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## OBJECTIVE

Some publications have evaluated mineral oils (MO) and paraffin oils based on labeling as light or heavy, terms which have been used to describe either density or viscosity, and as MO or liquid paraffin (LP). Such studies tested mouse embryo development, pH, osmolality, temperature, viscosity, density, and peroxide value. MO and light mineral oil (LMO), defined in the United States Pharmacopeia, and LP and light liquid paraffin (LLP), defined in the European Pharmacopoeia have requirements for viscosity and density but the defined ranges don't align exactly. All four are defined as mixtures of hydrocarbons. This work characterizes the hydrocarbon molecule sizes in the samples to evaluate similarities between MO, LMO, LP, and LLP with their overlapping definitions.

## MATERIALS AND METHODS

The oils for assisted reproductive technology (ART) evaluated include FUJIFILM Irvine Scientific Oil for Embryo Culture and Heavy Oil for Embryo Culture, Vitrolife Ovoil and Ovoil Heavy, LifeGlobal Paraffin Oil P.G., LifeGuard, and LiteOil, and Medicult Liquid Paraffin. Due to limited volumes, not every sample was used for every test. Viscosity was determined at room temperature, at 2–8°C, and at 37°C with an Ubbelohde viscometer. Density was determined at room temperature using a graduated cylinder and a laboratory balance. MEA was performed in a humidified CO box incubator using 1-cell B6C3F1 x B6D2F1 mouse embryos cultured for 96 hours. Carbon numbers were determined based on the ASTM D2887 Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography.

## RESULTS

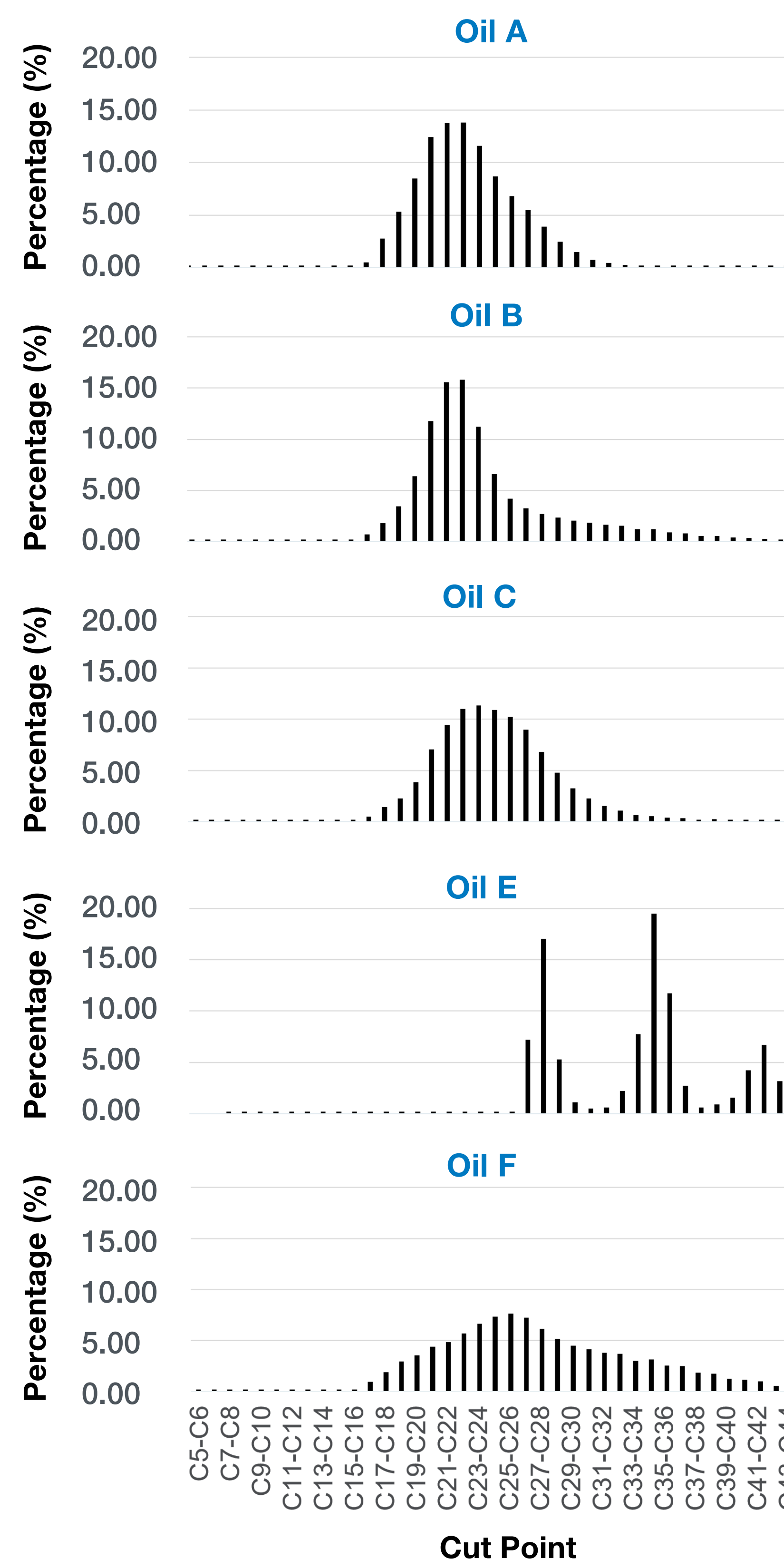
Viscosity centistoke (cSt) ranges were 55–622 (cold), 25–163 (room temperature), and 16–89 (37°C). Density ranged from 0.82 to 0.87 g/mL. All samples passed MEA. One of the more viscous oils had a wider spread of carbon numbers with percentages over 1%, with a peak of 8% at C25–C26, and skewed towards higher carbon numbers. A second oil tested was multimodal, with peaks of 17% at C27–C28, 19% at C34–C35, and 7% at C41–C42. Three lower viscosity oils had more similar carbon number profiles, with nearly normal distributions of carbon numbers for those with percentages over 1%, with peaks at 11–15% around carbon numbers C22–C24.

