Study of albumin conductivity in water and its reactions with transition metal ions

Banan A. Akrawi ,Ahlam M.J.Ali and Alaa Al-deen .M.Hani
Chemistry Department ,College of Science ,University of Mosul

(NJC)

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Abstract

The present work provide the measurement of the conductivity of bovine serum albumin in water at three different pH values (4,7 and 8.2) and at 37oC. The pH value which was chosen for this study is 7 which give almost straight line from the plot of specific conductivity against its concentration and because it is near the pH of blood serum.. Besides , the conductivities of albumin with each of solutions of some transition metal chlorides (MnCl2 ,FeCl2 ,CoCl2 , NiCl2 and CuCl2) at pH=7 were measured .The plot of specific onductivity against each mixture solution were discussed.

الخلاصة

في هذا البحث تم قياس توصيلية الألبومين أبقري في الماء وفي ثلاث قيم لل pH (4,7,and 8.2) ولقد تم اختيار قيمة pH=7حيث أعطت تقريبا خط مستقيم عند رسم العلاقة بين التوصيل النـوعي وتركيـز الألبومين للقيم المختلفة لل pH. فضلا عن هذا تم قياس توصيلية الألبومين مع كل مـن محاليـل كلوريـدات الفلزات الانتقالية($MnCl_2$,$FeCl_2$ $CoCl_2$, $NiCl_2$, $CuCl_2$) عند pH=7 وفي الماء عند درجة 37 مئوية . ولقد تم تفسير العلاقة بين تغير التوصيلية ضد تراكيز المحاليل بدلالة الامتصاص للكاتيونـات علـى سطح الألبومين.

Introduction

The interaction of proteins with small ions and molecules has become increasingly important to the chemist and the physician .Combination of proteins with small anions and cations [1-5] ,sulfonamides [6,7] , dyes [8] ,alkyl sulfates [9] ,faty acids [10] , and aromatic compounds [11] ,have been described.

The combination of chloride ion with human serum albumin has been investigated by two methods [12]. One is the measurement by conductance of the distribution of sodium chloride across a cellophane membrane with albumin on one side. The other utilized the electromotive force developed in a concentration cell with silver-silver chloride electrodes both half-cell of which contain sodium chloride, and one of which contains albumin.

The average number of chloride ions bound to each albumin molecule can be calculated.

The complexes formed by the interaction of the amphiphilic hydrochloride and human serum albumin (HAS)in aqueous solution at pH=3.2,4.9 (the isoelectric point) and 6.0 ,were investigated at 25oc using a range of physiochemical techniques [13] . The complexation process was investigated by conductometric measurements on HAS amitriptyline solutions of increasing drug concentration from which values of the critical concentration at which absorption of drug connected and also the critical micelle concentration of ampitriptyline in the presence of protein were determined .The number of adsorption sites was determined from the observed increases of the ξ – potential as a function of drug concentration.By static and dynamic light-scattering techniques the molar mass and hydrodynamic radius of the HAS –amitriptyline complexes were measured [13] .

The conformation of bovine serum albumin (BSA) as well as its interactions with negatively charged mica surface in saline solutions of different pH values ,have been studied by small-angle neutron scattering (SANS) and chemical force microscopy (CFM) [14] . The combination of the two techniques provides new insight to understand the interactions and conformation of BSA molecules.

The interaction of magnetic iron oxide nanoparticles with bovine serum albumin was investigated by fluorescence, ultraviolet visible absorption, Raman and circular dichroism spectroscopy. The result suggested that the interaction was spontaneous and the electrostatic interactions played key roles in the reaction process[15] .

The interactions between bovine serum albumin and gemini surfactants derived from cystine have been investigated and were compared with the conventional single – chain surfactant derived from cysteine . Effect of pH, temperature, electrical conductivity and surface tension measurements were used to obtain important system parameters such as critical aggregation concentration, degree of ionization, and the amount of surfactant binding to protein (M) [16].

The complexations between human serum albumin and the sodium perfluoroctanoate / sodium octanoate and sodium perfluorooctanoate / sodium dodecanoate systems have been studied by a combination of electrical conductivity , ion – selective electrode , electrophresis , and spectroscopy measurements . Positive cooperative binding has been found, thus revealing the importance of the hydrophobic interactions in both kinds of surfactants [17].

The present work undertaken in an attempt to clarify the general principles involved in protein – small ion interaction .For these purposes bovine albumin and some transition metal chlorides ($MnCl_2$, $FeCl_2$, $CoCl_2$, $NiCl_2$ and $CuCl_2$) were selected as a relatively simple system to study such a system in our laboratory using conductivity technique.

