This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Design of Nanoscopic Vehicles for Drug Targeting Based on Micellization of Amphiphiuc Block Copolymers

Kazunori Kataoka

To cite this Article Kataoka, Kazunori(1994) 'Design of Nanoscopic Vehicles for Drug Targeting Based on Micellization of Amphiphiuc Block Copolymers', Journal of Macromolecular Science, Part A, 31: 11, 1759 — 1769
To link to this Article: DOI: 10.1080/10601329408545880
URL: http://dx.doi.org/10.1080/10601329408545880

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J.M.S.—PURE APPL. CHEM., A31(11), pp. 1759–1769 (1994)

DESIGN OF NANOSCOPIC VEHICLES FOR DRUG TARGETING BASED ON MICELLIZATION OF AMPHIPHILIC BLOCK COPOLYMERS

KAZUNORI KATAOKA

Department of Materials Science;
Research Institute for Biosciences
Science University of Tokyo
2641 Yamazaki, Noda, Chiba 278, Japan
FAX: +81-471-23-9362

Downloaded At: 16:18 24 January 2011

ABSTRACT

Block copolymers in a selective solvent (a good solvent for one block but a nonsolvent for the other) form micellar structures through the association of the insoluble segments. Micelles formed through the association of amphiphilic block copolymers have several advantageous features as drug carrier systems due to their considerable thermodynamic stability as well as to the formation of a hydrophobic core separated from the outer aqueous milieu by the palisade of hydrophilic segments. The core acts as the microcontainer of hydrophobic drugs. This paper presents a concept and strategy for using a block copolymer micelle as a nanoscopic virus-mimicking carrier of anticancer drugs to treat solid tumors.

ADVANTAGEOUS FEATURES OF BLOCK COPOLYMER MICELLES AS VEHICLES FOR DRUG TARGETING

The selective delivery of anticancer drugs to malignant cells via drug carriers presents one approach to rationale drug therapy [1]. Since Paul Ehrlich's seminal perspectives on site specific delivery [2], many different drug carriers (e.g., monoclonal antibodies, soluble polymers, liposomes, and polymeric microspheres) have

Copyright © 1994 by Marcel Dekker, Inc.

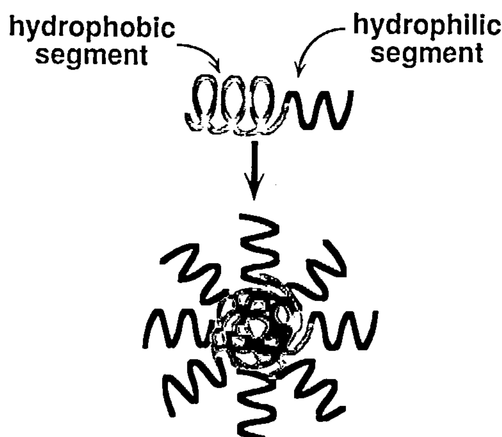

FIG. 1. Multimolecular micelle formation of AB block copolymers.

Downloaded At: 16:18 24 January 2011

been investigated. While an ideal case of drug targeting (i.e., Ehrlich's Magic Bullet) has not been realized, much has been learned about drug targeting (e.g., biological constraints) and in many cases greater targeting efficacy via drug carriers has been achieved.

We have been investigating micelle-forming polymer-drug conjugates as drug carriers [3-9]. Copolymers composed of hydrophobic and hydrophilic segments have the potential to form micellar structures in aqueous milieu [10, 11]. Although block copolymers of various types have the potential to form micellar structures, AB-type block copolymers are the most appropriate candidates for designing the size, the aggregation number, and the stability of the micelles formed due to their simple molecular architecture as schematically shown in Fig. 1. The hydrophobic segment forms the hydrophobic core of the micelle, while the hydrophilic segment surrounds the core as a hydrated outer shell. Since most drugs have a hydrophobic character, these drugs are easily incorporated in the inner core segment by covalent bonding or noncovalent bonding through hydrophobic interaction with core-forming segments. The features of block copolymer micelles relevant for drug delivery are summarized in Table 1.

