OCULAR IMPANTS: FORMATION AND CHARACTERISATION

A Thesis submitted in partial fulfilment of the requirements for the degree
of
Bachelor of Technology
in
Biotechnology
By

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Under the guidance of
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CERTIFICATE

This is to certify that the thesis entitled, **" OCULAR IMPLANTS: FORMATION AND CHARACTERISATION"** submitted by **Dheeraj Singh(110BT0035)** in partial fulfilment of the requirements for the award of **Bachelor of Technology Degree** in **Biotechnology** at National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ABSTRACT

The eye presents remarkable open doors and difficulties regarding the matter of the transport of Pharmaceuticals. While ingestion by this course is botching, there are few symptoms with routine visual dose structures like the eye drop and the eye suspension. A few polymeric frameworks have been utilized to create visual supplements for the better visual bioavailability and maintenance of medication for which the gelling frameworks have indicated focal points of advantageous organization and expanded contact time.

Solvent casting technique was used for preparing PVP/PVA ocular inserts. The arranged ocular inserts were portrayed by method for film thickness, weight variety, collapsing persistence Value, XRD, DSC, SEM.

These days, the utilization of polymers in therapeutic requisitions blankets an expansive assorted qualities of fields including inserts, prostheses, ophthalmology, dentistry and bone repair; they assume an imperative part as a provisional framework, a makeshift obstruction, and a medication conveyance framework. Poly(vinyl pyrrolidone) (PVP) and poly(vinyl Alcohol) (PVA) are incorporated in the rundown of engineered polymers which are utilized as a part of solution. PVP has a great notoriety because of its remarkable assimilation and buildings capacities, inasmuch as PVA presents imperative characteristics, for example, high hydrophilicity, perceived biodegradability, biocompatibility and great processability in film form shaping. In addition, these manufactured polymers are water dissolvable which is an exceptional trademark for film framing.

1.INTRODUCTION

A Ocular prosthesis or manufactured eye is a sort of craniofacial prosthesis that replaces a missing regular eye emulating an enucleation, destruction, or orbital exenteration. The prosthesis fits over an orbital insert and under the eyelids. Regularly alluded to as a glass eye, the visual prosthesis harshly takes the state of a curved shell and is made of restorative evaluation plastic acrylic. A couple of visual prostheses today are made of cryolite glass. A variant of the visual prosthesis is a slim hard shell known as a scleral shell which might be worn over a harmed or destroyed eye. Producers of visual prosthetics are known as ocularists. A visual prosthesis does not give vision; this might be a visual prosthesis. Somebody with a visual prosthesis is completely visually impaired on the influenced side and has monocular vision.

These days, the utilization of polymers in therapeutic requisitions blankets an expansive assorted qualities of fields including inserts, prostheses, ophthalmology, dentistry and bone repair; they assume an imperative part as a provisional framework, a makeshift obstruction, and a medication conveyance framework. Poly(vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) are incorporated in the rundown of engineered polymers which are utilized as a part of solution. PVP has a great notoriety because of its remarkable assimilation and buildings capacities, inasmuch as PVA presents imperative characteristics, for example, high hydrophilicity, perceived biodegradability, biocompatibility and great processability in film form shaping. In addition, these manufactured polymers are water dissolvable which is an exceptional trademark for film framing. By the by, this trademark could be a disservice on the grounds that the material might break up

in contact with liquids into the human body. Consequently, the proper combo of polymers and added substances could diminish the solvency in water and expand or adjust the mechanical properties, and in addition to get helpful films for a medium or long haul inserts.

The PVP ring holds a proton accepting carbonyl group, while PVA has hydroxyl molecules so hydrogen bond formation takes place preferably. These sorts of associations impact the mix in numerous angles, including the dissolvability and the mechanical properties. Additionally, the utilization of operators for crosslinking is a profitable method for get materials with some "perfect" aspects. The point of the present work was to plan and describe PVA/PVP mixes utilizing glutaraldehyde (GA) and lactic corrosive (LA) with a specific end goal to get suitable films with prospective restorative requisition[9]. Data about mechanical properties, level of swelling and solvency degree were acquired in the present exploration.

Polymer mixing is a helpful system for planning materials with a wide assortment of properties. A vital business preference is that polymer mixes offer a way to process new materials by utilizing effectively existing polymers, which in this way lessens advancement costs. Polyvinyl pyrrolidone (PVP) merit an exceptional consideration around the conjugated polymers as a result of great ecological solidness, simple processability, moderate electrical conductivity and rich physical science in control transport instrument. The nearby change of the compound structure affects extreme changes in electronic properties. The composite materials comprising of leading medium in the protecting polymer network give palatable mechanical and in addition electrical properties. Polyvinyl Alcohol is a potential material having a high dielectric quality[10], great

charge stockpiling limit and dopant-depending electrical and dopant depending optical properties. Both of the PVP and PVA are water-dissolvable and miscible in all extents, PVA was chosen as the part in the mix.

Poly (vinyl Alcohol) (PVA) is semicrystalline, water dissolvable, non lethal, better film and fiber structuring, biocompatible, amazing synthetic safety, great mechanical properties and biodegradable engineered polymer which is broadly utilized as a part of the biomedical field. Poly (vinyl pyrrolidone) (PVP) is a shapeless vinyl polymer which has wide provisions in biomedical field due to its properties including bond, phenomenal physiological similarity, low danger and sensible dissolvability in water and most natural solvents. At the point when these two polymers are blended, the cooperation between poly (vinyl Alcohol) and poly (vinyl pyrrolidone) are required to happen through intermolecular hydrogen holding between the hydroxyl gathering of PVA and carbonyl gathering of PVP[6].

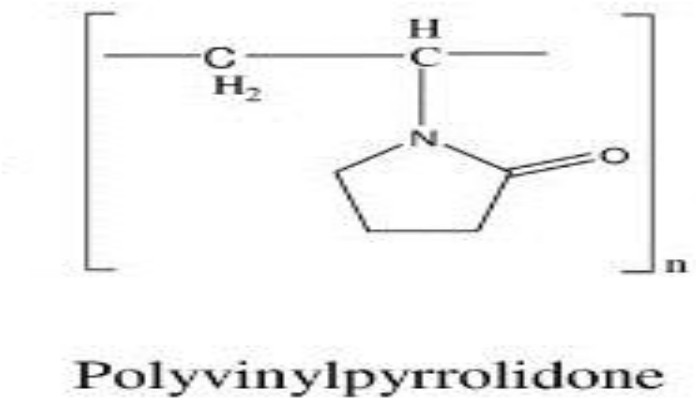
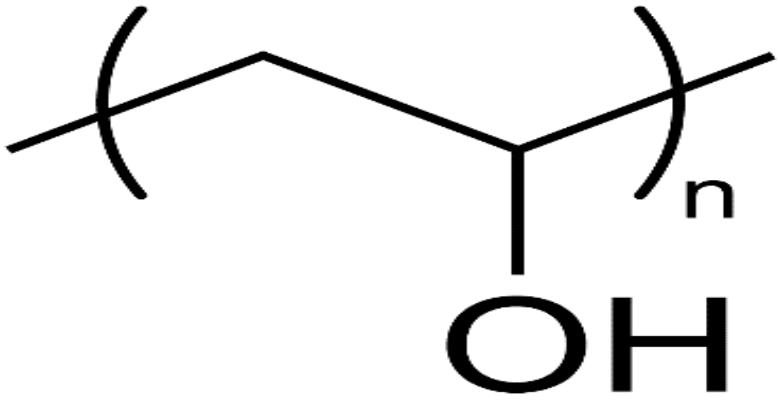


