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Synthesis, Characterization, Self-assembly and Anticancer Drug Delivery of Fluorescent Amphiphilic Homopolymers

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ABSTRACT

The self-assembly of amphiphilic polymers presents new possibilities in biomedical applications, particularly in drug delivery. This work focuses on synthesizing fluorescein-based amphiphilic homopolymers using ring opening polymerization and characterization for their structure and application. The Grubbs II generation catalyst is used to produce monodisperse polymers with low polydispersity index. The hydrophobicity of the polymers increases with the addition of methylenic carbon chains. The polymer was characterized fully using spectroscopy (UV-Vis, FT-IR and NMR), chromatography (GPC), microscopy (SEM) and light scattering (DLS). These homopolymers form aggregates, that are required as anticancer drug delivery vesicles. Doxil encapsulation and its release near cancer cells were investigated through the acidic and neutral physiological conditions. Microscopically, easy internalization of drug-loaded vesicles onto living cells has been proven.

Keywords: Homopolymer, Self-assembly, Barbiturate, Amphiphilic polymer, Drug-delivery.

INTRODUCTION

Self-assembly is an intriguing ability of polymers and other natural compounds that rivals other regular assembly forms including micelles, vesicles, helices, and tubes. Self-assembly has a wide variety of applications. There are numerous examples, most of which are beneficial in the biomedical and life sciences sectors, such as vesicles for medication, gene, and RNA delivery. It is very much usefulness in the realm of anticancer medication delivery.

The morphology of the polymers is playing very important role in the process of delivering drugs for the anticancer and other potent drugs such as skin problems[1,2,3,4,5].

For anticancer drug delivery applications, usual polymer forms used are worm-like or rod-like, or even as micelle or vesicles. Most important concept is drug delivery design is a structure for the tablet medicine containment is required to be more stabler than liposomes, for longlasting presence for minimum 24 h, and slowly soluble in acidic watery body fluids[6,7,8,9].

Current topic of interest is using medicine

as nanoparticles. The prime advantage of using nanoparticulate medicine for drug delivery is to avoid any coagulation or blockade inside small artery or veins. These injections (water soluble, biodegradable, longlasting and nanoparticulate forms) use particles of vesicular self-assembly containing drug molecules of diameter <200nm. It is aimed at good blood circulation and good accumulation in affected cells[8,9,10].

To respond well to external stimuli, non-covalent forces of interaction between hydrophilic and hydrophobic parts, metal ion and ligand by coordination, and finally host to guest interactions are commonly adopted for longlasting drug delivery applications[5,6].

Amphiphilic polymers in the form of micelles are most effective carrier for the drugs, especially cancer drugs. These homopolymers are good because of their poor solubility, limited stability, and low toxicity. Well-researched stimuli for drug release sometimes is using either light or enzyme also. Also, pH is also used. It is very important in biological systems of acidic pH values[3,4,5,6].

Hydrogen bonding helps in assisting self-assembly of polymers in aqueous media. It is very useful in supramolecular chemistry, and recognition-induced polymersomes. In this article, synthesis of vesicular structures from the newly synthesized polymer groups and its suitability for anticancer drug delivery are explored. Norbornene, barbiturate and thiobarbiturate functionality are used for creating new polymers, which are homopolymers, amphiboles, hydrogen bond forming, and most suitable for biomedical as drug delivery molecules. This work is residing on amphibole homopolymers that are less explored in the literature[5,8,9].

MATERIALS AND METHODS

Chemicals Required

Vinyl ethyl ether, stannic chloride, dichloromethane (DCM), methanol, Grubbs' generation II catalyst (G2), calcium hydride (CaH_2), dichloromethane (DCM), deutrated dimethyl sulphoxide (DMSO-d_6), deutrated chloroform ($CDCl_3$), heavy water (D_2O), and tetrahydro furan (THF).

Synthetic Schemes

Synthesis of Molecules-1

First of all, a solution of toluene with 10 g maleic anhydride and 8 g furan is prepared. The solution is stirred continuously for 48 h at ambient temperature. The resultant product is filtered, washed in toluene, and is then dried in vacuum. Yield obtained is 96% and proton NMR spectral method is used to get molecular features. MS (ESI) is used to get the molecular weight (166.02) that is close to observed value (166). This reaction is given in Figure 1.

