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A SOLUTION FOR THE CATIONIC$^+$/ANIONIC$^-$ BALANCE PROBLEM IN MINERAL NUTRITION STUDIES

RANDALL P. NIEDZ[1] AND TERENCE J. EVENS
U.S. Department of Agriculture
Agricultural Research Service
U.S. Horticultural Research Laboratory
2001 South Rock Road
Ft. Pierce, FL 34945

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Abstract. **Designing culture media for mineral nutrition studies is a complicated problem. In order to change the concentration of a single cation or anion it is generally necessary to change the complementary cat-/anion of a salt complex. Consequently, it often becomes necessary to simultaneously alter both the type and/or concentration of multiple salts to achieve a final set of media that differ only in the concentration of a single ion; when the objective is to alter the concentration of multiple ions the problem's complexity is increased exponentially. The cationic$^+$/anionic$^-$ balance problem is easily solved by linear programming (LP), a technique of applied mathematics devised specifically for solving a wide range of practical, complex, resource allocation problems such as scheduling, mixing, blending, and routing. The cationic$^+$/anionic$^-$ balance problem, how it is easily solved using LP techniques, and a public domain software program designed for nutrient formulation research will be presented.**

The role of mineral nutrition in horticultural science is of primary importance. Plants are capable of synthesizing organic molecules, via photosynthesis, from CO_2 in the air, H_2O in the soil, and energy obtained from the sun. However, they require a source of mineral nutrients, either from the soil or delivered in fertilizer. This is one difference between plants and animals; animals cannot make most of the organic nutrients needed for survival and must obtain them in their diet. The mineral nutrients required by plants are listed in Table 1 and are generally referred to as macro- and micronutrients; the difference between the two classes is in the relative quantities typically required for growth. For optimal growth, mineral nutrients must be available to the plant in proper concentrations and proportions regardless of whether the plant is grown in soil, hydroponics, or *in vitro* culture.

Mineral nutrients are available to the plant in the form of ions. For example, NH_4^+ and NO_3^- for nitrogen, PO_4^{3-} for phosphorous, K^+ for potassium, SO_4^{2-} for sulfur, Mg^{2+} for magnesium, and Ca^{2+} for calcium. The ions are formed when salts (in fertilizer or soil), which are made up of a positively charged ion and a negatively charged ion, are dissolved in water. Table 2 lists some salts commonly used for fertilizer, hydroponic nutrient solutions, and plant tissue culture media and the positive and negative ions they form when dissolved

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[1]Corresponding author.

Table 1. Nutrients required by plants.[a]

Macronutrients	
Carbon	C
Hydrogen	H
Oxygen	O
Nitrogen	N
Phosphorus	P
Potassium	K
Calcium	Ca
Magnesium	Mg
Micronutrients	
Iron	Fe
Manganese	Mn
Copper	Cu
Zinc	Zn
Molybdenum	Mo
Boron	B
Chlorine	Cl

[a]Cobalt (Co), nickel (Ni), sodium (Na), and silicon (Si) have been found to be essential for only some plant species.

in water. When a single salt is added to water the salt dissolves and what remains is a solution of the ions. For example, when KNO_3 is added to water it will dissolve and produce a solution of potassium ions, K^+, and nitrate ions, NO_3^-; KNO_3 no longer exists as a single salt. Understanding the relationship between the salts that make up a fertilizer and the nutrient solution that results when the salts (or fertilizer) are dissolved provides two perspectives on studying mineral nutrition. These two perspectives are illustrated in Table 3 using the macronutrient composition of the medium of Murashige and Skoog (1962), referred to as MS medium, and one of the most popular nutrient media for plant tissue culture. Notice that MS medium can be expressed in two different ways; 1) it can be expressed as the types and amounts of each salt used to make the medium or, 2) it can be expressed as the final concentration of ions that will result once all the salts are dissolved. The first expression by salt composition is generally referred to as the "recipe", and generally reveals how to make the medium. In a tissue culture laboratory the salts for MS medium would be weighed and dissolved in water to produce MS medium. The second expression, by ion composition, reveals the ions that dissociate from the salts and is therefore the only way to actually know what is in a medium. Knowing the ion composition is the only method for comparing media to determine how media differ and why, for example, one medium works

Table 2. Common salts and the types of positive and negative ions that they produce when dissolved in water.

