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# Nycodenz: A New Nonionic Iodinated Gradient Medium

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The properties of Nycodenz, a new nonionic iodinated density gradient medium, are described. This compound shares a number of characteristics with its related compound metrizamide. However, the stabler, more inert nature of Nycodenz endows it with a number of favorable properties as a gradient medium.

The introduction of metrizamide in 1973 not only allowed improved fractionations of particles previously separated in sucrose and ionic iodinated gradient media but also allowed one to carry out fractionations which were previously impossible. The applications of metrizamide are extremely diverse and have been reviewed in detail previously (1,2). In spite of its unique characteristics which made it a very useful gradient medium, it also presented some problems in that metrizamide solutions decompose when autoclaved and the presence of a sugar structure causes inteference in some chemical (3) and enzymatic assays (26).

In this report we describe the properties of a new nonionic iodinated gradient medium with the systematic name 5-(*N*-2,3-dihydroxypropylacetamido) - 2,4,6 - triiodo - *N*,*N*′ - bis(2,3 - dihydroxypropyl)-isophthalamide commercially available under the trade name of Nycodenz. Solutions of this gradient medium are stable to autoclaving and Nycodenz not only appears to interfere less with a number of assay techniques but also appears to be much less toxic to cells than other iodinated gradient media (4).

## MATERIALS AND METHODS

Nycodenz, systematic name 5-(*N*-2,3-dihydroxypropylacetamido) - 2,4,6 - triiodo - *N*,*N*′ - bis(2,3 - dihydroxypropyl)isophthalamide, and metrizamide were generously donated by Nyegaard & Company A/S (Oslo). All other chemicals were of analytical grade. Isotopically labeled compounds were purchased from Amersham International (Amersham, Bucks). Scintillation fluids were either prepared as described earlier (5,6) or purchased from commercial sources as described in the text.

The absorption spectra of Nycodenz solutions were measured using a Pye–Unicam SP 1800 spectrophotometer and osmolalities were measured using an MSE Advanced Digimatic osmometer Model 3DII.

All centrifugation runs were carried out on an MSE Pegasus DP ultracentrifuge in standard rotors as described in the text. At the end of all runs the rotors were allowed to coast to a halt. Gradients were fractionated by upward displacement with Maxidens (Nyegaard & Co. A/S) using an MSE gradient extractor; for the vertical rotor gradients a modified unloader cap was used to allow the gradients to be unloaded through the center of the tube cap (details on application). The density of fractions was deduced from their refractive indices measured using an Abbé refractometer (Bellingham & Stanley Ltd., London).

Isotopic quenching studies were carried out using duplicate samples of a minimum



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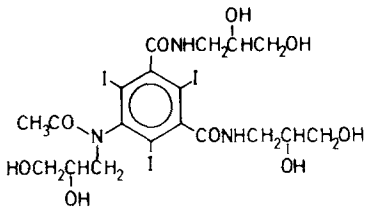

FIG. 1. Structure of Nycodenz.

of 0.1 μCi of isotopically labeled [$^{32}$P]orthophosphate, $^{14}$C-labeled acetate and glycogen, and $^{3}$H-labeled thymidine made up to 0.5 ml with either water (control samples) or Nycodenz solutions to give the required final concentration of 20% (w/v). Water-miscible scintillation fluid (4.5 ml) was added to each vial and the radioactivity of each vial was measured in a Packard 460C scintillation counter.

For assessing whether Nycodenz interfered with chemical assays solutions of Nycodenz were mixed with the reagents for assaying proteins (7–10), RNA (11), DNA (11,12), and sugars (13,14). The absorbance of each assay mixture was measured at the appropriate wavelength using an SP 1800 spectrophotometer. In parallel assays it was found that Nycodenz did not affect the linearity of the standard curve in any case.

The activities of the following marker enzymes in rat liver homogenates or subcellular fractions were assayed: $Mg^{2+}$-ATPase, $Na^+/K^+$-ATPase, 5′-nucleotidase (15), adenyl cyclase (16), NADPH-cytochrome *c* reductase (17), malate dehydrogenase (18), glucose-6-phosphatase (19), acid phosphatase (20), and catalase (21). Succinate dehydrogenase was measured by a modification of an earlier method (22) in that the reaction was terminated by the addition of a 0.1 vol of sodium dodecyl sulfate.

