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Research Article

Effect of Packaging Materials and the Leached Iron on the Stability of Butorphanol Tartrate Injection

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Abstract The aim of this study was to investigate the effect of various parameters on the stability of butorphanol tartrate injection and to screen the optimal packaging material. The effect of the headspace oxygen levels, ampoule color, manufacturer, and size on the stability of butorphanol tartrate formulation were evaluated. The headspace oxygen levels controlled by nitrogen purging were found to be particularly effective in improving stability of the butorphanol formulation, especially below 2%. Although it is a photolabile drug, butorphanol tartrate was getting degraded at much higher extent in amber color ampoules in comparison to clear ampoules. The degradation by oxidation was found to be a free radical-mediated process catalyzed by the presence of iron ions leached from the amber ampoules. The ampoule manufacturers also had a significant effect on the stability of butorphanol. Two-milliliter ampoules provided a better stability of the butorphanol tartrate injection than 1mL ampoules as 2-mL ampoules had the lower headspace oxygen level at the same level of oxygen content. The oxidation mechanism of the butorphanol tartrate injection was investigated under various conditions, which include iron powder spiking, removal of excipients, exposure to oxygen/nitrogen, exposure to stainless steel and at different pH. Iron powder spiking, presence of citric acid, exposure to oxygen, exposure to stainless steel, and high pH accelerated the oxidative degradation. The effect of oxygen, iron ion and citric acid is in agreement with a metal-catalyzed oxidation mechanism called Udenfriend reaction. Based on the formulation test results, limiting headspace oxygen level, ampoule color, manufacturer, size, controlling iron ion contamination, and pH are recommended for formulation development. In conclusion, it can be suggested that this study can lead to a better understanding of the degradation mechanism of butorphanol tartrate; hence, it would contribute to the development of butorphanol tartrate injection with improved stability.

KEY WORDS: butorphanol tartrate; stability; oxidation; leached iron; Udenfriend.

INTRODUCTION

Glass is an amorphous solid that has been widely used for packaging formulation drugs for a long time (1). Silicon dioxide (SiO_2) is a common fundamental constituent of glass and the most commonly used oxide for sterile dosage forms (2). Various minerals are added to the glass to modify its physicochemical properties according to its specific requirements. Based on minerals which are incorporated, glasses can be classified into broad families. Soda-lime and borosilicate glasses are commonly used for parenteral containers. In the soda-lime glass, soda, and lime refer to sodium oxide and calcium oxide, which are the primary modifiers and comprise roughly 25% of the composition by weight. Soda-lime glass has poor chemical resistance due to chances of leaching of mobile nature of sodium cations. Borosilicate glasses replacing some of the sodium and calcium with boron oxide (B_2O_3) were introduced between 1910 and 1920 to have exception chemical durability and heat resistance-including resistance to abrupt temperature changes and thermal shock (3). Borosilicate glasses are now commonly used for parenteral containers as a result of its high resistance to thermal processes such as depyrogenation, lyophilization, terminal sterilization and low alkali extractable (4). Aluminum oxide (Al_2O_3) is added to facilitate melting and to improve chemical durability of glass. Ferric oxide (Fe_2O_3) , titanium dioxide (TiO_2) and/or manganese oxide (MnO_2) can be added to produce amber glasses for providing light protection (3). Although glass containers are widely used in parenteral preparations, they cannot be considered completely inert. Various untoward events have occurred during the period of time with glass containers which led to a decrease in the effectiveness and safety of the medicine, including leaching, ion exchange, precipitation, glass dissolution, surface layer exfoliation, and corrosion (5). A variety of factors may cause metal ion to

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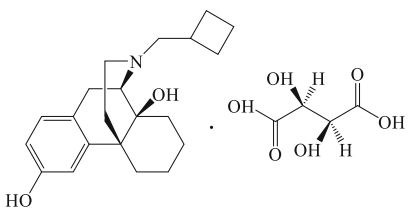
leach out of the glass structure, it is toxic if ingested in higher amount (6) or become catalysts to accelerate the degradation of the drug (7). Other important factors that affect the interaction between glass and drug products are type or processing, and formulation variables such as pH, buffer, drug characteristics, sterilization cycle, storage conditions, etc. (8).

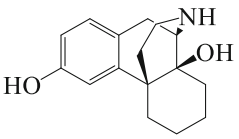
Butorphanol tartrate was first introduced in 1978 as a product directed towards moderate to severe pain such as postoperative, cancer, and biliary colic clinically (9). It is a synthetic opioid agonist-antagonist analgesic and its chemical structure formula is shown as Fig. 1A (10). The chemical name of butorphanol tartrate is 17-cyclobutylmethyl-3,14-dihydroxymorphi-nan and it is available in the US in an injectable form (STADOL®) by Bristol-Myers Squibb Co. Butorphanol is an opioid agonist-antagonists (OAA) of mixed opioid agonist-antagonists, clinically used as its tartrate (11, 12). Experimental results showed that Butorphanol was a partial agonist of μ receptor, had a dominant antagonist against δ receptor, and it also was κ receptor complete agonist (13, 14). *In vitro* experiments confirmed that the affinity ratio between butorphanol and opioid receptors (μ: δ: κ) was 1:4:25, so butorphanol is excited κ receptors primarily. Butorphanol has good analgesia while sedative effect and seldom tolerability. Due to its partial antagonism to μ receptor, there is little drug dependence in clinical use (15–17).

Although few studies assess the effect of packaging materials on the compound degradation (18–22), the influence of packaging materials and the leached iron on the stability of butorphanol tartrate formulation has not been evaluated. In addition, the degradation of butorphanol by Udenfriend-based processes to our knowledge has not been studied. The Udenfriend reaction was studied by Sydney Udenfriend *et al*. in 1954 who found that with the presence of adding Fe^{2+}, ascorbic acid, EDTA to aromatic compound aqueous solution and exposure to the air, aromatic compound can be hydroxylated effectively (23).