TABLE 1. Properties of Polymeric Micelles Relevant for Drug Delivery

Small size (~20 nm)
Apparent thermodynamic stability
Solubilization of hydrophobic drugs
Low RES uptake (i.e., stealth properties)
Modified biodistribution of drugs
Storage stability in freeze-dried form
Size of micelle may allow direct extravasation

The primal advantage of micelle formation is the resultant long-term circulation in the blood as renal filtration and reticuloendothelial system (RES) uptake are avoided. Since polymeric micelles have a diameter in the range of several tens of nanometers, they are expected to show low renal clearance. Even if the molecular weight of the constituting chains is lower than the critical molecular weight for renal filtration, these polymer chains can escape from renal excretion by forming the micelle structures. Further, polymeric micelles may have a low incidence of uptake by RES due to the low interfacial energy and high steric repulsion of the hydrophilic palisades surrounding the micelle-core. Their size may also contribute to decrease RES uptake, because RES recognition is believed to reduce for particles with diameter less than ~100 nm. In this context, it is of interest to notice the structural similarity of polymeric micelles with natural vesicular systems such as viruses and lipoproteins [12, 13]. These systems are all based on the supramolecular assembly of macromolecules to form nanoscopic vehicles having a microcontainer separated from the outer environment. Further, the nanoscopic size range (20–50 nm) of polymeric micelle is advantageous for extravasation, which should be an essential process for the carrier to reach the target (for example, a solid tumor) located outside of the capillaries [14]. It is to be noted that polymeric micelles have soft-shell/hard-core structures in which the densely packed core is surrounded by a highly hydrated and flexible shell. This flexible nature of the micelle palisade may contribute to its penetration through the vessel wall and interstitial space in the body.

Downloaded At: 16:18 24 January 2011

Although durability in the blood compartment might be achieved by other types of carrier including macromolecules with a considerable molecular weight as well as crosslinked particles in the nanoscopic size range, a polymeric micelle has a superior advantage which would be difficult for other carriers to achieve; that is, a controlled clearance through the programmed decay or dissociation of a micellar structure. Since the polymeric micelles are formed by intermolecular noncovalent interactions of single polymer chains (unimers), they can finally be decayed into unimers with a considerably low rate of dissociation, resulting in excretion from the renal route so far as the molecular weights of unimers are designed to be lower than the critical values for renal filtration. Therefore, polymeric micelles are expected to lower the toxicities associated with long-term accumulation in tissues, achieving controlled clearance through a balance with a prolonged half-life due to micelle formation.

From the pharmaceutical point of view, increased solubility is a great advantage of polymeric micelle systems. As mentioned before, many drugs are rather hydrophobic, and this may cause nonspecific tissue adsorption, resulting in an increase in the distribution volume. Inserting a hydrophobic drug into the core of the micelle will surely answer these problems and increase the applicability of the drugs in many dosage forms. Obviously, stability of drugs is also increased through micelle incorporation. Inactivation of the drug molecules can be avoided by decreasing contact with inactivating species in the aqueous (blood) phase (water and specific enzymes) [4]. Furthermore, a decreased dielectric constant in a hydrophobic environment affects the release rate of the bound drug, in most cases decreasing the release rate. The release rate is controlled by the stability of the micelles, the hydrophobicity of the micelle core, and the chemical species used for binding the drug to the polymer backbone.

Drug hydrophobicity often causes a serious problem in design of a drug-polymer conjugate due to precipitation of the conjugate during synthesis [15-17] or administration [18]. This is particularly the case when the hydrophilic functional groups (e.g., amino and carboxyl groups) of the drug and/or polymer are used for conjugation and are changed into more hydrophobic groups (e.g., amide group). In turn, this drawback can be dramatically changed into an advantage for the formation of micellar structures with hydrophobic inner-cores. Block copolymers composed of a hydrophilic segment and a drug-conjugating segment can maintain their water solubility by embedding a hydrophobic moiety in the core of the micelle to prevent progressive aggregation. These copolymers possess a high loading capacity for hydrophobic drugs per polymer chain.

The thermodynamic stability of block copolymer micelles is worth noticing. Block copolymers composed of hydrophilic and hydrophobic segments are known to form micellar structures at much lower critical micelle concentration (cmc) values [19, 20] than those for low molecular weight surfactants. The reason is that one block-copolymer molecule has more interaction sites for other polymers than do low molecular weight surfactants, resulting into a considerable increase in cohesive energy. Dissociation of polymeric micelles is extremely slow compared with that of surfactant micelles composed of low-molecular weight amphiphilies. In sharp contrast with a considerably higher dissociation rate of surfactant micelles, which is in the order of milliseconds, polymeric micelles are considered to dissociate with a rate in the order of hours or more (an example will appear in a later part of the text as well as in Fig. 3). These properties are advantageous for retaining stable micellar structures under in-vivo conditions for longer periods of time. Further, a micelle structure surrounded by a palisade of hydrophilic segment makes it possible to control in-vivo distribution of the block copolymers based solely on the properties of the palisade, which allows similar profiles of distribution to be achieved for micelles with inner-cores of different properties.

