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Effect of the nitrile group in extraction and bulk liquid membrane transport of amino acids

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Extraction and transport of amino acids through a liquid membrane system is an emerging field of supramolecular as well as biomedical science. In this piece of work, series of podands are used with triethylene and tetraethylene glycol chains having benzonitrile (PBT3, PBT4), anthraquinone (PAT3, PAT4) and naphthyl (PNT3,PNT4) end groups. Extraction and transport studies performed with amino acids: glycine, valine and threonine using these receptors. From the results, it is observed that the extraction efficiency of PBT3 and PBT4 is double than PNT3 and PNT4 podands, and also transport ability is double in PAT3, and PAT4. Thus we conclude that the nitrile group-containing podands (PBT3 and PBT4) are better extractants as well as a carrier than PAT3, PAT4, PNT3, PNT4, in which nitrile groups is a key component for molecular recognition of glycine, valine, and threonine, which are further used in the separation of amino acids.

Keywords: Nitrile group having podands, Bulk liquid membrane transport, Amino acids, Receptor-substate interaction

Amino acids play a key role in many biological processes, such as protein folding[1], bonding and catalytic transformation[2] of substrates by enzymes, and the expression and transfer of genetic information[3]. Classical examples of these interactions are the base stacking in DNA and the protein folding caused by phenylalanine and other aromatic amino acid side chain interactions[4]. The processes of molecular recognition are fundamental importance for the formation of higher organized chemical systems that result from the association of two or more chemical species[5]. These processes depend on weak but specific, noncovalent intermolecular interactions, such as hydrogen bonding,[6] ion pairing, hydrophobic interactions, etc., which are today frequently used for the programmed synthesis of supermolecules and biomimetic chemistry.

The development of selective receptors for extracting amino acids from an aqueous solution is of significant interest because of the important roles of these small protein molecules in many biological processes. There are many receptors like calixarene[7], cucurbituril[8], pillar arenes[9], and cyclic and non-cyclic receptors that are studied for the recognition of amino acids and show non-covalent interactions. We focus on multiple noncovalent interactions of amino acids in a small pocket thus synthesizing nitrile group containing receptors (shown in Fig. 1), in which two different binding sites are present-the hydrophobic exterior of the glycol chain (CH_2-O-CH_2) and terminal nitrile group have small, linear, polar, hydrophilic, strong electron-withdrawing nature properties. These multiple properties make them (PBT3, PBT4) an effective extractant as well as a carrier for amino acids compared to other receptors (PAT3, PAT4, PNT3, PNT4, and PQT4). The presence of hydrophilic (polar nitrile group) and hydrophobic (exterior of glycol chain) may show amphoteric nature and aggregate which will be applicable in separation science and bio-sensors.

Experimental Section

A magnetic stirrer, a Teflon-coated capsule for stirring, and a "U"- shaped glass cell (for BLM) were used for the transport studies. In addition, the reagents used for synthesis, extraction, and transport studies were of analytical grade and used without further purification.

Synthesis of receptors PBT3 and PBT4

Synthesis procedure of PBT3

Step -1: Tosylation of triethylene glycol[12] (T_3-DTOS)

A solution of sodium hydroxide (4.0 g, 1 mol) in distilled water was prepared (20.0 mL) and cooled to room temperature. The solution was placed in a two necked round-bottomed flask fitted with a

