RESEARCH ARTICLE

Studies of the Precipitation Pattern of Paclitaxel in Intravenous Infusions and Rat Plasma Using Laser Nephelometry

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Abstract

Cremophor EL (CrEL) is commonly used to solubilize paclitaxel (Ptx); a widely established anticancer agent used against many types of cancer. Using laser-based microplate nephelometry, in this work we assessed the precipitation kinetics of Ptx in CrEL-containing formulations upon dilutions with different infusion media or upon introduction into rat plasma. The precipitation profile of Ptx was assessed for a Taxol-like formulation and compared with an preparation with reduced CrEL content. These two formulations were diluted at various ratios in compatible infusion media and with or without rat plasma. The percentages of Ptx precipitated in dilution media and protein-binding in plasma were quantified using HPLC. The findings of turbidity measurements were in good agreement with HPLC. Despite the presence of albumin, it was possible to assess turbidity within infusion solutions and predict Ptx precipitation. Upon addition to plasma, no precipitation in Taxol-like formulation occurred after 2 hours. By contrast, precipitation occurred immediately in CrEL-reduced formulation. It is possible that the high percentage of protein-bound Ptx in plasma (98.5%-99.2%) has inhibited drug precipitation. Turbidity measurements using laser nephelometry can provide a rapid screening tool when developing intravenous formulations for poorly soluble drugs, such as Ptx and assess its stability upon dilution in animal plasma.

Keywords anticancer, compatibility, taxanes, cytotoxic, solubility

Introduction

Paclitaxel (Ptx) is a widely used antineoplastic taxane with established activity against a wide range of cancers. Ptx was approved by FDA in 1992 for the treatment of ovarian cancer[1, 2]. The use of Ptx has extended thereafter to include lung cancer[3], AIDS related Kaposi's sarcoma[4] and urologic, colon and head and neck cancers as well as other solid tumors[5]. However, Ptx is limited by its poor aqueous solubility (<0.01 mg/ml)[6, 7]. Ptx is commercially available in the market under the brand name of Taxol® which is an intravenous solution of Ptx in a solvent mixture of Cremophor EL (CrEL; ethoxylated castor oil) and dehydrated ethanol (1:1 v/v). The formulation is usually diluted by 5-20 times using isotonic solutions such as sodium chloride (0.9%) or dextrose (5%) prior to administration via intravenous infusion[8, 9].

The advantages of using CrEL as vehicle are compromised by its serious adverse effects such as myelosuppression, neuropathy, acute hypersensitivity, alopecia, neuropathy, nausea and vomiting [10, 11]. These toxicity manifestations might be ameliorated by the use of antihistamines and steroids prior to Taxol® administration[12, 13]. Importantly, the stability of Taxol® formulation in infusion media (Ptx 0.3-1.2 mg/ml) is a major concern as the drug may precipitate during parenteral infusion owing to reduced drug solubility upon dilution with aqueous phase [14, 15]. Despite the success of nanotechnology at solubilizing or efficiently dispersing Ptx (e.g. albumin-bound Ptx formulations). Moreover, Taxol® formulation is recognized to be cheaper than nanotechnology-based formulations of the drug. Therefore, Taxol® and CrEL formulations of Ptx are still justified for clinical use in many countries, and the stability of Ptx in Taxol® and CrEL-based formulations merits investigations.

The stability of CrEL-based formulations of Ptx has been evaluated in vitro[16], however, evaluation of Ptx formulation stability in environments that may mimic what happens in vivo or just prior to intravenous infusion are still needed, and reliable protocols to study the precipitation kinetics of Ptx in various media, including blood plasma should be established. Laser-based nephelometry has been widely used for studying microbial growth and effects of antifungal agents [17], analysis of protein concentration [18] and assessment of solubility and dispersion of drugs in formulations [19, 20]. Furthermore, nephelometry has been reported to correlate well with findings obtained using high performance liquid chromatography (HPLC)[21], and it may reduce the cost in product development

[22]. Thus, the potential application of laser nephelometry in evaluating the precipitation kinetics of poorly soluble drugs in physiologically relevant media such as plasma merits to be explored.

In this study, we have investigated the feasibility of laser nephelometry as a rapid screening tool to investigate the precipitation kinetics of Ptx in CrEL-based formulations using different infusion media as well as blood plasma. In order to gain further insight into the effect of CrEL on Ptx precipitation, we have compared the therapeutic doses of a Taxol-like formulation with an in-house formulation that contained a reduced content of CrEL.

Materials

Paclitaxel (Ptx) was provided by ChemieTek, USA. Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich, USA and Cremophor EL (CrEL, Kolliphor EL) was purchased from Sigma, Germany. Rat Plasma (Rcc Han Wistar male) in lithium heparin, pool of 115 animals and stored at -20˚C was purchased from Harlan Ltd, UK. All solvents and chemicals used for HPLC were of HPLC grade and obtained from Fisher Scientific, UK.

Methods

Preparation of Ptx formulations

Taxol-like formulations were prepared by dissolving Ptx in CrEL and ethanol (1:1 v/v) to constitute a drug concentration of 6 mg/ml. The same concentration of Ptx was prepared with reduced proportion of CrEL (CrEL and ethanol; 1: 9 v/v) or in complete absence of CrEL (i.e. using only ethanol). To mimic the concentrations commonly used for intravenous administration of Ptx, the drug solutions were diluted to 1.2, 0.6, 0.4, or 0.3 mg/ml using two infusion media, namely NaCl (0.9%) or dextrose (5%). Other samples of drug solution were diluted with deionized water which was used as control medium for comparing the precipitation behavior of the drug.

Scanning electron microscopy (SEM)

SEM imaging was utilized for assessing the habit and aggregation behavior of Ptx crystals as a result of 1:4 dilution with NaCl solution, dextrose solution or deionized water (18 hours after dilution). This was performed by centrifugation of the samples for 10 min followed by freeze-drying (Edwards Micro Modulyo freeze-dryer, IL, USA) of the sediment in order to completely remove water from the samples prior to SEM imaging. The Ptx specimens were then gold-coated

using a sputtering technique for 2 min using a JFC-1200 Fine Coater (JEOL, Tokyo, Japan). The crystals were viewed under SEM (JSM-6301F, JEOL) and images were taken at 3 kV. In case of Ptx crystals in dextrose, the samples were washed three times with an extra volume of distilled water and centrifuged for 5 min at 15 G before taking the images. This extra step was done to wash out the sugar deposited on the surface of the crystals, hence observation of the crystal habit is possible.

