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Studies on optimization and characterization of potato starch based hydrogels for enzyme immobilization in biosensor applications

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Abstract
The main objective of this work is to conduct optimization studies for preparation of potato starch based hydrogels to act as a matrix for screen-printed electrodes in biosensors. In this study, starch hydrogel cross-linked with citric acid (SHCC) at different concentrations (5%, 10%, 15%, and 20%) and citric acid modified porous starch hydrogel (CAM-PSH) were prepared and were characterized based on thickness (t), apparent density (AD), moisture content, swelling ratio (SR), and water uptake capacity (WUC). SEM analysis, EDX, and FTIR studies were conducted to study the morphological properties of porous starch hydrogels and to study the influence of citric acid modification on the porous starch hydrogels. The thickness and apparent density of CAM-PSH and SHCC samples had no significant difference. However, the moisture content, swelling ratio, and WUC of CAM-PSH were found to be higher compared to the SHCC. From the SEM analysis, it is evident that the CAM-PSH matrix has better porous space which is suitable for the immobilization of the enzymes. FTIR studies shows good binding of CAM-PSH to the screen printed electrodes. From these studies, it was optimized that CAM-PSH can be used as an immobilizing matrix for enzymes in biosensor applications.

Keywords: Porous starch hydrogels, citric acid, cross-linking, immobilization matrix

1. Introduction
Biosensor is an analytical device consisting of a transducer and a biological element, that interacts with the analyte or target molecule being tested and the biological response is converted into an electrical, optical or chemical signal by the transducer. The biological element (enzymes, antibody or an aptamer) acts as a sensor and is either immobilized or physically adsorbed onto the electrode surface depending on the properties of the electrode and the biological element. The transducer detects the response of the binding between the bio-component and the analyte and transmits the signal. The enzymes are widely used bio-receptor molecules owing to its specific binding ability, high selectivity, catalytic activity and high sensitivity to specific target molecule. However, as enzymes are mostly protein, they are naturally fragile, denatures when exposed to high temperature and are highly unstable resulting in reduced enzyme activity and high cost.
Enzyme immobilization is the method of confining the enzyme to a matrix that may be either natural polymers or inorganic materials. The characteristics of these matrices have to be different from that of the enzymes and should be capable of providing a stable environment for the activities of the enzymes. Immobilization enhances the stability, catalytic activity, selectivity and reusability of the enzymes. Enzyme immobilized biosensors has various advantages *viz*, biosensors based on enzyme immobilization has solved various challenges like enzyme stability, enzyme loss, reduced the time of enzymatic response, and offer disposable devices which can be easily used in stationary or in flow system. The matrices used for immobilizing the enzymes should have the following properties. 1) biocompatibility 2) thermal stability 3) high affinity to the enzymes 4) presence of functional groups 5) easy availability and 6) low cost. (Zdarta, 2018) [14].
Hydrogels are networks of natural polymers cross-linked with hydrophilic macromolecules that can absorb significant volumes of water or other biological fluids while also acting as an effective matrix for enzyme encapsulation. The networks can be made of homopolymers or copolymers, and the structure of the network is due to the presence of crosslinks, of chemical or physical nature. Absorption of water results in an increase in volume due to water retention, thus, forming an amorphous inner gel structure.

Wide range of organic and synthetic hydrogels are available for application in biomedicine, materials science and biosensors. The base materials for natural hydrogels include starch, cellulose, pectin, chitosan, etc. Hydrogel behavior is influenced by the stability of these crosslinks. If these bonds are capable of withstanding prolonged water exposure, the hydrogel will swell; otherwise, degradation of the crosslinks will result in hydrogel erosion and swelling. These materials' properties, which are primarily related to their ability to transport solvents, active molecules and their mechanical resistance, determine their application. As a result, a thorough understanding of the properties of hydrogels (as well as their structure–property relationships) is highly desirable in order to select an appropriate material (as well as its shape and size) for use in biosensor applications. (Kurita, 2001) [5].