## RESULTS

### *Properties of Nycodenz*

Nycodenz is an off-white solid with a melting point of 174–180°C. The structure of Nycodenz is shown in Fig. 1. Nycodenz dissolves readily in aqueous solutions and the absorption spectrum of a solution of 0.05 mg/ml is shown in Fig. 2. The absorption spectrum shows a maximum at 244 nm and an $E^{244\ nm}_{1\%1\ cm}$ of 342. The physicochemical properties of Nycodenz solutions are given in Table 1. At all densities Nycodenz solutions are marginally less viscous than metrizamide solutions and much less viscous than sucrose solutions. As expected the refractive index of solutions increases linearly with concentration and the osmolality also increases approximately linearly up to 60% (w/v). From the data in Table 1 the following relationship can be deduced:

TABLE 1

PROPERTIES OF NYCODENZ SOLUTIONS

| Percentage concentration (w/v) | Molar concentration | Refractive index ($\eta_{20°C}$) | Density (g/ml) at 20°C | Osmolality (mOsm) | Viscosity (cP) at 20°C |
|---|---|---|---|---|---|
| 0 | 0 | 1.3330 | 0.999 | 0 | 1.0 |
| 10 | 0.122 | 1.3494 | 1.052 | 112 | 1.3 |
| 20 | 0.244 | 1.3659 | 1.105 | 211 | 1.5 |
| 30 | 0.365 | 1.3824 | 1.159 | 299 | 1.8 |
| 40 | 0.487 | 1.3988 | 1.212 | 388 | 3.2 |
| 50 | 0.609 | 1.4153 | 1.265 | 485 | 5.3 |
| 60 | 0.731 | 1.4318 | 1.319 | 595 | 9.5 |
| 70 | 0.853 | 1.4482 | 1.372 | 1045 | 17.2 |
| 80 | 0.974 | 1.4647 | 1.426 | — | 30.0 |

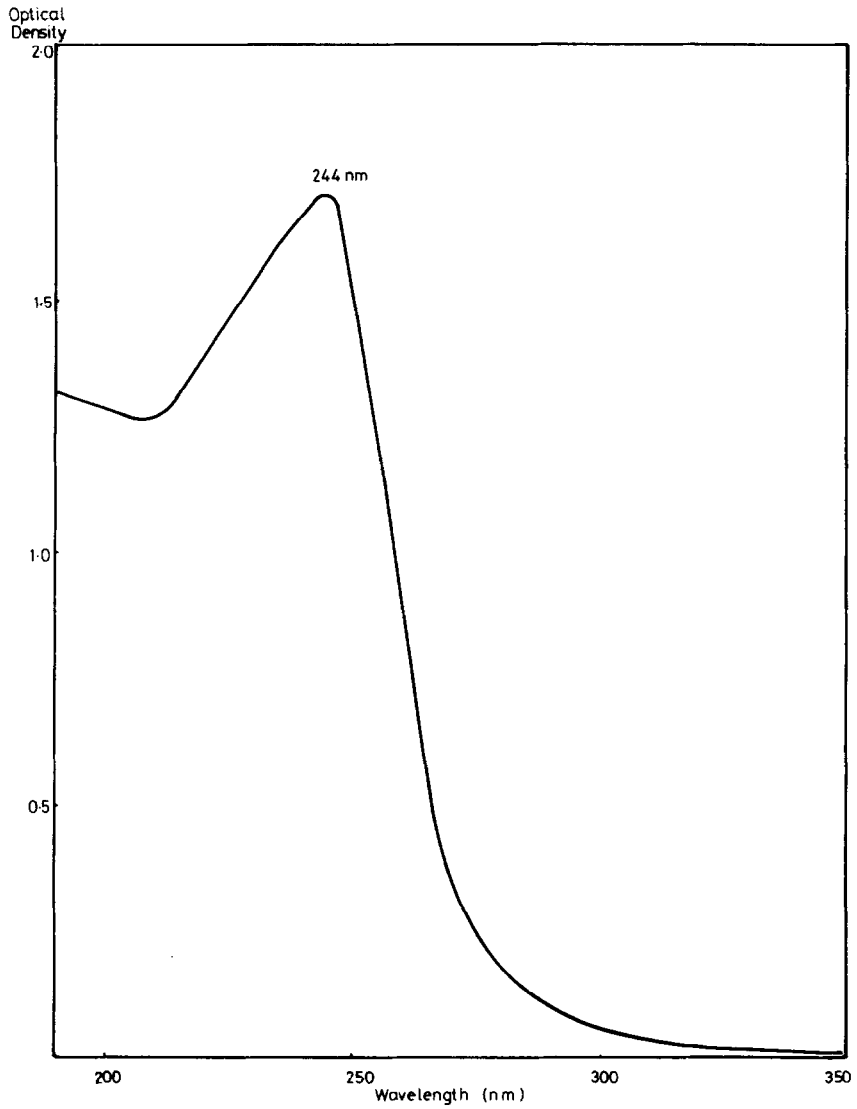


FIG. 2. Absorption spectrum of Nycodenz.

