Stability of Morphine Solutions in Plastic Syringes Determined by Reversed-Phase Ion-Pair Liquid Chromatography

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Abstract □ A reversed-phase ion-pair HPLC assay has been developed for quantitating morphine, codeine, apomorphine, and pseudomorphine in aqueous solutions. Using two types of plastic syringes, the effect of light (25 W) and temperature (22 and 3 °C) on the stability of morphine, over a 12-week period, has been investigated in the presence and absence of preservative and antioxidant. The leaching of contaminants from the plastic syringes to water stored in them, for a period of up to 12 weeks, has also been investigated. The results indicate that <3% of the morphine is degraded in both types of plastic syringes, stored in light at 22 ± 2 °C. The degradation is even less prominent in the dark or at 3 °C. Pseudomorphine has been identified as the major degradation product. Using 5% degradation of drug as the criterion for the determination of the shelf-life of morphine, it was found that in one brand of plastic syringes, morphine has a shelf-life of the order of 20 and 33 weeks, in the absence and presence of preservative and antioxidant, respectively. In the other brand of plastic syringe, the drug has a shelf-life of >1 year. Some unidentified leached contaminants have been detected in water stored in both brands of syringes.

Use of pre-filled morphine syringes for the treatment of chronic pains has become common for terminal outpatients as it precludes the possibility of ampule shattering and maintains the accuracy of dose and sterility of the preparation.[1-7] Epidural administration of morphine has also been practiced for post-operative analgesia.[2-6] Sterility of the formulation is of prime importance for epidural injections, in which preservatives cannot be included.[8]

Several reports have demonstrated the long-term stability of morphine solutions in glass ampules at room temperature.[9-11] However, conflicting reports have been published regarding the stability of preservative-free morphine solutions when stored in plastic syringes.[6,11,12] Whereas the studies by Bray et al.[12] and Gove et al.[6] have demonstrated adequate stability of these solutions for a period of several days, a report by Orr and McBride[11] has indicated that in plastic syringes, morphine degrades within 20 min at room temperature. This is of concern as morphine solutions are required to be stored in syringes for several hours when used as infusions, and for several days when dispensed as pre-filled syringes for cancer outpatients.

It has been suggested that upon storage, morphine degrades into apomorphine.[11] Other studies have identified pseudomorphine as the degradation product.[10,13-17] It is important to confirm the degradation product(s) because they have different therapeutic activity and hence may warrant corresponding scheduling of the doses of morphine.

Plastic containers are known to adsorb the components of the formulation stored in them.[18-25] Conversely, these containers may leach several undesirable components into the formulation.[20] Leaching of contaminants from the rubber plunger of disposable plastic syringes has also been reported.[23-25] In view of these reports, it is important to deduce the possibility of contamination of morphine solutions when stored over long periods in plastic syringes.

In this study, a reversed-phase ion-pair HPLC assay has been developed to quantitate morphine, its proposed degradation products, and a preservative chlorocresol. The assay also provides the opportunity to identify the decomposition products of morphine, following its long-term storage in two commonly used brands of plastic syringes. As a part of the stability study, the following aspects have been investigated: (*i*) the major decomposition product(s) of morphine; (*ii*) the shelf-life of morphine; and (*iii*) leaching of contaminants from the disposable plastic syringes.

Experimental Section

Apparatus—Chromatography was carried out using a Waters Associates M 6000A pump (Milford, MA), a M 441 selectable wavelength UV detector, and a rheodyne M 7125 injector fitted with a 20-μL sample loop. The same over-filling technique, as recommended by the manufacturer, was adopted for each injection. The chromatographic column was a 100 × 4.6-mm i.d. stainless steel, slurry packed with 5 μm Hypersil ODS (Shandon Southern Products, London). The chromatograms were recorded on a Rikadenki chart recorder MR 202 (Rikadenki Kogyo Company, Ltd., Tokyo). Infrared spectra were obtained using a Beckman Acculab 1 spectrophotometer (Beckman Instruments, CA). The ultraviolet spectra of the chromatographic peaks were obtained using the stop-flow scan technique with the Shimadzu UV−240 detector (Shimadzu Corp., Kyoto) equipped with a 8-μL flow cell. An electrothermal melting point apparatus (London) was used for the melting point determination of pseudomorphine.

