**American Journal of Chemistry and Applications**
2015; 2(1): 5-9
Published online January 20, 2015 (http://www.openscienceonline.com/journal/ajca)

# Formulation of dynamic buffer capacity for phytic acid

Anna Maria Michałowska-Kaczmarczyk[1], Tadeusz Michałowski[2], Agustin G. Asuero[3]

[1]Department of Oncology, the University Hospital in Cracow, Cracow, Poland
[2]Department of Analytical Chemistry, Cracow University of Technology, Cracow, Poland
[3]Department of Analytical Chemistry, the University of Seville, Seville, Spain

## Email address

michalot@o2.pl (T. Michałowski)

## To cite this article

Anna Maria Michałowska-Kaczmarczyk, Tadeusz Michałowski, Agustin G. Asuero. Formulation of Dynamic Buffer Capacity for Phytic Acid. *American Journal of Chemistry and Applications*. Vol. 2, No. 1, 2015, pp. 5-9.

## Abstract

The general formulation of dynamic buffer capacity for polyprotic acids and bases (and polyprotic acid and base salts) has been derived. Polyprotic acids show buffer capacity over a broad range of pH values, according to their successive protonation constants. Polyprotic acids with equidistant $pK_i$ values behave as universal buffers. The paper covers the dynamic buffer capacity for phytic acid, which posse's twelve acid groups. Phytic acid, the hexaphosphate ester of myo-inositol, has a great biological relevance, and shows antioxidant/anticancer properties.

## Keywords

Acid-Base Equilibria, Buffer Capacity, Phytic Acid, Titration

## 1. Introduction

Phytic acid $(CHOPO(OH)_2)_6$, known as inositol hexaphosphate, IP6) is an organic acid (Fig. 1); inositol = cyclohexane-1,2,3,4,5,6-hexol [1]. As a major phosphoric component of many seeds, it is extracted mainly from rice bran. In particular, phytic acid prevents oxidative stress in seeds. Moreover, phytic acid, by virtue of its ability to chelate Fe (+2), shows antioxidant action [2-6]. Thus it is a potent inhibitor of the iron-driven formation of reactive oxygen species that adversely affect the production or storage of various forms of food. It explains why seeds belonging to many plant species are viable for a long time; in spite of the fact they contain a potentially dangerous mixture of iron, oxygen, and unsaturated fatty acids. The splitting of phytic acid, lower inositol phosphate esters and inorganic phosphate can be affected by phytase that belongs to a special class of phosphomonoesterases [7-9]. Phytic acid has also striking anticancer properties, demonstrated in both *in vivo* and *in vitro* studies [10]. Phytic acid reduces melanin spots (brightening pigmentation), shrinks dilated vessels, and acts as antioxidant. When used as facial cream, it exhibits also mild moisturizing effect.

Phytic acid is also used in analyses as an acidulant for pH adjustment [11], e.g. in capillary electrophoresis (CE [12]). Because of its twelve acidic groups, phytic acid can be used as a buffer over a wide pH range 2-11). The use of phytic acid both as a modifier and as a pH buffer results in enhanced differences between the various protein mobilities when compared with the use of monoprotic buffers; it improves e.g. resolution in protein separations [13].

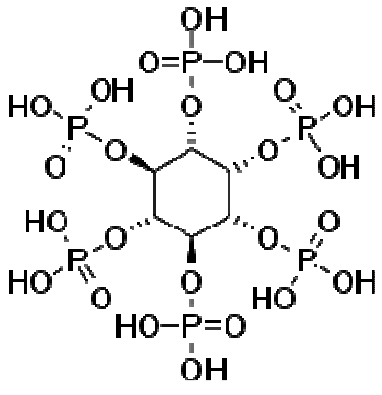


***Fig. 1.*** *Phytic acid [14].*

## 2. Acid-Base Properties of Phytic Acid

The 12-protic phytic acid can be denoted briefly as $H_{12}L$. Its acid-base properties can be expressed by pKi = -log$K_i$ (i = 1,…,12) values for successive protonation constants, $K_i$. An accurate knowledge of the $pK_i$ values is essential for a thorough understanding of its reactions in solution. However, large discrepancies among these data, found in literature were stated [15,16]. The protonated species are sometimes of very similar stability. From [17] we have: $pK_1$ = 1.92, $pK_2$ = 1.92, $pK_3$ = 1.92, $pK_4$ = 2.38, $pK_5$ = 2.38, $pK_6$ = 3.16, $pK_7$ = 5.20, $pK_8$ = 6.25, $pK_9$ = 7.98, $pK_{10}$ = 9.19, $pK_{11}$ = 9.53, $pK_{12}$ = 9.53. These $pK_i$ values are frequently put in context with stablility constants of complexes formed by anionic species of phytic acid with different cations, see e.g. [18]

