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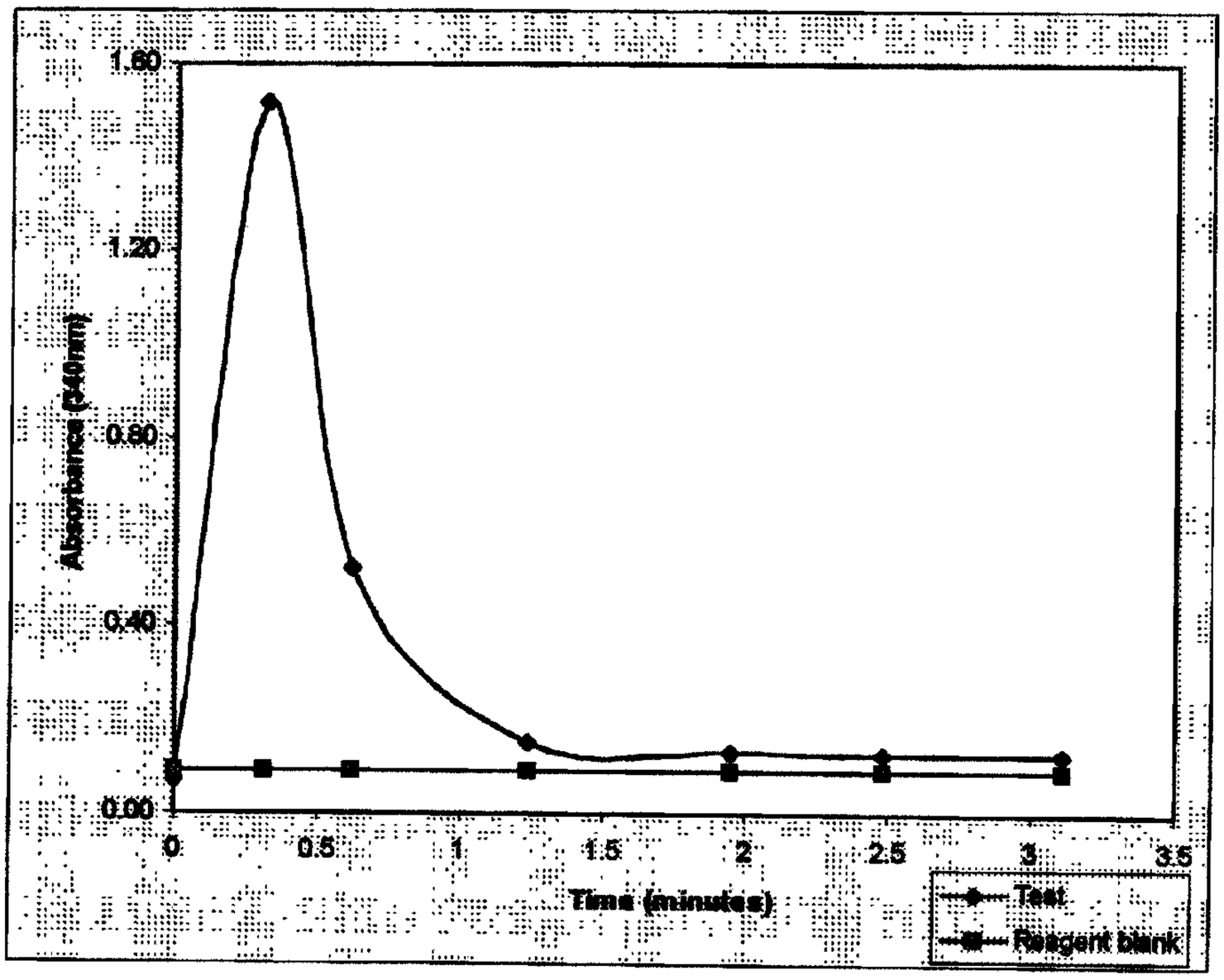


Removal of turbidity from lipemic sample.

(57) Abrégé/Abstract:
Agent for the removal of turbidity in biological samples comprising 0,5-10 mM Phenol, 0,5-15% polyoxyethylated triglyceride and at least one non-ionic tenside in a range of 0,5-15% capable in dissolving the polyoxyethylated triglyceride.

