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The viscous characterization of hydroxyethyl starch (HES) plasma volume expanders in a non-Newtonian blood analog

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Abstract. Although information pertaining to the viscous characterization of HES 130/0.4 Voluven® and HES 260/0.45 Pentaspan® is available, quantification is limited to 100% concentrations. We focus here on the quantification of their viscous behavior along with HES 130/0.4 Volulyte® in a shear thinning non-Newtonian blood analog of aqueous xanthan gum and glycerol. Dynamic viscosities of multiple batches of HES fluids were measured through capillary viscometry. The viscous behavior of 100%, 25% and 12.5% concentrations were then measured through a closed flow loop across physiologically relevant flow rates. Measured viscosities were 2.57 millipascal second (mPa·s) 6.52 mPa·s and 2.48 mPa·s for HES 130/0.4 Voluven®, HES 260/0.45 and HES 130/0.4 Volulyte®, respectively. Pipe flow analysis found that all HES fluids displayed Newtonian behavior at 100% concentrations. 25% concentrations of both HES 130/0.4 fluids decreased analog viscosity 23%–29% at a flow rate of 1.0 ml/s and 16%–21% at a flow rate of 22.5 ml/s. At a flow rate of 22.5 ml/s, 25% and 12.5% concentrations of HES 260/0.45 resulted in analog viscosity changes of 3.9%–4.5%. Capillary viscosity reductions of approximately 7% and 14.5% in HES 130/0.4 Voluven® and HES 260/0.45 suggest changes in molecular composition to batches previously measured. Maintenance of analog viscosity suggests that HES 260/0.45 would be suitable as a high viscosity plasma expander in extreme hemodilution through preservation of microcirculatory function and wall shear stress (WSS).

Keywords: HES fluids, hemodilution, microcirculation, shear thinning, non-Newtonian fluids

1. Introduction

Hydroxyethyl starch (HES) products represent an attractive option for the treatment of hypovolemia in patients presenting with severe trauma or those undergoing major surgery associated with large perioperative blood loss. Numerous fluids are available for plasma expansion to ensure adequate peripheral perfusion, however, HES fluids have been associated with favorable volume expansion ratios. Infusion of 6% HES and 10% HES have been shown to illicit plasma volume expansion ratios of 1.2:1 and

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1.45:1 times the infused volume and two to six times that of infused crystalloid solutions [10,41]. Although pharmacokinetic and risk benefit profiles for HES fluids have been well documented [17,35], until recently the reporting of simple mechanical properties such as viscosity has been limited for fluids available for use in Canada [39].

Walker et al. [39] quantified the viscous behavior of the two HES fluids available for volume expansion in Canada at the time of their study: 6% HES 130/0.4 Voluven® and 10% HES 260/0.45 Pentaspan® in both quasi-static capillary and dynamic pipe flow experiments. Pipe flow measurements quantified their viscous behavior across a range of flow rates, however, 100% concentrations of HES were run through the flow loop [39]. Given that volume expansion fluids are infused in whole blood, we focus here on the viscous characterization of 6% HES 130/0.4 (Voluven®, Fresenius Kabi, Bad Homburg, Germany), 10% HES 260/0.45 (Pentaspan®, Bristol Myers Squibb Canada, Montreal, QC, Canada) and 6% HES 130/0.4 (Volulyte®, Fresenius Kabi, Bad Homburg, Germany) in applicable concentration ratios [26,42] in a representative blood analog fluid of aqueous xanthan gum and glycerol.

The merits of maintaining elevated wall shear stress (WSS) and functional capillary density (FCD) through volume expansion has been well established [4,5,7,20,21,23,28,30,32,34]. Briefly, elevated WSS elicits an atheroprotective response from the endothelium, leading to flow alignment of endothelial cells and the expression of vasodilators [7,20,32] whereas maintenance of FCD through high viscosity volume expanders is critical to survival during prolonged hemorrhagic shock [5,20,30]. To gain an understanding of how infusion may alter blood viscosity and associated WSS, concentrations in conjunction with an appropriate blood analog are required. Newtonian blood analogs have a constant viscosity taken to represent that of blood at high shear strain rates $> 100\ s^{-1}$ [15,22]. Although applicable to the largest arteries where shear strain rates are high, whole blood displays considerable viscosity increases at low shear strain rates resulting from red blood cell aggregation [18]. This relationship is noted by Dammers et al. [9] who reported viscosities of 3.2 mPa · s and 5.0 mPa · s at mean shear strain rates of $400\ s^{-1}$ and $100\ s^{-1}$ in the carotid and brachial arteries, respectively. If we are to appreciate the viscous changes in whole blood upon dilution, it would be prudent to characterize this using a non-Newtonian analog fluid that displays representative shear thinning behavior.