$$\% \text{ w/v} = 607.746\ \eta_{20°C} - 810.126.$$

Solutions of Nycodenz are heat stable and they can be autoclaved; their stability to autoclaving is enhanced by the addition of millimolar concentrations of Tris and $Na_2Ca$–EDTA. Nycodenz is also very soluble in formamide and dimethylformamide; thus it is possible to prepare nonaqueous denaturing gradients up to 50% (w/v) with a maximum density of 1.4 g/ml for rate-zonal and isopycnic separations.

*Formation of Gradients*

When Nycodenz solutions are centrifuged in fixed-angle rotors gradients self-form quite rapidly. As shown in Fig. 3 the shape and the density of gradients are determined by the time, temperature, and speed of centrifugation as well as by the initial density. The rate at which gradients form is also dependent on the type of rotor used; gradients form fastest in vertical and shallow-angle, fixed-angle rotors and slowest in swing-out rotors (Fig. 4).

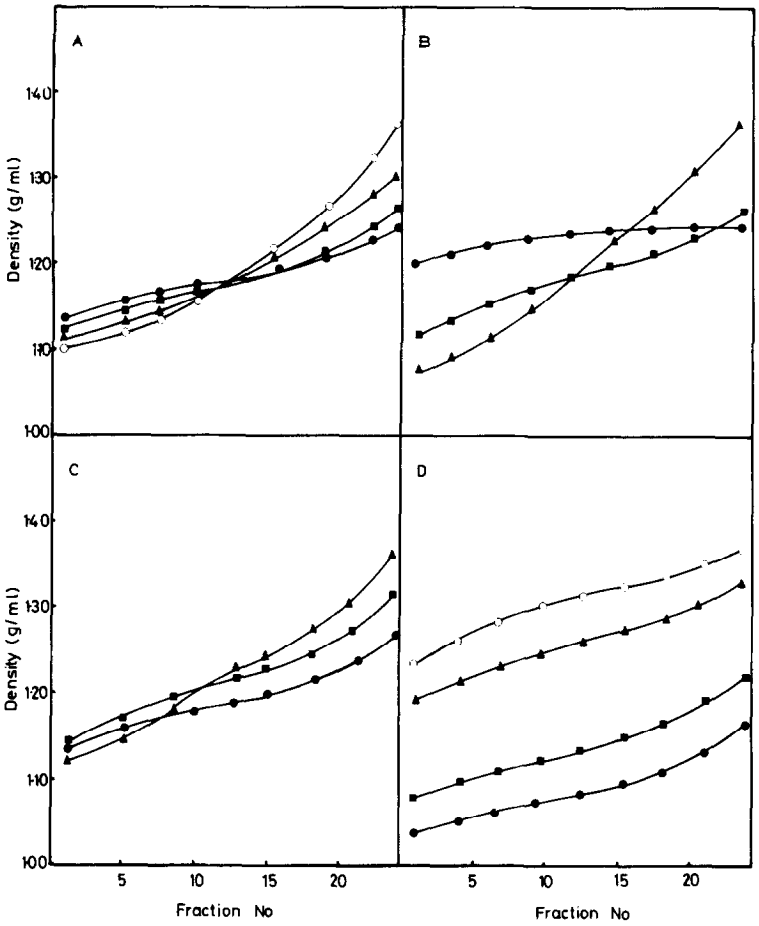


FIG. 3. Effect of centrifugation conditions and initial density on the formation of gradients. In all cases 5.0 ml of Nycodenz solution was centrifuged in a MSE 10 × 10-ml aluminum fixed-angle rotor. (A) Effect of centrifugation time on self-forming gradients: solutions of 40% (w/v) Nycodenz were centrifuged for 16 h (●), 25 h (■), 48 h (▲), and 75 h (○) at 30,000 rpm at 5°C. (B) Effect of centrifugation speed on self-forming gradients: solutions of 40% (w/v) Nycodenz were centrifuged for 24 h at 5°C at 15,000 rpm (●), 30,000 rpm (■), or 45,000 rpm (▲). (C) Effect of temperature on self-forming gradients: solutions of 40% (w/v) Nycodenz were centrifuged for 24 h at 30,000 rpm at 5°C (●), 15°C (■) or 25°C (▲). (D) Effect of initial density on self-forming gradients: solutions of 18% (●), 28% (■), 49% (▲), and 59% (○) were centrifuged for 24 h at 30,000 rpm at 5°C.

