



Pharmaceutics, Drug Delivery and Pharmaceutical Technology

## Pneumatic tube transport of trastuzumab in IV bags—Effect of headspace and surfactant on subvisible particle formation



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### ABSTRACT

In hospitals, IV bags can be prepared in advance for logistical and microbial safety reasons in a compounding unit and then transported to wards. Transport of protein drugs using a pneumatic tube system has been reported to result in high particle levels. In this study, pneumatic tube transport of trastuzumab in saline polyolefin bags was compared to delivery by hospital porters using an electric platform truck in an underground tunnel system. The transport was tracked using designed smart labels. Two strategies to prevent particle formation, removing headspace and adding the surfactant polysorbate 20 were evaluated. The transport by pneumatic tube had a higher level of shock and vibration than truck delivery. The total particle count measured using flow microscopy also increased more for pneumatic transport than for transport by vehicle. Removing the headspace decreased particle formation for both transports. Surfactant decreases particles over 10  $\mu\text{m}$  for trastuzumab in saline IV bags but increases the total particle levels. Pneumatic tube transport of saline in polyolefin bags resulted in high particle levels and surfactant increased the total particle count. Removing headspace is a measure that can be incorporated into compounding practices to cover for inadequate surfactant levels in IV bags.

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### Introduction

In recent years there has been a growing demand for in-use stability studies of protein drugs (Nejadnik et al., 2018, Blumel et al., 2023). This is based partly on observations made in clinical settings and partly on an increasing awareness of factors affecting protein stability during compounding, transport, and storage in a hospital setting.<sup>1,2</sup> Another concern regarding intravenous medication is reports of considerable numbers of subvisible particle levels from disposable syringes, IV line set-ups, and commercial diluents<sup>3,4</sup> which contribute to the total subvisible particle levels and potentially trigger aggregation. Subvisible particles resulting from mishandling of protein drugs have been shown to lead to immunogenic reactions leading to loss of efficacy or anaphylaxis.<sup>5,6</sup> Due to the risks related to subvisual particles, there are pharmacopeia standards for the limits of particles a product can contain.<sup>7,8</sup> Several handling steps are thought to lead to increases

in subvisual particles. For example, the increasing use of CSTDs such as syringes and vial adaptors has been reported to lead to protein aggregation and various subvisible particles deriving from polymers and the release of silicone oil.<sup>9–11</sup>

The transport of protein drugs is also seen as a critical step where the shaking and shocks could lead to the aggregation of proteins.<sup>12</sup> Centralized compounding and delivery of medication between hospitals is also emerging as a solution to cover medication shortages and increasing demands on quality and safety (van der Schors et al., 2021). Protein drugs prepared in IV bags are also exposed to additional stresses caused by dilution of excipient and exposure to material and the diluent. Surfactant level and headspace have previously been identified in in-use studies as two important factors in reducing particle levels during transport and subsequent storage of proteins in IV bags.<sup>12,13</sup> The importance of the air-liquid interface for the formation of protein aggregates is also known from agitation studies of protein solutions in vials with headspace.<sup>14,15</sup>

During a recent survey of the logistical handling of protein drugs in Uppsala University Hospital, the transport of IV bags from the

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pharmacy compounding unit to the wards was flagged as a risk priority as the likelihood of discovery after mishandling was ranked low. For example, a broken vial after transport is noticed which may not be the case for a dropped IV bag.<sup>16</sup> Uppsala University Hospital comprises multiple buildings and the longest distance is around 850 m. The drugs can be compounded either at the wards or the compounding unit. The pharmacy compounding unit is located close to the wards it serves with its main operations focusing on three areas: 1) handling hazardous drugs, 2) vial sharing of expensive drugs, and 3) safe handling of high-alert medications. The available transport across the hospital area is delivery by hospital porters using electric trucks in an underground tunnel system or a pneumatic tube system, Swisslog TranspoNet PTS. The PTS is currently not validated for the transport of medication.

While PTS has been validated and is commonly used for the transport of blood samples, there is more concern regarding its use with protein drugs. The transport of monoclonal antibodies and insulin in a clinical setting has been reported,<sup>17,18</sup> however, the existing studies show some contradictory results concerning the risks of pneumatic transport.<sup>19–22</sup> Transport of intravenous immunoglobulins (IVIG) using PTS has been demonstrated to give high levels of nano- and microparticles<sup>19,20</sup> while two other studies have shown a low increase in subvisual particles when protective measures have been in place.<sup>21,22</sup> For example, the addition of diluted surfactant was demonstrated to give protection in a study using mixed-transportation including PTS.<sup>21</sup> There has also been a concern that the analytical panel used in these studies does not cover all critical quality attributes necessary to claim stability.<sup>23</sup> This was addressed recently in a study using an extensive analytical panel including the identification of post-translational modification for 11 commercial mabs transported by PTS and here it was shown that the transport did not induce such post-translational modifications or subvisible particle formation provided headspace was removed.<sup>22</sup>

In this study, a non-commercial product of trastuzumab was chosen as a model protein for monoclonal antibodies. It is one of the top ten protein drugs used in Uppsala University Hospital.<sup>16</sup> The diluent chosen was 0.9 % saline since Trastuzumab has been found to aggregate when diluted in dextrose.<sup>24</sup> The choice was made to limit the material of the IV bags to polyolefin. PVC is not used in the European market and is being phased out in many others.<sup>25</sup> The risks associated with leachable substances from PVC during transport by PTS are also well documented.<sup>19,25</sup> Polyolefin bags on the other hand are more rigid, which has been proposed to contribute to particle formation.<sup>20</sup> The purpose of the study was to compare different alternatives for transport across the hospital area and evaluate measures to reduce subvisible particle formation. Two realistic alternatives for transport across the hospital area were chosen to model the impact of transport, PTS and delivery by hospital porters using electric platform trucks (trucks) in an underground tunnel system. To cover different scenarios, two factors identified in the literature were varied, the presence of headspace and the addition of surfactant.