This present study was undertaken to characterize the viscous behavior of HES 130/0.4 Voluven®, HES 260/0.45 and HES 130/0.4 Volulyte® at 25% and 12.5% concentrations across a range of shear strain rates that encompassed the non-Newtonian viscous behavior of a blood analog fluid. The non-Newtonian analog used replicated that suggested by Brookshier and Tarbell [3] consisting of aqueous xanthan gum and glycerol to mimic the shear thinning behavior of whole blood at physiologically representative hematocrit. This study further served to present what we believe is the first viscous quantification of HES 130/0.4 Volulyte® and to address limitations of our previous study, namely potential differences in intra-colloid viscosity and changes across date of expiration.

2. Methods

2.1. Capillary viscometry

The dynamic viscosities of 6% HES 130/0.4 (Voluven®, mean molecular weight 130 kilodaltons (kDa), molar substitution 0.4, expiry dates 04/13, 10/14), 10% HES 260/0.45 (mean molecular weight 264 kDa, molar substitution 0.45, expiry dates 07/12, 03/13) and 6% HES 130/0.4 (Volulyte®, mean

molecular weight 130 kDa, molar substitution 0.4, exp. date 03/14) were evaluated at room temperature (19°C–21°C) using a Cannon–Fenske routine calibrated CRFC (9721-B50) series size-50 capillary viscometer (Cannon Instrument Company, College Park, PA, USA). Batches of HES were stored at room temperature as per manufacturer specifications and all measurements were acquired prior to the date of expiration. Samples were drawn from two bags from each expiration date associated with HES 130/0.4 Voluven® (expiry 04/13, 10/14) and HES 260/0.45 (expiry 07/12, 03/13). Batches of HES 130/0.4 Volulyte® collected were of a common expiration date, limiting the measurement of samples from two bags at a single date of expiration. Three capillary measurements were acquired and averaged to represent the final dynamic HES viscosity. The dynamic viscosities of Newtonian control fluids of deionized water and 0.9% normal saline were also measured. All capillary measurements took greater than 230 s. The mean capillary shear strain rates ranged from 235 s^{-1} to 1500 s^{-1} depending on the efflux time of the fluid. The temperature difference between capillary measurements of HES bags with the same expiration date was limited to 0.7°C.

2.2. *Pipe flow*

A closed flow loop was constructed to observe the viscous behaviors of the HES fluids across a range of flow rates through a 0.635 cm diameter (d), 183 cm long rigid acrylic tube (Fig. 1) [39]. The diameter of the tube was representative of the brachial artery that is often interrogated to assess endothelial cell function [23]. Furthermore, the flow profile is close to parabolic that suggests nearly fully developed flow that would permit appropriate modelling using the Poiseuille (steady flow) or Womersley (pulsatile) equations [8,9,28]. Fluids were contained in an open reservoir and were driven through the loop by an ISMATEC Reglo-Z Digital gear pump (Cole Parmer, Montreal, QC, Canada) using a Micropump pump head (Cole Parmer) with a flow range of 0.27 ml/s to 26.7 ml/s at room temperature (19°C–21°C). An inlet length of 115d from the pump to the first pressure tap ensured fully developed flow at all flow rates measured. A Validyne model P305D differential pressure transducer (Northridge, CA, USA) was calibrated prior to the measurement of pressure drop between two pressure taps spaced 167 cm apart on the rigid tube.

100% concentrations (HES vol./total vol.) of HES were initially characterized to compare their viscous behavior to previously published results [39]. A blood analog fluid of aqueous xanthan gum and glycerol (40%) was used to mimic the non-Newtonian viscous behavior of whole blood [3]. 25% and 12.5% by

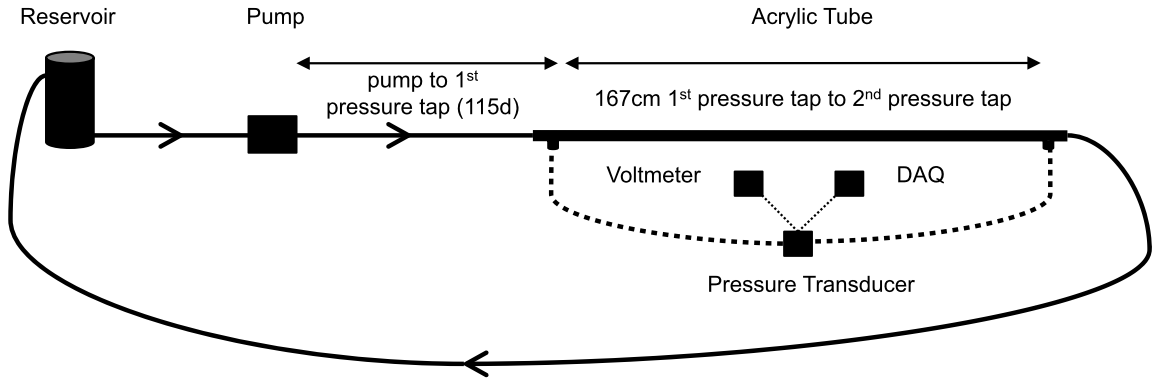

Fig. 1. Schematic representation of the flow loop design. An inlet length of 115d ensured fully developed flow at all flow rates measured.

