One of these permits measurement of the scattering in liquids such as pure benzene, within 2–3%, using 2–4 cc. of liquid.

2. The calibration of the apparatus in terms of several standard liquids is described. The molecular weight of bovine serum albumin, based on these standards, is in good agreement with values derived from osmotic pressure and from sedimentation and diffusion. Serum albumin may serve as a convenient working standard in the determination of molecular weights by turbidity.

3. Relations are stated for turbidity in multicomponent systems containing small ions or molecules and one or more macromolecular ions. The equations predict, among other terms, an effect corresponding to the Donnan factor for osmotic pressure, which becomes large for high net charge on the protein and at low ionic strength. This does not affect the extrapolated molecular weight values, but greatly affects the slope of the curves for c/τ as a function of c.

4. In dilute solutions, the turbidity, τ, of serum albumin obeys Debye's equation $Hc/\tau = (1/M_2) + 2Bc$. The interaction constant B agrees within the limits of error with the values independently obtained from osmotic pressures by Scatchard and his associates. However, the theoretical equations show that such agreement is not in general to be expected in multicomponent systems, and is found here only because some of the terms in the theoretical equations are numerically small under our conditions.

5. The interaction constant B is generally near zero for the isoelectric protein. When the valence (Z_2) of the protein, as determined from the acid-base titration curve, is large, B is large and positive at low ionic strength, but decreases as ionic strength increases.

6. The curves for B as a function of Z_2, at several different ionic strengths, have been studied in solutions of sodium chloride, sodium thiocyanate, and calcium chloride. The form of the curves clearly reveals effects of the specific binding of chloride and thiocyanate ions; the latter being bound much more tightly than the former, in agreement with Scatchard's findings. The curves become far more symmetrical when B is plotted as a function of Z_2^*, which is the net charge per albumin molecule, taking account of *all* ions bound by the protein.

7. Comparison of the curves for calcium and sodium chloride indicates binding of calcium by albumin, the binding increasing with increasing *p*H. Rough estimates of the amount of calcium bound agree reasonably with those of Hastings.

8. The curves for B, as a function of ionic strength and net charge (Z_2^*) on the protein, agree qualitatively with the predictions from the Donnan effect, but are numerically smaller. This indicates that the factor β_{22}^*—rate of change of activity coefficient of the protein component with its own concentration—is large and negative at low ionic strength, when the net charge on the protein is large. For the isoelectric protein, β_{22}^* is small and sometimes positive.

BOSTON 15, MASS. RECEIVED DECEMBER 15, 1949

[CONTRIBUTION NO. 496 FROM THE CHEMICAL LABORATORIES OF INDIANA UNIVERSITY]

A Study of Heterogeneous Equilibria in Aqueous Solutions of the Nickel Selenates at 30°[1]

BY C. S. ROHRER AND H. ROBERT FRONING

Few equilibrium studies have been made involving the hydrates of nickel selenate. The hexahydrate of nickel selenate was first prepared by E. Mitscherlich[2] and later by von Hauer.[3] Andre Klein[4] published an investigation of the two-component system nickel selenate and water, and reported solutions of the hexahydrate stable from the eutectic at −3 to 82.2°, and the tetrahydrate stable to the boiling point of its saturated solution. Dehydration curves gave breaks for the dihydrate at 105° and for the monohydrate at 170°. Mme. Demassieux[5] studied the dehydration of several nickel selenates and found no breaks which would correspond to the tetra-, di- or monohydrates. However, she stated that above 400° the anhydrous salt appeared.

The study on the three component system $NiSeO_4$–H_2SeO_4–H_2O was made at equilibrium to provide data on solubility of the hydrates in varying concentrations of selenic acid and to verify the existence of certain hydrates.

Experimental

Preparation of Selenic Acid.—The selenic acid used in this investigation was prepared by the method of Gilbertson and King.[6]

Preparation of Nickel Selenate.—A nickel carbonate was prepared in accordance with the procedure of Gagnon, Cloutier and Martineau[7] by adding solid C. P. potassium hydrogen carbonate in small quantities to a saturated solution of nickel nitrate, with stirring, until the filtered

---

[1] Taken in part from a thesis submitted by H. Robert Froning in partial fulfillment of the requirements for the Doctor of Philosophy Degree at Indiana University.

