High-Precision Measurement of the Swelling of Microspheres

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SYNOPSIS

For controlled release of drugs and other uses, the accurate and precise measurement of swelling of polymer microsphere carriers is often crucial. Most currently available techniques were designed to measure high degrees of swelling. We explored the use of digital light microscopy for the high precision measurement of small degrees of swelling of individual microsphere particles. By using a camera to acquire images of microspheres in a sample cell filled with solvent, accurate, precise, measurements of swelling were obtained. The technique has a precision of 0.52% change in the swelling of the volume of a microsphere. We compared this technique with a state-of-the-art gravimetric technique. Comparison of the two techniques revealed that as much as 84% of swelling as measured by the gravimetric method is excess fluid arising from confounding factors such as adsorption and capillary effects. Computer modeling shows that the inaccuracies are due entirely to liquid bridge formation between the microspheres. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Polymer microspheres have become popular as carriers for various bioactive agents during the last decade.[1] In applications for controlled release of drug agents and other critical end uses, an accurate and precise measurement of swelling of polymer carriers is often crucial.[2] Current techniques for the measurement of swelling in microspheres include the gravimetric determination of the solvent uptake after centrifugation[3] and the measurement of the height of a column of microspheres before and after swelling. Conceptually, it is easy to visualize situations in which both of these techniques would not be precise enough to provide reliable information due to confounding factors. For example, the gravimetric technique would include the adsorption of liquid medium to the surface of the spheres, selective absorption of ions from buffers, and capillary effects between spheres in the measurement of equilibrium weight changes. The weight gain of a polymer shown by this technique may be more indicative of the medium adsorbed to the spheres than that absorbed by them when the degree of swelling is very low. The human errors and low accuracy inherent in procedures in which the change in height of a column of microspheres is measured are easily grasped. The faults inherent in many less common methods are equally problematic.[4]

For applications that rely on low degrees of swelling, the low precision of these techniques presents a dilemma. The most obvious way of measuring the true degree to which a sphere swells is to measure its physical change in size.[4] We explored the use of digital light microscopy for the measurement of individual particle dimensions. In short, this technique involves attaching a digital television camera and a computer to the camera port of a standard microscope. By using the camera to acquire images of the microspheres digitally, results can be obtained almost instantaneously. Computer processing of the resulting digital data minimizes human errors, and, therefore, increases reproducibliity. The measurement of individual particles allows the study of differences between seperate members of an ensemble, as well as extrapolation to a larger population.

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The purposes of this study were: (1) to develop a protocol that uses light microscopy and digital imaging techniques to measure small amounts of swelling in pH sensitive microspheres, (2) to test the precision of the proposed technique with respect to a standard gravimetric method used for the same batch, and (3) to develop a mathematical model to examine the difference between the two methods.

MATERIALS AND METHODS

Preparation of Microspheres and Sample Cell

Poly(styrene-*co*-[2-vinylpyridine]-*co*-divinylbenzene) microspheres were prepared by suspension polymerization.[5] The composition, in molar ratio, was 9/90/1 of styrene, 2-vinylpyridine, and divinylbenzene, respectively. The size of the microspheres ranged from 100 to 400 μm.

Cel-Tek Grid Slides™ were coated with a thin coating of household polyurethane lacquer (M-Coat™). While the lacquer was still wet, microspheres were sprinkled onto the surface. The purpose of this step was to fix the spheres in place for easier identification and location. The resulting slides were allowed to cure for 7 days. Sample cells were constructed around the polyurethane bound spheres by laying a bead of epoxy (Devcon 2-Ton™) thickened with nano-particulate hydroxyapatite (approximately 5 parts epoxy to 1 part HA) around the edge of the slide. While the epoxy was still sticky, 27 gage needles were laid into the resin so that their tips were within the sample cell. A coverglass was used to seal the top of the sample cell (Fig. 1).

