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# Liquid-Liquid Extraction and Transport of Amino Acids through Membrane by Cucurbit[6]uril and its Derivatives

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Molecular recognition phenomenon of cucurbit[6]uril ($R_1$) and its derivatives perhydroxy cucurbit[6]uril ($R_2$), diphenylcucurbit[6]uril ($R_3$) and hemicucurbit[6]uril ($R_4$) towards aliphatic amino acids was studied. This article highlights the use of these macrocyclic receptors having variations in structure and shape for extraction and transport of amino acids using liquid membrane system. These receptors interact with amino acids by ion-dipole interaction. The observed sequence in supported liquid membrane (SLM) experiments for transport efficiency of different receptors for amino acids is serine > arginine > lysine > glycine by $R_1$, arginine ≈ serine > glycine > lysine by $R_2$, serine > glycine > lysine > arginine by $R_3$ and glycine > arginine > lysine > serine by $R_4$. In bulk liquid membrane (BLM) experiments the sequence of extraction and transport efficiency observed for amino acids using receptor $R_4$ is glycine > lysine > serine ≈ arginine. Receptor $R_2$ containing hydroxyl group at the outer surface of cucurbituril, increases ion dipole interaction between receptor and substrate and receptor $R_4$ having flexible cavity, emphasized better transport efficiency. Various parameters such as pH, time, concentration of amino acids and receptors were studied for extraction and transport of amino acids.

**Keywords: Cucurbit[6]uril, Molecular recognition, Amino acids, Supported liquid membrane, Bulk liquid membrane.**

## INTRODUCTION

Amino acids are one of the promising bioactive compounds and their demand is increasing in sensing and biomedical fields [1,2]. Therefore, the design and synthesis of receptors that can recognize and sense specific amino acids is necessary in diverse fields such as medical diagnostics, nutritional analysis and drug delivery [3]. The determination and separation of amino acids using bulk and supported liquid membrane system have become a very important goal of analytical chemistry. A wide range of supramolecular hosts have been developed to recognize amino acids [4,5].

Cucurbit[n]uril (n = 5, 6, 7, 10, 14) and its derivatives as new receptors in supramolecular chemistry are studied for a large area of applications in molecular recognition, catalysis, supramolecular vesicles, fluorescence sensing, drug delivery, separation science as well as in nanoscience [6-11]. The first member of cucurbituril family, the cucurbit[6]uril was synthesized by Kim *et al*. [12] using acid-catalyzed condensation of glycoluril and formaldehyde. In addition, the synthesis of functionalized derivatives of cucurbituril by enhancing their cavity size and their solubility opens the chances of large perspectives of these molecular containers to be used in several fields [13]. The curious potential of these receptors as high-affinity binding with their hydrophobic cavity and two polar portals towards various guests such as dyes, amino acids, peptides, nucleobases, drug molecules and even proteins attracted considerable attention of many research groups [14-17]. The cucurbit[n]uril family of synthetic macrocycles are very helpful in the area of molecular recognition due to high capacity to bind organic amines over a large range of affinities in aqueous solution. Recently extraction and transport of amino acids have been studied using bulk liquid membrane system by hemicucurbit[n]uril as carrier [18].

Carrier facilitated transport of biomolecules and metal ions through liquid membrane system using different receptors as an extractant as well as carrier plays a significant role in simulating biological membrane functions and separation technologies.

Liquid membranes are selective because of high transport efficiency, minimum sample consumption and economic superiority of liquid membrane over other separation techniques [19,20]. Supramolecular receptors like crown ethers, podands, lariat ethers and calixarenes have found applications for the selective transport of ions and biomolecules through bulk and supported liquid membrane systems [21-24].

In present work, the extraction efficiency and carrier ability of cucurbit[6]uril and its derivatives as synthetic receptors through liquid membrane system for amino acids (glycine, serine, lysine and arginine) is performed. These receptors have been prepared by reported method [25-28].

