# Investigation the Factors Affecting on Gatifloxacin Eye Drop Stability

**Saba Abdulhadee Jabir**
E-mail:sabahadee77@yahoo.com
Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Iraq.
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**ABSTRACT**.
This research included the study of different factors that may effect on gatifloxacin stability (anew quinolone synthetic antibacterial agent) in its aqueous solution in order to develop and optimize the best delivary of the drug to the eye (as eye drop) with maximum local concentration and minimum systemic absorption and toxicity.Different formulas of gatifloxacin solution for ophthalmic use (0.3%)w/v were prepared in citrate, acetate,citrate/phosphate and phosphate buffers,their tonicity adjusted with suitable quantity of sodium chloride.The effect of different factors that might affectthe stability of gatifloxacin in its prepared ophthalmic solution was studied and determined spectrophotometrically at 287 nm. The results showed that The use of disodium edetate as asequestering agent gave more stable formula and gatifloxacin undergoes hydrolysis at low pH with optimum stability at pH 6.0, which is the most suitable pH for this ophthalmic solution. The type of buffer significantlyaffects on the rate of hydrolysis of gatifloxacinspecially at low pH and optimum stability was obtained by using phosphate buffer. The concentration of phosphate buffer had a significant effect on the hydrolysis of gatifloxacin and the rate of hydrolysis increased as the concentration buffer increased. Ionic strength affects the hydrolysis rate of gatifloxacin and the hydrolysis increased as the ionic strength increased. Light had a significant effect on the rate of hydrolysis of the drug and the drug losses 10% of its potency after 10 monthes of light exposure at room temperature. The prepared formula **J ( gatifloxacin 0.3% in 0.1M phosphate buffer with sodium chloride 0.26% , xanthan gum 0.2% and disodium edetate 0.01%)** is thebest stable one and had no irritation on the eye of experimental animals, and it passes successfully quality control tests including: drug content, pH, clarity and sterility test and comply with united state pharmacopoeia for ophthalmic solutions.

## البحث في العوامل المؤثرة على ثبات قطرة العين كاتيفلوكساسين

**صبا عبد الهادي جابر**
فرع الصيدلانيات ، كلية الصيدلة، جامعة بغداد ، العراق
**الكلمات المفتاحية** : كتيفلوكساسين، المحلول العيني، محلول الفوسفيت الدارىء، الاس الهيدروجيني 6

**الخلاصة**
ان هذه الدراسة تهدف الى دراسة تاثير عوامل مختلفة على دواء الكاتيفلوكساسين في محلوله المائي لايجاد افضل صيغة تركيبية للدواء لاستعماله كقطرة للعين .لقد تم تحضير العديد من الصيغ التركيبية لدواء الكاتيفلوكساسين بتركيز 0.3% في محلول مائي باستعمال انواع متعددة من المحاليل الدارئه (Buffer solution) ذات الاس الهيدروجيني المختلف اضافة الى كونها تختلف في تراكيزها.وقد تم دراسة هذه التاثيرات على استقرارية الدواء في محلوله المائي باستعمال جهاز الاشعه فوق البنفسجية وعلى الموجة الضوئية **287 نانوميتر**. وقد اشارت النتائج الى ان استعمال مادة داي صوديوم اي دي تي اي كمادة حاجزة اعطى صيغة تركيبية ثابته لهدا المحلول ،كما اظهرت النتائج ان الدواء(كاتيفلوكساسين)يتحلل في المحيط الحامضي والقاعدي مع اقصى استقرارية عند الاس الهيدروحيني **6** والدي يعتبر الاكثر مناسبا لتحضير هده القطرة. كما وجد ان نوع المحاليل الدارئة يؤثر على سرعة تحلل الدواء واقصى استقرارية تم الحصول عليها باستعمال الفوسفيت كمحلول دارئ. اظهرت النتائج ان تركيز الفوسفيت كمحلول دارئ يؤثر بشكل كبير على تحلل كتيفلوكساسين وان ثابت سرعة التحلل يزداد بزيادة تركيز محلول الفوسفيت الدارئ. اظهرت النتائج ان القوة الايونية تؤثر على ثابت سرعة التحلل عند استخدام الفوسفيت كمحلول دارئ وان هدا التاثير يزداد بزيادة القوة

الايونية. اوضحت النتائج ان الضوء يؤدي الى زيادة ثابت سرعة التحلل للكتيفلوكساسين وان هدا الدواء يفقد 10% من فعاليته بعد عشرة اشهر من تعرضه للضوء بدرجة حرارة الغرفة. ان افضل صيغة تركيبية مستقرة وغير مخرشة لعيون الحيوانات المختبرية هي (J ) حيث انها اجتازت بنجاح كافة اختبارات السيطرة النوعية المتضمنة المحتوى الدوائي ، الاس الهيدروجيني ، الوضوح والنقاوة والتعقيم ومطابقة للدستور الدوائي الامريكي لمحاليل العيون .

## 1. INTRODUCTION

Medicinal ophthalmic preparations are products applied to the eye for the localized effect of the medication on the surface of the eye or to act interiory within the eye ball either for treatment of disease, relief of symptoms, diagnostic purposes or as adjuncts to surgical procedures.

Pharmaceutical preparation areapplied topically to the eye to treat surface or intraocular conditions including: bacterial, fungal and viral infections of the eye; allergic or infectious conjunctivitis, inflammation; elevated intraocular pressure; and dry eye due to in adequate production of fluids bathing the eye [1].

Ophthalmic preparation may be categorized into number of groups, which involve liquid preparation for application to the surface of the eye (eye drop and eye lotions), semisolid dosage forms for application to the margin of the eye lid or for introduction to the conjuctival sac (ointments, creams and gels), solid dosage form intended to be placed in contact with the surface of the eye to produce modified release of medication, devices for surgical implantation with in the eye to give modified release of medicament over aprolonged period, paranteral products for subconjunctival or intraocular injection and liquid products for irrigation of the eye during surgical procedures[2].

