Liquid membrane extraction and transport of amino acids using calix[6]arene

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Carrier-assisted transport through liquid membrane is one of the important application of supramolecular chemistry. Calix[6]arene was utilized as an extractant as well as carrier for amino acids through liquid membrane. The receptor forms complex with amino acids by CH-ni interactions, van der Waals interactions and hydrogen bonding. The host compound exhibited high fluxes for amino acids with high extraction efficiency i.e. valine and alanine due to its cyclic structure and cavity size. The sequence of extraction and transport efficiency observed was valine > alanine > glycine > threonine and valine > glycine > alanine > threonine respectively. In SLM experiments using cellulose nitrate, PTFE, Onion membrane and egg shell membrane support it was observed that receptor A transports glycine, valine and alanine. The trend of amino acid transported using PTFE and onion membrane support was glycine > alanine > valine > threonine and glycine > valine > alanine > threonine respectively. Among the membrane supports used, the egg shell membrane was found better support for transport of amino acids because of its hydrophobic nature and exhibits electrostatic interactions between positively charged –H_3N^+ group of amino acid and –COOH group of egg shell membrane. These results suggest that hydrophobicity of the guest molecule plays a key role in the extraction while the structure of receptor and pH governs transport efficiency of the amino acids.

Keywords: Amino acids, Calix[6]arene, Liquid membrane extraction, Egg shell membrane support

Liquid membrane studies of the biologically important molecules using synthetic receptors have gained importance in view of simulating biological membrane functions with certain distinct advantages over the conventional chemical techniques i.e. low cost, space requirement and low energy consumption\(^1\)\(^2\). The mobile carrier contained in the liquid membrane governs the efficiency and selectivity of liquid membrane transport and therefore a lot of novel carriers have been explored to create efficient transport systems\(^3\). Calix[n]arene and their derivatives are well known to have attractive host guest chemistry and have the potential to form interesting complexes with metal ions and amino acids by exhibiting extractability and selectivity\(^4\). Calix[6]arene and Calix[8]arene having relatively large cavity have become the useful receptors available for recognizing organic compounds\(^5\). Various applications of calix[n]arenes refers to purification, ion-selective electrodes, transport across membrane and ion channels\(^6\). Amino acids are one of the promising bioactive materials and their demand is increasing in the pharmaceutical and food industry\(^7\). Glycine is a precursor of porphyryns such as heme. Amino acids are under development as components of a range of biodegradable polymers. These materials have applications in environmental friendly packaging and in drug delivery systems. So their determination and separation by supported liquid membrane have become a very important goal of analytical chemistry. SLM technique comprises of three simultaneously occurring processes, molecule extraction from an aqueous source phase, diffusion through hydrophobic liquid membrane and release to aqueous receiving phase\(^8\). In the present work, the carrier ability (BLM and SLM) and extractability of calix[6]arene as a synthetic receptor for amino acids (glycine, alanine, threonine and valine) in organic membrane has been investigated.

Experimental Procedure

Analytical grade amino acids (glycine, valine, alanine and threonine) were purchased from S D Fine chemicals (India). Analytical grade calix[6]arene was purchased from Merck and used without further purification. The solvent CHCl_3 was obtained from Qualigens (India) and used without any further purification. Systronics-167 Visible
spectrophotometer was employed for the estimation of amino acids. The pH was measured by a digital Systronics pH-meter 335 with a combined electrode.

**Extraction studies**

10 mL of aqueous amino acid solution and 10 mL of receptor solution in chloroform were taken in a 50 mL beaker and stirred magnetically for 4 h at 30°C. After stirring, the mixture was allowed to stand for 5 min for the separation of two phases and the aqueous phase was analyzed for extracted amino acid by determining the difference in the concentration of amino acid in aqueous phase before and after extraction. The efficiency of extraction and transport was calculated by using Eq. (1) and distribution coefficient was calculated by Eq. (2) respectively.

\[
\text{Efficiency (\%)} = \frac{A_0 - A}{A_0} \times 100 \quad \text{...(1)}
\]

where \(A_0\) and \(A\) are the absorbance of the aqueous phases before and after extraction.

