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Effect of titrant concentration on pH-zero point of charge: verification by bootstrap technique

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Summary

Potentiometric titration provides a single value of the zero point of charge (ZPC) of which the confidence interval is difficult to assess. By applying bootstrap regression analysis, both the ZPC and its standard error could be determined empirically. The effect of titrant concentration (ranging from 0.025 to 2 N) could be verified. By using aluminum hydroxide gel as a model, the ionic strength contributed from titrant does not affect the calculated ZPC in practical aspects, even the values differ statistically. However, the invariant pH shift characteristics depend on the mathematical model used.

Introduction

A pH-zero point of charge, where feasible, is a valuable tool for studying pH-dependent adsorption of aluminum-base suspensions, which are currently used as adjuvants in vaccines (Callahan et al., 1991). The conventional potentiometric titration method required titrant concentrations in the range below 0.1 N. However, very dilute titrant may not be suitable for studying adsorption over a wider pH range due to the dilution effect from the titrant itself.

In previous communications (Wongpoowarak and Worakul, 1991, 1992), we stated that regression analysis of pH titration profiles (pH vs volume of titrant) could provide a confidence interval range of ZPC. However, only the confidence interval of pH on the *y*-axis could be computed directly, but not for the titrant volume (which is necessary for computation of the ZPC) on the *x*-axis. Furthermore, assessing the reliability of pH shift characteristics is also a formidable task. The bootstrap technique (or robust statistics), a recent concept in robust statistics, may be a valuable tool to provide an insight into these questions.

Theoretical

The potentiometric titration method can be used to determine the zero point of charge (ZPC).

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Theoretically, the ionic strength (μ) would exert no effect on the pH shift when the characteristic surface charge is at the ZPC (Feldkamp et al., 1981; Shah et al., 1982). The following expression should always be valid:

pH = pH at zero ionic strength

+ pH shift due to ionic strength effect

According to the Debye-Hückel concept of ionic strength, the pH of the system is affected by ionic strength. By fixing the titrant amount or titrant volume as V, the above statement should take the following form (Wongpoowarak and Worakul, 1991, 1992):

$$\mathrm{pH}(V, \mu) = \mathrm{pH}(V, \mu = 0) + S(\mu) \cdot h(V) \qquad (1)$$

$$S(\mu) \approx \sqrt{\mu} / \left(1 + \sqrt{\mu}\right) \qquad (2)$$

where $\mathrm{pH}(V,\mu = 0)$ is the pH at zero ionic strength at titrant volume equal to V. Henceforth, we refer to $\mathrm{pH}(V,\mu = 0)$ as the (extrapolated) reference pH. $S(\mu)$ is a function of ionic strength and $h(V)$ is a function of V. The product of $h(V) \cdot S(\mu)$ is a pH shift (from the pH at $\mu = 0$), contributed by the ionic strength at the same corresponding V.

By definition, V is an intermediate function of the reference pH and $h(V)$. Thus, at any fixed value of V, $h(V)$ can also be regarded as a function of the reference pH. In the mathematical aspect, $h(V)$ should become invariant with respect to both ionic strength (μ) and milliequivalent (meq) of titrant. We termed this as 'Invariant pH-shift characteristics' (Wongpoowarak and Worakul, 1992), by which the term invariant does not imply a temperature effect or any unexpected physicochemical interaction in the system.

In the titration process, the ionic strength of the system is not constant throughout the experimentation. This may violate the validity of the previous mathematical models for computing the ZPC. Practically, to maintain zero ionic strength throughout the experiment or to determine the true ionic strength of the charged system is very difficult. Also, the sample itself could contribute ionic strength to the system. Only a partial correction for the ionic strength from the titrant contribution is possible in our experiment.

To clarify this effect statistically, the standard error of ZPC must be known. We propose that bootstrap statistics should be used here to enumerate the standard error of the ZPC, even though its validity has not yet been fully explored (see Data analysis). This work aims at elucidating the effect of ionic strength contributed by the titrant on the ZPC and the invariant pH-shift characteristics.

Mathematical Model

As previously proposed, $h(V)$ can be computed (Wongpoowarak and Worakul, 1992). The underlying assumptions are: (1) Eqn 2 is valid; (2) μ is constant throughout the experiment; (3) no ionic strength contribution is made by the sample; (4) the sample concentration does not alter other physicochemical properties of the system, including the ZPC; and (5) the temperature is maintained at 25°C.