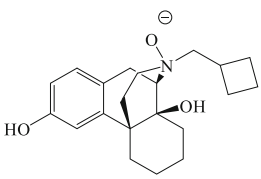
In this study, the influence of headspace oxygen levels and pharmaceutical glass packaging containers on butorphanol tartrate formulation has been explored from various aspects with the aim of improving the stability of butorphanol tartrate injection. A metal-catalyzed oxidative reaction mechanism called Udenfriend reaction has been proposed based on structural and formula information. The iron ion leached from the packaging material and the stainless-steel pipe can form a complex with the excipient citric acid to initiate the Udenfriend reaction, which can catalyze the oxidative degradation of butorphanol into a variety of products. The effect of formulation pH on stability was also investigated.



Butorphanol Tartrate



Imp-C



Imp-D

Fig. 1. Chemical structures of butorphanol tartrate and its degradants (Imp-C and Imp-D)

MATERIALS AND METHODS

Materials

The butorphanol tartrate (Fig. 1) was produced by the Process Optimization Center, China State Institute of Pharmaceutical Industry according to the method previously reported (24). Sodium citrate dihydrate was purchased from Hunan Huari Pharmaceutical Co., Ltd, citric acid monohydrate was from Hunan Erkang Pharmaceutical Co., Ltd. and NaCl was from Hebei Huachen Pharmaceutical Co., Ltd, Triethylamine KH_2PO_4, NaOH, NaCl and H_3PO_4 were obtained from Sinopharm Group acetonitrile was from Honeywell International and iron powder was from Macklin. All chemicals in these experiments are of analytical grade and all laboratory water was from Melin-Q. Five different ampoules from various manufactures were tested. Detailed information about the ampoules is shown in Table I. 316L stainless steel tube was provided by Guorui Pharmaceutical Co., Ltd.

Methods

Analysis of Butorphanol Impurities

The HPLC system Thermo DIONEX Ultimate 3000 comprised a LPG-3400SDN Ternary Pump, a WPS-3000TSL ANALYTICAL Autosampler, a TCC-3000RS Column Compartment, and a DAD-3000 Diode Array Detector. Thermo Scientific Chromeleon Chromatography Data System (CDS) was used as a processing module to obtain the results of this study. The chromatographic separations were conducted with a Thermo Hypersil Phenyl column (4.66mm*250mm, 5 μm), mobile phase A (0.025mol/L KH_2PO_4 solution-triethylamine, 100:2, v/v, pH adjusted to 3.00±0.05 by H_3PO_4) and mobile phase B acetonitrile. KH_2PO_4, triethylamine and H_3PO_4 were purchased from Sinopharm, acetonitrile was purchased from Honeywell. A 75-min gradient elution was performed using the method described in Table II. The injection volume was set at 60 μL. The flow rate was 1mL/min, the column temperature was kept at 40°C and the detection wavelength was 280nm. The performance has been monitored by the

Table I. Ampoules Studied in this Article

Manufacturers	Ampoules Color	Sizes
Manufacturer A	Clear	2mL
Manufacturer B	Clear	2mL
Manufacturer C	Clear	1mL, 2mL
Manufacturer D	Amber	2mL

Table II. Gradient Elution Method

Time(min)	Mobile phase A%	Mobile phase B%
0	95	5
45	50	20
55	40	60
65	40	60
65.1	95	5
75	95	5

analysis of a system suitability solution which contained 2μg Imp-B, 2μg Imp-C, 2μg Imp-D, 2μg Imp-F, 2μg Imp-G and butorphanol tartrate (1 mg/L). Parameters such as retention time, resolution, and symmetry factor verified the suitability of the system.

Analysis of Iron Ion

The concentration of iron ions was determined by Inductively Coupled Plasma Mass-Spectrometry (ICP-MS). The multi-element calibration standard and Fe calibration standard were purchased from Guobiao (Beijing) Testing & Certification Co., Ltd. An internal standard mix containing Y, Tb, Bi, Ge, In, Lu, Rh, and Sc was diluted to 0.1 mg/L in 1.36% HNO_3. Fe calibration standard was diluted to 10ppb, 20ppb, 50ppb 100ppb and 200ppb in 1.36% HNO_3 as standard solutions. Optima grade nitric acid (Shanghai Xu Word Chemistry Co., Ltd) and, Ultrapure water (Cascada™ I) were used. The samples were diluted 10 times with Ultrapure water, and then run on an Thermo Fisher iCAP-RQ ICP-MS. The peristaltic pump speed was 0.1rps, the radio-frequency power was 1550w, the atomizing chamber temperature was 2°C and the collection was repeated three times.

Preparation of the Butorphanol Tartrate Formulation

The butorphanol tartrate formulation was prepared as the prescription shown in Table III. The solution was sterile filtered with PES membrane (Unichro® Syring Filter, Diameter 25mm, Pore Size 0.22μm), filled into various ampoules, and purged with nitrogen gas. The filling volume was 1.1mL. This solution is hereby referred to as "formulation."

Headspace Oxygen (HO) Levels

To prepare the formulation with different oxygen headspace levels, the amber ampoules (Manufacturer D, 2mL) filled with formulation were purged with air, premixed oxygen/nitrogen gas (5%/95%) or high purity nitrogen (99.999%) at 20%, 5%, or 2% oxygen levels respectively and then sealed promptly. These samples were autoclaved at 121°C for 15min (25), stored at 60°C and analyzed for related substances by HPLC on day 0, day 5, day 10 and day 30 (Table IV). The final headspace oxygen level was determined using an oxygen analyzer from Oxysense® Testing Machines Inc.