Abstract

Agent for the removal of turbidity in biological samples comprising 0,5 - 10 mM Phenol, 0,5 - 15 % polyoxyethylated triglyceride and at least one non-ionic tenside in a range of 0,5-15% capable in dissolving the polyoxyethylated triglyceride.

Agent for the Removal of Turbidity in Biological Samples

The invention relates to an agent for the removal of turbidity in biological samples, especially in serum or plasma samples.

Turbidity typically occurs in plasma or serum samples (lipemic samples) which have an increased content of triglyceride-rich lipoprotein particles, e.g. chylomicrons.

Known agents for removal of turbidity are especially necessary for photometric analysis of lipemic samples in chemical or clinical diagnosis. If the component to be analysed absorbs at a wavelength corresponding to the absorbing wavelength of the triglycerides causing the turbidity, correct photometric analysis of the component in question will be difficult if not impossible. This applies especially when the concentration of the component to be determined is very low, e.g. in the case of the analyte Cell Reactive Protein (CRP).

Therefor a typical example for a photometric procedure in which interference with turbidity must be excluded is the assay of CRP. The assay uses anti-human CRP antibodies which specifically react with the CRP in the sample to form insoluble aggregates. The assay is initiated by the addition of the antibodies and the increase in CRP-antibody-aggregates is measured photometrically at 340nm. The triglycerides in lipemic samples also absorb at this wavelength so that there is the risk of overlapping extinction signals.

To resolve this problem with lipemic samples several agents are known in the prior art which can be added in order to remove turbidity. In this connection US patent 4708939

discloses an agent comprising polyethoxylated triglyceride and a secondary n-alkane sulphonate in aqueous solution. The known agent requires relatively high detergent concentrations which can affect certain assays, especially immunoturbidimetric assays. In addition n-alkane sulphonate is an agressive detergent which again can inhibit the reaction of interest. The known agent therefor cannot give total clearing and optimum antibody-antigen reaction.

The object of the present invention is to provide an agent for the removal of turbidity which is especially adapted to CRP assays, but can be used also for other assays.

An agent according to the invention comprises 0,5 to 10 mM phenol, 0,5 to 15 % of an polyoxyethylated triglyceride and 0,5 to 15 % of at least one further non-ionic tenside. Preferably a combination of two further non-ionic tensides is included.

In the agent according to the invention the polyethoxylated triglyceride (Triglyceridethoxylate) is necessary to obtain total clearing of the sample. A suited polyethoxylated triglyceride can have a HLB value in the range of 4-16, preferably 6. The preferred range of concentration is 0,5 to 2 %. Preferred polyoxyethylated triglycerides are available under the tradenames Mulsifan RT 163 (Zschimmer & Schwarz GmbH & Co.) or Tagat CH-40 (Goldschmidt AG).

The polyoxyethylated triglycerides alone cannot dissolve in the mixture resulting in a precipitate. Dissolution requires at least one further non-ionic tenside that assits in the solubilisation of the polyethoxylated triglycerides in the sample.

Typical examples of non-ionic tensides which can be used

include: Thesit, Tergitol, Triton, Brij, Nonidet P-40, Tween 20 (Sigma-Aldrich Ltd.).

The required non-ionic tensides are straight or branched polyoxyethylene ethers with a low degree of oxyethylation (2-10 oxyethylene units per molecule) with 10-18 carbon atoms.

Preferably the further non-ionic tenside is polyoxyethylene (8) isotridecylether which is available under the tradename Genapol X 080. This non-ionic tenside dissolves in the reaction mixture but alone does not have a clearing effect. It is required to solubilise the polyethoxylated triglycerides (as stated above). A preferred range of concentration in which the non-ionic tenside can be used is 0,5 to 2 %.

A further preferred non-ionic tenside is polyoxyethylene-10-tridecylether which can be purchased from Sigma. Also this tenside dissolves in the reaction mixture and assists the solubilisation of the polyethoxylated triglycerides. A preferred range of concentration is 0,5 to 2 %.

In a preferred embodiment a combination of polyoxyethylene (8) isotridecylether and polyoxyethylene-10-tridecylether is provided. Such combination allows a very effective dissolution of the polyoxyethylated triglycerides and thereby improves the clearing action of the agent according to the invention.

Phenol finally has no dissolving effect on the lipids but acts synergistically with the tensides to increase their clearing action and remove turbidity. The presence of phenol helps to reduce the effective concentrations of the tensides needed. In the absence of phenol higher tenside

concentrations would be necessary which would lead to samples having a too high viscosity.

