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Effect of Amino Acids on the Stability Properties of Nitrofurantoin Suspensions. Flocculation and Redispersion Compared with Interaction Energy Curves

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Abstract

The influence of the addition of amino acids, lysine, alanine and glutamic acid on the properties of nitrofurantoin suspensions was investigated. Flocculation and redispersability was investigated, taking into account the electrical properties of the nitrofurantoin/solution interface.

We conclude that lysine and glutamic acid, but not alanine, would be suitable amino acids for control of the stability of nitrofurantoin suspensions.

A fundamental problem in the preparation of pharmaceutical suspensions is the knowledge and prediction of their stability limits with the aim of controlling their aggregation. One of the most important physical properties of colloidal suspensions in general is the tendency of the particles to aggregate. As a consequence of the Brownian motion, frequent encounters take place between dispersed particles in a liquid medium. The suspension stability will be determined by the result of the interaction between the particles during such encounters.

When two colloidal particles approach the point at which their electric double layers overlap, they experience an electrostatic repulsive interaction; at the same time an attractive London–van der Waals interaction exists for any interparticle distance (Overbeek 1977). If the latter predominates, very compact sediments are originated (caking) which are difficult to redisperse, and are basically constituted by the drug responsible for the therapeutic activity.

The preparation of drug suspensions and the maintenance of their physical stability during long periods of time in variable conditions (temperature, transport, etc.) are still problems for the pharmaceutical industry. It is undoubtedly the characteristics of the particles (size, composition, etc.) and the liquid (mainly viscosity), and particularly the electrical properties of the solid/liquid interface, that control the stability of the whole system. For this reason, among the many factors that must be considered to predict the dispersion stability, the electrical characteristics of the double layer deserve special consideration. In particular, it is necessary to estimate the electrokinetic potential or zeta potential (ζ, see Hunter 1993). We have previously reported the values of ζ for nitrofurantoin (Delgado et al 1990a; Gallardo et al 1991).

The drug used in this work is nitrofurantoin, an important antibacterial agent used for the treatment of urinary tract infections. Due to its low solubility in water, it is administered as a suspension in a suitable vehicle. The analysis of the influence of the addition of amino acids on the properties of aqueous nitrofurantoin suspensions constitutes one of the essential aims of this work. Lysine, alanine and glutamic acid have been used; the choice has been made considering their potential usefulness in the control of the stability of suspensions.

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Lysine, being a basic amino acid, will be positively charged at pH 7, its isoelectric point being close to 9·47. As an acidic amino acid we used glutamic acid, which holds a net negative charge at pH 7 and has an isolectric point of 3·08. Finally, alanine is a non-polar or hydrophobic aminoacid, containing only one amine and one carboxylic group. Its isoelectric point is 6·11.

In this paper we report a study of the stability of nitrofurantion, based on flocculation and redispersability tests. We will take into account the electrical properties of the nitrofurantoin/solution interface, investigated by means of electrophoresis; from these, the interaction–energy curves between nitrofurantoin particles will be computed as a function of interparticle distance, according to the DLVO theory (Verwey & Overbeek 1948; Sonntag & Strenge 1972). Such curves will be correlated with the experimentally-determined stability of the suspensions.

Materials and Methods

Nitrofurantoin was supplied by Riedel–de Haen and amino acids used were from Merck. The water used in the preparation of suspensions was doubly distilled and then deionized through a mixed bed of ion exchange resins, and filtered through a 0·2 μm final membrane (Milli-Q Reagent Water System, Millipore). The stability of suspensions was experimentally characterized determining the volume of sedimented drug 24 h after the suspension was prepared. All tests were run in triplicate in suspensions containing 1% w/v concentration of particles. The suspensions were placed in 100-mL test-tubes with a 4 cm inner diameter, large enough to avoid wall effects during sedimentation (Rigamonti & Rugginenti 1969). The experimental error in the volume determination is 0·5 cm^3. The ratio V_s/V_o proposed by several authors (Matthews & Rhodes 1970) was used as a suitable value for quantifying flocculation; V_s is the volume of sedimented solids, and V_o is the initial total volume of the