Unfortunately, to cover all possible ranges of ZPC (Feldkamp et al., 1981), one must use a substantial amount of titrant. This violates the first and second assumptions. At very low values of μ or at high concentrations of titrant, the added titrant obviously affects the μ value. Titration by using a higher range of titrant concentrations is doubtful. The effect of uncontrollable μ on $h(V)$ and the ZPC should thus be assessed.

To compute the unbiased ZPC in Eqn 1, all regression parameters and the contributor functions in Eqn 1 should adapt themselves to the data set. By using subset regression, all ad hoc models and regression parameters for the contributor functions in Eqn 1 could be determined from experimental data.

From the previous communication, we found that $h(V)$ deviated from linearity (Wongpoowarak and Worakul, 1992). It should thus be approximated with a polynomial function of degree $\geqslant 2$. Also, both $\mathrm{pH}(V = 0,\mu)$ and $h(V)$ should be represented by high-degree polynomial functions (Wongpoowarak and Worakul, 1991). At the ZPC,

$h(V)$ is zero. Roots of this equation, V, could be solved iteratively by a fast convergent algorithm formula of Newton-Ralphson (Mathews, 1987):

$$V_{i+1} = V_i - \left[\frac{h(V_i)}{\frac{\mathrm{d}h(V_i)}{\mathrm{d}V_i}} \right] \tag{3}$$

Iteration stops when the size of $V_{i+1} - V_i$ is within tolerance limit.

This root of the equation, $h(V) = 0$, could be used to compute the corresponding ZPC by substitution into Eqn 1 and setting $\mu = 0$ (Wongpoowarak and Worakul, 1991). Partial subset regression was also performed to find an ad hoc model for each set of the bootstrap sample to mimic Eqn 1. Due to the time-consuming nature of calculation by this method, the calculation applies to only four different regression models, i.e., either polynomial of degree 3 or 4 for describing both $h(V)$ and $\mathrm{pH}(V, \mu = 0)$. From preliminary study, these models are suitable for most bootstrap data sets.

However, multiple roots of the equation, V at $h(V) = 0$, are inevitable. The root may converge to any unexpected value. This depends on the predefined initial value and the estimated regression parameters, which were varied in each bootstrap run. We should reject such a value as a numerical artifact. Such a value could readily be found, for it lies outside the experimentation range. In this study, such an occurrence is rare.

Materials and Methods

Instrument and materials

The pH meter model used was a SA 520 (Orion Research Inc.). Aluminum hydroxide gel was from Henkel (Germany). Other reagents (KCl and KOH) were purchased from Vidhayasorm, Thailand. Deionized water was used throughout this experiment.

Data analysis

The word 'bootstrap' denotes 'dependent origination'. It is used for describing any system or phenomenon that could feed back to strengthen itself, and emerges to existence. According to Efron and Tibshirani (1991), bootstrap statistics can be applied to almost any statistical estimation problem. In brief (Bustamante et al., 1991; Efron and Tibshirani, 1991), each element in a bootstrap sample of size n is a sample set which is randomly drawn with replacement from an original sample of the same size. Then, the selected statistical procedures are performed. Each bootstrap sample provides a set of regression parameter for each contributor function in Eqn 1. The ZPC could be computed by replacing V at $h(V) = 0$ into Eqn 1. By repeating this process B times, the mean and standard error of the ZPC could be established. The best-fit criterion of regression is R^2 adjusted.

Nowadays, some standard statistical softwares implement the bootstrap algorithm (Bustamante et al., 1991). This should facilitate a programming task on bootstrap regression where such software is available. Note that the bootstrap technique here is an ordinary weighted least-squares method with the weighing function conforming to the bootstrap method (Efron and Tibshirani, 1991). One can create such a program by writing a short program to create a bootstrap data file for performing regression analysis, and another short (piece of) program to compute the ZPC from the obtained regression parameters reported from the available statistical software. The whole process could be reduced to a single program or run in a batch-file mode by which many small programs may be arranged to perform the same task.

The ZPCs from various bootstrap runs were checked for normal distribution by a Kolmogorov plot. In brief, all ZPC values (N sets) were sorted from 1 to N of which its ordinal number (i) was transformed to a probability scale (p value) by a formula $(i-0.5)/N$. From the p value, the Z score could be computed automatically by using Derenzo's approximation. The normal distribution property could be illustrated by Kolmokorov's plot which is characterized by a linear trace for a normal distribution population. The confidence interval range of ZPC could be read directly from the projection on the x-axis scale within the corresponding Z score.

