BIOPHYSICAL STUDIES OF SOME DRUG/MICROBIAL CELL INTERACTIONS: AN OUTLINE

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ABSTRACT

This review will concentrate on the biophysical study of the interaction of a polyene antibiotic, nystatin (Figure 1), with sensitive and resistant cells of the yeast *Saccharomyces cerevisiae* (NCYC 239, Food Research Institute, Colney Lane, Norwich, UK). The procedure will be to outline a model of the interaction process and to illustrate the study, by physical chemical means, of the separate components of the model.

Nystatin is a member of the polyene group of antibiotics (Figure 1) and is active against fungal infections (1). Its properties both physical and biological have been well reviewed (2,3,4,5). Upon interaction with yeast cells it has been proposed that the nystatin complexes with sterol (ergosterol) present in the yeast cell membrane. This complexation results in loss of membrane integrity followed by loss of cytoplasmic constituents and, eventually, cell death (2,3,4,5).

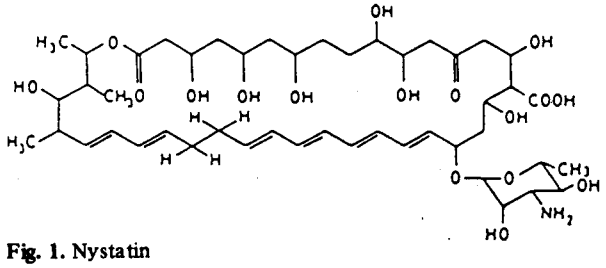

Fig. 1. Nystatin

The yeast cell, growth, morphology and metabolic detail has been extensively reviewed (6). For the purposes of this review it is only necessary to know that the membrane of the yeast is bounded by a wall and it is the surface of this wall which is in intimate contact with the aqueous suspension medium. Moreover membrane and wall properties and enzymic activity are quite dependent upon the presence of metal ions. The surface of the wall (the region where aqueous and lipid matrices are contiguous) is, necessarily therefore, charged. Nystatin has two protonatable sites in the molecule (7,8) of pK 4.5 and 8.6 respectively. These pK's are not declared here precisely because as nystatin has poor aqueous solubility the measured pK's depend upon the solvent system selected for study (DMF/H_2O, alcohol/H_2O systems). Thus simple inspection of the nystatin molecule indicates that, given the role of metal ions in the metabolism of yeast cells (9) and particularly of the role of Ca^{2+} in membrane rigidity (10) that both the pH dependence of the interaction and the possible complexation of nystatin with metal ions are worthy of investigation. The constitution of the yeast cell wall is 80-90% polysaccharide with the remaining fraction consisting mainly of lipid and protein (11). Groups present in the wall include fatty acids and fatty acid esters (12) and these are suggested (12) as being involved in the interaction of the nystatin with yeast cells. Thus the nature of both nystatin in solution and the fatty acids and fatty acid esters may well be important in determining the surface charge of the yeast cell surface.

The above outline of the gross features of the nystatin/yeast cell interaction indicates some of the features of the interaction which should be considered in the formulation of a model.

A "stepwise" sequence of events which describes the interaction is a follows;

a) electrostatic interactions between antibiotic in solution and cells in suspension;

b) adsorption of the drug on to the cell wall;

c) penetration of the drug through the cell wall;

d) interaction with membrane components and alteration of membrane properties;

e) leakage of intracellular materials in an, as yet, unknown sequence;

f) degradation of proteins and nucleic acids because of loss of membrane integrity;

g) secondary effects in cellular metabolism.

Study of these individual "steps" in the interaction process may be amenable to investigation provided that precise control of the yeast may be exacted so that comparison becomes possible. Control over the performance of the yeast (and bacteria) can be achieved through storage of inocula in liquid nitrogen (14). Inocula of *Sacch. cerevisiae* have been maintained in liquid nitrogen for periods in excess of 5 years without loss (+<1%) of performance characteristics (14).

