

# Sustainable Chrome Tanning System Using Protein Based Product Developed from Leather Waste: Wealth from Waste

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## Research Article

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# Abstract

Chrome tanning is one of the popular tanning methods followed globally that generates considerable level of chromium pollution in the resulting effluent. The existing conventional chrome tanning system has to be modified to increase the exhaustion level of chromium in the tanning process. In the present investigation, a protein based product has been developed from raw trimming wastes and applied in chrome tanning process to enhance the exhaustion level of chromium. The experiment conducted using the product at the level of 6% exhibited chromium exhaustion at the level of 93% in the process. FT-IR analysis revealed the presence of peaks for various functional groups namely carboxylic acids, aldehyde, amide and hydroxyl groups for the high exhaust chrome tanning. The shrinkage temperature of the experimental leather was found to be more than 100 o C. Results of microscopic studies from SEM-EDX revealed that enhanced adsorption of chromium up to a level of 5.24 weight % containing chromium with atomic % 1.27 in the sample of 6% co-polymer. AFM images also show increase in adsorption of chromium with increased amount of co-polymer in the solution. Increased interactions between active sites of collagen-complex and chromium resulted into improved exhaustion of chromium as revealed from higher intensities of XRD images. CD spectra revealed that the addition of protein based product increased the ellipticity of the collagen in the experimental sample as compared to control sample. The crust leather showed improved colour, organoleptic and comparable strength properties of the experimental leather.

## 1. Introduction

Chromium is one of the popular tanning material used in leather industry owing to its superior quality in transforming the skin into leather. Chromium is found in various forms in the environment according to the valencies however the safer form is chromium(III). This form is important to human being for maintaining balance in metabolism of hormones and glands. On the other hand, it is widely used in tanning industry for tanning the skins. The chromium used in the leather making is not fully absorbed by the skin/ pelt that emanate chromium in the level of 2000-4000 ppm (Dixit et al., 2015). This huge quantity of chromium generated in the tanning process causes severe pollution problems. The negative impacts of the chromium are that it affects the ground water and fertility of the soil because chromium attaches to the soil very strongly and sticks to the silt layer surrounding or within the groundwater reservoir.

Besides, increased uptake of chromium by the fish enhances the mortality rates in fish due to various reasons. This may be due to contamination, some time, chromium(III) gets converted into chromium(VI) which is carcinogenic. Chromium(VI) causes health problems to humans including allergic reactions, skin rash, nose irritations and damage kidney and liver (Shanker and Venkateswarlu, 2019; Zayed and Terry, 2003; Kanagaraj et al., 2020).

However, chromium(III) application in the leather industry is unavoidable for achieving the superior leather properties such as high shrinkage temperature, water resistance, softness, improved dyeing and

light fastness properties to the final leather. On the other side, conventional chrome tanning process discharges considerable amount of chromium (30-40% of the total chromium used) in the spent liquor which has to be addressed with suitable modifications in the in-house process control measures (Zhang and Chen, 2020). This is because of the weak cross-linking between chromium-collagen linkages.

The binding capacity of chromium with collagen in the tanning process can be enhanced by several ways. The primary way is through providing additional binding sites especially carboxylic acids during tanning process which is a proven and pragmatic method for enhancing the uptake of chromium. Several researchers have attempted to develop different aids to enhance the exhaustion in tanning process. These exhaust aids are prepared or synthesised by different ways to increase the binding capacity of chromium to the collagen matrix. Several reports are available such as application of special products namely, collagen hydrolysate, keratin hydrolysate, fleshing hydrolysate, nanoparticle dispersion during chrome tanning enhanced the exhaustion level was explored by various researchers (Cui et al., 2017; Anggriyani and Nugroho, 2018; Kanagaraj et al., 2008; 2005). It was inferred from the experiment that chromium exhaustion capacity can be enhanced to the level of 93% through novel system of Alutan-Basic Chrome Sulfate chrome tanning system (Rao et al., 2002). Literature findings also revealed that amphiphilic acrylic copolymer prepared from lauryl acrylate and acrylic acid, use of nanocomposites and novel cross-linking agent applied in chrome tanning enhanced the chromium uptake in tanning process (Liu et al., 2016; Kanagaraj et al., 2002). It has been found that application of silk hydrolysate at the level of 5% in chrome tanning improved the exhaustion level of chromium to 95% in tanning (Aslan et al., 2013). It was also found in the literature that the use of nanoparticle polymer at the level of 4% increased the chrome exhaustion level to 94% in chrome tanning process. The other researchers have also concluded that bio-adsorption of chromium using cellulose-montmorillonite composite material, bacillus pumulus, new hydrogels based on substituted anhydride, starch-graft acrylic acid/ sodium-montmorillonite superabsorbent, cationic polymer/ bentonite complex as adsorbents, modified collagen hydrolysate, cloisite-g-methacrylic acid copolymer nanocomposites as adsorbents for chromium and other applications have been reported. The literature also reports that microwave could enhance the chrome tanning process by promoting the chrome penetration and exhaustion level and bringing out better tanning effect by not damaging/ weakening the fibres of the leather (Zhang and Chen, 2020).

Recent studies have found that use of various copolymers namely novel collagenous based co-polymer using PEG, PVA also enhanced chrome exhaustion in tanning process (Kanagaraj et al., 2015a). Hydroxyl-terminated dendrimer (HTD) was used as a combination tanning agent for high chrome exhaustion (Yao et al., 2018). Salt-less pickling using cyanuric chloride and p-aminobenzoic acid increased the exhaustion level of chromium in tanning process. The final leathers obtained by this process showed comparable physico-chemical characteristics including shrinkage temperature, mechanical properties and organoleptic properties and reduced COD of the spent chrome liquor making the method a sustainable chrome tanning process (Cui et al., 2017). Similarly, materials rich in sulfonic acid groups was exploited in chrome tanning to improve the exhaustion of tanning. It was found that the exhaustion level of chromium was increased from 71.6% to 98.6%, in the chromium bath (Zhang et al., 2017).

It is of prime focus to develop technologies for mitigating or zero discharge of chromium in the leather making process. Several pioneers in leather research have paid attention on this topic and developed high exhaust chrome tanning systems that can reduce or eliminate chromium in the tanning process. With similar objectives, the present investigation focusses on development of protein based product from skin trimming wastes and application of the same in the chrome tanning process for providing additional binding sites to the collagen moieties in the leather tanning. The work also focusses on minimisation of solid proteinous wastes of leather industries by converting the raw trimming wastes into useful by-product.

## **2. Experimental Section**

### **2.1. Materials and methods**

Raw trimming waste was collected from the tannery division of CLRI. Protease enzyme, sodium hydroxide, sodium perborate, Basic Chromium Sulfate (BCS), sodium bicarbonate and sodium formate were purchased from Sigma-Aldrich. Raw goat skins were purchased from local vendors and the skins were converted into pickled pelt using the standard procedure in the tannery division of CSIR-CLRI and used for tanning process.

### **2.2. Synthesis of protein based product prepared from raw trimming wastes**

Protein based product (pbp) was prepared from the raw material namely raw trimming wastes obtained from tannery division of CSIR-CLRI. The waste weighing 1 kg was treated with 1 litre of water and mixed thoroughly for the period of 1 hour. To this, 1% of protease was added, stirred thoroughly and left over night. The protease hydrolyses the waste into the level of peptides. The protease converts the wastes into partial hydrolysate. It was further treated with 4% of sodium hydroxide and heated in the water bath for 150 minutes. The resultant product was completely hydrolysed. Then the hydrolysate was cooled and filtered thoroughly with filter cloth. To the filtrate, 0.5% of sodium perborate was slowly added in instalments with stirring mechanically for the period of 24 hrs and allowed to cool. The resultant product was adjusted to the pH of 4 and used in tanning process. The characteristic features of the product were analysed for various parameters using standard method.

### **2.3. Application of the product in the chrome tanning process**

Chrome tanning experiment was carried out using the pickled pelt. The pickled pelt (pH of 3) prepared earlier and ready to process was utilized (left sides for experiments and right sides for control experiments) for carrying out chrome tanning experiments. BCS at the level of 6% was applied to the pelt

and agitated in the drum for the period of 120 mins. After thorough penetration of the chromium, the basification process was carried out with 100% float for conventional method of tanning employing sodium formate at the level of 1% and sodium bicarbonate at the level of 1% (with 3 instalments) adopting standard procedure. The tanned spent liquor was collected, saved and later recharged in the same process after the addition of product in the experiments. Three sets of experiments were carried out using protein based product at the level of 2, 4 and 6%. The products were applied to the leather after the basification process, left sides for experiments and right sides for the control processes. The protein based product was treated with the leather by drumming for a period of 60 min and then the leather with the tanning bath was left for overnight. In the following day, the spent liquor that was collected earlier was recharged to the drum and the reaction was continued in the vessel for a duration of 60 min for thorough exhaustion of chromium and then the tanning process was completed. Similarly, the control experiments were carried out using right sides of pelts using conventional process without addition of the product and an another control experiment with addition of equivalent commercial product (Table 1).

