Eq. 1 employs $\sigma_m$. Table IV shows a strong intercorrelation between $\sigma_m$ and $\sigma_p$ (arc cos 0.75 = 41°) (20). Similarly, in Eq. 8, $\sigma_m$ replaces $\sigma_p$ of Eq. 4 in the treatment of electronic effects at position 6 (arc cos 0.84 = 33°). Another interesting fact derived from the correlation matrix is considerable autocorrelation of $\pi$, especially at position 2, with $\sigma_m$ and $\sigma_p$. A similar phenomenon was observed for some benzene derivatives (21).

The pKa values of 33 disubstituted and trisubstituted purines were calculated from Eqs. 7 and 8 and compared with measured values reported in the literature (Table VI). The agreement between observed and predicted values is reasonable considering the variation in experimental methods.

Studies are in progress on the correlation of the antitumor potency and toxicity of polysubstituted purines with the structural parameters described in this paper.

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# Clofibrate Microcapsules II: Effect of Wall Thickness on Release Characteristics


**P. L. MADAN**



Received July 24, 1980, from the *College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439.* Accepted for publication September 25, 1980.



**Abstract** □ The effect of wall thickness on the release characteristics of clofibrate from microcapsules prepared in gelatin–sodium sulfate was investigated. The wall thickness, calculated by recovering the wall material from the microcapsules and using the relationship between two concentric spheres, was related to the surface area of the droplets being encapsulated. Thinner walled microcapsules gave faster release and showed greater deviation from zero-order kinetics but followed the square root of time plots. Microcapsules having thicker walls approximated zero-order release but deviated from the square root of time plots. A theoretical model was developed to explain the release characteristics of the microcapsules. A linear correlation was found between the wall thickness and the *in vitro* $t_{50\%}$ release time.



**Keyphrases** □ Clofibrate—microcapsules, effect of wall thickness on release characteristics □ Microcapsules—clofibrate, effect of wall thickness on release characteristics □ Hypocholesterolemic agents—clofibrate, effect of microcapsule wall thickness on release characteristics


A recent investigation (1) reported the microencapsulation of clofibrate USP, a liquid hypocholesterolemic agent. Prepared by simple coacervation in gelatin–sodium sulfate, the microcapsules were recovered as discrete




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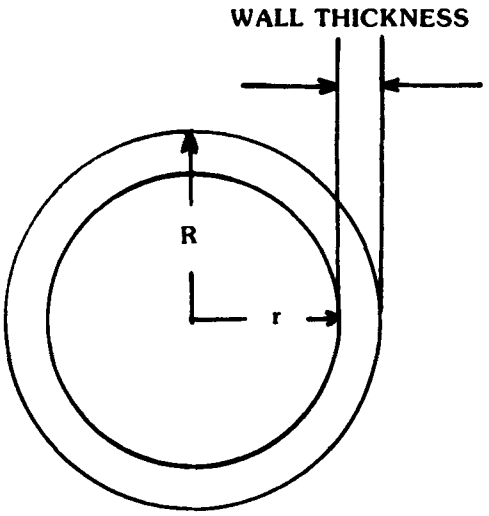


**Figure 1**—*Model of a microcapsule based on the concept of two concentric spheres.*

free-flowing particles and exhibited zero-order release at all hardening times studied. A linear correlation was found between the hardening time and the *in vitro* $t_{50\%}$ release time of the microcapsules. The microcapsules could not be hardened for longer than 8 hr without rupture, resulting in significant loss of clofibrate. Microcapsules hardened for 8 hr extended the drug release to a maximum of ~6 hr.

The effect of wall thickness on the release characteristics of clofibrate from the microcapsules is reported with a view to extending drug release from the microcapsules for longer periods.

## EXPERIMENTAL

**Materials**—All materials were similar to those reported in the earlier investigation (1).

**Microencapsulation**—The method used for the production of monodisperse spheres of clofibrate was similar to that reported previously (1). The spheres were microencapsulated by simple coacervation in gelatin–sodium sulfate and recovered as discrete free-flowing particles (1). To attain uniformity and reproducibility, all experiments were conducted under identical conditions.

**Dissolution**—Dissolution was followed by examining triplicate samples containing about 30 mg of drug using the modified flask method (1). Concentrations were determined spectrophotometrically at 226 nm.