For some features of some of the "steps" in the model techniques are available which enable their study. Firstly, particle microelectrophoretic studies (13,15) allow investigation of the electrochemistry of the yeast cell wall (16). The yeast cell electrochemistry has been, surprisingly, little studied (13,16) despite interest in flocculation (e.g. in beer fermentations). The limited studies available are conflicting, e.g. Eddy and Rudin (17) have suggested that

phosphate groups in the cell wall are responsible for cell surface charge whereas James (13) has referred to the presence of carboxyl groups on the yeast cell wall and hence described the yeast cell as a "carboxyl colloid". In the study by Beezer and Sharma (16) the electrophoretic behaviour of the yeast cell was studied as a function of pH, ionic strength, buffer composition, growth temperature of the culture, interaction with nystatin and interaction of the inoculum with Ca^{2+} ions.

Figure 2 shows electrophoretic mobility vs pH data for yeast cells suspended in acetate-veronal buffer solution. The pK of the yeast cell surface derived from these data is ~ 4 and refers (18) to groups situated very close (~ 2 nm) to the surface of the yeast cell. The isoelectric pH (pH at zero mobility) is shifted in the presence of nystatin (compare Figures 2 and 3) but the pK remains unaltered. This implies that the electrochemical consequences of nystatin adsorption on to the surface of the cell wall are small.

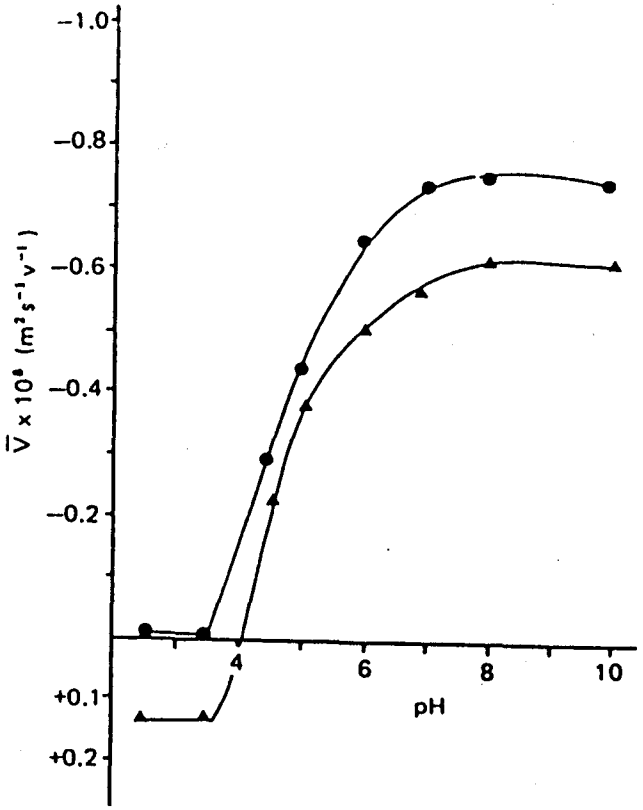

Fig. 2. Comparison of pH-mobility curves for *S. cerevisiae* grown in complex (●), and defined medium (▲) when suspended in acetate-veronal buffer pH 2.5-10.0 at an ionic strength = 0.1 mol dm^{-3}.

Effects of growth temperature on the subsequent electrophoretic behaviour of yeast cells are, however, very dramatic (Figure 4). Here the mobility is seen to increase abruptly at around 30° C. This temperature is the optimal growth temperature for yeasts (6). It is known (19) that alteration in growth temperature affects the sterol content of the yeast cell and concomitantly, it is supposed, though not examined, lipid content and hence lipid/sterol ratio. Changes in lipid composition are known to affect, markedly, membrane properties viz. sharp lipid phase transitions occur in membranes over narrow temperature ranges. These transitions are characteristic of a conversion of the membrane from an ordered crystalline state to a more random liquid crystalline state. Thus changes in electrophoretic mobility parallel changes in the state of fluidity of the

