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Determination of critical micelle concentrations of Pluronics

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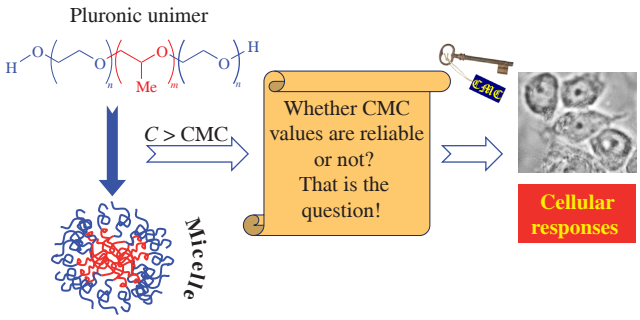
Critical micelle concentrations (CMCs) of Pluronics were determined by three techniques, namely 1,6-diphenyl-1,3,5-hexatriene fluorescence, sensitization of multidrug-resistant cancer cells with Pluronic unimers and a novel technique based on the migration of fluorophore-labeled lipid from liposomes into Pluronic micelles. The CMCs of each Pluronic determined by the three methods differ by no more than 30% and are consistent with data reported by different research teams. A literature review revealed a widely cited dataset of CMC values that differ significantly from data reported by other research groups, including ours.



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Pluronics are nonionic triblock copolymers with a central hydrophobic block of poly(propylene oxide) flanked on both sides with hydrophilic chains of poly(ethylene oxide). Such amphiphilic structure endows Pluronics with the properties of surfactants. In aqueous solution, the molecularly dispersed Pluronic chains (unimers) associate and form multimolecular aggregates (micelles) at appropriate concentrations and temperatures. The critical micelle concentration (CMC) defines the onset of micelle formation and corresponds to the maximum achievable concentration of unimers. CMC is an important parameter with regard to the difference in the effects of Pluronic unimers and micelles on cells.[1] Unimers with a hydrophilic/lipophilic balance <20 sensitize multidrug-resistant (MDR) cells[2,3] and make drug-resistant cancers sensitive to chemotherapy,[4,5] while Pluronic micelles affect cell viability.[1] Since knowledge of Pluronics CMCs is of importance it has been the subject of numerous publications. The aim of the present work was to compare our results with those published in the literature and to select reliable data for the correct interpretation of experiments on cells.

We first determined the CMC from the increase in fluorescence intensity of 1,6-diphenyl-1,3,5-hexatriene (DPH) resulting from its solubilization in the hydrophobic core of the micelles (for details, see Online Supplementary Materials, Section S1).[6] The CMC was derived at the intersection of a horizontal line passing through the points corresponding to low polymer concentration and the tangent of the ascending curve [Figure 1(*a*)]. Linear abscissa scales were used as in the original paper.[6] The CMC of Pluronic P85 determined in this way was 25 μM [see Figure 1(*a*)]. The analysis of Pluronics L61, L64, L81, F87 and P123 is given in Online Supplementary Materials, Section S2. The CMCs determined for the eight Pluronics are summarized in Table 1.

The CMC values determined by DPH fluorescence differed significantly from those published by Batrakova *et al.*[2] in 1999 and replicated in subsequent publications.[7–9] This discrepancy inspired us to validate our CMC data using a different approach based on the ability of unimers to inhibit MDR of cancer cells.[2,3]

To monitor MDR inhibition, NCI/ADR-RES cells were treated with varying amounts of Pluronic mixed with the anticancer drug doxorubicin (DOX, Verafarm, Russia) at a concentration that is non-toxic in the absence of polymers (see Online Supplementary Materials, Section S3). A representative result obtained with Pluronic P85 is shown in Figure 1(*b*). The S-shaped dependence of cell viability on polymer concentration revealed a range of P85 concentrations from 3 to 25 μM, in which cell numbers gradually decreased to ~50% and then stabilized. Since Pluronic P85 is