Downloaded At: 16:18 24 January 2011

PREPARATION OF MICELLE-FORMING POLYMERIC DRUG WITH BOUND ADRIAMYCIN

Not many studies have focused on the application of block copolymer micelles as drug carriers. Although Schmolka pointed out in his review the feasibility of using Pluronic (PEO-PPO block copolymer) micelles to increase the solubility of fluorocarbons as oxygen carrier in blood [21], research directed toward the application of polymeric micelles as drug carriers did not appear until 1984 when Ringsdorf et al. [22] reported work directed at an application of polymeric micelles for the sustained release of drugs. Their system was based on a block copolymer of poly(ethylene oxide) and poly(L-lysine) partially modified with long alkyl chains to increase hydrophobicity. An anticancer agent was conjugated to pendant amino groups of the poly(L-lysine) segment via an alkyl spacer with a labile ester linkage. Micelle formation of this polymer-drug conjugate was suggested based on data for dye solubilization [23], and its advantageous characteristics for drug formulations was shown through retardation in the release of a bound drug.

A similar approach to preparing a micelle-forming polymer-drug conjugate was developed by our group, starting from a block copolymer of poly(ethylene

oxide) (PEO) and poly(aspartic acid) (PEO/PAsp) [3-9]. In our case, the hydrophobicity of the conjugated drug itself was utilized as the driving force for micellization. Adriamycin (ADR), a hydrophobic anticancer drug, was chosen for this purpose. It was conjugated to the pendant carboxylic acid groups of PEO/PAsp through an amide bond, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as the coupling agent. The chemical structure of the conjugate, PEO-P[Asp(ADR)], is shown in Fig. 2. The synthesis of the conjugate has been described in detail elsewhere [3, 5, 7, 24]. The strong hydrophobicity and cohesive force of ADR molecules are favorable for micelle formation with sufficient stability in the blood compartment where such natural detergents as proteins and lipids exist in relatively high concentrations. From a pharmacological point of view, the cardiotoxicity of ADR is expected to be diminished upon incorporation into the micelle core due to a change in biodistribution.

Poly(aspartic acid) (PAsp) in PEO/PAsp as a drug-conjugating moiety has the advantage of biodegradability, which may contribute to reducing the chronic accumulation of carrier in the body. In polymeric micelle drugs, segments constituting the outer palisade region should be biologically inert to avoid foreign body reactions. For this purpose, PEO is quite suitable because it has low interaction with proteins and cells [25-27], and low toxicity.

Micelle formation of PEO/P[Asp(ADR)] conjugates thus prepared was confirmed by dynamic light scattering (DLS) [4, 5, 7] as well as by gel permeation chromatography (GPC) [6, 7, 28]. The hydrodynamic radius of the micelles determined by DLS was in the 15 to 60 nm range. It is to be noted that this size range approximately corresponds to that of viruses. Micelle diameter as well as micelle stability have a close correlation to the composition of the conjugate, including the molecular weight of both segments and the ADR content [7, 29]. In PEO/PAsp, with M_n ranging from 1,000 to 12,000 for PEO and from 900 to 8,100 for PAsp segments, most compositions formed micelles through ADR conjugation, but samples with short PEO segments and relatively long PAsp segments had a low tendency to form multimolecular micellar structure. The ratio of the two blocks seems to be a determining factor for micelle stability, as is the case with other block copolymer systems.

