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Pharma, Drug Discovery,
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Wines Antioxidant Activity

Steroids in the Youth

Highlights

Synthesis and Analytical

Coated Medical Devices

Discovering Thoughts, Inventing Future

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The Evaluation of the Wines Antioxidant Activity

By Florica Busuricu, Antoanela Popescu, Stela Zamfirescu
& Andreea Anghel

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Abstract - A new method for measuring the antioxidant activity is the method which using N, N' -di ethyl-pphenylendiamina (DMPD). In this paper, was verified of their effectiveness of the DMPD method on antioxidant foods. We used wine samples coming from different areas of Romania. Antioxidant action of wines is strictly related to the amount of polyphenols. To evaluate the sensitivity of the method, the system was tested by using of standard solution of TROLOX 1mg/mL and DMPD: FeCl₃ molar ratio of 10:1. Spectrofotometric measurements were recorded by using an UV-VIS Jenway 6300 at 505 nm. Antioxidant action was expressed as TEAC (TROLOX equivalent antioxidant capacity), using the calibration curves plated with different amounts of TROLOX. These results show that the red wine samples have a high antioxidant action, in conformed to the amount of polyphones. The method ensures sensibility and reproducibility in the measurement of antioxidant action of hydrolytic compounds.

Keywords : antioxidant activity, polyphones, DMPD method, wines.

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THE EVALUATION OF THE WINES ANTIOXIDANT ACTIVITY

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The Evaluation of the Wines Antioxidant Activity

Florica Busuricu ^α, Antoanela Popescu ^α, Stela Zamfirescu ^σ & Andreea Anghel ^σ

Abstract - A new method for measuring the antioxidant activity is the method which using N, N' -di ethyl-p-phenylendiamina (DMPD). In this paper, was verified of their effectiveness of the DMPD method on antioxidant foods. We used wine samples coming from different areas of Romania. Antioxidant action of wines is strictly related to the amount of polyphenols. To evaluate the sensitivity of the method, the system was tested by using of standard solution of TROLOX 1mg/mL and DMPD: FeCl₃ molar ratio of 10:1. Spectrofotometric measurements were recorded by using an UV-VIS Jenway 6300 at 505 nm. Antioxidant action was expressed as TEAC (TROLOX equivalent antioxidant capacity), using the calibration curves plated with different amounts of TROLOX. These results show that the red wine samples have a high antioxidant action, in conformed to the amount of polyphenols. The method ensures sensibility and reproducibility in the measurement of antioxidant action of hydrolytic compounds.

Keywords : antioxidant activity, polyphenols, DMPD method, wines.

I. INTRODUCTION

Cancer is a leading cause of death and may result from chronic injury to the epithelium by oxidants and other carcinogens¹. Epidemiological and experimental studies also offer strong evidence that implicates oxidative damage in the etiology of brain, heart and nervous system diseases². Although the body has effective defence systems that protect it against oxidative stress, the capacity of these protective systems decreases with aging creating a need to provide the body with a constant supply of phytochemicals through dietary supplements³. French people include in the daily diet a glass of red wine and this way, the cardiovascular accidents are 2,5 less than at the American consumers of alcoholic drinks⁴.

The analysis of the composition of wine demonstrated that it contains over 1000 benefic substances for the organism. Among the most important are the polyphenols, carbohydrates, mineral elements (K⁺, Ca²⁺, Mg²⁺), vitamins (A, B₂, B₅, B₆, C), organic acids, compound aromatics and proteins⁵. The phenols are found in a higher quantity in red wines (3-5 g/L) than in the white ones. Because of their antioxidant action, the phenols from the wine annihilate the negative action

of the free radicals, stopping the early aging and degenerative illnesses⁶.

The antioxidant protection is ensured by SO₂, which is used and accepted in all the countries for its multiple actions, amongst which we mention⁷: the antiseptic action, the action of inhibition of the enzymatic activity by blocking the activity of the complex of oxidative enzymes (polyphenoxidase, peroxidase and ascorbicoxidase). SO₂ are the action of reduction of the pH value and in this way, the solvability of the antocianes, the application of stabilization treatments and the increase of the antimicrobial efficiency are facilitated.

Romania is an important European country that produces wine, having an important historic past and rich cultural tradition, many of them related to viticulture. Nowadays, the country is in a period of great changes, building a future in European Union and aspires to become an appreciated member of the international community of the wine as producer of high quality wines. The researches made until now suggest that the Romanian wines present benefic vasodilators and ant sclerotic qualities, similar to those that stay at the base of the so called "French paradox"⁸.

In this context, in this paper it has been followed the antioxidant action of different Romanian and Italian wines-antioxidant action sustained by the antioxidant compounds of the wines - the polyphenols, as well as "active SO₂ " which is formed during keeping of the wines.

II. MATERIALS AND METHODS

The study was focalized in showing the antioxidant action of some types of Romanian and Italian wines and the following analysis were made:

- The quantity analysis of the polyphenols, the total, free and combined SO₂
- The measurement of ant oxidative ability by the DMPD method.

Chemicals Reagents. Folin-Ciocalteu phenol reagent, tanic acid, anhydrous sodium carbonate, anhydrous ferric chloride were purchased from Sigma Chemical Company; N,N-Dimethyl-p-phenylenediamine dihydrochloride (DMPD) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) were purchased from Aldrich, Germany; all solvents (methanol) and reagents (deionized water; acetate buffer pH 7, iodine) were purchased from local suppliers.

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Samples of wine nine red and eight white wine samples originating from different areas of Dobrogea, Romania and areas of Campania and Sicilia, Italia, were purchased from local and Italian markets.

Apparatus. Spectrophotometer measurements were recorded by using an UV-VIS Jenway 6300 apparatus.

Total Polyphenolic Content of Wine Samples. The phenolic content of the different wines was determined by Folin - Ciocalteu reagent⁹. Each sample (0.1 mL) was added to 4.2 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent (Sigma). After 1 min of mixing, 1 mL of an 80% solution of sodium carbonate and 4.2 mL of deionized water were added. The mixture was left 2 h at room temperature in the dark and the absorbance at 760 nm was measured. The concentration of the total phenolic content was determined by a comparison with the values obtained with a standard solution of tanic acid (0,01%). The total content of phenolic compounds in the extract in tanic acid equivalents was calculated by the following formula $T = CxV_1/V$, where: T = total content of phenolic compounds, $\mu\text{g/mL}$ wine, in tanic acid; C = the concentration of tanic acid established from the calibration curve ($\mu\text{g/mL}$); V = the volume of wine sample, milliliter; V_1 = the volum of product (1mL wine).

Sulfur Dioxide Determination. Total and free SO_2 content of wine samples was determined by the titrimetric method „Ripper” using solution of iodine 0.1N.

Scavenging Effect (%) by DMPD method ¹⁰. DMPD, 100 mM, was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water; 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer, pH 5.25, and the colored radical cation (DMPD^+) was obtained by adding 0.2 mL of a solution of 0.05 M ferric chloride (final concentration 0.1 mM). One milliliter of this solution was directly placed in a 1-mL plastic cuvette and its absorbance at 505 nm was measured. Standard solutions of the TROLOX were prepared as follows: 1 mg/mL of TROLOX was prepared by dissolving 0.1 g of TROLOX in 100 mL of methanol. Fifty microliters of standard antioxidants or of wine samples (diluted in water 1:20 for the red wines, undiluted for white wines) were added in the spectrometric cuvette and after 10 min at 25 °C under continuous stirring the absorbance at 505 nm was measured. The buffered solution was placed in the reference cuvette. A dose-response curve was derived for TROLOX, by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank) according to the equation:

$$\text{inhibition of } A_{505} (\%) = \left(1 - \frac{A_f}{A_0}\right) \times 100 \quad \text{where:}$$

A_0 is the absorbance of uninhibited radical cation and A_f is the absorbance measured 10 min after

the addition of antioxidant samples. Antioxidant ability of fish oil was expressed as TEAC (TROLOX equivalent antioxidant capacity) according to DMPD method, using the calibration curve plotted with different amounts of TROLOX.

Statistical Analysis. All data were expressed as mean \pm SD ($n=3$) by using Origin 8 test. Mean values do not differ significantly.

III. RESULTS AND DISCUSSION

Wine was widely studied for its antioxidative properties due to the wellknown health importance of its phenolic component. Antioxidant compounds in wine are mainly hydrophilic and their antioxidant activity could be well evaluated by the DMPD method.

Total Phenolic Content and Sulfur Dioxide of Wine Samples

The 17 wine samples were tested for their antioxidant ability. The concentration of the total phenolic content was determined by using calibration curve of tanic acid (see Fig.1). The standard deviation is very low and the dose-response curve is highly reproducible. The equation of calibration curve is: $C = 11,038 A - 0,269$; $Y = A * X$; $A = 0.122033$; Correlation Coefficient = 0.99519; Standard Error = 0.665321; $r = 0.99519$; $r^2 = 0,99040$.

The content of phenols are indicated in Table 1, the total and free SO_2 are indicated in Table 2, respectively Table 3. The present study shows the presence of the phenols in higher quantity in the red wines 900-1900 (ppm of tanic acid), than in the white ones 200-450 (ppm of tanic acid).

The obtained data are in concordance with the speciality literature; some of the red wines contain 1,72-1,91 g/L and the white ones contain between 0,43 and 0,46 g/L. The results are sustained by the content of total SO_2 which at the white wines is higher 70-188 ppm than at the red ones 46-90 ppm and higher at the types which have mentioned on the label “it contains sulfites”.

Fig. 1 : Calibration curve for tanic acid

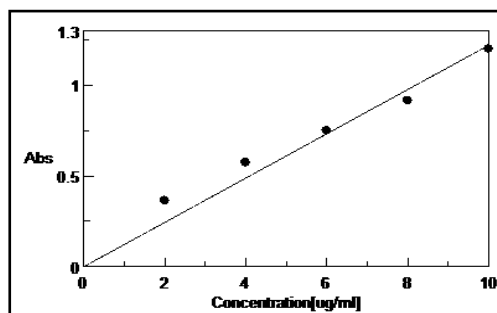


Table 1 : Amounts of polyphenols of tested wines

White wines			Red wines		
Samples	Type of wine	Amount of polyphenols (ppm tanic acid)	Samples	Type of wine	Amount of polyphenols (ppm tanic acid)
S ₁	C.*	288	S ₁	C.S ₁	1780
S ₂	M. ₁ .*	240	S ₂	C. S. ₂	1400
S ₃	M. ₂	378	S ₃	M. ₁ * ₂	1230
S ₄	S ₁	312	S ₄	M. ₂	1445
S ₅	S ₂	332	S ₅	B.	1920
S ₆	R ₁	255	S ₆	P. N. ₁	1675
S ₇	R. ₂	281	S ₇	P. N. ₂	1245
S ₈	R. ₃	445	S ₈	B.*	935
			S ₉	C.	1025

Table 2 : Amounts of free SO₂ of tested wines

Samples	Type of wines	Free SO ₂ ppm	Standard Deviation	Confidence Intervals for Mean 95%	
				Minimum	Maximum
White wines					
S ₁	C.*	25,39	0,190	25,23	25,60
S ₂	M. ₁ .*	23,46	0,344	23,24	23,86
S ₃	M. ₂	19,25	0,055	19,20	19,31
S ₄	S ₁	7,61	0,061	7,56	7,68
S ₅	S ₂	0,51	0,023	0,49	0,54
S ₆	R ₁	0,70	0,011	0,69	0,71
S ₇	R ₂	0,92	0,020	0,9	0,94
S ₈	R ₃	1,41	0,100	1,34	1,53
Red wines					
S ₁	C.S ₁	3,55	0,092	3,45	3,63
S ₂	C. S. ₂	2,83	0,050	2,79	2,89
S ₃	M ₁ * ₂	7,23	0,196	7,01	7,36
S ₄	M ₂	8,29	0,070	8,21	8,34
S ₅	B.	6,99	0,064	6,92	7,04
S ₆	P. N. ₁	4,47	0,017	4,45	4,48
S ₇	P. N ₂	0,69	0,078	0,64	0,78
S ₈	B.*	10,3	0,0721	10,24	10,38
S ₉	C.	9,10	0,0832	9,04	9,20

Each value is a mean of triplicate analyses \pm SD. Mean values do not differ significantly.