Gradients can also be easily preformed by freezing and thawing solutions in centrifuge tubes (Fig. 5). Gradients with more defined shapes can be generated by either using an appropriate gradient maker or by allowing step gradients to diffuse overnight. When preformed gradients are centrifuged in swing-out rotors the central portion of the gradient remains relatively stable (Fig. 6).

Isotonic gradients can be preformed using the recipes given in Table 2. The recipes are based on those originally devised for metrizamide (23). The gradient solutions can be sterilized by autoclaving prior to use if required. In the case of a 10–30% (w/v) Nycodenz gradient the deviation from isotonicity, taken as 290 mOsm (24), does not exceed 10 mOsm (Fig. 7).

### *Analysis of Gradients*

*Density of fractions.* As with most other gradient solutions the density of gradient

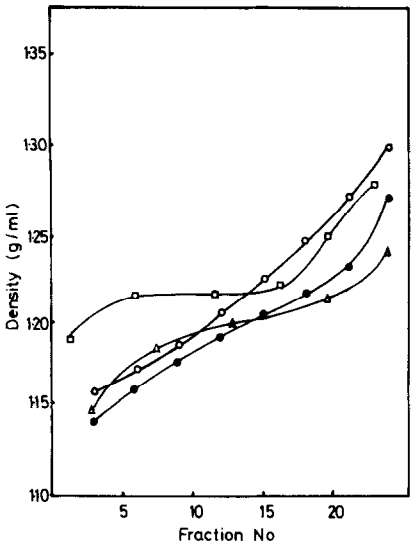


FIG. 4. Effect of rotor type on the formation of gradients in Nycodenz solutions. Solutions of 40% (w/v) Nycodenz were centrifuged for 24 h at a speed, in each case equivalent to 63,000$g_{av}$ in MSE 3 × 6.5-ml swing-out rotor (□), the MSE 10 × 10-ml titanium fixed-angle (35°) rotor (△), the MSE 10 × 10-ml aluminum fixed-angle (21°) rotor (●), and the MSE 8 × 35-ml vertical tube rotor fitted with 5.5-ml tubes and adaptors (○).

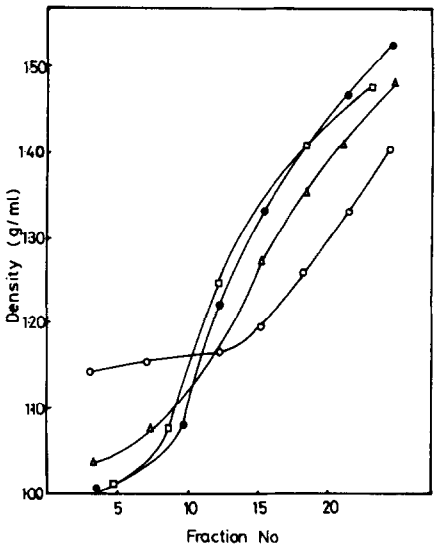


FIG. 5. Formation of Nycodenz gradients by freezing and thawing Nycodenz solutions, 5.0 ml of 40% (w/v), were placed in 10-ml centrifuge tubes and frozen in a −20°C freezer. Gradients were allowed to thaw out on the bench at room temperature (approx 21°C). The solutions were frozen and thawed one (○), two (△), three (□), or four (●) times before fractionation and analysis.

