SYNTHESIS, CHARACTERIZATION AND EVALUATION OF Gt-cl-poly(AA) SUPERABSORBENT AS A NEURO-BIOSENSOR

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Summary

In the present scenario the treatment of diseases depends upon the proper and appropriate diagnosis. Various diagnostic methods and tools are available in modern days for the same, but with the introduction of Green Pharmacy these tools are being successfully replaced. In the present work the diagnostic tool based on natural hydrocolloid (*Gum tragacanth*) is being synthesized, characterized and evaluated for its diagnostic prospect in neurotransmitter related ailments.

Keywords: Neurotransmitters, diagnostic tool, *Gum tragacanth,* UV-VIS spectroscopy

Introduction

Neurotransmitters are the endogenous chemicals which transmit the signals from neuron to the target cell across the synapse [1]. Any hyper or hypo conditions associated with neurotransmitter imbalance will affect the body's overall functioning. Amino acids, peptides and monoamines are among the neurotransmitters, which form complex with different transition metals [2-3] and chemical compounds [4] thereby, act as diagnostic tools. Hydrogels containing natural polysaccharides backbone can be utilized to form biologically active diagnostic tool. Ability of hydrogels to swell under biological conditions makes it an ideal material for their use in drug delivery [5-6] and immobilization [7] of therapeutically active compounds such as proteins and peptides. The pH sensitivity [8] of hydrogels minimizes the proteolysis of proteins and thus proves to be useful in quantitative analysis.

The reaction of amines with ninhydrin to form the colored reaction product known as Ruhemann's purple was discovered by Siegfried Ruhemann in 1910 [9]. The colorimetric or spectrophotometric measurement of the absorption produced by the purple reaction product of amino acids and ninhydrin has become one of the most widely used methods for the quantitative determination of small quantities of amino acids [10]. The reaction of amines, amino acids, peptides and related compounds with ninhydrin has got extensive applications in the qualitative and quantitative estimation of such compounds in biochemical, clinical, environmental, food, forensic, histochemical, microbiological, medical, agricultural, nutritional, plant and protein

sciences [11-13]. The sensitivity of the reaction, i.e. the colour intensity by a given amino compound produced under defined conditions, is significantly influenced by the composition of the solvent in which the reaction takes place. It is highest in organic solvents and decreases in solvent mixtures containing water [14].

The present work focuses on the synthesis and characterization of Gt-cl-poly(AA) by free radical polymerization method and utilizing it as a release device for ninhydrin dye for the identification of body neurotransmitters through UV-VIS spectrophotometry.

Material and Methods

Gum tragacanth (SD Fine Chemicals Pvt. Ltd.) and ascorbic acid-potassium persulphate (SD Fine Chemicals Pvt. Ltd.) were used as backbone and initiator, respectively. Glutaraldehyde (MERCK) and acrylic acid (MERCK) were used as crosslinker and monomer. Ninhydrin and amino acids (LOBA Chemicals Pvt. Ltd.) were used as received.

Instrumental Analysis

Scanning Electron Micrographs of the candidate polymers were taken on LEO-435VF, LEO Electron Microscopy Ltd. Thermal studies were carried-out on TGA/DTA 6300, SLL EXSTAR 6000 at a heating rate of 10 °C/min in air. UV-VIS analysis of ninhydrin-neurotransmitter complex was carried out on Systronics UV-VIS spectrophotometers.

Synthesis of superabsorbent

1.0 g of *Gum Tragacanth* was taken in a reaction flask containing 25 ml of distilled water. 0.5 mol L^{-1} of acrylic acid was added to the reaction mixture followed by the addition of ascorbic acid-KPS in 1:1.25 molar ratios as an initiating system and 0.42 mol L^{-1} of glutaraldehyde as a crosslinker. The reaction was carried out at pH 7.0 for 90 min at 40 °C. At the end of the reaction, the homopolymer was removed on washing with hot water and synthesized gel was allowed to stand for about 10-12 hours undisturbed for gelling process to take place. The product obtained was dried in oven at 60 °C till a constant weight was obtained. The percentage grafting (%G) and percentage swelling (%S) were calculated as per the following equation (1):

$$\%G \text{ or } \%S = \frac{F_W - I_W}{I_W} \times 100 \quad (1)$$

Where Iw = initial weight of the material taken; F_w = final weight of the material obtained

Ninhydrin release through bio-polymer and neuro-transmitter testing

Preparation of Standard Ninhydrin solution The 200ppm standard Ninhydrin solution was prepared by dissolving 0.2g of Ninhydrin in 1000ml of 95% ethanol. The solution was uniformly dissolved by boiling for 15-20 seconds and absorbance was measured immediately after mixing using UV-VIS spectrophotometer.

