Quantification of the Adsorption of Benzoates on Poly(dimethylsiloxane) Membrane

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Abstract

We present the first simple, yet successful, method to quantify compound adsorption onto a polymer based, skin mimic silicone membrane. Benzoate compounds were selected as adsorbants based on their known controversial safety within the healthcare market. We found that adsorption depends strongly upon the adsorbates chemical structure, more so than silicone membrane thickness. Quantification of adsorption was evaluated through reduction in solution concentration as the molecules adsorbed onto the poly(dimethylsiloxane) membrane. A direct correlation was observed between alkyl chain length and the number of molecules adsorbed per gram of membrane; as the adsorbate alkyl chain length increased, so does adsorption. This finding implies that the hydrophobicity of the adsorbate is directly governing the extent of adsorption. Calculation of the change in Gibbs free energy associated with the adsorption process (ΔG_{abs}) further confirmed a direct correlation between extent of adsorption and chain length. Our data highlights the importance of understanding, and more importantly quantifying, the adsorbate-membrane interaction if such systems are to be used to replace *in vivo* analysis.

Keywords: adsorption; aminobenzoates; hydroxybenzoates; PDMS; poly(dimethylsiloxane)

Declarations of Interest: None.

Introduction

Polydimethylsiloxane (PDMS) membrane (Figure 1) is frequently used in a variety of healthcare and pharmaceutical applications, including skin mimics for pharmaceutical and cosmetic testing [1, 2], more commonly known as topical and transdermal studies. This particular membrane is ideally suited to such an application as it is a reliable yet cheap model to determine a quantifiable measurement of absorption of compounds, i.e. it is often used to replace animal testing. In recent years, the variety of membranes suitable for transdermal studies to replace human skin and animal skin has widened, although PDMS remains the most commonly employed option based upon its suitability to provide reproducible data[3], along with recent modifications [4] widening its applications. PDMS-based membranes display many desirable properties including high free volume (leading to high solubility), facilitating calculation of gaseous diffusion coefficients [5].

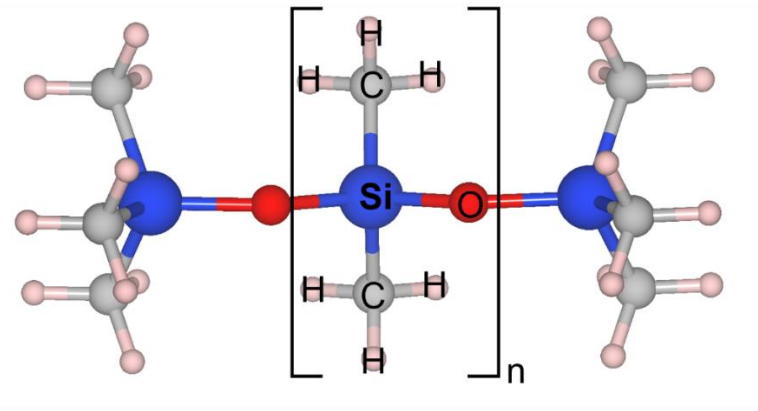

Figure 1. The chemical structure of poly(dimethylsiloxane)

PDMS membrane is also used for several other applications [6-8], including gaseous diffusion studied with respect to the rate and amount of absorption [9, 10], drug depot systems [11] and optical fibre coatings [12]. Several studies have considered the swelling effect on such membranes in the presence of solvents, for example the varied swelling observed in the presence of standard solvents [13]. We have shown in our previous studies the effects of