Calculation was performed on a 58 MHz IBM-AT PC compatible under a DOS 5 environment. Software was written by W.W. in QuickBASIC version 3 format (Microsoft Corp.). The time to compute a ZPC from each bootstrap sample (N about 60) is approx. 20 s in this study. For each data set, the bootstrap size (B) is 200, except for Fig. 3, in which the bootstrap size was much smaller due to the limitation of computer memory.

Method

Verification of Eqn 2 Aluminum hydroxide gel in deionized water (28°C) was titrated in a magnetically stirred beaker with 1.5 N KCl. The titration process covers the same ionic strength range encountered in the determination of the ZPC procedure. The effect of ionic strength on pH shift was recorded.

Verification of Eqn 1 Aluminum hydroxide gel was used as a model system to determine ZPC by the titration method of Feldkamp et al. (1981), with slight modification. In the procedure, aliquots of the gel containing approx. 1% equivalent aluminum oxide (Al_2O_3) were adjusted to the desired ionic strength with KCl. The sample suspensions were titrated with KOH or HCl exclusively, depending on the samples. The selected titrant concentrations were 0.025 N (Ortyl and Peck, 1991), 0.05, 0.1 N (Feldkamp et al., 1981), 0.25, 0.5, 1 and 2 N. Titrant was added by micropipette and stirred with a magnetic stirrer. In each titration step, the sample was allowed to equilibrate for not less than 2 min before each pH recording. To avoid the effect of temperature, all experiments were performed at 28°C, assuming that Eqn 2 is valid at this temperature. The μ value was always less than 0.15 throughout this study. Titrations were performed in three replicate runs.

Results and Discussion

From Eqn 2, we approximated $S(\mu)$ by $\sqrt{\mu}/(1+\sqrt{\mu})$ at 25°C. However, the temperature maintained in our laboratory is about 28°C. The precise form of Eqn 2 should also be verified. Assuming the ionic strength contribution from the sample is zero, the ionic strength contribution from the adding salt could be calculated. By titrating a blank sample suspension with a KCl solution of known concentration, the relationship between the pH shift and μ could be established. By using $S(\mu)$ as in Eqn 2, we found that;

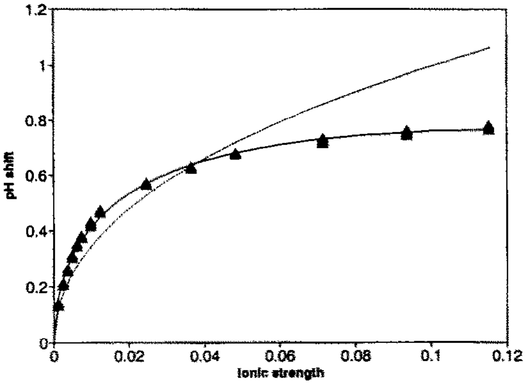

Fig. 1. Effect of added ionic strength on pH shift. (— — —) Using $S(\mu)$ in Eqn 2; (———) using $S(\mu)$ in Eqn 6.

$$\begin{gathered}\text{pH shift} = 3.6094 \cdot \sqrt{\mu}/\left(1+\sqrt{\mu}\right) \\ (n = 45;\ s = 0.076;\ F(1{,}44) = 374.4)\end{gathered} \tag{4}$$

However, the $S(\mu)$ in Eqn 2 is unsuitable and not representative of the actual relationship (see Fig. 1). This may due either to non-ideal experimental conditions or to the nature of Eqn 2 itself, or both. The modified form of $S(\mu)$ should compensate for all the aforementioned weak points, including the temperature effect and all other uncontrolled conditions. The empirical relationship between pH shift and μ in Fig. 1 could be determined by non-linear regression as in the following form:

$$S(\mu) \approx \sqrt{\mu}/\left(a+\sqrt{\mu}+b\cdot\mu\right) \tag{5}$$

where a and b are regression parameters.

From the experimental data,

$$\begin{gathered}\text{pH shift} = 4.715332 \\ \cdot\sqrt{\mu}/\left(0.977103+\sqrt{\mu}+6.72792\cdot\mu\right) \\ (n = 45,\ s = 0.014;\ F(3{,}42) = 12356)\end{gathered} \tag{6}$$

Obviously, the asymptote in Eqns 4 and 6 is not equal. Therefore, the parameters obtained from the modified form of $S(\mu)$ should not be applied to other conditions. They may also depend on several factors, namely, sample type, sample concentration, temperature effect and ionic impurities. However, it is sufficient for validation purposes in this study.

