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THE PHYSICO-CHEMICAL EFFECT OF COACERVATION CONDITIONS ON THE DIFFUSIONAL PROPERTIES AND SURFACE MORPHOLOGY OF BIODEGRADABLE MICROCAPSULES

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ABSTRACT

Microcapsules containing kaolin and hydrocortisone 21- acetate (HCAC) were produced through controlled coacervation of a biodegradable block copolymer of d-tartaric acid and 1,10-octanediol, under Plait and non-Plait compositions. Diffusional studies indicated that the permeability of Plait microcapsules was at least 40% greater than non-Plait microcapsules at the same polymer to core ratio. Scanning electron photomicrographs of the various microcapsules suggested surface morphologic differences among microcapsules produced under Plait and non-Plait solvent conditions.

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INTRODUCTION

Coacervation, a type of phase separation pertaining to macromolecules[1], is commonly used as a method to produce microcapsules[2-5]. Since the literature neglects or fails to consider the effect of the various solvent compositions on the macromolecular configuration during the coacervation process, it has therefore been assumed that equivalent coacervate phases are formed throughout the coacervating region irrespective of solvent and non-solvent composition. This study was initiated in order to investigate the effect of different polymer-mixed solvent compositions and the respective polymer conformation on the coacervation process and resultant properties for a particular polymer.

THEORETICAL CONSIDERATIONS

The effect of solvents on the molecular configuration and physical properties of long chain polymers has been studied by Flory[6] and others and recently reviewed by Kratochvil[7]. These investigators have shown that the interaction between a polymer and a solvent may be described using statistical thermodynamics. A detailed statistical thermodynamic development for polymer-solvent-non-solvent system used in this study has been developed by Shively[8] and for polymers in general[9]. In brief, the statistical thermodynamic description of macromolecules results in the following modified definitions of enthalpy and entropy, respectively:

$$\Delta H = RT \sum_{i=1}^{n} n_i \, v_i \, \chi_{ij} \qquad (1)$$

where,

ΔH = enthalpy of mixing
R = gas constant
T = absolute temperature
n_i = number fraction of molecules i
v_j = volume fraction of molecules j
χij = Flory-Huggins interaction parameter which is characteristic of solvent-solute pair.

and,

$$\Delta S_m = -R \sum_{i=1}^{n} n_i \ \text{nlog}\ v_i \qquad (2)$$

where, ΔS_m = entropy of mixing

The above modified enthalpy (Eq. 1) and entropy (Eq. 2) expressions were then used to determine the free energy of a polymer-solvent system using the familiar Gibbs-Helmholtz Free Energy relationship for each component. After making several substitutions and simplifications, the second derivative of the Gibbs-Helmoltz free energy relationship was determined using secular determinants. In doing this it was possible to describe the polymer-mixed solvent composition with the greatest free energy of mixing. The polymer-mixed solvent composition with the greatest free energy of mixing, the Plait composition, can be described by the following relationship:

$$0 = 1 - 2u_{13}V_1 - 2u_{23}V_2 + 2u_{12}V_1V_2 \qquad (3)$$

Where,

u_{13} = entropy factor + $\frac{Vo\chi_{13}}{RT}$

u_{23} = entropy factor + $\frac{Vo\chi_{23}}{RT}$

u_{12} = $\frac{Vo\chi_{12}}{RT}$

v_i = volume fraction of solvent (1), non- solvent (2), and polymer (3)
v_o = volume of unswollen polymer network

The Plait composition is experimentally observed using ternary phase diagrams as the polymer-mixed solvent composition in which the curvature of the ternary coacervation is zero.

Release of Hydrocortisone 21-acetate From Microcapsules

The release of hydrocortisone 21-acetate for microcapsules in which drug release is controlled by diffusion through the microcapsule wall is described by the following equation[11]:

$$\frac{dQ}{dt} = \frac{D`A}{l`} (C_s - C_o) \tag{4}$$

where,

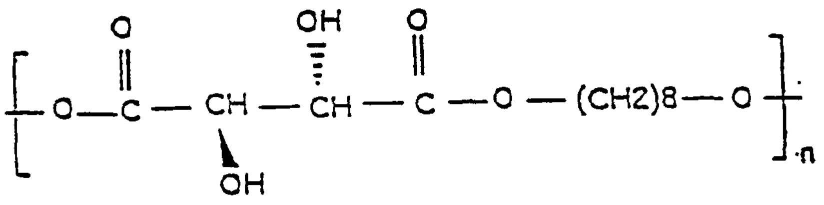
$\frac{dQ}{dt}$ = Rate of drug transfer (g/sec)
C_s = Solubility of drug
C_o = Concentration of drug in solution
D' = Effective diffusion coefficient
l' = Effective thickness of capsule wall
A = Area

Analysis of Equation (4) shows that the rate of release is proportional to the permeability $\frac{D'}{l'}$ of the system.