Starch is a widely available polysaccharide containing a large number of hydroxyl groups, thereby making it hydrophilic. It is made of amylase and branched amylopectin units. It is non-toxic, biocompatible and biodegradable. Hydrogels based on starch have certain benefits, but they also have significant drawbacks. Using starch as an adsorbent has a number of advantages: it is readily available and an abundant natural biopolymer; it is a renewable resource; it is cost-effective and easy to prepare with readily available and inexpensive reagents; it has an excellent water swelling capacity; it is an amphiphilic cross-linked adsorbent; and it can be used in a wide range of processes. (Amylomaize, 2019). The immobilisation of -amylase generated by *Bacillus amyloliquefaciens* was investigated using raw potato starch in conjunction with calcium alginate beads. The immobilised enzyme was more highly reactive than the free, unbound enzyme. Alginate beads preserved 40% of their effectiveness after seven cycles. The immobilisation of -amylase with starch was studied, and it was discovered that due to covalent immobilisation, it was more resistant to temperature inactivation. (Sharmeen *et al*., 2019).

(Heredia & Resto, 2020) [4] used starch hydrogels in which gold nanoparticles were immobilized for biosensor application. It was also reported that starch hydrogels enhanced the stability of the nanoparticles on its exposure to different environment by 400 percent. while also allowing for macromolecules to interact with the AuNPs surface. Crosslinking is the most widely used method for modification of starch. Citric acid, poly vinyl alcohol and glutaraldehyde are some of the commonly used crosslinkers. From the literature studies, it was observed that potato starch based hydrogels for enzymes immobilization has not yet been studied.

The objective of this work is to optimize and characterize potato starch-based hydrogels for their application as a matrix in biosensors. In this study, starch hydrogel cross-linked with citric acid (SHCC) at different concentrations (5%, 10%, 15%, and 20%) and citric acid modified porous starch hydrogel (CAM-PSH) are to be characterized.

2. Materials and Methods

2.1 Materials

Potato starch, anhydrous citric acid, deionized water and ethanol were purchased from Sigma Aldrich and supplied by M/s. Ponmani Scientific Instruments Pvt. Ltd.in Trichy, Tamil Nadu, India.

2.2 Preparation of immobilisation matrix using starch hydrogels cross-linked with citric acid (SHCC)

Potato starch (10 g) is dispersed into deionized water (100 ml) to obtain a starch solution. The potato starch solution was then heated at a temperature of 70°C with constant stirring for 120 minutes to complete starch gelatinization. To the hydrogels, citric acid (5%, 10%, 15% and 20% w/w on d.b. of potato starch weight) was added and the resulting dispersion was further mixed for 30 minutes. It is ensured that no air bubbles are entrapped in the solution. The above solution is poured onto a petri plate and dried at 50°C for 6 hours. These hydrogel films were stored at ambient temperature in a sealed pouch for further testing.

2.3 Preparation of citric acid modified porous potato starch hydrogel (CAM-PSH)

10 g of potato starch was added to 100 mL of deionized water. The mixture was heated to 70°C for 120 minutes for complete starch gelatinization. It was then stored at a temperature of 5°C for 48 hours to obtain a starch hydrogel. The gel was then cut into uniform layers and frozen at -10°C for 48 hours. The frozen layers were immersed in ethanol at room temperature for 3 hours. Fresh ethanol was replaced after every 1 hour. Then the layers were dried at 50°C for 6 hours followed by heating at 120°C for 2 hours.

Citric acid (30 g) was dissolved in 100 mL of ethanol. The potato starch cubes were immersed in the prepared solution for 12 hours at ambient conditions followed by drying at 130 °C for 90 minutes. Citric acid modified potato starch cubes were immersed in ethanol followed by washing for three to four times to remove the unreacted citric acid and then dried.

