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# EFFECTS OF POROUS SILICA AS DRUG CARRIER ON THE FORMULATION OF EFFERVESCENT NYSTATIN VAGINAL TABLETS

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تم فى هذا البحث تحميل النيستاتين على السيليكا ذات الفجوات بطريقة الترسيب من المذيب وتم دراسة تأثير هذه المادة على تبلور العقار من المخاليط الفيزيائية والمخاليط المحملة بطريقة المسح السعرى التفاضلى وتحليل تشتت الاشعة السينية ، وأوضحت نتائج التحليل هذه بأن النيستاتين قد تحول من الصورة البلورية إلى الصورة الغير بلورية عند تحميله على السيليكا معتمدا على نسبة العقار إلى السيليكا. وقد أعطت المخاليط المحملة معدل اتاحة للنيستاتين أعلى من المخاليط الفيزيائية أو العقار بمفرده وقد احتفظ العقار بفاعليته ضد الفطريات عند تخزين المخاليط المحملة فى درجة رطوبة صفر ودرجة حرارة ٢٥°م لمدة شهرين.

وقد تم صياغة هذه المخاليط المحملة من النيستاتين والسيليكا فى صورة أقراص فواره باستخدام طريقة الكبس المباشر. وقد تم تقييم الاقراص الفواره المحضرة طبقا لدستور الادوية الانجليزى ١٩٩٣ ووجد أنها تطابق المواصفات العيارية من حيث الوزن ومحتوى العقار ووقت التفتيت ومعدل الاتاحة بالاضافة إلى قوة الصلابة ودرجة الهشوشة. وقد أعطت الاقراص الفواره المحضرة باستخدام المخلوط المحمل معدل اتاحة للنيستاتين أعلى من نظيره من الاقراص المهبلية المتوافره فى السوق المحلى.

*Nystatin was loaded into porous silica by solvent deposition technique. The physical and loaded drug mixtures were examined by DSC and powder X-ray diffractometry. The stability of the drug at different relative humidities at 25° in these systems was evaluated by measuring the antimycotic effectiveness. The in-vitro dissolution of the drug from loaded mixtures was studied. The loaded mixtures were formulated into effervescent vaginal tablets. Physical Characteristics and dissolution behavior of the prepared effervescent vaginal tablets were evaluated according to B.P. 1993 limits. DSC and powder X-ray diffraction results revealed that the drug was changed from crystalline to amorphous state depending on drug to carrier ratio. The drug was stable and retained its activity in loaded mixtures stored at 0 % relative humidity for 2 months at 25°. Transformation of the drug to the amorphous state in the loaded mixtures lead to enhancement of drug dissolution. The prepared effervescent vaginal tablets complied with the standard requirements of uniformity of weight, drug content, disintegration time, dissolution, hardness and friability tests.*

## INTRODUCTION

Nystatin is a polyene macrolide antibiotic used in topical treatment of skin infections and vulvovaginal candidiasis[1,2]. It is only slightly water soluble, as a result of which the drug may exhibit poor absorption characteristics[3]. Dissolution properties of the drug are influenced by its physical state, including polymorphism, hydrate or solvate formation and degree of crystallinity[4].

Various physicochemical methods e.g. addition of surfactants and complexation have been used to improve aqueous solubility of

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poorly soluble drugs[5,6]. The mechanism of enhancing dissolution rate of these drugs dispersed in hydrophilic carriers was reported to be due to molecular dispersion [7] reduction of particle size[8] and solid solution[9].

Porous calcium silicate is widely used [10-12] as drug carrier in pharmaceutical preparations for its excellent flowability, mouldability, stabilizing and drug-release qualities. The use of colloidal and porous silica has recently attracted considerable attention in the pharmaceutical field of stability, enhancement of dissolution and bioavailability[13].

The aim of the present study was to evaluate the effect of porous silica as drug carrier on physicochemical properties of nystatin and its antimycotic effectiveness. Another goal of this work is to formulate nystatin into effervescent vaginal tablets and evaluation of their characteristics.

## EXPERIMENTAL

### Materials

Nystatin (Squibb co. England); Florite R (Tokuyama Soda, Tokyo, Japan) was used after drying in vacuum at 120° for 3h; Sabouroud dextrose agar (Sigma & Merck Sharp, Germany); Boric acid, anhydrous citric acid, sodium bicarbonate, and all other materials were of analytical grade.