Size analysis of Ptx crystals

Samples were measured using the Malvern 2000 laser diffraction size analyzer (Malvern Instruments Ltd., UK). Briefly, Ptx (0.3 g) was dissolved in 50 ml of CrEL and ethanol mixture (1:9 v/v), and Ptx samples in the solvent system were diluted with 50 ml deionized water within the dispersion cell. Crystal size and size distribution were respectively expressed as volume median diameter (VMD; 50% undersize) and span. Span = (90% undersize - 10% undersize) /VMD. Laser diffraction was used to accurately measure the size of Ptx crystals that are expected to have VMD values higher than 1 μm after 2, 4, 8, and 18 h of dilution. Measurements of this analysis were conducted in triplicate using three different batches with 10 min run for two times.

Turbidity studies in infusion media using laser nephelometry

All samples were measured by NEPHELOstar (BMG Labtech, Germany) using 96-well F-Bottom UV-Star Microplates (Greinerbioone, Germany). Throughout all experiments, microplates were prepared in triplicate for each sample. The run was carried out at 20˚C with a 61 cycles of 15 min each, and the total run time was approximately 15 h. Upon dilution with plasma, the turbidity measurement were repeated at 37 ˚C. Raw data and blank correction based on average of blanks/negative controls were exported from MARS Data Analysis Software 2011 (BMG Labtech, Germany) to Microsoft Excel Professional 2010 for further evaluation.

Ptx quantification in dilution media and serum via HPLC

In order to validate the outcomes of turbidity measurements, the same experiments were replicated and the amount of the precipitated Ptx was quantified using HPLC. The percentage of Ptx precipitation was estimated by adapting an HPLC method previously used by Vasantha et al. [23], using an Agilent 1200 HPLC system equipped with LC-2010HT HPLC spectrophotometer

detector (Agilent, Germany). The stationary phase used was Synergi Polar-RP C18 HPLC column (5µm, 250 × 4.6 mm) (Phenomenex, Germany). The injection volume was 50 µl and the flow rate of the mobile phase (acetonitrile and acetate buffer 60:40 v/v) was adjusted to 1 ml/min. The chromatographic run time of Ptx in the samples was fixed at 13 min, and the retention time of Ptx was found to be 7.7 min.

In order to assess Ptx concentration in Wistar rat plasma, the serum was separated via centrifugation at 2,000G for 4 h. After preparing methanolic solutions of Ptx at different concentrations (200-10,000 ng/ml), the solvent was evaporated (150 µL in 300 µL capacity HPLC vial with built-in inserts, Fisher Scientific, UK) at 40˚C for 45 min using a vacuum oven. The solutions were reconstituted with the same volume of serum which was added and mixed for 2 min using a vortex mixer. All samples were incubated at 37˚C for 2 h under continuous shaking followed by HPLC analysis. The Limit of Quantification (LOQ) of Ptx (based on peak-to-noise ratio >10) was defined as 2,500 ng/ml in mobile phase and 2,000 ng/ml in serum. The linearity was 0.999 in the range of 20-10,000 ng/ml and reproducibility was 0.66%.

Turbidity studies in infusion media and plasma using laser nephelometry

Taxol-like and formulations with reduced CrEL proportion were independently diluted at 1:4, 1:9, 1:14 and 1:19 ratios in each medium separately (dextrose 5% w/v, NaCl 0.9% w/v or deionized water). Wistar rat plasma (100 µl) was accurately placed into the 96-well plate with an equivalent volume of each one of the three media (blank samples). The pipetting of the samples was performed quickly and instantly subjected to nephelometric analysis at 37˚C with 61 cycles, a gain of 74 and a laser beam focus of 2 mm. The samples (n=3) were subjected to orbital shaking of 2 mm width, which lasted 3 sec before each cycle. No animals were used in our studies, and all animal plasma samples were purchased, as indicated in section 2.1.

Ptx protein binding studies

Four dilutions (1:4, 1:9, 1:14, and 1:19 v/v) were prepared with 5% w/v dextrose or 0.9% w/v saline solutions. After adding equal amounts of plasma (150 µl) in each solution, the samples were incubated with continuous shaking for 2 h at 37˚C followed by centrifugation in Centrifree® ultrafiltration tubes at 2,000 G for 3 h. The serum containing unbound drug was collected from

each sample and its volume was estimated by pipette measurement before conducting HPLC analysis.

Statistical analysis

One-way ANOVA was employed using SPSS Software (22.0.0.2) to analyse the results. Differences in results of $p < 0.05$ were considered to be significant.

Results and discussion

Morphology and size analysis of Ptx crystals

SEM images showed that Ptx crystals were precipitated after 18 h of dilution with various media at 1:4 ratio (Figure 1). However, the crystals formed in deionized water, saline and dextrose solution were needle-like. Ptx crystals had wide size distribution, and size growth of the crystals did not follow a trend with relevance to time following dilution (Table 1). In fact, size of Ptx crystals varied, and the measured VMD values after 2 h were 45.93, 83.23 and 215.35 μm along with span values of 2.151, 2.546 and 1.428 for samples diluted with dextrose, NaCl and deionized water, respectively. The large VMD and high span values indicated growth and concomitant aggregation of the crystals. It was expected that the crystal size will increase with time; however, the size of the crystals did not increase after 2 h. Size of Ptx crystal tended to be smaller in dextrose solution compared to NaCl solution and deionized water. It is possible that the higher concentration of dextrose in the solution has resulted in formation of sugar coat on the crystals and subsequent hindrance of attraction forces between the hydrophobic surfaces of the crystals; this retarded further enlargement of the crystals.

Laser nephelometry studies in infusion media

Laser nephelometry was used to investigate the precipitation profile of Ptx and following scenarios that mimic the dilution and administration conditions of the formulation. As shown in Figure 2, Ptx in pure ethanolic solvent (i.e. CrEL-free solution) precipitated immediately upon dilution with different infusion media. This reveals the advantage of CrEL as established vehicle for Ptx. Higher readings were observed with the least diluted solutions (1:4) because Ptx concentration was relatively high (Figure 2). By contrast, Taxol-like formulation (1:1 v/v CrEL: ethanol) showed no

increased levels of turbidity and the solution remained visually clear since no apparent precipitation occurred during the period of investigation (18 h) at room temperature, indicating that CrEL vehicle was effective at prevention of drug precipitation in Taxol-like formulation (Figure 3).

Ptx in reduced CrEL formulation (1:9 v/v, CrEL:ethanol) showed more turbidity in 5% dextrose solution when compared to samples diluted with saline solution or deionized water (Figure 4). It can also be seen that Ptx diluted in infusion media had higher turbidity when using higher drug concentrations (1:4). One possible explanation is that lower Ptx concentrations in infusion solutions reduced the level of supersaturation and hence the rate of precipitation over 18 h was further dropped compared to samples having higher Ptx concentrations. This is also reveals that if the decision in formulation development was to reduce the content of CrEL, then dilution with saline solution would be a better choice than using dextrose.

