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Influence of pH on the dissolution of folic acid supplements

Islam R. Younis[a], Mary K. Stamatakis[b], Patrick S. Callery[a], Paula Jo. Meyer-Stout[a,*]

[a] Department of Basic Pharmaceutical Sciences, West Virginia University, School of Pharmacy, Morgantown, WV 26506, United States
[b] Department of Clinical Pharmacy, West Virginia University, School of Pharmacy, Morgantown, WV 26506, United States

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ABSTRACT

The vitamin folic acid has received considerable attention because of its role in decreasing the risk of neural tube birth defects, and its potential role in reducing the risks of cardiovascular and psychiatric diseases. A significant concern is the quality of commercially available folic acid products. We evaluated the pharmaceutical performance of 15 currently available folic acid products in terms of meeting the USP standards for disintegration and dissolution, and showed that there has been significant improvement in the past decade in the quality of these products. However, at least one product failed to meet the requirement of each test performed. Since folic acid absorption is maximal at the proximal jejunum, dissolution was further evaluated in simulated gastric fluid. All the products failed to release more than 75% of the active ingredient in 60 min. While some excipient-related factors were preliminarily considered, it was ultimately proposed that the failure may be related to the pH-dependency of the solubility of folic acid, a premise supported by faster dissolution of laboratory prepared buffered folic acid tablets. The more limited solubility of folic acid in acidic medium should be taken into consideration in the required dissolution testing methods, as well as in product formulation to optimize release.

1. Introduction

The vitamin folic acid has received considerable attention because of its role in the prevention of disease. Pre-conception consumption of folic acid plays a major role in the prevention of neural tube defects, primarily anencephaly and spina bifida (Anon., 1991; Locksmith and Duff, 1998; Moore et al., 2003). In 1992, the U.S. Public Health Service, joined in 1993 by the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics Committee on Genetics, recommended that all women of childbearing age consume 400 micrograms of folate daily from food or supplements (Swain and St, 1997). It has also been suggested that folic acid may be effective in decreasing the risk for cardiovascular diseases (Stein and McBride, 1998; Rimm et al., 1998; Adank et al., 2003), colon cancer (Weir and Scott, 1998; La et al., 2002), neurological illnesses such as dementia and Alzheimer's disease (Reynolds, 2002; Miller, 2003), and hypertension (Forman et al., 2005).

Folate deficiency is a common finding and can be caused by a variety of factors such as malabsorption of folate in the diet and increased utilization of folic acid by the body. Folic acid obtained from pharmaceutical preparations is more bioavailable than dietary folate. Up to half of dietary folate is lost in the cooking process and requires hydrolysis for absorption (Suitor and Bailey, 2000; McNulty and Pentieva, 2004).

Folic acid absorption is facilitated by a carrier-mediated transport system and is maximal at the proximal jejunum, poor in the distal jejunum, and not absorbed from the ileum (Hepner et al., 1968; Butterworth et al., 1969), suggesting that complete dissolution of folic acid in the stomach would be preferred before it passes its absorption window. The failure of folic acid supplements to meet USP requirements for disintegration was reported earlier (Stout et al., 1996). Various laboratories have also shown dissolution failures: 6 out of 9 folic acid containing multivitamin supplements failed the USP dissolution requirements for folic acid (Hoag et al., 1997), dissolution levels less than 10% were observed (Giebe and Counts, 2000), and 4 out of 11 folic acid products tested in the UK failed to meet the British Pharmacopeia requirements for folic acid dissolution (Sculthorpe et al., 2001).

Given the significant role that available folic acid plays in healthy fetal development and this past history of folic acid tablet failure, the purpose of this study was to determine if there has been significant improvement in product performance of currently available folic acid supplements, both single entity and multivitamins, using the USP monograph testing standards for disintegration and dissolution. Secondly, products were tested in simulated gastric and simulated intestinal fluid, since these are more physiologically relevant than distilled water.

