



A comparative study of gelatin and dextran as sedimentation agents for neutrophil isolation during myeloperoxidase purification

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ABSTRACT

Myeloperoxidase (MPO), a haem-containing enzyme abundant in human neutrophils, significantly contributes to host defense and serves as a biomarker in cardiovascular and inflammatory diseases. Conventional purification methods employ dextran sedimentation for neutrophil isolation; however, dextran is costly and often unavailable. This study compared a gelatin-based sedimentation approach with the conventional dextran method to evaluate the suitability of gelatin as a cost-effective alternative for the purification of myeloperoxidase (MPO) from whole venous blood. Gelatin was used instead of dextran for neutrophil sedimentation before Ficoll centrifugation and hypotonic lysis of contaminating erythrocytes. A myeloperoxidase-rich fraction was prepared from whole neutrophil via selective salt precipitation, and the enzyme activity yield and specific activity were determined. The gelatin-based procedure produced MPO with a specific activity of 5.12 U/mg, 4.7-fold purification, and 42% yield, closely comparable to the 5.41 U/mg, 4.8-fold, and 43.2% values obtained using dextran. The Reinheit Zahl (RZ) value also showed parallel progression (0.39 vs. 0.43), confirming comparable purity. These findings demonstrate that gelatin is a viable, efficient, and economical substitute for dextran in neutrophil sedimentation during MPO purification. The method provides a reproducible, cost-effective approach for laboratories in developing economies to obtain high-quality MPO suitable for biochemical and diagnostic applications.

1.0 Introduction

Myeloperoxidase (donor: hydrogen peroxide oxidoreductase; EC 1.11.1.7) is a haem-containing enzyme that is highly abundant in human neutrophils (granulocytes), comprising about 5% of their weight [1]. It oxidizes a wide range of electron donors (reductants) using hydrogen peroxide [2]. Myeloperoxidase can exist as several spectroscopically distinguishable forms corresponding to the various redox states of the enzyme: native ferric form, compound I, compound II, and compound III [3].

The haem iron of the native enzyme exists in the ferric form and serves as the electron transfer centre. Like the other haem peroxidases,

myeloperoxidase combines with hydrogen peroxide to form the highly reactive redox intermediate called compound I. The high redox potential of this intermediate confers myeloperoxidase with the unusual ability to oxidize chloride ion to hypochlorous acid (a potent antimicrobial agent) with the concomitant regeneration of native enzyme [4].

Myeloperoxidase is centrally involved in host defense, but it is not of absolute necessity in killing microbial cells [5]. On this basis, much attention is focused on targeting myeloperoxidase for drug action to combat the inflammatory effect induced by oxidants without suppressing the host defense.

Neutrophil isolation is commonly performed by dextran sedimentation followed by density gradient

centrifugation, while the use of gelatin as a sedimenting agent has been only occasionally reported in earlier studies despite its potential advantages and has not yet been established as a standard practice. Dextran is a polysaccharide that is found in slimy layers around bacteria of the species *Leuconostoc mesenteroides*, where it is involved in the protection of the cell and the adhesion of the cell to surfaces [6]. Dextran is known to be uneconomical, especially in developing countries, considering its cost and non-availability as it has to be synthesized from a particular species of bacteria (*Leuconostoc mesenteroides*). Gelatin, in contrast, is a substantially pure protein food ingredient obtained by the thermal denaturation and partial hydrolysis of collagen, the most common structural protein in the animal kingdom [7,8]. It is an amphoteric protein rich in hydroxyproline, proline, and glycine [9]. Given its widespread use in food and pharmaceuticals and its generally greater accessibility and lower cost, gelatin is an attractive potential substitute for dextran in biochemical procedures.

A previous study by Jamal and Algirdas [10] employed gelatin sedimentation as part of a neutrophil preparation protocol to obtain plasma membranes for biochemical analysis; however, the use of gelatin was incidental and not evaluated as an alternative to dextran. Its potential as a substitute for neutrophil sedimentation therefore remains largely unexplored. This study, therefore, aimed to further compare the enzymatic activity and purification efficiency of the myeloperoxidase enzyme obtained through the use of gelatin for neutrophil sedimentation with that obtained through the use of dextran.

2.0 Materials and Methods

2.1 Chemicals and reagents

All chemicals and reagents were sourced from Sigma Chemical Co. Ltd., Poole, Dorset, U.K., and British Drug House. Venous blood samples were obtained from three healthy, drug-free volunteers in accordance with medical ethics and collected at Omosebi Hospital, Ilorin, Nigeria.

