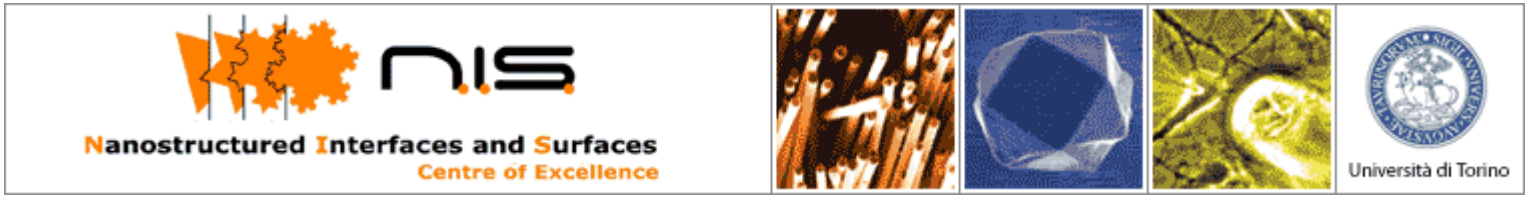


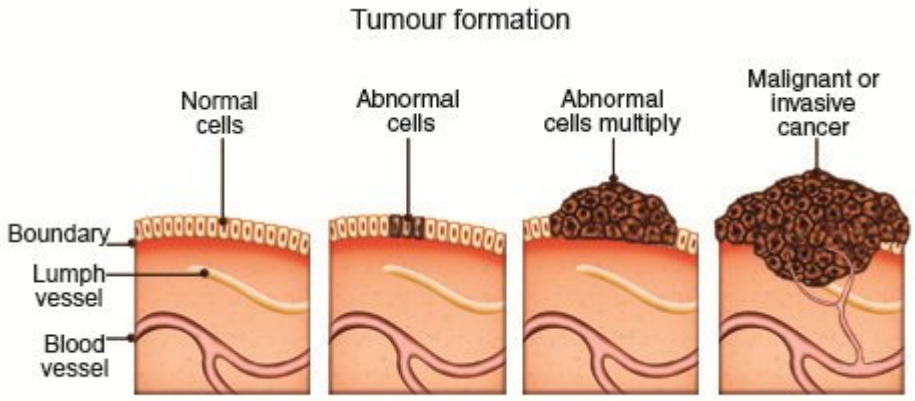
"pH-Sensitive conjugation of organic molecules on bioactive glasses for the development of stimuli-responsive biomaterials"

C. Magistris[1], V. Aina[1], G. Cerrato[1], G. Martra[1], G. Viscardi[1], G. Malavasi[2], G. Lusvardi[2]

1. Dept. of Chemistry, University of Torino, Centre of Excellence NIS (Nanostructured Interfaces and Surfaces) and Consortium INSTM, RU-Turin, Via P. Giuria 7, 10125 Turin, Italy.

2. Dept. of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Via Campi 183, 41125 Modena, Italy.

TUMORS and pH



_ inhomogeneous vessel distribution

_ increased aerobic and anaerobic glycolysis

normal tissue

intracellular	neutr. < 7.0
extracellular	7.0-8.1 (av. 7.5)[1]

tumor tissue

intracellular	neutr. < 7.0
extracellular	5.8-7.7 (av. 7.0)[1]

[1] http://www.cipherhealthcare.com/How_cancer_grows.aspx

pH Control in drug delivery

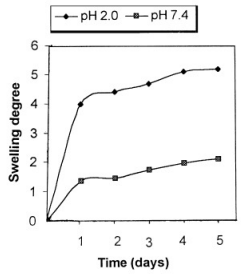


Fig. 1. Swelling behavior of the initial dry beads measured as a function of time at pH 2.0 and 7.4 at 37 °C.

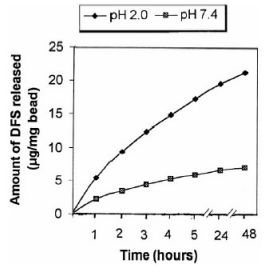


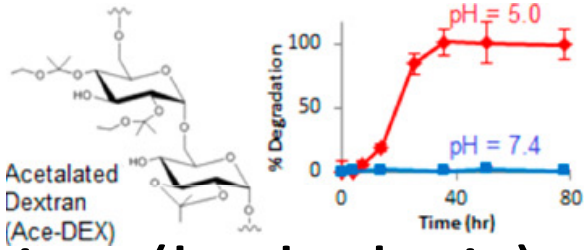
Fig. 3. Release of DFS from chitosan beads (56.25 μg DFS loaded/mg bead) versus time at pH 2.0 and 7.4 at 37 °C.

_ pH-responsive (swelling)

(**dextran-maleic acid hydrogel**[2]; polyurethane/ethylmetacrilate hydrogel[3]; chitosan[4])

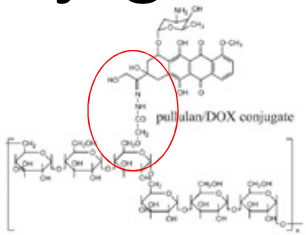
(polymer degradation)

(acetalated dextran[5])

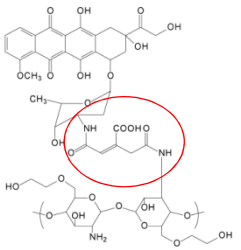


_ pH-sensitive conjugation (hydrolysis)

_ hydrazones



_ amides of α,β-**unsaturated anhydrides**



[2] Kim, S.H., Won, C.Y., Chu, C.C., 1999. Synthesis and characterization of dextran–maleic acid based hydrogel. J. Biomed. M Res. 46, 160–170

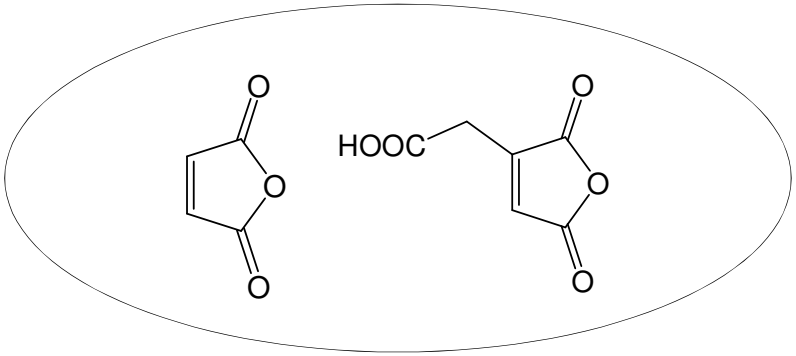
[3] Pardini J. APPL. POLYM. SCI. 2014 39799

[4] K.C. Gupta, M.N.V. Ravi Kumar, Biomaterials 21 (2000) 1115

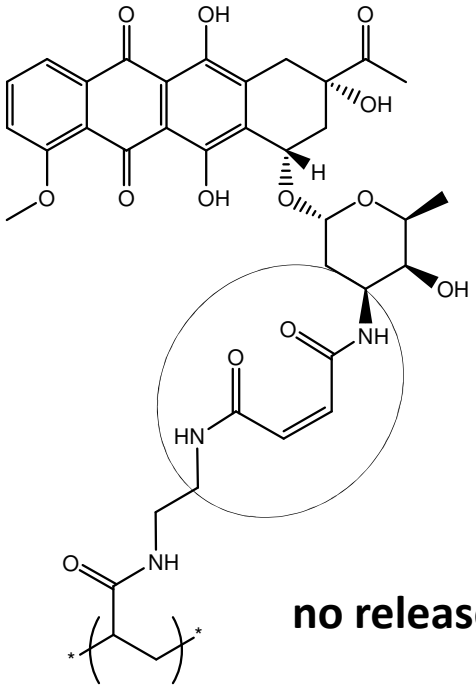
[5] Appl. Mater. Interfaces 2012, 4, 4149

_ 1981, Shen and Ryser[6]

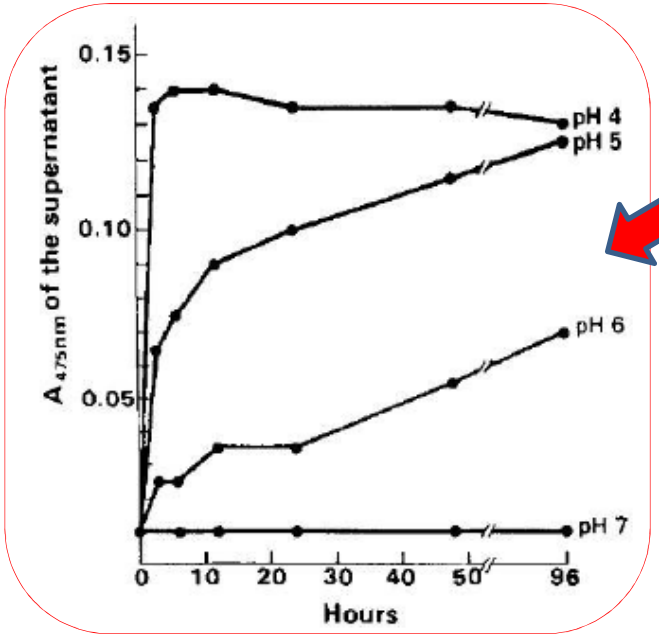
daunorubicin on aminoethyl polyacrylamide

