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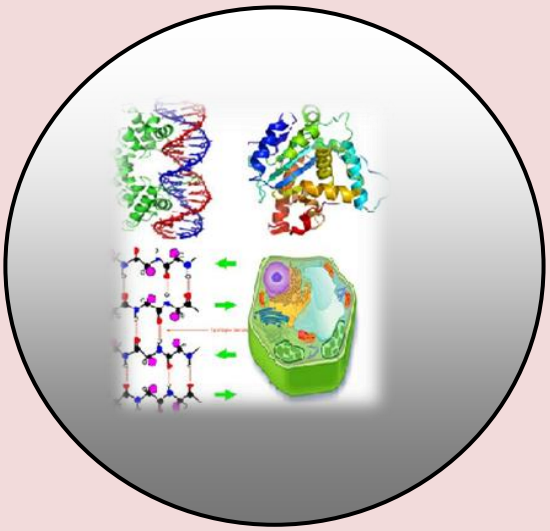

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RESEARCH PAPER

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Design, Synthesis and Biological Activity of α- and β-Cyclodextrin Dendrimers with Ciprofloxacin as Surface Moiety: A Study of Sustained Relax of Drugs

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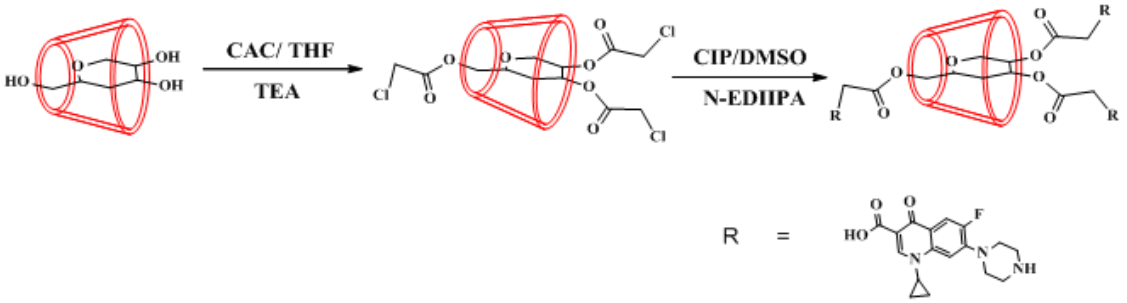
ABSTRACT

Novel biologically active macromolecule of α- and β-cyclodextrin drug delivery systems have been synthesized with ciprofloxacin drug as surface moiety in two steps for the sustained drug delivery demonstrations. Quantitative analysis of the omnipresent nature of ciprofloxacin drug formulations on different mode of drug release system is monitored using ultraviolet spectrophotometer at various physiological pH conditions. The results obtained undoubtedly evidenced that the drug ciprofloxacin release is pH dependant. Antibacterial performance of the spherical shaped macromolecule were investigated with Gram-positive and Gram-negative bacteria and the minimum inhibition concentration value of the title compounds were very lower at 100 µg,mL^{-1} when compared with standard ciprofloxacin which is at 500 µg,mL^{-1}. These compounds show nearly five times higher bactericidal activity than the standard ciprofloxacin. The structures of the synthesised α- and β-cyclodextrin giant molecules were characterized by means of MALDI-TOF, infrared, H^{1} and C^{13} NMR. The in-vitro studies and ciprofloxacin discharge from the spherical shaped macromolecules were also investigated.

Keywords: Ciprofloxacin, Cyclodextrin, Triethyl Amine, Dimethyl Sulfoxide and Tetrahydrofuran.



INTRODUCTION

Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quino- linecarboxylic acid) is a second-generation synthetic antibiotic of the fluoroquinolone antibacterial drug and most broadly prescribed in Europe (Ferech Scoenen et al. 2006, Drusano et al. 1986, Nelson et al. 2007, Oregon State University 2011). This is medicinally used for chronic bacterial prostatitis (recommended as a first-line antibiotic choice) (Schaeffer et al. 2004) lower respiratory tract infections (Zuger, 1998 and Vardakas et al 2008) skin and skin

structure infections, bone and joint infections and infectious diarrhea (Donaldson et al. 1994). A large number of 1-alkyl-7-(dialkylamino)-6-fluoro-1, 4-dihydro-4-oxoquinoline-carboxylic acids with various substituent at the phenyl ring are used as well-tolerated drugs in therapy (3rd-generation fluoroquinolone antibacterials; quinolone-quinolinone). Reactive arthritis (ReA) easily affected part of the body is heels, toes, low back, finger and joints. Aseptic arthritis which is the inciting infection of the gastrointestinal or genitourinary tract and this may be difficult by the development of recurrent acute anterior uveitis (AU). Different types of microorganisms responsible in the pathogenesis of ReA and AU (Valtonen et al. 1985; Wakefield et al. 1991). Although synovial fluid cultures are generally negative, several research groups have newly prepared bacterial antigens or known bacterial antigens (Granfors et al. 1989) bacteria-specific antibodies (Maki-Ikola et al. 1992) and cytotoxic CD81 T cells (Hermann et al. 1994) as well as polymerase chain reaction evidence of microbial (chlamydial) nucleic acid (Bas et al. 1995; Taylor-Robinson et al. 1992) in the synovium and joint fluid of patients with ReA. Several studies are explaining the role of antibiotics in ameliorating the usual course of ReA, but their findings have been inconclusive (Taylor-Robinson et al. 1993). The worth of ciprofloxacin in the treatment of ReA and AU, its broad range of activity against microorganisms with acceptable side effect profile and tissue-penetrating ability it is worthy to be extended in the level of its compound. The synthetic organic chemist and pharmaceutical scientists are at present concerned in developing an ideal sustained delivery system, which should have improvement of single dose for entire duration of the treatment and it be supposed to deliver the drug directly in specific infection site. The increased technical hitches like inevitable rise and down in the drug concentration in multiple dosing of conventional dosage and cost involved in the promotion to new drug entities, has gives greater attention on improvement of sustained delivery or controlled drug released systems called nanomedicine (Gwen et al. 2013). These controlled delivery systems contain a numeral of advantages over traditional drug systems such as improve the biological efficiency, reduced toxicity and improved patient convenience. The widely used matrix system for the purpose of sustained drug release is in fact prolongs and the controlled release of the drug from a well-mixed complex of one or more drugs with hydrophilic polymers.(Salsa et al. 1997). The main goal of controlled drug delivery systems is to improve the effectiveness of drug therapies.(Remington. 2006; Brahmankar et al. 1995; Lee et al. 2007; Brannon-Peppas et al 1999). Cyclodextrin is exclusive composite with lipophilic inner hollow space and hydrophilic outer surface that resembles a molecular storage place which holds non polar and non ionic guest molecules in its inner cavity called inclusion complex which change unique property (enhanced solubilization capacity) on guest molecules. This prospective lead has been neglect in many investigations of drug administration through different routes (Loftsson et al. 1996). This in fact explains that the adaptation of guest molecule within the inner cavity of CD depends upon polarity, size of the guest molecule and also the size of inner cavity (Morrison et al. 1996). Synthesized drug delivery system antibacterial activity analyzed by disc diffusion and minimum inhibition concentration (Ramya et al. 2017). Based on the previously mentioned findings, the aim of this research is to design and synthesize new and novel sustained drug delivery systems using ciprofloxacin and core α- and β-cyclodextrine. The biodegradable synthetic polymers are designed with well defined shape, monomer physicochemical properties and for long time activities. Cell based assay methods are important part of the synthetic organic field due to assess the ability of biological activity. In this study, we examined the drug release nature of omnipresent ciprofloxacin drug delivery system using Staphylococcus aureus(mtcc 737), (Gram positive) and Escherichia coli (mtcc-443),(Gram negative) microorganism. Synthesized polymeric giant molecules are expected to inhibit their growth patently due to the process sustain drug delivery.