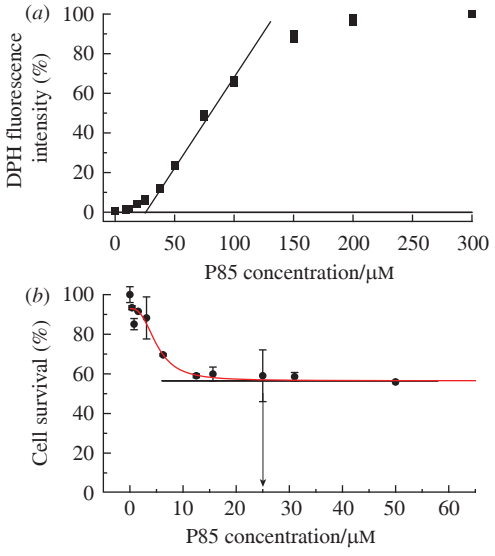

Figure 1 Determination of (*a*) the CMC of Pluronic P85 by DPH fluorescence and (*b*) the lowest concentration of Pluronic P85 sufficient to sensitize MDR cells.

Table 1 Compositions of Pluronics and their CMCs determined in this work and found in the literature.

Pluronic			CMC (this work)[a]/μM			CMC (Kabanov's group)[a]/μM		CMC (other researchers)[a]/μM	
Code	M_w/Da	Formula[b]	DPH method[c,d]	C^{MDR} method[e]	C_{12}NBD–PC method[d,f]	Pyrene method[g]	Reference	Various methods[h]	Reference
L61	1950	E_2–P_{31}–E_2	8	9–10	8–10	110	2	12.4 ± 1.2[i]	10
L64	2900	E_{13}–P_{31}–E_{13}	51	50	46	480	2	86[j]	11
F68	8400	E_{76}–P_{30}–E_{76}	1000			480	2	600–1000	12
						12000	13		
						1140	14		
L81	2750	E_3–P_{40}–E_3	1.4	2–4		23	2	3	7
						23	14		
P85	4600	E_{26}–P_{40}–E_{26}	24	25	18–23	65	2	13	13
								65[k]	13
								65[k]	14
								22	15
F87	7700	E_{61}–P_{40}–E_{61}	23			91	2	12	7
P123	5750	E_{20}–P_{69}–E_{19}	1	1.3	1	4.4	2	0.57[l]	16
								1.7[m]	16
								2.5[k]	17
								0.97	18
F127	12600	E_{100}–P_{65}–E_{100}	1.5			2.8	2	0.8[n]	19
			610					0.08	20
								500	20
								635	21
								800	22
								555–794	23

[a] Measured at 37 °C unless otherwise stated. [b] *E* = ethylene oxide unit, *P* = propylene oxide unit. [c] DPH fluorescence method. [d] In phosphate-buffered saline (PBS). [e] MDR cell sensitization method in DMEM. [f] Liposome–micelle C_{12}NBD–PC migration method. [g] Fluorescent probe (pyrene) method. [h] Fluorescence spectroscopy or surface tension method. [i] At 20 °C. [j] Measured by NMR method. [k] At 25 °C. [l] At 43 °C. [m] At 35 °C. [n] At 42 °C.

non-toxic up to 5% concentration,[4] the decrease in cell viability indicates MDR inhibition by the polymer and increasing cytotoxicity of DOX. Concentrations of Pluronic P85 above 25 μM did not enhance the effect [see Figure 1(*b*)]. Thus, a P85 concentration of 25 μM was the lowest concentration sufficient to reverse the MDR (C^{MDR}). The maximum possible inhibition of MDR is achieved at the highest concentration of unimers, which by definition corresponds to the CMC. This value can be seen in the graph at the beginning of the plateau. It was close to the CMC determined by DPH fluorescence (see Table 1). The same was true for the other Pluronics tested (see Table 1 and Online Supplementary Materials, Sections S2 and S4). The result is consistent with the literature data. The CMC of Pluronic P85 was previously determined[15] to be 22 μM, and exactly the same concentration of P85 induced suppression of MDR to the maximum level.[24] Thus, the CMC can be determined from the reversal of MDR by Pluronic.