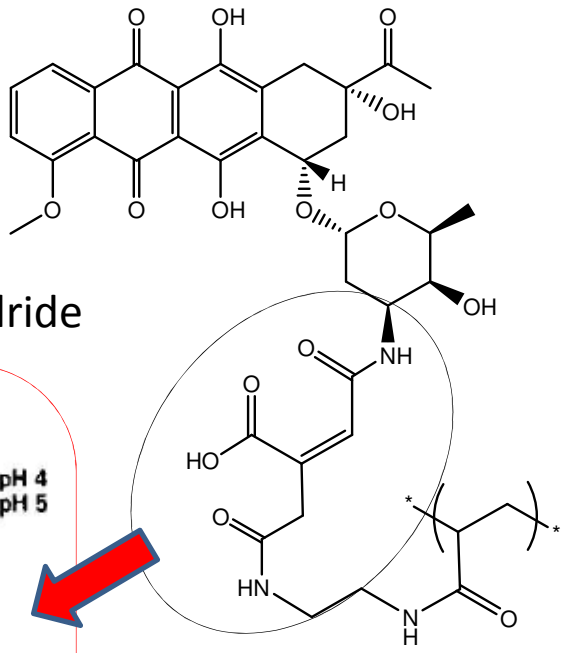


maleic anhydride vs. cis-aconitic anhydride

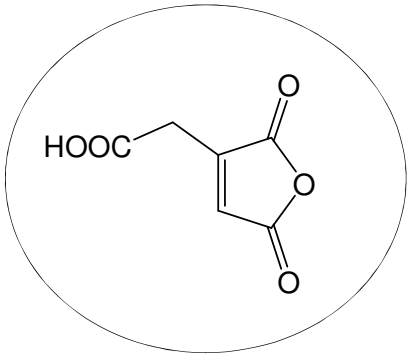


no release

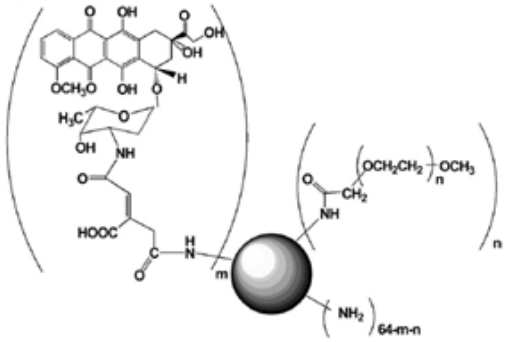


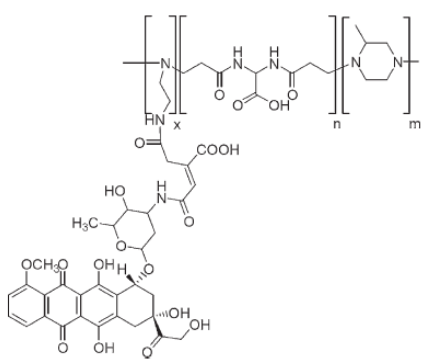


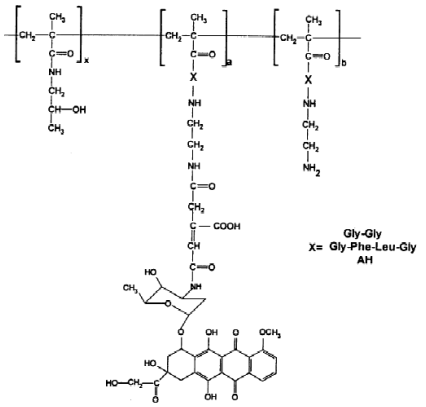
[6] W. C. Shen, H. J. P. Ryser, *Biochem. Biophys. Res. Commun.*, ***1981***, *102,* 1048–1054



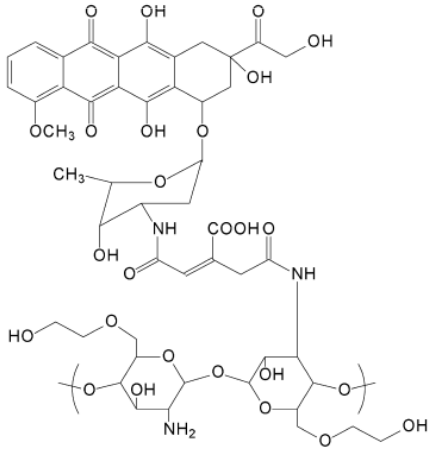
_ polyamidoamines[7,8,9]



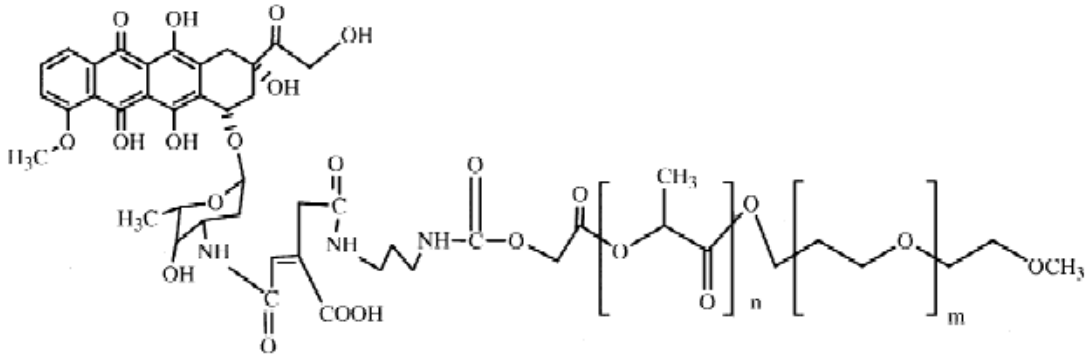




_ chitosan[10,11]

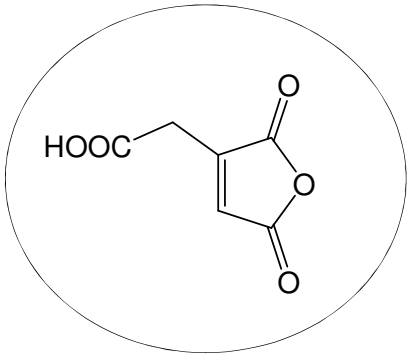


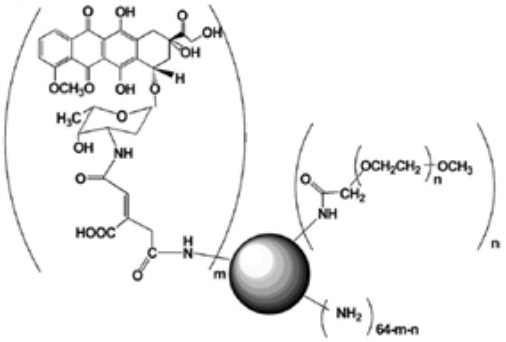
_ polylactic acid - polyethylene glycol[12]

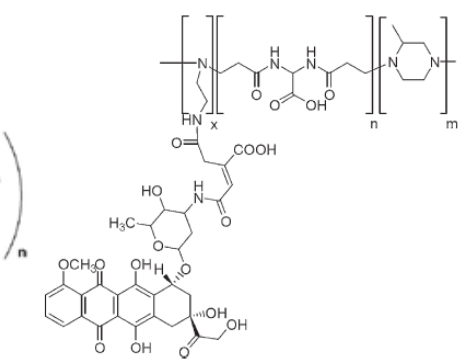


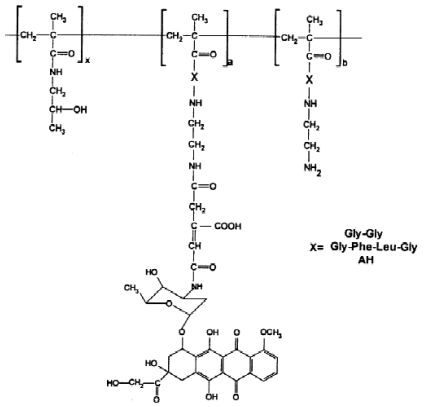
[7] S. Zhu, M. Hong, G. Tang, L. Qian, J. Lin, Y. Jiang, Y. Pei, *Biomaterials,* ***2010,*** *31,* 1360
[8] N. Lavignac, J. L. Nicholls, P. Ferruti, R. Duncan, *Macromolecular Bioscience,* ***2009,*** *9 (5), 480-487*
[9] K. Ulbrich, T. Etrych, P. Chytil, M. Jelınkova, B. Rıhovà, *J. Control. Release,* ***2003,*** *87 (1-3), 33-47*
[10] F.Q. Hu, L.N. Liu, Y.Z. Du, H. Yuan, *Biomaterials,* ***2009, 30, 6955-6963***
[11] S. B. Seo, C. R. Park, S. Y. Jeong, *J. Control. Release,* ***2003, 91, 135-145***
[12] Y. S. Yoo, E. A. Lee, T. G. Park, *J. Control. Release,* ***2002, 82, 17-27***

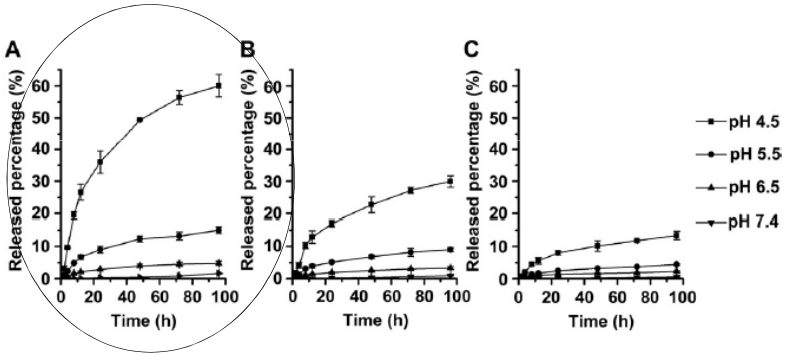
_ polyamidoamines[7,8,9]











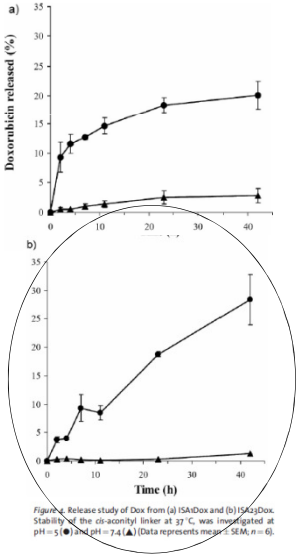


Figure 4. Release study of Dox from (a) ISA:Dox and (b) ISA:βDox. Stability of the *cis*-aconityl linker at 37 °C, was investigated at pH = 5 (●) and pH = 7.4 (▲) (Data represents mean ± SEM; $n = 6$).

