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# Microelectrophoretic behaviour of gelatin and acacia complex coacervates and indomethacin microcapsules

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## Summary

The electrophoretic behaviour of gelatin/acacia and of gelatin/gelatin complex coacervates is identical to that of equivalent mixtures of these polyions adsorbed onto a colloidal carrier. Encapsulated indomethacin particles, affected the electrophoretic behaviour of gelatin/gelatin microcapsules, but had no such effect on gelatin/acacia microcapsules.

## Introduction

Coacervates prepared by complexation of oppositely charged polyions can be used to prepare drug-containing microcapsules (Luzzi and Gerraughty, 1967; Madan, 1979). A knowledge of the charge carried by microcapsules and similar colloidal particles is essential, as the charge will affect the stability of these particles in suspension. This study compares the electrophoretic behaviour of gelatin/acacia and gelatin/gelatin coacervates with that of the individual polyions, and examines the possible effects of indomethacin core material on the electrophoretic behaviour of the coacervates.

Microelectrophoresis is commonly used to measure the charge carried by colloidal particles. It is also a widely accepted method of assessing the charge carried by polyions, by their prior adsorption onto colloidal particles (Abramson et al., 1942; Kragh and Langston, 1962; Wilkins and Myers, 1970). The microelectrophoretic behaviour of a mixture of two polyions may also be determined by this method (Burgess and Carless, 1984). The electrophoretic mobility of the mixture will depend on both polyions to varying extents, according to the degree of adsorption of each polyion.

The microelectrophoretic behaviour of coacervates and microcapsules should reflect their polyion composition. Encapsulated drug particles would not be expected to contribute to the electrophoretic mobility of microcapsules if the particles are completely surrounded by coating material. Takahashi and Konda (1979) have shown that the electrophoretic mobility of poly(phthaloyl L-lysine) microcapsules is dependent on the coating material, and is very sensitive to small charges in the composition of the coating. Shiba et al. (1971) studied the electrophoretic mobility of poly-

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phthalamide microcapsules containing aqueous solutions of bovine serum albumin and showed that the electrophoretic mobility was dependent on the bovine serum albumin present. They attributed this to incorporation of bovine serum albumin in the microcapsule coating.

Microelectrophoresis of gelatin/acacia coacervates and gelatin/acacia microcapsules containing micronized sulphamethoxazole particles has been studied by Takenaka et al. (1981). Takenaka and his co-workers concluded that the electrophoretic mobility of the sulphamethoxazole microcapsules was dependent on the core sulphamethoxazole particles.

## Materials and Methods

Two types of gelatin were obtained from Gelatin Products, (U.K.) Type A (acid processed) gelatin and Type B (alkali processed) gelatin. The gelatins had the following characteristics: Type A, Bloom No. 256, isoelectric pH 8.3, M.W. $4.7 \times 10^4$, and ash content 0.2% w/w; Type B, Bloom No. 250, isoelectric pH 4.8, M.W. $4.6 \times 10^4$, and ash content 1.1% w/w. The isoelectric pH values were measured by microelectrophoresis and by ion exchange. The M.W. was measured by membrane osometry using a Wecan Model 231 membrane osometer, Wescan Instruments. Acacia B.P. with an ash content of 3.2% w/w and a sulphated ash value of 4.9%, w/w was used. All gelatin and acacia solutions were deionized prior to use by mixing with Amberlite resins IRA-400 and IR-120 for 30 min at 40°C (method adapted from Janus et al., 1951). Micronized indomethacin B.P. was obtained from Nicholas Laboratories. Colloidal silica (Minusil) of particle size 2.7 $\mu$m (geometric weight-mean diameter) was obtained from Zeta-Meter, New York.

*Microelectrophoresis*

A Zeta-Meter was used in conjunction with a Plexiglas cell. Microelectrophoresis was conducted at 1 mM NaCl unless otherwise stated. 1 mM NaOH and 1 mM HCl solutions were used to maintain constant ionic strength as the pH was varied. The polyions were absorbed onto Minusil prior to microelectrophoresis. A 0.02% w/v polyion solution and a 0.1% w/v Minusil suspension were used. Where two polyions were to be adsorbed together, they were mixed together at 40°C before addition of Minusil.

*Manufacture of coacervates and microcapsules*

*Gelatin / acacia.* This method is adapted from Nixon and Nouh (1978). Gelatin/acacia complex coacervates were prepared by mixing 250 ml volumes of 2% w/v solutions of gelatin and acacia which had been held at 40°C for 1 h. Where drug was added, this was first triturated with glycerol, and the triturate was added to the acacia solution. The mixture was stirred at 45°C for 45 min. At the end of this period 10 ml of a 40% Formaldehyde Solution B.P. was added to harden walls of the microcapsules prior to rapid cooling of the suspension to 4–5°C. The microcapsules were collected and washed with cold isopropanol (5–10°C) three times, air dried, and finally dried over a stream of nitrogen gas to produce a free flowing powder.