Preparation of Solutions of Neurotransmitters

Different amino acids solutions (neurotransmitters) were prepared by dissolving 100mg of amino acid in 1000ml of distilled water, which gave the amino acids solutions of 100ppm. Absorbance of freshly prepared solutions was measured using UV-VIS spectrophotometer.

Loading of Ninhydrin onto Polymeric matrix

The loading of ninhydrin onto the polymeric matrix was carried out by swelling equilibrium method. The dried candidate polymers were immersed in known concentration of ninhydrin solution, and were allowed swelling for 24hour at 37°C. The swelled samples were dried at 40°C and were used as release device.

Ninhydrin release from polymeric matrix

The *in-vitro* release of the ninhydrin was carried out by placing dried and loaded polymeric sample in definite volume of different amino acids solutions (neurotransmitters) of known concentration for 30min, 60min and 90min time intervals at 37^{0}C. The amount of ninhydrin release and formation of complex was measured using UV-VIS spectroscopy.

Results and Discussion

Mechanism

Hydroxyl groups and $-CH_2$ present on the backbone are the active sites for graft copolymerization to take place. Various steps involved in the graft copolymerization of acrylic acid on *Gum tragacanth* are depicted in following scheme:

Ascorbic acid $\xrightarrow{-H^+}$ $\xrightarrow{-H^*}$ $\xrightarrow{-e^-}$ → Dehydro ascorbate

(2)

$$\text{Ascorbate} + {}^{-}O_3S\ O\ O\ SO_3^{-} \longrightarrow SO_4^{2-} + SO_4^{\bullet-} + \text{Dehydro ascorbate}$$

$$SO_4^{\bullet-} + H_2O \longrightarrow HSO_4^{-} + OH^{\bullet}$$

(3-4)

Initiation

$$\underset{\text{Monomer}}{H_2C{=}CH{-}COOH} + {}^{*}OH \longrightarrow {}^{*}H_2C{-}CH(COOH){-}OH$$

(5)

$$[\text{Gum Tragacanth}]_n + {}^{*}OH \longrightarrow H_2O + [\text{Gum Tragacanth radical}]_n$$

Gum Tragacanth

(6)

$$[\text{Gum Tragacanth}]_n + SO_4^{\bullet-} \longrightarrow HSO_4^{-} + [\text{Gum Tragacanth radical}]$$

Gum Tragacanth

(7)

Propagation

(8)

(9)

(10) **Termination**

Graft copolymer

(11-12)

Homopolymer

(13)

Initially, ascorbic acid ions react with the potassium persulphate to generate SO_4^{-*} (Eq. 3) which on further reaction with water molecules generates OH^* (Eq. 4). Interaction of OH^* and SO_4^{-*} with backbone and monomer resulted in the generation of active sites for grafting (Eqs. 5-7). Activated monomer and backbone molecules propagate further and give rise to three dimensional cross-linked networks in the presence of glutaraldehyde (Eqs. 8-10). However, chain termination takes place either by the reaction of OH^* with the live propagating macromolecular chains or reaction between two live chains (Eqs. 11-13).

Characterization

Scanning Electron Microscopy (SEM)

Scanning electron micrographs (SEM) were taken to differentiate the morphological differences on the surface of both *Gum tragacanth* and Gt-cl-poly(AA). Morphological changes in *Gum*

tragacanth were quite evident after grafting and crosslinking. Fig. 1b clearly showed that Gt-cl-poly(AA) has cross-linked networks, whereas *Gum tragacanth* has smooth and homogenous structure (Fig. 1a).

