POLYACROLEIN MICROSPHERES AS A NEW TOOL IN CELL BIOLOGY

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SUMMARY

Polyacrolein (PA) microspheres in sizes ranging from 0.04 μm to 40 μm were synthesized. Magnetic and fluorescent PA microspheres were formed by carrying out the polymerization process in the presence of appropriate ferrofluidic or fluorochromic compounds, respectively. The microspheres carry reactive aldehyde groups, through which various ligands, containing primary amino groups, were covalently bound at physiological pH values.

The potential use of these microspheres was demonstrated by the specific labelling of fresh human red blood cells (RBC) and by the separation of human RBC from turkey RBC by means of a magnetic field. PA microspheres were also bound covalently to the anti-allergic drug disodium chromoglycate (DSCG) and the conjugate was used for the labelling of rat basophilic leukaemia cells.

INTRODUCTION

Visualization and identification of specific sites on cell surfaces has great importance for the understanding of various biological phenomena, such as cell–cell recognition in development, cell communication, and differences between normal and tumour cells. There have been intensive efforts to find suitable techniques for labelling receptors on the cell surface. The best known involve the use of soluble fluorescent or radioactive antibodies or lectins (Pernis, Forni & Amante, 1970; Unanue, Perkins & Karnovsky, 1972; Yahara & Edelman, 1972). Other immunochemical methods make use of polystyrene lattices (Linthicum & Fell, 1975) or of biological macromolecules, such as ferritin (DePetris & Raff, 1973), haemocyanin (Smith & Ravel, 1972), viruses (Aoki et al. 1970) and peroxidase (Avrameas, 1970). However, all these techniques suffer from either lack of stability, restricted size or non-specific interactions. Recently, hydrophilic polymeric microspheres based on acrylic monomers were synthesized and used for cell labelling (Rembaum, Yen & Volkson, 1978a). These microspheres carried on their surfaces a variety of functional groups, such as carboxyl, hydroxyl, amide and/or pyridine groups. The functional groups were used for covalent binding of proteins to the microspheres by means of series of reactions (Molday, Dreyer, Rembaum & Yen, 1975). The last step of the microsphere derivatization technique, prior to antibody binding, consisted of a reaction with glutaraldehyde, designed to introduce reactive aldehyde groups. In order to simplify the derivatization procedure, polyglutaraldehyde (PGL) microspheres were synthesized, and were used for specific labelling of various types of cells (Rembaum, Margel & Levy, 1978b; Rembaum & Margel, 1978; Margel, Zisblatt & Rembaum, 1979).
In the present report, a novel synthesis of monodispersed polyacrolein (PA) microspheres and their potential use for cell labelling and cell separation are described (Margel et al. 1981). These microspheres were formed in sizes of 0.04 μm to 40 μm. Magnetic or fluorescent microspheres were formed by carrying out the polymerization reaction in the presence of ferrofluidic or fluorochromic compounds, respectively. The PA microspheres were designed to be hydrophilic and negatively charged, and to contain aldehyde groups.

Appropriate ligands, antibodies and drugs were covalently bound, at physiological pH values, to the microspheres through their aldehyde groups. The conjugates (microspheres-ligands) were used as a model for specific labelling of human red blood cells, for separating them from turkey red blood cells and for specific labelling of rat basophilic leukaemia cells.

