



## Research paper

## A comparative pH-dissolution profile study of selected commercial levothyroxine products using inductively coupled plasma mass spectrometry

Dimple Pabla, Fatemeh Akhlaghi, Hossein Zia \*

Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, R.I., USA

## ARTICLE INFO

## Article history:

Received 12 June 2008

Accepted in revised form 1 October 2008

Available online 1 November 2008

## Keywords:

Dissolution

ICP-MS

Levothyroxine

pH

Proton pump inhibitors

Bioequivalence

Synthroid®

Sandoz

Tirosint®

## ABSTRACT

Levothyroxine (T4) is a narrow therapeutic index drug with classic bioequivalence problem between various available products. Dissolution of a drug is a crucial step in its oral absorption and bioavailability. The dissolution of T4 from three commercial solid oral dosage forms: Synthroid® (SYN), generic levothyroxine sodium by Sandoz Inc. (GEN) and Tirosint® (TIR) was studied using a sensitive ICP-MS assay. All the three products showed variable and pH-dependent dissolution behaviors. The absence of surfactant from the dissolution media decreased the percent T4 dissolved for all the three products by 26–95% (at 30 min). SYN dissolution showed the most pH dependency, whereas GEN and TIR showed the fastest and highest dissolution, respectively. TIR was the most consistent one, and was minimally affected by pH and/or by the presence of surfactant. Furthermore, dissolution of T4 decreased considerably with increase in the pH, which suggests a possible physical interaction in patients concurrently on T4 and gastric pH altering drugs, such as proton pump inhibitors. Variable dissolution of T4 products can, therefore, impact the oral absorption and bioavailability of T4 and may result in bioequivalence problems between various available products.

© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

Levothyroxine (T4), administered orally as a sodium salt, is used exclusively for thyroid replacement therapy in patients with hypothyroidism and various forms of thyroid neoplasia [1]. It has a narrow therapeutic index such that doses merely 20–25% outside the therapeutic window can place patients at risk of severe adverse effects of hyper- or hypo-thyroidism, including harmful cardiac and/or metabolic effects [2,3]. Close clinical follow-up and careful dose titration are therefore essential for the safe and efficacious use of T4, which explains the importance of bioequivalence studies in verifying switchability between various marketed products.

Despite the efforts by Food and Drug Administration (FDA) of the United States to address these issues, bioequivalence of T4 products remains an on-going concern. Where the current methodology for bioequivalence determination has been criticized for insensitivity to distinguish between doses that differ by as much as 12.5% [4–9], the physico-chemical properties of the available

formulations have been somewhat ignored. Formulation characteristics including drug release, rate and extent of dissolution appear to be critical [10,11].

Oral absorption of drugs from solid dosage forms is dependent upon the release of the drug from the formulation, the dissolution of the drug, and the permeability of the drug across the gastrointestinal tract. Hence, *in vitro* dissolution is critical to the drug absorption *in vivo* and can serve as a surrogate for drug bioavailability. A dissolution methodology that is able to discriminate between formulations with different release patterns should, therefore, be used. *In vitro* dissolution tests have been successfully employed as a quality control tool to ensure inter-lot manufacturing reproducibility. Furthermore, they can be used as a sensitive method for differentiating between formulations of the same therapeutic agent, and in many circumstances, as an alternative to more expensive bioequivalence studies [12,13]. The dissolution tests for T4, as described in the United States Pharmacopeia (USP30), can be used as a one-point quality control measure, to estimate the amount of drug released (i.e. not less than 70% of the labeled amount of T4 is dissolved in 45 min according to test 1) [14]. However, these tests lack the ability to study and compare dissolution–time profiles of T4 in detail, or would require multiple tablets in one dissolution bath to detect the amount of drug dissolved. Use of a sensitive analytical technique to quantify the minute but significant differences in the dissolution profiles of T4

Abbreviations: T4, Levothyroxine; SLS, Sodium lauryl sulfate; SYN, Synthroid®; GEN, Levothyroxine sodium, generic; TIR, Tirosint®; PPI, Proton pump inhibitors.

\* Corresponding author. Biomedical and Pharmaceutical Sciences, University of Rhode Island, 125, Fogarty Hall, 41 Lower College Road, Kingston, RI 02881, USA. Tel.: +1 401 874 5022; fax: +1 401 874 2181.

E-mail address: [hzia@uri.edu](mailto:hzia@uri.edu) (H. Zia).

products is therefore critical in illustrating the possible contribution of dissolution to the bioequivalence problem.