The two most commonly used disposable syringes in this country, type A and type B, were obtained from Japanese Medical Supplies Company, Ltd. (Hiroshima) and Terumo Pty, Ltd. (Melbourne, Australia), respectively. The syringes were filled using a BD filling outfit (Becton Dickinson and Company, NJ), attached to Pharm-aide syringe tip connectors and fluid dispensing system (American Pharmaseal, CA). The syringes were capped with Pharm-aide syringe caps. Light was provided by a SL*25 (1200 lumen) Prismatic bulb (Philips, Holland). Illumination was monitored using a model 5511 luxmeter (East Kilbride Instruments, Ltd., Scotland). All pH measurements were made using a Solstat EPM 1500 pH meter (Solstat Industries, Ltd., Christchurch, NZ).

Materials—Morphine sulphate was supplied by Douglas Pharmaceuticals (Auckland, NZ). Apomorphine HCl was obtained from Sigma Chemicals (St. Louis, MO). Codeine phosphate was purchased from McFarlan Smith, Ltd. (Edinburgh, Scotland). Sodium dihydrogen orthophosphate, chlorocresol, potassium hydroxide, and sodium lauryl sulphate (99% pure) were from BDH (Poole, UK). Orthophosphoric acid, concentrated ammonia solution, methanol, and sodium metabisulphite were supplied by May & Baker Pty, Ltd. (Victoria, Australia). Potassium ferricyanide and acetonitrile (HPLC grade) were obtained from J. T. Baker (Phillipsburg, NJ). Pyridine (AR grade) was from Maehlinckrodt Chemical Works (St. Louis, MO). Water was glass distilled and MilliQ filtered. All glassware was

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silanized using Aquasil from Pierce Chemical (Rockford, IL). All reagents were AnalR or equivalent grade.

Synthesis of Pseudomorphine—Morphine sulphate (0.5 g) was dissolved in 50 mL of potassium hydroxide (0.4% w/v), to which 20 mL of potassium ferricyanide solution (2.9% w/v) was added slowly over a period of 50 min with constant stirring. The stirring was continued for an additional 30 min. The resulting precipitate was filtered and washed several times with hot methanol. About 10–20 mL of concentrated ammonia solution (~25% ammonia in water) was then added until the precipitate just dissolved. The volume of the soluble components was made up to 35 mL, heated to boiling, and then filtered. The precipitate obtained following this process was allowed to dry in a vacuum oven and then used for the infrared, ultraviolet, and melting point determinations.

Chromatography—The mobile phase consisted of 50% (v/v) acetonitrile in water, with 100 mM sodium lauryl sulphate, 40 mM sodium dihydrogen phosphate, and pH adjusted to 3 with orthophosphoric acid. A flow rate of 2 mL/min and a detection wavelength of 254 nm were employed.

Stability Study—One hundred and eight plastic syringes each of type A and type B were randomly divided into three equal groups. Group one, representing a formulation for epidural injections, was filled with a pure aqueous solution of morphine (2 mg/mL); group two, representing a formulation for intravenous administration, was filled with morphine solution (5 mg/mL) containing 0.2% (w/v) preservative (chlorocresol) and 0.1% (w/v) antioxidant (sodium metabisulphite); and group three was filled with distilled water. Within each group, the syringes were further randomly divided into three equal groups of 12 each. These were then placed in one of the following storage conditions: (*i*) ambient room temperature (22 ± 2 °C) under light; (*ii*) ambient room temperature (22 ± 2 °C) protected from light; and (*iii*) in a refrigerator (3 °C). For samples stored under light, a 25 W (1200 lumen) prismatic bulb was used continuously just above the syringes. The illumination varied between 5000 to 20000 lux due to the light entering through the windows. The syringes stored in the dark were wrapped in black polyethylene bags and placed in a box lined with black polyethylene.

From each group of 12 syringes, two syringes were randomly removed after 1, 2, 4, 6, 8, and 12 weeks. The contents of each syringe was immediately placed in a silanized scintillation vial and wrapped with aluminum foil to protect it from light. These samples were maintained at −15 °C until analyzed using HPLC. The samples were analyzed randomly by directly injecting the contents onto the HPLC column. Calibration curves for morphine and pseudomorphine were prepared each day, and peak heights were used for the quantitation.

