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W/O/W 三層膜 Microcapsules에 關한 硏究

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Three-ply Walled W/O/W Microcapsules Containing Furosemide and Reserpine

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Three-ply walled microcapsules containing furosemide and reserpine were prepared from multiple emulsion, and thd the appearance of multiple emulsion, the particle size distribution and the drug contents of microcapsules were studied. The microcapsule consisted of alternating three layer of acacia/ethyl cellulose/acacia, and the surface of microcapsules was not porous but wrinkles and had relatively elaborate structure and the particle size range is 4μm to 64μ m.

An ideal drug delivery device should be biostable, nontoxic, noncarcinogenic and should release the drug at constant, programmed rate for predetermined duration of medication. Synthetic polymers have been developed for controlled drug delivery system and microcapsules are an example of such polymeric delivery system.

Microcapsules are small capsule, usually spherical or spheroidal in shape and usually in the size range from several tenths of a micron to 5,000 microns in diameter.

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Microencapsulation is a process and technique in which solid material, liquid droplets or gases are completely enveloped by an intact membrane. The function of the capsular membrane are to protect the material and to control the flow of materials inside and outside, across the membrane. Generally, release of drug from microcapsules may be achieved via erosion, dissociation and/or diffusion.

Of various ways to manufacture microcapsules, those successfully employed include air suspension, coacervation, spray drying, orifice method and pan coating, interfacial polymerization and microencapsulation by phase separation. Several general reviews and patents of microencapsulation have been published.[1~8]

The first practical use of microencapsulation appears in U.S. patent issued to Green and Schleicher[9,10]. In 1967, Luzzi and Gerraughty developed a method for the evaluation of drug containing capsules prepared via complex coacervation.[11]

Most of microcapsules have been prepared from simple emulsion with one or two ply side walls enclosing the core. Such microcapsules have a limited range of retention of soluble material in the core. Multiple emulsions have themselves been used as controlled release delivery system[12~15]. However, such multiple emulsions are liquids, difficult to transport and prone to degradation.

In 1976, from interfacial rheological studies, Warburton had shown the formation of a rigid bipolymer film at the interface between an aqueous solution of a water-soluble polymer and a non-aqueous solution of an oil-soluble polymer[16]. In 1981, Warburton prepared three ply walled microcapsules by the multiple emulsion method[17] that is very similar to the drying liquid process[18~22]. In 1982, Morris et al. had shown three-ply walled microcapsules formed when the organic solvent was removed from the hydrophobic layer of multiple emulsion droplets.[23] They have suggested that if water-soluble active agents and organic-soluble active agents were added to aqueous polymer solution and organic polymer solution, respectively, the former was presented in the aqueous core(s) and the latter was encapsulated in the middle layer.

In this paper, it was the intent of this study to develope the preparation of microcapsules containing two or three drugs for the practical purpose. Authors prepared multilayer microcapsules containing furosemide and reserpine for the hypertensive treatment and examined its surface morphology by scanning electron microscopy. The size distribution of the microcapsules was measured by coulter counter.

Experimental

Materials—acacia (Junsei), ethyl cellulose (48.7% ethoxy content, Sigma), furosemide (Sigma), reserpine (Sigma), ethyl acetate (Kanto), seamless cellulose membrane (Union carbide).

Apparatus—homogenizer (Nissai model AM-7), HPLC (Waters Assoc. model-440), fluorometer (Shimadzu RF10), coulter counter (model TA Ⅱ), freeze dryer(Labcomco freeze dryer-18), scanning electron microscope (Nanolab 2100 Bausch & Lomb), microscope (Nikon Apophot x1500).

Preparation of Microcapsules—The basic procedure employed by Warburton[12] and Morris[13] for the preparation of microcapsules was used with some modification.