## Materials and method

### Preparation of samples

The monoclonal antibody trastuzumab was produced in the AdBIOPRO project as previously described<sup>26,27</sup> and kindly supplied by Bernt Nilsson, Department of Chemical Engineering, Lund University, Sweden. Trastuzumab was provided as an 11 mg/ml solution in a 25 mM Phosphate buffer, 25 mM sodium acetate, and 125 mM Sodium Chloride pH 6.2. The buffer was changed to a 25 mM histidine buffer (L-histidine, L-histidine monohydrochloride monohydrate (Merck KGaH, Darmstadt, Germany)) pH 6 using a dialysis cassette (Slide-A-Lyzer G2, Thermo Fischer Scientific, Rockford, USA)

following the instructions from the manufacturer. Polysorbate 20 (Tween 20, Croda, Sigma Aldrich,) was diluted in the histidine buffer to a stock concentration of 0.1 wt%. All chemicals used were of Eur. Pharm grade and the buffers were prepared from MilliQ Water (Millipore S.A., Molsheim, France). The buffer and stock solution of PS20 were filtered using a 0.2  $\mu$ m PES syringe filter (Fischer Scientific, Göteborg, Sweden). All the stock solutions were then frozen and thawed at room temperature at the site on the day of use. The samples for transport were prepared in 100 ml polyolefin IV bag containing 0.15 M sodium chloride (9 mg/ml, Fresenius Kabi, Uppsala, Sweden). The IV bags were prepared by removing the surplus solution to obtain a final volume of 100 ml after the addition of the trastuzumab stock solution. The final concentration in the IV bags of trastuzumab was 0.5 mg/ml and when Polysorbate 20 was added the final concentration was 0.01 wt%. The air headspace in the IV bags was approximately 30 ml after preparation. For some samples, the headspace was removed using a 10 ml siliconized syringe and 18-gauge needles when indicated.

### Study design

The study compared two types of transport used in Uppsala University Hospital, a pneumatic tube system (Swisslog Transpo Net PTS) or delivery by hospital porter using an electric platform truck (model VOLK EFW.03, Jungheinrich Svenska AB, Österhaninge, Sweden) in an underground tunnel system. For transport using PTS two factors, headspace in the IV bag and the addition of surfactant PS20 were varied in the samples. For truck transport samples only one factor was varied, removal of headspace. Each group had three replicates making the total number of IV bags 18 (12 using PTS and 6 using truck). In addition to this, IV bags where trastuzumab was replaced with buffer or PS20 stock solution were also transported by PTS. The full design is presented in Table 1. Before transport in the tube system the IV bags were wrapped in bubble foam and placed inside the pod, see SI Fig. 1. The route used was between the goods reception and the Department of Clinical Chemistry at Uppsala University Hospital, Uppsala, Sweden. The samples were sent forwards and back the same route using pneumatic transport and afterwards the samples were transported by foot one floor up to the laboratory. The transport time was around 6 minutes in each direction. For transport using the truck delivery service, the samples were placed in plastic carrier bags used for medication, see SI Fig. 2. The samples were then collected and delivered to the destination (Pediatric oncology ward) by a hospital porter and then returned to the sender using the same transport. The total duration for the truck transport was around 14 minutes.

### Datalogging of transport

A pod with a built-in accelerometer was kindly provided by Swisslog (Swisslog Transcheck, Partille, Sweden) and used to get an overview of the tube system. Data was obtained on g-forces and speed as a function of transport time and distance traveled in the tube making it possible to link the stress to events such as transfer between lines and arrival at receiving stations. A custom-designed “smart label” (CPI, Darlington, UK) was used to compare the two transports. The logger had a three-axis accelerometer (x,y,z) and also tracked temperature and light. The sampling rate was set to 100 Hz, sampling time 10 ms, and the maximum recorded force according to specifications, reached 8 G. The smart labels were placed with the IV bags inside the bubble wrapping before transport thus measuring the force inside the pod. On recommendation from the manufacturer, the z-led (lateral) was excluded due to noise levels above 1G, and the analysis was done using the x-axis (horizontal) and y-axis (vertical) only. The label data was further processed in MATLAB version

**Table 1**

A summary of the study design. The varied factors were the mode of transport between wards (PTS or electric platform truck), the volume of headspace in the IV bag, and the concentration of surfactant. In the last column, the number of 100 ml IV bags in each group and the total number for each transport is given.

Transport	Sample type	Headspace (HS)	Surfactant	IV bags
PTS	Trastuzumab (Mab)	30 ml	0.01% PS20	3
		0 ml	0% PS20	3
	No MAb added	0.01% PS20	3	
		0% PS20	3	
		Total:	12	
Truck	Trastuzumab (Mab)	30 ml	0.01% PS20	3
		0 ml	0% PS20	3
	No MAb added	0.01% PS20	3	
		0% PS20	6	
		Total:	6	

R2020b. A rolling average of 100 measuring points was used to establish a baseline for the smart labels data. For the comparison between transport, the normal vector was calculated as the square root of the sum of squares for x and y.

