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Physicochemical transfer functions to predict zone of precipitation between calcium and phosphate in simplified parenteral nutrition

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ABSTRACT

We use committee-vote via empirical transfer functions obtained from various regression models of actual physicochemical data for describing possible zone of hazardous precipitation of calcium and phosphate for particular amino acid brand name, i.e., Moripron-F® or Aminovenos-N-Paed®. The logistic regression with modified Hosmer-Lemeshow method is also presented. We use a slack variable for grouping data space near each original data point for calculating odd ratio of precipitation event and then extrapolate back to zero value of the slack variable in order to obtain the intrinsic odd ratio function that is independent from the slack variable. Committee-vote could reveal a nonlinear pattern with high accuracy and low health hazardous prediction for Moripron-F®. However, applying such algorithm with Aminovenos-N-Paed® was unsuccessful due to violation of the committee-voting rule, i.e., empirical transfer function has too weak prediction power.

Keywords: Modified Hosmer-Lemeshow logistic regression, Committee-vote decision, Transfer functions, Multiple pathway prediction.

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INTRODUCTION

Parenteral nutrition (PN) mixture is used as nutritional support for patients who are unable to eat. In the formulation of a PN mixture, precipitation between calcium and phosphate is a serious problem since venous line may be occluded. Moreover, it has been reported that the respiratory distress and sudden death are resulted from diffuse microvascular pulmonary emboli of calcium phosphate precipitates (Robinson and Wright, 1982; Breaux *et al.*, 1987; Knowles *et al.*, 1989; Hill *et al.*, 1996; McKinnon, 1996; Shay *et al.*, 1997). This problem can be found in both adult and pediatric patients; especially in preterm infants, since they require large amounts of calcium and phosphate from PN to maintain skeletal mineralization. The US Food and Drug Administration announced the Safety Alert in 1994 that cited two deaths and several cases of respiratory distress occurring in patients receiving PN containing calcium phosphate precipitates (Lumpkin, 1994.).

Such events usually reported from administering a three-in-one PN composed of carbohydrate, lipid and protein since it is cloudy and difficult to observe the precipitate. During storage, calcium phosphate precipitates are also a major threat to the stability of PN mixtures (Gellis *et al.*, 2000). In the case of conventional formula (i.e., lipid-free), one important factor affecting precipitation is the calcium-phosphate-amino acid composition. The precipitation patterns depend on the composition of amino acids in the solution; therefore, each brand name of amino acid solution has its own working chart for formulation of PN (Eggert *et al.*, 1982; Fitzgerald and Mackay, 1986; Fitzgerald and Mackay, 1987; Lenz and Mikrut, 1988; Dunham *et al.*, 1991; Mo-Suwan *et al.*, 1993; Mackay *et al.*, 1996; Mo-Suwan *et al.*, 1997). Usually, the experiment was performed at either room temperature (25°C) or at normal body temperature (37°C). However, there was a case report of precipitation which led to central venous catheter occlusion and it occurred late which was body-heat mediated although no precipitation before giving PN to a patient was noted (Robinson and Wright, 1982). Investigation for this precipitation in stress condition such as at body temperature or higher should be more preferable (Lenz and Mikrut, 1988). We performed *in vitro* experiment at 39°C in this study to imitate body temperature in fever condition in order to create in-house precipitation curve. Theoretically, precipitation is a result of perturbation in ionic equilibria of the mixed solutions (such as changes in pH and conductivity). However, the experiment for each amino acid solution or brand name consumes time and resources. We aimed to use committee-vote via empirical transfer functions obtained from various regression models of such information for describing possible zone of hazardous precipitation of calcium and phosphate when using two amino acid brand names, i.e., Moripron-F® and Aminovenos-N-Paed®. If such information could be used to describe precipitation, the methodology for data treatment used in this study may also be applicable to other kinds of problems as well.

MATERIALS AND METHODS

Materials

Moripron-F® (composed of l-isoleucine 0.56% w/v, l-leucine 1.25% w/v, l-lysine acetate 1.24% w/v, l-methionine 0.35% w/v, l-phenylalanine 0.935% w/v, l-threonine 0.650% w/v, l-tryptophan 0.13% w/v, l-valine 0.45% w/v, l-alanine 0.62% w/v, l-arginine 0.620% w/v, l-aspartic acid 0.380% w/v, l-cysteine 0.100% w/v, l-glutamic acid 0.650% w/v, l-histidine 0.600% w/v, l-proline 0.330% w/v, l-serine 0.220% w/v, l-tyrosine 0.035% w/v, aminoacetic acid 1.070% w/v, total nitrogen 15.2 mg/mL, and Na < 5 mEq/L) was purchased from Morishita Pharmaceutcal, Japan. Aminovenos-N-Paed® (composed of l-isoleucine 0.640% w/v, l-leucine 1.075% w/v, l-lysine monoacetate 1.000% w/w, l-methionine 0.462% w/v, l-cysteine 0.038% w/v, l-phenylalanine 0.457% w/v, l-threonine 0.515% w/v, l-tryptophan 0.183% w/v, l-valine 0.709% w/v, arginine 0.640% w/v, l-histidine 0.414% w/v, glycine 0.414% w/v, l-alanine 0.716% w/v, l-proline 1.619% w/v, l-serine 0.903% w/v, l-tyrosine 0.549% w/v, l-malic acid 0.150%