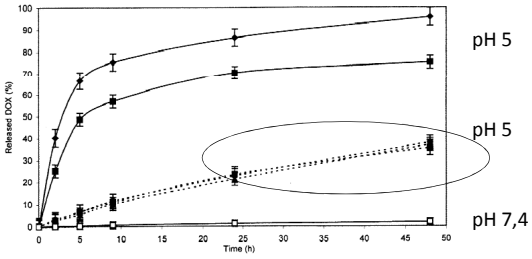
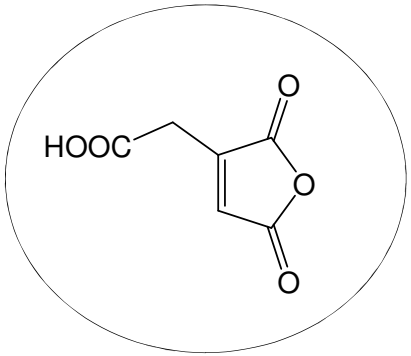


Fig. 2. Release of DOX from polymer–DOX conjugates incubated in 0.1 M acetate buffers pH 5 and pH 7.4 (containing 0.05 M NaCl) at 37 °C; substrate (DOX) concentration 0.5 mM: (■, ——) conjugate 16 (pH 5); (♦, ——) conjugate 18 (pH 5); (▲, - - -) conjugate 21 (pH 5); (■, - - -) conjugate 22 (pH 5); (●, - - -) conjugate 23 (pH 5); (□, ——) conjugate 22 (pH 7.4).

[7] S. Zhu, M. Hong, G. Tang, L. Qian, J. Lin, Y. Jiang, Y. Pei, *Biomaterials,* ***2010,*** *31,* 1360
[8] N. Lavignac, J. L. Nicholls, P. Ferruti, R. Duncan, *Macromolecular Bioscience,* ***2009,*** *9 (5), 480-487*
[9] K. Ulbrich, T. Etrych, P. Chytil, M. Jelınkova, B. Rıhovà, *J. Control. Release,* ***2003,*** *87 (1-3), 33-47*



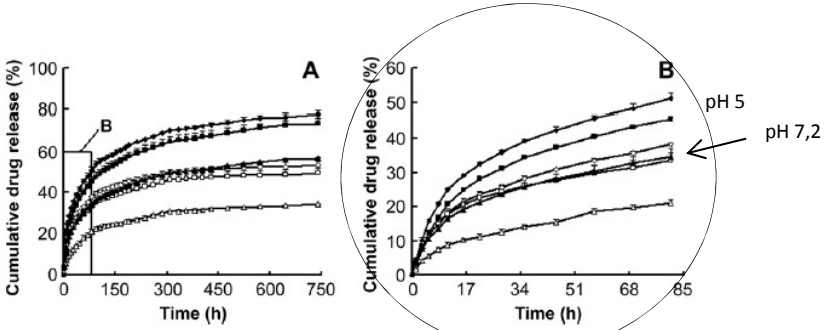


Fig. 5. Effect pH on the doxorubicin release profiles of from DOX–CSO–SA micelles. Release profile measured by fluorometer. (B) is the amplification of the part of (A). (-◇-) DOX-CSO-SA-3, pH 7.2; (-□-) DOX–CSO–SA-6, pH 7.2; (-△-) DOX–CSO–SA-10, pH 7.2; (-◆-) DOX–CSO–SA-3, pH 5.0; (-■-) DOX–CSO–SA-6, pH 5.0 and (-▲-) DOX–CSO–SA-10, pH 5.0. Each point is the mean of three replicates. Data represent the mean ± standard deviation ($n = 3$).

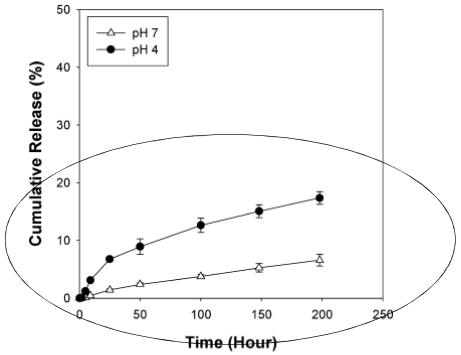
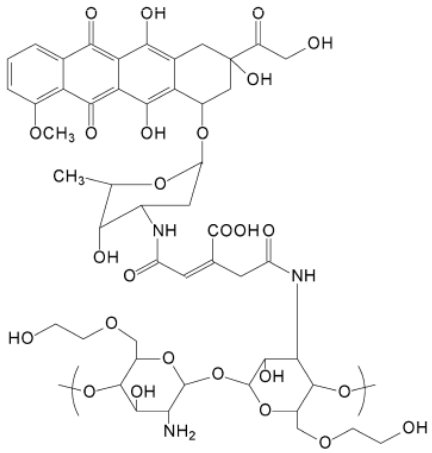
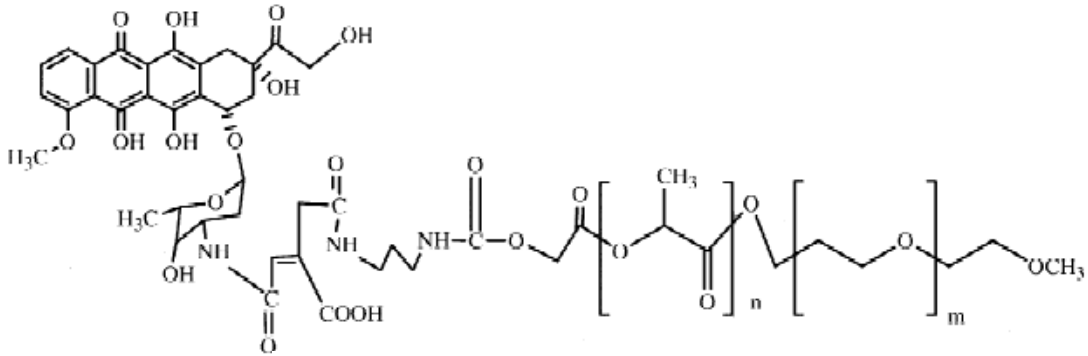


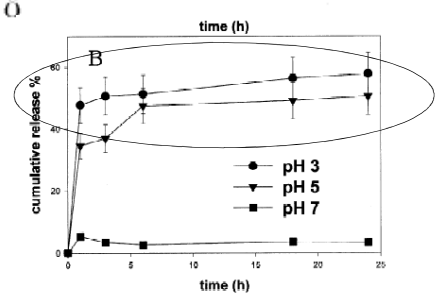
Fig. 6. Effect of pH on the release profile of doxorubicin from doxorubicin loaded GC-DOX (DOX/GC-DOX) nanoaggregates. Release profile measured by fluorometer. Each point is the mean

_ chitosan[10,11]



_ polylactic acid - polyethylene glycol[12]





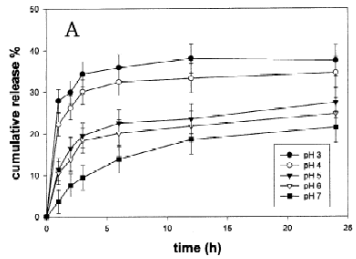
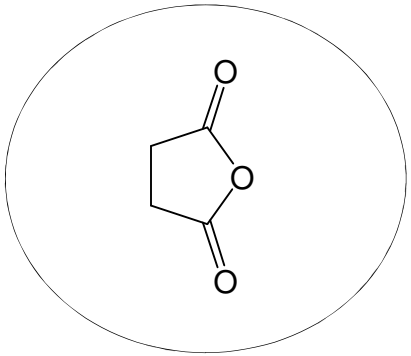
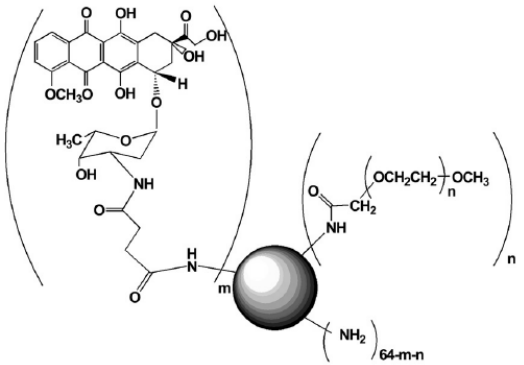


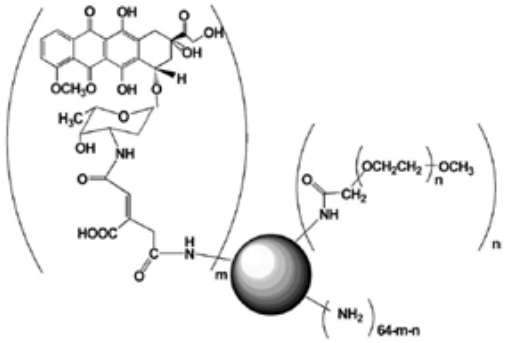
Fig. 4. Short-term release profiles of doxorubicin from doxorubicin-conjugated micelles at various pH values. (A) Doxorubicin-conjugated micelles with a hydrazone linkage and (B) those with a *cis*-aconityl bond.

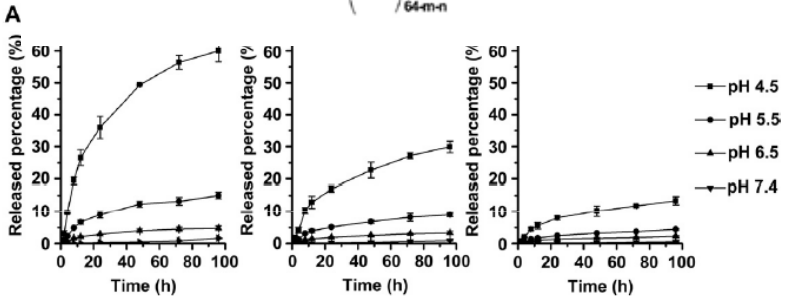
[10] F.Q. Hu, L.N. Liu, Y.Z. Du, H. Yuan, *Biomaterials,* ***2009, 30, 6955-6963***
[11] S. B. Seo, C. R. Park, S. Y. Jeong, *J. Control. Release,* ***2003, 91, 135-145***
[12] Y. S. Yoo, E. A. Lee, T. G. Park, *J. Control. Release,* ***2002, 82, 17-27***



_ polyamidoamines[7]