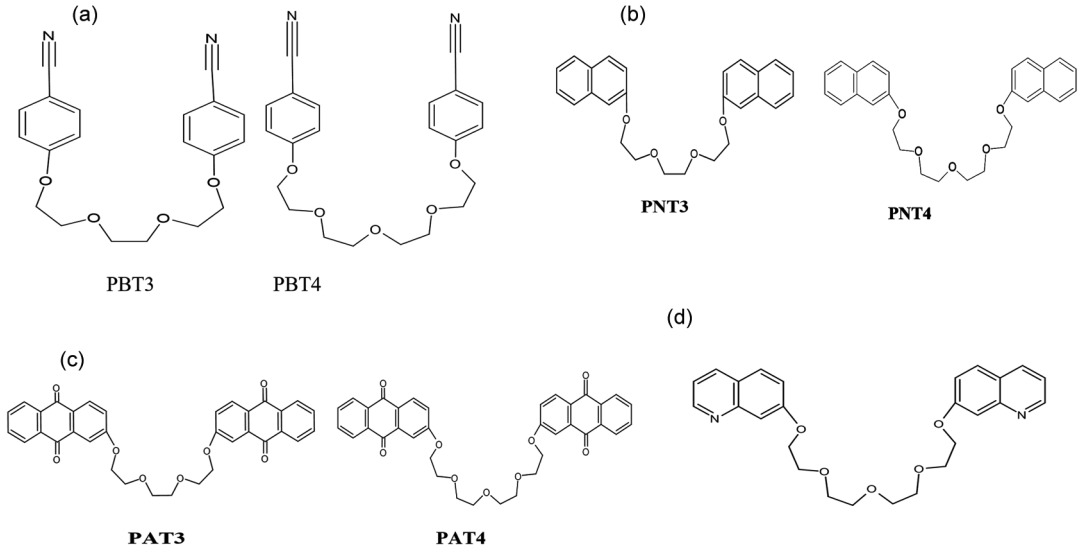


Fig. 1 — Structures of PBT3, PBT4, PAT3, PAT4, PNT3, PNT4 and PQT4

thermometer and a solution of triethylene glycol (5.68 g) in THF was added while stirring. The flask was put in an ice bath and cooled to 0°C. A solution of p-toluene sulphonyl chloride (145 g,7.6 mol) in THF in a pressure equalized addition funnel was placed and added dropwise to the stirred glycol solution over 3 h (temp. below 5°C) after that continue to stir the solution for further 1 h at below 5°C. Then the solution was poured onto a mixture of ice and water (250 g/250 mL) and continued to stir. After all the ice has melted, the product was filtered as a white powder.

Step-2: Synthesis of 1,10-Bis(p-cyanophenyl)-1,4,7,10-tetraoxadecane[13] (PBT3)

4-Cyanophenol (597 mg), triethylene glycol ditosylate (1.26 g) and potassium carbonate (1.4 g) were refluxed in acetonitrile for 44 h at 80°C. After cooling to room temperature, the mixture was filtered and concentrated in a vacuum, a white solid was obtained, which was dissolved in CH_2Cl_2. The organic layer was dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated to yield the crude product PBT_3 as a white solid.

Procedure for PBT4

Step-1: Tosylation of tetraethylene glycol (T_4-DTOS)

In the tosylation of tetraethylene glycol reagents, the solution of sodium hydroxide (4.0 g, 1 mol) in distilled water (20.0 mL), tetra ethylene glycol (6.0 mL, 035 mol) in THF, and solution of p-toluene sulphonyl chloride (14.5 g, 7.6 mol) in THF were taken. The reaction procedure and conditions are similar to those described for the synthesis of T_3-DTOS.

Step-2: 1,10-Bis(p-cyanophenyl)-1,4,7,10,13-pentaoxaun decane (PBT4)

PBT_4 was synthesized by the condensation of p-cyanophenol (597 mg, 5.01 mol), tetraethylene glycol ditosylate (1.20 g) and potassium carbonate (1.4 g) at 80°C for 44 h. The reaction procedure and conditions were similar as described for synthesis PBT3. The synthesis of PBT3 and PBT4 is shown in Schemes 1 and 2.

Synthesis of receptor PAT3 and PAT4

The synthesis of **PAT3 and PAT4** was carried out following a previously reported method (Awasthi *et al*.). The structures are shown in Fig. 1c.

Synthesis of receptor PNT3 and PNT4

The synthesis of **PNT3 and PNT4** was carried out following a previously reported method (Raizada *et al*.). The structures are shown in Fig. 1b.