w/v, and total nitrogen content 14.4 g/L) was purchased from Fresenius AG , Germany. The 50% dextrose in water for injection, 50% magnesium sulfate solution, and sterile water for injection were obtained from production facility of Songklanagarind Hospital. The 10% calcium gluconate solution was obtained from Government Pharmaceutical Organization, Thailand. The 8.71% dipotassium phosphate solution was obtained from Otsuka Pharmaceutical Factory, Japan. All chemicals were pharmaceutical grade and used without further modification.

Formulation of Simplified PN Mixtures

The composition range of each substance in the simplified PN mixtures was selected from its actual range of usage in real working formultions. Since high concentrations of micronutrients are used in neonates and newborns, it would be more appropriate that prescription review should be performed for these specific patient groups rather than in adult patients. By collecting and analyzing data of PN prescriptions from the previous reports (Mo-Suwan *et al.*, 1993; Mo-Suwan *et al.*, 1997), zinc concentration used was usually less than 0.1 mmole/L, magnesium concentration used was usually less than 4 mmole/L, and the median of dextrose concentration was 12%. Zinc was not included in this study, although there was a report that zinc forms complexes with higher kinetically stability than calcium (Csermely *et al.*, 1989), since its actual range is relatively very low in comparison with calcium or magnesium concentration. Because dextrose could reduce the calcium phosphate precipitation (Niermeyer *et al.*, 1986), usage below the median value would be regarded as stress condition. To simplify the experiment, the amount of dextrose was fixed at 10%. The amount of magnesium sulfate was fixed at 4 mmole/L. The concentrations of each amino acid solution were studied at 1%, 3%, or 5% while the concentrations of dipotassium phosphate and calcium gluconate solutions were varied in order to obtain phosphate and calcium ions in the concentrations of 0 to 40 mmolar. All simplified PN formulations were prepared in the identical size test-tubes by mixing and all ingredients with the same order, i.e., mixing amino acid solution with dipotassium phosphate solution and then adding dextrose solution, sterile water for injection, magnesium sulfate solution, and calcium gluconate solution, respectively.

Characterization of Simplified PN Mixtures

All samples were characterized for their appearance, pH and conductivity before and after passing storage in the stress condition. In the stress test, the samples were incubated at 39°C in a water-bath for 4 hours and then they were fast cooled to ambient temperature (≈27-30°C) before subsequently characterized. In our preliminary study, storage at 39°C with incubation time of 4 hours was the optimal condition for routine accelerated study under non-sterile condition. Incubation time longer than 10 hours leaded to turbidity due to microbial growth which would be indistinguishable from the chemical precipitation. However, the measurement at 39°C must be avoided since temperature increase would lead to increase in electrical conductivity approximately 2.5% per °C (Prentice, 1991). Hence, characterization of the samples in this

study was performed at ambient temperature. The samples were observed for their visual appearance against black and white background under bright light. The pH measurement was carried by a pH meter (Orion model 410A, Orion Research Laboratory Products Division, USA), and conductivity measurement was performed by a conductivity meter (Model CM-115, Kyoto Electronics Manufacturing Co., Ltd., Japan).

Data Analysis

Usually, the calcium-phosphate precipitation curves at various amino acid concentrations were prepared by drawing line between calcium and phosphate concentrations obtained from experimental data. However, to implement this relationship in software for convenient usage, prediction should be made via rules, algorithms, or functions. The safety of the prediction may be more important than the accuracy of prediction in PN preparation. The unbiased estimator describing the curve would lead to 50% chance of fatal precipitation in the patient's blood circulation. Good predictor in this case should be biased toward high power of prediction and maximize patient's safety. This is possible by redefining the objective functions of the regression analysis. Since empirical multivariate polynomial functions were not good enough to represent the experimental data in the preliminary data analysis, different approach was attempted, i.e., using multiple transfer functions to do the same task simultaneously. Since the dependent variable in this case is dichotomous data, i.e., clear solution or precipitation, the logistic regression is a reasonable method which can be used with other methods for providing a satisfactory prediction. The ionic equilibrium in the system is assumed to be perturbed only if precipitation occurs. Free ion loss via precipitation from a PN mixture should reflect in the change in pH, conductivity, and all other observable or measurable physicochemical properties. These data should be information-rich since it describes the real system behavior. Thus, it might be possible to use all information of physicochemical properties to serve as transfer functions in the prediction process in order to obtain the best prediction. However, the change in pH may not reflect the ion loss due to precipitation in the system when high buffer capacity is involved. The heavily perturbed ionic equilibrium may lead to only slight change in pH in the case of high buffer capacity. The opposite is true for low buffer capacity system. Since partial contribution effect from the known total phosphate species in the system could be calculated directly from Van Slyke's equation for related buffer species (Butler, 1964), we computed the buffer effect contributed from phosphate as "phosphate buffer index (PBI)". The PBI was defined as the area under the curve of calculated buffer capacity contributed from total phosphate species on y-axis versus pH on x-axis, from pH at time zero to final pH at 4 hours after keeping at 39°C. This PBI represent the capacity to resist pH change contributed to the phosphate species involved. The area under the curve is computed by stochastic numerical integration for ease of computation (Kalos and Whitlock, 1986). The parameters used in this study could be schematically summarized (Fig 1).
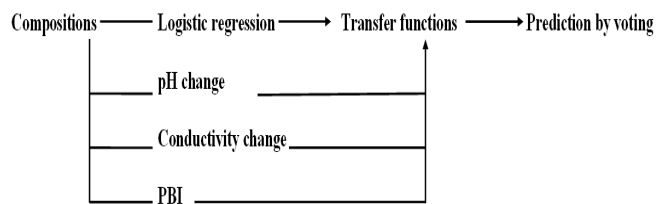