MATERIALS AND METHODS

Reagents

The following materials were purchased from commercial sources: rabbit immunoglobulin (IgG) and goat anti-rabbit IgG (G × R IgG) (Miles Yeda, Rehovot, Israel), lyophilized rabbit anti-human red blood cell (R × H RBC) (Cappel Lab. Inc., Cochranville, PA), tetramethyl rhodamine isothiocyanate (Research Organics Inc., Cleveland, Ohio), polyethylene oxide, M, 100 000 (Polysciences, Warrington, PA), cyanogen bromide, 2,4-dinitrophenyl-hydrazine, thioglycerol, sodium monophosphate, sodium diphosphate, 2-aminoethyl sulphuric acid, glutaraldehyde, acrolein, sodium hydrosulphite, glucose and hydroxylamine hydrochloride (Aldrich, San Leandro, CA), Ferrofluid (Ferrofluidics, Burlington, MA, no. A01; 5 %, w/v), Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). The drug disodium chromoglycate (DSCG) and its amino derivative was a kind gift from Dr I. Pecht, Rehovot, Israel. Acrolein was distilled at atmospheric pressure before use. PGL was obtained by polymerizing glutaraldehyde at pH 11-0 (Margel & Rembaum, 1980).

Synthesis of the surfactant PGL-NaHSO₃

This surfactant was prepared by the reaction of polyglutaraldehyde and sodium hydrogen sulphite (NaHSO₃), as follows: 12.5 g NaHSO₃ was dissolved in 30 ml H₂O; 5.0 g of PGL was then added and the solution was stirred until all the PGL was dissolved. The solution was dialysed extensively against H₂O and then lyophilized.

Synthesis of PA microspheres

PA microspheres were prepared in various ways. A description of the anionic and radiation procedures follows.

Anionic polymerization. NaOH (0.2 M) was added dropwise to an aqueous solution containing 8 % (w/v) acrolein and 0.5 % (w/v) of the surfactant PGL-NaHSO₃ until a pH of 10.5 was reached. The reaction continued for 2 h and the mixture was then dialysed extensively against distilled water and centrifuged 4 times at 2000 g for 20 min. The PA microspheres of average diameter 0.1 μm, as determined by scanning electron microscopy (SEM), could be redispersed in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) or in distilled water. By varying the concentration of the surfactant, acrolein, cosolvent or pH of the polymerization the size of the microspheres could be altered in a predictable way. Fluorescent microspheres were obtained by carrying out the polymerization in the presence of approximately 0.01 % (w/v) of an appropriate fluorochromic compound, e.g. tetramethyl rhodamine isothiocyanate (0.008 %, w/v). The latter compound was dissolved in an excess of ethylene diamine in a weight ratio of 1:20.

Magnetic PA microspheres were obtained by the addition of Ferrofluid (5 %, w/v) to the
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initial reaction mixture and then polymerizing it. The purification of the magnetic microspheres consisted of dialysis against distilled water and separation from diamagnetic impurities by means of a permanent magnet.

Radiation polymerization

An aqueous solution containing 9% (w/v) acrolein and 0.5% (w/v) polyethylene oxide as surfactant was deaerated with argon and then irradiated with a cobalt source (1 megarad). The microspheres produced (0.15 μm) were dialysed against distilled water, and washed by centrifugation 4 times at 2000 g for 30 min. Fluorescent microspheres were obtained by carrying out this polymerization in the presence of the desired fluorochromic compound, e.g. tetramethyl rhodamine isothiocyanate, fluorescein isothiocyanate or 9-aminoacridine.

Determination of aldehyde groups

The aldehyde content was determined as the percentage of nitrogen resulting from the oxime prepared by the heterogeneous reaction of PA with aqueous hydroxylamine hydrochloride solution (Sloan, Hofreiter, Mellies & Wolff, 1956; Borchert, 1961). Fifty mg of PA was stirred at room temperature for 24 h with 500 mg of hydroxylamine hydrochloride. The polymer was then filtered, washed several times with water and dried under vacuum at room temperature.

Synthesis of 2-aminoethyl disodium chromoglycate

The amino derivative of the drug DSCG had to be prepared to bind the drug covalently to the aldehyde groups of the PA microspheres. The amino-DSCG derivative (2-aminoethyl DSCG) used was synthesized by shaking DSCG with an excess of 2-aminoethyl sulphuric acid for 24 h at pH 9.0. The reaction proceeds as follows:

\[ \text{NaOOC} \text{O} \text{O} - \text{CH} - \text{CH} - \text{CH}_2 - \text{O} \rightarrow \text{NH}_2 \text{CH} - \text{CH} - \text{SO}_2 \text{H} \]

2-aminoethyl sulphuric acid

The solution was then acidified with HCl solution (pH 3.0) and the acid form of amino-DSCG precipitated was washed several times with HCl solution at pH 3.0. The sodium salt of the drug was obtained by dissolving the acid form of amino-DSCG in distilled water.