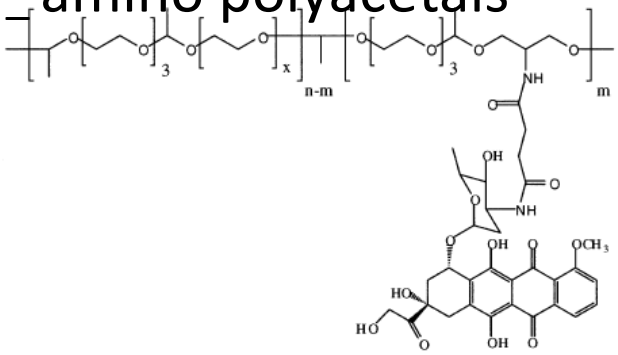


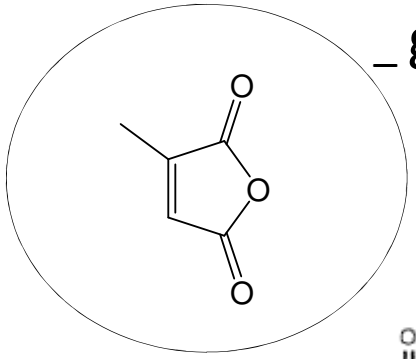




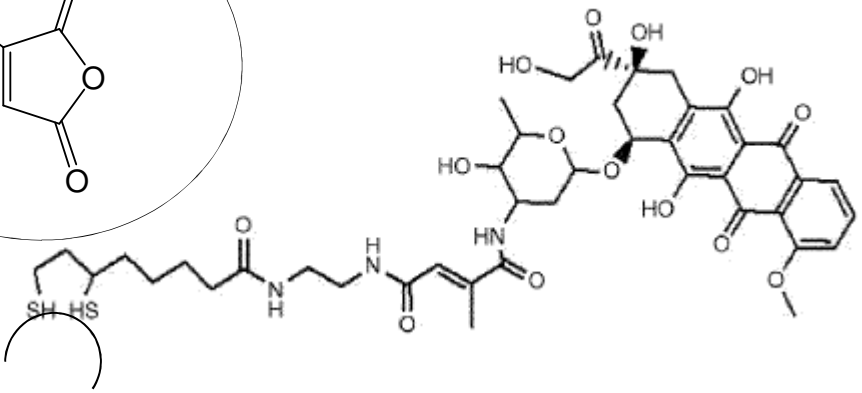
"PPSD conjugates released negligible amount of DOX (less than 1.0%) at any pH condition"

_ amino polyacetals[13]





_ gold nanoparticles[14]



release due to polyacetal degradation

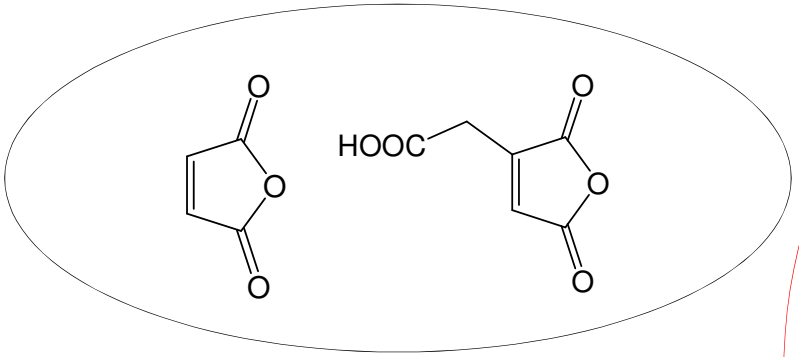
[7] S. Zhu, M. Hong, G. Tang, L. Qian, J. Lin, Y. Jiang, Y. Pei, *Biomaterials, **2010,** 31,* 1360

[13] R. Tomlinson, J. Heller, S. Brocchini and R. Duncan, *Bioconjugate Chem.* **2003**, *14,* 1096-1106

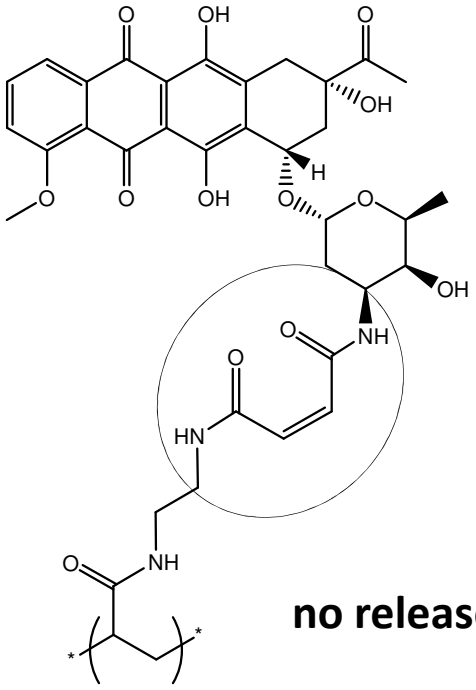
[14] S. Kim, J. Nam (Postech Academy-Industry Foundation Pohang-si, Gyeonsangbuk-do), EP2559429 **2013**

_ 1981, Shen and Ryser[6]

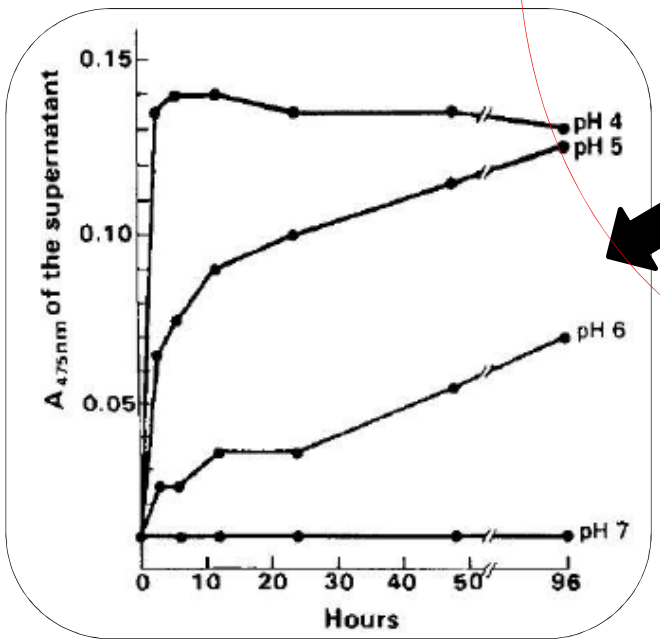
daunorubicin on aminoethyl polyacrylamide

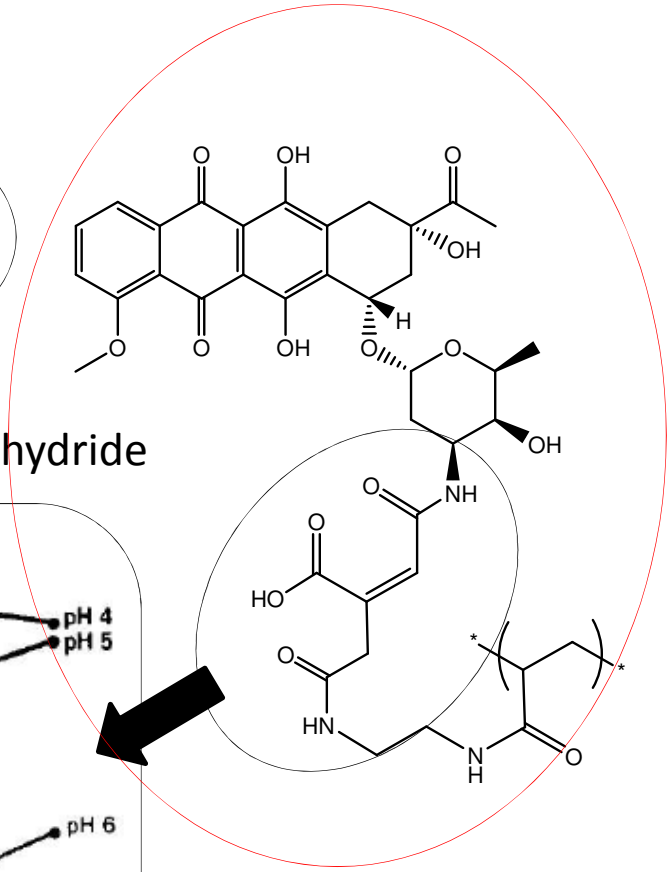


maleic anhydride vs. cis-aconitic anhydride



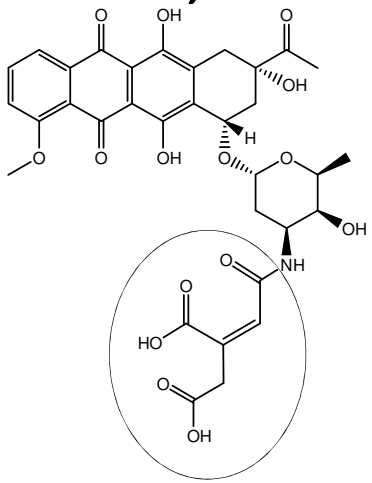
no release



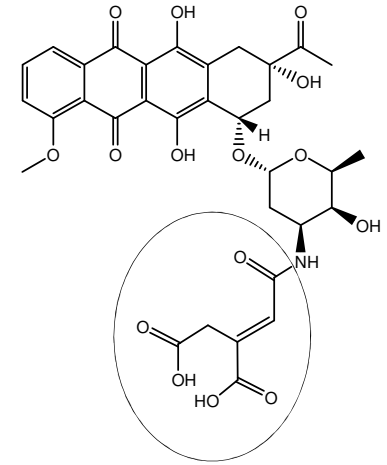


[6] W. C. Shen, H. J. P. Ryser, *Biochem. Biophys. Res. Commun.*, ***1981***, *102,* 1048–1054

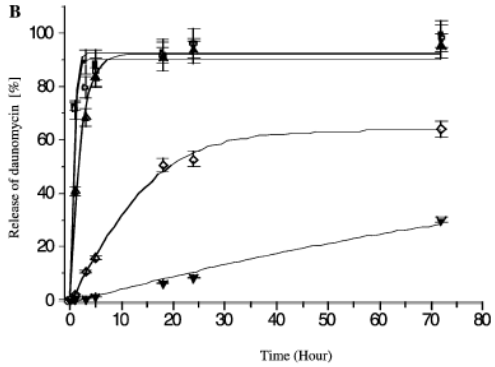
_ 2003, Hudecz[15]