suspension. Another parameter we consider interesting and which we determined is the flocculation ratio, β, defined by the following expression:

$$\beta = F/F^{\infty} \quad (1)$$

which gives the relation between the sediment volume of a flocculated suspension (F) and that of a stable suspension (F^{∞}). The redispersion properties of the sedimented suspensions were investigated using a simple and reproducible technique (Delgado et al 1990b). The samples were subjected to a 75 rev min^{-1} agitation for 2 min, and transmittance determined with a Perkin–Elmer spectrophotometer through the upper 30 mL of each test-tube at a wavelength of 410 nm. Redispersion was considered complete when the quantity of substance remaining sedimented was zero (minimum transmittance). Higher transmittances correspond to poorer redispersability of the suspensions.

Results and Discussion

Effect of pH and type of amino acid on sedimentation

Fig. 1 shows a group of typical curves in which the sediment volume increases with time (Rawlims & Kayes 1983), until reaching a maximum value, after 24 h, which corresponds to the situation in which all the particles have settled. There is a considerable effect of pH on the way the suspensions sediment.

Table 1. Solubility (mg L^{-1}) of nitrofurantoin in aqueous solutions of amino acids (10^{-2} M) at the pH values indicated. Values obtained in water are shown for comparison.

Amino acid (10^{-2} M)	pH 3	5	7	8	9
Glycine	60	80	240	780	4400
Lysine	80	60	160	900	4400
Glutamic acid	80	100	120	900	4000
Water	80	60	180	780	4200

At pH 3, a well defined compact sediment was obtained, which was difficult to redisperse, the supernatant being totally transparent. A similar result was obtained at pH 4 and 5; under these conditions practically 90% of the drug was sedimented, with final volumes of (compact) sediment of 5 and 4·8 mL respectively. By contrast, the characteristics of suspensions with pH close to neutrality are clearly different: sedimentation is much slower (about 4 h was required for the supernatant to be transparent) and the sediment volume is larger, reaching 9.2 mL at pH 6 and 11·7 mL at pH 7. A very noticeable feature, different from the previous cases, is the fact that the sediment remains spongey and easy to redisperse. At alkaline pH, the sediment volumes obtained were lower, and in all cases easier to redisperse than at acid pH. Cadwallader & Jun (1976) have shown

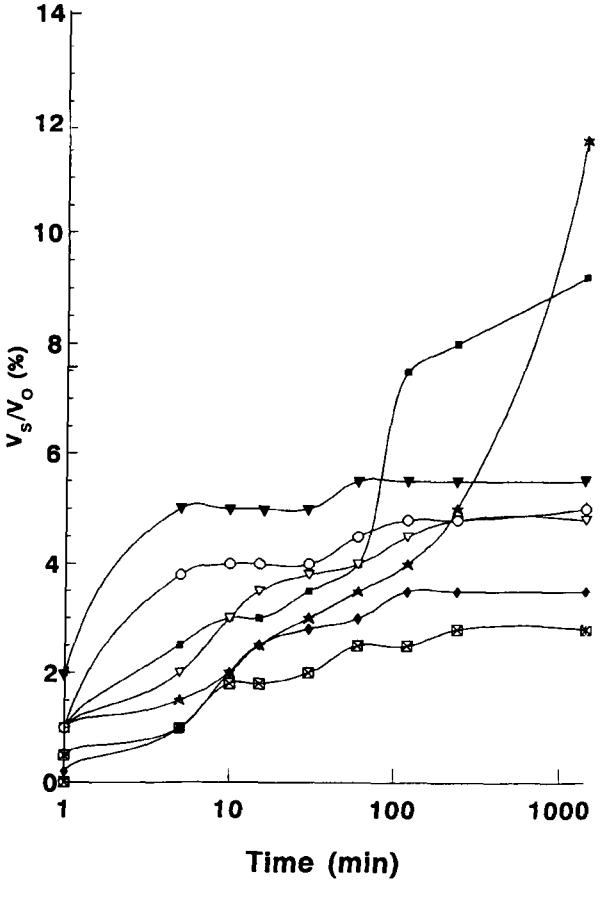

FIG. 1. Sedimentation of nitrofurantoin suspensions as a function of time for different pH values. (▼) pH 3; (○) pH 4; (▽) pH 5; (■) pH 6; (×) pH 7; (◆) pH 8; (□) pH 9.