MATERIALS AND METHODS

Materials The encapsulating macromolecule used was a biodegradable block copolymer originally synthesized and characterized by Bitritto et al.[12]. The polymer was prepared by the melt polymerization of tartaric acid and 1,10-decanediol (both of reagent grade, Aldrich Chemical

Company). The molecular weight of the polymer was approximately 1200 grams per mole with a melting point of 78-79°C. The block copolymer repeating unit, confirmed by NMR and IR[12], is shown below:



<u>Preparation of Titrants</u> Titrants of varying ionic strengths were made using sodium chloride (Reagent Grade, Aldrich Chemical Co.) and double distilled water (Barnstead, Fistill 4L,Newton, MA). The non-solvent ionic strengths evaluated were 0.0M, 0.01M, 0.05M, and 0.1M NaCl.

<u>Construction of Ternary Coacervation Profiles</u> Several dilutions of a stock ethanolic (solvent) polymer solution were made. Two hundred milliliters (200.0 ml) of the respective polymer solution were placed into a thermostatically controlled jacketed beaker at 25 ± 0.2 °C and stirred with a four blade paddle at a constant 400 Rpm (Hurst Manufacturing Co., Model CA, Princeton, IN). The solution was stirred for two hours under the above conditions. At this point, titration of non-solvent to the polymer/ethanol solution was initiated at the indicated ionic strengths. Non-solvent was titrated at a rate of 33 ml/hr using a peristaltic pump (ISCO, 3 Channel, Lincoln, NE) until the colloidal solution became turbid (cloud point) as determined visually. The concentrations of non-solvent,

solvent, and polymer at the cloud point were recorded and plotted on a ternary diagram.

Microencapsulation Procedure The desired ethanolic polymer solution was added to the thermostatically controlled jacketed beaker (25 ± 0.2 °C). A quantity of core material, kaolin (surface area of 48 M^2/G^8, USP Grade, Allied Chemical Company) or hydrocortisone 21-acetate (HCAC) (0.5-4 micrometer particles with a surface area of 82 M^2/G^8, Reagent Grade, Sigma Chemical Company), was added to the ethanolic polymer solution and stirred for two hours. It was important for comparison of different encapsulated core materials that there be the same average surface concentration of polymer coating for each core material. Therefore, a polymer to core ratio, normalized to kaolin, of two to one (2:1) was utilized in these and subsequent experiments. For example, 2 grams of polymer to one gram of kaolin establishes a calculated surface concentration of polymer of 0.085 grams of polymer per square meter of kaolin surface. This surface concentration was maintained for HCAC through the use of HCAC surface area and the appropriate calculations. At this point, the titration with non- solvent into the colloidal solution containing the suspended core materials was initiated at a rate of 33 milliliters per hour using a 3-channel peristaltic pump. The resulting microcapsules were filtered using sintered glass and dried at 50°C under vacuum (760mm Hg vacuum). The microcapsules were then stored in a vacuum desiccator.

Particle Size Determination The particle size of unencapsulated and encapsulated core materials was determined using polarized light microscopy in conjunction with computer image analysis (Quantimet 720, Cambridge Instruments, Ltd., Cambridge, U.K.). Samples were applied dry to standard microscope slides. The density of the sample on the slide was adjusted until particles were not touching. Data was collected until 5000 particles had been counted.

Determination of Polymer Surface Excess Polymeric solutions were prepared by dissolving predetermined quantities of polymer in 30% water-70% ethanol and 50% water-ethanol systems. The polymer concentrations studied for the above solvent systems ranged from 0.02 - 0.80 milligrams of polymer per milliliter.

The surface tensions of polymer solutions were determined using a surface tensiometer (Fisher Scientific, Plano, TX) via the du Nouy ring detachment method[13]. The experimentally determined surface tensions were corrected for the mass and shape of the suspended drop according to the method of Harkins and Jordan[14]. All reported surface tensions represent the averaged results of three trials (± 0.03 dynes/cm).

