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THE EFFECT OF MOLECULAR WEIGHT AND CONCENTRATION OF HYPERBRANCHED POLYGLYCEROL FOR SOLUBILISING HYDROPHOBIC DRUGS

RINGKASAN: *Polimer cabang poligliserol larut air dengan teras p-nitrophenol dengan lima berat molekul dan empat kepekatan yang berbeza bagi setiap polimer telah berjaya disintesis dan dicirikan. Poligliserol dengan teras ρ-nitrophenol telah disintesis menggunakan teknik pempolimeran pembukaan cincin anionik dengan menggunakan glisidol sebagai monomer. Lima berat molekul polimer cabang poligliserol yang telah disintesis adalah 4000 Da, 8000 Da, 12500 Da, 25000 Da and 50000 Da. Empat kepekatan yang berbeza bagi setiap polimer juga telah disediakan iaitu 1.0 x 10^{-4} M, 2.0 x 10^{-4} M, 4.0 x 10^{-4} M and 6.0 x 10^{-4} M. Polimer ini telah digunakan untuk melarutkan molekul hidrofobik iaitu naftalena dan ibuprofen. Keterlarutan kedua-dua molekul bertambah selepas pengkapsulan dengan polimer cabang poligliserol. Hasil kajian menunjukkan bahawa keterlarutan naftalena meningkat dari 0.62 x 10^{-4} M kepada 2.33 x 10^{-4} M bagi polimer cabang dengan berat molekul 4000 Da ke 50000 Da dengan kepekatan 1.00 x 10^{-4} M. Untuk ibuprofen, keterlarutan meningkat dari 5.30 x 10^{-4} M kepada 12.88 x 10^{-4} M untuk berat molekul polimer cabang dari 4000 Da ke 50000 Da dengan kepekatan 1.00 x 10^{-4} M.*

ABSTRACT: A water soluble hyperbranched polyglycerol with p-nitrophenol core with five different molecular weights and four different concentrations of each polymer has been synthesised and characterised. Polyglycerol with p-nitrophenol core was synthesized using anionic ring opening polymerization technique with glycidol as the monomer. Five different molecular weights of the hyperbranched polymer were produced i.e 4000 Da, 8000 Da, 12500 Da, 25000 Da and 50000 Da. Four different concentration of each molecular weight of hyperbranched polyglycerol were also prepared which were 1.0 x 10^{-4} M, 2.0 x 10^{-4} M, 4.0 x 10^{-4} M and 6.0 x 10^{-4} M. These hyperbranched polyglycerol were then used to solubilise hydrophobic molecules i.e. naphthalene and ibuprofen. Solubilisation of all molecules increased after encapsulation with the hyperbranched polyglycerol. The results showed that the solubilisation of naphthalene increased from 0.62 x 10^{-4} M to 2.33 x 10^{-4} M for hyperbranched polymer with molecular weight of 4000 Da to 50000 Da with concentration of 1.00 x 10^{-4} M. For ibuprofen, the solubilisation increased from 5.30 x 10^{-4} M to 12.88 x 10^{-4} M for hyperbranched polymer molecular weight of 4000 Da to 50000 Da with concentration of 1.00 x 10^{-4} M.

Keywords: Hyperbranched polymer, solubiliser, encapsulation.

INTRODUCTION

Dendritic polymers including dendrimers and hyperbranched polymers have gained widespread attention by researchers in biomedical fields due to their unique physical and chemical properties. Dendrimers are highly branched macromolecules that are monodisperse, possess a globular shape and have large numbers of controllable surface functionalities. Even though this carrier has perfect characteristics, it is not easy to synthesise. Synthesis is time consuming, and hence it is very expensive (Svenson, 2009). In contrast, hyperbranched polymers have high potential because they exhibits good hydrophilicity, excellent biocompatibility, low/absent immunogenity and high chemical stability (Mei *et al.*, 2012, Rajesh *et al.,* 2007).

Hyperbranched polymers are spherical, branched macromolecules possessing a specific architecture, which consists of the core, the interior branching units and terminal units (Sunder *et al.*, 2000; Frey *et al.*, 2002). The synthesis of controlled hyperbranched polymers was introduced in 1999, using anionic ring opening polymerization of glycidol, with slow monomer addition (Dirk *et al.,* 2011, Daniel, *et al.,* 2009). As a result, hyperbranched polymers with controlled molecular weight and high surface functionality was produced using these method.