### Equipment

Erweka tablet press type EKO (Apparatebau GM6H, Heusenstamm, Germany); Erweka hardness tester (Erweka Apparatabau); Roche friabilator (Erweka Apparatabau); USP dissolution apparatus (Erweka Apparatabau); UV spectrophotometer (Uvedic 320, Japan); pH meter (MV87 digital, Praci Tronic, Germany); Phillips diffractometer, PW1710, Netherlands; DSC calorimeter (DSC-50, Schimadzo, Japan).

### Methods

#### Preparation of the physical mixtures

Nystatin, which had a particle size of 90-125 $\mu$m and the dried porous silica (Florite R) were mixed in various concentration (10-40% w/w nystatin) using an agate mortar and pestle in dark and dry conditions avoiding oxidation of the drug. Mixing was done gently for five minutes avoiding any grinding effect on the mixture.

#### Preparation of nystatin loaded mixtures

Nystatin solutions in different concentrations (2-8 % w/v) in methanol were prepared. Fifty ml of drug solution were added to specified weight (6-9 g) of the dried Florite to give the same ratios as the physical mixtures. Methanol was evaporated at 25° using a rotary evaporator. The samples were dried for 3 days over $P_2O_5$ in desiccator under vacuum at 25° and kept in darkness until further investigations.

#### Stability studies

The physical and loaded drug mixtures were kept in desiccator at 25° over $P_2O_5$ under vacuum (0% relative humidity; RH) and over saturated solution of potassium bromide (79% RH) for a period of two months.

#### Powder X-ray diffraction studies

The powder X-ray diffraction patterns were recorded by using Phillips diffractometer PW1710, Cu K$\alpha$-radiation ($\lambda$ 15418); nickel filter, voltage 50 Kv, current 40 mA and scanning speed 3°.6/min.

#### Differential scanning calorimetry (DSC)

The DSC thermograms were recorded on a Schimadzo DSC-50 calorimeter with samples weight 3 mg. Each sample was scanned at 10°/min up to 200° under dry nitrogen stream.

#### Dissolution studies

The dissolution rates of nystatin, its physical and loaded mixtures were determined using USP XXI paddle apparatus. An amount of powder equivalent to 20 mg of nystatin was introduced into 250 ml of phosphate buffer pH 6.8 adjusted at 37 $\pm$ 0.5° and stirred at 50 rpm. At specified time intervals, an aliquot of 5 ml was withdrawn using pipette fitted with a filter and replaced by the same volume of dissolution medium equilibrated at 37°. The drug

concentration was determined spectrophotometrically at 320 nm [2] after appropriate dilution with phosphate buffer pH 6.8. Florite R being an insoluble silica, didn't interfere with the determination of the drug. The dissolution test was repeated three times for each sample and the results were averaged.

**Microbiological assay of nystatin by plate agar diffusion method**

The microbiological assay of nystatin samples was done adopting the plate agar diffusion method[14]. Candida albicans (reference strain) was cultured for 48 h on Sabouroud dextrose agar (SDA) at 37°. The cells were suspended in SDA (about 10 colony forming units per ml). Two hundred and fifty ml of the suspension were mixed with 18 ml melted (45°) SDA and poured into petri dishes (9 cm). Using a sterile cork borer aseptically one hole in the center of the plate (blank) and 7 holes in the periphery (for conc 100 $\mu$g/ml to 12.50 $\mu$g/ml) were made. The holes were filled with 50 $\mu$l of the solutions to be tested. In case of study of the efficiency of nystatin in loaded and physical mixtures, accurately weighed samples were suspended in buffer to give various concentrations listed in table 3. Inhibition zones were read after overnight incubation at 37°. Recorded values for zone sizes and concentrations of nystatin were computed from the average values of two independent runs each consisted of five measurements for every sample.

**Preparation of nystatin effervescent vaginal tablets**

The different formulae for preparation of nystatin effervescent vaginal tablets are presented in Table 1. The physical and loaded mixtures of 20 % w/w nystatin were used for preparation of effervescent tablets. Effervescent vaginal tablets were prepared using direct compression technique[15]. The components of effervescent base were mixed using a mortar and pestle then directly compressed with single punch tablet machine. The punches of machine are flatted surface. The compression of effervescent tablets was done under controlled conditions of humidity and light.

