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Effect of Temperature of Water Used for Reconstitution on Stability of Antibiotic Dry Suspension

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ABSTRACT

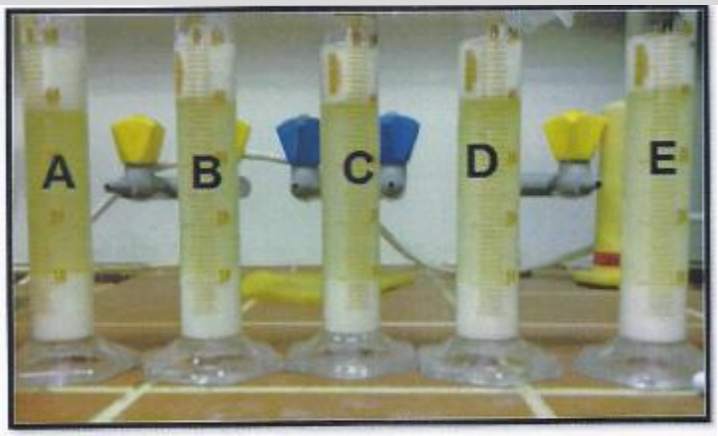
This study is carried out to test the stability of antibiotic dry suspensions reconstituted by various water temperatures. The testing included physical, microbial and chemical changes of samples reconstituted by water at 40, 60, 70 and 80°C. These changes were compared to control samples prepared by water at temperature 25°C. HPLC method was followed qualitatively to identify antibiotic active constituent in addition to quantitative analysis to evaluate antibiotic contents as compared to the control samples. The changes were assessed within one hour after reconstitution and after four days of reconstitution.

Physical tests showed changes of amoxicillin\clavulenic acid suspension's colourprepared at 80°C. Sedimentation ratio, sedimentation rate and sedimentation volume decreased as temperature increased. Besides, these parameters were tested only for amoxicillin suspension.Upon centrifugation, there was a decrease in sediment volume accompanied by an increase in supernatant volume resulting in changes in sediment/supernatant ratio.

Microbial study showed a marked decrease in antimicrobial activity for both amoxicillin and amoxicillin/clavulenic acid suspensions.

HPLC results showed a decrease in amoxicillin and clavulanate in samples prepared by heated water as compared to those prepared by cooled water at 25°C.

GRAPHICAL ABSTRACT



Introduction

Stability studies assess the changes in the physical, pharmacological and chemical properties of a drug which ultimately could lead to decrease in the effectiveness of these drugs. Antibiotics are of the most prescribed category of drugs. Antibiotics for pediatric use are commonly available as dry powders for reconstitution into oral suspensions. Once reconstituted, these oral suspensions should be refrigerated to preserve their potency and deliver optimal benefit to the patient. Possibility of degradation of these suspensions can arise either from temperature of water used for reconstitution or inappropriate storage conditions.[1, 2]

Essentially, successful treatment of infectious disease using antimicrobial therapy requires sufficient concentration of stable active drug at the site of action with respect to b-lactam antibiotics like amoxicillin. B-lactam ring is very sensitive and for these antibiotics, it must be intact to insure therapeutic effectiveness.[3-11]

Reconstitution of these antibiotics would base on boiling water firs to ensure sterility and as it cools down it is added to the antibiotic powder in a sufficient amount to be given to patients. However, some people would not cool this boiled water enough before adding.

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Thus, the aim of this study raised from the possible effect of temperature of water used for reconstitution is to cool enough to ensure the drug stability in case of amoxicillin and amoxicillin\clavulanate suspensions.

The tested parameters were physical, microbial and chemical parameters. Physical methods were used to assess any physical changes which in turn could lead to changes in suspension behaviours. After reconstitution with different temperature points, the antimicrobial activity is tested against susceptible strains to evaluate stability of antibiotic suspension. HPLC method is used qualitatively to evaluate antibiotic active constituents in comparison to a reference standard and quantitatively to measure any change in antibiotic content as compared to the control sample. The changes were assessed within one hour from reconstitution and after four days of reconstitution to identify any changes in antibiotic content through the shelf-life of the antibiotic suspension. Also, spectrophotometric method was used for the quantitative assay of antibiotic content.