2.4 Characterization of the starch based hydrogels:

2.4.1 Thickness and apparent density

The thickness of hydrogel was determined by measuring at five different positions using a screw gauge. It is represented as ‘t’ in mm. The apparent density (AD) of the samples was measured by the solvent displacement method. In this method, a solvent which is not absorbed by the sample is taken in known volume. A known weight of the sample is taken and then placed in the solvent. The amount of solvent displaced in the measuring cylinder is then noted. The apparent density is then calculated using the formula.

$$\text{Apparent Density, AD} = \frac{\text{mass of the sample (g)}}{\text{volume of the solvent displace (ml)}} \quad (1)$$

2.4.2 Moisture level

The hydrogels of known weight (W_i) were dried at 110 °C for 24 h and the final dried weight (W_d) is noted. The moisture content is calculated by using the following formula.

$$\text{MC}\ (\%) = \frac{W_i - W_d}{W_d} \times 100 \quad (2)$$

2.4.3 Water uptake capacity and swelling ratio

The swelling ratio (SR) was calculated by measuring the weight of the dried hydrogel initially and the weight of the swollen hydrogel after its immersion in distilled water at 25° C for 24 hours.

The SR is defined as follows

$$\text{SR} = \frac{SS_W - DS_W}{DS_W} \quad (3)$$

Where SS_W is the weight of the swollen hydrogel, and DS_W is the weight of the dried sample.

The water uptake capacity was measured by taking dried hydrogels of 1 cm^2 (n = 5) and weighing them (W_{DH}). It is then soaked in distilled water for 24 hours at room temperature. The hydrogels were then taken out, wiped slightly and their weights (W_{WAH}) were measured immediately. The water uptake (WUC) was calculated as

$$WUC = \frac{W_{DH} - W_{WAH}}{SA_H} \quad (4)$$

Where W_{DH} and W_{WAH} represent the weight of the dried hydrogel and the weight of the water absorbed hydrogel, and SA_H represents the surface area of the hydrogels.

2.5 Morphological studies

2.5.1 Scanning Electron Microscope

Scanning electron microscope (TESCAN-Vega 3, Czech Republic) was used to study the morphology, binding structure and the porous nature of the developed starch hydrogels. The samples were mounted in their dry state on an Al stub with a tape and then examined at 20 kV.

2.5.2 EDX

Energy dispersive X-ray analysis (EDX) is an analytical method used to determine the elements in a sample or to characterise its chemical composition. It is based on the interaction of an X-ray source and a sample. The fundamental working principle is based on the differences in the element's atomic structure and uniqueness of each element which results in a unique set of peaks on the element's X-ray emission spectrum. EDX analysis is done using a SEM instrument.

2.5.3 FT-IR analysis

The spectrum of the Fourier Transform Infrared analysis of samples PSH and CAM-PSH were measured using Attenuated Total Reflectance mode using Bruker Alpha T spectroscopy to characterise the possible reaction between potato starch hydrogel and citric acid. The samples were placed in the sample plate and the transmittance spectra regions were obtained between 4500 cm^{-1} and 500 cm^{-1}.

3. Results and Discussion

3.1 Characterization of the starch based hydrogels

The starch hydrogel cross-linked with citric acid (SHCC) and citric acid modified porous starch hydrogels (CAM-PSH) was prepared as shown in fig.2. The mechanism behind the cross-linking of starch with citric acid is based on the formation of cyclic anhydride intermediates (fig.1). The cyclic structure of starch breaks by the esterification action of -OH functional groups, which results in the formation of a new -COOH unit in the citric acid, forming a new intramolecular anhydride by reacting with the next –COOH unit. Considering that esterification occurs mostly at primary -OH groups, which are known to be highly reactive in a polysaccharide's structural unity when compared to secondary –OH groups. (Taylor *et al*., 2013) [11] reported that -COOH cross-linking is typically done in the dry conditions and requires higher temperature for the reaction to proceed. The hydrophobic nature of the substrate, on the other hand, facilitates water evaporation, which is the primary step for cross-linking with CA.