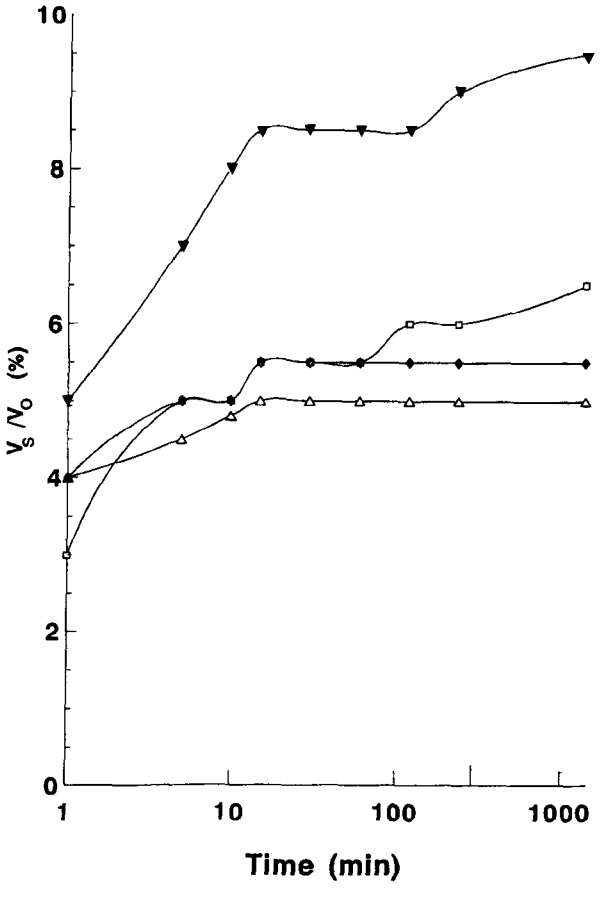

FIG. 2. Sedimentation of nitrofurantoin suspensions as a function of time for different pH values, in the presence of lysine. (◆) pH 3; (△) pH 5; (▼) pH 7; (□) pH 8.

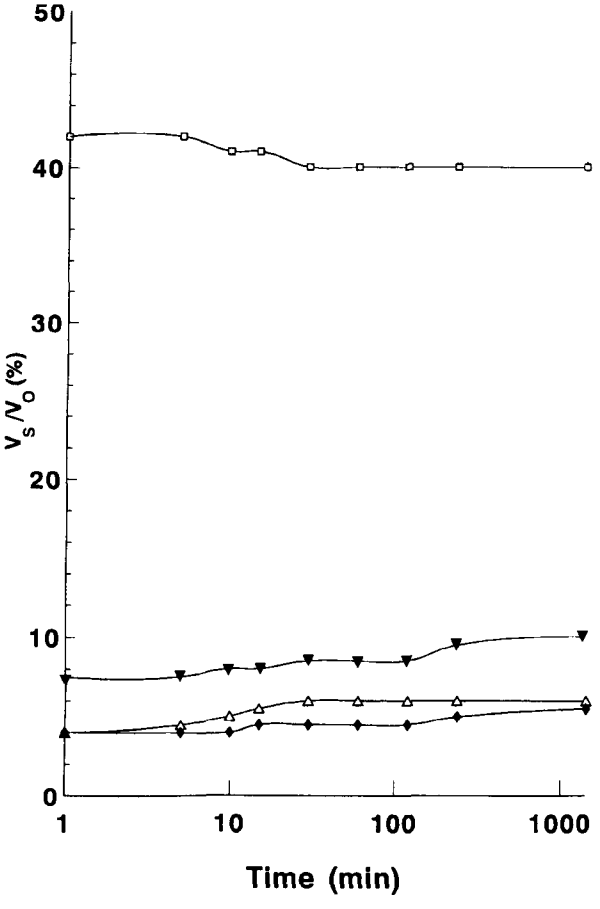

FIG. 3. Sedimentation of nitrofurantoin suspensions as a function of time for different pH values, in the presence of glutamic acid. (◆) pH 3; (△) pH 5; (▼) pH 7; (□) pH 8.