## 2.4. Estimation of chromium

Samples from the experimental and control samples containing the tanning effluent (spent-liquor) were collected and evaluated for quantity of chromium present. A known volume of the sample was digested using an acid mixture containing 11.5 ml of perchloric acid, 3.5 ml of sulfuric acid and 5 ml of nitric acid. The chromium present in the digested sample was determined using the standard procedure using a Perkin-Elmer Lambda 35 UV visible spectrophotometer and expressed as % exhaustion (Vogel, 1978).

## 2.5. Shrinkage temperature of leather

The shrinkage temperature of the leather sample was determined with a 'Theis shrinkage meter'. The sample of dimension 20 × 3 mm were hooked in the meter. The samples were then immersed in a glycereol–water solution (70:30). The temperature at which the specimen starts to shrink was noted as the shrinkage temperature of the particular leather (McLaughlin and Theis, 1945).

## 2.6. FT-IR analysis

The samples (control and experiment) after tanning were collected and dried in the water bath. They were mixed with potassium bromide (1:20; 0.02 g of sample with KBr at a final weight of 0.4g) separately. The samples were then ground, desorbed at 60 °C for 24 h and pressed to obtain IR-transparent pellets. The FT-IR was first calibrated for background scanning signal against a control sample of pure KBr. FT-IR spectra of the samples was recorded using an FT-IR spectrum 2000 Perkin-Elmer spectrophotometer within the scanning range of 400-4000  $\text{cm}^{-1}$ . Then the experimental sample was also scanned in similar way.

## 2.7. SEM-EDX studies

The control and experimental samples obtained from tanning experiments were dried and made into powder form and then all specimens were then coated with gold using JEOL JFC-1100E ion-sputtering device. A JEOL JSM-5300 scanning electron microscope was used for the analysis. The micrographs for the sample were obtained by operating the SEM at an accelerating voltage of 20 kV with different lower and higher magnification levels. The sample that showed clear views was presented in the present investigation. In a similar way, these samples were subjected to SEM-EDX studies to assess the effect of uptake of chromium. Both control and experimental samples were gold coated for 3 s and magnified in different magnification ranges. A JOEL JSM 5300 scanning electron microscope with EDX was used to study the experimental samples.

## 2.8. AFM study

AFM measurements using an Innova apparatus from Veeco Instruments (now Bruker Nano Surfaces Division) were carried out. Topography and deflection visualization were done in tapping mode in air. The feedback signal was optimized for each surface by matching the forward and backward signal of the height channel by varying the proportional and integral gain parameter. Chromium exhaustion studies by the protein based product for various experimental samples were imaged for studies on topography and deflection using standard method (Echlin, 1971; Lehner et al., 2011).

## 2.9. X-ray diffraction studies

The X-ray diffraction (XRD) studies were carried out using a Japan Science 2200PC X-ray diffractometer. The diffractograms were obtained at  $2\theta$ , in the range  $2^0 - 10^0$  by a Cu-K $\alpha$  beam ( $\lambda=0.1543$  nm) monochromated by a nickel filter. Under a scanning speed of  $1^0/\text{min}$  and current rating of 40KV and 20mA with X-ray tubes, the morphology of the prepared product was observed by H-600 transmission electron microscope.

## 2.10. Circular Dichroism (CD) spectroscopy

Far-UV CD spectra were recorded on a JASCO-810 automatic recording spectrophotometer at room temperature. A quartz cuvette with a 0.1 cm path length was used. CD spectra were accumulated at a scan rate of 50 nm/ min between a wavelength range of 190– 240 nm. 100  $\mu\text{l}$  of experimental and control sample is taken and 3 ml of distilled water is added where as for the protein sample, 0.2  $\mu\text{l}$  of the solution is dissolved in 3 ml distilled water. The protein secondary structure content was determined using the online DICHROWEB server. Each measurement was repeated for three times for which standard deviation from the mean value is represented by error.

## 2.11. Colour properties

The leather samples after tanning with protein based product were processed into leather and were subjected to study difference in colour properties using Gretag Macbeth Spectrolino Spectrophotometer with measurement geometry of  $45^{\circ}/0^{\circ}$ . L, a, b, c and H parameters of the measurement were obtained using the standard procedures.

## 2.12. Physical testing

The experimental and control crust leathers samples were performed for various physical tests and the data were obtained as per IULTCS method. Specimens were conditioned at  $80\pm 4^{\circ}\text{C}$  and  $65\pm 2\%$  RH. Over a period of 48 hours, physical properties such as tensile strength, % elongation at break and tear strength were examined for both experimental and control samples (IUP 6, 2000)

## 3. Results And Discussion

The tanning process is one of the important unit operations in leather processing where the tanned leather is obtained by the treatment of tanning agents. This operation provides permanent stability to the skin material through the form of leather. Chrome tanning process is widely employed and accepted method to achieve desirable properties in the final leather. The exhaustion property in tanning is enhanced by using protein based product in the tanning process. The protein based product that provides various functional groups enhance the crosslinking effect was characterised and used in the chrome tanning process. The chromium uptake in the tanning process and the exhaustion levels achieved by using the product for various experiments are discussed in the present investigation.

### 3.1. Characterisation of the product

The protein based product synthesised from skin trimming waste was characterised. The Table 2 and Figure 1 shows the characteristic features of the product. The pH of the product was 4.0, particle size was 1538 nm, relative viscosity was 1.006 cP and % solids were of 6.85. In overall the product showed that the low-molecular-weight polymer with the ability to interact with collagen molecules at molecular level provides the necessary functional groups for the crosslinking of chromium with collagen in the tanning process.

### 3.2. Chromium uptake in the tanning process

The newer chrome tanning experiment carried out using the product prepared from skin trimming wastes was evaluated and found out. Initially, the waste was hydrolysed and then modified to contain more functional groups responsible for enhanced uptake of chromium in the tanning experiments. The

influence of the product in the chrome tanning process was thoroughly studied. Three sets of the experiments were carried out using product at the level of 2, 4 and 6% in the tanning experiments. The uptake of chromium in the tanning bath was estimated and presented in the Table 3. It is seen from the table that the experiment carried out using the product at the level of 2% gave the chromium uptake (as  $\text{Cr}_2\text{O}_3$ ) of 0.55%. Similarly, the other chrome tanning experiments carried out with the product at the level of 4 and 6% exhibited the chromium ( $\%\text{Cr}_2\text{O}_3$ ) presence of 0.58 and 0.60% respectively against the conventional control sample of 1.90% and another control sample using commercial product of 0.59%. It is inferred from the results that the experiment carried out using the product at the level of 2% gave exhaustion of chromium of 90% in the tanning process. Similarly, the other experiments carried out using the product at the level of 4 and 6% showed the exhaustion of chromium of 90%, 93% respectively. The conventional chrome tanning experiment showed the exhaustion of chromium at the level of 79% and another one using equivalent commercial product exhibited exhaustion of 88%. The maximum chromium uptake was found in the experimental sample carried out using the product at the level of 6%. The reason for enhancing chromium uptake in the tanning process in the experimental samples may be due to the free functional carboxylic groups, aldehyde groups, OH groups and amide groups involved in cross-linking of chromium with collagen moieties. Several researchers reported that auxiliaries exhibiting functional groups such as carboxylic acid, amide groups, OH groups favour the uptake of chromium in chrome tanning process.