Sample preparation

Samples were prepared by reconstituting the suspension with water at different temperatures (40, 60, 70, and 80 °C). These samples were analyzed in comparison to control samples reconstituted with water at 25 °C.

Physical stability testing

Physical stability of a suspension is normally tested by the detection of any colour changes and measurement of rate of sedimentation where final volume or height of the sediment is assessed. Finally, centrifugation test also was carried out.

Colour

Method

Immediately after suspension reconstitution at different temperatures, the samples were visually observed for colour changes.

Results

For amoxicillin, there was no colour change among the samples. However, for amoxicillin\clavulanate, color of the samples ranged from white to orange as temperature increased as illustrated in Fig. 1.

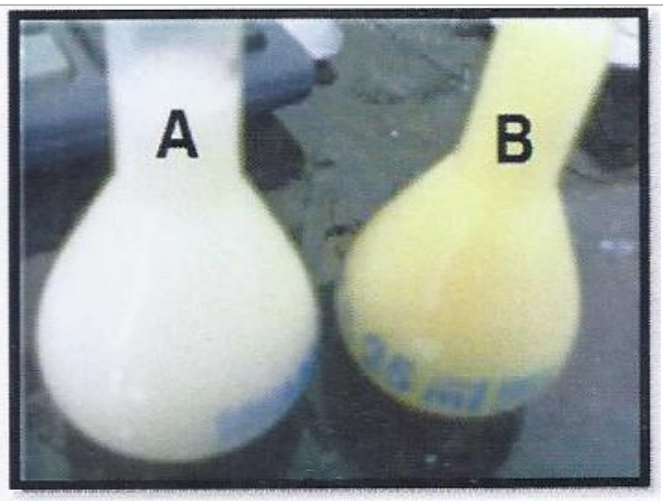

Fig. 1: Amoxicillin\clavulanate samples reconstituted with water at 25 °C (A) and 80 °C (B).

Discussion

Only amoxicillin\clavulanate samples' color was changed which suggests the different constituents are not stable at high temperatures. These changes may indicate chemical decomposition in either clavulanate or inerts or both.

Sedimentation rate, sedimentation ratio and sediment volume:

Method

Sedimentation properties were determined by taking a 50 mL of the reconstituted samples into a graduated cylinder and then keeping it undisturbed for four weeks. After each 7 days, sediment volume (V^o) was measured and the percentages of sediment were calculated as the ratio of sediment volume to the suspension volume, Fig. 2.

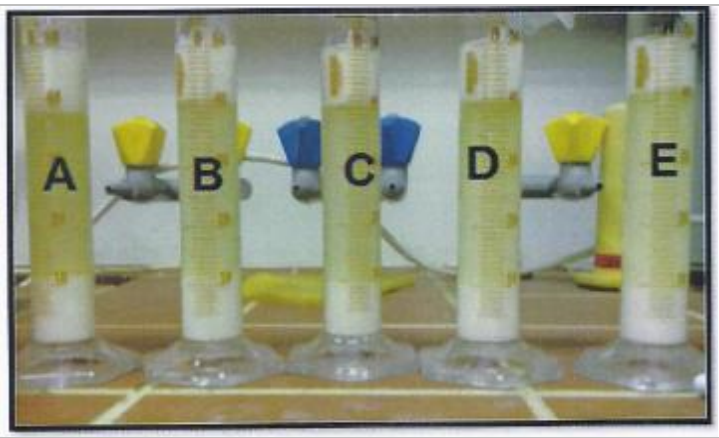

Fig. 2: sedimentation properties determination; A=25 °C, B=40 °C, C=60 °C, D=70 °C, E=80 °C.