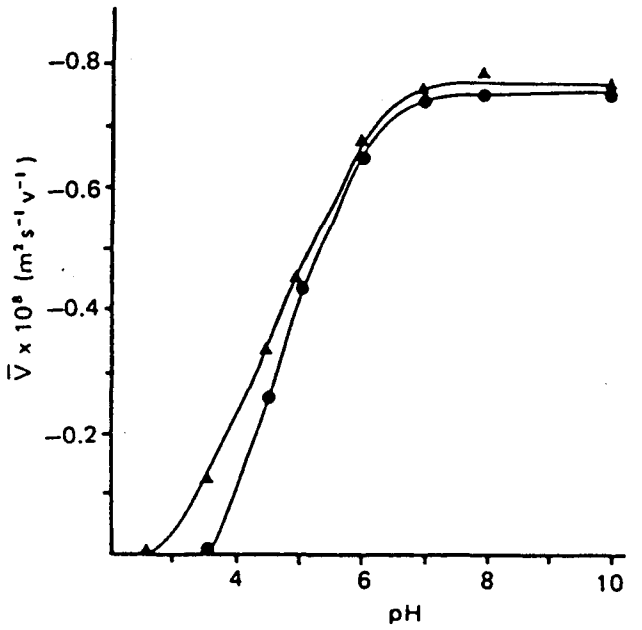

Fig. 3. Effect of nystatin (▲) on the pH-mobility curve for *S. cerevisiae* when suspended in acetate-veronal buffer pH 2.5-10.0 at an ionic strength of 0.1 mol dm^{-3}. The initial nystatin concentration was 10 $\mu g\ ml^{-1}$. Control, ●.

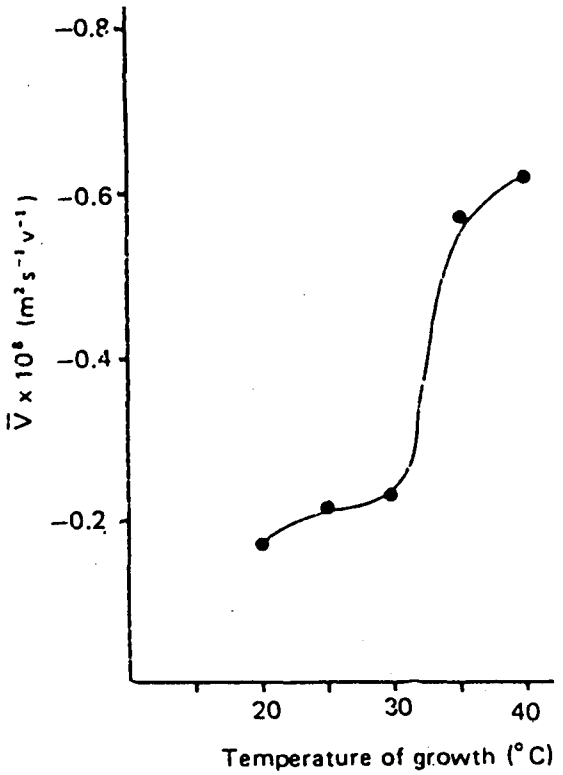

Fig. 4. The effect of growth temperature on the mobility of *S. cerevisiae* grown in defined medium and suspended in acetate-veronal buffer, pH 4.5 and at an ionic strength of 0.1 mol dm^{-3}.

membrane and it is inevitable therefore to conclude that the surface charge must have serious consequences for yeast growth metabolism and cell viability.

Study of the uptake of nystatin by yeast cells requires choice, initially, of the pH and nature of the suspending medium. The yeast cell itself is sensitive to the pH of the suspending medium not simply because of the charged nature of the cell surface but also because whole metabolism can be compromised by extremes of pH. The components of the selected buffer solution should have no significant effect upon the uptake process. Biphasic uptake vs times plots were observed (Figure 5) for buffer systems which contained metabolites or metabolisable constituents of yeasts whereas regular hyperbolic curves were obtained for phthalate and citrate buffers.

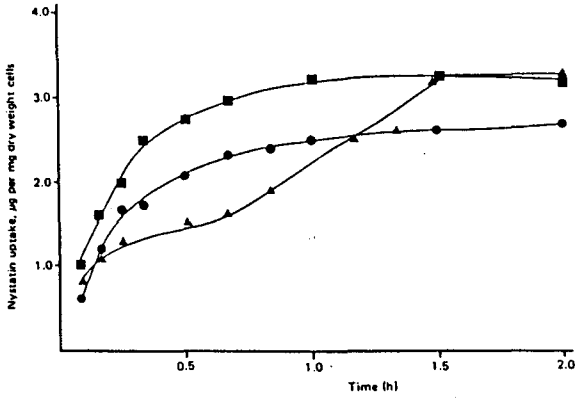

Fig. 5. Nystatin uptake by yeast cells suspended in buffer of different composition at pH 4.5: ■, phthalate; ●, citrate; ▲, acetate-veronal and acetate buffers.

