Nano-Bioconjugate Film from Aloe vera To Detect Hazardous Chemicals Used in Cosmetics

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ABSTRACT: It is of utmost importance to detect hazardous chemicals that affect human health. In this work, a simple method has been developed using a traditional medicinal herb Aloe vera as a carbon source to fabricate a nano-bioconjugate film. The nano-bioconjugate system comprises of A. vera gel itself and sodium alginate to form a fluorescent nano-bioconjugate film. The film was successfully used as an optical “turn-off” sensor in detecting analytes viz. para-Aminobenzoic acid (PABA), benzophenone, hydroquinone, and propylparaben, which are used in cosmetics and are listed as “red-listed” chemicals. The applicability of the fluorescent film in detecting these hazardous chemicals was even assessed with some locally purchased cosmetic samples. Mechanistic insight into the fluorescent quenching shown by nano-bioconjugate film is also discussed. Developments of such a detection system from sustainable sources make it an interesting option for fabricating sensors for hazardous chemicals.

INTRODUCTION

Nature manifests innumerable sources for the fabrication of new biomaterials to be utilized in varied desirable applications. One such source is the traditional medicinal herb, Aloe vera (Aloe barbadensis Miller), known as the “plant of immortality”, which has its usage not only in the health-care sector but also in the cosmetics and food industry.1 Such versatility of A. vera exists due to the wide range of chemical compounds with various biological properties found in the innermost jelly portion of the leaf.2 It has been reported that most of the beneficial effects of the aloe gel are attributed to the presence of aloe mucopolysaccharides.3 The goodness of A. vera has been further exploited in the field of nanotechnology in the form of hydrogels, nanoparticles, nanocomposites, and nanofibers to use in biomedical applications like drug delivery, tissue engineering, wound healing, etc. There are reports wherein the A. vera gel is blended with both synthetic and biopolymers, and such bioconjugate materials are used in wound-healing applications. Park et al. synthesized a hydrogel consisting of a mixture of A. vera gel, poly(vinyl alcohol), and poly(N-vinylpyrrolidone) by freeze-thaw and γ-irradiation techniques and applied as a wound dressing material.4 On the other hand, Pereira et al. fabricated a hydrogel using sodium alginate and A. vera to examine its wound-healing ability.5 In terms of nanoformulations, there are several reports of usage of A. vera; for example, electrospun polycaprolactum-A. vera nanofibers were designed as wound-healing material6 and nanofibrous scaffolds using PLACL, silk fibroin, and A. vera were designed for tissue regeneration.7 The addition of A. vera in all such cases exhibited improved adhesion and proliferation of cells.

Recently, carbon dots (CDs) derived from natural sources have been gaining attention among the research fraternity due to several advantages over other carbon sources. Natural products are economically viable, renewable, and biocompatible. Due to their rich functionality and occurrence of heteroatoms (N, S), there is no requirement of passivating and doping while synthesizing carbon dots, thereby making the route simpler and cost-effective.8 As per the literature, different varieties of natural products used in making CDs are being reported, viz. milk,9 green tea,10 honey,11 egg,12 coriander leaves,13 banana,14 and spices (cinnamon, black pepper)15 among a few. Moreover, their excellent fluorescence properties have been utilized in sensing biomolecules, synthetic food colorants, etc. For example, Baruah et al. used Tea CDs for dopamine sensors.10 Vandarkuzhali et al. prepared Nitrogen containing carbon dots (CDs) from the pseudostem of the banana plant and used as fluorescent sensors for Fe3+ and S2O3−− ions in cancerous cells.17

There are thousands of chemicals present in cosmetics and health-care products, many of which get absorbed into the skin. The common people, especially in underdeveloped countries, are ignorant of the fact that many of the ingredients cause skin irritation, endocrine disruption, and reproductive toxicity and hence are carcinogenic. Even a campaign for safe...
cosmetics in coalition with Breast Cancer Prevention Partner (BCPP) has been active since 2004 to create awareness of their adverse effects and they have been grouped into “red-listed” chemicals. For example, chemicals like para-aminobenzoic acid (PABA), parabens (used as preservative), benzophenone (BP), and hydroquinone (HQ), commonly present in creams/lotions, are known to easily penetrate the skin and enter the bloodstream after which they mimic and interfere with reproductive functions and influence the development of malignant melanoma, a form of skin cancer. Most of them even inhibit thyroxine, a hormone that regulates metabolism, resulting in hypothyroidism. Owing to such hazardous effects, there is a necessity to design a system that can detect the presence of such ingredients. Detection based on optical sensing strategies is quite convenient due to its faster and simpler methodology.

