

RESEARCH PAPER

Paper from Cotton Linters as Substrate for Ammonia Nanosensor using Electrospun Alginate Nanofibers

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Abstract

Nanosensor to detect ammonia was produced using electrospun nanofibers of sodium alginate (SA) blended with poly (vinyl alcohol) (PVA) by adding pH indicator dyes. During electrospinning, nanofibers were collected on a cellulosic paper prepared from cotton linters, at a flow rate of 0.5 mL/h, 15 kV voltage and 15 cm spinneret tip to collector distance. The conductivity and viscosity of the solutions were measured to understand the effect of pH indicator dyes in the solutions used for electrospinning. Optical microscopy, SEM and AFM analyses were performed to evaluate the morphology of nanofiber mats. The pH indicator dye loaded nanofiber mats were characterized by UV-visible spectroscopy, dye leaching test, contact angle measurement and CIE color space analyzer. This flexible nanosensor prepared on paper substrate can find potential applications in various fields like smart fish packaging, human breath analysis and agriculture as it can help to determine the nature of soil.

Keywords : Cotton linters, Cellulosic paper, Electrospinning, Nanofibres, Nanosensor.

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Introduction

Cellulose is found in large amounts in nature as it forms the cell wall of plants that occupy a large area on the earth. The estimated annual rate of production of cellulose per year is 0.85×10^{11} tons (Duchesne and Larson, 1989). Cotton linters, a major source of cellulose, are very short fibers remaining on the surface of cotton seeds after the process of ginning (removal of lint from cotton seed). They are removed from cotton seeds by a process called Delinting. Being a natural polymer of glucose, it is renewable, economical and bio-degradable thus causing no harm to the environment. Cellulose is mainly used in the production of paper & paperboard, textile materials, cellulose derivatives, microcrystalline cellulose (MCC), industrial sugar and biofuel. It is also used as rheological modifiers, stabilizers and water retention agent in pharma and food industries. It has been processed in the paper-pulping industry to form paper since a long time. The cellulosic paper finds immense applications in the field of printing, writing and packaging too (Xiaochuang *et al.*, 2016). Cellulosic paper is a preferred substrate for biosensors due to its remarkable mechanical strength making it stiff enough to hold the nanosensors firmly increasing the shelf life of the nanosensors. While wood pulp is mostly used for the production of printing grade paper,

cotton cellulose is a good raw material for production of high quality and durable paper like bond paper and security grade paper.

Sodium Alginate (SA) is a natural bio-degradable biopolymer of β -D-mannuronic acid and α -L-guluronic acid that dissolves in water slowly. Owing to its non-toxic nature, it has various applications in the medical field for tissue engineering, drug delivery, wound dressing etc. It is an extracellular polysaccharide produced by marine brown algae (Jian-Wei *et al.*, 2006). It has low molecular weight (216.12 g/mol) which causes bead formation during electrospinning. So to form the nanofibers successfully, combination with another material like poly ethylene oxide (PEO), poly (vinyl alcohol) (PVA) etc. is required. The second polymer would increase the viscosity making the aqueous alginate solution get electrospun to form bead-free nanofiber mats (Christopher *et al.*, 2011).

Electrospinning involves applying a very high voltage (20 to 80 kV) to a viscous polymer solution that is pumped through a syringe. The electric field causes the polymeric discharge to form into a Taylor cone and produces a continuous stable jet resulting in the formation of nanofibers (Christopher *et al.*, 2011). The electrospun nanofibers have great future as the nanofiber mats show very high surface area to volume ratio and porosity which increases the

sensitivity and reduces the response time of the product. These acquired properties could be immensely beneficial in the field of biosensors, composites, biomedicine, catalysis based reactions, smart food packaging and filtration (Huarong *et al.*, 2009). Electrospinning of biopolymers like polysaccharides (cellulose, starch, dextrose, chitosan), proteins (collagen, gelatin, silk) and DNA for diversified applications are getting momentum due to their biodegradability attribute (Schiffman and Schauer, 2008).

