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The Influence Of Reconstitution Vehicles On The Stability Of Ampicillin Oral Powders

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ABSTRACT

The effect of different reconstitution vehicles on the stability of ampicillin oral powders was studied. Distilled water, tap water and well water were used for powder reconstitution. The suspensions were stored at 35^0C that representing the average room temperature in Sudan. The stability of the drug in suspension was tested daily over a period of seven days. The drug contents in the samples were determined using the cup- plate agar diffusion technique and chemical spectrophotometric method. The obtained results, represented the means of 10-20 determinations. The study revealed a higher degradation extent in the ampicillin oral powders reconstituted with water obtained from wells, followed by tap water when compared with the slower rate of degradation in the oral antibiotic that reconstituted with distilled water. In an attempt to decrease the destabilizing effect of the reconstitution vehicles on the daily used oral antibiotic powders, the authors suggested to use such formulae in a single-dose sachet form to be used instantly after reconstitution.

ملخص

تمت دراسة الأثر المثبط للسوائل المختلفة المستعملة في حل بدرة الأمبسلين الفموية فى فعاليته ، إستعمل الماء المقطر ماء الصنبور ومياه الآبار لحل البدرة . تم حفظ المعلق في درجة حرارة 35 درجة مئوية والتي تمثل متوسط درجة حرارة الغرفة في السودان .

إختبرت درجة ثبات العينات يومياً لفترة سبعة أيام، تم حساب كمية المادة الفعّالة في العينات المختلفة بإستعمال تقنية الإنتشار الآقارى وطريقة قياس الضؤ الطيفى الكيميائية .

النتائج المتحصلة تمثل متوسط 10 – 20 تجربة لكل عينة . أوضحت الدراسة التدرك العالي في محتوى العينات التي تم حلها بواسطة مياه الآبار ثم مياه الصنبور عند مقارنتها بالمعدل البطئ للتدرك فى المضاد الحيوى عندما تم حله بواسطة المياه المقطرة . محاولة لتقليل اثر هذه السوائل على ثبات فاعلية المضادات الحيوية يقترح الباحثان أن تكون وحدات الجرعات مفردة فى شكل عبوات صغيرة لكل جرعة يتم تناولها مباشرة بعد الحل.

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Introduction. According to manufacturer's instructions, oral antibiotic powders have to be reconstituted with distilled water before use. However, it has been observed that it is rather a routine practice even in the principal hospitals in Sudan that, tap water is the common vehicle used for reconstitution of oral antibiotic powders. In most instances the tap water is obtained treated or chemically untreated from the Nile River and in some parts of the Sudan is obtained from wells. Therefore it is felt necessary to investigate the effect of these different vehicles on the stability of the oral reconstituted beta lactam antibiotic powders. Ampicillin was selected out of different oral Beta-lactam powders due to it's apparent high prescribing rate in a combination with cloxacillin in

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treating different paediatric bacterial infections. It is evident that intensive investigations have been carried out on the effect of vehicles on the stability of beta-lactam antibiotics, (Hou and Poole, 1971), as well as the catalytic degradation caused by metal ions contained in different types of water used to reconstitute the oral antibiotic powders. (Segelman and Farnsworth,1970; Martin, 1977 and Martindale, 1977). The effect of metal ion decomposition of ampicillin was also studied by Beard *et al*. (1992).

James and Riley (1985) studied the effect of infusion vehicles on the stability of intravenously administered ampicillins. Hogerzeil *etal*. And Ballereau *etal* (1992) had studied the effect of the storage temperature in the tropics on the stability of ampicillin and recommended not to store such drug more than one year in such regions.

Materials And Methods. Different brands of ampicillin containing oral powders were randomly selected from drug stores, to determine the influence of the reconstitution vehicles on their activities. Three different vehicles were used, distilled water, the treated River Nile tap water and well water. Standard preparations of Ampicillin, used as reference for assay, were 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 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 small volume of normal saline to produce a suspension containing about 10^8- 10^9 colony forming units (CFU) per ml. The prepared stock suspension was stored in the refrigerator at 4^0 C till used.

Two- fold serial dilutions of the tested antibiotic were freshly prepared in sterile distilled water at the time of the experiments. The concentrations of these dilutions ranged between 1.25 and 100 ug per ml. The assay was based on the use of double layer agar system, a 15- ml of uninoculated base agar, and 10-ml seed layer inoculated with standardized suspension of S*taphylococcus aureus.* The used petri-dishes were approximately 20 x 100 mm. Since differences in the thickness of the agar medium was reported to produce irregular inhibition zones, the plates were always prepared on a level surface (Kavanagh 1972). The seed agar prepared by adding 2 ml of the standardized suspension of *Staphyllococcus aureus* to 100 ml of seed agar, melted and cooled to 48^0 C, to give a final count of about 10^8 cell/ml. The seed layers were usually added after the base layers had solidified, and then the plates were refrigerated until used.