* Corresponding author. Tel.: +1 304 293 4827; fax: +1 304 293 2576.
E-mail address: pstout@hsc.wvu.edu (P.Jo. Meyer-Stout).

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2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Folic acid, 25% ammonium hydroxide solution in water, HPLC grade potassium monobasic phosphate, and p-aminobenzoyl-L-glutamic acid were obtained from Sigma (St. Louis, MO). Methylcellulose was obtained from City Chemical Corporation (New York, NY). HPLC grade methanol, hydrochloric acid, HPLC grade o-phosphoric acid, 85%, sodium hydroxide, sodium chloride, HPLC grade sodium perchlorate, HPLC grade tetrabutylammonium hydroxide 1 M in water, and potassium chloride were obtained from Fisher Scientific (Pittsburgh, PA).

2.1.2. Folic acid supplements

Samples of commonly used, commercially prepared single entity folic acid tablets (400 micrograms) and eight multivitamin supplements containing folic acid (800 micrograms) were obtained from local pharmacies in Morgantown, West Virginia. The multivitamin supplements were all tablets, with the exception of one capsule formulation. All products were available on a non-prescription basis.

2.2. Methods

2.2.1. Disintegration

Disintegration was performed according to section 701 of the USP (2003d) using the three basket disintegration apparatus (Vankel®). In addition to distilled water (pH 5–6), the test was also performed in simulated gastric fluid (0.034 M NaCl, pH 1.5) and simulated intestinal fluid (0.05 M KH_2PO_4 in 0.038N NaOH, pH 7.5).

2.2.2. Analytical methods

2.2.2.1. HPLC assay of folic acid in tablets containing only folic acid as active ingredient (2003c). The mobile phase was 96:4 mixture of 0.6 M sodium perchlorate with 0.02 potassium monobasic phosphate (1:1) and methanol, pH 7.2. The chromatographic system was equipped with Beckman® C_{18} ODS column (4.6 mm × 250 mm). Isocratic elution at 1.0 mL/min was applied and folic acid was detected by UV at 254 nm.

2.2.2.2. HPLC assay of folic acid as an ingredient of pharmacopeial preparations containing other active constituents (2003d). The mobile phase was made of 76:24 mixture of 0.02 M monobasic potassium phosphate with 25% tetrabutylammonium hydroxide (98.4:1.6) and methanol (pH 7.0). The chromatographic system used consisted of Nova-Pak Waters® C_{18} column (3.5 mm × 15 cm). The flow rate was set 1.0 mL/min and detection was at 280 nm.

2.2.2.3. HPLC assay of folic acid (2003a). The chromatographic system contained Agilent® Hypersil C_{18} ODS column (4.0 mm × 25 cm). Isocratic elution at 1.2 mL/min was applied using a 73:23 mixture of 0.02 M phosphate buffer with 0.05 M tetrabutylammonium hydroxide (98:2) and methanol (pH 5.0). The detector was set at 280 nm.

2.2.3. Dissolution

Dissolution was measured using USP Apparatus I for capsules and Apparatus II for tablets along with the test conditions specified for folic acid tablets and folic acid in multivitamin dosage units in the USP (2003b,c). For every product tested, dissolution was performed in simulated gastric fluid, simulated intestinal fluid, and distilled water. Dissolution was monitored at 15 min intervals for 1 h by taking 0.5 mL aliquots from the dissolution medium from each vessel of the dissolution apparatus. The aliquots were combined and immediately assayed for folic acid using the USP specified HPLC methods described above.

2.2.4. Folic acid solubility

Using a 50-mL volumetric flask, a saturated solution of folic acid was prepared in a buffer at pH values of 1, 3, 4, 7, and 10. The flasks were constantly shaken and maintained at 37 ± 1 °C in a shaking water bath (New Brunswick Scientific Co., NJ). Samples from each flask were withdrawn every day (for 5 days), until a constant assay value was obtained and assayed using the USP HPLC assay method for folic acid described above. The buffers used were composed of 0.2 M KCl (pH 1.0), 0.1 M potassium hydrogen phthalate (pH 3.0), 0.1 M potassium hydrogen phthalate (pH 4.0), 0.1 M potassium monobasic phosphate (pH 7.0), and 0.025 M borax (pH 10).