EXPERIMENTAL SECTION

Newly purchased chemicals are used as it is to synthesis the title target compound. 3-(4, 5-dimethyl-2-thiaozolyl)-2, 5-diphenyl-2H tetrazolium bromide (MTT) was purchased from Sigma chemical. The synthesized spherical shaped macromolecules were purified and structures of the α- and *β*-cyclodextrin polymeric molecule were elucidated by means of MALDI-TOF, IR, ^{1}H and ^{13}C NMR and spectral studies. The IR spectrum of the newly synthesized drug delivery system were recorded by KBr pellet method, the vibrational spectra ranging from 400-4000 cm^{-1} using AGILENT CARRY 650 spectrometer with global source, Ge/KBr beam splitter and a MCT detector. All the frequencies sharp bands are accurate to ± 2 cm^{-1}. Nuclear magnetic resonance spectrum were performed using BRUKER AVANCE III AMX -400 spectrometer (^{1}H and ^{13}C NMR spectra were recorded at 293K) operating with the frequencies of 400 MHz for proton and 100 MHz for carbon using DMSO-d6 as solvent. Samples were prepared by dissolving about 5mg of sample in 0.5 mL of DMSO-d6.

All NMR Measurements made using 10 cm NMR tubes and all the proton chemical shifts are reported in parts per million scales (δ scale) downfield from tetramethylsilane. MALDI-TOF mass spectra were performed by AB SCIEX TOF/TOF 5800. Sinapic acid was used as a matrix for the experiment and the matrix-sample ratio maintained at 100:1 throughout the experiment.

Synthesis of chloroacetyl β-cyclodextrin (compound-2) and chloroacetyl α-cyclodextrin (compound-5) and its spectral studies

The α-cyclodextrin 0.5g (0.001 mol) or β-cyclodextrin 0.5(0.0004 mol) and chloroacetyl chloride 1.0ml (0.01mol) were dissolved using tetrahydrofurane and refluxed for 4-6 hrs in the presence of a triethyl amine. After completion of reaction the mixture was poured in to ice water and the final product separated by low pressure distillation methods, compound used for further studies and characterization. Vibrational studies performed by Agilent carry 630 FT-IR spectrometer. Chloroacetyl chloride carbonyl group (νC=O) stretching frequency appeared at 1733cm^{-1} and absence of OH stretching frequency is conformed that all hydroxyl group of glucopyranose unit coupled with chloroacetyl chloride moiety.

Synthesis of β-cyclodextrin core drug delivery system (compound 3)

The chloroacetyl β-cyclodextrin 0.5g (0.2mol) and ciprofloxacin 1.27g (0.003mol) were dissolved using tetrahydrofuran and refluxed for 4-5 hrs in the presence of N-ethyldiisoprophyl amine. The product separated was filtered, dried and used for different characterization and biological studies. In the IR spectrum of synthesized drug delivery system the stretching frequencies of acetyl and cyclic ketone appeared at 1726 and 1629 cm^{-1} respectively. Similarly 3441 (ν-OH acid group), 1502 (νC=C aromatic ring), 3060 (νC=H aromatic ring) and 2852 - 2922 cm^{-1} (νC-H aliphatic) are also confirmed the title compound formation. ^{1}H NMR ($CDCl_3$, TMS, 27°C, 400 MHz), δ 3.33 (d, CH_2 protons of acetyl groups), δ 2.85-3.57 4H (d, CH_2 protons of piperidone ring). δ 4.07 2H (glucopyranose ring attached CH_2 protons), 15.03 (-OH Protons of acid groups) 1.86 (N-CH of cyclopropane), 1.22-140 (-$CH_{2\ of}$ of cyclopropane ring), 7.02- 8.76 (CH of aromatic protons) and 4.23 (CH of glucopyranose ring). ^{13}C NMR ($CDCl_3$, TMS, 27 °C, 100MHz), δ8.20 (CH2 carbons of cyclopropane in ciprofloxacin), δ 35.30 (N-CH carbon of cyclopropane ring). δ 49.62-52.57 (CH_2 carbon piperacine), δ 59.10 (CH_2 of acetyl group), δ 60.90 (O-CH_2 carbon attached with glucopyranose ring). δ 64.05 (CH of clucopyranose residue), δ 108.14-154.98 (CH of aromatic ring), δ 167.12 (C=O of acid group), δ 170.04(C=O of acetyl group) and δ 177.14 (C=O of six member ring). HSQC ($CDCl_3$, TMS, 27 °C,), δ 42.5 correlated with δ 4.11; δ 2.53 linked with δ 40.6; δ 207.1 and 170.1 are carbonyl carbon - no correlation; 8.28 ppm associated to 1.86 ppm; 52.57 ppm corlated with 2.86 ppm; 35.34 ppm corlated with 3.56 ppm; 49.67 ppm correlate with 3.47 ppm and 60.93 ppm correlated with 4.21 ppm. Physical properties such as melting point, λ-Max value, Colour, Nature, Micro analysis, Solubility, molecular weight are shown in the Table-1.

Synthesis of α-cyclodextrin core drug delivery system (compound 6)

About 0.5g (0.2 mole) of chloroacetyl α-cyclodextrin and 1.5g (4.5 mole) of ciprofloxacin were dissolved using tetrahydrofuran and refluxed for 4-5 hrs in the presence of N-ethyldiisoprophyl amine. The reaction mixture finally poured in to ice water. The product separated was filtered, dried and used to different charecterization and biological studies. Various stretching frequencies in IR spectrum authenticate that the different functional groups of α-cyclodextrine and ciprofloxacin combined to produce spherical shaped drug delivery system. Acetyl ketone and ciprofloxacin α, β unsaturated carbonyl group stretching frequencies appeared at 1721 and 1624 cm^{-1}. The aliphatic (C-H) stretching appeared at 2921 and 2851cm^{-1} is confirmed that the glucopyranose unit and methylene group of chloroacetyl chloride. 3054 cm^{-1} due to aromatic (C-H) stretching of ciprofloxacin and acid OH stretching frequency appeared at 3581 cm^{-1}. ^{1}H NMR (DMSO, TMS, 27 °C, 400 MHz) δ 1.21 and δ 1.32 are the signals of magnetically non-equivalent protons of cyclopropane CH_2 group, δ 2.75 piperazine ring CH_2 protons, δ 3.33 chloroacetyl chloride CH_2 protons, δ 3.81 to δ 4.12 glucopyranose ring protons, δ 7.88 ciprofloxacin eleventh carbon proton, δ 15.0 acid OH proton, δ 8.64 ciprofloxacin fourth carbon proton and δ 7.55 is due to ciprofloxacin 21st position proton. ^{13}C NMR (DMSO, TMS, 27 °C, 100 MHz) δ 8.0 propane ring CH_2 carbons, δ 36.3 piperazine ring CH_2 carbon, δ 14.6 cyclopropane ring CH carbon, δ 52.0 piperazine ring CH_2 carbon, δ 60.3 glucopyranose unit sixth position CH_2 carbons, δ 58.6 chloroacetyl chloride CH_2 carbon, δ 111.4 ciprofloxacin 21 th position CH carbon, δ 148.4 ciprofloxacin 4th position carbon and δ 106.8 is due to ciprofloxacin 21th position carbon. δ 152.2 fluorine attached ipso carbon, δ 139.6 ninth position ipso carbon, δ 170.3 chloroacetyl chloride carbonyl group, δ 166.4 Acid carbonyl group and δ 176.8 ciprofloxacin ring ketone.

In HSQC (DMSO, TMS, 27 ºC) δ 8.03 is correlated with δ 1.21 and 1.32 due to the magnetically non-equivalent protons on same carbon atom of cyclopropane CH_2 group. δ 35.8 correlated with δ 4.12 and δ 52.0 correlated with δ 2.75 is due to protons of piperazine ring two CH_2 groups.

The signal at δ 36.3 correlated with δ 3.81 due to another four protons correlation with two equivalent carbons. δ 60.3 correlated with δ 4.12 due to glucopyranose unit fourth carbon proton, δ 58.3 correlated with δ 3.33 is assigned to cloroacetyl chloride CH_2 group, δ 148.4 and 106.8 correlated with δ 8.64 and 7.55 due to the ciprofloxacin ring carbon and it's proton.

Table 1. physical properties of compound 3.

S. No	Physical Propertice	Values
1	λ-Max value	278 nm
2	Colours	Light yellow
3	Nature	Amorphous nature
4	Micro analysis calc / found	C = 59.29, H = 5.05, F = 4.47, N = 9.88, O = 21.31,
5	Solubility	CDCL3
6	Molecular weight	8933.97 g/ml

MALDI-TOF characterization of compound 3

MALDI-TOF MS was employed for the determination of molecular weights and the nature of end groups (Wachsen et al. 1997; Nielen. 1999) for the designed omnipresent natured ciprofloxacin drug delivery system. The most prominent peaks appeared are characterized by fragmentation of different units from the constructed compound 3. In total seven prominent peaks are appeared at 10984.92 Da, 8923.46 Da, 7129.15 Da, 6093.17 Da, 4220.62 Da, 3690.73 Da and 2573.56 Da, in that the molecular ion peak appeared at 8923.46 Da (equal to mother ion molecular mass) confirms the formation of title dendrimer. The expected molecular ion peak value is 8928.10 Da for the polymer. The monomer CIP-CAC mass is 372.35, on different fragmentation the twenty one CIP-CAC units with β-cyclodextrin through covalent bond are responsible for the peaks.