## CONCLUSION

Correlation between blastocyst rate and density or viscosity was weak, but there was a correlation between increased blastocyst rate and larger molecular size. There was no correlation between molecular size and labeling as either MO/LMO or LP/LLP, but labeling as light or heavy did correlate with smaller and larger sizes, respectively. Labeling as MO, LMO, LP, or LLP may not sufficiently convey the oil properties, such as density, viscosity, and potentially molecular size. Future studies could examine the makeup as paraffinic, naphthenic, or aromatic in ART oils, to further evaluate whether differences in molecular structures, performance or outcomes can be determined between products labeled as either mineral or paraffin oils.

## IMPACT STATEMENT

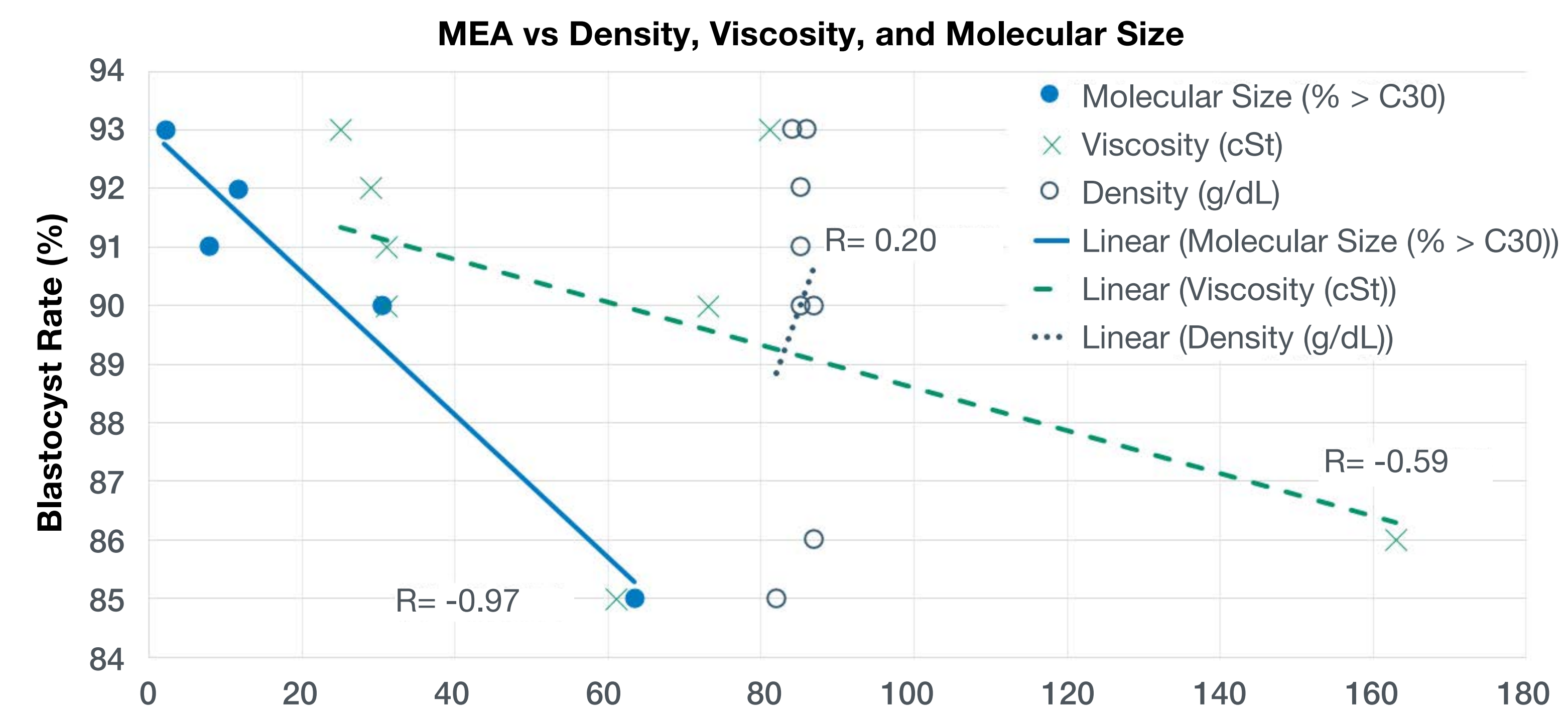
Further work remains to be done to continue characterizing oils for ART and to correlate differences in chemical properties to changes in performance.



**Figure 1. Molecular Size.** The percentage of molecules at a given size (carbon number) are shown for 5 oils. Most oils have a single peak of varying height and width in the middle of the range of 5 to 44 carbons in size, with little contribution from smaller molecules, and little to moderate contribution from larger molecules. One oil had three peaks, all at the top half of the range measured.

	Molecular Size	Density (g/dL)	Viscosity			Labeling Weight	Labeling: Mineral/Paraffin	% Blastocysts (StdDev)
			2-8 °C	20-25 °C	35-39 °C			