Fig. 1. Structure of PVP



Polyvinyl Alcohol

Fig. 2. Structure of PVA

Polymer mixes are physical mixtures of two or more structurally diverse homopolymer or copolymers and they cooperate through optional powers with no covalent interaction. Polymer mixes are ready by different systems and around them result mixing is exceptionally basic and fast in light of the fact that it obliges straight forward supplies, for example, glass plates just and not included any convoluted methodology [3]. Mixing of three or more polymers has turned into an inexorably critical strategy for planning materials with appropriately customized properties not the same as those of the constituent polymers. Mixing of polymers may bring about lessening their essential expense, enhancing their transforming and boosting their critical properties. The expansion in properties of the mix relies on upon the level of similarity or miscibility of polymers at the atomic level. Contingent on the level of sub-atomic blending, mixes may be considered good mixes or totally miscible, semicompatible or semi-miscible, incongruent or immiscible mixes. The degree of similarity or miscibility brings about inside and out diverse morphologies of

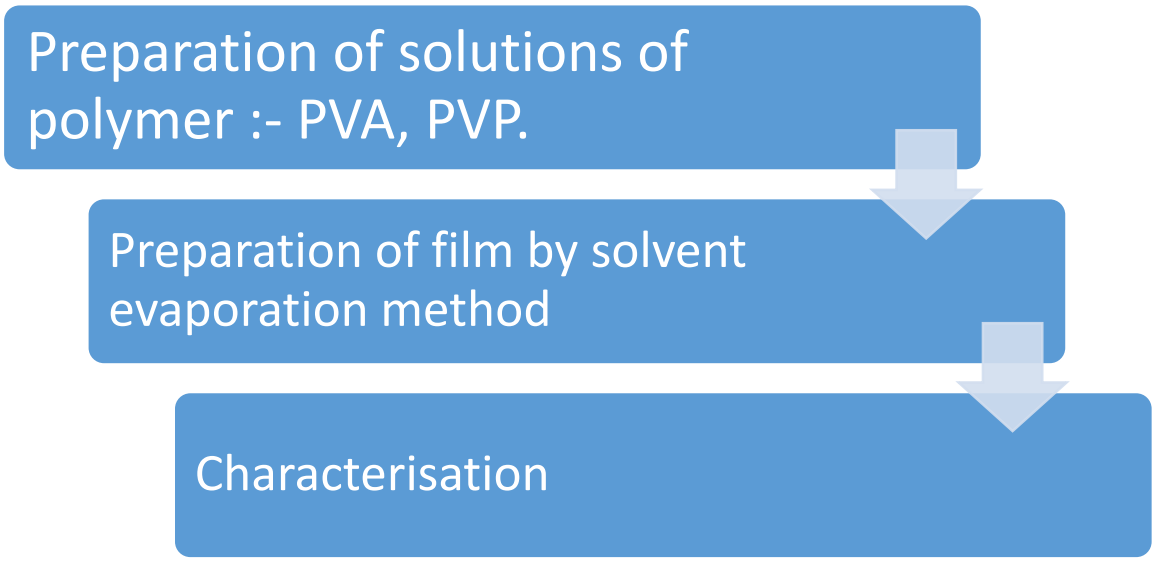
the mixes, extending from single stage to multiphase frameworks. Similarity of polymer mixes can likewise be anticipated utilizing differential checking calorimetric system. Miscible mix films shows one stage and single glass move temperature (Tg). Immiscible mixes are stage differentiated and show glass transition temperature (Tg) of every part.

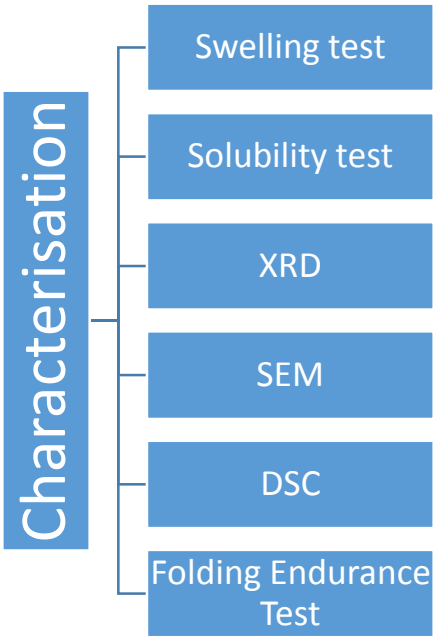
The eye as an entry for pill conveyance is largely utilized as local treatment against systematic treatment keeping in mind the end goal to stay away from the danger of eye harm from high blood concentration of the medicine, which is not required. The extraordinary life structures, physiology and organic chemistry of the eye render this organ impenetrable to outside substances, therefore displaying a steady test to the formulator to dodge the defensive boundaries of the eye without bringing on changeless tissue harm.

Most visual medicines like eye drops and suspensions cell for the topical organization of ophthalmically dynamic pills to the tissues around the visual cavity. These measurements structures are not difficult to impart yet experience the ill effects of the characteristic disadvantage that dominant part of the solution they hold is promptly weakened in the tear film when the eye drop result is imparted into the circular drive- and is quickly emptied away out of the percorneal hole by steady tear stream and lacrimo-nasal waste . Thus, just a little part of the ingrained measurements is assimilated by the target tissue hence, focused results and regular dosing are needed for the instillation to accomplish a satisfactory level of remedial impact. One of the new classes of medication conveyance frameworks, visual additions, which are picking up overall applause, discharge drugs at a prearranged rate for a more drawn out period by expanding the precorneal living arrangement time.

The novel structure of the human eye and also presentation of the eye straight forwardly to the environment makes it helpless against various phenomenal irresistible diseases brought about by growths. Host defences administered against these microorganisms, once anatomical hindrances are ruptured, are frequently lacking to avert misfortune of vision. Hence, the opportune distinguishing proof and medication of the included microorganisms are foremost. Case in point, filamentous contagious diseases of the eye are as a rule because of infiltrating trauma by items debased by vegetable matter of the cornea or globe or, by growth, of disease from nearby paranasal sinuses. Parasitic endophthalmitis and chorioretinitis, then again, are generally the consequence of precursor fungemia seeding the visual tissue. Candida spp are the most widely recognized reason for endogenous endophthlamitis, in spite of the fact that starting disease with the dimorphic organisms may prompt contamination and scarring of the chorioretina. Contact lens wear is connected with keratitis brought on by yeasts, filamentous growths, and Acanthamoebae spp[7].