“cis” isomer



“trans” isomer



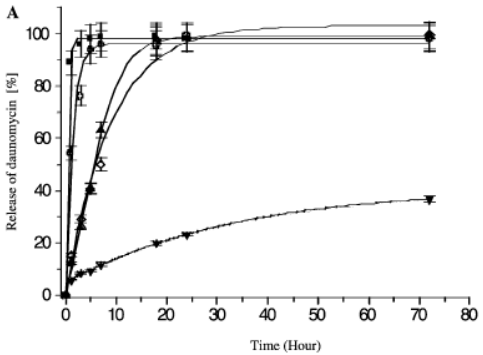
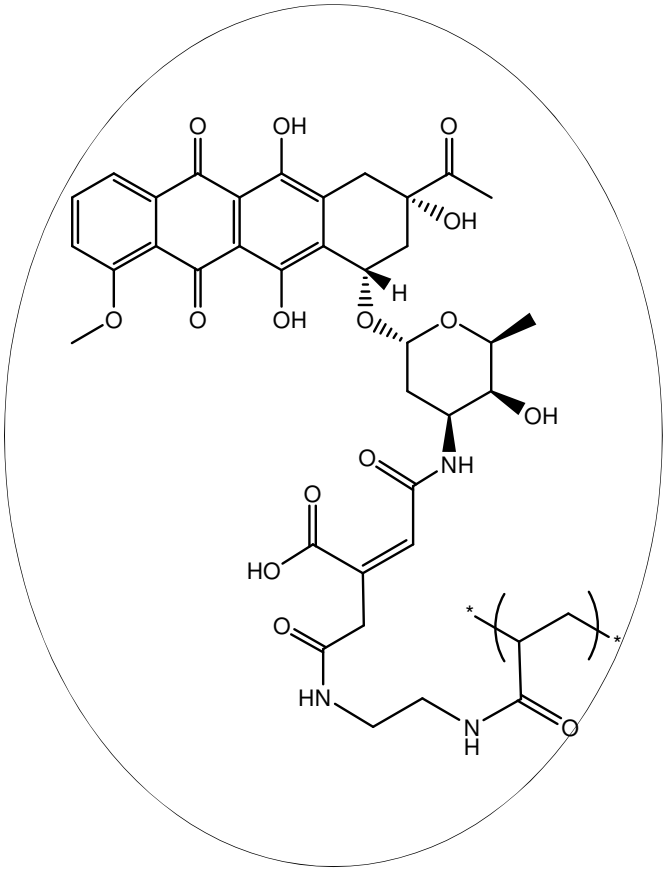


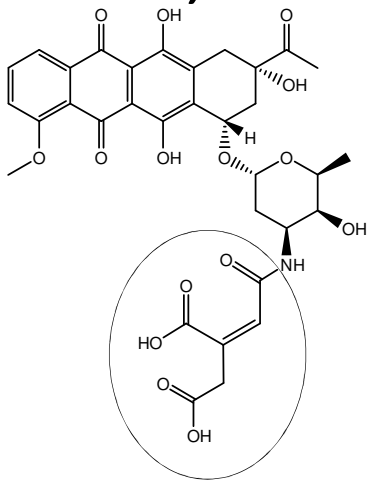
Fig. 3. pH dependent release of daunomycin from cAD-1 (A) and cAD-2 (B) isomers detected by analytical RP-HPLC. Elution was carried out as described in Fig. 2. Samples were dissolved in 0.1 M citrate–phosphate buffer ($c = 1$ mg/ml for daunomycin content) at pH 7 (▼), pH 6 (◇), pH 5 (▲), pH 4 (■), and pH 3 (○).



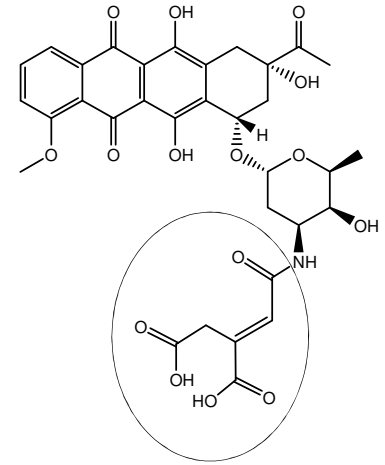
[15] J. Remenyi, B. Balazs, S. Toth, A. Falus, G. Toth and F. Hudecz, *Biochem. Biophys. Res. Commun.*, ***2003,*** *303,* ***556–561***

[16] A. Kakinoki, Y. Kaneo, Y. Ikeda, T. Tanaka, K. Fujita, *Biol. Pharm. Bull.*, ***2008****, 31,* 103-110

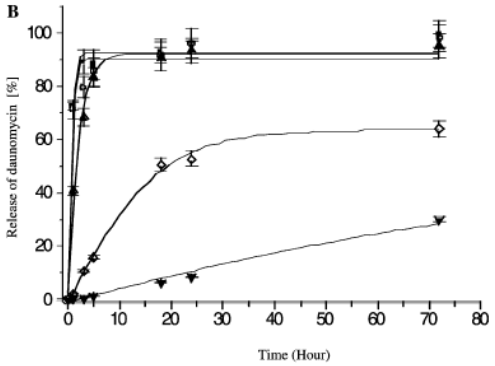
_ 2003, Hudecz[15]



"cis" isomer



"trans" isomer



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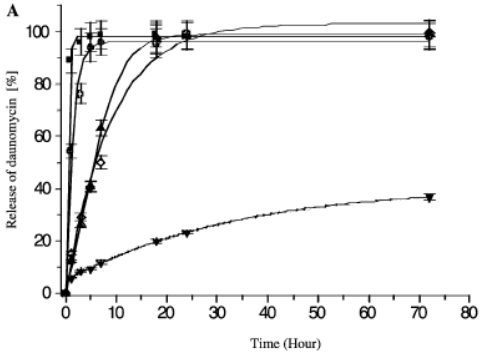
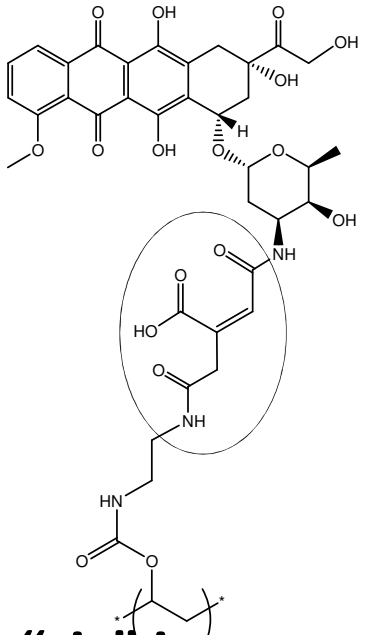
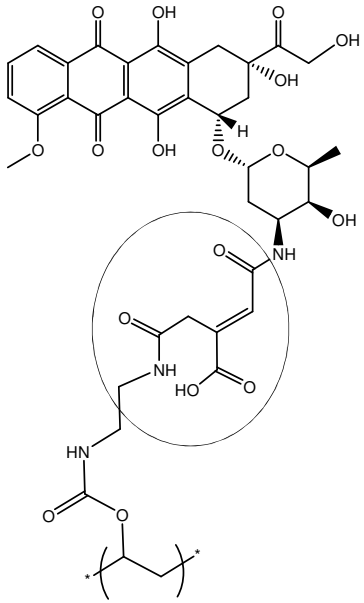


_ 2008, Kakinoki[16]



"cis" isomer



"trans" isomer

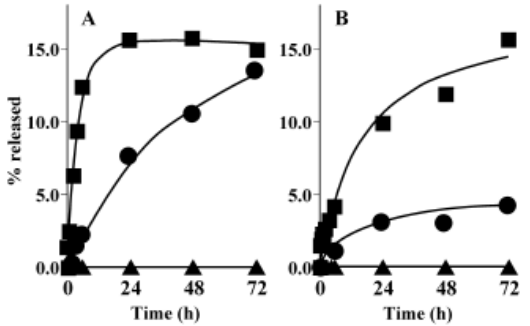


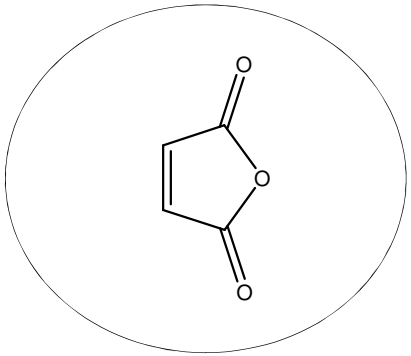
Fig. 5. Effect of pH on the Release of DOX from PVA–*cis*-ADOX (A) and PVA–*trans*-ADOX (B)

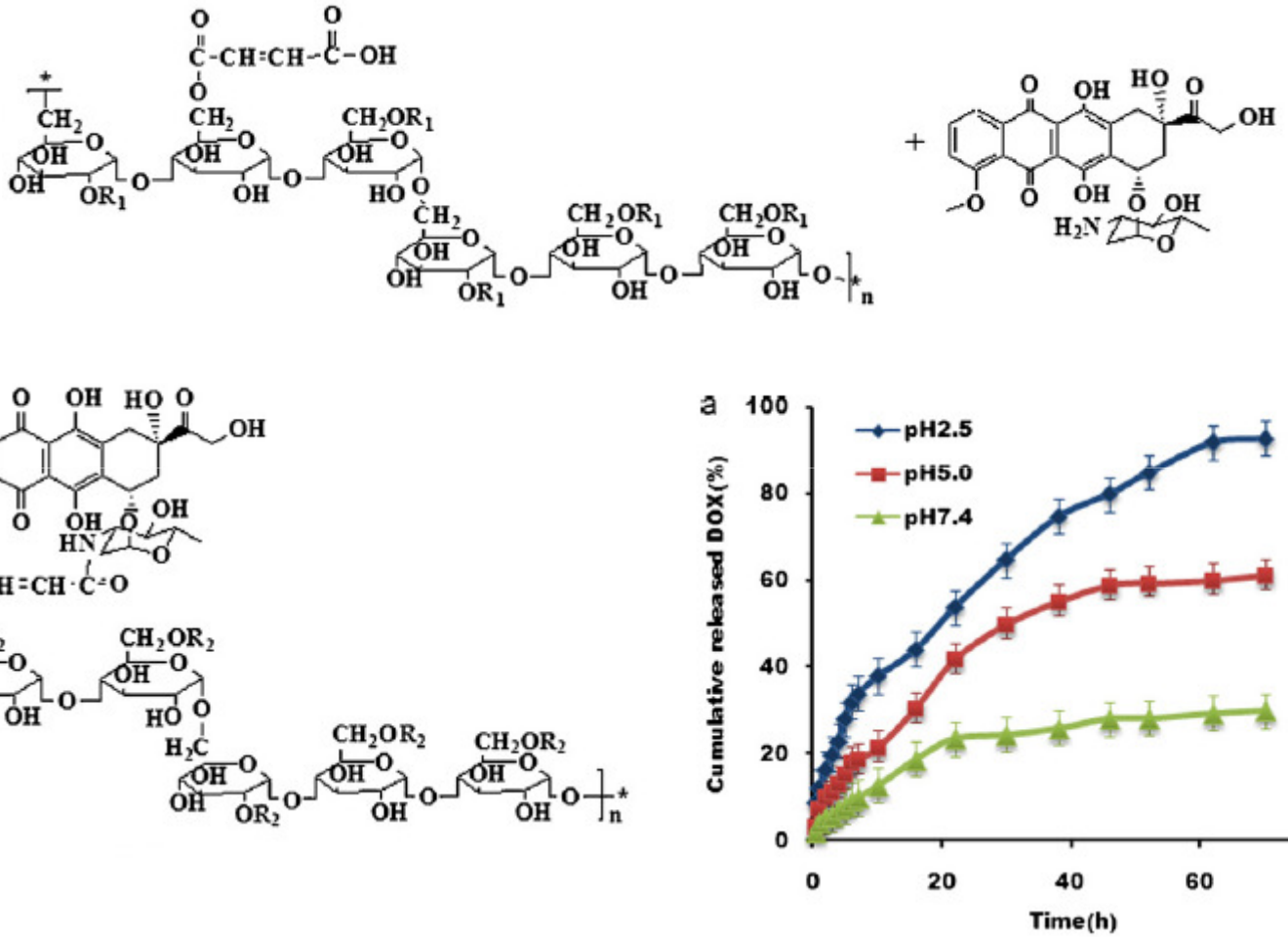
The release of DOX from the conjugates was determined in 0.1 M citrate buffer solution (μ=0.3) of pH 5.0 (■), 6.0 (●), and 7.0 (▲) at 37 °C.