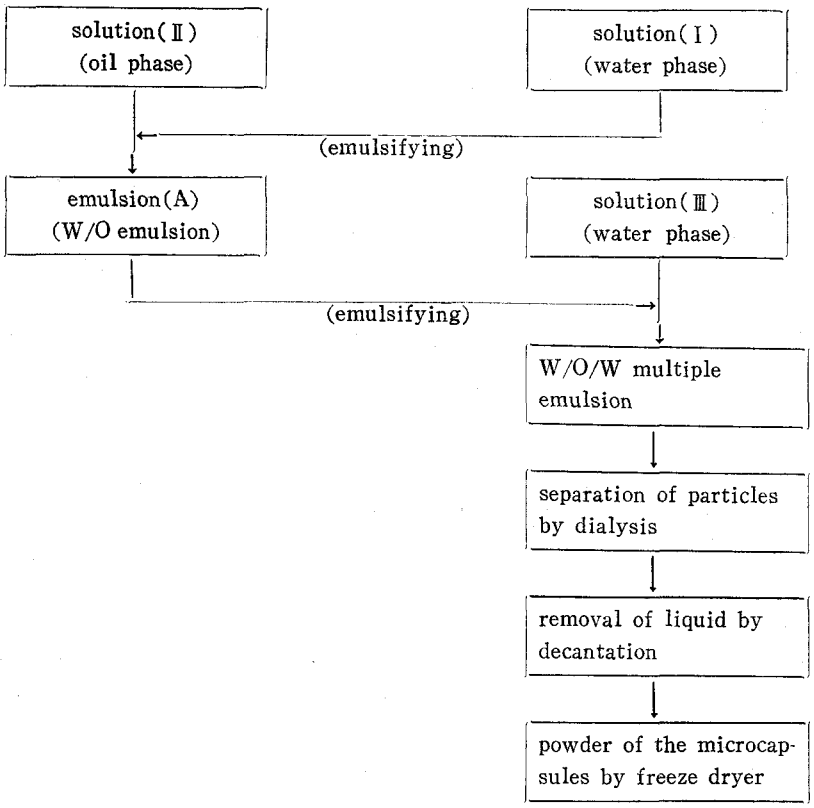
The primary encapsulating solution(Ⅰ) was made by dissolving 5.0gm of acacia and 0.2gm of furosemide in 100m*l* of 0.01N-NaOH solution at ambient temperature. Ethyl acetate solution(Ⅱ) was made by dissolving 0.1gm of reserpine and 4.0gm of ethyl cellulose, previously plasticized by the addition of 4.0gm of di-n-butyl phthalate in 100m*l* of ethyl acetate. Acacia solution(Ⅲ) was made by dissolving 5.0gm of acacia in 100m*l* of purified water.

20m*l* of solution(Ⅰ) was added slowly, drop-wise, to 40m*l* of solution(Ⅱ) and the mixtures were stirred by the homogenizer at 10,000 r.p.m. Stirring was continued till emulsion(A) was obtained. Then, 60m*l* of emulsion(A) was added slowly, drop-wise, to 60m*l* of solution(Ⅲ) and the mixtures were emulsified till an "water-in-oil"-in-water multiple emulsion was obtained. For microencapsulation, ethyl acetate was removed from multiple droplets by dialysis using seamless cellulose tubing. After dialysis, the system consisted of a sediment of microcapsules slurries and a supernatant liquid. Most of the supernatant was poured away and the sediment was transferred to freeze dryer in order to get free flowing powder of microcapsules. Their flow chart is shown in scheme 1.

Microscopy of Multiple Emulsion—It is the intent of this section to illustrate the general appearance of multiple emlsion droplets. The microscope slides were prepared for photography by placing one drop of multiple emulsion on slide and covering it with coverglass. Fig. 2 shows a dispersion of multiple emulsion droplets. It seems that the droplets are quite well dispersed.

Electron Microscopy—Surface morphology : microcapsules were mounted on to sample stubs with double sided adhesive tape and coated with gold. The surface morphology of microcapsules were investigated by scanning electron microscope and is shown in Fig.

Scheme 1. Procedure of multiple emulsion preparation



3a and 3b.

Particle Size Distribution—Microcapsules prepared by the above method were sized using the coulter counter fitted with a 140 micron orifice tube. The electrolyte was ISOTON II, buffered 0.9%(w/v) sodium chloride solution. The size distribution of microcapsules is shown in Fig.4.