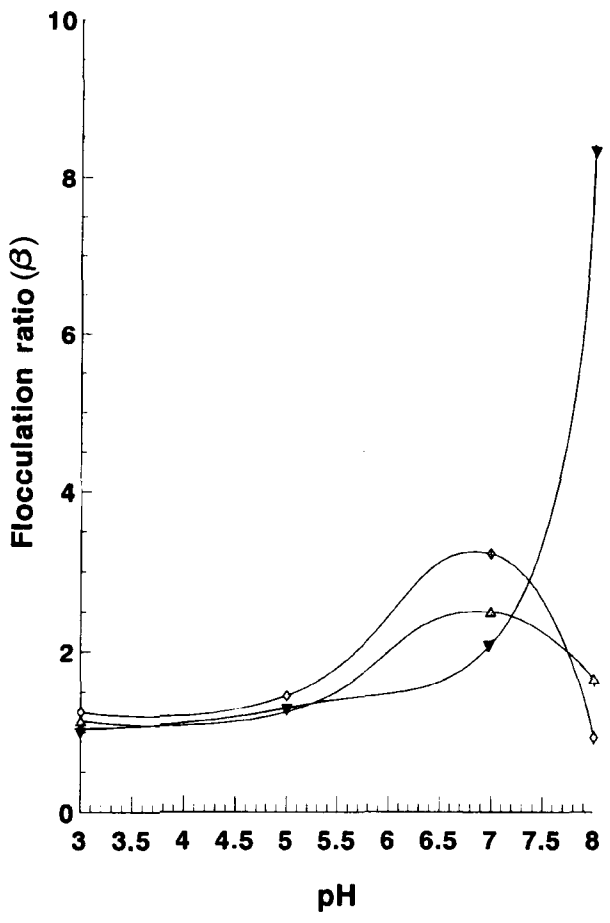

FIG. 4. Flocculation ratio of nitrofurantoin suspensions as a function of pH. (◇) Alanine; (△) lysine; (▼) glutamic acid.

that nitrofurantoin has an increased solubility in aqueous media above pH 6. In order to check for this possibility when amino acids are added to the dispersion medium, we determined the solubility of nitrofurantoin for pH values between 3 and 9 in different conditions; Table 1 shows that solubility increases considerably above pH 7. However, little or no effect was found on addition of the amino acids.

Figs 2 and 3 show the behaviour found for the sedimentation of suspensions in the presence of 10^{-2} M lysine and glutamic acid respectively. Larger sediment volumes were obtained at pH 7 in the presence of lysine, whereas in the presence of glutamic acid (pH 8) there is an initial large volume of sediment, decreasing with time towards a constant value, termed hindered sedimentation (Bueno 1979). It is more appropriate to use the β parameter (flocculation ratio), instead of the relative sedimentation volume V_s/V_o, for characterizing the stability of suspensions. To calculate this parameter we have taken the value of F^{∞} as the ratio V_s/V_0 for a suspension of nitrofurantoin in water (pH 5) without any other additions. The value of V_s, obtained as an average of three determinations carried out under the same conditions, was $4{\cdot}8 \pm 0{\cdot}2$ mL after 24 h sedimentation. Fig. 4 shows the dependence of β on pH for the three amino acids (10^{-2} M). In acidic conditions, the flocculation ratio reaches relatively small values, and a similar effect is observed at alkaline pH. However, at pH 7 β values were higher, which suggests that in these conditions the particles are aggregated forming flocculi that are easy to redisperse (Gallardo et al 1991). The suspensions are more unstable when the pH is close to neutrality. It must also be mentioned that the remarkably different effect again presented with glutamic acid at pH 8: at the concentration studied, β reaches values up to 8-fold those found for the other amino acids at any pH value.

So we can speak of flocculated nitrofurantoin suspensions: with a slight stirring, the system can be recomposed, which will be confirmed afterwards with the results obtained in redispersion tests.