Release of Hydrocortisone 21-acetate from Microcapsules The release of hydrocortisone 21-acetate from the respective microcapsules was accomplished by adding 0.01 grams of dried hydrocortisone 21-acetate microcapsules into a 500 milliliter thermostatically controlled jacketed beaker. Five hundred milliliters (500.0 ml) of double distilled water were added to the jacketed beaker and stirred at 400 RPM. Samples were removed (unfiltered) and assayed as a function of time.

The unfiltered microcapsule samples were added to a 1 cm pathlength cuvette which was placed within the sample compartment of a dual-wavelength spectrophotometer (DW-2, SLM/Aminco, Urbana IL) and assayed as previously reported[15]. The reference wavelength used for these studies was 277 nm and the sample wavelength for hydrocortisone 21-acetate was 256 nm. The corrected UV-absorption of each sample was determined and converted to concentration using a suitable Beer's law plot.

Sample Preparation for Scanning Electron Microscopy Microcapsules were coated with 150 angstroms of a gold-paladium mixture[8]. The coated microcapsules were brushed onto standard smooth graphite mounts.

RESULTS AND DISCUSSION

The cloud point compositions were determined and plotted using ternary diagrams, thus showing the relationship between the three components used to produce coacervation. The connection of cloud point compositions, for a titrant of given ionic strength, produced a coacervation profile, as shown in Figure (1). Those polymer-solvent compositions to the left of the coacervation profiles in Figure (1), represent the absence of a coacervated phase. In contrast, polymer-solvent compositions to the right of the coacervation profiles in Figure (1) represent those polymer-solvent compositions producing coacervate phases. Titration with non-solvent of differing ionic strengths revealed at least two diffuse regions along the coacervation profile in which the coacervate had different solvent interaction properties.

Analysis of Figure (1) indicates that at polymer concentrations greater than 550 ug/ml a noticeable region in each of the coacervation profiles was shown to be independent of the ionic strength of the titrant. For the purposes of this report, this general region of the coacervation profile is defined as the non-Plait region. The polymer in this region exhibited minimal or no solvent interaction. Under these conditions, polymer molecules in solution have been shown to approach a coiled configuration[7,16].

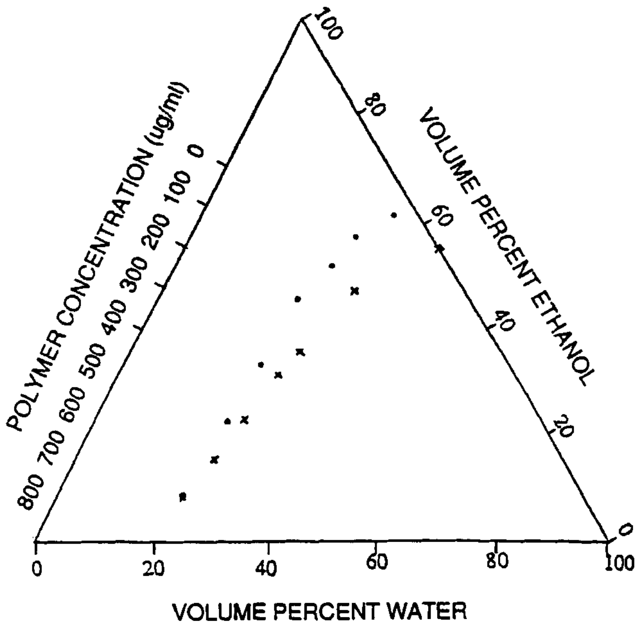


Figure 1. Ternary coacervation phase diagram for the block copolymer of d-tartaric acid and 1,10-decanediol at 25°C.

Key: (Δ)0.0 M NaCl;(X)0.1M NaCl.

Further analysis of Figure (1) indicates that when the coacervation profiles produced at various ionic strengths were compared to the coacervation profile for distilled water a second diffuse region which is dramatically altered by solvent properties, the Plait region, becomes evident at polymer concentrations below 130 ug/ml. Due to the dramatic effect of the non-solvent ionic strength on the coacervation process, it follows from the previous discussion that intermolecular adhesional forces are greater than polymer cohesional forces in this region. As a result of the decreased cohesive interactions in this region, the polymer adopts a more linear or extended configuration[16].

At the junction of these two diffuse regions there exists a single polymer/solvent/non-solvent composition in which cohesional interactions are equal to the adhesional interactions, termed the Plait composition.

The above results suggests the possibility that the coacervate phase is actually composed of coacervates having a range of solvent interaction characteristics or polymer configurations. For the above analysis to be correct, the molecular area should also vary as a function of solvent composition.

