

# Physico-Chemical and Microbiological Study for the Stability of Phenytoin Sodium Extemporaneously Compounded Suspension in Saudi Arabia Hospitals

Syed Ata ur Rahman<sup>1,2</sup>, Abdullah Alsaedi<sup>1</sup>, Abdulelah Alhusayni<sup>1</sup>, Abdulmalik Alqurshi<sup>1</sup>, Sameh Ahmed<sup>3</sup>, Yaser M. Alahmadi<sup>4</sup>, Alaa Omer M. Abdullaal<sup>5</sup>, Badr Ahmed A. Taher<sup>5</sup>, El-Sayed E. Habib<sup>1,6</sup>

<sup>1</sup>Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Taibah University, Madinah Munawwarah, Kingdom of Saudi Arabia

<sup>2</sup>Department of Pharmacy, College of Health Sciences and Nursing, AlRayan College, Madinah Munawwarah, Kingdom of Saudi Arabia

<sup>3</sup>Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Madinah Munawwarah, Kingdom of Saudi Arabia

<sup>4</sup>Department of Clinical and Hospital Pharmacy, College of Pharmacy, Taibah University, Madinah Munawwarah, Kingdom of Saudi Arabia

<sup>5</sup>Pharmacy Department, Madinah Maternity and Children Hospital, Madinah Munawwarah, Kingdom of Saudi Arabia

<sup>6</sup>Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt  
Email: saur9186@gmail.com, sa.urrahman@amc.edu.sa

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## Abstract

Epilepsy is a chronic and the fourth most common neurological disorder which affects people of all age groups. Recently research and awareness on epilepsy-related deaths have rapidly grown over the past two decades. Many previous studies are attributed to the guidelines that apprise health care professionals in handling these deaths, but there is a relative scarcity of information accessible for clinicians and pharmacists who are responsible for manufacturing or preparing the extemporaneous anti-epileptic suspensions in the hospitals. Mostly in partial seizures, phenytoin is one of the first-choice drugs. In Saudi Arabian hospitals, the extemporaneous preparation of phenytoin suspension is common, but the hot climatic weather in Saudi Arabia possesses stability problems that should be tackled as the prepared suspension should pass all the stability tests to ensure uniform dosage of the extemporaneous formulation. In the current study, the commercial capsules were used to prepare the oral phenytoin sodium extemporaneous suspension. The physical, chemical and microbiological stability of phenytoin sodium suspen-

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sion is analyzed at various temperatures.

## Keywords

Physical Stability, Phenytoin, Extemporaneous Preparation, Chemical Stability, Epilepsy

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## 1. Introduction

Epilepsy is a chronic, non-contagious disease of the brain which affects people of all ages. As stated by a World health organization (WHO) analysis, worldwide 50 million people suffer from epilepsy, thus making it one of the most common neurological disorders [1]. It is a noncommunicable chronic disease of the brain where electrical imbalances occur. If properly diagnosed and treated, almost 70% of patients affected by epilepsy could survive a convulsion-free life [1]. Due to epilepsy the mortality rate is impetuously increasing and is three times greater than the death rate of the general population [1].

Phenytoin is an efficacious anti-epileptic drug that is used to prevent focal and tonic-clonic seizures (grand mal seizure) [2]. It is commercially sold in both solid and liquid dosage form as a chewable tablet, extended-release capsule and oral suspension as well. Phenytoin is used in neonates, infants and children as an oral suspension form which is not readily available most of the time [3]. Thus, to overcome the non-availability most hospitals in Saudi Arabia prepare the extemporaneous oral suspensions from capsules by dissolving them in an alternative vehicle in the absence of suitable suspending vehicles. The suspension form allows the patient to intake when swallowing difficulties are observed. Thus, the stability of such formulations is crucial [4].

A pharmaceutical formulation needs vigilant determination to ensure a uniform drug distribution while administration as sedimentation may occur which will result in inhomogeneous dosage [5]. A standard formulation will maintain its stability under all relevant conditions. In many previous studies, the stability of such extemporaneous formulations has been conducted, but in the cold to moderate climates *i.e.*, temperature range from 4°C - 25°C only.

