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# Biomembrane Modelling in Planar Chromatographic Determination of Lipophilicity Using Olive and Castor Oils

**M. A. ADEYEMO [A, C, D], O. ADEYEYE[B], O. A. OKENIYI [B], S. O. IDOWU*[E, F]**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

## Abstract

**Background**

Lipophilicity is a crucial physicochemical parameter that predicts *in vivo* pharmacokinetics and should be reliably estimated in early stage drug discovery to reduce incidence of attrition. Previous methodologies for its measurement often lead to technically incorrect decisions due to simplistic architecture and poor biomimetic attributes. Significantly, a certain seed oil, used for biomembrane modelling on planar chromatographic platform, was reported to be sufficiently biomimetic and fit for purpose.

**Objectives**

To evaluate olive oil (OL) and olive-castor oil (OL-C) equi-mixture as lipids for biomembrane simulation on planar chromatographic platform.

**Material and Method**

Retention behavior of nabumetone, a model compound was used to optimize these potential lipid membranes using a thin film engineered from 5% Liquid paraffin (LP) as benchmark, while halofantrine, nabumetone , α-naphthol and β-naphthol representing varying molecular polarities, were used for validation studies. The validation involved 2-way analysis of variance (ANOVA) associated with variability in Basic lipophilicity parameter (Rmw), and Specific hydrophobic surface area (SHSA) for the optimized surfaces, relative to LP and octadecylsilane (ODS) Further validation entailed correlation of the lipophilicity descriptor i.e. isocratic chromatographic hydrophobicity index (ICHI) on OL, OL-C, ODS and LP with experimental Log $P_{(octanol/water)}$.

**Results**

Optimized film thicknesses were produced by 5% OL and 1.25% OL-C ($p > 0.05$). The 2-way ANOVA revealed great variability in performance characteristics of the surfaces ($p < 0.0001$), and the new surfaces also gave poorer correlation with Log P values ($R^2 = 0.502$ and 0.449 respectively).

**Conclusion**

The 1.25 % OL-C demonstrated a higher biomimetic attribute and warrants further validation studies to ascertain biorelevance, of lipophilicity measurement on this platform, in predicting oral drug absorption.

**Keywords:** Lipophilicity, Reversed-phase Thin Layer Chromatography, Retention behaviour, Olive oil, Castor oil

## INTRODUCTION

Lipophilicity is a critical physicochemical parameter that predicts *in vivo* pharmacokinetic and biopharmaceutical properties such as safety and efficacy (Arnott and Planey, 2012; Arnott, Kumar and Planey, 2013). Reports have thus shown that early optimization of this property especially for the lead molecules would help reduce attrition and failure during the clinical phase of drug discovery (Testa *et al*, 2000; Basavaraj and Betageri, 2014; Wang, Dong and Sheng, 2019). The time - honored approach and standard for estimating lipophilicity of new chemical entities during lead optimization stage of drug discovery process is the logarithm of octanol-water partition coefficient known as log P (Leo *et al*, 1971; Moreno *et al*., 2011). This model also forms an essential component of Lipinski's rule of five for determining drug likeliness (Lipinski, 2016). Unfortunately, despite its recorded successes, it has been criticized to be so simplistic in chemistry compared to the complex architecture of the biological membrane which is composed of cholesterols, integral and peripheral proteins, carbohydrates, glycoproteins and phospholipids. This structural complexity and amphiphilic chemistry of the cell membrane makes log P measurement insufficiently biomimetic and hence, incapable of accurately predicting in vivo lipophilicity (Giaginis and Tsantili-Kakoulidou, 2008); and has resulted in countless incidence of attrition reported by most pharmaceutical companies. In fact, its use alone in prediction of *in vivo* drug absorption has been reported to be an oversimplification of a biological complex process leading to inaccuracy in estimation; due to lack of real physiological conditions regulating in vivo membrane permeability (Balimane and Chong, 2008; Hermens, De Bruijn and Brooke, 2013).