HPLC analysis of Ptx precipitation

The results of turbidity measurements were cross-referenced with quantifiable HPLC analysis. In general, HPLC results were in agreement with the turbidity measurements and showed the same precipitation trend (Figures 5). For instance, Ptx precipitation at the dilution of 1:9 was accompanied with low turbidity measurements 2 h following dilution. Nevertheless, highest precipitation was revealed by HPLC to occur after 2 h of dilution (Figure 5) while the level of turbidity grew throughout the period of the test (Figure 4). The difference between HPLC and nephelometry findings can be attributed to the increased turbidity which is linked to crystal growth with time. In addition, there was limited precipitation of Ptx during the first 6 h for CrEL-reduced formulation (CrEL: ethanol 1:9) (Figure 5B). It is possible that for this formulation, the concentration of drug was reduced below its saturation limit, resulting in no drug precipitation. As the formulation was diluted with the infusion medium, the concentration of the solvent system was also reduced, resulting in a marked decrease in Ptx solubility and eventually supersaturation and drug precipitation. Overall, HPLC study correlates well with the nephelometry findings although some conflicting results might arise, possibly owing to the shape of Ptx crystals causing alterations in turbidity measurements.

Turbidity studies and protein binding in plasma

Precipitation of hydrophobic drugs has always been a concern during parenteral infusion of formulations. In our investigation, Ptx was directly introduced to plasma after dilution. Our study endeavored to mimic the injection conditions and to investigate the effect of plasma protein binding on Ptx solubility.

Higher baseline readings were observed in plasma because of the presence of large molecules such as albumin which might affect the turbidity profile of Ptx preparations (Figure 6). However, laser nephelometry has detected Ptx turbidity despite the presence of albumin in the samples. When plasma was used to dilute Ptx prepared in ethanolic solution, an immediate precipitation of the drug was found (data not shown). Taxol-like formulation was prepared in order to assess the potential precipitation of Ptx in the commercially available formulation following introduction to blood. It can be noticed that no significant increase of Ptx turbidity in Taxol-like formulation took place in the first 4 hours in plasma with the range of media used (Figure 6).

Similar to our findings of Ptx in infusion media only (Figure 4), the diminution of CrEL surfactant indicated a reasonable Ptx haziness which was increased with increasing the dilution ratio (1:9, 1:14, and 1:19) more than the highly concentrated Ptx ratio (1:4) (Figures. 7A, B & C). Indeed, no significant increase in turbidity with Taxol-like formulation (Figure. 6) occurred within 2 h while it took place instantly in CrEL reduced formulation (Figure. 7A). This will help in facilitating the screening of solubility enhancer for the intravenous formulations (1:1 v/v CrEL:ethanol).

On the other hand, the addition of reduced CrEL formulation led to a significant increase in turbidity for 1:4 dilutions when introduced to rats plasma (Figure 7). However, such an effect is less noticed in other dilutions particularly with dextrose solutions. Such an effect might be related to albumin binding to Ptx. A very high percentage of protein-bound Ptx was measured in plasma (98.5 to 99.2%) as shown in Figure 8. Similarly, it has also been demonstrated that bound Ptx to plasma proteins ranged from 76 to 97% using various animal species[24]. It is possible that protein binding to Ptx has reduced the amount of free drug available for crystal formation.

The difference in turbidity trends between dextrose and saline solution could be related to the large ionic strength of NaCl solution might reduce CrEL effect and alter the formulation stability by increasing the turbidity of the drug. Donyai & Sewell described the difference between the two dilutions after following the observed marginal increase in the physical stability of Ptx when 5% dextrose diluents was used in comparison to 0.9% NaCl solution[25]. It is possible that the ionic strength of the sodium chloride infusion would initiate rapid degradation of CrEL and ethanol micelles produced with Ptx.

However, the described turbidity study of Ptx in plasma might not be consistent with Ptx solubility results in large animals where plasma volume is much larger than rat blood volume of 10-25 ml depending on the size of the animal[26]. Further validation of these outcomes in the plasma of cancer patients is necessary to confirm their clinical applications.

Conclusion

Higher turbidity reading (1:9 v/v, CrEL:ethanol) was noted in dextrose solution compared to saline and deionized water. Despite the presence of albumin, it was possible to assess turbidity with 1:1 (v/v) dilution in infusion solutions and detect drug precipitation. Turbidity measurements were in good agreement with HPLC results, however the turbidity readings were sensitive to the nature of the aqueous media and did not allow accurate drug quantification. Owing to these limitations and the facile and fast nature of this analytical technique, turbidity measurement can provide a rapid initial screening tool when developing intravenous formulations for poorly soluble drugs and assessing its stability upon dilution or injection to animal circulation.

Conflicts of interest

Authors declare no conflicts of interest.

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Figure 2. Turbidity of Ptx in ethanolic solution only diluted in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution (n=3).

Figure 3: Turbidity of Ptx in Taxol-like formulation diluted in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution (n=3).

Figure 4. Turbidity of CrEl-reduced Ptx formulation (CrEL: ethanol, 1:9 v/v) diluted in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution (n=3).

Figure 5. Percentage of CrEl-reduced Ptx formulation (in CrEL: ethanol, 1:9 v/v) precipitation upon addition into 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution (n=3)

Figure 6. Turbidity diagrams of Taxol-like formulation (1:1 CrEL and ethanol) in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution; with Wistar rat plasma (n=3).

Figure 7. Turbidity diagrams of CrEl-reduced Ptx formulation (1:9 CrEL and ethanol) in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution; with Wistar rat plasma (n=3).

Figure 8. Percentage (%) of bound Ptx in rat plasma calculated from serum with 5% (w/v) dextrose (Dex) and 0.9% (w/v) NaCl at 1:4, 1:9, 1:14, and 1:19 infusion ratios (mean ± SD, n=3).

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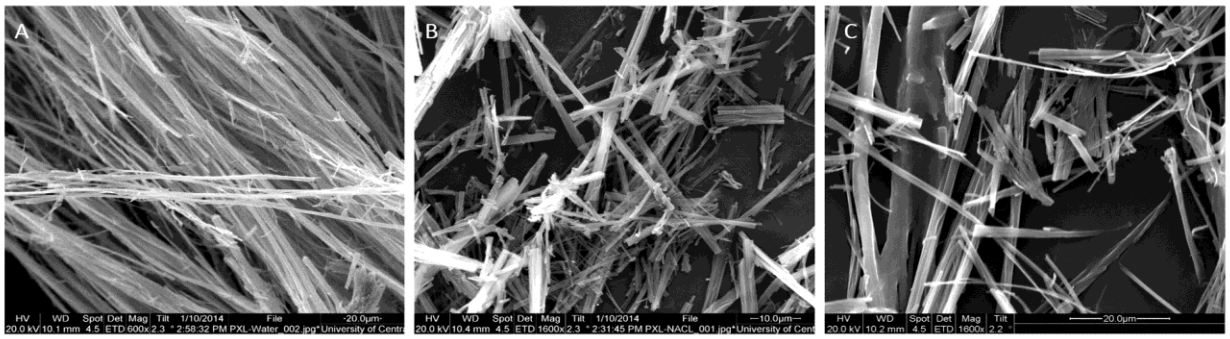
References