Purifications of G x R IgG

The antiserum was purified according to a procedure described previously (Cuatrecasas, 1970). The G x R IgG was bound to a column prepared by coupling rabbit IgG to Sepharose 4B. The bound G x R IgG was eluted from the column with glycine hydrochloride buffer (0.2 M,
The pH was adjusted to neutrality with 1 M-NaOH and any denaturated antibody was removed by centrifugation at 1500 g for 20 min. It was then dialysed against PBS at 4 °C and stored at −20 °C.

### Preparation of human and turkey RBC

Fresh human RBC were obtained by centrifuging fresh blood of a normal donor at 500 g for 10 min. The separated RBC were then washed with PBS by spinning the cells suspension four times at 500 g for 10 min. Turkey RBC were obtained by a similar procedure.

### Labelling of human RBC

PA microspheres were shaken for 2 h, at 4 °C, with purified G × R IgG (1 mg microspheres, 0.1 mg G × R IgG in a total volume of 0.15 ml PBS). Unbound antibody was then separated in two ways: (1) for microspheres of sizes larger than 0.6 μm, by spinning the microspheres suspension four times at 750 g for 20 min; (2) for microspheres of smaller size, by passing the suspension through a Sepharose 4B column, and monitoring the separation spectrophotometrically at 280 nm. The free aldehyde groups of the conjugate, microspheres-antibody, were quenched with 2 % (w/v) bovine serum albumin (BSA) solution for several hours at 4 °C.

Fresh human RBC were shaken for 50 min at 4 °C with R × H RBC (10⁶ human RBC with 0.8 μg R × H RBC in 0.1 ml PBS solution). The sensitized cells were separated and washed four times by spinning the cells suspension at 4 °C for 1 h with various concentrations of G × R IgG derivatized microspheres. The RBC were separated from unreacted derivatized microspheres by centrifugation three times at 500 g for 10 min. The labelled cells were resuspended in PBS and were examined in light and fluorescence microscopy, and with a fluorescence-activated cell sorter (FACS-II (Becton-Dickinson), photomultiplier 500 V, filters 550 nm).

Two types of control experiments were used and showed similar results. The first type consisted of G × R IgG derivatized microspheres and non-sensitized human RBC. The second control consisted of G × R IgG derivatized microspheres and human RBC pre-treated with rabbit serum. (10⁶ RBC were shaken for 30 min at 4 °C with 0.01 ml rabbit serum in 0.1 ml PBS. The cells were then separated and washed 4 times by spinning the cells suspension at 500 g for 10 min.)

### Separation of turkey RBC from human RBC

A mixture containing 10⁶ human RBC and 10⁶ turkey RBC was treated with magnetic microspheres by using the labelling procedure described above. A small magnet was then fitted on the outside wall of a vial containing 5 ml PBS solution of the mixture of cells. After 10 min, cells that were not attracted to the wall were isolated. The attracted cells were resuspended in PBS and the magnetic separation was repeated twice. The efficiency of the separation was then examined by light microscopy.

### Labelling of rat basophilic leukaemia cells

Fluorescent PA microspheres (0.1 μm size) were shaken for 12 h at room temperature with the amino-DSCG drug (1 mg microspheres, 0.5 mg 2-aminoethyl DSCG in a total volume of 0.15 ml H₂O). The free aldehyde groups that remained were quenched with thioglycerol (4 mg) for 2 h at room temperature. Thereafter, unbound antibody and excess of thioglycerol were separated by passing the microsphere suspension through a column of Sepharose 4B.

