

# Studies of XRD and FTIR on Synthesized Novel Hybrid Thin Film Made of Hydroxyapatite, Poly Vinyl Alcohol and Gelatin for Biomedical Application

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## Abstract

With the modern advancement of treatment approaches in medical science, the application of biomaterials in tissue engineering provides a remarkable opportunity to overcome graft rejection as well as proper wound healing. In this study, novel hybrid films have been synthesized by incorporation of polyvinyl alcohol (PVA), gelatin, and gelatin with glycerin along with different concentrations of pre-prepared hydroxyapatite (HAP) by solution casting method at room temperature in a biosafety cabinet. Glutaraldehyde has been added as a crosslinker in this whole procedure. Fourier-transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD) have been conducted to observe and compare the structural and chemical stability of the synthesized hybrid film properties. The FTIR results and X-Ray Diffraction analyses confirmed the chemical interactions between HAP, PVA, gelatin, and glycerin have occurred. The crystallinity of HAP also remains in all the prepared hybrid film samples that are observed in XRD. It is expected that these newly synthesized hybrid films could be a better opportunity for various sectors of tissue engineering such as skin, bone, tendon, and cartilage. These synthesized hybrid films can be suitable for wound healing covering. These studies could be a new scope for long-term drug delivery directly on wound sites in diabetic gangrene foot or burn patients as well as cartilage or joint replacement therapy.

## Keywords

XRD, FTIR, HAP, PVA, Gelatin

## 1. Introduction

Hydroxyapatite (HAP) is a nonstoichiometric carbonated multi-substituted apatite where calcium and phosphorus ratio varies between 1.50 to 1.67 [1]. Polyvinyl Alcohol (PVA) is biocompatible, water-soluble, and suitable for simulation of natural tissues. It has good oxygen permeability, shows no immunogenic effects, and features [2]. Gelatin (Gel) is a natural polymer composed of amino acids and peptides obtained after partial hydrolysis of the triple helix bonds in collagen. Among those amino acids, arginine, glycine, proline and aspartic acid (RGD sequence) provide necessary signals for cell proliferation, adhesion and differentiation. Repairment of large bone defects gelatin containing nanofibrous scaffolds is used [3]. Gelatin blends efficiently with both natural and synthetic polymers that can support high bio-affinity, cell viability, flexibility, bioactive, and biomechanical properties of the scaffolds [4] [5].

In this research work incorporation of HAP with polymeric biomaterials such as PVA and gelatin has been done that improved the localization of HAP and enhanced its biomedical application. Different percentage of HAP, PVA, and gelatin was used for the synthesis of hybrid film, and their comparative study was done accordingly. Individually these biopolymers have high degradation rate and poor mechanical strength and to overcome this drawback different crosslinking agents can be used among them one of the promising chemical crosslinking agents is glutaraldehyde. Because of its widespread obtainability, cost effectiveness, better reactivity, and strong stabilization properties, Glutaraldehyde (GTA) become one of the primary crosslinkers used in biomedical applications. It is a bifunctional chemical crosslinking agent which is more competent with collagen like fibrous proteins. Regarding the concern of cytotoxicity of GTA, numerous studies depict that using up to 8% GTA is considered safe and non-toxic as the cytotoxicity of GTA depends on concentration [6].

In this study, only 2.5% of glutaraldehyde has been used as crosslinker which is considered safe of biomedical use. To improve the synthesized film flexibility glycerine is used as plasticizer which reduces the film's roughness and enables the film softer. So, the plasticizer is added to the polymers as a film forming agent to make the polymer pliable, soft and enhance the flexibility in addition plasticity of the films. The mechanical and structural characteristics of films are affected by the addition of plasticizers as well which have been well established [7] [8]. Glycerin is also known as glycerol, as it possesses three hydroxyl groups (trivalent alcohols), a nanostructure with an extremely strong bonding effect that act as a plasticizer. It also induced reversible chelation and crosslinking with water that represented a molecular bridge for water and PVA. The self-healing ability and flexibility when incorporated with other materials were greatly improved [9] [10].