1. Trimble, E. L.; Adams, J. D.; Vena, D.; Hawkins, M. J.; Friedman, M. A.; Fisherman, J. S.; Christian, M. C.; Canetta, R.; Onetto, N.; Hayn, R.; Arbuck, S. G. Paclitaxel for Platinum-Refractory Ovarian-Cancer - Results from the First 1,000 Patients Registered to National-Cancer-Institute Treatment-Referral-Center-9103. *J Clin Oncol* **1993,** *11*, (12), 2405-2410.
2. Kumar, S.; Mahdi, H.; Bryant, C.; Shah, J. P.; Garg, G.; Munkarah, A. Clinical trials and progress with paclitaxel in ovarian cancer. *International journal of women's health* **2010,** *2*, 411-27.
3. Bergmann, T. K.; Green, H.; Brasch-Andersen, C.; Mirza, M. R.; Herrstedt, J.; Holund, B.; du Bois, A.; Damkier, P.; Vach, W.; Brosen, K.; Peterson, C. Retrospective study of the impact of pharmacogenetic variants on paclitaxel toxicity and survival in patients with ovarian cancer. *European journal of clinical pharmacology* **2011,** *67*, (7), 693-700.
4. Dongre, A.; Montaldo, C. Kaposi's sarcoma in an HIV-positive person successfully treated with paclitaxel. *Indian journal of dermatology, venereology and leprology* **2009,** *75*, (3), 290-2.
5. Kim, S. C.; Kim, D. W.; Shim, Y. H.; Bang, J. S.; Oh, H. S.; Kim, S. W.; Seo, M. H. In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. *J Control Release* **2001,** *72*, (1-3), 191-202.
6. Surapaneni, M. S. D., S. K.; Das, N. G. . Designing Paclitaxel Drug Delivery Systems Aimed at Improved Patient Outcomes: Current Status and Challenges. *ISRN Pharmacology* **2012,** *2012*, 1-15.
7. Konno, T.; Watanabe, J.; Ishihara, K. Enhanced solubility of paclitaxel using water-soluble and biocompatible 2-methacryloyloxyethyl phosphorylcholine polymers. *J Biomed Mater Res A* **2003,** *65A*, (2), 209-214.
8. Sznitowska, M.; Klunder, M.; Placzek, M. Paclitaxel solubility in aqueous dispersions and mixed micellar solutions of lecithin. *Chem Pharm Bull* **2008,** *56*, (1), 70-74.
9. Adams, J. D.; Flora, K. P.; Goldspiel, B. R.; Wilson, J. W.; Arbuck, S. G.; Finley, R. Taxol: a history of pharmaceutical development and current pharmaceutical concerns. *Journal of the National Cancer Institute. Monographs* **1993**, (15), 141-7.
10. van Zuylen, L.; Verweij, J.; Sparreboom, A. Role of formulation vehicles in taxane pharmacology. *Invest New Drug* **2001,** *19*, (2), 125-141.
11. Weiss, R. B.; Donehower, R. C.; Wiernik, P. H.; Ohnuma, T.; Gralla, R. J.; Trump, D. L.; Baker, J. R.; Vanecho, D. A.; Vonhoff, D. D.; Leylandjones, B. Hypersensitivity Reactions from Taxol. *J Clin Oncol* **1990,** *8*, (7), 1263-1268.
12. Szebeni, J.; Alving, C. R.; Savay, S.; Barenholz, Y.; Priev, A.; Danino, D.; Talmon, Y. Formation of complement-activating particles in aqueous solutions of Taxol: possible role in hypersensitivity reactions. *Int Immunopharmacol* **2001,** *1*, (4), 721-735.
13. Price, K. S.; Castells, M. C. Taxol reactions. *Allergy Asthma Proc* **2002,** *23*, (3), 205-208.
14. Constantinides, P. P.; Lambert, K. J.; Tustian, A. K.; Schneider, B.; Lalji, S.; Ma, W. W.; Wentzel, B.; Kessler, D.; Worah, D.; Quay, S. C. Formulation development and antitumor activity of a filter-sterilizable emulsion of paclitaxel. *Pharm Res-Dordr* **2000,** *17*, (2), 175-182.
15. Singla, A. K.; Garg, A.; Aggarwal, D. Paclitaxel and its formulations. *International journal of pharmaceutics* **2002,** *235*, (1-2), 179-192.
16. Gogate, U. S.; Schwartz, P. A.; Agharkar, S. N. Effect of unpurified Cremophor EL on the solution stability of paclitaxel. *Pharm Dev Technol* **2009,** *14*, (1), 1-8.
17. Fouda, M. M. G.; Knittel, D.; Hippler, U. C.; Elsner, P.; Schollmeyer, E. Antimycotic influence of beta-cyclodextrin complexes - In vitro measurements using laser nephelometry in microtiter plates. *International journal of pharmaceutics* **2006,** *311*, (1-2), 113-121.

18. Schmitzhuebner, U.; Nachbar, J.; Asbeck, F. The Determination of Anti-Thrombin-Iii, Alpha-2-Macroglobulin and Alpha-2-Antiplasmin in Plasma by Laser Nephelometry. *J Clin Chem Clin Bio* **1980,** *18*, (4), 221-225.
19. Mahida, J. P.; Antczak, C.; Decarlo, D.; Champ, K. G.; Francis, J. H.; Marr, B.; Polans, A. S.; Albert, D. M.; Abramson, D. H.; Djaballah, H. A synergetic screening approach with companion effector for combination therapy: application to retinoblastoma. *Plos One* **2013,** *8*, (3), e59156.
20. Pan, L.; Ho, Q.; Tsutsui, K.; Takahashi, L. Comparison of chromatographic and spectroscopic methods used to rank compounds for aqueous solubility. *Journal of pharmaceutical sciences* **2001,** *90*, (4), 521-529.
21. Bevan, C. D.; Lloyd, R. S. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates. *Analytical chemistry* **2000,** *72*, (8), 1781-7.
22. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **2001,** *46*, (1-3), 3-26.
23. Vasantha, J.; Kannan, G.; Goud, T.; Palani, T.; Vanitha, R.; Anitha, R.; Priya, J. Pharmacokinetic evaluation of Paclitaxel in South Indian cancer patients: a prospective study. *Journal of young pharmacists : JYP* **2011,** *3*, (4), 322-8.
24. Sparreboom, A.; van Tellingen, O.; Nooijen, W. J.; Beijnen, J. H. Preclinical pharmacokinetics of paclitaxel and docetaxel. *Anti-Cancer Drug* **1998,** *9*, (1), 1-17.
25. Donyai, P.; Sewell, G. J. Physical and chemical stability of paclitaxel infusions in different container types. *Journal of oncology pharmacy practice : official publication of the International Society of Oncology Pharmacy Practitioners* **2006,** *12*, (4), 211-22.
26. Sato, T.; Kamiyama, Y.; Kamano, T.; Rutkowski, J.; Adams Cowley, R.; Trump, B. F.; Jones, R. T. Pathophysiology of hemorrhagic shock. A model for studying the effects of acute blood loss in the rat. *Virchows Archiv. B, Cell pathology including molecular pathology* **1985,** *48*, (4), 361-75.



