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HALLOYSITE NANOTUBES: AS A TOOL FOR SUSTAIN RELEASE OF SACCHAROMYCES CEREVISIAE USING LAYER BY LAYER SELF ASSEMBLY APPROACH

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ABSTRACT

Natural tubules Halloysite are unique and versatile material formed by surface weathering of aluminosilicate minerals and comprises of different proportion of aluminum, silicon, hydrogen, and oxygen. It has a chemical formula of $Al_4Si_4O_{10}$ (OH) $_8.4H_2O$. Nano tubular geometry of halloysite exhibit nanoscale dimensions. Basically, this tubular arrangement varies with different regions. HNTs have high mechanical strength and modulus and these features make it an ideal material for preparing different polymer based composites. Halloysite Nanotubes (HNTs) are being used for so many varieties of biological and non-biological applications; remediation of environmental contaminants, act as a cargo for the delivery of drugs and various macromolecules, storage of molecular hydrogen and for catalytic conversion and processing of hydrocarbons. With this regard, the paper shows the layer by layer self-assembly approach by using polyelectrolytes (Polyallylamine hydrochloride and polystyrene sulphonate). The prolong release of saccharomyces cerevisiae for biotechnological point of view for the production of ethanol from layer by layer self-assembly was noted. The polyelectrolyte materialsshow the pH dependent release of saccharomyces cerevisiae. The pH dependent release was observed at pH 4.5, 7.0 and 10.5 which was represented by UV-Visible spectrophotometry.

KEYWORDS: Halloysite Nanotubes (HNTs), Saccharomyces Cerevisiae, Polyelectrolytes, Layer by Layer self-assembly, pH dependent, Ethanol Production.

INTRODUCTION

Halloysite Nanotube: Halloysite is defined as a two-layered aluminosilicate, chemically similar to kaolin, which has a predominantly hollow tubular structure in the sub micrometer range. As for most natural materials, the size of halloysite particles varies within 1-15 µm of length and 10-150 nm of inner diameter. However, at pH 8.5 the tubular lumen has a positive surface, promoting loading by negatively charged macromolecules and preventing their adsorption on the negatively charged outer surface. Halloysite nanotubes are capable of entrapping a range of active agents (drugs, nicotinamide adenine dineculeotide and marine biocides) within the inner lumen, as well as within the void spaces in the multi-layered Alumino-silicate shell, followed by their retention and release. Both hydrophobic and hydrophilic agents can be embedded after

appropriate pretreatment of the halloysite. Micro cylinders are an interesting alternative geometry for the entrapment and release of active agents in microencapsulation applications.

Saccharomyces cerevisiae: Saccharomyces cerevisiae has an extensive history of use in the area of food processing. Also known as Baker's Yeast or Brewer's Yeast, this organism has been used for centuries as leavening for bread and as a ferment of alcoholic beverages. With a prolonged history of industrial applications, this yeast has been either the subject of or model for various studies in the principles of microbiology. Saccharomyces cerevisiae, in addition to its use in food processing, is widely used for the production of macromolecular cellular components such as lipids, proteins, including enzymes, and vitamins (Bigelis, 1985; Stewart and Russell, 1985).

Applications of saccharomyces cerevisiae: Saccharomyces cerevisiae is one of the many model organisms studied in laboratories all over the world. Because its genome has been sequenced, its genetics are easily manipulated, and it is easy to maintain in the lab, this species of yeast has been an invaluable resource in the understanding of fundamental cellular processes such as cell division and cell death, as a source of vitamins and minerals. It is rich in B vitamins, chromium and selenium. Fermentation:Saccharomyces cerevisiae is commonly known as "baker’s yeast" or "brewer’s yeast". The yeast ferments sugars present in the flour or added to the dough, giving off carbon dioxide (CO_2) and alcohol (ethanol). The CO_2 is trapped as tiny bubbles in the dough, which rises.One yeast cell can ferment approximately its own weight of glucose per hour, giving rise to large volumes of CO_2. The same process occurs in bread dough - as the CO_2 from fermentation is trapped, the dough rises.

Layer By Layer Self Assembly: Emphasis is placed on the fabrication of nanostructured film based on a layer-by-layer (LBL) film approach. Here, discussion is regarding the theory and the mechanism of charge transfer in polyelectrolyte multilayer films (PEM), for the development of more sustain release formulation. Towards the utilization of LBL films, examples of several architectures and different electrochemical approaches demonstrate the potential of nanostructured LBL films for several applications that include the diagnosis, sustain release and monitoring of diseases.

Objective:

Our main aim in this research is to survey what can assist researchers by presenting various approaches currently used in the field of biotechnology utilizing supramolecular architectures based on an LBL approach for application in sustain release formulation.

MATERIALS AND METHODS

Instruments and Equipments used:

1. V-750 UV-Vis Spectrophotometer
2. Zetasizer Nano ZS (Malvern), Molecular size, Molecular weight, Particle size, Zeta potential-(Particle Size Range-0.3nm to 10µm)
3. pH meter (METTLER TOLEDO)
4. Scanning Electron Microscope (SEM) (Zeiss)
5. Waring Single Speed Drive Unit for Blender
6. Scientech SE-181 200x300x150 mm Rectangular Hot Plate With Cast Iron Top
7. Centrifuge (Sorvall™ Legend™ Micro 17 Microcentrifuge)
8. Magnetic Stirrer (Cole-Parmer StableTemp Hot Plates)
9. Vaccum Oven (Thermo Scientific™) (Achieve maximum temperatures of 220°C (428°F) with this flexible oven, while benefiting from two control configurations and display options)
10. High speed Homogenizers

Chemicals and Reagents:

Sodium poly(styrene sulfonate) (PSS, *M*w 70 000), poly(allylamine hydrochloride) (PAH, *M*w 50 000) Saccharomyces cerevisiae, Sucrose, Processed halloysite G was purchased from New Zealand China Clays Ltd., Auckland, New Zealand, Methyl Orange, Acetyl sulfate, DCE(Di-chloro Ethane), Methanol.

Methods:

Preparation of standard curve for Saccharomyces cerevisiae

Take 10 mg of saccharomyces cerevisiae and dissolved it in 10 ml water. Prepare the different concentrations of saccharomyces cerevisiae 20, 40, 60, 80, 100 ppm. Measure the concentration of saccharomyces cerevisiae by UV-Visible Spectrophotometer at 218 nm.

Loading of Saccharomyces Cerevisiae to Halloysite Nanotube

Take 10 mg of saccharomyces cerevisiae and dissolved it in 10 ml water.Add 25 mg of halloysite nanotube (HNT) and a pinch of methyl orange as a color indicatorstir it well on a magnetic stirrer for 1 hour at 2000-3000 RPM. The final solution of halloysite nanotube, saccharomyces cerevisiae and methyl orange were transferred to centrifuge tube and centrifuge it for 1 hour at 4000 RPM. After 1 hr check the loading capacity of the halloysite nanotube by using standard curve of saccharomyces cerevisiae and UV-Visible Spectrophotometer.

Preparation of Polystyrene Sulfonate

10.4 g of PS was dissolved in 50 cm^3 of dried DCE. The flask containing the solution was heated in the oil bath to 48-52 °C. Acetyl sulfate as sulfonating agent was prepared in a separate flask: 6.0 cm^3 of acetic anhydride was added to 50 cm^3 of DCE. The solution was cooled to about 5-10 °C and 3.0 cm^3 of 95-97 % sulfuric acid was carefully added.The prepared acetyl sulfate in solution of DCE was poured to the solution of PS in the DCE. The reaction mixture was heated to about 50 °C and stirred for 1 h.

Isolation of Polystyrene Sulphonate

For isolation of polystyrene Sulfonate three different methods were available: (1) take the above solution and poured it in water/ methanol. (2) If in water and methanol there is a formation of emulsion therefore to prevent this, solvent was evaporated and the solid polymer was ground to powder and washed with water. (3) If water soluble Polystyrene Sulfonate so, it could be isolated after evaporating the solvent and then purified with non-aqueous solvent like dichloroethane. Finally, the sulfonated polystyrene was dried in a Vaccum oven at about 500 °C for 3 days.

Preparation of Layer by Layer(LBL) Self Assembly

Alternate application of PAH and PSS solutions gives the formation of LBL self-assembly.

Application of 2 mg/ml polystyrene sulphonate (PSS) solution (Final zeta potential of layer was noted -21.5mV). Application of 2 mg/ml Polyallylamine HCL(PAH) solution to halloysite solution (Final zeta potential of layer was noted +35.8mV).

