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RESEARCH PAPER

Evaluation of a Novel Phase Separation Technique for the Encapsulation of Water-Soluble Drugs in Biodegradable Polymer

Tarun K. Mandal

Division of Pharmaceutics, College of Pharmacy,
Xavier University of Louisiana, New Orleans, Louisiana 70125

ABSTRACT

Biodegradable microcapsules of four water-soluble drugs (pentamidine, captopril, diltiazem, and metoprolol) were prepared using a novel phase separation technique. The microcapsules prepared by this method were irregular in shape. Particle size of the microcapsules was between 60 and 500 μm. The efficiency of encapsulation for all four drugs was more than 40% at 20% drug loading. The encapsulation increased up to 75% or higher when the drug loading was reduced to 5%. The in vitro dissolution was drug dependent. The microcapsules prepared with 5% drug loading showed the minimum dissolution at the end of 24 hr. The initial drug release increased significantly when the drug loading was increased up to 20%. Although the formulations containing 5% drug maintained a sustained-release profile up to 45 days, the formulations containing 10 and 20% drug released more than 50% drug within the first 2 days.

INTRODUCTION

Sustained-release formulations using biodegradable polymers have been developed for numerous therapeutic agents (1). One of the advantages of these formulations is that no followup surgical removal is required once the drug supply is depleted. The most widely investigated polymers are the aliphatic polyesters based on lactic acid and glycolic acid. These copolymers have attracted much attention because the biodegradation rate of the copolymer is easily controlled by altering the copolymer's composition. These polymers have been used with numerous drugs for parenteral delivery and have been shown to be biocompatible (2,3). A wide variety of drugs have been successfully encapsulated into poly(lactide/glycolide) (PLGA) for a sustained release (1,4). Once-a-month injectable microcapsules of leuprolide acetate using PLGA have been developed (5–

7). This product is now commercially available under the trade name Lupron-Depot®. Another commercially available similar product is Zoladez®, which contains goserelin acetate–PLGA (4,8).

Several methods have been developed for the microencapsulation of a wide variety of drugs (9–11). The double water-in-oil-in-water (w/o/w) emulsion method has been widely accepted for the encapsulation of a number of water-soluble drugs (2,12,13). The use of this double-emulsion method to encapsulate zidovudine (AZT) (water solubility 25 mg/ml) in PLGA resulted in less than 6% efficiency of encapsulation (14). The efficiency of encapsulation of AZT was increased up to 94% by using a novel phase separation technique (14–16).

The objective of this present investigation was to develop biodegradable delivery systems for water-soluble drugs. Four model drugs were used for this study: pentamidine, captopril, diltiazem, and metoprolol. Pentamidine is known to have activity against *Pneumocystis carinii*. It is available commercially as inhalation solution and injectable (17). Captopril is used for the treatment of hypertension. It is available commercially as a regular tablet (17). Diltiazem is a calcium channel blocker. It is available commercially in both oral and parenteral formulations. Currently, sustained-release oral formulations of diltiazem are dosed either twice daily or once daily (17). Metoprolol is a cardioselective β_1-blocker. It is available in both oral and parenteral formulations. Currently, a sustained-release oral formulation is dosed once daily (17).

MATERIALS AND METHODS

Materials

The copolymer poly(DL-lactic/glycolic acid), PLGA 50:50 (RG 506; inherent viscosity 0.8 dl/g), was obtained from Boehringer Ingelheim, Ingelheim, Germany. The surfactant L-α phosphatidylcholine was obtained from Avanti Polar-lipids, Inc., Albaster, AL Pentamidine, captopril, diltiazem, and metoprolol were obtained from Sigma Chemical Co., St. Louis, MO. Silicon oil, petroleum ether, polyvinyl alcohol (PVA), chloroform, and dichloromethane were also obtained from Sigma Chemical Co.

Experimental Methods

Preparation of Biodegradable Microcapsules

A specific amount of drug (as listed in Tables 1–4) was added to 5 ml of methylene chloride containing 500 mg of PLGA and sonicated for 30 sec. The polymer–drug mixture was added dropwise to a silicon oil/methylene chloride solution (20 ml; 1:6) while the solution was stirred continuously using a magnetic stirrer. Bath temperature was maintained at 8–10°C to control the evaporation rate. Five milliliters of petroleum ether was added slowly in portions (0.5 ml every 10 min). Stirring was maintained until the methylene chloride had completely evaporated. Microcapsules were then washed with cold petroleum ether, filtered, and freeze-dried to obtain a free-flowing powder.