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406 Figure 1. SEM images of Ptx crystals at ≥18 hours in (A) deionized water, (B) 0.9% (w/v) NaCl solution, (C)
407 crystals from 5% (w/v) dextrose solution.

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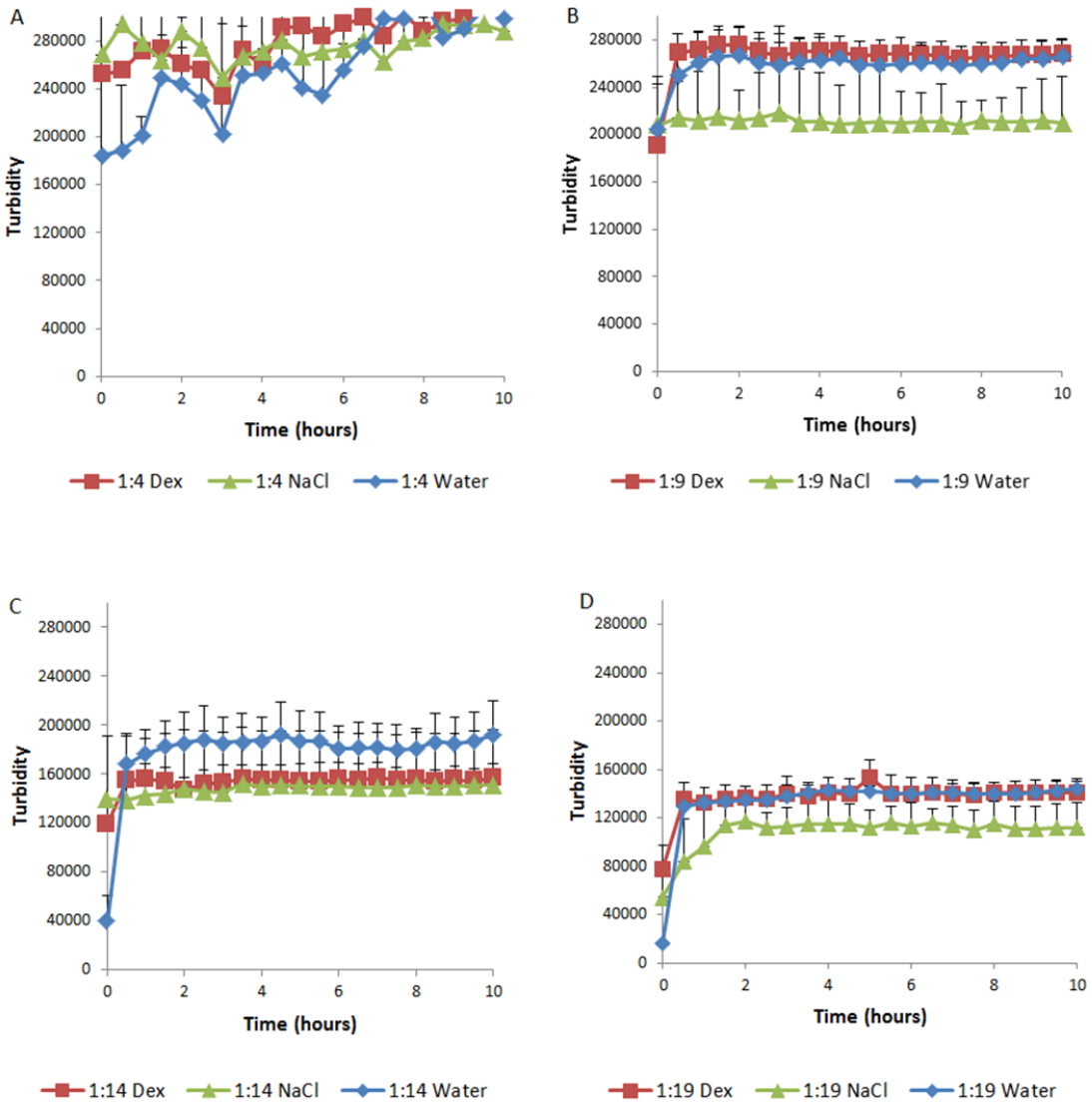

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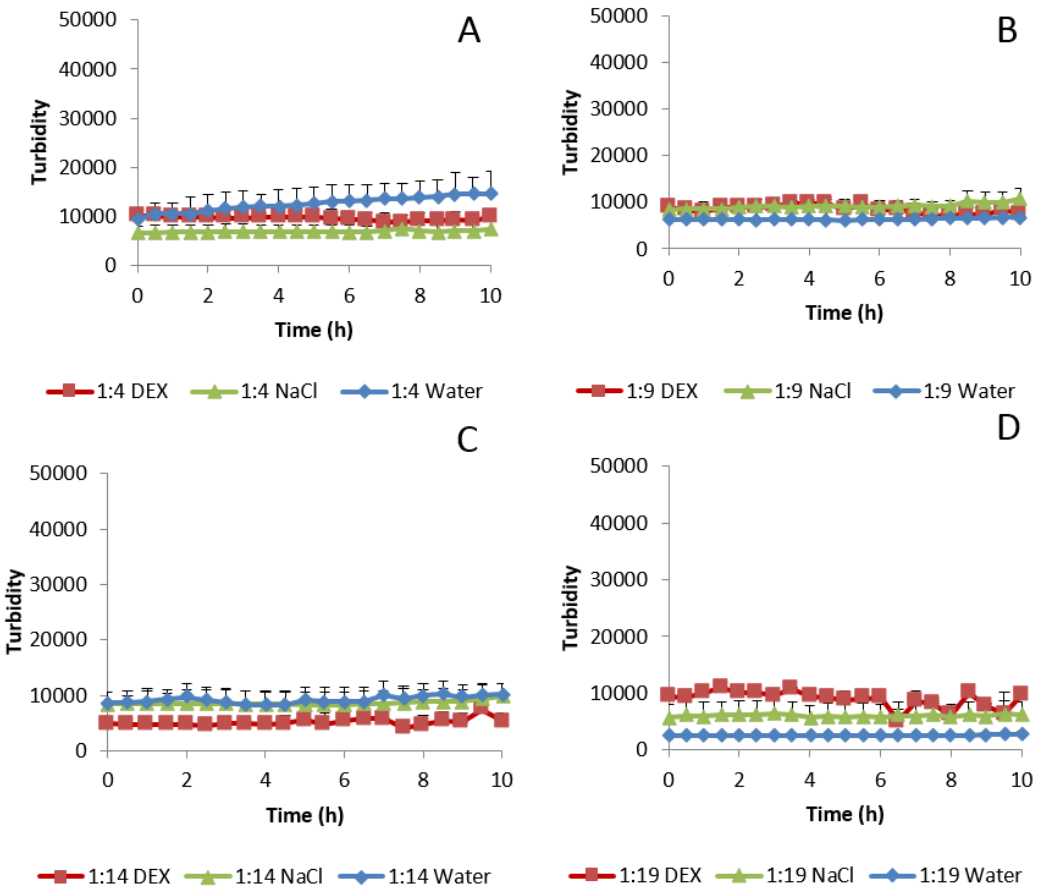
410 Figure 2. Turbidity of Ptx in ethanolic solution only diluted in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl
411 (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19
412 dilution (n=3).
413 423x396mm