Fig 1. Parameters for describing possible zone of hazardous precipitation between calcium and phosphate in PN mixtures.

Prediction via Logistic Regression

The precipitation probability could be described directly by logistic regression model. However, conventional logistic regression uses Hosmer-Lemeshow method for data pretreatment. In Hosmer-Lemeshow method, the data set are grouped (usually in 10 groups) and the frequency values of dichotomous data are counted in each interval as if they fall at the center of that interval before performing logistic regression (Hosmer and Lemeshow, 1989). We modified the Hosmer-Lemeshow schema by using an algorithm proposed by Wibul Wongpoowarak, one of the authors, i.e., by introducing slack variable into the empirical model describing odd ratio and then extrapolate back to zero value of the slack variable. In brief, by introducing a slack variable called "scanning range" (a point in the multidimensional vector space of the independent variables), a value of scanning range was arbitrarily selected and all data points contained within reach of that scanning range were grouped together. The odd ratio within that region could be computed. This odd ratio was a function of the independent variables at that center point and the added slack variable. This process could be repeated indefinitely. By varying the size of the slack variable and then fitting the log odd ratio with empirical multivariate polynomial functions (using original independent variables and the slack variable as the causative variables), the obtained function could be extrapolated back to where this slack variable was zero. The logistic function that was independent from that slack variable would be obtained.

Given X as the set of independent variables. Suppose that the actual function describing the odd ratio is:

$$\log(Odd) = f(X) \qquad (1)$$

the slack-variable model should be:

$$\log(Odd) = g(X, s) \qquad (2)$$

where s is a slack variable. If s is extrapolated to zero, this g function would be reduced its intrinsic form, f function. The most convenient empirical function describing $\log(Odd)$ is multivariate polynomial functions with best fit properties and definable at $s = 0$, i.e.,

$$f(X) = g(X, 0) \qquad (3)$$

At high value of s, the odd ratio would be converged to overall odd ratio of the system. To prevent this undesirable convergent, two alternatives are possible.

The first alternative is using mathematical model that could describe convergent properties at high value of s. The second alternative, which was used in this study, is by using small to moderate value of s. This would enable the use of empirical

multivariate polynomial function. In order to cope with extreme odd ratio in the sparse-data space from too few data points, empirical weighted regression analysis was used according to the following equations.

$$N = G + B \qquad (4)$$

$$Weight = \sqrt{(GB/N)} \qquad (5)$$

where:

G = number of preparation that is clear (good) after incubated at 39°C for 4 hours.

B = number of preparation that is precipitated (bad) after incubated at 39°C for 4 hours.

N = total data points.

By assigning the scanning range s for each data point, the G and B could be directly counted from the experimental data. The odd ratio (G/B) and its weighing function could be calculated before performing logistic regression. In this study a single value of scanning range was applied to both calcium and phosphate simultaneously for the range of both variables are similar. Since amino acid has only 3 different levels in this experiment, it would not involve with this scanning range. By performing logistic regression of odd ratio calculated by the described method with multivariate polynomial function, the intrinsic logistic function obtained (extrapolated to $s = 0$) will be used as a transfer function in committee-voting process.

Regression Analysis of Physicochemical Properties

The physicochemical properties included pH change, electrical conductivity change, and PBI. Regression analysis was performed with empirical multivariate polynomial functions of calcium, phosphate and amino acid. To use the all regression analysis results in a committee-voting process, they were compared to sets of criteria. If the value calculated from the regression function was higher (or lower) than a critical value obtained from trial and error, the prediction would be either "clear solution" or "precipitation" accordingly.

Committee-Voting Process

Predictors from one opinion and three opinions as described above were used to vote for precipitation tendency. If the vote count is higher than a predefined critical value, which was determined empirically by trial and error, the final vote will be either "clear solution" or "precipitation".