The molecular ion peak at 8923.46 Da corresponding to oligomers doped with H^+ of type $(C_{17}H_{17}FN_3O_3)_{21} – (C_2H_2O)_{21} – (C_{42}H_{49}O_{35})$. The fragment peak appeared at 7129.15 Da, corresponding to oligomers doped with H^+ ion of $(C_{17}H_{17}FN3O3)_{16} – (C_2H_2O)_{21} – (C_{42}H_{49}O_{35})$. The expected value for this fragment is 7272.81, the above values are confirmed that the ciprofloxacin fragment generated from the polymeric natured of giant molecule, the fragment value deviation may be due do removal of acetyl unit from ciprofloxacin.

The peak appeared at 6093.17 (expected value for the fragment is 6138.76) is corresponds to oligomers doped with H^+ fragment mass $[(C_{17}H_{17}FN_3O_3)_{13} – (C_2H_2O)_{21}– (C_{42}H_{49}O_{35})]$. The peak appeared at 4290.62 (expected value for the fragment is 4112.38) is corresponds to oligomers doped with H^+ fragment mass $[(C_{17}H_{17}FN_3O_3)_7 – (C_2H_2O)_{21} – (C_{42}H_{49}O_{35})]$. The peak ranging from 3690.73 Da, (expected value for the fragment is 3630.36) is corresponds to oligomers doped with H^+ fragment mass $[(C_{17}H_{17}FN_3O_3)_5 –(C_2H_2O)_{21}–(C_{42}H_{49}O_{35})]$. The peak appeared at 2573.56 (expected value for the fragment is 2639.97) is corresponds to oligomers doped with H^+ fragment mass $[(C_{17}H_{49}FN_3O_3)_2 – (C_2H_2O)_{21}– (C_{42}H_{49}O_{35})]$ Da.

MTT based cell viability assay for synthesized compounds

The MTT assay is performed by colorimetric methods for count the cell viability through increased metabolism of tetrazolium salt (Moshmann, 1983). Cultured *Escherichia coli* and *staphylococcus aureus* were taken into 96 well plates. Then the live cells were treated with various concentrations of the spherical shaped macromolecules (5, 15, 25, 50, 75 and 100µl) and ciprofloxacin (100 µl) were incubated at 37 °C for 12, 24, 48 and 72 hrs. The MTT (0.5 mg/ml) was added to the cells and then further incubated for another 3 h. The cells were centrifuged for 10 min and the supernatant was removed, 200 µl of dimethyl sulfoxide (DMSO) were added into each tubes. Absorbance was recorded in a microplate reader at 560 nm.

RESULTS AND DISCUSSION

Chemistry

Omnipresent nature of ciprofloxacin in narrower end primary (bonded to the C6 atoms) and broadest end secondary (bonded to the C2 and C3 atoms) hydroxyl groups in β-cyclodextrin core play an important role to design the spherical shaped macromolecule. In this, exactly twenty one piperazine of ciprofloxacin molecule react with twenty one chlorine groups of chloroacetyl β-cyclodextrin to form biodegradable ciprofloxacin dendrimer as the synthetic route is illustrated in Scheme 1.

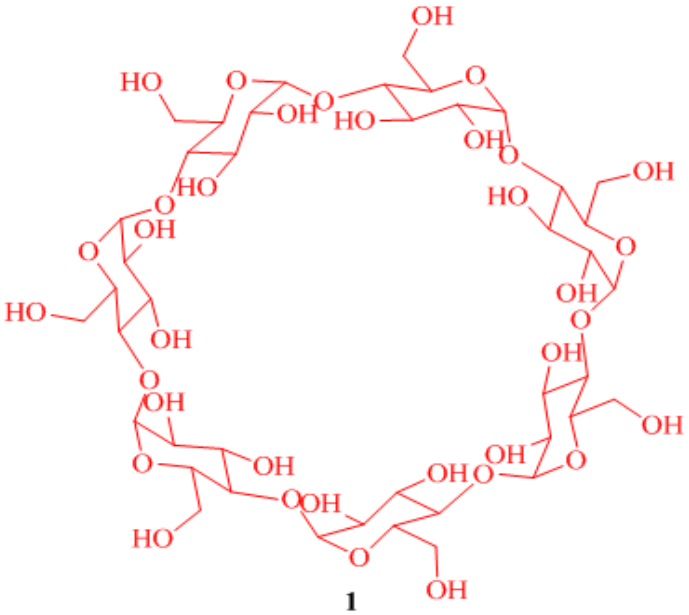
Scheme 1; Synthesis of chloroacetyl β-cyclodextrine(compound-2) and ciprofloxacin based drug delivery system(compound-3)

β-cyclodextrine (1) —CAC, N-EDIPA/THF, Reflux→ chloroacetyl β-cyclodextrine (2) —CIP, N-EDIPA/THF, Reflux→ β-cyclodextrine core dendrimer (3)

N-EDIPA - N-Ethyldiisopropylamine

CAC - chloroacetyl chloride

CIP - ciprofloxacin



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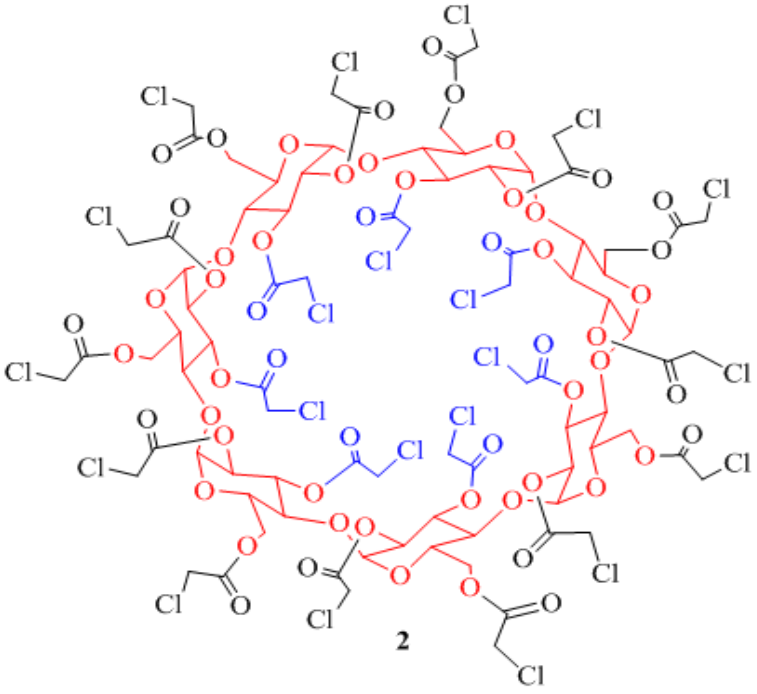

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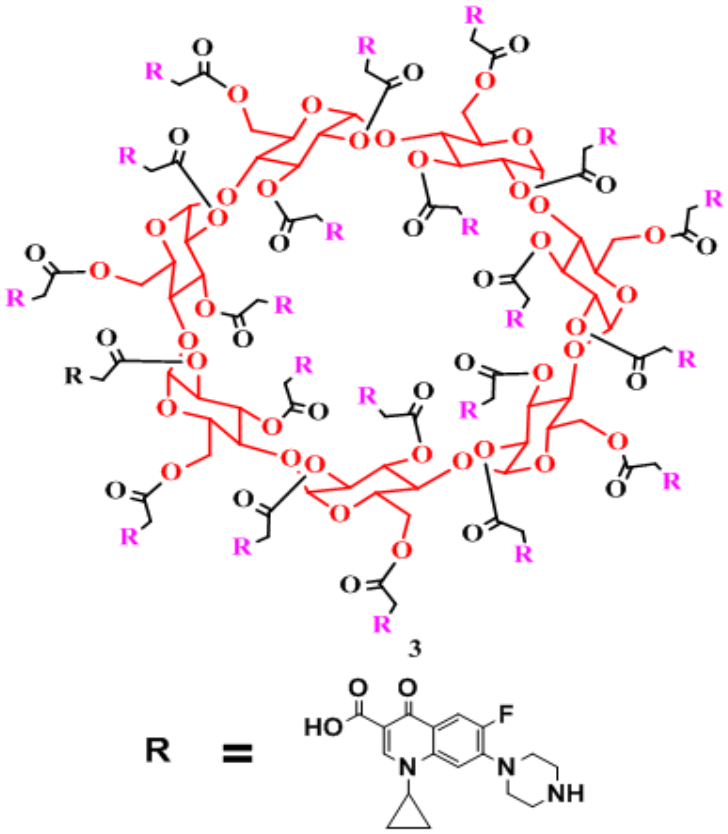