*Type A / Type B gelatin.* This method is described by Burgess and Carless (1985). 1% w/v solutions of Types A and B gelatin were held at 45°C and 250 ml volumes of each solution were then mixed together, stirring constantly. Where drug was added, this was first triturated with glycerol, and the triturate was added to the Type B gelatin solution. The mixture was stirred at 45°C for 1 h, following which the temperature was reduced to 25°C. Four hours were allowed for equilibration at this temperature. At the end of this period, 10 ml of a 16% Formaldehyde Solution B.P. was added to harden walls of the microcapsules, prior to rapid cooling of the suspension to 4–5°C. The microcapsules were centrifuged at low speed (1000–2000 rpm) for 10 min at 5°C.

The equilibrium fluid was decanted and the microcapsules were washed twice with cold water (5–10°C), once with a 1 : 1 mixture of isopropanol and water (5–10°C), and finally in cold isopropanol (5–10°C). The microcapsules were collected in silicone-coated evaporating dishes, air dried, and dried over a stream of nitrogen gas to produce a free flowing powder.

## Results and Discussion

*The effect of pH on the electrophoretic mobility of gelatin and acacia coacervates and microcapsules*

The effect of pH on the electrophoretic mobility of suspensions of the individual polyions adsorbed onto Minusil and of mixtures of the polyions adsorbed onto Minusil were determined. The pH–mobility profiles for Type A gelatin and acacia and their 1 : 1 and 2 : 1 mixtures are shown in Fig. 1a, and those of Type B gelatin and acacia and their 1 : 1 and 1 : 2 mixtures are shown in Fig. 1b. The electrophoretic mobility profiles obtained for Type A and B gelatin adsorbed onto Minusil are in general agreement with those of Kellaway and Najib (1981) for ossein gelatins adsorbed onto polystyrene latex.

The pH of zero mobility of each mixture coincides with the pH of electrical equivalence (EEP) of the two polyions i.e. the pH value where two polyions carry an equal and opposite charge (as determined from the electrophoretic mobility data). The pH–mobility profiles of each mixture fell between those of the singularly adsorbed polyions. This data suggests that the polyions have adsorbed onto the particle surfaces in the ratio in which they were mixed together.

Coacervates of gelatin and acacia were prepared and the electrophoretic mobility of the coacervate droplets was compared with that of gelatin/acacia mixtures adsorbed onto Minusil. The pH of preparation of the coacervates was the EEP of the particular mixture. At this pH value optimum coacervate yield is obtained (Burgess and Carless, 1984). Coacervates were prepared between Type A gelatin and acacia at a mixing ratio of 1 : 1 and between Type B gelatin and acacia at a mixing ratio of 1 : 2. As illustrated in Fig. 2, mixtures of gelatin and acacia adsorbed onto Minusil have electrophoretic mobility profiles which coincide with the electrophoretic mobility of coacervates prepared at the EEP of the polyion mixture.

Microelectrophoretic mobility profiles were determined for coacervate microcapsules containing indomethacin particles. Fig. 3 shows the electrophoretic mobility profiles of Type A gelatin/acacia and Type B gelatin/acacia, empty and indomethacin microcapsules (1 : 1 polyion mixtures); the profile of indomethacin alone is also shown. The pH mobility profiles of the indomethacin microcapsules is identical to that of the empty microcapsules. These results show that the en-

