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RESEARCH ARTICLE

A human milk-mimicking fluid for PIV experiments

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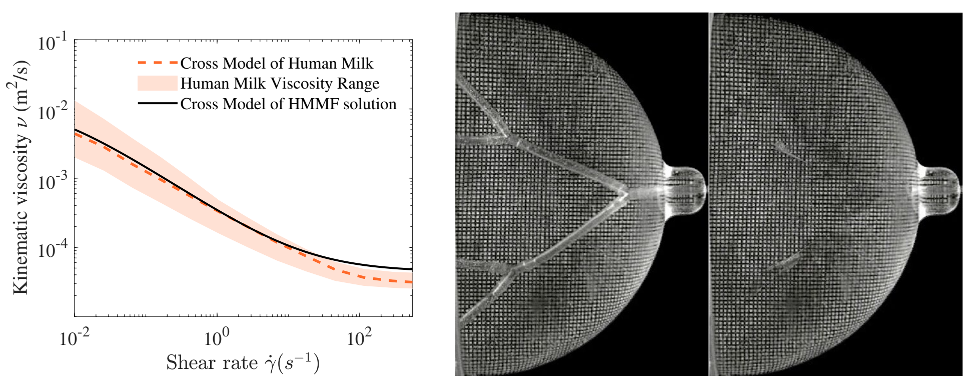
Abstract

It is essential to formulate a refractive index (RI)-matched fluid to use in silicone phantom models for particle image velocimetry (PIV) experiments. In this work, an extensive effort to develop a non-Newtonian human milk-mimicking fluid (HMMF) with different concentrations of sodium iodide (NaI), glycerol (Gly), xanthan gum (XG), and distilled water (DW) is conducted. Measurements of RI and density are fitted onto a linear empirical expression relating to the NaI and Gly concentration. The Cross model was utilized to find the nonlinear viscosity model of raw human milk. HMMF solution is generated from a multi-constrained optimization. The matched HMMF, suitable for use in the lactating human breast phantom with bifurcated ductal structures, is achieved with composition of 15.69% NaI, 30.27% Gly, 54.02% water and 0.02% XG by weight percentage resulting in a non-Newtonian viscosity matched with that of human milk.

Graphic abstract



(a) Cross model fit for non-linear human milk kinematic viscosity **(b)** Air in ducts **(c)** HMMF in ducts

1 Introduction

Over the past 2 decades, particle image velocimetry (PIV) systems have been developed to study the fluid dynamics of many types of flows (Schröder and Willert 2008). Optical clearness of the model and particle movement visualization in the fluids are important characteristics and requirements of a successful PIV experiment. However, for some applications, such as vascular models with complex geometry, these requirements cannot be easily satisfied (Yousif et al. 2011).

Refractive index (RI) matching is the key to solving distortion problems and allows more precise PIV measurements with high-speed cameras. Previous models used in PIV experiments on fluid dynamics have been built with transparent materials such as glass, acrylic, and polypropylene. Recently, PDMS and silicone are of particular interest in bio-fluid flow research due to their flexibility, excellent optical clarity, elasticity and tissue-mimicking abilities. In

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addition to RI matching fluids with solids in PIV experiments, matching the rheological properties of the non-Newtonian analog fluid in the PIV experiment requires further attention.

Studies in large vessels with shear rates above 500 s^{-1} use solutions of glycerin (Gly) with sodium iodide (NaI) in distilled water (DW) as a Newtonian blood-mimicking fluid (Najjari et al. 2016). When lower shear rates or complex geometries are considered, the addition of xanthan gum (XG) produces a shear-thinning fluid with viscoelastic properties (Sworn et al. 2011) and is considered the best choice for blood-mimicking fluids (Brookshier and Tarbell 1993). While the viscosity of an experimental fluid is adjusted by varying the Gly/DW ratio, the RI increases as the ratio increases (Yousif et al. 2011) and must be adjusted with NaI. The manipulation of these components to develop a RI matched, optically clear PIV fluid to imitate blood has been reported in multiple studies (Yousif et al. 2011; Brookshier and Tarbell 1993; Najjari et al. 2016). However, no human milk-mimicking fluid with refractive index, density and rheological match has been discussed. Furthermore, no empirical models of the viscosity curves were discussed to match the rheological properties of human milk.