volume of HES were added to the analog fluid simulating reductions in hematocrit to 30% and 35%, respectively. The nature of the pump head rotation restricted the acquisition of pipe flow measurements below a flow rate of 1.0 ml/s (wall shear strain rate of $\sim$40 s^{-1}) for concentrations of HES and blood analog and 2.0 ml/s (wall shear strain rate of $\sim$80 s^{-1}) for 100% concentrations of HES. The maximum flow rate was restricted to 22.5 ml/s (wall shear strain rate of $\sim$925 s^{-1}) to maintain Reynolds numbers (Re) < 2000, negating potential transition/turbulent effects. The upper limit wall shear strain rate was also selected to coincide with measured non-Newtonian viscous behavior of HES 130/0.4 Voluven® reported previously [39]. These flow rates equated to mean velocities of 3.2 cm/s and 71.1 cm/s with the highest velocities being physiologically representative of the large arteries [39].

Pressure drop measurements were acquired at 100 Hz using custom designed software in NI LabVIEW 2009 Version 9.0.1 (National Instruments, Austin, TX, USA, 2009). Four measurements were acquired and averaged at each flow rate for all concentrations. Using appropriate pressure calibrations, pressure in Pascals (Pa) was calculated. In a Newtonian regime, maintenance of a steady state, laminar flow would allow for viscosity calculation through Poiseuille's equation. However, non-Newtonian fluids often present with a higher shear strain rate in proximity to the wall. To account for this, the Weissenberg–Rabinowitsch correction as shown in Eq. (1) was applied to 25% and 12.5% HES concentration and analog measurements [31,33]:

$$\gamma_{\mathrm{c}} = \frac{4Q}{\pi R^3}\left[\frac{1}{4}\left(3 + \frac{\mathrm{d}\ln\gamma_{\mathrm{a}}}{\mathrm{d}\ln\tau}\right)\right], \tag{1}$$

where γ_{c} is the corrected shear strain rate, Q is the flow rate, R is tube radius, γ_{a} is the apparent shear strain rate and τ is the stress at the wall. Final viscosities presented were derived through division of calculated stress at the wall by the corrected non-Newtonian shear strain rate shown in Eqs (2) and (3):

$$\tau = \frac{\Delta P R}{2L}, \tag{2}$$

$$\mu_{\mathrm{c}} = \frac{\tau}{\gamma_{\mathrm{c}}}, \tag{3}$$

where τ is the stress at the wall, ΔP is the pressure drop, R is the radius, L is the distance between pressure taps, μ_{c} is the corrected viscosity and γ_{c} is the corrected shear strain rate.

2.3. Data analysis

Significant differences in the intra-colloid dynamic viscosity between the various HES capillary viscometer measurements were tested using paired t-tests assuming equal variance ($p < 0.05$) (Microsoft Excel 2007, Microsoft, Redmond, WA, USA, 2007). Initial intra-colloid comparisons were made between bags with the same expiration date (two bags collected per expiration date, $n = 3$ capillary measurements per drawn sample). HES bags with the same expiration dates were subsequently grouped (grouping of two bags of HES, $n = 6$ total capillary measurements per group) to determine if differences existed between different expiration dates. Common expiration dates of bags of HES 130/0.4 Volulyte® limited analysis to a comparison of intra-colloid dynamic viscosity at a single expiration date (two bags with common expiration data, $n = 3$ capillary measurements per bag).

Validation of pipe flow viscosities was assessed through the Refutas equation that is carried out in three steps and shown in Eqs (4)–(6):

$$\mathrm{VBI} = 14.534 \ln\bigl[\ln(\upsilon + 0.8)\bigr] + 10.975, \tag{4}$$

$$\mathrm{VBN} = [w_{\mathrm{a}} \times \mathrm{VBI}_{\mathrm{a}}] + [w_{\mathrm{b}} \times \mathrm{VBI}_{\mathrm{b}}], \tag{5}$$

$$\mu_{\mathrm{blend}} = \mathrm{e}^{\mathrm{e}(\mathrm{VBN}-10.975)/14.534} - 0.8, \tag{6}$$

where VBI is the viscosity blending index, υ is the kinematic viscosity, VBN is the viscosity blending number, w is the weight fraction (%/100) of each liquid component, μ_{blend} is the viscosity of the blended liquids and e is Euler's number. The Refutas equation is applicable to single-phase fluids with several liquid constituents in which the final viscosity is dependent on the proportion and the viscosity of each component [44]. Although pressure drop measurements for each liquid component were not acquired at precisely the same temperatures, the differences in temperature were marginal and likely had only a limited bearing on the resulting blended dynamic viscosity estimations.

3. Results

3.1. Capillary viscometry

Dynamic viscosities for all measured fluids are shown in Tables 1–3. Statistically significant intra-colloid viscosity differences were found between bags of HES 260/0.45 (expiry 07/12, $p = 0.03$) and HES 130/0.4 Volulyte® (expiry 03/14, $p < 0.001$); see Tables 2 and 3. Grouped mean dynamic viscosities of all bags of HES 130/0.4 Voluven® and HES 260/0.45 were 2.57 (0.06) mPa · s and 6.52 (0.08) mPa · s respectively.