These polymers are currently attracting much attention from the pharmaceutical industry as dendritic carriers in drug solubilisation and delivery applications. Recently, hyperbranched polyglycerols have shown potential as drug delivery vehicles for controlled dug delivery, solubilisation of inorganic drugs in organic media and dispersion of polar dyes in hydrophobic polymers (Uhrich, 1997). Studies conducted by *H. Mei et. al* showed that hyperbranched polyglycerol with ester linkages conjugated with methotrexate anticancer drugs micelles exhibited high anticancer efficacy (Mei *et al.*, 2012). Reports by Parag et. al revealed that hyperbranched polyglycerol conjugated with ibuprofen has high drug payloads (around 70%) and suitable as drug delivery carrier (Parag *et al.*, 2004). Studies done by Donald *et al* also showed that the hyperbranched polyglycerols are highly biocompatible in both in vivo and in vitro assay (Muhammad *et al.,* 2012, Donald *et al.,* 2009)

In this work, water soluble hyperbranched glycerol with p-nitrophenol core and glycidol as the monomer using anionic ring opening polymerisation technique was prepared with different molecular weights and concentrations. These hyperbranched polymers were then encapsulated with naphthalene and ibuprofen. The solubilisation effect of naphthalene and ibuprofen after encapsulation with hyperbranched polymers was studied.

EXPERIMENTAL PROCEDURE

Materials

Glycidol, p-nitrophenol, sodium hydride, tris(hydroxymethyl) amino methane, diethylene glycol dimethyl ether and methanol were purchased from Sigma-Aldrich and used as received. NMR samples were prepared using deuterated solvent supplied by Sigma Aldrich.

Hydrophobic model drug selection

The selected compound should be at least partially soluble in aqueous solution and be UV active. A model drug, naphthalene is a compound that consists of two benzene rings fused together and shows some solubility (0.58×10^{-4} M) in water. The second compound which is a nonsteroidal anto-inflammatory drug, ibuprofen, has a carboxylic functional group and shows some solubility (2.68×10^{-4} M) in water. These two compounds are available commercially. The chemical properties of both molecules were shown in Table 1 below.

Table 1. Chemical properties of the molecules used in encapsulation study

room temperature and the solvent discarded. The product, a brown polymer, was dissolved in methanol and then precipitated in 400 ml of acetone. The mixture was left for an hour and the solvent was disposed. ^{1}H NMR (D_2O, 250 MHz) δ_H = 8.16 (s, 1H), 7.05(s, 1H), 2.35-4.05 (b, polymer backbone), 2.16 (s, OH), ^{13}C NMR (D_2O 250 MHz) δ_H = 62.4, 70.8, 78.3; FTIR υ_{max} (cm^{-1}): 3420 (OH), 2873 (CH_2, CH), 1643 (C=C), 1065 (C-O-C); GPC (Mn) 4000

Different molecular weights of hyperbranched polymers were prepared using the same method above by varying the core to monomer ratio. Five different core to monomer ratios were used which were 1:5, 1:10, 1:25, 1:50 and 1:100.

Encapsulation studies

In this study, five different molecular weights (Mn) were used i.e. 4000 Da, 8500 Da, 12500 Da, 27500 Da and 50000 Da. For each molecular weight of the polymer, four different concentrations were prepared, which were 1.00 x 10^{-4} M, 2.00 x 10^{-4} M, 4.00 x 10^{-4} M and 6.00 x 10^{-4} M. Encapsulation studies were carried out using all the above samples. 10 mg of each drug was dissolved in 10 ml of methanol in a vial. The polymer was also dissolved in 10 ml of methanol in different vials. The drug solution was added to each polymer solution prepared. The solution was then physically mixed and the solvent removed under vacuo. Then, 10 ml of TRIS buffer pH 7.4 at 0.1 M was added to the sample. The solution was filtered to remove any undissolved drug molecule. The procedure was repeated at least twice. The absorbance of all samples were recorded at their characteristic wavelengths using UV-Vis spectrophotometer.