Determination of Total Content

For each formulation, a 20-mg sample was dissolved in 1 ml of dichloromethane. Four milliliters of methanol was added to the solution followed by ultracentrifugation (35,000 rpm at 15°C) to completely separate the precipitated copolymer. The amount of drug in each sample was determined by measuring the absorbance of clear supernatant in a spectrophotometer (DU 640, Beckman, Fullerton, CA) at λ_{max} (pentamidine: 266 nm; captopril: 212 nm; diltiazem: 240 nm; metoprolol: 223 nm). Each experiment was performed in triplicate.

In Vitro Dissolution Studies

For each formulation, a 40-mg sample was placed in a 10-ml tube and incubated in 5 ml of double-distilled water with constant shaking (20 rpm) at 37°C. Samples (600 μl) were collected at scheduled times using a filter pipet and centrifuged for 10 min at 10,000 rpm. The sample was spectrophotometrically analyzed for drug content. Fresh double-distilled water was added to the incubated sample (600 μl) to maintain sink conditions. Dissolution studies were performed independently in triplicate.

Particle Size and Morphology

Size, morphology, and surface appearance of microcapsules were examined by scanning electron microscopy (SEM) (Amray AMR 1000A, Bedford, MA). Samples for SEM were mounted on metal stubs and coated with gold to a thickness of 200–500 Å. Pictures were taken and the microcapsule sizes were determined according to a reference scale.

Statistical Analysis

The efficiency of drug encapsulation and the amount of drug released from the different formulations of microcapsules during the in vitro study were compared

using the SAS software package. A p value of <0.05 was considered as evidence of a significant difference.

RESULTS AND DISCUSSION

Pentamidine Microcapsules

Three different batches (batches A, B, and C) of pentamidine microcapsules were prepared to study the effect of pentamidine loading on the characteristics of the microcapsules. These microcapsules were evaluated and the results are listed in Table 1. A comparison of particle size reveals a similar average particle size in all three batches. The particles were all larger than 160 μm with a range of average sizes from 252 to 345 μm in diameter. This observation shows that the phase separation method used for the encapsulation of pentamidine in PLGA generally produced larger particles irrespective of the drug loading. The efficiency of encapsulation of pentamidine was determined by measuring the total amount of pentamidine present in each 20-mg sample of the microcapsules, i.e., core loading experimental, and comparing this value with the expected amount of pentamidine in each of the samples based on the drug loading during the preparation, i.e., core loading theoretical. The efficiency of encapsulation of pentamidine was high (75%) at 5% drug loading. The efficiency of encapsulation decreased significantly to 45% when the drug loading was increased to 20%. This observation shows that the phase separation method is more effective in the encapsulation of pentamidine when the drug loading is low.

The dissolution of pentamidine was compared to calculating the cumulative percentage of the drug released at a specific sampling time. Figure 1 shows the dissolution profiles of the pentamidine microcapsules. The amount of drug released from these microcapsules was at least 80% within the first 24 hr. This significantly high burst effect was due to the presence of unencapsulated drug on the surface of the microcapsules. During the process of phase separation, pentamidine precipitated on the surface of the preformed PLGA microparticles. A comparison of the scanning electron micrographs of the microcapsules (Fig. 2) also showed the presence of free pentamidine crystals on the surface, irrespective of the drug loading. However, the surface the microcapsules was smooth at 5 and 10% (batches A and B) drug loading. When the drug loading was increased to 20% (batch C), the microcapsules lost the smooth appearance completely and the surface was coated with free crystals. These unencapsulated free pentamidne crystals released immediately and produced a significantly high burst effect.