Table III. Prescription of the Butorphanol Tartrate Injection

Material	Concentration
Butorphanol tartrate	1mg/mL
Citrate monohydrate	0.2% w/w
Sodium citrate dihydrate	0.2% w/w
Sodium chloride	6.4mg/mL

Amber and Clear Ampoules

The formulation was filled into amber ampoules (Manufacturer D, 2mL) and clear ampoules (Manufacturer A, 2mL) respectively and purged with nitrogen gas. After sealed and sterilized by autoclaving at 121°C for 15min, the samples were kept at 60°C and analyzed for related substances on day 0, day 5, day 10 and day 30 (Table IV). Metal ion levels (*p*pb) in each solution were determined by ICP-MS (26).

Ampoules from Different Manufacturers

The prepared formulation was filled into 2mL Manufacturer A ampoules, 2mL Manufacturer B ampoules and 2mL Manufacturer C ampoules respectively, then purged with nitrogen gas and sealed immediately. The samples were autoclaved at 121°C for 15 min for sterilization, stored at 60°C for 30 days and analyzed for related substances by HPLC on day 0, day 5, day 10 and day 30 (Table IV).

Ampoules of Different Sizes (1mL and 2mL)

The formulation was filled into 1mL and 2mL clear ampoules (Manufacturer C) respectively and purged with nitrogen gas for 10 seconds. The ampoules were sealed immediately and autoclaved at 121°C for 15 min. The samples were stored at 60°C for 30 days and analyzed for related substances by HPLC on day 0, day 5, day 10 and day 3 (Table IV)0.

Effect of Iron Powder on Butorphanol Tartrate Stability (22)

The effect of iron powder on butorphanol tartrate stability was evaluated to study the relationship between Fe, O_2, citric acid, and butorphanol tartrate and deduce the possible degradation pathway of butorphanol tartrate with the leached iron.

Thirty milliliters of 1.0 mg/mL butorphanol tartrate solution was prepared in a glass vessel without citric acid, sodium citrate and sodium. Twenty milliliters of the solution was transferred to a 25-mL glass flask and spiked with 2.3 mg iron powder. The remainder of the solution was kept in the original glass vessel and not exposed to iron powder. Both solutions were exposed to air at room temperature and protected from light. Samples were collected at 0h, 1.5h, 3h and 6h and analyzed for related substances by HPLC after sterile filtered (Table IV).

Fifty milliliters 1.0 mg/mL butorphanol tartrate formulation was prepared with citric acid, sodium citrate and sodium chloride. Twenty milliliters of the formulation was

Table IV. Factors Tested in this Study

Factors		Butorphanol tartrate Conc.(mg/mL)	pH	Headspace Oxygen (%)	Storage Condition
Headspace oxygen		1	4.5	2.0/5.0/20.0	60°C, 30 days
Ampoules	Color (amber and clear)	1	4.5	2.0	60°C, 30 days
	Manufacturers	1	4.5	2.0	60°C, 30 days
	Sizes	1	4.5	2.0	60°C, 30 days
Iron powder	Oxygen	1	4.5	20.0	Room temperature, 6h
	Oxygen and citric acid	1	4.5	2.0	Room temperature, 6h
	Citric acid	1	4.5	2.0	Room temperature, 6h
Stainless Steel		1	4.5	2.0	60°C, 30 days
pH		1	2.5-6.0	2.0	60°C, 30 days

transferred to a 25-mL glass flask, spiked with 2.3 mg iron powder and exposed to air; 20 mL of the formulation was transferred to another 25 mL glass flask, spiked with 2.3 mg iron powder and protected by nitrogen gas. The remainder of the formulation was kept in the original glass vessel and not exposed to iron powder. All these solutions were store at room temperature and protected from light. Samples were collected at 0h, 1.5h, 3h and 6h and analyzed for related substances by HPLC after sterile filtered (Table IV).

Stainless Steel Exposure

The formulation was prepared in a glass vessel, sterile filtered, and analyzed by HPLC and ICP-MS initially. The formulation was then exposed to SS316L stainless steel tube at room temperature. The stainless steel exposed solution was filled into ampoules (Manufacturers A, 2mL) at 0h, 1h, 3h, 6h and 24h, purged with nitrogen gas and sealed promptly (25). The prepared sample ampoules were sterilized at 121°C for 15 min and stored at 60°C for 30 days. The samples were analyzed by HPLC and ICP-MS on day 0, day 5, day 10 and day 30 (Table IV).

Effect of pH on the Butorphanol Tartrate Formulation

The effect of pH on the formulation stability was studied to investigate the possible degradation pathway of butorphanol tartrate with the leached iron. The pH of the formulation was prepared and adjusted to 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 with aqueous solution of 1mol/mL NaOH and 1mol/mL HCl (27). These solutions were sterile filtered and filled into amber ampoules. The prepared sample ampoules were purged with nitrogen gas and sealed promptly. After autoclaved at 121°C for 15 min, the samples were stored at 60°C for 30 days and analyzed for related substances by HPLC (Table IV).

Statistical Analysis

To evaluate the statistical significance of the differences between groups, Student's *t*-test or one-way analysis of variance (ANOVA) was carried out by using Excel (Microsoft Office 2016, USA).