Study of the uptake of nystatin involved incubating (21) yeast cells with nystatin, sampling at known time intervals, removing the cells by centrifugation and analysis of the supernatant for nystatin content by UV spectroscopy. As phthalate interferes with this spectroscopic analysis citric acid/citrate, which is believed not to be metabolised by yeast (6), was selected as the buffer system. pH 4.5 was also selected for use in this study since at this pH yeast cells can grow and metabolise and also because this pH was found to be optimal in a microcalorimetric bioassay procedure (14,21). Furthermore study of the uptake of nystatin as a function of pH (22) showed that uptake was maximal at this pH. pH 4.5 indicates that half the carboxylic acid groups on the yeast cell surface will be charged (this pH is close to the pK of the surface): that the nystatin molecule will be protonated at the amino site and that half the carboxylic acid groups will be protonated. Thus the nystatin molecule will carry a net positive charge. This, reasonably will favour interaction between nystatin and the yeast cell. Nystatin uptake appears (Figure 6) to become asymptotic with pH as pH > 6 and this again indicates the importance of the charged nature of the cell and drug in mediating the interaction (22).

Uptake ($\mu g\ mg^{-1}$ dry weight cells) was studied as a function of nystatin concentration (Figure 7) and of temperature (Figure 8). In 6 these experiments "zero time" was taken as the time at which half the time of centrifutation had elapsed following instantaneous sampling of the prepared reaction mixture.

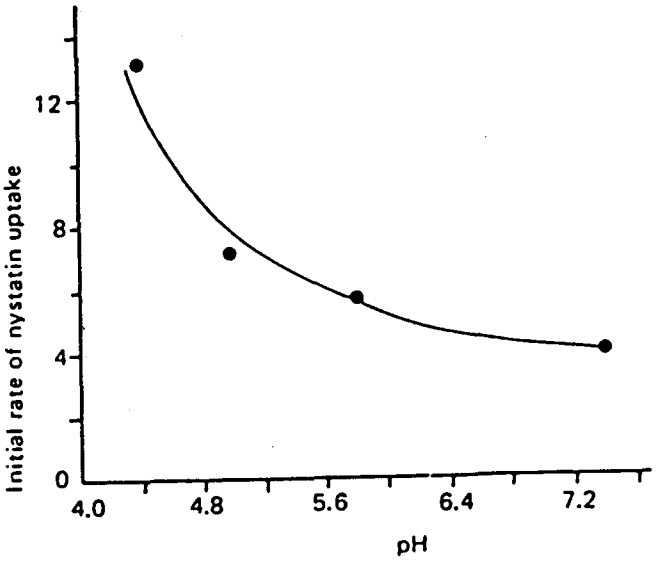

Fig. 6. The effect of pH on the initial rate of nystatin uptake by yeast cells.

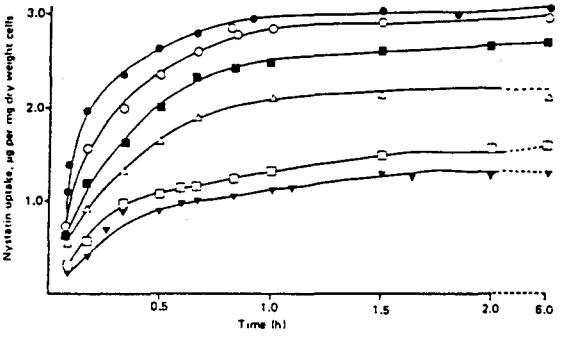

Fig. 7. Nystatin uptake as a function of initial nystatin concentration. Added nystatin concentratin ($\mu g\ ml^{-1}$): ▲, 4; □, 6; △, 8; ■, 10; ○, 12; ●, 14.

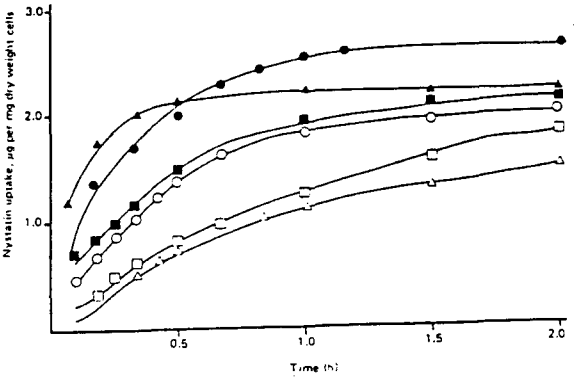

Fig. 8. Nystatin uptake as a function of temperature of incubation.