Therefore, the surface properties and the molecular area of the polymer under non-Plait and Plait coacervation conditions were determined. The molecular area was calculated in this investigation through the determination of the polymer surface excess (moles/cm^2). The corrected surface tension plots are shown for the 30% water-70% ethanol (surface tension = 30.92 dynes/cm) and 50% water-ethanol (surface tension = 34.6 dynes/cm) systems in Figures (2) and (3), respectively.

Analysis of Figure (2) shows that as the polymer concentration was initially increased, the surface tension decreased (negative slope) until a minimum surface tension or critical point was achieved. Further increases in the polymer concentration beyond the critical point resulted in an increased surface tension (positive slope). Further analysis of Figure (2) indicates that the surface tension of the plateau region was less than the surface tension of the initial solvent, (ie. polymer concentration of zero). Therefore, this suggests that the coacervated phase appears to be preferentially solvated by the solvent component which has the highest intrinsic surface tension, water (79 dynes/cm)[19].

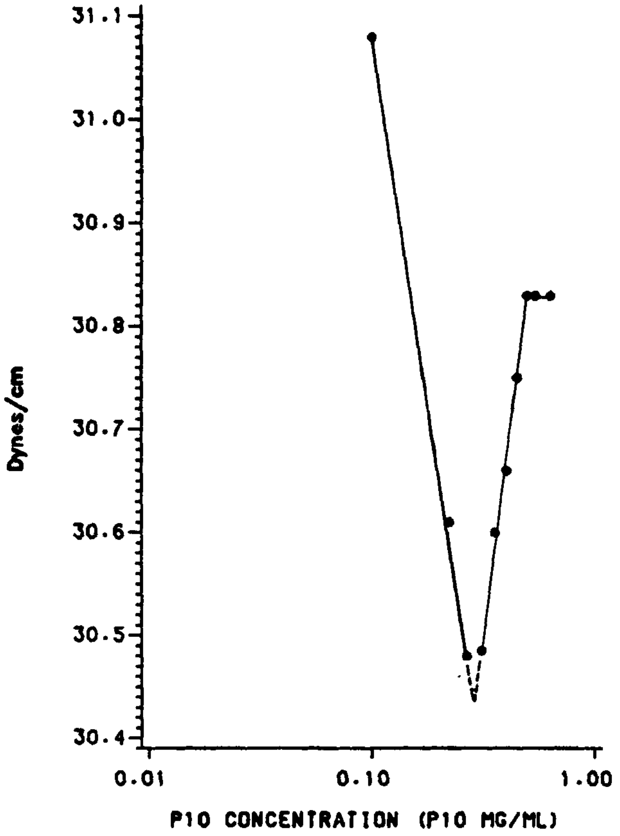


Figure 2. Surface tension at 25 °C as a function of the natural logarithm of the polymer concentration: 30% water-70% ethanol solvent composition.

The corrected surface tension versus polymer concentration plot for the 50% water-ethanol solvent composition is shown in Figure (3). Analysis of Figure (3) indicates that at the higher polymer concentrations, the polymer-solvent-vapor surface tension plateau was higher than the initial surface tension of the neat 50% water-ethanol solvent system. Although only three points of the plateau are shown in Figure (3), no change in the plateau was observed for a ten fold increase in polymer concentration. In this case the coacervate phase appears to be