Oral T4 is primarily absorbed in the jejuno-ileal segment of the small intestine [15]. A seminal study on T4 dose requirement was recently conducted by Centanni et al. on 248 patients with multinodular goiter. These patients also had impaired gastric acid secretion due to *Helicobacter pylori*-related gastritis and/or atrophic gastritis. It was revealed that hypothyroid patients with altered gastric acid secretion required an increase in T4 dose to achieve euthyroid state [16]. These findings suggest that normal gastric acid secretion is important for the intestinal absorption of T4. Sachmechi et al. also confirmed these results in a retrospective study in 92 patients receiving T4 in whom proton pump inhibitor (PPI) therapy was later initiated [17]. These studies further support the importance of gastric pH and therefore signify the influence of pH-dependent dissolution characteristics in achieving an optimal T4 effect.

In the present study, we have compared the pH-dissolution profiles of three marketed formulations of T4, *Synthroid*<sup>®</sup> (Abbott Laboratories, IL), levothyroxine sodium, generic (Sandoz Inc., NJ) and *Tirosint*<sup>®</sup> (Institut Biochemique, Switzerland), using a previously developed and validated, highly sensitive inductively coupled plasma mass spectrometry (ICP-MS) assay for T4 [18]. *Synthroid*<sup>®</sup> (SYN) and generic levothyroxine sodium (GEN) are available as tablets whereas *Tirosint*<sup>®</sup> (TIR) is a soft gelatin capsule for oral administration. Therefore, the objectives of this study were (a) to determine if dissolution of T4 could be a contributing factor to the bioequivalence problem of T4 products; and (b) to evaluate the effect of pH on the dissolution of these formulations.

## 2. Materials and methods

### 2.1. Materials

Levothyroxine sodium, ammonium acetate *TraceSelect*<sup>1</sup>, ammonium hydroxide *TraceSelect*<sup>1</sup>, acetic acid *TraceSelect*<sup>1</sup> and sodium lauryl sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standard, antimony (Ultra Scientific, North Kingstown, RI, USA), was supplied as a 1000 µg/ml stock solution in dilute nitric acid matrix. Hydrochloric acid was purchased from Fisher Scientific (Fairlawn, NJ, USA). *Synthroid*<sup>®</sup> (Abbott Laboratories, IL, USA) and generic levothyroxine sodium (Sandoz Inc., Princeton, NJ, USA), 0.15 mg each, were purchased from Rhode Island State Pharmacy (Cranston, RI, USA), and *Tirosint*<sup>®</sup>, 0.15 mg (Institut Biochimique, Pambio-Noranco, Switzerland), was purchased from Farma Mondo SA (Chiasso, Switzerland). Purified deionized water was prepared using Milli Q50 water purification system (Millipore, Bedford, MA, USA).

### 2.2. Dissolution studies

Dissolution studies were performed in a Vankel VK 4020 dissolution apparatus, a USP Type II instrument (Palo Alto, CA, USA), using a single 150 µg tablet per dissolution bath. The dissolutions were carried out in 0.05 M ammonium acetate buffers containing 0.05% w/v sodium lauryl sulfate (SLS) by adjusting the pH with acetic acid or ammonium hydroxide to 4.0, 5.0, 6.0, 7.0 or 8.0 as needed. Dissolution was also carried out in 0.1 N and 0.01 N hydrochloric acid (HCl), both containing 0.05% w/v SLS, representing pH 1.2 and 2.4, respectively. All the dissolution experiments were carried out at 75 rpm and at 37 °C taking samples at 10, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min replacing the dissolution media with fresh buffer equilibrated to 37 °C after each sample. Dissolution

was also carried out in 0.01 N HCl alone, without any surfactant, in order to study the inherent dissolution of the formulations in aqueous media. Also, dissolution in 0.1 N HCl was conducted at 50 rpm and at 37 °C to determine if the formulations met the criteria for rapid release drug products as set by the US FDA [19]. All the dissolution studies were performed in replicates of three.

### 2.3. Sample analysis

Levothyroxine concentrations in the dissolution samples were analyzed using a validated and published ICP-MS assay [18]. Samples withdrawn at each time point during the dissolution studies were diluted 1:5 with 0.5% ammonium hydroxide solution. An internal standard, antimony, was added to all the samples at a concentration of 10 ng/ml prior to dilution. The diluted samples were then directly infused into a Thermo electron X7 ICP-MS instrument acquiring the data in peak jumping mode at *m/z* of 126.90 for iodide (obtained from the breakdown of T4) and of 120.90 for antimony. Levothyroxine concentrations in the dissolution samples were calculated from calibration curves ranging from 0.3 to 100 ng/ml T4, run simultaneously with the unknown samples on the day of the analysis.

