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Influence of EDA-π interactions in drug encapsulation using nanospheres†

Sunil K. Sharma,[a,b,c] Rajesh Kumar,[a,b] Sumit Kumar,[c] Ravi Mosurkal,[a] Virinder S. Parmar,*[c] Lynne A. Samuelson,[d] Arthur C. Watterson*[b] and Jayant Kumar*[a]

[a] *Center for Advanced Materials (CAM), Departments of Chemistry and Physics, University of Massachusetts, Lowell MA 01854, USA*

[b] *Institute for Nano-Science Engineering and Technology (INSET), Departments of Chemistry and Physics, University of Massachusetts, Lowell MA 01854, USA*

[c] *Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi 110 007, India*

[d] *U.S. Army RDECOM, Natick Soldier Center, Kansas Street, Natick MA 01760, USA*

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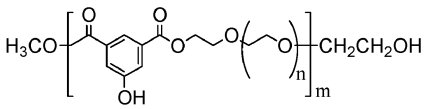
We have evaluated the influence of aromatic and hydrophobic interactions on the strength and selectivity of encapsulation using polymeric nanospheres.

Studies on inter- and intra-molecular interactions are becoming an increasingly active area of research for chemists and biologists. Chemists have learned to design and synthesize receptors where the nature and arrangement of their functional groups, their degree of flexibility, and their size and shape are in accordance with a rational plan.[1] The advantage of having available a variety of receptors with different properties for the study of molecular encapsulation phenomena is well emphasized in many recent papers.[2]

Nanomaterials including nanoscale composite materials have shown great promise in biological and medicinal applications such as drug delivery, gene delivery, and tissue engineering.[3] Our interest is in developing nano-structured materials for delivering drugs that have poor water solubility. Such lipophilic drugs are reported to have side effects due to their accumulation in several tissues other than the target tissue. Nanoparticles (NPs) represent a very promising approach to overcoming this problem.[4] Nanometer size drug carriers with hydrophilic surfaces are found to evade recognition and uptake by the RESs (reticulo-endothelial systems), and thus can circulate in the blood for a longer time. Furthermore, owing to their extremely small size, these nanoparticles extravasate at the pathological sites such as solid tumors through a passive targeting mechanism.

We have recently reported the synthesis and characterization of novel polymers based on poly(ethylene glycol)s (PEGs) and C-5 substituted isophthalates.[5] These polymers consist of hydrophilic groups (PEGs) and aromatic segments to promote aggregation tendency in aqueous medium. These amphiphilic polymeric systems were characterized by detailed spectroscopic analysis. Of these, we found polymer **1a** consisting of 5-hydroxy isophthalate and PEG units (average M_n 600) of particular interest.



1a

The 5-hydroxy isophthalate unit not only confers hydrophobicity to the polymeric system but also, owing to the symmetrical distribution of substituents along the aromatic moiety, keeps the steric effects to a minimum. Furthermore, the aromatic hydroxyl group can be used as an attachment site to link different functionalities. Attaching a decane chain at the 5-hydroxyl group of the polymer **1a** dictates a good balance between hydrophobic and hydrophilic groups and the resulting polymer **1b** (Fig. 1) was found to aggregate in aqueous solution forming nanospheric particles. The latter polymer has molecular weight (M_n) of 18 730 and an aggregation number of 8–10. The static root mean square radius (R_g, 17.3 ± 2.1 nm) and the hydrodynamic radius (R_h, 9.56 ± 1.2 nm) of the micelle were measured by light scattering techniques. The nanospheres consist of two spherical co-centric hydrophobic and hydrophilic regions (Fig. 1), where the inner hydrophobic region can be used for entrapping the hydrophobic compounds and the outer shell made up of hydrophilic PEGs confers solubility in aqueous systems. The inner core of our nanospheric system consists of two components, *i.e.* the electron-rich aromatic walls and hydrophobic long alkyl chains. We expect these nanospheres to be ideal for investigation of the influence of aromatic and hydrophobic interactions on the strength and selectivity of encapsulation in a hydrophilic surrounding. Molecular modeling studies using the HyperChem software suggest that aromatic units and alkyl chains would come close together forming the inner hydrophobic core. In this process, the phenyl rings would tend to stabilize the system by orienting themselves in nearly parallel positions so as to maximize the π–π interactions. The alkyl chains tend to line up in a nearly parallel fashion (Fig. 1). This was further supported by light scattering data as the nanosphere formed by **1b** has fairly good thermodynamic stability till 55 °C; however, replacing the aromatic unit by a non-aromatic aspartate unit in polymer **2** significantly reduces (to 40 °C) the nanosphere stability.