solvents [14], ionisation [15] and additives on diffusion through PDMS, including surfactants [16]. Interestingly, the presence of an anionic surfactant significantly (and unexpectedly) reduced membrane permeation, i.e. contradicting the well-known phenomenon of permeation enhancement in human skin in the presence of surfactants [17]. Upon further consideration it was postulated that the molecules were orientating themselves at the membrane surface so that the hydrophobic 'tails' were adsorbed within the membrane yet the hydrophilic 'head groups' remained within the aqueous solution. This creates a layer of adsorbed molecules on the membrane that should be acknowledged and fully understood, as it may influence the overall properties of the membrane. One important factor to determine is the extent to which a compound may spontaneously adsorb, i.e. quantifying compound adsorption from aqueous solution to within silicone membrane. Such information is critical if PDMS membrane is to be accepted as a credible option for analysis within the pharmaceutical and cosmetic industries. This study presents the first simple, yet effective, method to quantify compound adsorption within silicone membrane using model compounds that can undergo the adsorption process when in contact with PDMS. These specific compounds were selected based upon their frequent use within transdermal pharmaceutical and healthcare products yet the public controversy regarding their safety[18].

Experimental

PDMS membrane was used as purchased (Silex, UK) with a standard thickness of 0.1, 0.2 or 0.3 mm and cut to size as required (1.5 cm^2). The ten analytes and buffer components (to achieve pH 7.4) were used as received. 4-hydroxybenzoic acid (99+ %), ethyl 4-aminobenzoate (98 %), methyl 4-hydroxybenzoate (99 %), potassium phosphate dibasic (98+ %), potassium monobasic (99+ %), propyl 4-hydroxybenzoate (99+ %) and sodium chloride (99.5 %) were acquired from Thermofisher Acros Organics (Geel, Belgium). Butyl 4-hydroxybenzoate (99+ %), ethyl 4-hydroxybenzoate (99 %) and methyl 4-aminobenzoate (98

%) were acquired from Aldrich (St. Louis, Missouri, United States). Propyl 4-aminobenzoate (98 %) was acquired from Alfa Aesar (Heysham, Lancashire, UK). 4-aminobenzoic acid (99 %) was acquired from BDH Laboratory Reagents (Poole, Dorset, UK). Butyl 4-aminobenzoate (98+ %) was acquired from Fluka Analytical (Bucharest, Romania). The study was limited to these ten compounds as the solubility decreased to a level too low for detection when alkyl length (n) ≥ 5.

PDMS is known to leach impurities into solution and the PDMS used in these experiments was found to initially leach such impurities into the phosphate buffer thus requiring a washing process prior to each analyte being added. Once the PDMS had been thoroughly washed, analytes were added to determine the reduction in concentration through membrane adsorption. Phosphate buffered saline (PBS) was prepared by placing 8.766g NaCl, 7.385g K_2HPO_4 and 1.034g KH_2PO_4 into a 1 L volumetric, and made up to volume with ultra-pure water then adjusted to pH 7.4 using HCl. Standard solutions of the ten analytes in buffer (1 μg/mL, 2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL, 10 μg/mL, 15 μg/mL and, where appropriate 20 μg/mL) were used to identify the λ_{max} and from that a calibration graph was established using spectroscopy (Agilent Technologies Cary 60). 30 (10 x 0.1 mm, 10 x 0.2 mm or 10 x 0.3 mm) pieces of 1.5cm^2 PDMS were prepared along with 5 mL of PBS and placed in each vial. The buffer was removed and replaced after a total of 6, 24 and 30 h (to remove any leaching impurities), then left for a further 18 h before use. For each analyte a 500 μg/mL solution was prepared in PBS then sonicated for 1 h to ensure all the drug was dissolved then diluted to 10 μg/mL. Once the buffer had been removed from the PDMS-containing vials, 5 mL of 10 μg/mL benzoate solution was pipetted into three vials of each thickness of PDMS. Separately, one vial of each PDMS thickness had fresh PBS added and three vials had 5 mL of the analyte solutions added; these vials acted as controls. All vials were stored for a period of 6 h at room temperature then analysed using UV spectroscopy. Quantification of analyte adsorbed onto the PDMS was

calculated through subtraction of the absorbance generated by the vials containing PDMS and analyte from the absorbance of the analyte solutions without PDMS present. Absorbance changes were converted to concentration using calibration graphs and then to number of molecules removed from solution by adsorption through knowing the volume of solution used.