#### Measurements on site

After transport the samples were visually inspected in a photo box (Puluz Photo Studio Light Box, Dongguan City, China) using a light source between 2000 and 3750 lux as confirmed with an external light meter (Velleman DEM300 mini digital light meter, Gavere, Belgium). The samples were classified as i) no particles, ii) trace amount of particles (<5 particles), or iii) particles present (>5 particles). The presence of foam or bubbles was noted. A photograph was also taken in the photo box to document the visual appearance of the bags keeping the background and lighting constant.

Probe Drum (Labbot, Lund, Sweden) was used to measure absorbance, fluorescence, and light scatter. 1 ml was removed using silicone-free syringes from the IV bags before and immediately after transport and transferred to a disposable fluorescence cuvette (BRAND™, ThermoFisher Scientific Inc., Gothenburg, Sweden). The concentration was determined by measuring UV absorption at 280 nm and using an extinction coefficient 1.47 Lg-1cm-1 found in the literature for Trastuzumab. The samples were excited at 280 nm, and the fluorescence spectrum was then recorded during 200 ms. Light scattering at a 90° angle was measured using a laser at 636 nm during 200 ms.

#### Sampling for off-site analysis

After an initial evaluation was done on-site, samples were transported for analysis off-site at RISE (Research Institute in Sweden, RISE) in Stockholm (DLS and FlowCam) or at Lund University (SEC). For DLS and FlowCam samples were transported in refrigerated centrifuge tubes with screw caps. The tubes were filled to the brim to avoid headspace. The samples were then stored refrigerated and analyzed within 24 hours. To make sure that the effect was due to the transport studied, each sample was compared to a sample taken before the transport and submitted to the same handling afterward. IV bags without the addition of sample or buffer were transported to RISE, as they were, to minimize contamination from sampling procedures. For SEC the samples were immediately frozen and subsequently transported to Lund University.

#### Dynamic light scattering

The samples were analyzed on a Malvern Zetasizer ultra (Malvern, Worcestershire, UK) in acrylic disposable cuvettes (Sarstedt,

Nümbrecht, Germany). No pre-treatment was used. 1 ml of sample was transferred to the cuvette, allowed to equilibrate at 25°C for 120 s, and then measured in triplicate using a 173° backscatter. The material was set to protein using a refractive index of 1.45 and an absorbance of 0.001 and dispersant to water, using a refractive index of 1.33 and viscosity of 0.88 mPa · s. Before measurements, the equipment was calibrated with 60 nm latex beads (ThermoFisher Scientific, Fremont, CA). The results were analyzed using a general-purpose model in the instrument software ZS Xplorer to calculate size distribution, Z-average, polydispersity index (PDI), and peak mean size by intensity.

#### Flow imaging microscopy

FlowCam 8400 (Yokogawa Fluid Imaging Technologies, Inc., Maine, US) equipped with a FOV100 flow cell and a 10X magnifying lens. The equipment was calibrated using 15 μm polystyrene-based microparticles (Sigma-Aldrich, Missouri, US). The flow rate was set to 0.08 ml/min, the sample volume to 0.2 mL and the autoimage rate to 13 frames/s, giving an estimated efficiency of 72.5 percent. Each sample was analyzed in triplicates using a volume of 0.4 ml introduced sample by machine priming. Between each run, the flow cell was cleaned with 1 mL of Hellmanex 5% followed by 2 mL of MilliQ water at a flow rate of 1 mL/min. The collected data was analyzed using the instrument software VisualSpread sheet 5.9.0.74. Particle sizes were determined as area-based diameter or equivalent sphere diameter. Sphericity was defined as an Aspect Ratio larger than 0.85. The cumulative mass was determined using a method based on ellipsoidal volume calculated from the equivalent sphere diameter and aspect ratio.<sup>28</sup> In the calculation the protein density was set to 1.41 g/cm<sup>3</sup> protein content to 20% for the protein particles as described in the same publication. An AI-based classification of particle morphology was done by Sentry Sciences LLC (Longmont, Colorado, USA) using ParticleSentry<sup>AI</sup> software.<sup>29</sup>