### 3.3. Fourier Infrared (FT-IR) spectroscopic analysis

The control sample and experimental samples of tanning process with application of product (2%, 4% and 6% product) were subjected to FTIR analysis. Separate FTIR spectrum for each sample with characteristic wavenumbers may be viewed in Figure 2. In case of experimental sample (2%), a broad and strong band can be seen in Figure 2(a) at  $3388\text{ cm}^{-1}$  which appeared due to presence of stretching frequency of  $-\text{OH}$  group of product. The peak visible at  $1712\text{ cm}^{-1}$  is due to stretching of  $\text{C}=\text{O}$  which (aldehyde) mostly enhances adsorption of chromium and is responsible for improved uptake. The peak at  $1638\text{ cm}^{-1}$  has appeared due to presence of  $\text{N-H}$  bending of amide groups of product from the active sites. The peak at  $1104\text{ cm}^{-1}$  is due to  $\text{C-N}$  stretching of amide groups observed in the 2% sample and strong broad peak at  $2926\text{ cm}^{-1}$  is because of the presence of  $\text{O-H}$  stretching of carboxylic acid group. Thus, the spectra give evidences of existence of  $-\text{OH}$ ,  $-\text{C}=\text{O}$ ,  $-\text{NH}$ ,  $-\text{COOH}$  and amide active groups in the sample. A medium peak (in case of 4% sample, Figure 2b near  $832\text{ cm}^{-1}$  shows the presence of  $\text{C-O-H}$  bending at out-of-plane. Similarly, the experimental sample carried out using the product at 6% showed the presence at  $2929\text{ cm}^{-1}$  is because of the presence of  $\text{O-H}$  stretching of carboxylic acid group at  $1712\text{ cm}^{-1}$  is due to stretching of  $\text{C}=\text{O}$  which represents aldehyde group. It can be observed that  $-\text{COOH}$  group is absent in the control sample (Figure 2e). In the control sample (treating with commercial equivalent product at 2%), aldehyde moieties are almost saturated while peaks corresponding to  $-\text{COOH}$  groups are still available to take part in further adsorption of chromium. It is inferred from the FT-IR investigation that product prepared from skin trimmings contained the functional groups of aldehyde which may be

due to the conversion of amino acids in treating with sodium perborate in oxidation process. Besides, the product contained carboxylic acids, amide groups that favour the exhaustion of chromium in the tanning process. On the contrary, the control sample that was carried out using only BCS has not shown any indication of carboxylic acid groups and other control sample carried out using commercial equivalent product has clearly shown the presence of carboxylic acid group and not aldehyde group. Several researchers have made an attempts to increase the exhaustion of chromium in tanning process. Application of akovite–alumino silicate nano-composite (Ciuffi et al., 2012), sulphonic aromatic acid (Bcardit et al., 2008), oxazolidine derivatives (Covington, 1997), complexing agents such as sodium formate and disodium phthalate with nanoclay (Zhang et al., 2019), silk hydrolysate (Aslan et al., 2013), polycarboxylic material (Anggriyani and Nugroho, 2018), fleshing hydrolyzate (Kanagaraj et al., 2002), complexing agents such as sodium formate and disodium phthalate with nanoclay (Zhang et al., 2019), keratin hydrolyzate (Ramamurthy et al., 2015), methanosulfonic acid (MSA) (Zhang et al., 2018), gallic acid (Ramamurthy et al., 2014) in tanning process enhanced the exhaustion level of chromium to higher value.

### 3.4. Shrinkage temperature of the tanned leather

Shrinkage temperature of leather is one of the primary factor to be considered in tanning process because it gives the overall picture of the efficacy of tanning system in terms of type of cross-links formed, stability achieved and bonds formed, as simply, it can be defined as the measure of endothermic reaction resulting in the breakdown of hydrogen bonds in the polypeptide chain of collagen. The present work focuses on high exhaust tanning system with the protein based product developed from skin trimmings. The tanned leather was subjected to shrinkage temperature measurements and the results are given in Table 4. The results showed that the leather produced with various experiments showed better shrinkage temperature measurements. It can be seen form the table that experiment carried out with product at the level of 2% exhibited shrinkage temperature of  $107 \pm 0.5$  °C. Similarly, the leather obtained from other experiments using the product at the level of 4 and 6% showed shrinkage temperatures of  $110 \pm 1.0$  and  $112 \pm 0.7$  °C respectively. The leather produced from control experiments using commercial product showed shrinkage temperature of the leather with the value of  $107 \pm 0.5$  and  $106 \pm 0.5$  °C respectively. It is evident from the results that all the experimental leather showed shrinkage temperature of more than 100 °C which was due to multipoint hydrogen bonds and covalent cross-links between the product, collagen and chromium linkages. Similar results were reported from the other researchers also (Fuchs et al., 1993).

Gallic acid-graphene hydrogel (GA-GH) adsorbent with 3D porous architectures fabricated using a facile *in situ* reducing-assembly design effectively removes Cr(iii) and organic dye from tannery wastewater. Besides, GA-GH can be regenerated at pH 2.0 with hydro chloric acid and has the tendency to retain high adsorption capacity after multiple adsorption–desorption cycles. In addition, modified GA on

graphene sheets with benzene group also improves adsorption of organic dyes. This adsorption is due to the  $\pi$ - $\pi$  interactions between GA-GH and aromatic dyes (Liu et al., 2019).

Literature also reports that a new product of chrome syntan has been developed that showed more than 90% uptake of chromium in tanning process. This product act as both tanning and retanning agent which can be applied to the delimed pelts preventing the important step in leather processing namely pickling process that employs huge quantity of salt. The product helps in reducing chemical oxygen demand (COD), total dissolved solids (TDS) and chlorides to the level of 51, 81 and 99%, respectively in the tanning process (Suresh et al., 2001).

Gallic acid (GA: Trihydroxybenzoic acid) assisted chrome tanning system has been developed. It has been found from the experiment that the chrome uptake at the level of 93% has been achieved through this method. In addition to that, this method produced the leather with better organoleptic properties such as softness and fullness. This chrome tanning system reduces the pollution load especially TDS in the spent liquor (Ramamurthy et al., 2014).

In another study, application of polyamidoamine hyperbranched polymer (HPAM) as a pretanning agent for the depickled hides increased the chromium uptake in the tanning process. The optimum concentration of HPAM (5.0 wt.%) when applied to tanning process led to the increased chromium exhaustion up to 99.8% for the residual chrome concentration in the tanning bath of 2.15 mg/L. The leather produced with the tanning system exhibited better softness and shrinkage temperature of the leather (Ibrahim et al., 2013).

Pickle-free chrome tanning system using polymeric syntan has been attempted. Application of this syntan increases chromium uptake to the level of more than 90%. This system also reduces COD, TDS, chlorides in the tanning process to the level of 50, 80, 90% respectively (Rao et al., 2004).

Recycling and reusing of tanning floats by adopting masked chromium salts instead of commonly used chromium ones has been attempted. This process skips/ eliminates basification process and overcomes the change of pH in the process system thereby reducing the generation of considerable amount of neutral salts. The exhaust bath can be recycled and reused for several times. The present method helps in saving the water (90%) and also produces leather of better quality (Morera et al., 2011).

### **3.5. SEM-EDX analyses**

The experimental samples using the product at 2%, 4% and 6% levels in tanning processes were collected; specimen was prepared and imaged for SEM-EDX in JEOL JSM-5300 scanning electron microscope. The images were obtained at magnification of 100  $\mu\text{m} \times 315$ , 20  $\mu\text{m} \times 1.150$ , 20  $\mu\text{m} \times 1.360\text{K}$ , 20  $\mu\text{m} \times 1.59\text{K}$  respectively to study morphology of the final product. The results of SEM-EDX investigation are presented in Figure 3. The SEM micrographs with different resolutions show two types of grains. One type consists of thick conjugated fibrous and the other is of globular crystalline particles. The first one represents the

presence of protein based product and the second one shows the presence of chromium (Cr). In case of pure or product sample, the first type consists of longer fibrous structures representing free carboxylic acid moieties/ groups which functionally crosslinks chromium. The experimental sample with 4% product shows that chromium is adsorbed on fibrous particles; the active sites of the product are responsible for improved exhaustion as evident by the presence of globular grain particles on the fibrous structures having some unoccupied sites left also. The experimental sample with 6% product shows that reactive sites of the product are almost occupied by chromium. This image also reveals that chromium particles are adsorbed by active sites of product; almost complete occupation of active sites leaving no unoccupied structures representing complete exhaustion of chromium.

Chromium uptake by the product has also been confirmed and estimated by SEM-EDX studies. The Figure shows stratified layers of fibrous structure containing carbon and oxygen with an equivalent amount of chromium as weight % of 56.5 and 43.4 corresponding to atomic % of 63.4 and 36.5 respectively. Experimental sample with 2% product shows an adsorption of equivalent chromium of 4.48 weight % corresponding to an atomic % of 2.34. This indicates higher amount of un-adsorption leaving lower amount of chromium for exhaustion. Sample with 4% of product shows exhaustion of 2.31 weight % corresponding to atomic % of 0.75 indicating moderate amount of chromium present in the sample indicating higher amount of adsorption by the substrate. The images with 6% of product reveal exhaustion of 5.24 weight % corresponding to atomic % of 1.67 showing better exhaustion of chromium by the substrate. This confirms that the product is capable of increasing the exhaustion of chromium using its active binding sites such as carboxylic acid groups. The SEM figures 4 & 5 indicating the control samples containing globular crystalline particles and representing more amount of chromium in the control samples and the protein based product sample representing thick conjugated fibrous particles representing free carboxylic acid moieties / groups which functionally crosslinks chromium.