Sustain Release checking of Saccharomyces Cerevisiae

The solutions having saccharomyces cerevisiae and HNT with LBL self-assembly and HNT without LBL self-assembly were adjusted to acidic (4.5), neutral (7) and basic (10.5) pH.

Check UV absorbance of the solution at 218 λ max. Compare the data of HNT with LBL self-assembly and HNT without LBL self-assembly.

Loading capacity of HNT

Draw Std. calibration curve of Saccharomyces cerevisiae. Add 25 mg Halloysite Nanotube powder to the solution of saccharomyces cerevisiae. Put it for centrifugation for 1 hr. at 4000 rpm. Take the reading of stock solution and supernant solution at 218 nm in UV-Visible. Spectrophotometer and check loading capacity of halloysite nanotube.

RESULTS AND DISCUSSIONS

Table. Absorbance of Saccharomyces cerevisiae

Concentration (ppm)	Absorbance
20	0.08
40	0.14
60	0.18
80	0.22
100	0.33
Unknown	0.24

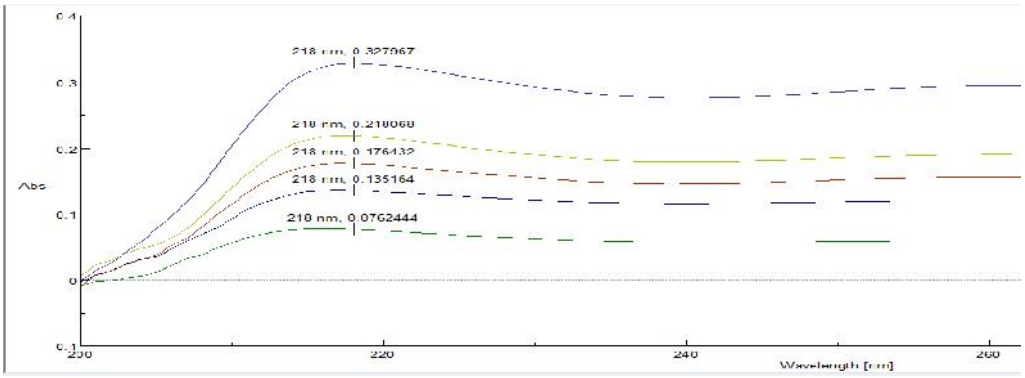

FIG.UV-Visible overlay of Saccharomyces cerevisiae forsolubility

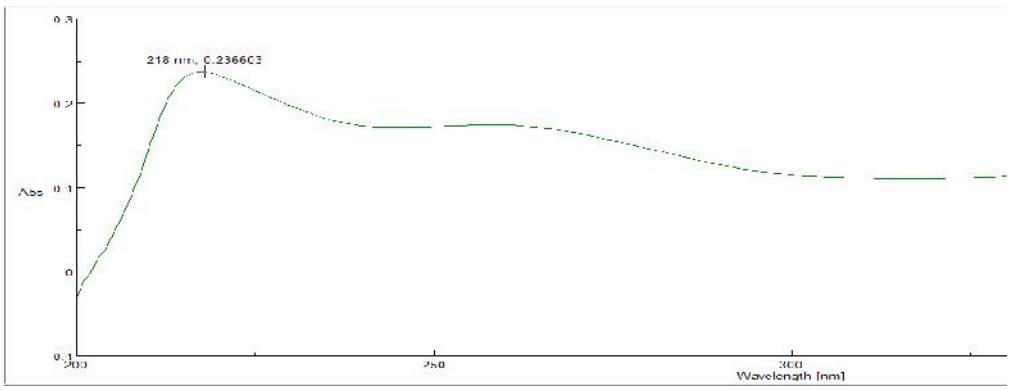

FIG. Absorbance of unknown solution

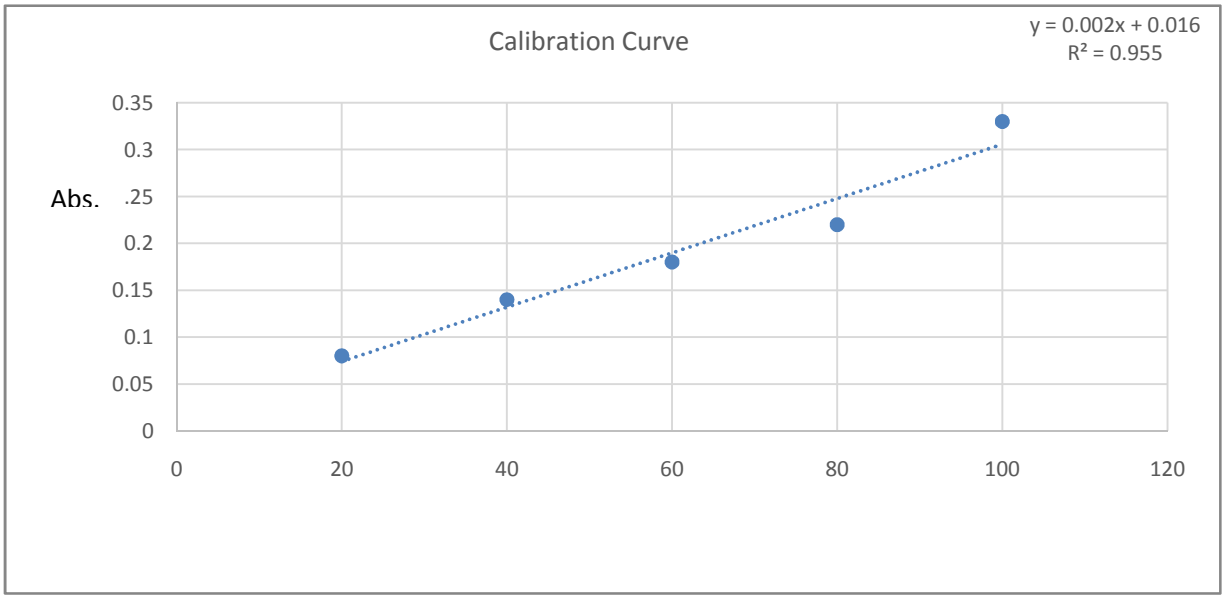

FIG. Standard calibration curve of Saccharomyces cerevisiae

Here, the calibration curve of 1,2,3,4,5 ppm 2,4,6,8,10 ppm, 10,20,30,40,50 ppm and 20,40,60,80,100 ppm has been plotted.The final calibration curve is 20,40,60,80,100 ppm because of linearity. The solubility of Saccharomyces cerevisiae is very less in water and the results shows the solubility of Saccharomyces cerevisiae is around 38.62µg/ml in water.

Table. Absorbance for checking of loading capacity

CONCENTRATION (ppm)	**ABSORBANCE**
20	0.09
40	0.16
60	0.22
80	0.27
100	0.39
Stock (y_1)	2.13
Supernant (y_2)	1.48
M	0.0036
C	0.013
x_1	588.06
x_2	407.5