*On the label is mentioned "it contains sulfites"

Table 3 : Amounts of total SO₂ of tested wines

Samples	Type of wines	Total SO ₂ ppm	Standard Deviation	Confidence Intervals for Mean 95%	
				Minimum	Maximum
White wines					
S ₁	C.*	187,95	0,155	187,78	188,08
S ₂	M ₁ .*	182,27	0,328	181,9	182,5
S ₃	M ₂	146,32	0,372	146	146,73
S ₄	S ₁	130,27	0,266	130,03	130,56
S ₅	S ₂	138,07	0,176	137,89	138,24
S ₆	R ₁	110,16	0,141	110	110,25
S ₇	R ₂	90,18	0,060	90,13	90,25
S ₈	R ₃	76,51	0,260	76,29	76,8
Red wines					
S ₁	C.S ₁	85,61	0,167	85,43	85,76
S ₂	C. S. ₂	90,76	0,196	90,54	90,88
S ₃	M ₁ .*	76,60	0,309	76,25	76,8
S ₄	M ₂	87,02	0,055	86,99	87,09
S ₅	B.	85,63	0,107	85,56	85,76
S ₆	P. N ₁	90,73	0,174	90,54	90,88
S ₇	P. N ₂	46,07	0,055	46,02	46,13
S ₈	B.*	71,70	0,115	71,57	71,78
S ₉	C.	87,02	0,130	86,89	87,15

Each value is a mean of triplicate analyses \pm SD. Mean values do not differ significantly.

*On the label is mentioned "it contains sulfites".

Regarding to the SO₂ analysis, the maximum admitted quantity is not higher in any of the samples, value registered by O.M.S. 975/1998 (Order of Health Minister) and C.E. (European Commission). Staying at the same quantity of SO₂ allows us to sustain that adding the preservative does not have the risk of modification the organoleptic and nutritive value of the product. The obtained results are given in Tables 2 and 3 and their analysis is made in according with the values accepted by O.M.S. 975/1998. This way:

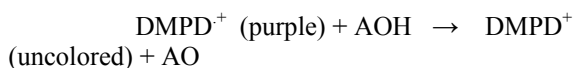
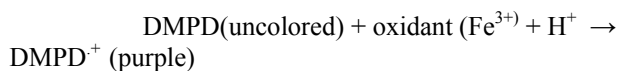
- The accepted quantity of total SO₂ in wines is: 160mg/L for the red wines with small quantity of carbohydrates; 260 mg/L for the white wines with small quantity of carbohydrates; 300mg/L for the wines with higher quantity of carbohydrates.
- The quantity of free SO₂ accepted in wines is 50 mg/L.

Comparing the values of the SO₂ total/combined from the white wines with its value from the red ones is observed that the assortments of white wines have more total/ combined SO₂, especially those which have mentioned on the label "it contains sulfites". The percentage of the "free active form" is very small,

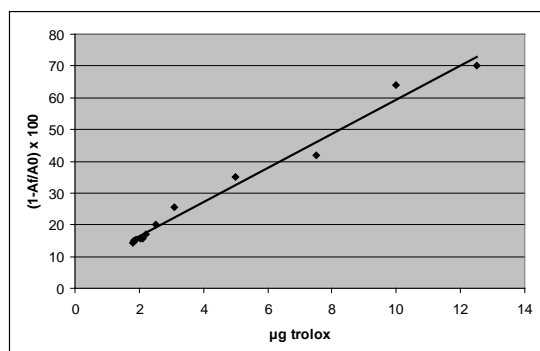
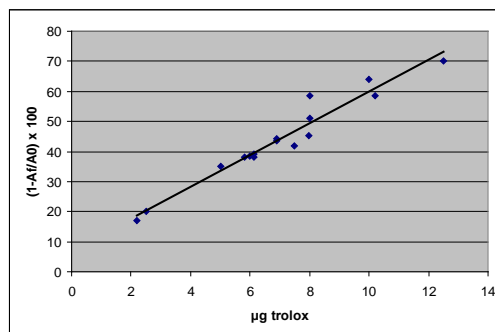
even sub unitary at some assortments; in the first three samples of white wines is found also in relatively small percentage 13%-15% for ensuring an antioxidant protection. Normally, the free SO₂ represents 15%-30% from the total SO₂, but the antiseptic and antioxidant actions have only 2%-10% free SO₂¹¹. From the ratio between the quantity of free SO₂ and total SO₂ is observed that not always the higher value of total SO₂ means a higher percentage of "active dioxide", which shows that the suffixation process is complex and has unexpected final effects.

a) The antioxidant ability of wine samples

The principle of the assay is that at an acidic pH and in the presence of a suitable oxidant solution DMPD can form a stable and colored radical cation (DMPD⁺) (Scheme 1, step 1)¹⁰. Antioxidant compounds (AO) which are able to transfer a hydrogen atom to DMPD⁺ quench the color and produce a decoloration of the solution which is proportional to their amount (Scheme 1, step 2). This reaction is rapid (less than 10 min) and the end point, which is stable, is taken as a measure of the antioxidative efficiency. Results are reported in Table 4.

Scheme 1

The antioxidative efficiency was expressed in TEAC (TROLOX equivalent antioxidant activity) according to method, using the calibration curve plotted with different amounts of TROLOX (see Figure 2 for white wines and Figure 3 for red wines). The standard deviation is very low and the dose-response curve is highly reproducible. Inhibition of the absorbance at 505 nm is linear between 0.2 and 11 μg of TROLOX. The relation ship calculated within this range for the standard compound is: $A_{505} \text{ (inhibition)} = 5.3 (\mu\text{g of TROLOX}) + 7.0$; $r^2 = 0.987$

Fig. 2: Antioxidant activity for white wines*Fig. 3:* Antioxidant activity for red wines

It is observed that the red wines have a higher antioxidative activity (between 5.80% -10,2%) than the white wines. The white wines have an antiradicalic efficiency lower than 3% (1,9% -3,10%). The difference of antioxidative activity is explained on the basis of the different contain of antioxidative compounds.

There is a correlation between the content of phenols and the TEAC of each red wine and a clear difference between the value of TEAC of red wine samples and the white ones. The total polyphenol content of the white wines is too low to account for their TEAC values (see Table 4). This finding could be related to the addition of antioxidants such as sulfur dioxide, which are widely used as preservatives, in white wines.

Table 4: Antioxidant Activity of tested wines

Samples	Type of wine	Amount of polyphenols (ppm tanic acid)	Antioxidant activity ($\mu\text{g trolox}$)	Inhibition effect (%)
<i>White wines</i>				
+	S ₁ C.*	288	1.8	14.2
	S ₂ M. ₁ *.	240	1.84	14.9
	S ₃ M. ₂	378	1.9	15.21
	S ₄ S ₁	312	2.05	15.50
	S ₅ S ₂	332	2.1	15.72
	S ₆ R ₁	255	2.1	17.08
	S ₇ R ₂	281	2.2	17.45
	S ₈ R ₃	445	3.1	25.7
<i>Red wines</i>				
S ₁	C.S ₁	1780	5.8	38,0
S ₂	C. S. ₂	1400	6.0	38,6
S ₃	M ₁ *	1230	6.12	39,0
S ₄	M ₂	1445	6.12	38,2
S ₅	B.	1920	6.9	43,6
S ₆	P. N. ₁	1675	6.9	44,2
S ₇	P. N. ₂	1245	7.97	45,3
S ₈	B.*	935	8.0	51,2
S ₉	C.	1025	10.2	58,7

*On the label is mentioned "it contains sulfites"

IV. CONCLUSIONS

In this paper a method to measure antioxidant power based on the DMPD colored radical cation is reported. The assay is particularly suitable for a largescale screening of white and red wines.

Studying the values of polyphenols and the sulf values, there are some samples in which the polyphenols are in the highest concentration, although the „active sulf” is found less-sb unitary values or a little after 1. This observation allows us to accept that there are found some sorts of the wines of higher quality than others.

The contain of polyphenols and of „active SO₂” shows the antioxidative action of the analysed wine samples.

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Synthesis and Analytical Characterization of Ester and Amine Terminated PAMAM Dendrimers

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GJMR-B Classification : NLMC Code: WJ 166



SYNTHESIS AND ANALYTICAL CHARACTERIZATION OF ESTER AND AMINE TERMINATED PAMAM DENDRIMERS

Strictly as per the compliance and regulations of:



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Surya Prakash Gautam ^α, Arun Kumar Gupta Anupama Sharma ^σ & Tapsya Gautam, Madhu ^ρ

Abstract - PAMAM dendrimers containing ethylene diamine core and methyl acrylate as repeating unit were synthesized by divergent approach. Analytical characterization of PAMAM dendrimers amine terminated full generation 4.0G and ester terminated half generation 3.5G were performed using UV-Vis spectroscopy, FT-IR spectroscopy, differential scanning calorimetry, NMR spectroscopy and MASS spectroscopy. The half generation dendrimers have the methyl ester terminating groups, which have the characteristic IR peaks for carbonyl at 1730-1750 cm⁻¹. For the full generation dendrimers, when the methyl ester groups were converted to amide groups, the corresponding carbonyl shifted to 1660 cm⁻¹. The characteristic methyl ester peak, which appeared in all the ¹H-NMR spectra of the ester terminating dendrimers, whereas it is absent in all the amine terminating dendrimers. The molecular weight was determined by ESI mass spectroscopy which further confirms the preparation of PAMAM dendrimers and provides information about the structural defects, polydispersity and purity.

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1. INTRODUCTION

Dendrimers are spherical, well defined, highly branched macromolecules with dense surface functional groups (Fig. 1) [1–3]. Ethylenediamine (EDA) core based Poly (amidoamine) (PAMAM) dendrimers synthesis needs repetitive Michael addition and amidation steps in which each iteration yields the next higher generation of the dendrimer. Multifunctional platform of dendrimers provides endless applications in drug delivery [4–8]. The synthesized PAMAM dendrimers are characterized for UV, FT-IR, NMR, DSC and MASS analysis. In the biomedical field dendrimers had been used for drug delivery, gene therapy, antigen conjugates, NMR contrast agents and synthetic vaccines [9–15]. UV-Vis spectrometry provides the proof of synthesis as well as the conjugation (surface modification) on dendrimers due to characteristic absorption maximum or shift in Lambda Max value due

to conjugation [16–20]. Appearance disappearance and reappearance of characteristic peaks in FTIR spectroscopy provides the proof of synthetic. Disappearance of nitrile groups in the synthesis of PPI dendrimers, disappearance reappearance of amine groups in PAMAM dendrimers generation, Pegylation of PAMAM dendrimers, disappearance of the aldehydes during the synthesis of PMMH dendrimers reflects the synthesis and surface modifications [21–23]. Nuclear magnetic resonance (NMR) spectroscopy permits determination of the structure and dynamics of molecules in solution. PAMAM dendrimers and complexed PAMAM are characterized by Rotational-Echo Double Resonance (REDOR) solid-state NMR spectroscopy [24]. Multidimensional NMR spectroscopy ((2D)-NMR, (3D)-NMR) is also acquiring increasing importance in the characterization of dendrimers [25]. NOESY experiments permit quantitative determinations of internuclear distances for nuclei in different parts of the dendrimer molecule [26]. The dynamics of dendritic branches can be investigated by measurement of ¹H- and ¹³C-spin-lattice relaxation times (T₁). Since the mobility of a dendrimer segment is proportional to its T₁ value, the change of mobility of the various dendrimer segments [27]. The DSC technique is generally used to detect the Glass Transition Temperature (T_g). The T_g is affected by the end group substitutions, and the molecular mass. DSC and Temperature Modulated Calorimetry (TMC) were also used to detect physical aging of PMMH dendrimers. Generation has practically no influence on the T_g values of liquid crystal dendrimers based on poly (phenyl acetylene) [28–30]. MALDI-TOF-MS and ESI-MS are among the few analytical methods suitable for detailed studies of structural defects in dendrimers on the basis of characteristic fragmentation patterns. The polydispersity and the purity of dendrimers explain the percentage of defect-free dendritic material [31–32]. The principal objective of the work presented here is to analytically characterize and investigate their structural characteristics by UV-Vis spectrometry, FT-IR spectroscopy, Differential Scanning Calorimetry (DSC), NMR spectroscopy and ESI Mass spectroscopy. The presented studies provide new insights into the understanding of the structure and properties of PAMAM

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dendrimer nanocomposites for future drug delivery and cancer treatments.