Several alternative methods such as reversed phase planar chromatography (RPTLC), reversed phase high performance chromatography (RP-HPLC), have been reported for lipophilicity determination of drugs (Ilijas *et al*., 2013; Hawryl *et al*. 2015; Ciura *et al*., 2019) but come with flaws of inaccurate measurements due to poor structural similarity of their stationary phase compared to the bio-membrane amphiphilicity. These have made bio-membrane modeling challenging and complex; necessitating use of stationary phase with more complex surface chemistry in reliable simulation of in vivo bio-partitioning process.

Newer chromatographic methods in attempt to solve this problem include immobilized artificial membrane chromatography (IAM) which involves tagging of chromatographic silica stationary support with phospholipids such as phosphatidylcholine, sphingomyelin etc. (Verzele *et al*., 2012; Valko, 2019); immobilized liposome chromatography (ILC) etc. (Dabrowska *et al*., 2011, Tang, Pu and Li, 2017). Thus, use of models with similar amphiphilic chemistry as the bio-membrane furnishes a better membrane simulation that helps to overcome notable flaw of inaccurate prediction and high attrition. However, this amphiphilic simulation has not been replicated on planar chromatographic platform until Idowu *et al*., (2009) reported the use of seed oil from *Leucaena leucocephala* as fit for purpose, biomimetic model for lipophilicity profiling of small molecule drugs. Olive oil was presumed to have amphiphilic properties based on its rich chemical constituents like triacylglycerol, flavonoids, phytosterols, polyphenols, terpenes etc. (Lopez *et al*., 2014); with reported fatty acid composition of palmitic acid (7.5 – 20%), palmitoleic acid (0.3 – 3.5%), stearic acid (0.5 – 5%), oleic acid (55 – 83%), linoleic acid (3.5 – 21%), linolenic acid (< 1%), arachidic acid (< 0.6%) and gadoleic acid (< 0.4%) (Gharby *et al*., 2018); while castor oil was reported to be composed largely of a polar constituent called ricinoleic acid (90%), linoleic acid (4.2%), oleic acid (3%), palmitic acid (1%), stearic acid (1%), dihydroxystearic acid (0.7%), eicosanoid acid (0.3%) and linolenic acid (0.3%) (Salimon *et al.*, 2010).

This study was thus conducted to investigate the potentials of these plant seed oils in simulation of biomembrane for lipophilicity profiling.

## METHODOLOGY

### Material and Method

#### Materials

Methanol (Merck), Liquid paraffin, Castor Oil (Technical grade; Bell, UK,with acid value 0.61), Olive Oil (Technical grade; Goya, Spain, with acid value of 0.95), n-hexane, distilled water, conical flasks, filter paper, pipette, measuring cylinder, volumetric flask, TLC tanks, precoated aluminum TLC plates GF254, Octadecylsilane (ODS) plates, Model compounds: α-naphthol (analytical grade; BDH, UK),β-naphthol (analytical grade; BDH, UK), nabumetone (chemical reference substance; Sigma, USA), halofantrine (Secondary reference; isolated and recrystallized with methanol from tablets of Glaxo SmithKline, Lagos; authenticated by Thin layer chromatography).

#### *Equipment*

Ultraviolet lamp (254/365nm, Gallenkamp, U.K.), Drying oven (Astell Hearson,U.K.), Water bath (Gallenkamp, UK), Vacuum pump (Oerlikon Leybold,

Germany), Analytical weighing balance (Mettler Toledo, China).

***Engineering and Optimization of the OL and OL-C lipid film thickness***

Varying concentrations of the olive oil and olive/castor oil (equi-mixtures) films i.e. 1.25, 2.5, 3.75 and 5 % of the oils in n-hexane were prepared and impregnated on 5 x 10 cm silica coated thin layer chromatographic plates. The film thickness for the new bio-membrane models was optimized by comparison of the retention behavior of nabumetone i.e. the model compound on these lipid surfaces with respect to 5 %w/v liquid paraffin as benchmark; and evaluation of the performance characteristics i.e. specific hydrophobic surface area (SHSA) and Basic lipophilicity parameter (Rmw) obtained from the linear regression plot of the retention behaviour using 1-way ANOVA.