### 2.4. Data analysis

The dissolution profiles were constructed by plotting the %T4 dissolved vs time, and were compared using a model independent approach as described by the FDA [19,20]. It uses a difference factor ( $f_1$ ) and a similarity factor ( $f_2$ ) to compare dissolution profiles. The difference factor calculates the percent difference between the two profiles at each time point (Eq. (1)) and is a measure of the relative error between the two curves:

$$f_1 = \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} * 100 \quad (1)$$

The similarity factor is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves:

$$f_2 = 50 * \log \left[ \frac{1}{\sqrt{1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2}} * 100 \right] \quad (2)$$

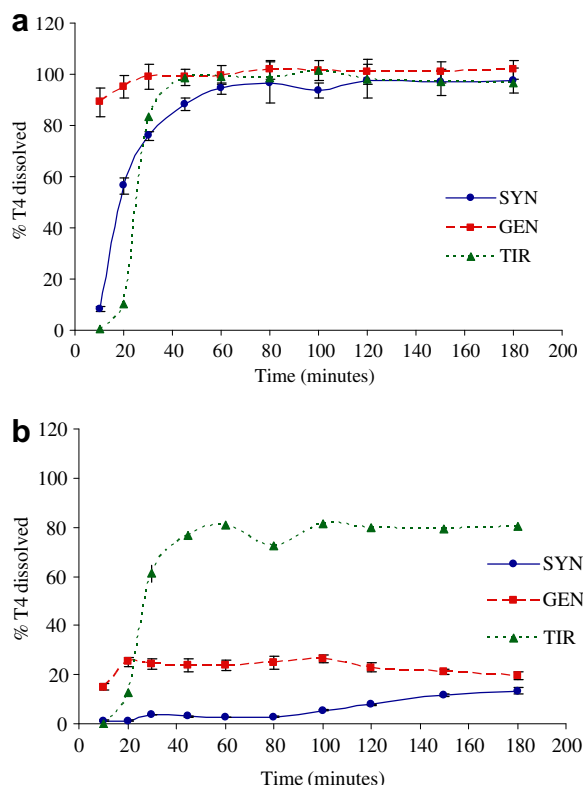
where  $n$  is the number of time points,  $R_t$  is the percent dissolution value of the reference product at time  $t$ , and  $T_t$  is the percent dissolution value of the test product at time  $t$ . For curves to be considered similar,  $f_1$  and  $f_2$  values should be close to 0 and 100, respectively. For the present study, SYN was used as the reference product as it is the most widely prescribed T4 product [21], and GEN and TIR were compared with it using  $f_1$  and  $f_2$ .

The effect of pH on dissolution of all the three products was studied by plotting the %T4 dissolved at 30, 60 and 120 min vs pH.

## 3. Results

The dissolution profiles for SYN, GEN and TIR in 0.01 N HCl in the presence as well as in the absence of 0.05% w/v SLS are shown in Fig. 1. It shows that SYN and TIR follow a more similar dissolution profile in the presence of SLS, whereas GEN has a significantly faster dissolution rate. The %T4 dissolved for SYN and TIR at 10 min were only  $8.18 \pm 0.88\%$  (mean  $\pm$  SD) and  $0.55 \pm 0.05\%$ , respectively, as compared to  $89.04 \pm 5.41\%$  for GEN. Table 1 shows the values of  $f_1$  and  $f_2$ . It is clear that neither GEN nor TIR is similar to SYN, further validating the above stated findings. Fig. 1 depicts the dissolution profiles performed in only 0.01 N HCl (without any SLS). It can

<sup>1</sup> *TraceSelect* is an elemental analysis grade.



**Fig. 1.** Dissolution of T4 products in 0.01 N HCl,  $n = 3$ ; (a) in the presence of 0.05% sodium lauryl sulfate and (b) in the absence of sodium lauryl sulfate.

**Table 1**

Difference factor ( $f_1$ ) and Similarity factor ( $f_2$ ) for *Tirosint*<sup>®</sup> and Generic levothyroxine sodium using *Synthroid*<sup>®</sup> as the reference product; dissolution carried out in 0.01 N HCl containing 0.05% sodium lauryl sulfate at 37 °C and 75 rpm.

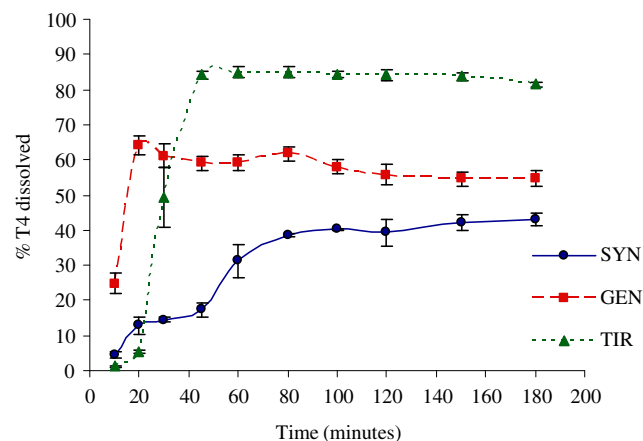
Time (minutes)	%T4 dissolved for SYN ( $R_t$ ) <sup>a</sup>	%T4 dissolved for test product ( $T_t$ ) <sup>a</sup>	Difference factor ( $f_1$ )	Similarity factor ( $f_2$ )
GEN vs SYN				
10	8.18	89.04	66.88	18.87
20	56.35	95.07		
30	76.01	98.98		
45	88.29	98.83		
TIR vs SYN				
10	7.49	0.55	31.08	37.21
20	56.35	10.47		
30	76.01	83.38		
45	88.29	98.54		

<sup>a</sup> Average of three replicates.