2.2.5. Formulation of self-buffering tablets

Two folic acid formulations were prepared in the laboratory. One formulation contained folic acid (400 micrograms) and methylcellulose, and the other formulation contained folic acid (400 micrograms), methylcellulose and 25% citrate buffer (sodium citrate and citric acid 2:1). In a separate experiment, the pH of this buffer was measured to be 4.5 at 37 °C. The dissolution of these two formulations was studied using the intrinsic dissolution testing methodology.

2.2.6. Intrinsic dissolution testing methodology

To study the intrinsic dissolution of folic acid from each formulation, the tablet (non-buffered and buffered) formulation was compressed in a 0.5 in. stainless steel flat face die (using a Carver laboratory press). The tablet was not ejected, but kept in the die so that one surface of the tablet was flush with the die face surface. Given that the methylcellulose filler in each formulation is non-disintegrating, non-dissolving, and that only one surface of the tablet is exposed, the study then measures folic acid dissolution from one flat, solid surface. With solid surface area held constant, the dissolution rate is then proportional to the drug's solubility and diffusional properties; thus, this methodology is more sensitive to a change in dissolution as a function of solubility than standard dissolution testing methods. It is also worth noting that, except for the buffering salts, the formulation of both tablets were the same. Therefore, the influence of the methylcellulose filler on folic acid's transport should be the same for both formulations. Also, chemically, methylcellulose is nonionic, neutral and, stable at most pH values (Parrott, 1970).

For each dissolution study, the die was suspended in 500 mL of non-stirred simulated gastric fluid at 37 °C, with only one face of the tablet exposed to the dissolution medium. Care was taken to position the die within the same position within the dissolution apparatus, as well as checking the surface of the tablet face to assure that no air bubbles formed during the course of the study. Samples (0.5 mL) from both the non-buffered and buffered formulations were withdrawn at 0, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min, filtered and analyzed by HPLC as described previously for folic acid. A total of three tablets were tested for each formulation.

3. Results

3.1. Disintegration

Table 1 shows the average disintegration time for the tested products. To meet the USP specification for disintegration, the dosage unit should completely disintegrate in 30 min. Fourteen out

Table 1
Folic acid dosage units disintegration time in minutes.

Product	Mean disintegration time[a,b] (min)		
	DW	SGF	SIF
S1[c]	138	18.0	267
S2	0.4	0.4	0.5
S3	8.0	9.7	8.6
S4	0.7	1.0	1.3
S5	0.25	0.2	0.2
S6	7.3	8.4	8.8
S7	10.5	12.9	8.8
MV1	8.12	8.7	10.5
MV2	10.0	8.5	9.5
MV3	8.4	8.5	10.9
MV4	19.3	18.8	22.3
NMV1	11.0	10.1	10.7
NMV2	4.3	4.4	3.8
MV7	14.5	14.5	5.0
CMV	25.5	22.2	24.2

Abbreviations: S, single entity folic acid tablets; MV, multivitamin tablets containing folic acid; CMV, multivitamin capsule containing folic acid; DW, distilled water; SGF, simulated gastric fluid; SIF, simulated intestinal fluid. To meet the USP requirement the dosage unit should completely disintegrate in 30 min.

[a] Each value represents the mean disintegration time of 18 dosage units.

[b] S.D. fell within the range of 0.1–3.4 min (on average CV, 10%), with exception of product S1.