Another novelty of our study was the investigation of the possibility of determining the CMC by measuring the migration of fluorophore-labeled lipid from liposomes into the hydrophobic core of Pluronic micelles. The idea is based on the ability of Pluronic unimers to bind to lipids of liposomes[25] and cell membranes,[3] as well as on the affinity of hydrophobic substances for the hydrophobic core of micelles.[26] In order to follow the Pluronic–lipid interaction using fluorescence spectroscopy, liposomes were prepared from egg yolk lecithin and 30% phosphatidylcholine labeled with the fluorophore 2-{12-[7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecano-yl}-1-palmitoyl-phosphatidylcholine (C_{12}-NBD-PC, Molecular Probes, USA). The initial fluorescence of the liposomes was negligible due to the self-quenching effect of C_{12}-NBD-PC. Low concentrations of Pluronics did not change the fluorescence intensity. However, it increased at higher polymer concentrations, indicating that the local fluorophore concentration decreased below the self-quenching threshold. This could occur if C_{12}-NBD-PC migrated from the liposomes into Pluronic micelles. We determined the polymer concentration corresponding to the onset of micelle appearance from the intersection of the two lines (Figure 2 and Online Supplementary Materials, Section S6).

The values obtained in this way are consistent with the CMCs determined by DPH fluorescence and C^{MDR} quantification (see Table 1). Thus, we developed a new method for determining the CMC based on the interaction of amphiphiles with liposomes containing 30% C_{12}-NBD-PC.

The CMC values of each Pluronic determined by the three approaches did not differ by more than 30% (see Table 1). The correlation of C^{MDR} (left *y*-axis) and CMC values determined using C_{12}-NBD-PC (right *y*-axis) with CMC values determined by DPH fluorescence is a straight line with a slope of approximately 1 (Figure 3). The correlation demonstrates the similarity of the CMC values determined by the three methods.

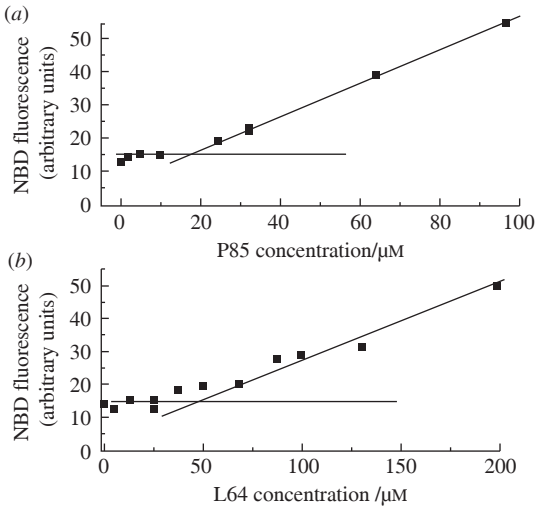

Figure 2 Determination of the CMC of Pluronics using NBD-labeled lipid incorporated into a liposomal membrane: (*a*) Pluronic P85 (CMC = 18 μM) and (*b*) Pluronic L64 (CMC = 46 μM).

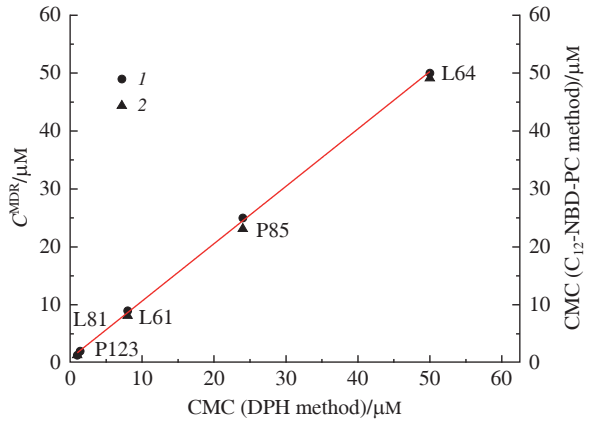

Figure 3 Correlation of (*1*) C^{MDR} values and (*2*) CMC values determined using C_{12}-NBD-PC with CMC values determined using DPH solubilization demonstrates the similarity of the three methods for determining CMC values of Pluronics.