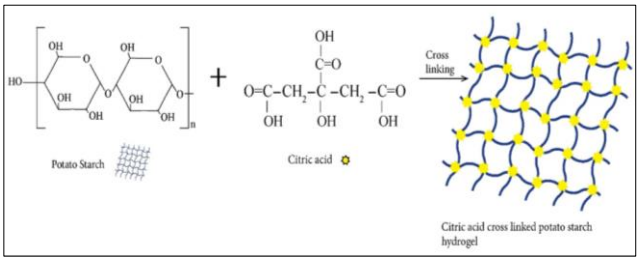

Fig 1: Citric acid cross-linked potato starch hydrogel

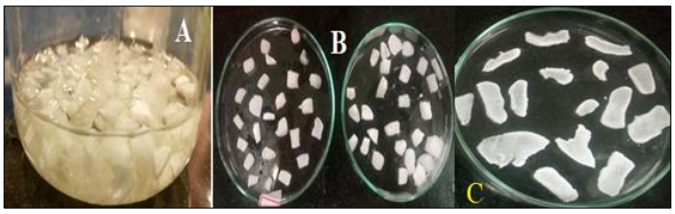

Fig 2: (A) Porous starch hydrogels immersed in citric acid solution (B) Citric acid modified porous starch hydrogels (C) Starch hydrogels cross-linked with citric acid.

3.2 Thickness and Apparent density

From the table1, it is observed that increase in the concentration of citric acid in SHCC increases its thickness up to a concentration of 15% citric acid and then it decreases the thickness of the hydrogel. The thickness of CAM-PSH This may be due to the effect of cross-linking action of the citric acid on the starch molecules. As the concentration of citric acid increases the degree of cross-linking thereby making the SHCC into a tight network. (Shi *et al*., 2008) reported that the plasticizing property of citric acid attributed to the reduced thickness of the films. (Matheus *et al*., 2020) [7] in his studies reported that hydrogels with higher concentration of citric acid when subjected to high pressure thermopressing resulted in lower thickness and large surface area. On the contrary,

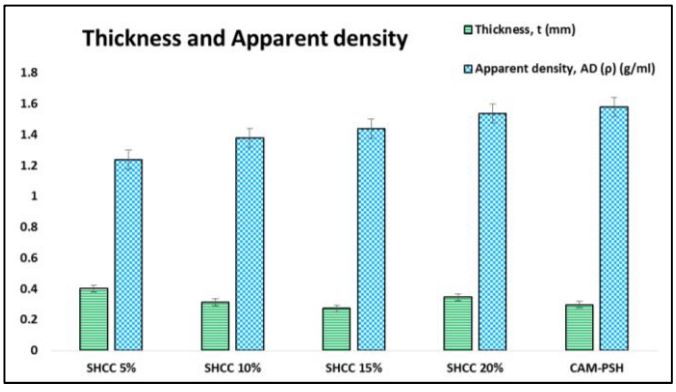

Fig 3: Thickness and Apparent density

(Li *et al*., 2013) reported an increase in film thickness with increase in citric acid concentration and explained that the amount of solid content increases with increasing concentration of citric acid thereby increasing the thickness of the film. The apparent density of SHCC samples increased with increasing concentration of citric acid, whereas for CAM-PSH it was found to be 1.376 g/ml. Similar trend was reported by (Wu *et al*., 2019) [19] and it was found that

increase in density with increase in citric acid concentration owing to the tight binding between the molecules in the matrix resulting in a high compact film.

Table 1: The moisture content of the SHCC samples were found to decrease with increasing concentration of citric acid up to 15% and then increased at a concentration of 20% citric acid.

S No.	Sample	Thickness, t (mm)	Apparent density, AD (ρ) (g/ml)	Moisture content (%)	Swelling Ratio (SR)	Water Uptake Capacity (WUC)
1	SHCC 5%	0.4	1.235	61.4384	2.034	16.19
2	SHCC 10%	0.312	1.376	38.43807	1.160	9.415
3	SHCC 15%	0.272	1.438	7.480173	0.556	8.54
4	SHCC 20%	0.344	1.536	14.07231	0.649	22.18
5	CAM-PSH	0.296	1.579	83.12301	2.345	24.6675

3.2.1 Moisture content

The moisture content of the SHCC samples were found to decrease with increasing concentration of citric acid up to 15% and then increased at a concentration of 20% citric acid. (Ghoshal & Goyal, 2019) [3] reported a reduction in the content of moisture with increase in citric acid content. The increase in the value of moisture content beyond 15% can be due to the availability of free space in the hydrogel matrix thereby permitting free movement of molecules. In case of CAM-PSH, the moisture content was found to be the highest compared to the SHCC at different concentration (Table -1). This may be attributed to the presence of higher porous structure which are retained by the modification of citric acid in the CAM-PSH than the starch hydrogels crosslinked with citric acid. (Wang *et al*., 2014) [12].