There are several experiments such as FTIR, XRD have been done of HAP, PVA, and Gelatin, individually as well as their combination such as PVA/HAP, Gelatin/PVA, Gelatin/HAP, and PVA/Gelatin/HAP has been a noted topic among researchers for the last couple of decades. In FTIR analysis the characteristic bands

of HAP were located most of the journal at 631,632, 3448, 3572  $\text{cm}^{-1}$  band due to structural  $\text{OH}^-$  and 875, 962, 1024,1041,1069, 1085  $\text{cm}^{-1}$  correspond to the stretching vibration  $\text{PO}_4^{3-}$  in HAP [11]-[14]. The O-H stretching band in the IR spectrum was the most distinctive feature of alcohols and phenols. PVA shows a broad and strong band around 3278 - 3280  $\text{cm}^{-1}$  for  $\text{OH}^-$  groups [11]. The absorption peaks at 2949, 2911.02, and 831  $\text{cm}^{-1}$  were the characteristic bands of asymmetric and symmetric C-H stretching respectively. The stretching band of C-O-C group was at 1095  $\text{cm}^{-1}$ , C=O at 1654.62  $\text{cm}^{-1}$  and there was also a visible peak at 1234  $\text{cm}^{-1}$   $\text{CH}_2$  wagging or CH wagging, OH wagging at 664.357  $\text{cm}^{-1}$  for PVA according to previous research [14] [15]. The absorption bands of FTIR spectra of gelatin related a number of characteristic bands such as at 3324  $\text{cm}^{-1}$ , 3433  $\text{cm}^{-1}$  (NH stretching), 1653  $\text{cm}^{-1}$  (amide I, C=O and CN stretching), 1241  $\text{cm}^{-1}$  (amide III), and  $\text{CH}_3$  bending vibration was at 1080  $\text{cm}^{-1}$ . In addition, alkyl group of gelatin represents  $\text{CH}_2$  stretching vibration of asymmetric and symmetric at 2949.59  $\text{cm}^{-1}$  and 2880  $\text{cm}^{-1}$  respectively [13] [15] [16]. In the case of the PVA/HAP hydrogel composites, slight shifts in the band of the phosphate groups, were located in 1045  $\text{cm}^{-1}$ , asymmetric stretching and bending vibration bands of  $-\text{CH}_2$  group shifted from 2943,828  $\text{cm}^{-1}$ , C-O group changed to 1094  $\text{cm}^{-1}$  [11] [14]. Gelatin-PVA Film at 3300  $\text{cm}^{-1}$  peak appeared due to  $-\text{NH}$  stretching of secondary amide of gelatin, peaks of PVA for hydroxyl group of PVA and a consequence of more intermolecular or intramolecular hydrogen bonds. C-H stretching at 2900  $\text{cm}^{-1}$  and peak at 2947  $\text{cm}^{-1}$  depicted the presence of a hydrocarbon chromophore in the esterified product whereas 1640  $\text{cm}^{-1}$  indicated C=O stretching. The acid initiated esterification of the polymers happened at around 1070  $\text{cm}^{-1}$  due to the presence of C-O bond of secondary alcoholic groups and the ester [17]. Another study of FT-IR spectra of pure PVA showed a peak at 3445  $\text{cm}^{-1}$  indicating stretching of O-H group. peak around 2800  $\text{cm}^{-1}$  was because of C-H stretching and 1382  $\text{cm}^{-1}$  was for C-O group. The characteristic FT-IR spectra of gelatin showed peaks at 3434  $\text{cm}^{-1}$  because of N-H stretching of amide and the C=O stretching vibration of carboxylate anion appeared at 1635  $\text{cm}^{-1}$  [18]. On the other hand, due to the presence of Glutaraldehyde as a cross-linker which is dialdehyde possesses aldehydic groups as well as other functional groups such as amines, phenols, and hydroxyl groups that are highly reactive. In 2013, research was done on neat electrospun PVA nanofibrous mat where GA was used as vapor cross-linking agent indicating that more of the  $-\text{OH}$  groups were involved in the formation of acetal bridge where two vibrational bands observed between 2730 and 2860  $\text{cm}^{-1}$  referred to the stretching vibration of C-H from alkyl and  $\text{O}=\text{C}-\text{H}$  from the aldehyde. The large, broad band observed at 3200 - 3650  $\text{cm}^{-1}$  was also associated with the stretching vibration of hydroxyl ( $-\text{OH}$ ) group from PVA and GA. The band observed at 1000 - 1140  $\text{cm}^{-1}$  with the gradual broadening of the peak width as O-C-O was attributed to vibration of the acetal group [19]. FTIR spectroscopy of Gelatin/HAP scaffolds preparation C=O stretching was reported at 1630 - 1695 for the amide I, the phosphate bands appeared in the range 900 - 1200  $\text{cm}^{-1}$  with

