Characterization of Paclitaxel and Albumin Oligomeric Status in Abraxane during Storage

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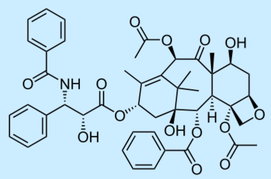
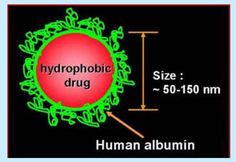
BACKGROUND





Paclitaxel (left) is a hydrophobic drug used in the treatment of cancer. At right, a schematic diagram of Abraxane is shown in which a paclitaxel particle is encapsulated in human albumin.

Abraxane for injectable suspension (paclitaxel protein-bound particles for injectable suspension) is an albumin-bound form of paclitaxel with a mean particle size of approximately 130 nanometers. It is used for the treatment of breast cancer. Abraxane is supplied as a lyophilized powder and each single-use vial contains 100 mg of paclitaxel and approximately 900 mg of human albumin. As the only excipient contained in the final product, the properties of albumin greatly impact the quality of the drug product. Change in the albumin aggregation status may affect the binding between paclitaxel and albumin. It is important to investigate albumin aggregate composition throughout its shelf life of drug product as changes may lead to the formation of more immunogenic aggregates or insoluble aggregates which may cause infusion set blockage.

OBJECTIVES

The objective of this study was to determine (a) the aggregate status of albumin in Abraxane drug product during storage; (b) any potential changes in aggregation status of albumin in the reconstituted suspension before use; (c) the soluble and total paclitaxel in the drug product during storage.

METHODS

Size exclusion HPLC was used for albumin analysis and reverse phase HPLC was used to analyze paclitaxel. A total of 20 vials of Abraxane drug products (100 mg) were used for this study, two vials for each sampling point. The drug product vials were placed in environmental chambers at 25°C/60%RH for 0, 3, 6, 12, 18, and 24 months, and at 40°C/75%RH for 0, 1, 2, 3, and 6 months, respectively. At each sampling point, drug product was freshly reconstituted with 20 mL of 0.9% sodium chloride injection USP and divided into 3 portions. Portion 1 of the suspension was used for the assay of the aggregate content/status and the total paclitaxel using two HPLC methods. Portion 2 was centrifuged at 24000g for 25 min. The supernatant was analyzed for soluble albumin and paclitaxel separately. Portion 3 of the suspension was refrigerated at 2°C to 8°C for 8 hours as instructed in the label for suspension storage, and then the suspension was analyzed for aggregate content/status as for portion 1 and soluble albumin/paclitaxel as for portion 2.

Table I. The aggregate status of albumin in Abraxane drug product during storage.
The uncertainties are standard deviations from 8 measurements.

	% monomer	% dimer	% unnamed	% polymer
40°C/75%RH				
0 month	78.02±0.43%	13.21±0.12%	4.70±0.17%	4.07±0.15%
1 month	74.07±0.43%	15.12±0.10%	6.66±0.21%	4.16±0.13%
2 month	71.93±0.39%	16.07±0.07%	7.82±0.19%	4.18±0.14%
3 month	69.21±0.57%	16.97±0.10%	9.58±0.33%	4.24±0.17%
6 month	64.15±0.49%	18.35±0.05%	13.01±0.29%	4.49±0.19%
25°C/60%RH				
0 month	78.02±0.43%	13.21±0.12%	4.70±0.17%	4.07±0.15%
3 month	77.29±0.44%	13.58±0.12%	5.03±0.18%	4.10±0.13%
6 month	76.50±0.43%	13.99±0.11%	5.28±0.16%	4.22±0.20%
12 month	74.49±0.64%	14.96±0.22%	6.42±0.34%	4.13±0.18%
18 month	73.80±0.65%	15.72±0.16%	7.24±0.21%	3.23±0.33%
24 month	74.16±0.51%	15.77±0.23%	6.86±0.36%	3.21±0.15%

RESULTS

Figure 1

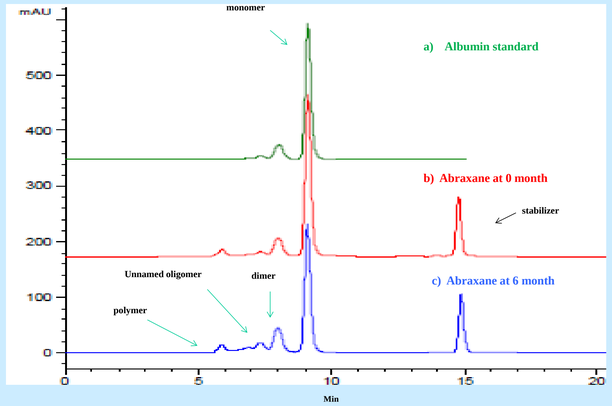


Figure 1 shows typical chromatograms of human albumin standard **(a),** abraxane sample at starting point **(b)** and 6 months at 40°C/75%RH **(c).** The oligomer composition changes can be seen by comparing chromatograms **b** and **c**. The results shown in **Table I** represent the average of 8 determinations including two vials each analyzed at 0 and 8 hours after reconstitution and their supernatants after centrifugation. The results from table and additional data (not shown here) analysis indicate that: 1. Aggregate compositions changed systematically with time, especially at accelerated conditions. 2. There was no significant difference in aggregate composition between soluble and total albumin. 3. Dimer and unnamed oligomers increased, but polymer remained almost constant under both conditions. 4. There was no significant change in aggregate composition after the suspension was stored at 4°C for 8 hours. 5. On average 44 mg out of 880 mg albumin per gram of drug product was bound to paclitaxel to form insoluble particles. 6. The percentage of total aggregates in the drug product was 22% when initiating the study (0 month), which was much higher than 5% as controlled for commercially available albumin protein.