Ophthalimic drug delivary is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The challenge to the formulator is to circumvent the protective barriers of the eye with outcausing permanent tissue damage. The most common method of ocular drug delivary is to instillation of drops in to the lower cul-de-sac of the eye.

One of the problems associated with the use of ophthalmic solutions is the rapid loss of administered drug due to the blinking of the eye and the flushing effect of lacrimal fluids [3].

Several adjuvants were added to aqueous drop formulations to enhance ocular bioavailability of drug[1], [4].

The model drug in this study is gatifloxacin, which is an antibiotic of fourth generation fluoroquinolone family, like other members of this family, inhibits the bacterial enzymes DNA gyrase and topoisomerase IV[5].

Gatifloxacin works by stopping the production of proteins that bacteria need to survive. So this drug is used for treating infections caused by certian bacteria, like conjunctivitis.

The purpose of this work is to study the effect of different factors on gatifloxacin in solution including: type of buffer, buffer pH, buffer concentration, ionic strength, addition of chelating agent, addition of thicking agent and effect of light in order to develop and optimize best delivary of the drug (as eye drop) with maximum local concentration and minimum systemic absorption and toxicity and apply quality control study on the best selected eye drop formula including:(drug content, clarity, pH and sterility test) and check its safety together with shelf life determination.

## 2. MATERIALS AND METHODS

### Materials

Gatifloxacinpowder(kanawatimedicalproduct,Syria),potassiumdihydrogenphosphate(FlukaAG.switzerland),disodium hydrogen phosphate (Gainland chemical company.U.K.),hydrochloic acid ,methyl cellulose ,sodium hydroxid ,acetic acid and sodium acetate (BDH limited pool, England),citric acid and sodium citrate both belong to (Seelze-Hannover), sodium chloride (Fluka - Garantine, AG) , benzalkoniumchloride,hydroxy propyl

methyl cellulose,xanthan gum and disodium edetate all supplied by samara drug industry (SDI)Iraq.
All other chemical are of analytical grad.

## Methods

### Formulation of gatifloxacin eye drop

Different formulas of gatifloxacin solution for ophthalmic use (0.3%) w/v were prepared in citrate, acetate, citrate/phosphate and phosphate buffers as shown in table 1 and 2. Their tonicity adjusted with suitable quantity of sodium chloride using the osmometer apparatus and then the solution filtered using 0.22 µm membrane filters and filled in sterile containers.

**Table 1 : Formulation of gatifloxacin as eye drop in different buffer at different pH.**

| Formulas | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Citrate buffer | Ci | Ci | Ci | Ci | Ci | | | | | | | | | | | | | |
| Citrae phosphate buffer | - | - | - | - | - | Ci/pho. | Ci/pho. | Ci/pho. | Ci/pho. | Ci/pho. | - | - | - | - | - | - | - | - |
| Acetate buffer | - | - | - | - | - | - | - | - | - | - | Acet. | Acet. | Acet. | Acet. | - | - | - | - |
| Phosphate buffer | - | - | - | - | - | - | - | - | - | - | - | - | - | - | pho | pho | pho | - |
| Distilled water(D.W) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | D.W |
| pH | 4.0 | 4.6 | 5.0 | 5.6 | 6.0 | 4.0 | 4.6 | 5.0 | 5.6 | 6.0 | 4.0 | 4.6 | 5.0 | 5.6 | 5.0 | 5.6 | 6.0 | 6.0 |
| Gatifloxacin (W/V)% | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Disodium edetate (W/V)% | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Benzalkonium chloride (W/V)% | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Sodium chloride (W/V)% | 0.22 | 0.2 | 0.18 | 0.21 | 0.51 | 0.12 | 0.02 | 0.003 | 0.002 | 0.001 | 0.49 | 0.41 | 0.36 | 0.31 | 0.26 | 0.28 | 0.26 | 0.86 |
| Final volume | 100 ml | | | | | | | | | | | | | | | | | |

**Table 2: Formulation of gatifloxacin as eye drop in phosphate buffer at pH 6.0.**

| Formulas | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phosphate buffer at pH=6.0 | Pho. | Pho. | Pho. | Pho. | Pho. | Pho. | Pho. | Pho. | Pho. | Pho. | Pho. |
| Buffer concentration(Molar) | 0.1 | 0.2 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| pH | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Ionic strength | 0.25 | 0.25 | 0.25 | 0.5 | 1.0 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gatifloxacin (W/V)%** | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| **Sodium chloride (W/V)%** | 0.26 | 0.002 | 0.001 | 1.8 | 4.68 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.28 |
| **Disodium edetate(W/V)%** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | - |
| **Benzalkonium chloride(W/V)%** | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| **Methyl cellulose (M.C) (W/V)%** | - | - | - | - | - | 0.3 | 0.5 | - | - | - | - |
| **Hydroxy propyl methyl cellulose (HPMC) (W/V)%** | - | - | - | - | - | - | - | 1 | - | - | - |
| **Xanthan gum (W/V)%** | - | - | - | - | - | - | - | - | 0.2 | 0.2 | 0.2 |

**Factors affect the stability of gatifloxacin**

In order to study the effect of different factors that might affect the stability of gatifloxacin in it's prepared ophthalmic solution, 0.05 ml of each prepared formula was with drawn at different time intervals and diluted to 20 ml with citrate, acetate, citrate/phosphate and phosphate buffer.

The decline in the concentration of gatifloxacin was followed spectrophotometrically at 287 nm and the observed first order rate constant (k) was obtained by linear regression analysis through utilizing the following equation [6]:-

$\text{Log } C_t = \text{Log } C_o - kt/2.303$

Where $C_t$= Concentration remaining at time (t)

$C_o$= Intial concentration.

