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THE INFLUENCE OF THE STORAGE TEMPERATURE ON THE ACTIVITYOFTHE RECONSTITUTED ORAL AMPICILLIN SUSPENSIONS

Mirghani Abd Elrahman Yousif *PhD* , **Asim Farouk** *PhD*
Faculty of Pharmacy – University of Gezira - Sudan

Abstract:

The following sets of experiments were conducted to investigate the extent of degradation of ampicillin antibiotic in suspension formulations stored at two different temperatures. The only variable in these experiments was the storage temperature. Oral ampicillin powders were reconstituted with distilled water and stored at two different temperatures of 5º and 35ºC respectively representing the refrigeration and average room temperature in Sudan. The reconstituted ampicillin suspensions were assayed for initial drug contents, and then daily for a period of seven days in order to determine the effect of the storage temperature on the stability of reconstituted oral ampicillin suspensions. The drug contents in the different samples were determined using the microbiological cup plate method and the chemical spectrophotometric technique. The results obtained showed a remarkable decrease in the activity of the oral ampicillin suspensions stored at room temperature, when compared with the refrigerated ones. Recommendation of a unit-dose sachet package of ampicillin oral powder to be reconstituted at the time of intake was suggested.

الخلاصة:

مجموعة من التجارب أُجريت للتحقق من مدى تلف مضــــاد الحيوية فى معلقات الأمبيســـــيللين المخزنة عند درجتى حرارة مختلفتين، المتغير الوحيد في هذه التجارب هو درجة حرارة التخزين. تم تعُليق مسحوق الأمبيســـــــيليين الفموي بالماء المقطر وتخزين المعلقات عند درجتي الحرارة 5 و 35 درجة مئوية والتي تمثل على التوالي درجة حرارة الثلاجة ومتوســـط درجة حرارة الغرفة في السودان. تم تحليل معلقات الأمبيسيللين لمعرفة المحتويات من مضـاد الحيوية الابتدائية يومياً لمدة سبعة أيام وذلك لمعرفة تأثير درجة حرارة التخزين على ثبات معلقات الأمبيســـيللين، كميات الدواء في العينات تم تحديدها باســتعمال ابارالآجار في الوسيط الزراعي والتقنية الكيميائية بالطيف الضوئي.النتائج المتحصل عليها بيّنت النقصان الواضح والمؤثر في فعالية معلق الأمبيسيللين المخزن في درجة حرارة الغرفة عند ما تمت مقارنته مع نفس معلق الأمبيسيللين المخزن في الثلاجة. تمت التوصية باستخدام نظام تعبئة الجرعة الاحادية من مسحوق الامتسيلين والتي تُعلق مباشرة عند الاستعمال.

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Introduction:

As recommended by the drug manufacturers, the reconstituted oral suspensions of beta-lactam antibiotics should be stored at a temperature not exceeding 30^0 C. It is evident that intensive investigations have been carried out on the effect of the storage temperature on the stability of beta-lactam antibiotic (1, 2, 3 and 4). Juste *et al*. (5) demonstrated the stability of ampicillin in solution at room temperature using the microbiological assay and high performance liquid chromatography (HPLC).

Since the refrigeration is not common in most parts of the Sudan, it is a routine practice to store the reconstituted oral suspensions of beta-lactam antibiotics at room temperature, which ranges from 15-47^0 C during different seasons of the year in the Central Region of the Sudan. The definition of room temperature is multifaceted and complex, and cannot be easily described as a simple range of acceptable, or even an average temperature. The out-of-hospital environment is notoriously uncontrolled, and one of the uncontrolled aspects is temperature (6), and this also was demonstrated by Allerga *et al*. (7). All these dictate the investigation of the effect of the storage temperature on the activity of the reconstituted oral beta-lactam antibiotic suspensions in the region.

Materials And Methods

Different brands of oral ampicillin powders were randomly selected from drug stores, to determine the influence of the storage temperature on their stability after reconstitution. A standard ampicillin used as a reference standard of the assay was obtained as pure chemical substance from the manufacturers. *Staphylococcus aureus* NCTC 6447 was used as test organism. One-mL aliquots of a 24-hour broth culture of the test organism were aseptically distributed into 8fl. Oz. Oxoid nutrient agar slopes, and incubated at 37^0 C for 24 hours. The bacterial growth was harvested and washed with sterile normal saline, and finally suspended in a small volume of normal saline to produce a suspension containing about 10^8 - 10^9 colony- forming-units (CFU) per mL, and the suspension was stored in the refrigerator at 4^0 C till used.