II. MATERIALS AND METHODS

a) Materials

Ethylenediamine (Merck Specialities (P) Ltd. Mumbai) and Methylacrylate (Loba Chem (P) Ltd., Mumbai) were used after distillation. Rest of the chemicals was purchased from Loba Chem (P) Ltd., Mumbai. For synthesis HPLC grade solvents were used.

b) Preparation of PAMAM Dendrimers

Dendrimers were prepared by a divergent synthesis scheme using the reagent excess method starting from Ethylenediamine (EDA) by consecutive Michael addition and ester amidation reaction. Dendrimers were prepared according to following step: Michael addition of primary amine (EDA in very first step) to methyl acrylate followed by amidation of formed multiester (tetra ester at very beginning) of EDA.

Michael addition reaction used ethylene diamine (EDA) as an initiator core for starting the synthesis of dendrimers by attaching four acrylate moieties on each amino group of EDA. The resulting compound referred to as "generation -0.5PAMAM tetra ester". This caused the branching in the structure of the dendrimer. The second step used is amidation of terminal carbomethoxy group (COCH_3) of methyl acrylate with EDA. This tetra ester with excess EDA gave "generation 0.0 PAMAM tetra amine". EDA was used in excess to about twenty to hundred times to avoid incomplete reactions and hence improved yield. The reaction was carried out using methanol as medium. The reactions were followed by removal of excess reagents by rotary vacuum evaporation at 55°C - 60°C , in every step. The whole reaction was carried out in dark at room temperature, using amber colored round bottom flask, which was corked tightly. Addition reaction was allowed to complete in two days, whereas amidation reaction complete in four days.

c) Evaluation of PAMAM Dendrimers

Synthesis of half and full generation dendrimers were confirmed through UV spectroscopy, FT-IR spectroscopy, DSC, ^1H -NMR and ESI-Mass spectroscopy.

III. RESULTS AND DISCUSSION

a) Identification of Dendrimers by UV-Spectroscopy

The change in λ_{max} values was obtained from half generation to full generation. The change in λ_{max} values from 284.0 (3.5G) to 277.5 (4.0G) nm revealed the change in structure of PAMAM dendrimers (Fig. 2 & 3). The λ_{max} of 4.0 G PAMAM dendrimers was 277.5 nm.

b) FT-IR Spectroscopy

The important peaks in FT-IR spectra of 3.5 G dendrimers were of Quaternary ammonium ion peak

3218.61 cm^{-1} , N-H stretch anti-symmetric sub. Primary amine 3021.91 cm^{-1} , C-H stretch 2402.87 cm^{-1} , 2834.22 cm^{-1} , C=O stretch of carbonyl group 1731.61 cm^{-1} , 1650.81 cm^{-1} , C-C bending 1215.91 cm^{-1} . The important peaks in FT-IR spectra of 4.0G dendrimers were of N-H stretch of primary amine at 3310.21 cm^{-1} , N-H stretch of anti-symmetric substituted primary amine at 3021.87 cm^{-1} , C-H stretch at 2947.66 cm^{-1} , C=O stretch of carbonyl group at 1668.12 cm^{-1} , N-H bending of N-substituted amide at $1511.92, 1417.42\text{ cm}^{-1}$, C-C bending at 1215.90 cm^{-1} . The results obtained are given in Table 1. The FT-IR spectra of 3.5G and 4.0G PAMAM dendrimers are shown in Fig. 4 & 5 respectively

c) Differential Scanning Calorimetry (DSC)

The changes in endothermic peak were analyzed. The changes in endothermic peak from 120.03 to 120.56°C were observed which shows the change in structure of PAMAM dendrimers. The results obtained are given in Table 2. The changes in endothermic peak are shown in Fig. 6 & 7 respectively.

d) NMR Spectroscopy

The PAMAM dendrimers was solubilized in deuterated methanol and analyzed at 300 MHz . Important shifts in NMR spectra of 3.5G dendrimers were 2.68 and 2.93ppm for carbonyl methylene proton - ($\text{CH}_2\text{C}=\text{O}$), 3.40ppm amide methylene proton - ($\text{CONHCH}_2\text{CH}_2\text{N}$). Important shifts in NMR spectra of 4.0G dendrimers were 3.03 ppm for carbonyl methylene proton - ($\text{CH}_2\text{C}=\text{O}$), 3.40 ppm amide methylene proton - ($\text{CONHCH}_2\text{CH}_2\text{N}$), 3.84 (var) ppm for $-\text{NH}_2$ terminal group (Fig. 8 & 9 and Table 3).

e) ESI Mass Spectroscopy

The ESI Mass spectra further confirm the preparation of PAMAM dendrimers. The molecular weight peak of 3.5G PAMAM dendrimers was 11944 Da and the molecular weight peak of 4.0G PAMAM dendrimers was 14483 Da. The ESI Mass spectra of 3.5G and 4.0G PAMAM dendrimers are given in Fig. 10 & 11 respectively, while data are shown in Table 4.

IV. CONCLUSIONS

The PAMAM dendrimers were synthesized using ethylenediamine as initiator core and methyl acrylate as repeating unit. Synthetic progress involves Michael addition and exhaustive amidation to complete cycle. Increasing amount of reactant in every progressive step was added to avoid incomplete reaction and hence to improve the yield. Completion of the reaction was confirmed by the copper sulphate solution reaction. The whole generation gave purple color, whereas half generation gave deep blue color, due to copper chelation at the terminal group of dendrimers. All the steps were found to be complete by the color reactions. Progress of Synthesis and

differentiation of 3.5G and 4.0G was confirmed by UV, IR, NMR and MASS spectroscopy.

The λ_{max} values were found out to be in range of 277-286 nm. The λ_{max} of 4.0G PAMAM dendrimers was found to be 277.5 nm. In IR spectrum peaks of N-H stretch for primary amine were obtained at 3310.21 cm^{-1} , which was due to NH_2 periphery of 4.0G PAMAM dendrimers. Half generation carboxyl terminated shows intense peaks in the C=O region while full generations shows intense peaks in the N-H stretch for primary amine. Appearance-disappearance reappearance of characteristic peaks provides the proof of synthesis. The changes in Endothermic peak from 120.03 to 120.56°C were observed which shows the change in structure of PAMAM dendrimers. In NMR spectra terminal amino group proton peaks ($-\text{CH}_2\text{NH}_2$) were obtained at 3.84 ppm and 2.68, 2.93, 3.03 ppm for carbonyl methylene proton ($-\text{CH}_2\text{C=O}$). Characteristic shifts in NMR spectra of 3.5G PAMAM dendrimers was due to terminal groups of $-\text{COOCH}_3$ at 3.73 ppm and 4.0G PAMAM dendrimers was for terminal groups of $-\text{NHCH}_2\text{CH}_2\text{NH}_2$ at 3.84 ppm. NMR spectral characteristic like shielding effects, deshielding effects, peak intensity, and integral value provides information about synthesis of dendrimers (Characteristic peaks in the spectra); conjugation chemistry (Shielding deshielding effects shifts in peaks); hydrodynamic radii (NMR pulse-field gradient spin echo); number of protons (Intensity of peaks and integral value); conformational changes (unique NMR signals from the core to the periphery); isomer populations observed by ^1H NMR reveal the onset of globular structure; NOE complexity emerges with globular structure: variable temperature NOESY studies show that the peripheral groups; Variable temperature coefficients for NH protons suggests that solvent is largely excluded from the interior of the dendrimer. Relaxation studies show that peripheral groups are more dynamic than groups at the core. The NMR data corroborated well with the FT-IR data to confirm the structure of the dendrimers.

The molecular weight peak of 3.5G PAMAM dendrimers was 11944 Da and the molecular weight peak of 4.0G PAMAM dendrimers was 14483 Da, which was approximate to theoretical molecular weight of PAMAM dendrimers. Deviation may be due to incomplete Michael addition causing the appearance of unsymmetrical dendrimeric structures, intramolecular cyclization, and the *retro*-Michael reaction. Synthesis of PAMAM dendrimers always generates "structural errors". Therefore it needs more attention for improving the synthesis and exploring the novel possible applications.

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Table 1 : IR Interpretation of 3.5 G dendrimers

Generation	S. No.	Peaks Value (cm ⁻¹)	Interpretation
3.5G	1	3218.61	Quaternary ammonium ion peak
	2	3021.91	N-H stretch anti-symmetric sub. Primary amine
	3	2834.22	C-H stretch
	4	1731.61, 1650.81	C=O stretch of carbonyl group
	5	1215.9	C-C bending
4.0G	1	3310.21	N-H stretch of primary amine
	2	3022.87	N-H stretch anti symmetric of sub. primary amine
	3	2947.66	C-H stretch
	4	1668.12	C=O stretch of carbonyl group
	5	1511.92, 1417.42	N-H bending of N- substituted amine
	6	1215.90	C-C bending

Table 2 : DSC spectra of 3.5 G and 4.0 G PAMAM dendrimers

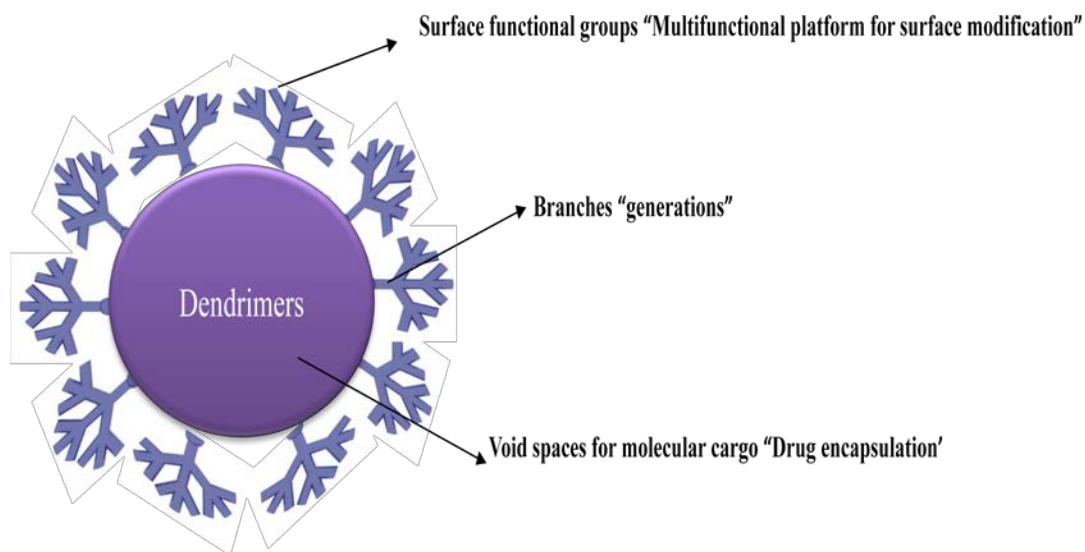
S. No.	Generation of dendrimers	Endothermic peak (°C)
1.	3.5G	120.03
2.	4.0G	120.56

Table 3 : NMR spectra chemical shifts and interpretation of 3.5 G and 4.0G PAMAM dendrimers

S. No.	Generation	δ values (ppm)	Interpretation
1	3.5G	2.44 and 2.46	$-\text{NCH}_2\text{CH}_2\text{N}-$
		2.52	$-\text{NHCOCH}_2\text{CH}_2\text{N}-$
		2.68	$-\text{NHCOCH}_2\text{CH}_2\text{N}-$
		3.40	$-\text{CONHCH}_2\text{CH}_2\text{N}-$
		2.93	$-\text{NCH}_2\text{CH}_2\text{COOCH}_3$
		3.73	$-\text{NCH}_2\text{CH}_2\text{COOCH}_3$
2	4.0G	2.93	$-\text{NCH}_2\text{CH}_2\text{N}-$
		2.98	$-\text{NHCOCH}_2\text{CH}_2\text{N}-$
		3.03	$-\text{NHCOCH}_2\text{CH}_2\text{N}-$
		3.40	$-\text{CONHCH}_2\text{CH}_2\text{N}-$
		2.95, 2.99	$-\text{CONHCH}_2\text{CH}_2\text{N}-$
		3.84	$-\text{CONHCH}_2\text{CH}_2\text{NH}_2$ (var)