RESULTS AND DISCUSSION

Degradation Products

Butorphanol tartrate could be degraded into many impurities in the stability studies. Two major degradation

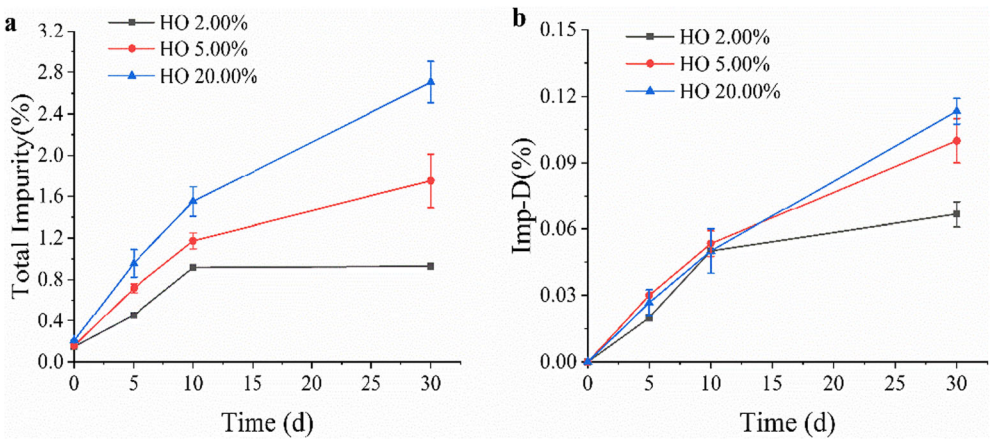


Fig. 2. Effect of oxygen headspace levels on the stability of the butorphanol tartrate formulation induced by stress condition at 60°C: (**a**) increase in total impurity content (%); (**b**) increase in Imp-D content (%)

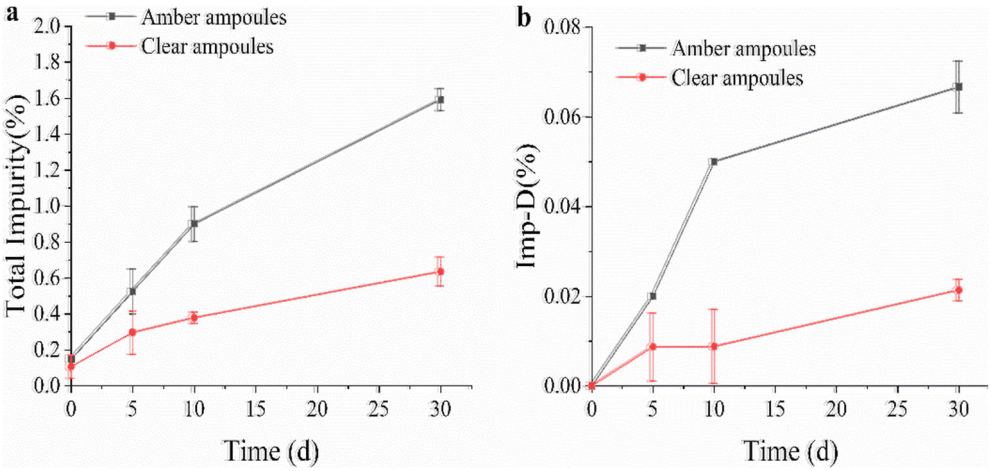


Fig. 3. Effect of amber and clear ampoules on the stability of the butorphanol tartrate formulation induced by stress condition at 60°C: (**a**) increase in total impurity content (%); (**b**) increase in Imp-D content (%)

products were identified as (-)-3, 14- Dihydroxymorphine (Fig. 1, Imp-C) and N- N-oxygen-(-) -cyclobutyl-methyl-3, 14-dihydromorphonone (Fig. 1, Imp-D), which are the focus of this study. Imp-C is an alkali-degraded impurity of butorphanol tartrate and Imp-D is an oxidative degradation product.

Headspace Oxygen (HO) Levels

Whether the headspace oxygen levels (%) under the control of nitrogen headspace effect the stability of the butorphanol tartrate formulation was investigated, and the results are shown in Fig. 2. As shown in Fig. 2a, after stored at 60°C for 30 days, the total impurity of samples under HO 2.00%, 5.00%, 20.00% are 0.93±0.02%, 1.75±0.26% and 2.71±0.19% respectively. The oxidative degradation was inhibited when headspace oxygen was reduced (p<0.0001). This result indicates that the headspace oxygen has a significant effect on the degradation of butorphanol tartrate formulation. The average HO of the samples collected at day 10 and 30 decreased from 2.0% to around 1.5%. It is evident that oxygen is directly involved in the degradation of the butorphanol tartrate formulation. There was no significant increase of the total impurity (%) of the samples when headspace oxygen was reduced to about 1.5% after stored at 60°C for 10 days and 30 days (p>0.05). Another phenomenon observed in this study as shown in Fig. 2b is that Imp-D was effectively controlled after purged with nitrogen gas (p<0.001). This study suggests that butorphanol tartrate formulation is oxidizable. In conclusion, purging nitrogen to control headspace oxygen can prevent oxidative degradation of butorphanol tartrate in amber ampoules efficiently.

Table V. Primary Components of Ampoules

Primary components	Weight (%)	
	Amber ampoules	Clear ampoules
SiO_2	70.5	75
B_2O_3	10	10.5
Al_2O_3	6	5
Fe_2O_3	1	-
Na_2O	7	7
K_2O		-
BaO	1.5	1.5
CaO	1	-
TiO_2	3	-

Amber and Clear Ampoules

The effect of the color of ampoules on butorphanol tartrate formulation is shown in Fig. 3. After high temperature test for 30 days, the total impurity of samples in amber ampoules is 1.59±0.06%, it is much higher than (p<0.0001) those in clear ampoules (0.64±0.08%). The Imp-D (%) of the butorphanol tartrate formulation in amber ampoules (0.07±0.006%) were also much more than (p<0.0001) those in clear ampoules (0.02±0.002%). Hence, the amber ampoules have a serious impact on the stability of butorphanol tartrate formulation. The results are like a previous study (19). Enever, *et al.* found that the degree of degradation of amitriptyline hydrochloride in amber ampules was much higher than that in clear ampules and this study shown that the oxidative degradation was a free radical-mediated process which was accelerated by the presence of metal-ion contaminants and the amber glass ampoules were the major source of metal-ion contaminants. In addition, metals are the one of common contaminants that contribute significantly to drug oxidation in dosage forms (28). The metals in dosage forms can affect the degradation rates even