#### Size exclusion chromatography

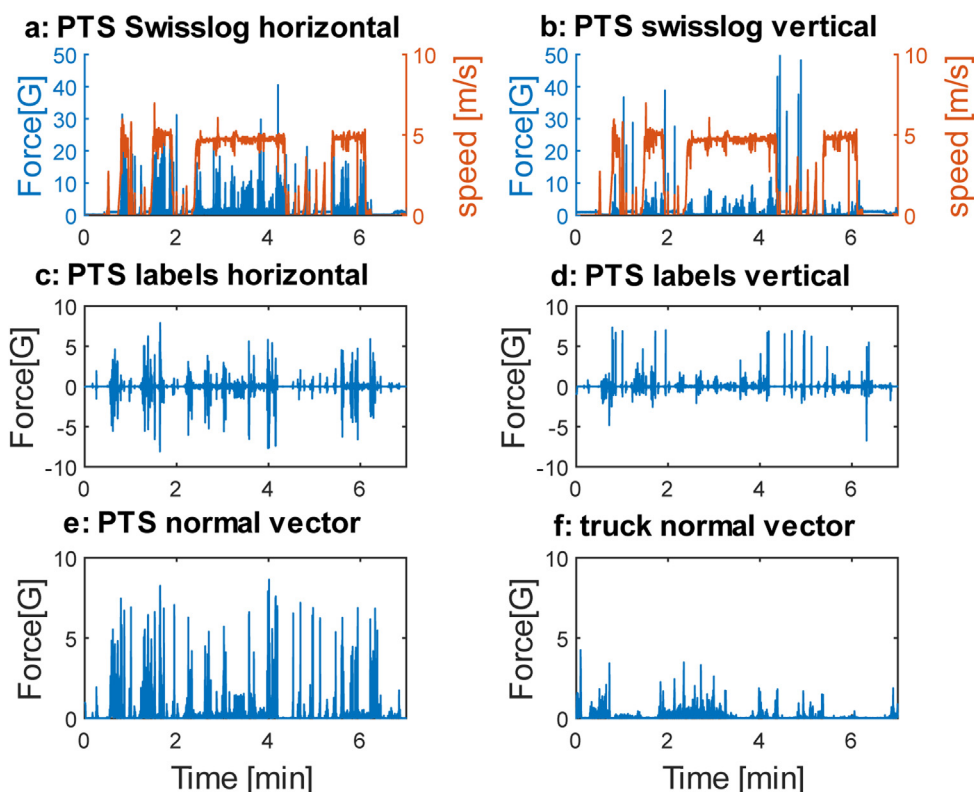
The samples were frozen in Eppendorf tubes and transferred frozen to the lab. The samples were thawed at RT the day of analysis and centrifugated for 10 minutes at 13400 RPM (12 000 G) using an Eppendorf minispin (Eppendorf SE, Hamburg, Germany). The analysis was run on a Thermo Scientific UltiMate 3000 HPLC system, with a guard column TSKgel SWXL Guardcolumn (Tosoh Bioscience GMBH, Stuttgart, Germany). Due to technical issues, two different SEC columns were used TSKgel G3000 SWXL (Tosoh Bioscience, GMBH, Griesheim, Germany) and Phenomex BioSep-SEC-S2000 (Torrance, CA, USA). The program used was 20 minutes at 1 ml/min using a mobile phase of 100 mM Sodium Phosphate, 100 mM Sodium Chloride, and 0.02% Sodium azide pH 7. The transported sample was compared to a sample taken before transport and the percentage loss of the native peak was calculated. The transported sample and the untreated control were analyzed in the same column. The loss was calculated according to Eq. (1), where A stands for peak absorbance at 280 nm.

$$\text{loss} = 100 \cdot \frac{A_{\text{after}} - A_{\text{before}}}{A_{\text{before}}} \quad (1)$$

## Results

#### Acceleration data

The journey through the pneumatic tube system was mapped using a pod with a built-in accelerometer. The distance travelled between the receiving stations was 870 m and the speed it moved with was 5 m/s. The time for the passage in one direction was



**Fig. 1.** Accelerometer data logged during transport with PTS and electric platform tuck. Row 1 shows SwissLog data given in the horizontal(1a) and vertical direction (1b). The force [G] is given on the left axis and speed [m/s] on the right. Row 2 shows measured G-forces for labels transported with IV bags in the horizontal(1c) and vertical (1d) directions. In row 3 the normal vector of the horizontal forces and the vertical forces[G] is used to compare PTS (1e) to truck transport (1f).

approximately 6 minutes. The exact time will vary between journeys depending on time spent in transfer stations. Representative graphs of accelerometer data are shown in Fig. 1. In the figure, horizontal is used for forces perpendicular to the movement and vertical in the direction of movement. The velocity of the pod can be seen on the right axes in the same plot. As can be seen in Fig. 1 the measured forces are more frequent in the horizontal, Fig. 1a, than in the vertical direction, Fig. 1b and coincide with the pod's movement often reaching between 10 G and 20 G. Notably, the number of shocks in the horizontal direction compared to the vertical direction for a typical journey was 1213 versus 69 in the 10G-20G range, 278 versus 78 in the 25G-50G range, and 8 versus 10 in the over 50G range. The shocks in the vertical direction are mostly linked to switching and receiving stations. There is also a consistent vibration in the under 2G range in both directions. The same overall picture is given from the labels attached to IV bags during transport, shown in Figs. 1c and 1d, but with lower acceleration recorded. The measuring limits for the labels are 8G and the IV bags were wrapped in bubble foam inside the pod which also affected the results. The label data is not given as absolute values here. It can be observed that the forces in the horizontal direction are more frequent than in the vertical direction. Using the labels to compare the two transports it is noticeable that the truck transport in Fig. 1f has fewer incidences of shock than the PTS transport in Fig. 1e, it also appears to be no continuous vibration during the transport. The magnitude of the shock is also much lower, with no shocks over 5G and few over 3G. The temperature was logged during transport with the smart labels and was  $22.9 \pm 0.2^\circ\text{C}$ .

#### Analyses on site

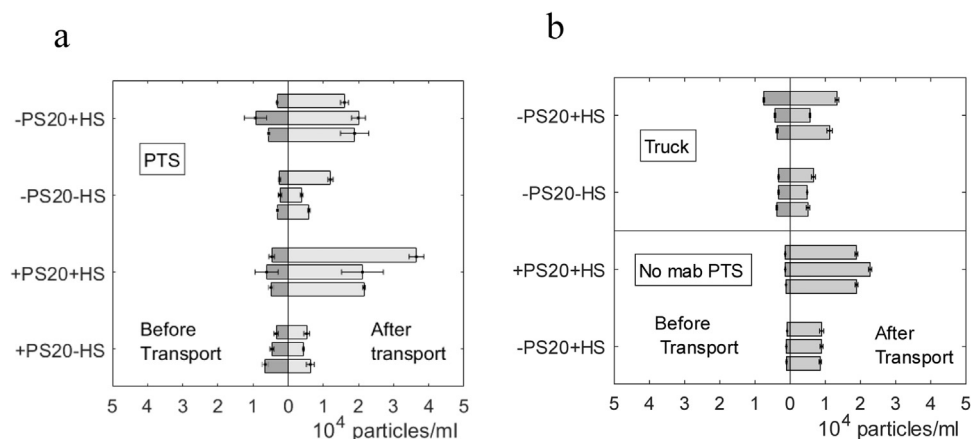
The IV bags were evaluated by visual inspection before and after transport regarding the presence of visible particles, bubbles, and

foam. Headspace was mostly removed completely but, in some cases, a singular or few air bubbles were observed before transport. Singular or few visible particles were observed in a few IV bags, but the amount did not change in these IV bags with either type of transport. For the group with headspace, there were bubbles and foam in some replicates, particularly in the presence of surfactant after transport. Records and representative photos are shown in SI Fig. 3 and SI Table 1.

