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Microwave initiated synthesis of polyacrylamide grafted Casein (CAS-g-PAM)–Its application as a flocculant

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ABSTRACT

Modification of the milk protein 'casein' has been carried out through *microwave initiated* graft copolymerization of acrylamide. The synthesis was optimized in terms of microwave irradiation time and monomer (acrylamide) concentration. The synthesized graft copolymers have been characterized by intrinsic viscosity measurement, elemental analysis, FTIR spectroscopy, scanning electron microscopy (SEM) and number average molecular weight determination through osmometry; taking the starting material (casein) as reference. Further, flocculation efficacy of various grades of the grafted product were studied and compared to that of the starting material 'casein' by *Jar test and settling test* procedure, in 1% coal-fine suspension.

1. Introduction

1.1. *Casein as a biomaterial*

Casein is the main protein constituent of milk. Proteins are biopolymers composed of a sequence of 20 α amino acids. They are large molecules with molecular masses ranging from 5000 up to several million Daltons. Casein has an appropriate amino acid sequence that is important for growth and development of the nursing young. Casein is an amphiphilic protein and its isoelectric point is 4.6. At pH above the isoelectric point casein is negatively charged and is soluble in water [1]. The conformation of casein is much like that of the denatured globular proteins. The high number of proline residues in casein cause particular bending of the protein chain and inhibit the formation of close-packed, ordered secondary structures. Casein lacks tertiary structure, which explains its stability against heat denaturation since there is very little structure to unfold. Also, without a tertiary structure there is considerable exposure of hydrophobic residues. This results in strong association reactions of casein and renders them partially soluble in water.

Major application of casein is as adhesives for wood, leather finishing, paper coating and in synthetic fibres as well as plastics for buckles, buttons etc. However, the principal use of casein products has been as an ingredient in food to enhance their physical properties such as foaming and whipping, thickening and water binding, emulsification and texture, and also to improve nutrition value.

1.2. *Coal washery effluent: a menace and an opportunity*

The mining and cleaning operations of coal generate large amounts of solid wastes, the latter being up to 90% of the total production. These wastes are scarcely reused [2,3] and most of it is disposed off in dumps and slurry ponds, causing major economic and environmental problems.

Moreover, owing to their content and size (<1 mm) which increases the surface area liable to be wet and oxidized, the disposal of coal fines wastes aggravates the risk of spontaneous combustion, leading in turn to the emission of noxious gases. Evidently, the recovery of coal from coal fine cleaning wastes and treatment or clarification of coal washery effluent is essential for the environment and also has its economical benefits.

1.3. *Flocculation for treatment of coal washery effluent*

Flocculation is a physical phenomenon of solid-liquid separation of colloidal suspension through aggregation. Flocculation is drastically enhanced by the presence of minute amount of branched polymeric substances called flocculant. A flocculant aids in floc formation by forming network between numerous colloidal particles. The process of flocculation involves minimal instrumentation and maintenance and hence is much more economical than other

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Table 1
Synthesis details of CAS-g-PAM.

Polymer Grades	Wt of casein (gm)	Wt of Acrylamide (gm)	Irradiation time (min)	% Grafting	Number Average Molecular weight (kDa)	Intrinsic viscosity (dl/g)
CASEIN	–	–	–	–	30	0.89
CAS-g-PAM 1	1	10	4	70.8	460	1.60
CAS-g-PAM 2	1	20	4	198	850	5.083
CAS-g-PAM 3	1	15	4	287	1072	10.96
CAS-g-PAM 4	1	15	3	153	800	4.52
CAS-g-PAM 5	1	15	5	218	890	7.99

better known separation processes (e.g., filtration). Evidently, the search for cost-effective flocculants as economic method of removing suspended particles has become the focus of many studies [4–8].

The separation of coal particles in coal washery effluent slurries (blackwater) is usually accomplished through filtration, sedimentation and flotation or some combination of them. Coal particles are colloidal in nature and this is the reason why coagulants or flocculants are usually added to destabilize the suspension and to promote the aggregation of particles before separation is carried out. Among various coal cleaning methods, flocculation was found to be one of the most effective and economically viable in promoting aggregation of colloidal coal particles [9]. Flocculation of coal fine suspension aided by organic flocculants has been the objective of many contemporary studies [4,10].

