INTRODUCTION

Biomaterials are either biologically derived materials from bacteria, fungi, plants and animals or engineered to interact with biological systems. They are used in biomedical applications such as to repair damaged body parts by interacting with living systems [4]. The most common biomaterials used to maintain or replace functions in the human body are metals, ceramics, or polymers [5, 8] and are used in the human body to replace or support physiological functions. Polymeric biomaterials are finding application in tissue regeneration and regenerative medicine. Medical implants, including heart valves, stents, and grafts, artificial joints, ligaments and tendons and hearing loss implants are examples of biomaterials that have demonstrated application in the field of biomaterials and regenerative medicine [14]. The polymeric classes of biomaterials can be fabricated into potential tissue engineering scaffolds for which CMC, alginate, fibrin gels, collagens, hyaluronic acid and other materials are commonly investigated [5, 14]. These polymeric materials can be converted into a variety of molecular forms, including nanofibrous scaffolds, foams, cryogels, hydrogels etc.

A gel with water as a dispersion medium is known as a hydrogel. As they resemble native tissue, they are also emerging as potential scaffolds. Their resemblance to the body cells due to their aqueous nature has brought them at the forefront of tissue engineering material research [4]. Hydrogels are highly porous in nature and allow material exchange through them. Agarose, alginate, chitosan, carboxy methyl cellulose, collagen, fibrin, gelatine and hyaluronic acid are some natural polymers used for fabrication of biomaterials as a tissue regenerative scaffold [11]. Carboxymethyl cellulose (CMC) is a linear polysaccharide and highly-soluble derivative of cellulose in water. It is commonly used derivative in biomaterials production. It has different characteristics such as mechanical strength, hydrophilicity and viscosity. Because of the raw material’s abundance and the low cost of the process, it has found applications in a variety of fields including cosmetics, pharma industries, food industries, chemical industries etc. CMC is powdery substance which is yellowish white in colour. It is hydrophilic but insoluble in organic solvents such as like acetone, ethanol, and benzol etc. CMC after being dissolved with binding agents leads to the fabrication of biomaterials [14].

From past two decades, various plant sources have been utilised for the isolation of cellulose which in turn used for synthesis of CMC. Hence cellulose
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is the root source of CMC synthesis. Pine straw is a most common forest waste found in Himalayan region of Uttarakhand, India and enormously present in forest areas. Cheer (Pine needles) is highly flammable and catches fire easily when they get dry in summer season. These dry needles are slippery also responsible for increasing pH of the surrounding soil. Scientific community nowadays is also looking for valorisation of waste for the production of potentially applicable commodities ranging from flavour, nutraceuticals to biomaterials [3, 13]. Scientifically they possess high cellulose content. In the view of availability and the cons of pine needles the present work focuses on the synthesis of CMC from pine needles that can be used as potential source for biomaterial fabrication for biomedical and regenerative medicine applications.

MATERIALS AND METHODS

Pine needles were collected from local forests of Ghurdauri, Pauri Garhwal, Uttarakhand India. Chemicals used during procedure were sodium hydroxide (Central drug house pvt Ltd), monochloroacetic acid (Central drug house pvt Ltd), standard CMC (Central drug house pvt Ltd), ethanol, methanol, glacial acetic acid (Hi-media laboratory), n-hexane (Loba Chemie Pvt Ltd.), sodium hypochlorite (Loba Chemie Pvt Ltd.), and sodium metabisulfite (Loba Chemie Pvt Ltd.).

Preparation of sample. Pine needles were collected from local regions of GBPIET, Pauri Garhwal and manually cut into small pieces and dried in the sun to remove moisture. Using a mixer grinder, the dried sample was ground into powder. The powdered sample was then sieved (passed through sieve) and stored under dry conditions for future use.

Isolation of cellulose. The powdered form of sample was treated with 10 % NaOH solution for 1 hour in a water bath at 100 degrees Celsius, followed by 1 hour stirring in a magnetic stirrer in a solid to liquor ratio of 1:20 at room temperature (RT). Filtration was used to separate the cellulose residue, which was then washed with 2 % acetic acid and distilled water. The cellulose was stirred with n-hexane at RT for 1 hour before being washed with 95 % ethanol. After filtration, then the obtained cellulose was heated for 90 minutes in a 5 % NaClO solution buffered at pH 4 in a solid to liquor ratio of 1:50 at 90-95 ºC. Filtering and washing with ethanol and distilled water separated cellulose. It was then treated for 15 minutes with a 2 % sodium metabisulphite solution, filtered, thoroughly washed with distilled water, and finally dried at 60 ºC until it reached a constant weight.

