EFFECT OF DIFFERENT BUFFERED AND UNBUFFERED DISPERSION MEDIUM ON THE PHYSICAL AND CHEMICAL STABILITY OF NYSTATIN SUSPENSIONS

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Abstract

Nystatin is a polyene macrolide which is widely used in the treatment of GI fungal infections. Different formulations of the drug including its suspension are available. From the bioavailability point of view, suspensions are usually superior to other dosage forms. The aim of this study was to investigate the influence of type and concentration of cellulosic suspending agents on the physical and chemical stability of nystatin in suspension. To this end, three grades of sodium carboxymethylcellulose (SCMC 358 cps, SCMC 1240 cps, and 1485 cps) as buffered vehicles (pH = 7) and an unbuffered microcrystalline cellulose (Avicel RC 591) vehicle were employed. All the formulations were passed physical stability tests in terms of sedimentation volume, redispersibility and freeze thaw tests. The formulations were further tested for chemical stability using the Arrhenius method. The test indicated that shelf lives of the buffered suspensions were more or less the same but much higher than that of unbuffered suspension (10 months vs. 1 month).

Key words: Nystatin, Suspension, Suspending agent, Stability

Introduction

Nystatin is a polyene macrolide which is widely used for the prophylaxis and treatment of oesophageal and intestinal candidiasis [1]. The bioavailability of drug, when administered as suspension is usually more than the tablet [2]. Oral suspensions are dosage forms that supply insoluble and often distasteful substances in a form that is pleasant to taste. Also degradation of a drug in the presence of water may preclude its use as an aqueous solution and in this case it may be possible to synthesize an insoluble derivative of the drug and to formulate it as a suspension. In addition, some materials are required to be present in the gastrointestinal tract in a finely divided form therefore formulation of them as suspensions will provide the desired high surface area [3]. Preparation of pharmaceutical suspensions is often associated with physical stability problems [4]. Sedimentation, caking, difficulty of sediment dispersibility and crystal growth often occur [5, 6]. The resuspendability associated with flocculation-deflocculation behavior of drugs and the polymer induced flocculation has been of great interest since most pharmaceutical suspensions contain hydrophilic polymers as suspending agents [7-9]. As nystatin degradation occurs in the presence of water [10,

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11], the chemical stability of the related suspensions should be evaluated. Nystatin in solution dosage form is highly unstable. Although the commercial suspensions of nystatin are available, but to the best of our knowledge, the effect of suspending agents on the physical and chemical stability of nystatin suspension has not been provided in the literature. In this study, attempts have been made to investigate the influence of type and concentration of cellulosic suspending agents on the physical and chemical stability of nystatin in suspension. Three grades of sodium carboxymethyl cellulose (SCMC 358 cps, SCMC 1240 cps, and 1485 cps) as buffered vehicles (pH = 7) and an unbuffered microcrystalline cellulose (Avicel RC 591) vehicle were employed. All the formulations were passed physical stability tests in terms of sedimentation volume, redispersibility and freeze thaw tests. The formulations were further tested for chemical stability by a first-order UV-derivative spectrophotometric assay using the Arrhenius method.

Materials and Methods

Materials

Nystatin powder (PIERREL, Italy), SCMC 358 cps, 1240 cps and SCMC 1485 cps (NYMA, Holland), Avicel RC 591 (FM, Brussels, Belgium) methylparaben, propylparaben and sodium saccharin (IRAN - DARU, Iran), cherry and orange essence (Quest - Holland), Glycerin (Caldic, Holland), disodium phosphate, monopotassium phosphate (Merck Germany).

Methods

Preparation of nystatin suspensions

As the maximum stability of nystatin has been observed at pH = 7 therefore it was necessary to use phosphate buffer in order to provide the mentioned pH [11, 12].

Suspensions which were investigated in this study contained 100000 units (2.059% w/v) of nystatin and different amounts of SCMC grades 358, 1240, 1485 cps and Avicel RC 591 as suspending agents (table 1). Methylparaben and propylparaben were used as preservative agents. The wetting agent (glycerin) was used at a concentration of 2% w/v. For the preparation of suspensions, the suspending agent was initially dispersed in prepared buffer (pH = 7) containing the preservative agents. In the next step, nystatin which was wetted by glycerin was added to the vehicle and dispersed by tumbling for 10 minutes. The prepared suspensions were transferred to 25 ml amber glass bottles with well sealed caps and stored at room temperature under static conditions.

Table1: Ingredients of nystatin suspensions (2.059 w/v %)

	A	B	C	D	E	F
Nystatin	2.059*	2.059	2.059	2.059	2.059	2.059
Methylparaben	0.2	0.2	0.2	0.2	0.2	0.2
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02
SCMC 358 cp	1	-	-	-	-	-
SCMC 1485 cp	-	0.3	0.4	-	-	-
SCMC 1240 cp	-	-	-	0.3	0.4	-
Avicell RC 591	-	-	-	-	-	1.75
Glycerin	2	2	2	2	2	2
Sodium saccharin	qs	qs	qs	qs	qs	qs
Flavor	qs	qs	qs	qs	qs	qs
Phosphate buffer pH = 7	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml
Water	-	-	-	-	-	Up to 100 ml

* w/v %

Physical stability tests

Sedimentation volume

The sedimentation volume (F) was measured in 100 ml graduated glass cylinders. Each suspension was shaken to ensure uniform dispersion prior to

the sedimentation study. The sedimentation volume was recorded at 1, 2 and 7 months storage periods for three samples of each formulation. Sedimentation volume was expressed as the ratio between the height of the sediment at the specified time ofz evaluation and the height of the suspension at the time of 0.

