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Purified and Bio-polished Cotton Fibers as DNA-Streptavidin Tags for Bio-applications,using a Novel Approach.

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Abstract

In our current focus,we researched into the applications of Cotton Fibers as probes and tags,we designed a simple experiment to tag DNA-Streptavidin Complex onto the surface of the bio-polished Purified Cotton Fiber(PCF) to develop a bio-polished PCF as a tag to detect or diagnose some target element. Also this kind of immobilization process helps us to design and develop future bio-medical textiles to help us in advancing medicine,using nano-bio materials. It is highly challenging to bind a heavy molecule,like DNA to Purified Cotton Fiber,without damaging its structure,we accomplished this task by using CBD. Hence we believe that our current novel research on bio-polished Purified Cotton Fibers,to turn them into probes and tags is one of the pioneering efforts in this promising domain of bio-medical textiles,for applications in Medicine and Space. To mention further,we adopted self assembly technique, of covalent bonding of materials,to accomplish our task. Self assembly techniques,are clean and environmentally friendly.

Key words: Cotton Fibers/CBD-Cellulose binding domain/DNA/Streptavidin/Bio-medical textiles/Self Assembly/Covalent bonding/Probes/Tags/Bio-sensors.

I. Introduction

Industries using advanced technologies - medicine,space,telecommunications, media, etc. - need innovative materials which can be integrated into intelligent and communicative textile structures. These materials in turn, require electronics and sensors which allow the transformation of physical phenomena, such as a strain deformation,into a measurable suitable electrical signal or optical signal.In this section we briefly introduce all the materials used in our research work to help the readers gain a fairly basic insight into the materials involved and their properties to understand our project wor k,hence we describe them,in the following small paragraphs,in the order shown below[1-10].

1.1 Cotton Fiber

Information on Cotton fabric: A natural matrix suitable for controlled release systems,probes,tags and bio-sensors.

"Mankind has been using the white hairs of cotton seeds,for several thousand years to produce many different kinds of textiles,sometimes elaborately decorated. Processing of the cotton hairs, the so-called lint, into textile fibers involves a number of stages: Cotton seeds with their attached cotton hairs are harvested on a large scale from the fields and then sent to factories where the lint is separated from the cotton seed. The cotton hairs are cleaned of debris, and then graded by physical properties such as length and strength. They are then blended with other cotton lint samples, and spun into cotton yarn, which is used to make cloth. The yarn or the cloth is dyed and processed to a particular finish, and is then fashioned into the desired textile product, like jeans or towels. The cotton hair used today has a unique curvature like a twisted ribbon. This structure makes it easy to spin cotton fibers together to form cotton yarn. The twisted curvature of the cotton fiber is a unique biological feature; it is an important characteristic that synthetic textile materials are still trying to mimic". [5-15]

"Cotton fiber consists of cellulose, a natural polymer composed of many molecules of the sugar glucose. Its unique structure is ideally suited for textile production. Each fiber is basically a hollow tube a few centimeters in length that, when spun and woven, provides the very special characteristic "feel" of cotton. The longer,finer and stronger the fiber, the better the quality of cotton. There is a continuous demand for improved cotton fiber quality. This demand has been met in the past to some extent by classical breeding". [5-14] "Today, molecular plant biologists are trying to unravel the process of cotton fiber development step

by step and identify the genes involved in determining the specific properties of the cotton fiber.

This knowledge is then used by both cotton breeders and biotechnologists' to explore possible modifications in key parts of the biochemical processes which could lead to improvements in cotton fiber quality."Since cotton fiber is the basis of textile industry we consider it is highly justifiable to consider cotton fibers to advance the research in bio-medical textiles for sensing and diagnostics to spearhead the revolution of intelligent and smart textiles.[7-21]

1.2 DNA

Information on DNA as building block in the Nano-Bio Revolution.