Experimental

Conductivity water was prepared by redistilling distilled water three times with the addition of a little amount of potassium permanganate and small pellets of KOH to obtain deionized water . The specific conductance of this water was less than 102×10^{-6} Siemens cm^{-1} . The transition metal chlorides ($MnCl_2$,$FeCl_2$,$CoCl_2$, $NiCl_2$ and $CuCl_2$) and Bovine albumin were purchased from B.D.H and used without farther purification .Conductivities of solutions of salts and salts / albumin complex were measured with Jenway PCM3 meter . A water bath (HAAKS NK22) was used to maintain the temperature at 25 ± 0.1^{o}c and the conductance was measured after thorough mixing and temperature equilibrium .The pH was measured by pH-meter by Hana instruments microprocessor.

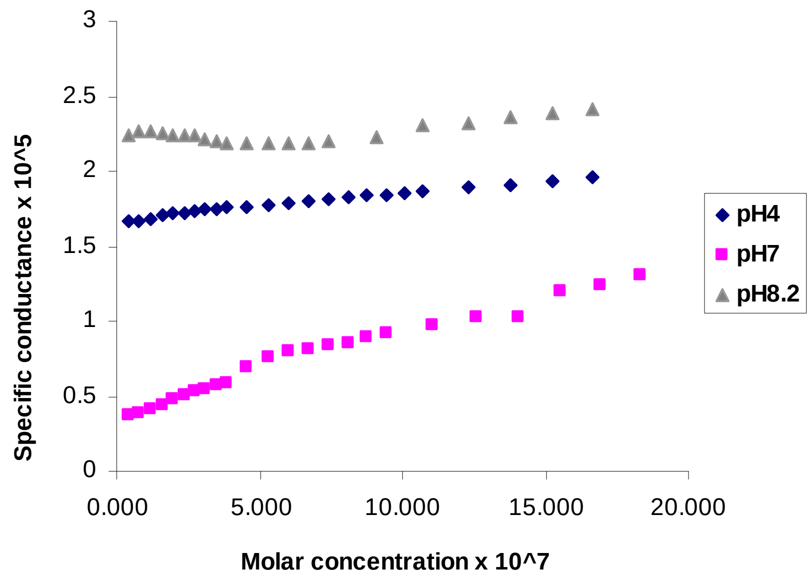
Stock solution of Bovine serum albumin of 1x10-5 M and stock solution of each salt of 1x10-3 M concentration were prepared for the experiments A certain amount (25 ml) of conductivity water was placed in the conductivity cell at constant temperature 37oc (±1) for a constant time (few minutes) and then the pH and the conductivity were measured .Then 5ml of stock solution of albumin was added to the conductivity cell and again the conductivity was measured .Finally ,small amount of stock solution of salt solution at 37oc was added to the cell then the conductivity of the solution was measured .The last procedure was repeated for about (20) times for each run.

Results and Discussion

The variation of specific conductivity of solutions of albumin (after subtraction of the contribution of the solvent to the conductivity) with its concentration at three different pH values (pH 4,7 and 8.2)are shown in Table 1 and Fig.1 .

Table (1): Variation of specific conductivity (L) of solutions of albumin with its molar concentration at three different pH values (pH 4, 7 and 8.2) .

pH 4		pH 7		pH 8.2	
Specific conductance $ohm^{-1}.cm^{-1}x10^{5}$ (L)	Concentration $M/Lx10^{7}$	Specific conductance $ohm^{-1}.cm^{-1}x10^{5}$ (L)	Concentration $M/Lx10^{7}$	Specific conductance $ohm^{-1}.cm^{-1}x10^{5}$ (L)	Concentration $M/Lx10^{7}$
1.66	0.398	0.377	0.398	2.23	0.398
1.67	0.794	0.388	0.794	2.26	0.794
1.68	1.19	0.418	1.19	2.26	1.19
1.7	1.57	0.439	1.57	2.25	1.57
1.71	1.96	0.479	1.96	2.24	1.96
1.72	2.34	0.51	2.34	2.23	2.34
1.73	2.72	0.53	2.72	2.23	2.72
1.74	3.1	0.551	3.1	2.21	3.1
1.74	3.47	0.571	3.47	2.2	3.47
1.75	3.85	0.592	3.85	2.19	3.85
1.76	4.58	0.694	4.58	2.19	4.58
1.77	5.3	0.765	5.3	2.19	5.3
1.79	6.02	0.796	6.02	2.19	6.02
1.81	6.72	0.816	6.72	2.19	6.72
1.82	7.41	0.836	7.41	2.2	7.41
1.83	8.09	0.857	8.09	2.22	9.09
1.84	8.76	0.898	8.76	2.31	10.7
1.85	9.42	0.918	9.42	2.33	12.3
1.86	10.1	0.979	11	2.36	13.8
1.87	10.7	1.03	12.6	2.39	15.3
1.89	12.3	1.19	14.1	2.42	16.7
1.91	13.8	1.24	15.5		
1.94	15.3	1.31	16.9		
1.96	16.7	1.36	1.83		



Figure(1):Plot specific conductance against molar concentration

Fig. 1 shows that at pH 7 the plot is almost straight line since there is some association between molecules of albumin.