Table 4 : ESI Mass spectra interpretation of 3.5 G and 4.0 G PAMAM dendrimers

S. No.	Generation of dendrimers	Theoretical molecular weight (Da)	Practical molecular weight (Da)
1.	3.5 G	12424	11944
2.	4.0 G	14215	14483

Figure 1 : Core structure of dendrimers

Core structure of Dendrimers

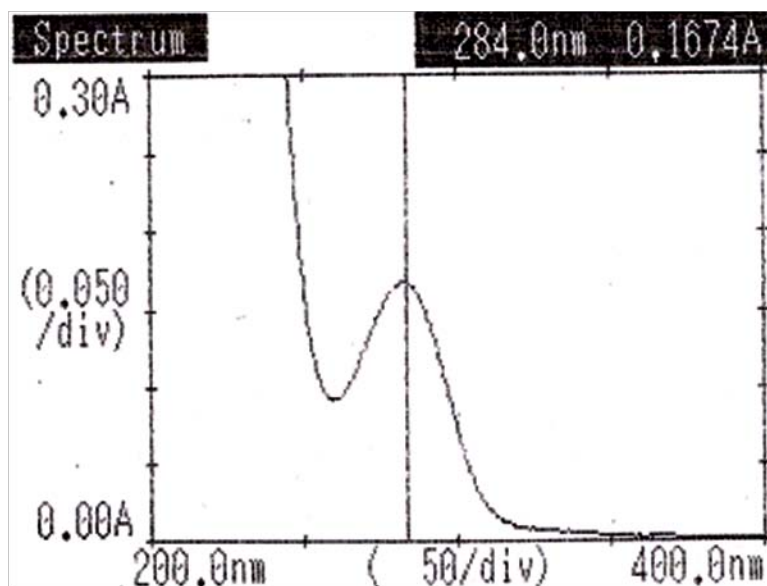
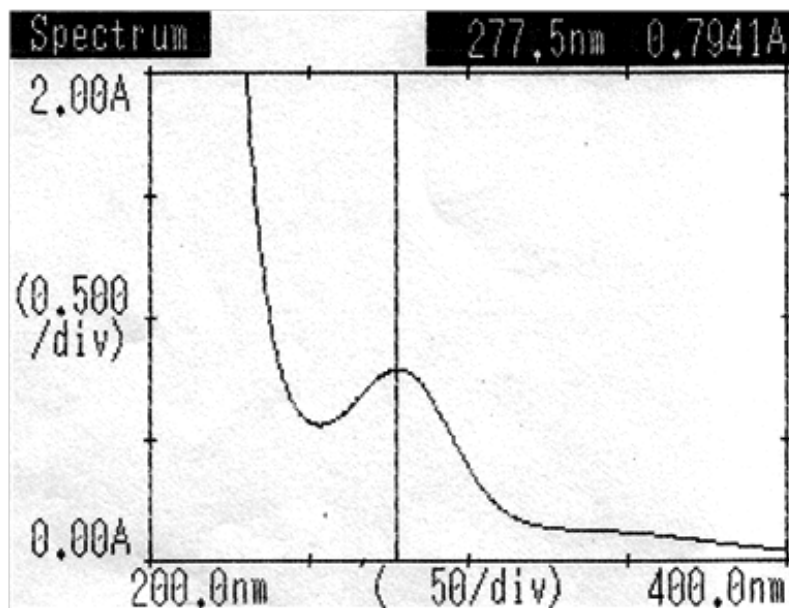
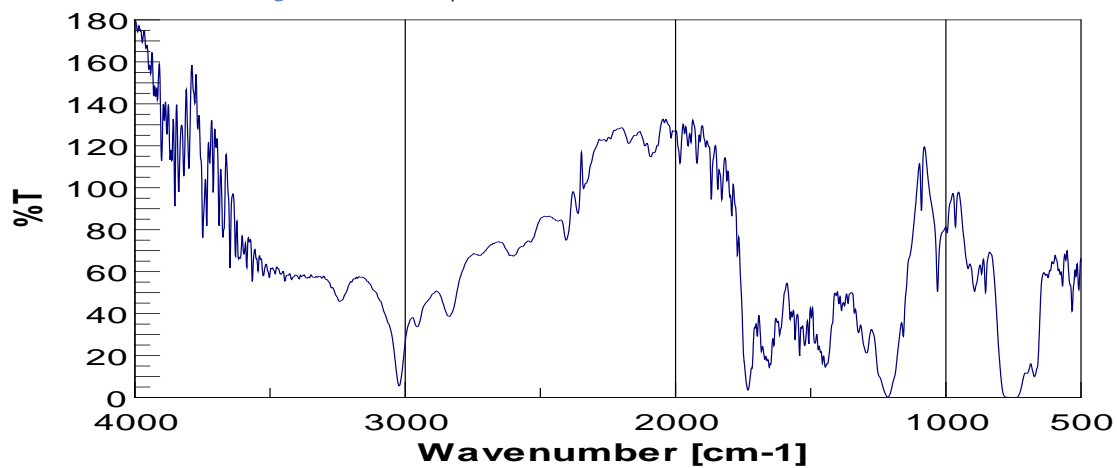
Figure 2 : UV spectra of 3.5 G PAMAM dendrimers*Figure 3 : UV spectra of 4 G PAMAM dendrimers**Figure 4 : FT-IR spectra of 3.5 G PAMAM dendrimers*

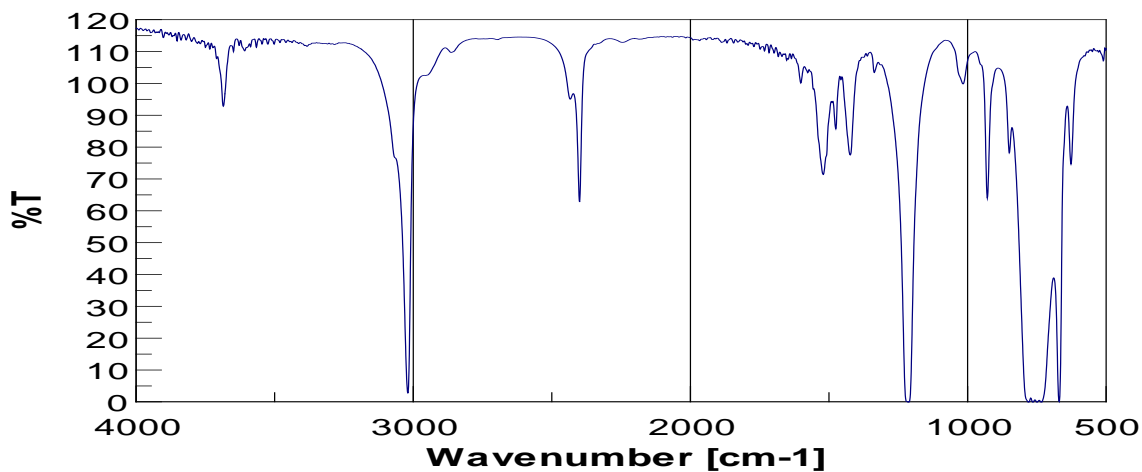
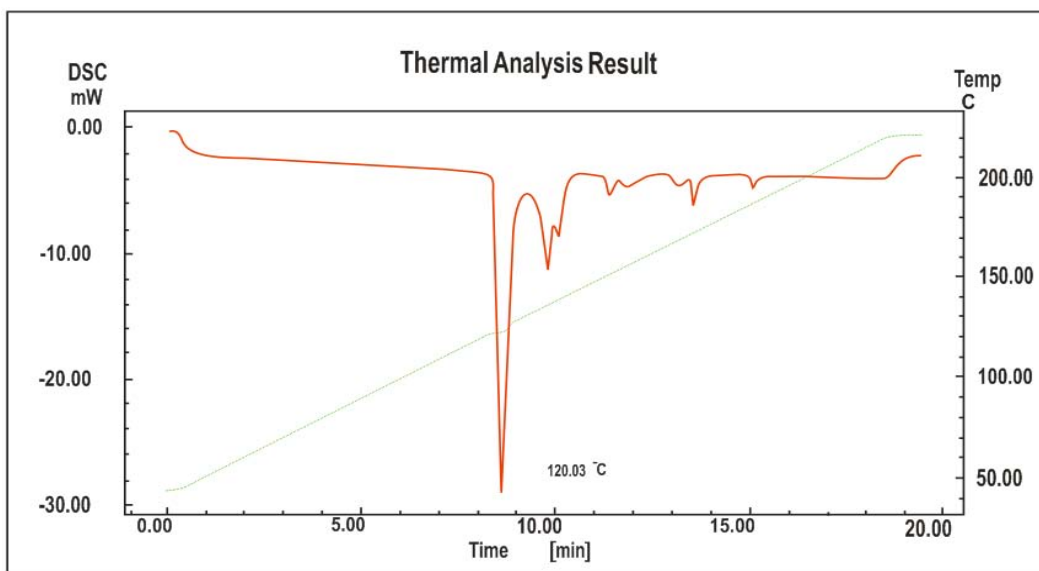
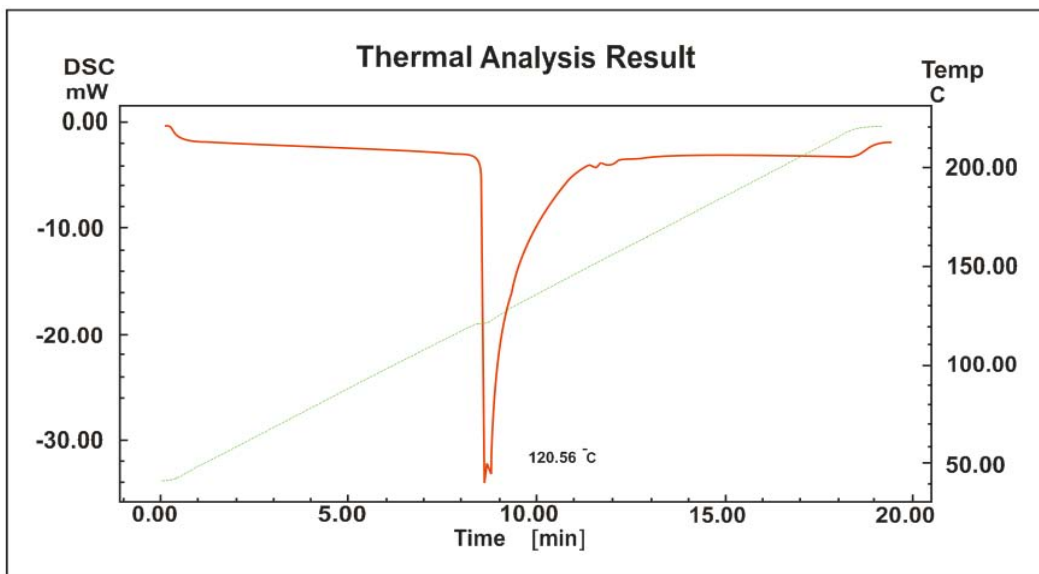
Figure 5 : IR spectra of 4.0 G PAMAM generation dendrimer*Figure 6* : DSC spectra of 3.5 G PAMAM dendrimers*Figure 7* : DSC spectra of 4.0 G PAMAM dendrimers