Table VI. Iron Element Concentration in Clear and Amber Ampoules

Color of ampoule	Fe concentration($p$pb)
Clear ampoules	74.74
Amber ampoules	1425.632

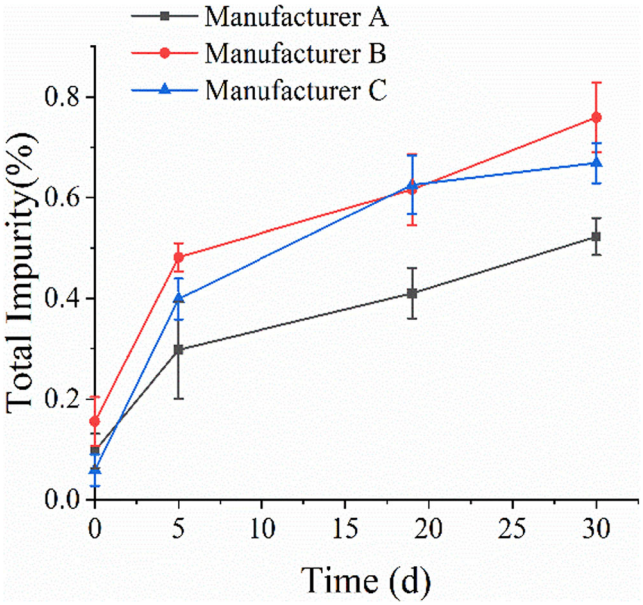


Fig. 4. Effect of ampoules from different manufacturers on butorphanol tartrate formulation degradation induced by stress condition at 60°C for 30d

at low levels because they are catalysts rather than consumed. The primary components of amber and clear ampoules provided by manufacturers is shown in Table V, the differences of these two kinds of ampoules are that amber ampoules have Fe_2O_3 and TiO_2. Therefore, it is important to detect the concentration of leached iron in the solution in different containers. As shown in Table VI, the leached iron content in the clear ampoules was much less than the leached iron content in amber ampoules, which again confirms that amber ampoules are not suitable to be inner packaging for butorphanol tartrate formulation.

Ampoules from Different Manufacturers

The effect of ampoules from different manufacturers on butorphanol tartrate formulation by stress condition at 60°C for 30 days is shown in Fig. 4. The average total impurity of ampoules from all three manufacturers above increased to 0.52±0.04%, 0.76±0.07%, and 0.67±0.04% respectively. It is obvious that ampoules from Manufacturer B and Manufacturer C both more than ampoules from Manufacturer A ($p<0.05$), suggesting that the manufacturers of ampoules could be a parameter for control the stability of butorphanol tartrate formulation and the optimal ampoules in this study is from Manufacturer a. According to the information provided by the manufacturers, the three Manufacturers' glass tube are

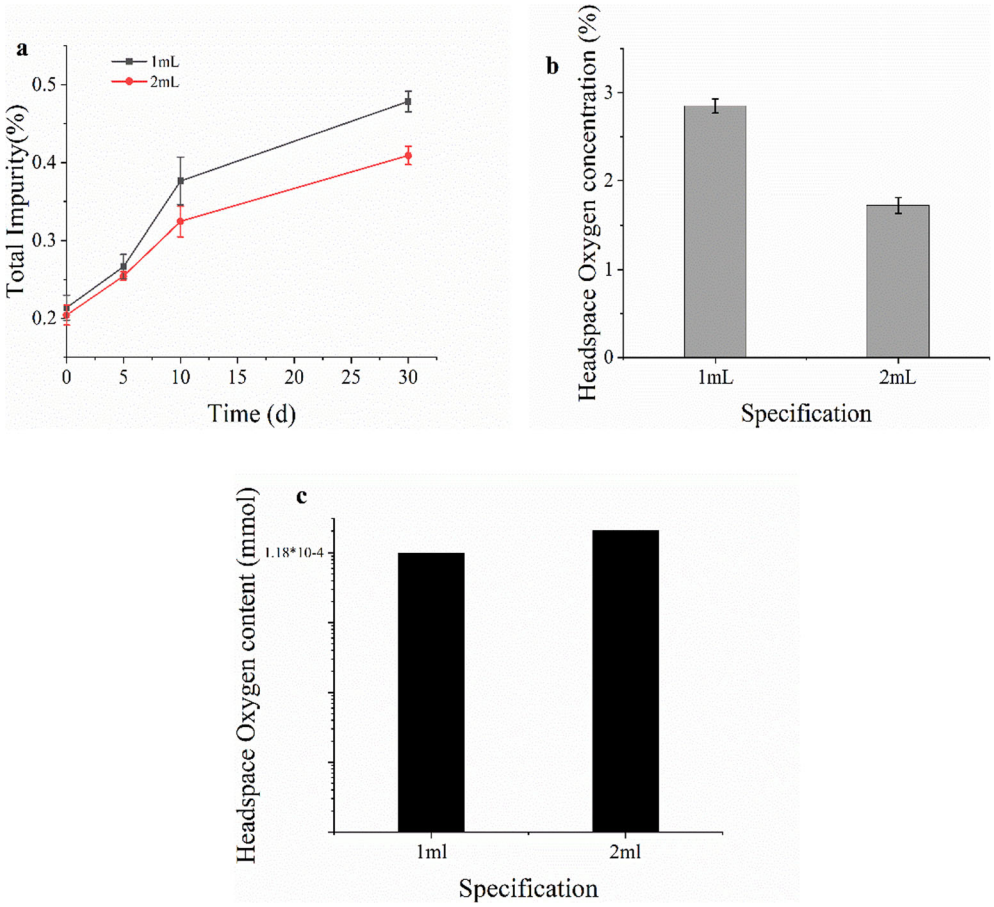


Fig. 5. (**a**) Effect of sizes of ampoules on the stability of butorphanol tartrate formulation degradation induced by stress condition at 60°C for 30 days; (**b**) Headspace oxygen concentration in 1mL and 2mL ampoules; (**c**) Headspace oxygen content in 1mL and 2mL ampoules