## 3.6. AFM analyses

Experimental and control samples were subjected to AFM study using different magnifications (2, 3, 8, 25  $\mu\text{m}$  and 800 nm) to analyse the topography and deflections of samples. The images obtained from AFM analyses of the experimental samples using product at the level of 2, 4 and 6% are provided in Figure 4. It can be seen that the product sample presented in Figure 4 portrays randomly scattered crystalline particles of the product.

It is seen from the figure that crystalline particles, spike, junk or fibrous particles are the protein based product (pbp) containing the functional groups. In other words, AFM images show two types of structures (i) smaller darker globular structures indicating presence of Cr(III) and other spots of linear fragments represent the protein based product. The non-uniformly distributed particles are ready for adsorption. This sample contains free functional groups of carboxylic acid which can adsorb chromium. However, deflection of mean of particles as represented by line graph indicates uniform distribution of chromium except at higher sizes (near 25  $\mu\text{m}$ ) where few spikes are visible with less spread of higher sizes of

particles. Isometric views of product as observed in Figure also confirm similar results/ findings. Generally during tanning, product combines with collagen fibres leaving less numbers of grooves of product – particles. From the deflection image it is evident that the product helped in enhancing the exhaustion of chromium from aqueous solution leading to better crosslink with collagen fibres giving rise to a complex structure.

Figure 4a shows AFM image for the case of adding the prepared product at the level of 2% in chrome tanning process. It can be observed from the Figure that there are two types of patches, one is due to the product-particles; whereas the other is due to chromium species appearing like small-darker globular structures. Presence of lesser number of functional groups (compared to other images) confirms that most of the product has been exhausted by the collagen fibres. Deflection image also shows that product has adsorbed the chromium from bath resulting into consequent binding with collagen matrix and forming a complex to stabilize the putrefaction of skin/ hide proteins further. Corresponding line graph of the experimental sample (with 2% product) also reveals uniform distribution over mean fit. Unadsorbed moieties give rise to few spikes; however, surface fit-line of deflection image describe overall uniform exhaustion.

In case of experiment with 4% product, the rate of exhaustion was found along with AFM images where the topography and deflections can be visible in Figure 4b. It can be found that whitish particles in the image are due to unused product moiety. Chromium particles are almost adsorbed at a slightly higher rate than the earlier case (with 4% product). The deflection pattern can be visualized as uniformly distributed around the mean value as it is evident from line graph of corresponding experimental sample. Improved exhaustion was further visualized in case of experimental sample with 6% product, as can be seen from AFM image as shown in Figure 4C. The line graph for the deflection image shows absence of chromium particles/ spots confirming complete exhaustion of chromium by active functional groups of protein and amino acids of collagen. The deflection image shows few left over particles of product in the experimental sample.

From the topography image, it can be stated that the exhaustion of chromium created some roughness in surface. The faded lighter portions reveal about scope for more exhaustion of chromium; while the darker spots hint about adsorbed particles of chromium. The experimental sample made-up with 6% product showed a line graph of mean fit to represent deflections which can be seen to be distributed evenly around the mean. This indicates improved exhaustion of chromium and smoothness of surface compared to cases with 2 and 4% product. Thus AFM studies conclude that increase in the amount of product improves exhaustion of chromium and smoothness surface of the film.

The control sample Figure 4d shows the presence of small darker globular structures indicating presence of chromium and the raw protein based product revealed the presence of more amount of crystalline structure representing functional groups of the product. Hence the AFM analysis pours some in-depth view of the interaction of product-chromium-collagen compounds favouring the enhanced uptake of chromium.

### 3.7. XRD analyses

The samples were subjected to XRD analyses to assess the morphology and distance among functional moieties of the product. The results can be seen in Figure 5 where the pattern of images for the product during tanning at different levels (2, 4 and 6%) can be viewed. In case of the experimental samples, two broad peaks at  $2\theta = 18^\circ, 22^\circ, 32^\circ$  and  $40^\circ$  are observed which slowly flatten out. These are similar to collagen samples collected from scrap leather of pure-collagen. The diffraction peaks at  $2\theta = 10^\circ - 80^\circ$  are due to functional groups of protein based product in tanning process. However, peaks were not visible at  $32^\circ$  in case of experimental sample which reveals that functional groups have cross-linked with Cr(III) and collagen increasing the uptake property of tannin. This also proves good compatibility among molecules of collagen, co-polymeric nature of the product and reagents. The increase in the intensity of peaks in case of the experimental sample shows that functional moieties are displaced by large angles decreasing the crystalline structure of  $-NH_2$  groups. Also, agglomeration of amide groups tries to extend the amorphous region due to exhaustion of chromium and its fixation with functional moieties.

### 3.8. Circular dichroism (CD) spectroscopy analyses

Circular dichroism spectroscopy is used to find chirality in the molecules of sample by measuring the difference in absorption between left and right circularly polarised lights in optically active substances. The results on CD spectroscopy are shown in Figure 6. It can be seen that mean residue ellipticity for the control sample (marked as O) is in the negative domain, indicating contamination in the sample during following standard protocol of the prepared sample. All other samples show chirality near by 210 nm revealing presence of other species around 210 nm with triple helical & denatured spectra of collagen matrix.

### 3.9 Other schemes of increasing uptake

Literature reports that a newer chrome tanning system using methanosulfonic acid (MSA) was developed. The chrome tanning was conducted at pH 5.0. The results showed that chromium uptake was enhanced from 81.0% to 95.8% in the tanning process. The chromium dosage was decreased by 26.7% and the residual chrome concentration was also decreased by 44-85% with various operations. Besides, the experiment also gave comparable organoleptic properties, area yields and mechanical properties (Zhang et al., 2016).

Literature report was available to enhance the exhaustion of chromium to more than 90% in tanning process. Sometime intrinsically modified chrome salts improve the exhaustion level of the chromium with rational pickling and tanning have been attempted by various researchers (Thanikaivelan et al., 2002). The mechanism behind high exhaust tanning system using modified tanning salt is that

preparation of tanning salts sans low-affinity species for increasing the exhaustion levels or affinity levels in the chrome tanning process.

The mechanism of chrome tanning process is a chemical process that involves formation of complex with salts and protein matrices at the molecular level by both inter and intra-chain crosslinks with triple helices, penta-fibril and fibrillary assemblies and fibre level hierarchies of skin structure. These crosslinks at the long range order of the collagen matrix provides functional stabilities such as shrinkage temperature and strength properties of the resultant leather (Gayathri et al., 1999).

In a similar work for enhancing the exhaustion of chromium in tanning process, reducing agents such as sodium bisulphite, sodium thiosulphate in conjunction with hydroxy carboxylic acids were employed. These reducing agents gave chrome exhaustion at the level of 93.51% and shrinkage temperature at the point of 121 °C in the tanned leather (EL-Shahat et al., 2019).

The other way to enhance the chromium uptake of the leather in tanning process is by carrying out tanning at an elevated pH using ethanolamine–formic acid auxiliaries. This experiment or study gave fruitful results mainly by achieving uniform distribution of chromium throughout the entire layers of the leather. However, the leather industries are not accepting the technologies because of the common problems encountered such as swelling and case hardening (Thanikaivelan et al., 2002).

In an another study of using polycarboxylic material was used during repickling and then chrome tanning was carried out that yielded better chrome exhaustion as compared to the conventional chrome tanning system. In addition, the shrinkage of the resultant leather showed higher value than the leather produced from the conventional tanning system (Anggriyani and Nugroho, 2018).

Utilisation of horn for making horn-meal yielded low molecular weight peptides with average particle size of 3,838 nm (3.84  $\mu$ m) and mean intensity of 5,243.7 nm (5.24  $\mu$ m) and mean volume of 6,228.1 nm (6.23  $\mu$ m). Application of these peptides in chrome tanning resulted in enhanced chromium exhaustion of more than 92%. The experimental leather showed expected level of softness, fullness, shrinkage temperature and strength properties with that of the conventionally produced leathers (Karthikeyan et al., 2008).

Chrome exhaustion was enhanced by increasing the cross-linking behaviour of chromium and collagen through pre-treating with complexing agents such as sodium formate and disodium phthalate with nanoclay (sodium montmorillonite) auxiliary. This complexing agent increases the exhaustion by distributing the chromium thoroughly in the various layers of collagen matrices. This technology directs the way forward for sustainable cleaner technology (Zhang et al., 2019; Yao et al., 2018).