In the current research, various temperature ranges have been covered *i.e.* temperature range from 4°C, 25°C, 40°C and 54°C, so that, in some middle east countries where the temperature rises up to 54°C during summer can also be observed [6]. These countries encounter issues in maintaining the stability of such pharmaceutical formulations during transportation and other various conditions [7]. Moreover, it will be unfavorable for middle wage earners to find a suitable storage condition *i.e.*, below 25°C to store these formulations.

This study aims to analyze the physicochemical and microbiological stability of extemporaneous suspension of an anti-convulsant drug *i.e.*, Phenytoin Sodium in various temperature range *i.e.*, temperature range from 4°C to 54°C, thus observing the stability in high climatic conditions.

## 2. Materials and Methods

This study was conducted at Taibah university research laboratory, Madinah Munawara, Saudi Arabia, between April 2019 to May 2020.

### 2.1. Chemicals and Reagents

Capsules containing 100 mg of phenytoin sodium (commercially available in Saudi Arabia) were used. Fluka analytical, US A supplied dextrose. Acetonitrile was of HPLC gradient grade obtained from Honeywell, CHROMASOLV® ≥ 99.9%, Germany. The standard pH calibration solutions (4.0 and 7.0) were purchased from Fisher Scientific, UK. All the other reagents and chemicals used were of analytical grade and purchased from Fluka analytical, USA and purified water was provided using a (Direct 8) Milli-Q® system of water purification, France.

### 2.2. Extemporaneous Preparation

The oral Phenytoin sodium extemporaneous suspension (20mg/mL) was prepared by suspending capsules. According to the USP 39/NF 34, the structured suspension vehicle was prepared which consist of dextrose (50% w/v) and purified water (1:3) [8]. The suspensions were filled in polyethylene plastic bottles and stored at 4°C, 25°C, 40°C and 54°C for up to 28 days. All samples were preserved from light.

#### 2.2.1. Physicochemical Stability Test

Physical characteristics, including color, odor, pH, viscosity and sedimentation volume were tested every week. Chemical stability was tested by a validated reverse phase HPLC assay (Shimadzu SIL-10AP), using a mixture of acetonitrile and 10 mM phosphate buffer (pH = 3.5) (40:60) as a mobile phase, while monitoring effluent at 220 nm.

#### 2.2.2. pH Measurement

The pH of phenytoin sodium suspension extemporaneous preparation was measured by pH meter (Hanna-pH 211, USA) on days 0 and 28. The pH meter was calibrated using a Fisher Scientific, UK standard buffer solutions (pH 4.0 and pH 7.0).

#### 2.2.3. Sedimentation

The phenytoin sodium extemporaneous suspension was stored in a 100 mL measuring cylinder at 4°C, 25°C, 40°C and 54°C after preparation. The sediment of suspensions was recorded on days 0 and 28. The volume of sedimentation was determined by  $F = Vu / Vo$ ; where  $F$  = volume of sedimentation,  $Vu$  = sediment volume (mL) and  $Vo$  = suspension volume (mL), respectively.

#### 2.2.4. Viscosity

The phenytoin sodium extemporaneous suspension was kept at 4°C, 25°C, 40°C and 54°C and the viscosity were determined on days 0 and 28. The viscosity was

recorded using Rotational Viscometer (Cannon-Model 2020) using spindle LV1 at various speeds of 20, 50, and 100 rpm, respectively.

