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Extraction and Bulk Liquid Membrane Transport of Charbohydrate ,Glucose, Fructose, Lactose Across Artificial Liquid Membrane System Using Anthraquinone Derived Podands

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Abstract- Redo switched anthraquinone derived podands 1-(1- anthraquinonloxy), 3, oxapentane – 5 – methane (T_1), 1,5 bis (2-(2-(2- hydroxyethoxy) ethoxy)ethoxy)anthracene-9-10 dione (T_2),1, 5 bis-(2-(2-hydroxyethoxy) ethoxy) ethoxy) anthracene-9-10 dione (T_3) have been synthesized by condensation of chloroanthraquinone,1,5dichloroanthraquinone with triethylene glycol, diethylene glycol,methoxy methanol in THF under N_2 atmosphere. The characterization has been done by m.p., TLC and IR, NMR spectral analysis. These receptors used for the liquid-liquid extraction, bulk liquid membrane transport of glucose, fructose, lactose. The trend observed for extraction of sugar with these receptors is lactose>glucose.>fructose and that for is fructose>glucose>lactose. Among all receptors T_3 shows higher extraction and transport than T_2, T_1 due to more number of donor sites in T_3 Bibracchial receptors are good extractant but poor carrier than single armed receptor. Extraction and transport ability enhanced in reduced state.

Keywords*-Bulk liquid membrane, Bibracchial* receptors

INTRODUCTION

The chemistry of saccharides plays a significant role in the metabolic pathways of living organisms. Recognition of charbohydrate is the challenging problem in supramolecular chemistry. The recognition of d-glucose is of particular interest, for example in the monitoring of diabeties.

Smith and coworkers[1] investigated ability of monoboronic acid to transport sugar thorough lipid bilayers. Takeuchi *et al.*[2] used boronic acid system for sugar extraction. Boronic acid-based saccharide receptors offer the possibility of creating saccharide sensors 'chemosensory' which are selective and sensitive for any chosen saccharide. With this importants of charbohydrate ,we tested charbohydrate as substrate.

The anthraquinone group[3] has proven to be the most versatile and useful redox active groupas its value in electrochemistry is more appreciated. Keeping this in view redox switched receptors based on anthraquinone derived podands were synthesized and used in the liquid membrane carrier facilitated transport studies of glucose, fructose, lactose.

MATERIALS AND METHODS

The reagents used for the synthesis of receptors are chloroanthraquinone, 1, 5Dichloranathraquinone (Lanchaster), Sodium hydride (MERCK)., diethylene glycol, triethylene, glycol ,methoxy methanol(Fluka). Solvents CH_2Cl_2, THF, CH_3OH, C_6H_6, $CHCl_3$, CH_2Cl_2 and CCl_4 were obtained from Qualigens.

Instruments –

Melting point was measured by the melting point apparatus (Boss 165), Elemental analysis (C, H,N) were obtained from CDRI Lucknow using elemental analyser (Carlo Erba 1108), IR Spectra were recorded in FTIR and ^{1}HNMR spectra recorded on NMR spectrophotometer (varian 350) at 400 MHz, Amino acids were estimated by spectrophotometer.

Preparation and Characterisation of Redox Switched - anthraquinone derived podands containing single arm (T_1)

1-(1- anthraquinoyloxy)-3 methoxy methane (T_1)

A solution[4]of methoxy methanol (0.8 mL) in THF (10 mL) was added to vigorously stirred suspension of NaH and the mixture was refluxed for 30 min. Then a solution of 1- chloroanthraquinone (2.42 g) in THF was added to it and refluxed for 10 hr. with stirring. This reaction was performed under N_2 atmosphere at 80^{o}C. This reaction mixture was concentrated and the residue was mixed with CH_2Cl_2 and then washed with water (twice) then with brine. The organic phase was separated and dried over $MgSO_4$, filtered and concentrated. Column chromatography followed by recrystalization with

CH_2Cl_2/ hexane then ethanol and characterization by m.p. and TLC.
m.p. – 125°C Yield – 80%
IR $\bar{\nu}$: 1680 cm^{-1} (C=O), 2923 cm^{-1}(C-H), 1266 cm^{-1} (Ar-O-CH_2) 1059 cm^{-1} (CH_2OCH_2), 801 cm^{-1} (Ar-H)
^{1}H NMR δ 3.93 (CH_2), 7.26 – 7.99 (Ar-H),
Elemental analysis – Calculated: C-76.32% H- 4.31%
found: C(77%),H(4.9%)