**Evaluation of prepared effervescent tablets**

Physical properties of prepared tablets such as, uniformity of weight, drug content, disintegration time, were determined according to B.P. 1993; uniformity of thickness, hardness and friability were also evaluated.

**Dissolution of nystatin from effervescent tablets**

The dissolution rate of effervescent tablets (formula 2 and formula 3) were carried out using USP XXI paddle apparatus under the same conditions aforementioned. In addition the dissolution behavior of nystatin from conventional nystatin inserts (Memphis Co., Egypt) was determined for comparison.

**Determination of pH**

One tablet from each batch was allowed to disintegrate in 200 ml distilled water at 25° and immediately the pH was determined. The experiment was repeated three times and the average values were recorded.

## RESULTS AND DISCUSSION

Powder X-ray diffractometry was utilized to study the crystallographic nature of solid dispersions[16-18]. The major X-ray diffraction peaks of nystatin were observed at $2\theta$ = 14.0°, 15.6°, 16.7°, 20.4° and 21.6° (Fig. 1). The X-ray diffraction pattern of the physical mixtures of nystatin and Florite R showed the same diffraction peak of low intensity. In the case of the loaded mixtures of 10-30 % w/w nystatin, the X-ray diffraction peaks due to the drug disappeared. In the case of the loaded mixture of 40 % w/w nystatin, X-ray diffraction peaks of the drug of low intensity were observed. It can be assumed from these results that loading of nystatin on porous silica resulted in its transformation to amorphous state (mixtures containing 10-30 % w/w nystatin) or reduction of its crystallinity (mixture containing 40 % w/w nystatin). The X-ray diffractograms of the loaded mixtures of 10-30 % w/w nystatin stored at either 0 % RH or 79 % RH for two months at 25° did not show any peak due to nystatin

**Table 1**: Composition of nystatin effervescent vaginal tablets.

| Ingredient | Formula 1 | Formula 2 | Formula 3 |
|---|---|---|---|
| Anhydrous citric acid | 18.0 % w/w | 18.0 % w/w | 18.0 % w/w |
| Boric acid | 21.6 % w/w | 21.6 % w/w | 21.6 % w/w |
| Sodium bicarbonate | 50.4 % w/w | 50.4 % w/w | 50.4 % w/w |
| Florite R | 10.0 % w/w | 8.0 % w/w | 8.0 % w/w |
| Nystatin | ---- | 2.0 % w/w | 2.0 % w/w |

Formula 1: plain base with 10 % w/w florite R.
Formula 2: medicated base containing 10 % w/w physical mixture of 20 % w/w drug.
Formula 3: medicated base containing 10 % w/w loaded mixture of 20 % w/w drug.

**Table 2:** Dissolution characteristics of nystatin from its physical and loaded mixture with florite R in phosphate buffer pH 6.8 at 37°.

| Time (min.) | Nystatin | % dissolved (physical mixtures)* | | | % dissolved (loaded mixtures) | | |
|---|---|---|---|---|---|---|---|
| | | 10 % | 20 % | 30 % | 10 % | 20 % | 30 % |
| 15 | 22.36 | 29.44 | 27.57 | 28.51 | 52.89 | 50.63 | 51.71 |
| 30 | 25.67 | 30.50 | 28.08 | 31.19 | 54.84 | 57.40 | 56.27 |
| 45 | 30.71 | 31.85 | 32.42 | 33.84 | 56.81 | 62.09 | 61.55 |
| 60 | 32.07 | 33.95 | 33.95 | 35.09 | 58.09 | 65.17 | 65.84 |
| 75 | 33.61 | 34.55 | 34.57 | 36.97 | 61.91 | 66.87 | 67.89 |
| 90 | 34.85 | 38.05 | 36.83 | 37.52 | 64.85 | 71.88 | 69.63 |
| 105 | 36.16 | 39.33 | 38.59 | 41.05 | 67.31 | 73.48 | 73.63 |
| 120 | 38.31 | 43.18 | 42.14 | 43.66 | 69.08 | 75.41 | 74.60 |

* The percent indicated the amount of drug in the mixture.