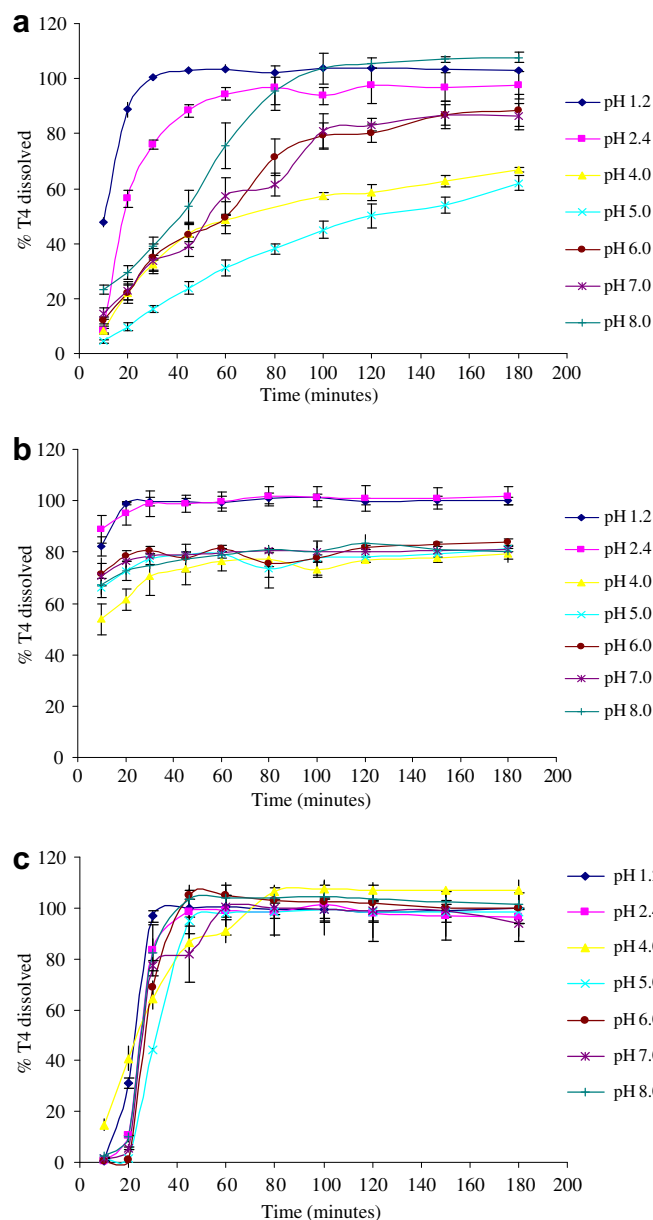
be seen that the dissolution of all the three products significantly reduced in the absence of SLS. Only about 13% and 20% of T4 dissolved from SYN and GEN, respectively, even after 180 min TIR performs better with about 80% T4 dissolved at the end of 180 min. Even though the profiles with and without SLS look similar with respect to shape, there is a great decrease in the magnitude of %T4 dissolved.

Fig. 2 shows the results for dissolution in 0.1 N HCl at 50 rpm. According to FDA, a drug product is considered to be a rapid release product if 85% of the drug is dissolved in 15–20 min, which corresponds to gastric emptying half-life (T50%) in fasting conditions [19]. The results show that none of the products meet these criteria with only  $12.78 \pm 2.51$ ,  $5.47 \pm 0.55$  and  $64.21 \pm 2.60\%$  T4 dissolved from SYN, TIR and GEN, respectively, in the first 20 min.

The dissolution profiles performed at various pH values are shown in Fig. 3. These profiles clearly show that pH plays an



**Fig. 2.** Dissolution of T4 products in 0.1 N HCl at 50 rpm, as indicated by FDA for immediate release drug products;  $n = 3$ .



