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Evaluation of Poly Hexade Cyanoacrylate (PHDCA) Nanoparticles with Enhanced Antimicrobial Potential

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Abstract

The present study focuses on the development of nanoparticle system for antimicrobial drug delivery. Nanoparticles particular have some unique physicochemical properties such as ultra-small and controllable size, large surface area to mass ratio, high reactivity and functional sable structure .This properties can be applied to facilitate the administration of antimicrobial drug. In this experiment we have evaluated the poly hexed cyanoacrylate (PHDCA) nanoparticle and studied its antimicrobial activity. In the experiment firstly PHDCA was dissolve in acetone then instant precipitation was occurred and then ultracentrifugation was done. After adding the distilled water PHDCA nanoparticles were observed. Nanoparticles tracking analysis confirmed the mean particle size for the entire nanoparticle within the range of 10nm to 54nm .The sequential reduced PHDCA nanoparticle incorporated in core shell arrangement were detected at 324nm nm with the aid of UV-Visible spectrophotometer. Poly hexade cyanoacrylate nanoparticle has gram negative charge and it inhibit/ kill the growth of the gram positive bacteria. The maximum lethality of nanoparticles was observed for *Streptococcus pneumonia* followed by *Staphylococcus aurous* while *Escherichia coli* found to be least susceptible organism for the PHDCA nanoparticle and for the bacteria *Pseudomonas aeruginosa* has no effect of PHDCA nanoparticle on it.

Keywords: Antibacterial, lethality, microorganism, nanotechnology

Introduction

Recently nanotechnology has become breathtakingly important in the pharmaceutical areas as alternative. Antimicrobial strategy due to re-emergence infectious disease and the appearance of antibiotic resistance strain especially within gram negative micro-organism NPs are typically smaller in size and biocide effectiveness is suggested to be owning to a combination of their small size, it have large surface to volume ratio, which enable interactions with microbial membrane. Emergence of infectious diseases in generous possess a serious threat to public health worldwide, especially with the

emergence on antibiotic resistant bacteria strain. Generally, both gram positive and gram negative bacterial strain are thought to present a major public health problem. Antimicrobial activity is related to compound that locally kill bacteria or slow down there growth, which is general toxic to surrounding tissue. PHDCA NPs is an effective antimicrobial agent against gram positive bacteria, perhaps action against the bacterial cell, similarly poly hexade cyanoacrylate monomer applied on to the *Streptococcus pneumonia* (both gram positive).possible explanation to the higher sensitivity of the gram positive bacteria might be the strong electro negative charge on the PHDCA monomer that react with the carbohydrate capsule of gram positive organism which have positively charge. While PHDCA have less effect on gram negative, PHDCA monomer in very less proportion did kill *E.coli* bacterium as compare with the *staphylococcus aureus* as it possess gram positive charge .One factor that can influence the tolerance of bacteria against PHDCA NPs is the rate of bacterial growth. Fast growing bacteria are susceptible than slow growing bacteria to antibiotics and NPs. This PHDCA NPs disrupt the integrity of the bacterial membrane by attaching to the membrane of bacteria by electrostatic interaction. Nano toxicity is generally triggered by the some reaction like induction of oxidative stress by the formation of free radical i.e. ROS, following the administration of NPs. PHDCA NPs are synthetic glues that have recently been many application due to several advantages such as colourlessness and transparency, fast curing property without any equipment at room temperature and high bond strength. In recent year Nano material receive more and more attention for their superior characteristics, there is to modify substance had gradually a hot research. The adhesive property of PHDCA NPs is due to they quickly form a strong polymer bond upon contact with weak bases and water, and solidify. By the polymerisation reaction PHDCA can bind to skin surface. Tissue adhesive has one important property which has a good tensile strength, are bactericidal and bacteriostatic, have negligible histotoxicity and they pill of spontaneously.

Materials and methods

Poly-hexa de cyanoacrylate was received from Hi bond chemical, Mumbai. Acetone and distilled water were received from Ganesh scientific lab, Amravati.

