed and wide-ranging properties dependent on groups involved and the degree of derivatization. Derivatizing cellulose interferes with the orderly crystal-forming hydrogen bonding, described above, so that even hydrophobic derivatives may increase the apparent solubility in water.

As mentioned before cellulose has hydrophilic character and it is not water soluble. Therefore for production of a water soluble polymer with cellulose chain as a backbone is necessary to break the bonds between the chains. It was investigated that sodium hydroxide penetrate into the cellulose and helps to produce cellulose derivatives.

Cellulose itself has three reactive hydroxyl groups, which react with inorganic and organic compounds. The result is the creation of cellulose inorganic and organic esters, ethers or alkoxides.

Cellulose derivatives are generally in granular or powder form. Pure grades are practically odourless and tasteless. Physical and chemical properties depends on their chemical structure, degree of substitution, physical form, their modifications and also on technology and other processing.

Cellulose derivatives are used in production of films and foils. The films and foils are based materials for extrusion or injection production. They are added to paints. They are used as thickeners in water-based adhesives, binders and glazes components in ceramics, binder agents for foundry chemicals, tobacco, plant protections and insecticides. Derivates are also based material for production of fibres. [21]

2.1.3 Oxycellulose (OK CEL)

When the cellulose fibres react with the oxidising agents in acid, alkaline or neutral solutions, the development of other characteristic properties associated with oxycellulose formation is caused. The type of these characteristics depends upon the conditions of oxidation. Oxycellulose prepared in neutral or acid solution may, in general, be described as the reducing type. This type has high reducing power, low methylene blue value and excessive loss of weight on boiling with alkalis. Non-reducing type of oxycellulose is prepared by reaction in alkali solution. This acidic type is characterized by great absorptive power for methylene blue and capacity for fixing alkalis, but with little reducing power.

Different types of oxycellulose are produced when it is blended with different types of oxidising agents: bromine-water, permanganate, nitric acid, dichromate, periodate, ozone and chromic acid oxycellulose. The oxidation could be caused by nitric acid, the action of hydrogen peroxide, ozone or the acting of electrolytes.[23]

2.1.4 Hydroxyethylcellulose (HEC)

The structure of HEC

HEC belongs to non ionic derivatives. It arises by the instrumentality of ethylene oxide or ethylene chlorohydrine on alkali cellulose. Not only the free cellulose hydroxy groups react during this reaction but also hydroxy group from hydroxyethyl's group, it results in different heterogeneity of HEC.

Thereinafter the general equation for HEC preparation, where R is anhydroglucose radical is written down. [22]

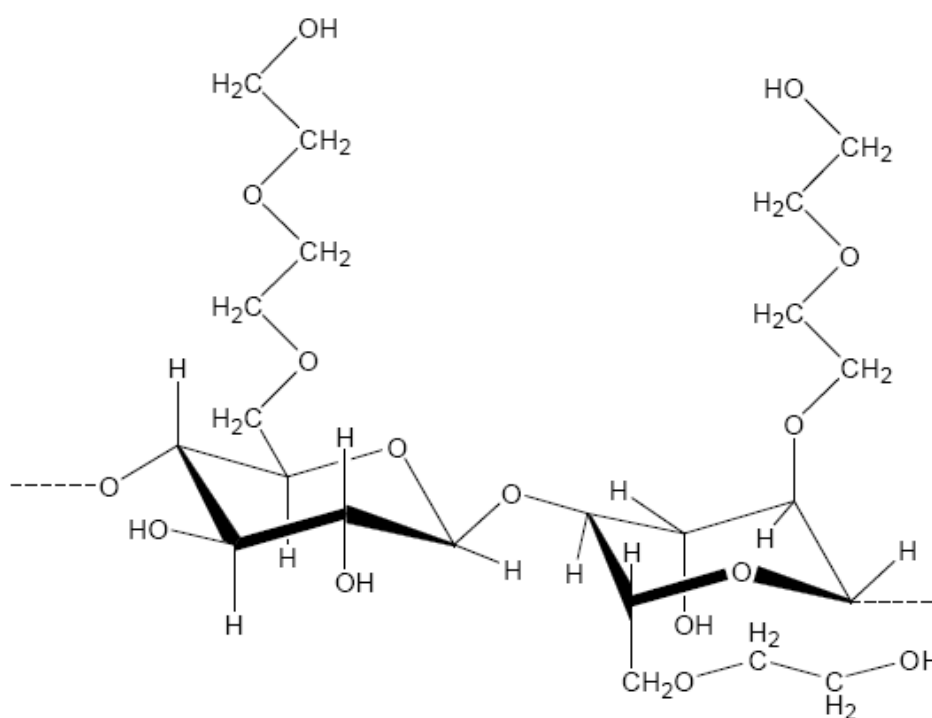
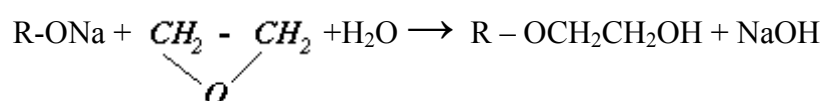
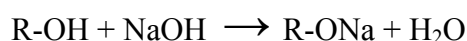


Fig. 4. This picture shows the structure unit of HEC. [24]

HEC properties

HEC with lower degree of substitution disperse in NaOH solutions. HEC with degree of substitution higher than 1,4 disperse in water.

HEC is completely soluble in dimethylamine and diethylformamide solutions. It is soluble in ethylene, propylene glycol and N-acetylene ethanolamin partially. It is insoluble in other organic solution. Adding of strong acid performs degreasing of viscosity of aqueous solutions of HEC.

HEC films prepared from aqueous solution are stable up to 100 °C. They are soluble in water but insoluble in organic solvent. Some ingredients as glycol, oil or glyoxal modulate elasticity and other properties of these films. [21]

Applications of HEC

HEC is used as thickener, stabilizator, and binder and for production of films and fibres. As thickener is widely used in paints, textile, paper, cosmetics and pharmaceutical industry. In the production of water based coatings is known as latex or emulsions paint. These could have interior and exterior applications, varying from matt to high sheen. HEC acts as a dispersant protective colloid, water retainer, thickener and film former.

A high volume of low-molecular-weight HEC has role as a protective colloid in the emulsion polymerisation of vinyl acetate and vinyl acetate copolymers. HEC improves their particle size distribution, mechanical and electrolyte stability. Very important application is in building and construction products. Here it is used to improve rheology, slump resistance of plasters and tile fixes, speed of solubility. HEC is also used as binder for graphite in pencil manufacture, battery manufacture, and ceramics and band tobacco. [21]

2.1.5 Carboxymethylcellulose (CMC)

The structure of CMC

This is most important derivative of cellulose. CMC is a semisynthetic water-soluble polymer in which CH_2COOH groups are substituted on the glucose units of the cellulose chain through an ether linkage.

A general equation for CMC preparation, where R is anhydroglucose radical is written down on this place. [22]

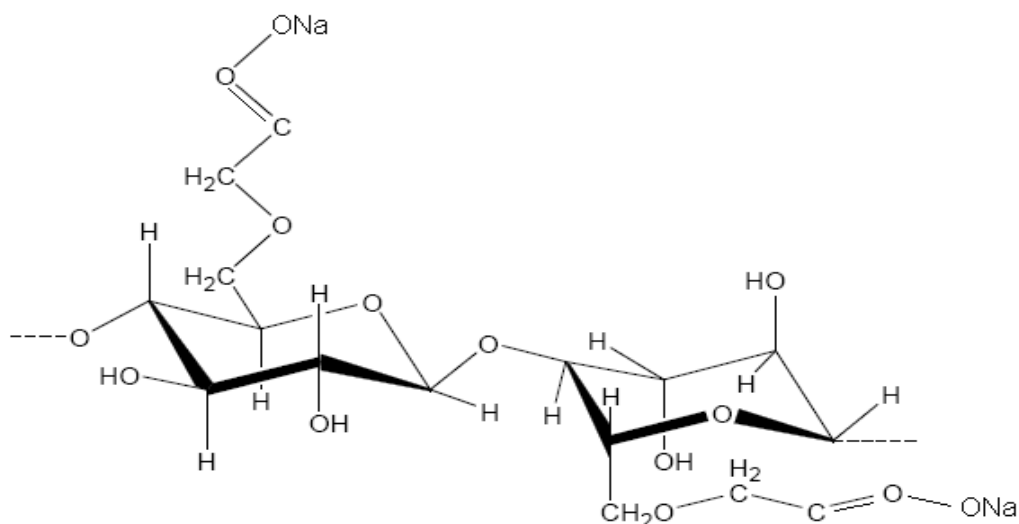
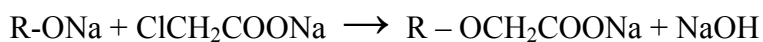
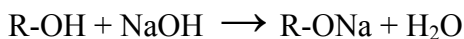


Fig. 5. The structure unit of CMC is presented on this figure.[24]

It is mixture of sodium carboxymethylcellulose, NaCl, sodium glycolate, NaOH, sodium chloracetate and water. CMC as this blend is used for technical purpose. It is Crude or technical CMC with purity about 80%. For special technical purposes partially refined forms are used. One form is refined with purity 97%. Others are pure or food grade, where CMC-Na content is minimum 99,5%. [21]

CMC properties

CMC is sodium carboxymethylcellulose. It is white or yellowish, flaky material with salty taste and without any odour. It is odourless, non-toxic, water-soluble powder or granules, pH 6,5-8,0, stable in pH range 2 – 10. It is insoluble in organic liquids, but very good soluble in water. It is linear electrolyte and shows out thixotropic behaviour.

The structure, concentration of solution, temperature, pH and properties of solvent influence its viscosity. The viscosity has maximum in pH range 6 – 9, and viscosity rapidly degrees abroad this interval. The viscosity decreases with increasing temperature. This

process is reversible till 50 °C. It reacts with heavy-metal salts to form films that are insoluble in water, transparent and unaffected by organic materials. [21]

Applications of CMC

Detergents this is major application of crude CMC. It is soil agent in powder detergents. CMC adsorbs on the fibre's surface to prevent redeposit ion of soils. Pure CMC is ingredient of food products, where it acts as water binder, thickener, suspending agent and emulsion stabilizers. Main applications are in convenience foods, baked foods, vending products as soft drinks and soups, ice creams and dessert products and also in dry pet foods. They are widely used in textile manufacturing (sizing). Crude CMC is also used for paper coating and boarding paper to lower porosity. It is used as an additive to prove mechanical strength and act as retention aid for fillers. Refined CMC has application as surface sizing and viscosity controller in paper, wallpaper-coating formulations and also as coating agent for carbonless copying papers. Crude CMC is used as thickener of drilling muds. CMC is ingredient for paints emulsion. Wide usage of CMC is in pharmaceuticals, cosmetics and toiletries (toothpastes, lotions, creams) for its thickening and binding properties. [21]

Skin surface wettability is an important factor of the skin protective functions as ecosystem preservation, smoothness and resiliency, barrier to chemicals. As mentioned before the very important function of human skin is to minimize water loss and entry of foreign matter. Its outermost layer, the statum cornem, creates this barrier property. Skin surface is hydrophobic but become more hydrophilic in the presence of skin surface lipids and that free fatty acids are responsible for this change. There is no relationship between the lipid level and surface tension. However, skin surface lipids (sebum) give the skin surface hydrophilic character.

It is very important to know skin surface properties, but it is also very important to investigate material, which could be applied on its surface. This could be done by the surface tension and contact angle measurements. [25]

3 THEORETICAL BACKGROUND FOR MEASUREMENT

3.1 The Surface Tension

3.1.1 The theoretical background

Liquids sometimes form drops, and sometimes spread over a surface and wet it. It all comes down to the forces between atoms or molecules, and the forces between them. The force between two atoms or molecules is generally repulsive if they are pushed too close together. The force increases so strongly as the distance is reduced. [28]

An equal attractive force in all directions balances the interactions of a molecule in the bulk of a liquid.

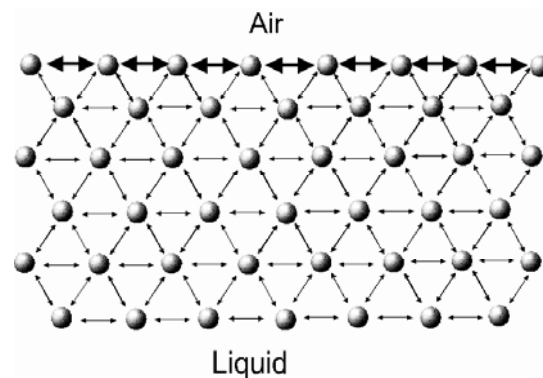


Fig. 6. The net represents the presence of free energy at the surface. [27]

The free energy at the surface is called surface free energy and can be quantified as a measurement of a force per unit length or may be thought of as the work required generating a unit area of surface. The usual unit for surface tension is the Newton per meter (N/m), although the dyne/cm is used on the surface.

This excess energy exists at the interface of two fluids. If one of the fluids is the vapour phase of a liquid being tested the measurement is referred to as surface tension. If the surface investigated is the interface of two liquids the measurement is referred to as interfacial tension. In either case the more dense fluid is referred to herein as the 'heavy phase' and the less dense fluid is referred to as the 'light phase'. Solids also may be described to have a surface free energy at their interfaces

Polar liquids, such as water, have strong intermolecular interactions and thus high surface tensions. In nature water is the liquid with the highest surface tension. At 20 degrees Celsius the surface tension of water is 72.8 mN/m.

Any factor, which decreases the strength of this interaction, will lower surface tension. Thus an increase in the temperature of this system will lower surface tension. Any contamination, especially by surfactants, will lower surface tension.

3.1.2 How to measure the surface tension of liquids

Surface tension is measured with tensiometers. Most tensiometers are instruments, which simply measure force. The most common ways to measure the surface tension of liquids are:

Wilhelmy Plate Method is standard method, where the thin plate (perimeter about 40 mm) is lowered to the surface of a liquid and the downward force directed to the plate is measured. Surface tension is directly the force divided by the perimeter of the plate.

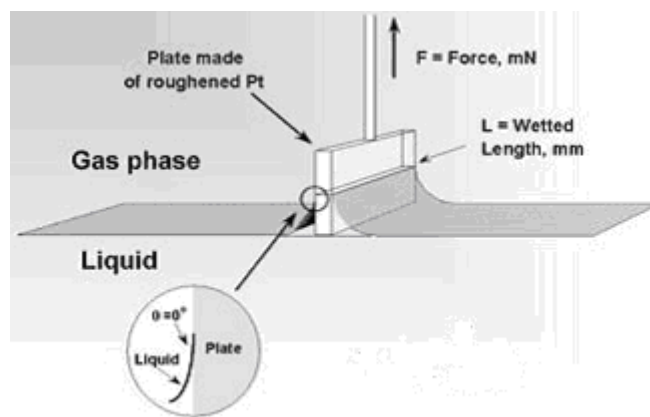


Fig. 7. This is the schematic picture of Wilhelmy Plate Method. [30]

The tension is calculated using the following equation:

$$\sigma = \frac{F}{L \cdot \cos \Theta} \quad (1)$$

σ surface or interfacial tension (N/m)

F force acting on the balance (N)

L wetted length (m)

θ contact angle ($^{\circ}$)

The plate is made of roughened platinum and is optimally wetted so that the contact angle is virtually 0° . This means that the term $\cos \theta$ has a value of approximately 1, so that only the measured force and the length of the plate need to be taken into consideration. Correction calculations are not necessary with the plate method.

DuNouy Ring Method is the method where a ring (standard perimeter about 60 mm) is pulled through the liquid/air interface and the maximum downward force directed to the ring is measured. [29]

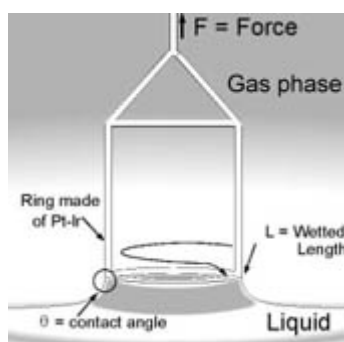


Fig. 8. The schematic picture of DuNouy Ring Method. [30]

Maximum Pull Force Method is method a combination of the above two methods. A thin rod is immersed into the sample and then pulled out and the maximum force is measured.

In **Pendant Drop Method** the liquid is injected from a needle so that it forms a drop on the tip of the needle. The drop is then optically observed and the surface tension is calculated from the shape of the drop.

Spinning Drop Method is a method when the surface tension values are so low that tensiometers, which use the above methods are not able to make the measurement.

A small drop of a sample is injected inside a thin tube with another liquid. The tube is then spinned with a high speed and the interfacial surface tension is calculated from the angular speed of the tube and the shape of the drop.

In **Bubble Pressure Method** a capillary tube is immersed in a sample liquid. A constant flow of gas is merged through the tube thus forming bubbles into the sample liquid. The pressure needed to form a bubble is measured. The surface tension of the sample is calculated from the pressure difference inside and outside the bubble and the radius of the bubble. [29]

The data from surface tension measurement could be used to calculate the activation energy. The equation (2) is used for this calculation.

$$\sigma = E_s + T(d\sigma / dT) \quad (2)$$

σ surface tension (N/m)

E_s surface free energy (N/m)

T absolut temperature (K)

3.2 The contact angle

3.2.1 The theoretical background

The Contact Angle is a quantative measure of the wetting of a solid by a liquid. It is defined geometrically as the angle formed by a liquid at the three-phase boundary where a liquid, gas and solid intersect.

If the angle is less than 90 degrees the liquid is said to wet the solid. If it is greater than 90 degrees it is said to be non-wetting. A zero contact angle represents complete wetting. [30]

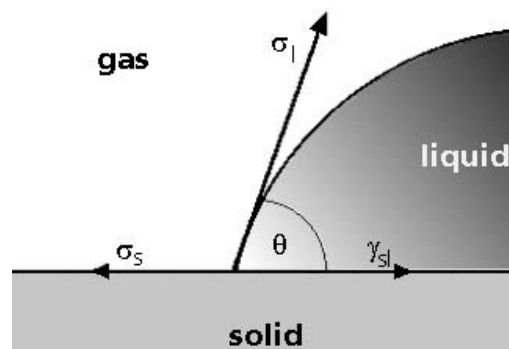


Fig. 9. Contact angle formation on a solid surface according to Young. [30]

The contact angle is a function of the liquid's surface tension and the surface 'free' energy of the substrate. At the heart of theory of Contact Angles, lies the Young equation:

$$\cos \Theta = \frac{\varphi_{sv} - \varphi_{sl}}{\varphi_{lv}} \quad (3)$$

φ surface tension, between the corresponding interfaces (N/m)

s solid

l liquid

v vapour

However, this equation is only valid for finite contact angles in case of mechanical equilibrium, so it does not apply when spreading takes place. Thus to encourage wetting γ_{sl} , γ_{lv} should be made as small as possible. The surfactant adsorbs to both the liquid-solid and liquid-vapour interfaces, lowering those interfacial tensions.

There are several methods, how the contact angle could be calculated: the Zisman method, Equation of State, the method according to Fowkes, the Owens, Wendt, Rabel and Kaelble method, WU method and the Schultz method. [30]

3.2.2 How the contact angle is measured

The contact angle can be measured on static drops, **static** measurement. The drop is produced before the measurement and has a constant volume during the measurement. The contact angle can be also measured on dynamic drops, **dynamic** measurement. The contact angle is measured while the drop is being enlarged or reduced; the boundary surface is being constantly newly formed during the measurement. Contact angles measured on increasing drops are known as “**advancing** angles”; those measured on reducing drops as “**receding** angles”.

Static contact angles

The drop is produced before the measurement and has a constant volume during the measurement, but interactions at the boundary surface can cause the contact angle to change considerably with time. Depending on the type of time effect the contact angle can increase or decrease with time.

These interactions could be evaporation of the liquid, migration of surfactants from the solid surface to the liquid surface, substances dissolved in the drop migrating to the surface (or in the opposite direction), chemical reactions between the solid and liquid or the solid being dissolved or swollen by the liquid.