Deviating from the conventional method, salt-less pickling using pre-tanning agent, Q 16, prepared by reacting cyanuric chloride and p-aminobenzoic acid was carried out. The results showed improvement in chromium uptake in the spent liquor, shrinkage temperature, mechanical properties in the resultant leather. This method also reduced COD of the chrome tanning process paving the strategy for sustainability in leather production (Cui et al., 2017).

An innovative idea of enhancing the chromium uptake in tanning process was attempted using auxiliary containing sulfonic acid groups. Induction of this auxiliary in tanning process enhanced chromium uptake level from 71.6% to 98.6%. The process also resulted in cost savings up to 46.9% in the processing. The other advantages of the methods were, comparable strength and organoleptic properties of the leather. This method also led to cleaner tanning process by generating lesser loads of Cr & Cl in the spent liquor to make sustainable leather processing (Zhang et al., 2017).

Chrome tanning supported by nano-composite gave increased chromium uptake in tanning process with a shrinkage temperature of the leather above 100 °C. The analytical findings using energy dispersive spectrometer study revealed that this novel type of system helped in distributing the chromium uniformly in the final leather. The specific system reduced the pollution loads namely BOD, COD of the effluent (Liu et al., 2016).

The environmental impact of the water used by leather industries could be minimised by recycling and reusing of waste water for the tanning operations. The experiment carried out at pilot and industrial scales reduced the risk of chromium and disposal of the wastewater in the tanning process (Kanagaraj et al., 2008; De Aquim et al., 2019).

Copolymer synthesised from collagen hydrolyzate and polyvinyl alcohol has been explored to attain high exhaust chrome tanning system. Application of copolymer at the level of 6% in chrome tanning process enhanced chromium uptake at the level of 94%. Analytical findings confirmed carboxylic acids, amide I and amide II, ester groups were responsible for increasing the chromium uptake level in the chrome tanning process (Kanagaraj et al., 2015b).

In a similar attempt, graft copolymer prepared from collagen hydrolysate and polyethylene glycol improved the uptake of chromium in the tanning process. Molecular modelling by gencollagen package study confirmed the reason for higher uptake of chromium to the level of 98%. The main reason for the higher uptake of chromium was the presence of functional group of carboxylic acids (Kanagaraj et al., 2015c).

Another novel way of carrying out chrome tanning was pickle free method by treating the pelt with sulphonic aromatic acid. This method completely sans use of formic and sulphuric acids in the pickling process that helps in reducing considerable amount of neutral salt and chrome tannin in the tanning process. Besides, this method has several advantages over conventional methods such as, reduction in float at the level of 75%, reduction in chromium at the level of 99% and chlorides at the level of 94% in the tanning process. The newer method was economical and cost saving up to 42% could be achieved (Bcardit et al., 2008).

The role of chromium in stabilising the collagen was investigated using real time small angle X-ray scattering, differential scanning calorimetry and natural cross-link analysis. The research indicated that the maximum stability of collagen can be achieved at concentrations as low as 1.8%. At lower

concentrations of this, the active functional amino acids are saturated via covalent bonding with collagen matrices (Zhang et al., 2018).

pH-sensitive and chromium-loaded mineralized nanoparticles (Cr-PPA NPs) were developed by self-assembly of (poly(ethylene glycol) methyl ether acrylate-co-acrylic acid) poly(PEG-co-AA) copolymers template  $\text{Cr}(\text{OH})_3$  mineralisation. Cr-PPA NPs showed excellent colloidal stability in water with high salt concentration, protein, and pH above 3.0 and provided uniform distribution of chromium in leather during chrome tanning process (Li et al., 2019).

Keratin hydrolysate from poultry feathers along with chrome shavings have been utilised for high exhaust chrome tanning and dyeing process. Application of the above constituents has increased the chrome exhaustion to the maximum level. In addition to the present work of eliminating amount of liquid wastes of chromium, a leather-like flexible sheet is also made that can be employed in leather goods and footwear industries (Ramamurthy et al., 2015).

An eco-friendly chrome-less tanning methodology using nanocomposite was developed. The nanocomposite applied in tanning process gave better hydrothermal stability, biodegradability and softness. In addition, SEM and AFM results showed the leather treated with nanocomposites exhibited well-dispersed fibrils and uniform fluff. The pollution loads such as BOD, COD were reduced to the considerable level in the tanning process (Ma et al., 2014).

### **3.10. Color measurement study of leather**

Color measurement study is one of the important analytical tool to know the effect of chrome tanning process. The main reason for carrying out color measurement study is that color properties reflect the rate of cross-linking phenomenon and chromium uptake in the tanning process. On the other hand, it is the indirect phenomenon to know the color chemistry behind the product-chromium-collagen interactions. It has been found from the several studies that the color properties were better in the case of enhanced chromium uptake in the tanning process. In other words, increased chrome uptake enhances the richness or brightness of the color of the leather. Hence the tanned leather was subjected to dyeing in the post-tanning process to manufacture the crust leather and the same was subjected to the color measurement property. The crust leathers were measured for color properties using CIELAB system. The Table 5 represents color measurement study of the crust leather. L, a, b, c are colour co-ordinates, 'h' is hue and 'c' is the chromaticity differences of the leather respectively. The experimental leather carried out with product at the level of 6% and control sample without product were subjected for colour studies. It is seen from the results that experimental leather showed lesser lightness of 'L' value indicating the increased trend of darker colour as that of control sample. The experimental samples also show relatively higher 'a' and 'b' value which is due to the presence of lesser greener and yellower component when compared with the control sample. It is also evident from that table that chromaticity and hue difference results were also lesser in the experimental sample compared to the control samples. It was confirmed from the results that the experimental sample showed better or increased colour uptake and produced darker

shade as compared to the control sample. The better colour uptake of the leather was due to better uptake of chromium as compared to the control sample. The functional groups of product namely carboxylic acid, aldehyde, amide, OH groups enhances the crosslinks in the tanning process and this may be the reason for increased exhaustion of chromium in the tanned leather.

### 3.11. Physical strength properties of leather

Physical strength properties are the most important and decisive factor in choosing the correct type of tanning process for attaining the sustainability in leather sector. The leathers obtained from the experimental processes were subjected to various physical strength properties namely tensile strength, % elongation and tear strength properties. The results are presented in Table 6. The experimental leathers exhibited analogous strength properties and values for control samples. Tensile strength property of the experimental leather sample obtained by the treatment of product at the level of 2 and 4% had strength of  $270 \pm 1.0$ ,  $275 \pm 3.0$  (kg/cm<sup>2</sup>) in comparison with the control sample of  $301 \pm 2.0$  (kg/cm<sup>2</sup>). Similarly, the other properties namely, elongation at break (%) and tear strength properties of experimental leathers were also comparable with that of control sample.

## 4. Conclusions

Chrome tanning process carried out with the protein based product at the various levels of 2, 4 and 6% were investigated for the effect of exhaustion of chromium and leather properties. It was found from the experiment that the application of the product at the level of 6% gave the exhaustion of chromium at the level of 93% in comparison with 79% for control sample. FT-IR analyses of the experimental samples confirmed the presence of active functional groups namely carboxylic acids, aldehyde, amide and hydroxyl groups in the samples which were absent in the control sample. These functional groups played an important role in improving the exhaustion of chromium and leather properties. These results were further supported by SEM-EDX analyses of the samples that indicated the presence of decreased level of chromium in the exhaust liquor. AFM study from topography and deflection showed clear pictures of the presence of lower level of chromium in the experimental samples as evidenced in SEM-EDX results. CD spectra revealed that the addition of protein based product increased the ellipticity of the collagen in the experimental sample as compared to control sample. The x-ray diffraction study showed peaks at  $2\theta = 10^\circ - 80^\circ$  which are responsible for improved uptake of chromium using protein based product in tanning process. The tanned leather also exhibited shrinkage temperature of more than 100 °C. The leathers were further processed into crust leather. The results showed that leather made with experimental processes excelled better colour properties with that of control sample. The strength and organoleptic properties of the crust leather were analogous to the conventionally produced control leathers. This present work aims to develop sustainable leather technologies by mitigating chromium and proteinous solid wastes problems of the leather industry.

## Declarations

## Authors Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Dr. James Kanagaraj], [Dr. Rames C Panda] and [Ramakrishna Prasanna]. The first draft of the manuscript was written by [Dr. James Kanagaraj] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors agree to publish and participate as per the norms of the journal.