Human milk is a complex non-Newtonian bio-fluid with similar rheological behavior as blood that must flow through a complex system of bifurcating ducts during breastfeeding (Alatalo and Hassanipour 2020). For in vitro flow experiments, a human milk-mimicking fluid (HMMF) must exhibit the same non-Newtonian characteristic of human milk while RI matching the phantom materials and maintaining optical clearness.

In this paper, a NaI–Gly–XG–DW solution is used to formulate a non-Newtonian HMMF for PIV experiments in a self-designed breast phantom (Jiang and Hassanipour 2020). The dynamic viscosity of HMMF is tested under room temperature and compared with human milk at body temperature. The effects of NaI, Gly and XG weight concentrations on the RI values, density and viscosity curve are investigated. A formula for HMMF at room temperature with a range of RIs matched to the silicone elastomers in the breast phantom and exhibiting a similar dynamic viscosity of the human milk is reproduced. Empirical models are used in the optimization tuning of RI, density and kinematic viscosity matching. This method can be applied to predict the best mixture of the elements in reproducing a non-Newtonian fluid for a target RI value in PIV experiments.

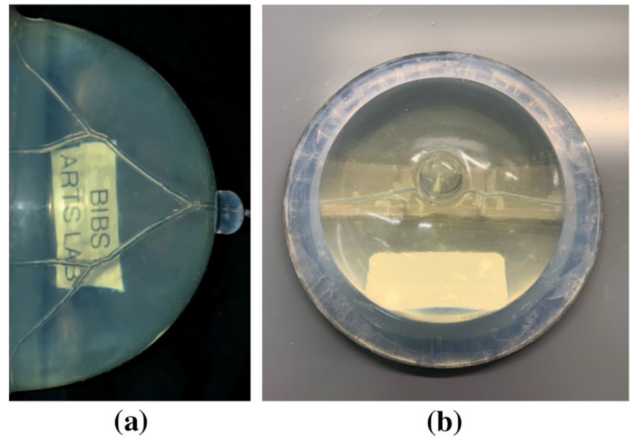

Fig. 1 The lactating human breast phantom **a** side view, and **b** top view

Table 1 Sample preparation for HMMF (w%w)

Item	Concentration range (%)	Step size (%)
NaI	5–20	5
Gly	10–50	10
XG	0.01–0.04	0.01

2 Methods

2.1 Viscosity, density, and RI measurements

Frozen human milk was donated by seven moms according to IRB 17-102 approved by The University of Texas at Dallas Internal Review Board. Human milk (HM) samples were thawed in warm water and tested at 37± 0.2 °C. HMMF samples were tested under room temperature at 22.5± 0.2 °C. Viscosity was tested on a rheometer (Anton Paar MCR 302) using a double-gap concentric cylinder geometry. After a 4 min pre-shear at 0.001 s^{-1}, a logarithmic shear ramp of 0.01–3000 s^{-1} was applied and data recorded 10 points/decade. A density meter with accuracy of ± 0.001 g/cm^3 (Anton Paar DMA 501) measured density of all samples at previously stated temperatures. The RI of PIV materials (solids and fluids) was measured with a refractometer at 532 nm wavelength (TORC 5000, Anton Paar) with an accuracy of ± 0.00002. RI and density of each HMMF sample were repeated five times with mean and standard deviation calculated. Kinematic viscosity curves were derived by dividing dynamic viscosity data by mean density of each sample for comparison.