OBJECTIVE


Preparation of solutions of polymer :- PVA, PVP.
Preparation of film by solvent evaporation method
Characterisation


Characterisation
Swelling test
Solubility test
XRD
SEM
DSC
Folding Endurance Test

2. LITERATURE REVIEW

Novel interpenetrating polymer system layers were produced out of poly(vinyl alcohol)/poly(vinyl pyrrolidone) mixes of diverse arrangements. The two polymer parts were freely crosslinked artificially with glutaraldehyde and photochemically with 4,4- diazostilbene-2,2-disulfonic corrosive disodium salt. The layer exhibitions were contemplated in pervaporation of tetrahydrofuran (THF)/water and THF/methanol mixtures. It was discovered that the layers were fabulous in THF parchedness, yet a great deal less proficient for the partition of THF/methanol mixtures. The pervaporation effects were steady with the layer swelling information[1].

The use of polymer mixes in medication conveyance frameworks has been carried out effectively as a result of the basic and productive systems for planning of polymer grids from mixes having craved blend of properties. Mixes of polyvinylalcohol (PVA) with common polymers e.g., hyaluronate,1 pullulan,2 chitosan,3–6 collagen,7 starch,8 and cellulose9 with enhanced biodegradability, mechanical and warm properties have been accounted for. A gathering of specialists prepared bioartificial materials focused around the mixes of poly (vinylalcohol-co-acrylic corrosive) and dextran[4].

Since (PVP + PVA) polymer mix is amorphous in nature, the dipolar atoms ought to have the capacity to arrange starting with one position then onto the next all the more effectively and will help assimilation over a wide recurrence also temperature range[2]. Changes in surgical methods, anesthesia, insert materials and design throughout the most recent decades have essentially enhanced clinical results and patient fulfillment. Moreover, the capability to all the more

successfully manage the long term complications of the anophthalmic attachment, for example, enophthalmos, the exposure and the lower lid laxity (ectropion), have enormously moved forward. Today, most patients can unhesitatingly come back to their day by day exercises with great nonessential effects emulating the evacuation of an eye[12].

Polymer-layer silicate nanocomposites have attracted much attention due to their improved properties, for example, increased mechanical strength and heat resistance, decreased gas permeability and flammability and compared to the base polymer[5].

The sonophoretic transport rates of monomeric insulin and vasopressin crosswise over human skin in vitro in the vicinity of a 20 khz ultrasound field are indicated to contrast considerably relying upon whether particles enter the skin from a saline result or from a thick ultrasonic coupling medium. Hypothetically, the decrease in sonophoretic transport brought about by the hydrogels might be illustrated by limit layers that structure inside the hydrogel owing to the generally fast rate of sub-atomic transport over the (ultrasonically) penetrated stratum corneum and additionally poor diffusive mass exchange between the skin and gel. The effects of in vitro trials performed with an air conditioner current going with the ultrasound indicate that the mass-exchange hindrance postured by the hydrogel could be disposed of for both vasopressin and insulin by smothering the diffusive limit layers, showing that moderately high rates of sonophoretic atomic transport crosswise over human skin are achievable when hydrogels are utilized as the ultrasound coupling medium as long as strategy is utilized to prompt sub-atomic blending inside the gel[8].

3. MATERIAL AND METHODS

3.1 MATERIAL REQUIRED

1. Blood
2. NACL
3. Trypan blue
4. 0.1N HCL
5. PVA (Poly Vinyl Alcohol)
6. PVP (Poly Vinyl Pyrrolidone)
7. PBS
8. KCL
9. EDTA
10. Distilled Water
11. Normal Saline (0.9 %)
12. Na2HPO4
13. KH2PO4

3.2 APPARATUS REQUIRED

1. Beaker
2. Falcon Tube
3. Petri Dish
4. Measuring Cylinder

5. Micropipette

3.3 INSTRUMENTS REQUIRED

1. Magnetic Stirrer
2. Incubator
3. Shaker
4. Magnetic Stirrer
5. Incubator
6. Shaker
7. Centrifuge
8. Hemocytometer
9. Spectrophotometer
10. Microscope
11. Weighing Balance
12. Differential Scanning Calorimeter
13. Scanning Electron Microscope
14. X Ray Diffracter

3.4 SOLUTION PREPARATION

PVA (Poly Vinyl Alocohol)

2 solutions of PVA was prepared of different concentrations.- 3%, 5%

5% PVA Solution –

1. Weigh 2.5 gm of PVA using weighing balance.
2. In beaker take 47.5 ml of distilled water.
3. Beaker containing water was kept on the magnetic stirrer at 150 C and 500 rpm.
4. The PVA is now slowly dropped inside the beaker.
5. Once the homogenous transparent solution is prepared, magnetic stirrer is switched off and the solution is ready for the test.

3% PVA solution:-

1.5gm of PVA + 48.5ml distilled water and mixed on the magnetic stirrer in a beaker till a homogenous transparent solution is prepared.

PVP (Poly Vinyl Pyrrolidone)

Similar to PVA two solution of PVP were prepared of 2% and 5% concentration respectively.

5% PVP = 2.5mg of PVP + 47.5 ml distilled water

2% PVP = 1 mg of PVP + 49 ml distilled water

Normal saline

0.9% normal saline was used for this experiment. Normal saline is used for blood dilution and as the negative control for hemolysis as the hemolysis is very less for Normal saline i.e. 0%.

0.9% solution = 0.45 gm of NACL + 50 ml of distilled water

3.5 HEMOCOPATIBILTY TEST

10 ml of 0.9% saline was added to 8 ml of blood. The diluation of blood was in the ratio 10:8. N ow in 9 ml of saline 0.5 ml of diluted blood was added and for test sample 0.5 ml of samples were added to that .For positive and negative control 0.5 ml of 0.1 NHCL and 0.5 ml of saline were added instead of test sample to the mixture of blood and salines (9 ml saline + 0.5 ml diluted blood). The samples were incubated along with positive and negative control for 60 min and the centrifuged at 1000 rpm for 5 min to settle all the blood components. Now the supernatent is taken and its OD is measured using spectrophotometer at 545nm.

% hemolysis = 100* (OD sample- OD-ve control)/ (OD= +ve control- OD-ve control)

3.6 TRYPAN BLUE EXCLUSION TEST

This exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cell posses intact cell membranes that exclude certain dyes like trypan blue.

A cell sample is mixed with dye and hen visually examined to determine whether cell take up or exclude dye.The visualization is done using microscope and dye mixture Is visualized on haemocytometer.