Synthesis of carboxymethyl cellulose. CMC was synthesised by alkalization and etherification reaction. With minor modifications, the alkalization reaction was carried out at 30 ºC [9]. First, 5 g of pure cellulose was infused with a 10 % (w/v) NaOH solution. Mechanical stirring for an hour produced a cellulose-to-liquor ratio of 1:2.7. In this step, 150 mL of ethanol was used as a solvent. Then 120 % (w/v) monochloroacetic acid (MCA) was added under constant stirring for 30 minutes to achieve a cellulose-liquor ratio of 1:1.2. This step was continued for 3.5 hours at 55 ºC. Filter the product and dissolve it in methanol. Glacial acetic acid was used to neutralise the slurry. The sample was then washed four times in 70 % ethanol solution and then with absolute ethanol to remove unwanted by products. Finally, the sample was filtered and dried in an oven set to 60 ºC temperature.
**Measurement of CMC yield.** CMC yield was calculated on the basis of dry weight. The yield value was calculated by dividing the net weight of dried CMC by the weight of cellulose:

$$\text{CMC yield (\%)} = \frac{\text{weight of CMC (in g)}}{\text{weight of cellulose (g)}} \times 100$$

**Determination of degree of substitution (DS):**

0.5 g of dried sodium CMC was gently ashed between for 24 hours between 450-600 °C and dissolved in 100mL of distilled water. 20mL of this solution was titrated with 0.1 N sulphuric acid with methyl orange as an indicator. The degree of substitution was used to calculate the carboxymethyl content:

$$\text{Degree of Substitution (DS)} = 0.162 \times \frac{B}{1 - 0.08 \times B}$$

$$B = 0.1 \times \frac{b}{G}$$

Where, \(b\) is the volume (in mL) of 0.1 N sulphuric acid and \(G\) is the mass (in grams) of pure CMC.

**Water retention capacity (WRC).** To 1.25 mL of distilled water, 50 mg of dry CMC sample was added, stirred, and incubated at 40°C for 1 hour. The residue was weighed after centrifugation, and the water retention capacity (WRC) was calculated as gram water per gram of dry sample.

**Oil retention capacity (ORC).** To 1.25 mL of olive oil, 50mg of dry CMC sample was added, stirred, and incubated at 40°C for 1 hour. The residue was weighed after centrifugation, and the oil retention capacity (ORC) was calculated as gram water per gram of dry sample.

**Ash content.** 0.5 g CMC sample was ashed in muffle furnace at 600 °C in the dried crucible for 6 h. The ash content was calculated using the following equation:

$$\text{Ash content (\%)} = \frac{\text{ash weight/sample weight}}{\times 100}$$

**CMC content.** The mixture of 1g CMC sample was mixed with 100 mL 80% and stirred for 10 minutes before being filtered. The pure CMC was obtained by washing the cake with 100mL of fresh 80% aqueous methanol which was then dried. The formula used to calculate the CMC content was:

$$\text{CMC content (\%)} = \frac{W}{W_o} \times 100$$

Where \(W_o\) (g) denotes the weight of the sample before washing and \(W\) (g) denotes the weight of the washed sample.

**RESULTS AND DISCUSSION**

Recent decades have seen significant increase in biomaterial fabrication from CMC for biomedical applications. Several plant sources have been utilized for CMC production till date. In the present study, we have utilized pine needles due to its abundance in Himalayan region. We have utilized ethanol as a solvent for the synthesis of CMC from pine needles (Fig. 1), whose reported CMC yield ranged between 46.5% and 120% [9, 10]. The yield is the amount of CMC obtained in relation to the amount of cellulose consumed. Extra cellulosic components are removed from the process by the action of NaOH. Our sample (Fig. 2) has a CMC yield of 110%. The DS is an important parameter of CMC characterization that indicates the solubility of CMC in water. It has been found that the standard CMC has DS value in the range 0.4-0.8. An increase in the DS value of the synthesized CMC indicates an increase in solubility [2]. Etherification process in CMC synthesis is primarily determined by the replacement of hydroxyl groups with carboxymethyl groups [1]. DS of CMC has also been found to be directly proportional to the intrinsic viscosity [9]. Although the yield of CMC in our process is in line with previous studies; however the DS of synthesised CMC was only 0.02, which suggested that sufficient hydrophobic carboxymethyl groups has not been replaced for the hydroxyl groups of the cellulose and further optimization of CMC synthesis is required.