The data suggest a rapid i.e. unquantifiable rate of uptake on to the surface followed by a slower, second phase of uptake. If the data for this slow phase (i.e. from 5 min incubation onward) are plotted as In (a-x) vs where a = the equilibrium value of the uptake; x = the uptake at any time t (min) then linear graphs are obtained from which apparent first order rate constants may be derived. Analysis of these rate constants as a function of temperature allows determination, via Arrhenius plots of lnK vs 1/T of the activation energy, Ea, for the uptake process. The value obtained is 41.6 kJ mol^{-1}.

Linearisation of the data illustrated in Figure 7 of uptake and its relation to initial solution phase concentra-

tion of nystatin can be achieved by use of reciprocal plots. Thus a plot of 1/(equilibrium, i.e. plateau value, uptake) vs 1/ (initial nystatin concentration) is linear and yields the monolayer capacity directly; this value is 10 $\mu g\ mg^{-1}$ dry weight of cells.

Consideration of the number of yeast cells per mg dry weight, their diameter, and hence the number of nystatin molecules associated with each yeast cell (1.62×10^8) together with the planar dimensions of the nystatin molecule permits calculation of the number of nystatin molecules taken up per unit surface area of the yeast cell ($4.10 \times 10^8\ m^{-2}$). These results together with the monolayer capacity indicate that ca. 13x more nystatin is taken up than can be accounted for by a monolayer at the yeast cell surface.

This result may be due to (i) a non-Langmuirian uptake or (ii) a second phase of nystatin uptake which is not true adsorption but may be transfer of nystatin from the outside surface through the cell wall to the cell membrane.

The calculted value of Ea is within the range found (22,23) for diffusion processes through viscous fluids. Thus is was suggested that two processes occur; (i) adsoprtion of the nystatin on to the cell wall followed by (ii) diffusion of the nystatin through the cell wall to the membrane/cytoplasm.

Again the influence of temperature upon the properties of the yeast cell surface are seen (Figure 8) to be profound. However the temperature referred to here is not the growth temperature (30) but the temperature at which the nystatin/cell interaction was studied. It has been shown (20) that the interaction of cell membranes and/or lipids with drugs results in a lowering of the lipid phase change temperature. It is presumed that this is the reason for the differing mechanisms of uptake revealed in Figure 8.

To investigate the role of the wall in the uptake process it is possible to repeat the experiments outlined above but to use, in the place of cells, protoplasts (i.e. cells from which the cell wall has been removed by enzymic digestion (25)). Results for the important parameters derived (Ea and apparent first order rate constants) are shown in Table 1. The results also describe the values of these parmeters for protoplasts prepared from sensitive and resistant *Saccharomyces cerevisiae*. The resistant cells were prepared (25,26) by repeated sub-culture of the yeast in medium of increasing nystatin concentration (the MIC of the resistant strain so derived was 35 ug ml^{-1} compared to an MIC of 2 ug ml^{-1} for the sensitive strain). No significant physiological differences could be discerned between the two cell lines. Some small variation was seen in cell size (~ 4%) but more particularly in the chain length of fatty acids found (26) and in amino acid content.