**Wall Thickness Determination**—The wall material was recovered from the encapsulated particles by extracting clofibrate with 2-propanol (2, 3). The wall thickness of the microcapsules was then determined from the volume relationship of two concentric spheres (Fig. 1) as follows.

When uniform, smooth, spherical particles are assumed, the average wall thickness is given by:

$$\text{wall thickness} = R - r \qquad \text{(Eq. 1)}$$

where $R$ is the radius of the large sphere (microcapsule) and $r$ is the radius of the small sphere (clofibrate droplet). If $W$ is the weight of the microcapsules, $W_w$ is the weight of the wall material recovered, $d_w$ is the density of the wall material, and $d$ is the density of clofibrate, then:

$$\text{volume of } n \text{ spherical clofibrate spheres} = n\frac{4}{3}\pi r^3 \qquad \text{(Eq. 2)}$$

and the weight of $n$ spherical spheres is:

$$W - W_w = n\frac{4}{3}\pi r^3 d \qquad \text{(Eq. 3)}$$

Similarly:

$$\text{volume of } n \text{ microcapsules} = n\frac{4}{3}\pi R^3 \qquad \text{(Eq. 4)}$$

From the relationship that the volume of $n$ microcapsules equals the volume of $n$ spheres and the volume of shell enclosing $n$ spheres:

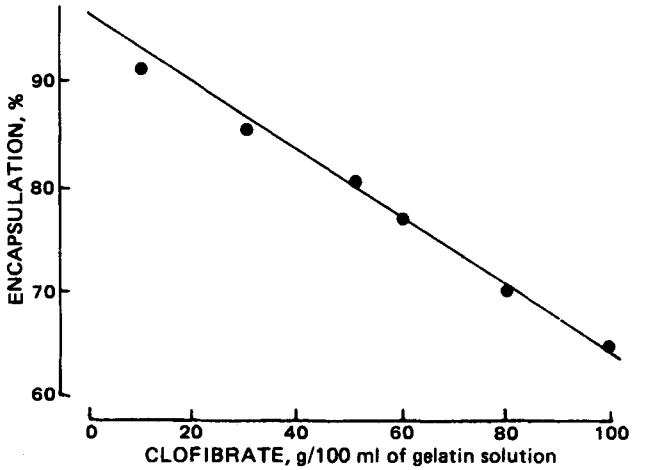


**Figure 2**—*Encapsulation efficiency of clofibrate at constant droplet size.*

shell volume enclosing $n$ spheres

$$= \left(n\frac{4}{3}\pi R^3\right) - \left(n\frac{4}{3}\pi r^3\right) = n\frac{4}{3}\pi(R^3 - r^3) \qquad \text{(Eq. 5)}$$

Therefore, the shell weight enclosing $n$ clofibrate spheres is:

$$W_w = n\frac{4}{3}\pi(R^3 - r^3)d_w \qquad \text{(Eq. 6)}$$

Dividing Eq. 6 by Eq. 3 gives:

$$\frac{W_w}{(W - W_w)} = \frac{n\frac{4}{3}\pi(R^3 - r^3)d_w}{n\frac{4}{3}\pi r^3 d} \qquad \text{(Eq. 7)}$$

which, upon rearranging, gives:

$$\frac{R^3 - r^3}{r^3} = \frac{W_w d}{(W - W_w)d_w} \qquad \text{(Eq. 8)}$$

$$\frac{R}{r} = \left[\frac{W_w d}{(W - W_w)d_w} + 1\right]^{1/3} \qquad \text{(Eq. 9)}$$

Subtracting 1 from both sides gives:

$$\frac{R - r}{r} = \left[\frac{W_w d}{(W - W_w)d_w} + 1\right]^{1/3} - 1 \qquad \text{(Eq. 10)}$$

Multiplying both sides by $r$ gives the wall thickness:

$$R - r = r\left\{\left[\frac{W_w d}{(W - W_w)d_w} + 1\right]^{1/3} - 1\right\} \qquad \text{(Eq. 11)}$$