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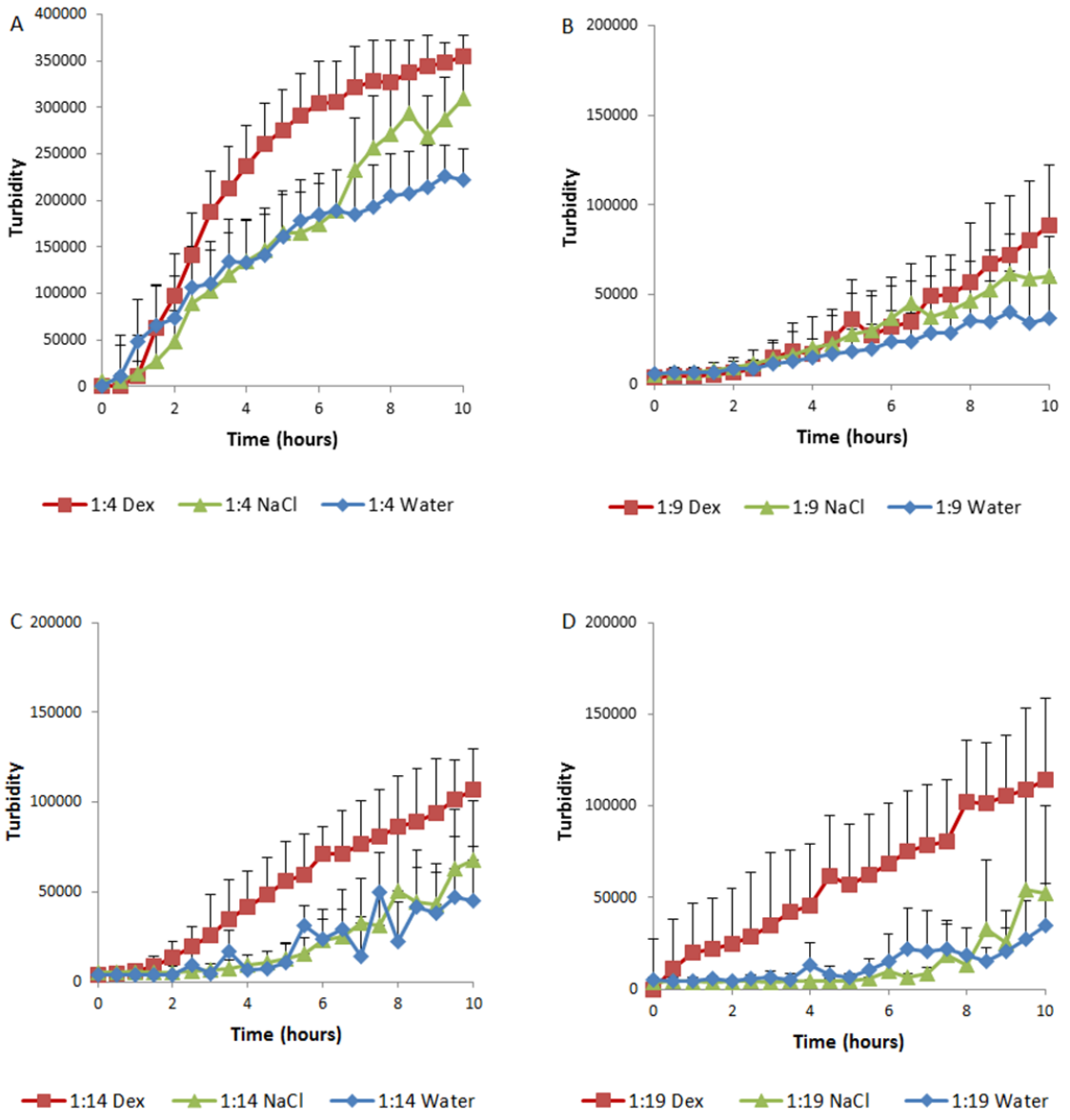
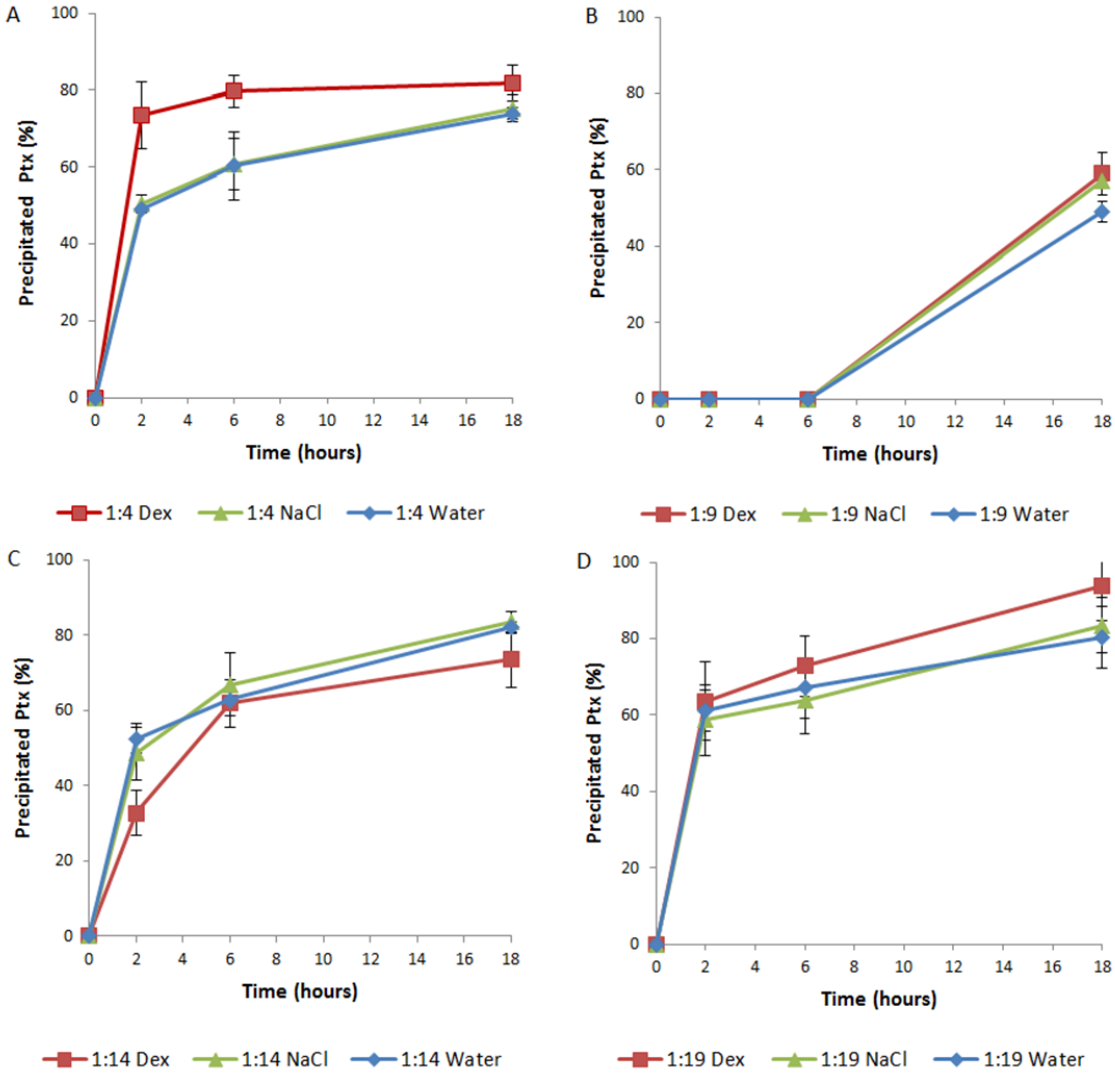
415 Figure 3: Turbidity of Ptx in Taxol-like formulation diluted in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl
416 (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19
417 dilution (n=3).