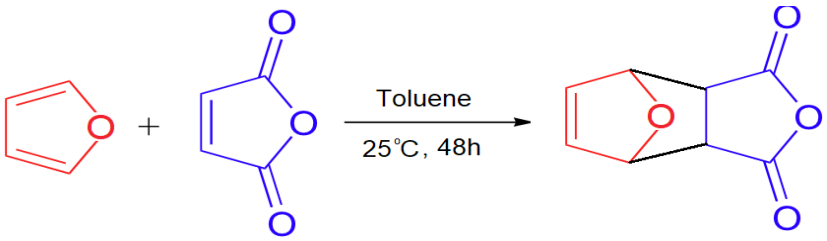

Fig. 1. Synthetic Scheme of Molecule-1

Synthesis of Molecules-2

To synthesize molecule 2, exactly 5 g of molecule 1 and 3.2 g benzyl amine are dissolved in 50 mL methanol. The resulting solution is continuously stirred for 72 h at 56°C to form a paste. Then, the pasty mass is then dissolved in dichloro-methane, and is then washed well in water. The organic portion layer so obtained is then dried using sodium sulphite (Na_2SO_3) in vacuum to get solid product. This reaction is given in Fig. 2. It is called molecule-2 (with only -CH_2- hydrophobicity group). Yield is 68% and proton NMR is used to get molecular features. MS (ESI) is used to get the molecular weight (255.17) that is very close to observed value (256.02). Benzyl amine contains methylene (CH_2) group between phenyl ring and amine. Amines with more -CH_2-, ie -$(CH_2)_0$-, -$(CH_2)_3$- and –$(CH_2)_4$- are used and the above procedure was repeated. Yields are 64%, 67% and 63% respectively for increasing

number of -CH_2-. Yields are 64%, 67% and 63% respectively for molecules-2 with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-. Proton NMR is used to get the molecular structure of these three molecules-2. MS (ESI) is used to calculate the molar masses of molecules-2 with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$- and respectively are 269.71, 283.12 and 297.14. The observed values of molar masses of molecules-2 with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$- respectively are 271.02, 284.28 and 298.53.

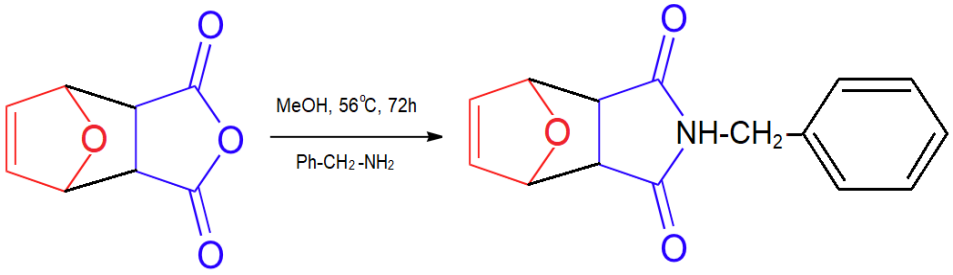

Fig. 2. Synthetic Scheme of Molecule-2

Synthesis of Molecules-3

To synthesize molecule-3 (with only -CH_2- hydrophobicity group), again in another round bottom flask, 500 mg molecule-2 (with only -CH_2- hydrophobicity group) is mixed with 7ml dichloro-methane. The RB flask is cooled in the ice-bath to stop fast reaction, 2 mL di-ethyl keto-malonate is added drop by drop using a syringe, followed by 2.25 mL stannous chloride addition drop by drop. The resultant mixture is stirred for 6 h at ambient temperature. The resulting product is concentrated in vacuum to obtain brownish precipitate. This reaction is given in Fig. 3. Yield is 65% and Proton NMR is used to get the molecular features. MS (ESI) is used to calculate the molar mass (429.14) that is close to observed value (430). Molecules-3 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) are synthesized by same procedure that is followed for molecule-3 (with only -CH_2- hydrophobicity group). Yields are 60%, 66% and 68% respectively for Molecules-3 (with hydrophobicity increasing groups -$(CH_2)_2$, -$(CH_2)_3$- and –$(CH_2)_4$-). Proton NMR is used to get the molecular structure of Molecules-3 (with hydrophobicity increasing groups -$(CH_2)_2$, -$(CH_2)_3$- and –$(CH_2)_4$-). MS (ESI) is used to calculate the molar masses of Molecules-3 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$) and respectively are 443.45, 457.47 and 471.5. The observed values of molar masses of Molecules-3 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) respectively are 444.31, 458.23 and 472.11