The characterizations of all the synthesized products are given in Table 1.

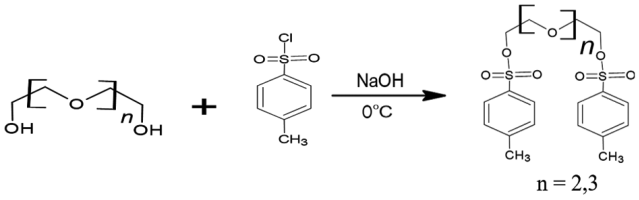
Procedure for liquid-liquid extraction studies[14]

In extraction studies, an equivalent volume (10 mL) of the aqueous solution of amino acids and receptors in $CHCl_3$ was stirred in a small beaker for 240 min on a magnetic stirrer. The amount of

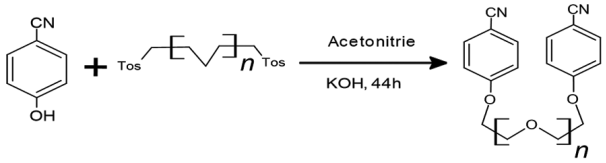
amino acids extracted by receptors was determined by the difference in the amount of substrate before and after the extraction.

Procedure for BLM transport studies[15]

Bulk liquid membrane transport experiments were performed in a "U" shaped glass cell, also known as "Pressman Cell" (Fig. 2b). 15 mL solution of receptor in $CHCl_3$ was placed at the bottom of the "U" tube, to serve as an organic layer with Teflon coated magnetic capsule, 10 mL of the aqueous solution of the substrate was placed in one limb of the U tube which serves as source phase/feed phase and 10 mL of double distilled water was placed in another limb of the U tube serves as receiving/striping phase. The organic phase was stirred for 24 h using a magnetic stirrer .The source and receiving phases were sampled and analyzed for substrate concentration using spectrophotometer. The "blank" experiments were carried out in which the organic phase is devoid of the receptor, which resulted there was no amino acid molecule/ion leakage observed from source phase to receiving phase. The BLM transport experiments were repeated twice to ensure their reproducibility.).



Scheme 1 — Synthesis of DTOS (T_3-DTOS & T_4-DTOS)



Scheme 2 — Synthesis of PBT (PBT3 & PBT4)

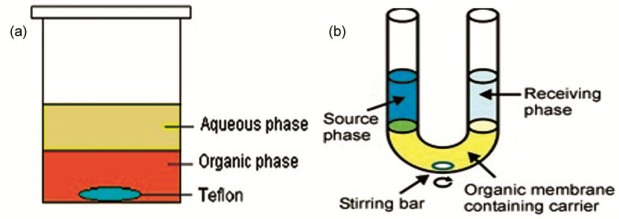


Fig. 2a — Experimental setup for extraction studies and (b) setup for BLM transport experiment

Optimization of receptors and amino acids concentration

The amino acid concentration was varied from 3.0×10^{-3} M to 6.0×10^{-3} M at keeping carrier concentration at 1.0×10^{-3} M and the optimal concentration 5.0×10^{-3}M was observed for all amino acids. and the receptor concentration was varied from 1.0×10^{-2} M to 1.0×10^{-4} M keeping amino acid concentration at 5.0×10^{-3} M and the optimum concentration1.0×10^{-3}M was observed for all receptors.

Results and Discussion

Extraction studies

The results of the extraction studies of amino acids with receptors are illustrated in Tables 2. The trend for extraction of amino acids is as follows;

Glycine: PBT3> PBT4 > PAT3> PAT4> PNT3 > PQT4 ≥ PNT4

Valine: PBT4> PBT3> PAT4> PAT3> PQT4 > PNT3> PNT4

Threonine: PBT4> PBT3> PAT4> PAT3> PQT4> PNT3> PNT4

Receptor PBT3 shows higher extraction for glycine and PBT4 shows higher extraction for valine and