\[
D_m = \frac{\text{Total concentration of amino acid in organic phase}}{\text{Total concentration of amino acid in aqueous phase}} \quad \text{...(2)}
\]

**Transport studies**

Transport studies were performed in a “U” tube glass cell at 25°C (Ref. 9). The 15 mL of chloroform containing appropriate concentration of receptor \(A_1\) was taken as membrane phase. The source phase was composed of 10 mL of different concentration of amino acid in one limb of the “U” tube and 10 mL of deionised water served as the receiving phase in the other limb. The membrane phase was constantly stirred for 24 h. Flux for transport studies was calculated by using Eq. (3).

\[
J_m = \frac{C \times V}{A \times t} \quad \text{...(3)}
\]

where \(C\) is concentration of amino acid in receiving phase (mol/dm³), \(V\) is volume of receiving phase (mol/dm³), \(A\) is effective area of membrane (m²) and \(t\) is time in s.

**Results and Discussion**

In the blank experiments for the extraction and transport study of each amino acid in which membrane was devoid of carrier, no leakage of amino acid from the source phase into the receiving phase was observed. All measurements were performed in triplicate to check reproducibility.

**Effect of receptor and amino acid concentration**

For optimization of the receptor concentration, its concentration was varied from \(1.0 \times 10^{-4}\) to \(1.0 \times 10^{-1}\) M at constant amino acid concentration \((5.0 \times 10^{-3}\) M). At optimal concentration of the receptor i.e. \(1.0 \times 10^{-1}\) M, amino acid interacts with the receptor and results in the formation of amino acid receptor complex in the membrane phase. The maximum distribution coefficient at optimal receptor concentration indicates the saturation capacity of the membrane phase for complex formation. Results in Table 1 show that an increase in the receptor concentration enhances the effectiveness of the liquid membrane in terms of increased transport rate of amino acids. Therefore, with further increase in the receptor concentration, the saturation capacity of the membrane phase for the complex is marginally

| Concentration of amino acid \((10^{-3}\) M) | Alanine \(J_m^{10^{-5}}\) mol/cm²s | \(D_m\) | Glycine \(J_m^{10^{-5}}\) mol/cm²s | \(D_m\) | Threonine \(J_m^{10^{-5}}\) mol/cm²s | \(D_m\) | Valine \(J_m^{10^{-5}}\) mol/cm²s | \(D_m\) |
|---|---|---|---|---|---|---|---|
| 2.0 | 10.65 | 39.56 | 13.20 | 58.43 | 8.38 | 33.40 | 12.53 | 43.85 |
| 4.0 | 13.48 | 49.00 | 15.89 | 62.00 | 10.43 | 45.67 | 16.84 | 56.42 |
| 5.0 | 16.73 | 54.33 | 14.45 | 65.34 | 12.75 | 48.53 | 17.14 | 71.09 |
| 6.0 | 12.90 | 53.64 | 14.35 | 56.67 | 10.38 | 46.32 | 16.23 | 62.34 |
| 7.0 | 11.45 | 51.02 | 13.87 | 53.98 | 9.76 | 38.11 | 13.20 | 47.74 |

Source phase: 10 mL amino acid solution.
Receptor concentration = \(1.0 \times 10^{-4}\) M in 10 mL chloroform.
Receiving phase: 10 mL deionised water (in transport studies).
Stirring speed = 200 rpm. Temperature = 30°C.
reduced due to the presence of more amount of receptor. However, the rate gradually slows down when the receptor concentration increase gradually more than $1.0 \times 10^{-4}$ M. The flux $J_m$ was controlled by the complex formation at the source receiving-membrane interface which was governed by the complexation and decomplexation at the optimal concentration. The amino acid concentration was varied from $2.0 \times 10^{-3}$ to $7.0 \times 10^{-3}$ M to study the influence on transport and extraction processes (Table 2). Both transport and extraction rates were maximal at $5.0 \times 10^{-3}$ M of amino acids (Figs 1 and 2).