**Table 3**: Efficiency of nystatin in 20% w/w nystatin loaded and physical mixtures.

| Drug conc. | Pure drug | Physical mixture | Loaded mixture |
|---|---|---|---|
| $\mu$g/ml* | Minimal inhibition zone (mm) | | |
| 100 | 23 | 23 | 26 |
| 50 | 20 | 20 | 24 |
| 25 | 18 | 18 | 22 |
| 12.5 | 12 | 12 | 18 |

* Concentration $\mu$g nystatin / ml of phosphate buffer (pH 6.8) suspension.

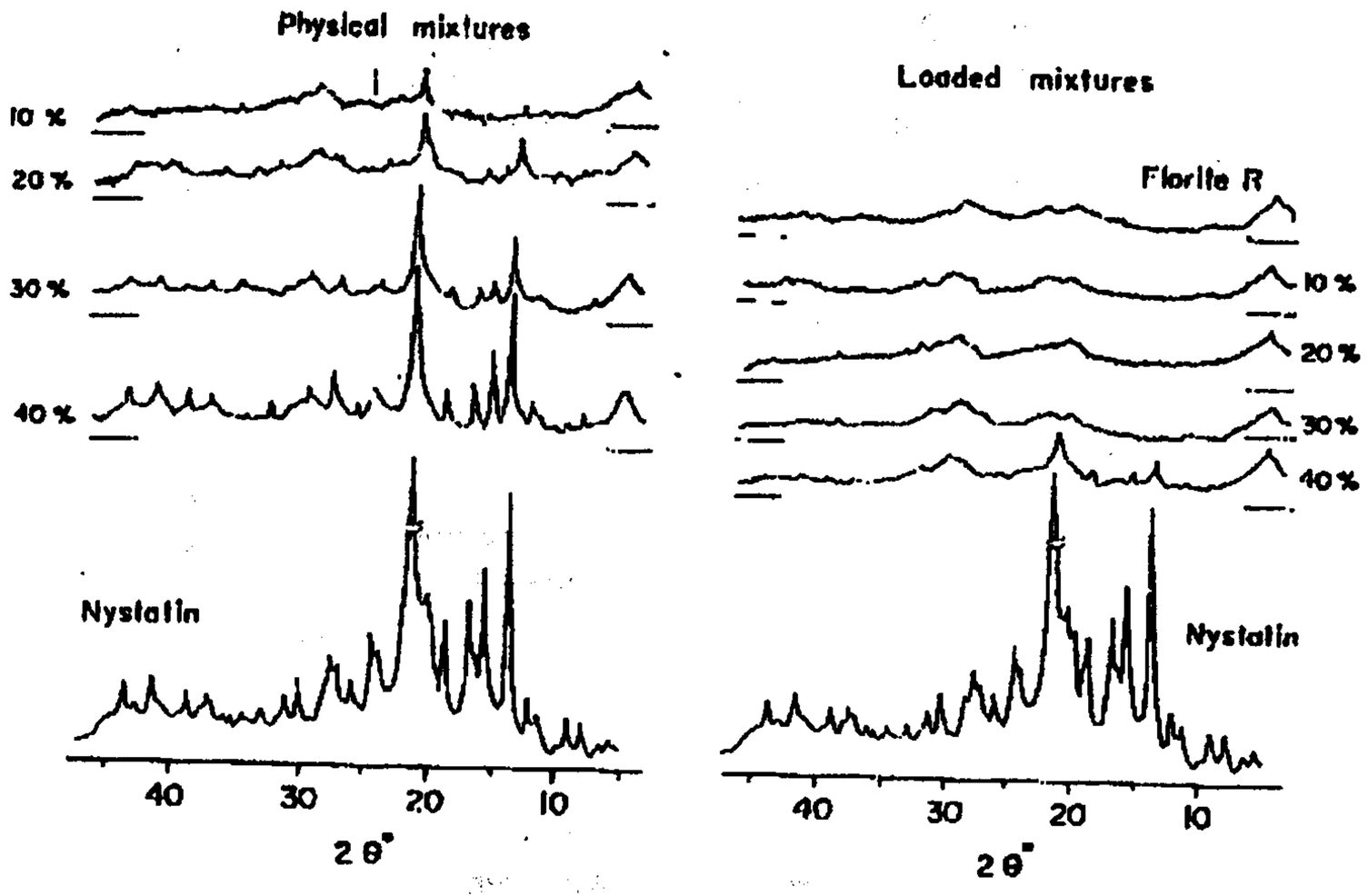


**Fig. 1:** X-Ray diffraction patterns of physical and loaded nystatin mictures in various ratios (%W/W nystatin).

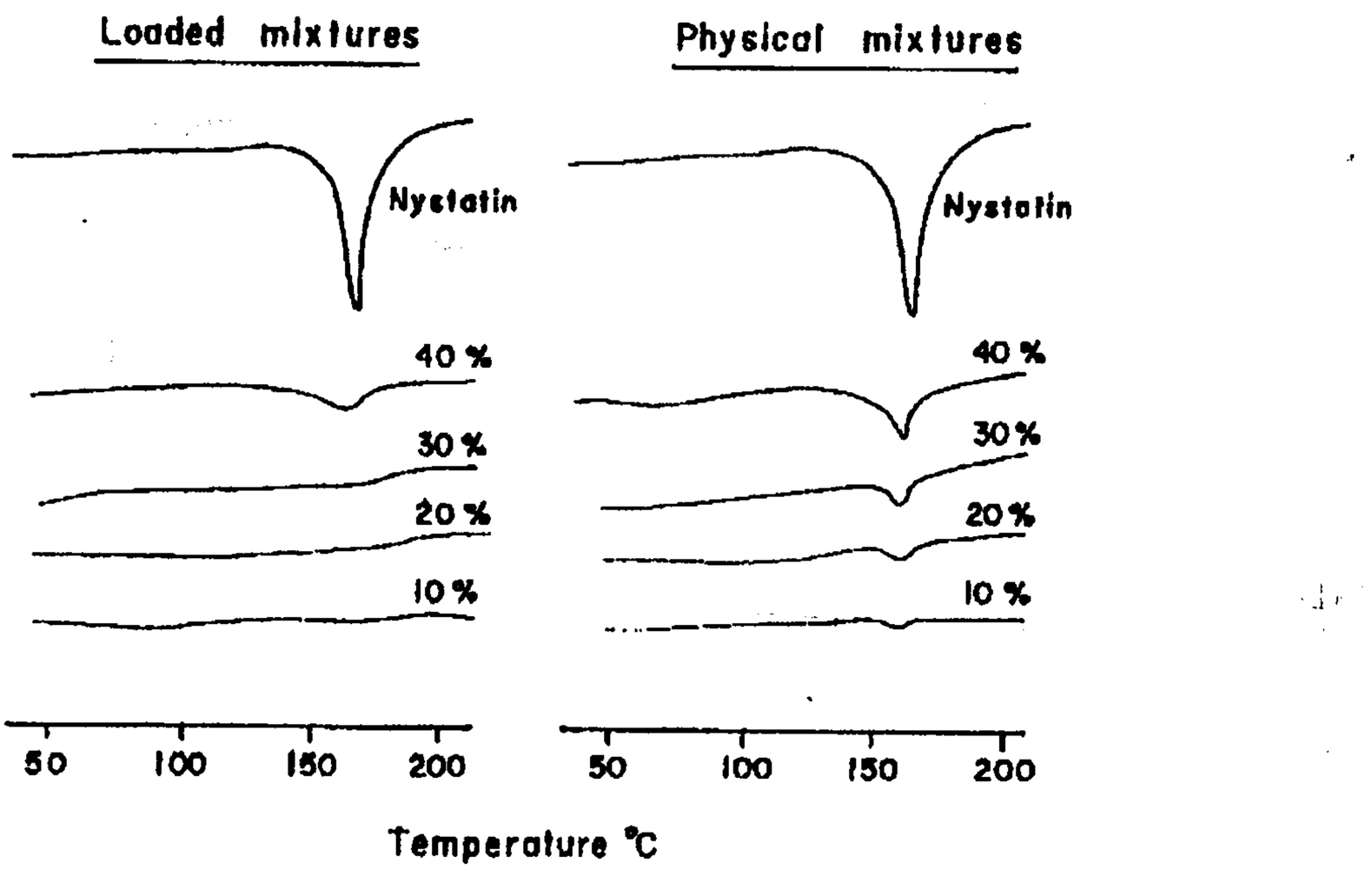


**Fig. 2:** DSC curves of the physical and loaded nystatin mixtures in various ratios (% W/w nystatin).

crystals (not shown). This indicated that the drug was retained in the amorphous state.