The conjugated-ADR content also has a crucial effect on micelle formation. Our recent study revealed that a 40-50% substitution of carboxylic acid residues in

Downloaded At: 16:18 24 January 2011

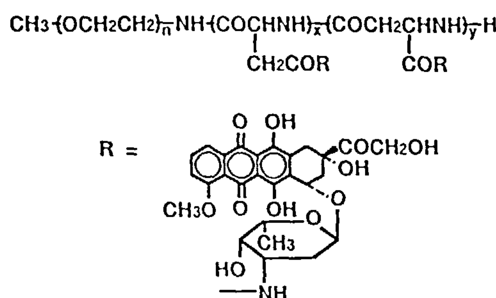

FIG. 2. Structural formula of adriamycin-conjugated PEO/PAsp block copolymer, PEO/[PAsp(ADR)].

the PAsp segment by ADR was required for PEO/P[Asp(ADR)] to form a stable micellar structure. The cohesive force due to conjugated ADR should be strong enough to overcome the electrostatic repulsion between carboxylate units along the PAsp segment. The conjugate prepared under controlled conditions with an appropriate composition [for example, the sample abbreviated 120-21(53) is PEO(M_n = 12,000)-PAsp(M_n = 2,100)-ADR(53% substitution)] formed a micelle with an essentially unimodal distribution (average diameter: 42 nm) judged by DLS in a gamma-averaged scale as well as by ultracentrifugation [30]. In GPC analysis, 120-21(53) has only a single peak at the gel exclusion volume ($>3 \times 10^5$ dalton), which is in line with the high stability of the micelle structure.

GPC is a suitable tool to study the micelle properties which depend on the composition. By reducing the substitution degree of ADR and the molecular weight ratio of PEO to PAsp, the conjugate gave two peaks with different elution volumes in GPC. The first peak at the gel exclusion volume corresponds to the micelle, and the second peak with a larger elution volume corresponds to single polymer chains (unimers). Apparently, this clear resolution of the micelle peak with the unimer peak suggests that micelle/unimer exchange might be very slow. Indeed, the release of unimers from the micelle can be followed by GPC after fractionation of these two peaks [28]. Figure 3 shows the release profile of unimers from the micelle of

Downloaded At: 16:18 24 January 2011

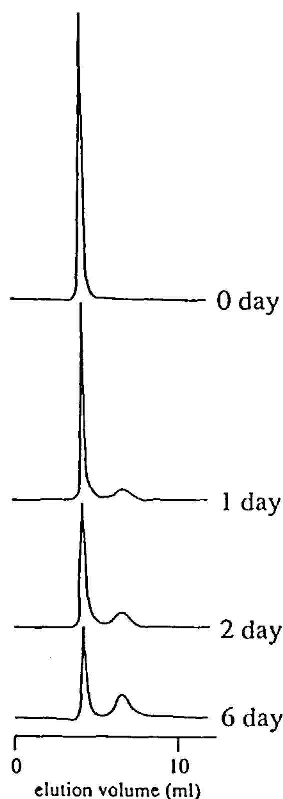

FIG. 3. Release of unimer from fractionated micelles. (Reprinted with permission from Ref. 28.)

43-17(30) in PBS after fractionation of the micelle and unimer peaks. There was obviously a slow dissociation of unimers from the micelle on a day-scale, indicating a very slow relaxation process of the polymeric micelle structure. The addition of 10% serum in PBS approximately doubled the dissociation rate. No detectable dissociation took place for the micelle prepared from 120-21(53), which demonstrates the feasibility of controlling the decaying time of the micelle by tailoring the chemical composition of the conjugate.

The associative nature of PEO/P[Asp(ADR)] was further demonstrated by fluorescence spectroscopy, where fluorescence due to bound ADR was strongly quenched in PEO/P[Asp(ADR)] solutions, consistent with the intermolecular association of ADR moieties within the micelle core [4]. Further, no characteristic peaks of protons bound to the P[Asp(ADR)] segment could be observed in the ^{1}H-NMR spectra of PEO/P[Asp(ADR)] in D_2O, indicating highly limited motions of the P[Asp(ADR)] segment due to the strong interactions of ADR within the micellar core. Because of this segregation of ADR moieties from the outer environment, the micellar solution remained clear even in concentrated form (>20 mg ADR equiv/mL). Further, it could be stored in lyophilized form and maintain its water solubility in the redissolving process.

Downloaded At: 16:18 24 January 2011

ANTICANCER ACTIVITY OF MICELLE-FORMING PEO/P[Asp(ADR)] CONJUGATES

In-vivo anticancer activity of micelle-forming PEO/P[Asp(ADR)] conjugates was first observed in P388 mouse leukemia by intraperitoneal (i.p.) injection [4]. The conjugate 43-19(30) showed even better survival for mice with low toxicity compared to free ADR judged from the value of the maximum median life-span over controls (T/C%). Further, PEO/P[Asp(ADR)] was found to have superior anticancer activity to the parent drug ADR against several murine and human solid tumors (e.g., C26, C38, M5076, MX-1) in advanced stages of development by intravenous (i.v.) injection, suggesting its feasibility in chemotherapy of human solid tumors [6, 8]. A complete regression of the tumor was observed in some cases. Though a large amount of research has been devoted to the targeting of anticancer drugs based on the concept of drug delivery systems, dramatic tumor regression by micelle-forming conjugates is one of only a few successful examples of a strategy of drugs combined with carrier systems. These studies on anticancer activity have been overviewed elsewhere [9].

