THE USE OF POLYMER SORBENTS IN RADIOIMMUNOASSAY

Antonín MIKAN[a] and Jiří ČOUPEK[b]

[a] *Agricultural Cooperative, 763 15 Slušovice*
[b] *Tessek Ltd., Křižovnická 3, 110 00 Prague 1*

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Several polymer sorbents were tested for use in radioimmunoassay. Separon EDMA, particle size 5—12 μm was found to have optimal properties. Its suspension behaves almost as a solution, pipette fouling never occurs. It can be centrifuged within one minute, thus eliminating the need for a refrigerated centrifuge. Assay results are the same as ith Norit A, but more reliable.

Radioimmunoassay of progesterone levels in milk is a powerful tool for monitoring the effectiveness of fertility control measures in cattle breeding[1]. The principle of the assay is the competition between ^{125}I-labeled and unlabeled (standard or endogenous) progesterone for binding sites on an antibody. Unbound progesterone is then removed by adsorption on a suitable sorbent. The supernatant activity, which is proportional to the amount of antibody-bound labeled progesterone, is then measured and evaluated. The uneven partitioning of progesterone between the aqueous and fat phases of milk samples is a source of problems in the assay[2]. It is possible to centrifuge the samples to get rid of the fat and to perform the assay in fat-free milk[3], but the progesteorne concentrations in fat-free milk are several times lower than in full-fat milk. This is why the relative standard deviation is higher and a systematic shifting in the course of the analysis becomes more significant, often rendering the results useless[4]. Since recent separation techniques, such as coated tubes or second antibody, are unavailable to many laboratories, sorbent separation continues to be the method of choice. Charcoal is widely used but there is sometimes a certain degree of imprecision in pipetting due to the coarseness of the particles. We were prompted, therefore, to search for a more suitable sorbent. The first step was the selection of a convenient sorbent with good hydro-lipophilic properties and a slow sedimentation rate in the reaction medium under conditions of natural gravity. Separon SE, poly(styrene-*co*-ethylene dimethacrylate) (35 : 65 mole %), particle size <10 μm, due to its low polarity, formed floating aggregates that did not settle during centrifuging. Separon SE, particle size 20—24 μm, and Separon EDMA, poly(ethylene dimethacrylate), particle size 15—19 μm, were too coarse and settled very quickly prior to centrifuging. The only sorbent with convenient properties proved to be Separon EDMA, particle size 5—12 μm. This sorbent was compared to Norit A.

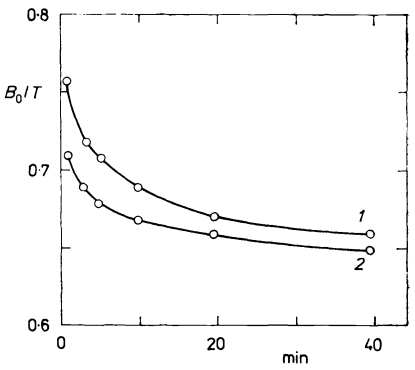

FIG. 1

Activity adsorption kinetics (0·5% (w/w) suspension of sorbents): *1* Separon EDMA, *2* Norit A

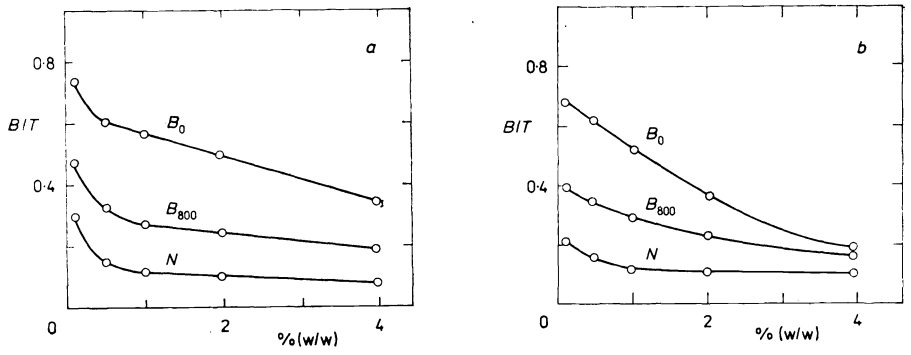

FIG. 2

Relative bound B/T (B is specified at curves) and nonspecific N/T activity as a function sorbent suspension concentration: *a* Norit A, *b* Separon EDMA

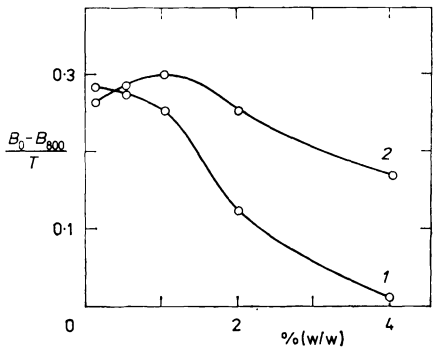

FIG. 3

Difference in activity between 0 and 800 pg progesterone per test tube standards as a function of Separon EDMA (*1*) and Norit A (*2*) suspension concentration