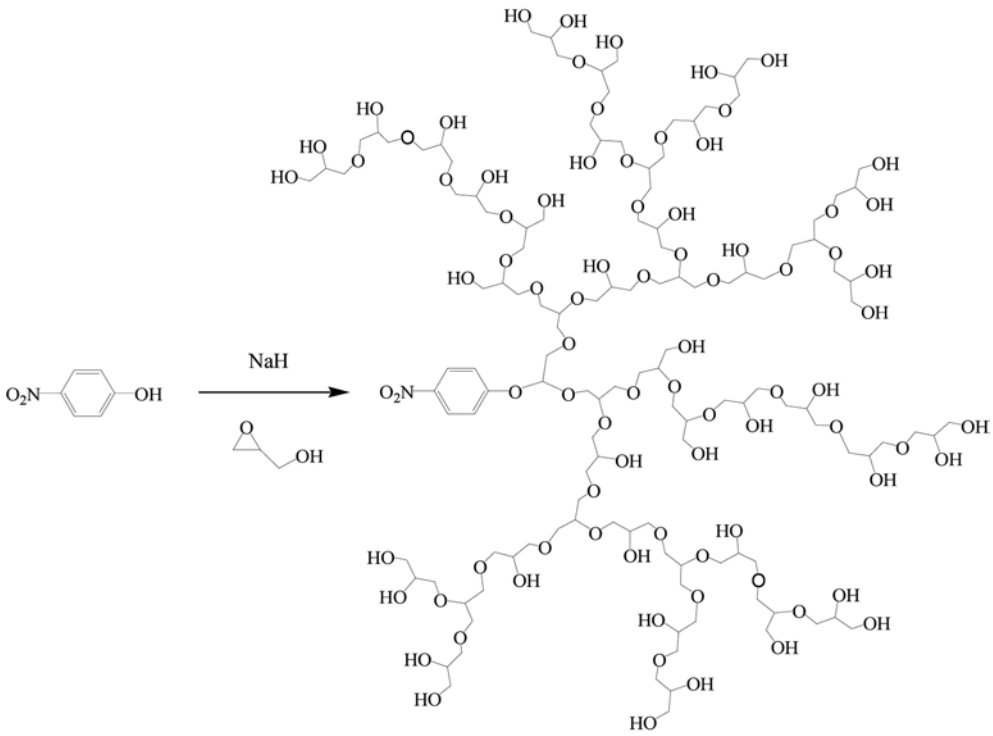
Characterisation

^{1}H NMR was performed at 250 MHz and ^{13}C NMR at 60 MHz using a Bruker AC-250. Aqueous GPC was carried out using Millipore Waters Lambda-Max 481 LC spectrometer with a LMW/HMW column. The eluent used was $NaNO_3$/NaH_2PO4 at pH 7. The machine was calibrated using polyethylene glycol-polyethylene oxide standards (Mn 220-1, 1,000,000 Da). The UV absorbance was assessed using Specord S 600 machine and analysed using WINASPECT spectroanalytical software. The FTIR spectroscopy was performed using Perkin Elmer Spectrum RX FT-IR System in the range of 400 to 4000 cm^{-1}.

RESULTS AND DISCUSSION

Synthesis of water soluble hyperbranched polyglycerol

The water soluble hyperbranched polymer was synthesised using p-nitrophenol as a core and glycidol as the branching monomer (Scheme 1). p-nitrophenol was chosen because it consists of an aromatic structure and an OH group which was easily detected by the ^{1}H NMR and to control the molecular weight of the polymers. Initially, the OH group was deprotonated by sodium hydroxide to form a phenoxide before further propagation with the monomer. This commercially available monomer is a reactive epoxide which represents a latent AB2 monomer (wherein the B groups of the monomer are only activated for polymerisation after the preceding reaction of the A group) (Sunder *et al.*,1999, 2000) that can be further polymerised to hyperbranched polyglycerol with numerous hydroxyl terminal groups. Slow monomer addition was crucial to minimise secondary polymerisation that can occur without the initiator or core. These macromolecules possess a hydrophobic interior and hydrophilic OH groups at the periphery (Danial *et al.*,2010).



Scheme 1. S ynthesis of hyperbranched polyglycerol

Analysis of the hyperbranched polymer produced using ^{1}H NMR revealed that the aromatic protons of the p-nitrophenol core was seen at 8.16 and 7.05 ppm (inset

in Figure 2). A broad peak in the range of 3.4 to 3.9 ppm was attributed to the remaining protons in multiple proton environments within the hyperbranched polymer, which were CH, CH_2 and OH groups (Figure 1).

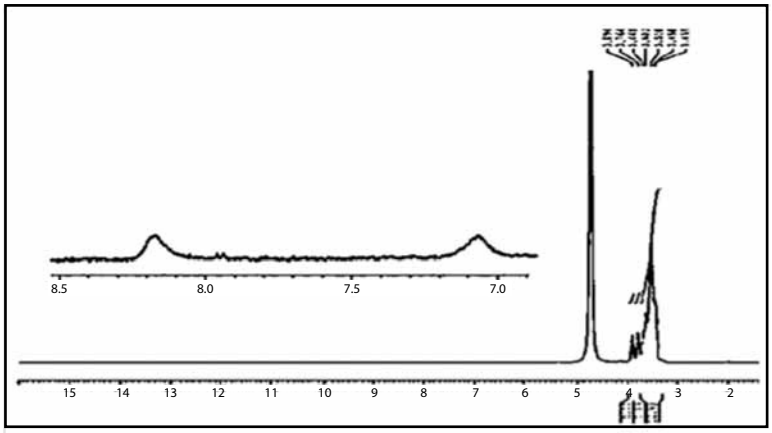


Figure 1. *The ^{1}H NMR spectra of hyperbranched polyglycidol. The spectrum was enlarged at 7.0 to 8.0 ppm indicating protons from ρ-nitrophenol .*