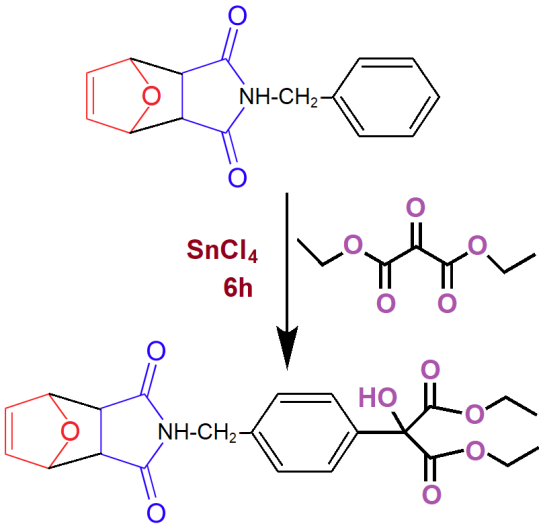

Fig. 3. Synthetic Scheme of Molecule-3

Synthesis of Molecules-4

To synthesize molecule-4 (with only -CH_2- hydrophobicity group), In another round bottom flask, 50mg molecule-3 (with only -CH_2- hydrophobicity group) is slowly dissolved in 5 mL THF. To the flask, 50 mg fresh prepared solution of sodium methoxide is added, followed by an addition of 50 mg thiourea. At ambient temperature, the mixture is well-stirred for 24 hours. The product is finally precipitated out by adding excess THF. The resulting product is concentrated to get a brown precipitate. This reaction is given in Fig. 4. Yield is 72% and proton NMR is used to get the molecular structure. MS (ESI) is used to calculate the molar mass (413.40) that is close to observed value (414.22). The Molecules-4 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) are synthesized by same procedure

that is followed for molecule-4 (with only -CH_2- hydrophobicity group). Yields are 69%, 70% and 64% respectively for Molecules-4 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-). Proton NMR is used to get the molecular structure of Molecules-4 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-). MS (ESI) is used to calculate the molar masses of 4b, 4c and 4d and respectively are 427.43, 441.46 and 455.48. The observed values of molar masses of Molecules-4 (with hydrophobicity increasing groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) respectively are 428.12, 442 and 456.72.

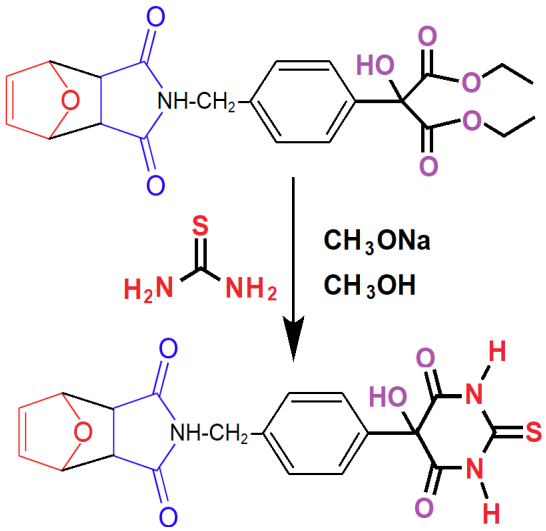

Fig. 4. Synthetic Scheme of Molecule-4

Synthesis of Polymers 1-4

In a round bottom flask, 2 g of one monomer molecules (from the list of monomer molecules, with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- or –$(CH_2)_4$-) is dissolved in dichloromethane-methanol solvent mixture in nitrogen inert environment. In another round bottom flask, 1 g Grubbs' II Generation catalyst is dissolved dichloromethane, in nitrogen atmosphere. Both the solutions are transferred to third round bottom flask, is stirred well at ambient temperature till polymer is formed. The polymerized mixture is finally quenched using vinyl ethyl ether. An aliquot of polymerized solution is analysed using GPC. The balance solution of third round bottom flask is used to extract the polymer by precipitation using pentane. Polymer is then purified using by dissolving in THF, by passing over alumina adsorbent to remove G2, and is precipitated out in pure form by adding pentane. This reaction is given in Fig. 5. GPC is used to get the molar mass of the polymer using THF solvent and polystyrene standard. This process is repeated for getting all four polymers, ie, Polymers 1-4 (with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-).