The pH which is chosen is 7 since it is the same pH of human body and the relation between specific conductivity and albumin concentration is more clear. In an attempt to study the nature of interaction between human serum albumin with different metal ions ,the data of conductivity of solutions of each of certain concentration of ($MnCl_2$,$FeCl_2$,$CoCl_2$, $NiCl_2$ and $CuCl_2$) at pH (4,7 and 8.2) are shown in Table 2a-e and Fig.2 a-e and each one with albumin at pH 7 are shown in table 3a-e and Fig.3a-e . Fig.2a-e show that the relation between specific conductivity and concentration for each metal chloride at the three different pH values show also a clear association between each metal ion itself.

Table(2a): Variation of specific conductivity (L) of solutions of $MnCl_2$ with its molar concentration at three different pH values (pH 4,7 and 8.2)

Concentration M/L x 10^5	Specific conductancex10^5		
	pH4	pH7	pH8.2
0.3322	0.0816	0.7854	0.0714
0.6622	0.1428	0.8568	0.153
0.9901	0.2346	0.9078	0.2346
1.315	0.3264	1.02	0.3264
1.639	0.408	1.1016	0.3876
1.9608	0.4896	1.1832	0.4692
2.2801	0.5712	1.2648	0.5406
2.5974	0.6426	1.3464	0.6222
2.9126	0.7242	1.4178	0.7038
3.2258	0.8058	1.5096	0.744
3.8462	0.969	1.6626	0.9078
4.4586	1.1118	1.8156	1.0506
5.0633	1.2546	1.9686	1.1934
5.6604	1.377	2.1216	1.3362
6.25	1.5198	2.2746	1.4688
6.8323	1.6728	2.4174	1.6218
7.4074	1.7952	2.5704	1.7544
7.9755	1.9584	2.7132	1.9074
8.5366	2.0808	2.856	2.0298
9.0909	2.2236	2.9988	2.142
10.448	2.5704	3.3354	2.4072
11.765	2.8968	3.57	2.7846
13.043	3.1824	4.0086	3.0906
14.286	3.468	4.3146	3.2742

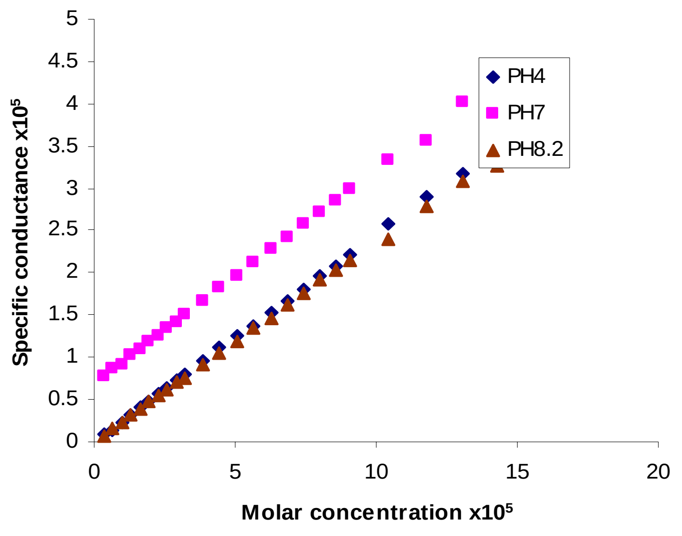
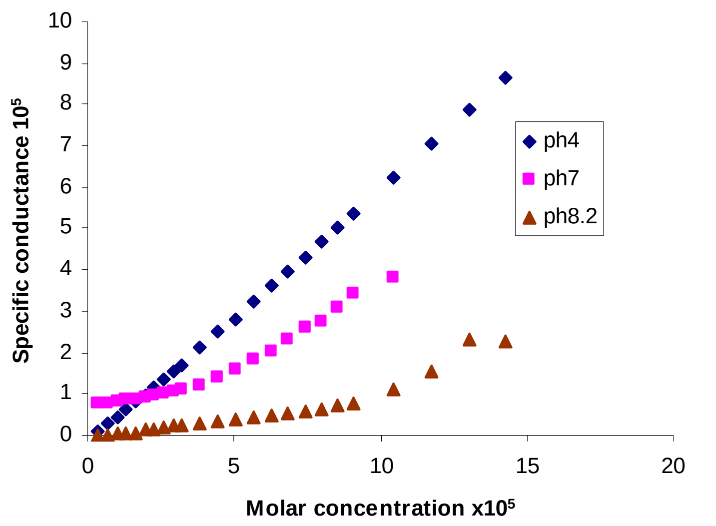