### 2.3. Microbial Stability Test

A microbiological test of the Phenytoin suspension was performed according to the United States Pharmacopeia monograph, Microbiological examination of non-sterile product [9]. Microbiological stability was performed on Phenytoin suspension stored for 4 weeks at 4°C, 25°C, 40°C and 54°C. Total aerobic microbial count (TAMC), total combined yeast and moulds (TYMC) and *E. coli* were examined. The suspension was diluted with Müller-Hinton Broth in a dilution of 1:10. A portion of diluted suspension was then streaked on Tryptic Soy Agar (TSA) plate for bacteria determination. The plates were incubated at 37°C for 24 - 48 hours to check the presence of bacteria. While, to detect the presence of yeast and mould, the diluted suspension was streaked on the Sabouraud-Dextrose Agar (SDA) plate. The plates were incubated at 25°C for 120 - 144 hours. For examination, the presence of *E. coli*, the diluted suspension was used to inoculate in 100 ml Tryptic Soy Broth (TSB) and incubated at 37°C for 24 hours, this broth sample was sub cultured on a MacConkey agar plate and incubated at 37°C for 24 - 48 hours, *E. coli* can be detected as the growing of brick-red colonies.

The Microbial count was considered to be the average number of colony-forming unit (cfu) growing on agar. The experiment was performed in a triplicate.

After stability storage, all samples were transferred to a validated 4°C storage unit scheduled for testing. On the testing day, samples were moved into a 25°C water bath allowing them to reach room temperature, following so samples were shaken well and tested for appearance and smell, pH level and sedimentation volume.

### 2.4. Ethical Consideration

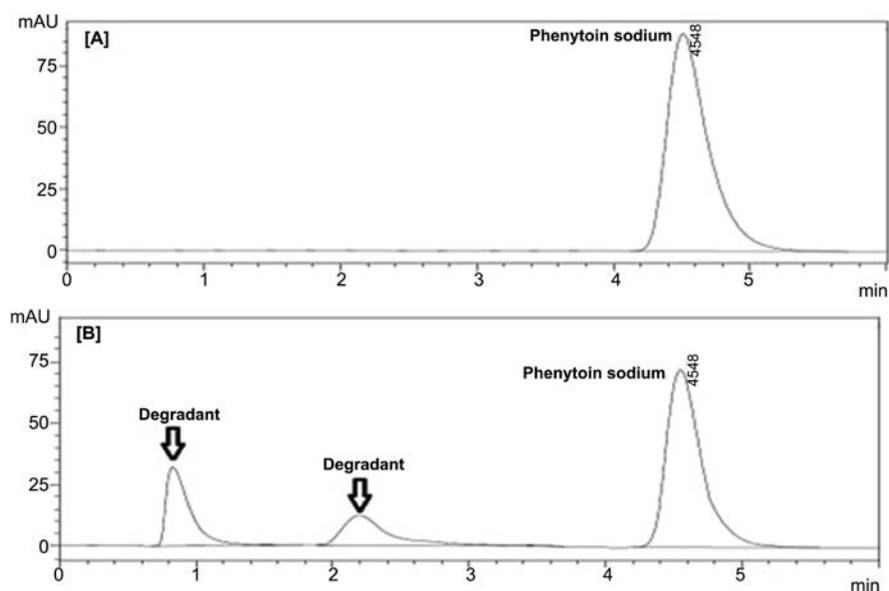
The ethical acceptance was sought and successfully obtained from the Taibah University ethics committee to carry out the research.