a typical peak of phosphate vibration at  $1019\text{ cm}^{-1}$ ; carbonate peaks  $\text{CO}_3^{2-}$  were present at the wavenumbers of  $740, 840, 876\text{ cm}^{-1}$ . An increase in peak intensity for P–O stretching at  $1019\text{ cm}^{-1}$  was clearly evident, and new bands related to  $\text{HPO}_4^{2-}$  ( $1229\text{ cm}^{-1}$ ) and P=O ( $1155\text{ cm}^{-1}$ ) were observed. The band at  $1337\text{ cm}^{-1}$  in gelatin was attributed predominantly to the wagging vibration of proline side chains.  $1400 - 1260\text{ cm}^{-1}$  that was ascribed to the presence of gelatin confirms the chemical interaction among carboxyl ions in gelatin and HA phases [20]. PVA-Gelatin/HAP composites with different composition Basak *et al.* represents O–H and P–O stretching at the peak  $3650\text{ cm}^{-1}$  and  $1100\text{ cm}^{-1}$  accordingly and attributed to the presence of aromatic C=C at  $1500$  and  $1600\text{ cm}^{-1}$  [21]. Furthermore, another study shows that a sharp peak at the wavelength of  $1650\text{ cm}^{-1}$  represents the carbonyl group due to using PVA/Glycerol/starch blend film [22].

Hydroxyapatite had strong characteristic diffraction peaks according to the different articles such as  $2\theta$  angles of  $25.86^\circ, 25.9^\circ, 31.8^\circ, 32.26^\circ, 32.9^\circ, 32.98^\circ, 39.9^\circ, 34.1^\circ, 46.7^\circ, 49.2^\circ, 49.48^\circ, 53.1^\circ, 53.4^\circ,$  and  $64.1^\circ$  respectively which consider as crystalline materials [14] [23]-[25]. In XRD studies, it was exhibited that gelatin possesses both crystalline and non-crystalline or amorphous regions in its structure with a sharp peak at  $11.1^\circ 2\theta$  and a broad peak at  $22.5^\circ 2\theta$  [26] [27]. On the other hand, the XRD pattern of PVA polymer is semicrystalline having a large peak at  $19^\circ - 20^\circ$  and a small peak at  $40^\circ - 42^\circ$  [14] [25]. The XRD pattern of PVA shows main characteristic peaks at  $11.2^\circ, 15.3^\circ, 16.1^\circ, 19.4^\circ, 20.1^\circ, 22.5^\circ,$  and  $40.3^\circ$  in another study which represents the semi-crystalline structure of PVA [26]. However, different research has been done where XRD was done to show the crystallinity of PVA/Gel scaffolds as composite or hydrogel. Some results suggested PVA/Gel composites were crystalline and some agreed with amorphous character. The reason behind this was the composition of PVA and gelatin [26] [28]-[30]. The crystallinity of the composite was reduced with an increase in gelatin [28] [30]. The evidence of crystalline regions of the individual polymers in PVA/Gel blend had been confirmed by two sharp peaks at  $11.2^\circ 2\theta$  with an intensity of 87 counts and  $23.2^\circ 2\theta$  with intensity of 206 counts. In addition, the blending of other polymers such as MWCNT observed the same peak but higher intensities 116 and 256 counts, individually [26]. In contrast, the characteristic intensity peaks in HAP/PVA composites were lowered, broadening the shape of peaks, and became more amorphous due to the interaction between HAP and PVA polymer [14] [25]. Another study on PVA hydrogels mentioned that the presence of very small degree of crystallinity coupled with a large amount of diffuse scattering represented the co-existence of small PVA nanocrystalline phase with the bulk amorphous phase. A relatively broad peak presented at  $2\theta = 22.5^\circ$  and a closer look at the diffraction profiles revealed a systematic increase in the sharpness of the diffraction peak with the increase in PVA concentration [31] [32]. Glycerin/glycerol plays an important role in XRD patterns. It decreased the degree of crystallinity and strength of peaks but improved the broad nature of the peaks [33].

The involvement of damaged wound tissue needed grafting in some cases which

are affluent for individuals. Therefore, there is more need for substitutes of different living tissues to repair wound damage. To meet this undeniable purpose various biomaterials, play important roles as these are engineered materials to support the processes of repair and regeneration by interacting with biological systems. There are multiple areas where polymeric material composites/films are used among them wound dressing, burn dressing, drug delivery is the important biomedical application for various tissue engineering sectors.

The objective of this research study is to synthesize hybrid films made of HAP, PVA and gelatin and study the structural and chemical stability of the novel synthesized hybrid film by XRD and FTIR. It is expected that these newly synthesized hybrid films could be a better opportunity for different tissue engineering sectors such as skin, bone, tendon, and cartilage as there are no previous experiments regarding this combination material. It will also be helpful for wound healing and burn dressing patients in head-neck surgery reconstruction, diabetic foot ulcer and orthopedic surgery.

