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ELECTROCHEMICAL BEHAVIOUR OF ION-EXCHANGING SUBSTANCES. IX.

ROOT POTENTIALS IN POTASSIUM CHLORIDE SOLUTIONS

BY

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Summary

Potential measurements of Lucerne clover, Medicago Sativa, were taken in KCl solutions. Regression equations were derived for the dependence of potential on KCl concentration. A statistical analysis of the data showed that the potentials do not depend on the age of seedlings. The measuring precision is discussed.

In this laboratory the electric potentials of plant roots in KCl solutions have been measured for a number of plant species. Purpose of this investigation was to extend preliminary measurements [1]) of Lucerne clover, Provence variety (Medicago Sativa).

Apparatus

The electric chain was essentially as described by TENDELOO, VERVELDE and ZWART VOORSPUY [2]). A few modifications were made from time to time. The final form as now used is shown in Figure 1.

On the day measurements were to be taken a whole seedling was mounted with a little isoelectric gelatin in a drawn out tube (B). From the reservoir of the holder (A) for the Ag/AgCl electrodes enough liquid (10^{-2} M KCl) to make electric contact was allowed to flow above the gelatin collar. The root itself was immersed in a series of KCl solutions (vessels C). The vessels with these solutions were placed on a microscope stage. The depth of immersion could be adjusted by raising or lowering the stage. The other electrode was a saturated calomel electrode (E). This electrode was mounted in a vessel (D) containing saturated KCl and carrying an agar bridge for making electric contact with the KCl solutions in the vessels (C).

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[1]) Measurements performed by E. G. KLOOSTERMAN, see G. J. VERVELDE, „Salt accumulation by plant roots", dissertation, Wageningen, 1952, page 42.

[2]) H. J. C. TENDELOO, G. J. VERVELDE and A. J. ZWART VOORSPUY, Rec. trav. chim. 63, 97 (1944).