Preparation and Characterisation of Redox Switched-anthraquinone derived podand containing double arm (A_2)

1,5 bis (2-(2-(2- hydroxyethoxy) ethoxy)ethoxy)anthracene-9-10 dione (T_2)

$A_2$was synthesized by condensation of 1,5-dichloroanthraquinone (2.77 g) with two ratio of triethylene glycol (2.68mL) in presence of NaH(0.48g.) under N_2 atmosphere.
m.p. – 90°C
Yield – 44%
IR Peak: 1675 cm^{-1} (C=O) 3074 cm^{-1} (C-H), 1265 (Ar-O-CH_2) 1202 cm^{-1}
(CH_2OCH_2), 803 cm^{-1} (Ar-H)
^{1}H NMR : δ 4.78 (OH), 3.70 (CH2), 7.06 – 7.41 (Ar-H)
Elemental analysis : Calculated:C-61.80%, H-6.84%,
found: C-(61.40%), H-(6.34%)

Preparation and Characterisation of Redox Switched-anthraquinone derived podand containing double arm (T_3)

1,5 bis -(2-(2- hydroxyethoxy) ethoxy)ethoxy)anthracene-9-10 dione (T_3)

T_2,T_3were synthesized by condensation of 1,5-dichloroanthraquinone (2.77 g) with two ratio of diethylene glycol (1.9mL),triethelene glycol in presence of NaH(0.48g.) under N_2 atmosphere as shown in scheme.
m.p. – 80°C Yield – 60%
IR $\bar{\nu}$: 3418 (O-H), 1676 cm^{-1} (C=O), 3093 cm^{-1} (C-H), 1265 (Ar-O-CH_2)
1131 cm^{-1} (CH_2OCH_2), 806 cm^{-1} (Ar-H).
^{1}H NMR δ 4.78 (OH), 3.70 (CH_2), 7.06 -7.41 (Ar-H)
Elemental analysis : Calculated: C-64.5%, H- 5.84%,
found: C-(63.46%), H-(5.76%)

Estimation of glucose, fructose and lactose by spectrophotometric method

Nelson Somogyis[5] method was used for glucose, fructose, Lactose estimation, using two solutions, Nelson solution (A) and Nelson solution (B).

Procedure:
Nelson solution (A) was prepared in four steps.

Step (I) – 24g Of sodium carbonate and 12gm of sodium potassium tartarate has been dissolved in 300mL distilled water to get solution(i).
Step (II) - 4g of copper sulphate has been dissolved in 40 mL distilled water to mget solution (ii).
Step (III) - solution (ii) was added to solution (i), mixed slowly with stirring and then 16gm of sodium bicarbonate was added to obtain solution.

Step (IV)- 180 g of sodium sulphate has been dissolved in 500 mL of hot water and then boiled to expel air then cooled to room temperature, this solution was then added to solution (iii) and the volume was made up to one liter. After 6-7 days this solution was used for estimation.
Nelson solution (B)- 25 gm of ammonium molybdate has been dissolved in 450 mL distilled water and then 21mL of concentrated H_2SO_4 has been added slowly and mixed with glass rod. To the above solution, 3 g Of sodium arsenate solution was mixed slowly (which has been already prepared in about 25 mL water) and the volume was made up to 500mL.

Extraction Procedure-

For extraction[6], 10 mL of sugar solution was vigorously stirred with 10 mL of receptor solution in an organic solvent ($CHCl_3$) in a small beaker using magnetic stirrer. The beaker was covered and stirred for 4 hours. After 4 hours, the mixture was allowed to stand for 5 minutes for the separation of two phases. The depleted aqueous phase was removed and the amount of sugar extracted by the receptor was determined by its difference in concentration in aqueous phase before and after extraction by spectrophotometer.

Bulk Liquid Membrane System (BLM)

Transport experiment[7] were performed in a U-tube glass cell in which 25 mL of receptor solution in $CHCl_3$ was placed in the bottom of the U-tube serving as the membrane. 10 mL of aqueous solution of sugar was placed in one limb of the U-tube serving as source phase and 10 mL of double distilled water was placed in another limb of the U-tube, which served as the receiving phase. The two aqueous phase i.e. source and receiving phase floating on the organic membrane phase respectively in two limbs of the U-tube. The membrane phase was constantly stirred using magnetic stirrer. The samples were withdrawn from source and receiving phase after 24 hours and amount of sugar transported was determined by Spectrophotometer.

