Formulation Development and Stability Testing of Oral Morphine Solution Utilizing Preformulation Approach

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ABSTRACT--Purpose. Prefomulation approach utilizing the fractional-ordered randomized blocked design was employed for the formulation development and stability testing of morphine solution. **Methods.** Factors expecting to affect the stability of morphine were evaluated, i.e., vehicle, antioxidant, chelating agent, and pH of the solution. Eight formulations of a possible 16 were prepared according to the block design. The stability of the preparations was tested after 35 days of storage. The data of preformulation study were used for formulation development. **Results.** The presence of glycerin and ethylenediamine-tetraacetic acid in the formulation, and the pH of the solution adjusted to 4, stabilized morphine. The concentration of morphine decreased drastically in the formulations containing sodium metabisulfite, and those pH adjusted to 6. After 35 days, only 65% of morphine was found in the formulation containing sodium metabisulfite and pH adjusted to 6. The results of preformulation study were used for preparing oral morphine preparations. Samples were kept in amber glass bottles and stored at 4°C and 25°C/75% RH for 13 months. No precipitation of the four formulations was detected. Only a decrease of odor and a small increase of pH value of the preparations (< 0.3 units) were observed. More than 97% of morphine remained in all samples. The samples were free from microbial contamination. **Conclusion.** Stable morphine solution formulations can be achieved with the utilization of the preformulation approach. They were stable more than 13 months when stored at 4°C and 25°C/ 75% RH**.**

INTRODUCTION

In drug formulation development, preformulation strategy plays a vital role in order to obtain a proper and stable formulation dosage form. This approach assists the formulator to reduce preparing unnecessary formulations leading to reduced cost and time effectiveness. In general, it reduces the number of experimental formulations while the effect of each factor to the stability of the formulation can still be achieved. These techniques have been used for several drugs such as phenol, pyridoxal hydrochloride and furosemide (1-4). The fractional-ordered randomized block design is one of widely used methods for studying the effect of each variable during the drug development (5). Attempting to study several variables simultaneously results in the need to prepare a number of formulations. If n is the number of such variables, 2^n is the number of formulations required for a two-level study (5). In this study the fractional-ordered randomized blocked design was conducted which was similar to the 2^{4-1} half-fractional factorial design (half-fractional factorial design) (1). In this preformulation study, these factors included antioxidant, co-solvent, chelating agent, and pH of the solution. According to the block design, the effects of the presence and absence of each factor in the preparation were investigated.

Morphine is recommended by the World Health Organization (WHO) for controlling moderate and severe pain, especially for cancer patients (6). Its molecule presents a phenolic group at 3-position leading to ready degradation by oxidation reaction (5). The pH of the solution is a major factor influencing morphine stability according to the pH-rate profile (6). It is rather stable in acidic solution. Moreover, morphine itself presents bitter taste. Therefore, in preparing oral morphine sulfate solution, apart from formulation of a stable preparation, bitter taste masking is another challenge.

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In Thailand, oral morphine solutions, e.g., Oramorph, are mainly used for pediatric patients. These preparations are not locally manufactured hence have to be imported. The cost and the possibility of shortage make the availability of the products an important issue. The objective of this study was to development an oral morphine solution. The physical, chemical and microbiological stability of formulations were tested after storage at standard storage conditions.

MATERIALS AND METHODS

Materials

Morphine sulfate was kindly supplied by the Narcotics Drug Control Division (Macfarlan Smith, Ltd, GlaxoWellcome, Boronia, Australia: Batch number 27342). Materials used in this study were as follows: sodium metabisulfite and sorbitol (70%) were obtained from Vidhyasom Co. Ltd, Bangkok, Thailand; ethylenediaminetetraacetic acid (EDTA) was the product of Riedel-de Haen, Seelze, Germany; glycerin was bought from S Tong Chemicals, Ltd., Bangkok, Thailand; methyl paraben and propyl paraben were the products of Namsiang Ltd, Bangkok, Thailand; *di*-sodium phosphate and sodium phosphate were the products of BDH laboratories supplies, Poole, England. Methanol (HPLC grade) was obtained from Merck, Bangkok, Thailand. The flavoring solutions were bought from Great Hill and Sereewat, Ltd, Bangkok, Thailand. Tryptic soy agar was the product of Difco laboratories, Detroit, USA. Other chemicals and reagents used were of analytical grade.