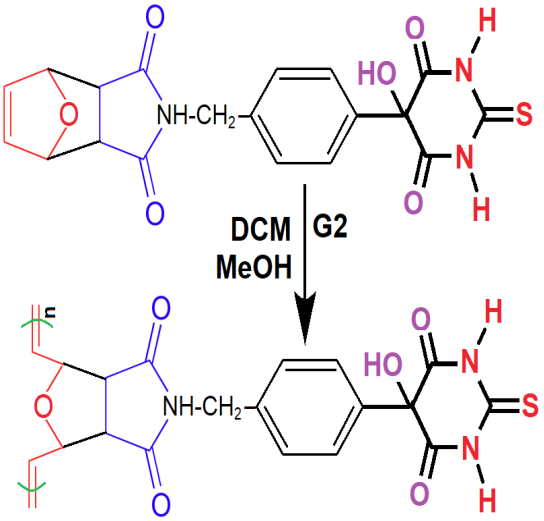

Fig. 5. Synthesis of Polymers FAH 1-4

Analytical Procedures

Spectral characterization

Proton NMR is recorded using 500MHz Bruker Advance III spectroscope. Deutrated solvents (DMSO-d_6, $CDCl_3$ and D_2O) are used. Intrument is calibrated to the reference compound tetramethyl silane (TMS). TMS is internal standard with δH 0.0). IR spectrum was recorded in Perkin-Elmer Spectrum-100 Optica FT-IR spectroscope. FT-IR spectrum is recorded in the nominal resolution (of 2 cm^{-1}). GPC is used to obtain molar mass and PDI (polydispersity index). Solvent THF and PMMA standard are used. GPC instrument contains 515 Model Waters HPLC pump-set and 2414 Model Waters RI Detector at ambient temperature in a flow rate 1.0mL/min. HR-MS analyser QTOF-YA263 is used to get molar mass. It is a high resolution instruments with positive mode electro-spray ionization (made by Waters Corporation). Fluorescent Emission is recorded in FluoroMax-3 spectroscope (Make: Horiba, Lamb: Xe 150W, Range 250-900nm). UV-Vis Absorption is done using Hitachi U-4100 spectrophotometer. Scan rate used is 500nm/min. Particle size is measured by Malvern Zetasizer Nano DLS instrument (lamb: 4.0mW He-Ne laser, laser beam λ = 633 nm). Particle shape was recorded in Zeiss HR-SEM microscope.

Polymerisation kinetics

Investigating the kinetics of polymerization is carried out to confirm the live character of ROMP of monomers (molecules-4 with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- or –$(CH_2)_4$-)). Aliquot of reaction sample is withdrawn in regular time intervals. To the aliquots withdrawn, stopping of polymerization is done by adding a vinyl ether (full name : vinyl ethyl ether). All the polymerization reactions are terminated with about complete monomer to polymer conversion. The above steps and procedures are repeated for varying molar ratios of monomer such as 10, 20 and 30 to unit dose of catalyst. As mentioned in analytical procedures, molecular weights (number average, Mn) are obtained using GPC chromatographic technique.

measurement

Fluorescence spectroscopy is used to obtain the critical aggregation concentration (CAC) of polymers. First, aggregation of polymer-1 (with only -CH_2-hydrophobicity group) is analysed using its fluorescence property. Molecule pyrene acts as the external probe. Concentration of pyrene compound is taken as constant, at C=0.2μM. Concentration of polymer-1 (with only -CH_2-hydrophobicity group)is ranging 0.01-1000μg/mL. Intensity of fluorescent emission is measured in 375nm, 382nm and 396nm. The experiments used an an excitation wavelength 339nm. In the emission spectra, intensity ratios of intensities for pyrene's first peak (375nm) and third peak (396nm) refers to surroundings polarity ratio. Ratio of fluorescent intensities at 396/375nm to measure polymer-1 (with only -CH_2-hydrophobicity group) concentration. CAC is nothing but the point of beginning of alteration of relative fluorescence intensity ratio. CAC value is 348μg/mL. This procedure is repeated for obtaining CAC values of other Polymers 2-4 (with hydrophobicity groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-).