"As bio- and nano-technology advances, the demand for writing information into DNA increases. Areas of immediate application are:– DNA computation which attempts to realize biological mathematics, i.e.,solving mathematical problems by applying experimental methods in molecular biology [1]. Because a problem must be first encoded in DNA terms, the method of encoding is of crucial importance. Typically, a set of fixed-length oligonucleotides is used to denote logical variables or graph components.[7-22]

DNA tag/antitag system which designs fixed-length short oligonucleotide tags for identifying biomolecules (e.g., cDNA), used primarily for monitoring gene expressions [2,3,4].DNA is responsible for the storage of biological information.DNA data storage,which advocates the use of bacterial DNA as a long-lasting high-density data storage, which can also be resistant to radiation [5].DNA signature which is important for registering a copyright of engineered bacterial and viral genomes.Steganography (an invisible signature hidden in other information) is useful for the exchange of engineered genomes among developers."**[1-10]**

[**"Writing Information into DNA",provided by Prof.(Dr) Masanori Arita,Graduate School of Frontier Sciences,University of Tokyo,Japan.Special Thanks**]

In our experiment,we used "dsDNA", to perform our tagging experiment with PCF,treated with CBD.No specific sequencing was used in our dsDNA specification just general purpose DNA was obtained and used."A gene is a segment of the DNA molecule that contains the information necessary for the synthesis of a functional,biological product,such as protein or RNA.Typically a cell has many thousands of genes,so DNA molecules are usually very big"[2].It is highly challenging,to bind DNA to Cotton Fiber and we were sucessful,in binding DNA a heavy molecule,to the purified Cotton Fiber without causing any damage,to the structure of the Cotton Fiber.[21-24]

1.3 Strepatvidin

Information about Streptavidin,SA

Streptavidin is used extensively in molecular biology and bionanotechnology due to the streptavidin-biotin complex's resistance to organic solvents, denaturants (e.g guanidinium chloride), detergents (e.g.SDS,Triton), proteolytic enzymes, and extremes of temperature and pH."Streptavidin is a tetrameric protein which binds very tightly to the small molecule, biotin. The binding constant for this interaction is very high and has made the streptavidin/biotin system the focus of a number of studies aimed at determining what particular intermolecular interactions give rise to the tight binding. If this strong binding can be understood, it should help in probing other systems where similar interactions are important. In particular, the design of new drugs and ligands for proteins and nucleic acids will benefit from having a detailed understanding of the interactions involved."The high affinity recognition of biotin and biotinylated molecules has made streptavidin one of the most important components in diagnostics and laboratory kits.

1.4 Cellulose Binding Domain

Information about Cellulose-binding domain

"Textile fabrics bio-polishing is one of the most important industrial application of celluloses. These are widely used to remove fibrils and fuzz fibers from cotton fabrics, or to produce the "stone-washed" look of denim garments. The depilling effect and the achievement of desirable touch properties are among the applications sought by the users. This process, although effective, is associated to a significant tensile strength loss. Interfacial properties are not considered in the removing of the pills. It is believed that the hydrolytic activity of celluloses is the only responsible process for the bio-polishing".[4-21]

The gene of CBD (Cellulose-binding domain) used in our experiment is from the gene of Thermobifida fusca WSH03-11, which is belong to Family Ⅱ of CBD Families. Then the gene of CBD is expressed in E. Coli's plasmid.

Since this paper,focuses mainly on using bio-polished purified cotton fibers for tagging or immobilization of bio-sensors ,tags or probes with bio functions, we are not describing the process of refining CBD.[8-20]

1.5 Biotin and its interactions with DNA and Streptavidin

"Vitamin H, more commonly known as biotin, is part of the B complex group of vitamins. All B vitamins help the body to convert food

(carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B complex vitamins, also help the body metabolize fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver".[2-25]The photoactivatable biotin can be incorporated randomly,in the DNA fragment double-stranded DNA and single-stranded DNA or RNA. Photoactivatable biotin is simply added to the sample and irradiated with UV light.DNA whether double stranded or single stranded,interacts with Streptavidin in the presence of biotin,forming a DNA-Streptavidin Complex. Photoactivatable biotins and related kits,are widely available from life sciences companies,such as Invitrogen USA,hence we are not going into the details of such materials.[23-26] We used biotin,DNA,Streptavidin in the ratio of 1:1 v/v to obtain biotinylated DNA-Streptavidin Complex.In our paper we refer to "biotinlyated DNA-Streptavidin complex" as "DNA-Streptavidin Complex".

II. Experimental Details-Materials and Methods

[All the materials in our current research were received and used as it is. Only Cellulose Binding Domain(CBD) and Cotton Fibers were purified using standard protocols,available from the published data.]