Table 1. the sedimentation rate*

Water temperature	V^f	W0		W1		W2		W3		W4	
		V^0	%F	V^0	%F	V^0	%F	V^0	%F	V^0	%F
80	50	-	-	-	-	-	-	-	-	10	20
70	50	-	-	-	-	-	-	-	-	10	20
60	50	-	-	-	-	-	-	11	22	10	20
40	50	-	-	-	-	-	-	12	24	11.5	23
25	50	-	-	-	-	14.5	29	12	24	12	24

*W = weeks (the volume is measured weekly), V^f = final suspension volume, V^0 = sediment volume, %F = percentage ratio of sediment to suspension volume; %F = 100 V^0/V^f.

Discussion

Sedimentation occurred at second week for control samples and at third week for samples prepared at 40 and 60 °C. However, samples prepared at 70 and 80 °C showed formation of sediment at week 4. In other words, sedimentation rate decreased as temperature increases. Sediment volume also decreased as temperature increase. This could indicate an increase of solubility of the constituent of suspension when temperature rises. Or the suspending agents lose their effect with preparation at high temperature. This test was done only for amoxicillin suspension.

Centrifugation

This method was used to study any changes in sediment and supernatant volume and ratio when the suspension centrifuged.

This method was used only to assess the final volume of sediment and supernatant layer to check any change in sediment and supernatant ratio. These parameters are not used to accurately predict the behavior of suspension under normal storage conditions because centrifugation might act to destroy the structure of the flocculated system especially that the formed sediment would become tightly packed and difficult to re-disperse whether or not the initial suspension is fluctuated or deflocculated. [6 Ebtihal]

Method

10 mL of the suspension was placed into a test tube and, then, centrifuged at 3000 rpm for 10 minutes (Function Line Labufuge 400, Germany). Afterwards, sediment and supernatant volume were measured. Amoxicillin suspension and Amoxicillin\clavulanate suspension samples were tested.

Results

Results are shown in Fig. 3 and Table 2.

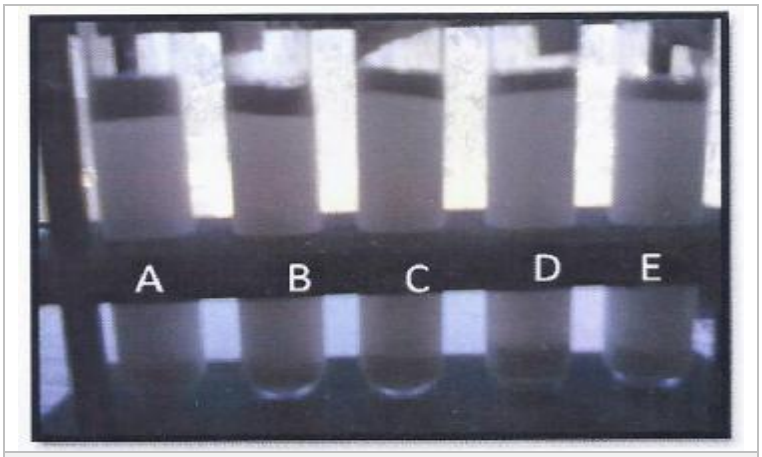

Fig. 3: Centrifuged samples samples of amoxicillin and their sediment and supernatant volume. A=25 °C, B=40 °C, C=60 °C, D=70 °C, E=80 °C.

Table 2. shows sediment and supernatant volume and their ratios for Amoxicillin*.

Water Temperature (°C)	Vs	V0	T	%Vs	%V0
80	2	5	7	28.6	71.4
70	2	5	7	28.6	71.4
60	2.5	4	6.5	38.5	61.5
40	3	4	7	42.9	57.1
25	4.5	2	6.5	69.2	30.8

*V_s = the volume of the supernatant layer, V_0 = the volume of sediment, T = the sum of Vs and V_0, $\%V_s$ = the percentage ratio of V_s to T, $\%V_0$ = the percentage ratio of V_0 to T.