## EXPERIMENTAL

Analytical grade of amino acids were purchased from S.D. Fine Chemicals (India). Glycoluril, diphenyl glycoluril, imidazolidine-2-one and paraformaldehyde purchased from Sigma Aldrich. Potassium persulphate and ninhydrin obtained from CDH. Solvents purchased from Qualigens (India). Cucurbit-[6]uril and its derivatives were synthesized according to reported methods [13]. Cellulose acetate membrane of 0.45 µm obtained from sartorious and durapore membrane 0.22 µm purchased from Millipore. Systronic-spectrophotometer 106 was used for the estimation of amino acids.

**Estimation of amino acids:** A 0.5 mL aqueous amino acid ($1 \times 10^{-3}$ M to $1 \times 10^{-1}$ M) was taken in 10 mL standard flask and then 0.2 mL ninhydrin solution (0.5%) was added and kept in the boiling water for 10 min Thereafter, 6 mL of 60% ethanol was added and makeup the mixture with double distilled water, results in blue coloured solution at $\lambda_{max}$ 570 nm. The calibration curve was obtained with various concentrations of amino acids and used for the estimation of amino acid in feed phase and stripping phase.

**Extraction studies:** A 10 mL of aqueous solution of amino acids ($1 \times 10^{-3}$ M to $1 \times 10^{-1}$ M) and 10 mL of chloroform solution of receptor $R_4$ were taken in a 50 mL beaker and stirred on a magnetic stirrer for 4 h at room temperature. 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 acids by determining the difference in the concentration of amino acids in aqueous phase before and after extraction.

**Transport studies:** Transport studies for bulk liquid membrane (BLM) system were performed in a "U" tube glass cell as shown in Fig. 1a [24]. A chloroform (15 mL) containing ($1 \times 10^{-4}$ to $1 \times 10^{-3}$ M) receptor $R_4$ was used as membrane phase, feed phase was composed of 10 mL of amino acids in one limb of the "U" tube and 10 mL of distilled water served as the stripping phase in another limb. The membrane phase was constantly stirred for 24 h and the striping phase was analyzed for the concentration of amino acids.

In supported liquid membrane (SLM) system synthetic membranes were impregnated with receptors ($R_1$-$R_4$), dipped overnight and used as membrane support for carrier-facilitated transport studies of amino acids. The supported liquid membrane was positioned between two cylindrical half-cells. One cell compartment (feed phase) was filled with water containing amino acids (50 mL) and the other cell compartment (stripping phase) filled with double distilled water (50 mL), separated by membrane as shown in Fig. 1b. Both phases were stirred with magnetic stirrer at 120 rpm at room temperature and the sample was withdrawn from the striping phase after 24 h and analyzed for the concentration of amino acids.

## RESULTS AND DISCUSSION

Blank experiments were performed with various concentrations of amino acids separately in which membrane was devoid of carrier. No detectable amount of amino acid across membrane could be observed in striping phase which proves that there was no leakage. All measurements were performed in duplicate and average values are shown in the tables.

**Effect of amino acids concentration:** In order to find out the optimum concentration of amino acids for extraction and transport in BLM system using receptor $R_4$, the concentration of amino acids from $1 \times 10^{-3}$ M to $1 \times 10^{-1}$ M were varied. At the lower concentration there was no considerable amount of amino acid extracted and transported, therefore the optimal concentration of amino acid was $1 \times 10^{-2}$ M. Receptor $R_4$ is chloroform soluble. In SLM system, the concentration of amino acids was taken $1 \times 10^{-3}$ M to $1 \times 10^{-1}$ M with various receptors ($R_1$-$R_4$).

**Effect of receptor concentration:** For optimization of receptor $R_4$ concentration, its concentration was varied from $1 \times 10^{-4}$ M to $1 \times 10^{-3}$ M at $1 \times 10^{-1}$ M concentration of amino