PART [A] -Purification procedure of raw cotton fibers

1. Experimental procedure (boiling experiment of raw cotton fibers)

Tab. 1 The Laboratory protocol for boiling experiment:

2. Boiling agent (o.w.f)	3. Cotton fabrics	4. Cotton fibers
5. Sodium hydroxide (NaOH)	6. 20	7. 10
8. Sodium silicate	9. 0.5	10. 0.5
11. Sodium bisulfite	12. 1	13. 1
14. Sodium phosphate	15. 1	16. 1
17. Synthetic detergent	18. 1	19. 1
20. Bath ratio	21. 1: 10-15	22. 1:20
23. Temperature	24. Boiling water	25. Boiling water
26. Time	27. 2 h	28. 1 h

Table 1

The procedure could be explained as follows:

Firstly, appropriate quantity of raw cotton fibers are weighed, then boiling agent was weighed according to the purification procedure or protocol of cotton fibers as shown in Tab. 1.

Secondly, the weighed raw cotton fibers and the boiling agent are placed in a glass beaker, then the glass beaker was immersed into the water-bath with boiling water,under atmospheric pressure for 1 hour, covered with Petri plate. During the process of boiling, the sample was stirred frequently,in order to merge the sample in the boiling solution.

Finally, after completion of the boiling process, the sample was rinsed with hot water for several times, then cold water was used again to rinse the sample until the pH value of the water used for rinsing the sample was about 7. There was no rumpling during the washing process. Then the sample was dried under 25℃,for over night in the oven.

※ 1. Bath ratio: weight of sample: volume of the working solution (g: mL).

2. o.w.f: : the percentage of the weight of the chemical agent and the weight of sample, with respect to the weight of sample.

3. In order to protect the structure of raw cotton fibers from damage during the boiling process, we used gentle recipe to purify the raw cotton fibers.

4. To prevent the damage of cotton fibers from oxygen with alkaline, the glass beaker was covered during the boiling process. [2]

** All the materials were received from Sigma Aldrich USA and Invitrogen USA.

PART [B] – Tagging purified Cotton Fiber with DNA-Streptavidin Complex.

In this section we performed the tagging of DNA-Streptavidin,to the bio-polished purified Cotton Fiber,so as to make the Cotton Fiber behave like a DNA-Streptavidin tag for biomedical applications in the near future. We certainly believe that PCF-CBD-DNA-Streptavidin has promising applications in bio-medical textiles, given the importance of DNA and Streptavidin.The PCF was dipped into the test tube containing DNA-Streptavidin Solution,prepared in 1:1

v/v ratio, for immobilization,based on the self assembly concept of covalent bonding. The results analyzed using SEM -Scanning Electron Microscopy,FTIR,DSC are described in the following sections.