Figure (2a):Plot of specific conductance against molar concentration

Table(2b): Variation of specific conductivity (L) of solutions of $FeCl_2$ with its molar concentration at three different pH values (pH 4,7 and 8.2)

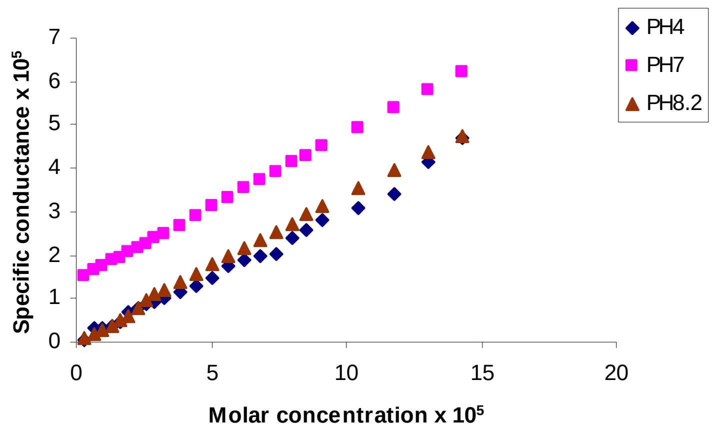
	pH4	pH7	pH8.2
0.3322	0.1122	0.765	0.0102
0.6622	0.2856	0.7854	0
0.9901	0.4488	0.816	0.0306
1.3158	0.6324	0.8466	0.0612
1.6393	0.8058	0.8874	0.0714
1.9608	0.9792	0.9282	0.1326
2.2801	1.1628	0.969	0.1632
2.5974	1.3464	1.02	0.1836
2.9126	1.5504	1.0608	0.2244
3.2258	1.7034	1.1118	0.255
3.8462	2.1114	1.2036	0.306
4.4586	2.4888	1.3872	0.3468
5.0633	2.7948	1.5912	0.408
5.6604	3.2436	1.8564	0.4488
6.25	3.6006	2.0298	0.4794
6.8323	3.9474	2.3256	0.5508
7.4074	4.3146	2.6316	0.5916
7.9755	4.6716	2.7744	0.6426
8.5366	5.0184	3.0804	0.7038
9.0909	5.3652	3.4068	0.7752
10.448	6.2118	3.825	1.1322
11.765	7.0482		1.5402
13.043	7.854		2.295
14.286	8.6394		2.2746



Figure(2b):Plot of specific conductance against molar concentration

Table(2c): Variation of specific conductivity (L) of solutions of $CoCl_2$ with its molar concentration at three different pH values (pH 4,7 and 8.2)

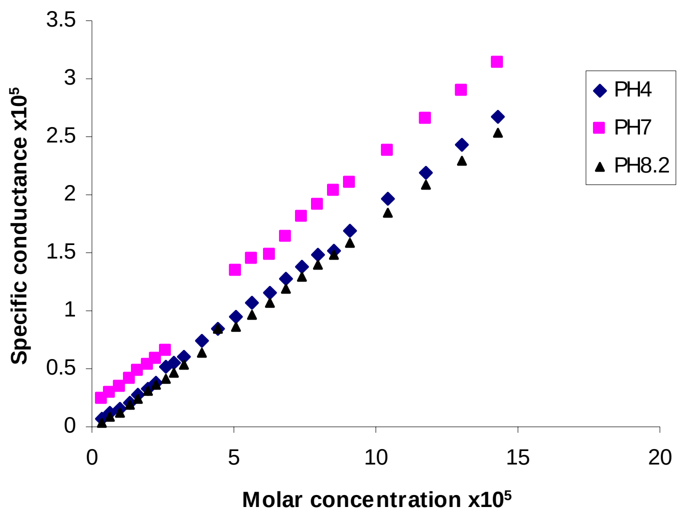
Concentration M/L x 10^5	pH4	pH7	pH8.2
	Specific conductancex10^5 (L)		
0.3322	0.051	1.4994	0.102
0.6622	0.306	1.6524	0.1836
0.9901	0.3366	1.7442	0.2856
1.3158	0.3876	1.8666	0.3876
1.6393	0.4794	1.9482	0.4896
1.9608	0.6834	2.0604	0.6018
2.2801	0.8058	2.1726	0.7956
2.5974	0.867	2.2746	0.9792
2.9126	0.9282	2.397	1.1016
3.2258	1.02	2.5092	1.2036
3.8462	1.1322	2.6826	1.3974
4.4586	1.2852	2.9172	1.581
5.0633	1.4688	3.1314	1.7952
5.6604	1.7442	3.3354	1.9992
6.25	1.8768	3.5292	2.1828
6.8323	1.989	3.723	2.3562
7.4074	2.04	3.9372	2.55
7.9755	2.4174	4.1412	2.7132
8.5366	2.5602	4.284	2.9376
9.0909	2.7948	4.4982	3.1212
10.448	3.0702	4.947	3.5292
11.765	3.4068	5.3754	3.9678
13.043	4.131	5.7834	4.3656
14.286	4.692	6.222	4.7532