Defining Objective Function and Parameters in Regression Analysis

The objective function is to maximize accuracy of prediction and to minimize wrong prediction affecting health hazard. Wrong prediction may be either one of two possibilities: predicting clear solution while the actual data shows precipitation or predicting precipitation while the actual data shows clear solution. The first type is very harmful for human health, i.e., fatal prediction.

The second type is safe for human health, at the expense of opportunity loss for maximizing the feeding of the important nutrient.

RESULTS AND DISCUSSION

Data Treatment

Mapping technique via transfer functions is an alternative to neural network (NN) or principal component analysis (PCA) which is well-known in pharmaceutical research (Bohidar *et al.*, 1975; Hussain *et al.*, 1991; Takayama *et al.*, 1999; Fox, 1984). In regression analysis, only the concentrations of all compositions were used as independent variables, while other physicochemical data were discarded in this process. However, physicochemical data may contain information that could also be used to describe precipitation chance. To improve the accuracy of such prediction, we attempted to use transfer functions for other physicochemical data to implement a rule that may improve the quality of prediction.

$$
\begin{aligned}
Calculated\ pH\ change &= p_1\ (compositions)\\
Calculated\ conductivity\ change &= p_2\ (compositions)\\
Calculated\ PBI &= p_3\ (compositions)\\
Calculated\ odd\ ratio\ of\ precipitation &= L_1\ (compositions)\\
Prediction &= f\ (p_1, p_2, p_3, L_1)
\end{aligned}
\qquad (6)
$$

The p_1, p_2 and p_3 are transfer functions described as multivariate polynomial functions that could be obtained empirically by ordinary least square method. The L_1 is obtained from logistic regression by using modified Hosmer-Lemeshow method as described in the corresponding section. Each regressed function acts as a transfer function. With its own empirical criteria, opinion on the precipitation status could be formed. All predictors vote to decide a single decision for each preparation: "clear solution" or "precipitation". Combining all predictor opinions, the vote according to empirical criteria would affect both prediction power and safety of usage.

One-Predictor System

Using Logistic Regression Alone

A vote was made by comparing the calculated odd ratio obtained from the regressed analysis with an arbitrary cut-off odd ratio to achieve the optimized result. The precipitation curve was drawn based upon such vote (Fig 2). When setting the cut-off odd ratio as 1 (as an unbiased estimator), the accuracy would be 58.8%, with fatal prediction 28.3%. However, by lowering the cut-off odd ratio below 0.04, fatal prediction would be 0%. The cut-off odd ratio in this study was set at 0.429 in order to optimize the practicality of the plot (Table 1). The graph is shown as bold solid lines for cut-off odd ratio at 1 and light lines for cut-off odd ratio at 0.04, the precipitation curve shift downward for the latter ratio (see dot arrow pointer).
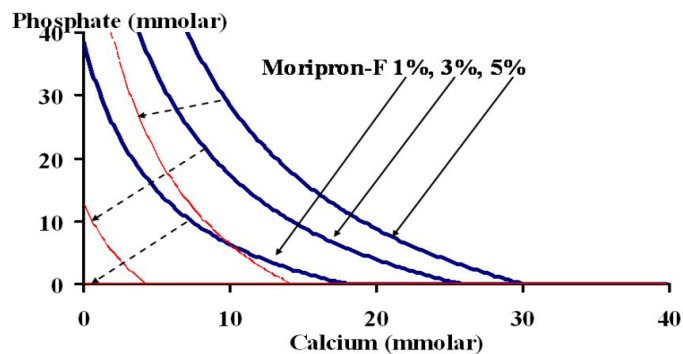
Fig 2. Precipitation charge when using logistic regression alone in 1 committee-vote (see explanation in the text).

Table 1. Accuracy and safety of usage in prediction process using various voting schemes.

	Precipitate if	Accuracy (%)	Fatal prediction (%)	P_{SOL}	Realistic
Conductivity change	< 0.048	76.2	4.6	0.140	No
pH change	< 0.004	61.6	1.2	0.076	No
PBI	< -0.605	72.9	11.7	0.196	No
Odd ratio	> 0.429	63.7	15.5	0.037	Yes
3-votes (without odd ratio)		76.7	12.5	0.237	No
4-votes (with odd ratio)		76.1	5.8	0.148	Yes

Using pH Alone

It shows unexpected pattern (Fig 3). In contrary with actual data, it does not permit high phosphate concentration.
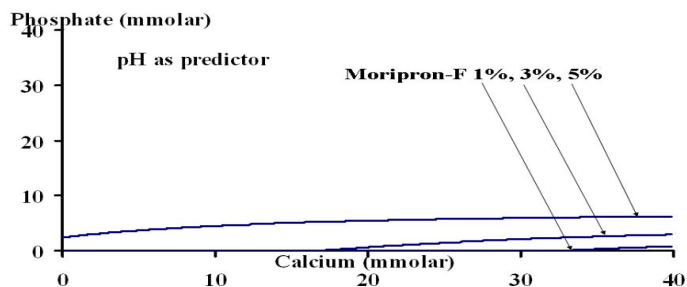

Fig 3. Precipitation charge when using pH alone in 1 committee-vote.