Herein, this work demonstrates a facile, cost-effective, and greener approach to obtain fluorescent CDs from A. vera via microwave treatment that are immobilized in a bioconjugate system comprising of A. vera gel itself and sodium alginate to form a fluorescent nano-bioconjugate film. In the process, the biopolymer, sodium alginate, known as a gelling agent, aids A. vera in film formation. The film could serve as an optical “turn-off” sensor in detecting all four structurally similar analytes, viz., PABA, benzophenone, hydroquinone, and propylparaben (PP), which are used in cosmetics and are listed as red-listed chemicals. Also, the practical applicability of the film for the detection of the presence of such harmful chemicals was tested using some cosmetic products locally available. A probable mechanism of fluorescence quenching is also discussed.

**RESULTS AND DISCUSSION**

This work illustrates the use of the popularly known medicinal plant A. vera in fabricating a bioconjugate film and synthesizing carbon dots from its leaf gel to form a nanocojugate system by immobilizing carbon dots prepared from A. vera leaf gel. First, carbon dots were synthesized from A. vera leaf gel. Scheme 1 shows the diagram portraying the method employed in preparing the A. vera carbon dot (CDAV) along with the fabrication of the bioconjugate film. In the process of fabrication, the biopolymer sodium alginate was used as a base material for film-casting since aloe gel itself could not form films and hence the resultant product is termed as a bioconjugate system. CDAV was prepared using the microwave technique described in detail in the Experimental Section.

Scheme 1. Schematic Representation of the Protocol Adopted in Preparing a Nano-Bio-Conjugate Film of Carbon Dot-Immobilized A. vera-Alginate Film

![Scheme 1. Schematic Representation of the Protocol Adopted in Preparing a Nano-Bio-Conjugate Film of Carbon Dot-Immobilized A. vera-Alginate Film](image-url)
scattering (DLS) measurement of CD$_{AV}$ shown in Figure 1C confirms the formation of carbon dots with the average particle size determined to be 3.5 nm. The particle size is in confirmation when correlated with the transmission electron microscopy (TEM) images of CD$_{AV}$. The TEM image shown in Figure 1D is in agreement with the DLS measurement, clearly showing the particles smaller than 5 nm and spherical.

A. vera carbon dot (CD$_{AV}$) could successfully exhibit antimicrobial activity against both Gram positive and Gram negative pathogenic bacterial strains Methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli, respectively. A significant inhibitory zone was formed for both strains for which the diameter measured was 22 mm for MRSA and 21 mm for E. coli (Figure 2). It is interesting to note that as per literature reports, A. vera gel aqueous extract has no activity against S. aureus and E. coli; however, our observation shows that when A. vera gel is converted to carbon dots, it demonstrates substantial activity against such strains.

The CD$_{AV}$ so formed was incorporated into the 3:1 aloe-alginate system to fabricate the nano-bioconjugate film (A. vera-alginate-carbon dot bioconjugate film, AV-Alg-CD$_{AV}$). It is interesting to note here that the nano-bioconjugate film using only A. vera could not be fabricated. AV-Alg-CD$_{AV}$ film was then subjected to different characterization techniques.

Figure 3A depicts the UV-visible spectrum of AV-Alg-CD$_{AV}$ bioconjugate film exhibiting two absorption maxima. The peak at 210 nm is due to n→σ* transition, requiring higher energy, showing the presence of –OH groups in the film, whereas the peak at 273 nm corresponds to n→π* transition occurring due to C=O groups. This confirms the successful conjugation of alginate and aloe system, which otherwise is not present in CD$_{AV}$. The AV-Alg-CD$_{AV}$ bioconjugate film is fluorescent. So photoluminescence studies were carried out using a solid setup (Figure 3B). It is evident from the emission spectra that with the increase in excitation wavelength from 340 to 370 nm, there is a gradual increase in its fluorescence intensity. The inset figure shows the photograph of AV-Alg-CD$_{AV}$ bioconjugate film when viewed under UV light. Scanning electron microscopy (SEM) images were recorded to have an insight into the topology of the nanoconjugate film. The representative SEM image is shown in Figure 3C. It is clear from the figure that CD$_{AV}$ is immobilized in the AV-Alg-CD$_{AV}$ bioconjugate film, resulting in making the film fluorescent.

