International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Phase Interactions of DL Alanine, hexa-glycine, NH_4Cl, NaCl, KCl, NaBr, KBr, and KI in Aqueous Two-Phase Systems of poly (ethylene glycol) 600 + dextranT(40) at 293.15 K

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Abstract: ***Apparent molar volumes, apparent molar expendability helps inphase interactions ofsalts (NH4Cl, NaCl, KCl, NaBr, KBr, and KI) with two amino acids (DL Alanine, Hexa-glycine) were measured in dextran 40+ poly(ethylene glycol)600 + water aqueous two-phase system. The design and basic engineering of such processes requires the knowledge of the liquid-liquid equilibrium, infractions and partitioning of small amount solute (approximately 0.0001 g solute per gram of solution). The coefficient for partition was correlated by using the osmotic viral equation. It uses surface fractions to encounter for the probability of interactions between solutes in solutions.The phase interaction coefficient in the equation can be calculated by hydrophilic group's parameters which are in fairly good agreement with the experimental data at 293.15K and room pressure (0.97atm).***

Keywords: Partition coefficient, Apparent Molar Volume and Expendability, Hydrophilic parameter

1. Introduction

A predictive method for the partition coefficients (Kp) of several amino acids [1,15] in the dextran [DEX] poly(ethylene glycol)[PEG] + water aqueous two phase system has been proposed(Zhou et al. 1997) by using the osmotic virial equation(Edmand and Ogston, 1968; King et al.1988). Salt effect on charged protein partitioning [13] in aqueous PEG+ DEX system by potential (non electrostatic from electrostatic effect) proposed by Weiyu Fan and Charles E. Glatz (1999). The liquid-liquid interaction also helps in salting out effects [3,4,5]. There the viral coefficient needs in calculation of KP were given by the hydrophilic parameters [9,10] . Solvation dynamics has been the focus of intense research activities for the past few years. Measurements of some bulk properties like viscosity, apparent molar volumes, apparent malar expansibility etc provide insight into intermolecular arrangement of the component with solutions. The hydrophilic group parameters helps in pharmaceutical industry for recovery with density behavior [6,7]. It is of interest to extend this procedure to larger molecules such as peptides. In this study KP values for two peptides (hexaglycine and DL alanine) in the same aqueous two phase system were measured to determine new hydrophilic group parameters [5,6] required for particles behavior and their solubility and salting out effect [13] in two phase system. Prediction performance of the present method for Kp,V0, E0 and related acoustic parameters has been determined at 1.0-10 milimole and Wt% w/vvalues for [11] peptide (hexaglycine and DL alanine) and some salts in PEG + DEX + water is presented and discussed.Aim was to develop a generalized molecular frame work to establish solubility behaviour and interaction kinetics which help as drug delivery, salting in and out effect in the field of medical bio-medical and pharmaceutical science.

2. Experimental

2.1Materials

Dextran (DEX) was purchased from Merck as DEX -T 40(MN=18800, mw=3800) ,and . Poly (ethylene glycol) (PEG 600) standard sample was purchased from E. Merck Pure Chemical Ind. as PEG600(MN= 550, MW=580). DL Alanine, Hexa-glycine, NH4Cl, NaCl, KCl, NaBr, KBr, and KIwere purchased from Sigma Chemical Company. All reagents were used A R grade and used without further purification. Triple distilled degased water was purified with a Millipore Milli-Q system in acidify KMnO4 solution.

2.2. Method

2.2.1Aqueous Two Phase System

Phase interactions were carried out in thermostat at 293.15K. For constant temperature, water was rotated by continuous starring. Reaction flask was kept within the thermostat for constant temperature. Preparation of aqueous two- phase systems were prepared in triple distilled water with concentration range 0.001 $molL^{-1}$ to 0.010 mol L^{-1} for stand for 24 hr. to avoid any precipitation. The aqueous two-three component solution were kept into thermostat for 15min for constant temperature. Stock solution of PEG`s (600), (50wt%) and salts (20.5wt%5) were prepared in triple distilled water. All ATPS had a fixed composition of 11.1 wt% PEG, 6.5 wt% salts and .001M amino acids were prepared and appropriate amount of water added to TPS and concentration range 2.5-1.9M. These concentrations were again measured. Experimental results are reported for the partitioning of small amounts (approximately 0.001 g solute per gram of solution) of low molecular combination peptides of glycine, L-glutamic acid, L-phenylalanine.

