Supplementary information for

pH-triggered solubility and cytotoxicity changes of malachite green derivatives incorporated in liposomes for killing cancer cells

Authors

Ryoko M. Uda,*† Nao Yoshida,† Tomoyuki Iwasaki,‡ and Keita Hayashi†

Affiliation

† Department of Chemical Engineering, National Institute of Technology, Nara College, 22 Yata-cho, Yamatokoritama, Nara 639-1080, Japan

‡ Division of Analytical Bio-medicine, Advanced Research Support Center, Ehime University, Shitsukawa, Toon, Ehime 791-0295, Japan

Corresponding author. Department of Chemical Engineering, National Institute of Technology, Nara College, 22 Yata-cho, Yamatokoritama, Nara 639-1080, Japan; TEL.: +81 0743 55 6164; fax: +81 0743 55 6169
E-mail address: ryoko@chem.nara-k.ac.jp (Ryoko M. Uda).

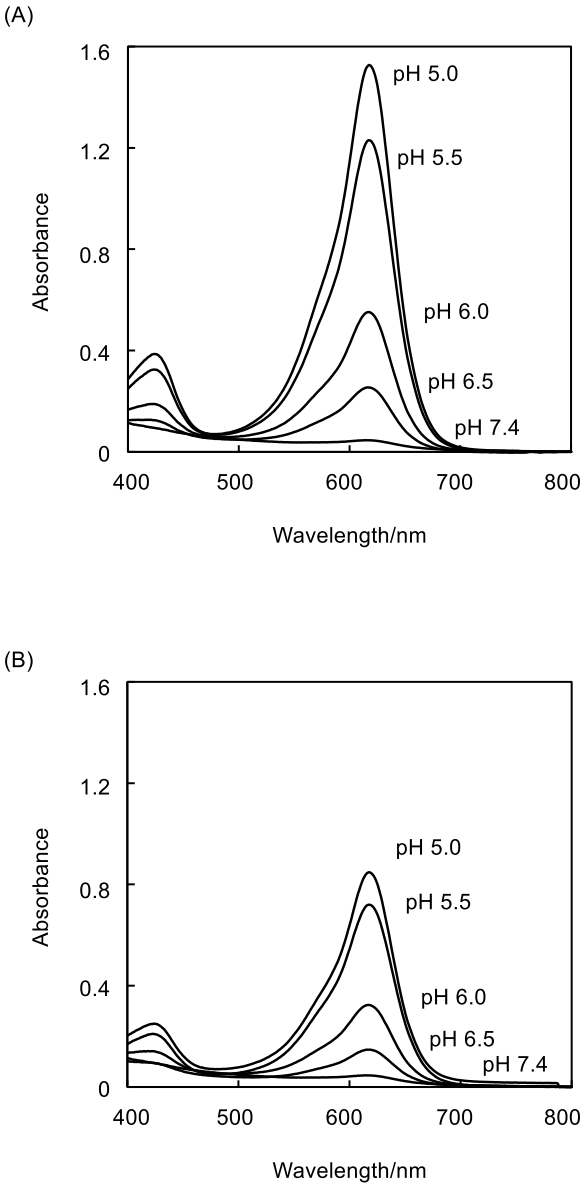

Figure S1. Absorption spectra of the MG-X liposomes after mixing with buffer solutions at various pH levels. (A) MG-OH/POPC liposome, (B) MG-OCH_3/POPC liposome.