The thermal stability of the AV-Alg-CD$_{AV}$ bioconjugate film was studied and compared with the bare AV-Alg film. The stacked thermogravimetric analysis (TGA) thermograms of AV-Alg-CD$_{AV}$ bioconjugate film and AV-Alg film are shown in Figure 4A. The thermograms showed mainly two degradation stages for both films. The first stage of degradation started at 36 °C and continued to 100 °C with 24% weight loss in AV-Alg film versus 15% weight loss in AV-Alg-CD$_{AV}$ film. Such a small weight loss could be due to the side chain breaking of alginate and glycerol. The second stage underwent maximum degradation, which started at 160 °C and continued up to 262 °C, in which there was 71% weight loss for AV-Alg film and 63% for AV-Alg-CD$_{AV}$ film. Therefore, the data clearly shows that the incorporation of CD$_{AV}$ results in improved thermal stability of the film. The better thermal stability of AV-Alg-CD$_{AV}$ can be due to the presence of uniform carbon cores of aloe mucopolysaccharides, which could be responsible for forming stronger interactions among the aloe-alginate conjugate system. Similarly, Fourier transform infrared Spectroscopy (FTIR) study was carried out on AV-Alg-CD$_{AV}$ and AV-Alg films to identify the functional groups present in the bioconjugate films and interaction of different units of the conjugate film, as shown in Figure 4B. The broadening of the
peak around 3300 cm$^{-1}$ in FTIR spectra clearly indicates hydrogen bonding between the −OH groups of alginate, glycerol, and the carbon dot system.$^{31}$ The peak around 2900 and 824 cm$^{-1}$ corresponds to C−H stretching and bending, respectively. The peak of strong intensity at 1613 cm$^{-1}$ indicates C=O stretching, whereas the peak around 1100 cm$^{-1}$ corresponds to C−O stretching. All such peaks suggest the presence of hydroxyl, carbonyl, and carboxylic moieties on both films. In fact, no notable differences in FTIR peaks are observed in both the films, thereby indicating the presence of similar functionalities. Mechanical properties were also investigated for AV-Alg-CD$_{AV}$ and AV-Alg films by recording their tensile strength (TS) (Figure 4C). It was observed that AV-Alg film has a TS of 5.9 MPa, which increased to 7.8 MPa on incorporation of carbon dots, for the film AV-Alg-CD$_{AV}$. This can be attributed to the increasing number of electrostatic interactions formed between the biopolymer and carbon dots, thereby indicating that the incorporation of CD$_{AV}$ improved the mechanical properties of the film.

Interestingly, the fluorescent film AV-Alg-CD$_{AV}$ could act as an optical sensor in detecting p-aminobenzoic acid (PABA), an ingredient used in sunscreens, termed as a red-listed chemical. Figure 5A represents the stacked PL plot of AV-Alg-CD$_{AV}$ on exposure to different concentrations of PABA.
observed that the intensity of the PL emission peak excited at 370 nm is quenched considerably in the presence of different concentrations of PABA solution ranging from $10^{-3}$ to $10^{-8}$ M. The quenching in fluorescence intensity is in the range of 37.7–71.5%. The corresponding nonlinear Stern–Volmer plot was obtained and is plotted as shown in Figure 5B.

Further, other fluorescence-quenching parameters, such as apparent $K_d$ values (dissociation constant) of different concentrations of the analyte PABA (Table S1) and a linear detection range (Figure S1) were evaluated and are provided in the Supporting Information. The linear detection range was plotted using few initial points as the system has a Stern–Volmer nonlinear plot.

Similarly, another structurally similar chemical used as an ingredient in cosmetics and also termed as a red-listed chemical is hydroquinone (HQ). The PL emission study of AV-Alg-CDAV in presence of different concentrations of HQ is shown in Figure 6A. The stacked PL spectra reveal that there is substantial quenching of PL intensity (more than 90%) when the film is in contact with HQ. In this case also, the concentration range of HQ was $10^{-3}$–$10^{-8}$ M. Another structurally similar chemical used as an ingredient in cosmetics is benzophenone (BP). On a similar line, the PL emission study of AV-Alg-CDAV in presence of different concentrations of BP is shown in Figure 6B. In this case too, quenching of PL intensity takes place when treated with BP. The concentration range for the study is $10^{-3}$–$10^{-8}$ M, and quenching of PL (30–50%) takes place in the presence of BP. The last structurally similar compound that is also a commonly used ingredient in cosmetics and termed as a red-listed chemical is propylparaben (PP). The stacked PL spectra of AV-Alg-CDAV in presence of PP are shown in Figure 6C. The PL intensity is like before; quenching of PL intensity takes place when treated with PP in the concentration range of $10^{-3}$–$10^{-8}$ M. The quenching % is also lower than that of PABA and HQ and is in the range of 20–40%.