**Effect of time, stirring and back extraction**

Figure 3 shows the time dependence of amino acid extraction through the liquid membrane containing calix[6]arene under the optimized experimental conditions. A rapid increase in extraction was observed during the first 75 min. A further increase in time results in decrease in extraction process. This decrease is due to the attainment of saturation of the extraction process. Above optimal reaction time, back extraction process also tends to reduce the efficiency of extraction process. The extraction efficiency for valine was 75% when both aqueous and organic phases were stirred and it dropped down to 26% without stirring (Figs 4 and 5). The above study indicates that the stirring of the both aqueous and organic phase significantly enhances the extraction as well as amount of transported amino acids by minimizing the diffusive layer, the aqueous organic interface.

For back extraction studies, aliquots from the loaded organic phase were withdrawn and subsequently back extracted for about 4 h with the same volume of the strippent which is deionised water in the present study (Fig. 6). The amount of back

<table>
<thead>
<tr>
<th>Receptor concentration</th>
<th>Alanine $J_m 10^{-5}$ mol/cm$^2$s</th>
<th>$D_m$</th>
<th>Glycine $J_m 10^{-5}$ mol/cm$^2$s</th>
<th>$D_m$</th>
<th>Threonine $J_m 10^{-5}$ mol/cm$^2$s</th>
<th>$D_m$</th>
<th>Valine $J_m 10^{-5}$ mol/cm$^2$s</th>
<th>$D_m$</th>
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<tr>
<td>$1.0 \times 10^{-2}$</td>
<td>10.00</td>
<td>24.45</td>
<td>12.86</td>
<td>48.67</td>
<td>9.67</td>
<td>31.94</td>
<td>11.45</td>
<td>38.88</td>
</tr>
<tr>
<td>$1.0 \times 10^{-3}$</td>
<td>12.87</td>
<td>36.47</td>
<td>11.32</td>
<td>54.32</td>
<td>11.34</td>
<td>43.84</td>
<td>12.47</td>
<td>48.97</td>
</tr>
<tr>
<td>$1.0 \times 10^{-4}$</td>
<td>16.73</td>
<td>54.33</td>
<td>14.45</td>
<td>65.34</td>
<td>12.75</td>
<td>48.53</td>
<td>17.14</td>
<td>71.09</td>
</tr>
<tr>
<td>$1.0 \times 10^{-5}$</td>
<td>13.75</td>
<td>47.45</td>
<td>14.56</td>
<td>50.21</td>
<td>11.00</td>
<td>42.61</td>
<td>15.90</td>
<td>66.35</td>
</tr>
<tr>
<td>$1.0 \times 10^{-6}$</td>
<td>11.21</td>
<td>42.69</td>
<td>13.20</td>
<td>48.43</td>
<td>8.54</td>
<td>30.51</td>
<td>14.56</td>
<td>46.47</td>
</tr>
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</table>

Concentration of amino acid in source phase = $5.0 \times 10^{-3}$ M
Receiving phase: 10 mL deionised water (in transport studies)
Stirring speed = 200 rpm. Temperature = 30°C

Fig. 1—Amount of amino acid transported after 24 h with calix[6]arene. Conc. of amino acid in source phase = $5.0 \times 10^{-3}$ M; Conc. of calix[6]arene in membrane phase = $1.0 \times 10^{-4}$ M in 15 mL chloroform; Stirring speed = 200 rpm; Temperature = 30°C

Fig. 2—Amount of amino acid extracted after 4 h with calix[6]arene. Conc. of amino acid in source phase = $5.0 \times 10^{-3}$ M; Conc. of calix[6]arene in receiving phase = $1.0 \times 10^{-5}$ M in 10 mL chloroform; Stirring speed = 200 rpm; Temperature = 30°C
extracted amino acid was optimal after 2 h of reaction time. Further increase in reaction time results in decrease in amount of back extracted amino acid.

**Effect of pH**

The pH of the source phase and receiving phase was varied to study the selectivity and fluxes of the transported amino acids in acidic form (pH, $s_i=4.00$ or 4.5) $(H_3N^+-R-COOH)$, zwitter ionic form (pH, $s_i=6.00$ or 6.5) $(H_4N^+R-COO^-$), and basic form (pH, $s_i=7.00$ or 7.5) $(H_2N-R-COO^-$), by macrocyclic calix[6]arene.

