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Pharmaceutical suspensions: micro electrophoretic properties

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The microelectrophoretic properties of the drugs griseofulvin, betamethasone, nalidixic acid and thiabendazole in aqueous dispersion have been examined and the zeta potentials calculated from the measured mobilities. Variation in magnitude of particle charge with pH of dispersion is reported and related to the chemical structure and surface characteristics. The effect of adding anionic (sodium dodecyl sulphate) cationic (dodecyl trimethyl bromide) and non-ionic (polyoxyethylene glycol monoethers of hexadecanol) surface-active agents, and mixtures of these ionic and non-ionic species, on the electrophoretic properties of the drug dispersions has been measured. The results reported agree with those found previously for a model polystyrene latex suspension system under the same conditions.

The value of measuring the zeta potential of dispersed particles in a suspension, when considering its stability, has been shown by, for example, Haines & Martin (1961), Matthews & Rhodes (1968, 1970) and Takamura, Kaneniwa & Imagawa (1974). However, to measure this parameter by microelectrophoresis, the drug has usually been dispersed with surface-active agents, thus altering the magnitude of the zeta potential. No attempt to measure the variation of charge with pH of the aqueous medium appears to have been made. This paper examines these charge characteristics in suspensions of betamethasone, griseofulvin, nalidixic acid and thiabendazole, both alone and in the presence of surface-active agents, the latter at a controlled pH.

MATERIALS AND METHODS

Materials

Betamethasone B.P., median diameter 1·6 μm, griseofulvin B.P. median diameter 1·9 μm were a gift from Glaxo Laboratories Limited, nalidixic acid B.P.C., mean particle diameter 36·5 μm, a gift from Winthrop Laboratories and thiabendazole B.P.C., median diameter 12 μm a gift from Merck Sharp and Dohme Limited. Particle size distributions of betamethasone and griseofulvin were obtained using a Joyce Loebl disc centrifuge, nalidixic acid by Fisher sub-sieve sizer and thiabendazole using an Andreasen pipette. All drugs were used as received.

Surface-active agents, sodium dodecyl sulphate (SDS), dodecyl trimethyl ammonium bromide (C_{12}TAB), and polyoxyethylene glycol monoethers of hexadecanol (the Texofor A series, as supplied by Glovers Chemicals Limited and containing an average of 10, 30 and 60 ethylene oxide units, $C_{16}E_{10}$; $C_{16}E_{30}$ and $C_{16}E_{60}$ respectively) have been described previously (Kayes, 1976).

Electrophoretic mobility measurements

Mobility determinations on the drug particles were made using a Rank Mark II Particle Microelectrophoresis Apparatus, (Rank Bros. Bottisham, Cambridge, U.K.) of a type as originally described by Seaman (1965). The flat cell assembly in which the cell is mounted laterally, was used; electrodes were of platinum black.

Solutions and dispersions were made in 10^{-3} mol dm^{-3} NaCl to give a solution of suitable conductance and to keep the ionic strength, as far as possible, constant. All measurements were made at 25°.

Mobility/zeta potential—pH plots. The drug was dispersed in 10^{-3} mol dm^{-3} sodium chloride solution. The pH of the dispersion was measured by pH meter, adjustment of pH being made by the addition of sodium hydroxide or hydrochloric acid. The dispersion was then transferred to the microelectrophoresis cell and velocity measurements made.

Effect of surface-active agents. The effect of the various surface-active agents (both alone and as mixtures of ionic and non-ionic species) on the mobility of the drug particles was examined. Dispersions of the drugs in solutions of the surface-active agents, pH adjustments being made where

necessary, were shaken for 24 h in a constant temperature bath to allow time for complete adsorption of the surface-active agent—Elworthy & Guthrie (1970) had found that 16 h was necessary for complete adsorption of non-ionic surfactants, C_xE_y, on griseofulvin. Velocity measurements were then made.

Calculation of zeta potentials

The theoretical relation between the electrophoretic mobility—the electrophoretic mobility, u, of a particle is its velocity over unit distance under the influence of an applied unit potential—and the zeta potential, ζ, is complicated by retardation and relaxation effects and by the magnitude of the zeta potential, such that it involves a power series in zeta potential the coefficients of which are κa (where a is the radius of the particle and $1/\kappa$ the Debye Huckel double layer thickness). However, Wiersema, Loeb & Overbeek (1966) have shown that for values of κa >100 at low potentials, the Smoluchowski equation, $u = \epsilon\zeta/\eta$, (where ϵ is the permitivity and η the viscosity of the dispersion medium) can be used without great error.