***Validation of the engineered OL and OL-C films***

The inherent complexity in architecture of these optimized new lipid surfaces and their performance attributes are evaluated against prior arts i.e. liquid paraffin and octadecylsilane (ODS) using the retention behaviors of additional model compounds with variable polarities namely α-naphthol, β-naphthol and halofantrine (Fig. 1). The 2-way ANOVA was used to delineate the variability in the performance of all these bio-membrane models. Finally, correlational analysis of the lipophilicity descriptor i.e. ICHI, of the model compounds obtained on all these lipid surfaces with experimental log P values obtained from literature was conducted.

**RESULTS AND DISCUSSION**

The considerations for the choice of nabumetone as model compound in the design optimization of the new lipid surface includes the presence of sufficient lipophilic core (i.e. naphthalene ring) and moderate hydrophilic moieties capable of hydrogen bond acceptance, comprising of ether and ketone functional groups. This structural attribute eliminates extensive specific interaction and promotes hydrophobic interaction as the dominant factor in the surface's partition dynamics (Idowu *et al*., 2009).

The retention behavior of the model compound, nabumetone on the lipid surfaces OL and OL-C created with varying film thicknesses are shown in figure 2. The optimization of the film thickness by statistical evaluation of the chromatographic parameters i.e. SHSA and Rmw showed that for the OL platform, 2.5, 3.75 and 5 % concentrations gave comparable SHSA value