Discussion

An increase in temperature causes a change in the ratio of sediment and supernatant. Since the decrease in sediment volume accompanied with increase in supernatant volume, the total volume of suspended constituents is the same. The reason of such changes could be related to a change in solubility of suspending materials. That was the case for amoxicillin suspension but for amoxicillin\clavulanate; sediment ratio was not affected by temperature.

Microbiology

In order to measure the biopotency, agar diffusion method was followed according to the British Pharmacopeia (BP 1995).

Method

For amoxicillin suspensions, the used bacterial strains are *Staph. aureus* (ATCC- 25922), and Eshirishia coli (ATCC – 29213). For amoxicillin\clavulanate supension *Pseudomonas aeruginosa* strain is used.

Procedure

Agar plates were cultured with the mentioned strains and a sterile cork borer was used to make four equal cups. Place one drop of melted agar into each cup and keep it to solidify. In each cup place equal volume (0.2 mL) of each antibiotic sample which contains an amount of antibiotic equivalent to (5.8 microgram of amoxicillin in case of Staph. aureus and 11.7 microgram for E. coli). For amoxicillin\clavulanate suspension an amount of 3.3 microgram of amoxicillin and 0.82 microgram of potassium clavulanate was used. Incubate the plate at 37 °C for 18 to 24 hours. After incubation measure the inhibition zone formed for each antibiotic.

Results

Zone of inhibition can be seen in Figures 4 and 5. Also values are represented in Table 3 and 4.

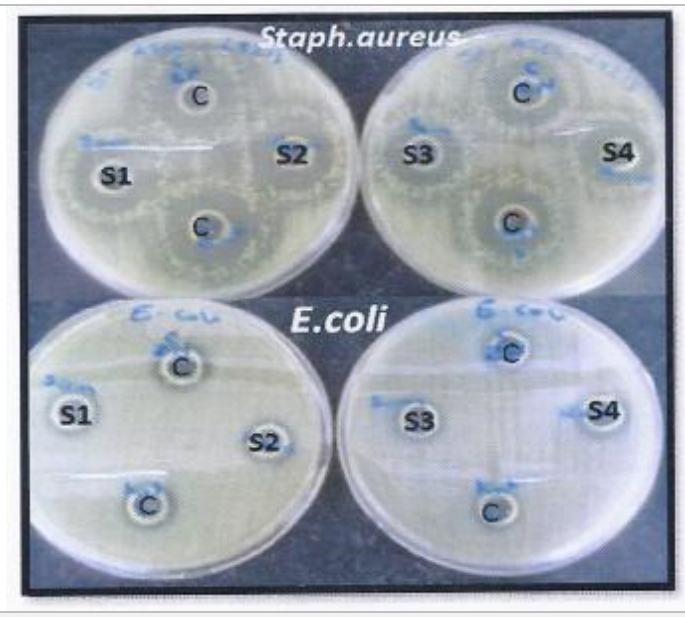

Fig. 4: shows the zone of inhibition of amoxicillin suspension where C is control, S1, S2, S3, S4 are the samples prepared at 80, 70, 60, 40 °C respectively.