Stain cells-dead cells

Unstained cells-live cells

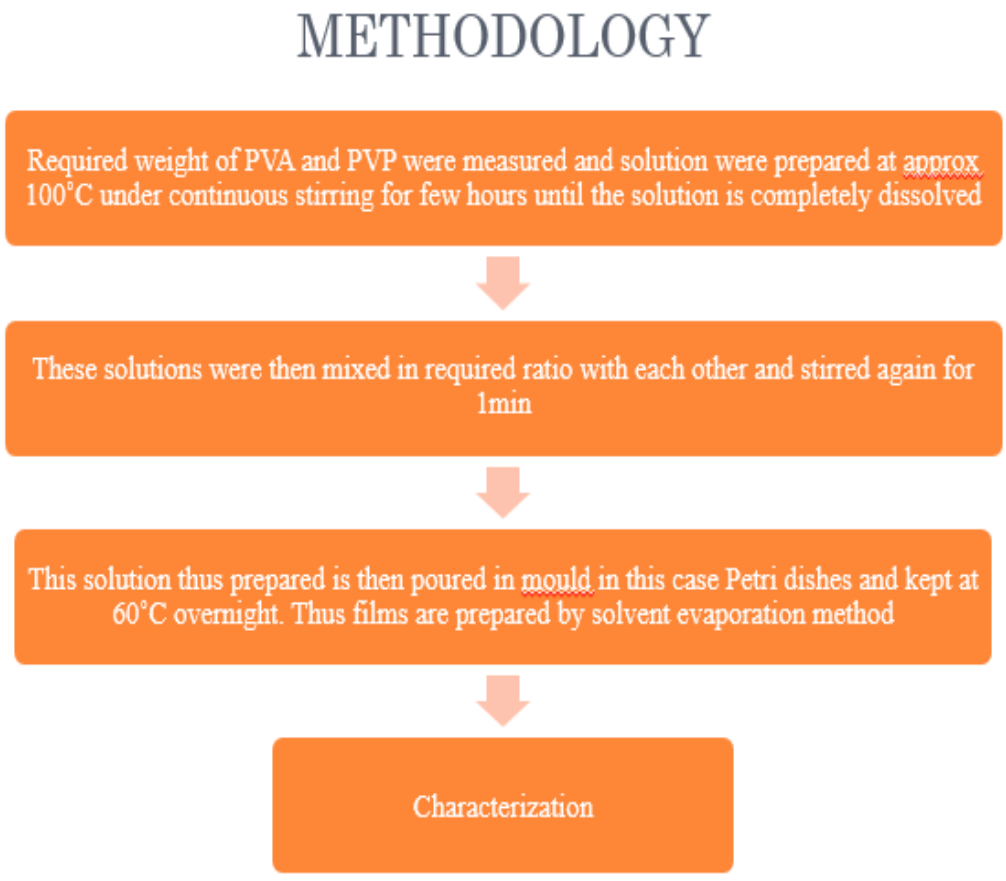
3.7 FILM FORMATION

Films formed by blends made from PVA and PVP are thermodynamically stable with excellent physical and mechanical properties which are non-toxic and biocompatible to biological samples. Both polymers have tendency to form complex with each other forming hydrogen bond.

The Solutions of PVA and PVP were mixed in required amount and stired for proper mixing. Then the solution was poured in petri dish and kept Overnight at 60˚C to evaporate solvent. This method of Formation of film is called Solvent evaporation Method[11].

Two samples were made:

- Sample 1: 5 % PVA + 5% PVP (1: 1)
- Sample 2 : 3% PVA + 2 % PVP (1:1)



3.8 SWELLING TEST

Tested by water absorption calculation

$$W_a = M_2 - M_1 \,/\, M_1$$

Where,

M_2 = weight of water soaked film

M_1 = weight of dry film (initial)

Squares of 1cm were cut and initial weight (M_1) is checked. Then, they were immersed into 5 ml of PBS .After every few hours, the samples were taken out and the surface moisture was carefully removed by paper napkin. They were weighted again (M_2).Finally, samples were allowed to dry until constant weight at 60ºC.

3.9 FOLDING ENDURANCE TEST

In Folding testing,folding endurance is characterized as the logarithm (to the base of ten) of the amount of two fold overlap that are obliged to make a test piece break under standardized conditions.

$$F = \log_{10} d,$$

where F is the folding endurance and d the number of double folds.

The folding endurance test measures a combo of elasticity, extend, and exhaustion properties. This test is likewise helpful for measuring the decay of polymer after maturing as it is delicate to switches which appear much sooner than there is a change in pliable, blast, or tearing safety. Slight expands in relative dampness cause a checked build in collapsing persistence. This, coupled with the amazingly little territory tried, brings about wide varieties for unique specimens. In light of this variability, collapsing continuance is not regularly utilized as a particular unless a tolerance of no less than 20%

is took into consideration high-review polymers and 30% for customary polymers. Checks the ability to withstand folding thus indicator of brittleness. Folding endurance test was done to calculate elasticity and the plasticity of blend films.

3.10 SEM STUDY

Film morphology , composition and measurements of the filaments were considered utilizing filtering electron magnifying lens with a quickening voltage of 20 kv . Got pictures were investigated utilizing Imagej programming for the figuring of the normal width of the film. An examining Scanning Electron Microscope (SEM) is a sort of electron magnifying instrument which prepares pictures of an example by filtering it with a centered light emission. The electrons from the machine interface with electrons in the sample, processing different indicators that are discovered and subsequently we get data in regards to the specimen's surface geography and structure. For SEM dissection, a little bit of specimen is taken which is given a platinum covering and afterward it is put on the SEM multi-holder. It was then embedded into the chamber and on provision of high quickening voltage , SEM pictures were gotten.

3.11 XRD ANALYSIS

To secure the crytallinity of the specimen this study was done. X-beam crystallography is a technique utilized for deciding the nuclear and sub-atomic structure of a gem, in which the crystalline particles cause a light emission-beams to diffract into numerous particular bearings and accordingly these diffracted beams gives a thought regarding the crystalline structure of the atom. In this strategy, the specimen was kept in the example holder and embedded in the

machine which from there on processed a chart with trademark crests for diverse examples consequently demonstrating their crystalline nature.

3.12 DSC (DIFFERENTIAL SCANNING CALORIMETRY)

The glass transition temperature, Tg, and other heat transition of the mix samples was measured on a differential filtering calorimeter (DSC), in an inactive environment, to minimize oxidative corruption, from room temperature to 600°c at an examining rate of 10°c/min. The mass of each one specimen was about 10 mg. The effects were recorded and investigated. The glass transition temperatures (Tg) were acquired from the midpoint of the transition and the melting temperatures (Tm) and decomposition temperature (Td) from the greatest of the dissolving crest.

3.13 SOLUBILITY TEST

Solubility factor

(SF)% = M_1-M_3 (100) **/M_1**

where M_3 = weight of film after drying

M_1 =weight of film(initial)

Squares of 1 cm^2 were cut and weighed (M_1).Then, they were immersed into 5 ml of PBS. Afterwards, the samples were taken out from the water and the surface moisture was carefully removed by paper napkin. Samples were allowed to dry until constant weight at 60ºC and weighted once more (M_3).