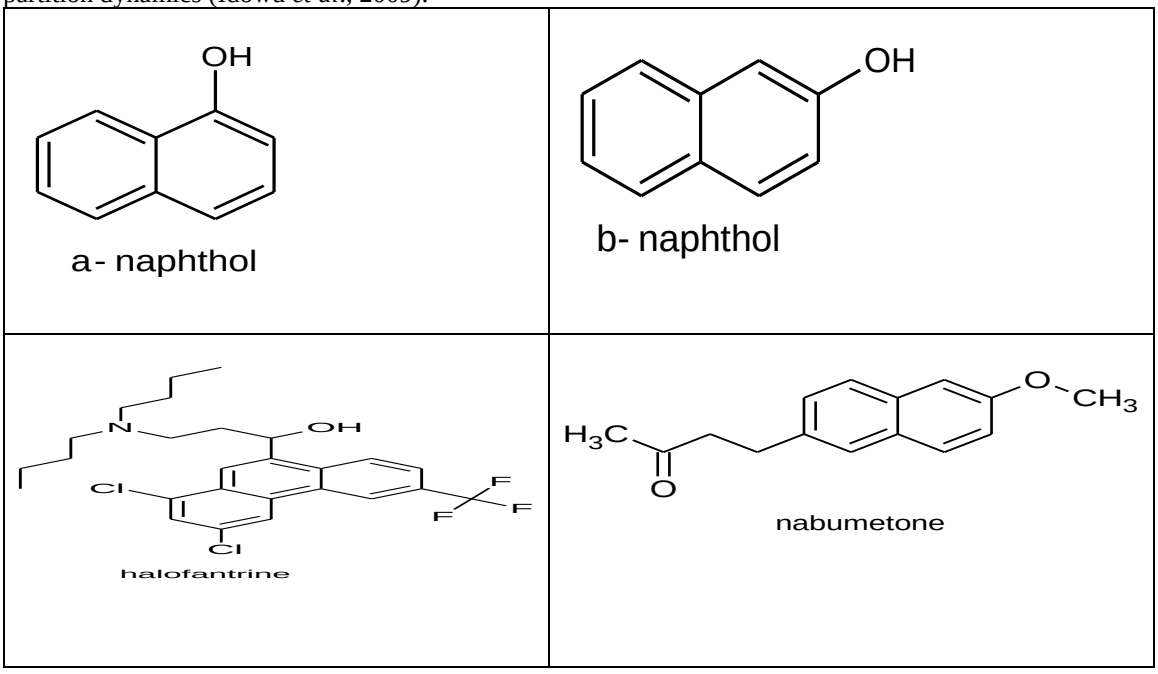


**Fig. 1: Chemical structure of model compounds**

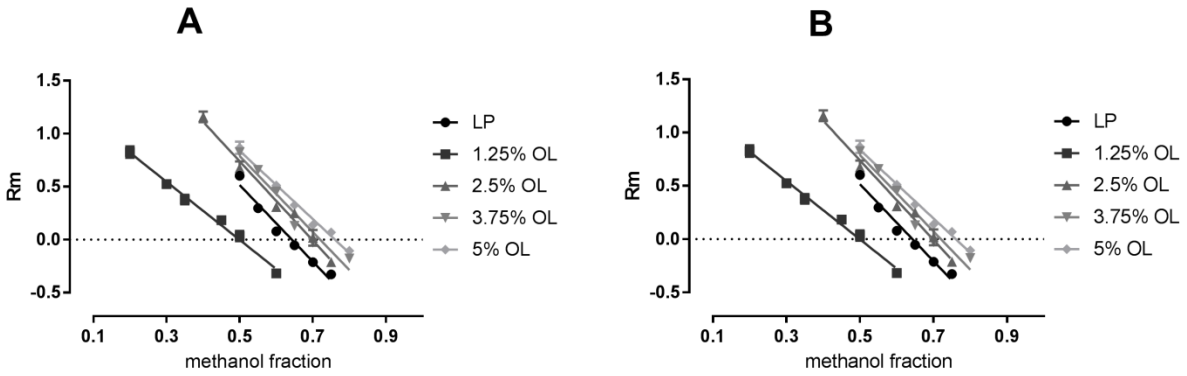


**Fig 2: Linear regression of Rm against methanol fraction showing the retention behaviour of nabumetone on (A) varying OL film thicknesses and (B) varying OL-C film thicknesses relative to LP benchmark**

as the benchmark while only 5 % concentration gave comparable Rmw value as benchmark (Fig. 3). Thus, since these two parameters are critical and used in overall description of the compound's lipophilicity index (Bieganowska *et al*., 1995; Liang and Lian, 2015), 5 % oil concentration is taken as the optimum film thickness for OL platform. However, for the OL-C platform, 1.25 % and 5 % oil concentration gave comparable SHSA value as LP benchmark while only 1.25 % concentration gave Rmw value equivalent to that obtained from the benchmark (Fig. 4), implying that the optimal concentration for the OL-C platform is 1.25 %. The retention behaviors of the compounds used for validation of these optimized lipid system 5 % OL and 1.25 % OL-C respectively against prior arts ODS and LP are documented in Fig 5.

The variability in the architectural complexity of these different biomembrane models is reflected by the different clustering of the data for individual model compounds (Fig. 6 and 7). The 2-way ANOVA shows that there is significant difference ($p < 0.0001$) in the performance attributes (i.e. Rmw and SHSA) captured by all the biomembrane models on account of the layer type (i.e. architectural complexity) and solute type (i.e. chemical diversity); which underscores the impact of the surface hydrophobicity in influencing the partition dynamics of these compounds (Idowu *et al*., 2009). The OL-C especially showed a wider scatter compared to the other biomembrane models with respect to the more polar compounds indicating the impact of its polar feature and capacity for facilitating better electrostatic interaction than other lipid surfaces. This is further corroborated by the pattern of hydrophobic interaction on all the surfaces; ODS > OL > LP > OL-C (Fig. 8). The goodness of fit for the correlation of the lipophilicity descriptor on these bio-membrane models with experimental log P values follows the sequence; LP > ODS > OL > OL-C with $R^2$ values 0.517, 0.510, 0.502 and 0.449 respectively (Table 1). This suggests that the partition dynamics at the LP-water, ODS-water and OL-water interfaces are closer to that of reference octanol-water than OL-C water interface. This poorer correlation depicted by OL-C further signifies a more complex architecture of this film surface compared to the other three models and underscores a greater amphiphilicity of its surface chemistry. Amphiphilicity of surface chemistry would afford a better simulation of the physiological unstirred water layer which exists between luminal contents and gut wall and contributes to oral drug absorption mechanics (Gun'ko *et al*., 2005).

