First step: *Preparation of crude extract*
1- (PMN) extracts were freezer and thawed several times[11].
2-Pellet suspended in 0.34 M sucrose
3-This is considered the crude.

Second step: *CM-Cellulose column chromatography*

The concentrated crude cell extract which contained all the Myeloperoxidase and then applied to a column of CM-Cellulose (3.5x15cm) was equilibrated with 0.02M sodium acetate and 0.1M NaCl (pH=5.0) overnight at 4^{o}C . The column of CMC washed with 60ml of the same buffer and the enzyme was eluted with a linear gradient in the same buffer from 0-0.5M, NaCl, total gradient was 150ml, fractions of 3ml were collected.

Three step: *Sephacryl S-200 column chromatography*

The active concentrated Myeloperoxidase was placed on column (2 x 70 cm) which had been equilibrated with 0.1 potassium phosphate (pH=7.3) and washed with the same solvent. Fractions of 3ml were collected.

Estimation of Myeloperoxidase activity:

Myeloperoxidase activity was determined by the method of Chance and Maehly[12]. The reaction mixture (3ml) contained 1ml of 50mM Sodium phosphate (pH=7.3). 2ml of 20mM guaiacol, 20ml of 40mM H_2O_2 and then enzyme. The reaction was started by adding H_2O_2 and increase in absorbance at 470nm was followed in Spectrophotometer. One unit Myeloperoxidase was defined as the amount of enzyme causing increase of 1 unit in the absorbance at 470nm in 1min. at 20^{o}C under these Conditions.

Protein Determination:

The protein content of the cell free extract was determined by the method of Barford *et al*.,[13]. With Bovine Serum Albumin (BSA) as the standard.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

This was parformd as described in Garfine[14] for the Lamemmli system using Bio-Red vertical slab gel cell. Electrophoresis utilized a 0.1M Tris-glycine buffer, pH=8.3 and 1% SDS and 2-mercaptoethanol. A 7.5% gel was employed.

Results

The purification of human (MPO) from (PMN) cells (Table I) included two steps, firstly, CM-cellulose ion exchange chromatography, in this step one peak was obtained (Figure1) which had fold and recovery of 2.96 and 92.12% respectively.

Table 1: Purification of Myeloperoxidase form human neutrophil

Sample	Volume	Enzyme activity u\ml	Total unit	Protein mg\ml	Specific activity u\mg	Fold	Yield
Crude	20	117.3	2346	1.35	86.9	1	100
CM-Cellulose	12	180.09	2161.08	0.7	257.27	2.96	92.12
Sephacryl S-200	9	105.5	949.5	0.32	329.69	1.281	43.94

The active product of the CMC step which was loaded on Sephacyl S-200 column gave one peak (Figure 2) and with fold and yield 1.281; 43.94% respectively. The specific activity of the purified human MPO was 234.44 unit/ mg.