The liquid-liquid equilibrium for PEG`s and Dxetran has recently been investigated. In order to determine the

partition co-efficients of the DL-Alanine and Hexaglycine in liquid system, two tie line were selected from each phase diagram. Presentation of the two phase samples were performed in 20 ml graduated glass bottles tightly closed. The total wt. of the component was about 7.5gm. The initial wt. of the DL-alanine and Hexaglycine added to system was $0.5X10^{-2}$gm. The mixtures were shaken for 20 min. and then placed in thermostatic water bath for at least 24hr. to ensure complete equilibrium. Syringes were used for samples approximately 5ml carefully removed for analysis from the top and bottom layer. The separated solutions were centrifuge for 20 m at 298.15K. Phase separating volume of each phases was marked tube and respected volume used for calculate mass of water for corresponding phase. Experiment repeated as tetraplicate and avg. volume of phase were calculated. Error be under 1.8%. and the uncertainty in the amino acid concentration for analysis of top and bottom phase was less than ±1.8 ml/L.

2.2.2 Partitioning Experiments

Solution of all component were prepared at room temperature in water at conc. 2-100mM depending their solubility(00,20,40,60,80,100μ L) of a given compound amt. (100,80,60,40,20,00μ L) were added to a fixed time. System kept for a day and centrifuges to accelerate phase settling. The partition co-efficient defined as the ratio of concentration of bottom and top phase, plotted as function of the conc. salt and polymer with deviation below4.5%.

3. Results and Discussion

The values of V_φ^o and Sv are obtained from the equation 1 are given in the table 1. (eq1,2 Masson Equation)

$$V\phi = V\phi^0 + Sv\sqrt{C} \qquad \text{eq-1}$$

Sv- Slope constant, C- conc., Apparent molar volume, AMV at ∞ conc.

The positive and large values for NH4Cl and alkali halides in aqueous DEX40 solution at the experimental temperature, indicates presence of strong ion-ion interaction and change with concentration. The ion-ion interaction was in more in aqueous DEX than water. As particular temperature Sv values decrease as concentration of DEX increases. $V\phi^0$values are negative for almost all salts indicate weak solute- solute interaction and provide evidence of electrostriction which increase at lower concentration . E^o and SE values obtained from equation 2 (table 1)were negative and excepting NaCl in 0.5 % and 1% .This indicates caging

$$E\phi = E\phi^0 + SE\sqrt{C} \qquad \text{eq-2}$$

$E\phi$ -apparent molar expansibility, SE – slope constant, C-Conc.

$$Sn = n_1 n_2^{-1} [\,1 - V\,Ks(n_1 V_1^o Ks^o)^{-1}] \qquad \text{eq-3}$$

V- volume solution, 1,2-mole solvent and solute, Ks incentric compressibility at infinite dilution & packing effect. Solvation number (Sn)eq3 values used for the ref. (17) were in order of NH4Cl >KBr>KCl>DL alanine >hexa-glycine in 0.5 -1.0 % DEX solution .

The partition coefficients of the two amino acids(DL-alanine and hexa-glycine) in DEX T40 + PEG600 + water, aqueous two phase systems are presented in Table 2,and concentration ranges were 1.0-2.0 mili mole/ litre. The partition coefficients values were calculated w.r.t. tie line lengths at particular concentration. These data were obtained from an arithmetic average of at least three –four measurements for each condition. The some unequal distributions of these small molecular compounds especially for amines with lower ratio were established despite the experimental errors. The TLL (tie-lines Length) data of the same aqueous two-phase systems have been reported elsewhere (Furuya et al., 1995a, 1996).

The partition coefficient, kp, and tie –line length are defined, respectively, as follows.

$$TLL = [(W1^{TOP} - W2^{BOT}) + (W1^{TOP} - W2^{BOT})]^{1/2} \qquad \text{eq- 4}$$

$$Kp = \frac{\text{The conc.of amino acids in PEG rich phase}}{\text{The conc.of amino acid in DEX rich top phase}} \text{mg/g} \qquad \text{eq- 5}$$

where W is the weight fraction and subscripts 1 and 2 indicate DEX and PEG, respectively. The accuracy of the experimental partition coefficient data is considered to be within ± 5% from the reproducibility.