Results and Discussion

Synthesis of Pseudomorphine—Due to the nonavailability of this compound from commercial sources, pseudomorphine was synthesized by oxidation of morphine with potassium ferricyanide. Previous methods for the synthesis of this compound involved the use of concentrated ammonia solution[26] or pyrridine,[27] to dissolve the precipitated pseudomorphine. However, the use of pyridine failed to dissolve the precipitate in our laboratory. Hence, the method of Bentley and Byke,[26] utilizing ammonia solution, was adopted. This method readily produced pseudomorphine as a fine white powder, with a melting point of 315 °C, which adequately resembles the published value of 327 °C.[28] The infrared and ultraviolet spectra of this compound also closely matched the published profiles.[29,30]

Chromatography—Several methods have been reported in the literature for the analysis of morphine and its congeners.[31,32] However, these reports do not provide sufficient information on the retention and separation of all four compounds of interest in the present study. Hence, an HPLC assay for the quantitation of these solutes in aqueous medium was developed as a part of this investigation. Morphine, codeine, apomorphine, and pseudomorphine are basic in nature. This will therefore permit their retention on C-18 silica support by the addition of an anionic hydrophobic pairing ion at low pH.[33,34] In this study, sodium lauryl sulphate was used as the pairing ion because of its adsorption characteristics on the C-18 support.[33,34] The organic modifier concentration, pH, and ionic strength of the eluant were empirically optimized as described previously.[35–37] The variation in k' for these compounds as a function of mobile phase pairing ion concentration is presented in Figure 1, where it can be seen that all the solutes pass through the predicted maxima.[33,34] This can be explained in the light of ion-exchange desolvation mechanism.[34] A mobile phase containing 50% (v/v) acetonitrile and 100 mM sodium lauryl sulphate was used as it provides adequate resolution and retention of all the solutes of interest, including the preservative chlorocresol. In addition, at this mobile phase composition, the retention of these ionic solutes is least sensitive to batch-to-batch variation of the eluant, and thus maintains the desired reproducibility. Figure 2 shows a representative chromatogram obtained under the optimal conditions. The linearity of the assay under different concentrations of ionic solutes is summarized in Table I. All standard curves were linear with correlation coefficient values >0.97. The within and between day coefficient of variation, based on six determinations of concentrations of 20, 70, 100, and 700 μg/mL, was ≤4%. Taking a signal-to-noise ratio of 3 as the criterion, the detection limit for the aqueous solutions of morphine, codeine, apomorphine, and pseudomorphine are 0.5, 1.0, 0.1, and 0.1 μg/mL, respectively.

Stability Study—Routine accelerated stability studies involve storage of test samples at elevated temperatures and fitting the Arrhenius equation to the relative degradation rate constants.[38] Such experiments are unsuitable for the present study as the use of high temperature may adversely affect the components of the syringe and induce reactions which normally do not take place at room temperature.[38] It was therefore decided to use those conditions in the stability study which are encountered in hospitals or the patient's home (i.e., ambient room temperature with light or darkness, or 3 °C, as in refrigerators). To minimize the contamination, the morphine samples from the pre-filled syringes were directly injected onto the column.