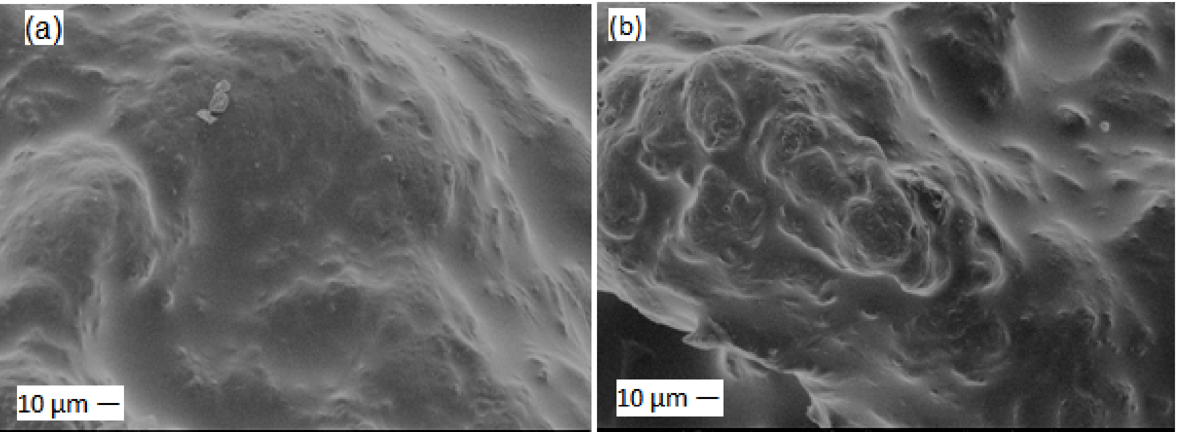

Fig. 1: SEM (a) *Gum tragacanth*, (b) Gt-cl-poly(AA)

Thermal Studies

Thermal studies of both the backbone and functionalized polymer were performed as a function of percent weight loss vs. temperature. In case of *Gum tragacanth*, three stage decompositions have been observed (Table 1). Primary decomposition reactions due to dehydration were observed upto 168.8 °C. First stage decomposition has been observed from 168.8 °C – 473.8 °C with 66.6 % weight loss. Second stage decomposition started at 473.8 °C and continues upto 577.5 °C with 3.3 % weight loss. Third stage decomposition was found from 577.5 °C to 644.5°C with 15.5 % weight loss. *Gum tragacanth* showed initial decomposition temperature (IDT) at 168.8 °C and final decomposition temperature (FDT) at 644.5 °C. Whereas Gt-cl-poly(AA) showed IDT at 211.4°C and FDT at 560.5 °C. Low thermal stability of Gt-cl-poly(AA) is due to morphological changes in *Gum tragacanth* during graft copolymerization of poly(AA), which disturbed its crystalline structure. In case of DTA *Gum tragacanth* showed sharp exothermic peaks at 580.8 °C (143.8 µV) and 646.6 °C (87.9 µV). On the other hand, Gt-cl-poly(AA) showed exothermic peak at 514.2 °C (136.6 µV). However, rate of weight loss per minute was found in agreement with TGA studies both in case of back bone and grafted samples.

Table 1: Thermal behavior of *Gum tragacanth* and Gt-cl-poly(AA)

Sample Code	**TGA**					**DTA**		**DTG**	
	IDT (^{0}C)	**1st stage Decomposit-ion, ^{o}C (% wt. loss)**	**2nd stage Decomposit-ion, ^{0}C (% wt. loss)**	**3rd stage Decompositi-on, ^{0}C (% wt. loss)**	**FDT, ^{0}C(residue left)**	**Exothermic peaks at different decomposition Temp.,°C (µV)**		**Decomposition Temp.,°C (Rate of wt. loss in mg/min)**	
						1st	**2nd**	**1st**	**2nd**
Gum tragacanth	168.8	168.8-473.8 (66.6 %)	473.8-577.5 (3.3 %)	577.5-644.5 (15.5 %)	644.5 (17.1 %)	580.8 (143.8)	646.6 (87.9)	579.7 (1.27)	646.6 (1.62)
Gt-cl-poly(AA)	211.4	211.4-350.5 (39.6 %)	350.5-516.5 (41.6 %)	516.5-560.5 (7.6 %)	560.5	514.2 (136.6)	---------	494.0 (0.901)	514.2 (0.785)

Ninhydrin release behavior through bio-polymer matrix and testing of **neuro-transmitters**

It has been observed that when candidate polymers were immersed in known concentration of ninhydrin solution it uptakes the ninhydrin solution and shows swelling behavior. These samples have been observed to show different release behavior depending on the type of neurotransmitter and type of ninhydrin - neurotransmitter complex formation. During the complex formation, different colours were observed, which indicates the transfer of charge during complex forming reaction. The UV-VIS absorbencies of different neurotransmitters, ninhydrin and their complexes have depicted in Tables 2-4.