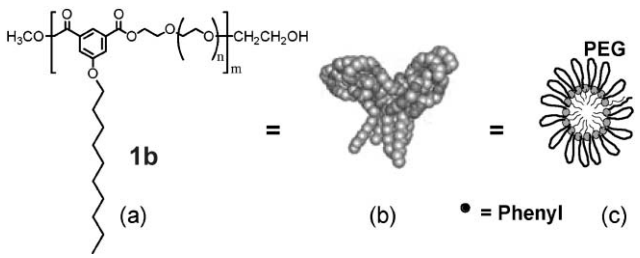
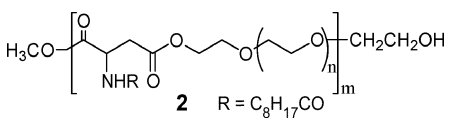


Fig. 1 (a) Molecular structure of polymer **1b**; (b) MM+ optimized geometry; (c) schematic view of formation of micellar nanoparticles.



2 R = $C_8H_{17}CO$

We have chosen a series of aromatic guests with different electronic properties to study their encapsulation. This would be helpful in understanding the factors that govern the encapsulation process at a molecular level. Preliminary investigations indicated that the polymer **1b** encapsulated electron deficient aromatic guests more efficiently. The observed variation in the chemical shifts of the protons of guest molecules provides clear evidence for encapsulation. The aromatic protons of the encapsulated guest molecule shifted upfield – this is characteristic of the aromatic shielding.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b4/b408993f/

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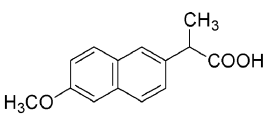
Various factors, *e.g.* π–π interactions, hydrophobic interactions and hydrogen bonding, seem to be operative in the encapsulation phenomenon. Initially we focused our study to evaluate the contribution of aromatic and hydrophobic effects, as under aqueous conditions the water molecules may compete for hydrogen bonding. Charge transfer (CT) interactions do not seem to contribute significantly since changes in the UV/visible region were not observed after addition of guests. To address the question whether or not hydrophobic effects are enough to establish encapsulation in water, we attempted the encapsulation of naproxen (**3**) and aspirin (**4**).



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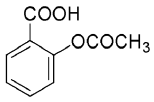

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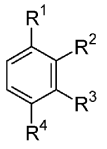
Although naproxen has a larger lipophilic area, it was encapsulated to a lesser extent (6%) compared with aspirin encapsulation (20%). This may be explained in terms of electronic structural differences, *i.e.* in aspirin the carboxylic group is attached to the aromatic ring and its negative inductive effect may reduce the electron density of the aromatic ring. However, in naproxen, the carboxylic group is in the side chain and thus does not have much effect on the aromatic electron density. These facts further suggest that electronic complementarity contributes significantly towards encapsulation. It has also been observed that all the protons of the encapsulated drug do not shift by the same magnitude on encapsulation, *e.g.* in case of aspirin (**4**), the protons at the C-3, C-4, and C-5 positions shifted upfield (Fig. 2); however, no shift was observed for the C-6 proton that is adjacent to carboxylic acid group (Table 1). Since the carboxylic acid group is hydrophilic it has the tendency to approach the aqueous phase and thus would tend to orient the aspirin molecule in the hydrophobic core of the nanosphere in such a way that the encapsulation occurs chiefly through the aromatic site with the ester group ($OCOCH_3$) as well as the C-3, C-4, and C-5 positions of the phenyl ring. Thus the proton at C-6 would not be significantly influenced by the polymer aromatic rings and would have negligible interaction with the inner hydrophobic core of the nanosphere.

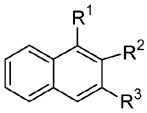
An identical pattern of ^{1}H NMR chemical shifts was observed while encapsulating *ortho*-, *meta*-, and *para*-nitrobenzoic acids (NBA, **6**–**8**), *i.e.* all the protons except the one adjacent to the carboxylic acid group shifted upfield while the one adjacent to the carboxylic acid group shifted least or not at all.