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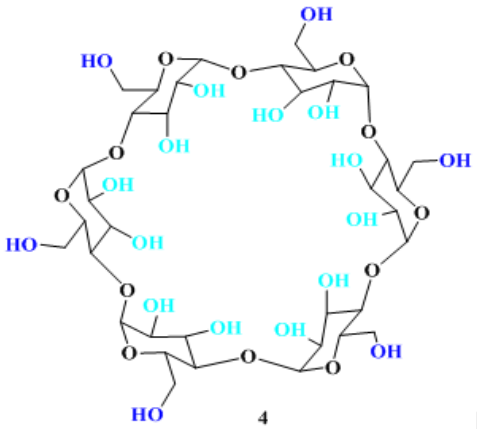
Figure 1. Chemical structure of β-cyclodextrine(1), chloroacetyl β-cyclodextrine(2) and β-cyclodextrin core dendrimer (3).

Scheme 2; Synthesis of chloroacetyl α-cyclodextrine(compound-5) and ciprofloxacin based drug delivery system(compound-6)

α-cyclodextrine (4) —CAC, TEA/THF, Reflux→ chloroacetyl α-cyclodextrine (5) —CIP, N-EDIPA/THF, Reflux→ α-cyclodextrine core drug delivery system (6)



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Similarly, the α-cyclodextrine glucopyranose units with eighteen primary and secondary hydroxyl groups used to design covalently bonded drug delivery system. The entire hydroxyl groups form ester link with chloroacetyl chloride, eighteen ciprofloxacin moiety performed drug delivery through the hydrolysis of ester bond and solubility of ciprofloxacin moiety. Piperazine N-H bond react with another terminal part of the chloroacetyl α-cyclodextrin. Biodegradable drug delivery system designed by eighteen ciprofloxacin moiety with α-cyclodextrine core.

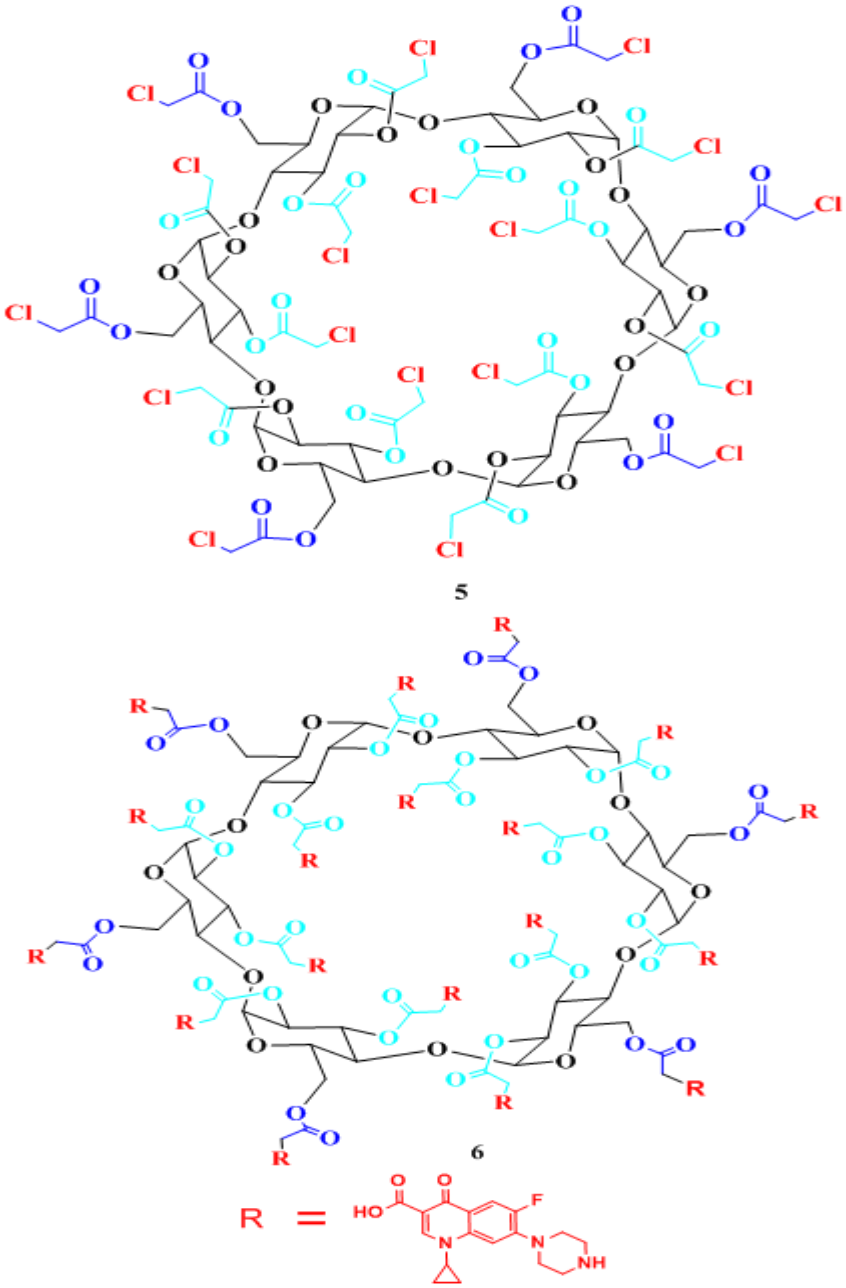

Figure 2. Chemical structure of α-cyclodextrine(4), chloroacetyl α-cyclodextrine(5) and α-cyclodextrin core dendrimer (6).

Mechanism of solubility

Solubility enhancement is important factor to improve desired therapeutic effectiveness and the concentration is important in systemic circulation for pharmacological response (Shine et al. 2007). The solubility of ciprofloxacin antibiotic determined by pH value, it is maximum insoluble in water and reveals highest solubility (40mg/mL) at pH 4–5, if the pH value is adjusted with hydrochloric acid (HCl). This is match up with the hydrochloride form of ciprofloxacin. However, the stability of the dry drug material is very high at moderate temperature. A few researchers were reported that the ciprofloxacin incorporated polymer structure and investigated their release studies (Tsou et al. 2005; Unnithan et al 2012). The release rates of ciprofloxacin from the spherical shaped macromolecules were determined by using absorption spectrometry.

Effect of *p*H and concentration bring effective change in properties and indeed, formulation development dependence the solubility factor, the maximum solubility of the drug help to significant formulations later in development.(Lipinski et al 1997; Prentis et al. 1988; Kim et al. 2004). Therefore screening and determining aqueous solubility is one of the most essential steps in drug discovery and development. Because of the solubility, effectiveness and permeability interrelate to persuade a compound's absorption; no single target solubility value can be defined for all compounds. Lipinski et al noted that from the commercially available drugs 87% drug solubility are greater than 65 µg/mL and this type of drugs have maximum bioavailability for an orally administered drug with a lowest concentration of 1 mg/kg if a drug has solubility greater than 65 µg/mL and average permeability. However, the bioavailability is likely to be inadequate if the solubility is less than 20 µg/mL. According to FDA protocols, the drug are soluble in 250 ml of aqueous solution at the pH range from 1 to 7.5, the drug are consider as a low soluble drugs. It is soluble less than the amount of aqueous solution the drugs are consider higher dosage. Also, according to Biopharmaceutical Classification System (BCS) guidelines, which is classified four types of drugs based on both solubility and permeability, the first class candidate have highest permeability, which greatly improve the bioavailability of drugs[42]. This class drugs is highly soluble (solubility N100 µg/mL, high permeability). Proper solubility capacity is performed during the drug discovery and expansion process and in fluoroquinolones that chemical reactions of the secondary amine in the piperazinyl ring. The synthesized polymeric structure of ciprofloxacin dendrimer retains comparable movement to the parent fluoroquinone, without the need of intracellular cleavage and release of antibiotic.