Figure 8 : NMR spectra of 3.5 G PAMAM dendrimers

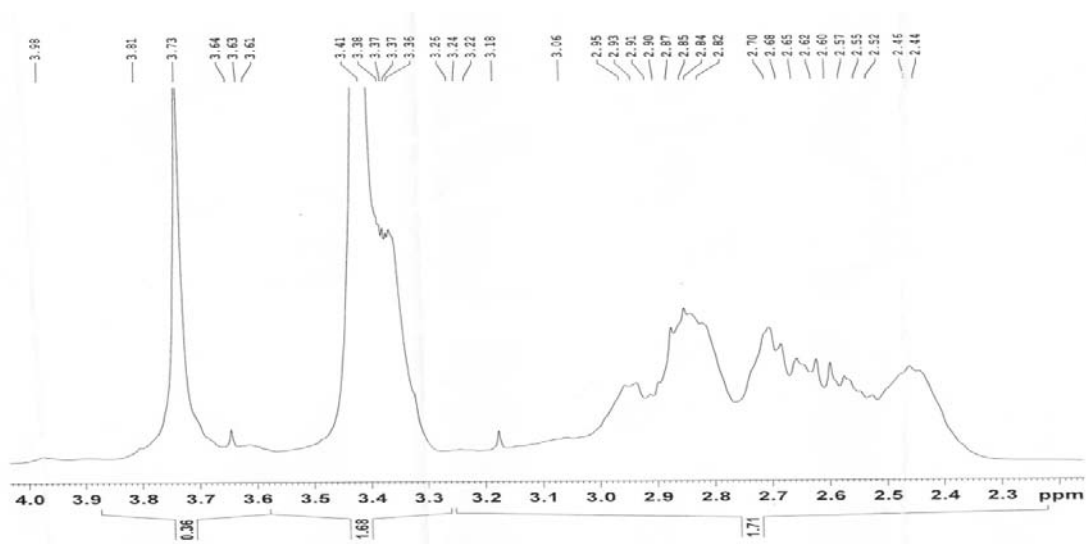


Figure 9 : NMR spectra of 4.0 G PAMAM dendrimers

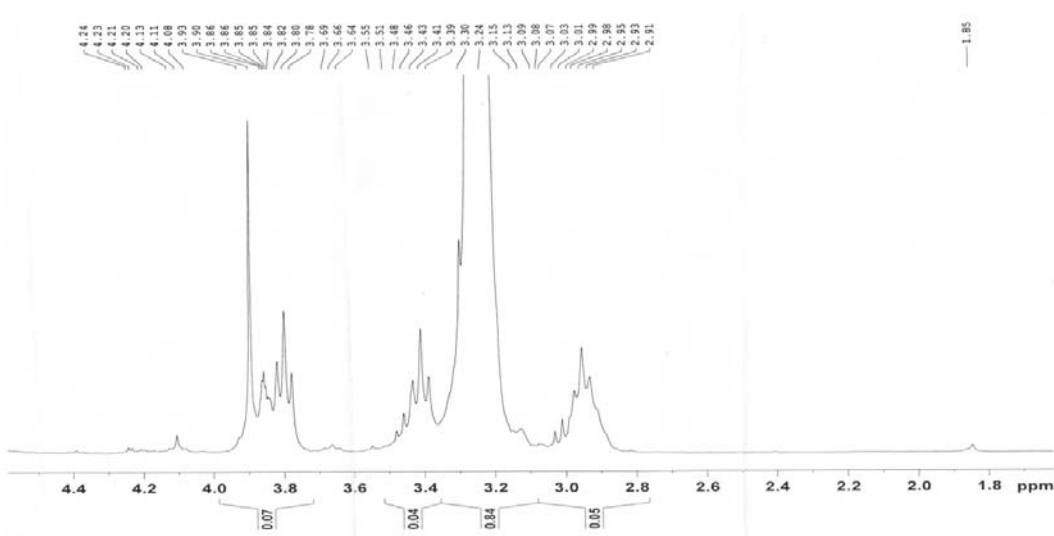


Figure 10 : ESI Mass spectra of 3.5 G PAMAM dendrimers

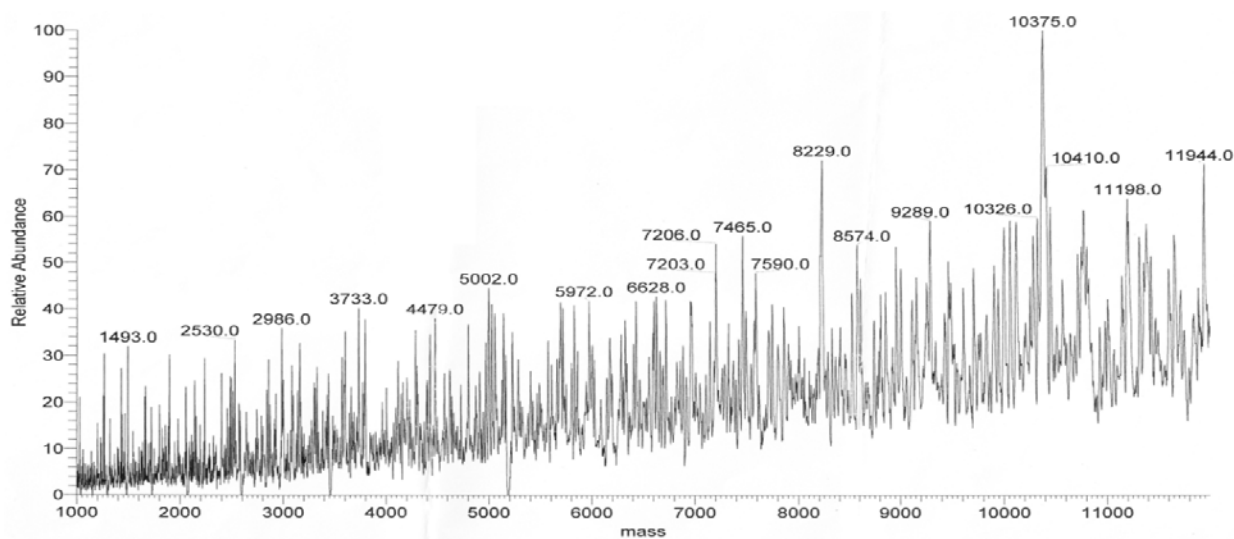
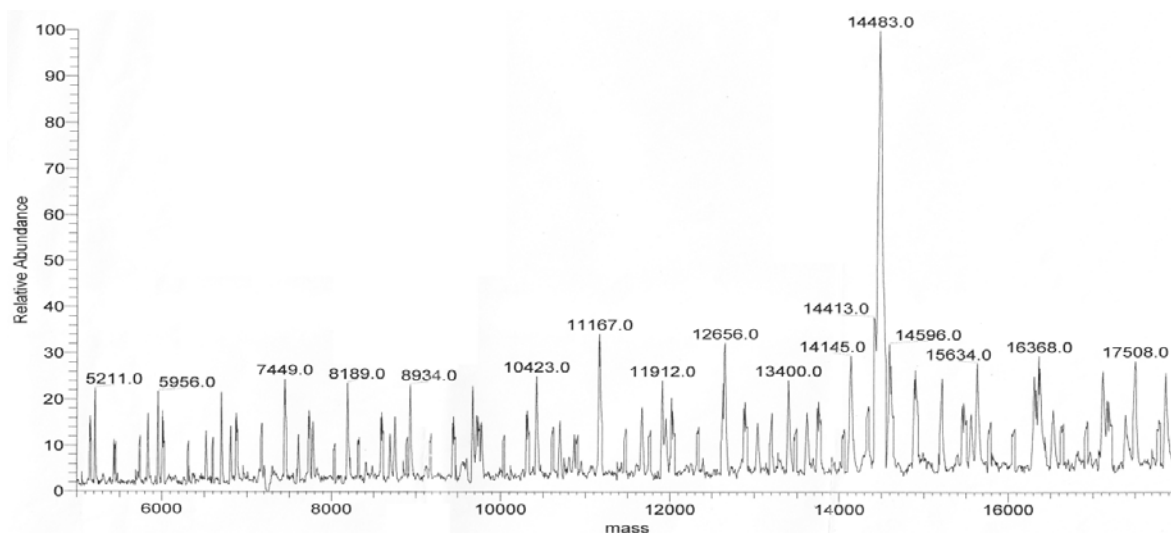


Figure 11 : ESI Mass spectra of 4.0 G PAMAM dendrimers

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Trends of Steroids in the Youth

By Waheed Zafar, Amir Razi, Abdul Rehman, Masroor Siddique,
Waleed Anwar, Danish Arif & Neelam Almas

University of Lahore

Abstract - Anabolic steroid usage has been recognized as a serious health and ethical problem in youth for several decades. Numerous examples of steroid usage rules violations have been highly publicized and have lead to the suspension .Youth, however, are not the only population of individuals that use steroids. The objective of this article is to determine the usage of steroids in the youth as a trend and to compare our findings with survey that why steroids are consumed and what are the advantages and disadvantages and also acknowledgement. To measure these attitudes, we conducted a survey of 200 people from different gyms. More than (83.5 %) percent of the study group reported that they heard about current or previous anabolic steroid usage. According to the previous eighty-eight (88%) percent had heard of anabolic steroids, but only 59.5% had heard their side effects explained to them. In survey, 88% had heard of anabolic steroids, 59.5% had heard the side effects explained to them, and 19.5% admitted to using steroids. 24.5% said that steroids should be promoted. Only 16.5% says that steroids are healthy and 29.5 % think that a steroid improves muscles and strength. Only 18.5% take steroids as a pain reliever. These results suggest that anabolic steroids remain a problem among young. Educational programs should be instituted during junior high school to increase the knowledge of anabolic steroids in this group. Information should come from qualified individuals including coaches, teachers, trainers, and especially parents.

GJMR-B Classification : NLMC Code: QV 55



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Trends of Steroids in the Youth

Waheed Zafar ^σ, Amir Razi ^α, Abdul Rehman ^σ, Masroor Siddique ^σ, Waleed Anwar ^σ, Danish Arif ^σ
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Abstract - Anabolic steroid usage has been recognized as a serious health and ethical problem in youth for several decades. Numerous examples of steroid usage rules violations have been highly publicized and have lead to the suspension .Youth, however, are not the only population of individuals that use steroids. The objective of this article is to determine the usage of steroids in the youth as a trend and to compare our findings with survey that why steroids are consumed and what are the advantages and disadvantages and also acknowledgement. To measure these attitudes, we conducted a survey of 200 people from different gyms. More than (83.5 %) percent of the study group reported that they heard about current or previous anabolic steroid usage. According to the previous eighty-eight (88%) percent had heard of anabolic steroids, but only 59.5% had heard their side effects explained to them. In survey, 88% had heard of anabolic steroids, 59.5% had heard the side effects explained to them, and 19.5% admitted to using steroids. 24.5% said that steroids should be promoted. Only 16.5% says that steroids are healthy and 29.5 % think that a steroid improves muscles and strength. Only 18.5% take steroids as a pain reliever. These results suggest that anabolic steroids remain a problem among young. Educational programs should be instituted during junior high school to increase the knowledge of anabolic steroids in this group. Information should come from qualified individuals including coaches, teachers, trainers, and especially parents.

I. INTRODUCTION

The term steroids normally are related to a class of drugs that are used to treat in many types of medical situations. These drugs are also used for supporting reproduction, regulation of the metabolism and immune functions. Recreational athletes also use steroids to enhance performance and to improve personal appearance

"Anabolic means use of something that causes a building up of tissue. The term anabolism refers more generally to an increase in lean tissue in particular muscle tissue".

1. Steroids are solvable in fats
2. Steroids are organic compounds

Testosterone is derived in the body from cholesterol, and like other steroid hormones, testosterone has its main effect on tissues. 1 ml Testoviron Depot contains 250 mg testosterone. Testosterone has main effect on tissues. Testosterone enter in body and attaches to a receptor which crosses in to cell nucleus where it activates production of

protein. Protein production leads to tissue repair and growth. **Muscle building steroids** have changed the way gaming events are being conducted in today's times. More and more sportsmen have shown intent to take on performance enhancing steroids with an aim to optimize their performance. The curiosity behind **steroids** has seen a following in demanding sports such as **weightlifting, swimming, baseball and football** like never before.

A steroid used in the treatment of medical conditions usually involves the use of only one type of steroid and medical patients are closely monitored and the doses used are approximately that which would be produced naturally by the human body.

Many steroids available on the black market are even of dubious quality and often only contain small amounts of the drug. Some of these drugs have even been reported to contain only water and a dye, or contain only normal peanut oil.

Uses of steroids:

1. Maintaining sexual characteristics in males
2. Increase mass of the muscles (Body Builders)
3. Increase mass of the bones
4. Used in the treatment of inflammation
5. Increase the effect of testosterone

Clinical particulars:

➤ In men

Hypogonadism; potency disorders; male climacteric, aplastic anemia

➤ In women

Supplementary therapy of progressive mammary carcinoma in the post menopause

Anabolic Steroids:

- Anadrol (oxymetholone)
- Dianabol (methandrostenolone)
- Winstrol (stanozolol)
- Deca-Durabolin (nandrolone)
- Oxandrin (Oxandrolone)
- Depot-Testosterone

Objectives:

- To see what are the steroids
- To see why steroids are consumed
- To see what are the advantages and disadvantages of steroids

Author ^α : Assistant professor: The University of Lahore.
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- Does young generation really have knowledge about the advantages and disadvantages of steroids before using it?