Dynamic contact angle

Dynamic contact angles describe the processes at the liquid/solid boundary during the increase in volume (advancing angle) or decrease in volume (receding angle) of the drop, i.e. during the wetting and dewetting processes.

Advancing angle

It is a result of the wetting process. Advancing angles always simulate a fresh surface for the contact angle; this is formed immediately after the creation of the contact between the liquid and the surface. As a result, advancing angles are normally measured in order to determine the surface free energy of a solid.

Receding angle

During the measurement of the receding angle the contact angle is measured as the size of the drop is being reduced, i.e. as the surface is being de-wetted. By using the difference between the advancing and the receding angles it is possible to make statements about the roughness of the solid or chemical inhomogeneties. [30]

Methods of evaluating the drop shape

The basis for the determination of the contact angle is the image of the drop on the drop surface. In the program the actual drop shape and the contact line (baseline) with the solid are first determined by the analysis. There exist several methods how the contact angle could be calculated. For example tangent method, height-width method, circle fitting method or Young-Laplace method.

Measuring the surface tension of pendant drops

A drop of liquid is hanging from a syringe needle then it will assume a characteristic shape and size from which the surface tension can be determined. A requirement is that the drop is in hydro mechanical equilibrium. [30]

3.3 The measurement of density

There exist many ways how the density of liquids can be obtained. One way is to measure weight of the particular standard with known weight and volume. The standard is weighted

in air and in liquid. Then the value of density is calculated by using modified Archimedes' equation:

$$F_b = \rho \cdot g \cdot V \quad (4)$$

F_b buoyancy force (N)

g gravitation acceleration (m^2/s)

V standard volume (m^3)

ρ density of liquid (g/m^3)

Aqueous polymer coatings are often applied to polymer films. Polymers commonly used in the pharmaceutical industry are acrylic polymers and cellulose derivatives. Aqueous film coatings often contain some electrolytes, organic acids or other additives, which help to control and improve some properties of the films. An important issue in this aqueous coating process is dispersion stability. An unstable dispersion results in aggregation of the colloidal particles, thereby affecting the film coating process. Additions of some electrolytes or acids into the disperse system for the aqueous film coating affects its colloidal stability. Interaction between pairs of colloidal particles changes by altering the salt concentration and pH. How the addition of electrolytes or the change of the pH affects the stability of colloidal dispersions is necessary to evaluate. UV Vis spectroscopy determined the molecule structure. Together with Zeta potential and particle size measurements it investigates the stability of colloidal dispersions, influence of the addition of electrolytes or the change of the pH. [32]

3.4 The UV VIS spectroscopy

3.4.1 The theoretical background

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorbance measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer

electrons to higher energy levels, and UV-Vis spectroscopy is usually applied to molecules or inorganic complexes in solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. Measuring the absorbance at some wavelength and applying **Beer-Lambert Law** can determine the concentration of an analyte in solution. [36]

$$A = \epsilon.b.c \quad (5)$$

ϵ absorbtivity, constant of proportionality (m^2/mol)

b path length (m)

c concentration of absorbing species (mol/m^3)

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule.

When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level. [35]

Absorbing species containing π , σ , and n electrons

Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (*chromophores*) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band.

Possible *electronic* transitions of π , σ , and n electrons are;

$\sigma \rightarrow \sigma^*$ Transitions

An electron in a bonding σ orbital is excited to the corresponding antibonding orbital. The energy required is large.

$n \rightarrow \sigma^*$ Transitions

Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of $n \rightarrow \sigma^*$ transitions. These transitions usually need less energy than $\sigma \rightarrow \sigma^*$

transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm. The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in the UV region is small.

$n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ Transitions

Most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π^* excited state. This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm). These transitions need an unsaturated group in the molecule to provide the π electrons. [35]

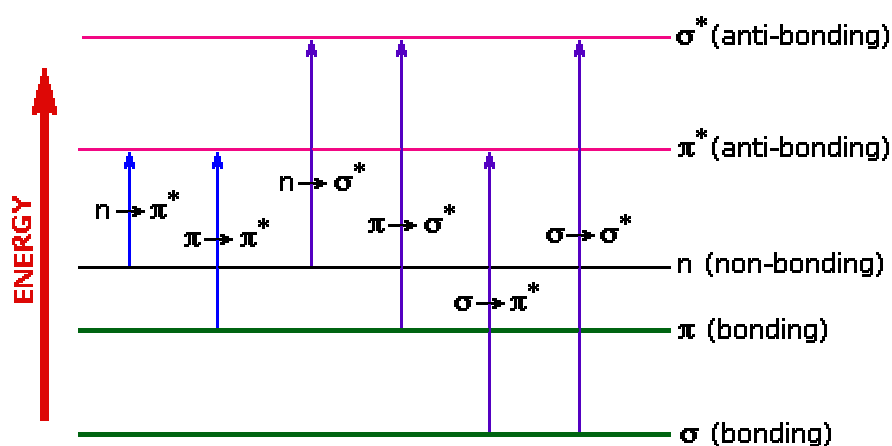


Fig. 10. Figure shows the types of electronic transitions.[37]

Experimental measurements are usually made in terms of transmittance, which is defined as :

$$T = \frac{\varphi}{\varphi_0} \quad (6)$$

T transmittance (1)

φ luminous flux density of light after it passes through the sample (lm)

φ_0 luminous flux density of incident light (lm)

The relation between absorbance and transmittance is:

$$A = -\log(T) = -\log\left(\frac{\varphi}{\varphi_0}\right) \quad (7)$$

A..... absorbance (1)

[36]

During absorption of light through an optical medium other processes that decrease the transmittance such as surface reflectance and scattering are running.

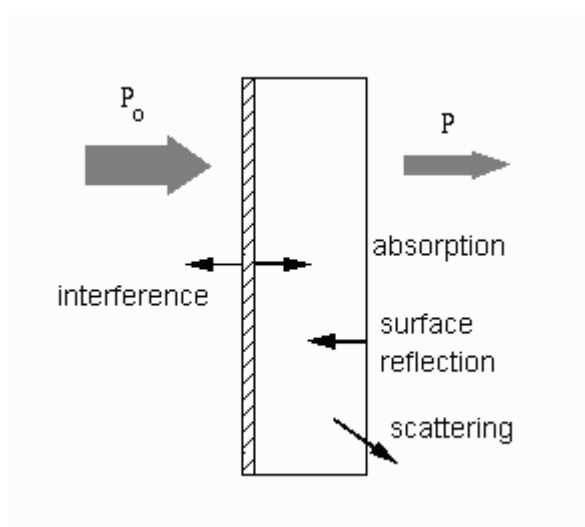


Fig. 11. This picture illustrates light absorption, transmittance, reflectance and scattering in optical medium. [36]

In analytical applications we often want to measure the concentration of an analyte independent of the effects of reflection, solvent absorption or other interferences and Lambert-Beer's equation is used for its calculation.

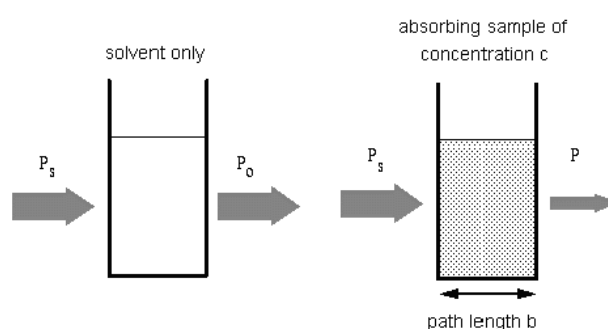


Fig. 12. The beam of light goes through solvent and concentrated sample. [36]

UV-Vis spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows, and filters. This more qualitative application usually requires recording at least a portion of the UV-Vis spectrum for characterization of the optical or electronic properties of materials.

3.4.2 How the UV VIS spectroscopy works

The UV/Vis spectrometer consists of a light source, a sample compartment, a diode-array detector, and a data acquisition computer. The sample compartment is between the light source and the detector. Typically liquid samples are used, contained in a transparent "cuvette" or "cell". The sample compartment in our spectrometer is made for 1 cm cuvettes. The spectrometer compares the amount of ultraviolet and visible light transmitted by a sample placed in the sample compartment with amount of light transmitted by cuvette with solvent.

3.5 The distribution of particle size

3.5.1 The theoretical background

The light dispersion in colloidal solution is known as Tyndall's effect. If the solution is diluted enough the total intensity of dispersion light is a sum of particular intensities dispergated by the colloidal particles. The intensity-diffused light on the particle surfaces depends on surface size, which reflects the beam of light. This surface size is equal to second power of effective diameter of the particle. Rayleigh described this theory. Than the equation for unpolarized light is:

$$R_g = \frac{\varphi_g \cdot r_s^2}{\varphi_0} = \frac{\pi^2 \cdot \alpha^2}{2 \cdot \varepsilon_0^2 \cdot \lambda^4} (1 + \cos^2 \vartheta) \quad (8)$$

R_v Rayleigh's diameter (m^2)

r distance from diffusive element (m)
 ν angle of light reflection (°)
 ϕ_0 luminous flux density of diffused light (lm)
 ϕ luminous flux density of incident light (lm)
 λ wavelength (nm)
 ϵ electric permittivity (F/m)
 α electric polarizability ($C^2 m^2/J$)
 [38]

3.5.2 Measurement principles

Particle size diameter could be measured by microscopic method, but this is very tedious and not so effective. In modern technology the better choice is to scatter sample by beam of light and recalculated it by computer. Particle size measurements could be provided on an instrument which measure Zeta potential. This measurement uses dynamic method of light diffusion and principle of detection the luminous flux density of diffused light, when autocollimator notes natural form of autocorrelation function of density of diffused light. Theory of diffused light is used to calculate a diffusivity, which is used to find natural particle diameter.

3.6 Zeta potential

3.6.1 The theoretical background

The presence of the particles in dispersion phase where is wide phase interfaces is the cause of the specific electric properties and originates electrokinetic effects. These effects are connected with specific charge distribution in phase boundary nearness during electrolyte absorption. There are two main forces acting between pairs of colloidal particles in an electrolyte solution: electrostatic double layer interaction and the van der Waals interactions. Specific behaviour during electrolyte absorption is banded with the specific character of electrostatic (Coulomb's) interactions.

Model of electrical double layer is used for understanding the ionic environment of the charged particle. The negatively charged particle polarizes the positives ions to offset its charge. These ions are called counter-ions. The ions charged just as the colloidal particle

are called co-ions. The layer of counter-ions line up to surround the surface of the colloidal particle. The layer is formed by electrostatic attraction. This layer is called Stern.[40]

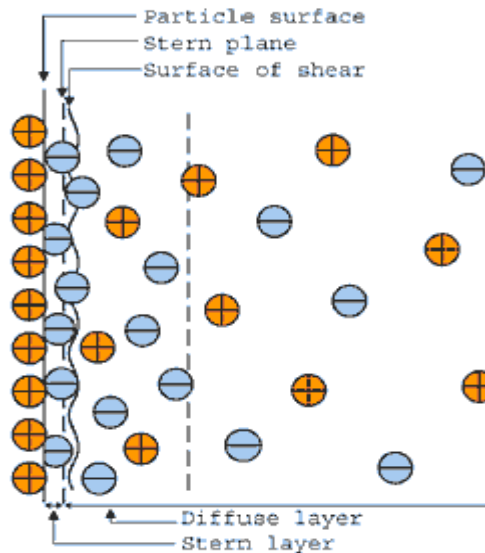


Fig. 13. On this picture the structure of the double layer is presented.[40]

Above the double layer is an outer region where a balance of electrostatic forces and random thermal motion determines the ion distribution. This layer is called diffusive. The potential in this region, therefore, decays with increasing distance from the surface until, at sufficient distance, it reaches the bulk solution value, conventionally taken to be zero. The zeta potential is the value at the surface of shear. The concentration of counter ions is higher near the surface and it decreases steadily to the concentration in the bulk liquid. Likewise, the co-ions are depleted near the surface, and their concentration increase until it reaches the level in the bulk liquid.

There are many different ways, how the charge can arise: ionisation of surface groups, adsorption of ions such as surfactants, multivalent ions or electrolytes, unequal dissolution of the ions comprising the surface molecules.

The charge changes with solution conditions such as: pH, ionic strength, addition of adsorbed ions, addition of surface active agents, addition of reagents that chemically bond to the surface and others. [40], [45]

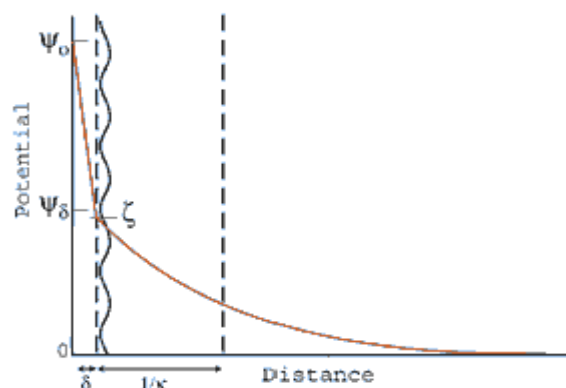


Fig. 14. The decay of the potential with increasing distance from particle surface is shown on this picture. [41]

Zeta potential

The electrical double layer arises to neutralize the charged colloidal particle and causes the potential difference between the particle and the outlying point in disperse solution. This potential difference is measured in millivolts (mV) and it is called surface potential (Nerst's potential).

Zeta potential is therefore a function of the surface charge of the particle, any adsorbed layer at the interface and the nature and composition of the surrounding medium in which the particle is suspended. It is usually, but not necessarily, of the same sign as the potential actually at the particle surface but, unlike the surface potential, the zeta potential is readily accessible by experiment. Moreover, because it reflects the effective charge on the particles and is therefore related to the electrostatic repulsion between them, zeta potential has proven to be extremely relevant to the practical study and control of colloidal stability and flocculation processes.

The zeta potential is the electrostatic potential at the surface of shear. It is not the potential at the surface of the particle. The surface of shear extends out from the particle surface. This surface separates the electrokinetic unit from the bulk of the solution. This kinetic unit moves.

Every shift of equalization of distributed charges is caused by electrokinetic effect. Mechanical or electrostatic forces damage spread of electrical double layer. When solid phase makes mechanical movement with regard to liquid phase, it forms measuring potential difference. This is called *streaming potential* or *sedimentation potential*. Electrostatic forces effect movement of solid or liquid phase. This effect is called *electrophoresis* or *electroosmosis*. [40], [45]

3.6.2 The Zeta potential measurement

Particles dispersed in a liquid often have a charge on the surface. If an electric field in the liquid is applied in the liquid then these particles will move towards either the positive or the negative pole of the applied field. The direction they select is a clear indication of the sign of the carried charge. The velocity with which they translate is proportional to the magnitude of the charge. Thus the velocity and the direction of the particles under the influence of a known electric field and then calculate mobility and zeta potential from this information.

The Zeta potential system has a laser beam which passes through the sample in a cell, which carries two electrodes to provide the electric field. Special electrode is put into the cuvette. Electrode consists of two parallel metal desks, which make the electric field in cuvette. The zeta potential measurement is based on electrophoretic and Doppler effects. The light which is scattered by the particles is Doppler shifted because the scattering particles are moving in the electric field and its velocity.

The principle of measurement is based on Doppler's effect. There frequency of incident light moves because of the direction and the velocity of the moving particle. The frequency of the laser is of the order of 10^{14} Hz and practical frequency shifts are of the order of a 10^2 Hz. It is very difficult to measure so small changes. The principle of optical heterodyning is used. A portion of the beam is split off and then re-combined with the scattered beam. [40], [45]

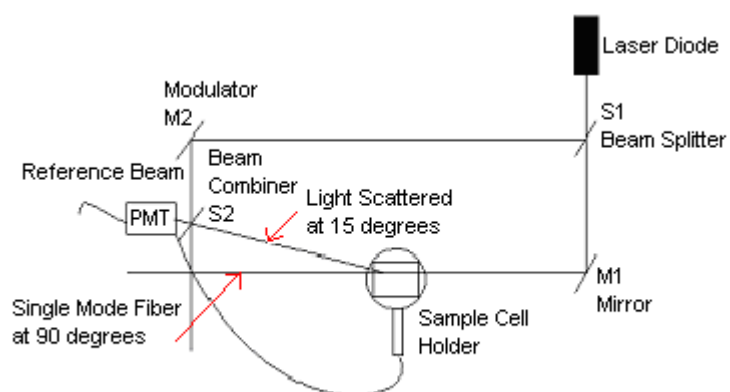


Fig. 15. This is the schema of the Zeta potential instrument with heterodyne optics. [40]

4 AIM OF THE MASTER THESIS

The aim of the master thesis is to evaluate cellulose derivates and make conclusions about their other evaluation and usage for wound healing dressing.

Their investigated properties are the density, the surface tension and the contact angle. Study of the temperature and concentration influence on these properties will be also investigated.

Next determination will be the measurement of the particle size distribution and the zeta potential. Particle size measurement will be investigated by the dynamic light diffusion. Influence of pH and ionic concentration on particle size and zeta potential will be determined.

The obtained data will be elaborated into the tables and the diagrams. The results will be concluded at discussion and conclusion.

II. EXPERIMENTAL PART

5 MATERIAL AND METHODS

5.1 Materials

5.1.1 Oxycellulose (OK CEL)

It was obtained from Synthesia a.s. from Pardubice, Czech Republic.

5.1.2 Hydroxyethylcellulose (HEC)

HEC was obtained from Hercules, Aqualon Division, in Alizay, France. The type 250LR, lot A-0749 with the technical name NATROSOL[®] was used. Molecular weight of Natrosol 250LR is about $9 \cdot 10^4$.

Natrosol non-ionic, water-soluble polymer is derived from cellulose. It is used in water-based paints for its action as a rheology modifier, stabilizer and/or suspending agent. And also plays a significant role in controlling rheology before, during, and after application.

Physically, HEC is a white, free-flowing powder that dissolves readily in hot or cold water. Available in a variety of types and grades, it can be used to produce solutions with a wide range of viscosity and pseudoplastic rheology. Upon drying, solutions of Natrosol form clear, gel-free films.

Natrosol is compatible with pigments, binders, surfactants, and preservatives used in water-based paint systems.

Natrosol is a hydroxyethyl ether of cellulose. Each anhydroglucose unit in the cellulose molecule has three reactive hydroxyl groups. The number of hydroxyl groups substituted in any reaction is known as the degree of substitution (DS). Theoretically, all three hydroxyls can be substituted. The product from such a reaction would have a DS of 3.

Type 250 LR has low molecular weight and it is treated to provide fast dispersion and easier dissolving in water.

Solutions of Natrosol are non-Newtonian in flow, since they change in viscosity with rate of shear. When Natrosol is dissolved in water, the viscosity of the aqueous solution increases rapidly with concentration. The viscosity-concentration relationship is nearly linear. The viscosity of solutions of Natrosol changes with temperature, increasing when cooled, decreasing when warmed. The viscosity of the solution is 10 Pa.s at 25 °C. [42]

5.1.3 Carboxymethylcellulose (CMC)

CMC was obtained from Hercules, Aqualon Division, in Alizay, France. Refined CMC type 7L, lot 20075 with the technical name BLANOSE[®] was used. Molecular weight of Natrosol 250LR is about $3 \cdot 10^5$.

It is purified sodium carboxymethylcellulose with minimum purity of 98%. Physically it is a light cream to white free-flowing powder or granulate, which readily dissolves in water to form clear, viscous solutions. It dissolves rapidly in cold or hot water. Refined CMC acts as a thickener, rheology control agent, binder, stabilizer, protective colloid, suspending agent and water retention aid forms films that are resistant to oils, greases and organic solvents and it is physiologically inert.

Refined CMC is cellulose ether, produced by reacting alkali cellulose with sodium monochloroacetate. The reaction is controlled in such a way that a predetermined substitution by sodium carboxymethyl groups ($-\text{CH}_2\text{COONa}$) is obtained. This is expressed as degree of substitution (DS) or the average number of sodium carboxymethyl groups per anhydroglucose unit on the cellulose chain. The substitution necessary to achieve optimum solubility and other desirable physical properties is much less than three, for commonly used type 7, as is used for measurements in this work is substitution in range 0,65-0,90.

