Supplementary information

Synergistic Combinations of Multiple Chemotherapeutic Agents in a High Capacity Poly(2-oxazoline)s Polymeric Micelles

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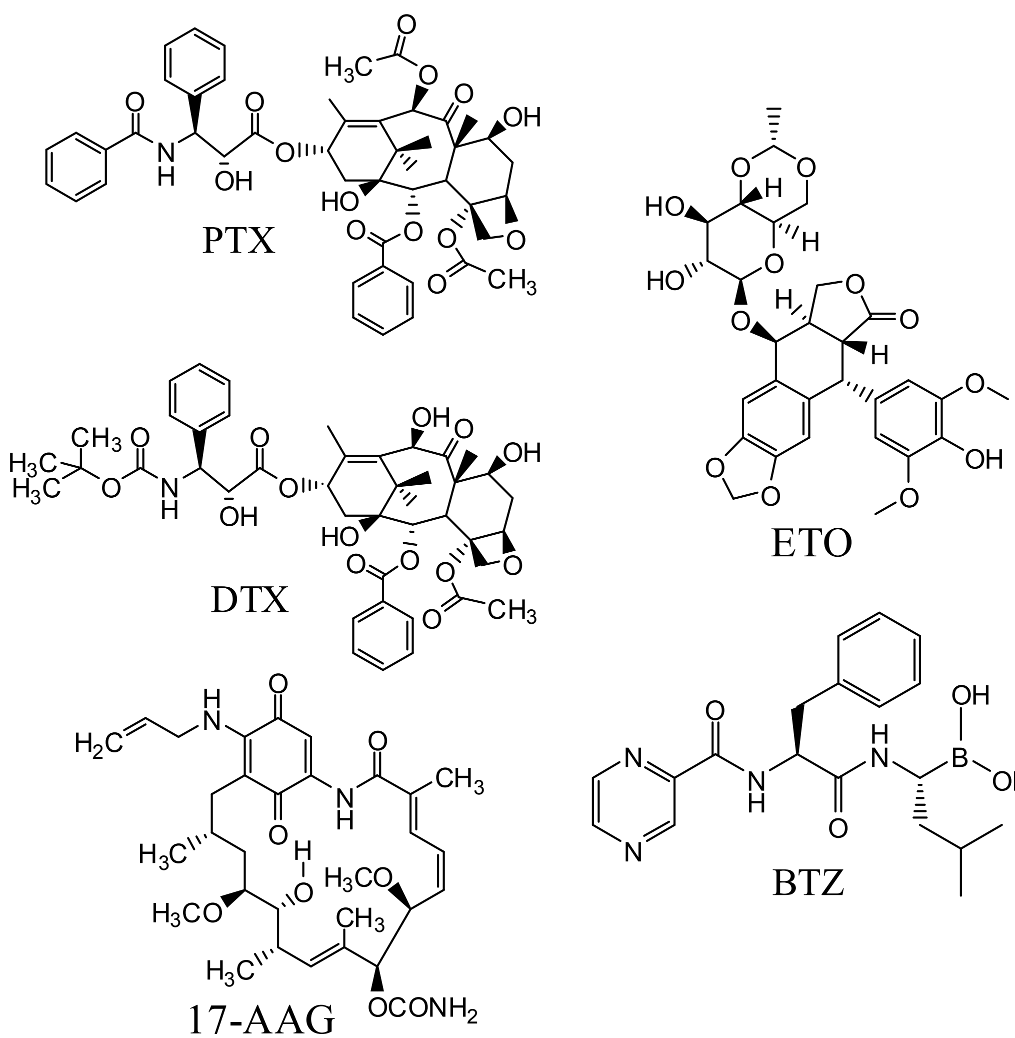

Fig. S1. Chemical structures of anti-cancer drugs.