While the particles of drugs used were not spherical, Overbeek & Wiersema (1967) have shown that, when the particle is insulating, and the thickness $1/\kappa$ of the double layer is small compared with the radius of curvature of any point, Smoluchowski's equation is valid irrespective of the form of the particle and thus applicable to the systems used.

RESULTS AND DISCUSSION

Mobility/zeta potential—pH plots

Griseofulvin. Fig. 1 shows a positive charge of $\zeta = +25$mV at pH 1·5 which rapidly decreases to zero at pH 2·4, there is then reversal of charge followed by an increase over the pH range 2·4 to 7·0. The zeta potential at the latter pH is -45mV. The potential then stays constant over the pH range 7 to 10. The positive charge at low pH can be attributed to the protonation of the α,β-unsaturated ketone at position 4′ aided by the positive donating mesomeric effect, (the +M effect) of the methoxyl group, as pH increases this effect will diminish. Above pH 2·4 there is a gradual increase in negative potential to pH 7·0, which then stays constant, this must be due to adsorption of hydroxyl ions. Because the zeta potential stays steady at pH 7·0 and above further studies were carried out at this pH.

Betamethasone (Fig. 1) shows little variation in mobility with pH. There is evidence that the particles are positively charged below pH 3·0, but movement was so slow even at high voltages that it was difficult to determine the mobility. Reversal of charge occurs at *ca* pH 3·0 and then from pH 3·0 to 5·0 there is a gradual increase in negative potential to a zeta potential of $-6{\cdot}5$ mV which then stays steady as pH is increased to 10·0. Such a pattern of mobility can only be due to ion adsorption as is expected from its structure. As a steady state mobility occurs above pH 5·0, further studies were carried out at *ca* pH 7·0.

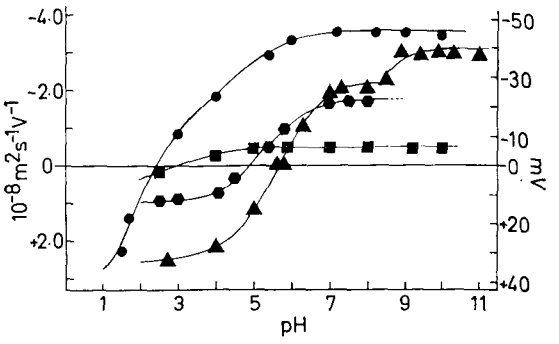

FIG. 1. pH—mobility (10^{-8} m^2 s^{-1}V^{-1})/zeta potential (mV). ●—griseofulvin, ▲—thiabendazole, ◆—nalidixic acid, ■—betamethasone.

For *nalidixic acid*, Fig. 1, the plot shows a positive zeta potential, virtually constant over the pH range 2–3·5, of +12 mV, this decreases with increasing pH to charge reversal at pH 4·9. The zeta potential then rapidly increases with pH to pH 7·0 to a steady state value of -22 mV. Above pH 8·0 the drug goes slowly into solution and it was not possible to achieve a steady pH above this value.

The positive charge at low pH is attributed to protonation of the immino group, =N–, at position 8, this effect would decrease as pH is increased. The gradual increase of negative potential over the pH range 4·9 to 7 is consistent with the ionization of the carboxyl group, –COOH. It has been shown (Ottewill & Shaw, 1967) that the bulk dissociation constant k_b of the carboxyl groups can be obtained using the equation

$$pk_b = pH_b \ (\zeta = \tfrac{1}{2}\zeta_o)$$

(ζ_o is the limiting zeta potential corresponding to complete ionization and pH_b ($\zeta = \frac{1}{2}\zeta_o$) is the pH at the zeta potential representing 50% ionization of the carboxyl groups).

Use of this equation here gives a pK_b for nalidixic acid of 5·75 which agrees well with the value of 6·0

reported by Winningham, Nemoy & Stamey (1968). For further study, due to the rapid change of mobility over the pH range 4·0 to 7·0, pH 7·0 was chosen as being the most suitable for the other additives.