Varying the length of the basic cellulose chain controls the viscosity of CMC. Viscosity of type 7L is in range 25 - 50 mPa.s at 25 °C. Viscosity is the most important property of CMC aqueous solutions. These solutions have a wide tolerance for fluctuations of pH, for monovalent salts, and for water-miscible organic solvents such as methanol, ethanol and glycerine. The pH of solutions is in range 5,0 - 8,5.

The CMC aqueous solutions had pseudoplastic behaviour and thixotropy character. Generally high viscosity CMC will show a stronger thixotropy, or a lower speed of return. Both properties depend on degree of substitution of the CMC.

Refined CMC is readily soluble in water at all temperatures right up to boiling point. Under normal conditions, the effect of temperature upon viscosity is reversible and rising or lowering the temperature has no permanent effect on the viscosity characteristics of the solution. However, long periods of heating at high temperatures may degrade the sodium carboxymethylcellulose and reduce its viscosity permanently.

CMC solutions are more resistant to microbiological attack than most other water-soluble gums they are not immune. If solutions have to be stored for any prolonged period, it is important to destroy or immobilize any cellulose (hydrolytic, viscosity destroying enzymes), which may have been introduced by microbial action. [43], [44]

5.2 Samples preparation

Every measurement, in this master thesis, was obtained on 2 and 4 weight % samples of each derivate (OK CEL, HEC, CMC).

Weighted amount of cellulose derivate powder was diluted in given amount of distil water. The cellulose powder was wetted by small volume of ethanol before dilution. Whole mixture was stirred till it forms clear, viscous solution. In case of OK CEL the solution was not clear but it was slightly yellowish and opalescent.

In case of particle size and zeta potential measurement prepared 2 and 4 weigh % samples were measured.

Measurement of pH influence was obtained on prepared samples. This prepared samples were poured into the beaker where the special electrode for ph measurement was dipped. Than the small amount of HCl or NaOH was adding subsequently under gently stirring to change pH of the solution. This was done till the desired pH was not obtained.

Measurement of ionic concentration influence was obtained on samples diluted by ($1 \cdot 10^{-2}$, $1 \cdot 10^{-3}$, $1 \cdot 10^{-4}$, $1 \cdot 10^{-5}$, $1 \cdot 10^{-6}$ mol/l) concentrated solutions of NaCl. The dilution was in ration 1:1. 2 ml of sample were poured into the cuvette, than 2 ml of NaCl solution were added into this cuvette and whole volume was shaken very gently.

UV-VIS measurement used the same samples as zeta potential and particle size measurements. But it was also measured on 1 weight % sample, this one was made by diluting 2 weight % sample by distil water in ratio 1:1 and whole mixture was shaken.

5.3 Chemicals

- sodium hydroxide (NaOH), solution ($c = 1 \text{ mol/l}$)
- hydrochloric acid (HCl), solution ($c = 1 \text{ mol/l}$)
- sodium chloride (NaCl), solutions
($c = 1 \cdot 10^{-2}, 1 \cdot 10^{-3}, 1 \cdot 10^{-4}, 1 \cdot 10^{-5}, 1 \cdot 10^{-6} \text{ mol/l}$)

5.4 Instruments

5.4.1 Tensiometr K12 (Krüss)

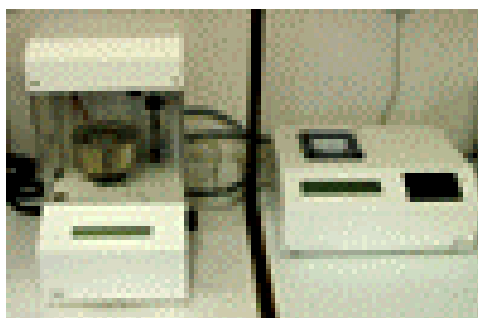


Fig. 16. Tensiometr K12. [46]

Density measurement:

- solid tester (Krüss standard) $\rho = 2,330 \text{ g/cm}^3$, hook for solid tester
- sensitivity 0,006g; time interval 5s

Surface tension measurement:

- Wilhely method
- standard plate: platinum, width = 19,9 mm, thickness 0,2 mm
- sensitivity 0,01g; immersion depth 2 mm; time interval 2s; 10 measured values

Contact angle measurement:

- Ring method, dynamic
- ring standard (Krüss): diameter 20 mm, thickness 0,2 mm
- sensitivity 0,0005g; time interval 2s; immersion depth 3 mm, 3 measured values

Samples were poured into the glass cup and established in the tensiometer. This cup is the standard for Tensiometer K12. Then it was let to temper on the requested temperature for 20 minutes.

As the first the density measurement was done. The software was set up for this measurement and the empty hook was installed and let to calibrate. Then we gently added the solid tester into the hook and start the density measurement.

When this measurement was finished the measuring extender was replaced by standard plate for surface tension measurement. Software was also changed and right setting data for surface tension measurement were set up. The measurement was repeated ten times just to have enough values for calculation of correct results and their standard deviation.

Last measurement on this device was the measurement of contact angle. The standard plate was replaced by standard ring and the software and settings were set up. Three values were measured.

The measured data were elaborated into the tables and the charts. Standard deviations were calculated and they are presented in the charts and tables also.

5.4.2 Doublebeam UV-VIS spektrofotometr (Helios Alpha - Thermo Spectronic)



Fig. 17. UV-VIS spectrometer.

[46]

- wavelength range 190 - 110 nm
- slot latitude 2 nm
- silicon cuvette, width 1 cm

Firstly, the distil water was poured into the two cuvette. Both cuvettes were placed into their compartments, one into the compartment for sample and the other into the solution compartment. Than the base line measurement was started.

The prepared samples were poured into the clean and dry silicon cuvette. Spectrophotometer settings were set up. It was set up whole wavelength range and medium smoothing.

5.4.3 ZetaPlus (Brookhaven Instruments Corporation)

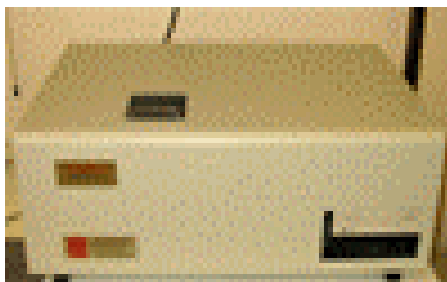


Fig. 18. ZetaPlus analyser. [46]

Zeta potential analyser with goniometry BI-9000AT, measured particle diameter is from 0,002 to 3 mm

Zeta plus and particle size software

special electrode for zeta potential measurement

special electrode for pH measurement

principle of laser-Doppler electrophoresis; particle size for electrophoresis 0.005-30 mm;

pH range: 2-12; sample volume: 1 ml-1.5 ml; conductive range: 0 - 700 mS/m; mobility range: 10^{-9} to 10^{-7} m²/V.s.

The prepared cellulose samples were poured into the clean and dry polystyrene cuvette, standard for ZetaPlus instrument. This cuvette was got inside the zeta potential analyser chest. The software for particle size measurement was dropped on and settings, such as sample name, number of rounds (5), spell of particular round (20 s).

When the particle size measurement was finished, the cuvette with sample was taken out. The special electrode for zeta potential measurement was plugged in this cuvette. The redundant sample amount was forced out and dried by a paper serviette. The clean and dry cuvette was got inside the chest again. In zeta potential software the optimal settings were selected, such as sample name, number of rounds (5), number of measurements in one round (5), concentration of solution, measured pH and the measurement was started.

6 RESULTS AND DISCUSSION

6.1 Density measurement

The density measurement was done, because density values are necessary for measurement of the surface tension and the contact angle. On the top of it the influence of concentration and temperature on density was investigated. The results from this measurement are elaborated in *Tab. 1*.

It is significant that increasing temperature influences density of the cellulose solutions. Density decreases slowly with increasing temperature. It is also shown in *Tab. 1*, that density is higher with higher concentration of the solution. This two conclusions are better shown in Fig. 19., where is shown how temperature and concentration influence density of samples. Density decreasing is gradual for both concentrations of each cellulose solution. Steeper decreasing is observable only in the case of OK CEL 4 w% sample, during initial increasing of temperature.

The low influence of solution concentration could be observed in case of HEC. Contrariwise, the higher influence of solution concentration is significant in case of CMC solution.

This is what was expected and the values of density are used to set up initial settings of the instrument for surface tension and contact angle measurements.

6.2 Surface tension measurement

Our imposition was to investigate surface tension of three cellulose derivate solutions OK CEL, HEC and CMC. These derivatives were investigated as different concentrated solutions (2 w%, 4 w%). The main point of this measurement was to investigate how the surface tension of these cellulose solutions reacts on increasing temperature and concentration of solutions. It was expected that surface tension could decrease with increasing temperature. The measured data are presented in *Tab. 2*. These data are also presented in more general charts on Fig. 20. In this table and charts is significantly shown that the surface tension has a decreasing trend with increasing temperature. The critical temperature, where the surface tension is equal to zero, was not evaluated in our measurement.

In case of OK CEL 2 w% solution it could be sum up that there is streamer decreasing of surface tension in temperature range 20 – 25 °C. Other decreasing is slow and gradual. Surface tension of OK CEL 4 w% varies with big changes in setting temperature range. The same behaviour could be observed in the case of both concentrations of HEC solutions. The decrease of surface tension is slow and gradual. Surface tension decrease, in case of CMC solutions, is slow in temperature range 20 – 25 °C. than surface tension decreases suddenly and rapidly. It is shown that values of surface tension of our investigated samples are much lower than surface tension of water.

We could not present any conclusions about solution concentration influence on surface tension. We do not have any definite result about this problem from this measurement.

The data from surface tension measurement were recalculated and results are shown in Tab. 3 and also presented in charts on Fig. 21. These data were elaborated by linear regression and the final equation was equalled with equation (2). The results of this elaboration are values of surface free energy. These results are presented in Tab. 3. The highest surface free energy is observed in case of CMC 2 w% solution.

6.3 Contact angle measurement

Another characteristic of cellulose derivatives is their contact angle. The advancing and receding contact angle was investigated by the Ring method on the Tensiometer K12. The effect of increasing temperature on contact angle was evaluated. The results from this investigation are presented in Tab. 4. The measurement was done for two weight concentrations (2 w%, 4 w%) of each cellulose sample (OK CEL, HEC, CMC). The temperature subsection is digestedly demonstrated at diagrams on Fig. 22. Here it is demonstrable shown that receding contact angle is smaller than advancing contact angle. On case of OK CEL and HEC solutions the advancing contact angle only slightly depends on increasing temperature or solution concentration. The receding angles have the same continuance. The two curves, which represent each weight concentration of one cellulose solution, slightly vary in the same range.

For every sample applies that the advancing angle is higher on case of 4 w% solutions and the receding angle is higher on the case of 2 w% solutions.

The great peak on advancing angle curve could be observe on case of 4 w% CMC solution at temperature range around 30 °C. It could be concluded that the solution is highly hydrophobic in this temperature point. On case of other samples it could be concluded that the wettability is not influenced by temperature of solution or its concentration.

6.4 UV-VIS spectroscopy

Another point of our work was to investigate how concentration of cellulose solution influences absorbance of ultraviolet and visible light. The measurement was done for three different weight concentrations (1 w%, 2 w% and 4 w%) of each derivate. The data obtained by this measurement are presented in Fig. 23. These three charts are clearer than tables. This measurement has so many values, because the wavelength range is from 190 to 1100 nm and the measurement step is 1.

The curves in these charts represent three different concentrations of cellulose solution. It is significant that the higher solution concentration the higher light absorbance.

The absorbance is much higher on case of OK CEL solutions. This is not observable on case of HEC and CMC solutions. For OK CEL it could be observe small absorbance of light in ultraviolet and the incipient phase of visible light. It could be cause by the $\pi \rightarrow \pi^*$ transitions, which are observable in the wavelength range 190 – 400 nm. And the absorbance of light in the incipient phase of visible light characterizes yellowish and opalesque character of OK CEL solution. It means that molecules in OK CEL solution refract the light beam and it causes that whole solution looks as slightly yellowish.

For HEC and CMC solutions this is not observable because they are clear. Here is also observable only the absorbance in UV part of spectrum which is cause by $\pi \rightarrow \pi^*$ transitions.

These data was elaborated for particular wavelength (400, 700 nm) to show the trend about solution concentration influence on light absorbance. These elaborated data are presented in Tab. 5 and more clearly at Fig. 24. In these charts the linear regression is used to elaborate data. From linear regression equations the extinction coefficient are calculated for particular cellulose solution.

These charts affirm the conclusion, that absorbance increase with increasing concentration of the solution.

Extinction coefficient is the highest for OK CEL solution, and low for HEC and CMC solutions.

Other aim of UV-VIS spectroscopy was to evaluate pH influence on light absorbance. This investigation was done in pH range 2 – 12. The data obtained in this measurement are presented at six diagrams at Fig. 25, Fig. 26 and Fig. 27. On case of OK CEL solutions it could be observe that light absorbance decrease with increasing pH till pH about 5. Than suddenly increase and at the end of pH range decrease again. This behaviour is much noticeable on case of solution which 2 w% concentration.

Regarding to HEC and CMC solutions, there is not any evidence of pH influence on light absorbance.

Data pertaining to wavelength 400 and 700 nm, which were obtained by this measurement, were selected and they are presented in *Tab. 6*, *Tab. 7*. The charts at Fig. 28, Fig. 29 are well arranged. Here is significantly shown that for OK CEL the dependence vary in whole pH range. There is apparent sudden decrease of absorbance in pH range 2 – 5 for both weight concentrations. Absorbance for HEC and CMC solutions is not almost subject to pH.

Third aim for UV-VIS spectroscopy was to evaluate ionic concentration influence on light absorbance. This investigation was done for concentration 0, 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} of NaCl. Cellulose solutions were diluted in ration 1:1, as described in part preparation of samples. It means that concentration of cellulose solutions is half-size (1 w%, 2 w%). The data obtained in this measurement are presented at six diagrams at Fig. 30, Fig. 31 and Fig. 32.

In these charts the indication of $\pi \rightarrow \pi^*$ transitions is also observable. But there is not any vestige of ionic concentration influence on light absorption.

For higher lucidity, data pertaining to wavelength 400 and 700 nm, were selected to show the trend of this influence. Data are presented in *Tab. 8*, *Tab. 9*. The charts at Fig. 33, Fig. 34 are again well arranged than tables. In these diagrams, when the curves represent different weight concentration, it is outward that the ionic concentration does not influence the light absorbance.

6.5 Measurement of the particle diameter

The measurement of the particle size was done for two weight concentrations (2 w%, 4 w%) of each cellulose derivate sample (OK CEL, HEC, CMC). The aim of this measurement was to evaluate the influence of pH and ionic concentration on size of the colloidal particle.

Data obtained by measurement of pH influence are presented in *Tab. 10*. These values are clearly elaborated as curves at Fig. 35.

For OK CEL solution is significantly observable that particle size consists in pH of cellulose solution. The diameter of particles in 2 w% solution is slightly contingent. But the diameter of particle of 4 w% solution decreases again at the pH range 2 – 4. The ability of molecules to swell is the lowest for pH about 4. From this point the diameter increase gradually.

These charts show that diameter of OK CEL particles is much lower than for HEC and CMC. It is caused by lower ability to absorb water from surrounding.

On case of HEC solution the diameter for 2 w% does not vary very significantly by the instrumentality of changing pH. For 4 w% solution of HEC, it could be concluded that the diameter decreases in pH range 2 – 7. Than the sudden increase is noticeable in pH range 7 – 10, which is followed by sweep decrease. So when solution pH is about 9, the conditions are good for molecules to absorb water from surrounding.

The curves, which represent 2 and 4 w% of CMC solution, show that particle size depends on pH of solution. The apparent increase of diameter size could be observe in pH range 2 – 5. It is much visible for 4 w% solution. It could be concluded that water absorbability is pretty well, when the solution has pH about 4 and 6.

The measurement of particle diameter also evaluated the influence of ionic concentration on diameter size. As previously mentioned the samples were prepared by diluting basic concentrated samples by NaCl solution in ratio 1:1. So new weight concentrations are 1 w% and 2 w%.

Measured values procured by this measurement are introduced in *Tab. 11*. The values are also visible on Fig. 36. Here it is apparent influence of ionic concentration on particle size. On case of OK CEL the influence is similar for both concentrations. It could be concluded

that the higher ionic concentration the higher amount of surrounded solution soak into the macromolecules, which boosts the particle size.

This behaviour is more apparent on case of HEC and CMC solutions. There is perceptible influence of ions, mainly for 1-weight % samples. The absorbability of molecules grows up with increasing ionic concentration. This particle size increasing is not possible for double concentrated solution. Molecules are very close each other and more marked swelling of molecules is not possible.

6.6 Measurement of zeta potential

The zeta potential measurement was provided on ZetaPlus instrument. The main point of these investigation was to evaluate the relation between pH of cellulose solution and zeta potential. Two weight concentrations (2 w%, 4 w%) of each cellulose derivate solution (OK CEL, HEC, CMC) were determined.

Data, which present solution pH influence on zeta potential, are presented in *Tab. 12*. But the charts on *Fig. 37* are more comprehensible than the table. It is visible that zeta potential of particles is generally negative. These values present that OK CEL and CMC cellulose solutions are stable in whole measured pH range. HEC cellulose solution is not stable at pH area around 2,5 for 2 w% solution and 7,5 for 4 w% one.

The second thing evaluated by zeta potential measurement was influence of ions. Samples were again diluted by NaCl solution in ration 1:1 (1 w%, 2 w%). Measuring data are presented in *Tab. 13*. and as curves on *Fig. 38*.

These curves prove that zeta potential for these solution particles is negative. OK CEL and CMC solutions are stable in whole ionic concentration range, which was measured. HEC solution is less stable with ability to coagulate for ionic concentration about $1 \cdot 10^{-5}$ g/ml. Otherwise it is stable in almost whole range.

Tab. 1. Values of temperature dependence of density for two different weight concentrations of each cellulose sample.

	OK CEL 2 w%	OK CEL 4 w%	HEC 2 w%	HEC 4 w%	CMC 2 w%	CMC 4 w%
Temperature	Density					
T	ρ					
(°C)	(g/cm ³)					
20	1,0042	1,0215	1,0059	1,0089	1,0060	1,0133
25	1,0039	1,0097	1,0047	1,0055	1,0045	1,0119
30	1,0018	1,0073	1,0023	1,0035	1,0037	1,0090
35	1,0010	1,0055	1,0013	1,0010	1,0025	1,0084
40	0,9992	1,0046	0,9999	0,9997	1,0024	1,0070

Tab. 2. Data from the surface tension measurement shows relationship between surface tension and temperature.

	OK CEL 2 w%	OK CEL 4 w%	HEC 2 w%	HEC 4 w%	CMC 2 w%	CMC 4 w%
Temperature	Surface tension					
T	σ					
(°C)	(mN/m)					
20	63,71	55,85	48,91	46,97	59,13	57,84
	0,51	0,18	0,02	0,14	0,06	0,36
25	55,27	60,23	46,04	45,70	57,78	56,87
	0,14	0,03	0,18	0,44	0,22	0,68
30	54,32	51,67	46,35	43,10	52,78	57,51
	0,03	0,03	0,19	0,16	0,08	0,84
35	54,73	47,69	46,61	44,08	47,55	55,77
	0,02	0,23	0,17	0,46	0,22	0,75
40	52,00	52,60	42,45	42,64	41,62	51,18
	0,03	0,02	0,13	0,53	0,45	0,40

Tab. 3. Re-counted data from the surface tension measurement. The dependence of the surface tension on temperature. The results of these functionality is surface energy for each sample and its two concentrations.