## Availability of data and materials

The authors declare that [the/all other] data supporting the findings of this study are available within the article [and its supplementary information files]

## Ethics approval and consent to participate

All procedures performed in studies involving tanning systems were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

## Consent for publication

All authors approve to publish their data in this journal leading to publication.

## Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare that There is no conflict of interest regarding this paper.

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## Tables

**Table 1**

### Process details of chrome tanning experiments

Steps involved	Chemical name	% of chemical/ water offered	Running time	Remarks
Chrome tanning	Pickle liquor	50%	Run 60 mins	2 feeds Check penetration
	BCS	6%		
Basification	Water	50%	20 mins	pH 3.8-4.0
	Sodium formate	1%	30 mins	
	Sodium bicarbonate	1.0%	90 mins	
	(3 feeds x 10 min + 60 min)			
Experiments	Protein based product	2, 4, 6 %	60 mins	The leathers after the treatment of the product were left over night in the same bath and drummed in the next day for 30 mins to complete the process.

**Table 2**

### Characteristics of the protein based product

Characteristics of Protein based product	Values
pH	4.0
Particle size	1538 nm
Relative viscosity (CP)	1.006 mpa.s @ 27.5 °C
% Solid level	6.85

**Table 3**

### % Exhaustion of chromium in the tanning process

S. No	Sample	% Cr <sub>2</sub> O <sub>3</sub> in the spent liquor	% Exhaustion
Experiment 1	Protein based product-2%	0.55	86
Experiment 2	Protein based product-4%	0.58	90
Experiment 3	Protein based product-6%	0.60	93
Control 1	Conventional based tanning system (without Protein based product)	1.90	79
Control 2	Control with equivalent commercial product-2%	0.59	88

**Table 4**

**Shrinkage temperature of the leather**

S. No	Name of the sample	Shrinkage temperature of the leather (°C)
1	Experiment with product 2%	105 ± 0.5
2	Experiment with product 4%	105 ± 1.0
3	Experiment with product 6%	107 ± 0.7
5	Control (without product)	106 ± 0.5
4	Control (commercial product-2%)	107 ± 0.5

**Table 5**

**Colour measurement study (tan colour) of the leather**

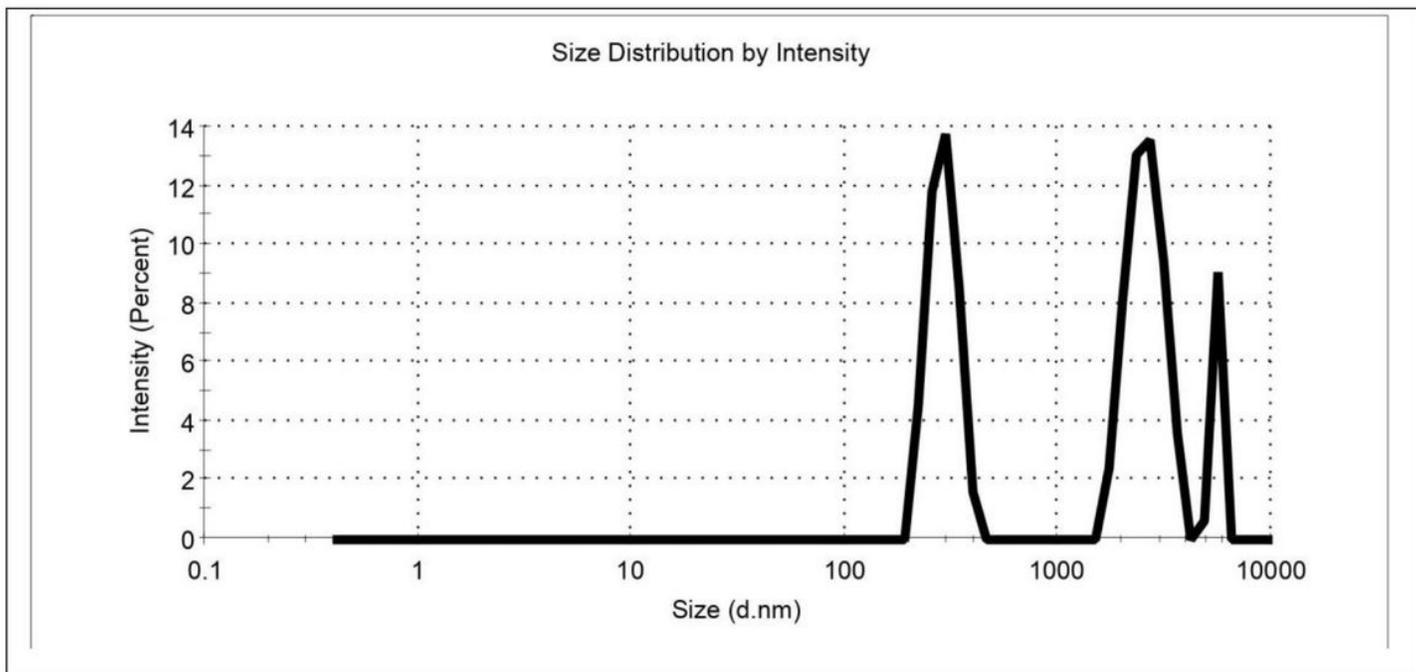
<b>Sample</b>	<b>L</b>	<b>a</b>	<b>b</b>	<b>c</b>	<b>h</b>
Experiment	57.13	20.49	36.74	42.07	60.86
Control	50.02	22.82	33.76	40.76	55.94
Experiment: Product-6%					
Control: Without product (conventional process)					

**Table 6**

**Physical strength properties of the leather**

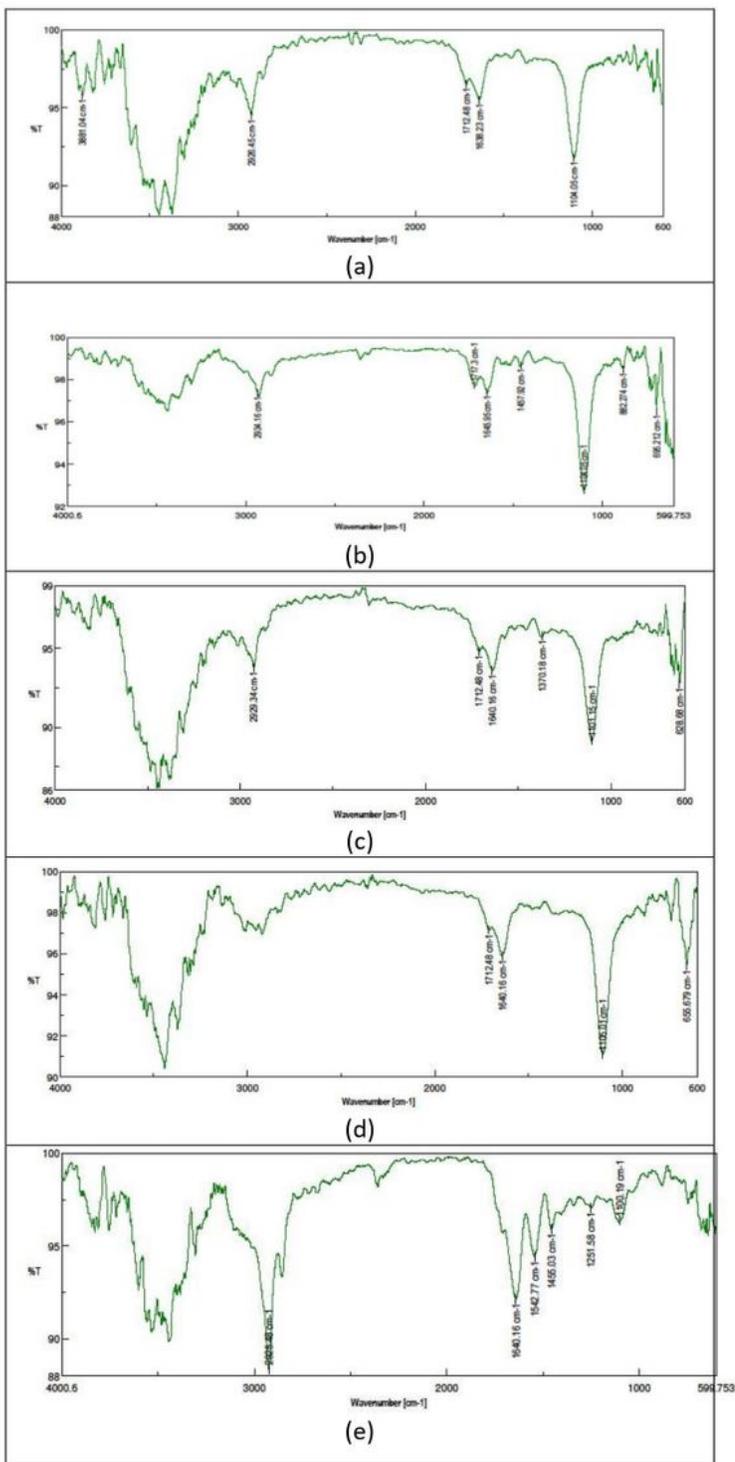
<b>Parameters</b>	<b>Product-4%</b>	<b>Product -6%</b>	<b>Control (No product)</b>
Tensile strength (kg/cm <sup>2</sup> )	270±1.0	275±3.0	280 ±2.0
Elongation at break (%)	58±1.0	62±1.0	60±1.0
Tear strength (kg/cm)	57±1.0	63±2.0	63±2.0

