An approach to the detection of synthetic milk in dairy milk: 2. Detection of detergents

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This work reports on methods to detect and estimate anionic detergents, which are important ingredients of synthetic milk formulations, based on the extraction of a detergent–Methylene Blue complex in chloroform which can be subsequently quantified by measuring the optical density at 653 nm. A base value of 0.52 for optical density was found to represent pure cows' milk. Any result above this value suggested the presence of an anionic detergent in milk.

INTRODUCTION

'Synthetic milk' is a well-designed combination of urea, salt, soda, sucrose, vegetable oil, detergent and water, which resembles cows' milk in colour and consistency. It is reported to be used for the adulteration of dairy milk from 5 to 10%. The problem is further complicated by a lack of analytical methods to detect the presence of synthetic milk in pure milk. The qualitative and quantitative detection of urea to indicate the presence of synthetic milk in dairy milk has recently been reported[1] and, in this communication, detection of synthetic milk based on the analysis of detergents present therein is discussed.

Detergents can be anionic, cationic and non-ionic, but the anionic types are the most commonly used industrially. Hence, the proposed method using Methylene Blue was designed for the detection and estimation of anionic detergents in milk. Methylene Blue is generally soluble in an aqueous phase but, with anionic detergents, forms a chloroform-soluble complex.[2] Therefore, the proposed test was based on the assumption that, if Methylene Blue and chloroform were mixed with milk, the concentration of Methylene Blue in a chloroform layer would be directly proportional to the concentration of detergent in the milk.

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TABLE 1
Qualitative detection of detergent by chloroform extraction of Methylene Blue in blends of synthetic milk and pure cows' milk

Pure milk:synthetic milk	*Observation*
100:0	Sight blue colour in chloroform layer
99:1	Sight blue colour in chloroform layer
98:2	Sight blue colour in chloroform layer
95:5	Perceptible blue colour in chloroform layer
90:10	Distinct blue colour in chloroform layer

MATERIALS AND METHODS

Materials

Samples of authentic cows' milk were procured from local sources and compared with a model 'synthetic milk' prepared in the laboratory, using local brands of liquid detergent (A) and powder detergents (B and C) and matching cows' milk with respect to its physicochemical and analytical profile. The formulation of the model is not disclosed for obvious reasons. All chemicals and solvents were of analytical reagent grade.

A Hitachi U-2001 spectrophotometer was used for the colorimetric estimations.

Methods

Qualitative detection of detergent in milk

To 2.5 ml of a suspect sample and 2.5 ml of pure milk in separate test tubes, 7.5 ml of ethanol was added to precipitate the protein, which was then filtered off. To 2 ml of the filtrate, 2 ml of Methylene Blue solution (25 mg/100 ml of water) was added and the mixture shaken well. Then 4 ml of chloroform was added and the mixture was shaken. The chloroform layer was allowed to separate. If the Methylene Blue colour extracted from a suspect sample into the chloroform layer was greater than that extracted from an authentic milk sample, it indicated the presence of detergent in milk.

Quantitative estimation of detergent in milk

The procedure was as described above but, once the chloroform had been allowed to separate, 1 ml of the chloroform layer was diluted with 9 ml chloroform and the optical density was read immediately at 653 nm. The optical density represented the concentration of the complex of Methylene Blue and detergent in the chloroform layer, which was proportional to the concentration of detergent present in the milk.

TABLE 2
Methylene Blue extractability of commercially available detergents

Samples	*Concentration of detergent (mg %)*	*Optical density at 653 nm*
Water	0.0	0.144
'Brand A' detergent	20	0.201
	400	1.224
'Brand B' detergent	20	0.195
	400	1.209
'Brand C' detergent	20	0.192
	400	1.194

For preparation of a standard curve, a detergent solution (0.4 g/100 ml of water) was used, which was diluted to give a range of 0–10 mg of detergent per 2.5 ml. This solution was treated in the same way as the milk samples described above.

RESULTS AND DISCUSSION

Since synthetic milk is being prepared unscrupulously, its composition has not so far been reported. However, from press reports, detergents are known to be an important ingredient in the formulation. The purpose of