[2] Mitscherlich, *Ann. Physik*, **11**, 326 (1827).

[3] von Hauer, *J. prakt. Chem.*, **80**, 217 (1860).

[4] Klein, *Ann. chim.*, **14**, 263 (1940).

[5] Demassieux, *Compt. rend.*, **221**, 557 (1945).

[6] Gilbertson and King, THIS JOURNAL, **58**, 180 (1936).

[7] Gagnon, Cloutier and Martineau, *Can. J. Research*, **19**, B, 179 (1941).

solution was almost colorless. The lime green solid was washed for two days or longer with repeated stirrings and filtrations before drying for 24 hours at 80°.

The solid nickel carbonate was added slowly to an approximately one molar solution of selenic acid which remained in slight excess. The solution was evaporated at a temperature of less than 80° to yield the emerald green tetragonal crystals of $NiSeO_4 \cdot 6H_2O$, which were then twice recrystallized before using. The final product gave a negative test for the nitrate ion, and potassium analysis with the Beckman Flame Photometer indicated the presence of less than 0.01% potassium.

Analysis of Nickel.—Nickel determinations were made according to a standard method using dimethylglyoxime.[8]

Analysis of Selenate.—A modified method of Soth and Ricci[9] was found applicable for determining the selenate content of nickel selenate.

Procedure.—Individual mixtures were prepared by adding a fine powder of the nickel selenate hexahydrate to solutions of water and selenic acid of definite ratios ranging from pure water to selenic acid monohydrate. The bottles were hermetically sealed with paraffin, placed in the constant temperature bath and stirred by end over end rotation for a period of time which exceeded that necessary for the attainment of equilibrium.

Three criteria were used to determine the establishment of equilibrium: first, when the density of the mother liquor had attained constancy; (this served as a preliminary criterion before analyses were made) second, when successive analyses of the mother liquor agreed within experimental error; third, when microscopic examination revealed the presence of a single solid phase. As a further test for the establishment of true equilibrium some of the mixtures of each hydrate were maintained at 80° for twenty-four hours with frequent stirring, others likewise maintained at 0°, and then agitated again at 30 ± 0.01° until equilibrium was maintained. This presented an opportunity for the appearance of any new solid phase and was also a means of determining the time necessary for the mixture to reach equilibrium.

Less than five days were required for solutions in contact with the hexahydrate to reach equilibrium, with the time ranging up to about two months for some of the viscous solutions of the monohydrate.

After equilibrium was attained, rotation of the samples was halted, and the solid phase was allowed to settle. Samples for density determinations and for analysis of the mother liquor were withdrawn by use of the pycnometer and sampling tube described by Kiehl and Manfredo.[10] Water was determined by difference.

All X-ray diffraction patterns were made with the Hayes diffraction unit. Chromium Ka radiation was used with the tube operated at a potential of 35 kv. and a current of 15 milliamperes. Since both the tetra- and monohydrates are hygroscopic, it was necessary to seal them off from the atmosphere. This was accomplished by placing the equilibrium liquid–solid mixture inside small capillaries, or by sealing the powdered solid in collodion films mounted on glass fibers.

Experimental Results.—Table I gives the results of the analyses for the mother liquor, and for the moist solid phase expressed in percentage by weight of nickel selenate, selenic acid and water. The density of the equilibrium liquid phase is also listed. The experimental results are depicted in Fig. 1.

Table II lists X-ray diffraction data for the hexa-, tetra- and monohydrates of nickel selenate. The *d* values are expressed in ångström units and listed in order of decreasing intensity.