Figure 2

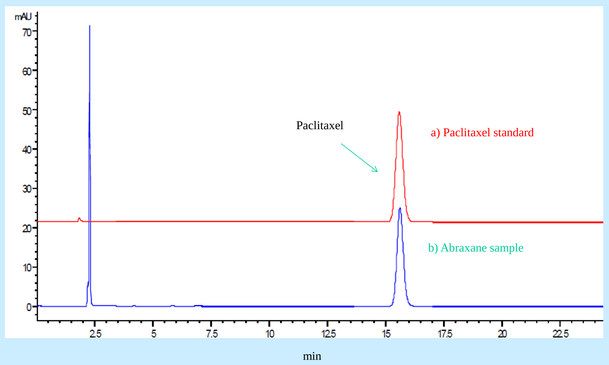


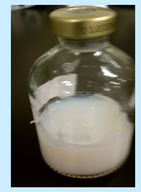
Figure 2 shows typical chromatograms of paclitaxel standard **(a) a**nd abraxane sample **(b)** for the characterization of paclitaxel. Standard solution was prepared freshly at each sampling point. At each sampling point, two vials were analyzed for total paclitaxel at 0 and 8 hours after reconstitution. The centrifuged supernatants were analyzed for amount of soluble paclitaxel. Results shown in **Table II** and additional data (not shown) analysis indicated that: 1. On average 93.2 mg paclitaxel was found per gram of drug (or 94.1 mg paclitaxel per vial of drug). Of that paclitaxel, 12.2 mg was found in solution (soluble) after centrifugation, which is 13% of total paclitaxel. 2. There was no significant change in soluble paclitaxel amount after the suspension was stored at 4°C for 8 hours. 3. There was no significant change in soluble pacilitaxel amount during storage.

Table II. The amount of paclitaxel and albumin in Abraxane drug product during storage.

	Total Paclitaxel (mg/g)	Soluble Paclitaxel (mg/g)	% Soluble Paclitaxel	Total Albumin (mg/g)	Soluble Albumin (mg/g)	% Soluble Albumin
40°C/75%RH						
0 month	91.23±1.27	10.63±0.29	11.7	880.50±5.91	834.85±7.46	94.8
1 month	94.63±0.53	12.73±0.22	13.4	880.12±6.84	839.15±3.70	95.3
2 month	95.47±1.09	14.03±0.22	14.7	884.66±3.35	837.68±4.50	94.7
3 month	89.48±1.61	11.06±0.23	12.4	876.37±1.78	821.71±1.14	93.8
6 month	93.97±1.20	14.20±0.31	15.1	872.31±3.80	832.23±4.22	95.4
25°C/60%RH						
0 month	91.23±1.27	10.63±0.29	11.7	880.50±5.91	834.85±7.46	94.8
3 month	93.78±0.42	11.61±0.19	12.4	875.83±2.57	831.68±5.06	95.0
6 month	89.27±0.77	10.31±0.23	11.5	867.16±3.97	825.02±7.50	95.1
12 month	96.80±1.84	12.51±1.08	12.9	892.30±6.49	852.32±4.74	95.5
18 month	92.32±0.33	11.50±0.69	12.5	892.14±7.63	845.38±3.29	94.8
24 month	94.68±0.82	13.75±0.10	14.5	880.93±7.12	841.06±7.11	95.5

Analysis of Uncertainty: The standard deviations given in Tables I and II are based on measurements taken on the same day using the same standard. Additional analytical uncertainty is expected between time points due to uncertainties in standard preparation, instrument response and other time-sensitive factors. The mean values in Table I show systematic variation over time, suggesting that the variation reflects changes in the albumin aggregation over time. The mean values in Table II show random variation over time, suggesting that the variation reflects random analytical error in the paclitaxel measurement.

The picture shows the Abraxane suspension in which drug product was freshly reconstituted with 20 mL of 0.9% sodium chloride injection USP. The suspension is stable for up to 8 hours at 4-8°C.



CONCLUSIONS

Albumin

- On average 44 mg out of 880 mg albumin per gram of drug product was bound to paclitaxel to form insoluble particles.
- The percentage of total aggregates in drug product was 22% at the beginning of the study (0 month), which was much higher than the 5% specification for commercially available human albumin.
- The percentage of albumin monomer decreased with storage time, especially under accelerated conditions. (See Table 1 for details.)
- There was no significant change in albumin aggregation status after the suspension was stored at 4°C for 8 hours.

Paclitaxel

- On average 93.2 mg paclitaxel was found per gram of drug (or 94.1 mg paclitaxel per vial of drug). Of this, 12.2 mg (13%) was found in solution after centrifugation.
- There was no significant change in soluble paclitaxel amount during storage.
- There was no significant change in soluble paclitaxel amount after the suspension was stored at 4°C for 8 hours.