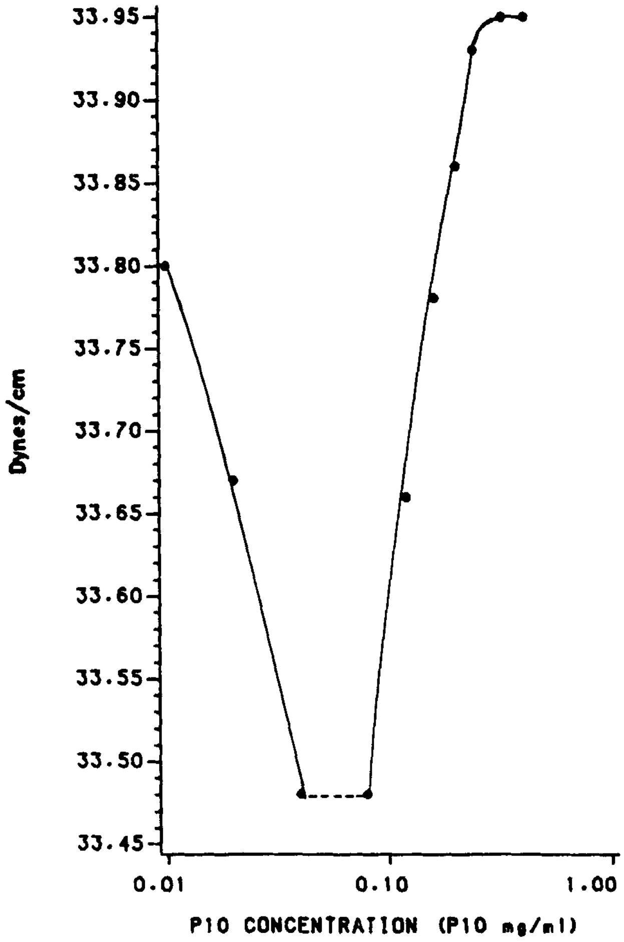


Figure 3. Surface tension at 25 °C as a function of the natural logarithm of the polymer concentration: 50% water-50% ethanol solvent composition.

preferentially solvated by the solvent component which has the lowest intrinsic surface tension of the two solvent components, ie. ethanol.

Additional information may be extracted from these studies by determining the surface excesses and surface areas from Figures (2 and 3). The results of such an analysis are tabulated in Table (1). Analysis of Table (1) indicates that the surface excesses and the calculated

Table I.

Surface Area of Polymer Molecule as a Function of Solvent Composition

Solvent Composition	Region	Surface Excess $(\frac{\text{mole}}{\text{cm}^2})$	Area per Molecule ($Å^2$)
30% water 70% alcohol (non-Plait)	Pre Coacervation	1.57x10E-11	1055
	Post Coacervation	-2.73x10E-11	608
50% water 50% alcohol (Plait)	Pre Coacervation	9.96x10E-12	1668
	Post Coacervation	-1.69x10E-11	986

"apparent" molecular surface areas were greater as anticipated for the polymer molecule in the (pre- coacervation) colloidal solution compared to the "apparent" molecular area in the coacervated phase for both solvent compositions. For example, at the 30% water-70% ethanol solvent composition, the polymer pre-coacervation surface area was 1055 $Å^2$/molecule compared to the post-coacervation surface area of 608 $Å^2$/molecule. Thermodynamically, these results agree with the theory that the coacervation process results in the reduction of the surface free energy of a system through the reduction of the molecular surface area [10].

It is reasonable to expect the molecular surface area to be smaller for a polymer molecule in a solvent composition where polymer cohesive interactions are stronger or more numerous than its adhesional interactions than in a polymer- solvent composition dominated by adhesional interactions. Analysis of Table I, indicates that the polymer-solvent composition dominated by polymer cohesional interactions, ie. non-Plait composition region (30% water-70% ethanol system), contained polymer molecules of smaller surface area compared to the solvent system dominated by polymer/solvent adhesional forces, ie. Plait composition region (50% water-ethanol system). These results are in complete agreement with theory and the results of others[20-21].

Analysis of Figures (2) and (3) shows that the coacervate phases resulting from the 30% water-70% ethanol and 50% water-ethanol solvent compositions were dramatically different. These studies reaffirm our speculation that the molecular configuration and interaction properties of coacervates at one solvent composition may not be equivalent to coacervates formed at other solvent compositions.

If this polymer molecule can have vastly different configurations, areas, or solvent adsorption properties within the coacervating region as suggested by the above analysis, it is logical to speculate that microcapsules made under different coacervation conditions may have different properties, ie. diffusional and/or morphological. This theory was investigated by studying the diffusional properties of hydrocortisone 21-acetate (HCAC) from microcapsules produced under Plait and non-Plait coacervation conditions as well as investigating the morphological characteristics through the use of scanning electron microscopy. When

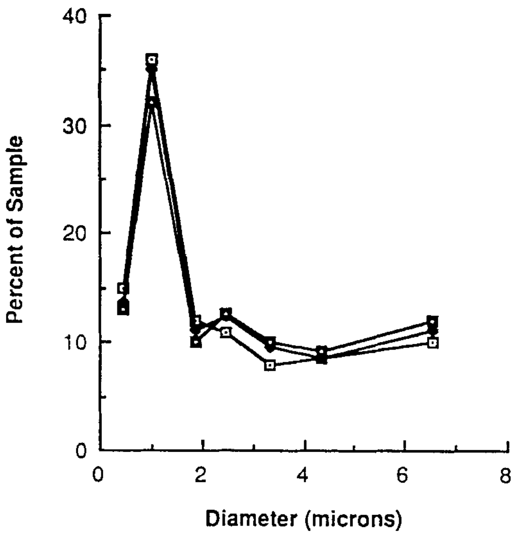


Figure 4. Particle size distribution of kaolin microcapsules with a polymer to core ratio of 2:1 (N=5000).