[15] J. Remenyi, B. Balazs, S. Toth, A. Falus, G. Toth and F. Hudecz, *Biochem. Biophys. Res. Commun.*, ***2003,*** *303,* ***556–561***

[16] A. Kakinoki, Y. Kaneo, Y. Ikeda, T. Tanaka, K. Fujita, *Biol. Pharm. Bull.*, ***2008****, 31,* 103-110

_ 2011, H. Wu[17]





[17] H. Zhang, F. Li, J. Yi, C. Gu, L. Fan, Y. Qiao, Y. Tao, C. Cheng, H. Wu, *European Journal of Pharmaceutical Sciences,* ***2011****, 42,* 517–526

our project

APTS-containing bioactive glass

APTS25SG423

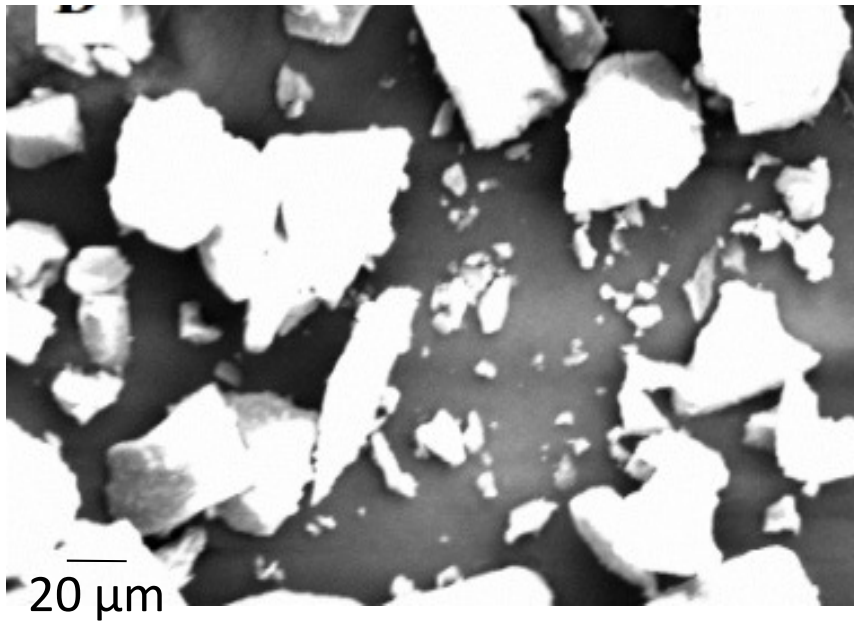
APTS is 25% of the precursors

synthesis via Sol-Gel

post-syntesis termic treatment (423 K)

free NH_2 on the surface(from titration): 3.5 mmol/g

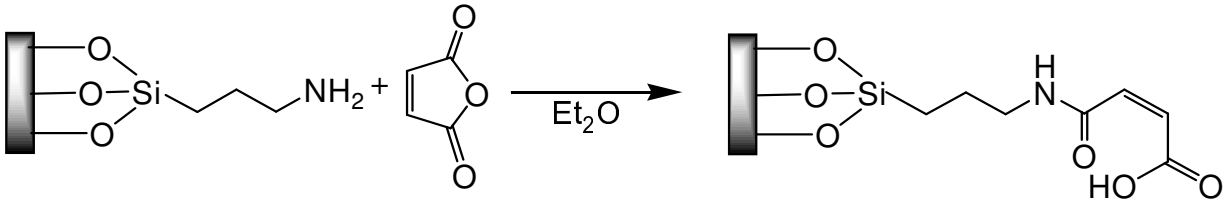
SEM Micrograph



EDS Microanalysis

ELEMENT	WEIGHT %
Si	26.42
O	40.18
Ca	7.26
P	3.11
C	19.56
N	3.43
TOTALS	100.00

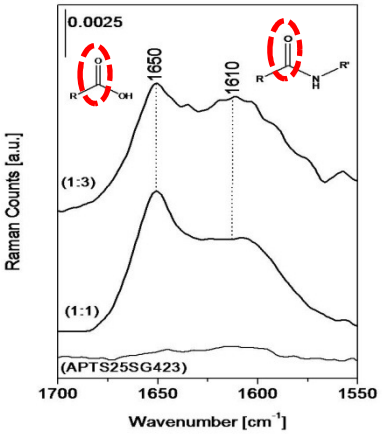
_ functionalization with maleic anh.[18]



NH_2: 3.5 mmol/g

0.9 mmol/g

Raman Spectroscopy



[18] G. Gupta and S. B. Wagh, *Indian Journal of Chemistry*, **2006**, *45B*, 697-702

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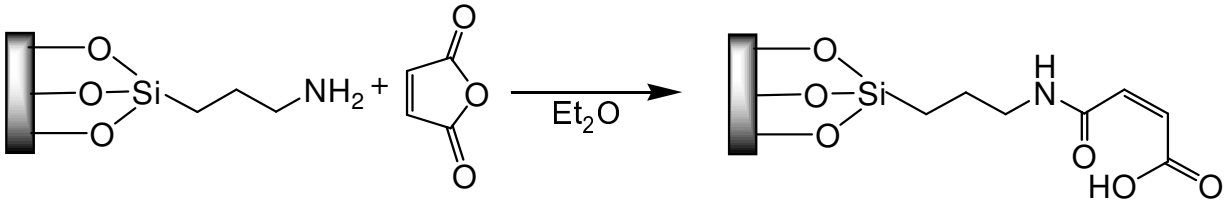
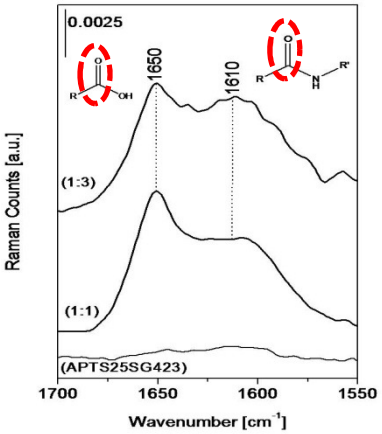
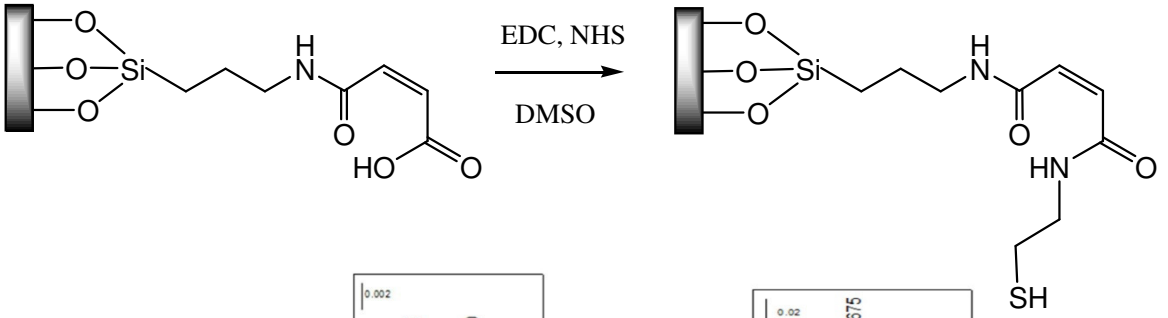


NH_2: 3.5 mmol/g 0.9 mmol/g

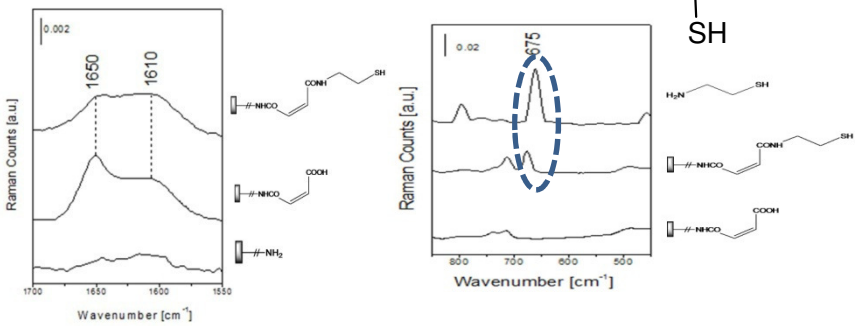
Raman Spectroscopy



_ conjugation with cysteamine

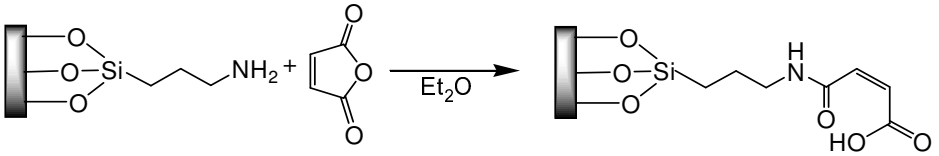


Elemental Analysis: N, 4.54; C, 8.3; H, 2.97; S, 3.64 (%wt); 8.85 g cysteamine /100 g bioglass