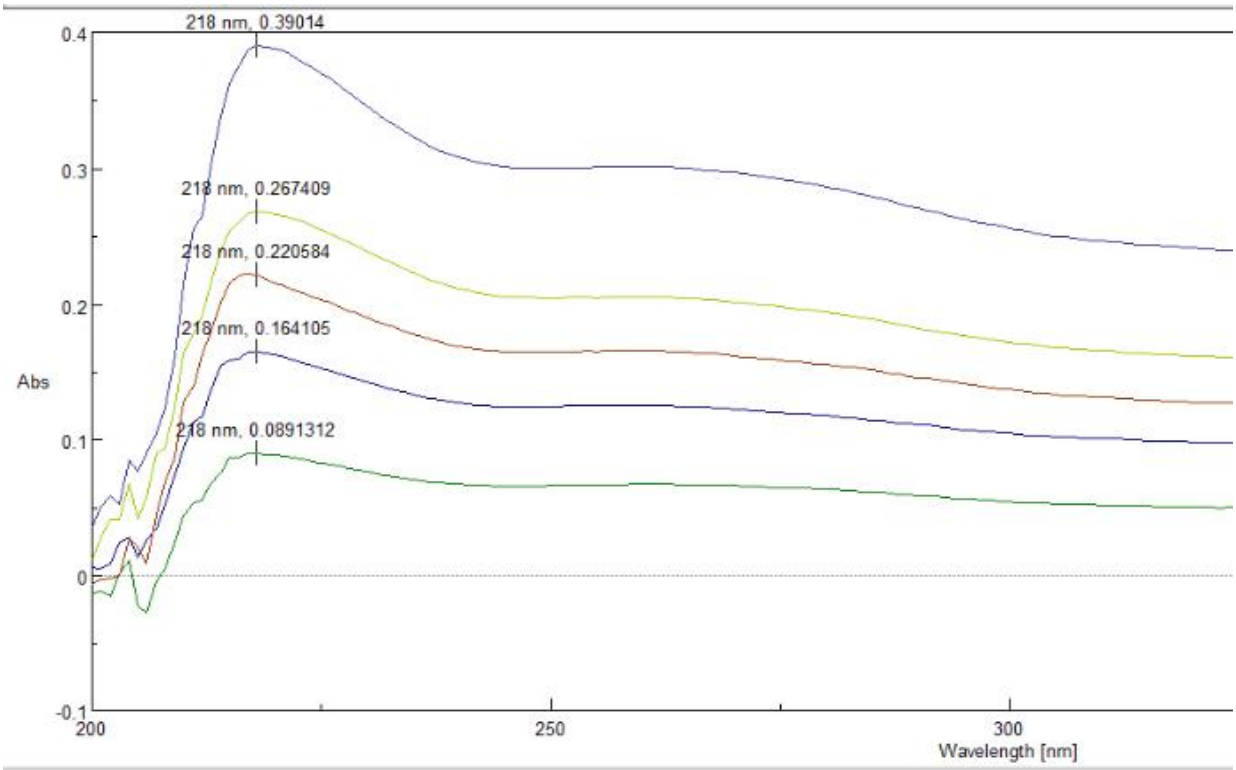

FIG.UV-Visible overlay of Saccharomyces cerevisiae for loading capacity of HNT

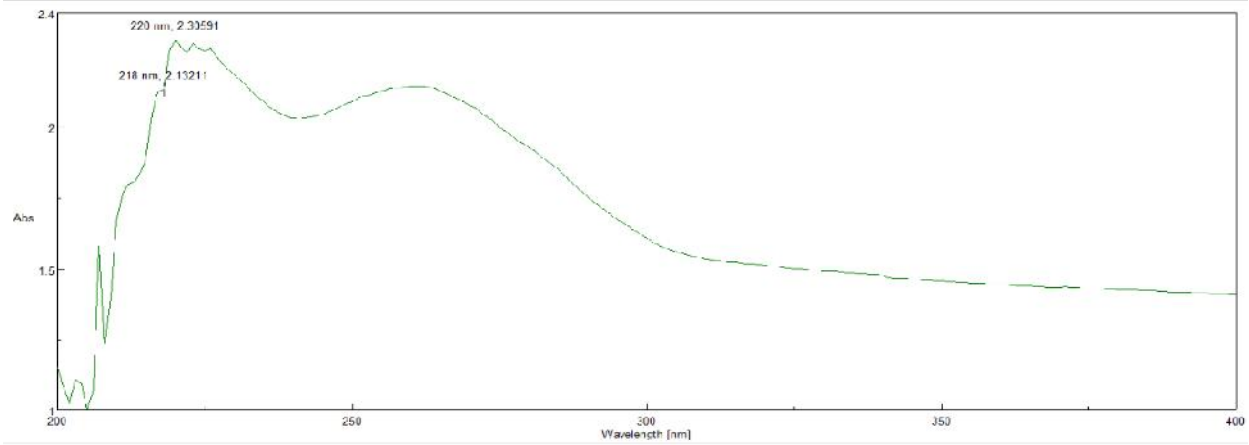

FIG. Absorbance of stock solution

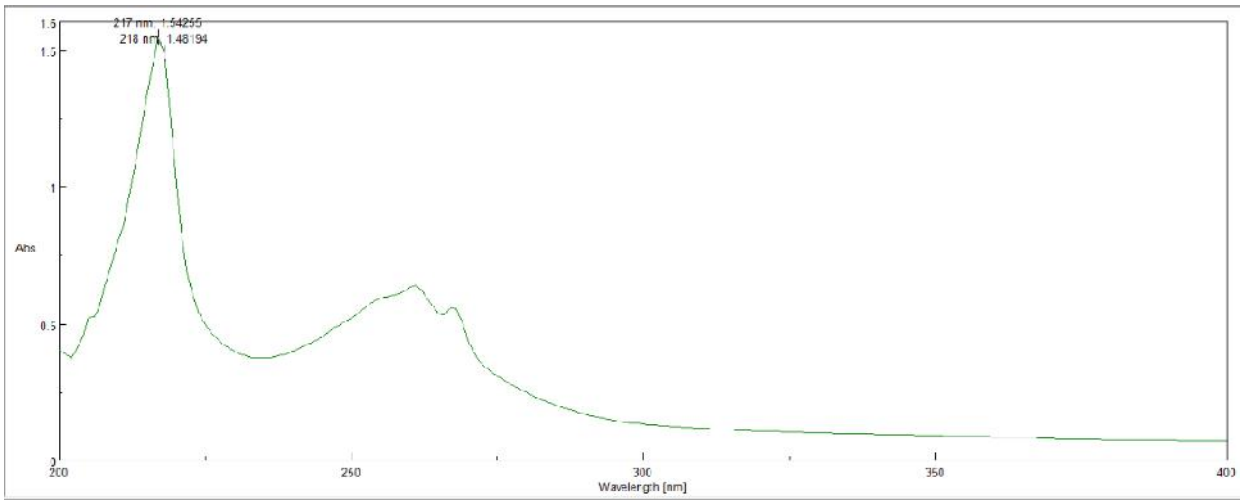

FIG. Absorbance of Supernant solution

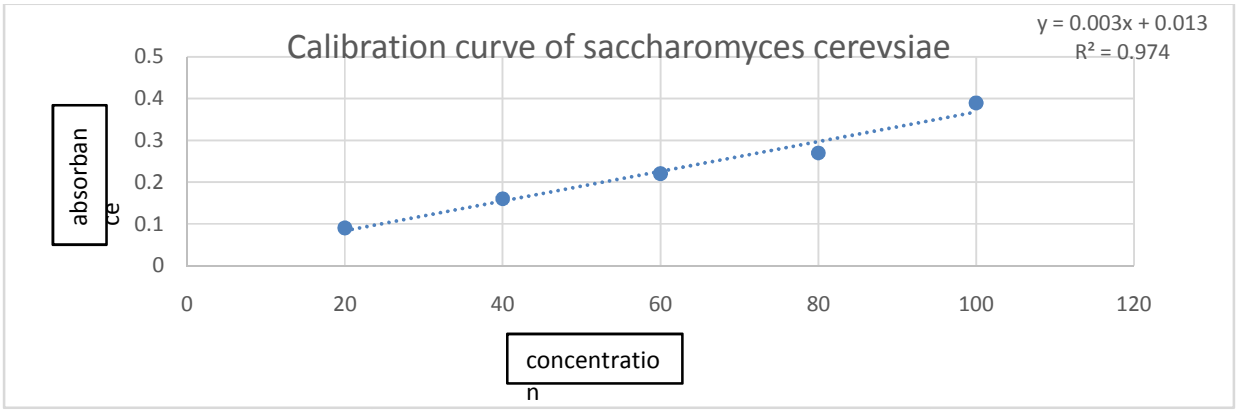

FIG. Standard calibration curve for loading capacity of HNT

The UV-Visible spectrophotometer result of loading capacity is 180.56 µg.Halloysite nanotubes are hollow structure that proved in SEM images and from the data 180.56µg saccharomyces cerevisiae can be loaded in halloysite nanotube lumen.