TABLE I

Density	Liquid phase percentage $NiSeO_4$	Liquid phase percentage H_2SeO_4	Solid phase percentage $NiSeO_4$	Solid phase percentage H_2SeO_4	Solid phase[a]	
1.365	28.87	0.11	49.46	0.09	a	
1.372	22.62	9.34	61.19	1.12	a	
1.415	16.12	20.26	47.32	7.40	a	
1.479	10.67	32.99	59.23	3.58	a	
1.537	8.81	38.99	55.90	6.71	a	
1.552	8.39	41.02	59.29	4.70	a	
1.723	8.68	49.64	55.84	8.08	a	m
1.756	9.36	51.18	56.77	10.75	a	m
1.761	9.37	51.16	62.29	9.73	b	m
1.794	8.89	52.94	52.68	17.06	b	m
1.816	8.87	53.38	53.61	17.28	b	m
1.831	8.65	55.57	57.00	14.90	b	m
1.893	8.91	58.84	56.01	16.30	b	m
1.646	8.03	47.22	37.19	30.94	c	
1.679	4.81	53.08	53.48	23.88	c	
1.694	4.19	55.16	58.23	21.25	c	
1.720	3.28	57.58	49.54	27.75	c	
1.814	0.96	64.71	55.91	25.53	c	
1.888	.45	68.44	44.62	35.20	c	
1.923	.27	70.98	52.23	31.34	c	
2.038	.23	74.70	43.38	39.42	c	
2.071	.15	76.46	42.57	40.83	c	
2.262	.04	83.85	40.80	46.45	c	
2.294	.03	86.22	38.00	50.93	c	

[a] a = $NiSeO_4 \cdot 6H_2O$; b = $NiSeO_4 \cdot 4H_2O$; c = $NiSeO_4 \cdot 1H_2O$; m = metastable.

TABLE II

X-RAY DIFFRACTION DATA FOR NICKEL SELENATES

$NiSeO_4 \cdot 6H_2O$ *d*	$NiSeO_4 \cdot 4H_2O$ *d*	$NiSeO_4 \cdot 1H_2O$ *d*
4.29	4.5	3.39
2.75	5.4	4.8
4.6	3.41	3.07
2.59	2.58	2.55
2.88	2.97	2.43
2.16	2.10	2.62
1.91	1.73	2.06
1.78	1.45	2.20
1.72		1.55
1.67		1.52
3.44		1.46
2.05		

Calculations

The composition of the equilibrium solid was determined by Schreinemakers[11] wet residue method, and verified by X-ray powder diffraction patterns. In order to eliminate the mechanical errors of plotting, the tie-line intersections in Fig. 1 were determined by the algebraic method of Roozeboom.[12] Since there are as many as 66 tie-line intersections for one solid phase, the method of least squares was used as the most satisfactory procedure for evaluating the composition of the pure solid compound for the monohydrate and the hexahydrate. The tie-lines for the

---

[8] Scott, "Standard Methods of Chemical Analysis," Vol. 1, D. Van Nostrand Co., Inc., New York, N. Y., 5th ed., 1939, p. 614.

[9] Soth and Ricci, *Ind. Eng. Chem., Anal. Ed.*, **12**, 328 (1940).

[10] Kiehl and Manfredo, THIS JOURNAL, **59**, 2118 (1937).

[11] Schreinemakers, *Z. physik. Chem.*, **11**, 76 (1893).

[12] Bakhuis Roozeboom, "Die heterogene Gleichgewichte vom Standpunkte der Phasenlehre," 1911, Vol. 3, Part 1, p. 149.