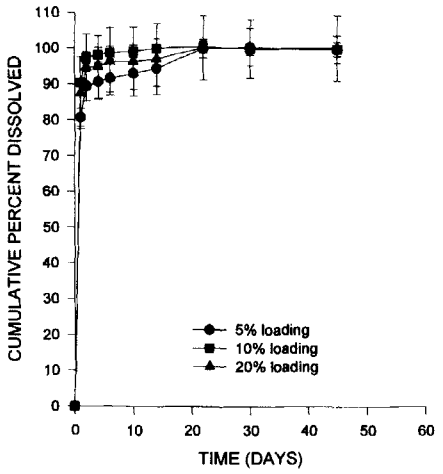

Figure 1. Dissolution profiles of pentamidine microcapsules: batch A (5% loading), batch B (10% loading), and batch C (20% loading).

Captopril Microcapsules

Three different batches (batches D, E, and F) of captopril microcapsules were prepared to study the effect of captopril loading on the characteristics of the microcapsules. These microcapsules were evaluated and the results are listed in Table 2. A comparison of particle

Table 1

Description of Pentamidine Microcapsules

Batch	Amount of Drug (mg)	% Loading	Particle Size (Range) (μm)	Encapsulation[a] (%) (±SD)
A	25	5	345 (204–500)	75.4 (3.13)
B	50	10	252 (167–418)	53.5 (0.94)
C	100	20	297 (238–400)	45.5 (2.71)

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) × 100.

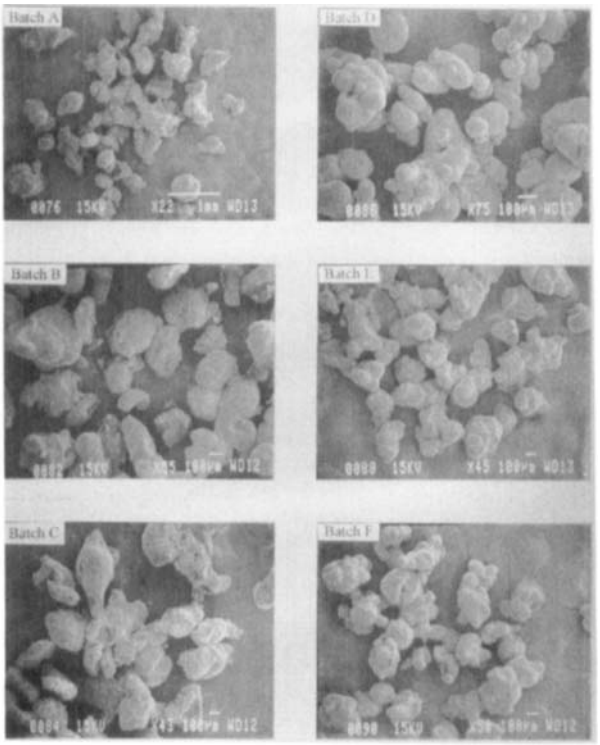

Figure 2. Typical SEM photographs of pentamidine and captopril microcapsules. Pentamidine: batch A (5% loading), batch B (10% loading), and batch C (20% loading); captopril: batch D (5% loading), batch E (10% loading), and batch F (20% loading).

size reveals a similar particle size in all three batches. The particles are all larger than 60 μm with a range of average sizes from 152 to 233 μm in diameter. This observation shows that the phase separation method used for the encapsulation of captopril in PLGA generally produced larger particles irrespective of the drug loading. The efficiency of encapsulation of captopril was more than 99% at 5% drug loading. The efficiency of encapsulation decreased to 73% when the drug loading was increased to 20%. However, the efficiency of encapsulation was still significantly high, more than 92%, at 10% drug loading.

Figure 3 shows the dissolution profiles of captopril microcapsules. The amount of drug released from these microcapsules was between 45 and 60% within the first 24 hr. However, no rank-order correlation was observed between the drug loading and initial drug release. The batch prepared with 20% captopril (batch F) showed the least initial burst effect, followed by a second burst effect between 6 and 10 days of dissolution. The initial slow release from this batch (batch F), compared with the other two batches (batches D and E), was due to the relatively smaller surface area, because of the agglomerated microcapsules (batch F) exposed to the dissolution media. However, the presence of high drug concentrations in these microcapsules initiates a second burst effect. The drug release continued up to 45 days. A comparison of the scanning electron micrographs (Fig. 2) showed that the microcapsules prepared with 5% drug loading were mixtures of spherical and irregularly