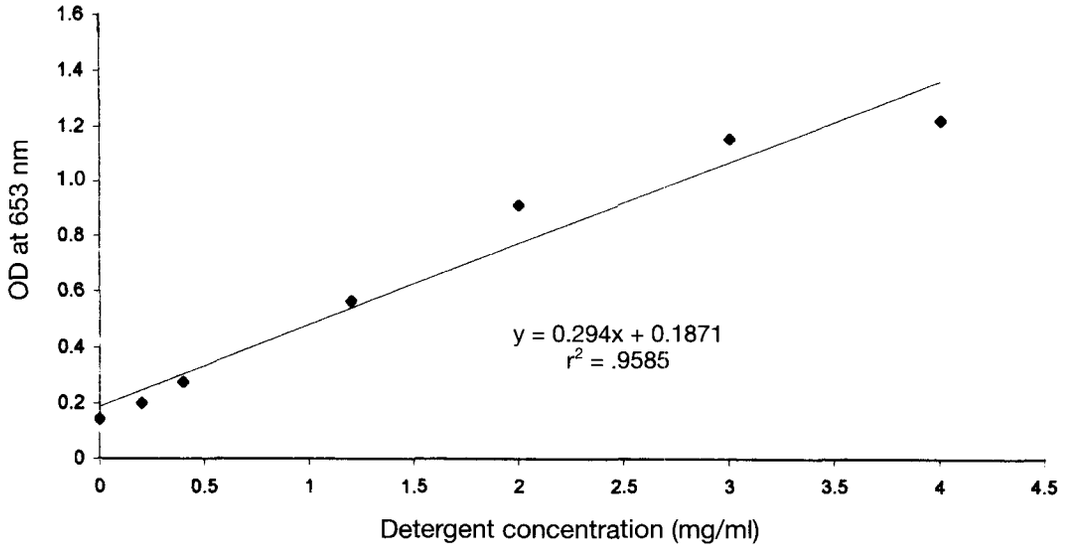

Fig. 1. Relationship between detergent concentration and optical density (OD) at 653 nm.

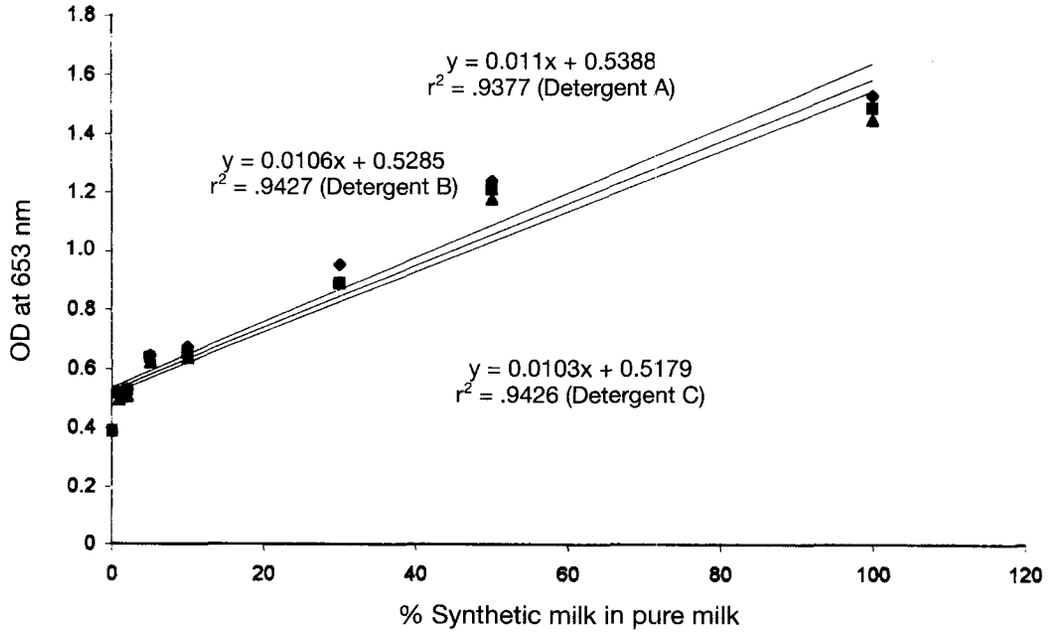

Fig. 2. Effect of the addition of detergent solution (synthetic milk) to pure milk on optical density (OD) at 653 nm.

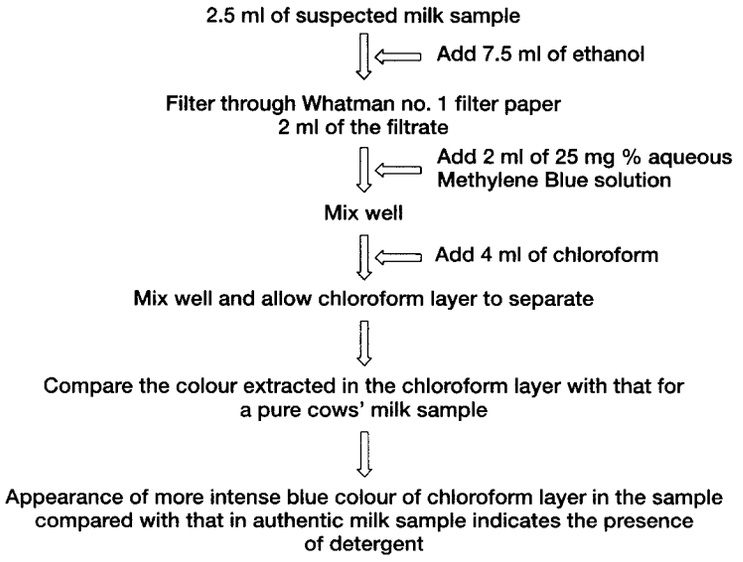

Fig. 3. Protocol for qualitative detection of detergent in milk.