RESULTS AND DISCUSSION

Extraction and transport studies of Sugar(Glucose, Fructose, Laqctose)by receptor (T_1 to T_3).

The result of extraction and transport studies of fructose, glucose and lactose with receptor (T_1 to T_3) through $CHCl_3$ organic bulk liquid membrane system have been studied and reported in Table 1 to 2. Blank experiments were carried out for extraction and transport studies for fructose, glucose and lactose in which the membrane was devoid of receptor. No leakage of fructose, glucose and lactose from source phase into organic layer was observed. All measurements were performed in duplicate to check the reproducibility.

Extraction and transport studies of substrate with redox switched receptors (T_1 to T_3) have been carried out with two different form of receptors i.e. oxidized state, and reduced state. The reduction of receptors was accomplished by using reducing agent Zn/NaOH.

Fructose, glucose and lactose concentration was varied from $1x10^{-3}$ M to $3x10^{-3}$ M and the concentration of receptor was kept constant in both oxidized as well as reduced state. Receptor concentration was varied from 1 x 10^{-2} M to 1 x 10^{-4} M keeping the concentration of fructose, glucose and lactose constant at $3x10^{-3M}$

T_3 shows higher extraction and transport than T_2, T_1, this can be explained on the basis of long chain length of tetraethylene glycol with more number of donor atoms in T_3 while T_2, T_1 having small chain length of diethelene glycol, methoxy methanol. Extraction increases with increased number of donor sites[8]

Maximum binding sites for interaction indicates formation of hydrogen bond[9] between amino groups of amino acid. The trend observed for extraction of sugar with receptor T_1-$T_3$is Lactose >Glucose> Fructose& for transport of sugar[10-11] with receptor fructose>glucose>lactose.

Lactose shows more extraction but poor transport than glucose, fructose this is explained on the basis of hydrophobicity of lactose is more than glucose, fructose so it shows more extraction. Glucose, fructose shows more affinity towards water so they get release at receiving phase and resulted in more amount of transport.

Double armed receptors are better extractant but poor carrier, which is explained as double arm receptor have two symmetric double arm make the conformation more stable and holding the substrate molecule than single arm receptor which resulted in more amount of extraction. The carrier ability of receptor for the transport of sugar is decreased in comparison to extraction ability. These results suggest that rate of transport depends on the release of molecules rather than uptake.

Counter transport of glucose, fructose, lactose with Na^+ion by receptor A_3 enhances transport amount of sugar as shown in Table No. 3.The amount of glucose lactose, fructose transported is found to be increased in the presence of Na^+ion as counter ion this is because of the development of the potential gradient due to Na^+ion transport from receiving to source phase as shown below.

