THERMAL CROSSLINKING IN MEMBRANES OF POLYVINYL ALCOHOL FOR USE AS CARTILAGE

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Abstract. *Cartilage is a type of dense tissue composed of collagen, elastic fibers and chondrocytes, embedded in a firm gel. The cartilage provides rigidity to vertebrates body and in the human body presents in three different forms: elastic cartilage, fibrocartilage and hyaline cartilage. These forms are due to fiber in the matrix, and due to the needs biomechanical (resistance to mechanical stresses and flexibility) to the locations where they are located. The pathologies in the cartilage of the joints interfere with movement and mobility, causing pain and many hardships for patients. A biomaterial to be used as artificial cartilage it should present similar mechanical behavior of cartilage: resist compression, possess the ability to exude fluids that act as a lubricant between the surfaces in contact, and provide resistance to shear movement. Among the options available poly vinyl alcohol (PVA) is studied by presenting these biomechanical characteristics. In this work was produced six membranes of PVA (MM 89000-98000) 15%: 3 membranes were prepared using 1% of the thermo initiator potassium persulfate (KPS) (w / v) and 3 membranes with 1% 4,4- Azobis (4-Cyanovaleric acid) (Azobis) (w / v). After thermal crosslinked processes the morphology of the membranes were analyzed by scanning electron microscopy (SEM) and optical microscope (OM) with polarized light, was possible to visualize the difference in coloration and structure of membranes. The thermal behavior of membranes were analyzed by differential scanning calorimetry (DSC) using two heating steps. The first using temperature between 25 ° until 250 ° C, after cooling until 0ºC and the second heating since 0ºC until 300ºC , the rate 10 º C / min in N_2 atmosphere, in this study was possible to identify the peak of glass temperature (Tg) and the melting temperature (Tm) peaks, in the membrane produced to azobis initiator. The membranes produced with KPS presented only graph part of PVA crosslinked, the graph nothing presents the peaks of Tg and Tm. The membranes also were performed testing fraction sol / gel , by heat of distilled water at 85 ° C for 3hours. The membranes produced with KPS not dissolved in this condition, but the membranes produced with Azobis dissolved into parts. Through this study it was possible to verify that the samples were thermal crosslinked using both thermo initiators.*

Keywords*: Biomaterial, Poly vinyl alcohol, Thermo initiators, Hydrogel, Cartilage*

1. INTRODUCTION

In this paper was studied the thermal crosslinking process of poly vinyl alcohol (PVA) with potassium persulfate $K_2S_2O_8$ (KPS) and 4,4 – Azobis (4- Cyanovaleric acid) (Azobis) as thermal initiator.

The PVA has excellent biocompatible and lubrication properties, it is a versatile biomaterial and among various applications has been studied for use in the treatment and replacement of cartilage "Scholten *et al*. (2011)", wound dressing "Hong and Sun (2010)" and as scaffolds "Varghese and Elisseeff (2006)".

The PVA is crosslinked to have different properties mechanical, chemical and physical. The mechanical properties are the most important parameters that biomaterial should have to be used in the treatment and replacement of cartilage, because the cartilage provides the load transmission through joints, and store and dissipate this load during activity. The cartilage must be capable to suffer: compress, deforms, stresses and strain during normal and daily activities "Flik *et al*. (2007)".

Recently there is necessity the regeneration and treatment of cartilages for offer a source of increasing demand of population. The most common disease that affects cartilage is osteoarthritis (OA), there are also the damage caused by trauma and damage due to practice sports. The AO is due to wear and tearing of the cartilage causing inflammation, chronic pain, stiffness and loss of mobility, can also be linked to processes of human aging. The sports practice usually involves damage to the knee joint, total or partial disruption occurring in the middle surface and the lateral femur and tibia "Gough (2007)".

The cartilage has poor capacity for regeneration and will not heal spontaneously because is avascularizated, have characteristic aneural, low cell density, low mitotic activity of cells, and the structural restriction that does not allow free migration of cells, therefore defects in cartilage represent a major problem in orthopedics "Swieszkowsi, Fray and Kurzydlowski (2008)".