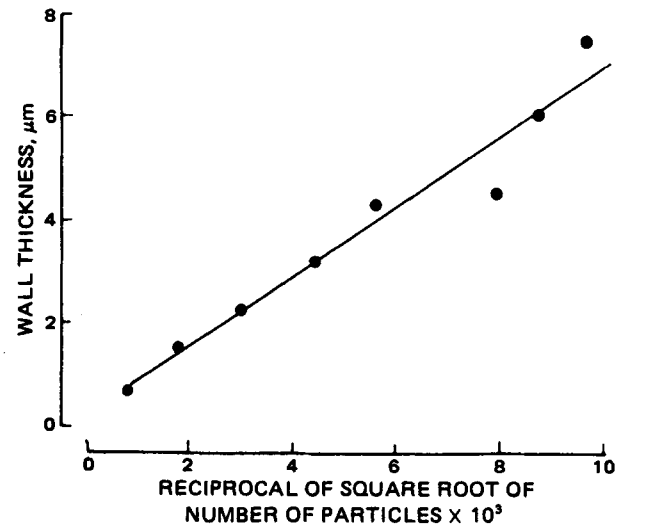


**Figure 3**—*Wall thickness as a function of the square root of the number of droplets.*

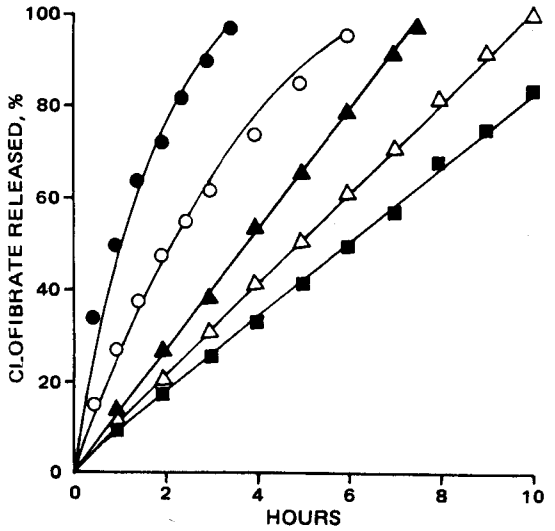


**Figure 4**—*Release of clofibrate from the microcapsules having wall thickness of 2.7 (●), 6.0 (○), 10.4 (▲), 13.5 (△), and 16.9 (■) μm.*

## RESULTS AND DISCUSSION

**Production of Monodisperse Spheres**—One method commonly used for preparing droplets of a liquid is its emulsification in another liquid in which it is immiscible. Although simple in operation, this method generally produces a heterogeneous mixture of droplets that may exhibit a large size distribution.

To maintain uniformity, the droplets being encapsulated must be monodisperse. The capillary method employed for generating clofibrate droplets produced essentially monodisperse spheres (1).

Two methods were used to study the effect of wall thickness. In the first method, the diameter of the clofibrate spheres being encapsulated was varied while the volume of clofibrate was kept constant. In the second method, the diameter of the spheres was held constant but the quantity of clofibrate encapsulated was varied.

**Microencapsulation of Clofibrate Spheres**—Encapsulation of all batches of clofibrate spheres was ascertained microscopically. The extent of clofibrate encapsulated was determined by briefly washing the dried microcapsules with 2-propanol. Unencapsulated clofibrate or partially encapsulated clofibrate spheres were dissolved by 2-propanol, leaving the encapsulated spheres intact. The percent of clofibrate encapsulated was then calculated from the original weight of clofibrate and the dried weight remaining following the brief 2-propanol wash.

The encapsulation efficiency was a function of the surface area of the core material being encapsulated. For example, at constant droplet size, the encapsulation efficiency decreased as the volume of liquid increased (Fig. 2). At constant volume, the encapsulation efficiency decreased with decreasing droplet size.

**Wall Thickness Determination**—The most direct method of determining the wall thickness of a microcapsule is by measurement using a microscope fitted with a micrometer after slicing the microcapsule with a microtome. The accuracy of such lengthy and tedious determinations is highly dependent on the precision of obtaining a section through the exact center of the microcapsule (Fig. 1). Any deviation from this center may produce a larger section of wall than exists (4). Since the microtome sections obtained are frequently at distances from the center, these readings represent an average that may exaggerate the true value. The wall thickness determined microscopically represents an average of the many determinations representative of various sections. Therefore, the volume relationship of two concentric spheres used for the calculation of wall thickness gives a better description of the average wall thickness without being time consuming and tedious.