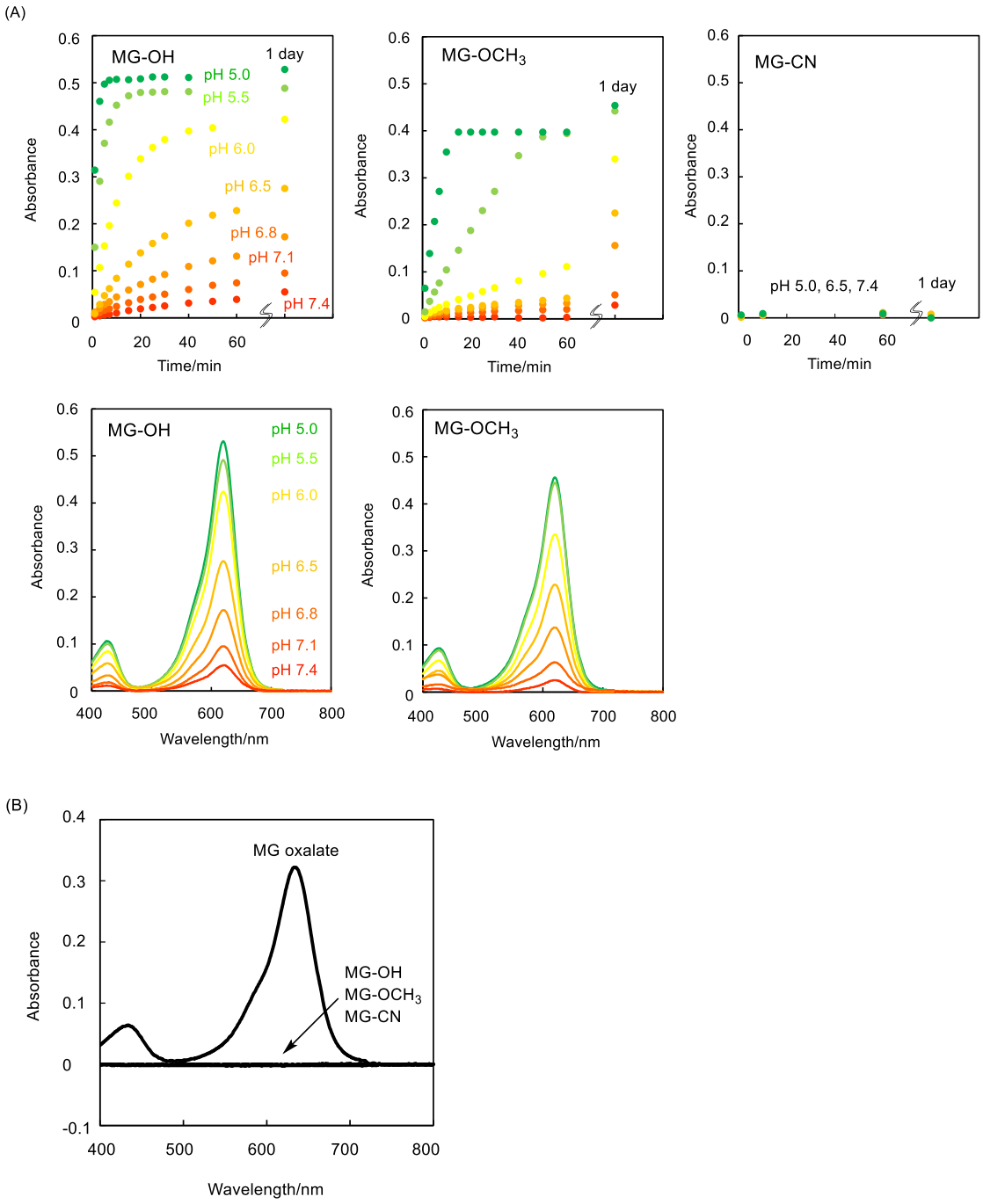

Figure S2. Time dependence of absorbance at 622 nm for DMSO solutions containing MG-X (0.1 mL) after mixing with buffer solutions at various pH levels (0.9 mL) and absorption spectra of the mixture at 1 day after mixing (A). The final concentration of MG-X was 50 μM. Absorption spectra of the DMSO solutions containing malachite green derivatives (50 μM) (B). The optical length of the cuvette for the measurement was 1 mm.