**The effect of different factors includes:**

- **Effect of buffer type and water**

  Three types of buffers were used to study the effect of buffer type and water on the rate of hydrolysis of gatifloxacin, these are citrate, acetate, citrate/phosphate and phosphate buffers. The concentration of buffered solutions was 0.1M at pH 6.0 (citrate, citrate/phosphate, phosphate and water) and pH 5.6 (citrate, acetate, citrate/phosphate and phosphate).

  The solutions were stored at $60^0$C for 120 days and assayed for their content at suitable time intervals[7].

- **Effect of pH**

  The effect of pH on the rate of hydrolysis of gatifloxacinin formula 1,2,3,4 and 5 was studied at pH 4.0, 4.6, 5.0, 5.6 and 6.0 using 0.1M citrate buffer.

  Formula 6,7,8,9 and 10 was studied at pH 4.0, 4.6, 5.0, 5.6 and 6.0 using 0.1M citrate/phosphate buffer.

  Formula 11, 12, 13 and 14 was studied at pH 4.0, 4.6, 5.0 and 5.6 using 0.1M acetate buffer.

  Formula 15, 16, 17 was studied at pH 5.0,5.6 and 6.0 using 0.1M phosphate buffer.

  The solutions were kept at $60^0$C for 120 days and assayed for their content at suitable time intervals.

- **Effect of buffer concentration**

The effect of buffer concentration on the stability of gatifloxacin was studied at pH 6.0, using phosphate buffer.

The buffer concentration was 0.1, 0.2 and 0.3M, the solution were stored at $60^0$C for 120 days and assayed for their content at suitable time intervals[8].

- **Effect of disodium edetate (chelating agent)**

  The effect of disodium edelate (0.1% w/v) as a chelating agent on the stability of gatifloxacin solution was studied using formula (J) in comparison with formula (k) in phosphate buffer at pH 6.0 and the ionic strength was odjusted at 0.25 using sodium chloride.

  The sample kept at $60^0$C for 120 days and assayed for their content at suitable time intervals [8].

- **Effect of ionic strength**

  The effect of ionic strength on the stability of gatifloxacin solution (formula A, D and E) was studied at pH 6.0, using 0.1M phosphate buffer.

  The ionic strength was adjusted to 0.25, 0.5, 1.0 by addition of sodium chloride as shown in table 2.

  The solutions were stored at $60^0$C for 120 days and assayed for their content at suitable time intervals.

- **Effect of thickening agent**

  Methyl cellulose (0.3 and 0.5% w/v)[9], hydroxy propyl methyl cellulose (1% w/v)[9], and xanthan gum (0.2% w/v) [10] were added as athickening agent to formula F,G,H,I and J in phosphate buffer to study its effect on the stability of gatifloxacin solution.

  The solutions were prepared by adding part of hot buffer solution (pH 6.0, ionic strength 0.25) to methyl cellulose, hydroxypropyl methyl cellulose and xanthan gum, disperse the mixture then cool.

  The content of the formulas were dissolved in the second part of the buffer solutions, and then added to the mixture of methylcellulose, hydroxy propylmethylcellulose and xanthan gum solutions withcontinous mixing.

  The pH of the final formula were adjusted to pH 6.0, then the volumes were adjusted, their viscosity were measured using viscometer at $25\pm0.1^0$C.

- **Effect of light**

  The effect of light was studied for formula (J) in phosphate buffer by placing it in a clear and ambered container then put close to the room window for sun light for 10 month.Samples were taken every month to be assayed for their drug content.

**Preservative efficacy (challenge test)**

This test is applied to the formulated medicin in its final container to determine whether it is adequantely protected against microbial spoilage.

The test was done for formula J according to U.S.P XXIII procedure.

**Irritation test**

The test was done by taking three adult albino rabbit weighing 2-2.5kg.

Animals were individually hosed in standard laboratory cages, at first each rabbit was examined specially the eye which was examined microscopically under a source of light to check the general condition of the eye at zero time.

Right eye of each rabbit was participated in the study. While the left eye serves as a control.

Formula J in phosphate buffer was tested and applied as follows: the lower lid was gently pulled away from the eye globe to form a pocket and 0.1 ml was instilled.

The procedure was repeated three times a day for three days. At various post instillation time, rabbits eye was examined under light for any change or reaction in the eye tissue that may result from the instillation of the test solution[7],[11] and these changes include:

- Redness of the eye that may result from irritation.
- Lacrimation that may result from irritation or foreign bodies.
- Purulent discharge, which may result from infection.

**Quality control**

Quality control of the eye drop includes checking of the following points:

- **Drug content**

Concentration of the active ingredients of the final formula was checked by using HPLC method for the assay of gatifloxacin[12].

- **pH:** The final product pH was measured by using pH-meter which is previously caliberated using stander buffer pH 4.01 and pH7.
- **Clarity test**: This test was done by shining focused beam of light through the solution, where undissolved particles scatter the light and the solution appears hazy.
- **Sterility test**: This test was carried out by using the direct introculation method. The following culture media have been found to be suitable for sterility test: Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria.The second one was soya-beancasein digest medium is suitable for the culture of both fungi and aerobic bacteria. Gatifloxacinwas directly inoculated in the above culture media. The preparation was diluted ten fold in these media and inoculation process has been done in asterile laminar flow cabinet[12]. Media intended for the growth of bacteria was incubated at 30-35$^0$C, then samples was incubated for 14 days and examined for their sterility at different time intervals during incubation period.

**Statistical analysis**

Student's t-test was used to examine the difference in the means of the results of parameters tested.A (P-value) of less than 0.5 was considered significant.