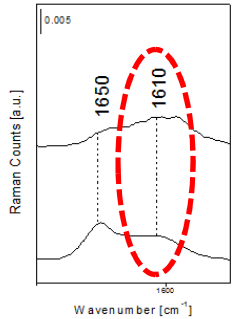


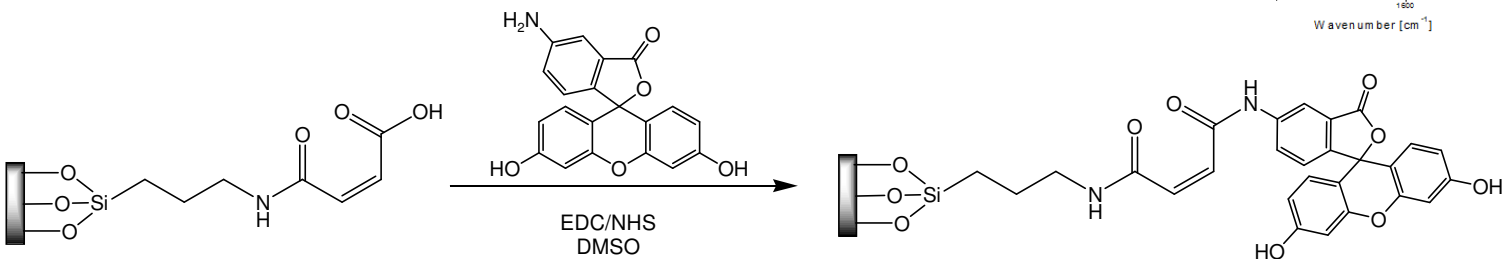
[18] G. Gupta and S. B. Wagh, *Indian Journal of Chemistry*, **2006**, *45B*, 697-702

_ functionalization with maleic anh.

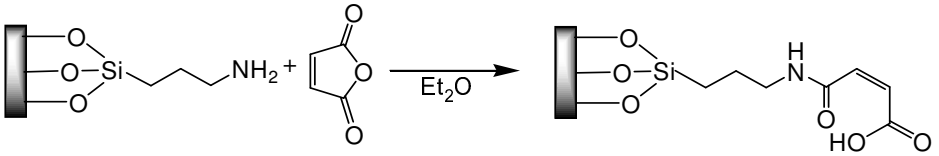


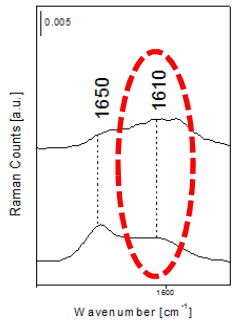
_ conjugation with 5-aminofluorescein





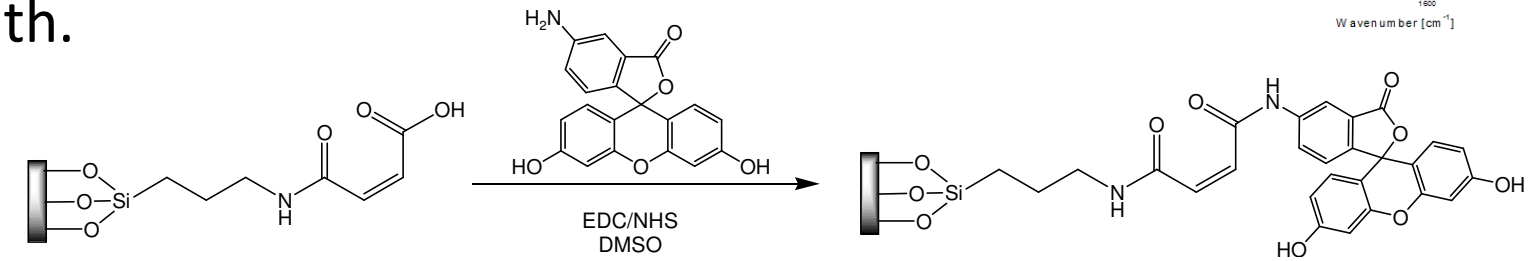
_ functionalization with maleic anh.



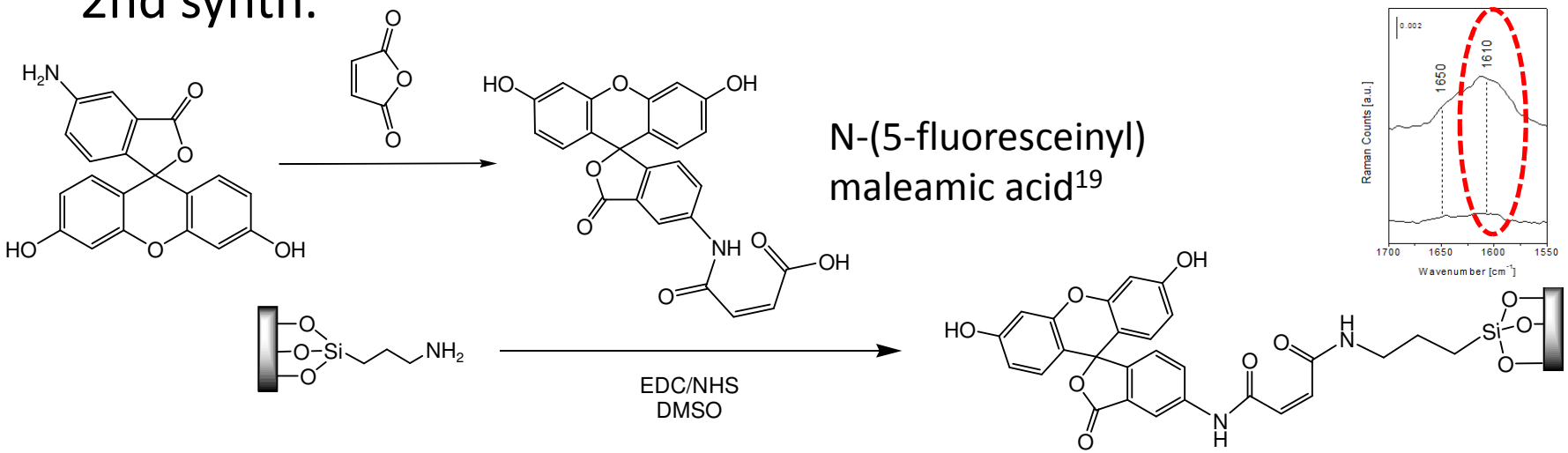


_ conjugation with 5-aminofluorescein

1st synth.



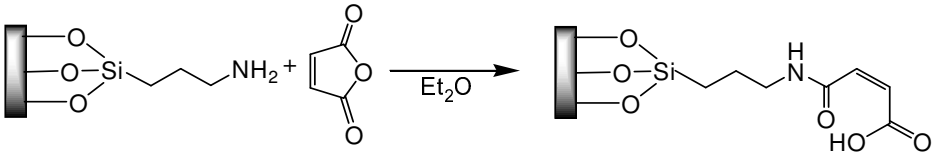
2nd synth.

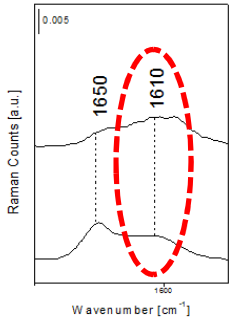


N-(5-fluoresceinyl) maleamic acid[19]

[19] P.Y. Reddy, S. Kondo, S. Fujuta, T. Toru. *Synthesis*. **1998**. 999-1002

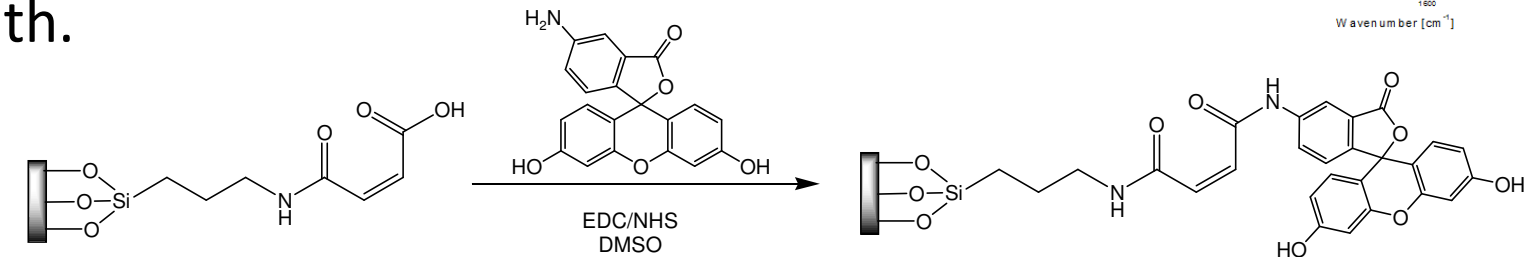
_ functionalization with maleic anh.





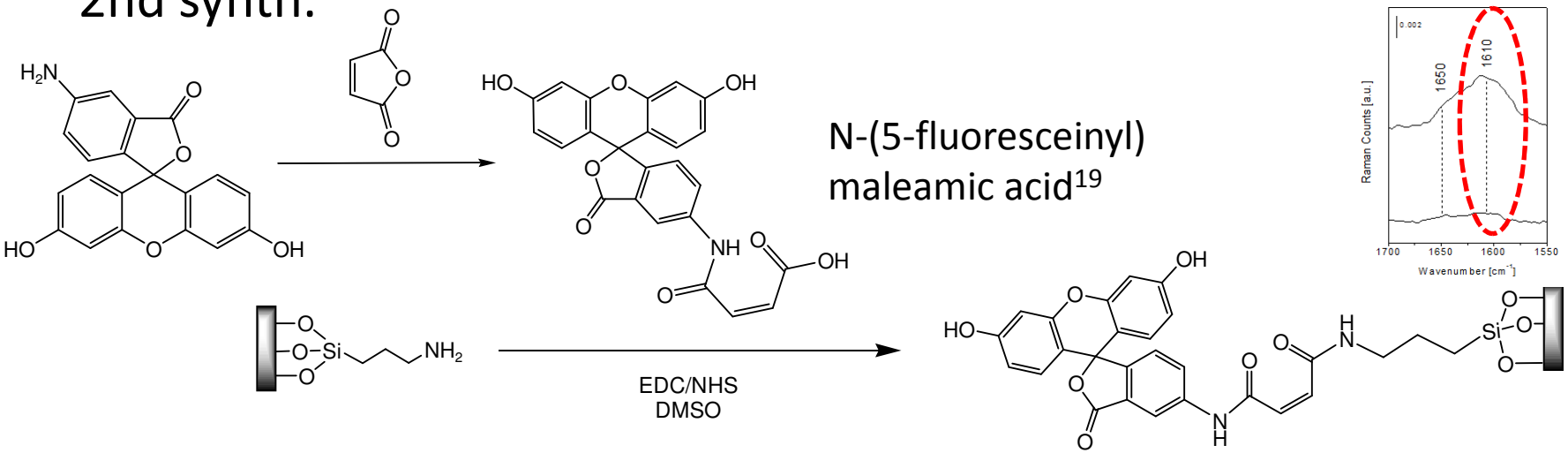
_ conjugation with 5-aminofluorescein

1st synth.