To prove that the hyperbranched polymers were fully synthesised, further characterisation using FTIR was performed. The FTIR result revealed a broad OH peak at 3420 cm^{-1}, CH_2 at 2873 cm^{-1}, a C=C peak at 1643 cm^{-1} and C-O-C at 1065 cm^{-1} as shown in Figure 2.

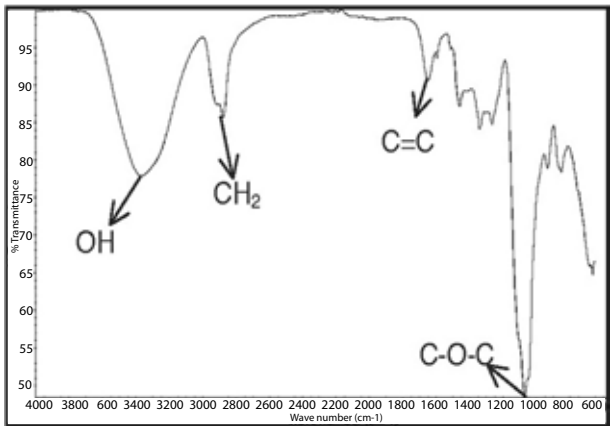


Figure 2. *FTIR spectra of hyperbranched polyglycidol .*

The molecular weight (Mn) of the hyperbranched polymer was determined using GPC. From the analysis, the Mn was 4000 Da. A solubility test showed that the hyperbranched polymer was easily dissolved in water. Taking all the spectral data together, it was concluded that the desired water soluble hyperbranched polyglycerol had been successfully synthesized. Further polymerisation with different core to monomer ratios which were 1:10, 1:25, 1:50 and 1:100 were

synthesised and different molecular weight of hyperbranched polymers were obtained as shown in Table 2.

Table 2. Different molecular weights of the hyperbranched polyglycerol

Polymer ratio	Molecular weight by GPC (Da)	Polydispersity
1:5	4000	1.8
1:10	8500	2.0
1:25	125000	2.3
1:50	27500	3.5
1:100	50000	6.0

Encapsulation studies

The encapsulation studies showed that even though the hyperbranched polymer easily dissolved in water, the guest molecules (naphthalene and ibuprofen) were hardly dissolved. So, a co-precipitation (Beezer *et al.*, 2003) method was used. This method involved the dissolution of water soluble hyperbranched polymer and drug separately in methanol. This is to ensure that the crystal lattice of both substances was fully collapsed. Methanol was then removed in vacuo to give the hyperbranched polymer/naphthalene or ibuprofen co-precipitate. On addition of TRIS buffer solution at 0.1 M and pH 7.4, a complex of infinite water solubility was formed (Beezer *et al.*, 2003). TRIS buffer was used so as to mimic the simulated body fluid in human. The concentration of guest molecules were determined by dividing the absorbance with the extinction coefficient (ε) value of the guest molecule. The extinction coefficient value was determined graphically from a Beer-Lambert plot. The ε value for naphthalene and ibuprofen was 41945 $M^{-1}cm^{-1}$ and 8387 $M^{-1}cm^{-1}$ respectively. The concentration of guest molecules encapsulated inside the hyperbranched polymer was calculated by taking the raw concentration result and substracting with 0.58×10^{-4} M for naphthalene and 2.68×10^{-4} M for ibuprofen (Table 1).

The concentration of naphthalene after encapsulation with a series of hyperbranched polymer of different molecular weights and concentrations are shown in Figure 3. From the bar graph, it was clearly shown that the solubility of naphthalene was zero (after free solubility of naphthalene was taken into account) and increased after encapsulation with hyperbranched polymers.