Salt	Cation⁺	Anion⁻
NH_4NO_3 (ammonium nitrate)	NH_4+	NO_3^-
KNO_3 (potassium nitrate)	K^+	NO_3^-
$MgSO_4$ (magnesium sulfate)	Mg^{2+}	SO_4^{2-}
$Ca(NO_3)_2$ (calcium nitrate)	Ca^{2+}	NO_3^-
KH_2PO_4 (potassium phosphate monobasic)	K^+ and H^+	PO_4^{3-}

Table 3. Common salts and the types of positive and negative ions that they produce when dissolved in water.

Murashige & Skoog (MS) Medium-Macronutrients			
Salt composition		Ion composition	
Salts	mg/L	Ions	mM
NH_4NO_3	1650	NH_4^+	20.61
KNO_3	1900	NO_3^-	39.40
$MgSO_4·7H_2O$	370	PO_4^{3-}	1.25
$CaCl_2·2H_2O$	440	K^+	20.04
KH_2PO_4	170	Ca^{2+}	2.99
		Mg^{2+}	1.50
		SO_4^{2-}	1.50
		Cl^-	5.99

better or worse than another medium. However, reporting the composition of a medium by ion composition has one major drawback—it is practically impossible to know what salts to use to make a particular medium from a list of the ion composition. This we call the anionic⁻/cationic⁺ balance problem. How this problem occurs, why it is such a difficult problem, and how to solve it are the topics of this report.

The cationic⁺/anionic⁻ Balance Problem

As illustrated in Table 2, each salt produces a positive ion and a negative ion when dissolved in water. The chemical terms for a positive and negative ion are "cation" and "anion", respectively. To understand the problem, consider the situation where it is necessary to understand the effects of potassium, K^+, on plant growth. It appears that a simple experiment would be adequate. We simply devise several treatments with low, medium, and high concentrations of K^+, keep the other ion concentrations constant, and measure the effects of each K^+ concentration on plant growth. Unfortunately, this is not what happens. Turning again to Table 2, what actually happens when we try and set up this simple experiment with potassium, K^+? Notice that because each ion comes from a particular salt, it is impossible to change one ion without changing the concentration of the associated ion in the salt. So, trying to change the K^+ level by reducing or increasing the KNO_3 will at the same time change the nitrate, NO_3^-, concentration and, if we try and adjust for the altered nitrate, NO_3^-, concentration by changing ammonium nitrate, NH_4NO_3, concentration than the ammonium, NH_4^+, concentration is changed, etc. The simple initial objective of just changing the potassium, K^+, while leaving the other ions unaltered turns out to be a very difficult problem. There has been no solution to the cationic⁺/anionic⁻ balance problem, until now, that defines what combination and concentration of salts to use that will allow for the changing of a single ion's concentration while leaving the other ion concentrations unchanged. The result of not having a solution to this problem is that researchers have been unable to change a single ion's concentration and, therefore, mineral nutrition experimental results in most studies are confounded due to the changing of multiple ion concentrations.