Key: (-◆-)Lot 1;(-□-) Lot 2;(-■-)Lot 3.

comparing microcapsules from different batches of different solvent conditions it is essential that the encapsulation process be reproducible. For this reason, the particle size distributions for three lots of encapsulated kaolin are shown in Figure (4). The diameter of unencapsulated kaolin ranged from 0.1 to 3 microns. Analysis of Figure (4) shows that the particle size distribution of kaolin microcapsules and therefore the encapsulation process was consistent from batch to batch (±5%).

The release profiles for hydrocortisone 21- acetate from Plait and non-Plait microcapsules are given in Figure (5). Analysis of Figure (5) shows that the slope, which is proportional to the permeability Equation (4), was approximately 40% greater for Plait microcapsules compared to

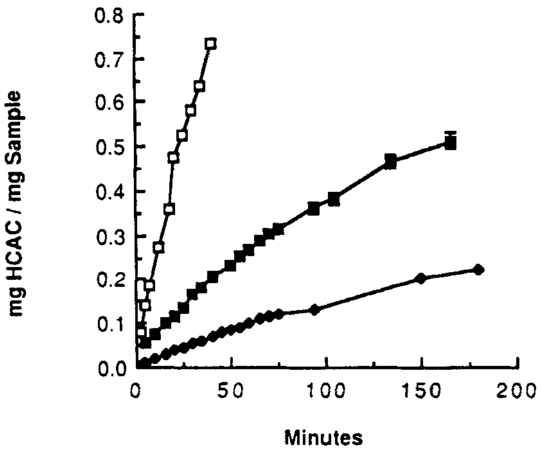


Figure 5. Release of hydrocortisone 21-acetate from microcapsules produced at 25 °C with a polymer to core ratio of 2:1.

Key: (-□-) un-encapsulated powder;(-◆-) non-Plait microcapsules;(-■-) Plait microcapsules.

the permeability for non-Plait microcapsules. Since the polymer to core ratio was accurately controlled, different surface concentrations of polymer are probably not the cause of any permeability differences between Plait and non-Plait microcapsules. Analysis of Figure (5) therefore indicates that the dissolution of unencapsulated HCAC was approximately 5 and 12 times faster that the release of HCAC from Plait and non-Plait microcapsules, respectively. By comparing the rates for the release of HCAC from Plait and non- Plait microcapsules, it is also evident that the rate of release of HCAC from Plait microcapsules is 40% greater than that from non-Plait microcapsules. Since C_s and C_0 in Equation (4) are constant as long as solid drug is present within

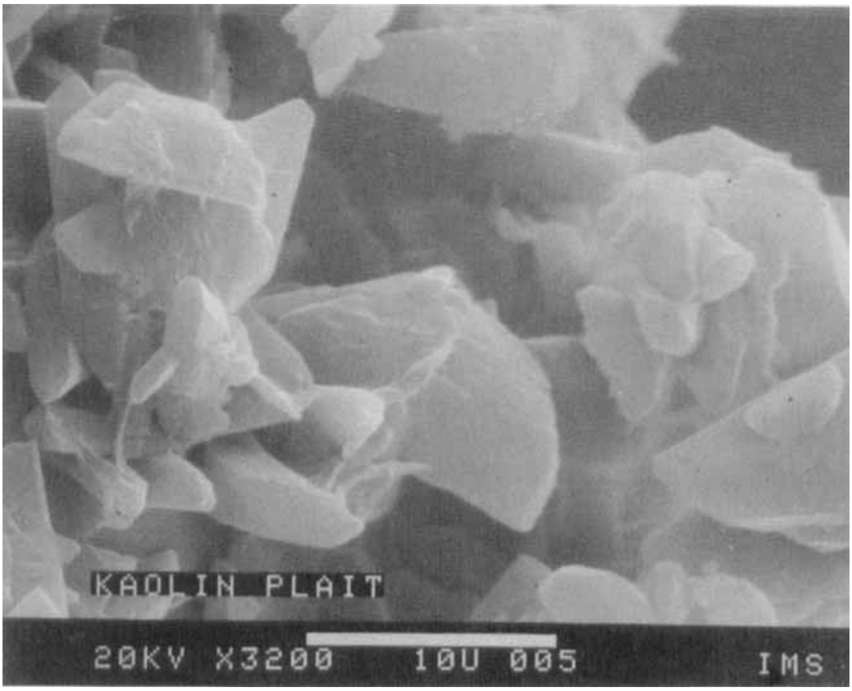


Figure 6. Scanning Electron Micrograph of Kaolin Plait Microcapsules with a polymer to core ratio of 2:1 at 25° C.

microcapsules and sink conditions are maintained, the permeabilities of Plait and non-Plait microcapsules appear to be different.