Literature review:

Abbas Yavari(2009):-

According to the International Olympic committee, the abuse of the anabolic steroids is found with ratio of 50% of positive testing. Steroids usage is not restricted to the sports man. Since it's remain unsolved public health problem. Although AASS use has been forbidden in organized sports nearly thirty years, their abuse remains one of the important problems as a widespread phenomenon in both athletic and nonathletic populations. The major motive for their abuse is to motivate physical fitness and appearance. Despite evidence of increased risks of AASS, abusers are simply mauve regarding the dangers of these substances

Trend among Youth to Buy Bulking Steroids (uncategorized):

Buying and following the bulking steroids cycle has become trend among the youth of our present society. In the domain of the third generation not only the body builder but also the youths have the craze for making their body to look like the super hero. They work out for countless hours to increase their muscular ability and strength in a shortest possible time. But the final result comes out to be negative.

Helen Keane (2005):

This article examines these two frameworks and their constitution of the male steroid user as psychologically disordered, drawing on a range of medical and psychological literature. The first framework understands steroid use as a form of illicit drug use, and constitutes the steroid user as an antisocial and excessively masculine subject. The second locates steroid use within the field of body image disorder, producing the steroid user as a damaged and feminized male.

Jay R. Hoffman (2006):

For almost half of this time no attempt was made by sports governing bodies to control its use, and only recently have all of the major sports governing bodies in North America agreed to banned from competition and punish athletes who test positive for anabolic steroids. Yet, controversy exists whether these testing programs deter anabolic steroid use t is of interest to understand why many athletes underestimate the health risks associated from these drugs.

Randall R. Wroble(2003):

Anabolic steroids remain a problem. Eighty-eight percent had heard of anabolic steroids, but only 64% had had their side effects explained to them. Only 47% stated that a parent, coach, teacher, or athletic

trainer was their primary source of information. Results were compared to a 1989 baseline study completed before legislation lead to the scheduling of anabolic steroids. In 1989, 78% had heard of anabolic steroids, 50% had had the side effects explained to them, and 2% admitted to using steroids

Andrew Berdahl (2003)

The first thing that comes to people's minds about steroids is the fact that steroids are 1dangerous drugs. However, there are many kinds of steroids that are beneficial to the body. The structure of steroid is used to simulate appetite and bone growth, and it also is used to cure chronic wasting conditions. Steroid increases protein within cells, and that builds up muscles rapidly. Initially, body builders were the first people who used steroids

Tumbler: (2005)

The abuse of steroid is used in modern society. The usage of steroids is also catching up with school and college-going children and even business professionals. In a survey in 1999, it was revealed that as much as 479,000 students worldwide or 2.9 percent of total student population had used steroid by the last year of high school. In their aim to do so, they forget that steroids can be harmful and may pose a danger to their healthy, body, and even life.

II. METHODOLOGY

a) Data type

- Primary data

b) Type of Research

We are going to do quantitative research. Type of our data is primary data. In quantitative research we collect the data by conducting survey, Depth interview, and questionnaire

c) Research tool

- Questionnaire

The questionnaire was modified from the one designed. Because of our type of data we have selected is primary questionnaire fill up we have chosen. On every questionnaire we mentioned age, sex, occupation, marital status to know about them. Our first two questions show the acknowledgement about steroids. In other questions we asked their perception about steroids. In the ending of questionnaire we asked about usage of steroids by them or other people they know.

d) Type of questionnaire

- Close ended questions

e) Targeted area

- Gym's
- Universities
- Colleges

f) *Sample size*

- o 200

We take 250 questionnaires for our research in which 30 questionnaires were ruined and 15 were uncompleted. We get the exact data from 205 people and we take exact figure of 200 questionnaires as our sample size.

Type of sampling:

Simple random sampling

g) *Findings*

	Frequency	Percent
16-20	88	44.0
21-25	103	51.5
26-30	9	4.5
Total	200	100.0

Table (a)

In our survey the majority of people from which we have collected data about 21-25 years old because we are targeting the youth. The age from 16-20 years old is 44% of the total population. The least one are from 26-30 years old from which we have collected the data.

	Frequency	Percent
Female	49	24.5
Male	151	75.5
Total	200	100.0

Table (b)

Our targets of the population are mostly male from where we collect the data. The majority is male with the 75.5% of the total population and the remaining part of population is female with the percentage of 24. The total population we have target is about 200 people including male and female.

Table:-

The responses are given below in the table

Have you ever heard of steroids (a drug taken to increase muscle size)		Frequency	Percent
	Yes	167	83.5
	No	27	13.5
	Don't know	6	3.0
Do you know side effects of it?	Yes	119	59.5
	No	41	20.5
	Don't know	40	20.0

Table 1

	Frequency	Percent
Single	188	94.0
Married	11	5.5
Widow	1	.5
Total	200	100.0

Table (c)

In our survey the marital status of most of the people are single. Mostly married people have no time they are much busy in their daily routine and busy life. 94 percent people of the total population are single in their marital status and 5.5 percent of our population are married remaining 0.5 percent have mentioned widow in the questionnaire.

	Frequency	Percent
Student	194	97.0
business man	5	2.5
Housewife	1	.5
Total	200	100.0

Table (d)

In the youth as we know mostly people are student. The 97% of the population have mentioned themselves as student, 2.5 % of our population from where we collect the data said that they are businessman rest of the population says that they are house wives with 0.5 percent of the population. So include all of them our total population become 200.

Corresponding to the objectives:

- Our first objective is to see what are steroids?

With perspective to our first objectives the questions in our questionnaire that fall in this category.

The response from the population with the reference to steroids is that 83.5% heard about steroids. Although 13.5% of population says that have not heard about steroids and 3% of targeted population says that they don't know about it.

It lies in our first objective to see what are steroids?

With perspective to the first objective 59.5 % of our population says that know about steroids. The

people who say no are 20.5% of the population we have targeted and rest of the 20% say that they don't know about steroids side effects. Majority knows about the side effects of steroids.

✚ Our second objective is to see why steroids are consumed.

Table:-

Responses in the perspective of this question are mentioned in the table as given below:

Questions		Frequency	Percent
Have you ever used steroids as pain reliever?	Yes	37	18.5
	No	107	53.5
	Don't know	56	28.0
Do you know steroids are often used in medicine these days?	Yes	103	51.5
	No	45	22.5
	Don't know	52	26.0
Have you ever used steroids?	Yes	39	19.5
	No	136	68.0
	Don't know	25	12.5

Table 2

As we have mentioned about the response of usage of steroids as pain reliever. The responses in the fever of yes were 18.5% of the population and the response in the fever of no were 53.5 % of total population. Rest of the people said that they don't know about steroids as a pain reliever. Majority is I saying that they not used steroids as a pain reliever.

Responses for the acknowledgment about usage of steroids in the medicine are given in the table.

Research says that about 51.5% people of total population are aware of usage of steroids in the medicines. And 22.5% people of the total population says that they have no knowledge about usage of steroids are being used in the medicines. Although the other 26% of our targeted population says that they don't know about this.

Response for the usage of steroids in the youth is given in the table

According to the research 19.5% of the targeted population said: that have used steroids. On other hand 68% people deny about the usage of steroids their response was negative for the steroid usage and rest of 12.5% people says that they don't know about usage of steroids either they used or not.

✚ Our third objective is to find out advantages or disadvantages of steroids.

The responses of steroids promotion in the view of youth is given below in the table:

Questions		Frequency	Percent
Do you think steroids should be promoted?	Yes	49	24.5
	No	122	61.0
	Don't know	29	14.5
To you steroids are healthy?	Yes	33	16.5
	No	109	54.5
	Don't know	58	29.0
Do you believe that steroids without proper nutrition and exercise will improve muscle size and strength?	Yes	59	29.5
	No	79	39.5
	Don't know	62	31.0
Do you feel that people take steroids to improve your chances for athletic success?	Yes	139	69.5
	No	32	16.0
	Don't know	29	14.5

Table 3

As the table show the views of youth for the promotion of steroid. 24.5% of the targeted population says that yes steroids should be promoted according to them but on other hand the majority says that steroids should not be promoted. 61% of the population says that steroids should not be promoted.

The perception of the people that steroids are healthy or not are given in the table:

Research says that 16.5% of the people are satisfied with the steroids that they are healthy. The population with majority 54.5% thinks that steroids are not healthy and 29% of the total population from where data is collected replied that they don't know about it either it healthy or injurious. It shows the advantages or disadvantages of steroids in the view of people.

Steroids without proper nutrition and exercise will improve muscle size who agrees with it and

response yes is 29.5% and on other side people who disagree with it that steroids cannot improve muscle without proper nutrition and exercise is about 39.5. Majority falls in this segment that have said no and remaining says that they don't know

As the result shows that 69.5% feel think that steroids can improve chance for athletic success but 16% feel that people don't take steroids to improve their chances of success for athletics. The part consist of 14.5% of the targeted population feel that they have no idea or they don't know that people take steroids or not take steroids to improve their chances for athletic success.

Our forth objective is to see awareness among youth about steroids.

The table given below shows the results from the targeted population:

Question		Frequency	Percent
Is it safe, if steroids used carefully will not harm an athlete?	Yes	54	27.0
	No	69	34.5
	Don't know	77	38.5

Table 4

The result shows that 27% of the population agrees with it that if steroids are taken carefully with proper dose they don't harm you. 34.5% says that steroids are harmful either they taken proper or not. There is also existences of people that don't know about steroids are harmful or not if they are taken carefully 38.5% of the total represents this.

III. CONCLUSION

This research was conducted to gauge the perception of steroids as a trend in the youth. Approximately one percent of 10 to 14 year-old youth sports participants are using or have used anabolic steroids. Even though usage has decreased by over

50% since 1989, steroid use is still a serious problem. Insufficient knowledge and inappropriate attitudes regarding the benefits and risks of using anabolic steroids is also a major concern. About 51% have acknowledgement about steroids rather 49% rest of the steroids users take steroids without acknowledgement. Research impacts positive on the steroids as trend in the youth. Steroids should not be promoted 86% of the people elect it.

IV. RECOMMENDATION

Educational programs have shown to be effective against other forms of drug use. New educational and intervention efforts against anabolic steroids likewise should be instituted. These programs should start before junior high and continue through high school. Informational sources about steroids should come from qualified individuals including teachers, coaches, and trainers. Parents should also be involved and educated to help inform their children about steroids. It should be prescribed according to the Doctors advice.

Limitations:

The hurdles we have to face in our research are:

- University class timing
- Fuel consumption
- Lectures missing
- Problem in questionnaire fill up

- Miss match the timings of gym

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APPENDIX

Please answer every question on the appropriate line.

The University of Lahore

We the students of LBS are interested in learning more about anabolic steroids. Our objective is to help expand the body of knowledge about how steroids effects the young generation.

Topic: trend of steroids in the youth

QUESTIONNAIRE

Age Sex Marital status..... Occupation

❖ Have you ever heard of steroids (a drug taken to increase muscle size and/or strength)?

☐ Yes ☐ No ☐ don't know

❖ Do you know side effects of it?

☐ Yes ☐ No ☐ don't know

❖ Do you know steroids are often used in medicines these days?

☐ Yes ☐ No ☐ don't know

❖ Do you think steroids should be promoted?

☐ Yes ☐ No ☐ don't know

❖ To you, steroids are healthy?

☐ Yes ☐ No ☐ don't know

❖ Do you believe that steroids without proper nutrition and exercise will improve muscle size and strength?

☐ Yes ☐ No ☐ don't know

❖ Have you ever used steroids as a pain reliever?

☐ Yes ☐ No ☐ don't know

❖ Do you feel that people take steroids to improve your chances for athletic success (college scholarships, world championships, professional contracts, etc.)?

☐ Yes ☐ No ☐ don't know

❖ Is it safe, if steroids used carefully will not harm an athlete?