	OK CEL 2 w%	OK CEL 4 w%	HEC 2 w%	HEC 4 w%	CMC 2 w%	CMC 4 w%
Temperature	Surface tension					
T	σ					
(K)	(N/m)					
293,15	0,064	0,056	0,049	0,047	0,059	0,058
298,15	0,055	0,060	0,046	0,046	0,058	0,057
303,15	0,054	0,052	0,046	0,043	0,053	0,058
308,15	0,055	0,048	0,047	0,044	0,048	0,056
310,15	0,052	0,053	0,042	0,043	0,042	0,051
Surface energy E_s (J/m²)	0,221	0,210	0,150	0,115	0,352	0,145

Tab. 4. The values from measuring of temperature dependence of advancing and receding contact angle for both weight concentrations of each cellulose sample.

	OK CEL 2 w%	OK CEL 4 w%	HEC 2 w%	HEC 4 w%	CMC 2 w%	CMC 4 w%
Temperature	Advancing contact angle					
T						
(°C)						
	(°)					
20	89,00	89,90	90,30	90,60	105,00	91,30
	0,10	0,08	0,19	0,50	17,00	1,50
25	88,90	91,30	90,70	101,00	94,30	90,10
	0,22	0,22	0,33	13,00	0,65	0,00
30	88,80	90,40	90,30	105,00	90,60	127,00
	0,12	0,40	1,15	13,00	0,00	12,00
35	89,20	90,10	90,10	91,50	89,30	90,00
	0,12	0,00	0,25	1,60	0,55	1,00
40	89,60	91,00	88,00	90,20	89,90	92,80
	0,50	0,95	0,00	2,10	0,00	0,40
	Receding contact angle					
	(°)					
20	82,50	79,20	69,60	60,40	67,60	72,90
	1,00	4,80	6,00	9,80	5,20	1,10
25	84,90	77,70	82,50	60,90	82,50	79,50
	1,50	6,10	3,90	17,30	0,85	0,75
30	81,50	72,60	78,40	80,70	77,40	69,40
	4,50	0,35	4,80	6,05	0,00	0,65
35	74,80	71,20	82,30	85,00	78,30	83,00
	2,40	0,00	4,40	1,00	4,10	0,85
40	81,00	80,80	67,80	82,00	71,90	73,60
	0.97	1.90	0.00	3.60	0.00	1.80

Tab. 5. The values from the measurement of absorbance and its dependence on concentration of cellulose solution, where wavelength was 400 or 700 nm. The extinction coefficient was calculated from linear regression of this relation. These measurement was done for three different concentrations of each cellulose sample.

Wavelength	Absorbance								
λ	A								
(nm)	(1)								
	OK CEL			HEC			CMC		
	Concentration								
	10	20	40	10	20	40	10	20	40
	kg/m ³								
400	1,016	1,538	2,145	0,019	0,037	0,122	0,013	0,026	0,053
700	0,497	0,823	1,204	0,005	0,011	0,078	0,002	0,001	0,006
	Extinction coefficient								
	ϵ								
	(m ² /kg)								
400	6,03			0,28			0,13		
700	3,31			0,16			0,01		

Tab. 6. The measured values of pH dependence of absorbance for two different concentrations of each cellulose solution. The measurement was obtained on wavelength 400 nm.

Wavelength					
λ					
400 nm					
OK CEL 2 w%		OK CEL 4 w%		HEC 2 w%	
pH	Absorbance	pH	Absorbance	pH	Absorbance
	A		A		A
(1)	(1)	(1)	(1)	(1)	(1)
2,59	1,996	2,71	1,472	2,50	0,042
2,98	1,681	3,20	2,145	3,11	0,032
3,20	1,538	5,07	0,426	4,19	0,032
3,40	1,334	7,24	0,852	5,70	0,056
4,02	0,515	9,10	1,125	6,40	0,039
4,20	1,568	10,39	1,380	7,36	0,040
5,57	1,056	11,20	1,557	8,35	0,053
6,82	0,458				
8,56	1,497				
9,98	0,652				
HEC 4 w%		CMC 2 w%		CMC 4 w%	
pH	Absorbance	pH	Absorbance	pH	Absorbance
	A		A		A
(1)	(1)	(1)	(1)	(1)	(1)
3,32	0,036	2,51	0,018	2,35	0,046
4,09	0,035	3,31	0,012	3,27	0,033
5,16	0,055	4,45	0,012	4,31	0,036
6,45	0,040	5,33	0,013	5,19	0,051
8,26	0,049	6,37	0,016	6,20	0,053
9,47	0,063	7,20	0,016	7,25	0,045
10,52	0,095	8,25	0,022	8,44	0,063
11,15	0,168	9,53	0,029	9,17	0,077
		10,56	0,024	10,70	0,046
		11,25	0,029	11,29	0,071

Tab. 7. The measured values of pH dependence of absorbance for two different concentrations of each cellulose solution. The measurement was obtained on wavelength 700 nm.

Wavelength					
λ					
700 nm					
OK CEL 2 w%		OK CEL 4 w%		HEC 2 w%	
pH	Absorbance	pH	Absorbance	pH	Absorbance
	A		A		A
(1)	(1)	(1)	(1)	(1)	(1)
2,59	1,403	2,71	0,894	2,50	0,034
2,98	1,064	3,20	1,204	3,11	0,026
3,20	0,823	5,07	0,081	4,19	0,027
3,40	0,779	7,24	0,089	5,70	0,034
4,02	0,205	9,10	0,302	6,40	0,028
4,20	0,976	10,39	0,280	7,36	0,028
5,57	0,367	11,20	0,103	8,35	0,032
6,82	0,114				
8,56	0,954				
9,98	0,134				
HEC 4 w%		CMC 2 w%		CMC 4 w%	
pH	Absorbance	pH	Absorbance	pH	Absorbance
	A		A		A
(1)	(1)	(1)	(1)	(1)	(1)
3,32	0,012	2,51	0,002	2,35	0,004
4,09	0,011	3,31	-0,001	3,27	-0,001
5,16	0,029	4,45	-0,002	4,31	0,002
6,45	0,022	5,33	-0,002	5,19	0,004
8,26	0,007	6,37	0,005	6,20	0,005
9,47	0,010	7,20	-0,002	7,25	-0,003
10,52	0,008	8,25	0,001	8,44	0,028
11,15	0,016	9,53	0,003	9,17	0,014
		10,56	-0,002	10,70	0,004
		11,25	-0,002	11,29	0,000

Tab. 8. The values from measurement of ionic concentrations dependence of absorbance for two different concentrations of each cellulose sample. The measurement was done on wavelength 400 nm.

	Wavelength					
	λ					
	400 nm					
	OK CEL 1 w%	OK CEL 2 w%	HEC 1 w%	HEC 2 w%	CMC 1 w%	CMC 2 w%
Salt concentration (NaCl)	Absorbance					
c	A					
g/ml	(1)					
0	1,996	2,145	0,037	0,122	0,037	0,053
1,00E-06	1,568	1,923	0,025	0,019	0,003	0,008
1,00E-05	0,515	2,033	0,027	0,022	0,004	0,017
1,00E-04	1,334	1,582	0,028	0,024	0,002	0,005
1,00E-03	1,538	1,964	0,028	0,024	0,003	0,007
1,00E-02	1,681	1,976	0,035	0,028	0,018	0,012

Tab. 9. The values from measurement of ionic concentrations dependence of absorbance for two different concentrations of each cellulose sample. The measurement was done on wavelength 700 nm.

	Wavelength					
	λ					
	700 nm					
	OK CEL 1 w%	OK CEL 2 w%	HEC 1 w%	HEC 2 w%	CMC 1 w%	CMC 2 w%
Salt concentration (NaCl)	Absorbance					
c	A					
g/ml	(1)					
0	1,402	1,204	0,011	0,078	0,005	0,002
1,00E-06	0,975	1,327	0,026	0,004	0,004	0,002
1,00E-05	0,205	1,424	0,027	0,009	0,007	0,005
1,00E-04	0,778	0,880	0,027	0,007	0,008	0,001
1,00E-03	0,823	1,369	0,027	0,006	0,010	0,003
1,00E-02	1,062	1,378	0,029	0,010	0,009	0,003

Tab. 11. The values obtained by measuring of particle diameter and its dependence on ionic concentration in cellulose solution. The measurement was done for two different concentrations of each cellulose sample.

	OK CEL 1 w%	OK CEL 2 w%	HEC 1 w%	HEC 2 w%	CMC 1 w%	CMC 2 w%
Salt concentration	Diameter					
C	D					
(g/ml)	(nm)					
0,00E+00	440	610	5600	21400	20700	74400
	40	30	700	3800	3100	7400
1,00E-06	430	340	4600	6300	13900	21000
	50	100	1800	1200	2900	4100
1,00E-05	600	460	1300	8600	5800	8700
	40	80	210	2000	1000	780
1,00E-04	550	300	15700	4200	13300	9600
	10	100	4000	910	4300	590
1,00E-03	470	520	20200	6500	36500	11600
	10	60	9800	570	13000	1300
1,00E-02	570	350	11400	5200	10500	10412
	70	50	1200	1500	1200	950

Tab. 12A. The results of zeta potential measurement, which investigated pH dependence of potential. This measurement was done for two different concentrations of each cellulose sample.

OK CEL 2 w%		OK CEL 4 w%		HEC 2 w%	
pH	Zeta potential	pH	Zeta potential	pH	Zeta potential
	ζ		ζ		ζ
(1)	(mV)	(1)	(mV)	(1)	(mV)
2,37	-0,21	2,71	-8,17	2,35	-0,36
	3,60		3,90		0,80
2,59	-6,30	3,05	-10,78	2,50	1,63
	4,00		2,30		0,50
2,98	-10,68	4,02	-21,63	3,11	0,00
	2,60		4,80		0,00
3,10	-8,49	5,07	-4,21	4,19	-1,59
	1,70		3,30		0,40
3,42	-29,85	6,30	-16,11	5,70	-1,77
	1,20		1,30		0,00
3,82	-23,54	7,24	-21,25	6,40	-2,96
	2,50		3,10		0,80
4,02	-35,12	8,07	-12,31	7,36	-1,77
	1,20		2,90		0,90
6,82	-29,40	9,10	-17,21	8,35	-3,24
	2,80		2,30		0,80
8,56	-13,74	10,39	-24,98	9,27	-4,84
	8,60		2,90		1,00
9,85	-36,19	11,20	-1,04	10,78	-3,66
	4,00		3,60		1,00
11,72	-24,87			11,34	-4,10
	4,50				0,90

Tab. 12B. The results of zeta potential measurement, which investigated pH dependence of potential. This measurement was done for two different concentrations of each cellulose sample.

HEC 4 w%		CMC 2 w%		CMC 4 w%	
pH	Zeta potential	pH	Zeta potential	pH	Zeta potential
	ζ		ζ		ζ
(1)	(mV)	(1)	(mV)	(1)	(mV)
2,50	-0,84	2,51	-2,21	2,35	-2,10
	0,40		0,30		0,01
3,32	-1,95	3,31	-15,91	3,27	-15,15
	0,00		1,00		0,50
4,09	-2,18	4,45	-31,85	4,31	-25,07
	0,00		0,90		2,30
5,16	-3,40	5,33	-17,02	5,19	-25,56
	0,40		7,10		0,20
6,45	-2,83	6,37	-35,20	6,20	-29,03
	0,30		4,30		1,00
7,26	0,94	7,20	-10,99	7,25	-24,13
	1,10		6,80		2,30
8,26	-0,55	8,25	-30,91	8,44	-31,49
	0,30		5,00		0,80
9,47	-2,29	9,53	-41,01	9,17	-30,71
	0,01		0,90		0,90
10,52	-1,97	10,56	-36,75	10,70	-34,99
	0,00		0,90		1,10
11,15	-2,05	11,25	-37,96	11,29	-34,17
	0,20		1,50		0,90

Tab. 13. The values obtained from z eta potential measurement and measurement of relation between ionic concentration in cellulose solution and measured potential. The measurement was done for two different concentrations of each cellulose sample.

	OK CEL 1 w%	OK CEL 2 w%	HEC 1 w%	HEC 2 w%	CMC 1 w%	CMC 2 w%
Salt concentration	Zeta potential					
C	ζ					
(g/ml)	(mV)					
0,00E+00	-8,49	-10,78	-1,77	-2,33	-35,20	-29,03
	1,70	2,30	0,00	0,30	4,30	1,00
1,00E-06	-17,11	-16,27	-2,91	-0,26	-39,82	-20,32
	0,90	1,50	0,70	0,60	1,40	5,80
1,00E-05	-15,21	-19,74	0,20	0,32	-13,57	-37,54
	1,50	3,30	0,00	1,20	9,50	2,50
1,00E-04	-19,05	-14,83	-1,56	-3,01	-41,90	-36,50
	1,80	0,40	0,50	0,60	4,10	1,00
1,00E-03	-21,41	-11,44	-5,06	-2,59	-32,91	-25,28
	0,80	3,30	1,30	0,40	4,30	6,10
1,00E-02	-19,10	-13,26	-1,45	-2,39	-32,89	-31,06
	0,70	3,60	2,20	1,00	4,02	0,60

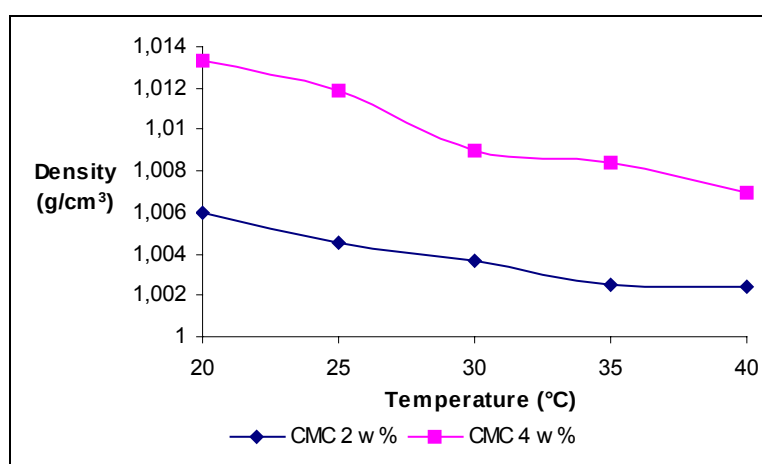
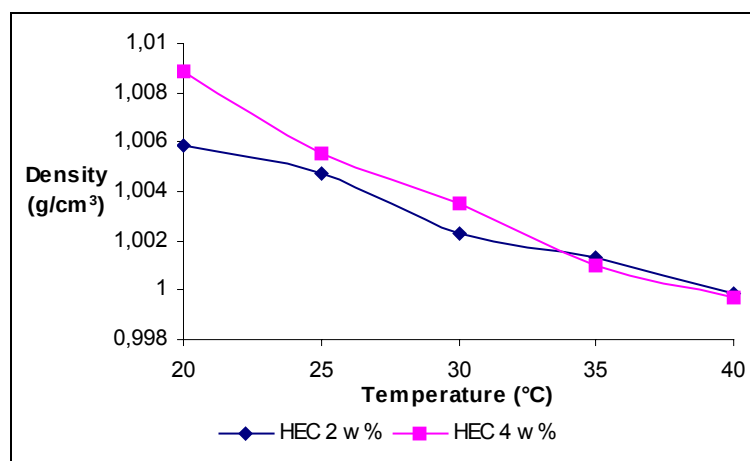
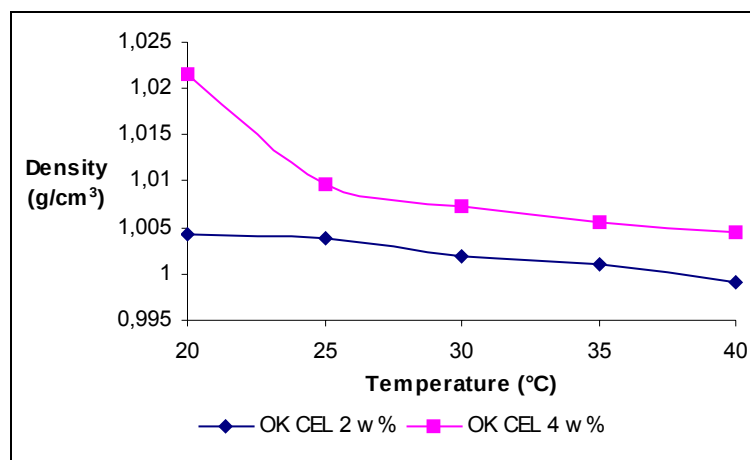


Fig. 19. These three diagrams present the temperature functionality of density. These charts also show how density depends on weight concentrations of cellulose solutions.

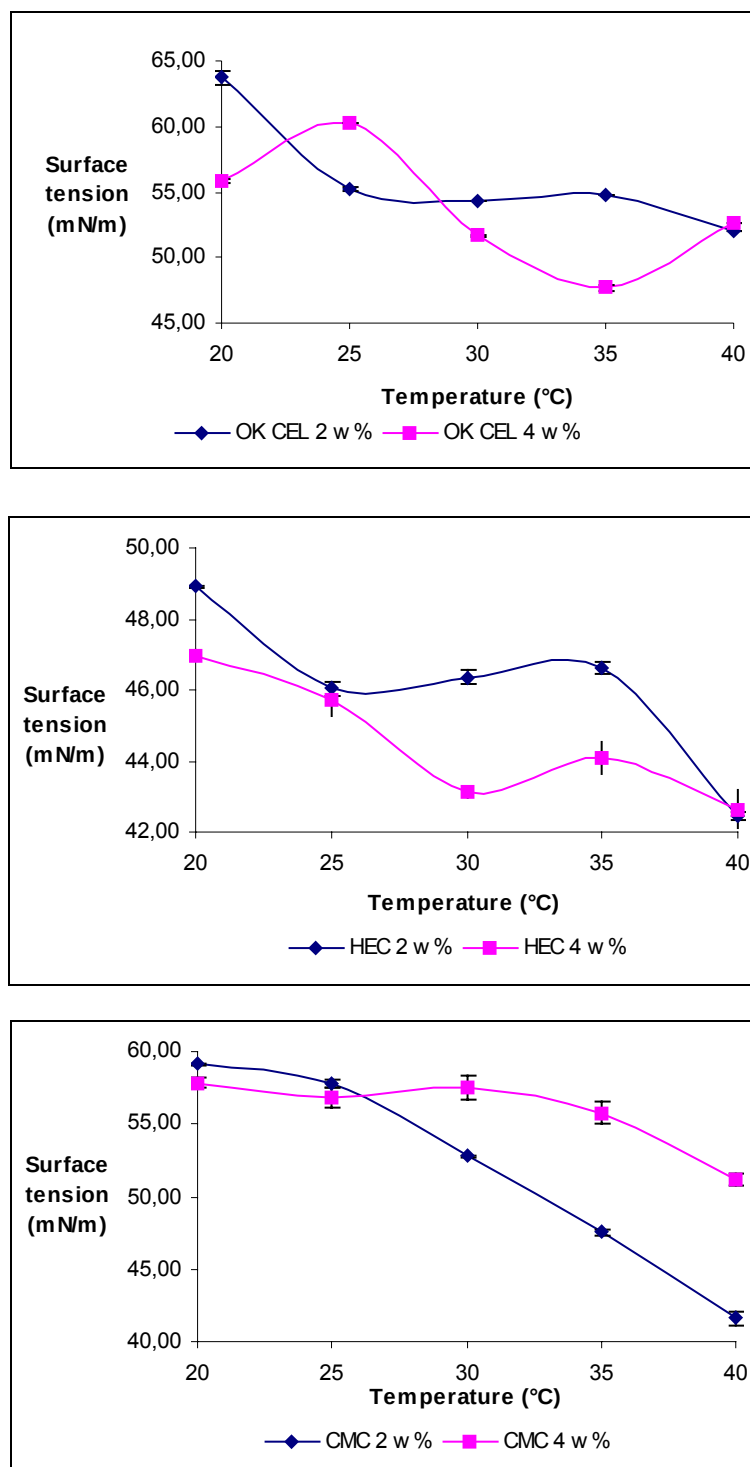


Fig. 20. These three graphs present the temperature dependence of surface tension. These charts also show how the surface tension depends on weight concentrations of cellulose solutions.