## 3. RESULTS and DISCUSSION

This study indicated that the minimum rate of hydrolysis of gatifloxacin was achieved using 0.1M phosphate buffer, since it has lower rate constant ($0.075x10^{-3}day^{-1}$) in comparsion to that in citrate/phosphate buffer ($0.15x10^{-3}$ $day^{-1}$) and citrate buffer ($0.166x10^{-3}$ $day^{-1}$) and water ($0.1x10^{-3}$ $day^{-1}$) as shown in table 3.

**Table 3: Effect of buffer type and water on the degredation rate of gatifloxcin solution at pH 6.0 and 60°c.**

| Type of buffer | K×10$^{-3}$ day$^{-}$ |
|---|---|
| **Citrate** | 0.166 |
| **Citrate /Phosphate** | 0.15 |
| **Phosphate** | 0.075 |
| **D.W** | 0.1 |

This may suggest that phosphate buffer is a suitable buffer to be used to prepare gatifloxacin solution, hence neither citrate nor citrate/phosphate buffer can be used since a significant difference is obtained and this is agreed with the previous reported data [10].

Table 4, figure 1,2,3 and 4 show the effect of pH on the rate of hydrolysis of gatifloxacin in formula (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16 and 17) at pH 4,4.6,5.0,5.6,6.0 respectively in 0.1M citrate buffer and pH 4.0, 4.6, 5.0, 5.6 in 0.1M acetate buffer and pH 5.0, 5.6 and 6.0 in 0.1M citrate/phosphate buffer keeping the ionic strength 0.25 and the temperature 60$^0$C.

The results from the data indicated that the optimum pH for gatifloxacin was pH 6.0 where minimum rate of hydrolysis was obtained with rate constant of $0.166x10^{-3}$ day$^{-}$ for 0.1M citrate buffer,$0.15x10^{-3}$ day$^{-}$ for 0.1M citrate/phosphate buffer and $0.075x10^{-3}$ day$^{-}$ for 0.1M phosphate buffer.

The data indicates that any change in pH lead to slight change in the rate constant of hydrolysis of gatifloxacin and the hydrolysis can be described in terms of acid catalyzed reaction[13].

The stability of the drug in phosphate buffer was higher than that in citrate, acetate, citrate/phosphate buffers for all the studied pH.

**Table 4: Effect of pH on the degredation rate of gatifloxacin solution in formulas from 1 to 17 at pH rang( 4.0 -6.0) and ionic strength 0.25 at 60°c.**

| pH | K×10$^{-3}$ day$^{-}$ | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Citrate buffer | | | | | Citrate /Phosphate buffer | | | | | Acetate buffer | | | | Phosphate buffer | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| **4.0** | 6.1 | | | | | 1.66 | | | | | 1.8 | | | | | | |
| **4.6** | | 5.7 | | | | | 0.66 | | | | | 0.33 | | | | | |
| **5.0** | | | 0.3 | | | | | 0.3 | | | | | 0.166 | | 0.167 | | |
| **5.6** | | | | 2.3 | | | | | 0.16 | | | | | 0.133 | | 0.16 | |
| **6.0** | | | | | 0.166 | | | | | 0.15 | | | | | | | 0.075 |

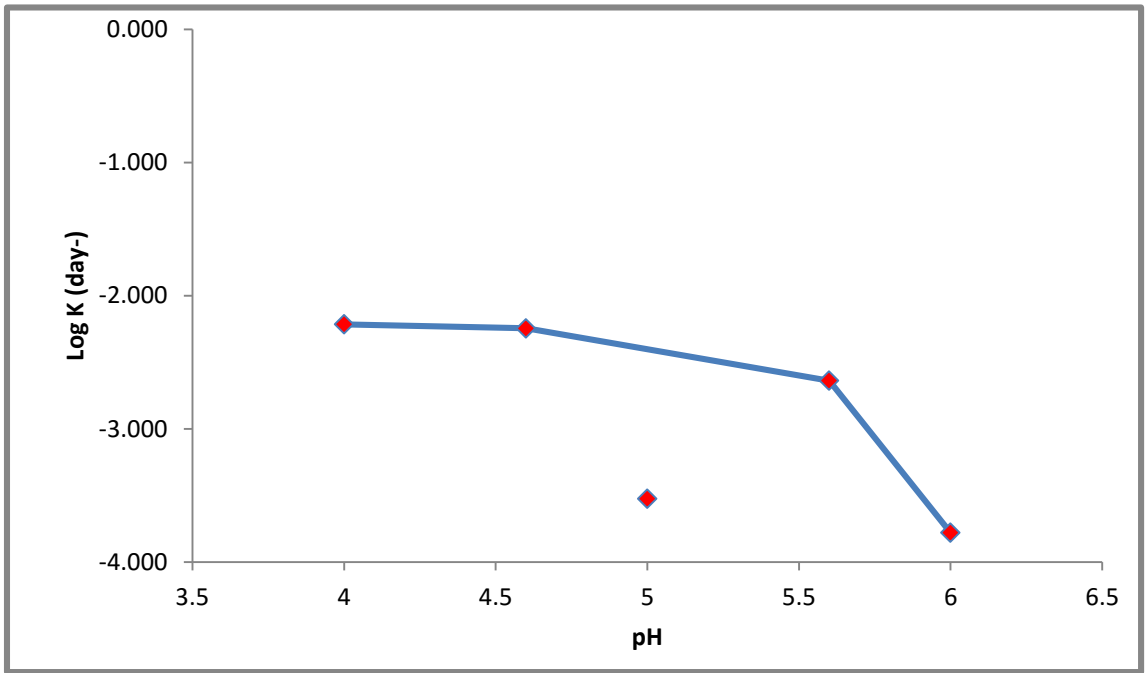


**Figure 1: pH – degredation rate of gatifloxacin solution in citrate buffer for formula 1,2,3,4 and 5 at pH rang (4.0-6.0) and 0.25 ionic strength at 60$^{0}$C.**