Methods

Preformulation study

The block design and sample preparation

The master formula of morphine solution consisted of 0.2% (w/v) morphine sulfate, 50% (v/v) syrup USP, 1% (w/v) paraben concentrate, and purified water. In this study, 4 factors (sodium metabisulfite, EDTA, glycerin and pH of the solution) were chosen to be evaluated. Thus, the total number of the experimental formulations was 16 (2^n), where *n* was the number of factors studied. The design was similar to that described by Connors et al (5). However, when the fractional-ordered randomized blocked design was applied, the number of formulations reduced to 8 (Table 1). Sodium metabisulfite, EDTA and glycerin were added (if presented) at concentrations of 1% (w/v), 0.1% (w/v) and 15% (v/v), respectively. The plus or minus designations in the block referred to the presence or absence of the variable in the formula or referred to the higher or the lower of particular parameter (5). For example, apart from ingredients specified in the master formula, formulation 2 comprised sodium metabisulfite, EDTA and the pH of the preparation was adjusted to 4 (Table 1). Table 2 summarizes the eight formulations prepared according to the design given from Tables 1. Morphine sulfate was dissolved in part of the purified water. Ingredients as listed in each formulation were then added and mixed with a magnetic stirrer. The total volume and the pH were then adjusted. Three bottles of each formulation were prepared and kept in amber glass bottles (n=3), stored at room temperature for 35 days.

Physical and chemical evaluation

The physical and chemical stability testing of morphine preparations were performed at day 0, 7, 14, 28, and 35. pH of the samples was measured after calibration using standard buffer solutions pH 4 and 7. (Corning, model 200, Ciba corning diagnostics, Ltd, Sadbury, England) and precipitation of the preparations was also visually observed. Morphine remaining was analyzed using a HPLC system (described below). Samples (5.0 ml) were pipetted, diluted with mobile phase in a 50 ml volumetric flask, and then filtered through Whatman filter paper No 1. Filtered solution was kept in a test tube before HPLC assay.

High-performance liquid chromatography system

HPLC analysis was performed with a system comprising a solvent delivery pump (Perkin Elmer, model 200, US instrument division, Norwalk, USA), a manual injector (Rheodyne, model 7125i, California, USA) equipped with a 20 µl loop and a UV/visible spectrophotometer (Perkin Elmer, model 785A, US instrument division, Norwalk, USA) set at 240 nm. A 3.9×150 mm C_{18} reversed-phase column (Symmetry®, 5-µm, Waters, Milford, USA) was used. Chromatograms were recorded with an integrator (Perkin Elmer, model 1022, US instrument division, Norwalk, USA). The mobile phase for the HPLC assay consisted of 0.79 g sodium 1-heptane sulfunate monohydrate (Sigma Chemical Co, St. Louise, USA) in methanol, water and glacial acetic acid at volume ratios of 300:700:10. The pH of mobile phase was adjusted to 4. It was filtered through a 0.45 µm pore size

Table 1: Fractional-ordered randomized blocked showing factors studied and percent morphine remaining (values in parentheses; mean±SD) after 35 days of storage at room temperature (n=3)

		pH 4		pH 6	
		Sodium metabisulfite (–)	**Sodium metabisulfite (+)**	**Sodium metabisulfite (–)**	**Sodium metabisulfite (+)**
Glycerin –	**EDTA –**	F1 (102.28±1.19)			F5 (65.65±0.32)
	EDTA +		F2 (96.18±0.48)	F6 (97.28±3.25)	
Glycerin +	**EDTA –**		F3 (80.68±3.19)	F7 (100.38±1.87)	
	EDTA +	F4 (101.39±3.33)			F8 (87.78±0.19)

(+), presence; (–), absence

Table 2: Eight formulations of morphine solution in preformulation study in relevant to the fractional-ordered randomized blocked design (n = 3)

Component	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Morphine sulfate (mg)	200	200	200	200	200	200	200	200
Syrup USP (ml)	50	50	50	50	50	50	50	50
Paraben concentrate (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sod. metabisulfite (g)	-	1.0	1.0	-	1.0	-	-	1.0
Glycerin (ml)	-	-	15	15	-	-	15	15
EDTA (g)	-	0.1	-	0.1	-	0.1	-	0.1
Purified Water (ml)	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100
pH adjusted to	4	4	4	4	6	6	6	6

membrane and degassed before use. The flow rate used was 0.9 ml/min. The assay was performed at a room controlled temperature of 25°C. Standard solutions of morphine were prepared on each day of sample analysis by diluting a stock solution with mobile phase to the concentrations of 50-250 μg/ml. To calculate the drug concentrations, peak areas were determined and compared to the standard curve. The retention time of morphine was 3.2 min.

Data analysis

The morphine remaining in each formulation was compared to its initial concentration. Morphine remaining in all formulations at day 35 was used to investigate the effect of each factor utilizing the block design approach.