## Figures



**Figure 1**

Graph showing particle size distribution of the protein based product



**Figure 2**

(a) Experiment with 2% product, (b) Experiment with 4% product, (c) Experiment with 6% product, (d) Control without product, (e) Commercial equivalent product 2%

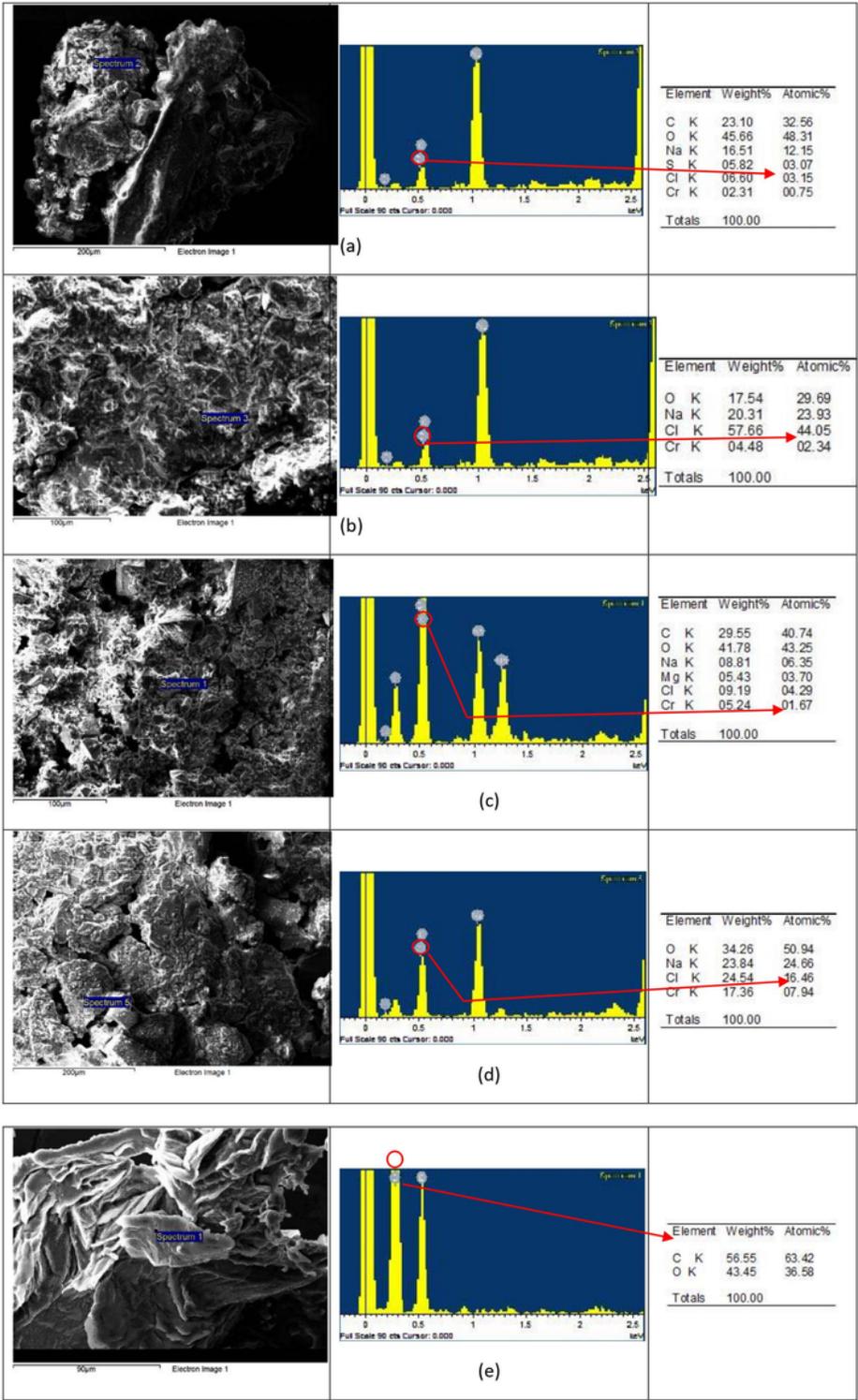


Figure 3

SEM-EDX images of (a) Experiment with 2% product, (b) Experiment with 4% product, (c) Experiment with 6% product, (d) Control without product, (e) Protein based product