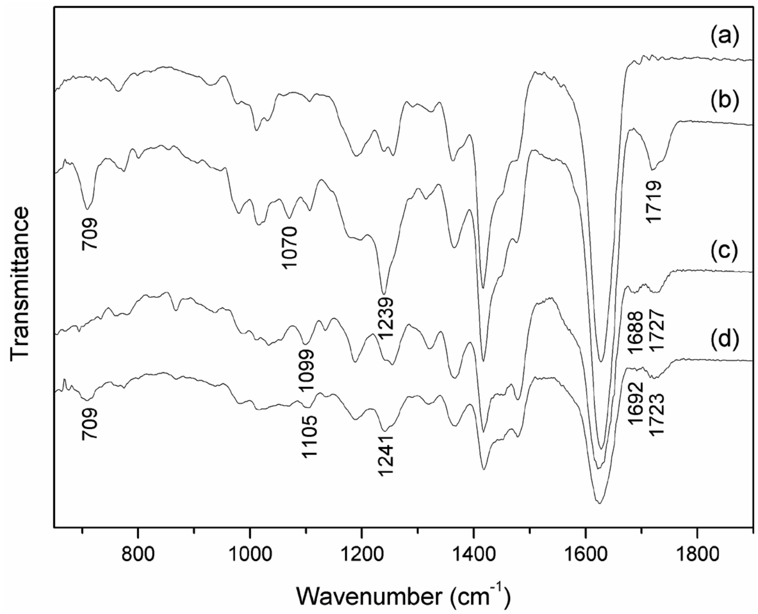

Fig. S2. Attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectra of **(a)** amphiphilic block copolymer alone, and POx block copolymer micelles loaded with **(b)** PTX, **(c)** 17-AAG, and **(d)** combination of PTX and 17-AAG. Incorporation of drugs into POx micelles was confirmed by ATR FT-IR spectra. For example, compared to the spectrum of POx polymer alone (a), additional vibrational bands at 709 cm^{-1}, 1070 cm^{-1}, 1239 cm^{-1} and 1719 cm^{-1} for PTX loaded POx micelles were observed (b). These bands represented characteristic group vibrations of PTX such as the out-of-plane ring bending of Ph-R group, in-plane C-H bending of Ph-R group, C-O-C stretching of -COO- group, and C=O stretching of PhCOO- group respectively. Accordingly, the spectrum of 17-AAG loaded micelles exhibited characteristic vibrational bands of 17-AAG. For example, the vibrational band at 1727 cm^{-1} was attributed to C=O stretching of NH2COO- group; the vibrational band at 1688 cm^{-1} to C=O stretching in quinine; and the vibrational band at 1099 cm^{-1} to C-O stretching of $(R)_2$CH-OH group (c). For PTX/17-AAG co-loaded micelles, typical vibrational bands from both PTX and 17-AAG were observed including 709 cm^{-1}, 1105 cm^{-1}, 1241 cm^{-1}, 1692 cm^{-1}, and 1723 cm^{-1} (d). 2.2.5. ATR FT-IR spectra of dried POx powders and freeze-dried drug loaded micellar formulations were obtained on a Nicolet 380 system (Thermo Scientific, Waltham, MA, USA) with a SilverGate™ Evolution ATR accessory (Specac, Cranston, RI). The spectra were recorded at room temperature between 400 cm^{-1} and 4000 cm^{-1} at 4 cm^{-1} spectral resolution and compared to verify the incorporation of drugs into POx micelles.