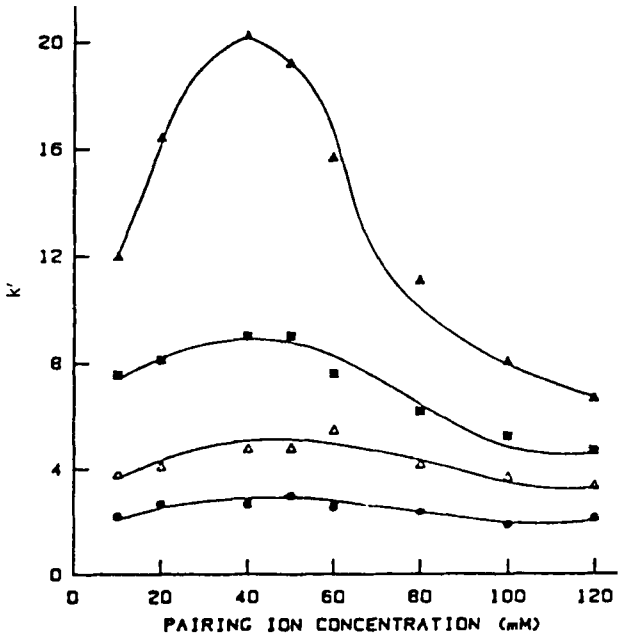

Figure 1—*Variation in k' of morphine (●), codeine (△), apomorphine (■), and pseudomorphine (▲) with the concentration of sodium lauryl sulphate. The mobile phase was 50% (v/v) acetonitrile buffer containing 40 mM disodium hydrogen phosphate at pH 3. The flow rate was 2 mL/min.*

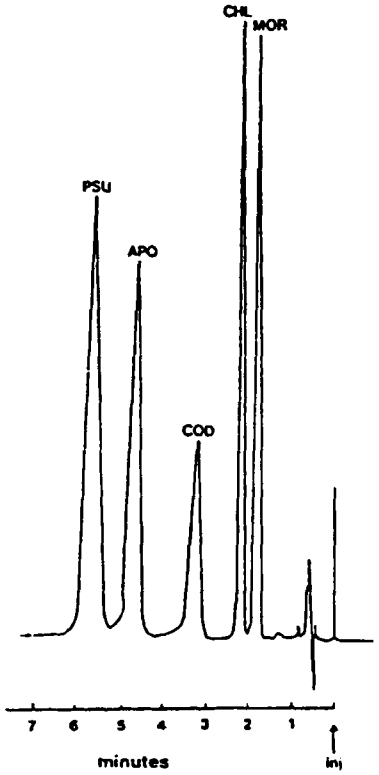

Figure 2—*A representative chromatogram of an aqueous mixture containing 0.1 mg/mL of morphine* (MOR), *0.5 mg/mL of chlorocresol* (CHL), *0.05 mg/mL of codeine* (COD), *0.025 mg/mL of apomorphine* (APO), *and 0.025 mg/mL of pseudomorphine* (PSU). *The chromatographic conditions are given in the Experimental Section.*

No discoloration or precipitation was observed in the solutions stored in syringes under different conditions over the 12-week period. Analysis of these solutions identified pseudomorphine as the major degradation product, in the preservative- and antioxidant-free, as well as the preservative- and antioxidant-included samples. A minor peak, 'X', which had retention time and UV spectrum similar to codeine, was also detected. However, this peak was also observed in the fresh morphine samples. In addition, no significant increase in the height of this peak was observed with increase in the storage time over 12 weeks. Hence, it appears that codeine is present as a contaminant in morphine samples.

In preservative- and antioxidant-free morphine solutions, which were stored in light for >4 weeks, another small degradation peak, 'Y', was detected. The retention time of this peak (3.8 min) did not correspond to any other compound used in this study. Because of its minute quantity in the sample, the UV spectrum for this peak could not be recorded accurately. This peak was less evident in solutions containing preservative and antioxidant, even when they demonstrated equivalent amounts of pseudomorphine. Typical chromatograms of the morphine solutions, after twelve weeks of storage in light in the two brands of syringes, in the absence and presence of preservative and antioxidant, are displayed in Figures 3 and 4, respectively. It can be seen that a greater amount of pseudomorphine is formed in solutions stored under light and in type A syringes, compared with that in type B syringes under the same conditions. It appears that the higher degree of opacity offered by type B syringes may have allowed greater protection to the drug from light,