## 3. Formulation of Dynamic Buffer Capacity

### 3.1. General Notations

The dynamic buffer capacity is defined as follows [19,20]

$$\beta_V = \left|\frac{dc}{dpH}\right| \tag{1}$$

where

$$c = C \cdot \frac{V}{V_0 + V} \tag{2}$$

denotes current concentration of a reagent R in a D+T mixture obtained after addition of V mL of C mol/L solution of the reagent R (considered as titrant, T) into $V_0$ mL of a solution named as titrand (D). If the additivity in volumes of D and T is assumed, then the volume of D+T is $V_0$+V mL, at this point. In particular, the reagent R can be a strong base, MOH, a strong acid, HB, a weak polyprotic acid $H_nL$ or its salt of $M_mH_{n-m}L$ (m = 1,…,n) or $H_{n+m}LB_m$ type [19,21]. From (1) and (2) we have

$$\beta_V = \left|\frac{dc}{dV}\cdot\frac{dV}{dpH}\right| = \frac{C\cdot V_0}{(V_0+V)^2}\cdot\left|\frac{dV}{dpH}\right| \tag{3}$$

The buffer capacity $\beta_V$ is an intensive property, expressed in terms of molar concentration, i.e., intensive variable. The expressions for $dV/dpH$ in (3) will be formulated in further parts of the paper.

For the sake of simplicity in notation, the charges of particular species $X_i^{z_i}$ can be omitted when put in square brackets, expressing molar concentration, [ $X_i$ ].

Let us assume that V mL of MOH (C, mol/L) is added, as reagent R, into $V_0$ mL of $K_mH_{n-m}L$ ($C_0$, mol/L) + HB ($C_{0a}$, mol/L) + MOH ($C_{0b}$, mol/L). The concentration balances are as follows:

$$[M] = \frac{C_{0b}V_0 + CV}{V_0+V},\ [B] = \frac{C_{0a}V_0}{V_0+V},$$

$$[K] = \frac{m\cdot C_0V_0}{V_0+V},\ \sum_{i=0}^{q}[H_iL] = \frac{C_0V_0}{V_0+V} \tag{4}$$

Denoting

$$[H_iL] = K_i^H\cdot[H]^i\cdot[L] \tag{5}$$

$$b_i = K_i^H\cdot[H]^i = 10^{\log K_i^H - i\cdot pH} \tag{6}$$

$$f_i = \frac{b_i}{\sum_{j=0}^{q} b_j} \tag{7}$$

$$\alpha = [H] - [OH] = 10^{-pH} - 10^{pH-pK_W} \tag{8}$$

$$\Delta_0 = C_{0b} - C_{0a} \tag{9}$$

and applying the formula for mean number of protons attached to $L^{-n}$ [19]

$$\begin{aligned}\bar{n} &= \frac{\sum_{i=1}^{q} i\cdot[H_iL]}{\sum_{i=0}^{q}[H_iL]} = \frac{\sum_{i=0}^{q} i\cdot K_i^H\cdot[H]^i}{\sum_{j=0}^{q} K_j^H\cdot[H]^j}\\ &= \frac{\sum_{i=0}^{q} i\cdot b_i}{\sum_{j=0}^{q} b_j} = \sum_{i=0}^{q} i\cdot f_i = \sum_{i=1}^{q} i\cdot f_i\end{aligned} \tag{10}$$

in the charge balance

$$\alpha + [M] + [K] + \sum_{i=0}^{q}(i-n)[H_iL] = 0 \tag{11}$$

we get, by turns,

$$\alpha + \frac{C_{0b}V_0 + CV}{V_0+V} - \frac{C_{0a}V_0}{V_0+V} + m\cdot\frac{C_0\cdot V_0}{V_0+V} = (n-\bar{n})\cdot\frac{C_0\cdot V_0}{V_0+V} \tag{12}$$

$$\alpha V_0 + \alpha V + \Delta_0 V_0 + CV_0 = (n-m-\bar{n})\cdot C_0\cdot V_0 \tag{13}$$

$$V = V_0\cdot\frac{(n-m-\bar{n})\cdot C_0 - \Delta_0 - \alpha}{C+\alpha} \tag{14}$$

$$\begin{aligned}V_0 + V &= V_0\cdot\frac{(n-m-\bar{n})\cdot C_0 - \Delta_0 + C}{C+\alpha}\\ &= ((n-m)\cdot C_0 - \Delta_0 + C)\cdot V_0\cdot\frac{1}{C+\alpha} - C_0\cdot V_0\cdot\frac{\bar{n}}{C+\alpha}\end{aligned} \tag{15}$$

