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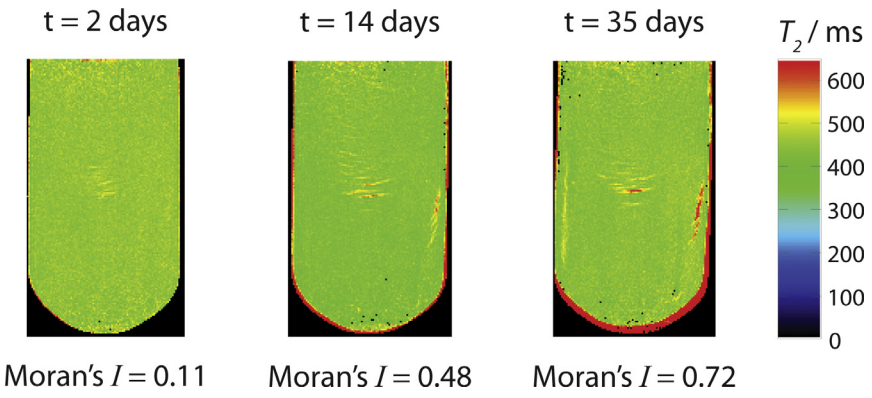
Regular Article

Characterisation of heterogeneity and spatial autocorrelation in phase separating mixtures using Moran's *I*

Emma S. Thompson [a], Pieter Saveyn [b], Marc Declercq [b], Joris Meert [b], Vincenzo Guida [b], Charles D. Eads [c], Eric S.J. Robles [d], Melanie M. Britton [a,*]

[a] School of Chemistry, University of Birmingham, Birmingham B15 2TT, UK
[b] Procter & Gamble Brussels Innovation Center, 1853 Strombeek Bever Temselaan 100, Belgium
[c] Procter & Gamble Company, Mason, OH 45040, USA
[d] Procter & Gamble Company, Newcastle Innovation Center, Newcastle-Upon-Tyne NE12 9TS, UK

GRAPHICAL ABSTRACT



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ABSTRACT

In complex colloidal systems, particle-poor regions can develop within particle-rich phases during sedimentation or creaming. These particle-poor regions are overlooked by 1D profiles, which are typically used to assess particle distributions in a sample. Alternative methods to visualise and quantify these regions are required to better understand phase separation, which is the focus of this paper. Magnetic resonance imaging has been used to monitor the development of compositional heterogeneity in a vesicle-polymer mixture undergoing creaming. T_2 relaxation time maps were used to identify the distribution of vesicles, with vesicle-poor regions exhibiting higher T_2 relaxation times than regions richer in vesicles. Phase separated structures displayed a range of different morphologies and a variety of image analysis methods, including first-order statistics, Fourier transformation, grey level co-occurrence matrices and Moran's *I* spatial autocorrelation, were used to characterise these structures, and quantify their heterogeneity. Of the image analysis techniques used, Moran's *I* was found to be the most effective at quantifying the degree and morphology of phase separation, providing a robust, quantitative measure by which comparisons can be made between a diverse range of systems undergoing phase separation. The sensitivity of Moran's *I* can be enhanced by the choice of weight matrices used.

* Corresponding author.
E-mail address: m.m.britton@bham.ac.uk (M.M. Britton).

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1. Introduction

Many consumer and pharmaceutical products are based on colloidal suspensions [1–3], but density differences between the particles and fluid can lead to sedimentation or creaming which will affect the "shelf-life" of such products. Polymers are frequently added to suspensions to enhance the shelf-life of the product, as well as its efficacy or dispensability [2]. However, adding a non-adsorbing polymer can cause particles to aggregate, via depletion flocculation, causing a gel to form and changing the rate and mechanism of phase separation [3]. By observing suspensions over time, it is possible to better understand phase separation processes, enabling the sedimentation and re-suspendability of a system to be assessed and predicted [1]. For example, creaming in vesicle-polymer mixtures has been studied by using visual inspection [3,4] and optical characterisation [5] to monitor the development of the vesicle-poor, vesicle-rich interface over time. However, visualising phase separation in these suspensions is often difficult, as they are frequently opaque [1].

Magnetic resonance imaging (MRI), however, provides a means by which sedimentation and creaming can be observed, non-invasively, in opaque systems [1,2,6–13]. Previously, ^{1}H MR images of spin density have been used to study sedimentation of polymer particles [2,6] and paliperidone palmitate particles [1], and T_2 relaxation time maps have been used to study sedimentation of polymer particles [10,11], glass beads [10] and rayon fibres [9] as well as separation of asphaltenes from crude oil [7] and phase separation in moisturising creams [8]. Chemical shift imaging has also been used to investigate sedimentation and creaming in biodiesel [12] and multinuclear (^{1}H and ^{19}F) MRI has been used to study sedimentation of polymer particles [13]. In these studies, phase separation has been predominantly probed using vertical profiles of MRI signal intensity, relaxation time or volume fraction, identifying regions either *rich* or *poor* in suspended particles. By monitoring these profiles over time, the rate of phase separation can be determined [1,2,7]. However, this approach assumes that the volume fraction of particles only varies vertically, controlled by the direction of gravity, and that there is a complete separation of the different phases, which may not be the case [3,6]. Particle-poor regions within the particle-rich phase have been identified in sedimentation [6] and creaming [3,4] in colloidal suspensions, particularly in suspensions with viscoelastic properties [6]. Yet, these regions are overlooked in 1-dimensional (1D) profiles and analysis, but must be visualised and quantified in order to gain meaningful insight into phase separation processes [6].