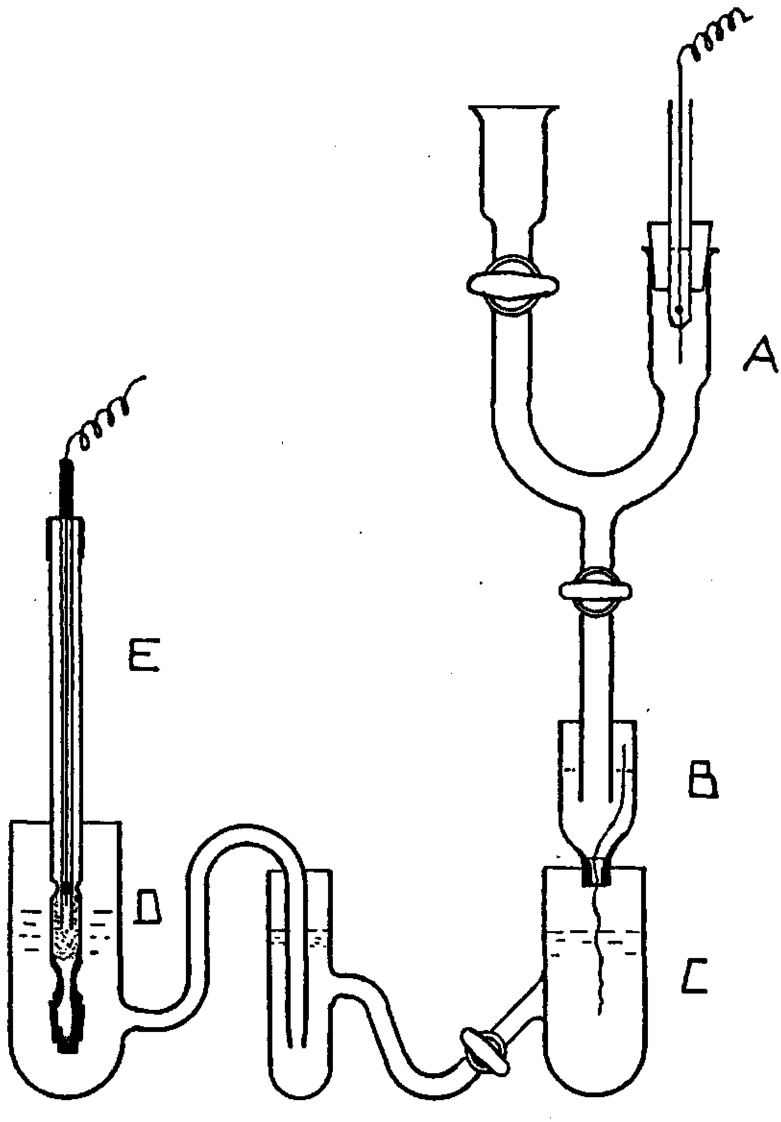

Fig. 1. Electrolytic Chain. A. Electrode holder with two Ag/AgCl electrodes (one electrode is shown). B. Tube with seedling held in place with a gelatin collar. C. Vessel containing x-molar KCl. D. Vessel containing saturated KCl solution and carrying an agar bridge. E. Saturated calomel electrode

All units were so constructed and arranged that the chain potential could be measured while keeping all stopcocks open. The electric measurements were made with a Cambridge pH-electrometer.

Experimental results. Statistical analysis of data

A first series of potential measurements was taken with 14 roots. The seeds were germinated on gauze discs. After a few days the seedlings, when grown large enough, were transferred and placed loose in the drawn out tubes; the roots were hanging in a nutrient solution [3]).

Of each solution two samples were taken. Usually measurements were started in the most dilute solution. It was found possible also to start measurements in 0.1 M KCl and to go through the series of solutions in reversed order.

The measured potentials are represented in Table I. One measurement was not duplicated. The same value −99 was filled in the open space in order to have a complete rectangular set of data.

TABLE I

Root potentials. Germination started on 27 VII

root	date of measurements	Molarity KCl							
		10^{-4}		10^{-3}		10^{-2}		10^{-1}	
1′	31 VII	− 60	− 63	− 30	− 30	− 2	+ 5	+ 22	+ 15
4	1 VIII	− 52	− 61	− 30	− 33	+ 6	+ 4	+ 33	+ 35
4′	1	− 54	− 60	− 30	− 33	+ 5	+ 1	+ 27	+ 23
8	3	− 67	− 79	− 45	− 49	− 9	− 9	+ 24	+ 22
8′	5	− 58	− 52	− 30	− 15	+ 7	+ 6	+ 42	+ 37
2′	5 VIII	− 61	− 59	− 30	− 31	+ 1	+ 2	+ 32	+ 39
11′	6	− 42	− 61	− 31	− 24	− 1	− 1	+ 11	+ 14
14	6	− 68	− 74	− 40	− 42	− 1	− 1	+ 13	+ 10
14′	6	− 41	− 53	− 18	− 19	+ 16	+ 16	+ 32	+ 37
13′	7	* − 99	− 99	− 68	− 68	− 27	− 26	+ 6	+ 14
18′	7 VIII	− 56	− 57	− 25	− 24	+ 3	+ 4	+ 15	+ 18
21″	7	− 75	− 77	− 38	− 37	− 1	+ 9	+ 22	+ 23
12′	12	− 72	− 72	− 37	− 36	+ 8	+ 11	+ 19	+ 17
12	12	− 68	− 75	− 35	− 36	+ 6	+ 7	+ 33	+ 31

* estimated value.

A statistical analysis of the data yielded the following results. Besides a pronounced dependence of potential on concentration, a marked difference between individual seedlings was noted. This difference is not merely a variation of general level of potentials, but also a degree of variation in response of individual roots to KCl concentration. The variance estimates for these sources of variation of potential are given in Table II.

TABLE II

Source of variation	Sum of squares	Dimension	Variance estimate
Concentration	128,329.61	3	42,776.54
Root mean	10,375.46	13	798.11
Interaction (concentration × root). . .	3,165.89	39	81.18
Differences between duplicate readings .	839.00	55	15.25

[3]) See VERVELDE, page 86.