Synthesis of PHDCA Nanoparticles:

Typically, for the controlled nanosphere, 300mg of PHDCA was dissolved in slightly warm 15ml acetone. The organic phase was poured rapidly through a syringe in 30ml milliq water under magnetic stirring. Nano sphere precipitation occurs instantaneously. The acetone was evaporated under reduce pressure. The colloidal suspension of Nanosphere was purified by ultra-centrifugation (12000 RPM, 145, 00g, 4°c) for 1.5 hr and the resulting Nano sphere pellet was suspended in distilled water under reduced pressure. The final suspension obtained by filtration through a sintered glass membrane (millexap 20 Millipore)

UV-Visible absorbance spectroscopy:

The reduction of PHDCA was confirmed by subjecting diluted aliquots of the poly- hexa de cyanoacrylate, poly- hexa de cyanoacrylate-acetone NPs to UV-Visible spectrophotometer (model-shimadzu UV-1700, Japan) in the range of 300nm to 800nm.

Nanoparticle tracking analysis (NTA):

Nanoparticle tracking analysis was performed through Nano sightLM 20 (UK) to measure the size of the NPs. For these, NPs samples (5µl) were diluted with distilled water (2ml) and injected onto the

sample chamber and observe through camera fixed with the instrument.

Fourier transforms infrared spectroscopy studies (FTIR):

Is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. For determination of surface functionalities of PHDCA nanoparticles, FTIR studies were performed on Perkin-Elmer FTIR -1600, USA. Sample was prepaid by mixing potassium bromide powder (10mg) with purified nanoparticle. Potassium bromide powder has functioned to dried and remove the moisture content.

Antibacterial activity of PHDCA nanoparticle:

Antibacterial activity of PHDCA NPs against some pathogenic bacteria including *Staphylococcus aureus* (ATCC-33591), *Escherichia coli* (ATCC14948), *Streptococcus pneumonia* (ATCC-49619), and *Pseudomonas aeruginosa* (ATCC-27853) were assayed by disc diffusion method and evaluated for minimum inhibitory concentration (MIC).

Disc diffusion assay:

The standard Gentamycin loaded antibiotic discs were purchased from HI-media, Mumbai, India.Kierby-Bauerdisc diffusion assay was performed on Muller Hinton agar plates against *S.aureus, P.aeruginosa, E.coli, and S.pneumoniae*. A single colony forming unit of individual test organism was grown over night in Muller-Hinton broth at 37°c .100µl of inoculums (approx. concentration $1X10^5$ CFU /ml) of each bacterium was streaked on to the agar plate and then standard and prepaid disk impregnated with PHDCA NPs, PHDCA-acetone NPs with standard antibiotic disc were place onto the agar surface. The prepared disc with acetone was place as a control upon incubation at 37°c for 24hr, the zone of inhibition were measured in terms of mm.

Determination of MIC of PHDCA Nanoparticles:

Broth dilution method was used to determine MIC of PHDCA NPs against bacteria .The inocula from the broth containing 1×10^2 CFU/ml was taken into wells of microtiter plate accordingly ,to make the final cell number up to 1×10^6 .To these 8 wells ,PHDCA NPs concentration is 2,4, 6, 8 and 60µg ml^{-1}against bacteria were added. According to these concentrations, the stroke solution of broth was added to the wells containing test organism .for the visual observation, the microtiter plates were incubated at 37°c for 24hr. On completion of incubation period, 40µl of tetrazolium salt prepared in distilled water to each micro well and observe no colour changes.

Result and discussion

UV-Visible Spectrophotometry:

Nanoparticles play an important role in changing the entire properties of materials due to its size. The most widely technique used to examine the optical properties of Nano sized particles is UV-visible absorption spectroscopy. The primary reductions of poly-hexa de cyanoacrylate ion into PHDCA nanoparticle were primarily detected by UV-Visible spectrophotometry as shown in figure 1. The transformation of ion into nanoparticle was further confirmed by UV-Visible spectra with strong absorbance peak at 324nm.This peak indicate that nanoparticles formation was take place at 324nm.

Nanoparticle tracking analysis (NTA):

Nanoparticle tracking analysis reveals size of nanoparticles by analysing and tracking the Brownian motion of freely suspended particle in colloidal solution. The rate of movement is related only to the viscosity and temperature of the liquid;

particle density or refractive index has no influenced on it. The approximately diameter 10-100nm which is a small particle for this NTAallows the determination of a size distribution profile in liquid suspension. Mean size of nanoparticles was calculated by tracking minimum 1000 nanoparticles active in Brownian motion. NTA revealed quite polydispersed population of PHDCA nanoparticle of mean size 40±54nm .The size histograms of PHDCA NPs are evident from fig-2(a,). Here the particle size vs. relative intensity 3D plot is shown in fig-(d).