Figure(2c)Plot of specific conductance against molar concentration

Table(2d): Variation of specific conductivity (L) of solutions of $NiCl_2$ with its molar concentration at three different pH values (pH 4,7 and 8.2)

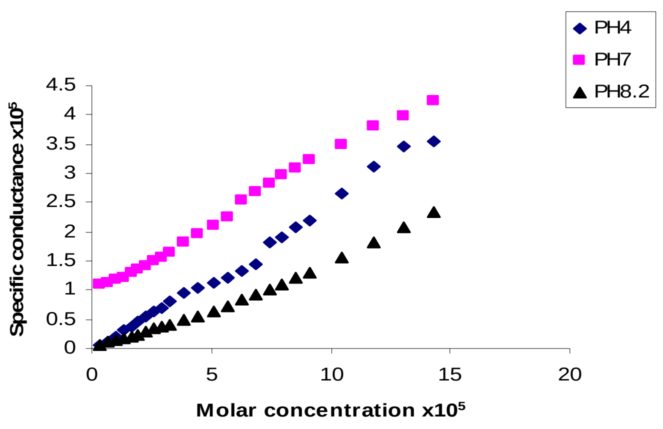
Concentration M/L x 10 ^{5}	pH4	pH7	pH8.2
	Specific conductancex10^5 (L)		
0.3322	0.0612	0.2346	0.0306
0.6622	0.1122	0.2958	0.0918
0.9901	0.153	0.3468	0.1224
1.3158	0.2142	0.4182	0.1938
1.6393	0.2754	0.4794	0.2448
1.9608	0.3264	0.5406	0.306
2.2801	0.3774	0.5916	0.3672
2.5974	0.51	0.663	0.4182
2.9126	0.5508		0.4692
3.2258	0.612		0.5304
3.8462	0.7344		0.6426
4.4586	0.8364		0.8364
5.0633	0.9486	1.3464	0.867
5.6604	1.0608	1.4484	0.969
6.25	1.1526	1.4892	1.071
6.8323	1.275	1.6422	1.1832
7.4074	1.377	1.8054	1.2954
7.9755	1.479	1.9074	1.3974
8.5366	1.5096	2.0298	1.4892
9.0909	1.683	2.1114	1.581
10.448	1.9584	2.3868	1.8462
11.765	2.193	2.652	2.0808
13.043	2.4276	2.8968	2.295
14.286	2.6724	3.1416	2.5398



Figure(2d):Plot of specific conductance against molar concentration

Table(2e): Variation of specific conductivity (L) of solutions of $CuCl_2$ with its molar concentration at three different pH values (pH 4,7 and 8.2)

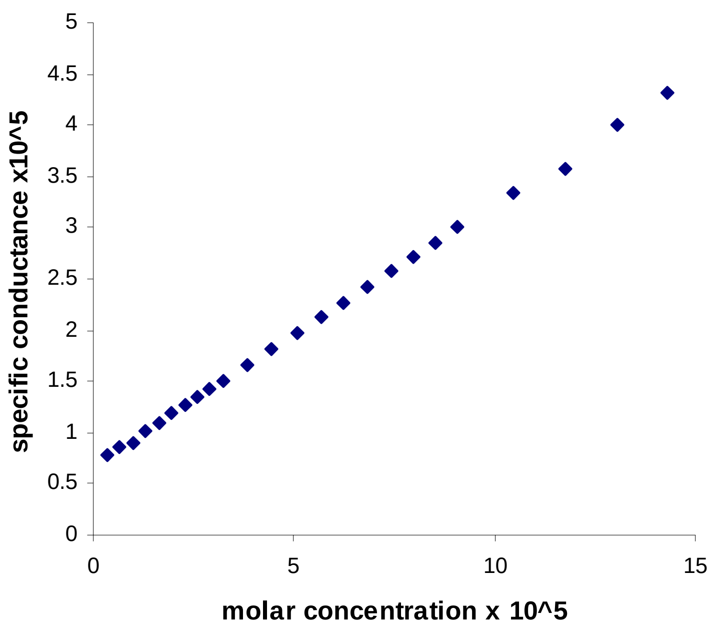
Concentration M/L x 10^5	pH 4	pH 7	pH 8.2
	Specific conductancex10^5 (L)		
0.3322	0.0612	1.091	0.0612
0.6623	0.1224	1.132	0.102
0.9901	0.2142	1.173	0.1326
1.316	0.306	1.224	0.1734
1.639	0.3774	1.285	0.2142
1.961	0.4692	1.346	0.2448
2.28	0.561	1.418	0.2856
2.597	0.6324	1.489	0.3468
2.913	0.7038	1.561	0.3774
3.226	0.7956	1.632	0.408
3.846	0.9588	1.805	0.4794
4.459	1.051	1.958	0.5508
5.063	1.112	2.111	0.6426
5.66	1.224	2.254	0.7344
6.25	1.336	2.53	0.8364
6.832	1.428	2.683	0.9282
7.407	1.826	2.825	1.02
7.975	1.907	2.978	1.102
8.537	2.081	3.091	1.214
9.091	2.183	3.223	1.306
10.45	2.642	3.488	1.561
11.76	3.111	3.794	1.826
13.04	3.468	3.978	2.071
14.29	3.56	4.233	2.336