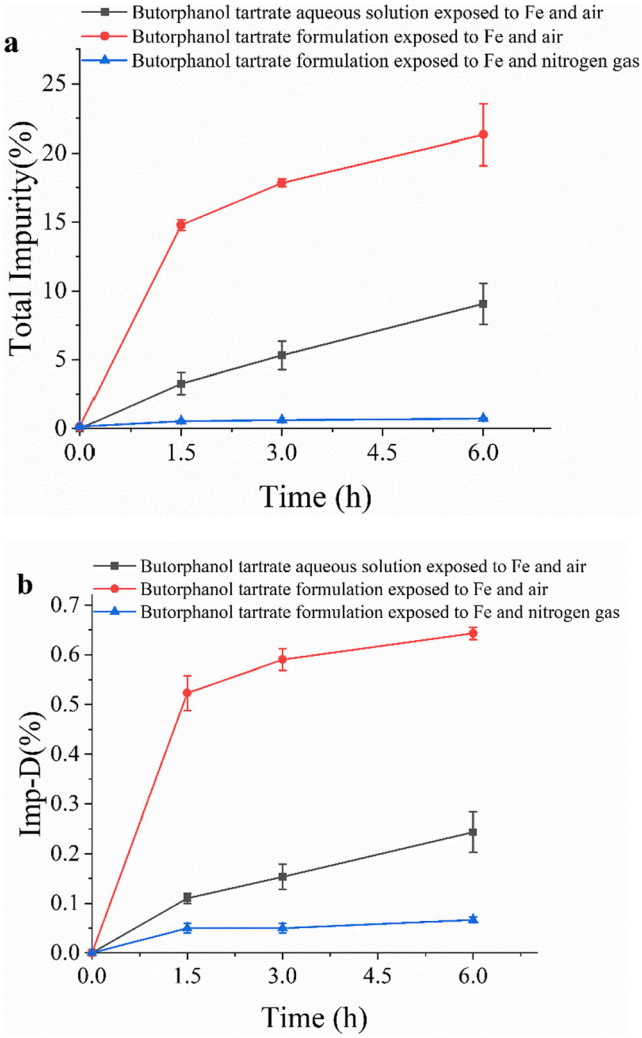


Fig. 6. Effect of iron powder on the stability of the butorphanol tartrate in aqueous solution and the butorphanol tartrate formulation under air exposed and the protection of nitrogen condition: (**a**) increase in total impurity content (%); (**b**) increase in Imp-D content (%)

purchased by Schoot, it means that the primary components of these clear ampoules are the same (Table VI). Hence, the different effect of ampoule manufacture may be due to different production processes. For ampoules, in the process of forming the neck of the container, especially the bottom, because of the evaporation of alkali metals and borate caused by local heat, the chemical tolerance of the inner surface of the hot processed part is lower than that of the unheated part in the glass container (7). In summary, we should make comparison between ampoule manufacturers in order to improve product stability.

Ampoules of Different Sizes (1mL and 2mL)

The effects of different sizes of ampoules on the stability of butorphanol tartrate formulation were investigated. As shown in Fig. 5a, there is significant difference of the total impurity content observed in the accelerated testing effect by ampoules of different sizes (0.48±0.01% in 1mL ampoules and 0.40±0.01% in 2mL ampoules) ($p<0.01$). Figure 5b shows the headspace oxygen level of these samples was 2.93±0.08% in 1ml ampoules and 1.74±0.09% in 2ml ampoules ($p<0.01$). However, it had been calculated that the headspace oxygen content in 1mL and 2mL ampoules were $1.18*10^{-4}$ mmol and $1.48*10^{-4}$ mmol (Fig. 5c). Based on these results and the effect of headspace oxygen leave in 2mL ampoules, the content of total impurity of butorphanol tartrate formulation was not only influenced by headspace oxygen content but also headspace oxygen concentration. More oxygen could be consumed at the high headspace oxygen concentration with the same headspace oxygen content. Therefore, different sizes of ampoules do have effects on the stability of butorphanol tartrate formulation.

Effect of Iron Powder on Butorphanol Tartrate Stability

As mentioned above in amber and clear ampoules, the leaching iron from amber ampoules may initiate the free radical-mediated oxidative degradation process. To investigate the reaction mechanism of the oxidative degradation of butorphanol tartrate, the effect of iron power on the stability of butorphanol tartrate was studied and the results are shown as Fig. 6. By comparing the total impurity of the three solutions within 6h, the fastest degradation of butorphanol tartrate was present with iron powder, O_2 and citrate acid. The total impurity of the butorphanol tartrate formulation exposed to Fe and O_2 (21.32±2.24%) is much more than ($p<0.001$) the butorphanol tartrate aqueous solution exposed to Fe and O_2 (9.06±1.49%). It is demonstrated that the degradation process needs the participation of citric acid. Reed, *et al*. had determined the photochemical degradation of citrate buffered formulations of phenyl ether-based drug, they found that the combination of citrate from the formulation and light contributed to reduction of Fe (21). However, there were no significate degradation occurred without O_2 ($p>0.05$). In addition, the increase trends of Imp-D of the three samples within 6h were the same as the total impurity. Thus, it is deduced that molecular oxygen is involved directly in the oxidative degradation of butorphanol tartrate and the degree of oxidative degradation will be deepened with the present of citric acid.