Drug encapsulation

Doxil is drug selected for polymer drug release studies. Doxorubicin hydrochloride is the chemical name of doxil drug. In a vial, a solution of 1 mg polymer-1 (with only -CH_2-hydrophobicity group) is 1 mL deionised water is prepared. Similarly, in another vial, a solution of 1mg of doxil in deionised water is prepared Mixture of these two solutions are agitated for 30 min allowing encapsulation of the medication in polymer-1 (with only -CH_2-hydrophobicity group) vesicles. The non-encapsulated doxil molecules are removed by dialysis. The dialysis tube has 1500Da cut-off. The dialysis is performed againt 100 mL deionized water. Amount of non-encapsulated drug is identified by taking aliquot of the sample and analyzing under 552nm and 587nm in fluorescence spectroscope. This helps us to to determine the release of non-encapsulated doxil from the periphery of the polymer polymer-1 (with only -CH_2-hydrophobicity group) vesicles. This method is repeated many times until the doxil emission at 552nm and 587nm disappears completely. A similar experiments are done using other Polymers 2-4 (with hydrophobicity groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-). The drug loading efficiency (DLE) is equal to 100x(mass of drug molecules in vesicles/mass of drug encapsulated vesicles)[2,3,5,6].

Drug release

Doxil encapsulated with polymer is analysed for drug release by the dialysis technique. In the dialysis tube having cut off 1500Da, polymer encapsulated doxil polymer-1 (with only -CH_2-hydrophobicity group) vesicle solution is taken and dialysis study is done. It is done against 50 mL phosphate buffer of varying pH's (7.4 and 5.5) in regular varying time intervals. The doxil drug release monitoring is done using fluorescence spectroscopy. From dialysis instrument, the aliquots are withdrawn in fixed regular time intervals. Fluorescence emission spectral studies are done for dialysis sample aliquots. Wavelength used are 552nm and 587nm. These are doxil emission wavelengths. The quantity of drug released is plotted against time. Instrumental measurement errors are monitored using standard deviation or variance. The above procedure is repeated for other three variants, Polymers 2-4 (with hydrophobicity groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$).

RESULTS AND DISCUSSION

Formation of Poymer Molecules

NMR spectroscopy is used to study all raw chemical components, as well as synthetic compounds and monomers. In ^{1}H NMR spectroscopy of monomer molecule 3, the proton signal ranges from 8.6ppm to 9.6ppm. This indicates these are due to hydrogen atoms attached to nitrogen atom of cyclo-imide (-NH) protons. This is nothing but the proof of the formation of a cyclic ring of thio-

barbiturate. The signal at 180-181 ppm in ^{13}C NMR spectra denoted the synthesis of monomer with norbornane derivative and thiobarbiturate moieties. Finally, polymerization gives monodisperse amphiphilic polymers "Polymers 1-4 (with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-)". The ring opening method with metathesis and polymerization are applied to produce the Polymers 1-4 (with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) with monodisperse and amphiphilic nature.

To impart polymerization to our aimed synthesis, first the monomer solid is dissolved in a solvent mixture of pure dichloromethane and methanol in 9:1 volume %ratio. Meanwhile the second-generation variety catalyst of Grubbs' is dissolved in dichloromethane (anhydrous). The solution of monomer is transferred to the flask containing the solution of Grubb's II generation catalyst, with constant stirring and also by maintaining room temperature during mixing of monomer solution and catalyst solution. The polymerization reaction is terminated by adding ethyl vinyl ether is used to stop the polymerization reaction. Polymer is purified using precipitation and dialysis before being used in future research. The formation of amphiphilic polymers is validated by the GPC method. Fig. 6 gives normalized GPC traces for polymers.

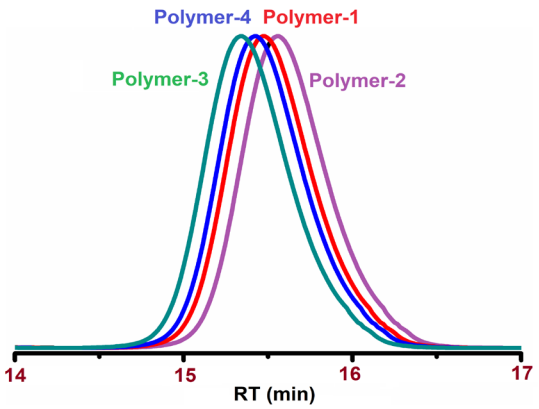

Fig. 6. Normalized GPC traces for Polymers

The determination of molar mass of the polymer is estimated using the polystyrene as standards and the solvent used is tetrahydrofuran. The GPC data indicate that the Polymers 1-4 (with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) have a restricted distribution of molecular weights. The life aspects of ROMP are then explored for Polymers 1-4 (with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-). Kinetic investigations are carried out on monomers at various ratios of monomer to catalyst (ie, M:I ratios = 10:1, 20:1 & 30:1). The number average molecular weights (Mn) are calculated using GPC. Table 1 displays the molecular weights and PDIs of several polymers.