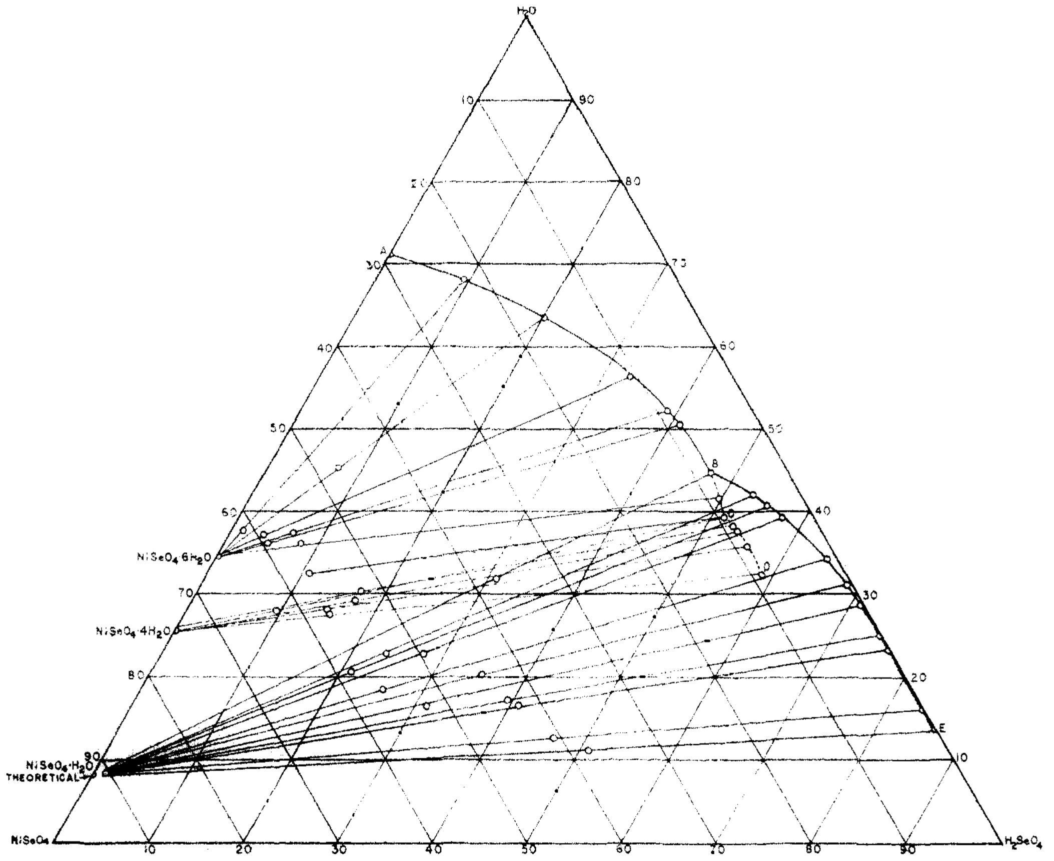

Fig. 1.—System $NiSeO_4$-H_2SeO_4-H_2O. 30° isotherm data in weight percentage.

tetrahydrate were very close together. Therefore the method of tie-line pairs having the greatest possible angle was chosen to determine the composition of the solid phase.

Discussion of Results

The isothermal diagram Fig. 1 shows three solid phases: $NiSeO_4 \cdot 6H_2O$, $NiSeO_4 \cdot 4H_2O$ and $NiSeO_4 \cdot 1H_2O$ at equilibrium with their isothermally univariant liquid phase, respectively, in contact. There was no evidence of the existence of either a dihydrate or the anhydrous salt in the concentration range from water to 86.22% selenic acid. From the shape and composition of the solubility curve for the monohydrate, it would appear unlikely that a stable dihydrate could exist in selenic acid solutions at 30°. Klein[4] obtained a break corresponding to the composition of the dihydrate, in his dehydration curves at 105°.

The Solubility Curve ABC for the Solid Phase $NiSeO_4 \cdot 6H_2O$.—AB is that portion of the curve over which the hexahydrate represents the stable phase as a function of increasing selenic acid concentration up to 47.2%. The solubility of the hexahydrate over this range varies from 28.87% at A to approximately 8.0% at B. The invariant point B was determined by intersection and is practically identical with the composition as determined for the first point on the solubility curve for the monohydrate.

The portion BC represents the metastable range of the hexahydrate. The invariant point C was determined by direct analysis of a solution at equilibrium with the two solid phases $NiSeO_4 \cdot 6H_2O$ and $NiSeO_4 \cdot 4H_2O$. The solubility of the metastable hexahydrate increases from its minimum value of 8.0 at B to 9.36% at point C where the selenic acid concentration is 51.18%.