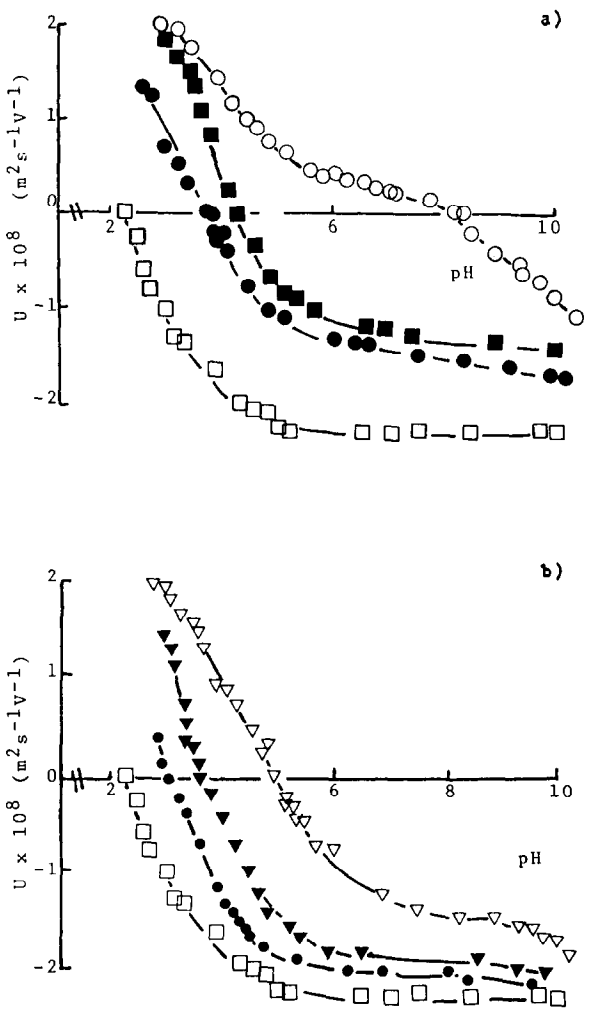


Fig. 1. The effect of pH on the electrophoretic mobility of gelatin and acacia and gelatin/acacia mixtures. (a) Type A gelatin/acacia, (b) Type B gelatin/acacia.
Key: □, acacia, I.E.P. = pH 2.2
○, Type A gelatin, I.E.P. = pH 8.3
●, 1 : 1 Type A gelatin/acacia, I.E.P. = pH 3.8
■, 2 : 1 Type A gelatin/acacia, I.E.P. = pH 4.3
▽, Type B gelatin, I.E.P. = pH 4.8
▼, 1 : 1 Type B gelatin/acacia, I.E.P. = pH 3.6
●, 1 : 2 Type B gelatin/acacia, I.E.P. = pH 2.9
I.E.P. is the isoelectric pH value.

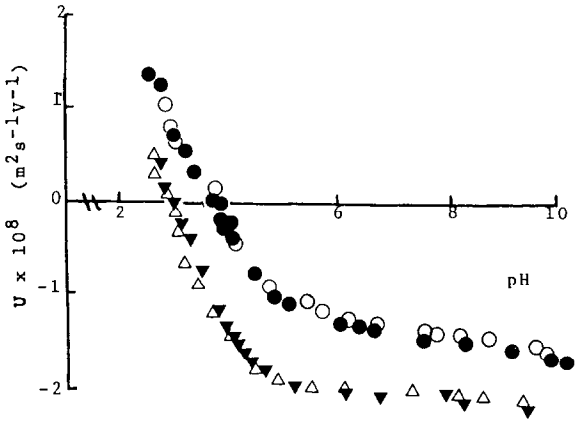


Fig. 2. The effect of pH on the electrophoretic mobility of gelatin/acacia coacervates, and gelatin/acacia mixtures adsorbed onto Minusil.

Key: ○, Type A gelatin/acacia (1 : 1) coacervates, I.E.P. = pH 3.8
●, Type A gelatin/acacia (1 : 1) mixtures, I.E.P. = pH 3.8
△, Type B gelatin/acacia (1 : 2) coacervates, I.E.P. = pH 2.9
▼, Type B gelatin/acacia (1 : 2) mixtures, I.E.P. = pH 2.9

I.E.P. is the isoelectric pH value.

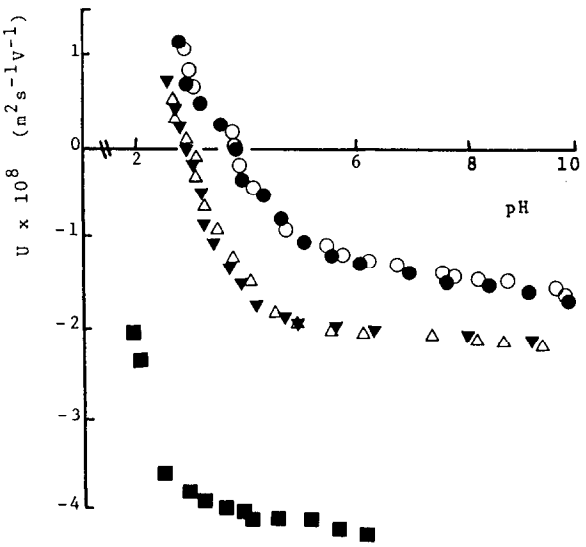


Fig. 3. The effect of pH on the electrophoretic mobility of gelatin/acacia microcapsules and of indomethacin.