The last source of variation is a measure of precision, or of possible error (about 4 mV) of potential measurements for this type of electric chain comprising a seedling. The Cambridge pH-meter can be read to 0.5—1.0 mV. The third source of variation may be considered an inherent source of varying results for potential measurements on a *series* of roots. This apparently unavoidable source of variation is more marked than the chance errors of an individual measurement. Thus, the estimate 81.18 for this source is used as a basis of comparison as to significance for other variance estimates. It is seen that not only the variance due to concentration is significant, but also the variance caused by differences between roots on account of different potential levels.

The question arose whether the differences between roots might be attributed to the age of seedlings. Thus, the variance of differences between the seven different age groups was compared with the variance of potential differences within each age group (Table III). It was found that differences in age of a few days offer no explanation for the observed differences between roots. Roots show these differences regardless of age.

TABLE III

Source of variation	Sum of squares	Dimension	Variance estimate
Differences between age groups	4,117.50	6	686.25
Differences within age groups	6,257.96	7	893.99
Differences between roots	10,375.46	13	798.11

A further analysis of data showed that the measuring errors at the four different concentrations were not uniform. The estimates of variance are listed in Table IV.

TABLE IV

log C_{KCl}	Sum of squares	Dimension	Variance estimates
— 4	452.50	13	34.81
— 3	159.00	14	11.36
— 2	91.50	14	6.54
— 1	136.00	14	9.71
all concentrations	839.00	55	15.25

The Bartlett test [4]), as modified by Hartley and Pearson, was used. On account of this criterion, it is not permissible to use the variance estimate 15.25 as a uniform base of reference. This makes no difference with regard to the other conclusions arrived at above, as all the estimates for the different concentrations are smaller than the variance estimate 81.18, the preferred basis of reference.

[4]) Hartley, Biometrika 31, 249 (1940).

With a second series the seeds were allowed to germinate in the folds of a strip of moist filter paper. This strip was supported on a slanting glass plate. The lower end of filter paper and glass plate reached in a shallow trough with distilled water. In this way the filter paper was kept moist. Seedlings were transferred to the drawn out tubes, as before, when grown large enough and before root hairs could develop too much. The roots were hanging either in distilled water or nutrient solution.

Each root after mounting was pretreated a while in a KCl solution until the potential remained reasonably constant. The depth of immersion was adjusted so as to minimize variation of potential with depth of immersion, preferably at the level where the observed potential attained an extreme value.

A greater range of concentrations was used. It was found, however, that the potentials were established more slowly in 0.4 and 1.0 M KCl and tended to become irreversible. No further measurements were taken in these high concentrations.

TABLE V

Root potentials. Set A germination started on 17 VIII. Set B germination started on 24 VIII

Root	Date of measurements	Germinating medium[1])	Pretreatment [2]) solution	Molarity KCl									
				10^{-5}		10^{-4}		10^{-3}		10^{-2}		10^{-1}	
A 1′	31 VIII	+	— —	* — 83	* — 83	— 40	— 59	— 38	— 40	— 7	— 5	+ 20	+ 19
A 2′	1 IX	+	— 4	— 54	— 67	— 62	* — 62	— 28	— 33	+ 7	+ 3	+ 27	+ 28
A 5′	2	+	— 5	— 87	— 87	— 71	— 75	— 47	— 56	— 15	+ 6	+ 20	+ 16
A 6′	2, 3	+	— 2										
A 7′	3	—	— 2	—137	—133	— 95	— 95	— 45	— 52	— 2	— 5	+ 27	+ 27
A 8′	1 IX	—	— 5	— 63	— 56	— 50	— 51	— 20	— 20	+ 19	+ 20	+ 40	+ 39
A 9′	7	+	— 3	— 78	— 81	— 69	— 70	— 28	— 30	+ 1	— 2	+ 15	+ 14
A 10′	7, 8	+	— 3	— 59	— 60	— 48	— 44	— 22	— 23	+ 1	+ 1	* + 13	+ 13
A 11′	8	—	— 3	* — 81	— 81	— 64	— 69	— 37	— 37	— 1	— 4	+ 15	+ 13
B 2	15, 16	+	— 3	— 91	— 86	— 77	— 77	— 40	— 47	— 4	— 8	+ 4	+ 3
B 3	16 IX	—	— 3	— 64	— 62	— 48	— 48	— 17	— 17	— 1	0	+ 4	+ 5
B 4	17	—	— 4	— 80	— 71	— 73	— 73	— 38	— 37	+ 6	+ 3	+ 21	* + 21
B 5	18	+	— 4	—119	—111	— 90	— 92	— 46	— 43	+ 2	+ 7	+ 36	+ 35
B 6	18	+	— 4	— 83	— 84	— 73	— 71	— 27	— 22	+ 12	+ 13	+ 19	+ 19
B 7	19	—	— 4	—102	—100	— 95	— 98	— 62	— 62	— 32	— 16	0*	0*
B 8	21 IX	—	— 4	—105	— 99	— 96	— 95	— 60	— 62	— 21	— 8	+ 32	+ 21
B 9	21	+	— 4	— 77	— 78	— 72	— 77	— 36	— 36	+ 7	— 2	+ 23	+ 22
B 10	22	+	— 4	— 95	— 99	— 80	— 83	— 36	— 35	+ 6	+ 3	+ 27	+ 27
B 11	22	—	— 3	—121	—119	—103	—102	— 55	— 56	— 7	— 4	+ 27	+ 25
B 12	23	—	— 3	—103	— 89	— 82	— 79	— 37	— 39	— 4	— 1	+ 30	+ 25