The concentration of trastuzumab in the IV bags before transport was determined using absorbance at 280 nm to  $0.51 \pm 0.05$  mg/ml. The concentration was constant after transport and there was not any systematic difference in absorbance at 350 nm indicating turbidity. To evaluate subvisible particle formation, the Right Angle Light Scatter (RALS) was measured before and after transport at 636 nm. The peak for IV bags without trastuzumab added was close to the baseline and did not increase by transport. For all other groups, there was weak light scattering before and after transport possibly originating from proteins, micelles, or some particulate matter. For the group without PS20 and with headspace, the light scattering doubled after transport with PTS but for the other groups, the difference was within one standard deviation. Finally, fluorescence was measured before and after transport. The fluorescence spectrum did not show a red shift indicating structural changes to the protein. Data for absorbance, fluorescence, and LS can be found in SI Table 2-5.

#### Flow imaging microscopy

The total count of subvisible particles measured before and after transport is given in Fig. 2 and Table 2. The data is presented for individual IV bags, to show variation between replicates, and there are clear differences in particle levels after transport depending on the type of transport and protective strategies used. The transport using PTS resulted in higher particle levels than transport using an electric



**Fig. 2.** The total subvisible particle count above  $2 \mu\text{m}$ . The data presented is the mean and standard deviation of three replicates for each IV bag. In 2a the IV bags with Trastuzumab are transported by PTS. In 2b top the IV Bags without trastuzumab (-mab) added are transported with PTS and in 2b bottom trastuzumab in IV bags transported with a truck. To the left in each plot is the particle level before transport and to the right is the level after transport. Groups are based on the addition of polysorbate (+PS20) or not (-PS20) or headspace in the IV bag (+HS) or not (-HS).

platform truck for IV bags without surfactant and in both cases, the particle levels were higher when there was also an air headspace in the IV bag. Transport using a truck resulted in close to 10,000 particles per ml when transported with headspace, and for transport with PTS, the total particle level was close to 20,000 particles per ml. When 0.01% PS20 was added to the IV bags with trastuzumab, there was minimal particle formation after transport without headspace, but after transport with headspace, there was an almost five-fold increase in total particle levels after transport resulting in even higher particle levels than without surfactant. IV bags without trastuzumab added and transported with PTS had over 9000 particles per ml after transport and a similar increase was observed upon the addition of 0.01% PS20.

Looking at particles divided in different size bins in Fig. 3, the same trend is observed regarding the effect of both transport type and headspace in the IV bag. Removing headspace reduced particle formation in all size bins for both types of transport. For transport using PTS with headspace in the IV bag, there was also a marked difference depending on whether there was surfactant added. IV bags with trastuzumab and 0.01% PS20 had a substantial increase for particles in the  $2\text{--}5 \mu\text{m}$  and  $5\text{--}10 \mu\text{m}$  range but a minimal increase in particles over  $10 \mu\text{m}$ , whereas without surfactant there was a considerable increase in all size bins. Transport of IV bags without trastuzumab resulted in a similar increase in subvisible particles in the range of  $2\text{--}5 \mu\text{m}$  and  $5\text{--}10 \mu\text{m}$  following the addition of PS20 to the IV bags but a minimal increase in particles over  $10 \mu\text{m}$ . An aspect ratio factor

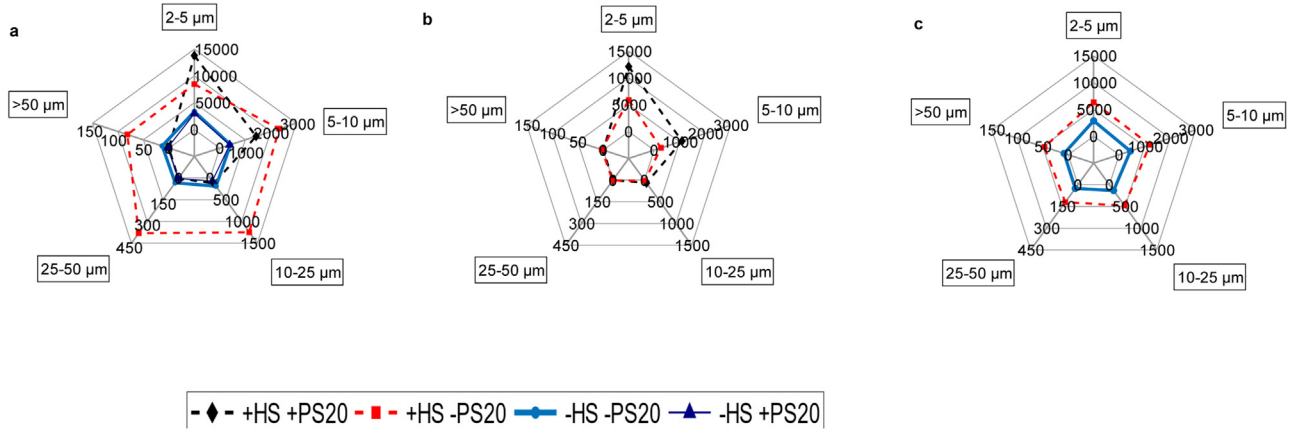
of 0.85 was used to divide the particles over  $5 \mu\text{m}$  into spherical and non-spherical. As can be seen in Fig. 4, after transport of trastuzumab without surfactant in IV bags with headspace there is an increase in mainly non-spherical particles. This is also the case but to a lesser extent for some but not all IV bags where trastuzumab is transported without surfactant but with headspace removed. For IV bags with added surfactant, there is an increase in the size range of  $5\text{--}10 \mu\text{m}$ , and the quotient spherical to non-spherical is higher. Images showing a representative selection of particles are shown in Fig. 5.