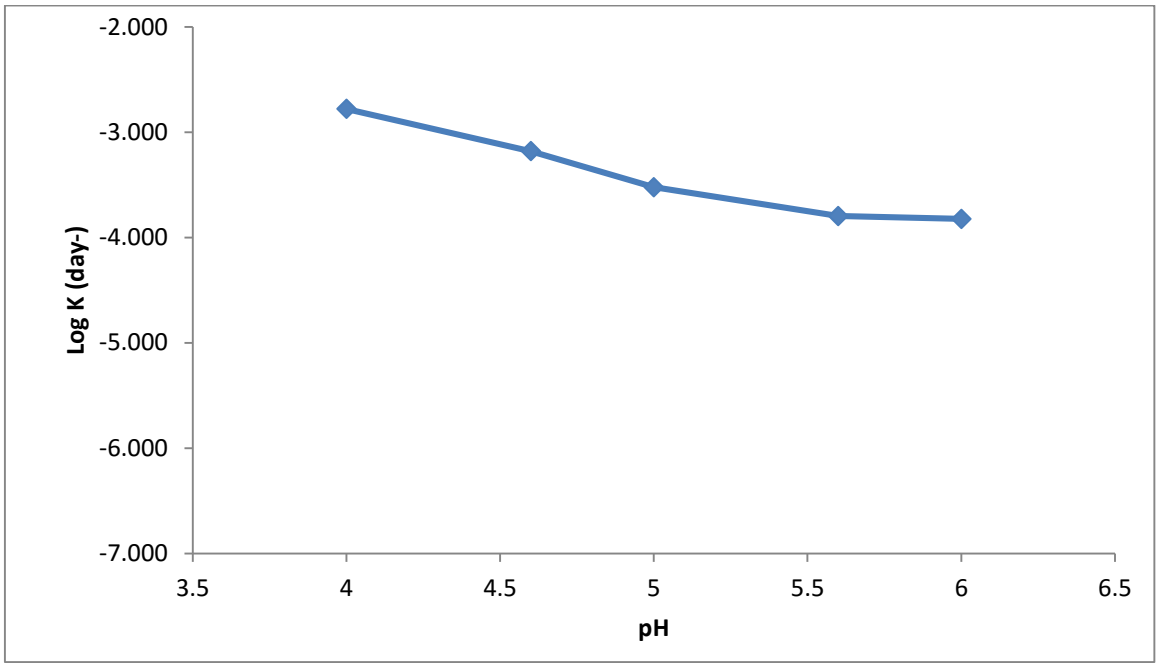


**Figure 2: pH-degredation rate of gatifloxacin solution in citrate/phosphate buffer for formula 6,7,8,9 and 10, at pH rang (4.0-6.0) and 0.25 ionic strength at $60^0$C.**

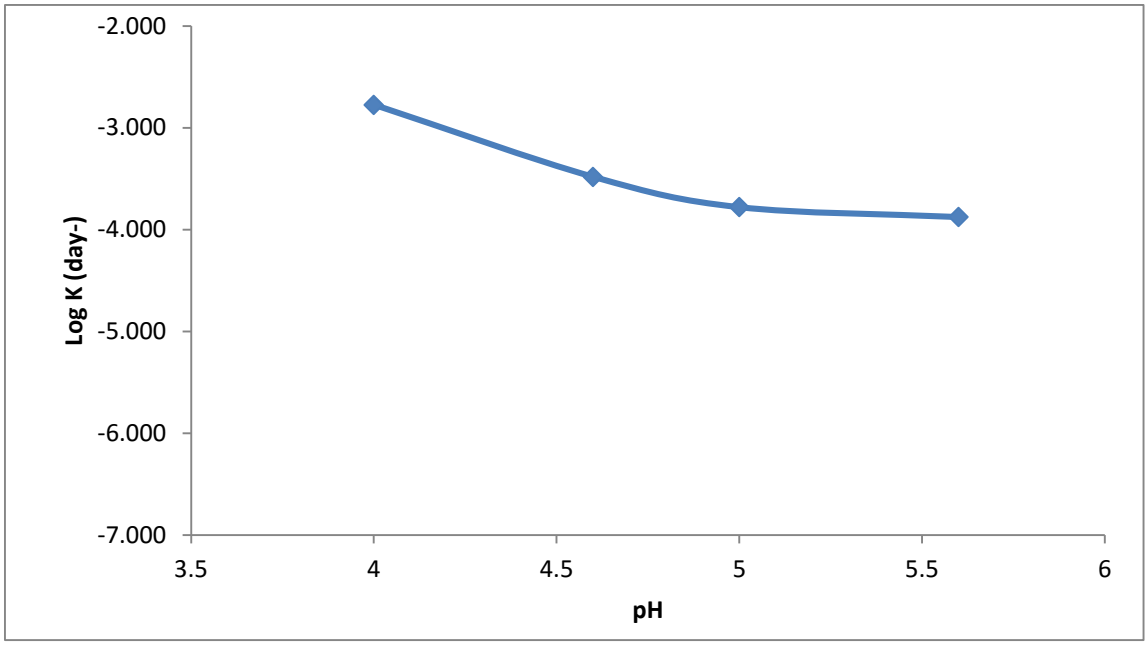


**Figure 3: pH-degredation rateof gatifloxacin solution in acetate buffer for formula 11,12,13 and 14 at pH rang (4.0-5.6) and 0.25 ionic strength at $60^0$C.**