Sensors based on different sensing principles (optical, electrochemical, piezoelectric, catalytic and field effect gas sensors) have been employed to sense gases with biopolymers. The response time of chemical gas sensors is largely determined by the specific surface area of sensing material under study. More the specific surface area of the sensing material, higher will be the sensitivity factor of the sensor. Thus, making use of electrospun nanofibers would greatly enhance the efficiency of the nanosensor (Anwane *et al.*, 2018; Kriegel *et al.*, 2008).

The simplest method to determine for fish freshness is a physical assessment to check the colour, texture and odour of fish meat. A more sophisticated laboratory method is to analyze the gas emitted from fish meat for the presence of total volatile basic nitrogen (TNB-N) by GC-MS instrument. But, these methods are not practically viable under field conditions. Recently, *in situ* methods using sensors, lasers and electrometric techniques are being developed to monitor the quality of packed fish meat (Hedwig *et al.*, 2019). A visual change in color can be observed if a pH indicator dye gets immobilized in a biopolymer film to detect amine products coming out of the packaged fish during spoilage. The ammonia vapors can be considered equivalent to the amine products released by the enzyme catalyzed decomposition of trimethylamine oxide present in the fish muscles (Crowley *et al.*, 2005). The objective of this study was to develop ammonia nanosensor from natural, non-toxic polysaccharide, SA and impregnation of pH indicator dye into it using cotton based paper as a substrate

Materials and Methods

Materials

Sodium Alginate was purchased from MP Biochemicals, PEO was purchased from Sigma-Aldrich and PVA was purchased from Merck. The pH indicator dyes, Phloxine B (Phl B) was purchased from Himedia, India and Bromophenol blue (BPB) was purchased from The British Drug House Ltd., England. Deionized water used in all solutions was produced using a Milli Q Water purification system. Cellulosic paper was prepared from cotton linters. Bleached cotton linter was beaten in a beater (Hollander beater) followed by refining in tri-disc refiner to 50 degree SR and handmade sheets were made with the thickness of 70 GSM.

Methods

Solution preparation

Different concentrations of SA (2% to 5%) and the binder polymers, PEO (5% to 8%) and PVA (8% to 12%) were prepared and mixed in different proportions. Solutions of SA and binder polymers were separately made using a magnetic stirrer (IKA-RCT Basic) at room temperature for 1 h and then mixed by the magnetic stirrer for another 1 h. Since PVA is hot water soluble, 80 °C temperature was maintained during its preparation in a hot plate for 20 min. The solution which gave bead-free nanofibers was subjected to further characterization by addition of 6% (w/w) relative concentration of pH indicator dyes like Phl B and BPB in the solutions. The dissolution was carried out at room temperature under magnetic stirring for 2-3 h.

Electrospinning

The electrospinning apparatus consisted of a fume hood housing the equipment, an external syringe pump (New Era Pump Systems, Inc.) with a syringe attached to metallic needle, a controllable high-voltage source connected to the needle with range of 0 to 50 kV, and the cellulosic paper was attached on a grounded copper foil wrapped collector plate for collection of the fibrous mats. Solutions containing different concentrations of SA with binder polymers were checked for electrospinning capability. Syringes of 10 mL capacity carried the solutions and were attached to the spinneret during the electrospinning process. The conditions used for electrospinning of the solutions were as follows: 5-20 cm from the spinneret tip to collector distance (TCD); 0.1 to 1.0 mL/h flow rate; and voltage 10 to 30 kV depending on fiber quality to optimize the successful nanofiber formation.

Solution characterization

Viscometer (DV- III Ultra Programmable Rheometer, Brookfield) and Conductivity meter (CON 2700, Conductivity /°C/°F meter, Eutech Instruments) were used to measure viscosity and electrical conductivity of the electrospinning solutions with and without pH indicator dye, respectively. Spindle no. 63 (LV-3) rotating at three different speeds (10, 20 and 30 rpm) was used to carry out the viscosity and torque measurements at 22 °C. All the parameters were analyzed in triplicates and were expressed as average ± standard deviation (S.D.).