Drug Contents of Micrcapsules—Furosemide in microcapsules was determined by HPLC [24,25]

In the case of reserpine 100mg of microcapules were accurately weighed, and dissolved in 50m*l* of chloroform a suitable dilution was made with ethyl alcohol, and it was assayed by fluorometer(activation 400nm and emission 500nm).

Results and Discussion

Theoretical—When droplets of solution(Ⅰ) were dispersed in solution(Ⅱ), the films adsorbed at the interface between the aqueous droplet and the continuous phase of ethyl acetate were formed from acacia and ethyl cellulose. Then, acacia oriented

to the inner aqueous side and ethyl cellulose to the outer organic side(Fig. 1a). The continuous phase is ethyl acetate with ethyl cellulose dissolved. When this W/O emulsion was mixed with solution(Ⅲ) in order to form a multiple emulsion, a fresh water/oil interface was created. Again, ethyl cellulose and acacia molecules are drawn to it, and a double polymer film is formed from them, which can be visualized as possessing two walls, an inner and an outer, each composed of two polymer (Fig. 1b). Figure 1b shows ethyl acetate to lie in a layer between layers of ethyl cellulose. When ethyl acetate is removed by diaysis, the outer layer of ethyl celllulose is contracted and eventually come into contact with the inner, the two ethyl cellulose films coming together so that two layers meet and one mono-layer is formed(Fig. 1c).

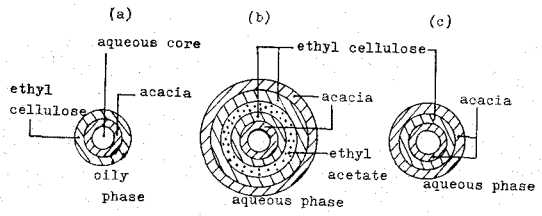


Figure 1—Representations of the structures
Key : (a), W/O emulsion droplet;
(b), W/O/W multiple emulsion droplet;
(c), microcapsule

Appearance of Multiple Emulsion—Photomicrograph of the multiple emulsion, taken prior to preparing microcapsules, were made to determine any visual peculiarities of the various conditions. Figure 2 shows that one of the multiple emulsion droplets has none of multi-cores as its inner aqueous core.

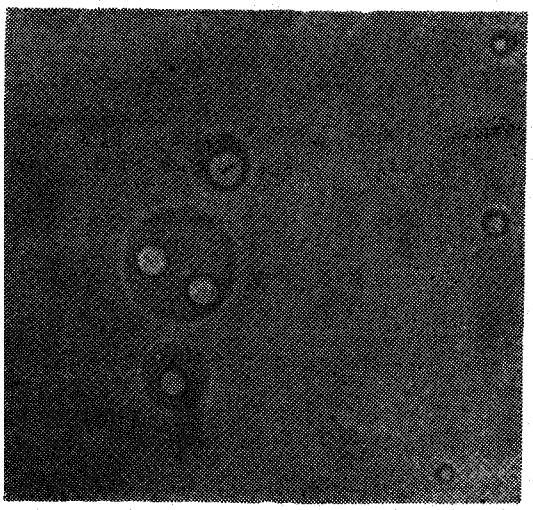


Figure 2—Photomicrograph of the multiple emulsion.

Electron Microscopy of Microcapsules—Figure 3 shows shapes and surface characteristics of the microcapsules. Their shapes are usually spherical form(Fig. 3a) and the surface of microcapsules is not porous, but it seems wrinkles and has relatively elaborate structure(Fig. 3b). It is considered the surface film of microcapsules to be formed by simultaneous diffusion from two different polymer system.
The surface is acacia layer.