4. RESULTS AND DISCUSSION

4.1 HEMOCOMPATIBILITY TEST

The hemocompatibility of the PVA and PVP samples have been tabulated in Table 1 and 2. The results indicate that the samples are highly hemocompatible in nature indicating its biocompatibility. Hence the PVP/PVP films may be tried as Ocular implant.

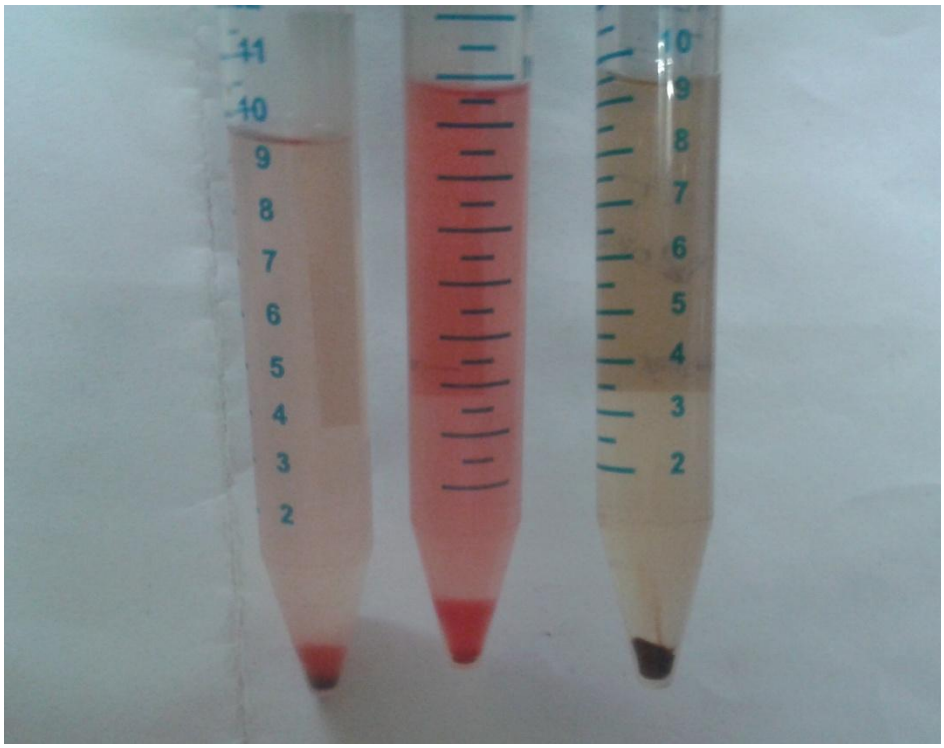


Fig. 3. Hemocompatibility test

FOR PVA :-

Sample	OD	%hemolysis
+ve control	0.838	
_ve control	0.095	
5% pva	0.104	1.21
3% pva	0.102	0.94

For PVP :-

Samples	OD	%hemolysis
+ve control	0.901	
-ve control	0.164	
5% pvp	0.190	3.53
2% pvp	0.171	0.94

4.2 TRYPAN BLUE ASSAY

After the cell suspension was mixed trypan blue dye and visualized under microscope on haemocytometer , We see that the cell which are not dead remain unstained as their cell wall is intact. The cells whose cell wal has been compromised were stained blue .