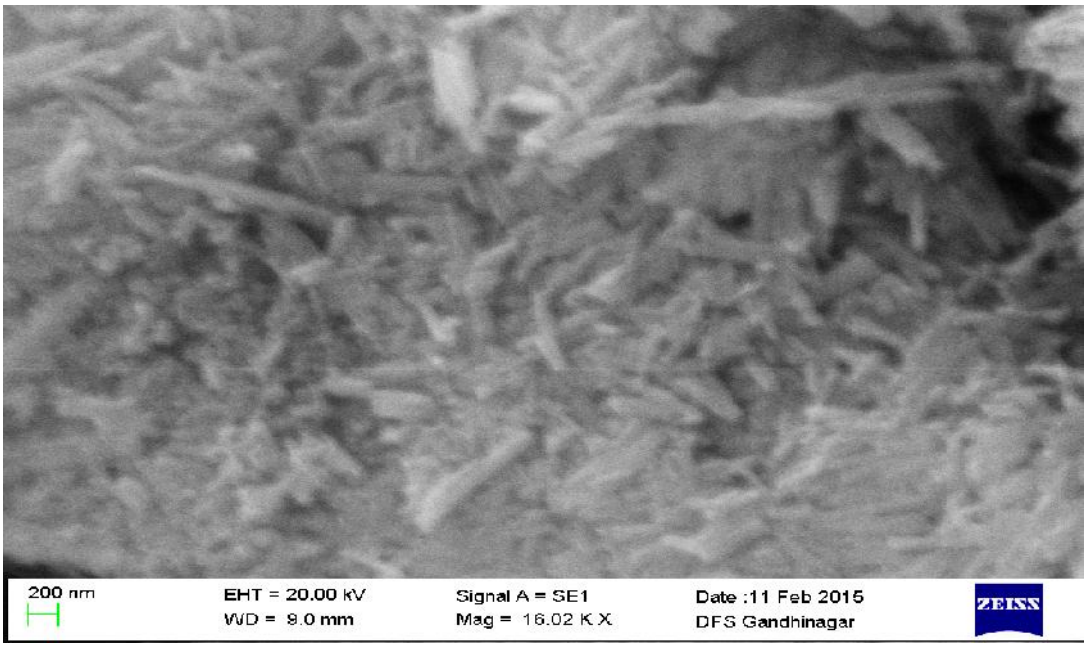

FIG. SEM image of HNT incorporated with Saccharomyces cerevisiae

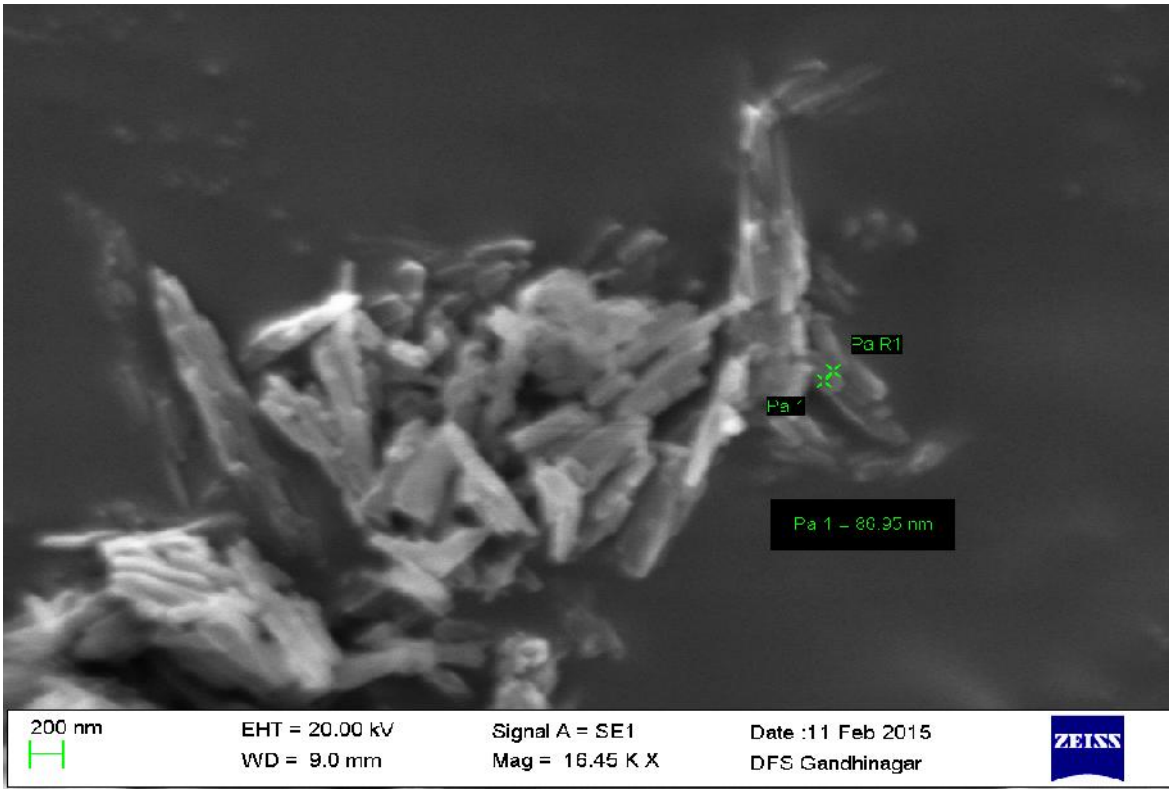

FIG. SEM image of HNT

Layer by Layer self-Assembly

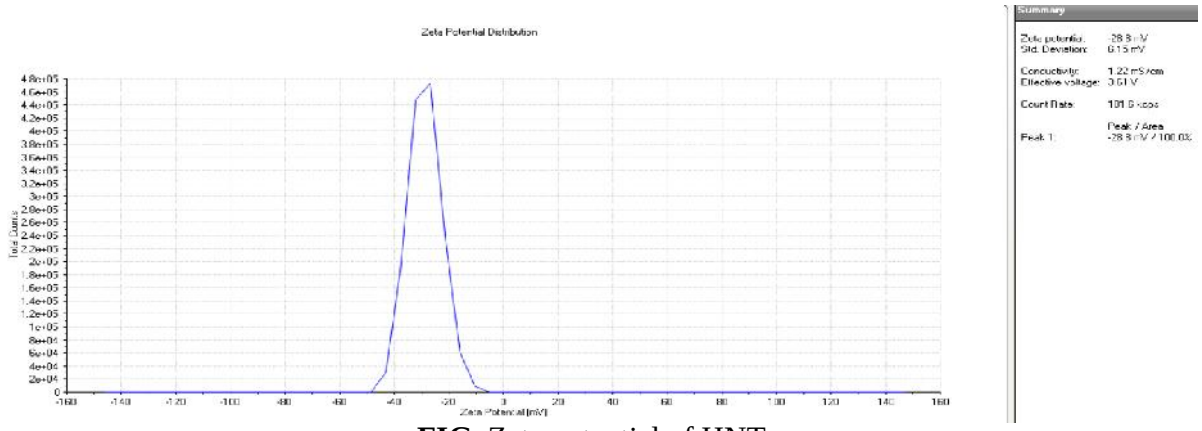

FIG. Zeta potential of HNT

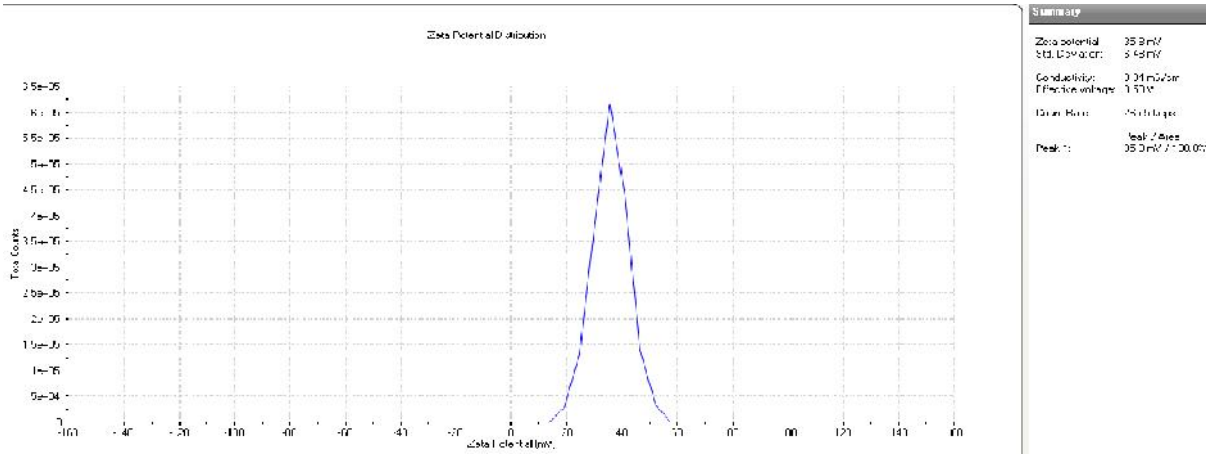

FIG. Zeta potential of Polyallylamine Hydrochloride (PAH)