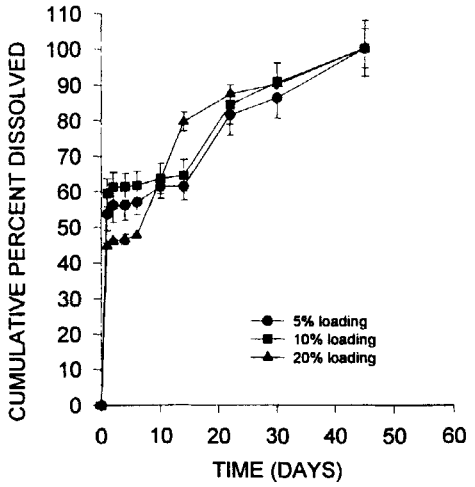

Figure 3. Dissolution profiles of captopril microcapsules: batch D (5% loading), batch E (10% loading), and batch F (20% loading).

Table 2

Description of Captopril Microcapsules

Batch	Amount of Drug (mg)	% Loading	Particle Size (Range) (μm)	Encapsulation[a] (%) (±SD)
D	25	5	152 (67–266)	99.3 (0.48)
E	50	10	233 (133–355)	92.8 (0.52)
F	100	20	182 (80–320)	73.7 (0.09)

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) × 100.

shaped agglomerated particles. The relative proportion of spherical particles decreased as the drug loading was increased to 10%. The surface of the microcapsules also contained a few crystals at 10% drug loading. When the drug loading was increased to 20%, the surface of the microcapsules was covered with free crystals and the microcapsules were mostly irregularly shaped, agglomerated particles.

Diltiazem Microcapsules

Three different batches (batches G, H, and I) of diltiazem microcapsules were prepared to study the effect of diltiazem loading on the characteristics of the microcapsules. These microcapsules were evaluated and the results are listed in Table 3. A comparison of particle size reveals a similar particle size in all three batches. The particles are all larger than 60 μm with a range of average sizes from 123 to 180 μm in diameter. This observation shows that the phase separation method used for the encapsulation of diltiazem in PLGA generally produced larger particles irrespective of the drug loading. The efficiency of encapsulation of captopril was more than 90% at 5% drug loading. The efficiency of encapsulation did not change when the drug loading was increased to 10%. The efficiency of encapsulation decreased to 63% when the drug loading was increased to 20%. However, the efficiency of encapsulation was still significantly high.

Figure 4 shows the dissolution profiles of diltiazem microcapsules. The drug loading significantly influenced the initial burst effect. The amount of drug released from these microcapsules was between 8 and 60% within the first 24 hr. The microcapsules prepared with 5% drug (batch G) showed the minimum dissolution at the end of 24 hr. The initial drug release increased more than threefold when the drug loading was increased to 10% (batch H). The initial release increased further to 60% when the drug loading was increased to 20% (batch I). Practically, the microcapsules prepared with 20% drug loading showed very poor sustained effect. However, drug release from all three batches continued up to 45 days. A comparison of the scanning electron micrographs (Fig. 5) showed that the surfaces of the microcapsules prepared with 5% drug loading were very smooth and the microcapsules were all spherical shaped. A few of the microcapsules formed irregularly shaped particles because of agglomeration as the drug loading was increased to 10%. As the drug loading was increased further to 20%, the microcapsules lost their spherical shape completely. These microcapsules were all irregularly shaped particles and the surface was coated with free crystals.

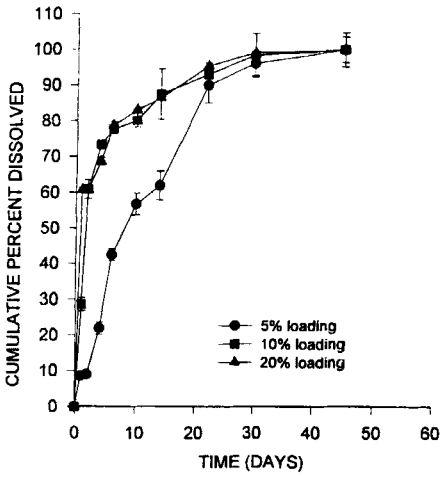

Figure 4. Dissolution profiles of diltiazem microcapsules: batch G (5% loading), batch H (10% loading), and batch I (20% loading).

