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Belinostat loaded lipid-polymer hybrid nanoparticulate delivery system for Breast Cancer: Improved Pharmacokinetics and Biodistribution in tumor model

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Methods

1) mPEG-PLA synthesis

The lactide was weighed and recrystallized using ethyl acetate to obtain crystals of lactide. Thus, obtained lactide and m-PEG were accurately weighed, and the mixture was melted using monowave at 120 ℃ for 10 minutes. Later to the melted mixture the catalyst, tin(II) 2-ethylhexanoate was added in presence nitrogen environment. Subsequently, the reaction was carried out at 135 ℃ for 45 minutes. Thus, obtained viscous mass of polymer was dissolved in the chloroform and precipitated using ice cold isopropyl alcohol. Later, the product was centrifuged at 8000 rpm for 10 minutes to obtain a settled product which was further dissolved in chloroform and precipitated in diethyl ether, twice. The polymer thus obtained was dried and stored.

2) Quantification of Belinostat

Quantification of belinostat in analytical, bioanalytical and tissue samples was performed using previously developed RP-HPLC method. Briefly, for all the samples chromatographic separation of drug was performed using Inertsil® ODS column (250 v 4.6 mm, 5 µm). While mobile phase consisted of acetonitrile and 0.01% ortho phosphoric acid (43:57, v/v) for

analytical drug determinations it was acetonitrile and 0.01% orthophosphoric acid (29:71, v/v) for bioanalytical and tissue samples. For all the samples the separation was achieved at a flow rate of 1.0 ml/min with a detection wavelength of 268 nm.

3) Solubility study

The solubility study for determining the release media and its volume was conducted in four different surfactants. Briefly, various surfactants at different concentrations were prepared to which Belinostat was added in excess amounts and were subjected to shaking for 48 h at 37 ^{o}C. Subsequently the solutions were centrifuged, and the supernatants were analyzed for the drug concentrations.

4) Sample preparation for plasma and biodistribution samples

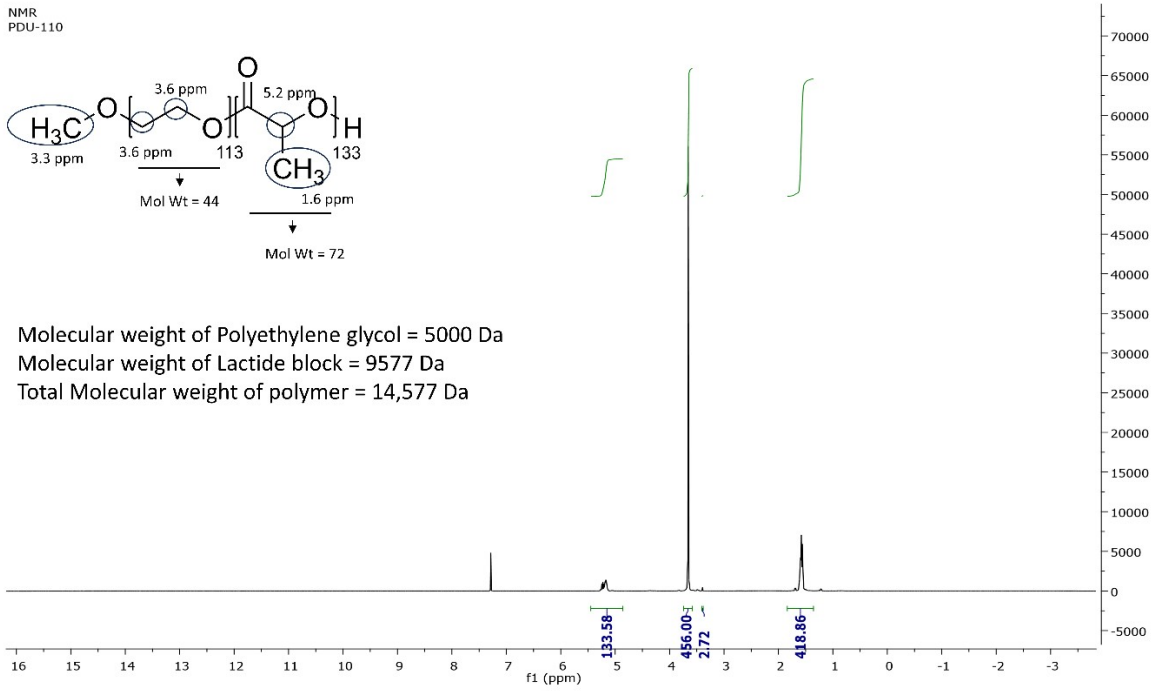
For plasma samples the samples were thawed and to 100 µl of the samples internal standard was added and vortexed properly. To this, acidified methanol was added and vortexed for 10 minutes to extract the drug. Subsequently the samples were centrifuged, and the supernatants were collected and dried at 37 ^{o}C. Thus, dried samples were redispersed in methanol and were subjected to HPLC analysis. For tissue samples, the samples were accurately weighed and homogenized completely in PBS. To the homogenized samples internal standard was added in equivalent quantities and the drug was extracted using acidified methanol. Thus, obtained samples were dried at 37 ^{0}C and redispersed in 100 µl methanol and evaluated for drug concentrations using HPLC method.

