while transitioning solvents during flow. Polystyrene resin loading is limited to about 300 µmol/g, because of the need to limit resin swelling and thus limit pressure drop through the resin bed.

Accordingly, it would be an improvement to find a new way to use SPOS, that would address one or more of these deficiencies. It would be a special improvement to find a SPOS system that could be used at a commercial scale for oligonucleotides for large volume products, for example multiple metric tons per year. It would be a further advancement if such a system could be more environmentally friendly and reduce manufacturing costs and overall be more efficient. The present embodiments solve one or more of these deficiencies.

SUMMARY

In an aspect of the present disclosure, there is provided a method of adding an oligonucleotide to a solid support within a bed reactor, the method comprising:

removing a protecting group from the 5’ position of an oligonucleotide that is attached to the solid support;

adding an activated amidite solution to the bed reactor, wherein the activated amidite solution comprises an amidite and flows up and down within the bed reactor or fluidizes with nitrogen bubbling or other agitation and reacts at the 5’ position of the oligonucleotide, wherein the phosphorous linkage found within the amidite comprises a P atom that is in an oxidation state of III; and

converting the P atom from an oxidation state of III to an oxidation state of V.

In another aspect of the present disclosure, there is provided a system for adding an oligonucleotide to a solid support comprising a bed reactor and an activated amidite solution, wherein the activated amidite solution comprises an amidite and flows up and down within the bed reactor or fluidizes with nitrogen bubbling or other agitation.

The present examples involve a method of adding a phosphoramidite to a solid phase resin within a bed reactor in which a protecting group is removed from the 5’ position of an oligonucleotide and the coupling an activated amidite solution to the unprotected group, wherein the activated amidite solution comprises an amidite and fluidizes the resin in the reactor. Fluidization may occur by forcing the liquid to flow up and down within the bed reactor, bubbling an inert gas, or other type of agitation to create a slurry. The amidite reacts at the 5’ position of the oligonucleotide.

In addition to the coupling reaction, reagent solutions for deblocking, oxidizing, thiolating, and capping may each be fluidized with the resin to provide complete liquid/solid contacting and re-set the resin bed with no channels. The fluidization may be followed by plug flow reaction with reagent flow in the downward direction through the resin bed as is typical of conventional SPOS. The same fluidization portion followed by plug flow portion may be done for the solvent washes after each reaction. In this manner, the majority of the resin swelling and shrinking may take place during the fluidization portion of the solid/liquid contacting, where it is advantageous to overcome pressure drop and eliminate channeling.

BRIEF DESCRIPTION OF THE DRAWINGS

The features and advantages of the present disclosure will become more apparent to those skilled in the art upon consideration of the following detailed description taken in conjunction with the accompanying figures.