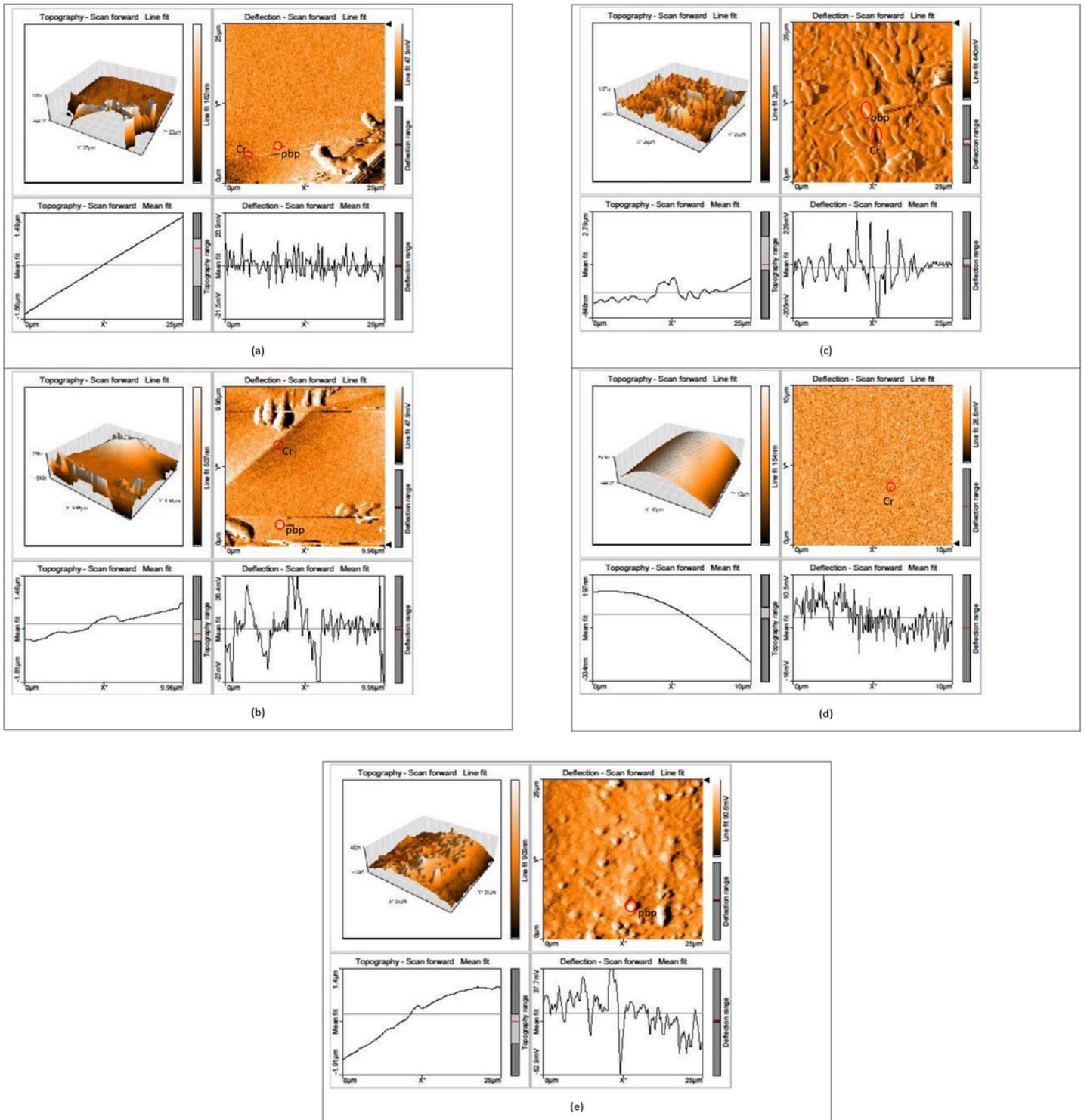
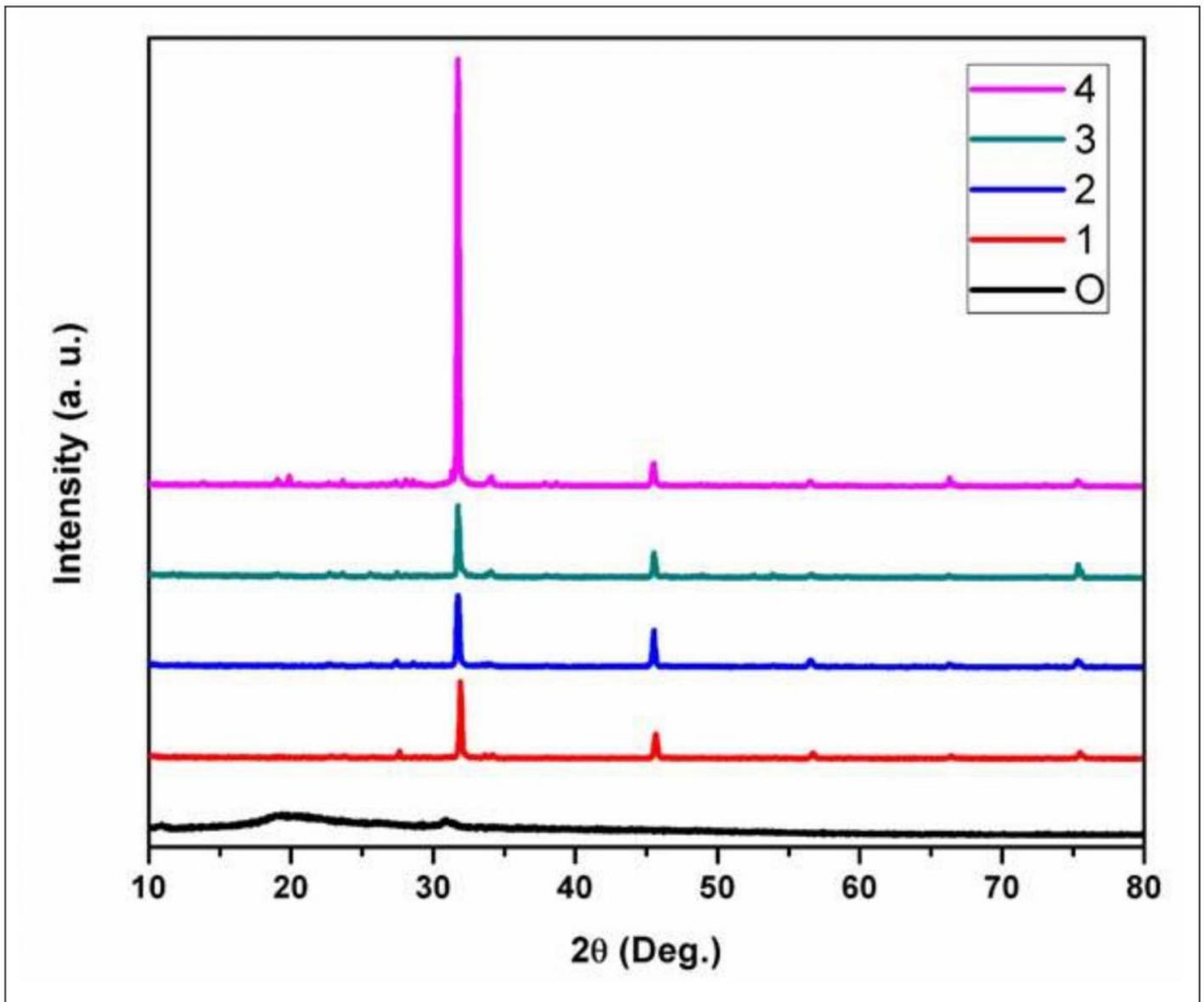


Figure 4

AFM images of (a) Experiment with 2% product, (b) Experiment with 4% product, (c) Experiment with 6% product, (d) Control without product, (e) Protein based product #Cr: Chromium #Cp: Protein based product



**Figure 5**

Plot of XRD images showing intensity with respect to deflection for; (0) control without product, (1) experiment with 2% product, (2) experiment with 4% product, (3) experiment with 6% product and (4) Protein based product

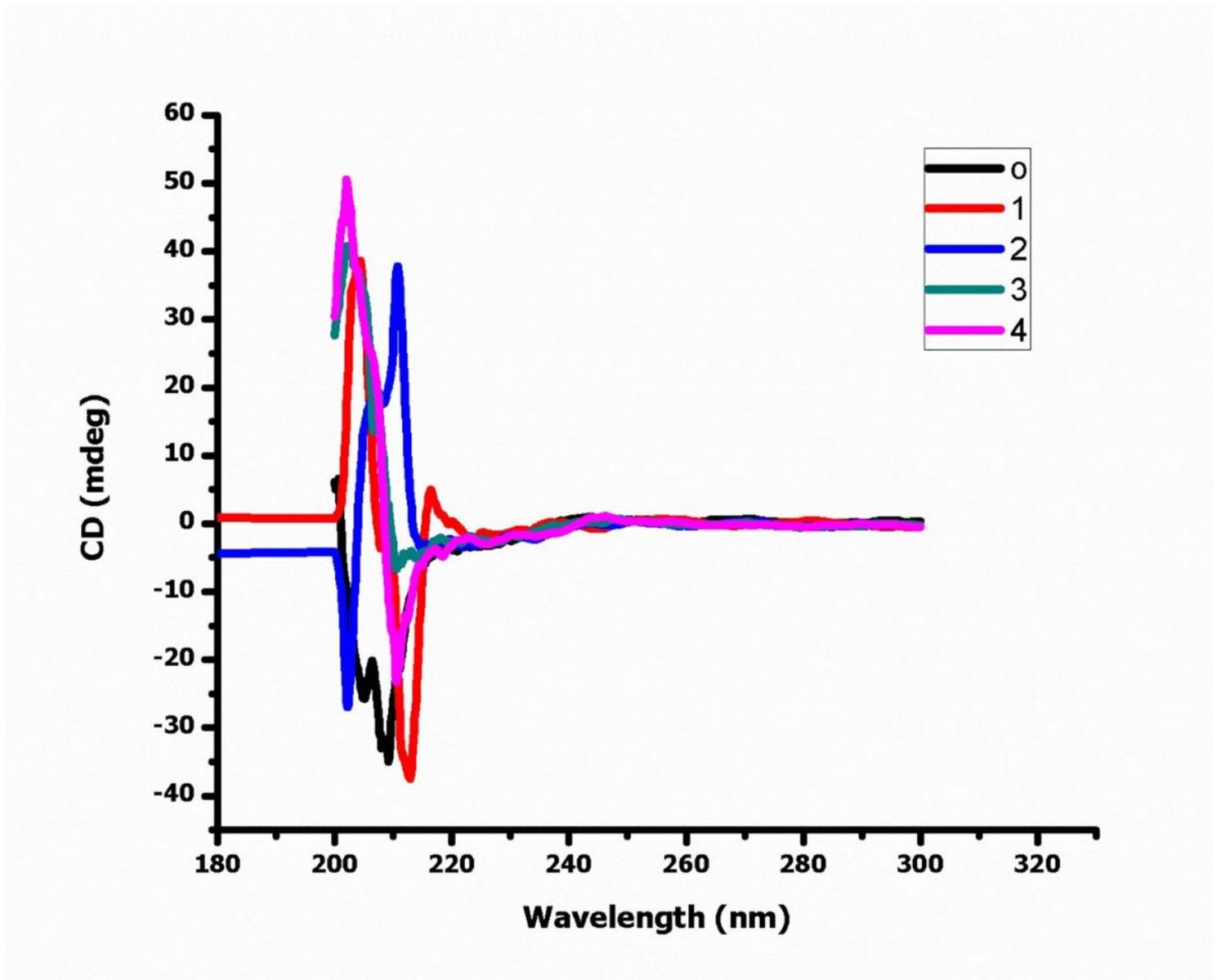


Figure 6

Plot of CD graph showing intensity with respect to deflection for; (0) control without product, (1) experiment with 2% product, (2) experiment with 4% product, (3) experiment with 6% product and (4) Protein based product

## Supplementary Files

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