One tool that can be used to produce of artificial cartilage is the rapid prototyping (RP). The RP is a technology that allows construction of complex models with internal details that could not be manufactured by other manufacturing methods. The models are constructed from a virtual model obtained by 3D CAD, computed tomography scan , and among others. The RP has allowed construct the precise and detailed biomaterial to use in medicine, this method is characterized by an additive process, where models are built layer by layer "Souza and Ulbrich (2009)". The RP has been developed to use in medicine because aims to produce the specific devices implantable for each patient.

In this work was studied the thermal crosslinking process of PVA with two thermo initiators (KPS and Azobis). The PVA is a biomaterial that has been studied as replacements "plugs" implantable cartilaginous defect to stop the advance of wear and prevent total disruption of the cartilage. The PVA hydrogel also have been studied for use as wound dressing, the wound dressing are used to promote a physical barrier that prevents contamination of the wound by exogenous organisms, thereby facilitating healing and minimizes the scar production "Liu *et al*.(2010)". During the wound healing process, the biomaterial should have antifungal and bactericidal character; permeability of oxygen; and the capacity of adapt to the countour of deep or irregularly shaped of wounds "Sirousazar and Yari (2010)".

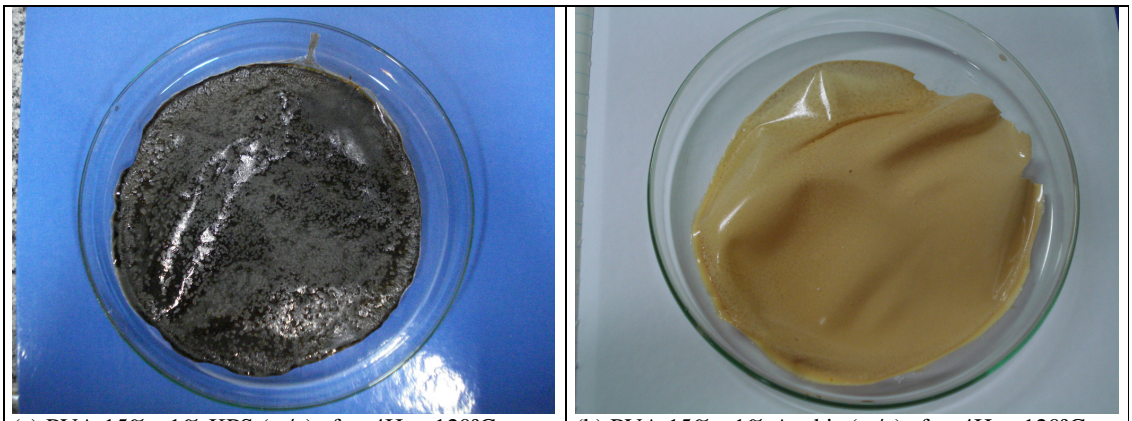
The KPS is a strongly oxidizing radical producer, and promotes coupling between polymer radicals leading to a crosslinked structure "Bolto *et al*. (2009)". The KPS is most used like initiator for polymerization in aqueous medium. It is generates sulfate ion radicals by it is thermal or photochemical decomposition. The persulfate ion induces a radical on the PVA back bone chain and the vinyl monomer. As possible that it can oxidizing some of the generated radicals (secondary alcohol) into carbonyl group "Gholap, Jog and Manohar (2004)". Using the Azoinitiator is possible to produce free radical polymerization, the polymerization reaction occur due to rate temperature "Lyoo *et al*. (2006)".

The structure of PVA is usually crystalline due to the strong intermolecular interaction between PVA chains with intermolecular hydrogen bonding. The intensity of peak in the diffraction graph and also the size of the crystals of PVA are determined by the number of PVA chains packing together "Ma *et al*. (2002)". In this study was analyzed the crystalline structure of PVA using the x-ray diffraction technique.

2. MATERIALS AND METHODS

2.1. Production and thermal crosslinking process in the membranes

In this work was produced six membranes of PVA (MM 89000-98000) 15%: 3 membranes were prepared using 1% of the thermo initiator KPS (w / v) and 3 membranes with 1% Azobis (w / v). The membranes were dried at room temperature and then crosslinked with heating at about 120°C using a ventilated oven. The permanence times of the membranes in the ventilated oven were: 3 hours, 4 hours "Fig. 1 (a , b)" , and 5 hours.