Rat basophilic leukaemia cells, 2H₃ (10⁶ cells in 0.3 ml Tyrode solution (Mazurek, Berger & Pecht, 1980) containing 0.5 % (w/v) BSA), were shaken for 1 h at room temperature with various concentrations of the microsphere suspension. The labelled cells were separated from unreacted microspheres by centrifugation four times at 400 g for 10 min, resuspended in Tyrode solution and were examined by light and fluorescence microscopy, and with the FACS.

The control used in each experiment consisted of microspheres that were not bound to the drug and rat basophilic leukaemia cells.
Fig. 1. SEM photomicrographs of PA microspheres of various sizes. A, 0.2 μm; B, 0.4 μm; C, 0.8 μm; D, 1.9 μm; E, 2.7 μm; F, 4.2 μm.
RESULTS AND DISCUSSION

Polyacrolein powder as well as microspheres were formed in aqueous media by polymerizing acrolein by either anionic or radicalic (radiation) mechanisms. In the radicalic mechanism acrolein polymerized through its double bond to yield a polymer with the following ideal structure, \((\text{CH}_2\text{-CH(CHO)})_n\) (Schulz, 1967). The anionic mechanism for the polymerization is more complicated and related research is presently under way. However, the anionic microspheres obtained were found to be the most suitable for cell labelling, since they fulfil the essential conditions necessary for specific cell labelling; hydrophilicity and negative charge (Rembaum & Yen, 1979).

![Graph showing effect of pH on size of PA microspheres.](image)

Fig. 2. Effect of pH on the size of PA microspheres. Acrolein, 15% (w/v); PGL-NaHSO\(_3\), 0.5% (w/v); 23 °C.

Fig. 1 presents SEM photomicrographs of monodispersed PA microspheres in various sizes, obtained by anionic polymerization. Figs. 2-4 illustrate the size variation of PA microspheres as a function of pH, surfactant and acrolein concentration, respectively. This behaviour is in agreement with results obtained previously (Rembaum et al. 1978a; Margel et al. 1979).

The presence of aldehyde groups was ascertained by several methods. The infrared spectra indicate absorption bands at 1720 cm\(^{-1}\) and 2740 cm\(^{-1}\) due to the stretching of non-conjugate aldehyde and CH of aldehyde groups, respectively. Additional evidence for the presence of aldehyde groups was shown by means of 2,4-dinitrophenylhydrazine, which yields yellow microspheres. Quantitatively, the aldehyde content of PA was determined by nitrogen analysis of the product resulting from the interaction of PA with hydroxylamine hydrochloride (Table 1). The results of the
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nitrogen analysis show that the number of aldehyde groups decreases as the pH at which the polymerization is carried out increases. This can be explained by the Cannizzaro reaction, which occurs under basic conditions between each two aldehyde groups to yield a carboxyl group and a hydroxyl group (Schulz, 1962). Further evidence for the occurrence of the Cannizzaro reaction is the asymmetric stretching band at 1600 cm⁻¹ of the carboxylate group obtained in the infrared spectra, and the
Table 1. Nitrogen analysis of the products obtained by the reaction of PA prepared at different pH with hydroxylamine hydrochloride

<table>
<thead>
<tr>
<th>pH</th>
<th>% N in reaction product</th>
<th>No. of aldehyde groups /g (x 10^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0†</td>
<td>15.4</td>
<td>7.0</td>
</tr>
<tr>
<td>10.0</td>
<td>4.6</td>
<td>2.1</td>
</tr>
<tr>
<td>10.5‡</td>
<td>4.4</td>
<td>2.0</td>
</tr>
<tr>
<td>11.0</td>
<td>4.2</td>
<td>1.9</td>
</tr>
<tr>
<td>12.7</td>
<td>2.5</td>
<td>1.1</td>
</tr>
<tr>
<td>13.5</td>
<td>1.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Corrected by reducing % N found before the reaction.
† Radiation polymerization.
‡ PA microspheres.

observed increased water solubility of PA synthesized at high pH, e.g. at pH above 13.5 a water-soluble PA is obtained.

