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Original Article

The Effect of Beta-Cyclodextrin on the Iodometric Determination of Some Penicillins Reported in USP and BP

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Abstract

This work summarizes how the efficiency of pharmacopeias (USP and BP) procedures for the iodometric determination of pencillins can be significantly enhanced through the use of beta-cyclodextrin (β-CD). In the commonly used iodometric method, titration takes about 30 min; 15 min to hydrolyze the antibiotics and 15 min for reaction between iodine and hydrolyzed species. The experimental results indicate that β-CD accelerates both hydrolysis of amoxicillin, ampicilline and cloxacilline and the reaction of their hydrolyzed products with iodine. Effect of different parameters such as sodium hydroxide, hydrochloric acid concentrations, and the waiting times in the presence of β-CD on the total titration times reported in pharmacopeia are evaluated. Finally, it is shown that the analysis times of iodometric assay for ampiciline an d amoxicilin in the presence of 0.108 $mg.^{ml-1}$ β-CD are reduced from 30 to 5 min and those of cloxaciline is reduce to 10 min. The iodometric titration in the presence of β-CD is referred to as modified USP. Student t-tests at 95% level shows that there are no significant differences between USP and Modified USP. It is also shown that β-CD increases the stability of penicillin-G relative to USP buffer No.1. The reliability of the proposed method is evaluated by analysis of the above mentioned antibiotics in different pharmaceutical forms. The results show that the modified method can be successfully applied for the assay of cloxaciline injection vial, amoxicillin and ampicillin capsules and suspensions.

Keywords: β-Cyclodextrin; Iodometric assay; Penicillins; Pencilin G Stability.
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1. Introduction

Qualitative and quantitative control of drugs after production is performed by the standard methods described in United States pharmacopeia (USP) and British pharmacopeia (BP). Iodometry is described in USP as one of the standard methods for determination of ampicillin, amoxicillin, penicillin-G, and cloxacillin [1, 2]. In this method, penicillins are hydrolyzed by NaOH and then the products are oxidized with I_2. Both hydrolyzing and oxidizing steps are time

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consuming and each step takes about 15 min, hence a total waiting time of 30 min is required to report the analysis results.

Applications of cyclodextrins (CDs), especially β-CD, and their derivatives cover various areas of pharmaceutical researches including improved drug loading and solubility, encapsulation and controlled release of drugs [3-10]. CDs are cyclic oligosaccharides with a non-polar cavity and a polar surface. This particular configuration allows complexation of non-polar guest and a good solubility of the host-guest complex in water and in biological medium. These CDs are well known because of their capabilities in forming stable host-guest inclusion complexes [11]. Several weak intermolecular interactions including dipole-dipole, electrostatic, Van der Waals, hydrophobic and hydrogen bonding interactions are suggested between host and guest molecules [3, 4]. Therefore, complex formation changes the properties of both host and guest molecules.

Catalytic activity of CDs on a variety of reactions is well known [12]. Hydrolysis of phenyl acetates is one of the first works on catalytic actions of CDs [13]. All the reactions catalyzed by the CDs and their derivatives proceed via complexation. β-CD in alkaline media accelerates the breakage of β-lactam ring of penicillins 20 to 90 times faster than alkaline hydrolysis in its absent [14, 15].

In the iodometric determination of penicillins an alkaline hydrolysis occurred prior to reaction with iodine molecules. However, in this study catalytic effect of β-CD on the chemical reactions is used to minimize the iodometric reaction times of ampicilline, amoxicillin, and cloxacillin. Finally, a modified iodometric method for the above mentioned penicillins is introduced and referred to as modified USP (MUSP).

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical reagent grades and were purchased from Merck (Darmstadt, Hess, Germany). Ampicillin, amoxicillin, penicillin-G and cloxacillin standards are received as a gift from Kosar and Jaberebn-e-Hayan Pharmaceutical Companies Iran. Double distilled water is used throughout.

Ampicillin and amoxicillin capsules (500 mg) and suspensions (250 mg/5 ml), penicillin G (1000000 unit) and cloxacillin (500 mg) injection vials and capsules are prepared from local drug stores.