To compute for the range of the confidence interval of ZPC, there should be only one mathematical model that could be used consistently throughout the calculation process, with the assumption that the model must be correct. However, only Eqn 1 could be regarded as an ideal model in this meaning. Eqn 1 should thus be approximated by ad hoc empirical model for each bootstrap data set. We found that, in this study, the worst case of adjusted R^2 is higher than 0.9939, indicating that the ad hoc model is reasonable.

From bootstrap computation, all bootstrap ZPCs have a normal distribution with very low standard error (Fig. 2; and Table 1). The bootstrap mean of the ZPC is slightly different from the unbiased ZPC computed from only one best-fit model. Although some lines in Fig. 2 differ statistically ($p < 0.05$), the differences are very small from a practical aspect. By comparing the ZPC values obtained from different methods, both ZPCs partially corrected for μ and without any correction for μ could be compared. The μ correction was calculated from the known amount of titrant volume and its concentration. It was found that the ZPCs calculated from both methods are approximately the same, even at very high concentrations of titrant (2 N). At the same titrant

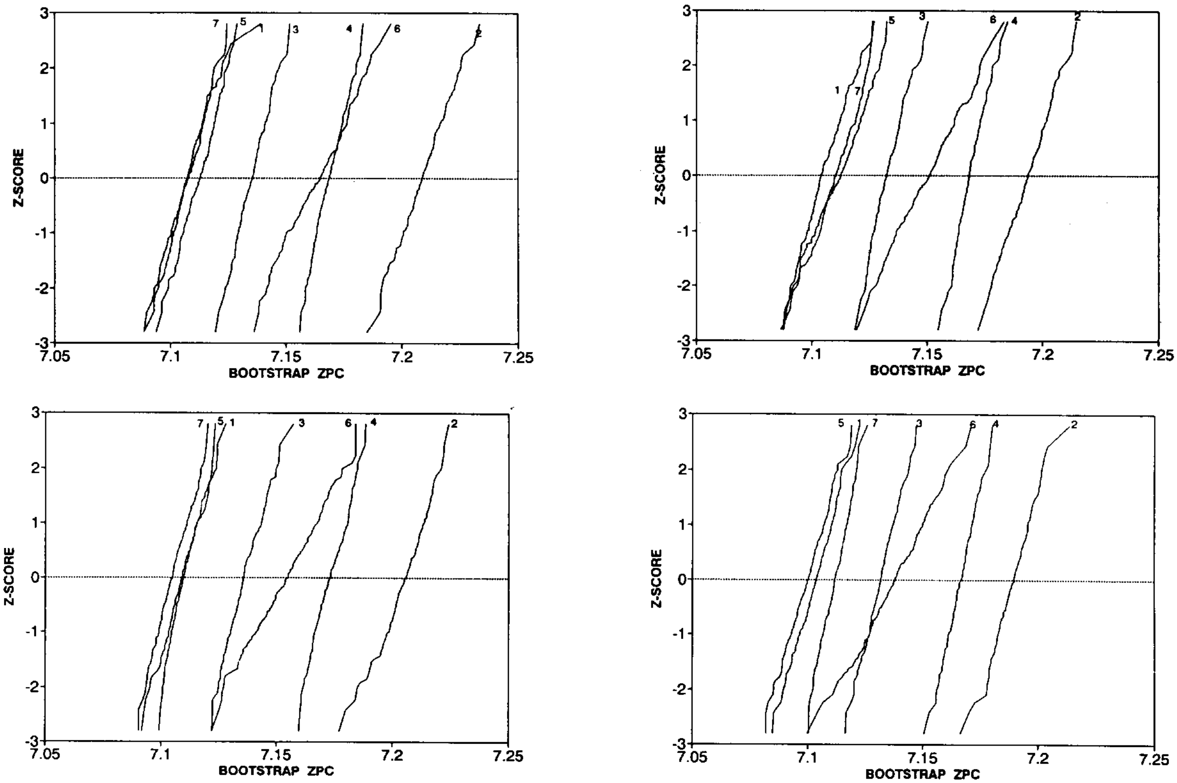

Fig. 2. Normal distribution of bootstrap ZPC. Kolmokorov's plot characterized by linearity indicates normal distribution properties. The bootstrap size is 200 for all lines. (a, top left) Using $S(\mu)$ in Eqn 2 without μ correction; (b, top right) using $S(\mu)$ in Eqn 2 with partial μ correction; (c, bottom left) using $S(\mu)$ in Eqn 5 without μ correction; (d, bottom right) using $S(\mu)$ in Eqn 5 with partial μ correction. Concentrations of KOH were in the following order: (1) 0.025 N; (2) 0.05 N; (3) 0.1 N; (4) 0.25 N; (5) 0.5 N; (6) 1 N; (7) 2 N.