Key: ○, Type A gelatin/acacia (1 : 1) empty, I.E.P. = pH 3.8
●, Type A gelatin/acacia (1 : 1) indomethacin, I.E.P. = pH 3.8
▽, Type B gelatin/acacia (1 : 2) empty, I.E.P. = pH 2.9
▲, Type B gelatin/acacia (1 : 2) indomethacin, I.E.P. = pH 2.9
■, Indomethacin

I.E.P. is the isoelectric pH value.

capsulated indomethacin has no effect on the electrophoretic mobility of the microcapsules and indicates that the drug is completely surrounded by the polyion coacervate coating. If the drug was associated with the microcapsule, either by adherence to the outside of the wall, or present within the wall, then the charge on the drug could affect the electrophoretic behaviour of the microcapsules.

Takenaka et al. (1981) suggested that the electrophoretic property of gelatin/acacia coacervates was affected by encapsulated sulphamethoxazole particles, since the pH mobility curve of the sulphamethoxazole microcapsules was almost the same as that of the sulphamethoxazole alone. Confusion may have arisen in this case since the electrophoretic properties of the drug are very similar to those of the polyion mixture used to form the coacervate coating.

*The effect of pH on the electrophoretic mobility of type A / Type B gelatin coacervates and microcapsules*

The effect of pH on the electrophoretic mobility of the two types of gelatin and on their 1 : 1 and 2 : 1 (A : B) mixtures was determined (Fig. 4). As occurred for the gelatin/acacia mixtures, the

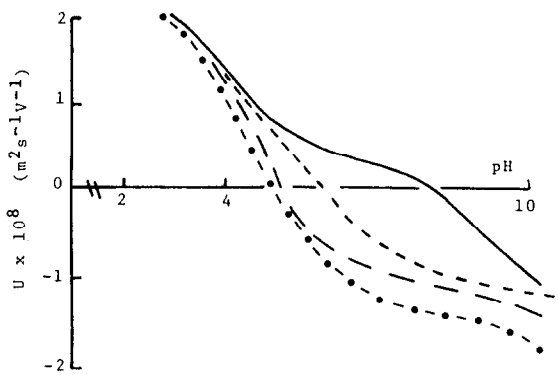


Fig. 4. The effect of pH on the electrophoretic mobility of Types A and B gelatin and of mixtures of these two gelatins, adsorbed onto Minusil.

Key: ———, Type A gelatin, I.E.P. = pH 8.3
●—●, Type B gelatin, I.E.P. = pH 4.8
— —, Type A/B gelatin (1 : 1) mixture, I.E.P. = pH 5.4
– – – –, Type A/B gelatin (2 : 1) mixture, I.E.P. = pH 6.0

I.E.P. is the isoelectric pH value.

pH–mobility profiles of the mixtures fell between those of the individually adsorbed gelatin and the pH of zero mobility of each mixture coincided with the EEP of the two polyions. The data suggest that the polyions adsorbed onto the colloidal carrier in the ratio at which they had been mixed.

The pH–electrophoretic mobility profiles of gelatin/gelatin coacervates are shown to be identical to those of equivalent mixtures of the gelatins adsorbed onto Minusil (Fig. 5). The pH–electrophoretic mobility profiles of Type A/Type B gelatin indomethacin microcapsules were determined and compared with those of empty coacervates (Fig. 6). Refer to Fig. 3 for the pH–mobility profile of indomethacin alone. The presence of indomethacin has a significant influence on the electrophoretic mobility of these microcapsules. This is probably a consequence of the method of manufacture of Type A/Type B gelatin microcapsules which is more complicated than that for gelatin/acacia microcapsules. Gelatin/gelatin microcapsules are prone to wall defects (Burgess and Carless, 1985). And as a consequence, drug particles may be present within the capsule wall or adhere to the outside of the microcapsule.

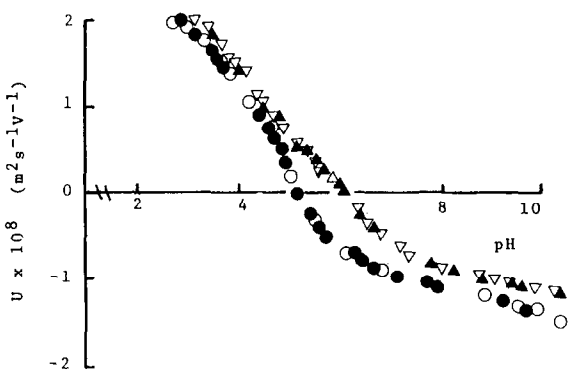


Fig. 5. The effect of pH on the electrophoretic mobility of gelatin Type A/B mixtures and coacervates.
Key: ●, 1:1 mixture, I.E.P. = pH 5.4
○, 1:1 coacervates, I.E.P. = pH 5.4
▲, 2:1 mixture, I.E.P. = pH 6.0
▽, 2:1 coacervates, I.E.P. = pH 6.0
I.E.P. is the isoelectric pH value.