Using PBI Alone

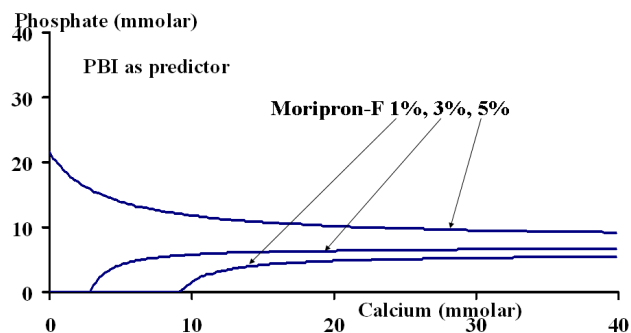This one (Fig 4) also showed somewhat similar pattern as that predicted with pH alone.

Fig 4. Precipitation charge when using PBI alone in 1 committee-vote.

Using Electrical Conductivity Alone

This function has desirable properties: maximum accuracy of prediction while minimizing wrong and health hazardous prediction. However, it is impractical to use this graph, since it still overestimates the precipitation in high phosphate region (Fig 5).
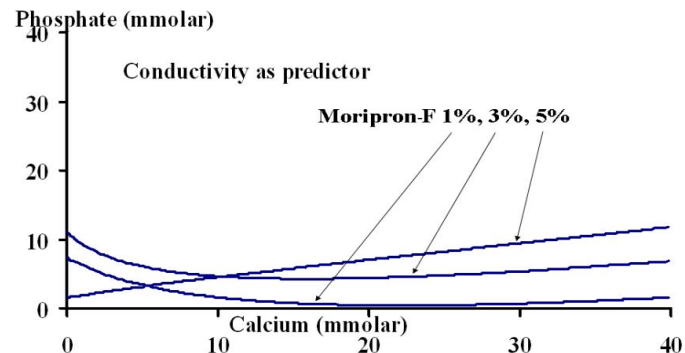

Fig 5. Precipitation charge when using conductivity alone in 1 committee-vote.

It could be observed that logistic regression provides a sensible precipitation curve, while other physicochemical properties when used alone could not. In brief, logistic regression as a single predictor could provide the most reasonable prediction among other single predictor.

Three-Predictors System (Without Logistic Regression)

Precipitation is expected if all 3 predictors predict so. Using 1 committee voting, the performance is poor, but in combination they could improve both accuracy and safety of predictions. With 3-predictors system (Fig 6), high phosphate concentration (>22 mmolar) is totally prohibited. This could not reconcile with actual experimental data, where preparation of 25 mmolar phosphate at 3% amino acid is still possible. Hence, this prediction system is still not satisfactory, even though it looks more favorable than using each predictor alone.

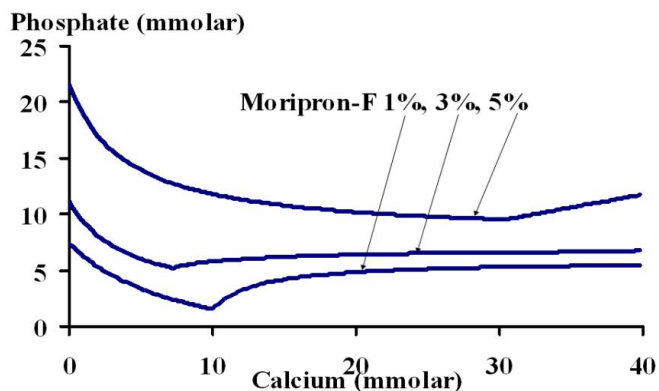**Fig 6.** Precipitation charge when using pH, PBI and conductivity in 3 committee-voting.

Four-Predictors System (With Logistic Regression)

Precipitation is expected if and only if 3 or 4 predictors predict so. By using 4-predictor system, it provides more reliable and safer prediction than using any vote alone in terms of accuracy, safety, and maximizing solubility of preparation (Fig 7).
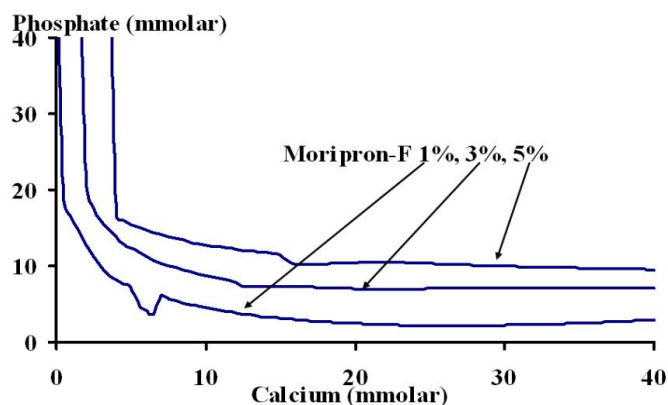
Fig 7. Precipitation charge when using logistic regression of visual inspection, pH, PBI and conductivity in 4 committee-voting.