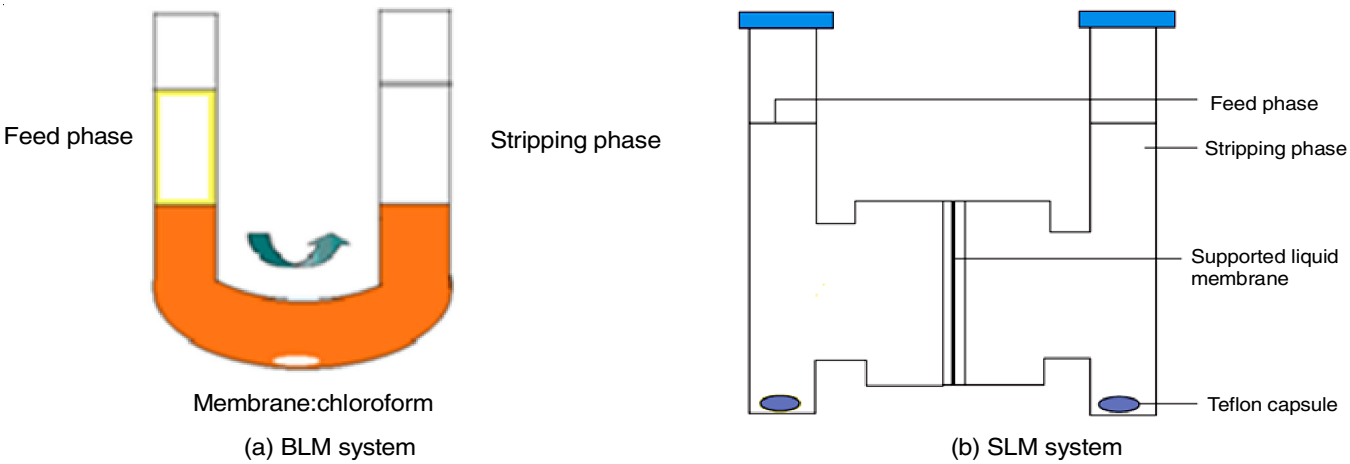


Fig. 1. (a) BLM system and (b) SLM system used in experiment for extraction and transport of amino acids

acid for extraction and transport in BLM system. The amount of amino acids extracted and transported is given in Table-1. From the results, it is observed that the amount of amino acid extracted increases with increase in the concentration of receptor while the transported amount was high at lower concentration of receptor (Fig. 2).

The results of transport of amino acids through SLM system with various receptors ($R_1$-$R_4$) having concentration ($1 \times 10^{-3}$ M to $1 \times 10^{-2}$ M) after 24 h are shown in Tables 2 and 3. It is observed that the amount of transport of amino acid increases with increase in concentration of receptors as well as concentration of amino acids (Figs. 3 and 4). Serine is polar in nature having additional hydroxyl group and most transported amino acid by these receptors except $R_4$. Arginine is hydrophilic in

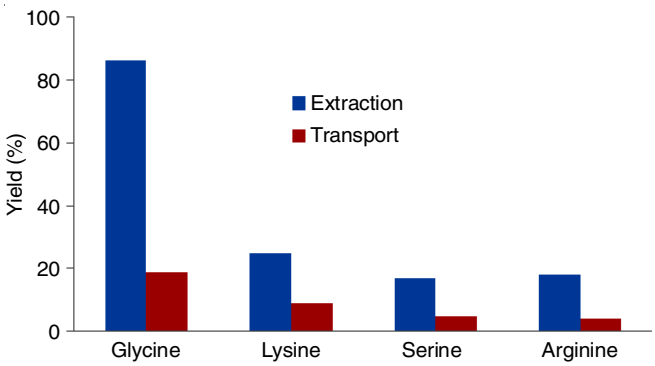


Fig. 2. Extraction and transport (%) of amino acids through BLM system with receptor $R_4$ [concentration of receptor: $1 \times 10^{-3}$ M; concentration of amino acids: $1 \times 10^{-2}$ M]

TABLE-1
AMOUNT OF AMINO ACID EXTRACTED AND TRANSPORTED THROUGH BLM SYSTEM USING CHLOROFORM LIQUID MEMBRANE WITH RECEPTOR $R_4$

| Amino acids ($1 \times 10^{-2}$ M) | Efficiency (%) for amino acids extracted by $R_4$ [$10^{-3}$ M] | Efficiency (%) for amino acids extracted by $R_4$ [$10^{-4}$ M] | Efficiency (%) for amino acids transported by $R_4$ [$10^{-3}$ M] | Efficiency (%) for amino acids transported by $R_4$ [$10^{-4}$ M] |
|---|---|---|---|---|
| Glycine | 86 | 71 | 17 | 19 |
| Lysine | 25 | 22 | 8 | 9 |
| Serine | 17 | 16 | 4 | 5 |
| Arginine | 18 | 13 | 4 | 4 |