[1]) + indicates root grown in nutrient solution.
— indicates root grown in distilled water.

[2]) $-n$ indicates pretreatment in 10^{-n} M KCl solution.

* estimated values.

This second series comprised a set of 8 seedlings measured first and another set of 11 seedlings measured later. These two sets were treated as one series of measurements. Results are listed in Table V.

The starred values were not measured, but are estimated values. Where a measurement was not duplicated, the same value as measured was filled in the open space. Where no measurements were taken suitable values were estimated by the missing plot technique. Indicated also are manner of growing the seedlings, whether in distilled water or nutrient solution, and the concentration of the pretreatment KCl solution. Again, the same main sources of variation are accounted for as shown in Table VI.

TABLE VI

Source of variation	Sum of squares	Dimension	Variance estimate
Concentration	323,400.08	4	80,850.02
Root mean	21,786.45	18	1,210.36
Interaction (concentration × root). . .	18,154.92	70	259.36
Differences between duplicate readings .	1,334.00	89	14.99

It is seen that the variance caused by chance errors is practically the same as for the first series of measurements. The other variance estimates follow the same pattern; they are a little higher. Hence, the same conclusions hold as before.

Again, the variance estimates due to reading errors may not be considered uniform for the five different concentrations. The estimates now are as shown in Table VII.

TABLE VII

log C_{KCl}	Sum of squares	Dimension	Variance estimate
− 5	338	17	19.88
− 4	241	18	13.39
− 3	129	19	6.79
− 2	537	19	28.26
− 1	89	16	5.56
all concentrations	1334	89	14.99

The same conclusion obtains as for the first series. The present pattern of estimates, it may be noted, is somewhat different from the pattern for the first series of measurements. The relatively large variance for 10^{-2} M KCl is striking. This modified pattern might be attributed to two causes, a pretreatment in a KCl solution up to an hour, and (or) a slightly modified technique with respect to depth of immersion.

As a possible means of improving the technique of measuring root potentials, the seedlings, whether grown in water or a nutrient solution, were placed in a dilute KCl solution. They were left there until an apparently stationary potential distribution along the root was obtained. In this way the roots were preconditioned so to say for the measurements in the series of KCl solutions, when mainly changes in direct response to the varying KCl concentrations will then occur.

It was noticed, as just mentioned, that a more or less pronounced distribution of potential may be found on varying the depth of immersion. In so far as true Donnan equilibria might be established at membranes showing selective permeability, more pronounced potential differences might be established than might be on account of steady diffusion. Thus, it is natural to look for a depth of immersion giving a maximal potential difference. From a more experimental point of view, adjusting the depth of immersion so as to obtain, if possible, a potential extreme has the advantage that then the exact depth of immersion would be least critical. Thus, the potentials measured might be best reproducible. Once a certain depth of immersion of a root had been decided on, all further measurements were made at this same depth.