Effect of pH and type of amino acid on the redispersion of suspensions

In the previous section, we have studied the sedimentation process of nitrofurantoin suspensions, a very interesting aspect in the physicochemical system that we are examining. However, it is clear that the most important factor, from the pharmaceutical point of view, is that the resulting sediment exhibits the quality of being easily redispersable by simple agitation in such a way that at the moment of administration, a homogeneous system might allow an exact dosage of the preparation. In spite of this, there are few reports on redispersability of sedimented particles. Most authors confine themselves to assert that flocculated systems redisperse easier than nonflocculated suspensions. Thus, it seemed of interest to verify experimentally this possible relation in our suspensions. The study of redispersa-

Table 2. Transmittance (%) of nitrofurantoin suspensions after redispersion.

pH	Amino acid		
	Alanine	Lysine	Glutamic acid
3	92	97	100
5	76	76	100
7	45	0	90
8	50	22	0

bility was, therefore, parallel to the sedimentation and flocculation studies, the tests being performed after 24 h (estimated time for sedimentation to be complete). Table 2 summarizes the experimental results; redispersability data are presented (expressed in percent transmittance) as a function of pH for the three amino acids at 10^{-2} M constant concentration. It is shown that, in general, for high values of β (glutamic acid, pH 8) transmittances are low; that is to say, a better redispersion is achieved. In contrast, high values of transmittance correspond to low values of β, for the acid pH range, probably indicative that sedimentation of the particles has practically been individual, forming a compact sediment at the bottom of the container, where van der Waals attractive forces are likely to be very significant.

Interaction energy curves

Our first aim was to predict (or at least, to explain) the stability properties of nitrofurantoin suspensions. The classical theory of stability of colloidal suspensions (DLVO) states that the main variables controlling stability are: average particle size, ionic characteristics (concentration and type of ions) of the dispersion medium and, above all, the surface electrical characteristics of the particles. According to the DLVO model, the total energy of interaction between particles as a function of the distance H that separates their surfaces has two components:

$$V_T(H) = V_{LW}(H) + V_{EL}(H) \quad (2)$$

where V_T is the total energy, V_{LW} is the Lifshitz–van der Waals attractive contribution, and V_{EL} the component due to electrostatic repulsion between double layers. The expression of the first of these contributions is (Sonntag & Strenge 1972):

$$V_{LW}(H) = -Aa/12H \quad (3)$$

where a is the radius of the particles, assumed spherical (4 ± 1 μm in our case) and A is the so-called Hamaker constant, characteristic of the particles and the medium. This constant has been previously determined (Bolívar 1993), and its values are $1{\cdot}7 \times 10^{-20}$ J, $1{\cdot}8 \times 10^{-20}$ J, and $1{\cdot}3 \times 10^{-20}$ J, in 10^{-2} M solutions of alanine, lysine, and glutamic acid, respectively.

The electrostatic component of the interaction energy is given by (Sonntag & Strenge 1972):

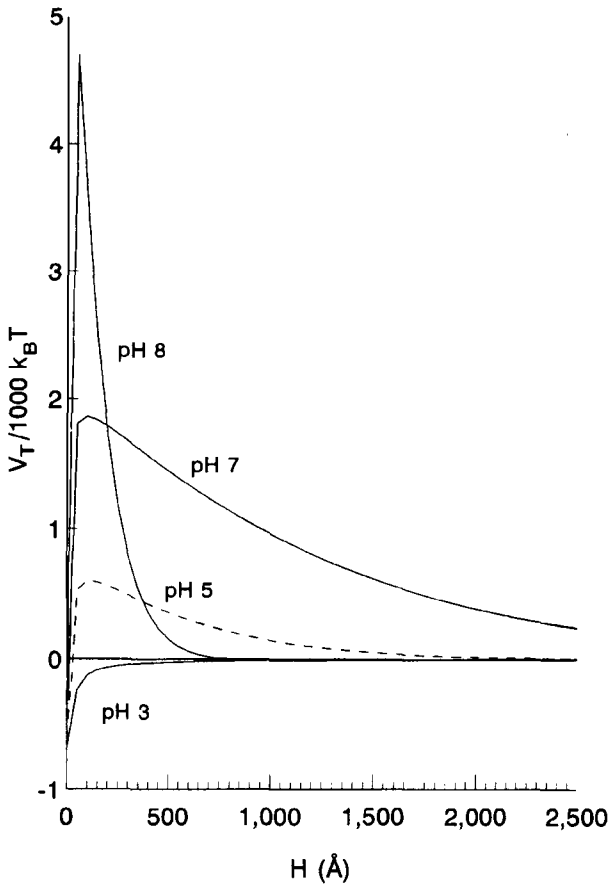

FIG. 5. Potential energy of interaction plotted as a function of distance for different pH values. Amino acid: alanine.