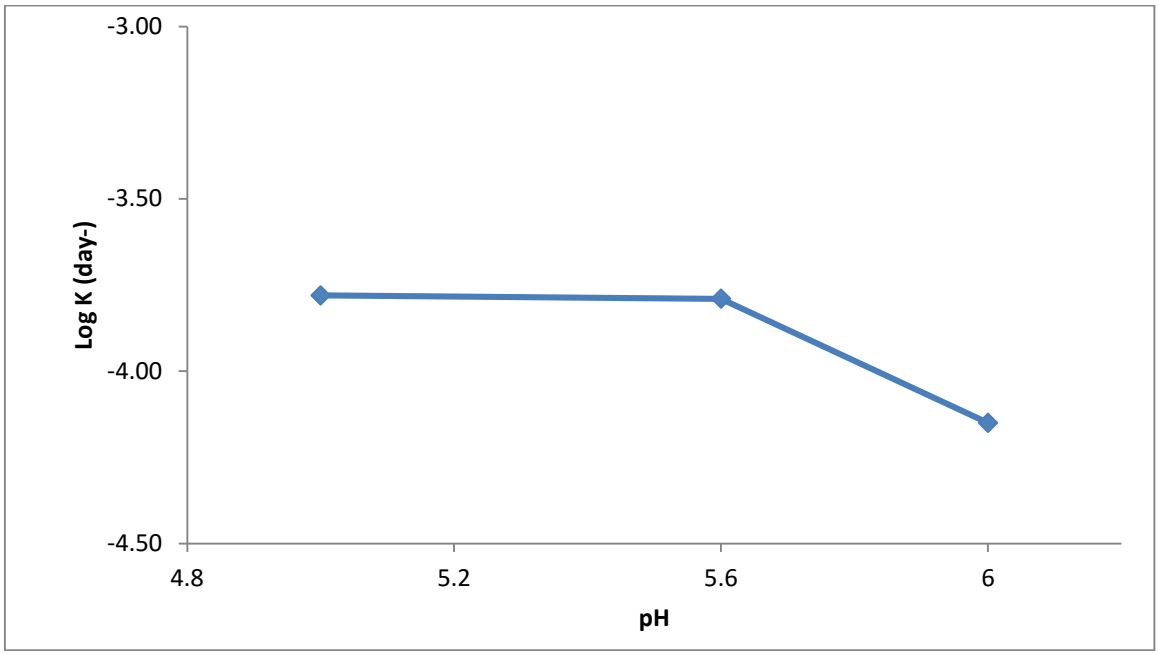


**Figure 4: pH-degredation rateof gatifloxacin solution in phosphate buffer for formula 15,16 and 17 at pH rang (5.0-6.0) and 0.25 ionic strength at $60^0$C.**

The effect of buffer concentration on the rate of hydrolysis of gatifloxacin for formula (A,B and C) in phosphate buffer is shown in figure 5, at pH 6.0, $60^0$C and ionic strength 0.25, the result indicates that the rate of hydrolysis of gatifloxacin increases as the concentration of buffer increases and the optimum stability was obtained at 0.1M phosphate buffer where the rate constantof degredationis equal to $0.075x10^{-3}$ $day^{-1}$and this is agreed with Naaijkens and van [14].

The effect of disodium edetate (0.1%w/v) on the stability of gatifloxacin solution using formula J and K in phosphate buffer is shown in figure 6.

The result indicates that the rate of hydrolysis of gatifloxacin in the presence of disodium edetate formula J decreases in comparison to that of formula K where no edetate added and this agreed with reported data [15].

Effect of ionic strength on the rate of hydrolysis of gatifloxacin in formula (A,D and E) was studied at pH 6.0 using 0.1M phosphate buffer as shown in table 5. The result indicated that as the ionic strength increased the rate of hydrolysis is increased.

Therefore the lowest value of ionic strength (0.25) was chosen to be used in all the prepared formulas.

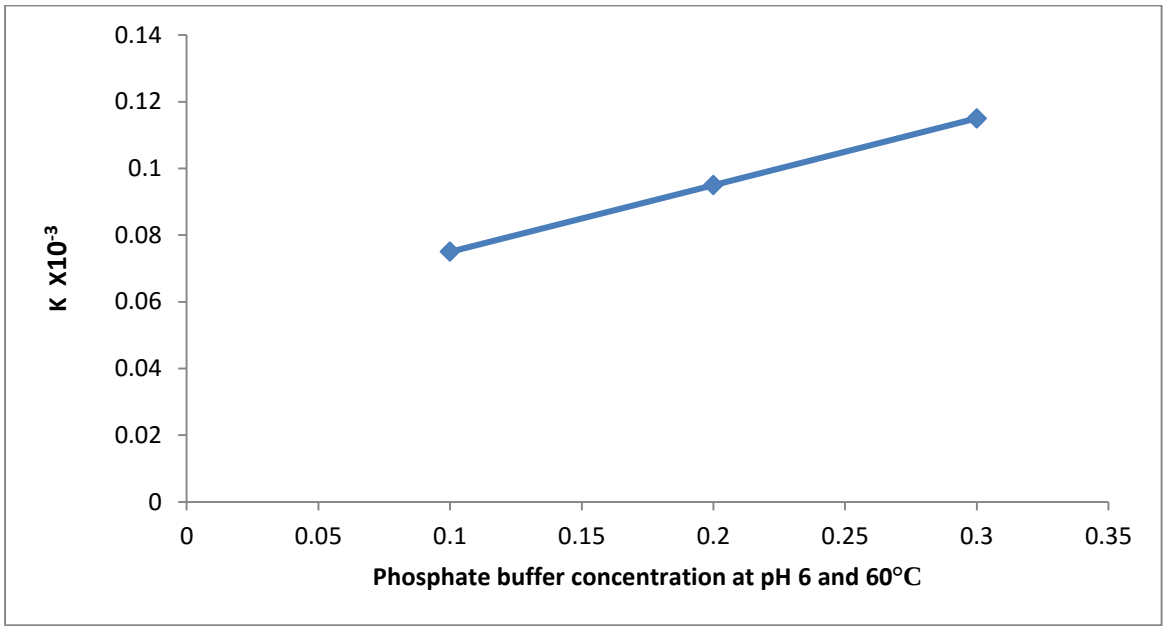


**Figure 5: Effect of buffer concentration on the rate of hydrolysis of gatifloxacin solution in formula (A,B and C) in phosphate buffer at pH 6.0 and $60^0$C.**