[c] S.D. is 11.9 min in SGF, 138 min in DW, and 208 min in SIF.

of the 15 products tested passed the USP requirement for disintegration (less than 30 min). One product, a single entity folic acid tablet formulation (S1), failed to pass the USP requirements and exhibited unacceptable disintegration behavior (ranging from 11 to more than 480 min). Few products exhibited increased or decreased disintegration time with increasing pH. In general, disintegration was faster in acidic conditions and slower in neutral conditions. Disintegration showed sensitivity to pH which likely reflects the influence of the different excipients in the tested formulations.

3.2. Dissolution

3.2.1. Single entity folic acid tablets

All the tested products met the USP requirement of folic acid dissolution in distilled water (pH 5–6) which requires that more than 75% of the folic acid labeled amount is released in 45 min. The dissolution range was 85–143% (Table 2). It is interesting to note that, while product S1 failed the disintegration test, it did not fail the USP test for dissolution. All the tested products released more than 75% of the labeled amount in 45 min in simulated intestinal fluid at pH 7.5 (Table 3). The dissolution profile of folic acid in simulated intestinal fluid for each product was similar to the dissolution profile obtained in distilled water. The dissolution range was 89–135%.

Table 2
Folic acid (in single entity folic acid tablets) percent release in distilled water, USP monograph methodology.

Product	Percent released		
	Average[a]	CV[a]	Pass/fail[b]
S1	85.7	2.9	Pass
S2	143.8	3.7	Pass
S3	93.5	1.0	Pass
S4	91.9	4.1	Pass
S5	106.2	2.0	Pass
S6	97.9	5.8	Pass
S7	97.3	0.1	Pass

[a] The mean percent release and coefficient of variation (CV) obtained from three runs, six tablets at each time-point for each brand.

[b] All the tested products met the USP requirements for folic acid dissolution from single entity folic acid tablets (>75% of label claim released in 45 min).

Table 3
Folic acid (in single entity folic acid tablets) percent released in 45 min in different media.

Product	Percent released in 45 min		
	SGF[a]	DW[a]	SIF[a]
S1	23.6	85.7	89.7
S2	56.6	143.8	135.8
S3	38.0	93.5	112.3
S4	46.5	91.9	101.3
S5	51.9	106.3	101.5
S6	68.1	97.9	116.1
S7	34.1	97.4	110.1

Abbreviations: S, single entity folic acid tablets; DW, distilled water; SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

[a] The mean percent release obtained from three runs, six tablets at each time-point for each brand. On average, the coefficient of variation, CV, <10%.

All the tested products failed to release 75% of the labeled folic acid in 45 min in simulated gastric fluid at pH 1.5. Dissolution as low as 23% was observed, where the highest percent release obtained was 68% (Fig. 1 and Table 3).

3.2.2. Multivitamins dosage units containing folic acid

Dissolution was measured using USP Apparatus II for tablets or Apparatus I for capsules. Seven of the tested products released more than 75% of folic acid in distilled water in 1 h. One of the products (the capsule formulation) failed to meet the USP requirement for folic acid dissolution in multivitamin dosage units (Table 4). The dissolution range was 38–145%. The tested products generated similar dissolution profiles in simulated intestinal fluid. The dissolution range was 47–130% (Table 5).

All the tested products failed to release more than 75% of folic acid in 60 min in simulated gastric fluid. The dissolution range was 15–61% (Fig. 2 and Table 5).

3.3. Folic acid pH-solubility profile

The saturation solubility of folic acid at different pH values was measured and a solubility-pH profile was generated (Fig. 3). Folic acid exhibited an increased solubility with increasing pH at 37 °C. No signs of major degradation of folic acid in the studied pH values were observed. Folic acid solution concentrations were stable