Characterization of nanofiber mats

Optical microscopy was performed to confirm the production of nanofibers. Light microscope (Nikon Eclipse 80i, Japan) attached with a Digital Camera (Evolution VF Cooled Color, Mediacybernetics) to aid in capturing images was put to use. The

samples were imaged at 400 \times magnification. Emission was collected through a Fluorescein isothiocyanate (FITC) filter giving rise to an epifluorescence image for the fluorescent pH indicator dye, Phl B. The UV lamp used was Nikon Intensilight C-HGFI with HP Compaq desktop and Image Pro Plus Version 6.3 software.

The morphology of electrospun nanofibers was studied using a scanning electron microscope (SEM), Philips XL-30, with an accelerating voltage of 12 kV. Each sample was coated with a thin layer of conducting material (gold/palladium) using a sputter coater (Polaron®) before SEM analysis. In atomic force microscopy (AFM), the surface of the sample was detected with the tip, moving in close proximity to the sample. The three-dimensional model of the sample surface was reconstructed from individual positions of the tip. Using AFM, fiber diameter, roughness and surface area were effectively measured. The samples were prepared by electrospinning directly on the mica sheets stuck with the stub on the collector and the mica sheets were then scanned in AFM. AFM analysis was carried out in diInnova® SPM (Veeco, Santa Barbara, CA, US) equipped with a 90 μm scanner by tapping mode in ambient condition. The silicon nitride cantilever with a spring constant of 40 N/m was used for scanning. The scan rate of 1.0 Hz and 512 lines per 10 μm were used to optimum contrast. No filtering was done during scanning. All the parameters were analyzed in triplicates and were expressed as average \pm S.D.

Ammonia gas sensing

The ammonia gas sensing property of the blended nanofiber mat incorporated with pH indicator dyes was tested by collecting the electrospun nanofibers onto glass slides and determining the absorbance variation measurements at room temperature. The absorption spectra of nanofiber mats were recorded on a UV-vis spectrometer (UV-1700 Pharma Spec, Shimadzu, Japan). The light source was a tungsten halogen lamp powered with a 12V DC source. The monochromator allowed selecting the working wavelength. The spectrometer system was connected to the

computer with windows-based UV Probe Version 2.21 software that was used for data acquisition and analysis. At the beginning of the experiment, the zero point of the spectrometer was carried out using electrospun the blended solution without any dye. Ammonia vapours from liquor ammonia were used to test the electrospun nanofiber mats of both the pH indicator dyes, Phl B and BPB to check for the change in the color developed.

Dye leaching test

Dye leaching of the nanofiber mats was analyzed by placing 0.012 g of the samples in 10 mL of deionized water at pH 8. After 24 h the absorbance of the water solution with possible released dye was measured by UV-vis spectroscopy, at 536 nm for Phl B and 579 nm for BPB. Finally the dye release was converted into percentage release with respect to the original amount of dye present in the samples (Schueren *et al.*, 2013).

Water contact angle and CIE color space analysis

Water contact angle was measured using GBX ILMS Version 3.6 instrument to study the hydrophilic nature of the electrospun nanofiber mats. For determining the grey values of developed electrospun nanofiber mats, the CIE (International Commission of Illumination) color space coordinates were determined. A color is defined by its RGB values which give the amount of red, green and blue in a particular color. In CIE L*a*b* color space values, L* stands for lightness, a* and b* for the green-red and blue-yellow color components and ΔL represents brightness difference between samples. The magnitude of color difference was quantified as ΔE .

Results and Discussion

Electrospinning process

Table 1 summarizes the results obtained from different combinations of blended solutions of SA with PEO and PVA after electrospinning.