Performance of Different Protocols

By using predictor functions obtained from all physicochemical properties, the safety of prediction could be improved dramatically while retaining satisfactory prediction accuracy (Table 1). In order to assess the applicability of the working chart, a new parameter P_{SOL} was used to represent an area fraction of the left side portion of the working chart (clear solution zone). The P_{SOL} is defined as a probability that the solution would stand clear within the range of amino acid concentration between 1% and 5%, calcium and phosphate concentrations between 0 and 40 mmolar. Monte Carlo integration method was used to compute the probability of clear solution by assign a random formulation and compute the chance of falling below the curve predicted by this method. High value of P_{SOL} permits high calcium and high phosphate in the preparation. It is obvious that this value in the case of 4-predictors system is lower than in the 3-predictors system, but it is far better than using logistic regression alone. However, since P_{sol} was not used as an objective function, it was used only as additional information.

There is a computational concept that majority committee voting could reduce error rate even in weak learning algorithm (Freund, 1995), under the condition that the individual vote is better than random guessing and is diverse, i.e., independently different from other votes (Hansen and Salamon, 1990). Such framework is in good agreement with this finding, for each individual predictor function has accuracy more than 50% (accuracy of each vote lies between 61.6% and 76.2%) and they predict in partially independent manner (Fig 1 to 4). The committee votes improve both accuracy and reduce health risk as expected (Table 1).

The precipitation/clear regions as shown in the graphs are not smooth. Since each predictor function acts as a constraint for other predictor functions, this would result in highly nonlinear behavior. However, one could observe that each line has roughly 3 distinct regions: horizontal region, vertical region, and the middle region. This may be simply computational coincidence or may represent actual relationship. If the latter case is true, this plot would suggest complicate mechanisms of precipitation involved worth exploring. In the vertical region (at low calcium concentration), one could see that if calcium concentration is less than a specific critical level, excessive amount of phosphate could be added without precipitation. This critical level changes as amino acid concentration change.

In ecological (waste water management) and mineralogical studies, it is well known that calcium and phosphate could precipitated into several different salts, such as octacalcium phosphate or $Ca_4H(PO_4)_3.3H_2O$ (molar ratio Ca:P 1.33), tricalcium phosphate or amorphous calcium phosphate or $Ca_3(PO_4)_2$ (molar ratio Ca:P 1.50), calcium hydroxy apatite or $Ca_5OH(PO_4)_3$ (molar ratio Ca:P 1.67), and dicalcium phosphate dihydrate or $CaHPO_4.2H_2O$ (molar ratio Ca:P 1.00) (House, 2007). Since each salt has its own solubility product or K_{sp} (Butler, 1964), the log-log plot between concentrations of calcium and phosphate should reveal different slopes for different salts. However, vertical or horizontal region is not reconciled with solubility product concept. Both regions should be regarded as an anomalous deviation. Exploring both regions may elucidate the possible mechanisms of precipitation. Many researchers (Eggert *et al*., 1982; Schuetz and King, 1978) explained that amino acids reduce the pH of the system, leading to lesser risk of precipitation. On the contrary, we found from this experiment (Table 2), that pH reduced ($p < 0.001$) for most solutions when precipitation occurred due to stressed test. Since PBI would always have the same sign as the changes in pH, the pH reduction would always lead to negative PBI. We found that conductivity has statistical significant reduction in precipitated system ($p < 0.001$) while it increased in the case of clear solution ($p < 0.001$). Conductivity is expected to be reduced from precipitation since ionized species are eliminated from a solution, but in clear solution the change may be due to several possible reasons, including different ionization at different pH.

Table 2. Change in physicochemical properties within 4 hours at 39°C.

Properties	PN Appearance	Average	SD	N	t-test	*p*
pH change	Clear	-0.063	0.121	308	-9.1	< 0.001
	Precipitation	-0.175	0.176	449	-21.0	< 0.001
PBI (baseline is zero)	Clear	-0.188	0.345	308	-9.5	< 0.001
	Precipitation	-0.582	0.739	449	-16.7	< 0.001
Conductivity change	Clear	0.066	0.247	308	4.7	< 0.001
	Precipitation	-0.081	0.246	449	-7.0	< 0.001

However, it is not obvious why pH in clear solution change in respect with time, with both practical and statistical significance. Approximately 55% of clear preparations (169 in 308 preparations) have pH changes (within 4 hours at 39°C) more than 0.10 pH unit which could not be regarded as trivial. From this anomaly, there are many possibilities that could not be overlooked: unobservable precipitation, chemical stability of amino acid in the solution, glycation of amino acid, solubility of calcium phosphate salt in the unionized form, or soluble complex of amino acid with calcium (amino acid chelation) or with phosphate (if such mechanism exist).