Oil A	2.04	84	55	25	16	light	mineral	93 (4.19)
Oil B	11.64	85	80	29	19	light	paraffin	92 (1.70)
Oil C	7.76	85	81	31	20	light	paraffin	91 (6.94)
Oil D	NT	85	86	31	20	light	paraffin	90 (2.83)
Oil E	63.39	82	166	61	38	heavy	NA	85 (1.41)
Oil F	30.42	87	219	73	38	heavy	mineral	90 (5.44)
Oil G	NT	86	253	81	46	medium	mineral	93 (1.89)
Oil H	NT	87	622	163	89	heavy	mineral	86 (4.64)

**Table 1. Comparison of molecular size, density, viscosity, and labeling.** The percentage of molecules greater than C30 are shown for five oils. Density varies between these oils from 0.84–0.87 g/mL. Viscosity is temperature dependent with higher viscosity at lower temperatures. Labeling as mineral or paraffin did not relate to molecular size, density, or viscosity. Labeling as light or heavy more closely reflected relative viscosities or molecular sizes, but not strictly density. Differences in molecular size, density, and viscosity were found among oils labeled as either light or heavy. Mouse embryo assay blastocyst rates were all comparable and >80% (85–93%). With standard deviation, StdDev, (1.70–6.94), there were no notable differences in blastocyst rates. NT = not tested, NA = not applicable.



**Figure 2. MEA Correlations.** Correlations between MEA vs density, viscosity, and molecular size were evaluated. MEA results were correlated very weakly with density ( $R = 0.20$ ) and moderately with viscosity ( $R = -0.59$ ), but there was a very strong correlation between MEA and molecular size ( $R = -0.97$ ).