High tumoricidal activity against solid tumors of micelle-forming conjugates might be due to their effective accumulation in the tumor, thereby increasing the local concentration of anticancer agents at the tumor sites. Indeed, in-vivo activity of a micelle-forming polymeric anticancer drug strongly depends on the composition, while in-vitro cytotoxic activity was found to be almost the same regardless of the composition [8]. Figure 4 shows tumor growth inhibition of the conjugates at a dose of 100 mg ADR equiv/kg of mouse body weight. Result obtained for free ADR at a dose to give maximum growth inhibition without toxic death (10 mg/kg) is also shown. As described elsewhere [6], the toxic score of ADR equivalents in the conjugate was estimated to be less than 1/10 that of free ADR, permitting a higher dose with fewer side effects compared to free ADR. In Fig. 4 it is obvious that the

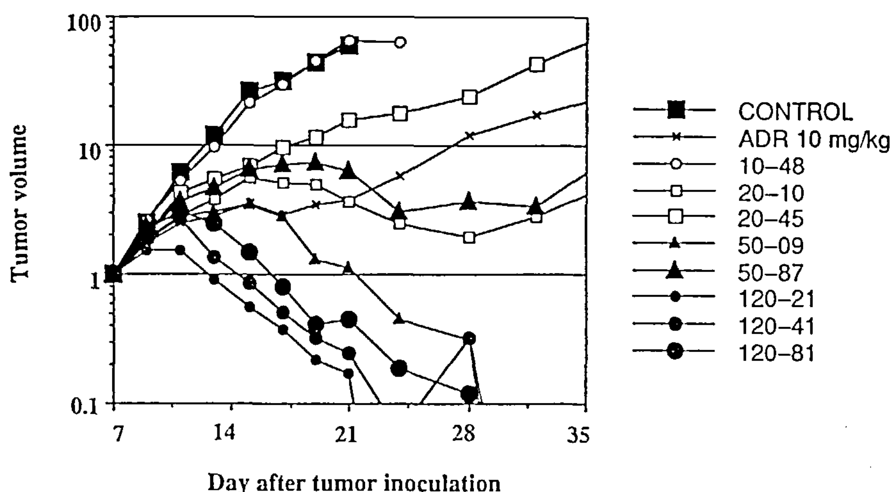

FIG. 4. In-vivo antitumor activity of micelle-forming conjugates against C26.

Downloaded At: 16:18 24 January 2011

composition of the conjugates crucially affects the growth inhibition of Colon 26 (C26) tumor in mice. Complete disappearance of tumors by intravenous injection of the conjugates with such long PEO chains as 120-21(104), 120-41(78), and 120-81(84) was observed. On the other hand, a conjugate with a short PEO chain [10-48(12)] did not show any inhibition of tumor growth. Correlation of in-vivo activity with micelle stability as evaluated by GPC is worth noticing; a micelle with a longer PEO chain showed increased stability and higher tumoricidal activity. We recently confirmed that micelles with appropriate compositions can circulate stably through blood, and are preferentially taken up and retained by the tumor [31, 32].

Surprisingly, at 4 and 24 hours, 120-21(104) exhibited 68 and 10%, respectively, of the original dose in blood [31]. This is in a sharp contrast with conventional colloidal carriers which are rapidly and efficiently removed from circulation within minutes [1]. This prolonged circulation in the blood is in line with a relatively low uptake by the liver and spleen, the representative organs of RES. For 10-48(12), very low blood circulation times were observed (1% injected dose at 4 hours); this is consistent with the poor micellar stability of this conjugate [7]. The stability of 120-21(104) in blood is strongly correlated with its enhanced accumulation in tumors. Tumor accumulation ratios to normal tissue (muscle) at 24 hours for 120-21(104) (tumor/muscle ratio = 40) showed an order of magnitude increase in comparison to free ADR (tumor/muscle ratio = 1.5). An increase in the tumor accumulation ratio to the heart was also significant for the micelle-forming conjugate, suggesting a low incidence of cardiac toxicity in the conjugate system. Accumulation of the conjugate at tumor sites, possibly through direct extravasation, might be due to enhanced vascular permeability and retention effects in a tumor, known as the EPR effect [33, 34]. To achieve this effect at a sufficient level, it will be important to emphasize the core-shell structure of the micelle in order to inhibit nonspecific interactions of the hydrophobic core of the micelle with the biocomponents (e.g., RES) by covering the core with a hydrated outer shell which will provide higher stability of the micelle structure in blood, as schematically shown in Fig. 5.