Table 1 — Characterization of PBT3 and PBT4

Receptors	Molecular formulae	M. P. (°C)	Selected FTIR absorption bands	Characteristic ^{1}HNMR chemical shift values	Elemental analysis % Calculated (Found)		
					C	H	N
PBT3	$C_{20}H_{20}N_2O_4$	108	2240-2273 cm^{-1}(CN), 1603cm^{-1} Aromatic (C-C), 1126 cm^{-1} (Ar-O-R), 835 cm^{-1} (Ar-H)	3.512-3.582(Ar -O-CH_2),3.3-4.2(CH_2-O-CH_2),6.221-9.05(Ar-H)	68.17 (68.02)	5.72 (5.69)	7.95 (7.87)
PBT4	$C_{22}H_{24}N_2O_5$	48	2230 cm^{-1} (CN), 1576 cm^{-1}, Aromatic(C-C), 1126cm^{-1}(CH_2-O-CH_2) , 836 cm^{-1} (Ar-H)	3.481-3.551(Ar-O-CH_2),3.575-4.359(CH_2-O-CH_2), 7.370-8.306(Ar-H)	66.65 (66.35)	6.10 (5.95)	7.07 (7.00)

threonine as compared to the others. Selectivity can be explained on the bases of the structure of receptors under following points.

Effect of end groups

Podands have different end groups (benzonitrile, naphthyl, and anthraquinone) and similar chain lengths.

Podands having trimethylene glycol (T3EG) chain (PBT3, PNT3, PAT3)

On comparing the results of extraction with PBT3, PAT3, and PNT3, it is clear that PBT3 is a good extractant as compared to PNT3 and PAT3 (Table 2). The value of extraction of three amino acids (Gly, Val, Thr) by PBT3 is almost double than PNT3.the ratio of extraction efficiencies of PBT3 & PNT3 for Gly, Val, and Thr are 1.62, 1.60, and 1.56, respectively, and the ratio of extraction efficiency of PBT3 & PAT3 is 1.47, 1.5, and 1.41, respectively (as shown in Fig. 3).

It is clear from the structure of PBT3 that it possesses a nitrile group (-CN) at the terminals of the T3EG chain and it is a ditopic receptor (nitrogen and oxygen as binding sites) that forms non-covalent interaction (hydrogen bonding) with amino acids[16]. As in the nitrile group, an sp hybridized 'C' atom is present that is triply bonded to an "N" atom with a lone pair that forms hydrogen bonding as a hydrogen bond acceptor with amino acids. The unique structural characteristics of nitrile groups (having linear shape) enable them to radially interact with amino acids.

Podands having tetra ethylene glycol (T4EG) chain (PBT4, PNT4, PQT4, PAT4)

The podands having tetraethylene glycol chain (T4EG), used in the present study are PBT4, PNT4, PNT4, PAT4. On comparing the results of extraction of Gly, Val, Thr with these podands, the sequence of extraction ability is in the order: PBT4> PAT4 >PQT4> PNT4 (Fig. 4). PBT4 and PQT4 are ditopic, and PAT4 and PNT4 are monotopic receptors. The value of extraction of three amino acids (Gly,Val,Thr) by PBT4 is almost triple for PNT4. The ratio of transport efficiency of PBT4/PNT4 for Gly, Val and Thr is 2.922, 3,.20, and 3.14, respectively, and the ratio of transport efficiency of PBT4/PQT4 for Gly, Val, Thr is 2.30, 1.95, and 2.02, respectively. The best extractant among these receptors is PBT4 which further proves the role of nitrile groups and their interaction with amino acids (protein).

Effect of chain length

Podands (PBT3 and PBT4) (PNT3 and PNT4) (PAT3,PAT4)

On comparing the results of extraction Gly, Val, Thr based on the chain length of podands (PBT3 and PBT4) (PNT3 and PNT4), (PAT3, PAT4), it is observed that podands (PBT3, PAT3) having T3EG chain, are selective for Gly and podands (PBT4, PAT4) having T4EG chain is selective for Val (Fig. 5) and in PNT3 & PNT4 no significant change is observed[17] (Fig. 6).