Table 1
Capillary viscometry measured dynamic viscosities (mPa · s ± SD) of deionized water and normal saline at room temperature

	Water	Normal saline
Viscosity (∼20°C)	0.99 ± 0.01	1.02 ± 0.02

Table 2
Capillary viscometry measured dynamic viscosities (mPa · s ± SD) of HES 130/0.4 (Voluven®) and HES 130/0.4 (Volulyte®)

	HES 130/0.4 Voluven®	HES 130/0.4 (Volulyte®) #1 Exp. 03/14	HES 130/0.4 (Volulyte®) #2 Exp. 03/14
Viscosity (≅ 20°C)	2.57 ± 0.06[#]	2.41 ± 0.02[*]	2.54 ± 0.01[*]

[*]Statistical significance between samples of HES 130/0.4 (Volulyte®) exp. 03/14.
[#]Dynamic viscosities of all samples of HES 130/0.4 (Voluven®) were combined as viscosity differences were <0.1 mPa · s.

Table 3
Capillary viscometry measured dynamic viscosities (mPa · s ± SD) of HES 260/0.45

	HES 260/0.45 #1 Exp. 07/12	HES 260/0.45 #2 Exp. 07/12	HES 260/0.45 Exp. 03/13
Viscosity (≅ 20°C)	6.45 ± 0.07[*]	6.63 ± 0.05[*]	6.49 ± 0.04[#]

[*]Statistical significance between samples of HES 260/0.45 exp. 07/12.
[#]Dynamic viscosities of samples of HES 260/0.45 exp. 03/13 were combined as viscosity differences were <0.1 mPa · s.

3.2. Pipe flow

All 100% HES concentrations presented a Newtonian viscous behavior. Mean pipe flow dynamic viscosities were within 5% of capillary measurements. The non-Newtonian analog displayed shear-thinning behavior, the dynamic viscosities decreasing by 29% from 7.77 mPa · s to 5.54 mPa · s corresponding to an increase in shear strain rate (Fig. 2). Viscosity approached its asymptotic value at a shear strain rate of $\sim$500 s^{-1} after which viscosity displayed a marginal decrease of 2.6% from 5.69 mPa · s to 5.54 mPa · s (Fig. 2).

The viscous behavior of 25% and 12.5% HES concentrations are presented in Figs 3–5. Concentrations of HES 130/0.4 (Voluven® and Volulyte®) decreased the analog dynamic viscosity at all shear strain rates (Figs 3 and 4). The largest decreases occurred with 25% HES that lowered analog dynamic viscosities between 22.3% and 29.1% at $\sim$40 s^{-1} and between 15.5% and 20.5% at $\sim$925 s^{-1} (Figs 3 and 4). Non-Newtonian viscous behavior in both concentrations of HES 130/0.4 transitioned to shear independent viscous behavior between $\sim$333–500 s^{-1}; see Figs 3 and 4. At high shear strain rates, no appreciable shear thickening was observed at both concentrations of HES 130/0.4. In contrast, all 25% concentrations of HES 260/0.45 increased analog dynamic viscosities with the lone exception at $\sim$40 s^{-1} (Fig. 5). At $\sim$925 s^{-1}, analog viscosity increased to between 5.57 mPa · s and 5.8 mPa · s (Fig. 5). The 12.5% concentrations presented with an increased range of viscous values compared to 25% HES 260/0.45 (Fig. 5). Similar to concentrations of HES 130/0.4, non-Newtonian viscous behavior transitioned to shear independent behavior as shear strain rate increased (Fig. 5).

For brevity, dynamic viscosity curves of 12.5% HES 130/0.4 Voluven® #2 (expiry 10/14), 25% HES 260/0.45 #1 (expiry 03/13) and HES 130/0.4 Volulyte® #1 (expiry 03/14) are presented with comparisons to their perspective blended dynamic viscosity estimates (Fig. 6). Maximum discrepancies were $< 10\%$ with the greatest divergence found at the lowest shear strain rates associated with maximum pressure error.

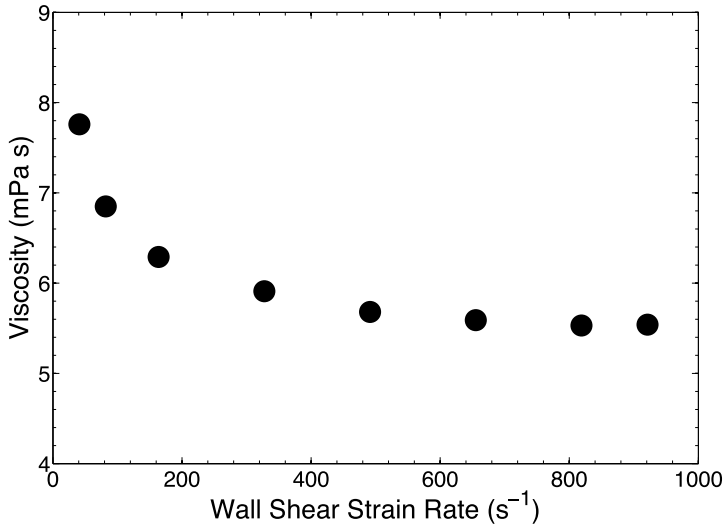

Fig. 2. Viscous characterization of the representative shear thinning non-Newtonian blood analog as a function of corrected wall shear strain rate (s^{-1}).