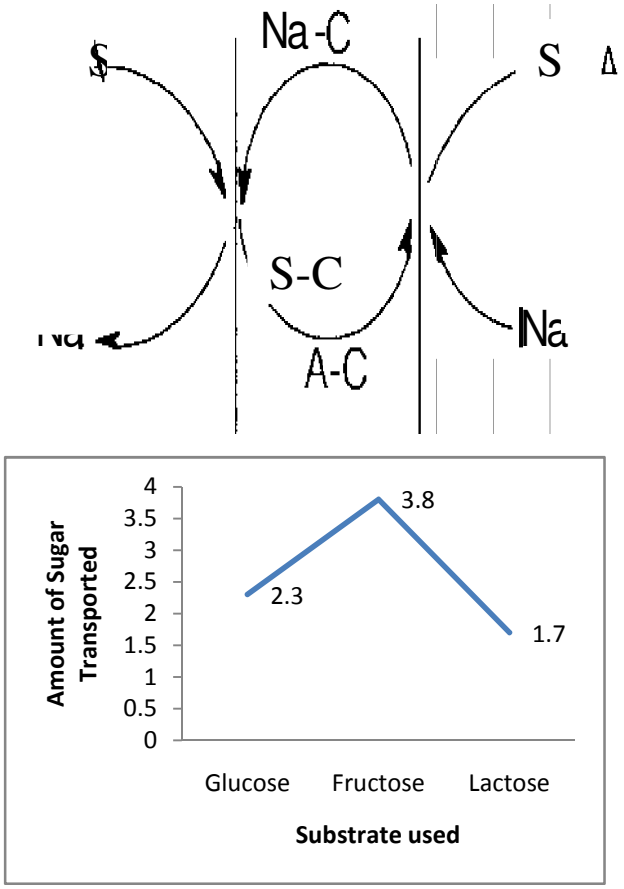

Figure 1 -Counter transport Mechanism of Sugar

Anthraquinone derived podands have been used for extraction and transport studies of glucose fructose and lactose and performed in reduced as well as in oxidized state of the receptor. In reduced state, anthraquinone undergoes one and two electron reduction to form either radical anion or dianion which co-operate to bind with substrate as compared to oxidized state of the receptor.

The amount of fructose, glucose and lactose extracted and transported mainly depends upon the structure of receptor and their concentration and also on the concentration of fructose, glucose and lactose. Results are listed in Table 1,2,3

CONCLUSION

Extraction ability enhanced in the reduced state.The experimental results suggest that extraction and transport of sugar essentially controlled by the structure of receptor, the nature of sugar, concentration of receptor and sugar.

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Table No:1
Amount of sugar extracted after 4h into an organic phase conc. of receptor – 1x 10^{-2}M

Conc. of sugar (M)	Amount of sugar extracted in 24h (1x10^{-3}M)					
	T_1		T_2		T_3	
	Oxidized state	Reduced state	Oxidized state	Reduced state	Oxidized state	Reduced State
Glucose						
3x 10^{-3}	1.2	2.9	3.3	5.6	4.7	6.3
2x 10^{-3}	0.9	1.7	2.6	4.5	3.6	5.8
1x 10^{-3}	0.7	1.6	1.9	3.2	2.4	4.0
Fructose						
3x 10^{-3}	0.8	2.5	4.1	4.5	4.8	5.1
2x 10^{-3}	0.5	1.6	3.0	3.4	3.7	4.0
1x 10^{-3}	-	1.0	2.5	2.7	2.9	3.2
Lactose						
3x 10^{-3}	2.6	3.5	4.8	6.4	5.3	7.5
2x 10^{-3}	1.7	2.2	3.5	4.6	4.3	6.2
1x 10^{-3}	1.2	1.7	2.6	3.2	3.7	4.9

Table No :2

Amount of sugar transported after 24h into an organic phase

conc. of receptor – 1x 10^{-2}M

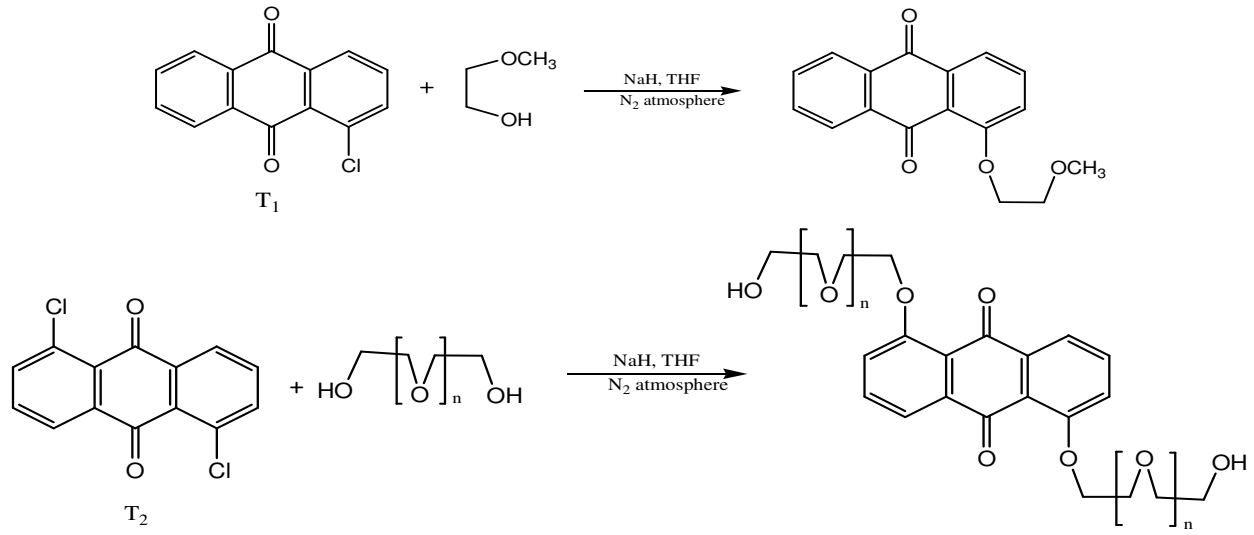
Conc. of sugar (M)	Amount of sugar transported in 24h (1x10^{-3}M)					
	T_1		T_2		T_3	
	Oxidized state	Reduced state	Oxidized state	Reduced state	Oxidized state	Reduced State
Glucose						
3x 10^{-3}	0.9	1.9	1.4	2.0	1.01	2.74
2x 10^{-3}	-	-	1.0	1.2	0.80	1.32
1x 10^{-3}	-	-	0.9	1.0	0.43	1.10
Fructose						
3x 10^{-3}	1.2	1.8	1.5	2.9	2.12	3.96
2x 10^{-3}	-	0.8	-	0.7	0.98	1.55
1x 10^{-3}	-		-	0.4	0.77	1.34
Lactose						
3x 10^{-3}	-	0.6	-	1.0	0.8	1.90
2x 10^{-3}	-	0.1	-	0.2	0.69	0.39
1x 10^{-3}	-	-	-	-	0.41	-