It may be remarked further that with this second series the individual variance estimates for the different concentrations all are somewhat smaller than the corresponding estimates for the first series, excepting the variance estimate for 10^{-2} M KCl. This is still true if the two subsets of 8 and 11 roots are examined separately. In table VIII all estimates for the different concentrations are listed.

TABLE VIII

Variance estimates due to chance errors

	log KCl concentration				
	– 5	– 4	– 3	– 2	– 1
Series I	—	34.81	11.36	6.54	9.71
Seires II	19.88	13.39	6.79	28.26	5.56
Series II_A	20.33	30.00	10.25	30.50	1.71
Series II_B	19.64	2.91	4.27	26.64	8.55

The estimates for the two subsets are rather different for three of the KCl concentrations used. Hence, it appeared desirable to probe a little deeper and to examine the point, whether the two subsets should be considered distinct in some manner. The seedlings of the first subset were measured from 14–22 days after they started to grow, while the roots of the second subset were measured when they were from 23–30 days old. Hence, there might be a difference between the two age groups. This was tested along analogous lines as for the first series of measurements. The variance estimates found and the dimensions are given in Table IX.

TABLE IX

Source of variation	Sum of squares	Dimensions	Variance estimate
Differences between the two periods. .	3.154.79	1	3,154.97
Differences within the two periods . .	18,631.48	17	1,095.97
Differences in level between roots . . .	21,786.45	18	1,210.36

The first variance estimate is larger than the second estimate. The quotient is not that large that the subsets may be considered different on account of age.

Another question has to be considered whether the seedlings grown in nutrient solution and those grown in distilled water might show different characteristics. The roots of each subset and the entire series may be divided into two groups accordingly. This gives the following results (Table X).

TABLE X

Source of variation	Sum of squares	Dimension	Variance estimate
Subset II_A			
Effect of nutrient solution	178.64	1	178.64
Differences otherwise	8,688.35	6	1,448.06
	8,866.99	7	1,266.71
Subset II_B			
Effect of nutrient solution	798.22	1	798.22
Differences otherwise	8,966.27	9	996.25
	9,764.49	10	976.45
Entire series II			
Effect of nutrient solution	1,545.61	1	1,545.61
Differences otherwise between roots . .	20,240.84	17	1,190.64
	21,786.45	18	1,210.36

As far as can be concluded from the present data, growing seedlings in nutrient solution does not contribute to the differences existing between roots.

The differences between roots, as measured, occur regardless of age of seedlings and of manner of growing them, whether in nutrient solution or distilled water. The two subsets II_A and II_B did differ with respect to precision of measurement, as we saw already.

The measurements of II_B at the KCl concentrations 10^{-4} and 10^{-3} M were much more precise. After the pretreatment as indicated in Table I, the potentials were always measured first in the most dilute solution and then in turn in the more concentrated solutions. With several roots, after the standard series of measurements was finished, additional readings were taken in the reversed order. A selection of these data will be given elsewhere.

These check measurements gave evidence, that the potentials as measured are reversible with respect to KCl concentration. They were quickly reversible if the order was reversed after measuring in 10^{-2} M KCl. All this tends to indicate that simple, quickly established equilibria are determining the potentials, as one would expect for a Donnan equilibrium at a membrane with small inner volume. Time factors due to life processes hardly seem to interfere.

From observation of the dependence of potential on time in 0.4 and 1.0 M KCl, the conviction was already gained that Provencer Lucerne clover was adversely affected by these higher concentrations of KCl. The potentials became erratic and sluggish.

The peculiar lack of precision in 10^{-2} M KCl is considered the paradoxial result of an improved technique. It is suggested, as an explanation, that time factors are hard to avoid altogether. With the improved techniques described, it apparently was possible to reach stationary potentials quickly before time factors made themselves felt. This then was so in 10^{-4} M KCl and still in 10^{-3} M KCl. By the time measurements in 10^{-2} M KCl are taken, the experimenter is overtaken by slow but still small time factors.