Swelling Measurements

After injecting a phosphate/citrate pH buffer into the sample cell, images were acquired with a Nikon Labphot™ microscope equipped with a Sony CCD video camera (Model XC-77) and an Apple LCII computer. Images were processed using an Apple LCII and Image 1.44 software.[6] The total area of the projected cross-section of the sphere was measured by counting the number of pixels in the digitized image. Results of these measurements were processed by converting the measured areas into volumes and computing the ratio of the volume at time (t) to the initial volume. Results are converted to % vol swelling (Q) by the following equation:

$$Q = \left(\frac{A_t^{3/2}}{A_0^{3/2}} - 1\right) \times 100, \qquad (1)$$

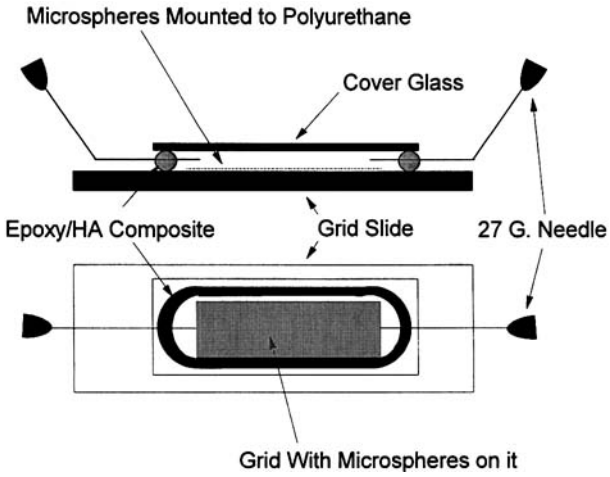

Figure 1 The sample cell preparation used in this experiment. This sample preparation is useful in situations where basement layer/solvent compatibility is adequate and the sphere size is small.

where
A_t = area measured at time t, and
A_o = initial area immediately after injecting buffer solution.

The precision of the technique was determined through the use of multiple focus/defocus cycles on a single object. A microsphere 300 microns in diameter was focused on. The operator upset the focus and then refocused a total of five times, acquiring an image at each repetition. This process was repeated for two more spheres.

During experimental measurements, five images were acquired at each sampling time, refocusing between image acquisitions. Acquisitions were made just after injection of the pH buffer, then every 15 min for 2 h and once more when swelling equilibrium was reached. A total of 10 acquisition time periods were scheduled for each experiment. The spheres were studied in four different pH environments, namely, 4.5, 5.0, 6.0, and 7.0 phosphate citrate buffers. A total of 1000 data points were collected during the study. The buffer in the sample cell was replaced with fresh buffer solution at least three times during the period of the experiment. The magnification in all images was 100×.

Weight changes were measured by Pepper's[3] technique by placing approximately 0.5 g of microspheres into the same buffers as those used in the digital image measurements until equilibrium swelling was reached. Swollen microspheres were placed in centrifuge tubes with glass frits at the bottom to eliminate excess liquids from the spheres. After centrifugation, the microspheres were reweighed and

the percent swelling with respect to initial dry weight computed. Controls of empty, fritted tubes were run at the same time. The effects of retained fluid in the glass frits was eliminated by subtraction of the weight gain of the frits from the weight gain of the microsphere/frit combination. The results will be compared with those obtained with the digital technique.

Mathematical Model of Equilibrium Swelling by Gravimetric Method

Confounding factors that could contribute to the excess weight gain associated with the gravimetric technique include: (1) liquid retained by polymer porosity, (2) defect air bubbles within the polymer, (3) surface adsorption of liquid, and (4) liquid bridge formation between spheres. Assume that any changes around one single sphere caused by the known confounding factors can represent the ensemble of microspheres of various sizes. Thus, the following model is proposed to assess the excess swelling expected from a single sphere when the gravimetric method is used,

$$Q_e = \frac{V_p + V_d(Q+1) + \frac{4}{3}\prod[(r+a)^3 - r^3] + 12\prod[r^2 - (r-b)^2]\,b - 4\prod b^2(3r-b)}{\frac{4}{3}\prod r^3}, \quad (2)$$

where

Q_e = excess swelling with respect to that measured by the digital imaging technique,
V_p = initial pore volume,
V_d = initial defect bubble volume,
Q = swelling ratio by eq. (1),
r = radius of the swollen sphere,
a = thickness of adsorbed buffer on the sphere, and
$2b$ = length of the meniscus between spheres (Fig. 2).