## 2. Materials & Methodology

### 2.1. Materials

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) was synthesized by chemical precipitation from Calcium nitrate tetra-hydrate ( $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ ) with Disodium hydrogen phosphate ( $(\text{NH}_4)_2\text{HPO}_4$ ) where Ca/P molar ratio of 1.67 and pH 10 - 11 was maintained by small amount of ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) [34]. Calcium nitrate tetra hydrate, Di ammonium Hydrogen phosphate and Gelatin Purified were purchased from Merck, India, while Ammonium Solution 25%, Glutaraldehyde Solution 25%, Ethanol was purchased from Merck, Germany. Poly vinyl alcohol (PVA), molecular weight of 115,000 kDa, with a polymerization degree of 1700 - 1800 and a hydrolysis degree of 98.99% was purchased from Qualikems Fine Chem Pvt. Ltd., India. Acetic Acid with 99.5% purity from BDH chemical, England and Glycerin pure from UNITAD Product, Bangladesh was used including Phosphate buffered solution (PBS) pH 7.4, purchased from New Mumbai for experiment. All the chemical were used without further purification. Deionized water was used throughout the experiment.

### 2.2. Methodology to Prepare HAP/PVA/GEL Hybrid Film

HAP/PVA/GEL Hybrid film was made by two different methods, one is adding humectants and another is without humectants. In this experiments, Glycerin/Glycerol (Gly) was used as humectants which preserve the moisture of synthesized hybrid film. Two different amounts of synthesized HAP were chosen 5%, and 10% and this HAP solution was prepared individually by adding acetic acid that helped to dissolve HAP properly. At 70 °C with continuous stirring by magnetic stirrer for around 1 hour 5% PVA solution was prepared and add with individual HAP solution. On the other hand, Gelatin solution was prepared by two ways. In one solution only deionized water was used to produce 5% Gelatin solution and on

another, Glycerin was used along with gelatin solution where Gelatin: Glycerin: Deionized water ratio was maintained 2:1:2 and prepared 10% Gelatin solution. These two Gelatin solutions was mixed with HAP and PVA mixtures separately. Stirring and heating was continued during the whole procedure. After 30 minutes of continuous stirring and heating, 1 mL of 2.5% Glutaraldehyde was added for chemical crosslinking of the materials. During this procedure temperature was maintained 40°C - 50°C. After another half an hour of continuous heating and stirring, the final solution transferred into petridish covering a thermal paper and keep it in room temperature for 5 - 6 days to obtain desired film.

A total four sample was synthesized and used for further characterization and study.

In the following chart, four samples and the composition ratio is mentioned accordingly.

Sample No.	Composition Ratio
S1/5 HPG	5% HAP: 5% PVA: 5% Gelatin
S2/10 HPG	10% HAP: 5% PVA: 5% Gelatin
S3/5 HPGGly	5% HAP: 5% PVA: 10% Gelatin
S4/10 HPGGly	10% HAP: 5% PVA: 10% Gelatin

## 2.3. Studies of Chemical and Structural Properties

### 2.3.1. FTIR

The chemical analysis was performed by the Fourier-transform infrared (FTIR) spectroscopy (FTIR; Spectrum Two, PerkinElmer, USA) over a wavenumber range between 400 and 4000  $\text{cm}^{-1}$  at room temperature.

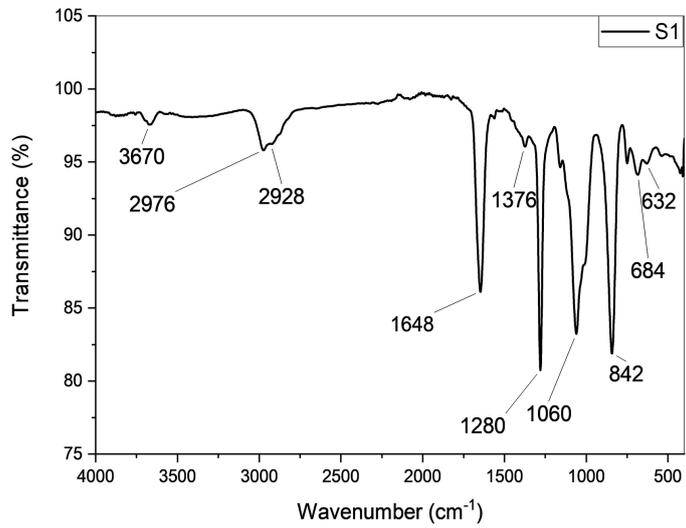
### 2.3.2. XRD

X-ray diffraction patterns (XRDs) were recorded on an X-ray diffractometer (SmartLab Studio, Rigaku, Japan) to confirm the crystallinity of synthesized materials. comprising a source of Cu  $K\alpha$ , 30 mA current, and 40 kV voltage with a scan rate of 10°/min.