Differentiating Eq. (15) gives

$$\frac{d(V_0+V)}{dpH}=\frac{dV}{dpH}=-((n-m)C_0-\Delta_0+C)\cdot V_0\cdot\frac{1}{(C+\alpha)^2}\cdot\frac{d\alpha}{dpH} - C_0\cdot V_0\cdot\frac{\frac{d\bar{n}}{dpH}\cdot(C+\alpha)-\bar{n}\cdot\frac{d\alpha}{dpH}}{(C+\alpha)^2} \quad (16)$$

Applying the relation

$$\frac{dz}{dpH}=\frac{dz}{d[H]}\cdot\frac{d[H]}{dpH}=-\ln 10\cdot[H]\cdot\frac{dz}{d[H]} \quad (17)$$

for z = α (Eq. (8)) and $\bar{n}$ (Eq. (10)), we get [20,21]

$\frac{d\alpha}{dpH}=-\ln 10\cdot([H]+[OH])=-\ln 10\cdot(\alpha^2+4K_W)^{1/2}$, where

$$K_W=[H][OH] \quad (18)$$

$$\frac{d\bar{n}}{dpH}=-\ln 10\cdot\sum_{j>i=0}^{q}(j-i)^2\cdot f_i f_j \quad (19)$$

and then from Eq. (17) we have

$$\frac{dV}{dpH}=\frac{V_0\cdot\ln 10}{(C+\alpha)^2}\cdot(((n-m)\cdot C_0-\Delta_0+C-C_0\cdot\bar{n})\cdot([H]+[OH])+C_0\cdot(C+\alpha)\cdot\sum_{j>i=0}^{q}(j-i)^2\cdot f_i f_j) \quad (20)$$

$$\sum_{j>i=0}^{12}(j-i)^2\cdot f_i f_j = f_0f_1+f_1f_2+f_2f_3+f_3f_4+f_4f_5+f_5f_6+f_6f_7+f_7f_8+f_8f_9+f_9f_{10}+f_{10}f_{11}+f_{11}f_{12}$$
$$+4(f_0f_2+f_1f_3+f_2f_4+f_3f_5+f_4f_6+f_5f_7+f_6f_8+f_7f_9+f_8f_{10}+f_9f_{11}+f_{10}f_{12})$$
$$+9(f_0f_3+f_1f_4+f_2f_5+f_3f_6+f_4f_7+f_5f_8+f_6f_9+f_7f_{10}+f_8f_{11}+f_9f_{12})$$
$$+16(f_0f_4+f_1f_5+f_2f_6+f_3f_7+f_4f_8+f_5f_9+f_6f_{10}+f_7f_{11}+f_8f_{12})$$
$$+25(f_0f_5+f_1f_6+f_2f_7+f_3f_8+f_4f_9+f_5f_{10}+f_6f_{11}+f_7f_{12})$$
$$+36(f_0f_6+f_1f_7+f_2f_8+f_3f_9+f_4f_{10}+f_5f_{11}+f_6f_{12}) \quad (24)$$
$$+49(f_0f_7+f_1f_8+f_2f_9+f_3f_{10}+f_4f_{11}+f_5f_{12})$$
$$+64(f_0f_8+f_1f_9+f_2f_{10}+f_3f_{11}+f_4f_{12})$$
$$+81(f_0f_9+f_1f_{10}+f_2f_{11}+f_3f_{12})$$
$$+100(f_0f_{10}+f_1f_{11}+f_2f_{12})$$
$$+121(f_0f_{11}+f_1f_{12})$$
$$+144f_0f_{12}$$