The first term of the equation addresses the porosity within the polymer sphere. The second accounts for the volume of any large voids (bubble defects) within the microspheres. The third term, involving a, takes a measure of the contributions due to surface layers of liquid on the microspheres. Finally, the last two terms address the volume trapped in the liquid bridge between spheres. To simplify the calculation, the meniscus stretching between spheres was assumed to be flat as apposed to the normally hyperbolic shape (Fig. 2). The first of these last two terms is a computation of the volume of a cylinder of liquid that is imagined to extend between the two spheres, its straight sides being of height **2b** and cross-sectional area of $\pi \mathbf{r}_2^2$ (Fig. 2). The final term is a computation of the volume of the spheres that intersects with the volume described by the aforementioned cylinder.

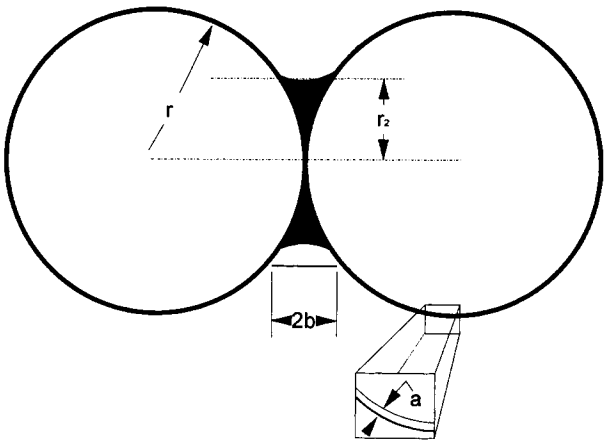

Figure 2 The model system. Variables used in the mathematical model of differences seen in swelling measured by the digital imaging technique and the gravimetric technique are displayed.

There are four unknown variables in the proposed model, $\mathbf{V_p}$, $\mathbf{V_d}$, **a**, and **b**. Parameters $\mathbf{V_p}$ and $\mathbf{V_d}$ were measured by conducting BET analysis and true density measurements on a Quantachrome Autosorb™ and a Quantachrome Mycropyc™ 40 pycnometer, respectively. The values of **a** and **b** can be determined for each pH environment by fitting the values $\mathbf{Q_e}$ and respective radii of microspheres into the equation.

The number of liquid bridges around a sphere depends on the packing of spheres after centrifugation. To keep the number of unknown variables low, the model shown in eq. (2) assumes hexagonal close packing with a coordination number of 12. If a simple cubic cell is assumed, the coefficients should be 6 and 2 for the last two terms of the equation.

The volume of the liquid bridge can also be calculated by employing the reduced neck radius, $\mathbf{R_2}$, which is defined as the neck radius, $\mathbf{r_2}$, divided by the radius of the sphere, **r** (Fig. 2). The following equation represents the volume of one-half of a liquid bridge with respect to the volume of a sphere.[7]

$$Q_e = \frac{3k_pR_2^4}{16}, \quad (3)$$

Table I Equilibrium Swelling Values for Polymer Microspheres at Various pHs as Measured by the Digital Imaging Technique and Pepper's Gravimetric Technique

pH	Q_A	Q	Q_W	Q_E	$[(Q_W - Q)/Q_W] \times 100$
4.5	84.5	77.7	217.5	139.8	64.3
5.0	20.6	9.4	59.0	49.6	84.1
6.0	18.0	8.8	36.6	27.8	76.0
7.0	11.7	7.5	33.5	26.0	77.0

Q_A = Average area gain (%) by digital image technique.
Q = Average volume gain (%) by digital image technique.
Q_W = Average weight gain (%) by gravimetric technique.
Q_E = Average excess swelling (%).

where
k_p = coordination number of spheres and
R_2 = reduced neck radius.