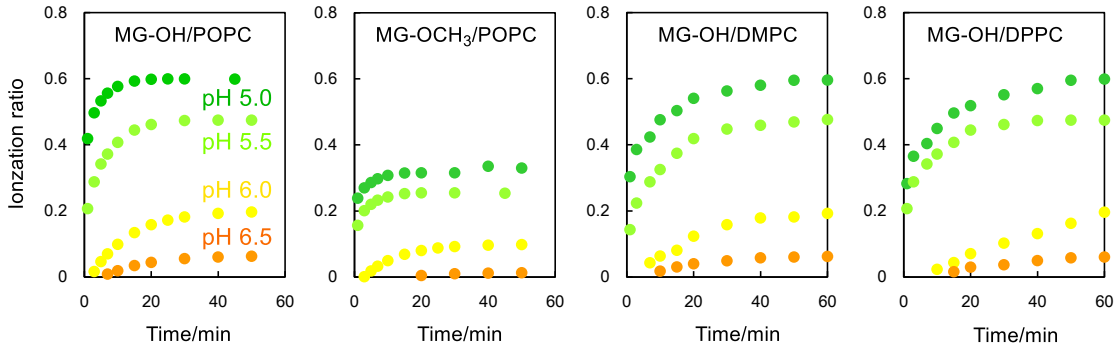

Figure S3. Ionization ratio of MG-OH and MG-OCH_3 in liposomes after mixing with buffer solutions at various pH levels (5.0-6.5). The ionization ratio of MG-CN was not obtained because the absorbance of MG-CN was too small to be applied to the calibration curves prepared for MG oxalate.