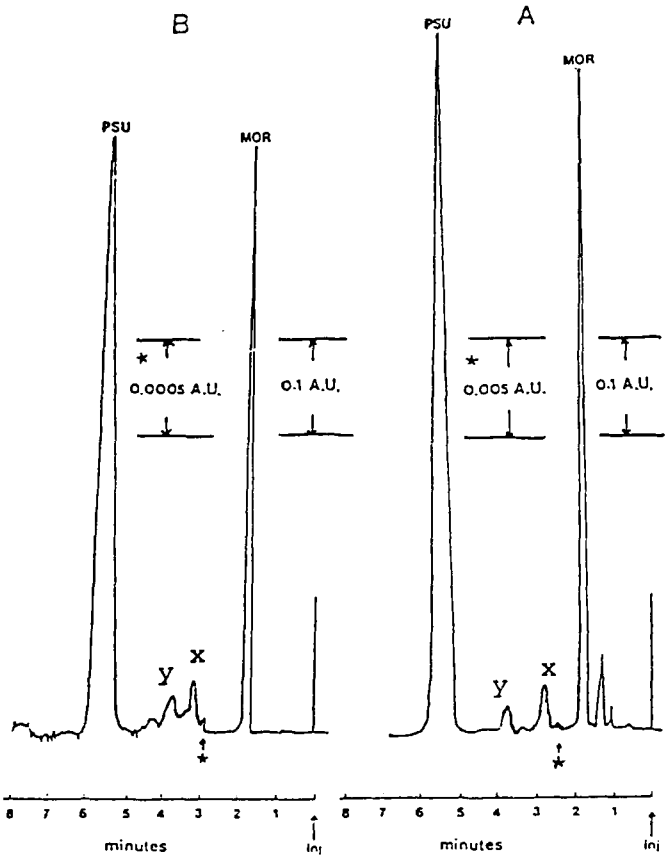

Figure 3—*Representative chromatograms of preservative- and antioxidant-free morphine solutions stored under light at 22 ± 2 °C for 12 weeks in* (A) *type A syringes and* (B) *type B syringes. Key:* 'X' *the peak which has retention time similar to that of codeine;* 'Y' *the unidentified peak with a retention time of 3.8 min;* (*) *the time during elution when the detector response was switched to a higher sensitivity level.*

Table I—Regression Data for the Calibration Curves of Morphine, Pseudomorphine, Codeine, and Apomorphine

Compound	*t*, min[a]	Concentration Range, μg/mL	Slope[b]	Intercept[b]	r^2
Morphine	1.80	500–5000	6.934 (0.319)	0.007 (0.0003)	0.999
		10–500	0.018 (0.001)	−0.014 (0.0008)	0.996
Pseudomorphine	5.55	0.2–5	4.823 (0.201)	0.014 (0.0007)	0.999
		5–100	2.157 (0.151)	0.079 (0.0063)	0.999
Codeine	3.15	1–10	0.094 (0.004)	−0.028 (0.0014)	0.997
Apomorphine	4.60	10–100	0.060 (0.003)	−0.016 (0.0010)	0.999

[a] Retention time for different solutes. [b] Mean of five within-day calibration curves; the data in parentheses represent standard deviations.

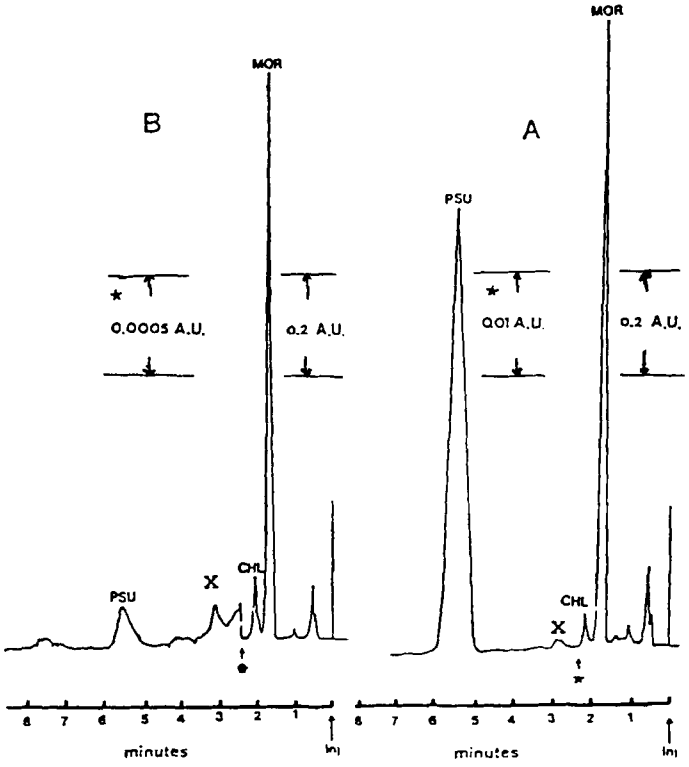

Figure 4—*Representative chromatograms of morphine solution containing preservative and antioxidant, stored under light at 22 ± 2 °C for 12 weeks in* (A) *type A syringes and* (B) *type B syringes. The symbols 'X' and* (*) *are as defined in Figure 3, and* CHL *and* PSU *are defined in Figure 2.*

and thus may have restricted the formation of pseudomorphine.