Some explanation needs the formulation of $\log K_i^H$ (i = 1,…,12) values in Equations (5) – (7) and then (24) and (25). The relations between $\log K_i^H$ and $pK_i$ (i = 1,…,12) are as follows:

$$\log K_1{}^H = pK_{12},\ \log K_2{}^H = pK_{11}+pK_{12},\ \dots,$$

$$\log K_{12}{}^H=\sum_{i=1}^{12}pK_i \quad (25)$$

## 3.2. Buffer Capacity in the System Phytic Acid + NaOH

Considering the titration of $V_0$ mL of $C_0$ mol/L $H_{12}L$ with V mL of C mol/L NaOH and applying the formulae derived above, we have: M = Na, q= n=12, $C_{0b} = C_{0a} = 0$, i.e., $\Delta_0 = 0$, and then from Equations (14) and (20) we have:

$$V=V_0\cdot\frac{(12-\bar{n})\cdot C_0-\alpha}{C+\alpha} \quad (21)$$

$$\frac{dV}{dpH}=\frac{V_0\cdot\ln 10}{(C+\alpha)^2}\cdot((12\cdot C_0+C-C_0\cdot\bar{n})\cdot([H]+[OH])+C_0\cdot(C+\alpha)\cdot\sum_{j>i=0}^{12}(j-i)^2\cdot f_i f_j) \quad (22)$$

where (Eq. 10)

$$\bar{n}=\sum_{i=1}^{12}i\cdot f_i=f_1+2f_2+3f_3+4f_4+5f_5+6f_6+7f_7+8f_8+9f_9+10f_{10}+11f_{11}+12f_{12} \quad (23)$$

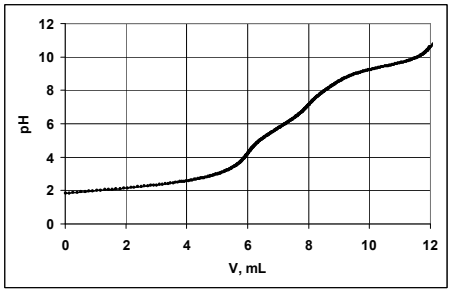
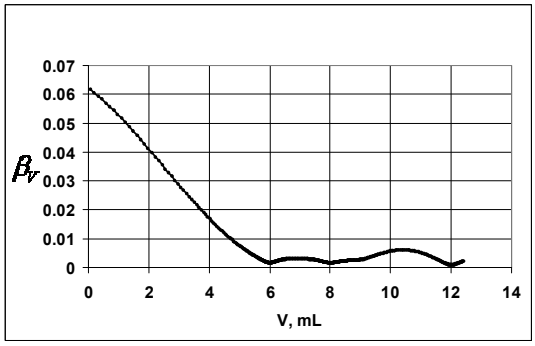
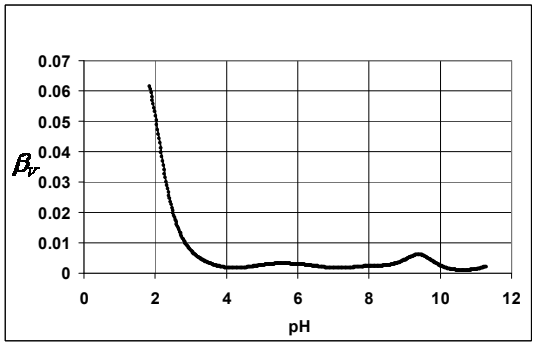



***Fig. 2.*** *The pH titration curve; for details – see text.*

The pH titration curve, pH = pH(V), plotted for $V_0$ = 10 mL, $C_0$ = 0.01 mol/L $H_{12}L$, C = 0.1 mol/L NaOH, is presented in Fig. 2. The $\beta_V$ vs. V and $\beta_V$ vs. pH relationships are plotted in Figures 3a and 3b.




(3a)




(3b)

***Fig. 3.*** *The plots for (3a) $\beta_V$ vs. V and (3b) $\beta_V$ vs. pH relationships; for details – see text.*

## 4. Final Comments

The buffer capacity $\beta_V$ for any polyprotic acid or base (of polyprotic acid or base salt) may be readily derived from the concentration and charge balance equations. Phytic acid (inositol hexaphosphate) with twelve acidic groups and spacing $pK_i$ values behaves nearly as a universal buffer. Giving the low values of the first successive protonation constants the buffer capacity of phytic acid increase rapidly from pH 4 downwards. The buffer strength is high and relatively constant between pH 4 and 10.

## References

[1] P. Yang, P.P.N. Murthy, R.E. Brown, Synergy of Intramolecular Hydrogen-Bonding Network in myo-Inositol 2-Monophosphate: Theoretical Investigations into the Electronic Structure, Proton Transfer, and pKa, *Journal of American Chemical Society*, 127 (2005) 15848-15861.