## 3. Results and Discussion

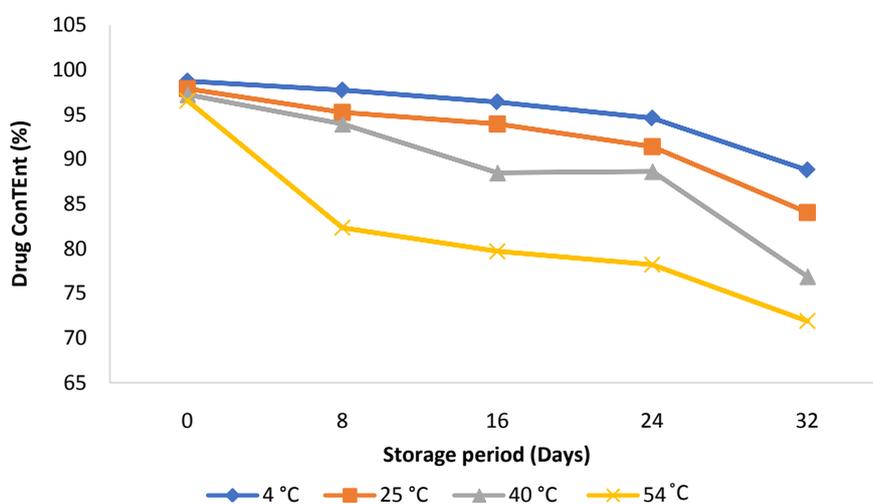
### 3.1. Physico-Chemical Stability

#### 3.1.1. Drug Analysis

The HPLC technique was adopted to determine Phenytoin sodium. The retention duration of phenytoin sodium was 4.2 - 4.6 mins respectively (Figure 1). The results displayed that the percent concentration of phenytoin sodium constantly remained above 90% of the initial concentration after storage for 28 days at 4°C, 25°C, 40°C and 54°C in polyethylene plastic containers. The initial concentration percentage was in a range of 72.64% - 99.78% as illustrated in Figure 2.



**Figure 1.** HPLC Chromatograms of phenytoin sodium extemporaneous suspension. [A] QC sample. [B] After storage at 54°C for 28 days.



**Figure 2.** Monitoring of drug content for 32 days.

Drug content percentage of 20 mg/ml phenytoin sodium extemporaneous preparation after storage in polyethylene plastic container at 4°C, 25°C, 40°C and 54°C on days 0, 8, 16, 24 and 32.

### 3.1.2. pH Measurement

The results concluded that the pH of phenytoin sodium suspension structured vehicle was about 4.2 - 4.3. The 20 mg/mL oral phenytoin sodium extemporaneous suspension was observed white foamy and readily re-dispersible suspension. After freshly prepared, the pH of phenytoin sodium extemporaneous suspension increased to 10.57, this elevation of pH was caused due to the sodium salt of the drug. The pH values were rather constant in a range of 4.56 - 10.57 after storage for 28 days (Table 1).

**Table 1.** Appearance, smell and pH of phenytoin sodium after storage at 4°C, 25°C, 40°C and 54°C for 28 days.

Storage period (days)	Sample	Appearance & smell	Storage Temp.	pH Reading	Avg. pH	STD
0	-	White foamy	4°C	10.73	-	-
28	A	No change in color or odor	4°C	10.56	10.57	0.01
	B			10.56		
	C			10.59		
28	A	Color changed to light Caramel—Sweet smell	25°C	8.47	8.69	0.16
	B			8.83		
	C			8.76		
28	A	Color changed to Caramel—Sweet smell	40°C	5.58	5.59	0.03
	B			5.56		
	C			5.63		
28	A	Color changed to dark Caramel—Post sweet smell	54°C	4.72	4.56	0.14
	B			4.37		
	C			4.58		

### 3.1.3. Sedimentation Volume

The sedimentation volumes (F) were between 0.16 and 0.72 (**Table 2**). However, prominent caking and clumping of suspended particles was observed at 40°C and 54°C. This result attributes that the structured vehicle of this suspension was a desirable vehicle for excellent physical stability.

### 3.1.4. Viscosity

After storing for 28 days at 4°C, 25°C, 40°C and 54°C, the viscosities were significantly constant from the freshly prepared suspensions at the same speed of needle (**Figure 3**).