III. Results and Discussion

SEM Analysis

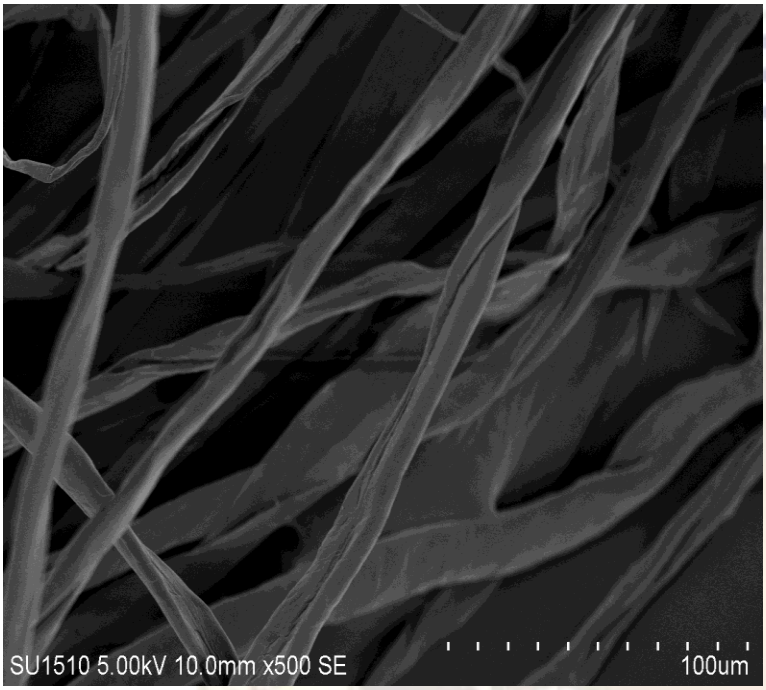

Figure 1:SEM of RCF-Raw Cotton fiber

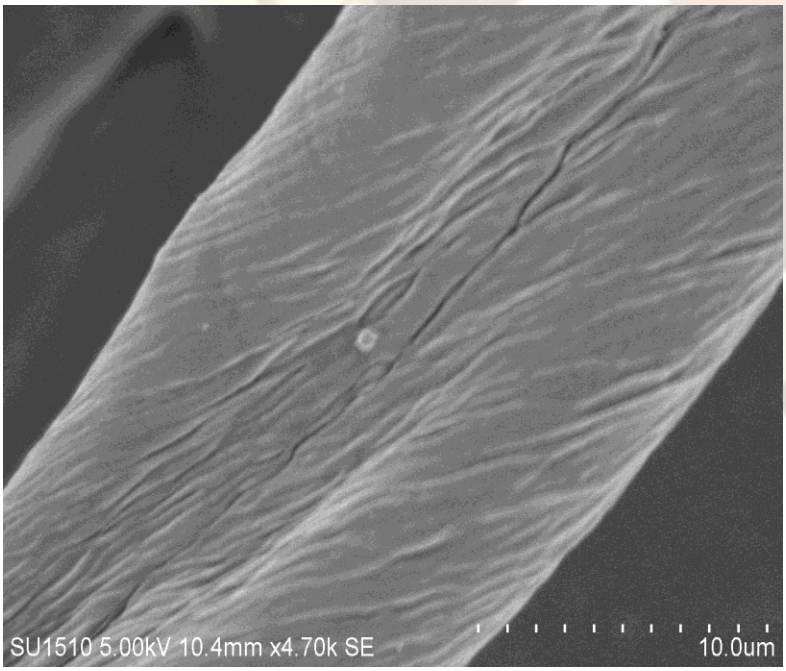

Figure 2 :SEM of PCF-Purified Cotton Fiber

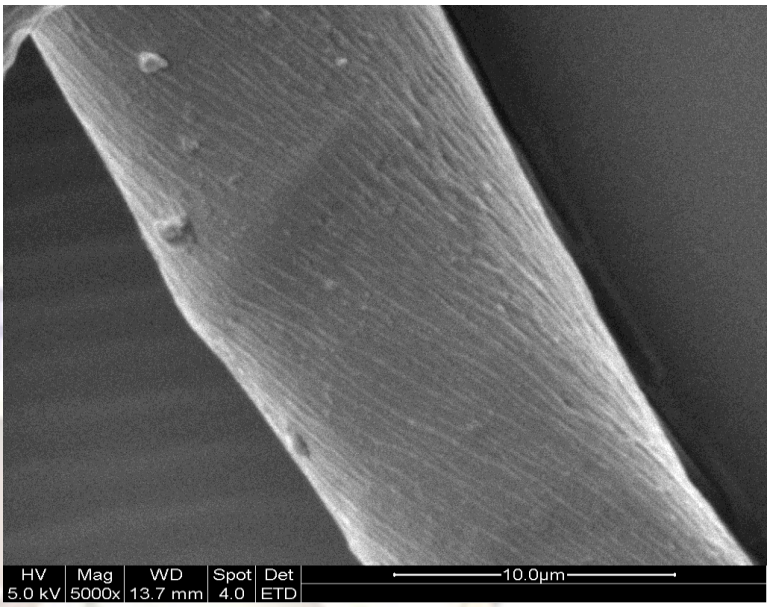

Figure 3: SEM of PCF treated with CBD

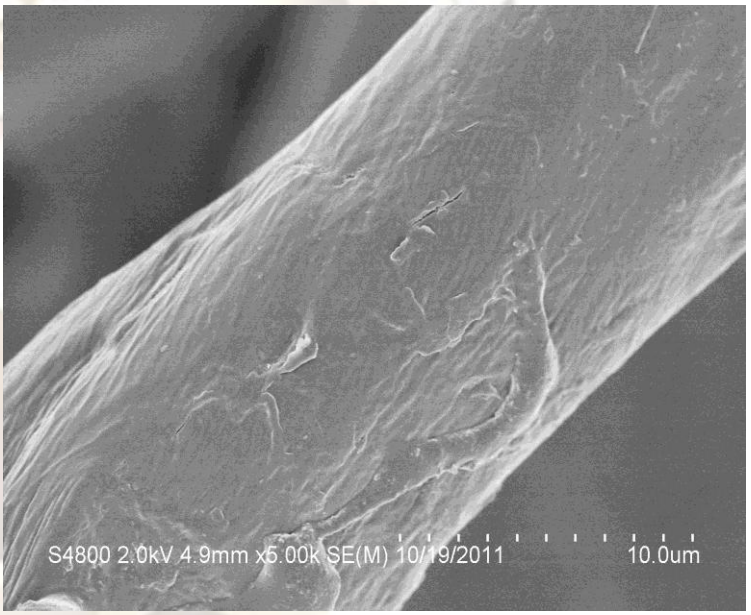

Figure 4: SEM of bio-polished PCF with DNA-Streptavidin Tag.