[2] E. Graf, J.R. Mahoney, R.G. Bryant, J.W. Eaton 1984. Iron-catalysed hydroxyl radical formation. *Journal of Biological Chemistry* 259, 3620–3624.

[3] E. Graf, K.L. Empson, J.W. Eaton 1987. Phytic acid. A natural antioxidant. *Journal of Biological Chemistry* 262, 11647–11650.

[4] E. Graf, J.W. Eaton. Antioxidant functions of phytic acid, *Free Radicals in Biology and Medicine* 8(1) (1990) 61–69.

[5] E. Graf, K.L. Empson, J.W. Eaton, Phytic acid. A natural antioxidant. *The Journal of Biological Chemistry*, 262(24) (1987) 11647-11650.

[6] M.J. Burkitt, B.C.Gilbert, The autoxidation of iron(II). in aqueous systems: the effects of iron chelation by physiological and therapeutic chelators on the generation of reactive oxygen species and the nducement of biomolecular damage. *Free Radical Research Communications* 14 (1991) 107–123.

[7] X.G. Lei, J.M. Porres, Phytase enzymology, applications, and bio-technology. *Biotechnology Letters* 25(21) (2003)1787-1794.

[8] V. Kumar, A.K. Sinha, H.P.S Makkar, K. Becker, Dietary roles of phytate and phytase in human nutrition. *Food Chemistry* 120 (2010) 945-959.

[9] L.R. Wilken, Z.L. Nikolov, Evaluation of alternatives for human lysozyme purification from transgenic rice: Impact of phytic acid and buffer, *Biotechnology Progress* 26(5) (2010) 1303–1311.

[10] I. Vuvenik, A.M. Shamsuddin, Cancer inhibition by inositol hexaphosphate (IP6) and inositol: From laboratory to clinic, *Journal of Nutrition* 133 (2003) 3778S-3784S.

[11] T. Kornfelt, A. Vinther, G.N. Okafo, P. Camilleri, Improved peptide mapping using phytic acid as ion-pairing buffer additive in capillary electrophoresis, *Journal of Chromatography A*, 726(1) (1996) 223-228.

[12] J.R. Veraart, Y. Schouten, C. Gooijer, H. Lingeman , Evaluation of phytic acid as a buffer additive for the separation of proteins in capillary electrophoresis, *Journal of Chromatography A* 768(2) (1997) 307-313.

[13] G.N. Okafo, A. Vinther, T. Kornfelt, P. Camilleri, Effective ion-pairing for the separation of basic proteins in capillary electrophoresis, *Electrophoresis* 16(1) (1995) 1917-1921.

[14] http://en.wikipedia.org/wiki/Phytic_acid.

[15] N. Li, O. Wahlberg, I. Puigdomenech, L.-O. Ohman, Equilibrium Studies of Phytate ions. 1. Equilibria Between Phytate Ions and Protons in 3 M $NaClO_4$ Medium, Acta Chemica Scandinavica 43 (1989) 331-339. http://actachemscand.org/pdf/acta_vol_43_p0331-0339.pdf

[16] F. Crea, C. De Stefano, D. Milea, S. Sammartano, Formation and stability of phytate complexes in solution, *Coordination Chemistry Reviews* 252 (2008) 1108–1120.

[17] W.J. Evans, E.J. McCourtney, R.I. Shrager, Titration studies of Phytic Acid, *Journal of American Oil Chemists Society* 59 (1982) 189-191

[18] C. De Stefano, D. Milea, A. Pettignano, S. Sammartano, Speciation of phytate ion in aqueous solution. Alkali metal complex formation in different ionic media, *Analytical and Bioanalytical Chemistry* (2003) 376, 1030–1040.

[19] T. Michałowski, Calculations in analytical chemistry with elements of computer programming (in Polish) PK, Cracow 2001 http://suw.biblos.pk.edu.pl/resourceDetails&rId=3974.

[20] A.G. Asuero, T. Michałowski, Comprehensive formulation of titration curves referred to complex acid-base systems and its analytical implications, *Critical Reviews in Analytical Chemistry* 41(2) (2011) 151-187.

[21] T. Michałowski, A.G. Asuero, New approaches in modelling the carbonate alkalinity and total alkalinity, *Critical Reviews in Analytical Chemistry*, 42 (2012) 220-244.