(a) PVA 15% +1% KPS (w/v) after 4H at 120ºC (b) PVA 15% +1% Azobis (w/v) after 4H at 120ºC

Figure 1- Samples after 4 hours at 120ºC (a) Sample with KPS (b) Sample with Azobis – Images by digital camera.

2.2. Visual and morphology analysis

The samples were visual analyzed by photograph digital camera, model DSC-W35 Cyber-shot, Sony trademark; scanning electron microscopy (SEM), JEOL trademark, model JXA 840A plus EDS Noram System Six, and optical microscope (OM) with polarized light – GX 51 model, mettalurgical microscope, Olympus trademark.

2.3. Fraction sol/gel analysis

The fraction sol/gel was realized using the distilled water; analytical balance model BK 400, trademark Gehaka; magnetic stirrer with heating, model RH basic 1, Ika trademark; and erlenmeyer flask with cap. The extraction was

realized with distilled water heated at 85ºC for 3 hours. The PVA hydrogel was weighed and then immersed in distilled water heated at 85ºC by 3 hours. After the extraction the specimens was removed, dried and reweighed. The amount of gel extracted was calculated by "Eq. 1".

$$\text{Gel Content } \% = Wf / Wo \quad (1)$$
$$\text{Sol Content } \% = 1 - \text{Gel Content}$$

Where "Wo" is the original weight dry PVA, and "Wf " is the weight of the PVA after extraction and drying.

2.4. Thermal analysis

The thermal analyses were realized by differential scanning calorimetric (DSC) technique, using equipment DSC Mettler Toledo model 823e. The samples analyzed were heated and cooling: initially heat 25 at 250ºC, cooling 250ºC at 0ºC and after heat 0ºC at 300ºC.

2.5. Analysis of crystallinity

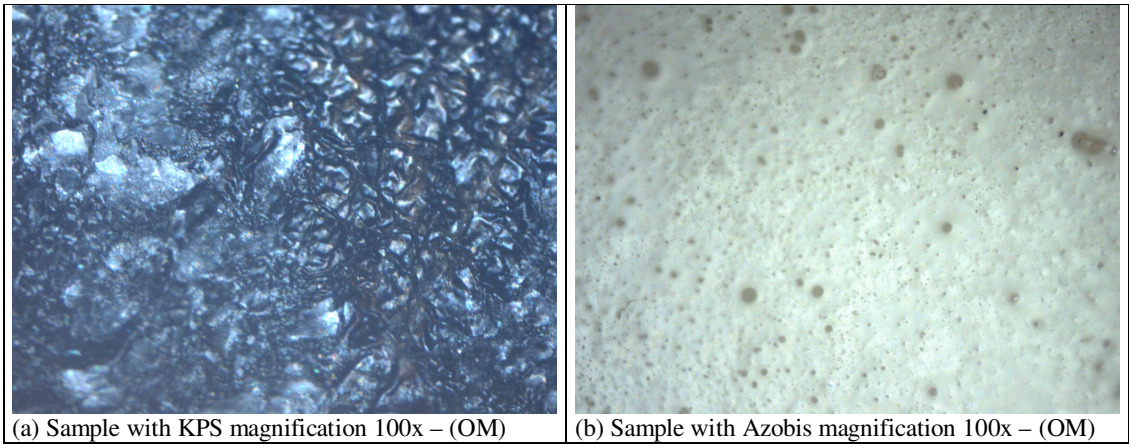
For the analyses of x-ray diffraction were used the equipment Rigaku trademark, model DMAX 2200, with radiation CuKα, filter of Ni, 20kV, 20mA. Was used step of 0,02° and time of integration of 1s.

3. RESULTS AND DISCUSSIONS

3.1. Morphology analysis

After the thermal crosslinking processes the morphology and appearance of samples become differences, the samples produced with KPS were dark coloration, and the thermo initiator Azobis produced the milky color in the samples "Fig. 2"

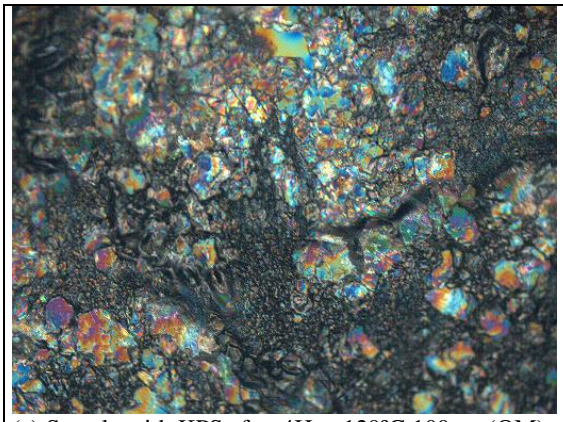
In the (OM) with polarized light was possible to visualize the difference in the structure of membranes. The visual appearance of the 6 membranes are like a brittle material, but the three membranes that were produced with the KPS are more brittle than those produced with Azobis.