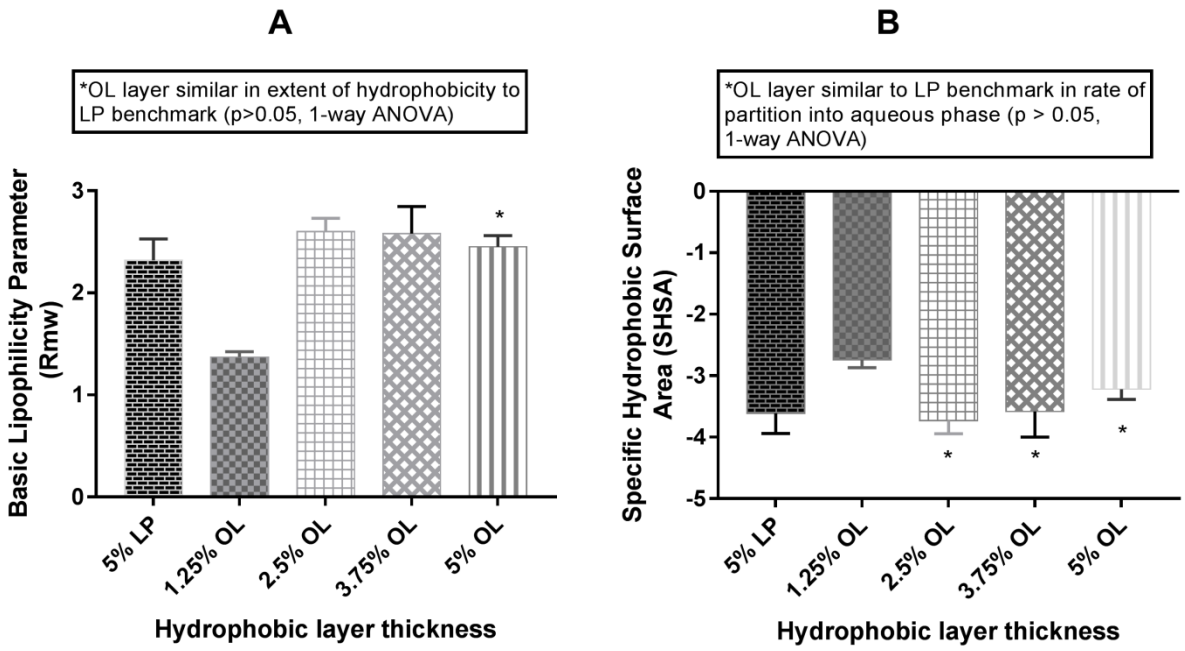


**Fig. 3: Optimization of OL film thickness relative to liquid paraffin film on (A) using extent of hydrophobicity (Rmw) and (B) using rate of partition into aqueous phase (SHSA) as benchmark parameter. 5 % OL was chosen as optimal film thickness being similar to the benchmark with respect to both parameters.**