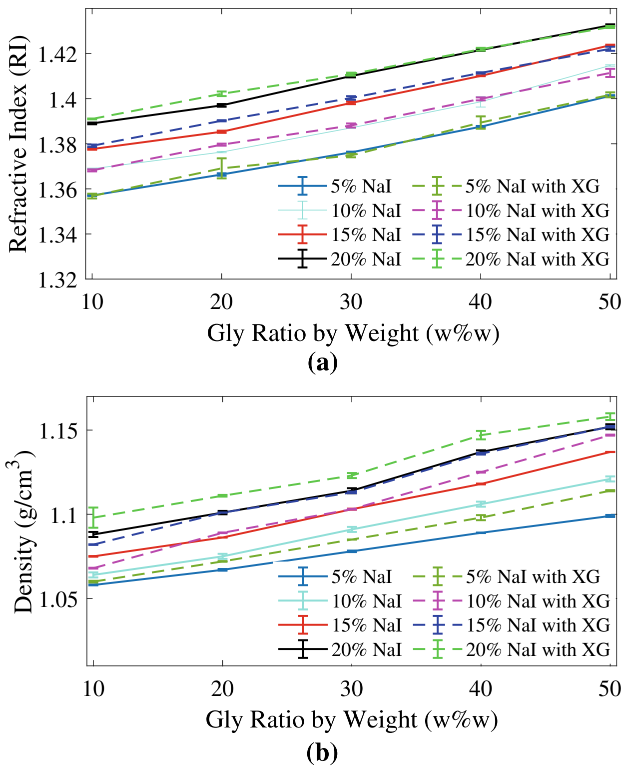

Fig. 2 Variations in **a** RI and **b** density for different formulations of HMMF, both with (dashed lines) and without (solid lines) XG

2.2 Lactating human breast phantom

In this work, an optically clear, flexible, and elastic breast phantom was designed to mimic a human breast in the setup (see Fig. 1) for studying the fluid dynamic of human milk in the milk ducts during breastfeeding. The breast phantom contains a breast shell (or skin), transparent filling water-based gel (Zerdine gel, CIRS, Norfolk, VA, USA), and the ductal structure as outlined in Jiang and Hassanipour 2020.

2.3 Materials for the development of HMMF

Based on the rheological profile of the raw human milk samples, Gly and DW provided a Newtonian solution to approximate viscosity as shear rate approaches infinity while the addition of XG enabled the HMMF to imitate the non-Newtonian property at relatively low shear rates ($< 10^2\ s^{-1}$). NaI enabled matching the target RI of the breast phantom. Materials were weighed by electronic balance with 0.0001 g resolution (Analytical Balances ML204T, Mettler Toledo, Switzerland) and mixed by a mechanical stirrer (Overhead Stirrer VWR230, VWR international, LLC) as percentages of weight to total fluid mixture weight (w%w). Each formulation required approximately 30 min for the solids to completely dissolve in the Gly before being added to DW.

An initial solution composed of 5% NaI, 10% Gly w%w and 0.01% XG was prepared toward a RI of 1.357 (the lower bound RI of the materials used in breast phantom). Four groups of different concentration levels of NaI were generated from 5 to 20% with step size of 5%. The Gly and XG ratios vary from 10 to 50%, and 0.01 to 0.04% with each RI concentration level, respectively. Table 1 presents the concentration range of the 100 samples and the step size used in the experiments.

3 Results and discussion

All RI are measured under 532 nm wavelength (specific for PIV laser wavelength). The breast ducts are made of Slygard-184 silicone PDMS material (Dow Corning Corp., Midland, MI, USA). Reported RI for PDMS mixed ratios of the base to the curing agent 10:1 is 1.414 $\pm$ 0.0008. The measured RI for the Zerdine Gel and the breast skin are 1.363 $\pm$ 0.011 and 1.396 $\pm$ 0.021, respectively. The breast phantom is assembled with breast skin, Zerdine gel, and the breast ducts. The RI of the assembled breast phantom

Table 2 Empirical expression of RI, density and dynamic viscosity in terms of NaI, Gly and XG concentrations