Resuspendability

The resuspendability of suspensions was evaluated after 1, 2 and 7 months storage periods qualitatively. The test was performed by shaking the cylinder manually at 180° movement, after sedimentation was completed (for three samples). Based on the numbers of shaking required to convert the sediment to uniformly dispersed suspension, the formulations were evaluated. Cake formation was also evaluated qualitatively.

Freeze-thaw cycling test

All formulations were frozen at -4°C for 24 hours and thawed at +20°C for the next 24 hours, the number of cycles was 6 and the crystal growth was evaluated by direct microscopic observation using an ordinary microscope (Ernst, USA).

Chemical stability test

Arrhenius accelerated stability test (an isothermal stability test) was employed to determine shelf life of the prepared formulations. The formulations stored in thermostatically controlled ovens at 40, 45, 50, 55 and 60°C. Then samples were taken after predetermined time intervals and the amount of nystatin remaining intact was assayed by first derivative UV spectrophotometry at 325.4 nm using double beam UV spectrophotometer (Shimadzu, Japan). The first derivative UV spectrophotometry was developed for determination of nystatin. In order to obtain calibration curve, a stock solution of nystatin was prepared by dissolving 100 mg of the drug in 100 ml mixture of dimethylformamide methanol (1:1 ratio). Standards for the calibration graph ranging from 0.2 to 1.6 mg/100ml were prepared from stock solution and dimethylformamide methanol (1:1 ratio) was used as blank.

Results and discussion

Sedimentation volume, resuspendability and freeze-thaw cycling

Redispersibility is one of the major considerations in the assessment of a suspension, and after formation should be easily dispersed by moderate shaking to yield a homogenous system. Measurement of the sedimentation volume (F) and the ease of the redispersion (Res.) form are two methods of the most common basic evaluative procedures. The values of F for the suspensions of table 1, 7 month after formulation were between 0.9 and1. At the end of the same time Res. values were between 2 to 10 indicating ease of dispersion. No cake formation was observed during the storage period. Microscopic examination revealed no crystal growth in the suspensions following freeze - thaw cycling.

Thus the formulations were acceptable from physical stability point of view.

Arrhenius accelerated stability test and shelf life

By processing the data obtained from the stress test namely the concentration of undecomposed drug during the test time at various temperatures, it was revealed that the kinetic of degradation for nystatin is zero order. This was assured after fitting the data to the first and zero order kinetic models and subsequent error analysis. The error with the zero order kinetic was much less than the first order one. This is in line with the theory that the kinetic of drug degradation in suspensions is zero order (14). The representative zero order plots at different temperatures for formulation C are shown in Fig 1. The zero order rate constants, k_0, values for each temperature were obtained from the corresponding linear plot. The Arrhenius equations were then obtained by

regression of log k_0 against reciprocal absolute temperature (1/T). The representative Arrhenius eq. for formulation C is as follows:

Logk = 8.4645 - 3684.62 (1/T) r^2 = 0.9626

and the corresponding plot is shown in Fig 2. From the equation the shelf life at temperature 25 °C was calculated to be about 10 months. For the other suspensions the shelf lives were slightly less than 10 months except for formulation G whose shelf life was 1 month. The later formulation was unbuffered whereas the others were buffered at the optimum pH of stability for nystatin.

Fig.1 Undecomposed drug Time curves at different temperatures for formulation C.

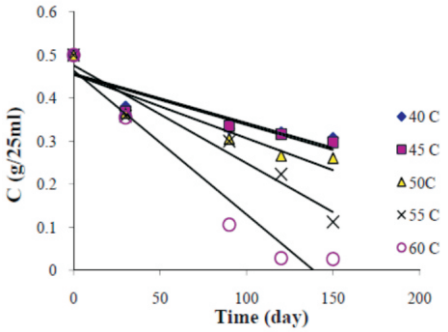
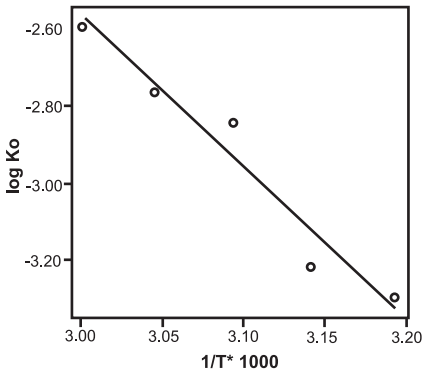


Fig. 2 log K_0 against absolute temperature for formulation



Conclusions

The cellulosic suspending agents at concentrations used in this study produced physically stable nystatin suspensions regardless of their chemical nature and the drug in the buffered vehicles at optimum pH of 7 gave chemically stable suspensions as compared with that of unbuffered vehicle.

Acknowledgment

The authors wish to thank financial support provided by Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz,Iran.

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