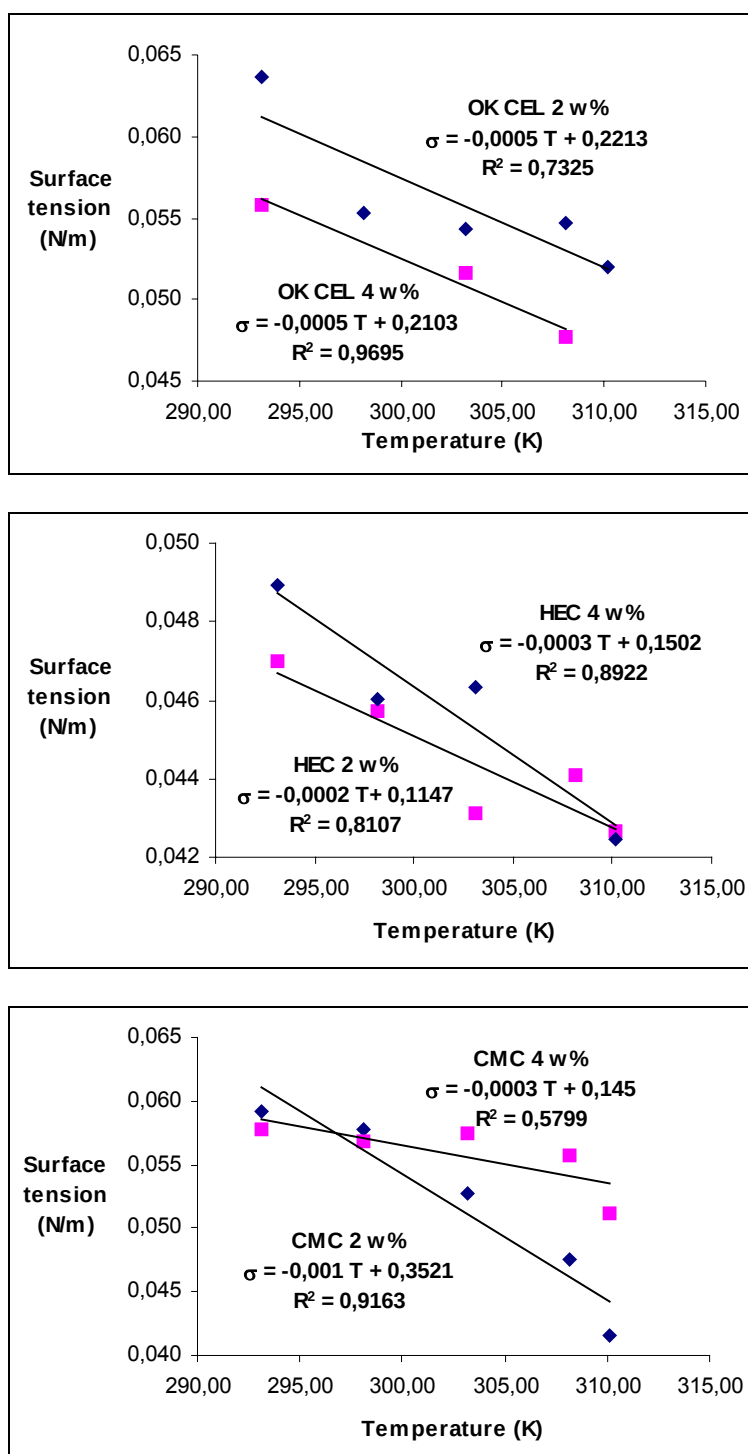


Fig. 21. The diagrams present the temperature relation of the surface tension. These linearity and their equations were used to calculate the surface free energy.

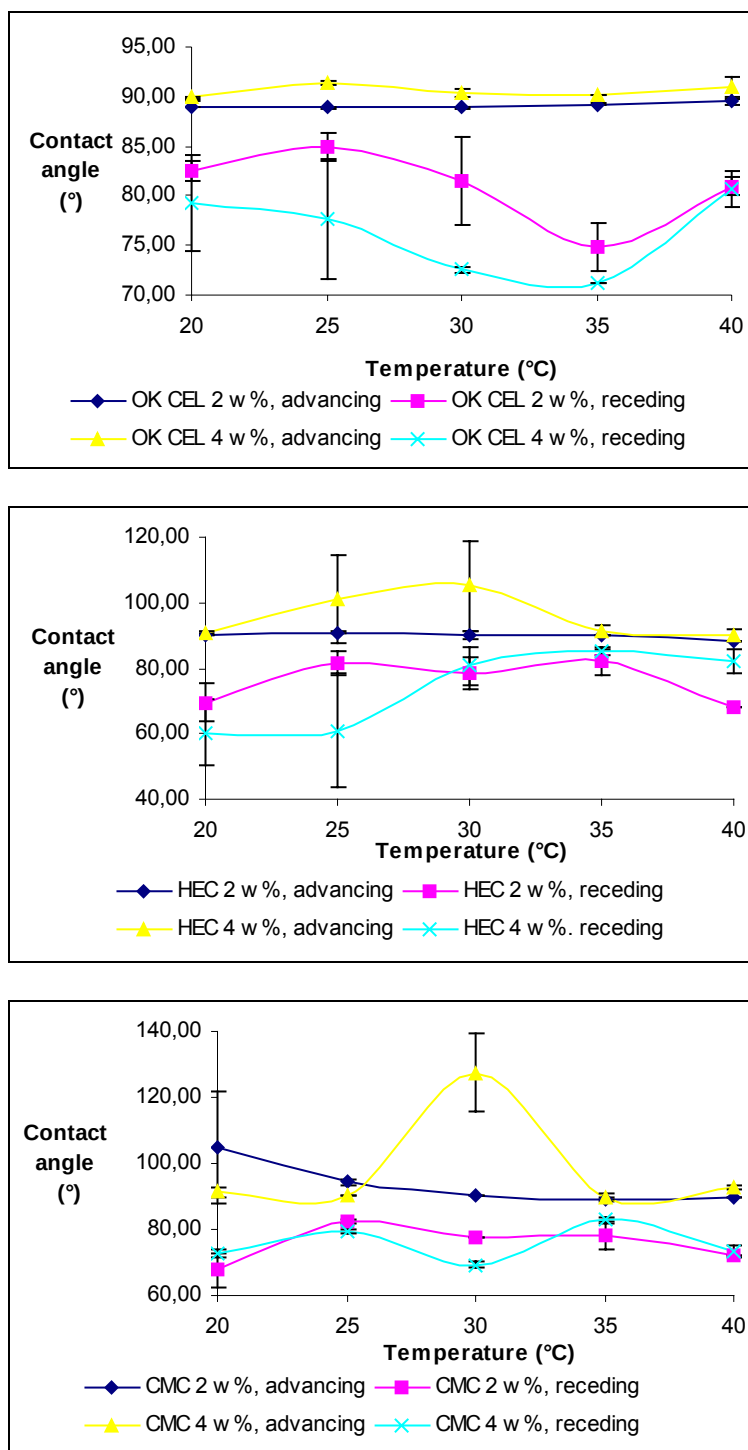


Fig. 22. These charts show temperature dependence of advancing and receding contact angle. They also show differences cause by different weight concentration of cellulose solution.

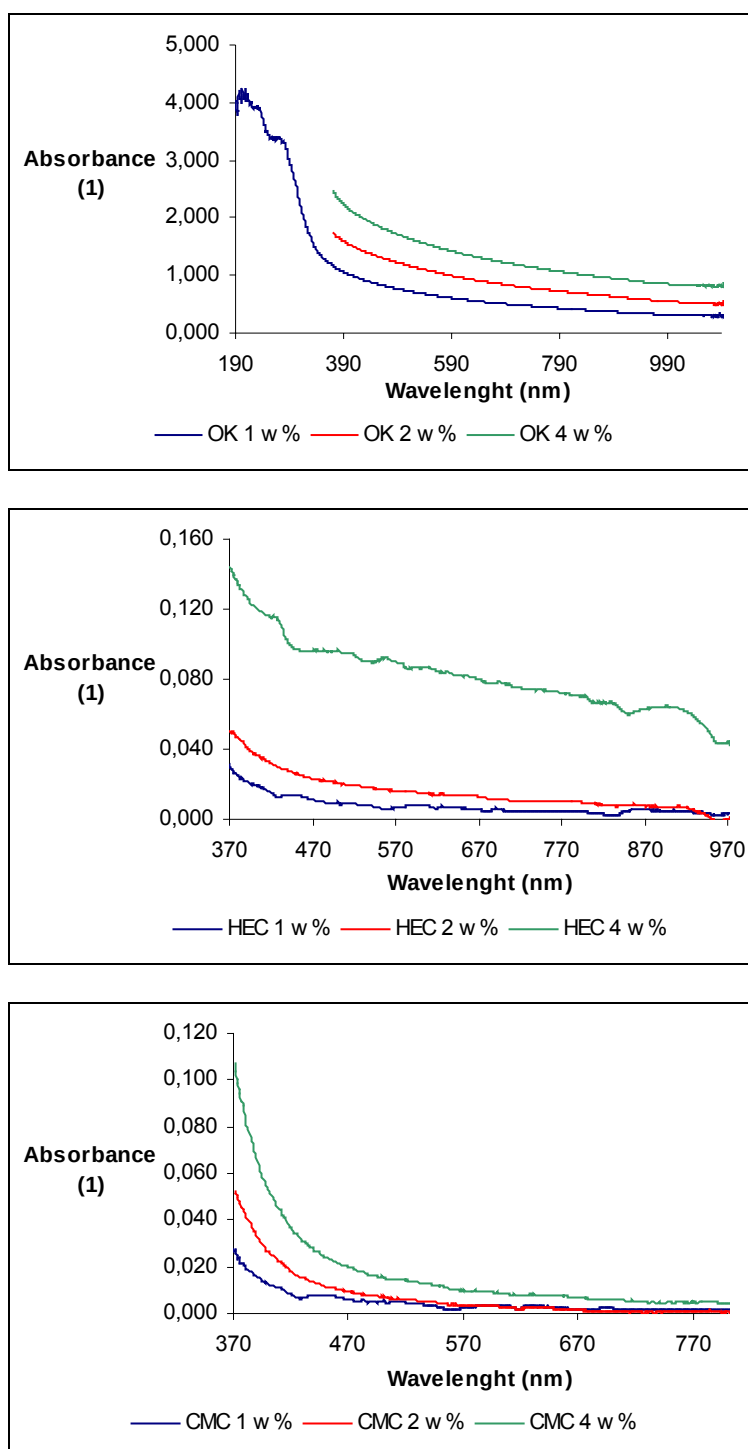


Fig. 23. These graphs are obtained from absorbance measurement. They show absorbance dependence on cellulose solution concentration.

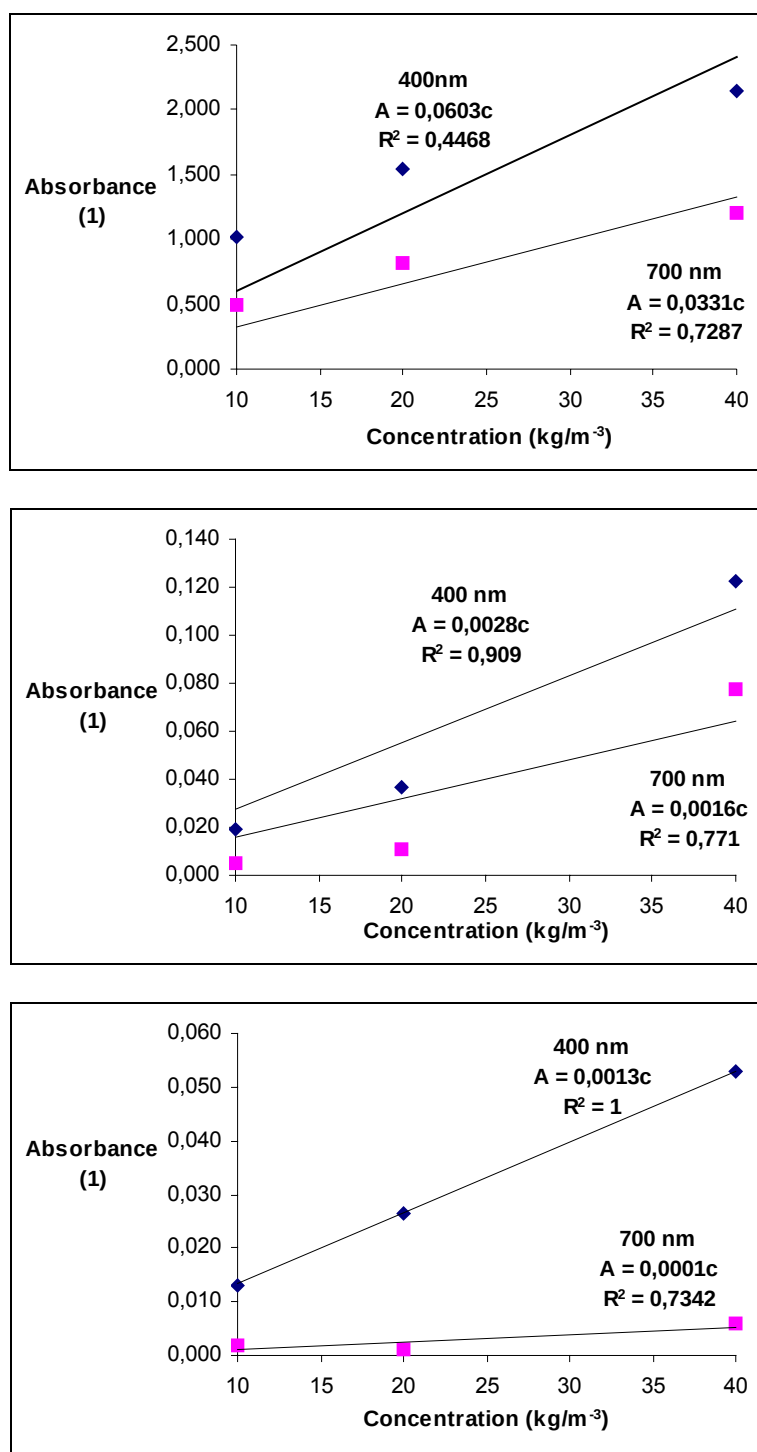


Fig. 24. These charts show the relation between cellulose solution concentration and absorbance. The equations of linear regression were used to calculate extinction coefficient.

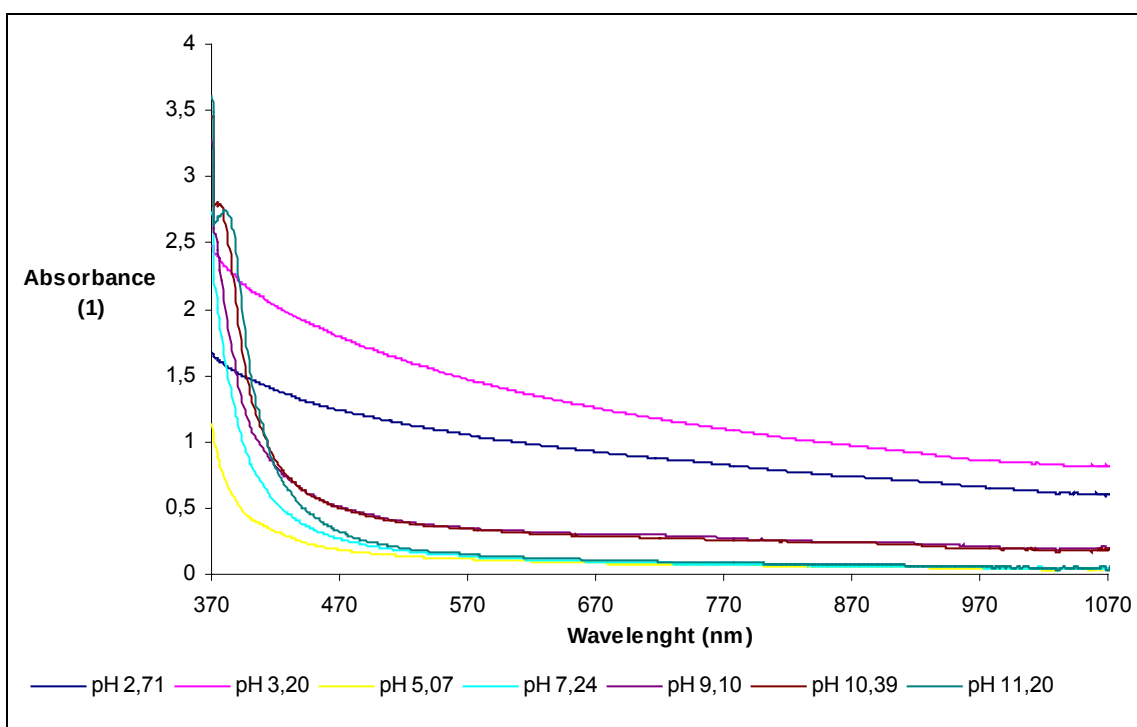
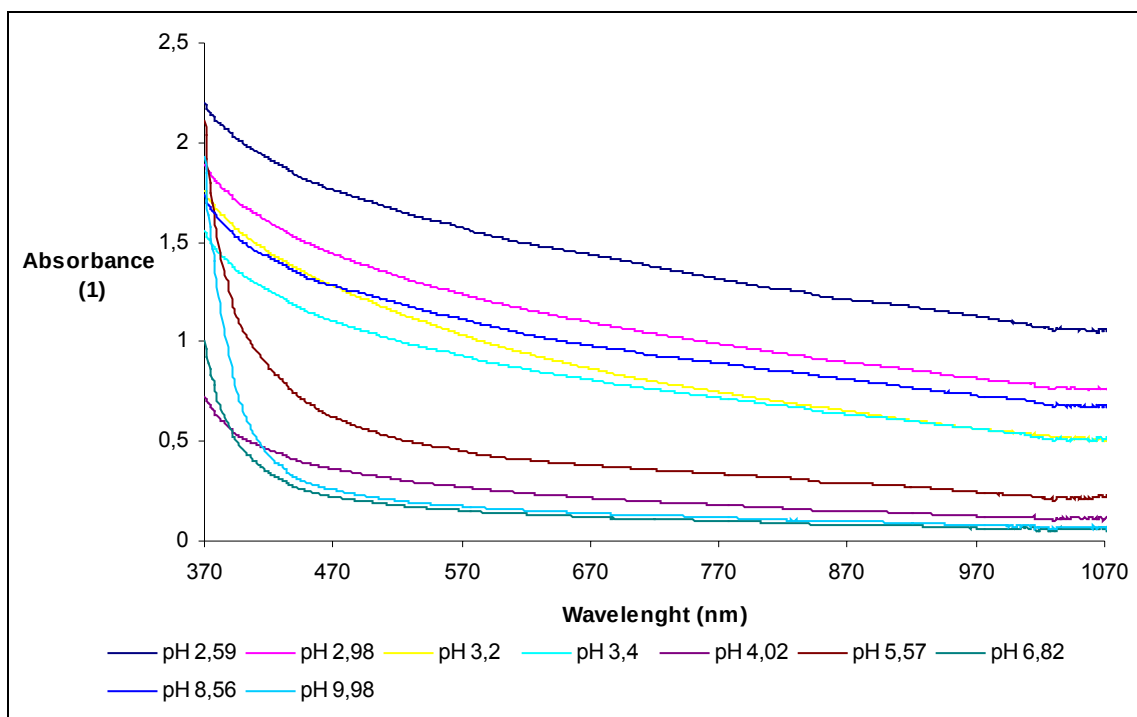


Fig. 25. These two charts show the values of pH dependence of absorbance. These values represent two different weight concentrations (2 w%, 4 w%) of OK CEL.