Thiabendazole. The mobility/zeta potential—pH plot for thiabendazole, Fig. 1 shows a positive potential at low pH of +33 mV which gradually decreases to the pH for charge reversal at 5·65. The potential then increases to a value of −27 mV at pH 7·5, is steady over the range 7·5 to 8·5 and again increases to −37·5 mV at pH 9·0, the zeta potential stays steady at this value over the pH range 9 to 11. Thiabendazole has two immino groups –N= in its structure at positions 3 and 3′ and these would account for the positive charge at low pH. The fact that the zeta potential at pH 2·5 is *ca* +33 mV, with two immino groups in the structure, whilst with nalidixic acid at pH 2·5 it is +12 mV with one immino group, supports this suggestion. The increase in negative potential which occurs over the pH range 5·65 to 7·5 is attributed to hydroxyl ion adsorption as with griseofulvin, and the stepwise increase between pH 8·5 and 9·0 to ionization of the =NH, secondary amino group. Again the pH for further studies was chosen to be in the steady state region close to pH 7·0, here pH 7·5.

Effect of surface-active agents on mobility and zeta potential

Effect of non-ionic surface active agents. The effect of non-ionic surfactant on the mobility of the drug particles was similar to that found with the polystyrene latex model system previously examined (Kayes, 1976), one of the non-ionics C_{16} E_{30} was used with all four drug systems. Results are shown in Fig. 2 (the results for polystyrene latex included for comparison). In all cases the plots show the characteristic fall off in mobility with increasing concentration of non-ionic to a steady state value. This suggests that the adsorption pattern of the non-ionic surfactant is similar to that of the non-ionic onto polystyrene latex, i.e. that there is adsorption of the alkyl chain onto a hydrophobic portion of the surface, by the hydrophobic effect, and also association of the ethylene oxide groups with some polar group at the surface probably by hydrogen bonding. Elworthy & Guthrie (1970) have shown that such adsorption does occur with these non-ionics on griseofulvin.

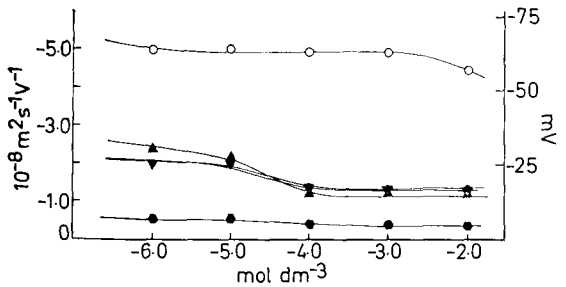

FIG. 2. Mobility (10^{-8}m^{2}s^{-1}V^{-1})/zeta potential (mV)—$\log_{10}$ concentration $Ce_{16}E_{30}$ (mol dm^{-3}). ○—polystyrene latex, ◆—betamethasone, ▲—griseofulvin, ●—nalidixic acid, ▼—thiabendazole.

Although the surfaces of the particles are to some extent hydrophobic they also possess polar groups and are still different from each other; this is typified by the fact that the percentage displacement of mobility at the steady state is not equal:—griseofulvin steady state mobility $-3{\cdot}5 \times 10^{-8}$ m^2 s^{-1} V^{-1} displaced to $-1{\cdot}20 \times 10^{-8}$ m^2 s^{-1} V^{-1}, a 66% displacement. Betamethasone $-0{\cdot}45$ to $-0{\cdot}35 \times 10^{-8}$ m^2 s^{-1} V^{-1} −22%; nalidixic acid $-1{\cdot}68$ to $-1{\cdot}25 \times 10^{-8}$ m^2 s^{-1} V^{-1} −26%; thiabendazole $-2{\cdot}10$ to $-1{\cdot}25 \times 10^{-8}$ m^2 s^{-1} V^{-1} −40%.

Differences would be expected from the differences in chemical composition but the results point to the non-ionics being more strongly adsorbed onto griseofulvin and thiabendazole; this point is suggested by the mobility–pH plots which show a rise to a fairly high negative value apparently due to hydroxyl ion adsorption. Some difference in adsorption energies is also shown with anionic and cationic surfactants.

Effect of dodecyl trimethyl ammonium bromide. The adsorption of C_{12}TAB at the particle-liquid interface is dependent on the chemical structure of the particle and its charging mechanisms. At the pH's used the charging mechanism of griseofulvin, betamethasone and thiabendazole is ion adsorption. Nalidixic acid acquires its charge by ionization of carboxylic acid groups. Results for griseofulvin, typical of all four drugs, are shown in Fig. 3. Nalidixic acid behaves analogously to a polystyrene latex (Kayes, 1976), i.e. adsorption is a two stage process, with neutralization of the –COO′ of the acid and then adsorption of the C_{12}TAB in reverse orientation to give rise to a positive zeta potential which will increase to a maximum as the concentration of cationic increases.