Results and Discussion

For all ten analytes a significant reduction in concentration within the buffer solution was observed after 6 h, indicating that the PDMS membrane had adsorbed a significant amount of each of the compounds. Analysis of the control vials further supported that the PDMS membrane acted as an adsorbate, as concentration of the compounds remained unchanged during the experiment, i.e. the compounds did not interact with the internal vial surface or underwent degradation. The reduction in concentration, along with knowledge of the buffer volume, was used to calculate the number of molecules bound to the PDMS present. The ten analytes consisted of two 'groups' of compounds – five based upon 4-hydroxybenzoic acid and five based upon 4-aminobenzoic acid, both groups including compounds with an increasing alkyl chain length from 0 to 4. The observed reduction in adsorption in the presence of membrane was deemed significant, i.e. beyond experimental error, with percentages in concentration reduction ranging from 10 % for 4-hydroxybenzoic acid up to 86 % for butyl 4-aminobenzoate. Although it is unknown how the molecules bind to the PDMS it can be theorised that the alkyl 'tail' section of the compound adsorbs onto the hydrophobic PDMS surface with the more polar head groups remaining in aqueous solution, much like that previously seen for surfactants [15]. This theory correlates well with the fact that the number of molecules removed from solution increases with 'tail' (carbon chain) length in a linear manner for both sets of compounds (i.e. hydroxybenzoates and aminobenzoates). This finding indicates that there is a stronger binding between the PDMS and the analyte molecules with an increase in carbon chain length. As the hydrophobicity of the molecules increases with

121 increasing alkyl chain length this is further proof that it is the tail that adsorbs to the PDMS
122 surface. Researchers have previously considered the effect of solvent choice on permeation
123 through PDMS [19], whereby propanol sorption was proposed to have occurred within PDMS,
124 yet quantification of this effect has not previously been quantified, such as achieved in this
125 study.

126 Although there is a small difference between the two sets of compounds regarding the
127 extent of adsorption as reflected in number of molecules bound per gram of PDMS, there is a
128 general increase regarding alkyl chain length with respect to extent of adsorption. Compounds
129 from each series, which do not contain an alkyl chain, i.e. when n = 0, display an almost
130 identical behaviour on PDMS (2.18×10^{16} molecules for 4-aminobenzoic acid and 2.19×10^{16}
131 molecules for 4-hydroxybenzoic acid), which is further evidence of the length of the alkyl
132 chains to be the main driving force for adsorption on PDMS. The small difference in adsorption
133 behaviour between the two series of compounds is likely a consequence of the differences in
134 ionisation that are present at the experimental pH (7.4). Although 4-hydroxybenzoic acid itself
135 is almost completely ionised, the remaining four compounds in the series are predominantly
136 unionised at this pH. In contrast, all of the compounds in the aminobenzoate series are
137 completely ionised at this pH. This suggests that compounds in their ionised form more
138 favourably interact with the membrane, which is unexpected as the membrane is hydrophobic
139 and therefore it could be assumed that unionised species would display a more favourable
140 interaction. As the difference between the two series is small in comparison to the overall
141 change in adsorption with alkyl chain length, we infer that chain length is a more influential
142 factor compared with the degree of ionisation in the adsorption of these compounds. Ideally,
143 further compounds within the alkyl series would have been studied yet the increasing
144 hydrophobicity (as the chain length increases) resulted in a reduction in solubility which
145 prevented analytical detection at acceptable levels.

To investigate if membrane thickness affected the adsorption of compounds on PDMS, experiments were repeated using 0.2 mm and 0.3 mm PDMS. To allow for comparisons between all three thicknesses, the total mass of membrane remained constant thus as the thickness increased, the volume increased but the surface area decreased. The surface to volume ratio decreased from 1.31 x 10^{-3} for 0.1 mm, to 3.14 x 10^{-4} for 0.2 mm, and to 1.44 x 10^{-4} for 0.3 mm. Calculation of the number of molecules adsorbed per gram of PDMS (i.e. normalised to allow for volume differences) for all ten compounds with all three thicknesses can be seen in Figure 2.