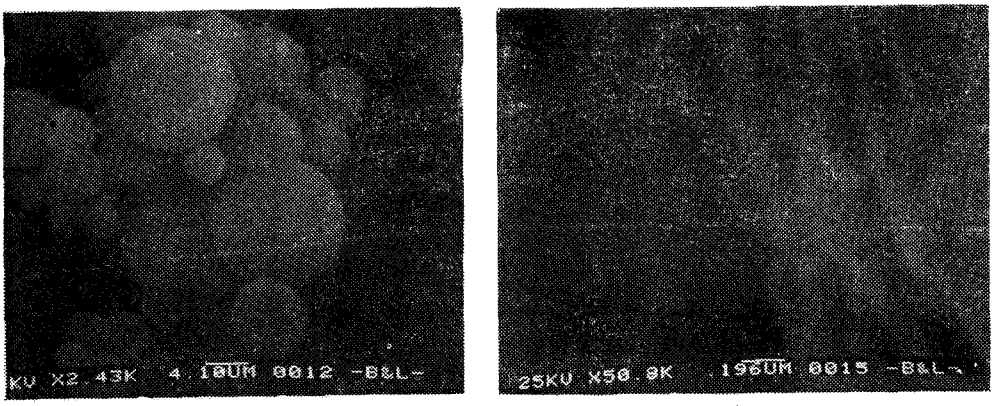


Figure 3—Scanning electron micrographs of the microcapsules.
Key : (A), 2,430X; (B), 50,800X

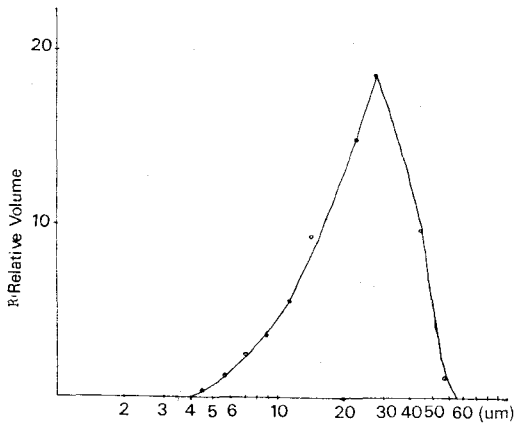


Figure 4—Size distribution curve of microcapsules

Particle Size of Microcapsules—Figure 4 shows the particle size distribution of microcapsules measured by coulter counter. The size range is from 4 μm to 64μm.

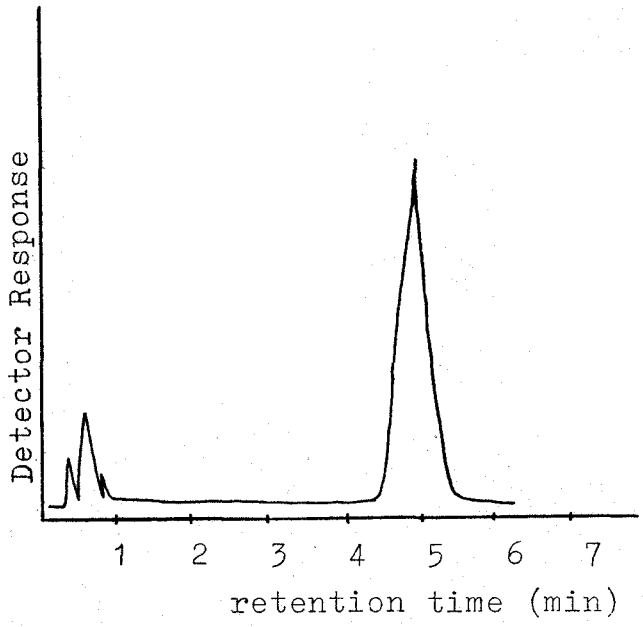


Figure 5—Chromatogram of standard solution of furosemide

Drug Contents of Microcapsules—In the case of furosemide, the calibration curve was obtained from standard solutions of 5,10,15 and 20 mcg/m*l* and the concentrations of furosemide were directly related to the height of Y-axis. The chromatogram of standard solution is presented in Fig. 5. Furosemide 0.75mg is contained in 100mg of microcapsules prepared by the multiple emulsion method.

In the case of reserpine, the calibration curve was obtained from standard solutions of 0.5, 1, 1.5 and 2mcg/m*l* and concentrations of reserpine were directly related to the intensities. Reserpine 1.005mg is contained in 100mg microcapsules prepared by the above method.

Conclusion

1. Three-ply walled microcapsules containing furosemide and reserpine were prepared from multiple emulsion and their free flowing powders were obtained from freeze drying method.
2. The surface of microcapsules is not porous, but it seems wrinkles and has relatively elavorate structure and the particle size range is 4 μm to 64μm.

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