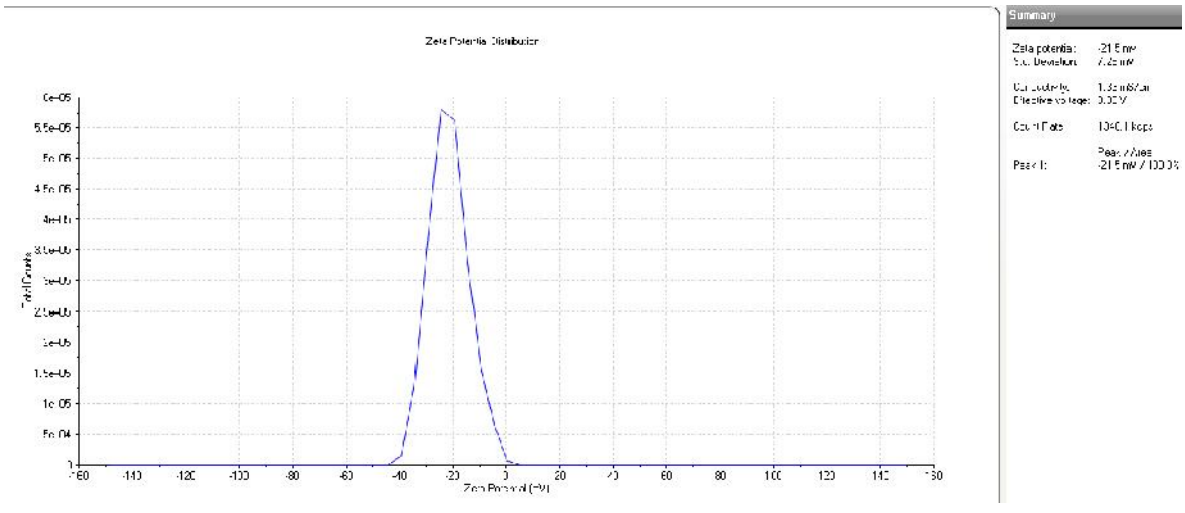

FIG. Zeta potential of prepared Polystyrene Sulphonate (PSS)

The zeta potential of Halloysite Nanotube is negative on outer side due to SiO_2 functional group. So, we can easily modify outer lumen with anionic and cationic polyelectrolyte.The

polyelectrolytes used for the modification were: Polyallylamine Hydrochloride (PAH), Polystyrene Sulphonate (PSS).