Metoprolol Microcapsules

Three different batches (batches J, K, and L) of metoprolol microcapsules were prepared to study the effect of metoprolol loading on the characteristics of the microcapsules. These microcapsules were evaluated and

Table 3

Description of Diltiazem Microcapsules

Batch	Amount of Drug (mg)	% Loading	Particle Size (Range) (μm)	Encapsulation[a] (%) (±SD)
G	25	5	125 (67–160)	90.8 (1.63)
H	50	10	123 (86–157)	90.2 (1.95)
I	100	20	180 (100–272)	63.4 (2.56)

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) × 100.

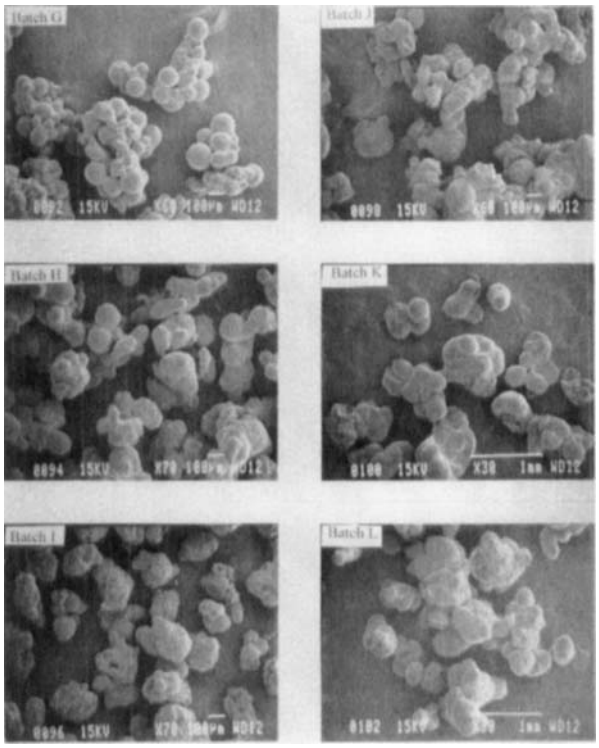

Figure 5. Typical SEM photographs of diltiazem and metoprolol microcapsules. Diltiazem: batch G (5% loading), batch H (10% loading), and batch I (20% loading); metoprolol: batch J (5% loading), batch K (10% loading), and batch L (20% loading).

the results are listed in Table 4. A comparison of particle size shows a significant effect of drug loading on the size of the microcapsules. The average particle size increases from 150 to 357 μm as the drug loading was increased from 5 to 20%. The sizes of the microcapsules prepared with 5% drug loading were between 72 and 229 μm in diameter. A comparison of the efficiency of encapsulation showed a significant effect of drug loading on the total amount of metoprolol encapsulated. The efficiency of encapsulation decreased from 99 to 87% when the drug loading was increased from 5 to 10%. The efficiency of encapsulation continued to decrease to 52% when the drug loading was increased to 20%.

Figure 6 shows the dissolution profiles of metoprolol microcapsules. The drug loading significantly influenced the initial burst effect. The amount of drug released from these microcapsules was between 26 and 50% within the first 24 hr. The microcapsules prepared with 5% drug (batch J) showed 26% dissolution at the end of 24 hr. The initial drug release increased to 32% when the drug loading was increased to 10% (batch K). The initial release increased further to 50% when the drug loading was increased to 20% (batch L). A rank-order correlation was observed between the cumulative percent drug dissolved at any time and the drug loading throughout the dissolution. The microcapsules prepared with a higher amount of metoprolol always showed higher dissolution at any sampling time. A comparison of the scanning

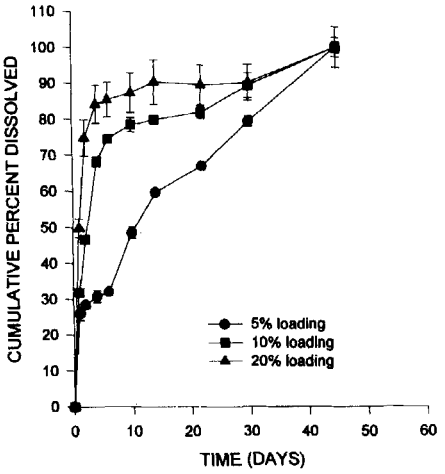

Figure 6. Dissolution profiles of metoprolol microcapsules: batch J (5% loading), batch K (10% loading), and batch L (20% loading).