Table 1: GPC values : Molar mass and Polydispersity

M/I ratio	Polymer-1		Polymer-2		Polymer-3		Polymer-4	
	Mn	PDI	Mn	PDI	Mn	PDI	Mn	PDI
10:1	4900	1.06	4200	1.03	4100	1.07	4300	1.03
20:1	6200	1.10	8100	1.09	8500	1.04	7600	1.11
30:1	11300	1.13	10700	1.10	12000	1.12	11500	1.14

Self-Assembly of Polymer Molecules

The self-assembly behavior of the polymer molecule (Polymers 1-4 (with hydrophobicity groups -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-)) is also examined. This polymer molecule in aqueous solution leads to the formation of vesicles. After finding out the vesicle structure formation, increase of hydrophobicity is done by adding extra methylene groups in the polymer molecule. This addition of methylene group is done between norbornane moiety and thiobarbiturate moiety. After adding hydrophobicity increase in polymer molecules, morphological changes are also envisaged due to the shifts in the hydrophobic and hydrophilic balance. To investigate more on these changes on self-assembly propety, a variety of polymers are examined to obtain clearly the significance of the thiobarbiturate moiety in the self-assembly arising out of hydrogen bonding[1,2,3,4,10].

Another study uses scanning electron microscopy to examine the amphiphilic assemblages generated by polymers in an aqueous phase. The critical aggregation concentration (CAC) of these polymers is determined using a pyrene-based probe. The ratio of emission intensities of first peak (at 375 nm) and third peak (at 396 nm) peaks are mainly considered, as these peaks offer specific features about the polarity of its surroundings. The polymer concentration is utilized to change the relative emission intensities at 396 and 375nm. The reported CAC value for polymer-1 (with only one-CH_2- hydrophobicity group) is 348 µg/mL (Figure 7).

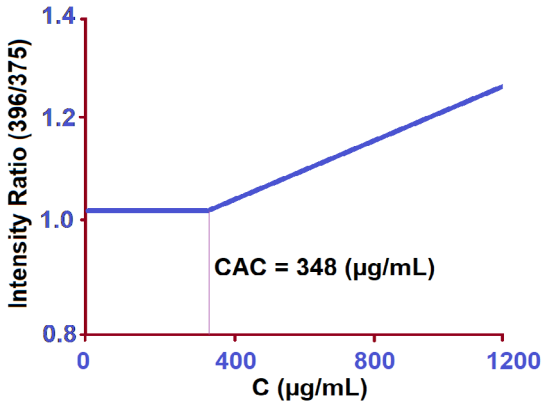

Fig. 7. Critical Aggregation Concentration by Pyrene probe

The numerical values of CAC for the three selected polymers are 354 µg/mL, 365 µg/mL and 370 µg/mL. However, by increasing the chain length, ie, (-CH_2-)n where n ranges from 2 to 4, the size of the aggregation increases from 127nm to 229nm. Amphiphilic homopolymers with vesicle-like structures form spontaneously in water solution due to the formation of strong hydrogen bonded interactions that balance between hydrophobic and hydrophilic interactions, arising between between the norbornene moiety backbone and thiobarbiturate moiety. DLS and SEM data are used to determine the self-assembly behavior of homopolymer in water medium. DLS studies demonstrate that the polymer-1 (with only one -CH_2- hydrophobicity group) has a unimodal distribution of polymer particle sizes. This normal distribution shows mean value of hydrodynamic diameters 127 nm (Figure 8).