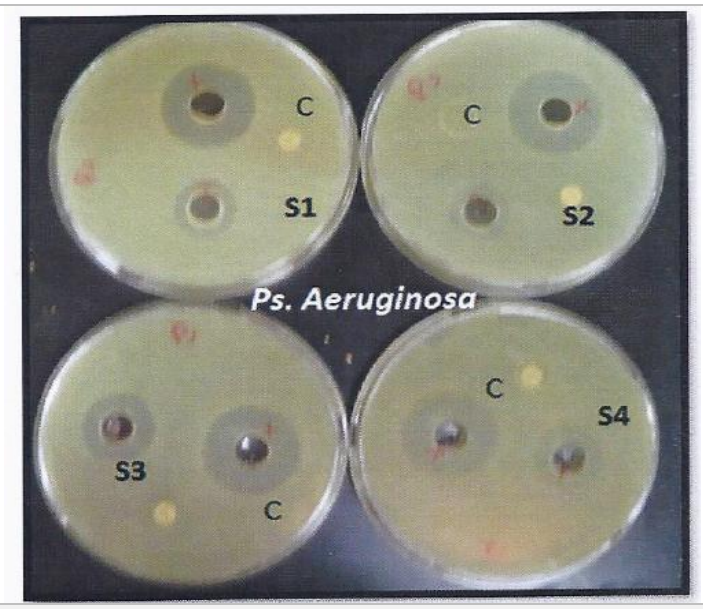

Fig. 5: zone of inhibition of amoxicillin\clavulanate where C is control, S1, S2, S3, S4 are the samples prepared at 80, 70, 60, 40 °C respectively.

Table 3. Zone of inhibition of amoxicillin *

Water Temperature (°C)	Zone of inhibition in mm					
	E. coli			Staph. aureus		
	S	C	%loss	S	C	%loss
80	23	25	8	18	19	5.3
70	23	25	8	18	19	5.3
60	20	21	4.8	22	23	4.3
40	21	21	0	22	22	0

Table 4: Zone of inhibition of Amoxicillin\clavulanate suspension*

Water Temperature (°C)	Zone of inhibition in mm		
	Ps. Aeruginosa		
	S	C	%loss
80	18	28	35.7
70	18	28	35.7
60	21	29	27.6
40	20	28	28.6

*S = sample, C = control sample at 25 °C, %loss of activity compared to control.

Discussion

There was a decrease in antibacterial activity for both amoxicillin and amoxicillin\clavulanate antibiotics where the decrease was more significant for amoxicillin\clavulanate, fig. 9 B. There is more than 35% decrease in its antibacterial activity against *Ps. Aeruginosa* even at lower temperature of 40 °C.

Chemical Content Determination

Antibiotic suspension contents were determined using either spectrophotometric or HPLC methods.

Spectrophotometric method

Method

Spectrophotometer (Jenway 6505 UV/Vis, UK) was used to measure amoxicillin content by adding accurate amount of amoxicillin trihydrate to prepare a solution containing 20µg/mL in a citro-phosphate buffer pH 7.2. Solution was sonicated and filtered. Absorbance was measured at 231nm against solvent blank.

Table 5. absorbance results for amoxicillin suspension

Water temperature (°C)	absorbance
80	0.470
25	0.546

Calculating concentration of the amoxicillin suspension sample reconstituted with water whose temperature is 80 °C. The concentration decreased from 20 µg/mL to 17 µg/mL which means 15% decrease in amoxicillin content when prepared with water at 80 °C.

High performance liquid chromatography (HPLC)

Method

Samples of amoxicillin and amoxicillin\clavulanate and their corresponding standards were prepared according to USP 2000. The analysis was run into HPLC instrument (Shemadzu coupled with SPD-20 AV detector, Japan) to compare their antibiotic content within 1hr of reconstitution and after 4days of reconstitution. The eluted samples used mobile phase of methanol : buffer pH 4.4, 5:95, at flow rate of 1 mL/min. The used column was Supel CosilC18 (250x4.6 mm). Oven temperature was 30 °C and at 220 nm. The resulted areas under the curve were taken and the percentage loss calculated for the samples was compared to that of the control sample (prepared at 25 °C).