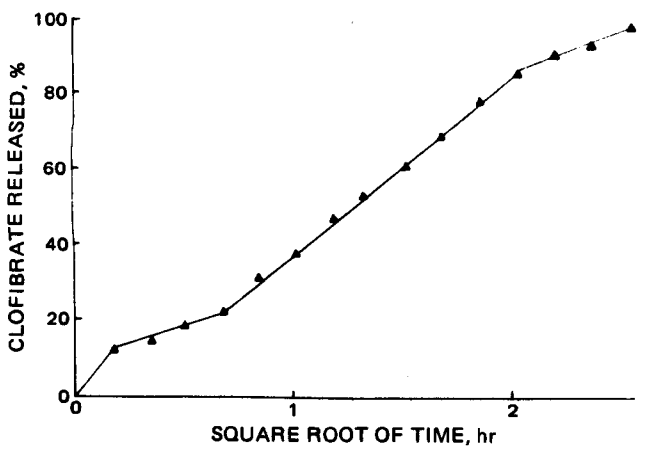


**Figure 5**—*Square root of the time plot of microcapsules having a wall thickness of 10.4 μm.*

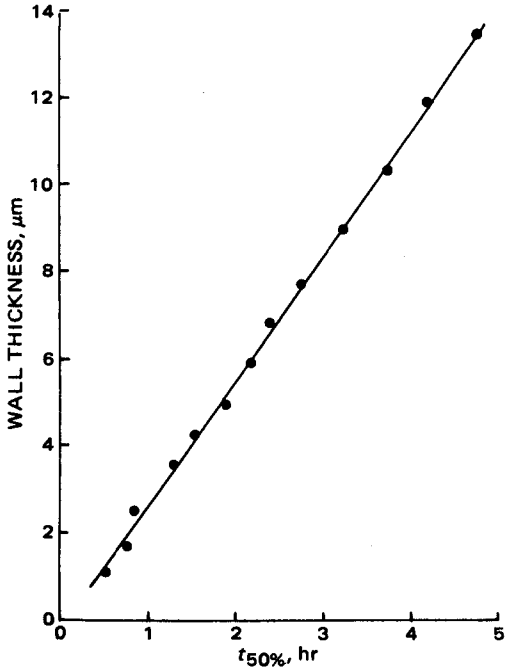


**Figure 6**—*Wall thickness of microcapsules as a function of* in vitro $t_{50\%}$ *release time.*

The wall thickness of the microcapsules also was dependent on the surface area of the core material. At a constant volume of clofibrate, smaller diameter droplets gave thinner walls than those with larger diameter. Similarly, reducing the quantity of the core material while maintaining a constant sphere diameter gave thicker walls. Although the wall thickness decreased with increasing surface area of the encapsulated droplets, a linear relationship could not be established. However, the wall thickness was inversely related to the square root of the number of droplets present when either the droplet size or the volume of clofibrate encapsulated was varied (Fig. 3). This observation seems to confirm the hypothesis that, in a coacervation system where part of the total colloid present is used in coating the core material, the coating thickness is influenced by the total number of particles present in the system (3).

**Dissolution**—The release profiles of the drug from the microcapsules were different from those observed previously (1). Microcapsules with thinner walls did not follow zero-order kinetics; as the wall thickness increased, the tendency toward zero-order release also increased (Fig. 4).

Various release mechanisms were considered, but no single mechanism could explain all of the data completely. Other investigators (5) also reported difficulty in explaining the dissolution data by any one type of treatment.

The square root of time plots characteristic of release through simple diffusion or through leaching by dissolution into the permeating fluid was linear, or nearly so, only for the thin-walled microcapsules. As the wall thickness was increased, the tendency toward linearity decreased. The release from the microcapsules having much thicker walls appeared to follow four stages (Fig. 5): (*a*) an initial large surge of release of drug, which was retained on or near the surface of the microcapsules; (*b*) slow release due to the lag time required for the dissolution medium to wet the microcapsules and to penetrate into the microcapsules; (*c*) relatively faster release for the major portion of the release profile, indicating a steady state; and (*d*) slow release toward the end, suggesting an insufficient quantity of drug remaining in the microcapsules to sustain the required concentration gradient.

The release of the drug from the microcapsules was related directly to the wall thickness (Fig. 6). Increasing the wall thickness retarded the release of the drug for longer than 12 hr from the unhardened microcapsules. Since the drug release rate from the hardened microcapsules is substantially lower than from the unhardened microcapsules (1), this procedure has potential for the development of a dosage form that may substantially reduce the frequency of administration, thus resulting in fewer missed doses.