The components in the agent according to the invention are known substances which already are used in agents for the removal of turbidity known in the prior art.

The use of phenol as synergistically acting component is known from EP 0004857. From EP 0004857 it is also known to use Genapol as non-ionic tenside. The use of further non-ionic tensides is known from the US patent 4708939 cited above. The prior art, however, discloses only the use of the single components or sub-combinations thereof. None of them shows the combination of all of them as in the agent according to the invention.

It surprisingly turned out that all components included in the agent according to the invention are necessary to effectively remove turbidity with minimal effects to a possible antibody-antigen reaction. Theoretically Genapol and polyoxyethyl

ene-10-tridecylether together could effect clearing. However, the concentrations required for clearing are so high that any antibody-antigen reaction is totally inhibited. If according to the invention Mulsifan and Phenol are added the concentrations of Genapol and polyoxyethylene-10-tridecylether can be reduced which allows clearing without affecting immunological reactions.

In principle the agent according to the invention can be used in all immunoassay formats where there is a likelihood of interaction of contributing to lipemia.

The example given below uses an immunoturbidimetric format. Those skilled in the art would be aware of associated benefits for other formats including (but not limited to) latex-enhanced assays, magnetic particle chemiluminescent immunoassays, immunofluorescent assays (polarised and non-polarised), ELISA's, immunochromatographic assays, or any assay format requiring reduction of lipemic and/or other associated non-specific binding problems where the benificial maintenance of antibody binding characteristics is mediated by the combination of reactants in the present invention.

In the following a typical formulation of a clearing agent according to the invention to be used in an assay for the detection of CRP is described. Furthermore an example is added showing the removal of turbidity effected by agent according to the invention in lipemic samples.

Formulation of a clearing buffer (R1) for a CRP-assay:

Reagent	**Range**	**Function**
Purified water		
Tris (100mM)/Liter	50-300 mM	Buffer
NaCl (100mM)/Liter	50-150mM/	Assists clarification & reaction.
PEG 6000 (2%)(w/w)	1-3%	Req. for optimisation of reaction rate
Phenol (2Mm)(Liter)	0.5-10mM	Lipid clearing component
Polyoxyethylene 10 -tridecylether (1.0%)(v/v)	0.5-2%	Lipid clearing component
Genapol X 80 (1.0%)(v/v)	0.5-2%	Lipid clearing component
Mulsifan RT 163 (1.1%)(v/v)	0.5-2%	Lipid clearing component
NaN_3		Anti microbial agent
4M HCl		pH reagent to 7.5
Gentamicin sulphate soln.		Stabilizer

The buffer R1 is adjusted to pH 7,5 (possible range pH 3-9) and is preferably used at a temperature of 37°C (possible range 15-40°C).

For the CRP-assay 250ml of R1 are mixed with 18ml of the sample. Then 30ml of an antiserum solution (R2; includes anti CRP antibodies) are added. After mixing of the sample with R1 buffer and R2 antiserum solution, the CRP in the sample reacts specifically with the anti-human CRP antibodies of R2 to yield insoluble aggregates. The absorbance of these aggregates is proportional to the CRP concentration in the sample.

The purpose of the R1 buffer is to dissolve lipids in lipemic samples and to provide optimum conditions for the immunogical reaction.

Removal of turbidity in lipemic samples

In this test a clearing buffer (R3) was used with the following formulation:

100mM Tris buffer adjusted to pH 7.5 with HCl, 100Mm NaCl, 2% Polyoxyethylene glycol 6000, 2mM Phenol, 1,0% Polyoxyethylene 10 tridecylether, 1,0% Genapol X 80 and 1,0% Mulsifan RT 163.

In a cuvette 18ml of strongly lipemic serum were mixed at 37°C with 250ml of R3. The change of absorbance at 340nm was determined against dependence on time. The results are illustrated graphically in Fig 1.

The course of the change of absorbance illustrated in Fig 1 shows that in the case of a sample/reagent ratio of 18ul: 250ul a turbidity of about 1.5 is reduced within about 1.5 minutes to a cleared level of about absorbance 0.1 in comparison with the reagent blank. Therefore, after 1.5 minutes complete removal of turbidity has been achieved.