**Fig. 3.** Dissolution profiles in various pH buffers containing 0.05% sodium lauryl sulfate,  $n = 3$ : (a) *Synthroid*<sup>®</sup>, (b) Generic levothyroxine sodium, (c) *Tirosint*<sup>®</sup>.

important role in the dissolution of T4 products with %T4 dissolved changing with change in the pH. The effect of pH on dissolution can be further studied from plots of %T4 dissolved vs pH at a given time (Fig. 4). As seen in Fig. 4a, the dissolution of all three products decreases with an increase in pH. Although the dissolution goes through a minimum at around pH 4–5, it increases again with further increase in the pH. However, the magnitude of change in dissolution with change in pH is clearly different for all the three products, with SYN appearing to be the most sensitive. As time proceeds, the percentage of T4 dissolved increases as expected. However, the effect of pH on dissolution diminishes with time, as seen in Fig. 4b and c, which show the dissolution after 60 and 120 min, respectively. GEN and TIR, in particular, show more consistent dissolution pattern over the entire pH range after 60 min and 120 min with the percent drug dissolved being close to 100%. The pH for the first 30 min, however, appears to be critical for TIR. Furthermore, unlike for GEN and TIR, for SYN the pH drastically affects the dissolution in spite of longer dissolution times, with the percent T4 dissolved dropping to as low as 50% at pH 5, even after 120 min of dissolution (Fig. 4c).

#### 4. Discussion

This study used a highly sensitive ICP-MS assay of T4 to show that the dissolution patterns of three widely prescribed T4 prod-

ucts are significantly different and that the dissolution of T4 from these products is sensitive to the pH of the dissolution media. Dissolution is one of the most important factors that along with permeability governs the oral absorption of a drug. The dissolution tests for T4, as described in USP, are a one-point quality control means. However, the high amounts of surfactant present in the dissolution medium (0.2% sodium lauryl sulfate in 0.01 N HCl) often result in extremely rapid dissolution, thus making it ineffective to study dissolution profiles of different formulations. It has been shown by Volpato et al. that the current USP dissolution test conditions for T4 (USP 30) are unable to discriminate between T4 products with different bioavailabilities and lead to a poorer IVIVC when compared to the previous milder conditions (USP 24) [22]. Moreover, the concentration of T4 in the dissolution studies is typically measured using HPLC with ultraviolet detection, as described by USP, which suffers from the inherent lack of sensitivity. Collectively, these drawbacks make it ineffective to discriminate between the subtle, but significant, differences in the patterns of *in vitro* dissolution of T4 products, which can further lead to differences in their absorption profiles [14]. ICP-MS is an excellent analytical technique for the determination of trace elements because of its exceptional sensitivity and elemental specificity [23]. T4 is a good candidate for ICP-MS analysis as it contains iodine, an element not found in other commonly used chemicals including pharmaceutical excipients (Fig. 5). ICP-MS has already been successfully employed for the determination of iodine in other mediums including natural and tap water, food products and urine samples [24–26]. It has also been used to analyze iodinated X-ray contrast agents in water [27]. For the present studies, a previously developed and validated, highly sensitive ICP-MS assay was, therefore, used for quantification of T4 [18].

Bioequivalence is an imperative concern for various available T4 products and as stated above, dissolution plays an important role in the absorption and bioavailability of a drug, and hence can be a determinant for bioequivalence. Our experiments demonstrate that the three products studied here exhibit very different dissolution behaviors (Fig. 1). Evidence that the dissolution of GEN and TIR is different from that of SYN is that the values of  $f_1$  and  $f_2$  (Table 1) do not fall within the required limits. Also, it is shown that dissolution from GEN is almost instantaneous with about 89% T4 dissolving in the first 10 min compared to only about 7% and 0.5% for SYN and TIR, respectively. The extremely fast dissolution of GEN can be explained by the presence of sodium starch glycolate among the tablet's inactive ingredients [28]. Sodium starch glycolate is a very strong disintegrating agent, which causes GEN tablets to disintegrate instantaneously, increasing the surface area tremendously, leading to rapid dissolution. No such disintegrating agent is present in SYN [29], which explains its relatively slower dissolution.

The dissolution of all the three products significantly reduces with the removal of SLS from the dissolution media (Fig. 1). These results imply that the inherent solubility of T4 products is rather