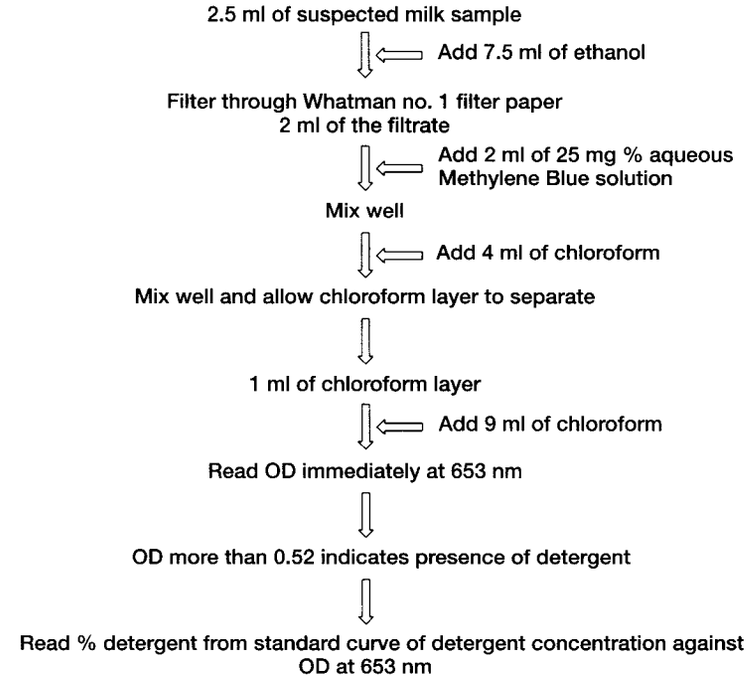

Fig. 4. Protocol for quantitative estimation of detergent in milk.

this addition is to emulsify and stabilize the oil-in-water emulsion, and to simulate the colour of dairy milk. Most of the commercial detergents are anionic in nature and, based on the fact that if such a detergent is present in 'synthetic milk' a Methylene Blue–detergent complex can be extracted from the milk phase into chloroform,[2] the qualitative detection and quantitative estimation of detergents in milk was attempted.

Table 1 shows the results of qualitative detection of detergent following different levels of adulteration of pure cows' milk with synthetic milk. The method was sensitive enough to detect 20 mg of detergent/100 ml of milk. A greater extraction of blue colour into the chloroform layer of a suspect sample compared with an authentic sample indicates the presence of synthetic milk.

A standard curve of optical density (at 653 nm) of chloroform-extracted complex of Methylene Blue and detergent against concentrations of the detergent was plotted (Fig. 1). A linear relationship between the concentration of detergent (0–4 mg/ml) and optical density at 653 nm with $r^2 = .958$ was obtained. The lowest level of detection was found to be 20 mg of anionic detergent/100 ml of milk.

To substantiate the suggestion that any detergent (which could be used in synthetic milk formulation) could extract Methylene Blue from an aqueous phase and transfer it to a layer of chloroform, Methylene Blue extractions of three popular commercial brands of detergents were performed. As shown in Table 2, all the detergents could extract Methylene Blue from the aqueous phase into the chloroform layer. The lowest level of detergent detectable by this method was again found to be 20 mg/100 ml.

Blends of cows' milk and synthetic milk (prepared with detergents A, B and C) in various proportions were then estimated colorimetrically at 653 nm after complexing with Methylene Blue and subsequent extraction of the complex into chloroform (Fig. 2). During this trial, it was observed that some component(s) of pure cows' milk were able to extract Methylene Blue from the aqueous layer to the chloroform layer and hence contribute to the optical density. This reading of optical density should, therefore, be treated as a 'blank' and the value should be deducted during the estimation of detergent. A good linear relationship was observed between the percentage of synthetic milks (prepared with detergents A, B and C) in pure milk and the optical density at 653 nm (Fig. 2) with $r^2 = .937$, .942 and .942, respectively. Since the minimum value of the intercepts was 0.52, which represents pure cows' milk, any reading above this point should be taken to indicate presence of detergent. Therefore, a cut-off value of 0.52 for optical density at 653 nm can be taken as indicating the absence of detergent-containing synthetic milk in pure cows' milk.

The protocols developed for the qualitative detection and quantitative estimation of synthetic milk based on analyses for detergent components are summarized in Figs. 3 and 4, respectively.

Conclusions

A quantitative method based on the extractability of the Methylene Blue–detergent complex into chloroform was sufficiently

sensitive to detect 20 mg of detergent in 100 ml of milk. This approach to the detection of synthetic milk in dairy milk offers a sensitive and rapid method for routine laboratory testing. This test is recommended for use in public health laboratories as well as at milk distribution and collection centres.

REFERENCES

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