Two-fold serial dilutions of the tested antibiotic suspensions were freshly prepared in sterile distilled water at the time of the experiments. The concentrations of these dilutions ranged between 1.25 and 100 μg per mL. The assay was based on the use of a double-layer agar system, with fifteen-mL of un-inoculated base agar and ten-mL seed layer inoculated with standardized

suspension of *Staphylococcus aureus*. The Petri dishes used were approximately 20 x 100 mm. The seed agar was prepared by adding two mL of the standard suspension of *S. aureus* to 100 ml of seed agar, melted and cooled to 48^0C to give a final count of about 10^8cells/mL. The plates were always prepared on a levelled surface to ensure an even layer thickness. The seed layer was usually added after the base layer had solidified, and then the plates were refrigerated until used.

The cups were made by using a flamed and cooled 8-mm cork borer. Five discs were removed out of each of the seeded agar plates by means of Pasteur pipettes. Aliquots of 0.1 mL of each of the antibiotic solutions were added to appropriate cups at random, with an average of 10-20 cups used for each dilution. After filling the reservoirs with the appropriate dilutions, the plates were allowed to stand at 25^0C for 2 hours for the antibiotic to diffuse across the agar, and then incubated in an upright position at 37^0 C for 16-18 hours. The diameters of the resultant growth inhibition zones were carefully measured, the readings were statistically analyzed, and the final results were tabulated.

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The spectrophotometric method adopted was based on the method reported by Smith *et al.* (8). Buffered copper sulphate solution used in the technique was prepared by dissolving 3.93 gm of $CuSO_4.5H_2O$ in distilled water and diluted to one litre; to 15 mL of the copper sulphate solution was added citrate- phosphate buffer, pH 5.2, to one litre giving a final concentration of 15 µg Cu/mL. For the preparation of the citrate phosphate buffer, pH 5.2, 464 mL of 0.1M citric acid solution was mixed with 536 mL of di-sodium hydrogen phosphate solution (0.2M solution), and the pH adjusted to 5.2 with citric acid or di-sodium hydrogen phosphate solutions.

One-mL aliquots of the different antibiotic concentrations were suitably diluted with 9 mL quantities of a buffered copper sulphate solution. The resulting solutions, contained in stoppered glass tubes, were heated in a water-bath at 75^0C for 30 minutes. The tubes were then rapidly removed and cooled in an ice-bath. The optical densities of the respective solutions were determined at 320 mµ, in 1-cm cell, with unheated buffer ampicillin solution in the reference cell. The calibration curve of the antibiotic was prepared using different concentrations of ampicillin.

The suspensions could be considered stable if they maintained at least 90% of their initial concentration as shown by Stella (9).

Results And Discussion

The initial drug content of ampicillin suspensions was 97.6% by the microbiological method, and 98.6% by the chemical method, with no significant difference between the two methods (Table1).

Table 1: Comparison of Drug Contents of Oral Ampicillin Suspensions, Reconstituted and Stored at Different Temperatures, Using Microbiological Cup-plate and Chemical Spectrophotometry Assay Techniques.

Temperature (^{o}C)	Time (Days)	Microbiological Cup- plate	Spectrophotometry	Statistical Significance of Difference (P)**
		Drug content (mg) + SE *		
0		244.1 (3.1)	246.7 (2.7)	NS
1		240.8 (3.7)	243.5 (2.6)	NS
2		238.7 (3.9)	240.2 (2.7)	NS
3		227.5 (3.2)	235.6 (2.6)	NS
5^0	4	207.4 (3.1)	224.9 (2.7)	< 0.05
5		187.5 (2.9)	211.8 (2.6)	< 0.01
6		172.3 (2.8)	193.6 (2.7)	< 0.01
7		155.1 (2.6)	181.4 (2.1)	< 0.01
1		232.1 (3.6)	237.4 (2.9)	NS
2		211.6 (3.6)	221.2 (2.8)	< 0.05
3		192.5 (3.2)	212.6 (2.7)	< 0.05
35^0	4	173.4 (3.3)	195.7 (2.8)	< 0.05
5		135.9 (3.0)	172.6 (2.9)	< 0.01
6		120.7 (2.8)	143.8 (2.3)	< 0.01
7		105.4 (2.6)	121.5 (2.7)	< 0.01

*: Drug content of Ampicillin 250mg/5ml-suspension (average of 10-20 determinations)
    ± Standard Error of the Means.
**: Probability Values express results of T-test between Means of Drug content
     determined by the two assay techniques.
NS: Not Significant.

In the first three days of the experiment, the respective drug contents of ampicillin suspensions

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stored at 5ºC were 96.5 %, 95.5% and 91.0 % by the microbiological method, and 97.4%, 96.1% and 94.2 % by the chemical method, with no significant differences between the two methods of analysis.

The respective drug contents of ampicillin suspensions stored at 35^0C in the first three days were 92.8%, 84.6% and 77.0% by the microbiological method, and 94%, 88.5% and 85.0% by the chemical method.

On the fourth day of the experiment, the drug contents of ampicillin suspensions stored at 5^0C were 82.9% by the microbiological method and 89.9% by the chemical method, with a significant difference (P<0.05) between the two methods of analysis.