CLAIMS

1. Agent for the removal of turbidity in biological samples comprising

- 0,5 - 10 mM Phenol
- 0,5 - 15 % by volume polyoxyethylated triglyceride;
- 0,5 - 15 % by volume polyoxyethylene-10-tridecylether; and
- 0,5 - 15 % by volume polyoxyethylene (8) isotridecylether

2. Agent according to claim 1, characterised in that the concentration of polyoxyethylene-10-tridecylether is 0,5 - 2% per volume.

3. Agent according to one of the claims 1 to 2, characterised in that the concentration of polyoxyethylene (8) isotridecylether is 0,5 - 2% by volume.

4. Agent according to one of the claims 1 to 3, characterised in that the concentration of the polyoxyethylated triglyceride is 0,5 - 2%.

5. Use of the agent according to one of the claims 1 to 4 in an assay to detect Cell Reactive Protein (CRP).

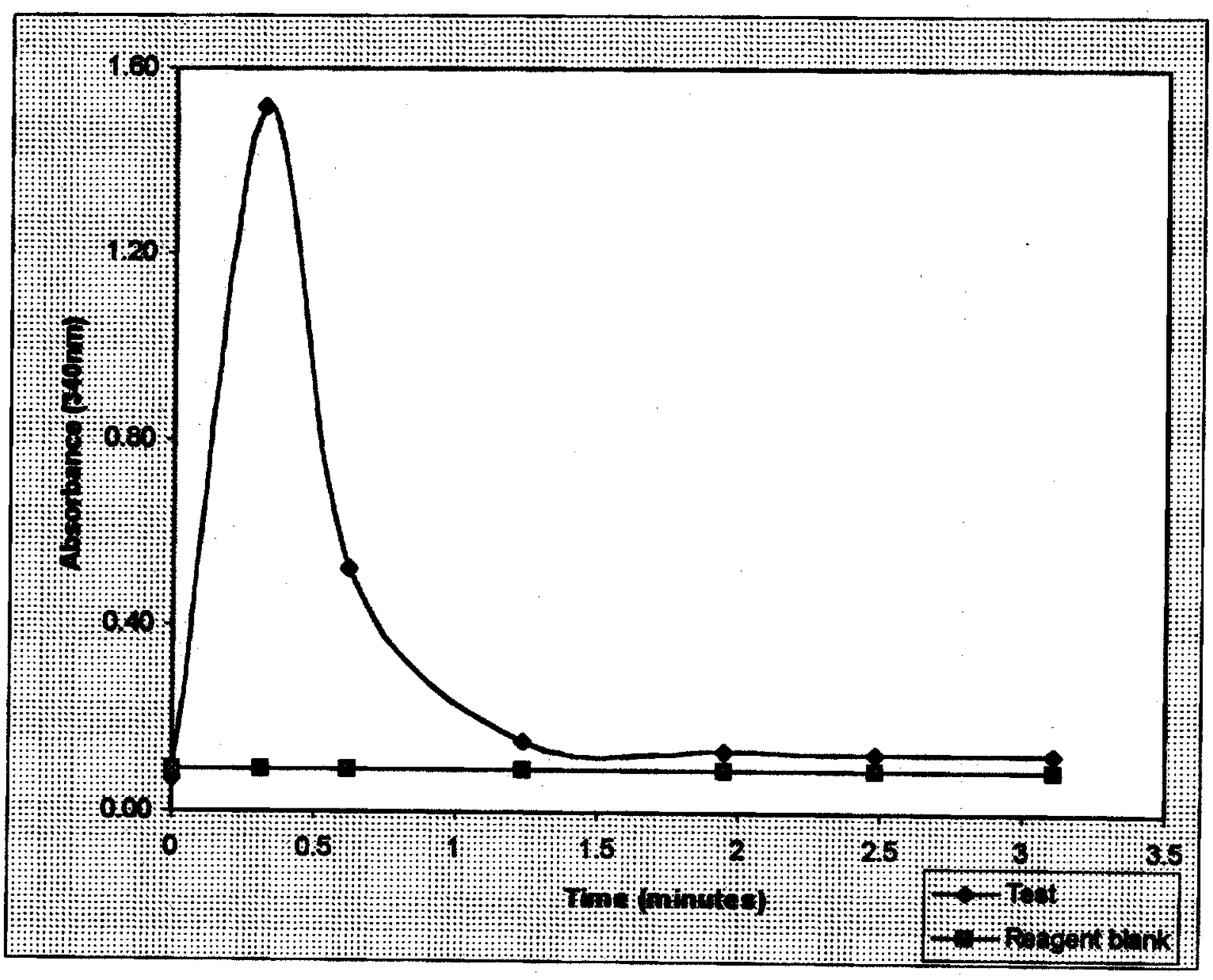
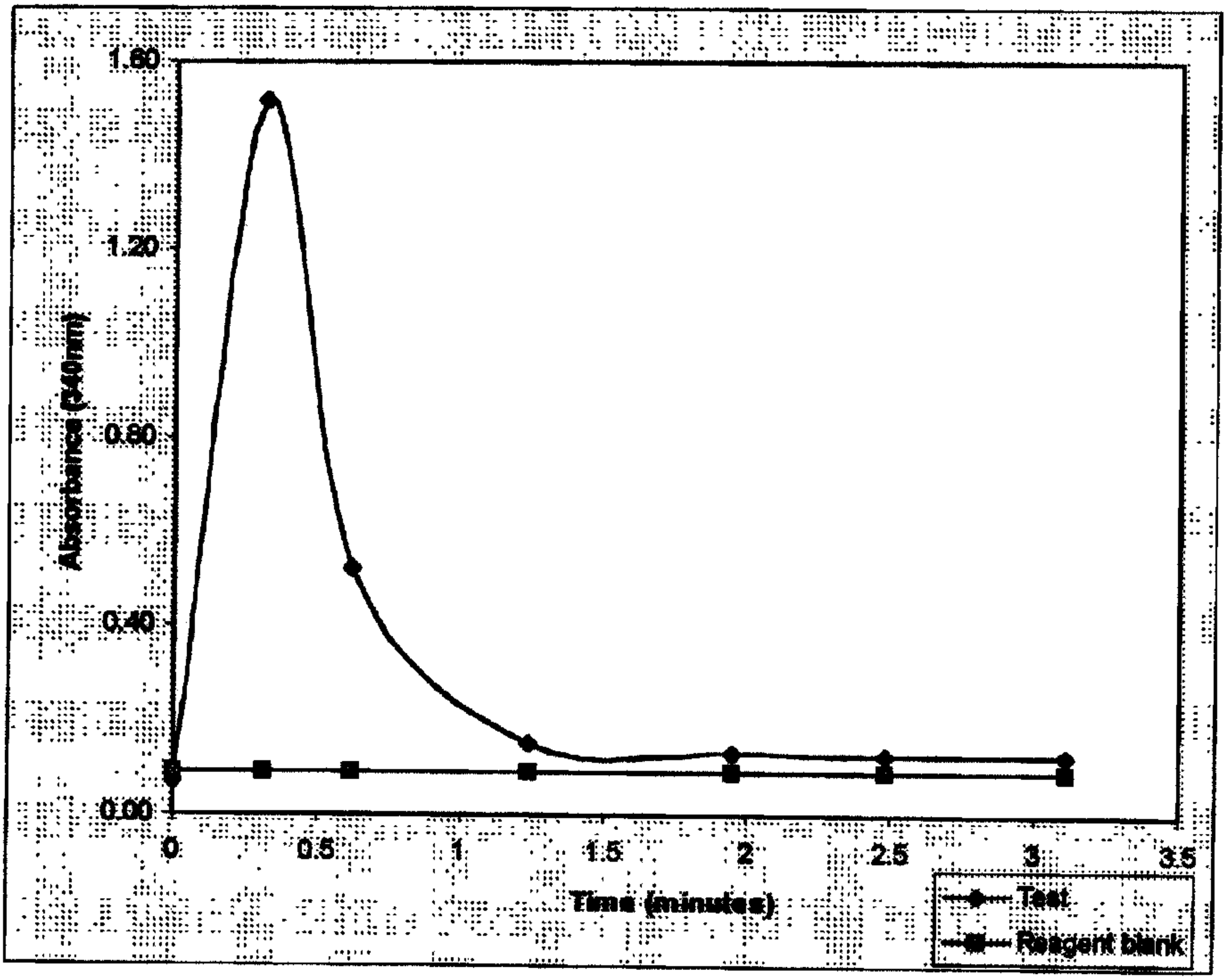


Fig 1 Removal of turbidity from lipemic sample.



Removal of turbidity from lipemic sample.