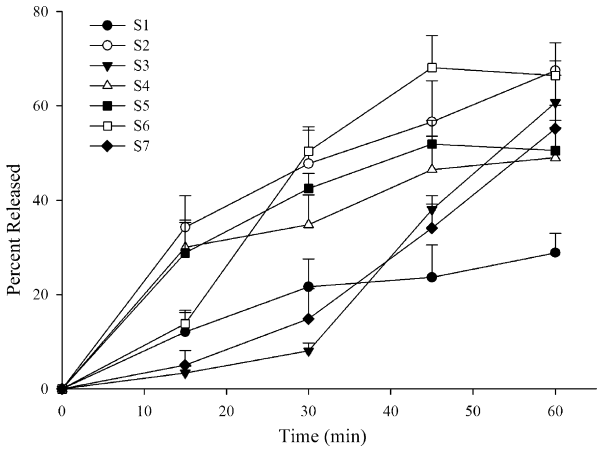

Fig. 1. Folic acid (single entity folic acid tablets) dissolution profile in simulated gastric fluid. Each point represents the mean dissolution concentration of six tablets at each time-point for each brand, with error bars representing standard deviation. All the tested products failed to release more than 75% of their label claim in 60 min.

Table 4
Folic acid (in multivitamin dosage units) percent released in distilled water, USP monograph methodology.

Product	Percent released		
	Average[a]	CV[a]	Pass/fail[b]
MV1	100.6	4.1	Pass
MV2	145.5	2.4	Pass
MV3	106.1	1.5	Pass
MV4	108.8	2.5	Pass
MV5	112.4	3.0	Pass
NMV1	121.9	2.3	Pass
NMV2	102.5	5.0	Pass
CMV	38.5	4.6	Fail

Abbreviations: MV, multivitamin tablets containing folic acid; CMV, multivitamin capsule containing folic acid.

[a] The mean percent release and coefficient of variation (CV) obtained from three runs, six dosage units at each time-point for each brand.

[b] To meet USP requirements 75% of the label claim should be released in 60 min.

Table 5
Folic acid (in multivitamin dosage units) percent released in 60 min in different media.

Product	Percent Released in 60 min		
	SGF[a]	DW[a]	SIF[a]
MV1	61.2	100.6	102.5
MV2	28.7	145.5	130.2
MV3	52.9	106.1	86.8
MV4	47.1	108.8	99.5
MV5	45.8	112.4	86.3
NMV1	42.2	121.9	85.9
NMV2	32.8	102.5	104.5
CMV	15.2	38.5	47.4

Abbreviations: MV, multivitamin tablets containing folic acid; CMV, multivitamin capsule containing folic acid; DW, distilled water; SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

[a] The mean percent release and coefficient of variation (CV) obtained from three runs, six tablets at each time-point for each brand. On average CV < 10%.

throughout the time course of the experiment (5 days). Folic acid solubility was relatively low in acidic fluid and significantly higher in basic solutions. Solubility of folic acid at pH 7 was 183-fold more than the solubility of folic acid at pH 1.

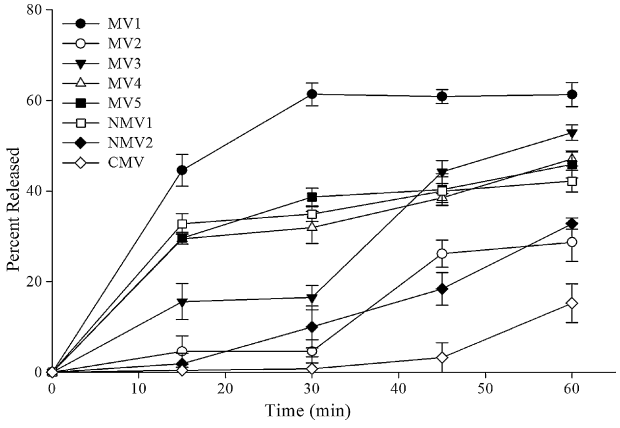

Fig. 2. Folic acid (multivitamin dosage units) dissolution profile in simulated gastric fluid. Each point represents the mean dissolution concentration ($n = 6$ dosage units), with error bars representing standard deviation. Dissolution was carried using USP Apparatus I for capsules (CMV) and USP Apparatus II for tablets. All the tested products failed to release more than 75% of their label claim in 60 min.