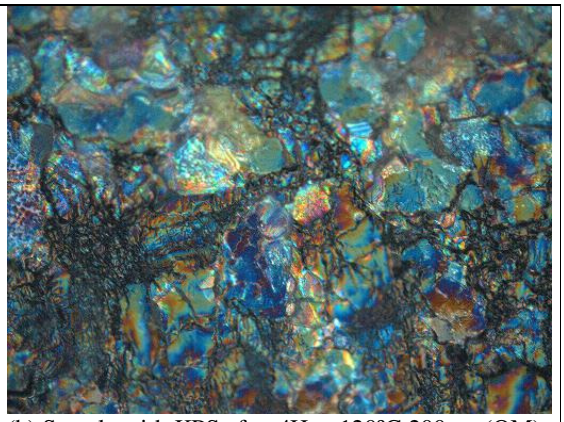
(a) Sample with KPS magnification 100x – (OM) (b) Sample with Azobis magnification 100x – (OM)

Figure 2 – Membranes of PVA 15% + 1% of thermo initiators after thermal crosslinked process – (OM) (a) KPS (b) Azobis.

In the (OM) the samples were visualized with polarized light, was possible to view the presence of thermo initiator in the matrix of PVA.

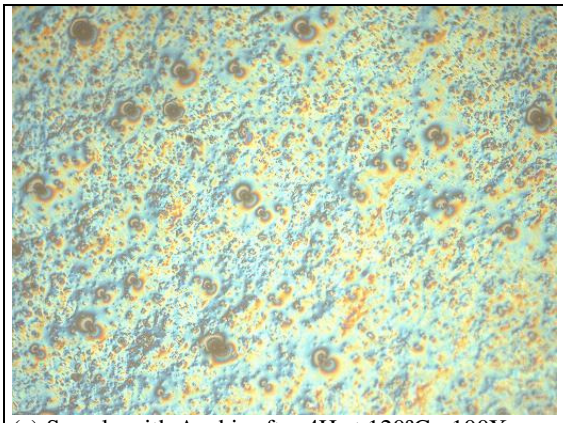


(a) Sample with KPS after 4H at 120ºC 100x – (OM) polarized light

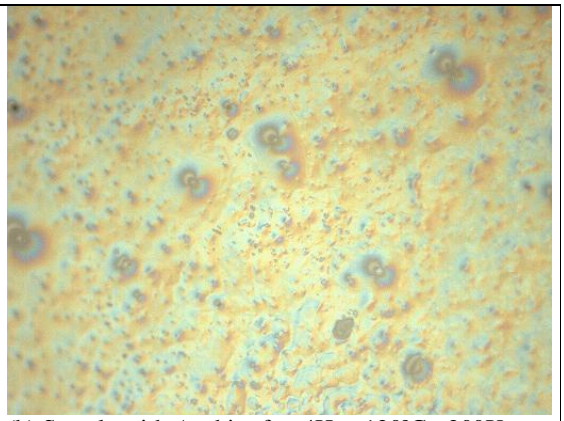


(b) Sample with KPS after 4H at 120ºC 200x – (OM) polarized light

Figure 3 – Membrane of PVA 15% + 1% KPS (w/v) analyzed of (OM) polarized light (a) 100x magnification (b) 200x magnification



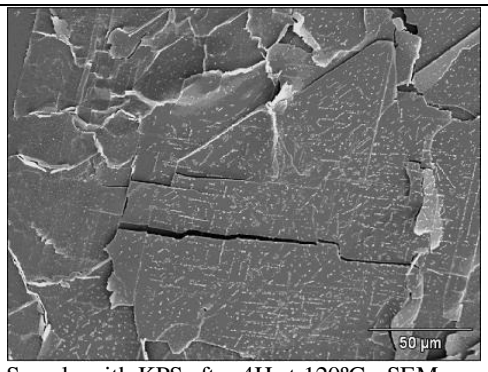
(a) Sample with Azobis after 4H at 120ºC - 100X (OM) polarized light