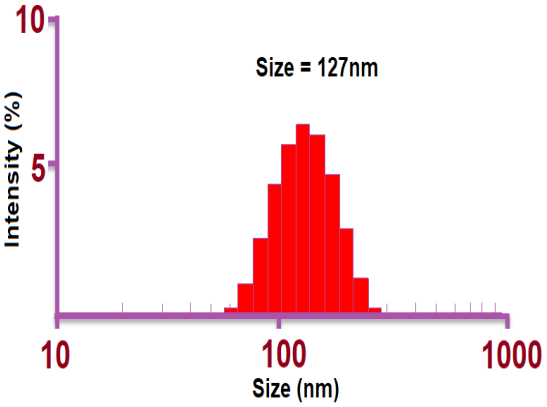

Fig. 8. Hydrodynamic radius by DLS

Again, the normal distribution of particle sizes for the three different versions of the polymers (Polymers 2-4 (with hydrophobicity groups -$(CH_2)_2$, -$(CH_2)_3$- and –$(CH_2)_4$-)) are determined and are found to be 147nm, 151nm, and 229nm, respectively. The summary of these results are given in Table 2. The average diameter of aggregate rises and the length of the hydrophobic chain are proportional. SEM indicates that the particles are spherical (Fig. 9). Interestingly, when hydrophobicity was inserted between the thiobarbiturate and norbornene backbones, the spatial organization and shape of the amphiphilic polymer remained unchanged. As a result, it is argued that the thiobarbiturate moiety is critical for the self-assemblage and vesicle formation by the hydrogen bonding assistence.

Table 2: Properties of amphiphilic homopolymers

Code	CAC (µg/mL)	Size by DLS (nm)	DLE(%)
Polymer-1	348	127	68
Polymer-2	354	147	63
Polymer-3	365	151	61
Polymer-4	370	229	55

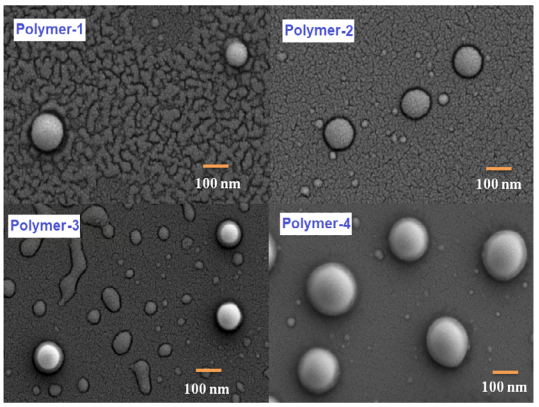

Fig. 9. SEM images of the amphiphilic homopolymers

Doxil Encapsulation and Release

The next step is to initiate the drug encapsulation capacity of polymer molecules. The anti-cancer medication doxorubicin (Doxil) is chosen. It is a preclinical anti-cancer medication. The research aims to determine the polymer molecules' ability to encapsulate doxil. Doxil, in its salt form, is water-soluble. It is held up by polymer assemblies. Doxil exhibits fluorescence characteristics. Thus, the drug encapsulation experiment is monitored using fluorescence spectroscopy. The polymer-1 (with only one -CH_2- hydrophobicity group) and Doxil solutions in water are combined and agitated for 30 minutes. The solution was then transferred to a dialysis apparatus whose cutoff value is 3500Da cutoff and dialyzed five times using 100 mL of water. The total absence of the emission intensity of Doxil at 552nm and 587nm indicates that all non-encapsulated Doxil had been eliminated.

The drug-loading efficiency (DLE) is calculated and is 68% by weight-weight ratio. It is polymer polymer-1 (with only one -CH_2- hydrophobicity group) that has the highest value (68% by weight) for drug loading efficiency, in comparison to the other three polymer variants, for Polymers 2-4 (with hydrophobicity groups -$(CH_2)_2$-, -$(CH_2)_3$- and –$(CH_2)_4$-) are 63%, 61%, and 55% w/w respectively. The DLE values are reported in Table 2. Other drug delivery properties, such as stimulus sensitivity and amphiphilic polymer breakdown in acidic environments, are discussed in the section that follows. The disintegration of the polymeric vesicles are then studied by quantifying the release of doxil encapsulated into the polymer-1 (with only one -CH_2- hydrophobicity group) polymeric solution. Doxil-packed polymer vesicles are dissolved in water and gets dialyzed in 50mL PBS buffer solution of pH 5.5. An aliquot of the solution of polymeric material are examined under the fluorescence emission at 552nm and 587nm to measure and to determine release of Doxil from the vesicles.

Repetition of this process is done for every 15 minutes. The experimental results show that the change in intensity of emission at 582nm and 587nm is notably high after 120 minutes. A comparable approach is employed under pH 7.4, human physiological conditions. Doxil's emission at 582 and 587 nm shows no significant change, indicating that the reservoir remained steady (Fig. 10). Doxil release peaks at 92% at pH 5.5, but drops to 5% at pH 7.4 (Fig. 11). The drug release experiment demonstrates that the polymer assemblies with amphiphilic character possessing a well-known burst release mechanism.