A Solution to the cationic⁺/anionic⁻ Balance Problem

To convert a medium formulation expressed as its ion composition to a salt formulation required to actually make that medium requires a method to solve the cationic$^+$/anionic$^-$ balance problem. Therefore, mineral nutrition research can be planned using ion composition formulations that are readily compared and studied, and simultaneously converted into the salts required to make that particular medium. Because of the problem's complexity it is necessary to utilize a tool of applied mathematics to solve a specialized system of simultaneous linear equations. The tool is called linear programming or LP, and was developed to efficiently solve a general class of problems where the objective is to optimize the allocation of scarce resources. Linear programming techniques are widely utilized in a broad set of applications such as those listed by Pannel (1997) and include "allocating classes to classrooms in schools, selecting the combination of crop and livestock enterprises that will maximize profits to a farm, choosing efficient water treatment methods to meet pollution standards in a river, selecting salary levels for staff, formulating least-cost mixtures of various components such as livestock feeds, planning advertising strategies, energy planning, forest management and planning, financial planning and portfolio optimization, scheduling of petroleum refining operations, and military defense planning."

To illustrate the use of LP consider Table 4 where MS medium was modified by rounding off the concentration of each of the component ions. The modification is a trivial example that represents some simple changes that result in a problem impossible to solve without LP. The salts and their respective concentrations required to make both the original and reformulated MS are listed in Table 5. Notice that it was necessary to change the concentration of every salt in the original MS medium and to substitute a new salt $K_4P_2O_7$, potassium pyrophosphate, for the KH_2PO_4, potassium phosphate monobasic, used in the original formulation. It is not possible to reformulate the modified MS ion composition from the original MS salts even though the modifications were very minor. Another attribute of the LP solution is that this is not the only solution, just the most mathematically efficient solution based on the list of salts specified in the equations. Many additional formulations are possible; an example is illustrated in Table 5 (column 3) using K_2HPO_4, potassium phosphate dibasic, rather than $K_4P_2O_7$, potassium pyrophosphate.

Table 4. MS medium reformulated by rounding off the original ion concentrations.

Ions	Original (mM)	Reformulated (mM)
NH_4^+	20.61	21
NO_3^-	39.40	39
PO_4^{3-}	1.25	1
K^+	20.04	20
Ca^{2+}	2.99	3
Mg^{2+}	1.50	2
SO_4^{2-}	1.50	2
Cl^-	5.99	6

Table 5. LP solution of the salts required to make the reformulated MS medium specified in Table 4. Both reformulations satisfy the modified ion concentrations.

Salts	Original (mg/L)	Reformulation 1 (mg/L)	Reformulation 2 (mg/L)
NH_4NO_3	1650	1681	1681
KNO_3	1900	1820	1820
$MgSO_4{\cdot}7H_2O$	370	493	493
$CaCl_2{\cdot}2H_2O$	440	441	441
KH_2PO_4	170	—	—
$K_4P_2O_7$	—	165	—
K_2HPO_4	—	—	174

A Tool for Mineral Nutrition Research

To assist researchers conducting experiments in mineral nutrition research we have developed a software application, ARS-MEDIA, which will eliminate the need to learn complex linear programming mathematics. The software is specifically designed for the formulation of nutrient media used in such applications as plant tissue culture, hydroponics, algal culture, fertilizer formulations, microbial culture, and any application that requires the definition of a specific culture media by its ion composition and the component salts necessary to make that culture media. The desired ion composition is entered and the software automatically constructs and solves the appropriate equations using an internal LP algorithm. The output to the user is a simple list of what salts to use and in what quantities. Additionally, the user has the capability of specifying what salts to use or not use. This has the advantage that a user can specify only the salts available in the laboratory rather than generating a solution that requires the purchase of additional salts. Features to be added in the future include data mining capabilities using an extensive and expandable historical database of plant, algal, microbial, and animal media. Some simple thermodynamic capabilities may be considered for future versions such as pH calculations, alkalinity and possible precipitates.

Conclusions

The cationic$^+$/anionic$^-$ balance problem, a major constraint to mineral nutrition research has been solved using the applied mathematical technique of linear programming. Additionally, a software tool, ARS-MEDIA, has been developed to assist researchers conducting mineral nutrition research in the design of experiments where single and multiple ion concentrations can be varied independently of all other ions in the composition.

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