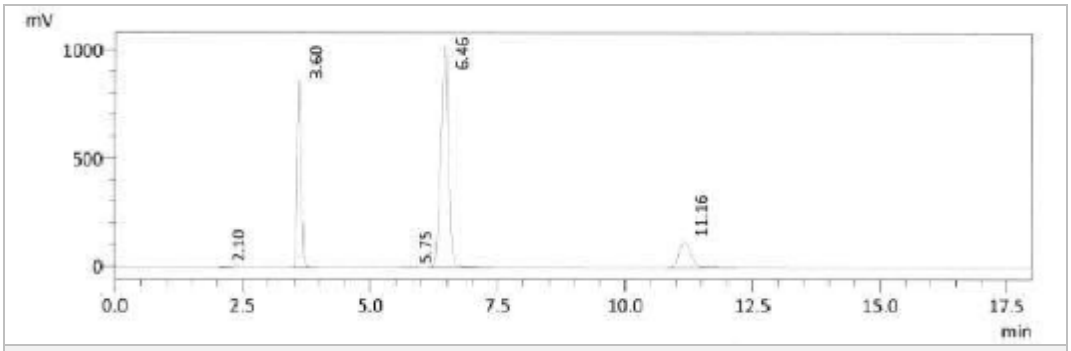

Fig. 6: retention times for control sample at 25 °C

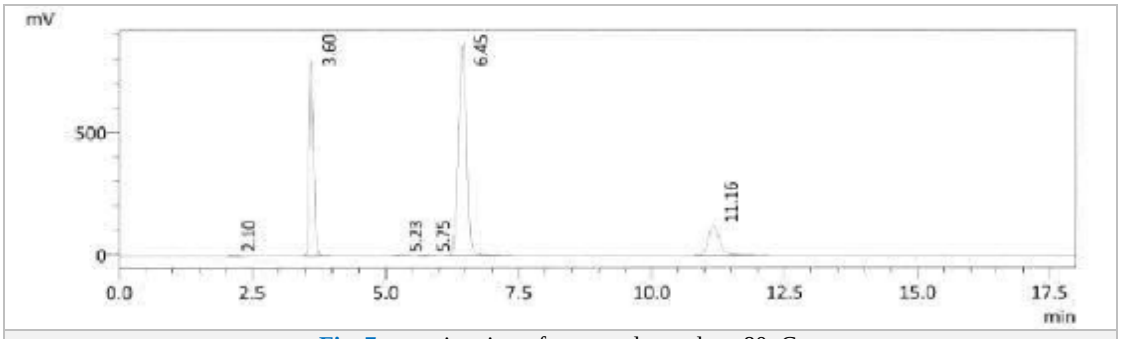

Fig. 7: retention times for control sample at 80 °C.

Table 6. retention times (RT) and AUCs for amoxicillin samples and %loss refer to control.

4Sample prepared with water at x °C	RT (min)	AUC within 1hr	%loss refer to 25 °C	AUC after 4 days	%loss refer to 25 °C
80	6.46	13476080	5.6	13313736	10.7
25	6.46	14899088	-	14674259	1.5

Table 7. retention times RT and AUCs for amoxicillin\clavulanate samples and %loss refere to control.

Sample prepared with water at x °C	RT (min)	AUC within 1hr	%loss refer to 25 °C	AUC after 4 days	%loss refer to 25 °C
Amoxicillin 80	6.46	9321074	15.6	9126649	17.4
Amoxicillin 25	6.46	11044675	-	10766368	2.5
Clavulanate 80	3.60	4580823	8.1	4250898	14.8
Clavulanate 25	3.60	4986124	-	4579176	8.2

Discussion

The analysis indicated a marked decrease in the content of both amoxicillin and clavulanate in the samples which were prepared with hot water and compared to control (samples prepared with water at 25 °C). After 4 days of reconstitution, there was about 17% loss in the content of amoxicillin as compared to 14% in clavulanate. However, there was not any decomposition compound on HPLC chromatogram which might not be detected at the same used wavelength

Conclusion

All the obtained results showed that reconstituting the suspension with heated water which is not cooled down enough to 25 °C will lead to changes in physical properties as sedimentation behavior will in turn lead to inaccuracy in dose measurement. Warm water can also affect effectiveness of the antibiotic. These changes would also be accompanied by chemical changes that might lead to adverse effects on patient's health. Eventually, all these changes in therapeutic effect of the antibiotic will develop antibacterial activity. Therefore, reconstitution conditions must be firm to ensure optimum therapeutic outcome from the use of the antibiotic suspension.

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