The spherical shaped polymeric compound solubility is strongly depends on *p*H and it is almost insoluble in water and alcohol. However, if we use buffer solution as solvent to inspect the solubility of the drug it is found to decreasing the *p*H range from 7 to 1 the drug solubility increases. In this study the buffer solutions are prepared from 0.1M HCl and 0.1M sodium bicarbonate solution and due to the high solubility of hydrochloride form of ciprofloxacin the pH value is adjusted with hydrochloric acid (HCl).

Sustain drug release studies of ciprofloxacin based α, β-cyclodextrin core drug delivery system (compound 3 and 6) in Different *p*H

The drug discharge activities are monitored by UV absorption spectrometry by following different intensity of drug in the presence of various *p*H of the solution. We may also gather information concerning influence of acidic and basic condition in drug release. Different *p*H solution prepared such as *p*H1, *p*H2 *p*H3, *p*H4, *p*H6 and pH7 and adjusted with 0.1M $NaHCO_3$ solution. The *p*H1 buffer solution is giving higher absorption intensity due to increasing drug solubility with increasing the acidity of buffer solution. The microbial evaluation obvious that the release of ciprofloxacin, antimicrobial activity and sustain drug delivery tendency of the spherical shaped drug delivery system was studied to reveal potential application as a drug delivery system. The result shows that the drug delivery system controls the release rate of the ciprofloxacin drug from the polymeric nature of giant molecule, which is controlled by the release medium. The development of a biodegradable ciprofloxacin system, based on omnipresent nature of ciprofloxacin, should be of great interest in drug delivery systems. Hydrolysis studies were carried out to evaluate the ciprofloxacin release from macrocycle α- and β-cyclodextrin under different conditions. The study was performed by incubation in different buffer solution of pH 1-7 and followed the *in-vitro* drug release. Drug releases is a hydrolytic degradation of dendrimer, it is involved the chemical scission at ester linkages by water molecules. The cumulative drug delivery of spherical macromolecule is shown in Figure 3. The ciprofloxacin based drug delivery system undergoes faster ciprofloxacin release at lower *p*H with respect to time. At *p*H-7, only 20% of drug was released in 14 hrs and about the 50–60% of drug has been released in *p*H-4 and 6. Mean while, at the *p*H-1 the maximum amount (98%) of drug was released within 14 hrs. At very lower pH the hydrolysis is extremely fast and percentage of drug discharge is increases rapidly. So the behavior of minimum quantity of drug can fully functionalized with in 14 hrs. The above results revealed that near Gastric fluid *p*H (1.5) the drug release is maximum and also in a controlled manner. Quantity of β-cyclodextrin core spherical molecule drug releases was studied with various pH conditions by a UV–Vis spectrophotometry (UV-1202 Shimadzu) at the absorption maximum of the free drug in aqueous buffered solutions (kmax 277 nm) using a 1 cm quartz cell. A standard curve was obtained by plotting concentration versus absorption. Drug releases rout of twenty one ciprofloxacin molecules containing in the spherical molecule surface are illustrated in the Figure 3. In conclusion, the discharge rate is very much reliant on the ester segment in the polymer and *p*H of the medium. Kinetics of the biodegradation of polyester is still under investigation and we intend to present those findings in a future report. The cumulative drug delivery of α-cyclodextrin core polymeric giant molecule is also shown in Figure 4.

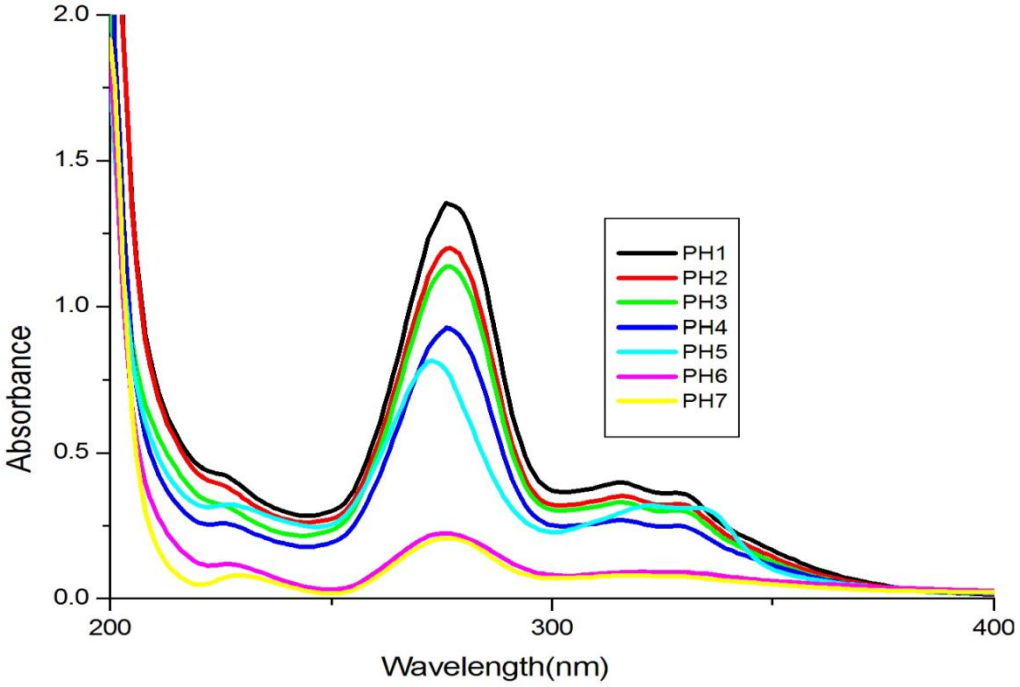

Figure 3. Cumulative % release of ciprofloxacin from β-cyclodextrine core dendrimer (3).

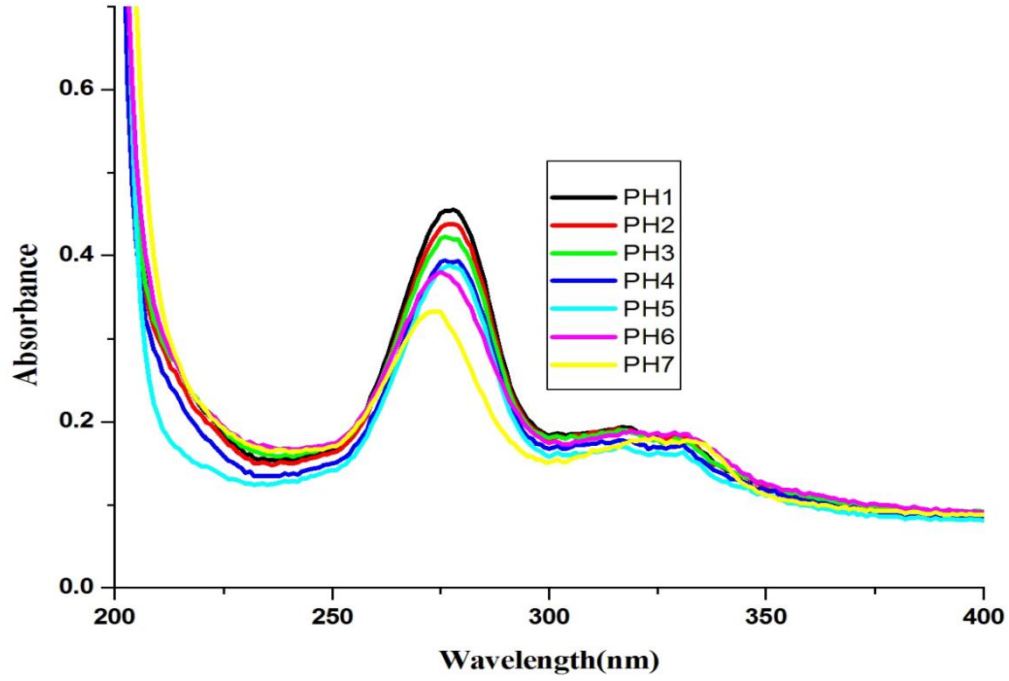

Figure 4. Cumulative % release of ciprofloxacin from α-cyclodextrin core dendrimer (6).