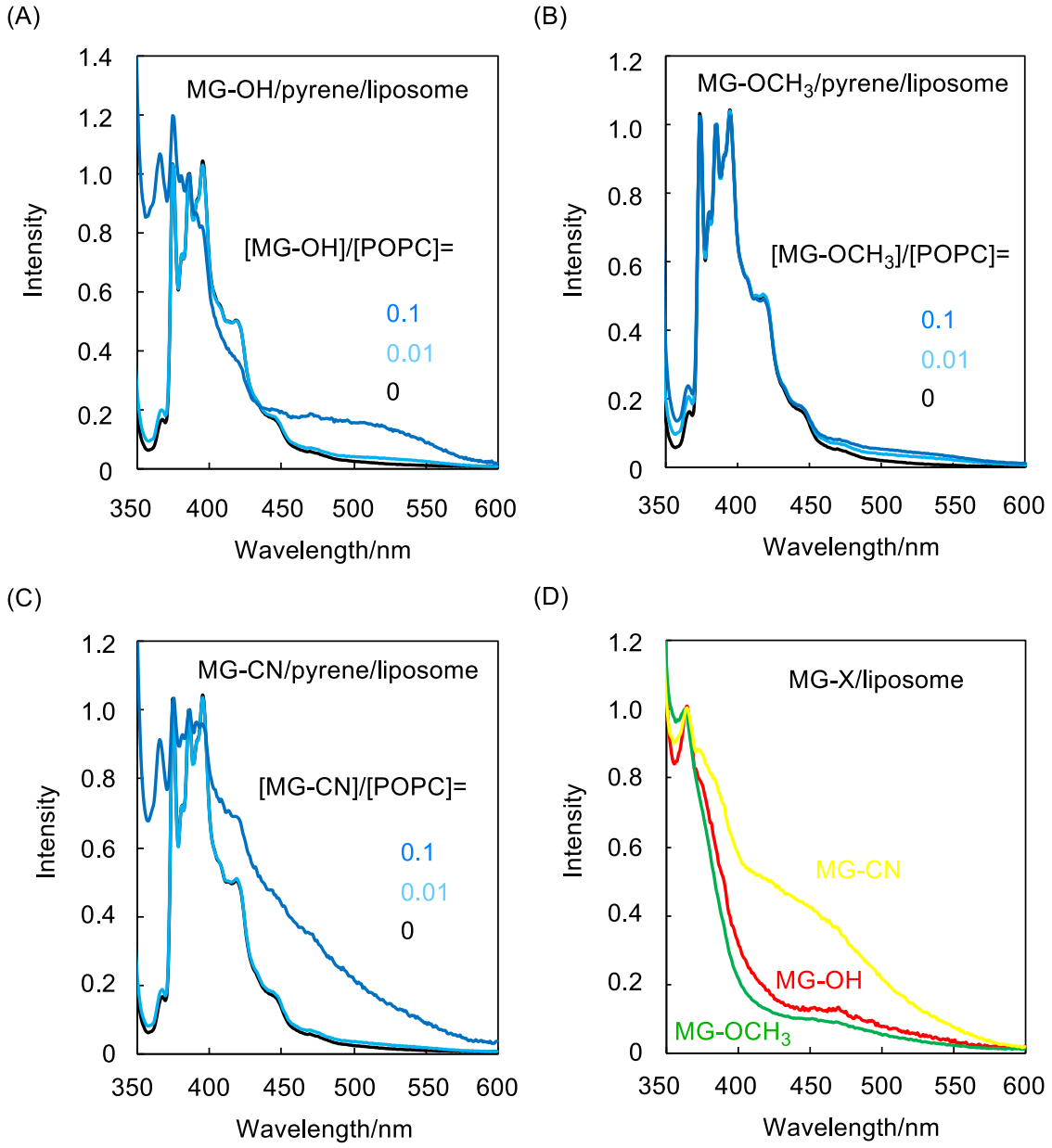

Figure S4. Fluorescence spectra of MG-X/pyrene/POPC liposome. (A) MG-CN, (B) $MG\text{-}OCH_3$, (C) MG-CN. [POPC] = 9.3-9.8 mM, [pyrene] = 0.5 μM. λ_{ex} = 337 nm. Fluorescence intensity was calibrated to the third vibronic peak of pyrene (385 nm) which is essentially insensitive to polarity.[1)] Fluorescence spectra of MG-X/POPC liposomes (D). [POPC] = 9.3-9.8 mM, [MG-X]/[POPC] = 0.1. λ_{ex} = 337 nm. Fluorescence intensity was calibrated to the peak at 363 nm which was assigned to the emission from MG-X.[2)]

Pyrene is a lipophilic compound solubilized inside the lipid membrane. The fluorescence intensity ratio of the first and third vibronic peaks of pyrene (I1/I3) has an extreme sensitivity to the polarity of an environment. The I1/I3 ratio in POPC liposome was observed to be around 1.03, which is comparable to the reported values in phospholipid bilayers.[3] Pyrene has also been known to form an exciplex with *N*,*N*-dimethylaniline which was observed at 520 nm in the fluorescence spectrum.[4] The fluorescence intensity at 520 nm increased with the MG-OH concentrations in the MG-OH/pyrene/POPC liposome (Figure S4(A)), while the MG-OH/POPC liposome did not present an emission peak around 520 nm (Figure S4(D)). MG-OH is considered to be located at the same lipophilic conditions as pyrene, i.e. lipid membrane. MG-OCH_3/pyrene/POPC liposome exhibited a slight increase in fluorescence intensity around 520 nm (Figure S4(B)).

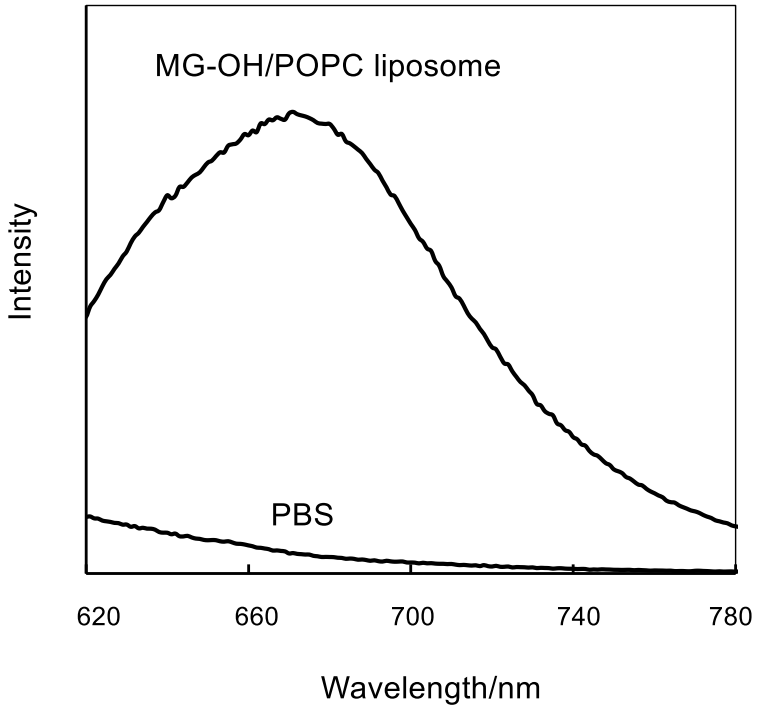

Figure S5. Fluorescence spectra of Colon 26 cells after incubation for 2 h treated with MG-OH/POPC liposomes and PBS. λ_{ex} = 590 nm.