The values for the activation energies for the uptake process by sensitive cells (Table 1) at a nystatin concentration of 10 ug ml^{-1} is very close to that (41.6 kJ mol^{-1}) reported by Beezer and Sharma (16) for uptake of nystatin (from 10 ug ml^{-1} nystatin concentration) onto yeast cells grown in complex medium. This value it was suggested is consistent with diffusion through the cell wall being the rate determining step (27). However the activation energy for uptake from 5 $\mu g\ ml^{-1}$ nystatin solutions is much lower (Table 1). In contrast the activation energies for uptake by resistant cells are inverted (i.e. *higher* for uptake from 5 ug ml^{-1} solution than from 10 $\mu g\ ml^{-1}$ solution). Furthermore the effects of the presence of glucose differs between sensitive and resistant cells. The sensitive cells show marked differences (e.g. ca. 20% increase for uptake from 10 μg ml^{-1} solutions). Thus both rate constants and the activation energies to which they lead indicate the differences between sensitive and resistant cells and are reflected in physical chemical parameters of the nystatin/yeast interaction. The k_1 values for uptake of nystatin by resistant cell derived prothoplasts are consistently lower (Table 1) than those for uptake by sensitive cell derived protoplasts. Moreover the values of k_1 all more closely resemble the values of k_1 found for uptake by whole cells. This is in contrast to the values of k_1 for uptake by sensitive cell derived protoplasts which show increases rather larger, although of the same magnitude, as their whole cell equivalents. There appears to be little difference between the k_1 values for uptake by sensitive cell derived protoplasts in the presence and absence of glucose. However on comparing values of activation energies for uptake rather more marked differences are revealed. Also in sensitive cell derived protoplasts Ea is increased by increasing nystatin concentration, whereas in resistant cell derived protoplasts Ea values show much greater consistency both with regard to nystatin concentration and presence of glucose. The values of Ea for resistant cell derived protoplasts are similar to those found for uptake by whole resistant cells. However the values of Ea for uptake by sensitive cell derived protoplasts are, under all conditions, approximately half the values found for their whole cell equivalents. Thus the protoplast surface appears to present a more simple barrier to interaction (lower Ea, larger k_1). These data seem therefore to support the suggestion that the cell wall is crucial in mediating nystatin/cell interactions. This analysis, of course, supposes that the nature of the protoplast surface is not dramatically altered by the enzymic procedures leading to its preparation, and hence that comparison of cell and protoplast is justified.

Table 1

Apparent first order rate constants for nystatin uptake by sensitive(S) and resistant(R) yeast cells at different temperatures for added nystatin concentrations of 5 $\mu g\ ml^{-1}$ and 10 $\mu g\ ml^{-1}$ at pH 4.0.

	Apparent first order rate constant (sec^{-1}) x 10^4			
T (K)	nystatin S	5 $\mu g\ ml^{-1}$ R	nystatin S	10 $\mu g\ ml^{-1}$ R
288	4.7_6	5.1_1	3.2_8	4.0_2
293	5.0_0	5.2_3	3.4_5	5.2_6
298	5.9_2	6.2_5	5.7_3	6.3_8
303	7.6_8	9.5_8	6.5_8	6.6_4
313	8.5_0	26.8_2	12.5_3	10.4_7

Apparent first order rate constants for nystatin uptake by sensitive (S) and resistant (R) yeast cells at different temperatures in the presence of glucose (10 mM) for added nystatin concentrations of 5 $\mu g\ ml^{-1}$ and 10 $\mu g\ ml^{-1}$ at pH 4.0.

	Apparent first order rate constant (sec^{-1}) x 10^4			
T (K)	nystatin 5 $\mu g\ ml^{-1}$ S	R	nystatin 10 $\mu g\ ml^{-1}$ S	R
288	5.0_9 [v]	3.5_9	3.3_9	5.3_4
293	7.1_0	5.9_5	3.6_7	5.7_5
298	7.2_4	7.3_1	3.9_6	6.7_5
303	8.7_5	7.7_0	5.9_6	9.1_5
313	10.7_4	15.8_0	12.1_6	10.0_0

Apparent first order rate constants for uptake of nystatin (5 $\mu g\ ml^{-1}$ or 10 $\mu g\ ml^{-1}$ added concentration) by sensitive (S) and resistant (R) yeast protoplasts as a function of temperature in the presence of 10 mM glucose (pH 4.0).

	k_1 (sec^{-1}) x 10^4			
T (K)	nystatin 5 $\mu g\ ml^{-1}$ S	R	nystatin 10 $\mu g\ ml^{-1}$ S	R
288	8.1_1	5.4_0	7.5_6	5.6_4
293	9.3_6	6.6_9	7.6_7	5.9_4
298	10.3_7	6.9_9	8.5_3	7.5_5
303	10.4_3	9.2_0	8.9_2	9.6_0
313	10.5_9	9.2_4	12.7_7	9.9_5

Apparent first order rate constants (k_1) for uptake by sensitive (S) and resistant (R) yeast protoplasts as a function of temperature (pH 4.0; added nystatin concentration 5 $\mu g\ ml^{-1}$ or 10 $\mu g\ ml^{-1}$)

	k_1 (sec^{-1}) x 10^4			
T (K)	nystatin 5 $\mu g\ ml^{-1}$ S	R	nystatin 10 $\mu g\ ml^{-1}$ S	R
288	8.1_3	6.19	6.28	5.8_3
293	8.8_9	7.2_5	8.0_8	5.8_6
298	9.4_7	7.3_5	8.1_4	7.5_9
303	9.9_7	10.2_6	9.7_4	9.0_7
313	11.2_1	13.3_2	10.2_0	16.1_4