3.2.3 Swelling ratio and Water uptake capacity

(Matheus *et al*., 2020) [7] reported that the addition of citric acid as crosslinker reduced the swelling ratio. The decrease in the swelling ability of SHCC may be due to the development of crosslinks between the –OH in starch molecules and –COOH in citric acid molecule thereby making it difficult for the penetration of water molecules into the hydrogel matrix. The increase in concentration of citric acid reduced the swelling ability of SHCC which can be attributed to the increase in the percentages of crosslinks and the esterification reaction. Similar results were reported by (Ghosh & Netravali, 2012). (Shi *et al*., 2008) reported that addition of citric acid at concentrations above 0.75 (g/100 g) may act as a plasticizer in the polymer matrix. In case of CAM-PSH the occurrence of the porous structure helps in holding up water molecules in the porous space thereby increasing its swelling ability. (Seligra *et al*., 2015) [9] observed that the intergrity of the starch films improved when its cross-linked using citric acid.

The swelling ratio explains the swelling behavior which is the most important feature of hydrogels. The mechanism behind the swelling process is as follows: when the dry polymeric hydrogel material is immersed in water, the first molecules that imbibes through the hydrogel matrix hydrates the most hydrophilic groups, resulting in its swelling due to absorption of water. When the hydrophilic groups are saturated by the absorption of water, the next hydrophilic group begins to absorb the water and this process continues till the attainment of equilibrium.

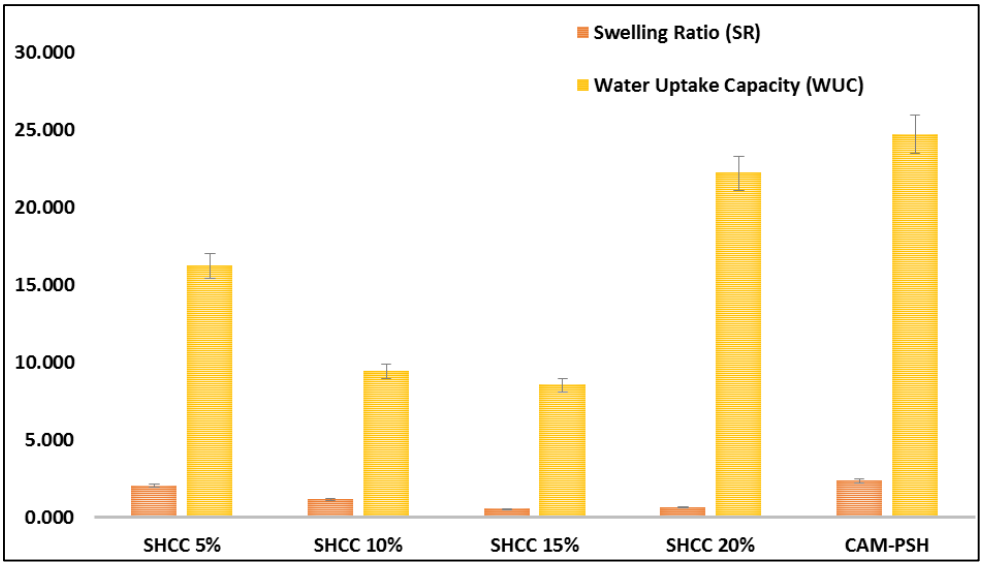

Fig 4: Swelling Ratio and Water Uptake Capacity

3.3 Morphological studies

3.3.1 SEM: This technique is quite useful for determining the film's characteristics. Citric acid's effect on the hydrogel's microstructure after cross linking was studied using SEM images. Micrographs of native starch films revealed a partly smooth surface that was less dense and pore-free (Thomas, 2017). While starch films treated with citric acid exhibited an uniform and compact network devoid of pores or cracks, untreated starch films did not (Salihu & Wsoo, n.d.)2018. Additionally, it was discovered that films treated with citric acid exhibited a denser and uniform network than original starch films (Zaman *et al*., 2019). A similar study revealed that the cross-linked structure of whey protein exhibited a denser network than native films. (2019, Ghoshal & Goyal) [3]