Bulk liquid membrane transport studies

The sequence for transport of amino acids through a bulk liquid membrane system is given below.

Table 2 — Extraction studies of amino acids with podands

Receptors	Glycine	Valine	Threonine
PBT3	2.87	2.70	1.16
PAT3	1.95	1.80	0.52
PNT3	1.78	1.68	0.74
PBT4	2.63	3.14	1.38
PAT4	1.46	2.00	0.96
PNT4	0.90	1.00	0.43
PQT4	1.14	1.61	0.68

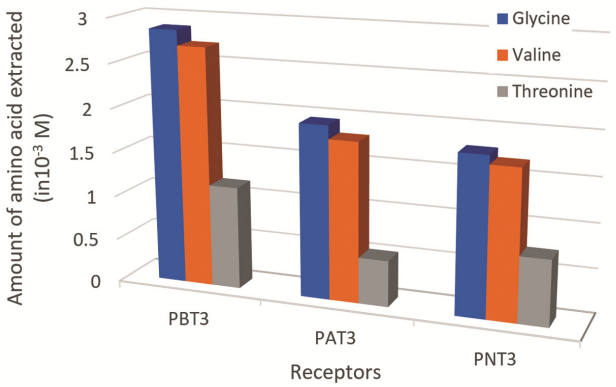


Fig. 3 — Extraction of amino acids (Gly,Val,Thr) by PBT3, PA,T3 and PNT3

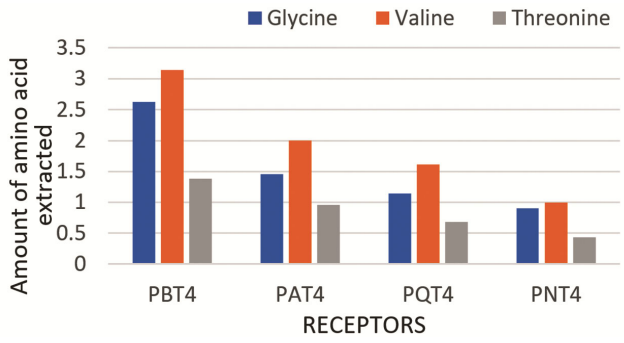


Fig. 4 — Comparing extraction of amino acids by PBT4, PAT4, PQT4 and PNT4

Glycine:PBT3>PBT4>PAT4>PQT4>PNT3>PNT4>PA3
Valine: PBT4> PBT3> PAT4>PAT3> PQT4> PNT4> PNT3
Threonine: PAT4 > PAT3 > PBT4 > PBT3 > PQT4 ≥ PNT4 > PNT3

The carrier ability of receptors was studied considering different parameters such as end group, chain length, etc. The important parameters in the transport of amino acids are discussed under the following points.

Structure of Receptors/Carriers

Effect of end groups

Podands have different end groups (benzonitrile, naphthyl and anthraquinone) and similar chain length.

Podands having triethylene glycol T3EG (PBT3, PAT3, PNT3): On comparing transport results of PBT3, PAT3, and PNT3, it is clear that PBT3 showed good transport ability than PNT3. The value of transport of three amino acids (Gly,Val,Thr) by PBT3 is double that of PNT3 the ratio of transport ability of PBT3 & PNT3 for Gly, Val, and Thr 1.5, 2.34, and 2.62, respectively, and the ratio of transport ability of PBT3 & PAT3 is 2.0, 1.81, and 2.17, respectively, for all the three amino acids (Fig. 7).

In the structure of PBT3 (Fig. 1a) a nitrile group is present, which a is strong hydrogen bond acceptor and this nitrile group is para-substituted, which shows excellent inductive property and helps in hydrogen bonding. Strong polar nature exhibit hydrophilic property therefore easily release amino acids to receiving phase and illustrate good carrier ability as compared to PAT3 and PNT3 as shown in Fig. 7.