An analysis of changes in morphology was also done using Particle-Sentry<sup>AI</sup> software where the different particles can be separated based on morphological features in the images and mapped using Euclidean distance to visualize the morphological differences between samples, for full analysis see SI2. For transport using PTS, there were slight differences in morphology between before and after samples for IV bags transported without headspace. For transport using PTS of trastuzumab with no addition of PS20 in IV bags with headspace, there was a shift in mode and morphologies mapped. When PS20 was added to the IV bag there was more overlap, and the mode for the particles was unchanged, reflecting that there were fewer morphological changes between samples before and after transport. For IV bags without trastuzumab added and transported with headspace, there were also indications of a shift in particle morphology, reflecting an increase in small spherical particles which were observed visually. For particles transported using a truck, there were few morphological changes observed before and after transport.

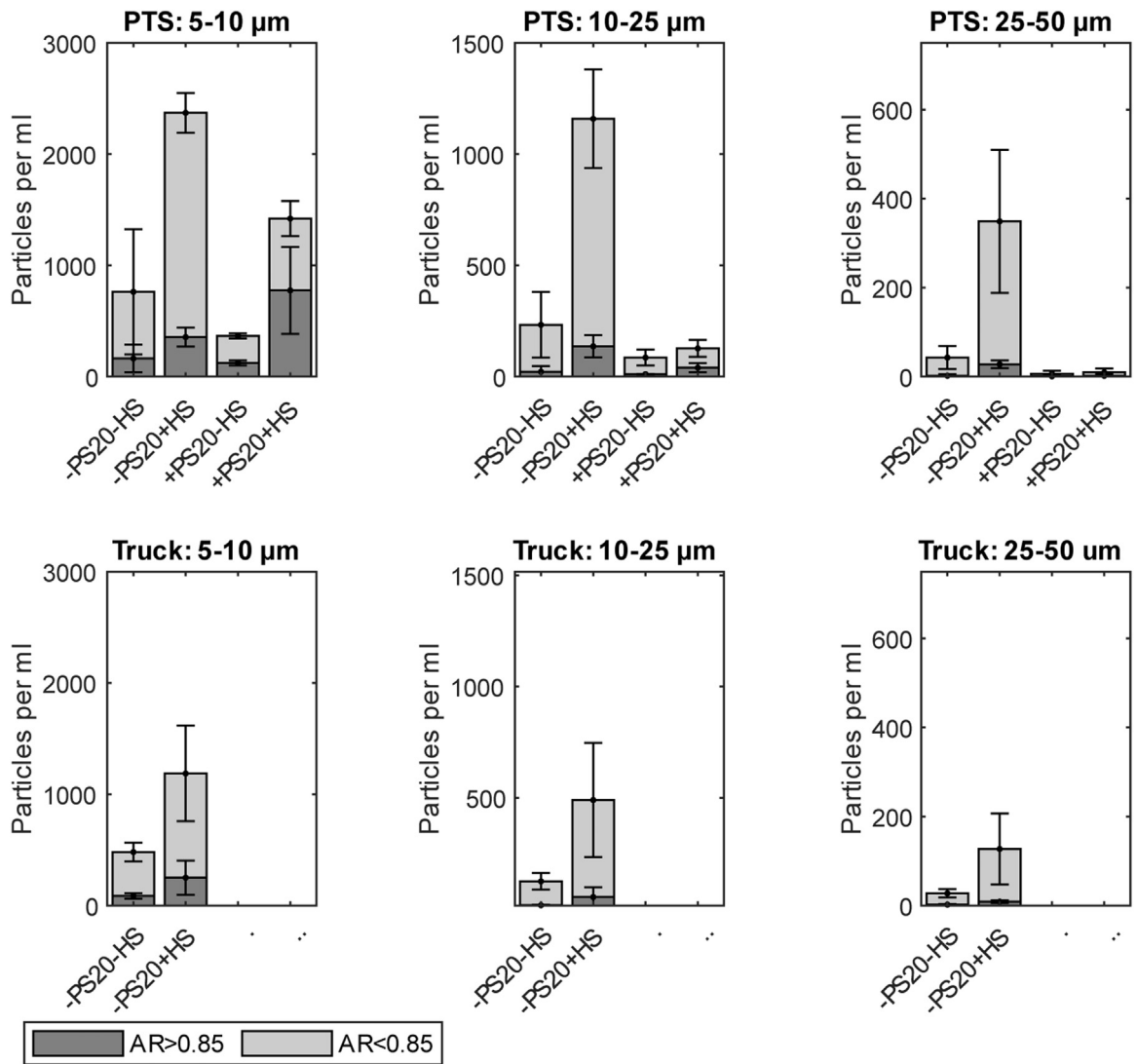
**Table 2**

Subvisible particle formation before and after transport measured by FlowCam given as total particle counts, and particles over  $10 \mu\text{m}$  and over  $25 \mu\text{m}$ .