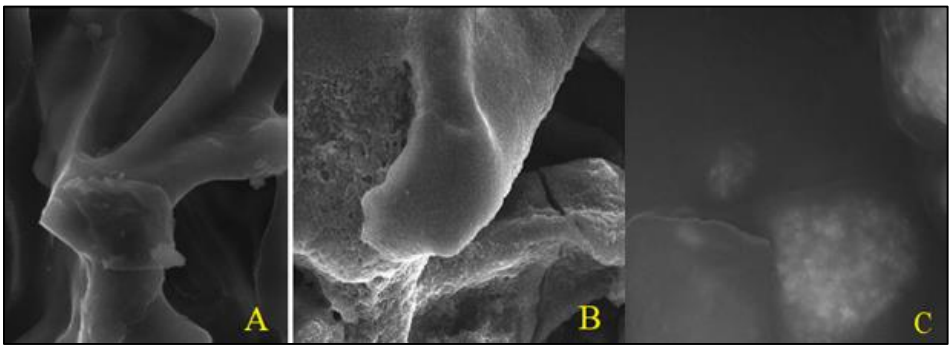

Fig 5: EDX spectra

3.2.2 EDX

The EDX spectra in Fig.5 represents the elemental composition (Carbon and Oxygen) typical of organic hydrogel matrix, while the elemental and atomic weight percentages of SHCC and CAM-PSH samples were presented in Table 2.

Table 2: The elemental and atomic weight percentages of SHCC and CAM-PSH

SAMPLES	ATOMIC %		WEIGHT %	
	C	O	C	O
SHCC	75.51	Ab	43.77	Ab
CAM-PSH	76.02	21.9	70.14	26.71

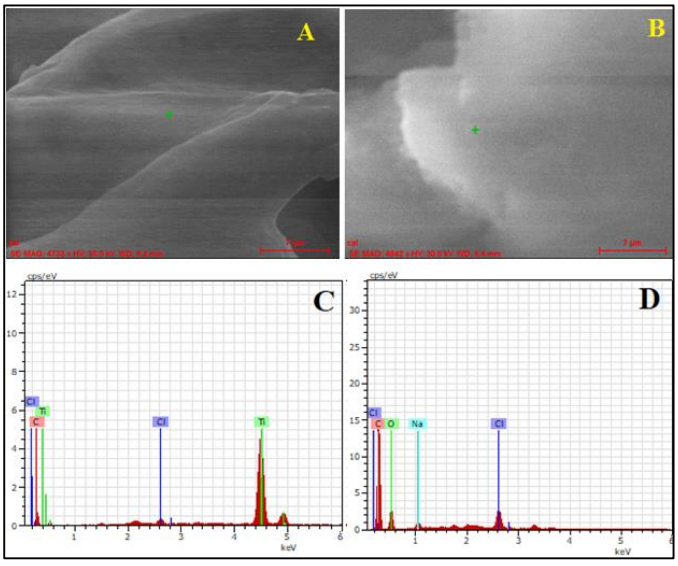

Fig 6: EDX Spectra (A) distribution of SHCC (B) distribution of CAM-PSH (C) elemental mapping of SHCC (D) elemental mapping of CAM-PSH

The slight shift of the elemental and atomic weight percentage (Table 2) of carbon in the SHCC sample in comparison with CAM-PSH indicates the presence of CA in both the samples. Citric acid with the chemical formula ($C_6H_8O_7$), has increased the percentage of oxygen atoms in the CAM-PSH than SHCC. In SHCC sample, the absence of oxygen content may be due to the effect of crosslinking of citric acid with the starch hydrogel matrix, where as in CAM-PSH, the porosity of the starch hydrogel and the modification with citric acid in the porous starch hydrogel matrix has retained the amount of oxygen thereby increasing its elemental and atomic weight. Swelling studies also shows an increase in the swelling ratio and water uptake capacity of CAM-PSH which is due to its increased porosity.