For the cup-plate method, using a flamed and cooled 8-mm cork borer, 5 cups were cut out of each of the seeded agar plates, using of Pasteur Pipettes, 0.1 ml aliquots of each of the antibiotic solutions were added to appropriate cups at random; an average of 10-20 cups were used for each dilution. After filling the reservoirs with the appropriate dilution, the plates were allowed to stand at 35^0 C for 2 hours, and then incubated in the up-right position at 37^0 C for 16-18 hours. The diameters of the resultant inhibition zones were carefully measured, the readings were statistically analyzed, and the final results were tabulated.

The spectrophotometric method adopted was based on the method reported by Smith *et al.* (1967). Buffered copper sulphate solution used in the technique was prepared by solving 3.93 g of

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CuSO4. 5 H2 O in distilled water and diluted to one litre; to 15 ml of the copper sulphate was added citrate- phosphate buffer, pH 5.2, to one litre giving a final concentration of 15 ug Cu /ml. For the preparation of the citrate-phosphate buffer, pH 5.2, 464 ml of 0.1 M citric acid solution was mixed with 536 ml of disodium hydrogen phosphate solution (0.2 M solution), and the pH adjusted to 5.2 with citric acid or disodium hydrogen phosphate solutions.

One-ml aliquots of the different antibiotic concentrations were suitably diluted with 9-ml quantities of buffered copper sulphate solution. The resulting solutions contained in stoppered glass test tubes, were heated in a water bath at 75^0 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 mu, in 1- cm cell, with unheated buffer ampicillin solution in the reference cell. The calibration curves of the antibiotic were prepared using different concentrations.

Results. The initial drug contents of ampicillin oral powders reconstituted with different vehicles were 96.7%-98.2% by the microbiological method, and 98.5%-98.9% by the chemical method, with no significant differences between the two methods of analysis(Table)

On the first day of storage at 35^0 C, the drug contents of ampicillin suspension reconstituted with different vehicles were 92.8% - 96.7% by the microbiological method , and 96.6% - 97.8% by the chemical method. There were significant differences between the two methods.

On the second day of storage at 35^0 C, the drug contents of ampicillin oral powders reconstituted with distilled water, tap water and well water were respectively 87.8%, 82.7% and 77.8% by the microbiological method , and 90.7% , 86.7% and 83.7% by the chemical method , with significant differences ($p < 0.05$) in the drug contents of syrups reconstituted with different vehicles , and in the results of the two methods of analysis.

The drug contents of the suspensions on the third day at 35^0 C as determined by the microbiological method were 79.5 % , 70.6% and 62.9% , and by the chemical method were 85.5%, 77.5% and 72.8% for the oral powders reconstituted with distilled water , tap water and well water respectively . Significant differences ($p < 0.05$) were observed between the drug contents of the syrups reconstituted with the different vehicles , and the results of the two methods.

The drug contents of the suspensions on the 4th day of storage at 35^0 C , were as follows : for the oral powders reconstituted with distilled water , 70.5 % by the microbiological method and 79.4 % by the chemical method ; with tap water , 58.3 % by the microbiological method and 67.6 % by the chemical method ; and with well water , 49.8 % by the microbiological method and 58.2 % by the chemical method . The differences in drug contents of the oral powders reconstituted with different vehicles were significant ($p < 0.01$), and the results of the two analytical methods were significantly different ($P < 0.05$ - $P < 0.01$).

Ampicillin suspensions stored at 35^0 C, for 5 days had their drug contents as follows: 58.9%, 46.7 % and 36.7% as determined by the microbiological method, and 70.2 %, 55.4% and 47.7% by the chemical method , for the oral powders reconstituted with distilled water , tap water and well water, respectively. The drug contents of the syrups reconstituted with the different vehicles were

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significantly different ($P < 0.01$), and the differences between the results of the two methods of analysis were significant ($P < 0.01$).

On the 6th day of storage at 35^0 C, the drug contents of the suspensions were as follows: 50.6%, 39.8% and 29.4% by the microbiological method and 62.9%, 48.1% and 35.9% by the chemical method for the syrups reconstituted with distilled water , tap water and well water respectively .

On the 7th. day of storage at 35^0C, the drug contents of the syrups reconstituted with distilled water , tap water and well water were respectively : 44.6%, 28.7% and 20.2% by the microbiological method , and 57.4% , 36.9% and 14.8% by the chemical method, the differences between the drug contents of the syrups reconstituted with different vehicles in the 6th and 7th days of experiment were very significant ($P < 0.001$), and also significant differences ($P < 0.01$- $P < 0.001$) were observed in the results of the two analytical methods.