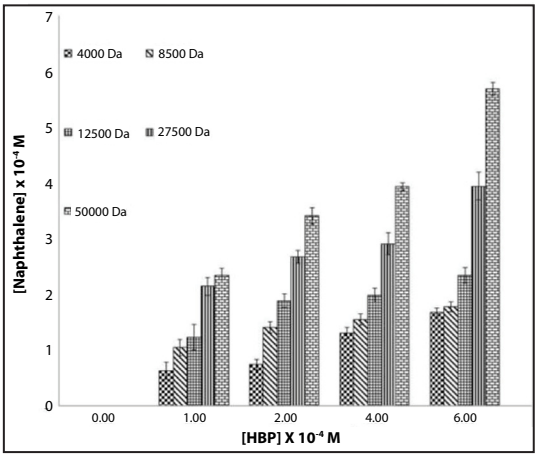


Figure 3. *Increasing molecular weight and concentration of hyperbranched polymers with increasing concentration of encapsulated naphthalene.*

For example, when using hyperbranched polymer with molecular weight of 4000 Da and concentration of 1.00 x 10^{-4} M, the solubility of naphthalene increased from 0.62 x 10^{-4} M to 2.33 x 10^{-4} M at hyperbranched polymer molecular weight of 50000 Da. Similar trend applied to hyperbranched polymer concentration of 6.00 x 10^{-4} M, the naphthalene solubility increased from 1.66 x 10^{-4} M for hyperbranched polymer molecular weight of 4000 Da to 5.70 x 10^{-4} M at 50000 Da. Increased in polymer concentration also increased the solubility of naphthalene which was from 0.62 x 10^{-4} M at polymer concentration of 1.00 x 10^{-4} M to 1.66 x 10^{-4} M at polymer concentration of 6.00 x 10^{-4} M. From the results above, it showed that the hyperbranched polymers have the ability to encapsulate hydrophobic molecules within their hydrophobic voids of the polymer. The hydrophobic interior was developed during the synthesis of the polymer through anionic ring opening polymerisation technique of the monomer (Daniel *et al*., 2010).

Encapsulation with ibuprofen

Encapsulation using ibuprofen was carried out using the same method as previously described. The concentration of ibuprofen after encapsulation was calculated by dividing the actual absorbance by the extinction coefficient (ibuprofen. The ε value was obtained graphically from a Beer-Lambert plot as 8387 $M^{-1}cm^{-1}$. The final data after substracting with 2.68 x 10^{-4} M (Table 1) is shown in Figure 4.

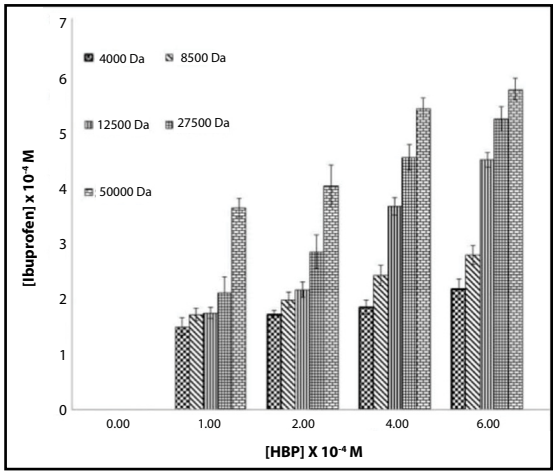


Figure 4. *Increased concentration of encapsulated ibuprofen after encapsulation with different concentrations and molecular weights of hyperbranched polymer.*

The results showed that the concentration of ibuprofen increased as the molecular weight and concentration of the hyperbranched polymer increased. For example, at hyperbranched polymer molecular weight of 4000 Da, the ibuprofen solubility was 5.30 x 10^{-4} M and it rose to 12.88 x 10^{-4} M at polymer molecular weight of 50000 Da. The same pattern was observed where increased in hyperbranched polymer concentration will increase the solubility of ibuprofen. This was clearly seen at polymer molecular weight of 8500 Da, where the solubility of ibuprofen rose from 6.07 x 10^{-4} M to 9.89 x 10^{-4} M.

CONCLUSION

Water soluble hyperbranched polymer with p-nitrophenol core and glycidol as monomer were successfully synthesised using anionic ring opening polymerisation. These polymers were used to solubilise naphthalene and ibuprofen. The results clearly demonstrated that hyperbranched polymers are capable of solubilising the above molecules. For naphthalene, increased in polymer molecular weight from 4000 Da to 50000 Da, increased the naphthalene solubility from 0.62 x 10^{-4} M to 2.33 x 10^{-4} M for polymer concentration of 1.00 x 10^{-4} M. The same results were observed for ibuprofen. For polymer molecular weight of 4000 Da to 50000 Da with polymer concentration of 1.00 x 10^{-4} M, the solubility of ibuprofen increased from 5.30 x 10^{-4} M to 12.88 x 10^{-4} M.

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