Formulation and long-term stability testing

Preparation of morphine solution

The resulting data obtained from preformulation the study was used for preparing morphine solutions. Therefore, syrup, glycerin and purified water were the selected vehicles (see results session). Following several trials, 4 formulations of morphine solution at a concentration of 10 mg/5 ml were prepared (Table 5). In brief, morphine sulfate was dissolved with a part of purified water in a beaker. Subsequently, ingredients as listed in each formulation were added and mixed thoroughly using magnetic stirrer. pH was adjusted to approximately 4. Samples were equally divided, transferred into 3 amber glass bottles (n=3, each of 180 ml) and kept at 4°C (Aqualytic AL 180, Liebherr, Germany). Similar procedure was conducted for samples which were kept at 25°C/ 75%RH (Termaks, series 6000, Bergen, Norway).

Table 3: pH of morphine solution after 35 days of storage at room temperature

Day	pH of morphine sulfate oral solution (mean±S.D, n=3)			
	F1	**F2**	**F3**	**F4**
0	4.16±0.02	4.03±0.01	4.03±0.01	4.07±0.02
7	3.90±0.06	3.53±0.03	3.41±0.02	3.96±0.03
14	4.21±0.16	3.55±0.03	3.34±0.02	4.17±0.04
28	3.96±0.10	3.46±0.03	3.16±0.01	4.00±0.04
35	4.18±0.01	3.64±0.01	2.97±0.02	3.97±0.06
Day	**pH of morphine sulfate oral solution (mean±S.D.; n=3)**			
	F5	**F6**	**F7**	**F8**
0	6.00±0.00	6.05±0.03	6.02±0.02	5.97±0.03
7	5.73±0.01	6.08±0.03	6.05±0.02	5.86±0.05
14	4.02±0.03	6.20±0.03	6.05±0.03	5.87±0.03
28	3.64±0.03	6.10±0.07	5.97±0.01	5.89±0.03
35	3.25±0.05	6.03±0.07	5.96±0.02	5.89±0.02

Physical evaluation

General appearance (precipitation observation), color and odor of each sample were observed at month 0, 0.75, 2, 4.5, 6, 7.5, 9, 11 and 13 and the pH was measured and recorded.

Chemical evaluation
Sample preparation

Percent morphine remaining in each solution was determined using HPLC system at the times specified above. After shaking, 5.0 ml of the sample was pipetted into a 50 ml volumetric flask. The mobile phase was added to adjust the final volume, and then filtered using Whatman filter paper no 1 prior to injection into the HPLC. Samples stored at refrigerator were left to room temperature before the analysis procedure was undertaken.

HPLC system

HPLC system in this experiment was similar to that described in the preformulation session.

Biological stability

Total microbial count tests were performed to determine the microbial contamination of the samples at the same time of physical and chemical testing. Tryptic soy agar was used as the media. Testing was performed in laminar air flow using aseptic techniques. The samples were compared to positive control utilizing *S. aureus, P. aeruginosa and E. coli* as test microorganisms and negative control (7). Briefly, 5 ml of the sample was diluted with 45 ml of phosphate buffer pH 7.2 and mixed. 1 ml of the mixture was transferred onto a plate containing tryptic soy agar. The mixture was mixed gently, followed by incubation at 35°C for 24-48 h, and the microbial contamination was counted. Each sample was run in duplicate.

RESULTS

Preformulation study
Stability testing and the block design

No precipitation was observed in any of the samples during the storage period. The pH of F1 and F4 was reasonably steady (Table 3). A decrease of pH of F2 and F3 was observed, especially that of F3. The pH of F6-F8 was fairly constant whereas that of F5 decreased drastically from 6 to 3.25 after 35 days of storage. The change of pH of F5 was found to relate to the decrease of morphine concentration.

Table 1 demonstrates the fractional-ordered randomized blocked design showing the percentage of morphine remaining (in parentheses) after 35 days of storage at room temperature. In general, preparations with pH 4 demonstrated better stability than those of pH 6, regardless of substances added in the preparation. This result complied with the pH-rate profile of morphine (5) and previously reported (8-9). F1, F4, and F7 were most stable as more than 99% of morphine remained in the solution. Very interestingly, samples with sodium metabisulfite (F2, F3, F5 and F8) presented stability problems, F5 (pH adjusted

to 6) in particular, as only 65 % of morphine was retained.