The performance of natural polymers varies widely due to variation in composition from source to source [11]. Synthetic polymers are highly efficient and can be tailored to the needs of a particular application [12] however; their biggest disadvantage is their shear degradability [13]. It has been observed that by grafting flexible polyacrylamide chains onto polysaccharides, it is possible to develop efficient flocculant for the treatment of various wastewaters and industrial effluents [14–16]. Presently, graft copolymers are of interest in algae harvesting from seawater through flocculation for augmenting the biomass needs of the society [17].

Graft copolymerization is the most effective synthesis route of modifying natural polymers (e.g. polysaccharides) [18–20]. The process of graft copolymer synthesis starts with a preformed polymer (Protein in this case). An external agent is used to create free radical sites on this preformed polymer, once the free radical sites are formed on the polymer backbone (i.e. preformed polymer), the monomer (i.e. vinyl or acrylic compound) can get added up through the chain propagation step, leading to the formation of grafted chains. The various methods of graft copolymer synthesis actually differ in the ways of generation of the free radical sites on the preformed polymer. The most contemporary technique in graft copolymer synthesis involves the use of *microwave radiation* to initiate the grafting reactions. Superiority of this technique over others has been well discussed in earlier studies [6,21–30].

1.4. Plan of investigation

The paper details the grafting of polyacrylamide chains (PAM) onto the backbone of casein, thus resulting in formation of polyacrylamide grafted casein (CAS-g-PAM). The synthesis has been carried out by *microwave initiated method* i.e. using only microwave irradiation for generating free radical sites on the backbone of casein. The synthesized grafted product has been investigated for flocculation efficacy in coal fine suspension towards its applicability in coal washery effluent treatment. The synthesis of CAS-g-PAM and its subsequent application as flocculant has not been reported earlier, to the best of our knowledge.

2. Materials and methods

2.1. Materials

Casein was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Acrylamide was procured from Sisco Research Laboratory, Mumbai, India. Acetone was purchased from Rankem, New Delhi, India. All the chemicals were used as received, without further modification.

2.2. Synthesis of graft copolymer

2.2.1. Microwave initiated synthesis of polyacrylamide grafted casein (CAS-g-PAM)

A suspension of 1 g of casein in 40 ml of distilled water was prepared. Desired amount of acrylamide diluted in 10 ml water was added to the casein suspension. The reaction mixture was transferred to the microwave reactor (Catalyst^{TM} systems CATA 4 R) (supplementary Fig. 1), stirred well and irradiated with microwave radiation (700 W) for the intended amount of time (3–5 min), keeping the irradiation cut-off temperature at 70 °C. The irradiation time was strictly maintained, compensating for the cut off time. Once the microwave irradiation procedure got complete, the gel like mass left in the reaction vessel was cooled and poured into excess of acetone. The precipitated grafted polymer was collected and was dried in hot air oven. Subsequently, it was pulverized and sieved. The synthesis details of various grades of the graft copolymer have been shown in Table 1. For each of the grades thus synthesized, the percentage grafting was evaluated as:

$$\%\text{Grafting} = \frac{\text{Wt. of graft copolymer} - \text{Wt. of Protein}}{\text{Wt. of Protein}} \times 100$$

2.2.2. Purification of the graft copolymer by solvent extraction method

Any occluded PAM formed due to competing homopolymer formation reaction was removed by extraction with acetone for 24 h. The synthesis procedure of the graft copolymer has been summarized in Scheme 1(a).

2.3. Characterization

2.3.1. Intrinsic viscosity measurement

Viscosity measurements of the polymer solutions were carried out with an Ubbelodhe viscometer (Constant: 0.003899) at 25 °C. The viscosities were measured in aqueous solutions at neutral pH. The time of flow for solutions was measured at four different concentrations (0.1%, 0.05%, 0.025% and 0.0125%). From the time of flow of polymer solutions (t) and that of the solvent (t_0, for distilled water), relative viscosity ($\eta_{rel} = t/t_0$) was obtained.Specific