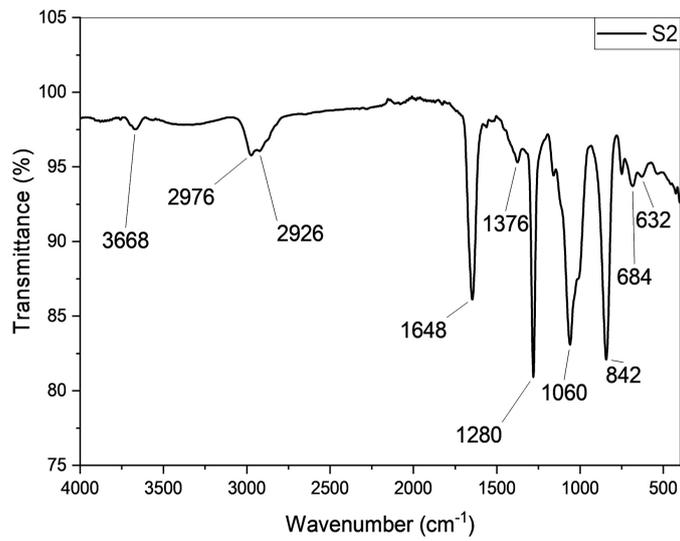
## 3. Results and Discussions

### 3.1. FTIR (Fourier Transform Infrared Radiation)

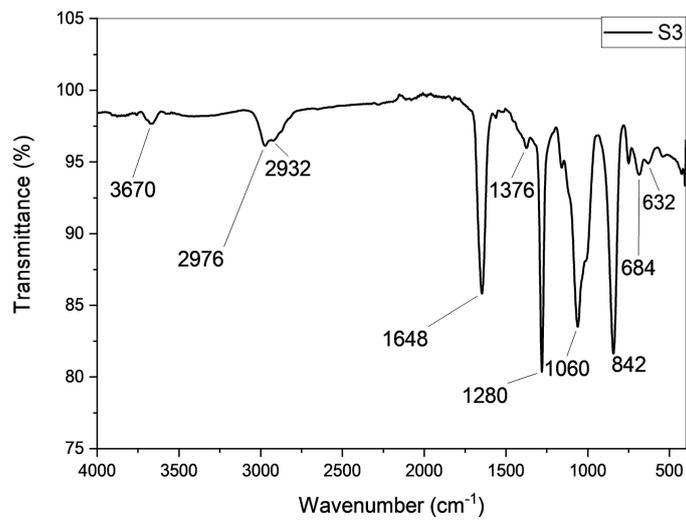
FTIR spectroscopy was performed to analyze the presence of functional groups of synthesized hybrid films that were composed of hydroxyapatite, polyvinyl alcohol, gelatin, glycerin, and crosslinker glutaraldehyde. Two or more polymers are mixed with a reliable approach to developing novel biomaterials revealing the unifications of properties that could not be obtained by individual polymers. The possible interaction which was taking place in these synthesized hybrid films are analyzed by FTIR spectra in **Figure 1**. All four sample represents almost similar distinguished peak due to the blending of several components together.



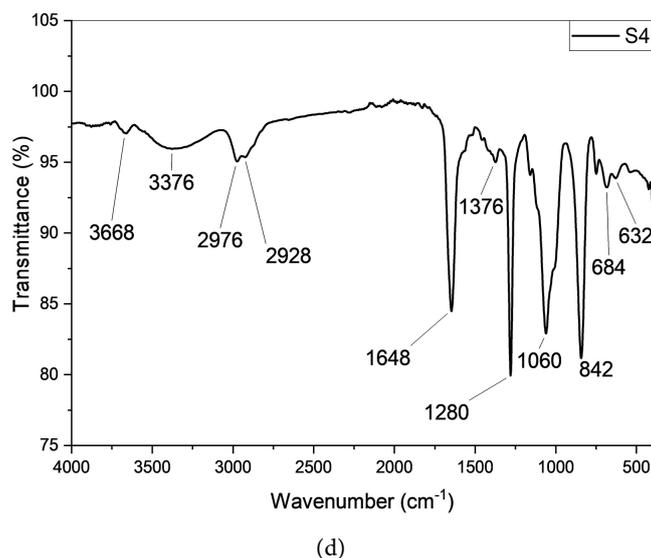
(a)



(b)



(c)