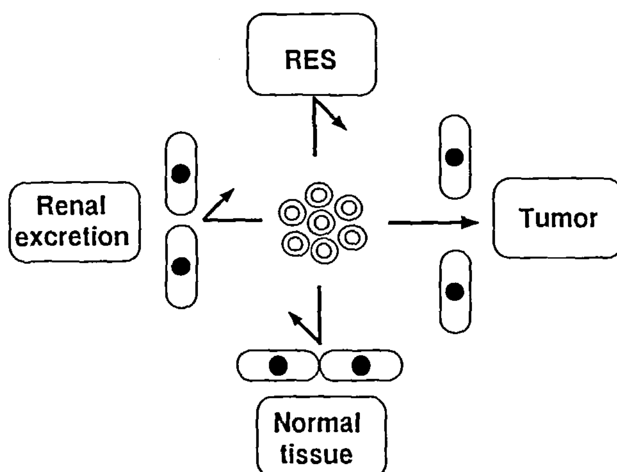

FIG. 5. Accumulation of micelle-forming conjugates in a tumor utilizing enhanced permeability of tumor vasculature.

Downloaded At: 16:18 24 January 2011

We recently extended our research to the design of block copolymer micelles capable of installing drugs in the core through noncovalent interactions. For this purpose, block copolymers of PEO and poly(β-benzyl-L-aspartate) (PEO/PBLA) is quite promising because of its high stability (cmc < 10 mg/L) and high capability of entrapping such hydrophobic molecules as pyrene [35, 36]. Indeed, ADR was found to be stably entrapped in PEO/PBLA micelles while keeping its high anticancer activity [37]. Current studies are investigating the feasibility of using PEO/PBLA micelles with physically-loaded ADR in the chemotherapy of solid tumors.

ACKNOWLEDGMENTS

A part of the work cited in this paper was supported by a Grant-in-Aid for Scientific Research (05455022); The Ministry of Education, Science, and Culture, Japan; and by a grant from Mochida Science Foundation. The author would like to acknowledge his colleagues in the International Center for Biomaterials Science (ICBS) for their contribution to the research highlighted in this paper.

REFERENCES

[1] E. Tomlinson, *Adv. Drug Deliv. Rev.*, *1*, 87 (1987).
[2] P. Ehrlich, *Collected Studies on Immunity*, Wiley, New York, 1906.
[3] M. Yokoyama, S. Inoue, K. Kataoka, N. Yui, T. Okano, and Y. Sakurai, *Makromol. Chem.*, *190*, 2041 (1989).
[4] M. Yokoyama, M. Miyauchi, N. Yamada, T. Okano, Y. Sakurai, K. Kataoka, and S. Inoue, *Cancer Res.*, *50*, 1693 (1990).