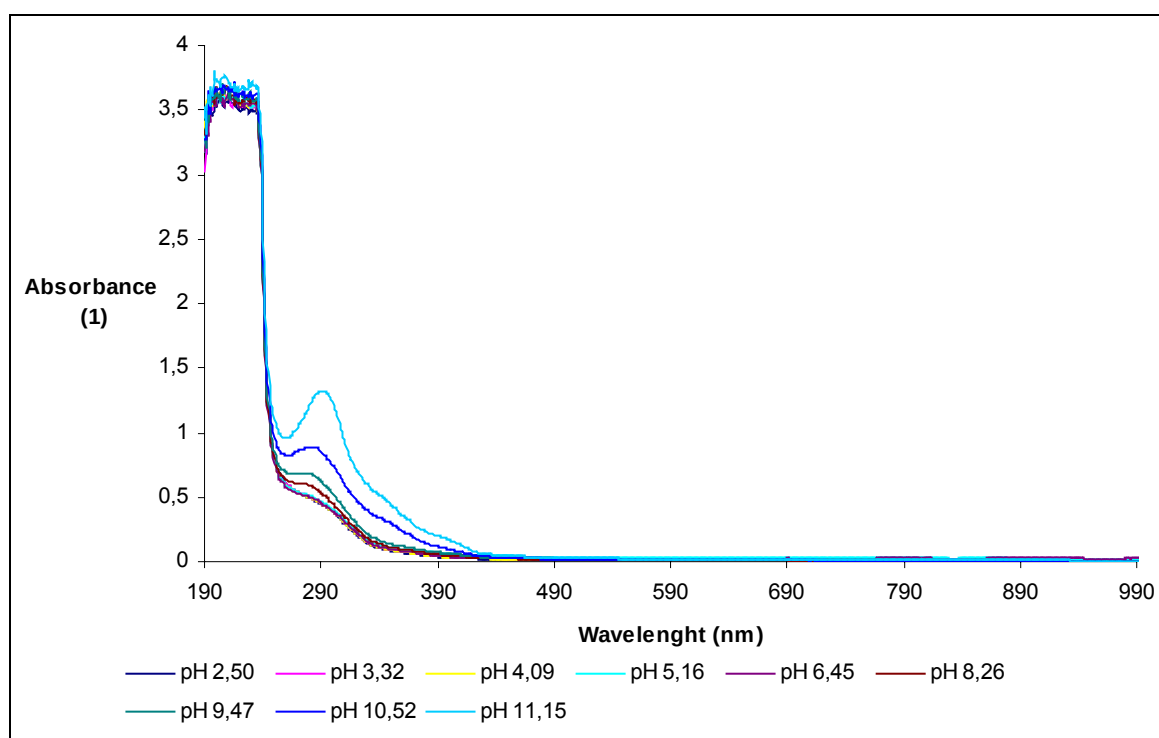
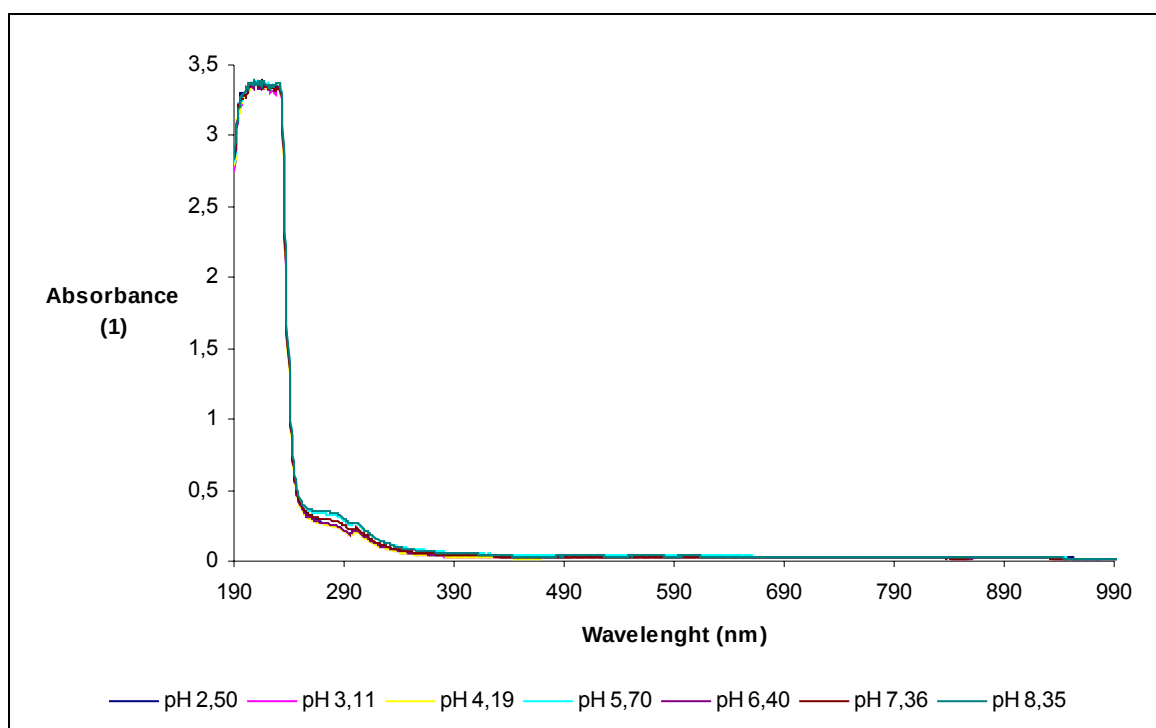


Fig. 26. These two charts show the values of pH dependence of absorbance. These values represent two different weight concentrations (2 w%, 4 w%) of HEC.

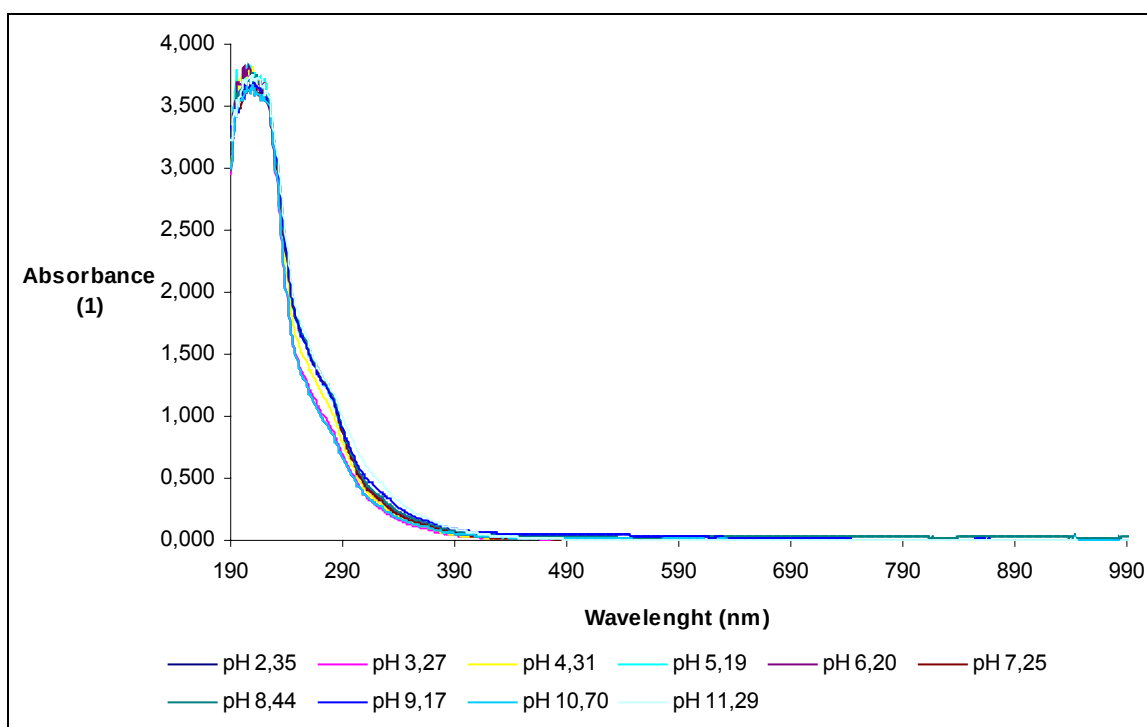
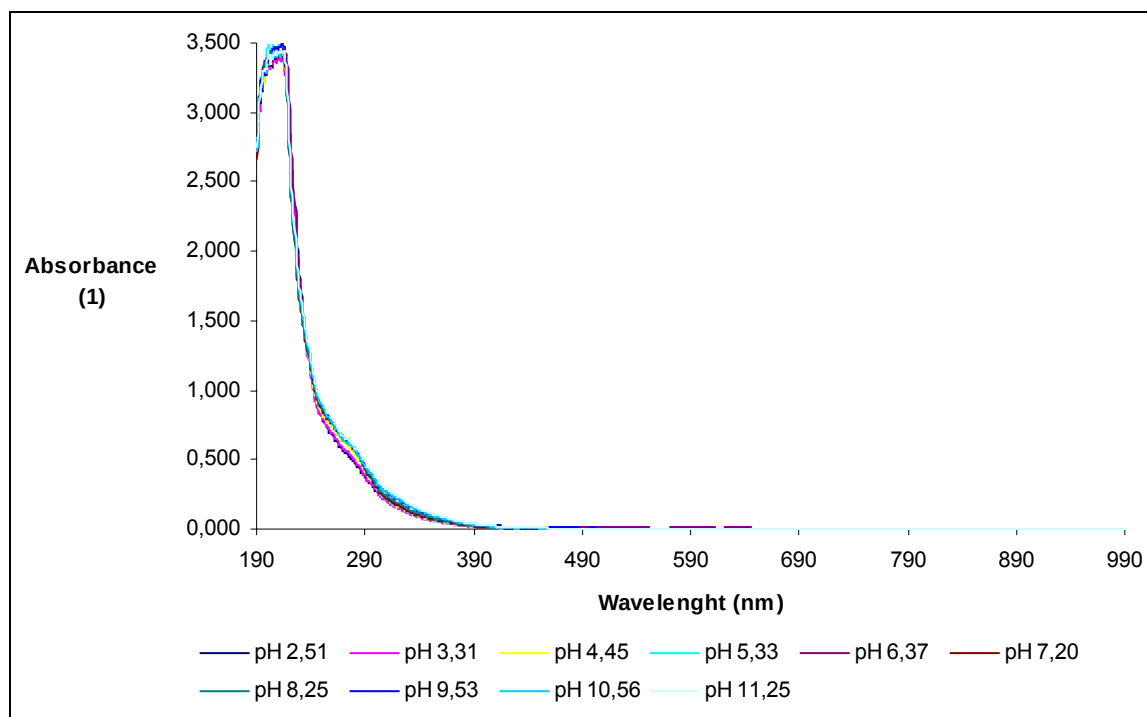


Fig. 27. These two charts show the values of pH dependence of absorbance. These values represent two different weight concentrations (2 w%, 4 w%) of CMC.

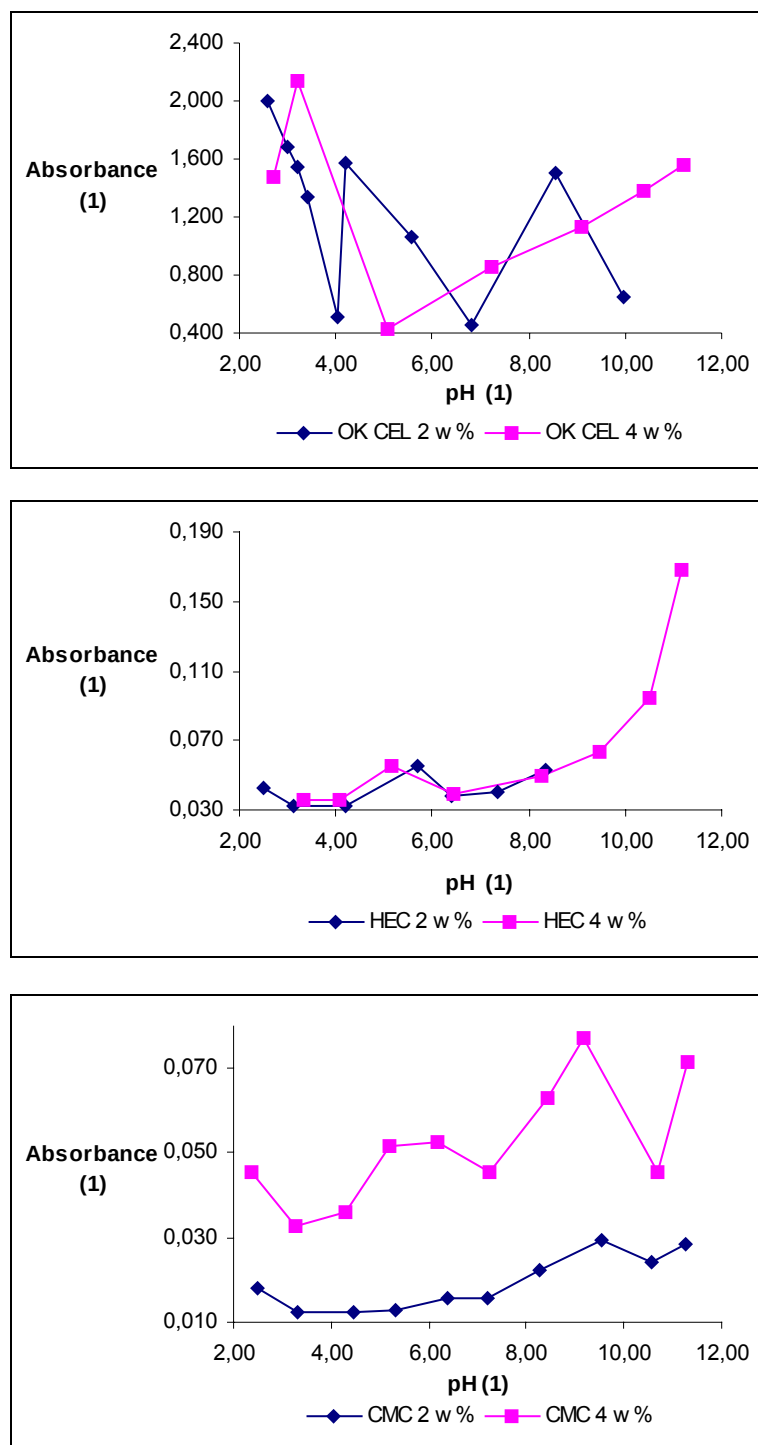


Fig. 28. These diagrams present pH dependence of absorbance. These values belong to the wavelength 400 nm.

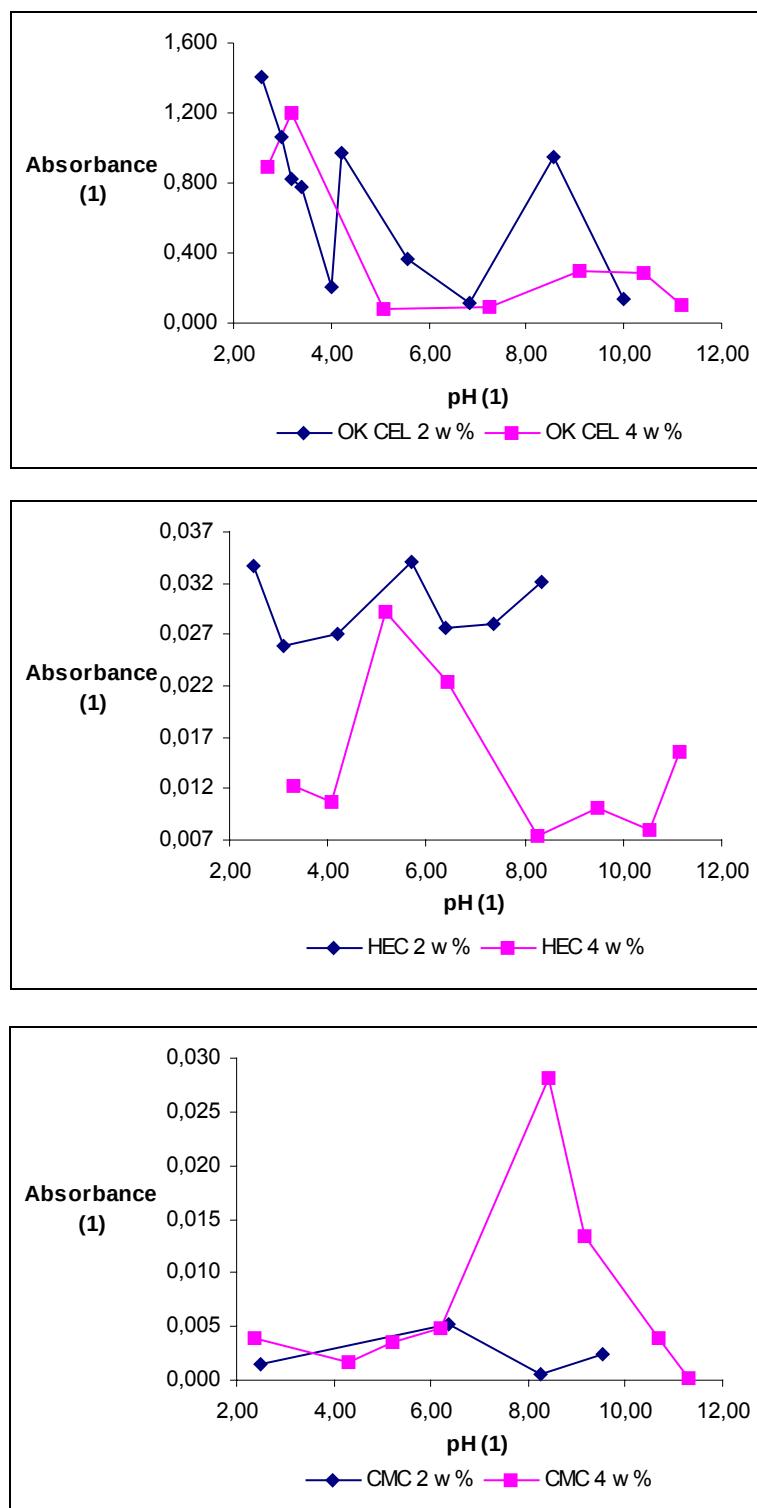


Fig. 29. These diagrams present pH dependence of absorbance. These values belong to the wavelength 700 nm.