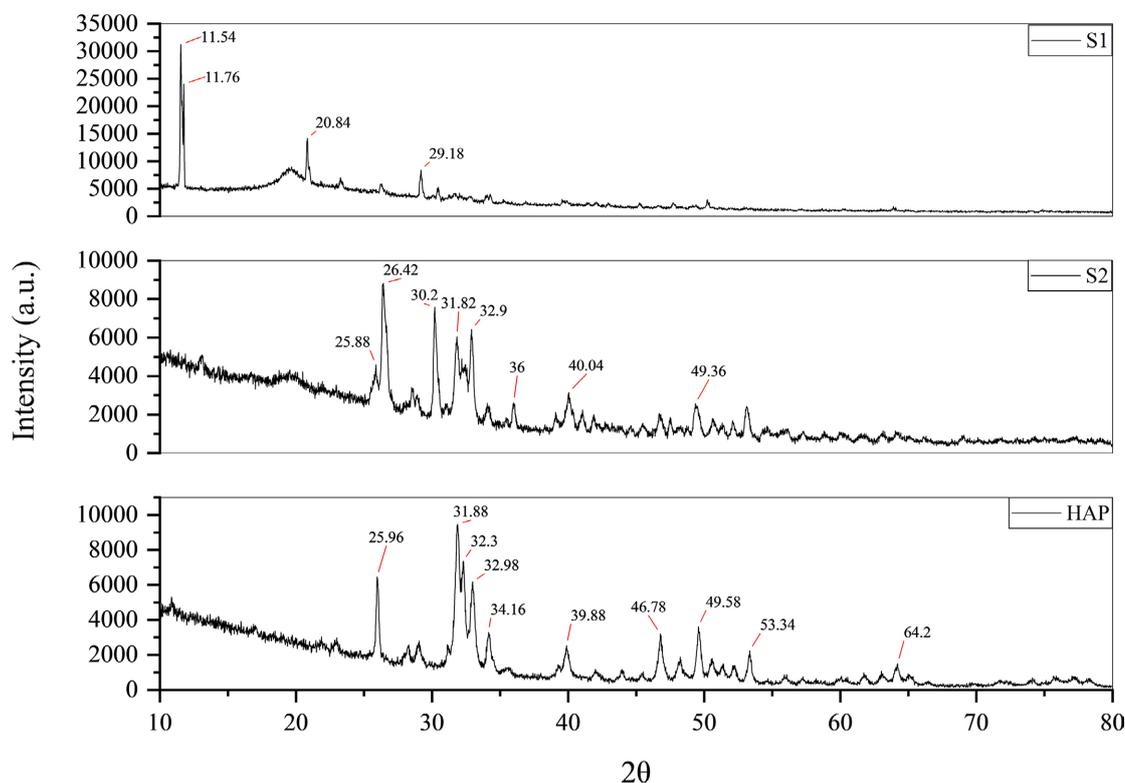
**Figure 1.** FTIR analysis of HAP/PVA/GEL Film (S1, S2) and of HAP/PVA/GEL/Glycerin Film (S3, S4).

The first small peak was at  $3670\text{ cm}^{-1}$  in S1 and S3 and  $3668\text{ cm}^{-1}$  in S2 and S4 which indicated the presence of O–H, phenol, and N–H stretching due to the blending of HAP, PVA, gelatin, and GA. At  $3376\text{ cm}^{-1}$  a broad round peak appeared due to the presence of glycerin in S4 that also present the abundance O–H functional group. At  $2976\text{ cm}^{-1}$  and  $2926 - 2932\text{ cm}^{-1}$  wavenumber there could be the formation of asymmetric and symmetric C–H stretching vibration of C–H from alkyl and O=C–H from the aldehyde as glutaraldehyde was used as the cross-linking agent. A sharp peak at  $1648\text{ cm}^{-1}$  represents C=O stretching vibration of carboxylate anion. The presence of proline in gelatin structure and  $\text{HPO}_4^{2-}$  in hydroxyapatite attributed to a wagging vibration of proline side chain at  $1376\text{ cm}^{-1}$  and a sharp peak visible at  $1280\text{ cm}^{-1}$  confirm the chemical interaction among C–N stretching vibration of amide III of gelatin, as well as  $\text{CH}_2$  wagging or CH wagging of PVA. The next sharp wide peak at  $1060\text{ cm}^{-1}$  was described as the presence of O–C–O attributed to vibration of the acetal group as acetic acid was used during synthesis as a dissolving agent of hydroxyapatite. Moreover, the typical phosphate bond vibration of HAP and the presence of C–O bond of secondary alcoholic groups, and the ester of acid-initiated esterification of the polymers could be the reason for the widening of the peak. Afterward, there was another high peak intensity at  $842\text{ cm}^{-1}$  wavenumber which was for P–O stretching and carbonate peaks  $\text{CO}_3^{2-}$  of hydroxyapatite. Lastly, at  $684$  and  $632\text{ cm}^{-1}$  relatively small peak band appeared probably due to overlapped structural  $\text{OH}^-$  of HAP and OH wagging of PVA.