Given that Plait and non-Plait microcapsules were produced with the same surface concentration of polymer, (ie. the same polymer to core ratio) and appeared to have different permeabilities, the surface morphology of the various microcapsules was determined using scanning electron microscopy. Analysis of kaolin Plait (Figure 6) and hydrocortisone 21-acetate (HCAC) Plait microcapsules (Figure 8) indicates that their surface morphologies were similar. The same was true for kaolin non-Plait microcapsules and HCAC non-Plait

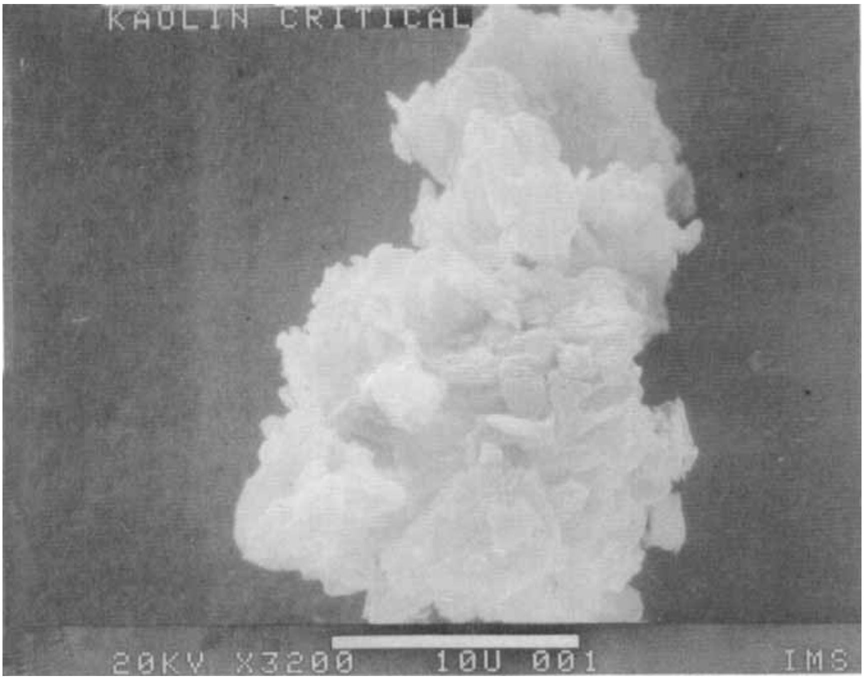


Figure 7. Scanning Electron Micrograph of Kaolin Non-Plait (critical) Microcapsules with a polymer to core ratio of 2:1 at 25°C.

microcapsules, as shown in Figures (7) and (9), respectively. Comparison of the surface morphology between Plait and non-Plait microcapsules, independent of the core material, revealed that the surface morphology of Plait microcapsules was relatively smooth and regular compared to the rough and irregular surfaces of non-Plait microcapsules.

SUMMARY

Based on the analysis of ternary phase diagrams, and the apparent differences in polymer surface areas and polymer- solvent