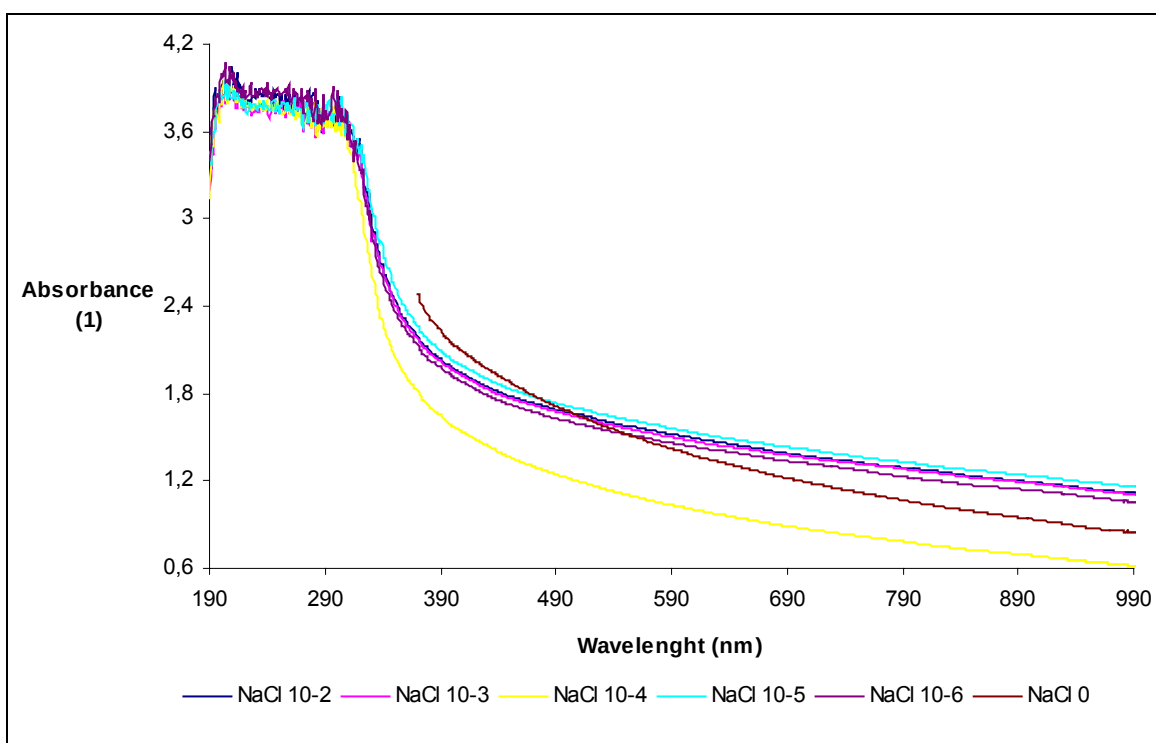
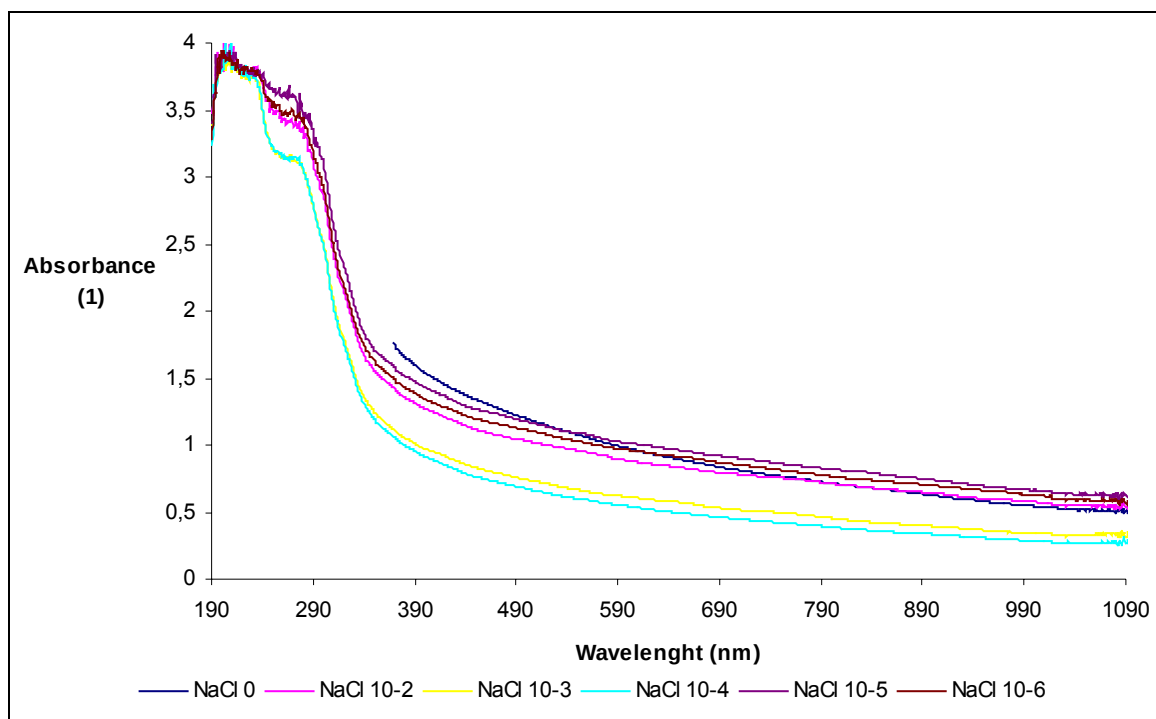


Fig. 30. These two diagrams represent relation between absorbance and increasing ionic concentration in cellulose solution. These values belong to OK CEL.

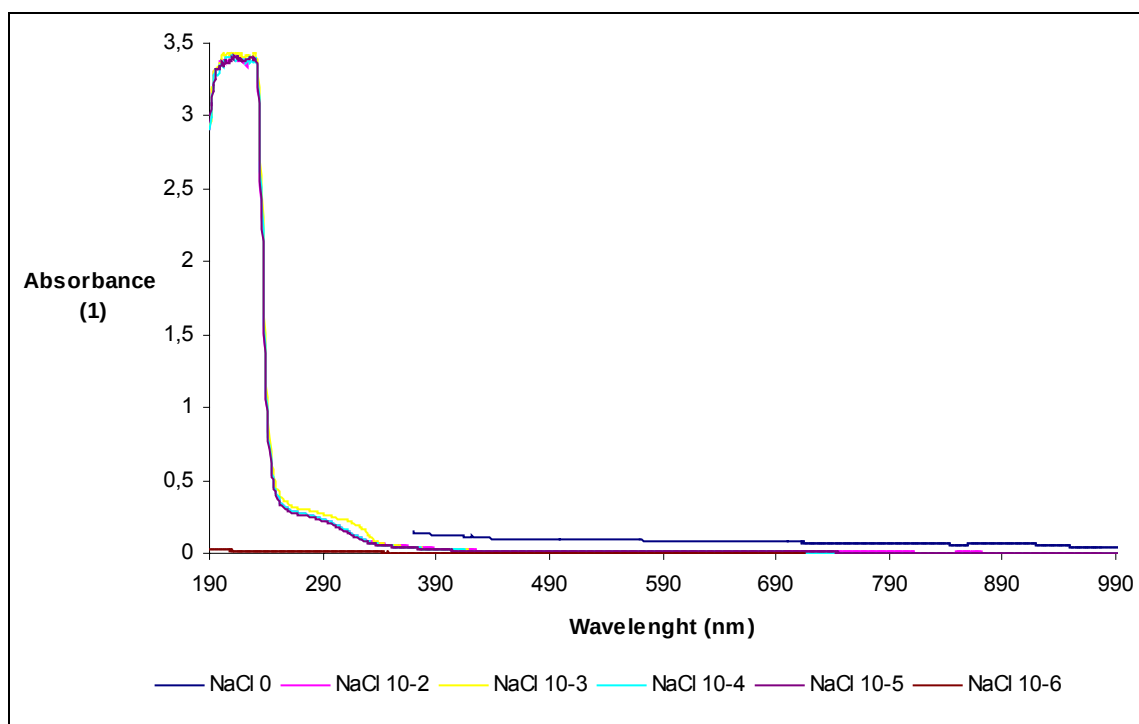
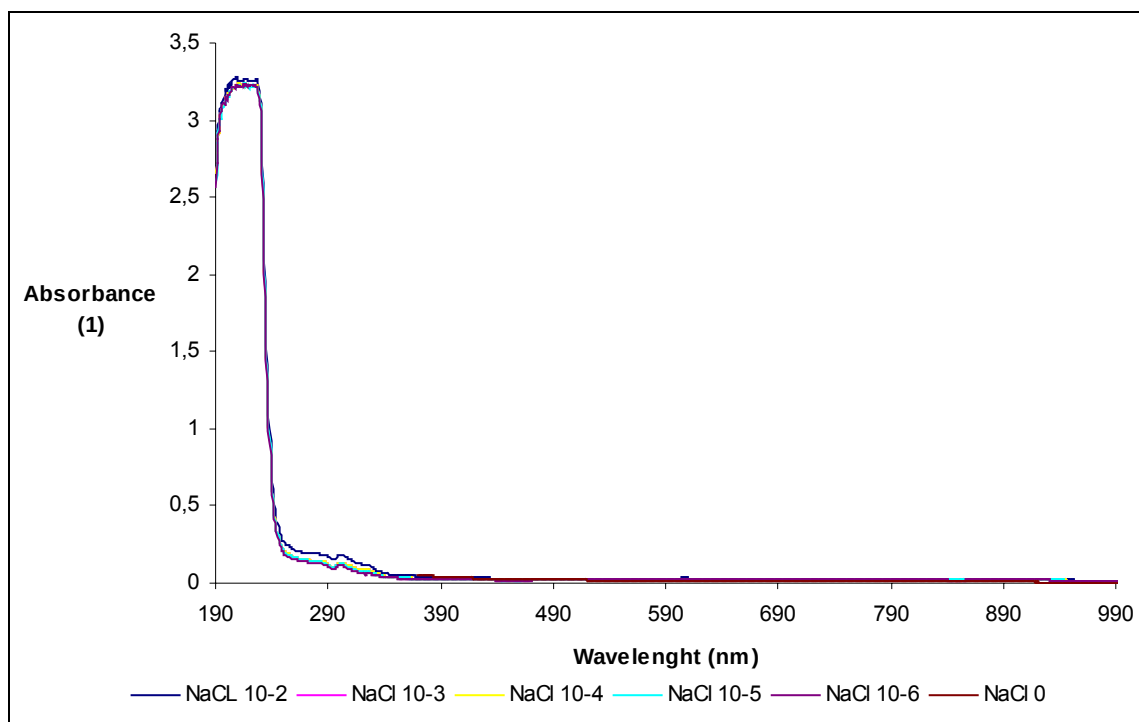


Fig. 31. These two diagrams represent relation between absorbance and increasing ionic concentration in cellulose solution. These values belong to HEC.

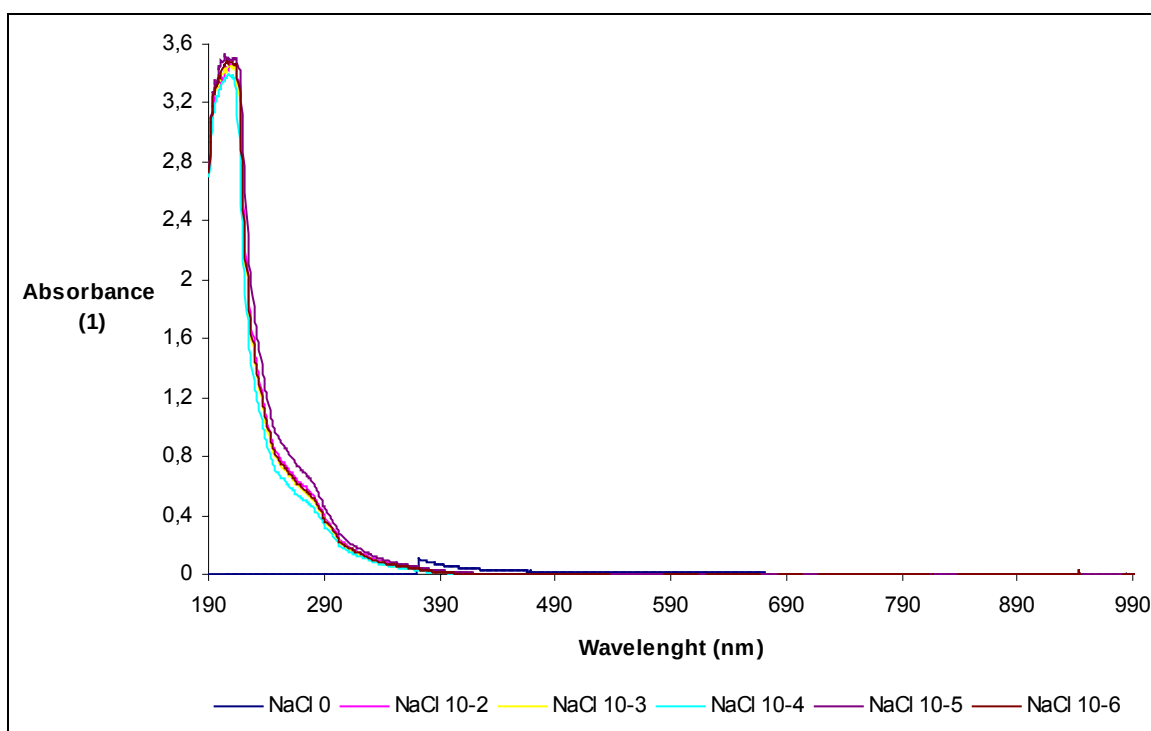
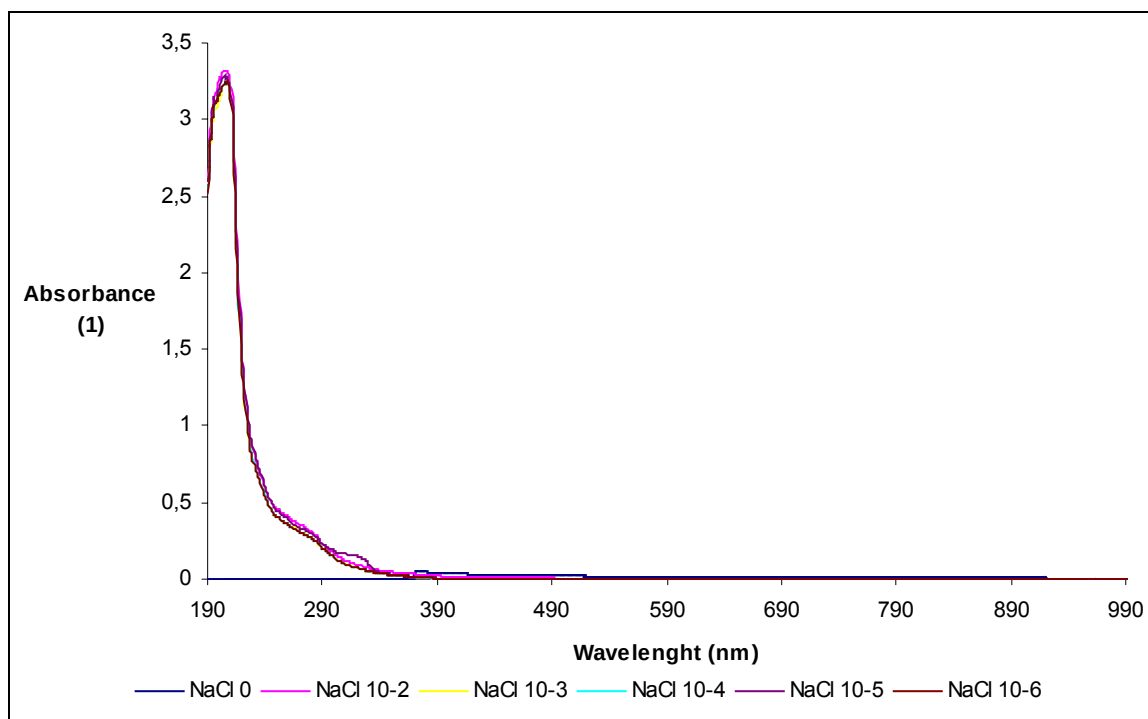


Fig. 32. These two diagrams represent relation between absorbance and increasing ionic concentration in cellulose solution. These values belong to CMC.

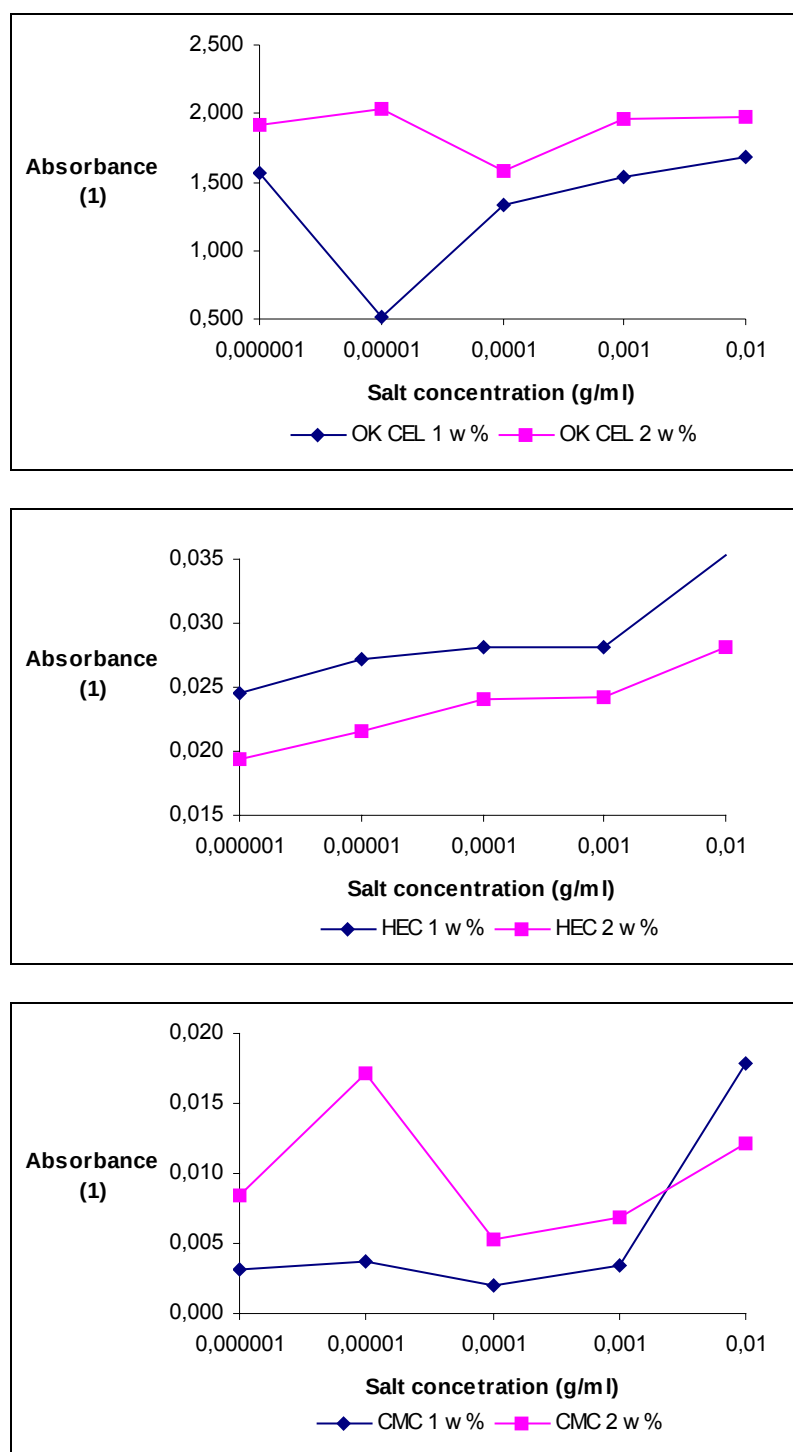


Fig. 33. These three charts present ionic concentration dependence of absorbance. These values belong to measurement with wavelength 400 nm.

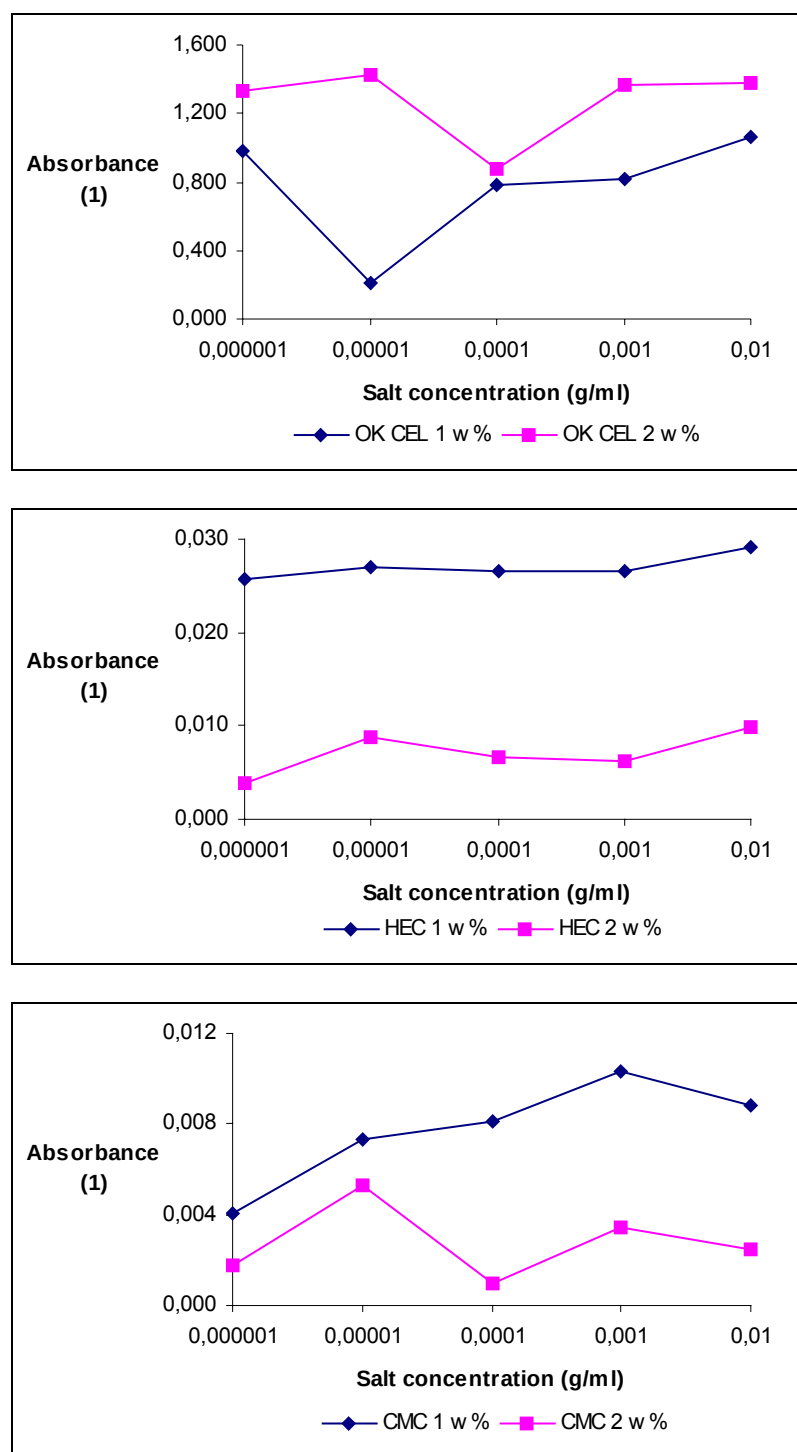


Fig. 34. These three charts present ionic concentration dependence of absorbance. This values belong to measurement with wavelength 700 nm.