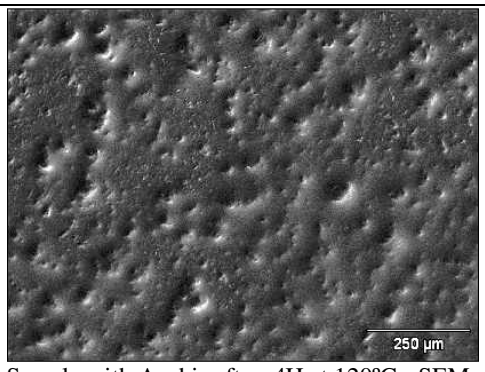
(b) Sample with Azobis after 4H at 120ºC - 200X (OM) polarized light

Figure 4 – Membrane of PVA 15% + 1% Azobis (w/v) analyzed of (OM) polarized light (a) 100x magnification (b) 200x magnification

In this study was possible view that, the two thermal initiators produce the different morphologies in the structure of the membranes. The KPS produced the brittle surface and the dark color in the membranes "Fig. 5a". In the morphology of the membrane produced with the Azobis, the thermal initiator is not scattering in the surface of membranes "Fig. 5b".



(a) Sample with KPS after 4H at 120ºC - SEM



(b) Sample with Azobis after 4H at 120ºC - SEM

Figure 5 - Membranes of PVA 15% + 1% of thermo initiators after the thermal crosslinking process – SEM (a) KPS thermal initiator (b) Azobis thermal initiator.

3.2. Fraction sol/gel analysis

The density of crosslinking produced by thermal crosslinking, was calculated by determination of the gel content in the PVA hydrogel (insoluble fraction). In this study was possible to observe that the KPS is more efficient during the thermal crosslinking process, because produced the gel fraction major than sol fraction .

Table 1. Results of performed testing fraction sol / gel

Fraction gel/sol	PVA 15% + 1% Azobis (w/v)	PVA 15% + 1% KPS (w/v)
Gel Fraction	0,15 ± 0,01	0,80 ± 0,16
Sol Fraction	0,85 ± 0,01	0,20 ± 0,16

3.3. Thermal analysis

In the analyses of DSC were possible view that the membranes produced with KPS were completely thermal crosslinked using the parameters cited (heat 120°C and time of 3H, 4H and 5H) "Fig. 6", but the Azobis not obtained the same results "Fig 7". The Azobis is less efficient for thermal crosslinked the PVA using these parameters (heat 120°C and time of 3H, 4H and 5H). These results confirm the obtained during the fraction sol/gel.