Terms		Fitted equation	R^2	RMSE
RI		$n = 1.336 + 2.180 \times 10^{-3}\,\text{NaI} + 1.107 \times 10^{-3}\,\text{Gly} + 5.201 \times 10^{-3}\,\text{XG}$	0.9779	0.691×10^{-3}
Density (g/cm^3)		$\rho = 1.024 + 2.714 \times 10^{-4}\,\text{NaI} + 1.714 \times 10^{-3}\,\text{Gly} + 2.167 \times 10^{-2}\,\text{XG}$	0.9712	5.180×10^{-3}
Viscosity (Pa·s)	η_0	$\eta_0 = 2.209\,\text{NaI}^{1.4} - 77.49\,\text{Gly} \times \text{XG} - 1.548 \times 10^3\,\text{XG} + 0.625$	0.8944	1.4270
	η_∞	$\eta_\infty = 9.406 \times 10^{-3} \times e^{0.07\times\text{NaI}} + 0.206\,\text{Gly} \times \text{XG} + 4.740 \times 10^{-3}\,\text{Gly} - 0.064$	0.9344	0.0756
	K	$K = 0.022\,\text{NaI}^{2.89} - 2.149\,\text{Gly} + 6.428\,\text{Gly}^2 \times \text{XG} - 7.11$	0.9218	0.4942
	r	$r = 0.534\,\text{NaI}^{0.18} - 0.0654\,\text{Gly} + 52.7\,\text{XG} + 1.342$	0.9415	0.1376

is 1.4012 $\pm$ 0.0007, which is set as the target RI matching system for HMMF. RI was found to vary linearly with NaI concentration and Gly concentration as shown in Fig. 2a. XG failed to significantly impact RI.

Similarly, densities of samples were measured to find the appropriate match for HMMF. Figure 2b shows the mean density from five times of the measurements of different NaI, Gly, XG and DW concentrations. Density was also found to be linearly related to the Gly and NaI concentrations for both Newtonian and non-Newtonian fluids. Additionally, adding XG in the NaI–Gly mixture increases the overall density of the fluid by 1.81 $\pm$ 0.32.

As we observed the linear correlations of both RI and density versus concentration ratio, simple predictions of the RI and density tuning on a multi-component fluid were made using the empirical Arago–Biot (AB) equation (Arago and Biot 1806), which is based on linear volumetric addition for each component. The AB equation is expressed as $n = \Sigma_i \phi_i n_i$, where n is the RI, ϕ is the volume fraction, and i is the indicate number of components. A linear 2D regression fit for RI and density in terms of NaI and Gly concentration of the HMMF was described by equations shown in Table 2, where the units for NaI and Gly are weight percentages to total fluid mixture weight (w\%w). Accurate fit was achieved in all linear empirical expressions as outlined by R^2 (R-squared) and RMSE (root mean square error) results. A similar regression equation for RI was presented by Yousif et al. (2011). To compare the two equations, data sets when XG = 0 were used in the 2-D equation for RI by Yousif et al. (2011), which predicted the RI within $\pm$ 0.02 difference. The primary differences between our equation for RI and Yousif et al. involve the use of XG to imitate the non-Newtonian behavior of the fluid and the % by weight of Gly. Our equation allows for the addition of XG to imitate non-Newtonian viscosity of human milk, and the % by weight of Gly is in relation to the total fluid weight, which is consistent with how NaI is measured. Whereas, in Yousif et al. 2-D RI equation, only 2 components are considered, NaI and Gly, and the Gly is % by weight in relation to % by weight of water only, while the NaI is % by weight in relation to the total fluid weight.