TABLE-2
AMOUNT OF AMINO ACID TRANSPORTED INTO RECEIVING PHASE THROUGH SLM SYSTEM USING VARIOUS MEMBRANES SUPPORTS AFTER 24 h WITH RECEPTOR $R_1$, $R_2$, $R_3$ AND RECEPTOR $R_4$ [Concentration of receptors: $1 \times 10^{-2}$ M]

| Amino acids | Conc. (M) | Efficiency (%) for amino acids transported by $R_1$ | Efficiency (%) for amino acids transported by $R_2$ | Efficiency (%) for amino acids transported by $R_3$ | Efficiency (%) for amino acids transported by $R_4$ |
|---|---|---|---|---|---|
| Glycine | $1 \times 10^{-1}$ | – | 23 | 10 | 36 |
| | $1 \times 10^{-2}$ | 0.7 | 7 | 4 | 3 |
| | $1 \times 10^{-3}$ | – | – | 2 | 3 |
| DL-Serine | $1 \times 10^{-1}$ | 38 | 36 | 39 | 11 |
| | $1 \times 10^{-2}$ | 0.6 | 1.8 | 3.3 | – |
| | $1 \times 10^{-3}$ | – | – | 0.8 | – |
| L-Arginine | $1 \times 10^{-1}$ | 5 | 38 | 3 | 24 |
| | $1 \times 10^{-2}$ | 8.5 | 0.5 | 0.2 | 1.4 |
| | $1 \times 10^{-3}$ | – | – | – | – |
| L-Lysine | $1 \times 10^{-1}$ | 3.8 | 11 | 3 | 8.5 |
| | $1 \times 10^{-2}$ | – | 2.5 | 0.9 | 0.4 |
| | $1 \times 10^{-3}$ | – | – | – | – |

TABLE-3
AMOUNT OF AMINO ACID TRANSPORTED INTO RECEIVING PHASE THROUGH SLM SYSTEM USING VARIOUS MEMBRANES SUPPORTS AFTER 24 h WITH RECEPTOR $R_1$, $R_2$, $R_3$ AND RECEPTOR $R_4$ [Concentration of receptors: $1 \times 10^{-3}$ M]

| Amino acids | Conc. (M) | Efficiency (%) for amino acids transported by $R_1$ | Efficiency (%) for amino acids transported by $R_2$ | Efficiency (%) for amino acids transported by $R_3$ | Efficiency (%) for amino acids transported by $R_4$ |
|---|---|---|---|---|---|
| Glycine | $1 \times 10^{-1}$ | – | 21 | 14 | 12 |
| | $1 \times 10^{-2}$ | – | 7.2 | 3 | 1.2 |
| | $1 \times 10^{-3}$ | – | – | 1.2 | 0.5 |
| DL-Serine | $1 \times 10^{-1}$ | 10.5 | 1.7 | 23 | 4.2 |
| | $1 \times 10^{-2}$ | – | 4 | 2.5 | – |
| | $1 \times 10^{-3}$ | – | – | 8.3 | – |
| L-Arginine | $1 \times 10^{-1}$ | 12 | 27 | 4 | 7.1 |
| | $1 \times 10^{-2}$ | – | 10 | 0.7 | 0.7 |
| | $1 \times 10^{-3}$ | – | – | – | – |
| L-Lysine | $1 \times 10^{-1}$ | 4 | 11 | 4 | 6 |
| | $1 \times 10^{-2}$ | – | 1.4 | 0.4 | 0.5 |
| | $1 \times 10^{-3}$ | – | – | – | – |