5 $R^1 = COOCH_3$; $R^2 = OAc$; $R^3 = R^4 = H$
6 $R^1 = COOH$; $R^2 = NO_2$; $R^3 = R^4 = H$
7 $R^1 = COOH$; $R^3 = NO_2$; $R^2 = R^4 = H$
8 $R^1 = COOH$; $R^4 = NO_2$; $R^2 = R^3 = H$
9 $R^1 = COOCH_3$; $R^4 = NO_2$; $R^2 = R^3 = H$
10 $R^1 = COOH$; $R^4 = OCH_3$; $R^2 = R^3 = H$
11 $R^1 = COOH$; $R^4 = OAc$; $R^2 = R^3 = H$
12 $R^1 = OH$; $R^3 = NO_2$; $R^2 = R^4 = H$

On encapsulation, the *ortho* protons (C-2H and C-6H) do not shift for *p*-NBA (**8**), they shifted by 0.01 ppm for *o*-NBA (**6**) and by 0.03 ppm for *m*-NBA (**7**). Interestingly, the proton adjacent to the nitro group shifted to the greatest extent, *i.e.* for *o*-NBA the C-3 proton shifts by 0.13 ppm, in the case of *m*-NBA encapsulation, the C-2 and C-4 protons shifted by 0.17 and 0.19 ppm, respectively. A maximum shift of 0.33 ppm was observed for the C-3 and C-5 protons for encapsulated *p*-NBA (Table 1). These data suggest that the electron withdrawing nitro group leads to a stronger interaction with the aromatic groups of the polymeric nanosphere. To further support our hypothesis, we studied the ^{1}H NMR chemical shifts for 4-methoxybenzoic acid (**10**) and 4-acetoxybenzoic acid (**11**) and observed a similar phenomenon in these two cases, *i.e.* the protons adjacent to the carboxylic group shift by the least amount in comparison with the protons adjacent to methoxy (in **10**) or acetoxy (in **11**) groups (Table 1). However, the magnitude of chemical shift was less than those for nitro compounds. In order to further establish the effect of the carboxylic group on the orientation of encapsulated molecule, we derivatized aspirin to its methyl ester **5**. The carbomethoxy moiety is relatively lipophilic as compared to carboxylic acid, the former should have no affinity for aqueous region and thus it may not impose any restriction on the orientation of the encapsulated molecule. The observed chemical shift values for the encapsulated **5** are in accordance with our hypothesis as all the aromatic protons shifted upfield (Table 1). Also, we observed a similar phenomenon in the case of methyl 4-nitrobenzoate (**9**), *i.e.* in the methyl ester of **8**, we did not observe any preference of orientation on encapsulating compound **9** and all the protons shift towards lower δ-values in comparison to the unencapsulated sample (Table 1). In the case of compound **12** that lacks the carboxylic acid group, all the protons were observed to shift on encapsulation.

To further confirm our hypothesis of orientation of the guest, we have studied the chemical shifts of naphthoic acids **13**–**17** after encapsulating them in the same polymeric system **1b**.



13 $R^1 = COOH$; $R^2 = R^3 = H$
14 $R^1 = R^3 = H$; $R^2 = COOH$
15 $R^1 = OH$; $R^2 = COOH$; $R^3 = H$
16 $R^1 = COOH$; $R^2 = OH$; $R^3 = H$
17 $R^1 = H$; $R^2 = COOH$; $R^3 = OH$

On comparing the chemical shift differences of different aromatic protons in the native naphthoic acids **13** and **14** and their encapsulated forms, low chemical shift differences were observed (0.02–0.06 ppm) for **13**, as compared to **14** (0.04–0.11 ppm). This may be due to the difference in positioning of the carboxylic group. In compound **13**, the acid group may have pulled the entire aromatic ring cluster out of the nanosphere; however, in 2-naphthoic acid (**14**), the carboxylic group is so positioned that after encapsulation, still a part of the aromatic moiety remains in the nanosphere. The observance of significant chemical shifts

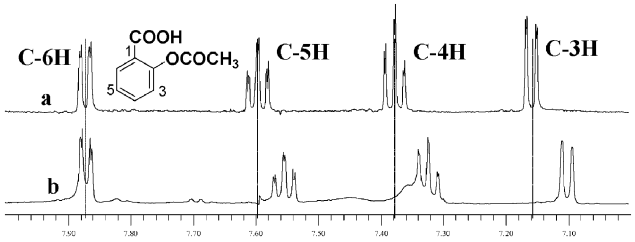


Fig. 2 ^{1}H NMR spectra of (a) aspirin in D_2O; (b) aspirin encapsulated in the nanosphere **1b** (Fig. 1).