Results

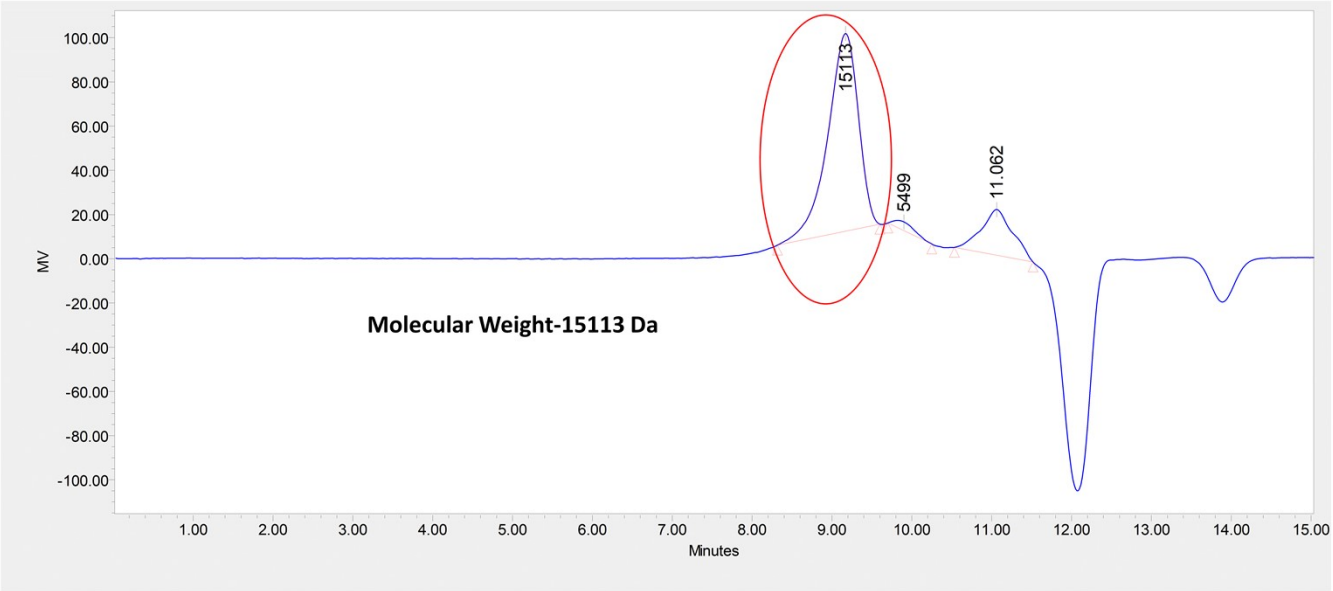
2.1) mPEG-PLA Synthesis

The polymer was characterized using ^{1}H NMR and it was found that the molecular weight of the polymer was 14, 577 Da **(Supplementary Figure 1)**. Furthermore, when the polymer was

characterized by GPC, the molecular weight obtained was found to be 15,113 Da **(Supplementary Figure 2)**.



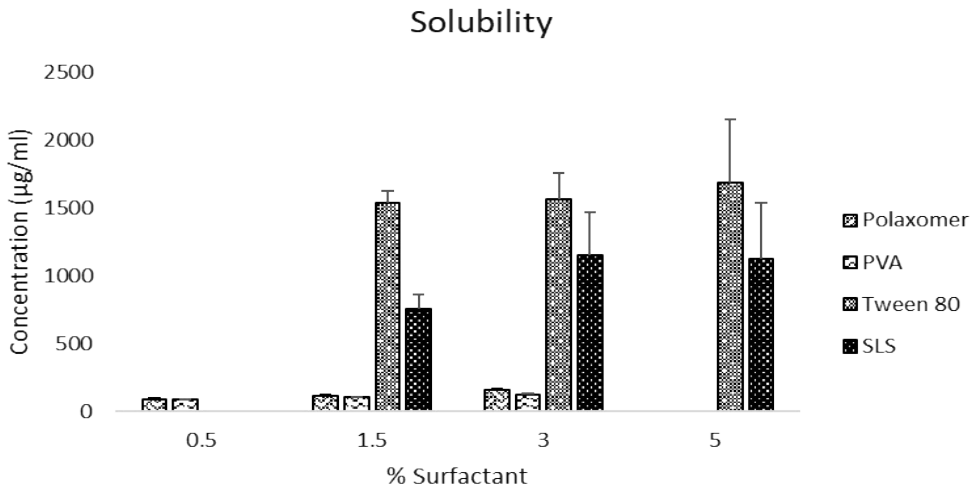
Supplementary Figure 1. NMR results of mPEG-PLA polymer