2.2 Isolation of neutrophils

Polymorphonuclear neutrophils (PMN) were prepared from whole venous blood using a modified procedure involving gelatin sedimentation, Ficoll centrifugation, and hypotonic lysis of contaminating erythrocytes [11,12].

2.3 Partial purification of myeloperoxidase

A myeloperoxidase-rich fraction was prepared from the isolated neutrophils using selective salt precipitation with ammonium sulfate, based on a

modified method of Andrews and Krinsky [13] and Takeyama *et al.* [14]. The crude neutrophil lysate was brought to 50% ammonium sulfate saturation and stirred at 4 °C for 30 min. The precipitate was removed by centrifugation (15,000 × g, 15 min, 4 °C). The supernatant was then adjusted to 70% saturation and centrifuged again. The resulting pellet was dissolved in 50 mM sodium phosphate buffer (pH 6.0) and dialyzed overnight against the same buffer at 4 °C. Peroxidase activity was assayed at each purification step, and the purified enzyme was stored at -20°C.

2.4 Determination of myeloperoxidase peroxidation activity

The peroxidation activity of myeloperoxidase was determined using 0.5 mM tetramethylbenzidine as the donor substrate, following methods modified from Suzuki *et al.* [15] and Bozeman *et al.* [16]. 1mM hydrogen peroxide was added to the reaction mixture containing 50 mM sodium phosphate buffer (pH 5.4) and the appropriate enzyme aliquot. One unit of MPO activity is defined as the amount of enzyme producing an increase in extinction of 1.0 unit per minute at pH 5.4 and 37°C.

2.5 Determination of enzyme concentration and purification factors

At each step of the purification, the yield, specific activity, and fold purification achieved for each fraction were determined. One unit of myeloperoxidase activity was defined as the amount of enzyme that produced an increase in extinction of 1.0 unit per minute at pH 5.4 and 37°C, calculated from the initial rate of reaction using 0.5 mM tetramethylbenzidine as substrate. The absolute concentrations of the enzyme solution were determined by taking the extinction at 430 nm. Native myeloperoxidase, owing to its haem content, absorbs at 430 nm, and the concentration was determined by direct spectrophotometric determination at this wavelength. The molar extinction coefficient (ϵ°_{430}) used was 91000 M⁻¹cm⁻¹ [17].

$$[MPO] = \frac{A_{430}}{\epsilon \times l}$$

where A_{430} = absorbance at 430 nm and l = 1 cm path length.

Another index used in assessing the degree of purification is the Reinheit Zahl (RZ) value [13]. The RZ value is defined as the ratio of absorbance at 430 nm and 280 nm ($\epsilon_{430}/\epsilon_{280}$). It is analogous to specific activity, as it indicates the ratio of total enzyme (haem content) to total protein present.

2.6 Determination of protein concentration

The protein content of samples was determined by the method of Lowry *et al.* [18] using 1 mL of the sample and 1 mL of alkaline copper reagent (2% Na₂CO₃ in 0.1 M NaOH containing 0.5% CuSO₄·5H₂O and 1% Na-tartrate, mixed freshly at 50:1:1 ratio). The mixture was incubated for 10 min at room temperature, followed by the addition of 0.1 mL of Folin–Ciocalteu reagent (1 N) diluted 1:1 with water. After 30 min incubation in the dark, absorbance was read at 750 nm. Protein concentration was extrapolated from a BSA calibration curve.

2.7 Statistical analysis

All assays were performed in triplicate for each of three independent biological samples (n = 3). Results are presented as mean ± standard deviation (SD). Statistical significance between gelatin- and dextran-isolated enzyme preparations was assessed using paired Student's t-test (p < 0.05)

3.0 Results

The various purification metrics of myeloperoxidase extracted from gelatin-sedimented neutrophils are presented in Table 1. The initial crude myeloperoxidase fraction had a low peroxidase activity. The first supernatant fraction (Fraction I) showed a specific activity of 1.16 U/mg, a purification fold of 1.1, and a yield of 84%. Sequential fractionation with (NH₄)₂SO₄ further purified the enzyme, yielding two fractions (Fraction II and Fraction III). Fraction II had a specific activity of 1.65 U/mg, while Fraction III yielded the most purified fraction with a specific activity of 5.12 U/mg and a purification factor of 4.7. However, this last step resulted in a yield reduction to 42% of the initial total activity, representing a 26% loss from the previous step. The purification metrics of myeloperoxidase purified from dextran-sedimented neutrophils are presented in Table 2 for direct comparison. The final fraction (Fraction III) for the dextran-sedimented sample had a specific activity of 5.41 U/mg and a purification factor of 4.8, with a final yield of 43.2%.