### 3.2. XRD

**Figure 2** and **Figure 3** show the XRD patterns of the HAP powder and HAP/PVA/GEL Film (S1 and S2), HAP/PVA/GEL/Glycerin (S3 and S4) respectively. It can be seen from **Figure 1** that the characteristic peaks of HAP were located at  $25.96^\circ$ ,  $31.88^\circ$ ,

32.3°, 32.98°, 34.16°, 39.88°, 46.78°, 49.58°, 53.34°, and 64.2° respectively which are matched with the diffraction peaks of standard stoichiometric HAP (JCPDS No. 9-0432) [14] [23]-[25]. These HAP patterns in X-ray diffraction suggest low crystallinity which plays an important part during the bioresorption processes [23].

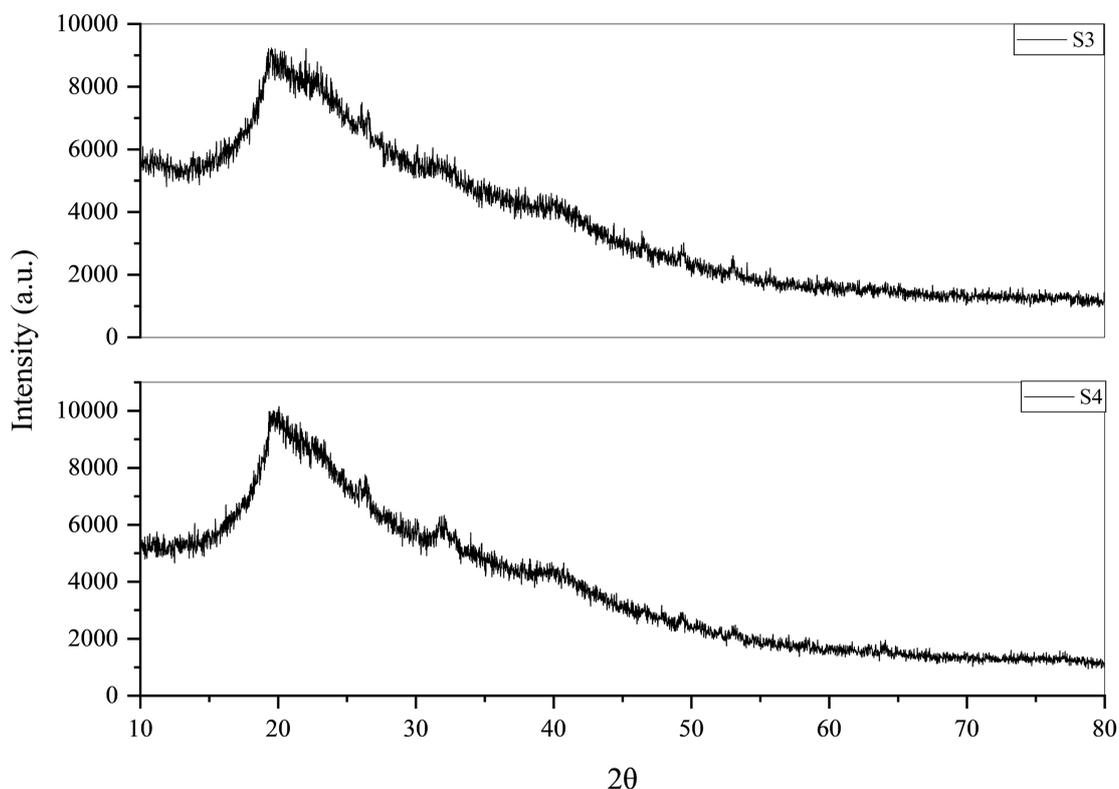


**Figure 2.** XRD analysis of HAP and HAP/PVA/Gelatin film (S1 and S2).

**Figure 2** also presents the XRD pattern of synthesized hybrid film HAP/PVA/GEL film whereas S1 and S2 contain 5% and 10% HAP respectively which included characteristic peaks of both HAP, PVA, and gelatin. It shows two sharp peaks at 11.54° and 11.76° with high intensity along with two sharp but small peaks at 20.84° and 29.18° with relatively low intensity for S1. 11.54°, 11.76° and 20.84° peaks correspond with the characteristic peak of PVA, gelatin, and PVA/Gel scaffolds whereas 29.18° represents the presence of HAP in S1. Alternatively, in S2 where HAP concentration was increased, the visible peaks of S1 disappeared rather new peaks appeared that were more corresponding with the characteristic pattern of HAP molecules such as 25.88°, 31.82°, 32.9°, and 49.36°. And these peaks confirmed the presence of HAP molecules in S2.