Kinetics of Swelling

The dimensional changes of the spheres were also studied as a function of time. The following equation,[8] which is based on Fickian diffusion in spheres, can be used to describe such dynamic changes,

$$\frac{Q}{Q_\infty} = 1 - \exp\left(-\prod^2 \left(\frac{Dt}{r_0^2}\right)^n\right), \qquad (4)$$

where
Q = value of swelling at time t,
Q_∞ = value of swelling at equilibrium,
D = diffusion coefficient of the solvent in the polymer,
n = a dimensionless exponent, and
r_o = initial sphere radius.

RESULTS

The measured precision of the microscopy technique was high. The standard deviation of the volume of an individual sphere, as a percent of the mean, ranged from 0.10 to 0.50%, with a mean of 0.26%. If a 2σ rule is adopted, then the precision of this technique is better than 0.52% of total swelling.

Table I shows the percent swelling increase at equilibrium by the digital image technique and the gravimetric technique. The results have validated our concern that gravimetric methods will yield excess swelling. It is surprising that the excess value actually constitutes as much as 84% of the total weight gain.

Nitrogen BET interpenetration did not reveal the presence of any significant internal porosity. Additionally, the true density of the microspheres as measured by nitrogen micropycnometry exactly matched the theoretically predicted density of 1.19 g/cc. Therefore, it is unlikely there were significant voids within the spheres.

For the present polymeric system then, the null values of porosity ($\mathbf{V_p}$) and bubble volume ($\mathbf{V_d}$) led to the elimination of the first two terms of the model. Initial runs of a two parameter model involving both **a** and **b** indicated that the value of **a** was of the order 10^{-7} μm. A null value for **a** was assumed. True and bulk density measurements also show that the packing efficiency of the microspheres in this study was 38%, close to the 43% seen in simple cubic (SC) cells. This indicates that the actual packing of microspheres used in the gravimetric technique would be close to a simple cubic cell after centrifugation. The proposed model can then be reduced as follows:

$$Q_e = \frac{k_p b^2 (3r - 2b)}{4r^3} \qquad (5)$$

Finally, the value of the meniscus length, **b**, was determined using the radius and excess volume calculated for each condition. The parameter $\mathbf{k_p}$ was posited at 6, assuming simple cubic packing. Results are shown in Table II.

Based on the values of b, which was the half-length of the assumed flat meniscus, the reduced neck radius can be calculated by the following equation,

$$R_2 = \frac{\sqrt{r^2 - (r - b)^2}}{r} \qquad (6)$$

The value of $\mathbf{R_2}$ was then used to calculate $\mathbf{Q_e}$ by eq. (3). Results for the simple cubic case are given

Table II The Values of b Calculated for Different Coordination Numbers in Various pH Buffers

pH	Average Radius, r (μm)	Average Swelling (% volume)	b (HCP) (μm)	b (SC) (μm)
4.5	70.9	77.7	33.8	59.6
5.0	60.3	9.4	15.6	23.2
6.0	60.2	8.8	11.3	16.5
7.0	59.9	7.5	10.9	15.9

in Table III. A nearly perfect match between eqs. (2) and (3) was observed.

The kinetic data fit the Fickian model described in eq. (4) to within > 99% in all cases studied. From this we can conclude that the diffusion mechanism in the spheres we studied was, indeed, Fickian. An example curve is shown for a 9/90/1 (styrene/2-vinyl pyridine/divinylbenzene) copolymer at pH 5.0 in Figure 3.

