

WhitePaper: Passive dissolution of Coumarin-6 into water by Polyvivo polymers

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Background

A classic drug delivery problem is difficulty with IV injections of poorly soluble drugs. The requirement for large quantities of saline to dissolve such materials limits their clinical use. One common solution for this problem is to dissolve the drug into a surfactant micelle. Here testing was performed to assay the capability of a variety of Polyvivo polymers for solubilizing drugs by simple passive micelle uptake at room temperature with gentle shaking. Note this is different than co-dissolution to form nanoparticles or other methods such as sonication which notable aide the emulsification of the material so in no means should these results be considered the maximum possible loading. Rather this is simply exposing the poorly soluble substance to an aqueous solution of the dissolved polymer.

Drug Model: Coumarin-6 (C6)

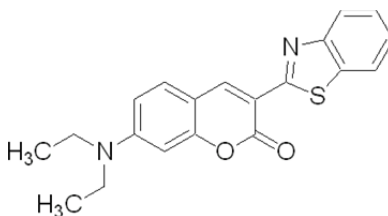


Figure 1. Coumarin-6 structure

Coumarin-6 (Fig 1) is traditionally used as a laser dye due to its highly fluorescent nature however it is commonly used as a model hydrophobic drug for studies involving release and tracking of localized delivery. The native solubility of Coumarin-6 in water is low which makes it a good model for hydrophobic drugs such as paclitaxel, everolimus, and others. Visibly this material is bright yellow making it easy to see even to the naked eye.

Method

Dissolution

Five ml of distilled water was added to a 22ml scintillation vial. To the water a pre-weighed quantity of PolyVivo polymer indicated below was added so as to generate a 1,2, or 5% w/v solution respectively. An excess quantity of C6 was added to the scint vial and the vial was incubated overnight with gentle shaking at room temperature with the exemptions noted below of materials which were incubated at 4C.

Screening

Following this each solution was loaded into a syringe and pushed through a 0.45µm (450nm) PVDF filter to eliminate any particulate matter. The filtered solution was considered to be effectively dissolved as anything larger than 450nm had been excluded (Fig 2).

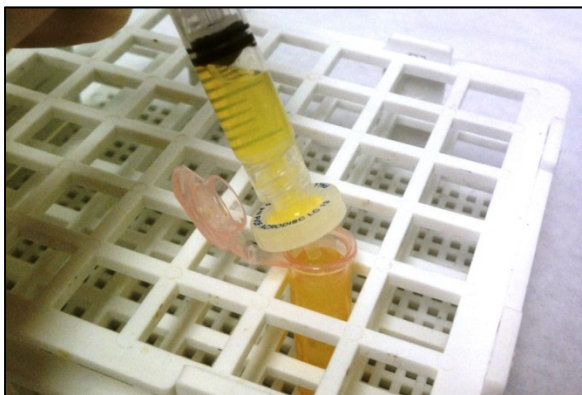


Figure 2. example image of screening out particulates.

In some cases, the dissolved polymer in water turned into a gelatinous solution and therefore could not be passed through the 0.45 μ m filter. To ensure a successful screening, these solutions were centrifuged twice (10,000RPM, 5-min) so that the Coumarin 6 particles would fall to the bottom and the non-particulate solution could be extracted for testing. These cases are noted below.

Testing

Solution was combined as 1 ml of C6 loaded solution with 1 ml ethanol (USP) and tested via UV-Vis in a Genesys-6 spectrophotometer. This was compared to a blank consisting of 1:1 distilled water and ethanol. The absorbance at 469nm was recorded and compared to a previously established calibration curve for coumarin-6 to obtain mg/ml concentration in the solution. This was then corrected for the dilution factor and converted to μ g/ml. This was compared to previously measured μ g/ml content of coumarin-6 in water as “fold-increase” in solubility due to the polymer.

Sometimes during dilution, the polymer solution precipitated with the ethanol. In these cases, instead of using ethanol to dilute the solution the 1 ml polymer solution was added to 1 ml water for dilution and the blank used was 2ml distilled water. These cases are noted below.