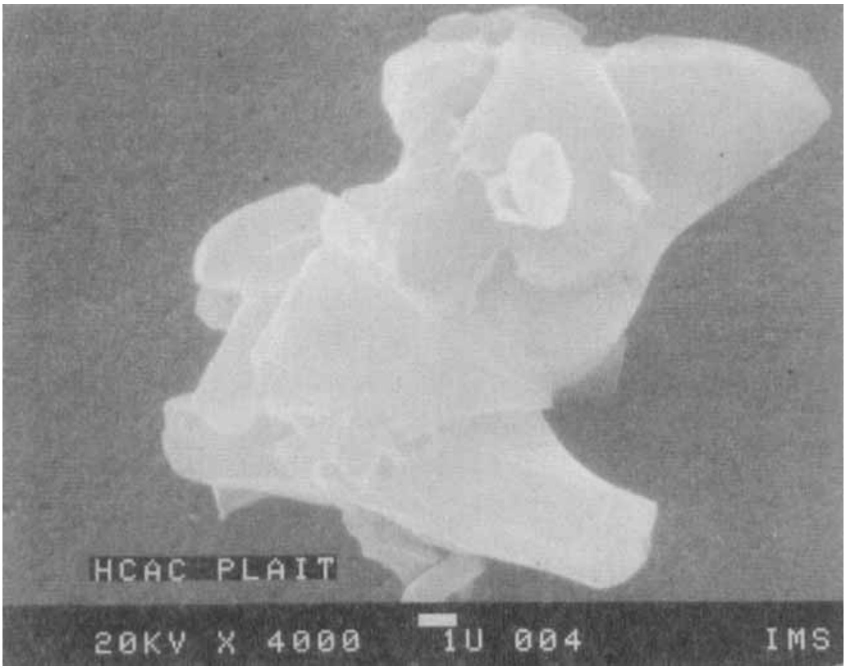


Figure 8. Scanning Electron Micrograph of Hydrocortisone 21-acetate Plait Microcapsules with a polymer to core ratio of 2:1 at 25° C.

preferential adsorption, diffusional properties, and surface morphologies, coacervates generated under different solvent conditions should not be considered equivalent. For example, under non-Plait conditions the polymer exhibited minimal solvent interactions (Fig. 1), and the preferential sorption of water compared to ethanol (Fig. 2). In addition, the diffusion of HCAC from non-Plait microcapsules was approximately 40% less than Plait microcapsules (Fig. 5) and the surface morphology was rough and irregular (Figs. 7,9). This is in contrast to Plait

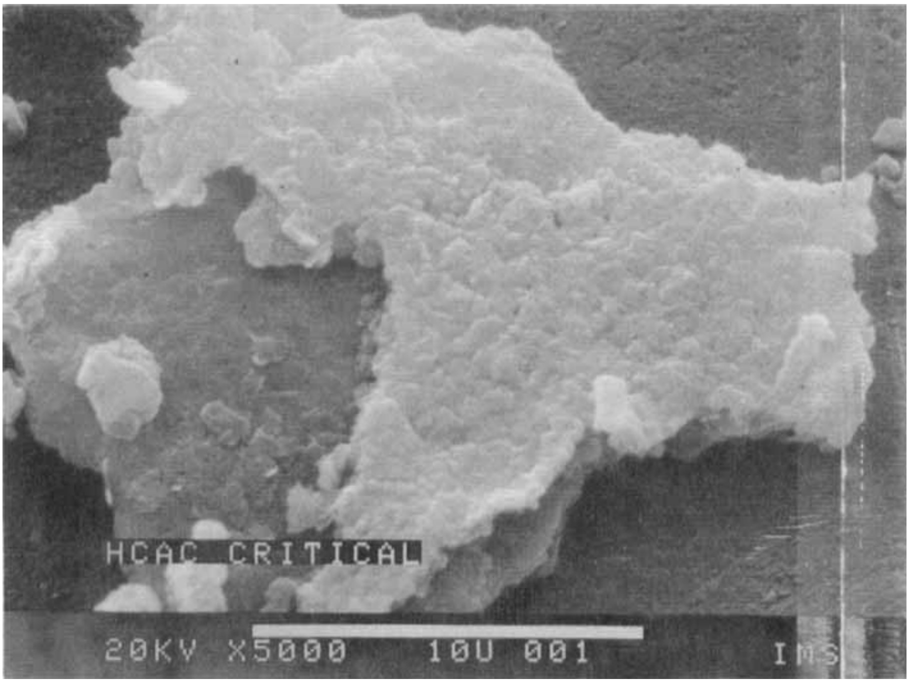


Figure 9. Scanning Electron Micrograph of Hydrocortisone 21-acetate Non-Plait (critical) Microcapsules with a polymer to core ratio of 2:1 at 25° C.

coacervation conditions where the polymer is theoretically in a more linear configuration and preferentially sorbs ethanol (Fig. 3), and results in microcapsules with a smooth morphology (Figs. 6,8).

Although this study presents potentially important implications to microencapsulation via coacervation, these authors consider these findings preliminary and plan additional studies to validate our early findings. For example, additional studies are planned using other surface analysis techniques such as diffuse reflectance and X-ray diffraction in order to validate our hypothesis introduced in this communication.

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