$$V_{EL}(H) = 2\varepsilon_r\varepsilon_0 a\zeta^2 \times \ln(1 + e^{-\kappa H}) \quad (4)$$

where ε_r is the dielectric constant of the solution, ε_0 is the permittivity of a vacuum, and κ is the inverse of the Debye length (or double layer thickness), that depends on the molar concentration c_i, and the valency z_i on the ions of the medium, as follows:

$$\kappa = \sqrt{\left(\frac{10^3 e^2 N_A \sum C_i z_i^2}{\varepsilon_r \varepsilon_0 k_B T}\right)} \quad (5)$$

where e is the electron charge, N_A is the Avogadro number, k_B is the Boltzmann constant, and T is the absolute temperature. To calculate c_i, use was made of the pK value of each amino acid. Table 3 summarizes the data employed for the three amino acids, at the different pH values studied. The zeta potentials included in Table 3 were determined from electrophoresis measurements (Gallardo et al 1991; Bolivar 1993).

Table 3. Zeta potential (ζ) and reciprocal Debye length (κ) for the suspensions studied.

	Amino acid (10^{-2} M)											
	Alanine				Lysine				Glutamic acid			
pH	3	5	7	8	3	5	7	8	3	5	7	8
ζ (mV)	− 5	− 17	− 27	− 48	− 19	− 21	− 23	− 28	− 13	− 17	− 13	− 21
$\kappa(10^{-3}$ Å$^{-1})$	20	1·7	0·9	8	32	23	23	22	15	23	30	33

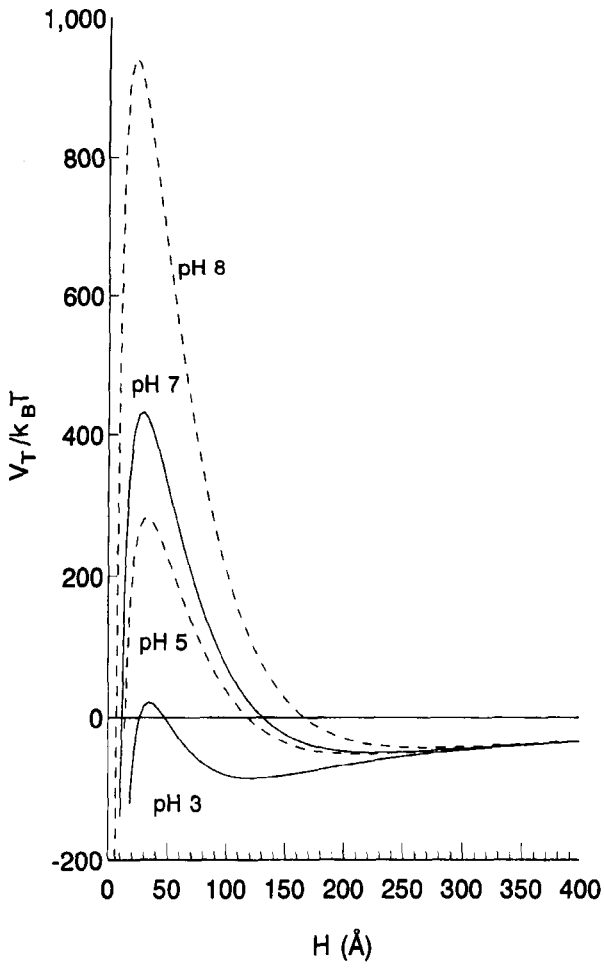

FIG. 6. Potential energy of interaction plotted as a function of distance for different pH values. Amino acid: lysine.