Differential scanning calorimetry has been used for characterization of the state of the drug and studying its interaction with carriers[19]. Figure 2 shows the DSC curves of nystatin and its physical and loaded mixtures with Florite in various ratios. Nystatin showed an endothermic peak at 166° due to melting of drug crystals. The physical mixture showed the endothermic peak due to melting of drug crystals at lower temperature. In the case of the loaded mixtures

of 10-30 % w/w nystatin the DSC curves showed no endothermic peak at the melting region of drug crystals. This confirms the presence of the drug in the amorphous state[20]. In the case of the loaded mixture of 40 % w/w nystatin a small broad endothermic peak was observed due to fusion of excess drug crystals.

Dissolution data of nystatin, its physical and loaded mixtures in different ratios with Florite R were shown in Table 2. The physical mixtures showed slightly faster drug release than drug alone. The loaded mixture showed a markedly faster dissolution rate of the drug (Figure 3). The mixing ratio of nystatin to Florite R in either physical or loaded mixtures has no significant effect on the dissolution behavior of the drug. These results indicated that the dissolution of nystatin from these systems depended on its crystalline state. When the loaded mixture was placed in the dissolution medium, drug molecules existed in amorphous state and can be released immediately into the dissolution medium. This finding was in agreement with the attribution of Carli *et al.*[21].

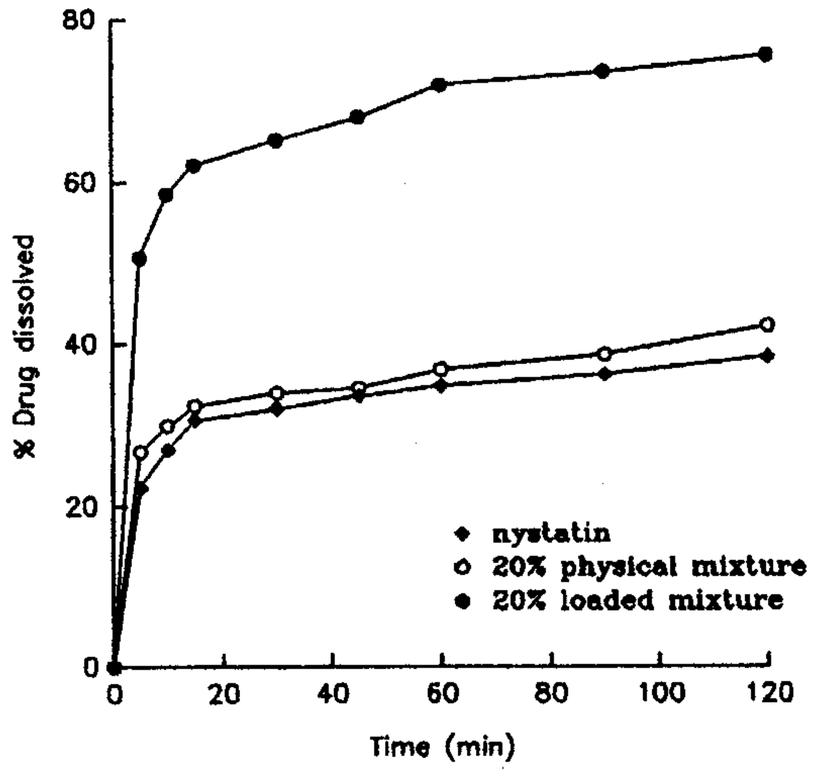


**Fig. 3: Dissolution profiles of nystatin from the physical and loaded mixtures with florite R (containing 20% w/w nystatin).**

Table 3 showed the efficiency of nystatin physical and loaded mixtures with porous silica. Minimum inhibition zones were recorded as an indication of the antimycotic activity of the drug. It is obvious that loaded mixture exhibited higher efficiency of the drug as indicated by larger minimum inhibition zones. These findings are in good agreement with dissolution results.