TABLE 1

Comparative results of bootstrap ZPC [a] *with and without partial correction for* μ *by* $S(\mu)$ *and modified* $S(\mu)$

N KOH	Initial pH of antacid ($n = 2$)	ZPC by $S(\mu)$ [b]		ZPC by modified $S(\mu)$ [c]	
		With partial μ correction	Without μ correction	With partial μ correction	Without μ correction
0.025	5.75	7.104 ± 0.007 (7.106)	7.107 ± 0.006 (7.108)	7.103 ± 0.006 (7.104)	7.109 ± 0.006 (7.109)
0.05	5.75	7.193 ± 0.007 (7.192)	7.208 ± 0.008 (7.209)	7.189 ± 0.006 (7.188)	7.205 ± 0.008 (7.205)
0.1	5.72	7.132 ± 0.005 (7.132)	7.135 ± 0.005 (7.136)	7.131 ± 0.005 (7.132)	7.136 ± 0.006 (7.137)
0.25	5.70	7.168 ± 0.004 (7.167)	7.168 ± 0.005 (7.167)	7.166 ± 0.005 (7.166)	7.173 ± 0.006 (7.179)
0.5	5.70	7.111 ± 0.009 (7.107)	7.112 ± 0.006 (7.111)	7.099 ± 0.006 (7.099)	7.110 ± 0.005 (7.114)
1	5.72	7.150 ± 0.011 (7.149)	7.163 ± 0.011 (7.157)	7.137 ± 0.012 (7.138)	7.154 ± 0.012 (7.150)
2	5.72	7.110 ± 0.007 (7.113)	7.107 ± 0.006 (7.107)	7.111 ± 0.004 (7.112)	7.104 ± 0.006 (7.104)

[a] Data represent bootstrap mean of ZPC ± bootstrap S.E. with (unbiased mean of ZPC) calculated from the best-fit model, using here three decimal places instead of two for statistical purposes only.

[b] Using $S(\mu)$ as $\sqrt{\mu}(1+\sqrt{\mu})$: minimum R^2adj, 0.9939; maximum root mean square error, 0.0368.

[c] Using $S(\mu)$ as $\sqrt{\mu}/(0.977103+\sqrt{\mu}+6.72792\cdot\mu)$; minimum R^2adj, 0.9951; maximum root mean square error, 0.0284.

concentration, the maximum difference of the mean ZPC determined from both methods (partial correction and no correction; intra-sample, both methods) is about 0.02 pH unit, which is very small compared to the pH measurement error. The maximum difference of the bootstrap mean among various titrant concentrations (inter-sample, same method) is only 0.11 pH unit, and the difference between the highest and lowest bootstrap ZPC (pooled samples, both methods) is 0.15 pH unit. These results verify the validity of the assumptions for Eqn 1, and clearly demonstrate that titrant concentrations in the range 0.025–2 N do not affect the value of the ZPC to any significant extent. From the fluctuating initial pH value (of approx. 1% antacid suspension), the influence of instrumental calibration, temperature variation and different equili-