Table 2: UV-VIS Absorptions of Standard Ninhydrin Solution and Neurotransmitters (amino acids)

S.No.	**Reagents**	**Chemical Structure**	**Absorbance at Λ_{max} (nm)**	**Absorbance**
1	Ninhydrin		345.6	2.413

2	L-Proline		225.2	2.268
3	Glycine		214.0	2.081
4	Histidine		219.6	2.041
5	DL-dopa		272.8	2.262

Table 3: UV-VIS Absorptions of Ninhydrin - Neurotransmitter complex formation

S.No.	Complexes	Λ_{max} (nm)	Absorbance at different time intervals (min)		
			30	60	90
1	L-Proline-Ninhydrin complex	275.6	2.477	2.484	2.510
2	Glycine-Ninhydrin complex	270.0	2.196	2.480	2.538
3	Histidine-Ninhydrin complex	222.4	2.478	2.508	2.541
4	DL-Dopa-Ninhydrin complex	270.0	2.468	2.484	2.516

Table 4: Colour Observations during Ninhydrin - Neurotransmitter complex formation

S.No.	Neurotransmitters	Dye	Colour Observed
1	L-Proline	Ninhydrin	Yellow
2	Glycine	Ninhydrin	Blue
3	Histidine	Ninhydrin	Blue
4	DL-Dopa	Ninhydrin	Red

As is evident from the Table 3, that the release of ninhydrin through Gt-cl-poly(AA) and its reaction with L-proline was found to form complex. Ninhydrin was found to give absorption at λ_{max}=345.6nm and L-proline exhibited absorption at λ_{max}= 225.2nm with 2.413 and 2.268 absorbance, respectively. However, L-proline-ninhydrin complex was found to give absorbance at λ_{max}= 275.6nm with absorbance 2.477, 2.484 and 2.510 at 30min, 60min and 90min time interval, respectively. Thus the absorbance was found to increase with increase in complex concentration and time interval. This clearly shows that ninhydrin formed complex with the L-proline which again was evident with the yellow colouration of the amino acid solution, alongwith absorbance pattern of UV-VIS spectrum. Proline is one of the main collagen component found in the tendons, ligaments and connective tissues of body, which assist in bone, skin and cartilage formation and in proper functioning of joints and tendons. Its presence could be detected easily with the sustained release of ninhydrin through biocompatible polymers [15].

Similarly the release of ninhydrin through Gt-cl-poly(AA) was found to form deep blue coloured complex with glycine. The complex exhibited absorption at λ_{max}= 270.0nm with 2.477, 2.484 and 2.510 absorbance at 30min, 60min and 90min respectively. However, glycine was found to give absorbance at λ_{max} = 214.0nm. Moreover, glycine is an inhibitory neurotransmitter used by the nervous system to prevent epileptic seizures and is used in the treatment of manic depression, hyperactivity and progressive muscular dystrophy. Additionally the amount of glycine is vital for prostrate and pituitary gland function [15].

Release of ninhydrin through Gt-cl-poly(AA) was found to form deep blue coloured complex with histidine. Histidine exhibited absorption at λ_{max}= 219.6nm with 2.413 and 2.041absorbance. However, histidine -ninhydrin complex was found to give absorbance 2.478, 2.508 and 2.541 at λ_{max}= 222.4nm at 30min, 60min and 90min time interval, respectively. Histidine is a compound released by immune system during allergic reaction. Being a precursor of histamine, it protects the nerve cells by repair of tissues and maintenance of the myelin sheath. It is also required for the synthesis of both red and white blood cells [15].

DL-Dopa on release of ninhydrin through Gt-cl-poly(AA) matrix formed a red coloured complex which gave the absorption at λ_{max}= 270.0nm with 2.468, 2.484 and 2.516 absorbance at 30min, 60min and 90 min, respectively.Dopamine a brain neurotransmitter has a significant role in the inhibition of prolactin hormone and thus proving useful in treatment of Parkinson's disease. Its presence could be detected easily by use of such biocompatible polymers. Hence, conclusion can be drawn that biopolymer matrix could be used for the detection of different types of neurotransmitters through the release of ninhydrin. Moreover, such bio-polymeric matrices have biocompatibility [15].

Conclusion

The candidate polymer synthesized was found to show colour sensitivity on release of ninhydrin towards different neurotransmitters. This showed that the candidate polymer can be used as a sensor for the detection of type of neurotransmitter and their concentration in the body. Moreover, the intensity of colour and the results of UV-VIS spectroscopy make the candidate polymer an appropriate diagnostic tool for the diagnosis of various ailments (hypo or hyper) associated with the deficiency of neurotransmitters.

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