The effect of storage at different relative humidities (RH) and 25° on the efficiency of 20 % w/w nystatin physical and loaded mixtures is shown in table 4. The antimycotic activity of the drug was retained in the physical and loaded mixture stored at 0 % RH for two months. In case of storage at high RH, the physical mixture showed partial loss of activity while the loaded mixtures showed complete loss of activity. It was reported that inactivation of amorphous nystatin when exposed to atmospheric oxygen, is greatly enhanced by presence of more than 9% moisture[2].

**Table 4:** Effect of storage at different relative humidities (RH) and 25° on the effeciency of 20% w/w nystatin loaded and physical mixtures.

| | Drug concentration ($\mu$g/ml)* | | | |
|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 |
| physical mixture stored at 0% RH | + | + | + | + |
| physical mixture stored at 79% RH | + | + | - | - |
| loaded mixture stored at 0% RH | + | + | + | + |
| loaded mixture stored at 79% RH | - | - | - | - |

* concentration $\mu$g nystatin/ml of methanol solution.
\+ inhibition of growth.
\- growth observed.

**Table 5**: Physical characteristics of different formulations of effervescent nystatin vaginal tablets.

| | Weight (g) mean(C.V. %) | Thickness(mm) mean(C.V. %) | Hardness Kg | Friability % | Disintegration time min. | pH |
|---|---|---|---|---|---|---|
| Formula 1 | 1.030 (3.34) | 5.721 (0.37) | 3.08 | 0.74 | 3.0 | 6.31 |
| Formula 2 | 0.959 (3.13) | 5.278 (0.32) | 3.37 | 0.72 | 3.5 | 6.38 |
| Formula 3 | 0.815 (2.59) | 5.404 (1.24) | 3.42 | 0.46 | 4.0 | 6.19 |

Formula 1: plain base with 10% w/w florite R.
Formula 2: tablets contain 10% w/w physical mixture of 20% w/w drug.
Formula 3: tablets contain 10% w/w loaded mixture of 20% w/w drug.

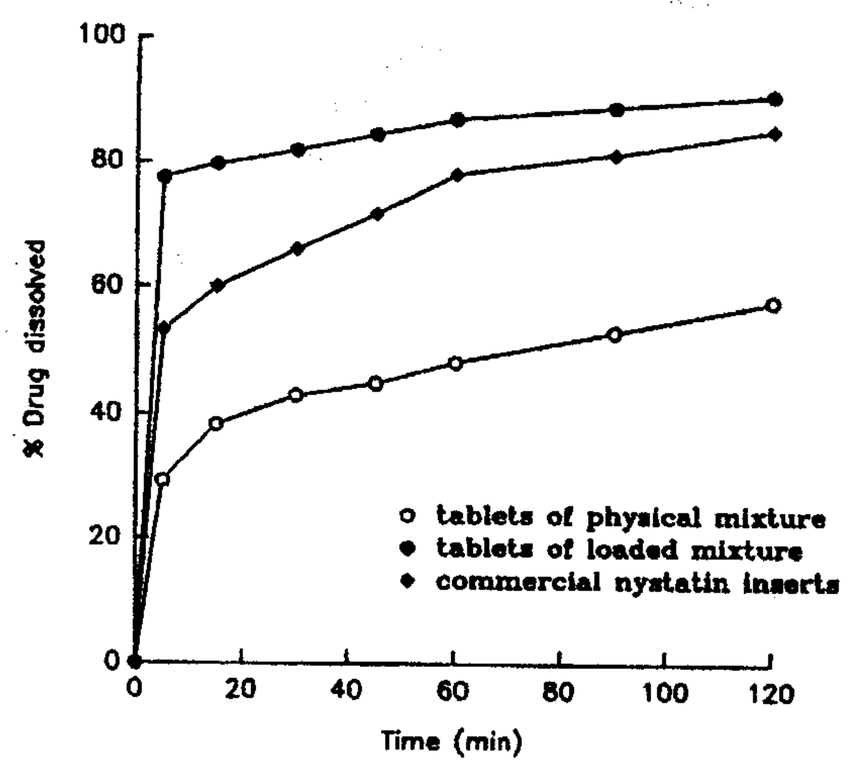


**Fig. 4: Dissolution profiles of nystatin from effervescent tablets containing 10% w/w of physical or loaded mixtures of 20% w/w drug and from commercial nystatin inserts.**

Table 5 summarize the physical characteristics of different formulations of effervescent nystatin vaginal tablets. Uniformity of weight, drug content and disintegration time of prepared tablets were found to comply with the requirements of B.P. 1993[22]. All effervescent tablets showed an acceptable values of hardness (diameter of tablet 13 mm). Moreover, low friability values were obtained in all formulae as indicated by the small values of % loss (less than 1.5 %). From these results it was concluded that the prepared effervescent vaginal tablets have good mechanical properties which facilitate their handling and durability.