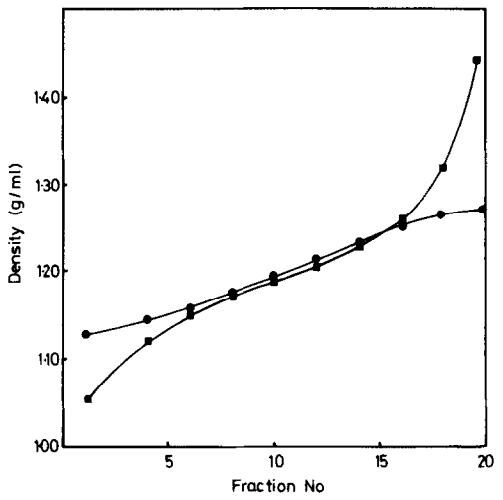


FIG. 6. Stability of preformed gradients during centrifugation. Preformed 25–50% (w/v) Nycodenz gradients were prepared by allowing step gradients to diffuse overnight at 5°C. The gradient profile before (●) and after (■) centrifugation in an MSE 3 × 6.5-ml swing-out rotor at 50,000 rpm for 24 h was analyzed.

fractions is most conveniently measured from their refractive indices. From the data in Table 1 it can be shown that

$$\text{density (g/ml)} = 3.242\ \eta_{20°C} - 3.323.$$

Before using this equation the refractive index must be corrected to take into account the actual temperature and the presence of buffer or salt in the gradient solution.

*Effect of Nycodenz on isotopic measurements.* Table 3 shows the degree of quench-

TABLE 2

RECIPES FOR ISOTONIC GRADIENT SOLUTIONS OF NYCODENZ

| Solution | Osmolality (mOsm) | Recipe |
|---|---|---|
| Isotonic Nycodenz solution | 290 | 27.6% Nycodenz dissolved in 0.3 mM $Na_2Ca$-EDTA, 5 mM Tris-HCl (pH 7.6), 3 mM KCl |
| Gradient diluent | 251 | 0.75% NaCl dissolved in 0.3 mM $Na_2Ca$-EDTA, 5 mM Tris-HCl (pH 7.6), 3 mM KCl |

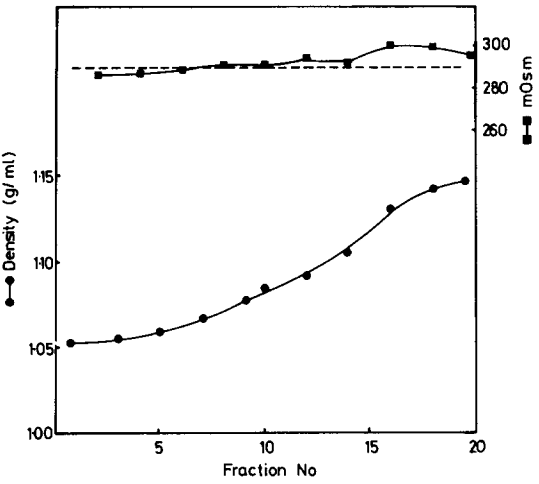


FIG. 7. Formation of an isotonic Nycodenz gradient. Using the recipes given in Table 2 solutions containing 27.6, 18.4, 13.8, and 9.2% (w/v) Nycodenz were prepared and 2.0 ml of each solution was layered into an MSE 6 × 14-ml tube (Beckman equivalent SW40 tube). The tube was sealed with Parafilm and laid on its side for 1.5 h at 7°C.

ing caused by Nyocdenz on the radioactivity of isotopes used in biological work. It can be seen that using PPO/POPOP/toluene/Triton scintillator (5) the amount of quenching is minimal in all cases. A number of other commercially available scintillation fluids have been tried and all those tested appeared compatible with Nycodenz. However, Nycodenz caused significant quenching of Bray's scintillant although stronger beta emitters such as $^{32}P$ are quenched less than $^{3}H$ (Table 3). This is similar to the quenching behavior of metrizamide as described previously (25).

*Effect of Nycodenz on some common chemical assays.* Table 4 shows that Nycodenz, unlike metrizamide, does not interfere with a number of common assays. However, Nycodenz does interfere with both the Folin phenol procedure (7) and microbiuret (8) assays of protein. In our experience extremely accurate assays of protein can be carried out using either the amido-black filter assay (9) or the Coomassie blue assay (10). In the case of nucleic acids the common assays for DNA and RNA, diphenylamine, and orcinol are compatible with Nycodenz. In addition the methyl green assay for DNA (12) can also be used in the presence of Nycodenz. Nycodenz was found to interfere significantly in the assays for polysaccharides using the anthrone procedure (13) in that, after prolonged heating at 100°C in concentrated $H_2SO_4$, iodine appeared to be released into the assay solution. However, it was found that Nycodenz was stable to the phenol/$H_2SO_4$ reagent used in the assay for sugars (14).