From Table 2, it can be seen that KP of DL-alanine and hexa-glycine decrease with increasing tie-line length and KP of DL-alanine are reported to be smaller than those of hexa-glycine. At lower concentration of DL-alanine and hexa-glycine in DEX T40 + PEG600 + water were large difference in Kp values due to larger difference in TLL (Table2), it is shown that solvent –solute interaction are stronger than solvent-solvent interactions, which correlated KP values with the more hydrophilic groups (Table 3). : Effect of conc. on location of binodal curve at 293.15K (Table 3) were reported for PEG 600 with DL-alanine and hexaglycine. These effects on salt distribution are the result of change in solvent structure and of polymer-salt (Zwitter ion) and polymer-polymer interactions. Long chain of peptide bond will reduce PEG solubility in the Dex phase and salt solubility in the PEG phase, which leads to a more uneven distribution of polymers and amino acid between two phases.

Interaction Parameters of Osmotic Virial Equation

To predict of the KP values, the osmotic virial equation proposed by Edmond and Ogston (Edmond and Ogstomn, 1968; King et al. 1988) can be applied. The partition coefficient in the infinite dilute condition can be derived as:

$$\frac{\ln Kp^m}{m_2^{BOT} \square m_2^{TOP}} = a_1^4 \frac{\left[m^{1\,BOT} \square m^{1\,TOP}\right]}{\left[m^{2\,BOT} \square m^{2\,TOP}\right]} + a_2^4 \; eq \qquad 6$$

by plotting lnkp(m)/ (m2BOT-m2TOP) vs. (m1 BOT-m1TOP)/(m2 BOT-m2TOP), the interaction parameters a14 and a24 can be determined. The partition coefficient, Kp(m), expressed by the ratio of molalities can be transformed as follows by the experimental results of Kp,

$$Kp^{\infty}\frac{m_4^{TOP}}{m_4^{BOT}} = \left[\frac{1000 + M_1 m_1^{TOP} \square M_2 m_2^{TOP}}{1000 + M_1 m_1^{BOT} \square M_2 m_2^{BOT}}\right] \quad eq-7$$

where η is the hydrophilic parameter and is defined as follows:

$$\eta = \frac{\Sigma_i^{-1} \eta k N k}{\text{the number of total atoms of amino acids or peptide except hydrogen}}$$

$$eq-8$$

the number of total atoms of amino acid or peptide except hydrogen in equation, nk is the hydrophilic parameter of group k, and Nk is the number of group k contained in amino acid or peptide. The authors used the same values of ηi4 and βi4 (i=1, 2) proposed by Zhou et al. (1997) and determined the new values of nk by the data of Kp for salts and peptides obtained in this work (Table 2).The values of nk determined by Zhou et al.(1997) and obtained additionally in this work are shown in Table 4.

4. Conclusion

The partition coefficients shows for two amino acids hexa-glycine and DL- alanine in dextran+ poly (ethylene glycol) + water aqueous two phase were higher due to transition state accompanied by rupture of intermolecular interactions. The partition coefficients of amino acids and alkali salts were correlated by using the osmotic virial equation. The interaction coefficients are estimated by using hydrophilic group parameters and successful correlation results are obtained. The tie line lengths/ composition were correlated by hydrophilic group parameters and salting out ability. Present investigation on halides of alkali metals shows higher ion – ion interaction and minimum ion- solvent interaction. Furthermore, by using the same procedure, the partition coefficients of amino acids are predicted show fairly good agreement.

5. Acknowledgement

I am thankful to Deshbandhu college and their staff for proving space and valuable support.

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Tables

Table 1: Values parameters V_φ^o(m^3M^{-1}),Sv($m^{9/2}M^{3/2}$), E^o($m^3M^{-1}K^{-1}$) SE ($m^{9/2}M^{3/2}K^{-1}$) at 293.15K

Wt%	V_φ^o m^3M^{-1}	Sv $m^{9/2}M^{3/2}$	E^o $m^3M^{-1}K^{-1}$	SE $m^{9/2}M^{3/2}K^{-1}$
NH4Cl				
0.1	-23.00	804.816	-0.078	63.967
0.3	7.14	693.301	0.024	21.282
0.7	-45.45	505.455	-0.155	112.272
1.0	-95.85	576.788	-0.327	21.262