Discussion. The drug contents of ampicillin syrups reconstituted with distilled water decreased from an initial drug content of 98.2 % to 44.6 % on the 7th. Day ; those of the syrups reconstituted with tap water were 97.0 % and 28.7 % ; and those of the syrups reconstituted with well water were 96.7 % and 20.2 % .

Examination of the stability curves of ampicillin syrups clearly indicated that the plotting of the drug concentration versus time gave almost straight lines in most parts of the curves , indicating significant linearity and showing that the degradation of the drug followed zero order kinetics to a very significant extent (Fig.). The slopes of these curves were as follows : for ampicillin syrups reconstituted with distilled water tap and well water were respectively : 0.0225 , 0.0169 , and 0.0311 , 0.0250 , 0.0350 and 0.0302; the former results for the microbiological method and the latter results for the chemical method (Fig). The slopes of the linear **Table:** Comparison of drug contents of ampicillin oral powders, reconstituted with different vehicles and stored at 35^0C using micorbiological Cup-Plate and Chemical Spectrophotometry assay techniques.

Time Days	Distilled water Cup-plate spect Drug content (mg)±SE*	Tap- Water cup-plate spect Drug content (mg)±SE*	Well -Water Cup-plate spect Drug content mg)±SE*
0	245.6±3.2 247.3± 2.9 ---------NS--------	242.6±3.8 246.9±2.6 ---------NS----------	241.7±3.6 246.2±2.4 ----------NS**------
1	241.8±3.1 244.5±2.7 ----------NS----------	238.6±3.3 243.1±2.4 ----------NS----------	231.9±2.9 241.5±2.2 ----------NS----------
2	219.5±2.6 226.8±2.8 -------p<0.05---------	206.8±3.1 216.7±2.5 -------p<0.05---------	194.6±2.1 209.7±2.3 --------p<0.05---------
3	198.7±2.2 213.7±2.6 --------p<0.05---------	176.4±2.7 193.8±2.3 --------p<0.05---------	157.3±1.8 181.9±2.0 --------p<0.05---------
4	176.3±2.2 198.5±2.5	145.7±2.3 168.9±2.2	124.5±1.3 145.6±2.0

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	---------P<0.05--------	--------p<0.01---------	--------p<0.01---------
5	147.4±2.3 175.6±2.2 --------p<0.01---------	116.8±2.1 138.5±1.9 -------p<0.01---------	91.8±1.1 119.2±1.7 --------p<0.01---------
6	126.6±2.1 157.4±2.3 -------p<0.01---------	99.5±1.8 120.3±2.0 -------p<0.001---------	73.5±1.8 89.8±1.8 -------p<0.001---------
7	111.5±1.8 143.5±2.1 --------p<0.01---------	71.8±1.3 92.4±1.9 -------p<0.001---------	50.5±1.7 36.9±1.7 -------p<0.001---------

*Drug content of Ampicillin 250mg/5ml - syrups
(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.

portions of the stability curves of the drug in suspension formulations , represented the velocity constant of ampicillin degradation when it's respective syrups were reconstituted with distilled water , tap water and well water . The velocity constants of degradation of the drug were highest with well water , followed by tap water and the least with distilled water.

It is quite evident that the use of distilled water for the reconstitution of oral powders of beta-lactam antibiotics is of paramount importance in order to avoid unnecessary increased degradation of these drugs catalyzed by the metal ions present in reconstitution vehicles rather than distilled water.

The metal ions present in tap water, whether from the Nile or from the wells would be most likely responsible for the higher degradation extents of the beta-lactam antibiotics in the preparations reconstituted by tap water or well water compared to the relatively lower degradation rate detected in the antibiotic preparations reconstituted by distilled water . Metal ions such as zinc, copper etc. .... were reported to catalyze the degradation of beta-lactam antibiotics. (Segelman and Farnsworth 1970; Martindale , 1977) .

The above results clearly demonstrate the drastic effect of the different reconstitution vehicles on the stability and therefore the activity of ampicillin oral powders. The authors related the remarkable degrading effect of the vehicles on ampicillin to the high contents of minerals specially that obtained from wells, this was in agreement with similar studies (Segelman and Farnsworth, 1970). The results also show a relative agreement of the outcomes of the two methods of analysis.

Based on the obtained results, distilled water could be considered as the only suitable vehicle to be used in the reconstitution of Beta-lactam oral powders.

In order to decrease the destabilizing and the hydrolytic effect of the different reconstitution vehicles; the authors in an attempt to lessen the contact time between the antibiotic active moiety and the used vehicle are recommending the use of a sachet unit dose formula to be taken instantly after reconstitution.

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