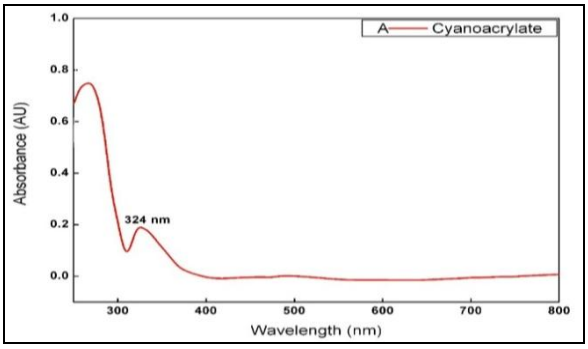

Fig. 1: UV-Visible absorbance spectra recorded for PHDCA NPs.

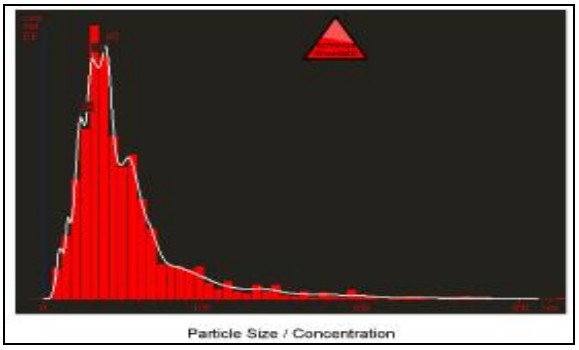

Fig. 2 (a)

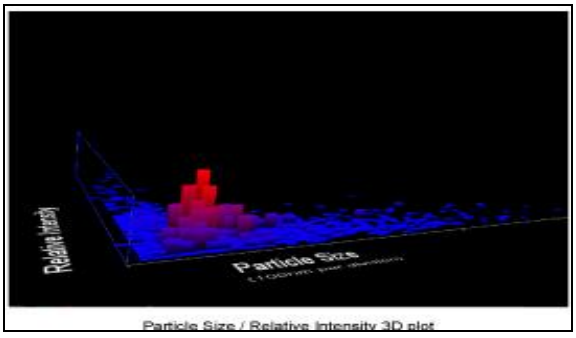

Fig. 2 (b)

Fourier transforms infrared spectroscopy studies (FTIR):

FTIR spectroscopic analysis was carried out to study chemistry of small molecules adsorbed on the surface of nanoparticles. As described in experimental section, centrifuged and purified nanoparticles solutions were used for FTIR analysis, which ensures elimination of interference from free biomass. FTIR spectra of purified PHDCA nanoparticle were analyse .The main peak that appear in spectra of Poly hexa de cyanoacryalate (fig. 3a) were at ~3270 cm^{-1} can be assigned to O-H (H-bonded) usually broad alcohol, phenol group. The weak absorbance peak appeared at ~1640 cm^{-1}can be assigned to C=C (symmetry reduced intensity) alkenes group. And the absorbance peak appeared at ~1089 cm^{-1} can be assigned to C-N amines group. The FTIR spectra of PHDCA nanoparticles (fig. 3b) was recorded some major peaks~1036.456~1084.527, ~1238.966, ~1370.714, ~1636.645, ~1998.378, ~2106.cm^{-1}.The band around~1036.456, 1084.527, 1238.966 cm^{-1} can be ascribed C-N stretch of aliphatic amines. The band around ~1370.714 cm^{-1} C_H rock of alkanes .The absorbance peak appeared at ~1636.645 cm^{-1} can be assigned to N-H bending of 1°amines or C=C- or aromatic groups . The weak absorbance peak appeared at cm^{-1}~ 2106.485 cm^{-1} can be assigned to alkynes C≡C stretching of aliphatic amines .The main band around~3284 cm^{-1} Can be assigned to can be assigned to C(triple bond) C-H:C-H stretch of alkynes terminal. And the band around at ~ 3326.262 cm^{-1} assigned to N-H stretch of primary, secondary amines, amides.It may be noted that some of the absorbance peak recorded for poly hexa de cyanoacrylate and all the nanoparticle remains almost unchanged hinting towards the role common surface functionalities as capping ligands. We suggest Alcohol and phenol abundantly present in poly hexa de

cyanoacrylate as the responsible bio constituents for reduction of metal ions into nanoparticle could be due to the strong binding of some of the functional groups over the surface of the nanoparticles including amine, alkenes.