With the other drugs the C_{12} TAB ions will adsorb on to the particle surface in reverse orientation by the hydrophobic effect, charging ions will thus be displaced from the Stern plane, the net effect will be reductions of the Stern potential to zero followed

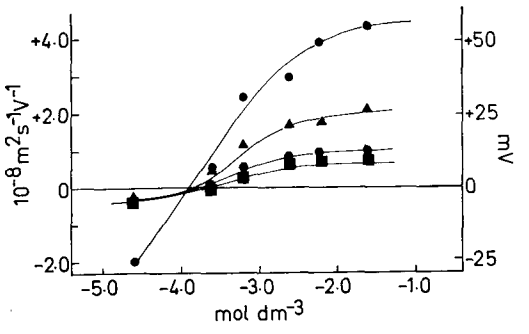

FIG. 3. Mobility ($10^{-8}m^2s^{-1}V^{-1}$)/zeta potential (mV) griseofulvin—$\log_{10}$ concentration (mol dm^{-3}). ●—C_{12} TAB, ■—with 10^{-2} mol dm^{-3} $C_{16}E_{60}$, ◆—10^{-2}mol dm^{-3} $C_{16}E_{30}$, ▲—10^{-2} mol dm^{-3} $C_{16}E_{10}$.

by charge reversal to a maximum positive value as the concentration of C_{12}TAB increases.

A similar pattern of results was reported by Takamura & others (1974) with sulphadiazine, sulphaphenazole and sulfisomidine.

The concentrations of C_{12}TAB required to bring about charge reversal with the four drugs (the reversal of charge concentration RCC) are shown in Table 1 together with their steady state zeta potentials in the absence of C_{12}TAB.

Table 1. *Reversal of charge concentrations (RCC) and adsorption free energy for C_{12} TAB on drug particles.* Steady state zeta potentials in the absence of C_{12}TAB.

Drug	RCC of C_{12}TAB mol dm^{-3}	Zeta potential mV (without C_{12}TAB)	$-\Delta G$ kJ mol^{-1}
Griseofulvin	$1{\cdot}3 \times 10^{-4}$	−45	29·7
Betamethasone	$4{\cdot}0 \times 10^{-5}$	−6·5	26·4
Nalidixic acid	$3{\cdot}2 \times 10^{-4}$	−22	28·9
Thiabendazole	$9{\cdot}0 \times 10^{-5}$	−27	29·4

RCC's follow the original zeta potentials for griseofulvin, betamethasone and thiabendazole as might be expected from the ion adsorption charge mechanism. Nalidixic acid is different, presumably because of the charge neutralization required.

The maximum values for zeta potentials found at $2{\cdot}5 \times 10^{-2}$ mol dm^{-3} C_{12}TAB are:— griseofulvin +54·6 mV; betamethasone +46·6 mV; nalidixic acid +25 mV; thiabendazole +44·3 mV. These results suggest that approximately the same amount of C_{12}TAB adsorbs onto griseofulvin, betamethasone and thiabendazole but that nalidixic acid, because of the presence of –COOH groups, is not able to take up the same quantity of C_{12} TAB molecules as those molecules adsorbed with their charge groups towards –COO′ will have their alkyl chains towards the aqueous phase consequently the 'saturation' potential will be lower than if the molecules were all orientated with their charge group towards the aqueous phase.

The slope of the zeta potential–$\log_{10}$ concentration plot can be used to calculate the free energies of adsorption ΔG of cationic surface-active agents, Table 1. The values of ΔG are of the same order, however it is interesting that the values for griseofulvin and thiabendazole are slightly higher than the other two drugs and that this is similar to the results with non-ionic surfactants.

Effect of mixtures of dodecyl trimethyl ammonium bromide and non-ionic surface-active agents. The effect of adding non-ionic surface-active agents to the C_{12}TAB drug suspension systems was examined with constant concentrations of the non-ionic and variation of the concentration of C_{12}TAB. The mixtures used were griseofulvin with C_{12}TAB and C_{16} E_{10}, C_{16} E_{30} and C_{16} E_{60}; and betamethasone, nalidixic acid, thiabendazole with C_{12}TAB and C_{16} E_{30}.

Fig. 3 shows results for griseofulvin which are typical of the four drugs.