[5] M. Yokoyama, M. Miyauchi, N. Yamada, T. Okano, Y. Sakurai, K. Kataoka, and S. Inoue, *J. Control. Rel.*, *11*, 269 (1990).
[6] M. Yokoyama, T. Okano, Y. Sakurai, H. Ekimoto, C. Shibazaki, and K. Kataoka, *Cancer Res.*, *51*, 3229 (1991).
[7] M. Yokoyama, G. S. Kwon, T. Okano, Y. Sakurai, T. Seto, and K. Kataoka, *Bioconj. Chem.*, *3*, 295 (1992).
[8] M. Yokoyama, G. S. Kwon, T. Okano, Y. Sakurai, H. Ekimoto, K. Okamoto, T. Seto, and K. Kataoka, *Drug Deliv.*, *1*, 11 (1993).
[9] K. Kataoka, G. S. Kwon, M. Yokoyama, T. Okano, and Y. Sakurai, *J. Control. Rel.*, *24*, 119 (1993).
[10] Z. Tuzar and P. Kratochvil, *Adv. Colloid Interface Sci.*, *6*, 201 (1976).
[11] G. Riess, G. Hurtrez, and P. Bahadur, in *Encyclopedia of Polymer Science and Engineering*, Vol. 2, 2nd ed., Wiley-Interscience, New York, 1985, p. 324.
[12] A. R. Rees and M. J. E. Sternberg, *From Cells to Atoms*, Blackwell Scientific, Oxford, 1984, p. 8.
[13] R. E. Connsell and R. C. Pohland, *J. Med. Chem.*, *25*, 1115 (1982).
[14] J. N. Weinstein and W. van Osdol, *Int. J. Immunopharmacol.*, *14*, 457 (1992).
[15] C. J. T. Hoes, W. Potman, W. A. R. van Heeswijk, J. Mud, B. G. De Grooth, J. Grave, and J. Feijen, *J. Control. Rel.*, *2*, 205 (1985).
[16] R. Duncan, P. Kopeckova-Rejmanova, J. Strohalm, I. Hume, H. C. Cable, J. Pohl, B. Lloyd, and J. Kopecek, *Br. J. Cancer*, *55*, 165 (1987).
[17] N. Endo, N. Umemoto, Y. Kato, Y. Takeda, and T. Hara, *J. Immunol. Methods*, *104*, 253 (1987).
[18] F. Zunino, G. Pratesi, and A. Micheoloni, *J. Control. Rel.*, *10*, 65 (1989).
[19] C.-L. Zhao, M. A. Winnik, G. Riess, and M. D. Croucher, *Langmuir*, *6*, 514 (1990).
[20] M. Wilhelm, C.-L. Zhao, Y. Wang, R. Xu, M. A. Winnik, J.-L. Mura, G. Riess, and M. D. Croucher, *Macromolecules*, *24*, 1033 (1991).
[21] I. R. Schmolka, *J. Am. Oil Chem. Soc.*, *54*, 110 (1977).
[22] J. H. Bader, H. Ringsdorf, and B. Schmidt, *Angew. Makromol. Chem.*, *123*, 457 (1984).
[23] M. K. Pratten, J. B. Lloyd, G. Horpel, and H. Ringsdorf, *Makromol. Chem.*, *186*, 725 (1985).
[24] M. Yokoyama, S. Inoue, K. Kataoka, N. Yui, and Y. Sakurai, *Makromol. Chem., Rapid Commun.*, *8*, 431 (1987).
[25] A. Abuchowski, J. R. McCoy, N. C. Palczuk, T. van Es, and F. F. Davis, *J. Biol. Chem.*, *252*, 3582 (1977).
[26] F. Fuertges and A. Abuchowski, *J. Control. Rel.*, *11*, 139 (1990).
[27] S. I. Jeon, J. H. Lee, J. D. Andrade, and P. G. de Gennes, *J. Colloid Interface Sci.*, *142*, 149 (1991).
[28] M. Yokoyama, T. Sugiyama, T. Okano, Y. Sakurai, M. Naito, and K. Kataoka, *Pharm. Res.*, *10*, 895 (1993).
[29] M. Yokoyama, G. S. Kown, T. Okano, Y. Sakurai, M. Naito, and K. Katraoka, *J. Control. Rel.*, *28*, 59 (1994).
[30] M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka, *Ibid.*, In Press.
[31] G. S. Kown, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka, *Pharm. Res.*, *10*, 970 (1993).

Downloaded At: 16:18 24 January 2011

[32] G. S. Kwon, S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka, *J. Control. Rel.*, *27*, 17 (1994).
[33] Y. Matsushima and H. Maeda, *Cancer Res.*, *46*, 6387 (1986).
[34] H. Maeda, L. W. Seymour, and Y. Miyamoto, *Bioconj. Chem.*, *3*, 351 (1992).
[35] G. S. Kwon, M. Naito, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka, *Langmuir*, *9*, 945 (1993).
[36] G. S. Kwon, M. Naito, K. Kataoka, M. Yokoyama, Y. Sakurai, and T. Okano, *Colloids Surf., B: Biointerfaces*, *2*, 429 (1994).
[37] G. S. Kwon, M. Naito, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka, *Pharm. Res.*, Submitted.

Downloaded At: 16:18 24 January 2011