At pH 6.00-7.2, both, the transported and extracted amount of amino acid was maximal. At high pH range, the carrier diffusion process seems to govern the transport phenomenon. On the contrary, at low pH range 2.5-3.00, very little amino acid was extracted and transported. When the pH of the source phase was acidic, the transport of amino acid was dependent on the nature of amino acid side chain. The amino acid was transported by the complexation of their $H_4N^+$ by the macrocyclic cavity of calix[6]arene. This can be explained by the nature of the amino acids.

Fig. 3—Amount of amino acid extracted after 4 h with calix[6]arene. Conc. of amino acid in source phase = $5.0 \times 10^{-3}$ M; Conc. of calix[6]arene in receiving phase = $1.0 \times 10^{-4}$ M in 10 mL chloroform; Stirring speed = 200 rpm; Temperature = 30°C

Fig. 4—Efficiency of amino acids extracted without stirring. Conc. of amino acid in source phase = $5.0 \times 10^{-3}$ M; Conc. of calix[6]arene in receiving phase = $1.0 \times 10^{-4}$ M in 10 mL chloroform; Temperature = 30°C

Fig. 5—Efficiency of amino acids extracted with stirring. Conc. of amino acid in source phase = $5.0 \times 10^{-3}$ M; Conc. of calix[6]arene in receiving phase = $1.0 \times 10^{-4}$ M in 10 mL chloroform; Stirring speed = 200 rpm; Temperature = 30°C

Fig. 6—Efficiency of amino acids back extracted after 4 h with calix[6]arene. Conc. of amino acid in source phase = $5.0 \times 10^{-3}$ M; Conc. of calix[6]arene in receiving phase = $1.0 \times 10^{-4}$ M in 10 mL chloroform; Stirring speed = 200 rpm; Temperature = 30°C
(hydrophobicity) because the distribution coefficient (D) depends on the hydrophobicity, which determines the partition coefficient of the amino acid\(^{10}\). Therefore, the largest increase in efficiency was observed for valine and alanine (Fig. 7).

Tables 1 and 2 display the results of transport and extraction studies of amino acids using calix[6]arene as a carrier at different concentrations of receptor and amino acids. The trend of extraction and transport efficiency of receptor was observed to be valine > alanine > glycine > threonine at optimal concentration of amino acids and receptor. The receptor exhibits high extractability with valine and alanine because it forms complex due to entrapment of amino acid into its cup like cavity. The active sites at the rim of receptor are favourable for recognition of H\(_4\)N\(^+\) moiety of amino acid. The inclusion effect of the cyclic receptor due to CH-\(\pi\) interactions is also one of the driving forces allowing the transfer of the amino acid from aqueous to organic phase thus resulting in good extractability\(^{5,11}\). Besides, the extraction behaviour of receptor with amino acids strongly depends on the hydrophobicity of amino acids (Fig. 8). The more hydrophobic valine and alanine were carried faster in comparison to the less hydrophobic glycine and threonine. Higher distribution coefficient values were observed for hydrophobic amino acids. The sequence of transportability of calix[6]arene for amino acids was observed to be similar to the trend of extractability. The hydrophobic receptor with suited conformation permits the passage of amino acid receptor complex from the organic phase and releases the amino acid at the membrane-receiving phase. The results of supported liquid membrane (SLM) studies of amino acids with calix[6]arene are shown in Table 3. The observed trend for the transport of amino acids by receptor across cellulose nitrate, onion, PTFE membrane support was found to be glycine> valine > alanine> threonine and for egg shell membrane(ESM) support, the order was glycine > alanine > valine > threonine, respectively. Calix[6]arene loaded egg shell membrane emerges as best carrier for the transport of investigated amino acid through supported liquid membrane. Egg shell membrane possess collagen, glycoprotein and proteoglycan like proteins with high content of amide, carboxylate and amine; surface functional groups exhibit electrostatic interactions between positively charged –H\(_4\)N\(^+\) group of amino acid and –COOH group of egg shell membrane (Fig. 9). Figures 10 and 11 show the SEM images of unloaded egg shell membrane and receptor loaded egg shell membrane system with high cross linking and homogeneity. No significant change in the morphologies of calix[6]arene loaded egg shell membrane system have been observed before and after the transport of amino acids (Figs 11 and 12). This indicates the better carrier ability of calix[6]arene loaded egg shell membrane as compared to other membrane supports for the transport of selected amino acid.
Thus, the structural and physical parameters i.e. macrocyclic cavity, size of the cavity, time of stirring and pH play an important role in the transport of amino acids, while nature and hydrophobicity of amino acid decides the extraction ability of amino acids.