3.2.3 FTIR

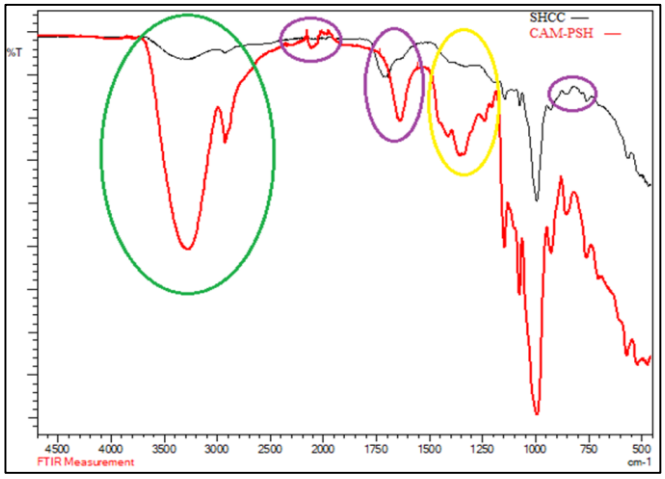

Fig 7: FTIR Spectra of SHCC (starch hydrogel cross-linked with citric acid and CAM-PSH (citric acid modified porous starch hydrogel)

The FT-IR spectrum was used to study the changes in the structure of potato starch hydrogel on a molecular level when the potato starch hydrogel was modified with citric acid. Fig. a and b shows the FTIR spectra of the SHCC and CAM-PSH respectively. The FT-IR spectrum of SHCC ranging from 3244 to 3311 cm^{-1} with higher intensity compared to the FTIR spectra of CAM-PSH can be attributed to the stretching of intermolecular and intramolecular H bonds of the alcohol –OH group. The peak at 2931 cm^{-1} in SHCC corresponds to –C-H stretch absorption band for linear long chain aliphatic compounds. The peak at 2100 in SHCC corresponds to C=C=O stretching which is absent in the CAM-PSH. This peak followed by the spectra at 1600– 1300 cm^{-1}, 1200–1000 cm^{-1} and 800–600 cm^{-1} indicates the absorption band of C≡C in the triple bond region. The peak at 1639 cm^{-1} in PSH shifted towards 1700 cm^{-1} in CAM-PSH. This peak in CAM-PSH at around 1700 cm^{-1} can be due to the ester group (Buchanan, 2008) [4]. This confirms an esterification reaction between the functional group –OH (starch) and –COOH (citric acid), thereby forming an ester group. Ma *et al.* (2018) reported a similar modification due to cross-linkages by citric acid in soy residue. A similar observation has been noted by (Pornsuksomboon *et al.*, 2016) [8] in cross-linking the film using citric acid. The reduced intensity of the -OH peaks on the citric acid modified porous starch hydrogel can be due to the result of the chemical interaction with citric acid. The low intense OH peaks from 3244 to 3311 cm^{-1} and 1700 cm^{-1} on CAM-PSH, indicates that the successful modification of PSH with citric acid. (Lipatova & Yusova, 2021) [6]. The peaks at

1305, 1336, 1340, 1357 in the SHCC spectra can be correlated with the bending structure of O–C–H, C–C–H, and C–O–H and is absent in CAM-PSH (Mei *et al*. 2015). In the fingerprint region, at 758cm^{-1} the intensity of the peak in PSH is less compared to the intensity in CAM-PSH.