2.2. Steps in iodometric titration of penicilines reported in USP and BP

The iodometric procedure for determination of the above mentioned penicillins, are described in BP and USP as follow:

1. Pour 2 ml of antibiotic solution in an Erllen Myer and add 2 ml of 1 M NaOH solution
2. Wait 15 min to hydrolyze the penicillines
3. Add 2 ml 1.2 M HCl solution and 10 ml 0.01 M I2 solution
4. Wait 15 min for the reaction between iodine molecule and hydrolyzed products to become complete
5. Titrate with 0.01 M sodium thiosuphate solution till a pale yellow color is observed then add 10 drops of starch solution, and continue titration till the end point is reached.

In each experiment all of the antibiotic

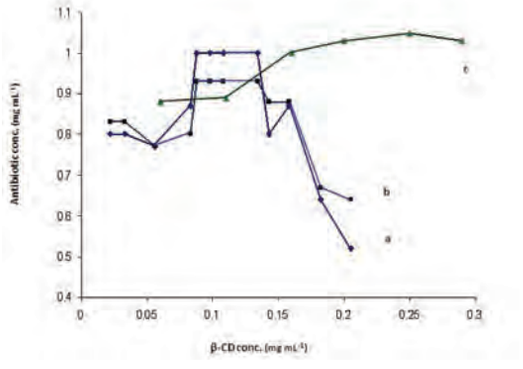

Figure 1. The effect of β-CD concentration on the iodometric determination of a) Amoxicillin, b) Ampicillin and c) Cloxacillin.

Table 1. Comparison of the results obtained by both USP and modified USP (MUSP) methods.

	USP	MUSP
Amoxicillin (1 mg.mL^{-1})	0.92 ±0.001	0.93 ±0.009
Ampicillin (1.11 mg.mL^{-1})	0.99 ±0.017	1.00 ±0.009
Cloxacillin (1.25 mg.mL^{-1})	1.11 ±0.016	1.03 ±0.011

The results are mean of six measurements.

concentrations were calculated as reported in USP [2].

2.3. Modified USP (MUSP)

The extent of the effect of β-CD on the determination of each antibiotic is determined separately and a detailed procedure which is described below is referred to as MUSP.

2.4. Assay of amoxicillin and ampicillin

Two milliliter of the antibiotic solution was transferred to an Erllen Myer and 1 ml of 1.85 mg.ml^{-1} β-CD was added to give a final concentration of 0.108 mg.ml^{-1}, then 2 ml NaOH (1 M), 2 ml 1.2 N HCl solution and 10 ml 0.01 M I_2 solution was added, and waited for 5 min. The resulting solution was titrated with 0.01 M sodium thiosulphate solution.

Blank titrations are also performed with the same procedure but 2 ml distilled water is added instead of the antibiotic solution. Blank and antibiotics titration results are needed to calculate the penicillins concentration as is reported in USP [2].

2.5. Assay of cloxacillin sodium

Two milliliter of antibiotic solution was transferred to an Erllen Myer and 2.5 ml of 1.85 mg.ml^{-1} β-CD was added to reach a final concentration of 0.27 mg.ml^{-1}, then, 2 ml 1 M NaOH was added. After 5 min, 2 ml 1.2 N HCl and 10 ml 0.01 M I2 was transferred to the Erllen, and waited another 5 min, and titrated with 0.01 M sodium thiosulphate solution. Blank titrations were prepared with the same procedure by adding 2 ml distilled water instead of the antibiotic solution.

3. Results and discussion

The effect of β-CD in two delay steps (hydrolysis of the penicillines and oxidation of hydrolyzed product by iodine) is studied and modified USP or BP methods (MUSP or MBP) are proposed.

To check the necessity of the total 30 min waiting times in the iodometric determination of the mentioned penicillines, repeated experiments were performed using different waiting times for hydrolysis of the selected penicillines and the iodine reaction and analyzing the results. As the results show, 30 min waiting times is necessary for ampicilline, amoxicilline, and cloxacilline sodium determination. Penicillin G shows a different behavior.

In USP method, peniciline-G is prepared in NaOH and hydrolysis is started immediately after solution preparation. The first 15 min, waiting time reported in USP and BP is not necessary and β-CD had no effect on the hydrolysis step. By analyzing replicate titrations, it is also found that the reaction rates of hydrolyzed products with iodine are also fast and reaction time can be set to 5 min without β-CD. So, no β-CD is needed to be added.