Amino acid could interact with glucose, known as Maillard reaction, or glycation of amino acid, causing browning reaction which could be identified with various methods such as immunofluorescence or ELISA (Hudson *et al*., 2001). Usually this

reaction requires much higher temperature as in confectionery production. In this study the solution color did not change by visual inspection, this mechanism is unlikely. But its possibility could not be totally eliminated. If solubility of calcium phosphate according to K_{sp} product in the unionized form is the main mechanism, the vertical and horizontal regions should not exist at all. Also, it could not explain why the critical levels (in the horizontal/vertical regions) show dependency on the amino acid concentration. Hence, this mechanism is very unlikely. Another possibility is a metal ion complex formation. The amino acid and metal ion complex, or amino acid chelate, is water soluble with neutrally charge (Ashmead, 1986; Ashmead, 1989). Another patent stated that its solubility is greatly improved especially in the desecrated water and in the presence of other organic acids (Hsu, 2001). The latter patent is in good agreement with the well known fact that calcium phosphate precipitate differently from calcium gluconate and calcium chloride, as found to be due to different degree of ionization (Henry *et al*., 1980). The cupric-glycine complex is also a well known example in such metal-amino acid complex (Sinko, 2010). There is an evidence of calcium and certains amino acid (aspartic acid, glutamic acid, glycine, alanine and lysine) forming matrix protein in renal stones with the hypothesis that soluble complex may be the involved mechanism (Kohri *et al*., 1989). If soluble complex is involved in preventing calcium phosphate precipitation, it could rationalize the pH reduction and the abrupt change of different regions in the working chart. The horizontal and vertical lines would indicate the saturated limit of binding capacity between amino acid and calcium (or with phosphate). However, the supportive evidence of amino acid and phosphate soluble complex is still unavailable, only that phosphate could form hydrogen bond with amino acid side chain (histidine, lysine, glutamic acid) (Carmona and Rodriguez, 1986). The soluble complex hypothesis requires stoichiometric relationship, one would expect to see concentration relationship between this critical limit and amino acid concentration, which reconcile with this experimental data. It is reasonable to expect similar pattern (3 distinct plot regions) in different brandnames of amino acid solutions. There are several works that show such characteristics and support this hypothesis (Fitzgerald and Mackay, 1986; Fitzgerald and Mackay, 1987; Lenz and Mikrut, 1988; Prinzivalli and Ceccarelli, 1999). The work by Dunham and co-workers (Dunham *et al*., 1991) shows 5 distinct regions, but since the criteria for precipitation in that work is the 10% reduction in free calcium concentrations, the curve is drawn according to the free calcium loss, not visual inspections. Hence, the graph in that work could not be used to compare with other studies.

CONCLUSIONS

This study is in good agreement with using algorithm framework for each individual predictor function to have accuracy more than 50% (accuracy of each vote lies between 61.6% and 76.2%) and also in diverse manner. The committee votes improve both accuracy and reduce health risk as expected in formulation of PN containing Moripron-F® as amino acid solution. The prediction details could be described by a code of program (see Appendix).

However, when applying logistic regression to predict for another brand name of amino acid solution (Aminovenos N-paed®), we found that the prediction was meaningless for practical purpose due to excessive prediction of precipitation and preparation was nearly impossible at all (i.e., P_{SOL} is practically zero). According to the majority committee voting concept, the vote will be meaningful when all individual votes are better than random guessing and with diversification. The method of majority vote is inapplicable to Aminovenos-N-Paed® experiment since prediction from pH, buffer capacity and electrical conductivity could not provide good voting quality in itself.

Further basic research to elucidate the true mechanisms of precipitation is also necessary for a priori prediction of precipitation curve in the case of new amino acid brand names. This suggested that the applicability of this technique is still very limited. Due to the highly nonlinear behavior of the chart, we did not use this chart in actual practice, although it is very interesting in computational aspect. It shows that transfer function based upon real-world phenomena is an interesting methodology and it may hint a clue to the underlying interesting real-world phenomena.