Supplementary Figure 2. Chromatogram of mPEG-PLA polymer by GPC

2.2) Solubility studies

The results for solubility studies have been depicted in **Supplementary figure 3**.



Supplementary Figure 3. Solubility of Belinostat in various surfactants

2.3) Invitro release

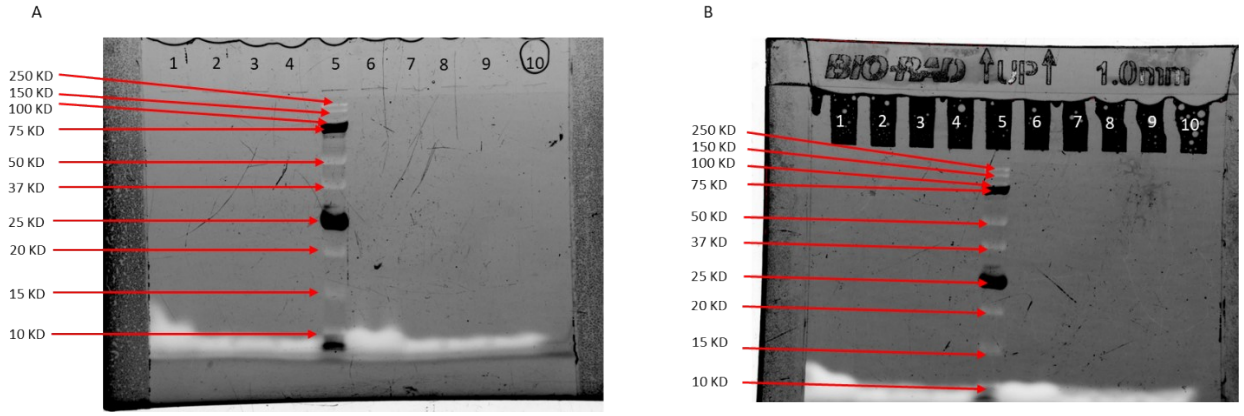
The R-square values for different release kinetic models observed when time vs cumulative drug concentration profiles were fitted in them are enlisted in **Supplementary Table 1**

Supplementary Table 1
R-square values for different release kinetic models.

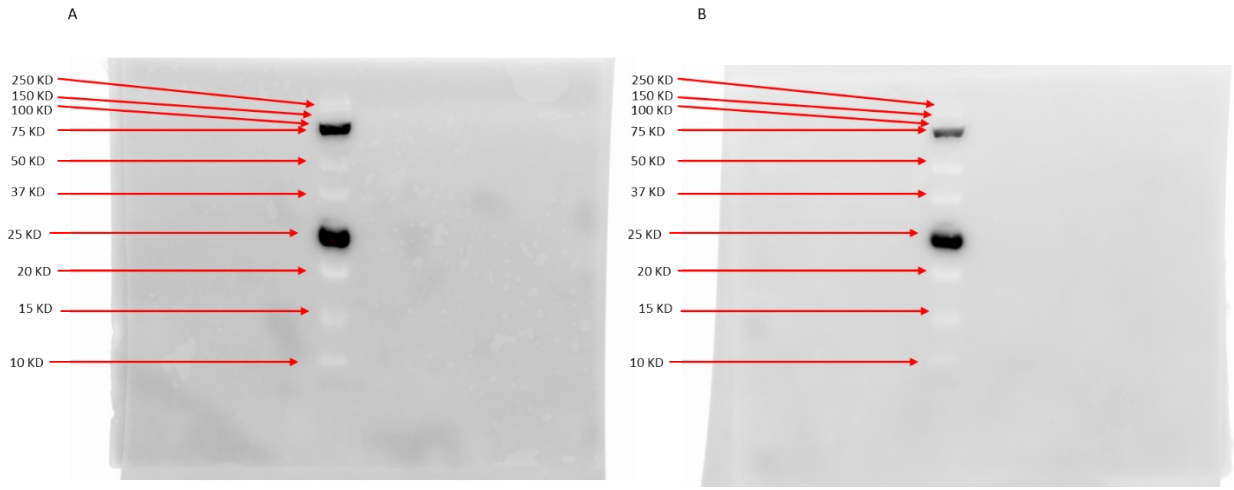
	Free Drug	B1	B2	B4
Zero Order	-5.9	-1.5	-3.4	-2.5
First Order	0.9	0.5	0.9	0.02
Higuchi	-0.8	0.4	-0.1	0.3
Korsmeyer peppas	0.8	0.8	0.8	0.8
Hixon Crowell	-0.2	0.3	0.3	-0.4