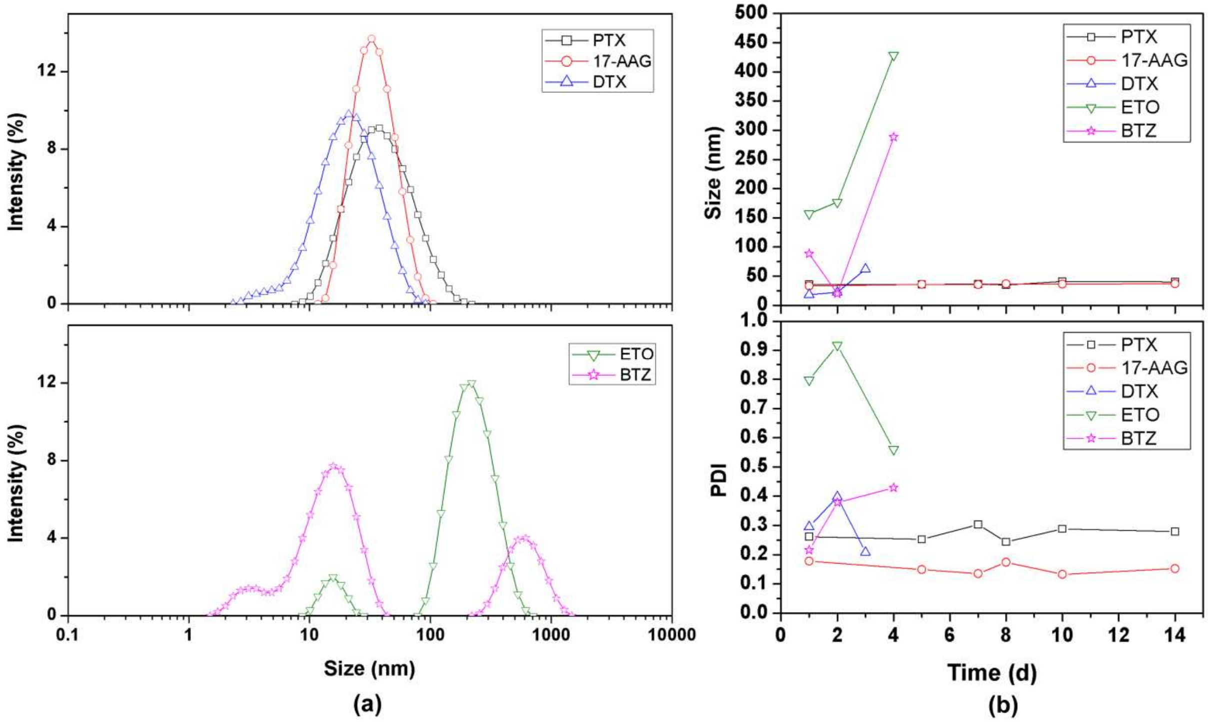

Fig. S3. **(a)** Size distribution (as determined by DLS) of POx micelles loaded with single drugs: PTX (□), 17-AAG (○), DTX (△), ETO (▽), and BTZ (☆); **(b)** Stability studies of POx micelles loaded with single drugs as in (a) by plotting average size (nm) and PDI over consecutive time points (days). (b) Measurement ended at 14 days. Lines between data points are for illustration purpose only.

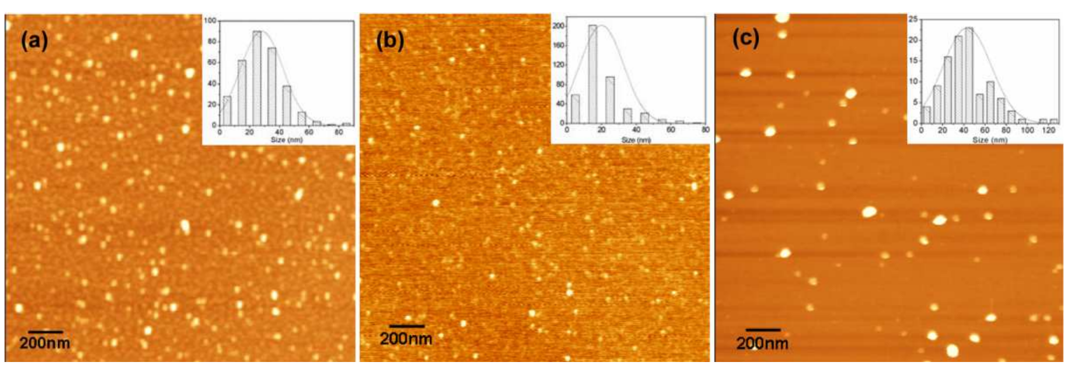

Fig. S4. AFM topography scans of drug-loaded micelles of POx along with size distribution analysis of micelles (size (nm) vs. count) from an arbitrary area. **(a)** POx with PTX; **(b)** POx with 17-AAG; **(c)** POx with PTX and 17-AAG. Scan area is 2 μm^2. Morphological studies on dried samples by AFM suggest that PTX/17-AAG co-loaded POx micelles retain spherical shape resembling those of single drug PTX or 17-AAG micelles.