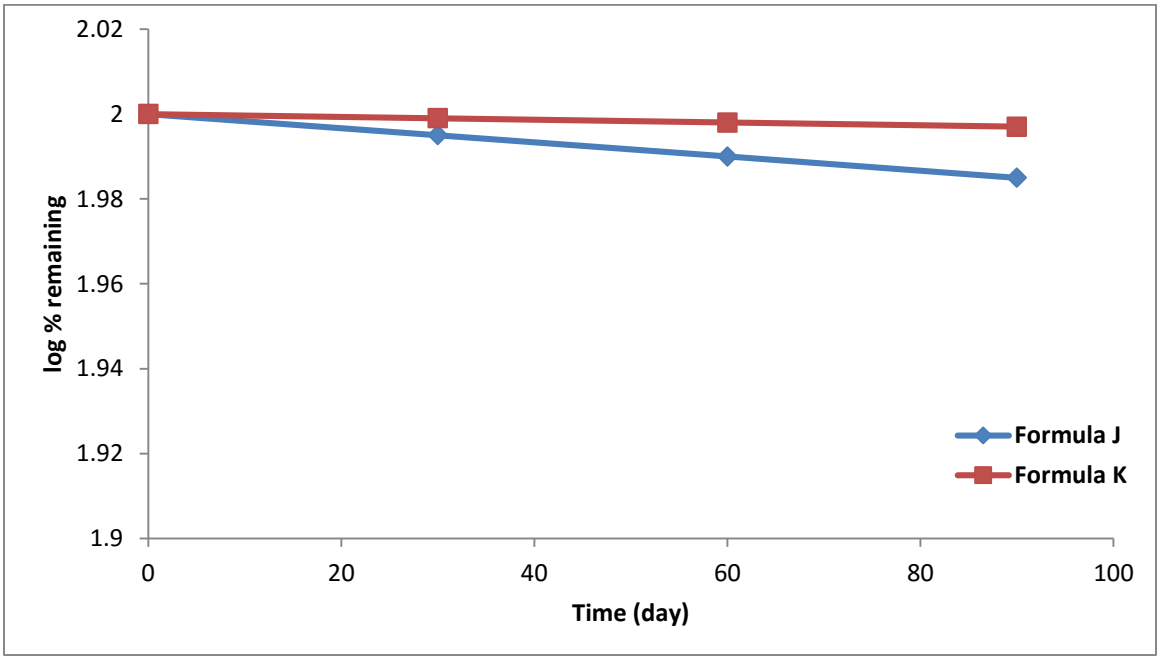


**Figure 6 : Effect of chelating agent (disodium edetate) on the rate of hydrolysis of gatifloxacin eye drop for formula (J and K).**

**Table 5: Effect of ionic strength on the rate of hydrolysis of gatifloxacin in formula (A,D and E) in phosphate buffer at pH 6.0, 0.1M and $60^0$C.**

| Formula in phosphate buffer | Ionic strength | Kx$10^{-3}$ day$^{-}$ |
|---|---|---|
| A | 0.25 | 0.075 |
| D | 0.5 | 0.105 |
| E | 1.0 | 0.12 |

The effect of addition of thickening agent on the viscosity of gatifloxacin ophthalmic solution is shown in table 6, the results showed that the viscosity of formula H is significantly

higher than that of formula A($P<0.05$), where neither hydroxy propyl methyl cellulose, methylcellulose nor xanthan gum added.

**Table 6: Effect of thickening agents [M.C(0.3%, 0.5%), HPMC 1% and xanthan gum 0.2%] on the viscosity and the rate of hydrolysis of gatifloxacin in formula (F, G, H, I and J) in phosphate buffer, 0.1M at pH 6.0 and $60^0$C.**

| Formula | Viscosity (mpa.s) | $Kx10^{-3}$ $(day^-)^-$ |
|---|---|---|
| A | 1 | 0.075 |
| F (0.3%M.C) | 6.4 | 0.078 |
| G (0.5% M.C) | 14 | 0.088 |
| H (1% HPMC) | 20 | 0.081 |
| I (0.2% xanthan gum) | 19.6 | 0.08 |
| J (0.2% xanthan gum) | 23.3 | 0.07 |

In addition to that the addition of methyl cellulose (as in formula F and G) also increases the viscosity of the solution but to a less extent than hydroxy propyl methyl cellulose and xanthan gum this is due to the chemical nature of the two polymers.

The results also show that the viscosity of formula H is no significantly differences than that of formula I but significantly differences to formula J ($P<0.05$).

The addition of xanthan gum to the gatifloxacin containing ophthalmic aqueous liquid preparation having improved the viscosity that can gives enough contact time of the drug with the eye and so improving it's ocular bioavailability as well as the intraocular peneteration of gatifloxacin can be provided [16].

When sodium chloride is added to the aqueous liquid preparation, the formation of any precipitate and the reduction in viscosity in the aqueous liquid preparation can be suppressed[10].

The effect of light on the hydrolysis of gatifloxacin was studied by placing the selected formula (formula J) in clear, dark containers at room temperature for 10 months.

As shown in figure 7, there is a linear relationship for logarithmic plot of the percent remaining of gatifloxacin versus time when the drug was stored in a clear containers indicating that gatifloxacin follows first order kinetics.