The labelling of cell surface receptors by means of PA microspheres was found to be simple and efficient as evidenced by numerous tests using human RBC and rat basophilic leukaemia cells. The labelling procedure was based on the possibility of distinguishing between cell subpopulations that have different receptors on their surfaces. Fig. 5 presents the direct and the indirect procedure employed for the labelling. In the direct procedure the guided microsphere, i.e. a microsphere to which a specific ligand is covalently bound, selects its target site and binds to it (Fig. 5A), and in the indirect method (Fig. 5B) an intermediate ligand is employed.

In the labelling of RBC the indirect procedure was applied, ligand 1 was G × R IgG and ligand 2 was R × H RBC. The labelling was carried out with various sizes of PA microspheres. Fig. 6 demonstrates the results obtained by using 4 μm microspheres. The diameter of these microspheres is close to that of the cells and therefore each cell can be labelled with a few microspheres only. Specific labelling of the cells was obtained when the ratio, no. of microspheres/no. of cells, was 0.6. In these optimal

Fig. 5. Schematic representation of direct (A) and indirect (B) labelling of cells.
Fig. 6. Photomicrograph of human RBC labelled with various concentrations of PA micropHERs of 4 μm size. x 420. 5 × 10^8 RBC labelled with 3 × 10^9 micropHERs: A, control cells; B, experimental cells.

Fig. 7. Photomicrograph of human RBC labelled with 5 × 10^9 micropHERs: A, control cells; B, experimental cells.
Fig. 7. Fluorescence and phase micrographs of labelled human RBC with 0.6 μm fluorescent PA microspheres. ×1200. a, Phase picture of control cells; b, phase picture of the experimental cells; c, fluorescence picture of the experimental cells.
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Fig. 8. Fluorescence histograms obtained with the FACS showing human RBC (10^6 cells) labelled with 0.1 μm fluorescent PA microspheres (10^11 microspheres). Curve 1, fresh human RBC; curve 2, control cells; curve 3, experimental cells.

Table 2. Labelling of human RBC with various concentrations of fluorescent microspheres monitored by the FACS

<table>
<thead>
<tr>
<th>No. of microspheres</th>
<th>Treatment</th>
<th>Fluorescent cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>4.0*</td>
</tr>
<tr>
<td>1.0 x 10^11</td>
<td>Control</td>
<td>4.4</td>
</tr>
<tr>
<td>1.0 x 10^11</td>
<td>Experiment</td>
<td>60</td>
</tr>
<tr>
<td>2.0 x 10^11</td>
<td>Control</td>
<td>5.0</td>
</tr>
<tr>
<td>2.0 x 10^11</td>
<td>Experiment</td>
<td>60</td>
</tr>
<tr>
<td>4.0 x 10^11</td>
<td>Control</td>
<td>5.0</td>
</tr>
<tr>
<td>4.0 x 10^11</td>
<td>Experiment</td>
<td>89</td>
</tr>
<tr>
<td>1.0 x 10^12</td>
<td>Control</td>
<td>6.5</td>
</tr>
<tr>
<td>1.0 x 10^12</td>
<td>Experiment</td>
<td>97</td>
</tr>
</tbody>
</table>

* Autofluorescence of fresh RBC.
Fig. 9. SEM photomicrographs of labelled human RBC in a mixture of human RBC and turkey RBC.