Slightly acidic pH values (pH 6.19-6.50) were obtained with effervescent tablet from all batches. This acidic pH is intended for better stability of nystatin[2] and compatibility with vaginal fluids.

Figure 4 shows the dissolution behavior of nystatin from effervescent vaginal tablets. Effervescent tablets formulated with 20 % nystatin loaded mixture showed higher dissolution rate than tablets formulated with 20 % nystatin physical mixtures. Rapid disintegration and effervescence of tablets allowed the dispersion of the drug particles in the dissolution medium and prevent floating or aggregation of powder. This may explain why the dissolution of nystatin from effervescent tablets is higher than from corresponding physical or loaded mixtures (Figure 3). It is worth mentioning that the dissolution rate of nystatin from effervescent tablets, prepared using loaded mixtures, is higher than that from conventional nystatin inserts.

In conclusion, the present results are promising for preparation of nystatin in effervescent vaginal tablets for enhancing the effectiveness of the drug.

Stability of the drug in the prepared effervescent vaginal tablets and its clinical evaluation will be the subject of the next research.

## REFERENCES


1- O.Arthur, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania, p.1175 (1980).
2- F. Klaus, Analytical Profiles of Drug Substances, Vol 6, Academic Press, New York, San Francisco, London, p. 344 (1977).
3- H.Van Doorne and E.H.Bosch, Int. J. Pharm, 73, 43 (1991).

4- T.Sato, A.Okada, K.Sekiguchi and Y.Tsuda, Chem Pharm. Bull., 29, 2675 (1981).
5- L.Thorsteinn, F.Hafrun, J.O.Birna and G.Orn, Acta Pharm. Nord., 3, 215 (1991).
6- E.Shefter and C.Chengk, Int. J. Pharm., 6, 179 (1980).
7- O.I.Corregan, C.A.Murphy and R.F.Timony, ibid., 4, 67 (1979).
8- M.Gibaldi, S.Feldman and T.R.Bates, J. Pharm. Sci., 57, 708 (1968).
9- A.Sheth and C.I.Jarowski, Drug Develop. Ind. Pharm., 16, 769 (1990).
10- K.Unger, H.Rupprecht and W.Kircher, Pharm. Ind., 42, 1027 (1980).
11- K.Unger, J.Chromatogr. Lib., Vol. 16, Amsterdam, Oxford, NewYork, (1979).
12- H.Y.Abdallah, N.Khalafalla and S.A.Khalil, Drug Develop. Ind. Pharm., 9, 795 (1983).
13- A.M.El-Sayed, A.S.Ali and A.A.Assi, S.T.P. Pharma, 3, 319 (1993).
14- T.B.Platt, J.D.Levin, J.Gentile and M.A.Leitz, in F. Kavanagh, ed., Analytical Microbiology, Vol II, Academic press, Inc., New York, London, P. 147 (1972).
15- E.Mendell, J. Manuf. Chem. & Aersol New, 34, 43 (1972).
16- W. L. Chiou and S. Niazi, J. Pharm. Sci., 62, 498 (1973)
17- P.C.Schmidt and K.Benke, Pharm. Ind., 46, 259 (1984).
18- S.M.Safwat, S.S.Tous and M.M.Mohamed, ibid, 53, 1144 (1991).
19- E.C.Signoretti, A.Dell'Utri, A.De Salvo and L.Donini, Drug Develop. Ind. Pharm., 12, 603 (1986).
20- Y.Nakai, E.Fukuka, S.Nakajima and K.Yamamoto, Chem. Pharm. Bull., 25, 3340 (1977).
21- F.Carli, I.Colombo L.Magarotto and A.Motto, 4th Pharm Technol. conf., Edimb., U. K., (1984).
22- British Pharmacopoeia, London, p. 753 (1993).