*Effect of Nycodenz on enzyme activity.* At high concentrations (in excess of 25%) all gradient media inhibit enzyme activities significantly. In all enzyme assays, however, the gradient sample is normally diluted by the reagents at least by a factor of 5, so that the final concentration is of the order of 10–15% at most. At these lower concentrations the degree of inhibition depends on the gradient medium and the individual enzyme. Table 5 shows that many of the important marker enzymes can be assayed in the presence of low concentrations of Nycodenz. Of all the

TABLE 3

EFFECT OF NYCODENZ ON THE QUENCHING OF RADIOISOTOPES

| Scintillation fluid | Percentage quenching in the presence of 20% (w/v) Nycodenz | | |
|---|---|---|---|
| | $^{3}H$ | $^{14}C$ | $^{32}P$ |
| PPO/POPOP/toluene/Triton (5) | 10 | 5 | 3 |
| Bray's scintillator (6) | 62 | — | 20 |
| Hydroluma (Lumac Systems, Switzerland) | 3 | — | — |
| Rialuma (Wallac, Finland) | 5 | — | — |
| Ready Solv (Beckman, United States) | 7 | — | — |
| L. S. C. (Hopkin & Williams, United Kingdom) | 12 | — | — |

TABLE 4

COMPATIBILITY OF NYCODENZ WITH CHEMICAL ASSAYS

| Assay for | Method | Reference | Interference from Nycodenz |
|---|---|---|---|
| DNA | Diphenylamine | 11 | No |
| | Methyl green | 12 | No |
| RNA | Orcinol | 11 | No |
| Protein | Folin phenol | 7 | Yes |
| | Microbiuret | 8 | Yes |
| | Amido black | 9 | No |
| | Coomassie blue | 10 | No |
| Polysaccharides (determined as hexoses) | Anthrone | 13 | Yes |
| | Phenol/$H_2SO_4$ | 14 | No |

enzymes studied, only $Na^+/K^+$-ATPase was inhibited significantly by 5% Nycodenz. In the enzyme assays there are two important practical points worth noting. First, the catalase assay (21) involves determination of unreacted $H_2O_2$ by titration with $KMnO_4$: since all of the gradient media decolorize $KMnO_4$, sufficient of the latter must be added to take this into account. Second, the adenyl cyclase measurement involves estimation of the 3',5'-cyclic AMP in a competitive binding protein assay with cyclic [$^3$H]AMP, followed by removal of unbound cyclic AMP by adsorption to charcoal. The amounts of cyclic AMP involved are very small (1–8 pmol) and their adsorption to the charcoal is inhibited by both Nycodenz and metrizamide but not by sucrose. Normally the method involves the addition of 0.1 ml of a suspension of 10% Norit in buffer containing 2% bovine serum albumin (25); the problem is overcome by increasing the

TABLE 5

EFFECTS OF GRADIENT MEDIA ON THE ACTIVITIES OF MARKER ENZYMES

| | | Relative activity in the presence of | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5% Gradient medium | | | 15% Gradient medium | | |
| Cell fraction | Marker enzyme | Nycodenz | Metrizamide | Sucrose | Nycodenz | Metrizamide | Sucrose |
| Plasma membranes | 5'-Nucleotidase | 91 | 108 | 80 | 80 | 100 | 42 |
| | Adenyl cyclase | 100 | 105 | 100 | 64 | 73 | 87 |
| | $Na^+/K^+$-ATPase | 48 | 48 | 10 | 55 | 52 | 0 |
| Endoplasmic reticulum | NADPH–cytochrome *c* reductase | 82 | 64 | 95 | 50 | 53 | 47 |
| | Glucose-6-phosphatase | 102 | 97 | nd[a] | 84 | 96 | nd |
| Mitochondria | Succinate dehydrogenase | 93 | 95 | 108 | 107 | 94 | 121 |
| | Malate dehydrogenase | 81 | 77 | nd | 36 | 44 | nd |
| | $Mg^{2+}$-ATPase | 86 | 91 | 106 | 86 | 93 | 110 |
| Lysosomes | Acid phosphatase | 96 | 101 | 105 | 94 | 100 | nd |
| Peroxisomes | Catalase | 110 | 105 | 100 | 114 | 118 | 115 |

*Note.* Results are percentages of activity in the absence of any added gradient medium.

[a] Not determined.