For +ve Control

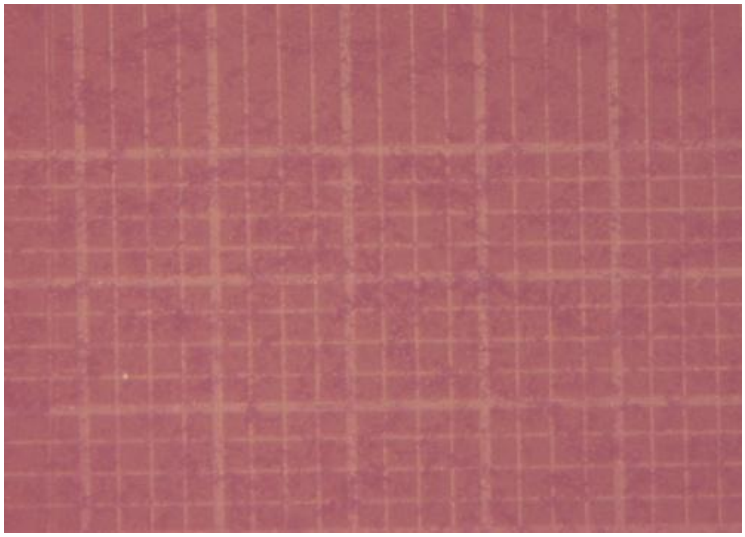


Fig. 4. Trypan blue assay for +ve Control

For –ve Control

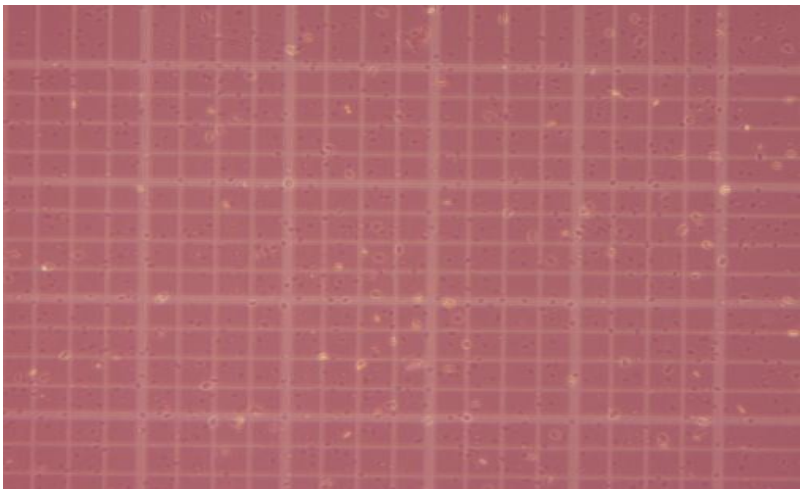


Fig. 5.Trypan Blue Assay for –ve Control

For PVA

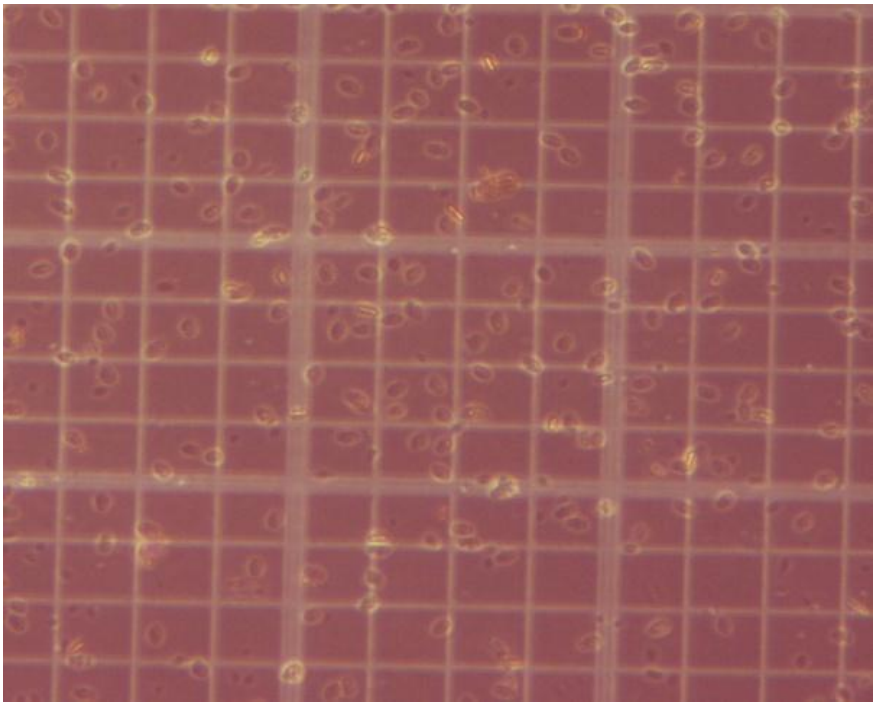


Fig. 6. Trypan Blue Assay for PVA

For PVP

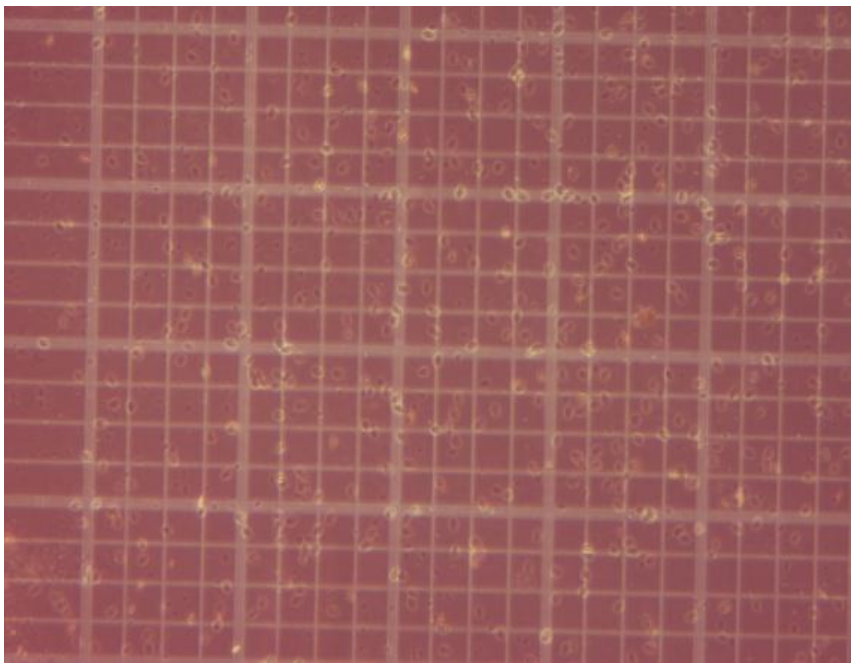


Fig. 7. Trypan Blue Assay for PVP

4.3 SWELLING TEST

PVA crystallites in the membranes play a double role:

(1) reduce the absorbed solvent and

(2) restrict the swelling of the membrane by a physical crosslinking effect.

The degree of swelling is higher for PVA/PVP blends than just for PVA. It could be explained because PVP reduces the crystallinity of the blend.

