Novel Drug Release Profiling Method for Albumin-Bound Paclitaxel Nanoparticle Drug Products

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Introduction

ABRAXANE is an albumin-bound paclitaxel nanoparticle drug product approved by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic breast cancers and non-small cell lung cancers. There have been challenges to characterize the *in vitro* release of paclitaxel from the nanoparticles due to hydrophobic nature of paclitaxel and difficulty in separating the released drug from albumin. Solution phase NMR spectroscopy is available for directly monitor the released paclitaxel without separation. However, the NMR instrumentation is not readily available, therefore, not suitable for routine *in vitro* release tests.

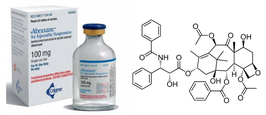


Fig 1. Abraxane and Paclitaxel

Here, we developed a drug release method using a USP type 2 dissolution apparatus hyphenated with autosampler and a bi-phasic release media containing aqueous and organic phase (**1-octanol**). This bi-phasic release media helps the released paclitaxel to partition rapidly from the aqueous phase into the organic phase, providing a 'sink' condition. This approach enabled an accurate determination of the released paclitaxel from albumin-bound paclitaxel nanoparticles.

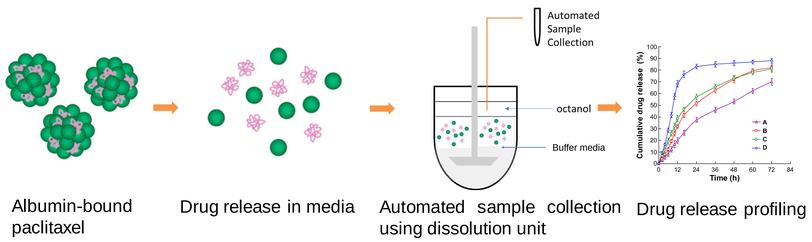
Dissolution Unit and Bi-phasic Method

Advantages of developing a two-phase methods for drug release profiling

- Conventional methods such as dialysis cassette or float-a-lyzer methods give inaccurate quantitation values due to drug adsorption on membrane and/or plastic as a result of paclitaxel hydrophobicity and additionally give delayed time based on diffusion trough semi-permeable membrane.
- The paclitaxel (active pharmaceutical ingredient (API)) has a high affinity for human serum albumin (HSA), resulting in the drug release profiling with quantitation of released paclitaxel in the buffer media difficult.
- Two-phase method consisting of an organic phase (1-octanol) and the aqueous phase, provides the released drug to be partitioned into the organic layer, preventing the saturation and precipitation of paclitaxel in the aqueous phase.
- Further, the insolubility of HSA in the organic phase prevents the interference of plasma protein in the accurate determination of released paclitaxel partitioned into organic phase.

Experimental Procedure

- ✓ The release media was a 100 mM NaCl aqueous solution buffered at 7.4 pH using a 5 mM sodium dihydrogen phosphate and disodium hydrogen phosphate buffers.
- ✓ A 1000 ppm Abraxane and paclitaxel suspensions were prepared by dispersing the drug product or API in 1 mL of the release media and transferred into a dissolution container comprising 700 mL of release buffer solution and 200 mL of 1-octanol at 7.4 pH and 37^{0}C.
- ✓ After that, the release profile of paclitaxel was determined by liquid chromatography mass spectrometry (LCMS) of the samples taken from organic layer at different time intervals.
- ✓ The stirring paddle speed was 75 rpm while the temperature was maintained at 37^{0}C and the experiment was triplicated in three containers at the same conditions. Both the aqueous and organic phases were collected to determine the released paclitaxel via liquid chromatography-mass spectroscopic (LC-MS) analysis through Triple-Quadrapole mass spectrometry (MRM mode).
- ✓ Six-unit, 900 mL Distek Select 2500 bathless dissolution unit hyphenated with Distek Eclipse 5300 Automated Dissolution Sampler was used for the drug release testing. The samples were collected for 9 hours or 48 hours at 37^{0}C from the organic layer. Flush volume was 5 mL while the flushing rate was 12 mL/min and the sample collection volume was 1 mL.



Albumin-bound paclitaxel | Drug release in media | Automated sample collection using dissolution unit | Drug release profiling

Fig 2. Experimental outlook of the Abraxane drug release profiling

LCMS Methods

Instrumentation:
LC: Agilent 1260 infinity UPLC system
MS: Sciex QTRAP 5500 mass spectrometer

Column:
Acquity UPLC CSH C18 column (100×2.1 mm×1.8 µm)
HPLC: Mobile Phase: A, water; B, MEOH both with 0.1% formic acid and 10 mM ammonium formate. Doxorubicin was spiked as the internal standard in final sample preparation.
MS and LC conditions:
ESI source in Positive mode.
Column Temperature 50°C, Multiple Reaction Mode (MRM) in Mass Spectrometer
The binary pump gradient was initiated at 75% A and allowed to run for 1 min, decreased to 40% until 3 mins and further taken down to 30% until 10 mins. At 10.10 mins B% was brought up to 100% and maintained until 16 mins before changing immediately to 25% B at 16.10 mins which was allowed to equilibrated up to 20 mins.