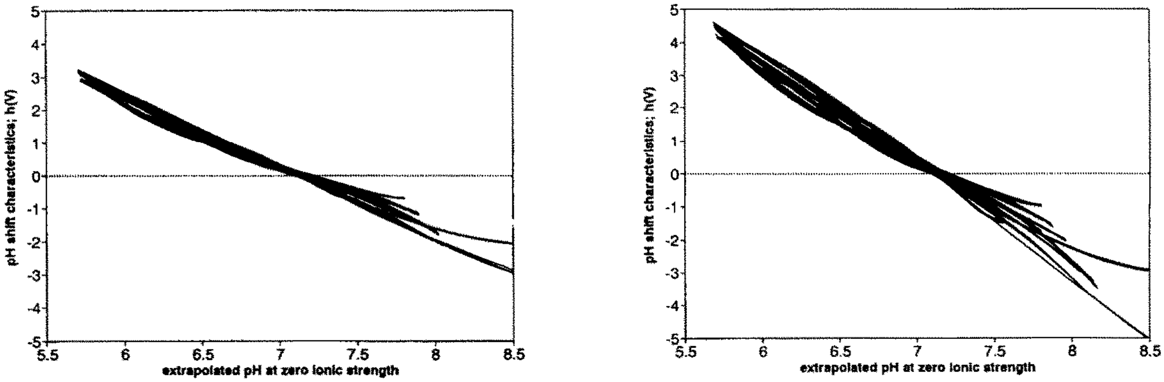

Fig. 3. Invariant pH shift characteristic plot by different form of $S(\mu)$, with and without correction for μ. (a) Using $S(\mu)$ in Eqn 2; (b) using $S(\mu)$ in Eqn 5.

bration time of titration task on the reliability of ZPC may be greater. These results suggest that the range of titrant concentration may be extended from the suggestion of Feldkamp et al. (1981). Later studies which prefer very low titrant concentration, for example, using 0.025 N titrant for finding the ZPC of titanium dioxide (Ortyl and Peck, 1991), may not be suitable for observing the pH-shift characteristics over a wider pH range.

It was found that the different forms of $S(\mu)$ changed the plot of invariant pH-shift characteristics as illustrated in Fig. 3. All pH-shift characteristic profile from various titrant concentrations were pooled together so as to demonstrate the overall similarities. The pH($V,\mu = 0$) on the x-axis should be regarded as the extrapolated value of pH at zero ionic strength which was computed from Eqn 1. Fig. 3 indicates that the invariant pH-shift characteristics computed directly from Eqn 1 could be regarded as practically independent of titrant concentration. This plot is consistent with the mathematical deduction (Wongpoowarak and Worakul, 1992).

It should be noted that the plot of extrapolated pH at zero ionic strength vs $h(V)$ is unreliable near the end of the titration process. In Fig. 3, the right-hand tails (near the end of titration) of the plot are dubious. This may due to various factors, most probably the titrant volume error or the numerical nature of Eqn 1 itself. At high titrant concentration, any small error in titrant volume could adversely affect the reliability of the measured pH. Very accurate measurement together with all possible subset regression of Eqn 1 should be useful in minimizing this uncertainty. Using a different form of $S(\mu)$ in Fig. 3a and b, both profiles are also different. Our previous assumptions about the invariant property of $h(V)$ should then be strictly confined to the standardized environment, experimental protocol, and any comparison between different sample batches must be performed by consistently using the same form of $S(\mu)$.

Conclusion

The results indicate that, when using aluminum hydroxide gel as a model system, the ZPC is independent of titrant concentration, over a wide range of titrant concentrations (0.025–2 N). Eqn 1 is thus a well-behaved equation for calculating the ZPC. It provided the same range of ZPC even when the assumptions about the validity of $S(\mu)$ failed and ionic strength correction was not made. This observation is not true in the case of pH-shift characteristics. The different form of $S(\mu)$ influences the pH-shift characteristic plot. Only when the experimentation protocol is standardized and using consistently the same form of $S(\mu)$, should the reliability of the pH-shift characteristics be ensured.

Appendix A

Derenzo's approximation (Hoaglin, 1991) for transforming p to Z score has a relative error of less than 0.00013% for $1.0E{-}7 < p < 0.5$, i.e., this formula correct to four decimal places is sufficient for visualizing the normal distribution property of ZPC. The algorithm is written according to QuickBasic compiler (Microsoft Corp.) format. The input value (p) must be between $1.0E{-}7$ and $1{-}1.0E{-}7$,

```
DEF fnP2Z(p)                        'p-value is between 1E-7 to 1-1E-7
    STATIC y, a, b UpperHalf, Q
    IF p = 0.5 THEN                 'the routine cannot be used
       fnP2Z = 0                    'but, by definition, it is zero
       EXIT DEF
    END IF
    UpperHalf = (p > 0.5)           'check if value of p higher than 0.5
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```
    IF UpperHalf THEN p = 1-p           'If yes, then transform it to a proper range
    y = -LOG(2*p)                       'LOG is natural log (base e) in Basic Language
    a = ((4*y + 100)*y + 205)*y*y       'temporary variable
    b = ((2*y + 56)*y + 192)*y + 131    'temporary variable
    Q = SQR(a/b)
    IF UpperHalf THEN fnP2Z = Q ELSE fnP2Z = -Q
    'Check for a proper sign for Z from p-value, set it to positive value
    'for p > 0.5, and to negative value for p < 0.5)
END DEF
```

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