Transport	Group	Before transport			After transport		
		total particle	>10 $\mu\text{m}$	>25 $\mu\text{m}$	total particle	>10 $\mu\text{m}$	>25 $\mu\text{m}$
PTS IV bags without protein	Buffer + No PS20 HS	1001±116	19±18	1±2	8804±220	26±9	5±3
	Buffer + PS20 HS	1334±123	23±5	6±6	20 148±2243	89±22	2±3
	HS	4005±792	1±1	1±1	13 218±279	1±1	1±1
PTS Trastuzumab	No PS20 no HS	2616±394	80±27	17±9	7246±4272	446±257	99±82
	No PS20 HS	5983±3095	313±263	64±64	18 298±2051	2524±563	657±208
	PS20 no HS	4852±1565	180±41	17±7	5312±974	179±74	17±15
Truck transport Trastuzumab	PS20 HS	5239±784	215±65	19±3	26 392±8745	224±64	18±20
	No PS20 no HS	3467±287	128±50	23±18	5513±967	206±114	53±4
	No PS20 HS	5173±2023	206±114	29±17	10 056±3968	921±567	234±143

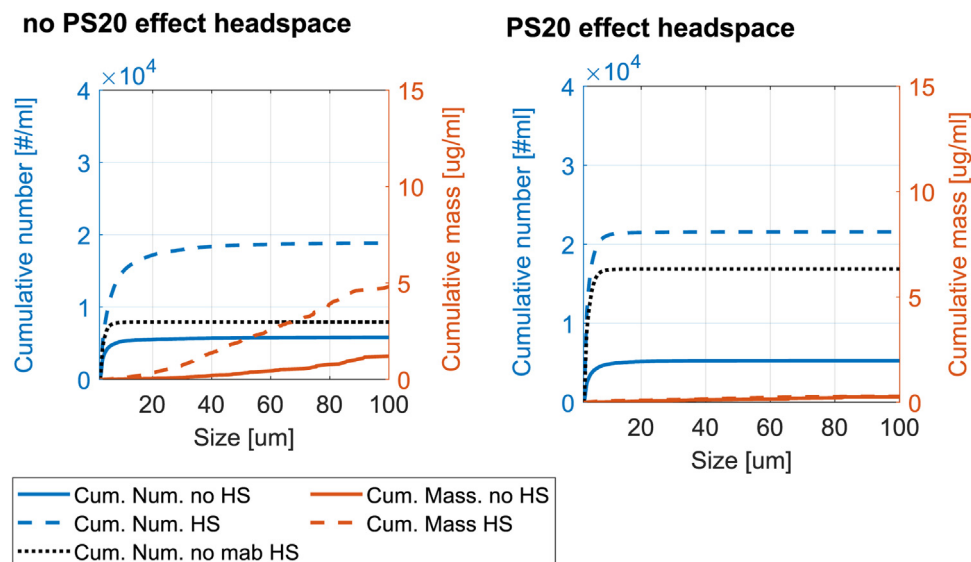


**Fig. 3.** The number of subvisible particles per ml over different size bins. The data presented is the median of three IV bags in each group after transport of trastuzumab in IV bags with mab using PTS (2a), no mab using PTS(2b), and mab using truck (2c). The groupings are based on headspace in IV bag (+HS) or removed headspace (-HS) and added polysorbate 20 (+PS20) or not (-PS20).



**Fig. 4.** The mean particle count (n=3) for particles over 5 μm divided into three size bins and categorized using an aspect ratio of 0.85 after transport using PTS (above) or electric vehicle(below). The different groups are designated based on headspace in the IV bag (HS) or polysorbate 20 added (PS20).





**Fig. 6.** The effect of headspace is shown for the IV bags without PS20 added (6a) and with PS20 added (6b) transported with a pneumatic tube. The cumulative number distribution is in blue, and the cumulative mass distribution is in red and dotted lines indicate headspace in the IV bags. The cumulative distributions are shown up to 100  $\mu\text{m}$ . The cumulative number for the IV bags without mab is added in black.

particle levels compared to the non-transported sample levels including an increase of particles over 10  $\mu\text{m}$ . The higher mechanical impact level translated to a higher subvisible particle level for PTS than for the delivery service using a truck, although the low-level stress from the truck, resulted in increased particle levels compared to before transport. PTS has previously been compared to walking but here there is also a difference compared to vehicular delivery and the results confirm the strong impact previously seen using unprotected protein formulations.<sup>20</sup> Two different protective measures, the removal of headspace and the addition of PS20, as well as the combination of the two were investigated. The removal of headspace is an important protective measure in that it can be incorporated into a compounding practice, while the protection of residual surfactant will depend on the product and dose. The increase in subvisible particles after transport using a truck of IV bags with headspace removed was negligible. Removing headspace in IV bags during PTS transport lowered the total particle count, but contrary to what has been reported<sup>17,22</sup> did not offer complete protection on subvisible particles over 10  $\mu\text{m}$ . In most cases, headspace has been tested for commercial products having residual surfactant which could explain the difference since the combination of adding a surfactant and removing headspace effectively reduced particle formation when tested. A recent study indicates that residual surfactants after dilution give sufficient protection.<sup>21</sup>

To understand the relative effect of headspace and surfactants the cumulative mass was calculated based on the volumetric distribution and compared to the number-based distribution. The cumulative mass has been proposed as a method to estimate small losses due to protein aggregation not usually detected by for example SEC.<sup>28,35</sup> The loss in mass after transport using PTS was estimated using a method by Kalonia et al.<sup>28</sup> to be between 40 ng/ml and 12  $\mu\text{g}/\text{ml}$ . The accuracy of the disposable syringes used in compounding, according to ISO 7886-1:2017, is 4% for 10 ml syringes and 5% for below 5 ml. The variation for the dose used in the study, 50 mg, would be 2.5 mg, meaning that the highest loss for a 100 ml IV bag, 1.2 mg, would be in the same range as the error during preparation. Subvisible particles are therefore better indication of quality after transport than changes in concentration.