Table 1: Solutions tested for electrospinning

Electrospinning solution	Flow rate	Voltage (mL/h)	TCD (kV)	Result (cm)
2% SA + 8% PEO (40:60)	0.25 - 1	10-25	10	Beads observed
2% SA + 8% PVA (40:60)	0.25 - 1	12-24	10	Beads observed
5% SA + 5% PEO (50:50)	0.2-1	15-30	16	Beads observed
2% SA + 12% PVA (1:3)	0.1-1	17-25	5-15	Fibers with few beads
2% SA + 12% PVA (1:1 & 2:3)	0.1-1	17-25	5-15	Beads observed
5% SA + 9% PVA (1:3)	0.1-1	17	10	Beads observed
4% SA + 8% PVA (1:3)	0.1-1	17	10	Beads observed
4% SA + 10% PVA (1:3)	0.5	20	15	Fibers with few beads
5% SA + 10% PVA (1:3)	0.1-1	17	10	Beaded fibers

Solution characterization

Conductivity results as shown in the **Table 2** indicate that the solution containing SA alone has a higher conductivity as compared to its blended solutions. The reduced amount of conductivity after addition of binder polymer PVA can be attributed to the interactions between SA and PVA which has led to decrease in the degree of ionization. High amount of anionic repulsions of like charges caused major hindrance to electrospinning of SA. This has been overcome with the addition of binder polymer in the electrospinning solution as evident from the conductivity values (Jian-Wei *et al.*, 2006). Total Dissolved Solids (TDS) and salinity of blended solutions were increased on addition of pH indicator dyes. The conductivity also increases with addition of pH indicator dyes due to the charges brought into the solution by the dye molecules. Thus, TDS and salinity were found to be directly proportional to conductivity when the effect of pH indicator dye was checked.

Viscosity of SA blended with PVA was reduced on addition of 6% (wt/wt) relative concentration of the pH indicator dyes. Torque of the solutions was directly proportional to the speed of spindle (**Table 3**). Viscosity range of around 400 to 600 cP was found to be appropriate for electrospinning.

It was neither possible to electrospin SA solutions at lower concentration of 2% (wt/vol) nor electrospun at higher concentration of 5% (wt/vol) as too low or too high viscosities prevented formation of a drop at the needle opening to initiate electrospinning. Also, the diameters of the fibers increased due to the increased viscosity (Jian-Wei *et al.*, 2006). 4% concentration of SA with 10% PVA in 1:3 proportion was found to be ideal for electrospinning to get nanofibers. Both the pH indicator dyes at 6% relative concentration successfully got electrospun at the above electrospinning conditions (**Table 1**).

Table 2: Conductivity measurements of electrospinning solutions

Solution	Conductivity (mS)	TDS (ppt)	Salinity (ppm)	Resistance (Ω)
4% SA + 10% PVA (1:3)	1.282	0.901	672.7	398.9
4% SA + 10% PVA (1:3) + 6% Phl B	2.274	1.628	1244	223.7
4% SA + 10% PVA (1:3) + 6% BPB	1.819	1.292	967.9	281.8
4% SA	4.050	3.267	2.675 ppt	105.6

Characterization of nanofiber mats

Optical microscopy was used for preliminary step of confirming the formation of uniform bead free nanofibers and imaged at 400× magnification (**Fig. 1**). Diameters of the nanofibers of SA with Phl B and BPB were 371 nm and 524 nm, respectively as measured in the images using the software. Epifluorescent images confirm uniform dye distribution of fluorescent dye like Phl B along the fibers.

The SEM images showed smooth bead free nanofibers for SA blended with PVA without any pH indicator dye and with Phl B nanofiber mats (**Fig. 2**). This shows that the pH indicator dyes did not restrict the formation of the SA blended nanofiber mats. While the SA blended with PVA and with Phl B had the similar morphology of fibers joining in the crossing and merging together, the nanofiber mat with BPB showed slightly beaded fibers due to the chemical nature of pH indicator dye. AFM images at the magnification of 10µm were used to determine the fiber diameter, roughness and surface area (**Table 4**).

The diameter of SA blended with PVA nanofibers obtained with and without pH indicator dyes were in the range of around 400 to 1000 nm. The roughness of the nanofiber mat of SA blended with PVA was increased due to the addition of pH indicator dyes. But the morphology of the fibers was not affected (**Fig. 2**). This shows that the pH indicator dyes do not interfere with the property of nanofiber formation of SA blended with PVA solutions.