The effectiveness of gelatin as a sedimentation agent relative to dextran is presented in Figures 1, 2, and 3, which compare their specific activity, purification yield, and RZ values, respectively. According to Figures 1, 2, and 3, the values of these parameters for myeloperoxidase purified from both gelatin-sedimented neutrophils and dextran-sedimented neutrophils show no significant difference (p > 0.05) across all purified fractions.

4.0 Discussion

Myeloperoxidase (MPO) holds significant biomedical importance as a diagnostic and prognostic biomarker in cardiovascular, inflammatory, and hematologic disorders. Elevated MPO levels have been associated with increased cardiovascular risk and mortality [19]. Also, the immunohistochemical detection of MPO is very essential in diagnosing acute myeloid leukemia and differentiating it from lymphoid malignancies [20]. Furthermore, owing to the unique ability of MPO to break down carbon nanotubes, the isolation and purification of MPO is useful for advanced applications such as nanomaterial degradation and safety research, in addition to diagnostic testing and mechanistic studies [21].

Because of these broad clinical and research applications, the reliable purification of myeloperoxidase in sufficient yield and purity is essential, and this necessitates the development of cost-effective and reproducible methods for the isolation of MPO. Traditionally, dextran sedimentation followed by density gradient centrifugation (e.g., Ficoll) has been the preferred approach for neutrophil isolation. However, the production of dextran depends on specialized microbial fermentation (e.g., *Leuconostoc mesenteroides*), making it costly and less accessible, especially in resource-limited settings [6]. Gelatin, on the other hand, is inexpensive, biocompatible, suitable for sedimentation without cytotoxicity, and widely available as a by-product of the food and pharmaceutical industries [7,8]. Moreover, previous studies have shown that gelatin-isolated neutrophils occupy a slightly “primed” cellular state, distinct from the “non-activated” state of dextran-isolated neutrophils [10]. This unique feature may be advantageous for *in vitro* studies that require partially activated neutrophils. Also, gelatin may help maintain the structural stability of myeloperoxidase (MPO) during purification because of its ability to form a protective colloidal matrix around proteins. This property can minimize enzyme denaturation or loss of catalytic activity, which possibly contributes to the comparable yield and purity observed between the gelatin and dextran methods [22]. Furthermore, another valuable advantage of gelatin is that it can facilitate the preparation of plasma membranes free of secretory organelles in biochemical and membrane studies [10].

The findings of this study confirm that gelatin can effectively substitute dextran for neutrophil sedimentation without compromising the yield, purity, or activity of purified myeloperoxidase. The purification metrics of myeloperoxidase obtained using gelatin sedimentation compare highly favourably (p > 0.05) with those obtained using the conventional dextran sedimentation method,

Table 1: Purification metrics of gelatin-sedimented neutrophil myeloperoxidase

PARAMETERS	CRUDE	FRACTION I	FRACTION II	FRACTION III
Volume (ml)	6.40	6.00	4.20	1.00
Activity (U/ml)	5.60 ± 0.34	5.00 ± 0.30	5.80 ± 0.35	15.00 ± 0.90
Total Activity	35.84 ± 2.15	30.00 ± 1.80	24.36 ± 1.46	15.00 ± 0.95
Protein Content (mg/ml)	5.10 ± 0.41	4.32 ± 0.35	3.52 ± 0.28	2.93 ± 0.23
RZ Value	-	0.08 ± 0.00	0.13 ± 0.01	0.39 ± 0.02
Specific Activity (U/mg)	1.10 ± 0.07	1.16 ± 0.07	1.65 ± 0.10	5.12 ± 0.31
Purification Factor	1.00 ± 0.06	1.10 ± 0.07	1.50 ± 0.09	4.70 ± 0.28
Yield (%)	100.00 ± 6.00	84.00 ± 5.00	68.00 ± 4.10	42.00 ± 2.50

Values represent mean ± SD (n = 3)