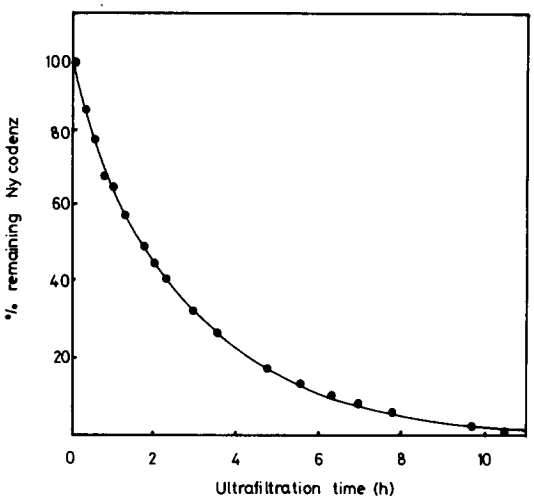


FIG. 8. Removal of Nycodenz by ultrafiltration. Nycodenz solution (18%) was placed in a 100-ml Bio-Rad fiber dialysis beaker (Type bHFD, fiber area 1000 $cm^2$, $M_r$ cutoff limit 5000). The ultrafiltration cell was stirred at room temperature and distilled water was passed through the fibers under gravity at an average flow rate of 15 ml/min. At intervals the refractive index of the Nycodenz solution in the ultrafiltration beaker was measured and the concentration of Nycodenz was calculated from the equation given in the text.

amount of Norit by 50% and halving the amount of bovine serum albumin.

The other practical problem common to both Nycodenz and metrizamide is that is is necessary to avoid using enzyme assays which involve spectrophotometric measurements in the ultraviolet range. If this is unavoidable, it is preferable to remove the Nycodenz prior to assay as described in the following section.

*Removal of Nycodenz from samples.* Large particulate samples, for example, cell organelles, can be easily separated from Nycodenz by centrifuging the appropriate pooled fractions after they have been diluted by the addition of 2 vol of a suitable buffer. The pelleted material should then be washed at least once by resuspension and recentrifugation to remove any remaining Nycodenz.

The relatively low molecular weight of Nycodenz ($M_r$ 821) enables it to be removed from most samples by dialysis, ultrafiltration, or gel filtration on Sephadex G-25. Figure 8 shows an example of such a separation using a fiber ultrafiltration apparatus.

Nycodenz is also readily soluble in both acidic and ethanolic media. Thus in some cases samples can be isolated free of Nycodenz simply by precipitating the samples by the addition of trichloroacetic acid or ethanol.

## DISCUSSION

Nycodenz is a new nonionic gradient medium which has a number of distinct advantages over existing gradient media. Many features of Nycodenz are similar to those of metrizamide as a result of the presence of a similar triiodobenzene ring. However, Nycodenz is much more stable to heat in that its solutions can be autoclaved without decomposition.

Gradients readily self-form when solutions of Nycodenz are centrifuged in fixed-angle or vertical rotors. As in the case of metrizamide gradients the fractionation of samples can be optimized by adjusting the centrifugation conditions to manipulate the shape of the gradients. Preformed gradients also behave similarly to metrizamide gradients in that they are stable when centrifuged in swing-out rotors. Isotonic gradients can be prepared with a density up to 1.15 $g/cm^3$ which is sufficient for the isopycnic banding of most cell types.

While the degree of quenching of scintillation fluids by Nycodenz and metrizamide is similar the absence of a sugar structure means that Nycodenz does not interfere with a number of standard assays which involve the spectrophotometric assays for sugars, for example, the orcinol and diphenylamine assays. Similarly, yeast hexokinase which is inhibited by the deoxyglucose moiety of metrizamide (26) is likely to be unaffected by Nycodenz. Overall, it appears that Nycodenz is not significantly more inhibitory to enzymes than other gradient media, at least as judged from the results of studies of the

activities of marker enzymes in the presence of Nycodenz.

One important feature of Nycodenz is the ease with which it can be removed from samples after the fractionation is completed, unlike some media based on colloidal silica. After appropriate dilution of gradient fractions even the smallest particles can be pelleted by centrifugation without any risk of contaminating the pellet with Nycodenz. Like sucrose, Nycodenz can be readily removed by dialysis and ultrafiltration. Unlike Percoll and even ionic iodinated media, Nycodenz is not precipitated at low pH and hence samples can be isolated from gradient fractions by acid precipitation.