By using 2-dimensional (2D) MRI, phase separation can be better monitored and, through image analysis, quantified. An example of such image analysis is segmentation [14], where regions of similar composition can be identified within a sample by comparing the signal intensity or T_2 relaxation time, for a pixel, to a threshold value. This method could provide a means by which the amount of phase separated material may be quantified and can lead to an increase in image contrast and a simplification of features within the MR image [14,15]. Other forms of segmentation include cluster analysis [16], which looks for regions of homogeneous signal intensity, and independent component analysis (ICA) [16,17], which identifies statistically independent groups of pixels. However, the information available by segmentation can be limited and is dependent on the technique chosen. Alternative image analysis techniques include methods based on Fourier transformations or wavelet transformations [18], which determine the spatial frequency of heterogeneity in pixel intensities. These methods can distinguish both fine (high frequency) and coarse (low frequency) features in an image [19]. For example, wavelet transformation allows spatial information to be extracted at specific length scales [20] and can be monitored over time. However, these methods do not easily provide a quantifiable measure by which samples can be compared.

Image analysis methods based on first order statistics have been employed in medical imaging [21], and can be used to describe the distribution of pixel intensities and differentiate between homogeneous and heterogeneous images. Unfortunately, they are unable to provide information about the relative position of features or their connectivity [21]. First order statistics of MRI data have been used to distinguish between healthy and tumorous brain tissue [22]. Other statistical methods include grey level run length matrices (GLRLM) and grey level co-occurrence matrices (GLCM) which assess the probability that specific grey levels occur within a specified spatial relationship and allow the calculation of various parameters, such as local homogeneity, contrast and entropy of the pixel intensities [21,23,24]. However, the number of grey levels chosen in these analyses may affect the result. While using fewer grey levels makes the calculation less computationally demanding, it can also result in a loss of image detail [25].

Image analysis methods using segmentation, transformation and statistical methods have yielded useful information from MR images, however, they can discard useful information about the spatial localisation of the features, be difficult to interpret or over simplify features within an image. An approach used to overcome the limitations of these image analysis methods, is autocorrelation, which quantifies the heterogeneity or clustering in an image. Spatial autocorrelation determines whether an observed variable, at a particular location, is significantly dependant on the value of that variable in a neighbouring region [26]. By quantifying the spatial autocorrelation of a parameter, it is possible to determine whether the data is clustered, as well as quantify how strongly it is clustered [16]. There are a variety of measures of spatial autocorrelation including Moran's I [27], Geary's C [28] and Getis and Ord Gi^* [29], enabling the spatial distribution of a variable to be quantified using a single number. The most widely used of these measures is Moran's I, which has been applied to analyse optical images [30], X-ray CT images [31–33] and clinical MR images [16,30,31,34,35]. This method provides a simple means of assessing the degree of spatial autocorrelation, with values ranging from −1, for negative correlations, to 1, for positive correlations. As shown in the images in Fig. 1, increased clustering leads to higher values of Moran's I. Where pixel intensities are randomly distributed, Moran's I is equal to 0, and more alternating features lead to lower, more negative, values of Moran's I [34,36].

In the MRI studies, Moran's I has been used to assess noise levels [30,31,35], study neural networks [16] and distribution of fat in muscles [34]. In the study by Derado et al. [16], Moran's I was used to investigate neural networks, which were identified using segmentation techniques. Spatial clustering of fat, in MR images of muscles, has also been quantified using Moran's I [34]. However, Moran's I has not yet been employed to quantify the amount of heterogeneity within an MR image or characterise the compositional heterogeneity of complex fluids.

In this paper, a vesicle-polymer mixture undergoing creaming was visualised over time using 2D MRI, which revealed vesicle-poor regions with the vesicle-rich phase. The resulting 2D MR images were analysed using first-order statistics, Fourier transformations, GLCM and Moran's I. The results were compared between all image analysis methods and their potential for quantifying phase separation and spatial heterogeneity was assessed. A detailed description of the Moran's I calculation is presented and explained. Different spatial weight matrices were evaluated and it was found that careful selection of the spatial weight matrix made it possible to quantify smaller structures than have been previously accessible [34] using this method.