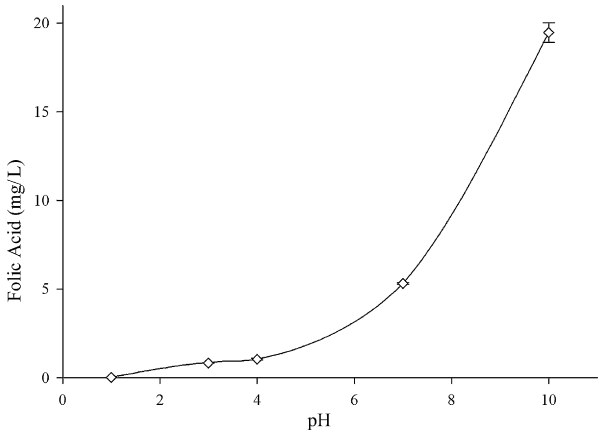

Fig. 3. Folic acid solubility-pH profile. Each point represents the mean ($n = 3$) with the error bar representing the standard deviation. Solubility study was carried out at pH values of 1, 3, 4, 7, and 10. Folic acid solubility increased as a function of pH.

3.4. Intrinsic dissolution of self-buffering tablets

Fig. 4 shows the amount of folic acid released from each of the buffered and non-buffered laboratory formulated folic acid tablets ($n = 3$), when dissolution proceeds from a single face of the tablet, a non-disintegrating flat surface of constant area. The results indicated a significant difference ($p < 0.05$) between the average amount of folic acid released from the buffered and non-buffered tablets, with the buffered tablets showing a faster rate of release. While not shown here, an analysis was performed to determine if the increase in dissolution from buffered tablets could be predicted on an a priori basis based on folic acid's pH-related solubility change. It was found that a priori rate predictions could not be made; the dissolution rate change as a function of pH-dependent solubility change was lower than what would be predicted.

4. Discussion

The pharmaceutical quality of commercially available folic acid products had been a concern in the last decade. Because of the growing role of folic acid in prevention of many medical conditions, and due to earlier concerns regarding the pharmaceutical quality, potency, and efficacy of commercially available folic acid products, the current study sought to determine product perfor-

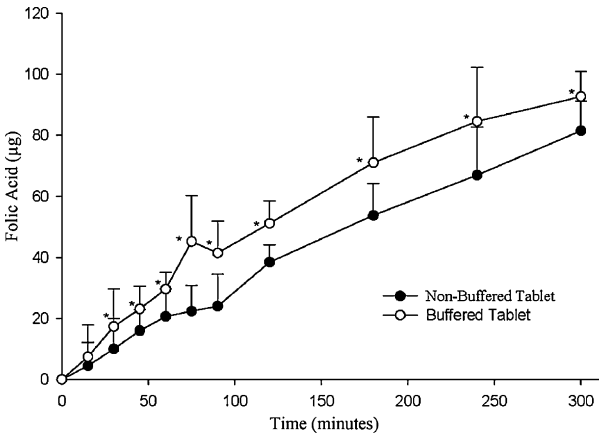

Fig. 4. Constant surface area folic acid release. Each point represents the mean ($n = 3$) with error bars representing standard deviation. *Significant difference between the average amount of folic acid released from the laboratory formulated buffered and laboratory formulated non-buffered tablets ($p < 0.05$).

mance of currently available folic acid supplements. While vitamins need to be safe, the Food and Drug Administration (FDA) does not require that they undergo the same requirements as drug products, which are required to be both safe and effective. Thus, there is no assurance that a vitamin supplement performs effectively. However, the United States Pharmacopoeia (USP) addressed this matter of public health through the establishment of monograph standards for folic acid (1993).