Table VII. Three Points of Udenfriend Reaction

The three point of Udenfriend	Exemplification
A transition metal ion with oxidation reduction ability	Fe^{2+}, Cu^{+} etc.
Chelating agent	EDTA, Citric acid (29), DTPA etc.
Reducing agent	ascorbic acid (30), phenolic etc.

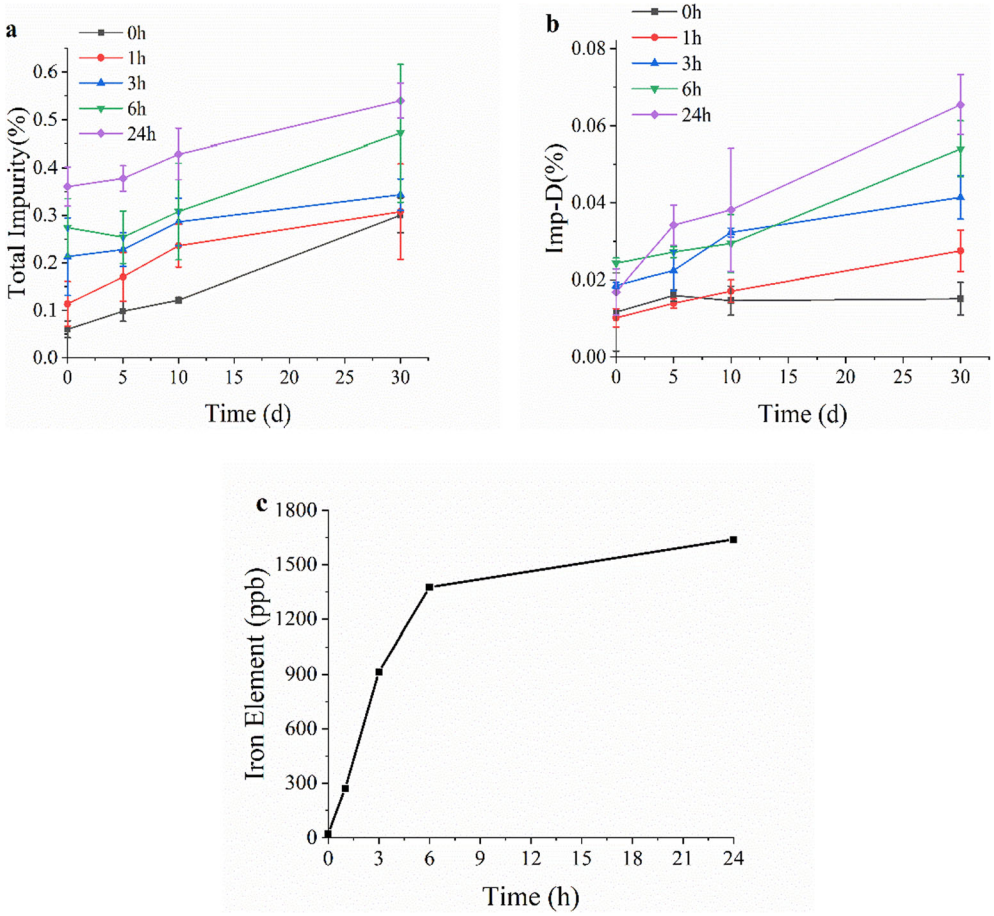


Fig. 7. Effect of stainless steel on the stability of the butorphanol tartrate formulation induced by stress condition at 60°C: (**a**) increase in total impurity content (%); (**b**) increase in Imp-D content (%); (**c**) increase in iron element content (%)

These results suggested that the effect of oxygen, iron ion and citric acid is in agreement with a metal-catalyzed oxidation mechanism called Udenfriend reaction, which has three key components: a transition metal ion with redox ability, chelating agent and reducing agent. The details are shown in Table VII. Butorphanol tartrate formulation in amber ampoule also has these three key components due to the presence of iron ion (transition metal ion), citric acid (chelating agent) and butorphanol with phenolic hydroxyl (reducing agent). The reaction process deduced is shown as Eq. 1 below.

Stainless Steel Exposure

The effect of stainless-steel tube on the butorphanol tartrate degradation is shown in Fig. 7. As shown in Fig. 7a, the total impurity had a marked increase after exposed to S316L Stainless-Steel tube since 3h ($p<0.05$). The results indicate that stainless steel tube have a certain effect on the stability of butorphanol tartrate formulation. Figure 7b shows that before accelerated test, there no significate diffidence of the content of Imp-D after exposing to stainless steel tube for 24h ($p>0.05$). Under the stress condition of 60°C, Imp-D was very little in 0h treated samples, and did not increase on day 30 ($p>0.05$); the Imp-D content of samples which was exposed to stainless steel tube after 1h increased rapidly ($p<0.001$) in 30 days at stress condition. It is suggesting that stainless steel tube was also involved in the oxidative degradation of butorphanol tartrate formulation. As Fig. 7c shows that the content of iron ions in the samples above grew rapidly in the first 6 h, but increased gradually later. This result indicated that the leaching rate of iron from stainless steel tube is limited. Consequently, the leaching iron of stainless-steel tube have significant effect on the stability of butorphanol tartrate formulation and the negative concentration of leaching iron is 1500 ppb (the same as amber ampoules). Hence, the optimal time range of butorphanol tartrate formulation exposed to stainless steel tube is within 6h.

pH as a Parameter for Control of Formulation in Amber Ampoules

As shown in Fig. 8a, there was a significant increase observed in the total impurities (%) before and after the accelerated testing (60°C) for 30 days over the pH range of 3.0-6.0 ($p<0.001$), and the degradation rate grew rapidly with the increase of pH value. This implies that more degradation occurred when pH value was above 3.0. Figure 8b and c shows the content of the degradation products Imp-C and Imp-D also increased significantly with pH value ($p<0.001$, $p<0.001$). Imp-C is an alkaline degradation, it is produced from butorphanol by dealkylation reaction in alkaline