Activation energies (kJ mol^{-1}) for uptake of nystatin by sensitive (S) and resistant (R) yeast cells.

	no glucose		10 mM glucose	
Added nystatin	5 $\mu g\ ml^{-1}$	10 $\mu g\ m^{-1}$	5 $\mu g\ ml^{-1}$	10 $\mu g\ ml^{-1}$
S	19.9	42.3	21.2	40.4
R	30.6	25.1	39.7	21.5

Activation energies (kJ mol^{-1}) for uptake of nystatin by sensitive (S) and resistant (R) yeast protoplasts at two added nystatin concentrations (5 $\mu g\ ml^{-1}$ and 10 $\mu g\ ml^{-1}$) and the absence and presence of glucose.

	Activation Energy (kJ mol^{-1})			
	No glucose		10 mM glucose	
Added nystatin	5 $\mu g\ ml^{-1}$	10 $\mu g\ ml^{-1}$	5 $\mu g\ ml^{-1}$	10 $\mu g\ ml^{-1}$
S	9.66	19.88	12.41	16.06
R	24.29	23.21	24.62	27.14

The consequences of nystatin action upon yeast cells is, as outlined above, the loss of membrane integrity and eventually the loss of cytoplasmic constituents. By standard analytical methods it is possible to analyse for some of these components of the yeast cytoplasm in the supernatant from yeast/nystatin interaction. Such studies have been performed and, again, study of the rates of these processes as a function of temperature allows the derivation of the values for Ea associated with each discrete process. The components investigated are shown in Table 2 together with their associated values of Ea. In addition it is possible from the plots of $\ln k_1$ vs.1/T to evaluate the entropy of activation (31) and hence the value of the Gibbs Function for the activation process (Table 3).

The proposal is, therefore, that if the yeast cell wall can be described as a viscous fluid then the mechanism of cellular ingress or egress via the membrane is rapid and that it is the non-specific diffusion process through the *cell wall* which is rate determining. To further examine this conclusion it is appropriate to assemble the kinetic results and attempt to analyse these data for enthalpy/entropy compensation. The purpose of such an examination is to establish causality i.e. are all processes controlled by some common feature? A plot of lnA (related to $\Delta S^{\ddagger}$ **vs** Ea is linear (correlation coefficient 0.9920). However following recent analyses (32) this plot of $\Delta S^{\ddagger}$ related function and an entalpy related function is inadequate proof of the existence of a real correlation and could arise through error propogation from the original data. A plot of $\Delta S^{\ddagger}$ vs $\Delta H^{\ddagger}$, the more appropriate test for compensa-

tion, for each of the 5 processes is not linear (correlation coefficient 0.1769), but when the uptake data point is omitted then the four remaining points lie on a straight line whose correlation coefficient is 0.8904.

The omission of the data point for uptake is reasonable on the grounds that uptake onto the surface represents an entirely different reaction from the release of "pool" materials from the yeast cytoplasm, for example, Mg^{2+} is unlikely to be present as the free ion. It is rather surprising that these data show such correlation for such differing materials.

TABLE 2

Kinetic data for the interaction of nystatin with *Saccharomyces cerevisiae* NCYC 239

Reaction	lnA*	k(s^{-1})	E_a (kJ mol^{-1})	Ref.
Release of Mg^{2+}	1.40	5.5×10^{-4}	22.1	30
Release of amino acids	2.56	3.62×10^{-4}	26.0	28
Release of Rb^+	6.58	2.5×10^{-4}	36.9**	29
Release of 260 nm absorbing material	7.43	2.83×10^{-4}	38.7	30
Uptake of nystatin	9.56	7.37×10^{-4}	41.6	22

* A is the pre-exponential factor in the Arrhenius equation $k = Ae^{-E_a/RT}$.