Table 3—Amount of amino acids transported into receiving phase through SLM using various membrane supports after 24 h with receptor (A<sub>1</sub>) in chloroform

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Amino acids (mM)</th>
<th>Egg shell membrane (×10&lt;sup&gt;-3&lt;/sup&gt; M)</th>
<th>J&lt;sub&gt;m&lt;/sub&gt; (mol/cm&lt;sup&gt;2&lt;/sup&gt;s)</th>
<th>PTFE membrane (×10&lt;sup&gt;-3&lt;/sup&gt; M)</th>
<th>J&lt;sub&gt;m&lt;/sub&gt; (mol/cm&lt;sup&gt;2&lt;/sup&gt;s)</th>
<th>Onion Membrane (×10&lt;sup&gt;-3&lt;/sup&gt; M)</th>
<th>J&lt;sub&gt;m&lt;/sub&gt; (mol/cm&lt;sup&gt;2&lt;/sup&gt;s)</th>
<th>Cellulose nitrate membrane (×10&lt;sup&gt;-3&lt;/sup&gt; M)</th>
<th>J&lt;sub&gt;m&lt;/sub&gt; (mol/cm&lt;sup&gt;2&lt;/sup&gt;s)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Alanine</td>
<td>1.52</td>
<td>0.0142</td>
<td>0.80</td>
<td>0.0116</td>
<td>1.00</td>
<td>0.0097</td>
<td>1.32</td>
<td>0.0135</td>
</tr>
<tr>
<td>2</td>
<td>Glycine</td>
<td>2.90</td>
<td>0.2143</td>
<td>1.45</td>
<td>0.1531</td>
<td>2.05</td>
<td>0.2025</td>
<td>2.56</td>
<td>0.2110</td>
</tr>
<tr>
<td>3</td>
<td>Threonine</td>
<td>1.35</td>
<td>0.0124</td>
<td>0.40</td>
<td>0.0078</td>
<td>0.78</td>
<td>0.0100</td>
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<tr>
<td>4</td>
<td>Valine</td>
<td>1.60</td>
<td>0.0153</td>
<td>0.92</td>
<td>0.0112</td>
<td>1.20</td>
<td>0.0121</td>
<td>1.43</td>
<td>0.0143</td>
</tr>
</tbody>
</table>

Concentration of amino acid in source phase = 5.0 × 10<sup>-3</sup> M
Receptor concentration in membrane source = 1.0 × 10<sup>-4</sup> M in 10 mL chloroform
Receiving phase: 10 mL deionised water (in transport studies)
Stirring speed = 200 rpm. Temperature = 30°C

Fig. 9—Schematic representation of liquid membrane studies of amino acids using calix[6]arene as carrier in chloroform liquid membrane system. AA means amino acid: (a) represents interaction between NH<sub>4</sub><sup>+</sup> and active OH site of calix[6]arene.; (b) represents CH-n interaction between alkyl part of amino acid and n electrons of calix[6]arene.

Thus, the structural and physical parameters i.e. macrocyclic cavity, size of the cavity, time of stirring and pH play an important role in the transport of amino acids, while nature and hydrophobicity of amino acid decides the extraction ability of amino acids.
Conclusions

The liquid-liquid extraction and transport studies with calix[6]arene have been carried out in order to investigate the molecular recognition properties of calix[6]arene for various amino acids. The high extractability of calix[6]arene for amino acids is created by the inclusion effect and the CH-π interactions between the cyclic receptor and the guest molecule. The results here led to conclude, that the receptor shows better extraction efficiency but poor transportability. The receptor is selective extractant and proves to be a better carrier for valine and alanine. Thus, the calix[6]arene is expected to be a novel recognition tool for various biologically important amino acids and in future its derivatives can be synthesized and used for liquid membrane studies of biologically important peptides and nucleobases.

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