Figure(2e):Plot of specific conductance against molar concentration

Table(3a): Variation of specific conductivity (L) of solutions of $MnCl_2$ with its molar concentration at pH =7

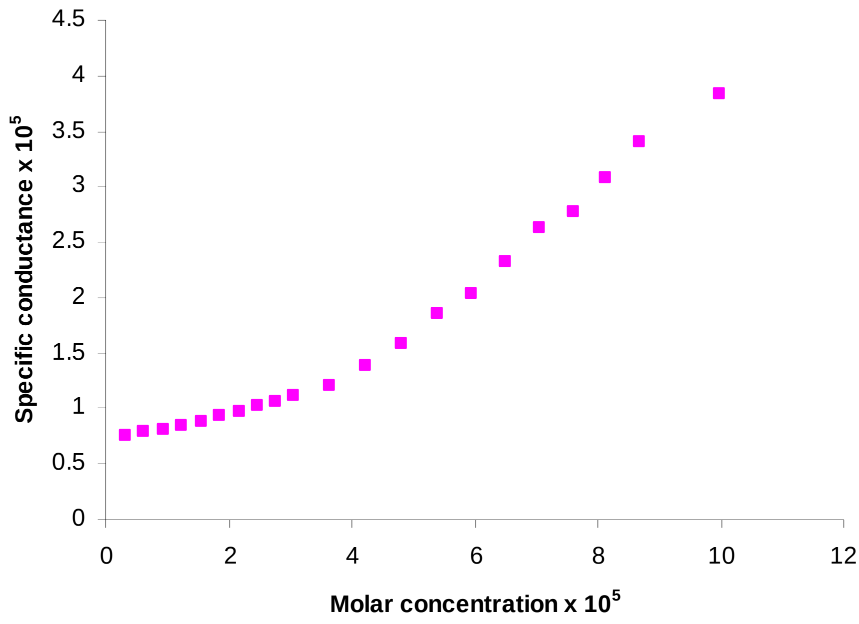
Concentration M/L x 10^5	Conductivity Siemens x 10^5	Specific conductance Siemens Cm-1x 10^5
0.33222	1.42	0.785
0.6622	1.49	8.57
0.99009	1.54	9.08
1.3157	1.65	1.02
1.6393	1.73	1.1
1.9607	1.81	1.18
2.2801	1.89	1.26
2.5974	1.97	1.35
2.9126	2.04	1.42
3.2258	2.13	1.51
3.8461	2.28	1.66
4.4586	2.48	1.82
5.0632	2.58	1.97
5.6603	2.73	2.12
6.25	2.88	2.27
6.8323	3.02	2.42
7.4074	3.17	2.57
7.9754	3.31	2.71
8.5365	3.45	2.86
9.0909	3.59	3
10.4478	3.92	3.34
11.7647	4.15	3.57
13.0435	4.58	4.01
14.2857	4.88	4.31



Figure(3a):Plot of specific conductance against molar concentration

Table(3b): Variation of specific conductivity (L) of solutions of $FeCl_2$ with its molar concentration at pH =7

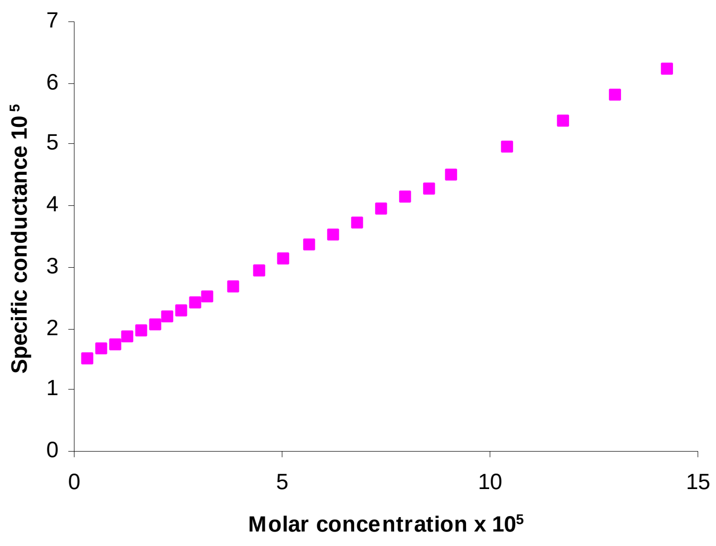
Concentration	Conductivity	Specific conductance
M/L x 10^5	Siemens x 10^5	Siemens Cm^{-1} x 10^5
0.3154	1.4	0.765
0.6289	1.42	0.785
0.9404	1.45	0.816
1.25	1.48	0.847
1.5576	1.52	0.887
1.8633	1.56	0.928
2.1671	1.6	0.969
2.469	1.65	1.02
2.7692	1.69	1.06
3.0674	1.74	1.11
3.6585	1.83	1.2
4.2424	2.01	1.39
4.8192	2.21	1.59
5.3892	2.47	1.86
5.9523	2.64	2.03
6.5088	2.93	2.33
7.0588	3.23	2.63
7.6023	3.37	2.77
8.1395	3.67	3.08
8.6705	3.99	3.41
9.9715	4.4	3.83