Figure 5 compares the formation of pseudomorphine in light in both types of syringes, in the absence and presence of preservative and antioxidant. The plots reinforce the fact that greater amounts of pseudomorphine are formed in the type A syringe than in the type B syringe. Of particular interest is the observation that the presence of preservative and antioxidant in type A syringes favors the formation of pseudomorphine. There is no ready explanation for this observation.

Conventionally, the quantitation of the shelf-life of a drug involves determination of loss of drug as a function of time

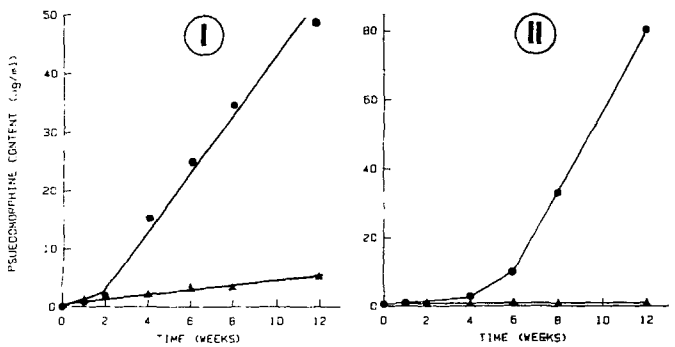

Figure 5—*Plots showing pseudomorphine formation, under light at 22 ± 2 °C, as a function of storage time in preservative- and antioxidant-free morphine solution* (I), *and in morphine solution containing preservative and antioxidant* (II). *Key:* (●) *type A syringes;* (▲) *type B syringes.*

and computation of the degradation rate constant at ambient room temperature or other prescribed temperature.[38] However, in the present study, very minute changes in the morphine content were detected under all test conditions over the 12-week storage period. In addition, the coefficient of variation of the analysis was greater than the percentage of the drug decomposed. Recently, Taylor et al.[39] have suggested that the quantitative measurement of a major decomposition product, rather than that of the undecomposed drug, provides a better evaluation of the stability of a drug. Hence, this approach was adopted in the present study.

Since all the peaks emerging from degradation product(s), except that for the pseudomorphine, were very small, morphine was assumed to decompose solely to pseudomorphine. The mean pseudomorphine content in type A and type B syringes, over a 12-week storage period under different conditions and in the absence and presence of preservative and antioxidant, is listed in Table II. The table also includes the data for percentage morphine degradation under these conditions. It can be seen that in the absence of preservative and antioxidant, the maximum morphine degradation at room temperature and in the presence of light is <3%. In the absence of light or at 3 °C, the degradation is of the order of 0.02 to 0.13%. When preservative and antioxidant are present, the maximum degradation at room temperature and in the presence of light is <2%, and this figure reduces to ≤0.01% in the absence of light or at 3 °C. It must be noted that in type A syringes, regardless of the presence of preservative and antioxidant, the samples stored in light tend to exhibit a lag phase before the degradation of morphine or the formation of pseudomorphine acquires a steady rate. This may be attributed to the presence of an antioxidant in plastic used for the fabrication of type A syringes. It is also important to note that in general, type B syringes offer higher stability to morphine solutions than type A syringes.