☐ Yes ☐ No ☐ don't know

❖ Have you ever used steroids?

☐ Yes ☐ No ☐ don't know



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Anti Biofilm Effect of Biogenic Silver Nanoparticles Coated Medical Devices against Biofilm of Clinical Isolate of Staphylococcus Aureus

By S. Karthick Raja Namasivayam, Beninton. B. Christo, S. M. Karthigai Arasu
K. Arun Muthu Kumar & K. Deepak

Sathyabama University, Chennai

Abstract - Biofilm represents the most prevalent type of virulent factor of most of the pathogenic microorganism and involved in crucial development of clinical infection and exhibit resistance to antimicrobial agents. Now the biofilm is considered as major target for the pharmacological development of drugs. A biofilm serves to promote bacteria persistence by resisting antibiotic treatment and host immune responses. Antibiotics are rendered ineffective when biofilms form due to their relative impermeability, the variable physiological status of microorganisms, subpopulations of persistent strains, and variations of phenotypes present. Metal nanotechnology chemistry has the potential to prevent the formation of these life-threatening biofilms on life supporting devices. In the present study, anti biofilm effect of silver nanoparticles coated catheter against clinical isolate of Staphylococcus Aureus was studied. Silver nanoparticles synthesized by leaf extract broth of Azadirhacta indica were coated on the catheter chara-cterized by scanning electron microscopy which reveals complete dispersion of the nanoparticles on the fibre surface of the catheter and the size, shape of the particles shows uniform spherical particles with the size of 50-60 nm. Distinct effect of biofilm inhibition was recorded in the nanoparticles coated catheter and maximum inhibition was observed during 72 hour of incubation. Biochemical composition of biofilm matrix mainly total carbohydrates and total protein was highly reduced. The present study would suggests the development of anti microbial coated medical devices against pathogenic microorganism.

Keywords : biogenic silver nanoparticles, biofilm, catheter.

GJMR-B Classification : NLMC Code: QY 21, QW 90



Strictly as per the compliance and regulations of:



Anti Biofilm Effect of Biogenic Silver Nanoparticles Coated Medical Devices against Biofilm of Clinical Isolate of *Staphylococcus Aureus*

S. Karthick Raja Namasivayam^a, Beninton. B. Christo^o, S. M. Karthigai Arasu^p, K. Arun Muthu Kumar^o & K. Deepak[¥]

Abstract - Biofilm represents the most prevalent type of virulent factor of most of the pathogenic microorganism and involved in crucial development of clinical infection and exhibit resistance to antimicrobial agents. Now the biofilm is considered as major target for the pharmacological development of drugs. A biofilm serves to promote bacteria persistence by resisting antibiotic treatment and host immune responses. Antibiotics are rendered ineffective when biofilms form due to their relative impermeability, the variable physiological status of microorganisms, subpopulations of persistent strains, and variations of phenotypes present. Metal nanotechnology chemistry has the potential to prevent the formation of these life-threatening biofilms on life supporting devices. In the present study, anti biofilm effect of silver nanoparticles coated catheter against clinical isolate of *Staphylococcus aureus* was studied. Silver nanoparticles synthesized by leaf extract broth of *Azadirhacta indica* were coated on the catheter characterized by scanning electron microscopy which reveals complete dispersion of the nanoparticles on the fibre surface of the catheter and the size, shape of the particles shows uniform spherical particles with the size of 50-60 nm. Distinct effect of biofilm inhibition was recorded in the nanoparticles coated catheter and maximum inhibition was observed during 72 hour of incubation. Biochemical composition of biofilm matrix mainly total carbohydrates and total protein was highly reduced. The present study would suggest the development of anti microbial coated medical devices against pathogenic microorganism.

Keywords : biogenic silver nanoparticles, biofilm, catheter.

1. INTRODUCTION

Biofilms are universal, complex, interdependent communities of surface associated microorganisms. The organisms are enclosed in an exopolysaccharide matrix occurring on any surface, particularly aquatic and industrial water systems as well as medical devices. As such, biofilms are highly relevant for public health (Donlan and Costerton, 2002). Biofilm, likely the predominant mode of device related microbial

infection exhibit resistance to antimicrobial agents (Adonizio *et al.*, 2008). They can serve as hides for disease and are often associated with high level antimicrobial resistance of the associated organisms. Biofilms create an environment that enhances antimicrobial resistance. The EPSs of biofilms contain considerable amounts of polysaccharides, proteins, nucleic acids and lipids which are responsible for maintaining structural integrity of the biofilm and provide an ideal matrix for bacterial cell growth. Intermolecular interactions between the functional groups within these macromolecules serve to strengthen the overall mechanical stability of the EPSs and the survivability of the microorganisms. During the past 20 years it has been reported that between 6 and 14% of patients that enter general hospitals develop a nosocomial infection (Vazquez-Argon *et al.*, 2003), i.e., an infection that was not present or incubating at the moment of patient admission at a hospital. Over-all, a large percentage of biofilm-related infections are associated with indwelling medical devices: about 1 million cases- an estimated 60% of nosocomial infections are due to biofilms that have formed on indwelling devices (Darouiche, 2004). Medical implants that are more prone to biofilm formation include: artificial voice prostheses, replacement joints, prosthetic heart valves, cardiac pacemakers cerebrospinal fluid shunts, endotracheal tubes, urinary catheters, contact lenses, dental implants (Hall-Stoodley *et al.*, 2004; von Eiff *et al.*, 2005). When a biofilm infection occurs, the microorganism establishes an immune response to antigens released from the biofilm.

The main microorganisms responsible for biofilm formation on indwelling medical devices are: Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus viridans*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) bacteria as well as yeasts (Davey and O'Toole, 2000). *Candida* species are in fact emerging as important nosocomial pathogens, and approximately 80% of patients with candidemia possess a CVC, a fact

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which highlights the importance of yeasts from *Candida* spp., as common causes of CVC related infections (Ben-Ami *et al.*, 2008). Inhibition of biofilm is considered as drug target and the pharmacological inhibition of biofilm development is now extensively studied for the treatment of various bacterial and fungal infections (Karthick Raja Namasivayam and Allen Roy, 2013). In the present study, anti biofilm effect of biogenic silver nanoparticles coated catheter against clinical isolate of *Staphylococcus aureus* has been discussed.

II. MATERIALS AND METHODS

a) Synthesis of Biogenic Silver Nanoparticles

Biogenic silver nanoparticle was synthesized from leaf extract broth of *Azadiracta indica* (neem) 100gm of dried leaf material was homogenized finely in domestic mixer and 1gm of homogenized material was dissolved in 100ml deionised water and filtered through crude filter paper 50ml of collected filtrate was transferred to 100 ml of beaker and 100ml of 0.1mM silver nitrate was added and the preparation was kept under magnetic stirrer. Conversion of reaction mixture from pale green to dark brown indicates synthesis of silver nanoparticle and further conformation was carried out by uv- visible spectroscopy, Scanning Electron Microscopy and Energy Dispersive Atomic spectroscopy (EDX) the characterized particle was used for further studies.

b) Bacterial Stains

Clinical isolate of *Staphylococcus aureus* was obtained from Madurai medical college hospital, Tamil Nadu, India. Bacterial strain was maintained on slope of nutrient agar slant. (Hi media, Mumbai). Nutrient broth was used for inocula preparation. Cultures was inoculated from fresh slopes and incubated with shaking at 37°C for 24 hours. Cells were collected by centrifugation and the collected cell debris washed twice in phosphate buffer saline and suspended to OD520 prior to use in biofilm experiments.

c) Biofilm Inhibition Assay

Biofilm inhibition carried out in 96 well plates adopting modified method of biofilm spectrophotometric assay (Toole and Kolter, 1998). 100µL of cell suspension of the strain thus prepared was added in to 96 well time plate and different concentration of nano particle added and incubated at 37°C for three days after the incubation the liquid culture was removed and 100µL of 1% weight/ volume aques solution of crystal violet was added. Following staining at room temperature for 30 minutes the dye was removed and wells were washed thoroughly, 95% ethanol was added and incubates for 15 minutes the reaction mixture was read spectrophotometrically at 590 nm. Biofilm inhibition (%) was calculated by the following formula

$$\% \text{ of inhibition} = \frac{\text{OD in control} - \text{OD in treatment}}{\text{OD in control}} \times 100$$

Catheter was obtained from local medical shop (romo10) the catheter was cut in to 1x1 cm² surface and the cut pieces (5 nos) were transferred to a beaker containing 20mL of silver nanoparticles suspension with 100µg concentration kept in ultrasonicator for three hours at room temperature, Coating of nanoparticles was confirmed by color change of the catheter surface fine dispersion of particle by scanning electron microscopy and Fontier transform infra red spectroscopy (FTIR) these pieces were used for biofilm inhibition study.

d) Biofilm Inhibition Study

The cut pieces was transferred to a test tube containing 5mL of 24 hour culture, the inoculated tubes were kept in 37°C for 3 days (72 hrs) after the incubation period the whole content was aspirated and 5mL of 1% crystal violet was added and incubated at room temperature for 10mins. Crystal violet was removed and successive washing was made using sterile phosphate buffer saline to remove unbound cells or free planktonic cells. After washing, 5mL of ethanol was added kept at room temperature for 15 minutes the reaction mixture was read at 590 nm and the biofilm inhibition was determined as described earlier.

III. EVALUATION OF BIOCHEMICAL COMPOSITION OF BIOFILM MATRIX

a) Isolation of Biofilm Matrix

Isolation of biofilm matrix material from the microtitre plate and catheter was carried out by standard method. Adherent biofilms were transferred to screw cap bottles containing 10 ml distilled water. The bottles were sonicated for 5 min in an ultrasonic water bath and vortexed vigorously for 1 min to disrupt the biofilms. Cell suspensions were then pooled and centrifuged. The collected supernatant used as source for studying biochemical composition mainly protein by Lowry *et al*/and total carbohydrate by Dubois *et al*.

IV. RESULT AND DISCUSSION

Biogenesis of silver nanoparticle from leaf extract broth of *Azadiracta indica* was primarily confirmed by colour change of the reaction mixture from green to brown, plasmon absorption maxima at 420nm by U.V spectrophotometer (Figure 1). Particles morphology was studied by Scanning electron microscopy (SEM). SEM images were recorded by using a Carlzeiss Supra 55 field emission scanning electron microscope equipped with an energy-dispersive spectrum (EDS, oxford instruments) capability. In a SEM setup, the nanoparticulate sample, coated to be conductive (e.g.

gold, palladium), is scanned in a high vacuum chamber with a focused electron beam. The scanning electron microscopy study reveals uniform spherical particles with the size of 50-60nm and the presence of silver in the reaction mixture was further confirmed by EDAS.

Biofilm inhibition study clearly revealed all the tested concentration inhibited biofilm of *Staphylococcus aureus*. Results were represented as inhibition percentage of biofilm development (Table 1). In microtitre plate assay, anti biofilm effect was observed as dose dependent manner. As presented in table 1, silver nanoparticles with 100µg/ml recorded maximum anti biofilm effect with 84.0 followed by 75.5, 69.0, 59.4 and 42.1 % inhibition at the respective concentration.

Coating of biogenic silver nanoparticle was easily identified by color change of catheter (Figure 3) dispersion of nanoparticle on the catheter surface was confirmed by scanning electron microscope which reveals the uniform spherical particles were embedded on the catheter surface with the size of 50 to 60nm (Figure 4). Frontier transform infra red spectroscopy (FTIR) reveals the characteristic changes in the vibrational peaks of coated and non coated catheter (Figure 5). Biofilm inhibition study revealed 87.0 % inhibition during 72 hours of incubation period. Surface topography with SEM reveals complete degeneration of biofilm with weakened cell masses (Figure 6). Similar anti biofilm effect of chemogenic silver nanoparticles coated catheter against clinical isolate of *Staphylococcus aureus* has been reported (Karthick Raja Namasivayam *et al*, 2012). Biochemical composition of biofilm matrix total carbohydrate and total protein was also highly reduced. The matrix is one of the most distinctive features of a microbial biofilm. It forms a three dimensional, gel- like, highly hydrated and locally charged environment in which the microorganisms are largely immobilized. Matrix-enclosed micro colonies, sometimes described as stacks or towers, are separated by water channels which provide a mechanism for nutrient circulation within the biofilm the composition of the matrix varies according to the nature of the organism and reduction of the biochemical composition of the biofilm matrix leads to weakening of the biofilm thus facilitate entry of the drugs. In respective concentration of nanoparticles treatment, 70.0, 57.0, 31.9, 14.5 and 8.5 µg of total carbohydrates was recorded under microtitre plate assay Similarly, 79.0, 45.0, 30.0, 22.0 and 17.5 µg of protein were recorded. Similar reduction of carbohydrate as 5.0 and 13.0 µg of protein was observed in nanoparticle coated catheter (Table 2).