Biological activity

Preparation of media

Nutrient broth became used to bacteria cultivate. Agar media prepared by addition of 24% w/v agar within the nutrient broth to form agar slants. Bacteria were sub-cultured on the nutrient slants. The inoculums become organized through moving lapful of the corresponding organism from the stock way of life into the sterile broth and incubated at 37°C for bacterial traces inclusive of Staphylococcus aureus 260 (mtcc 737),

Bacillus subtilis (mtcc 2063), Escherichia coli (mtcc-443), Pseudomonas aeruginosa (mtcc 741) and Proteus mirabilis (mtcc425). 20 mL of sterile nutrient agar media became brought to every Petri dish and 2mL of 24 h broth lifestyle if micro organism have been, then introduced to the respective plates and combined thoroughly by way of rotatory movement of the plates. The respective test compounds had been dissolved in DMSO within the attention of 10 mg/mL. The solution becomes maintained as inventory answer. The unique concentrations (100, 2 hundred and 500 ppm) were organized from the stock solution. A sterile paper disk of 5 mm diameter turned into saturated with agar plates. The 270 Petri plates had been incubated at 37°C and zones of inhibitions had been measured with the exception of the diameter of the paper discs (5 mm) manipulate discs had been completed with sterile water.

Antibacterial activity

A fascinating, biologically relevant construction of stable higher generation spherically shaped macromolecule from β-cyclodextrin and ciprofloxacin is screened their in-vitro antibacterial activity with various six types of strains (three Gram positive and three Gram negative). The measured biological ability of synthesised compound 3 as shown in Figure 5 is very high (49.6 mm) then that of ciprofloxacine (28.2) against vipro cholerae. Similarly, the synthesized molecule shown significant zones of inhibition values for various bacterial organisms are as shown in the Table 2. The macromolecule 3 exhibited good potent activity against *Staphylococcus aureus* (mtcc 737), Vibrio cholerae (mtcc-3906), *Bacillus subtilis* (mtcc 2063) (Gram positive) and Escherichia coli (mtcc-443), Klebsiella pneumonia, Salmonella typhi (Gram negative). The compound 3 was found to display outstanding antibacterial activity against entire tested microorganisms. Figure 5 indicates that the minimum inhibition properties are at low concentration of 0.050 μg/mL^{-1} against Vibreo cholerae, at 0.075 μg/mL^{-1} against Bacillus subtilis, at 0.025 μg/mL^{-1} against Escherichia coli, at 0.075μg/mL^{-1} against *Klebsiella pneumonia* at 0.100 μg/mL^{-1} against Salmonella typhi and at 0.100 μg/ml against *staphylococcus aureus* due to the twenty one molecules of ciprofloxacin surface moiety in synthesized drug delivery system. From minimum inhibition concentration values the test compound have excellent activities against various bacterial species, the MIC efficiency are given the Table 3 and it is reveal that the inhibition effects of the compound 3 more potent than the standard.

Table 2. In vitro antibacterial activity of compound (3) (Zone of inhibition in mm).

Name of the organism	ppm	Staphylococcus aureus	Vibreo cholera	Bacillus subtilis	Escherichia coli	Klebsiella pneumonia	Salmonella typhi
Compound 3	100	47.4	49.6	38.6	48.4	47.1	46.4
Ciprofloxacin	500	26.4	27.5	26.8	28.2	27.6	27.8

Table 3. In vitro antibacterial activity of compound (3) (minimum inhibition concentration (μg/mL^{-1})).

Name of the organism	Staphylococcus aureus	Vibreo cholerae	Bacillus subtilis	Escherichia coli	Klebsiella pneumonia	Salmonella typhi
Compound 3	0.100	0.050	0.075	0.025	0.075	0.100
Ciprofloxacin(std)	2.903	2.901	2.920	2.902	2.904	2.902

Synthesized compound 3 was screened *in-vitro* antimicrobial activity against gram-positive bacterial strains like *Staphylococcus aureus* (mtcc 737), Vibrio cholerae (mtcc-3906) and Bacillus subtilis (mtcc 2063) and gram-negative bacteria strains like Escherichia coli (mtcc-443), *Klebsiella pneumonia, Salmonella typhi* and *Proteus mirabilus* at a concentration of 100 μg/mL by standard cup plate method and the results, in fact, were compared with a standard drug Ciprofloxacin. Approximately 20 mL of liquid agar medium freshly prepared by using distilled water was poured into each petridish plate and then dried in an incubator at 37° C for the duration of 1 hrs. The standardized culture of microorganism was spread for all petridish plate using by L-shaped spreader was used to spread. Cups of approximately 6 mm diameter were made in petridishes using sterile cork borer and then labelled. A solvent control was also tested to measure the effect of solvent against the growth microbes. The 0.1 percent of Dimethylsulfoxide (DMSO) was used to prepare the test solutions of synthesized compounds and ciprofloxacin (100 μg/mL). The prepared solutions were treated to each cup in petridishes and kept a side in an aseptic area for 1hrs to allow diffusion of the drug/sample, followed by incubation at 37° C for 24 h. the zone of inhibition (in mm) was measured and the results are given in Table 2.

Broth dilution technique was performed to determine the MIC of synthesized compounds that inhibited growth of at least any one microorganism according to the standard procedure (Amidon et al. 1995). The mean zone of inhibition produced by synthesized compound in agar diffusion methods against tested bacterial strains ranged 38 to 50 mm. the negative control (0.1 % DMSO) did not produce zone of inhibition for all the bacterial strains tested. Ciprofloxacin standard drug used as antibacterial positive control and it is produced inhibition ranged between 26 to 29 mm in our study the gram-positive bacteria were more susceptible than the gram-negative species. It is very interesting to observe that the antimicrobial activities depend upon the increasing nature of the ciprofloxacin surface moieties and position of the substituent in the β-cyclodextrin-ciprofloxacin drug delivery system, it is explain from Figure 5 that outstanding activity was revealed to title compound with increasing ciprofloxacin moiety against gram-positive and gram-negative bacteria species. The minimum inhibitory concentration (MIC) values of the tested compounds were also displayed in Figure 5. The observation of the results revealed that the compound with 0.025µl/ml^{-1} concentration shown significant MIC values with *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*.

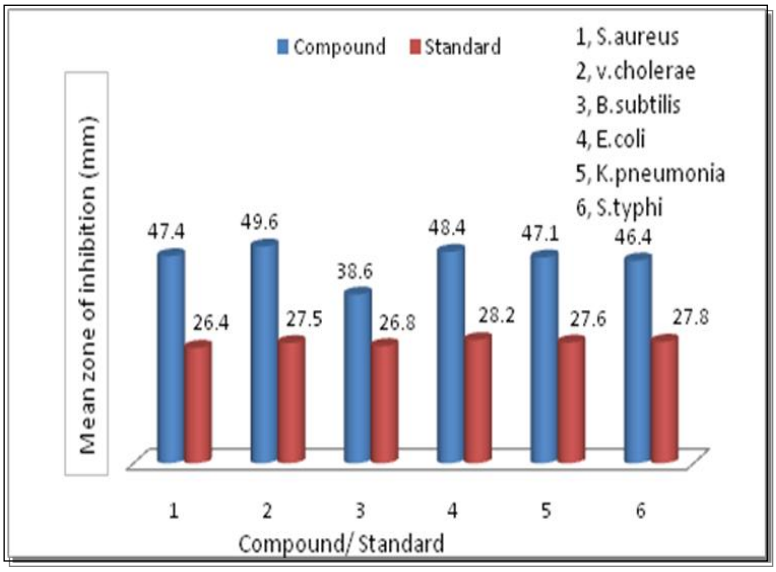

Figure 5. Zone of inhibition and Minimum inhibition concentration (ppm) of compound (3).

MTT assay studies to β-cyclodextrin core drug delivery system(compound-3)

Biological ability of ciprofloxacin based drug delivery system was measured by MTT assay in E.Coli cells. Table 4 and Figure 6 shows that the cytotoxic affect of various concentrations (5, 15, 25, 50, 75 and 100µl) of β-cyclodextrin core drug delivery system in E. coli cells. Among the four different time duration, the 72 hrs time interval experiment the ciprofloxacin delivery showed a maximum antibacterial activity when compared to other three in E. coli cells. The IC50 value was found to be 50, 75 and 100 µL, concentrations.