A 10% loss of active substance in any drug formulation is the usual accepted level for the prediction of shelf-life.[38] For morphine injections, the dose is critical and hence, 5% loss is acceptable for the shelf-life estimations.[10] On the basis that two moles of morphine are required to form one mole of pseudomorphine, 5% degradation of morphine occurs when pseudomorphine levels reach 85 μg/mL for the 2-mg/mL preservative- and antioxidant-free solutions, and 213 μg/mL for the 5-mg/mL morphine solutions containing preservative and antioxidant. Assuming zero-order degradation kinetics,[5,9,39] it was found that, when stored in light at ambient room temperature, the shelf-life of preservative- and antioxidant-free morphine solutions in type A syringes is ~20 weeks. Inclusion of preservative and antioxidant increases the shelf-life to ~33 weeks. However, regardless of the presence of preservative and antioxidant, the morphine solutions in type B syringes, stored under light at room temperature, exhibit a shelf-life of >1 year.

Several unidentified peaks at 254 nm were observed in the water samples stored in both types of syringes over a period of 12 weeks. Storage of syringes in the dark or 3 °C did not prevent the occurrence of these peaks. Whereas the prominence of these peaks increased with an increase in storage time in type A syringes, the samples from type B syringes exhibited a constant peak height as a function of time. These results confirmed the leaching of certain unknown components from the syringes. Occurrence of these peaks at different positions, however, indicated the presence of different contaminants in the two types of syringes. In view of the possibility of toxic action due to the leached components from plastic containers,[22,40,41] further work is required to isolate and investigate the pharmacological activity of these components. This is particularly important for type A syringes,

Table II—Effect of Temperature (22 and 3 °C) and Light (25 W) on the Degradation of a Morphine Solution

Storage Condition	Storage Time, weeks	Pseudomorphine Content, μg/mL[a]		Pseudomorphine Content, μg/mL[b]	
		Type A	Type B	Type A	Type B
Light and 22 °C	0	0.09 (0.005)	0.09 (0.005)	0.61 (0.01)	0.61 (0.01)
	1	0.84 (0.05)	1.06 (0.06)	0.99 (0.02)	0.57 (0.01)
	2	1.89 (0.11)	1.61 (0.09)	0.76 (0.02)	0.59 (0.01)
	4	15.21 (0.89)	2.05 (0.12)	3.02 (0.07)	0.63 (0.01)
	6	24.63 (1.23)	3.18 (0.15)	10.84 (0.07)	0.52 (0.01)
	8	34.51 (2.03)	3.30 (0.19)	33.14 (0.78)	0.56 (0.01)
	12	47.72 (2.80)	5.17 (0.30)	80.32 (1.90)	0.63 (0.01)
Dark and 22 °C	0	0.09 (0.005)	0.09 (0.005)	0.61 (0.01)	0.61 (0.01)
	1	0.66 (0.04)	0.36 (0.02)	0.68 (0.02)	0.43 (0.01)
	2	0.46 (0.03)	0.41 (0.02)	0.30 (0.005)	0.52 (0.01)
	4	0.58 (0.03)	0.98 (0.06)	0.40 (0.01)	0.48 (0.01)
	6	0.61 (0.04)	1.14 (0.10)	0.53 (0.01)	0.46 (0.01)
	8	0.64 (0.04)	2.13 (0.13)	0.66 (0.02)	0.48 (0.01)
	12	0.83 (0.05)	2.16 (0.13)	0.42 (0.01)	0.55 (0.01)
Dark and 3 °C	0	0.09 (0.005)	0.09 (0.005)	0.61 (0.01)	0.61 (0.01)
	1	0.29 (0.02)	0.44 (0.02)	0.48 (0.01)	0.63 (0.01)
	2	0.26 (0.02)	0.36 (0.03)	0.77 (0.02)	0.53 (0.01)
	4	0.41 (0.02)	0.56 (0.03)	0.45 (0.01)	0.47 (0.01)
	6	0.51 (0.02)	0.47 (0.03)	0.53 (0.01)	0.38 (0.01)
	8	0.58 (0.03)	0.32 (0.02)	0.38 (0.01)	0.24 (0.005)
	12	0.32 (0.02)	0.46 (0.03)	0.62 (0.01)	0.44 (0.01)

[a] Pseudomorphine content in absence of preservative and antioxidant; the figures in parentheses represent percent morphine degradation under similar conditions. [b] Pseudomorphine content in the presence of preservative and antioxidant; the figures in parentheses represent percent morphine degradation under similar conditions.

where the proportion of leached components increases with storage time.

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