V. ACKNOWLEDGEMENT

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Figure 1 : UV vis spectra of synthesized silver nanoparticles

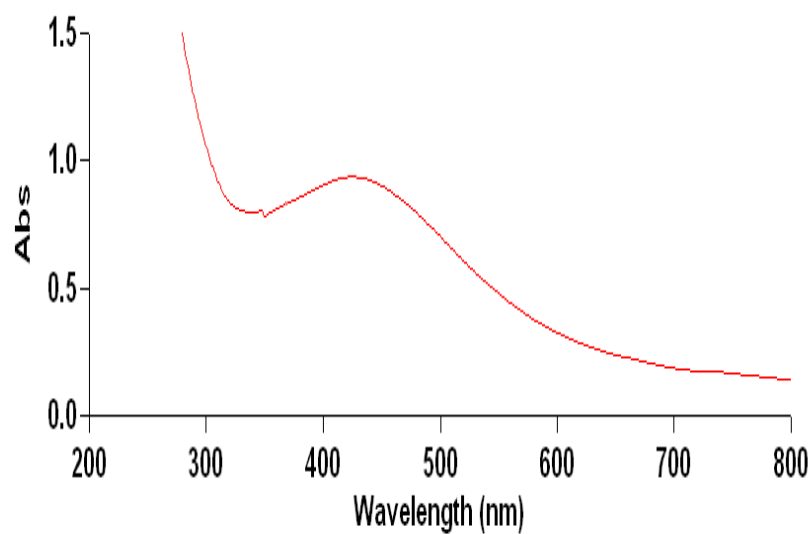


Figure 2 : SEM image of synthesized nanoparticles

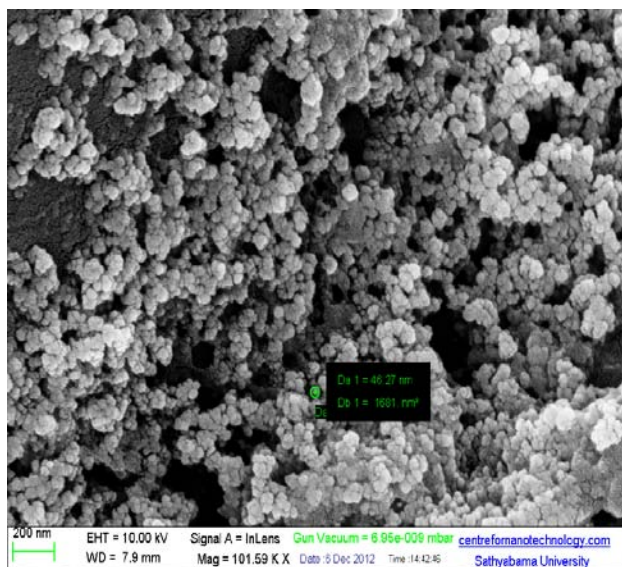


Figure 3 : Silver nanoparticles coated catheter



Figure 4 : FTIR spectra of un coated coated catheter

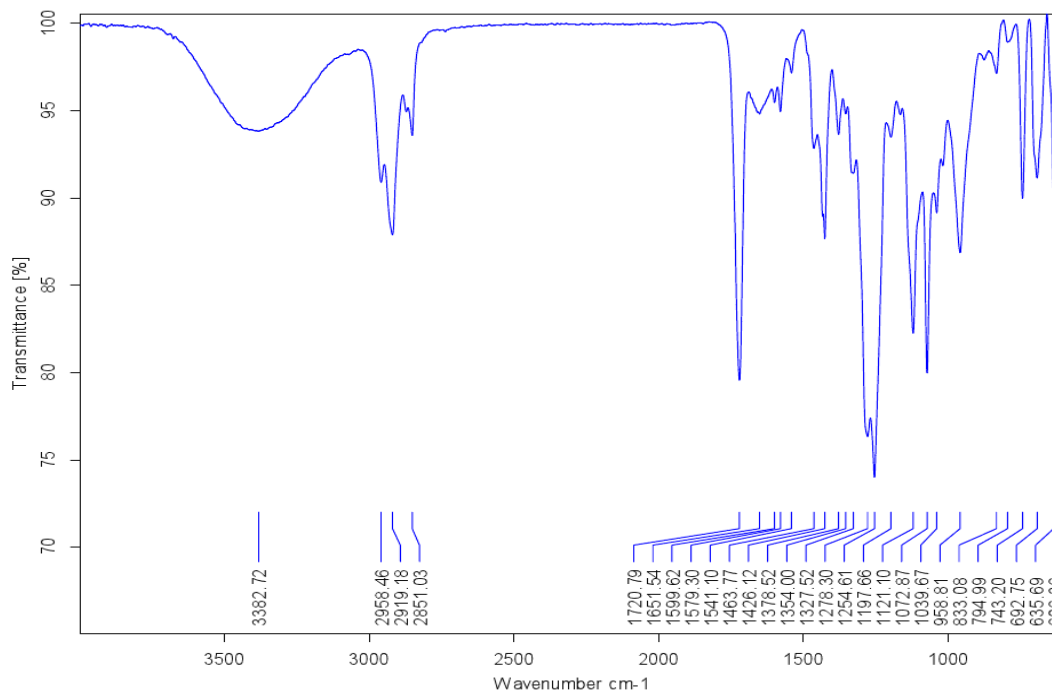


Figure 5 : FTIR spectra of silver nanoparticles coated catheter

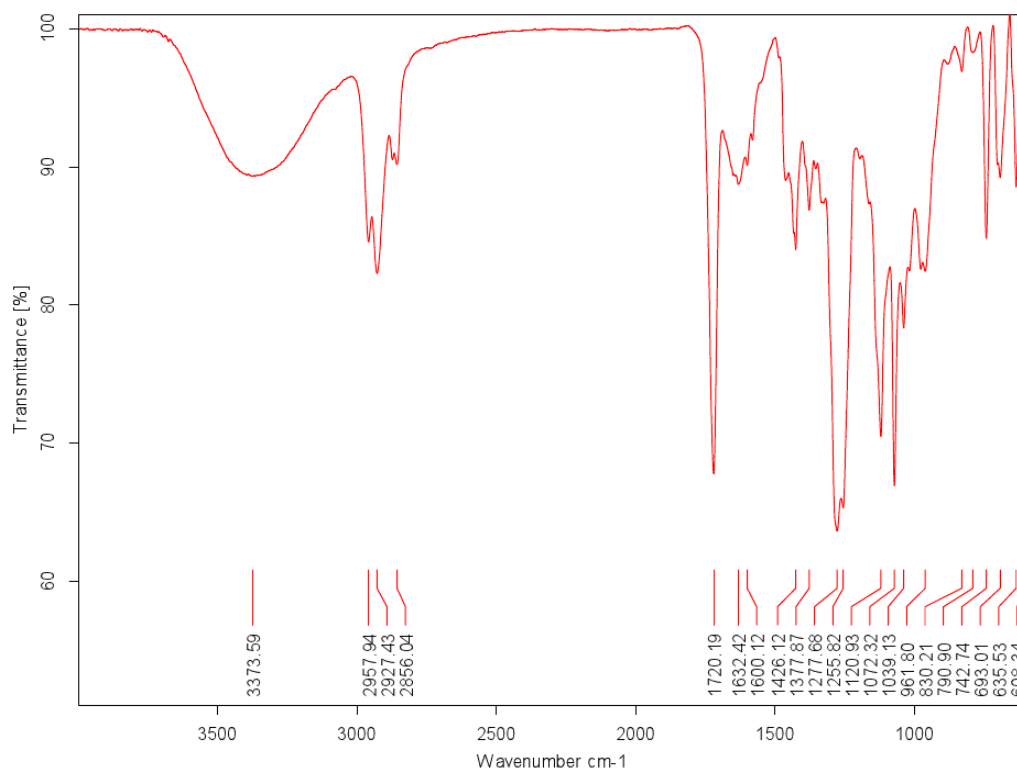


Figure 6 : SEM image of loose cell disrupted *Staph.aureus* biofilm on the silver nanoparticles coated catheter

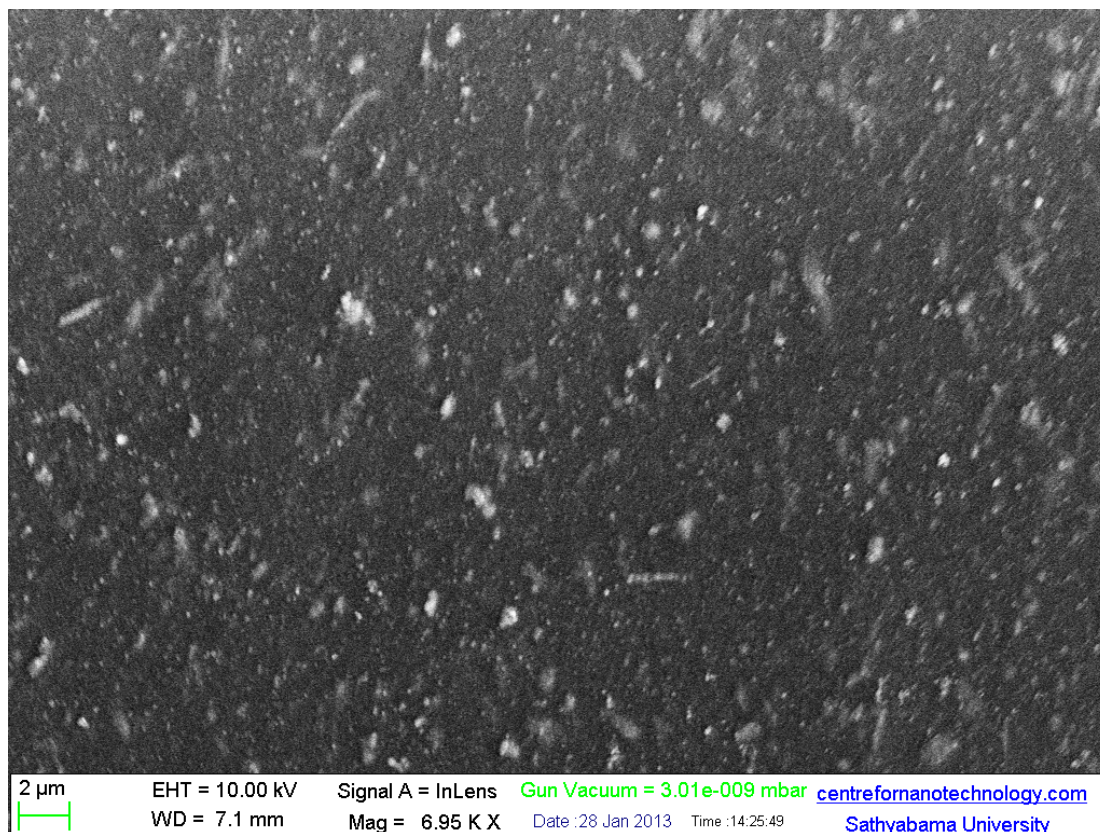


Table 1 : Biofilm inhibition (%) of *Staphylococcus aureus* by nanoparticles

S.No	Concentration (µg)	Biofilm inhibition (%)
1	10	42.1
2	25	59.4
3	50	69.0
4	75	75.5
5	100	84.0

Table 2 : Effect of nanoparticles on biochemical composition of biofilm matrix of *Staphylococcus aureus*

S.No	Concentration	Total Protein (µg)	Total carbohydrate (µg)
1	10	79.0	70.0
2	25	45.0	57.0
3	50	30.0	31.9
4	75	22.0	14.5
5	100	17.5	8.5
6	Catheter	13.0	5.0

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