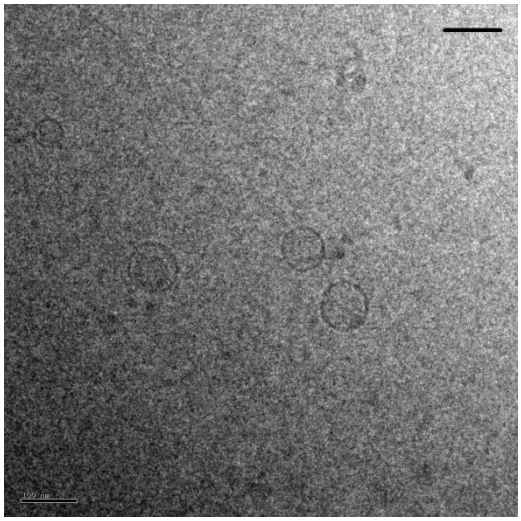

Figure S6. Cryo-transmission electron micrograph of MG-OH/POPC liposomes ([MG-OH]/[POPC] = 0.1). Bar = 100 nm.

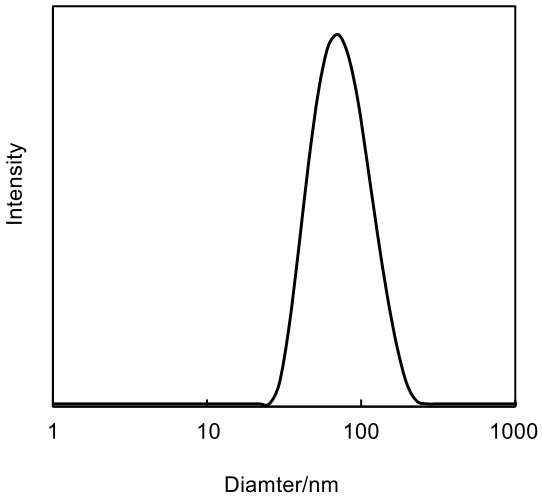

Figure S7. Size distribution of MG-OH/POPC liposomes measured using DLS ([MG-OH]/[POPC] = 0.1).

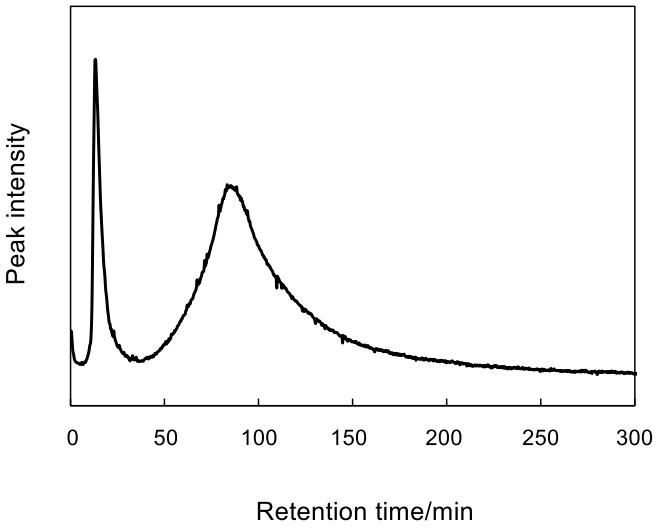

Figure S8. Typical chromatogram of the MG-OH/POPC liposomes ([MG-OH]/[POPC] = 0.1) in phosphate buffer solutions at pH 6.0.

References

1) K. Kalyanasundaram, J. K. Thomas, *J. Am. Chem. Soc.* 1977, **99**, 2039; D. S. Karpovich, G. J. Blanchard, *J. Phys. Chem.* 1995, **99**, 3951.

2) R. M. Uda, D. Yamashita, Y. Sakurai, K. Kimura, *Langmuir* 2007, **23**, 7936.

3) D. Arrais, J. Martins, *Biochim.Biophys. Acta*, 2007, **1768**, 2914; E. A. Lissi, E. Abuin, M. Saez, A. Zanocco, A. Disalvo, *Langmuir* 1992, *8*, 348.

4) K. Sen, S. Bandyopadhyay, D. Bhattacharya, S. Basu, *J. Phys. Chem. A* 2001, **105**, 9077.