Table 2: Purification metrics of dextran-sedimented neutrophil myeloperoxidase

PARAMETERS	CRUDE	FRACTION I	FRACTION II	FRACTION III
Volume (ml)	6.40	6.00	4.20	1.00
Activity (U/ml)	5.80 ± 0.35	5.50 ± 0.33	6.00 ± 0.36	15.80 ± 0.95
Total Activity	36.24 ± 2.17	32.00 ± 1.92	25.36 ± 1.52	16.00 ± 0.95
Protein Content (mg/ml)	6.10 ± 0.49	5.30 ± 0.42	4.12 ± 0.33	2.95 ± 0.24
RZ Value	-	0.09 ± 0.01	0.13 ± 0.01	0.43 ± 0.02
Specific Activity (U/mg)	1.16 ± 0.07	1.20 ± 0.07	1.89 ± 0.11	5.41 ± 0.32
Purification Factor	1.20 ± 0.07	1.40 ± 0.08	1.60 ± 0.10	4.80 ± 0.29
Yield (%)	100.00 ± 6.00	84.50 ± 5.00	68.60 ± 4.10	43.20 ± 2.60

Values represent mean ± SD (n = 3)

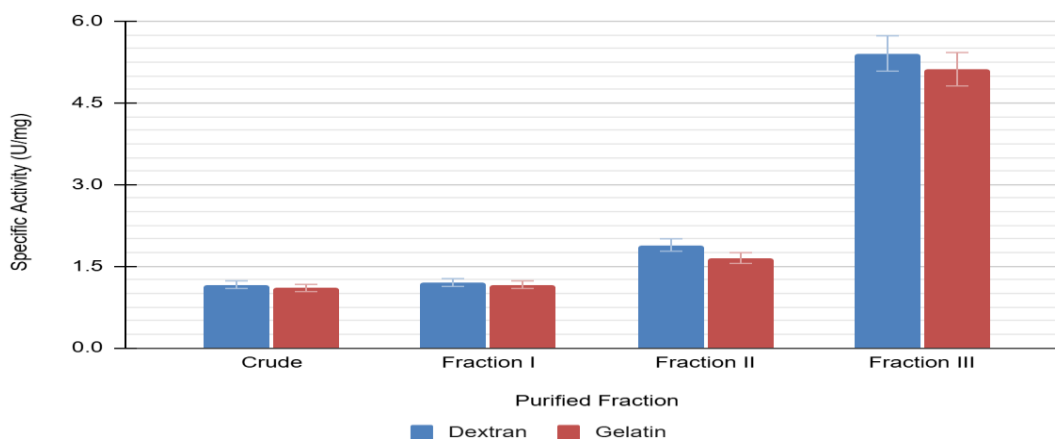


Figure 1: Specific activity of dextran-derived and gelatin-derived myeloperoxidase in the crude and purified fractions. Error bars represent ± SD (n = 3)

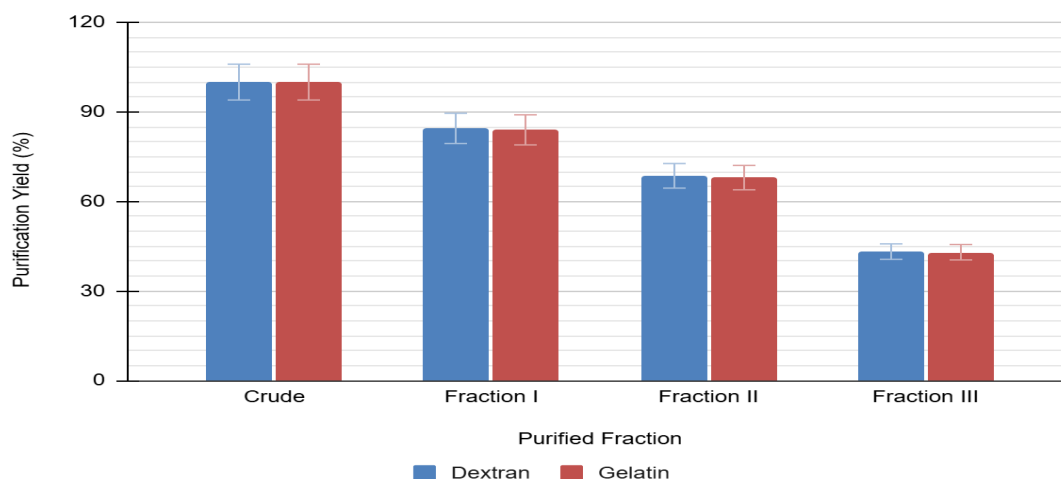


Figure 2: Purification yield of dextran-derived and gelatin-derived myeloperoxidase in the crude and purified fractions. Error bars represent \pm SD (n = 3)