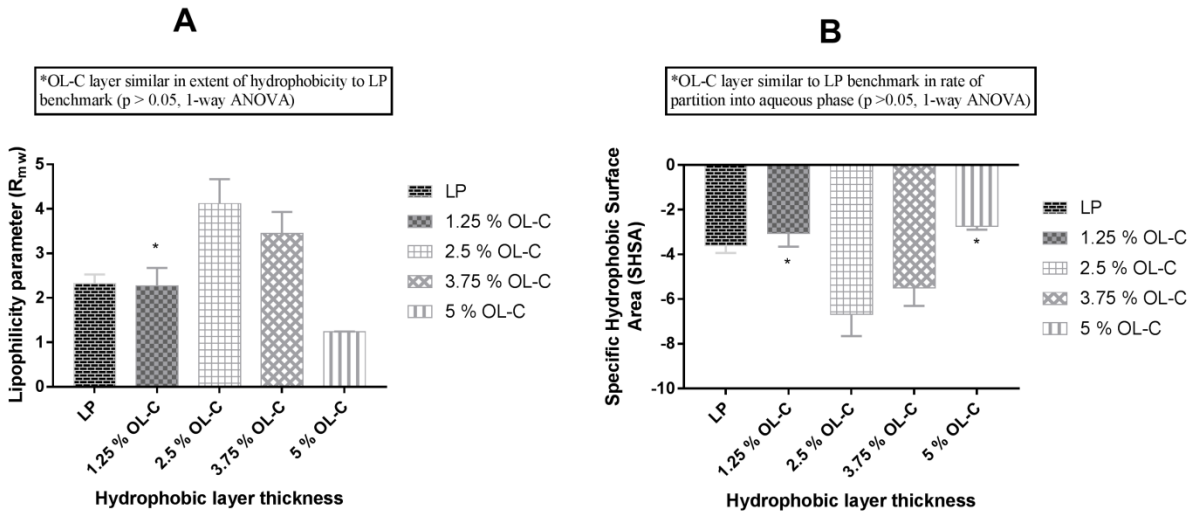


**Fig. 4: Optimization of OL-C film thickness relative to liquid paraffin film on (A) using extent of hydrophobicity (Rmw) and (B) using rate of partition into aqueous phase (SHSA) as benchmark parameter. 1.25 % OL-C was chosen as optimal film thickness being similar to the benchmark with respect to both parameters.**

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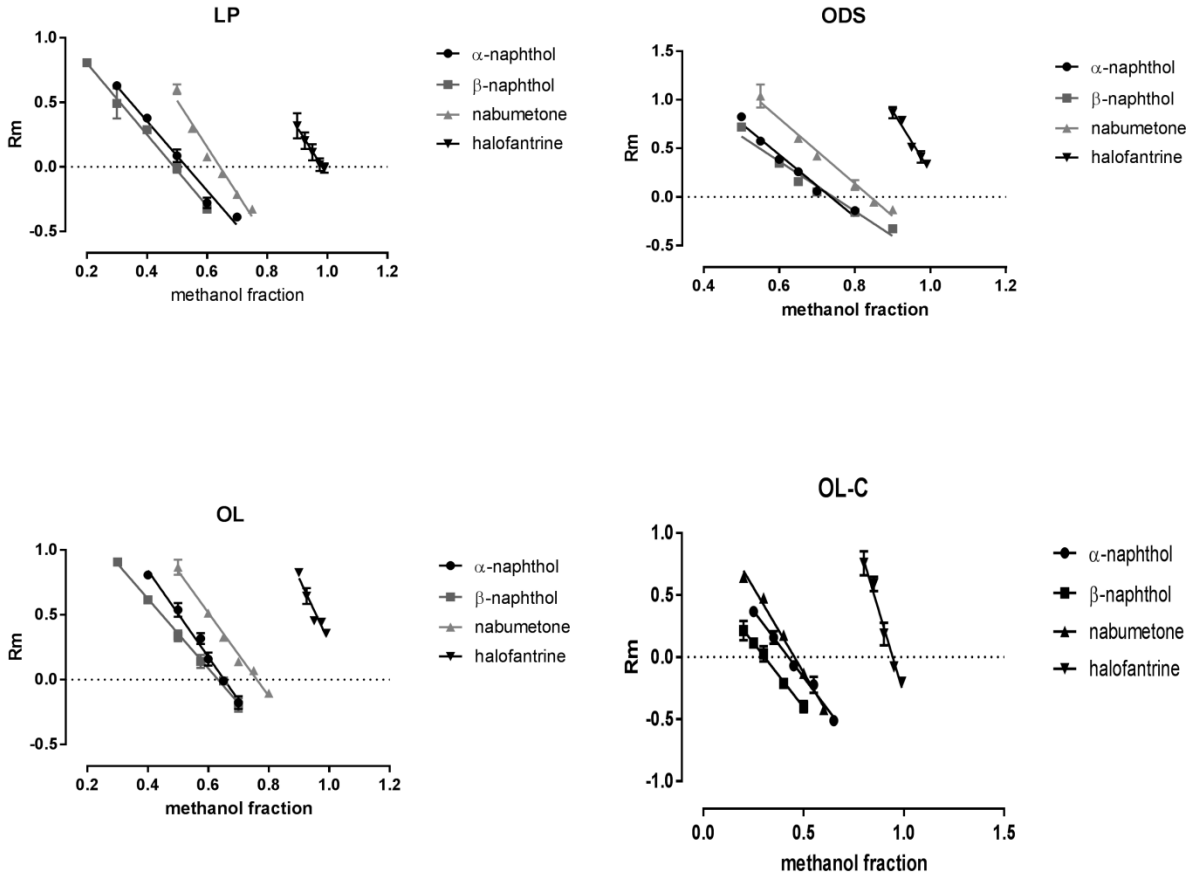