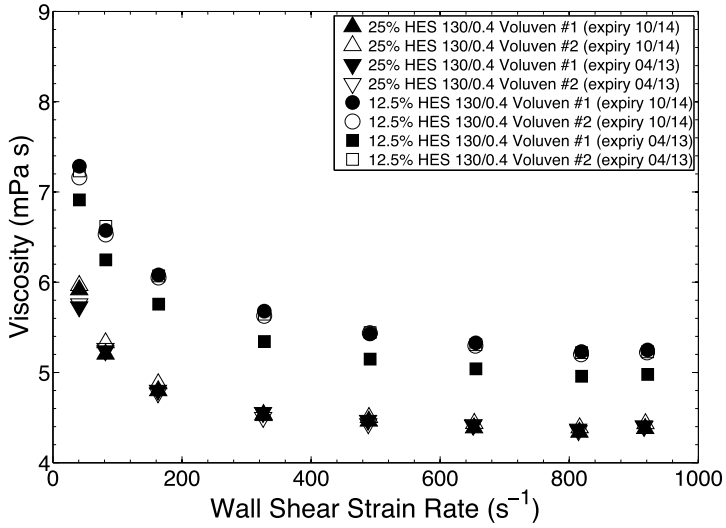

Fig. 3. Pipe flow measured dynamic viscosities (mPa · s) of 25% and 12.5% concentrations of HES 130/0.4 Voluven® with expiration dates of 04/13 and 10/14 in a non-Newtonian blood analog across a range of physiologically representative wall shear strain rates (s^{-1}).

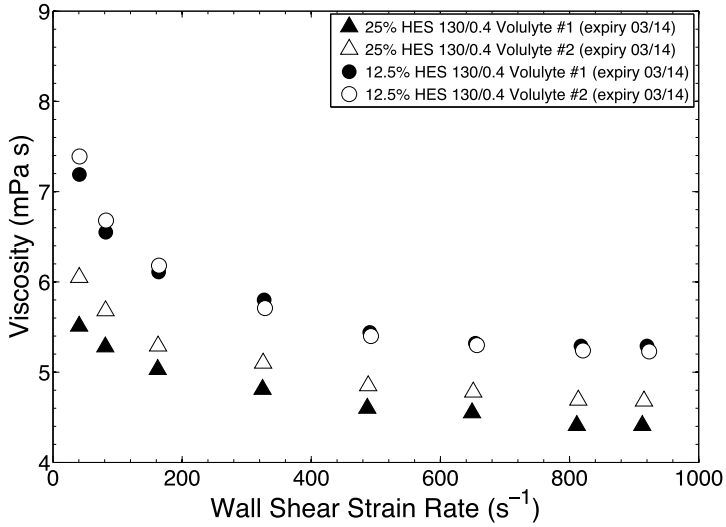

Fig. 4. Pipe flow measured dynamic viscosities (mPa · s) of 25% and 12.5% concentrations of HES 130/0.4 Volulyte® with an expiration date of 03/14 in a non-Newtonian blood analog across a range of physiologically representative wall shear strain rates (s^{-1}).

4. Discussion

The main focus of this work was the viscous characterization of the three HES fluids available in Canada for volume expansion (HES 130/0.4 Voluven®, HES 260/0.45 and HES 130/0.4 Volulyte®) at appropriate concentrations in a non-Newtonian blood analog fluid. Furthermore, through viscous quantification by capillary viscometry, we sought to determine if dynamic viscosities of the volume expansion fluids remained unchanged across a range of expiration dates and whether the viscosities of these sam-

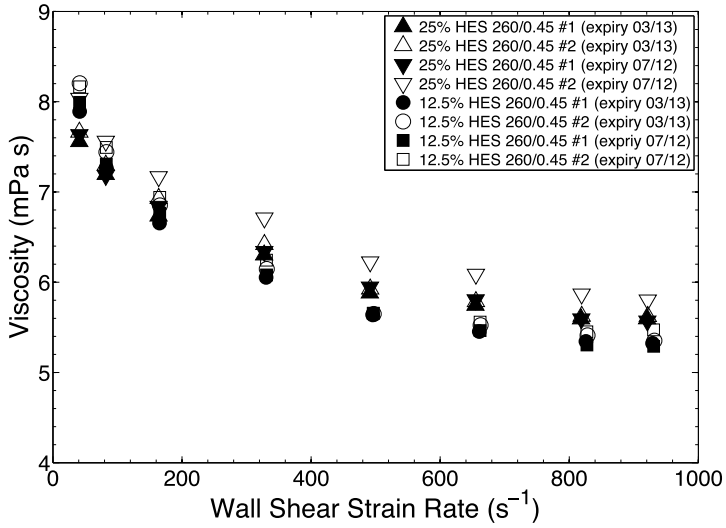

Fig. 5. Pipe flow measured dynamic viscosities (mPa · s) of 25% and 12.5% concentrations of HES 260/0.45 with expiration dates of 07/12 and 03/13 in a non-Newtonian blood analog across a range of physiologically representative wall shear strain rates (s^{-1}).