USP suggests preparation of penicillin-G solution in buffer No. 1 to stabilize it for assay purposes. The stability of penicillin-G in USP buffer No. 1 is compared with that of β-CD. The results indicate that penicillin-G solution prepared in USP buffer No. 1 show 3.2% hydrolysis after 24 h while just 1.7% hydrolysis is occurred in β-CD after 72 h. This stability probably is due to inclusion complex formation at the inset.

In order to establish MUSP method for the three mentioned antibiotics, optimum concentration of β-CD, NaOH, HCl and reaction time are determined. The sequence

Table 2. Analysis of amoxicillin, ampicillin and cloxacillin in different pharmaceutical forms.

	Amoxicillin (5mg mL^{-1})	Ampicillin (5mg mL^{-1})	Cloxacillin (mg)
Suspension[a] (USP)	211.25±9.97	226.25 ±13.26	-
Suspension[a] (MUSP)	198.75 ±13.06	212.50 ± 9.58	-
capsules[b] (USP)	315.00 ±1.02	398.00 ±4.04	521.25 ± 4.01
Capsules[b] (MUSP)	287.50 ±2.04	356.00 ±2.07	527.50 ±1.09
injection vial[b] (USP)	-	-	539.17 ±3.05
injection vial[b] (MUSP)	-	-	538.33 ±4.06

The results are mean of six measurements.; [a]250 mg per 5 mL; [b]500 mg; a & b are lable amounts

of the addition of reagents is also checked. Finally, MUSP method is used to determine the considered penicillins.

3.1. Effect of β-CD in hydrolysis of penicillins

In order to investigate the effect of β-CD on hydrolysis of the mentioned pencillines β-CD is added in hydrolysis step (step 1, section 2.3) and results for a series of consecutive titrations with different hydrolysis times (step 2, section 2.3) are compared. The iodine reaction time is kept constant as set in USP. It is concluded from the results, that β-CD immediately hydrolyzed amoxicillin and ampicillin. So, in the presence of β-CD hydrolysis waiting time can be omitted for these two penicillins. As for cloxacillin sodium, hydrolysis time is reduced from 15 to 5 min. However, β-lactam ring cleavage is accelerated in the presence of β-CD as expected and reported [15].

3.2. Effect of β-CD on reaction of I_2 with hydrolyzed products of the penicillins

In another set of titrations, β-CD is added just before the iodine addition (step 3 of section 2.3) and the solutions are titrated with varied time intervals (step 4, section 2.3). The obtained results show that β-CD can also affect the oxidation reaction between iodine and hydrolyzed products. It seems that inclusion complexes of β-CD with iodine [16] could be the reason for such increase in the rate of oxidation of these compounds. The reaction time was reduced to 5 min instead of 15 min for ampicillin, amoxicillin and cloxacilline sodium.

Also the obtained results illustrate that β-CD should be added in hydrolysis step.

3.3. Effect of β-CD concentration on the titration results

Figure 1 shows the effect of β-CD concentration on the accuracy of the titration results. As it is illustrated from this Figure, the optimized β-CD concentrations for ampicilline, amoxicillin, and cloxacilline sodium are 0.108, 0.108, and 0.27 $mg.mL^{-1}$, respectively.

3.4. Effect of NaOH and HCl on measurement of penicillines

At optimized time and β-CD concentrations, the variation in NaOH and HCl concentrations on the titration results are investigated. For this purpose the concentrations below and above those suggested in the USP are tested. But no considerable changes in the results are observed and USP concentrations namely, 1 M NaOH and 1.2 M HCl, are used throughout the study.

3.5. Comparison of USP and MUSP methods

Detailed of modified USP method for the above mentioned penicillins are summarized in section 2.3.1 and 2.3.2. In order to show the reliability of the proposed methods the mentioned standards and some of their pharmaceutical forms are analyzed by both USP and MUSP iodometric procedures.

The results of analysis of standard solutions of the three mentioned penicillins by two methods are presented in Table 1. The

methods are comparable. The analysis of variance at 95% level also showed that there are no significant differences between these methods.