**Analysis of Real Samples.** The potential applicability of AV-Alg-CDAV film was assessed with two locally available cosmetic products. It was found that when the fluorescent film was subjected to the extract prepared from the real samples, there occurred a substantial quenching of the film as observed from the PL emission spectra at an excitation wavelength of 370 nm. Figure 7 demonstrates the PL emission spectra of two real samples, namely, RS-1 and RS-2, and is thereby successful in detecting the presence of harmful chemicals in cosmetics. RS-1 and RS-2, as per the chemical composition provided with the product, contain PABA and PP among others.

Figure 8 gives the representative histogram plot comparing the change of fluorescence intensity of AV-Alg-CDAV film in the presence of all analytes, viz., PABA, HQ, BP, and PP, at $10^{-3}$ M and the two real samples RS-1 and RS-2 (arbitrary concentration). The histogram plot clearly shows that the quenching efficiency is higher for PABA and HQ. However, when compared with PABA and HQ, BP and HQ show lesser efficiency. Thus, AV-Alg-CDAV bioconjugate film is able to successfully detect chemicals that are used in cosmetics and are listed as red-listed chemicals.

**Mechanistic Insight.** We tried to understand the mechanism of fluorescence quenching as shown by AV-Alg-CDAV bioconjugate film in the presence of PABA, HQ, BP, and PP. The surface immobilization of fluorescent CD$_{AV}$ onto the AV-Alg film renders the bioconjugate film fluorescent. There is still no agreement on the exact reason for the origin of fluorescence in carbon dots. However, radiative recombination...
of photogenerated electron–hole pairs, quantum confinement, emissive traps, free zigzag sites, edge defects, surface states, surface passivation, and inner-filter effects are some of the reasons underlined for emission properties of carbon dots.32–34 In presence of analytes (PABA, HQ, BP, and PP) as in our case, $\text{CD}_{AV}$ acts as an electron donor producing excited electrons after being irradiated by excitation light and then transfers electrons to the analyte, which causes the fluorescence quenching of the CDs. The schematic representation of the electron transfer (ET) process is shown in Scheme 2.

Scheme 2. Schematic Representation of ET Taking Place between AV-Alg-CD$_{AV}$ Bioconjugate Film and Different Analytes

| CONCLUSIONS |
| --- |
| There is a need to detect carcinogenic chemicals used in cosmetics, viz. PABA, benzophenone, hydroquinone, and propylparaben. In this work, we fabricated a fluorescent nano-bioconjugate film of A. vera -alginate incorporating carbon dots obtained from A. vera. The nano-bioconjugate film was then used successfully to detect carcinogenic chemicals (PABA, HQ, BP, and PP) used in cosmetics. The feasibility of using AV-Alg-CD$_{AV}$ bioconjugate film in a real situation was demonstrated by detecting these chemicals in cosmetic products purchased from the local market. The development of such a nano-bioconjugate film pave the way for using greener materials for detecting hazardous materials. |

| EXPERIMENTAL SECTION |
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| **Materials.** Alginic acid sodium salt was purchased from Sigma-Aldrich, India. A. vera was collected from IASST campus, Guwahati, India. $p$-Aminobenzoic acid (PABA) and benzophenone were purchased from SRL. Hydroquinone, propylparaben, glycerol anhydrous, ethanol, and acetic acid were all purchased from Merck, India. All chemicals mentioned were used without any further purification. Distilled water was used throughout for conducting the experiments.  

**Methods.** Synthesis of A. Vera Carbon Dot. A. vera leaves collected were washed thoroughly with water. The fleshy inner gel was peeled off and oven-dried. The inner gel consists of 99% water, so it was allowed to remove water completely such that only the dried aloe flakes were left over. The aloe flakes mainly consisted of mucopolysaccharides along with a very small amount of amino acids, vitamins, and minerals.1 Dry aloe flakes (0.5 g) were then dispersed in 10 mL of water and subjected to microwave treatment at 200 °C for 30 min. The pressure and power of the microwave were maintained throughout at 13.5 bar and (20–30) W, respectively. The resultant solution was yellowish-brown. This was then followed by centrifugation (10,000 rpm for 15 min) and filtration to discard the residual part so as to collect the supernatant liquid having a concentration of 40 mg/mL. 