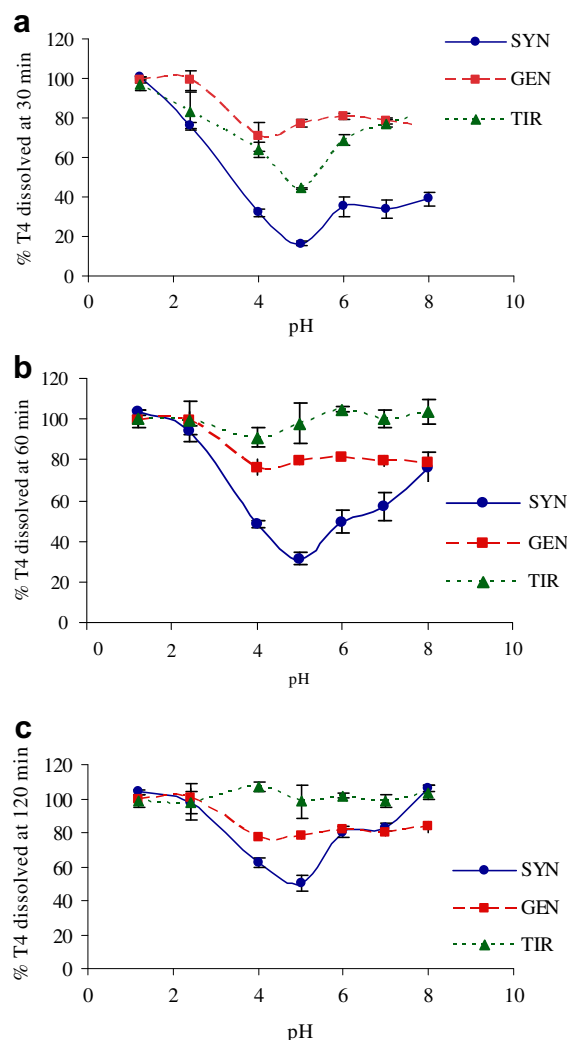


Fig. 4. Comparison of %T4 dissolved from various products at pH ranging from 1.2 to 8 at the end of (a) 30 min, (b) 60 min, and (c) 120 min;  $n = 3$ .

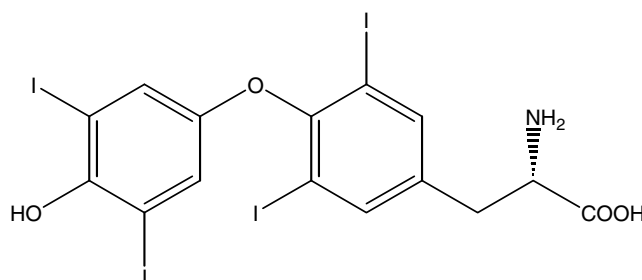


Fig. 5. Chemical structure of levothyroxine (T4).



low. To further evaluate this hypothesis, dissolution of all the three products was performed in 0.1 N HCl at 50 rpm, the results for which show that none of the products meet the criteria for an immediate release dosage form (Fig. 2). According to FDA's guidelines for Dissolution Testing of Immediate Release Solid Oral Dosage Forms [19], a rapidly dissolving drug product is one that shows 85% dissolution in 15 min under mild test conditions of 0.1 N HCl and 50 rpm when using USP apparatus II. Rapid dissolution of 85% in 15 min corresponds to T50% gastric emptying time, which is the time it takes to empty 50% of ~200 ml of water from the stomach under fasting conditions. Such a drug product is generally believed to behave as a solution and can ensure that the bioavailability of the drug is not limited by dissolution. The results obtained in our study, therefore, show that dissolution of T4 from these products is limited and can be a rate-limiting step in its absorption, and hence, can affect its bioavailability. Further implication of these results is that dissolution might be a contributing factor to the bioequivalence problems between various T4 products.

According to FDA's orange book, GEN is AB2 rated, which means it is therapeutically equivalent to SYN [30]. However, supplemental data on bioequivalence studies submitted to FDA by Sandoz, Inc. show that the relative bioavailability of GEN is 12.5% higher than that of SYN [31]. Despite being declared bioequivalent, a 12.5% difference in the bioavailability of T4 can lead to clinically relevant changes in the TSH levels of patients, which has raised serious concerns regarding the interchangeability of these products [32–34]. Our results show that the dissolution of GEN is significantly higher than that of SYN. A 92% higher dissolution (at 10 min) of GEN explains its higher bioavailability when compared to SYN. These results further confirm the above-stated inference that dissolution is one of the plausible causes of T4 bioequivalency problems.

The second objective of the present study was to examine the effect of pH on the dissolution of T4 products. T4 is absorbed from the jejuno-ileal part of the gastrointestinal tract. However, it has been shown that patients receiving PPIs require a higher dose of T4, indicating that gastric pH is critical for the subsequent intestinal absorption of T4 [16,17]. Our study shows that the dissolution of T4 from all the three products, SYN, GEN and TIR, is sensitive to pH (Fig. 3). The %T4 dissolved decreases with an increase in pH to a minimum at pH 5, and then increases again with further increase in pH (Fig. 4). The solubility of levothyroxine sodium is pH dependent and shows a similar behavior, which explains the dissolution of T4 products being pH dependent [35]. However, the effect of pH is different on the three T4 products studied. As time progresses, the effect of pH on GEN and TIR diminishes but not on SYN (Fig. 4b and c). Both SYN and GEN are tablet formulations of T4, but GEN contains a strong disintegrating agent, sodium starch glycolate, which causes instantaneous disintegration of GEN tablets, increasing the surface area manifolds resulting in rapid dissolution. Absence of such disintegrant in SYN formulation explains its slower dissolution, as the tablet surface slowly erodes in the dissolution bath instead of disintegrating. Therefore, the inherent dissolution of T4 from GEN is higher than that of SYN.