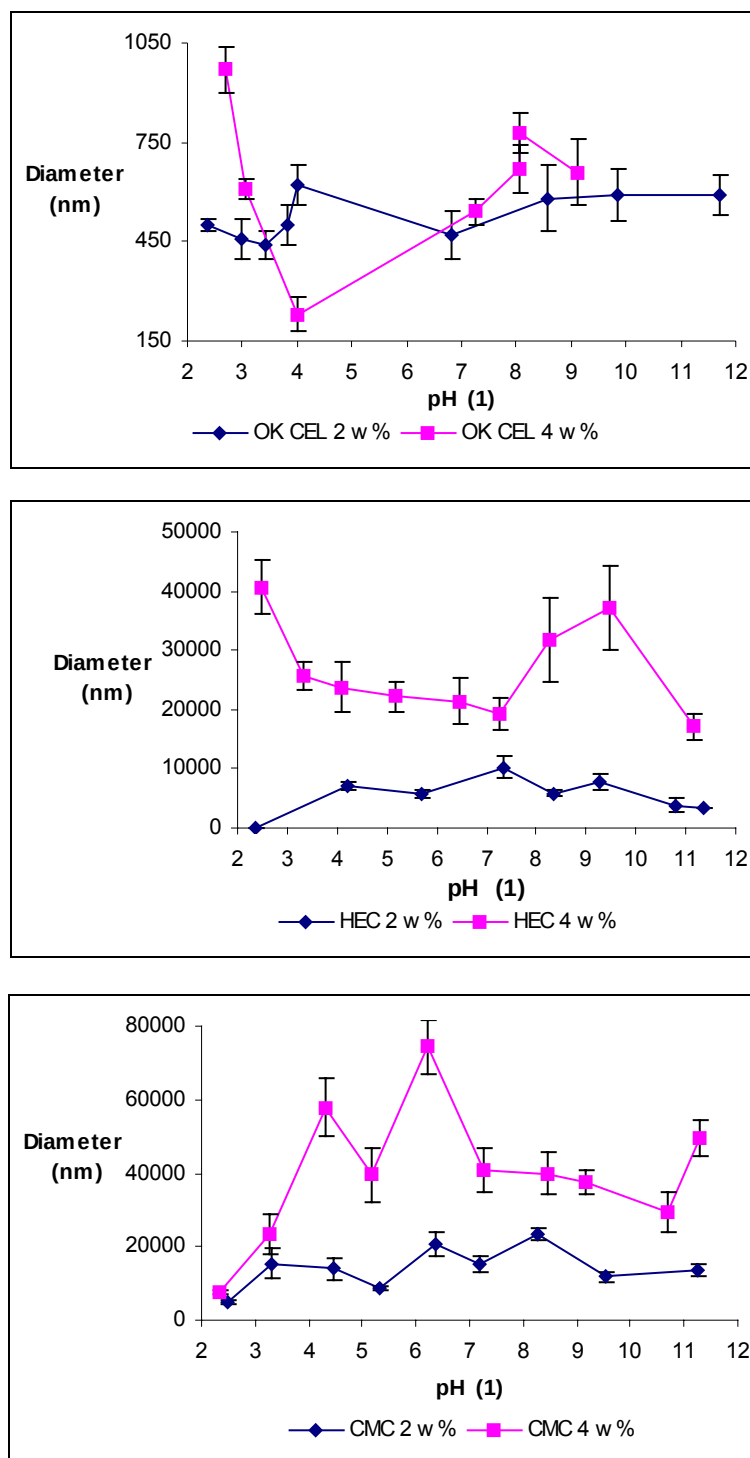


Fig. 35. These three diagrams represent data of particle diameter and their dependence on pH of cellulose solution.

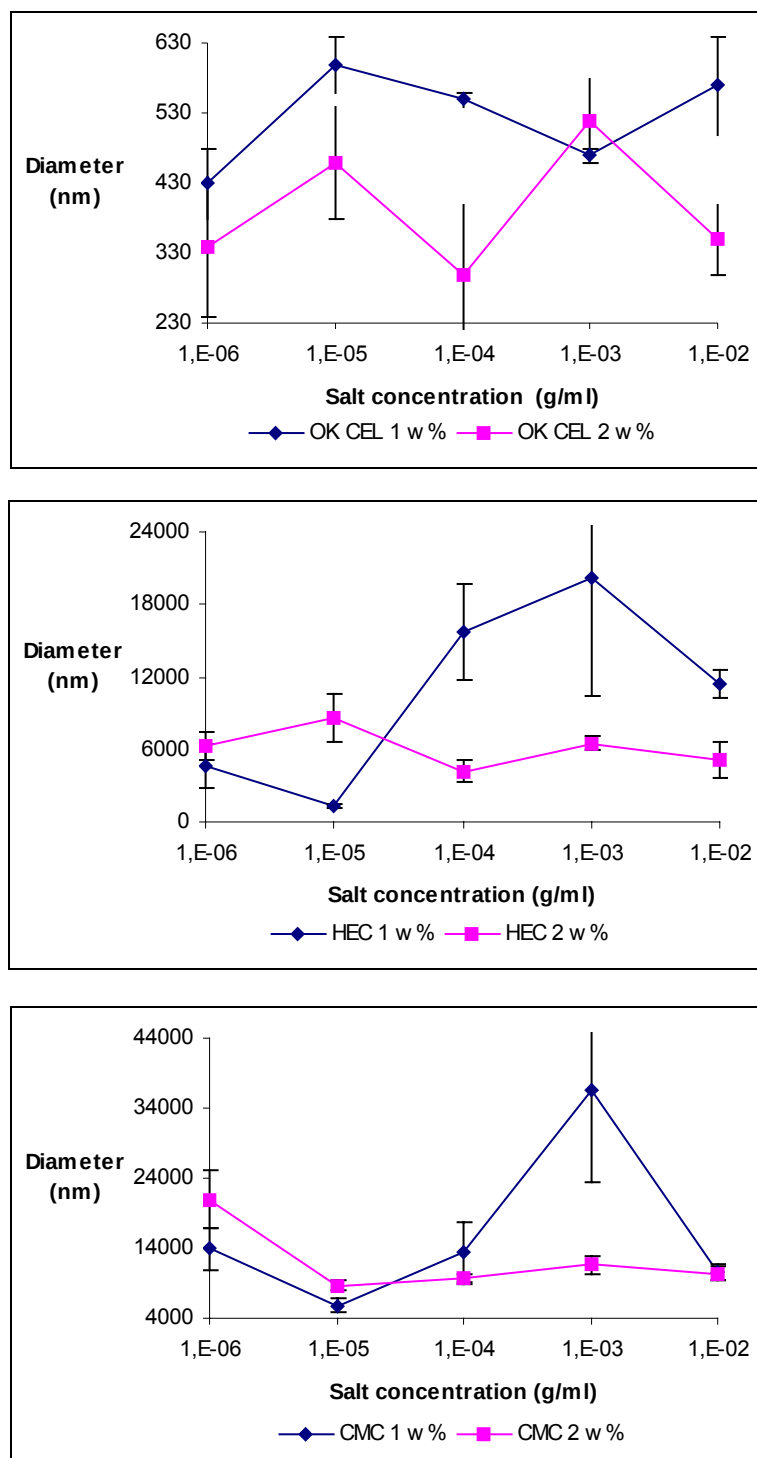


Fig. 36. These three diagrams represent data of particle diameter and their dependence on ionic concentration in cellulose solution.

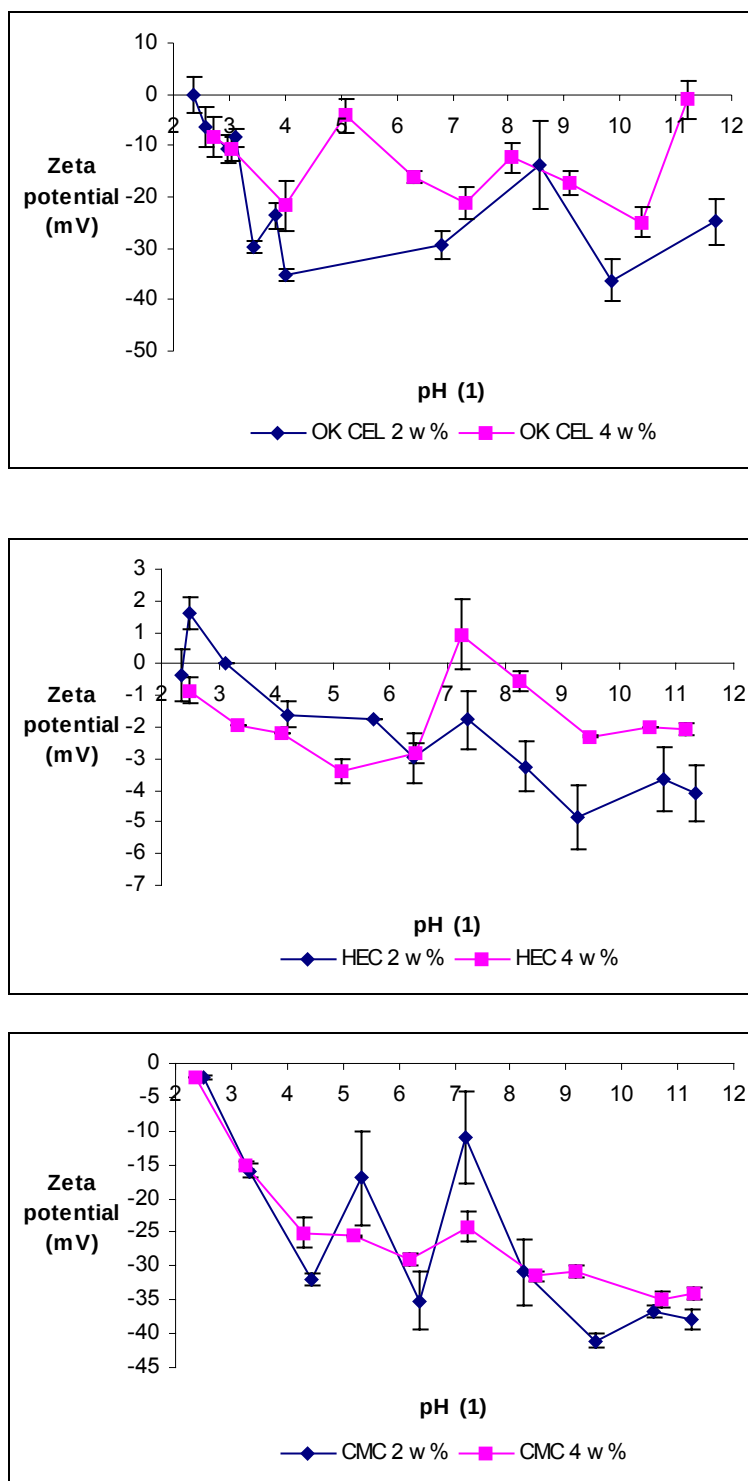


Fig. 37. These three graphs show the functionality of zeta potential and pH of cellulose solution.

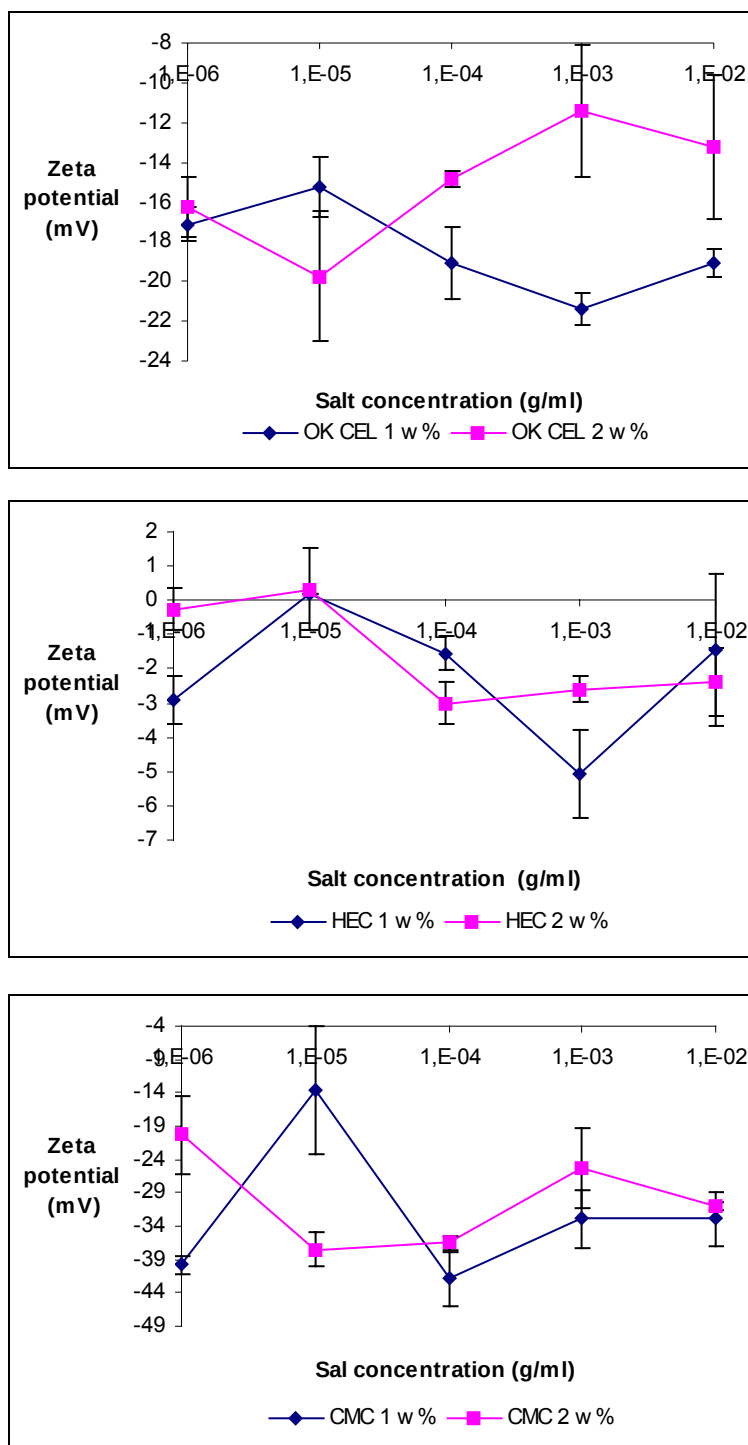


Fig. 38. These three graphs show the functionality of zeta potential and ionic concentration in cellulose solution.

CONCLUSION

The aim of this master thesis was to determine basic physico-chemical properties of sorted cellulose derivates (OK CEL, HEC, CMC). The evaluated properties were: density, surface tension, contact angle, UV-VIS light absorbance, particle size and zeta potential.

The other study was focused on temperature and concentration dependences of selected physical parameters (density, surface tension and contact angle of wetting) of cellulose derivatives in aqueous solutions. Similarly the effect of the latter conditions on UV VIS light absorbance was investigated to allow the calculation of the extinction coefficient. Last part of our work was focused on determination of the influence of counter ion concentration and pH of solution on particle size and zeta potential.

From our measurement we can conclude that density of solutions depends on solution temperature. The higher temperature means the lower density as comes from the basic theory. On the other hand higher solution concentration is the cause of higher density of solution.

Surface tension decreases with increasing temperature. But we do not have any definite conclusions for concentration of solution influence on surface tension. Obtained values of the surface tensions of cellulose aqueous solutions was around 50 mN/m, the derivatives act as the surfactants.

The measurement of contact angle brings determinations that this is only slightly dependent on concentration and temperature.

The measurement on UV VIS spectrometer determined that absorbance increases with increases solution concentration. Ionic concentration and pH influence only the light absorbance by OK CEL solution. Other cellulose derivates are stolid to these influences.

That the particle size varies by influence of varying pH of solutions or ionic concentration in cellulose solution was determined by measuring of particle diameter. This is more significant for HEC and CMC solutions; their molecules have higher absorbability.

Investigations of zeta potential brings conclusions that zeta potential is almost negative for cellulose determined in our work and that these cellulose solutions are stable in whole determined pH range and that adding of NaCl does not influence this behaviour.

We did the basic investigations of selected cellulose derivates. If we want to conclude that these derivates are suitable for applications in wound healing dressing, than other evaluations of these materials are needed.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree of Celsius
A	Absorbance (1)
b	Path length (m)
C	Carbon
c	Concentration of absorbing species (mol/m ³)
cm	Centimetre
CMC	Carboxymethylcellulose
cos	Cosine
DNA	Deoxyribonose nuclei acid
DS	Degree of substitution
E _s	Surface free energy (N/m)
F	Force acting on the balance (N)
F _b	Buoyancy force (N)
Fig.	Figure
g	Gravitation acceleration (m ² /s)
g	Gram
h	An hour
HCl	Hydrochloric acid
HEC	Hydroxyethylcellulose
K	Kelvin
L	Wetted length (m)
l	Liquid
m	Meter
ml	Millilitre

mm	Millimetre
NaCl	Sodium chloride
NaOH	Sodium hydroxide
nm	Nanometre
OK CEL	Oxycellulose
PMN	Polymorphonuclear leucocytes
ρ	Distance from diffusive element (m)
R_v	Rayleigh's diameter (m^2)
σ	Surface tension (N/m)
s	Solid
s	Second
T	Absolute temperature ($^{\circ}\text{C}$) or (K)
T	Transmittance (1)
Tab.	Table
UV	Ultraviolet
UV-VIS	Ultraviolet and visible
v	Vapour
V	Standard volume (m^3)
w	Weight (g)
w%	Weight percentage (%)
α	Electric polarizability ($\text{C}^2 \text{ m}^2/\text{J}$)
ε	Electric permittivity (F/m)
υ	Angle of light reflection ($^{\circ}$)
ϕ	Surface tension, between the corresponding interfaces (N/m)
ϕ	Luminous flux density of light after it passes through the sample (lm)
ϕ	Luminous flux density of incident light (lm)

φ_0	Luminous flux density of incident light (lm)
φ_v	Luminous flux density of diffused light (lm)
ε	Absorbitivity, constant of proportionality (m^2/mol)
λ	Wavelength (nm)
θ	Contact angle (°)
ρ	Density of liquid (g/m^3)
σ	Surface or interfacial tension (N/m)

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