Podands having tetraethylene glycol (T4EG) chain (PBT4, PNT4,PQT4,PAT4): The podands having T4EG chain, used in the present study are PBT4, PNT4, PQT4,PAT4 as shown in Fig. 1a. On comparing the results of transport ability of Gly, Val,Thr with these podands, the sequence of transport ability is: PBT4> PAT4 >PQT4> PNT4. PBT4 and PQT4 are ditopic and PAT4, and PNT4 are monotopic receptors. The value of transport of three amino acids (Gly, Val, Thr) by PBT4 is almost double for PNT4. The ratio of extraction efficiency of PBT4/PNT4 for Gly, Val and Thr is 2.34, 3.4, and 2.25, respectively, and the ratio of an extraction efficiency of PBT4/PQT4 for Gly Val and Thr is 1.91,2.95, and 1.65, respectively (Fig. 8).

The best carrier among these receptors is PBT4 which has hydrophobic exterior because of glycol chain (CH_2-O-CH_2) and hydrophilic nitrile (-CN) group. Easily show complexation and decomplexation of amino acids. The nitrile group interacts with the –COOH group of amino acids, and the glycol chain interacts with $–NH_3^+$ of amino acids. The benzonitrile group is more flexible than the anthraquinone moiety. Thus, PBT_4 exhibit high transport of amino acids and is selective for Val.