Adsorption kinetics were studied using a 0·5% (w/w) suspension of Separon EDMA, particle size 5–12 μm, and Norit A (Fig. 1). B_0, B_{800}, and N indicate the activity of supernatant from the test samples without unlabeled progesterone, with 800 pg of unlabeled progesterone per test tube, and without both unlabeled progesterone and antiserum, respectively. T, the so called total activity, is the activity of a 500 μl aliquot from the probes into which only buffer instead of a suspension of sorbent was added. Activity decreased rapidly during the first minutes of separation, corresponding to the removal of bound activity from weak binding sites. The stripping of weak binding sites generally leads to an improvement in the specificity of the assay and finishes after approximately twenty minutes for both sorbents. No difference in the speed of adsorption between Norit A and Separon EDMA was found.

The ratios between bound or non-specific activity and total activity versus concentration of sorbent are plotted in Fig. 2. The supernatant activity at lower concentrations (less than 0·5%) of sorbents is higher due to incomplete adsorption of free activity, as shown by the non-specific activity curves. This phenomenon is more pronounced in the case of Norit A than Separon EDMA. The gradually decreasing activity of both B_0 and B_{800} is caused by the adsorption of bound activity at higher concentrations of sorbents. The decrease is larger in Separon EDMA than in Norit A.

For the assay, the difference between B_0 and B_{800} should be as high as possible while the non-specific activity, N, should be kept reasonably low. It may be inferred from Figs 2 and 3 that a compromise to meet both conditions can be reached in the 0·5%–1% concentration range for both sorbents.

Real samples of milk were analyzed with Norit A and Separon EDMA simultaneously. The differences between the results were subjected to the statistical analysis by Student's t-test for paired values. At a confidence level of 95%, no significant differences were found between the two methods of separation.

The major advantage of Separon EDMA is its spherical shape together with a narrow particle size distribution. Its suspension behaves almost as a solution. It is easy to handle and pipette fouling never occurs. Although it does not settle quickly under conditions of natural gravity, it can be completely centrifuged within one minute, thus eliminating the need for a refrigerated centrifuge. In the laboratory of Agricultural Cooperative Slušovice, several years of trouble-free routine employment of Separon EDMA has confirmed the conclusions of this work.

EXPERIMENTAL

Reagents

Progesterone standard, ^{125}I-progesterone and antiserum were supplied by JZD Potěhy, Czechoslovakia. Progesterone-free milk was obtained from cows in the oestrus phase. Solutions were made in 0·05M phosphate buffer, pH 7·5, unless otherwise stated. Sorbents: Norit A by SERVA,

Separon SE (spec. surface 70 $m^2 g^{-1}$; particle size <6, <10, and 20—24 μm) and Separon EDMA (300 $m^2 g^{-1}$; particle size 5—12 and 15—19 μm) both by TESSEK Ltd., Czechoslovakia.

The standard of 40 ng progesterone per ml of milk (800 pg per test tube in the assay) was prepared from a stock solution (1 μg progesterone per ml of methanol). A solution of ^{125}I--progesterone in buffer was prepared for an activity of 20 000 c.p.m. per 100 μl of the solution. The concentration of antiserum solution in buffer was maintained to a sufficiently high level so as to bind at least 50% of total activity in the absence of unlabeled progesterone.

Procedure

An amount of 0·5 ml of milk (progesterone-free or containing 40 ng of progesterone per ml) was diluted with 2 ml of phosphate buffer at 40°C and 100 μl of diluted milk was transferred to a polystyrene test tube and cooled to 4°C. Then 100 μl solution of ^{125}I-labeled progesterone and 100 μl of buffer with or without antiserum was added. The resulting mixture was incubated overnight in a refrigerator at 4°C. The following day, 500 μl of suspension of sorbent was pipetted while stirring thoroughly. Pure buffer instead of sorbent suspension was pipetted into the test tubes for measurement of the total activity. After twenty minutes all were centrifuged at a temperature of 4°C for ten minutes at 3 000 r.p.m. The activity of 500 μl of supernatant was measured representing the bound or non-specific activity, while 500 μl aliquot of the solution without sorbent represented total activity. A ^{125}I RIA counter with 16 wells produced by Radioecology and Nuclear Technology Institute, Košice, Czechoslovakia was used for measurement. All samples were carried out in duplicate.

REFERENCES

1. Lamming G. E., Bulman D. C.: Br. Vet. J. *132*, 507 (1976).
2. Pennington J. A., Sphar S. L., Lodge J. R.: Br. Vet. J. *132*, 487 (1976).
3. Pope G. S., Majzlik I., Ball P. J. H., Leaver J. D.: Br. Vet. *J.* 132, 497 (1976).
4. Mikan A., Mikan V.: Agrochemia (Bratislava) *25*, 183 (1985).

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