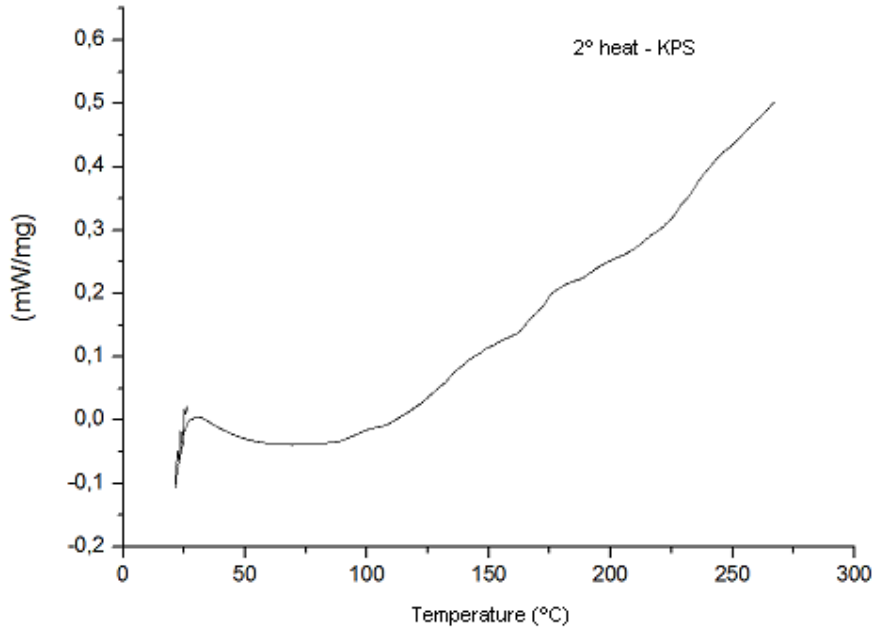


Figure 6- DSC graph of the membrane produced with KPS thermal initiator

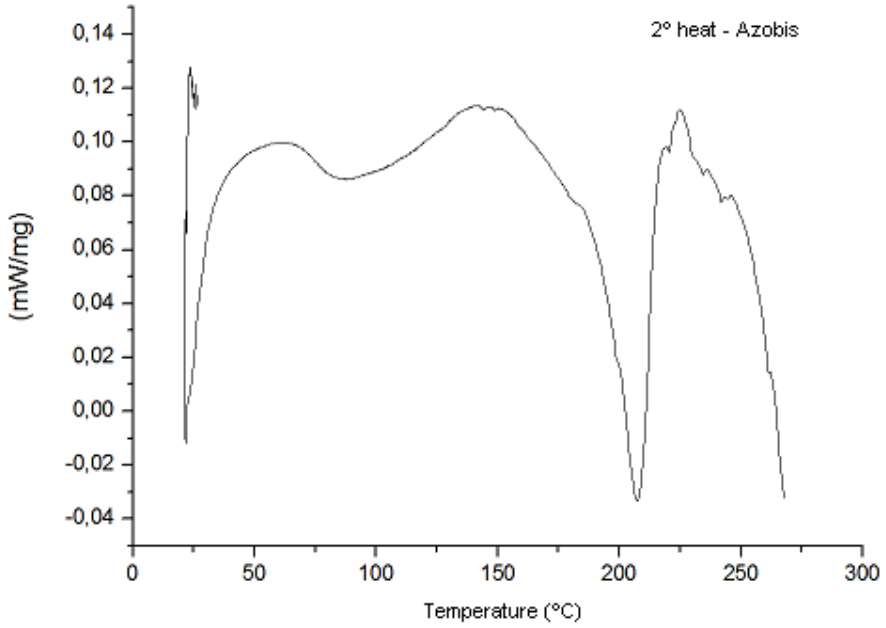


Figure 7- DSC graph of the membrane produced with Azobis thermal initiator

3.4. Analysis of crystallinity

In the analyses of X-ray diffraction the peak of cristallinity of membranes produced with KPS is bigger than peak produced wih Azobis. This indicated that the structure formed by KPS is more crystalline "Fig. 8".