Table1: Experiment parameters optimized in the release media for paclitaxel release profile

Parameter	Range	Effect on drug release rate	Selected condition
Paddle speed	50, 75, 100, 125 rpm	Increased release rate with the increase of RPM	75 rpm
Aqueous media to octanol volume ratio	8:1, 7:2, 6:3	Highest release rate obtained at 7:2 ratio	7:2
Excess HSA in the aqueous media (10-, 100- and 1000-fold excess)	12.8, 128.5, 1285 mg/L	Slightly decrease with the increase of HSA concentrations. All achieved above 80% release at 9 hours	n/a
NaCl concentration in 5 mM phosphate buffer	100 mM, 150 mM	No effect	100 mM
Human serum/plasma and bovine serum added in the buffer media	5%	Release rates decreased and about 20-70% released at 9 hours	n/a

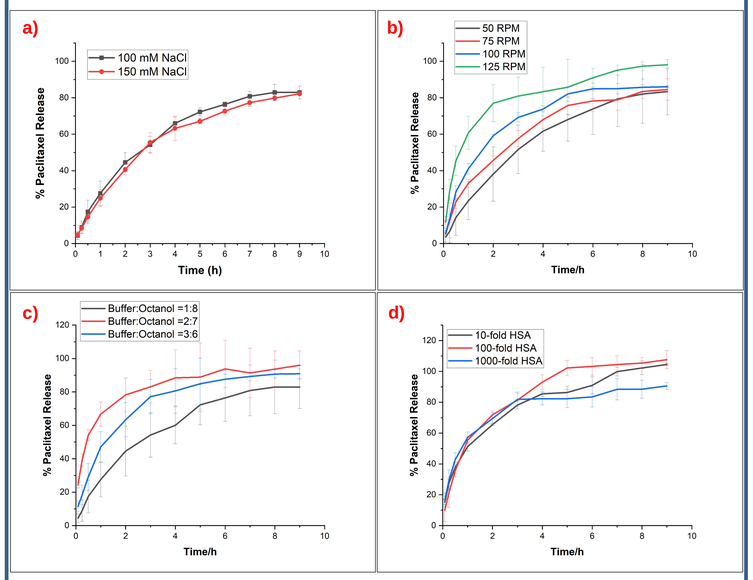


Fig 3. Average paclitaxel release from dispersed Abraxane in 5 mM PBS solution at 7.4 pH at 37^{0}C, a) Effect of different NaCl concentrations b) Effect of different RPM values of the paddle c) Paclitaxel release at different buffer:octanol ratio and d) Paclitaxel release at different human serum albumin concentrations in the PBS buffer (Mean ± SD, n = 3).

Results

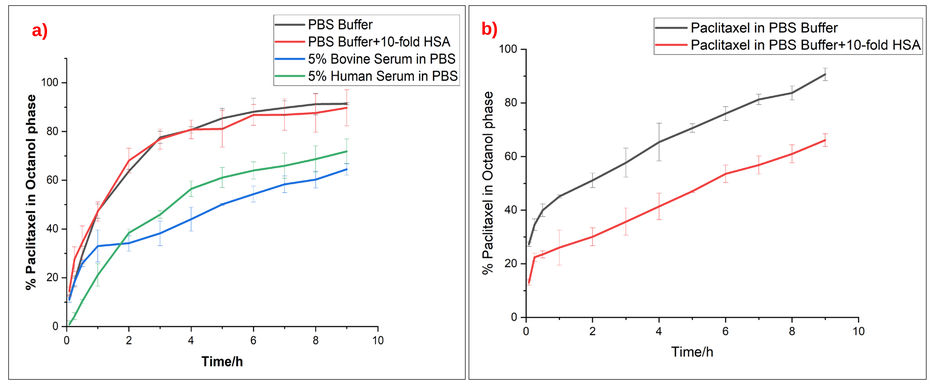


Fig 4. Average paclitaxel release from 1.28 mg/L a) Abraxane and b) Paclitaxel suspended in 700 mL of 5 mM PBS solution at 7.4 pH at 37^{0}C, at different buffer conditions (Mean ± SD, n=3)

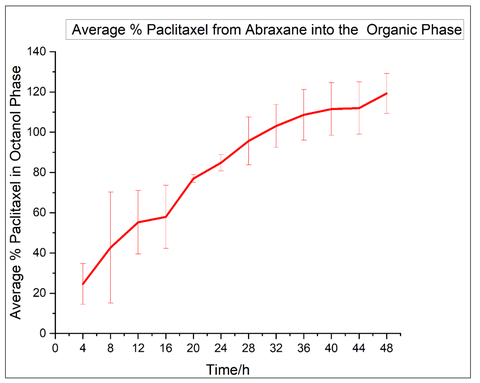


Fig 4. Paclitaxel release by 1.28 mg/L Abraxane dissolved in 700 mL of 5 mM PBS solution at 7.4 pH at 37^{0}C, in 5% Human plasma over 48 h (Mean ± SD, n=3)

Summary and Conclusion

1. An *in vitro* release method using convenient setup, USP 2 dissolution apparatus and bi-phasic media, was developed and optimized for albumin-bound paclitaxel nanoparticles.
2. The *in vitro* release data suggested that the Abraxane had a burst release of paclitaxel at the beginning and the drug release reached 100% over the period of 9 hours at the optimized 'sink' condition. The presence of HSA in the release media showed a minimum effect on drug release but the presence of 5% human plasma reduced the drug release rate significantly, probably due to the presence of hydrophobic lipoprotein in the plasma.
3. This *in vitro* release method can also be explored for other emulsion or liposome formulations containing hydrophobic drug substances.

References

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2) Characterization and in vitro/in vivo dissolution of nab-paclitaxel nanoparticles, Cancer Res May 1 2008 (68) (9 Supplement) 5624

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