418

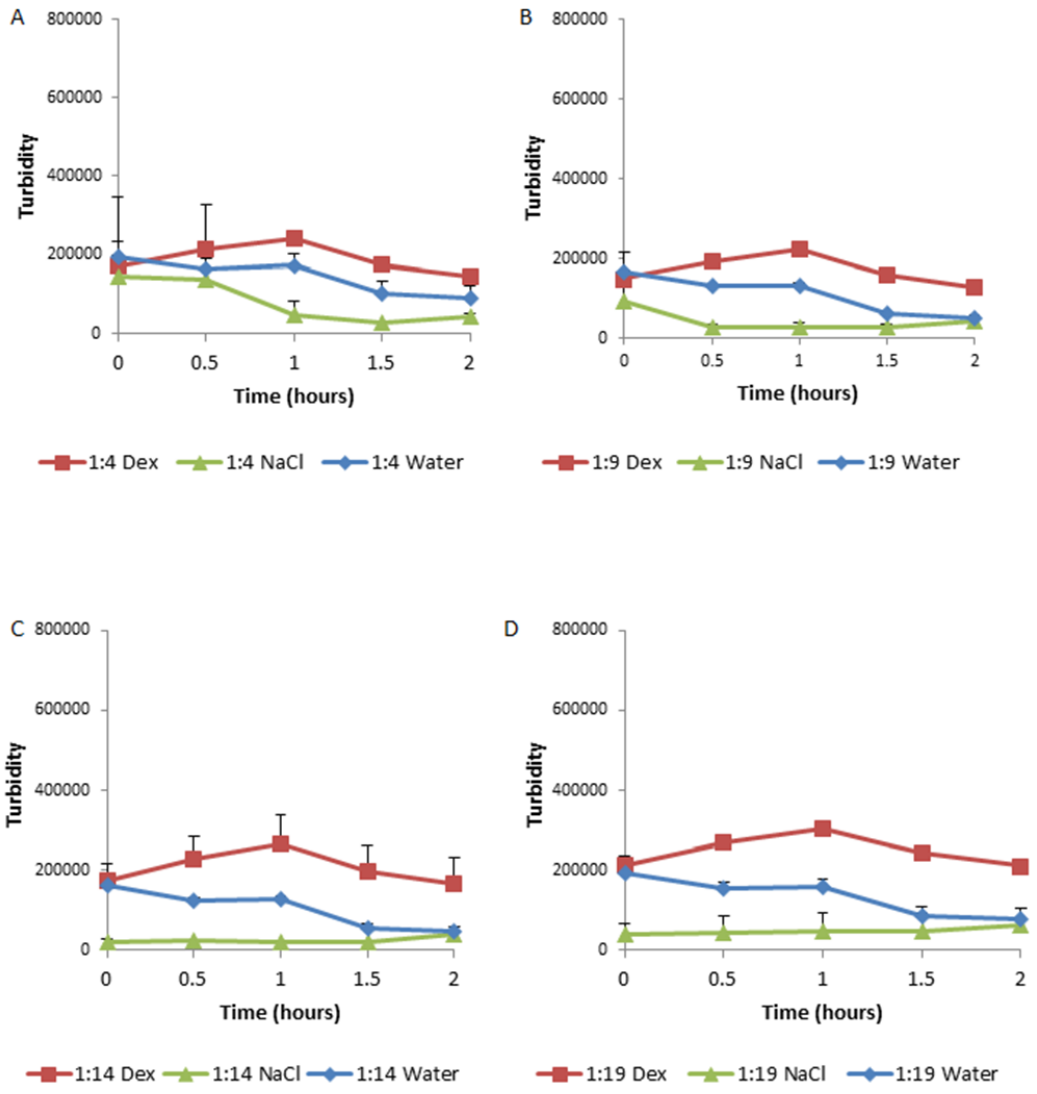
419 Figure 4. Turbidity of CrEl-reduced Ptx formulation (CrEL: ethanol, 1:9 v/v) diluted in 5% (w/v) dextrose
420 (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14
421 dilution and (D) 1:19 dilution

422



423

424 Figure 5. Percentage of CrEl-reduced Ptx formulation (in CrEL: ethanol, 1:9 v/v) precipitation upon addition
425 into 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution,
426 (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution (n=3)



427

428 Figure 6. Turbidity diagrams of Taxol-like formulation (1:1 CrEL and ethanol) in 5% (w/v) dextrose (Dex) ,
429 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution
430 and (D) 1:19 dilution; with Wistar rat plasma (n=3).