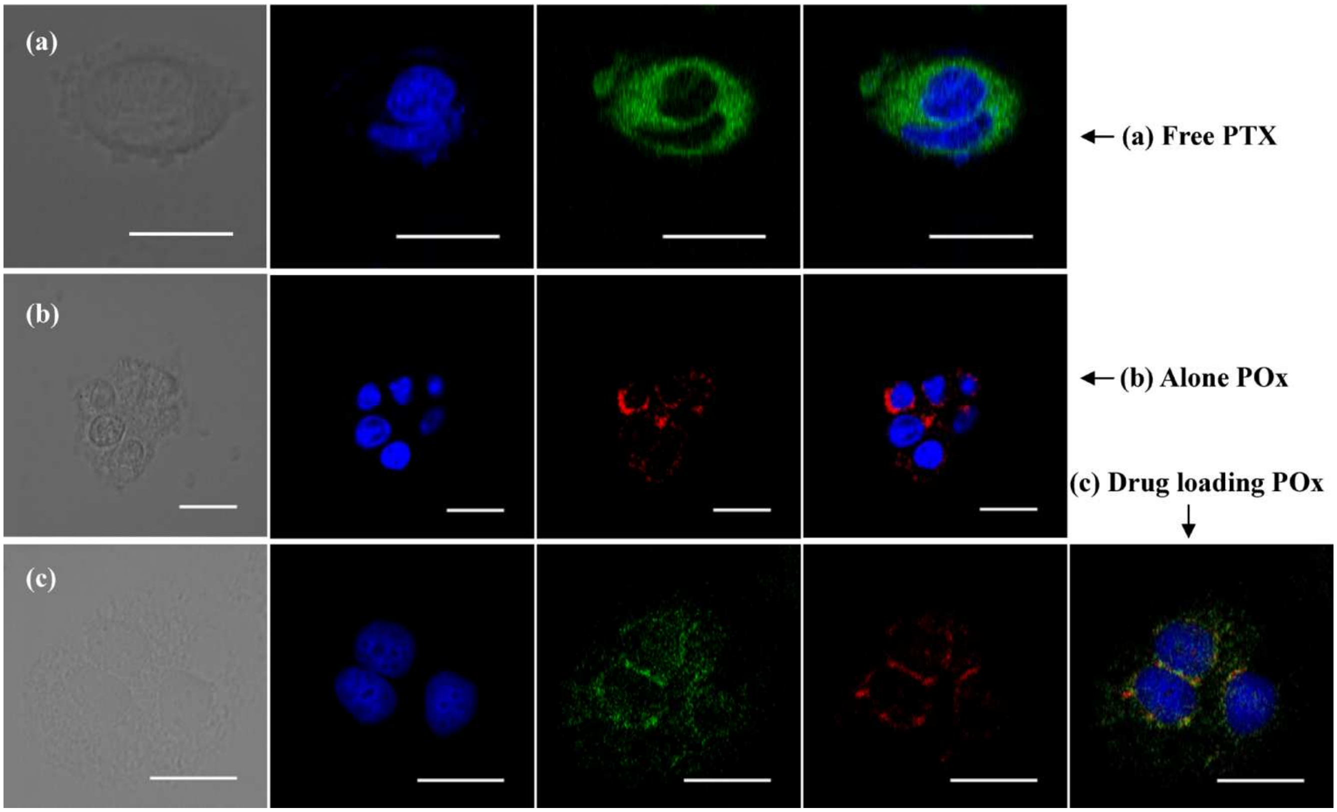

Fig. S5. CLSM images of MCF-7 cells incubated with **(a)** free PTX, **(b)** POx block copolymer alone and **(c)** POx micelles co-loaded with PTX and 17-AAG for 4 h. Blue: Cell nuclei staining by Hoechst 33342; Green: BODIPY® FL PTX; Red: AF647 labeled POx. Scale bars are 20 µm.

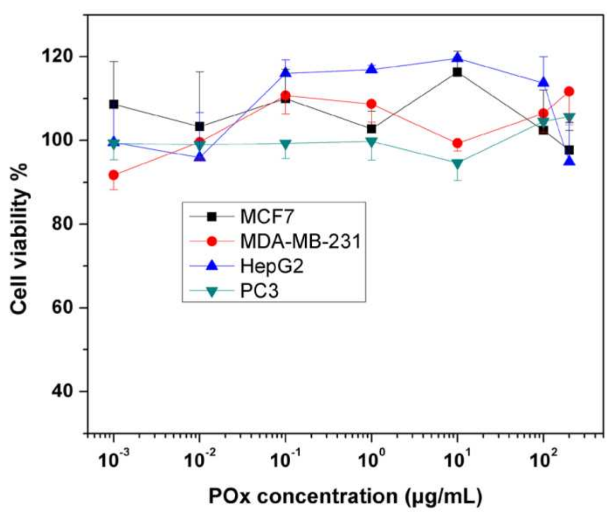

Fig. S6. The viability of the MCF7, MDA-MB-231, HepG2 and PC3 cells after their treatment for 24 h to different concentrations of amphiphilic POx block copolymer. Data are mean ± SEM (n = 6).