TGA: 65.5 g 5-amminofluorescein/100 g

2nd synth.



N-(5-fluoresceinyl) maleamic acid[19]

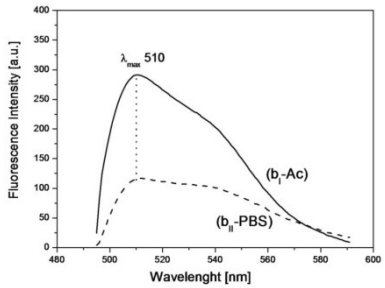
TGA: 35.2 g 5-amminofluorescein/100 g

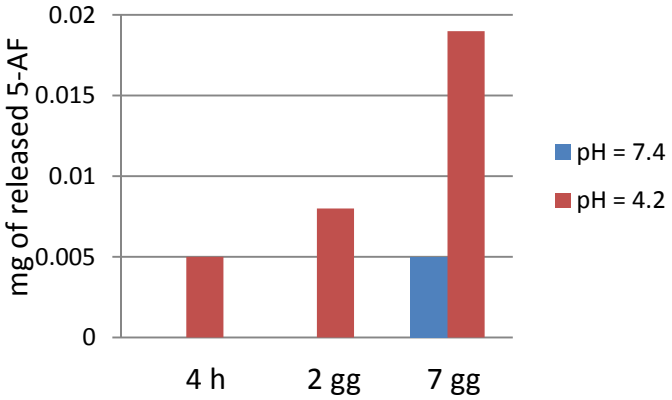
UV-Vis: 37.1 g 5-amminofluorescein/100 g

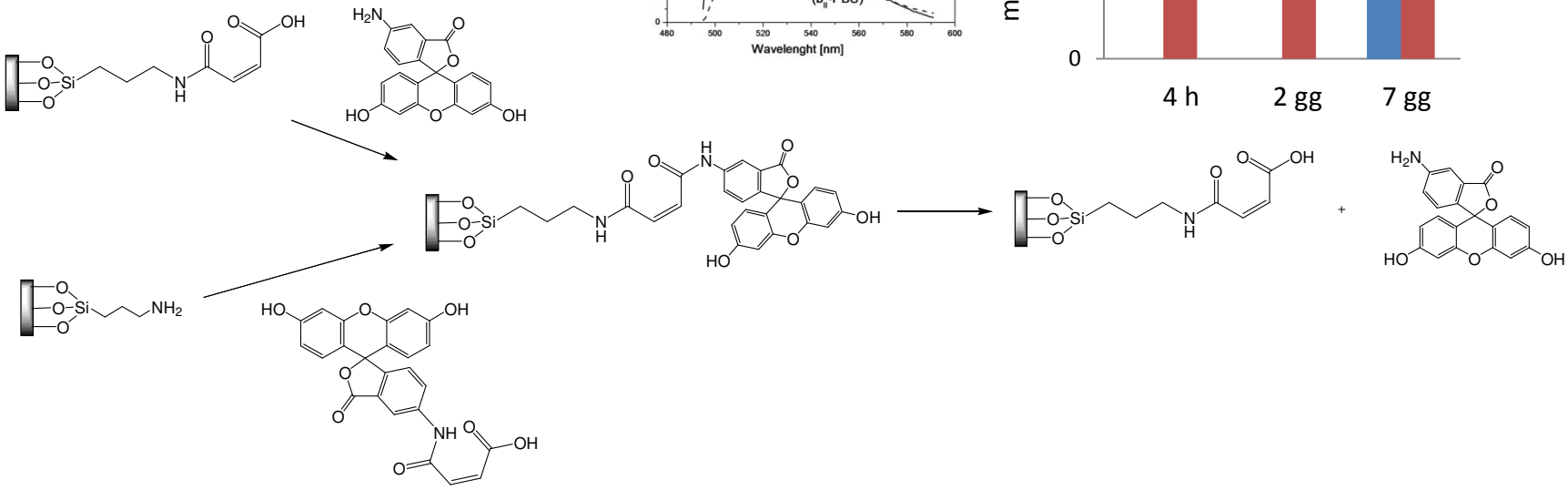
[19] P.Y. Reddy, S. Kondo, S. Fujuta, T. Toru. *Synthesis*. **1998**. 999-1002

release tests

- phosphate buffer (pH 7.4)
- acetate buffer (pH 4.2)







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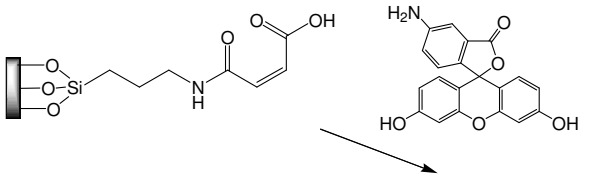

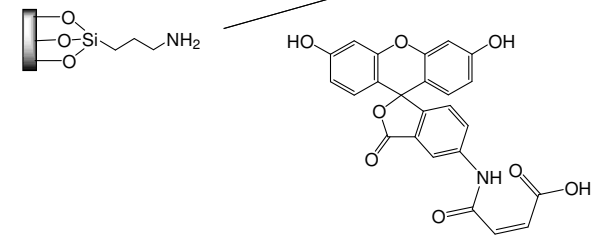

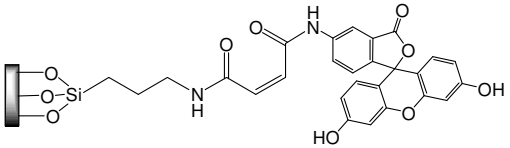

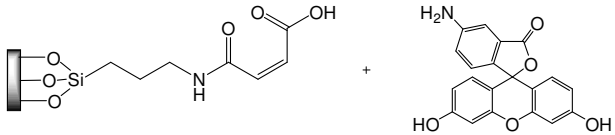

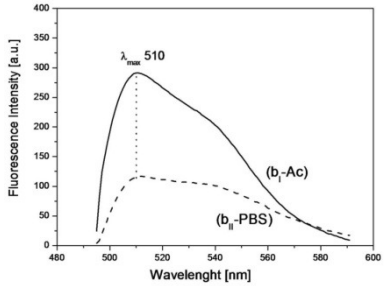

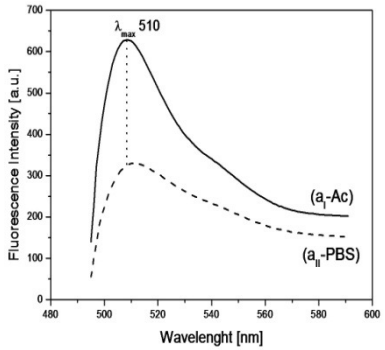

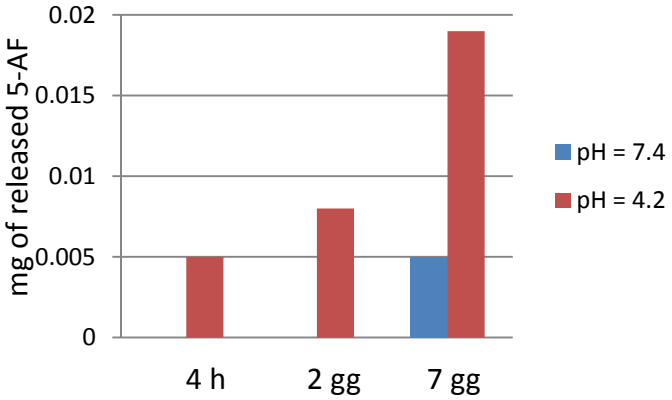

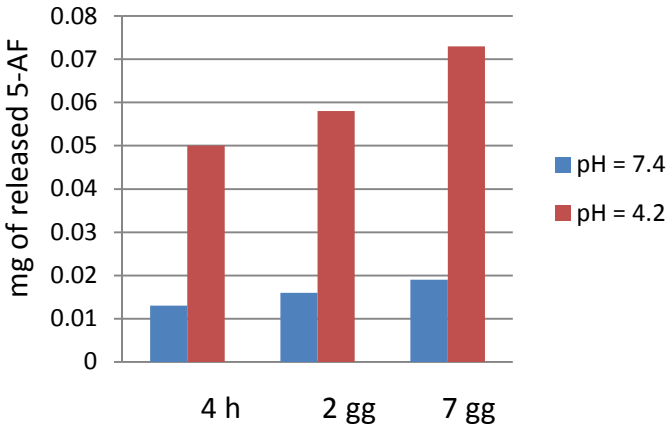

Conclusions and perspectives

_ functionalization of ATPS-containing bioglass (Raman; titration; elemental analysis; TGA, UV)

_ release tests (pH 7.4 and pH 4.2)

- _ pH-sensitive
- _ low amount released (comparable with previous studies; concentration on the tumor site)

_ cis-aconitic anhydride

_ conjugation with doxorubicin

_ application to TiO_2-chiosan hybrid materials

Thanks

prof. Gianmario Martra, dott. ssa Giuseppina Cerrato, dott. ssa Valentina Aina
prof. Guido Viscardi
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prof. ssa Gigliola Lusvardi, dott. Gianluca Malavasi
Dept. of Chemical and Geological Sciences, University of Modena and Reggio Emilia

prof.ssa Michela Signoretto, dott.ssa Elena Ghedini
Department of Chemistry, University of Venice

Francesca Malafronte and Walter Intelisano