Overall, TIR shows the most consistent and highest dissolution compared to both SYN and GEN (Figs. 1–4). TIR is a soft gelatin capsule formulation containing T4 dissolved in glycerin. T4 already being in solution form enhances its dissolution, the only rate-limiting step being the dissolution of the soft gelatin capsule shell [36]. These results imply that an increase in gastric pH with the use of PPIs might lead to a decrease in dissolution of T4 products. Furthermore, a decrease in dissolution of T4 due to changes in the pH can lead to a decrease in its absorption. Hence, patients on PPIs might require a higher dose of T4 to achieve euthyroid state, as demonstrated by Centanni et al. and Sachmechi et al. [16,17]. However, an important point to

be noted here is that the magnitude of decrease in dissolution with pH is different for various T4 products. Hence, in speculation, patients on SYN, for example, might require a much higher increase in dose of T4 than those on TIR.

In summary, we have shown that the dissolution pattern of three commercial T4 products is significantly different and that the dissolution decreases with increase in pH.

## 5. Conclusion

Dissolution is one of the most important characteristics of a drug directly affecting the drug absorption and bioavailability. The analytical assays for T4 determination, including immunoassays and HPLC–UV, suffer from the lack of specificity and sensitivity and/or from lengthy extraction or derivatization procedures and therefore, fail to detect and quantify subtle but significant differences between various formulations. The use of a rapid and highly sensitive ICP–MS assay helped us quantify such differences between three T4 products, *Synthroid*<sup>®</sup>, generic levothyroxine sodium by Sandoz Inc. and *Tirosint*<sup>®</sup>. The three products had drastically different dissolution profiles with respect to both shape and %T4 dissolved. The extremely high dissolution of the Sandoz generic tablets explains the observed 12.5% higher bioavailability when compared to *Synthroid*<sup>®</sup>. Furthermore, the dissolution is sensitive to pH, which can lead to a higher T4 dose requirement in patients with higher gastric pH. *Tirosint*<sup>®</sup>, a soft gelatin capsule containing T4 dissolved in glycerin, showed the most consistent dissolution pattern. Such a capsule formulation might work better *in vivo*, when compared to tablets, owing to the enhanced dissolution due to the presence of T4 in solution form. In conclusion, dissolution of T4 products is critical for its bioavailability and is possibly one of the contributing factors to the bioequivalence problem between various T4 products available.

## Acknowledgments

This research was made possible, in part, by the RI-INBRE research core facility supported by Grant # P20RR16457 from NCRR, NIH.

## References

- [1] P.A. Singer, D.S. Cooper, E.G. Levy, P.W. Ladenson, L.E. Braverman, G. Daniels, F.S. Greenspan, I.R. McDougall, T.F. Nikolai, Treatment guidelines for patients with hyperthyroidism and hypothyroidism. Standards of Care Committee, American Thyroid Association, *JAMA* 273 (1995) 808–812.
- [2] D. Carr, D.T. McLeod, G. Parry, H.M. Thornes, Fine adjustment of thyroxine replacement dosage: comparison of the thyrotrophin releasing hormone test using a sensitive thyrotrophin assay with measurement of free thyroid hormones and clinical assessment, *Clin. Endocrinol. (Oxf.)* 28 (1988) 325–333.
- [3] I. Klein, K. Ojamaa, Thyroid hormone and the cardiovascular system, *N. Engl. J. Med.* 344 (2001) 501–509.
- [4] V. Blakesley, W. Awni, C. Locke, T. Ludden, G.R. Granneman, L.E. Braverman, Are bioequivalence studies of levothyroxine sodium formulations in euthyroid volunteers reliable?, *Thyroid* 14 (2004) 191–200.
- [5] V.A. Blakesley, Current methodology to assess bioequivalence of levothyroxine sodium products is inadequate, *AAPS J.* 7 (2005) E42–E46.
- [6] J.V. Hennessey, Levothyroxine a new drug? Since when? How could that be?, *Thyroid* 13 (2003) 279–282.
- [7] I. Klein, S. Danzi, Evaluation of the therapeutic efficacy of different levothyroxine preparations in the treatment of human thyroid disease, *Thyroid* 13 (2003) 1127–1132.
- [8] G.H. Mayor, T. Orlando, N.M. Kurtz, Limitations of levothyroxine bioequivalence evaluation: analysis of an attempted study, *Am. J. Ther.* 2 (1995) 417–432.
- [9] L. Wartofsky, Levothyroxine: therapeutic use and regulatory issues related to bioequivalence, *Expert Opin. Pharmacother.* 3 (2002) 727–732.
- [10] G. Oliveira, M.C. Almaraz, F. Soriguer, M.J. Garriga, S. Gonzalez-Romero, F. Tinahones, M.S. Ruiz-de Adana, Altered bioavailability due to changes in the formulation of a commercial preparation of levothyroxine in patients with differentiated thyroid carcinoma, *Clin. Endocrinol. (Oxf.)* 46 (1997) 707–711.
- [11] A.P. Shroff, J.K. Jones, Standards for levothyroxine preparations, *JAMA* 244 (1980) 658–659.