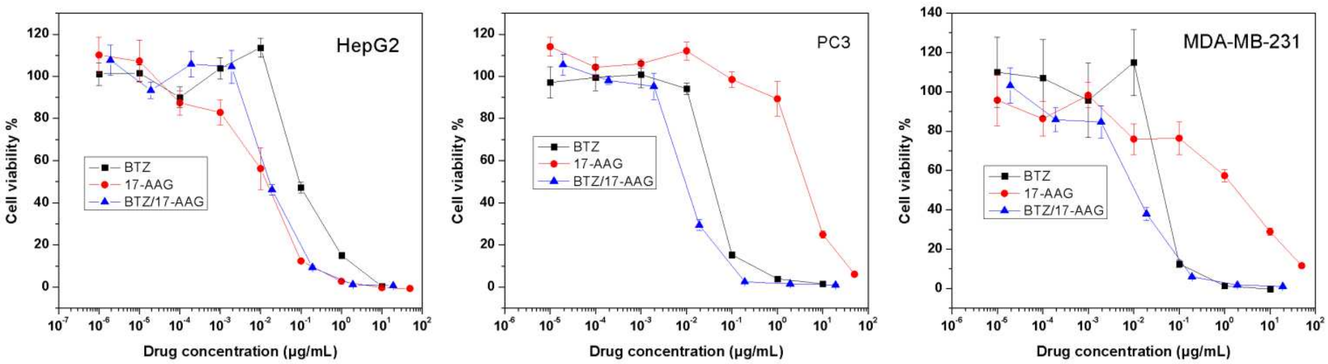

Fig. S7. The viability of the HepG2, PC3, and MDA-MB-231 cells after their treatment for 24 h with the micellar BTZ, 17-AAG and BTZ/17-AAG combination. Data are mean ± SEM (n = 6).

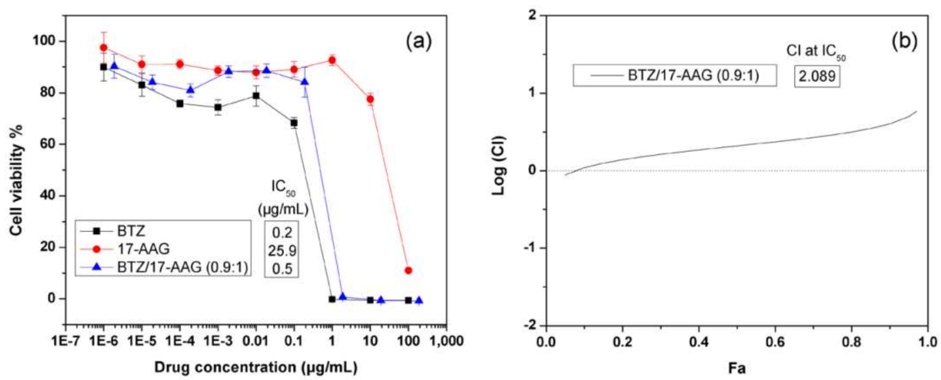

Fig. S8. (a) Cytotoxicity of single drugs and two-drug combination, BTZ/17-AAG solublized in POx micelles in MCF7/ADR cells (left, mean ± SEM, n = 6) and **(b)** the corresponding log CI vs. F_a plot (right). **(a)** The total drug concentration is presented for two drug combination.

Table S1. Yields of drug loaded POx micelles.