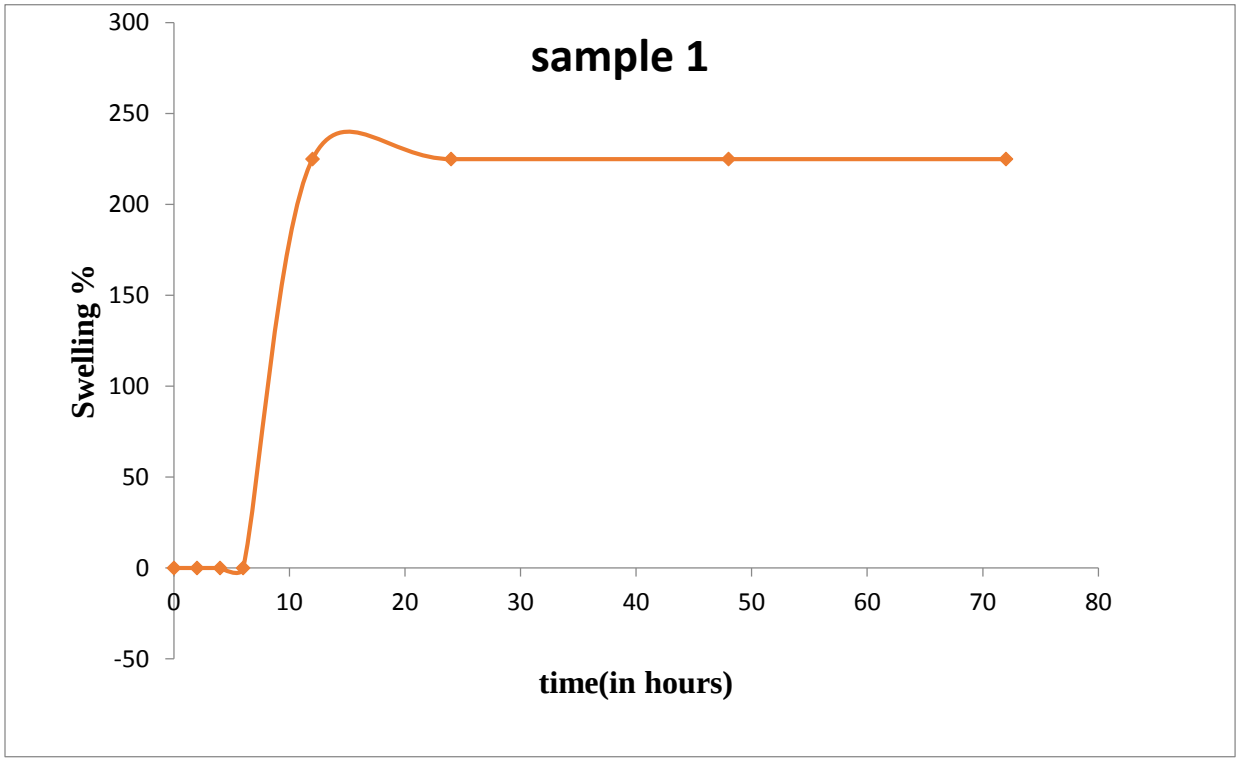


Fig. 8. Graph of swelling For Sample 1

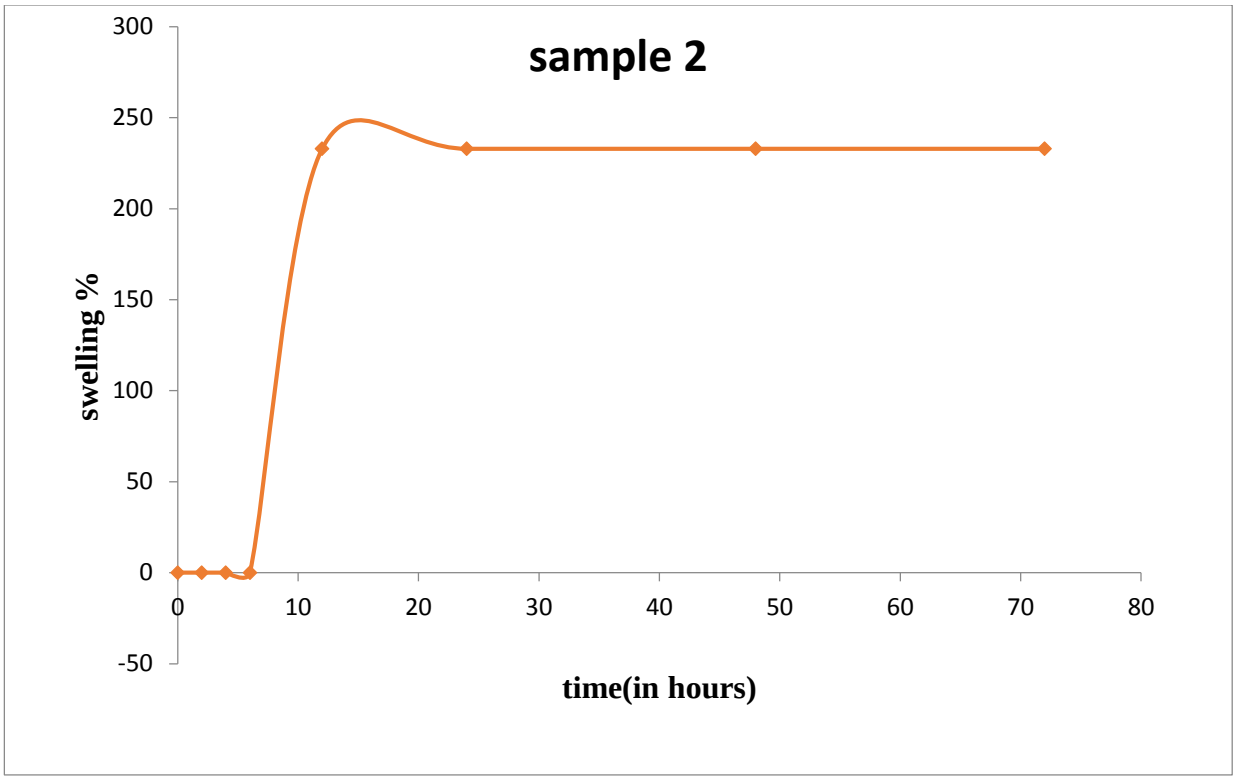


Fig. 9. Graph of Swelling for Sample 2

4.4 FOLDING ENDURANCE TEST

The sample was folded in the mid point, between the thumb and the finger and after that it was opened. The procedure is redone till the supplement demonstrated breakage or split. The aggregate collapsing operation is Folding endurance value.

- NO CRACKS OR BREAKS UNTIL D = 198
- The films that contains both pva and pvp showed more endurance coz of their regular polymeric structure which is difficult to be broken easily.

4.5 DSC (DIFFERENTIAL SCANNING CALORIMETRY)

The Thermal Behavior of PVA/PVP mix doped were researched by differential scanning calorimetry to gauge heat transition of the arranged mix Film. The glass transition temperature,

T mix increments with increase in mixing of PVP. This is come about because of intermolecular communication (hydrogen bonding) shaped around PVA, PVP.

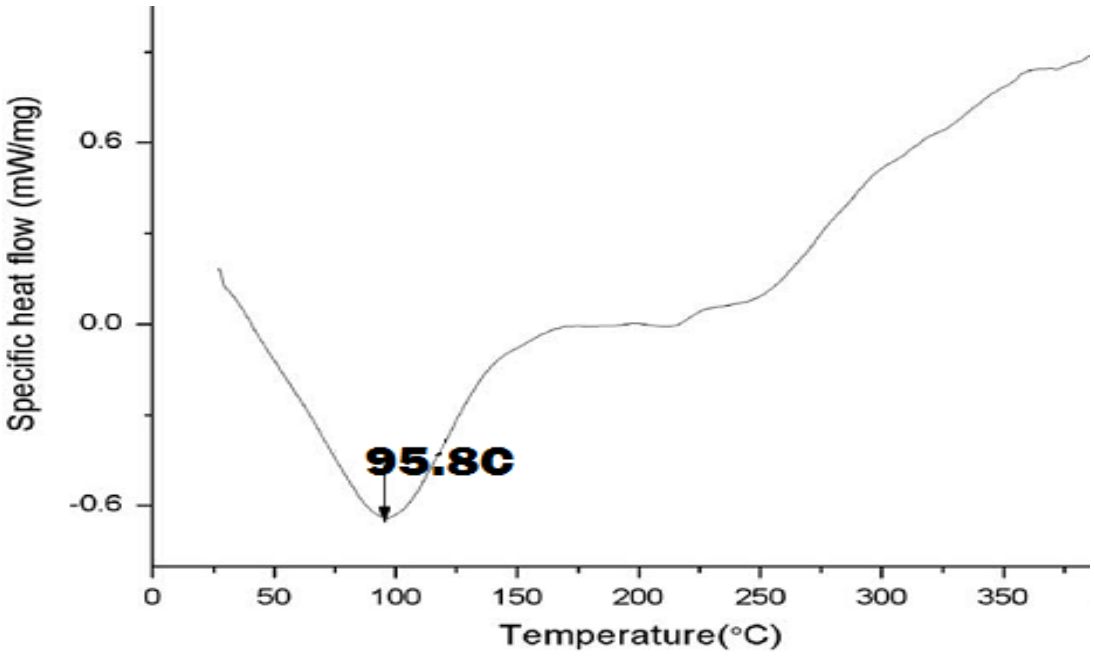


Fig. 10. Graph generated by DSC for PVA/PVP film

4.6 SOLUBILITY TEST

- Sample 1- 35%
- Sample 2- 26%
- Solubility increases with the PVP content in the film.

4.7 SEM (SCANNING ELECTRON MICROSCOPE)

By utilizing the SEM procedure, the morphology of PVP/PVA mix was concentrated on. It is an uniform sort, yet with varying degrees of harshness. Which recommend that the PVP particles may scatter in the delicate-fragment stage with little impact on the microphase partition and blending of the hard and delicate fragments.