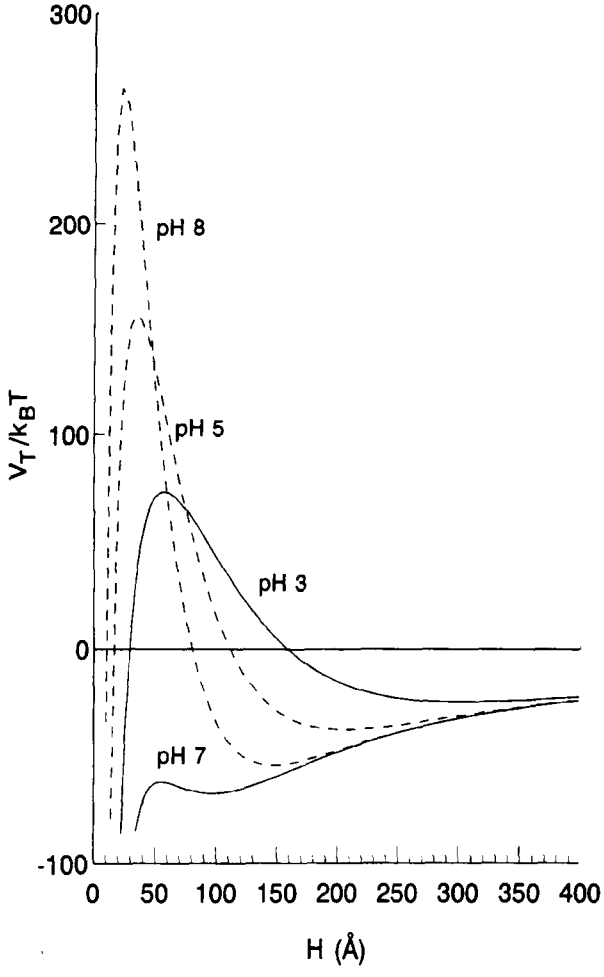

FIG. 7. Potential energy of interaction plotted as a function of distance for different pH values. Amino acid: glutamic acid.

The results are shown in Figs 5 (alanine), 6 (lysine) and 7 (glutamic acid). Fig. 5 indicates that nitrofurantoin suspensions in the presence of alanine should be slightly unstable at pH 3, whereas at pH 5, 7 and 8 the potential energy barrier would avoid aggregation. However, the fact that the double layer is so extended (high values of κ^{-1}) in this case makes the variation of potential energy with distance so slow that particles will not practically exert any net force between them (dV/dH;0 for a wide interval of H). Only at pH 7 there is a net repulsion that would give slightly more stable suspensions, as in fact observed (Fig. 4).

The stability of suspensions in the presence of lysine should be affected in a very different way by pH, according to predictions based on the curves $V_T(H)$ (Fig. 6). At pH 3, a very pronounced secondary minimum ($\sim 100\ k_BT$) is found, which is compatible with the instability of the suspensions and their difficult redispersion, in agreement with data in Fig. 4 and Table 2; the aggregates formed would not be so compact, but the great energy of the minimum makes redispersion difficult, as shown in Table 2. At pH 5 and 7, the minimum is less significant, so suspensions redisperse easily, according to experimental data. The slight increase of transmittance observed at pH 8 does not correspond, however, with the tendency of variation of V_T with H of Fig. 6: it is probably just an effect associated with the solubilization of the drug particles under these pH conditions (Table 1): transmittance increases as a consequence of the decrease of solids concentration in the medium.

The variation of V_T with respect to H in the presence of 10^{-2} M glutamic acid is shown in Fig. 7. There is a secondary minimum (H;150 Å, V; $-$ 60 k_BT), at pH 8; the experimental situation should correspond to large sedimentation volumes (high β) and very redispersable suspensions. Both phenomena were observed, as shown in Fig. 4 and Table 2. At pH 7, the curve shows a situation of irreversible aggregation (low β value and difficult redispersion), whereas at pH 3 and 5 the system should be stable; also, in this case (due to the particle size, sedimentation will always be present), low values of sedimentation volume will be attained. Redispersion will only be made more difficult, as the data show, by the particles forming compact sediments in which the distances between them are short enough to make van der Waals attractive forces significant.

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