Assay results of the selected pharmaceuticals presented in Table 2 demonstrate good agreement between MUSP and USP method. Also statistic tests show that there is no significant difference between USP and proposed modified method.

4. Conclusion

In conclusion, β-CD catalyzed both hydrolysis and reaction of hydrolyzed products with iodine. The inclusion complexes formed in the presence of β-CD promote the reaction between iodine and the hydrolyzed products. So, assay times, an important factor in chemical analysis, are reduced from 30 to 5 and 10 min for amoxicillin, ampicilline, respeactively and cloxacillin sodium. It is indicated that structural differences of penicillins can affect the extent of β-CD catalytic action.

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References

[1] British Pharmacopia, London, (2010); (2006); 1(2002) 164, 372; 1(1998) 90-101.

[2] The United States Pharmacopia, Newyork, (2007); 1(2000), 129-132; 2(2003), 1961-3.

[3] Pariot N, Edwards-Le´vy F, Andry MC, Le´vy MC. Cross-linked β-cyclodextrin microcapsules. II. Retarding effect on drug release through semi-permeable membranes. *Int J Pharm* 2002; 232: 175-81.

[4] Lin SZ, Wouessidjewe D, Poelman MC, Duchene D. *In vivo* evaluation of indomethacin/cyclodextrin complexes gastrointestinal tolerance and anti-inflammatory activity. *Int J Pharm* 1994; 106: 63-7.

[5] Fetzner A, Bohm S, Schreder S, Schubert R. Degradation of raw or film-incorporated β-cyclodextrin by enzymes and colonic bacteria. *Eur J Pharm Biopharm* 2004; 58: 91-7.

[6] Pose-Vilarnovo B, Perdomo-Lo´pez I, Echezarreta-Lo´pez M, Schroth-Pardo P, Estrada E. Torres-Labandeira JJ. Improvement of water solubility of sulfamethizole through its complexation with β- and hydroxypropyl-β-cyclodextrin: characterization of the interaction in solution and in solid state. *Eur J Pharm Sci* 2001; 13: 325-31.

[7] Boudad H, Lebas G, Cheron M, Duche^ne D, Ponchel G. Combined hydroxypropyl-□-cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir. *Int J Pharm* 2001; 218: 113-24.

[8] Loftsson T, Masson M. Cyclodextrins in topical drug formulations: theory and practice. *Int J Pharm* 2001; 225: 15-30.

[9] Shanmugam M, Ramesh D, Nagalakshmi V, Kavitha R, Rajamohan R, Stalin T. Host-guest interaction of l-tyrosine with β-cyclodextrin. *Spectrochim Acta Part A* 2008; 71: 125-32.

[10] Scondo A, Dumarcay F, Marsura A, Barth D. Tandem Staudinger-Aza-Wittig reaction in supercritical CO2: synthesis of a pharmaceutical interest compound. *J Supercrit Fluids* 2010; 53: 60-3.

[11] Roth HJ, Eger K, Troschutz R. *Pharmaceutical Chemistry*. Ellis Horwood, 2, 1991, pp. 252-3.

[12] Dodziuk H. *Cyclodextrin and their complexations: chemistry, analytical methods, applications*. Wiley-VCH Verlag GmbH & Co., 2006, pp. 93-105.

[13] VanEtten RL, Sebastian JF, Clowes GA, Bender ML. Acceleration of phenyl ester cleavage by cycloamyloses. A model for enzyme specificity. *J Am Chem Soc* 1967; 89: 3242-53.

[14] Tutt DE, Schwartz MA. Specificity in the cyclohept-amylose-catalysed hydrolysis of penicillins. *J Chem Soc Chem Commun* 1970; 2: 113-4.

[15] Schwartz MA, Tutt DE. Model catalysts which simulate penicillinase. V. The cyclohepta-amylose-catalyzed hydrolysis of penicillins. *J Am Chem Soc* 1971; 93: 767-72.

[16] Diaz D, Vargas-Baca I, Gracia-Mora J. Beta-cyclodextrin inclusion complexes with iodine: an advanced and inexpensive undergraduate chemistry experiment. *J Chem Educ* 1994; 71: 708.