Thus, verification of our CMCs by the three methods did not reveal any errors in our experiments that could explain their contradiction with the data reported by Kabanov's group[7–9] (see Table 1) and often cited by other authors without verification.[27–29]

Therefore, we reviewed previously published papers and found that our data were in good agreement with those reported by other research groups (see Table 1).

The choice of reliable CMCs for the analysis of cellular experiments is particularly important. This can be illustrated, for example, by the work of Redhead *et al.*,[30] who investigated the cytotoxicity of Pluronics. The dose–response curves they obtained began to slope at certain points. The authors concluded that 'no change in the trend of the dose–response curve was observed above CMC', which implied that both unimers and micelles were toxic to cells. This conclusion is reasonable if based on the CMC values reported by Kabanov's group. However, using data from other research groups, including ours, the authors would have been able to find that only micelles were cytotoxic.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7689.

References

1 I. D. Grozdova and N. S. Melik-Nubarov, *ACS Appl. Polym. Mater.*, 2022, **4**, 8764; https://doi.org/10.1021/acsapm.2c01179.
2 E. Batrakova, S. Lee, S. Li, A. Venne, V. Alakhov and A. Kabanov, *Pharm. Res.*, 1999, **16**, 1373; https://doi.org/10.1023/A:1018942823676.
3 A. Zhirnov, E. Nam, G. Badun, A. Romanyuk, A. Ezhov, N. Melik-Nubarov and I. Grozdova, *Pharm. Res.*, 2018, **35**, 205; https://doi.org/10.1007/s11095-018-2484-4.
4 V. Yu. Alakhov, E. Yu. Moskaleva, E. V. Batrakova and A. V. Kabanov, *Bioconjugate Chem.*, 1996, **7**, 209; https://doi.org/10.1021/bc950093n.
5 E. V. Batrakova and A. V. Kabanov, *J. Controlled Release*, 2008, **130**, 98; https://doi.org/10.1016/j.jconrel.2008.04.013.
6 A. Chattopadhyay and E. London, *Anal. Biochem.*, 1984, **139**, 408; https://doi.org/10.1016/0003-2697(84)90026-5.
7 M. Yu. Kozlov, N. S. Melik-Nubarov, E. V. Batrakova and A. V. Kabanov, *Macromolecules*, 2000, **33**, 3305; https://doi.org/10.1021/ma991634x.
8 E. V. Batrakova, S. Li, V. Yu. Alakhov, D. W. Miller and A. V. Kabanov, *J. Pharmacol. Exp. Ther.*, 2003, **304**, 845; https://doi.org/10.1124/jpet.102.043307.
9 A. V. Kabanov, E. V. Batrakova and D. W. Miller, *Adv. Drug Delivery Rev.*, 2003, **55**, 151; https://doi.org/10.1016/S0169-409X(02)00176-X.
10 L. Sanderson, M. da Silva, G. N. Sekhar, C. Rachel, R. C. Brown, H. Burrell-Saward, M. Fidanboylu, B. Liu, L. A. Dailey, C. A. Dreiss, C. Lorenz, M. Christie, S. J. Persaud, V. Yardley, S. L. Croft, M. Valero and S. A. Thomas, *PLoS Neglected Trop. Dis.*, 2021, **15**, e0009276; https://doi.org/10.1371/journal.pntd.0009276.