To evaluate the presence or absence of each factor influencing morphine stability, the fractional-ordered randomized blocked was conducted. Table 4 demonstrates average percent morphine remaining with each factor after 35 days of storage at room temperature. The data are from Table 1. For example, to examine the presence of EDTA in the preparation, it can be achieved by obtaining the average value (morphine concentration) under the glycerin + block (Table 1). These values are 80.68 (F3), 101.39 (F4), 100.38 (F7) and 87.78 (F8). The resulting average value is therefore 92.56 (Table 4). On the other hand, the value for the absence of glycerin is 90.35. Thus, in this case the presence of glycerin in the formulation was preferred as it gave a higher average value (morphine remaining) than that of the absence. A similar procedure was applied to the other factors. Continuing the analysis indicated that the presence of EDTA and glycerin, in addition to the pH of the solution adjusted to 4, stabilized morphine in the solution system.

Table 4. Percent morphine remaining of each factor after 35 days (n=4)

Factors		**Maen ± SD**
pH	**4**	95.13 ± 10.0
	6	87.77 ± 15.7
Sodium metabisulfite	**–**	100.3 ± 12,2
	+	82.57 ± 12.9
Glycerin	**–**	90.35 ± 16.7
	+	92.56 ± 10.1
EDTA	**–**	87.25 ± 17.4
	+	95.66 ± 5.71

The long-term stability study

Formulation of morphine solution

For HPLC assay, it was found that the lower limit of detection was 1 µg/ml. The standard solution was prepared to obtain the solution of concentration of 25-125% of the sample concentration. The standard curve was freshly prepared and used for each analysis. All samples were analyzed with HPLC within a day of each analysis. The linearity of the standard curves between the morphine concentrations (x) and the peak area (y) of each assay was obtained (e.g. y = 8709x+7758). Correlation coefficients of the standard curves were greater than 0.999. It was found that the %CV of slope of standard curves (n=9) was 1.34 indicating high accuracy of the assay.

Formulations of morphine solution at a concentration of 10 mg/5 ml (0.2% w/v) are shown in Table 5. They were formulated based on the results obtained from the preformulation study. As pH was a major factor influencing morphine stability, the pH of all formulations was adjusted to approximately 4. After several trials, sorbitol, sodium chloride, sodium citrate buffer, tartaric solution and 4 different flavors at concentrations as shown were added for taste masking of morphine and for adjusting the pH of the preparation. Sodium metabisulfite was excluded according to the results obtained from the preformulation study. Morphine sulfate was first dissolved in a part of purified water. Ingredients as listed in each formulation were added and mixed using a magnetic stirrer, followed by pH and volume adjustment. It was found that clear solutions was observed, however, a slight bitter taste of morphine was still noticed. The color of the samples was very slightly yellowish. The stability of these samples was investigated under normal storage conditions, i.e., refrigerator and 25°C/75% RH.

Physical and chemical stability

No precipitation and color change were observed in any of the samples during the 13 months of storage in both storage conditions. The taste was virtually the same throughout the study period. Initial pH of the samples ranged 3.8-4.0. A slight increase of pH was observed in all samples (less than 0.3 units). The viscosity of the samples, however, was not measured in this study.

The percent morphine remaining in the 4 formulations over a 13 month period was greater than 97%. No difference was observed between formulations and storage conditions. The average pH of the samples (between 3.8 and 4.3) was able to minimize the degradation of morphine (5, 8-9).

Microbial stability

Total viable count test was used in this experiment to investigate microbial contamination. During 13 months of the study, no microbial contamination was observed in all samples in both storage conditions. From the positive control test, colonies of microorganisms studied were observed. This

Table 5: Four formulations of morphine sulfate oral solution

Component	F1	F2	F3	F4
Morphine sulfate	0.36 g	0.36 g	0.36 g	0.36 g
Glycerin	67.5 ml	81 ml	27 ml	90 ml
Syrup USP	67.5 ml	54 ml	90 ml	40.5 ml
Sorbitol	22.5 ml	36 ml	13.5 ml	22.5 ml
EDTA (w/v)	0.1%	0.1%	0.1%	0.1%
Paraben concentrate (v/v)	1 %	1 %	1 %	1 %
Sodium chloride solution[#]	2.4 ml	2.2 ml	2.20 ml	2.7 ml
Sodium citrate buffer (0.05M)	9 ml	13.5 ml	9 ml	9 ml
Tartaric acid solution[*]	2.5 ml	2.0 ml	2.2 ml	1.40 ml
Purified water to	180 ml	180 ml	180 ml	180 ml
pH adjusted to	3.82	3.98	3.87	4.00

Sodium chloride stock solution[#], 20 g in 50 ml purified water; Tartaric acid stock solution[*], 10 g in 35 ml purified water

demonstrated that the media support the growth of the microorganisms. However, amount of colony from positive test was not counted. Whereas those of negative control tests were free of microbial contamination. Similar results of negative and positive control tests were found in every test of microbial study during the study period.