The effect of headspace on subvisible particle formation with and without surfactant in the IV bag is illustrated in Fig. 6. The removal of

headspace leads to a similar decrease in the number-based population in both cases. However, with headspace, there is a difference in shape for the number-based population, with a more gradual increase in the cumulative number over the size range for the protein drugs transported in IV bags without surfactant added. With surfactant, the number-based population is dominated by particles under 5  $\mu\text{m}$  and there is close to no loss in mass due to large particles. The loss occurs primarily without surfactant and reflects the formation of larger aggregates observed previously. These larger aggregates had an aspect ratio over 0.85 and the morphology from flow imaging can be described as amorphous having fibrous filaments consistent with what is described for protein aggregates after mechanical stress.<sup>36</sup> Furthermore, an AI-based method was used to compare the particle morphology of samples before and after transport indicated a clear shift in morphology associated with the transport of IV bags with headspace. The effect of headspace in the absence of surfactant indicates that the aggregation is to a large extent surface mediated. There is some particle formation when headspace is removed which could be either disrupted protein film from the surface of IV bags or resulting from cavitation. PS20 could also affect growth and agglomeration following stress, by binding to exposed hydrophobic residues although usually, polysorbates act by competing for surfaces.<sup>37</sup> However, considering the mass balance for particles over and under 10  $\mu\text{m}$ , the contribution of the particles under 10  $\mu\text{m}$  to the cumulative mass with surfactant makes it unlikely that the difference in larger aggregates that is observed without surfactant results from agglomeration.

An AI-based method was used to compare the particle morphology of samples before and after transport indicating a clear shift in morphology associated with transport of IV bags with headspace. The effect of headspace in the absence of surfactant indicates that the aggregation is to a large extent surface mediated. There is some particle formation when headspace is removed which could be either disrupted protein film from the surface of IV bags or resulting from cavitation. PS20 could also affect growth and agglomeration following stress, by binding to exposed hydrophobic residues although usually, polysorbates act by competing for surfaces.<sup>37</sup> However, considering the mass balance for particles over and under 10  $\mu\text{m}$ , the contribution of the particles under 10  $\mu\text{m}$  to the cumulative mass with surfactant, makes it unlikely that the difference in larger aggregates that is observed without surfactant results from agglomeration.

To elucidate to what extent the particles were generated by protein or by particles deriving from handling and the IV bags themselves two tests were done. One where IV bags were injected with buffer or stock solution of PS20, and one where IV bags were transported without any addition. The latter was done to eliminate the risk of particles deriving from stock solutions used, syringes or tubes used for sampling. IV bags with no addition were transported using PTS and transported directly to the lab to avoid contamination during sampling. A substantial number of particles were obtained in all cases. However, the total particle number was considerably higher with PS20 in the IV bags, and the levels were similar for IV bags with and without trastuzumab added, indicating that the particles seen for the trastuzumab with PS20 group derived from the IV bag and not from aggregated protein.

No further work was done on the identification of the particles, but some observations can be made. The particles were mostly in the size range 2–5  $\mu\text{m}$  and remained in solution when measured after 24 hours and the number increased by transport and by the addition of PS20. The surface pressure that an air bubble of that size has makes it unlikely that it was air. Transport by PTS has previously been linked to the formation of subvisible particles in IV bags of PVC where particles from di(2-ethylhexyl) phthalate (DEHP) were found to accelerate protein aggregation as well as being immunogenic in in vitro assays using complement activation in human serum.<sup>19</sup> While transport using PTS involves an elevated level of force, long-distance road trips are also reported to have a considerable level of force<sup>31,38</sup> which could contribute to the high particle levels observed in IV saline.<sup>3,4</sup> Thus, transports of IV bags merits further investigations. The increase in particle number following the addition of surfactant is particularly important given numerous reports of subvisible particles in IV components and the fact that silicone oil can be released during compounding from syringes and CSTDs used.<sup>4,39,40</sup> The effect of PS20 could be either increasing the release of water-immiscible compounds or stabilization of the drops or particles in solution. Therefore, it is also important measure to remove headspace even if surfactants are present in the formulation as surfactants can lead to increased levels of particles from the material and these can be a cause of problems both as triggers of degradation of the drug and a potential risk for patient safety.

## Conclusion

PTS involves higher stress levels than the porter delivery using an electric truck, which translates to significantly higher levels of subvisible particles during transport of trastuzumab in IV bags with headspace and without adequate surfactant levels. The mechanical impact from PTS also results in higher levels of particles for the polyolefin IV bags used in this study and surfactants can exacerbate this problem. Subvisible particle formation during PTS transport can be reduced by the addition of surfactant and removal of headspace. Surfactant reduced particle levels over 10  $\mu\text{m}$  but did not reduce total particle levels. Therefore, it is strongly recommended to remove headspace during the transport of IV bags, especially during transport using PTS.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

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