**Fig. 5: Linear regression of Rm against methanol organic modifier for the validation of OL and OL-C against LP and ODS using 4 model compounds**

| Source of Variation | % of total variation | P value |
|---|---|---|
| **Interaction** | 6.16 | <0.0001 |
| **Layer type** | 11.7 | <0.0001 |
| **Solute type** | 79.8 | <0.0001 |

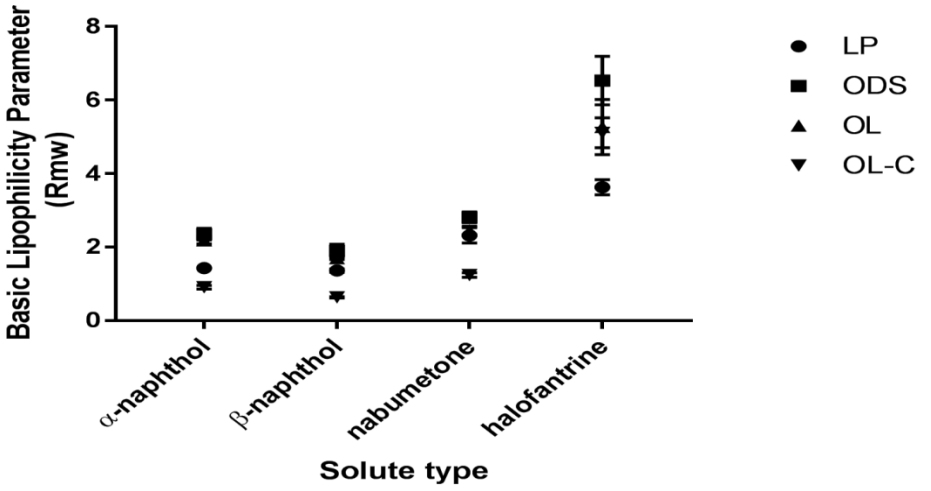


**Fig. 6: Graph showing the pattern of variation in the Rmw values for the model compounds on account of the Solute type and Layer type.**

| Source of Variation | % of total variation | P value |
|---|---|---|
| **Interaction** | 16.2 | <0.0001 |
| **Layer type** | 6.38 | <0.0001 |
| **Solute type** | 71.2 | <0.0001 |

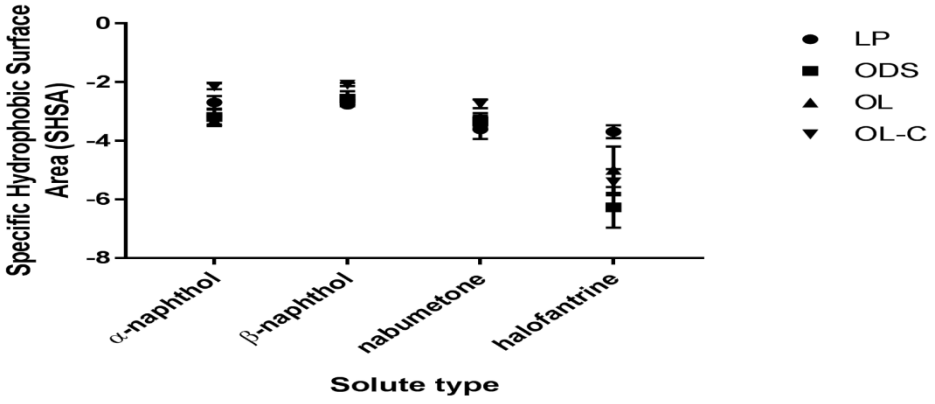


**Fig. 7: Graph showing the pattern of variation in the SHSA values for the model compounds on account of the Solute type and Layer type.**