Figure(3b):Plot of speciicf conductance against molar concentration

Table(3c): Variation of specific conductivity (L) of solutions of $CoCl_2$ with its molar concentration at three different pH =7

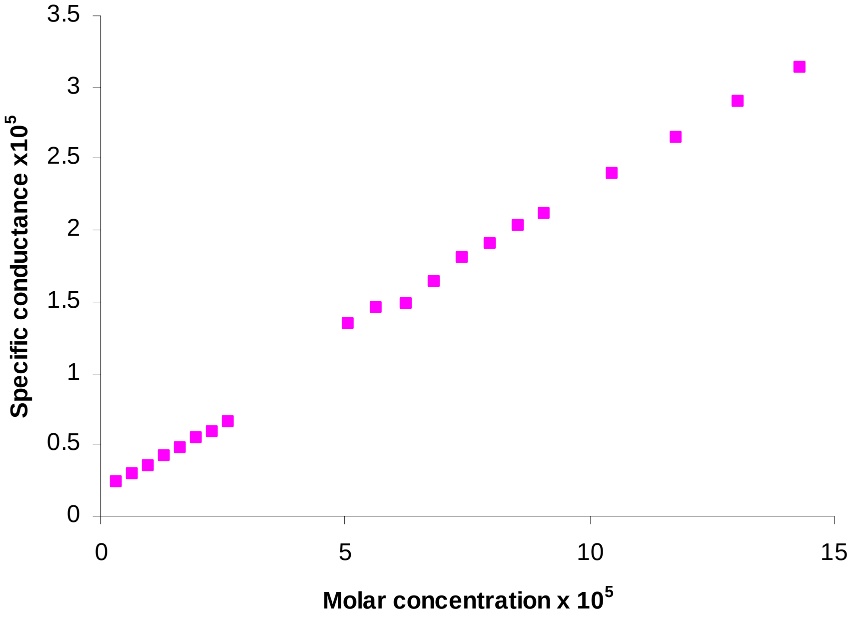
Concentration M/L x 10^5	Conductivity Siemens x 10^5	Specific conductance Siemens Cm^{-1}x 10^5
0.3322	1.47	1.5
0.6622	1.62	1.65
0.99009	1.71	1.74
1.3157	1.83	1.87
1.6393	1.91	1.95
1.9607	2.02	2.06
2.2801	2.13	2.17
2.5974	2.23	2.27
2.9126	2.35	2.4
3.2258	2.46	2.51
3.8461	2.63	2.68
4.4586	2.86	2.92
5.0632	3.07	3.13
5.6603	3.27	3.34
6.25	3.46	3.53
6.83323	3.65	3.72
7.4074	3.86	3.94
7.9754	4.06	4.14
8.5365	4.2	4.28
9.0909	4.41	4.5
10.4478	4.85	4.95
11.7647	5.27	5.38
13.0435	5.67	5.78
14.2857	6.1	6.22



Figure(3c):Plot of specific conductance against molar concentration

Table(3d): Variation of specific conductivity (L) of solutions of $NiCl_2$ with its molar concentration at pH =7