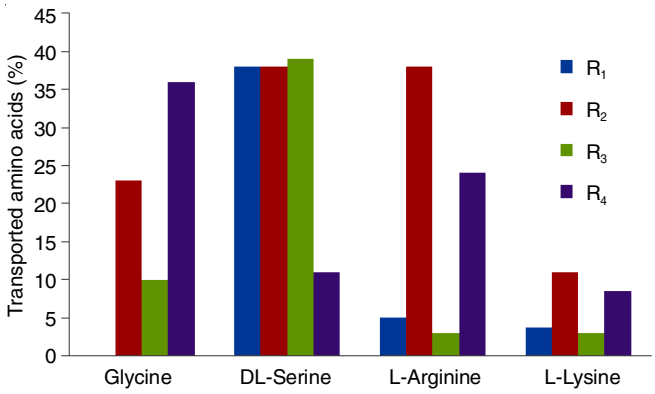


Fig. 3. Amount of amino acid transported into receiving phase through SLM using various membranes supports with receptors $R_1$, $R_2$, $R_3$ & $R_4$ [concentration of receptors: $1 \times 10^{-2}$ M; concentration of amino acids: $1 \times 10^{-1}$ M]

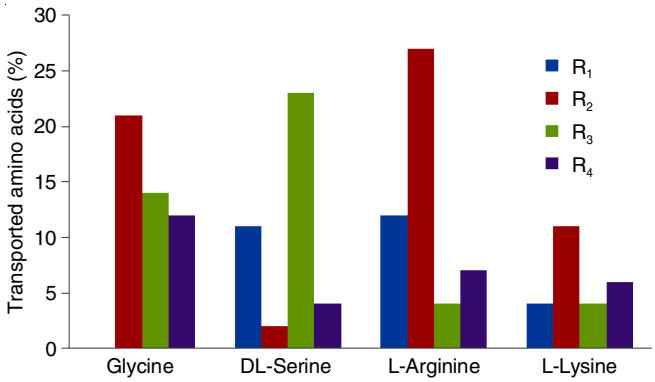


Fig. 4. Amount of amino acid transported into receiving phase through SLM using various membranes supports with receptors $R_1$, $R_2$, $R_3$ & $R_4$ [concentration of receptors: $1 \times 10^{-3}$ M; concentration of amino acids: $1 \times 10^{-1}$ M]

nature, which is selectively transported at $1 \times 10^{-1}$ M concentration by $R_2$.

The sequence of extraction and transport of amino acids observed is glycine > lysine > serine ≈ arginine using receptor $R_4$ in BLM system. In SLM system, the sequence of transport of amino acids observed is serine > arginine > lysine > glycine by $R_1$, arginine ≈ serine > glycine > lysine by $R_2$, serine > glycine > lysine > arginine by $R_3$ and glycine > arginine > lysine > serine by $R_4$ as carrier. Receptor $R_2$ and receptor $R_4$ exhibited better transport efficiency in SLM system. The results showed that the structure of macrocyclic receptors (Fig. 5) is one of the important parameters for recognition of amino acids. Receptor $R_1$ possess pumpkin shape rigid structure consisting of six glycouril units. Receptor $R_2$ having the two hydroxyl group at each glycouril unit of cucurbit[6]uril and these groups oriented at the outer surface of cucurbit[6]uril, which increases the ion-dipole interaction between receptor and substrate. Receptor $R_3$ having two phenyl ring on the convex phase of cucurbit[6]uril. Interior cavity is hydrophobic and exterior portal is hydrophilic in nature for these three receptors. Structure of receptor $R_4$ having alternate conformation of six imidazolidine-2-one unit, each unit connected *via* single methylene bridge, which is flexible in nature, facilitate the transport of amino acids. These receptors interact with amino acids by ion

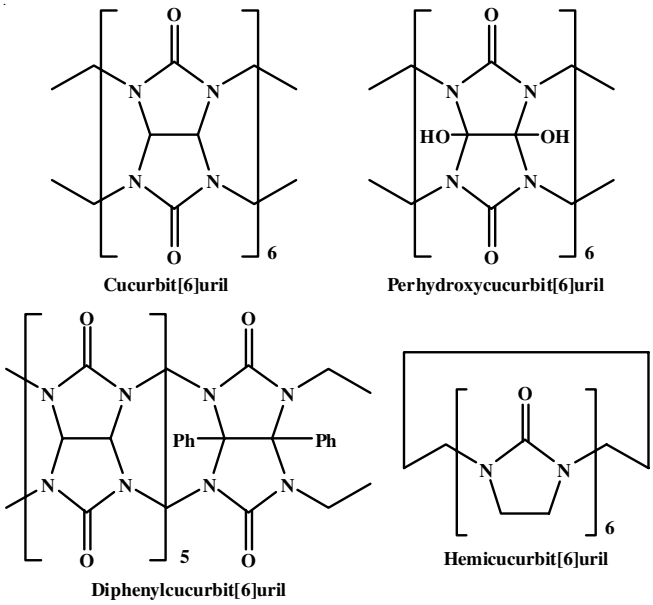


Fig. 5. Chemical structure of macrocyclic receptors used throughout the experiments

dipole interaction between the $NH_3^+$ group of amino acids and carbonyl moiety of receptors.

