STUDIES OF SELF-ASSEMBLED SUBSTITUTED POLY(ACRYLATE) NETWORKS AS POTENTIAL SUSTAINED DRUG DELIVERY SYSTEMS AND OF FLUORESCENT CONJUGATED POLYMER NANOPARTICLES IN CELL IMAGING

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ABSTRACT

Polymer networks are promising biomaterials for drug delivery as they have porous structures and are often biocompatible. The general aspects of the host-guest complexation capability of polymer networks containing cyclodextrins as well as their application in drug delivery are considered in Chapter 1. The introduction of cyclodextrins into polymer networks has the potential to improve drug loading capacity and modulate subsequent drug release behavior due to the host-guest complexation by cyclodextrins of drug molecules. Thus, Chapter 2 and Chapter 3 are concerned with new research on water soluble β-cyclodextrin, adamantyl and octadecyl substituted poly(acrylate) networks, respectively, as potential sustained drug delivery systems.

In Chapter 2, research into self-assembled poly(acrylate) networks cross-linked through host-guest complexation between β-cyclodextrin, β-CD, substituents and adamantyl, AD, substituents as potential sustained drug delivery systems is described. A poly(acrylate) (PAA) 8.8% randomly substituted with β-CD through an ethyl tether, PAAβ-CDen, is synthesized as a host poly(acrylate). Poly(acrylate)s 3.3%, 3.0% and 2.9% randomly substituted with AD substituents, respectively, through ethyl, hexyl and dodecyl tethers in the PAAADen, PAAADhn and PAAADddn are synthesized as guest polymers. The host-guest complexation of PAAADen, PAAADhn, and PAAADddn by PAAβ-CDen in aqueous solution produce three self-assembled poly(acrylate) networks. These complexations are characterized by isothermal titration calorimetry, ITC, 2D NOESY ^{1}H NMR spectroscopy, and rheology. It is found that the length of the tether between the AD group and the poly(acrylate) backbone has a substantial influence on the complexation constants, K_{ITC}, as well as the associated enthalpy change, ΔH, and entropy change, ΔS. The smallest and largest K_{ITC} occur for PAAADen with the shortest tether and PAAADddn with the longest tether, which coincides with the lowest

and highest viscosities occurring for the aqueous PAAβ-CDen/PAAADen and PAAβ-CDen/PAAADddn networks. The complexation of three different dye molecules, acting as drug models, by the β-CD substituents in these networks is characterized by UV-Vis spectroscopy and 2D NOESY ^{1}H NMR studies. The results suggest that dye complexation by the β-CD substituents in the three poly(acrylate) networks is weaker by comparison with the complexation by native β-CD and PAAβ-CDen, as indicated by decreased complexation constants. The poly(acrylate) networks exhibit complexation-controlled dye release behavior, and thereby sustained dye release profiles. Thus, the three poly(acrylate) networks studied, which form hydrogels at higher concentrations, have substantial potential as sustained drug delivery systems.

In Chapter 3, the complexation and release behavior of six dyes in a β-CD- and octadecyl-substituted poly(acrylate) network is explored to further extend the understanding of the host-guest complexation between β-CD substituents and guest molecules within a fourth polymer network system and its influence on the release of guest molecules from the polymer network. Thus, β-CD substituents are 9.3% randomly substituted onto poly(acrylate) through an ethyl tether to give PAAβ-CDen and octadecyl, C18, substituents are 3.5% randomly substituted onto poly(acrylate) to give PAAC18. The network forms through the host-guest complexation between the β-CD substituents and C18 substituents, and is characterized by a complexation constant of $K = 1.13 \times 10^{4}$ dm^{3} mol^{-1}, associated with ΔH = −21.55 kJ mol^{-1} and $T\Delta S$ = 1.59 kJ mol^{-1}. The complexation of the dyes by the β-CD substituents in the PAAβ-CDen/PAAC18 network is characterized by UV-Vis absorption and fluorescence spectroscopy and 2D NOESY ^{1}H NMR studies. The results suggest that the complexation of dyes by the β-CD substituents in the PAAβ-CDen/PAAC18 network is weaker by comparison with the complexation by native β-CD and PAAβ-CDen, as indicated

by decreased complexation constants. The PAAβ-CDen/PAAC18 network exhibits complexation-controlled dye release behavior and thereby sustained dye release profiles. Thus, the PAAβ-CDen/PAAC18 network, or hydrogel at higher concentration, is a potential sustained drug delivery system.

Conjugated polymer nanoparticles are promising fluorescent probes as a consequence of their high brightness and photostability. Chapter 1 introduces the general methods of preparing conjugated polymer nanoparticles and their wide ranges of biological applications. However, conjugated polymer nanoparticles exhibit large-scale aggregation and precipitation at the high ionic strengths encountered under physiological conditions, which presents an impediment to their biological applications. In seeking to address this issue, the research described in Chapter 4 and Chapter 5 addresses stabilization of conjugated polymer nanoparticles using hydrophobic linear alkyl group substituted poly(acrylate)s and bovine serum albumin and explores their deployment in cell imaging applications.