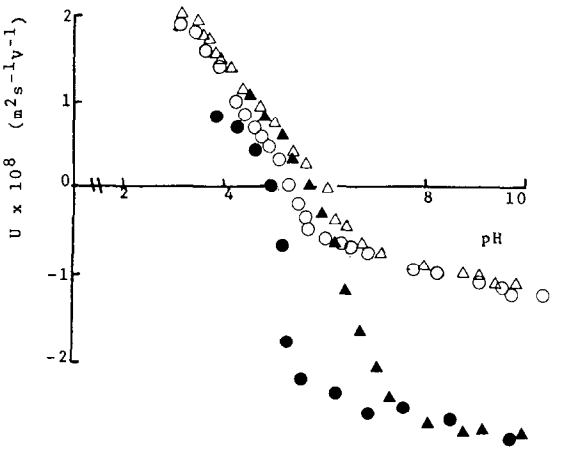


Fig. 6. The effect of pH on the electrophoretic mobility of Type A/B gelatin microcapsules.
Key: ○, Type A/B gelatin (1:1) empty, I.E.P. = pH 5.4
●, Type A/B gelatin (1:1) indomethacin, I.E.P. = pH 5.1
△, Type A/B gelatin (2:1) empty, I.E.P. = pH 6.0
▲, Type A/B gelatin (2:1) indomethacin, I.E.P. = pH 5.7
I.E.P. is the isoelectric pH value.

## Conclusion

The electrophoretic mobility of gelatin/acacia and gelatin/gelatin mixtures are representative of coacervates prepared from these polyion mixtures. The charge carried by gelatin/acacia coacervates is not affected by encapsulated indomethacin particles which indicates that the drug is completely encapsulated and is not present in any significant amount in the capsule wall. This contrasts with the behaviour of the gelatin/gelatin microencapsulated indomethacin which indicates that the drug particles are associated in some way with the capsule wall and produce a change in electrophoretic mobility.

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## References


Abramson, H.A., Moyer, L.S. and Gorin, M.H., Dissolved and adsorbed proteins and related surfaces. In Abramson, H.A., *Moyer, L.S., and Gorin, M.H. (Eds.), Electrophoresis of* Proteins, Reinhold, New York, 1942, pp. 80–104.

Burgess, D.J. and Carless, J.E., Microelectrophoretic studies of gelatin and acacia for the prediction of complex coacervation. J. Colloid Interface Sci., 98 (1984) 1–8.

Burgess, D.J. and Carless, J.E., Manufacture of gelatin/gelatin coacervate microcapsules. Int. J. Pharm., 27 (1985) 61–70.

Janus, J.W., Kenchington, A.W. and Ward. A.G., A rapid method for the determination of the isoelectric point of gelatin using mixed bed deionization. Research (London), 4 (1951) 247–248.

Kellaway, I.W. and Najib, N.W., The effect of hydrophilic polymers on the electrophoretic mobility of suspended particles. Int. J. Pharm., 7 (1981) 285–292.

Kragh, A.M. and Langston, W.B., The flocculation of quartz and other suspensions with gelatin. J. Colloid Sci. 17 (1962) 101–123.

Luzzi, L.A. and Gerraughty, R.J., Effects of additives and formulation techniques on controlled release of drugs from microcapsules. J. Pharm. Sci. 56 (1967) 1176–1177.

Madan, P.L., Methods of preparing microcapsules: coacervation or phase separation. Pharm. Technol. Int., (1979) 51–56.

Takenaka, H., Kawashima, Y. and Lin, S.L., Electrophoretic properties of sulphamethoxazole microcapsules and gelatin–acacia coacervates. J. Pharm. Sci. 70 (1981) 302–305.

Shiba, M., Kawano, Y., Tomioka, S., Koishi, M. and Kondo, T., Studies on microcapsules. X.1. Electrophoretic behaviour of polyphthalamide microcapsules containing aqueous solutions of bovine serum albumin. Bull. Chem. Soc. Jap. 44 (1971) 2911–2915.

Wilkins, D.J. and Myers, P.A., The anomalous electrophoretic behaviour of certain adsorbed protein derivatives (344450). Proc. Soc. Exp. Biol. Med., 133 (1970) 255–259.