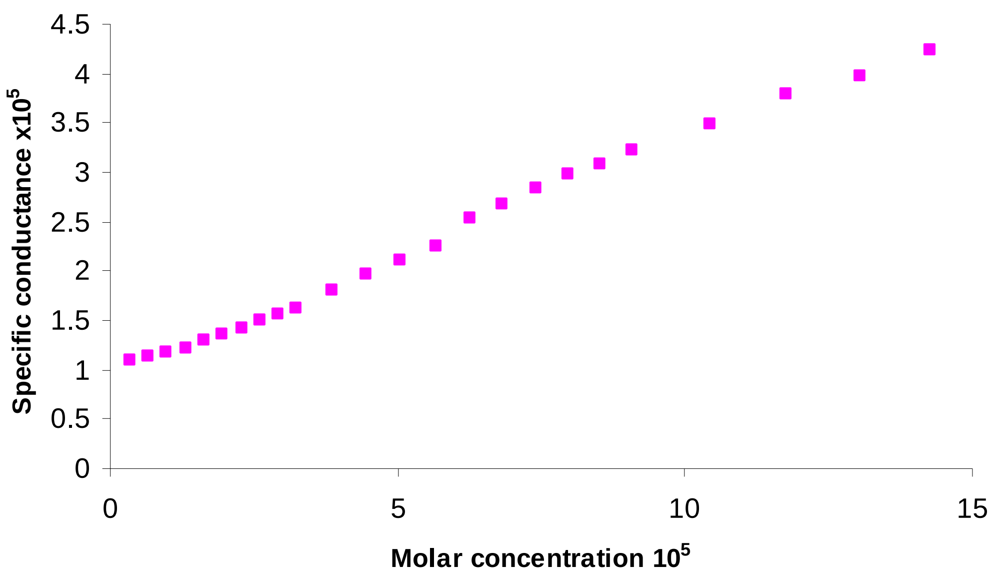
Concentration M/L x 10^5	Conductivity Siemens x 10^5	Specific conductance Siemens Cm^{-1}x 10^5
0.3322	0.23	0.235
0.6622	0.29	0.296
0.99009	0.34	0.347
1.3157	0.41	0.418
1.6393	0.47	0.479
1.9607	0.53	0.541
2.2801	0.58	0.592
2.5974	0.65	0.663
5.0632	1.32	1.35
5.6603	1.42	1.45
6.25	1.45	1.49
6.8323	1.61	1.64
7.4074	1.77	1.81
7.9754	1.87	1.91
8.5366	1.99	2.03
9.0909	2.07	2.11
10.4478	2.34	2.39
11.7647	2.6	2.65
13.0435	2.84	2.9
14.2857	3.08	3.14



Figure(3d):Plot of specific conductance against molar concentration

Table(3e): Variation of specific conductivity (L) of solutions of $CuCl_2$ with its molar concentration at pH =7

Concentration M/L x 10^5	Conductivity Siemens x 10^5	Specific conductance Siemens Cm^{-1}x 10^5
0.3322	1.07	1.09
0.6622	1.11	1.13
0.99009	1.15	1.17
1.3157	1.2	1.22
1.6393	1.26	1.29
1.9607	1.32	1.35
2.2801	1.39	1.42
2.5974	1.46	1.49
2.9126	1.53	1.56
3.2258	1.6	1.63
3.8461	1.77	1.81
4.4586	1.92	1.96
5.0632	2.07	2.11
5.6603	2.21	2.25
6.25	2.48	2.53
6.8323	2.63	2.68
7.4074	2.77	2.83
7.9754	2.92	2.98
8.5365	3.03	3.09
9.0909	3.16	3.22
10.4478	3.42	3.49
11.7647	3.72	3.79
13.0435	3.9	3.98
14.26857	4.15	4.23



Figure(3e):Plot of specific conductance against molar concentration

Fig.3 (a-e) show (the plot of conductivity vs. concentration) show different regions give rise some break points. Generally the curvatures of the plots can be explained by the following : The first break point especially is more clear for $FeCl_2$ +Albumin solution ,is interpreted as formation of metal ion / protein complex or as the critical aggregation which describe the adsorption The second inflection corresponds to the saturation of the protein surface .The third break corresponds to an unfolding or conformational change of the protein complex. At pH7 the albumin and metal ion monomers have opposite charge and there is electrostatic interaction between metal ions and albumin resulting in more extensive adsorption of the metal ions. The fourth inflection may be corresponds to hydrophobic interaction occur at higher concentrations. This hydrophobic interaction between albumin and metal ions can give rise to a conformational change even when the concentration of the metal ions are remarked low concentrated [13].

The same effect have been noticed by Elena-et-al [17] that positive cooperative binding has revealing the importance of the hydrophobic interactions between albumin and surfactant.

Generally the changes in the conductivity of metal ions / serum albumin as a function of metal ion concentration especially at higher concentrations cause an increase of conductivity.

Generally the changes in the conductivity of metal ions / serum albumin as a function of metal ion concentration especially at higher concentrations cause an increase of conductivity.

Pablo-et-al [18] found also that the interactions of the amphiphilic antidepressant drug clomiparmine hydrochloride with the blood plasma protein observe the gradual increase of conductivity of higher concentrations which is typical of saturation rather than a denaturation process [5] .

Conductivity measurements of the complex formed by the interaction of amphiphilic penicillin drugs cloxacillin and dicloxacillin and human serum albumin in water at 25 $^{\circ}$ have shown hydrophobic adsorption of the drug on the protein surface ,leading to surface saturation .

The results indicate also increasing conductivity with increase in the concentration of added drug [19] .

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