ACKNOWLEDGEMENTS

APPENDIX

Prediction function for Moripron-F® which is abbreviate written as Moripron. This function was written in Visual Basic Module in Microsoft Access. Moripron unit is percentage, calcium unit is millimolar, phosphate unit is millimolar. The function MoripronPrediction will return 0 for "clear solution is expected" and 1 for "precipitation is expected". This function is a basis for creating working chart as shown above.

```
Function PredictConduct(Moripron, Calcium, Phosphate)
x = Moripron
y = Calcium
z = Phosphate
  Rem ******* Best Model of Conduct difference
  ' N  Mean.Err Mean.Sq.Err  Adj.R2  F(9,750)  p-value
  ' 760  1.07E-01 4.61E-02   0.29749  36.71  < 0.00001
P1 = 0.2666442              'SE 8.196537E-02
P2 = 0.04716392            'SE 2.908783E-02
P3 = -0.01679295           'SE 6.88953E-03
P4 = -0.0266121            'SE 9.335472E-03
P5 = -0.01354405           'SE 4.796559E-03
P6 = 0.0002885588          'SE 1.962441E-04
P7 = 0.0003867525          'SE 2.735034E-04
P8 = 0.004440316           'SE 8.866511E-04
P9 = -0.001336355          'SE 2.599645E-04
P10 = 0.003339687          'SE 1.035683E-03
```

```
PredictConduct =        P1 + P2 * x + P3 * y + P4 * z + P5 * x ^
                        2 + P6 * y ^ 2 + P7 * z ^ 2 + P8 * x * y
                        + P9 * y * z + P10 * x * z

End Function


Function PredictpH (Moripron, Calcium, Phosphate)
x = Moripron
y = Calcium
z = Phosphate
  Rem ******* Best Model of pHdifference
  ' N Mean.Err Mean.Sq.Err Adj.R2  F(8,751)  p-value
 '760 1.17E-01 2.20E-02   0.20413  25.33  < 0.00001
  P1 = -0.1020892                  'SE 5.055044E-02
  P2 = -0.01630997                 'SE 2.001708E-02
  P3 = 0.005759205                 'SE 2.530894E-03
  P4 = -0.001814379                'SE 6.289742E-03
  P5 = 0.0007748762                'SE 5.957884E-04
  P6 = -0.001283003                'SE 1.618223E-04
  P7 = -0.004185784                'SE 7.129899E-04
  P8 = 0.01373154                  'SE 3.312856E-03
  P9 = 0.0006524337                'SE 1.887098E-04
  PredictpH =        P1 + P2 * x + P3 * y + P4 * z + P5 * x * y +
                     P6 * y * z + P7 * x * z + P8 * x ^ 2 + P9 * z ^
                     2
End Function


Function PredictPBI(Moripron, Calcium, Phosphate)
x = Moripron
y = Calcium
z = Phosphate
  Rem ******* Best Model of PBI
  ' N Mean.Err Mean.Sq.Err Adj.R2  F(7,752)  p-value
 '760 7.19E-01 8.89E-01   0.42751  81.97  < 0.00001
  P1 = -1.048903                   'SE .2490174
  P2 = -0.2596395                  'SE .1223362
  P3 = 0.07780069                  'SE 1.385819E-02
  P4 = 0.1375185                   'SE 1.626869E-02
  P5 = 0.007577323                 'SE 3.705814E-03
  P6 = -0.01453173                 'SE 8.977548E-04
  P7 = -0.03882334                 'SE 4.495297E-03
  P8 = 0.1267296                   'SE 2.048023E-02
  PredictPBI =       P1 + P2 * x + P3 * y + P4 * z + P5 * x * y +
                     P6 * y * z + P7 * x * z + P8 * x ^ 2
End Function


Function Predictodd(Moripron, Calcium, Phosphate)
x = Moripron
y = Calcium
z = Phosphate
u = 0
  P1 = -0.9515043                  'SE .194194
  P2 = -0.965557                   'SE 8.257813E-02
  P3 = 0.0848798                   'SE 6.567539E-03
  P4 = 0.04995269                  'SE 1.258034E-02
  P5 = 0.3126601                   'SE .1219488
  P6 = 0.02145674                  'SE 2.422878E-03
  P7 = 0.008600611                 'SE 6.888767E-04
  P8 = -0.04997348                 'SE 7.731848E-03
  P9 = 0.3886348                   'SE 5.097514E-02
  P10 = 0.000315277                'SE 7.534163E-05
  P11 = -0.008776718               'SE 8.771035E-04
```

```
Predictodd =        Exp(P1 + P2 * x + P3 * y + P4 * z + P5 * u
                    + P6 * x * y + P7 * y * z + P8 * z * u + P9
                    * u * x + P10 * x * y * z * u + P11 * u ^ 2 *
                    x * y)

End Function


Function MoripronPrediction(Moripron, Calcium, Phosphate) 'This
prediction is declared to be unsafe for real usage
If PredictpH(Moripron, Calcium, Phosphate) > 0.004 then
        Predictor1=0
    Else
        Predictor1=1
    End if

    If  PredictConduct(Moripron, Calcium, Phosphate) > 0.048
      then
        Predictor2=0
    Else
        Predictor2=1
    End if

    If PredictPBI(Moripron, Calcium, Phosphate) > -0.605  then
        Predictor3=0
    Else
        Predictor3=1
    End if

    If  PredictOdd(Moripron, Calcium, Phosphate) <   0.42857
      then
        Predictor4=0
    Else
        Predictor4=1
    End if

    If Predictor1+Predictor2+Predictor3+Predictor4 >= 3 then
          MoripronPrediction=1
          'Prediction: This formula should precipitate.
    Else
          MoripronPrediction=0
          'Prediction: This formula should provide clear
      solution.
    End if
End function
```

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