The observed rate constant (k) was calculated from the slop of the line was found to be 0.015 $month^{-1}$, accordingly, the $t_{10\%}$ of gatifloxacinis equall to 7.0 months.

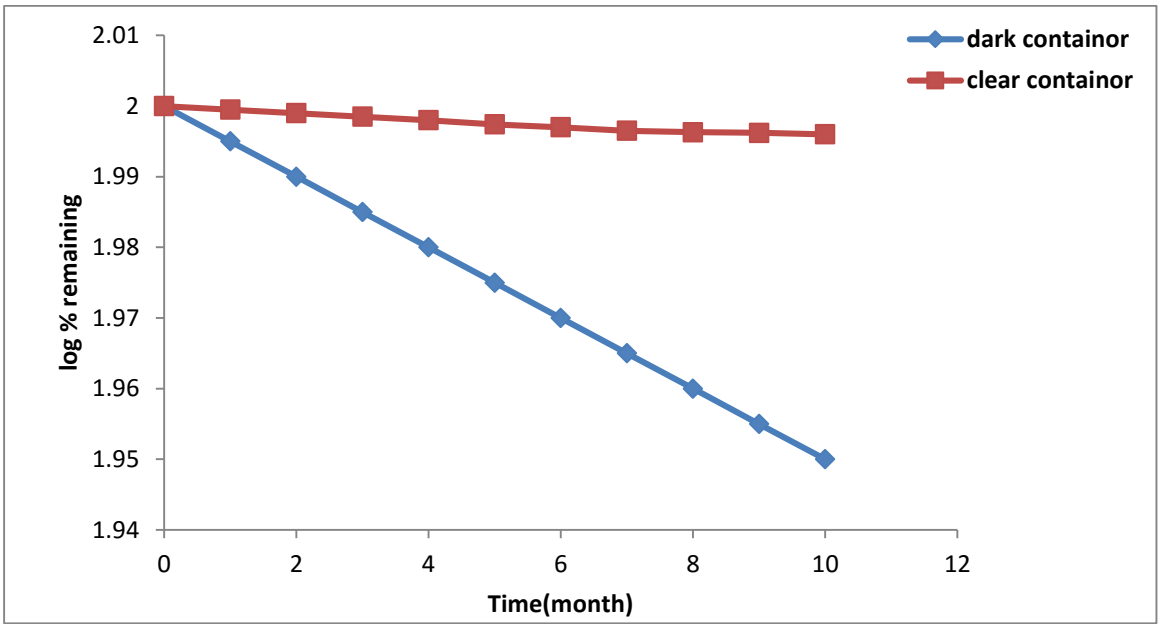

**Figure 7: Effect of light on the rate of hydrolysis of gatifloxacin on formula J in phosphate buffer at pH 6.0 and room temperature.**

On the other hand, the formula which stored in dark containers showed no detectable changes in the percent remaining when exposed to the same condition. The results suggest that gatifloxacin solution should be kept in dark container since the drug affected by light [17].

The results of preservative efficacy on formula J indicated that the preservative used benzalkonium chloride was very effective and it cause 100% reduction in the growth of all tested micro-organisms after 15 minutes of the inoculation.

The same results were obtained after 14 and 28 days which fit the U.S.P requirements [18].

The results showed that there are no signs for ocular irritation, lacrimation or redness of the eye of the tested animals, also there is no signs of eye infection or purulent discharge after three consecutive days of instillation of gatifloxacin to the rabbits eyes and this indicates that the additives used were compatible with the eye tissue and the formula free from foreign particles or fibers which may cause irritation or redness of the eye may stimulate lacrimation[19].

The best selected formula J passed all quality control test including drug content, pH, clarity and sterility test and complied with the U.S.P requirements as shown in table7.

**Table 7: Results of quality control tests for the selected (formula J in phosphate buffer) of gatifloxacin.**

| Type of test | Results | |
|---|---|---|
| | Formula J | U.S.P requirements |
| Drug contents | 98% | 90-110% |
| pH | 6.0 | 6.0-7.5 |
| Clarity test | The solution was very clear and no particles were detected in the product. | (comply) |
| Sterility test | The product was steril no growth of micro-organism was seen after 14 days. | (comply) |

The overall results suggested that gatifloxacin can be optimized in it's aqueous solution inorder to develop stable formulation that complies the non-official and official requirements in order to be used as effective non irritant ophthalmic eye drops.

## 4. CONCLUSION

After conducting this investigation on gatifloxacin as an aqueous ophthalmic solution, one canconclude the following:-

1. It was found that there was an effect of buffer type on the rate of hydrolysis of this drug.On the other hand,the buffer concentration also had an effect on the rate of hydrolysis of drug.Maximum stability was achieved by using phosphate as buffering agent with concentration 0.1 M.
2. The optimum stability of gatifloxacin aqueous solution was obtained at pH 6.0 and it was found that the rate of hydrolysis icreased at low and high pH.
3. The use of disodium edetate as asequestering agent gave more stable formula.
4. The addition of methyl cellulose to the gatifloxacin ophthalmic solution (formula F&G) increases the viscosity but to aless extent than hydroxyl propyl methyl cellulose(formula H) and xanthan gum(formula I&J).

5. The addition of xanthan gum to the gatifloxacin ophthalmic solution(formula I&J) having improved the viscosity that can give enough contact time to the eye and so improved ocular bioavailability.
6. When sodium chloride is added to the aqueous liquid preparation (formula J 0.9%),the formation of any precipitate and the reduction in the viscosity can be suppressed.
7. Light had an effect on the hydrolysis of gatifloxacin ophthalmic solution (formula J) so shoud be kept in dark containor.
8. The selected formula J passes all the quality control tests successfully.

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