11 C. Roques, K. Bouchemal, G. Ponchel, Y. Fromes and E. Fattal, *J. Controlled Release*, 2009, **138**, 71; https://doi.org/10.1016/j.jconrel.2009.04.030.
12 P. Prasanthan and N. Kishore, *RSC Adv.*, 2021, **11**, 22057; https://doi.org/10.1039/d1ra03770f.
13 A. V. Kabanov, I. R. Nazarova, I. V. Astafieva, E. V. Batrakova, V. Yu. Alakhov, A. A. Yaroslavov and V. A. Kabanov, *Macromolecules*, 1995, **28**, 2303; https://doi.org/10.1021/ma00111a026.
14 E. V. Batrakova, H.-Y. Han, V. Yu. Alakhov, D. W. Miller and A. V. Kabanov, *Pharm. Res.*, 1998, **15**, 850; https://doi.org/10.1023/A:1011964213024.
15 V. I. Slepnev, L. E. Kuznetsova, A. N. Gubin, E. V. Batrakova, V. Yu. Alakhov and A. V. Kabanov, *Biochem. Int.*, 1992, **26**, 587; https://pubmed.ncbi.nlm.nih.gov/1610369/.
16 G. Wanka, H. Hoffmann and W. Ulbricht, *Macromolecules*, 1994, **27**, 4145; https://doi.org/10.1021/ma00093a016.
17 G. Wanka, H. Hoffmann and W. Ulbricht, *Colloid Polym. Sci.*, 1990, **268**, 101; https://doi.org/10.1007/BF01513189.
18 U. Ashraf, O. A. Chat, M. Maswal, S. Jabeen and A. A. Dar, *RSC Adv.*, 2015, **5**, 83608; https://doi.org/10.1039/C5RA13002F.
19 S. R. Croy and G. S. Kwon, *J. Controlled Release*, 2004, **95**, 161; https://doi.org/10.1016/j.jconrel.2003.11.003.
20 E. Hecht and H. Hoffmann, *Langmuir*, 1994, **10**, 86; https://doi.org/10.1021/la00013a013.
21 Y. Zhang and Y. M. Lam, *J. Colloid Interface Sci.*, 2007, **306**, 398; https://doi.org/10.1016/j.jcis.2006.10.073.
22 J. R. Lopes and W. Loh, *Langmuir*, 1998, **14**, 750; https://doi.org/10.1021/la9709655.
23 P. Linse and M. Malmsten, *Macromolecules*, 1992, **25**, 5434; https://doi.org/10.1021/ma00046a048.
24 D. W. Miller, E. V. Batrakova, T. O. Waltner, V. Yu. Alakhov and A. V. Kabanov, *Bioconjugate Chem.*, 1997, **8**, 649; https://doi.org/10.1021/bc970118d.
25 K. Kostarelos, Th. F. Tadros and P. F. Luckham, *Langmuir*, 1999, **15**, 369; https://doi.org/10.1021/la971052d.
26 C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd edn., Wiley, New York, 1980; https://books.google.com/books?id=079pAAAAMAAJ.
27 T. M. Krupka and A. A. Exner, *Int. J. Hyperthermia*, 2011, **27**, 663; https://doi.org/10.3109/02656736.2011.599828.
28 S. S. Kulthe, N. N. Inamdar, Y. M. Choudhari, S. M. Shirolikar, L. C. Borde and V. K. Mourya, *Colloids Surf., B*, 2011, **88**, 691; https://doi.org/10.1016/j.colsurfb.2011.08.002.
29 Z. Wei, S. Yuan, J. Hao and X. Fang, *Eur. J. Pharm. Biopharm.*, 2013, **83**, 266; https://doi.org/10.1016/j.ejpb.2012.09.014.
30 M. Redhead, G. Mantovani, S. Nawaz, P. Carbone, D. C. Gorecki, C. Alexander and C. Bosquillon, *Pharm. Res.*, 2012, **29**, 1908; https://doi.org/10.1007/s11095-012-0716-6.

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