A DS value below 0.4 indicates that the polymer is not soluble but swellable [10], which is clearly seen when we compared the WRC of prepared sample to that of standard CMC. The WRC of our sample was 8.4 g/g, which was 4 g/g for standard CMC. Higher WRC is an indication of hydrophilicity of the prepared CMC. The ORC of the prepared sample was compared to a standard CMC. Similarly, the ORC of our sample is 4 g/g, whereas standard CMC has an ORC of 4.4 g/g. The ash content is directly proportional to the degree of substitution [7]. In line with previous studies, our prepared sample has an ash content of 6%, compared to 8% for standard CMC. Purity of CMC was widely measured by treating prepared CMC with methanol [9, 10]. This washing step removes the reaction by-products, which are mainly sodium chloride and sodium glycolate [9]. We found that our test sample has a CMC content of nearly 10%, compared to 50% for the standard CMC. Our results indicated that prepared CMC contains more impurities, which might be the reason for its lower DS.

Figure 2 – Cellulose (a) and CMC (b) obtained from pine needles
CONCLUSION

Pine needles have been investigated as a root cause for fire hazards and responsible for acidic pH of soil. In fact they have potential to be used as source of cellulose and CMC was synthesised by cellulose with NaOH and monochloroacetic acid. Though the prepared CMC does meet the standard CMC considerations in terms of DS and % CMC content; however, our proof of concept study shows that pine needle could be utilized to prepare CMC by using more robust biochemical techniques.

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REFERENCES


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СИНЕЗ И ХАРАКТЕРИСТИКА КАРБОКСИМЕТИЛЦЕЛЛЮЛОЗЫ ИЗ СОСНОВОЙ ХВОИ ДЛЯ ПРИМЕНЕНИЯ В БИОМЕДИЦИНЕ И РЕГЕНЕРАТИВНОЙ МЕДИЦИНЕ

Факультет биотехнологии Инженерно-технологического института Говинда Баллабха Панта, Паури Гархвал-246194 (Уттаракханд, Индия)

Карбоксиметилцеллюлоза (КМЦ) является одним из наиболее широко используемых компонентов гидрогелей для биомедицинских применений, поскольку ее предшественник целлюлоза является самой распространенной биологической макромолекулой на Земле. В последние годы наблюдается заметный рост синтеза КМЦ из многих растительных источников. В настоящем исследовании использовали хвою бодрящей сосны (Pinus spp.) из-за ее обилия и легкой доступности в гималайских регионах по всей Индии. Целлюлоза была извлечена из сосновых иголок. Затем процесс этерификации был использован для получения КМЦ из целлюлозы сосновых иголок с гидроксидом натрия и монохлоруксусной кислотой. Выход КМЦ, степень замещения, влагоудерживающая способность, маслоудерживающая способность, зольность и содержание КМЦ определяли путем сравнения полученной КМЦ со стандартной КМЦ. Полученная КМЦ имела чистоту 10 % и выход 110 %. Степень замещения, влагоудерживающая способность и маслоудерживающая способность были определены как равные 0,02, 8,4 г/г и 4 г/г соответственно. Содержание золы в синтезированной КМЦ, по расчетам, составляло 6 %. Большинство характеристик были либо эквивалентны коммерческой КМЦ, либо превосходили ее, поэтому синтезированный продукт может быть использован в качестве потенциального компонента для изготовления биоматериалов для биомедицинских применений.

Ключевые слова: карбоксиметилцеллюлоза; сосна; биомедицина, целлюлоза.

Суреш Чандра Фулара – доцент кафедры биотехнологии Инженерно-технологического института Говинда Баллабха Панта, Гурдаури, Паури Гархвал (Уттаракханд, Индия). E-mail: phulara.biotech@gmail.com

БИОМЕДИЦИНАДА ЖӘНЕ РЕГЕНЕРАТИВТІ МЕДИЦИНАДА ҚОЛДАНУ УШІН ҚАРАҒАЙ ИНЕЛЕРІНІҢ КАРБОКСИМЕТИЛЦЕЛЛЮЛОЗА ЖӘНЕ СИПАТТАМАСЫ

Говинда Баллабха Панта инженерлік-технологиялық институтының биотехнология факультеті, Паури Гархвал-246194 (Уттаракханд, Үндістан)

Карбоксиметилцеллюлоза (КМЦ) биомедицинадағы қолдану үшін гидрогельдің ең көп қолданылатын компоненттерінің бірі болып табылады, өйткені оның прекурсоры целлюлоза жер бетіндегі ең көп таралған биологиялық макромолекула болып табылады. Сондықтан әртүрлі ертүрлі есімдік кезедерінен КМЦ синтезі тұрғындықты есім бақытталды. Бул зерттеуде қуатталықтарың қарғай інелері қолданылды (Pinus spp.) оның көптеген мен Үндістаның Гималай аймақтарында оңай қол жетімділігіне байланысты. Целлюлоза қарғай інелерінен алынды. Синтезленген Карбоксиметилцеллюлозаның синтезі және сипаттамасы.

Қілт сөздер: карбоксиметилцеллюлоза; қарағай; биомедицина, целлюлоза.