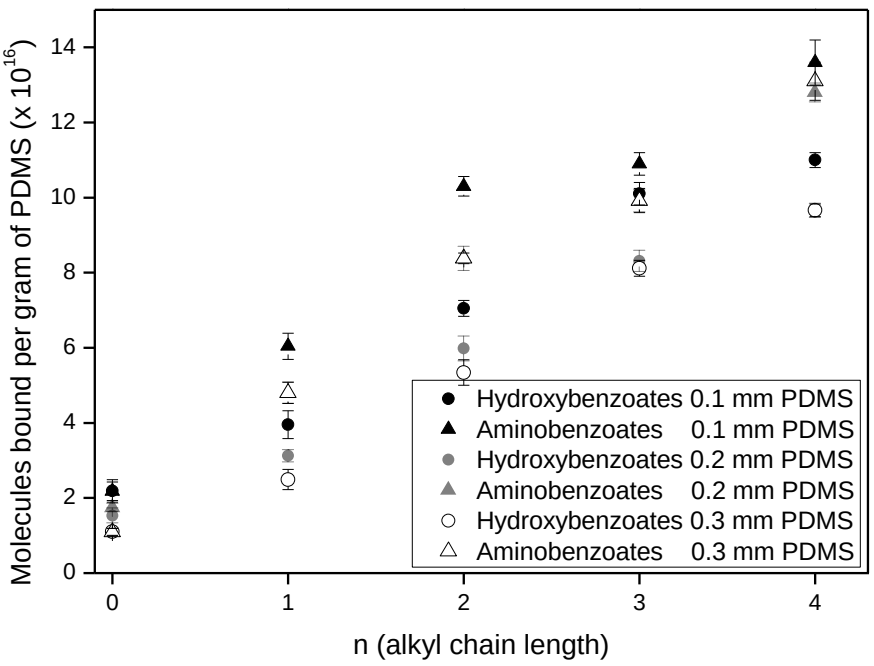

Figure 2. Number of molecules removed from solution through adsorption to PDMS (0.1, 0.2 mm and 0.3 mm thickness) for two groups of compounds (hydroxybenzoates and aminobenzoates) with increasing alkyl chain length (n = 6, error = ±SD)

The number of molecules bound to the PDMS slightly decreases with membrane thickness (Fig 2). For example, for methyl 4-aminobenzoate 6.04 x 10^{16} molecules bound to the 0.1 mm thickness PDMS whereas 4.80 x 10^{16} molecules of the same compound bound to the 0.2 mm thickness PDMS. However, this is a small change in comparison with the influence of alkyl

chain length on the extent of adsorption. These findings suggest that membrane thickness (and therefore also membrane volume) do not play a significant role in dictating the extent of binding of aminobenzoates or hydroxybenzoates to PDMS compared with the effect of alkyl chain length. Such findings also confirm that surface area does not dictate the extent of adsorption as the former varies between thicknesses yet the latter is not affected. Data presented includes calculations based on three concentrations for each compound; the total concentration present, the concentration remaining in aqueous solution after the addition of PDMS and the concentration adsorbed onto the membrane. Knowledge of these three values allows a partitioning term (*P*) to be described as shown in Equation 1.

Equation 1. $$\textit{Membrane Surface Partitioning (P)} = \frac{\textit{Concentration of compound in PDMS}}{\textit{Concentration of compound in solution}}$$

P is a reflection of a compounds preference to adsorb onto the PDMS. Knowing thermodynamically that the change in Gibbs free energy (ΔG) is related to partitioning (*P*) by $\Delta G = -RT\ln P$, where R is the gas constant (8.314 J K^{-1} mol^{-1}) and temperature (T) is 293 K, it is possible to calculate a change in Gibbs free energy for the absorption process (ΔG_{abs}) for each compound (Figure 3).