Ammonia gas sensing property and UV-visible spectroscopy

When a pH indicator dye is placed in an environment that is basic enough so that deprotonation occurs, a shift in the wavelength maximum (λ_{max}) of the dye absorption spectrum takes place. For Phl B, a shift from 360 nm to 567 nm occurred and for BPB, a shift

Table 3: Viscosity and torque measurements of pullulan solution

Solution	Speed of spindle (rpm)	Average Viscosity (cP) ± SD	Average Torque (%) ± SD
4% SA + 10% PVA (1:3)	10	572.9 ± 18.5	4.3 ± 0.15
4% SA + 10% PVA (1:3) + 6% Phl B	20	526.9 ± 30.6	8.9 ± 0.62
4% SA + 10% PVA (1:3) + 6% BPB	30	541.3 ± 28.5	13.2 ± 0.72
4% SA + 10% PVA (1:3) + 6% Phl B	10	407.6 ± 20.5	3.5 ± 0.15
4% SA + 10% PVA (1:3) + 6% BPB	20	431.3 ± 23	7.1 ± 0.4
4% SA + 10% PVA (1:3) + 6% Phl B	30	419.6 ± 23	10.4 ± 0.67
4% SA + 10% PVA (1:3) + 6% BPB	10	438.6 ± 23	3.7 ± 0.2
4% SA + 10% PVA (1:3) + 6% Phl B	20	455.3 ± 24	7.6 ± 0.37
4% SA + 10% PVA (1:3) + 6% BPB	30	473.6 ± 22	11.8 ± 0.51

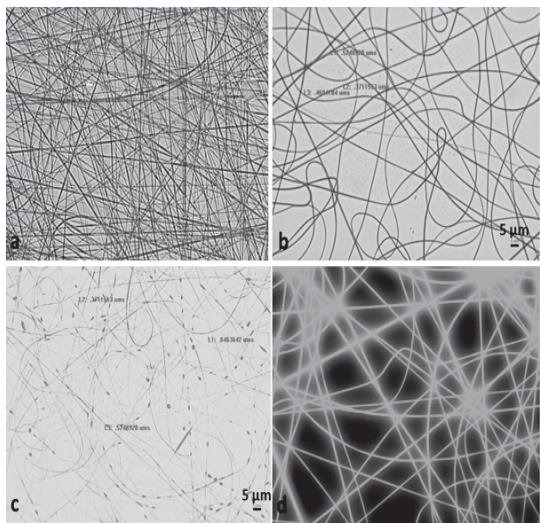


Fig. 1: Optical microscopic images of electrospun nanofiber mat containing 4% SA + 10% PVA (1:3) (a), with Phl B (b), with BPB (c) and epifluorescent image with Phl B (d) at 400x magnification

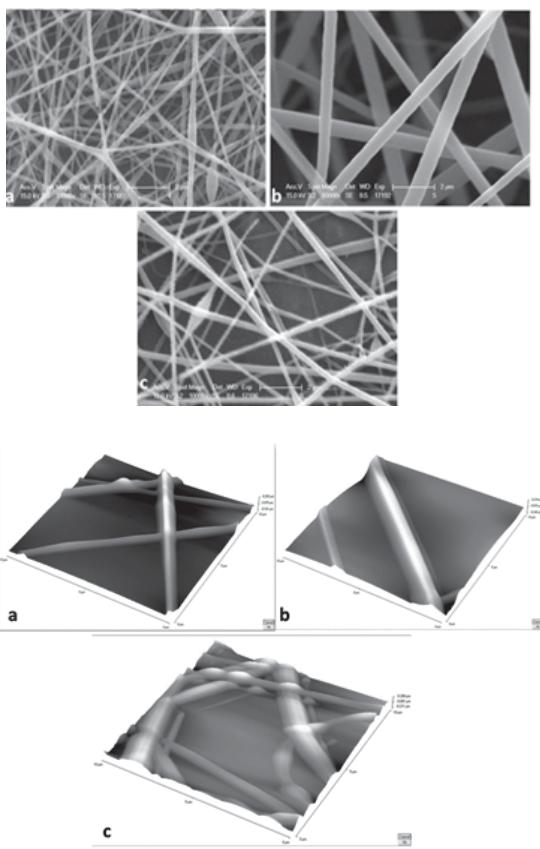


Fig. 2: SEM (top) and AFM (bottom) images of electrospun nanofiber film containing 4% SA + 10% PVA (a), with Phl B (b), with BPB (c) at 10,000 \times magnification. Scale bars represent 2 μ m in SEM and 10 μ m \times 10 μ m scan area in AFM.