Results

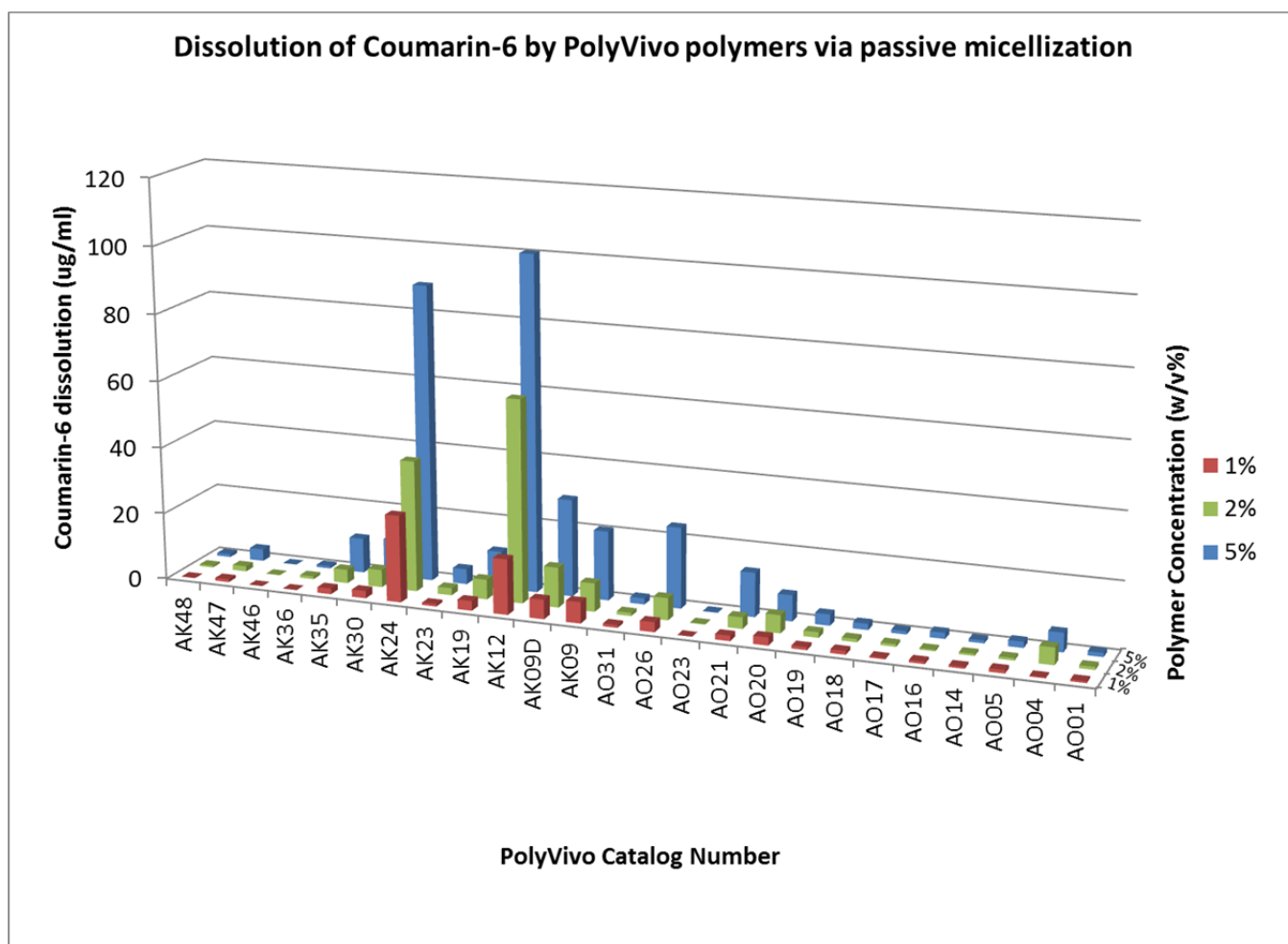
Table 1 shows screening results from screening of Poly-vivo products for coumarin-6 solubility. This is for testing in singlicate.

Product (description)	(w/v%)	Dissolved μ g/ml	Solubility fold improvement
Water	NA	0.087	NA
AK48 (mPEG-PLLA (Mw ~5,000:500 Da)	1%	0.11	1.31
	2%	0.53	6.11
	5%	0.87	10.04
AK47 (mPEG-PLLA (Mw ~5,000:1,000 Da) (83% PEG)	1%	0.87	10.04
	2%	1.55	17.90
	5%	3.60	41.49
AK46 (PLA-PEG-PLA (1,000-1,000-1,000 Da) ^d	1%	0.00	0.00
	2%	0.08	0.87
	5%	0.04	0.44
AK44 (mPEG-PS (2,000-2,000 Da) ^e	1%	14.40	165.95
	2%	34.91	402.21
	5%	83.24	959.01
AK36 (mPEG-PCL	1%	0.08	0.87