Yet, the average potentials calculated from the duplicate measurements for 10^{-2} M KCl may conform reasonably close to the stationary potentials of the same simple membrane equilibrium. It is felt, that this is not true any more for the potentials in 0.1 M KCl.

The discussion presented gives an account of known experimental factors and parameters. Concentration of KCl is the dominant factor. For a theoretical interpretation of the measured potentials it is desirable to ascertain *how* the potentials vary with KCl concentration. The average potentials found are listed in Table XI A.

TABLE XI A

Potential averages

	log KCl concentration				
	− 5	− 4	− 3	− 2	− 1
First series.		− 64.8	− 34.4	+ 1.4	+ 23.8
Second series	− 87.6	− 73.9	− 39.1	− 0.8	+ 20.3

In the beginning of this investigation, a special reference potential on the potentiometer viz. 921 and 920 mV was taken for each series.

For future reference it is desirable to adopt a common reference potential for wich we took 900 mV. A Weston cell was placed in series. The revised averages for these preliminary series are given in Table XI B.

TABLE XI B

Potential averages

	log KCl concentrations				
	− 5	− 4	− 3	− 2	− 1
First series.		− 85.8	− 55.4	− 19.6	+ 2.8
Second series	− 107.6	− 93.9	− 59.1	− 20.8	+ 0.3

The effect of KCl concentration was analyzed in the usual way by means of orthogonal polynomia in terms of linear, second and higher degree components. The relative importance for each series of measurements can be judged from the variance estimates listed in Table XII by comparison with the reference estimates as discussed above (see Tables II and V).

TABLE XII

First series

Degree components	Sum of squares	Dimension	Variance estimate
First.	127,383.78	1	127,383.78
Second	448.00	1	449.00
Third	497.83	1	497.83
	128,329.61	3	

Second Series

Degree components	Sum of squares	Dimension	Variance estimate
First.	317.033.09	1	317.033.09
Second	818.80	1	818.80
Third	5,548.17	1	5,548.17
Fourth	0.02	1	0.02
	323,400.08	4	

For the first series all three possible components are significant. The linear and third degree component are significant for the second series. No fourth degree component is indicated. Statistically the second degree component is not significant. Since a second degree component is significant for the first series, such a component will be retained for the second series as well.

By means of these orthogonal components the regression equations for potential (y) on logarithm of concentration (x) were obtained. These equations are (potentials referred to the new common reference potential)

First Series	. . . .	$y = -\ 7.11 - 32.33x - 25.57x^2 - 3.14x^3$
Second series	. . . .	$y = -14.27 - 38.81x - 27.41x^2 - 3.18x^3$

In connection with a theoretical interpretation, the location of the inflection point of the empirical equations is of interest. The point of inflection is easily obtained from the regression equations.

	log molarity KCl	Potential at inflection point
First series	− 2.71	− 44.84
Second series	− 2.87	− 53.43

It should be noted that both estimates fall at nearly the same KCl concentration. To decide whether the two points of inflection could be different, the confidence intervals for each of the two series were determined. The confidence interval here is the stochastic region such that the probability that it covers the true abscissa of the inflection point is equal to 0.95. These intervals with limits x_1 and x_2 come out fairly large.

Limits of confidence ranges for abscissae of points of inflection

First series	$x_1 = -3.73$	$x_2 = -2.53$
Second series	$x_1 = -3.03$	$x_2 = -2.64$

It is seen that the confidence interval of x for the second series falls entirely inside the confidence interval of x for the first series. The two abscissae in question for the two series fall both well within the shorter confidence range for the second series.

Conclusions

1. It was found that stationary potentials are established in KCl solutions. The potentials are reasonably reproducible and reversible.
2. The potentials are not dependent on the age of young seedlings. The manner of growing, whether in nutrient solution or distilled water, appears to have no influence.
3. The regression equations for the dependence of potential on KCl concentration for the first and second series of roots agree fairly well. The KCl concentrations at the inflection points of the two graphs agree closely.

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