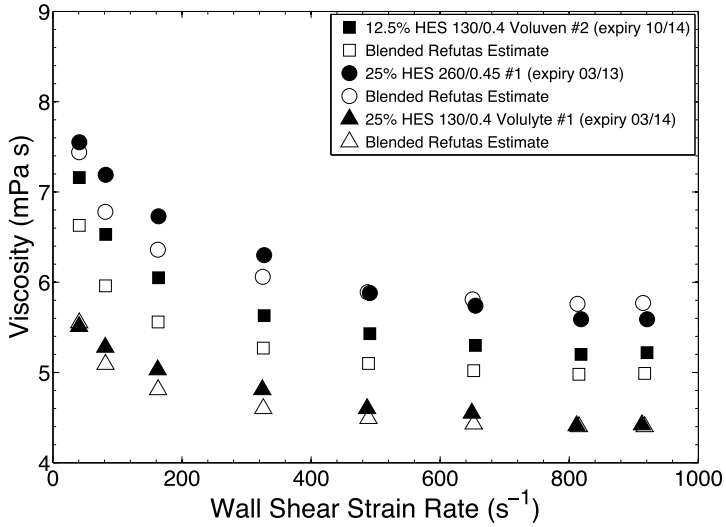

Fig. 6. HES concentration viscosity curves of 12.5% HES 130/0.4 Voluven® #2 (expiry 10/14), 25% HES 260/0.45 #1 (expiry 03/13) and 25% HES 130/0.4 Volulyte® #1 (expiry 03/14) and their corresponding blending dynamic viscosity (mPa·s) estimate using the Refutas equation.

ples varied with previously published results. Our findings represent not only the quantification of the viscous behavior of these fluids in appropriate concentrations with a non-Newtonian blood analog, but also the first characterization of intra-colloid dynamic viscosity across a range of expiration dates and to our knowledge the first quantification of the viscous properties of HES 130/0.4 Volulyte®.

4.1. Capillary viscometry

Mean dynamic viscosities of all samples of HES 130/0.4 Voluven®, HES 260/0.45 and HES 130/0.4 Volulyte® were 2.57 (0.06) mPa·s, 6.52 (0.08) mPa·s and 2.48 (0.07) mPa·s respectively. Similar to HES 130/0.4 Voluven®, it can be inferred that HES 130/0.4 Volulyte® will lower blood viscosity upon infusion. Statistically significant differences between intra-colloid dynamic viscosities were noted for HES 260/0.45 (expiry 07/12) and HES 130/0.4 Volulyte® (expiry 03/14). Differences in temperature at time of measurement could partially account for the differences noted here. However, all intra-colloid sample measurements from bags with the same expiration date were acquired with maximum temperature differences limited to 0.7°C. Although a temperature difference of 0.7°C was noted for capillary measurements of samples from bags of HES 260/0.45 (expiry 07/12), the highest temperature and viscosity was associated with sample two that presented with the highest dynamic viscosity (Table 3). Temperature differences between capillary measurements of HES 130/0.4 Voluven® and HES 130/0.4 Volulyte® samples from bags with the same expiration dates were limited to 0.3°C and 0.1°C respectively. Despite the statistically significant differences noted, intra-colloid dynamic viscosities varied less than 5%, while sample size was limited to three for statistical analysis between bags having the same expiration date.

Appreciable differences were noted in viscosities between samples quantified here and those previously reported [39]. HES 130/0.4 Voluven® and HES 260/0.45 presented with approximately 7% and 14.5% reductions in viscosity respectively despite measurements, with the exception of the second sample of HES 260/0.45 (expiry 07/12, 21.4°C), acquired at temperatures below 21°C [39]. Capillary measurements of Newtonian control solutions of deionized water and normal saline were within approximately 2% of accepted viscosity values, confirming the reliability of our HES measurements. Although expired, a sample of HES 260/0.45, quantified previously (expiry 10/10) was measured [39]. A viscosity of 7.58 ± 0.03 mPa · s was obtained that was within 0.5% of the viscosity reported [39]. Given this and the validity of the Newtonian control measurements, the findings presented here suggest that the molecular composition of starches between the batches tested here and those previously may have changed.

HES 130/0.4 Volulyte® presented with dynamic viscosities lower than HES 130/0.4 Voluven®. The composition of the electrolytes in HES 130/0.4 Volulyte® is isotonic with that of normal plasma, which increases the pH of the carrier solution compared to HES 130 Voluven® [12,13]. Whether the increased acidic content of HES 130/0.4 Voluven® is responsible for the increased viscosity is not known. However, if the two HES 130/0.4 fluids follow the pH/viscosity relationship for blood, the decreased viscosity associated with HES 130/0.4 Volulyte® would be expected [27].