A marked lack of toxicity of Nycodenz has also been observed in earlier studies. In the case of mammalian cells, they do not appear to metabolize Nycodenz (27) and cells exposed to Nycodenz retain a more normal morphology than in other iodinated media or Percoll (28,29) and in the case of mast cells appear less damaged as judged by histamine release (28).

In conclusion, the introduction of Nycodenz facilitates the analysis of biological particles and this new nonionic iodinated gradient medium is likely to prove extremely useful in a wide range of applications.

## REFERENCES


1. Rickwood, D., and Birnie, G. D. (1975) *FEBS Lett.* **50,** 102–110.
2. Rickwood, D. (1978) *in* Centrifugal Separations in Molecular & Cell Biology (Birnie, G. D, and Rickwood, D., eds.), pp. 219–250, Butterworths, London.
3. Hinton, R. H., Mullock, B. M., and Gilhuus-Moe, C. Chr. (1974) *in* Methodological Developments in Biochemistry (Reid, E., ed.), Vol. 4, pp. 103–110, Longmans, London.
4. *Acta Radiol. Suppl.* **362,** (1980).
5. Rickwood, D., and Klemperer, H. G. (1971) *Biochem. J.* **123,** 731–738.
6. Bray, G. A. (1960) *Anal. Biochem.* **1,** 279–285.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
8. Itzhaki, R. F., and Fill. D. M. (1964) *Anal. Biochem.* **9,** 401–410.
9. Schaffner, W., and Weissmann, C. (1973) *Anal. Biochem.* **56,** 502–510.
10. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
11. Schneider, W. C. (1957) *in* Methods in Enzymology (Colowick, S. P., and Kaplan, N. O., eds.), Vol. 3, pp. 680–684. Academic Press, New York.
12. Peters, D. L., and Dahmus, M. E. (1979) *Anal. Biochem.* **93,** 306–311.
13. Fong, J., Schaffer, F. L., and Kirk, P. K. (1953) *Arch. Biochem. Biophys.* **45,** 319–326.
14. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) *Anal. Chem.* **28,** 350.
15. Avruch, J., and Wallach, D. F. H. (1971) *Biochim. Biophys. Acta* **233,** 334–347.
16. Wisher, M. H., and Evans, W. H. (1975) *Biochem. J.* **146,** 375–388.
17. Williams, C. H., and Kamin, H. (1962) *J. Biol. Chem.* **237,** 587–595.
18. Ochoa, S. (1955) *in* Methods in Enzymology (Colowick, S. P., and Kaplan, N. O., eds.), Vol. 1, pp. 735–751, Academic Press, New York.
19. Hubscher, G., and West, G. R. (1965) *Nature (London)* **205,** 799–800.
20. Sloat, B. J., and Allen, J. M. (1969) *Ann. NY Acad. Sci.* **166,** 574–601.
21. Cohen, G., Dembiec, D., and Marcus, J. (1970) *Anal. Biochem.* **34,** 30–38.
22. Brown, B. L., Ekins, R. P., and Albano, J. D. M. (1972) *Advan. Cyclic Nucleotide Res.* **2,** 25–40.
23. Chambers, J. A. A., and Rickwood, D. (1978) *in* Centrifugation: A Practical Approach (Rickwood, D., ed.), pp. 33–46, Information Retrieval Ltd., London.
24. Seglen, P. O. (1976) *in* Biological Separations in Iodinated Density-Gradient Media (Rickwood, D., ed.), pp. 107–121, Information Retrieval Ltd., London.
25. Loos, J. A., and Roos, D. (1976) *in* Biological Separations in Iodinated Density-Gradient Media (Rickwood, D., ed.), pp. 97–105, Information Retrieval Ltd., London.
26. Ridge, D. (1978) *in* Centrifugal Separations in Molecular & Cell Biology (Birnie, G. D., and Rickwood, D., eds.), pp. 33–61, Butterworths, London.
27. Bertoni, J. M., and Alexander, G. M. (1981) *Biochem. Pharmacol.* **30,** 1135–1138.
28. Aakhus, T., Sommerfelt, S. C., Stormorken, H., and Dahlstrom, K. (1980) *Acta Radiol. Suppl.* **362,** 131–134.
29. Mutzel, W., Siefert, H. M., and Speck, U. (1980) *Acta Radiol. Suppl.* **362,** 111–115.
30. Aspelin, P., Birk, A., Almen, T., and Kiesewetter, H. (1980) *Acta Radiol. Suppl.* **362,** 117–122.