Table No :3

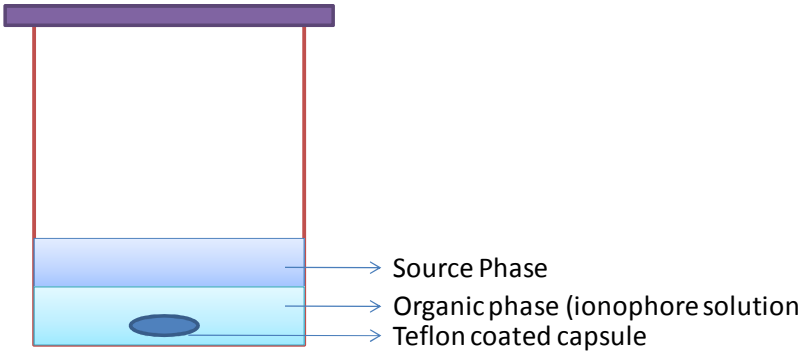
Amount of sugar transported with Na^+ after 24h into an organic phase by receptorT_3

conc. of receptor – 1x 10^{-2}M
Conc. of sugar– 3x 10^{-3}M

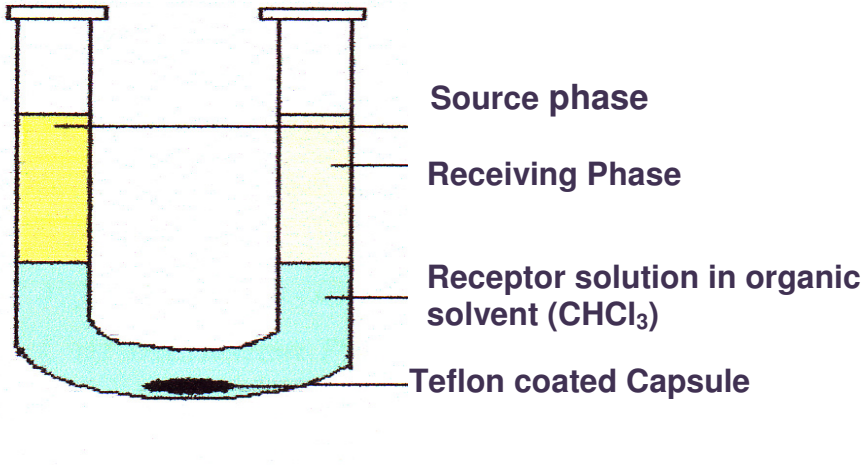
Sugar	Amount of sugar transported with Na^+ after 24h in(1x 10^{-2}M)
Glucose	2.3
Fructose	3.8
Lactose	1.7



Scheme



Apparatus set up for Extraction fig. 1



Apparatus for bulk liquid membrane transport fig. 2