Many studies in the last decade showed the failure of marketed folic acid supplements to meet the USP disintegration and dissolution specifications for folic acid (Stout et al., 1996; Hoag et al., 1997; Giebe and Counts, 2000; Sculthorpe et al., 2001). To date, those tests serve as the only tool to assess the quality of commercially available folic acid products. One can speculate that poor disintegration and/or dissolution may affect absorption and hence bioavailability; however, the in vivo bioavailability of folic acid from commercial products has not been reported in the literature yet nor have studies comparing these products in vitro, in vivo performance.

Our data showed improvement in the pharmaceutical quality (disintegration and dissolution) of folic acid products compared to the previous cited studies, when tested by USP standards (in distilled water). However, the most striking finding in our results was the failure of all tested products to release more than 75% of their label claim when tested in simulated gastric fluid in 60 min. When compared with the percent of folic acid dissolved in distilled water or simulated intestinal fluid (Tables 3 and 5), each with a higher pH than simulated gastric fluid, it appears that folic acid product dissolution may be pH dependent. This is of concern since folic acid should be readily dissolved in the acidic stomach before it passes its absorption window in the upper small intestine; failure to do so may result in limited absorption and reduced bioavailability of folic acid in the proximal jejunum.

While it was not within the scope of this research to study in vivo behavior, the in vitro data reported here demonstrate the need for thorough in vivo studies of this class of products, particularly bioavailability. Although the majority of the tested products passed the USP standards for dissolution, the obtained results in simulated gastric fluid raise the question of possible failure to achieve the recommended folate levels in women consuming commercially available supplements which in turn could increase the risk of neural tube defects. Other patients who demonstrate altered gastric acid secretions might fail to achieve the required folate levels by consuming marketed folic acid products. Investigators have shown that basal acid output is significantly higher in patients undergoing dialysis compared to controls (Milito et al., 1983), a patient population that routinely receives folic acid supplementation. Product failure could also worsen anemia associated with chronic kidney disease.

The failure of folic acid dissolution in acidic media could be attributed to many factors. Du and Hoag reported that certain excipients adversely affect folic acid dissolution. Bulk filler solubility and disintegrant amount were found to be the two major formulation factors affecting folic acid tablets dissolution (Du and Hoag, 2003). Our findings suggest that this failure could also be attributed, but not limited to, the pH-dependent solubility of folic acid. In a preliminary study (Fig. 4) the release of folic acid was higher from laboratory prepared buffered folic acid tablets compared to laboratory formulated non-buffered tablets, when tested using an intrinsic dissolution method. Dissolution occurred from a non-disintegrating constant surface area without mixing, which eliminates other variables that might affect the dissolution process. Normally, the pH of the bulk fluid will reflect the pH of the stationary diffusion layer upon the tablet's surface, with surface pH affecting drug solubility, the driving force for the drug's dissolution. However, in the case of the buffered folic acid tablet, as the buffer dissolves at the surface, it will alter the pH of the microenvironment of the stagnant diffusion layer to a relatively more basic pH (pH ≈ 4), while the bulk fluid pH (pH 1.5) remains the same (pH 1.5). In theory, this increased surface pH should increase folic acid's solubility, favoring a more rapid dissolution of the folic acid from the buffered tablet. Our results show a faster release of folic acid from the buffered tablets, supporting the premise that pH effects do contribute to some extent to folic acid dissolution.

In conclusion, the poor dissolution of commercially available folic acid products in simulated gastric fluid could significantly affect product efficacy. Until the in vitro/in vivo correlations for folic acid are established, consideration should be given to consumption of commercially available folic acid tablets in conditions where the stomach pH is highly acidic. It may be recommended to consume folic acid tablets after a meal or with antacid, which may promote dissolution. Consideration should also be given to the relevance of the current USP testing standard for predicting folic acid product performance in physiological conditions. Finally, given the results of a more rapid dissolution from self-buffered tablets, formulators of folic acid-based products should consider this design approach, which allows the product to perform somewhat independent of the gastric conditions, in order to optimize folic acid release.

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