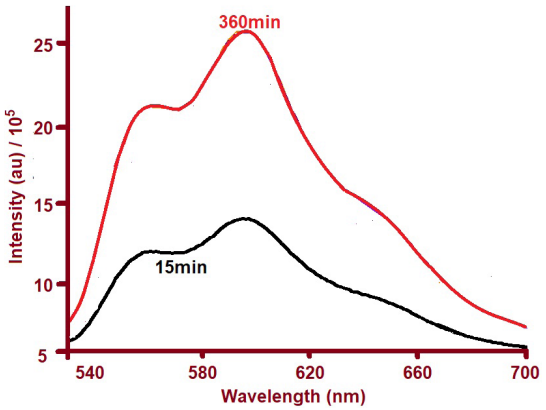

Fig. 10. Fluorescence spectra for Doxil release by Polymer-1 at pH 5.5

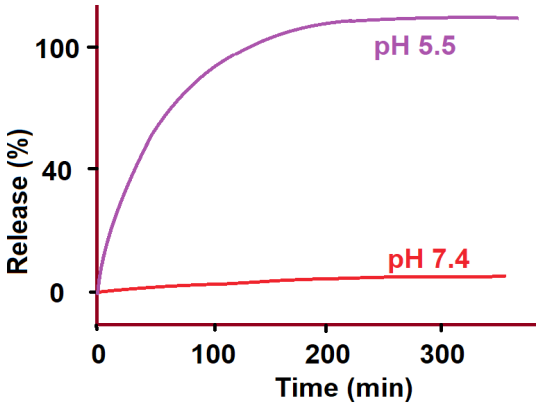

Fig. 11. Cumulative drug release by Polymer-1 at pH 5.5 and pH 7.4.

As per the currently assumed hypothesis, around pH 5.5, hydrogen bonding between thiobarbiturate moieties are disrupted, leading to an important role in aggregation formation. Fig. 12 depicts the overall self-assembly and medicine administration process with a cartoon illustration. A previous study examined the cellular absorption of FAH (the control molecule) from this amphiphilic homopolymer series. In this study, the 4T cell line of a cancer cells of mouse mammary glands are used. It shows that the absorption of drug-loaded vesicles are very rapid in living cells[2,3,5,6].

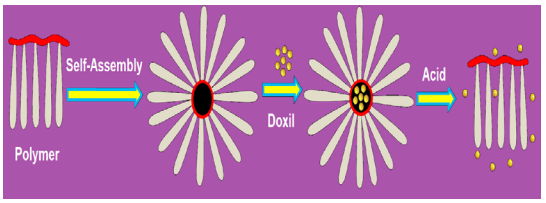

Fig. 12. Overall self-assembly and medicine administration process

CONCLUSION

Using ring opening polymerization of fluorescein-derived norbornene, four fluorescent amphiphilic homopolymers, are synthesized. All so produced polymers are evaluated for cancer-drug delivery purpose. It is proven good for opening a new avenue for designing of vesicle-superstructures for biomedical cancer-drug delivery applications. The hydrophobicity of polymer is increased by adding more number of methylenic carbon chains, -[CH_2]n- with n ranging from 1 to 4, between the norbornene part and the thiobarbiturate part. It displays self-assembly in an aqueous environment. With the help of GPC, molecular weight and polydispersity index are obtained. External pyrene

probe gives the critical aggregation concentrations. DLS measurement gives aggregate size, 127-229nm, whilst FE-SEM provides morphology of vesicles. It is very clear that the starting materials thiobarbiturate and fluorescein creates the vesicle shape of all four polymers. Vesicle aggregates are used to for drug-delivery application of an anti-cancer commercial drug Doxil (chemical name:doxorubicin hydrochloride). Out of the four polymers, it is polymer-1 (hydrophobicity increasing -[CH_2]n- with n values 1) that shows high drug loading efficiency 68%. But, other 3 polymers show little less, ie, 63%, 61%, and 55%, respectively for hydrophobicity increasing -[CH_2]n- with n values 2, 3 and 4 respectively. Furthermore, as far as drug-release efficiency is concerned, again it is polymer-1 that shows more than 92% at pH of around 5.5 and less than 5% at neutral physiological pH of around 7.4. It is proven that the polymer-1 conforms well for the durability of assembly under physiological settings. These findings make these four newly synthesized polymers as promising stimuli-responsive nanocarrier for effective cancer therapy.

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Conflict of Interests

There is no conflict of interests in this work.

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