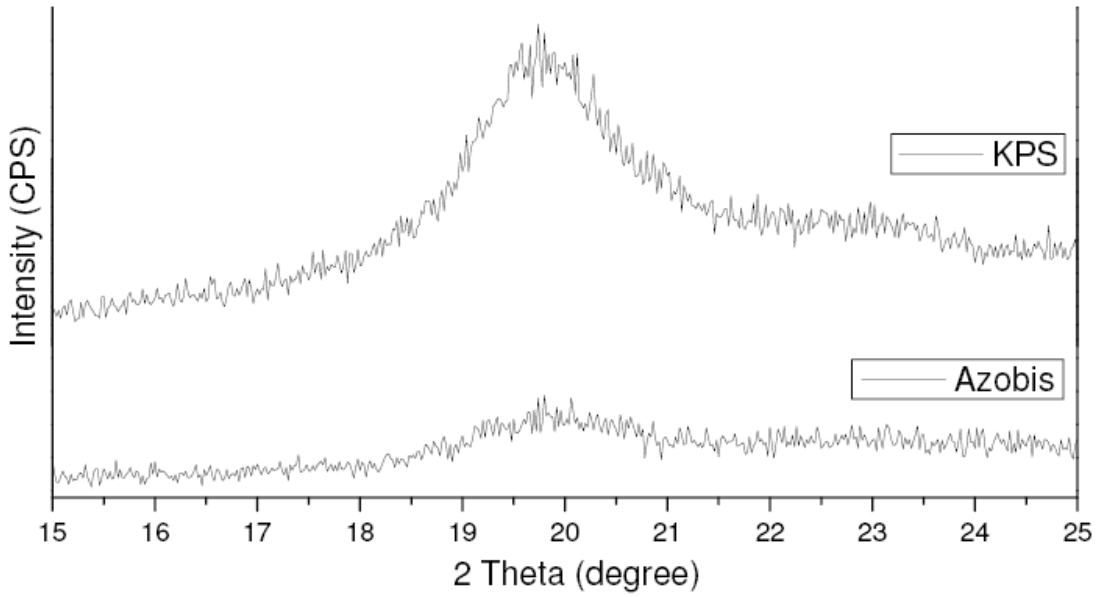


Figure 8 – X-ray graphs of membranes.

4. CONCLUSIONS

Through this study it was possible to verify that the samples were thermal crosslinked using both thermo initiators. The KPS thermo initiator was more efficient in the thermal crosslinking process, the Azobis crosslinked only parts of PVA. But as the samples were brittle couldn't be used as a medical device for treating cartilage. The samples can be used like dressing wound , because nothing presents the mechanical resistance necessary to applied like medical device for treatment for cartilage.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

BOLTO, B. et al, 2009. " Crosslinked poly (vinyl alcohol) membranes". Progress in polymer science, Australia Vol. 34, pp. 969-981.

FLIK, K. R. et al., 2007, " Articular Cartilage Structure, Biology, and Function" ed. Riley J Williams Humana Press Inc., New Jersey, p.12

GHOLAP, S. G.; JOG J. P. ; MANOHAR V. B., 2004 . "Synthesis and characterization of hydrophobically modified poly(vinyl alcohol) hydrogel membrane". Polymer Science and Engeneering Group, India, Vol. 45, pp. 5863-5873.

GOUGH, J. E., 2007, "Cartilage tissue engineering", ed Woodhead Publishing Limited, United Kingdom, p. 21

HONG, K. H.; SUN, G., 2010 "Photoactive antimicrobial PVA hydrogel prepared by Freeze-Thawing process for wound dressing". Journal of Applied Polymer Science, USA, Vol. 116, pp. 2418-2424.

LIU, X. et al, 2010. "In vivo wound healing and antibacterial performances of electrospun nanofibre membranes". Journal of biomedical materials research part A, Australia, Vol. 94A, pp. 499-508.

LYOO, W.S. et al, 2006. " Preparation of High-Molecular-Weight Poly(vinyl alcohol) with High Yield by Solution Polymerization of Vinyl Acetate in Methanol Using 4,40-Azobis(4-cyanovaleric acid)". Journal of Applied Polymer Science, Korea, Vol. 102, pp. 4831–4834.

MA, X. D. et al, 2002, "Preparation and characterization of polyvinyl alcohol–selenide nanocomposites at room temperature". Journal Materials Chemistry, Republic of China, Vol. 12, pp. 663–666.

SCHOLTEN, P. M. et al, 2011, "A semi-degradable composite scaffold for articular cartilage defects". Journal of biomedical materials research part A, USA, Vol. 97A, pp. 8-15.

SIROUSAZAR, M., YARI, M., 2010, " Dehydration kinetics of polyvinyl alcohol hydrogel wound dressing during wound healing process". Chinese Journal of Polymer Science, Iran, Vol. 28, pp. 573−580.

SOUZA, A. F.; ULBRICH, C. B. L. Engenharia integrada por computador e Sistemas CAD/CAM/CNC – Princípios e aplicações. São Paulo: Editora Artliber Ltda , 2009. 332p.

SWIESZKOWSKI, W.; FRAY, M.; KURZYDLOWSKI, K. J, 2008, "Biomaterials for cartilage reconstruction and repair", Ed. Taylor e Francis Group, 678p.

VARGHESE, S., ELISSEEFF, J. H., 2006 "Hydrogels for musculoskeletal tissue engineering". Adv. Polymer Science, USA, Vol. 203, pp. 95-144.

7. RESPONSIBILITY NOTICE

The following text, properly adapted to the number of authors, must be included in the last section of the paper:

The authors Lonetá Lauro Lima, Cecília Amélia de Carvalho Zavaglia and Vanessa Petrilli Bavaresco are the only responsible for the printed material included in this paper.