In Chapter 4, the synthesis of hydrophobic linear alkyl group substituted poly(acrylate)s, PAAC*n*, is described as is their employment as conjugated polymer nanoparticle stabilizers. (When n = 18, 16 and 10 the alkyl groups are octadecyl, hexadecyl and decyl, respectively.) The carboxylate groups of PAAC*n* increase the surface charge of the conjugated polymer nanoparticles and thereby stabilize them in phosphate buffered saline, PBS. Nanoparticles of the green-yellow emitting conjugated polymer, F8BT, stabilized with PAAC*n*, F8BT-PAAC*n*, are prepared using a nano-precipitation method. In contrast to the significant aggregation with a negligible yield (~ 0%) of bare F8BT nanoparticles in PBS, high yields approaching 90% are observed for F8BT nanoparticles stabilized with PAAC18 at 1%, PAAC16 at 3%, and PAAC10 at 10% substitution. The F8BT-PAAC*n* nanoparticles have small sizes ranging from 50 to 70 nm in diameter, highly negative surface charge and high colloidal stability over 4 weeks in PBS. These properties pave the way for the deployment of F8BT-PAAC*n*

nanoparticles in biological applications. Spectroscopic results indicate the PAAC*n* has no adverse effect on the UV-Vis absorptivity and fluorescence brightness of F8BT-PAAC*n* nanoparticles relative to bare F8BT nanoparticles. In addition, F8BT-PAAC*n* nanoparticles are internalized by HEK 293 cells and exhibit negligible cytotoxicity. Thus, PAAC*n* are versatile and robust stabilizing materials that facilitate the application of F8BT-PAAC*n* nanoparticles as fluorescent probes in cell imaging.

The research described in Chapter 5 shows that bovine serum albumin, BSA, stabilizes conjugated polymer nanoparticles in phosphate buffered saline, PBS, evidently due to the combined effects of the negatively charged surfaces arising from the BSA carboxylate groups and the steric effect of the bulk 3D structure of BSA. Three multicolored conjugate polymers, PDOF, F8BT, and MEHPPV, are employed to prepare their corresponding nanoparticles using a nano-precipitation method. In contrast to the significant aggregation with negligible yields (~ 0%) of bare conjugated polymer nanoparticles occurring in PBS, high yields approaching 100% are observed for conjugated polymer nanoparticles stabilized with BSA, CP-BSA nanoparticles, in PBS. These CP-BSA nanoparticles have small sizes ranging from 20 to 60 nm, negative surface charges and high colloidal stability. Spectroscopic results indicate the BSA has no adverse effect on the UV-Vis absorptivity and fluorescence brightness of the CP-BSA nanoparticles relative to bare conjugated polymer nanoparticles. These properties potentially pave the way for the deployment of these CP-BSA nanoparticles in biological applications.

DECLARATION

This is to declare that the work presented within this thesis is original and was carried out at the University of Adelaide during the period of 2011-2016. This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is given.

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ABBREVIATIONS

General

ΔH	enthalpy change
ΔS	entropy change
ITC	isothermal titration calorimetry
UV-Vis	ultraviolet/visible
A	observed absorbance
ε	molar absorbance (mol^{-1} dm^3 cm^{-1})
F	observed fluorescence
f	molar fluorescence
et al.	et alia
Hz	Hertz
I	ionic strength (mol dm^{-3})
K	complexation constants (dm^3 mol^{-1})
NMR	nuclear magnetic resonance
δ	chemical shift (ppm)
NOESY	nuclear overhauser enhancement spectroscopy
ROESY	rotating frame overhauser enhancement spectroscopy
pH	$-\log[H^+]$
ppm	parts per million
T	temperature
wt	weight
Φ	fluorescence quantum yield
η	refractive index

Chemicals

α-, β-, γ-CD	α-, β-, γ-cyclodextrin
AD	adamantyl
en	ethyl

hn	hexyl
ddn	dodecyl
β-CDen	6^A-(2-aminoethyl)amino-6^A-deoxy-6^A-β-CD
ADen	1-(2-aminoethyl)amidoadamantyl
ADhn	1-(6-aminohexyl)amidoadamantyl
ADddn	1-(12-aminododecyl)amidoadamantyl
PAAβ-CDen	β-CDen randomly substituted poly(acrylate)
PAAADen	ADen randomly substituted poly(acrylate)
PAAADhn	ADhn randomly substituted poly(acrylate)
PAAADddn	ADddn randomly substituted poly(acrylate)
MR	sodium salt of methyl red
MO	sodium salt of methyl orange
EO	sodium salt of ethyl orange
C18	octadecyl
PAAC18	octadecyl randomly substituted poly(acrylate)
ANS	sodium 8-anilinonaphthalene-1-sulfonate
TNS	sodium 6-(*p*-toluidino)naphthalene-2-sulfonate
BNS	sodium 6-(*p*-*t*-butylphenylamino)naphthalene-2-sulfonate
C6	hexyl
C10	decyl
C12	dodecyl
C14	tetradecyl
C16	hexadecyl
PAAC6	hexyl randomly substituted poly(acrylate)
PAAC10	decyl randomly substituted poly(acrylate)
PAAC12	dodecyl randomly substituted poly(acrylate)
PAAC14	tetradecyl randomly substituted poly(acrylate)
PAAC16	hexadecyl randomly substituted poly(acrylate)
F8BT	poly[(9,9-dioctylfluorenyl-2,7-diyl)-alt-co-(1,4-benzo-[2,1,3]-thiadiazole)]
PDOF	poly(9,9-dioctylfluorenyl-2,7-diyl)-End capped with DMP
MEHPPV	poly[2-methoxy-5-(2-ethylhexyloxy)-1-4-phenylenevinylene]-End capped with DMP

BSA	bovine serum albumin