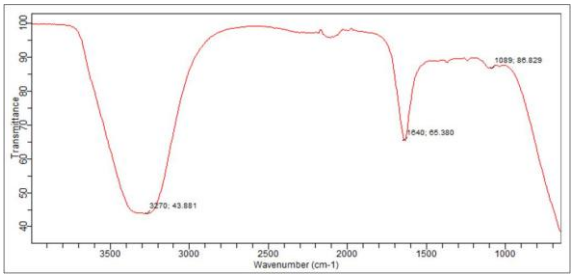

Fig. 3a

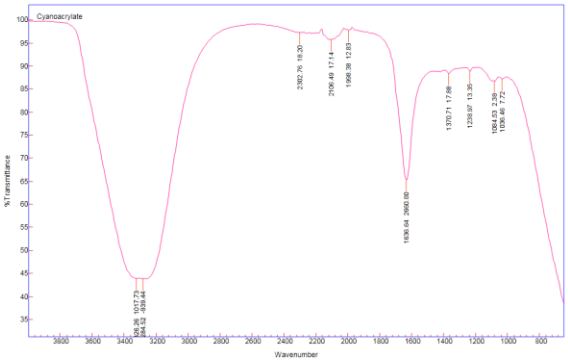

Fig. 3b

Antibacterial assay of PHDCA nanoparticles:

The growth inhibitory activity of PHDCA nanoparticle was assayed against *S.aureus, S.peumoniae, E.coli, and P.aeruginosa*. The values of zone of inhibition and minimum inhibitory concentration (MIC) of the NPs against tested organism are given in Table (1 and 2). The more or less equivalent growth inhibitory effect in terms of zone of inhibition was observed for PHDCA nanoparticles against all tested organisms (Table 1).

Minimum inhibitory concentration of PHDCANPs against bacterial strain:

The significant increase in the zone of inhibition was observed for PHDCA nanoparticles in combination with standard antibiotics. Among the tested bacterial strain, *S.pneumoniae* and *S.aureus* were found to be most sensitive to PHDCA nanoparticles resulting into growth inhibition at concentration 8µg ml^{-1}. Lowest MIC values were recorded for *P.aeruginosa* followed by *E.coli*. From the table 2 it is evident that, the activity of PHDCA nanoparticle was significantly accelerated when combination with standard antibiotics, the considerable synergistic antimicrobial effect of PHDCA nanoparticle and standard antibiotic was observed .The present study deals with the novel method, seed mediated growth technique for the synthesis of PHDCA nanoparticles. This is the first report of poly- hexade cyanoacrylate nanoparticles give antibacterial activity from our opinion. From the point of view of Nano biotechnology, this is an innovative approach towards synthesizing advanced nanomaterial's with control over their structure and surface properties.

Table 1: Antibacterial activity of nanoparticles.

Name of the strain	Diameter of zone of inhibition in mm			
	Control	PHDCA NPs	Ab	PHDCA NPs & Ab
S.aureus	Nil	11	17±0.5	20±1
S.pneumoniae	Nil	14±1	18	23
E.coli	Nil	10	14	16
P.aeruginosa	Nil	Nil	12	12

Note: (All values are significant at the 0.05% level of significance)

Table No. 2

Name of the strain	Minimum inhibitory concentration in$\mu g ml^{-1}$ (PHDCANPs)
S.aureus	6
S.pneumoniae	8
E.coli	20
P.aeruginosa	25

Note: AB, standard antibiotics (gentamycin)
(All values are significant at the 0.05% level of significance)

Conclusion

The present study reports sequential reduction reaction as a novel approach for bio fabrication of PHDCA nanoparticles. PHDCA nanoparticles reveal quite polydispersity as it shows particle size within the range of 10-54nm.This nanoparticle were evaluated for their antibacterial effect, which showed enhanced antimicrobial bioactivity against *S.pneumoniae* and *S.aureus*.The present study provides new possibility of synthesizing predetermined nanoparticles without using stabilizing agent's .In future these nanoparticles can be used as advanced nanomaterial's for catalysis, antimicrobial activity, and drug delivery purpose. These studies are really important in our fight against hospital acquired infections, which are prevalent due to steel clad surfaces where microbes can thrive.

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