Preparation of A. vera-Alginate Bioconjugate Film. Solvent-casting method was used for fabricating the bioconjugate film. This process was followed by the preparation of sodium alginate solution and A. vera solution separately. Sodium alginate solution (5% (w/v)) was prepared whereby during the process, glycerol (50% w/w, wrt the mass of alginate) was added to the solution. On the other hand, a 5% w/v solution of A. vera gel was prepared. The two solutions were then mixed in the ratio of 3:1 (optimized) and were subjected to magnetic stirring for 2 h. The resulting mixture was cast into a Petri dish and was kept in a hot-air oven at approximately 55 °C. The dried films were then peeled off from the Petri dish and stored in desiccator under vacuum. The bioconjugate film so formed was termed as AV-Alg. 

Preparation of Nano-Bioconjugate Film. This method involves the immobilization of A. vera carbon dot in the bioconjugate film. A similar solvent-casting method was followed. In this process, A. vera carbon dots (1%) were added to the solution of a 3:1 mixture of A. vera gel solution and sodium alginate solution, subjected to magnetic stirring for 3 h. The homogenized solution was then cast into a Petri dish and put in a hot-air oven, maintaining the temperature around 55 °C. After complete drying, the film was formed and peeled off from the Petri dish; it was then stored in a desiccator at room temperature. The nano-bioconjugate film so obtained was termed as AV-Alg-CD$_{AV}$.

Fluorescence Quenching of the Nano-Bioconjugate Film by Different Analytes: Four structurally similar analytes, such as $p$-aminobenzoic acid (PABA), hydroquinone (HQ), benzophenone (BP), and propylparaben (PP) were used for this purpose. Different concentrations of each of the analyte solutions were taken in which PABA solution was prepared with 10 M acetic acid, HQ in water, and BP and PP in ethanol. Uniformly sized films ($1 \times 1 \text{ cm}^2$) were dipped into the analyte solution of different concentrations for 10 min and then taken out and dried, after which PL measurement of the films was then recorded to see the difference. 

Preparation of the Extract from the Cosmetic Products (Real Samples). To a small amount of the real sample, 10 mL of ethanol was added and magnetically stirred for 2 h to form a suspension. The extract was then collected by filtering to separate it off from the residue.

 Fluorescence quenching of the AV-Alg-CD$_{AV}$ film with that of the prepared extract from real samples was checked following the same protocol that of the four structurally similar analytes. 

Characterization. The synthesized carbon dots from A. vera were characterized using different experimental techniques. Particle size was determined using dynamic light scattering (DLS) measurement on Malvern ZS90. Optical measurements were carried out by recording the UV–visible spectrum using a Shimadzu UV spectrophotometer UV-2600, and emission spectra, using a Jasco spectrofluorometer, FP-8300. The
transmission electron microscopy (TEM) image was taken in a JEOL TEM-2100 plus model by the drop-casting method on a carbon-coated copper grid. Characterizations were carried out for the as-prepared films (with and without carbon dots) to get an insight into the changes occurring after the incorporation of the nanomaterial. To study the changes in the chemical environment, FTIR spectra were recorded using the Nicolet 6700 FTIR instrument using KBr pellets. Thermogravimetric analysis (TGA) of both films was carried out with a PerkinElmer 4000 instrument in the range of 35–800 °C, maintaining the heating rate at 10 °C/min under a nitrogen flow rate of 20 mL/min. Tensile strength (TS) measurement of the films was done on Universal Testing Machine (Tinius Olsen SST) using a 2.5 kg load cell at a speed of 5 mm/min. Also, scanning electron microscopy (SEM) images of the film containing the carbon dot were recorded on a Carl Zeiss Sigma VP instrument.

**Antimicrobial Test.** The antimicrobial activity of the synthesized *A. vera* carbon dots was determined by the agar-well diffusion method. For this experiment, two model organisms were selected, namely, Gram positive Methicillin-resistant *S. aureus* (MRSA) and Gram negative *E. coli*. MRSA and *E. coli* cells were inoculated in a sterilized nutrient broth medium and incubated overnight in a shaking incubator at 37 °C. Both the bacterial strains were then transferred and cultured on sterilized nutrient agar plates. During the process, wells were punched in the plates using a sterile stainless steel borer. Three hundred microliters of *A. vera* carbon dot (CDAV) was filled into the wells of each of the plates and was incubated for 24 h at 37 °C. The diameters of the inhibited zone were measured in millimeters. Sterilized distilled water was used as control.

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**Author Contributions**
Film fabrication along with all characterization and sensing was done by A.D., R.S., and D.C. A.D. and D.C. conceived the idea and wrote the paper.

**Notes**
The authors declare no competing financial interest.

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**ABBREVIATIONS**
PABA,p-aminobenzoic acid; HQ,hydroquinone; BP,benzophenone; PP,propylparaben; AV-Alg-CD$_{AV}$,Aloe vera-alginate-carbon dot bioconjugate film

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