4.2. Pipe flow

100% concentrations of HES displayed Newtonian behavior with marginal variation in viscosity across the range of shear strain rates. HES 130/0.4 Voluven® did not display the shear thickening properties measured previously at high shear strain rates [39]. Although discrepancies can be expected in measured viscosity between pipe flow and capillary methods, one would expect that if manual calibrations were solely responsible for the shear thickening previously observed, a consistent offset in viscosity would be present across all values measured. The highlighted viscosity differences within and between samples measured here and those previously suggest that the shear thickening observed may be related to isolated fluid properties between samples.

The use of a non-Newtonian blood analog allowed for the characterization of HES fluids in appropriate concentrations when mixed with a representative shear thinning fluid. Although the importance of HES

viscous behavior at 100% concentration should not be overlooked, these fluids are administered in whole blood. A cautious approach must be taken in interpreting results at the lowest shear strain rates. The calibration procedure was associated with an error of ±0.01 volts (V), equating to a pressure error of ±17.2 Pa. Although marginal at high pressure drops, an error of ±0.01 V does become appreciable for pressure measurements at shear strain rates of 40 s^{-1} (5%–7.25% dependent on the viscosity of the diluted HES fluid).

Concentrations of 25% HES 130/0.4 Voluven® and HES 130/0.4 Volulyte® presented with the largest drop in analog dynamic viscosities from 7.77 mPa · s to between 5.51 mPa · s and 6.05 mPa · s (approximately 22–29%) at ∼40 s^{-1} and from 5.54 mPa·s to between 4.38 mPa·s and 4.68 mPa·s (approximately 16–21%) at ∼925 s^{-1}. The spread between high and low viscosity at low and high shear strain rates was greater for 12.5% concentrations of HES 260/0.45 compared to 25% concentrations. This was expected as one would predict the concentration to tend towards the Newtonian viscosity of the HES fluid as the volume fraction of HES is increased [11]. A similar pattern was noted for 25% and 12.5% concentrations of HES 130/0.4 Voluven® and Volulyte®. Although the analog fluid mimicked the shear thinning behavior of whole blood, the combined mixture of aqueous xanthan gum and glycerol did not account for the potential interaction of these fluids with acute phase reactants that could alter viscosity *in vivo*.

4.3. Molecular behavior

At shear strain rates lower than measured here, Neff et al. [24] found that whole blood viscosity decreased in relation to HES 130/0.4 concentration at a shear strain rate of 0.1 s^{-1}. This suggests that HES 130/0.4 decreases erythrocyte aggregation as a result of their macromolecule size, whereas Neff et al. [24] noted that HES 200/0.5 increased whole blood viscosity at low shear strain rates (0.1 s^{-1}) in relation to concentration suggesting enhanced erythrocyte aggregation through molecular bridging. Given the large macromolecule size of HES 260/0.45, a similar viscosity increase at low shear strain rates and concentrations tested here would be expected. This does present an area of future study however, as to our knowledge, erythrocyte aggregation resulting from concentrations of HES 260/0.45 and HES 130/0.4 Volulyte® have yet to be reported.

4.4. Carrier solution

Past work has also focused on the importance of the carrier solution on resulting patient safety [1,41]. Specifically, Base et al. [1] examined the efficacy and safety of HES 130/0.4 in a 0.9% saline solution (Voluven®) to that of HES 130/0.4 in a balanced electrolyte solution (Volulyte®). Safety and efficacy between the two fluids were comparable although hyperchloremic acidosis was more prevalent in patients infused with HES 130/0.4 Voluven® [1]. Wilkes et al. [43] showed that the use of a balanced carrier dampened chloride levels and prevented hyperchloremic metabolic acidosis while improving gastric mucosal perfusion that reduced postoperative incidence of nausea and vomiting. Furthermore, Ondiveeran and Fox-Robichaud [25] noted that 6% pentastarch in a balanced solution may suppress hepatic inflammation through reduced leukocyte recruitment. Base et al. [1] concluded that the use of balanced solutions is likely unnecessary at moderate infusions, but could prove beneficial at higher doses to reduce the prospect of hyperchloremic acidosis.

4.5. Microcirculation considerations

The relationship between viscosity, wall shear and the maintenance of microcirculatory function has been discussed at length elsewhere [39]. Vascular compensation in response to decreased blood viscosity

through moderate levels of hemodilution (up to 50% hematocrit reduction) is achieved through elevating cardiac output that increases blood flow and maintains adequate tissue oxygen perfusion [29,36]. Despite the reduction in viscosity, elevated shear stress is maintained, permitting the continued production of vascular compensatory mechanisms [37]. Extreme hemodilution (>60% hematocrit reduction) passes a critical threshold where reduced cardiac output and flow decreases WSS that restricts compensatory mechanisms of the endothelium leading to microvascular constriction and reduced FCD [29,36].