Table. 4. Cytotoxicity effect of compound 3 in E. coli cells by MTT assay.

S. No.	Time (hrs)	control	Cip	Concentration					
				5	15	25	50	75	100
1	12	100	96.2747	99.9755	96.2747	98.5149	97.1088	97.0208	96.3051
2	24	100	89.2072	99.1506	99.1152	98.7091	89.5751	84.1331	75.2651
3	48	100	89.0027	98.4428	92.9237	85.7702	78.7361	73.1265	58.3112
4	72	100	90.0263	93.9618	83.4797	56.9134	37.0219	34.5789	29.6311

Values are expressed as the mean ± SD of three experiments in each group.

Data expressed as mean ± SD of three experiments in each group

The ciprofloxacin containing macromolecule 3 exhibited significant antibacterial property in *staphylococcus aureus*measured by MTT assay.

Cytotoxicity effect of 3 in *staphylococcus aureus* showed in different manner against the concentration (Table 5 and Figure 7). It has been noticed that the 72 and 48 hrs time duration showed a maximum Cytotoxicity against *staphylococcus aureus*. The IC_{50} value of ciprofloxacin was found to be 75 and 100 µL concentrations.

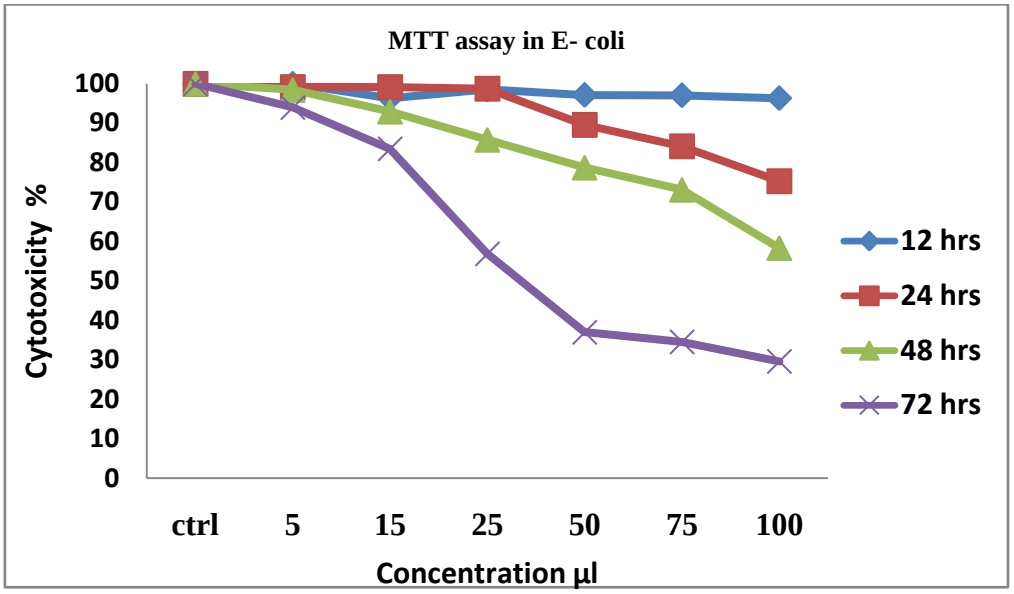

Figure 6. Cytotoxicity effect of compound 3 in E. coli cells by MTT assay.

Table 5. Cytotoxicity effect of compound 3 in *Staphylococcus aureus* cells by MTT assay.

S. No.	Time (hrs)	Control	cip	Concentration µl					
				5	15	25	50	75	100
1	12	100	89.8201	97.9995	94.2548	89.0796	85.3758	79.2184	69.8473
2	24	100	90.0213	96.3741	94.0029	89.0228	84.2767	77.7386	67.2520
3	48	100	91.4521	94.4057	90.3837	87.4153	82.6056	74.1239	59.9039
4	72	100	90.8763	91.5748	87.6378	82.9316	75.1193	49.8143	37.8735

Values are expressed as the mean ± SD of three experiments in each group.

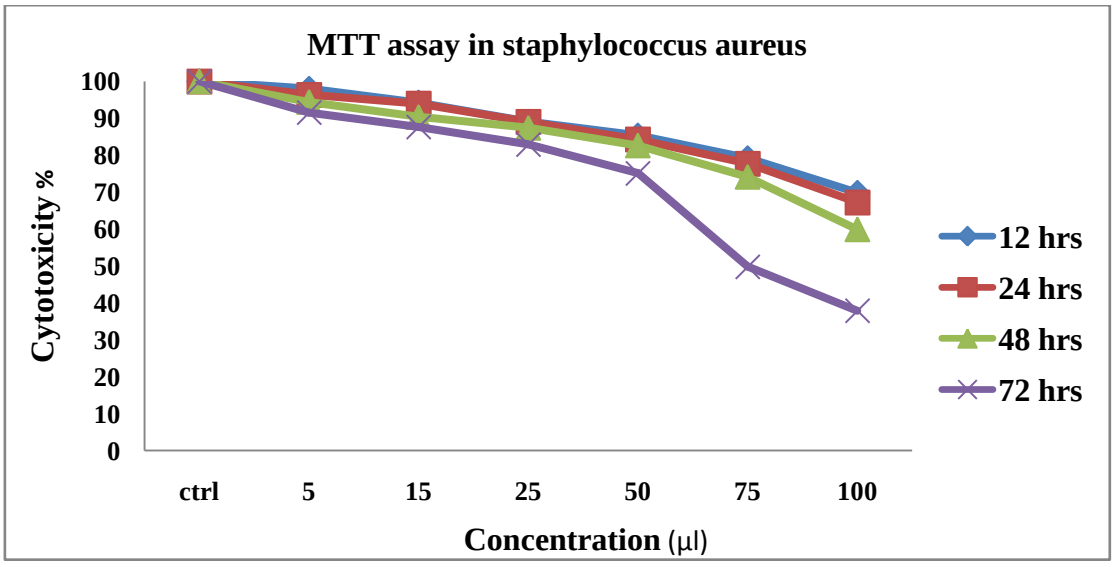

Figure 7. Cytotoxicity effect of compound 3 in *Staphylococcus aureus* cells by MTT assay.

Values are expressed as the mean ± SD of three experiments in each group

The results of cell viability were calculated by MTT assay technique, showed that the synthesized test compound 3 has remarkable activities against *Escherichia coli* and *staphylococcus aureus*. Therefore, they could be introduced as newly synthesized antibacterial agents against commonly occurring microorganism. Moosavy et al., 2008, Fazeli et al., 2007, Misaghi and Akhondzadeh (Sobczak et al. 2010; NCCL Standards. 2000). Were previously reported that the antibacterial activities of some compound. Antibacterial agent tested with various microorganisms such as *Salmonella. typhimurium*, *Staphylococcus aurous* and *Bacillus cereus* in a food model system and in brain heart infusion (BHI) broth the current study was illustrate that sustain drug delivery of newly synthesized compound 3 by MTT assay technique. Viable cell percentage change with respect different hours and various concentration, this result conform the sustain delivery of compound 3. E coli cell viability calculated after incubated with compound 3 at 12, 24, 48 and 72 hrs and the data are given Table.4 after 12 hrs incubation the cell viability is 96.30 for 100µl only 3.7 percentage bacteria killed by test compound. After 24 hours incubation the cell viability of E coli 75.27 only 24.73 percentage killed. For 48 hours 100µl show 58.31 cell viable 41.69 percentage only killed. For 72 hours 100µl show 29.63 percentage cell viable 70.37 percentage are killed. The slowly decrease the cell viability this result conformed sustain delivery of synthesized delivery system. Drug delivery time duration and concentration is show in the Figure 6. Result are compared with standard ciprofloxacin, which is killed approximately 10% for each hours.

Compound 3 treated with *Staphylococcus aureus* to prove the control drug delivery of polymer nature of giant molecule. It is incubated on 12, 24, 48 and 72 hrs with different concentration 5, 15, 25, 50, 75 and 100µl, viable cell percentage differ with respect to the treated drug concentration. For 12 hrs 100µl give 69.85 percentage viable cells, 30.15 percent only killed by compound 3. After 24 hrs incubate at 100 µl show 67.25% viable cells, 32.8 % only killed. After 48 hrs at 100 µl show 59.90 percent viable cell 40.1 % are killed by drug releases. For 72 hrs at 100 µl show 37.87 percent viable cells, 62.13 percent are killed, this result explicit sustain drug delivery of compound 3. Viable cell reduced with increasing incubation time and increasing concentration and these results are given in Table 5 and Fig 7.