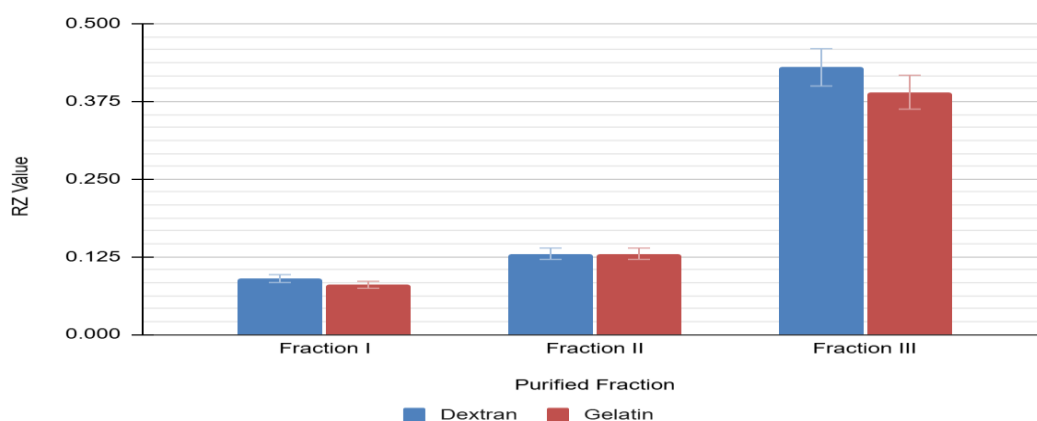


Figure 3: RZ Value progression of dextran-derived and gelatin-derived myeloperoxidase in the crude and purified fractions. Error bars represent \pm SD (n = 3)

according to Figures 1, 2, and 3. Figure 1 clearly indicates that gelatin follows the same purification trend as dextran. The final specific activity of 5.12 ± 0.31 U/mg for the gelatin-derived myeloperoxidase is closely comparable to the 5.41 ± 0.32 U/mg for the dextran-derived myeloperoxidase, confirming that gelatin does not interfere with enzyme integrity or recovery any more than dextran. These values are also consistent with the previously reported specific activity of 5.6 U/mg obtained by Andrews and Krinsky [13], whose method was the basis for the salt precipitation step, thereby validating the reproducibility of this method. Furthermore, the final purification yield of 42% for the gelatin method is almost identical to the 43.2% yield for the dextran method (Figure 2). Also, Tables 1 and 2 show that there is no distinctive difference ($p > 0.05$) between the final purification factor of the gelatin-derived MPO (4.7) and that of dextran-

derived MPO (4.8). These results imply that both methods demonstrated comparable enzyme recovery.

Figure 3 shows the progression of the Reinheit Zahl (RZ) value across purification fractions. The RZ value reflects the relative haem content (enzyme concentration) versus total protein, and thus serves as an index of purity. For the gelatin-derived MPO, the RZ increased from 0.08 ± 0.00 (Fraction I) to 0.39 ± 0.02 (Fraction III), while the dextran-derived MPO showed a similar progression from 0.09 ± 0.01 to 0.43 ± 0.02 . This steady increase indicates efficient removal of contaminating proteins and enrichment of haem-containing MPO during successive purification steps. The close correspondence of RZ values further confirms that gelatin sedimentation yields an enzyme of comparable spectral and structural purity to that obtained with dextran.

The strong comparability of all parameters studied suggests that a myeloperoxidase-rich fraction of high purity can be reliably obtained by exploiting the sedimentation property of gelatin.

5.0 Conclusion

The study validates gelatin as a viable, cost-efficient substrate for dextran in the purification of myeloperoxidase from human blood. The established methodology provides a reliable and cost-effective approach for researchers, especially those in developing economies to prepare high quality myeloperoxidase-rich fractions for biochemical research and diagnostics.

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Disclosure of conflict of interest

None

Disclosure of source of funding/grant

None

Authors Contributions

ROA - Conceptualization, Data curation, Formal analysis, Resources, Methodology, Supervision, Writing – review & editing; JTO - Investigation, Writing – original draft, review & editing.

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