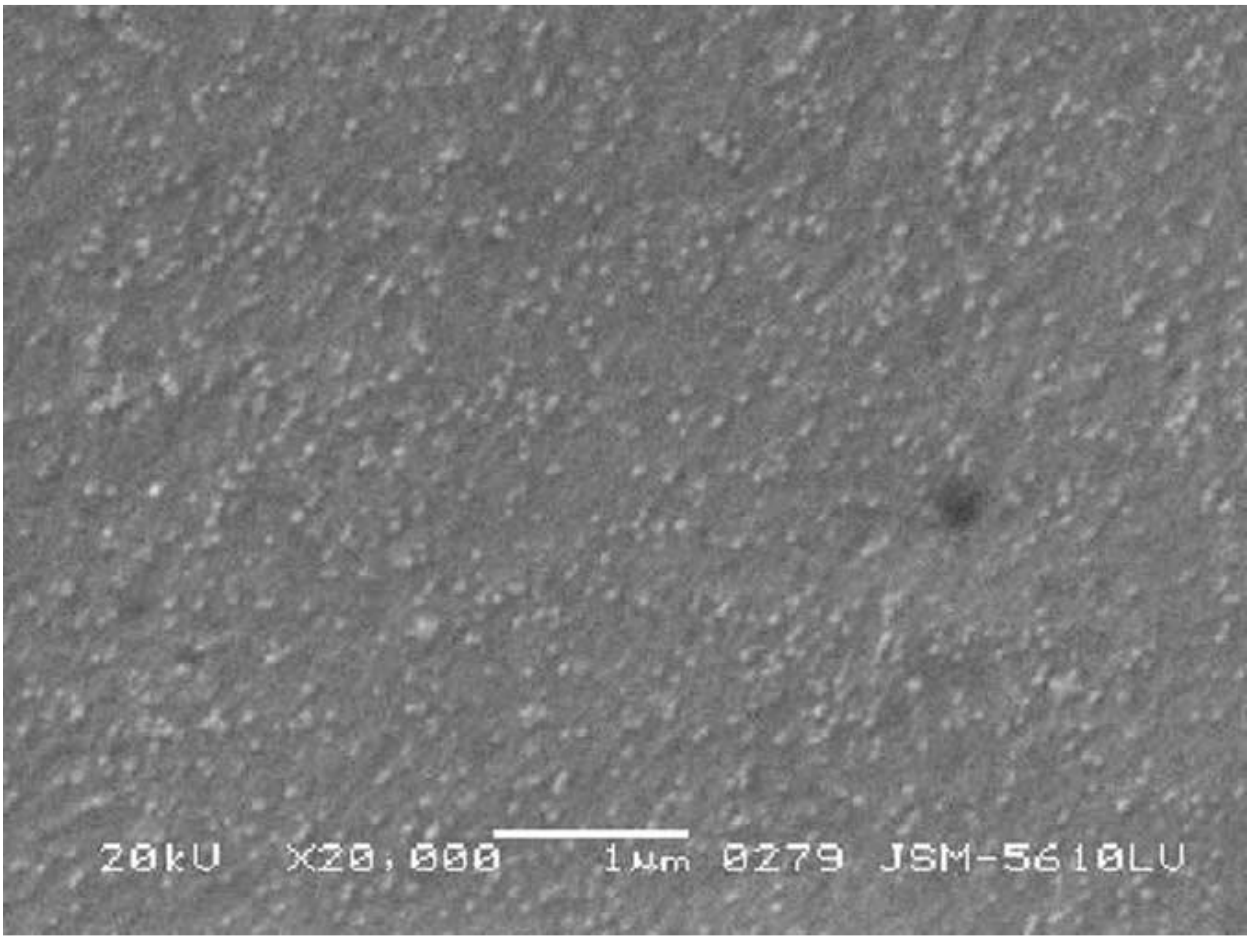


Fig. 11. Image generated by SEM for PVA/PVP Film

4.8 XRD (X RAY DIFFRACTION)

The Uniformity in the PVP/PVA blend was determined by X ray diffraction method.

The following Graphs were generated for different samples. Peak increases with increase in concentration of PVP in blend.

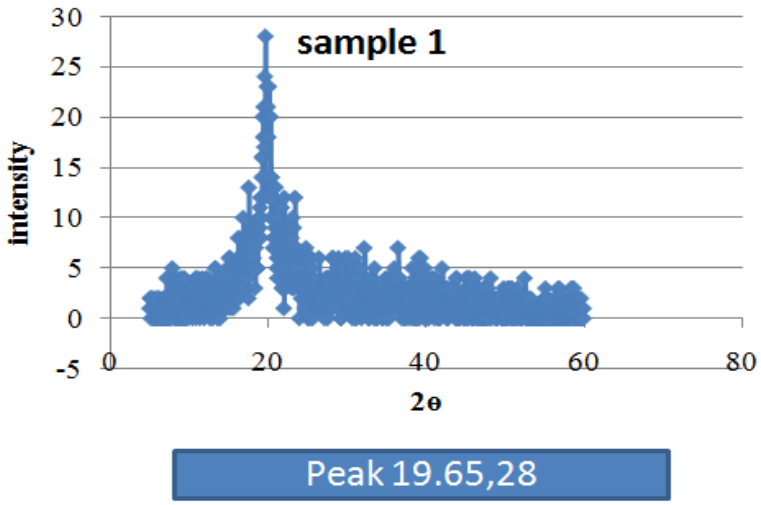


Fig. 12. XRD Graph for Sample 1

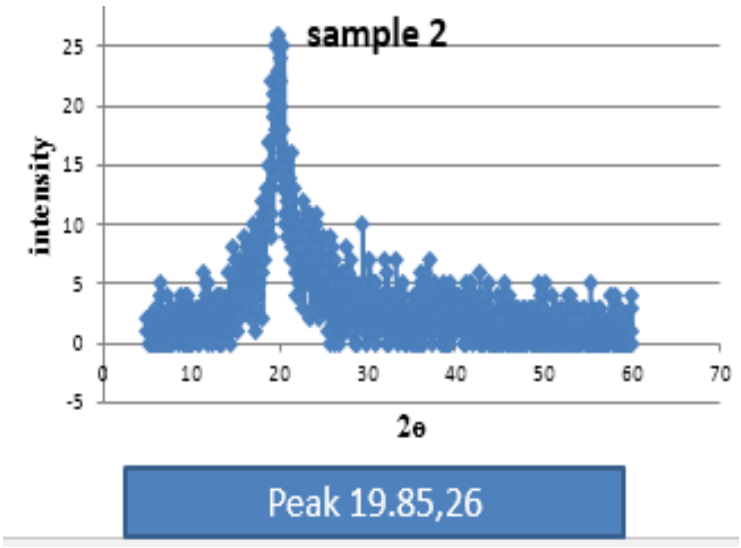


Fig. 13. XRD Graph for Sample 2

5.CONCLUSION

Both PVA and PVP are highly Hemocompatible which make them perfect to be used for ocular inserts formation. Films formed by blends made from PVA and PVP are thermodynamically stable with excellent physical and mechanical properties which are non-toxic and biocompatible to biological samples. Both polymers have tendency to form complex with each other forming hydrogen bond.

Characterization of PVA/PVP films was done using various techniques like XRD, DSC , SEM, Swelling Test etc. and results were generated . These results show that these polymer films made of PVA/PVP can be incorporeted with drugs. These films release the drug at the site at required rate for long period .

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