The kinematic viscosity ν is described by $\nu = \frac{\eta}{\rho}$, where η is the dynamic viscosity and ρ is density. The kinematic viscosity for human milk, HMMF samples varying XG concentration only, and HMMF samples with different concentrations of NaI and Gly were reported in Fig. 3. For XG ratios above 0.04%, the shear rate-viscosity curve was beyond the scope of human milk as shown in Fig. 3b. The uncertainties for kinematic viscosity results for all measured data points were estimated using Kline and McClintock Method (Kline and McClintock 1953). Results are presented in Table 4. Maximum percentage kinematic viscosity uncertainty is

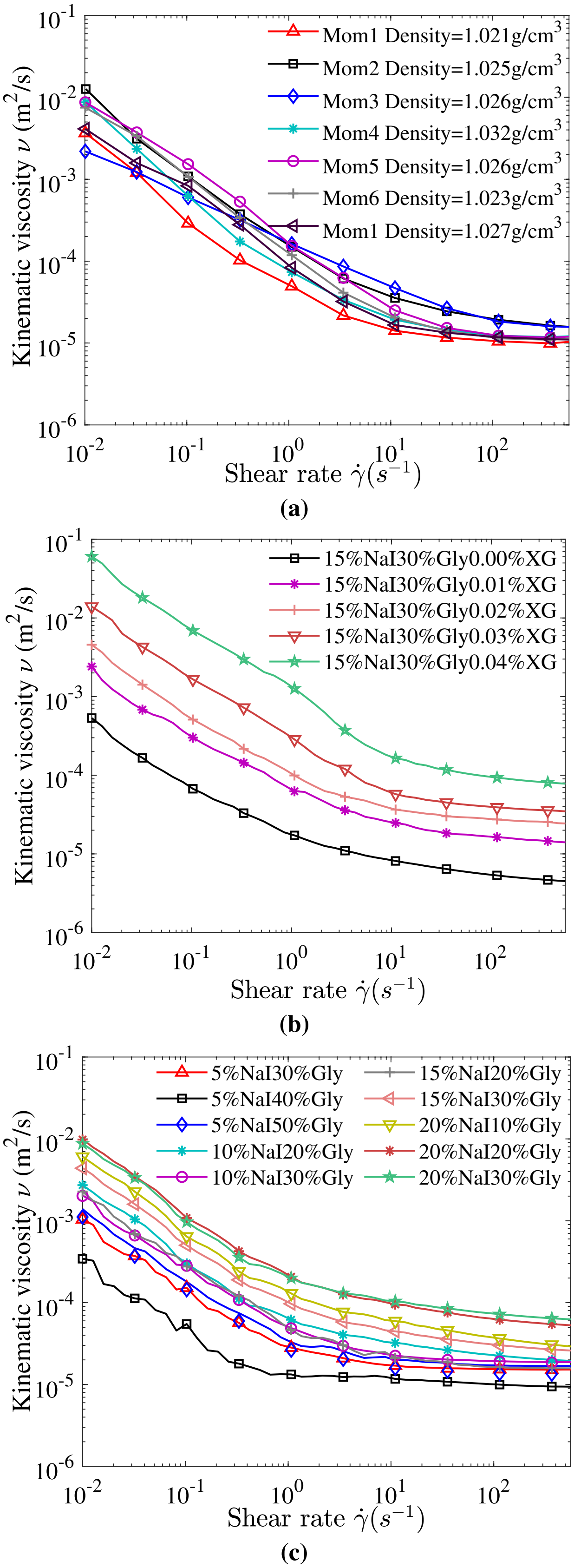

Fig. 3 Kinematic viscosity curves of **a** human milk samples, **b** HMMF in XG control group, and **c** HMMF in NaI and Gly control groups at 0.02% XG

Table 3 Fluid properties of HMMF and human milk (HM) matched by cross model

Fluid	Density (g/cm^3)	Cross model viscosity (Pa s)			
		η_0	η_∞	K	r
HM	1.0602	15.75	0.137	96.44	1.09
HMMF	1.1204	16.81	0.209	93.01	1.11