The X-ray diffraction data listed in Table II give additional crystallographic information on the hexahydrate. A search of the literature revealed that no previous determination of the *d* distances for the hydrate had been made. It is interesting to note that five of the first seven lines listed for this hydrate agreed within experimental error with values recorded[13] for nickel sulfate hexahydrate.

The Solubility Curve C–D for the Solid Phase $NiSeO_4 \cdot 4H_2O$.—This hydrate is metastable over its entire solubility range. The invariant point C is the point of intersection of the solubility curve for the metastable tetrahydrate with the metastable portion of the hexahydrate curve as previously indicated. The solubility of this hydrate

---

(13) American Society for Testing Materials, "Alphabetical Index of X-Ray Diffraction Patterns," A. S. T. M., Philadelphia, 1945.

decreases from a value of 9.36% at point C to about 8.6% at a selenic acid concentration of 55.6% and then increases slightly to a value of 8.91% at point D. It was not necessary to seed the solutions to induce the formation of the tetrahydrate, and less than ten days were required for these solutions to reach equilibrium.

The solid phase was composed of small light green crystals which settled readily. The crystals were found to exhibit oblique extinction and low birefringence. The crystals were euhedral with an almost equidimensional rectangular form. The surface of the crystals became opaque within ten or fifteen minutes after mounting the crystals for microscopic examination, apparently due to the change from the tetrahydrate to the hexahydrate. It was also noted that if any mother liquor was present on the slide, crystals would shortly begin to form that could be identified as those of the hexahydrate.

Klein[4] published a semi-quantitative diagram for the *d* values of the tetrahydrate. His most intense line appeared to be the 4.29 Å. line observed in this study for the hexahydrate. Other values also appeared to be due to the transition to the hexahydrate. In this study numerous pictures were taken using various precautions to make sure that the lines reported were those due to the tetrahydrate. The values are listed in Table II.

The Solubility Curve BE for the Solid Phase $NiSeO_4 \cdot 1H_2O$.—Nickel selenate monohydrate is found to be stable over the entire range from its invariant point B with the hexahydrate to 86.22% selenic acid point E. At point E only 0.03% of nickel selenate is present in the liquid phase; from the shape of the curve in this concentrated range, it is evident that the concentration of nickel selenate will show a further decrease at still higher concentrations of selenic acid.

Equilibrium was reached much more slowly for solutions of the monohydrate. About one month was required for the more viscous solutions in the region from about 70 to 86% selenic acid; while in approaching equilibrium from solutions in the metastable region along BC it was necessary to seed the solutions. More than two months were required for complete conversion from the hexahydrate to the monohydrate.

The monohydrate dissolves very slowly in water as indicated by the fact that the process of dissolving samples of 6 to 10 g. each of the moist solid in a liter volumetric flask required about twenty-four hours for complete solution. Samples of the moist solid were found to undergo no hydration when left for several hours on a microscope slide or upon a glass fiber. The monohydrate is less soluble over the entire range from B to E than is nickel sulfate monohydrate at corresponding sulfuric acid concentrations.

Klein[4] reported the formation of the monohydrate on heating the hexahydrate for five or six hours at 170°, while Demassieux[5] found no evidence for its existence in her heating curves of the hexahydrate. In this investigation the monohydrate crystals were observed to exist in aggregates of fine granular particles which varied in color from a pale green to almost white in the concentrated regions depending upon the particle size. Little difficulty was encountered in obtaining sharp consistent X-ray diffraction powder photographs of the 1-hydrate. Values for the *d* spacings are listed in Table II and are markedly similar to those recorded[13] for the corresponding sulfate.

Summary

Solubility relationships in the ternary system $NiSeO_4$–H_2SeO_4–H_2O have been studied at 30°. Nickel selenate hexahydrate and monohydrate are the only solid phases found to exist in equilibrium with their solutions. The tetrahydrate is metastable over its entire solubility range.

X-Ray diffraction studies were made and the lattice plane spacings, *d* value reported for each of the hydrates.

A method for preparing high purity nickel selenate is given.

BLOOMINGTON, INDIANA RECEIVED NOVEMBER 18, 1949