Translation of findings to the microvasculature must incorporate the Fåhraeus–Lindqvist effect. Assuming the analog mimics red cell behavior, a cell free-layer would exist adjacent to the tube wall. In the vessels of the microvascular bed, the cell-free layer encompasses a greater portion of the lumen to that of the systemic arteries, reducing hematocrit and viscosity. The high shear strain rates of the peripheral vasculature and relative expansion of the cell-free layer suggests analog viscosity in tubing comparable to the arterioles would be reduced to that measured here at high shear strain rates [19]. Upon increasing hemodilution however, hematocrit in arterioles tends towards equilibrium to that of systemic vasculature leading to maintenance of viscosity in the peripheral network [19,30]. The use of a high viscosity expander would generate pressure redistribution that expands capillary diameter and maintains adequate blood flow [37]. For the starches evaluated here, this suggests that extreme hemodilution would be best served through the use of HES 260/0.45 that would preserve microcirculation function through maintenance of FCD and WSS [36,37]. Consideration must also be given to the reduced coagulation impairment and lowered tissue accumulation associated with tetrastarches (HES 130/0.4) in relation to pentastarches (e.g. HES 260/0.45) despite the perceived benefits of FCD maintenance through high viscous HES fluids [41].

5. Limitations

Although a representative non-Newtonian blood analog was used for HES concentrations, we were unable to incorporate the precise effect of acute phase reactants on the resulting concentration viscosities. Furthermore, we were unable to replicate the behavior of α-amylase on HES that immediately degrades starch molecules into smaller fragments upon infusion that restricts precise representation of *in vivo* metabolism of starch molecules [41].

Gear pump and micropump head restrictions prevented the collection of data at very low shear strain rates where erythrocyte aggregation is accelerated [14,24]. The importance of this was noted by Neff et al. [24] where whole blood viscosity decreased or increased in relation to the concentrations of HES 130/0.4 and HES 200/0.5 respectively, suggesting opposing actions on erythrocyte aggregation. This poses an interesting question moving forward. Xanthan gum was unable to replicate neither the specific cell–cell interactions nor the zeta potential of red blood cells that is linked to aggregation. Salt concentration, divalent cations and ionic strength have been shown to inversely affect zeta potential [16]. Given the difference in electrolyte composition between the HES 130/0.4 fluids, namely the addition of Mg^{++} and acetate ($CH_3CO_2^-$) to Volulyte® suggests dissimilar zeta potentials and aggregations responses. This supports the development of a miniaturized flow loop system where only very small amounts of blood would be required that would permit the tracking of circulating red cells and subsequent cell–cell interaction with HES molecules. Furthermore, Valant et al. [38] noted shear thinning of HES 130/0.4 Voluven® at shear strain rates below 10 s^{-1}. This warrants further investigation to determine if this represents consistent non-Newtonian behavior or an isolated response.

Ideally, pressure drop measurements should be made at 37°C to replicate internal body temperature. However, the spatial requirement for our closed flow loop necessitated data acquisition at room temperature. Despite this constraint, the analog gave a reasonable approximation of asymptotic whole blood viscosity in patients presenting with elevated hematocrit at 37°C [2,40]. Furthermore, the plot in Fig. 2 showing the decrease of analog fluid viscosity with increasing wall shear strain rate is similar to that of the decrease of the viscosity of whole human blood with increasing shear rate [6]. Although all aggregates of red cells likely have broken up at shear rates $> 100\ s^{-1}$, the viscosity continues to decrease due to red cell deformation, but at a progressively decreasing rate until the curve flattens out at shear strain rates $> 500\ s^{-1}$.

Peculiar results were noted at low flow rates for both concentrations of HES 260/0.45 where viscosity values increased in relation to the non-Newtonian analog. Given the lower viscosity of HES 260/0.45 compared to the analog at these flow rates, an increase in viscosity of the concentrations was not expected. As mentioned previously, precision error associated with the transducer could contribute to the results presented here. Despite this, however, the largest discrepancies between measured viscosities and Refutas estimated viscosities were restricted to $<10\%$.

6. Conclusions

In summary, this study presented the viscous quantification of the three HES fluids available for volume expansion in Canada (HES 130/0.4 Voluven®, HES 260/0.45 Pentaspann®, HES 130/0.4 Volulyte®) across a range of shear strain rates, at appropriate concentrations in a non-Newtonian blood analog. Initial capillary measurements presented dynamic viscosities approximately 7% and 14.5% lower than previously measured for HES 130/0.4 Voluven® and HES 260/0.45 respectively, suggesting a change in molecular composition between batches measured here and those measured previously. The decrease in viscosity would diminish the vascular and microvascular compensatory mechanisms associated with high viscous expanders including the maintenance of elevated wall shear stress and functional capillary density. Viscous characterization in a non-Newtonian analog showed that both HES 130/0.4 fluids decreased analog dynamic viscosity at both concentrations across all shear strain rates while 25% HES 260/0.45 increased viscosity. Although the analog fluid presented with rheological behavior that was not an exact replication of the non-Newtonian viscous behavior of blood, our findings do provide an appreciation of resulting viscosity changes in patients with elevated hematocrit and within vessels associated with low shear strain rates.

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