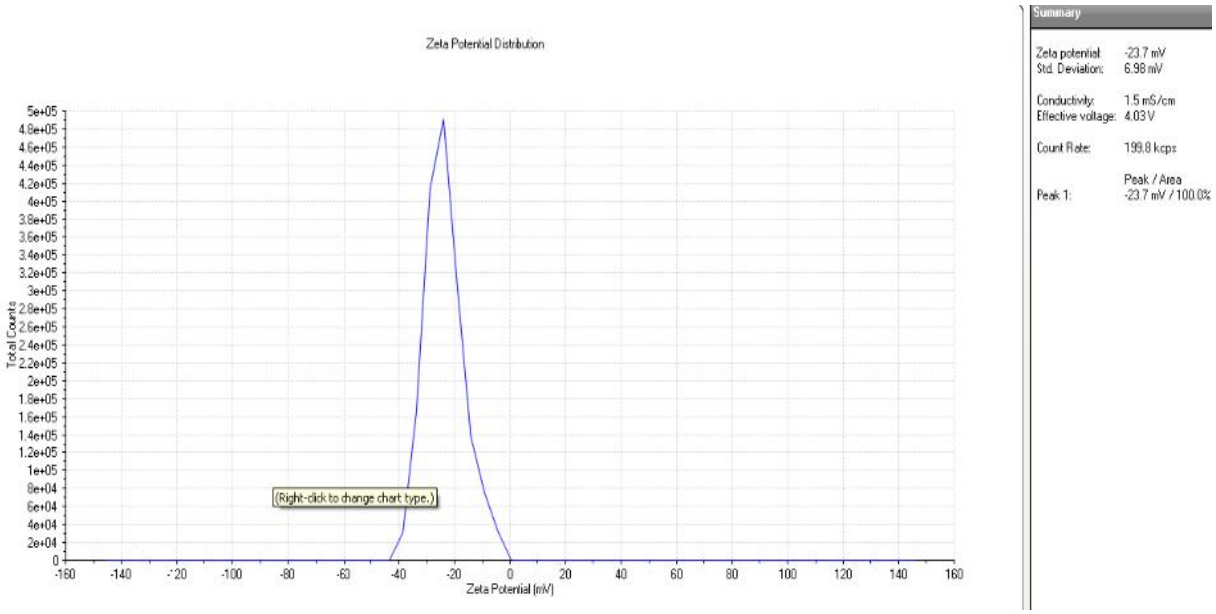

FIG. Zeta potential of HNT

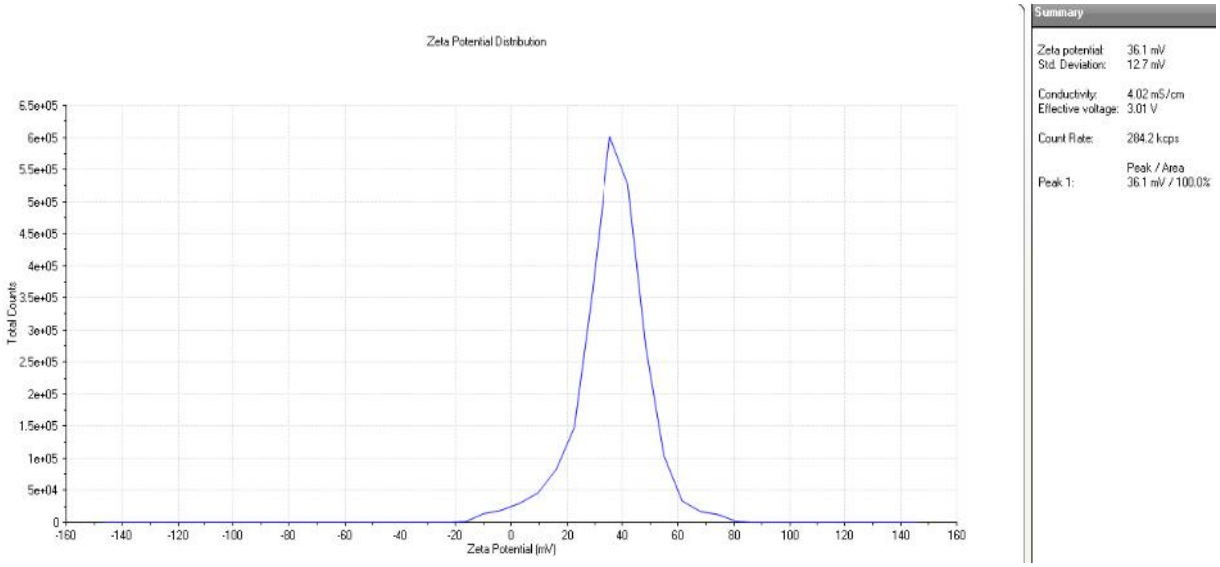

FIG. Zeta potential of HNT+PAH

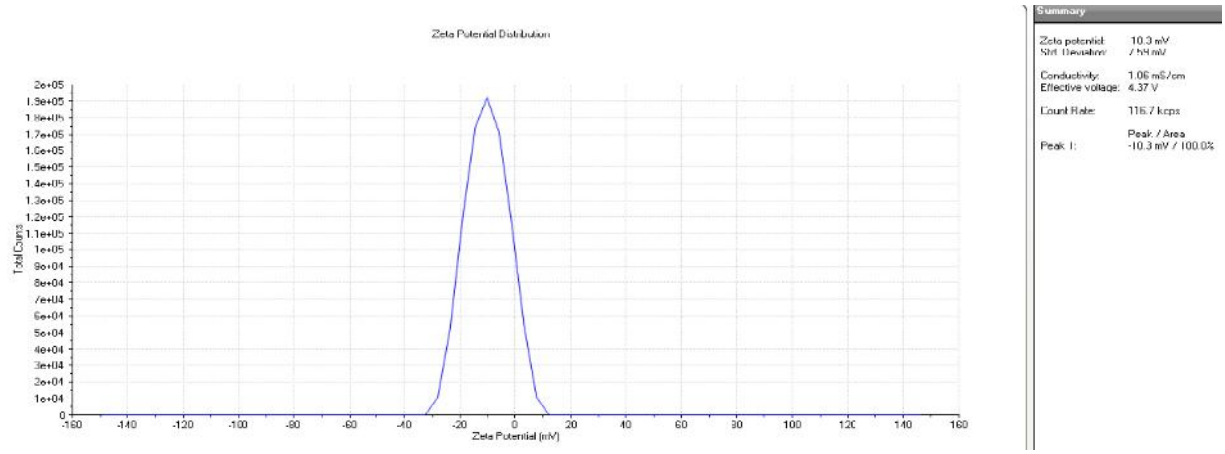

FIG. Zeta potential of HNT+PAH+PSS

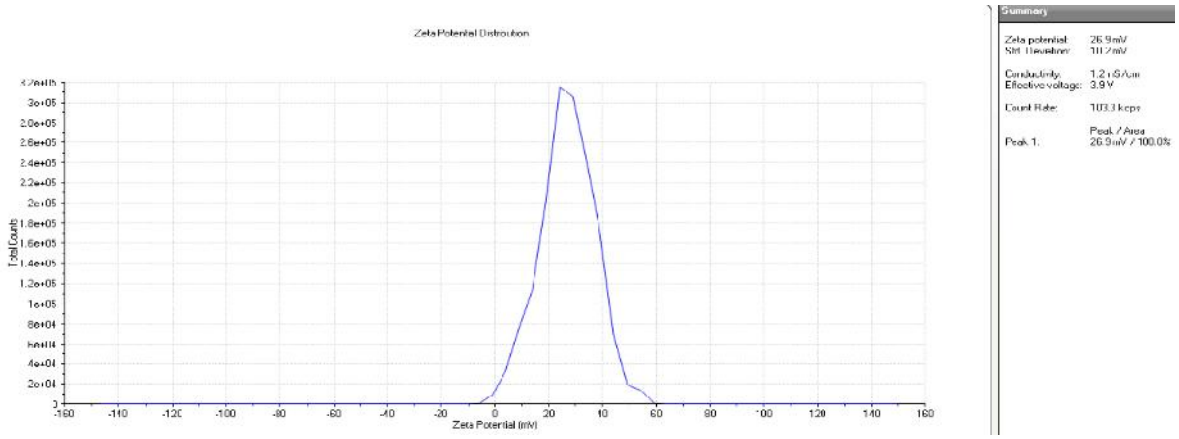

FIG. Zeta potential of HNT+PAH+PSS+PAH

Table. Zeta potential of layer by layer self-assembly by Malvern Zetasizer

No.	Layerby Layer	Zeta Potential [mV]
1	Halloysite Nanotube (HNT)	-23.7
2	HNT+PAH	36.1
3	HNT+PAH+PSS	-10.3
4	HNT+PAH+PSS+PAH	26.9

Release of saccharomyces cerevisiae at different pH as mentioned in below table (Without layer by layer self-assembly)

TABLE. Release data of saccharomyces cerevisiae without layer by layer self-assembly

No.	pH	Absorbance	Concentration (µg/10 ml)
1	4.5	1.288	354.17
2	7.0	0.808	220.83
3	10.5	1.458	401.39

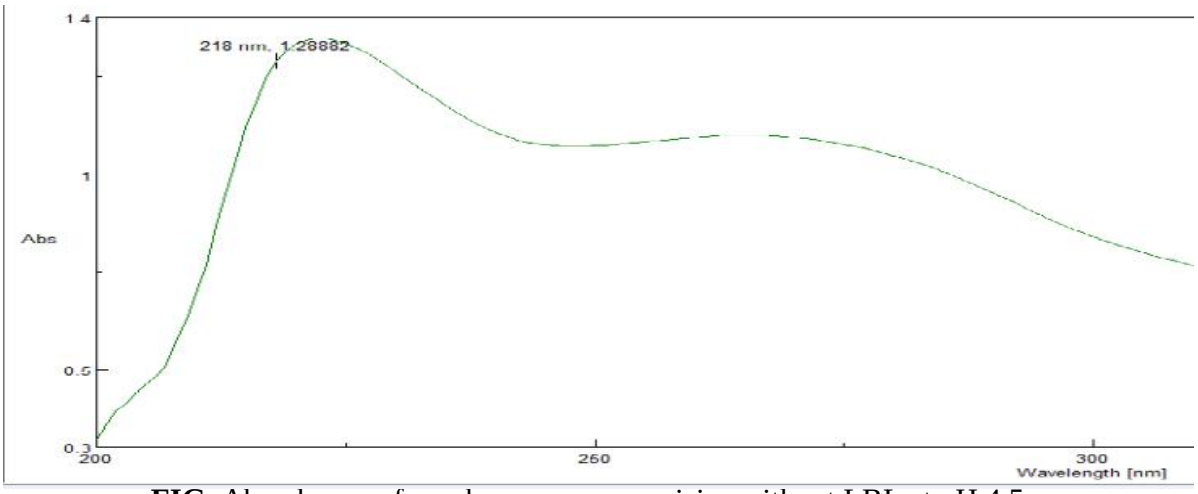

FIG. Absorbance of saccharomyces cerevisiae without LBL at pH 4.5

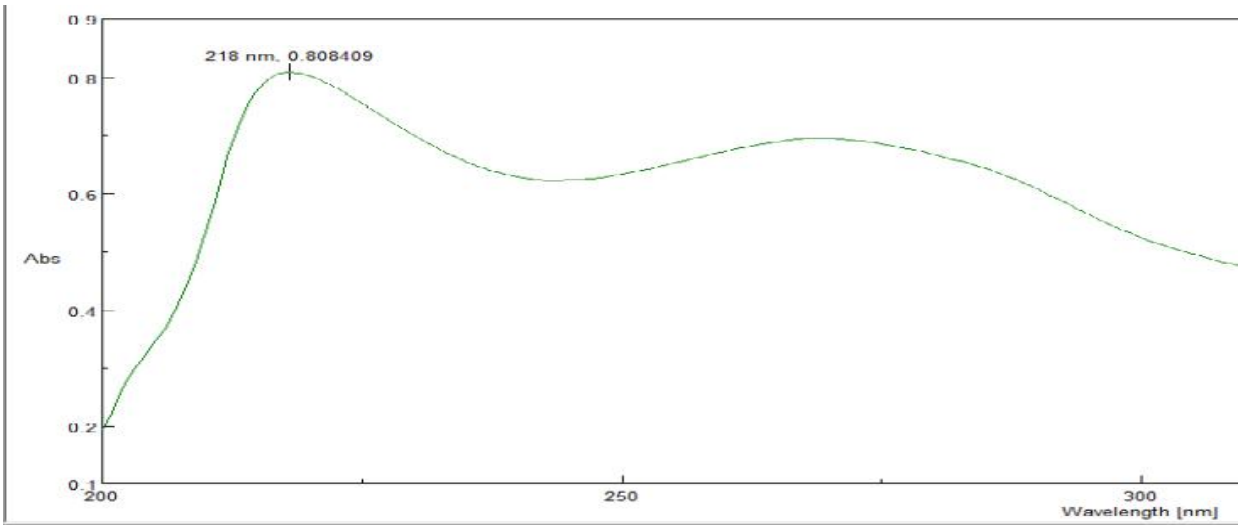

FIG. Absorbance of saccharomyces cerevisiae without LBL at pH 7.0

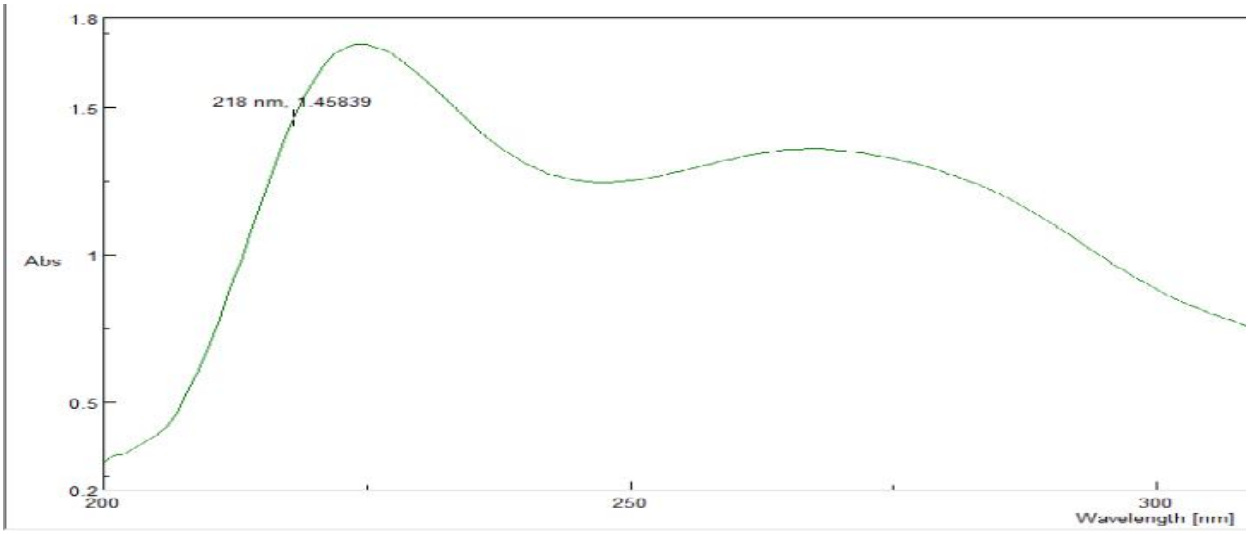

FIG. Absorbance of saccharomyces cerevisiae without LBL at pH 10.5

Sustain release of saccharomyces cerevisiae at different pHas mentioned below (With layer by layer self-assembly)