from 396 nm to 605.5 nm occurred for the peak absorption. When fish or seafood product begins to spoil, basic spoilage volatiles would gradually be produced in the package headspace resulting in a pH increase and the sensor color would change from colorless to pink (for Phl B) or yellow or green to blue (BPB), easily visible to the naked eye. This color change was observed when the sensing nanofiber mat was exposed to ammonia gas which is one of the spoilage volatiles. The absorption spectra showed the expected shift in the peak (λ_{max}). The dye functionalized nanofiber mat will act as a nanosensor for rapid colorimetric detection of ammonia emitted from spoiling packaged food products like fish. Earlier work reported similar colorimetric detection of volatile nitrogen compounds associated with fish spoilage using plastic film containing BPB indicator (Wells *et al.*, 2019).

Dye leaching test

The percentage release of dye was found to be 45.22% for Phl B and 86.27% for BPB after placing the nanofiber mats in water for 24 h. This shows 54.78% of Phl B and only 13.73% of BPB were retained in the mats. The probable reason for low dye release for Phl B could be attributed to the stronger interactions of the dye with the polymer. Thus, Phl B is found to be a more promising sensing dye for incorporation in electrospun nanofiber mats. While similar work is not reported in the literature, BPB is commonly demonstrated as a promising sensing dye for detection of volatile gas associated with fish spoilage (Wells *et al.*, 2019).

Water contact angle and CIE color space analysis

The electrospun nanofiber mat of SA blended with PVA had a contact angle of 113.7° showing its hydrophobic nature. After addition of Phl B and BPB in SA blended with PVA nanofiber mat, the water contact angle decreased to 76.6° and 54.6°, respectively making the nanofiber mat hydrophilic, aiding the increase in the

Table 4: Characteristics of nanofibers mats analyzed using AFM images

Solution	Average Diameter	Average Roughness (nm) \pm SD		Surface area (μm) ²
	(nm) \pm SD	Ra*	RMS	
4% SA + 10% PVA (1:3)	460 \pm 45	10.3858 \pm 1.5	17.6416 \pm 2.1	102.4
4% SA + 10% PVA (1:3) + 6% Phl B	804 \pm 32	42.74 \pm 12.36	57.6925 \pm 21.2	102.6
4% SA + 10% PVA (1:3) + 6% BPB	944 \pm 20	88.4 \pm 9.37	108.2 \pm 17.94	104.4

* Ra is Roughness average and RMS is Root Mean Square of the measured surface

compatibility of the sensor. Addition of the dyes showed decrease in the brightness (L^*) of the mats by 5% and 25% for Phl B and BPB, respectively. Since Phl B shows a visible color change from colorless to pink, a^* was measured. The a^* value was 6.823, when incorporated by electrospinning in SA blended with PVA nanofiber mat, which changed to 63.398 after exposure to ammonia vapors indicating the increase in intensity of redness. The blueness/yellowness color component, b^* , negative values indicate blueness while positive values indicate yellowness. Since, BPB shows a visible color change from yellow to blue, its b^* was measured. Its b^* value was 21.259, when incorporated by electrospinning in SA blended with PVA nanofiber mat, which changed to -14.917 after exposure to ammonia vapors indicating the color change from yellow to blue.

Conclusions

SA blended with PVA nanofibrous mats and having pH indicator dyes (Phl B and BPB) were fabricated *via* electrospinning technique. Incorporation of the pH indicator dyes did not interfere with the electrospinning process or with the nanofiber formation process. Phl B and BPB dyes were successfully used as they were sensing ammonia gas with visible color change. However, BPB dye leached out by 55% more than the Phl B dye. Cotton based cellulosic paper supported the formation of nanofibers and stabilized the nanosensor formed on it. This system can be potentially used in smart packaging of fresh foods, monitoring exhaled ammonia in human breath for disease diagnosis and for ammonia estimation in water.

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