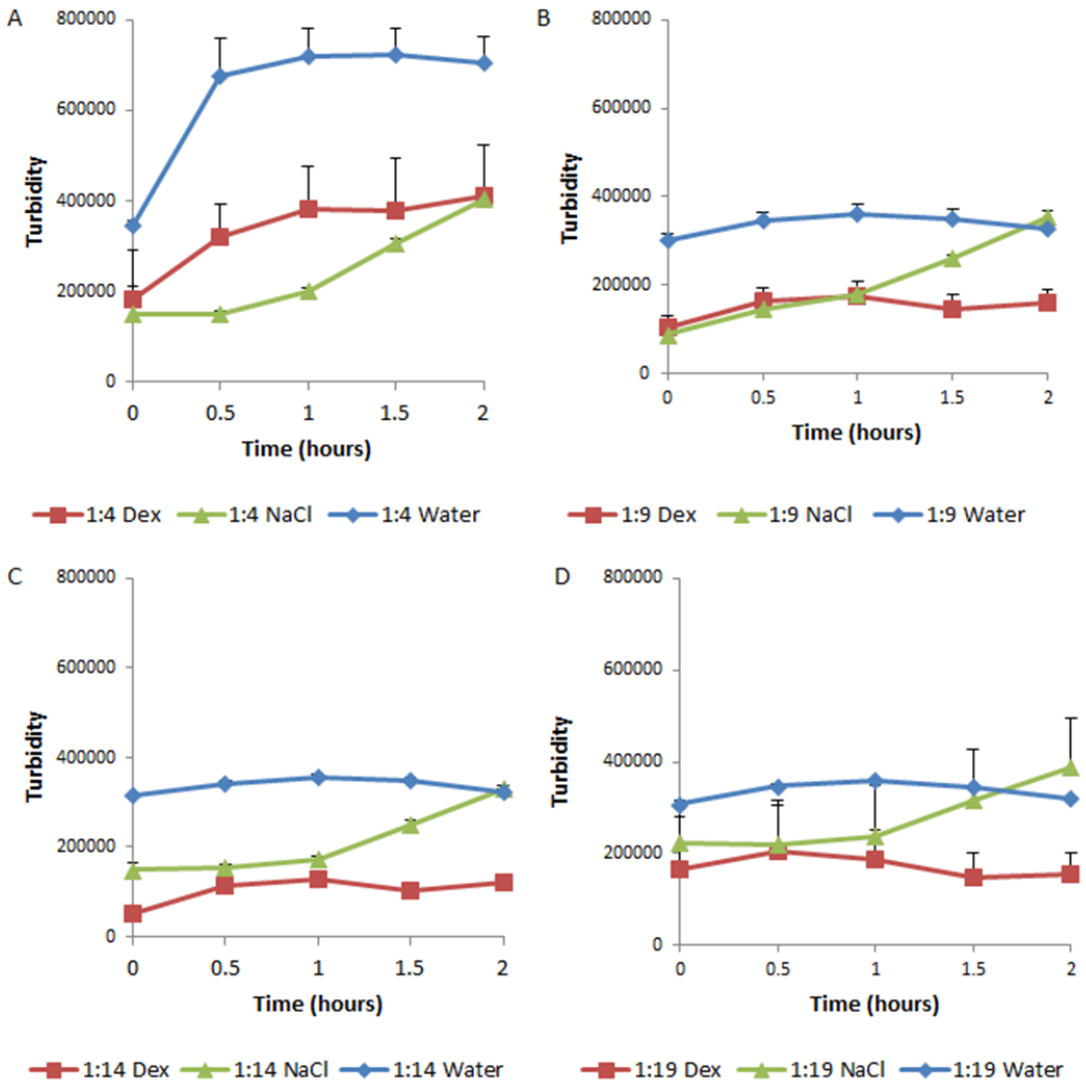
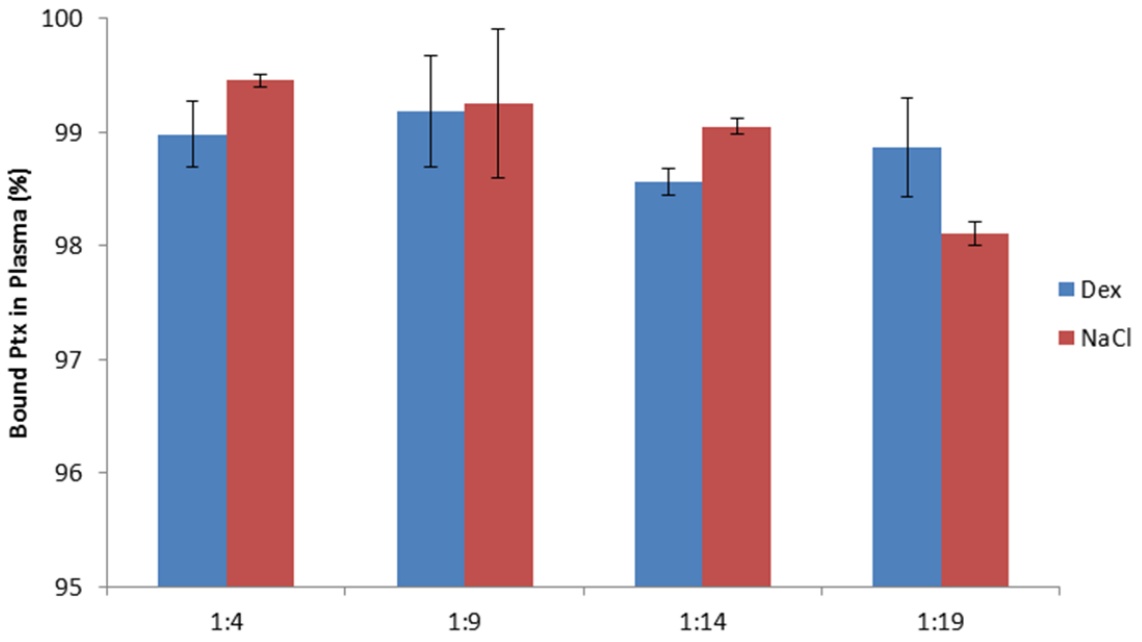


Figure 7. Turbidity diagrams of CrEl-reduced Ptx formulation (1:9 CrEL and ethanol) in 5% (w/v) dextrose (Dex) , 0.9% (w/v) NaCl (NaCl) and deionized water (Water) with (A) 1:4 dilution, (B) 1:9 dilution, (C) 1:14 dilution and (D) 1:19 dilution; with Wistar rat plasma (n=3).

435



436

437 Figure 8. Percentage (%) of bound Ptx in rat plasma calculated from serum with 5% (w/v) dextrose (Dex)
438 and 0.9% (w/v) NaCl at 1:4, 1:9, 1:14, and 1:19 infusion ratios (mean ± SD, n=3).