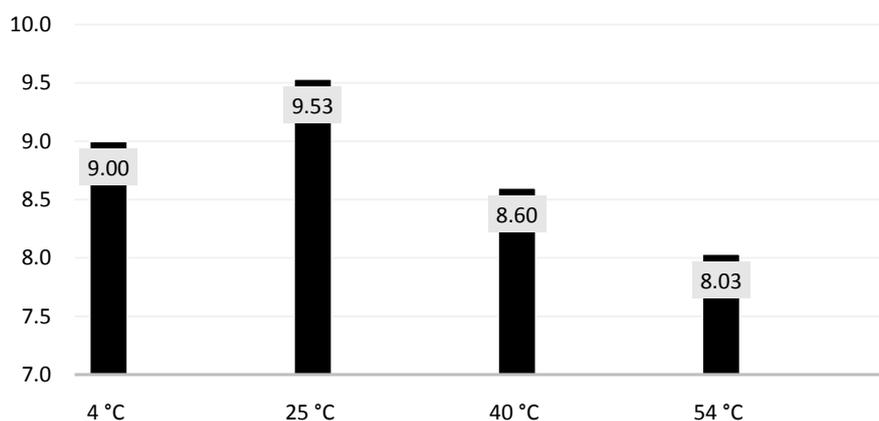
## 3.2. Microbial Stability

In nonsterile liquid formulations, the microbial contamination may generate turbidity, bad odor, and also affect appearance and palatability. Specifically, in immunocompromised patients, the elevated level of microorganisms may be hazardous to health. By-product of microorganism metabolism may induce a change in the pH of the preparation and reduce the chemical stability. To use extemporaneous preparation after its beyond-use date, a preservative is required. In this current study, potassium sorbate was used as a preservative in the structured suspension vehicle.

The microbial stability study indicated that the total aerobic microbial count (TAMC) was less than 8 cfu/ml, while the total yeast and mold count (TYMC) was less than 3 cfu/ml in the storage conditions at 40°C and 54°C in the 4th week. Furthermore, there was no *E. coli* or bacterial contamination detected in the MacConkey media plates of any samples. This result attributes that phenytoin

**Table 2.** Sedimentation volume of phenytoin sodium extemporaneous suspensions after 28 days at 4°C, 25°C, 40°C and 54°C.

Sedimentation (F)			
Storage period (days)	Storage Temp.	Avg. F.	STD
0	4°C	0.28	-
28	4°C	0.33	0.02
28	25°C	0.72	0.44
28	40°C	0.19	0.05
28	54°C	0.16	0.01

**Figure 3.** Viscosity of phenytoin sodium extemporaneous suspension after 28 days at 4°C, 25°C, 40°C and 54°C.

sodium extemporaneous suspension met the USP specifications in the microbial examination of nonsterile product throughout 28 days [9].

The stability of a pharmaceutical formulation is defined as the ability of a product in a particular storage condition to maintain and retain within its physicochemical, toxicological, therapeutically and microbiological specifications [10]. The storage condition which is based on the climatic conditions is the most crucial aspect in the analysis of a stability study. Stability testing of pharmaceutical formulations consists of complex procedures that involve time consumption, considerable cost, and scientific expertise to build in efficacy, quality, and safety in a drug product [7].

The stability testing evaluates the effect of environmental factors on the quality of a drug substance or a formulated product that is utilized for prognosis of its shelf life and it will also determine the adequate storage conditions and moreover it will suggest labeling specifications [11]. The data produced during the stability study is an essential to get regulatory approval for any formulation or drug [12].

The phenytoin sodium extemporaneous suspension showed no remarkable changes in pH, color, odor or sedimentation volumes after 16 days of storage at 4°C - 25°C. However, storage at 40°C - 54°C caused a prominent caramelization

in color and odor, as well as observable caking and clumping of suspended particles. Phenytoin sodium was in the list of top 200 drugs of 2015 with more than 1.6 million prescriptions written in 2015 in the United States alone [13].

#### 4. Conclusion

Phenytoin sodium (20 mg/ml) suspension was stable for a period of up to 16 days when stored in polyethylene plastic containers between 4°C and 25°C. Avoiding exposure to temperatures > 25°C is strongly recommended to ensure physical and chemical properties intact. The appearance and pH observations were intact throughout the study. The microbial stability of Phenytoin suspension showed that the TAMC was less than 8 cfu/ml, while the TYMC was less than 3 cfu/ml in the storage conditions at 40°C and 54°C on the 28th day. Moreover, there was no *E. coli* contamination detected in the MacConkey media plates of any samples. Furthermore, in the microbial examination of nonsterile products, USP specifications were met by phenytoin sodium extemporaneous suspension, but it was advised to use a buffer system for controlling the final pH of the prepared suspension to reduce the bitter taste of drug and ensure good microbial stability.

#### Conflicts of Interest

The authors declare that they have no conflict of interests and no financial support that might affect the study.

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