No effective transport was observed with varying the concentration of receptors ($1 \times 10^{-4}$ to $1 \times 10^{-2}$ M) with a constant concentration of amino acid $1 \times 10^{-3}$ M in SLM system. As shown in Figs. 2 and 3, one can observed that structure and concentration of receptors govern the transport efficiency for amino acids. Receptor $R_1$, $R_2$ and $R_3$ having more transport efficiency for serine, due to the presence of additional -OH group in serine. Receptor $R_4$, transports more amount of glycine due to small size.

Moreover, it is observed that there may be retention of receptor on supported liquid membrane, which inhibits the transport efficiency with respect to extraction (Tables 2 and 3).

**Effect of pH:** Fig. 6 shows results of transport of amino acids at variant pH. The transport of amino acids in acidic form (pH = 4.00 or 4.5) ($NH_3^+$-R-COOH), zwitterionic form (pH = 6.00 or 6.5) ($NH_3^+$-R-$COO^-$) and basic form (pH = 7.00 or 7.5) ($NH_2$-R-$COO^-$) were studied. The optimum pH for transport of amino acid was 6. At this pH, the carrier diffusion governs the transport phenomenon. The amino acids were transported by the ion-dipole interaction between > C=O group of the uridyl moiety of receptor, which is hydrophilic and $NH_3^+$ group of amino acid. The results of transport revealed that interaction of amino acids with receptor is pH sensitive.

**Effect of time:** Fig. 7 shows the time dependence of amino acids transport through supported liquid membrane system using macrocyclic receptor. We estimated the amount of amino acid transported in every 60 min and 90 min interval. In both conditions, the results revealed that the amount of amino acid gradually decreases then increases with time and after 24 h the amount of amino acid transported decrease. Source and receiving phase both have same behaviour with respect to time. This is due to the container type structure of receptors in which amino acid shows tumbling like behaviour.

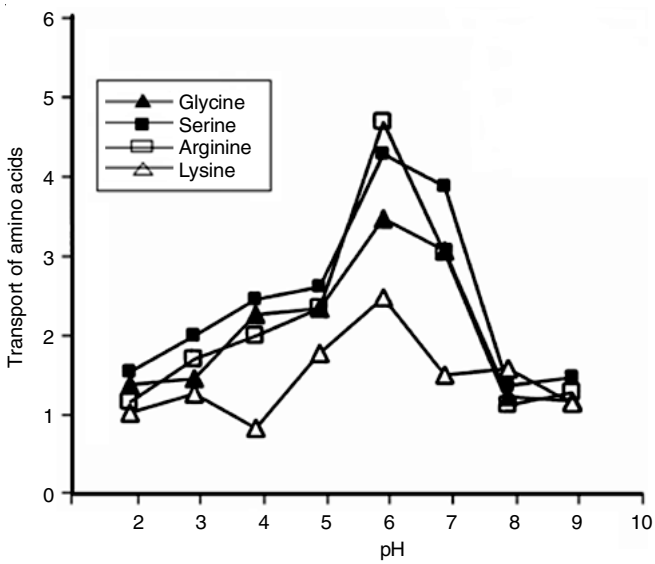


Fig. 6. Effect of pH [concentration of receptors: $1 \times 10^{-2}$ M; concentration of amino acids: $1 \times 10^{-1}$ M]