Table 4

Description of Metoprolol Microcapsules

Batch	Amount of Drug (mg)	% Loading	Particle Size (Range) (μm)	Encapsulation[a] (%) (±SD)
J	25	5	150 (72–229)	99.4 (0.95)
K	50	10	307 (167–500)	87.4 (7.10)
L	100	20	357 (167–800)	52.0 (4.55)

[a]Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) × 100.

electron micrographs (Fig. 5) showed that a change in the drug loading did not change the surface morphology of the microcapsules. All three batches of the microcapsules were a mixture of agglomerated, irregular, and spherical particles with a smooth surface.

In conclusion, the novel phase separation technique was highly efficient for the encapsulation of the four highly water-soluble drugs: pentamidine, captopril, diltiazem, and metoprolol. In general, the efficiency of encapsulation decreased significantly when the drug loading was increased from 5 to 20%. However, the efficiency of encapsulation was drug dependent. Scanning electron micrographs of the microcapsules showed that the surface of the particles was coated with free crystals at higher drug loading. The microcapsules prepared by this method were mostly irregular in shape. The phase separation method produced relatively larger microcapsules. The average particle size of the microcapsules was between 123 and 357 μm. The release profiles of the microcapsules were dependent on the drug loading. In general, the initial drug release increased significantly when the drug loading was increased to 20%. Pentamidine microcapsules failed to maintain a sustained drug release. More than 80% drug released within the first 24 hr, irrespective of the drug loading. However, the microcapsules of the other three drugs (captopril, diltiazem, and metoprolol) maintained a sustained drug release up to 45 days.

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REFERENCES

1. D. H. Lewis, in *Biodegradable Polymers as Drug Delivery Systems* (M. Chasin and R. Langer, eds.), Marcel Dekker, New York, 1990, p. 1.
2. M. J. Alonso, S. Cohen, T. G. Park, R. K. Gupta, G. R. Siber, and R. Langer, Pharm. Res., 10, 945 (1993).
3. M. Kanke, G. H. Simmons, D. L. Weiss, B. A. Bivins, and P. P. DeLuca, J. Pharm. Sci., 69, 755 (1980).
4. J. L. Cleland and R. Langer, in *Formulation and Delivery of Proteins and Peptides* (J. L. Cleland and R. Langer, eds.), American Chemical Society, Washington, DC, 1994, p. 1.
5. H. Okada, Y. Ogawa, and T. Yashiki, US Patent 4652441, 1987.
6. H. Okada, Proc. Int. Symp. Controlled Release Bioact. Mater., 16, 12 (1989).
7. H. Okada, Y. Inoue, T. Heya, H. Ueno, Y. Ogawa, H. Toguchi, Pharm. Res., 8, 787 (1991).
8. B. J. Furr and F. G. Hutchinson, J. Controlled Release, 21, 117 (1992).
9. N. Nihant, S. Stassen, C. Grandfils, R. Jerome, and Ph. Teyssie, Polym. Int., 32, 171 (1993).
10. R. Arshady, Polym. Eng. Sci., 30, 195 (1990).
11. R. Alex and R. Bodmeier, J. Microencapsulation, 7, 347 (1990).
12. C. X. Song, H. F. Sun, and X. D. Feng, Polym. J., 19, 485 (1987).
13. Ch. Schugens, N. Laruelle, N. Nihant, Ch. Grandfils, R. Jerome, and Ph. Teyssie, J. Controlled Release, 32, 161 (1994).
14. T. K. Mandal, A. Lopez-Anaya, E. Onyebueke, and M. Shekleton, J. Microencapsulation, 13, 257 (1996).
15. T. K. Mandal, M. Shekleton, L. Washington, E. Onyebueke, and T. Penson, Proc. Int. Symp. Controlled Release Bioact. Mater., 22, 768 (1995).
16. T. K. Mandal, Pharm. Sci., 2, 99 (1996).
17. *Physicians' Desk Reference*, 49th ed., Medical Economics, Montvale, NJ, 1995.