A, x 4000; B, x 7500.
conditions the control cells (Fig. 6A) do not bind to the microspheres at all, while the experimental cells (Fig. 6B) form small aggregates with the microspheres, indicating their specific labelling. Under the same conditions, but in a ratio: no. of microspheres/no. of cells = 10, the experimental cells form large aggregates (Fig. 6B') while the control cells form small ones (Fig. 6A'), indicating some non-specific adherence between the cells and the microspheres. Fig. 7 illustrates the results obtained by labelling human RBC with 0.6 μm fluorescent PA microspheres. The control experiment (Fig. 7A) shows very few microspheres attached to the cells, while the labelled cells (Fig. 7B) are covered completely by microspheres that show intense fluorescence (Fig. 7C). Visualization of cell receptors with 0.1 μm beads is achieved using
microspheres with fluorescent properties. The degree of the specificity of the \(0.1 \mu m\) fluorescent PA microspheres towards RBC was measured with the FACS (Fig. 8). Fig. 8, curve 1 represents the autofluorescence histogram of fresh human RBC. The fluorescent properties of the control cells (Fig. 8, curve 2) are similar and due mainly to their autofluorescence. On the other hand, the experimental cells (Fig. 8, curve 3) show much higher fluorescence, indicating their labelling with fluorescent microspheres. Table 2 presents the percentage of fluorescent cells obtained by labelling fresh human RBC with increasing concentrations of microspheres; 4% of untreated fresh human RBC were found to be fluorescent due to their autofluorescence. The number of experimental cells becoming fluorescent increased by increasing the concentration of microspheres, e.g. in the presence of \(1.0 \times 10^{11}\) microspheres per \(10^6\) RBC 60% of the cells were found to be fluorescent, while in the presence of \(1.0 \times 10^{12}\) microspheres per \(10^6\) cells 97% of the cells were fluorescent. In contrast, the percentage of fluorescent control cells was very low and did not increase significantly by increasing the concentration of microspheres. The high specificity of the \(0.1 \mu m\) PA microspheres was also shown by specifically labelling human RBC in a mixture containing human RBC and turkey RBC. The SEM photomicrograph describing this experiment (Fig. 9) shows labelling of the human RBC and no labelling at all of the turkey RBC. The fluorescence and phase micrograph shown in Fig. 10 provides further evidence for the high specificity of these microspheres towards RBC.

In order to illustrate the potential use of magnetic PA microspheres the same system including a mixture of human RBC \((10^6)\) and turkey RBC \((10^6)\) was applied. The separation of labelled human RBC from unlabelled turkey RBC by means of a magnetic field was found to be very simple and effective; approximately 95% of the cells attracted to the magnet were human RBC. This approach to cell separation with magnetic microspheres may be very promising and further research is needed.

Polymeric microspheres can also be bound to various drugs in order to study the mechanism of their action on certain membrane receptors. The potential use of PA microspheres for this purpose was illustrated by labelling rat basophilic leukaemia cells with the conjugate, disodium chromoglycate-PA microspheres. DSCG is an anti-allergic drug that has been shown to have specific binding site on the membranes of mast cells and basophils through which its pharmacological activity is expressed (Mazurek et al. 1980). DSCG has been found to act by inhibiting the degranulation and secretion processes of mast cells and basophils. Accumulated evidence suggests that this inhibition takes place by blocking the calcium influx into the cells (Foreman & Garland, 1976; Mazurek et al. 1980). DSCG is a chelating drug that forms a complex with the calcium ion. This complex, drug-Ca\(^{2+}\), acts on the cells by forming a ternary complex, drug-Ca\(^{2+}\)-receptor, while the Ca\(^{2+}\) gates in the membrane are part of the receptor. The formation of this ternary complex prevents the Ca\(^{2+}\) influx and hence the degranulation-secretion events are inhibited. Visualization of Ca\(^{2+}\) gates on mast cells and basophils was achieved by means of fluorescent polyglutaraldehyde microspheres (Mazurek et al. 1980). In the present research PA microspheres were also checked for their potential use as specific markers for mapping Ca\(^{2+}\) gates on rat
Fig. 11. Fluorescence histograms obtained with the FACS showing rat basophilic leukaemia cells (10⁶) labelled with various concentrations of the conjugate: DSCG-fluorescent PA microspheres. Curve 1, fluorescence histograms of fresh cells; curve 2, fluorescence histograms of the control cells; curve 3, fluorescence histograms of the experimental cells. A. Labelling with 2.9 x 10¹¹ microspheres; B, labelling with 4.0 x 10¹¹ microspheres; C, labelling with 1.0 x 10¹¹ microspheres.
basophilic leukaemia cells. Fig. 11 illustrates the FACS results obtained by labelling $10^8$ cells in Tyrode medium with various amounts of 0.1 μm fluorescent PA microspheres. Specific labelling was obtained when $2.0 \times 10^{11}$ microspheres were used during the labelling procedure. Curve 1 in Fig. 11A presents the autofluorescence histogram of fresh rat basophilic leukaemia cells. The fluorescent properties of the control cells (curve 2, Fig. 11A) are similar and due mainly to their autofluorescence.