In addition, the broad diffuse peaks at 40.04° equivalent to the characteristic peaks of PVA proved that PVA possessed a semi-crystalline structure. Besides, the sharp peaks at 26.42°, 30.9°, and 36° depicted the appropriate incorporation of HAP, PVA, and Gelatin in S2. Nevertheless, the peak intensity of S1 was also higher which confirms the uniform distribution of HAP with the rise of crystallinity

whereas in S2 the intensity declined as the HAP concentration was increased [14] [26]. The sharp peak denotes molecular arrangement was in order after the incorporation of HAP in PVA/Gel and improved interfacial adhesion [26]. There was some insignificant and amorphous peak visible due to the presence of gelatin in the sample and the hydrogen-bonding interaction between PVA polymer and HAP.



**Figure 3.** XRD analysis of HAP and HAP/PVA/Gelatin/Glycerin film (S3 and S4).

Conferring to **Figure 3** XRD curves, there were numerous small sharp peaks visible in the diffraction peaks of S3 and S4 exhibiting both the crystalline and amorphous nature of the synthesized hybrid film. A broad peak between  $2\theta = 19^\circ - 26^\circ$  could be ascribed to indicate the semi-crystalline and crystalline nature of PVA and hydroxyapatite to the polymer network. A closer observation of the diffraction patterns of S3 and S4 attributed the systematic increase in the sharpness of the diffraction peak along with increase in HAP concentration from 5% to 10%. All the samples including the HAP sample intensity peak commenced at  $\sim 5000$  a.u. except for S3 which appeared at  $\sim 6000$  a.u. The intensity peak of S1 and S2 was reached at  $\sim 31,000$  and  $\sim 8800$  respectively whereas in other samples where glycerin is added as a plasticizer in S3 and S4 the intensity reached a maximum  $\sim 9200$  and  $\sim 10,150$  respectively. Then a gradual decrease of intensity was seen without any significant broad or sharp peak up to  $\sim 1000$  for S3 but there was a small broad peak visible around between  $2\theta = 31^\circ - 33^\circ$  which might be due to the presence of 10% HAP in the S4 with intensity  $\sim 6600$  and then gradual reduction

of intensity had been seen upto ~1000 a.u. These varieties of diffraction patterns are characterized by the presence of a small degree of crystallinity coupled with a large amount of diffuse scattering indicating the co-existence of HAP, PVA, and gelatin nanocrystalline phase with the bulk amorphous phase. In S3 and S4 the addition of the glycerin plasticizer plays a crucial role such as improving the broad nature but decreasing the strength of peaks which was well established in S3 and S4 [33]. The overall intensity of the peaks was enhanced in the S3 and S4 film after adding the glycerin plasticizer, which may recommend the formation of a new interaction that may lead to a slightly crystalline form and also peaks to diminish that considered as a sign of the formation of a new network with a change in the internal structure of the synthesized hybrid film. The amorphous phase of gelatin was aggravated due to the addition of glycerin in S3 and S4. This XRD pattern also indicated that the HAP/PVA/GEL and HAP/PVA/GEL/Glycerin film may participate in biomedical applications [10] [35].

#### 4. Conclusion

In this research study, HAP/PVA/GEL and HAP/PVA/GEL/Gly films containing different HAP percentages (5%, and 10%) were developed by the solution casting method. FTIR analysis showed that O–H, phenol, and N–H stretching, C–H from alkyl and O=C–H from the aldehyde, C=O stretching vibration of carboxylate anion, C–N stretching vibration of amide III, wagging vibration of proline, CH<sub>2</sub> or CH wagging and OH wagging, O–C–O acetal group, HPO<sub>4</sub><sup>2-</sup>, P–O stretching, carbonate peaks CO<sub>3</sub><sup>2-</sup>, and OH<sup>-</sup> in all four samples of synthesized hybrid film. XRD results in the presence of hydroxyapatite and polyvinyl alcohol with defined crystallinity. The characterized sharp diffraction peaks were observed in both HAP/PVA/GEL films and with increasing HAP percentage the number of sharp peaks and crystallinity was increased. On the other hand, due to the presence of glycerin and increasing the percentage of gelatin, HAP/PVA/GEL/Gly samples there were no significant sharp diffraction peaks were observed rather XRD results manifested broad peak that characterized the presence of a small degree of crystallinity coupled with a large amount of diffuse scattering indicating the co-existence of HAP, PVA, and gelatin nanocrystalline phase with the bulk amorphous phase. Both FTIR and XRD results evident the proper blending of all the components of hybrid films. However, it is recommended to do biocompatibility tests for future medical applications to see the cell proliferation and antibiotic resistance. Lastly, it could be said that in this study newly developed HAP/PVA/GEL and HAP/PVA/GEL/Gly are demonstrated as a new opportunity for drug delivery, wound healing, and grafting in different tissue engineering sectors. There is wide scope in the future to experiment with these synthesized hybrid films for the proper biomedical applications.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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