Table 1 Comparison of the ^{1}H NMR chemical shift values (δ) of the aromatic compounds before and after (in parentheses) encapsulation

Compound	C-2H	C-3H	C-4H	C-5H	C-6H
2-Acetoxybenzoic acid (**4**)	—	7.16	7.38	7.60	7.87
	—	(7.10)	(7.32)	(7.55)	(7.87)
Methyl 2-acetoxybenzoate (**5**)	—	7.22	7.43	7.68	8.00
	—	(7.15)	(7.36)	(7.62)	(7.93)
2-Nitrobenzoic acid (**6**)	—	8.06	7.78	7.65	7.67
	—	(7.93)	(7.72)	(7.61)	(7.66)
3-Nitrobenzoic acid (**7**)	8.76	—	8.43	7.72	8.33
	(8.59)	—	(8.24)	(7.62)	(8.30)
4-Nitrobenzoic acid (**8**)	8.09	8.29	—	8.29	8.09
	(8.09)	(7.96)	—	(7.96)	(8.09)
Methyl 4-nitrobenzoate (**9**)	8.33	8.20	—	8.20	8.33
	(8.03)	(7.91)	—	(7.91)	(8.03)
4-Methoxybenzoic acid (**10**)	7.85	7.01	—	7.01	7.85
	(7.82)	(6.90)	—	(6.90)	(7.82)
4-Acetoxybenzoic acid (**11**)	7.96	7.20	—	7.20	7.96
	(7.94)	(7.12)	—	(7.12)	(7.94)
3-Nitrophenol (**12**)	7.67	—	7.77	7.45	7.25
	(7.55)	—	(7.63)	(7.35)	(7.17)

Table 2 Comparison of the ^{1}H NMR chemical shift values (δ) of naphthoic acids before and after (in parentheses) encapsulation

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8
1-Naphthoic acid (**13**)	—	8.18	7.48	7.92	7.58	7.55	7.55	7.92
	—	(8.16)	(7.46)	(7.86)	(7.56)	(7.53)	(7.53)	(7.86)
2-Naphthoic acid (**14**)	8.36	—	7.92	7.90	7.90	7.56	7.56	7.99
	(8.32)	—	(7.86)	(7.79)	(7.79)	(7.45)	(7.45)	(7.89)
1-Hydroxy-2-naphthoic acid (**15**)	—	—	7.77	7.36	7.84	7.59	7.54	8.23
	—	—	(7.66)	(7.20)	(7.71)	(7.44)	(7.38)	(8.1)
2-Hydroxy-1-naphthoic acid (**16**)	—	—	7.12	7.80	7.86	7.35	7.52	8.75
	—	—	(7.05)	(7.70)	(7.77)	(7.28)	(7.46)	(8.85)
3-Hydroxy-2-naphthoic acid (**17**)	8.38	—	—	7.22	7.71	7.33	7.50	7.88
	(8.24)	—	—	(6.99)	(7.47)	(7.14)	(7.29)	(7.68)

(0.11–0.24) in hydroxynaphthoic acids **15** and **17** may be due to the fact that in both of these compounds, the hydroxyl group is at an adjacent position with respect to the C-2 carboxylic acid group and thus would bring the substituted part of the aromatic ring towards bulk solvent, while the remaining aromatic part would remain in the nanosphere thus enhancing the aromatic–aromatic interactions. However, the substituents in compound **16** are so placed that they would try to bring the entire aromatic moiety out of the nanosphere and this leads to weaker aromatic interactions between the guest and the nanosphere. Interestingly, in all the naphthoic acids, the proton adjacent to the carboxylic group undergoes the minimum shift in its ^{1}H NMR spectrum upon encapsulation (Table 2).

Further we investigated the effect of temperature on the chemical shift (^{1}H NMR) values of encapsulated aspirin and noticed a chemical shift reversal towards low field for the protons at C-3, C-4, and C-5 on gradually increasing the temperature (30–50 °C). This further substantiates that weak, non-covalent interactions must be operating during encapsulation.

In conclusion, the polymer **1b** is the first studied member of a new type of amphiphilic nanospheres which were designed to achieve encapsulation of aromatic guests mainly by EDA-π interactions. A modification in either the aromatic or aliphatic moieties will allow the preparation of a variety of nanospheres with distinctive encapsulation properties. Our investigation would enable the design and development of a model polymer system that has high drug affinity, controlled release profile for the incorporated drug, and good compatibility between the core forming block and incorporated drug.

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