The drug contents of ampicillin suspensions stored at 35^0C on the fourth day of the storage were 69.4% by the cup-plate microbiological method, and 78.3% by spectrophotometric method, with a significant difference (P<0.05) between the two analytical methods.

On the 5th, 6th and 7th days of storage at 5^0C, the respective drug contents of ampicillin suspensions were 75.0%, 68.9% and 62.0% by the microbiological method, and 84.7%, 77.4% and 72.6% by the chemical method, with significant differences (P < 0.01) between the results of the two methods of the analysis.

In the last three days of the experiment, the respective drug contents of ampicillin suspensions stored at 35^0C, were 54.4%, 48.3% and 42.2% by the microbiological method, and 69.0%, 57.5% and 48.6% by the chemical method, with significant differences (P< 0.01) between the results of the two methods of analysis. Nevertheless, it is necessary to state that estimation of antibiotic activity by the microbiological methods is subject to error due to the inherent variability of the biological responses, thus the estimations are subject to considerable error unless based on a large number of observations; this was agreed upon with Chapi and Edberg (10). However, the obtained results by the different methods of analysis indicated a good correlation between different techniques as shown by Verdon *et al.* (11).

Drug contents of ampicillin suspensions stored at 5^0C decreased from an initial content of 96.5% to 62.0% on the 7th day, and the drug contents of ampicillin suspensions stored at 35^0C were reduced from an initial content of 92.8% to 42.2% on the 7th day. The loss in drug contents of ampicillin suspensions at 5^0C for the seven days of the study was about 34%, whereas the decrease in drug contents of ampicillin suspensions stored at 35^0C for the same period was about 51.0%. Also according to these results, it was evident that by the 7th day, the drug contents of ampicillin suspensions stored at 5^0C were 20%-24% higher than the equivalent ampicillin suspensions stored at 35^0C.

Examination of Figure (1) showed that in most parts of the stability curves there were some linearity between logarithms of drug concentrations and time, particularly in the last 3-4 days of the experiments, and this reflected that the degradation of ampicillin might to a considerable extent follow first order kinetics.

Fig 1: Comparison of drug contents of Ampicillin suspension, reconstituted and stored at different Temperatures, Using Microbiological cup-plate and chemical Spectrophotometry Assay Techniques.
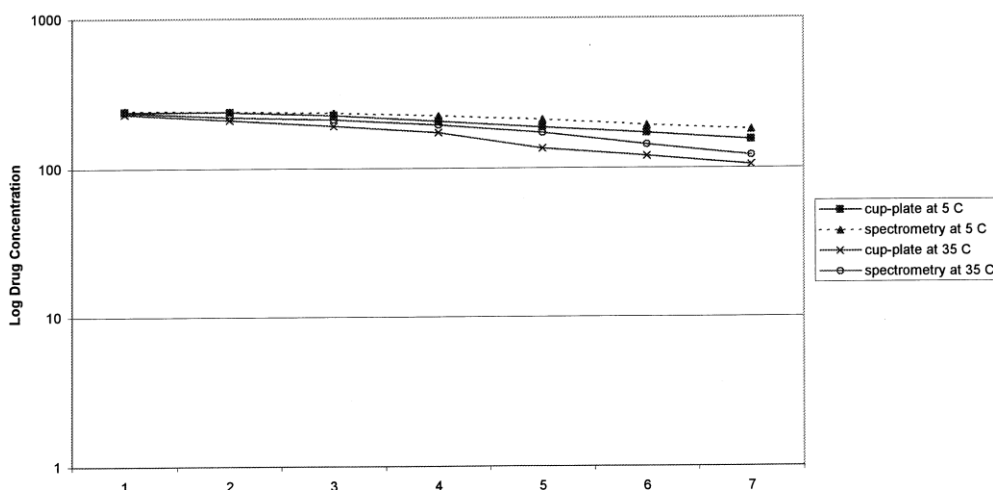
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The steepest parts of the curves were those of the time interval of the 4th/5th days, and the least steep parts of the curves were those of the first 2-3 days. Also, the stability curves indicated that the rates of degradation of ampicillin were minimum at 5^0 C in the first 2-3 days and maximum at 35^0 C in the last 4-5 days of the experiments.

The obtained results showed a remarkable decrease in the activity of oral ampicillin suspensions stored at room temperature when compared with the refrigerated ones.

The above results clearly confirmed the influence of the storage temperature on the stability and therefore on the activity of the reconstituted oral beta-lactam antibiotic suspensions. It is evident that even the refrigerated suspensions had their drug contents decreased to less than 90% by the fifth day of the study, and since antibiotic preparations are considered stable if they maintained at least 90% of their initial concentrations (5), other necessary measures should be taken to decrease the impact of storage temperature on the stability of such thermolabile drug formulae. The authors recommended the formulation of a unit dose sachet pack of such drugs, to be immediately reconstituted at the time of administration.

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