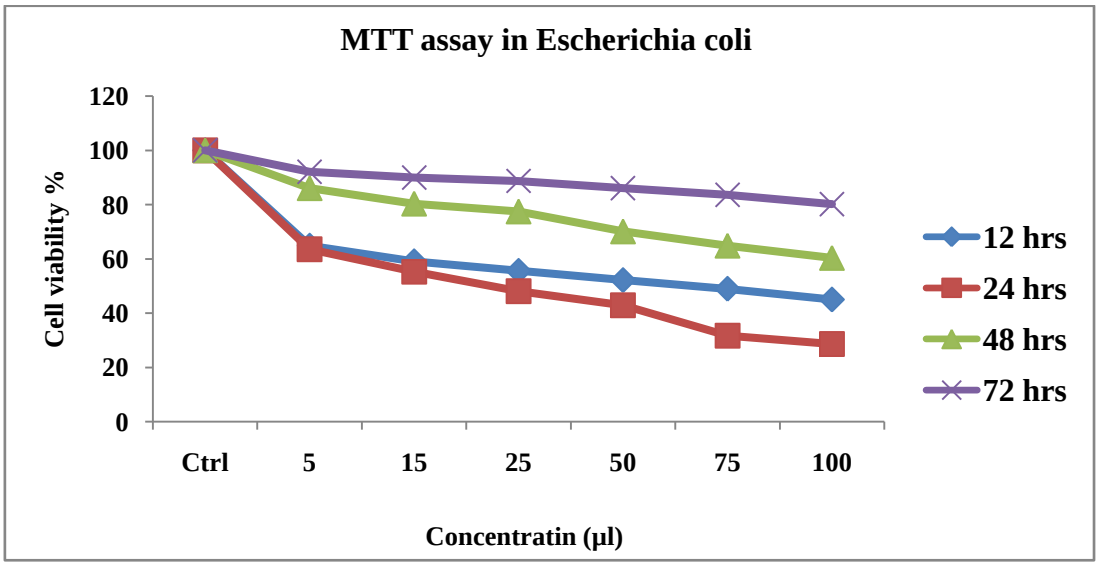

Figure 8. cytotoxicity effect of compound 6 in E. coli cells by MTT assay.

MTT assay study of α-cyclodextrin core drug delivery system (compound-6)

Biological activity of α-cyclodextrin core spherical macromolecule was assessed by MTT assay method. Percentage of cell viability was measured to various amount (5, 15, 25, 50, 75, 100 µl) with different hour duration (12, 24, 48, 72 hrs) against two bacteria species. Synthesized compound 6 treated with *staphylococcus aureus* and *Escherichia coli*. 75µl and 100µl have maximum cytotoxicity effect against *Escherichia coli* and *staphylococcus aureus*. The IC_{50} value found to be amount of 25, 50, 75 and 100 µl to 24 hrs. 75 and 100 µl to 12hrs against E coli, the IC_{50} values against staphylococcus found to be 75 and 100 µl at 24 hrs. Synthesized compound reduced the cell viability of E coli until 48 hrs after that cell viability are increases to 48 and 72 hrs due to activity of synthesized compound only for 24 hrs.

Table 6. α-cyclodextrin core drug delivery system activity is only less in durability against E coli.

Table. 6. cytotoxicity effect of compound 6 in *Escherichia coli* cells by MTT assay.

T(hrs)	Ctrl	strd	Concentration (µl)					
			5	15	25	50	75	100
12	100	61.7339	64.8841	59.1236	55.5915	52.1789	48.9835	45.0325
24	100	56.9142	63.5793	55.2163	48.0727	42.9156	31.6319	28.6025
48	100	87.7287	86.0286	80.3188	77.4302	70.0695	64.8047	60.2936
72	100	89.3856	92.1436	89.9614	88.7249	86.1179	83.5451	80.1666

Values are expressed as the mean ± SD of three experiments in each group.

Data expressed as mean ± SD of three experiments in each group

It is shown in Fig 8 and α-cyclodextrin core drug delivery system activity against staphylococcus are shown in the Table 7 and Fig 9. The activity of synthesized compound is compared against E coli and S aureus, the 75 and 100µl give good result. The maximum cell viability percentage is reducing in E coli than staphylococcus cells.

Table 7. Cytotoxicity effect of compound 6 in *Staphylococcus aureus*cells by MTT assay.

T(hrs)	Ctrl	Strd	Concentration (µl)					
			5	15	25	50	75	100
12hrs	100	68.9145	65.4503	61.6654	59.8951	56.4795	53.1796	50.8105
24hrs	100	47.6965	61.5906	58.0199	54.7191	51.7721	47.7528	44.1030
48hrs	100	53.2860	74.1249	71.1704	67.5615	64.4596	60.9318	58.6230
72hrs	100	73.0493	95.8183	92.3439	85.9049	79.2453	72.5054	69.7000

Values are expressed as the mean ± SD of three experiments in each group.

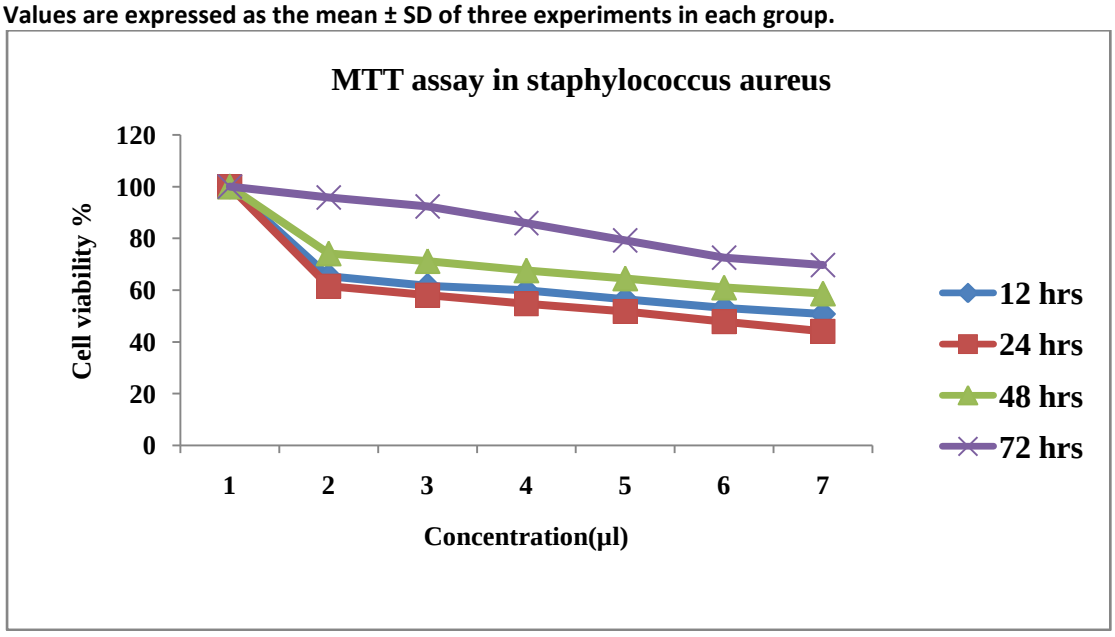

Figure 9. cytotoxicity effect of compound 6 in *Staphylococcus aureus* cells by MTT assay.

Data expressed as mean ± SD of three experiments in each group

Comparison of α and β-cyclodextrin core drug delivery system

From the MTT assay result β-cyclodextrin core drug delivery system have long time activity due to their sustain releases of ciprofloxacin unit. MTT assay prove that β-cyclodextrin drug delivery system reduced the cell viability of Escherichia coli and staphylococcus aureus species. α and β cyclodextrin delivery system effectively reduced the E coli cell viability than staphylococcus aureus cells.

The synthesized β-cyclodextrin core system long time activity against both species, which is slowly reduce the live cells fron 12 to 72 hrs. α-cyclodextrin core drug delivery system activity against both cells, which reduced the cell viability from 12 to 24 hours after that cell viabilities are increased to 48 and 72 hrs. From this result β-cyclodextrin core system act as very good sustain drug delivery system. β-cyclodextrin is more convenient to design the covalent bonded sustain drug delivery system.

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