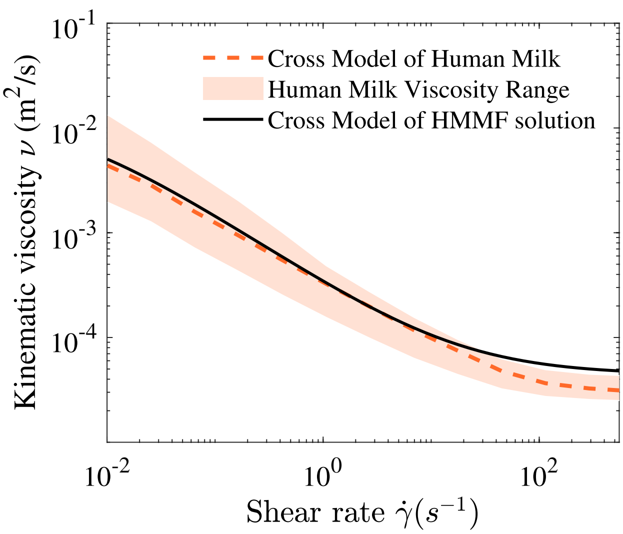

Fig. 4 HMMF viscosity matching of human milk

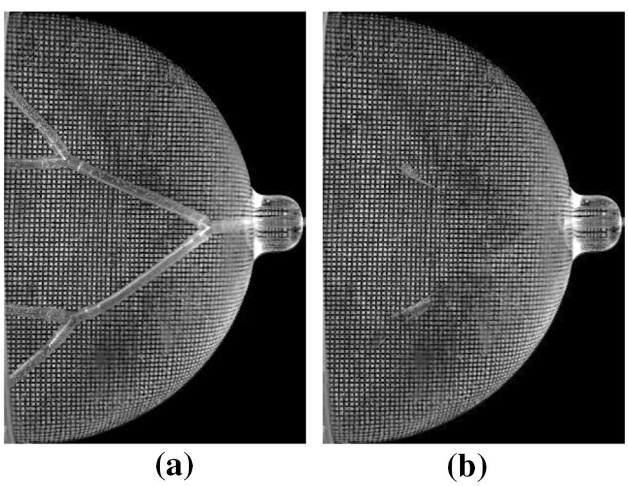

Fig. 5 Impact of HMMF in breast phantom. **a** Air in the duct structure illuminates ducts. **b** RI matched HMMF ($n = 1.4018$) in the Y-shaped ducts eliminates visibility of ducts in the breast model

± 0.275%. Non-Newtonian, shear-thinning flow behavior was clearly displayed by human milk and imitated in the HMMF (see Fig. 3). Both Gly and NaI increased kinematic viscosity with increasing concentrations. The impact of NaI

Table 4 The model uncertainty and the experimental measurement uncertainty of each model

Results	Components	Measurement uncertainty (%)	Model uncertainty (%)
RI	NaI	0.109	0.269
	Gly		0.292
	XG		0.403
ρ	NaI	0.214	0.179
	Gly		0.331
	XG		0.583
Viscosity	NaI	0.275	1.017
	Gly		1.123
	XG		2.079

on non-Newtonian flow behavior was originally reported to decrease viscosity, elasticity, and shear-thinning (Najjari et al. 2016). However, this finding was not supported by our results. A closer look at the methods reported by Najjari et al. shows that as the concentration of NaI increased, the concentration of both Gly and XG decreased. In this work, we maintained the concentration of Gly and XG so only DW concentration decreased as NaI increased. Thus, the impact of NaI on viscosity flow behavior depends on the concentration and interaction of other components, which is supported by Sworn et al. (2011).

A Cross model fit (Cross 1965) was used to find the nonlinear model of dynamic viscosity (η) with regards to shear rate ($\dot{\gamma}$) through nonlinear 2D regression approach. The Cross model is given by:

$$\eta(\dot{\gamma}) = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + K\dot{\gamma}^r}, \quad (1)$$

where η_0 and η_∞ refer to the viscosities as shear rate $\dot{\gamma}$ approaches zero and infinity, respectively. K and r are constants representing the degree and rate of shear thinning. Each parameter in the Cross model is a function of NaI, Gly, and XG concentrations as denoted in Table 2.