Table. Release data of saccharomyces cerevisiae from layer by layer self-assembly

Monthly Study Report						
Day	**pH [Absorbance]**			**pH [Concentration (µg/ml)]**		
	4.5	**7.0**	**10.5**	**4.5**	**7.0**	**10.5**
1	-0.18	-0.45	-0.29	0	0	0
2	0.04	0.01	-0.17	7.5	0	0
3	0.057	0.09	-0.05	12.22	21.39	0
4	0.06	0.1	0.04	13.06	24.17	7.5
5	0.07	0.1	0.05	15.83	24.17	10.28
6	0.09	0.11	0.09	21.39	26.94	21.39
7	0.099	0.14	0.091	23.89	35.28	21.67
8	0.11	0.144	0.117	26.94	36.39	28.89
9	0.13	0.16	0.12	32.5	40.83	29.72
10	0.142	0.164	0.13	35.83	41.94	32.5
11	0.15	0.1795	0.14	38.06	46.25	35.28
12	0.17	0.1954	0.166	43.61	50.67	42.5
13	0.18	0.24	0.18	46.39	63.06	46.39
14	0.21	0.285	0.19	54.72	75.56	49.17
15	0.22	0.319	0.2	57.5	85	51.94
16	0.223	0.32	0.2002	58.33	85.28	52
17	0.226	0.344	0.206	59.17	91.94	53.61
18	0.23	0.414	0.2323	60.28	111.39	60.92
19	0.2796	0.421	0.2324	74.06	113.33	60.94
20	0.3	0.44	0.24	79.72	118.6	63.06
21	0.308	0.461	0.249	81.94	124.44	65.56
22	0.31	0.469	0.25	82.5	126.67	65.83
23	0.312	0.51	0.289	83.06	138.06	76.67
24	0.359	0.57	0.314	96.11	154.72	83.61
25	0.413	0.617	0.39	111.11	167.78	104.72
26	0.42	0.62	0.454	113.6	168.61	122.5
27	0.4889	0.623	0.4	132.19	169.44	107.5
28	0.49	0.63	0.459	132.5	171.39	123.89
29	0.654	0.732	0.524	178.06	199.72	141.94
30	0.891	0.732	0.625	243.89	199.72	170