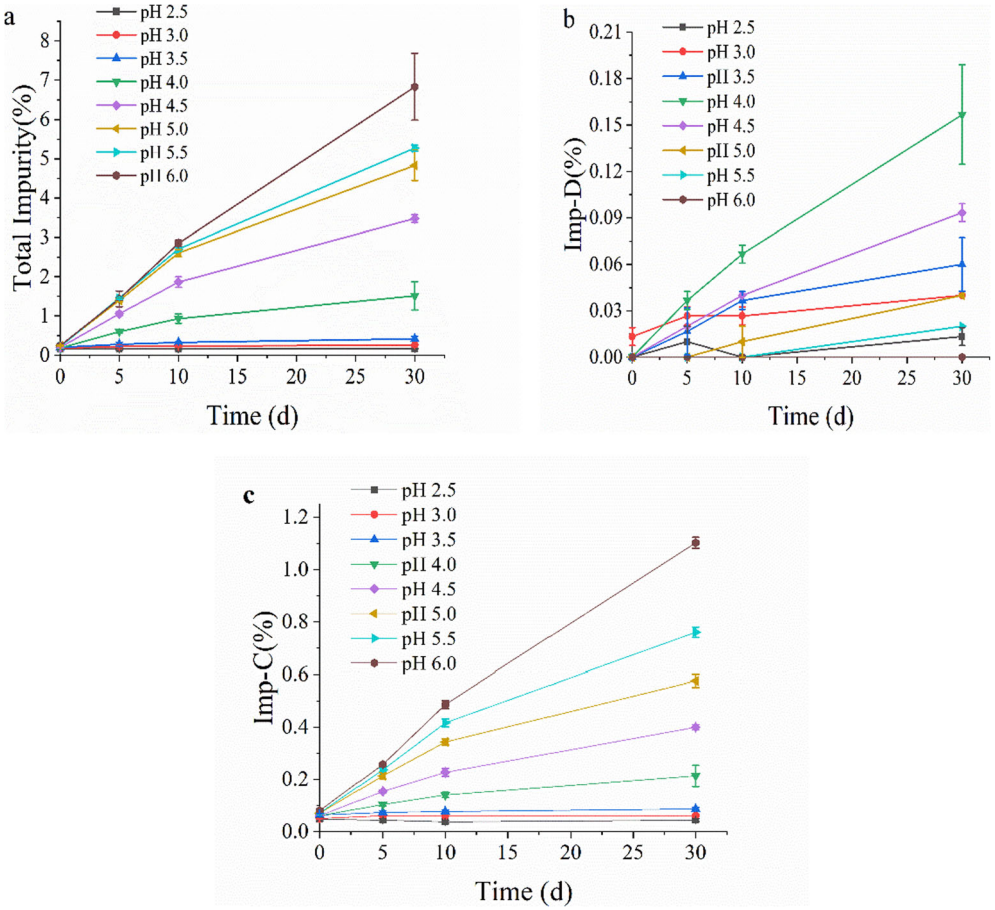


Fig. 8. Effect of pH value on the stability of the butorphanol tartrate formulation induced by stress condition at 60°C: (**a**) increase in total impurity content (%); (**b**) increase in Imp-C content (%); (**c**) increase in Imp-D content (%)

condition. Hence, lower pH value can effectively inhibit the degradation of butorphanol to Imp-C. As mentioned above in Degradation Products, Imp-D is an oxidation degradation. Freed *et al.* investigated the effect of pH on the degradation of N-oxides in the preparation study, they found that oxidation of tertiary alkyl amines was inhibited by lowering the pH or adding citric acid, and when the pH of the solution is much lower than the pKa of tertiary amine, the oxidation of tertiary amine can be effectively inhibited (31). Consequently, high pH can accelerate the oxidative degradation and these results also confirm the presence of Udenfriend reaction again.

CONCLUSION

In this study, the effect of headspace oxygen levels, ampoule color, ampoules manufacturer and ampoules size on the stability of butorphanol tartrate injection were investigated to screen the optimal packaging material. We used two methods (Student's t-test or ANOVA) to analyze the statistical significance of the differences between groups. It was shown that the headspace oxygen below 2% levels controlled by nitrogen purging particularly improved stability of the butorphanol formulation. Butorphanol tartrate was getting degraded at much higher extent in amber color ampoules in comparison to clear ampoules. It was found that the oxidative degradation is a free radical-mediated process catalyzed by the presence of iron ions leached from the amber ampoules. The ampoule manufactures also had a significant effect on the stability of butorphanol. Two-milliliter ampoules provided a better stability of the butorphanol tartrate injection than 1mL ampoules as 2mL ampoules had the lower headspace oxygen level at the same level of oxygen content. The oxidation mechanism of the butorphanol tartrate injection was investigated under various conditions, which include iron powder spiking, removal of excipients, exposure to oxygen/nitrogen, exposure to stainless steel and at different pH. Iron powder spiking, presence of citric acid, exposure to oxygen, exposure to stainless steel and high pH accelerated the oxidative degradation. The effect of oxygen, iron ion and citric acid is in agreement with a metal-catalyzed oxidation mechanism called Udenfriend reaction. Furthermore, the leaching iron content from amber ampoules and stainless-steel tube was detected by ICP-MS, this result deduced that there will be significate effect the oxidative degradation of butorphanol tartrate formulation when leaching iron content up to 1500 ppb. Based on the results obtained above, the optimal packaging material is suggested as follows: 2mL clear ampoules from Manufacturer a;

headspace oxygen level below 2%; and the time of exposed to stainless steel below 6h. Therefore, it can be concluded that packaging materials and the leached iron can lead to a better understanding of the degradation mechanism of butorphanol tartrate; hence, it would contribute to the development of butorphanol tartrate injection.

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