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# Synthesis and Preliminary Pharmacology of an Internal Standard for Assay of Neostigmine


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Received August 5, 1980, from the **Division of Pharmacology and the* ‡*Division of Medicinal Chemistry, College of Pharmacy, University of South Carolina, Columbia, SC 29208.* Accepted for publication September 19, 1980.



**Abstract** □ The synthesis of the diethyl analog of neostigmine, its preliminary pharmacology, and its use as an internal standard for the GLC assay of neostigmine are described. Both the diethyl analog and neostigmine undergo thermal demethylation in the injection port. The column selected produced satisfactory resolution and short retention times for neostigmine and the diethyl analog. The diethyl analog apparently possesses acetylcholinesterase-inhibiting properties, as evidenced by potentiation of the contractile response to acetylcholine on the ileum. In addition, acetylcholine levels in the brain were elevated slightly. Water solutions of the diethyl analog appeared to lose biological activity with time. The diethyl analog appears to be suitable for use as an internal standard for the GLC assay of neostigmine.



**Keyphrases** □ Neostigmine—acetylcholine analog, synthesis and preliminary pharmacology of neostigmine analogs, quantification by GLC using flame-ionization detection □ GLC, flame ionization—analysis, neostigmine and analogs, synthesis, preliminary pharmacological studies in rats □ Cholinergics—neostigmine and analogs, synthesis, pharmacological activity evaluated in rat brain and smooth muscle, quantification by GLC


Neostigmine, a quaternary ammonium compound, has been measured following isolation from biological fluids by many methods, including chemical modification of the molecule followed by polarography (1), photocolorimetry (2), or spectrophotometry (3, 4). Other quaternary ammonium compounds have been isolated from biological fluids using ion-pair extraction (5–7). Neostigmine also has been analyzed using counterion complexation followed by liquid scintillation spectrometry (8), GLC (9, 10), or GLC–mass spectrometry (11).

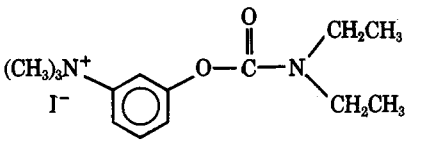
Recent assays for neostigmine used pyridostigmine as an internal standard followed by thermal dimethylation in the injection port and GLC separation. Neostigmine and pyridostigmine are both employed clinically, and their combined therapy presents a problem with currently available assays. Since GLC is widely used with substantial economic advantages over GLC–mass spectrometry, it was necessary to synthesize an analog of neostigmine applicable to the assay of quaternary ammonium cholinesterase inhibitors.

Since neostigmine possesses cholinomimetic properties, the structural similarity of the diethyl analog suggested that it may possess similar pharmacological actions. The present study reports the synthesis of the diethyl analog of neostigmine, its use as an internal standard in the quantification of neostigmine, and preliminary pharmacological findings.

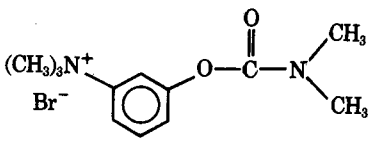
## EXPERIMENTAL

**Synthesis[1] of Diethyl Amino and Tertiary Analogs of Neostigmine**—The preparation of the diethyl analog of neostigmine required the synthesis of an intermediate, 3-[[(diethylamino)carbonyl]oxy]-*N,N*-dimethylaniline (I), which subsequently was converted to the diethyl analog, 3-[[(diethylamino)carbonyl]oxy]-*N,N,N*-trimethylbenzenaminium iodide (II).

*Synthesis of I*—Compound I was synthesized by a modified procedure of Yanagisawa (12). A solution of 3-dimethylaminophenol (10.0 g, 0.073 mole) in anhydrous tetrahydrofuran (100 ml) was added dropwise with stirring to phosgene in 12.5% benzene (192 ml) in an ice bath. After 24




II




IV

[1] IR spectral data were determined on a Beckman Acculab 4 spectrophotometer using the potassium bromide technique. NMR spectra were determined on a Varian EM 360A high-resolution spectrometer with tetramethylsilane as the internal reference. Melting points were obtained using a Thomas-Hoover capillary apparatus and are uncorrected. TLC was performed using Eastman chromatogram sheets, type 6060 (silica gel); the sheets were developed in an iodine chamber. Carbon, hydrogen, nitrogen, and iodide values were obtained from analyses performed by Atlantic Microlabs, Atlanta, Ga.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