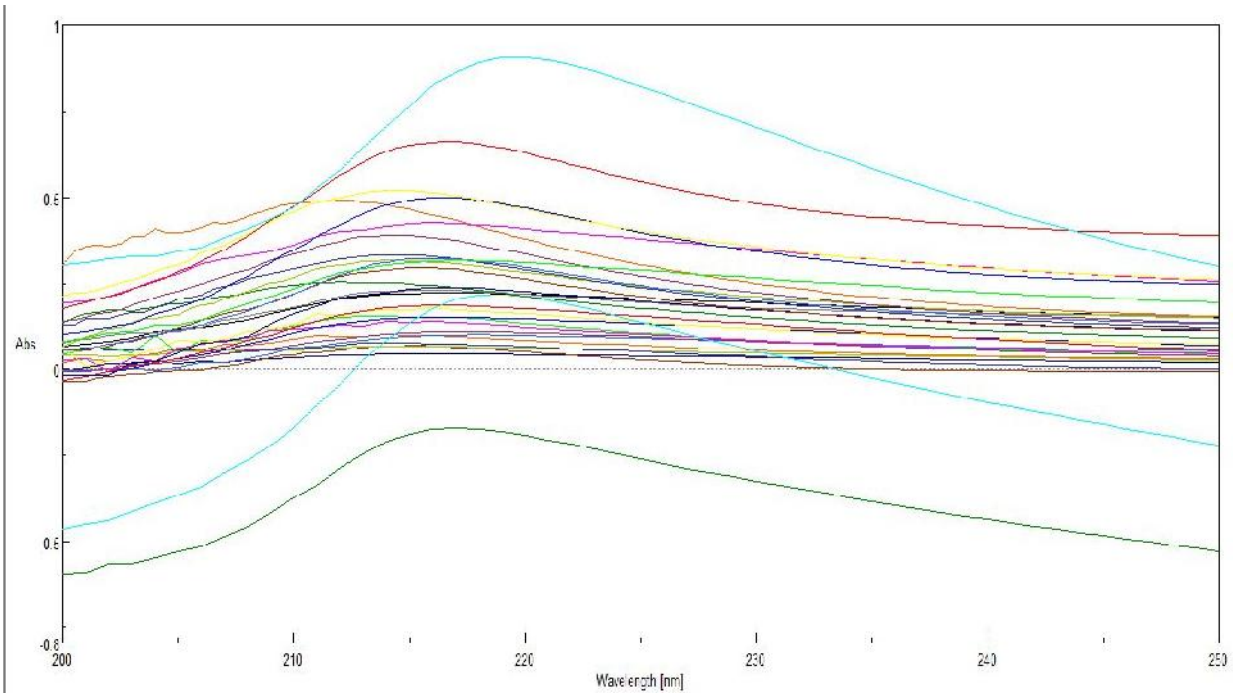

FIG. Overlay graph of pH 4.5 for 30 days

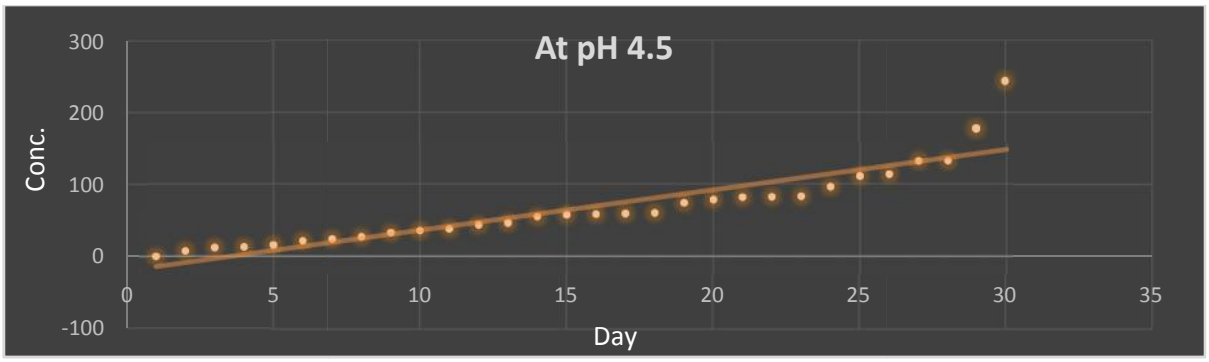

FIG. Sustain release of saccharomyces cerevisiae at pH 4.5

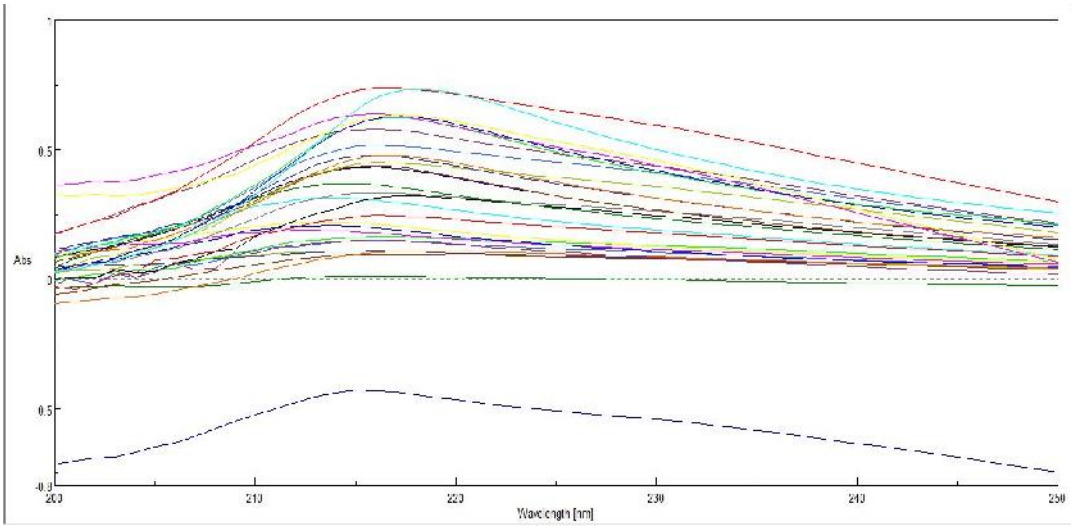

FIG. Overlay graph of pH 7.0 for 30 days

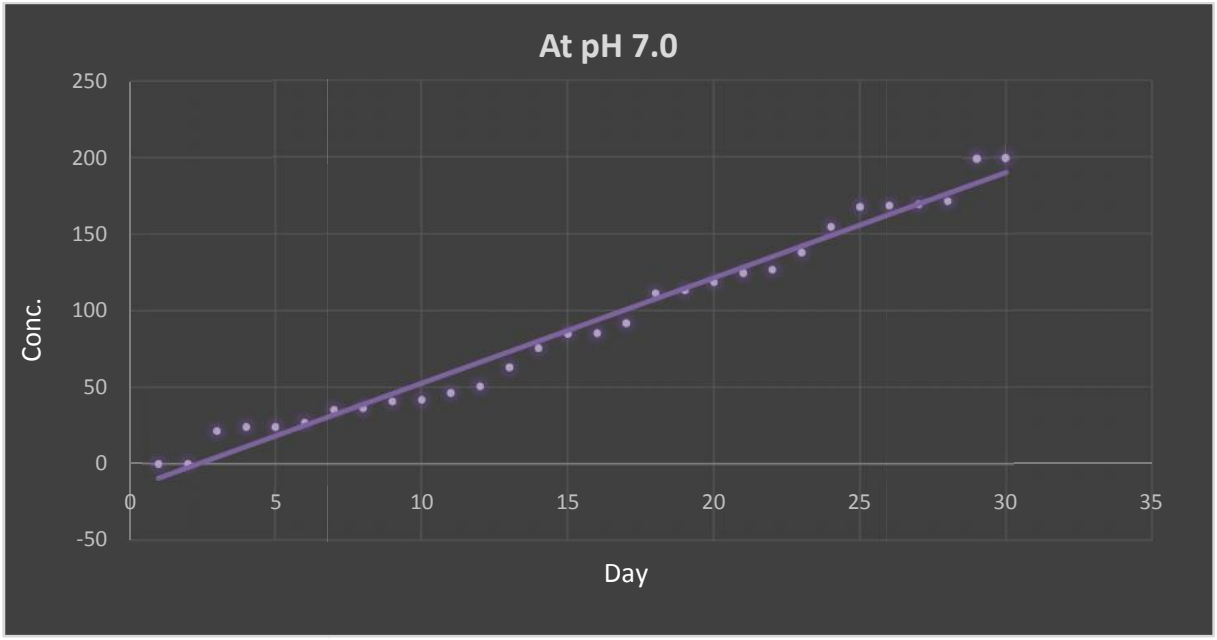

FIG. Sustain release of saccharomyces cerevisiae at pH 7.0

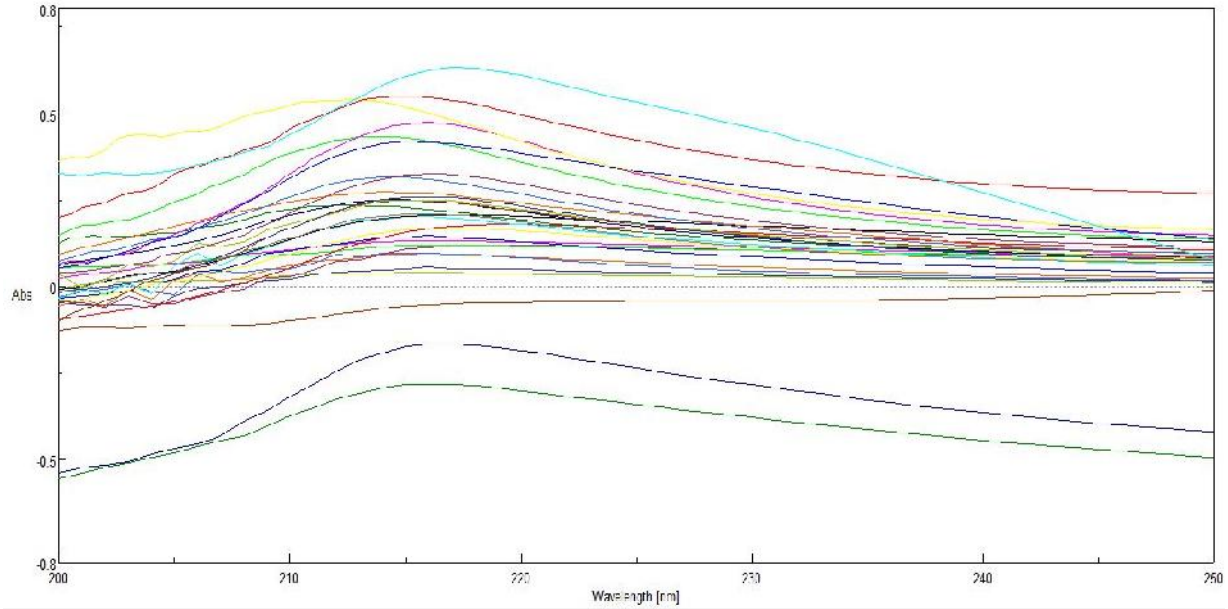

FIG. Overlay graph of pH 10.5 for 30 days

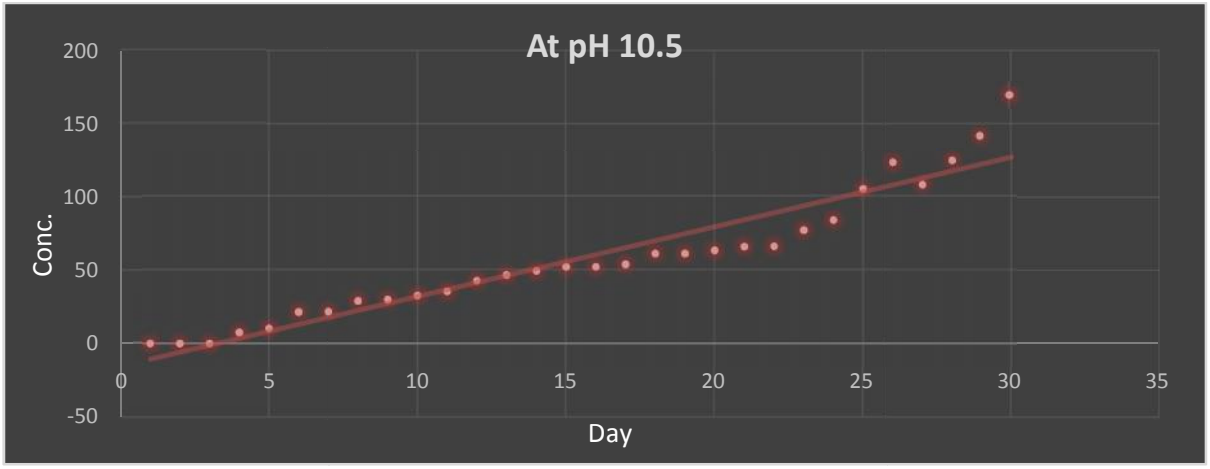

FIG. Sustain release of saccharomyces cerevisiae at pH 10.5

CONCLUSION

From the results we can conclude that the nanomaterial (halloysite nanotube) for the sustain release of the saccharomyces cerevisiae. Halloysite nanotube is a natural clay material which is having hollow tubular like structure in which we can easily incorporate saccharomyces cerevisiae. The halloysite nanotube is used for various types of materials like drugs, enzymes, anti-corrosion materials. Halloysite nanotube is covered with polyelectrolytes like Polyallylamine hydrochloride (PAH) & polystyrene sulphonate (PSS). By this way layer by layer self-assembly is prepared on halloysite nanotube. This layer by layer self-assembly is giving sustain release dependent on pH. The study of various pH is carried out like pH 4.5 (acidic), 7.0 (neutral), 10.5 (basic). The result shows the pH dependent release of saccharomyces cerevisiae.

Abbreviations

PAH (Polyallylamine hydrochloride), PSS (polystyrene sulphonate), HNT (Halloysite Nanotube).

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