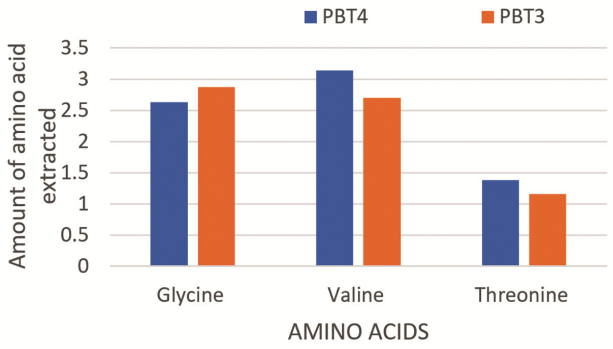


Fig. 5 — Extraction of amino acids (Gly,Val,Thr) by PBT3 and PBT4

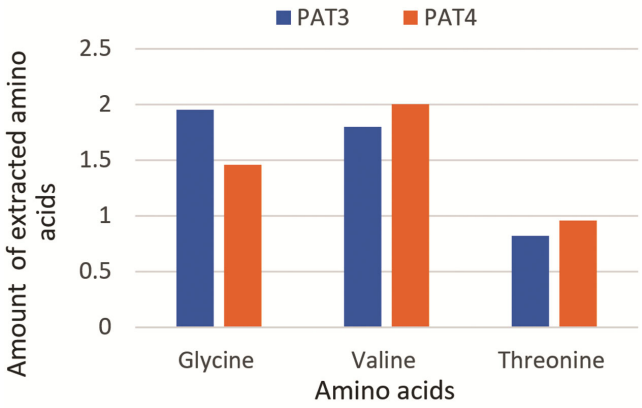


Fig. 6 — Extraction of amino acids (Gly,Val,Thr) by PAT3 and PAT4

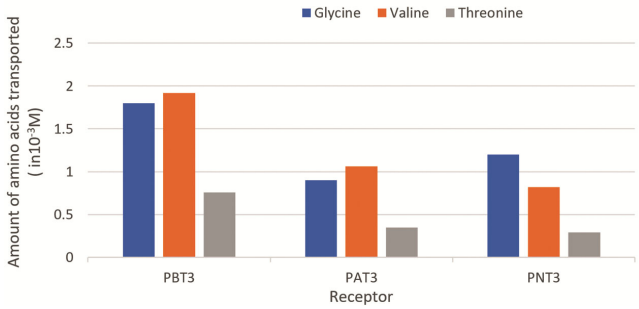


Fig. 7 — Transport of amino acids by PBT3, PAT3 and PNT3

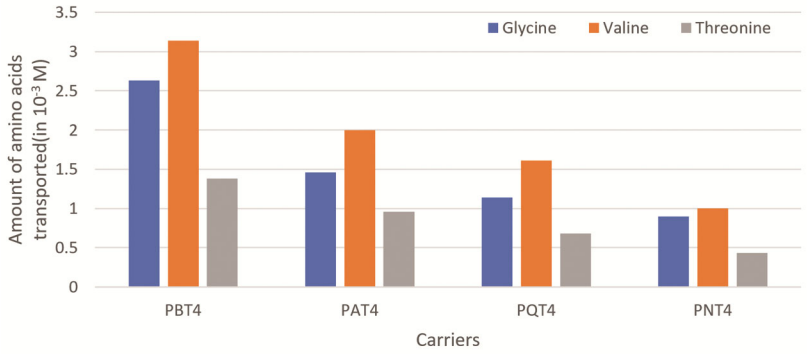


Fig. 8 — Comparison of transport of amino acids by PBT4, PAT4, PQT4 and PNT4

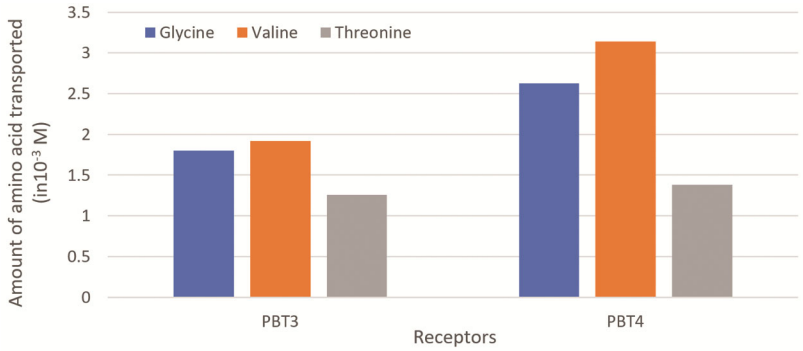


Fig. 9 — Transport of amino acids (Gly, Val, Thr)by PBT3 and PBT4

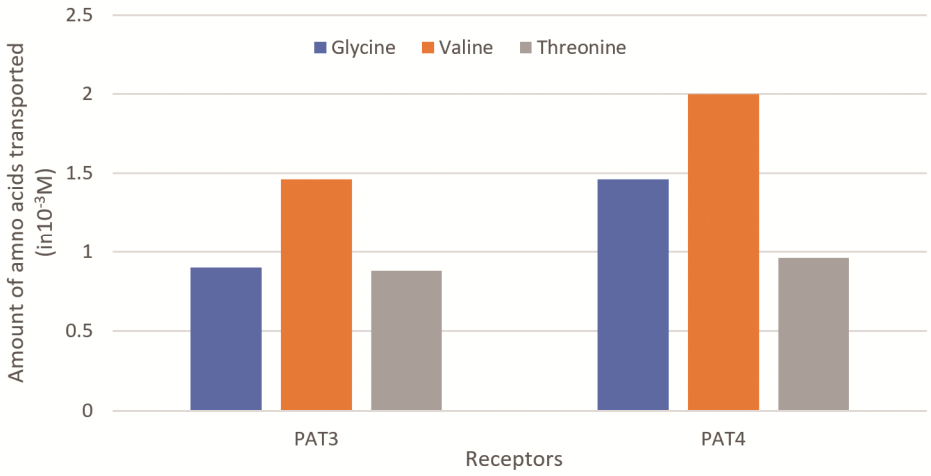


Fig. 10 — Transport of amino acids (Gly,Val,Thr) by PAT3 and PAT4

***Effect of chain length*:**

Podands (PBT3 and PBT4) (PNT3 and PNT4) (PAT3, PAT4): On comparing the results of transport ability on the basis of chain length of podands (PBT3 and PBT4), (PNT3 and PNT4), (PAT3, PAT4), it is observed that PBT3, having T3EG chain, is selective for Val and PBT4, having T4EG chain is selective for Gly. Podands having anthraquinone end groups (PAT_3 and PAT_4) shows poor carrier ability. (Figs 9 and 10).