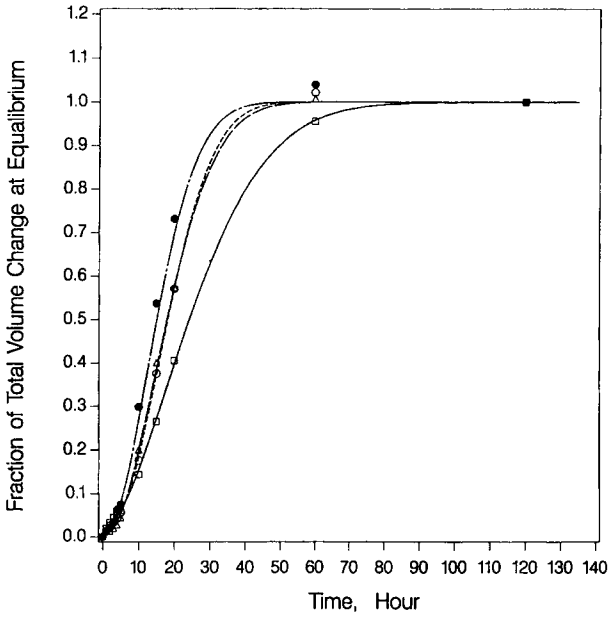

Figure 3 Kinetic data for 9/90/1 (styrene/2-vinylpyridine/divinyl benzene) spheres from the same lot in pH 5.0 buffer. The solid lines represent the Fickian model. The dots and squares represent actual data. Note the differences between the various spheres measured and the good agreement between the calculated and measured values.

DISCUSSION

The optical technique described here shows promise as a means of obtaining accurate and precise swelling measurements of spheres in a variety of sizes and solvent systems. Both equilibrium and kinetic data can be obtained with relative ease. Because single spheres are studied, this leads to the possibility that behavior normally hidden in the averaged ensembles studied by traditional techniques may be revealed. In addition, one can choose from a number of modifications of the technique.

In situations where very small spheres are being studied and a basement attachment membrane (we used polyurethane in this study) compatible with the swelling solvent is available, the sample preparation and technique described here is useful. In situations where larger spheres are being studied and an inverted microscope is available, a sample preparation utilizing a Petri dish with one microsphere in it is both simple and convenient. The sphere may be observed with an inverted microscope as it rests on the bottom of the Petri dish. This technique has the advantages of easy sample preparation and a large reservoir volume that doesn't need changing as does the reservoir in the closed cell preparation.

The modeling attempts have demonstrated that the difference seen between the digital imaging technique and Pepper's gravimetric technique for the measurement of swelling in microspheres is almost entirely due to liquid bridge formation. This liquid bridge volume cannot easily be corrected for and so leads to both inprecise and inaccurate measurements when low values of swelling are being measured via Pepper's technique. At very high degrees of swelling, the liquid bridge effect may, or may not be significant. In this study the swelling as measured by Pepper's technique due to liquid bridge volume ranged from 64 to 84%. Hence, the researcher should be aware of the errors inherent in these aggregate measurements.

Two models of liquid bridge formation were presented and compared to one another. These two models yield nearly identical results. The difference between the two models is greatest at high degrees of swelling [140% actual swelling vs. 107%, as shown by eq. (3)]. Presumably, this is where the assumption of a flat meniscus in eq. (2) breaks down.

Each of the two models for liquid bridge formation were derived from separate approaches. The model in eq. (2) was derived from a purely geometrical standpoint. The model distilled from the works of Kousaka in eq. (3) were derived from physical chemistry arguments. Their equivalence is not exact, as has been shown in the modeling data. This is to be expected, because a brief mathematical analysis shows that the two models are only equivalent when

Table III The Values Obtained for the Excess Volume Eqs. (2) and (3) Are Almost Exactly the Same

pH	Radius, r	b (μm)	R_2	Q_e (%)	Q_e [eq. (3)] (%)
4.5	70.9	59.6	0.99	1.40	1.07
5.0	60.3	23.2	0.79	0.50	0.44
6.0	60.2	16.5	0.69	0.28	0.25
7.0	59.9	15.6	0.68	0.26	0.24

$\mathbf{r_2}$ is equivalent to $\mathbf{4/3r}$—a physical impossibility. Each model, however, does aid in the visualization of the phenomenon of excess fluid retention during swelling experiments.

The kinetic model presented has shown that the kinetic behavior of these spheres is Fickian and easily modeled. This modeling attempt may be considered a case study in the kinetic measurement of swelling via digital imaging techniques.

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