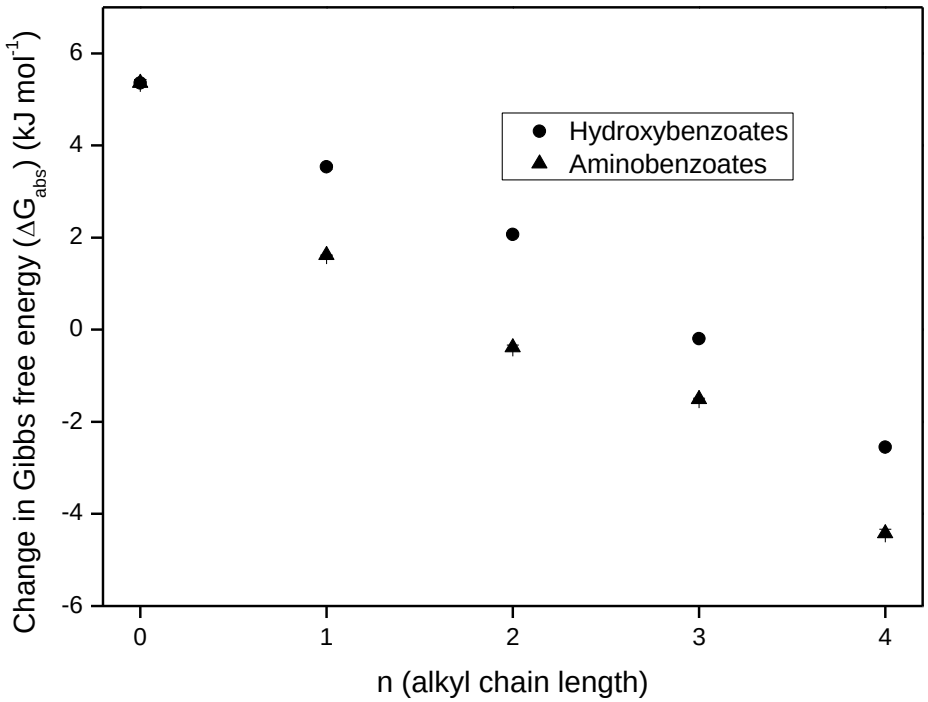

Figure 3. Calculated changes in Gibbs free energy of absorption (ΔG_{abs}) for two groups of compounds (hydroxybenzoates and aminobenzoates) with increasing alkyl chain length (n = 6, error = ±SD, 0.1 mm PDMS)

ΔG_{abs} becomes more favorable as the alkyl chain length increases, which further indicates that the alkyl chain drives the adsorption process into the hydrophobic membrane. Furthermore, the change in ΔG_{abs} with alkyl chain length is generally consistent implying there is a direct correlation between adsorption and chain length and that the membrane surface had not become saturated during the study. The values obtained for ΔG_{abs} are comparable with those seen previously in literature for similar studies whereby a transfer process was observed for similar compounds partitioning between organic (octanol/cyclohexane) and aqueous phases, further confirming the validity of the method presented in this study [20]. Such behaviour (although known for liquid-liquid interfaces, PDMS droplet-water interfaces [21] and solvent solubility thermodynamics [22]), has not been previously acknowledged for solute-PDMS interfaces, such as the results presented in this study.

Conclusions

In conclusion, we present the first analytical quantification of physical adsorption onto PDMS membrane through determining the decrease in concentration from solution using ten model compounds. It was found that the number of molecules bound to the PDMS membrane increased as alkyl chain length increased, which is a direct consequence of the overall interaction between the hydrophobicity of the compound (increasing as the number of carbons in the alkyl chain increased) and the hydrophobic membrane surface. Only small differences were observed regarding the extent of adsorption between the two sets of compounds and between the three different thicknesses of membrane. Finally, the changes in Gibbs free energy associated with the adsorption process was determined, which opens avenues to acquire thermodynamic data for other compounds adsorbed at solid-liquid interfaces.

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Data Availability: The raw data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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