DL Alanine				
0.1	-48.857	329.27	-0.167	24.302
0.3	-38.837	243.13	-0.132	92.512
0.7	-75.25	324.120	-0.257	90.125
1.0	-93.003	51.022	-0.317	81.022
NaCl				
0.1	-34.65	214.67	-0.118	4.793
0.3	-102.95	505.455	-0.351	112.27
0.7	-63.458	324.120	-0.216	65.250
1.0	-112.011	576.789	-0.382	21.263
KCl				
0.1	-65.250	392.952	-0.222	24.292
0.3	-85.652	343.130	-0.292	92.512
0.7	-55.285	496.376	-0.186	81.796
1.0	12.450	501.022	0.042	81.022
KBr				
0.1	-168.336	584.65	-0.574	170.752
0.3	-48.725	284.141	-0.166	23.589
0.7	-75.25	109.207	-0.257	-5.538
1.0	21.396	47.365	0.030	-5.555
NaBr				
0.1	-42.365	284.141	-0.144	23.589
0.3	-15.281	534.25	-0.052	32.215
0.7	2.585	576.789	0.008	21.263
1.0	-0.756	496.376	-0.003	80.125
KI				
0.1	45.949	329.952	0.157	24.292
0.3	-53.443	234.130	-0.182	92.515
0.7	-185.23	534.25	-0.632	32.215
1.0	-293.445	1610.386	-1.001	47.521

Table 2: Experimental. Partition coefficients of D L Alanine and Hexa-glycine in aqueous two phase at 293.15 K.

Aqueous two phase	Conc. Mol L^{-1}	Tie- line Length Wt %	Kp							
			DL Alanine	Hexa-glycine	NH4Cl	NaCl	KCl	NaBr	KBr	KI
DEX T 40 + PEG 200 + Water	1.oX 10^{-3}	13.33	0.84	0.83	0.99	0.89	0.90	0.86	0.95	0.91
	1.3X 10^{-3}	18.16	0.74	0.69	0.97	0.85	0.89	0.88	0.93	0.90
	1.7X 10^{-3}	21.98	0.69	0.65	0.92	0.81	0.88	0.83	0.93	0.89
	2.0X 10^{-2}	24.84	0.68	0.63	0.94	0.72	0.89	0.81	0.91	0.91
DEX T 40 + PEG 600 + Water	1.oX 10^{-3}	08.53	0.90	0.84	1.01	0.91	0.92	0.89	0.96	0.92
	1.3X 10^{-3}	09.15	0.81	0.87	0.99	0.89	0.90	0.86	0.95	0.91
	1.7X 10^{-3}	10.48	0.78	0.63	0.99	0.85	0.89	0.88	0.93	0.90
	2.0X 10^{-2}	11.37	0.73	0.62	0.91	0.81	0.88	0.83	0.93	0.89
DEX T 500 + PEG 200 + Water	1.oX 10^{-3}	11.94	0.85	0.84	1.02	0.78	0.80	0.75	0.86	0.92
	1.3X 10^{-3}	13.33	0.80	0.81	1.01	0.75	0.81	0.74	0.88	0.91
	1.7X 10^{-3}	17.00	0.77	0.76	1.04	0.77	0.79	0.79	0.90	0.88
	2.0X 10^{-2}	21.98	0.72	0.65	0.95	0.72	0.89	0.81	0.91	0.91
DEX T 500 + PEG 600 + Water	1.oX 10^{-3}	06.62	0.89	0.86	1.05	0.91	0.98	0.95	0.97	0.89
	1.3X 10^{-3}	09.56	0.80	0.78	1.02	0.92	0.96	0.96	0.96	0.85
	1.7X 10^{-3}	12.48	0.79	0.76	1.02	0.89	0.92	0.92	0.88	0.87
	2.0X 10^{-2}	14.48	0.74	0.69	0.98	0.77	0.99	0.91	0.90	0.82

Table 3: Effect of conc. on location of binodal curve at 293.15K

PEG200 Wt%	DL Alanine Ws%	Hexa-glycine Wt%	PEG600 Wt%	DL Alanine Ws%	Hexa-glycine Wt%
39.12	10.50	9.85	41.23	9.00	7.51
38.90	11.32	9.96	38.44	9.23	7.94
38.10	11.85	10.22	36.45	9.82	8.12
37.15	12.15	10.45	34.65	10.10	8.45
36.56	12.45	10.87	33.05	10.55	8.78
34.67	12.82	11.05	31.51	11.24	9.11
32.35	12.95	11.48	30.15	11.28	9.35
30.00	13.05	11.83	28.92	11.52	10.00
28.85	13.40	12.25	27.80	11.74	10.30

Table 4: Hydrophilic group parameters Ƞn at 303.15K (Reference values)

Group K	OH Alcohol	ACH	C-	NH	CHn	CONH	COOH	NH2	O-	N-
ηK	0.949	0.104	O.O79	0.062	0.0	2.3	0.75	0.432	0.57	1.13