DISCUSSION

Preformulation study

Apart from morphine sulfate, the master formula comprised syrup USP, paraben concentrate, and purified water (Table 1). Factors chosen in the preformulation study were sodium metabisulfite, glycerin, EDTA and pH of the solution. Since the major problem of morphine is oxidation reaction, thus, sodium metabisulfite was a recommended antioxidant as it has been reported to prevent oxidation of morphine (5). Glycerin was chosen as it has been stated to stabilize morphine (5). EDTA has been found to give affiliate effect with sodium metabisulfite for prevention of oxidation (5, 8). The two pH values of the solution (4 and 6) were selected to investigate the effect of pH according to the pH-rate profile of morphine (5, 8).

Samples containing EDTA have shown to stabilize morphine, especially, when compared to those with and without sodium metabisulfite (F3, F4, F7 and F8). This finding was similar to a previous report, that EDTA and sodium metabisulfite gave a synergistic effect to stabilize morphine (5, 8). It is noticeable that samples adjusted pH to 4 (F1) without addition of other ingredients were also chemically stable during the study period. Thus it can be concluded that pH was one of the major factors influencing the stability of morphine in the solution as previously described (5, 8-9).

The presence of sodium metabisulfite in the formulation, on the other hand, decreased the stability of morphine. This study gave opposite results to previous reports where the use of sodium sulfite, sodium bisulfite, sodium pyrosulfate stabilized morphine in solution systems (5, 8). However, this study gave similar results to several researches in that sodium metabisulfite has shown incompatibility problems with several drugs such as epinephrine (11-15), furosemide (16) and amitryptylline (17). In the case of epinephrine, it suggested to omit bisulfite in the preparation. Obvious degradation was observed in the presence of light as demonstrated. Isoprenaline and dopamine were also reported to present a similar degradation process to epinephrine (14). The degradation mechanism of these drugs has also been suggested. This is very interesting as the molecule of morphine and those reported, epinephrine (as an example), present a similar chemically reactive oxidizable group (-OH). Photostability is also a problematic degradation of both epinephrine and morphine. To prove the mechanism of bisulfite influencing the stability of morphine, further study must be undertaken.

From the preformulation study, it can be summarized here that pH is a major factor influencing morphine stability in solution system. The presence of EDTA and glycerin assisted morphine stability. In contrary, sodium metabisulfite can be problematic and is suggested to be excluded.

Long-term stability study

A decrease of odor was observed in all samples after 3 months of storage. This may be because of the small amount of flavor was added. A slight increase of pH was observed in all samples, it can be therefore concluded here that the solvent system and the buffering agents used were capable of controlling the pH of the solution. In addition, the reasonably constant pH of approximately 4 was related to morphine stability as higher than 97% of the drug was found.

The difference of the percentage of the vehicles between the formulations did not affect the stability of morphine. Other ingredients added such as tartaric acid and sodium citrate did not affect the stability of morphine, and in fact, improved the taste. It can be concluded that these 4 formulations of morphine sulfate solution were stable and can be kept in refrigerators or at the 25°C/75%RH for at least 13 months.

Sodium metabisulfite is known to prevent the oxidation reaction of many compounds, especially with phenolic groups (5, 8-10). Given this, many investigators have studied the use of sodium metabisulfite for stabilization of drugs. However, we found that morphine was less stable in the presence of sodium metabisulfite, similarly to that previously reported with other compounds (11-17). The results obtained from preformulation and long-term formulation studies gave similar results. The results therefore indicated that the use of sodium metabisulfite and high pH of the solution can be problematic for preparing morphine sulfate oral solution. Other factors assisted morphine stability, such as EDTA and glycerin, similar to other reports (5, 8). These two compounds are recommended to be added in the formulation. Finally, it is recommended that oral morphine solution must be kept from light exposure as recommended by other reports (5, 8). From the microbial stability test, no contamination of the samples was observed. Therefore 1% paraben concentrate was a suitable antimicrobial agent used in oral morphine sulfate solution.

CONCLUSION

This study demonstrates that a preformulation study using the fractional-ordered randomized block design can be used successfully to evaluate the factors affecting morphine stability. pH was proved to be a major factor influencing the stability of morphine since the change of ratios of the solvents used or the addition of other ingredients did not show any difference in the stability of morphine. It is strongly recommended that sodium metabisulfite not be included in the formulation. This finding also provides significant data to formulate stable oral morphine sulfate solution for at least 13 months storage with acceptable taste.

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