In contrast, the histogram of the experimental cells (curve 3, Fig. 11A) indicates higher fluorescence, which is due mainly to their specific labelling with the fluorescent microspheres. Fig. 11B presents the results obtained by using $4.0 \times 10^{11}$ microspheres in the labelling process. The histogram of the control cells (curve 2, Fig. 11B) indicates some non-specific labelling but still the histogram of the experimental cells (curve 3,
Fig. 11 B) shows much higher fluorescence, indicating the heavier labelling of the cells with fluorescent microspheres. Labelling of the cells with a higher concentration of microspheres ($1 \times 10^{18}$) resulted in higher non-specific labelling, as shown in Fig. 11 C. The histogram of the control cells (curve 2, Fig. 11 C) is similar to that of the experimental cells (curve 3, Fig. 11 C), indicating non-specific labelling with approximately the same amount of fluorescent microspheres. Fig. 12 illustrates fluorescence and phase micrograph results obtained by using the optimal conditions necessary for specific labelling of rat basophilic leukaemia cells. In the fluorescence mode the control cells do not appear, while the experimental cells do; indicating the specific labelling of the cells with the fluorescent microspheres.

CONCLUSIONS

A novel synthesis of monodispersed PA microspheres is described. The microspheres obtained by anionic polymerization fulfil the necessary conditions for specific cell labelling: hydrophilicity and negative charge. However, the microspheres formed by radiation polymerization contain approximately four times more aldehyde groups per unit weight (Table 1); therefore, for applications that do not require high specificity such as enzyme immobilization the radiation microspheres may be preferred. Research that may improve the specificity properties of the radiation microspheres is being carried out in our laboratory.

PA microspheres as well as PGL contain high concentrations of aldehyde groups on their surface. The aldehyde groups permit covalent binding with antibodies, enzymes and other proteins in a single step. Therefore, these microspheres may eliminate the intermediate steps used previously, in which the cyanogen bromide and carbodiimide reaction were used (Molday et al. 1975). PGL microspheres have several disadvantages such as the instability of microspheres in sizes larger than 0.7 µm and the instability of the magnetic microspheres in PBS solution. The PA microspheres do not have these disadvantages and they can be considered as more advanced polyaldehyde microspheres.

The control that one has on synthesizing PA microspheres with a variety of fluorescent and magnetic properties, along with their size and mechanical characteristics, makes them a very useful tool in biology. In the present paper the potential use of these microspheres for specific cell labelling and cell separation has been demonstrated; it shows that sometimes optimal conditions have to be found in order to prevent non-specific interaction between cells and microspheres. PA microspheres may also be used in some other potential clinical applications such as diagnostic tests (Malin & Edwards, 1972) and phagocytosis (Palzer, Walton & Rembaum, 1978). Special attention may also be given to the magnetic microspheres and their potential use for cell separation by means of a magnetic sorter, drug delivery (Widder, Senyei & Scarpelli, 1978), immobilized enzymes (Munro, Dunnill & Lilly, 1977) and enzyme immunoassay (Guesdon, Thierry & Avrameas, 1978).

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