** Experiments conducted with *Saccharomyces cerevisiae* SQ 1600.

TABLE 3

Thermodynamic parameters for the processes described

Reaction	E_a (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta G^{\ddagger}$ (kJ mol^{-1})	Ref.
Release of Mg^{2+}	22.1	−276	103.8	30
Release of amino acids	26.0	−267	105.1	28
Release of Rb^+	36.9	−233	105.9	29
Release of 260 nm absorbing material	38.7	−226	105.6	30
Uptake of nystatin	41.6	−209	103.5	22

The compensation observed, and indeed the close similarity in the values of the derived Gibbs Functions for activation suggests that the same mechanism underlies the release of these very different cytoplasmic constituents from yeast cells challenged by nystatin. If indeed the rate determining step is a diffusion process through the cell wall then consideration should be given to improving this aspect of interaction in the design and synthesis of new, and more effective, antifungal drugs.

Metal ion complexes of nystatin have indeed been prepared (8). The metals involved are those which are most commonly found in cellular systems – both in structures and associated with enzyme activity viz. Mg(II), Ca(II), Ni(II), Cu(II) and Zn(II). Not only have these complexes been prepared but they have been characterised structurally and by determination of their stability constants. Examination of the bioactivity of the prepared complexes showed them all to be lower than the parent nystatin but because of improved solubility their achievable overall bioactivity in aqueous solution may well be an improvement on that of nystatin.

The existence of such complexes and their reduced bioactivities requires reassessment of the role of species other than sterol in describing the interaction of nystatin with yeast cells – and furthermore the identification of the cell wall as crucial in determining the *rate* of the interaction both contribute to a revision of the molecular properties of effective antifungal drugs.

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EDUCAÇÃO

EXPERIÊNCIAS E ANALOGIAS SIMPLES PARA O ENSINO DE CONCEITOS EM QUÍMICA. II – RENDIMENTO DE PROCESSOS FÍSICOS E QUÍMICOS

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ABSTRACT

Simple Experiments and Analogies for the Teaching of Concepts in Chemistry. II – Yields of Physical and Chemical Processes.

The clear perception of the physical meaning of theoretical, actual and percentage yields of chemical reactions or of physical processes is not always easy for high-school students and also for first-year college students. Thus, an analogy between yields and election results is presented; this analogy has been found to facilitate much the understanding of the different yield concepts. The analogy is versatile and perennial, since it may be employed with the freshes election results of greater interest to the students.

Ao nível do ensino universitário de Química Geral, ao se introduzir o conceito de rendimento de uma reação, nota-se que grande parte dos alunos ficam perdidos, sem entender o significado físico do novo conceito introduzido. Esta dificuldade é agravada se este conceito for utilizado juntamente com o de reagente limitante; dificuldades com cálculos estequiométricos envolvendo este último conceito foram recentemente mencionadas por Kalantar[1], o qual relata um procedimento que facilita a sua sobrepujança. Estas dificuldades também são notadas, num grau mais exacerbado, no 2º Grau.

Ainda ao nível de Química Geral Universitária, porém experimental, também notou-se recentemente grande dificuldade por parte de muitos alunos para entender o conceito de rendimento percentual de um processo físico, especificamente um processo de recristalização de um composto orgânico.

Reações químicas nem sempre ocorrem dando produtos como esperado com base em suas estequiometrias. Às vezes, somente uma fração dos reagentes reage, e outras vezes, embora a reação ocorra, uma fração dos produtos obtidos é de produtos secundários devido a reações competitivas. Assim, o *rendimento real* de um produto desejado pode ser bem menor que o seu *rendimento teórico.* Do mesmo modo, a qualquer processo físico (recristalização, destilação etc.) também pode ser associado um rendimento teórico e um rendimento real.

Cabe definir o que aqui se entende pelos diversos tipos de rendimento. Assim:

RENDIMENTO TEÓRICO: corresponde à massa ou quantidade de matéria de um produto que seria obtida a partir de uma massa ou quantidade de matéria de reagente se a reação fosse única e ocorresse totalmente; seu cálculo é feito considerando a estequiometria da reação e a presença de eventuais reagentes limitantes (não se leva em conta um possível equilíbrio químico, isto é, considera-se que a constante de equilíbrio da reação é infinita). Por *exemplo,* para a reação

$$H_2(g) + \frac{1}{2} O_2(g) \rightarrow H_2O(\ell)$$

ao se fazer reagir 2,0 g de hidrogênio com 20,0 g de oxigênio, o rendimento teórico em água é 18,0 g (note-se que o hidrogênio é reagente limitante).