The zeta potential–$\log_{10}$ concentration plots follow a pattern similar to that for polystyrene latex/CxTAB/non-ionic systems. There is one main difference in that the RCC is not displaced to the same extent as with the polystyrene systems (see Table 2).

Table 2. *Reversal of charge concentrations (RCC) for C_{12} TAB on drug particles and a polystyrene latex, both alone and in the presence of C_{16} E_{30}.* Displacement of the reversal of charge concentration due to the C_{16} E_{30}.

Drug	RCC with C_{12} TAB mol dm^{-3}	RCC with C_{12}TAB and C_{16} E_{30} mol dm^{-3}	RCC Dispalcement
Griseofulvin	$1{\cdot}3 \times 10^{-4}$	2×10^{-4}	0·7
Betamethasone	$4{\cdot}0 \times 10^{-5}$	2×10^{-4}	1·6
Nalidixic acid	$3{\cdot}2 \times 10^{-4}$	$4{\cdot}5 \times 10^{-4}$	1·3
Thiabendazole	$9{\cdot}0 \times 10^{-5}$	$1{\cdot}2 \times 10^{-4}$	0·3
(Polystyrene latex (Kayes 1976)	$1{\cdot}0 \times 10^{-5}$	$6{\cdot}0 \times 10^{-4}$	5·9)

It is significant that the displacement values follow the adsorption energies of C_{12}TAB on the various drugs as reported in Table 1, showing that the form of the plot produced depends on the relative adsorption energies of the two surfactants on any particular drug system.

The effect of altering the ethylene oxide chain length with griseofulvin, Fig. 3, was similar with C_{16} E_{10}, C_{16} E_{30} and C_{16} E_{60} and C_{12}TAB, the mobilities lying within $-0{\cdot}5$ to $+1{\cdot}5 \times 10^{-8}$ m^2 s^{-1} V^{-1}. A similar result was found with polystyrene systems.

Effect of sodium dodecyl sulphate: at the pH's at which investigations were made all particles carry a negative charge—griseofulvin, betamethasone and thiabendazole by ion adsorption and nalidixic acid by ionization of carboxylic acid groups. As with the polystyrene system (Kayes, 1976) the driving force for adsorption is the hydrophobic effect, the molecules of SDS being adsorbed 'between' the –COOH groups of nalidixic acid, and onto the surface of the other drugs replacing charging ions already within the Stern layer. The zeta potential should consequently increase with increasing concentration of surface-active agent until the surface is 'full' and should then remain constant. Results shown for griseofulvin, Fig. 4 are typical. The plot shows the same pattern as found with anionic surface-active agents and polystyrene latex, i.e. a small increase in negative zeta potential as the concentration of SDS is increased, probably due to replacement of ions in the Stern layer by the SDS, followed by a fairly rapid rise to a maximum. The zeta potential then stays constant with increasing concentration of SDS or falls off slightly due to ionic strength effects. In all cases the maximum in potential occurs at about 10^{-2} mol dm^{-3} SDS which is in the region of its cmc in water at 25°. A similar concentration for maximum zeta potential was found with the polystyrene latex/SDS system.

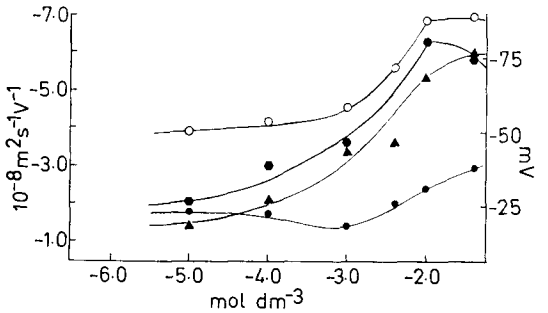

FIG. 4. Mobility (10^{-8}m^{2}s^{-1}V^{-1})/zeta potential (mV griseofulvin—$\log_{10}$ concentration SDS (mol dm^{-3}).) ○—SDS, ●—$C_{16}E_{30}$ 10^{-2} mol dm^{-3}, ▲—$C_{16}E_{30}$ 10^{-3} mol dm^{-3}, ◆—$C_{16}E_{30}$ 10^{-4} mol dm^{-3}.

The maximum values for zeta potentials found with the four drugs when it can be assumed that the surface of the particles is covered with vertically orientated molecules of SDS, taken at 10^{-2} mol dm^{-3} SDS are given in Table 3; it would be expected that all results would be of the same order at maximum adsorption—the –COO′ groups of nalidixic acid contributing an effect of the same magnitude as the $-SO_3'$, group of SDS.