2.4) Immunoblot assay

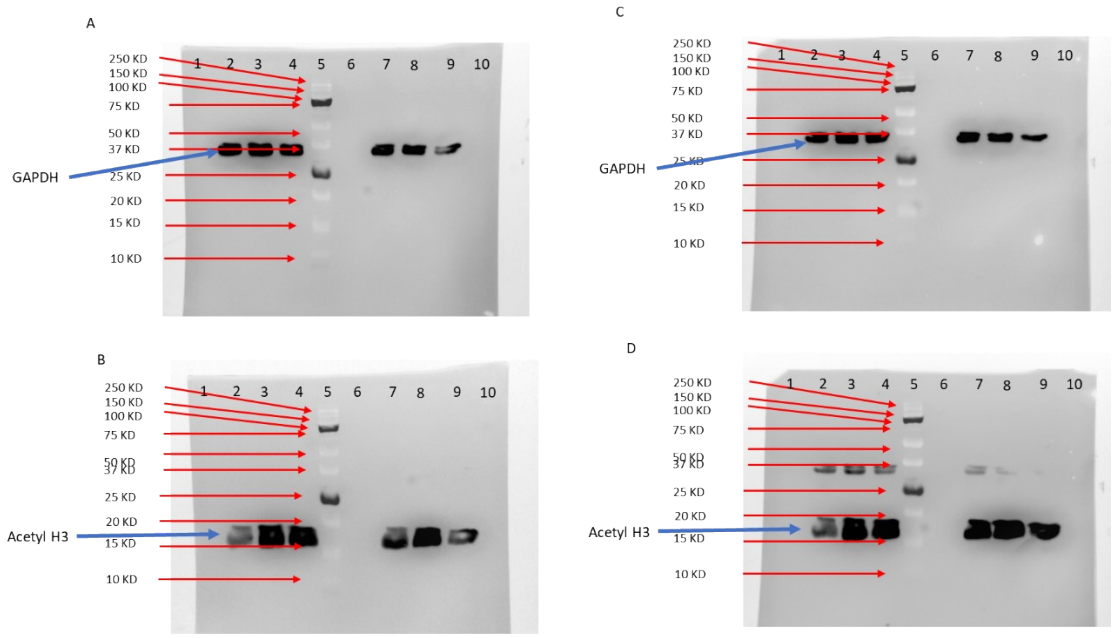
After the imaging of the uncropped and unprocessed gel and transfer of proteins onto the blots they were further processed according to the procedure described in the main manuscript to determine the expression of the proteins GAPDH and Acetyl H3 (Lys9/Lys14) harbouring molecular mass of 37 kDa and 17 kDa respectively. All the final results of the blots are illustrated and discussed in the main manuscript. Further the raw data, for the blots have been depicted in **Supplementary figure 4, 5 and 6.**



Supplementary Figure 4. Representative images of unprocessed Gel. A and B) Images of uncropped stain free gel for n=1 and n=2 respectively (marked against the respective molecular mass markers). *Lane 5: Molecular mass markers; Lanes 2,3 and 4: Control, Free drug and B4 treatment groups respectively of MCF 7 cells; Lanes 7,8 and 9 Control, Free drug and B4 groups respectively of 4T1 cells



Supplementary Figure 5. Representative image of unprocessed blot. B) Images of stain free blots for n=1 and n=2 respectively (marked against the respective molecular mass markers).



Supplementary Figure 6. Raw data for immunoblots. A and B) Protein expression of GAPDH and Acetyl H3 levels respectively for n=1. C and D) Protein expression of GAPDH and Acetyl H3 levels respectively for n=2.
*Lane 5: Molecular mass markers; Lanes 2,3 and 4: Control, Free drug and B4 treatment groups respectively of MCF 7 cells; Lanes 7,8 and 9 Control, Free drug and B4 groups respectively of 4T1 cells.