Podands having quinoline moiety as an end group (PQT4) exhibits better transport efficiency for amino acids due to their flexible nature that permits effective conformational changes in the binding and releasing process. The flexible ether chain can adopt itself $-NH_3^+$, whereas the insertion of the aromatic end group reduces the flexibility of the receptor responsible for transport ability. The CH-π interactions take place with quinoyl which stabilize

the conformation of the amino acid receptor complex.

The transport of amino acids across organic membranes takes place through carrier-facilitated active or uphill models. The amino acid (substrate) combines with the carrier (receptor) to form a complex at the source membrane interface. Amino acid-receptor complex diffuses towards the receiving phase and the decomplexation of the complex at the membrane receiving phase takes place. The amino acid diffuses in the receiving phase and the receptor molecule diffuses back towards the source-membrane phase and retains it is original conformation.

The sequence of carriers' abilities of all selected receptors (lariat ethers and podands) at optimal concentration of amino acids (Gly, Val, Thr) and receptors can be summarized as:

Gly: PBT_4 >PBT_3 >$LEAT_4$>$LEAT_3$> PAT_4>PQT_4> PNT_3>PNT_4>PAT_3
Val: PBT_4 > PBT_3>$LEAT_3$ >$LEAT_4$ >PAT_3> PAT_4> PNT_3> PQT_4> PNT_4
Thr: PBT_4 > PBT_3 > PAT_4 >$LEAT_4$ > PQT_4 >$LEAT_3$ >PNT_4 > PAT_3 > PNT_3

Nature of amino acids

Participation of amino acids in a great variety of metabolic and biochemical processes makes them the most studied compounds, especially regarding their transport through biological membranes. In the transport of amino acids, receptors are required to recognize them using biological interactions such as hydrogen bonding, π-stacking, and hydrophobicity reactions across artificial bulk liquid membrane systems. The most hydrophobic Val was great extent as compared to the Gly and Thr amino acids. The sequence of the amount of amino acids (Gly,Val,Thr) transported at optimal concentration can be summarized as:

LEAT3, PBT3, PAT3, PNT3, PNT4: Gly>Val>Thr
LEAT4, PBT4, PQT4: Val >Gly>Thr

Conclusion

On comparing the results of extraction, BLM podands PBT3 and PBT4 show higher extraction and carrier ability among all the podands in the structure of receptors PBT3 and PBT4 both have unique characteristics because of the nitrile group on both the terminals of glycol chain which affect the extraction and carrier ability of the receptors. PBT3 and PBT4 both have a hydrophobic exterior (CH_2-O-CH_2) and nitrile group is sp hybridized 'C' atom is present that is triply bonded to an "N" atom with a lone pair forms hydrogen bonding as a hydrogen bond acceptor with amino acids. and, the unique structural characteristics of nitrile groups (having linear shape) enable them to radially interact with amino acids.and nitrile group is also polar in nature which makes it hydrophilic therefore it easily releases also amino acids from the source phase to receiving phase thus it is also a better carrier for amino acids. In the case of podands having anthraquinone, it is observed that podands having anthraquinone end groups (PAT3, and PAT4) are good extractants but poor carrier and naphthyl end groups having podands (PNT3, PNT4) showed less extraction efficiency, but better carrier ability then PAT3 and PAT4. It is also observed from the results extraction of glycine is more in PBT3 while transport is less. and also,the extraction of valine is more in PBT4 while transport is less.which proves that more interaction less transport phenomenon. In our studies, it is found that valine is selective for PBT4 thus it can be used in the separation of valine, and also studies its dimer and tetramer formation[19] which is a useful application in chemical technology[20].

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