(Mw ~ 750:2,500 Da) (23% PEG)	2%	0.83	9.61
	5%	0.72	8.30
AK35 (PCL-PEG-PCL (Mw ~ 1,000:1,000:1,000 Da) (33% PEG)	1%	1.71	19.65
	2%	4.06	46.73
	5%	10.39	119.66
AK30 (mPEG-PLGA (5,000-4,000 Da, 1:1 LA:GA) (56% PEG)	1%	2.20	25.33
	2%	5.23	60.27
	5%	11.07	127.52
AK24 (PLGA-PEG-PLGA (Mn~1,100:1,000:1,100 Da, 3:1 LA:GA) (25% PEG) ^{a, b}	1%	26.00	299.58
	2%	39.23	451.99
	5%	88.81	1023.20
AK23 (mPEG-PLGA (2,000:1,500 Da, 1:1 LA:GA) (57% PEG)	1%	0.72	8.30
	2%	2.12	24.46
	5%	4.55	52.40
AK19 (PLGA-PEG-PLGA (1,500:1,500:1,500 Da, 1:1 LA:GA) (33% PEG)	1%	2.92	33.63
	2%	5.91	68.13
	5%	11.07	127.52
AK12 (PLGA-PEG-PLGA (Mn~1,000:1,000:1,000 Da, 1:1 LA:GA) (25% PEG) ^{a, b}	1%	16.56	190.84
	2%	60.73	699.60
	5%	100.49	1157.71
AK09D (PEG-PDLLA-Decyl (2,000:2,200 Da) Decyl (48% PEG Plus Decyl Endcap)	1%	5.80	66.82
	2%	11.98	138.00
	5%	29.04	334.52
AK09 (mPEG-PDLLA (2,000:2,200 Da) (48% PEG)	1%	6.37	73.37
	2%	8.49	97.82
	5%	20.73	238.88
AO31 (PNVCL)	1%	0.80	9.17
	2%	0.95	10.92
	5%	1.86	21.40
AO26 (PF(MDI) -50)	1%	2.96	34.06
	2%	6.60	75.99
	5%	24.18	278.62
AO23 (P(NIPAM-AM) (95:5)	1%	0.00	0.00
	2%	0.19	2.18
	5%	0.15	1.75
AO21 (P(NIPAM-AM) (75:25)	1%	1.59	18.34
	2%	3.45	39.74
	5%	13.19	151.97
AO20 (P(PF407) (10:1) (5,000 Da) endcap (nominal 10 poloxamer chains/mPEG endcap)	1%	2.39	27.51
	2%	5.34	61.58
	5%	7.77	89.52
AO19 (P(DMEAMA-mPEG) (95:5)	1%	0.87	10.04
	2%	1.63	18.78
	5%	3.34	38.43
AO18 (P(VCL-mPEG) (95:5)	1%	1.06	12.23
	2%	0.95	10.92
	5%	1.86	21.40
AO17 (P(NIPAM-mPEG) (95:5) ^{b, c}	1%	0.42	4.80
	2%	0.76	8.73
	5%	0.95	10.92

AO16 (P(NIPAM-AM) (50:50) ^c	1%	0.68	7.86
	2%	0.45	5.24
	5%	1.78	20.53
AO14 (P(NIPAM-AA) (95:5) ^{b, c}	1%	0.57	6.55
	2%	0.61	6.99
	5%	0.87	10.04
AO05 (PASP (Mw~21,000 Da) ^b	1%	0.99	11.35
	2%	0.72	8.30
	5%	1.82	20.96
AO04 (PSPAK (Viscosity 10-100 cP 1% in water at 60 RPM)	1%	0.27	3.06
	2%	5.08	58.52
	5%	5.72	65.94
AO01 (PAM (Viscosity 25-150 cP 1% in water at 60 RPM) ^{b, c}	1%	0.53	6.11
	2%	0.87	10.04
	5%	1.18	13.54

Figure 3 summarizes the screening results by showing the $\mu\text{g/ml}$ coumarin-6 dissolution achieved by passive micellization uptake.



To further study these polymers a selection of 4 polymers were re-tested in triplicate and the average and standard deviation of all tests (N=4) is listed in the table below.

Product (description)	(w/v%)	Dissolved $\mu\text{g/ml}$ ($\pm\text{STDEV}$)	Solubility fold improvement ($\pm\text{STDEV}$)

Water	NA	0.087	NA
AK09 (mPEG-PDLLA (2,000:2,200 Da) (N=4))	1%	3.0 ± 2.3	34.3 ± 26.2
	2%	5.0 ± 2.3	57.4 ± 27.0
	5%	12.2 ± 5.7	140.3 ± 65.8
AK24 (PLGA-PEG-PLGA (Mn~1,100:1,000:1,100 Da, 3:1 LA:GA) (N=4) ^{a,b})	1%	12.6 ± 9.2	145.3 ± 105.5
	2%	24.8 ± 9.6	285.9 ± 111.0
	5%	43.5 ± 30.8	501.7 ± 355.4
AK30 (mPEG-PLGA (5,000-4,000 Da, 1:1 LA:GA) (N=4))	1%	1.3 ± 0.6	14.7 ± 7.1
	2%	2.9 ± 1.5	33.7 ± 17.8
	5%	6.5 ± 3.4	75.3 ± 36.0
AK35 (PCL-PEG-PCL (Mw ~ 1,000:1,000:1,000 Da) (N=4))	1%	1.1 ± 0.4	12.4 ± 4.8
	2%	2.6 ± 1.0	29.6 ± 11.5
	5%	6.5 ± 2.6	75.4 ± 29.7

Notes

- a-Products AK12 and AK24 were incubated at 4C over night to ensure complete dissolution in water.
- b-Products which precipitated when ethanol added during dilution include AO01, AO05, AO14, AO17, AK24, and AK12. For these water was added for dilution instead of ethanol.
- c - Products which were gelatinous when dissolved in water include AO01, AO14, AO16, AO17. These materials were centrifuged to remove solids as they could not pass through filter.
- d – Product AK46 observed to only partially dissolve in water.
- e- Product AK44 has slight green color due to residual catalyst which may interfere with absorption.

Conclusion

The results of this screening assay indicate several different PolyVivo polymers have the capacity to aid the solubility of coumarin-6 dye in water via passive micellization.