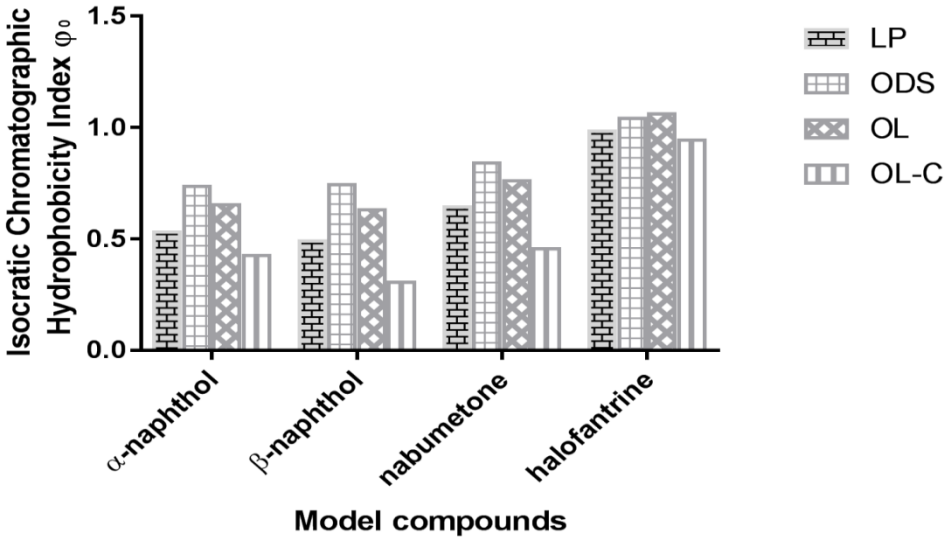


**Fig. 8: Lipophilicity profiling of the model compounds showing the effect of solute type and layer type on overall variation in isocratic chromatography hydrophobicity index ($\varphi_o$)**

**Table 1: Goodness-of-correlation of derived hydrophobicity descriptor (ICHI) with experimental Log P for the different biomembrane models**

| | | | | | Coefficient of Determination ($R^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Experimental Data/Model Compounds | | | | | | | |
| | αNP | BNP | NBT | HF | LP | ODS | OL | OL-C |
| LogP | 2.98 | 2.70 | 3.27 | 3.25 | 0.517 | 0.510 | 0.502 | 0.449 |
| LPφ | 0.532 | 0.490 | 0.643 | 0.983 | - | - | - | - |
| ODSφ | 0.736 | 0.743 | 0.841 | 1.040 | - | - | - | - |
| OLφ | 0.653 | 0.632 | 0.761 | 1.060 | - | - | - | - |
| OL-Cφ | 0.424 | 0.305 | 0.454 | 0.944 | - | - | - | - |

This study is intended to be a proof-of-concept, which confirms the hypothesis that the mechanism of partition dynamics across the lipid water interface is quite different from what happens at liquid paraffin - water interface. Sequel to this a bigger study including a larger library of structurally diverse small molecules will be undertaken to assess the potential of this new biomembrane model for general utility.

## CONCLUSION

Two potentially useful artificial membranes for lipophilicity profiling of small molecules were engineered from optimized 5 % olive oil (OL) and 1.25 % equi-mixture of olive and castor oil (OL-C) using 5 % liquid paraffin (LP) as benchmark. The statistical evaluation of their retention performance and validation analyses show that the 1.25 % OL-C has better biomimetic attributes, which warrants further studies to ascertain the biorelevance of lipophilicity measures on this new platform.

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*Address for correspondence: Sunday Olakunle Idowu

Department of Pharmaceutical Chemistry,
Faculty of Pharmacy,
University of Ibadan,
Nigeria
Telephone: +234 805 842 7072
E-mails: olakunleid@yahoo.com; so.idowu@ui.edu.ng

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