4. Conclusion

In this study SHCC and CAM-PSH were prepared and was characterized based on thickness, apparent density, moisture content, swelling ratio and water uptake capacity. The amount of solid content increases with increasing concentration of citric acid thereby increasing the thickness of the hydrogel. The apparent density of SHCC samples found to be lower than that of CAM-PSH. The swelling ratio of CAM-PSH was higher than SHCC, which is the most important feature of hydrogels. Citric acid's effect on the hydrogel's microstructure after cross linking was studied using SEM images. Studies have shown that the hydrogel treated with citric acid exhibited a denser and uniform network. SEM studies confirmed the high porosity of CAM-PSH than the SHCC. FTIR spectra confirmed the binding ability of CAM-PSH to the surface of the screen printed electrodes. Therefore, CAM-PSH can be used as a matrix for immobilizing the enzymes on the screen printed electrodes for use in biosensor applications.

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6. References

1. Buchanan CM. Rapid and environmentally friendly preparation of starch esters 2008;74:137-141. https://doi.org/10.1016/j.carbpol.2008.01.013
2. Ghosh T, Netravali AN. ‘ Green ’ crosslinking of native starches with malonic acid and their properties. Carbohydrate Polymers 2012;90(4):1620-1628. https://doi.org/10.1016/j.carbpol.2012.07.041
3. Ghoshal GG, Goyal M. Moth bean starch (*Vigna aconitifolia*): isolation, characterization, and development of edible / biodegradable films 2019. https://doi.org/10.1007/s13197-019-03959-4
4. Heredia FL, Resto PJ. Fast Adhesion of Gold Nanoparticles (AuNPs) to a Surface Using Starch Hydrogels for Characterization of Biomolecules in Biosensor Applications, 2020.
5. Kurita K. Controlled functionalization of the polysaccharide chitin 2001, 26.
6. Lipatova IM, Yusova AA. International Journal of Biological Macromolecules Effect of mechanical activation on starch crosslinking with citric acid. International Journal of Biological Macromolecules 2021;185:688-695. https://doi.org/10.1016/j.ijbiomac.2021.06.139
7. Matheus B, Cagnin C, Yamashita F, Bonametti J, Salomão P, Oliveira D. LWT - Food Science and Technology Citric acid as crosslinking agent in starch / xanthan gum hydrogels produced by extrusion and thermopressing. LWT - Food Science and Technology, 2020;125:108950. https://doi.org/10.1016/j.lwt.2019.108950
8. Pornsuksomboon K, Barta B, Mészáros K, Kaewtatip K. Properties of baked foams from citric acid modified cassava starch and native cassava starch blends. Carbohydrate Polymers 2016;136:107-112. https://doi.org/10.1016/j.carbpol.2015.09.019
9. Seligra PG, Jaramillo CM, Famá L, Goyanes S. Ac ce p te us t. 2015. *Carbohydrate Polymers*. https://doi.org/10.1016/j.carbpol.2015.11.041
10. Shi R, Bi J, Zhang Z, Zhu A, Chen D, Zhou X. The effect of citric acid on the structural properties and cytotoxicity of the polyvinyl alcohol / starch films when molding at high temperature 2008;74:763-770. https://doi.org/10.1016/j.carbpol.2008.04.045
11. Taylor P, Uliniuc A, Claude U, Lyon B, Polymères M, Villeurbanne UMR. (N.D.). Modified Starch-Based Hydrogels Cross-Linked with Citric Acid and their use as Drug Delivery Systems for Modified Starch-Based Hydrogels Cross-Linked with Citric Acid and their use as Drug Delivery Systems for Levofloxacin. November 2014, 37-41. https://doi.org/10.1080/1539445X.2012.710698
12. Wang S, Ren J, Li W, Sun R, Liu S. Properties of polyvinyl alcohol / xylan composite films with citric acid. Carbohydrate Polymers 2014;103:94-99. https://doi.org/10.1016/j.carbpol.2013.12.030
13. Wu H, Lei Y, Lu J, Zhu R, Xiao D, Jiao C *et al*. Food Hydrocolloids E ff ect of citric acid induced crosslinking on the structure and properties of potato starch / chitosan composite fi lms. Food Hydrocolloids, 2019, 97(July), 105208. https://doi.org/10.1016/j.foodhyd.2019.105208
14. Zdarta J. A General Overview of Support Materials for Enzyme Immobilization: Characteristics, Properties, Practical Utility 2018. https://doi.org/10.3390/catal8020092.