- [12] CDER, Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations, US FDA, 2002.
- [13] CDER, Guidance for Industry: Waiver of in Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, US FDA, 2000.
- [14] USP, Levothyroxine sodium tablets, in: The United States Pharmacopoeia, United States Pharmacopoeia Convention Inc., Rockville, MD, 2007, pp. 2470–2471.
- [15] M.T. Hays, Localization of human thyroxine absorption, *Thyroid* 1 (1991) 241–248.
- [16] M. Centanni, L. Gargano, G. Canettieri, N. Viceconti, A. Franchi, F.G. Delle, B. Annibale, Thyroxine in goiter, *Helicobacter pylori* infection, and chronic gastritis, *N. Engl. J. Med.* 354 (2006) 1787–1795.
- [17] I. Sachmechi, D.M. Reich, M. Aninyei, F. Wibowo, G. Gupta, P.J. Kim, Effect of proton pump inhibitors on serum thyroid-stimulating hormone level in euthyroid patients treated with levothyroxine for hypothyroidism, *Endocr. Pract.* 13 (2007) 345–349.
- [18] D. Pabla, F. Akhlaghi, A. Ahmed, H. Zia, Development and validation of an inductively coupled plasma mass spectrometry method for quantification of levothyroxine in dissolution studies, *Rapid Commun. Mass Spectrom.* 22 (2008) 993–996.
- [19] CDER, Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, US FDA, 1997.
- [20] J.W. Moore, H.H. Flanner, Mathematical comparison of dissolution profiles, *Pharm. Tech.* 20 (1996) 64–74.
- [21] Top 200 brand drugs by unit in 2007. Available from: <<http://www.drugtopics.com>>.
- [22] N.M. Volpato, R.L. Silva, A.P. Brito, J.C. Goncalves, M. Vaisman, F. Noel, Multiple level C in vitro/in vivo correlation of dissolution profiles of two l-thyroxine tablets with pharmacokinetics data obtained from patients treated for hypothyroidism, *Eur. J. Pharm. Sci.* 21 (2004) 655–660.
- [23] A. Montaser, *Inductively Coupled Plasma Mass Spectrometry*, Wiley-VCH, New York, 1998.
- [24] M. Haldimann, B. Zimmerli, C. Als, H. Gerber, Direct determination of urinary iodine by inductively coupled plasma mass spectrometry using isotope dilution with iodine-129, *Clin. Chem.* 44 (1998) 817–824.
- [25] Y.S. Takaku, T. Masuda, Y. Igarashi, Iodine determination in natural and tap water using inductively coupled plasma mass spectrometry, *Anal. Chem.* 11 (1995) 823–827.
- [26] H. Vanhoe, A.F. Van, J. Versieck, R. Dams, Effect of solvent type on the determination of total iodine in milk powder and human serum by inductively coupled plasma mass spectrometry, *Analyst* 118 (1993) 1015–1019.
- [27] F. Sacher, B. Raue, H.J. Brauch, Analysis of iodinated X-ray contrast agents in water samples by ion chromatography and inductively-coupled plasma mass spectrometry, *J. Chromatogr. A* 1085 (2005) 117–123.
- [28] Sandoz Inc., Levothyroxine Sodium Tablets, USP (Package Insert), 2007.
- [29] Abbott Laboratories, *Synthroid*<sup>®</sup> (Levothyroxine Sodium Tablets, USP) 03-5443-R2-Rev. (Package Insert), 2006.
- [30] CDER, Orange Book, 2008. Available from: <<http://www.fda.gov/cder/>>.
- [31] Center for Drug Evaluation and Research, Application Number(s) 21–342/S-003, 2007. Available from: <<http://www.fda.gov/cder/>>.
- [32] Request that the Commissioner of Food and Drug Take Action Described Methodology for Oral Levothyroxine Sodium Drug Products, 2007. Available from: <<http://www.fda.gov/>>.
- [33] Equivalence of Levothyroxine Sodium Products: Joint Public Meeting, 2007. Available from: <<http://www.fda.gov/cder/>>.
- [34] American Thyroid Association, Thyroid Experts Warn of Clinically Important Differences in Potency of FDA-Approved Levothyroxine Products, 2007. Available from: <<http://www.thyroid.org/index.html>>.
- [35] C.M. Won, Kinetics of degradation of levothyroxine in aqueous solution and in solid state, *Pharm. Res.* 9 (1992) 131–137.
- [36] Institut Biochimique SA, Tiroint<sup>TM</sup>, 2007. Available from: <<http://www.doctorathand.com/>>.