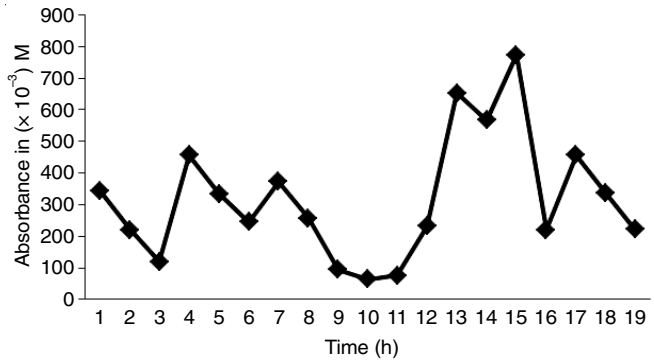


Fig. 7. Amount of amino acid transported after 24 h into receiving phase through SLM system using receptor $R_1$ [concentration of receptors: $1 \times 10^{-2}$ M; concentration of amino acids: $1 \times 10^{-1}$ M]

Transport of amino acids is clearly shown in SEM images of membranes (Fig. 8). It is observed that membrane plays an important role before and after transport experiment. It has been observed that receptors interact with amino acids and bind on membrane surface and some of them release in receiving phase. From these results, it is concluded that the amount of transport of amino acids is less than that of interactive (extracted) amount.

## Conclusion

The extraction and transport efficiency through bulk and supported liquid membranes of macrocyclic receptors for various amino acids (glycine, serine, lysine and arginine) were studied. The obtained results suggested that cucurbit[6]uril ($R_1$) and its derivatives perhydroxy cucurbit[6]uril ($R_2$) and diphenyl cucurbit[6]uril ($R_3$) can act as carrier for amino acid and hemi-cucurbit[6]uril ($R_4$) can acts as extractant as well as carrier for amino acids aiming their separation. Receptors $R_1$, $R_2$ and $R_3$ exhibited better transport efficiency for serine while receptor $R_4$ is good carrier for glycine. In the SLM system, receptors $R_2$ and $R_4$ exhibited the good transport efficiency. Receptor $R_2$ having the two hydroxyl group at each glycouril unit of cucurbit-[6]uril and these groups oriented at the outer surface of cucurbit-[6]uril, which increases the ion-dipole interaction between receptor and substrate and structure of receptor $R_4$ having alternate conformation of its monomer and each unit connected *via* single methylene bridge, which is flexible in nature, facilitate the transport of amino acids. The structure and design of receptors/carrier play an important role in separation and hence these results may help in designing of more specific carrier for the substrate. Some parameters, such as the pH and time also influenced the transport of amino acids through membrane.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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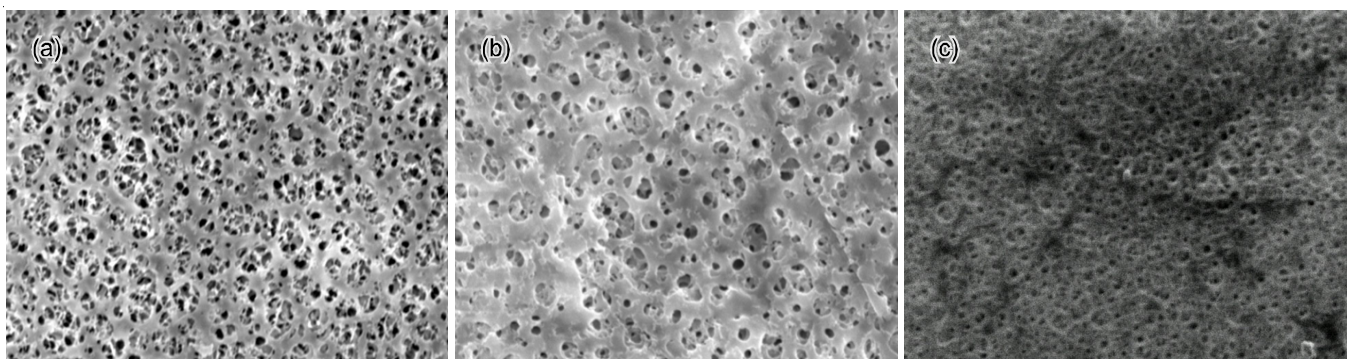


Fig. 8. SEM images of unloaded cellulose acetate membrane. (a), receptor loaded cellulose acetate membrane before transport process (b) and receptor loaded cellulose acetate membrane after transport process (c)

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