An optimized solution for the concentration ratio of NaI, Gly, XG and DW in HMMF was generated using the genetic algorithm (GA) optimization approach for nonlinear constraints (Homaifar et al. 1994). RI, density and viscosity curves were input as constraints in the optimization process.

Due to the complexity and nonlinearity of the cost function and constraints, the optimization procedure cannot reach a single optimal point for all equations. Optimal density was sacrificed to obtain an optimal RI and dynamic viscosity for HMMF. The optimal aqueous solution for HMMF used in this work was validated by the visual results of RI matching in the breast phantom (see Fig. 5). The resultant HMMF is composed of 15.69% NaI, 30.27% Gly, 0.02% XG and 54.02% DW (w%w) with a density of 1.120 ± 0.075 g/cm^3 and RI of 1.4018. Fluid properties of HMMF and human milk are presented and compared in Table 3. viscosity curves of two fluids are shown in Fig. 4. Based on the geometry of flow channels, the camera exposure time and camera frame size, the fluorescent red polyethylene microspheres tracer particle chosen for PIV experiments is 45 μm in diameter (CoSpheric. LLC, Santa Babara, CA, USA) and 1.12 g/cm^3 in density.

The HMMF is the first transparent liquid that mimics human milk density and the non-Newtonian viscosity properties while matching the RI in the complex silicone breast phantom. The HMMF is inexpensive, non-flammable, and not silicone solvable according to the Material Safety Data Sheet of the PDMS material. The one limitation to be noted is that the HMMF shows a yellowing over time, especially after exposure to air or light for several hours (Narrow et al. 2000). However, this can be resolved by requiring a rinse with DW after use in phantom. Since all the measurements are performed at room temperature, a detailed future study of NaI, Gly, and XG based solutions with regard to temperature fluctuations is required to fully characterize their dependence on temperature and their solubility under heating.

4 Model uncertainty

An estimate of the model error[1] can be performed using Bayesian Monte Carlo technique (Dilks et al. 1992). The model uncertainty was evaluated over the whole input space for the 100 prepared samples. In each iteration, only one parameter was given a variance value while others remained constant. We assume 10% fluctuation with a random distribution of 1000 points at each step of NaI, Gly and XG's percentage weight concentration. Averaged percentage uncertainty caused by the fluctuation in each component (NaI, Gly and XG) for RI, density and viscosity are presented in Table 4.

Model uncertainties of all fitted models are below 2.079% for a 10% fluctuation. The maximum uncertainty, as shown in Table 4, is observed in viscosity model. This model is predicted by a set of parameters that are estimated by three components concentration, where the related concentration fluctuations influence the chosen parameters in the Cross model and increases the uncertainty. Changes in XG concentrations were found more sensitive to each model with higher model uncertainties compared to NaI and Gly.

5 Conclusion

In this paper, a sodium iodide (NaI), glycerin (Gly), xanthan gum (XG), and distilled water (DW) solution is used to formulate a non-Newtonian human milk-mimicking fluid (HMMF) for use in a PIV fluid dynamic experiment with a Y-shaped, bifurcated ductal structure in a silicone lactating breast phantom. Characterization of the RI, density and kinematic viscosity as functions of the NaI, Gly, and XG concentrations enable the adjustment of the aqueous solution to match the RI of the phantom while maintaining an appropriate density and non-Newtonian viscosity profile of the target biological fluid, human milk.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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[1] For any parametric model, the relationship of its model outputs and experimental measurements can be expressed as:

$$y_E(x) = y_R(x, \theta) + \delta(x) + \epsilon, \quad (2)$$

where θ stands for empirical parameters and x stands for design variables. $y_E(x)$ is the experimental measurement, $y_R(x, \theta)$ is the results from the predicted model (fitted equation in this work), $\delta(x)$ is the model uncertainty, and ϵ is the measurement uncertainty (Arendt et al. 2012).

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