FTIR spectra

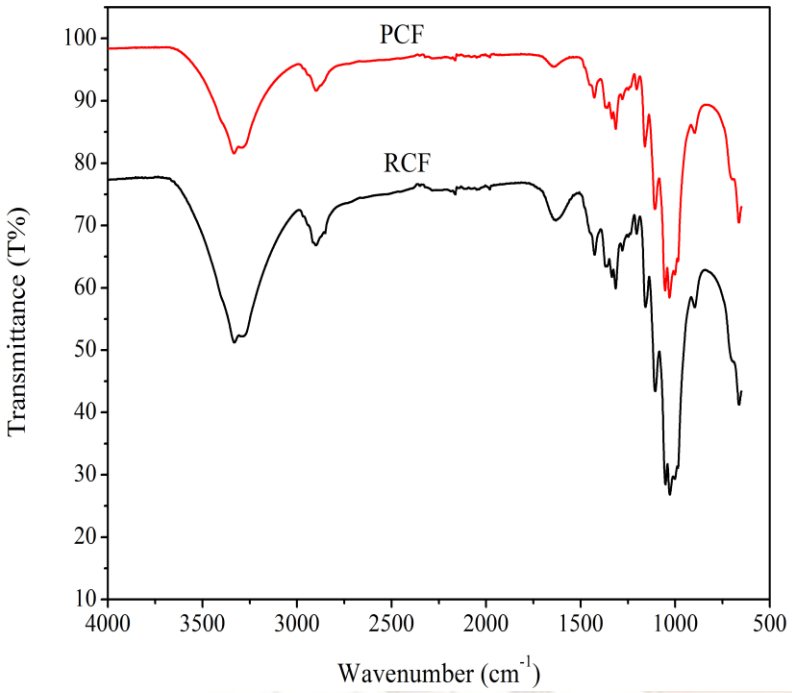

Figure 5. FTIR Cotton Fiber(RCF) and Purified Cotton Fiber(PCF)Comparison

We compare and analyze our experimental work on purified cotton fibers using FTIR,to compare the IR transmission of untreated and purified cotton fibers. Very useful in understanding the bio-polishing mechanisms for surface treatment of cotton fibers.

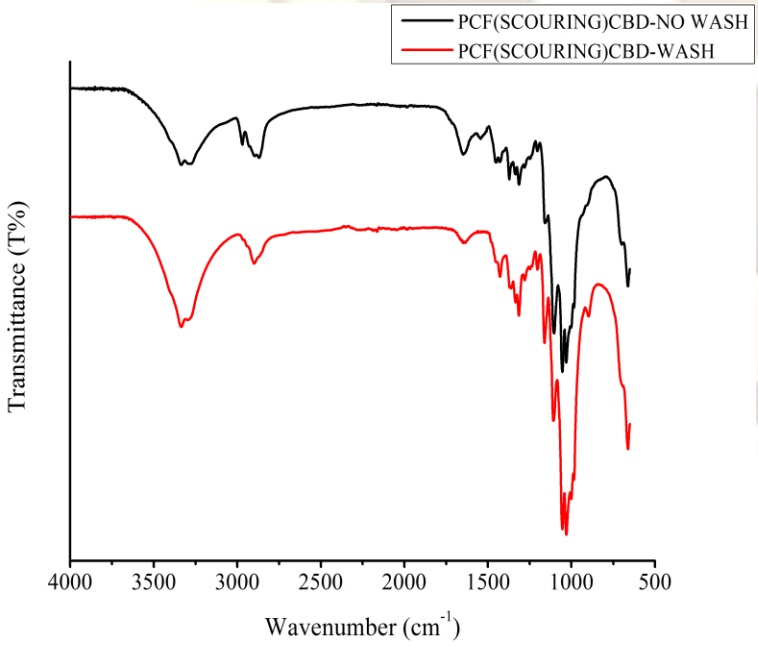

Figure6. FTIR -for Purified Cotton Fiber

Here we explain our experiment based on PCF treated with CBD. Purified Cotton Fiber was examined after it was treated with CBD,under two conditions,i.e "with out washing" and "washing".The IR spectra were obtained to compare the effectiveness,of CBD binding to the PCF,It was observed that damage was not induced on the surface of the PCF.