Table 3. *Zeta potential at maximum adsorption and adsorption free energy for SDS on drug particles.*

	Zeta potential, mV, at maximum absorption of SDS, 10^{-2} mol dm^{-3}	$-\Delta G$ kJ mol^{-1} taken from SDS conc/zeta potential slope at 10^{-3} mol dm^{-3} SDS
Betamethasone	−54	30·6
Griseofulvin	−89	31·8
Nalidixic acid	−67	31·7
Thiabendazole	−59	31·3

The calculation of the free energies of adsorption of anionic surface-active agents onto the surface of negatively charged (due to ionizing carboxylic acid groups) polystyrene latex particles has been discussed by Kayes (1976). The situation would seem to be analagous with nalidixic acid. However for griseofulvin, betamethasone and thiabendazole, where the negative charging mechanism is due solely to ion adsorption, there will not be any contribution due to electrostatic repulsion and the adsorption energies will be due solely to the hydrophobic effect. The slope of the zeta potential-$\log_{10}$ concentration SDS plot was taken at the point where rapid rise in potential occurred i.e. 10^{-3} mol dm^{-3} SDS in all cases. The results, Table 3, show good agreement between the values for the four drugs with those for C_{12}TAB (Table 1) and confirm the findings for C_{12}TAB and SDS on polystyrene latex that it is the alkyl chain which is the factor controlling the adsorption of these surfactants onto a hydrophobic surface.

Effect of mixtures of sodium dodecyl sulphate and nonionic surface-active agents. The effect of adding non-ionic surface-active agents to the SDS—drug systems used in the previous sub-section was examined. A constant concentration of non-ionic

surfactant was used, while the concentration of SDS was varied and the change in mobility measured. The systems used were griseofulvin with SDS and C_{16} E_{10}, C_{16} E_{30} and C_{16} E_{60}; and betamethasone, nalidixic acid and thiabendazole with SDS and C_{16} E_{30}.

The results for the SDS non-ionic mixtures follow the pattern found with polystyrene/SDS/C_{16} E_y systems. Fig. 4, which gives results for griseofulvin, SDS with concentrations of C_{16} E_{30}, shows that displacement of the zeta potential-$\log_{10}$ concentration plot occurs. The magnitude of the displacement depending on ethylene oxide chain length. The increased lowering of potential found with slightly higher concentrations of SDS with C_{16} E_{30} and C_{16} E_{60}, is similar to that found with polystyrene latex/SDS/C_{16} E_{30} and C_{16} E_{60}, and is due to complex formation between the surfactants, as discussed by Kayes (1976). As expected no such complexation effect was found with SDS/C_{16} E_{10}.

In earlier work with polystyrene latex systems, the effect of ethylene oxide chain length on the zeta potential of the system polystyrene latex/SDS/C_{16} E_y was investigated and a direct proportionality between chain length and lowering of zeta potential produced was found.

Fig. 5 shows a plot of zeta potential lowering produced by 10^{-2} mol dm^{-3} C_{16} E_y, against ethylene oxide chain length in C_{16} E_y for the griseofulvin/SDS/C_{16} E_y system, results for the polystyrene latex/SDS/C_{16} E_y System are included. All results taken at a concentration of SDS of 10^{-4} mol dm^{-3}. There is reasonable agreement between the real and model systems.

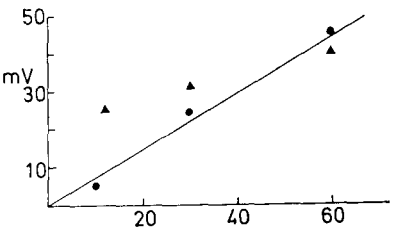

FIG. 5. Variation in zeta potential with increase in ethylene oxide chain length of $C_{16}E_y$. ●—polystyrene latex/SDS/$C_{16}E_y$, ▲—griseofulvin/SDS/$C_{16}E_y$. Ordinate–Zeta potential lowering by 10^{-2} mol dm^{-3} $C_{16}E_y$, mV. Abscissa—Number of ethylene oxide units in $C_{16}E_y$.

It is concluded that, because of the variation of zeta potential with pH that occurs with drugs in aqueous dispersion, investigation should be made of these characteristics before a drug is formulated as a suspension. Further, the effects of surface active agents on the electrokinetic properties of drug systems composed of comparatively large particles, are similar to those produced with a model polystyrene latex suspension system.

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