Drugs	Yield (%) [a]
PTX	99.2±1.5
DTX	99.1±1.0
17-AAG	96.1±1.5
ETO	97.4±1.3
BTZ	93.7±0.9
PTX 17-AAG	97.7±2.0
DTX 17-AAG	97.9±1.1
PTX ETO	95.2±2.7
ETO 17-AAG	94.8±1.4
PTX BTZ	93.2±3.9
BTZ 17-AAG	91.8±1.1
PTX 17-AAG ETO	88.6±1.8
PTX 17-AAG BTZ	88.1±1.2
DTX	90.6±3.6[b]
PTX	88.7±2.6[c]

[a] The formulations presented in this table are same as those presented in **Table 1**. Unless stated otherwise the POx block copolymer [P($MeOx_{40}$-b-$BuOx_{21}$-b-$MeOx_{34}$)] was used and its concentration in the dispersion was 10 g/L. The yield of drug loaded micelles was determined as the ratio (initial POx weight + loaded drug weight) / (initial POx weight + feeding drug weight), (n = 3 ± SD) [b] 50 g/L POx and DTX was used in this experiment. [c] 50 g/L POx and PTX was used along with a different batch of POx copolymer [P($MeOx_{33}$-b-$BuOx_{26}$-b-$MeOx_{45}$)].

Table S2. Characteristics of the drug release from POx micelles.

Drugs in POx micelles	$t_{1/2}$ (h)	Drug released at 24 h (%)	Drug released ratio at 24 h[a]
PTX	18	67	n.a.
17-AAG	6	96	n.a.
BTZ	4	99	n.a.
ETO	3	99	n.a.
PTX 17-AAG	15 7	68 95	1.0 : 1.4
PTX BTZ	20 2	60 99	1.0 : 1.6
17-AAG BTZ	6 3	96 99	1.0 : 1.0
PTX ETO	18 5	63 98	1.0 : 1.5
17-AAG ETO	7 5	96 98	1.0 : 1.0
PTX 17-AAG BTZ	18 8 3	61 95 97	1.0 : 1.6 : 1.6
PTX 17-AAG ETO	21 8 4	55 92 98	1 : 1.7 : 1.8

[a] Determined as the fraction of the drug released relative to the slowest released drug in the drug combination.

Table S3 Comparison of our results with others for drug formulation in LC and DL values

Drugs	Our results[a]			G.S. Kwon group's results		
	Solution drug concentration (g/L)	*LC* (%)	*DL* (%)	Solution drug concentration (g/L)	*LC*[d] (%)	*DL*[e] (%)
PTX	3.88	27.9	38.8	3.54	9.3	10.3
DTX	3.87	27.9	38.7	4.27	10.3	11.5
17-AAG	3.45	25.6	34.5	3.90	10.2	11.3
ETO	3.62	26.6	36.4	3.31	8.7	9.6
BTZ	3.12	23.8	31.2	/	/	/
PTX 17-AAG	3.66 3.93	43.1	75.9	3.92 3.88	20.6	25.9
DTX 17-AAG	3.92 3.70	43.3	76.3	4.62 4.01	20.5	25.8
PTX ETO	3.59 3.54	41.6	71.4	/	/	/
ETO 17-AAG	3.69 3.38	41.1	70.7	3.49 4.21	20.0	25.0
PTX BTZ	3.27 3.52	40.3	67.8	/	/	/
BTZ 17-AAG	3.27 3.25	39.5	65.2	/	/	/
PTX 17-AAG ETO	3.01 3.19 3.27	48.7	94.8	3.50 3.61 3.17	26.2	35.6
PTX 17-AAG BTZ	3.18 3.03 3.17	48.4	93.8	/	/	/
DTX[b]	40.6	44.8	81.2	/	/	/
PTX[c]	38.71	43.6	77.4	/	/	/

[a] Unless stated otherwise the POx copolymer P($MeOx_{40}$-b-$BuOx_{21}$-b-$MeOx_{34}$) was used and its concentration in the dispersion was 10 g/L. [b] 50 g/L POx and DTX was used in this experiment. [c] 50 g/L POx and PTX was used along with a different batch of POx copolymer P($MeOx_{33}$-b-$BuOx_{26}$-b-$MeOx_{45}$). [d] Recalculated value according to LC equation. [e] Original reported data. (H.C. Shin, A.W.G. Alani, D.A. Rao, N.C. Rockich, G.S. Kwon, Multi-drug loaded polymeric micelles for simultaneous delivery of poorly soluble anticancer drugs, J. Control. Release 140 (2009) 294-300)

$$LC = \frac{m_{drug}}{m_{drug} + m_{excipient}} \cdot 100\% \qquad (1)$$

$$DL = \frac{m_{drug}}{m_{excipient}} \cdot 100\% \qquad (2)$$