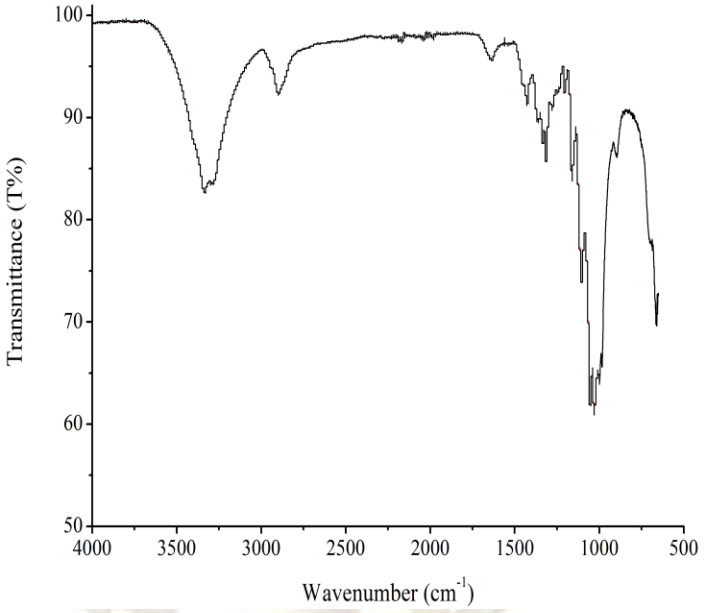

Figure 7. FTIR Spectral response of PCF-CBD-DNA-SA

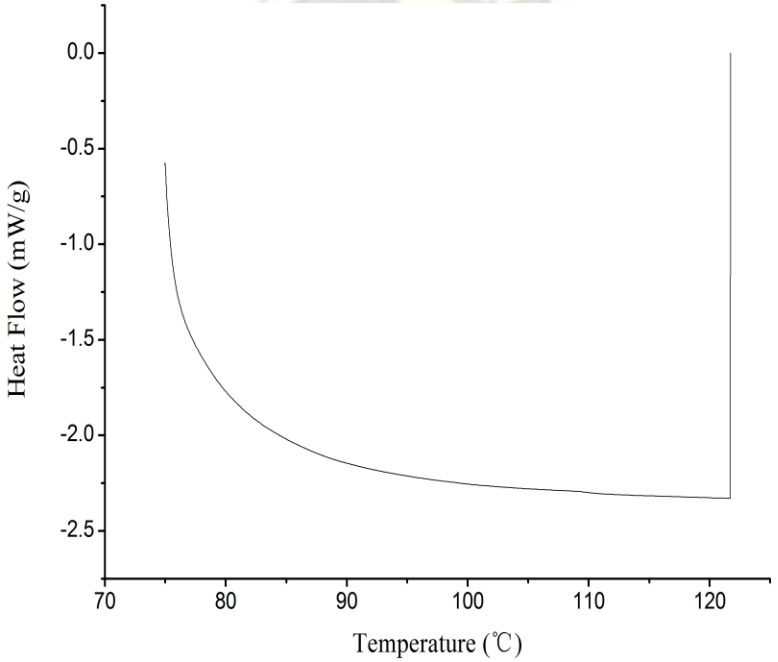

Figure 8. DSC analysis of PCF-CBD-DNA-SA Material System

The effect of biotin binding on streptavidin (SA)structure and stability was studied using differential scanning calorimetry,

The data provide a rationale for previous suggestions that biotin binding induces an increase in protein tightness (structural cooperativity) leading, in turn, to a higher thermostability.

IV. Conclusion with Future Perspectives

The results confirm the viability of using cotton fibers[cotton],as a natural bio-polished matrix for controlled released systems,bio-sensing,probes,tags etc.This is one of the pioneering papers on CBD interaction with Cotton Fibers to highlight the "tagging" or "immobilization" of DNA-Streptavidin Tag.In future these immobilization or tagging novel concepts of biosensors or tags,to the textiles could revolutionize the important domain of Smart and Intelligent textiles for Space,Medical,Nuclear or other challenging hi-tech industries to propel the nano-biorevolution.

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